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Biomimetic surfaces for greener coastal infrastructures: an analysis of factors that contribute to make artificial structures more "natural"

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Abstract

The growing ecological awareness for impacts due to the proliferation of marine infrastructure is promoting the adoption of eco-engineering strategies to make these novel substrates more valuable habitats to natural communities. Biomimicry, as a tool of eco-engineering, aims to design infrastructure more suitable for wildlife by manipulating structural factors to mimic natural habitats. However, little is known about the extent to which natural and artificial substrates differ in their structure and to what extent such differences affect the biota.

To fill these knowledge gaps, in the Second Chapter, I quantified the divergence in physical habitat structure (quantified in terms of inclination, exposure, roughness, abundance and diversity of morphological elements) across a combination of natural and artificial habitats of regular and irregular morphologies (seawalls = artificial regular; cliffs = natural regular; breakwaters = artificial, irregular; and boulder fields = natural irregular) in the North Adriatic Sea. Results revealed a similarity among substrates greater than expected. Only inclination was consistently steeper in artificial than in natural habitats, while seawalls tended to provide a greater abundance of landward sheltered habitats. Other minor differences in roughness or in the abundance of some surface microelements were related to the general morphology (regular or irregular) of the habitat rather than to its artificial or natural identity.

In the Third Chapter, I related the characteristics of physical structure in each habitat to the composition and abundance of epibenthic communities by performing a field experiment. Differently from what was found for the physical components of the habitats, epibenthic communities showed marked differences between natural and artificial substrates, and were particularly distinct in seawalls. Overall the physical structure of the habitat explained a significant but limited (<10 %) proportion of the differences in the epibenthic community structure, which, consistently with the findings of Chapter 2, was mostly related to the effects of inclination and exposure. These findings suggested that other factors could play a major role in producing the observed differences in the biota.

As physical structure explained only a limited proportion of the differences in biota between artificial and natural habitats, I explored how much the differences in material composition between artificial and natural habitats could contribute to structure the epibenthic communities in the Fourth Chapter, by using a systematic review along with a meta-analysis. I compared the material types most commonly used to build infrastructures (i.e. natural rocks, concrete, metal, plastic, ceramic, rubber and wood), aiming to identify which building material is associated with the greatest abundance and diversity of colonizing organisms. Although the overall influence of material type was quantitatively limited, rocky-type substrates, including concrete in its various formulas, consistently supported more abundant and diverse epibenthic communities than materials such as metal, plastic, and rubber. The effects were particularly evident for fouling species (algae and sessile invertebrates), while vagile taxa did not respond significantly to the material composition. The choice of building material adds up as another factor to consider to obtain more sustainable infrastructure, since an informed choice can promote ecological benefits at multiple levels, combining the ecological enhancement *in situ* with the reduction of other environmental impacts (raw material demand, energy use, carbon footprint) during the production process.

In the Fifth Chapter, I combined the previous findings to design, in collaboration with a company involved in the additive manufacturing sector (Desamanera), a new improved 3D printed biomimetic artificial surface and tested its performance in the field with an experiment, currently in the preliminary results stage. The experiment tested the combined role of three factors, which included the shape of the tiles (biomimetic vs flat), the brightness of the material (dark vs light), and the inclination at which tiles were installed (sloped vs vertical). The experiment aimed to explore how combining multiple factors, each explaining a limited but significant proportion of the structure of epibiota, can produce benefits in several areas, including the increase in ecological value, the reduction of impacts during the production process, and the improvement of the aesthetic value. Preliminary results are reporting weak differences in total cover between shapes and between brightnesses, although the early stages of colonization are widely covering each tile type.

Overall, this thesis suggests that biomimicry has the potential to increase the ecological value of artificial habitats especially when a wide range of aspects is simultaneously considered. Indeed, none of the

structural factors, individually, can fully mimic the “natural conditions” to effectively improve the ecological performance of the artificial substrates. This emphasizes the need to include in future works a multi-level perspective to fully achieve the great potential of biomimicry.

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Chapter 1 – General Introduction

1.1 Ocean sprawl –a global problem

Human beings have always settled and prospered near the water, including coastal areas, exploiting the considerable advantages here provided for livelihood and commercial purposes. The growing demands of modern society have led to a progressive acceleration of the exploitation of the ecosystem services in coastal areas (Costanza et al., 1997; Airoldi and Beck, 2007; Beaumont et al., 2007). To support these growing demands, coastal areas have seen natural habitats replaced by a rapid proliferation of anthropogenic infrastructures, which is expected to continue in the future (Bugnot et al., 2021; Floerl et al., 2021). Marine infrastructure comes in a variety of designs, materials and spatial configurations to perform a range of functions including coastal protection, commercial support, boat shelter, tourism and freight transport (Dugan et al., 2011; Firth et al., 2016; Strain et al., 2019). This rapid coastal urbanization, and the related proliferation of artificial structures, is called "ocean sprawl" (*sensu* Duarte et al., 2013; Firth et al., 2016; Bishop et al., 2017). Today, coastal urbanization affects more than two million km^2 of seabed (Bugnot et al., 2021), has armored more than 50% of the shoreline (Floerl et al., 2021) and hosts more than 40% of the world population as well as 60% of the large megacities (Martínez et al., 2007; McGranahan et al., 2007).

Over the years, coastal urbanization has had strong negative repercussions on the natural environment, which has seen its ecosystem services substantially jeopardized (Costanza et al., 2014). Interacting with the consequences of global climate change (e.g. rise in the average sea level, increase in the frequency of extreme climatic phenomena; IPCC, 2014), the loss of ecosystem services in coastal environments is also having dramatic consequences on the world population which directly or indirectly relies on marine and coastal environments for their livelihood and well-being.

1.2 Ecological impacts of ocean sprawl

Coastal urbanization and related anthropogenic activities are burdening marine ecosystems due to the little ecological consideration for the exploitation of resources, the production of pollutants and the destruction and fragmentation of natural habitats by infrastructure (Todd et al., 2019). The impact produced by coastal infrastructure on the marine ecosystems is diversified (Bruun, 1972). First of all, the construction of coastal infrastructures produces an environmental impact at every stage of the construction process, from obtaining raw materials (Padmalal et al., 2008), through all the industrial processes, up to the final energy cost of the whole process (Cooke et al., 2020). Overall, the carbon footprint on a global scale associated with the construction of concrete structures, both marine and terrestrial, amounts to 8-9% of CO_2 emissions (Cooke et al., 2020). The infrastructure also affects directly the marine ecosystems, causing a direct loss of native habitat and associated biodiversity, by fragmenting it, destroying it or transforming it (Airoldi et al., 2005, 2009). Alteration of natural habitats by infrastructures is particularly emphasized in sedimentary environments (Heery et al., 2017) since artificial substrates support a community of organisms completely different from pre-existing environments (Bacchiocchi and Airoldi, 2003) and alter the connectivity of populations by acting both as barriers and corridors (Airoldi et al., 2015; Bishop et al., 2017). Furthermore, the impacts of infrastructures reverberate beyond the place in which they are located through a “halo” effect (Posey and Ambrose, 1994; Bugnot et al., 2021), affecting neighboring ecosystems in different ways (i.e. hydrodynamics, spilling of contaminants, noise and light pollution) and thus extending to wider spatial scales (Bulleri and Chapman, 2010; Todd et al., 2019). Finally, even if the infrastructure is placed on a hard-bottom environment, it will rarely result similar to native habitats in terms of biodiversity (Moschella et al., 2005), presence of non-indigenous species (Airoldi et al., 2015), genetic diversity (Fauvelot et al., 2009) or beta diversity (Porter et al., 2018). Overall, the way marine infrastructure is currently designed limits their ecological performance as novel habitats, making them poor surrogates for the natural habitats they replace.

1.3 Causes of the poor ecological performance of artificial structures

Many structural and non-structural drivers could contribute to the poor ecological performance of artificial infrastructures, limiting their capability in supporting biodiversity and ecosystem services that are as close as possible to those provided by natural hard habitats. Artificial substrates differ from natural habitats for many factors, including composition, morphology, location, or age (Bulleri and Chapman, 2010). Some of these factors (e.g. location) are established because of the primary engineering scope of the structure (e.g. coastal defence; Airoldi et al., 2005) and could be more difficult to modify, however other components could be more easily adjusted so that structures could be designed targeting additional multifunctional goals and supporting valuable ecosystem services (Dafforn et al., 2015). Wide differences were observed between the biota settled on natural rocky substrates and that on infrastructures built with non-natural materials (i.e. plastic, metal, concrete; Connell and Glasby, 1999) or that are intrinsically different in shape and position (i.e. floating pontoons; Connell, 2000). On the other hand, on infrastructure built with local natural materials (i.e. sandstone seawall along sandstone shore; Chapman, 2003), or characterized by more heterogeneity of microhabitats (Bulleri and Chapman, 2004; Sedano et al., 2020), communities resulted less dissimilar to those settled on natural rocky reefs.

Scalable differences in observed communities as structural differences increase (Bulleri and Chapman, 2004; Sedano et al., 2020) suggest that manipulation of these factors in the eco-design of artificial substrates could be an effective tool to promote their ecological performance (Pioch et al., 2018) and, hence, to enhance their multifunctionality (Dafforn et al., 2015).

1.4 Ecological amelioration of grey infrastructures: the biomimicry

Growing awareness about the impacts of ocean sprawl (Lotze et al., 2018; Strain et al., 2019) is leading to a greater interest by stakeholders in eco-engineering countermeasures (Morris et al., 2016; Evans et al., 2017; Kienker et al., 2018). Eco-engineering aims to integrate societal and conservation targets via an improved design of the built environments (Mitsch and Jorgensen, 1989). Due to the expansion of marine infrastructure and considering that many older infrastructures need to be upgraded or renewed to face the effect of both aging and global climate change (i.e. sea level rise; Mamo et al., 2020), the near future is a crucial moment to promote this new and more sustainable way of urbanizing. Eco-engineering strategies promote the ecological value of infrastructures while meeting engineering standards, with eco-designs inspired by or mimicking nature (i.e. biomimicry, *sensu* Benyus, 1997; Pioch et al., 2018). Enhancement of the morphological complexity with prefabricated panels (Borsje et al., 2011; Loke and Todd, 2016), modification of substrate slope or the addition of small to large horizontal elevations (Chapman and Underwood, 2011), the retrofitting of key microhabitats (e.g. crevices, pools, etc.; Firth et al., 2014; Evans et al., 2016; Strain et al., 2021), or the use of materials with physiochemical properties similar to natural substrates (Dennis et al., 2018) are, to date, the types of manipulations most commonly reported in eco-engineering biomimetic interventions (reviewed in Schoonees et al., 2019 and O'Shaughnessy et al., 2020). These manipulations aim to reduce the stressors related to the lack of "natural conditions" by providing, for example, water retention and shading against thermal and desiccation stresses (Evans et al., 2016; Loke et al., 2019), by increasing the availability of niches and shelters against predators (Coombes et al., 2015; Loke and Todd, 2016), by damping waves action against dislodgment of organisms (Loke and Todd, 2016; MacArthur et al., 2019), or by attracting the settlement through chemical and physical cues (Anderson, 1996). Overall, these interventions have been demonstrated to support biodiversity on artificial structures, although the reported benefits were highly variable and context-dependent (Strain et al., 2018).

1.5 Gap in knowledge

This variability in the ecological outcomes of current eco-designs raises questions about their generalizable effectiveness and highlights the need for a critical analysis of the structural drivers that can contribute to make artificial structures more “natural”. It is plausible that most biomimetic designs, albeit based on correct ecological principles (i.e. the increase of available niches; Hutchinson, 1957), rely on generalized or assumed considerations about what “natural conditions” are and how man-made structures have to mimic them. For example, the lack of the structural complexity inherent to natural reefs in artificial substrates is frequently considered one of the main factors causing the lack of biodiversity in the artificial habitats (Bulleri and Chapman, 2004; Moschella et al., 2005; Burt et al., 2009; Firth et al., 2014). However, there have been only a few comparisons of the differences in complexity among natural and artificial habitats (Aguilera et al., 2014), which showed variable differences depending on the considered type of artificial habitat (Lawrence et al., 2021). In addition, the effect of structural drivers on organisms was mostly investigated by comparing extreme conditions (i.e. vertical vs. horizontal substrates; landward vs seaward orientations; flat vs rough surfaces; Moschella et al., 2005), possibly overweighting the role of these factors. Conversely, the more realistic variability in the morphology and structure of the artificial habitats, as well as the cumulative contribution of different structural factors, have not been considered. Filling in these knowledge gaps would help provide site-specific information on how to effectively upgrade infrastructures to achieve multifunctionality and define more clearly the goals of biomimicry.

1.6 Thesis aims and thesis structure

The purpose of my PhD was to develop knowledge to design new biomimetic surfaces to increase the ecological potential as habitats of the artificial infrastructures along the Italian coasts of the northern Adriatic. To achieve this goal, I first focused on identifying the extent of the structural differences, in terms of morphology, spatial complexity and composition, that exist between artificial and natural substrates, and how these differences contribute to shaping the associated epibenthic communities. After that, I combined the knowledge to develop novel biomimetic surfaces that I am currently testing in the field to quantify the ecological and aesthetic benefits.

The work has been organized into five chapters, structured as follows.

Chapter 2: I quantified the physical structure of different morphologies of natural and artificial habitats along the Monte Conero promontory, and compared it across habitat types (artificial vs natural) and morphologies (regular vs irregular) and across multiple spatial scales. The physical structure was assessed by using five descriptors: inclination, exposure, roughness, and abundance and diversity of surface morphological microelements. This study tested the widespread assumption that artificial habitats lack the physical structure proper to natural habitats, and quantified the differences in physical structure between various natural and artificial coastal habitat types, thus providing information on how to include this structural component during future biomimetic applications. This chapter has been published in the journal Frontiers in Marine Science (Grasselli and Airoldi, 2021)

Chapter 3: I quantified the composition, abundance and structure of epibenthic communities that grow on different morphologies of natural and artificial habitats along the Monte Conero promontory, and related them to the physical structure of these habitats, previously characterized in Chapter 2, to define the contribution of this structural component to the differences in the epibiota. By identifying the role played by the physical structure in shaping the biota, we provided information on the benefits expected by biomimetic approaches focusing on structural habitat enhancement only. This chapter is a manuscript in preparation for Journal of Applied Ecology.

Chapter 4: Due to the Covid-19 pandemic which precluded me from performing an original experiment, I conducted a systematic review, supported by a meta-analysis, on the effects of material composition in shaping the biodiversity of epibenthic organisms across a variety of environmental settings. I extracted data from 76 selected studies, to test the effects of the most common materials used to build infrastructures (i.e. natural rocks, concrete, metal, plastic, ceramic, rubber and wood) in affecting the epibiota. The findings defined which type of material has the most similar performance to natural rock substrates. This chapter is a manuscript in preparation for Coastal Engineering.

Chapter 5: I developed a manipulative experiment, combining the knowledge acquired in the previous chapters, to outline the response of the epibenthic community in a highly urbanized context (the Ravenna port) to the manipulation of three different factors in artificial habitats, specifically inclination, surface morphology and color/brightness. The materials used to cast the experimental surfaces were chosen to mimic as much as possible some of the characteristic features of the natural rocks in the region, as well as to reduce some other impacts in the production process by recycling local natural materials in a circular economy context. The aim is to explore whether and to what extent biomimicry can combine benefits at different levels: in ecological enhancement, in reduction of the impacts of the production process and in aesthetics amelioration. This study is currently ongoing. The chapter reported preliminary results.

Chapter 6: I reported a synthesis of the main results obtained during the PhD project as well as the conclusions and the considerations to which these results led me and the possible future directions.

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Chapter 2 – How and to what degree does physical structure differ between natural and artificial habitats? A multi-scale assessment in marine intertidal systems [1]

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Abstract

Marine infrastructures are increasing, generating a variety of impacts and introducing artificial habitats which have low ecological value and support assemblages that differ significantly from those on natural rocky coasts. While in the past there was little ecological consideration as to how artificial structures were built, now the trend is to look for “greener” designs inspired by or mimicking nature. These greening efforts have had a strong focus on enhancing physical habitat structure to support more diverse assemblages, driven by the untested assumption that artificial habitats lack the physical structure proper to natural habitats. We tested this assumption by comparing five descriptors of physical structure (inclination; exposure; roughness; abundance, and diversity of surface morphological microelements) across a combination of natural and artificial habitats of regular and irregular morphologies (seawalls = artificial regular; cliffs = natural regular; breakwaters = artificial, irregular; and boulder fields = natural irregular) in the North Adriatic Sea. Most structural descriptors were similar between artificial and natural habitats. Only inclination was consistently steeper in the artificial than in the natural habitats. Other minor differences in roughness or in the abundance of some surface microelements were related to the general morphology (regular or irregular) of the habitat rather than to its artificial or natural identity. The outcomes challenge the widespread assumption that artificial habitats lack the physical structure proper to natural habitats and stimulate renewed consideration about other structural and non-structural elements that could enhance the performance and sustainability of artificial marine structures, such as construction material, environmental setting or maintenance. They also encourage a wider reflection about what makes an artificial building surface “greener”: structural complexity is an important ecological parameter, and its deliberate increase will lead to responses in the biota, however, this may not necessarily match “more natural” conditions.

Keywords: artificial marine structure, greening of gray infrastructure, habitat complexity, intertidal rocky shore, spatial variability, structure from motion, surface design, topography

2.1 Introduction

Urban waterfronts are replete with hard built infrastructure for coastal protection, trade, transportation, and recreation (e.g., seawalls, jetties, breakwaters, groins, docks, pontoons; Airoldi and Beck, 2007). This infrastructure comes in a variety of designs, materials and spatial configurations, depending on the primary function (Firth et al., 2016). It has a clear construction footprint on native habitats, causing permanent modification in the abiotic and biotic state around and on the structure (Heery et al., 2017), and it provides habitat that consistently performs poorly compared to natural reefs (Moschella et al., 2005; Perkol-Finkel et al., 2006; Carvalho et al., 2013), supporting distinct assemblages with low species and genetic diversity (Fauvelot et al., 2009) and dominance by opportunistic and invasive species (Bulleri and Airoldi, 2005; Airoldi et al., 2015). Recent estimates suggest that marine infrastructure globally modifies 2,072,603 km^2 of the seafloor, which makes up 1.5% of the global coverage of the ocean exclusive economic zones (Bugnot et al., 2021).

While in the past there was little ecological consideration as to how marine artificial infrastructure was built (Airoldi et al., 2005), now the trend is to look for "greener" designs inspired by or mimicking nature, that introduce less alterations to the natural environment, support wildlife, or recover critical ecosystem functions (Firth et al., 2014b; Dafforn et al., 2015; Airoldi et al., 2021). These greening efforts have had a strong focus on physical habitat structure as a key factor for enhancing natural communities (Loke et al., 2015; Morris et al., 2017). Ongoing pilot projects have increased surface area, microhabitats available and/or topographic complexity by including for example pools, pits, and ledges in concrete walls, or retrofitting planters and panels with microhabitats on existing infrastructure (reviewed in Schoonees et al., 2019; O'Shaughnessy et al., 2020). A recent meta-analysis has shown that these enhancements often increased the abundance and number of species, yet the outcomes were inconsistent across species and studies, with overall uncertain benefits (Strain et al., 2018b).

The explicit and/or implicit assumption of greening projects that focus on structural enhancements is that artificial infrastructure lacks the physical structure proper to natural reefs, which would impair a variety of

species (Bulleri and Chapman, 2004; Burt et al., 2009; Firth et al., 2014b; Dafforn et al., 2015). However, an extensive search in the scientific literature revealed a lack of formal quantitative comparisons of structural physical characteristics between natural and artificial habitats, with the only exception of Aguilera et al. (2014). We also noticed that topographic heterogeneity was especially emphasized as a limiting factor for the biota by papers dealing with morphologically regular seawalls (Bulleri and Chapman, 2004; Bulleri, 2005; Morris et al., 2017). Conversely, papers dealing with irregular infrastructures (i.e., built with quarried rocks or molded concrete elements) highlighted other potentially limiting factors for the biota, including greater pressures from fish (Ferrario et al., 2016; Gianni et al., 2018), greater disturbances (Airoldi and Bulleri, 2011), or a greater influence of the surrounding sedimentary environment (Franzitta and Airoldi, 2019; Komyakova et al., 2019). Finally, although physical habitat structure affects organisms differently at multiple spatial scales (Kovalenko et al., 2012; Loke et al., 2015), multi-scale measurements are still rare in artificial habitats, and most studies have focused on very small spatial scales from centimeters to meters (Strain et al., 2018b).

Here we present a multi-scale, quantitative comparison of physical structure among a variety of artificial and natural hardbottom intertidal habitats along the Monte Conero promontory (North Adriatic Sea, Italy) aiming to test the general hypothesis that physical structure differs between artificial and natural habitats. Physical structure is an emergent property that can be associated to numerous key physical components of the habitat. Different terms have been used interchangeably to describe it (e.g., “heterogeneity” or “complexity”), and there are not univocal metrics to quantify it (Stein et al., 2014; Loke et al., 2015). We focused on a group of descriptors of physical surface properties that are most often claimed to be limiting for the growth of biota in artificial habitats and that could be more easily translated into design enhancement in ecoengineering applications (sensu Morris et al., 2019), specifically: (i) inclination; (ii) exposure; (iii) roughness; and (iv) abundance, and (v) diversity of surface morphological microelements like crevices, holes, grooves and pits. We tested the hypothesis that these descriptors differed between natural and artificial habitats not only in their average values but also in their variance distributions (Benedetti-Cecchi, 2003). Specifically: the inclination of artificial structures could be more vertical and more uniform

than that of rocky shores to minimize construction materials and the footprint on the seafloor; the exposure of artificial habitats could face prevailing winds and be more uniform than that of natural habitats to provide shelter from waves to coastal assets; and the roughness and abundance and diversity of surface morphological microelements of artificial habitats could be lower and more uniform than those of natural habitats due to design constraints.

Because in our search of the scientific literature we had observed that papers dealing with morphologically regular seawalls placed greater emphasis on the limiting role of physical structure than papers dealing with irregular infrastructures, we formally tested if the five structural descriptors differed across two broad morphologies of artificial and natural habitats: regular (artificial seawalls and rocky cliffs) and irregular (block armored infrastructures and rocky boulder fields). We hypothesized that the differences between artificial and natural habitats per se could be smaller than those observed between regular and irregular habitat morphologies.

We finally tested the hypothesis that structural differences between habitats and morphologies were consistent across different spatial scales. In landscape analysis, the spatial scale consists of two components, grain and extent, which both affect estimates of physical structure (Wiens, 1989). Here we focused on a relatively small and fixed grain size (a small sample quadrat that fitted the size of the blocks that are used for the construction of marine infrastructures), while tested the variations in structural descriptors across different spatial extents: small (centimeters to meters); meso (meters to 10 s of meters); and large (100 s meters to kilometers).

2.2 Materials and methods

2.2.1 Study Area and Field Sampling

This study took place over ≈ 8 km of coastline at Monte Conero promontory (Adriatic Sea, Italy, Figure 2.1). Here artificial rock-mold groins and breakwaters (hereafter both referred to only as "breakwaters") and concrete-mold seawalls, built to protect small marinas and tourist beaches, are interspersed with marl and limestone subvertical cliffs and boulder fields. A more detailed description of the characteristics of both artificial and natural habitats can be found in Perkol-Finkel et al. (2012). The study focused on the low-intertidal (≈ 0 to C 20 cm relative to mean low-water level, average tidal amplitude ≈ 30 cm), as this is where most eco-engineering tests are in progress (O'Shaughnessy et al., 2020; Strain et al., 2021).

In April 2019, we selected 12 interspersed sites, 100 s meters to kilometers apart (Figure 2.1), three for each of four habitats: seawalls (type artificial, morphology regular), cliffs (type natural, morphology regular), breakwaters (type artificial, morphology irregular), and boulder fields (type natural, morphology irregular). At each site, with the aid of a tape measure, we located four replicated areas 200 cm x 20 cm and 10 m apart. Within each area, we further located four replicated 10 cm x 10 cm sampling quadrats (hereafter plots), 50 cm apart. In total, we sampled 192 plots (4 plots per area, and 4 areas per each of 12 sites) to provide comparisons of physical structure across orthogonal combinations of two habitat types (artificial and natural) and two morphologies (irregular and regular), and across three spatial scales (small: differences among plots; meso: differences among areas; and large, differences among sites).

Before sampling, we removed any species from the plots, as we focused on the physical structure of the primary substrata. Then, in each plot we quantified (i) Inclination; (ii) Exposure; (iii) Roughness; and (iv) Abundance, and (v) Diversity of morphological elements. Inclination was estimated using a handheld clinometer positioned in the center of the plot. Exposure (i.e., aspect) was estimated using a compass positioned in the center of the plot and facing the sea. Exposure was not measured when Inclination was <10, as exposure of flat substrates is meaningless, which occurred at 9 plots in boulder fields.

Figure 2.1. Map with site locations for each combination of habitat Type (Artificial = black symbols, Natural = white symbols) and Morphology (Irregular = circle symbols, Regular = star symbols). The dashed cross represents the Due Sorelle site.

To estimate Roughness as well as Abundance and Diversity of morphological elements, we filmed each plot with a digital camera (Canon PowerShot G12 or S95 equipped with underwater housing, resolution 1280 x 720 pixels) as from Figueira et al. (2015), doing two 360 passes around the plot at distances of 30 and 15 cm, and then two perpendicular arc passes over the top of each plot. This allows to record the plot from every angle, and obtain high-quality overlapping stereo-photos for the subsequent reconstruction of high-resolution 3D structure model (Westoby et al., 2012).

2.2.2. Photogrammetric Analysis

The videos were analyzed using the Structure-from-Motion (SfM) photogrammetric technique (Westoby et al., 2012). The SfM workflow requires the selection from the video of an adequate number of partially overlapping and not-fuzzy photos to represent the surface from each angle. Preliminary tests identified in 25–30 the adequate number of stereo-photos to obtain a high-resolution 3D-model without slowing down

excessively the whole SfM procedure. For 12 plots (6 from breakwaters, 4 from boulder fields and 2 from cliffs) we did not obtain sufficient high-quality photos for further SfM analysis due to wave splashing.

A textured digital surface model of the plot was created from the stereo-photos using Agisoft Metashape Standard v. 1.5 software, setting the workflow as in Supplementary Table S2.1. Briefly, photos were aligned based on the shared invariant features at the highest accuracy possible. The position of each feature was triangulated to recreate a high-quality 3D dense point cloud, which was used to build a mesh of triangular polygons and obtain a continuous surface. The "height field (2.5D)" surface type was preferred to the "arbitrary (3D)" as recommended for planar surfaces. On this continuous surface, a fitted texture was applied from the original stereo-photos. When occasional misaligned points occurred, i.e., when waves splashed the plots, we performed a post-processing cleaning to correct the error and avoid a complexity overestimation (Bayley et al., 2019). Finally, the x-y-z orientation of each model was done manually, levelling the base of the plot on the x-y plane. Each digital 3D plot model was exported from Agisoft as a 3-columns XYZ ASCII file and imported in R v.3.5.3 to quantify surface Roughness, and Abundance and Diversity of morphological elements. Each ASCII file, representing a 3D plot model, was rasterized at a spatial resolution (cell size) of 1 mm^2.

Surface Roughness was computed as the ratio between the real plot surface (3D area), calculated with the "computeAreaRaster" function ("mkde" package), and its orthogonal planar projection (2D area). In this way, Roughness values ranged between 1 to a theoretical infinite maximal. We preferred surface roughness to other correlated measures (i.e., linear roughness, random roughness or fractal; Shepard et al., 2001; Kovalenko et al., 2012) because it is not biased by any specific placement of the linear paths (Figueira et al., 2015).

The Abundance and Diversity of discrete surface morphological elements were estimated using a landform classification based on the Topographic Position Index (TPI). TPI is an algorithm that measures the relative topographic position of each cell of the rasterized 3D plot models as the difference between the elevation at this cell and the average elevations of the adjacent cells within a predetermined neighborhood (Weiss,

2001). TPI was calculated with the "tpi" function in the "spatialEco" package (scale 0.5 cm, window type "circle," and normalization "TRUE"). A range of 0.5 cm was chosen as the best compromise between the average dimension of the colonizing organisms (and consequently of the structural elements perceived by them; Warfe et al., 2008), and the loss of assessed area at the edge of the model after the TPI is computed. Normalization was set to "TRUE" as suggested by De Reu et al. (2013) because TPI classification in complex surfaces could be biased by roughness. Thereafter, each cell was classified into one of six morphological elements (ridges, upper slopes, middleslopes, flat slopes, lower slopes and valleys, see Supplementary Figure S2.1) by thresholding their continuous TPI values using standard deviations units and checking the slope of the cell for TPI values near 0 (see Weiss, 2001 for details). The abundances of the morphological elements were expressed as percentage over the total number of cells, and a variation of the Shannon Diversity Index, the Shannon's Entropy 3D (H-3D), reported in the CASU software (Loke et al., 2014), was applied to provide an estimate of the surface morphological diversity. H-3D measures the relative abundance (pi) of the morphological elements as proportion of the surface area rather than of the total number of elements. This provides more meaningful H' values when the dimensions of the elements are heterogeneous, as in our case. Because we identified six Topographic "forms" using the TPI, the maximal value that H-3D could assume was 2.58 (Eq. 1), with maximal relative abundance (pi) of each element equal to 1/6.

$$Shannon\ 3\mathrm{D}\max = -\sum_{1}^{6}\frac{1}{6} * log_2\frac{1}{6}$$

2.2.3 Statistical analyses

Differences in the average descriptors of physical structure were analyzed using a four-way univariate permutational analyses of variance (PERMANOVA), including factors habitat Type (artificial vs. natural, fixed), Morphology (regular vs. irregular, fixed) crossed to Type, Site (three levels, random) nested in Type x Morphology, and Area (four levels, random) nested in Site. For Inclination, there were 4 replicates for each combination of factors. For Exposure replicates varied between 2 and 4, due to 9 missing values for horizontal substrata. Similarly, for Roughness and Abundance and Diversity of morphological elements

replicates varied between 2 and 4, due to missing values from 12 photos that could not be analyzed via SfM. We used PERMANOVA instead of classical ANOVA because of ease of use with unbalanced replication and because of lack of normality assumptions (Anderson et al., 2008).

