UNIVERSITÀ POLITECNICA DELLE MARCHE

DIPARTIMENTO SCIENZE DELLA VITA E DELL'AMBIENTE

Corso di Laurea Magistrale in Biologia Marina

Marine ecosystem regeneration, analysis of the early benthic colonization on artificial substrates: Case study along Catalan coast, (Spain)

Rigenerazione dell'ecosistema marino, analisi delle fasi inziali di colonizzazione bentonica di substrati artificiali: Caso studio lungo la costa Catalana, (Spagna)

Tesi di Laurea Magistrale di:

Agnese Pestillo

Relatore:
Chiar.mo Prof.
Carlo Cerrano

Correlatore:
Gent.mo Prof.
Sergio Rossi

Sessione straordinaria febbraio 2025

Anno accademico: 2023/2024

Index

RIASSUNTO	5
ABSTRACT	7
1. INTRODUCTION	8
1.1 Marine ecosystem regeneration	8
1.2 Different basic materials for artificial reefs	9
1.3 Early benthic colonization	11
1.4 Host organization – UGI	12
1.5 Project proposal: marine benthic colonization	13
2. AIMS OF THE STUDY	17
3. MATERIALS AND METHOD	18
3.1 Study area	18
3.2 Tile's study methods	19
3.3 Statistical analysis	23
4. RESULTS	25
4.1 Biodiversity	25
4.2 Colonization status	31
4.3 Carbon immobilization	32
4.4 Relationship between structural complexity and number of decapods	33
5. DISCUSSION	35
6. CONCLUSIONS AND FUTURE PERSPECTIVES	39
7. SUPPLEMENTARY ACTIVITY: Monitoring program Port Olímpic, Barcelona (Spain)	40
8. BIBLIOGRAPHY	43

RIASSUNTO

Questo studio si è concentrato sull'analisi di 42 piastrelle (substrati artificiali) posizionate in una struttura sommersa lungo la Costa Dorada (Catalogna, Spagna) per un periodo di tre anni (2020–2023). La ricerca ha incluso diverse valutazioni, tra cui la biodiversità associata, lo stato di colonizzazione (inteso come le fasi di successione ecologica sulle piastrelle), una stima del carbonio immobilizzato (g $CaCO_3$ / m^{-2}) dagli organismi, analisi della complessità strutturale (intesa come nulla, bidimensionale e tridimensionale) e della relazione di questo fattore con il numero di decapodi. L'obiettivo principale è stato analizzare l'efficacia delle piastrelle come struttura unica in questi aspetti. Sono state utilizzate diverse metodologie, che spaziano dall'analisi visiva delle fotografie delle piastrelle per esaminare la biodiversità, a indagini in laboratorio per quantificare il carbonio immobilizzato. Tutti i dati risultanti sono stati sottoposti ad analisi statistica (RStudio) per confermare e validare le conclusioni riguardanti le piastrelle, tranne che per la valutazione sullo stato di colonizzazione che è avvenuta esclusivamente in modo visivo. I risultati hanno evidenziato un forte successo nella colonizzazione e nella promozione della biodiversità. Dopo tre anni di immersione, il numero totale di individui osservati è stato di 2.344, con una densità di 1.055 individui per metro quadrato, appartenenti a 41 taxa diversi (compresi tra generi e specie), con una predominanza in termini di numero di specie di Bryozoa e Chordata (Ascidiacea). L'indice di Shannon (*H'*), utilizzato per la valutazione della diversità, ha mostrato valori che hanno variato da un minimo di 0,84 *H'* a un massimo di 2,17 *H'*, con un *H'* totale considerando tutte le piastrelle insieme pari a 1,8. La copertura media delle piastrelle è stata dominata dai Polychaeta, che hanno coperto il 40% della superficie totale (2.22m^{-2}). Considerando il tempo di immersione di tre anni, le piastrelle sono state trovate in un buono stato di colonizzazione e in linea con il tempo trascorso in mare ma ancora in una fase di crescita senza raggiungere una fase stabile di "climax". Per quanto riguarda il carbonio immobilizzato, sottoforma di $CaCO_3$ (materia inorganica) è stato ottenuto un deposito di 67.56 - 940.62 g $CaCO_3/m^{-2}$, con una media di ~ 536.86g $CaCO_3/m^{-2}$ (sd ± 197.9), in 3 anni di immersione. In seguito ad opportuni calcoli effettuati è stato anche raggiunta la quantità di carbonio puro in $CaCO_3$ immobilizzato, ~ 112.56g C/m^{-2}. Le piastrelle hanno supportato un notevole numero di decapodi, 103ind/m^{-2} e l'analisi statistica ha confermato una correlazione positiva, seppur debole, con la complessità strutturale, suggerendo che una maggiore complessità favorisce la presenza di questi animali, in particolare nelle fasi giovanili

della loro vita offrendo maggior rifugio per questi organismi. In conclusione, i risultati indicano quindi che le piastrelle sono state un substrato efficace nel favorire la biodiversità, con un particolare impatto positivo sul supporto ai decapodi e sul fissaggio del carbonio. Questa tesi ha incluso anche attività esterne supplementari allo studio, riguardanti monitoraggi in immersione relativi a strutture artificiali per un progetto ancora in fase di studio, di proprietà di Underwater Gardens International (UGI), presso il Port Olímpic di Barcellona, in collaborazione con il team di biologi marini di UGI. Lo studio ha avuto un riscontro positivo considerando il tempo breve di impianto delle strutture, da aprile 2024, in cui è stato evidente come in sei mesi gli organismi abbiano colonizzato nei tempi giusti, coerenti con le fasi di successione ecologica i substrati artificiali.

ABSTRACT

This study focused on the analysis of 42 tiles (artificial substrates) distributed in a submerged structure along the Costa Dorada (Catalonia, Spain) over a three-year period (2020-2023). The research involved several assessments, including the evaluation of the associated biodiversity, the colonization status (understood as the colonization stages of the organisms on the tiles), an estimate of the carbon immobilized (g $CaCO_3$ m^{-2}) by the organisms that colonized the tiles and analyses of the structural complexity (zero, two-dimensional and three-dimensional) and its relationship with the number of decapods. The main objective was to analyze the effectiveness of the tiles as a unique structure in these aspects. Various methodologies were used, ranging from visual assessments of photographs of the tiles for biodiversity assessment to laboratory analyses to quantify the carbon sequestered. All the resulting data were subjected to statistical analysis (RStudio) to confirm and validate the conclusions related to the tiles. The results revealed a significant biodiversity, confirmed by the Shannon index, with a particular abundance of polychaetes (Annelida). The colonization status of organisms on the tiles was found to be aligned with the immersion period. Statistical analysis of the relationship between complexity and decapod abundance confirmed an increase in the number of decapods related to greater complexity. In conclusion, the various assessments demonstrated that the tiles were an efficient substrate, attracting a remarkable diversity of species and showing a strong potential for carbon sequestration. This study also included monitoring activities related to artificial structures for a project still under study owned by Underwater Gardens International (UGI), at the Port Olímpic in Barcelona, in collaboration with their team of marine biologists.

1. INTRODUCTION

1.1 Marine ecosystem regeneration

Marine regeneration is different from the concept of marine restoration. Regeneration of marine ecosystems is now one of the main missions of the EU Biodiversity Strategy 2030, which supports the Green Deal – the European agreement set to address the climate crisis by protecting and restoring natural ecosystems and related capital (EU, 2019). Restoration is derived from the Latin word “restaurare”, meaning “to repair, give back, or build up again”. This term is often used to describe actions that aim to bring something back to its original state or condition, often focuses on individual organisms or specific aspects of an ecosystem. Regeneration, on the other hand, comes from the latin word "generare," meaning "to give birth or generate." It typically refers to the process of renewal or growth, often involving the replacement of lost or damaged parts, can apply to entire ecosystems or even biological processes (Morseletto, 2020). Regeneration represents a form of upgrade from restoration and while both terms involve renewal or rebuilding, restoration implies a more deliberate and often human-driven process, while regeneration emphasizes natural processes of growth and renewal. The rapid deterioration of marine and coastal ecosystems, caused by the combined effects of various human activities, hinders the success of conservation efforts. To prevent further damage and promote ocean recovery, we need to enhance conservation planning, maintaining conservation plans. This includes improving the effectiveness of regeneration strategies, ensuring that sustainability goals are met, and strengthening marine spatial planning as a comprehensive tool for addressing climate change (Manea et al., 2023). Marine ecosystem regeneration and restoration are a relatively new fields, perceived by many to be expensive and prone to failure, in part explaining its low rates of implementation compared with terrestrial ecosystems (Saunders et al., 2020) but the loss of marine biodiversity is now widely described. Given the complexity of the marine environment, it is difficult to curb because the damage is sometimes irreversible, such as the artificialization of the coastline (Boissery et al., 2022). A solid understanding of the mechanisms that influence the functioning of key marine ecosystems and reliable restoration programmes is needed (Rossi & Rizzo, 2021), for this reason and more solutions are urgently needed and will require leadership, trans-disciplinary approaches, international frameworks and national roadmaps, political and financial commitments, and strong governance (Waltham et al., 2020). The host organization UGI, where I did the internship to my thesis and which will be discussed later, tries to implement habitat regeneration through sea garden, that are «Underwater Gardens» that respond in an intelligent and integrated way to the specific regeneration needs of a damaged marine ecosystem through Smart Enhanced Reefs (SER®), a new generation of intelligent artificial reefs that allow marine life to regenerate, increase local biodiversity and marine biomass, mitigate the environmental impact of human activity and the effect of climate change, create permanent carbon immobilization and offering unique diving experience.

1.2 Different basic material for artificial reef

Recovery degraded marine coastlines and enhancing natural habitats is crucial for the health of both marine and terrestrial life. Artificial reefs (ARs) offer a potential solution for revitalizing damaged ecosystems and can play a significant role in rehabilitating damaged marine areas, creating new habitats that benefit marine biodiversity (Alistair Becker et al., 2018,Hammond et al., 2020) and replicating the ability of natural reefs to support marine life (Paxton et al., 2020). ARs can also contribute to the social and economic prosperity of local fishing communities (Lima et al., 2020, Ramos et al., 2021), are used to stabilise marine coasts, protect the seabed from trawling methods (Cardenas-Rojas et al., 2021), and promote diving tourism (Hammond et al., 2020). ARs can be used as a tool for restoring marine ecosystems, trying to mimic natural hard substrates and creating underwater gardens covered with species typical of confined environments (Rossi & Rizzo, 2021,Giangrande et al., 2021) that's the main goals of Underwater Gardens International. This includes analysing the materials used in construction, their design, and the building process itself (Yoris-Nobile et al., 2023). Artificial reefs have been deployed in coastal waters around the globe for many years, using a variety of materials, including discarded vehicles, shipwrecks, tires, concrete blocks, and more recently, specifically designed structures that mimic natural environments (Cardenas-Rojas et al., 2021, Pickering et al., 1999,Vivier et al., 2021). Research suggests that concrete, due to its surface texture, can be a suitable material for artificial reefs and may enhance biological colonization (Paxton et al., 2020,Vivier et al., 2021). The design elements of artificial reefs, including their overall shape, the size and configuration of their holes, their surface texture, and the materials used, significantly influence the types of marine life that are attracted to and thrive in a particular area (Riera et al., 2018, Xu et al., 2019, MacArthur et al., 2020, Sempere-Valverde et al., 2018). Test results can help promote future research in this field and test different combinations and heterogeneities of substrates for more environmentally sustainable surfaces in coastal structure design (Sempere-Valverde et al., 2018) because it is assumed that the type of material will determine the development of the first steps of the benthic community (Burt et al., 2009). Identify suitable materials for artificial reefs is a key focus of marine restoration efforts. These materials should be able to provide nutrients while minimizing pollutants and the risk of disease in marine ecosystems. One of the most suitable materials for the construction of artificial barriers is optimized (pH lower then 9) concrete, due to its similarity to rock substrates, its easy commercial availability and knowledge of construction techniques, which can be mixed with other materials for different purposes. Biochar is an example of this material, with organic origin, has been shown to be a material with optimal stability in seawater, demonstrating greater ability to colonize marine organisms in less time (Bracho-Villavicencio et al., 2024) . Biochar has been used for soil remediation, carbon sequestration, composting of organic solid wastes, water decontamination, enhance surface area (Wang & Wang, 2019) and usually has a high resistance to biodegradation due to its highly condensed aromatic structure (Thomas A. J. Kuhlbusch, 1998). The study of this material has been done mainly for agricultural applications, as a fertilizer (Cui et al., 2022) but few studies have evaluated the performance of Biochar material as a component part of artificial reefs and despite its potential benefits, biochar has not been thoroughly evaluated for its ability to promote marine organism colonization, particularly with restoration purposes during the early stages of succession (Gann et al., 2019), and due to the interest in the velocity of the regeneration action on the ecosystem.

In this study, carried out during the internship period at the Underwater Gardens International (UGI), from beginning of June until the end of October 2024, for a total of 5 months. During this time the project of the Universitat Rovira i Virgili was followed (description below) and consisted of creating an artificial structure formed by different tiles, composed of a mixture of materials with a cement base and some of these with the addition of these introduced material Biochar, trying to understand the effectiveness of these structures, to promote colonization by organisms with fast regeneration times.

The Mediterranean Sea faces mounting anthropogenic pressures that threaten its distinctive biodiversity. To effectively manage and protect this marine environment, spatially detailed information about the ecological health of its ecosystems is crucial. Despite the rapid decline of Mediterranean biodiversity due to climate change and human activities, conservation efforts, whether focused on specific species or entire ecosystems, remain limited (Bevilacqua et al., 2020; Bianchi & Morri, 2000). The restoration, with the concept of regeneration, of Mediterranean marine ecosystem can represent a valuable solution and is recommended where the historical presence is recorded and the impacts that led to its loss are no longer acting (a reasoned recovery could be considered also where historical data are not available) (Mangialajo et al., 2013) and it's one of the main objectives from the host organization Underwater Gardens (UGI).

