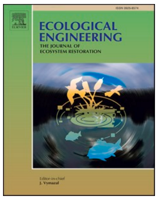
Ecological Engineering 183 (2022) 106765

Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng



Implementation of artificial substrates for *Dendropoma cristatum* (Biondi 1859) reef restoration: Testing different materials and topographic designs.

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ARTICLE INFO

Keywords:
Biogenic reefs
Settlement
Artificial substrates
Restoration ecology
Mediterranean Sea

ABSTRACT

Vermetid reefs are intertidal key habitats that strongly increase the biological and ecological value of the coastal ecosystems, providing ecological goods and services. In some parts of the Mediterranean Sea, this habitat is experiencing an increasing decline, due to environmental and anthropogenic pressures and the lack of officially coordinated strategies promoting its conservation at the basin scale.

Moreover, experimental data testing for vermetid reef restoration is presently scant. Here we tested the potential to achieve new vermetid colonies on engineered substrates, aiming for their further transplantation to depleted reefs for restoration purposes. We investigated in the field the settlement rate of the central-Mediterranean vermetid species *Dendropoma cristatum* on artificial tiles made of different concrete mixtures (geopolymer concrete, pozzolanic concrete mixed with fine sand, magnesian concrete, pozzolanic concrete mixed with magnesium and coarse sand) and one plastic material (expanded polyvinyl chloride), engineered with a range of interstice shapes and sizes (i.e., holes and crevices of 1 and 2 mm), obtaining on each tile four different topographic designs. Also, the benthic fauna colonization on these engineered tiles has been monitored.

While the material typology had a relative effect on the settlement of *D. cristatum*, the presence of specific topographic designs played an important role, not only for the reef-builder species but also for the benthic fauna. A significantly higher vermetid density and invertebrate taxonomic richness and individual abundance were associated with the hole presence on the tiles, confirming to be the preferred interstice type among those texted. Furthermore, geopolymer concrete was the material that showed higher resistance to the intertidal conditions and durability after the experiment.

The production of geopolymer tiles engineered with hole interstices may be a valid option for future studies aiming at the restoration of this intertidal key habitat. However, while our achievements correspond to a higher vermetid and benthic invertebrate settlement within a few weeks of field experiment, post-settlement processes need to be considered in the perspective to restore the functionality of a degraded reef.

1. Introduction

Vermetid reefs are intertidal bioconstructions widely distributed on sub-tropical and warm-temperate rocky coasts, built by the association of gregarious sessile gastropods, crustose coralline algae (CCA) and less frequently found encrusting organisms (Calvo et al., 2009; Chemello, 2009; Milazzo et al., 2016). Due to their high horizontal width and three-dimensionality, these habitats modify the mesolittoral zone, increasing the topographic complexity, thus supporting high levels of marine biodiversity (Consoli et al., 2008; Colombo et al., 2013; Milazzo et al., 2016; Ape et al., 2018). Furthermore, vermetid reefs address ecologically relevant services and goods for human well-being, providing protection from coastal erosion processes and locally modulating the hydrodynamic conditions, regulating the sediment and nutrient transport and accumulation, acting as carbon sinks and supplying refuges, repairs, nursery and feeding areas to a wide variety of

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https://doi.org/10.1016/j.ecoleng.2022.106765
Received 21 February 2022; Received in revised form 26 July 2022; Accepted 31 July 2022

intertidal fish and benthic assemblages (Chemello, 2009; Chemello and Silenzi, 2011; Milazzo et al., 2016; Donnarumma et al., 2018). In the Mediterranean Sea, the main reef-builders are the encrusting coralline red algae *Neogoniolithon brassica-florida* (Harvey) Setchell and Mason (1943) and different vermetid species belonging to the *Dendropoma petraeum* spp. complex which includes at least four cryptic species with no overlying distribution (Templado et al., 2016). In particular, in the central-Mediterranean Sea, *D. cristatum* (Biondi, 1859) is the main vermetid bio-engineer, which creates bioconstructions along the Sicilian and the Maltese rocky coasts and in the central-Tyrrhenian Sea.

In some parts of the Mediterranean, in the last decades, vermetids are facing an increasing mortality leading to local extinctions and ecological shifts, with consequences for the bioconstruction persistence (Rilov et al., 2020; Albano et al., 2021; Badreddine et al., 2019; Galil, 2013).

Some field studies performed in Sicily and Israel showed that future temperature and pH conditions may reduce the settlement rate of *Dendropoma* reef-builder species and the photosynthetic and calcification performance of the red algae *Neogoniolithon brassica-florida*, leading to the reef decline (Fine et al., 2016; Milazzo et al., 2014, 2019).

Due to this acknowledge ecological interest and the sensitivity to persistent environmental and anthropogenic disturbances (Di Franco et al., 2011; Galil, 2013; Milazzo et al., 2014; Terradas-Fernández et al., 2020), European legislation currently invokes the conservation of these Mediterranean biogenic habitats and bioconstructor organisms (European Council, 1979; Barcelona Convention Protocol concerning Specially Protected Areas and Biological Diversity in the Mediterranean, 1995; Council Directive, 1992; the IUCN Red List of Mediterranean Habitats, Gubbay et al., 2016). However, the lack of official strategies promoting coordinated actions at the basin scale for the protection of these biogenic reefs is contributing to the ineffectiveness of local conservation efforts. Where local pressures may be removed or mitigated by an active conservation policy, restoration actions should be included in an integrated approach to the management of this habitat.

To date, there is no mention of official guidelines for vermetid reef restoration, although, globally, habitat restoration is an emerging focus of both conservation policies and ecological studies (Miller and Hobbs, 2007; Perring et al., 2015). Although a natural re-colonization of bare rocks by vermetids has been observed (Antonioli et al., 1999) and the achievement of artificially cultured vermetid surfaces has been reported by recent research (Terradas-Fernández et al., 2020; La Marca et al., 2018; Spotorno-Oliveira et al., 2015), there is a lack of empirical studies testing localised re-population strategies.

Thus, information on habitat selection by bioconstructor species at the stage of settlement (i.e., the larval ability to reach a site suitable for their survival and recruit into adults, Harrington et al., 2004) represents a cornerstone to understand the dynamic of colony development.

The larval development of Mediterranean vermetid species is entirely intracapsular with crawling juveniles (i.e., post-larvae ranging in size from 580 to 830 μm, Templado et al., 2016), leaking out the maternal shell and chasing a site for their permanent cementation onto the substratum (Hughes, 1989; Calvo et al., 1998; Klerman et al., 2004).

Current research demonstrates that site-specific biological cues promote the settlement of different species of vermetid bioconstructors (i.e., crustose coralline algae, Spotorno-Oliveira et al., 2015; mature microbial biofilms, La Marca et al., 2018, 2021), while the mineralogical substratum properties do not seem to be determinant for the establishment of a colony (Terradas-Fernández et al., 2019; Donnarumma et al., 2018).

Moreover, the substratum physical complexity (i.e., topography) is essential for the survivorship of benthic bioconstructors at the settlement stage, sheltering the new settlers from predation and other physical disturbances (Walters et al., 1997; Spieler et al., 2001; Underwood, 2004; Smith et al., 2014; Loke and Todd, 2016; Montalto et al., 2020).

Therefore, the consideration of appropriate interstices is fundamental to design artificial substrates for the establishment of a vermetid colony. The translocation of these artificially colonised substrates to promote vermetid replenishment in target sites (i.e., outplanting) could be a more sustainable approach than the collection of natural reef fragments from a pristine reef (Spotorno-Oliveira et al., 2015; La Marca et al., 2018; Terradas-Fernández et al., 2019).