The use of ANOVA is discouraged in circular statistics (Mardia and Jupp, 2000; Jammalamadaka and SenGupta, 2001), therefore Exposure could not be directly analyzed due to the vectorial essence of these complex numbers ($a + bi$, where a is the real part and bi the imaginary part). As a proxy, we quantified the angle formed between the Exposure of each plot and the prevailing exposure of the Monte Conero coastline. This was estimated from the map as 30° and 90° for the sites north to and south to the "Due Sorelle", respectively (Figure 2.1). We obtained non-vector values ranging from 0 (maximal concordance with coastline direction, seaward exposure) to 180 (maximal discordance with coastline direction, landward exposure), which expressed the plot deviations from the prevailing coastline Exposure. We used PERMANOVA C for PRIMER (Anderson et al., 2008) to partition the variability and obtain F-statistics on a matrix of Euclidean distances. P-values were calculated using 9,999 random permutations of the appropriate exchangeable units and Type III sums of squares. In case of few unique permutations (<100), Monte Carlo (MC) p-values replaced the permuted p-values in the analysis.

Differences in the variance distributions of the descriptors of physical structure at each spatial scale (small, meso and large) were analyzed following Dal Bello et al. (2017). For this analysis, we considered seawalls, breakwaters, cliffs and boulder fields as four levels of a single factor "Habitat". While this procedure did not test for the interaction Type x Morphology, it considerably simplified the analysis allowing to run a fully nested model. Briefly, for each physical descriptor, the "VarCor" function of "lmerTest" package (in R v.3.5.3) extracted the bias-corrected variance components associated to the three spatial scales for each habitat. We then calculated the maximal differences among these variance components (VarDIF-small, VarDIF-meso, VarDIF-large), which were each compared with a null distribution of values generated through bootstrapping (randomly reallocating samples 10,000 times) given that the null hypothesis is true (variance at each spatial scale is the same for all habitats). The observed variance components were deemed different across habitats if VarDIF at each scale exceeded the critical threshold (a = 0.05) obtained

from the null distribution (for details see Dal Bello et al., 2017). In case of significant differences, post hoc pairwise comparisons were performed, using a threshold of 0.01.

2.3 Results

2.3.1. Inclination

Artificial habitats were on average more vertical than natural habitats, and regular habitats were on average more vertical than irregular habitats (Figure 2.2A and Table 2.1). This made the seawalls the most vertical and the boulder fields the least vertical of the four habitat combinations explored, with an average plot inclination of 92.6 and 53.9, respectively. At the small and meso scales, regular habitats had more homogeneous inclinations than irregular habitats, and artificial habitats had more homogeneous inclinations than natural habitats (Figure 2.2B). Overall, seawalls were the most homogeneous at both the small and meso scales, while boulder fields had the greatest variability in inclination at the meso-scale (Supplementary Table S2.2). These differences, however, became negligible at the large scale, where all habitats showed consistent (i.e., low variance) inclinations at all sites (Figure 2.2B).

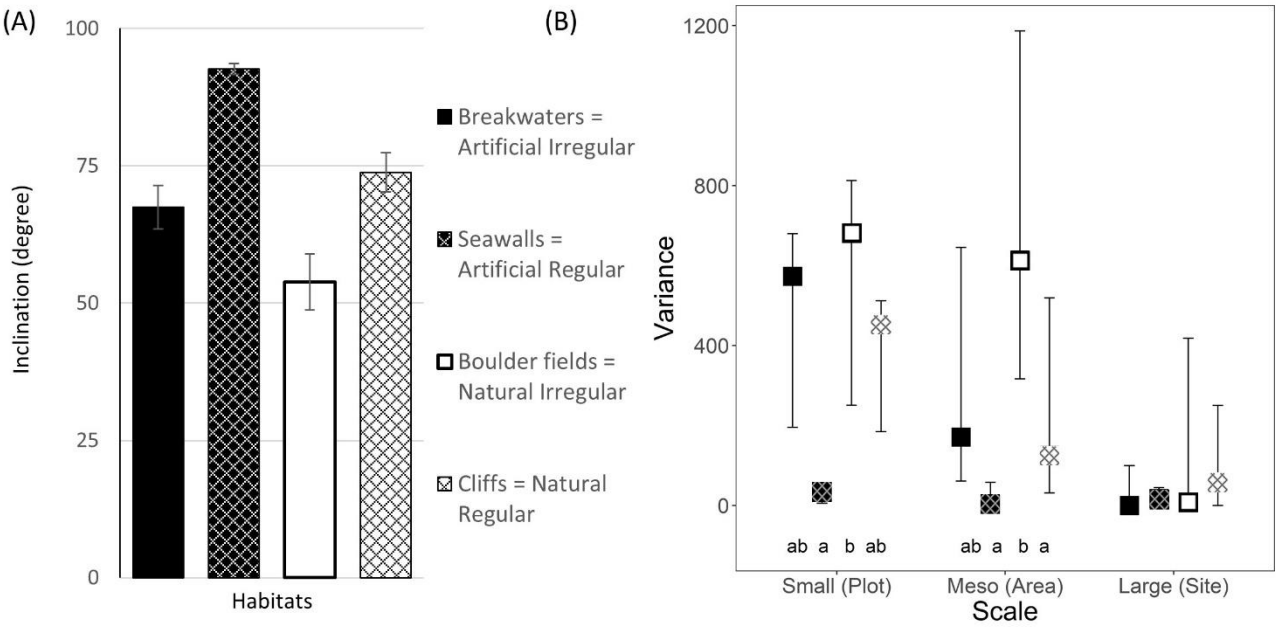

Figure 2.2. (A) *Mean inclination (±1 SE; data are averages of 4 plots at each of 4 areas at each of 3 sites, n = 48) and* (B) *estimated variance in inclination (±95% CI from bootstrapping) at each scale for the four combinations of habitat Types and Morphologies. Letters indicate significant differences from pairwise-tests (Supplementary Table S2.2).*

Table 2.1. Four-way PERMANOVA results testing the effects on Inclination, Exposure, Roughness and Diversity of morphological microelements. In bold are p-values <0.05.

	Inclination				Exposure			
Source	df	MS	Pseudo-F	p-value	df	MS	Pseudo-F	p-value
Type =T	1	12534	8.51	**0.023**	1	14864	1.38	0.267
Morphology = M	1	24207	16.44	**0.004**	1	29689	2.75	0.132
T x M	1	332	0.23	0.646	1	5317	0.49	0.493
Site (T x M) = S	8	1473	1.06	0.407	8	10919	2.78	**0.018**
Area (S)	36	1388	3.20	**<0.001**	36	3953	2.34	**<0.001**
Residual	143	434			134	1690		
	Roughness				Diversity of morphological elements			
Source	df	MS	Pseudo-F	p-value	df	MS	Pseudo-F	p-value
Type =T	1	0.036	0.52	0.488	1	0.031	0.71	0.415
Morphology = M	1	0.663	9.65	**0.022**	1	0.120	2.69	0.161
T x M	1	0.058	0.85	0.382	1	0.046	1.03	0.347
Site (T x M) = S	8	0.069	1.00	0.452	8	0.045	1.03	0.432
Area (S)	36	0.069	1.43	0.081	36	0.044	1.39	0.093
Residual	132	0.048			132	0.031		

2.3.2 Exposure

When the compass point directions faced by each plot surface were grouped into the 16 major cardinal and intercardinal directions, we found that plots faced a broad range of directions, the only exception being represented by seawalls which mostly faced North-West or South (Figure 2.3). When the compass degrees were transformed into deviations from the prevailing coastline exposure, no differences were observed between habitat Types or Morphologies (Table 2.1). Although seawalls had almost 50% more landward microhabitats (represented by the greatest average deviation from prevailing seaward exposure, Figure 2.4A) than any other habitat, this difference was not significant, probably due to the large variability among both areas and sites (Table 2.1) and to loss of power from the lack of the horizontal replicates. At the small scale, seawalls had virtually no variation in exposure among plots (Figure 2.4B). This differed from all other habitats which showed high variability, with the greatest small-scale variation in exposure for boulder fields (Supplementary Table S2.2). At the meso and large scales, the trend was opposite, with seawalls showing the greatest variation in exposure among areas and sites. At the meso scale, this difference was not detected as significant. At the large scale, exposure was more variable among sites for regular than

irregular habitats, and for artificial than natural habitats, which resulted in the greatest and lowest variability for seawalls and boulder fields, respectively (Figure 2.4B and Supplementary Table S2.2).

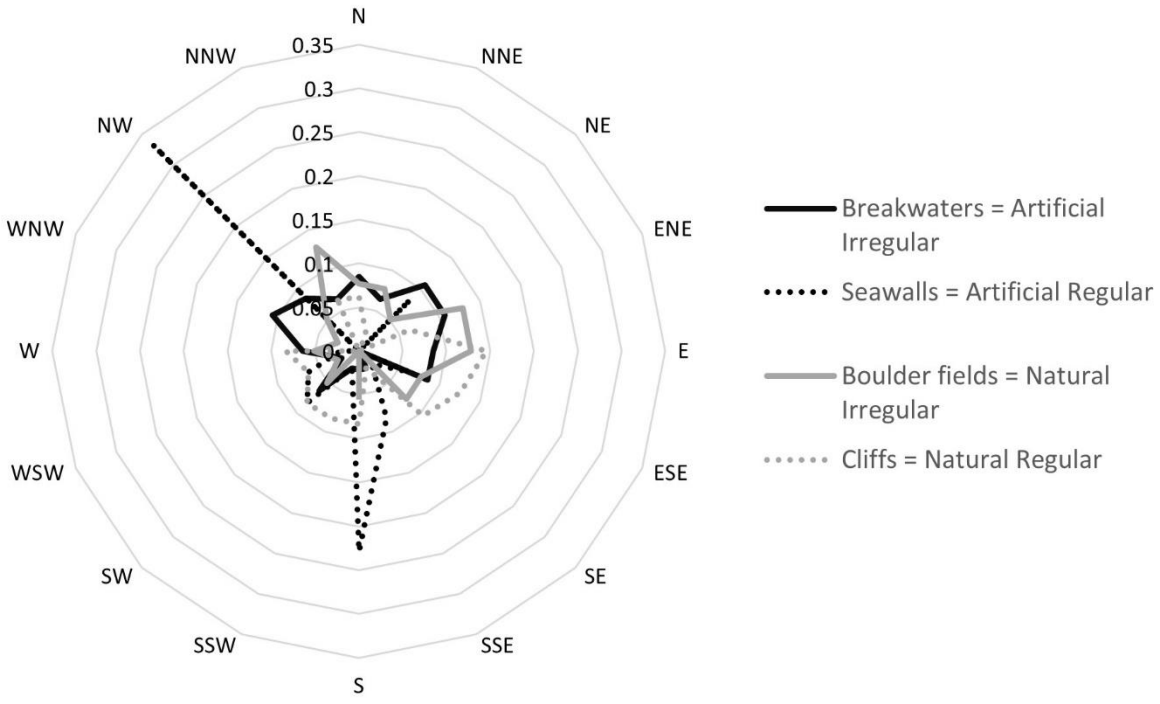

Figure 2.3. Radar graph representing the frequency (observed/total plots per habitat) of the exposures for the four combinations of habitat Types and Morphologies. The compass values were grouped into 16 major cardinal and intercardinal directions by approximating each point value to the nearest cardinal direction

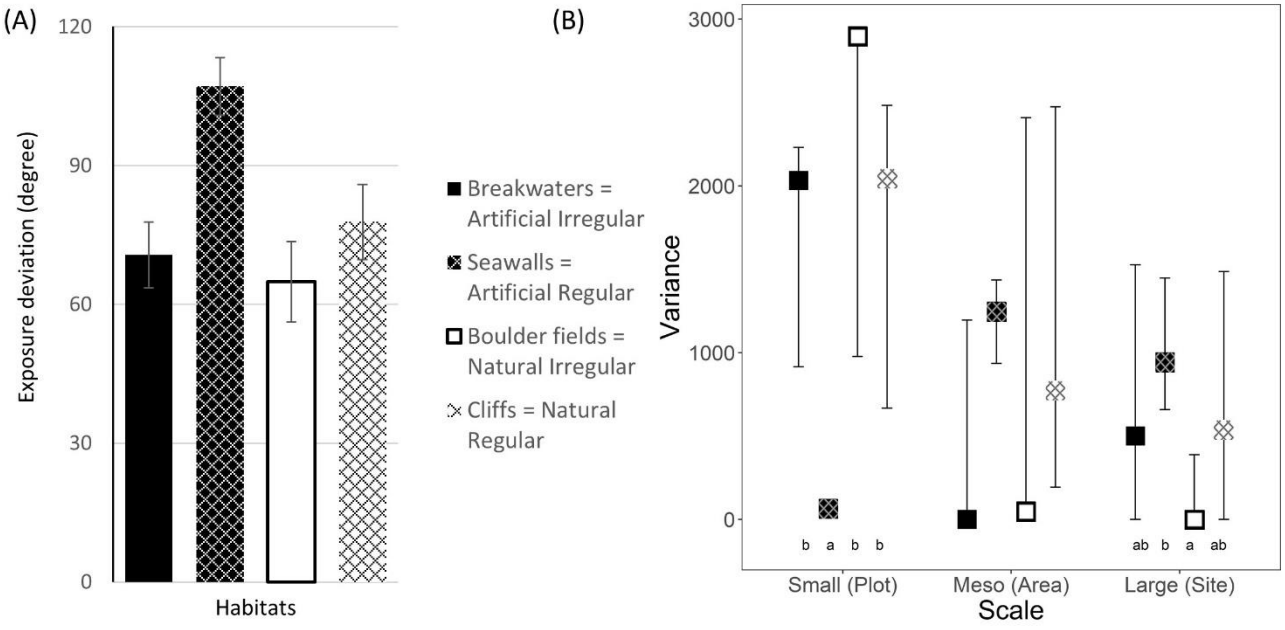

Figure 2.4. (A) *Mean exposure deviation (±1 SE; data are averages of 2–4 plots at each of 4 areas at each of 3 sites, n = 39–48) and* (B) *estimated variance in Exposure (±95% CI from bootstrapping) at each scale for the four combinations of habitat Types and Morphologies. Letters indicate significant differences from pairwise-tests (Supplementary Table S2.2).*

2.3.3 Roughness

Irregular habitats were significantly rougher than regular habitats, with an average roughness of 1.25 and 1.14, respectively. Conversely, there were no differences between artificial and natural habitats, which showed comparable rugosity (Figure 2.5A and Table 2.1). Roughness was homogeneous for all habitats at all spatial scales considered (Figure 2.5B). The only exception was represented by breakwaters, which showed some variation in roughness at the small scale among plots, but the difference was not significant (Figure 2.5B and Supplementary Table S2.2).

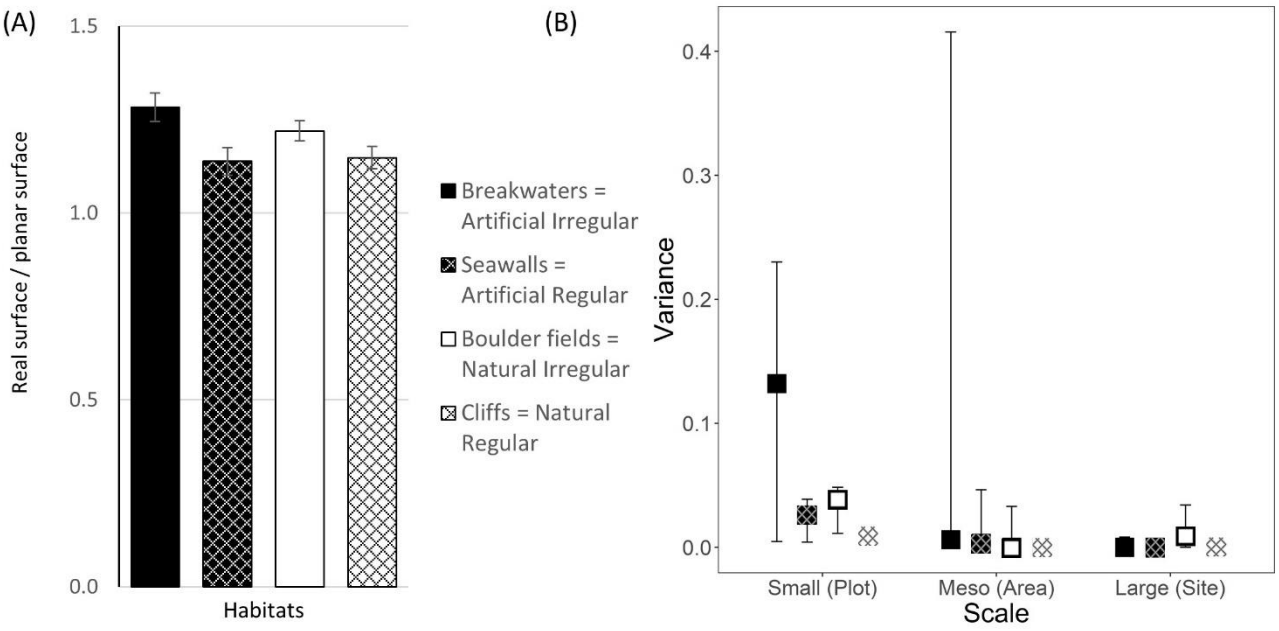

Figure 2.5. (A) *Mean surface roughness (±1 SE; data are averages of 2–4 plots at each of 4 areas at each of 3 sites, n = 42–48) and* (B) *estimated variance in roughness (±95% CI from bootstrapping) at each scale for the four combinations of habitat Types and Morphologies. Letters indicate significant differences from pairwise-tests (Supplementary Table S2.2).*

2.3.4 Abundance and diversity of morphological microelements

Except for sparse "Flat slopes" (<4% cover), the other morphological microelements were evenly represented, with abundances between 16% for "Lower slopes" in breakwaters to 25.9% for "Valleys" in cliffs (Figure 2.6). The abundances of the microelements were comparable among habitat Types (Figure 2.6). Significant differences were detected only between habitat Morphologies, with "Lower slopes" more abundant in the regular than in the irregular habitats and, vice versa, with "Ridges" more abundant in the irregular than in the regular habitats (Table 2.2). In addition, "Valleys," equally represented in regular and

irregular artificial habitats (Breakwaters: 20.9%, Seawalls: 20.7%), were more abundant in irregular (19.7%) than regular (25.9%) natural habitats (significant interaction Type x Morphology, Table 2.2).

Table 2.2. Four-way PERMANOVA results testing the effects on the abundances of six morphological microelement (valleys, lower slopes, flat slopes, middle slopes, upper slopes, ridges). In bold are p-values <0.05.

	Valleys				Lower slopes			
Source	df	MS	Pseudo-F	p-value	df	MS	Pseudo-F	p-value
Type =T	1	0.0194	2.54	0.126	1	0.0000	0.02	0.898
Morphology = M	1	0.0423	5.54	**0.024**	1	0.0192	9.22	**0.008**
T x M	1	0.0418	5.47	**0.024**	1	0.0005	0.23	0.671
Site (T x M) = S	8	0.0076	1.29	0.262	8	0.0021	0.56	0.804
Area (S)	36	0.0059	0.97	0.516	36	0.0038	1.46	0.067
Residual	132	0.0061			132	0.0026		
Pairwise	T x M	Artificial:	Irregular = Regular					
		Natural:	Irregular < Regular					
		Irregular:	Artificial = Natural					
		Regular:	Artificial = Natural					
	Flat slopes				Middle slopes			
Source	df	MS	Pseudo-F	p-value	df	MS	Pseudo-F	p-value
Type =T	1	0.0053	2.76	0.089	1	0.0151	2.96	0.124
Morphology = M	1	0.0056	2.88	0.081	1	0.0028	0.55	0.489
T x M	1	0.0053	2.76	0.092	1	0.0007	0.14	0.712
Site (T x M) = S	8	0.0019	2.83	**0.018**	8	0.0051	0.98	0.462
Area (S)	36	0.0007	1.51	0.054	36	0.0052	1.73	**0.014**
Residual	132	0.0005			132	0.0030		
	Upper slopes				Ridges			
Source	df	MS	Pseudo-F	p-value	df	MS	Pseudo-F	p-value
Type =T	1	0.0016	0.16	0.721	1	0.0006	0.09	0.768
Morphology = M	1	0.0034	0.34	0.586	1	0.0946	15.16	**0.009**
T x M	1	0.0018	0.18	0.700	1	0.0090	1.43	0.250
Site (T x M) = S	8	0.0100	3.60	**0.003**	8	0.0062	0.36	0.935
Area (S)	36	0.0028	1.29	0.153	36	0.0172	1.32	0.128
Residual	132	0.0022			132	0.0130		

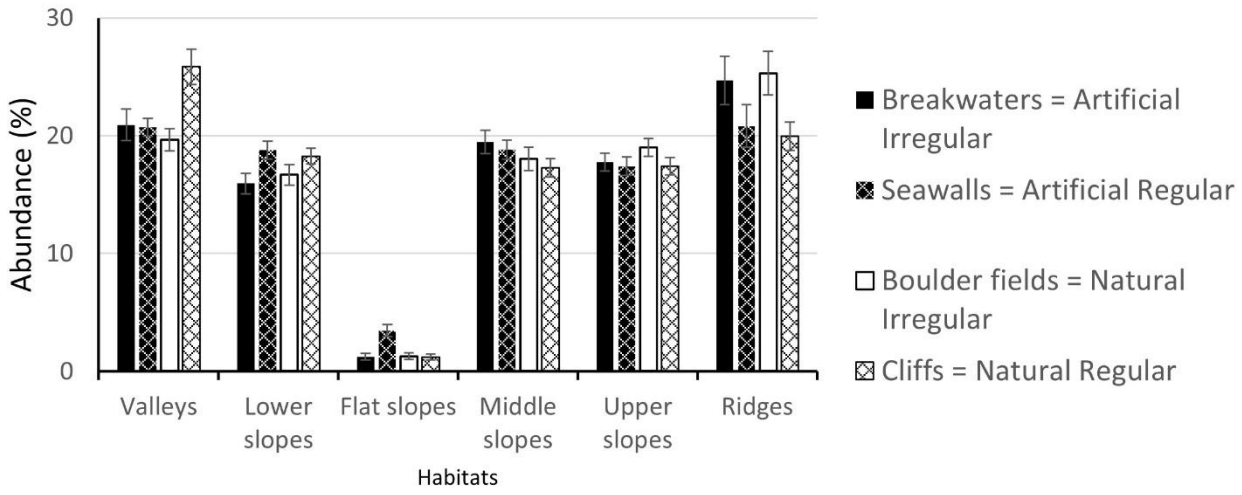

Figure 2.6. Mean abundances (±1 SE; data are averages of 2–4 plots at each of 4 areas at each of 3 sites, n = 42–48) of each of the six morphological microelements in the four habitats.

No differences were detected in the Diversity of morphological elements which was comparable among habitats (Figure 2.7A and Table 2.1). Furthermore, Diversity of morphological elements was homogeneous for all habitats at all scales considered (Figure 2.7B and Supplementary Table S2.2).

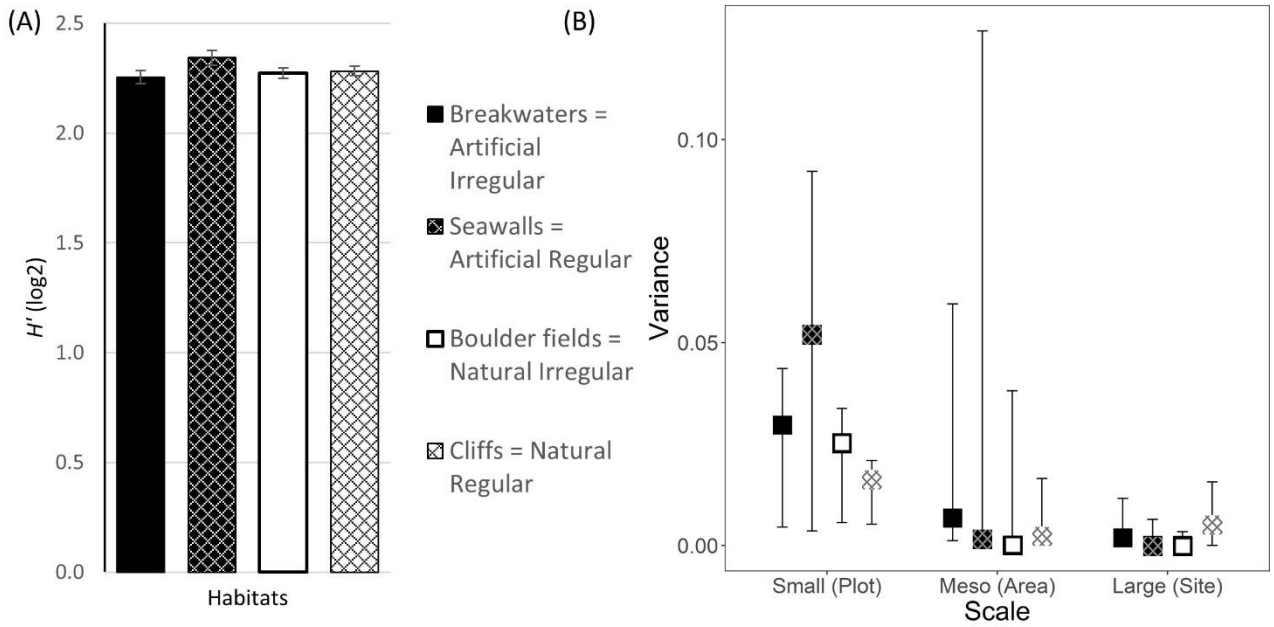

Figure 2.7. (A) *Mean diversity of morphological microelements (±1 SE; data are averages of 2–4 plots at each of 4 areas at each of 3 sites, n = 42–48) and* (B) *estimated variance in diversity of morphological microelements (±95% CI from bootstrapping) at each scale for the four combinations of habitat Types and Morphologies. Letters indicate significant differences from pairwise-tests (Supplementary Table S2.2).*

2.4 Discussion

We provide the first formal quantitative comparison of several descriptors of physical structure among a variety of artificial and natural marine habitats. Contrary to what generally assumed, at the scales covered by this study most descriptors were relatively similar between artificial and natural habitats. The only notable structural difference was inclination, which was consistently steeper in the artificial than in the natural habitats. Other minor differences observed, for example in roughness or in the abundance of some textural elements–frequently controlled in eco-engineer enhancements–were related to the general morphology (regular or irregular) of the habitat rather than to its artificial or natural identity.

Our analysis formally confirmed previous observations that artificial habitats are consistently steeper than natural shorelines (Rilov and Benayahu, 2000; Knott et al., 2004; Perkol-Finkel et al., 2006). Slope is an important factor in the design of artificial structures (Burcharth et al., 2007), affecting the stability and reflection properties of the artificial structures, as well as the costs, as milder slopes require larger volumes of construction material. Inclination is also an important determinant of the structure of assemblages of hard bottom substrata (Glasby, 2000; Glasby and Connell, 2001; Knott et al., 2004; Virgilio et al., 2006; Hanlon et al., 2018), affecting the abundance and distribution of species via changes in light, temperature, water flow, sedimentation, larval behavior and access of predators. In general seaweeds are more abundant on subvertical or horizontal surfaces, while invertebrates tend to be more abundant on steeper surfaces. Steeper surfaces also quantitatively decrease the area of intertidal habitat compared to subvertical or horizontal surfaces (Vaselli et al., 2008). As such, inclination should be a crucial factor that is considered also for an ecologically informed, “greener” design of artificial structures. In principle, efforts should be made to match more closely the milder slope of the natural habitats (Chapman and Underwood, 2011). At the same time, steeper artificial structures not only minimize material, space use and costs, but also reduce the physical footprint of the construction (i.e., Bugnot et al., 2021) on the native, often sedimentary, habitats (Schoonees et al., 2019). Any eco-engineering approach would probably require a balanced consideration of the costs and benefits of modifications in the inclination of the artificial habitats. An alternative approach could be to focus on the small- and meso-scale heterogeneity in the inclination of

artificial habitats to match more closely that of natural habitats. At the small- and meso-scales artificial habitats were more homogeneous in inclination than natural habitats of comparable morphologies, and this was particularly notable for seawalls. Potential greening options to reproduce the milder slopes of natural habitats could include stepped seawalls, such as those used at Barangaroo in Sydney Harbor (Airoldi et al., 2021), or construction blocks of less uniform shapes.

Despite artificial structures are often built to provide landward shelter along wave-exposed shorelines for mooring and coastal protection, we could not detect significant differences in exposure between artificial and natural habitats, possibly due to the missing horizontal replicates. Seawalls, however, had the greatest number of plots deviating from the prevailing coastline exposure compared to all other habitats, and virtually no variations in exposure at the smallest scale. This suggests that the extensive and accelerating construction of artificial habitats such as seawalls along urbanized shorelines (Bugnot et al., 2021) might introduce considerable amounts of sheltered hard-bottom habitats at the regional scales (Airoldi et al., 2015). Exposure is a crucial determinant of rocky shore assemblages (Denny, 1988; Burrows, 2012), and shelter could facilitate the spread of non-indigenous species in artificial habitats (Floerl and Inglis, 2003; Bulleri and Airoldi, 2005). Research on the effects of the sheltered habitats introduced by artificial structures relative to natural reefs at the regional scales would be required to establish the importance of exposure as a moderator factor in eco-engineering efforts.

Increasing surface topographic complexity generally has positive effects on biodiversity in both natural and artificial habitats (Stein et al., 2014; Loke and Todd, 2016; Strain et al., 2021). These effects are related to both increased surface area and supply of microhabitats which can differ in light, temperature, humidity and predation access (Strain et al., 2018a). A recent meta-analysis of eco-engineered surfaces (Strain et al., 2018b) and a global experiment (Strain et al., 2021) have both highlighted, however, that the outcomes of topographic enhancements can be extremely variable among locations, tidal levels, latitudes and studies, warning about generalizations. Our results raise additional questions about the generalized benefits provided by topographic enhancements of artificial surfaces, as this may not always reproduce relevant natural features. In fact, in our region, we found little differences in surface structure complexity between

artificial and natural habitat both when using rugosity as a proxy for topographic complexity (Shumway et al., 2007; MacArthur et al., 2019) and when applying TPI to quantify the abundance and diversity of morphological microelements. The only minor, yet significant, differences in the abundance of ridges and lower slopes were related to habitat morphology (regular and irregular) and not to habitat type, which suggests that some differences attributed so far to the artificial nature of the habitat might be in fact confounded by differences related to the morphology.