1.3 Early benthic colonization

Ecological succession is a fundamental process in marine ecosystems, involving significant modifications in community structure and function (Odum, 1993;Antoniadou et al., 2010) Besides environmental factors (e.g., temperature, salinity, light, depth), substratum and relative position to the seafloor also play an important role in the settlement, recruitment and growth of benthic organisms. The material used in construction of artificial reefs can strongly affect fouling assemblage development (Spagnolo et al., 2014). The entire process is slow, continuous and perceptible over a long-time scale, commonly interrupted by local natural disturbances of varying magnitude (Olabarria, 2002;Bulleri & Benedetti-Cecchi, 2006;Antoniadou et al., 2010). Sampling to monitor the first stages of benthic colonization are performed on a long-time scale because especially in the first months there is a low level of species composition. In particular, the first two months represent the pioneering phase and differ from the other sampling periods in having a low and limited number of species. The following 2 years will correspond to the period of dominance of molluscs and polychaetes (Ardizzone G. D. et al., 1989). Specifically, during these phases of primary biological or ecological succession, it is possible to initially observe the formation of a "primary film" or "biofilm", composed of debris and bacteria, is formed by a matrix of natural polymers secreted by these organisms, to which also adhere different microbes, fungi, algae and debris (Liu et al., 2017) (Lozano-Cortés & Zapata, 2014) and has a very rapid cycle. However, it cannot be considered a true colonization process, since it does not involve any multicellular organism, but it is an important and indispensable phase because the deposit of organic material, which influences the formation of this primary film. A second colonization phase follows during which the "microfouling" process begins, during which bacteria and debris, protozoa, diatoms, nematodes and microalgae accumulate together. In the study (Kim et al., 1987) reports that the pioneer algae in this phase are mainly formed by blue-green algae, diatoms, green filamentous algae and red coralline algae. Subsequently, in the third phase, colonization occurs by sessile biotas such as bryozoans (fast growing) and molluscs, polychaetes, sponges and ascidians (slow growing), a process called "macrofouling" (Hirata, 1987; Khalaman, 1989). These organisms settle in the available substrate and serve to increase the complexity of the habitat, providing shelter and nutrients to the fauna that subsequently colonize the structure. In the end, the communities of organisms present reach a phase called the "climax". During climax, interactions between species become more complex and stable, which facilitates the coexistence of different life forms in a balanced environment (Connell & Slatyer, 1977). It has been proven that up to the first 5 years of colonization, in artificial structures, a series of biotic changes are present over time without reaching a climax state that is not yet completely stable (Ardizzone G. D. et al., 1989;Antoniadou et al., 2011). Seasons influence the settlement of marine organisms and subsequent (Watson & Barnes, 2004; Cifuentes et al., 2010;Antoniadou et al., 2011). Therefore, the best time to install artificial structures is spring, since the conditions of photoperiod, temperature and availability of resources are higher. However, this effect seems to gradually decreases as succession proceeds (Pacheco et al., 2011).

1.4 Host organization

Underwater Gardens

Figure 1 - Underwater Gardens International (UGI) log. [1]

From June 1 to October 25, 2024, has been done an internship in preparation for this thesis at the host organization Underwater Gardens International (UGI) (*Figure 1*) based in Barcelona, Spain. UGI was founded by Marc García-Durán Huet in 2016, is a company formed by a multidisciplinary network of professionals and experts with broad international recognition that integrates both a scientific and business perspective (including scientists, marine biologists, architects, and many others). Their project participation and solutions aim to regenerate marine life enable increased biodiversity with used of new basic material, combat climate change,

[1] Underwater Gardens Internation logo (UGI). From the website www.underwatergardens.com

mitigate the environmental impact of human activities, create carbon sinks, promoting "Blue Growth" and "Circular Economy". All this is done through the implementation of regeneration strategies, a different concept from just restoration activities, a topic that will be explored later. Regeneration refers to restarting the functionality of habitats and the interconnections between them. When carrying out restoration activities we often focus on a single habitat, starting with a single species. UGI has internally developed its own technology and software called reefhopper®. A software to provide solutions to highly complex problems by creating smart enhanced reefs (SER®) (Underwater Gardens) that enhance and accelerate the regeneration of marine life. UGI also provides innovative socio-economic models to finance the maintenance of marine ecosystems so that they thrive in a healthy, resilient and fast-growing environment, and provides comprehensive control and monitoring for each solution thanks to digital monitoring systems, underwater gardening programs and general public involvement (citizen science), ensure the success of each marine regeneration action. During the internship period at the UGI office, there was the opportunity to participate and be involved in their activities as an active part of the team. UGI participates and supports many marine restoration and regeneration projects, one of which was the subject of this thesis and is explained in the following chapter.

1.6 Project proposed: Finding materials for marine benthonic colonization

UNIVERSITAT ROVIRA i VIRGILI

Figure 2 - Universitat Rovira i Virgili (URV) log. [2]

The Universitat Rovira i Virgili (URV) (*Figure 2*), in February 2019 submitted a call for funding from the European Maritime Affairs and Fisheries Fund (EMAFF) (under reference BDNS 429552) under priority A3, operations that contribute to better management conservation of biological resources. The application for the above European funds was approved on July 16, 2019. In general terms, the project proposed by URV, with the collaboration of external companies and public administrations, aims to characterize the conservation status of marine habitat in a short coastal area of Costa Dorada (including along the Catalan coast). The samples proposed for the study are tiles composed of cement-based mortar, a material that makes it

[2] Universitat Rovira i Virgili (URV) logo. From the web site https://www.urv.cat/en/

possible to adequately reproduce the physicochemical properties of concrete and, at the same time, to make small tiles that are not possible with concrete alone, due to the composition of the mix that disintegrates. Were immersed 42 tiles, 12 of Control (C) (cement only) and 30 composed of cement with addition of Biochar (B). Each tiles have a size of 230x230 mm, a thickness of 30 mm and a weight of 3.65 kg (*Figure 3*).

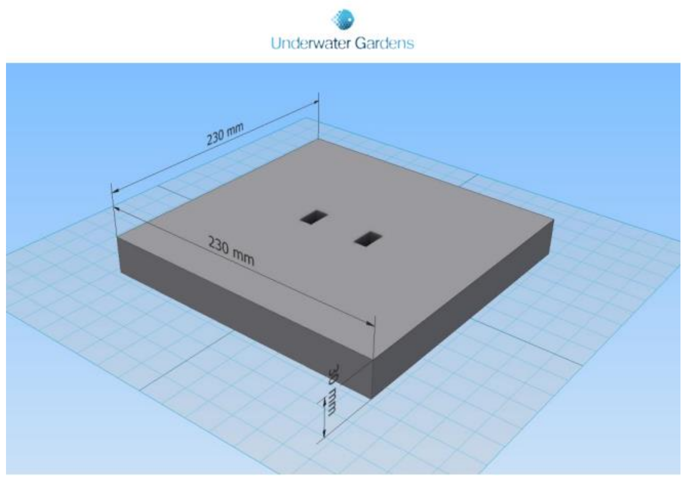

Figure 3 - Descriptive image of the shape and size of one of the tiles. [3]

The installation of the tiles in the marine environment was carried out with a light and removable structure that allowed the positioning of the tiles in equal conditions and without altering the current marine environment. The installation of the proposed structure was temporary and determined by the duration of the study, 3 years. Once the field phase was completed, the complete dismantling of the structure was carried out. A flat structure was proposed consisting of a frame and a metal mesh that generates the necessary support and support for the tiles. This metal frame, to avoid the interference of the tiles with the seabed and at the same time to remain fixed, was fixed to concrete modules, each of these having an approximate mass of 100 kg. The frame could accommodate a total of 7 groups of 6 tiles (42 tiles) as in the following figure (*Figure 4*).

[3] Descriptive image of the shape and size of one of the tiles. From Underwater Gardens International document "Descripicó tècnica - Proves materials FEMP"

1.1	2.1	3.1	4.1	5.1	6.1	7.1
1.2	2.2	3.2	4.2	5.2	6.2	7.2
1.3	2.3	3.3	4.3	5.3	6.3	7.3
1.4	2.4	3.4	4.4	5.4	6.4	7.4
1.5	2.5	3.5	4.5	5.5	6.5	7.5
1.6	2.6	3.6	4.6	5.6	6.6	7.6

Figure 4 – Personal elaboration. Representation of the distribution of tiles in the structural grid under water. [4]

After the tile's assembling the complete structure was placed at a depth of between -20m and -25m depth, horizontally, on sandy bottom with no signs of existence of rhizomes and devoid of seagrass (*Figure 5*).

Figure 5 – Complete tile structure and positioned under water [5]

The structure remained on site for 3 years to give the organisms enough time to attach and have significant data for the studies. In October 2023, the entire structure was dismantled, taking special care of the colonized tiles and taking a photo of the colonized part of each tile. The tiles had two substrates available for colonization but only the lower part had a high level of colonization compared to the upper part which did not (*Figure6*). This result was attributed to a high level of sedimentation which did not allow the upper part of the tiles to be colonized easily.

[4] Descriptive image of the shape and size of one of the tiles. From Underwater Gardens International document "Descripicó tècnica - Proves materials FEMP"
[5] Complete tiles structure and position under water. From Underwater Gardens International website www.underwatergardens.com

TOP
(poor colonization)

BOTTOM
(high colonization)

Figure 6 - This image represent the two sides of the tiles. Upper part (TOP), lower part (BOTTOM) [6]

[6] This image represent the two sides of the tiles. Upper part (TOP), lower part (BOTTOM). From Underwater Gardens International document “ANOVA experiment summary”

2. AIMS OF THE STUDY

The final goal of this study is to verify whether, after three years of immersion, these tiles, designed as a complete artificial structure, have generated a microhabitat with high biodiversity, showing a good level of colonization. Additionally, we aim to understand if, through this process, a significant amount of carbon has been stored over time. To achieve this, the first step was to evaluate the biodiversity present in the tiles and as a whole structure and whether the average % coverage varied between different phylum in the tiles. With the scope also to understand whether one phylum prevailed over the others, particularly the polychaetes, to confirm the hypothesis in their role as providers of natural habitat for subsequent colonizations and main carbon sinks organisms.

Another step was to make a primary observation of the photos to have a brief description of the state of colonization in the tiles and provide an effectiveness of the structure as a whole (Watson & Barnes, 2004). Succession is the process that develops from the beginning of a time sequence until a state of minimal change in species composition is reached, called a series. Each set of species that can be distinguished along the series is called a serial stage.

The third step was to evaluate the effectiveness of the structure in immobilizing carbon ($gCaCO_3m^{-2}$) by calculating it starting from the biomass and obtaining the ash weight following the methodology of (Chiantore et al., 2003; Steven Nelson Murray et al., 2002), considering the immersion time of 3 years. And then also to consider if there could be relationships between the biomass (dry weight) and the Shannon index (*H*'). It was chosen to consider this because there is considerable societal benefit from benthic carbon storage and immobilization through its mitigation value buffering climate change. This service is often termed "blue carbon" and is that retained within marine ecosystems (mainly coastal) (Barnes et al., 2019) since it's a highly efficient mechanism, present in nature and manage (Duarte et al., 2005), the scientific profile of blue carbon measurement has increased rapidly.

In the end, a further study was carried out to analyze the complexity through the evaluation of the relationship between the structural complexity and the number of crustaceans (decapods), following the hypothesis that complexity determines the presence of a greater number of decapods (Kramer et al., 2017; Pallas et al., 2006).

3. MATERIALS AND METHOD

3.1 Study area

The site where the structure was positioned in the littoral along the Catalan coast, Spain (*Figure7*).

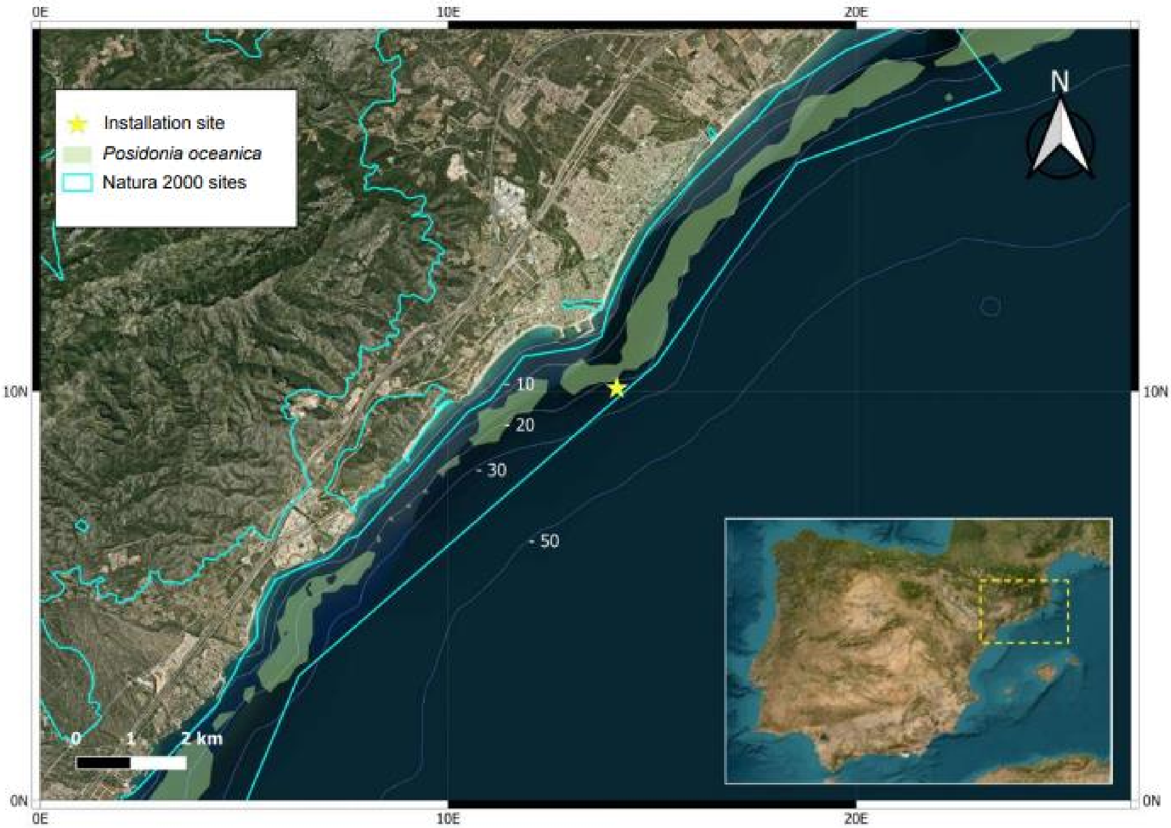

Figure 7 - Personal elaboration. Installation site. This map in the figure represents the installation site of the tile structure under water, along littoral zone of Catalan coast. [7]