In this study, we aim to identify a combination of material and topography suitable for the production of artificial settlement substrates designed for vermetid reef restoration.

Specifically, we carried out a field-based settlement experiment on tiles made of four concrete mixtures and one plastic material engineered with six topographic designs to assess: i) the vermetid settlement success on artificial substrates differing in material and surface topography, ii) the benthic invertebrate assemblage colonizing these substrates.

We expect the *Dendropoma cristatum* settlement and the benthic invertebrate early colonization to differ between concrete mixtures and plastic tiles and to reach a higher rate on complex topographic designs.

2. Materials and methods

2.1. Study area

The study was carried out in the locality of “Addaura” (North Western Sicily, geographical coordinates: 30°11′28”N; 13°20′55″E; Fig. 1a) between April and September 2021. Here, a continuous vermetid reef develops on a limestone wave-cut platform (Fig. 1c), approximately covering 202 ± 1.0 m (mean ± SE) of linear coastline. The average reef width is 4.05 ± 0.31 m and the outer and the inner rims (i.e., respectively the seaward and the coastward platform profiles) measure 334 ± 25 and 365.5 ± 11.5 m (data obtained by satellite imagery analysis and field measurements; Table 1).

Here the natural vermetid density on the outer and the inner reef rims (the average values ± SE are reported in Table 1) and the absence of clear signs of reef degradation suggest a good ecological status of this vermetid platform, despite the proximity of a small marina and fishers and summer tourist frequentation.

Throughout the study period, the seawater temperature of the study area was monitored by placing a HOBO thermo-logger (HOBO Pendant® MX2201 Water Temperature) on the outer reef rim, where the artificial tiles have been deployed during the settlement experiment (Fig. 1b). Here, crawling vermetid availability is supposed to be higher compared to the inner rim, since a higher recruitment rate has been found (Franzitta et al., 2016).

2.2. Tile topographic design

Artificial tiles of 10 × 10 × 2 cm have been used as substrates for *D. cristatum* settlement and the benthic invertebrate colonization. Two different 10 × 10 × 2 cm tile models (hereafter named A and B; Fig. 2) have been produced, each including four 5 × 5 cm topographic designs (surface types) engineered by means of interstices of different shapes and sizes. Round holes, square holes, linear and crossed crevices have been chosen as the best shapes that mimic the natural refugia find by crawling vermetids in their environment.

Since the size of *D. cristatum* post-larvae ranges between 740 and 830 μm (Templado et al., 2016), each interstice has been designed both 1 and 2 mm in width and 3 mm in depth, considering the effect that the microtopographic scale has on larval attachment during the settlement phase (Whalan et al., 2015).

In particular, the tile model A included the following surface types: 1_A: round 2 mm and 1 mm holes; 2_A: 1 mm crevices; 3_A: 2 mm crevices; $CTRL_A$: one flat surface, as control, while the tile model B included the surface types: 4_B: square 2 mm and 1 mm holes; 5_B: 1 mm crossed crevices; 6_B: 2 mm crossed crevices; $CTRL_B$: the flat control (Fig. 2).

2.3. Tile production

Four different concrete mixtures and one plastic material have been

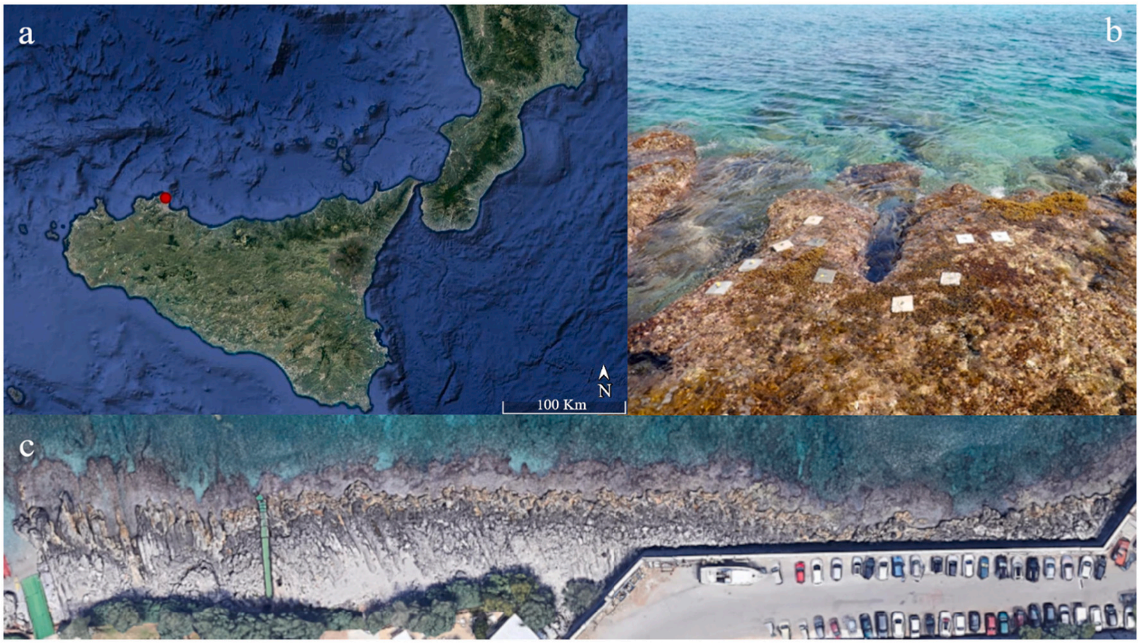

Fig. 1. a) Map of the study area, the red dot indicates the experimental site; b) artificial tiles deployed in the field during the settlement experiment; c) satellite imagery of the whole vermetid reef in the study area.

Table 1
Macrostructure measurements of the vermetid reef and average adult and juvenile *D. cristatum* density (mean ± SE/100 cm^2) on both reef rims, measured by photographic visual estimates.

Reef macrostructure data (m)	Mean	SE
Linear length	202.0	1.0
Reef width	4.2	0.3
Outer rim length	334.0	25.0
Inner rim length	365.5	11.5
Density of *D. cristatum* (ind./100 cm^2)	Mean	SE
Juveniles on the outer rim	203.0	37.6
Adults on the outer rim	174.6	19.5
Juveniles on the inner rim	122.7	15.3
Adults on the inner rim	428.6	30.5

selected for the experimental tile production.

While the different surface types were achieved on the plastic tiles by milling and drilling the tile top surface, concrete tiles were prepared by three-dimensional (3D) printing by D-Shape technology (Monolite UK LTD). Firstly, the tile CAD file was converted into a physical model made of strength nylon by a Binder Jetting 3D printer (Fig. 2a). Onto the 3D printed nylon model, a flexible negative silicon mould was realized (Fig. 2b), where the concrete mixtures were poured. Once cured, the casted tiles were extracted from the mould and all the geometrical features designed in the CAD file were accurately reproduced on the substrate (Fig. 2c).

2.4. Concrete mixture features

All the concrete mixtures selected for tile production are featured by the reduced amount of energy necessary for their production compared to the traditional building cement. Each mixture is described below and additional detail is reported in Table A1 in the Supplementary material.

2.4.1. Geopolymer concrete (GP)

Geopolymer concrete, also named “green concrete” (Das et al., 2013), is considered a new sustainable alternative to the traditional concretes, since its production entail a lower carbon dioxide footprint (Hassan et al., 2019). Geopolymers are covalently-bonded aluminosilicate structures that form long-range, non-crystalline (amorphous) networks, obtained by mixing an aluminosilicate powder and an aqueous solution of alkaline hydroxides or silicates (respectively “precursor” and “activator”, Davidovits, 1976, 1991). This cement type finds application in severe construction and industrial fields, having excellent mechanical strength, high fire, chemical and radiation resistance (Hassan et al., 2019; Zaidi et al., 2021). This concrete has been selected to confer high resistance to breakage and to the chemical-physical weathering to the settlement tiles, given their exposure to the high thermal and solar irradiation along the intertidal shores and the potential presence of corrosive solvents in the seawater.