At the scales covered by this study, most descriptors of physical structure were relatively similar between artificial and natural habitats. Yet studies showed that assemblages associated to artificial and natural habitats consistently differ (Bulleri, 2005; Perkol-Finkel et al., 2006; Carvalho et al., 2013), including works in the study region (Moschella et al., 2005; Airoldi et al., 2015). This suggests that eco-engineering tests aiming to enhance the ecological performance of artificial habitats should also pay attention to other structural and non-structural elements that have been reported to differ between artificial and natural habitats, but which so far have been largely neglected (Airoldi et al., 2021). These elements include among others: construction material (Glasby, 2000; Sella and Perkol Finkel, 2015; McManus et al., 2018); careful planning of maintenance (Airoldi and Bulleri, 2011); distance from pollution sources (Strain et al., 2021); position (Glasby and Connell, 2001; Moschella et al., 2005); and the environmental (Franzitta and Airoldi, 2019), biotic (e.g., predation pressure Ferrario et al., 2016; Gianni et al., 2018), and social (Strain et al., 2019) settings. It is also important to consider that our study covered only about 8 km of coastlines in a region characterized by small tides and moderate wave action, which may affect the design of artificial structures. It is possible that in other geographical regions structural differences among artificial and natural habitats might be greater than those observed in our study region. For example, in other macrotidal regions rock pools (which were virtually absent at our natural sites) might be a relevant element causing structural differences between artificial and natural structures at small/medium scales (Aguilera et al., 2014), which would justify their engineered enhancement (Firth et al., 2014a). Also the scales covered by the study should be expanded in future studies, in order to identify at which scale structural differences between the two habitats might become more relevant.

In conclusion, our results challenge the widespread simplified assumption that artificial habitats lack the physical structure proper to natural reefs. The structural difference between a seawall and a rocky reef could be less than that between a seawall and a rubble-mound breakwater, and at small and meso-scales a rubble-mound breakwater could be more heterogeneous (i.e., in inclination) than a subvertical rocky cliff. This encourages a wider reflection about what makes a construction surface "greener": surface topographic complexity is an important ecological parameter, and its deliberate increase will likely lead to responses in the biota. However, if the intervention is not designed to mimic structural natural patters at the adequate scale, increasing habitat complexity may not lead to assemblages that converge toward a "more natural" state.

Data availability statement

The original data presented in the study are publicly available on figshare: doi: 10.6084/m9.figshare.1691846

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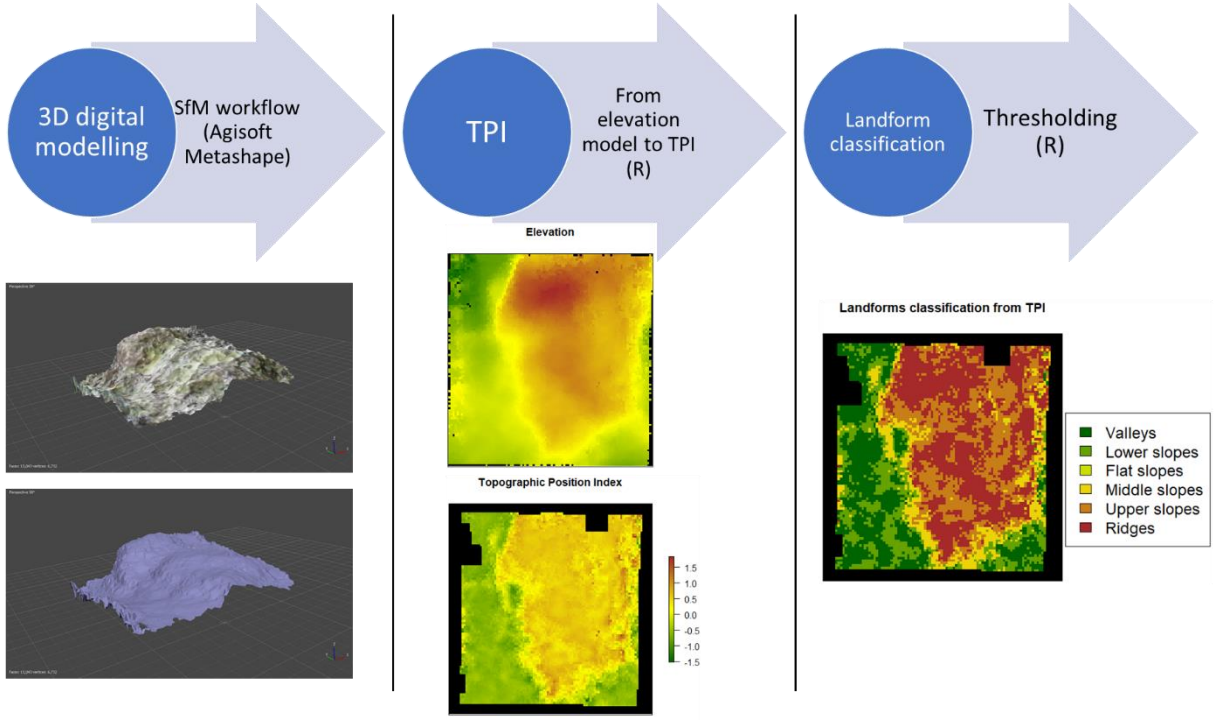
Supplementary materials



Supplementary Figure S2.1. Workflow process to identify discrete morphological microelements in 10x10cm plots. From 3D modelling to classification of TPI values into one of the six microelements (ridges, upper slopes, middle slopes, flat slopes, lower slopes and valleys).

Supplementary Table S2.1. Agisfot Metashape Standard v 1.5 setting for the reconstruction of the 3D models. If the photos failed in a good alignment with these initial settings, key point limits were increased to 70,000

Process	Settings
Photos alignment	**Highest** accuracy, generic preselection disabled, **40,000** key point limit, **1,500** tie point limit.
Optimize camera alignment	(use all the ones selected by the software.)
Build dense cloud	**High** quality, **moderate** depth filtering, reuse depth maps disabled.
Build mesh	**Height field (2.5d)** surface type, **high** face count, interpolation enabled, calculate vertex colours enabled.
Build texture	Keep uv mapping mode, mosaic blending mode, texture size/count 4,096, enable hole filling, enable ghosting filter

Supplementary Table S2.2. Test of differences in estimated components of variance for Inclination, Exposure, surface Roughness and Diversity of morphological elements at each of the three spatial scales (small, meso, large) as a function of 4 habitat levels (Breakwater = AR-IR, Seawall = AR-RE, Boulder field =NA-IR, Cliff = NA-RE). In bold significant p-values (α = 0.05 for main test, α = 0.01 for post-hoc pair-wise comparisons). NP = Not Performed.

	INCLINATION			**EXPOSURE**		
MAIN TEST	Small (Plot)	Meso (Area)	Large (Site)	Small (Plot)	Meso (Area)	Large (Site)
P-VALUE	**0.018**	**0.004**	0.648	**<0.001**	0.107	**0.020**
	Inclination			**Exposure**		
PAIRWISE	Small (Plot)	Meso (Area)	Large (Site)	Small (Plot)	Meso (Area)	Large (Site)
AR-IR VS. AR-RE	0.014	0.270	NP	**0.002**	NP	0.070
AR-IR VS. NA-IR	0.639	0.013	NP	0.167	NP	0.051
AR-IR VS. NA-RE	0.591	0.704	NP	0.984	NP	0.410
AR-RE VS. NA-IR	**0.002**	**0.001**	NP	**<0.001**	NP	**0.010**
AR-RE VS. NA-RE	0.060	0.397	NP	**0.001**	NP	0.083
NA-IR VS. NA-RE	0.312	**0.006**	NP	0.176	NP	0.045
	ROUGHNESS			**DIVERSITY OF MORPHOLOGICAL ELEMENTS**		
MAIN TEST	Small (Plot)	Meso (Area)	Large (Site)	Small (Plot)	Meso (Area)	Large (Site)
P-VALUE	0.217	0.546	0.065	0.449	0.452	0.162
	Roughness			**Diversity of morphological elements**		
PAIRWISE	Small (Plot)	Meso (Area)	Large (Site)	Small (Plot)	Meso (Area)	Large (Site)
AR-IR VS. AR-RE	NP	NP	NP	NP	NP	NP
AR-IR VS. NA-IR	NP	NP	NP	NP	NP	NP
AR-IR VS. NA-RE	NP	NP	NP	NP	NP	NP
AR-RE VS. NA-IR	NP	NP	NP	NP	NP	NP
AR-RE VS. NA-RE	NP	NP	NP	NP	NP	NP
NA-IR VS. NA-RE	NP	NP	NP	NP	NP	NP

Chapter 3 – Physical structure explains a significant but relatively small proportion of the differences in the biota between natural and artificial shorelines

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Abstract

The widespread loss of biodiversity related to ocean sprawl makes it desirable for coastal artificial structures to be designed so as to provide novel yet valuable habitats for natural communities, by providing niches for a wide range of species that use the structures as mimic surrogate of the natural habitat. These "greening" efforts have had a strong focus on physical habitat structure as a key factor for enhancing natural communities. However, few studies have explicitly quantified the differences in physical structure between natural and artificial habitats, and how these differences contribute to the currently limited growth of epibiota on most artificial structures. In this study we evaluate how much some of the most common physical parameters manipulated in small-scale greening efforts of marine artificial structures (inclination, exposure, roughness, diversity of microhabitats) contribute to the differences observed in the abundance and structure of intertidal epibiota between natural (boulder fields and cliffs) and artificial (breakwaters and seawalls) habitats of different morphologies. Although the epibenthic communities significantly differed between the artificial and natural substrates, and were particularly distinct on the concrete seawalls compared to all other habitats, the descriptors of the physical structure were not as different between artificial and natural habitats and explained a significant but quantitatively limited (< 10%) proportion of the variability of the epibiota. Inclination and exposure were the only descriptors that played a role in predicting this proportion of the variability of the epibiota, while Roughness and Diversity of microhabitats were not related to the epibiota variability. These findings lead to the conclusion that physical structure is not the main ecological driver controlling differences in intertidal epibiota between natural and artificial habitats, and that other co-occurring factors could directly or indirectly contribute to the differences in biota. Biomimetic efforts aiming to enhance the ecological value of the artificial habitats should consider the context in which the artificial substrates are placed and identify which combinations of structural and non-structural elements could be enhanced to better recreate the natural conditions and promote native biodiversity.

Keywords: coastal infrastructure, complexity, epibenthic community, hard substrates, intertidal habitats, rocky reef, rugosity

3.1 Introduction

The physical structure of habitats and how it affects natural communities has long been a central theme of ecology (MacArthur and MacArthur, 1961). It is generally acknowledged that more complex habitats tend to support greater species richness and abundance, across a range of functional groups, than less complex habitats (McCoy and Bell, 1991; Stein et al., 2014). This concept has acquired significant applied value in restoration and reconciliation efforts (Abelson et al., 2021), where (re)building complexity of natural habitats in hybrid as well as in entirely novel ecosystems (Nordstrom, 2014; Schoonees et al., 2019) is considered one way to support wildlife and help to reinstate lost ecological functionalities and services such as biodiversity support, carbon storage, flood regulation, recreation, spiritual fulfilment and education (Miller and Hobbs, 2007; Loke et al., 2015).

The importance of habitat physical structure for natural processes and functions has gained particular momentum in marine restoration efforts aiming at renaturing armored urban waterfronts (Airoldi et al., 2005; Chapman and Blockley, 2009; Chapman and Underwood, 2011; Firth et al., 2014; Dafforn et al., 2015; Airoldi et al., 2021). Coastal armoring (represented by seawalls, breakwaters, dykes, groins, jetties, pilings, bridges, artificial reefs, and other marine constructions; Dugan et al., 2011) has already modified up to 1.5% of the global seafloor coverage in the exclusive economic zones and is sprawling at unprecedented rates (Bugnot et al., 2021), displacing native habitats and species (Bulleri and Chapman, 2004; Airoldi and Beck, 2007; Heery et al., 2017), and causing loss of biodiversity and ecosystem functions at local to regional scales (Chapman, 2003; Airoldi et al., 2015; Bishop et al., 2017; Mayer-Pinto et al., 2018). The recognition of these impacts has stimulated a greener, biomimetic, multifunctional concept of coastal artificial structures (Chapman and Blockley, 2009; Chapman and Underwood, 2011; Firth et al., 2014; Dafforn et al., 2015; Pioch et al., 2018; Airoldi et al., 2021), that combines their utilitarian function with the support to a variety of flora and fauna that use the structures as novel (*sensu* Hobbs et al., 2009) surrogate of their natural habitat along built urban waterfronts.

Communities on and around artificial structures have been shown to be distinct and of lower diversity than those of natural rocky reefs (Perkol-Finkel et al., 2006; Burt et al., 2009; Fauvelot et al., 2009; Franzitta and Airoldi, 2019), with limited growth of many native taxa and a prevalence of opportunistic and invasive species (Bulleri et al., 2006; Glasby et al., 2007; Airoldi et al., 2015). These differences are particularly evident when structures are built along sedimentary coastlines, but they tend to persist even when structures are built in close proximity to natural rocky shores (Chapman, 2003; Airoldi et al., 2015; Franzitta and Airoldi, 2019), which suggests that the use of these structures by many species could be limited by environmental as well as structural factors. Efforts at greening this currently gray infrastructure have mostly focused on small-scale structural enhancements of topographic complexity to provide more surface area, microhabitats, or refugia from predators, desiccation or wave action, to species on and around the structures (Loke et al., 2015; Morris et al., 2017; O'Shaughnessy et al., 2020). However, few studies have explicitly quantified the differences in physical structure between natural and artificial habitats and the scales at which these differences are displayed (but see Aguilera et al., 2014 and Lawrence et al., 2021). Even less is known about how any structural differences contribute to the known significant biotic differences between artificial and natural habitats (but see Sedano et al., 2020a, 2020b). This information is crucial to guide effective greening approaches and weigh their benefits, which so far have been inconsistent and not universally positive (Strain et al., 2018b, 2021).

In this study, we quantified variations in the biotic structure of the epibenthic communities across a combination of nearby artificial and natural hard habitats of regular and irregular morphologies (seawalls = artificial regular; cliffs = natural regular; breakwaters = artificial, irregular; and boulder fields = natural irregular) along the Monte Conero promontory (North Adriatic Sea), and across three different spatial extents: small (centimeters to meters); meso (meters to 10 s of meters); and large (100 s meters). We hypothesized that distinct biotic differences exist between artificial structures and natural rocky shores independently of their morphologies (Bulleri and Chapman, 2004; Sedano et al., 2020c), with natural rocky reefs hosting assemblages richer and more diverse than artificial substrates at all spatial scales (Moschella et al., 2005; Carvalho et al., 2013; Firth et al., 2016). We then used multivariate techniques to quantify how

much of the observed variation in biotic structure could be related to variations in five descriptors of the physical structure of the same habitats, specifically inclination, exposure roughness abundance and diversity of surface morphological microelements. A previous analysis of these structural descriptors (Grasselli and Airoldi, 2021) found that only inclination was consistently steeper in the artificial than in the natural habitats, while other minor differences in roughness or in the abundance of some surface microelements were related to the general morphology (regular or irregular) of the habitat rather than to its artificial or natural identity. We therefore hypothesized that inclination would be the structural factor, among those considered, explaining most of the observed biotic variability between artificial and natural habitats. We expect that effect of inclination would vary among the functional groups of organisms, in particular driving the dominance of macroalgal community in more gently sloping natural substrata and of sessile invertebrates in steeper artificial substrata (Dafforn et al., 2012; Chee et al., 2020).

3.2 Materials and methods

3.2.1. Study site and sampling design

The research was carried out in April 2019 along 8 km of the Monte Conero promontory (central-western Adriatic Sea, Italy), between the localities of Numana (43°30’31’’N, 13°37’28’’E) and Portonovo (43°33’55’’N, 13°35’27’’E). Along this jagged coastline, marl and limestone natural rocky outcrops, forming both sub-vertical platforms (hereafter called cliffs) and boulder fields, are interspersed with artificial substrates built to protect small marinas and tourist beaches. These artificial structures are represented by breakwaters and groins (hereafter jointly referred to as “breakwaters”), made by large blocks of quarried rock, and by concrete seawalls.

We sampled the low-intertidal (≈ 0 to +20 cm relative to mean low-water level, average tidal amplitude ≈ 30 cm) epibiota across two broad morphologies of artificial and natural habitats, regular (concrete seawalls and rocky cliffs) and irregular (breakwaters and rocky boulder fields). For each of these 4 substrates, three sampling sites, 100s meters to kilometers apart, were randomly selected (Figure 3.1). With the aid of a tape measure, we located four sampling areas 2 m long and 10 m apart at each sampling site, and within each area four replicated 10x10 cm plots 50 cm apart. In total there were 192 replicated plots which allowed to compare intertidal epibiota across the orthogonal combinations of two habitat types (artificial and natural) and two morphologies (irregular and regular), and to test for the spatial variability across three different spatial extents: small (centimeters to meters); meso (meters to 10s of meters); and large (100s meters to kilometers).

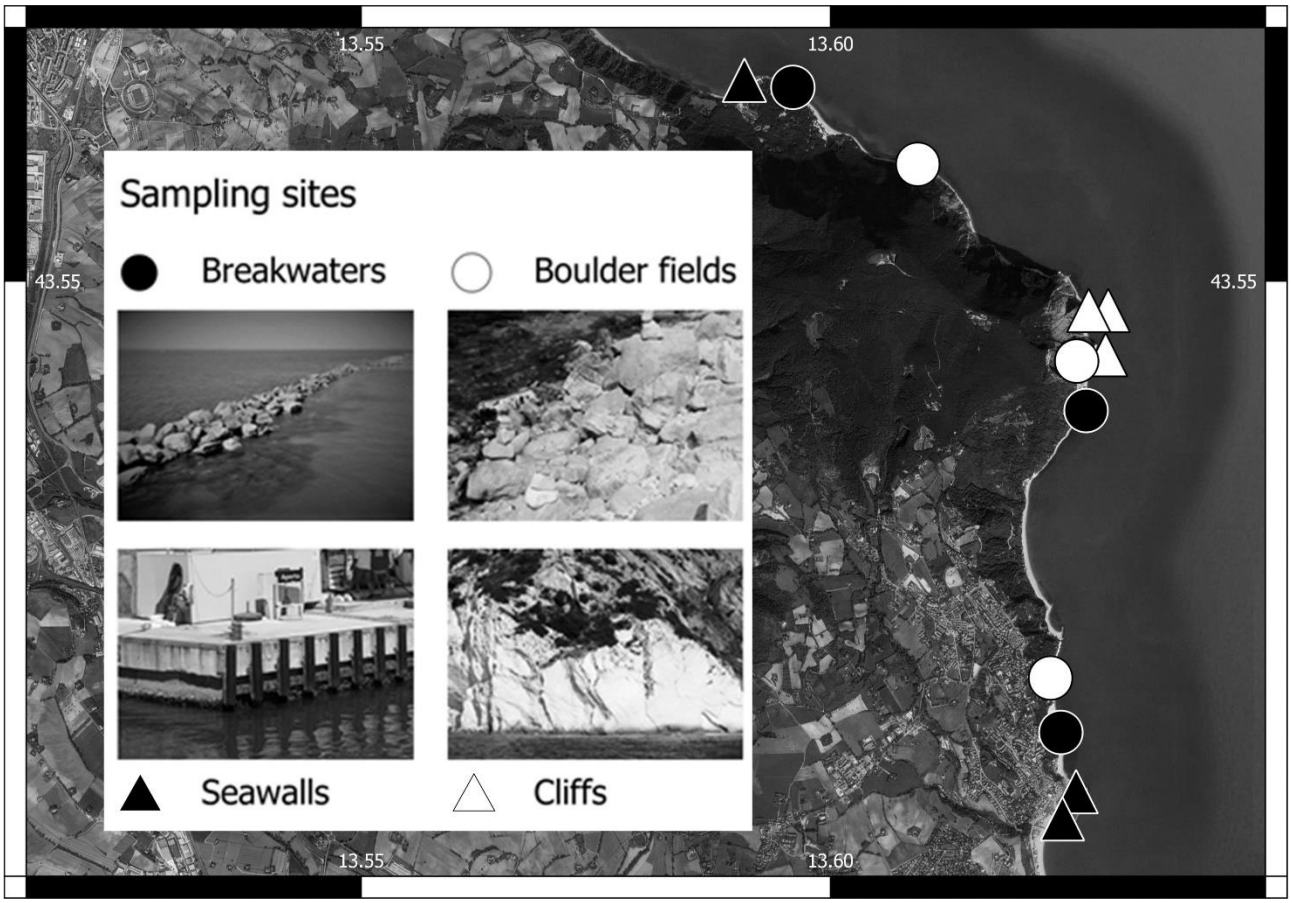

Figure 3.1. Location of sampling sites for each combination of habitat Type (Artificial = black symbols, Natural = white symbols) and Morphology (Irregular = circle symbols, Regular = triangle symbols).

3.2.2 Field sampling and benthic assessment

To explore the differences in epibenthic communities between habitat types and morphologies at the three spatial scales, we photographed the 192 intertidal plots at low tide, using digital cameras Canon PowerShot G12 or S95, equipped with underwater housings. For seven plots (six from breakwaters and one from cliffs), photo-quadrats of sufficient quality were not captured due to wave splashing.

The percentage cover of each sessile taxa was quantified for each photo-quadrat using photoQuad version 1.4 software (Trygonis and Sini, 2012): each quadrat was divided into 200 sub-quadrats of 0.5 cm^2, and the sub-quadrats where each taxon was occurring were summed. The same procedure was used to determine the amount of space unoccupied by macroscopically visible organisms. The density of vagile invertebrates was also quantified by counting the number of specimens of each taxon in each photo quadrat. Organisms were identified as species or morphospecies, and then fouling organisms were classified into three coarser level groups including biofilm, macroalgae and sessile invertebrates.

3.2.3 Structural descriptors

After each photograph, we removed any species from the plots and quantified five descriptors of the physical structure of the primary substrata that are most often claimed to be limiting for the growth of biota in artificial habitats and that are most often manipulated in greening applications, specifically: (i) Inclination; (ii) Exposure; (iii) Roughness; and (iv) Abundance, and (v) Diversity of morphological elements. Methods for these descriptors have been reported in Grasselli and Airoldi (2021) and will be briefly summarized here.

Inclination was estimated using a hand-held clinometer positioned in the center of the plot. Exposure (i.e., aspect) was estimated using a compass positioned in the center of the plot and facing the sea. Exposure was not measured when Inclination was <10°, as exposure of flat substrates is meaningless, which occurred at 9 plots in boulder fields. Thereafter, since Exposure could not be directly analyzed due to its vectorial essence, the angle formed between the Exposure of each plot and the prevailing exposure of the Monte Conero coastline was considered (see Grasselli and Airoldi, 2021 for details). To estimate Roughness as well as Abundance and Diversity of morphological elements, we filmed each plot with a digital camera as from Figueira et al. (2015). The videos were then analyzed using the Structure-from-Motion (SfM) photogrammetric technique (Westoby et al., 2012) and a textured digital surface model of the plot was created from the stereo-photos using Agisoft Metashape Standard v. 1.5 software, using the workflow described in Grasselli and Airoldi (2021). For 12 plots (6 from breakwaters, 4 from boulder fields and 2 from cliffs), we did not obtain sufficient high-quality photos for further SfM analysis due to wave splashing.

From each digital surface model, surface Roughness was computed as the ratio between the real plot surface (3D area) and its orthogonal planar projection (2D area). The Abundance and Diversity of discrete surface morphological elements were estimated using a landform classification based on the Topographic Position Index (TPI, i.e. the difference in elevation between each cell of the digital surface model and the mean of the adjacent cells within a predetermined neighborhood; Weiss, 2001). Thresholding of TPI values permitted to classify each cell into one of six morphological elements (ridges, upper slopes, middle slopes, flat slopes, lower slopes and valleys). The abundances of the morphological elements were expressed as

percentage cover of the total number of cells, and a variation of the Shannon Diversity Index, the Shannon's Entropy 3D (H-3D), reported in the CASU software (Loke et al., 2014), was applied to provide an estimate of the surface morphological diversity.

3.2.4 Statistical analysis

3.2.4.1 Differences in the structure of benthic assemblages

Differences in the six response datasets obtained from benthic photo-assessment (i.e., total cover, biofilm cover, macroalgal cover, sessile invertebrate cover, vagile invertebrate density and multivariate community structure) were analyzed using 4-way permutational analyses of variance (PERMANOVA) including factors habitat Type (artificial vs natural, fixed), Morphology (regular vs irregular, fixed, crossed to Type), Site (three levels, random, nested in Type x Morphology), and Area (four levels, random, nested in Site). Although tests for total cover, biofilm cover, macroalgal cover, sessile invertebrate cover and vagile invertebrate density were univariates, we performed all analyses using the statistical package PERMANOVA+ for PRIMER instead of classical ANOVA because of ease of use with unbalanced design (replicates varied between 2 and 4 due to 7 missing photos) and to avoid the normality assumptions (Anderson et al., 2008). To partition the variability and obtain F-statistics of total cover, we used PERMANOVA on a matrix of Euclidean distances calculated from the original untransformed data and calculated all P-values using 9,999 random permutations of the appropriate exchangeable units and Type III sums of squares. In case of too few unique permutations (<100), Monte Carlo (MC) p-values replaced the permuted p-values in the analysis (Anderson et al., 2008). The same analysis was applied to the multivariate community structure: variability was partitioned from a Bray-Curtis dissimilarities matrix calculated from the dataset previously standardized in this case. The standardization was necessary due to differences in metric scales as density was used for vagile invertebrates and percentage cover for sessile organisms. A dummy variable (value of 1) was added to the standardized dataset due to the presence of samples with zero values only (absence of any associated organisms). No further transformations were applied because test on homogeneity of variance (PERMIDSP) did not improve after data-transformation,

and therefore untransformed data were used. Differences in assemblage composition among habitat types and morphologies were visualized using a principal coordinates analysis (PCO) plot.

3.2.4.2. Relationships between descriptors of physical structure and epibiota

To identify the effect of physical structure of the substrate in shaping the epibenthic community, we used the descriptors of physical structure as a set of explanatory variables. Since both the explanatory dataset (descriptors of physical habitat structure) and the response datasets (total cover, biofilm cover, macroalgal cover, sessile invertebrates cover, vagile invertebrates density and community structure) reported missing values, the combination of the two data sets reduced the final number of plots on which the analysis was performed to 170. We used Redundancy Analysis (RDA) to model the variability of response datasets explained by the descriptors of the physical structure. Before performing RDA, Variance Inflation Factor (VIF) was performed in R (version 3.5.3) using function 'vif' of package 'car' to check for collinearity among explanatory variables (Neter et al., 1996). As no explanatory variable reported high collinearity (VIF < 5), the complete set of explanatory variables (Inclination, Exposure, Roughness and Diversity of morphological microelements) was retained for subsequent RDA. Consistently with Legendre (Legendre and Gallagher, 2001), we performed simple RDAs and a transform-based RDA (tb-RDA) on un-transformed univariate response datasets and on standardized Hellinger-transformed epibiota community, respectively. The explanatory variable selection and the subsequent RDA were performed in R using function 'ordiR2step' of package 'vegan'. Within the function, the number of permutations to provide a null distribution was set to 9,999 and the 'R2scope' argument to 'TRUE'. In this case, adjusted R^2 was preferred to unadjusted R^2 to provide an unbiased estimation of the variation of community explained by physical structure (Pered-Neto et al., 2006) and the strict procedure in the forward selection set by the 'R2scope' allows to avoid Type I error (Blanchet et al., 2008). In the graphical representation of tb-RDA result, species vectors were superimposed to identify the highest correlation between species and the set of environmental variables.

3.3 Results

3.3.1 Assemblage composition

A total of five taxa of invertebrates were observed. The filter feeders *Mytilus galloprovincialis*, Ostreidae (a mix of *Ostrea edulis* and *Magallana gigas*, hardly distinguishable by photo-quadrats and so classified together) and Cirripedia (barnacles) represented the community of sessile invertebrates, while the grazers *Monodonta mutabilis* and *Patella* spp. were the vagile invertebrates. The macroalgal community was more diverse, including *Ulva* sp., *Scytosiphon* sp., *Cystoseira* sp., leafy Bangiaceae, filamentous Rhodomelaceae and articulate coralline algae (ACA), as well as all those mixes of species that are difficult to identify from photos and, therefore, classified based on their thickness in green filamentous algae (GFA) and dense algal turf. Further, the epilithic biofilm, commonly formed by bacteria, cyanobacteria and diatoms, was observed (Supplementary Table S3.1).

Overall, total cover resulted highly different among habitat Types interacting with Morphologies (Table 3.1). Indeed, seawalls reported the higher mean total cover (91.4%), while cliffs the lower (29.4%; Figure 3.2a). Individually, the various morpho-functional groups showed different trends. Although not supported by statistical significance (Table 3.1), biofilm and macroalgae reported greater cover on substrates with 'irregular' morphologies (Figure 3.2b-c). On the other hand, both sessile and vagile invertebrates resulted to differ among habitat Types interacting with Morphologies (Table 3.1), with sessile invertebrates dominant on seawalls and virtually absent from breakwaters and boulder fields and vagile invertebrates more abundant on cliffs and poorly represented on seawalls and boulder fields (Figure 3.2d-e). Altogether, community structure reported strong differences in habitat Type interacting with Morphology with seawall hosting the most different assemblages compared to the other substrates (Table 3.1). As clearly reported by the superimposition of species vectors (with Pearson correlations > 0.2) in the PCO plot (explaining the 40.1% of total variation), samples clustered along X-axis (23.6%) between seawalls and the other three substrates because of different abundances of algal turf and of the taxa of invertebrates *Patella* spp., Ostreidae and Cirripedia (Figure 3.2f). Algal turf, Cirripedia and Ostreidae resulted more abundant on seawalls while *Patella* spp. was more abundant on natural habitat types and on breakwaters.

Table 3.1. Four-way PERMANOVA results testing the effects on total cover, biofilm cover, macroalgal cover, sessile invertebrate cover, vagile invertebrates density (Euclidean Distances on untransformed data) and on the multivariate community structure of epibiota (Bray–Curtis dissimilarities on standardized data). In bold are p-values <0.05.

