# PHOTOGRAMMETRIC AND FLUORESCENCE SOLUTIONS FOR MONITORING OF HABITAT FORMING ORGANISMS

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**ABSTRACT:**

The development and testing of innovative technologies and automated data analysis methodologies offer tools for the monitoring of complex marine ecosystems and the direct and indirect effects of climate change on natural heritage. Photogrammetric methods allow precise mapping of the underwater landscape as well as detailed three-dimensional (3D) reconstruction of marine structures, improving the study of complex marine ecosystems. Moreover, fluorescence analyses can provide critical information about the health status of marine organisms. Analysing the variations in their self-fluorescence, allow for early detect changes in their physiological state. These applications provide very useful data to evaluate the health state of biodiversity-rich 3D biogenic structures and make measurements of fine-scale changes, with greater precision than existing methodologies. This contribution shows a multidisciplinary approach to the design, development, and implementation of a technological solution based on the above-mentioned optical measuring systems. Such a system is characterized by a reflex camera, LED-based light sources, and filters to allow the analysis of the self-fluorescence signal. The proposed solution aspires to improve the standardization of monitoring plans through non-destructive fine-scale accurate data collection for image analysis and multi-temporal comparisons, providing challenging stepping-stones for habitat-forming anthozoan management and restoration activities. Initial results of tests carried out in controlled conditions are shown. The photogrammetric approach resulted in 3D reconstructions that allow the monitoring of deformations at millimetre scale. The fluorimetry methodology allowed to obtain high-resolution images with great repeatability, which enabled the identification of stressful status even in absence of geometric deformations. The proposed approaches and obtained results are discussed, together with potential issues related to their implementation in a real-world context adopting remotely operative vehicles.

## 1. INTRODUCTION

The Mediterranean Sea is recognized as a biodiversity hotspot and it is one of the most affected areas by the underway climate alterations (Katsanevakis et al., 2014). Impacts on benthic habitats threaten an elevated number of species, including ecosystem engineers such as the habitat formers. These species play an important ecological role in generating and maintaining marine biodiversity and provide critical ecosystem services to the global economy. Seabed communities dominated by sponges, bivalves, gorgonians, and corals have a high naturalistic and cultural value and are actually threatened by climatic and anthropogenic pressures acting on local and global scales (Guarnieri et al., 2016). In particular, the ongoing seawater warming is altering the structure of benthic habitats with cascade effects on the entire associated communities (Heron et al., 2016; Kersting et al., 2013). Among the species at risk in the Mediterranean, habitat-forming anthozoans are widely distributed in infralittoral rocky areas, where they form a biogenic subtidal seascape. Despite their relevant ecological role and conservation interest, they are very vulnerable to climate anomalies and human pressures, which also influence the recovery rate of populations (Otero et al., 2017; Piazzi et al. 2021). The scleractinian *Cladocora caespitosa* is the sole endemic reef-builder coral in the Mediterranean Sea, and it is morphologically similar to tropical counterparts, appearing as isolated massive colonies, small patches or forming rare extensive banks (e.g., in the Adriatic Sea; Kružić and Benković, 2008). Thanks to symbiotic zooxanthellae, it can colonize rocky bottoms from shallow waters to about 40 m depth and emit green fluorescence. Ongoing seawater warming and thermal anomalies can cause stress and increase mass mortality events in *C. caespitosa* populations, acting in synergy with other factors, such us pathogen infections, to affect necrosis tissue rate (Kersting et al., 2013; Heron et al., 2016).

The study and analysis of habitat formers need the implementation of new observation and measuring systems, and the definition of high-resolution mapping procedures based on innovative survey methodologies. Given the threats posed by climate changes and human impacts on stony corals (e.g., recreational fishing, anchoring, and SCUBA diving), the implementation of robust monitoring methods is central in tracking changes in their health status. Such changes can be observed in terms of physiological stress (perceived as a reduction in fluorescence), and modifications to the carbonate structure (e.g. anthropogenic impacts causing the detachment or breaking of colonies). Photogrammetry and fluorimetry techniques allow non-invasive and non-destructive investigations capable of returning metric and colorimetric

information of the detected object (Drap et al., 2015; Storlazzi et al., 2016).