2.4.2. Pozzolanic concrete mixed with fine sand (CP)

The pozzolanic concrete is a hydraulic cement which becomes adhesive through a chemical reaction between the water and the dry ingredients (a mixture of approximately 50–65% of Portland concrete Clinker and 40% of Natural Pozzolana rock).

It has been selected for its known resistance to seawater (Massazza, 1993; Mehta and Monteiro, 2006) and concrete type CEM IV/B (P—V) 32,5 R UNI – EN 197/1 was used for tile production of this study. To create the mixture cast into the silicone mould, a small amount of fine sand was also added to the mixture (river sand originated from the dissolution of the Alpine and Apennine massifs of the Northern Italy, specifically).

2.4.3. Magnesian concrete (CM)

Magnesian concrete (also known as “sorel cement”) was selected since natural rocks containing magnesium are ideal substrates for biomass attachment (Manso et al., 2014). This material is a non-hydraulic cement prepared by mixing finely divided MgO powder with a concentrated solution of MgCl. The set cement consists of a mixture of magnesium oxychlorides and hydroxide in varying proportions, depending on the initial cement formulation and other variables, such as the setting time.

To improve the resistance of magnesian concrete to seawater, a longer tile dry-time was necessary, allowing the oxychlorides to react with the atmospheric carbon dioxide and to form magnesium chloro-carbonates (Cole and Demediuk, 1955).

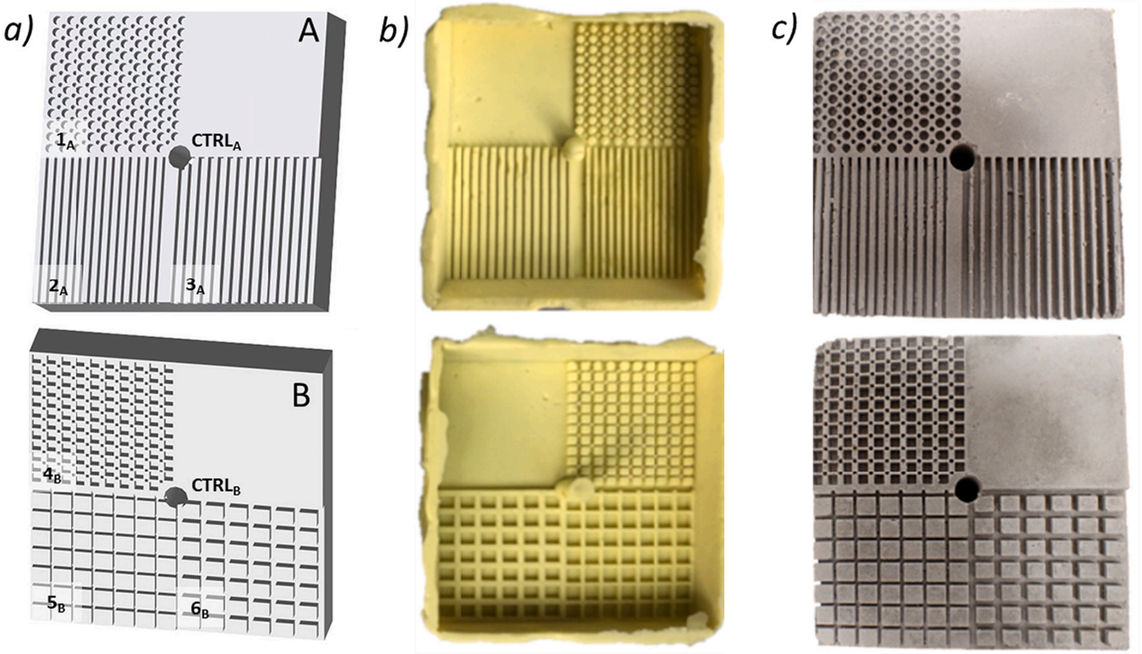

Fig. 2. Steps of concrete tile production (above: A model, below: B model): a) the three-dimensional digital model; b) the silicon mould reproducing the negative tile model; c) the casted tiles obtained from the mould. Each tile measured 10 × 10 × 2 cm and included 3 different 5 × 5 cm topographic designs (surface types) and one flat control (1_A: round 2 mm and 1 mm holes; 2_A: 1 mm crevices; 3_A: 2 mm crevices; 4_B: square 2 mm and 1 mm holes; 5_B: 1 mm crossed crevices; 6_B: 2 mm crossed crevices; $CTRL_A$ and $CTRL_B$: flat control surfaces).

2.4.4. Pozzolanic concrete mixed with magnesium and coarse sand (CS)

To confer immediate hardness to the magnesian concrete, 10% of pozzolanic concrete and 10% of coarse sand (by weight) were added to the previously described mixture. This mixture was empirically made, trying to increase the resistance of the magnesian tiles.

2.5. Plastic tile features

Plastic materials are usually employed as settlement surfaces in ecology, since they are rapidly colonised by biota, they cope with biological and physical weathering and they are easily reachable with limited costs (Pinochet et al., 2020). Among the plastic materials traditionally used in ecological research, forex has been selected to be employed in this study. This material consists of expanded or semi-expanded polyvinyl chloride (PVC) and it is very light, elastic and easily shaped. For this reason, it tolerates cutting or milling and it is particularly suitable for direct printing.

2.6. Dendropoma cristatum settlement assay

The experiment has been repeated four times during the *D. cristatum* breeding season (T-I, T-II, T-III and T-IV, Table 2). At each time three sets of ten randomly allocated artificial tiles (two tile models × five materials) have been fastened on the outer rim of the vermetid reef, spaced 20 m apart from each other along the reef.

Taking into account the reproductive output variation of the species during the whole experimental period, tile permanency in the field differed among sampling times, as reported in Table 2.

Table 2
Duration of each experimental time from the day of tile deployment to the day of tile collection.

Sampling times	Deployment	Collection
T-I	01/04	24/05
T-II	24/05	11/06
T-III	11/06	23/06
T-IV	01/09	15/09

Thus, the first experimental time was the longest, since the early of April coincides with the beginning of the reproductive season of *D. cristatum* (Calvo et al., 1998) and the following experimental times were progressively shorter.

The experiment has been stopped between July and August to avoid the tourists frequenting the area during this period to influence the study (e.g., through the human trampling on the reef rim where the tiles have been placed or through the direct tile harvesting). At the end of each time, the artificial substrates were collected, photographed, singularly placed in a plastic bag to avoid the loss of the associated fauna, transferred to the laboratory and stored at −20 °C. Subsequently, the number of *D. cristatum* settlers on each 5 × 5 cm tile surface type was counted under the stereomicroscope (Leica MDG41) and compared either among topographic designs (5 × 5 cm surface type) and materials (10 × 10 cm surface) during each experimental time.

2.7. Benthic fauna colonization assay

Simultaneously to the visual count of the *D. cristatum* settlers, the non-encrusting benthic fauna associated with each 5 × 5 cm tile surface type was sorted and stored within 2 ml tubes filled with 70% marine water and ethanol for further identification at the highest taxonomic level.

During each experimental time, the abundance of organisms, the taxonomic richness and the assemblage structure have been compared either among the different materials (10 × 10 cm surface) and among the 5 × 5 cm surface types.