The Catalan coast, located in northeastern Spain, is a coastal stretch bathed by the Mediterranean Sea, which extends for approximately 580 kilometres starting in the north with Cabo de Creus (a promontory that marks the border with France) and ending in the south with the delta of the Ebro River. The coast is characterized by a great variety of landscapes, ecosystems and

---

[7] Installation site. Created by the author using QGIS. ETRS89 UTM 31N coordinate system. Data: Coordinates x, y of points for the installation site from UGI document: " Descripicó tècnica - Proves materials FEMP". *Posidonia oceanica* data: UNEP-WCMC, Short FT (2021). Global distribution of seagrasses (version 7.1). Seventh update to the data layer used in Green and Short (2003). Cambridge (UK): UN Environment World Conservation Monitoring Centre. Data DOI: https://doi.org/10.34892/x6r3-d211. Natura 2000 sites data: public database, download on the EMODnet web portal (Human Activities, https://emodnet.ec.europa.eu/en/human-activities). Bathymetric data: from https://agricultura.gencat.cat/ca/serveis/cartografia-sig/bases-cartografiques/pesca-maritima-proteccio-litoral/batimetria/index.html

biodiversity. The northern part called Costa Brava, is characterized by rugged cliffs and less sandy seabed than the central part of the coast, Costa Dorada, and ends with the Costa del Embro. The waters along the entire coast are characterized by a rich variety of marine species, the meadows of *Posidonia oceanica* are ecosystems of great ecological value, which host a wide range of organisms. The installation site fall in an area of Natura 2000 network, established in 2006. This area is Protected under the Habitats Directive, Special Area of Conservation (SIC Habitats Directive) with a marine area of 4593.91 ha. In the area around the installation site, there is *Posidonia oceanica,* a type of Habitat of Community Interest (Annex I Directive 92/43/EEC, code 1120), which covers the largest number of ha (1908.81) (EEA)[8]. The substrate in the coast in the interested area is mainly composed of fine sand, although there are phanerogam meadows, but not at the point of installation of the structure
(-20m/-25m).

3.2 Tile's study methods

Biodiversity assessments were performed at the UGI headquarters from photographic samples. The photos were taken the day the tiles were removed from the water, with the digital camera Olympus E-M5 Mark III. Following that, visual observations of photographic records can be used in the laboratory to list species, count individuals, or visually estimate percent cover (Murray S. et al., 2002) and also photo analysis is the easiest means of obtaining a great deal of data and allows to consult references and colleagues if identifying organisms proves difficult (Lessios H.A., 1996). A list of species was then created to quantify the diversity of the community created over time of 3 years. The upper part of the tiles was not considered because it was not colonized. Based on Linnean model of taxonomic classification of (Verma & Prakash, 2020), the benthic organisms present and recognized were classified into their respective Phylum, reported with a name code, the name of the genus of each organism, the species that were recognized with certainty and the respective quantities as number of individuals. The identification of some organisms at genus and species level was possible thanks to the use of guide's books and specialized websites of the marine environment along the Spanish coast [9] [10]

[8] EEA European Environment Agency. Information regarding the Natura 2000 zone specific to the study area. https://eunis.eea.europa.eu/sites/ES5140001
[9] Guia Participativa Marina del Barcelonès (zenodo.org). Marcombo (Salvador et al., 2021)
[10] Club d'Immersió Biologia: Guia d'espècies. https://www.cibsub.cat/guia.php

[11] [12] [13]. About the number of individuals, has been done for each Phylum but for polychaetes the counting proved more difficult due to the structural compactness of these in the tiles. Therefore, the estimate of the numerical quantity of polychaetes (Fam. Serpulidae) was initially based on the study of (Sandonnini et al., 2021) where an estimate of the number of individuals was reached through a proportion considering total sampled area, conversion of the average total percentage coverage and average body length of the polychaetes. Following this formula and considering that, the area per m^{-2} covered by the tiles in total, without considering spaces between them in the grid, was ~2 m^{-2} (2.22 m^{-2}), that the average total coverage of the polychaetes was 40% (converted 0.4%) and that the average length of the polychaetes examined and reached is 1cm, also taking into account the study on the average body lengths of (Çinar, 2006), the result of 55,545 individuals for the study area was obtained. Currently the study of (Sandonnini et al., 2021) confirms this result by stating that an average between 24 thousand and 84 thousand/m^{-2} have been calculated, so the results achieved in this study are more than truthful. But in the same study the calculation is done considering a three-dimensional surface, completely covered by polychaetes, without overlying colonization stages, instead in this study in the first place only one face of the tiles was analysed, the part facing the seabed (BOTTOM) because the upper face did not show any colonization, for this reason the result could represent an overestimate. Then the calculation continued going to reach an estimate of individuals per tile including in the calculations the total number of serpulids (55,545). First by calculating the area of serpulids covered in each tile, using the available data: area of the tiles (529 cm^{-2}) multiplied by the percentage coverage of serpulids of each of them. Subsequently, the areas were added together to reach the average density by dividing the sum of the areas (8993 cm^{-2}) / total number of individuals (55,545). Finally, the density obtained (0.15) was multiplied again by the area covered by serpulids on each tile, thus obtaining a more realistic and precise estimate, for this case study, of the number of serpulids individuals present on each tile, for a total of 1435ind/0,89m^{-2}. This quantity of individuals is possible and present in the study of (Lozano-Cortés & Zapata, 2014) where artificial tiles are also analysed.

Percentage coverage is the percentage of the surface of the sample unit covered by bodies of the organisms (Steven Nelson Murray et al., 2002), in this case we estimate it at the phylum level,

[11] La vida marina del mar Mediterráneo. Brau Edicions S L (Ballesteros E., 2015)
[12] Guía visual de Fauna y Flora del Mediterráneo. Anthias Editorial (Llamas A., 2015)
[13] iNaturalist online platform. https://www.inaturalist.org

and it was evaluated through computer analysis using a square placed over the photo of each tile, with a grid of 100 squares inside and choosing 20 of these, previously selected randomly (*Figure8*). The percentage cover values of each organism were provided considering the percentage present of each organism in each of the 20 squares. Considering that a common difficulty in estimating the cover of intertidal organisms is the stratification or multiple occupation of the space directly above the primary substrate (Murray S. et al., 2002), we considered the organism at the last stage of colonization as the only cover. However, it has also been considered that visual methods can be subjective, and it has been shown that observer bias can influence the accuracy of the data (Bullimore B. & Hiscock K., 2001).

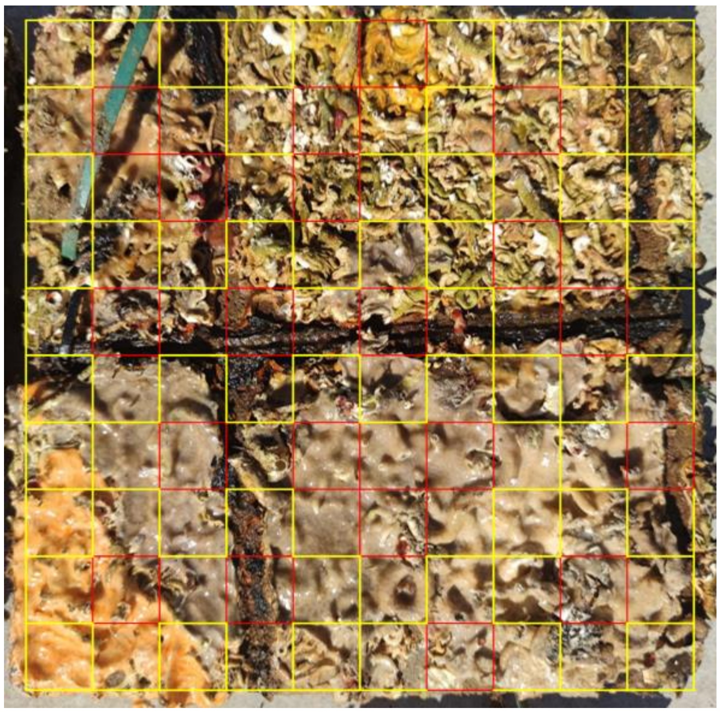

Figure 8 – Personal elaboration. Ex. of Tile 4.4 with the grid of 100 squares and the 20 random squares shown in red [14]

For the evaluation of the colonization status a general assessment was made following the basis of information shared with UGI team[15].

For the carbon immobilized (inorganic matter) in the structures of the organisms, it was decided to proceed with the use of the methodology reported by (Chiantore et al., 2003; Steven Nelson

[14] UGI Photo took by Clara Garcia Duran (Ottobre 2023). Personal elaboration grid added by author using PowerPoint 2016. Ex. of Tile 4.4 with the grid of 100 squares and the 20 random squares shown in red

[15] Information shared with UGI team from document “Sucesión ecológica en comunidades bentónicas”

Murray et al., 2002), through the calculation of the biomass. Initially, a 10cm x 10cm superficial portion of each tile (for a total of 42 samples) was removed by scraping (*Figure* 9).

Figure 9 – Personal elaboration. Ex. photo of tile 4.1 showing the removal of the surface portion through scraping (10cm x 10cm) [16]

In the laboratory (at the Università Politecnica delle Marche), the dry weight (biomass) was determined directly, placing the fabrics in the heater at 60°C for 24 hours, depending on the quantity of material and the water content of the fabrics, until a constant weight was reached. So, to obtain the inorganic component, the operation was carried out by placing the samples in a muffle at 500-550 °C for 4 hours. What remains is made up of the so-called ash: the weight of the inorganic part of the samples which is the fundamental part that includes the stored carbon, which is no longer lost over time (Chiantore et al., 2003). To obtain the accurate quantity of pure immobilized carbon from this data, it was done by calculating the atomic mass of $CaCO_3$, considering the mass of carbon (C) = 12, the mass of calcium (Ca) = 20 and the mass of oxygen (O) = 24 (8x3), resulting in 56. We then continued by calculating the percentage of carbon actually present in the $CaCO_3$ through a proportion = total mass / mass of C×100 and multiplying the result by the average of ash weight. The ash weight, was also subtracted from the dry weight, giving the organic matter (Chiantore et al., 2003), which in this case includes the organic carbon

[16] Personal photo during tile's analysis (09/10/2024). Ex. photo of tile 4.1 showing the removal of the surface portion through scraping (10cm x 10cm)

component that is lost over time, which falls within the carbon cycle (respiration, replacement with other organisms in ecological succession).

To evaluate the relationship between the number of crustaceans (decapods) and structural complexity, the methodology of (Ory et al., 2012) was followed and adapted to our case. Both data, crustacean count and structural complexity evaluation, were taken from the photo-square analysis. Were considered only the crustaceans present in the 20 random little squares. For the structural complexity, substrate composition by organisms with the greatest coverage in each little squares were used as an independent variable in the analysis of decapod presence and abundance and then was visually assessed using 3 categories of complexity. The score 0 correspond to a small square composed mostly of a flat substrate, so null complexity, the score 1 corresponds to a two-dimensional complexity where the small square was composed for the greatest coverage by organisms that corresponded to this property: encrusting bryozoans and sponges while the third and final score 2 corresponds to a more complex three-dimensional complexity composed of polychaetes, that can provide small spaces and shelters to the juvenile decapods identified in the tiles (Ory et al., 2012), molluscs, calcareous filamentous algae (*Liagora* sp.) and erect bryozoans and colony ascidians.

3.3 Statistical analysis

Statistical analyses were conducted using RStudio version 4.4.2 to test various relationships between the data. First, the relationship between percentage cover and the different Phyla present on the plates was examined. Finally, the relationship between structural complexity (null, two-dimensional, three-dimensional) and the number of decapods was explored. For each relationship, a normality test was initially performed using the Shapiro-Wilk test to check for normal or non-normal distribution of the data. Subsequent analyses proceeded with the use of the non-parametric Kruskal-Wallis test, as the data did not meet the assumptions for ANOVA. In one case, Levene's test was also applied to check for homogeneity of variance between groups. In the case of the relationship between percentage cover and Phyla, a post-hoc Pairwise test was chosen, using the Dunn test with Bonferroni correction method, to specifically identify where the differences between groups occurred. In the evaluation of the relationship between biomass and Shannon index (H') both Pearson's test and the linear regression model were performed. In the analyses of the relationship between structural complexity and decapod number, Kendall's Tau correlation was added to measure the strength and direction of the relationship with the tau value (-1 - negative correlation, +1 positive correlation, 0 no correlation) and a linear regression-correlation model was performed.

4. RESULTS

4.1 Biodiversity

After three years the 42 tiles were characterized by the presence of a high quantity of organisms which included 2.344 individuals (1.055ind/m^{-2}). A total of 41 sessile and mobile taxa (genera and species) belonging to 11 main phyla were identified (*Table1*). In term of number of genera, Bryozoa were the most abundant one (9 taxa), followed by Chordata (Ascidiacea) (7 taxa) and then other taxa: 4 Mollusca (Bivalvia), 4 Porifera, 3 Rhodophyte, 3 Arthropoda (Decapods), 2 Cnidaria, 2 Mollusca (Gastropoda), 1 Heterokontophyta and 1 Echinodermata.