	Total cover				Biofilm cover			
Source	df	MS	Pseudo-F	p-value	df	MS	Pseudo-F	p-value
Type =T	1	1.11	1.72	0.228	1	0.13	0.53	0.511
Morphology = M	1	0.34	0.52	0.499	1	0.36	1.44	0.262
T x M	1	10.04	15.63	**0.006**	1	0.01	0.05	0.828
Site (T x M) = S	8	0.65	3.85	**0.003**	8	0.25	2.32	**0.040**
Area (S)	36	0.17	2.51	**<0.001**	36	0.11	2.38	**0.001**
Residual	137	0.07			137	0.05		
Pairwise T x M	Irr: Art = Nat		Art: Irr < Reg					
	Reg: Art > Nat		Nat: Irr = Reg					
	Macroalgal cover (primary producers)				Sessile invertebrate (filter feeders)			
Source	df	MS	Pseudo-F	p-value	df	MS	Pseudo-F	p-value
Type =T	1	0.49	0.75	0.418	1	8.30	46.38	**0.006**
Morphology = M	1	2.83	4.31	0.072	1	13.67	76.36	**<0.001**
T x M	1	1.00	1.52	0.261	1	8.29	46.30	**0.006**
Site (T x M) = S	8	0.66	4.30	**0.003**	8	0.18	3.04	**0.012**
Area (S)	36	0.15	4.12	**0.000**	36	0.06	2.63	**<0.001**
Residual	137	0.04			137	0.02		
Pairwise T x M					Irr: Art = Nat		Art: Irr < Reg	
					Reg: Art > Nat		Nat: Irr = Reg	
	Vagile invertebrates (grazers)				Multivariate community structure			
Source	df	MS	Pseudo-F	p-value	df	MS	Pseudo-F	p-value
Type =T	1	17	2.33	0.150	1	49871	3.62	**0.014**
Morphology = M	1	21	2.82	0.131	1	64955	4.72	**0.003**
T x M	1	129	17.46	**0.006**	1	79220	5.75	**0.001**
Site (T x M) = S	8	7	3.92	**0.002**	8	13845	3.67	**<0.001**
Area (S)	36	2	1.01	0.439	36	3788	2.05	**<0.001**
Residual	137	2			137	1852		
Pairwise T x M	Irr: Art = Nat		Art: Irr = Reg		Irr: Art = Nat		Art: Irr ≠ Reg	
	Reg: Art < Nat		Nat: Irr < Reg		Reg: Art ≠ Nat		Nat: Irr ≠ Reg	

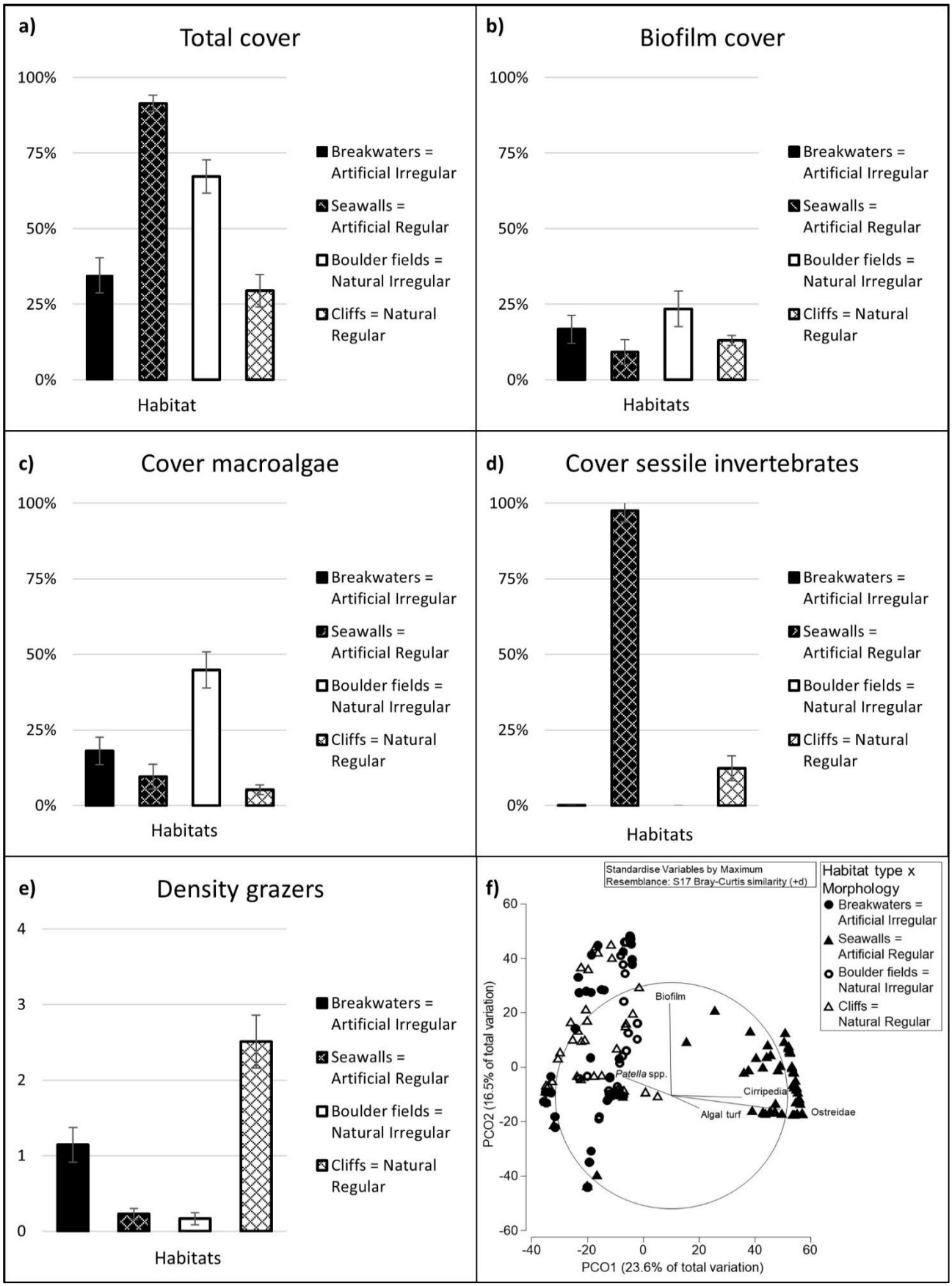

*Figure 3.2. For each orthogonal combination of factors habitat type and morphology: **a-e**) Mean (± 1 SE) Total cover, Biofilm cover, Macroalgae cover, sessile invertebrates cover and vagile invertebrates density. Data are averages of 2–4 plots at each of 4 areas at each of 3 sites, n = 42–48. **f**) Principal coordinates analysis (PCO) plot of standardized community structure with vectors overlay showing the length and direction of each taxon whose correlation was > 0.2 with the PCO axes.*

3.3.2 Effect of descriptors of physical structure on epibiota

A weak effect of the various descriptors of physical structure on epibiota was found. Indeed, total cover of organisms, as well as density of vagile invertebrates, was not correlated to any of the four descriptors of physical habitat structure (Table 3.2). When the covers of the various morpho-functional groups were individually considered, the models provided by the simple RDAs explained the 9.1% of biofilm variance, the 9.9% of macroalgal variance and the 21.6% of sessile invertebrates variance (Table 3.2). The model selected for biofilm cover retained Exposure and Roughness descriptors, which respectively explained a significant 5.5% and 4.3% of the total variance according to the marginal effects (Table 3.2). Exposure was negatively correlated with higher levels of biofilm cover (r = -0.72), while Roughness was positively correlated to biofilm (r = 0.63; Figure 3.3a). Macroalgal cover was negatively related to only Inclination (r = -1), which alone explained all the variability of the model. On the other hand, sessile invertebrates cover included three out of four physical structure descriptors (i.e. Inclination, Exposure and Roughness), which respectively explained a significant 7.0%, 6.6% and 3.5% of the total variance according to marginal effects (Table 3.2). Moreover, ordination of data showed that Inclination and Exposure were positively correlated with higher covers of invertebrates while Roughness was negatively correlated (Pearson's r: +0.77, + 0.65 and - 0.44, respectively; Figure 3.3c). Transformed-based RDA on community structure reported that physical structure significantly constrained just the 9.2% of the total inertia of the intertidal epibiota and, according to the marginal effects, only Inclination and Exposure explained a highly significant 4.6% and 3.2% of the total inertia, respectively (Table 3.2). Accordingly, the ordination triplot was composed by two RDA axes only which explained the 8% and the 1.2% of the total inertia (Figure 3.3d). Triplot reported a clear separation of samples, driven by increases in Inclination (r = -0.85) and Exposure (r = -0.72) in seawall sites, just along the first axis (RDA 1) of the ordination while no pattern was evident along the second axis. Moreover, organism scores suggest that Ostreidae was the taxon contributing more to the inertia among organisms' groups because further from the origin of the ordination plot. Ostreidae and Cirripedia resulted strongly correlated with RDA1 axis that, indirectly, means a high positive correlation with the physical

descriptors Inclination and Exposure. Conversely, an opposite relation was observed for the taxa leafy Bangiaceae, filamentous Rhodomelaceae and articulate coralline algae (ACA) and for biofilm (Figure 3.3.d).

*Table 3.2. Results of the permutation test for simple-RDA (modelling total cover, biofilm cover, macroalgal cover, sessile invertebrates cover and vagile invertebrates density), and for tb-RDA (modelling multivariate community structure) are provided. For each test, the constrained models and the marginal effects of each descriptor of physical structure selected by the models reported the proportion and amount of the inertia/variance explained, the pseudo-F and the level of significance; *: p < 0.05; **p < 0.01; ***p < 0.001; ns: not significant.*

	Total cover					Biofilm cover				
Model	df	% Explained	Inertia	F	p-value	df	% Explained	Inertia	F	p-value
Constrain	4	0.5%	0.001	0.21	ns	2	9.1%	0.005	8.40	***
Residual	165	99.5%	0.176	---	---	167	90.9%	0.054	---	---
Marginal	df	% Explained	Variance	F	p-value	df	% Explained	Variance	F	p-value
Inclination	---	---	---	---	---	---	---	---	---	---
Exposure	---	---	---	---	---	1	5.5%	0.003	10.23	**
Roughness	---	---	---	---	---	1	4.3%	0.003	7.98	**
Diversity of morphological microelements	---	---	---	---	---	---	---	---	---	---
Residual	---	---	---	---	---	167	91.2%	0.054	---	---
	Macroalgal cover (primary producers)					Sessile invertebrates (filter feeders)				
Model	df	% Explained	Inertia	F	p-value	df	% Explained	Inertia	F	p-value
Constrain	1	9.9%	0.010	18.49	***	3	21.6%	0.047	15.22	***
Residual	168	90.1%	0.094	---	---	166	78.4%	0.171	---	---
Marginal	df	% Explained	Variance	F	p-value	df	% Explained	Variance	F	p-value
Inclination	1	9.9%	0.010	18.49	***	1	7.0%	0.014	13.92	***
Exposure	---	---	---	---	---	1	6.6%	0.014	13.29	***
Roughness	---	---	---	---	---	1	3.5%	0.007	7.01	**
Diversity of morphological microelements	---	---	---	---	---	---	---	---	---	---
Residual	168	90.1%	0.094	---	---	166	82.9%	0.171	---	---
	Vagile invertebrates (grazers)					Multivariate community structure				
Model	df	% Explained	Inertia	F	p-value	df	% Explained	Inertia	F	p-value
Constrain	4	2.7%	0.086	1.15	ns	2	9.2%	0.063	8.46	***
Residual	165	97.3%	3.099	---	---	167	90.8%	0.624	---	---
Marginal	df	% Explained	Variance	F	p-value	df	% Explained	Variance	F	p-value
Inclination	---	---	---	---	---	1	4.6%	0.031	8.29	***
Exposure	---	---	---	---	---	1	3.2%	0.021	5.71	***
Roughness	---	---	---	---	---	---	---	---	---	---
Diversity of morphological microelements	---	---	---	---	---	---	---	---	---	---
Residual	---	---	---	---	---	167	92.2%	0.624	---	---

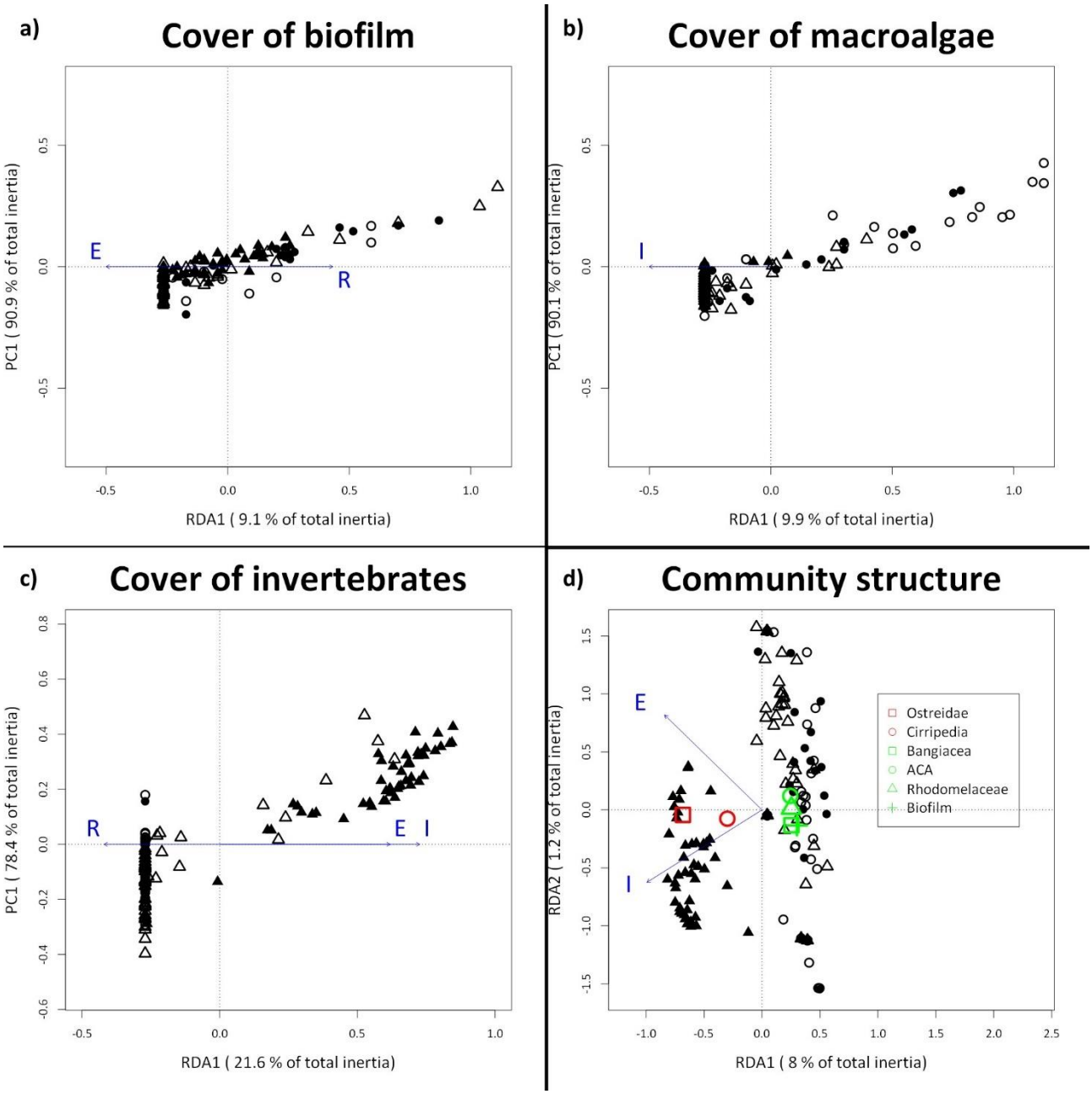

*Figure 3.3. Simple-RDA plots of the relationships between the biofilm cover (**a**), macroalgal cover (**b**) and sessile invertebrate cover (**c**) and the descriptors of the physical structure selected by the models and represented by blue arrows (Inclination = I; Exposure = E; Roughness = R, Diversity of morphological microelements = D). **d)** tbRDA triplot of the relationships between the multivariate community structure and the descriptors of the physical structure selected by the model and represented by blue arrows (see above); each taxa whose correlation was > 0.2 with the RDA axes was overlayed (red symbols for invertebrates, green symbols for macroalgae and biofilm). For sample symbols of the four plots please refers to Figure 3.2f legend.*

3.4 Discussion

Ecological engineering can help greening currently grey marine infrastructure, increasing their similarity with natural rocky habitats (Dafforn et al., 2015; Pioch et al., 2018). Our study provides critical quantitative information on the role of physical structure in driving differences in the communities between built and natural habitats. We found that different components of habitat structure contribute in different ways to differences in taxa distributions between the two habitats. Total cover differed between artificial and natural substrata of regular morphologies, being significantly larger on seawalls than on cliffs, but these differences were not related to the physical structure of the substrates. When the various morpho-functional groups of organisms were individually considered, further specific responses emerged. Sometimes these responses depended on differences between artificial and natural substrata of regular morphologies, with filter feeders more abundant on seawalls and grazers denser on cliffs, while at other times the biotic responses were moderately related with differences in inclination, exposure and, mildly, roughness. Therefore, topographic differences did not contribute much to the biotic differences, consistently with their limited observed differences between artificial and natural habitats (Grasselli and Airoldi, 2021).

We found greater differences in epibiota cover and composition between seawalls and natural substrates than between breakwaters and natural substrates, and weak relationships with habitat structure. Comparable responses were observed by Bulleri and Chapman (2004) and by Sedano et al. (2020b) in Tyrrhenian and Alboran seas, respectively, where the biggest differences with rocky reef epibiota were observed in seawalls than in other more irregular substrates (i.e. breakwaters or rip-raps, respectively). In our region, this difference was driven by a dense multi-strata cover of oysters in the seawalls, which covered almost all available space. The cover was more articulated in the other habitats, with a greater abundance of seaweeds, despite the presence of higher densities of grazers, as well as bare uncovered rock. Previous work has suggested a positive relationship between habitat complexity and oyster coverage (Strain et al., 2018a), but mostly at sites with high predator pressure, which was not our case.

We found the lack of any relation of total cover of organisms with physical structure and a moderate effect related to inclination and exposure of the substrates in shaping the epibiota composition between artificial and natural structures. This weak effect and the exclusion from the predictive model of those descriptors most commonly used as proxies of structural complexity (i.e. roughness and diversity of microhabitats; Shumway et al., 2007; Loke et al., 2015) are in contrast with what is commonly reported in the literature about the importance of physical structure as an ecological driver (Moschella et al., 2005; Bulleri and Chapman, 2010). However, when the covers of biofilm, macroalgae and sessile invertebrates were considered separately, their responses to physical structure resulted to be greater but dissimilar. Sessile invertebrates were more abundant in steeper and more landward plots, which represent the structural conditions proper of the artificial substrates, and in particular of seawalls, here (Grasselli and Airoldi, 2021) as elsewhere (Chapman and Underwood, 2011). As in a mirror image, biofilm on the one hand and macroalgae on the other were lightly more abundant, the former, in more seaward plots and, the latter, in more gently sloping plots, typical conditions of these (Grasselli and Airoldi, 2021) and other (Perkol-Finkel et al., 2006) natural rocky habitats. In addition, biofilm resulted slightly favored on rougher surfaces while sessile invertebrates were favored on smoother surfaces.

It is important to acknowledge that our findings refer to one-time point. Therefore, the results could be different in magnitude, direction and/or significance through seasons. While confirming the differences already observed previously in the communities settled on natural and artificial substrates (Moschella et al., 2005; Perkol-Finkel et al., 2006), our study revealed an extremely mild effect played by the physical structure. Neither the roughness nor the diversity of microhabitats, which represent the factors most manipulated at the investigated grain size during biomimetic eco-engineering interventions (*sensu* Chapman and Underwood, 2011), explained the differences in the intertidal community observed between artificial and natural habitats. The two factors were relatively similar in the two habitats, at least at the scale under study here (Grasselli and Airoldi, 2021), and differences in community structure were not correlated to these microhabitat variations. This suggests that in our area other features could play a major role in affecting differently the biological community between natural and artificial habitats, especially on

seawalls, such as material composition. Man-made infrastructures and natural substrates are known to usually differ in term of material composition (Bulleri and Chapman, 2010), therefore they also differ in the related physiochemical properties (e.g. porosity, color, hardness, surface chemistry, etc.) which are known to affect colonizing organisms (Terlizzi and Faimali, 2010). This could be our case as seawalls, where the epibenthic communities differed most from natural substrates, were the only substrate made by concrete, while breakwaters and the surrounding natural reefs were both rocky substrates.

In conclusion, our results suggest that in our study region physical structure is not the main ecological driver controlling differences in intertidal epibiota between natural and artificial habitats, but other co-occurring factors could directly or indirectly contribute to the differences in biota. Biomimetic efforts aiming to enhance the ecological value of the artificial habitats should consider the context in which the artificial substrates are placed. Thus, identifying which combinations of structural and non-structural elements could be enhanced as a whole, and not individually, to better recreate the natural conditions and promote native biodiversity.

Data availability statement

The original data presented in the study will be uploaded to public repositories as soon as the work is published.

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Supplementary materials

Taxa (metric)	Morpho-functional group	Datasets	Artificial Irregular (Breakwater)	Artificial Regular (Seawall)	Natural Irregular (Boulder field)	Natural Regular (Cliff)
***Mytilus galloprovincialis* (%)**	Filter feeder	Sessile invertebrates and Community structure	0.0 ± 0.0	8.0 ± 2.3	0.0 ± 0.0	12.2 ± 4.1
Ostreidae (%)	Filter feeder	Sessile invertebrates and Community structure	0.0 ± 0.0	77.0 ± 3.7	0.0 ± 0.0	0.0 ± 0.0
Cirripedia (%)	Filter feeder	Sessile invertebrates and Community structure	0.0 ± 0.0	12.6 ± 1.9	0.0 ± 0.0	0.2 ± 0.1
***Monodonta mutabilis* (n)**	Grazer	Vagile invertebrates and Community structure	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.1
***Patella* spp. (n)**	Grazer	Vagile invertebrates and Community structure	1.1 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	2.2 ± 0.3
***Ulva* sp. (%)**	Primary producer	Macroalgae and Community structure	0.2 ± 0.2	0.2 ± 0.1	3.0 ± 1.2	0.0 ± 0.0
***Scytosiphon* sp. (%)**	Primary producer	Macroalgae and Community structure	0.2 ± 0.2	0.0 ± 0.0	0.2 ± 0.2	0.0 ± 0.0
***Cystoseira* sp. (%)**	Primary producer	Macroalgae and Community structure	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Leafy Bangiacea (%)	Primary producer	Macroalgae and Community structure	3.2 ± 1.1	0.0 ± 0.0	7.4 ± 3.0	2.7 ± 1.1
Articulate coralline algae (%)	Primary producer	Macroalgae and Community structure	10.5 ± 3.7	0.0 ± 0.0	4.1 ± 2.1	2.1 ± 1.0
Filamentous Rhodomelaceae (%)	Primary producer	Macroalgae and Community structure	3.0 ± 2.0	0.0 ± 0.0	25.3 ± 5.1	0.2 ± 0.2
Green filamentous algae (%)	Primary producer	Macroalgae and Community structure	0.8 ± 0.8	0.0 ± 0.0	5.0 ± 2.5	0.1 ± 0.1
Dense algal turf (%)	Primary producer	Macroalgae and Community structure	0.0 ± 0.0	9.3 ± 4.0	0.0 ± 0.0	0.1 ± 0.1
Biofilm (%)	Biofilm	Biofilm and Community structure	16.7 ± 4.1	9.1 ± 1.2	23.5 ± 5.3	13.1 ± 3.5

Supplementary Table S3.1. Taxa/groups identified of intertidal biota (and the measurement metric). For each taxon/group, reference functional group, dataset in which they were included and mean abundance (±ES) in each orthogonal combination of habitat type and morphology were reported.

Chapter 4 – Material texture influences the abundances of particular functional groups of organisms on artificial structures

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Abstract

Marine urbanization and economic growth are resulting in the proliferation of artificial structures. These structures are primarily built with artificial materials which could impact their ecological performance. We conducted a qualitative synthesis and a meta-analysis on 76 studies to determine the effects of material composition in shaping the biodiversity of key functional groups of epibenthic organisms across a variety of environmental settings, by comparing rocky-type substrates (i.e. natural rocks, concrete) to each other and to other material types commonly used to build infrastructures (i.e. metal, plastic, ceramic, rubber and wood). Overall, there were no differences in the diversity, abundances, and biomass of colonizing organisms among substrate types, regardless of the environmental settings. However, there were more frequently greater abundances of epibiota (grouping fouling and vagile organisms), or of just fouling taxa (algae and sessile invertebrates), on the rocky-type substrates than on metal, plastic, and rubber materials, and concrete supported more diverse communities than metal and rubber. Our results indicate that the material composition has relatively little effect in shaping epibenthic communities, so eco-engineering interventions should prioritize other types of manipulations to increase the ecological performance of artificial habitats. Nevertheless, we believe that the choice of the material type is still crucial because eco-friendly materials produce important benefits lowering raw materials demand, energy cost or CO_2 emissions. Thus, when the construction of marine infrastructure is unavoidable, the environmental sustainability of materials should be considered along with other engineering considerations.

Keywords: review, epibenthic community, urban infrastructures, artificial material, physicochemical properties, eco-engineering, green material

4.1 Introduction

The marine ecosystems are being occupied by artificial infrastructure (Bugnot et al., 2021), which is built to support population growth and related anthropogenic activities (McGranahan et al., 2007). This infrastructure is made from a variety of materials and used for different purposes including coastal protection, support for trading, resource extraction, and shelter for coastal infrastructure and communities (Strain et al., 2019). The replacement of native ecosystems with manmade infrastructure reduces connectivity among habitats (Goodsell, 2009; Bishop et al., 2017), β-diversity of communities (Porter et al., 2018), and abundances and diversity of colonizing organisms (Chapman, 2003; Moschella et al., 2005). As consequence, marine urbanization represents a major threat to key ecological functions and ecosystem services (Bulleri and Chapman, 2010; Costanza et al., 2014; Heery et al., 2017).To overcome these problems, a range of solutions are being applied to promote sustainability in urban ecosystems through the reduction of anthropic pressures, the restoration of natural habitats, and the eco-engineering of artificial structures (Airoldi et al., 2021). Marine infrastructures have lower diversity than natural habitats because of their steeper angles (Chapman, 2003; Lam et al., 2009), different structural features (Perkol-Finkel et al., 2006), lower complexity (Lawrence et al., 2021), and artificial materials (Chapman and Bulleri, 2003). Eco-engineering design is one of the strategies implemented to improve the ecological benefits of artificial hard structures (*sensu* Bergen et al., 2001; see O'Shaughnessy et al., 2020 for a review), when other types of interventions are not feasible (i.e. habitat restoration; Chee et al., 2017). Eco-engineering designs seek to manipulate fine to large scale physical and chemical features of artificial hard infrastructures (Pioch et al., 2018), as those characteristics are considered to impair their ecological performances (Bulleri and Chapman, 2010).

The choice of the material is a critical step during eco-engineering design because, besides the cost and the engineering performance, material type has consequences on several key considerations for stakeholders like the carbon footprint and the net ecological impacts (Evans et al., 2017). The material choice affects the cost of the production process in terms of raw materials demand, energy cost for processing and transport, and CO_2 emission (Cooke et al., 2020). Greening this process by replacing traditional materials with other

eco-friendlier (i.e. mixing with by-products of industrial processes) would promote sustainability by reducing carbon footprint and lowering the use of raw materials (Meyer, 2009), although maintaining engineering performance (Huang et al., 2016; Becker et al., 2020). The material type, representing a substrate for the colonization of marine communities, affects also the settlement of organisms in various ways due to its physio-chemical properties (see Terlizzi and Faimali, 2010 for a review). Properties like texture (i.e. microroughness) affect the retention of spore and larvae (Fletcher and Callow, 1992; Berntsson et al., 2000), color affects the thermal stress in intertidal habitats (Lathlean and Minchinton, 2012) and the phototactic response in subtidal habitats (James and Underwood, 1994), or surface chemistry affects attraction of organisms by mean of chemical cues (Anderson, 1996). The identification, or development (Dennis et al., 2018), of building materials with optimal properties for the settlement and the post-settlement survival of organisms biota would promote an increase in the ecological value, therefore in the multifunctionality, of artificial structures.

However, consistent information about how material type affects the colonizing biota is still lacking. Some studies demonstrated that different epibenthic communities develop on various artificial materials (Brown, 2005; Dennis et al., 2018) or rocky substrates (Herbert and Hawkins, 2006; Canessa et al., 2019). Others did not observe differences at all (Perkol-Finkel et al., 2012; Foley et al., 2014), suggesting there is heterogeneity in the differences between material types (Dodds et al., manuscript submitted). Distinctness in physiochemical properties between rocky-type substrates (i.e. concrete; Waters and Zalasiewicz, 2018), whose porosity, surface chemistry, and texture most closely resemble the properties of rocky reefs to which organisms have evolved to adapt, and the other construction materials, more unnatural in their properties (i.e. metal, plastic or rubber; Chase et al., 2016), may have driven the observed heterogeneity in biota responses. The effects of different materials could also differ through time (Vaz-Pinto et al., 2014; Sempere-Valverde et al., 2018), between functional groups (Sedano et al., 2020) and environmental settings. Weathering and bioturbation alter the properties of the substrates over time (Coombes et al., 2011), and fouling organisms, covering the bare substrate, weaken its effect by creating a secondary substrate for other organisms (Hanlon et al., 2018). Fouling taxa, including algae and sessile invertebrates,

are more impacted by material properties than vagile taxa since microhabitat selection is almost irreversible after metamorphosis, thus influencing post-settlement mortality (Osborn, 2003). Indeed, the ability of vagile organisms in escaping from unfavorable conditions to more appropriate microhabitats results in a general lower effect of substrate composition (Guidetti et al., 2004). Environmental stressors in intertidal (i.e. hydrodynamics and desiccation) differ from subtidal habitats, hence substrate features such as porosity and thermal capacity are in the former environment of greater importance (Carrasco et al., 2019). Laboratory tests can isolate the effect of materials but often cannot be extrapolated to field conditions, whereas field studies cannot disentangle the effects of materials from other site-specific abiotic and biotic conditions, leading to discrepancies between laboratory and field experiments (Anderson, 1996). Testing the effects of materials on the colonizing biota is important for eco-engineering artificial structures and enhancing their ecological value.