After lab work, all the tiles were dried out, scraped with a soft brush and rinsed with freshwater, removing the associated colonization. Persistent organisms and settlers observed under the stereomicroscope were manually removed by a tweeze. Cleaned tiles were stored to be reused for the following experimental times and missing tiles were replaced with new ones if available, except for T-II, where new sets of experimental tiles were used.

After the last experimental time, furthermore, tile deterioration signs on each tile have been quantified by a visual assessment, to compare the effect of intertidal environmental factors on substrates of different materials and to assess their durability in the field (the method and the results are reported in the section “Material degradation assay” of the Supplementary material).

2.8. *Statistical analysis*

During the four experimental times, some tiles have been lost as a consequence of their degradation during the field deployment and their replacement with new ones was not possible. This resulted in a different number of replicates for each sampling time (ranging between 30 and 21) and in an unbalanced experimental design.

Differences in the *D. cristatum* settlement rate and the benthic fauna assemblage composition among materials and topographic designs were analysed through univariate and multivariate distance-based permutational non-parametric analyses of variance (PERMANOVA; Anderson, 2001; McArdle and Anderson, 2001). The total number of *D. cristatum* settlers was considered as a response variable for the settlement assay, while for the benthic fauna assemblage the total abundance of organisms, the taxonomic richness and the assemblage composition have been considered.

To detect the differences among materials, we analysed the number of the considered organisms on the whole tile surface (10 × 10 cm). The experimental design included two fixed factors: “Material” (with five levels: GP, CP, CM, CS, FO during T-I and four levels: GP, CP, CM, FO during the other times) and “Model” (with two levels: tile model A and tile model B). Each sampling time was individually analysed and CS tiles were considered only during T-I because of their weakness and then excluded from the experiment.

To analyse the differences in the number of organisms among the four surfaces type of each tile (5 × 5 cm), we considered two fixed factors: “Material” (with five levels: GP, CP, CM, CS, FO during T-I and four levels: GP, CP, CM, FO during the other times) and “Design” (with eight levels: 1_A, 2_A, 3_A, 4_B, 5_B, 6_B, $CTRL_A$ and $CTRL_B$).

The analyses were performed on square-root transformed data, using the Bray Curtis dissimilarity (adding a dummy value when resemblance between two samples was undefined), 9999 permutations of residuals under a reduced model with a Type III (partial) sum of squares and the Monte Carlo test (Anderson, 2001).

When significant differences were revealed for one of the considered factors (i.e., “Material”, “Model” and “Design”), the differences among all the pairs of levels within the factor were explored by *a-posteriori* pairwise comparison.

Moreover, with the aim of annulling the possible non-independence of the data recorded on the four 5 × 5 cm surface types within the same 10 × 10 cm tile, thus reinforcing the results of our analysis, we performed an additional analysis following the approach of Loke and Todd (2016). The detailed analysis and the results are reported in the Supplementary material (Tables A7 – A10). Statistical analyses were performed using the PRIMER-E v6+ statistical software with the PERMANOVA + extension (Plymouth Marine Laboratory; Clarke and Warwick, 1994; Clarke and Gorley, 2006).

3. Results

3.1. *Dendropoma cristatum settlement*

Differences in the *D. cristatum* settlement rate on the artificial tiles were found across the whole experimental period and a peak of settlement was registered at the third sampling time.

On average, the number of settlers (mean ± SE) varied between 1.66 ± 0.54 ind./100 cm^2 and 51.08 ± 6.43 ind./100 cm^2, respectively registered at the first and at the third sampling time (Fig. 3, primary Y axes). Over the entire experiment, the environmental temperature increased from 19.15 ± 0.03 °C to 28.38 ± 0.07 °C (Fig. 3, secondary Y axes).

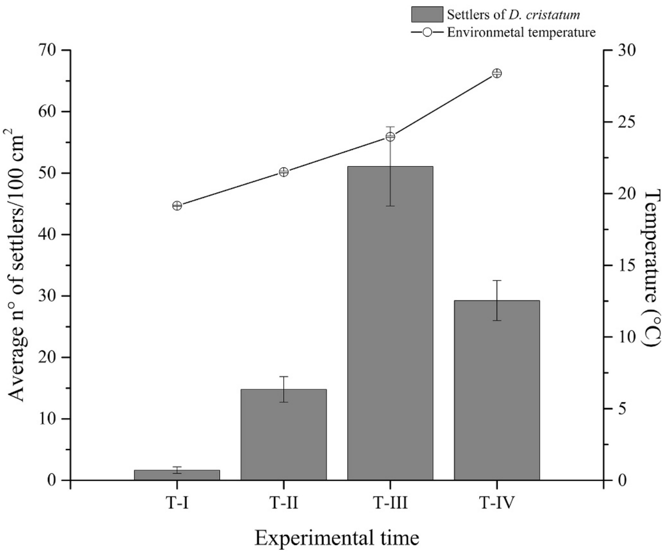

Fig. 3. Number (mean ± SE) of *D. cristatum* settlers/100 cm^2 (main Y axes) and mean environmental temperature (secondary Y axes) registered on the outer reef rim during each experimental time.

3.1.1. *D. cristatum settlement on different materials*

The average number of *D. cristatum* settlers (i.e., settlers/100 cm^2) did not differ among the tested materials (PERMANOVA, Table A2 in Supplementary material).

However, CP and GP tiles hosted the highest number of *D. cristatum* settlers during T-III and T-IV, reaching a maximum value of 53 ± 9.57 ind./100 cm^2 and of 60.17 ± 18.82 ind./100 cm^2, respectively, when the settlement peaked (T-III; Fig. 4).

The average vermetid settlement on plastic forex tiles was comparable to concrete mixture tiles throughout the whole experimental period, ranging between 0.81 ± 0.81 ind./100 cm^2 and 45.66 ± 11.43 ind./100 cm^2, respectively at the T-I and at the T-III (Fig. 4).

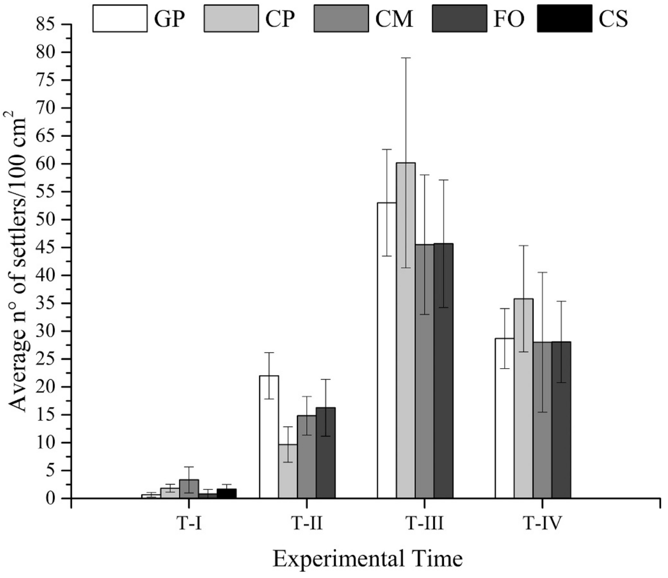

Fig. 4. Comparison of the number (mean ± SE) of *Dendropoma cristatum* settlers across the five tested materials (10 × 10 cm surface) during the whole experimental period. GP: geopolymer concrete; CP: pozzolanic concrete; CM: magnesian concrete; FO: forex; CS: pozzolanic concrete mixed with magnesium and coarse sand.

PERMANOVA did not show significant differences among A and B tile models for any of the tested materials (Table A2 in Supplementary material).