Code	Phylum (Class)	Taxa in the tiles	n. Ind
(Rho)	**Rhodophyta (Florideophyceae)**	*Hildebrandia* sp.	1
		Liagora sp.	19
		Peyssonelia sp.	3
(Het)	**Heterokonta (Phaeophyceae)**	*Padina pavonica* (Linnaeus, 1960)	2
(Por)	**Porifera (Demospongiae)**	*Chondrosia reniformis* (Nardo, 1847)	12
		Crambe crambe (Schmidt, 1862)	2
		Petrosia ficiformis (Poiret, 1789)	3
		Chondrilla nucula (Schmidt, 1862)	2
(Cni)	**Cnidaria (Anthozoa)**	*Polycyanthus muellerae* (Abel, 1959)	26
		Actinia equina (Linnaeus, 1758)	1
(Ane)	**Anellida (Polychaeta)**	*Janua* sp. *Serpula* sp. *Spirobranchus* sp. *Vermiliopsis* sp.	1.435
(MolG)	**Mollusca (Gastropoda)**	*Bittium* sp.	6
		Hexaplex sp.	2
(MolB)	**Mollusca (Bivalvia)**	*Anomia ephippium* (Linnaeus, 1758)	77
		Crassostrea gigas (Thunberg, 1793)	6
		Ostrea edulis (Linnaeus, 1758)	60
		Rocellaria dubia (Pennant, 1777)	107
(Art)	**Arthropoda (Malacostraca)**	*Parapenaeus* sp.	102
		Phasifaea sp.	81

		Not Identified Brachyura genus	45
(Bry)	**Bryozoa (Gymnolaemata)**	*Beania* sp.	2
		Cellepora pumicosa (Pallas, 1766)	38
		Patinella sp.	70
		Reteporella mediterranea (Hass, 1948)	12
		Rosselliana sp.	42
		Schizobrachiella sanguinea (Norman, 1868)	40
		Schizomavella sp.	3
		Scrupocellaria delilii (Audouin, 1826)	4
		Turbicellepora sp.	15
(Ech)	**Echinodermata (Ophyuroidae)**	*Ophiothrix* sp.	3
(Cho)	**Chordata (Ascidiacea)**	*Aplidium* sp.	24
		Cystodyte sp.	3
		Didemnum fulgens (Milne Edwards, 1841)	5
		Didemnum maculosum (Milne Edwards, 1841)	15
		Diplosoma sp.	16
		Pseudodistoma obscurum (Pérès, 1959)	41
		Not Identified colony ascidia genus 1	19

Table 1 – Personal elaboration. Table representing the list of identified organisms with their quantities and the identification code per phylum.

The identified organisms were reported in the list above, with relative quantity and identification code for each Phylum. Identification was performed down to genus level, species where possible, and organisms with low certainty were labelled Not Identified.

Sessile benthic organisms dominated in terms of covered surface with an average of 78% coverage. Specifically, the Polychaeta were more dominant with an average of 40% coverage with Serpulidae being the most abundant family, in line with the colonization status at which the tiles were analysed (Ardizzone G. D. et al., 1989) (*Figure10*).

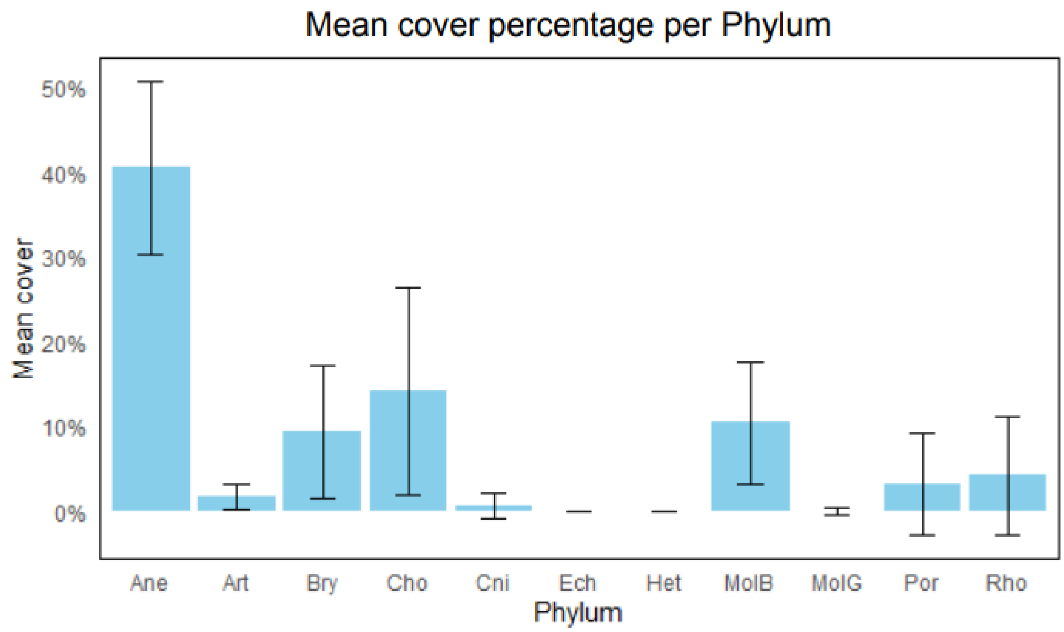

Figure 10 - Personal elaboration. Histogram graphic representation of the mean percentage coverage for each Phylum with the standard deviations. (Ane) Anellida,(Art) Arthropoda, (Bry) Bryozoa, (Cho) Chordata, (Cni) Cnidaria, (Ech) Echinodermata, (Het) Heterokonta, (MolB) Mollusca Bivalvia, (MolG) Mollusca Gasteropoda, (Por) Porifera, (Rho) Rhodopytha.

Followed by Chordata with mean 14%, Mollusca (Bivalvia) with mean 10% and Bryozoa with mean 9% with encrusting species in the majority. Porifera characterized only 3% of coverage being present only in the initial development stage above the serpulids with particular abundance of *Chondrosia reniformis* (Nardo, 1847). A small percentage of Cnidaria coverage of 1% was also identified, with the largest number of individuals covered by the single-bodied coral species *Polycyanthus muellerae* (Abel, 1959) (*Figure11*), with identification and their possible presence also confirmed by studies in literature (Gili J.M. et al., 1982; Corriero et al., 2019).

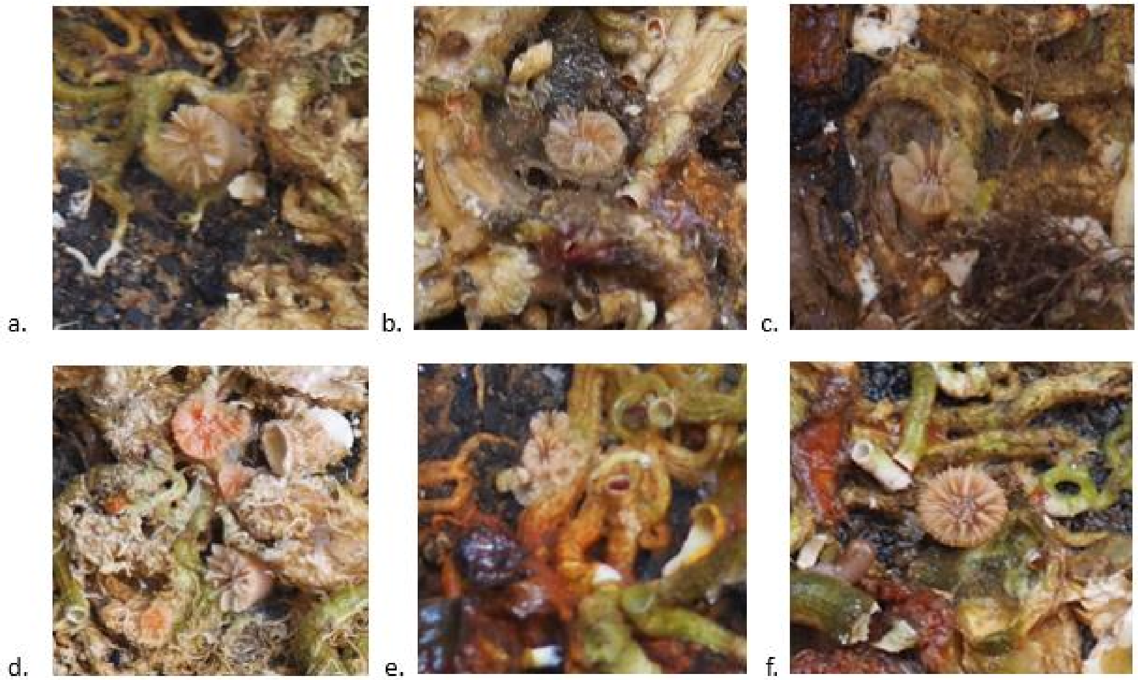

Figure 11 - Personal elaboration. Examples of cnidarians Polycyanthus muellerae (Abel, 1959) present in the tiles. a. Tile 2.1; b. Tile 2.2; c. Tile 2.3; d. Tile 3.1; e. Tile 6.2; f. Tile 7.6.

They were counted and identified in 13 tiles for a total of 27 individuals, including one *Actinia equina* (Linnaeus, 1758) (Ros J. et al., 1982). Algae contributed only for 4%, with a particular presence of calcified algae of the genus *Liagora* sp and in sporadic cases also the encrusting algae of the genus *Peyssonelia* sp.

For other benthic invertebrates, a particular abundance in number of Crustaceans, represented by 3 groups, was found, exclusively decapods such as shrimps and crabs at the juvenile life stage, *Parapenaeus* sp. and *Phasifaea* sp. Overall a total of 228 decapods were found, all tiles have at least 1 decapod, with a minimum of 1 and a maximum of 25 in the same tile (tile 6.6). A low quantity of Molluscs (Gasteropods) was also identified in 4 tiles (3.3, 3.5, 7.3, 7.4) and in addition Echinodermata (Ophyuroidae) were found in 3 tiles (1.6, 2.5, 3.4) (*Figure 12*).

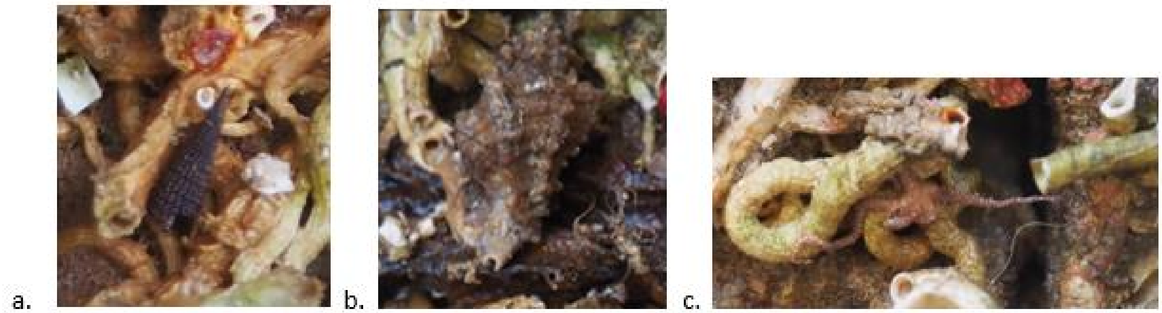

Figure 12 - Personal processing. Examples of Mollusca (Gastropoda) and Echinodermata Ophiothrix sp. of some tiles. to. Tile 3.3; b. Tile 7.4; c. Tile 2.5

Additionally, during the calculation of the percentage coverage, the substrate parts of the tiles covered with Microfouling with an average of 7% were also considered. As it is a colonization by unicellular autotrophic and heterotrophic eukaryotes, as a first phase of colonization and in the long term until the stabilization of the organisms (climax), the process of microfouling begins, which includes, in addition to bacteria and debris, also protozoa, diatoms, nematodes and microalgae. It has been observed by (Kim et al., 1987) that the pioneer organisms involved in these phases are the first to adhere to the artificial surfaces.

The following graph shows the total coverage colonized by organisms (Colonized), percentage of uncolonized substrate (Empty) and percentage of broken tiles (Broken) (*Figure 13*).

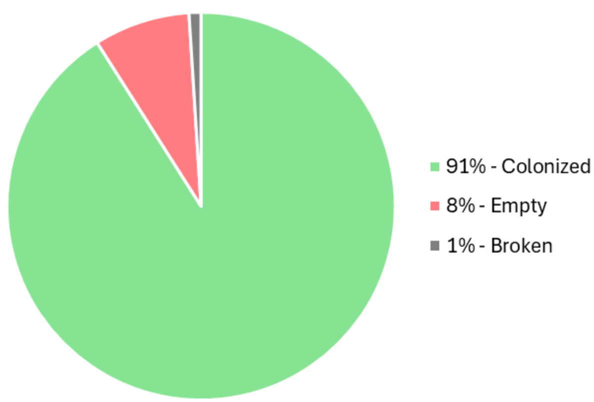

Figure 13 - Personal elaboration. Pie chart representing the percentage of total colonization, the percentage of empty substrate without organisms (Empty) and the percentage of broken tiles (Broken).

To evaluate the diversity for each tile and overall in total, the Shannon diversity index (H') was calculated. The results oh Shannon index (*H'*) show a variability of the index from a minimum value of 0.84 *H'* to a maximum value of 2.17 *H'* (*Figure14*), with a total Shannon index considering all the tiles together of 1.8.