Hence, we conducted a qualitative synthesis and meta-analysis to determine the effects of material type on the colonizing organisms (i.e. algae, sessile and vagile invertebrates). We hypothesized that colonizing organisms will differ in terms of abundance, diversity, or biomass among rocky-type substrates, either natural (indigenous or imported/manufactured rocky substrates) or artificial (i.e. concrete; Waters and Zalasiewicz, 2018), and between these substrates and other construction materials (i.e. metal, ceramic, rubber, plastic, and wood) due to the different physio-chemical properties (i.e. micro-texture, porosity, hardness, color, mineralogic content, pH, etc.). We tested the effects of local rocky substrates (H1), imported/manufactured rocky substrates (H2), and Portland concrete (H3). We expected to find decreasing effect sizes on colonizing organisms from H1 to H3 due to an increasing divergence between these control substrates and the substrates found in the natural environment. Moreover, for each hypothesis, we expected the effects of the materials to differ between functional groups of organisms (epibiota, algae, sessile and vagile invertebrates), based on their behavioral and morphological traits, and between environmental settings due to the interaction of material type with other site-specific conditions. Finally, we expected the effects of material type to decline through time due to changes in surface promoted by

weathering and colonization of sessile taxa. The overall aim was to inform engineers and managers what material types support the highest biodiversity of colonizing organisms.

4.2 Methods

4.2.1 Literature screening

We conducted a literature search to identify manipulative and mensurative studies which compared, the effects of different materials on the diversity, abundances and biomass of colonizing organisms on built infrastructure. We conducted the literature searches in both Scopus and Web of Science databases, running two equivalent strings, on the 16th of November 2020. The strings were developed trying to maximize their sensitivity, losing in specificity, on the other hand, to include a higher number of documents as ecology lacks proper mesh-heading indexes and integrated databases (Pullin and Stewart, 2006). The search strings were developed, and then trained, using 21 eligible papers.

The two equivalent search strings were:

- For Scopus: *TITLE-ABS-KEY ((("rock" OR "substra*" OR "material*") W/2 ("type*" OR "composit*" OR "colour*")) OR ("chemi*" W/2 ("substr*" OR "rock" OR "composit*")) OR "concret*") AND TITLE-ABS-KEY ("intertida*" OR "subtida*" OR "estuarin*" OR "marin*" OR "coast*" OR "artific*") AND TITLE-ABS-KEY ("biodivers*" OR "assemblag*" OR "recruit*" OR "coloni?at*" OR "benth*")*
- For Web of Science: *((("rock" OR "substra*" OR "material*") NEAR/2 ("type*" OR "composit*" OR "colour*")) OR ("chemi*" NEAR/2 ("substr*" OR "rock" OR "composit*")) OR "concret*") AND ("intertida*" OR "subtida*" OR "estuarin*" OR "marin*" OR "coast*" OR "artific*") AND ("biodivers*" OR "assemblag*" OR "recruit*" OR "coloni?at*" OR "benth*")*

A total of 4496 documents were initially identified, of which 2478 were from Scopus, 29 from Scopus secondary documents (i.e. Scopus not indexed documents) and 1989 from Web of Science (Moher et al. 2009; Figure 4.1). These papers were initially screened by title to remove any duplicates and off-topic studies, and 237 were retained for further investigation. Thereafter, the remaining papers were screened by the abstract only, or by the full text if necessary, and excluded based on differences in the surrounding environment (i.e. communities on hard substrates were compared to communities located on soft sediment or living biogenic substratum, or substrates coated with antifouling paints), differences in the

types of built infrastructures (i.e. pontoon vs breakwater, seawall vs piling), when the substrates compared were expressly placed in different geographic location, or the documents reported a study conducted in freshwater environment.

The final selection included 102 documents; 79 of which were obtained by the string selection and the other 23 documents were identified throughout “snowballing” process (Wohlin, 2014). This process consisted of using the reference list of a paper or the citations to that paper for each of the 79 documents initially selected to identify additional papers. This process was iterated until no new papers were found.

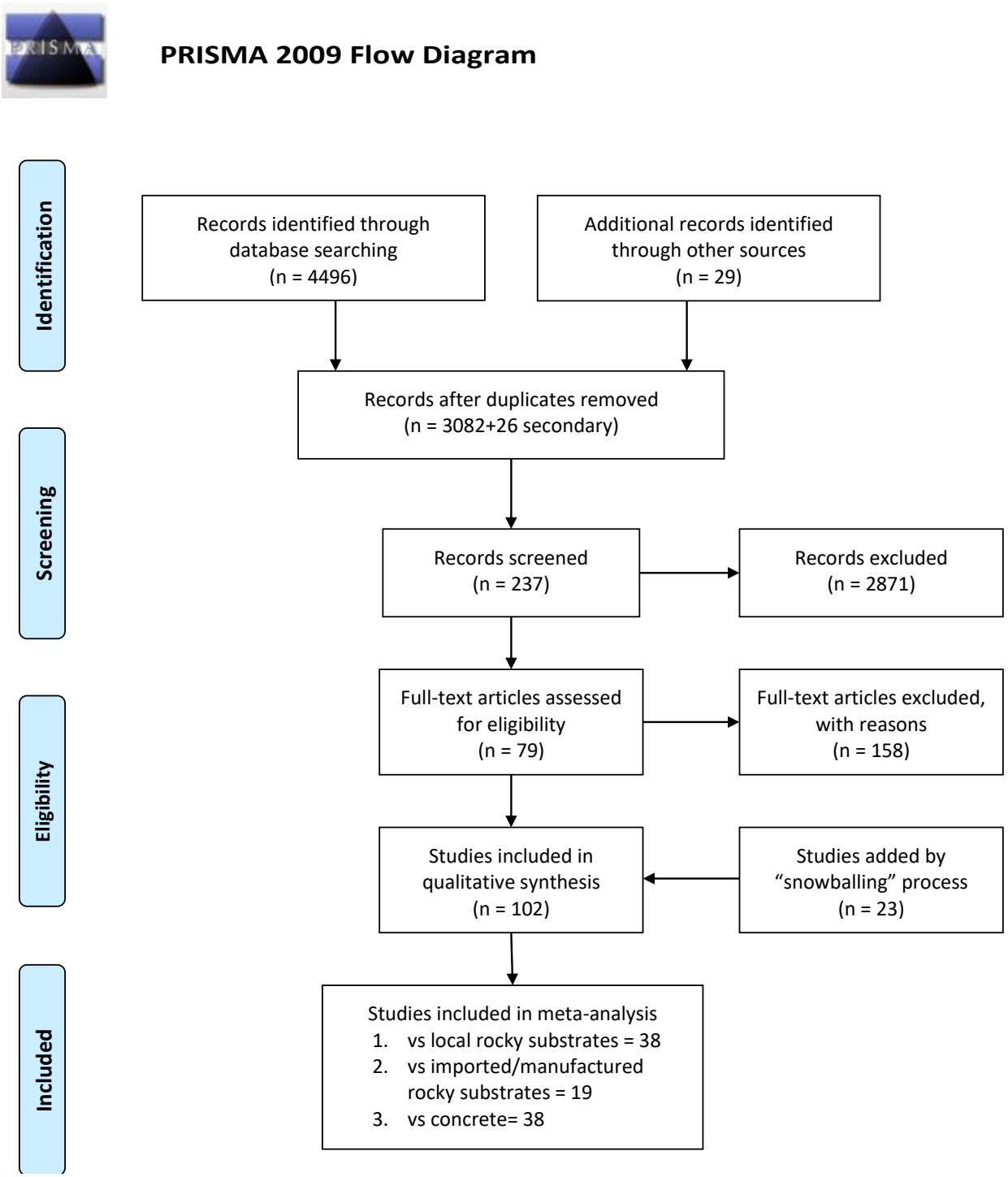

Figure 4.1. Flow of information through the different phases of the systematic review. For each of the four phases, the number of relevant publications is reported. From Moher et al. (2009).

4.2.2 Data extraction

Of the 102 studies retained for the qualitative synthesis, we recorded the types of tested materials, the type of local substrate (checked on national geochemical atlases when not reported by the paper, Supplementary Table S4.1), the functional groups (i.e. algae, sessile invertebrates, vagile invertebrates or all these groups combined forming the benthic assemblage, hereafter 'epibiota') and the phylogeny of the

assessed organisms, the type and size of manipulation, depth, duration of the study, and any additional relevant notes that might be important for understanding conditions of the study (e.g. possible conflicts of interest, etc.). Since the information provided by the studies about the tested materials was heterogeneous, the materials were classified according to their typology into seven groups (i.e. rock, concrete, metal, ceramic, rubber, plastic, and wood; Table 4.1). We also recorded metadata related to the publication date, the geographic location (e.g. country, ocean basin, latitude) and the environmental context when reported.

Table 4.1. List of the seven material groups used in the study with their respective included material types.

Material type	Materials included
Ceramic	Brick, Ceramic, Porcelain, Terracotta
Concrete	Portland cement, ECOncrete, cement with added biological components or with added recycled industrial wastes
Metal	Aluminum, Iron, Metal, Steel, Zinc
Plastic	Acrylic – Fiberglass – Plastic – PVC – Polyethylene – Polystyrene – Polycarbonate
Rock	Asbestos – Basalt –Coral reef – Gabbro – Gneiss – Granite – Limestone – Marble – Marl – Mudstone – Quartzite – Rhyolite – Sandstone – Shale – Siltstone – Slate
Rubber	Rubber – Tire
Wood	Plywood – Wood

Seventy-six studies were used in the meta-analysis from which it was possible to determine mean, standard deviation (SD) and sample size of colonizing organisms per material type. In particular, the metrics of interest were abundance (percentage cover and density), diversity (taxa richness and Shannon diversity) and biomass. In each of these studies, colonizing organisms were compared between at least a rocky-type substrates, used in our meta-analysis as control substrate, and another material, either artificial or natural, used as treatment (Table 4.1). Based on the type of rocky-type substrate assessed, these studies were all included in at least one of three hypotheses: H1 (vs. local rocky substrates), H2 (vs. imported or manufactured rocky substrates) or H3 (vs. Portland concrete). Hence, the same study could be eligible to test for more than one hypothesis if more of these rocky-type substrates were simultaneously compared to other construction materials. From the 76 studies, 38 reported data suitable to test H1, 19 to test H2 and 38 to test H3 by mean of a meta-analysis (Figure 4.1; Supplementary Table S4.2).

Where the data were depicted in figures, we used GetData Graph Digitizer version 2.26 (www.getdata-graph-digitzer.com) to extract means and SDs. Data extraction was not always straightforward as some studies were complex and tested the differences between multiple material types and manipulated numerous extra conditions (i.e. substrate complexity, inclination, sampling period, etc.). To avoid confusion, in such cases, we considered only data from comparable manipulated conditions. We recorded them as individual datapoints in the spreadsheet and linked them using an Experimental identification number (or ID). In cases where studies reported separately multiple measures from the same substrate within the same conditions (e.g. three replicas from three random sites within the same location), we pooled the means and weighted them as follow:

$$\bar{X} = \frac{\sum_i n_i * \bar{x}_1}{\sum_i n_i}$$

And then we pooled the SDs, first identifying the deviance (SS) within and between groups as follow:

$$SS_{within} = \sum_i^g (n_i - 1) * SD_i$$

$$SS_{between} = \frac{\sum_i^g n_i * (\bar{x}_i - \bar{X})}{g - 1}$$

And then square rooting the total deviance divided for the total number of samples minus one:

$$\sqrt{\frac{SS_{within} + SS_{between}}{N - 1}}$$

4.2.3 Imputation of missing SD

When the SD was missing or other measurements of the sample variances were recorded (i.e. confidence intervals or standard errors), estimations or conversions were necessary. The missing SD were estimated by fitting a linear model between the known means and SDs, both log10 transformed, from similar studies (Higgins et al., 2019). Imputation of missing SD is suggested over the exclusion of data to promote robustness of the results, including as much information as possible (Weir et al., 2018).

4.2.4 Meta-analysis

We calculated the effect sizes for the numerous comparisons using Standardized Mean Difference (SMD) (Cohen, 1988) which was preferred over logarithmic response ratio due to the presence of zeros in the dataset (Çoğaltay and Karadağ, 2015). SMD was calculated as:

$$SMD = \frac{\overline{X_C} - \overline{X_T}}{S_{within}}$$

In the numerator, X_C and X_T are the sample means of rocky-type substrates (control) and other tested materials (treatment), respectively. In the denominator, S_{within} is the within-groups standard deviation, pooled across "Control" and "Treatment" groups, calculated as:

$$S_{within} = \sqrt{\frac{(n_C - 1) * S_C^2 + (n_T - 1) * S_T^2}{n_C + n_T - 2}}$$

We checked for publication bias using funnel plots, while the number of studies needed to increase p-value over 0.05 was assessed with Rosenthal's failsafe number.

We fitted a multilevel model that included both categorial and continuous moderators. The moderators extracted from the metadata were: functional groups (hereafter 'FG'; i.e. algae, sessile invertebrates, vagile invertebrates and epibiota), material types (hereafter 'TY'; i.e. rock, concrete, metal, ceramic, rubber, plastic and wood), duration of the experiment (in month), ocean basin (i.e. Atlantic, Coral Triangle, Indian, Mediterranean, Pacific, Red Sea or Laboratory), absolute latitude (as covariate), environmental context (natural, built infrastructure or laboratory), tidal elevation (intertidal, subtidal, or laboratory). In addition, we tested the interaction only between TY and FG since the amount of available data to test the interaction among other moderators was insufficient. The random effects included in the model were: the Experiment ID nested the respective study identities, the year of publication and the phylogeny of the assessed organisms. The moderators were included in the analyses only if assessed in at least two studies. All analyses were performed on R v.3.5.3 using packages 'metaphor' (Viechtbauer, 2010).

4.3 Results

The studies used in the meta-analysis encompasses more than four decades and were widespread, across the globe (Figure 4.2). Rock and concrete substrates were tested in 67% and 63% of the studies, respectively the other materials (i.e. metal, plastic, ceramic, rubber and wood) were assessed in 13% to 22% of the studies. Abundance was the metric more frequently measured (87%), followed by diversity (33%) and, biomass (16%). The funnel plots produced for each metric and each hypothesis, H1, H2 or H3, did not indicate the presence of any publication bias as there were no notable asymmetries (Supplementary Figure S4.1). In addition, the data extracted for each hypothesis came from studies performed in multiple laboratories, years and countries, suggesting the results are not strongly biased towards an individual country or time point.

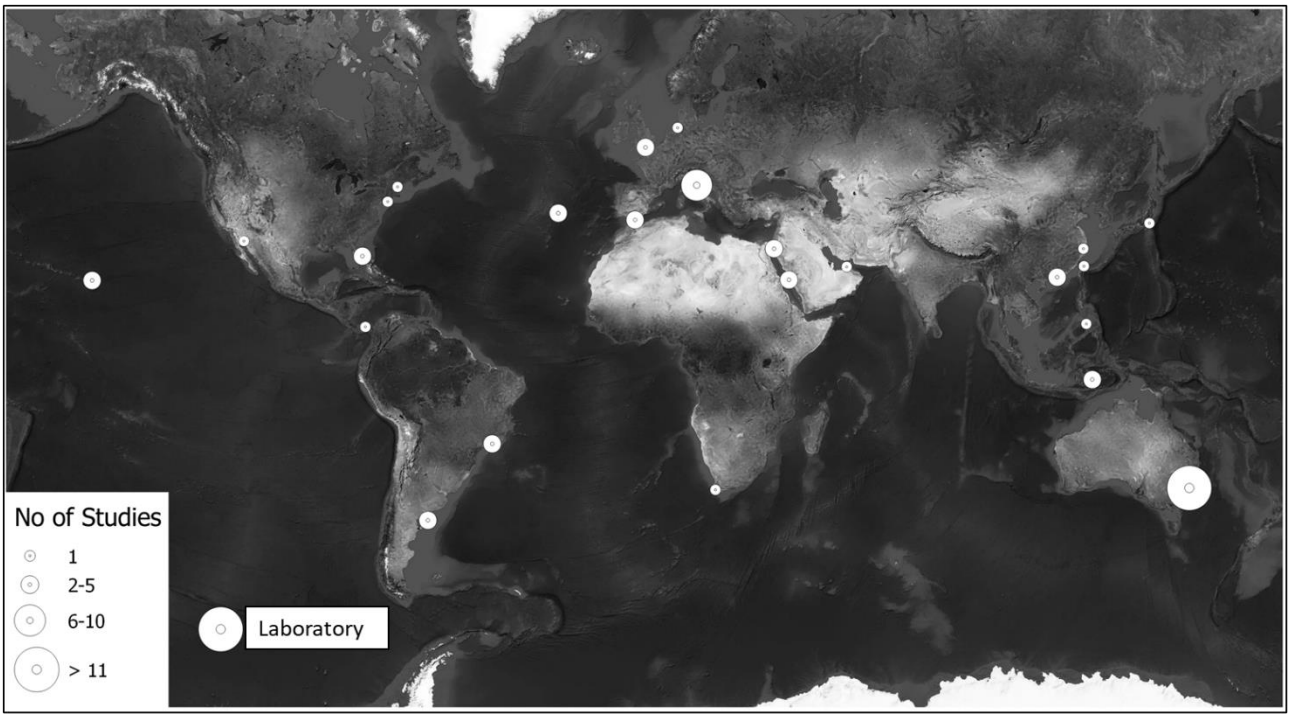

Figure 4.2. The map shows the geographical location of the studies. The circle dimension represents the number of studies performed at each location. The circle representing the studies conducted in laboratories (n = 12) were grouped altogether and placed at 60°S 115°E and labelled.

Overall, our meta-analysis revealed the lack of significant differences in colonizing organisms between control (i.e. rocky-type substrates) and treatment materials in each of the three hypotheses for all the metrics of interest (Figure 4.3). Moreover, the addition of moderators in the models rarely affected the response of the colonizing organisms (i.e. not significant heterogeneity of the moderator (QM); Supplementary Table S4.3).

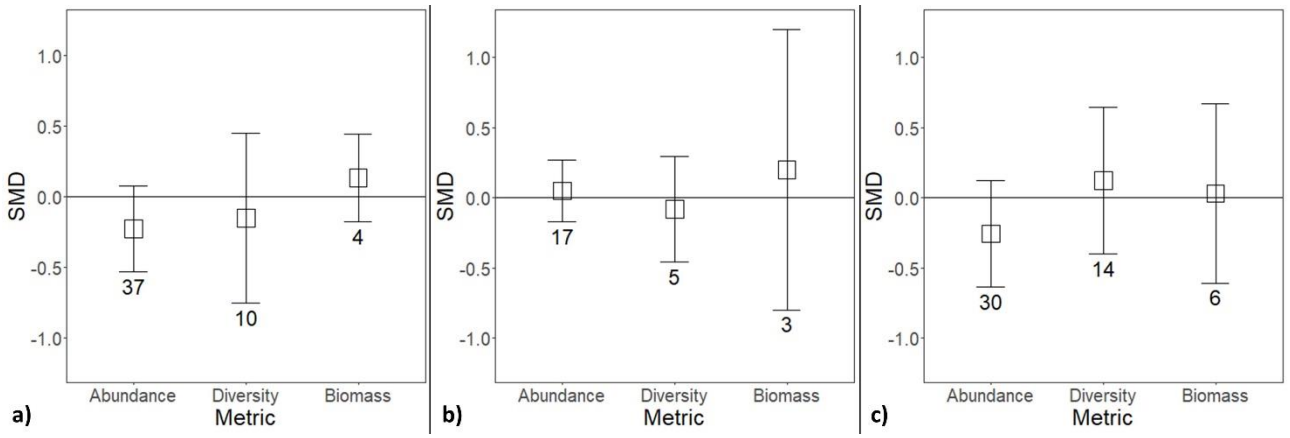

Figure 4.3. Effect sizes (+/-CI) of metrics of colonizing organisms on control rocky-type substrates vs. other materials for the three hypotheses: a) H1, b) H2 and c) H3. Effect sizes were calculated as Standardized Mean Difference (SMD) estimated with random-effects models including: 'study', 'ID' nested in 'study', 'phylogeny' of the organisms and 'year' of the study. Positive SMD indicates that the metric reported higher values on treatment materials rather than control substrates, and a negative SMD means the opposite. If error bars overlap with zero, then effect size is not significant. Numbers report the number of studies involved in each comparison.

Overall, there were no differences between the indigenous rocky substrates, imported rocky materials, Portland cement and the other construction materials. There were however significant effects of the moderators. Below, we detailed our findings separately for each hypothesis. When reported, positive values of Cohen's *d* effect size indicate that a community metric is higher on artificial materials than on control substrates (i.e. indigenous rocky substrates for H1, imported rocky substrates for H2, Portland concrete for H3). In contrast, a negative Cohen's *d* means the opposite. 95% confidence intervals (CI) overlapping with zero mean the difference between artificial and control substrates is not significant.

4.3.1 H1: vs. indigenous rocky substrates

The epibiota was more abundant on indigenous rocky substrates relative to other material types (i.e. concrete, plastic and rubber) and there were higher abundances of sessile invertebrates on indigenous rocky substrates compared with rubber materials (Figure 4.4a; Supplementary Table S4.4). In contrast, the abundances of algae and vagile taxa were unaffected by material type (Figure 4.4a; Supplementary Table S4.4). The indigenous rocky substrates supported higher abundances of colonizing organisms than other materials through time (Figure 4.4b; Supplementary Table S4.3). Across the environmental context, there were higher abundances of colonizing organisms on indigenous rocky substrates than other material types in the laboratory studies but not in the field studies (Figure 4.4c; Supplementary Table S4.3).

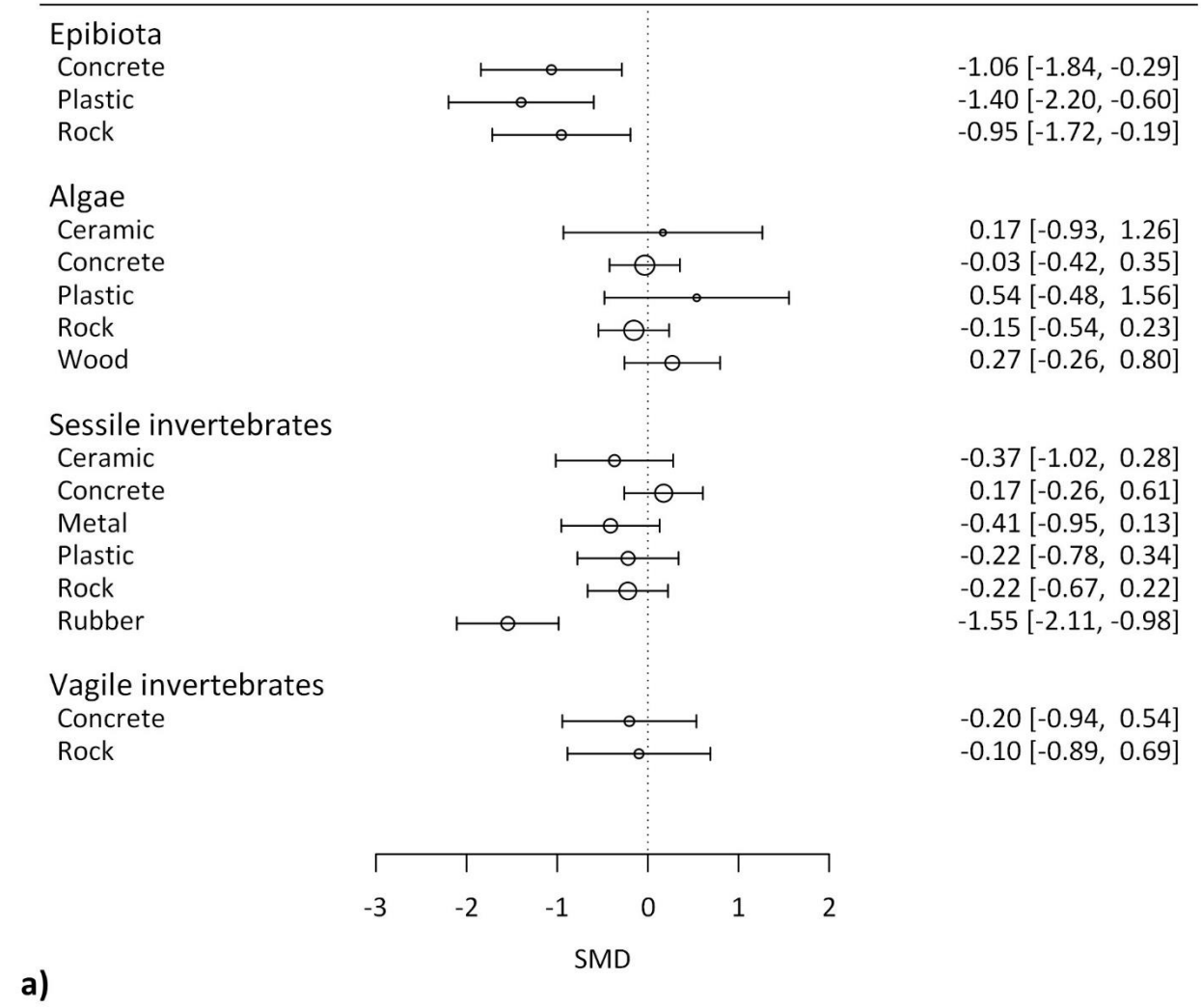

Figure 4.4. a) Forest plot of effect sizes (+/-CI) of abundance of colonizing organisms on local rocky substrates (the control substrate) vs. other materials for each tested combination of functional group and material type, used as moderators. "Rock" group includes only imported rocky substrates (i.e., different type of rocks than the substrate reported by the geological atlases in that same study area; Supplementary Table S4.1). b) Scatter plot reports the Standardized Mean Differences (SMDs) of all the data points of

abundance of organisms (circles) against the duration of exposition to substrates. The red dotted line indicates the zero, the blue line and the dotted blue lines indicate, respectively, the effect size and the 95% CI of abundance of colonizing organisms on control rocky-type substrates vs. other materials. c) Effect sizes (+/-CI) of abundance of colonizing organisms on the control substrate vs. other materials for each experimental context, used as moderator. Numbers report the number of studies involved in each.

4.3.2 H2: vs. exotic rocky substrates

The epibiota was more abundant on imported/manufactured rocky substrates relative to other material types (i.e. metal, rubber and wood; Figure 4.5a; Supplementary Table S4.5). Algae reported lower abundances on imported/manufactured rocky substrates compared with concrete, but higher compared to the other materials (i.e. ceramic, metal, plastic, rubber and wood; Figure 4.5a; Supplementary Table S4.5). In contrast, the abundances of sessile and vagile invertebrates were unaffected by material types with the exception of ceramic which supported higher abundances of sessile invertebrates than imported/manufactured rocky substrates (Figure 4.5a; Supplementary Table S4.5). The imported/manufactured rocky substrates supported higher diversity of colonizing organisms than other materials through time (Figure 4.5b; Supplementary Table S4.3).

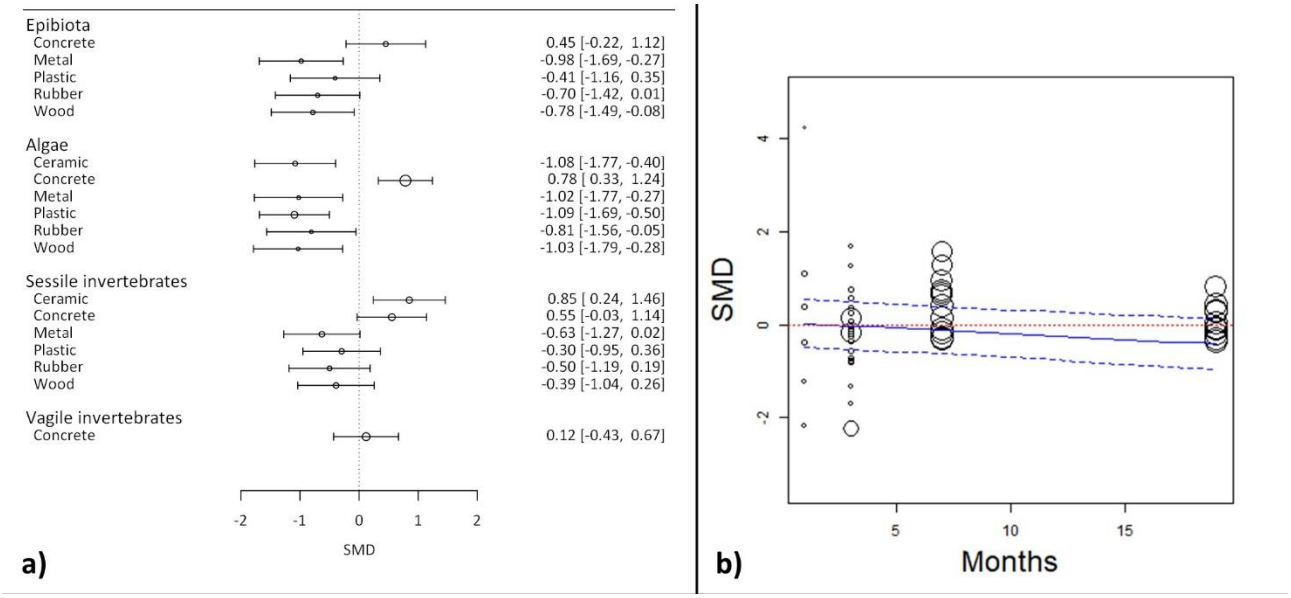

Figure 4.5. a) Forest plot of effect sizes (+/-CI) of abundance of colonizing organisms imported/manufactured rocky substrates (the control substrate) vs. other materials for each tested combination of functional group and material type, used as moderators. b) Scatter plot reports the Standardized Mean Differences (SMDs) of all the data points of diversity of colonizing communities (circles) against the duration of exposition to substrates. The red dotted line indicates the zero, the blue line and the dotted blue lines indicate, respectively, the effect size and the 95% CI of diversity of colonizing communities on the control substrate vs. other materials.

4.3.3 H3: vs. Portland cement

Algae were more abundant on wood compared to Portland concrete (Figure 4.6a; Supplementary Table S4.6). In contrast, epibiota, sessile and vagile invertebrates, and algae reported similar abundances when comparing other types of material. (Figure 4.6a; Supplementary Table S4.6). The Portland concrete supported communities of colonizing organisms more diverse than metal and rubber, but there were no differences between Portland concrete and other types of concrete, plastic, and wood (Figure 4.6b; Supplementary Table S4.3). The Portland concrete supported also higher levels of diversity and biomass of colonizing organisms than other materials through time (Figure 4.6c-d; Supplementary Table S4.3).