3.1.2. *D. cristatum settlement on different topographic designs*

Regardless of the tile material, the number of *D. cristatum* settled on each engineered surface type (i.e., settlement/25 cm^2) was significantly higher compared to the number of settlers recorded on the flat controls (PERMANOVA, $p < 0.001$, Table 3 and Table A7 in Supplementary material), except for T-I.

Both the hole surface types represented the topography that best fits habitat selection by the species and, through the time, the number of settlers on these interstice types was progressively higher compared to the other surface types (Fig. 5). In particular, the square holes (4_B surface type) showed the highest average number of settlers/25 cm^2 during T-II and T-III (6.36 ± 0.83 and 23 ± 4.8 ind./25 cm^2, respectively; Fig. 5), the round holes (1_A surface type) showed a peak of settlement at the last experimental time (16.1 ± 3.16 ind./25 cm^2; Fig. 5) and both hole shapes showed significant differences with the other surface types (*a-posteriori* pair-wise, Table A3 and A8 in Supplementary material).

Furthermore, crevice interstice typology does not seem to represent a good design for crawling *D. cristatum* snails, especially when crossed and regardless of crevice size (Fig. 5).

3.2. *Benthic fauna colonization*

3.2.1. *Benthic fauna on different materials*

The taxonomic identification of benthic fauna was performed at class or, when possible, at order level and a list of the discriminated taxa with their % abundance on each material during the four experimental times is reported in Table 4.

A general dominance of Amphipoda, Bivalvia and Copepoda was recorded during the first, the second and the fourth experimental times, while during T-III the class Gastropoda was dominant on all the materials (Table 4).

The average abundance of benthic invertebrates (ind./100 cm^2 ± SE), the taxonomic richness (n. taxa/100 cm^2 ± SE) and the assemblage composition did not show clear patterns among materials during the experiment and significant differences were observed only during the last two sampling times (PERMANOVA, Table 5). After the first sampling, fauna abundance was lowest on CP and peaked on GP and CM with maximum values recorded during T-IV (97.5 ± 6.5 and 102.0 ± 15.0 ind./100 cm^2 respectively; Fig. 6), and significant differences with the other materials were revealed during T-III and T-IV (*a-posteriori* pair-wise, $p < 0.05$, Table A4 in Supplementary material).

Table 3
Results of the PERMANOVA analysis comparing the total number of *D. cristatum* settlers (ind./25 cm^2) on the tile sub-surfaces (5 × 5 cm) among the tested materials (GP: geopolymer concrete; CP: pozzolanic concrete; CM: magnesian concrete; CS: pozzolanic concrete mixed with magnesium and coarse sand; FO: forex) and the topographic designs (1_A: round hole pattern; 2_A: 1 mm crevices; 3_A: 2 mm crevices; 4_B: square hole pattern; 5_B: 1 mm crossed crevices; 6_B: 2 mm crossed crevices; $CTRL_A$ and $CTRL_B$: flat controls) during the four sampling times. Statistically significative values are in bold. df = degrees of freedom; MS = mean square; Pseudo-F = F statistic; P(MC) = Monte Carlo probability level.

	Source	df	MS	Pseudo-F	P (MC)
T-I	Material	4	145.80	0.52	0.7244
	Design	7	334.34	1.20	0.3129
	Material × Design**	27	158.58	0.57	0.9572
	Residuals	78	279.59		
	Total	116			
T-II	Material	3	762.26	2.11	0.0922
	Design	7	3726.20	10.30	**0.0001**
	Material × Design**	20	367.35	1.02	0.4601
	Residuals	62	361.65		
	Total	92			
T-III	Material	3	277.76	0.80	0.5246
	Design	7	7690.80	22.10	**0.0001**
	Material × Design**	20	274.45	0.79	0.7646
	Residuals	62	347.94		
	Total	92			
T-IV	Material	3	232.12	1.44	0.2189
	Design	7	6494.60	40.21	**0.0001**
	Material × Design**	16	185.93	1.15	0.3092
	Residuals	50	161.50		
	Total	76			

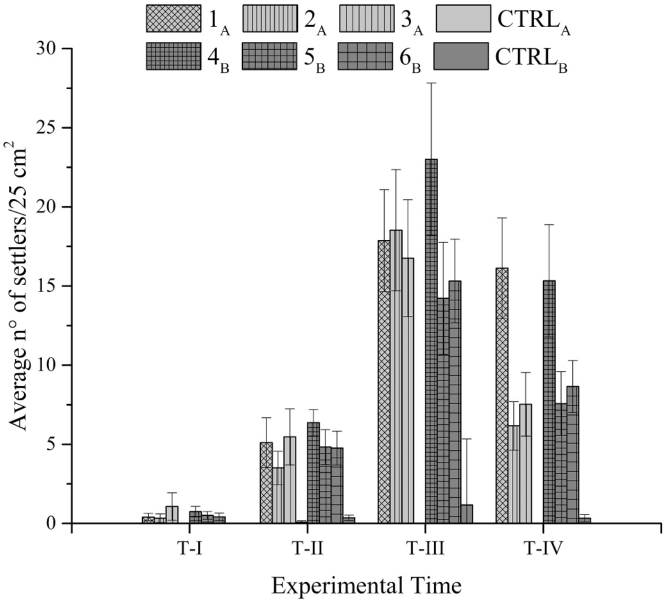

Fig. 5. Comparison of the number (mean ± SE) of *Dendropoma cristatum* settlers across the six tested topographic designs (5 × 5 cm surface type) during the whole experimental period. 1_A: round holes (2 mm and 1 mm); 2_A: 1 mm crevices; 3_A: 2 mm crevices; 4_B: square holes (2 mm and 1 mm); 5_B: 1 mm crossed crevices; 6_B: 2 mm crossed crevices; $CTRL_A$ and $CTRL_B$: flat controls.

The taxonomic richness (Fig. 7) and the assemblage composition did not differ among materials, except during T-III (PERMANOVA, $p < 0.05$, Table 5). At this time, *a-posteriori* pair-wise comparison showed a significantly higher number of taxa on concrete tiles (from 5.66 ± 0.21 taxa/100 cm^2 on CP to 5.83 ± 0.16 taxa/100 cm^2 on CM) compared to FO (3.00 ± 0.63 taxa/100 cm^2) (*a-posteriori* pair-wise, $p < 0.05$, Table A4 in Supplementary material) and significant differences of the assemblage composition between CM and FO ($p < 0.05$, Table A4 in Supplementary material).

3.2.2. *Benthic fauna colonization on different topographic designs*

During all the experimental times, the benthic fauna abundance (ind./25 cm^2), the taxonomic richness (n. taxa/25 cm^2) and the assemblage composition were significantly different for all the topographic designs compared to the flat controls (PERMANOVA, Table 6 and Table A9 in Supplementary material) and differences between surface types were also revealed during specific sampling times (*a-posteriori* pair-wise, Table A5 and A10 in Supplementary material).

Specifically, the benthic fauna abundance and the taxa richness were higher on the hole interstices with the maximum value respectively recorder on the surface type 1_A during T-IV (38.2 ± 3.06 ind./25cm^2 Fig. 8) and on the surface type 4_B during T-II (4.6 ± 0.3 taxa/25 cm^2; Fig. 9).

In the comparison among surface types of each tile, significant differences were detected also among materials (*a-posteriori* pair-wise, Table A6 in Supplementary material).

Table 4
Benthic fauna community structure during the four experimental times on the artificial materials (GP: geopolymer concrete; CP: pozzolanic concrete; CM: magnesian concrete; CS: pozzolanic concrete mixed with magnesium and coarse sand; FO: forex). The highest taxon % abundances on each material and during each time are in bold.