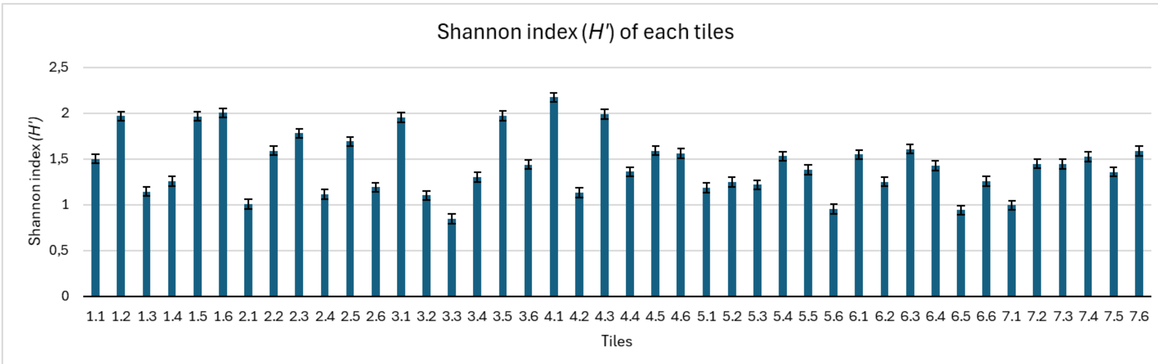

Figure 14 - Personal elaboration. Graphic representation of the Shannon index (H') of each tiles.

The analysis of percentage cover data for each phylum was performed using various statistical tests. The Shapiro-Wilk test with statistic $W = 0.66$ and p-value = 2.2e-16 ($p < 0.05$) confirmed that the data do not follow a normal distribution, so the null hypothesis (normal distribution of the data) was rejected. The result of Levene's test showed an F statistic of 30.74 with a p-value < 2.2e-16, indicating that the variances between the groups are not homogeneous ($p < 0.05$). This suggests that at least one of the groups has a variance significantly different from the others. The Kruskal-Wallis test was performed to compare the rank means between the groups. The result yielded a statistic with a value H (Chi-squared χ^2) = 282.53, with 10 degrees of freedom (df), and a p-value < 2.2e-16, indicating that there are statistically significant differences between at least two of the groups ($p < 0.05$). Therefore, the null hypothesis of equal ranks between the groups is rejected. The results of the Dunn post-hoc Pairwise comparisons with Bonferroni correction revealed that the phylum Ane is significantly more abundant compared to other phyla, as confirmed by the comparisons between Ane and the other groups. Specifically, the differences between Ane and other groups were statistically significant, with very low corrected p-values (all below 0.05), indicating that the superiority of Ane in terms of percentage coverage is not due to chance. These results support the hypothesis that Ane represents the most abundant and prominent group among those analysed, highlighting a clear difference in its distribution compared to the other phyla.

4.2 Colonization status

In the tiles of the present study several benthic species were identified that are characteristic of the third colonization phase, such as several species of sponges, ascidians, cnidarians and bryozoans (*Figure 15*).

Figure 15 - Photo from UGI. Examples of well-colonized tiles. a. Tile 1.4; b. Tile 2.3; c. Tile 5.4; d. Tile 7.1

In some cases, the tiles were characterized by the presence of microfouling visible through various colorations of the substrate from yellow to dark colours to black (Remple et al., 2021), and it is known how these organic formations contribute to the attachment of new larvae and organisms, which leads to a structural complexity that favours refuge areas for other mobile benthic species (Schembri & Zammit, 2022). This good condition, of the substrate and the presents of microorganisms, such as microfouling, is also confirmed by the presence of single-bodied cnidarians which, although still small in size, demonstrate that the larvae have found a safe habitat with the necessary components during the settlement phase, fundamental and necessary elements during this phase of the life cycle (Brandl et al., 2014). Furthermore, a high number of crustaceans (decapods) were identified in the tiles, which further confirms not a complete, but a sufficient structural complexity that may allow small prey, such as decapods present at a juvenile stage, to maintain a higher abundance reducing the intensity of predation (Denno et al., 2005) because detection is more difficult and many shelters of different sizes are available (Canion & Heck, 2009). Another factor that was taken into consideration is that the tiles were taken from the water in October (year 2023), because as previously mentioned, seasonality is a factor to consider since, during a spring/summer season there are favourable conditions of temperature, photoperiod and nutrients that coincide with the reproductive period and therefore the habitats and new substrates can be colonized and enriched with new species. Instead, during the winter seasons they are characterized by periods of growth in terms of space and individual, the organisms grow, there is no new recruitment.

4.3 Carbon immobilization

After appropriate analyses, it was found the inorganic matter immobilized (ash weight) by the organisms for each tiles, with a deposit of 67.56 – 940.62 g $CaCO_3$ /m^{-2} in 3 years of immersion, with an average of ~ 536.86g $CaCO_3$/m^{-2} (sd ± 197.9). Following the formulas explained previously, from which a percentage of carbon of ~ 21% (0.21) was obtained within the $CaCO_3$ and multiplying it by the average of ash weight, from this, the pure C actually immobilized by the tiles in 3y is ~ 112.56g C/m^{-2}. The analyses continued with a relationship to see if species diversity (Shannon index) affects biomass. The results of the Shapiro-Wilk test for the biomass variable showed that there was not enough evidence to reject the null hypothesis that the data are normally distributed (p-value biomass $0.07 > 0.05$) while maintaining an uncertainty in the distribution, with a p-value slightly above 0.05. Regarding the Shannon index (H') result, the test confirmed a normal distribution of the data with a p-value $0.17 > 0.05$. A Pearson correlation test was performed to assess the relationship between species diversity, measured by the Shannon index, and biomass. The results showed a correlation coefficient of $r = -0.0093$, with a p-value = 0.95 (p value > 0.05) indicating that there is no significant linear correlation between the two variables. The 95% confidence interval for the correlation coefficient includes zero (-0.3124, 0.2954), further supporting the hypothesis that there is no significant relationship. Therefore, these results suggest that species diversity does not have a significant linear impact on biomass in the analysed sample. Then through a scatter plot (*Figure16*) was obtained the visualization of the relationship.

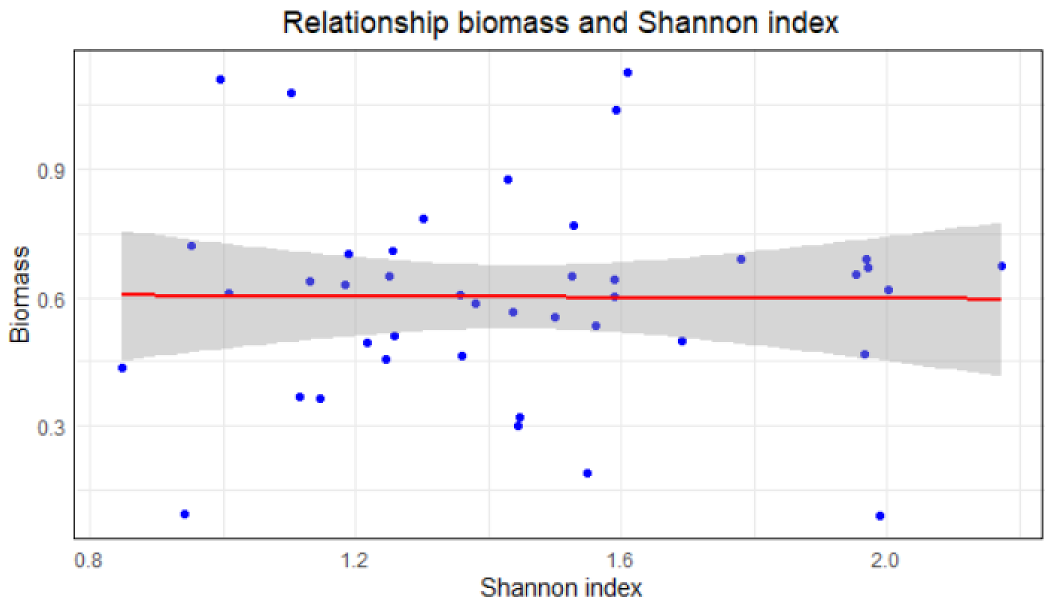

Figure 16 - Personal elaboration. Relationship between biomass and Shannon index (H').

The linear regression model revealed a significant intercept with a p-value of 0.000632, suggesting that when species diversity (Shannon index - *H'*) is zero the biomass is expected to be 0.610 (SE = 0.165). However, the coefficient for *H'* of -0.006564 has a p-value of 0.9533, which is significantly greater than 0.05, indicating that species diversity does not have a significant effect on biomass. The t-value for *H'* is very close to zero, confirming that the effect of index on biomass is negligible. Additionally, the R^2 value of 8.687e-05 suggests that the model explains an extremely small amount of the variability in biomass. The F-statistic value of 0.003475 and the associated p-value of 0.9533 confirm that the model, as a whole, is not statistically significant. It is not possible to conclude that species diversity affects biomass in our sample.

4.4 Relationship between structural complexity and number of decapods

The Shapiro-Wilk test (p-value of $2.2\text{x}10^{-16} < 0.05$) demonstrated that the data do not follow a normal distribution, so non-parametric tests were performed. In an initial analysis, the Kendall's

Tau test provided a p-value of 0.4 > 0.05, indicating the absence of a correlation between the two variables and an inaccurate p-value due to a high number of identical data points. Through data visualization, neither the scatter plot nor the ggplot provided a satisfactory resolution or trend. A different approach was then attempted. The mean complexity for each tile, referred to as "accurate complexity," was calculated and compared with the number of decapods per tile. Another Kendall's Tau test was performed with these data, and in this case, the p-value of 0.02 < 0.05 allowed the rejection of the null hypothesis, confirming a significant correlation between accurate complexity and the number of decapods. The tau value of 0.266 indicated a weak positive correlation, suggesting a tendency for the number of decapods to increase with complexity, although the relationship is weak, it is still present. Data visualization with a scatter plot (ggplot) provided a satisfactory resolution *(Figure17*). The linear regression model showed that the coefficient of accurate complexity is positive (3.069), suggesting that for every 1-unit increase in average complexity, the number of decapods will increase by an average of 3 units. The p-value of 0.00458 < 0.05 associated with this variable indicates that there is a relationship between complexity and the number of decapods. However, the strength of the relationship is not particularly high ($R^2 = 0.09$), suggesting that other factors may have a stronger influence on the number of decapods.

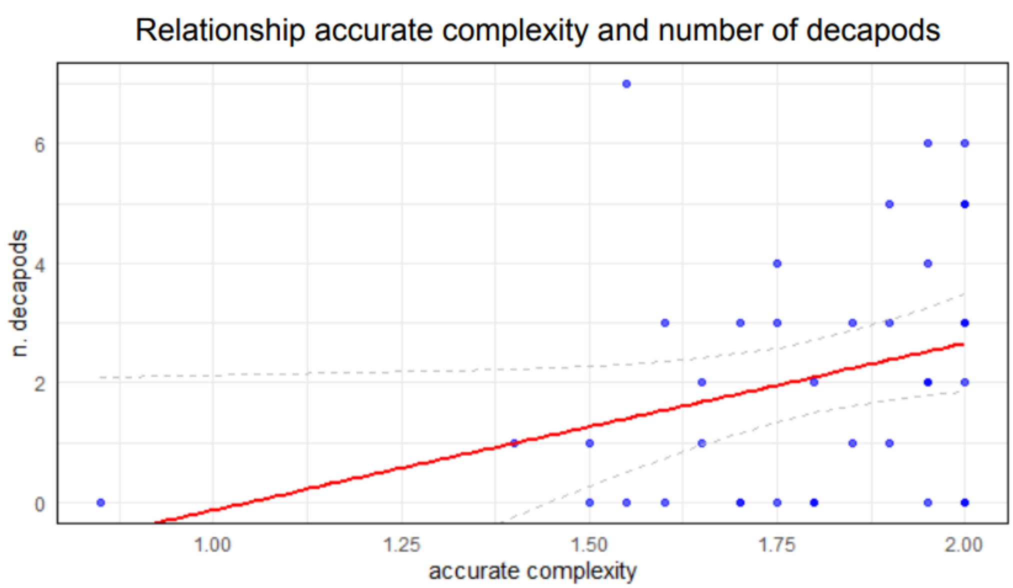

Figure 17 - Personal elaboration. Relationship between accurate complexity and number of decapods.

5. DISCUSSIONS

Artificial substrate tiles were colonized by a diverse assemblage of organisms. In general, sessile organisms look for new substrates suitable for their space occupation, relating its first stages to the detection of a material that allows them to settle (Babcock & Mundy, 1996). This colonization of newly available habitat has an important effect on the subsequent patterns of succession (Chapman, 2002). The calculation of the diversity index (*H*') in the present study showed a variability between the tiles with values mostly above 1, which demonstrates a significant diversity between the species in the tiles, with a predominance of Bryozoa and Chordata (Ascidiacea) in terms of number of identified genera and species. While polychaetes may dominate in terms of biomass or surface cover (especially in the early stages of colonization), bryozoans and ascidians may have greater diversity at the species level, even if they occupy a smaller portion of the surface (Fariñas-Franco & Roberts, 2014; Lavender, 2017). The mean coverage of Ane was found to be significantly higher, suggesting that this group is particularly prevalent, being only four genera identified as primary builders. The results obtained from statistical analyses and the histogram support our initial hypothesis that the Ane phyla have a higher average coverage compared to other groups, in line with the colonization status at which the tiles were analysed (Ardizzone G. D. et al., 1989). In fact, serpulids and capitellids rapidly promote a change in the substrate characteristics, adding $CaCO_3$ that may be attractive for other organism (Terlizzi et al., 2000). The validity of this result is reinforced by the outcome of the Kruskal-Wallis test and post-hoc tests, which indicated significant differences between Ane and other phyla. it is therefore possible to confirm the hypothesis that polychaetes are the prevalent group, which favours the structural base by colonizing the substrates first as confirmed by other studies (Ardizzone G. D. et al., 1989; López et al., 2011; Bracho-Villavicencio et al., 2024).