Underwater digital image collection and processing to calculate 3D reconstructions via Structure from Motion (SfM) photogrammetry have progressed in recent years (Guo et al., 2016). Generally, close-range photogrammetry has been employed to obtain measures of tropical coral colony dimensions and surface area (Burns et al. 2015; Lavy et al. 2015), even at the polyp scale (Gutierrez-Heredia et al., 2016). These studies have supported the use of photogrammetry and 3D modeling to track critical physical and biological features of corals (Burns et al., 2020; Nocerino et al., 2020; Rossi et al., 2021). Furthermore, a previous literature review highlights that 3D models using close-range digital photogrammetry have been obtained mostly for tropical coral communities, while their application in Mediterranean environments remains largely unexplored. Moreover, the application of remotely operated vehicles (ROV) for coral monitoring is largely unexplored as well, and the exploitation of such technology in common scientific practice still needs to be validated with respect to the traditional and more innovative diver-based approaches (Palma et al., 2017). A first attempt to quantify 3D coral reef geometry using an ROV-based photogrammetric approach is provided by Price et al. (2019).

Fluorimetry has been largely used in multiple applications as an indicator of the health status of organisms containing fluorescent biomolecules (O'Malley-James and Kaltenneger, 2018). The phenomenon occurs when part of an organism (e.g. specific pigments) absorbs electromagnetic radiation and then almost immediately reemits light (usually at a longer wavelength). Fluorescence can thus be used for the classification of coral reef organisms (Xu et al., 2018; Bollati et al., 2021) or to provide information about their health status (Teague et al., 2022). Specifically, fluorescence analyses on GFP (green fluorescent protein)-like proteins, present in *C. caespitosa*, can be used as intrinsic optical marker useful to assess its health status (Teague et al., 2019). Generally, in natural conditions corals display a gentle colour, however, when exposed to blue or UV light, a brilliant green fluorescence is observable. Fluorescence could constitute an important marker to assess coral susceptibility to bleach (i.e., the loss of the photosynthetic endosymbionts) and necrosis, phenomena that lead to critical effects on the state of corals, increasing their mortality (Roth and Deheyn, 2013; Caldwell et al., 2017). In fact, healthy organisms usually tend to display full fluorescence, while the absence of the fluorescence phenomenon indicates the presence of dead, damaged, or stressed polyps retracted inside the corallite (Ramesh et al., 2019).

In the present study, natural fluorescence and photogrammetric 3D reconstructions were used as a non-invasive proxy to assess *C. caespitosa* health status. High-resolution photogrammetry is employed to generate 3D reconstructions of a living *C. caespitosa* colony and derive measures of fine-scale changes in its structure, aiming at obtaining greater precision and accuracy than existing methodologies (Ferrari et al., 2017; Rossi et al., 2021). The analyses of *C. caespitosa* colony geometrical and optical features were performed in controlled laboratory conditions before and after the application of physical damages resembling those occurring in the natural environment.

## 2. METHODS

Laboratory tests were performed in a 300 L tank of a 950 L recirculating seawater aquarium system with controlled conditions (temperature 17 ± 2 °C, salinity 36-38, photoperiod 12 h light/ 12 h dark). When *C. caespitosa* colonies were not used in the experiments, they were lit with a LED lamp (Easy LED Aqualantis) and fed ad libitum with nauplii of the crustacean *Artemia franciscana*.

Experimental tests on damaged colonies were conducted in order to recreate mechanical injuries occurring in the natural environment due to anthropic pressures (e.g., impacts of recreational SCUBA divers, fishing gears, and boating). A coral colony was placed inside a plastic container and a small anchor was left fall on it from 20-30 cm. The sample was hurt hard breaking the carbonate skeleton. The aim was to look for a change in the fluorescence pattern and test the ability of the 3D model to identify the damage that occurred.

