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From individuals to communities.
Innovative methodologies to assess multiscale structural complexity of marine benthic habitats.

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A Maria, Pepi y Bernabé

Contents

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This thesis is the product of an intense three-years period in which, after facing many challenges, did nothing but confirm my curiosity and passion for marine sciences. During that time, I had the opportunity to get in touch with interesting research topics, to apply new emergent techniques and to do one of the most wonderful field work of my live. Nonetheless, I must recognize that at the end what I valorised the most is the network of amazing people that helped me to open up to new perspectives, supported me and contributed to my work in one way or another, building the researcher and the person that I am today.

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Summary

Over the past decades, thanks to the continuous development of new technologies, innovative techniques have raised becoming the new standard in many disciplines. The rapid evolution of computer vision, the appearance of powerful non-professional working stations and the increasing interest of the scientific community for a proper assessment of three-dimensional (3D) features have fuelled the emergence of new approaches. Although structural complexity has been long recognized as one of the main factors driving biotic interactions and influencing the functioning and resilience of ecosystems, until only recently we did not have the proper tools to approach it. From a biological point of view, these techniques open new interesting possibilities to capture and represent in a more complete and realistic way both ecological and biological processes.

In this context, this PhD project approached different ecological levels, from communities to individuals, through the coupling of innovative and traditional methods. Depending on the scale of the study, different techniques were implemented to properly capture the structural complexity at hand. Therefore, a variety of studies are hereby presented with a common objective: the inclusion of 3D features in the assessment of different biological scales by the implementation of cutting-edge techniques (i.e., Structure from Motion photogrammetry and Micro-computed tomography).

Photogrammetry – defined as the art, science, and technology of obtaining reliable information about physical objects – has a long history behind, nonetheless several technological and methodological advancements allowed its *Renaissance* over the past decades, widening its application to different environments and disciplines. In this context, an extensive literature review was conducted on Elsevier's Scopus database to explore the application of this technique through time, paying special attention to the transfer of methods between terrestrial and underwater approaches. A total of 1,923 were included in the analyses, underlying a rising interest in the survey of natural scenarios by means of photogrammetry in the considered period (1985-2021). The acquired information helped to identify trends during its development and to highlight the urgency to widen the range of its applications in aquatic habitats to fill the current gap of knowledge on their structure and species distribution, delaying the design of proper conservation strategies. To this end, as an output of the international collaborative effort performed during the 3DSeaFor workshop, methodological and technological gaps were highlighted for the application of optical-based methods in the assessment of marine animal forest's structural complexity, driving the next steps for the implementation of optical-based approaches in the monitoring of these crucial habitats.

Understanding distribution patterns of a species is vital for the development of effective management plans. This is especially true when addressing species with mesophotic distributions.

One useful tool to address this challenge are species distributional models (SDM). In our study, we aimed to assess the distribution of a long living parasitic zoanthid, *Savalia savaglia* (Bertoloni, 1819), a crucial habitat-former of the mesophotic zone. Even if it is included in the Annex II of Barcelona Convention, no effective protection measures or monitoring plan have been implemented so far. To build the model, known occurrences of this species were compiled and harmonized in a single dataset, and a presence-only Maxent Ecological Niche Model was developed, including a variety of seafloor complexity descriptors in the list of predictors candidates in the analysis. Bathymetry irregularity was identified as one of the two co-variates that most influenced the distribution of the species. This study provided the most updated distribution for *S. savaglia* across the Mediterranean Sea, as well as a preliminary assessment at basin-level of its potential distribution, which may support future monitoring or management efforts in deep environments.

Together with mesophotic ecosystems, marine caves can be considered other biodiversity hotspots still largely underexplored. Even though they are considered priority habitats by the European legislation (Habitats Directive, 92/43/EEC), they are still largely unexplored due to safety and logistical constrains. The benthic community of a small semi-submerged cave Grotta Azzurra (North Adriatic Sea) was assessed through SfM-photogrammetry, comparing its results with more traditional methods (50x50 cm and 20x20cm quadrats). Traditional approaches managed to obtain an accurate general picture of this habitat; however, they underestimated organism's densities as well as some of the calculated seascape metrics. SfM was suggested as a new tool for the monitoring of these environments, allowing for the first time to implement a seascape approach in this type of habitat.

Among all benthic organisms identified in Grotta Azzurra, sponges (Porifera) were undoubtedly the most represented phylum. Porifera represents an essential component of marine ecosystems, affecting the benthic community composition through competitive interactions. The rising of commercial interest for this taxon as a source of natural compounds creates the urge to properly evaluate sponge growth and regeneration rates. In this context, three Mediterranean demosponges (*Axinella polypoides* Schmidt, 1862, *Chondrosia reniformis* Nardo, 1847 and *Sarcotragus foetidus* Schmidt, 1862), presenting different morphotypes were used as model organisms to test the suitability of SfM in the evaluation of growth rates and biomass production of different sponge growth habits. SfM proved to be an effective approach for the long-term sponge growth's monitoring, representing a strong candidate for the establishment of a one size fits-all 3D methodology to assess different sponge morphologies.

Sponges together with other benthic taxa, such as polychaetes and molluscs, can deeply modify their surrounding environment, representing both fundamental ecosystem engineers and bioeroders. Up until today, studies on boring organisms have been mainly based on destructive

methods, considering only bidimensional approaches or geometric approximations, with very little 3D-contributions. In this framework, traditional approaches were coupled with innovative methods (i.e., Micro-CT and SEM microscopy) for the description and quantification of the boring activity of different microborers. The pioneer excavating organisms occurring in the North Adriatic Sea were investigated by the deployment of limestone experimental panels for a period of 2 years. Additionally, samples of *Corallium rubrum* (Linnaeus, 1758) collected at Cape Verde were analysed to explore the boring community hosted in them. Our study is the first assessment of the pioneer erosive community in the North Adriatic, represented by a complex of the polychaete species *Polydora*, obtaining the first estimation of their bioerosive activity in the Adriatic; on the other hand, in the case of *C. rubrum*, three already known sponge species were found boring into the coral skeleton (*Alectona millari, Dotona pulchella mediterranea* and *Thoosa armata*), and a new species of the genus *Alectona* was described (*A. ricardi* sp. nov.).

A collection of seven manuscripts is included in this PhD thesis. Four of them have been published on international journals (with Q1 and Q2 ranking), while one paper is currently under review and another one just submitted. Additionally, the paper on sponge growth rates is still in preparation. All these manuscripts provide a new three-dimensional perspective for the assessment of different biological and ecological aspects of benthic species, giving new insights to law makers in the implementation of new conservation and management strategies and policies. In addition, at the end of the thesis, I listed other products and publications not concerning the topic of this thesis but performed during the PhD period.

Keywords: 3D, SfM, photogrammetry, seascape, marine cave, SDM, conservation, Micro-CT, bioerosion.

Introduction

Although we live in a complex world and our binocular vision let us perceive its structure from a tree-dimensional (3D) perspective, traditional scientific approaches have been constrained by bidimensional (2D) methods, being accepted as a standard by many disciplines until the end of the 20th century (Lepczyk et al., 2021). Over the past two decades, significant technological and methodological developments allowed the emergence of innovative approaches, unlocking new possibilities, and impacting our ability to capture and conceptualize reality (Mesoudi et al., 2013). Among the series of cutting-edge techniques that raised in recent years a recurrent topic has been identified: the shift from 2D to 3D perspective to observe, analyse, and quantify the structural complexity of patterns, systems, and processes (Bagrov et al., 2020). Although pioneer applications of 3D emergent methods were mainly led by a few disciples, such as medicine, biotechnology, or engineering, the increased accessibility to these pieces of technology is now contributing to widen their implementation (Höhne et al., 2012; Westoby et al., 2012), reaching new branches of science (e.g., molecular biology, medicine, biotechnology, engineering, architecture, or cultural heritage) (Sansoni et al., 2009). This phenomenon is mainly related to the rapid evolution of computer vision, the appearance of powerful non-professional 3D graphic processors and working stations, as well as to a constant change in modern society, which day-to-day get more familiar with virtual environments. The fact that these developments became gradually engrained in our daily lives may create the illusion that they are impactless or they simply passed unnoticed. Yet nowadays, 3D technology plays a significant role in our society, just to mention its wider presence in digital media, the emergence of ID recognition systems in our personal devices and public environments (e.g., airports, train stations, banks, among others), the increased presence of 3D printed components on commercial products, or the rise of virtual/augmented reality (VR/AR) spaces or experiences in general (Cipresso et al., 2018). In this technological and sociological context, new methods appear every day to record or emulate the real world into its digital twin, the so-called Metaverse (Dwivedi et al., 2022). In a more scientific framework, likewise, the rapid development of acoustic-, laser- and image-based techniques allowed to approach mid to large-scales scenarios, while the rise of other techniques, such as micro computed tomography (Micro-CT) or single-molecule localization microscopy, make possible to reach down to nanometric or even molecular scales (Barbieri and da Silva, 2019; Keklikoglouk et al., 2019; Thiele et al., 2022). Therefore, the exploration and testing of this pool of innovative tools should be especially encouraged in disciplines where it remains unexplored in order to uncover its full potential.

In biological sciences, although structural complexity has been recognized for a long time to be one of the main drivers of ecosystem's functioning, biodiversity, and resilience, only recently we had access to techniques to properly assess this feature (Ferrari et al., 2016). From the vertical structure given by a tree community in a forest, to the one provided by corals in a reef, we now have a pool of methodologies to better approach environments complexity. However, while terrestrial ecology benefited from the early appearance of aerial surveys and satellite-remote sensing (Lepzic et al., 2021), 3D complexity of underwater environments had been overlooked for many years. Acoustic methods, such as multibeam or SWATH (Small Waterplane Area Twin Hull) had been largely applied to approach this feature in medium and large-scale studies, obtaining bathymetric records with resolutions down to the centimetre (Cameron et al., 2014). Nevertheless, these techniques measure changes in the seafloor elevation with no substrate-type discrimination, limiting its application for ecological and biological studies.

Until recent years the standard method to quantify habitat complexity *in situ* was the "chain-and-tape" approach, an invasive technique able to estimate topographic complexity by comparing the linear distance of a transect with the length of a chain moulded to the surface of the substrate (Graham et al., 2013). Luckily, over the past two decades, emergent techniques are revolutionizing the way to monitor underwater ecosystems. One of these techniques is Structure from Motion (SfM) photogrammetry, a method that, fuelled by the late advancements in image-matching algorithms and computing systems, became the versatile, cost-effective approach it is today (Ferrari et al., 2016; Bayley and Mogg, 2020). In a nutshell, this technique allows to obtain accurate 3D digital reconstructions from a set of overlapping images, and, due to its relatively simple and low-cost application, it became the tool of choice for many disciplines (Remodino et al. 2010; Burns et al., 2015; Hackl et al., 2018; Caravaca et al., 2020; Pierdicca et al., 2021; Urbina-Barreto et a., 2021). In ecological studies, SfM opened the opportunity to obtain multi-scale accurate baselines of both biological spatial patterns and structural complexity, representing a huge step forward in terms of monitoring methods (Ferrari et al., 2016, 2021). Considering that the 2021-30 decade has been defined as the Decade for Ecosystem Restoration by the United Nations, and that during this period, landscape approaches will be promoted to prevent and reverse ecosystem degradation, huge monitoring efforts will be necessary to assess environments' status and the effectivity of restoration actions (Waltham et al., 2020). Structural complexity should be, thus, included among the considered metrics, and photogrammetrical approaches must be considered for small and mid-scale approaches as a suitable candidate to establish a proper baseline and identify possible community shifts during these times tainted by anthropogenic and climatic pressures. Moreover, the relatively simple application of the SfM-protocol (Bayley and Mogg, 2020), together with the increasing access to high quality underwater cameras (e.g., action cameras) (Raoult et al., 2016), makes this technique a powerful tool to be considered by citizen science initiatives. Which, by training recreational divers to implement a standardized protocol, sampling efforts could be increased, reducing monitoring costs and supporting the assessment of areas of interest, as already happening in the framework of the MPA Engage project, where divers were trained in the application of the technique for the monitoring of Mediterranean Marine Protected Areas (MPAs) (Garrabou et al., 2022).

Photogrammetry is not the single technique in biological sciences under the spotlight for the assessment of 3D feature. In fact, depending on the scale or scales aimed to be addressed it is crucial to choose the right method. The inclusion of a 3D perspective in sub-millimetric-scales studies also contributed to increase our knowledge in many disciplines. Nowadays it is possible to assess molecules' 3D structure and have a deeper understanding of its interactions thanks to Hi-C/ChIAPET (Zhang and LI, 2020) or new X-ray crystallography approaches (Jang et al., 2022), a major step in biotechnology, genomics, and proteomics. Marine biology has not been left behind in terms of the implementation of 3D sub-millimetric assessments, being Micro-CT one of the main techniques currently applied in this field (Keklikoglouk et al., 2019, 2021). Although photogrammetry has been also tested and applied at those scales, by coupling it with electron microscopy (Barbieri and da Silva, 2019), Micro-CT provides a unique advantage. Since tomographic 3D imaging techniques are based in the processing of X-ray planar images, it is not only possible to study the external surface of a sample, but also to explore and segment its internal structure (Keklikoglouk et al., 2019). This methodological advance represented a step forward in biological studies, allowing the production and inspection of high-resolution 3D anatomical and morphological data (Kerbl et al; 2013; Rawson et al., 2020; Chatzinikolaou and Keklikoglou, 2021). Additionally, Micro-CT has also contributed to a better understanding of some ecological processes, such as bioerosion – defined as the mechanism by which organisms breakdown a hard substrate, a ubiquitous process that transforms habitats all over the world (Davidson et al., 2018) – (Färber et al., 2016). Traditionally, bioerosive assessments entailed the creation of silicon or resin embedding casts which required the dissolution of the studied substrate in the process. Thanks to Micro-CT, the 3D analysis of internal structures is now possible, without damaging the analysed samples, and allowing not only to quantify erosion rates, but also producing accurate 3D digital casts from the intricate cavities left by the microborers (Beuck et al., 2006; Schönberg and Shields, 2008; Färber et al., 2016; Pulido Mantas et al., 2022b).

The potentialities of all the aforementioned approaches rely on their ability to capture 3D features and on the possibility to transfer this information to the public. Although 3D virtual environments were already a hot topic in the early 21st century (Keklikoglou et al., 2016; Scopigno et al., 2017; Donnelly, 2019), COVID-19 emergency made us realize the lack of digital material and platforms able to support educational need during prolonged lock-down conditions (Bashir et al., 2021). Since then, huge investments have been made to digitalize a wide range of collections (e.g., libraries, museums, zoological collections) and to develop of 3D virtual repositories (Jacobs, 2022; Banfi et al., 2023; Hutson and Hutson, 2023). The no longer need of a highly performant computer for interacting with 3D environments, have contributed to the establishment of virtual and augmented reality applications as a useful instrument for education and awareness (Aristov et al., 2021; Anastasovitis and Roumeliotis, 2023; Fatimah and Ningsih, 2023; Hutson and Hutson, 2023). All these facts emphasize a clear product gap that will need to be addressed in the following years.

Aim of the study

Technical advancements toward a three-dimensional (3D) science continue to fundamentally shift the way we view, explore, and conceptualize the natural world, allowing the scientific community to capture a more complete vision of the ecosystems and organisms.

Along this PhD thesis, there is a general aim linking the various studies contained within this research: the implementation of emergent approaches that include a 3D perspective into the benthic communities' assessment. Through the different chapters different ecological levels were addressed, from single organisms at µm scale by means of Microcomputed tomography (Micro-CT), to population scale and seascape approaches by the application of underwater photogrammetry and ecological niche models.

Depending on the study, different objectives were settled, among them:

i) To identify the current trends in 3D monitoring by means of optical methods, summarising the evolution of the technique in both terrestrial and underwater scenarios (**Addressed in Chapter 1 – paper 1 and 2**)
ii) To explore the potential of SfM-based seascape approach to assess the abundance and spatial distribution patterns of the sessile benthic assemblages of a marine cave (**Addressed in Chapter 2 – paper 3**)
iii) To assess the Mediterranean distribution of *Savalia savaglia,* a rare parasitic species listed as near-threatened by the IUCN (**Addressed in Chapter 3 – paper 4**)
iv) To approach the sponge growth of three Mediterranean demosponges of biotechnological and ecological interest by means of SfM (**Addressed in Chapter 4 – paper 5**)
v) To quantify, and characterize the bioerosive patterns of different microborers through the application of Micro-CT (**Addressed in Chapter 5 – paper 6 and 7**)

All the representatives 3D outputs produced in each study were made available online on Sketchfab (https://sketchfab.com/zoomar), currently the largest platform available for sharing and interacting with 3D data, following the FAIR (findability, accessibility, interoperability, and reusability) principles, and contributing to the dissemination of scientific 3D material for educational purposes.

Chapter 1

Photogrammetry: an emergent tool to assess 3D structural complexity

Even though photogrammetry has more than 170 years of history, several technological and methodological advancements allowed its *Renaissance* over the past decades and, consequently, its application to different environments and disciplines. In this first chapter, two papers are presented, in which the emergence of optical-based methods is analysed, together with the current gaps and future perspective its application in underwater scenarios.

In paper 1, "**Photogrammetry, from the land to the sea and beyond: a unifying approach to study terrestrial and marine scenarios**", the evolution of photogrammetrical survey applications was explored, paying special attention to the transfer of methods between terrestrial and underwater approaches. During the review effort of more than 2,000 articles, its coupling with other techniques together with spatial and temporal trends on its application were analysed.

Paper 2, "**Needs and Gaps in Optical Underwater Technologies and Methods for the Investigation of Marine Animal Forest 3D-Structural Complexity**" is the output of an international collaborative effort performed during the 3DSeaFor workshop, which aimed to build a network between marine ecologists and engineers, promoting a large interdisciplinary team to drive the next steps in the 3D monitoring of marine animal forest by means of optical-based approaches.

These two manuscripts contributed to summarise the optical techniques application' trends and identify possible future trends and research hot topics related to its application in ecosystems' monitoring efforts.

Paper 1

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Photogrammetry, from the land to the sea and beyond: a unifying approach to study terrestrial and marine scenarios

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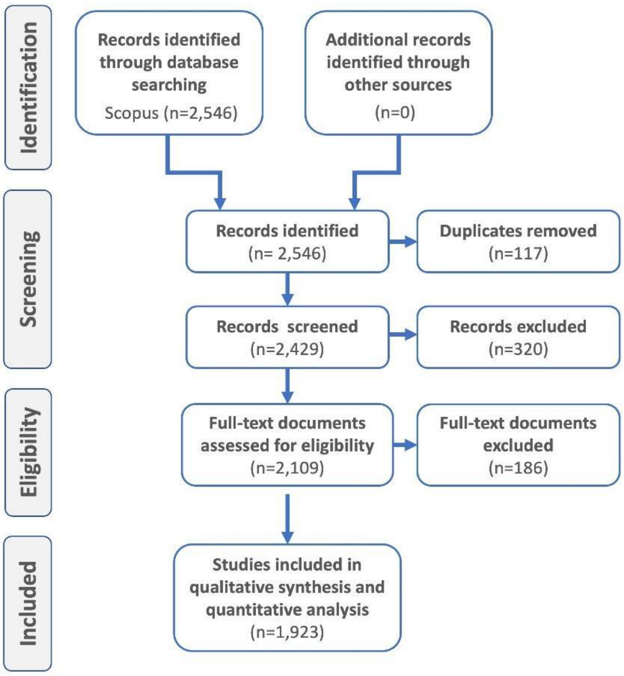

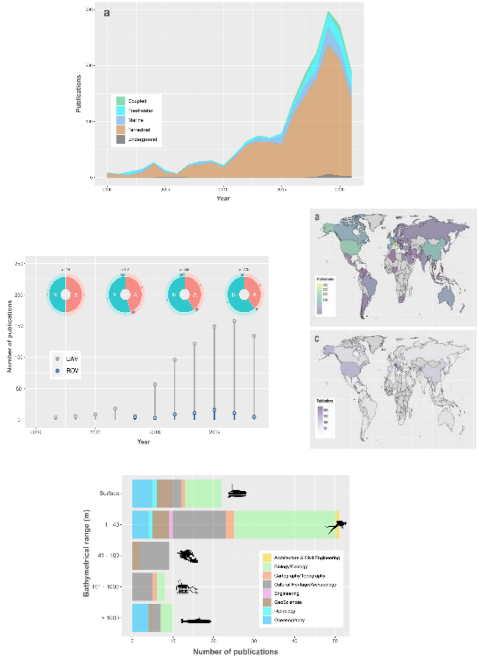

Supplementary material available at:

Review article

Photogrammetry, from the land to the sea and beyond: a unifying approach to study terrestrial and marine environments

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Abstract

The series of technological advances that occurred over the past two decades allowed photogrammetry-based approaches to achieve their actual potential, giving birth to one of the most popular and applied procedures: structure from motion (SfM). The technique expanded rapidly to different environments, from the early ground-based and aerial applications in terrestrial scenarios, to underground and underwater surveys. Nevertheless, the transfer through different media required a period of adaptation that could take anything from years to decades. Only recently, thanks to the emergence of low-cost versatile imaging systems, have airborne and underwater photogrammetry became approachable to a wide range of research budgets, resulting in a popular cost-effective solution for many disciplines. Although numerous review efforts have already been made to resume the current knowledge on photogrammetry, this review summarizes the evolution of the technique in both terrestrial and underwater environments, paying special attention to the transfer of methods and techniques between the two environments. The acquired information helped to identify trends during its development and to highlight the urgency to widen the range of its applications in aquatic habitats in order to fill the current gap of knowledge on their structure and species distribution, delaying the design of proper conservation strategies.

Keywords: optical methods, Structure from Motion, three-dimensional approaches, multidisciplinary, survey.

1. Introduction

The description of habitat complexity is the method we use to define the surrounding environment and to quantify the structural key elements of an ecosystem [1,2]. From the traditional bi-dimensional (2D) survey techniques to the relatively recent three-dimensional (3D) approaches, every method has accomplished its objective in its own historical and technological context. Among the pool of techniques available is photogrammetry, defined as “the art, science, and technology of obtaining reliable information about physical objects and the environment through processes of recording, measuring, and interpreting images and patterns of electromagnetic radiant energy and other phenomena” [3]. Although it has more than 170 years of history, various technological and methodological advancements have allowed for photogrammetry “Renaissance” over the past decades [4]. In fact, since its first documented cartographical applications by Laussedat in 1949, a series of turns of events pushed photogrammetry from its highly parameterized origins towards more optimized procedures, transforming it into the versatile tool it is today [5]. At its start, the rise of stereoscopy and the development of the airplane popularised the technique defining what is now called analogue photogrammetry [6]. In the 1940s, thanks to the arrival of computers and their increasingly widespread availability, fully analytical procedures were developed, leaving graphical solutions outdated and establishing the basis of modern photogrammetry. However, it was not until the appearance of digital imagery, together with the design of state-of-the-art algorithms (e.g., scale-invariant feature transform), that photogrammetry became such a powerful tool [7,8,9,10,11]. The subsequent optimization of pattern recognition algorithms and the exponential increase in computing power since the late 1990s finally allowed the methodology to achieve its actual potential, giving birth to one of the most popular and applied procedures: structure from motion (SfM) photogrammetry. With the appearance of SfM workflow, photogrammetric sampling and processing were facilitated considerably. Even though this procedure is based on stereoscopic principles, its main asset is its highly automated nature: the whole 3D geometry of the scene, including the camera orientation and positions, is solved through the implementation of a series of algorithms over a dataset of overlapping images [12].

The advent of more powerful workstations favoured the development of tailored commercial and open-source software, leading to easier access to the technique. With its increased potential and accessibility, it was only a matter of time before photogrammetry caught the attention of different disciplines, such as paleosciences [13,14,15], geological sciences [16,17,18], architecture [19,20,21], civil and industrial engineering [22,23,24], cultural heritage [25,26,27], and life sciences [1,28,29]. Photogrammetry’s main purpose was no longer cartography and topography, but instead to record and explore the world from a 3D perspective. Consequently, the technique expanded rapidly to different environments, from the early ground-based and aerial applications in terrestrial habitats [30,31,32], to underground [33,34] or underwater surveys [35,36], and even outer space [37]. Generally, this transfer through different media required a period of adaptation that could take years to decades, firstly to adapt the existing apparatus to these new environments, and secondly to develop equipment affordable to the different discipline's budget [38,39].

In the case of submerged environments, the first underwater photographs were taken in the 1850s [40], but we had to wait until 1978 for the first major photogrammetric underwater survey [41]. This delay corresponded to the development of tools that allowed for a reliable underwater implementation of the technique, such as the appearance of the diving apparatus and the rise in compact water-proof housing. However, only recently underwater imaging systems became economically affordable to the broad public, making underwater photogrammetry approachable to a wide range of research budgets and resulting in a popular cost-effective solution for many disciplines (e.g., archaeology, marine biology, oceanography, or engineering) [42,43,44].

Nowadays, similar patterns as the ones described below can be observed in the coupling of photogrammetry with various emerging technologies developed in the first place for terrestrial applications. One clear example is the merging of photogrammetry and laser-scanning data, which, through its combination, allow for overcoming some of the techniques' individual limitations (e.g., increase in accuracy for photogrammetry and include colour information in the case of laser-scanning) [18,26,27]. Although numerous review efforts have already been made to resume the current knowledge on photogrammetry [45,46,47,48,49,50,51,52,53], the main aim of this work is to summarise the evolution of the technique in both terrestrial and underwater environments, paying special attention to the transfer of methods and techniques between the two environments. The acquired information will help to identify trends during its development and to highlight the urgency needed to widen the range of applications in the aquatic habitats to fill the still current gap of knowledge on their structure and species distribution, delaying the design of proper conservation strategies.

2. Material and Methods

The review effort was conducted using the search procedure present in Table 1, following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement as a guide [54], and limiting it to the cut-off date of 31 January 2022. Documents complying with the inclusion criteria and containing the search term combination in the title, keywords, or abstract were included in the screening. Only publications written in English, Italian, French, or Spanish were considered for eligibility. Publications not containing any information on photogrammetric surveys were excluded. This strategy resulted in 2546 documents being found. To select which papers to include in the analysis, a three-step process was implemented, as follows: (i) duplicates were excluded, (ii) abstract screening was performed to identify potentially relevant manuscripts (Figure 1), and (iii) a full-text screening was conducted. Finally, a total of 1923 publications were retained for the quantitative analysis (Table S1).

Table 1. Research procedure and inclusion-exclusion criteria.

Years	1950 - 2021
Search terms	"Photogrammetry" AND ("mapping" OR "survey") AND ("terrestrial" OR" underwater")
Database	Scopus
Inclusion criteria	· Peer reviewed studies, conference papers and books · Studies testing photogrammetric applications · Published in English, Italian, French, or Spanish
Exclusion criteria	· Duplicated manuscripts · Studies not including photogrammetric surveys

From each of the included manuscripts, different information was obtained (Table 2A) and is presented as Supplementary Material Table S1. Regarding the specific details from each of the photogrammetric surveys types, the following details were extracted: (i) the branch of science in which the survey was framed; (ii) the environment in which the technique was applied; (iii) the specific habitat, structure, or object addressed; (iv) the nature of the surveyed item; (v) the type of implemented photogrammetry; (vi) if underwater, the maximum surveyed depth; (vii) the coupled methodology; and (vii) the location system (Table 2B; see Table S1 for categories). In all the analyses conducted on the literature, each publication could be included in one or more categories.

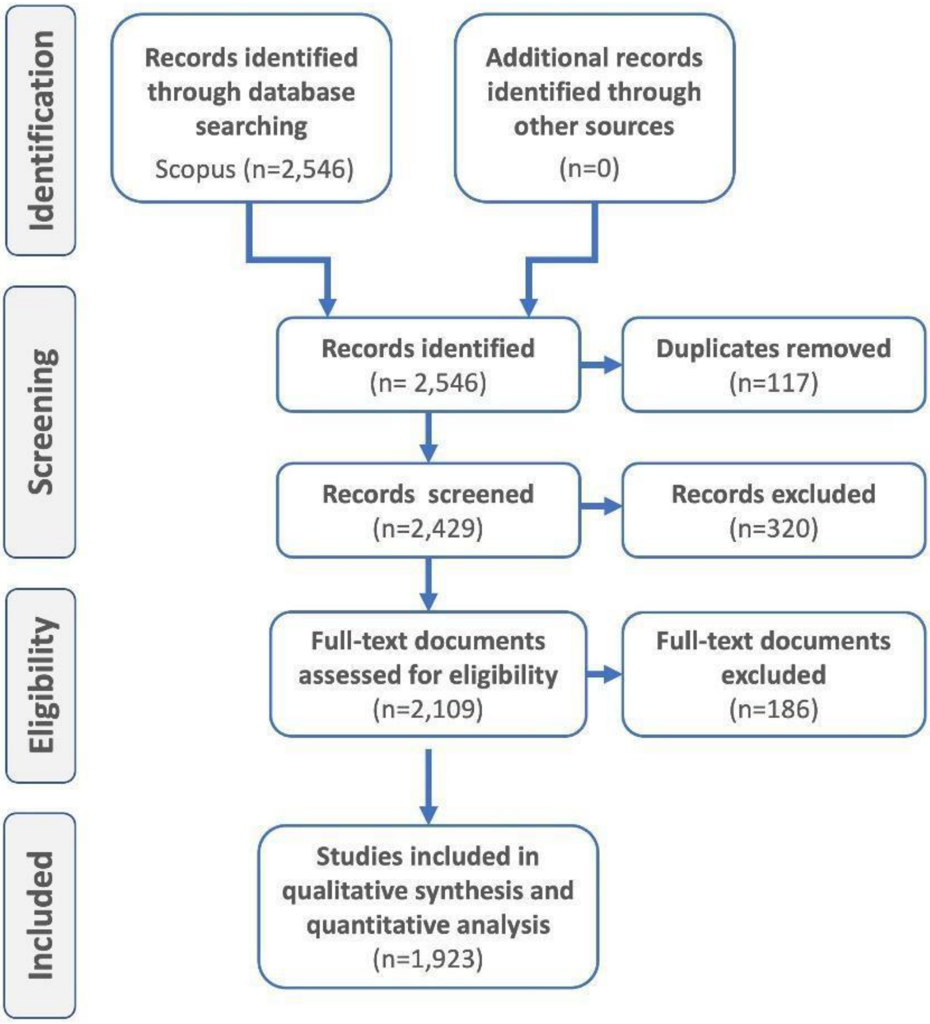

Figure 1. Flow diagram of the method and selection process implemented for this review effort.

Table 2. Parameters used to classify photogrammetric surveys during the data extraction.

Category	Definition
A) General features of the manuscript	
Year	Year of publication
Authors	Authors of the publication
Title	Title of the publication
DOI	DOI of the publication
Country	Country who funded the study

B) Features extracted from each survey	
Branch of science	Discipline in which it is applied the survey
Environment	Environment in which the survey has been performed: i) terrestrial; ii) marine; iii) freshwater; iv) terrestrial-aquatic; v) underground
Specific environment	Specific type of environment/structure/object surveyed
Nature scenario	Nature of the surveyed scenario: i) natural; ii) artificial
Sampling approach	Type of photogrammetry implemented: i) airborne; ii) ground-based; iii) underwater; iv) space-borne
Depth	If underwater, maximum depth at which the survey was performed
Coupled techniques	If applicable, complementary approach with which have been coupled the photogrammetry: i) laser-scan; ii) multi-spectral imaging; iii) thermal imaging; iv) acoustic techniques; v) tomography; vi) radiation; vii) machine learning
Coupled location system	If applicable, location system with which have been coupled the photogrammetry: i) global positioning system (GPS) & global navigation satellite system (GNSS); ii) mobile mapping system (MMS); iii) post-processing kinetics (PPK); iv) real time kinetics (RTK); v) simultaneous location and mapping (SLAM)
Vehicles	If applicable, vehicle used for the photogrammetry survey: i) ROV; ii) UAV; iii) Satellite

3. Results and Discussion

3.1.Photogrammetric surveys through time

The history of photogrammetry can be seen as a constant process of development and optimization [10], with a continuous widening of its application through time (Figure 2). Before 1960, only three articles were recorded, mainly for cartographical purposes [54,55,56]; however, since the 1960s the number of publications started to fluctuate until 2000, representing a breakpoint. From this year on, the growth of computing power and the emergence of new technologies [57] allowed for the spread of the technique. Among all types of photogrammetry, ground-based and airborne implementations were the ones that prevailed in the reviewed literature. Conversely, underwater applications started to be noticed just after 2010, with a peak in publications in 2019 (n = 35), likely thanks to the increasingly available low-cost waterproof compact cameras [58]. The general drop observed after this year (Figure 2) can probably be traced to two main reasons: (i) not all published manuscripts were already added to SCOPUS when the online research took place and (ii) the effects of the 2020 COVID-19 emergency in terms of field-work and mobility restrictions [59].

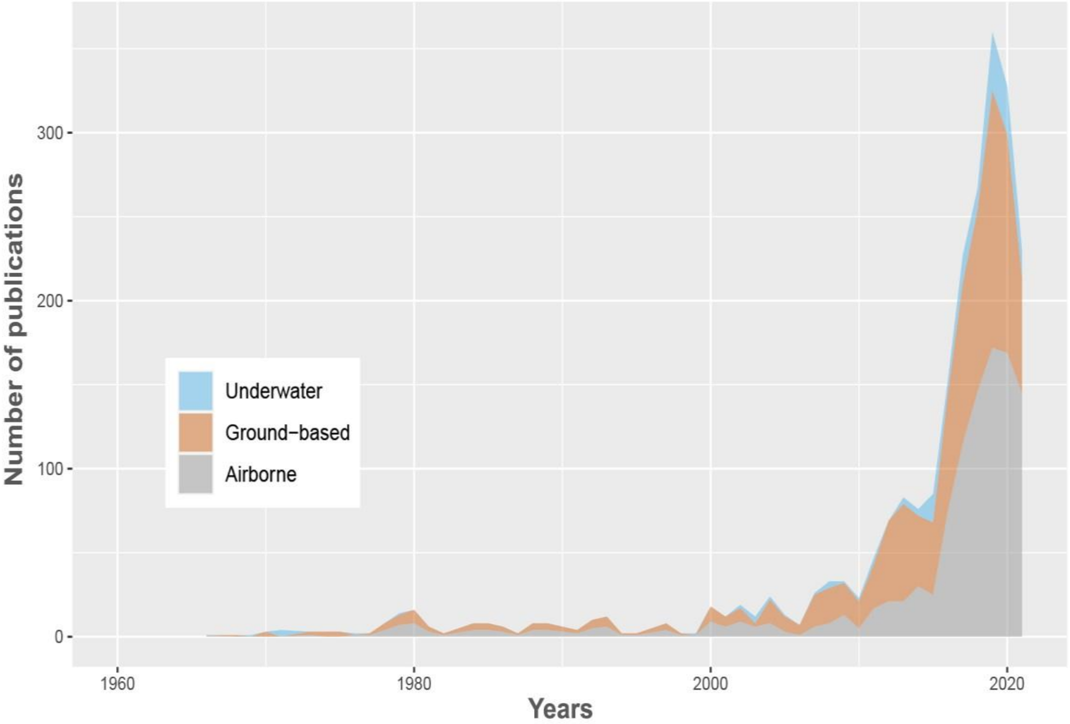

Figure 2. Area plot representing the number of publications per year in function of the type of photogrammetry applied.

3.2. Worldwide application of photogrammetry

In the past, the application of photogrammetry was polarised towards developed countries; however, since 2010, the accessibility and thus use of this technique became increasingly frequent [11]. Considering the total number of publications and the types of photogrammetry considered here, Italy was the country with more research groups focused on photogrammetric approaches (with 454, 296, 231, and 47 publications in general, ground-based, airborne, and underwater applications, respectively) (Figure 3a–d). Other countries highly involved in photogrammetric surveys were the USA, the UK, China, France, Spain, and Germany (Figure 3a–d). While airborne and ground-based studies showed a similar distribution worldwide, underwater implementations still seem to be mainly focused on Europe and the USA (Figure 3d). The almost complete lack of studies in developing countries regarding underwater approaches could be related to the relatively high implementation costs until recent years [58]. However, it may also be explained as an artefact of this analysis, as only the first author's affiliation was recorded to define the geographical distribution of the publications [60]. With the current commercialization of low-budget solutions [58,61], the number of publications in all three photogrammetry types is expected to increase, especially in developing countries.

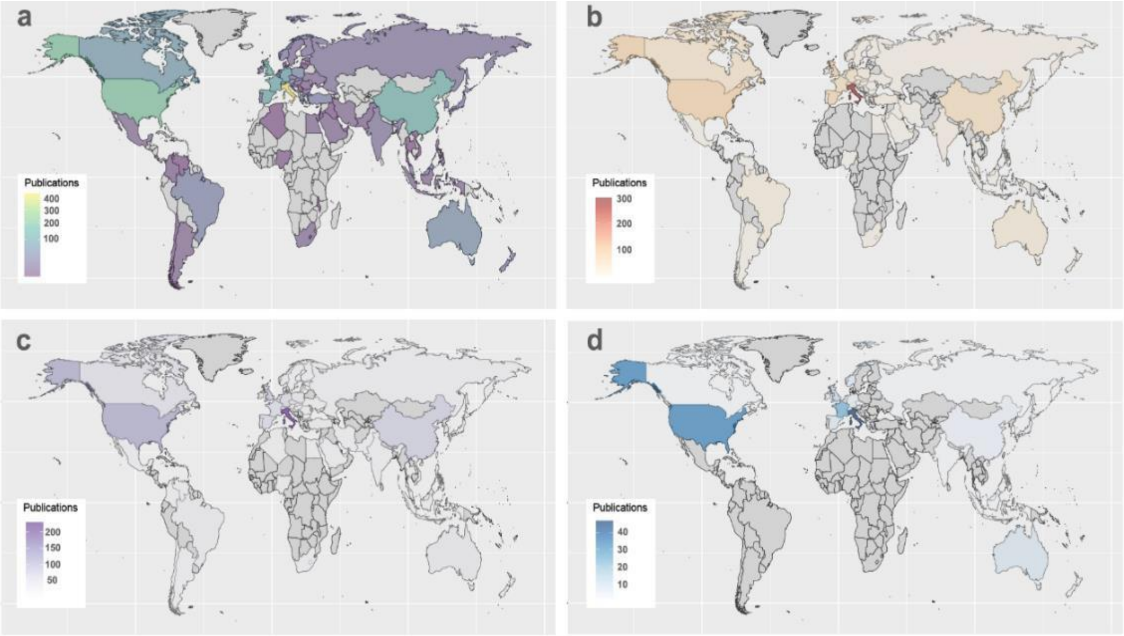

Figure 3. Geographical distribution of the countries who funded the study in the analysed publications: (a) total number of publications, (b) ground-based applications, (c) airborne-photogrammetry applications, and (d) underwater applications.

3.3.Photogrammetric surveys among disciplines

In agreement with Figure 2 and Figure 4, some of the disciplines have a longer history applying photogrammetry. Taking a close look to the heatmaps, it is possible to identify some of the "pioneer-disciplines", such as photogrammetry/geomatics, geosciences, and cartography/topography, which were already implementing all three photogrammetry types in the 1970s (Figure 4a–c). These early applications are clearly marked by the testing of the technique's accuracy or its potential uses, especially for mapping purposes e.g., [62,63].

Even though it is evident how all three technique typologies benefited from an increase in the number of studies after 2010, airborne approaches showed the most marked growth in this latest period, reaching a wider range of disciplines (Figure 4b). This is directly related with the development and popularisation of small unmanned aerial vehicles (UAVs), which drastically decreased the cost of aerial surveys, previously performed by manned aircrafts [64]. The other two typologies, on the other hand, seemed to have been applied in more specific contexts so far, with a few areas of science monopolising their implementation (Figure 4a,c). In fact, the disciplines showing a predilection for ground-based approaches are cultural heritage/archaeology (164), geosciences (87), and architecture/civil engineering (78) (Figure 4a), which benefited from the opportunity to monitor scenarios from a 3D perspective, a useful approach in terms of structure integrity assessments [65,66,67]. Concerning cultural heritage, photogrammetry also allowed for the possibility for the development of virtual repositories, an emerging tool in constant evolution thanks to its huge potential in terms of science transfer and education [68,69]. In the matter of underwater applications, Biology/Ecology (46) and Cultural Heritage/Archaeology (28) were the disciplines mostly exploiting the technique (Figure 4c). This fact reflects the increasing interest of Life Sciences in the study and monitoring of underwater habitats' structural complexity and organisms' distributions [1,35,70,71]. Additionally, in terms of underwater cultural heritage, as well as land-based approaches, the popularisation of image-based techniques for the digitalization of historical sites also occurred. In fact, the number of 3D reconstructions and immersive experiences of archaeological sites skyrocketed over the past years [42,72], with a special emphasis on wreck scenarios [73,74,75,76].

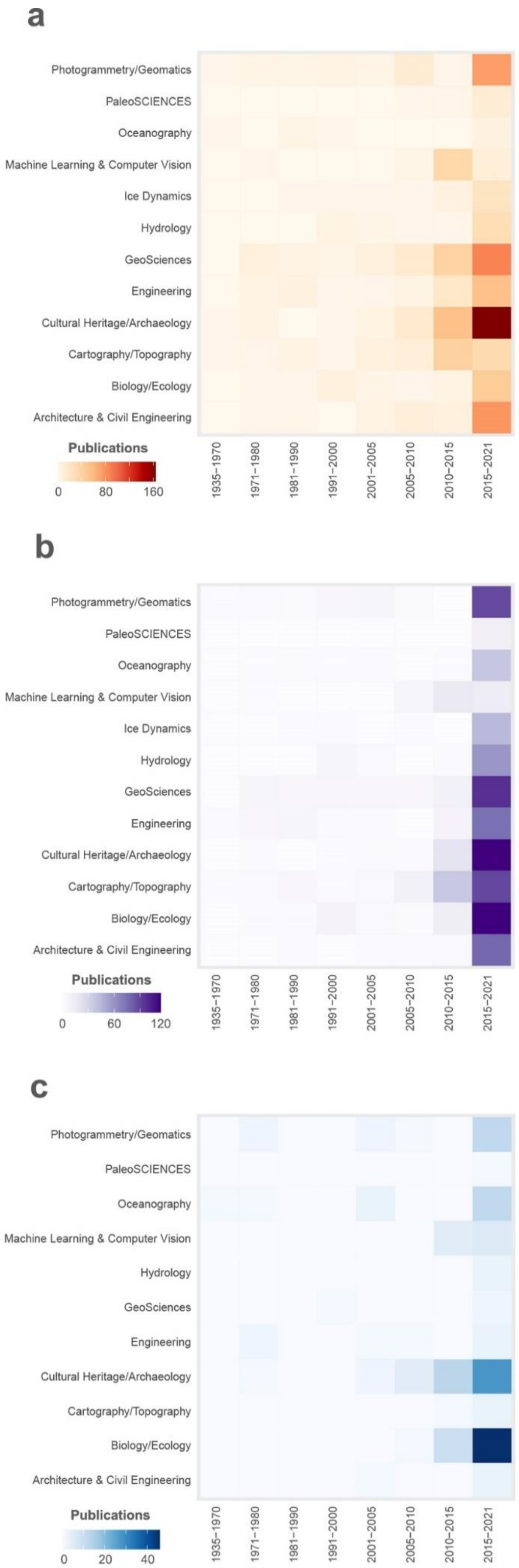

Figure 4. Number of publications per year and the discipline in which the study was framed. (a) Ground-based applications, (b) airborne-photogrammetry applications, and (c) underwater.

3.4. Environments surveyed by photogrammetry

As photogrammetry allows for cost-effectively evaluating a wide range of scenarios at different scales, it has rapidly expanded through different environments along history [1,77,78]. To analyse the scenarios covered by photogrammetric surveys, we focussed our analysis during the period containing most of the publications (2000–2021). A clear general dominance on terrestrial environments' surveys have been recorded (Figure 5a). Although after 2012 a promising increase occurred in the number of studies covering aquatic environments, the total number of works is still quite small (n = 209) (Figure 5a). Coupled and underground approaches are instead still marginal, a fact possibly linked to the purposes of these studies, which mainly focussed on coastal areas [79,80] and cave systems [81,82], respectively.

A deeper analysis was also performed considering the two decades separately, in order to understand which scenarios were assessed (Figure 5b,c). Construction surveys prevailed in terrestrial environments for both decades, driven by its rising application in cultural heritage and architectural studies (Figure 5b,c). Even though the ratio terrestrial–aquatic did not change significantly among the decades, a higher representation of natural scenarios could be detected (Figure 5b,c), with the emergence of forests monitoring activities in land, of ice dynamic research in fresh-water media, and coral reefs in marine habitats (Figure 5c).

Environmental sciences have always acknowledged the importance of 3D complexity as a main driver of ecosystem functionality, but it is only now that we have the tools to properly quantify it [83,84,85]. The current decade (2021–2030) has been defined by the United Nations as the Decade for Ecosystem Restoration, during which landscape approaches will be promoted to prevent and reverse ecosystem degradation in terrestrial, freshwater, and marine environments [86]. Huge monitoring efforts will be necessary to properly assess their status and the effectiveness of the restoration actions [86].

The marked increase in the number and diversity of photogrammetric approaches in life and environmental sciences suggests the technique as a suitable candidate to cost-effectively survey wide natural areas, monitoring changes from both a 2D and a 3D perspective and assessing features previously oversighted, such as structural complexity.

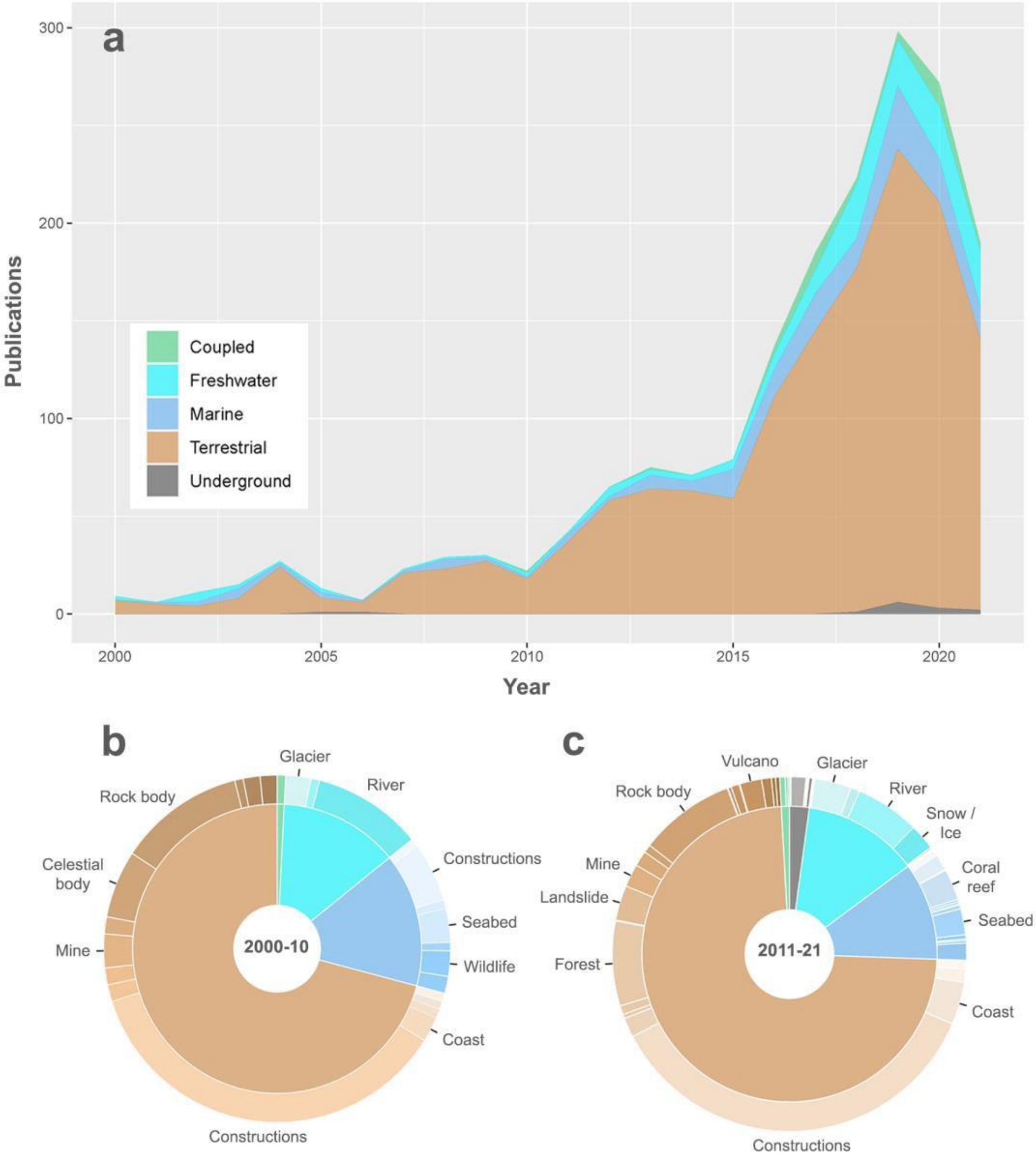

Figure 5. Type of environments addressed during the surveys over the past two decades: (a) temporal trend; (b) ring chart representing the percentage of surveys performed per environment from 2000 to 2010; (c) ring chart representing the percentage of surveys performed per environment from 2011 to 2021.

3.5. Bathymetric distribution of underwater surveys

Most of the studies assessing aquatic systems mainly address depths accessible by recreational diving [87], leaving our oceans largely unexplored, with only a few sparse pieces of information collected about the deep sea [88]. Although huge international efforts are being made to cover these gaps [89], there is still much work to do before we have a complete map of our oceans' floors. Indeed, we observed how most of the underwater surveys were performed between the surface and 40 m depth (78), leaving deeper areas highly underrepresented (Figure 6), with the deepest survey reaching 3659 m [78]. Biological studies (36) were the most abundant from the surface down to 40 m depth, mainly tackling coral reef ecology (see Table S1 for references). Below this bathymetry, archaeological surveys were the ones prevailing in the literature, mostly addressing wreck remains (see Table S1 for references). However, up to 45% of the total documents assessing underwater scenarios could not be considered for the creation of Figure 6 as they did not provide any specific information about the depth range covered by the survey's activities (Table S1). Nonetheless, the highlighted gaps of knowledge are expected to be gradually filled by a combination of: (i) the continuous reduction in the operational costs of remote operated vehicles (ROVs) [90,91]; (ii) the increased frequent involvement of the technical diving community through citizen science projects [87,91,92,93,94]; (iii) the international legislations (e.g., Marine Life 2030 and other various Ocean Decade programs) aiming to increase the number of monitoring plans worldwide to create a global network and community of practice for the observation and forecasting of marine life [95]; and (iv) the international effort being performed in the framework of the GEBCO Seabed 2030 project, which aims to map the whole oceans by 2030 [89].

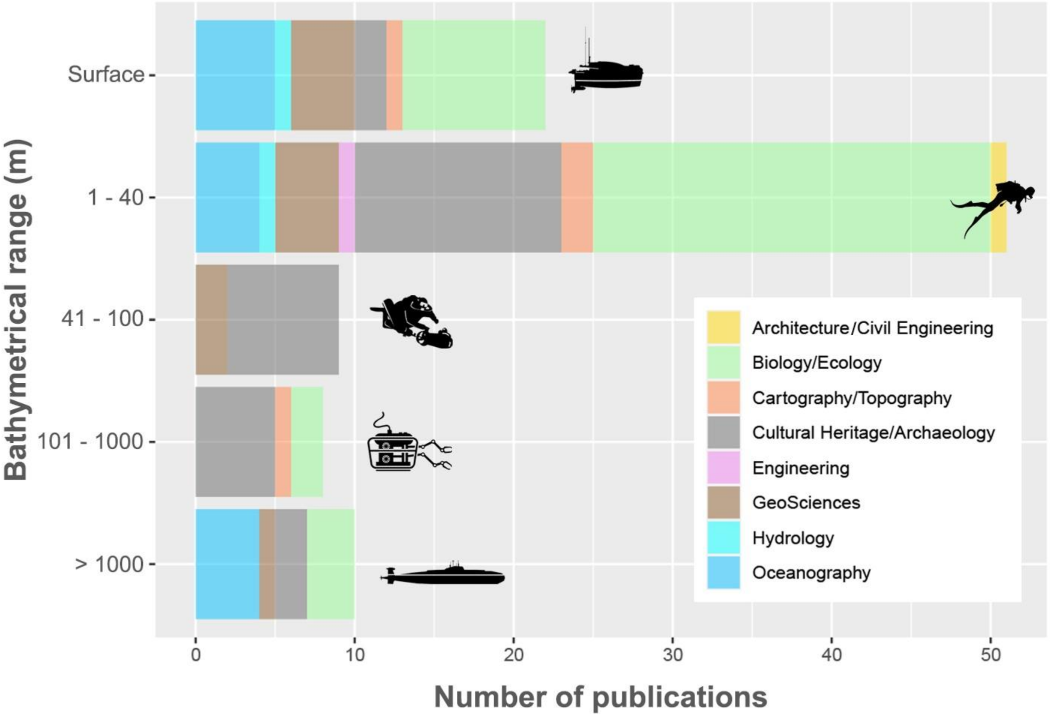

Figure 6. Bathymetric distribution of underwater photogrammetric approaches with the relative disciplines of implementation. Icons indicate the main but not exclusive tools applied at each depth range.

3.6. The revolution of unmanned vehicles

Thanks to the development of powerful micro-computers along with the downsizing of remote sensing devices, the survey costs of remotely control systems have dropped continuously in the past years, persuading more and more disciplines towards the everyday use of these robotic systems [60,90,96]. To analyse this phenomenon, we decided to focus on the period from 2010 to 2021, as our research strategy only included six studies before 2010 (see Table S1 for references). The growing interest in the use of unmanned vehicles is reflected in Figure 7. Unmanned aerial vehicles (UAVs) found rapid popularization and implementation, substituting the more traditional airborne approaches and considerably reducing the sampling costs [97]. The drastic increase after 2015 of aerial drone-based surveys (Figure 7) was related to the relatively rapid commercialization of low-cost systems [63,97], while their underwater equivalent (i.e., ROVs) was still struggling with the development of more accessible systems due to the intrinsic challenges and logistic constraints of underwater robotics [91,98]. For this reason, the total number of ROV surveys found by our review strategy was quite low (n = 59), even though a slight growth in their use was observed in the last few years (Figure 7). In fact, ROVs are mainly used by offshore oil and gas companies for inspection, maintenance, and repair of their infrastructures [98]. Nonetheless, the release of more approachable priced apparatus allowed for predicting a rise in the application of ROVs by the scientific community, and with it, an opportunity to increase the knowledge on deep environments, helping with the implementation of nature-based management and conservation strategies.

In terms of the type of surveyed scenarios in the last decade, an increase in the number of studies addressing natural environments was observed compared with artificial scenarios, especially when UAVs were used (Figure 7). Nowadays, the coupling of cutting-edge technologies (e.g., multi-spectral imagery, laser-scan, and thermal imagery) with UAVs, have opened new possibilities, becoming the tool of choice for small and medium scale surveys for many different user groups [51,99,100].

Autonomous underwater vehicles (AUVs) were not considered for plotting Figure 7 due to the small number of studies included in this review (5), even though they are now a hot topic in research [46,101], especially for monitoring purposes. It is expected that the same technological advances contributing to the appearance of low-cost ROVs will price down these high-budget pieces of equipment as well [91]. In fact, these systems, together with machine learning approaches and real time photogrammetry, will be a promising research line for the forthcoming years.

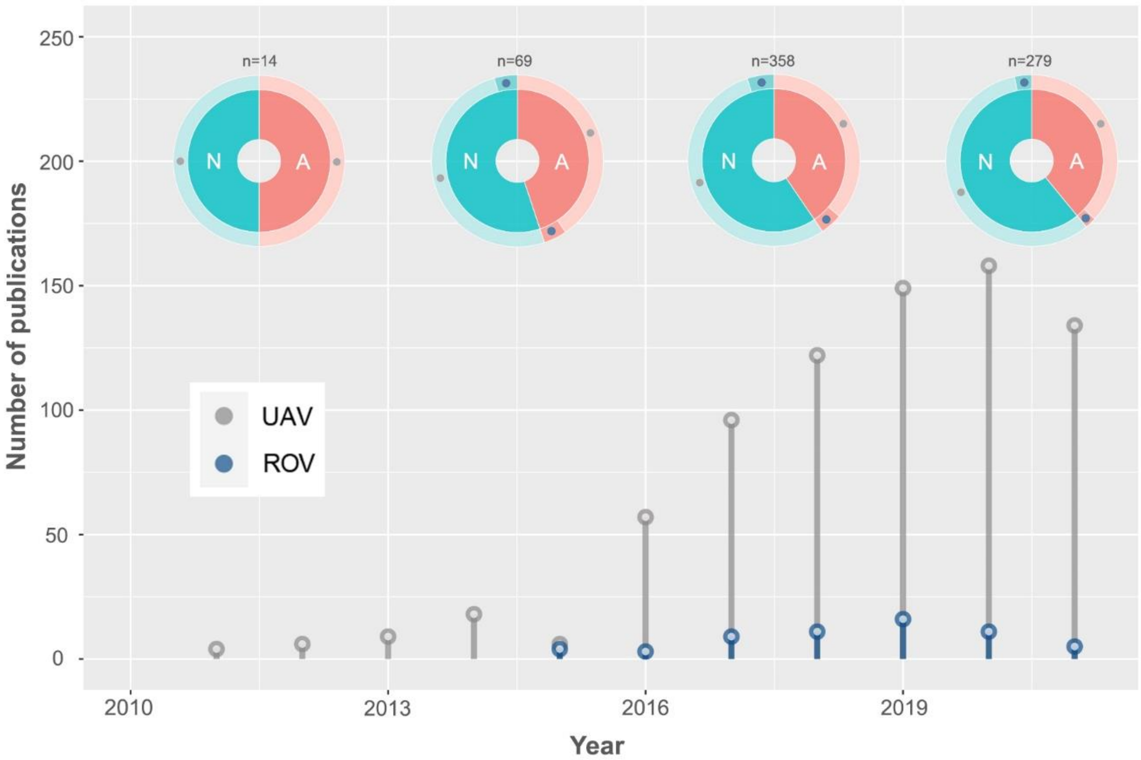

Figure 7. Lolly plot presenting the use of unmanned aerial vehicles (UAVs) and remotely operated vehicles (ROVs) in the past 21 years. The ring plots on the upper part of the figure show the percentage of studies covering natural (N, in bluish) or artificial scenarios (A, in red) for each three-year period. n = number of total studies.

3.7.Coupling of Photogrammetry with other techniques

Another interesting aspect of photogrammetry is its versatility to be coupled with a wide range of complementary approaches (Figure 8). By analysing the literature, a repeated pattern could be identified (Figure 8), in which the first trials on technique-coupling were often performed to control the accuracy of photogrammetry or to test a possible data merging among techniques [102,103]. Over the years, these combinations raised some interesting approaches, occasionally ending up in the following:

(i) The development of new methodologies, such as multispectral photogrammetry [100,104] or thermal photogrammetry [105];

(ii) The increase in the accuracy and resolution of the technique (such as laser-scanner or real time kinetics) [106,107,108,109];

(iii) The approach to new environments through the use of ROVs or AUVs [27,34,110,111].

After 2016, a great increase in the number of surveys using coupled approaches could be identified, with laser-scanning being the most paired technique (Figure 8), reaching great results through merging the textures obtained from the RGB imagery with the depth maps produced by the laser-scanning [112]. Its coupling with location systems (e.g., real time kinetics, simultaneous localization, mapping, and Global Navigation Satellite Systems) also gained some adaptions over time (Figure 8), representing a great improvement in the georeferencing process of digital reconstructions [21,113,114,115,116]. Conversely, coupling with other techniques, such as acoustic systems and multispectral or thermal imagery, still nowadays remains low (Figure 8), mainly due to their specificity, thus reducing their application to a few disciplines [99,115].

Regarding the transfer of these coupled approaches to the underwater domain, while some of them are already widely applied (e.g., multibeam, radar, and sonar) [76,117], others are still poorly implemented due to the high costs of the required equipment (e.g., multispectral imagery or underwater location systems) or the limitations of specific techniques (e.g., the fast extinction of near-IR wavelengths in underwater environments for thermal imagery) [118,119]. There are still some challenges to overcome before they can become suitable candidates for low-budget underwater survey plans. Nonetheless, the rapid development of new technologies and the growing interest in aquatic environments create the perfect framework to keep investing in the implementation of non-invasive, low-cost, multi-sensor approaches, thus contributing to the establishment of standardised monitoring techniques in a multidisciplinary scenario.

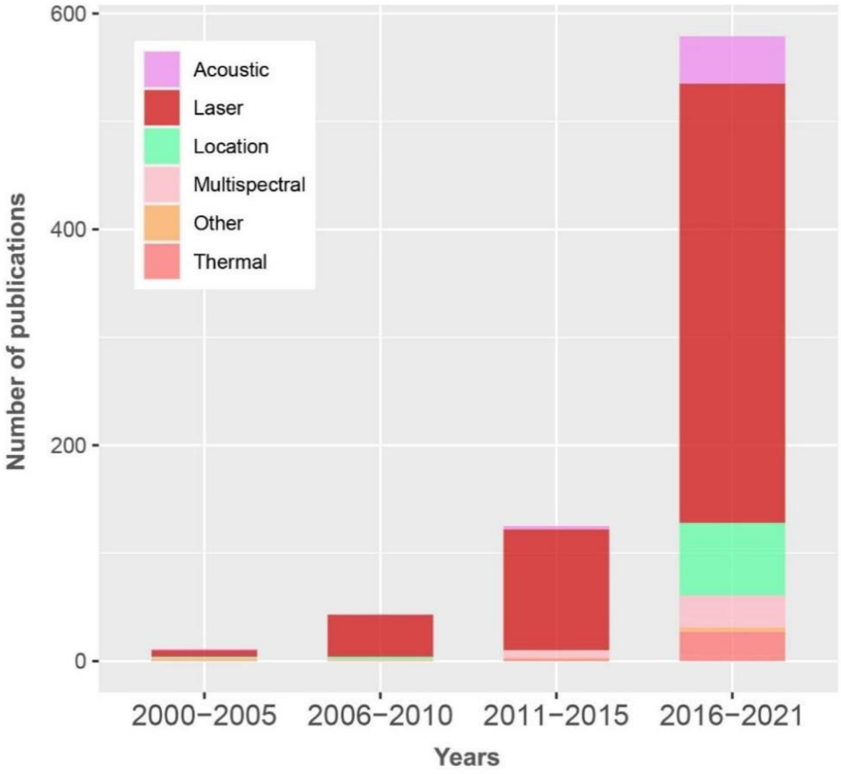

Figure 8. Stacked bar plot showing the number of publications in which photogrammetry was coupled with other techniques during survey activities.

4. Conclusions

This study summarises past and current trends in photogrammetry applications in both terrestrial and aquatic environments. Over the past decades, this technique has provided a cost-effective solution for different disciplines to approach a wide range of scenarios that are more and more frequently represented by natural environments. In this context, the inclusion of SfM photogrammetry in monitoring programs should be considered to accurately describe habitats' structural complexity, creating temporal baselines fundamental to understanding and measuring possible changes over time, and thus possibly helping lawmakers in designing ad hoc nature-based conservation and protection plans. The continuous development of new technologies has allowed for couple photogrammetry with other techniques, capturing global complexity as never before. Given the current tendencies, an exponential increase in its application both in terrestrial and aquatic environments can be expected. Advances in the field of machine learning classifiers, cloud computing, and unmanned vehicles will play a key role in upscaling and automatization of the technique.

Underwater photogrammetric approaches still have a long road to go before reaching their full potential. The appearance of more economic underwater location systems and ROVs will contribute to the popularization of photogrammetric surveys for medium and large-scale assessments. Nonetheless, at local scale, the wide-spread use of low-cost compact cameras allows photogrammetry to be included in marine citizen science programs (e.g., protocol 11 of the Interreg MED MPA Engage project, [120]), highlighting the high versatility of the technique and its huge potential in public engagement.

To conclude, there is an urgent need for the implementation of non-invasive, cost- effective techniques, and SfM photogrammetry represents a valuable example that could allow us to look at marine ecosystems from a multiscale integrative perspective, which is fundamental for the exploration of seascapes and the design of effective and tailored conservation measures.

References

1. Burns, J.H.R.; Delparte, D.; Gates, R.D.; Takabayashi, M. Utilizing underwater three-dimensional modeling to enhance ecological and biological studies of coral reefs. Int. Arch. Photogramm. Remote Sens. Spat. Inf. Sci. 2015, XL-5/W5, 61–66.
2. Monfort, T.; Cheminée, A.; Bianchimani, O.; Drap, P.; Puzenat, A.; Thibaut, T. The Three-Dimensional Structure of Mediterranean Shallow Rocky Reefs: Use of Photogrammetry-Based Descriptors to Assess Its Influence on Associated Teleost Assemblages. Front. Mar. Sci. 2021, 8, 639309.
3. McGlone, C.; Mikhail, E.M.; Bethel, J.S.; Mullen, R. Manual of Photogrammetry, 5th ed.; American Society for Photogrammetry and Remote Sensing: Bethesda, MD, USA, 2004.
4. Kovalenko, K.E.; Thomaz, S.M.; Warfe, D.M. Habitat complexity: Approaches and future directions. Hydrobiologia 2012, 685, 1–17.
5. Ferrari, R.; McKinnon, D.; He, H.; Smith, R.N.; Corke, P.; González-Rivero, M.; Mumby, P.J.; Upcroft, B. Quantifying Multiscale Habitat Structural Complexity: A Cost-Effective Framework for Underwater 3D Modelling. Remote Sens. 2016, 8, 113.
6. Polidori, L. On Laussedat's contribution to the emergence of photogrammetry. ISPRS-Int. Arch. Photogramm. Remote Sens. Spat. Inf. Sci. 2020, XLIII-B2-2020, 893–899.
7. Konecny, G. The International Society for Photogrammetry and Remote Sensing—75 Years Old, or 75 Years Young. Photogramm. Photogramm. Eng. Remote Sens. 1985, 51, 919–933.
8. Gosh, S. History of Photogrammetry; Laval University: Quebec City, QC, Canada, 1981.
9. Lowe, D.G. Object recognition from local scale-invariant features. In Proceedings of the Seventh IEEE International Conference on Computer Vision, Kerkyra, Greece, 20–27 September 1999; Volume 2, pp. 1150–1157.
10. Ghosh, S.K. Fundamentals of Computational Photogrammetry; Concept Publishing Company: Delhi, India, 2005.
11. Paris, L. Fotogrammetria e/o fotomodellazione. Geom. Desc. Rapp. Dig. 2012, 55–62.
12. Westoby, M.; Brasington, J.; Glasser, N.F.; Hambrey, M.J.; Reynolds, J.M. 'Structure-from-Motion' photogrammetry: A low-cost, effective tool for geoscience applications. Geomorphology 2012, 179, 300–314.
13. Nyimbili, P.H.; Demirel, H.; Seker, D.Z.; Erden, T. Structure from Motion (SfM)—Approaches & applications. In Proceedings of the International Scientific Conference on Applied Sciences, Antalya, Turkey, 27–30 September 2016.
14. Remondino, F.; Rizzi, A.; Girardi, S.; Petti, F.M.; Avanzini, M. 3D Ichnology-recovering digital 3D models of dinosaur footprints. Photogramm. Rec. 2010, 25, 266–282.
15. Bonali, F.L.; Tibaldi, A.; Corti, N.; Fallati, L.; Russo, E. Reconstruction of Late Pleistocene-Holocene Deformation through Massive Data Collection at Krafla Rift (NE Iceland) Owing to Drone-Based Structure-from-Motion Photogrammetry. Appl. Sci. 2020, 10, 6759.
16. Li, X.; Xiong, B.; Yuan, Z.; He, K.; Liu, X.; Liu, Z.; Shen, Z. Evaluating the Potentiality of Using Control-free Images from a Mini Unmanned Aerial Vehicle (UAV) and Structure-from-Motion (SfM) Photogrammetry to Measure Paleoseismic Offsets. Int. J. Remote Sens. 2021, 42, 2417–2439.
17. Haneberg, W.C. Using close range terrestrial digital photogrammetry for 3-D rock slope modeling and discontinuity mapping in the United States. Bull. Eng. Geol. Environ. 2008, 67, 457–469.