Analysing the photographs it was evident that the organisms of the 3rd colonization phase (molluscs, ascidians, sponges) had found a solid base for their settlement and colonization, so the polychaetes played the key role of habitat providers, providing a natural substrate for the colonization of the subsequent organisms (Giangrande et al., 2020) and the data resulting from the analysis and their evident abundance confirm this. The added three-dimensionality is an important cue to understand the subsequent colonization. Overgrowing organisms may cover and interfere in Ane behaviour and feeding needs (Kupriyanova et al., 2001), but its opportunistic settlement at the beginning is compensated with the fast occupation of space (Callaway, 2003). It is not the first case study on tiles of this type in which polychaetes have a greater and marked presence compared to other groups (Bracho-Villavicencio et al., 2024). Considering also the fact that the tiles were colonized only on the side facing the seabed, thus creating a shadow zone, on a fine bottom and with little wave movement, (Wouters et al., 2018) confirms that a high diversity of polychaetes can occur in areas under water, with fine grain sizes, with little exposure to the waves and low slope. Another benthic community with high diversity and very frequent in the tiles were the bryozoans, (Birkeland, 1977) proposed that the dominance of these taxa was related to a suite of r-strategy life history traits present in these organisms (small size, fast growth, early reproductive maturity and short generation times). The analyses for the colonization status and to understand the temporal succession were based on studies in literature (Ardizzone G. D. et al., 1989; Watson & Barnes, 2004; Cifuentes et al., 2010; Antoniadou et al., 2011; Pacheco et al., 2011). This suggests that community structure in the tiles changed during the 3 years of immersion due to the replacement of some species or the addition of new ones, with the achievement of the third phase of colonization, demonstrated by the presence of bryozoans, molluscs, ascidians, porifera, sponges and cnidarians. Although we did not carry out temporal monitoring and therefore on the time of the various colonization phases, the tiles subject to this study reached a good state of colonization, consistent with the year in which they were collected and analysed (Ardizzone G. D. et al., 1989). Also considering the winter season in which the collection was carried out, it can be deduced that at that time the organisms in the tiles were at the beginning of a growth phase and not of reproduction. In any case, considering the immersion time and what was stated previously, it is evident and correct to state that the final climax phase was not reached, since the species identified especially in the secondary phase, with slow growth (macrofouling process) were present at the initial stages of growth, especially the sponges (Watson et al., 2004). Instead, ascidians, which were the second group with the largest covered surface, after the polychaetes, were also present at a more advanced level of growth (Ardizzone G. D. et al., 1989; Antoniadou et al., 2010). Overall, it can be said that the tiles had a good state of colonization still growing.

About carbon immobilization, the results obtained showed an average fixation of 536.86 g $CaCO_3$ m^{-2} over 3 years, which it was consider the annual average about 178.95 g/m^{-2}/year but this data is very uncertain because three years are too few to estimate the annual fixation, since this study talking about organisms in their first stages of colonization. In any case, the average value over 3 years is still lower than the estimates of infralittoral $CaCO_3$, which vary between 680 g/m^{-2}/year and 1,000 g/m^{-2}/year, reported by other studies in the southwestern Mediterranean Sea (Cebrián E. et al., 2000). However, the data is closed to the range of $CaCO_3$ fixation for the rocky sea beds of the Balearic platform, which go from 150 to 470 g/m^{-2}/year (Canals M. et al. 1997). It is important to consider that the immersion period used in this study (3 years) is relatively short and that the organisms studied are still growing. This could explain the lower production compared to other studies, which generally refer to more mature and stable ecosystems. Therefore, the results of this study, although lower than other estimates in the literature, are consistent with the specific temporal and environmental context, suggesting that $CaCO_3$ production in the artificial substrate could increase in the future, as the organisms develop further and reach a stable production. However, calcium carbonate fixation remains lower than most values in tropical marine systems where the combined action of more complex communities increases carbon fixation in much higher amounts (Rossi S. et al., 2021). Statistical analyses provided the results of a lack of relationship between biomass and Shannon index (H'), this lack of relationship is entirely possible in nature, it is not said that there is always a linear relationship (Bolam S.G. et al., 2002) and the variability could be due to other factors. Could be explained by the fact that the community, in this case, despite the three years is still in the early colonization phase. So, the low correlation could derive from the fact that the ecological system is not yet sufficiently mature to show a clear and strong relationship between biomass and diversity (Noël L.M. et al., 2009). Furthermore, in this study the high presence of polychaetes could explain the high biomass even in the presence of few species, since polychaetes are known to be organisms that contribute significantly to the biomass of an environment (Montefalcone M. et al., 2022) so even with a low diversity (few species), if polychaetes are abundant, the biomass could be high.

In visual analysis decapods were particularly present in places where the level of complexity was high (three-dimensional) and absent in places where the complexity was null (empty). During statistical analysis between the mean complexity and the number of decapods, the Kendall-Tau correlation and linear model results suggested a moderate positive relationship between the mean complexity and the number of crustaceans, although not very strong. The very low R^2 suggests that the complexity explains only a small part of the variability in decapods so there may be other variables not included in this study that influence the number of decapods. Our results confirm those of previous studies (Ory et al., 2012; Kramer et al., 2017) highlighting a positive correlation between habitat complexity and decapod density. In particular, decapods are present in their juvenile stage were identified, including shrimps and crabs. Recent studies suggest that the complexity of the forests (biofouling) is a key factor to explain zooplankton abundance (Borghese J. et al., 2025). The presence of an alive three-dimensional structure enhances the presence of other vagile organisms. Previous studies suggest that the higher percentage of juveniles found for most species (crustacean decapods) suggests that the sampled habitats meet the requirements of a nursery habitat (Pallas et al., 2006). The high juvenile densities found may be explained by the high levels of larval settlement taking place during summer in temperate latitudes (Pfister, 1997) and the number of adults decreases for those species with larger size at maturity (Pallas et al., 2006). Since there is evidence of adult presence at deeper depths (Freire et al., 2002; Bernárdez et al., 2002), in this study is possible to hypothesise that these shallow habitats play an important nursery.

6. CONCLUSIONS AND FUTURE PERSPECTIVES

In conclusion, the various evaluations demonstrated that the tiles were an efficient substrate, attracting a remarkable diversity of species, showing significant carbon immobilization potential and reached a good state of colonization coherent with the three years of immersion. Statistical analyses of the mean coverage per phylum confirm that the phylum Ane (polychaetes) has a significantly higher mean coverage than the other phylum analysed and this result suggests that the phylum Ane could have a prevalent ecological role in the studied environment, as builders of a natural substrate (Ardizzone G. D. et al., 1989; López et al., 2011), although further studies may be necessary to explore the factors that contribute to this difference. The statistical analysis highlighted a positive relationship between complexity and the number of decapods. Further research could deepen this relationship, exploring other factors that could influence the number of crustaceans. The preference for this structure by the juvenile decapods, also giving the structure a nursery role (Pallas et al., 2006, Borghese J. et al., 2025).

To further investigate the results obtained, it would be interesting to analyze the effectiveness of the materials used through monitoring at different temporal scales, with a particular focus on the early stages of colonization. In this context, it would be useful to examine whether there are differences in growth rates and larval settlement times, as well as in the growth of organisms, in relation to the composition of the artificial substrates. Some studies suggest that while the type of material can influence species abundance, it does not have a significant impact on species richness (Dodds K.C. et al., 2022). A further step forward could be to more thoroughly examine this relationship to understand whether and how the substrate composition might influence species abundance. In parallel, the studies could be extended to include monitoring that also considers mobile species, such as fish, to assess how they may interact with and influence the colonization of sessile organisms. Another future goal could be to modify the orientation of the tiles by positioning the entire structure at a different angle relative to the seabed. This strategy could reduce the effects of sedimentation, promoting greater colonization of organisms, even on the upper portions of the tiles. This approach is already being tested, thanks to a proposal put forward by the UGI marine biologists, who have suggested this possible solution to further enhance the ongoing study.

7. SUPPLEMENTARY ACTIVITY: Monitoring program Port Olímpic, Barcelona (Spain)

UGI is known for its active involvement in marine regeneration projects, which is also their core focus. By combining parametric design with ecological insights, their team creates smart reefs that adapt to local environments, promoting biodiversity and combating the impacts of climate change. These custom underwater landscapes not only regenerate marine life but also create sustainable models for future ocean conservation initiatives.

"We are the gardeners of the sea, promoting biodiversity and advancing a regenerative model for marine ecosystems." [17]

At the heart of their innovative approach is Reefhopper®, a sophisticated software platform that uses advanced parametric modelling to design Smart Enhanced Reefs (SER®). This powerful tool allows the team to meticulously map and understand the biotic assemblages that form underwater ecosystems, considering critical factors such as soil composition and the presence of suspension feeders such as corals and sponges. This multidisciplinary approach has led to five pilot projects in Norway, Denmark, Israel, Catalonia and the Canary Islands, each adapted to the unique ecological needs of the region. The data collected from these projects flows into Reefhopper, building a growing catalogue of projects for different ecozones and advancing the field of underwater landscaping, as explained and described in "Projects in Action: Creating Smart Reefs and Improving to Regenerate Marine Life" by Marc García-Durán Huet (Founder of UGI) in: https://www.wipo.int/en/web/ip-advantage/w/stories/hague-system/underwater-gardens. December 13, 2024. In fact, at the same time as the tile study that is the subject of this thesis, an additional activity was carried out. As mentioned at the beginning of this study, the analyses were carried out at the headquarters of Underwater Gardens International, Barcelona (Spain) during a 5-month internship activity (from the beginning of June to the end of October 2024) and during this period participation was taken in one of the active and under-study projects on SER located at Port Olímpic in Barcelona. Contribution and active participation in two days

[17] Marc García-Durán Huet (founder of UGI). Web article "Projects in Action: Creating Smart Reefs and Improving to Regenerate Marine Life". Web site WIPO. From link: https://www.wipo.int/en/web/ip-advantage/w/stories/hague-system/underwater-gardens.

of sea diving was provided, which provided additional data to determine the progress and state of colonization of these structures positioned in the sea since the spring of April 2024 at a depth of 8 - 10 m on a sandy bottom, prevalent in the coastal area of the port. The SERs are made of ecological materials, including concrete, geopolymers and calcium carbonate-based agglomerates, weighing between 1.2 and 1.6 tons. Once anchored to the seabed, they provide vital habitats for marine organisms. Their designs feature complex shapes that mimic natural shelters and dynamics found in various marine habitats, enhancing natural regenerative processes. Monitoring dives were then carried out at different times of distance to monitor the state and colonization of the structures in which the evolution was observed from the biofilm phase, created in the first months after installation, to the second phase of microfouling. Subsequently, two other dives were carried out, in which I participated, on 8 and 24 October 2024 and the activities focused on the collection of photographic data of the structures, on the monitoring of transient species (water column) in the area and stationary species (benthic) present in the substrate and inside the cavities (*Figure18*).

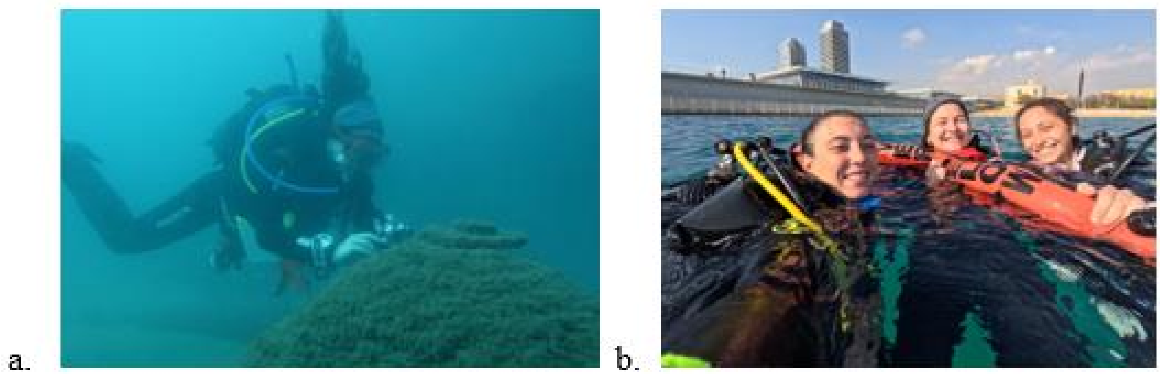

Figure 18 - Photos taken during the last monitoring activity.
a. Me during monitoring activity b. Monitoring team

In these last monitoring, surprising was the sight of an octopus hidden inside one of the structures only after 6 months from the installation of the structures on the site and then the presence of coralline red algae, pioneer algae was found, and mobile species (fishes) indicate that the ecological succession is occurring according to the times dictated by the environmental characteristics of the area (*Figure19*).