TAXA % ABUNDANCE																	
	T-I					T-II				T-III				T-IV			
	GP	CP	CM	CS	FO	GP	CP	CM	FO	GP	CP	CM	FO	GP	CP	CM	FO
Acarina	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.5	0.0	0.0	0.0	0.0	0.3	0.7
Amphipoda	**47.3**	25.2	22.5	17.5	**61.9**	**44.7**	**29.5**	12.3	6.1	27.6	15.7	14.0	22.2	**52.1**	**44.3**	36.3	40.2
Bivalvia	16.4	**33.3**	**41.4**	**45.8**	12.5	7.3	18.8	**36.8**	9.3	10.7	11.3	10.6	0.0	1.4	8.1	6.6	3.9
Copepoda	3.3	1.0	1.0	1.8	3.3	4.8	6.0	23.3	24.6	2.0	5.9	9.7	0.8	37.8	41.2	**49.7**	**46.5**
Decapoda	0.6	0.3	0.0	0.0	0.5	0.3	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.5	0.0	0.5	0.5
Gastropoda	16.1	23.7	25.4	27.0	14.5	8.7	20.4	17.2	20.1	**42.2**	**44.8**	**52.2**	**32.9**	5.2	4.1	3.8	5.3
Isopoda	15.6	15.4	9.6	7.5	5.5	17.8	10.1	9.0	0.6	8.0	8.9	7.6	26.3	1.1	0.9	1.1	1.7
Ostracoda	0.0	0.7	0.0	0.0	1.1	16.3	15.2	0.7	**39.3**	8.5	11.0	6.0	17.7	1.8	1.4	1.7	1.0
Polychaeta	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tanaidacea	0.2	0.5	0.0	0.4	0.7	0.1	0.0	0.7	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.1

Table 5
Results of the PERMANOVA analysis comparing the benthic fauna colonization (in terms of: total Abundance of individuals, Taxa Richness and Assemblage composition) on the whole tile surface (10 × 10 cm) among the tested materials (GP: geopolymer concrete; CP: pozzolanic concrete; CM: magnesian concrete; CS: pozzolanic concrete mixed with magnesium and coarse sand; FO: forex) and the two tile models (A and B) during the four sampling times. Statistically significative values are in bold. df = degrees of freedom, MS = mean square, Pseudo-F = F statistic, P(MC) = Monte Carlo probability level.

	Source	df	MS	Pseudo-F	P (MC)	MS	Pseudo-F	P (MC)	MS	Pseudo-F	P (MC)
			Abundance			Taxa Richness			Assemblage composition		
T-I	Material	4	362.68	0.82	0.5586	353.60	0.87	0.5085	1337.80	1.82	0.066
	Model	1	320.37	0.72	0.4538	321.32	0.79	0.3963	788.77	1.07	0.3505
	Material × Model	4	364.08	0.82	0.5494	338.55	0.83	0.533	494.54	0.67	0.7647
	Residuals	18	444.17			406.55			735.48		
	Total	27									
T-II	Material	3	236.09	0.48	0.7636	58.99	0.92	0.4623	2049.40	1.36	0.2209
	Model	1	1510.80	3.07	0.0885	43.10	0.67	0.4191	1310.80	0.87	0.4502
	Material × Model	3	166.98	0.34	0.8715	64.55	1.01	0.4274	506.11	0.33	0.9788
	Residuals	13	491.47			64.05	0.92		1512.30		
	Total	20									
T-III	Material	3	1447.70	4.60	**0.0065**	515.00	8.95	**0.0005**	1831.00	2.18	**0.0282**
	Model	1	274.45	0.87	0.3959	24.12	0.42	0.5473	619.36	0.74	0.5814
	Material × Model	3	374.66	1.19	0.3332	82.69	1.44	0.2627	776.28	0.92	0.5332
	Residuals	16	314.42			57.56			841.14		
	Total	23									
T-IV	Material	3	77.66	3.31	**0.0496**	19.35	0.44	0.7315	298.99	1.19	0.3153
	Model	1	35.54	1.51	0.2364	13.44	0.30	0.5866	250.52	1.00	0.4133
	Material × Model	2	257.27	10.95	**0.0017**	57.30	1.30	0.3048	465.22	1.85	0.0835
	Residuals	13	23.49			44.20			250.87		
	Total	19									

4. Discussion

For bioconstructor species, the use of artificial surfaces for juvenile cultivation is a valid option to minimize a destructive impact on the donor population, allowing the manageable transplantation of a new colony to the target area for restoration. Our field experiment aimed at testing the effectiveness of artificial substrates for vermetid colonization and revealed that the substrate material does not affect the settlement of the species *D. cristatum*, while an important role is played by specific topographic designs.

All the artificial substrates employed in our study are suitable for *D. cristatum* early colonization and, when the settlement of the species was greater (i.e., T-III and T-VI), geopolymer and pozzolanic concrete tiles showed the highest settlement success. The average settlement was higher than the rate measured on non-engineered artificial limestone blocks employed in a companion paper (i.e., La Marca et al., 2018, 1.41 ± 0.22 ind./25 cm^2 after 5 days of substrate field-deployment), although two different years of settlement are considered.

Our results confirm that engineered concretes, successfully used in the field of habitat restoration and ecological engineering (Spieler et al., 2001; Coombes et al., 2015; MacArthur et al., 2019) may represent a promising option also for vermetid reef restoration.

Further, the ability of *Dendropoma cristatum* to colonize plastic surfaces is here reported for the first time and could entail consequences for the dispersion of the species. The benthic stage of crawling vermetids, indeed, limits their colonization ability to the closeness substrates, while floating plastic could transport the settlers to new sites, with positive implications for the range of the species distribution.

The choice of appropriate topographic reliefs fosters the habitat physical structure and functionality for this species, confirming that the interstices produced on our tiles effectively match the vermetid size at the crawling stage.

We preferred to produce four surface types on the same tile with the main intent to reduce the sampling impact on this protected Mediterranean coastal key habitat, although we were aware that this may have limited the independence among topographic designs. However, the

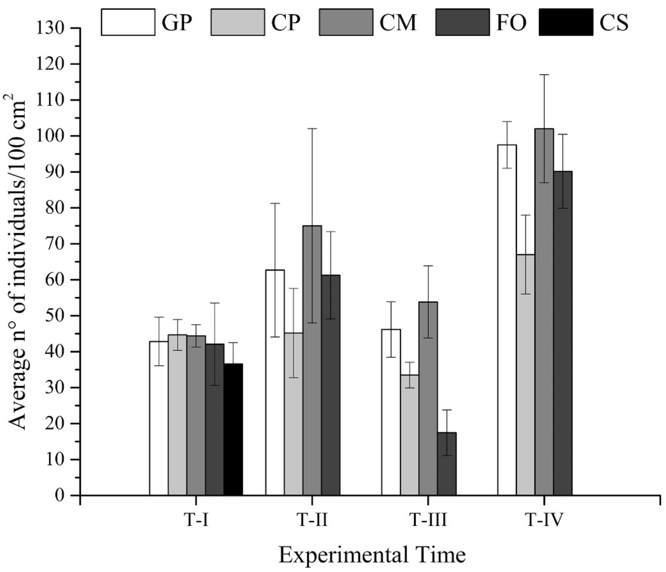

Fig. 6. Comparison of the number (mean ± SE) of benthic organisms across the five tested materials (10 × 10 cm surface) during the whole experimental period. GP: geopolymer concrete; CP: pozzolanic concrete; CM: magnesian concrete; FO: forex; CS: pozzolanic concrete mixed with magnesium and coarse sand.