18. Kolzenburg, S.; Favalli, M.; Fornaciai, A.; Isola, I.; Harris, A.J.L.; Nannipieri, L.; Giordano, D. Rapid Updating and Improvement of Airborne LIDAR DEMs Through Ground-Based SfM 3-D Modeling of Volcanic Features. IEEE Trans. Geosci. Remote Sens. 2016, 54, 6687–6699.
19. Caravaca, G.; Le Mouélic, S.; Mangold, N.; L'Haridon, J.; Le Deit, L.; Massé, M. 3D digital outcrop model reconstruction of the Kimberley outcrop (Gale crater, Mars) and its integration into Virtual Reality for simulated geological analysis. Planet. Space Sci. 2020, 182, 104808.
20. Carnevali, L.; Ippoliti, E.; Lanfranchi, F.; Menconero, S.; Russo, M.; Russo, V. Close-range mini-UAVs photogrammetry for architecture survey. Int. Arch. Photogramm. Remote Sens. Spat. Inf. Sci. 2018, XLII-2, 217–224.
21. Picon-Cabrera, I.; Garcia-Gago, J.M.; Sanchez-Aparicio, L.J.; Rodriguez-Gonzalvez, P.; Gonzalez-Aguilera, D. On the Use of Historical Flights for the Urban Growth Analysis of Cities Through Time: The Case Study of Avila (Spain). Sustainability 2020, 12, 4673.
22. Martinez, J.G.; Albeaino, G.; Gheisari, M.; Volkmann, W.; Alarcón, L.F. UAS Point Cloud Accuracy Assessment Using Structure from Motion–Based Photogrammetry and PPK Georeferencing Technique for Building Surveying Applications. J. Comput. Civ. Eng. 2021, 35, 05020004.
23. Rogers, J.D. Hoover dam: Evolution of the dam's design. In Hoover Dam: 75th Anniversary History Symposium, Proceedings of the Hoover Dam 75th Anniversary History Symposium, Las Vegas, NV, USA, 21–22 October 2010; Las Vegas, NV, USA, 21–22 October 2010, American Society of Civil Engineers: Reston, VA, USA, 2012; pp. 85–123.
24. Hackl, J.; Adey, B.T.; Woźniak, M.; Schümperlin, O. Use of Unmanned Aerial Vehicle Photogrammetry to Obtain Topographical Information to Improve Bridge Risk Assessment. J. Infrastruct. Syst. 2018, 24, 04017041.
25. Balsa-Barreiro, J.; Fritsch, D. Generation of 3D/4D photorealistic building models. The testbed area for 4D Cultural Heritage World Project: The historical center of Calw (Germany). In Advances in Visual Computing, Proceedings of the 11th International Symposium, ISVC 2015, Las Vegas, NV, USA, 14–16 December 2015; Springer International Publishing: Cham, Switzerland, 2015; Volume 11, pp. 361–372.
26. Balsa-Barreiro, J.; Fritsch, D. Generation of visually aesthetic and detailed 3D models of historical cities by using laser scanning and digital photogrammetry. Digit. Appl. Archaeol. Cult. Heritage 2018, 8, 57–64.
27. Owda, A.; Balsa-Barreiro, J.; Fritsch, D. Methodology for digital preservation of the cultural and patrimonial heritage: Generation of a 3D model of the Church St. Peter and Paul (Calw, Germany) by using laser scanning and digital photogrammetry. Sens. Rev. 2018, 38, 282–288.
28. Pierdicca, R.; Intrigila, C.; Piccinini, F.; Malinverni, E.S.; Giannetti, I.; Caruso, G. Multidisciplinary Approach for the Analysis of Structural Heritage at Risk: The Case Study of Stylite Tower at Umm ar-Rasas (Jordan). Int. J. Arch. Heritage 2021, 1–25.
29. Laba, M.; Tsai, F.; Ogurcak, D.; Smith, S.; Richmond, M.E. Field Determination of Optimal Dates for the Discrimination of Invasive Wetland Plant Species Using Derivative Spectral Analysis. Photogramm. Eng. Remote Sens. 2005, 71, 603–611.
30. Urbina-Barreto, I.; Garnier, R.; Elise, S.; Pinel, R.; Dumas, P.; Mahamadaly, V.; Facon, M.; Bureau, S.; Peignon, C.; Quod, J.-P.; et al. Which Method for Which Purpose? A Comparison of Line Intercept Transect and Underwater Photogrammetry Methods for Coral Reef Surveys. Front. Mar. Sci. 2021, 8, 636902.
31. Hagan, T. A case for terrestrial photogrammetry in deep-mine rock structure studies. Int. J. Rock Mech. Min. Sci. Géoméch. Abstr. 1980, 17, 191–198.

32. Kappenberger, G.; Steinegger, U.; Braun, L.N.; Kostka, R. Recent changes in glacier tongues in the Langtang Khola basin, Nepal, determined by terrestrial photogrammetry. IAHS Publ.-Publ. Int. Assoc. Hydrol. Sci. 1993, 218, 95–102.
33. Rodríguez, M.S.C.; Maidagan, D.G.; González-Aguilera, D.; Nieto, L.M.; Gómez-Lahoz, J.; Herrero, J.S. Nouveaux regards sur la Grotte de La Peña (San Román de Candamo, Asturies, Espagne). L'anthropologie 2011, 115, 384–424.
34. Alessandri, L.; Baiocchi, V.; Del Pizzo, S.; Di Ciaccio, F.; Onori, M.; Rolfo, M.F.; Troisi, S. Three-dimensional survey of Guattari cave with traditional and mobile phone cameras. ISPRS-Int. Arch. Photogramm. Remote Sens. Spat. Inf. Sci. 2019, XLII-2/W11, 37–41.
35. Pizarro, O.; Eustice, R.; Singh, H. Large area 3D reconstructions from underwater surveys. In Oceans' 04 MTS/IEEE Techno-Ocean'04 (IEEE Cat. No. 04CH37600); IEEE: New York, NY, USA, 2004; Volume 2, pp. 678–687.
36. Palma, M.; Casado, M.R.; Pantaleo, U.; Pavoni, G.; Pica, D.; Cerrano, C. SfM-Based Method to Assess Gorgonian Forests (Paramuricea clavata (Cnidaria, Octocorallia)). Remote Sens. 2018, 10, 1154.
37. Liu, Z.; Peng, M.; Di, K. A continuative variable resolution digital elevation model for ground-based photogrammetry. Comput. Geosci. 2014, 62, 71–79.
38. Rebikoff, D. History of underwater photography. Photogramm. Eng. 1967, 33, 897–904.
39. Capra, A.; Castagnetti, C.; Dubbini, M.; Gruen, A.; Guo, T.; Mancini, F.T.; Neyer, F.; Rossi, P.; Troyer, M. High Accuracy Underwater Photogrammetric Surveying. In Proceedings of the 3rd IMEKO International Conference on Metrology for Archeology and Cultural Heritage, Lecce, Italy, 23–25 October 2017.
40. Martínez, A. "A souvenir of undersea landscapes:" underwater photography and the limits of photographic visibility, 1890–1910. História Ciências Saúde-Manguinhos 2014, 21, 1029–1047.
41. Welsh, N.; Leadbetter, I.K.; Cheffins, O.W.; Hall, H.M. Photogrammetric procedures for a North Sea oil rig leg repair. Int. Arch. Photogramm. 1980, 23, 474–483.
42. Lucarelli, A.; Brandolini, P.; Corradi, N.; De Laurentiis, L.; Federici, B.; Ferrando, I.; Lanzone, A.; Sguerso, D. Potentialities of integrated 3D surveys applied to maritime infrastructures and to the study of morphological/sedimentary dynamics of the seabed. In Proceedings of the IMEKO TC-19 International Workshop on Metrology for the Sea, Genoa, Italy, 3–5 October 2019; pp. 3–5.
43. Barrile, V.; Pucinotti, R.; Bilotta, G. Geomatics and Virtual Reality Techniques for Underwater Heritage. In INTERNATIONAL SYMPOSIUM: New Metropolitan Perspectives; Springer: Cham, Switzerland, 2020; pp. 1598–1607.
44. Cahyono, A.B.; Wibisono, A.C.; Saptarini, D.; Permadi, R.I.; Budisusanto, Y.; Hidayat, H. Underwater Photogrammetry Application for Coral Reef Mapping and Monitoring. Int. J. Adv. Sci. Eng. Inf. Technol. 2020, 10, 293–297.
45. Bemis, S.P.; Micklethwaite, S.; Turner, D.; James, M.R.; Akciz, S.; Thiele, S.T.; Bangash, H.A. Ground-based and UAV-Based photogrammetry: A multi-scale, high-resolution mapping tool for structural geology and paleoseismology. J. Struct. Geol. 2014, 69, 163–178.
46. Stone, C.; Webster, M.; Osborn, J.; Iqbal, I.A. Alternatives to LiDAR-derived canopy height models for softwood plantations: A review and example using photogrammetry. Aust. For. 2016, 79, 271–282.
47. Menna, F.; Agrafiotis, P.; Georgopoulos, A. State of the art and applications in archaeological underwater 3D recording and mapping. J. Cult. Heritage 2018, 33, 231–248.

48. Aicardi, I.; Chiabrando, F.; Lingua, A.M.; Noardo, F. Recent trends in cultural heritage 3D survey: The photogrammetric computer vision approach. J. Cult. Herit. 2018, 32, 257–266.
49. Iglhaut, J.; Cabo, C.; Puliti, S.; Piermattei, L.; O'Connor, J.; Rosette, J. Structure from Motion Photogrammetry in Forestry: A Review. Curr. For. Rep. 2019, 5, 155–168.
50. Chemisky, B.; Menna, F.; Nocerino, E.; Drap, P. Underwater Survey for Oil and Gas Industry: A Review of Close Range Optical Methods. Remote Sens. 2021, 13, 2789.
51. Deliry, S.I.; Avdan, U. Accuracy of Unmanned Aerial Systems Photogrammetry and Structure from Motion in Surveying and Mapping: A Review. J. Indian Soc. Remote Sens. 2021, 49, 1997–2017.
52. Śledź, S.; Ewertowski, M.W.; Piekarczyk, J. Applications of unmanned aerial vehicle (UAV) surveys and Structure from Motion photogrammetry in glacial and periglacial geomorphology. Geomorphology 2021, 378, 107620.
53. Zahari, N.M.; Karim, M.A.A.; Nurhikmah, F.; Aziz, N.A.; Zawawi, M.H.; Mohamad, D. Review of unmanned aerial vehicle photogrammetry for aerial mapping applications. In Proceedings of the International Conference on Civil, Offshore and Environmental Engineering; Springer: Singapore, 2021; pp. 669–676.
54. Page, M.J.; McKenzie, J.E.; Bossuyt, P.M.; Boutron, I.; Hoffmann, T.C.; Mulrow, C.D.; Shamseer, L.; Tetzlaff, J.M.; Akl, E.A.; Brennan, S.E.; et al. The PRISMA 2020 Statement: An Updated Guideline for Reporting Systematic Reviews. BMJ 2021, 372, n71.
55. Medina, M. Surveys in Aguascalientes by photogrammetric methods. Trans. Am. Geophys. Union 1935, 16, 67–70.
56. Wolff, N. Air Survey and Colonial Cadastral Mapping. Emp. Surv. Rev. 1938, 4, 281–290.
57. Powell, J.R. The Quantum Limit to Moore's Law. Proc. IEEE 2008, 96, 1247–1248.
58. Raoult, V.; David, P.A.; Dupont, S.F.; Mathewson, C.P.; O'Neill, S.J.; Powell, N.N.; Williamson, J.E. GoPros™ as an underwater photogrammetry tool for citizen science. PeerJ 2016, 4, e1960.
59. Hobday, A.J.; I Browman, H.; Bograd, S.J. Publishing and peer reviewing as indicators of the impact of COVID-19 on the productivity of the aquatic science community. ICES J. Mar. Sci. 2020, 77, 2439–2444.
60. Roveta, C.; Annibaldi, A.; Afghan, A.; Calcinai, B.; Di Camillo, C.; Gregorin, C.; Illuminati, S.; Mantas, T.P.; Truzzi, C.; Puce, S. Biomonitoring of Heavy Metals: The Unexplored Role of Marine Sessile Taxa. Appl. Sci. 2021, 11, 580.
61. Karatas, İ. The evolution of unmanned aerial vehicles (UAVs) and their similarities with proxy groups. Akade-Mik Hassasiyetler 2020, 7, 53–69.
62. Faig, W. Calibration of close-range photogrammetric systems: Mathematical formulation. Photogramm. Eng. Remote Sens. 1975, 41, 1479–1486.
63. Fryer, J.G.; Done, T.J. An underwater trilateration. Aust. Surv. 1982, 31, 7–12.
64. Watts, A.C.; Perry, J.H.; Smith, S.E.; Burgess, M.A.; Wilkinson, B.E.; Szantoi, Z.; Ifju, P.G.; Percival, H.F. Small Unmanned Aircraft Systems for Low-Altitude Aerial Surveys. J. Wildl. Manag. 2010, 74, 1614–1619.
65. Guarnieri, A.; Milan, N.; Vettore, A. Monitoring of Complex Structure for Structural Control Using Terrestrial Laser Scanning (Tls) And Photogrammetry. Int. J. Arch. Heritage 2013, 7, 54–67.
66. Fassi, F.; Achille, C.; Fregonese, L. Surveying and modelling the main spire of Milan Cathedral using multiple data sources. Photogramm. Rec. 2011, 26, 462–487.
67. Salvini, R.; Francioni, M.; Riccucci, S.; Fantozzi, P.L.; Bonciani, F.; Mancini, S. Stability analysis of "Grotta delle Felci" Cliff (Capri Island, Italy): Structural,

engineering–geological, photogrammetric surveys and laser scanning. Bull. Eng. Geol. Environ. 2011, 70, 549–557.

68. Dall'Asta, E.; Bruno, N.; Bigliardi, G.; Zerbi, A.; Roncella, R. Photogrammetric techniques for promotion of the archaeological heritage: The archaeological museum of Parma (Italy). ISPRS-Int. Arch. Photogramm. Remote Sens. Spat. Inf. Sci. 2016, XLI-B5, 243–250.
69. Esmaeili, H.; Thwaites, H.; Woods, P.C. Workflows & Challenges Involved in Creation of Realistic Immersive Virtual Museum, Heritage, and Tourism Experiences. In Proceedings of the 13th International Conference on Signal-Image Technology & Internet-Based Systems (SITIS), Jaipur, India, 4–7 December 2017.
70. Palma, M.; Magliozzi, C.; Casado, M.R.; Pantaleo, U.; Fernandes, J.; Coro, G.; Cerrano, C.; Leinster, P. Quantifying Coral Reef Composition of Recreational Diving Sites: A Structure from Motion Approach at Seascape Scale. Remote Sens. 2019, 11, 3027.
71. Rossi, P.; Ponti, M.; Righi, S.; Castagnetti, C.; Simonini, R.; Mancini, F.; Agrafiotis, P.; Bassani, L.; Bruno, F.; Cerrano, C.; et al. Needs and Gaps in Optical Underwater Technologies and Methods for the Investigation of Marine Animal Forest 3D-Structural Complexity. Front. Mar. Sci. 2021, 8, 591292.
72. Gambin, T.; Hyttinen, K.; Sausmekat, M.; Wood, J. Making the Invisible Visible: Underwater Malta—A Virtual Museum for Submerged Cultural Heritage. Remote Sens. 2021, 13, 1558.
73. Costa, E. The progress of survey techniques in underwater sites: The case study of Cape Stoba Shipwreck. ISPRS-Int. Arch. Photogramm. Remote Sens. Spat. Inf. Sci. 2019, XLII-2/W10, 69–75.
74. Firth, A.; Bedford, J.; Andrews, D. HMS Falmouth: 3D Visualization of a First World War Shipwreck. In Coastal Research Library; Springer: Berlin/Heidelberg, Germany, 2019; Volume 31, pp. 187–196.
75. Prado, E.; Gómez-Ballesteros, M.; Cobo, A.; Sánchez, F.; Rodriguez-Basalo, A.; Arrese, B.; Rodríguez-Cobo, L. 3D modeling of Rio Miera wreck ship merging optical and multibeam high resolution points cloud. ISPRS-Int. Arch. Photogramm. Remote Sens. Spat. Inf. Sci. 2019, XLII-2/W10, 159–165.
76. Secci, M.; Beltrame, C.; Manfio, S.; Guerra, F. Virtual reality in maritime archaeology legacy data for a virtual diving on the shipwreck of the Mercurio (1812). J. Cult. Heritage 2019, 40, 169–176.
77. Mogstad, A.A.; Ødegård, Ø.; Nornes, S.M.; Ludvigsen, M.; Johnsen, G.; Sørensen, A.J.; Berge, J. Mapping the Historical Shipwreck Figaro in the High Arctic Using Underwater Sensor-Carrying Robots. Remote Sens. 2020, 12, 997.
78. Ozaki, Y. The Accuracy of the Contour Lines I. J. Geod. Soc. Jpn. 1959, 5, 92–95.
79. Gerdes, K.; Arbizu, P.M.; Schwarz-Schampera, U.; Schwentner, M.; Kihara, T.C. Detailed Mapping of Hydrothermal Vent Fauna: A 3D Reconstruction Approach Based on Video Imagery. Front. Mar. Sci. 2019, 6, 96.
80. Kalacska, M.; Chmura, G.L.; Lucanus, O.; Bérubé, D.; Arroyo-Mora, J.P. Structure from motion will revolutionize analyses of tidal wetland landscapes. Remote Sens. Environ. 2017, 199, 14–24.
81. Karkani, A.; Evelpidou, N.; Vacchi, M.; Morhange, C.; Tsukamoto, S.; Frechen, M.; Maroukian, H. Tracking shoreline evolution in central Cyclades (Greece) using beachrocks. Mar. Geol. 2017, 388, 25–37.
82. Maté-González, M.; Sánchez-Aparicio, L.J.; Blázquez, C.S.; García, P.C.; Álvarez-Alonso, D.; de Andrés-Herrero, M.; García-Davalillo, J.C.; González-Aguilera, D.; Ruiz, M.H.; Bordehore, L.J.; et al. On the Combination of Remote Sensing and

Geophysical Methods for the Digitalization of the San Lázaro Middle Paleolithic Rock Shelter (Segovia, Central Iberia, Spain). Remote Sens. 2019, 11, 2035.

83. Pukanská, K.; Bartoš, K.; Bella, P.; Gašinec, J.; Blistan, P.; Kovanič, Ľ. Surveying and High-Resolution Topography of the Ochtiná Aragonite Cave Based on TLS and Digital Photogrammetry. Appl. Sci. 2020, 10, 4633.
84. Ferrari, R.; Bryson, M.; Bridge, T.; Hustache, J.; Williams, S.B.; Byrne, M.; Figueira, W. Quantifying the response of structural complexity and community composition to environmental change in marine communities. Glob. Chang. Biol. 2016, 22, 1965–1975.
85. Reichert, J.; Backes, A.R.; Schubert, P.; Wilke, T. The power of 3D fractal dimensions for comparative shape and structural complexity analyses of irregularly shaped organisms. Methods Ecol. Evol. 2017, 8, 1650–1658.
86. Jackson, T.D.; Williams, G.J.; Walker-Springett, G.; Davies, A.J. Three-dimensional digital mapping of ecosystems: A new era in spatial ecology. Proc. R. Soc. B Boil. Sci. 2020, 287, 20192383.
87. 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.; et al. UN Decade on Ecosystem Restoration 2021–2030—What Chance for Success in Restoring Coastal Ecosystems? Front. Mar. Sci. 2020, 7, 71.
88. Cattaneo-Vietti, R.; Mojetta, A. The essential role of diving in Marine Biology. BELS-Bullet. Environ. And Life Sci. 2021, 3, 1–44.
89. Kennedy, B.R.C.; Cantwell, K.; Malik, M.; Kelley, C.; Potter, J.; Elliott, K.; Lobecker, E.; Gray, L.M.; Sowers, D.; White, M.P.; et al. The Unknown and the Unexplored: Insights into the Pacific Deep-Sea Following NOAA CAPSTONE Expeditions. Front. Mar. Sci. 2019, 6, 480.
90. Mayer, L.; Jakobsson, M.; Allen, G.; Dorschel, B.; Falconer, R.; Ferrini, V.; Lamarche, G.; Snaith, H.; Weatherall, P. The Nippon Foundation—GEBCO Seabed 2030 Project: The Quest to See the World's Oceans Completely Mapped by 2030. Geosciences 2018, 8, 63.
91. Teague, J.; Allen, M.J.; Scott, T.B. The potential of low-cost ROV for use in deep-sea mineral, ore prospecting and monitoring. Ocean Eng. 2018, 147, 333–339.
92. Willners, J.S.; Carlucho, I.; Katagiri, S.; Lemoine, C.; Roe, J.; Stephens, D.; Łuczyński, T.; Xu, S.; Carreno, Y.; Pairet, È.; et al. From market-ready ROVs to low-cost AUVs. In OCEANS 2021: San Diego–Porto; IEEE: New York, NY, USA, 2021; pp. 1–7.
93. Turicchia, E.; Ponti, M.; Rossi, G.; Milanese, M.; Di Camillo, C.G.; Cerrano, C. The Reef Check Mediterranean Underwater Coastal Environment Monitoring Protocol. Front. Mar. Sci. 2021, 8, 620368.
94. Pulido Mantas, T.; Varotti, C.; Roveta, C.; Palma, M.; Innocenti, C.; Giusti, M.; Benabdi, M.; Trainito, E.; Mačić, V.; Gambi, M.C.; et al. Mediterranean Sea shelters for the gold coral *Savalia savaglia* (Bertoloni, 1819): An assessment of potential distribution of a rare parasitic species. Mar. Environ. Res. 2022, 179, 105686.
95. Viduka, A.; Edney, J. GIRT scientific divers citizen science programme: Volunteer motivations and characteristics. J. Community Archaeol. Heritage 2021, 9, 143–160.
96. Estes, M.; Anderson, C.; Appeltans, W.; Bax, N.; Bednaršek, N.; Canonico, G.; Djavidnia, S.; Escobar, E.; Fietzek, P.; Gregoire, M.; et al. Enhanced monitoring of life in the sea is a critical component of conservation management and sustainable economic growth. Mar. Policy 2021, 132, 104699.
97. Purzycki, M.; Komorowska, A.; Ilnicka, A.; Papiez, J.; Szymanska, E. From ROVs to AUVs–Optimization and Analysis of Underwater Vehicles Design. In Proceedings of the 2022 45th Jubilee International Convention on Information, Communication and

Electronic Technology (MIPRO), Opatija, Croatia, 23–27 May 2022; IEEE: New York, NY, USA, 2022; pp. 1517–1521.

98. Nex, F.; Remondino, F. UAV for 3D mapping applications: A review. Appl. Geomat. 2014, 6, 1–15.
99. McLean, D.L.; Parsons, M.J.G.; Gates, A.; Benfield, M.C.; Bond, T.; Booth, D.J.; Bunce, M.; Fowler, A.; Harvey, E.S.; Macreadie, P.I.; et al. Enhancing the Scientific Value of Industry Remotely Operated Vehicles (ROVs) in Our Oceans. Front. Mar. Sci. 2020, 7, 220.
100. Frodella, W.; Elashvili, M.; Spizzichino, D.; Gigli, G.; Adikashvili, L.; Vacheishvili, N.; Kirkitadze, G.; Nadaraia, A.; Margottini, C.; Casagli, N. Combining InfraRed Thermography and UAV Digital Photogrammetry for the Protection and Conservation of Rupestrian Cultural Heritage Sites in Georgia: A Methodological Application. Remote Sens. 2020, 12, 892.
101. Puce, S.; Cerrano, C.; Marques, A.C.; Bavestrello, G. Eudendrium klausi (Cnidaria, Hydrozoa), a new species of hydroid from Belize. J. Mar. Biol. Assoc. U. K. 2005, 85, 291–305.
102. Qin, J.; Yang, K.; Li, M.; Zhong, J.; Zhang, H. Real-Time Positioning and Tracking for Vision-Based Unmanned Underwater Vehicles. ISPRS-Int. Arch. Photogramm. Remote Sens. Spat. Inf. Sci. 2022, XLVI-3/W1, 163–168.
103. Balletti, C.; Beltrame, C.; Costa, E.; Guerra, F.; Vernier, P. Photogrammetry in maritime and underwater archaeology: Two marble wrecks from Sicily. In Optics for Arts, Architecture, and Archaeology, V; SPIE: Bellingham, WA, USA, 2015; Volume 9527, pp. 127–138.
104. Kurz, T.H.; Buckley, S.J.; Howell, J.A. Close range hyperspectral imaging integrated with terrestrial LiDAR scanning applied to rock characterization at centimeter scale. Int. Arch. Photogramm. Remote Sens. Spat. Inf. Sci. 2012, 39, 417–422.
105. Sebar, L.E.; Lombardo, L.; Parvis, M.; Angelini, E.; Re, A.; Grassini, S. A metrological approach for multispectral photogrammetry. Acta IMEKO 2021, 10, 111–116.
106. Patrucco, G.; Cortese, G.; Tonolo, F.G.; Spanò, A. Thermal and Optical Data Fusion Supporting Built Heritage Analyses. ISPRS-Int. Arch. Photogramm. Remote Sens. Spat. Inf. Sci. 2020, XLIII-B3-2, 619–626.
107. Dlesk, A.; Vach, K.; Pavelka, K. Photogrammetric Co-Processing of Thermal Infrared Images and RGB Images. Sensors 2022, 22, 1655.
108. Baltsavias, E.P. A comparison between photogrammetry and laser scanning. ISPRS J. Photogramm. Remote Sens. 1999, 54, 83–94.
109. Moussa, W. Integration of Digital Photogrammetry and Terrestrial Laser Scanning for Cultural Heritage Data Recording. Master’s Thesis, University of Stuttgart, Stuttgart, Germany, 2014.
110. Roosevelt, C. Mapping site-level microtopography with Real—Time Kinematic Global Navigation Satellite Systems (RTK GNSS) and Unmanned Aerial Vehicle Photogrammetry (UAVP). Open Archaeol. 2014, 1, 29–53.
111. Ekaso, D.; Nex, F.; Kerle, N. Accuracy assessment of real-time kinematics (RTK) measurements on unmanned aerial vehicles (UAV) for direct geo-referencing. Geo-Spat. Inf. Sci. 2020, 23, 165–181.
112. Remondino, F.; Barazzetti, L.; Nex, F.; Scaioni, M.; Sarazzi, D. UAV photogrammetry for mapping and 3d modelling–current status and future perspectives. Int. Arch. Photogramm. Remote Sens. Spat. Inf. Sci. 2011, 38, 25–31.
113. Sakagami, N.; Hirayama, K.; Taba, R.; Kobashigawa, S.; Arashiro, S.; Takemura, F.; Takahashi, S. Development and field experiments of a human-portable towed ROV for high-speed and wide area data acquisition. Artif. Life Robot. 2020, 26, 1–9.

114. Abdelhafiz, A.; Mostafa, Y. Automatic texture mapping mega-projects. J. Spat. Sci. 2018, 65, 467–479.
115. Dorozhynskyy, O. Influence of GPS data fixed during the flight for aerial survey and aerial triangulation. Int. Arch. Photogramm. Remote Sens. 2000, 33 Pt 4, 238–246.
116. Rau, J.-Y.; Teo, T.-A.; Chen, L.-C.; Tsai, F.; Hsiao, K.-H.; Hsu, W.-C. Integration of gps, gis and photogrammetry for texture mapping in photo-realistic city modeling. In Pacific-Rim Sym-Posium on Image and Video Technology; Springer: Berlin/Heidelberg, Germany, 2006; pp. 1283–1292.
117. Cooper, I.; Hotchkiss, R.; Williams, G. Extending Multi-Beam Sonar with Structure from Motion Data of Shorelines for Complete Pool Bathymetry of Reservoirs. Remote Sens. 2020, 13, 35.
118. Biass, S.; Orr, T.R.; Houghton, B.F.; Patrick, M.R.; James, M.R.; Turner, N. Insights Into Pāhoehoe Lava Emplacement Using Visible and Thermal Structure-From-Motion Photogrammetry. J. Geophys. Res. Solid Earth 2019, 124, 5678–5695.
119. Lathlean, J.; Seuront, L. Infrared thermography in marine ecology: Methods, previous applications and future challenges. Mar. Ecol. Prog. Ser. 2014, 514, 263–277.
120. Garrabou, J.; Bensoussan, N.; Di Franco, A.; Boada, J.; Cebrian, E.; Santamaria, J.; Guala, I.; Grech, D.; Cerrano, C.; Pulido, T.; et al. Monitoring Climate-Related Responses in Mediterranean Marine Protected Areas and Beyond: ELEVEN STANDARD PROTOCOLS; Institute of Marine Sciences, Ed.; Spanish Research Council ICM-CSIC: Barcelona, Spain, 2022; pp. 37–49.

Paper 2

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Needs and Gaps in Optical Underwater Technologies and Methods for the Investigation of Marine Animal Forest 3D-Structural Complexity

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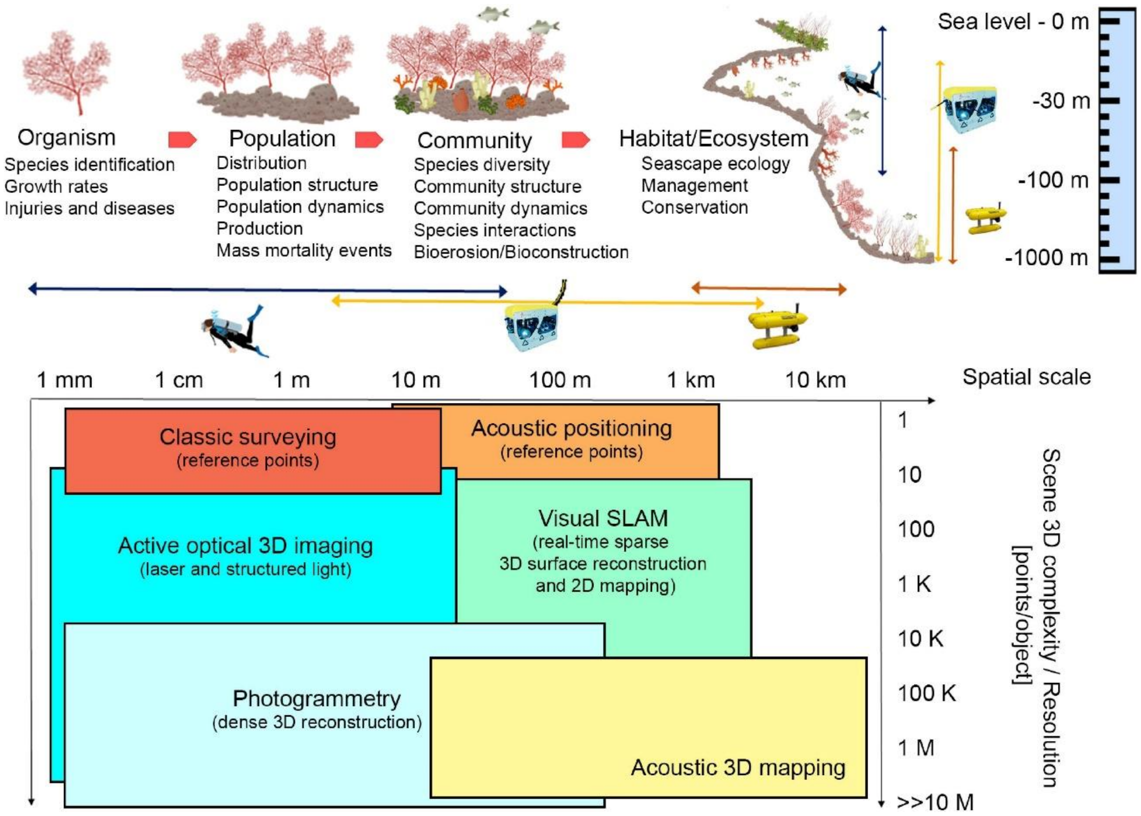

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Abstract

Marine animal forests are benthic communities dominated by sessile suspension feeders (such as sponges, corals, and bivalves) able to generate three-dimensional (3D) frameworks with high structural complexity. The biodiversity and functioning of marine animal forests are strictly related to their 3D complexity. The present paper aims at providing new perspectives in underwater optical surveys. Starting from the current gaps in data collection and analysis that critically limit the study and conservation of marine animal forests, we discuss the main technological and methodological needs for the investigation of their 3D structural complexity at different spatial and temporal scales. Despite recent technological advances, it seems that several issues in data acquisition and processing need to be solved, to properly map the different benthic habitats in which marine animal forests are present, their health status and to measure structural complexity. Proper precision and accuracy should be chosen and assured in relation to the biological and ecological processes investigated. Besides, standardized methods and protocols are strictly necessary to meet the FAIR (findability, accessibility, interoperability, and reusability) data principles for the stewardship of habitat mapping and biodiversity, biomass, and growth data.

Keywords: biodiversity, 3D monitoring, semantic segmentation, underwater photogrammetry, biogenic reefs, conservation.

1. Introduction

Precise maps are nowadays easily available on the land surface, providing location, extent, and topography of terrestrial ecosystems. Interestingly, it is roughly calculated that only 5% of the oceans floor is accurately mapped at the ecological community level (Rossi and Orejas, 2019). Thus, a complete 3D and semantically enriched description of benthic habitats is required through a technologically improved and methodologically robust approach.

Marine animal forests are benthic communities dominated by sessile suspension feeders (such as sponges, gorgonians, scleractinian corals, and bivalves) able to generate 3D frameworks with high structural complexity (Rossi et al., 2017). These organisms act as habitat-forming species, since they "modulate the availability of resources (other than themselves) to other species by causing physical state changes in biotic or abiotic materials" (Jones et al., 1994). The complex structures resulting from their growth significantly enhance the heterogeneity in most environmental factors (such as light, current, food availability, or sediment suspension), providing habitats for other species, enhancing species diversity, and directly or indirectly participating in or promoting building of biogenic reefs (Rossi et al., 2017).

The overall bioconstruction rate of the biogenic reefs made or promoted by animal forests varies according to different prevailing species, but has often low values: for example, growth in height is estimated to range from less than a millimeter to few centimeters per year, depending on species and environmental conditions (Coma et al., 1998; Kružić and Benković, 2012; Ordoñez et al., 2019). Higher rates are typical of healthy tropical coral reefs dominated by branching corals, while lower rates occur in temperate and cold seas dominated by sponges, gorgonians or bivalves. Negative growth rates of biogenic structures may arise when biological and chemical-physical erosion processes dominate over the bioconstruction ones (Davidson et al., 2018). For example, in the Mediterranean biogenic reefs, the integrity of gorgonian populations is threatened by: (i) mechanical damage due to fishing lines and nets, anchorages, and recreational divers; (ii) suffocation by mucilaginous benthic aggregates; (iii) increased water turbidity and sedimentation rates as a result from poor land management (Ponti et al., 2018 and references therein); (iv) the invasion from non-indigenous and/or predator species (Maldonado et al., 2013; Righi et al., 2020). Moreover, climate change is one of the most concern threat since seawater warming, and higher frequency and severity of marine heatwaves have increased the mass mortalities of animal forests in both tropical and temperate seas (Garrabou et al., 2019). The vast decline of erect sponges and gorgonians in the Mediterranean Sea (Cerrano et al., 2000; Turicchia et al., 2018) is reducing considerably the structural complexity of these ecosystems, with important consequences for ecosystem biodiversity, functioning, and the services they provide (Verdura et al., 2019).

The study of marine animal forests down to the mesophotic zone is usually performed by scuba-diving surveys (Cerrano et al., 2019), using visual estimations, photo-sampling, and direct assessments of variables (e.g., colony size, percent cover, presence of necrosis/bleaching). Yet, accuracy of measurements and spatial replication are often not taken into consideration and some direct measures (e.g., annual growth rates) may still be based on destructive sampling (Peirano et al., 2001; Marschal et al., 2004). Since many invertebrates and their symbiotic algae contain fluorescent pigments, fluorometry and photographic image analysis are increasingly employed as a non-invasive proxy for organism health state and pathogenesis patterns (see Caldwell et al., 2017 for references), and to analyze their population dynamic; however, these studies are labor-intensive and require manual interpretation (Zweifler et al., 2017; Montero-Serra et al., 2019).

Technological developments have made optical and acoustic techniques practical to provide non-destructive measurement estimates of large sections of tropical coral reefs in 3D (House et al., 2018; Rossi et al., 2020), but their application in cold and temperate animal marine forests is still limited (Palma et al., 2018). Moreover additional efforts are still needed to obtain high definition reconstructions that range from aggregations to single organisms as well as subsequent reconstructions to allow tracing of a possible structural shift over time. Indeed, ranging from small to broad spatial scale of marine animal forests (Figure 1), the use of photogrammetry for georeferencing the samples and for spatial data acquisition is a promising tool to study many aspects of these communities and evaluate ecological key parameters, from organism health to the species interactions at the basis of bioconstruction and bioerosion processes. Within this viewpoint paper we mainly focus on optical techniques, in particular photogrammetry. Acoustic techniques are effective and widely used, but at present they do not guarantee to reach the same accuracy and resolution as photogrammetry (Czechowska et al., 2020). At the scale of single organisms, accurate reconstructions of 3D individual morphology may considerably enhance the capability for species recognition, as well as the accurate quantification of survival and growth rates under variable environmental conditions (Olinger et al., 2019). At the population level, morphological information about organisms may allow for a precise description of the population size structure, its dynamic variation through time (Montero-Serra et al., 2019) and its functioning (Ledoux et al., 2010). At the community level, the careful reconstruction of the framework built up by the structural and the associated species may allow to precisely describe the community structure in terms of species composition, abundance, and evenness. Finally, at the large scale of habitat or ecosystems, 3D georeferenced reconstructions may allow for the application of landscape indexes and descriptors to the analysis of benthic communities.

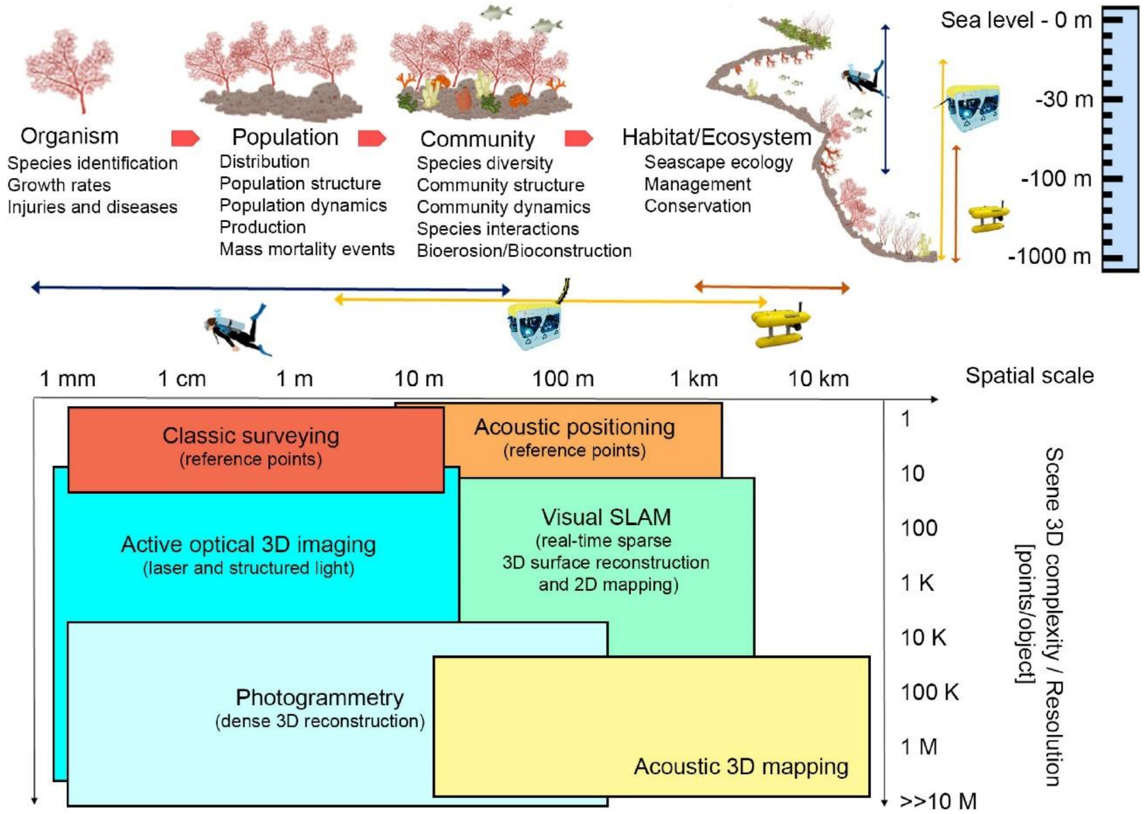


Figure 1. Ecological key aspects of marine animal forests and different technological approaches that can be applied at different spatial scales, depth, and 3D complexity of investigated area. In the table at the bottom, SLAM stands for Simultaneous Localization And Mapping. Illustrations modified from Montero-Serra (2018), and NOAA. Source: IAN Symbols Library, University of Maryland.

2. Technologies and Methodologies

2.1. Data Acquisition

The underwater environment poses multiple challenges to 3D reconstruction-oriented explorations based on optical methodologies, such as low visibility, turbidity, light attenuation, and low data transfer rates. It is still challenging to acquire spatial data through photogrammetric methods at a wide area (wider than few square meters) at a few centimeters level of accuracy, which could be limiting for the study of large benthic species. New technologies and automation are required both in data acquisition and analysis to quantify the structural complexity of animal forests. Besides, survey planning and adaptation strategies that are tailored for optical and acoustic 2D/3D maps reconstruction, dynamically adapted to local conditions, and oriented toward the optimal coverage of a site, need to be explored.

Scuba divers are still widely employed for surveying tasks at accessible diving depths, in clear warm and turbid/cold waters complying with safety limitations (Piazza et al., 2018; Nocerino et al., 2019). However, remotely operated vehicles (ROVs) and autonomous underwater vehicles (AUVs) have become increasingly reliable tools and have led to inspections at depths and across spatio-temporal scales hostile both to divers and crewed vehicles (Figure 1; Gori et al., 2011; Shihavuddin et al., 2013; Montseny et al., 2019). In both cases, reference information is required to support spatial continuous methodologies and produce a georeferenced 3D model. The position of stable reference points, installed on the sea floor, or the position of sensors can be performed through classic survey technique or acoustic positioning, depending on the dimension and depth of the investigated site (Bruno et al., 2019; Rossi et al., 2020; Figure 1). Moreover, acoustic positioning systems, combined with underwater tablet, may allow divers to plan specific paths to be followed during the survey (Scaradozzi et al., 2018), thus optimizing the acquisition phase and guaranteeing repeatable and complete data. For deeper surveys, acoustic positioning and ROV/AUV navigation data may only provide approximate georeferencing information that may be too coarse for adequately scaling the 3D models when these are created from a single camera. In such cases, the use of laser calipers can solve this problem (Istenič et al., 2020).

Undoubtedly, automatic optical and acoustic imagery classification, as well as 2D mapping (Franchi et al., 2018), would speed up and enhance animal forests investigation allowing non-destructive visual surveys, measuring morphological variables, and paving the way to more specific 3D reconstruction-oriented explorations. Acoustic based techniques are well-suited in large areas of interest (from 10 m to 10 km), and acoustic 3D mapping is successfully employed to map a wide portion of the sea floor at various depths, but in case of organism biometry, higher accuracy and resolution techniques are required. These systems operate in the long-range acquisition and do not suffer from turbidity, but the resulting 3D reconstructions have low resolution and accuracy compared to the optical ones (Lagudi et al., 2016; Figure 1).

On the other hand, optical systems are well-suited for close-range acquisition (a few centimeters to tenths of meters). Active optical 3D imaging techniques project different patterns on the investigated area. The triangulation is computed by intersecting the laser beam that passes through a pixel lit up by the pattern, and the projected pattern itself (Palomer et al., 2019). These tools allow to obtain accurate and high-resolution 3D reconstructions, but results are influenced by visibility conditions and acquisition distance (Figure 1). Passive techniques (photogrammetry) are based on multiple views of a scene and take advantage of structure from motion (SfM) and Multi View Stereo (MVS) algorithms (Burns et al., 2015). Natural or artificial light can be used to illuminate the scene. The triangulation is done by intersecting the optic rays that pass through a pixel in each image where the same feature is automatically identified and allows the generation of an accurate, dense 3D reconstruction (Westoby et al., 2012). Underwater photogrammetry has long been used because of its flexibility and low cost, potentially providing high-resolution imagery in the underwater context under well-planned protocols of data collection (Nocerino et al., 2019). RGB sensors are the most used in underwater applications because they allow achieving measurable and photorealistic products. Other sensors may offer new opportunities to visualize the internal and external morphology of bioconstructions, such as multispectral acquisitions (Nocerino et al., 2017) and high-resolution x-ray computed tomography, which can be applied to collected samples (Farber et al., 2016). For shallow water applications, surface autonomous vehicles equipped with acoustic and optical sensors (Stanghellini et al., 2020), and low altitude Unmanned Aerial Vehicle (UAV)-based photogrammetry (Casella et al., 2017; Agrafiotis et al., 2020) can provide important information outlining bathymetric profile, filling the gap between satellite and underwater imagery. In the latter case, compensation for water refraction is required (Agrafiotis et al., 2020). In addition, underwater multispectral and hyperspectral imagery is an emerging technique for applications in shallow and deep-sea environments. It is a passive technique in shallow waters; instead, at high depth, when solar illumination is not available, an active light source is necessary. Anyway, this technique works properly at an acquisition distance of 1–5 m, because of the strong absorption caused by the water column (Liu et al., 2020).

Simultaneous localization and mapping (SLAM) methodologies have proved to be effective for underwater explorations and subsea metrology applications. SLAM relies on data integration from navigation sensors (e.g., global positioning system—GPS, inertial measurements unit—IMU) and remote sensing or perception (e.g., camera, lidar, sonar) systems; it comprises a wide range of algorithms designed to provide an autonomous vehicle with the ability to move in an uncharted environment while producing a map (or model) of the scene (Menna et al., 2019).

3D animal forest formations can occur in flat and gently sloping seabeds, or even in vertical slopes, often with crevices, terraces and overhangs. This poses major difficulties in surveying from the surface and requires complex acquisition paths from ROVs/AUVs/divers to collect images for proper 3D reconstruction. When utilizing underwater photogrammetry (or other optical methodologies) to survey marine animal forests, there is an important trade-off, as well as specific drawbacks and limitations that need to be considered. The spatial resolution of final maps depends on the quality of input images: underwater images suffer from wavelength-specific attenuation and scattering, which cause image degradation to increase significantly with distance. Thus, the distance to the object has a strong effect on the quality of the obtained images and consequently the 3D reconstruction. Additionally, in very shallow waters, rippling caustic are adversely affecting SfM and MVS methods, leading to less accurate matches (Agrafiotis et al., 2018) and causing issues in the SLAM based navigation of the ROVs and AUVs (Trabes and Jordan, 2017). A full reconstruction of the 3D complexity of underwater animal forests is challenging, occlusions can occur, and different view angles must be designed. Additionally, high data redundancy is required. Accurately designed acquisition paths (Vidal et al., 2020) and/or omnidirectional cameras (Bosch et al., 2019) could be used as well as forward-looking cameras and modular survey designs, adaptable in real-time. This requires serious navigation abilities from the AUV and is substantially unenforceable with human divers. New navigation technologies are needed, including visibility enhancement (Berman et al., 2020) for visual SLAM and obstacle avoidance to enable close-range photogrammetry of specific features. Neural networks might offer a solution to improve the AUV capabilities in terms of obstacle avoidance and real time decision-making. Computer simulations may help in training the neural networks and achieve such improvement. The photogrammetric approach has been successfully applied to hard corals, while gorgonians, soft corals and erect sponges pose important challenges due to their passive movement with water flow. However, a SfM technique has recently been tested on images from a gorgonian forest (Palma et al., 2018).

2.2.Image and Data Analysis

The collection of underwater imagery for the monitoring of marine animal forests became a widespread resource, leading to the accumulation of millions of images each year. However, the rate of image acquisition and the amount of the produced 2D orthophotos dramatically outpaces the human ability to extract information from them (Beijbom et al., 2015). The most commonly required data are the organisms' size and abundance, the spatial distribution of populations on a broader landscape, and their evolution in relation to the changes of the underwater environment. Detailed information about 2D statistical methods for assessing these parameters, but also to evaluate growth and mortalities using ortho-mosaics are summarized in Edwards et al. (2017), Pedersen et al. (2019), and Sandin et al. (2020). These large data collections consisting of images can also be coupled with previous experimental or field work observations and quantifications in single species or communities, enabling a validation of ex situ analysis and a large scale investigation. For example: crossing the numbers of carbon input and output of a single organism together with its distribution, density, population structure and biomass in large spatial approaches, may be the key to understand the role of the organism as a carbon immobilizer. Terrestrial tools (used to study land communities acting as carbon immobilizers for example) in marine systems are in implementation thanks to the advances in 3D-image analysis and quantification. Acquired digital data (images and 3D models) are also perfectly suited for the sharing within the scientific community and the public. This could enhance a multidisciplinary and shared research, in accordance with FAIR principles.

Regardless of the biological purpose, spatial analysis, such as detecting changes in organisms, implies the need to segment target species with a high degree of accuracy. Manual 2D segmentation, e.g., a per-pixel classification, is an extremely time-consuming process that can be accelerated using deep learning-based methodologies (Pavoni et al., 2020).

The information on the depth axis is fundamental to evaluate the volumetric change caused by the structural growth or erosion. Additionally, many of the sessile organisms that constitute marine animal forests are hardly recognizable from a simple orthographic top-down view. The main prospect is to extend the semantic segmentation from images to height fields (2.5D) or 3D models. Open source segmentation software specifically targeted to marine species that handling 2.5D data have been already developed and distributed.

For example, TagLab1 enables assisted and automatic annotation, leveraging a human-in-the-loop approach to improve the accuracy of automatic predictions. Besides, it supports the comparison of multi-temporal surveys, automatically extracting useful historical information from segmented regions.

3D automatic semantic segmentation is the open challenge yet to face. As highlighted by a comprehensive survey paper (Han et al., 2019), available deep learning architectures might fail in producing segmentation that meets the standards of ecological analysis. Moreover, the limits of architectures working on point clouds, such as the PointNet++ (Qi et al., 2017) and the one by Çiçek et al. (2016), that is a variant of the U-Net architecture originally developed for biomedical data analysis, are not clearly assessed in this application field due to lack of benchmark dataset. Multi-modal learning approaches, combining oriented images, RGB and depth information, might help to fill this gap and solve the task (Dai and Nießner, 2018).

3. Discussion

In the context of animal forests mapping and 3D structural complexity analysis, the integration of different technologies could support multi-resolution and multi-scale surveying and monitoring. AUVs have the potential to efficiently investigate vast unexplored underwater regions, while ROVs allow for a higher resolution and more detailed mapping of smaller areas of interest that exceed the scuba-diving depth range.

Even if the scientific community is developing new approaches for improving “low” cost surveying and monitoring of the marine environment, their adoption is still not widespread since further testing and optimization of methodologies are needed. In addition, the limited funding for research, and the low interest of the general public in the seabed, slow these developments. Broad scale 3D reconstructions could improve the communication about the importance and diversity of marine environment and increase the public interest.

The main needs and gaps regarding the study of the 3D complexity of underwater animal forests are related to data collection and analysis (Figure 2), with concern to the improvement of diver-based or mobile robots (ROV/AUVs) methods in terms of positioning, guidance, and communication toward the surface. Adequate surveying performances of data collection platforms should be improved, depending on the level of robotic and further developments on real time decision capabilities (e.g., real time evaluation of the quality of the data gathered). Likewise, rigorous modeling of sensors, evaluation of metric accuracies and other methodological errors along with the standardization of data formats and analysis tools will be crucial focuses.

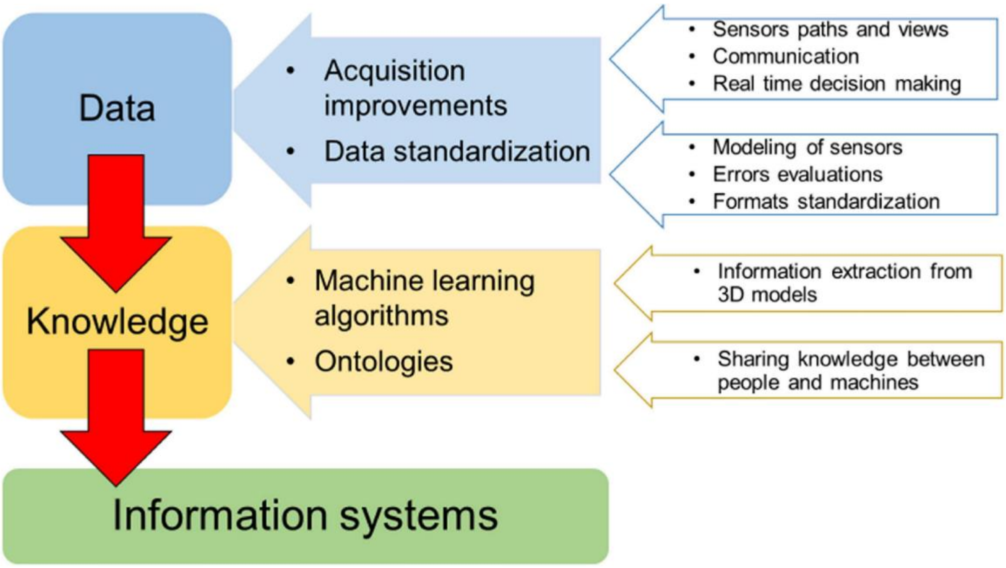


Figure 2. Main needs and gaps for 3D complexity investigations of underwater marine animal forests. The generation of exchangeable knowledge (information system) starts from standardized data.

The efficiency and quality of collected data, including multiple sensors, data acquisition at multiple scales, and data fusion from multiple platforms and times, need to be supported by the development of machine learning algorithms and methods for biodiversity classification/segmentation and 3D models definition of underwater changing objects. Indeed, benthic populations display 3D behaviors in time whose accurate analysis on the high-resolution 3D representation of these habitats is still beyond modern solutions based either on geometry processing or on deep learning. Fully automated solutions dramatically reduce the human effort in 2D/3D analysis; however, the accuracy of deep learning-based methodologies in the recognition/segmentation of benthic organisms is still lower than human experts, and the dense marine animal forests with a large 3D development pose even more difficulties in the discrimination of organisms. An efficient analysis tool must consider a human in the loop approach, leaving the experts the chance to edit the automatic results thus improving the overall accuracy. In addition, the analysis of large image sets can directly or indirectly benefit from public engagement, through citizen science programs, as was the case with plankton (Robinson et al., 2017). Citizen science data need to be carefully pre-analyzed in terms of accuracy and reliability to evaluate their effective use for mapping and 3D reconstruction applications.

Finally, the development of an information system to better retrieve and manage information on 3D complexity from collected data through time should be addressed. The integration of data belonging to various expeditions, methodologies and purposes is an important need too. The integration of all the existing habitat mapping data in a common and open information system could provide a more accurate, spatially representative assessment of habitat degradation and a proper design and management of restoration actions (Gerovasileiou et al., 2019). Ecological data are highly heterogeneous and complex to manage, therefore providing a shared and common understanding of data and processes will improve data accessibility and analysis. Ontologies (a knowledge graph-based Web tool, see Guarino et al., 2009) have a meaningful role in supporting knowledge sharing expectations. A common understanding facilitates communication between people and information systems (machines) and enhances the ability to search for information across different knowledge repositories, in light of meeting the FAIR (i.e., findability, accessibility, interoperability, and reusability) data principles (Wilkinson et al., 2016).

References

Agrafiotis, P., Karantzalos, K., Georgopoulos, A., and Skarlatos, D. (2020). Correcting image refraction: towards accurate aerial image-based bathymetry mapping in shallow waters. Remote Sens. 12:322. doi: 10.3390/rs12020322

Agrafiotis, P., Skarlatos, D., Forbes, T., Poullis, C., Skamantzari, M., and Georgopoulos, A. (2018). Underwater photogrammetry in very shallow waters: main challenges and caustics effect removal. Int. Arch. Photogramm. Remote Sens. Spatial Inf. Sci. XLII-2, 15–22. doi: 10.5194/isprs-archives-XLII-2-15-2018

Beijbom, O., Edmunds, P. J., Roelfsema, C., Smith, J., Kline, D. I., Neal, B. P., et al. (2015). Towards automated annotation of benthic survey images: variability of human experts and operational modes of automation. PLoS One 10:e0130312. doi: 10.1371/journal.pone.0130312

Berman, D., Levy, D., Avidan, S., and Treibitz, T. (2020). Underwater single image color restoration using haze-lines and a new quantitative dataset. IEEE Trans. Pattern Anal. Mach. Intell. doi: 10.1109/tpami.2020.2977624

Bosch, J., Istenič, K., Gracias, N., Garcia, R., and Ridao, P. (2019). Omnidirectional multi-camera video stitching using depth maps. IEEE J. Oceanic Eng. 99, 1–16. doi: 10.1109/JOE.2019.2924276

Bruno, F., Barbieri, L., Muzzupappa, M., Tusa, S., Fresina, A., Oliveri, F., et al. (2019). Enhancing learning and access to underwater cultural heritage through digital technologies: the case study of the “Cala Minnola” shipwreck site. Digit. Appl. Archaeol. Cult. Herit 13:e00103. doi: 10.1016/j.daach.2019.e00103

Burns, J. H. R., Delparte, D., Gates, R. D., and Takabayashi, M. (2015). Integrating structure-from-motion photogrammetry with geospatial software as a novel technique for quantifying 3D ecological characteristics of coral reefs. PeerJ 3:e1077. doi: 10.7717/peerj.1077

Caldwell, J. M., Ushijima, B., Couch, C. S., and Gates, R. D. (2017). Intra-colony disease progression induces fragmentation of coral fluorescent pigments. Sci. Rep. 7:14596. doi: 10.1038/s41598-017-15084-3

Casella, E., Collin, A., Harris, D., Ferse, S., Bejarano, S., Parravicini, V., et al. (2017). Mapping coral reefs using consumer-grade drones and structure from motion photogrammetry techniques. Coral Reefs 36, 269–275. doi: 10.1007/s00338-016-1522-0

Cerrano, C., Bastari, A., Calcinai, B., Di Camillo, C., Pica, D., Puce, S., et al. (2019). Temperate mesophotic ecosystems: gaps and perspectives of an emerging conservation challenge for the Mediterranean Sea. Eur. Zool. J. 86, 370–388. doi: 10.1080/24750263.2019.1677790

Cerrano, C., Bavestrello, G., Bianchi, C. N., Cattaneo-Vietti, R., Bava, S., Morganti, C., et al. (2000). A catastrophic mass-mortality episode of gorgonians and other organisms in the Ligurian Sea (North-western Mediterranean), summer 1999. Ecol. Lett. 3, 284–293. doi: 10.1046/j.1461-0248.2000.00152.x

Çiçek, Ö, Abdulkadir, A., Lienkamp, S. S., Brox, T., and Ronneberger, O. (2016). "3D U-Net: learning dense volumetric segmentation from sparse annotation," in MICCAI Medical. Image Computing and Computer-Assisted Intervention, Vol. 424–432, eds S. Ourselin, W. S. Wells, M. R. Sabuncu, G. Unal, and L. Joskowicz (New York, NY: Springer). doi: 10.1007/978-3-319-46723-8_49

Coma, R., Ribes, M., Zabala, M., and Gili, J. M. (1998). Growth in a modular colonial marine invertebrate. Estuar. Coast. Shelf Sci. 47, 459–470. doi: 10.1006/ecss.1998.0375

Czechowska, K., Feldens, P., Tuya, F., de Esteban, M. C., Espino, F., Haroun, R., et al. (2020). Testing side-scan sonar and multibeam echosounder to study black coral gardens: a case study from Macaronesia. Remote Sens. 12:3244. doi: 10.3390/rs12193244

Dai, A., and Nießner, M. (2018). "3dmv: joint 3d-multi-view prediction for 3d semantic scene segmentation," in Proceedings of the 15th European Conference, Munich, Germany, September 8–14, 2018, Munich, 452–468.

Davidson, T. M., Altieri, A. H., Ruiz, G. M., and Torchin, M. E. (2018). Bioerosion in a changing world: a conceptual framework. Ecol. Lett. 21, 422–438. doi: 10.1111/ele.12899

Edwards, C., Eynaud, Y., Williams, G. J., Pedersen, N. E., Zgliczyn-ski, B. J., Gleason, A. C. R., et al. (2017). Large-area imaging reveals biologically driven non-random spatial patterns of corals at a remote reef. Coral Reefs 36, 1291–1305. doi: 10.1007/s00338-017-1624-3

Farber, C., Titschack, J., Schonberg, C., Ehrig, K., Boos, K., Illerhaus, B., et al. (2016). Long-term macrobioerosion in the Mediterranean Sea assessed by micro-computed tomography. Biogeoscience 13, 3461–3474. doi: 10.5194/bg-13-3461-2016

Franchi, M., Ridolfi, A., and Zacchini, L. (2018). "A forward-looking sonar-based system for underwater mosaicing and acoustic odometry," in Proceedings of the IEEE/OES Autonomous Underwater Vehicle Workshop (AUV), Porto. doi: 10.1109/auv.2018.8729795

Garrabou, J., Gómez-Gras, D., Ledoux, J. B., Linares, C., Bensoussan, N., López-Sendino, P., et al. (2019). Collaborative database to track mass mortality events in the Mediterranean Sea. Front. Mar. Sci. 6:707. doi: 10.3389/fmars.2019.00707

Gerovasileiou, V., Smith, C. J., Sevastou, K., Papadopoulou, N., Dailianis, T., Bekkby, T., et al. (2019). Habitat mapping in the European Seas-is it fit for purpose in the marine restoration agenda? Mar. Policy 106:103521. doi: 10.1016/j.marpol.2019.103521

Gori, A., Rossi, S., Berganzo, E., Pretus, J. L., Dale, M. R. T., and Gili, J. M. (2011). Spatial distribution patterns of the gorgonians Eunicella singularis, Paramuricea clavata, and Leptogorgia sarmentosa (Cap de Creus, northwestern Mediterranean Sea). Mar. Biol. 158, 143–158. doi: 10.1007/s00227-010-1548-8

Guarino, N., Oberle, D., and Staab, S. (2009). What is an Ontology? Handbook on Ontologies. Heidelberg: Springer, 1–17.

Han, X. F., Laga, H., and Bennamoun, M. (2019). Image-based 3d object reconstruction: state-of-the-art and trends in the deep learning era. IEEE Trans. Pattern. Anal. Mach. Intell, 1. doi: 10.1109/tpami.2019.2954885

House, J. E., Brambilla, V., Bidaut, L. M., Christie, A. P., Pizarro, O., Madin, J. S., et al. (2018). Moving to 3D: relationships between coral planar area, surface area and volume. PeerJ 6:e4280. doi: 10.7717/peerj.4280

Istenič, K., Gracias, N., Arnaubec, A., Escartín, J., and Garcia, R. (2020). Automatic scale estimation of structure from motion based 3D models using laser scalers in underwater scenarios. ISPRS J. Photogramm. Remote Sens. 159, 13–25. doi: 10.1016/j.isprsjprs.2019.10.007

Jones, C. G., Lawton, J. H., and Shachak, M. (1994). Organisms as ecosystem engineers. Oikos 69, 373–386. doi: 10.2307/3545850

Kersting, D. K., and Linares, C. (2019). Living evidence of a fossil survival strategy raises hope for warming-affected corals. Sci. Adv. 5:eaax2950. doi: 10.1126/sciadv.aax2950

Kružić, P. S., and Benković, L. (2012). The impact of seawater temperature on coral growth parameters of the colonial coral Cladocora caespitosa (Anthozoa, Scleractinia) in the eastern Adriatic Sea. Facies 58, 477–491. doi: 10.1007/s10347-012-0306-4

Lagudi, A., Bianco, G., Muzzupappa, M., and Bruno, F. (2016). An alignment method for the integration of underwater 3D data captured by a stereovision system and an acoustic camera. Sensors 16:536. doi: 10.3390/s16040536

Ledoux, J. B., Frias-Vidal, S., Montero-Serra, I., Antunes, A., Casado, B. C., Civit, S., et al. (2020). Assessing the impact of population decline on mating system in the overexploited mediterranean red coral. Aquatic Conserv. Mar. Freshw. Ecosyst. 30, 1149–1159. doi: 10.1002/aqc.3327

Ledoux, J. B., Garrabou, J., Bianchimani, O., Drap, P., Féral, J. P., and Aurelle, D. (2010). Fine-scale genetic structure and inferences on population biology in the threatened mediterranean red coral, Corallium rubrum. Mol. Ecol. 19, 4204–4216. doi: 10.1111/j.1365-294X.2010.04814.x

Liu, B., Liu, Z., Men, S., Li, Y., Ding, Z., He, J., et al. (2020). Underwater hyperspectral imaging technology and its applications for detecting and mapping the seafloor: a review. Sensors 20:4962. doi: 10.3390/s20174962

Maldonado, M., López-Acosta, M., Sánchez-Tocino, L., and Sitjà, C. (2013). The rare, giant gorgonian Ellisella paraplexauroides: demographics and conservation concerns. Mar. Ecol. Prog. Ser. 479, 127–141. doi: 10.3354/meps10172

Marschal, C., Garrabou, J., Harmelin, J. G., and Pichon, M. (2004). A new method for measuring growth and age in the precious mediterranean red coral Corallium rubrum (L.). Coral Reefs 23, 423–432. doi: 10.1007/s00338-004-0398-6

Menna, F., Nocerino, E., Nawaf, M. M., Seinturier, J., Torresani, A., Drap, P., et al. (2019). "Towards real-time underwater photogrammetry for subsea metrology applications," in Proceedings of the OCEANS 2019, Marseille, 1–10. doi: 10.1109/OCEANSE.2019.8867285

Montero-Serra, I. (2018). Resilience of Long-Lived Mediterranean Gorgonians in a Changing World: Insights From Life History Theory and Quantitative Ecology. Ph.D. thesis, University of Barcelona, Barcelona.

Montero-Serra, I., Garrabou, J., Doak, D. F., Ledoux, J. B., and Linares, C. (2019). Marine protected areas enhance structural complexity but do not buffer the consequences of ocean warming for an overexploited precious coral. J. Appl. Ecol 56, 1063–1074. doi: 10.1111/1365-2664.13321

Montseny, M., Linares, C., Viladrich, N., Olariaga, A., Carreras, M., Palomeras, N., et al. (2019). First attempts towards the restoration of gorgonian populations on the Mediterranean continental shelf. Aquat. Conserv. Mar. Freshwater Ecosyst. 29, 1278–1284. doi: 10.1002/aqc.3118

Nocerino, E., Dubbini, M., Menna, F., Remondino, F., Gattelli, M., and Covi, D. (2017). Geometric calibration and radiometric correction of the MAIA multispectral camera. Int. Arch. Photogramm. Remote Sens. Spat. Inf. Sci. 42, 149–156. doi: 10.5194/isprs-archives-xlii-3-w3-149-2017

Nocerino, E., Neyer, F., Grün, A., Troyer, M., Menna, F., Brooks, A. J., et al. (2019). Comparison of diver-operated underwater photogrammetric systems for coral reef monitoring. Int. Arch. Photogramm. Remote Sens. Spat. Inf. Sci. 42, 143–150. doi: 10.5194/isprs-archives-xlii-2-w10-143-2019

Olinger, L. K., Scott, A. R., McMurray, S. E., and Pawlik, J. R. (2019). Growth estimates of Caribbean reef sponges on a shipwreck using 3D photogrammetry. Sci. Rep. 9:18398. doi: 10.1038/s41598-019-54681-2

Ordoñez, A., Wangpraseurt, D., Lyndby, N. H., Kühl, M., and Diaz-Pulido, G. (2019). Elevated CO2 leads to enhanced photosynthesis but decreased growth in early life stages of reef building coralline algae. Front. Mar.Sci. 5:495. doi: 10.3389/fmars.2018.00495

Palma, M., Casado, M., Pantaleo, U., Pavoni, G., Pica, D., and Cerrano, C. (2018). SfM-based method to assess gorgonian forests (Paramuricea clavata (Cnidaria. Octocorallia). Remote Sens. 10:1154. doi: 10.3390/rs10071154

Palomer, A., Ridao, P., Forest, J., and Ribas, D. (2019). Underwater laser scanner: Ray-based model and calibration. IEEE/ASME Trans. Mechatron. 24, 1986–1997. doi: 10.1109/tmech.2019.2929652

Pavoni, G., Corsini, M., Fiameni, G., Callieri, M., Edwards, C., and Cignoni, P. (2020). On improving the training of models for the semantic segmentation of benthic communities from orthographic imagery. Remote Sens. 12:3106. doi: 10.3390/rs12183106

Pedersen, N. E., Edwards, C. B., Eynaud, Y., Gleason, A. C. R., Smith, J. E., and Sandin, S. A. (2019). The influence of habitat and adults on the spatial distribution of juvenile corals. Ecography 42, 1703–1713. doi: 10.1111/ecog.04520

Peirano, A., Morri, C., Bianchi, C. N., and Rodolfo-Metalpa, R. (2001). Biomass, carbonate standing stock and production of the mediterranean coral Cladocora caespitosa (L.). Facies 44, 75–80. doi: 10.1007/bf02668168

Piazza, P., Cummings, V. J., Lohrer, D. M., Marini, S., Marriott, P., Menna, F., et al. (2018). Divers-operated underwater photogrammetry: applications in the study of antarctic benthos. Int. Arch. Photogramm. Remote Sens. Spat. Inf. Sci. 42, 885–892. doi: 10.5194/isprs-archives-XLII-2-885-2018

Ponti, M., Turicchia, E., Ferro, F., Cerrano, C., and Abbiati, M. (2018). The understorey of gorgonian forests in mesophotic temperate reefs. Aquat. Conserv. 28, 1153–1166. doi: 10.1002/aqc.2928

Qi, C. R., Yi, L., Su, H., and Guibas, L. J. (2017). “Pointnet++: deep hierarchical feature learning on point sets in a metric space,” in Proceedings of the 31st International Conference on Neural Information Processing Systems, 5099–5108.

Righi, S., Prevedelli, D., and Simonini, R. (2020). Ecology, distribution and expansion of a mediterranean native invader, the fireworm Hermodice carunculata (Annelida). Mediterr. Mar. Sci. 21, 575–591. doi: 10.12681/mms.23117

Robinson, K. L., Luo, J. Y., Sponaugle, S., Guigand, C., and Cowen, R. K. (2017). A tale of two crowds: public engagement in plankton classification. Front. Mar. Sci. 4:7. doi: 10.3389/fmars.2017.00082

Rossi, P., Castagnetti, C., Capra, A., Brooks, A. J., and Mancini, F. (2020). Detecting change in coral reef 3D structure using underwater photogrammetry: critical issues and performance metrics. Appl. Geomatics 12, 1–15. doi: 10.1007/s12518-019-00263-w

Rossi, S., Bramanti, L., Gori, A., and Orejas, C. (2017). Marine Animal Forests. The Ecology of Benthic Biodiversity Hotspots. Cham: Springer International Publishing.