### 2.1 Photogrammetry and 3D reconstruction

The investigate sample was fixed over small support (rock, seashell, or small piece of tile) to guarantee the stability of the sample during the acquisition of images. Underwater imaging was performed using a CANON 2000D reflex camera and an 18-55 mm EFS lens, coupled with an underwater housing (Easy Dive LEO3) equipped with a dome port. The dome port allows for a reduction of image distortion and the achievement of accurate results (Menna et al., 2016, 2017). The camera resolution was 24.1 Mpixels and a short acquisition distance was kept (about 50 cm), allowing the detection of numerous details and the generation of high-resolution reconstructions (resolution of about 0.1 mm). Images were acquired with a 35mm focal length, in automatic and autofocus mode to achieve the best image quality. A colour checker was placed near the organism being surveyed for colour balance correction. The issue of colour correction in underwater imaging (Bianco et al., 2015; Akkaynak and Treibitz, 2019) is not addressed in this paper because colour analyses are under investigation.

Photogrammetric processing requires external references to reduce distortion effects due to water medium and obtain a scaled and accurate 3D reconstruction (Rossi et al., 2020). A set of 12 targets with known coordinates (in a local reference system) was used to constrain the photogrammetric reconstruction. The targets were printed over a reference plate and distributed around the sample (see Fig. 1). The plate housed the sample during the survey: its surface displays a heavily textured design consisting of geometric shapes in grey colour scale to facilitate image matching (Gutierrez-Heredia et al., 2016), as shown in Fig. 1. The camera was mounted over stable support, while the reference plate was manually rotated. With this configuration, four sets of images were acquired performing full circles around the sample with viewing angles ranging from sub-horizontal to sub-nadiral (approximately 20°, 45°, 60°, 80°), ensuring a strong geometry, accurate results, and reducing occlusion areas (Gutierrez-Heredia et al., 2016). Around 30 images were acquired for each set and no zooming images were included, for a total of 120 images.

Image sets were then processed using the photogrammetric software Agisoft Metashape (v. 1.7), which is largely used by the scientific community in underwater investigations (Bayley and Mogg, 2020; Ventura et al., 2022; Gutierrez-Heredia et al., 2015; Burns et al., 2020). The software uses automatic feature recognition to register images. These features (blue points in Fig. 1B) serve as tie points for the acquired dataset and allow to generate a sparse point cloud, to obtain cameras poses and orientation, and to estimate internal cameras parameters in an arbitrary reference system. External metric references were then added to the project and optimization of images registration was performed to obtain results in the local reference system of the targets. The calculated outputs were then used for the dense point cloud computation (a medium quality with original resolution halved and aggressive depth filter were set). Removal

of low confidence points (value < 2) was applied before performing the textured-mesh calculation.

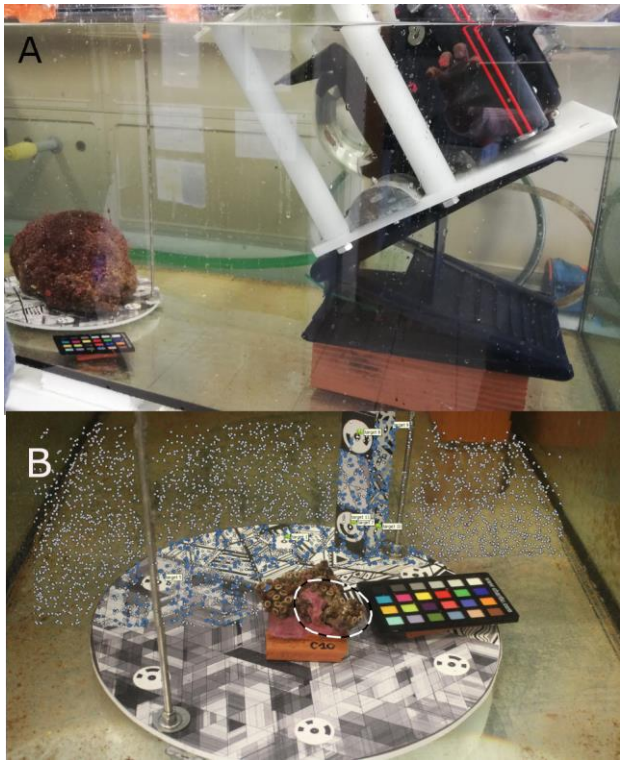


**Figure 1**. A- Set-up of photogrammetric acquisition. B- view of the sample and the colour checker positioned over the support with targets; The white and blue points, only depicted in a portion of the image, represent the identified keypoints and tie points, respectively; the dashed circle highlights the area affected by mechanical stress.