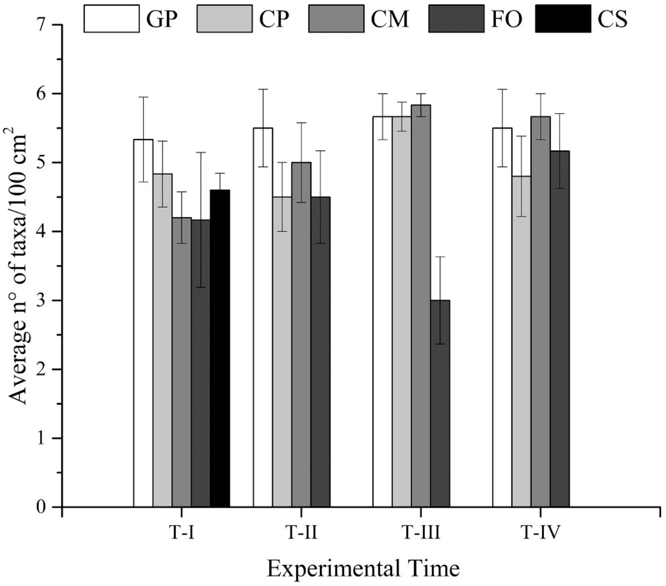

Fig. 7. Comparison of the benthic fauna taxa richness (mean ± SE) across the five tested materials (10 × 10 cm surface) during the whole experimental period. GP: geopolymer concrete; CP: pozzolanic concrete; CM: magnesian concrete; FO: forex; CS: pozzolanic concrete mixed with magnesium and coarse sand.

statistical analysis we performed to annull the non-independence of the data recorded on the surface types within the same tile confirmed our results (see Tables A7 – A10 in the Supplementary material).

From the second experimental period, the holes represent the surface type that better promote the vermetid settlement, independently from their geometry. These differences become consistent when the settlement rate is higher, thus when the availability of interstices (i.e., 1 and 2 mm holes) represents a limiting factor for the vermetid attachment.

Hole design, compared to the crevices, may limit the entry and the mobility of predators, may retain a higher sea-water amount during the low-tide, reducing desiccation stress and solar irradiation for *D. cristatum* settlers, especially during the summer diurnal low tides. These stressful intertidal conditions are hypothesized as factors that limit the settlement of this vermetid species (Franzitta et al., 2016), being also responsible for the benthic colonization on intertidal substrates subjected to extended periods of emersion (Fletcher and Callow, 1992; Coombes, 2011; Firth et al., 2013).

Moreover, attached initial colonists may enhance later settlement and recruitment occurrence, reinforcing the initial topographic influence.

Therefore, the use of topographic designs that match the *D. cristatum* juvenile requirements can optimize the establishment of the species on artificial surfaces by multiple ways, likewise for other sessile benthic invertebrates on intertidal rocky shores (Coombes et al., 2015; Loke and Todd, 2016; Strain et al., 2018; MacArthur et al., 2019; Hartanto et al., 2021).

The effectiveness of a reef-restoration action is not limited to the recovery of the target species but it should aim to promote the whole biological assemblage, as a proof of the habitat functionality re-achievement (Brown et al., 2014; Graham et al., 2017).

Artificial materials employed in ecological studies may have adverse effects on biological diversity, lowering the natural abundance and diversity of the species (Chapman and Underwood, 2011; Coombes et al., 2015; Hsiung et al., 2020).

Except for the third experimental time, our substrates host a benthic fauna composition similar to the invertebrate assemblage reported on the outer vermetid reef rim during the same experimental period, with a dominance of crustaceans (i.e., amphipods and copepods) and bivalves (mainly mussels) (Chemello et al., 2000; Donnarumma et al., 2014; Ape et al., 2018). The presence of these taxa is probably correlated to the high wave-energy and the input of allochthonous resources on the seaward reef profile and to the overlap of the reproductive period of these taxa with the vermetid breading season (e.g., Netto et al., 1999; Gosling, 2022).

Although the differences among material types were not consistent among the four sampling times, the fauna abundance and the taxa richness were higher on the concrete mixtures, in particular on the geopolymer and magnesian tiles compared to the forex substrates.

The selectivity for the topographic designs was confirmed throughout the whole experiment also by the benthic fauna assemblage, suggesting that the holes are the preferred interstice type also for the pioneer sessile and vagile invertebrates. Hole design may compensate for the absence of the vermetid cover on our artificial tiles, improving the quality and the availability of microhabitats for the intertidal organisms. The rate and the success of settlement and recruitment of the early colonists, furthermore, are limiting factors in the development of more complex and diverse intertidal assemblages. Attaining a higher biodiversity value at the beginning of the colonization may favour the intertidal assemblage development, fostering the complexity and the diversity of the reef-associated community (Anderson and Underwood, 1994; Connell, 1985; Farrell, 1991; Gaines and Roughgarden, 1985).

The physical degradation of the artificial tiles employed in our study was also estimated, since the substrate resistance requires accurate consideration in the context of restoration research (Spieler et al., 2001). Based upon our visual assessment of the deterioration signs on each tile, the geopolymer concrete represents the artificial mixture that better copes with the environmental conditions. This concrete type is made of finest aggregates which can reduce the inter-mixture void space, improving the mixture cohesiveness (Unsworth et al., 2021). Furthermore, geopolymers are made by a non-crystalline (amorphous) network, likely conferring a lower physical vulnerability and higher durability to the tiles (Hassan et al., 2019).

By contrast, concrete mixtures which include grain particles, such as the coarse sand (used in the CS mixture), exhibited the worst resistance probably due to the higher inter-mixture void space (Mehta and Monteiro, 2006) which reduces the cohesiveness and increases the material

Table 6

Results of the PERMANOVA analysis comparing the benthic fauna colonization (in terms of: total Abundance of individuals, Taxa Richness and Assemblage composition) on the tile sub-surfaces (5 × 5 cm) among the tested materials (GP: geopolymer concrete; CP: pozzolanic concrete; CM: magnesian-concrete; CS: pozzolanic concrete, magnesium and coarse sand; FO: forex) and the topographic designs (1_A: round holes; 2_A: 1 mm crevices; 3_A: 2 mm crevices; 4_B: square holes; 5_B: 1 mm crossed crevices; 6_B: 2 mm crossed crevices; $CTRL_A$ and $CTRL_B$: flat controls) during the four sampling times. Statistically significative values are in bold. df = degrees of freedom, MS = mean square, Pseudo-F = F statistic, P(MC) = Monte Carlo probability level.

	Source	df	MS	Pseudo-F	P (MC)	MS	Pseudo-F	P (MC)	MS	Pseudo-F	P (MC)
			Abundance			Taxa Richness			Assemblage composition		
T-I	Material	4	59.43	0.14	0.9907	113.89	0.46	0.7726	3007.20	3.21	**0.0004**
	Design	7	4630.60	10.88	**0.0001**	2135.40	8.70	**0.0001**	7512.60	8.03	**0.0001**
	Material × Design**	27	212.96	0.50	0.9882	136.31	0.55	0.965	547.97	0.59	0.9965
	Residuals	70	425.66			245.52			935.74		
	Total	108									
T-II	Material	3	425.44	0.92	0.4494	227.44	1.21	0.3051	2895.60	1.91	**0.0313**
	Design	7	4178.30	9.08	**0.0001**	1646.90	8.74	**0.0001**	5544.40	3.66	**0.0001**
	Material × Design**	20	365.41	0.79	0.7586	209.71	1.11	0.3609	803.65	0.53	0.9991
	Residuals	50	460.10			188.49			1514.30		
	Total	80									
T-III	Material	3	4373.90	13.21	**0.0001**	3386.60	21.94	**0.0001**	7630.10	9.68	**0.0001**
	Design	7	5545.90	16.75	**0.0001**	2956.00	19.15	**0.0001**	7786.20	9.88	**0.0001**
	Material × Design**	20	260.24	0.79	0.7798	157.54	1.02	0.4416	893.88	1.13	0.2548
	Residuals	62	330.99			154.36			788.43		
	Total	92									
T-IV	Material	3	391.17	5.02	**0.0003**	323.24	6.42	**0.0005**	765.92	2.13	**0.0153**
	Design	7	8544.50	109.61	**0.0001**	3179.20	63.14	**0.0001**	12,059.00	33.56	**0.0001**
	Material × Design**	16	276.19	3.54	**0.0001**	186.04	3.69	**0.0001**	503.36	1.40	**0.0424**
	Residuals	50	77.95			50.35			359.37		
	Total	76									