Rossi, S., and Orejas, C. (2019). “Approaching cold-water corals to the society: novel ways to transfer knowledge,” in Proceding of the Mediterranean Cold-Water Corals: Past, Present and Future, (Cham: Springer), 473–480. doi: 10.1007/978-3-319-91608-8_39

Sandin, S. A., Edwards, C. B., Pedersen, N. E., Vid, P., Gaia, P., Esmeralda, A., et al. (2020). Considering the rates of growth in two taxa of coral across Pacific islands. Adv. Mar. Biol. 87, 167–191. doi: 10.1016/bs.amb.2020.08.006

Scaradozzi, D., Zingaretti, S., Ciuccoli, N., Costa, D., Palmieri, G., Bruno, F., et al. (2018). Lab4Dive mobile smart lab for augmented archaeological dives. IOP Conf. Ser. Mater. Sci. Eng. 364:012054. doi: 10.1088/1757-899x/364/1/012054

Shihavuddin, A. S. M., Gracias, N., Garcia, R., Gleason, A., and Ginter, B. (2013). Image-based coral reef classification and thematic mapping. Remote Sens. 5, 1809–1841. doi: 10.3390/rs5041809

Stanghellini, G., Del Bianco, F., and Gasperini, L. (2020). OpenSWAP, an open architecture, low cost class of autonomous surface vehicles for geophysical surveys in the shallow water environment. Remote Sens. 12:2575. doi: 10.3390/rs12162575

Trabes, E., and Jordan, M. A. (2017). A node-based method for SLAM navigation in self-similar underwater environments: a case study. Robotics 6:29. doi: 10.3390/robotics6040029

Turicchia, E., Abbiati, M., Sweet, M., and Ponti, M. (2018). Mass mortality hits gorgonian forests at montecristo island. Dis. Aquat. Org. 131, 79–85. doi: 10.3354/dao03284

Verdura, J., Linares, C., Ballesteros, E., Coma, R., Uriz, M. J., Bensoussan, N., et al. (2019). Biodiversity loss in a mediterranean ecosystem due to an extreme warming event unveil the role of an engineering gorgonian species. Sci. Rep. 9:5911. doi: 10.1038/s41598-019-41929-0

Vidal, E., Palomeras, N., Istenič, K., Gracias, N., and Carreras, M. (2020). Multisensor online 3D view planning for autonomous underwater exploration. J. Field Rob. 37, 1–25. doi: 10.1002/rob.21951

Westoby, M. J., Brasington, J., Glasser, N. F., Hambrey, M. J., and Reynolds, J. M. (2012). Structure-from-motion’ photogrammetry: a low-cost, effective tool for geoscience applications. Geomorphology 179, 300–314. doi: 10.1016/j.geomorph.2012.08.021

Wilkinson, M. D., Dumontier, M., Aalbersberg, I. J., Appleton, G., Axton, M., Baak, A., et al. (2016). The FAIR guiding principles for scientific data management and stewardship. Sci. Data 3:160018. doi: 10.1038/sdata.2016.18

Zweifler, A., Akkaynak, D., Mass, T., and Treibitz, T. (2017). In situ analysis of coral recruits using fluorescence imaging. Front. Mar. Sci. 4:273. doi: 10.3389/fmars.2017.00273

Chapter 2

Observing marine caves from a seascape perspective

Although marine caves are only a small fraction of Mediterranean marine environments, they are considered biodiversity hotspots, acting as a reservoir for many endemic species. Even though they are included in the Habitats Directive (92/43/EEC), and their threats and urge to be properly assessed have been recently highlighted, no special efforts have been made so far to monitor these crucial ecosystems. The rise of new technologies, such as SfM-photogrammetry, opened new possibilities for the monitoring of these unique environments.

In this context, paper 3, "**Photogrammetry in marine caves: unveiling hidden seascapes**", explores the potential of SfM to assess the abundance and spatial distribution patterns of the sessile benthic assemblages associated with a semi-submerged marine cave, considering, as case study, the small Grotta Azzurra (North Adriatic Sea). The results obtained by photogrammetry were compared with a more traditional approach by the virtual deployment of 20 x 20 cm and 50 x 50 cm quadrats.

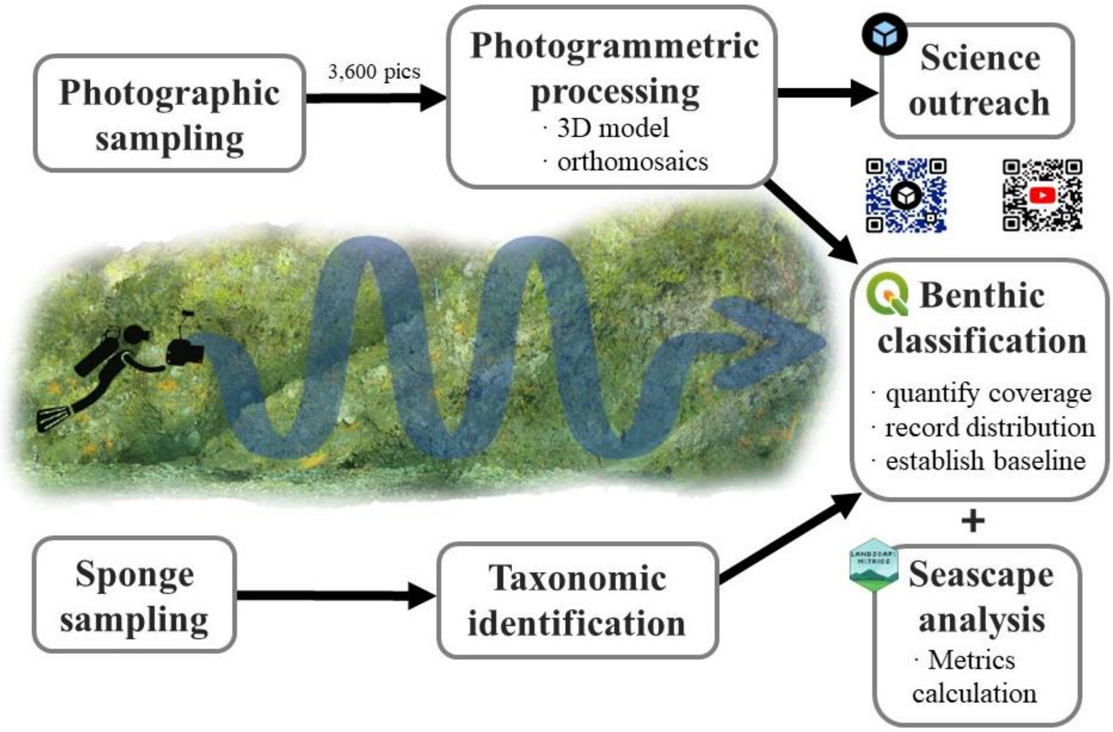
SfM demonstrated to be a suitable non-invasive technique to record cave assemblages, establishing a baseline in terms of biological and structural patterns of the habitat. Moreover, the calculation of seascape indices proved to be a comprehensive way to describe the spatial patterns of biological distributions, useful to identify potential future community shifts.

Paper 3

Scientific Reports, *accepted for publication*

Photogrammetry as a promising tool to unveil marine caves' benthic assemblages

Torcuato Pulido Mantas, Camilla Roveta, Barbara Calcinai, Martina Coppari, Cristina Gioia Di Camillo, Veronica Marchesi, Teo Marrocco, Stefania Puce and Carlo Cerrano



Supplementary material available at:

3D material available at:

Photogrammetry as a promising tool to unveil marine caves' benthic assemblages

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Abstract

Traditionally, monitoring approaches to survey marine caves have been constrained by equipment limitations and strict safety protocols. Nowadays, the rise of new approaches opens new possibilities to describe these peculiar ecosystems. The current study aimed to explore the potential of Structure from Motion (SfM) photogrammetry to assess the abundance and spatial distribution of the sessile benthic assemblages inside a semi-submerged marine cave. Additionally, since impacts of recent date mussel *Lithophaga lithophaga* illegal fishing were recorded, a special emphasis was paid to its distribution and densities. The results of SfM were compared with a more "traditional approach", by simulating photo-quadrats deployments over the produced orthomosaics. A total of 22 sessile taxa were identified, with Porifera representing the dominant taxa within the cave, and *L. lithophaga* presenting a density of 88.3 holes/m^2. SfM and photo-quadrats obtained comparable results regarding species richness, percentage cover of identified taxa and most of the seascape metrics, while, in terms of taxa density estimations, photo-quadrats highly overestimated their values. SfM resulted in a suitable non-invasive technique to record marine cave assemblages. Seascape indexes proved to be a comprehensive way to describe the spatial pattern of distribution of benthic organisms, establishing a useful baseline to assess future community shifts.

Keywords: Imaging technology, seascape ecology, dark habitats, Mediterranean Sea.

Introduction

Caves represent a unique pocket of biodiversity in the oceans as on land. The stability of their peculiar environmental conditions can trigger important isolation processes and lead to the evolution and selection of rare endemic species. In the marine environment these conditions, when occurring in the photic zone, offer scientists the opportunity to explore and to study, in accessible "natural laboratories", the mesophotic and the deep-sea ecosystem biodiversity and functioning [1,2], where they can be transposed and studied along a horizontal gradient instead of a vertical one. Moreover, caves can create refuges for various species and reveal unexpected trophic webs [1]. Strong environmental gradients (e.g., hydrodynamics, light, nutrient concentrations, among others) occur towards the inner parts of the caves, shaping their biotic communities and defining different ecological zones [3-6]. And even though the ecological uniqueness and importance of these habitats have been repeatedly acknowledged by the scientific community [7-10], only a relatively small number of marine caves have been explored and assessed in terms of their biotic component [e.g., 4, 10-25].

Global and local stressors strongly affect coastal ecosystems [7, 26], and marine caves are not an exception. In fact, these habitats are characterised by highly stable and fragile biocenosis [9,27], and different studies showed how the synergy of climate change and human activities can deeply modify their communities [e.g., 7,28]. Illegal harvesting (e.g., of *Corallium rubrum* (Linnaeus, 1758), *Lithophaga lithophaga* (Linnaeus, 1758) among others), spearfishing, unregulated visit of divers and tourist boats, and the deep modification of coastal areas, together with the more and more frequent marine heat waves [29], are also threatening the sessile benthic communities of marine caves, which are known to have a low recovery potential [7,9,27,30]. In the Mediterranean Sea, marine caves are listed as priority habitats by the Habitat Directive (92/43/ECC, code 8330) and included in the Dark Habitats Action Plan [31]. Effective management plans or protection/conservation measures are still lacking [10,32]. Although some studies have addressed the vulnerability characterising marine caves [7, 9, 33], it is difficult to generalise the main threats of this habitat at a local scale. For the proper development of targeted and effective measures, *ad-hoc* surveys are crucial to identify the main pressures affecting them [7,10].

Traditionally, monitoring approaches to survey marine caves have been constrained by equipment limitations and the strict safety protocols of cave diving [34]. Different methods have been used to describe the biodiversity hosted in these enclosed habitats, including among others, photo-quadrats, linear transects or video surveys [10,25,35-38]. Nowadays, the development of new technologies and the minimization of underwater equipment contributed to the emergence of new tools: such as vehicles (e.g., Autonomous Underwater Vehicles (AUVs)), sensors (e.g., compact echosounders), and methods (e.g., Structure from Motion (SfM) photogrammetry), which opened new possibilities to assess changes also occurring inside caves [39-41]. Thanks to its ability to digitally reconstruct a whole scenario from a series of overlapping images, SfM-photogrammetry is considered a cost-effective technique to monitor a great variety of underwater environments from a three-dimensional (3D) perspective [42-56]. Additionally, it offers the possibility to better assess organisms with complex architectures, constituting a suitable method for in-situ non-invasive evaluations [57]. The feasibility of this approach has been confirmed in different habitats, not only by its increased number of applications in the scientific literature, but also by its implementation in citizen science initiatives [44], as its recent inclusion among citizen science protocols for the monitoring of Marine Protected Areas in the Mediterranean (e.g., protocol 11 of the Interreg MED MPA Engage project) [56].

In this context, the objective of the current study was to explore the potential of SfM-photogrammetry to assess the abundance and spatial distribution patterns of the sessile benthic assemblages associated with a semi-submerged marine cave, considering, as a case study, a cave in the North Adriatic Sea. We focussed our attention mainly on the sponge diversity, since Porifera represents the predominant taxon within the study cave, thus constituting an essential component in this environment, and more generally acting as ecosystem engineers, driving substrate and nutrient availability [58-60]. Additionally, a special emphasis was paid to *L. lithophaga*'s hole distribution and densities, due to the still current illegal fishing activities occurring in the area (Figure S1), despite being strictly protected under international directives, agreements, and conventions [27]. The obtained results using photogrammetry were compared with those achieved through a more traditional approach, by simulating photo-quadrats deployments over the produced orthomosaics, to assess their efficiency in capturing biodiversity.

Results

Benthic sessile community assessment

Thanks to the photogrammetric approach it was possible to obtain a 3D digital reconstruction for the whole cave (Video S1). Additionally, two orthomosaics were produced from the orthoplane of each of the walls (Fig. 1a), corresponding to a total projected area of 61.2 m^2 (24.5 m^2 and 36.7 m^2 for the semidark and dark zone, respectively) (Table 1). Overall, by coupling photogrammetry and the sponge samples analysis, a total of 22 sessile taxa were identified, including Rhodophyta (2), Porifera (13), Hydrozoa (1), Serpulidae (1), Bilvavia (1) and Tunicata (4) (Table 2). In addition, 2 vagile taxa, the shrimp *Palaemon serratus* and the echinoid *Paracentrotus lividus*, were observed (Table 2). Among all identified taxa, only two (*Aplysina aerophoba* and *P. lividus*) are under protection, being listed in the Annex II and III of the SPA/BD Protocol of Barcelona Convention.

Table 1 – Sampling protocols applied for the cave community characterization. Q20 = 20 x 20 cm quadrats; Q50 = 50 x 50 cm quadrats; SfM = Structure from Motion photogrammetry.

Protocol	**Sampling units**	**Sampling area**	**Description**	**References**
SfM	Whole cave	61.2 m^2	Reconstruction of the whole cave habitat	Palma et al., 2017; Bayley & Mogg, 2020
Q20	60	2.4 m^2	Random deployment of 15 quadrats per cave zone	Bianchi et al., 2004; Leujack & Ormond. 2007
Q50	40	10.0 m^2	Random deployment of 10 quadrats per cave zone	Bianchi et al., 2004; Leujack & Ormond, 2007

Table 2 – List of identified species and benthic categories with the corresponding coverage per category, expressed as total cover for Structure from Motion (SfM) photogrammetry and average cover (av) (± standard deviation, sd) for 50 x 50 cm quadrats (Q50) and 20 x 20 cm quadrats (Q20). * = listed in the Annex II and III of the SPA/BD Protocol of Barcelona Convention.

Categories	**Group composition**	**SfM (m^2)**			**Q50 (area ± sd, m^2)**			**Q20 (area ± sd, m^2)**		
		Semidark	Dark	Whole	Semidark	Dark	Whole	Semidark	Dark	Whole
Porifera										
AI group	*Aaptos aaptos* (Schmidt, 1864) *Ircinia variabilis* (Schmidt, 1862)	0.013	0.434	0.447	0.010 ± 0.005	0.012 ± 0.019	0.012 ± 0.019	–	0.027 ± 0.047	0.027 ± 0.047
Aplysina aerophoba (Nardo, 1833)*		1.103	5.31	6.413	0.008 ± 0.018	0.020 ± 0.034	0.015 ± 0.028	0.035 ± 0.047	0.09 ± 0.13	0.07 ± 0.11
Chondrosia reniformis (Nardo, 1847)		0.031	0.024	0.055	0.0002	–	0.0002	–	–	–
Clathria (Microciona) armata (Bowerbank, 1862)		0.606	0.744	1.35	0.004 ± 0.006	0.005 ± 0.008	0.004 ± 0.007	0.018 ± 0.027	0.018 ± 0.031	0.018 ±0.029
EP group	*Erylus mammilaris* (Schmidt, 1862) *Penares euastrum* (Schmidt, 1868)	2.921	4.008	6.929	0.049 ± 0.079	0.026 ± 0.057	0.037 ± 0.069	0.12 ± 0.11	0.11 ± 0.15	0.11 ± 0.13
Haliclona (Haliclona) michelei Van Soest & Hooper, 2020		1.071	0.319	1.39	0.007 ± 0.009	0.004 ± 0.005	0.006 ± 0.008	0.035 ± 0.034	0.03 ± 0.03	0.034 ±0.033
Hymedesmia (Hymedesmia) pansa Bowerbank, 1882		0.324	0.033	0.357	0.008 ± 0.140	0.002 ± 0.050	0.007 ± 0.013	0.023 ± 0.040	–	0.02 ± 0.04
Oscarella lobularis (Schmidt, 1862)		0.047	0.005	0.052	0.001 ± 0.001	0.005 ± 0.007	0.002 ± 0.002	0.007 ± 0.008	–	0.007 ± 0.008
Rhabderemia topsenti Van Soest & Hooper, 1993		0.836	0.666	1.502	0.023 ± 0.026	0.016 ± 0.042	0.021 ± 0.033	0.070 ± 0.068	0.08 ± 0.13	0.08 ± 0.11
Tedania anhelans (Vio in Olivi, 1792)		0.294	0.167	0.461	0.007 ± 0.010	0.011 ± 0.015	0.008 ± 0.012	0.030 ± 0.024	0.04 ± 0.09	0.031 ± 0.061

Timea bifidostellata Pulitzer-Finali, 1983		0.483	0.09	0.573	0.006 ± 0.014	0.012 ± 0.042	0.008 ± 0.023	0.029 ± 0.046	–	0.029 ± 0.046
Others										
Crustose Coralline Algae (CCA)		0.757	–	0.757	0.007 ± 0.011	–	0.007 ± 0.011	0.027 ± 0.032	–	0.027 ± 0.032
Folious red algae		1.974	–	1.974	0.06 ± 0.15	–	0.06 ± 0.15	0.25 ± 0.25	–	0.25 ± 0.25
Hydroid assemblages		1.776	1.311	3.087	0.051 ± 0.092	0.09 ± 0.12	0.063 ± 0.11	0.19 ± 0.19	0.13 ± 0.13	0.16 ± 0.17
Tunicates	Didemnidae Giard, 1872 Ascidiidae Herdman, 1882 Styelidae Herdman, 1881 Pyuridae Hartmeyer, 1908	0.153	0.441	0.594	0.003 ± 0.004	0.002 ± 0.002	0.002 ± 0.003	0.011 ± 0.012	0.013 ± 0.012	0.012 ± 0.012
Bare substrata		11.876	21.735	33.611	0.045 ± 0.120	0.61 ± 0.23	0.10 ± 0.22	0.22 ± 0.23	0.57 ± 0.23	0.33 ± 0.28
Not considered for cover calculation										
Lithophaga lithophaga (Linnaeus, 1758)*		–	–	–	–	–	–	–	–	–
Serpulids		–	–	–	–	–	–	–	–	–
Palaemon serratus (Pennant, 1777)		–	–	–	–	–	–	–	–	–
Paracentrotus lividus (Lamarck, 1816)*		–	–	–	–	–	–	–	–	–
Not applicable		0.284	1.44	1.724	–	–	–	–	–	–

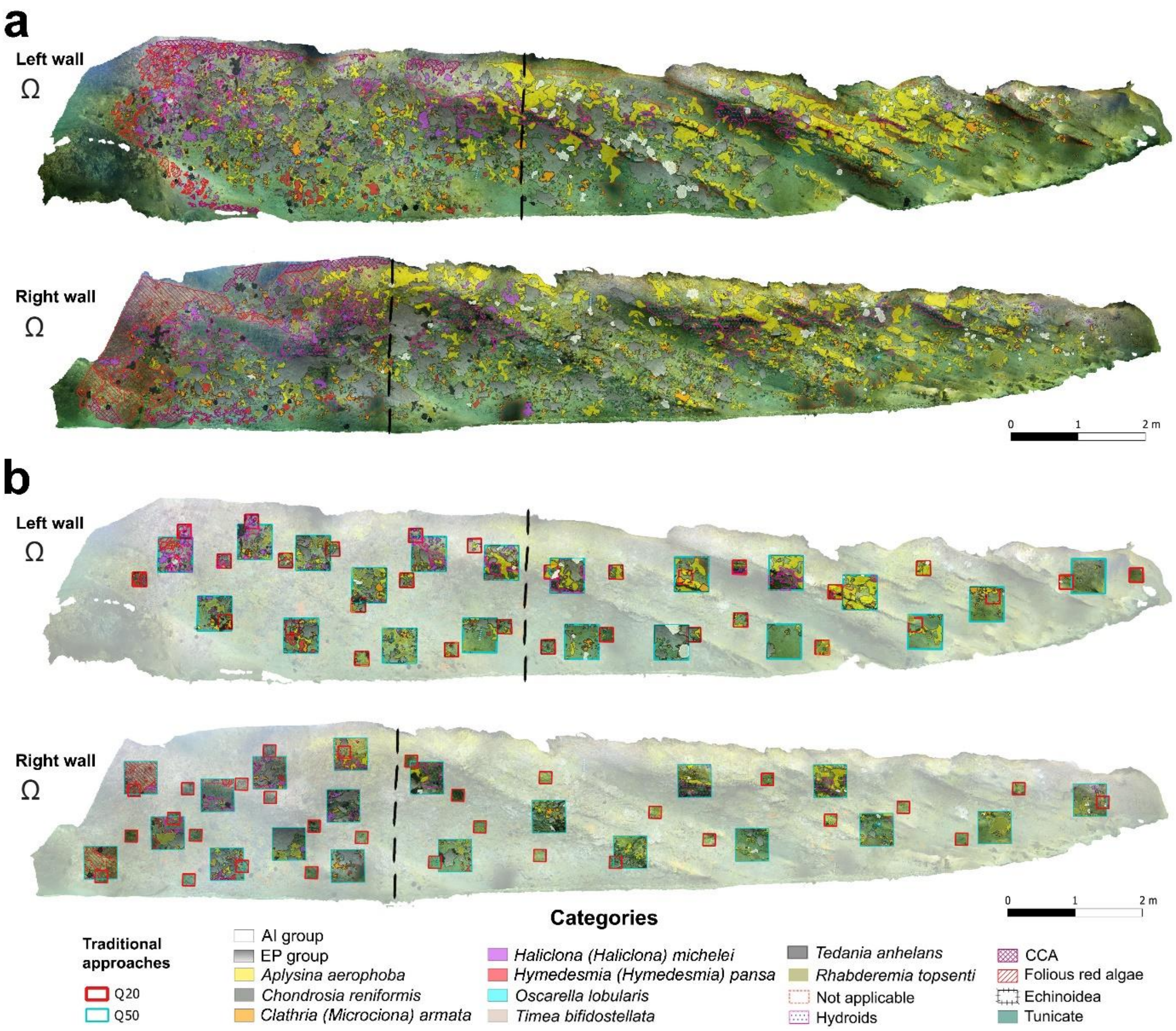

Fig. 1 – (a) Cave walls orthomosaics segmented by the categories defined in Table 2; (b) distribution of the randomly deployed quadrat approaches. Dashed lines delimit the semidark and dark areas. Q20 = 20 x 20 cm quadrats; Q50 = 50 x 50 cm quadrats. The symbol **Ω** indicates the cave entrance. For categories' acronyms see Table2.

Since it was not possible to differentiate some of the sponge species by simple imagery, for the analyses, several species were clustered into groups, allowing to identify a total of 16 benthic categories (Table 2). The area covered by each category was extracted separately for each eco-zone (Table 2; Fig. 1a and 2a). Apart from the Rhodophyta (crustose coralline algae (CCA) and folious red algae), all the other categories were shared between the two biocoenosis, with a gradual decrease in the biotic cover towards the inside part of the cave, where the percent coverage of bare substrate reached the 55 % (Fig. 1a and 2a). In both cave biocoenoses, Porifera resulted as the prevalent phylum, with a total of 31.4% and 32.2% in the semidark and dark zone, respectively (Fig. 1a and 2a,b). The semidark area was dominated by the EP (*Erylus mammillaris* and *Penares euastrum*) group (2.91 m^2), followed by folious red algae (1.97 m^2) and hydroid assemblages (1.78 m^2), while the less represented taxa were the AI (*Aaptos aaptos* and *Iricinia variabilis*) group (0.013 m^2), *Chondrosia reniformis* (0.031 m^2) and the only homoscleromorphan sponge recorded, *Oscarella lobularis* (0.047 m^2) (Table 2; Fig. 1a and 2a). *A. aerophoba* was instead the dominant component of the dark biocenosis (5.31 m^2), together with EP group (4.01 m^2) and hydroids (1.31 m^2) (Table 2; Fig. 1a and 2a). Again *O. lobularis* and *C. reniformis* were among the taxa with the lowest coverage (0.005 and 0.024 m^2, respectively), along with *Hymedesmia (Hymedesmia) pansa* (0.033 m^2) (Table 2; Fig.1a and 2a). Additionally, up to a total cover of 1.7 m^2 (2.8%) of the cave was classified as “Not applicable” due to the low quality of orthomosaic caused by the particulate present in the water column (Table 2; Fig. 1a and 2a).

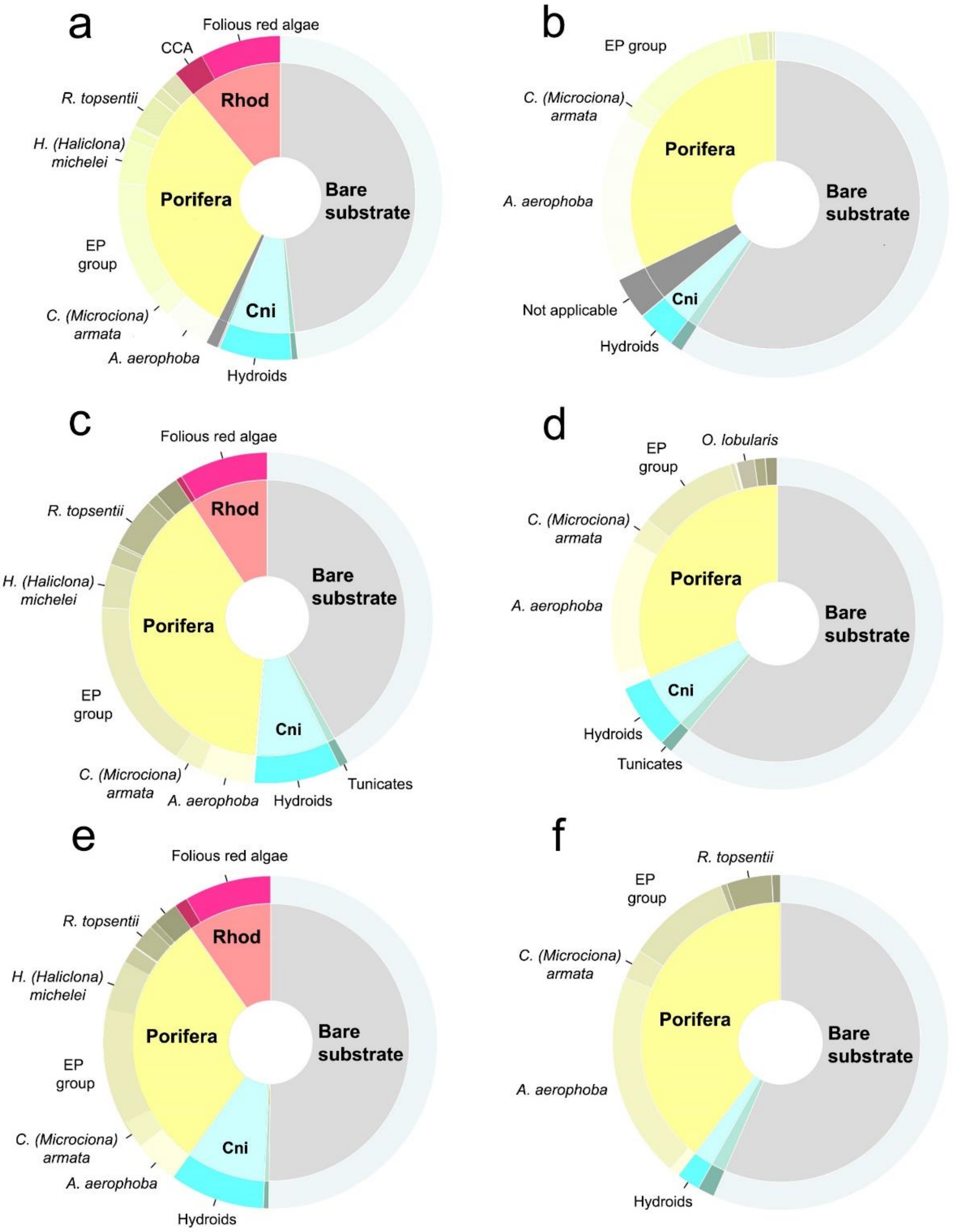

Fig. 2 – Ring-charts representing the percentage cover of the sessile benthic taxa identified and of the bare substrate per: Structure from Motion Photogrammetry in the (a) semidark and (b) dark zones; 50 x 50 cm quadrat approach in the (c) semidark and (d) dark zones; and 20 x 20 cm quadrat approach in the (e) semidark and (f) dark zones. For categories’ acronyms see Table1.

Overall, the sampling method using the Q50 (n=40), covering an area of only 10 m^2 (Table 1; Fig. 1b; Figure S2), allowed to detect of all the 16 benthic categories, while the deployment of Q20 (n=60, surveyed area = 2.4 m^2) recorded a total of 15 categories, missing only *C. reniformis* (Table 1 and 2; Fig. 1b; Figure S2). Analysing each biocenosis separately, *C. reniformis* was not found in the dark area by the Q50, while the Q20 approach failed to detect *H. (H.) pansa, O. lobularis* and *Timea bifidostellata* in the dark area and the AI group in the semidark one (Table 2). However, the results obtained by each approach in terms of percentage cover do not seem to greatly differ (Fig. 2a-f), although some discrepancies can be observed: the CCA cover being underrated by both quadrat approaches in the semidark zone (Fig. 2c,e), or the hydroid assemblages of the dark biocoenosis being slightly underestimated by the Q20 (Fig. 2f) while overrated by the Q50 ones (Fig. 2d).

In Figure 3a,b the effect of the sampling effort was investigated better to explore the possible differences among the three approaches. In the semidark area, only the Q50 reached to identify all categories after 19 deployments, managing to detect 15 out of 16 benthic categories after deploying 10 sampling units (Fig. 3a). On the other hand, the Q20 identified 13 categories after 10 deployments, and still missed three at the maximum effort (n=30) (Fig. 3a). In the case of dark biocoenosis, the applied sampling effort was not sufficient to reach the same number of categories as the SfM. Even so, the Q50 recorded 11 out of 13 categories after the deployment of 10 sampling units, while the Q20 only identified 7 for the same number of deployments (Fig. 3b).

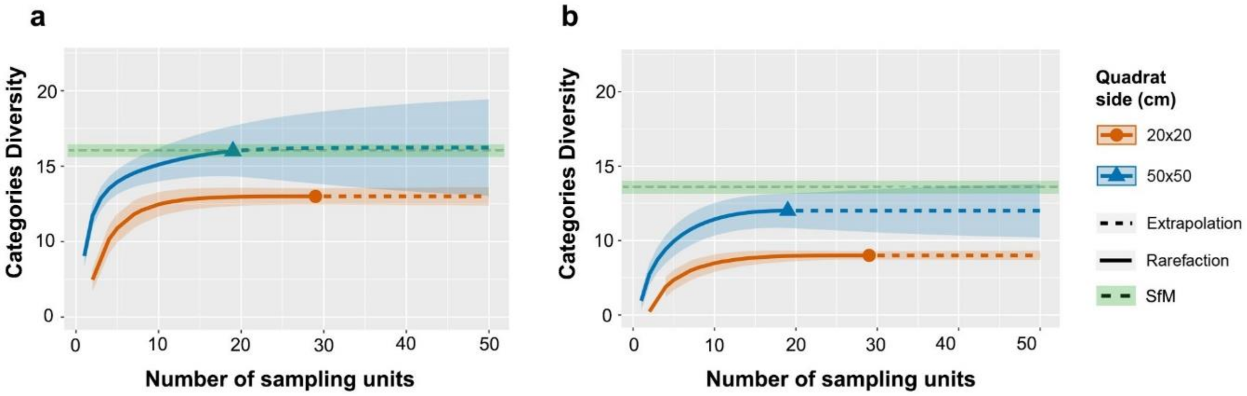

Fig. 3 – Sampling-unit-based rarefaction and extrapolation curves with 95% confidence intervals (shaded areas) for benthic diversity data considering the three applied methodologies in the (a) semidark and (b) dark areas of the cave. Q20 = 20 x 20 cm quadrats; Q50 = 50 x 50 cm quadrats; SfM = Structure from Motion photogrammetry.

Assessment of Lithophaga lithophaga *holes' density and distribution*

Thanks to SfM-photogrammetry, *L. lithophaga* hole densities were recorded through the walls of the cave, obtaining an average density of 88.3 holes / m^2 at cave scale (Fig. 4). Considering the two ecozones separately, a general decrease on the date mussel average densities was detected towards the inner parts of the cave, with a density of 102.95 holes / m^2 in the semidark area against 78.65 holes / m^2 in the dark one. Differences between the cave walls were also evident, being the semidark area of the left wall the ecozone holding the higher density (139.8 holes / m^2) (Fig. 4). In addition, taking a close look, a preference of this species for boring into the vertical and sub-vertical parts of the walls may be observed (Fig. 4; Figure S3).

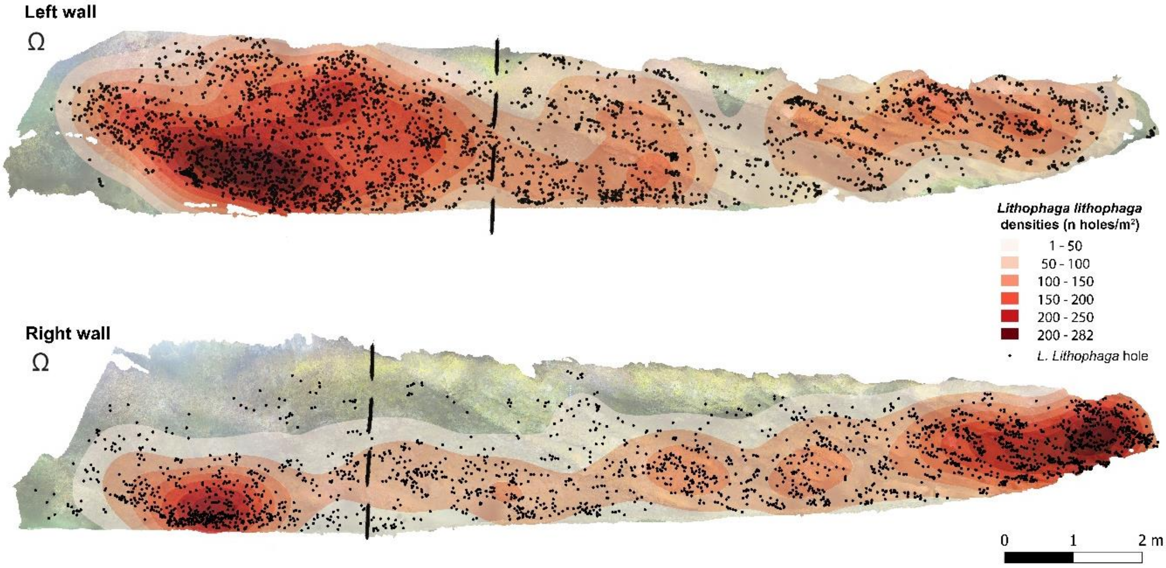

Fig. 4 – Distribution and density of date mussel (*Lithophaga lithophaga*) holes through the cave walls obtained by the analysis of Structure from Motion photogrammetry results. Dashed lines delimit the semidark and dark areas. The symbol Ω indicates the cave entrance.

Both Q50 and Q20 methods overestimated *L. lithophaga* hole densities at cave scale (106.8 and 129.17 holes / m^2, respectively). Considering the different areas separately, in the semidark zone the Q20 obtained very similar results to the SfM approach (105 holes / m^2) whilst the Q50 method underestimated hole densities (83.7 holes / m^2). Conversely, in the dark area it occurs just the opposite, obtaining a density value of 88.2 holes / m^2 with the Q50 and 100.83 holes / m^2 with the Q20.

The influence of sampling effort on the determination of *L. lithophaga* hole densities for the two considered quadrat sizes is presented in Figure 5 a,b. In the semidark area both quadrat approaches underestimated *Lithophaga* species with a relatively small number of sampling units, however the Q20 managed to obtain similar results that the SfM when reached the maximum sampling effort (Fig. 5a). On the other hand, in the dark area, with the deployment of 10 sampling units both quadrat approaches came quite close to the ground truth values, slightly overestimating hole's density at its maximum sampling effort (Fig. 5b).

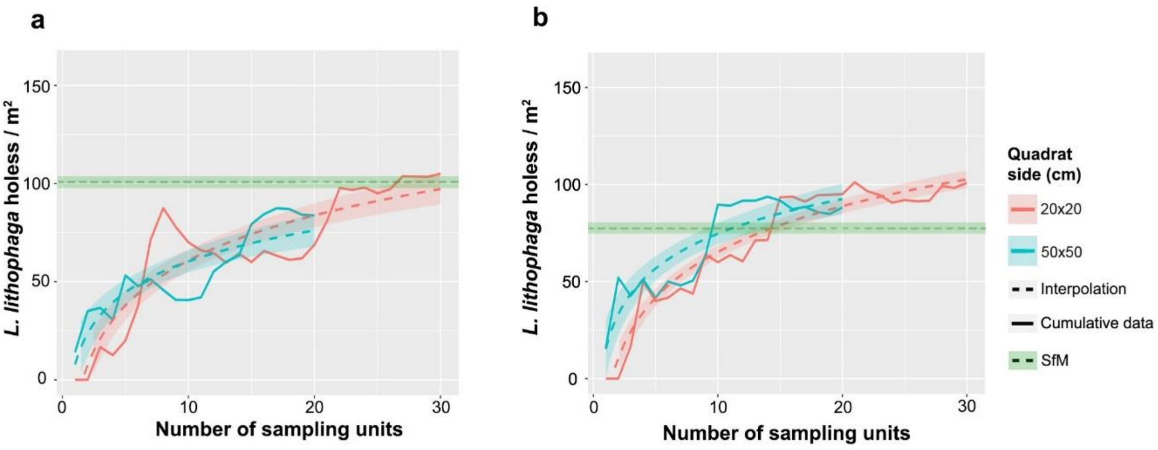

Fig. 5 – Interpolation curves for *Lithophaga lithophaga* holes' density data in function of the sampling effort for (a) semidark and (b) dark area. Green line = results obtained by the Structure from Motion (SfM) approach analysis of the whole cave is presented as the green line. Q20 = 20 x 20 cm quadrats; Q50 = 50 x 50 cm quadrats.

Seascape Metrics

Indices values at seascape level were calculated, according to Table 3, and presented as supplementary material in Table S1. Comparing the results obtained applying SfM, an increase in the abundance of PN and in the PARA index towards the inside of the cave was observed (Fig. 6). On the other hand, LPI and SDPS presented higher values in the semidark area, while MPS was comparable among the two zones (Fig. 6). Differences have also been recorded among sampling methodologies: (i) both quadrat approaches obtained lower values for PN, MPS and SDPS than SfM, (ii) quadrats recognized more significant patches (> LPI) in the dark biocoenosis, and, in terms of shape, (iii) SfM found more complex patch shapes (> PARA) in the dark area, Q20 in the semidark area, while Q50 registered similar values for both ecological zoned (Fig. 6).

Table 3 – Seascape descriptors calculated at category and seascape levels for both ecological zones of Grotta Azzurra. All metrics not presenting units in the table are dimensionless.

Index		Brief description
Patch number, size, and shape	Patch Number (PN) (n patch)	Number of patches. It describes the fragmentation of a category, however, does not necessarily contain information about the configuration or composition of the category.
	Largest Patch Index (LPI) (%)	Correspond to the percentage of the seascape interested at the single largest patch; where 100 would mean that the seascape consists in a single large patch and approach 0 as this dominant patch decreases in size.
	Mean of Patch Size (MPS) (m^2)	The metric summarises each category as the mean of all patch areas belonging to category i
	Standard Deviation of Patch Size (SDPS) (m^2)	The metric summarises each category as the standard deviation of all patch areas belonging to category i
	Mean Patch Area Perimeter Ratio (PARA)	It summarises each category as the mean of each patch belonging to category i. The perimeter-area ratio describes the patch complexity in a straightforward way. As PARA increases, patches become more complex.

Dispersion / Aggregation	Patch Density (PD) (n patch / 1 m^2)	Number of patches per area of the landscape, please notice that it is standardized to area in order to be comparable among landscapes with different areas.
	Landscape Shape Area Index (LSI) (%)	This index measures the perimeter-to-area ratio for the whole. It ranges from 1 to infinite; where 1 would mean that the seascape consists in a single patch and increases as the patches become more disaggregated.
	Division Index (DIVISION) (ratio)	Correspond to the probability that two pixels chosen randomly in the seascape are not located inside the same patch; it presents higher values when a seascape is highly segmented in separate patches.
	Cohesion Index (COHESION) (%)	It characterises the connectedness of patches belonging to a category. It assesses if patches of the same category are located aggregated or rather isolated and thereby gives information about the landscape configuration.
Diversity	Shannon's Diversity Index (SHDI)	This index is based on the information theory (Shannon & Weaver, 1949) and its value represents the amount of "information" per category. Takes both into account the number of categories and the abundance of each category, thus a high value corresponds to a higher number of category types and/or evenness.
	Simpson's Diversity Index (SIDI)	The value of Simpson's index reflects the probability that two random patches belong to the same category. It is less sensitive to rare categories than SHDI. The higher the value, the greater the diversity.
	Shannon's Evenness Index (SHEI)	This index is based on the proportion of max. SHDI on the distribution of area among patch types; its value is 1 when the area covered by the different patch types is evenly distributed. It is understood as a measure of dominance.
	Simpson's Evenness Index (SIEI)	This index is based on the ratio between the actual SIDI and the theoretical max. SIDI. Its value is 1 when the area covered by the different patch types is evenly distributed.

Regarding aggregation indices, no great discrepancies were found between the two cave zones by any of the methods (Fig. 6), yet Q20 and Q50 overestimated the average patch density present in the cave (Fig. 6). Finally, the results obtained with SfM for diversity and evenness found higher SHDI and SIEI values in the semidark area, indicating a more diverse and equally distributed community than the dark area (Fig. 7). Additionally, even though Q20 slightly underestimated both SHDI and SIEI, the values obtained with the three methods are similar (Fig. 7).

Seascape indices were also calculated at category level, with all three approaches obtaining comparable results (see Table S2). Regarding patch number size and shape indices, for PN and PD, *Clathria (Microciona) armata* and *H. (H.) michelei* were identified as the more abundant and dense species in the semidark area, while in the dark one they were tunicates and *A. aerophoba*; on the other hand, *C. reniformis*, *O. lobularis* and AI group hold the lower values in both biocoenosis (Table S2). Red folious algae and hydroid assemblages were the categories presenting the higher LPI, MPS and SDPS in the semidark area, while EP group, *A. aerophoba* and again hydroids where the highest in the dark zone. The categories with the smallest patch, average size and size variability were *C. (M.) armata*, EP group, *H. (H.) pansa*, *O. lobularis*, *Rhabderemia topsenti* and tunicates in both biocenosis (Table S2).

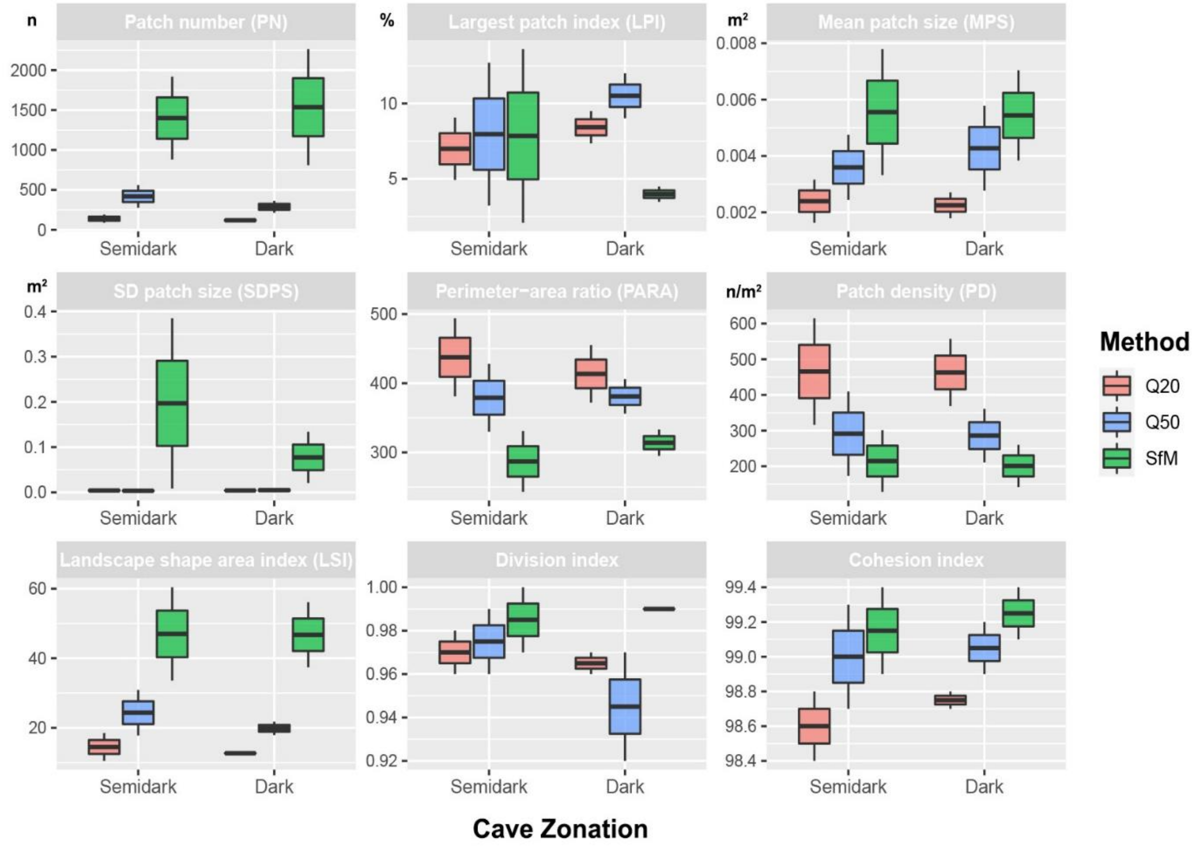

Fig. 6 – Seascape metric scores obtained for the three methodologies applied in the two cave zones. Q20 = 20 x 20 cm quadrats; Q50 = 50 x 50 cm quadrats; SfM = Structure from Motion photogrammetry.

Looking at aggregation indices, LSI, division, and cohesion gave us an idea of the configuration and connectedness of the patches. *H. (H.) michelei*, *C. (M.) armata* and *O. lubularis* resulted in the categories more disaggregated and less connected in the semidark areas, while AI group, hydroids and red folious algae were the categories holding the most compact and aggregated distributions. Towards the dark area, hydroids, and *H. (H.) michelei* were the more aggregated categories against tunicates, *O. lobularis* and *A. aerophoba* which were highly disaggregated and less compact (Table S2). The PARA index was the only exception in the observed trend. In fact, the three methods considered showed highly different results: SfM recognized *O. lobularis, H. (H.) pansa* and *Tedania anhelans* as the species with the most complex patches shapes (>PARA), while both quadrat approaches identified *C. reniformis*, tunicates, *C. (M.) armata* and *O. lobularis* (Table S2).

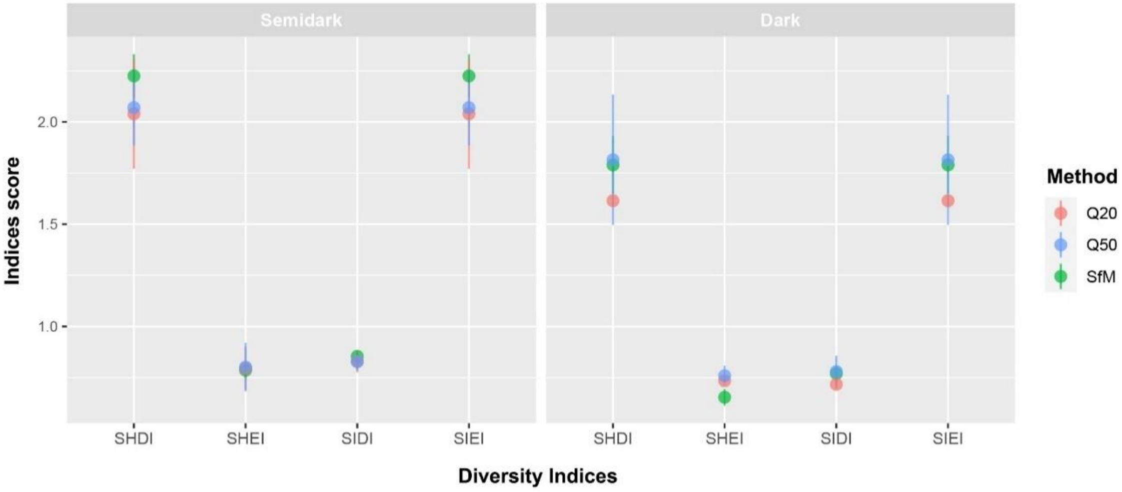

Fig. 7 – Diversity Indices scores obtained for the three different methodologies inspected in the two cave zones. SHDI = Shannon Diversity Index; SIDI = Simpson Diversity Index; SHEI = Shannon Evenness Index; SIEI = Simpson Evenness Index; Q20 = 20 x 20 cm quadrats; Q50 = 50 x 50 cm quadrats; SfM = Structure from Motion photogrammetry.

Discussion

All taxa identified in Grotta Azzurra are well-known and typical of Mediterranean marine caves [9,31,61], and their abundance and distribution along the two cave zones agree with the previous literature [3,4,6,62]. Porifera was the most representative group, defining Grotta Azzurra as a "sponge realm", as previously observed for other caves [37,60]. Overall, the most abundant sponges in terms of coverage and number of patches along the entire cave (*Aplysina aerophoba* and the EP group, formed by *Erylus mammillaris* and *Penares euastrum*) are also described in the literature among the most common species and genera of Mediterranean marine caves [31,61,63]. Additionally, *A. aerophoba* specimens presented an unusual growth habit characterised by "cushion-shaped individuals connected by abundant thin branching processes and forming large encrusting plates" (Figure S4), a type of growth only recently described by Costa et al. [63,64] in four semi-submerged Italian caves.

Hydroids were another abundant taxon in both biocenosis, and the only class of Cnidaria found in Grotta Azzurra. Cnidarian species (e.g., *Leptopsammia pruvoti* Lacaze-Duthiers, 1897, *Parazoanthus axinellae* (Schmidt, 1862)*, Astroides calycularis* (Pallas, 1766), among others) are typical components of cave communities, especially on the ceilings and walls of the entrance and semi dark zones. However, their presence is highly related to various abiotic factors, such as depth, water movement and sedimentation [5,65]. The peculiar environmental constraints of this semi-submerged cave (i.e., tunnel-shaped morphology, high hydrodynamic conditions) strictly select the species that can exploit this challenging niche. Endolithic, encrusting and soft-bodied species can face the high-water turbulence, dominating the benthic assemblage [66,67]. These features allowed hydroids to proliferate and to form significant patches along the walls, and their presence also in the deeper parts of the cave points out that high rates of water movement are not limited to the cave entrance [61,68,69] but also to the waves' reflection inside the cave. Additionally, hydroid patches presented a peculiar and marked distribution, occupying almost exclusively the overhangs of the walls' roughness, a pattern possibly driven to intercept the maximum intensity of water movement [68,69].

In terms of species richness, percentage cover of identified taxa and most of the seascape metrics at category level, the three sampling methodologies here applied (SfM, Q50 and Q20) obtained comparable results, highlighting the reliability of traditional approaches in the general characterization of a cave community, and suggesting the comparability of data obtained by traditional and more innovative methods. Nevertheless, it must be considered that the sampling effort implemented in this study for Q50 and Q20 was particularly high compared to the typical sampling effort applied in caves [35,70], usually related to bottom time and other logistic constraints.

The results of random quadrat deployments can strongly vary depending on the quadrat size and sampling effort, limiting its potential to capture uncommon species distributions (Table 4) [35,36]. The Q50 seemed to be a more cost-effective solution than Q20, obtaining more similar results to SfM. Even so, a limitation on its implementation in these habitats should be acknowledged; in fact, caves often present narrow passages where carrying a frame of such dimensions could not be possible or its manipulation risks to damage organisms protruding from the surface, especially species with fragile skeletons (Table 4) [71]. Additionally, in terms of taxa density estimations both quadrats' approaches overestimated their values at cave and category levels (Table 4). A similar situation occurred for the estimation of *Lithophaga lithophaga* hole densities. Conversely, the application of SfM-photogrammetry allowed not only to define density values accurately, but also to record their distribution along the cave walls. The density patterns of *L. lithophaga* holes could possibly be explained by the hydrodynamics occurring in the cave: in fact, by comparing its distribution with the structural complexity of the walls, it is clear how the morphology and orientation of the substrate play a crucial role on the settlement of this species, which usually thrives in sub-vertical to vertical carbonate substrates, thus avoiding high sedimentation rates [72,73]. Due to the high price and demand of the date mussel in the Mediterranean Sea, this species is still intensively collected despite the laws and marine policies forbidding it. For its extraction, the rock is heavily damaged, causing a dramatic simplification of the biotic and structural composition of the substrate, leading to an impoverishment of the entire community [74,75], a phenomenon widely spread in the Conero Riviera (Figure S1).

Table 4 – List of advantages and disadvantages of the compared methodologies inspected in this study. Q20 = 20 x 20 cm quadrats; Q50 = 50 x 50 cm quadrats; SfM = Structure from Motion photogrammetry.

Protocol	Estimated time consumption (h)		Advantages	Disadvantages
	Sampling	Analysis		
SfM	1	100	· Establish a complete baseline · Capture 3D structural complexity · Multiscale approach · Science outreach application	· Require longer bottom times · Require longer analysis times · Depending on the extension and scale, possible need of a powerful workstation.
Q20	0.5	15	· Shorter bottom times · No need of a powerful workstation	· May miss uncommon species · Densities overestimation
Q50	0.3	30	· Shorter bottom times · No need of a powerful workstation	· May miss uncommon species · Densities overestimation · Size of the frame

In this context, when applicable, SfM photogrammetry should be considered as an additional and complementary tool for marine cave monitoring. Applied over time, short, medium, and long-term changes could be recorded from seascape to individual level, down to a mm scale [48,76]. By the 3D reconstruction of the substrate morphology, a more complete picture of the biological distribution patterns and the processes driving them can be provided [47,77]. The implementation of systematic monitoring plans including photogrammetry would also help lawmakers in the creation of ad-hoc management and protection measures targeting specific threats affecting marine caves locally [46]. Furthermore, the obtained 3D models represent a powerful ally for public outreach activities, raising awareness by developing interactive experiences where people can explore these hidden habitats (e.g., Figure S5) [78,79].

Nonetheless, given the intrinsic risks related to cave diving [34], some considerations must be made before implementing the technique in underwater caves, especially in terms of cave’s depth, size and morphology to ensure SfM applicability. Moreover, depending on the main objective of the study, the desired spatial and taxonomical resolution of the digital outputs, and the extension of the area to be mapped, the sampling, processing and annotation times may increase exponentially (Table 4). Thus, a cost-benefits evaluation must be made to evaluate the different techniques available, reducing operators’ risks and maintaining a cost-effective monitoring effort [9,31]. In the assessment of large areas, a more powerful working station or cloud-based processing solutions may be needed to process the imagery dataset [47]. Additionally automated and semi-automated tools must be considered to support and speed up manual annotation process, improving the cost efficiency of the method [80-84].

Although the rise of compact waterproof cameras provides a high-quality, reasonably-price solution, which suggest photogrammetry as an affordable and attractive method for both recreational and scientific scuba divers [57], a proper training and the implementation of a standardized protocol is essential for a good application of the technique and the acquisition of accurate reconstructions [49, 56].

In conclusion, even if quadrats work great detecting the main coverages and trends in homogeneously distributed benthic communities, when it comes to sparse heterogeneous communities (e.g., dark cave biocoenosis) or rare organisms, they may overlook some of its components. Underwater photogrammetry demonstrated to be a suitable non-invasive technique to record the cave benthic assemblages, besides the calculation of a group of carefully selected seascape indices proved to be a comprehensive way to describe the present spatial pattern. A first baseline was established for Grotta Azzurra: the definition of the current distribution of its biotic component represents a crucial information in these times of rapid community shifts due to the synergy of climate change and human stressors [10,29]. A bright future awaits photogrammetry in the field of marine environments monitoring thanks to its huge potential and versatility, together with the exponential progress of computer vision, robotics, and machine learning [80-84].

Materials and methods

Study site

The Conero Riviera (Ancona, Italy) is a shoreline that suffered from strong artificialization over the last century, with some of its natural substrates modified or replaced by cemented structures [85]. A series of shallow marine semi-submerged caves are present along its coastal cliffs [86]. In this study the bigger of these caves was explored, locally known as Grotta Azzurra (43°37'17.2"N, 13°31'38.4"E) (Fig. 8a). Its opening represents a big fissure facing north into the calcareous walls, starting a few metres above the sea surface (Fig. 8b), and continuing down to 4.5 m depth. With 15 m in length, this semi-submerged tunnel-shaped cave reaches its maximum depth at its entrance (4.5 m), presenting a floor mainly composed of gravels and small rock aggregations. The dominant wave direction in the area is E – SE (Fig. 8c), leaving the cave partially sheltered from the direct action of waves. Nevertheless, the lack of thin sediment accumulation inside the Grotta Azzurra, despite the high sediment charge that characterises the bottoms of the Conero Riviera [85], suggests a moderate to high hydrodynamic regime.

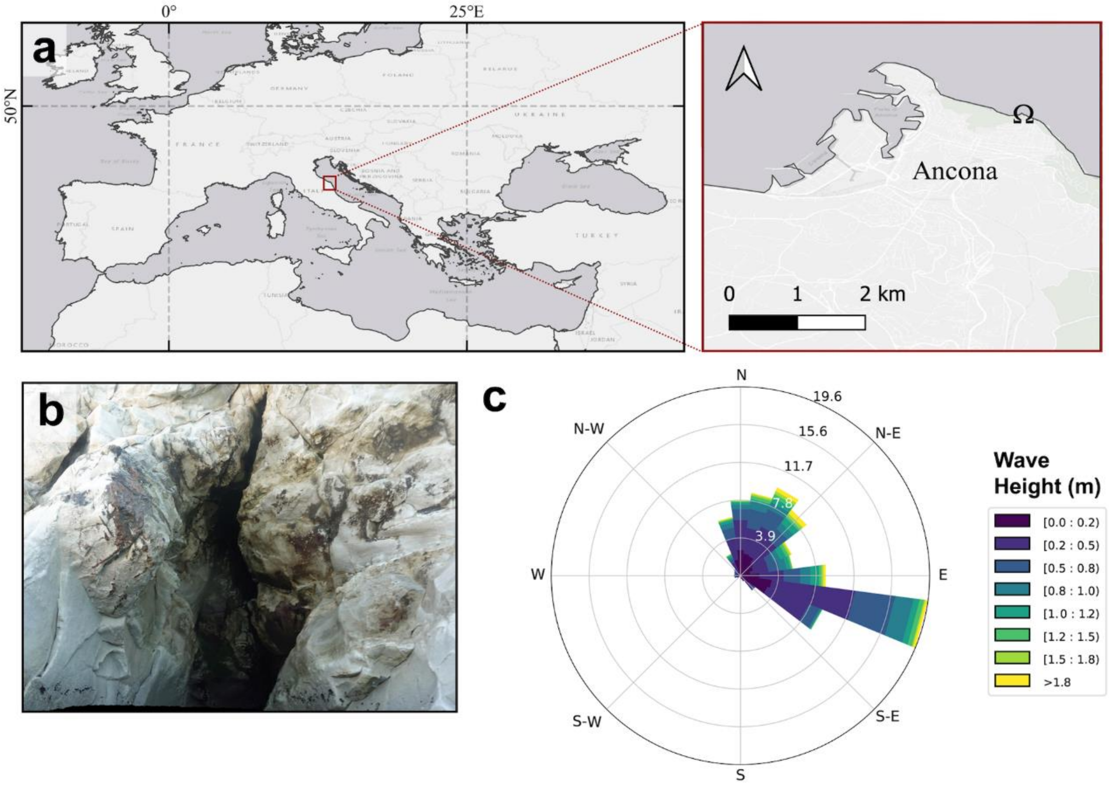

Fig. 8 – (a) Location map of the Grotta Azzurra (Ancona, Italy), marked with an **Ω**. (b) Detail of the emerged part of Grotta Azzurra entrance. (c) Wave rose of significant wave height, corresponding to the for 2021 full year period at the near-shore waters in front of the cave entrance.

Data acquisition and photogrammetric processing

The sampling strategy for the characterization of the sessile benthic community present inside Grotta Azzurra consisted of the coupling of two sampling methodologies, each one implemented during a dedicated dive. In the first place, a photographic sampling of the cave was performed during early September 2021. A GoPro HERO8 Black (Woodman Labs, Inc., San Mateo, CA, USA) equipped with an artificial lighting system composed of two AKKIN 5000 underwater lights was used. Along the walls and the floor of the cave, a series of metric references were placed to scale-up and control the accuracy of the digital reconstructions. The camera was set to time-lapse mode at 2 frames per second, and the diving operator adapted the sampling path to capture the whole cave topography by carrying out a vertical boustrophedonic pattern, ensuring a minimum of 60% overlapping among consecutive images [45,47]. Maintaining an average distance from the substrate of around 40 cm, the images were homogeneously illuminated. A total of 3,600 images were collected and controlled to ensure picture quality before being imported into Metashape v. 1.8.2. (Agisoft LLC, St. Petersburg, Russia). Images alignment was performed using high accuracy generic pair selection settings to produce the point clouds, limiting the key points identification to 100,000 and the tie point limit to 10,000 common feature points. Meshes were produced by the arbitrary three-dimensional surface type from the depth maps data, medium face count and interpolation disabled. To scale up the model, 5 metric references were manually detected in the imagery dataset and used to create scale bars in the reference settings. Finally, an orthomosaic of each wall was produced by mosaic blending mode from the mesh surface data and exported as tiled tiff in a local coordinate system (m). The overall photogrammetric process to generate both the digital reconstruction of the cave and the couple of orthoimages took 16 h of processing time using a Lenovo Legion laptop (Beijin, China) with an Intel Core i7-9750H 2.60-GHz processor (Intel Corporation, Santa Clara, CA, USA), 32 Gb RAM and a graphic card NVIDIA GeForce RTX 2060 (NVIDIA Corporation, Santa Clara, CA, USA).

After identifying the organisms occurring in the orthomosaic, cover percentages of the various sessile organisms were calculated to assess the abundance and the distribution patterns of benthic species in the two ecological zones (semidark and dark), following the cave zonation defined by Pérès and Picard [3]. When the sponges were not identifiable at species level, the individual's location in the digitised cave walls was noted using the orthomosaics. To increase the taxonomic resolution, a second dive was performed to collect a fragment as small as possible of each unidentified sponge. The collected samples were then fixed in ethanol 95% and processed as described by Rützler [87] for the preparation of slides. Specimens were then identified at species level using a Nikon Eclipse Ni compound microscope. Slides were stored as a reference and deposited at the Zoology Laboratory Collection (Department of Life and Environmental Sciences), Polytechnic University of Marche (Ancona, Italy). Taxonomic identifications were carried out based on Systema Porifera [88], "Proposal for a revised classification of the Demospongiae (Porifera)" [89] and the World Porifera Database [90].

In this study, no live vertebrates and/or higher invertebrates have been used for experimental purposes.

Digitalization and single patch analyses

The orthomosaics produced for both cave walls were imported into QGIS software v. 3.12 [91], the different mega-epibenthic sessile taxa were manually digitised into scaled polygons or patches and classified at the lowest taxonomic level possible. Nonetheless, in some cases, it was not possible to differentiate between some of the identified species, therefore they were clustered into a group of species (Table 2). In particular, the AI group included the sponge species *Aaptos aaptos* (Schmidt, 1864) and *Ircinia variabilis* (Schmidt, 1862), and the EP group consisted in the aggregation of the sponges *Erylus mammillaris* (Schmidt, 1862) and *Penares euastrum* (Schmidt, 1868) (Table 2). Serpulids and vagile species were not considered for the analysis. The whole digitization process required 10 days (i.e., 70 h). Both walls were fully segmented and classified, including the bare substrate, and each segmented category's surface area (m^2) was calculated. *Lithophaga lithophaga* occurrence was accounted for and digitised throughout the entire walls. The fact that among all holes inspected, only *L. lithophaga* individuals, or shell fragments, were found inside the holes, made us assume that the date mussel was only responsible for all the holes present in the cave. However, to maintain the non-invasive character of the approach and to contain bottom time during sampling activities, it was not possible to confirm if each of the recorded holes was currently inhabited by the bivalve. *L. lithophaga* density was calculated in each cave zone by dividing the number of holes with the surface of each zone and expressed as *L. lithophaga* holes/m^2. Additionally, a heatmap (Fig. 5) was produced in QGIS using a 1m radius to visualize density changes through the cave walls.

Two ecological zones were considered for the analysis, the semidark (i.e., entrance of the cave, with sciophilous macroalgae) and dark (i.e., inner section of the cave) biocoenoses, applying the model of cave zonation defined by Pérès and Picard [3]. To compare the results obtained by photogrammetry and to explore the effects of different sampling efforts, a second approach was also applied implementing a virtual simulated quadrat deployment in QGIS. Two quadrats' sizes were considered, 20x20 cm (Q20) and 50x50 cm (Q50), with 60 and 40 deployments respectively (Table 1). To this end, the tool "Random Points Inside Polygon" was applied to place the quadrats' centroids over the walls, discarding those ending up partially out of the orthomosaics or overlapping [45]. Secondly, the already classified vectorial layers were clipped by each digital quadrat to extract species' surface covers and the *L. lithophaga* holes densities.

Seascape metric estimations

Key seascape metrics were calculated using *landscapemetrics* R package [92], a drop-in replacement for FRAGSTATS software [93]. Using a rasterized version of our digitised cave community as an input, this package allowed the quantification of seascape characteristics at a multispatial level by considering patches size, shape, and distribution. A series of seascape descriptors (Table 3) were selected based on the literature and its ecological relevance [45,46,82,83], and estimated at both category and seascape levels for each ecological zone and sampling approach. For the analysis, the category "bare substrate" was not considered, to focus our spatial analytical efforts on the biotic components of the cave.