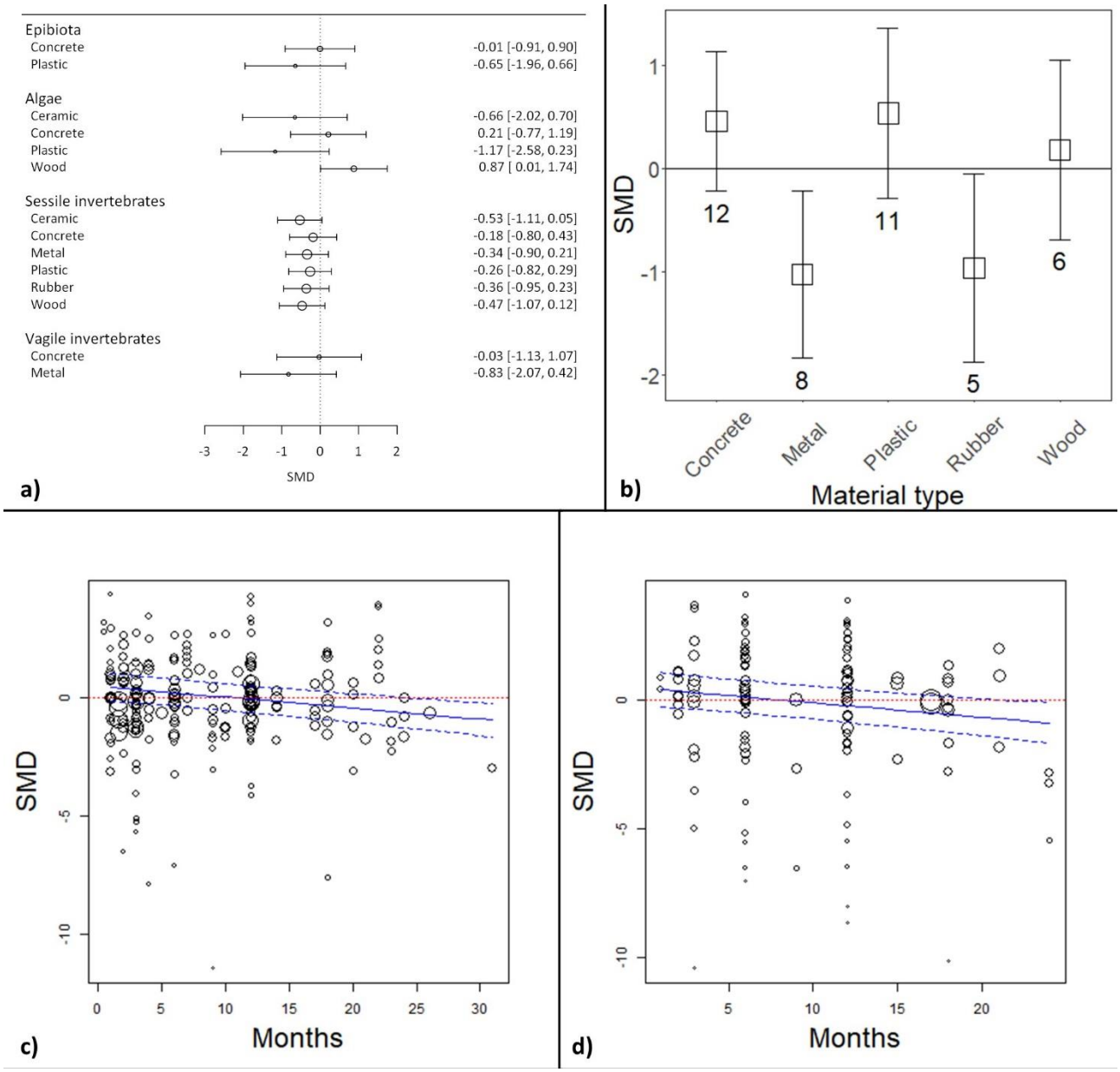

Figure 4.6. a) Forest plot of effect sizes (+/-CI) of abundance of colonizing organisms on Portland concrete (the control substrate) vs. other materials for each tested combination of functional group and material type, used as moderators. "Concrete" group includes only concrete made by a blend different than Portland (i.e. ground granulated blast-furnace slag concrete, fuel-ash concrete, coal-ash concrete). b) Effect sizes (+/-CI) of diversity of colonizing organisms on the control substrate vs. other materials for material type, used as moderator. Numbers report the number of studies involved in each. c) Scatter plot reports the Standardized Mean Differences (SMDs) of all the data points of diversity of organisms (circles) against the duration of exposition to substrates. The red dotted line indicates the zero, the blue line and the dotted blue lines indicate, respectively, the effect size and the 95% CI of abundance of colonizing organisms on control rocky-type substrates vs. other materials. d) Same as "c)" but data refers to biomass of organisms.

4.4 Discussion

Our study provided useful information about to what extent material types affect colonizing biota. The synthesis of the 76 studies included in our analysis demonstrated that material types affected the colonizing organisms in some specific situations and their effect is variable through time. Material type affected the abundances of specific functional groups (i.e. algae and sessile invertebrates) and the diversity of communities. In both cases, rocky-type substrates performed better than other artificial materials such as metal, plastic or rubber. These findings only partially agreed with our hypotheses. Indeed, we expected to observe more generalized and pronounced differences between rocky substrates, especially local rocky substrates, and the other construction materials. Moreover, we expected various environmental settings to play a major role in shaping the community responses. So, our findings were not able to fully disentangle from the variability reported by literature as the different responses of the various functional groups did not follow any consistent pattern and just once the laboratory conditions affected differently organisms compared to field conditions.

Nevertheless, our results recommend preferring rocky-type substrates (natural rock or concrete; Waters and Zalasiewicz, 2018) over other construction materials. Greater abundances of organisms, although with differences between functional groups, were observed in rocky-type substrates compared to other artificial materials (i.e. metal, plastic and rubber), and communities more diverse were observed on Portland concrete than on metal and rubber. The better performance of rocky-type substrates against these other artificial materials is probably caused by a better match of the behavioral and morphological traits of some of the colonizing organisms with substrates more textured and porous (Anderson and Underwood, 1994; Reyes and Yap, 2001). Hence, physical properties of substrates (i.e. texture, hardness, porosity and specific heat) could be the main cause of the observed differences, affecting for example attachment (Hartanto et al., 2022) or thermal stress in the intertidal environment (Lathlean and Minchinton, 2012). On the other hand, chemical properties are relegated to a minor role since they produced insignificant or no effect on organisms when the physical properties were standardized (Hixon and Brostoff, 1985; McManus et al., 2018; Hsiung et al., 2020). Differences in behavioral and morphological traits, again, might have produced

the greater effect of materials observed on fouling (i.e. sessile invertebrates and algae) compared to vagile organisms. In fact, the formers are directly and more affected by physiochemical properties which then reverberate, usually dampening, at higher trophic levels through bottom-up processes (Guidetti et al., 2004; Dennis et al., 2018), especially when macrofouling organisms form a secondary substrate for the vagile fauna (Sedano et al., 2020).

In addition, the perception of material types by colonizing organisms has proved to change through time and, sometimes, to differ between laboratory and field experiments. The rapid colonization by microorganisms forming a biofilm layer in the early stages of colonization can alter the physiochemical properties of the primary substrate (Qiu et al., 2003; Coombes et al., 2011), altering the perception for later colonizing organisms (Lozano-Cortés and Zapata, 2014). Furthermore, the presence of biofilm promotes the subsequent settlement of macrofouling organisms (Su et al., 2007; Li et al., 2014). These organisms can further alter the perception of the substrate by forming biogenic substrates, thus masking completely the substrate properties (Terlizzi and Faimali, 2010). Perhaps not surprisingly then, in cases where these and other factors were removed (i.e. under short-term laboratory experiments using a single model species), we observed differences in the abundance of single taxa among materials (Chase et al., 2016; Pinochet et al., 2020). However, these so perfectly isolated conditions are hardly representative of, thus not comparable to, field conditions, hence producing heterogeneity in the outcomes between these experimental contexts (i.e. field vs. laboratory; Anderson, 1996; Foley et al., 2014).

Overall, our results demonstrated that artificial structures built with man-made and natural materials frequently support similar epibenthic communities and that the use of rocky-type substrates is already enough to promote ecological value as much as possible through material type manipulation. This relatively weak effect of material type on the biodiversity of colonizing organisms indicates that eco-engineering strategies should focus on other types of manipulations to better improve the ecological value of artificial marine infrastructures. For example, the addition of complexity through microhabitats or seeding with habitat-forming species (Strain et al., 2018) appears to have greater benefits for the biodiversity of artificial structures than material types. Nonetheless, the choice of the material type still provides crucial ecological

benefits by reducing environmental impacts (raw materials demand, energy cost or CO_2 emission) during the production process (Cooke et al., 2020), especially as that coastal hardening is forecasted to expand over the next decades (Floerl et al., 2021). In this direction, greening concrete industry is able to provide these ecological benefits by replacing quarry virgin aggregates and Portland cement with recycled by-products (i.e. shell fragments, fly ash or ground granulated blast furnace slag; Meyer, 2009). Since the growing interest for these types of secondary benefits (Evans et al., 2017), the development of eco-friendlier materials is increasing and the body of works testing greener concrete is already reporting both an ecological enhancement *in situ* and a carbon footprint reduction during production processes while meeting engineering standards (Dennis et al., 2018; McManus et al., 2018).

Although our study was able to answer some questions regarding the effect produced by the type of material on the epibenthic community, others remained unsolved mainly due to the small number of available studies. We were not able to fully explore how environmental settings change the response of colonizing organisms to material type because moderator sub-levels were rarely assessed in at least three primary studies, especially when dealing with diversity and biomass of organisms. This scarcity of primary studies assessing diversity and biomass of organisms may have also reduced the power of these analyses, hiding the true effect driven by the material type. For the same reason, the response of organisms towards the type of material is likely more affected by some of the moderators considered (e.g. environmental contexts) even if, when tested, they seldom reported a significant effect on the epibenthic community. To support this, significant differences between materials were observed only when many primary studies were involved. This was the case of abundance, where the studies involved were always at least 30 for each hypothesis, and of diversity for that only hypothesis (H3) of the three in which the primary studies were more numerous (14 studies in H3, against 10 in H1 and 5 in H2). Additionally, most of the primary studies rarely extended beyond 12 months. Thus, it is not possible to be sure that the trend observed through time, in which prolonged exposures favor communities settled on rocky-type substrates, can be extrapolated even for longer durations. Therefore, given these limitations, our understanding of the extent to which

material types affect biodiversity is only partially resolved by this study, and the effects produced by material types in any situation and for longer durations remain largely unknown.

Our results demonstrate that, although the choice of material weakly affects the colonizing organisms, its effect in shaping epibenthic communities was not strong as expected (Falace and Bressan, 2000; Perkol-Finkel et al., 2006; Bulleri and Chapman, 2010; Terlizzi and Faimali, 2010). Thus, other types of eco-engineering interventions could be more effective in eco-engineering artificial structures (Strain et al., 2018). Nevertheless, the choice of the material type is still a crucial step because eco-friendly materials (i.e. concrete made with recycled by-products) are able to promote equally important benefits in the early phases of the marine urbanization process by reducing carbon footprint or raw materials demand (Dennis et al., 2018; Cooke et al., 2020). The union of these benefits with those provided by interventions aimed at enhancing ecological performance (i.e. adding or retrofitting of microhabitats, seeding of habitat-forming species) is, in our opinion, capable of improving the sustainability of marine urbanization. Despite this, we warn against the consideration that these manipulations can fully compensate for all the types of impact, and that approaches that aim to avoid it, preserving or restoring natural habitats, have always to be prioritized when possible (Arlidge et al., 2018).

Data availability statement

The original data presented in the study will be uploaded to public repositories as soon as the work is published.

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Supplementary materials

Supplementary Table S4.1. Source reporting information about the geology of each location involved in the systematic review. In case national geochemical atlas was checked, link is provided.

Locality	Geological information	Link atlas
Argentina	Info reported on paper	
Australia	Info reported on both papers and atlas	https://portal.ga.gov.au/restore/38ed09a9-9e23-45eb-9016-dbe1dc92531d
Azores	Info reported on papers	
Brazil	Info reported on atlas	http://www.cprm.gov.br/publique/Hidrologia/Estudos-Hidrologicos-e-Hidrogeologicos/Atlas-Hidrogeologico-do-Brasil-ao-Milionesimo-4267.html
Canada	Info reported on atlas	https://geoscan.nrcan.gc.ca/starweb/geoscan/servlet.starweb?path=geoscan/fulle.web&search1=R=208175
China/ Hong Kong/ Taiwan	Info reported on atlas	http://www.geoscience.cn/UploadFiles/2016_10_28/hgm03.JPG
Colombia	Info reported on paper	
Croatia	Info reported on atlas	https://www.hgi-cgs.hr/osnovna-geoloska-karta-republike-hrvatske-1100-000/
Emirates	Info reported on paper	
German	Info reported on atlas	https://produktcenter.bgr.de/terraCatalog/DetailResult.do?fileIdentifier=f1780a06-69b6-44b0-855e-dc0f8da9a1d4
Great Britain	Info reported on both papers and atlas	https://mapapps.bgs.ac.uk/geologyofbritain/home.html?&_ga=2.66747310.1275203152.1620816072-2023875979.1620816072
Hawaii	Info reported on paper	
Indonesia	Info reported on paper	
Israel	Info reported on both papers and atlas	https://egozi.gsi.gov.il/webapps/geology/geo50k_keymap/
Italy	Info reported on both papers and atlas	https://www.isprambiente.gov.it/Media/carg/
Japan	Info reported on atlas	https://gbank.gsj.jp/geonavi/geonavi.php#11,35.47400,139.77199
Mexico	Info reported on paper	
Panama	Info reported on paper	
Philippines	Info reported on paper	
Saudi Arabia	Info reported on atlas	https://www.researchgate.net/figure/Geological-map-of-Jeddah-city_fig3_332181342
South Africa	Info reported on paper	
Spain	Info reported on both papers and atlas	https://igme.maps.arcgis.com/home/webmap/viewer.html?webmap=92d3a8e400b44daf911907d3d7c8c7e9
USA	Info reported on atlas	https://ngmdb.usgs.gov/ngmdb/ngmdb_home.html

Supplementary Table S4.2. Details of the studies included in the meta-analysis. For each study, the author(s), year, study title, journal name, location(s) and tested materials are provided. The inclusion or the exclusion of the studies from the three hypotheses (H1, H2, H3) is indicated with '1' or '0', respectively. Metrics of colonizing organisms (abundance, diversity, biomass) reported in the studies are indicated with a '1' and absence with a '0'.

Autor(s)	Year	Title	Journal	Location(s)	Materials tested	H1	H2	H3	Abundance	Diversity	Biomass
Long	1974	Marine fouling studies off Oahu, Hawaii	The Veliger	Hawaii	Rock, Metal, Plastic	0	1	0	0	0	1
Caffey	1982	No effect of naturally-occurring rock types on settlement or survival in the intertidal barnacle, *Tesseropora rosea* (Krauss)	Journal of Experimental Marine Biology and Ecology	Australia	Rock	1	0	0	1	0	0
Hixon & Brostoff	1985	Substrate characteristics, fish grazing, and epibenthic reef assemblages off Hawaii	Bulletin of Marine Science	Hawaii	Rock, Plastic	1	0	0	1	1	1
Woodhead & Jacobson	1985	Epifaunal settlement, the processes of community development and succession over two years on an artificial reef in the New York bight	Bulletin of Marine Science	USA	Concrete	0	0	1	1	1	0
Raimondi	1988	Rock type affects settlement, recruitment, and zonation of the barnacle *Chthamalus anisopoma* Pilsbury	Journal of Experimental Marine Biology and Ecology	Mexico	Rock	1	0	0	1	0	0
Fitzhardinge & Bailey-Brock	1989	Colonization of artificial reef materials by corals and other sessile organisms	Bulletin of Marine Science	Hawaii	Rock, Concrete, Metal, Rubber	1	0	1	1	0	0
McGuinness	1989	Effects of some natural and artificial substrata on sessile marine organisms at Galeta Reef, Panama	Marine Ecology Progress Series	Panama	Rock, Concrete, Plastic, Wood	1	1	1	1	0	0
Wells et al.	1989	Substrate as a determinant of marine intertidal algal communities at Smitswinkel Bay, False Bay, Cape	Botanica Marina	South Africa	Rock	1	0	0	1	1	0
Anedrson & Underwood	1994	Effects of substratum on the recruitment and development of an intertidal estuarine fouling assemblage	Journal of Experimental Marine Biology and Ecology	Australia	Concrete, Metal, Plastic, Wood	0	0	1	1	1	0
James & Underwood	1994	Influence of colour of substratum on recruitment of spirorbid tubeworms to different types of intertidal boulders	Journal of Experimental Marine Biology and Ecology	Australia	Rock	1	0	0	1	0	0
Nelson	1994	A comparison of the fouling community development on stabilized oil-ash and concrete reefs	Bulletin of Marine Science	USA	Concrete	0	0	1	1	1	0
Sampaolo & Relini	1994	Coal ash for artificial habitats in Italy	Bulletin of Marine Science	Laboratory	Concrete	0	0	1	0	0	0
Bombace et al.	1995	Benthic communities on concrete and coal-ash blocks submerged in an artificial reef in the central Adriatic Sea	Proceedings of the 30th European marine biological symposium.	Italy	Concrete	0	0	1	0	1	0

			University of Southampton.								
Anderson	1996	A chemical cue induces settlement of Sydney rock oysters, *Saccostrea commercialis*, in the laboratory and in the field	Biological Bulletin	Australia/Laboratory	Rock, Concrete	1	0	1	1	0	0
Vose & Nelson	1998	An assessment of the use of stabilized coal and oil ash for construction of artificial fishing reefs: Comparison of fishes observed on small ash and concrete reefs	Marine Pollution Bulletin	USA	Concrete	0	0	1	0	0	0
Connell	2000	Floating pontoons create novel habitats for subtidal epibiota	Journal of Experimental Marine Biology and Ecology	Australia	Rock, Concrete	1	0	0	1	0	0
Glasby	2000	Surface composition and orientation interact to affect subtidal epibiota	Journal of Experimental Marine Biology and Ecology	Australia	Rock, Concrete, Wood	1	0	1	1	0	0
Lam	2001	Algal and sessile invertebrate recruitment onto an experimental PFA-concrete artificial reef in Hong Kong	Asian Marine Biology	Hong Kong	Concrete	0	0	1	1	0	0
Reyes & Yap	2001	Effect of artificial substratum material and resident adults on coral settlement patterns at Danjugan Island, Philippines	Bulletin of Marine Science	Philippines	Rock, Concrete, Rubber	1	0	1	1	0	0
Cattaneo-Vietti et al.	2002	Can rock composition affect sublittoral epibenthic communities?	Marine Ecology	Italy	Rock	1	0	0	1	1	0
Lin & Shao	2002	The development of subtidal fouling assemblages on artificial structures in Keelung Harbor, northern Taiwan	Zoological Studies	Taiwan	Concrete, Metal	0	0	1	0	1	1
Walker	2002	Fish assemblages associated with artificial reefs of concrete aggregates or quarry stone offshore Miami Beach, Florida, USA	Aquatic Living Resources	USA	Rock, Concrete	0	1	1	1	0	0
Groppelli et al.	2003	Observations on the settlement of *Phallusia mammillata* larvae: Effects of different lithological substrata	Italian Journal of Zoology	Laboratory	Rock	1	0	0	1	0	0
Nandakumar et al.	2003	Microfouling studies on experimental test blocks of steelmaking slag and concrete exposed to seawater off Chiba, Japan	Biofouling	Japan	Concrete	0	0	1	1	1	1

Qiu et al.	2003	Development of a marine subtidal epibiotic community in Hong Kong: Implications for deployment of artificial reefs	Biofouling	Hong Kong	Concrete, Metal, Rubber, Wood	0	0	1	0	0	1
Schiaparelli et al.	2003	Can mineralogical features affect the distribution patterns of sessile gastropods? The Vermetidae case in the Mediterranean Sea	Journal of the Marine Biological Association of the United Kingdom	Italy	Rock	1	0	0	1	0	0
Faimali et al.	2004	The interplay of substrate nature and biofilm formation in regulating *Balanus amphitrite* Darwin, 1854 larval settlement	Journal of Experimental Marine Biology and Ecology	Laboratory	Rock, Concrete	0	1	0	1	0	0
Guidetti et al.	2004	Living on the rocks: Substrate mineralogy and the structure of subtidal rocky substrate communities in the Mediterranean Sea	Marine Ecology Progress Series	Italy	Rock	1	0	0	1	1	0
Brown	2005	Epifaunal colonization of the Loch Linnhe artificial reef: Influence of substratum on epifaunal assemblage structure	Biofouling	United Kingdom	Concrete, Metal, Plastic, Rubber, Wood	0	0	1	1	1	0
Azevedo et al.	2006	Colonization of benthic organisms on different artificial substratum in Ilha Grande Bay, Rio de Janeiro, Brazil	Brazilian Archives of Biology and Technology	Brazil	Concrete, Metal, Rubber	0	0	1	1	1	0
Chapman & Clynick	2006	Experiments testing the use of waste material in estuaries as habitat for subtidal organisms	Journal of Experimental Marine Biology and Ecology	Australia	Rock, Metal, Rubber, Wood	0	1	0	1	1	0
Herbert & Hawkins	2006	Effect of rock type on the recruitment and early mortality of the barnacle *Chthamalus montagui*	Journal of Experimental Marine Biology and Ecology	United Kingdom	Rock	1	0	0	1	0	0
Moreira	2006	Patterns of occurrence of grazing molluscs on sandstone and concrete seawalls in Sydney Harbour (Australia)	Molluscan Research	Australia	Rock, Concrete	0	1	0	1	0	0
Creed & De Paula	2007	Substratum preference during recruitment of two invasive alien corals onto shallow-subtidal tropical rocky shores	Marine Ecology Progress Series	Brazil	Rock, Concrete, Metal, Ceramic	1	0	1	1	0	1
Totti et al.	2007	Seasonal variations of epilithic diatoms on different hard substrates, in the northern Adriatic Sea	Journal of the Marine Biological Association of the United Kingdom	Italy	Rock	1	0	0	1	0	1
Tyrrell & Byers	2007	Do artificial substrates favor nonindigenous fouling species over native species?	Journal of Experimental Marine Biology and Ecology	USA	Rock, Metal, Plastic, Rubber, Wood	0	1	0	1	0	0

Burt et al.	2009	Coral recruitment and early benthic community development on several materials used in the construction of artificial reefs and breakwaters	Journal of Experimental Marine Biology and Ecology	Emirates	Rock, Concrete, Ceramic	0	1	1	1	0	0
Guarnieri et al.	2009	Local vs regional effects of substratum on early colonization stages of sessile assemblages	Biofouling	Italy	Rock, Concrete	1	1	0	1	0	0
Neo et al.	2009	Can artificial substrates enriched with crustose coralline algae enhance larval settlement and recruitment in the fluted giant clam (*Tridacna squamosa*)?	Hydrobiologia	Laboratory	Concrete	0	0	1	1	0	0
Ivesa et al.	2010	Differential patterns of distribution of limpets on intertidal seawalls: Experimental investigation of the roles of recruitment, survival and competition	Marine Ecology Progress Series	Australia	Rock, Concrete	1	0	0	1	0	1
Pozzolini et al.	2010	Influence of rocky substrata on three-dimensional sponge cells model development	In Vitro Cellular and Developmental Biology - Animal	Laboratory	Rock	1	0	0	0	0	0
Savoya & Schwindt	2010	Effect of the substratum in the recruitment and survival of the introduced barnacle *Balanus glandula* (Darwin 1854) in Patagonia, Argentina	Journal of Experimental Marine Biology and Ecology	Argentina	Rock, Ceramic	1	0	0	1	0	0
Salinas de León et al.	2011	Scleractinian settlement patterns to natural cleared reef substrata and artificial settlement panels on an Indonesian coral reef	Estuarine, Coastal and Shelf Science	Indonesia	Concrete, Ceramic	0	0	1	1	0	0
Green et al.	2012	Ecological consequences of the type of rock used in the construction of artificial boulder-fields	Ecological Engineering	Australia	Rock	1	0	0	1	0	0
Lathlean & Minchinton	2012	Manipulating thermal stress on rocky shores to predict patterns of recruitment of marine invertebrates under a changing climate	Marine Ecology Progress Series	Australia	Rock, Plastic	1	0	0	1	0	0
Perkol-Finkel et al.	2012	Conservation challenges in urban seascapes: Promoting the growth of threatened species on coastal infrastructures	Journal of Applied Ecology	Italy	Rock, Concrete, Ceramic	1	0	0	1	0	0
Foley et al.	2014	Ecological engineering considerations for coral reefs in the design of multifunctional coastal structures	Proceedings of the Coastal Engineering Conference	Hawaii/Laboratory	Concrete	0	0	1	1	0	0

Liversake et al.	2014	Associations of benthic fauna with different rock types, and evidence of changing effects during succession	Marine Ecology Progress Series	Australia	Rock	1	0	0	1	0	0
Perkol-Finkel & Sella	2014	Ecologically active concrete for coastal and marine infrastructure: Innovative matrices and designs	From Sea to Shore - Meeting the Challenges of the Sea	Israel	Concrete	0	0	1	1	0	1
Vaz-Pinto et al.	2014	Invasion success and development of benthic assemblages: Effect of timing, duration of submersion and substrate type	Marine Environmental Research	Azores	Rock, Concrete, Plastic	1	0	1	1	1	0
Nenadović et al.	2015	Development of periphytic diatoms on different artificial substrates in the Eastern Adriatic Sea	Acta Botanica Croatica	Croatia	Rock, Concrete, Metal, Plastic, Rubber, Ceramic, Wood	1	1	1	1	1	0
Cacabelos et al.	2016	Material type and roughness influence structure of inter-tidal communities on coastal defenses	Marine Ecology	Azores	Rock, Concrete	1	1	0	1	1	0
Chase et al.	2016	The influence of substrate material on ascidian larval settlement	Marine Pollution Bulletin	Laboratory	Rock, Concrete, Plastic	1	0	1	1	0	0
Cuadrado Rica et al.	2016	Colonisation et détérioration des bétons incorporant des coquilles pour récifs artificiels	Materiaux et Techniques	Laboratory	Concrete	0	0	1	0	0	0
Martins et al.	2016	Ecology of a key ecosystem engineer on hard coastal infrastructure and natural rocky shores	Marine Environmental Research	Azores	Rock, Concrete	1	1	0	1	0	0
Ushiama et al.	2016	The effects of substratum material and surface orientation on the developing epibenthic community on a designed artificial reef	Biofouling	Australia	Rock, Metal, Plastic, Wood	0	1	0	1	1	0
Davis et al.	2017	Ecological performance of construction materials subject to ocean climate change	Marine Environmental Research	Laboratory	Rock, Concrete, Plastic	0	1	1	1	0	0
Kennedy et al.	2017	Comparison of recruitment tile materials for monitoring coralline algae responses to a changing climate	Marine Ecology Progress Series	Australia	Rock, Plastic, Ceramic	0	1	0	1	1	0
Quan et al.	2017	Long-term oyster recruitment and growth are not influenced by substrate type in China: Implications for sustainable oyster reef restoration	Journal of Shellfish Research	China	Rock, Ceramic	0	1	0	1	0	1
Bavestrello et al.	2018	Differences in composition of shallow-water marine benthic communities associated with two ophiolitic rock substrata	Estuarine, Coastal and Shelf Science	Italy	Rock	1	0	0	1	1	0

Dennis et al.	2018	Reefcrete: Reducing the environmental footprint of concretes for eco-engineering marine structures	Ecological Engineering	United Kingdom	Concrete	0	0	1	1	1	1
Janetzki et al.	2018	Assemblages on limestone and siltstone boulders diverge over six years in a primary-succession transplant experiment	Marine Ecology Progress Series	Australia	Rock	1	0	0	1	1	0
McManus et al.	2018	Partial replacement of cement for waste aggregates in concrete coastal and marine infrastructure: A foundation for ecological enhancement?	Ecological Engineering	United Kingdom	Concrete	0	0	1	1	1	0
Perkol-Finkel et al.	2018	Seascape architecture – Incorporating ecological considerations in design of coastal and marine infrastructure	Ecological Engineering	Israel	Concrete	0	0	1	0	1	0
Sempere-Valverde et al.	2018	Substratum type affects recruitment and development of marine assemblages over artificial substrata: A case study in the Alboran Sea	Estuarine, Coastal and Shelf Science	Spain	Rock, Concrete	0	1	0	0	0	1
Al-Sofyani et al.	2019	Recruitment patterns of the solitary ascidian *Phallusia nigra* Savigny, 1816 on artificial substrates submerged in the central Red Sea, Saudi Arabia	Oceanological and Hydrobiological Studies	Saudi Arabia	Concrete, Metal, Plastic, Ceramic	0	0	1	1	0	0
Canessa et al.	2019	The influence of the rock mineralogy on population density of *Chthamalus* (Crustacea: Cirripedia) in the Ligurian Sea (NW Mediterranean Sea)	European Zoological Journal	Italy	Rock	1	0	0	1	0	0
Carrasco et al.	2019	Effects of different intertidal hard substrates on the recruitment of *Crassostrea gigas*	Hydrobiologia	Argentina	Rock	1	0	0	1	0	0
Mos et al.	2019	Building global change resilience: Concrete has the potential to ameliorate the negative effects of climate-driven ocean change on a newly-settled calcifying invertebrate	Science of the Total Environment	Laboratory	Rock, Concrete	1	1	0	1	0	0
Siddik & Satheesh	2019	Interactive effects of light and substrate colour on the recruitment of marine invertebrates on artificial materials	Community Ecology	Saudi Arabia	Concrete, Metal, Plastic, Ceramic	0	0	1	1	0	0
Tuhumena et al.	2019	The effect of type and duration of substrate collector placement to the coral genus recruitment in Saleo Beach area, Dampier strait, Raja ampat, Indonesia	AACL Bioflux	Indonesia	Rock, Metal, Ceramic	0	1	0	1	0	0

Becker et al.	2020	The role of artificial material for benthic communities – Establishing different concrete materials as hard bottom environments	Marine Environmental Research	Germany	Concrete	0	0	1	1	1	0
Canessa et al.	2020	Lithology could affect benthic communities living below boulders	Journal of Marine Biological Association of the United Kingdom	Italy	Rock	1	0	0	1	0	0
Pinochet et al.	2020	Marine invertebrate larvae love plastics : Habitat selection and settlement on artificial substrates	Environmental Pollution	United Kingdom	Concrete, Plastic, Wood	0	0	1	1	0	0
Preston et al.	2020	Interactions of larval dynamics and substrate preference have ecological significance for benthic biodiversity and *Ostrea edulis* Linnaeus , 1758 in the presence of *Crepidula fornicata*	Wiley	United Kingdom	Concrete, Plastic	0	0	1	1	0	0
Sedano et al.	2020	Do artificial structures cause shifts in epifaunal communities and trophic guilds across different spatial scales?	Marine Environmental Research	Spain	Rock, Concrete	1	0	0	1	1	0