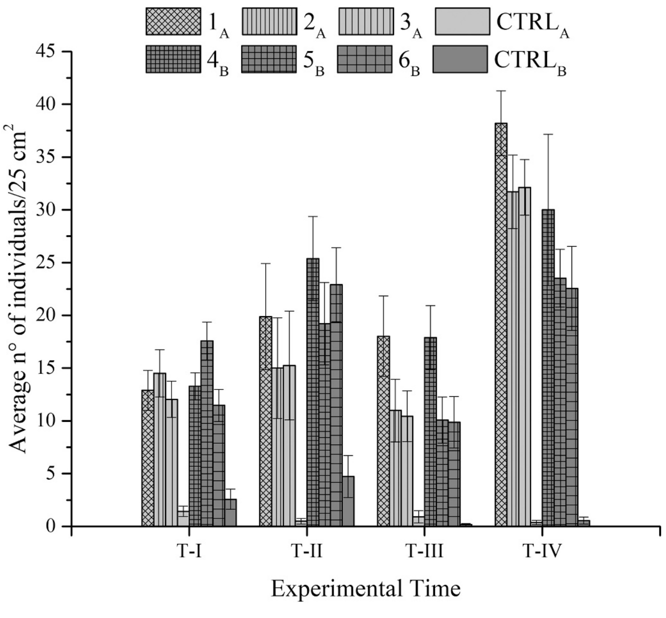

Fig. 8. Comparison of the numbers of benthic organisms (mean ± SE) across the six tested topographic designs (5 × 5 cm surface types) during the whole experimental period. 1_A: round holes (2 mm and 1 mm); 2_A: 1 mm crevices; 3_A: 2 mm crevices; 4_B: square holes (2 mm and 1 mm); 5_B: 1 mm crossed crevices; 6_B: 2 mm crossed crevices; $CTRL_A$ and $CTRL_B$: flat controls.

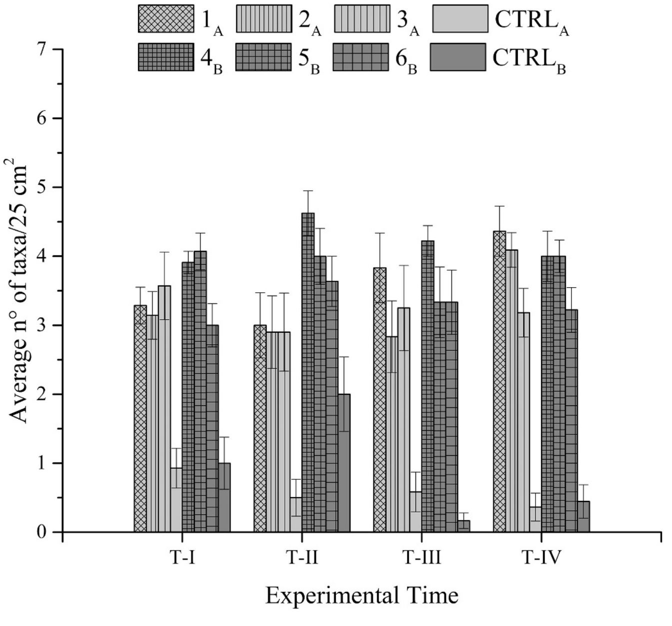

Fig. 9. Comparison of the benthic fauna taxonomic richness (mean ± SE) across the six tested topographies (5 × 5 cm surface types) during the whole experimental period. 1_A: round holes (2 mm and 1 mm); 2_A: 1 mm crevices; 3_A: 2 mm crevices; 4_B: square holes (2 mm and 1 mm); 5_B: 1 mm crossed crevices; 6_B: 2 mm crossed crevices; $CTRL_A$ and $CTRL_B$: flat controls.

permeability to seawater (Ge et al., 2015).

Forex tiles apparently showed 100% of integrity after the field experiment. However, considering the wide temporal span to address a reef restoration, the use of forex may represent a source of microplastic, implicating an impact on the multiple ecosystem components and being environmentally ineffective.

Additional studies may consider the production of artificial substrates with a different shape, further reducing their vulnerability to the wave impact (e.g., a round shape rather than a square), and allowing the study of the colonization pattern over a longer temporal scale than herein considered.

5. Conclusion

Our study represents the first attempt to identify an engineered substrate for the vermetid colonization and then translocation to depleted reefs for restoration purposes, addressing some relevant methodological issues. We recognize the high potential of geopolymer concrete for the production of these artificial substrates, due to the good results obtained in terms of i) reef-builder and invertebrate settlement performances on this material, ii) the enhanced energy consumption and generation of greenhouse gases during the production of this mixture (Hassan et al., 2019) and iii) the higher tile resistance under intertidal conditions (see the Fig. A1 of the Supplementary material).

The creation of hole interstices matching the body size of the settlers increases the multi-functionality of the engineered substrates, enhancing the potential for vermetid establishment, especially during their reproductive peak, and for pioneer benthic invertebrate colonization.

The vermetid colonised surfaces could be employed as habitat reconstruction tools, for example coping with the structural simplification of reefs presenting damaged and abraded patches due to physical disturbances, or re-densifying the natural vermetid populations and increasing their resistance and resilience to disturbances where the bioconstruction persistence is impaired. Certainly, the post-settlement and post-recruitment processes on these artificial substrates remain to be tested and further attempts to operate over a larger spatial and temporal scale than herein considered are decisive to define the later steps of this research and to advance the awareness on vermetid reef restoration opportunities.

Author contributions

ELM, FA and SM conceived this research; ELM wrote the manuscript, with a contribution from FA, MM, VM and AR; ELM, FA, MM, AR, VM and ES conducted the fieldwork; ELM and FA conducted the laboratory and data analysis; FA and AR performed the statistical analyses; ED and SM contributed to the manuscript revision and discussion. SM provided funds to support the experiment. All the authors commented on the first draft of the manuscript.

Declaration of Competing Interest

We wish to draw the attention of the Editor to the following fact which may be considered potential conflicts of interest. One of the authors is employed by the company producing the concrete tiles employed in the study (Monolite UK LTD). Nevertheless, the research did not receive any financial support from the Monolite UK and the author employed in the company did not influence the research outcomes. He only helped us in choosing the most suitable materials for our study and provided technical indications on the different tested materials (e.g., hardness, composition, resistance in seawater).

Data availability

Data will be made available on request.

Acknowledgements

This work received the financial support of the Italian Ministry of the University and Research (MUR), within the framework of the PRIN project MARHES - Marine habitats restoration in a climate change-impaired Mediterranean Sea (PRIN - cod. 2017MHHWBN_003 Linea C).

The authors are also grateful to the Vela Club staff for the facilitation provided during the fieldwork.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoleng.2022.106765.

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