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References

1. Vacelet, J. & Boury-Esnault, N. Carnivorous sponges. *Nature* **373,** 333-335 (1995). 10.1038/373333a0
2. Harmelin, J.G. & Vacelet, J. Clues to deep-sea biodiversity in a nearshore cave. *Vie et Milieu* ***47*(4)**, 351-354 (1997).
3. Pérès, J.M., & Picard, J. Nouveau manuel de bionomie benthique de la Mer Méditerranée. *Recueil des Travaux de la Station Marine d'Endoume* **31(47)**, 5-137 (1964).
4. Riedl, R. Biologie der Meereshöhlen. Hamburg: Paul Parey (1966).
5. Benedetti-Cecchi, L., Airoldi, L., Abbiati, M. & Cinelli, F. Spatial variability in the distribution of sponges and cnidarians in a sublittoral marine cave with sulphur-water springs. *J. Mar. Biolog. Assoc.* ***78*(1)**, 43-58 (1998). 10.1017/S0025315400039953
6. Morri, C., & Bianchi, C.N. Zonazione biologica. In Grotte Marine: Cinquant'Anni di Ricerca in Italia. Rome: Ministero dell'Ambiente e della Tutela del Territorio (eds. Cicogna, F., Bianchi, C.N., Ferrari, G., Forti, P.), 257–265 (2003).
7. Montefalcone, M., *et al.* Thirty-year ecosystem trajectories in a submerged marine cave under changing pressure regime. *Mar. Environ. Res.* **137**, 98-110 (2018). 10.1016/j.marenvres.2018.02.022
8. Mačić, V., Panou, A., Bundone, L., Varda, D., & Pavićević, M. First inventory of the semi-submerged marine caves in South Dinarides Karst (Adriatic coast) and preliminary list of species. Turkish *J. Fish Aquat. Sci.* **19(9)**, 765-774 (2019). 10.4194/1303-2712-v19_9_05
9. Gerovasileiou, V. & Bianchi, C.N. Mediterranean marine caves: A synthesis of current knowledge. *Oceanogr. Mar. Biol.*, 1-87 (2021)
10. Digenis, M., Arvanitidis, C., Dailianis, T. & Gerovasileiou, V. Comparative Study of Marine Cave Communities in a Protected Area of the South-Eastern Aegean Sea, Greece. *J. Mar. Sci. Eng.* **10(5)**, 660 (2022) 10.3390/jmse10050660
11. Pérès, J.M., & Picard, J. Notes sommaires sur le peuplement des grottes sous-marines de la région de Marseille. *C. R. Somm. Séances. Soc. Biogéogr.* **227**, 42-45 (1949).
12. Sarà, M. Distribuzione ed ecologia dei Poriferi in acque superficiali della Riviera ligure di Levante. *Arch Zool Ital* **49**,181–248 (1964).
13. Sket, B. & Iliffe, T.M. Cave fauna of Bermuda. Internationale *Revue. Der. Gesamten. Hydrobiol. Hydrogr.* **65**, 871-882 (1980). 10.1002/iroh.19800650610
14. Yager, J. Remipedia, a new class of Crustacea from a marine cave in the Bahamas. *J. Crustac. Biol.* **1**, 328-333 (1981). 10.2307/1547965
15. Harmelin, J.G. Bryozoan dominated assemblages in Mediterranean cryptic environments. In Bryozoa: Ordovician to recent (eds. Nielsen, C., & Larwood, G.P.). Olsen and Olsen, Fredensborg, pp 135-143 (1985).
16. Hart, C.W., Manning, R.B., & Iliffe, T.M. The fauna of Atlantic marine caves: evidence of dispersal by sea floor spreading while maintaining ties to deep water. *Proc. Biol. Soc. Wash.* **98(1)**, 288–292 (1985).
17. Manning, R.B., Hart, C.W., & Iliffe, T.M. Mesozoic relicts in marine caves of Bermuda. *Stygologia* **2(1/2)**, 156-166 (1986).
18. Bianchi, C.N, Cattaneo-Vietti, R., Cinelli, F., Morri, C. & Pansini, M. Lo studio biologico delle grotte sottomarine: conoscenze attuali e prospettive. *Boll. Mus. Ist Biol. Univ. Genova* **60-61**, 41-69 (1996).
19. Cicogna, F., Bianchi, C.N., Ferrari, G. & Forti, P. Grotte marine: cinquant'anni di ricerca in Italia. Roma: Ministero dell'Ambiente e della Tutela del Territorio. (2003)
20. Heiner, I., Boesgaard, T.M., & Kristensen, R.M. First time discovery of Loricifera from Australian waters and marine caves. *Mar. Biol. Res.* **5(6)**, 529-546 (2009). 10.1080/17451000902933009
21. Iliffe, T.M. & Kornicker, L.S. Worldwide diving discoveries of living fossil animals from the depths of anchialine and marine caves. Smithson *Contrib. Mar. Sci.* **38**, 269-280 (2009).

22. Jørgensen, A., Boesgaard, T.M., Møbjerg, N., & Kristensen, R.M. The tardigrade fauna of Australian marine caves: With descriptions of nine new species of Arthrotardigrada. *Zootaxa* **3802(4)**, 401-443 (2014). 10.11646/zootaxa.3802.4.1
23. Ereskovsky, A.V., Kovtun, O.A. & Pronin, K.K. Marine cave biota of the Tarkhankut Peninsula (Black Sea, Crimea), with emphasis on sponge taxonomic composition, spatial distribution and ecological particularities. *J. Mar. Biol. Assoc.* UK **96(2)**, 391-406 (2016). 10.1017/S0025315415001071
24. Perez, T., *et al.* How a collaborative integrated taxonomic effort has trained new spongiologists and improved knowledge of Martinique Island (French Antilles, eastern Caribbean Sea) marine biodiversity. *PloS one* **12(3)**, 0173859; 10.1371/journal.pone.0173859 (2017).
25. Cardone, F., Mazzetti, M., Sorci, A., Cesaretti, A., Cimmaruta, R. & Gravina, M.F. First Speleological and Biological Characterization of a Submerged Cave of the Tremiti Archipelago Geomorphosite (Adriatic Sea). *Geosci. J.* **12(5)**, 213 (2022). 10.3390/geosciences12050213
26. Nepote, E., Bianchi, C.N., Morri, C., Ferrari, M., & Montefalcone, M. Impact of a harbour construction on the benthic community of two shallow marine caves. *Mar. Pollut. Bull.* **114(1)**, 35-45 (2017). 10.1016/j.marpolbul.2016.08.006
27. La Mesa, G., Paglialonga, A., & Tunesi, L. Manuali per il monitoraggio di specie e habitat di interesse comunitario (Direttiva 92/43/CEE e Direttiva 09/147/CE) in Italia: ambiente marino. ISPRA, Serie Manuali e linee guida 190/2019 (2019).
28. Chevaldonné, P. & Lejeusne, C. Regional warming-induced species shift in north-west Mediterranean marine caves. *Ecol. Lett.* **6(4)**, 371-379 (2003) 10.1046/j.1461-0248.2003.00439.x
29. Garrabou, J., *et al*. Marine heatwaves drive recurrent mass mortalities in the Mediterranean Sea. *Glob. Change Biol.* **58**, 5708– 5725 (2022). 10.1111/gcb.16301
30. Iliffe, T.M., Jickells, T.D., & Brewer, M.S. Organic pollution of an inland marine cave from Bermuda. *Mar. Environ. Res.* **12(3)**, 173-189 (1984). 10.1016/0141-1136(84)90002-3
31. Gerovasileiou, V., Aguilar, R. & Marín, P. Guidelines for Inventorying and Monitoring of Dark Habitats in the Mediterranean Sea; SPA/RAC-UNEP/MAP, OCEANA: Tunis, Tunisia, 2017; pp 1–40 (2017)
32. Petricioli, D., Buzzacott, P., Radolović, M., Bakran-Petricioli, T. & Gerovasileiou, V. Visitation and conservation of marine caves. In Book of Abstracts of the International Symposium "Inside and outside the Mountain", Custonaci: Comune di Custonaci and Centro Ibleo di Ricerche Speleo-idrogeologiche, pp. 29-30 (2015).
33. Gerovasileiou, V., Bancila, R.I., Katsanevakis, S. & Zenetos, A. Introduced species in Mediterranean marine caves: an increasing but neglected threat. *Mediterr. Mar. Sci.*, **23(4)**, 995-1005 (2022). 0000-0002-9143-7480
34. Iliffe, T.M., & Bowen, C. Scientific Cave Diving. *Mar. Technol. Soc. J.* **35(2)**, 36-41 (2001). 10.4031/002533201788001901
35. Benedetti-Cecchi, L., Airoldi, L., Abbiati, M. & Cinelli, F. Estimating the abundance of benthic invertebrates: a comparison of procedures and variability between observers. *Mar. Ecol. Prog. Ser.* **138**, 93-101 (1996). 10.3354/meps138093
36. Bianchi, C.N., *et al.* Hard bottoms. *Biol. Mar. Mediterr.* **11(1)**, 185-215, (2004).
37. Gerovasileiou, V. & Voultsiadou, E. Sponge diversity gradients in marine caves of the eastern Mediterranean. *J. Mar. Biol. Assoc. UK*, **96(2)**, 407-416 (2016) 10.1017/S0025315415000697
38. Spaccavento, M., *et al*. A non-invasive monitoring method to assess the composition of megabenthic communities in semi-submerged marine caves. In 2022 IEEE International Workshop on Metrology for the Sea; Learning to Measure Sea Health Parameters (MetroSea), pp 257-261 (2022) 10.1109/MetroSea55331.2022.9950848

39. Gerovasileiou, V., Trygonis, V., Sini, M., Koutsoubas, D., & Voultsiadou, E. Three-dimensional mapping of marine caves using a handheld echosounder. *Mar. Ecol. Prog. Ser.* **486**, 13-22 (2013). 10.3354/meps10374
40. Chemisky, B., *et al.* Les fonds marins accessibles à tous avec la restitution tridimensionnelle haute resolution. In *proceedings of the MerlGéo*, de la côte à l'océan, l'information géographique en movement. Brest, 24-26 November. pp 57-60 (2015).
41. Vallicrosa, G., Fumas, M.J., Huber, F. & Ridao, P. Sparus II AUV as a Sensor Suite for Underwater Archaeology: Falconera Cave Experiments. In 2020 IEEE/OES Autonomous Underwater Vehicles Symposium (AUV). St. Johns, NL, Canada, 1-5, (2020). https://doi.org/10.1109/AUV50043.2020.9267935 (2020)
42. Burns, J.H.R., Delparte, D., Gates, R. D., & Takabayashi, M. Integrating structure-from-motion photogrammetry with geospatial software as a novel technique for quantifying 3D ecological characteristics of coral reefs. *PeerJ*, **3**, e1077 (2015). 10.7717/peerj.1077
43. Burns, J.H.R., *et al.* Assessing the impact of acute disturbances on the structure and composition of a coral community using innovative 3D reconstruction techniques. *Methods Oceanogr.*, **15**, 49-59 (2016). 10.1016/j.mio.2016.04.001
44. Raoult, V. *et al.* GoPros™ as an underwater photogrammetry tool for citizen science. *PeerJ*, **4**, p.e1960 (2016). 10.7717/peerj.1960
45. Palma, M., Rivas Casado, M., Pantaleo, U., & Cerrano, C. High resolution orthomosaics of African coral reefs: A tool for wide-scale benthic monitoring. *Remote Sens.* **9(7)**, 705 (2017). 10.3390/rs9070705
46. Palma, M., *et al.* Quantifying coral reef composition of recreational diving sites: A structure from motion approach at seascape scale. *Remote Sens.* **11(24)**, 3027 (2019). 10.3390/rs11243027
47. Bayley, D. T., Mogg, A. O., Koldewey, H., & Purvis, A. Capturing complexity: field-testing the use of 'structure from motion'derived virtual models to replicate standard measures of reef physical structure. *PeerJ,* **7**, e6540 (2019). 10.7717/peerj.6540
48. Piazza, P. *et al*. Underwater photogrammetry in Antarctica: Long-term observations in benthic ecosystems and legacy data rescue. *Polar Biology*, **42**, 1061-1079 (2019). 10.1007/s00300-019-02480-w
49. Bayley, D.T. & Mogg, A.O. A protocol for the large-scale analysis of reefs using Structure from Motion photogrammetry. *Methods Ecol. Evol.* **11**, 1410-1420 (2020). 10.1111/2041-210X.13476
50. Fukunaga, A. & Burns, J.H. Metrics of coral reef structural complexity extracted from 3D mesh models and digital elevation models. *Remote. Sens.* **12(17)**, 2676 (2020). 10.3390/rs12172676
51. Furlani, S. Integrated observational targets and instrumental data on rock coasts through snorkel surveys. *Mar. Geol.*, **245**, 106191 (2020). 10.1016/j.margeo.2020.106191
52. Casoli, E. *et al.* High spatial resolution photo mosaicking for the monitoring of coralligenous reefs. *Coral Reefs*, **40(4)**, 1267-1280 (2021). 10.1007/s00338-021-02136-4
53. Giordan, D. *et al.* R. Survey solutions for 3D acquisition and representation of artificial and natural caves. *Appl. Sci.*, **11(14)**, 6482 (2021). 10.3390/app11146482
54. Ventura, D. *et al*. Integration of close-range underwater photogrammetry with inspection and mesh processing software: a novel approach for quantifying ecological dynamics of temperate biogenic reefs. *Remote. Sens. Ecol. Conserv.*, **7(2)**, 169-186 (2021). 10.1002/rse2.178
55. Furlani, S. & Antonioli, F. The swim-survey archive of the Mediterranean rocky coasts: Potentials and future perspectives. *Geomorphology*, **421**, 108529 (2023). 10.1016/j.geomorph.2022.108529
56. Garrabou, J., *et al*. Monitoring Climate-related responses in Mediterranean Marine Protected Areas and beyond: ELEVEN STANDARD PROTOCOLS. 74 pp. Edited by: Institute of Marine Sciences, Spanish Research Council ICM-CSIC, Passeig Marítim de la Barceloneta 37-49, 08003 Barcelona, Spain (2022) 10.20350/digitalCSIC/14672

57. Bell, J.J. The functional roles of marine sponges. *Estuar. Coast. Shelf. Sci.* **79**, 341–353 (2008). 10.1016/j.ecss.2008.05.002
58. Voultsiadou, E., Kyrodimou, M., Antoniadou, C. & Vafidis, D. Sponge epibionts on ecosystem-engineering ascidians: The case of *Microcosmus sabatieri*. *Estuar. Coast. Shelf.* **86**, 598-606 (2010). 10.1016/j.ecss.2009.11.035
59. Gerovasileiou, V. & Voultsiadou, E. Marine caves of the Mediterranean Sea: a sponge biodiversity reservoir within a biodiversity hotspot. *PLoS One* **7(7),** 39873 (2012) 10.1371/journal.pone.0039873
60. Bussotti, S., Terlizzi, A., Fraschetti, S., Belmonte, G. & Boero, F. Spatial and temporal variability of sessile benthos in shallow Mediterranean marine caves. *Mar. Ecol. Prog. Ser.* **325**, 109-119 (2006). 10.3354/meps325109
61. Bianchi, C.N. & Morri, C. Studio bionomico comparativo di alcune grotte marine sommerse: definizione di una scala di confinamento. *Ist Ital. Speleol.* **6(II)**: 107-123 (1994).
62. Costa, G., *et al*. Sponge community variations within two semi-submerged caves of the Ligurian Sea (Mediterranean Sea) over a half-century time span. *Eur. Zool. J.* **85(1)**, 381-391 (2018). 10.1080/24750263.2018.1525439
63. Costa, G., Violi, B., Bavestrello, G., Pansini, M. & Bertolino, M. *Aplysina aerophoba* (Nardo, 1833) (Porifera, Demospongiae): an unexpected, miniaturised growth form from the tidal zone of Mediterranean caves: morphology and DNA barcoding. *Eur. Zool. J.* **87(1)**, 73-81 (2020). 10.1080/24750263.2020.1720833
64. Kruzic, P., Zibrowius, H., & Pozar-Domac, A. Actiniaria and Scleractinia (Cnidaria, Anthozoa) from the Adriatic Sea (Croatia): first records, confirmed occurrences and significant range extensions of certain species. *Ital. J. Zoo.* **69(4)**, 345-353 (2002). 10.1080/11250000209356480
65. Pansini, M., Pronzato, R., Fresi, E., Cinelli, F., Mazzella, L., & Ponticelli, M. Evoluzione delle biocenosi bentoniche di substrato duro lungo un gradiente di luce in una grotta marina superficiale. In Atti del IX Congresso della Società Italiana di Biologia Marina (eds. Fresi, E., & Cinelli, F.). La Seppia, Firenze, pp 315–330 (1977).
66. Boero, F., Cicogna, F., Pessani, D. & Pronzato, R. In situ observations on contraction behaviour and diel activity of *Halcampoides purpurea* var. *mediterranea* (Cnidaria, Anthozoa) in a marine cave. *Mar. Ecol.* **12(3)**, 185-192 (1991). 10.1111/j.1439-0485.1991.tb00252.x
67. Boero, F. The ecology of marine hydroids and effects of environmental factors: a review. *Mar. Ecol.* **5(2)**, 93-118, (1984). 10.1111/j.1439-0485.1984.tb00310.x
68. Boero, F. Hydroid zonation along a marine cave of the Penisola Sorrentina (Gulf of Naples). *Rapp. Comm. Int. Mer. Médit.* **29**, 135-136 (1985).
69. Bell, J.J. The distribution and prevalence of sponge species in a semi-submerged temperate sea cave. *Ir. Nat. J.* **27(7)**, 249-265 (2003).
70. Costello, M.J., *et al*. Methods for the study of marine biodiversity. In The GEO handbook on biodiversity observation networks (eds. Walters, M., & Scholes, R.). Springer, Cham, pp 129-163 (2017) 10.1007/978-3-319-27288-7_6
71. Fanelli, G., Piraino, S., Belmonte, G., Geraci, S. & Boero, F. Human predation along Apulian rocky coasts (SE Italy): desertification caused by *Lithophaga lithophaga* (Mollusca) fisheries. *Mar. Ecol. Prog. Ser.* **1–8** (1994). 10.3354/meps110001.
72. Trigui El-Menif, N., Kefi, F.J., Ramdani, M., Flower, R. & Boumaiza, M. Habitat and associated fauna of *Lithophaga lithophaga* (Linné 1758) in the bay of Bizerta (Tunisia). *J. Shellfish Res.* **26**, 569-575 (2007). 10.2983/0730-8000(2007)26[569:HAAFOL]2.0.CO;2.
73. Devescovi, M. & Ivesa, L. Colonization patterns of the date mussel *Lithophaga lithophaga* (L., 1758) on limestone breakwater boulders of a marina. *Period. Biol.* **110(4)**, 339-345 (2008).
74. Colletti, A., *et al*. The date mussel *Lithophaga lithophaga*: Biology, ecology and the multiple impacts of its illegal fishery. *Sci. Total Environ.* **744**, 140866 (2020). 10.1016/j.scitotenv.2020.140866

75. Palma, M., Rivas Casado, M., Pantaleo, U., Pavoni, G., Pica, D., & Cerrano, C. SfM-based method to assess gorgonian forests (*Paramuricea clavata* (Cnidaria, Octocorallia)). *Remote Sens.* **10(7)**, 1154 (2018). 10.3390/rs10071154
76. Quiles-Pons, C., *et al*. Monitoring the complex benthic habitat on semi-dark underwater marine caves using photogrammetry-based 3D reconstructions. In 3rd Mediterranean Symposium on the conservation of Dark Habitats. Genoa, Italy, 21-22 September 2022, pp 113-114 (2022).
77. Nocerino, E., Menna, F., Farella, E., & Remodino, F. 3D Virtualization of an underground semi-submerged cave-system. In International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences XLII-2/W15. 27th CIPA International Symposium "Documenting the past for a better future" (eds. Gonzalez-Aguilera, D., Remondino, F., Toschi, I., Rodriguez-Gonzalvez, P., Stathopoulou, E.), 1–5 September 2019, Ávila, Spain, pp 857-864 (2019). 10.5194/isprs-archives-XLII-2-W15-857-2019
78. Marroni, L., Brandt, P., Gaertner, P., & Marassich, A. Citizen science and 3D modelling to study and protect Mediterranean marine caves: a real application in the caves of the Gulf of Orosei (Sardinia, Italy) (No. EGU22-3817). Copernicus Meetings (2022). 10.5194/egusphere-egu22-3817
79. Olinger, L.K., Scott, A.R., McMurray, S.E., & Pawlik, J.R. Growth estimates of Caribbean reef sponges on a shipwreck using 3D photogrammetry. *Sci. Rep.* **9(1)**, 1-12 (2019). 10.1038/s41598-019-54681-2
80. Teague, J. & Scott, T. Underwater photogrammetry and 3D reconstruction of submerged objects in shallow environments by ROV and underwater GPS. *J. Mar. Sci. Technol.* **1**, 5 (2017).
81. Menna, F., *et al*. Towards real-time underwater photogrammetry for subsea metrology applications. In OCEANS 2019-Marseille, pp 1-10 (2019). 10.1109/OCEANSE.2019.8867285
82. Mohamed, H., Nadaoka, K., & Nakamura, T. Towards benthic habitat 3D mapping using machine learning algorithms and structures from motion photogrammetry. *Remote Sens.* **12(1)**, 127 (2020). 10.3390/rs12010127
83. Rossi, P., Ponti, M., Righi, S., Castagnetti, C., Simonini, R., Mancini, F., *et al.* Needs and gaps in optical underwater technologies and methods for the investigation of marine animal forest 3D-structural complexity. *Front. Mar. Sci.* **8**, 591292 (2021) 10.3389/fmars.2021.591292
84. Pavoni, G., *et al*. TagLab: AI-assisted annotation for the fast and accurate semantic segmentation of coral reef orthoimages. *J. Field Robot* **39(3)**, 246-262 (2022). 10.1002/rob.22049
85. Rindi, F., Gavio, B., Díaz-Tapia, P., Di Camillo, C.G., & Romagnoli, T. Long-term changes in the benthic macroalgal flora of a coastal area affected by urban impacts (Conero Riviera, Mediterranean Sea). *Biodivers. Conserv.* **29**, 2275–2295 (2020). 10.1007/s10531-020-01973-z
86. Furlani, S. *et al.* M. Tn (tidal notches) at the Conero area (W Adratic coast): implications for coastal instability. *Geogr. Fis. e Din. Quat.*, **41**, 33-46 (2018). 10.4461/GFDQ.2018.41.11
87. Rützler, K. Sponges in coral reefs. In: Stoddart DR, Johannes RE, editors. Coral reefs: Research methods. Monographs on oceanografic methodologies. Paris: UNESCO. pp. 5, (1978).
88. Hooper, J.N.A., & Van Soest, R.W.M. Systema Porifera. A guide to the classification of sponges. Boston, MA (Springer 2002).
89. Morrow, C., & Cárdenas, P. Proposal for a revised classification of the Demospongiae (Porifera). *Front. Zool.* **12**, 7 (2015). https://doi.org/10.1186/s12983-015-0099-8
90. de Voogd, N.J., *et al.* World Porifera database. (2022) Available: http://www.marinespecies.org/porifera. Accessed Jan 2022.
91. QGIS.org (2022) QGIS Geographic Information System. QGIS Association. http://www.qgis.org

92. Hesselbarth, M.H.K., Sciaini, M., With, K.A., Wiegand, K., & Nowosad, J. landscapemetrics: an open-source R tool to calculate landscape metrics. *Ecography* **42**, 1648-1657 (2019). 10.1111/ecog.04617
93. McGarigal, K., Cushman, S.A., & Ene, E. FRAGSTATS v4: Spatial Pattern Analysis Program for Categorical and Continuous Maps. Computer software program produced by the authors at the University of Massachusetts, Amherst. Available at the following website: https://www.umass.edu/landeco/ (2012).
94. Garrabou, J., Riera, J. & Zabala, M. Landscape pattern indexes applied to Mediterranean subtidal rocky benthic communities. *Landsc. Ecol.* **13(4)**, 225-247 (1998). 10.1023/A:1007952701795
95. Teixidó, N., Garrabou, J. & Arntz, W.E. Spatial pattern quantification of Antarctic benthic communities using landscape indexes. *Mar. Ecol. Prog. Ser.* **242**, 1-14 (2002). 10.3354/meps242001

Chapter 3

Considering seabed irregularity in the assessment of the Mediterranean distribution of a rare species

Understanding distribution patterns of a species is crucial to develop effective management plans and implement proper monitoring efforts. To do so, one of the most applied tools are species distributional models (SDM), which have already allowed to assess geographic ranges and environmental niches of a wide range of terrestrial and marine species (Robinson et al., 2022). Even though approaching marine deep environments by SDM is still a great challenge to face, in this chapter the first Mediterranean basin distribution assessment of a long living parasitic zoanthid, *Savalia savaglia*, is presented. The knowledge about the species is scarce and, even if it is considered as Near Threatened by the IUCN and included in the Annex II of Barcelona Convention, no monitoring plan or protection measures have been implemented so far.

In paper 4, "**Mediterranean Sea shelters for the gold coral *Savalia savaglia* (Bertoloni, 1819): An assessment of potential distribution of a rare parasitic species**", the occurrence of this species was compiled and harmonized in a single dataset, and an Ecological Niche Model was developed to identify the species main environmental drivers (Breiner et al., 2015), including seafloor complexity descriptors as predictors candidates in the analysis.

This study provided (i) the most updated distribution for *S. savaglia* across the Mediterranean Sea, harmonizing in a single occurrence database from previous studies as well as new validated observations, and (ii) a preliminary assessment at basin-level of continuous potential distribution map based on a presence-only MaxEnt niche model, identifying bathymetry irregularity as one of the two co-variates that most influenced the distribution of the species. The results of our study provide useful information that may support future monitoring or management efforts and contribute to the development of better *S. savaglia* distributional models in the future.

Paper 4

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Mediterranean Sea shelters for the gold coral *Savalia savaglia* (Bertoloni, 1819): An assessment of potential distribution of a rare parasitic species

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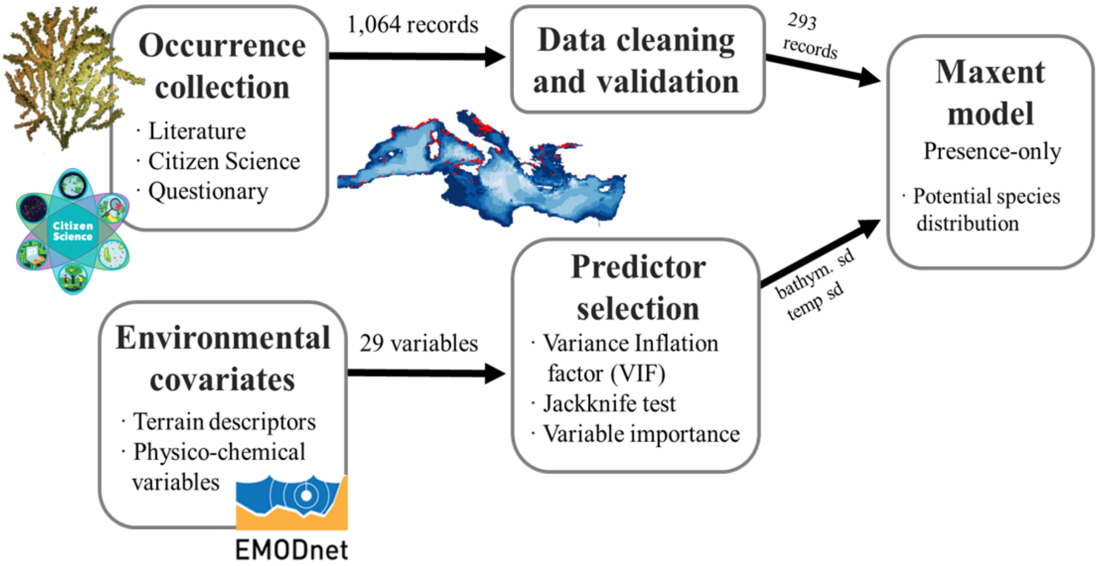

Supplementary material available at:

Mediterranean Sea shelters for the gold coral *Savalia savaglia* (Bertoloni, 1819): An assessment of potential distribution of a rare parasitic species

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Abstract

Savalia savaglia is an ecosystem engineer listed as Near-Threatened by the IUCN, even though effective management and proper monitoring efforts to assess its distribution is still lacking. The record of large, long-established colonies can indicate the occurrence of areas with limited human local pressure. These areas may be considered as proxies for the creation of baselines of reference useful to design restoration strategies. The aim of this work was to update the distribution of *S. savaglia* Mediterranean populations to develop an Ecological Niche Model, highlighting potential areas for future monitoring programs. Occurrence data were collected and harmonized into a single dataset using the scientific literature and validated observations to feed a presence-only MaxEnt model, obtaining a basin-level potential distribution of the species. The results of our study can support decision-makers in marine spatial planning measures including the preservation of mesophotic environments and prioritizing areas for conservation.

Keywords: hexacorallia, zoanthidea, benthic ecology, mesophotic populations, predictive model, conservation, fishing, marine protected areas, citizen science.

1. Introduction

Temperate Mesophotic Ecosystems (TMEs) have been defined only recently as habitats with upper limits being the depth reached by 1% of surface irradiance and lower limits being the deepest extent of benthic primary producers (Cerrano et al., 2019). Until a few years ago, TMEs have been among the less studied marine habitats, partly due to the challenge of exploring such depths (Menza et al., 2007). But, thanks to the increasing accessibility to advanced techniques – e.g., technical SCUBA diving (since the late 1990s) (Pyle, 2019), acoustic and imaging techniques (Reimer et al., 2019), as well as Remotely Operated and Automated Underwater Vehicles (ROV and AUV, since the 2000s) (Turner et al., 2017; Armstrong et al., 2019) – they are gaining more and more attention among the scientific community (Cerrano et al., 2019). Their exploration has contributed to a better understanding of their main environmental characteristics as well as the biodiversity they host, but due to the huge extension covered by these ecosystems we are still left with important gaps.

In this study, we will focus our efforts on one of the key inhabitants of TMEs, *Savalia savaglia* (Bertoloni, 1819), a parasitic parazoanthid presenting an Atlanto-Mediterranean distribution. This long-living parazoanthid generally displays an aggressive parasitic behavior (Bell, 1891; Zibrowius, 1985; Cerrano et al., 2006; Sinniger et al., 2010; Previati et al., 2010; Giusti et al., 2015) using different gorgonian species (e.g., *Paramuricea* spp., *Eunicella* spp. and *Leptogorgia* spp) as substrate to grow on, even though it can also be found associated with anthipatharians (Cerrano et al., 2010). *S. savaglia* and its hosts are considered ecosystem engineers, organisms that are able to modify the physical features of the surrounding habitat, increasing spatial heterogeneity and facilitating many species, creating the so-called "animal forests" (Fig. 1a) (Cerrano et al., 2010; Rossi et al., 2017; Paoli et al., 2017). Mainly owing to their three-dimensional frameworks, these ecosystems provide important ecological services such as carbon storage, stabilization of the substrate, reduction of re-suspension, and the creation of a wide range of diverse ecological niches, thus enhancing biodiversity, and supplying nursery and feeding areas for many organisms (Cerrano et al., 2019; Rossi and Rizzo, 2021). These ecological functions, in the presence of *S. savaglia*, can be prolonged for hundreds of years thanks to its much longer lifespan compared to that of gorgonians (Cerrano et al., 2010).

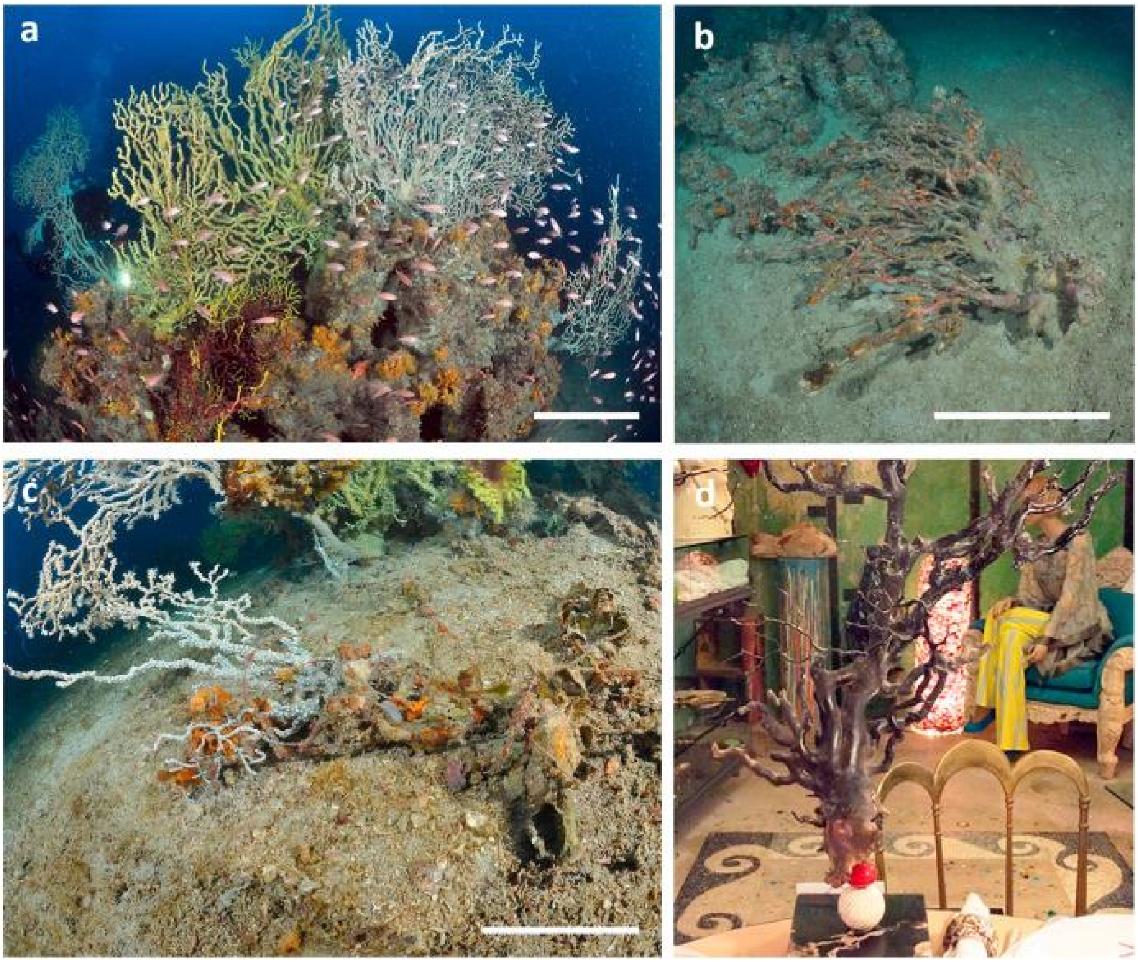


Figure 1. Image composite illustrating healthy *Savalia savaglia* colonies and some of their threats: a) healthy *S. savaglia* forest; b) and c) show *S. savaglia* colonies impacted by fishing lines; d) *S. savaglia* for sale as ornamental coral. Scale bars: a-b = 50 cm; c = 25 cm.

Despite the fact that *S. savaglia* is a species long believed to be worthy of protection (Cerrano et al., 2010; Giusti et al., 2014, 2015) – being included in the Annex II of Specially Protected Areas of Mediterranean Importance (SPAMI-Barcelona Convention) for the protection of threatened species, the Appendix II of the Berne Convention on the Conservation of European Wildlife and Natural Habitats, and currently considered as Near-Threatened by the International Union for Conservation of Nature (IUCN) Red List since 2015 (IUCN, 2021) – it still lacks effective management as well as monitoring protocols. It has been observed that *S. savaglia* specimens may be particularly impacted by fishing activities (Fig. 1b–c), such as trawling or ghost-nets (Bavestrello et al., 1997; Roberts and Hirshfield, 2004; Bo et al., 2014). Moreover, especially in the past, specimens have been collected as souvenirs (Fig. 1d) (Barrajón Domenech et al., 2008; Artüz et al., 1990; Watling and Norse, 1998; Previati et al., 2010), an exploitation that, today can still be locally active (Andrea Costantini, personal communication). These pieces of evidence call for an increase in our efforts to raise awareness on the urgency of targeted monitoring and protection measures, to provide a detailed and updated distribution map, and to include this species in the design of current and future conservation strategies.

Ecological Niche Models (ENM) have been highlighted, in the recent decades, as a useful tool in analyzing environmental factors that drive for species distribution by identifying the ecological niche used by species within an area of interest (Breiner et al., 2015). One challenging task for the application of this method is the collection of an adequate distributional dataset, especially in the marine realm, where biological sampling is a time-consuming and expensive process, and data are often biased towards shallow and coastal areas (Phillips et al., 2019). This problem is aggravated for species hosted in habitats of difficult accessibility, for which scattered presence-only data are often the only available information (Leverette and Metaxas, 2005). As for the environmental data, despite the continuous progress in data acquisition and analytic power, those of marine ecosystems generally have a relatively low spatial resolution and are scattered among non-homogeneous, local datasets, precluding a smooth integration; this is particularly true for benthic layers (Reiss et al., 2015). In recent years, however, the advent of cutting-edge spatial interpolation led to the creation of public marine datasets containing environmental layers in uniform extent and resolution at vast spatial scales, such as Bio-Oracle (Tyberghein et al., 2012; Assis et al., 2018).

In this context, the aims of this study were, firstly, to compile and update the occurrence dataset of *S. savaglia* in the Mediterranean basin to synthesize its current spatial knowledge and, secondly, to develop an Ecological Niche Model as a cost-effective approach to identify the species main environmental drivers (Breiner et al., 2015). These findings will be useful to decision-makers to identify the species potential distribution over a wide area, helping to address future data collection and management decisions, such as those related to marine spatial planning.

2. Material and methods

2.1.Study area

Defining the study area is a critical step when modelling the geographical distribution of a species. For this study, a few considerations had to be made. First of all, most of the records of *Savalia savaglia* in the literature are localized in the Mediterranean basin with very few coming from the Canary Islands, Madeira, Cape Verde, and the Western Moroccan coasts (Johnson, 1899; Ocaña et al., 2007), and only two papers reporting the presence of colonies on the Atlantic coasts of the Iberian Peninsula (Altuna et al., 2010; Boavida et al., 2016). In light of this, we decided not to consider the occurrences in the Eastern Atlantic, as much more data, collected through targeted monitoring activities, are necessary to better understand the extent of *S. savaglia*'s populations in these areas. Focusing our efforts on the Mediterranean Sea also presents some advantages. Being an enclosed basin with specific policies and jurisdictions, and a certain degree of cooperation between neighbouring nations, it was easier to both access homogenous environmental data and engage coordinated actions in the management of the marine space. Secondly, other constraints were applied to the study area, not considering the modelling efforts in the South-eastern Mediterranean (below the 34° parallel North), where no occurrences were recorded. Boka Kotorska Bay in Montenegro and the Corinthos Strait in Greece host important populations but were also left out of the modelling results as the available environmental predictors do not cover these areas.

2.2. Occurrence data collection

In order to update the current knowledge on *S. savaglia*'s geographic distribution, presence-only data were collected. To do so, a multi-source approach was implemented for this study. Sources include:

- A review of all published scientific and grey literature, considering all existing records from the oldest (Bell, 1891) to the most recent ones (UNEP/MAP-PAP/RAC-SPA/RAC and MSDT, 2019). Relevant scientific papers were retrieved using the Web of Science research engine, by searching the query "*Savalia savaglia*" OR "*Gerardia savaglia*" in the "topic" field (last access 06/09/2021): references of the papers returned from this search were then screened for other relevant studies. Annual and regional reports published by the SPA/RAC (Specially Protected Areas/Regional Activity Centre) and Oceana were also checked for information on the distribution.

- Datasets from Citizen Science (CS) projects, namely the Reef Check web-based geographic information system for the Mediterranean Sea (RCMed, www.reefcheckmed.org) and other observational networks indexed by the Global Biodiversity Information Facility (GBIF, www.gbif.org).

- An online survey specifically tailored for this study (Document S1 Supplementary Material). The questionnaire was created using the Google Forms software and mainly spread on social media, and among SCUBA diving centers and communities of the Mediterranean region.

For each occurrence record, in addition to the coordinates, other information - depth range and the number of colonies, when available - was collected to allow more in-depth considerations on *S. savaglia*'s distribution. All the obtained records were harmonized in a single dataset and subjected to a cleaning and validating process to ensure data quality. Firstly, all records with coordinates falling on land were eliminated. Then, separate observations from the same source matching coordinates were clustered together in a single record. For data obtained from the online survey, occurrences were validated through a taxonomic analysis using photographical evidence provided by the source, and, when possible, they were cross-referenced with close records from the previously listed sources.

2.3.Environmental data acquisition and processing

Covariates of interest for the modelling of *S. savaglia*'s distribution were identified in literature, based on the species known ecology and on what is commonly known to affect the distribution of erect sessile suspension feeders (Rossi, 1958; Ocaña et al., 2007; Giusti et al., 2015; Reiss et al., 2015; Topçu et al., 2019). This ended up on the following list of candidate-predictors: temperature, salinity, dissolved oxygen, current velocity, primary production and bathymetry derivatives. The predictors used for this study can be clustered in three different groups depending on their source (Table 1):

- Terrain Descriptors were derived from the spatial analysis of a Digital Terrain Model (DTM) of the whole Mediterranean basin, obtained from the EMODnet bathymetry portal (Thierry et al., 2019). The native resolution of this bathymetric raster is 1/16 arc min., corresponding to a grid cell of around 115 m in latitude. It was originally downloaded in 12 tiles in.asc format, which then were merged in a single file using the raster package (Hijmans and van Etten, 2012) in the R open-source software (RCore Team, 2020). Terrain analysis was performed using the terrain function included in the raster package to calculate the following terrain descriptors: slope, aspect, northness, eastness, profile curvature, planform curvature, Terrain Roughness Index (TRI), standard deviation (sd), and Bathymetric Position Index (BPI). A neighboring cell of 8 was defined for the calculation of DTM sd, slope, and aspect, while an additional predictor was produced classifying BPI into 6 categories, following the method applied by De Reu et al. (2013).

- Physico-Chemical variables (temperature, salinity, current velocity, dissolved oxygen, primary production) were acquired from EMODnet Mediterranean Sea Biogeochemistry & Physics Analysis and Forecast (Clementi et al., 2019; Bolzon et al., 2020). In this case, the variables presented a native resolution of 1/24°, the equivalent of around 4 km in the latitude grid cell and a temporal resolution spanning from 2018– to 07 to 2020–07. These variables originally included different depth levels but given the sessile character of the species, only the deepest level was considered for the analysis. Finally, minimum, maximum, mean, and standard deviations were extracted for each variable along the two years' time series, and the variables were resampled by bilinear interpolation method using the R package raster to equal the terrain descriptors resolution (1/16 arc min.) before being used as covariates on the model.

Table 1. Pool of variables obtained along with its correspondent units, descriptors quantified, sources and native resolution.

Variables	Description	Unit	Descriptors	Source	Native resolution
Bathymetry	Digital bathymetry	m	Slope, Aspect, Curvature, Roughness, TRI, BPI, sd	EMODnet[1]	1/16 arc min. (ca. 115m)
Temperature	Monthly mean	ºC	min, mean, max, sd	EMODnet[2]	1/24º (ca. 4 km)
Salinity	Monthly mean	psu	min, mean, max, sd	EMODnet[2]	1/24º (ca. 4 km)
Current velocity	Monthly mean	$m\ s^{-1}$	min, mean, max, sd	EMODnet[2]	1/24º (ca. 4 km)
Dissolved oxygen	Monthly mean	$mmol\ m^{-3}$	min, mean, max, sd	EMODnet[3]	1/24º (ca. 4 km)
Primary Production	Monthly mean	$mg\ m^{-3}\ day^{-1}$	min, mean, max, sd	EMODnet[3]	1/24º (ca. 4 km)

EMODNet Portals: [1] Bathymetry; [2] Physics; [3] Biogeochemistry.

2.4. Model development

Since a common issue in modelling species distributions is that models are fed with a high number of predictors and relatively few occurrences, leading to model overfitting (Breiner et al., 2015; Vaughan and Ormerod, 2005; Parviainen et al., 2008; Innocenti et al., 2020), a pre-selection of key environmental covariates has been performed. In our case, the level of correlation among covariates was tested by the Variance Inflation Factor coefficient (VIF), which was calculated using the usdm R package (Naimi, 2015) for all 29 predictor candidates. Those presenting values higher than 2.5 were considered as strongly correlated (Martin et al., 2014) and thus not considered for our models.

Many different techniques can be applied to develop ENMs depending on the type of available occurrence data. Maximum Entropy (MaxEnt) has been selected as a suitable modelling approach for *S. savaglia*, recognized in the literature as a useful tool to determine distributions of marine sessile taxa using presence-only data, obtaining robust predictive accuracy (Reiss et al., 2011; Zapata-Ramirez et al., 2014; Melo-Merino et al., 2020; Kanki et al., 2021). MaxEnt method calculates a species potential distribution by seeking the more uniform distribution, while respecting environmental constraints defined by the species known distribution. MaxEnt analysis was performed by the open-source software MaxEnt 3.4.4 (Phillips et al., 2006, 2017) using default model parameters with the exception of: a) the regularization multiplier, which was set to 2 (Innocenti et al., 2020; Radosavljevic & Ancerson, 2014) as a trade-off among complexity and simplicity, relaxing some of the constraints imposed by the environment and obtaining smoother models; b) the number of background points, which was increased to 100,000 given the great heterogeneity of conditions in the study area (Barbet-Massin et al., 2012); and c) a final model was produced by subsampling the dataset, using the 85% of the occurrences for training and 15% for testing. Testing subset was homogeneously, randomly sampled by using the pp.subsample function inside spatialEco R package (Evans, 2021) to avoid possible spatial bias during validation process. As model output, clog-log transformation was chosen, which can be expressed from 0 to 1 as potential habitat suitability. Finally, to assess the model efficiency, two performance metrics were chosen: (i) the Receiver Operating Characteristic (ROC) curve, which evaluates the trade-off between prediction sensitivity and specificity, and (ii) the associated Area Under the Curve (AUC), which presents values of around 0.5 in the case of a random prediction, getting closer to 1 on better performing models. To identify the variables to be used in the model, an initial screening was conducted by eliminating those with higher collinearity (VIF >2.5). With the obtained set of variables, a first model was built, and the contribution of each predictor was analyzed using the Jackknife test. The variable with the lowest contribution was eliminated and a new model was calculated. Proceeding by successive steps, all variables that contributed less than 5% to the model were eliminated. In addition, histograms of some of the variables not selected as predictors for the model were produced to better describe the environmental features present in areas recognized as potentially suitable for the species.

To improve the data visualization and further analyze *S. savaglia*'s ecological niche, a threshold was settled at 0.122 clog-log, suggested by MaxEnt as the threshold value to maximize the model test sensitivity plus specificity. Areas above this threshold were considered as "potentially suitable". To understand whether the presence of *S. savaglia* in the Mediterranean might be endangered by trawling activities, potentially suitable areas were presented together with (i) areas subject to trawling and (ii) areas subject to protection measures that may prevent this type of impact. Data on trawling activities were obtained from the EMODnet Human Activities Portal (Natale et al., 2015), resulting from the records of passages of trawling vessels collected during the year 2014. Data on protection measures were retrieved from the Marine Protected Areas in the Mediterranean (MAPAMED, 2019) database, from which national Marine Protected Areas (MPAs), SPAMIs and Fishery Restricted Areas (FRAs) were selected as zones with effective protection measures, due to the general restrictions on the circulation of fishing vessels implemented on these areas.

3. Results

3.1. Occurrence's source distribution

New observations of *Savalia savaglia*'s colonies were reported in areas where their presence was not known before: along the Algerine coasts, in Provence (France), the south of Turkey, the Corinthos Strait (Greece), Malta, and the Sicilian islands (Italy) (Fig. 2). Most of the collected records were in the Western Mediterranean (126) and Adriatic Sea (94), while no occurrences were detected in the Tunisian Plateau. Records reporting the higher number of colonies come from the Boka Kotorska Bay (Montenegro) (500 colonies from a single observation), the Croatian coasts (around 350 colonies from 10 records), and several locations along the coasts of Italy (around 400 colonies from 25 records) (Fig. 2). From the record collection, emerged that the Sea of Marmara, not considered in the model, hosts large populations of *S. savaglia*.

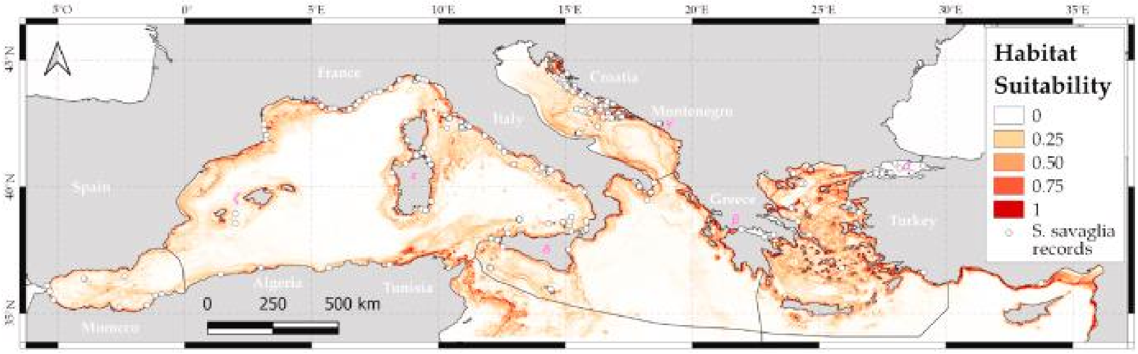


Figure 2. Collected occurrences (white circles) of *Savalia savaglia* and estimated potential suitability calculated by MaxEnt for the species.Mentioned locations marked in pink as follow: α) Sea of Marmara; β) Corinthian Strait; γ) Boka Kotorska Bay; δ) Sicily; ε) Sardinia and ζ) Balearic archipelago.

Our search for occurrences resulted in the collection of a total of 1064 records over 10 countries (Table S2, Supplementary Material). During the processes of cleaning and validation of the dataset, the number of records was drastically reduced to 293 confirmed and unique occurrences (Table S2).

The Web of Science database selected 25 papers, of which only 10 mentioned *Savalia* and just 3 provided occurrences of the species. However, the subsequent scanning of their references and the analysis of relevant grey literature allowed us to retrieve 35 documents producing useful records of *S. savaglia*'s distribution. The search of scientific literature provided 111 of our records. More than 40% of them were retrieved from just two papers, the work by Giusti et al. (2015), and the one by Di Camillo et al. (2018). CS projects provided 118 of our records, with the RCMed Web-GIS (Turicchia et al., 2021) database alone accounting for 79 occurrences. Finally, the ad hoc Google survey provided a total of 100 records of *S. savaglia* in the Mediterranean Sea, 64 of which were validated (Fig. 3a).

Looking at the depth range of our records (Fig. 3b), most of the reported colonies of *S. savaglia* (60% of collected observations) were found between 15 and 40 m, the shallowest record being a vast population in the Boka Kotorska Bay, Montenegro, located at 8–22 m depth (UNEP/MAP-PAP/RAC-SPA/RAC and MSDT, 2019), and the deepest observation being located on the Ses Olives seamount in the Balearic Islands (UNEP/MAP-RAC/SPA, 2016), between 500 and 900 m. The number of records is smaller as the depth grows, and we could observe a significant reduction in the contribution from Citizen Science projects and the survey; almost all the occurrences below 70 m were obtained from the scientific literature.

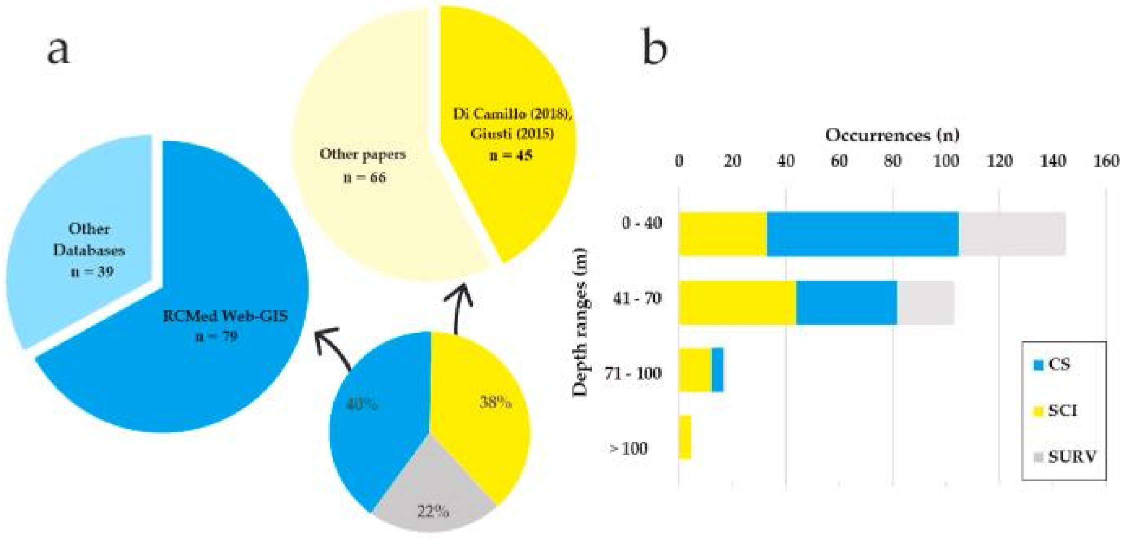
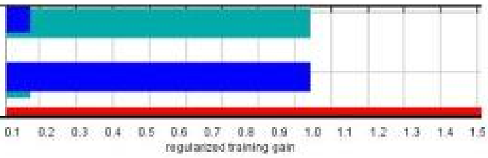


Figure 3. *Savalia savaglia* occurrence-dataset analysis: (a) Percentage of records per source type; (b) Number of records per depth range. Presented in blue the observations coming from Citizen Science databases, in yellow the ones from the Scientific Literature and in grey the ones obtained by means of the online survey.

*3.2.*Savalia savaglia *distribution model*

Of the initial 29 variables, only two variables were retained to build the model: the standard deviation of bathymetry and the standard deviation of water temperature at the bottom (Table 2, Fig. S3).

Table 2. Table containing MaxEnt estimated contribution, permutation importance of each predictor variable as well as the Jackknife test of variable importance of training data.

Variables	Variable % contribution	Permutation importance (%)	Jackknife of regularized training gain from *Savalia savaglia*[1]
Bathymetry sd	26.6	21.4	
Temperature sd	73.4	78.6	

a Presented in blue the model gain using only that variable, in green the result of removing that variable from the model and red with all variables.

When feeding the cleaned dataset to MaxEnt, the number of occurrences suffered an additional reduction. During the model calculation, due to both the clustering of sparse observations falling into the same predictor cell and the fact that environmental predictors were not always defined near the coast, leaved the dataset with 175 records to feed the model. Of these 175 observations, MaxEnt used 149 of the occurrences for training and 26 for testing, obtaining for the training asset an averaged relative AUC score of 0.912, and a 0.825 score for the test (Fig. 4). When analyzing the contribution of each environmental variable to the model, the standard deviation of temperature contributed the most with 73.6%, while the standard deviation of bathymetry contributed with 26.6% (Table 2).

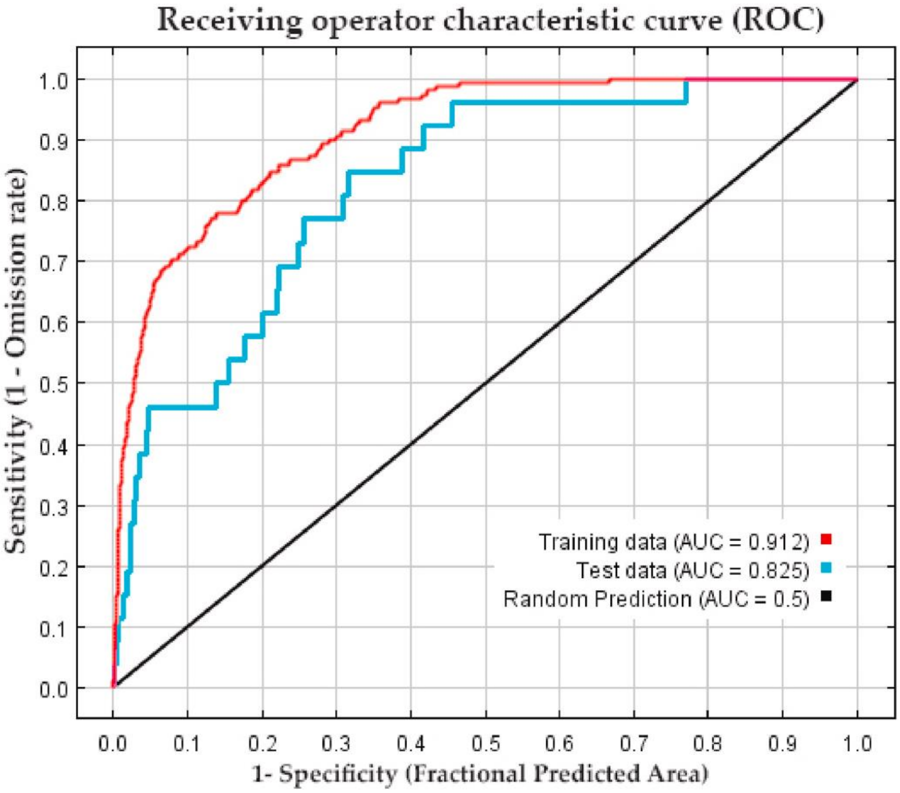


Figure 4. Receiver operating characteristic curve of the maxent model.

The relative importance of the variables was confirmed by the Jacknife test, which showed that the temperature standard deviation was the covariate contributing the most to the training gain when used as the single variable in the model and was the one decreasing the most the training gain when omitted from the model. (Table 2).

The MaxEnt variable response curves gave us a picture of how the predictors influenced the estimated suitability for this species (Fig. 5), by varying one predictor at a time and keeping the others at their average sample value. We could observe how *S. savaglia* more likely grows in areas having moderately to highly irregular bottoms, since potential suitability increases drastically as bathymetry sd increases; in addition, in relation to temperature, the species prevails in areas with small thermal variations throughout the year (temperature sd between 0.5 and 5 °C).

For a more complete characterization of *S. savaglia*'s ecological niche, histograms were produced for some of the variables discarded during the covariate selection process (Fig. 6). In these figures, we can observe the general conditions present on the whole area considered by the model and compare them to the conditions occurring on the areas identified as potentially suitable (≥0.122clog-log). Fig. 6a clearly shows the marginality of *S. savaglia* ecological niche and the species' prevalence over areas presenting relatively shallow depths (100–300m); it would seem

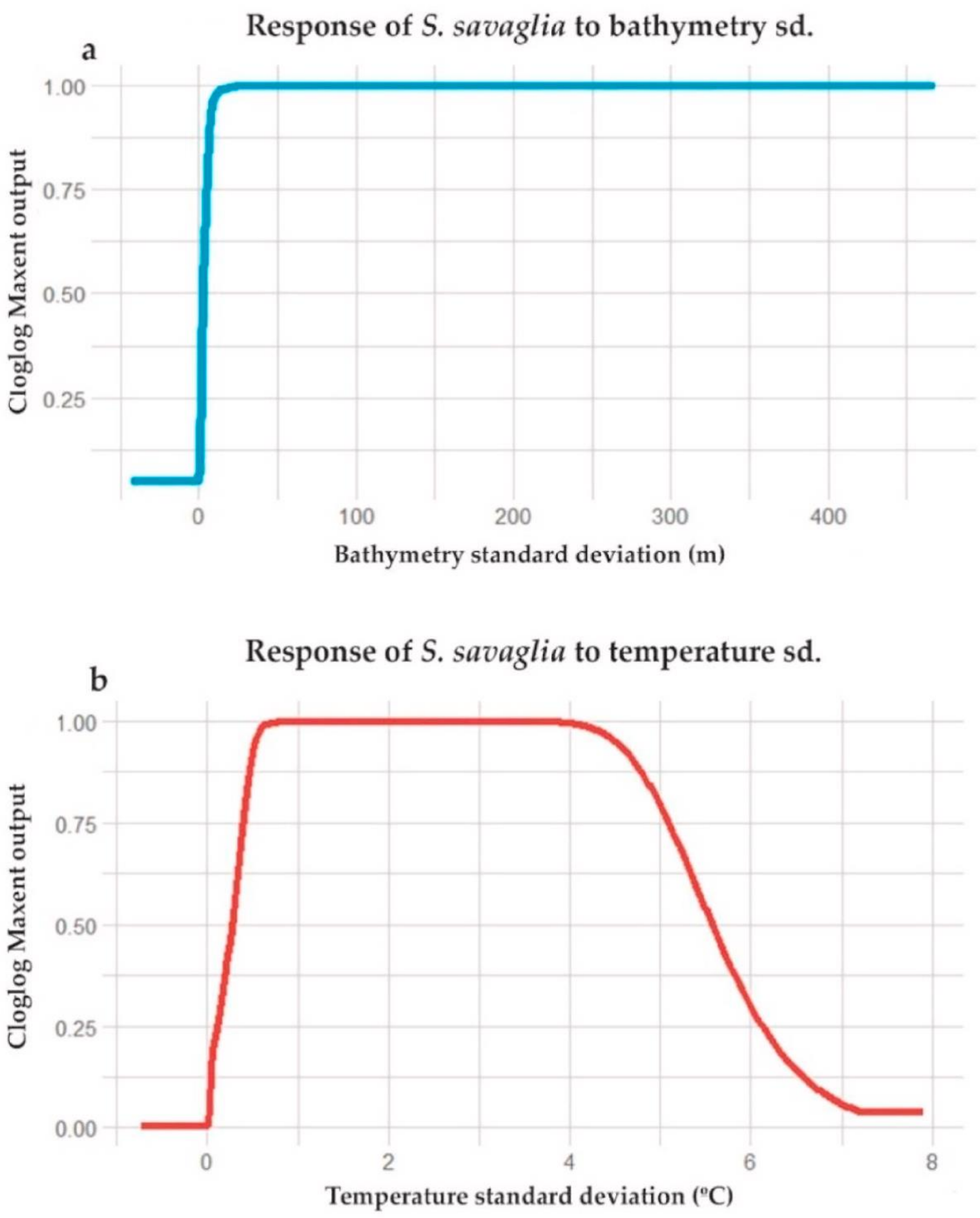


Figure 5. Response curves of the predictors used by the model.

that, in relation to depth, the suitability first decreases rapidly (from 0 to 80m) and then gradually up to about 800–900m. In terms of dissolved oxygen (Fig. 6b), although there is a slight preference for minimum oxygenation values around 230 mmol m^{-3}, the curve seems very flat from 180 to 250 mmol m^{-3}, indicating a possible adaptability of *S. savaglia* to a wide range of oxygenation values. Concerning temperature maximum (Fig. 6c), *S. savaglia* seems to prevail in areas with a maximum between 13 and 20 °C; although it seems to tolerate higher temperatures, up to 24 °C. Lastly, Fig. 6d shows the species preference for oligotrophic areas holding relatively low primary production values (<6 mg m^{-3} day^{-1}).

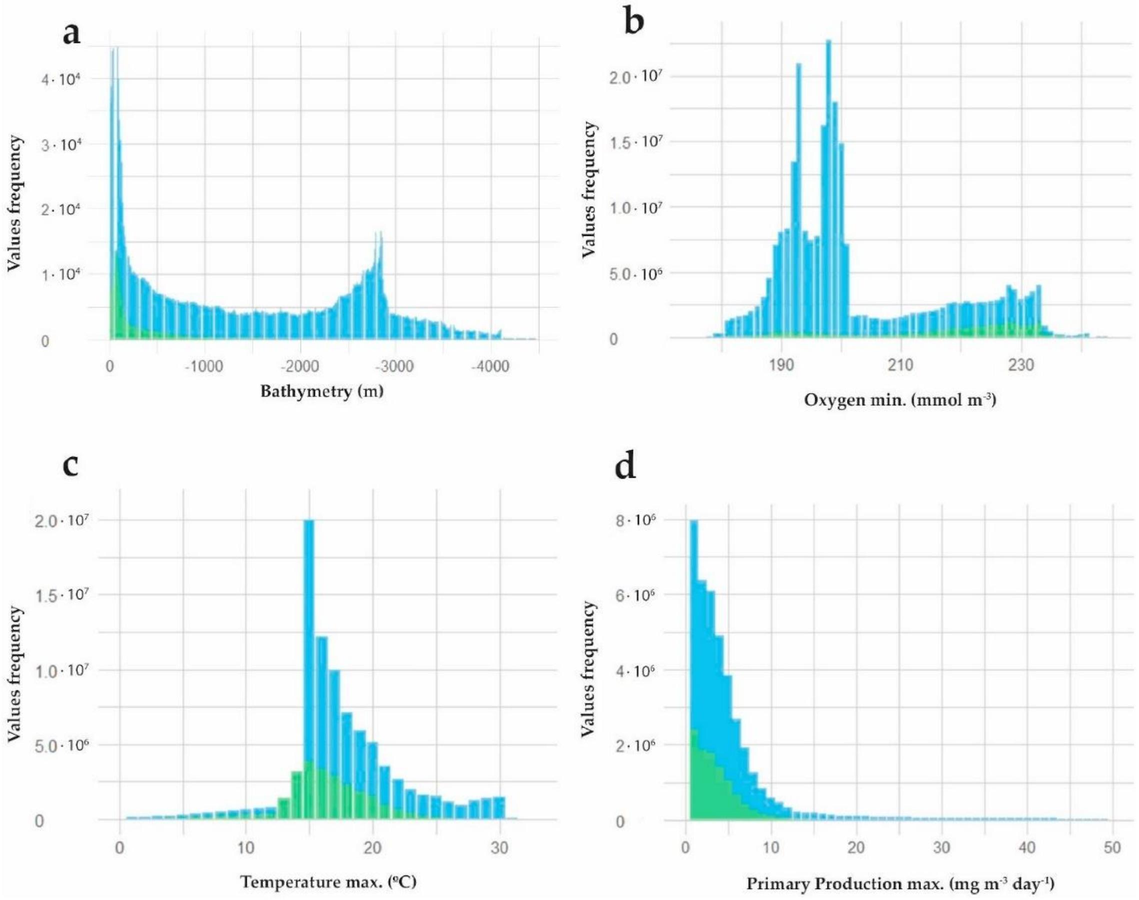


Figure 6. Histograms comparing the frequency of values of different environmental parameters along the whole study area in blue and the frequencies of values present on potentially suitable areas for *Savalia savaglia* in green.

A potential suitability map is plotted on Fig. 2, along with the collected presence records of the species. Most of the occurrences of *S. savalia* are found in the areas highlighted by the model. However, some areas where no occurrences for *S. savaglia* have been recorded yet are also highlighted as highly potentially suitable (Fig. 2), such as the southern coasts of Sicily and Sardinia (Italy), the Balearic Islands, numerous Greek (i.e., Cyclades and Ionian Islands) and Croatian islands (i.e., Lastovo Archipelago Nature Park and Mljet Island), and some areas along African coasts (i.e., Parc national de Jebel Chitana-Cap Négro, in Tunisia, Skikda in Algeria and Marsdal Beach in Morocco). In Fig. 7, the results of our model are plotted together with MPAs currently adopting some kind of protection against fishing activities and with data on trawling impacts over the basin. We focused on 4 areas of interest (Fig. 7b–e) as an example to characterize the general situation occurring in the Mediterranean (Fig. 7a). The first case (Fig. 7b) considered the coasts of Sardinia, Apulia, Calabria, Sicily, Malta, and Tunisia. In the latter region it is possible to observe how along the African coasts, although long traits are highlighted as potentially suitable, only two Tunisian islands (Zembra and Gallite) hold fishery restrictions.

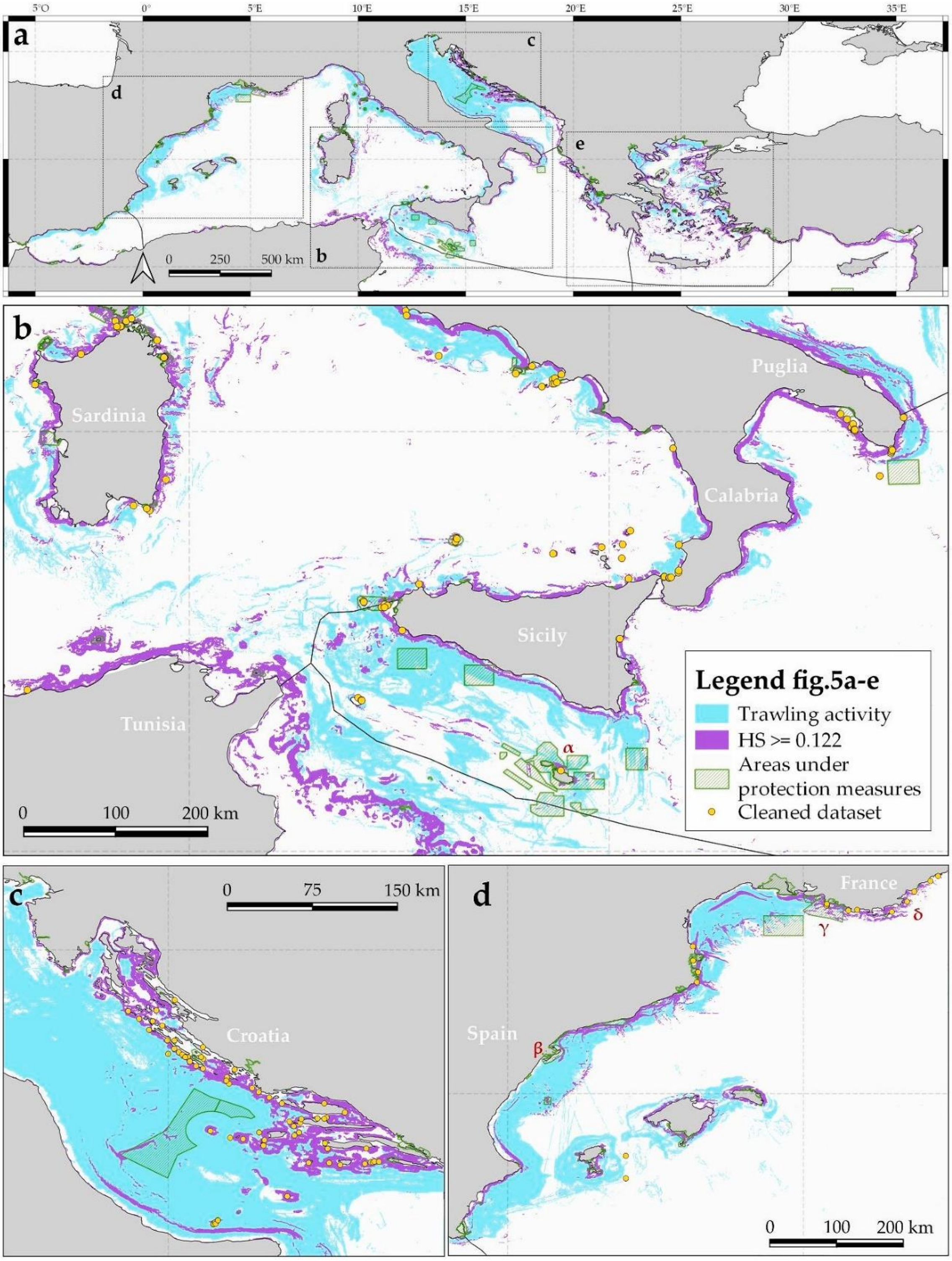


Figure 7. Potential suitability for *Savalia savaglia* (≥0.122clog-log) plotted along with estimated areas under i) trawling impacts and ii) effective protection measures against fishing. This figure cover: a) the whole study area; b) the Tyrrhenian Sea; c) central Adriatic Sea; d) Balearic Sea and e) Aegean Sea (continue the following page). [1]Mentioned locations marked in red as follow: α) Malta; β) Ebro Delta Natural Park; γ) Les Calanques MPA; δ) St. Tropez Peninsula. 2 Maps at higher resolution are available in the FigS4.