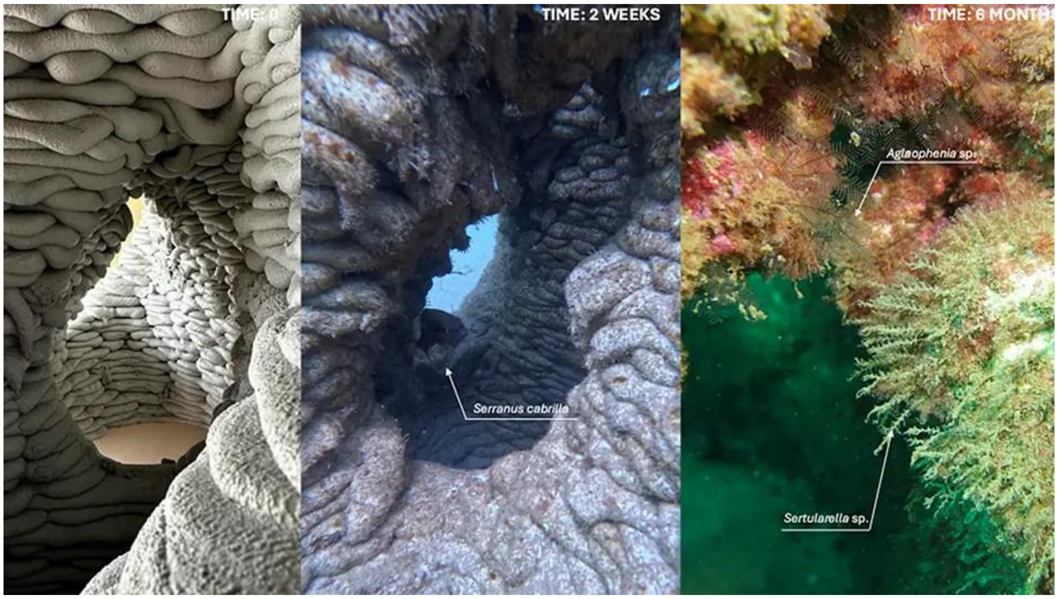

Figure 19 - Photo by Underwater Gardens International. Series of photos of the time scale of colonization in the artificial structure [18]

[18] Photo by Underater Gardens Internation. Present in the web article "Projects in Action: Creating Smart Reefs and Improving to Regenerate Marine Life". From WIPO web site (World Intellectual Property Organization)

8. BIBLIOGRAPHY

Alistair Becker, Matthew D. Taylor, Heath Folpp, & Michael B. Lowry. (2018). Managing the development of artificial reef systems: The need for quantitative goals. *Fish and Fisheries* (4th ed., Vol. 19, pp. 740–752). DOI:10.1111/faf.12288

Antoniadou, C., Voultsiadou, E., & Chintiroglou, C. (2010). Benthic colonization and succession on temperate sublittoral rocky cliffs. *Journal of Experimental Marine Biology and Ecology*, *382*(2), 145–153. https://doi.org/10.1016/j.jembe.2009.11.004

Antoniadou, C., Voultsiadou, E., & Chintiroglou, C. (2011). Seasonal patterns of colonization and early succession on sublittoral rocky cliffs. *Journal of Experimental Marine Biology and Ecology*, *403*(1–2), 21–30. https://doi.org/10.1016/j.jembe.2011.04.001

Ardizzone G. D., Gravina M. F., & Belluscio A. (1989). Temporal Development of Epibenthic Communities on Artificial Reefs in the Central Mediterranean Sea. *University of Miami - Rosenstiel School of Marine, Atmospheric & Earth Science*, *44*(2), 592–608. https://www.researchgate.net/publication/233562801_Temporal_Development_of_Epibenthic_Communities_on_Artificial_Reefs_in_the_Central_Mediterranean_Sea#:~:text=Patterns%20of%20epibenthic%20species%20settlement%2C%20growth%20and%20turnover,12-14%20m%20depths%20along%20the%20Tyrrhenian%20coast%20%28Italy%29.

Aurelle, D., Thomas, S., Albert, C., Bally, M., Bondeau, A., Boudouresque, C., Cahill, A. E., Carlotti, F., Chenuil, A., Cramer, W., Davi, H., De Jode, A., Ereskovsky, A., Farnet, A., Fernandez, C., Gauquelin, T., Mirleau, P., Monnet, A., Prévosto, B., … Fady, B. (2022). Biodiversity, climate change, and adaptation in the Mediterranean. *Ecosphere*, *13*(4). https://doi.org/10.1002/ecs2.3915

Babcock, R., & Mundy, C. (1996). Coral recruitment: Consequences of settlement choice for early growth and survivorship in two scleractinians. *Journal of Experimental Marine Biology and Ecology*, *206*(1–2), 179–201. https://doi.org/10.1016/S0022-0981(96)02622-6

Ballesteros Sagarra E. (2015). *La vida marina del mar Mediterráneo*. Brau Edicions S L.

Barnes, D. K. A., Sands, C. J., Richardson, A., & Smith, N. (2019). Extremes in Benthic Ecosystem Services; Blue Carbon Natural Capital Shallower Than 1000 m in Isolated, Small, and Young Ascension Island's EEZ. *Frontiers in Marine Science*, *6*. https://doi.org/10.3389/fmars.2019.00663

Bernárdez, C., Freire, J., & González-Gurriarán, E. (2002). Migratory Patterns of Female Spider Crabs Maja Squinado Detected Using Electronic Tags and Telemetry. *Journal of Crustacean Biology*, *22*(1), 91–97. https://doi.org/10.1163/20021975-99990212

Bevilacqua, S., Katsanevakis, S., Micheli, F., Sala, E., Rilov, G., Sarà, G., Malak, D. A., Abdulla, A., Gerovasileiou, V., Gissi, E., Mazaris, A. D., Pipitone, C., Sini, M., Stelzenmüller, V., Terlizzi, A., Todorova, V., & Fraschetti, S. (2020). The Status of Coastal Benthic Ecosystems in the Mediterranean Sea: Evidence From Ecological Indicators. *Frontiers in Marine Science*, *7*. https://doi.org/10.3389/fmars.2020.00475

Bianchi, C. N., & Morri, C. (2000). Marine Biodiversity of the Mediterranean Sea: Situation, Problems and Prospects for Future Research. *Marine Pollution Bulletin*, *40*(5), 367–376. https://doi.org/https://doi.org/10.1016/S0025-326X(00)00027-8

Boissery, P., Lenfant, P., Lecaillon, G., Gudefin, A., Fonbonne, S., Selfati, M., El Ouamari, N., Brunet, R., Espinosa, F., & Bazairi, H. (2022). The ecological restoration: A way forward the conservation of marine biodiversity. In *Coastal Habitat Conservation: New Perspectives and Sustainable Development of Biodiversity in the Anthropocene* (pp. 171–191). Elsevier. https://doi.org/10.1016/B978-0-323-85613-3.00002-5

Bolam S.G., Fernandes T.F., Huxham M. (2002). Diversity, biomass, and ecosystem processes in the marine benthos. *Ecological monographs, 72(4),* 599-615. https://doi.org/10.1890/0012-9615(2002)072[0599:DBAEPI]2.0.CO;2

Borghese J., Giangrande A., Arduini D., Trani R., Doria L., Anglano M., Rizzo L., & Rossi S. (2025). Seasonal variations in biological and environmental variables of an innovative IMTA system in the Mar Grande of Taranto (Mediterranean Sea). *Aquaculture*.

Bracho-Villavicencio, C., Matthews-Cascon, H., García-Durán, M., Vélez, X., Lago, N., Busquier, L., & Rossi, S. (2024). Benthic Colonization on New Materials for Marine Ecosystem Restoration in Porto Cesareo, Italy. *Journal of Marine Science and Engineering*, *12*(1). https://doi.org/10.3390/jmse12010169

Brandl, S. J., Hoey, A. S., & Bellwood, D. R. (2014). Micro-topography mediates interactions between corals, algae, and herbivorous fishes on coral reefs. *Coral Reefs*, *33*(2), 421–430. https://doi.org/10.1007/s00338-013-1110-5

Bulleri, F., & Benedetti-Cecchi, L. (2006). Mechanisms of recovery and resilience of different components of mosaics of habitats on shallow rocky reefs. *Oecologia*, *149*(3), 482–492. https://doi.org/10.1007/s00442-006-0459-3

Bullimore B., & Hiscock K. (2001). Quantitative surveillance of sublittoral rock biotopes and species using photographs. In *Marine Monitoring Handbook. Joint Nature Conservation committee. Procedural Guideline* (12th ed., Vol. 3, pp. 315–326). https://www.academia.edu/6243936/Procedural_Guideline_No_3_12_Quantitative_surveillance_of_sublittoral_rock_biotopes_and_species_using_photographs#:~:text=This%20procedural%20guideline%20outlines%20a%20method%20for%20conducting,sublittoral%20rock%20biotopes%20and%20species%20through%20photographic%20techniques.

Burt, J., Bartholomew, A., Usseglio, P., Bauman, A., & Sale, P. F. (2009). Are artificial reefs surrogates of natural habitats for corals and fish in Dubai, United Arab Emirates? *Coral Reefs*, *28*(3), 663–675. https://doi.org/10.1007/s00338-009-0500-1

Callaway, R. (2003). Long-term effects of imitation polychaete tubes on benthic fauna: they anchor Mytilus edulis (L.) banks. *Journal of Experimental Marine Biology and Ecology*, *283*(1–2), 115–132. https://doi.org/10.1016/S0022-0981(02)00474-4

Calvo, E., Simó, R., Coma, R., Ribes, M., Pascual, J., Sabatés, A., Gili, J., & Pelejero, C. (2011). Effects of climate change on Mediterranean marine ecosystems: the case of the Catalan Sea. *Climate Research*, *50*(1), 1–29. https://doi.org/10.3354/cr01040

Canals M., E. Ballesteros † (1997). Production of carbonate particles by phytobenthic communities on the Mallorca-Menorca shelf, northwestern Mediterranean Sea. In book: *Deep Sea Research Part II: Topical Studies in Oceanography*. Volume 44, Issues 3–4,611-629. https://doi.org/10.1016/S0967-0645(96)00095-1

Canion, C., & Heck, K. (2009). Effect of habitat complexity on predation success: re-evaluating the current paradigm in seagrass beds. *Marine Ecology Progress Series*, *393*, 37–46. https://doi.org/10.3354/meps08272

Cardenas-Rojas, D., Mendoza, E., Escudero, M., & Verduzco-Zapata, M. (2021). Assessment of the performance of an artificial reef made of modular elements through small scale experiments. *Journal of Marine Science and Engineering*, *9*(2), 1–18. https://doi.org/10.3390/jmse9020130

Cebrián E., Ballesteros E., Canals M. (2000). Shallow rocky bottom benthic assemblages as calcium carbonate producers in the Alboran Sea (southwestern Mediterranean). *OCEANOLOGICA ACTA, 23(3),* 311-322. https://doi.org/10.1016/S0399-1784(00)00131-6

Chapman, M. G. (2002). Early colonization of shallow subtidal boulders in two habitats. *Journal of Experimental Marine Biology and Ecology*, *275*(2), 95–116. https://doi.org/10.1016/S0022-0981(02)00134-X

Chiantore, M., Santangelo, G., & Bavestrello, G. (2003). *Monitoraggio delle popolazioni animali naturali*. https://www.researchgate.net/publication/303466814

Cifuentes, M., Krueger, I., Dumont, C. P., Lenz, M., & Thiel, M. (2010). Does primary colonization or community structure determine the succession of fouling communities? *Journal of Experimental Marine Biology and Ecology*, *395*(1–2), 10–20. https://doi.org/10.1016/j.jembe.2010.08.019

Çinar, M. E. (2006). Serpulid species (Polychaeta: Serpulidae) from the Levantine coast of Turkey (eastern Mediterranean), with special emphasis on alien species. *Aquatic Invasions*, *1*(4), 223–240. https://doi.org/10.3391/ai.2006.1.4.6

Connell, J. H., & Slatyer, R. O. (1977). Mechanisms of Succession in Natural Communities and Their Role in Community Stability and Organization. *The American Naturalist*, *111*(982), 1119–1144. https://doi.org/10.1086/283241

Corriero, G., Pierri, C., Mercurio, M., Nonnis Marzano, C., Onen Tarantini, S., Gravina, M. F., Lisco, S., Moretti, M., De Giosa, F., Valenzano, E., Giangrande, A., Mastrodonato, M., Longo, C., & Cardone, F. (2019). A Mediterranean mesophotic coral reef built by non-symbiotic scleractinians. *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-019-40284-4

Cui, K., Chang, J., Feo, L., Chow, C. L., & Lau, D. (2022). Developments and Applications of Carbon Nanotube Reinforced Cement-Based Composites as Functional Building Materials. In *Frontiers in Materials* (Vol. 9). Frontiers Media S.A. https://doi.org/10.3389/fmats.2022.861646

Denno, R. F., Finke, D. L., & Langellotto, G. A. (2005). Direct and Indirect Effects of Vegetation Structure and Habitat Complexity on Predator-Prey and Predator-Predator Interactions. In *Ecology Of Predator-Prey Interactions* (pp. 211–239). Oxford University PressNew York, NY. https://doi.org/10.1093/oso/9780195171204.003.0010

Duarte, C. M., Middelburg, J. J., & Caraco, N. (2005). Major role of marine vegetation on the oceanic carbon cycle. In *Biogeosciences* (Vol. 2). www.biogeosciences.net/bg/2/1/

Fariñas-Franco, J. M., & Roberts, D. (2014). Early faunal successional patterns in artificial reefs used for restoration of impacted biogenic habitats. *Hydrobiologia*, *727*(1), 75–94. https://doi.org/10.1007/s10750-013-1788-y

Freire, J., Bernárdez, C., Corgos, A., Fernández, L., González-Gurriarán, E., Sampedro, M. P., & Verísimo, P. (2002). Management strategies for sustainable invertebrate fisheries in coastal ecosystems of Galicia (NW Spain). *Aquatic Ecology*, *36*(1), 41–50. https://doi.org/10.1023/A:1013350723445

Gann, G. D., McDonald, T., Walder, B., Aronson, J., Nelson, C. R., Jonson, J., Hallett, J. G., Eisenberg, C., Guariguata, M. R., Liu, J., Hua, F., Echeverría, C., Gonzales, E., Shaw, N., Decleer, K., & Dixon, K. W. (2019). International principles and standards for the practice of ecological restoration. Second edition. *Restoration Ecology*, *27*(S1), S1–S46. https://doi.org/10.1111/rec.13035

Giangrande, A., Gambi, M. C., & Gravina, M. F. (2020). Polychaetes as Habitat Former: Structure and Function. In *Perspectives on the Marine Animal Forests of the World* (pp. 219–237). Springer International Publishing. https://doi.org/10.1007/978-3-030-57054-5_8

Giangrande, A., Gravina, M. F., Rossi, S., Longo, C., & Pierri, C. (2021). Aquaculture and restoration: Perspectives from mediterranean sea experiences. *Water (Switzerland)*, *13*(7). https://doi.org/10.3390/w13070991

Gili, J.M. (1982). Fauna of the benthic cnidaria_Catalonia. Treballs de l'Institució Catalana d'Història Natural, vol 10, 175 pp. https://www.researchgate.net/publication/236222085_Gili_JM-1982_Fauna_de_Cnidaris_de_les_illes_Medes_Treballs_de_l'Institucio_Catalana_d'Historia_Natural_vol_10_175_pp

Hammond, M., Bond, T., Prince, J., Hovey, R. K., & McLean, D. L. (2020). An assessment of change to fish and benthic communities following installation of an artificial reef. *Regional Studies in Marine Science*, *39*, 101408. https://doi.org/https://doi.org/10.1016/j.rsma.2020.101408

Kramer, M. J., Bellwood, D. R., Taylor, R. B., & Bellwood, O. (2017). Benthic Crustacea from tropical and temperate reef locations: differences in assemblages and their relationship with habitat structure. *Coral Reefs*, *36*(3), 971–980. https://doi.org/10.1007/s00338-017-1588-3

Kupriyanova, E., Hove, H., Nishi, E., & Rzhavsky, A. (2001). Life-history patterns in serpulimorph polychaetes: Ecological and evolutionary perspectives. *Oceanography and Marine Biology*. https://www.researchgate.net/publication/258022286

Lavender, J. (2017). Ecological processes influencing the composition of subtidal marine hard-substrate communities. *School of Biological, Earth and Environmental Sciences*.