*Supplementary Table S4.3. Table reports, separately for the three hypotheses and the three metrics, the outcomes of each model processed during the meta-analysis. If a moderator was included in the model, then categories of the moderator were reported. Effect sizes for each moderator's categories were reported if the moderator's heterogeneity was significant. The effect size was calculated using SMD (Standardized Mean Difference). NP = test not performed due to insufficient data. Significance levels: NS = not significant, * = p<0.05, ** = p<0.01, *** = p<0.001.*

H1	**Abundance**			**Diversity**			**Biomass**		
Moderator included	No of studies	Level	Heterogeneity	No of studies	Level	Heterogeneity	No of studies	Level	Heterogeneity
Random model	37	//	-0.23 NS	10	//	-0.15 NS	4	//	0.13 NS
Functional group (FG)	37	Epibiota Algae Sessile inv. Vagile inv.	QM = 7.12 NS	9	Epibiota Algae	QM = 0.26 NS	NP	NP	NP
Artificial material (TY)	37	Ceramic Concrete [1] Metal Plastic Rock [2] Rubber Wood	-0.50 NS -0.08 NS -0.70** -0.45* -0.16 NS -1.72*** -0.28 NS	10	Concrete [1] Plastic Rock [2]	QM = 3.49 NS	NP	NP	NP
FG * TY	37	All interactions if reported in two studies at least. See Supplementary Table S4.4		NP	NP	NP	NP	NP	NP
Duration (month)	27	covariate	-0.02**	4	covariate	QM = 1.17 NS	NP	covariate	NP
Basin	37	Atlantic Indian Lab. Medit. Pacific	QM = 8.44 NS	8	Atlantic Medit.	QM = 0.07 NS	NP	NP	NP
Latitude	37	covariate	QM = 0.35 NS	10	covariate	QM = 0.23 NS	4	covariate	QM = 0.37 NS
Environ. context	32	Artificial Laboratory Natural	0.61 NS -1.37* -0.26 NS	8	Artificial Natural	QM = 1.08 NS	NP	NP	NP
Tidal Zone	37	Intertidal Laboratory Subtidal	QM = 6.23 NS	10	Intertidal Subtidal	QM = 1.15 NS	NP	NP	NP
H2	**Abundance**			**Diversity**			**Biomass**		
Random model	17	//	0.05 NS	5	//	-0.08 NS	3	//	0.20 NS
Functional group (FG)	17	Epibiota Algae Sessile inv. Vagile inv.	QM = 2.13 NS	NP	NP	NP	NP	NP	NP
Artificial material (TY)	17	Ceramic Concrete[1] Metal Plastic Rubber Wood	0.31* 0.30* -0.71*** -0.39* -0.47** -0.57**	5	Ceramic Concrete[1] Metal Plastic Rock Rubber Wood	QM = 11.99 NS	NP	NP	NP
FG * TY	17	All interactions if reported in two studies at least. See Supplementary Table S4.5		NP	NP	NP	NP	NP	NP
Duration (month)	13	covariate	QM = 0.68 NS	4	covariate	-0.03**	3	covariate	QM = 0.07 NS
Basin	15	Atlantic Lab. Medit.	QM = 0.94 NS	NP	NP	NP	NP	NP	NP

		Pacific							
Latitude	17	covariate	QM = 0.05 ^{NS}	5	covariate	QM = 0.03 ^{NS}	3	covariate	QM = 1.41^{NS}
Environ. context	14	Artificial Laboratory Natural	QM = 1.25 ^{NS}	4	Artificial Natural	QM = 0.14 ^{NS}	NP	NP	NP
Tidal Zone	17	Intertidal Subtidal	QM = 1.11 ^{NS}	5	Intertidal Subtidal	QM = 0.54 ^{NS}	NP	NP	NP
H3	**Abundance**			**Diversity**			**Biomass**		
Random model	30	//	-0.26 ^{NS}	14	//	0.12 ^{NS}	6	//	0.03 ^{NS}
Functional group (FG)	30	Epibiota Algae Sessile inv. Vagile inv.	QM = 7.05 ^{NS}	14	Epibiota Algae Sessile inv.	QM = 4.83 ^{NS}	6	Epibiota Algae	QM = 5.50 ^{NS}
Artificial material (TY)	30	Ceramic Concrete[3] Metal Plastic Rubber Wood	QM = 9.74 ^{NS}	14	Concrete[3] Metal Plastic Rubber Wood	0.45 ^{NS} -1.03* 0.53 ^{NS} -0.97* 0.18 ^{NS}	6	Concrete Metal	QM = 0.34 ^{NS}
FG * TY	30	All interactions if reported in two studies at least. See Supplementary Table S4.6		NP	NP	NP	NP	NP	NP
Duration (month)	29	covariate	QM = 1.60 ^{NS}	14	covariate	-0.05***	6	covariate	-0.06***
Basin	28	Atlantic Lab. Medit. Pacific Red Sea	QM = 2.96 ^{NS}	14	Atlantic Medit. Pacific	QM = 5.90 ^{NS}	5	Atlantic Pacific	QM = 0.05 ^{NS}
Latitude	30	covariate	QM = 0.22 ^{NS}	14	covariate	QM = 0.23 ^{NS}	6	covariate	QM = 0.71 ^{NS}
Environ. context	29	Artificial Laboratory Natural	QM = 1.55 ^{NS}	14	Artificial Natural	QM = 1.38 ^{NS}	6	Artificial Natural	QM = 0.09 ^{NS}
Tidal Zone	30	Intertidal Laboratory Subtidal	QM = 1.70 ^{NS}	14	Intertidal Subtidal	QM = 0.47 ^{NS}	NP	NP	NP

[1] "Concrete" class in H1 and H2 analyses includes Portland cement and concrete made by different mixtures of aggregates or cements (i.e. ground granulated blast-furnace slag concrete, fuel-ash concrete, coal-ash concrete, shell concrete, ECOncrete®).

[2] "Rock" class in H1 analyses includes only imported rocky substrates (i.e., different types of rocks than the substrate reported by the geological atlases in that same study area; Supplementary Table S4.1).

[3] "Concrete" class in H3 analyses includes only concrete made by different mixtures of aggregates or cements than Portland (i.e. ground granulated blast-furnace slag concrete, fuel-ash concrete, coal-ash concrete).

Supplementary Table S4.4. Results of meta-analyses testing the interaction between functional group (FG: rows) and material type (TY: columns) on the abundance of colonizing organisms for hypothesis 1. NS = not significant. NP *= not performed because of insufficient data (i.e. less than two studies).*

H1: Abundance	Ceramic	Concrete [1]	Metal	Plastic	Rock [2]	Rubber	Wood
Epibiota	NP	**-1.07****	NP	**-1.40*****	**-0.95***	NP	NP
Algae	0.17 NS	-0.03 NS	NP	0.54 NS	-0.16 NS	NP	0.27 NS
Sessile	-0.37 NS	0.17 NS	-0.41 NS	-0.22 NS	-0.22 NS	**-1.55*****	NP
Vagile	NP	NP	NP	NP	NP	NP	NP

[1] "Concrete" class in H1 and H2 analyses includes Portland cement and concrete made by different mixtures of aggregates or cements (i.e. ground granulated blast-furnace slag concrete, fuel-ash concrete, coal-ash concrete, shell concrete, ECOncrete®).

[2] "Rock" class in H1 analyses includes only imported rocky substrates (i.e., different types of rocks than the substrate reported by the geological atlases in that same study area; Supplementary Table S4.1).

Supplementary Table S4.5. Results of meta-analyses testing the interaction between functional group (FG: rows) and material type (TY: columns) on the abundance of colonising organisms for hypothesis 1. NS = not significant. NP *= not performed because of insufficient data (i.e. less than two studies).*

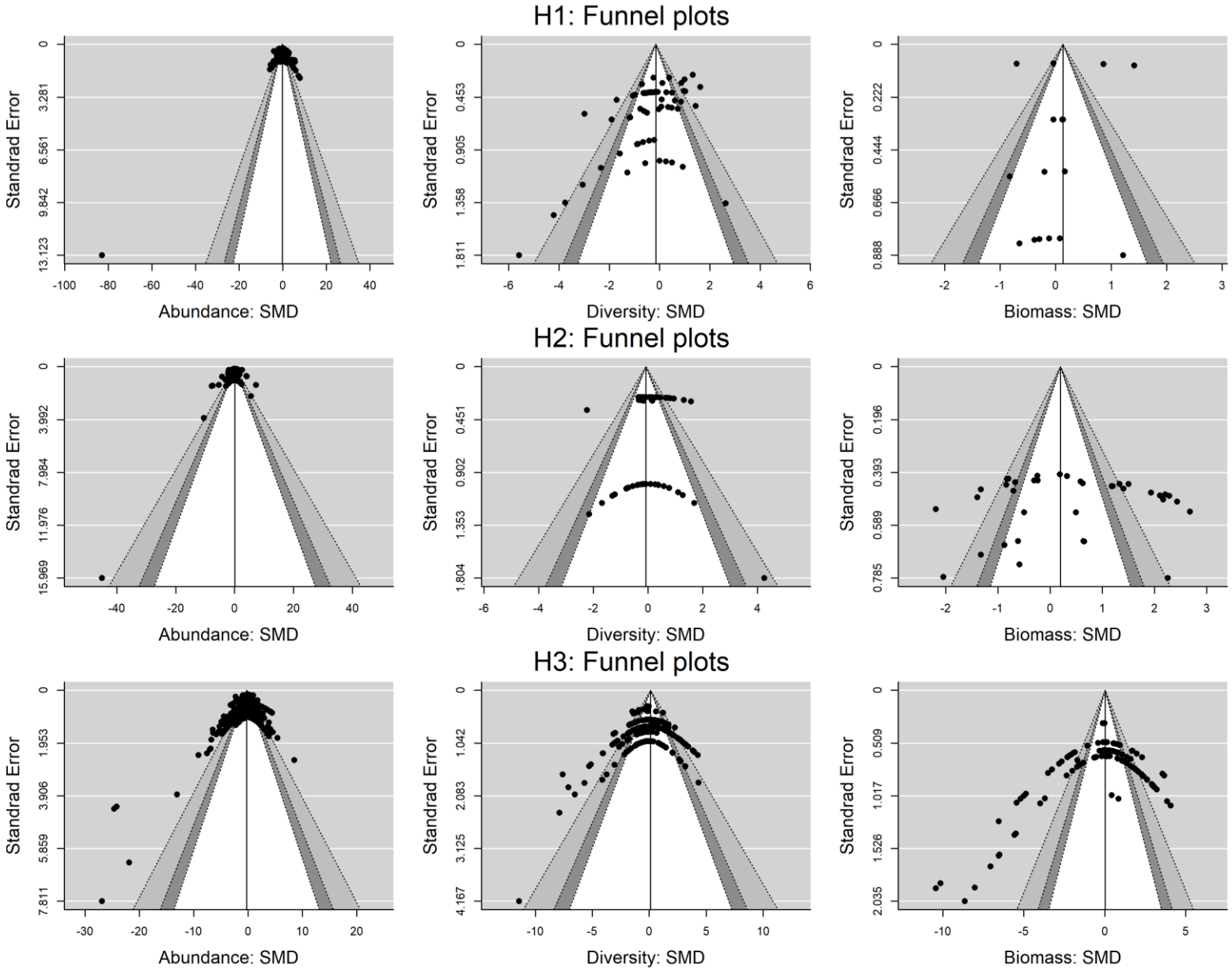
H2: Abundance	Ceramic	Concrete [1]	Metal	Plastic	Rubber	Wood
Epibiota	NP	0.45 NS	**-0.98****	-0.41 NS	-0.70 NS	**-0.78***
Algae	**-1.08****	**0.78*****	**-1.02****	**-1.10*****	**-0.81***	**-1.03****
Sessile	**0.85****	0.55 NS	-0.63 NS	-0.30 NS	-0.50 NS	-0.39 NS
Vagile	NP	0.12 NS	NP	NP	NP	NP

[1] "Concrete" class in H1 and H2 analyses includes Portland cement and concrete made by different mixtures of aggregates or cements (i.e. ground granulated blast-furnace slag concrete, fuel-ash concrete, coal-ash concrete, shell concrete, ECOncrete®).

Supplementary Table S4.6. Results of meta-analyses testing the interaction between functional group (FG: rows) and material type (TY: columns) on the abundance of colonising organisms for hypothesis 1. NS = not significant. NP *= not performed because of insufficient data (i.e. less than two studies).*

H3: Abundance	Ceramic	Concrete [1]	Metal	Plastic	Rubber	Wood
Epibiota	NP	-0.01 NS	NP	-0.65 NS	NP	NP
Algae	-0.66 NS	0.21 NS	NP	-1.17 NS	NP	**0.87***
Sessile	-0.53 NS	-0.18 NS	-0.34 NS	-0.26 NS	-0.36 NS	-0.47 NS
Vagile	NP	-0.03 NS	-0.83 NS	NP	NP	NP

[1] "Concrete" class in H3 analyses includes only concrete made by different mixtures of aggregates or cements than Portland (i.e. ground granulated blast-furnace slag concrete, fuel-ash concrete, coal-ash concrete).



Supplementary Figure S4.1. Funnel plots produced for each metric during analyses for Hypothesis 1, Hypothesis 2 and Hypothesis 3.

Chapter 5 – Design of biomimetic surfaces: promote sustainability of coastal infrastructures through multiple benefits.

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Abstract

The use of biomimicry in eco-engineering strategies is increasing nowadays to provide multifunctional marine infrastructures and thus overcome the negative repercussions provided by the rapid urbanization of coastal habitats. However, much of the potential of biomimicry will remain unexplored until the benefits of an aesthetic value improvement and an environmental impact reduction (lowering carbon footprint and raw material demand) are not considered along with the ecological performance enhancement. We carried out a pilot study in a highly anthropized environment, the Ravenna port, where greener concrete blends (using shell fragments and magnesium potassium phosphate cement) were used to cast tiles of different complexity and brightness using additive manufacturing technology (i.e. 3D printing). The intertidal epibenthic communities have been evaluated in a 12-month monitoring program to estimate the colonization and the biodiversity among the types of tiles. After three months, the early stages of colonization have been observed on each tile type. Weak differences in total cover were observed between complexity levels, with flat tiles more covered by biofilm than complex tiles, especially in steeper positions, and between brightness levels, with dark tiles more covered by biofilm than light tiles. Despite is still too early to draw solid conclusions from these preliminary results, the greener concrete blends resulted easily colonizable by organisms and can withstand the harsh intertidal conditions since no tiles have been lost, or irremediably damaged. Whether community response to the manipulated factors remains the same, intensifies or reverses, it will only be shown over time. At the end of the experiment, the most the epibiota will differ among levels of complexity, the less the aesthetics of artificial structures will be manipulable in terms of shapes and colors without compromising the ecological performance.

Keywords: Biomimicry, Aesthetic value, Eco-engineering, Eco-friendly material, Greener concrete, Intertidal, Complexity

5.1 Introduction

The rapid urbanization of coastal habitats is causing negative repercussions on marine ecosystems, on native biodiversity and, consequently, is worsening the ecosystem services here developed (Costanza et al., 2014). To overcome these negative repercussions, multifunctional eco-engineering designs are applied to coastal infrastructures (Dafforn et al., 2015; O'Shaughnessy et al., 2020). Eco-engineering is used to pursue the enhancement of the ecological value of coastal infrastructures by manipulating both the substrate composition and the substrate complexity at various scales (Pioch et al., 2018). This manipulation is performed since these two features differ between natural and artificial substrates (Lawrence et al., 2021), thus contributing to the lower ecological performance of the coastal infrastructures (Bulleri and Chapman, 2010). With biomimicry more frequently integrated into eco-engineering designs (Airoldi et al., 2021), the trend is now to introduce fewer alterations to the environment by emulating nature (Benyus, 1997). Since many older infrastructures need urgent upgrades to cope with the effects of ocean climate change (i.e. sea level rise, more frequent storm surge; IPCC 2014; Mamo et al., 2020), this situation represents a unique historical moment not only to promote the adoption of biomimicry but also to enhance its effectiveness and explore new directions. In fact, to date, the eco-engineering projects and the biomimetic techniques often aim to enhance ecological value *in-situ* only, ignoring, on the other hand, that greater overall sustainability could be achieved by a combined effect produced at multiple levels.

Among these, the choice of building material represents a further step as it contributes to enhancing several priorities of stakeholders (Evans et al., 2017). Indeed, the replacement of the traditional "grey" materials with "greener" (i.e. recycled) ones would produce benefits in sustainability reducing the footprint of their construction by lowering both the use of energy and the use of raw materials. Nowadays, concrete, the most used material to build infrastructure and responsible for 8-9% of the global anthropogenic CO_2 emissions and the 9% of global industrial water use (Cooke et al., 2020), could be made more sustainable by replacing, partially or totally, traditional cement binder (e.g. Portland) or aggregates with more ecological-friendly materials. Utilization of by-products, such as fly-ash and ground granulated blast-furnace slag (GGBS) as cement compounds, or hemps, fibers or fragments of shells as aggregates, represents today

the most common way to use alternative materials in the greener application in marine engineering (Meyer, 2009; Dennis et al., 2018; McManus et al., 2018).

Despite largely encouraged, marine greening designs still found scarce application, especially in the smallest projects, and they are mostly limited to the experimental field. It is likely that making these interventions more aesthetically appealing, without compromising their structural integrity or their ecological performance, could promote their wider adoption boosting their efficiency in short and long-term perspectives. So, the improvement of the aesthetic value of the infrastructures to recall the cultural life as well as the artistic and landscape heritage of the place in which they are inserted (Nordstrom, 2014) represents a further, more ambitious, and therefore still little explored step compared to trying to "simply" reduce the changes in the identity of the surrounding environment (Moschella et al., 2005). This said, understanding how and to what extent it is possible to manipulate the aspect of coastal infrastructures to combine aesthetic improvement with ecological enhancement represents an important knowledge gap toward greater sustainability of marine infrastructures.

In this direction, understanding how color manipulation affects organisms is the first step to fulfill this knowledge gap. However, the paucity of studies dealing with color effect on marine organisms does not provide robust guidelines about how to manipulate the appearance without compromising the ecological performance since these studies rarely last longer than few months (but see Satheesh & Wesley, 2010), they focused mainly on single taxa response rather than on the whole community response (but see Dobretsov et al., 2013), or they rarely manipulated color on substrates other than plastic materials (but see James & Underwood, 1994). Nevertheless, it was noticed that, among the color components, color brightness (the amount of light reflected) plays a greater role in affecting fouling organisms than color hue (its spectral composition; Ells et al., 2016). In intertidal systems, this produces greater thermal stress on organisms (i.e. barnacles) associated with darker surfaces than with lighter surfaces (Lathlean and Minchinton, 2012; Leal et al., 2020).

Consequently, we carried out an experiment in a highly anthropized environment (i.e. the Ravenna port, Italy: 44°29’ N, 12°18’ E) where the colonization of intertidal epibiota was monitored monthly for a year on experimental surfaces following a sampling design with three fixed and orthogonal factors: inclination (two levels: sloping vs vertical), complexity (two levels: biomimetic vs flat) and brightness (two levels: light vs. dark). If the manipulation of inclination and complexity was used to investigate the ecological effect produced by the imitation of natural substrates, brightness manipulation allowed to investigate to what extent it would be possible to act on the appearance of artificial structures to aesthetically ameliorate coastal infrastructures. Considering a null hypothesis that there would be no differences between epibiotic communities settled on the various experimental surfaces, this experiment will provide useful information about how to direct future biomimetic interventions whether this hypothesis is rejected or accepted. In the first case scenario, the experiment will define which combination of factors generates the greatest ecological improvement, limiting on the other hand the manipulation of the appearance for aesthetic purposes. While in the second case scenario, if the two brightness levels will support comparable communities, it will be possible to promote the aesthetic value of artificial coastal structures (i.e. using a wider range of colors) during eco-engineering applications as this would not compromise their ecological performance.

5.2 Materials and Methods

5.2.1 Choice of 'natural best candidate'

Initially, it was necessary to identify which shape represented a biomimetic surface. The identification of the biomimetic surface representing the 'best natural candidate' followed the selective process reported in Evans et al. (2021). Briefly, the natural topography, obtaining the 3D digital surface model, and the associated epibiota were assessed from 90 10x10 cm sampling plots during a baseline survey conducted in 2019 of the nearest intertidal rocky reef (i.e. along Monte Conero promontory, Italy: 43°32' N, 13°36' E). The ecological ranking of the 90 sampling plots based on the epibiota settled allowed to identify the subset of sampling plots supporting the 'best' and the 'worst' communities (Supplementary Figure S5.1a). Thereafter, these 'best' and 'worst' communities were modelled on descriptors (inclination, surface roughness, diversity of morphological microelements; for details see Grasselli and Airoldi 2021) of the plot topography they belonged to identify and select those topographic descriptors which varied more between 'best' and 'worst' plots (Supplementary Figure S5.1b). This allowed to perform a second selection on the 'best' plots solely, scoring them according to the values of the topographic descriptors, and thus to shortlist the 'best candidates'. Finally, the identification of the ideal biomimetic surface among the 'best candidates' relied on the inspection of their digital 3D models and the choice of that most suitable to be 3D cast in experimental units and installed in the field (for detail see Evans et al., 2021). At the end of the selective process, the 'natural best candidate' reported an inclination of 55° and a surface roughness equal to 1.19 (Supplementary Figure S5.2).

5.2.2 Greener concrete blends

The concrete blends used in this study to cast the different types of experimental surfaces were different compared to the canonical Portland concrete both in the cement binder and in the type of aggregates used. The printing powder was prepared by homogeneously mixing natural recycled aggregate and alternative binder, magnesium potassium phosphate cement, that guarantees fast setting and hydraulic properties of the concrete (Park et al., 2016). Burned clamshells were used as aggregate in substitution of the traditional aggregate (river sand, rocks, etc.), while magnesium oxide (MgO, CCM grade, RHI Magnesita GmbH, Vienna,

Austria) and monopotassium phosphate (KH_2PO_4, MKP, agricultural grade, Agri 2000 Italia Srl, Ferrara, Italy) were selected as powder binder. The traditional aggregate replacement by mussel shell was possible thanks to an inertisation and crushing process that made them suitable in particle size (< 4 mm), shape (not too long elongated), and volume. Two different types of MgO (from the same supplier) were used to obtain two different levels of brightness of the final product: a "light" blend (light reflectance of the wet surface = 40%) and a "dark" blend (light reflectance of the wet surface = 19%; Table 5.1). Technical characteristics and the total amount of MgO powder added in the formulations was the same, the brightness variation was related to a small different quantity of impurities (i.e. FeO %).

Table 5.1. Features of the two greener concrete blends used to cast the tiles.

Mix Name	***Materials used***		***Final color***	
	Hydraulic binder	*Natural aggregates*	*Average colour (HEX code)*	
Dark mix	MKPC (higher FeO impurity)	Clamshell	Light brown (#837563)	
Light mix	MKPC (lower FeO impurity)	Clamshell	Light grey (#AAAAAA)	

5.2.3 Tile casting and deployment

The concrete blends were used to cast 40 tiles 250 x 250 x ~40 mm^3 each. Tiles were cast using a large-scale powder bed binder jetting technology, the 3D printer Desamanera1 150.150 with a cubic voxel resolution of 5.7×5.7×5.7 mm^3 and developed by Desamanera Srl (Rovigo, Italy). Jetting technology is an Additive Manufacturing (AM) technology that can bind fluids to bond powdered materials in layers. In recent

decades, the use of AM techniques is growing and establishing itself as a new and revolutionary manufacturing method because it offers great advances in terms of customization, design freedom, flexibility and scalability, reduction of waste and on demand production (Conner et al., 2014; Paris et al., 2016). The printer permits to deposit the powder to prepare an appropriate thick printing layer, then, to selectively spray the reaction liquid to activate the binding reaction ($MgO + KH_2PO_4 + H_2O$) and, finally, to recoat the printed area with new material for creating the next layer. This process is repeated till the end of the piece.

Ten tiles were created for each of the four combinations of the two factors Complexity and Brightness using AM technology: Biomimetic-Light (BL hereafter), Biomimetic-Dark (BD hereafter), Flat-Light (FL hereafter) e Flat-Dark (FD hereafter). After curing for 10 days, the tiles were deployed the 3rd week of September 2021 inside the port of Ravenna on a rocky breakwater facing East in the lower intertidal area (max amplitude 90cm; Strain et al. 2021). Five tiles for each of the four types created (BL, BD, FL, FD) were attached to substrates with an inclination between 50° and 65° (Sloping, S hereafter: SBL, SBD, SFL, SFD), similar to the average inclination found in the natural substrates along Monte Conero (Grasselli and Airoldi, 2021), and five at inclinations steeper than 70° (Vertical, V hereafter: VBL, VBD, VFL, VFD), similar to the average inclination of seawalls. Due to the lack of enough vertical spots along the breakwater, only four VBL and four VBD tiles were deployed and, therefore, there were six of each SBL and SBD tiles. An area of about 30 cm in diameter has been cleaned of any organisms previously settled and then tiles were bolted to the breakwater with stainless steel screws.

5.2.4 Data collection and photo assessment

Epibiota colonization monitoring is still ongoing and is performed by taking an orthogonal snapshot of each tile monthly for a year (i.e. 12 sampling times) during low tides. Epibiota is assessed by plotting 40 randomly stratified points in each photo quadrat in PhotoQuad software (Trygonis and Sini, 2012) and labelling each organism morphologically relying on the standardized classification system CATAMI (Althaus et al., 2015). An arbitrary coverage value of 0.5% is assigned for species present but not intercepted by the

plotted points. This preliminary draft shows the results of the first three sampling times (after one, two and three months, respectively time 1, time 2 and time 3), all of which occurred in the autumn period.

5.2.5 Statistical analyses

Epibiota total cover and multivariate composition were compared, separately for each sampling time to focus solely on the effect of tile types during the colonization, among the orthogonal combination of the three fixed factors using PERMANOVA (Anderson et al., 2008). We used PERMANOVA instead of classical ANOVA because of ease of use with unbalanced replication and because of lack of normality assumptions (Anderson et al., 2008). To partition the variability and obtain F-statistics, PERMANOVA was used on a matrix of Euclidean distances calculated from the original untransformed data for epibiota total cover, and on a matrix of Bray-Curtis dissimilarities calculated from the original untransformed dataset for the multivariate epibiota composition. For both applications, PERMANOVA calculated all P-values using 9,999 random permutations of the appropriate exchangeable units and Type III sums of squares. The evolution of the multivariate assemblage composition during the colonization was visualized plotting centroids only of the eight tile types at each sampling time in a principal coordinates analysis (PCO) plot.

5.3 Results

1st sampling: 19-21 October. After one month, tiles resulted almost completely covered by a thin layer of biofilm although with inconsistent differences between inclinations and complexities (Table 5.2a). The pairwise testing for the interaction Inclination×Complexity identified significant greater total cover of biofilm on vertical flat tiles than on vertical biomimetic tiles ($t_{1,14}$ = 7.54, p < 0.001; Figure 5.1a), and on sloping biomimetic tiles than on vertical biomimetic tiles ($t_{1,15}$ = 4.14, p = 0.002; Figure 5.1a). No other taxa were observed so an analysis of multivariate composition was not performed. Only one tile detached during this period, hence it was excluded from the analyses. The lost tile was re-attached to the rock substrate to be included in the following monitoring events and to avoid information loss.

Table 5.2. ***a)*** *Permutational analysis of variance comparing the total cover between Inclination (vertical vs. horizontal), Complexity (biomimetic vs. flat), Brightness (light vs. dark) and their interaction, separately for each monitoring event.* ***b)*** *Permutational analysis of variance comparing the epibiota multivariate composition between Inclination (vertical vs. horizontal), Complexity (biomimetic vs. flat), Brightness (light vs. dark) and their interaction, separately for each monitoring event.*

a)		Time 1			Time 2			Time 3		
Source	df	MS	Pseudo-F	p-value	MS	Pseudo-F	p-value	MS	Pseudo-F	p-value
Inclination = I	1	0.020	10.858	**0.003**	0.000	0.003	0.953	0.030	2.150	0.162
Complexity = C	1	0.071	37.475	**<0.001**	0.049	5.957	**0.018**	0.011	0.790	0.408
Brightness = B	1	0.000	0.122	0.730	0.004	0.465	0.514	0.194	13.814	**<0.001**
I x C	1	0.036	19.064	**<0.001**	0.000	0.040	0.841	0.062	4.406	**0.031**
I x B	1	0.004	2.147	0.150	0.007	0.831	0.372	0.032	2.298	0.152
B x C	1	0.001	0.521	0.474	0.010	1.238	0.284	0.029	2.054	0.174
I x C x B	1	0.000	0.155	0.696	0.003	0.306	0.591	0.038	2.734	0.115
Residual	32	0.002			0.008			0.014		
b)		Time 1			Time 2			Time 3		
Source	df	MS	Pseudo-F	p-value	MS	Pseudo-F	p-value	MS	Pseudo-F	p-value
Inclination = I	1	--	--	--	385.14	2.478	0.084	192.51	0.642	0.681
Complexity = C	1	--	--	--	437.71	2.816	0.065	103.83	0.346	0.878
Brightness = B	1	--	--	--	256.70	1.652	0.180	914.25	3.050	**0.015**
I x C	1	--	--	--	86.01	0.553	0.598	574.69	1.917	0.101
I x B	1	--	--	--	8.78	0.057	0.969	270.94	0.904	0.485
B x C	1	--	--	--	244.05	1.570	0.193	314.22	1.048	0.391
I x C x B	1	--	--	--	429.23	2.762	0.069	545.53	1.820	0.115
Residual	32	--			155.42			299.78		

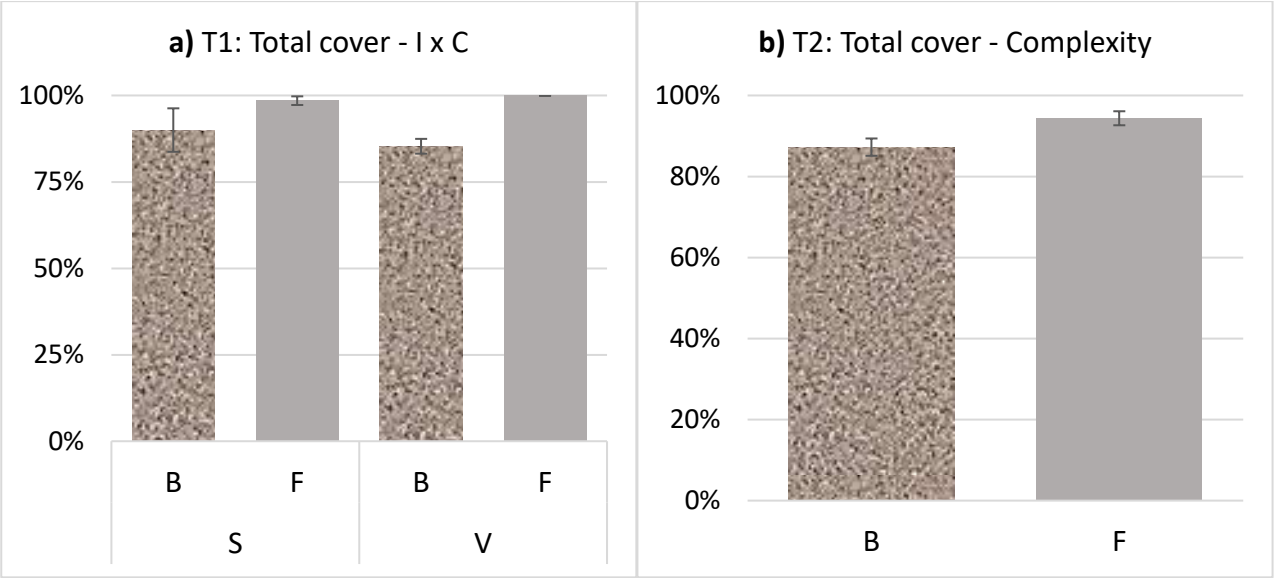

*Figure 5.1. **a)** Total cover (±1SE) among brightness and replicas for each combination of inclination and complexity (n = 8–12). **b)** Total cover (±1SE) among inclinations, brightness and replicas (n = 20). The initials S and V refer to the two levels of inclination: Sloping and Vertical. The initials B and F refer to the two levels of complexity: Biomimetic and Flat.*

2nd sampling: 18-19 November. After two months, groups of macroalgae were observed from photographs. They were labelled as: 'sheet-like/membranous green algae', frequently observed, 'filamentous/filiform red algae' and 'sheet-like/membranous brown algae', both rarer. 'Sheet-like/membranous green algae' tended to start their colonization at the edge of the upper half of the tiles or near the upper two bolts, while 'filamentous/filiform red algae' was observed in the lower half of the tiles, starting its colonization from above the tiles. In general, the presence of these macroalgae was not associated with a particular combination of factors as reported by the not-significant PERMANOVA results (Table 5.2b). On the other hand, despite the biofilm appeared generally thicker, empty space was still present, especially in the more exposed area of the biomimetic tiles where the 3D model created the more marked elevations. This led to a significant lower total cover in biomimetic tiles than in flat tiles (Table 5.2a; Figure 5.1b).