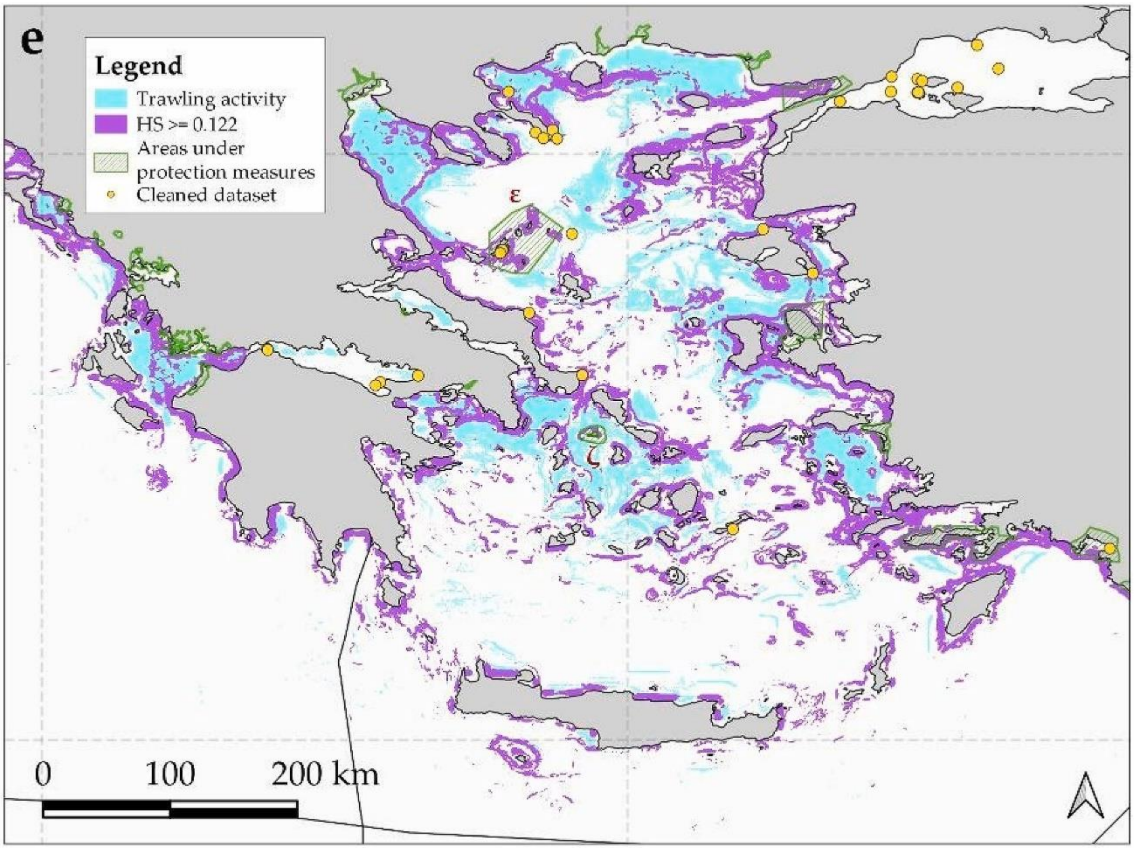


Figure 7 (continuation). Potential suitability for *Savalia savaglia* (≥0.122clog-log) plotted along with estimated areas under i) trawling impacts and ii) effective protection measures against fishing. This figure cover: a) the whole study area; b) the Tyrrhenian Sea; c) central Adriatic Sea; d) Balearic Sea and e) Aegean Sea. [1]Mentioned locations marked in red as follow: α) Malta; β) Ebro Delta Natural Park; γ) Les Calanques MPA; δ) St. Tropez Peninsula. 2 Maps at higher resolution are available in the FigS4.

On the other hand, along the Italian coasts (especially in Apulia and Sicily) many sites estimated as suitable are exposed to trawling impacts, in some cases even those falling in protected areas. The second zoomed map (Fig. 7c) was focused on the Middle Adriatic basin, where a massive trawling impact is evident, potentially threatening vast populations of the target species. The third detail from the suitability map showed Spanish and French waters (Fig. 7d), where a similar situation is taking place: trawling activities surround areas with high probability of encountering the species, with some exceptions around protected areas such as the Ebro Delta Natural Park (Catalonia, Spain), Les Calanques MPA (Marseille, France) and the waters in front of St. Tropez Peninsula (Provence-Alpes-Côte d'Azur, France). Lastly, looking at the Aegean Sea (Fig. 7e), a few suitable areas are under protection measures, as Sporades Islands (Greece), Gyaros Island (Greece) and some zones along the Turkish coast, but still many highlighted areas are unprotected and close to or directly impacted by trawling threats.

4. Discussion

Increasingly frequent episodes of mass mortality among Mediterranean gorgonian forests are being recorded everywhere, even in areas unaffected by local anthropogenic impacts, being mainly linked to thermal anomalies (Bavestrello et al., 1994; Cerrano et al., 1999; 2008; Garrabou et al., 2019; Garrabou et al., 2022) and disease outbreaks (Calvo et al., 2011). It is thus evident that the ongoing climate crisis represents a direct threat to these key habitat-forming organisms, which are becoming fragmented and are considered in strong regression (Linares et al., 2005; Ponti et al., 2019). Animal forests are evolving towards rapid cycling systems (Rossi et al., 2017), characterized by reduced resilience and lower biomass. Recent studies addressing the importance of animal forests in the blue Carbon (C) budget (Coll et al., 2010; Coppari et al., 2019; Rossi and Rizzo, 2021) suggest that gorgonians have high levels of C sequestration. Every lost colony represents a loss in C retention capability, which is in turn thought to be crucial to fight against the climate crisis. The role of *S. savaglia* in the C sequestration and benthic-pelagic coupling is yet to be addressed, but given its longer lifespan, compared to that of gorgonians (hundreds vs tens of years) and the stiffness of its skeleton, it is reasonable to believe it gives a considerable contribution in a long-term perspective. Moreover, the occurrence of more stable environmental conditions inside gorgonian forests (Cerrano et al., 2010) can enhance the C uptake process in the surrounding sediments and facilitate the growth of coralline algae and bryozoans (Scinto et al., 2009; Ponti et al., 2018). Considering these findings, the need to map and monitor this peculiar species, along with the delicate and vital ecosystem hosting it, is urgent and compelling.

The ecoregions hosting most of the *S. savaglia* observations were the Western Mediterranean, the Adriatic Sea, and the Ionian Sea, where most of the field campaigns and studies have been focused (Table S2), and where CS projects have been successfully implemented. The main sources of our data, scientific literature and CS projects, are strongly biased towards coastal and shallower areas (within reach of SCUBA divers) of the Western basin. Additionally, it is impossible to tell whether the lack of occurrence data from the Levantine Basin is due to a genuine absence of *S. savaglia* in its waters, or to a lack of knowledge of the biodiversity and low sampling effort of benthic communities in these areas an issue that has been extensively discussed in the literature (Coll et al., 2010; Bianchi et al., 2012; Di Camillo et al., 2018; Ponti et al., 2019).

Our study offers, for the first time, an estimate of the potential distribution of *S. savaglia* in almost the entire Mediterranean basin through the development of a MaxEnt model; a demonstrated cost-effective method for the design of sampling schemes (Franklin, 2010; Phillips et al., 2017; Smith et al., 2021), the guiding of surveys for exploration (Georgian et al., 2019) and the development of conservation areas (Kinlan et al., 2020). Due to the rarity of the species and the nature of our data (presence-only), it is not possible to talk about probability of occurrence. Nevertheless, we can refer to the clog-log MaxEnt output as an estimation of suitability (Putra and Mustika, 2021) or as encounter probability for a given sampling program (Grüss and Thorson, 2019). In the past the distribution of this species has been modelled at lower spatial scale (Giusti et al., 2014), while this is the first attempt to add physio-chemical variables along with substrate types to the list of predictors, aiming for a full-basin assessment. Although environmental variables at high resolution are becoming more and more available thanks to remote sensing, even at large scales, we faced some limitations on their application. For instance, the current data resolution is not high enough to ensure a good coverage of coastal areas, which translates in the partial loss of occurrences that could have fed the model. Moreover, some of the ecologically important environmental variables lack a full-basin cover, especially in areas where relatively few marine habitats have been surveyed and mapped relatively less. Updating and completing the layers for such ecologically important facies could lead to more performant distributional models for cryptic species associated to them.

ROC curves and relative AUC values were quite similar in terms of training and test data. This, along with AUC estimates close to 1, suggests that our model captured the variation existing in our training dataset, and thus possessed a theoretical good discrimination ability with respect to our test data (Innocenti et al., 2020). Areas identified as potentially suitable (≥0.122clog-log) for *S. savaglia* covered an extension of approximately 71,353.91 km^2, distributed in agreement with the known observations of the species. Most of the occurrences that could not be considered by the model, also ended up in the immediate vicinity of highlighted “suitable zones”, which also indicates a good level of reliability of our results. In addition, some areas that did not host occurrence data were designated as potentially suitable: these could be considered as candidate study sites for ground-truthing surveys.

Only 6.74% (4,809.24 km^2) of the potentially suitable areas are currently under some kind of protection measures (see Fig. 7a). Considering that not always these measures are respected (Tickler et al., 2019), we face the reality that vast areas hosting this species are still exposed to the interlinked effects of human impacts and climate change. In addition, since most MPAs in the Mediterranean are developed to protect other ecosystems, rather than coralligenous assemblages, in many cases the *S. savaglia* colonies fall into B or C zones, which do not provide adequate protection for the species as boating and even fishing activities, both artisanal and recreational, are usually allowed. To our knowledge, the only MPA specifically targeting animal forests for protection is in Montenegro, in the Boka Botorska Bay (UNEP/MAP-PAP/RAC-SPA/RAC and MSDT, 2019). These ecosystems are threatened by both commercial and artisanal fishing. In particular, as reported by Martin et al. (2014), a single passage of a trawling vessel can be the cause of a complete loss of the entire ecosystem integrity. Moreover, many studies report strong mechanical damage from abandoned fishing gear (see Fig. 1b–c) and high level of bycatch affecting animal forests (Consoli et al., 2019; Enrichetti et al., 2019).

Looking at the updated species distribution, it is reasonable to think that *S. savaglia* was once more common at relatively shallow depths (30–50 m) but has declined sharply due to its collection in the past decades through bycatch, fishing impacts (Bo et al., 2014), or just to use as decorations (Fig. 1d). At these depths, most findings report few scattered colonies, which could prove that there was once a coral forest that disappeared (Gaglioti et al., 2019). An exception occurs in the Sea of Marmara, where very dense *S. savaglia* populations are present at relatively shallow waters (30–60m). Considering the very peculiar environmental conditions present in this basin (Beşiktepe et al., 1994; Artüz et al., 2018; Topçu et al., 2019) an ad-hoc model of this area should be developed in future studies. And more recently, more abundant populations of *S. savaglia* were found in the Skerki (Sicilian Strait, Italy) ET, personal observation), Ustica (Sicily, Italy) (Gaglioti et al., 2019) and Capo Carbonara (Sardinia, Italy) (Egidio Trainito, personal observation) at these depths, however all these populations are somehow protected from fishing impact by the geomorphology of the site, rich in crevices or other sheltered habitats. Considering these observations, an appropriate protection for *S. savaglia* must include no-take zones, as coherent data on artisanal and recreational fishing, especially at this large scale, is impossible to obtain. As a starting point we compared the potential distribution areas given by our model with areas exposed to trawling impacts. Fig. 7 shows that areas considered suitable for the species are generally surrounded and, in some cases, overlapped by trawling impacts. Furthermore, since the use of Automatic Identification System transmitters on trawling-fleets, although mandatory, is still poorly implemented in some countries (especially over African coasts) (Ferrà et al., 2018; Armelloni et al., 2021), we can only assume that the areas subjected to this activity are greatly underestimated. Additionally, the general lack of data in terms of trawling activities over African coasts together with the absence of occurrences recorded for *S. savaglia* call for urgent monitoring efforts on the area.

S. savaglia is not an exceptional case regarding the lack of information on the marine species. In fact, a general gap of knowledge can be observed when considering the available information on threatened species in the marine realm, where 23.2% of them are listed in the Data Deficient (DD) Category of the International Union for Conservation of Threatened Species (IUCN, 2021). To help solving this issue, the next cycle of implementations of the Marine Strategy Framework Directive (2008/56/EC), together with the European Biodiversity Strategy for 2030 (COM/2020/380) and the European Green Deal (COM/2019/640) established some common goals, such as increasing of our knowledge of the sea and its inhabitants, and the identification of their potential threats. These goals should be obtained by increasing the harmonization in data collection and archives, emphasizing the importance of better data accessibility and information transferability for marine policy makers. In addition, the evolution of new digital tools such as

ENMs will play an important role in reaching this goal. Furthermore, the involvement of the public in scientific endeavors through CS projects, under the condition that standardized trainings and protocols are followed to ensure the data quality (Kullemberg et al., 2016), is increasingly recognized by the scientific community as a suitable method of acquiring data for research (Dickinson et al., 2010).

5. Conclusions

This is the first study updating the distribution of *Savalia savaglia* across the entire Mediterranean Sea, harmonizing in a single database, occurrences from previous studies and new validated observations, providing a first assessment, at basin-level, of continuous potential distribution map. Although a large-scale model does not give the level of knowledge obtainable with targeted local-scale studies, the result of this work allows us to identify the potential distribution of *S. savaglia* throughout the overfished Mediterranean Sea, and cross-reference it with data on trawling fisheries, that are potentially more impactful for benthic species. That said, some facts need to be considered:

- since the model also identified suitable areas where the presence of *S. savaglia* is not ascertained by direct observations, a ground-truthing will be necessary to define regional and local conservation policies. Priority should be given to sites that already fall under some level of protection, as it will be easier to ask for the development and implementation of animal forests' targeted protection measures;
- the results of our study provide a map of potential habitats where ROV studies or explorations by technical divers can be conducted to verify the model, and this will help to correct the biological sampling bias towards coastal and shallow areas, by conducting monitoring campaigns that focus on deep and under-sampled sea-bottoms;
- experimental studies are needed to fully understand which environmental drivers actually influence *S. savaglia*'s growth. This knowledge will help to increase the quality of distribution models. In addition, as ecological variables for the marine environment are constantly improving in terms of both coverage and resolution, in the future, it will be possible to increase the predictive capability of the model.

In conclusion, other interesting perspectives for further studies are (i) to include in the model co-occurrence patterns of *S. savalia* and its typical hosts (e.g., gorgonians and antipatharians), and (ii) to project the model to future climate scenarios, which will allow to focus conservation efforts on areas less affected by the current climate crisis, and therefore more likely to be resilient in the long-term.

References

Altuna, Á., Sinniger, F., Aldrey, J.M., 2010. Occurrence of *Savalia savaglia* (Anthozoa: Zoantharia) in the Ría de Arousa (Galicia, north-western Spain, north-eastern Atlantic), Mar. Biodiver. Rec. 3, 1–5. https://doi.org/10.1017/S1755267210000965

Armelloni, E.N., Tassetti, A., Ferrà, C., Galdelli, A., Scanu, M., Mancini, A., Fabi, G., Scarcella, G., 2021. AIS data, a mine of information on trawling fleet mobility in the Mediterranean Sea. Mar. Policy, 129, 104571. https://doi.org/10.1016/j.marpol.2021.104571

Armstrong, R.A., Pizarro, O., Roman, C., 2019. Underwater Robotic Technology for Imaging Mesophotic Coral Ecosystems, in: Loya, Y., Puglise, K.A., Bridge, T.C.L. (Eds.) Mesophotic Coral Ecosystems. Springer, Cham. 2019, pp. 973-988. https://doi.org/10.1007/978-3-319-92735-0_51.

Artüz, M.İ., Artüz, M.L., Artüz, O.B., 1990. Mercan türlerine getirilen yasaklar ile ilgili görüşler. TC Çevre Bakanligi Raporu KKGM Su Ürünleri, Sirküleri Düzenlemeleri. https://doi.org/10.13140/2.1.2608.0326.

Artüz, M.L., Artüz, O.B., Sakınç, M., Yalçın, B., Erdoğan, B.E., 2018. Fewer Species but More Existing Individuals: Testing the Hypothesis 'Pessimum Conditions Rule' Based on Long-Term Data of Species Composition of Benthic Fauna and Environmental Variables in the Sea of Marmara, Turkey. Res. J. Environ. Toxicol. 2(2): https://doi.org/10.16966/2576-6430.116

Assis, J., Tyberghein, L., Bosch, S., Verbruggen, H., Serrão, E.A., De Clerck, O., 2018. Bio-ORACLE v2. 0: Extending marine data layers for bioclimatic modelling. Glob. Ecol. Biogeogr. 27(3), 277-284. https://doi.org/10.1111/geb.12693

Barbet-Massin, M., Jiguet, F., Albert, C.H., Thuiller, W., 2012. Selecting pseudo-absences for species distribution models: how, where, and how many? Methods Ecol. Evol. 3(2), 327-338. https://doi.org/10.1111/j.2041-210X.2011.00172.x

Barrajón Domenech, A., Moreno Lampreave, D., Arroyo Tenorio, M.C., López-González, P.J., 2008. *Gerardia savaglia* Bertoloni, 1819. In: Barea-Azcón, J. M., Ballesteros-Duperón, E., Moreno, D. (Eds.). *Libro Rojo de los Invertebrados de Andalucía*. 4 Tomos. Consejería de Medio Ambiente, Junta de Andalucía, Sevilla. 2008, pp 251-255

Bavestrello, G., Bertone, S., Cattaneo-Vietti, R., Cerrano, C., Gaino, E., Zanzi, D., 1994. Mass mortality of *Paramuricea clavata* (Anthozoa, Cnidaria) on Portofino Promontory cliffs, Ligurian Sea, Mediterranean Sea. Mar. Life, 4(1), pp.15-19.

Bavestrello, G., Cerrano, C., Zanzi, D., Cattaneo-Vietti, R., 1997. Damage by fishing activities to the Gorgonian coral *Paramuricea clavata* in the Ligurian Sea. Aquat. Conserv. 7(3), 253-262. https://doi.org/10.1002/(SICI)1099-0755(199709)7:3<253::AID-AQC243>3.0.CO;2-1.

Bell, F. J., 1891. Contributions to our knowledge of the antipatharian corals, Proceedings of the Proc. Zool. Soc. Lond. 8, 142–142.

Bianchi, C.N., Morri, C., Chiantore, M., Montefalcone, M., Parravicini, V.; Rovere, A., 2012. Mediterranean Sea biodiversity between the legacy from the past and a future of change. In: Stambler, N. (Ed.) Life in the Mediterranean Sea: A Look at Habitat Changes, Nova Publisher 2012, 1, p. 55. SBN: 978-1-61209-644-5

Bo, M., Bava, S., Canese,S., Angiolillo, M., Cattaneo-Vietti, R., Bavestrello, G., 2014. Fishing impact on deep Mediterranean rocky habitats as revealed by ROV investigation. Proc. Zool. Soc. Lond. 171, 167-176. https://doi.org/10.1016/j.biocon.2014.01.011

Boavida, J., Assis, J., Reed, J., Serrão, E. A., Gonçalves, J. M., 2016 Comparison of small remotely operated vehicles and diver-operated video of circalittoral benthos, Hydrobiologia 766(1), 247–260. https://doi.org/10.1007/s10750-015-2459-y.

Bolzon, G., Cossarini, G., Lazzari, P., Salon, S., Teruzzi, A., Feudale, L., Di Biagio, V., Solidoro, C., 2020. “Mediterranean Sea Biogeochemical Analysis and Forecast (CMEMS MED-Biogeochemistry 2018-Present).” Copernicus Monitoring Environment Marine Service (CMEMS). https://doi.org/10.25423/CMCC/MEDSEA_ANALYSIS_FORECAST_BIO_006_014_MEDBFM3.

Beşiktepe S.T., Halil, İ. S., Emin Özsoy, M.A.L., Temel Oğuz, Ü.Ü., 1994 The circulation and hydrography of the Marmara Sea, Prog. Oceanogr. 34(4), 285-334, ISSN 0079-6611, https://doi.org/10.1016/0079-6611(94)90018-3.

Breiner, F.T., Guisan, A., Bergamini, A., Nobis, M.P., 2015. Overcoming limitations of modelling rare species by using ensembles of small models. Methods Ecol. Evol. 6(10), 1210-1218. https://doi.org/10.1111/2041-210X.12403

Calvo E., Simo R., Coma R., Ribes M., Pascual J., Sabates A., Gili J.M., Pelejero C., 2011. Effects of climate change on Mediterranean marine ecosystems: The case of the Catalan Sea. Clim. Res. 50(1): 1-29. https://doi.org/10.1111/2041-210X.12403

Cerrano, C., Bavestrello, G., Bianchi C.N., Cattaneo-Vietti, R., Bave, S., Morganti, C., Morri, C., Picco, P., Sara, G., Schiaparelli, S., Siccardi, A., Sponga F.A., 1999. Catastrophic mass-mortality episode of gorgonians and other organisms in the Ligurian Sea (Northwestern Mediterranean), summer. Ecol. Lett., 3(4): 284-293. https://doi.org/10.1046/j.1461-0248.2000.00152.x

Cerrano, C., Previati, M., Palma, M., Arillo, A., 2006. Distribuzione di *Gerardia savaglia* (Bertoloni, 1819) (Cnidaria, Zoanthidea) nell'Area Marina Protetta di Portofino. Biol. Mar. Mediterr. 13, 164-165.

Cerrano, C., Bavestrello, G., 2008. Medium-term effects of die-off of rocky benthos in the Ligurian Sea. What can we learn from gorgonians? Chem. Ecol. 24, 73-82. https://doi.org/10.1080/02757540801979648

Cerrano, C., Danovaro, R., Gambi, C., Pusceddu, A., Riva, A., Schiaparelli, S., 2010. Gold coral (*Savalia savaglia*) and gorgonian forests enhance benthic biodiversity and ecosystem functioning in the mesophotic zone. *Biodivers. Conserv.* 19(1), 153-167. https://doi.org/10.1007/s10531-009-9712-5

Cerrano, C., Bastari, A., Calcinai, B., Di Camillo, C., Pica, D., Puce, S., Valisano, L., Torsani, F., 2019. Temperate mesophotic ecosystems: gaps and perspectives of an emerging conservation challenge for the Mediterranean Sea. Eur. Zool. J. 86(1), 370-388. https://doi.org/10.1080/24750263.2019.1677790

Clementi, E., Pistoia, J., Escudier, R., Delrosso, D., Drudi, M., Grandi, A., Lecci, R., Cretí, S., Ciliberti, S., Coppini, G., Masina, S., Pinardi, N., 2019. Mediterranean Sea Analysis and Forecast (CMEMS MED-Currents, EAS5 system) (Version 1) [Data set]. Copernicus Monitoring Environment Marine Service (CMEMS). https://doi.org/10.25423/CMCC/MEDSEA_ANALYSIS_FORECAST_PHY_006_013_EAS5

Coll, M., Piroddi, C., Steenbeek, J., Kaschner, K., Lasram, F.B.R., Aguzzi, J., Ballesteros, E., Bianchi, C.N., Corbera, J., Dailianis, T., Danovaro, R., Estrada, M., Froglia, C., Galil, B.S., Gasol, J.M., Gertwagen, R., Gil, J., Guilhaumon, F., Kesner-Reyes, K., Kitsos, M.S., Koukouras, A., Lampadariou, N., Laxamana, E., Cuadra, C.M.L., Lotze, H.K., Martin, D., Mouillot, D., Oro, D., Raicevich, S., Rius-Barile, J., Saiz-Salinas, J.I., Vicente, C.S., Somot, S., Templado, J., Turon, X., Vafidis, D., Villanueva, R., Voultsiadou, E., 2010. The biodiversity of the Mediterranean Sea: Estimates, patterns, and threats. PLoS ONE 5(8), e11842. https://doi.org/10.1371/journal.pone.0011842

Consoli, P., Romeo, T., Angiolillo, M., Canese, S., Esposito, V., Salvati, E., Scotti, G., Andaloro, F., Tunesi, L., 2019. Marine litter from fishery activities in the Western Mediterranean

Sea: The impact of entanglement on marine animal forests. Environ. Pollut. 249, 472-481. https://doi.org/10.1016/j.envpol.2019.03.072

Coppari, M., Zanella, C., Rossi, S., 2019. The importance of coastal gorgonians in the blue carbon budget. Sci. Rep. 9(1), 1-12. https://doi.org/10.1038/s41598-019-49797-4

De Reu, J., Bourgeois, J., Bats, M., Zwertvaegher, A., Gelorini, V., De Smedt, P., Chu, W., Antrop, M., De Maeyer, P., Finke, P., Van Meirvenne, M., Verniers, J., Crombé, P., 2013. Application of the topographic position index to heterogeneous landscapes. Geomorphology 186, 39-49. https://doi.org/10.1016/j.geomorph.2012.12.015

Di Camillo, C.G., Ponti, M., Bavestrello, G., Krzelj, M., Cerrano, C., 2018. Building a baseline for habitat-forming corals by a multi-source approach, including Web Ecological Knowledge. Biodiv. Conserv. 27(5), 1257-1276. https://doi.org/10.1007/s10531-017-1492-8

Dickinson, J.L., Zuckerberg, B., Bonter, D.N., 2010. Citizen science as an ecological research tool: challenges and benefits. Annu. Rev. Ecol. Evol. Syst. 41, 149-172. https://doi.org/10.1146/annurev-ecolsys-102209-144636

Enrichetti, F., Bava, S., Bavestrello, G., Betti, F., Lanteri, L., Bo, M., 2019. Artisanal fishing impact on deep coralligenous animal forests: a Mediterranean case study of marine vulnerability. Ocean Coast. Manag. 177, 112-126. https://doi.org/10.1016/j.ocecoaman.2019.04.021

Evans, J.S., 2021. *spatialEco*. R package version 1.3-6. https://github.com/jeffreyevans/spatialEco.

Ferrà, C., Tassetti, A.N., Grati, F., Pellini, G., Polidori, P., Scarcella, G., Fabi, G., 2018. Mapping change in bottom trawling activity in the Mediterranean Sea through AIS data. Mar. Policy 94, 275-281. https://doi.org/10.1016/j.marpol.2017.12.013

Franklin, J., 2010. Mapping species distributions: spatial inference and prediction. Cambridge University Press. https://doi.org/10.1017/CBO9780511810602

Gaglioti, M., Dieli, T., Gambi, M., Cerrano, C., 2019. What am I doing here? An unusual ecological context of a population of *Savalia savaglia* (Anthozoa) at the Ustica island (Italy). Biol. Mar. Medit. 26(1), 310-311. https://doi.org/10.13140/RG.2.2.30943.51362.

Garrabou, J., Gómez-Gras, D., Ledoux, J.B., Linares, C., Bensoussan, N., López-Sendino, P., Bazairi, H., Espinosa, F., Ramdani, M., Grimes, S., Benabdi, M., Souissi, J.B., Soufi, E., Khamassi, F., Ghanem, R., Ocaña, O., Ramos-Esplà, A., Izquierdo, A., Anton, I., Rubio-Portillo, E., Barbera, C., Cebrian, E., Marbà, N., Hendriks, I.E., Duarte, C.M., Deudero, S., Díaz, D., Vázquez-Luis, M., Alvarez, E., Hereu, B., Kersting, D.K., Gori, A. , Viladrich, N. , Sartoretto, S., Pairaud, I., Ruitton, S., Pergent, G., Pergent-Martini, C., Rouanet, E., Teixidó, N., Gattuso, J.P., Fraschetti, S., Rivetti, I., Azzurro, E., Cerrano, C., Ponti, M., Turicchia, E., Bavestrello, G., Cattaneo-Vietti, R., Bo, M., Bertolino, M., Montefalcone, M., Chimienti, G., Grech, D., Rilov, G.;, Tuney Kizilkaya, I., Kizilkaya, Z., Topçu, E.N., Gerovasileiou, V., Sini, M., Bakran-Petricioli, T., Kipson, S., Harmelin, J.G., 2019. Collaborative database to track mass mortality events in the Mediterranean Sea. Front. Mar. Sci. 6, 707. https://doi.org/10.3389/fmars.2019.00707

Georgian, S.E., Anderson, O.F., Rowden, A.A., 2019. Ensemble habitat suitability modeling of vulnerable marine ecosystem indicator taxa to inform deep-sea fisheries management in the South Pacific Ocean. Fish. Res. 211, 256-274. https://doi.org/10.1016/j.fishres.2018.11.020

Giusti, M., Innocenti, C., Canese, S., 2014. Predicting suitable habitat for the gold coral *Savalia savaglia* (Bertoloni, 1819) (Cnidaria, Zoantharia) in the South Tyrrhenian Sea. Cont. Shelf Res. 81, 19-28. https://doi.org/10.1016/j.csr.2014.03.011

Giusti, M., Cerrano, C., Angiolillo, M., Tunesi, L., Canese, S., 2015. An updated overview of the geographic and bathymetric distribution of *Savalia savaglia*. Mediterr. Mar. Sci. 16(1), 128-135. https://doi.org/10.12681/mms.890

Grüss, A., Thorson, J.T., 2019. Developing spatio-temporal models using multiple data types for evaluating population trends and habitat usage. ICES J. Mar. Sci. 76(6), 1748-1761. https://doi.org/10.1093/icesjms/fsz075

Hijmans, R.J., van Etten, J., 2012. Raster: Geographic analysis and modeling with raster data. R package version 2.0-12. Department of Ecology and Evolution, University of Lausanne. http://CRAN.R-project.org/package=raster

Innocenti, C., Battaglini, L., D'Angelo, S., Fiorentino, A., 2020. Submarine landslides: mapping the susceptibility in European seas. Q. J. Eng. Geol. Hydroge. 54(1), qjegh2020-027. https://doi.org/10.1144/qjegh2020-027

IUCN, 2021. The IUCN Red List of Threatened Species. Version 2020-2. https://www.iucnredlist.org. Downloaded on 15 May 2021.

Johnson, J.Y., 1899. Notes on the antipatharian corals of Madeira, with description of a new species and new variety with remarks on a specimen from the West Indies in the British Museum. Proc. Zool. Soc. Lond. 4, 813-824.

Kanki, T., Nakamoto, K., Hayakawa, J., Kitagawa, T., Kawamura, T.A., 2021. New Method for Investigating Relationships Between Distribution of Sessile Organisms and Multiple Terrain Variables by Photogrammetry of Subtidal Bedrocks. Front. Mar. Sci. 8, 654950. https://doi.org/10.3389/fmars.2021.654950

Kinlan, B.P., Poti, M., Drohan, A.F., Packer, D.B., Dorfman, D.S., Nizinski, M.S., 2020. Predictive modeling of suitable habitat for deep-sea corals offshore the northeast United States. Deep Sea Res. Part I: Oceanogr. Res. 158, 103229. https://doi.org/10.1016/j.dsr.2020.103229

Kullenberg, C., Kasperowski, D., 2016. What is citizen science? - A scientometric meta-analysis. PLoS ONE 11(1), E0147152. https://doi.org/10.1371/journal.pone.0147152

Leverette, T.L., Metaxas, A., 2005. Predicting habitat for two species of deep-water coral on the Canadian Atlantic continental shelf and slope. in: Freiwald, A., Roberts, J.M. (Eds.). Cold-Water Corals and Ecosystems. Erlangen Earth Conference Series. Springer, Berlin, Heidelberg, pp. 467-479. https://doi.org/10.1007/3-540-27673-4_23

Linares, C., Coma, R., Diaz, D., Zabala, M., Hereu, B., Dantart, L., 2005. Immediate and delayed effects of a mass mortality event on gorgonian population dynamics and benthic community structure in the NW Mediterranean Sea. Mar. Ecol. Prog. Ser. 305, 127-137. https://doi.org/10.3354/meps305127

MAPAMED, the database of MArine Protected Areas in the MEDiterranean. 2019. © 2020 by SPA/RAC and MedPAN. Licensed under CC BY-NC-SA 4.0. Available at: https://www.mapamed.org/

Martin, C.S., Giannoulaki, M., De Leo, F., Scardi, M., Salomidi, M., Knittweis, L., Pace, M.L., Garofalo, G., Gristina, M., Ballesteros, E., Bavestrello, G., 2014. Coralligenous and maërl habitats: predictive modelling to identify their spatial distributions across the Mediterranean Sea. Sci. Rep. 4(1), 1-9. https://doi.org/10.1038/srep05073

Melo-Merino, S.M., Reyes-Bonilla, H., Lira-Noriega, A., 2020. Ecological niche models and species distribution models in marine environments: A literature review and spatial analysis of evidence. Ecol. Modell. 415, 108837. https://doi.org/10.1016/j.ecolmodel.2019.108837

Mengersen, K., Peterson, E.E., Clifford, S., Ye, N., Kim, J., Bednarz, T., Brown, R., James, A., Vercelloni, J., Pearse, A.R., Davis, J., 2017. Modelling imperfect presence data obtained by citizen science. *Environmetrics* 28(5), e2446. https://doi.org/10.1002/env.2446

Menza, C., Kendall, M., Hile, S., 2007. The deeper we go the less we know. Rev. Biol. Trop. 56, 11–24. https://doi.org/10.15517/RBT.V56I0.5575

Naimi, B., 2015. usdm: uncertainty analysis for species distribution models. R package version 1.1–15. R Documentation. http://www. rdocumentation. org/packages/usdm. https://doi.org/10.1111/j.1600-0587.2013.00205.x

Natale, F., Gibin, M., Alessandrini, A., Vespe, M., Paulrud, A., 2015. Mapping Fishing Effort through AIS Data. *PLoS ONE* 10(6), e0130746.https://doi.org/10.1371/journal.pone.0130746

Ocaña, O., Brito, A., González, G., Herrera, R., 2007. Additions in relation to Gerardiidae from the Macaronesian waters and the Mediterranean Sea (Anthozoa: Zoantharia). Vieraea 35, 163–168.

Paoli, C., Montefalcone, M., Morri, C., Vassallo, P., Bianchi, C.N., 2017. Ecosystem functions and services of the marine animal forests. in: Rossi, S., Bramanti, L., Gori, A., Saco, O., del Valle, C. (Eds.) Marine animal forests: the ecology of benthic biodiversity hotspots. Springer, Cham, pp.1271-1312.

Parviainen, M., Luoto, M., Ryttäri, T., Heikkinen, R.K., 2008. Modelling the occurrence of threatened plant species in taiga landscapes: methodological and ecological perspectives. J. Biogeogr. 35(10), 1888-1905. https://doi.org/10.1111/j.1365-2699.2008.01922.x

Phillips, S.J., Anderson, R.P., Schapire, R.E., 2006. Maximum entropy modeling of species geographic distributions. Ecol. Modell. 190(3-4), 231-259. https://doi.org/10.1016/j.ecolmodel.2005.03.026

Phillips, S.J., Anderson, R.P., Dudík, M., Schapire, R.E., Blair, M.E., 2017. Opening the black box: an open-source release of Maxent. Ecography 40(7), 887–893. https://doi.org/10.1111/ecog.03049

Phillips, S.J., Dudík, M., Elith, J., Graham, C.H., Lehmann, A., Leathwick, J., Ferrier, S., 2019. Sample selection bias and presence-only distribution models: Implications for background and pseudo-absence data. Ecol. Appl. 19(1), 181–197. https://doi.org/10.1890/07-2153.1.

Ponti, M., Turicchia, E., Costantini, F., 2019. Mediterranean gorgonian forests: Distribution patterns and ecological roles', Proceedings of the 3rd Mediterranean symposium on the conservation of coralligenous and other calcareous bio-concretions. ISBN 978-9938-9574-5-7

Ponti, M., Turicchia, E., Ferro, F., Cerrano, C., Abbiati, M., 2018. The understory of gorgonian forests in mesophotic temperate reefs. Aquat. Conserv.: Mar. Freshw. Ecosyst. 28(5), pp.1153-1166. http://dx.doi.org/10.1002/aqc.2928

Previati, M., Palma, M., Bavestrello, G., Falugi, C., Cerrano, C., 2010. Reproductive biology of *Parazoanthus axinellae* (Schmidt, 1862) and *Savalia savaglia* (Bertoloni, 1819) (Cnidaria, Zoantharia) from the NW Mediterranean coast. Mar. Ecol. 31(4), 555–565. https://doi.org/10.1111/j.1439-0485.2010.00390.x.

Putra, M.I.H., Mustika, P.L.K., 2021. Maximum entropy model: estimating the relative suitability of cetacean habitat in the northern Savu Sea, Indonesia. Mar. Mammal Sci. 37(1), 6-28. https://doi.org/10.1111/mms.12719

Pyle R.L., 2019. Advanced Technical Diving. in: Loya Y., Puglise K., Bridge T. (Eds.). Mesophotic Coral Ecosystems. Coral Reefs of the World. Springer, Cham. https://doi.org/10.1007/978-3-319-92735-0_50.

R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

RAC/SPA - UNEP/MAP, 2011. Rapid assessment survey of coastal habitats to help prioritize the suitable new areas needing a status of protection for the development of a network of Marine and Coastal Protected Areas in Montenegro. By Badalamenti F., Garcia Charton J.A., Treviño-Otón J., Mačić V., Cebrian D. (Ed.). RAC/SPA - MedMPAnet Project, Tunis.

Reimer, J.D., Kise, H., Santos, M.E.A., Lindsay, D.J., Pyle, R.L., Copus, J.M., Bowen, B.W., Nonaka, M., Higashiji, T., Benayahu, Y., 2019. Exploring the biodiversity of understudied benthic taxa at mesophotic and deeper depths: examples from the order Zoantharia (Anthozoa: Hexacorallia). Front. Mar. Sci. 6, 305. https://doi.org/10.3389/fmars.2019.00305.

Reiss, H., Cunze, S., König, K., Neumann, H., Kröncke, I., 2011. Species distribution modelling of marine benthos: a North Sea case study. Mar. Ecol. Prog. Ser. 442, 71-86. https://doi.org/10.3354/meps09391

Reiss, H., Birchenough, S., Borja, A., Buhl-Mortensen, L., Craeymeersch, J., Dannheim, J., Degraer, S., 2015. Benthos distribution modelling and its relevance for marine ecosystem management. ICES J. Mar. Sci. 72(2), 297-315. https://doi.org/10.1093/icesjms/fsu107

Roberts, S., Hirshfield, M., 2004. Deep-sea corals: out of sight, but no longer out of mind. Front. Ecol. Environ. 2(3), 123-130. https://doi.org/10.1890/1540-9295(2004)002[0123:DCOOSB]2.0.CO;2

Rossi, L., 1958. Primo rinvenimento di *Gerardia savaglia* (Bert.) (Zoantharia) nei mari italiani (Golfo di Genova). *Doriana*, 2(85), 8.

Rossi, S., Bramanti, L., Gori, A., Saco, O., del Valle, C., 2017. Marine animal forests: the ecology of benthic biodiversity hotspots. Springer, Cham.

Rossi, S., Coppari, M., Viladrich, N., 2017. Benthic-pelagic coupling: new perspectives in the animal forests. in: Rossi, S., Bramanti, L., Gori, A., Saco, O., del Valle, C. (Eds.) Marine animal forests: the ecology of benthic biodiversity hotspots. Springer, Cham, pp. 855-885. https://doi.org/10.1007/978-3-319-21012-4_23

Rossi, S., Rizzo, L., 2021. The Importance of Food Pulses in Benthic-Pelagic Coupling Processes of Passive Suspension Feeders. Water 13(7), 997.. https://doi.org/ 10.3390/w13070997

Scinto, A., Bertolino, M., Calcinai, B., Huete-Stauffer, C., Previati, M., Romagnoli, T., Cerrano, C., 2009. Role of a *Paramuricea clavata* forest in modifying the coralligenous assemblages. In Proceedings of the 1st Mediterranean symposium on the conservation of the coralligenous and others calcareous bio-concretions. RAC/SPA publ., Tunis, pp. 136-140.

Sinniger, F., Reimer, J.D., Pawlowski, J., 2010. The Parazoanthidae (Hexacorallia: Zoantharia) DNA taxonomy: description of two new genera. Mar. Biodiv. 40(1), 57-70. https://doi.org/10.1007/s12526-009-0034-3.

Smith, J.N., Kelly, N., Renner, I.W., 2021. Validation of presence-only models for conservation planning and the application to whales in a multiple-use marine park. Ecol. Appl. 31(1), e02214. https://doi.org/10.1002/eap.2214

Thierry, S., Dick, S., George, S., Benoit, L., Cyrille, P., 2019. EMODnet Bathymetry a compilation of bathymetric data in the European waters. OCEANS 2019 – Marseille IEEE, pp. 1-7, doi: 10.1109/OCEANSE.2019.8867250.

Tickler, D.M., Carlisle, A.B., Chapple, T.K., Curnick, D.J., Dale, J.J., Schallert, R.J., Block, B.A., 2019. Potential detection of illegal fishing by passive acoustic telemetry. Anim. Biotelem. 7(1), 1-11. https://doi.org/10.1186/s40317-019-0163-9

Topçu, N.E., Turgay, E., Yardımcı, R.E., Topaloğlu, B., Yüksek, A., Steinum, T.M., Karataş, S., Öztürk, B., 2019. Impact of excessive sedimentation caused by anthropogenic activities on benthic suspension feeders in the Sea of Marmara. J. Mar. Biol. Assoc. U.K. 99(5), 1075-1086.. https://doi.org/10.1017/ S0025315418001066.

Turner, J.A., Babcock, R.C., Hovey, R., Kendrick, G.A., 2017. Deep thinking: a systematic review of mesophotic coral ecosystems. ICES J. Mar. Sci. 74(9), 2309-2320. https://doi.org/10.1093/icesjms/fsx085.

Turicchia, E., Ponti, M., Rossi, G., Milanese, M., Di Camillo, C.G., Cerrano, C., 2021. The Reef Check Mediterranean Underwater Coastal Environment Monitoring Protocol. Front. Mar. Sci., 1086. https://doi.org/10.3389/fmars.2021.620368

Tyberghein, L., Verbruggen, H., Pauly, K., Troupin, C., Mineur, F., De Clerck, O., 2012. Bio-ORACLE: a global environmental dataset for marine species distribution modelling. Glob. Ecol. Biogeogr. 21(2), 272-281. https://doi.org/10.1111/j.1466-8238.2011.00656.x

UNEP/MAP-RAC/SPA, 2016. Montenegro: Platamuni and Ratac areas. Mapping of marine key habitats and initiation of monitoring network. By Torchia G., Pititto F., Rais C., Trainito E., Badalamenti F., Romano C., Amosso C., Bouafif C., Dragan M., Camisassi S., Tronconi D., Macic V., Sghaier Y.R., Ouerghi A. (Eds.). RAC/SPA - MedKeyHabitats Project, Tunis.

UNEP/MAP-PAP/RAC-SPA/RAC and MSDT, 2019. Investigation of hard bottom habitats with special attention given to Anthozoa and their taxonomy in Boka Kotorska Bay, Montenegro. By: Trainito, E. (Ed.). PAP/RAC – GEF Adriatic project. https://doi.org/10.13140/RG.2.2.20367.61605

Vaughan, I.P., Ormerod, S.J., 2005. The continuing challenges of testing species distribution models. J. Appl. Ecol. 42(4), 720-730. https://doi.org/10.1111/j.1365-2664.2005.01052.x

Watling, L., Norse, E.A., 1998. Disturbance of the Seabed by Mobile Fishing Gear: A Comparison to Forest Clearcutting. Conserv. Biol. 12(6), 1180-1197. https://doi.org/10.1046/j.1523-1739.1998.0120061180.x.

Zapata-Ramirez, P.A., Huete-Stauffer, C., Coppo, S., Cerrano, C., 2014. Using Maxent to understand and predict the distribution of coralligenous environments. Proceedings 2nd Mediterranean Symposium on the Conservation of Coralligenous and Other Calcareous Bio-concretions, Portoroz, pp. 183-188. https://doi.org/10.13140/2.1.4602.9763

Zibrowius, H., 1985. Comportement agressif du Zoanthaire *Gerardia savaglia* contre le Gorgonaire *Paramuricea clavata* (Cnidaria: Anthozoa), Rapports et Proces-verbaux des Reunions. Commission Internationale pour l’Exploration Scientifque de la Mer Méditerranée, pp. 351–353.

Chapter 4

Photogrammetry, a promising non-invasive approach to monitor sponge growth

Along the past decades, sponge populations have been decreasing due to a synergy of climate change and human induced pressures. In addition, the rising of commercial interest for this taxon as a source of natural compounds creates the urgency to properly evaluate sponge growth and regeneration rates. Traditional approaches to estimate sponges' growth and biomass production are traditionally destructive, and often fatal for the organism (Olinger et al., 2019). In addition, they are not usually adapted to all growth forms. Nowadays, the rise of new techniques open new possibilities for a non-invasive monitoring.

In this context, in paper 5, "**A photogrammetric approach to estimate growth in Porifera: *Axinella polypoides* Schmidt, 1862, *Chondrosia reniformis* Nardo, 1847 and *Sarcotragus foetidus* Schmidt, 1862 study cases**", three Mediterranean demosponges presenting different morphotypes were used as model organisms to test the suitability of SfM in the evaluation of growth rates and biomass production of different sponge growth habits.

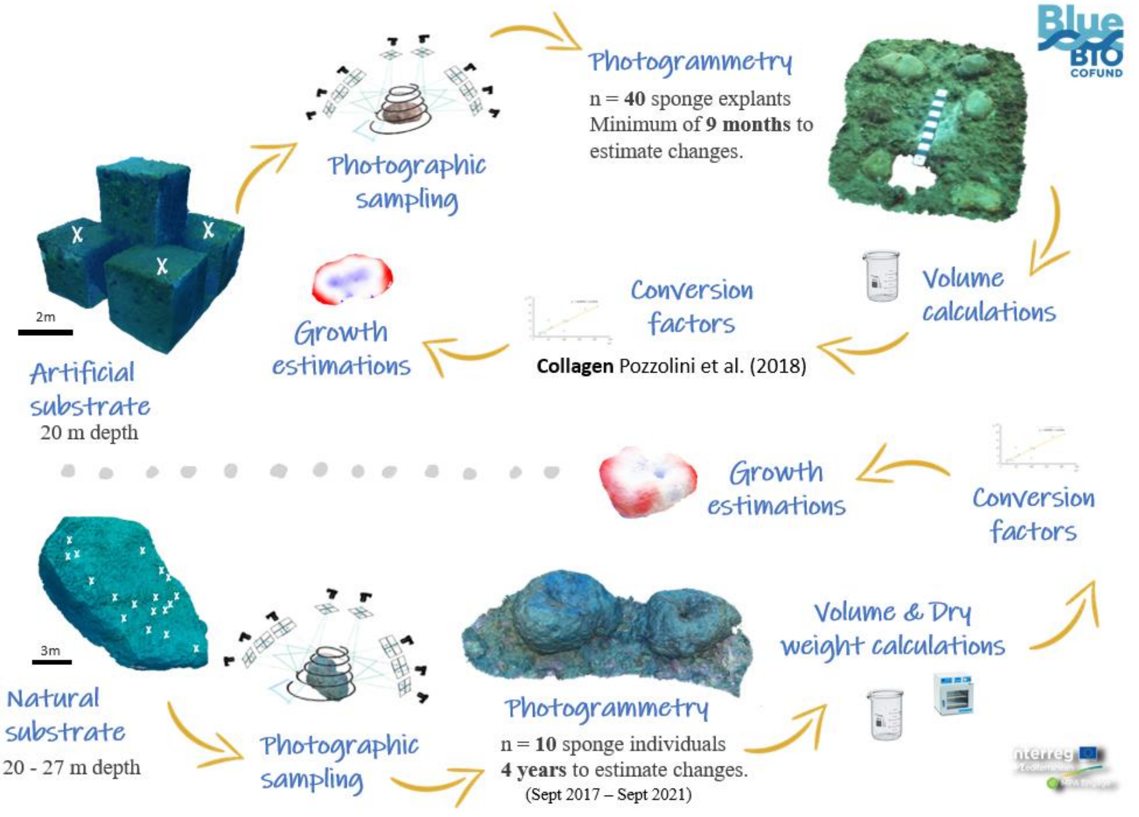
Growth rates of all three species were approach here for the first time by SfM, proving to be an effective approach for the long-term sponge growth's monitoring, and representing a strong candidate for the establishment of a one size fits-all 3D methodology to assess different sponge morphologies. Moreover, for both *Axinella polypoides* and *S. foetidus*, this study represents the first growth estimations for these species in the literature.

Paper 5

In preparation

A photogrammetric approach to estimate growth in Porifera: *Axinella polypoides* Schmidt, 1862, *Chondrosia reniformis* Nardo, 1847 and *Sarcotragus foetidus* Schmidt, 1862 study cases

Torcuato Pulido Mantas, Camilla Roveta, Barbara Calcinai, Cristina Gioia di Camillo, Martina Coppari, Stefania Puce and Carlo Cerrano



Supplementary material available at:

3D material available at:

A photogrammetric approach to estimate growth in Porifera: *Axinella polypoides* Schmidt, 1862, *Chondrosia reniformis* Nardo, 1847 and *Sarcotragus foetidus* Schmidt, 1862 study cases

Torcuato Pulido Mantas, Camilla Roveta, Barbara Calcinai, Cristina Gioia di Camillo a, Martina Coppari , Stefania Puce , Carlo Cerrano

Abstract

Along the past decades, sponge populations have been decreasing due to a synergy of climate change and human induced pressures. In addition, the rising of commercial interest for this taxon as a source of natural compounds creates the urgency to properly evaluate growth and regeneration rates before considering any kind of industrial exploitation to preserve wild populations. Being modular organisms, sponges could be partially and repeatedly collected in situ, however, to avoid their depletion over time, it would be necessary to wait for a complete regeneration of the animal before detaching a new fragment. Approaches to estimate the sponge biomass increment are traditionally destructive (i.e., wet weight, dry weight, radiocarbon dating methods), or not fully suited to certain sponge morphologies (i.e., 2D photo-samplings and area determination). Nowadays, thanks to the emergence of innovative techniques, it is possible to approach sponge production in a non-invasive way. In this study, three sponge species presenting different three-dimensional architectures, *Axinella polypoides* (erect, branching), *Sarcotragus foetidus* (massive) and *Chondrosia reniformis* (submassive-lobate), were monitored using Structure-from-Motion (SfM) photogrammetry. By applying this technique, it was possible to estimate growth and regeneration rates, observing also spatial growth patterns of each specimen. Moreover, changes in biomass were estimated by studying the relationship between the volume and the dry weight of each species. *A. polypoides* recorded an increase in volume of 63.17 % · year $^{-1}$, while for *S. foetidus* and *C. reniformis,* an increase of 38.2 ± 5.9% and 120 ± 63.14% · year $^{-1}$, respectively, was observed. The estimated biomass production was 0.047 g DW·year $^{-1}$·dm^{-3} for *A. polypoides*, 13 ± 5 g DW·year $^{-1}$·dm^{-3} for *S. foetidus* and of 130 ± 82 g DW·year $^{-1}$·dm^{-3} for *C. reniformis*. Overall, SfM photogrammetry proved to be a cost-effective approach for long-term sponge growth assessment. Although it can hold some limitations linked to water turbidity and sponge structural complexity, it is here proposed as a suitable non-invasive compromise for in-situ volume and biomass estimations.

Keywords: growth assessment, mariculture, photogrammetry, biomass, conservation

1. Introduction

Porifera represents an essential component of marine ecosystems, affecting the benthic community composition through competitive interactions, representing both fundamental ecosystem engineers and bioeroders (Diaz & Rutzler, 2001, Rohde & Schupp, 2011, Cerrano et al., 2007; Van Soest et al., 2012; Maldonado et al., 2017). Being sedentary filter feeders, sponges play valuable ecological functions, taking part in a variety of processes, such as the benthic-pelagic coupling of inorganic nutrients, the carbon flux, or the seawater filtration, processing rates up to 35 ml · min^{-1} · (cm sponge) $^{-3}$ (Milanese et al., 2003; Weisz et al., 2008; Maldonado et al., 2012; Pham et al., 2019; Maggioni et al., 2022). In addition to ecological role, the role of this group as a precious source of bioactive compounds has called the attention of the scientific community, rising their commercial value and adding a new stressor to their already impacted populations (Di Cesare Mannelli et al., 2021). In fact, over the past decades, sponge assemblages have been decreasing worldwide due to a synergy of climate change and human induced pressures (Wulff, 2006; Cebrian et al., 2011; Di Camillo et al., 2013; Garrabou et al., 2022). Although the exploitation of certain sponge species have been occurring for centuries (e.g., the bath sponge *Spongia officinalis*), an uncontrolled harvest together with heavy outbreak diseases could end in the regional extinction of certain species (Bierwirth et al., 2022). To avoid that, before considering any kind of industrial exploitation, a proper evaluation of their growth and regeneration rates should be conducted, in order to preserve wild populations.

Overall, little is known about marine sponge life traits (e.g., life spam, growth rates, reproductive cycles, among others) (Garrabou and Zabala, 2001; McMuray et al., 2010; Riesgo and Maldonado, 2008; Rohde and Schupp, 2011), and the variations in morphology and growth rates, that can occur among locations, populations, or seasons, contribute to the lack of knowledge regarding this topic (Pronzato et al., 1998; Rohde and Schupp, 2011). Although growth rates have been defined for some species in both tropical and temperate environments (Rohde and Schupp, 2011), clear indicators of their annual growth, useful to easily determine the growth rates and age, are lacking (Olinger et al., 2019).

Approaches to estimate sponge growth and biomass are traditionally destructive (i.e., wet weight, dry weight, radiocarbon dating methods) (e.g., Roche et al., 2001; Çelik et al., 2011; Freeman et al., 2015), often fatal for the organism, and not adapted to all sponge growth habits (e.g., encrusting, rope-like, tubular, or massive) (Olinger et al., 2019). In fact, they mainly record two-dimensional (2D) metrics (e.g., planar projection photography) (e.g., Garrabou and Zabala 2001; de Caralt et al., 2008), even though sponges often present complex three-dimensional (3D) structures (Olinger et al., 2019). Luckily, technological and methodological advancements are helping to develop new techniques to capture 3D features in underwater environments (Ferrari et al., 2021). While earlier 3D imaging methods, such as stereo-photogrammetry, required dualcamera equipment and technical expertise for its implementation (Koopmans & Wijffels, 2008), the increase of computational power along with the advancements in computer vision algorithms resulted in the arrival of Structure from Motion (SfM) photogrammetry, one of the most popular and used procedures nowadays. This technique can be applied using a single camera and allows to obtain accurate 3D digital reconstructions from a series of overlapping images (Olinger et al., 2019).

In this context, the current study aimed to test the feasibility of a SfM-photogrammetry to assess the growth rates of various Mediterranean demosponges (*Axinella polypoides* Schmidt, 1862, *Chondrosia reniformis* Nardo, 1847 and *Sarcotragus foetidus* Schmidt, 1862) during three different conditions: the regeneration of donor specimens, the growth of sponge explants and the natural growth of sponge individuals. The development of 3D cost-effective approaches to monitor sponge growth can represent a step forward in the monitoring of sponges, constituting a powerful tool in the fields of conservation and sponge mariculture.

2. Materials & Method

Target species

During this study three Mediterranean demosponge species were approached, each one presenting a different growth habit.

Axinella polypoides is an erect, branching, long-living marine sponge with a spicular skeleton (Figure 1a). Due to its erect growth habit, it is extremely sensitive to mechanical damage, specially by entanglement in fishing gears, mucilage filaments or anchoring impacts. Even though its ecological importance is well recognized as habitat-former, playing a major role in the benthic-pelagic coupling, little is still known about its distribution, conservation status or life traits (Azzola et al., 2021). In addition to this general lack of knowledge, the relatively recent isolation of biotechnologically interesting compounds in the metabolome of the species (Aiello et al., 2010; Menna et al., 2012), creates the urge to assess its growth rates, until now unknown.

Sarcotragus foetidus is an irregularly massive sponge with a skeleton made of spongin fibres and commonly found in Mediterranean coralligenous assemblages (Figure 1b). In this case, the ecological relevance of the species is not only due to the structural complexity that their individuals provide to the surrounding habitat, but also to the wide aquiferous system within the sponge, acting as refuge for many organisms (Cerrano et al., 2006; Gerovasileiou et al., 2016; Goren et al., 2021). Its major threats are mainly represented by artisanal fishing activities, which eradicate them from the substrate by the entanglement in their gears, and the increase in frequency of mass mortality events linked to periods of extreme temperature (Gerovasilieu et al., 2018; Garrabou et al., 2022). Additionally, this species chemo-diversity called the attention of the biomedical community, due to the antitumoral activities of some of its secondary metabolites (Chaudhari & Kumar, 2020; Di Cesare Mannelli al., 2021). No information regarding growth rates of this species is currently present in the literature.

Chondrosia reniformis is a peculiar massive lobate sponge (Figure 1c) entirely made of collagen fibers, which makes this organism of great interest for pharmaceutical and nutraceutical industries (Pozzolini et al., 2018). Even though different approaches for the cultivation of this species are under current development in several international laboratories (Orel et al., 2021; Gökalp et al., 2022), the implementation of 3D monitoring methods would significantly contribute to the proper quantification of its regeneration and growth rates, a crucial aspect in the evaluation of production and sustainability of marine culture plants.

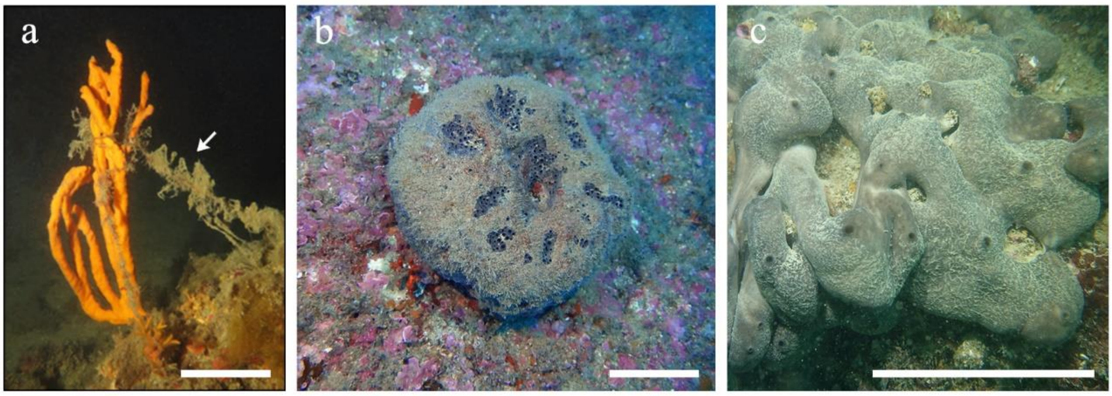


Figure 1. Pictures of the three sponge species assessed by this study: a) *Axinella polypoides*; b) *Sarcotragus foetidus*; and c) *Chondrosia reniformis*. Scale bars: 10 cm. Arrow in a) points out the presence of a fishing line impacting the sponge.

All three species present different levels of protection. *A. polypoides* and *S. foetidus* are both included in the list of endangered or threatened species of Annex II of the Barcelona convention (UNEP/MAP-SPA/RAC, 2018). Additionally, *A. polypoides* is also included in the list of the Bern convention (1987) and considered as endangered in Italy, according to the International Union for Conservation of Nature red list (IUCN, 2022). The third species, *C. reniformis,* is not currently included in any convention or considered as an endangered species, even though its shallow populations have been repeatedly affected by mass mortality events over the past years, calling for a re-evaluation of its conservation status (Di Camillo & Cerrano, 2015). Moreover, the discovery of all these three species as promising sources of compounds of medical and nutraceutical interest (Menna et al., 2012; Pozzolini et al., 2018; Di Cesare Mannelli al., 2021) demand a proper assessment of natural growth and regeneration rates before even considering them for mariculture purposes (Bierwirth et al., 2022). Within this framework, *A. polypoides* and *C. reniformis* assessments were performed in regenerating specimens after the production of explants, while *S. foetidus* assessment was carried out in an undisturbed wild population.

Study sites

Three locations were monitored for the growth assessment of the considered sponge species. In the case of *A. polypoides*, the monitoring of three donor specimens took place around 30 m depth, over a natural rocky platform located in Punta Sciusciaù (Gallinara Island, Albenga) (Figure 1a). Unluckily, just after only two months of monitoring efforts, two of the three individuals surveyed were impacted by fishing activities occurring in the island (one was completely ripped out from the substrate and the second one was found collapsed on the seafloor and lost completely after four months) (see detail Figure 1a).

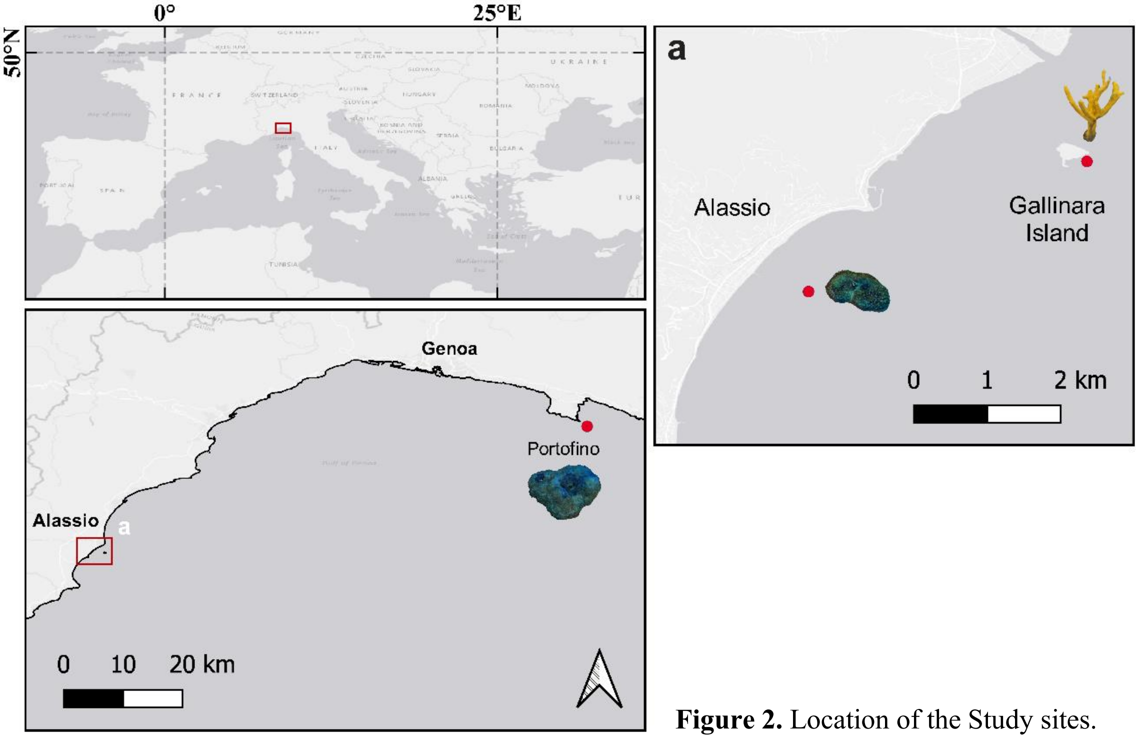


Figure 2. Location of the Study sites.

The natural population of *S. foetidus* monitored during this study is located at Punta del Faro (Portofino Marine Protected Area) (Figure 2 and 3). The rock hosting all *S. foetidus* individuals (n=20) is at a depth range between 15 and 27 meters, representing a “cross-road” of the local system of currents that characterize the area. From the initial 20 individuals surveyed in September 2017, 5 of them were not found in the second survey, ending up in the assessment of 15 individuals in November 2021.The age of all sponges is approximately the same since they are from the same recruitment event occurred in 2014 (Carlo Cerrano personal communication). The presence of a population with known age provided a unique opportunity to assess the growth rates of this species.

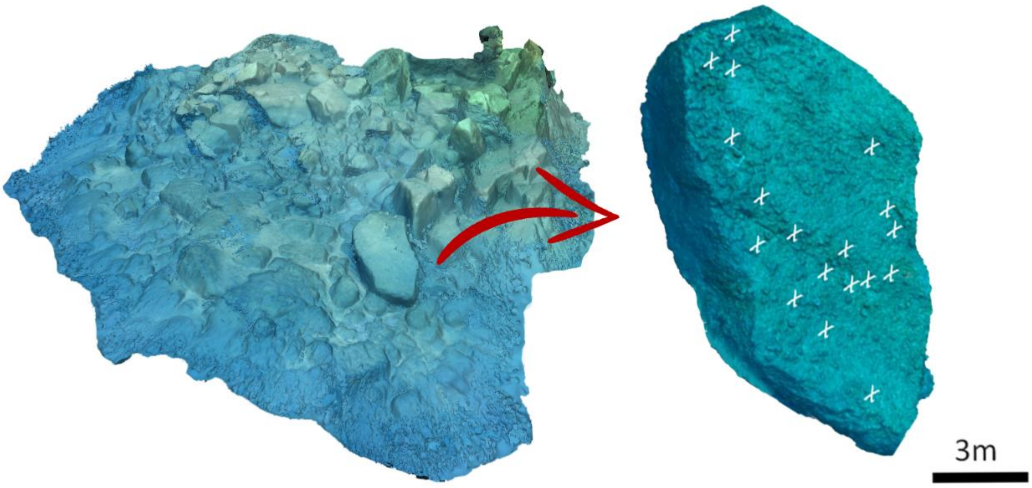


Figure 3. 3D digital reconstruction of Punta del Faro dive spot (Portofino MPA), the boulder where the *Sarcotragus foetidus* population was monitored is highlighted and the exact location of each of the individuals is marked with a white "X" in the detail of the rock at right.

Regarding *C. reniformis*, the sponge explants were monitored over old artificial reefs along the coast of Alassio at 20 m depth (Figure 2a and 4). Each module consisted of a concrete cube of 2 m side, and the modules are organized in groups of five blocks, forming a pyramidal system (Relini et al., 2007). A total of 85 *C. reniformis* fragments were transplanted and monitored on the horizontal faces of 16 blocks (Figure 4).

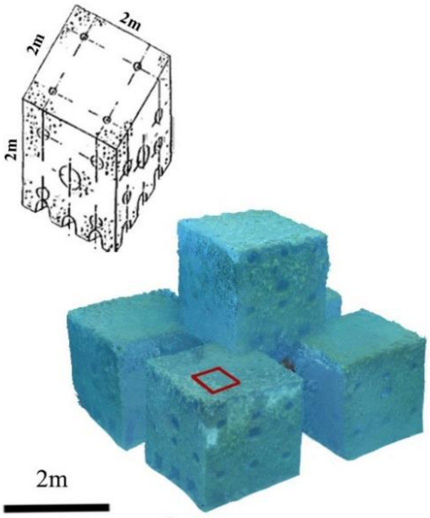


Figure 4. Artificial structures used as substrate for mariculture implants of *Chondrosia reniformis*.

Images acquisition and photogrammetric processing

A Sony RX100 V camera system equipped with an underwater housing and a couple of Akkin 5000, as artificial lighting system, was used for all photographic samplings. Periods and number of individuals surveyed for the three study cases are defined in Table 1. The photographic protocol consisted, in a first place, in the placement of metric references close to the sponge individuals to be surveyed, and subsequently a series overlapping images (40 to 60 pictures, Figure 5) were taken around each sponge at an approximate distance of 0.5m, covering in the process any possible perspective and ensuring a minimum of 60% overlapping among consecutive images (Palma et al., 2017; Bayley and Mogg, 2020). from the initial 20 individuals surveyed in September 2017,

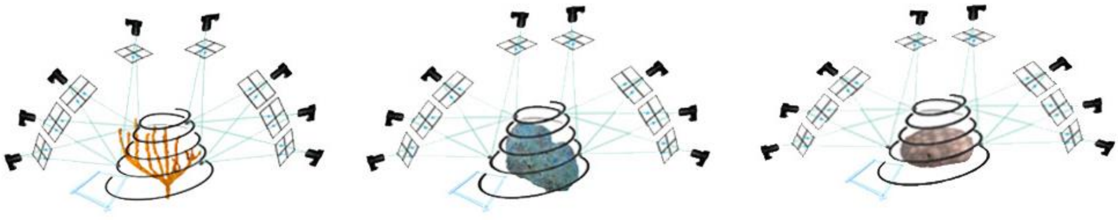


Figure 5. The scheme of the image acquisition strategy for all three approached species.

To produce the digital reconstructions of the sponges, the software Agisoft Photoscan (Agisoft LLC, St. Petersburg, Russia) was used. In the first place, a set of images was aligned using high accuracy generic pair selection settings to produce the point clouds, limiting the key points identification to 100,000 common features. From this sparse cloud, the dense point cloud was obtained, and a mesh was produced by the arbitrary 3D surface type, high face count. Once the 3D models of the sponges were texturized, the digital reconstructions were scaled up using the deployed metric references as scale, and substrate and references were removed from the model during the cleaning process. Subsequently, by using the “close holes” tool inside the mesh toolbox of Agisoft, it was possible to close the mesh of the reconstructions, allowing volume calculations. The overall photogrammetric process to generate, clean and measure each 3D reconstruction took around 30 minutes of processing time using a Lenovo Legion laptop (Beijin, China) with an Intel Core i7-9750H 2.60-GHz processor (Intel Corporation, Santa Clara, CA, USA), 32 Gb RAM and a graphic card NVIDIA GeForce RTX 2060 (NVIDIA Corporation, Santa Clara, CA, USA).