Lessios, H. A. (1996). METHODS FOR QUANTIFYING ABUNDANCE OF MARINE ORGANISMS. In book: M.A. Lang and C. C. Baldwin (eds.) Methods and techniques of underwater research, pp. 149-175. *Smithsonian Institution, Washington.* https://stri-sites.si.edu/docs/publications/pdfs/AAUS%201996.pdf

Lima, J. S., Sanchez-Jerez, P., dos Santos, L. N., & Zalmon, I. R. (2020). Could artificial reefs increase access to estuarine fishery resources? Insights from a long-term assessment. *Estuarine, Coastal and Shelf Science*, *242*, 106858. https://doi.org/https://doi.org/10.1016/j.ecss.2020.106858

Llamas A. (2015). *Guía visual de Fauna y Flora del Mediterráneo*. Anthias Editorial .

López, D. R., Cavallero, L., Brizuela, M. A., & Aguiar, M. R. (2011). Ecosystemic structural-functional approach of the state and transition model. *Applied Vegetation Science*, *14*(1), 6–16. https://doi.org/10.1111/j.1654-109X.2010.01095.x

Lozano-Cortés, D. F., & Zapata, F. A. (2014). Invertebrate colonization on artificial substrates in a coral reef at Gorgona Island, Colombian Pacific Ocean. In *Rev. Biol. Trop. (Int. J. Trop. Biol. ISSN* (Vol. 62).

MacArthur, M., Naylor, L. A., Hansom, J. D., & Burrows, M. T. (2020). Ecological enhancement of coastal engineering structures: Passive enhancement techniques. *Science of The Total Environment*, *740*, 139981. https://doi.org/https://doi.org/10.1016/j.scitotenv.2020.139981

Manea, E., Agardy, T., & Bongiorni, L. (2023). Link marine restoration to marine spatial planning through ecosystem-based management to maximize ocean regeneration. *Aquatic Conservation: Marine and Freshwater Ecosystems*. https://api.semanticscholar.org/CorpusID:260754083

Mangialajo, L., Gianni, F., Airoldi, L., Bartolini, F., Francour, P., Meinesz, A., Thibaut, T., & Ballesteros, E. (2013). *CONSERVATION AND RESTORATION OF CYSTOSEIRA FORESTS IN THE MEDITERRANEAN SEA: THE ROLE OF MARINE PROTECTED AREAS*.

Montefalcone Monica, Oprandi Alice, Azzola Annalisa, Morri Carla, Bianchi Carlo Nike (2022). Chapter One - Serpulid reefs and their role in aquatic ecosystems: A global review. Editor(s): Charles Sheppard. *Academic Press, Advances in Marine Biology, 92*, 1-54. https://doi.org/10.1016/bs.amb.2022.06.001.

Morseletto, P. (2020). Restorative and regenerative: Exploring the concepts in the circular economy. *Journal of Industrial Ecology*, *24*(4), 763–773. https://doi.org/10.1111/jiec.12987

Noël, L. M. L. J., Griffin, J. N., Moschella, P. S., Jenkins, S. R., Thompson, R. C., & Hawkins, S. J. (2009). Changes in diversity and ecosystem functioning during succession. *In M. Wahl (Ed.), Marine Hard Bottom Communities* (Chapter 15). Springer-Verlag Berlin Heidelberg. https://doi.org/10.1007/b76710_15

Odum, E. P. (1993). *Ecology and our endangered life-support systems* (2nd ed.). Sinauer Associates, Inc. Publishers.

Olabarria, C. (2002). Role of colonization in spatio-temporal patchiness of microgastropods in coralline turf habitat. In *Journal of Experimental Marine Biology and Ecology* (Vol. 274). www.elsevier.com/locate/jembe

Ory, N. C., Dudgeon, D., Dumont, C. P., Miranda, L., & Thiel, M. (2012). Effects of predation and habitat structure on the abundance and population structure of the rock shrimp Rhynchocinetes typus (Caridea) on temperate rocky reefs. *Marine Biology*, *159*(9), 2075–2089. https://doi.org/10.1007/s00227-012-1994-6

Pacheco, A. S., Laudien, J., Thiel, M., Oliva, M., & Heilmayer, O. (2011). Succession and seasonal onset of colonization in subtidal hard-bottom communities off northern Chile. *Marine Ecology*, *32*(1), 75–87. https://doi.org/10.1111/j.1439-0485.2010.00398.x

Pallas, A., Garcia-Calvo, B., Corgos, A., Bernardez, C., & Freire, J. (2006). Distribution and habitat use patterns of benthic decapod crustaceans in shallow waters: a comparative approach. *Marine Ecology Progress Series*, *324*, 173–184. https://doi.org/10.3354/meps324173

Paxton, A. B., Shertzer, K. W., Bacheler, N. M., Kellison, G. T., Riley, K. L., & Taylor, J. C. (2020). Meta-analysis reveals artificial reefs can be effective tools for fish community enhancement but are not one-size-fits-all. In *Frontiers in Marine Science* (Vol. 7). Frontiers Media S.A. https://doi.org/10.3389/fmars.2020.00282

Pfister, C. (1997). Demographic consequences of within-year variation in recruitment. *Marine Ecology Progress Series*, *153*, 229–238. https://doi.org/10.3354/meps153229

Pickering, H., Whitmarsh, D., & Jensen, A. (1999). Artificial Reefs as a Tool to Aid Rehabilitation of Coastal Ecosystems: Investigating the Potential. *Marine Pollution Bulletin*, *37*(8), 505–514. https://doi.org/https://doi.org/10.1016/S0025-326X(98)00121-0

Ramos, J., Tuaty-Guerra, M., Almeida, M., Raposo, A. C., Gaudêncio, M. J., Silva, A. D., Rodrigues, N., Leandro, S. M., & Caetano, M. (2021). An artificial reef at the edge of the deep: An interdisciplinary case study. *Ocean & Coastal Management*, *210*, 105729. https://doi.org/https://doi.org/10.1016/j.ocecoaman.2021.105729

Remple, K. L., Silbiger, N. J., Quinlan, Z. A., Fox, M. D., Kelly, L. W., Donahue, M. J., & Nelson, C. E. (2021). Coral reef biofilm bacterial diversity and successional trajectories are structured by reef benthic organisms and shift under chronic nutrient enrichment. *Npj Biofilms and Microbiomes*, *7*(1). https://doi.org/10.1038/s41522-021-00252-1

Riera, E., Lamy, D., Goulard, C., Francour, P., & Hubas, C. (2018). Biofilm monitoring as a tool to assess the efficiency of artificial reefs as substrates: Toward 3D printed reefs. *Ecological Engineering*, *120*, 230–237. https://doi.org/https://doi.org/10.1016/j.ecoleng.2018.06.005

Rossi, S., & Rizzo, L. (2021). Marine animal forests as carbon immobilizers or why we should preserve these three-dimensional alive structures. In *Perspectives on the Marine Animal Forests of the World* (pp. 333–400). Springer. https://doi.org/10.1007/978-3-030-57054-5_11

Salvador, X., Sánchez, L., Domingo, O., Comaposada, A., Soacha, K., & Piera, J. (2021). *Guia Participativa Marina del Barcelonès*. Marcombo .

Sandonnini, J., Del Pilar Ruso, Y., Cortés Melendreras, E., & Giménez Casalduero, F. (2021). Massive Aggregations of Serpulidae Associated With Eutrophication of the Mar Menor, Southeast Iberian Peninsula. *Frontiers in Marine Science*, *7*. https://doi.org/10.3389/fmars.2020.531726

Saunders, M. I., Doropoulos, C., Bayraktarov, E., Babcock, R. C., Gorman, D., Eger, A. M., Vozzo, M. L., Gillies, C. L., Vanderklift, M. A., Steven, A. D. L., Bustamante, R. H., & Silliman, B. R. (2020). Bright Spots in Coastal Marine Ecosystem Restoration. In *Current Biology* (Vol. 30, Issue 24, pp. R1500–R1510). Cell Press. https://doi.org/10.1016/j.cub.2020.10.056

Schembri, S., & Zammit, G. (2022). The Biodiversity of Epilithic Microalgal Communities Colonising a Central Mediterranean Coastline. *Journal of Coastal Research*, *38*(2), 249–260. https://doi.org/10.2112/JCOASTRES-D-21-00052.1

Sempere-Valverde, J., Ostalé-Valriberas, E., Farfán, G. M., & Espinosa, F. (2018). Substratum type affects recruitment and development of marine assemblages over artificial substrata: A case study in the Alboran Sea. *Estuarine, Coastal and Shelf Science*, *204*, 56–65. https://doi.org/https://doi.org/10.1016/j.ecss.2018.02.017

Spagnolo, A., Cuicchi, C., Punzo, E., Santelli, A., Scarcella, G., & Fabi, G. (2014). Patterns of colonization and succession of benthic assemblages in two artificial substrates. *Journal of Sea Research*, *88*, 78–86. https://doi.org/10.1016/j.seares.2014.01.007

Steven Nelson Murray, Richard F. Ambrose, & Megan N. Dethier. (2002). *Methods for Performing Monitoring, Impact, and Ecological Studies on Rocky Shores* (University of California, Ed.). Minerals Management Service.

Terlizzi, A., Conte, E., & Giangrande, A. (2000). Settlement patterns of two Spirorbidae (Annelida, Polychaeta) species in the harbour of Ischia (Gulf of Naples, Mediterranean Sea). *Italian Journal of Zoology*, *67*(3), 303–306. https://doi.org/10.1080/11250000009356328

Thomas A. J. Kuhlbusch. (1998). Black Carbon and the Carbon Cycle. *Science* , *280*(5371), 1903–1904. https://www.science.org/doi/10.1126/science.280.5371.1903#core-collateral-purchase-access

Verma, A. K., & Prakash, S. (2020). STATUS OF ANIMAL PHYLA IN DIFFERENT KINGDOM SYSTEMS OF BIOLOGICAL CLASSIFICATION. *International Journal of Biological Innovations*, *02*(02), 149–154. https://doi.org/10.46505/ijbi.2020.2211

Vivier, B., Dauvin, J.-C., Navon, M., Rusig, A.-M., Mussio, I., Orvain, F., Boutouil, M., & Claquin, P. (2021). Marine artificial reefs, a meta-analysis of their design, objectives and effectiveness. *Global Ecology and Conservation*, *27*, e01538. https://doi.org/https://doi.org/10.1016/j.gecco.2021.e01538

Waltham, N. J., Elliott, M., Lee, S. Y., Lovelock, C., Duarte, C. M., Buelow, C., Simenstad, C., Nagelkerken, I., Claassens, L., Wen, C. K. C., Barletta, M., Connolly, R. M., Gillies, C., Mitsch, W. J., Ogburn, M. B., Purandare, J., Possingham, H., & Sheaves, M. (2020). UN Decade on Ecosystem Restoration 2021–2030—What Chance for Success in Restoring Coastal Ecosystems? *Frontiers in Marine Science*, *7*. https://doi.org/10.3389/fmars.2020.00071

Wang, J., & Wang, S. (2019). Preparation, modification and environmental application of biochar: A review. In *Journal of Cleaner Production* (Vol. 227, pp. 1002–1022). Elsevier Ltd. https://doi.org/10.1016/j.jclepro.2019.04.282

Watson, D. I., & Barnes, D. K. A. (2004). Temporal and spatial components of variability in benthic recruitment, a 5-year temperate example. *Marine Biology*, *145*(1), 201–214. https://doi.org/10.1007/s00227-003-1291-5

Wouters, J. M., Gusmao, J. B., Mattos, G., & Lana, P. (2018). Polychaete functional diversity in shallow habitats: Shelter from the storm. *Journal of Sea Research*, *135*, 18–30. https://doi.org/10.1016/j.seares.2018.02.005

Xu, Q., Ji, T., Yang, Z., & Ye, Y. (2019). Preliminary investigation of artificial reef concrete with sulphoaluminate cement, marine sand and sea water. *Construction and Building Materials*, *211*, 837–846. https://doi.org/https://doi.org/10.1016/j.conbuildmat.2019.03.272

Yoris-Nobile, A. I., Slebi-Acevedo, C. J., Lizasoain-Arteaga, E., Indacoechea-Vega, I., Blanco-Fernandez, E., Castro-Fresno, D., Alonso-Estebanez, A., Alonso-Cañon, S., Real-Gutierrez, C., Boukhelf, F., Boutouil, M., Sebaibi, N., Hall, A., Greenhill, S., Herbert, R., Stafford, R., Reis, B., van der Linden, P., Gómez, O. B., … Lobo-Arteaga, J. (2023). Artificial reefs built by 3D printing: Systematisation in the design, material selection and fabrication. *Construction and Building Materials*, *362*. https://doi.org/10.1016/j.conbuildmat.2022.129766