3rd sampling: 16 December. After three months, the first groups of sessile invertebrates, although rarely observed, started to colonize the tiles. Among them, 'tube worms', 'hard encrusting Bryozoa' and 'soft foliaceous Bryozoa' joined the previous groups of macroalgae, with the only exception of 'sheet-like/membranous brown algae' which was no longer observed. Significant more total cover was observed on dark tiles than on light tiles (Table 5.2a; Figure 5.2a) and, within the interaction Inclination×Complexity, on sloping flat tiles than on vertical flat tiles ($t_{1,16}$ = 2.00, p = 0.038; Figure 5.2b). Epibiota multivariate composition resulted affected by brightness too but not by inclination nor complexity (Table 5.2b). The

PCoA also highlighted this difference in epibiota composition between dark and light tiles, mainly along the second axis (explaining the 20.5% of total variation), which seems to be attributable to greater biofilm abundance on dark tiles (Figure 5.2c).

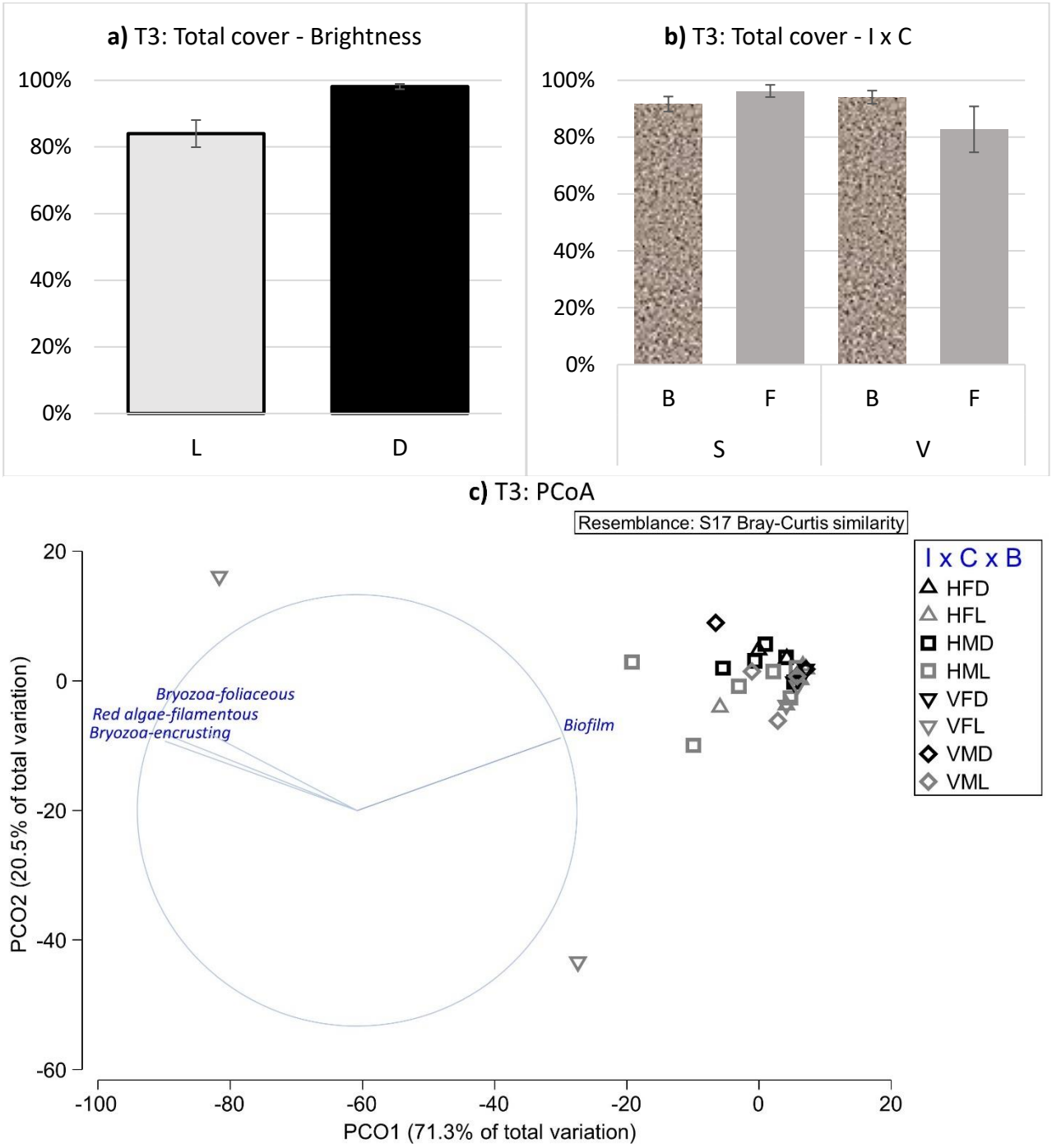

Figure 5.2. ***a)*** *Total cover (±1SE) among inclinations, among complexities and replicas (n = 20).* ***b)*** *Total cover (±1SE) among brightness and replicas for each combination of inclination and complexity (n = 8–12).* ***c)*** *Principal coordinates analysis (PCoA) plot of epibiota multivariate composition with vector overlay showing the length and direction of each CATAMI group whose correlation was > 0.3 with the PCO axes. The initials S and V refer to the two levels of inclination: Sloping and Vertical. The initials B and F refer to the two levels of complexity: Biomimetic and Flat. The initials D and L refer to the two levels of brightness: Dark and Light. For the PCoA symbols meaning please refer to the section "Tile casting and deployment".*

In the first three months, coinciding with the autumn season, the early successional stages were monitored. Indeed, after that biofilm initially almost completely colonized the empty tiles but more the flat ones than the biomimetic ones, macroalgae then started to grow on them but not where the space was unoccupied. However, with the beginning of the cold weather, the succession reversed with few exceptions, as evident from the PCoA plot where the centroids for each tile type resulted further between time 1 and time 2 than between time 1 and time 3 (Figure 5.3). Indeed, the macroalgae reduced in their number and abundance during the third month, uncovering the underlying biofilm, and making the communities at this stage similar to those of the first sampling time.

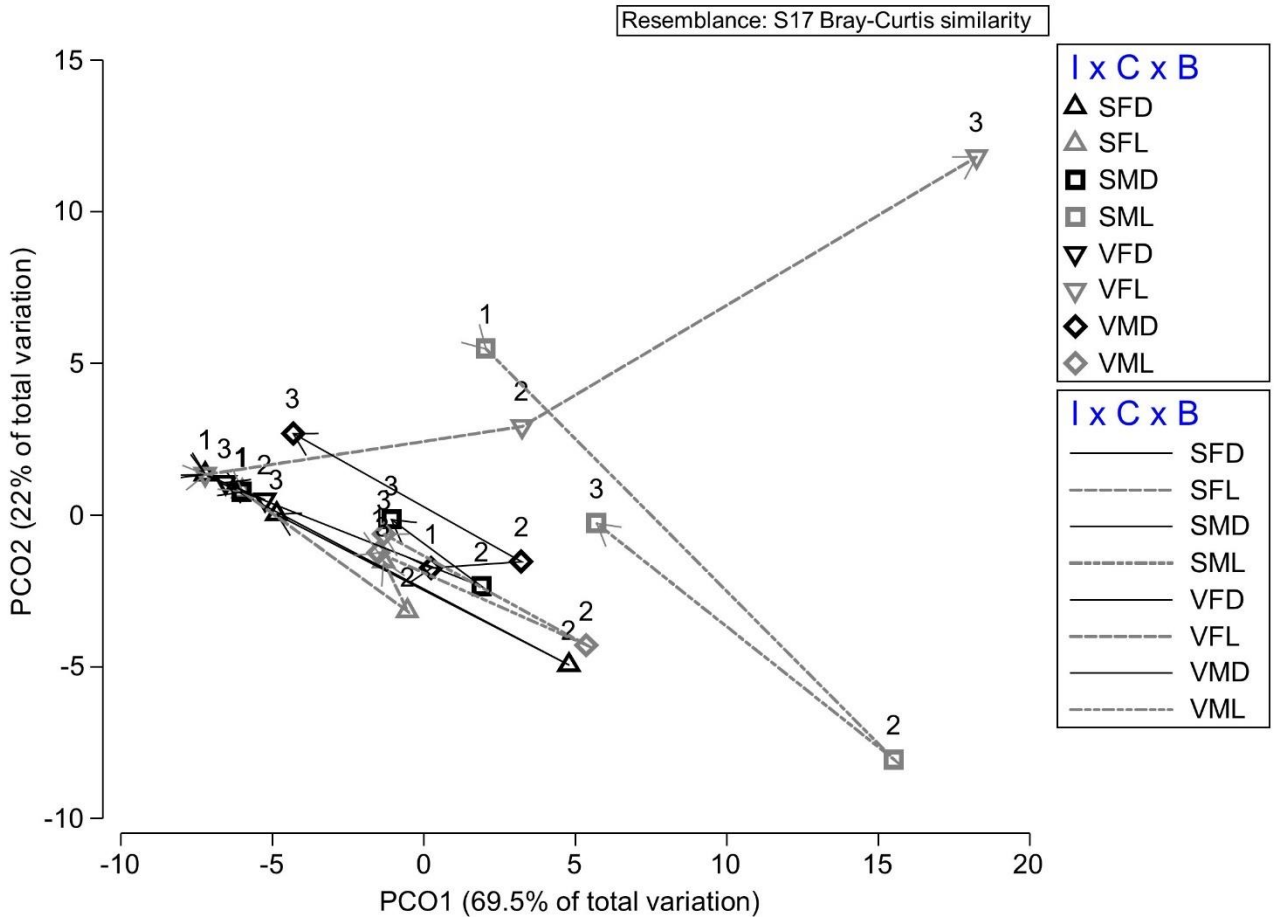

Figure 5.3. Principal coordinates analysis (PCoA) plot of centroids for each combination of inclination (I), complexity (C) and brightness (B) calculated in Bray-Curtis space from the untransformed epibiota multivariate composition. The lines connect the centroids referring to the same combination of fixed factors (i.e. I, C and B) and the number refers to the sampling period. For the PCoA symbols meaning please refer to the section "Tile casting and deployment".

5.4 Discussion

In this study, we are trying to evaluate the possibility of combining the lowering of the ecological footprint, using alternative materials, with ecological enhancement and aesthetic improvement of artificial structures, through a biomimetic manipulation of some key factors. The results obtained so far, albeit preliminary, are showing how at least the first stages of a primary succession can develop on the construction material tested here, with few differences among the manipulated factors of inclination, complexity or brightness. In fact, an almost uniform layer of biofilm quickly formed on all the tile types, probably thanks to the texture of the material used (Kerr et al., 1999; Köhler et al., 1999). Then, larger organisms like macroalgae found the conditions to start their growth (Park et al., 2011), albeit slowed down by the advance of the cold weather.

Among the few differences observed so far, the epibiota covered more the flat tiles than the biomimetic ones. This difference was due to a thinner, somewhere absent, biofilm layer on the ridges (i.e. the most exposed areas) of the biomimetic tiles, especially if the tile was attached vertically (Grasselli F. pers. obs.). It is likely that in these more exposed areas, drying faster during low tides, the thermal capacity is reduced, exposing the organisms to greater temperature excursions and desiccation stress than in areas that remain wet for longer. Furthermore, the epibiota resulted different, both in terms of community structure and total coverage, among the brightness conditions after the third month. Here, the dark tiles supported a community characterized by greater biofilm coverage than the light ones. It is likely that the dark tiles, by heating more than the light tiles, reduced the effect of the cold days of late autumn on the organisms during the emersion periods. This result, although predictable, contrasts with what was observed during the hot season, when the growth of intertidal organisms (barnacles) was greater on lighter, hence cooler, surfaces (Lathlean and Minchinton, 2012).

Despite is still too early to draw solid conclusions or to forecast how to effectively manipulate biomimicry to enhance ecological performance and increase aesthetic value in extremely anthropized coastal environments (i.e. ports and marinas), some preliminary considerations could already be discussed about the used greener concrete blends. Indeed, the material used here to build the tiles is showing several

strengths, beyond being able to host the epibiota. First, it can withstand the harsh intertidal conditions since no tiles were lost, or irremediably damaged, during the monitored period, even if we have not tested the engineering properties yet. In addition, the production of this material reduces the carbon footprint by 72-91% and requires less raw materials, when compared with Portland concrete, by replacing the aggregates with shell fragments (Dennis et al., 2018). Finally, the leaching of heavy metals is absent here as these toxic substances are not present in our material, although the leaching of heavy metals is already low in the various types of available concrete for marine infrastructures (McManus et al., 2018). These strengths make our blends an effective greener alternative to substantially reduce the environmental footprint, especially in the case of a scaled-up use.

The second consideration concerns the manipulations performed. On the one hand, the use of biomimicry, accurately imitating natural substrates in terms of complexity and inclination, has not currently led to an increase in biodiversity. This may be due both to the limited time monitored and, probably, to the low morphological difference that exists between natural and artificial substrates (Grasselli and Airoldi, 2021) which would not accentuate the rise of differences between communities. On the other hand, since the response of organisms towards brightness is opposite to that hypothesized during hotter seasons, this seems to support that the moment a structure is built affects the settled community, timing differently with the life cycles of organisms (Qiu et al., 2003; Brown, 2005).

Whether community response to the manipulated factors remains the same, intensifies or reverses, for example when the body size of the settled organisms, increasing, will be affected by the scale of complexity here manipulated (Tews et al., 2004; Warfe et al., 2008), it will only be shown over time. However, if the differences between communities that we are observing among the brightness levels remain, or increase, this will indicate that, in order not to compromise the ecological enhancement, aesthetic manipulation will have to be limited to the range of colors within a narrower brightness range. In any case, we will be able to determine how and to what extent it will be possible to combine manipulation for aesthetic purposes with that for ecological purposes.

Data availability statement

The original data presented in the study will be uploaded to public repositories as soon as the work is completed and published.

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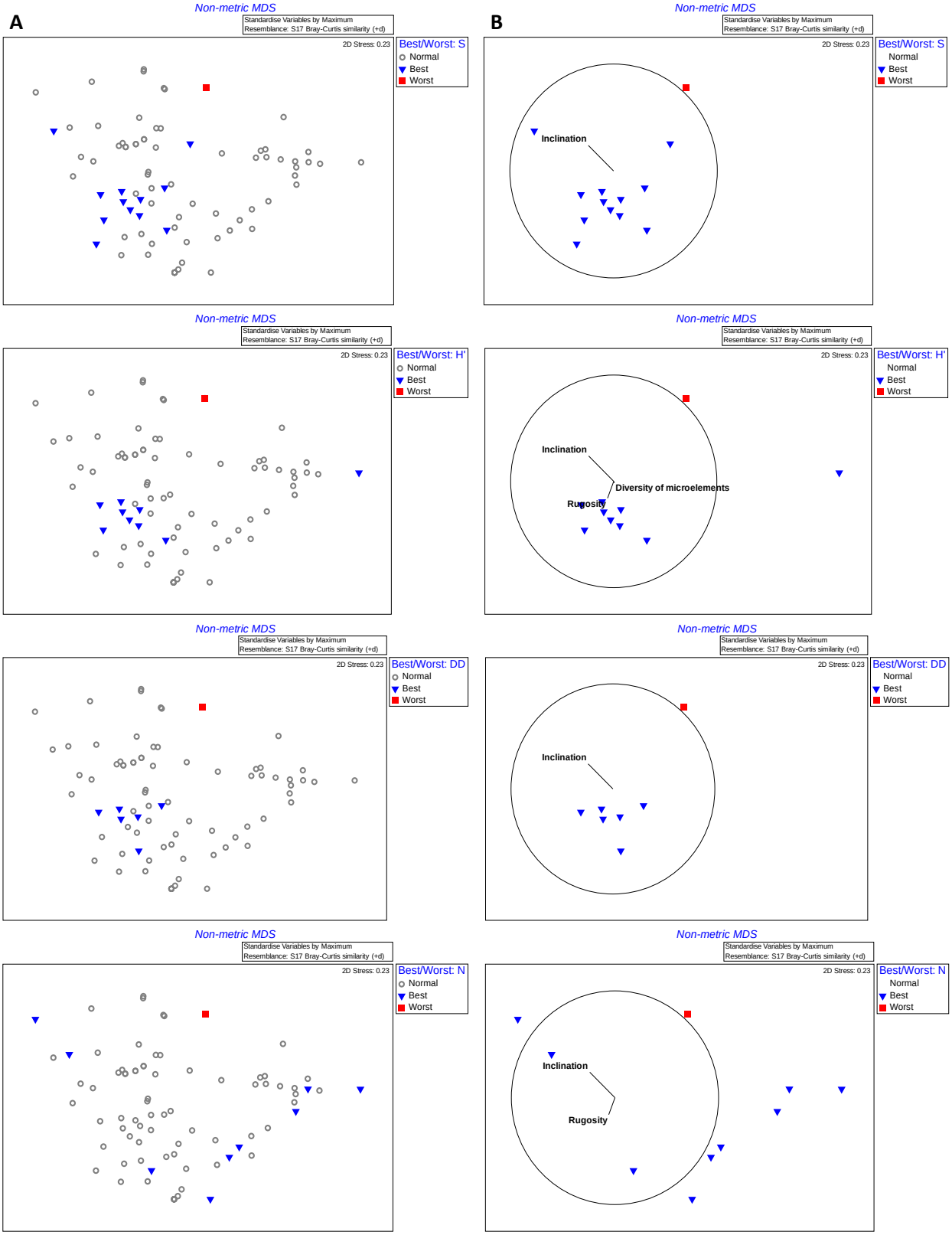
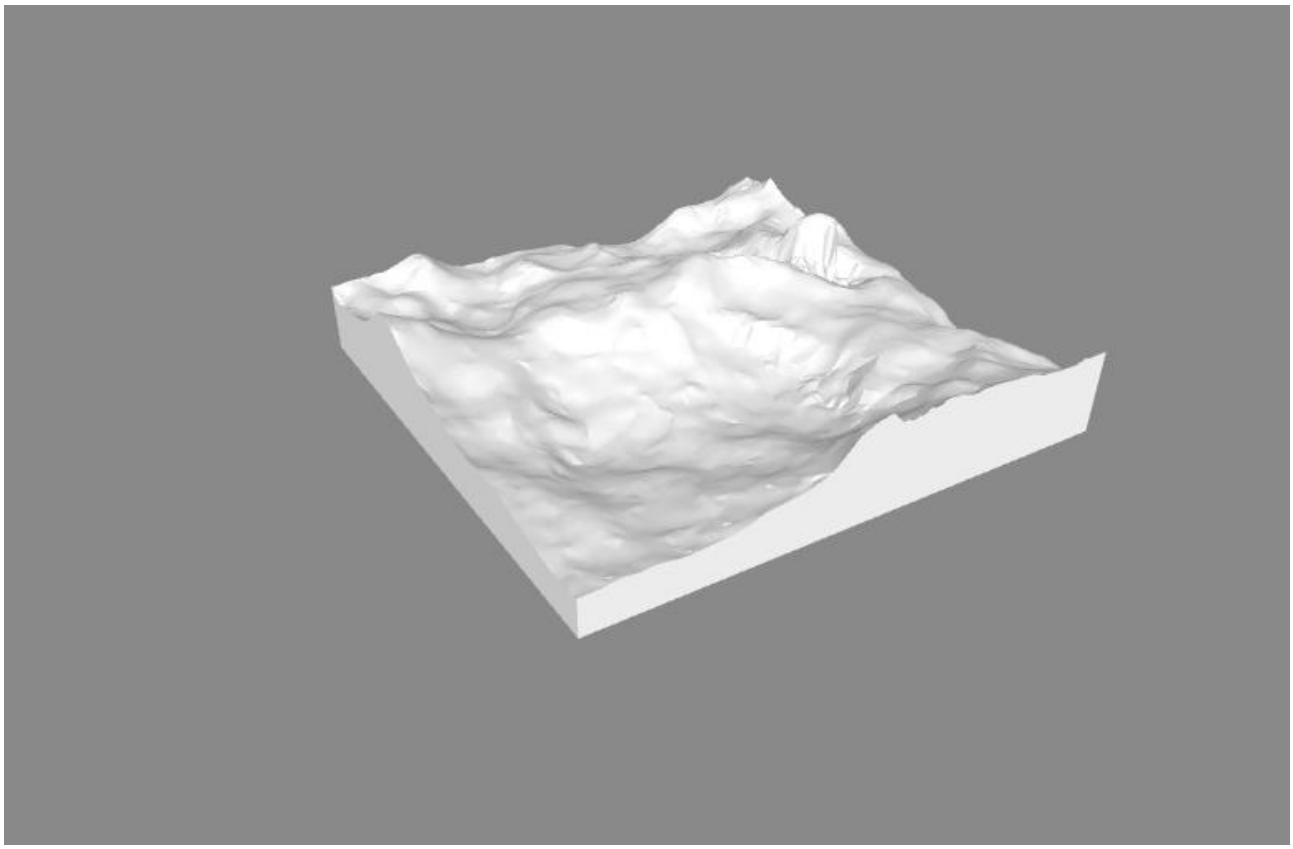
Supplementary materials



Supplementary Figure S5.1. A) nMDS ordination of multivariate species composition in 90 natural rocky reef quadrats. The 'best' and 'worst' quadrat subsets for four biodiversity indices (from top to bottom: Species richness (S), Shannon diversity (H'), Diversity deficit (DD), Total coverage (N)) are highlighted. B) Vectors represent the direction and strength of multiple Pearson correlations between community composition and the topographic variables modelled for each of the four biodiversity indices. Outer circles represent correlation of 1. For all plots, ordinations are based on Bray-Curtis similarities of standardized un-transformed abundances of taxa. Analyses carried out in PRIMER v7.



Supplementary Figure S5.2. 'Natural best candidate' 3D digital model retained by the Evans et al. (2021) selective process.

Chapter 6 – General discussion

Promoting multifunctional marine infrastructure that introduces fewer alterations to the state of the natural environment is crucial to face the expected increase in marine urbanization and its impacts (Dafforn et al., 2015; Airoldi et al., 2021). Biomimicry, through approaches and designs inspired by or mimicking nature (Benyus, 1997), represents a tool to support wildlife and critical ecosystem service along with the engineering functionalities (Schoonees et al., 2019; Airoldi et al., 2021). In my thesis, I produced missing knowledge on factors that structure assemblages of epibenthic species in artificial infrastructures of different morphologies and materials, with the final purpose to inform a biomimetic design of artificial infrastructures along the Italian coasts of the northern Adriatic. The primary aims consisted in:

1) quantifying the differences in physical structure between natural rocky shores (boulder fields and cliffs) and artificial infrastructures (i.e. breakwaters and seawalls);
2) quantifying the contribution of physical structure to differences in the epibiota between natural rocky shores and artificial infrastructures;
3) identifying which of the material types used to build marine infrastructures best performs in supporting marine wildlife and reducing natural resource use and carbon footprint, while also meeting engineering standards;
4) developing and testing new artificial surfaces for a greener, biomimetic design of marine infrastructures along the North Adriatic sea.

In **Chapter 2**, I characterized and compared the habitat physical structure between artificial and natural hard substrates. I tested the widespread assumption that physical complexity is lower in the artificial substrates, thus causing the poor ecological performance of artificial infrastructures in terms of providing habitat for epibenthic species (Moschella et al., 2005; Firth et al., 2014a; Dafforn et al., 2015). I contrasted five descriptors of physical structure (inclination; exposure; roughness; abundance and diversity of surface morphological microelements) across different spatial scales in regular and irregular morphologies of artificial and natural habitats in the North Adriatic Sea, and found that most descriptors were more similar between artificial and natural habitats than generally assumed. Artificial habitats were consistently steeper than natural habitats, independently of their morphologies, and had homogeneous inclinations and exposures at all spatial scales considered. Other descriptors were similar between artificial and natural

habitats. Such minor differences challenged the widespread assumption that artificial infrastructure lacks the physical habitat complexity needed to support rich and diverse epibenthic assemblages.

Chapter 3 was closely related to Chapter 2. Here, I focused on quantifying how much the differences in the structure of benthic assemblages between artificial and natural habitats were explained by the descriptors of physical structure. I observed significant differences in epibenthic communities between natural and artificial substrates which were particularly high on seawalls. However, physical structure explained only a minor proportion (< 10%) of these differences, which was mostly related to differences in inclination and exposure between artificial and natural habitats. Surface roughness and diversity of microhabitats, which are the factors most often manipulated in green engineering approaches, did not explain any relevant variation, at least at the scales covered by this study. These outcomes stimulated the consideration of other structural elements that could enhance the performance of artificial marine structures as biomimetic habitats, such as construction material. They also encouraged a wider reflection about what makes an artificial building surface "greener": structural complexity is an important ecological parameter, and its deliberate increase will lead to responses in the biota, however, this may not necessarily match "more natural" conditions.

In **Chapter 4**, I quantified the effects related to the type of material used for marine buildings in influencing the colonization and growth of sessile benthos and vagile fauna by using a systematic review of the literature and a meta-analysis. I contrasted rocky-type substrates (i.e. natural rocks and Portland concrete), which are the most common materials used for coastal armoring (Gittman et al., 2015), to other types of materials commonly used to build infrastructures (i.e metal, plastic, ceramic rubber and wood) and found effects that varied across functional groups and material types. There were greater abundances of fouling (algae and sessile invertebrates), but not vagile taxa, on the rocky-type substrates than on metal, plastic and rubber materials, and concrete supported communities more diverse than smoother materials like metal, plastic and rubber. The outcomes suggest that material alone, again, explains a significant but quantitatively limited proportion of the community composition and abundance on marine infrastructures. Material choice, however, can also affect other important ecological outcomes, such as the possibility of lowering carbon footprint and raw material demand, for example using concrete blends made with recycled by-products.

In **Chapter 5,** I combined the knowledge acquired to create biomimetic surfaces by mean of additive manufacturing technology (i.e. 3D printing). Every single factor tested in the previous chapters explained only a limited proportion of the biotic differences observed between artificial and natural habitats, however, the variable combination of different factors could provide new artificial surfaces with much better ecological performance as habitats. The tiles were tested in the port of Ravenna. These biomimetic surfaces were designed to provide and explore benefits at multiple levels: the physical structure (in terms of inclination and complexity) and the material composition were delineated to be more suitable for the colonization of fouling organisms; material type, in addition, was an eco-friendly concrete whose carbon footprint and raw material demand were lowered by using recycled aggregates and alternative binder; and aspect (in terms of brightness) was manipulated to test their ecological as well as aesthetic effects, which could be important in seascapes of cultural value (Nordstrom, 2014). As the experiment started later than planned due to Covid constraints, I presented here only preliminary results. The tiles were rapidly colonized, with minor differences in biofilm coverage related to complexity (flat or biomimetic) and brightness (dark or light).

The knowledge produced in this thesis can support future improved biomimetic designs of marine infrastructure which are better integrated into the natural and cultural marine landscapes (Firth et al., 2014b; Dennis et al., 2018). I showed that a greener design of the structures should take into account and combine multiple factors, such as inclination, exposure, surface complexity, material composition and color, as each component individually explained only a small proportion of the structure of the epibiota. The ultimate goal should be to produce structures that mimic as close as possible the local natural habitats, to provide better surrogate habitats for native biodiversity. Therefore, the “natural condition” is an emergent trait of the ecosystem, and the mimicry of the various drivers should occur simultaneously during biomimetic interventions to take advantage of the multiple benefits associated with their combination.

The next step would be to understand to what extent biomimicry can be successfully implemented in urban areas across the globe and in different socio-cultural, economic and ecological contexts, providing an opportunity for the conservation of marine species in urbanized marine environments (Airoldi et al., 2021).

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Ravenna, 12 Maggio 2022

Ferrante Grasselli