Table 1. Summary of the monitoring activities from the three study cases.

Species of interest	**Location**	**Start date**	**Final date**	**N individuals surveyed**
Axinella polypoides	Gallinara Island	June 2021	Sept 2022	3 specimens
Chondrosia reniformis	Alassio coast	May 2021	Sept 2022	85 explants
Sarcotragus foetidus	Portofino MPA	Sept 2017	Nov 2021	20 individuals

While the volume calculations were obtained by the same commercial software used for the photogrammetric processing, the distance-based mesh comparisons (Figure 6a_3,b_3,c_3) were performed by CloudCompare v2.12 (2022) open-source software. To do so, firstly the 3D models to be compared (t_0 and t_1) were imported into the software, and a preliminary mesh alignment was performed manually, to be finished using the “fine registration” tool limiting the number of iterations to 99. Once the two model meshes were aligned, distances between the two meshes were calculated by the “cloud/mesh dist.” tool, using as a reference the model from the initial (t_0) monitoring step.

Biomass and collagen estimations

Sponge samples of individuals present in the surrounding area of each study site, at a similar depth and of similar size to the population assessed in the study were collected in the field. The samples were taken to the laboratory, and its volume was calculated by water displacement method (ml of displaced water in a graduated cylinder). Secondly, all sponge fragments were dried at 60ºC for 48 hours inside an oven and weighted to obtain the dry weight biomass of each of the samples. Finally, the relationship between the volume values and the dry weight was investigated by performing a linear regression, calculated using the *stats* package in R software. Additionally, *C. reniformis* collagen production was estimated using the average yields already defined by Pozzolini et al. (2018), based on the volume changes obtained by SfM-photogrammetry:

$$\text{Collagen content (DW)} = \text{Biomass sponge (DW)} \cdot 0.30 \text{ (Pozzolini et al., 2018)}$$

3. Results

Changes in the estimated volume

Thanks to the application of SfM- photogrammetry, it was possible to estimate the volume changes of the different specimens at hand. All the individuals surveyed displayed an increase in volume along the monitoring period. The single donor specimen of *Axinella polypoides* regenerated their apical parts with an increase in height ranging from 1 to 2.5 cm between June 2021 and September 2022 (Figure 6a$_3$), corresponding to a total increase in volume of 68.35% year $^{-1}$. Regarding *Sarcotragus foetidus*, 10 specimens were selected for the growth calculations (the models holding the higher accuracy and lower levels of epibiosis), and sponge changes were measured in terms of volume and mesh distances. This resulted in an increase in height ranging from 2.8 to 6.8 cm (Figure 6b$_3$), corresponding to an average increase in volume of 38.2 ± 5.9% year $^{-1}$. At last, in the case of *Chondrosia reniformis*, from the actual 85 sponge explants currently monitored, 40 were used for the yearly growth assessments (the individuals surveyed for a minimum of 9 months), obtaining linear growth values up to 5 cm (Figure 6c$_3$), corresponding to an average increase in volume of 120 ± 63.14 % year $^{-1}$. In addition, the cloud-distance based model comparisons (Figure 6 a$_3$,b$_3$,c$_3$) allowed to identify spatial growth patterns of the three species, highlighting in which areas the sponge individuals grew or shrank. Showing how *A. polypoides* growth the most in its apical ramifications, while *S. foetidus* and *C. reniformis* focus its growth in their peripheral areas.

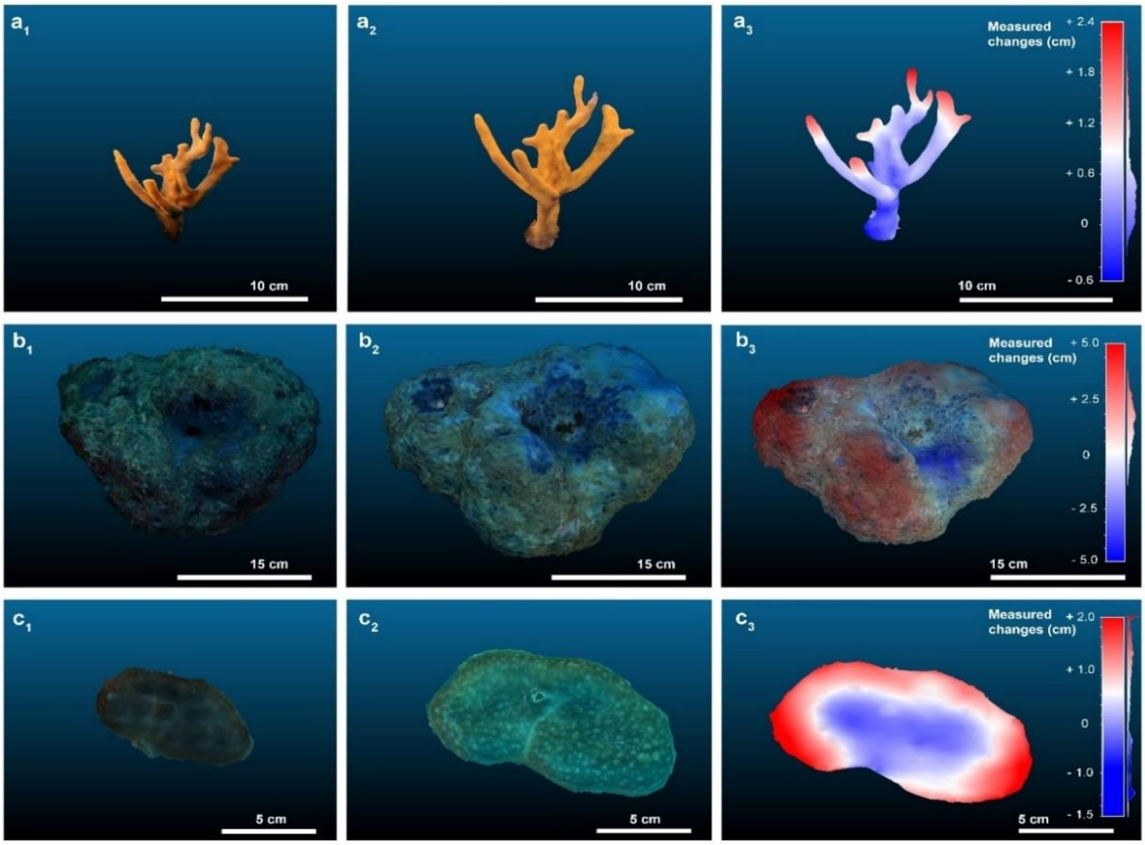


Figure 6. Examples of the 3D digital reconstructions of a) *Axinella polypoides*, b) *Sarcotragus foetidus* and c) *Chondrosia reniformis*, obtained from t_0 (a_1,b_1,c_1) and t_1 (a_2,b_2,c_2), together with the result obtained by the models comparisons performed by CloudCompare software (a_3,b_3,c_3)

Biomass and collagen estimations

From the volume values obtained from the photogrammetric approach, and thanks to the study of volume – biomass relationships on the three species of interest (Figure 7), the biomass yearly productions were obtained. *A. polypoides* donor individual produced 0.047 g DW·year^{-1}·dm^{-3}, while *S. foetidus* population volume changes corresponded to an average biomass increase of 13 ± 5 g DW·year^{-1}·dm^{-3} (Figure 8).

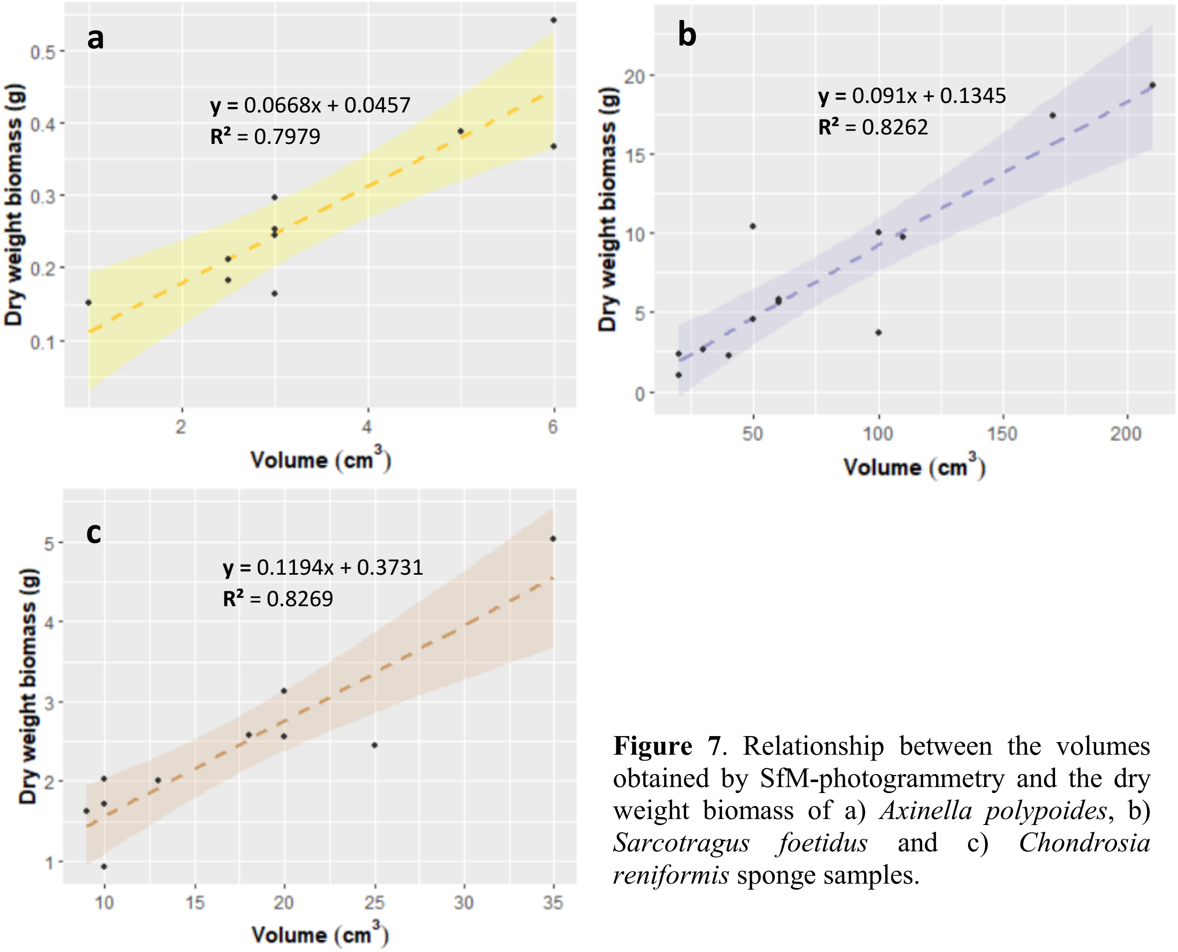


Figure 7. Relationship between the volumes obtained by SfM-photogrammetry and the dry weight biomass of a) *Axinella polypoides*, b) *Sarcotragus foetidus* and c) *Chondrosia reniformis* sponge samples.

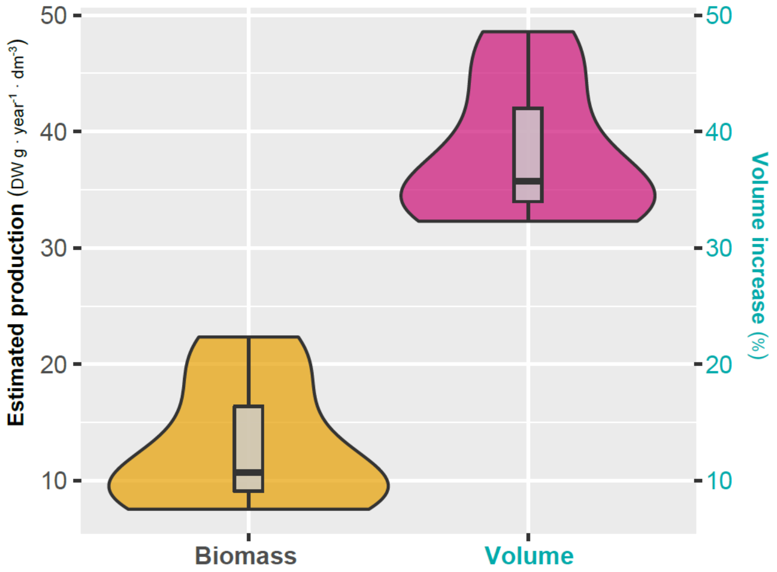


Figure 8. *Sarcotragus foetidus* estimated yearly production (dry weight, d.w., of biomass) and volume increase. Please note that the colour of the axis match the colour of the variable related to it.

On the other hand, *C. reniformis* explants presented an average estimated biomass production of 130 ± 82 g DW dm^{-3} year^{-1}. Furthermore, an average collagen production was estimated, representing a rate of 39 ± 25 g DW of collagen dm $^{-3}$ year $^{-1}$ (Figure 9).

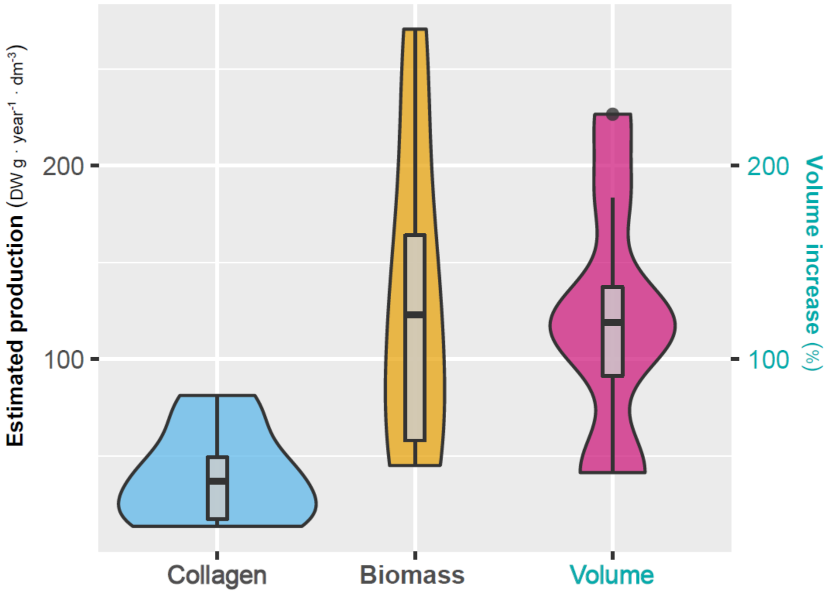


Figure 9. *Chondrosia reniformis* estimated yearly production (dry weight, d.w., of biomass and collagen) and volume increase. Please note that the colour of the axis match the colour of the variable related to it.

4. Discussion

In the Mediterranean Sea Porifera is a well-represented phylum, reaching its higher diversity rates in coralligenous and marine caves assemblages (Bertolino et al., 2013; Gerovasileiou and Voultsiadou, 2014; Calcinai et al., 2015). In these habitats, many sponges are considered key-stone species due to their effects in terms of competition for the substrate, bioerosive processes or filtration rates (Rohde & Schupp, 2011), while others are important habitat formers thanks to their complex 3D morphologies (Kružić, 2014; Gerovasileiou et al., 2016). However, the more and more frequent occurrence of marine heat waves in the last decade contributed to the increase, in number and severity, of mass mortalities events, causing devastating effects in ecosystems resilience (e.g., Perez et al., 2000; Garrabou et al., 2009; Cebrian et al., 2011; Di Camillo et al., 2013; Garrabou et al., 2022). In addition to climatic threats, the fact that every day new sponges are identified as promising sources of compounds of biotechnological interest (Di Cesare Mannelli al., 2021), makes imperative a proper evaluation of the distribution and conservation status of sponge populations. To do so, the implementation of long-term, non-invasive studies assessing growth patterns and rates would greatly contribute to a better understanding of the funcional role and health of sponge assemblages worldwide (Olinger et al., 2019).

In this framework, this study proposed SfM-photogrammetry as a cost-effective approach for the assessment of different sponge growth habits, highlighting the technological advantages of 3D morphometric evaluations. Even though this method can hold some limitations linked to water turbidity and sponge structural complexity (Lochhead and Hedley, 2020), it constitutes a suitable compromise for in-situ non-invasive volume and biomass estimations, with only an average sum of square error of ± 1.4 mm, considering all 3 morphotypes. Overall, SfM greatly assessed sponges' volumes and only individuals presenting high degree of epibiosis (especially for *Sarcotragus foetidus*) were not considered for the volume change analysis, since it would have represented a considerable overestimation of the real sponge volume. In each study site, the photographic sampling was performed in a single dive by a couple of underwater operators, resulting in diving times between 40 and 90 minutes. Even if the application of this approach considerably increases operational bottom times, mainly due to the higher number of pictures required compared to 2D approaches, it would be a small price to pay for a better evaluation of the sponge growth.

For both *Axinella polypoides* and *S. foetidus*, this study represented the first estimations of regeneration and growth rates in the literature. Nonetheless, a reference of relative monthly growth was found in the grey literature, consisting in an increase of 1.53 ± 0.47 % in height per month (Lisi, 2012 unpublished data). Since this study considered seasonal growth of primary, secondary, and tertiary ramifications it is not possible to directly compare our results. But considering that the initial height of the *A. polypoides* surveyed here (10.5 cm) and after 14 months (12 cm). We could say that Lisi 2D approach was not far from the height changes obtained by SfM. Nonetheless, the results here presented for the species are based in the growth analysis of a single specimen and a higher number of replicates and a longer monitoring period should be performed before considering this growth rate as a reference. In the case of *S. foetidus*, only growth rates of congeneric species were found. Respectively, *S. spinosulus* Schmidt, 1862 and *S. fasciculatus* (Pallas, 1766) presented increases in volume of 100% year^{-1} (Perez-Lopez et al., 2017) and 40.03 ± 4.81% year^{-1} (Turon et al., 2013), only this last presenting comparable values with the ones found for *S. foetidus*. Additionally, we took advantage of a unique opportunity in this study, assessing the growth of a population with known age (currently 6 years). Opening the possibility during future monitoring efforts to study growth dynamics through the life cycle of the species, a still quite unexplored topic in sponge research.

Chondrosia reniformis growth had already been addressed in the past (Wilkinson and Vacelet, 1979; Garrabou and Zabala, 2001; Gökalp et al., 2019; Orel et al., 2021; Gökalp et al., 2022). However, *C. reniformis* growth estimations have been previously expressed solely as % of 2D surface increase per time, hindering the comparability of our results, some of the previous studies obtained an increase in area between 70 and 120% (Wilkinson and Vacelet, 1979; Gökalp et al. 2019, 2022), within the range of % volume increase obtained in this study. Additionally, by observing the documented growth patterns of the species using the cloud-distance of the digital reconstructions (t_0-t_1), it is depicted a general growth behavior: *C. reniformis* explants firstly attach to the substrate, shrinking during a first phase, in which increases in height, to later grow in surface, loosing height especially on its central area (Figure 6a$_3$). These observations suggest that the growth assessment of this species could end up in an overestimation if conducted only by 2D approaches, especially during these first phases of explant re-attachment and growth.

Our study also highlighted how sponge growth habits and structural composition may affect growth rates and have important implications in substrate competition. In fact, our results agree with previous works (e.g., Turron et al., 1996; Becerro et al., 1994; Olinger et al., 2021) which demonstrated how highly competitive species, such as *C. reniformis* in this study, usually present higher growth rates and plasticity. On the other hand, habitat former sponge species, such as *S. foetidus* and *A. polypoides*, as well as other habitat former's taxa (e.g., octocorals and bryozoans, among others), are usually characterized by lower growth rates, limiting their potentiality for mariculture. In these cases of low individual biomass production, the creation of primmorphs - and thus of cultivable cellular lines –, when possible (Sipkema et al., 2003), should be considered for biotechnological purposes instead of in situ cultivation.

The application of photogrammetry for sponge monitoring it has more than 15 years now, from its early applications using a stereovision system for sponge volumes estimations (Abdo et al., 2006; Koopmans and Wijffels, 2008), to its late applications using SfM to approach sponge distributions (Prado et al., 2021), age and growth estimations (Olinger et al., 2019) as well as its interactions with other organisms (Olinger et al., 2021). In fact, the technique had always managed to capture biological processes in a more realistic and complexive way. Its systematic application for the assessment of volume changes in sponges, could constitute the establishment of a non-invasive, one size fits all monitoring tool. This would contribute to the standardization of methodologies and units for growth assessments, and would increase the comparability among studies, a recurrent issue found in the literature. Moreover, recreational divers now have access to low-cost high resolution underwater camera equipment, due to the arrival of action cameras, such as GoPros (Raoult et al., 2016). By the involvement and training of volunteers, photogrammetry could be added to the toolbox of many citizen science projects, reducing monitoring costs and supporting scientific research' efforts in the assessment of our precious and impacted underwater environments (Garrabou et al., 2022b).

References

- Abdo, D.A., Seager, J.W., Harvey, E.S., McDonald, J.I., Kendrick, G.A. and Shortis, M.R., 2006. Efficiently measuring complex sessile epibenthic organisms using a novel photogrammetric technique. Journal of Experimental Marine Biology and Ecology, 339(1), pp.120-133. https://doi.org/10.1016/j.jembe.2006.07.015
- Aiello, A., Fattorusso, E., Luciano, P., Menna, M. and Vitalone, R., 2010. Polyaxibetaine, an amino acid derivative from the marine sponge *Axinella polypoides*. Journal of natural products, 73(4), pp.620-622. https://doi.org/10.1021/np900759n
- Azzola, A., Bavestrello, G., Bertolino, M., Bianchi, C.N., Bo, M., Enrichetti, F., Morri, C., Oprandi, A., Toma, M. and Montefalcone, M., 2021. You cannot conserve a species that has not been found: The case of the marine sponge *Axinella polypoides* in Liguria, Italy. Aquatic Conservation: Marine and Freshwater Ecosystems, 31(4), pp.737-747. https://doi.org/10.1002/aqc.3492
- Bertolino, M., Cerrano, C., Bavestrello, G., Carella, M., Pansini M. and Calcinai, B., 2013. Diversity of Porifera in the Mediterranean coralligenous accretions, with description of a new species. ZooKeys, (336), p.1. https://doi.org/10.3897/zookeys.336.5139
- Bierwirth, J., Pulido Mantas, P., Villechanoux, J. and Cerrano, C., 2022. Restoration of Marine Sponges—What Can We Learn from over a Century of Experimental Cultivation? Water, 14(7), p.1055. https://doi.org/10.3390/w14071055
- Calcinai, B., Bertolino, M., Bavestrello, G., Montori, S., Mori, M., Pica, D., Valisano, L. and Cerrano, C., 2015. Comparison between the sponge fauna living outside and inside the coralligenous bioconstruction. A quantitative approach. Mediterranean Marine Science, 16(2), pp.413-418. https://doi.org/10.12681/mms.900
- Cebrian, E., Uriz, M.J., Garrabou, J. and Ballesteros, E., 2011. Sponge mass mortalities in a warming Mediterranean Sea: are cyanobacteria-harboring species worse off?. PLoS One, 6(6), p.e20211. https://doi.org/10.1371/journal.pone.0020211
- Çelik, İ., Cirik, Ş., Altınağaç, U., Ayaz, A., Çelik, P., Tekeşoğlu, H., Yılmaz, H. and Öztekin, A., 2011. Growth performance of bath sponge (*Spongia officinalis* Linnaeus, 1759) farmed on suspended ropes in the Dardanelles (Turkey). Aquaculture Research, 42(12), pp.1807-1815. https://doi.org/10.1111/j.1365-2109.2010.02781.x
- Cerrano C., Calcinai B., Pinca S., Bavestrello G., 2006. Reef sponges as hosts of biodiversity: cases from North Sulawesi. Xth Int. Coral Reef Symposium Proceedings
- Cerrano, C., Sambolino, P., Azzini, F., Calcinai, B., Bavestrello, G., 2007. Growth of the massive morph of *Cliona nigricans* (Schmidt 1862)(Porifera, Clionaidae) on different mineral substrata. Italian Journal of Zoology, 74(1), pp.13-19. https://doi.org/10.1080/11250000600929370
- Chaudhari, S. and Maushmi S.K., 2020. Marine sponges *Sarcotragus foetidus*, *Xestospongia carbonaria* and *Spongia obscura* constituents ameliorate IL-1 β and IL-6 in lipopolysaccharide-induced RAW 264.7 macrophages and carrageenan-induced oedema in rats. Inflammopharmacology, 28(4), pp. 1091-1119. https://doi.org/10.1007/s10787-020-00699-2
- De Caralt, S., Uriz, M.J. and Wijffels, R.H., 2008. Grazing, differential size-class dynamics and survival of the Mediterranean sponge *Corticium candelabrum*. Marine Ecology Progress Series, 360, pp.97-106. https://doi.org/10.3354/meps07365
- Di Camillo, C.G. and Cerrano, C., 2015. Mass mortality events in the NW Adriatic Sea: phase shift from slow-to fast-growing organisms. PloS one, 10(5), p.e0126689. https://doi.org/10.1371/journal.pone.0126689.g006

- Diaz, M. C. and Rützler, K., 2001. Sponges: an essential component of Caribbean coral reefs. Bulletin of Marine Science 69(2), pp.535–546.
- Di Camillo, C.G., Bartolucci, I., Cerrano, C., and Bavestrello, G., 2013. Sponge disease in the Adriatic Sea. Marine Ecology, 34(1), pp.62-71. https://doi.org/10.1111/j.1439-0485.2012.00525.x
- Di Cesare Mannelli, L., Palma Esposito, F., Sangiovanni, E., Pagano, E., Mannucci, C., Polini, B., Ghelardini, C., Dell'Agli, M., Izzo, A.A., Calapai, G. and de Pascale, D., 2021. Pharmacological activities of extracts and compounds isolated from mediterranean sponge sources. Pharmaceuticals, 14(12), p.1329. https://doi.org/10.3390/ph14121329
- Ferrari, R., Lachs, L., Pygas, D.R., Humanes, A., Sommer, B., Figueira, W.F., Edwards, A.J., Bythell, J.C. and Guest, J.R., 2021. Photogrammetry as a tool to improve ecosystem restoration. Trends in Ecology & Evolution, 36(12), pp.1093-1101. https://doi.org/10.1016/j.tree.2021.07.004
- Freeman, C.J., Baker, D.M., Easson, C.G. and Thacker, R.W., 2015. Shifts in sponge-microbe mutualisms across an experimental irradiance gradient. Marine Ecology Progress Series, 526, pp.41-53. https://doi.org/10.3354/meps11249
- Garrabou, J., Bensoussan, N., di Franco, A., Boada, J., Cebrian, E., Santamaria, J., Guala, I., Grech, D., Cerrano, C., Pulido Mantas, T., Jou, M., Marambio, M. and Azzurro, E., 2022b. Monitoring Climate-related responses in Mediterranean Marine Protected Areas and beyond: ELEVEN STANDARD PROTOCOLS. 74 pp. Edited by: Institute of Marine Sciences, Spanish Research Council ICM-CSIC, Passeig Marítim de la Barceloneta 37-49, 08003 Barcelona, Spain. (2022) https://doi.org/10.20350/digitalCSIC/14672
- Garrabou, J., Coma, R., Bensoussan, N., Bally, M., Chevaldonné, P., Cigliano, M., Díaz, D., Harmelin, J.G., Gambi, M.C., Kersting, D.K. and Ledoux, J.B., 2009. Mass mortality in Northwestern Mediterranean rocky benthic communities: effects of the 2003 heat wave. Global change biology, 15(5), pp.1090-1103. https://doi.org/10.1111/j.1365-2486.2008.01823.x
- Garrabou, J., Gómez-Gras, D., Medrano, A., Cerrano, C., Ponti, M., Schlegel, R., ... and Harmelin, J. G., 2022a. Marine heatwaves drive recurrent mass mortalities in the Mediterranean Sea. Global change biology, 28(19), pp.5708-5725. https://doi.org/10.1111/gcb.16301
- Garrabou, J. and Zabala, M., 2001. Growth dynamics in four Mediterranean demosponges. Estuarine, coastal and shelf science, 52(3), pp.293-303. https://doi.org/10.1006/ecss.2000.0699
- Gerovasileiou, V., Chintiroglou, C.C., Konstantinou, D. and Voultsiadou, E., 2016. Sponges as "living hotels" in Mediterranean marine caves. Scientia Marina, 80(3), pp.279-289. https://doi.org/10.3989/scimar.04403.14B
- Gökalp, M., Wijgerde, T., Sarà, A., De Goeij, J.M. and Osinga, R., 2019. Development of an integrated mariculture for the collagen-rich sponge *Chondrosia reniformis*. Marine Drugs, 17, p.29, https://doi.org/10.3390/md17010029
- Gökalp, M., Kooistra, T., Rocha, M.S., Silva, T.H., Osinga, R., Murk, A.J. and Wijgerde, T., 2020. The effect of depth on the morphology, bacterial clearance, and respiration of the Mediterranean sponge *Chondrosia reniformis* (Nardo, 1847). Marine Drugs, 18(7), p.358. https://doi.org/10.1111/raq.12516
- Gökalp, M., Wijgerde, T., Murk, A. and Osinga, R., 2022. Design for large-scale maricultures of the Mediterranean demosponge *Chondrosia reniformis* Nardo, 1847 for collagen production. Aquaculture, 548, p.737702. https://doi.org/10.1016/j.aquaculture.2021.737702

- Goren, L., Idan, T., Shefer, S. and Ilan, M., 2021. Macrofauna inhabiting massive demosponges from shallow and mesophotic habitats along the Israeli Mediterranean coast. Frontiers in Marine Science, 7, p.612779. https://doi.org/10.3389/fmars.2020.612779
- Koopmans, M. and Wijffels, R.H., 2008. Seasonal growth rate of the sponge *Haliclona oculata* (Demospongiae: Haplosclerida). Marine Biotechnology, 10(5), pp.502-510. https://doi.org/10.1007/s10126-008-9086-9
- Kružić, P., 2014. Bioconstructions in the Mediterranean: Present and Future. In: Goffredo, S., Dubinsky, Z. (eds) The Mediterranean Sea. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-6704-1_25
- Lisi, F., 2012. Struttura e dinamica di popolazione di *Axinella polypoides* (Schmidt, 1862) (Porifera, Demospongiae) presso l'Isola Gallinara (SV). Laurea magistrale, Università di Bologna, Corso di Studio in Biologia marina [LM-DM270] - Ravenna http://amslaurea.unibo.it/view/cds/CDS8024/
- Lochhead, I., and Hedley, N., 2020. 3D modelling themperate waters: building rigs and data science to support glass sponge monitoring monitoring efforts in coastal British Columbia. International Archives of the Photogrammetry, Remote Sensing & Spatial Information Sciences 43. https://doi.org/10.5194/isprs-archives-XLIII-B2-2020-969-2020
- Maggioni, F. Bell, J., Pujo-Pay, M., Shaffer, M., Cerrano, C., Hugues, L., Letourneur, Y., Rodolfo-Metalpa, R., 2022. Sponge Organic Matter Recycling: Reduced Detritus Production Under Extreme Environmental Conditions. SSRN 4299920. http://dx.doi.org/10.2139/ssrn.4299920
- Maldonado, M., Ribes, M., van Duyl, F.C., 2012. Nutrient fluxes through sponges: Biology, budgets, and ecological implications. Advances in Marine Biology 62: pp.113–182. https://doi.org/10.1016/B978-0-12-394283-8.00003-5
- Maldonado, M., Aguilar, R., Bannister, R., Bell, J., Conway, J., Dayton, P., Díaz, C., Gutt, J., Kelly, M., Kenchington, E. and Leys, S., 2017. Sponge grounds as key marine habitats: a synthetic review of types, structure, functional roles, and conservation concerns. In: Rossi S, Bramanti L, Gori A, Orejas Saco del Valle C (eds). Marine animal forests: The ecology of benthic biodiversity hotspots. Springer, Cham., pp. 1-39. https://doi.org/10.1007/978-3-319-17001-5_24-1
- McMurray, S. E., T. P. Henkel, and J. R. Pawlik. 2010. Demographics of increasing populations of the giant barrel sponge *Xestospongia muta* in the Florida Keys. Ecology 91: pp.560– 570. https://doi.org/10.1890/08-2060.1
- Menna, M., Aiello, A., D'Aniello, F., Fattorusso, E., Imperatore, C., Luciano, P. and Vitalone, R., 2012. Further investigation of the mediterranean sponge *Axinella polypoides*: Isolation of a new cyclonucleoside and a new betaine. Marine drugs, 10(11), pp.2509-2518. https://doi.org/10.3390/md10112509
- Milanese, M., Chelossi, E., Manconi, R., Sara, A., Sidri, M., Pronzato, R., 2003. The marine sponge *Chondrilla nucula* Schmidt, 1862 as an elective candidate for bioremediation in integrated aquaculture. Biomolecular engineering, 20(4-6), pp.363-368. https://doi.org/10.1016/S1389-0344(03)00052-2
- Morganti, T.M., Ribes, M., Yahel, G. and Coma, R., 2019. Size is the major determinant of pumping rates in marine sponges. Frontiers in physiology, p.1474. https://doi.org/10.3389/fphys.2019.01474
- Olinger, L. K., Scott, A. R., McMurray, S. E., & Pawlik, J. R. (2019). Growth estimates of Caribbean reef sponges on a shipwreck using 3D photogrammetry. Scientific reports, 9(1), 1-12. https://doi.org/10.1038/s41598-019-54681-2

- Olinger, L.K., Chaves-Fonnegra, A., Enochs, I.C. and Brandt, M.E., 2021. Three competitors in three dimensions: photogrammetry reveals rapid overgrowth of coral during multispecies competition with sponges and algae. Marine Ecology Progress Series, 657, pp.109-121. https://doi.org/10.3354/meps135794
- Orel, B., Giovine, M. and Ilan, M., 2021. On the path to thermo-stable collagen: Culturing the versatile sponge *Chondrosia reniformis*. Marine Drugs, 19(12), p.669. https://doi.org/10.3390/md19120669
- Pansini, M. and Pronzato, R., 2009. *Axinella polypoides* Schmidt, 1862. In: Relini, G. and Tunesi, L. (Eds.) Biologia Marina Mediterranea, 6: pp. 77– 80. Protected species according to the SPA/BIO Protocol (Barcelona Convention) present in Italy. Identification sheets. Genova: Erredì Grafiche Editoriali.
- Perez, T., Garrabou, J., Sartoretto, S., Harmelin, J. G., Francour, P., and Vacelet, J., 2000. Massive mortality of marine invertebrates: an unprecedented event in northwestern Mediterranean. Comptes rendus de l'Academie des sciences. Serie III, Sciences de la vie, 323(10), pp. 853-865. https://doi.org/10.1016/s0764-4469(00)01237-3
- Perez-Lopez, P., Ledda, F.D., Bisio, A., Feijoo, G., Perino, E., Pronzato, R., Manconi, R. and Moreira, M.T., 2017. Life cycle assessment of in situ mariculture in the Mediterranean Sea for the production of bioactive compounds from the sponge *Sarcotragus spinosulus*. Journal of Cleaner Production, 142, pp.4356-4368. https://doi.org/10.1016/j.jclepro.2016.11.137
- Pham, C.K., Murillo, F.J., Lirette, C., Maldonado, M., Colaço, A., Ottaviani, D. and Kenchington, E., 2019. Removal of deep-sea sponges by bottom trawling in the Flemish Cap area: conservation, ecology and economic assessment. Scientific reports, 9(1), pp.1-13. https://doi.org/10.1038/s41598-019-52250-1
- Pozzolini, M., Scarfì, S., Gallus, L., Castellano, M., Vicini, S., Cortese, K., Gagliani, M.C., Bertolino, M., Costa, G. and Giovine, M., 2018. Production, characterization and biocompatibility evaluation of collagen membranes derived from marine sponge *Chondrosia reniformis* Nardo, 1847. Marine drugs, 16(4): p.111. https://doi.org/10.3390/md16040111
- Prado, E., Cristobo, J., Rodríguez-Basalo, A., Ríos, P., Rodríguez-Cabello, C. and Sánchez, F., 2021. In situ growth rate assessment of the hexactinellid sponge *Asconema setubalense* using 3D photogrammetric reconstruction. Frontiers in Marine Science, p.26. https://doi.org/10.3389/fmars.2021.612613
- Pronzato, R., Bavestrello, G. and Cerrano, C., 1998. Morpho-functional adaptations of three species of *Spongia* (Porifera, Demospongiae) from a Mediterranean vertical cliff. Bulletin of Marine Science, 63(2), pp.317-328.
- Raoult, V., David, P.A., Dupont, S.F., Mathewson, C.P., O'Neill, S.J., Powell, N.N. and Williamson, J.E., 2016. GoPros™ as an underwater photogrammetry tool for citizen science. PeerJ, 4, p.e1960. https://doi.org/10.7717/peerj.1960
- Relini, G., Relini, M., Palandri, G., Merello, S. and Beccornia, E., 2007. History, ecology and trends for artificial reefs of the Ligurian sea, Italy. In Biodiversity in Enclosed Seas and Artificial Marine Habitats (pp. 193-217). Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-6156-1_17
- Riesgo, A. and Maldonado, M., 2008. Differences in reproductive timing among sponges sharing habitat and thermal regime. Invertebrate Biology, 127(4), 357-367. https://doi.org/10.1111/j.1744-7410.2008.00128.x
- Roche, S., Ronziere, M.C., Herbage, D. and Freyria, A.M., 2000. Native and DPPA cross-linked collagen sponges seeded with fetal bovine epiphyseal chondrocytes used for cartilage tissue engineering. Biomaterials, 22(1), pp.9-18. https://doi.org/10.1016/S0142-9612(00)00084-3

- Rohde, S., Schupp, P.J., 2012. Growth and regeneration of the elephant ear sponge *Ianthella basta* (Porifera). In: Maldonado, M., Turon, X., Becerro, M.A., Uriz, M.J. (eds) Ancient Animals, New Challenges. Sponge Research Developments. Hydrobiologia, 687, pp.219-226. https://doi.org/10.1007/s10750-011-0774-5
- Sipkema, D., Van Wielink, R., Van Lammeren, A.A.M., Tramper, J., Osinga, R. and Wijffels, R.H., 2003. Primmorphs from seven marine sponges: formation and structure. Journal of biotechnology, 100(2), pp.127-139. https://doi.org/10.1016/S0168-1656(02)00253-5
- Turon, X., Garriga, A. and Erwin, P.M., 2013. Lights and shadows: growth patterns in three sympatric and congeneric sponges (*Ircinia* spp.) with contrasting abundances of photosymbionts. Marine biology, 160, pp.2743-2754.
- UNEP/MAP-SPA/RAC, 2018. SAP/RAC : SPA-BD Protocol - Annex II: List of endangered or threatened species.
- Van Soest, R.W., Boury-Esnault, N., Vacelet, J., Dohrmann, M., Erpenbeck, D., De Voogd, N.J., Santodomingo, N., Vanhoorne, B., Kelly, M. and Hooper, J.N., 2012. Global diversity of sponges (Porifera). PLoS one, 7(4), p.e35105. https://doi.org/10.1371/journal.pone.0035105
- Gerovasileiou, V., and Voultsiadou, E., 2015. Mediterranean marine caves as biodiversity reservoirs: a preliminary overview. In Proceedings of the 1st Mediterranean Symposium on the Conservation of Dark Habitats; Langar, H., Bouafif, C., Ouerghi, A., Eds, pp. 45-50. https://doi.org/10.13140/2.1.4063.3281
- Webster, N.S., 2007. Sponge disease: a global threat? Environmental Microbiology, 9(6): pp.1363-1375. https://doi.org/10.1111/j.1462-2920.2007.01303.x
- Weisz, J.B., Lindquist, N. and Martens, C.S., 2008. Do associated microbial abundances impact marine demosponge pumping rates and tissue densities?. Oecologia, 155(2), pp.367-376. https://doi.org/10.1007/s00442-007-0910-0
- Wilkinson, C. V. and Vacelet, J. 1979 Transplantation of marine sponges to different conditions of light and current. Journal of Experimental Marine Biology and Ecology 37, 91–104. https://doi.org/10.1016/0022-0981(79)90028-5
- Wulff, J.L., 2006. Rapid diversity and abundance decline in a Caribbean coral reef sponge community. Biological Conservation, 127(2), pp.167-176. https://doi.org/10.1016/j.biocon.2005.08.007

Chapter 5

Micro-CT supporting bioerosive assessments, adding a new perspective

Bioerosion processes, although highly overlooked in the past, nowadays they have been recognized as important drivers on marine environments, modifying and structuring coastal ecosystems. Up until today, studies on boring organisms have been mainly based on destructive methods, considering only bidimensional approaches or geometric approximations, with very little 3D-contributions. Along the present chapter, Micro-CT was coupled with traditional approaches for the description and quantification of the boring activity of different microborers.

In the paper 6, "**Bioerosion features of boring polydorid polychaetes in the North Adriatic Sea**", experimental carbonate tiles were inspected by Micro-CT and resin-embedding cast technique to quantify the erosion rates during the pioneer stages in the Adriatic Sea.

While, in paper 7, "**A 3D Innovative Approach Supporting the Description of Boring Sponges of the Precious Red Coral *Corallium rubrum***", red coral samples from Cape Verde were inspected by electron microscopy and Micro-CT to characterize the boring sponge community hosted inside the *C. rubrum* scleraxis.

Both coral scleraxis bioerosion and polychaetes boring activity was approached for the first time by Micro-CT. The results of these studies, on the one hand, identified the pioneer erosive community in the North Adriatic as a complex of *Polydora* spp. and obtained the first estimates of its bioerosive activity in the Adriatic; on the other hand, in the case of *C. rubrum* bioerosive assessment, three already known sponge species were found boring into the coral (*Alectona millari*, *Dotona pulchella mediterranea* and *Thoosa armata*), and a new species of the genus *Alectona* was described (*A. ricardi* sp. nov.).

Paper 6

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https://doi.org/10.1007/s10750-022-04839-3

Bioerosion features of boring polydorid polychaetes in the North Adriatic Sea

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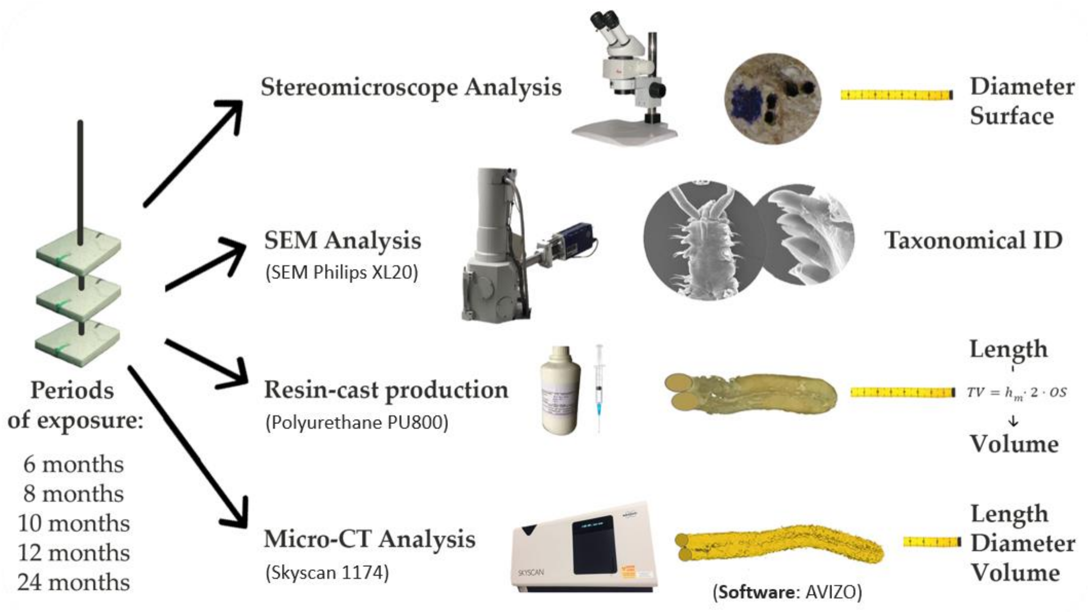

Supplementary material available at:

Bioerosion features of boring polydorid polychaetes in the North Adriatic Sea

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Abstract

Considering the pivotal role played by erosive organisms in the marine habitat and the scanty knowledge of this phenomenon in the Mediterranean Sea, the present study aimed to identify the pioneer excavating organisms occurring in the first stages of bioerosion, providing the first estimation of their rate of erosion along the North Adriatic Sea. Bioerosion activity was investigated by deploying sets of limestone panels for a period of 2 years. Moreover, micro-computed tomography was used for the first time to study polychaete erosion, confirming it is a very useful tool for the description of erosion processes. Blocks were placed in the touristic harbor Marina Dorica (Ancona) in July 2017 and periodically collected, the first time after a 6-month period, then bimonthly. Two species of the polychaete genus *Polydora* were the only boring organisms that settled on the experimental substrates. Erosion rate was estimated by measuring the holes produced by both worm species, and the eroded volumes obtained by resin casts of the holes. *Polydora* appeared after 6 months of exposure significantly changing its density rates (holes·100 cm^{-2}·month^{-1}) during the exposure, being comparable with densities reported from tropical areas. The erosion rate on the contrary is lower, reaching values of 0.053 g·cm^{-2}·year^{-1}.

Keywords: *Polydora*, boring worms, erosion rates, experimental blocks, micro-CT , resin casts.

1. Introduction

Destruction of carbonate substrates due to organism activities, or bioerosion (Neumann, 1966), is a common process in several marine ecosystems from temperate (Cerrano et al., 2001a; Wisshak et al., 2005; Schönberg & Wisshak, 2014), to tropical (Hutchings et al., 1992, 2013; Hutchings & Peyrot-Clausade, 2002; Pari et al., 2002; Tribollet et al., 2002; Hutchings, 2008) and even polar regions (Cerrano et al., 2001b; Meyer et al., 2021). Boring organisms participate to a wide range of regulation and maintenance ecosystem services: in fact, they enhance habitat complexity offering refuge to several taxa and contribute to the carbonate cycle, with the erosion of coastal areas and the production of fine sediments (Naylor et al., 2012; Davidson et al., 2018). Moreover, considering that their boring activity can be influenced by a variety of environmental factors (e.g., nutrients, salinity, temperature, pH), in the context of the current climate crisis, the study of the bioerosion processes became a timely topic (Chazottes et al., 2008; Schönberg & Wisshak, 2014; Schönberg et al., 2017).

Schönberg & Wisshak (2014) reported that only the 11% of the research on bioerosion were conducted in the Mediterranean Sea; since then, only 7 papers have been found on SCOPUS (https://www.scopus.com) using “bioerosion,” “boring species,” and “Mediterranean” as search terms. In particular, studies about the rates of bioerosion in Mediterranean basin are even scantier (Calcinai et al., 2008, 2011; Färber et al., 2015, 2016) and exclusively about sponges and polychaetes.

Several organisms belonging to different taxa are involved in calcareous-boring activities; they can be microborers as cyanobacteria (Tribollet et al., 2011; Pica et al., 2016) and fungi (Gleason et al., 2017), or macroborers as sponges (Bavestrello et al., 1991; Calcinai et al., 2008, 2011), molluscs (Ćurin et al., 2014; Peharda et al., 2015), echinoderms (Belaústegui et al., 2017), and polychaetes (Martin & Britayev, 1998; Hutchings, 2008). Experimental studies for the evaluation of bioerosion rates are principally conducted using experimental blocks made of dead coral substrate (Tribollet et al., 2002; Hutchings, 2008), limestone panels (Wisshak et al., 2005; Casoli et al., 2019), shells (Calcinai et al., 2007), and wood (Reish et al., 2018). These experimental blocks also provide essential information about successional stages of the boring communities. In the first place, the local pattern of currents influences the larval dispersion (Tribollet et al., 2002) affecting the recruitment of boring organisms. The following successional stages, mainly facilitated by the borers, lead to the achievement of mature boring communities (Hutchings, 2008). Polychaetes have been reported as the pioneer-dominant borers on newly available submerged substrates and considered among major bioeroders along with sponges and molluscs (Hutchings et al., 1992, 2011). Nevertheless, studies about values of bioerosion of polychaetes are not abundant. Numerous researches assessed this topic in coral reefs ecosystems, obtaining a great variation among their estimates: Tribollet and Golubic (2005) (0.010–0.130 kg

CaCO3·m^{-2}·year^{-1}) together with Yeung et al. (2021) (0.013–0.162 kg CaCO$_3$·m^{-2}·year^{-1}) presented the lowest values, followed by Kiene and Hutchings (1994) (0.104 kg·m^{-2} ·year^{-1}), Hutchings (2008) (0.356 kg·m^{-2}·year^{-1}), Chazottes et al. (2002) (0.050–0.400 kg CaCO$_3$·m^{-2} ·year^{-1}), and Davies and Hutchings (1983) which presented the highest value in the literature (1.800 kg·m^{-2}·year^{-1}). Generally, this group present lower values than sponges, considered as the principal erosive organisms in later succession stages (Pari et al., 2002). Only recently the colonization dynamics of boring polychaetes has been studied in the Mediterranean Sea (Tyrrhenian Sea), using experimental substrates (Casoli et al., 2019; Gravina et al., 2019), but still no data are available regarding their erosion rates.

Several families of polychaetes are involved in bioerosion processes, such as Eunicidae, Cirratulidae, Sabellidae, and Spionidae. These worms exhibit a variety of feeding preferences but are in general considered as filter and surface deposit feeders. Boring polychaetes use both chemical secretions and mechanical excavation for dissolving the rocks and making their holes (Hutchings, 2008); some sabellids, for example, although limited to a few species, have developed glandular areas along the ventral part of the body, while in some polydorids (Spionidae) the erosion of the carbonate substrate is possible thanks to the modified chaetae present on the 5th chaetiger, in addition to the production of chemicals compounds (Zottoli & Carricket, 1974; Sato-Okoshi & Okoshi, 1993; Hutchings, 2011). Polydorid is one of the most studied boring taxa and this is principally due to the serious damages these polychaetes inflict on oysters and abalone shells in aquaculture systems (Sato-Okoshi et al., 2008; Walker, 2011). These polychaetes are easily recognizable on the substrate for their typical 8-shaped holes, due to the two closed openings they produce divided by a thin mucous layer, allowing palps to move in the surrounding ground looking for food particles. The longitudinal section of the hole, where the worm lives in, is U-shaped, and each 8-shaped hole corresponds to a single boring individual. Not all the genera belonging to this taxon have been recognized as boring (Sato-Okoshi, 1999); sometimes, it's not even easy to attribute the species to the correct taxon because of the similar morphological characteristics shared by a group of species, thus making identification a difficult task (see for example Simon & Sato-Okoshi, 2015; Bertasi, 2016; Çinar & Dagli, 2021), such as the case of the species complex Polydora ciliata (Johnston, 1838) (Mustaquim, 1986, 1988; Manchenko & Radashevsky, 1998).

Micro-computed tomography (micro-CT) has been extensively applied for studying internal and external anatomical features of several taxa such as corals (Morales Pinzón et al., 2014; Enochs et al., 2016; Urushihara et al., 2016), sponges (Heim & Nickel, 2010), or polychaetes (Faulwetter et al., 2013; Parapar et al., 2017, 2019, 2021), and in terms of analysis of bioerosion traces produced by micro- and macroborers (Beuck et al., 2007). Regarding annelids, micro-CT has been implemented to visualize, from a three-dimensional perspective, bioturbation in polychaetes

(Pennafirme et al., 2019) and the boring pattern produced by the *Siboglinid Osedax* (Higgs et al., 2010). To the best of our knowledge, this is the first time micro-CT is used to visualize the development of boring polychaete cavities.

Considering the role that bioerosion plays in the marine habitat and the scanty knowledge of this phenomenon in the Mediterranean Sea, this study aims to assess on experimental limestone panels: (i) the pioneer, bioerosive organisms occurring in the first stages of bioerosion along the North Adriatic Sea and (ii) the annual rate of bioerosion during this first colonization phase. A diagram summarizing the different techniques implemented along the study was produced and added as Supplementary Material (Online Resource 1).

2. Methods

2.1.Study site, experimental design, and sample treatment

Marina Dorica (43°36.65′ N; 13°28.91′ E) is a touristic harbor in the northern-west coast of the Ancona Promontory (Adriatic Sea, Italy) (Fig. 1). In this site, five sets of three experimental blocks (15 in total) were placed under a wharf (W3) at a depth of 1 m. These blocks were made of Pietra Leccese (Margiotta, 2006), a common calcareous formation along Italian coasts consisting in a compact biomicrite made of planktonic foraminifera with its origin in the Miocene (Bossio et al., 2002).

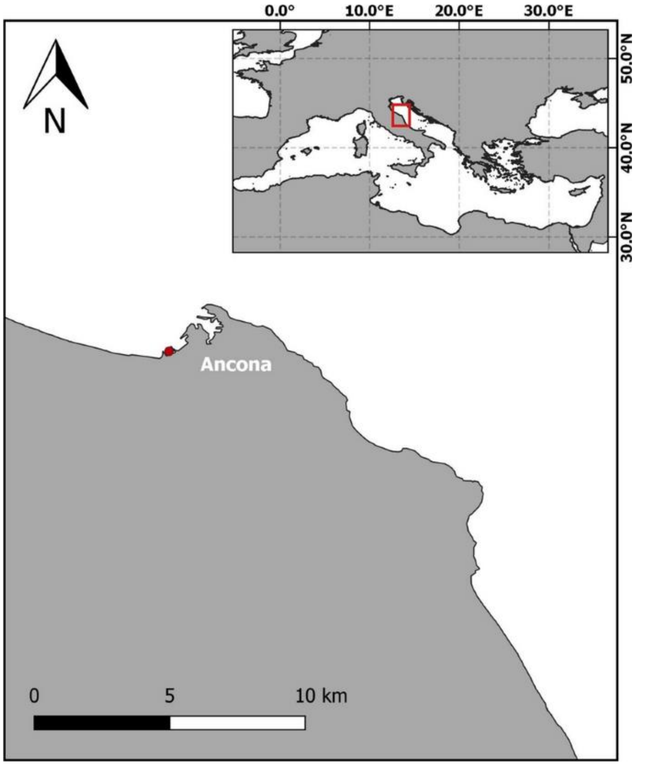

Figure 1. Map of the study site, located in the touristic harbor of Marina Dorica (Ancona, Italy)

Blocks were positioned between the poles using a line and kept separated by plastic tubes 20 cm long. The wharf is in proximity of the canal port where the water exchange, with the open sea, is higher with respect to the inner port. The calcareous blocks were submerged from July 2017 to July 2019, and the first set of three blocks was collected in January'18 after 6 months from the positioning. Later, the other sets were collected bimonthly, up to July'18; the last three blocks were collected after 24 months of immersion (in total), in July 2019. When removed from the structure, the blocks were separately placed in plastic bags with sea water and transferred to the laboratory; at this point, all the encrusting organisms were carefully removed from the blocks and the blocks were kept in aquaria, under a continuous sea-water circulation. The cleaning procedure did not affect the integrity of boring worms and keeping them alive in aquaria allowed to precisely localize their holes. Magnesium chloride (7%) was added to the water to relax polychaetes and to facilitate their extraction from the substrate; right after the worms were extracted, forcing them out of their holes with the help of a Pasteur pipette, fixed in alcohol solution (95%) and identified. For scanning electron microscopy (SEM) analyses, several specimens were primary fixed in ethanol 20%, then dehydrated through a graded ethanol series (50%, 70%, 90%, 100%), and dried with Hexamethyldisilazane 98% until complete evaporation following Shively & Miller (2009). Finally, they were attached on stubs, sputtered with gold-platinum and observed under SEM Philips XL20.

2.2.Density and erosion parameters assessment

For each block, the 8-shaped *Polydora*'s holes were counted, calculating their average density (holes·100 cm^{-2}). The two openings, creating an 8-shaped sign on the rock surface, approximately have equal diameter (d) (Fig. 2a); for this reason, only one diameter (of the single opening) per each hole was measured under stereomicroscope; the surface of each opening (OS) (Fig. 2a) was considered circular in shape and calculated with the formula $OS = (d/2)^2\pi$. The value of the surface erosion produced by a single worm (8-shaped hole) was obtained doubling the OS values.

To assess the volume of carbonate material removed by the polychaetes, a polyurethane resin (PU800) (www.antichitabelsito.it) was injected into the holes of the blocks, with a surgical syringe. Then, the blocks were dissolved in acid chloride (37%) and the hardened resin casts were removed. For this purpose, the blocks of March, May, and July 2018 were used. The resin casts reproduced worm excavations (Fig. 2b), whose regular form allowed us to approximate its shape to two paired cylinders (Fig. 2a) to calculate the total volume (TV) excavated by a single worm; we applied the formula TV = $h_m 2OS$, where 2OS is the average value of the excavated area (i.e., the double openings; see above) calculated for each period of sampling, and h stands for the height of the cast; the value h_m was obtained by calculating the average height of 15 casts for each period (Fig. 2a, b). The total volume excavated in July 2018 was then used to estimate the erosion rate of polychaetes as mm^3 of excavated substrate per mm^{-2} of substrate, per $year^{-1}$. To evaluate the weight (g) of the substrate eroded by polychaetes per surface and $year^{-1}$, the volumes removed by these after one year of exposure (July 2018), were multiplied by the specific gravity of the "Leccese" rock (2.577 $g \cdot cm^{-3}$) and by the specific gravity of the micritic limestone of the maiolica formation that mainly characterizes the Conero substratum (2.5 $g \cdot cm^{-3}$) (Calcinai et al., 2011).

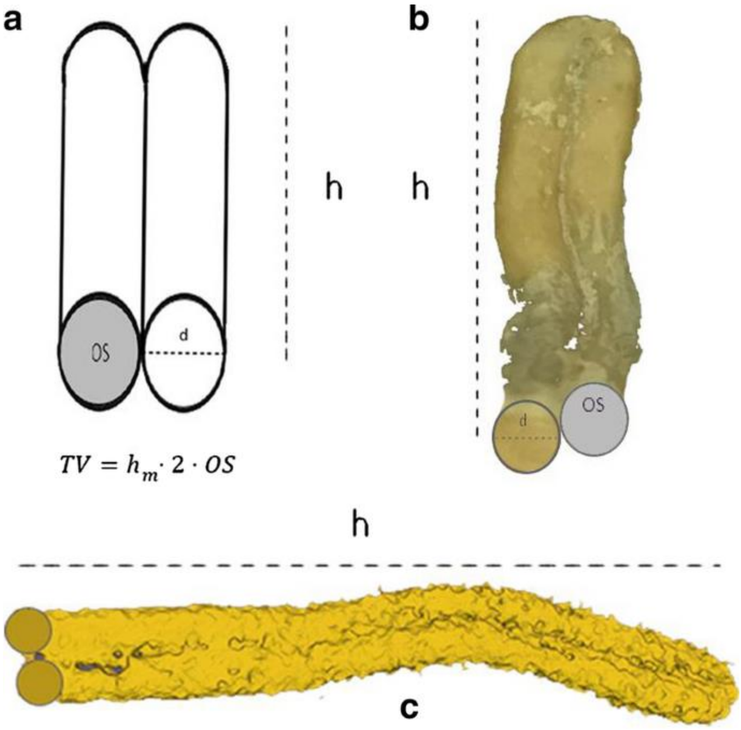

Figure 2. Three methodologies used to extract polydorid's morphometrics. (a) Model used as a proxy for cavity volume; (b) Resin cast; (c) 3D reconstruction of Polydora's cavities after micro-CT processing. (d diameter, h height, OS surface of a single opening).

In order to compare the density of excavating worms (= holes) on the blocks between the different times of exposure, it was standardized by dividing its density values by their time of exposure.

Parallel to the approach made with resin casts, *Polydora* samples from January and July 2018 were micro-CT-scanned at Centro di Ricerca e Servizio di Microscopia delle Nanostrutture (CISMiN) at the Polytechnic University of Marche, using a Skyscan 1174. During scanning, the sample was rotated in 0.20° angular increments reaching a total of 180° rotation, acquiring an X-ray absorption radiograph was digitally recorded at each rotation step. The X-ray source was set at 50 kV and 800 μA and an aluminum filter was placed between the sample and the camera in order to maximize the energy transmission through the sample. Scans were performed at a maximum pixel size of 25.25 μm. Initial processing of the projection images was performed with cone-beam reconstruction software (Nrecon v.1.7.3.1., Skyscan), resulting in a series of axial cross-sections. Post-processing of the cross-sections was conducted using the Amira software edition from the Zuse Institute Berlin, ZIB Amira 5 version 2019.1 (Stalling et al., 2005), where, in the first place, a Gaussian smoothing filter using a 3 × 3 × 3 voxel kernel was applied to reduce image noise. Then, bioerosive pores were differentiated from the rocky substrate by the following: (i) cross-sections segmentation by an interactive thresholding, defining the solid substrate; (ii) then, ambient occlusion module was applied to the binarized cross-sections in order to allow erosion pores to be visualized separately, as digital 'casts' in a greyscale depending on the occlusion of the same; and (iii) a final interactive thresholding was applied again to allow the software to perform volume calculations. Once single cavities were individualized on the 3D reconstructions, dimensions such as diameter, length, and volume values were extracted for each reconstructed erosion channel (Fig. 2c). Two sampling periods were assessed by the tomographic approach in order to test the technique, January and July 2018.

2.3.Statistical analysis

Given the proximity among the three blocks extracted at each time period, it was not possible to consider them as independent replicates. This is the reason why a repeated-measures ANOVA test was conducted to compare groups of samples exposed to different periods of immersion. The data were previously checked for homogeneity of variance using the Levene's test, and whenever data did not meet the assumptions for parametric analyses, they were logarithmically transformed. Moreover, when the assumptions for parametric analyses were not met, even after logarithmical transformation, data were compared by the equivalent non-parametric analysis, the Friedman test.

All statistical analyses were performed using PAST 4 (Hammer et al., 2001) and graphical outputs with R software (R Core Team, 2020).

3. Results

3.1.Boring organism, density, and erosion traces

Boring species recorded into the experimental blocks belonged to two species: *Polydora ciliata* complex (Annelida: Spionidae) (Walker, 2011) and *Polydora* sp.. The *P. ciliata* complex specimens had the typical characters of the species (Fig. 3), but since the first description of the species refers to tubicolous non-boring forms on mud sediments (Johnston, 1838), for the boring sibling species of this complex, only genetic analyses can distinguish among different taxa (Manchenko & Radashevsky, 1993, 1998). Polydora sp. is a form showing some of the characters of the *P. ciliata* complex but differs in having (i) dark bar pigmentation of the palps, (ii) the pigmentation present on the prostomium and dorsal side of the 3rd–4th segments, and (iii) the stout special chaetae of the 5th segment without a spur or secondary tooth. In fact, the pigmentation on both palps and prostomium, as well as in the anterior segments, closely resembles that of *Polydora haswelli* Blake & Kudenov, (1978) (see figure in Sato-Okoshi & Abe, 2013); however, the shape of the stout modified chaetae of the 5th segment differs from *P. haswelli*, in lacking any clear lateral accessory flange (Radashevsky et al., 2006). The form present in our samples resembles the worms collected by Boscolo and Giovanardi (2002) in the external coloration; these worms were found as borers infesting the bivalve *Ruditapes philippinarum* (A. Adams & Reeve, 1850) (cited as *Tapes philippinarum*) shells in the northern Adriatic and attributed by the Authors to *P. ciliata*. Therefore, considering that *P. haswelli* is actually not reported for the Mediterranean Sea, our specimens deserve further taxonomic analyses, possibly matched with molecular ones, since the 18S gene sequencing is already available for some Japanese specimens of *P. haswelli* (Sato-Okoshi & Abe, 2013).

However, from an ecological and functional point of view, both species found in our samples seem to exert the same role as boring pattern and activity, and therefore, their effects can be considered as they were a single taxonomic entity. Bearing this in mind, signs of bioerosion made by *Polydora* spp. have been present since the first sampling in January 2018, where after six months of immersion, we could already count a total of 80 specimens in all three blocks. Moreover, their density changed along the periods of immersion, reaching the highest value in July 2018, after 1 year of submersion ($n = 290$ in three blocks with a density of 32.8 ± 15.8 holes·cm^{-2}).

Considering exposure time, the density of excavating worms (= holes) was comprised between 1.51 ± 0.51 worms 100 cm^{-2}·month^{-1} in January 2018 (after 6 months of exposure) and 2.74 ± 1.32 worms 100 cm^{-2}·month^{-1} in July 2018, after 12 months (Fig. 4). Repeated-measures ANOVA test revealed significant differences in relation to the periods of immersion ($P < 0.05$), specially between July 2018 and July 2019 (Tukey's test $P = 0.01296$).

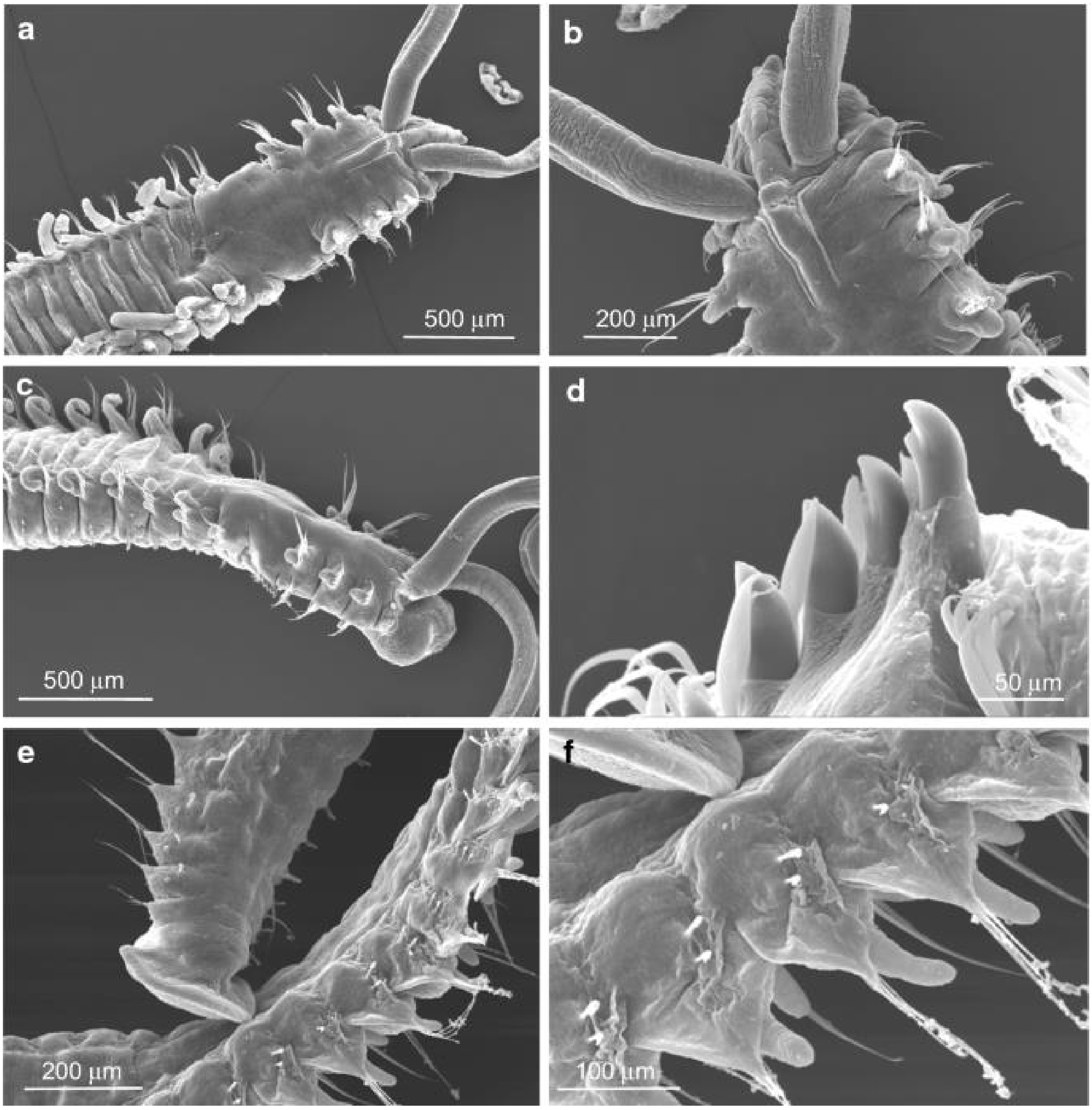

Figure 3. SEM pictures of a specimen of *Polydora ciliata* complex, showing the main taxonomic characters. a–c Dorsal and lateral views of the prostomium and first few segments showing lack of notochaetae at the 1st segment, and the start of neuropodial uncini and branchiae at the 7th segment. d Modified stout chaetae of the 5th segments with a typical spur (sometimes a small tooth). e–f posterior part of the body showing the lack of both special chaetae and branchiae in the last segments, and the plate-like pygidium.

The average size of diameters of a single opening is shown in Fig. 5. The smallest value of 0.70 ± 0.19 mm was recorded in May'18 (after 10 months of immersion) while the higher value was 0.81 ± 0.13 mm in July'19, after 24 months of immersion. The Friedman test showed no significant differences ($P > 0.05$) between diameters among periods of immersion.

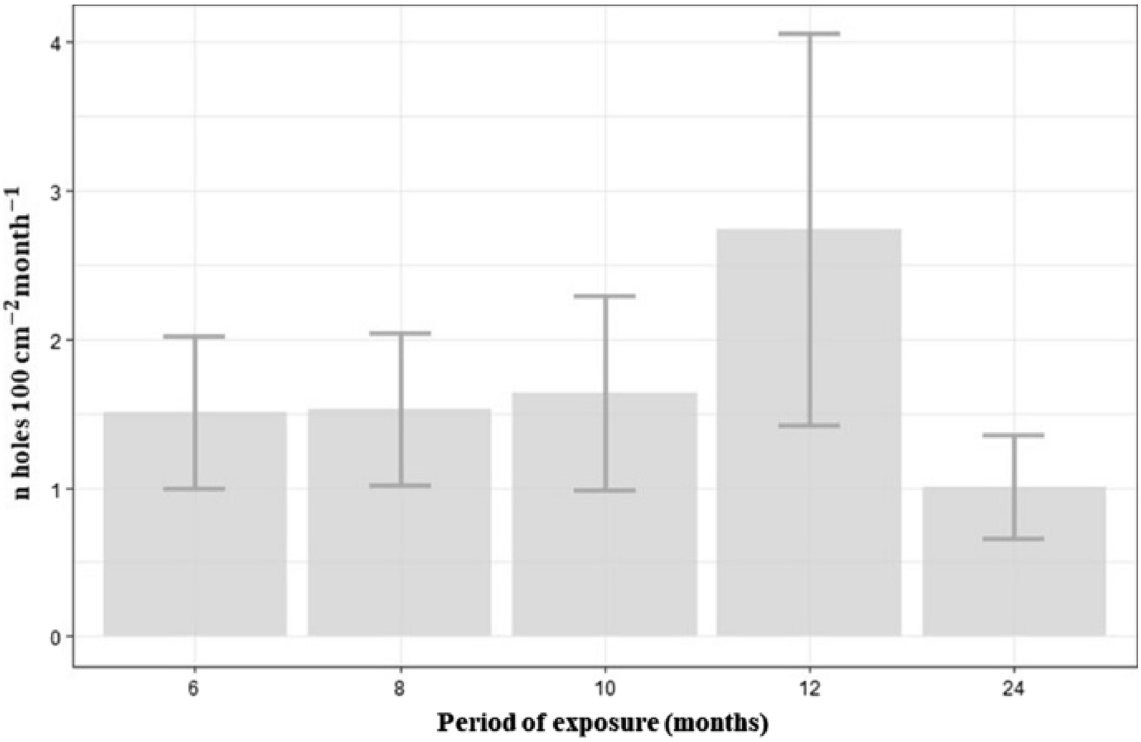

Figure 4. Density rate of the excavating worms (holes) (average ± SD) along the period of exposure.

The heights of the resin casts were evaluated for holes produced in March, May, and July 2018. They are between 18.4 ± 4.5 mm in May and 19.2 ± 4.1 mm in March. Repeated-measures ANOVA test revealed no significant differences ($P > 0.05$) in the heights of the holes, reproduced by the casts.

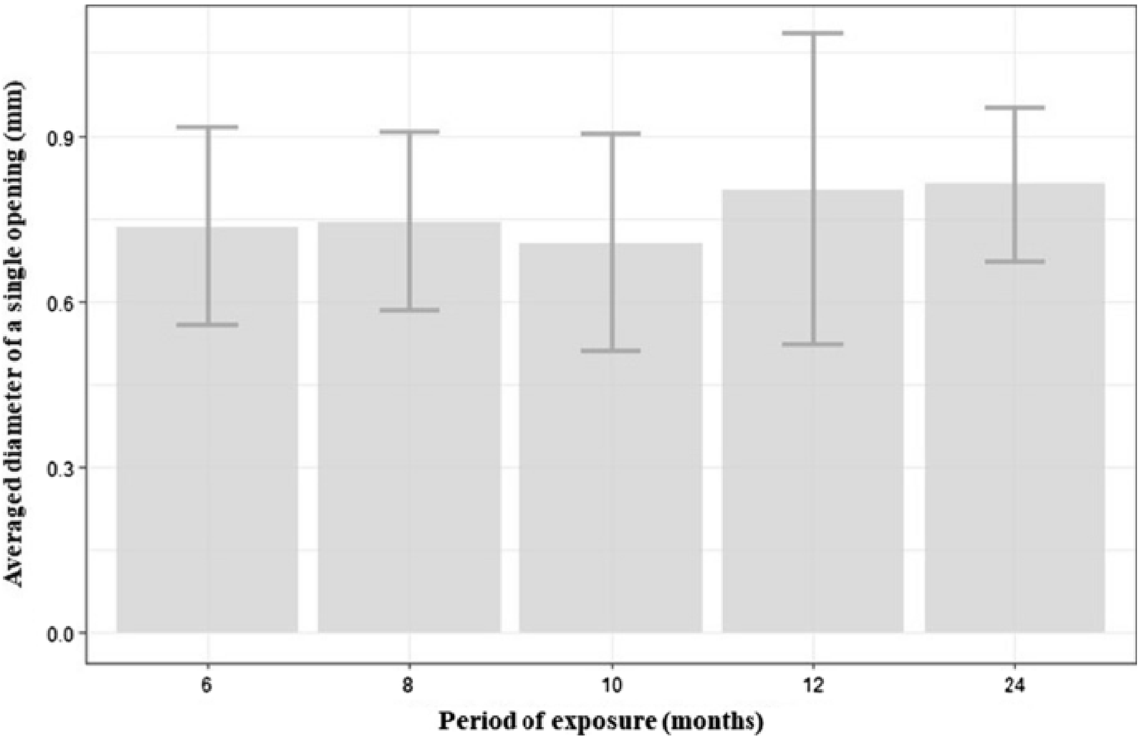

Figure 5. Average diameter (± SD) of Polydora's hole (single opening) along the period of exposure.

3.2.Erosion rates assessment

The eroded volumes were ranged from 6.4 $mm^3 \cdot cm^{-2}$ of substrate (March'18, after 6 months of exposure) to 21 $mm^3 \cdot cm^{-2}$ of substrate (July'18, after a year). Assuming that substrate erosion is constant over time and considering the periods of blocks' immersion, the eroded volume rates varied between 9.788 $mm^3 \cdot cm^{-2} \cdot year^{-1}$ (March '18) and 21.086 $mm^3 \cdot cm^{-2} \cdot year^{-1}$ (July'18). The rates obtained using the full-year period eroded volume was considered as a more complete estimate, possibly accounting for seasonal variations. In this context, erosion rate was calculated by multiplying the yearly eroded volume by the specific gravity of the Leccese rock, obtaining a rate of 0.0543 $g \cdot cm^{-2} \cdot year^{-1}$. If we consider the specific gravity of the Conero rock present along Ancona Promontory, the erosion rates on the Conero area would be around 0.053 $g \cdot cm^{-2} \cdot year^{-1}$.

3.3.Micro-CT characterization

Micro-CT was applied for two of the experimental time steps: January '18, where two subsamples were scanned resolving three fully reconstructed holes, presenting an averaged diameter of 0.71 ± 0.09 mm and a length of 8.32 ± 3.08 mm; while for July '18, a single subsample resolved three holes, with an averaged diameter of 0.78 ± 0.14 mm and an average length of 9.39 ± 3.29 mm. The 3D reconstructions obtained from the scanning allowed also to calculate the volume for each of the holes, obtaining averaged eroded volumes of 7.52 ± 4.73 mm^3 for January '18 and 8.51 ± 5.52 mm^3 for July '18.

4. Discussion

The aim of this study was to describe the first phases of bioerosion processes along the North Adriatic Sea coast and provide the first estimation of the relative bioerosion rates. The North-Italian Adriatic coast is characterized almost entirely by sandy beaches while the Conero Promontory, where Ancona is located, is a massive calcareous promontory colonized by both epilithic and endolithic organisms as sponges and bivalves (Calcinai et al., 2009). Up until today, only sponge bioerosion has been studied on this peculiar ecosystem (Calcinai et al., 2011). At Mediterranean level, knowledge regarding polychaete erosion rates is still absent. For some borers, as sponges (*Cliona adriatica* Calcinai et al., 2011) and bivalves *(Rocellaria dubia* (Pennant, 1777)), it has been possible to estimate the erosion with non-destructive methods, allowing to calculate the volume of the internal cavities by mean of its external openings (Schiaparelli et al., 2005; Calcinai et al., 2011). For polydorids, only destructive methods, such as cracking the substrates, have been used (Simon & Sato-Okoshi, 2015; Radashevsky et al., 2017); considering that heights of the excavations did not vary significantly with the time of exposure, we suggest to estimate the volume eroded by *Polydora* spp. directly by measuring the diameter of the openings, considering the erosion cavity as a double cylinder, and avoiding destructive approach; moreover, we also propose, for the first time, micro-CT three-dimensional reconstructions as a non-destructive method for evaluating polydorids' erosion cavities. The erosion pattern in *Polydora* seems different in respect of other taxa as the bivalve *R. dubia* (see Schiaparelli et al., 2005), the date mussel (*Lithophaga lithophaga*, (Linnaeus, 1758)), or the piddock, (*Pholas dactylus* Linnaeus, 1758) where both the erosion cavities and the superficial openings enlarge following the mollusks' growth.

This study shows that, after 6 months, the polydorid *Polydora* spp. appeared into the blocks, and no other excavating organisms were detected during the following 2 years of experiment. No evidence was recorded for boring sponges, bivalves, or echinoderms, widely considered as the principal taxa involved in boring processes both in tropical and temperate seas (Schönberg & Wisshak, 2014). These organisms are known to appear in late successional stages of the boring community (after 2 years, e.g., Chazottes et al., 1995), after worms that, in the coral reefs, are always reported as pioneers (Hutchings & Peyrot–Clausade, 2002; Hutchings, 2008; Hutchings et al., 2013). In this study, blocks were immersed only for 2 years but, although in the short term, this experiment highlights that also in the Mediterranean Sea, the first succession phase in the boring community mirrors that of the tropical areas (see, e.g., Hutchings et al., 1992; Tribollet et al., 2002).

The density of *Polydora* spp. significantly changed with the time of exposure, as reported for other bioeroders in studies conducted in the tropical areas (Chazotte et al., 1995; Hutchings et al., 2002; Hutchings et al., 2013). Recently, Casoli et al. (2019) registered the presence of only 10 specimens of *Polydora ciliata* and of another excavating worm (*Dodecaceria concharum* Örsted, 1843), in experimental blocks in the Tyrrhenian Sea. In contrast, our experimental blocks presented a high abundance of *Polydora* spp. specimens, reaching its maximum densities after one year. This sequence coincides with the results of Casoli et al. (2019) and Gravina et al. (2019) that found *Polydora* exclusively present in the first period, in blocks immersed for 1 year, substituted later by *D. concharum*, the only worm present in 2- and 3-year-old blocks. Consequently, species of the genus *Polydora* may be considered as pioneer species characterizing the first period of succession also in the Adriatic Sea.

In terms of eroded volumes, the higher values recorded in July 2018 are probably due to the settlement of a new cohort of *Polydora* spp., that, as reported by Blake (1969), it is characterized by one or two reproductive events in a single season, usually in late spring. In fact, during the observation in vivo, done on the samples of May 2018, several mature specimens were recorded.

Regarding the density, per unit of volume, of the boring polychaetes, our data varied between 10 ± 4.5 and 36.25 ± 23.3 worms$\cdot$100 cm^{-3}. Values comparable to those reported in coral reefs by Hutchings and Peyrot-Clausade (2002) which assessed abundances of some coral-boring polydorids in the French Polynesia, obtaining densities from 0.5 ± 0.8 to 36.3 ± 33.7 ind$\cdot$100 cm^{-3}; higher values as those reported by Hutchings et al. (2013) for *Polydora* spp. (303.0 ± 72.75

ind·100 cm^{-3}). On the other hand, bioerosion rates, here assessed, are much lower than those reported in tropical areas (see data in the introduction). This is not surprising, as it is known that the bioerosion rate in the tropics exceeds the ones occurring in temperate and cold waters (Wisshak, 2006). Nevertheless, the comparison between present data and those from literature allows only some very general considerations; in literature, the erosion rates are often calculated without discriminating among the taxa, i.e., grouping polychaetes and sipunculans as “worms,” and being this group mainly clustered together with bivalves, as macroborers. Moreover, differences observed in terms of density and erosion rates may be due to the different lithologies used during the experiments; in fact, it has been demonstrated that the bioerosion rate is higher in porous substrates (corals) in respect of denser material (Leccese rock) (Calcinai et al., 2007).

Thanks to the micro-CT analysis, a clear three-dimensional view of the erosion patterns produced by *Polydora* spp. was obtained, showing a unified “8-shaped hole” which occasionally gets separated into two single funnels along its development. Measurements taken from the digital casts obtained similar values that the ones extracted from resin casts; yet the tomography did not only enabled us to extract measures, but also to observe the position and orientation of the cavities into the substrate, as well as to explore bioerosion pattern as a whole, otherwise impossible to obtain by more invasive approaches (e.g., Online Resource 2, where two worms appeared to have avoided perforating each other cavities by slightly changing direction during their erosion activity). Nevertheless, even if micro-CT has proved its value on the assessment of erosive patterns, its application on high-density substrates can cause artifacts during the scanning, thus limiting the maximum volume of sample to be analyzed. A posterior subsampling would make the technique to lose its non-invasive character, risking to damage the cavities inside the sample (as observed on Online Resource 2, where the cut on the substrate damaged two cavities). Moreover, operational costs for a high number of samples can be considerable, for which the coupling of more cost-effective techniques, such as resin casts, should be considered.

5. Conclusions

This is the first study evaluating bioerosion rates produced by polychaetes, organisms which have a dominant role in the bioerosion processes affecting calcareous substrates, both natural and artificial, in the Mediterranean Sea. The study has shown how the spionid polychaetes *Polydora* spp. trigger the earlier stages of bioerosion in the North Adriatic Sea, and that it is possible to estimate the etched volume with non-destructive methods. In the sea, the bioerosion affects any kind of calcareous substrate, playing a relevant role in several marine ecosystem dynamics. Bio-erosive successions in temperate climates are poorly studied. Moreover, in the near future, due to the increasing of organic pollution in coastal waters together with a general warming, an increment of the bioeroders’ activity is expected. Long-term experiments using substrates having different lithologies, at different depths and considering waters with different contents of organic matter, are needed to provide a wider and more precise picture of the bioerosion trends of the Mediterranean Sea.

References

Bavestrello, G., Calcinai, B. & M. Sarà, 1991. Delectona ciconiae sp. nov. (Porifera, Demospongiae) boring in the scleraxis of *Corallium rubrum*. Journal of the Marine Biological Association of the United Kingdom 76: 867–873. https://doi.org/10.1017/S0025315400040844

Belaústegui, Z., Muñiz, F., Nebelsick, J.H., Domènech, R. & J. Martinell, 2017. Echinoderm ichnology: bioturbation, bioerosion and related processes. Journal of Paleontology 91: 643–661. https://doi.org/10.1017/jpa.2016.146

Bertasi, F. 2016. The occurrence of the alien species *Polydora cornuta* Bosc, 1802 (Polychaeta: Spionidae) in North Adriatic lagoons: an overlooked presence. Italian Journal of Zoology, 83(1), 77-88. https://doi.org/10.1080/11250003.2016.1140839

Beuck, L., Vertino, A., Stepina, E., Karolczak, M., & O. Pfannkuche, 2007. Skeletal response of *Lophelia pertusa* (Scleractinia) to bioeroding sponge infestation visualised with micro-computed tomography. Facies 53: 157–176.

Blake, J.A., 1969. Reproduction and larval development of *Polydora* from Northern New England (Polychaeta: Spionidae). Ophelia 7: 1-63. http://dx.doi.org/10.1080/00785326.1969.10419288.

Blake, J.A. & J.D. Kudenov, 1978. The Spionidae (Polychaeta) from southeastern Australia and adjacent areas with a revision of the genera. Memoirs of the National Museum of Victoria 39: 171–280.

Boscolo, R. & O. Giovanardi, 2002. *Polydora ciliata* shell infestation in *Tapes philippinarum* Manila clam held out of the substrate in the Adriatic Sea, Italy. Journal of invertebrate pathology 79: 197-198. http://dx.doi.org/10.1016/S0022-2011(02)00029-0

Bossio A., Mazzei R., Monteforti B. & G. Salvatorini, (2002). Note illustrative della carta geologica dell'area di Lèuca (con Appendice biostratigrafica a cura di Foresi L.M., Mazzei R., Salvatorini G.). Atti della Società Toscana di Scienze Naturali, Memorie Serie A 107: 97-163.

Calcinai, B., Azzini, F., Bavestrello, G., Gaggero, L. & C. Cerrano, 2007. Excavating rates and boring pattern of *Cliona albimarginata* (Porifera: Clionaidae) in different substrata. Porifera Research: Biodiversity, Innovation & Sustainability: 203-210.

Calcinai, B., Cerrano, C., Iwasaki, N. & G. Bavestrello, 2008. Sponges boring into precious corals: an overview with description of a new species of *Alectona* (Demospongiae, Alectonidae) and a worldwide identification key for the genus. Marine Ecology 29: 273–279. https://doi.org/10.1111/j.1439-0485.2008.00246.x

Calcinai, C., Bavestrello, G., Betti, B., Bo, M., Cerrano, C., Di Camillo, C.G., Martinelli, M., Puce, S. & S. Tazioli, 2009. Storie vitali peculiari del benthos di substrato duro nei SIC marini marchigiani. Biology Marine Mediterranean 16: 42–45. https://doi.org/10.1111/j.1439-0485.2008.00246.x

Calcinai C., Bavestrello G., Cuttone G., C. Cerrano, 2011. Excavating sponges from the Adriatic Sea: description of *Cliona adriatica* sp. nov. (Demospongiae: Clionaidae) and estimation of its boring activity. Journal of the Marine Biological Association of the United Kingdom 91: 339–346. https://doi.org/10.1017/S0025315410001050

Casoli, E., Ricci, S., Antonelli, F., Perasso, C.S., Ardizzone, G. & M.F. Gravina, 2019. Colonization dynamic on experimental limestone substrata: the role of encrusting epilithics favouring boring polychaetes. Hydrobiologia, 842: 101-112. https://doi.org/10.1007/s10750-019-04028-9

Cerrano, C., Bavestrello, G., Bianchi, C.N., Calcinai, B., Cattaneo-Vietti, R., Morri, C., M. Sarà, 2001a. The role of sponge bioerosion in Mediterranean coralligeneous accretion. Mediterranean Ecosystems: 235-240. https://doi.org/10.1007/978-88-470-2105-1_30

Cerrano, C., Bavestrello, G., Calcinai, B., Cattaneo-Vietti, R., Chiantore, M., Guidetti, M. & A. Sarà, 2001b. Bioerosive processes in Antarctic Sea. Polar Biology 24: 790-792. https://doi.org/10.1007/s00300100294

Chazottes, V., Le Campion-Alsumard, T. & M. Peyrot-Claused, 1995. Bioerosion rates on coral reef: interaction between macroborers, microborers and grazer (Moorea, French Polynesia). Palaeogeography, Palaeoclimatology, Palaeoecology 113: 189-198. https://doi.org/10.1016/0031-0182(95)00043-L

Chazottes, V., Le Campion-Alsumard, T., Peyrot-Clausade, M., P. Cuet, 2002. The effects of eutrophication-related alterations to coral reef communities on agents and rates of bioerosion (Reunion Island, Indian Ocean). Coral Reefs 21, 375–390. https://doi.org/10.1007/s00338-002-0259-0

Chazottes, V., Reijmer J.J. & Cordier, E. (2008) Sediment characteristics in reef areas influenced by eutrophication-related alterations of benthic communities and bioerosion processes. Marine Geology 250:114–127. https://doi.org/10.1016/j.margeo.2008.01.002

Çinar, M. E., & E. Dagli, 2021. Bioeroding (boring) polychaete species (Annelida: Polychaeta) from the Aegean Sea (eastern Mediterranean). Journal of the Marine Biological Association of the United Kingdom, 1-10. https://doi.org/10.1017/S002531542100031X

Ćurin, M., Peharda, M., Calcinai, B. & S. Golubić, 2014. Incidence of damaging endolith infestation of the edible mytilid bivalve Modiolus barbatus. Marine Biology Research 10: 179-189. https://doi.org/10.1080/17451000.2013.814793

Davidson, T.M., Altieri, A.H., Ruiz, G.M. & M.E. Torchin, 2018. Bioerosion in a changing world: a conceptual framework. Ecology letters 21: 422–438. https://doi.org/10.1111/ele.12899.

Davies, P. J., & Hutchings, P. A. (1983). Initial colonization, erosion and accretion of coral substrate. Coral Reefs, 2(1), 27-35. https://doi.org/10.1007/BF00304729

Enochs, I.C., Manzello, D.P., Wirshing, H.H., Carlton, R. & J. Serafy, 2016. Micro-CT analysis of the Caribbean octocoral Eunicea flexuosa subjected to elevated pCO2. ICES Journal of Marine Science 73: 910–91. https://doi.org/10.1093/icesjms/fsv159

Färber, C., Wisshak, M., Pyko, I., Bellou, N. & A. Freiwald, 2015. Effects of water depth, seasonal exposure, and substrate orientation on microbial bioerosion in the Ionian Sea (Eastern Mediterranean). PLoS ONE 10. https://doi.org/10.1371/journal.pone.0126495.

Färber, G., Titschack, J., Schönberg, C., Ehrig, K., Boos, K., Baum, D., Illerhaus, B., Asgaard, U., Bromley, R.G., Freiwald, A. & M. Wisshak, 2016. Long-term macrobioerosion in the Mediterranean Sea assessed by micro-computed tomography. Biogeosciences 13: 3461–3474. https://doi.org/10.5194/bg-13-3461-2016

Faulwetter, S, Vasileiadou, A, Kouratoras, M., Dailianis, T., & C. Arvanitidis, 2013. Micro-computed tomography: introducing new dimensions to taxonomy. ZooKeys 263: 1–45. https://doi.org/10.3897/zookeys.263.4261

Gleason, F.H., Gadd, M.G., Pitt, J.I & A.W.D. Larkum, 2017. The roles of endolithic fungi in bioerosion and disease in marine ecosystems. I. General concepts. Mycology. 8: 205-215. https://doi.org/10.1080/21501203.2017.1352049.

Gravina, M.F., Antonelli, F., Sacco Perasso, C., Cesaretti, A., Casoli, E. & S. Ricci, 2019. The role of polychaetes in bioerosion of submerged mosaic floors in the Underwater Archaeological Park of Baiae (Naples, Italy). Facies: 65(2): 19.

Hammer, Ø., Harper, D.A.T. & P.D. Ryan, 2001. Past: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica 4: 4-9.

Heim, I. & M. Nickel, 2010. Description and molecular phylogeny of *Tethya leysae* sp. nov. (Porifera, Demospongiae, Hadromerida) from the Canadian Northeast Pacific with remarks on the use of microtomography in sponge taxonomy. Zootaxa. 2422: 1–21. https://doi.org/10.5281/zenodo.194503

Higgs, N.D., Glover, A.G., Dahlgren, T.G., C.T.S. Little, 2010. Using computed-tomography to document borings by *Osedax mucofloris* in whale bone. Cahiers de biologie marine 5: 401-405. https://doi.org/10.21411/CBM.A.19160A8F

Hutchings, P.A., 2008. Role of polychaetes in bioerosion of coral substrate. In Tapanila, L. & M. Wisshak (eds), Current Developments in Bioerosion. Springer Publishing as part of the Erlangen Earth Conference Series: 249–264. https://doi.org/10.1007/978-90-481-2639-2_5

Hutchings, P.A., 2011. Bioerosion. In Hopley, D. (ed.), Encyclopedia of modern coral reefs: structure, form and process. Springer, Dordrecht: 139–156. http://dx.doi.org/10.1007/978-90-481-2639-2

Hutchings, P.A., Kiene, W.E., Cunningham, R.B. & C. Donnelly, 1992. Experimental Investigation of bioerosion at Lizard Island, Great Barrier Reef. Part 1. Patterns in the distribution and extent of non-colonial, boring communities. Coral Reefs 11: 23–31.

Hutchings, P.A. & M. Peyrot–Clausade, 2002. The distribution and abundance of boring species of polychaetes and sipunculans in coral substrates in French Polynesia. Journal of Experimental Marine Biology & Ecology 269: 101–121. https://doi.org/10.1016/S0022-0981(02)00004-7

Hutchings, P., Peyrot-Clausade, M. & A. Stuken, 2013. Internal macrobioerosion on five species of *Acropora* following the 1998 bleaching event: Implications for the long-term impact of bleaching on the Great Barrier Reef. Pacific Conservation Biology 19: 409–417. https://doi.org/10.1071/PC130409

Kiene, W.E. & P.A. Hutchings, 1994. Bioerosion experiments at Lizard Island, Great Barrier Reef. Coral Reefs 13: 91-98. https://doi.org/10.1007/BF00300767

Martin, D. & T.A. Britayev, 1998. Symbiotic polychaetes: review of known species. Oceanography & Marine Biology 36: 217-340.

Manchenko, G.P. & V.I. Radashevsky, 1993. Genetic differences between two sibling species of the *Polydora ciliata* complex (Polychaeta: Spionidae). Biochemical Systematics and Ecology 21: 543-548

Manchenko, G.P. & V.I. Radashevsky, 1998. Genetic evidence for two sibling species within *Polydora* cf. *ciliata* (Polychaeta: Spionidae) from the Sea of Japan. Marine Biology 131: 489-495. https://doi.org/10.1017/S0025315402005350

Margiotta, S., 2006. Bio-cronostratigrafia a foraminiferi planctonici dei sedimenti miocenici nell'area di Strudà (Lecce, Puglia). Geologica Romana 39: 1-14.

Meyer, N., Wisshak, M. and Freiwald, A., 2021. Bioerosion ichnodiversity in barnacles from the Ross Sea, Antarctica. Polar Biology, 44(4), pp.667-682. https://doi.org/10.1007/s00300-021-02825-4

Morales Pinzón, A., Orkisz, M., Rodríguez Useche C.M., Torres González J.S., Teillaud S., Sánchez, J.A. & M. Hernández Hoyos, 2014. A Semi-Automatic Method to Extract Canal Pathways in 3D Micro-CT Images of Octocorals. PLoS ONE. 9. https://doi.org/10.1371/journal.pone.0085557

Mustaquim, J., 1986. Morphological variation in *Polydora ciliata* complex (Polychaeta: Annelida). Zoological Journal of the Linnean Society 86: 75-88. https://doi.org/10.1111/j.1096-3642.1986.tb01808.x

Mustaquim, J., 1988. Electrophoretic variation of isozymes in *Polydora ciliata* complex (Polychaeta Spionidae). Comp. Biochemestry & Physiology 91: 197-205. https://doi.org/10.1016/0305-0491(88)90135-6

Naylor, L.A., Coombes, M.A. & H.A. Viles, 2012. Reconceptualising the role of organisms in the erosion of rock coasts: a new model. Geomorphology 157: 17–30.

Neumann, A.C., 1966. Observations on coastal erosion in Bermuda and measurements of the boring rate of the sponge, Cliona lampa. Limnology & Oceanography 11: 92–108. https://doi.org/10.4319/lo.1966.11.1.0092

Osorno, A., 2005. Impact d'une eutrophisation experimentale sur les processes de la bioerosion recifale, One Tree Island, Grande Barriere d'Australie. Docteur en sciences de l'Université de la Méditerranée, Aix-Marseille II, pp. 130.

Parapar, J., Al-Kandari, M., Cands, M., & Moreira, J. (2021). A new species of *Polyophthalmus* (Annelida, Opheliidae) from the Arabian Gulf, with an insight on internal anatomy and diversity of the genus. Zootaxa, 5052(4), 501-528. https://doi.org/10.11646/zootaxa.5052.4.3

Parapar, J., Candás, M., Cunha-Veira, X., & J. Moreira, 2017. Exploring annelid anatomy using micro-computed tomography: A taxonomic approach. Zoologischer Anzeiger, 270, 19-42. https://doi.org/10.1016/j.jcz.2017.09.001

Parapar, J., Caramelo, C., Candás, M., Cunha-Veira, X., & J. Moreira, 2019. An integrative approach to the anatomy of *Syllis gracilis* Grube, 1840 (Annelida) using micro-computed X-ray tomography. PeerJ, 7, https://doi.org/e7251. 10.7717/peerj.7251

Pari, N., Peyrot–Clausade, M. & P.A. Hutchings, 2002. Bioerosion of experimental substrates on high islands and atoll lagoons (French Polynesia) during 5 years of exposure. Journal of Experimental Marine Biology & Ecology 276: 109–127. https://doi.org/10.1016/S0022-0981(02)00243-5

Pica, D., Tribollet, A., Golubic, S., Bo, M., Di Camillo, C.G., Bavestrello, B. & S. Puce, 2016: Microboring organisms in living stylasterid corals (Cnidaria, Hydrozoa). Marine Biology Research 12: 573-582. https://doi.org10.1080/17451000.2016.1169298./

Peharda, M., Calcinai, B., Puljas, S., Golubic, S., Arapov, J. & J. Thèbault, 2015. Endoliths in *Lithophaga lithophaga* shells – variation in intensity of infection and species occurrence. Marine environmental research 108: 91–99. https://doi.org/10.1016/j.marenvres.2015.05.002

Pennafirme, S., Machado, A.S., Machado, A.C., Lopes R.T., Lima, I.C.B., M.A.C. Crapez, 2019. Monitoring bioturbation by a small marine polychaete using microcomputed tomography Micron 121: 77-83. https://doi.org/10.1016/j.micron.2019.03.004

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Radashevsky, V.I., Lana, P.C & R.C. Nalesso, 2006. Morphology and biology of *Polydora* species (Polychaeta: Spionidae) boring into oyster shells in South America, with the description of a new species. Zootaxa 1353: 1–37. https://doi.org/10.11646/zootaxa.1353.1.1

Radashevsky, V.I., Choi, J-W., M.C. Gambi, 2017. Morphology and biology of *Polydora hoplura* Claparède, 1868 (Annelida: Spionidae). Zootaxa 4282: 543–555. https://doi.org/10.11646/ZOOTAXA.4282.3.7

Reish, D.J., Gerlinger, T.V. & R.R. Ware, 2018. Comparison of the *Polychaetous* Annelids Populations on Suspended Test Panels in Los Angeles Harbor in 1950-1951 with the Populations in 2013-2014. Bulletin, Southern California Academy of Sciences 117: 82–90.

Sato-Okoshi & Okoshi. K. 1993. Microstructure of scallop and oyster shells infested with boring *Polydora*. Nippon Suisan Gakkaishi 59: 1243–1247. https://doi.org/10.2331/suisan.59.1243

Sato-Okoshi, W. 1999. Polydorid species (Polychaeta: Spionidae) in Japan, with descriptions of morphology, ecology and burrow structure. 1. Boring species. Journal of the Marine Biological Association of the United Kingdom 79: 831−848. https://doi.org/10.1017/S0025315498001003 https://doi.org/10.3160/soca-117-01-82-90.1

Sato-Okoshi, W., Okoshi, K. & J. Shaw, 2008. Polydorid species (Polychaeta: Spionidae) in south-western Australian waters with special reference to *Polydora uncinata* and *Boccardia knoxi*. Journal of the Marine Biological Association of the United Kingdom 88: 491−501. https://doi.org/10.1017/S0025315408000842

Sato-Okoshi, W. & H. Abe, 2013. Morphology and molecular analysis of the 18S rRNA gene of oyster shell borers, *Polydora* species (Polychaeta: Spionidae), from Japan and Australia. Journal of the Marine Biological Association of the United Kingdom 93: 1279–1286. https://doi.org/10.1017/S002531541200152X

Schiaparelli, S., Franci, G., Albertelli, G. & R. Cattaneo-Vietti, 2005. A non-destructive Method to Evaluate Population Structure and Bioerosion Activity of the Boring Bivalve *Gastrochaena dubia*. Journal of Coastal Research 21: 383-386. https://doi.org/10.2112/03-0054.1

Shively, S. & W.R. Miller, 2009. The use of HMDS (hexamethyldisilazane) to Replace Critical Point Drying (CPD) in the Preparation of Tardigrades for SEM (Scanning Electron Microscope) Imaging. Transactions of the Kansas Academy of Science 112: 198-200. https://doi.org/10.1660/062.112.0407.

Schönberg, C.H.L. & M. Wisshak, 2014. Marine Bioerosion, in: Goffredo, S., Dubinsky, Z. (Eds.). The Mediterranean Sea: its history and present challenges. Springer Science+Business Media Dordrecht. https://doi.org/10.1007/978-94-007-6704-1_26.

Schönberg, C.H.L. Fang, J. K. H., Carreiro-Silva, M., Tribollet, A. & M. Wisshak, 2017. Bioerosion: the other ocean acidification problem: Contribution to the Themed Issue: 'Ocean Acidification'. ICES Journal of Marine Science 74: 895–925.

Simon, C.A. & W. Sato-Okoshi, 2015. Polydorid polychaetes on farmed molluscs: distribution, spread and factors contributing to their success. Aquaculture Environment Interactions 7: 147–166. https://doi.org/10.3354/aei00138.

Stalling, D., Westerhof, M. & H.C. Hege, 2005. Amira: a highly interactive system for visual data analysis, in: Hansen, C. D., Johnson, C. R. (Eds.), The Visualization Handbook. Elsevier: 749–767. https://doi.org/10.1016/B978-012387582-2/50040-X

Tribollet, A., Decherf, G., Hutchings, P.A. & M. Peyrot-Clausade, 2002. Large-scale spatial variability in bioerosion of experimental coral substrates on the Great Barrier Reef (Australia): importance of microborers. Coral Reefs 21: 424–432. https://doi.org/10.1007/s00338-002-0267-0.

Tribollet, A., & Golubic, S. (2005). Cross-shelf differences in the pattern and pace of bioerosion of experimental carbonate substrates exposed for 3 years on the northern Great Barrier Reef, Australia. Coral reefs, 24(3), 422-434. https://doi.org/10.1007/s00338-005-0003-7

Tribollet, A., Golubic, S., Radtke, G. & J. Reitner, 2011. On microbiocorrosion. In Advances in Reitner, J., Quéric, N.-V. & G. Arp (eds), Stromatolite Geobiology. Lecture Notes in Earth Sciences 131. Berlin: Springer: 265–76.

Urushihara, Y., Hasegawa, H. & N. Iwasaki, 2016. X-ray micro-CT observation of the apical skeleton of Japanese white coral *Corallium konojoi.* Journal of Experimental Marine Biology and Ecology 475: 124–128. https://doi.org/10.1016/j.jembe.2015.11.016

Walker, L.M., 2011. A review of the current status of the *Polydora* - complex (Polychaeta: Spionidae) in Australia and a checklist of recorded species. Zootaxa. 2751: 40−62. https://doi.org/10.11646/ZOOTAXA.2751.1.3

Wisshak, M. 2006. High-Latitude Bioerosion: The Kosterfjord Experiment - Lecture Notes in Earth Sciences, Springer, Berlin. https://doi.org/10.1007/978-3-540-36849-6

Wisshak, M., Gektidis, M., Freiwald, A. & C. Lundälv, 2005. Bioerosion along a bathymetric gradient in a cold-temperate setting (Kosterfjord, SW Sweden): an experimental study. Facies 51: 93–117. https://doi.org/10.1007/s10347-005-0009-1.

Yeung, Y. H., Xie, J. Y., Zhao, Y., Yu, H. Y., Chen, C., Lu, W. W., & Qiu, J. W. 2021. Rapid external erosion of coral substrate in subtropical Hong Kong waters. Marine Pollution Bulletin, 169, 112495. https://doi.org/10.1016/j.marpolbul.2021.112495

Zottoli, K. A. & Carriker. M. R. 1974. Burrow morphology, tube formation, and microarchitecture of shell dissolution by the spionid polychaete *Polydora websteri*. Marine Biology 27: 307–316. https://doi.org/10.1007/BF00394366

Paper 7

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A 3D Innovative Approach Supporting the Description of Boring Sponges of the Precious Red Coral *Corallium rubrum*

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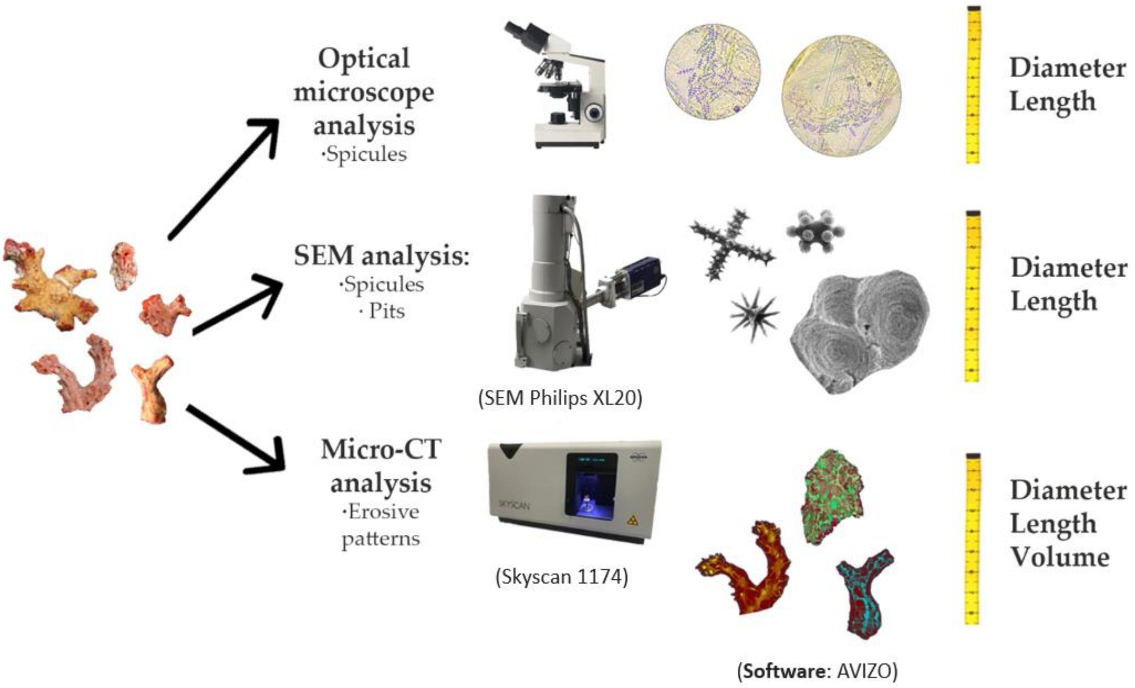

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Abstract

The carbonatic scleraxis of *Corallium rubrum* (L.), commonly known as red coral, is often found infested by excavating sponges. These boring organisms produce galleries inside the compact axis of the coral in a variety of shapes compromising the integrity of the skeleton and reducing its commercial value. Three sponge species, already known to bore into *Corallium rubrum*, have been identified in colonies collected from Cape Verde Archipelago—*Alectona millari* (Carter, 1879); *Dotona pulchella mediterranea* (Rosell and Uriz, 2002); and *Thoosa armata* (Topsent, 1888)—together with a new species belonging to the genus *Alectona* and here described. SEM analysis provided evidence of the microerosion patterns of these species, confirming the presence of radial scars overlapped with the concentric ones, in *T. armata*. For the first time, microcomputed tomography was employed to obtain three-dimensional reconstructions of sponge excavations inside the red coral scleraxis and to estimate the eroded volume.

Keywords: bioerosion, Porifera, *Alectona*, *Thoosa*, *Dotona*, new species, micro-CT, Cape Verde

1. Introduction

Boring sponges are among the main actors shaping coral reefs and calcareous substrates in general [1]. Impacts of sponge erosion are widely studied in tropical areas, while few data are available for temperate and mesophotic corals. In particular, regarding octocorals, the scleraxis of the precious corals belonging to the family Coralliidae from the Mediterranean Sea and the Pacific Ocean is frequently damaged by excavating sponges which produce galleries of various sizes and shapes [2,3,4], compromising the integrity of the skeleton, likely limiting colony stability and reducing its commercial value [5,6]. In particular, the red coral skeleton is the preferential substrate for several species belonging to the genera *Thoosa*, *Alectona*, and *Dotona* [4].

The genus *Alectona* is characterised by the presence of diactinal (sometimes polyactinal) spicules, fusiform amphiasters, and by the lack of tylostyles [7]. Its bioerosive activity produces erosion marks, the so-called pitting pattern, characterised by concentric grooves overlapped by radiating scars [8,9]. Species of Alectona, known so far as excavating into red coral, are 6 out of 11 [4,10]: *A. millari* (Carter, 1879); *A. sorrentini* (Bavestrello, Calcinai, Cerrano, and Sarà, 1998); *A. triradiata* (Lévi and Lévi, 1983); A. *wallichii* (Carter, 1874); *A. verticillata* (Johnson, 1899); and *A. sarai* (Calcinai, Cerrano, Iwasaki, and Bavestrello, 2008).

Dotona species are characterised by styles or strongyles as main spicules, and by spiny microstrongyles and diplasters as microscleres [7]. This genus includes two species, *D. pulchella* (Carter, 1880), which is known to excavate into the red coral [4], and *D. davidi* (Kirkpatrick, 1900), documented only inside the scleractinian coral *Echinopora* sp.

In the case of the genus *Thoosa*, tylostyles or oxeas may be present as megascleres (missing in some species), and amphiasters and oxyasters as typical microscleres. Out of 16 known species of *Thoosa* [10], *T. armata* (Topsent, 1888), *T. bulbosa* (Hancock, 1849), *T. midwayi* (Azzini et al. 2007), and *T. mollis* (Volz, 1939) have been recorded boring into the red coral from the Mediterranean Sea and the Indo-Pacific Ocean [4].

Until today, studies on boring sponges are mainly based on destructive methods to measure endolithic sponge biomass and consequently the volume of eroded substrate [11]. In recent years, microcomputed tomography (micro-CT) has begun to be used to analyse, describe, and quantify sponge erosion, avoiding the destruction of the material [11,12,13]. Erosion chambers are, in general, well-visualised with this technique but, depending on the resolution power of the CT scanner, fine details, such as pioneer filaments used by the sponge to go forward in the substrate, or erosion scars cannot be well-resolved [11].

In some colonies of red coral sampled at the Cape Verde Archipelago, we detected the presence of three already known excavating sponges together with a new species, here described. Coupled with the traditional taxonomic approaches, we propose the application of the micro-CT on the coral samples to observe the three-dimensional architecture of sponge erosion, obtaining a more complete and detailed morphological description of the chambers and the volume eroded inside the scleraxis.

2. Material and Methods

Five samples of *Corallium rubrum* (Linnaeus, 1758) collected at Cape Verde, kindly supplied by the Museo Civico di Storia Naturale di Genova (Genoa, Italy), were analysed. The collection consists of old colonies stored in the Museum, and data about the exact sampling location(s), depth(s), or date(s) of the collection are not available.

The sponge spicule complement was analysed according to Rützler [14]. Spicule size range, mean and standard deviation (SD) were obtained from 15 measurements per spicule type. Spicules were also analysed with a scanning electron microscope (SEM); thus, spicule dissociations were transferred onto stubs and sputtered with gold. Erosion chambers and excavation scars were also studied using both SEM and optical microscopy. For SEM observation, fragments of *C. rubrum* were put on stubs and sputtered with gold. Pit dimensions (maximal diameter, n = 15) were measured on SEM images using ImageJ 1.37, avoiding pioneer areas of the erosion chambers. The SEM studies were carried out using a Philips XL 20 SEM.

Sponges' erosive patterns were assessed via micro-CT. The scanning of the red coral samples was performed with the Skyscan 1174 of the Centro di Ricerca e Servizio di Microscopia delle Nanostrutture (CISMiN) at the Università Politecnica delle Marche (Italy). The tomograph was equipped with an X-ray source (20–50 kV) and a flat panel detector with 1304×1024 pixels. Additionally, an aluminium filter was placed between the sample and the X-ray source to maximise the energy transmission through the sample. During scanning, the X-ray source was set at 50 kV and 800 µA, and the sample was rotated a total of 180° by 0.36° angular increments, acquiring an X-ray absorption radiograph at each rotation step. Raw data were preprocessed with cone-beam reconstruction software (Nrecon v.1.7.3.1., Skyscan), resulting in a series of axial cross-sections with a range of pixel sizes from 9.6 to 28.5 µm.

Postprocessing of the cross-sections was conducted using the AMIRA-Avizo software by Thermo Fisher Scientific. Firstly, a 3D-mode iterative median filter was applied to reduce image noise. Then, sponge cavities were segmented from the coral substrate by the following workflow: (1) the interactive thresholding module binarised the cross-sections, defining the coral skeleton as solid material; (2) the production of "digital casts" from the empty cavities inside the coral were obtained applying ambient occlusion module over the binarised images allowed; (3) a second interactive thresholding module was implemented to calculate the eroded volumes; (4) lastly, species designation to the digital casts were validated by coupling spicules and pits observations.

All dimension metrics extracted from the three-dimensional reconstructions were obtained with the help of the axial cross-sections and the measurements module. When a single sponge species was present inside the sample, the volume eroded by the sponge was calculated; when it was not possible to assign the erosion traces at the level of the single species, the volume of sponge erosion was expressed as the total erosion volume referring to all of the sponges present in the sample. Three-dimensional models of the erosive patterns obtained from the five analysed samples are provided as Supplementary Material in File S1, and metrics from the eroded traces are presented in Table S1.

The type material of the new species, together with the entire collection, is deposited at the Museo Civico di Storia Naturale "G. Doria" of Genova (MSNG), Italy.

3. Results

3.1.Erosion Pattern and Excavated Volutme

Four species of boring sponges—*Alectona millari*, *Alectona ricardi* sp. nov., *Thoosa armata*, and *Dotona pulchella mediterranea*—were detected in the five samples of *Corallium rubrum* and herein described (Figure 1, Figure 2, Figure 3, Figure 4, Figure 5, Figure 6, Figure 7, Figure 8 and Figure 9). The coral samples presented different percentages of eroded volume. In the first sample (CT3, Figure 1), D. pulchella mediterranea and *A. millari* were detected, eroding 40.15% of the total volume of the sample. In the second sample (CTX, Figure 4), three species were identified: A branch of CTX was excavated by *D. pulchella mediterranea* and *A. ricardi* sp. nov. (Figure 4b–d) that, in the complex, were responsible for the erosion of 22.14% of the total volume, while the second branch included *D. pulchella mediterranea* and *A. millari* (Figure 1d–f) that eroded up to the 30.40% of the volume. The third and the fourth samples (CT5 and CT6, Figure 5) were exclusively bored by *T. armata*, eroding 20.08% and 13.48% of the samples' volume, respectively. Finally, the fifth sample (CT2, Figure 8) was exclusively excavated by *D. pulchella* mediterranea, which removed 7.79% of scleraxis.

3.2.Taxonomy

Order Tetractinellida Marshall, 1876
Suborder Thoosina Carballo, Bautista-Guerrero, Cárdenas, Cruz-Barraza, and Aguilar-Camacho, 2018
Family Thoosidae Cockerell, 1925
Genus *Alectona* Carter, 1879
Alectona millari Carter, 1879
Material

This sponge was present in a small red coral sample (CT3, Figure 1a) of 1.7 cm high and 0.8 cm in diameter, together *with D. pulchella mediterranea*, and in a second larger branched colony of *C. rubrum* (CTX, Figure 1d) of about 5.5 cm high and 2.5 cm in diameter at its base, where *D. pulchella mediterranea* and *A. ricardi* sp. nov. were also detected.

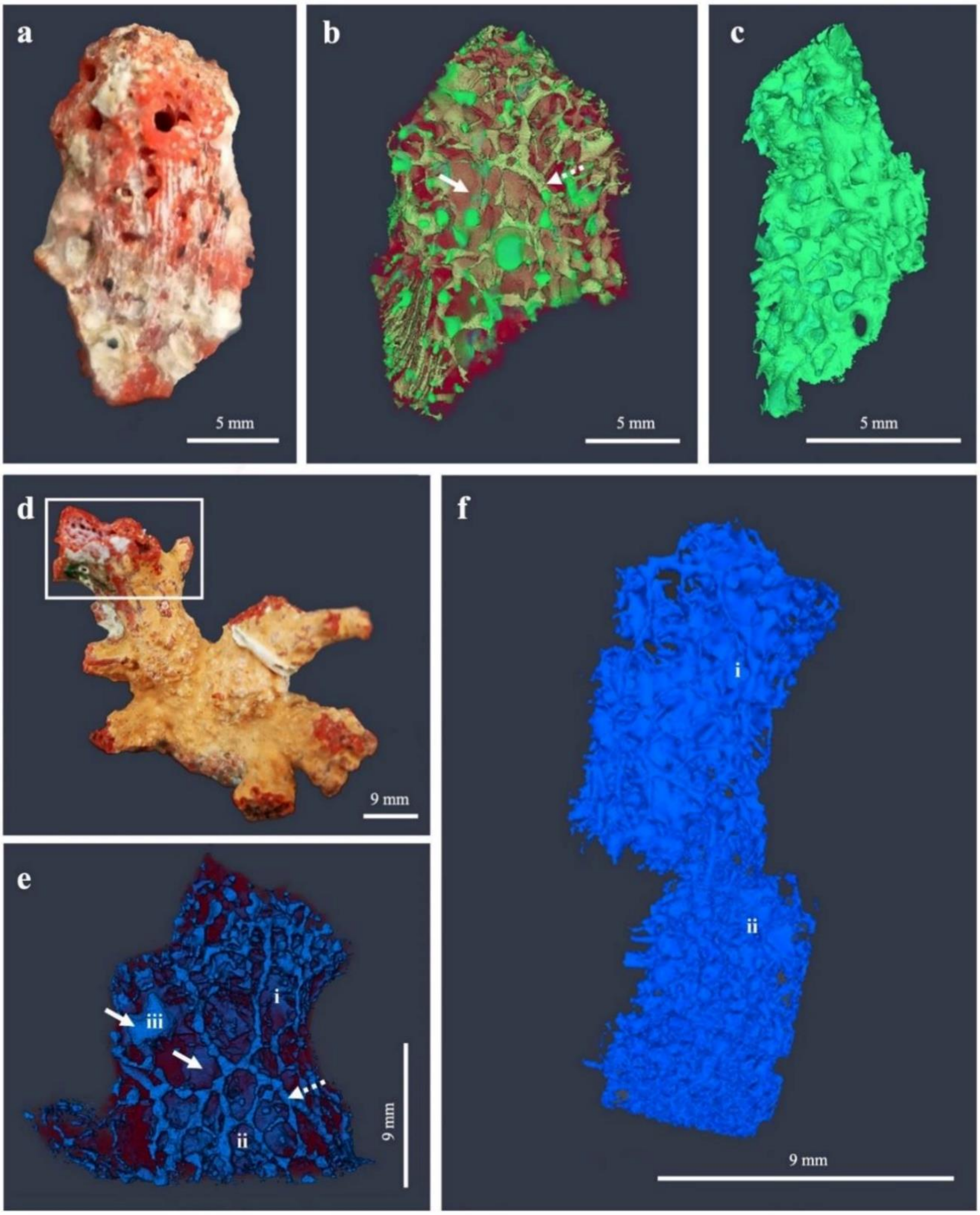

Figure 1. Three-dimensional visualisation of the red coral samples excavated by *Alectona millari*, from micro-CT reconstructions: (a) red coral sample CT3 showing the sponge papillae; (b) eroded cavities are in green and coral in transparent red, the continuous arrow points to the chamber of *A. millari*, surrounded by the chambers of *Dotona pulchella mediterranea* (dotted arrow); (c) system of cavities of *A. millari* after a manual cleaning of most of *D. pulchella mediterranea* surrounding erosive activity; (d) red coral sample CTX; the area inside the rectangle is the location where the scanning efforts were focused; (e) digital cast showing the sponge eroded systems in blue and the coral skeleton in transparent red, the continuous arrows mark two of the chambers of *A. millari*, while the dotted arrow marks the chambers of *D. pulchella mediterranea*; (f) erosive activity of *A. millari* after a manual cleaning of *D. pulchella mediterranea* erosive cavities. Chambers i and ii correspond to the ones shown in (e).

Taxonomic description

Spicules: Long acanthoxeas sometimes are shown as bent or curved, with blunt spines at the apex, scattered along the shaft (Figure 2a–d). Some spicules are only slightly spiny (Figure 2c), while others are bifurcated (Figure 2e). Measurements are 225 (245.5 ± 10.1) 260 × 15 (19.4 ± 2.5) 22.5 µm. Smooth oxeas sometimes have a central knob or are curved (Figure 2f,g). Measurements are 165 (198 ± 19) 225 × 12 (14.6 ± 2.2) 18 µm. Amphiasters are characterised by spiny knobs and are irregularly arranged (Figure 2h–k). Some are thinner and short with pointed extremities. Measurements are 20 (44 ± 14.4) 65 µm. Numerous armed larvae were detected inside the chambers (Figure 2l). A layer of discotriaenes covers the larva with sinuous, thin styles (450 (534.1 ± 93.8) 664 × 2.5−5 µm) radiating from its centre (Figure 2l,m). The discotriaenes are flattened and triangular (Figure 2l,n,o); measurements of the diameter are 107.5 (125 ± 9.8) and 137.5 µm. The outer surface has, sometimes, a sort of triangular depression and is rough (Figure 2l,n), while the inner surface is convex and smooth, with a central, short rhabd (Figure 2o).

Erosion pattern: *A. millari* developed mainly in the central section of the coral axis, excavating large subspherical erosion chambers. In the first sample (CT3, Figure 1a) where *A. millari* was present along with *D. pulchella mediterranea* (Figure 1b; File S1), the only detected chamber was 5.19 × 11.13 mm wide (Figure 1c), while in the sample where *A. millari* shared the substrate with *D. pulchella mediterranea* and *A. ricardi* sp. nov., it was possible to recognise three chambers: a small one of 1.38 mm × 2.89 mm and two large irregular cavities 4.9–5.3 × 13–14 mm separated from each other by a short connecting duct of 2 mm in length (Figure 1e,f; File S1; Table S1). Only in one sample (CTX) was it possible to detect papillary channels of 0.13–1 mm in length, reaching the coral surface through papillae of 0.12 to 0.30 mm in diameter (Table S1).

The SEM analysis of the walls of the erosion chambers showed pits of 77.5 ± 10.8 µm decorated with concentric and radial lines (Figure 3a).

Remarks This species has been described as boring into several kinds of substrates such as scleractinians, shells, rocks, and red coral, as reported in, for instance, [4,17]. The description of the *A. millari* cavities system obtained via micro-CT observations agreed with the literature [17], where the erosion pattern is described as a network of massive central chambers from which pioneer filaments extend. Among our samples, this is the species that presented a wider variation in terms of chamber morphology, possibly due to the co-occurrence with two other sponge species in the same colony (Figure 1b,e); in fact, it has been documented for other boring sponges that their characteristic erosive pattern may be strongly modified by the interactions with other bioeroders competing for the same substrate [13,18,19].

No description of ichnotaxa holding similar erosion patterns was found in the literature.

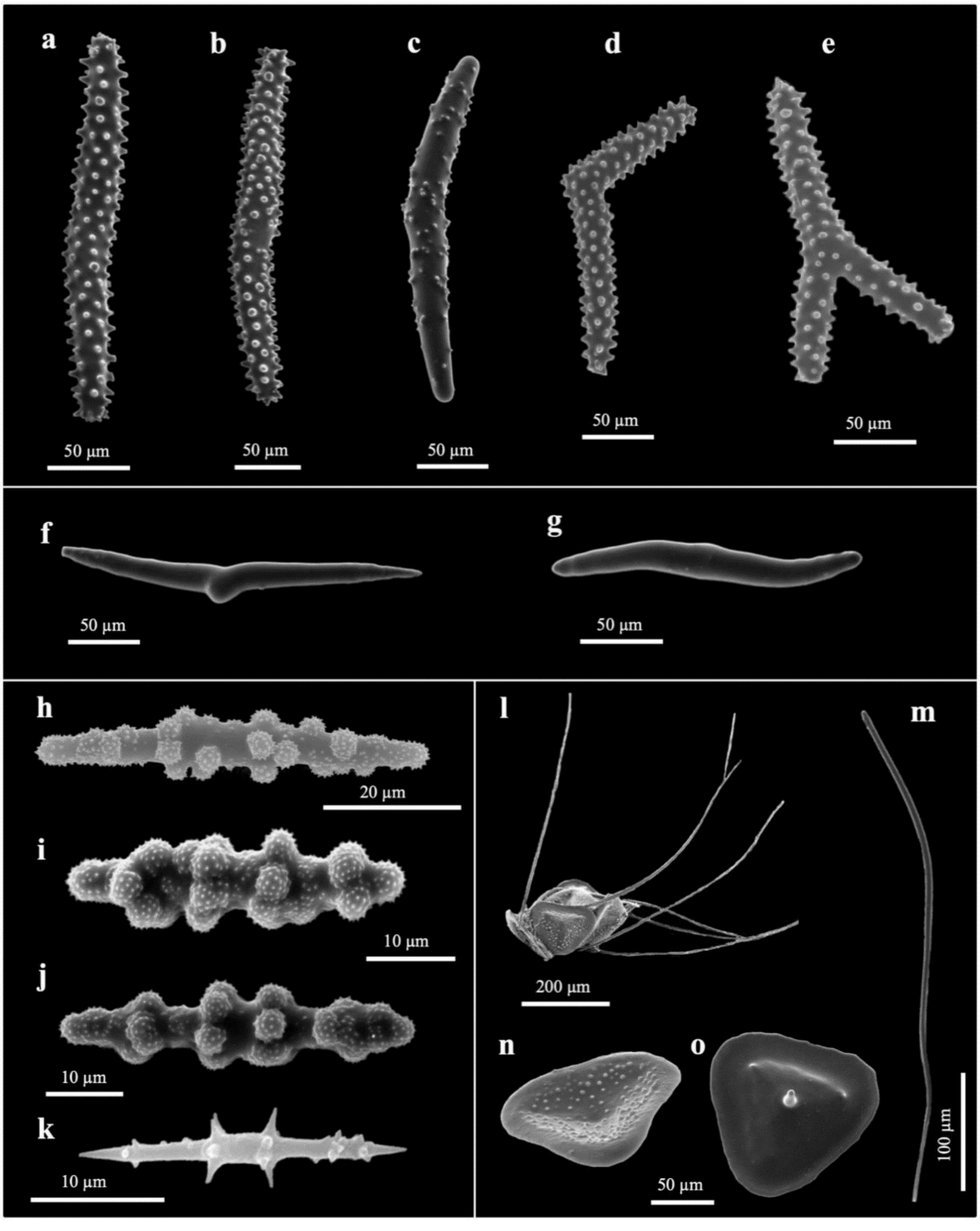

Figure 2. Spicule composition of *Alectona millari*. SEM images: (a–d) long acanthoxeas, with flattened spines scattered along the shaft; (e) acanthoxea showing a lateral ray, with a similar appearance to that of spined triactine; (f,g) curved, small, and smooth oxeas; (h–k) irregular spiny amphiasters; (l) armed larva; (m) thin sinuous style from larva surface; (n,o) outer and inner faces of the discotriaenes covering the larvae, respectively.

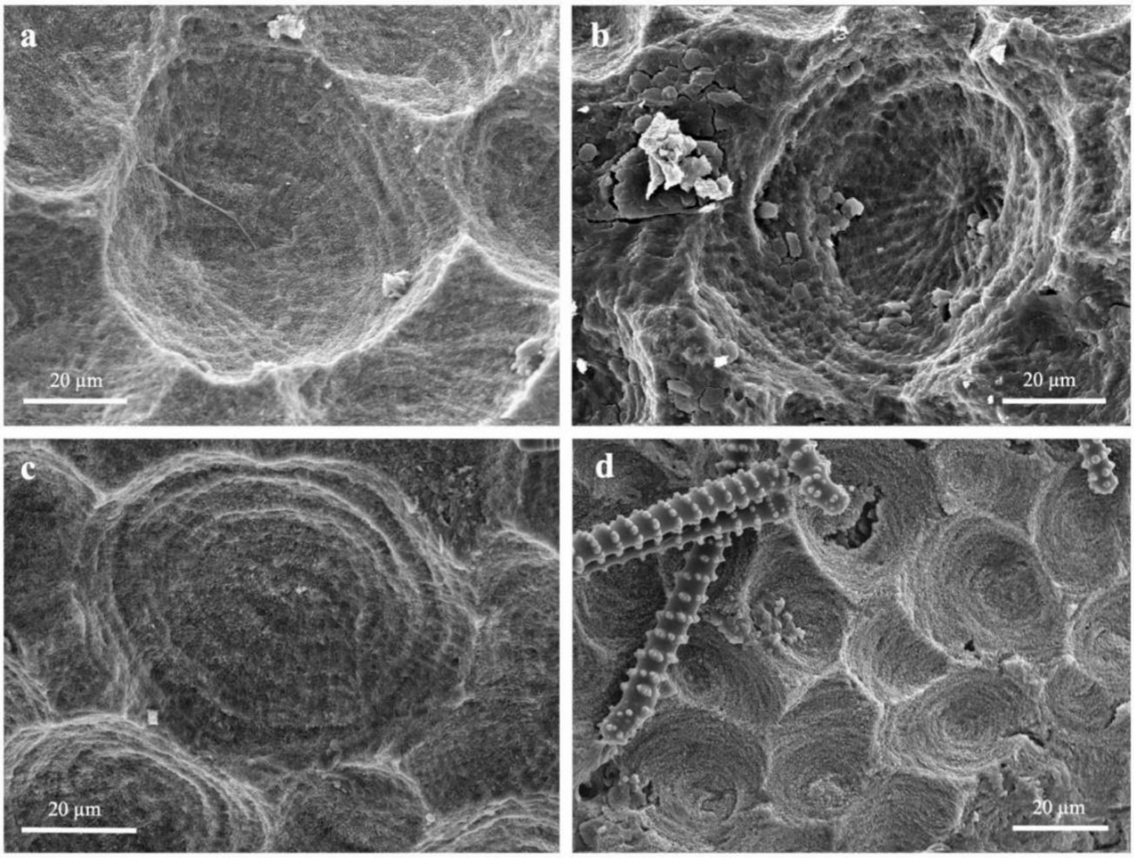

Figure 3. SEM images of sponge-generated bioerosion chambers, showing in the walls the characteristic pits of the different species recorded in the red coral samples: (a) *Alectona millari*; (b) *A. ricardi* sp. nov.; (c) *Thoosa armata*; (d) *Dotona pulchella mediterranea*. In c, the presence of radial scars superimposed on concentric lines was previously only reported by [15,16].

Order Tetractinellida Marshall, 1876
Suborder Thoosina Carballo, Bautista-Guerrero, Cárdenas, Cruz-Barraza, and Aguilar-Camacho, 2018
Family Thoosidae Cockerell, 1925
Genus *Alectona* Carter, 1879
Alectona ricardi Calcinai and Bertolino sp. nov.
urn:lsid:zoobank.org:pub:5A5AAF18-5929-4938-A6B0-D86B570FCED0

Diagnosis

This species is characterised by spiny triactines and thin nodular amphiasters (Figure 5).

Material Holotype: MSNG 62433.

Alectona ricardi sp. nov. occupied one of the ramifications of a *C. rubrum* colony of about 5.5 × 2.5 cm (CTX, Figure 4a), along with *A. millari* and *D. pulchella mediterranea* (Figure 4b). No data about sampling coordinates and depth are available.

Taxonomic description

Spicules: Triactines (Figure 5a–e) are covered by long simple or compound spines arranged in verticils along the rays. Some spicules show reduced spines or are completely smooth (Figure 5d,e). Rays are generally straight, but triactines with one or more curved rays are also common (Figure 5a,e). Some rare forms with four rays are also present (Figure 5f). Ray measurements: 75 (86.3 ± 6.6) 100 × 17.5 (20.9 ± 3.5) 25 µm. Nodular amphiasters have six short rays organised in verticils (Figure 5g,h); spines are organised in knobs at the extremities. The axis is 15 (20 ± 4) 25 µm long and the rays are 2.5 (5 ± 1.4) 7.5 µm. Longer and thinner amphiasters (Figure 5h) have short spines scattered along the rays and are more gathered at the extremities. The axis is 21 (25.3 ± 4) 32.5 µm long, and the short, conical, spiny rays are 7.5 (10 ± 2.8) 12.5 µm. Rare, very small, straight amphiasters with spines concentrated at the extremities and rare spines along the shaft (Figure 5i), about 7.5 µm long.

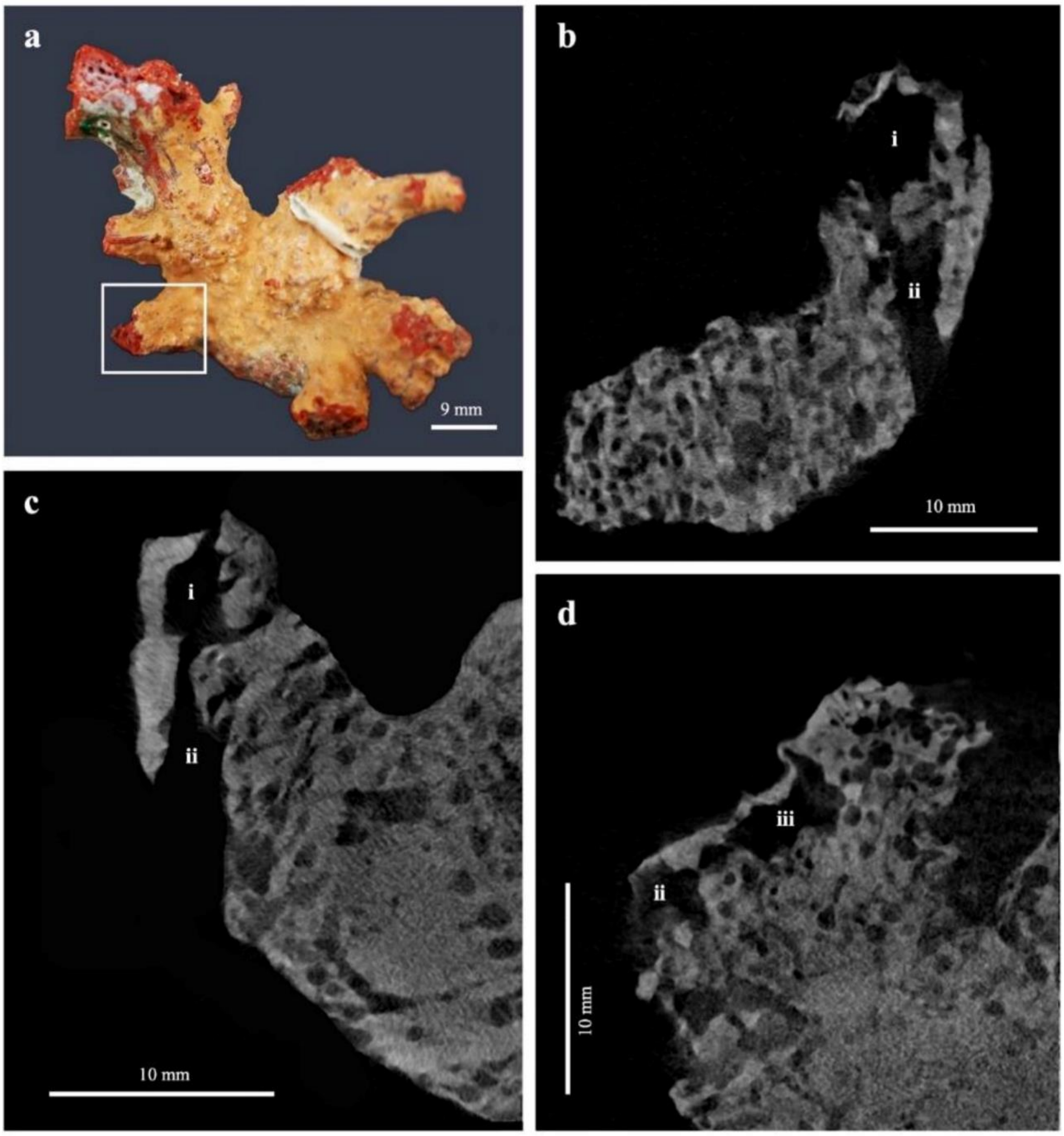

Figure 4. Red coral sample excavated by *Alectona ricardi* sp. nov. along with cross-sections from micro-CT scans: (a) red coral sample CTX; the marked area is the part of the sample where the new species was detected; (b) perpendicular (xz) cross-section showing two of the three cameras (i,ii) of A. ricardi sp. nov.; (c) perpendicular cross-section (xy) showing the same two cameras (i,ii) with a different perspective; (d) transversal cross-section (yz) showing the second and the third cameras (ii,iii) of this species. The camera occupying the more basal part of the ramification (ii) resulted in an opening to the exterior due to secondary erosion.

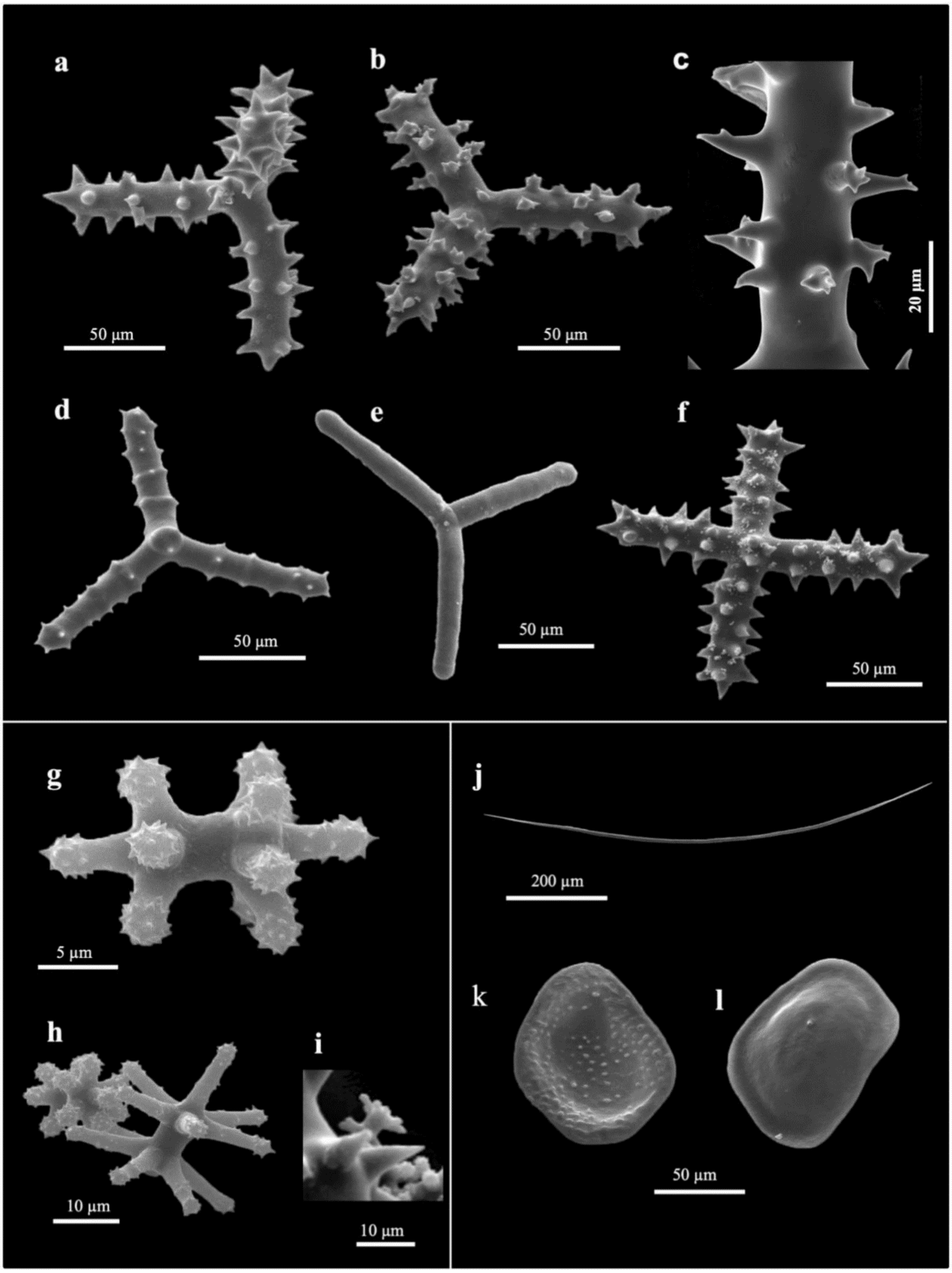

Figure 5. Spicule composition of *Alectona ricardi* sp. nov. present in the red coral sample. SEM images: (a,b) triactines covered by triangular spines; (c) magnification of a ray showing the compound spines; (d) slightly spined triactine with spines clearly organised in verticils; (e) smooth triactine; (f) triactine with four rays; (g) nodular amphiaster; (h) nodular amphiaster (in the background) and thinner amphiaster; (i) very small amphiaster; (j) oxea of an armed larva; (k) outer surface of a quadrangular discotriaene; (l) inner surface of a quadrangular discotriaene.

In the tissue, numerous armed larvae covered with discotriaenes (Figure 5k,l) and slightly curved oxeas (Figure 5j), often with rounded extremities were found (several broken, n = 4, 740–1230 µm). The discotriaenes are flattened and with an irregular quadrangular shape and measure 82.5 (94.6 ± 10.8) 112.5 µm. The outer surface has a depression with a rough surface (Figure 5k), while the inner surface is smooth and convex (Figure 5l).

Erosion pattern: This species eroded mainly the central section of the coral ramification (Figure 4b), excavating three large irregular cavities 2.4–5.1 × 4.1–7.8 mm separated from each other by a relatively short connecting duct of 2.9 ± 0.25 mm in length (Figure 4c,d; File S1; Table S1). From the cavities, some papillary channels, 0.4–0.9 mm long, developed to the coral surface opening in papillae, with diameters ranging from 0.1 to 0.3 mm (Table S1). SEM observation of the chambers provided evidence of the presence of the typical pits with superimposed radial etchings usually produced by the species of the genus Alectona (Figure 3b). The diameter of the pits is on average (±SD) 53.3 ± 8.6 µm (Table S1).

Remarks The spicule set, and in particular the presence of triactines, is unusual for species of this genus, and it has been found exclusively in *Alectona triradiata* (Lévi and Lévi, 1983), described for the Pacific Ocean also boring in the scleraxis of *Pleurocorallium elatius* (Ridley, 1882) [20]. The new species differs from *A. triradiata* in having characteristic nodular amphiasters *Thoosa*-like and in the absence of thin, slender amphiasters, with pointed extremities.

Etymology It is named after prof. Riccardo Cattaneo-Vietti, who prematurely passed away, in consideration of his research on the Mediterranean red coral.

Order Tetractinellida Marshall, 1876
Suborder Thoosina Carballo, Bautista-Guerrero, Cárdenas, Cruz-Barraza, and Aguilar-Camacho, 2018
Family Thoosidae Cockerell, 1925
Genus *Thoosa* Hancock, 1849
Thoosa armata Topsent, 1888

Material

Two small fragments of *C. rubrum* 2.1 × 0.5 cm and 1.5 × 1 cm, respectively (CT5, Figure 6d), were infested by this species.

Taxonomic description

Spicules: Small nodular amphiasters have two verticils of six, spiny knobs (Figure 7a) sometimes with thinner axis and longer rays (Figure 7b); measurements are 10.9 (24.2 ± 7.8) 38.4 × 2.3 (8 ± 4.1) 14.2 µm. Large amphiasters with six, straight, thin, and long rays are arranged in two verticils; spines are grouped at the extremities giving them a knob-like appearance (Figure 7c). Some spines are also scattered along the axis and the rays. Measurements are 40 (49.6 ± 7.2)

59.5 × 2-4 µm. Larger amphiasters, with six, straight, long, and thick rays are arranged in two verticils and end with pointed tips surrounded by small spines (Figure 7d). Measurements are 41.5 (51.5 ± 5.2) 66 × about 14 µm. Oxyasters, biradiate (Figure 7e), sometimes have three- or four-radiated (Figure 7f,g), are wing-like, ending with pointed tips and presenting a central swelling. The rays measure 40 (56.2 ± 9.1) 74 × about 2–3 µm. Ovoid accessory microscleres (Figure 7h); their longer diameter measure 18–28.2 µm (only four measured).

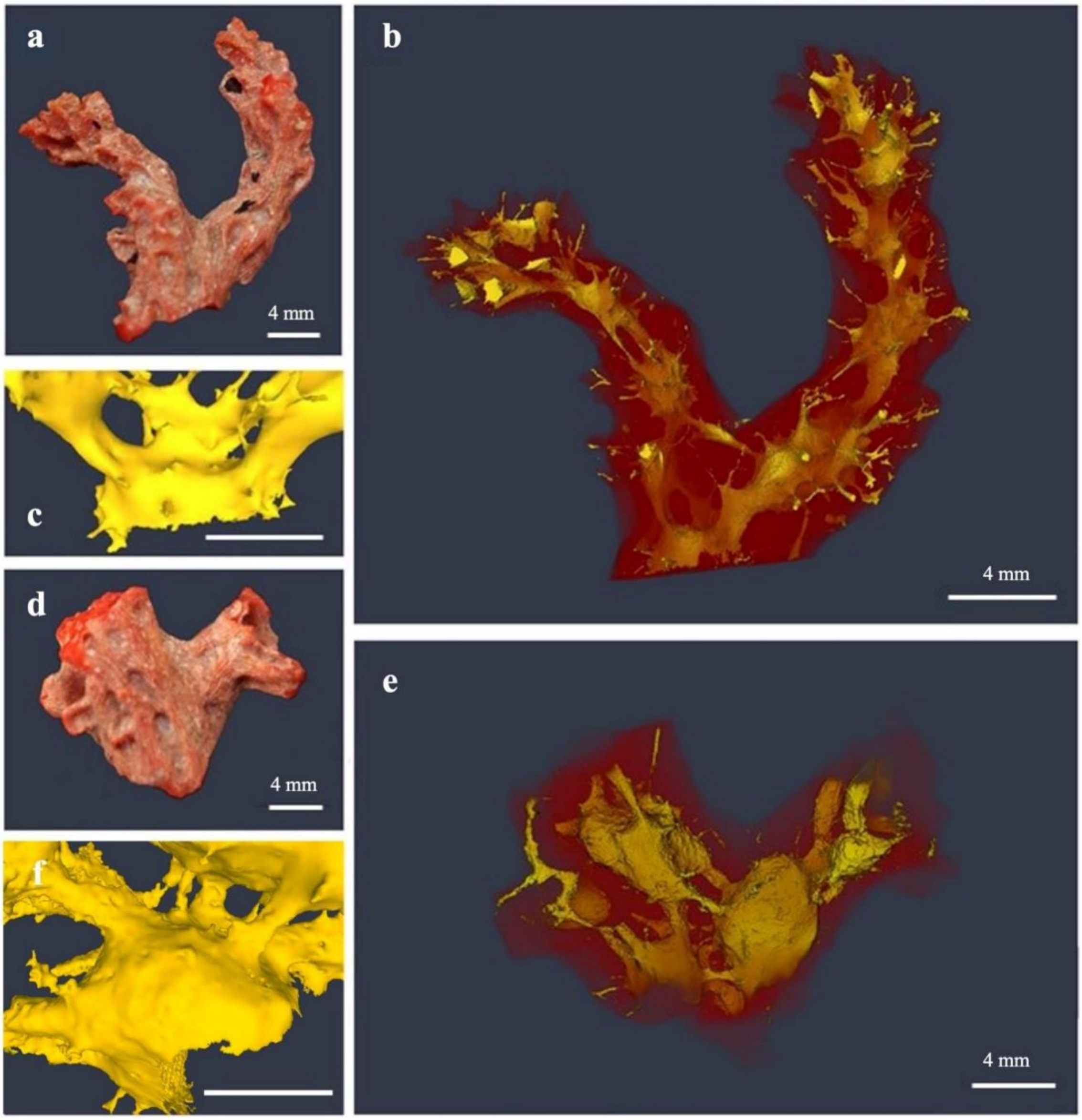

Figure 6. Three-dimensional visualisation of the two red coral samples excavated by *Thoosa armata* from micro-CT reconstructions: (a,d) red coral samples CT6 and CT5, respectively; (b,e) eroded cavities represented in yellow and coral in transparent red; (c,f) details of T. armata erosive pattern. Scale bars in (c,f) equal to 2 mm.

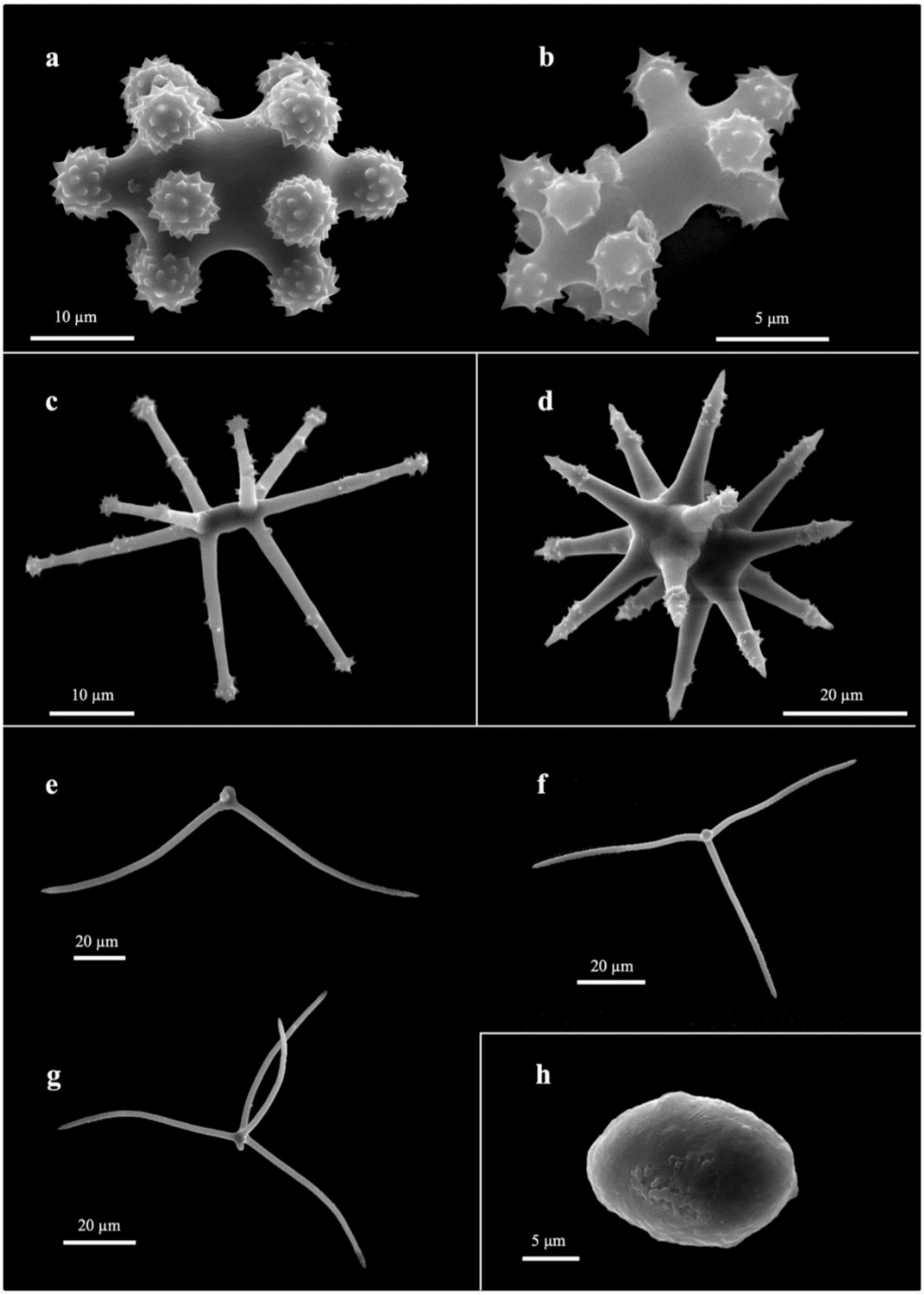

Figure 7. Spicule composition of *Thoosa armata*. SEM images: (a) typical nodular amphiaster; (b) nodular amphiaster with thinner axis; (c) amphiaster with six, straight and long rays arranged in two verticils; (d) larger amphiaster with pointed tips surrounded by small spines; (e) biradiate oxyaster; (f) biradiate oxyaster becoming triradiate; (g) oxyaster with four rays; (h) ovoid accessory microsclere.

Erosion pattern: *T. armata* eroded mainly the central section of the coral axis, producing irregular chambers from ellipsoidal to subspherical shapes, with diameters ranging from 1.8 to 3.9 mm, and lengths from 0.6 to 1.8 mm (Figure 6b,e; File S1; Table S1). Cameras were connected by numerous short ducts of 1.3 ± 0.4 mm in length, often found fused together, presenting thicker eight-shaped channels (Figure 6c,f; Table S1). Abundant papillae were observed on micro-CT reconstructions, with a diameter of 0.09–0.2 mm; papillary channels were 0.57–1.58 mm long (Table S1). In the SEM images, the walls of the erosion chambers showed ovoid pits of diameters of 60 ± 9 µm, on average (±SD), and with the surface incised by concentric marks and superimposed radial incisions (Figure 3c; Table S1).

Remarks: *T. armata* has been described, for the first time, in Gabon (West Africa), and our sample fully agrees with the original description. This species showed erosion scars characterised by circular and radial ornamentations typical of the species of the genus *Alectona*, as already reported in the same samples by Pica et al. [15] and Carballo et al. [16] in *Thoosa* spp. from the Mexican Pacific Ocean.

Morphometrics and erosive patterns obtained from Thoosa were compared with previously described ichnotaxa, and among these, the trace marker of *Entobia geometrica* (Bromley and d'Alessandro, 1984) resembled those produced by the living species *T. armata.* The traces left by this species fit the typical description of *E. geometrica* [18] in the size and shape of its erosive patterns; additionally, in our samples, it is possible to recognise the growth phases B and C, stages characterised by oval to subspherical shaped chambers (size from 1.0 to 3.7 mm) (Figure 6b,e), connected by two-to-three connecting ducts (Figure 6c,f), frequently fused together (Figure 6c), and presenting numerous pioneer filaments (Figure 6a).

Order Clionaida Morrow and Cárdenas, 2015
Family Clionaidae d'Orbigny, 1851
Genus *Dotona* Carter, 1880
Dotona pulchella mediterranea Rosell and Uriz, 2002

Material

Three different fragments of *C. rubrum* were infested by this species. One fragments (CT2, Figure 8), 1.5 cm high and 0.6 cm in its basal diameter, was exclusively bored by D. pulchella mediterranea; a second colony 5.5 cm high with a diameter of 2.5 cm at its base, was also excavated by *A. millari* and *A. ricardi* sp. nov. (CTX, Figure 1d and Figure 4a; File S1). In the third sample (CT3, Figure 1a; File S1), 1.7 cm in height and 0.8 cm in diameter, *A. millari* was also present.

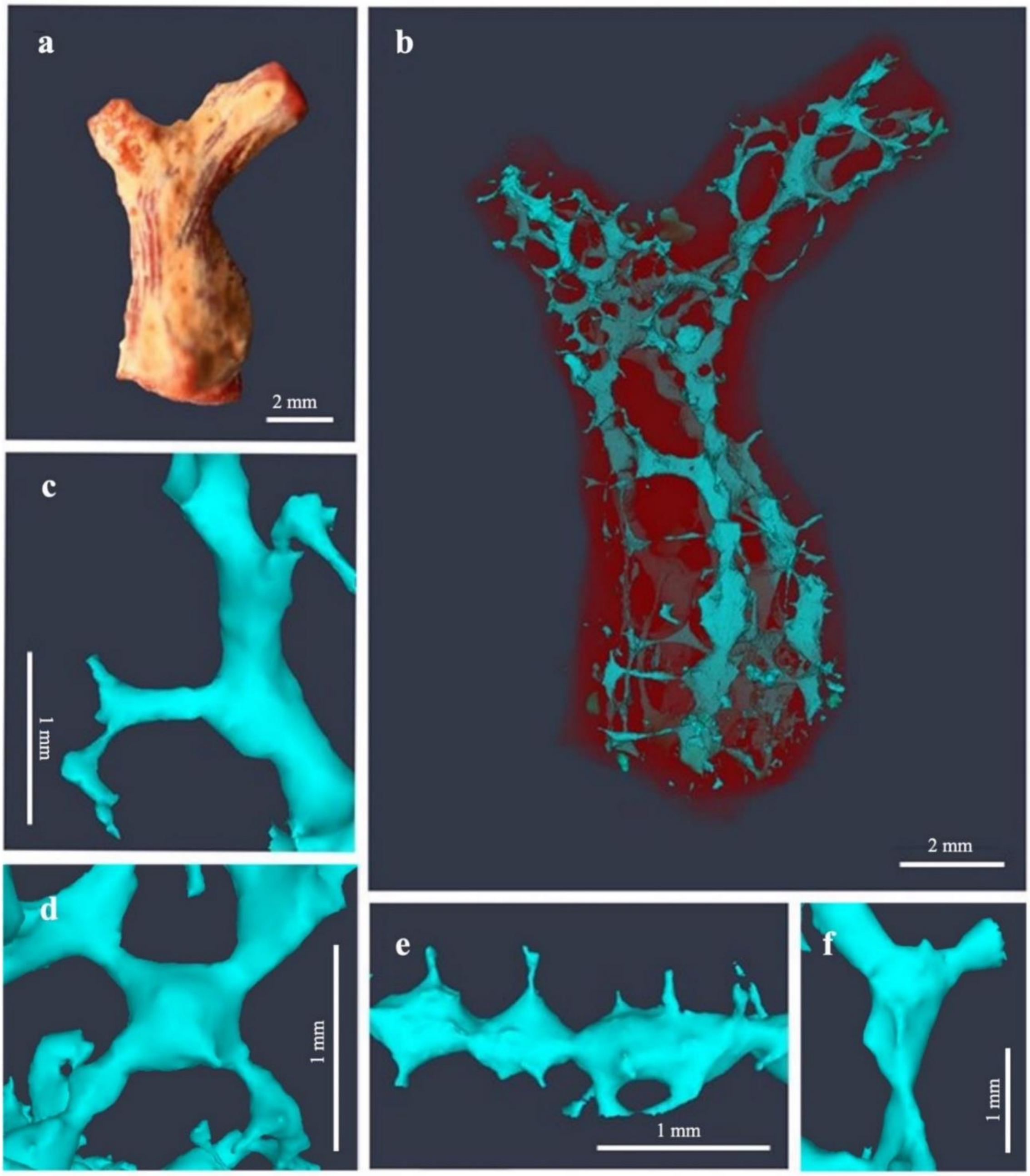

Figure 8. Three-dimensional visualisation of the red coral sample excavated by *Dotona pulchella mediterranea*, from micro-CT reconstructions: (a) red coral sample CT2; (b) *D. pulchella mediterranea* cavities represented in blue and coral in transparent red; (c–e) different shape of chamber intersections: L, X, and T-shaped, respectively; (f) moniliform perforation pattern produced by *D. pulchella mediterranea*.

Taxonomic description

Spicules: Microstrongyles with rounded ends and microspined tubercles are spirally arranged. They are often curved or bent especially towards one extremity (Figure 9a–c). They measure 48.8 (63.6 ± 6.8) 72.5 × 5 (6.1 ± 0.7) 7.5 µm, with very thin styles having 95 (108.5 ± 12.3) 117.5 x <2 µm (Figure 9d).

Spiny diplasters measure 12.5 ± 15 µm (only four detected and measured, not illustrated in Figure 9).

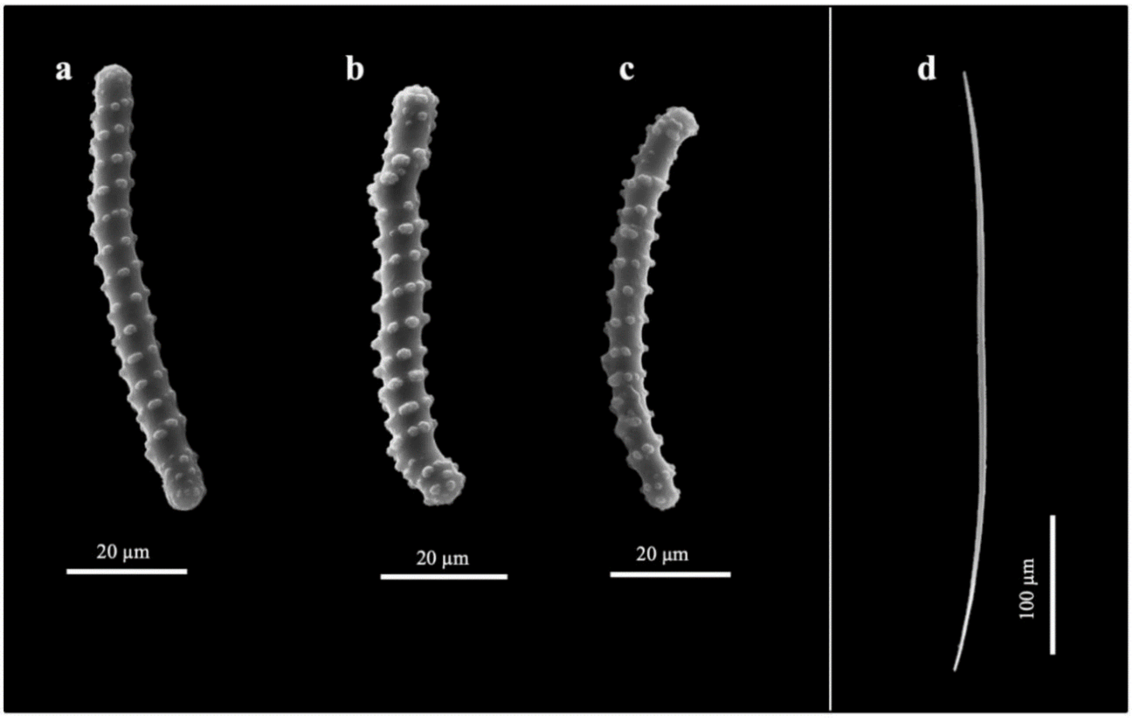

Figure 9. Spicule composition of *Dotona pulchella mediterranea*. SEM images: (a–c) microstrongyles with microspined tubercles, spirally arranged; (d) style.

Erosion pattern: In all samples, the erosion of *D. pulchella mediterranea* developed mainly in the external part of the coral skeleton, with a moniliform perforation pattern (Figure 8b,e), characterised by numerous chambers organised in strings, and connected by constrictions in advanced erosion phases or long thin connecting ducts in the initial stages; commonly, these intersections present an L-, X-, or T-shape (Figure 8c–f). The species produced chambers measuring 0.6–0.9 × 1.5–9.56 mm (Table S1). Papillae were not observed. SEM analysis showed small pits characterised by concentric incisions, which are more evident towards the peripheral part and less evident in the centre; they have an averaged diameter (±SD) of 31.2 ± 6.5 µm (Table S1).

Remarks: Our specimen fits the description of the subspecies from the Mediterranean [21], differing only in the absence of macroscleres.

From the comparison of the erosive patterns and dimensions with the ichnotaxa described in the literature, we found great similarities between *D. pulchella mediterranea* and *Entobia cateniformis* (Bromley and d'Alessandro, 1984), in which systems were reported as a complex of cylindrically elongated chambers, connected by constrictions and organised in rows with different types of intersections. In the present samples eroded by

D. pulchella mediterranea, it was possible to identify resemblances within all *E. cateniformis* growth phases (A-D)—from the thin pioneer filaments characteristic of phase A to the network of interweaving chains from phase D, passing through the intermediate phases defined by the development of the cylindrical chains and the creation of the intersections (T, L, X) by anastomosis and dichotomy [16].

4. General Remarks

Traditionally, studies on the three-dimensional architecture of sponge bioerosion have been implemented by the application of invasive methods, mainly based on substrate removal [22,23]. Here, for the first time, the bioerosion in the red coral was approached via micro-CT to better understand the development of channels and chambers of the sponge inside the coral scleraxis and to quantify the percentage of the eroded substrate. The erosion activity here evaluated varied in the examined species. *Dotona pulchella mediterranea* removed a lower percentage of coral scleraxis (7.79%) than Thoosa armata (20.08% and 13.48%), a sponge usually producing chambers larger than *D. pulchella mediterranea*. Unfortunately, it was not possible to extrapolate the percentage of substrate removed by the single Alectona species but to calculate only the total eroded volume, due to the close presence of several species in the same coral sample, producing an intricate boring system. Using micro-CT, Schönberg and Shields [11] evaluated the bioerosion in the spar produced by *Cliona celata* (Grant, 1826) and *Cliona orientalis* (Thiele 1900), two Australian boring species belonging to the same family as Dotona, obtaining higher values (up to 48%) of the eroded substrate. The different value of bioerosion is not surprising, considering that it strongly varies in function of the species, the nature of the substrate, its growth phase/age, and the different environmental conditions to which samples were subjected [24]. Moreover, the limited size of the substrate (as is the case of the studied samples), and the presence of several species excavating the same sample, affect the erosion activity of the boring species [16]. In fact, *A. millari* excavated chambers of different sizes and shapes, even inside the same sample (CTX, Figure 1 and Figure 4; Table S1), which is probably a consequence of the competition among the boring sponges for the available substrate. These alternative patterns have been previously defined as stenomorphic boring patterns [16], occurring when a sponge is constricted by physical substrate restrictions [15]. In addition, galleries produced by *A. millari*, *A. ricardi* sp. nov. and *D. pulchella mediterranea* seemed to merge in some areas, even though boring sponges are believed to avoid contact within the same substrate [25]. The observed pattern may be related to the different moments of colonisation after the primary chambers have remained empty, as it has been already observed for other clionaid species, which colonised and enlarged empty cavities during their development into the substrate [26].

Based on the three-dimensional reconstructions of the sponge chambers' systems and morphometric data extracted from the samples, our results were compared with the descriptions of different *Entobia* species (i.e., sponge bioerosion traces). The change in boring behaviour of some species during their growth, the wide range of factors influencing it, and the similar erosive patterns produced by different species make it difficult to attribute a boring pattern to a single species. Throughout the literature, many Entobia have been attributed to different species of clionaids considering the morphological characteristics of the cavities [17]; an example is *Entobia cateniformis*, ichnospecies firstly attributed to *Cliona vermifera* (Hancock, 1867) by Bromley and D'Alessandro [17], while Färber et al. [13] assigned it to *Cliona schmidtii* (Ridley, 1881), and more recently, Turicchia et al. [27] attributed it to *Cliona viridis*. Conversely, our results in this study suggested *E. cateniformis* as a possible trace marker for *D. pulchella mediterranea*.

The analysis and description of endolithic bioerosion patterns have always been challenging tasks. Currently, owing to the application of innovative techniques such as micro-CT, new possibilities arise, allowing researchers to record, from a three-dimensional perspective, the erosive traces present in the substrate. Through this process, a scaled digital cast is obtained, facilitating the storage and sharing of erosive patterns. The development of public repositories to properly store, categorise, and virtually manipulate these casts should be promoted, contributing to the shift from two- to three-dimensional approaches in bioerosion studies. In addition, the creation of labelled three-dimensional database of erosive patterns can be useful for the development of tailored algorithms, which would help in the close future with the semi-automated segmentation of erosive patterns, especially when more than one entity is present in the same sample.

Even though micro-CT represents an innovative tool in the study of boring organisms, it is important to acknowledge its limitations: (1) the need for a powerful scanner to approach very small features (<10 µm) as, for example, papillae channels, sponge spicules, pioneer filaments or chips; and (2) the separation of erosion traces of different sponges when more than one species is present in a sample (as observed in samples CT3 and CTX; Figure 1 and Figure 4). In our case, resolution restrictions were overcome by the coupling of SEM observations to the study, while, by analysing the spicule set present in the traces left behind by sponges, together with the characterisation of the pits eroded in the chamber walls, it was possible to assign the erosive systems to each of the species identified inside the coral scleraxis.

A remarkably high number of species have been recorded so far infesting the red coral scleraxis [28], increasing habitat biodiversity [6,29] but reducing the commercial value of the coral skeletons on the jewellery market [5]. In the following decades, a general increment of the bioeroders' activity is expected, caused by the warming trend, along with the organic pollution affecting coastal areas [30,31]. Despite the apparently low percentage of skeleton removal, the analysed boring sponges may compromise, both the economic value and the coral structural integrity. The impact of boring sponges was already observed in tropical reefs, where they promote scleractinian corals fragmentation [32,33]. Also in temperate habitats, despite their lower bioerosion rates, boring sponges represent the main corals' macroborers, affecting corals' adhesion and persistence on the substrate, likely facilitating their detachment [1,34,35]. In the Mediterranean Sea, where *Corallium rubrum* is endemic and generally grows on coralligenous bioconstruction, it has been documented that the sponge diversity is higher inside respect outside in coralligenous accretions [36]. In this habitat, boring sponges can be considered as intermediate disturbers, shaping coralligenous assemblages' composition and complexity [34], the same action they perform in coral reefs. The three-dimensional structure and the architectural complexity of coralligenous epibenthic assemblages may be considered an effective proxy of coralligenous health status [37], the level of boring sponge infestation of erect calcareous colonies can clearly affect the long-term stability of this vertical layer. As for tropical reefs, the variability in bioerosion rates and bioeroder abundances severely impacts many reef attributes (i.e., habitat heterogeneity, net reef accretion, and framework integrity) [1]. Therefore, the mapping of the epi- and endolithic organisms can represent a useful tool in the monitoring and definition of coral reefs' ecological status [15].

To conclude, considering all these aspects, together with the general lack of knowledge on the red coral bioerosion, studies such as the one presented in this paper are necessary to better understand the balance between bioconstruction and bioerosion acting in temperate reefs and the many factors affecting these fundamental but neglected processes.

References

1. Weinstein, D.K.; Maher, R.L.; Correa, A.M.S. Bioerosion. In Mesophotic Coral Ecosystems. Coral Reefs of the World; Loya, Y., Puglise, K., Bridge, T., Eds; Springer: Cham, 2015; Volume 12, pp. 829-847. https://doi.org/10.1007/978-3-319-92735-0_43

2. Calcinai, B.; Cerrano, C.; Bavestrello, G.; Milanese, M.; Sarà, M. Il popolamento di spugne perforatrici di Corallium rubrum e di alcuni madreporari del Promontorio di Portofino. Boll. Mus. Ist. Biol. Univ. Genova 2002, 64, 53-59.

3. Azzini F.; Calcinai, B.; Iwasaki; N., Bavestrello, G. A new species of Thoosa (Demospongiae, Hadromerida) excavating precious coral Corallium sp. From Midway. Ital. J. Zool. 2007, 74, 405-408. https://doi.org/10.1080/11250000701632790

4. Calcinai, B.; Cerrano, C.; Iwasakei, N.; Bavestrello, G. Sponges boring into precious corals: an overview with description of a new species of Alectona (Demospongiae, Alectonidae) and a world-wide identification key for the genus. Mar. Ecol. 2008, 29, 273-279. https://doi.org/10.1111/j.1439-0485.2008.00246.x

5. Liverino, B. Il corallo: esperienze e ricordi di un corallaro. Analisi 1984.

6. Corriero, G.; Abbiati, M.; Santangelo, G. Sponges inhabiting a Mediterranean red coral population. Mar. Ecol. 1997, 18(2), 147-155. https://doi.org/10.1111/j.1439-0485.1997.tb00433.x

7. Rützler, K. Family Clionaidae D'Orbigny, 1851. In Systema Porifera; Springer, Boston, MA, 2002, pp. 173-185 https://doi.org/10.1007/978-1-4615-0747-5_19

8. Vacelet, J. Planktonic armoured propagules of the excavating sponge Alectona (Porifera: Demospongiae) are larvae: evidence from Alectona wallichii and A. mesatlantica sp. nov.. Mem. Queensl. Mus. 1999, 44, 627-642

9. Calcinai, B.; Bavestrello, G.; Cerrano, C. Bioerosion micro-patterns as diagnostic characteristics in boring sponges. Boll. Mus. Ist. Biol. Univ. Genova. 2004, 68, 229-238.

10. de Voogd, N.J.; Alvarez, B.; Boury-Esnault, N.; Carballo, J.L.; Cárdenas, P.; Díaz, M.-C.; Dohrmann, M.; Downey, R.; Hajdu, E.; Hooper, J.N.A.; Kelly, M.; Klautau, M.; Manconi, R.; Morrow, C.C.; Pisera, A.B.; Ríos, P.; Rützler, K.; Schönberg, C.; Vacelet, J.; van Soest, R.W.M. World Porifera Database 2022. Accessed at https://www.marinespecies.org/porifera on 2022-02-03. https://doi.org/10.14284/359

11. Schönberg, C.H.; Shields, G. Micro-computed tomography for studies on Entobia: transparent substrate versus modern technology. In Current Developments in Bioerosion; Springer, Berlin, Heidelberg, 2008, pp. 147-164. https://doi.org/10.1007/978-3-540-77598-0_8

12. Beuck, L.; Vertino, A.; Stepina, E.; Karolczak, M.; Pfannkuche, O. Skeletal response of Lophelia pertusa (Scleractinia) to bioeroding sponge infestation visualised with micro-computed tomography. Facies 2007, 53(2), 157-176. https://doi.org/10.1007/s10347-006-0094-9

13. Färber, C.; Titschack, J.; Schönberg, C.H.L.; Ehrig, K., Boos, K.; Baum, D.; Wisshak, M. Long-term macrobioerosion in the Mediterranean Sea assessed by micro-computed tomography. Biogeosciences 2016, 13(11), 3461-3474. https://doi.org/10.5194/bg-13-3461-2016

14. Rützler, K. The burrowing sponges of Bermuda. Smithson. Cont. Zool. 1974, 165, 32. https://doi.org/10.5479/si.00810282.165

15.Beuck, L.; Freiwald, A. Bioerosion patterns in a deep-water Lophelia pertusa (Scleractinia) thicket (Propeller Mound, northern Porcupine Seabight). In Cold-water corals and ecosystems. Springer: Berlin, Heidelberg, 2005; pp. 915-936. https://doi.org/0.1007/3-540-27673-4_47

16.Bromley, R.G.; D'Alessandro, A. The ichnogenus Entobia from the Miocene, Pliocene and Pleistocene of southern Italy. Riv. Ital. Paleontol. Stratigr. 1984, 90(2).

17.Bromley, R.G.; D'Alessandro, A. Ichnological study of shallow marine endolithic sponges from the Italian coast. Riv. Ital. Paleontol. Stratigr. 1989, 95(3).

18.Bavestrello, G.; Calcinai, B.; Cerrano, C.; Sara, M. Alectona species from North-Western Pacific (Demospongiae: Clionidae). J. Mar. Biol. Ass. U. K. 1998, 78, 59-73. https://doi.org/10.1017/S0025315400039965

19.Pica, D.; Calcinai, B.; Bertolino, M. Excavating sponges boring into the precious red coral from Cape Verde Archipelago. Biol. Mar. Mediterr. 2011, 18 (1), 278-279.

20.Carballo, J.L.; Bautista-Guerrero, E.; Cárdenas, P.; Cruz-Barraza, J.A.; Aguilar-Camacho, J.M. Molecular and morphological data from Thoosidae in favour of the creation of a new suborder of Tetractinellida. Syst. Biodivers. 2018, 16(5), 512-521. https://doi.org/10.1080/14772000.2018.1457100

21.Rosell, D.; Uriz, M.J. Excavating and endolithic sponge species (Porifera) from the Mediterranean: species descriptions and identification key. Org. Divers. Evol. 2002, 2(1), 55-86. https://doi.org/10.5194/bg-2015-653

22.Golubić, S.; Friedmann, I.; Schneider, J. The lithobiontic ecological niche, with special reference to microorganisms. J. Sediment. Res. 1981, 51, 475-478. https://doi.org/10.1306/212f7cb6-2b24-11d7-8648000102c1865d

23.Wisshak, M. In Microbioerosion; Knaust, D.; Bromley, R.G. Eds., Trace Fossils as Indicators of Sedimentary Environments: Dev. Sedimentol; Elsevier, Amsterdam. 2012, 64, pp. 213-243. https://doi.org/10.1016/B978-0-444-53813-0.00008-3

24.Davidson, T.M.; Altieri, A.H.; Ruiz, G.M.; Torchin, M.E. Bioerosion in a changing world: a conceptual framework. Ecol. Lett. 2018, 21(3), 422-438. https://doi.org/10.1111/ele.12899

25.Bromley, R.G.; Tendal, O.S. Example of substrate competition and phobotropism between two clionid sponges. J. Zool. 1973, 169, 151-155.

26.Hoeksema, B.W. Excavation Patterns and Spiculae Dimensions of the Boring Sponge Cliona celata from the SW Netherlands. Senckenb. marit. 1982, 15, 55-85.

27.Turicchia, E.; Abbiati, M.; Bettuzzi, M.; Calcinai, B.; Morigi, M.P.; Summers, A.P.; Ponti, M. Bioconstruction and Bioerosion in the Northern Adriatic Coralligenous Reefs Quantified by X-Ray Computed Tomography. Front. Mar. Sci. 2022, 8. https://doi.org/10.3389/fmars.2021.790869

28.Calcinai, B.; Bavestrello, G.; Cerrano, C.; Sarà, M. Boring sponges living into precious corals from the Pacific Ocean. Ital. J. Zool. 2001, 68(2), 153-160. https://doi.org/10.1080/11250000109356400

29.Bavestrello, G.; Calcinai, B.; Sarà, M. Two new species of Cliona (Porifera, Demospongiae) boring the scleraxis of Corallium elatius from the western Pacific. Ital. J. Zool. 1995, 62(4), 375-381. https://doi.org/10.1080/11250009509356092

30. Webb, A.E.; van Heuven, S.M.; de Bakker, D.M.; van Duyl, F.C.; Reichart, G.J.; de Nooijer, L.J. Combined effects of experimental acidification and eutrophication on reef sponge bioerosion rates. Front. Mar. Sci. 2017, 4, 311. https://doi.org/10.3389/fmars.2017.00311

31. Fang, J.K.; Mello-Athayde, M.A.; Schönberg, C. H.; Kline, D. I.; Hoegh-Guldberg, O.; Dove, S. Sponge biomass and bioerosion rates increase under ocean warming and acidification. Glob Chang Biol. 2013, 19(12), 3581-3591. https://doi.org/10.1111/gcb.12334

32. Tunnicliffe, V. Breakage and propagation of the stony coral Acropora cervicornis. Proc. Nad. Acad. Sci. 1981, 78, 2427-2431. https://doi.org/10.1073/pnas.78.4.2427

33. Highsmith, R.C. Reproduction by fragmentation in corals. Mar. Ecol. Prog. Ser. 1982, 7(2), 207-226. http://dx.doi.org/10.3354/meps007207

34. Cerrano, C.; Bavestrello, G.; Bianchi, C.N.; Calcinai, B.; Cattaneo-Vietti, R.; Morri, C.; Sarà, M. The role of sponge bioerosion in Mediterranean coralligenous accretion. In Mediterranean Ecosystems; Faranda, F.M., Guglielmo, L., Spezie, G.C. Eds. Springer, Milano, 2001; pp. 235-240. https://doi.org/10.1007/978-88-470-2105-1_30

35. Grace, S. Winter quiescence, growth rate, and the release from competition in the temperate scleractinian coral Astrangia poculata (Ellis & Solander 1786). Northeastern Nat. 2017, 24, B119-B134. https://doi.org/10.1656/045.024.s715

36. Calcinai, B.; Bertolino, M.; Bavestrello, G.; Montori, S.; Mori, M.; Pica, D.; Valisano, L.; Cerrano, C. Comparison between the sponge fauna living outside and inside the coralligenous bioconstruction. A quantitative approach. Mediterr. Mar. Sci. 2015, 16(2), pp.413-418.

37. Valisano, L.; Palma, M.; Pantaleo, U.; Calcinai, B.; Cerrano, C. Characterization of North–Western Mediterranean coralligenous assemblages by video surveys and evaluation of their structural complexity. Mar. Pollut. Bull. 2019, 148, pp.134-148.

Concluding remarks

The various studies contained in this PhD project highlighted the advantages of applying emergent 3D approaches, providing a collection of successful methodological applications at different ecological scales. SfM-photogrammetry demonstrated to be a suitable cost-effective approach to support underwater monitoring from single organisms to entire communities. Despite the unavoidable constrains of the technique as the need of good visibility conditions for its accurate application, other limitations, such as the limiting bottom times or the need of high performant computers for big data processing, are expected to be overcome in the upcoming years. In fat, the coupling of optical-based methods with other emergent technologies, like machine learning (e.g., automated orientation, real time segmentation), underwater robotics (e.g., ROV and AUV) or cloud computing systems, represents a hot topic in many scientific fields, with an exponentially growing market. Nonetheless, the rise of 3D techniques should not imply the complete replacement of traditional approaches; in fact, as also presented in this thesis, the importance of traditional approaches and taxonomical knowledge is still crucial, being impossible to reach a species-level resolution only by imagery analysis.

Ecological Niche Modelling proved once again their utility to draw distributional range estimations of a species. Although the scale of our approach limited the local application of its results, it may drive future monitoring activities to localize and protect Mediterranean marine animal forests. Thanks to the continuous improvement of horizontal and vertical resolution of satellite bio-geo-physico-chemical variables, together with the increasing knowledge of the species inhabiting our oceans, SDMs will get more accurate in the future and continue supporting species/habitat-based management plans.

Micro-CT implementation also proved to be a powerful tool to characterize benthic species bioerosive behaviour, even though it still needs to reach its full potential. By the development of more powerful scanners, together with tailored algorithms to assist traces-segmentation, they may overcome its current constrains, mainly linked to samples' size-density, processing times, and scanning costs. Therefore, depending on the objectives of the study, a costs-benefits evaluation considering alternative methods it is still suggested.

Although 3D innovative methods are reshaping the classic biologist toolbox, some of the new metrics that have been developed for 3D analyses and their ecological meaning are still largely unexplored, especially in underwater environments. Therefore, the development of easily applicable procedures to consider 3D complexity into the seascape ecology remains a challenging task for marine biologist and ecologists. This aspect is, in fact, fundamental for marine environments' conservation and resilience, and its inclusion in marine policies could have major implications in economic activities threatening seafloor structural complexity, such as the fishing industry and the deep sea mining.

This PhD research represents a great example of how the current shift towards a 3D science may provide a more complete or representative perspective of a studied phenomenon. The coupling of 3D emergent approaches with more traditional ones should be, thus, encouraged and promoted in the following years, as well as the development of new integrative procedures to describe the structural complexity of our environments.

3D approaches have a bright future ahead.
Why not stop trying to understand a three-dimensional world
with a two-dimensional thinking?

References

Anastasovitis, E. and Roumeliotis, M., 2023. Transforming computed tomography scans into a full-immersive virtual museum for the Antikythera Mechanism. Digital Applications in Archaeology and Cultural Heritage, p.e00259.

Aristov, M.M., Moore, J.W. and Berry, J.F., 2021. Library of 3D visual teaching tools for the chemistry classroom accessible via Sketchfab and viewable in augmented reality. ACS Publications, pp.3032-3037. https://doi.org/10.1021/acs.jchemed.1c00460

Banfi, F., Pontisso, M., Paolillo, F. R., Roascio, S., Spallino, C., and Stanga, C., 2023. Interactive and Immersive Digital Representation for Virtual Museum: VR and AR for Semantic Enrichment of Museo Nazionale Romano, Antiquarium di Lucrezia Romana and Antiquarium di Villa Dei Quintili. ISPRS International Journal of Geo-Information, 12(2), p.28. https://doi.org/10.3390/ijgi12020028

Bashir, A., Bashir, S., Rana, K., Lambert, P. and Vernallis, A., 2021. Post-COVID-19 adaptations; the shifts towards online learning, hybrid course delivery and the implications for biosciences courses in the higher education setting. Frontiers in Education, p. 310. https://doi.org/10.3389/feduc.2021.711619

Bagrov, A.A., Iakovlev, I.A., Iliasov, A.A., Katsnelson, M.I. and Mazurenko, V.V., 2020. Multiscale structural complexity of natural patterns. Proceedings of the National Academy of Sciences, 117(48), pp.30241-30251. https://doi.org/10.1073/pnas.2004976117

Barbieri, G. and da Silva, F.P., 2019. Acquisition of 3D models with submillimeter-sized features from SEM images by use of photogrammetry: a dimensional comparison to microtomography. Micron, 121, pp.26-32. https://doi.org/10.1016/j.micron.2019.02.013

Bayley, D.T. and Mogg, A.O., 2020. A protocol for the large-scale analysis of reefs using Structure from Motion photogrammetry. Methods in Ecology and Evolution, 11(11), pp.1410-1420. https://doi.org/10.1111/2041-210X.13476

Beuck, L., Vertino, A., Stepina, E., Karolczak, M. and Pfannkuche, O., 2007. Skeletal response of *Lophelia pertusa* (Scleractinia) to bioeroding sponge infestation visualised with micro-computed tomography. Facies, 53, pp.157-176. http://dx.doi.org/10.1007/s10347-006-0094-9

Breiner, F.T., Guisan, A., Bergamini, A. and Nobis, M.P., 2015. Overcoming limitations of modelling rare species by using ensembles of small models. Methods in Ecology and Evolution 6(10), pp.1210-1218. https://doi.org/10.1111/2041-210X.12403

Burns, J.H.R., Delparte, D., Gates, R.D. and Takabayashi, M. (2015). Utilizing underwater three-dimensional modeling to enhance ecological and biological studies of coral reefs. International Archives of Photogrammetry, Remote Sensing and Spatial Information Sciences - ISPRS Arch., 40(5), p.61. https://doi.org/10.5194/isprsarchives-XL-5-W5-61-2015

Cameron, M.J., Lucieer, V., Barrett, N.S., Johnson, C.R. and Edgar, G.J., 2014 Understanding community-habitat associations of temperate reef fishes using fine-resolution bathymetric measures of physical structure. Marine Ecological Progress Series, 506, pp.213–229. https://doi.org/10.3354/meps10788

Caravaca, G., Le Mouélic, S., Mangold, N., L'Haridon, J., Le Deit, L. and Massé, M., 2020. 3D digital outcrop model reconstruction of the Kimberley outcrop (Gale crater, Mars) and its integration into Virtual Reality for simulated geological analysis. Planetary and Space Science, 182, p.104808. https://doi.org/10.1016/j.pss.2019.104808

Chatzinikolaou, E. and Keklikoglou, K., 2021. Micro-CT protocols for scanning and 3D analysis of Hexaplextrunculus during its different life stages. Biodiversity Data Journal, 9. https://doi.org/10.3897/BDJ.9.e71542

Cipresso, P., Giglioli, I.A.C., Raya, M.A. and Riva, G., 2018. The past, present, and future of virtual and augmented reality research: a network and cluster analysis of the literature. Frontiers in psychology, p.2086. https://doi.org/10.3389/fpsyg.2018.02086

Davies, A.B. and Asner, G.P., 2014. Advances in animal ecology from 3D-LiDAR ecosystem mapping. Trends in ecology & evolution, 29(12), pp.681-691. https://doi.org/10.1016/j.tree.2014.10.005

Davidson, T.M., Altieri, A.H., Ruiz, G.M. and Torchin, M.E., 2018. Bioerosion in a changing world: a conceptual framework. Ecology letters, 21(3), pp.422-438. https://doi.org/10.1111/ele.12899

Donnelly, I., 2019. Microscopia: A Virtual Museum of Organisms Created with SEM Photogrammetry. Honors College. 476. https://digitalcommons.library.umaine.edu/honors/476

Dwivedi, Y.K., Hughes, L., Baabdullah, A.M., Ribeiro-Navarrete, S., Giannakis, M., Al-Debei, M.M., Dennehy, D., Metri, B., Buhalis, D., Cheung, C.M. and Conboy, K., 2022. Metaverse beyond the hype: Multidisciplinary perspectives on emerging challenges, opportunities, and agenda for research, practice and policy. International Journal of Information Management, 66, p.102542. https://doi.org/10.1016/j.ijinfomgt.2022.102542

Fatimah, S. and Ningsih, T.Z., 2023, January. Through Virtual Field Trip Technology Intervention, Can Museums Be a Source of Historical Learning?. In Unima International Conference on Social Sciences and Humanities (UNICSSH 2022), Atlantis Press, pp. 1275-1283. https://doi.org/10.2991/978-2-494069-35-0_154

Ferrari, R., McKinnon, D., He, H., Smith, R.N., Corke, P., González-Rivero, M., Mumby, P.J. and Upcroft, B., 2016. Quantifying multiscale habitat structural complexity: a cost-effective framework for underwater 3D modelling. Remote Sensing, 8(2), p.113. https://doi.org/10.3390/rs8020113

Ferrari, R., Lachs, L., Pygas, D.R., Humanes, A., Sommer, B., Figueira, W.F., Edwards, A.J., Bythell, J.C. and Guest, J.R., 2021. Photogrammetry as a tool to improve ecosystem restoration. Trends in Ecology & Evolution, 36(12), pp.1093-1101. https://doi.org/10.1016/j.tree.2021.07.004

Garrabou, J., Bensoussan, N., di Franco, A., Boada, J., Cebrian, E., Santamaria, J., Guala, I., Grech, D., Cerrano, C., Pulido Mantas, T., Jou, M., Marambio, M. and Azzurro, E., 2022b. Monitoring Climate-related responses in Mediterranean Marine Protected Areas and beyond: ELEVEN STANDARD PROTOCOLS. 74 pp. Edited by: Institute of Marine Sciences, Spanish Research Council ICM-CSIC, Passeig Marítim de la Barceloneta 37-49, 08003 Barcelona, Spain. (2022) https://doi.org/10.20350/digitalCSIC/14672

Gerovasileiou, V. and Bianchi, C.N., 2021. Mediterranean marine caves: A synthesis of current knowledge. Oceanography and Marine Biology, CRC Press, pp.1-87. http://dx.doi.org/10.1201/9781003138846-1

Graham, N.A.J. and Nash, K.L. The importance of structural complexity in coral reef ecosystems. Coral Reefs 32, pp.315–326 (2013). https://doi.org/10.1007/s00338-012-0984-y

Hackl, J., Adey, B.T., Woźniak, M. and Schümperlin, O., 2018. Use of unmanned aerial vehicle photogrammetry to obtain topographical information to improve bridge risk Assessment. Journal of Infrastructure Systems, 1, p.04017041. https://doi.org/10.1061/(ASCE)IS.1943-555X.0000393

Höhne, K.H., Fuchs, H. and Pizer, S.M. eds., 2012. 3D imaging in medicine: algorithms, systems, applications. Springer Science & Business Media, 60. https://doi.org/10.1007/978-3-642-84211-5

Hutson, J. and Hutson, P., 2023. Museums and the Metaverse: Emerging Technologies to Promote Inclusivity and Engagement. https://doi.org/10.5772/intechopen.110044

Jacobs, H.L., 2022. SketchUp and Sketchfab: Tools for Teaching with 3D. Journal of the Society of Architectural Historians, 81(2), pp.256-259. https://doi.org/10.1525/jsah.2022.81.2.256

Jang, K., Kim, H.G., Hlaing, S.H.S., Kang, M., Choe, H.W. and Kim, Y.J., 2022. A Short Review on Cryoprotectants for 3D Protein Structure Analysis. Crystals, 12(2), p.138. https://doi.org/10.3390/cryst12020138

Kerbl, Alexandra, Stephan Handschuh, Marie-Therese Nödl, Brian Metscher, Manfred Walzl, and Andreas Wanninger. "Micro-CT in cephalopod research: investigating the internal anatomy of a sepiolid squid using a non-destructive technique with special focus on the ganglionic system." Journal of Experimental Marine Biology and Ecology 447 (2013): 140-148. https://doi.org/10.1016/j.jembe.2013.02.022

Keklikoglou, K., Arvanitidis, C., Chatzigeorgiou, G., Chatzinikolaou, E., Karagiannidis, E., Koletsa, T., Magoulas, A., Makris, K., Mavrothalassitis, G., Papanagnou, E.D. and Papazoglou, A.S., 2021. Micro-CT for biological and biomedical studies: A comparison of imaging techniques. Journal of imaging, 7(9), p.172. https://doi.org/10.3390%2Fjimaging7090172

Keklikoglou, K., Faulwetter, S., Chatzinikolaou, E., Wils, P., Brecko, J., Kvaček, J., Metscher, B. and Arvanitidis, C., 2019. Micro-computed tomography for natural history specimens: a handbook of best practice protocols. European Journal of Taxonomy, 522, pp.1-55

Lepczyk, C.A., Wedding, L.M., Asner, G.P., Pittman, S.J., Goulden, T., Linderman, M.A., Gang, J. and Wright, R., 2021. Advancing Landscape and Seascape Ecology from a 2D to a 3D Science. BioScience, 71(6), pp.596-608. http://dx.doi.org/10.1093/biosci/biab001

Mesoudi, A., Laland, K.N., Boyd, R., Buchanan, B., Flynn, E., McCauley, R.N., Renn, J., Reyes-García, V. and Shennan, S., 2013. The cultural evolution of technology and science. In: Cultural Evolution: Society, Technology, Language, and Religion. Peter J. Richersonand Morten H. Christiansen (Eds.). Strüngmann Forum Reports, vol. 12, J. Lupp, series editor. Cambridge, MA: MIT Press: pp. 193-216. ISBN 978-0-262-01975-0

Nielsen, J., 1998. Nielsen’s law of internet bandwidth. Online at http://www. useit. com/alertbox/980405. html, 1, p.2.

Pierdicca, R., Intrigila, C., Piccinini, F., Malinverni, E.S., Giannetti, I. and Caruso, G., 2021. Multidisciplinary Approach for the Analysis of Structural Heritage at Risk: The Case Study of Stylite Tower at Umm ar-Rasas (Jordan). International Journal of Architectural Heritage, pp.1-25. https://doi.org/10.1080/15583058.2021.1966554

Raoult, V., David, P.A., Dupont, S.F., Mathewson, C.P., O’Neill, S.J., Powell, N.N. and Williamson, J.E., 2016. GoPros™ as an underwater photogrammetry tool for citizen science. PeerJ, 4, p.e1960. https://doi.org/10.7717/peerj.1960

Rawson, S.D., Maksimcuka, J., Withers, P.J. and Cartmell, S.H., 2020. X-ray computed tomography in life sciences. BMC biology, 18(1), pp.1-15. https://doi.org/10.1186/s12915-020-0753-2

Remondino, F., Barazzetti, L., Nex, F., Scaioni, M. and Sarazzi, D., 2011. UAV photogrammetry for mapping and 3d modeling–current status and future perspectives. The International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences - ISPRS Arch, 38(1), p.C22. https://doi.org/10.5194/isprsarchives-XXXVIII-1-C22-25-2011

Robinson, N.M., Nelson, W.A., Costello, M.J., Sutherland, J.E. and Lundquist, C.J., 2017. A systematic review of marine-based species distribution models (SDMs) with recommendations for best practice. Frontiers in Marine Science, 4, p.421. https://doi.org/10.3389/fmars.2017.00421

Sansoni, G., Trebeschi, M. and Docchio, F., 2009. State-of-the-art and applications of 3D imaging sensors in industry, cultural heritage, medicine, and criminal investigation. Sensors, 9(1), pp.568-601. https://doi.org/10.3390/s90100568

Schaller, R.R., 1997. Moore's law: past, present and future. IEEE spectrum, 34(6), pp.52-59. https://doi.org/10.1109/6.591665

Schönberg, C.H. and Shields, G., 2008. Micro-computed tomography for studies on Entobia: transparent substrate versus modern technology. Current developments in bioerosion, pp.147-164. https://doi.org/10.1007/978-3-540-77598-0_8

Scopigno, R., Callieri, M., Dellepiane, M., Ponchio, F. and Potenziani, M., 2017. Delivering and using 3D models on the web: are we ready?. Virtual Archaeology Review, 8(17), pp.1-9. https://orcid.org/0000-0002-7457-7473

Thiele, J.C., Jungblut, M., Helmerich, D.A., Tsukanov, R., Chizhik, A., Chizhik, A.I., Schnermann, M.J., Sauer, M., Nevskyi, O. and Enderlein, J., 2022. Isotropic three-dimensional dual-color super-resolution microscopy with metal-induced energy transfer. Science Advances, 8(23), p.2506. https://doi.org/10.1126/sciadv.abo2506

Urbina-Barreto, I., Garnier, R., Elise, S., Pinel, R., Dumas, P., Mahamadaly, V., Facon, M., Bureau, S., Peignon, C., Quod, J.P. and Dutrieux, E., 2021. Which method for which purpose? A comparison of line intercept transects and underwater photogrammetry methods for coral reef surveys. Frontiers in Marine Science, 8, p.636902. https://doi.org/10.3389/fmars.2021.636902

Waltham, N.J., Elliott, M., Lee, S.Y., Lovelock, C., Duarte, C.M., Buelow, C., Simenstad, C., Nagelkerken, I., Claassens, L., Wen, C.K., Barletta, M., 2020. UN decade on ecosystem restoration 2021–2030—what chance for success in restoring coastal ecosystems? Frontiers in Marine Science, p.71. https://doi.org/10.3389/fmars.2020.00071

Westoby, M.J., Brasington, J., Glasser, N.F., Hambrey, M.J. and Reynolds, J.M., 2012. Structure-from-Motion photogrammetry: A low-cost, effective tool for geoscience applications. Geomorphology, 179, pp.300-314. https://doi.org/10.1016/j.geomorph.2012.08.021

Zhang, Y. and Li, G., 2020. Advances in technologies for 3D genomics research. Science China Life Sciences, 63, pp.811-824. https://doi.org/10.1007/s11427-019-1704-2

Additional contributions

Publications on international journals

- Roveta, C., Pulido Mantas, T., Bierwirth, J., Calcinai, B., Coppari, M., Di Camillo, C.G., Puce, S., Villechanoux, J. and Cerrano, C. Can colony resizing represent a strategy for octocorals to face climate warming? The case of the precious red coral *Corallium rubrum*. Coral reefs, 42, 535-549. http://dx.doi.org/10.1007/s00338-023-02365-9
- Di Camillo, C., Roveta, C., Pulido Mantas, T., Gravili, C., Cerrano, C., Calcinai, B., Coppari, M., and Gregorin, C., Marrocco, T., Riccardi, A. and Puce, S. (2023). Guests or pests? Eirenid hydroids living on the soft tissue of bivalves. Reviews in Aquaculture, 1-15. http://dx.doi.org/10.1111/raq.12823
- Garrabou, J., Gómez-Gras, D., Medrano, A., Cerrano, C., Ponti, M., Schlegel, R., Bensoussan, N., Turicchia, E., Sini, M., Gerovasileiou, V., Teixido, N., Mirasole, A., Tamburello, L., Cebrian, E., Rilov, G., Ledoux, J.B., Souissi, J.B., Khamassi, F., Ghanem, R., Benabdi, M., Grimes, S., Ocaña, O., Bazairi, H., Hereu, B., Linares, C., Kersting, D.K., la Rovira, G., Ortega, J., Casals, D., Pagès-Escolà, M., Margarit, N., Capdevila, P., Verdura, J., Ramos, A., Izquierdo, A., Barbera, C., Rubio-Portillo, E., Anton, I., López-Sendino, P., Díaz, D., Vázquez-Luis, M., Duarte, C., Marbà, N., Aspillaga, E., Espinosa, F., Grech, D., Guala, I., Azzurro, E., Farina, S., Gambi, M.C., Chimienti, G., Montefalcone, M., Azzola, A., Pulido Mantas, T., Fraschetti, S., Ceccherelli, G., Kipson, S., Bakran-Petricioli, T., Petricioli, D., Jimenez, C., Katsanevakis, S., Kizilkaya, I.T., Kizilkaya, Z., Sartoretto, S., Elodie, R., Ruitton, S., Comeau, S., Gattuso, J.P. and Harmelin, J.G., 2022. Marine heatwaves drive recurrent mass mortalities in the Mediterranean Sea. Global change biology 28(19): 5708-5725. https://doi.org/10.1111/gcb.16301
- Bierwirth, J., Pulido Mantas, T., Villechanoux, J. and Cerrano, C., 2022. Restoration of Marine Sponges—What Can We Learn from over a Century of Experimental Cultivation? Water 14(7): 1055. https://doi.org/10.3390/w14071055
- Roveta, C., Annibaldi, A., Calcinai, B., Girolametti, F., Illuminati, S., Pulido Mantas, T., Truzzi, C. and Puce, S., 2022. Distribution of mercury inside the Mediterranean sponge *Chondrosia reniformis*: A study case from the Tuscan Archipelago National Park (Tyrrhenian Sea). Journal of Sea Research 184: 102206. https://doi.org/10.1016/j.seares.2022.102206
- Roveta, C., Marrocco, T., Calcinai, B., Pulido Mantas, T., Pica, D., Valisano, L. and Puce, S., 2022. Unravelling the sponge diversity of the Tuscan Archipelago National Park (Tyrrhenian Sea, Italy). The European Zoological Journal 89(1): 310-323. https://doi.org/10.1007/s12526-021-01254-0
- Roveta, C., Marrocco, T., Pica, D., Pulido Mantas, T., Rindi, F., Musco, L. and Puce, S., 2022. The effect of substrate and depth on hydroid assemblages: a comparison between two islands of the Tuscan Archipelago (Tyrrhenian Sea). Marine Biodiversity 52(1): 1-20. https://doi.org/10.1080/24750263.2022.2042406
- Villechanoux, J., Bierwirth, J., Pulido Mantas, T. and Cerrano, C., 2022. Testing Transplantation Techniques for the Red Coral *Corallium rubrum*. Water 14: 1071. https://doi.org/10.3390/w14071071
- Roveta, C., Annibaldi, A., Afghan, A., Calcinai, B., Di Camillo, C.G., Gregorin, C., Illuminati, S., Pulido Mantas, T., Truzzi, C. and Puce, S., 2021. Biomonitoring of toxic elements: the

unexplored role of marine sessile taxa. Journal of Applied Sciences 11(2): 580. https://doi.org/10.3390/app11020580

- Afghan, A., Cerrano, C., Luzi, G., Calcinai, B., Puce, S., Pulido Mantas, T., Roveta, C. and Di Camillo, C.G., 2020. Main Anthropogenic Impacts on Benthic Macro-fauna of Sandy Beaches: A Review. Journal of Marine Science and Engineering 8: 405. https://doi.org/10.3390/jmse8060405
- Roveta, C., Annibaldi, A., Vagnoni, F., Pulido Mantas, T., Domenichelli, F., Gridelli, S. and Puce, S., 2020. Short-term effects of environmental factors on the asexual reproduction of *Aurelia* sp. polyps. Chemistry and Ecology: 1-7. https://doi.org/10.1080/02757540.2020.1735375

Publications on conference proceedings

- Pulido Mantas, T., Calcinai, B., Coppari, M., De Min, A., Di Camillo, C.G., Puce, S., Roveta, C. and Cerrano, C. A photogrammetric approach to estimate growth in Porifera: *Sarcotragus foetidus* Schmidt, 1862 and *Chondrosia reniformis* Nardo, 1847 study cases. Proceedings of the 11th World Sponge Conference. Leiden (NL), 10-14 October 2022. p.63.
- Pulido Mantas, T., Di Camillo, C.G., Calcinai, B., Coppari, M., Gregorin, C., Henjes, J., Schiefenhövel, K., Marrocco, T., Puce, S., Riccardi, A., Roveta, C. and Cerrano, C. Sustainable sponge farming to obtain bioactive compounds: *Chondrosia reniformis* Nardo, 1847 as an innovative source of collagen. Proceedings of the Aquaculture Europe 22, Innovative solutions in a changing world. Rimini (IT), 27-30 September 2022. pp.1081-1082.
- Pulido Mantas, T., Calcinai, B., Coppari, M., Di Camillo, C.G., Marchesi, V., Marrocco T., Puce, S., Roveta, C. and Cerrano C. 3D mapping of a marine cave, the importance of establishing a baseline. Proceedings of the 3rd Mediterranean Symposium on the Conservation of the Dark Habitats. Genoa (IT), 19-23 September 2022. p.111.
- Pulido Mantas, T., Calcinai, B., Coppari, M., Di Camillo, C.G., Marchesi, V., Marrocco, T., Coppari, M; Di Camillo, G., Marrocco, T., Roveta, C. and Cerrano C. Photogrammetry, an emergent tool for Marine Conservation. Proceedings of the MER Community Summit 2022. Bilbao (ES), 7-11 September 2022. p.74.
- Pulido Mantas, T., Varotti, C., Palma, M., Benabdi, M., Trainito, E., Macic, V., Gambi, M.C. and Cerrano, C. Is it possible to predict the distribution of a rare parasitic species? The challenging case of *Savalia savaglia* (Bertoloni, 1819) (Cnidaria, Anthozoa). Proceedings of the EVOLMAR 2020: Marine Evolution. Online. 23-25 November. p.62
- Roveta, C., Annibaldi, A., Calcinai, B., Coppari, M., Costa, R., Di Camillo, C.G., Fernandes Couceiro, J., Girolametti, F., Puce, S., Pulido Mantas, T. and Cerrano, C. Heavy meals for a light sponge: mercury bioaccumulation in the sponge *Chondrosia reniformis* Nardo, 1847 and its prokaryotic community. Proceedings of the 11th World Sponge Conference. Leiden (NL), 10-14 October 2022. p.251.
- Roveta, C., Bierwirth, J., Calcinai, B., Coppari, M., Di Camillo, C.G., Pulido Mantas, T., Villechanoux, J. and Cerrano, C. Mitigation strategies of the red coral *Corallium rubrum* (Linnaeus, 1758) to face climate warming. Proceedings of the 4th Mediterranean Symposium on the Conservation of Coralligenous and Other Calcareous Bio-Concretions. Genoa (IT), 19-23 September 2022. p.169.

- Di Camillo, C.G., Storari, A., Scarpa, C., Ponti, M., Pulido Mantas, T., Roveta, C., Coppari, M., Calcinai, B., Puce, S. and Cerrano, C. A citizen-based protocol to assess vulnerability of narrow passages to scuba diving. Proceedings of the 3rd Mediterranean Symposium on the Conservation of the Dark Habitats. Genoa (IT), 19-23 September 2022.
- Roveta, C., Annibaldi, A., Calcinai, B., Girolametti, F., Illuminati, S., Pulido Mantas, T., Truzzi, C. and Puce, S. Annual variations of Hg levels in two Mediterranean sponge species. Proceedings of the XVII Italian-Hungarian Symposium on Spectrochemistry. Turin (IT), 14-18 June 2021. p.185.

List of other publications

- Garrabou, J., Bensoussan, N., Di Franco, A., Boada, J., Cebrian, E., Santamaria, J., Guala, I., Grech, D., Cerrano, C., Pulido Mantas, T., Jou, M., Marambio, M. and Azzurro, E., 2022. Monitoring Climate-related responses in Mediterranean Marine Protected Areas and beyond: ELEVEN STANDARD PROTOCOLS. 74 pp. Edited by: Institute of Marine Sciences, Spanish Research Council ICM-CSIC, Passeig Marítim de la Barceloneta 37-49, 08003 Barcelona, Spain. https://doi.org/10.20350/digitalCSIC/14672