### 2.2 Fluorimetry

The instrumentations used in this work to observe natural fluorescence include a Canon 2000D (the same used for the photogrammetric acquisition) equipped with a microscopy emission filter (model FST001 – Alexa 488 FMF001) and a blue LED-based light source (UltraFire H-B3 – 470nm) that is properly filtered with a microscopy excitation filter (model FST001 – Alexa 488 FXF001). Although LEDs sources generally have an almost narrow emission spectrum, it is still advisable to introduce an excitation filter to remove the residual wavelengths on the extremes (spectral tails) that could fall within the region of the fluorescence signal, that is, that will be transmitted by the emission filter. The acquisition set-up used to acquire fluorescence imagery (shown in Fig. 2) was kept fixed for the whole acquisition phase. Both, the camera and the light source were positioned outside the tank and mounted on two small tripods to allow stable acquisition and unvaried illumination conditions. They are placed at about 50 cm from the sample and their axis converged on it. Camera settings and acquisition were controlled remotely from a laptop avoiding operator interferences in the shooting phase.
Experiments were designed to obtain acquisitions in dark conditions (the system was covered with a black cloth to avoid ambient light effects). The excitation filter (transmission band specs are shown in Fig. 2), applied to the artificial light source, allowed to illuminate the organisms with the blue component of the light, triggering the fluorescence phenomenon. The emission filter (specs in Fig. 2), applied to the lens of the Canon camera allowed to observe the green light emitted by fluorescent proteins and any residual ambient green light. Two consecutive images were acquired with the illumination source powered on and off: the first image (blank image) obtained with no excitation of the light source (only the ambient light contribution was captured); the second image with excitation light (fluo image), thus, ambient light contribution and fluorescence contribution were both captured. To remove the contribution related to ambient green light, a pixel-by-pixel subtraction between the two images was implemented. Two datasets (each one composed of ten images with a ten-second rate) had been acquired before and some minutes after the stress event. Since the intensity value of the individual pixels of the image also depends on camera settings such as aperture stop, exposure time, gain (ISO), camera settings were set at the start of the test and then kept unchanged for all acquisitions. In this way, on the one hand, it was possible to directly subtract the blank image from the fluo one. On the other hand, it was possible to make a direct comparison of the fluorescence images acquired in the two epochs (before and after the application of the stress). A 3D printed support was used to ensure that the sample position was the same for all epochs.

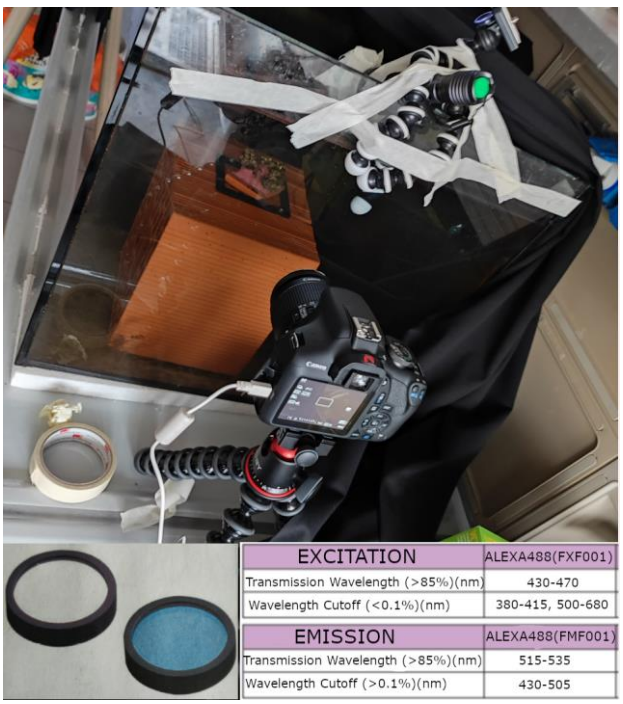


**Figure 2**. Fluorimetry set-up: camera over a tripod, light-source (top centre), used filters (emission-left and excitation-right) and their specs (bottom).

### 2.3 Multitemporal analysis

The monitoring of geometric deformations (i.e., loss of 3D complexity, portion removal) was carried out through a direct comparison of 3D models generated at two subsequent epochs: before the stress (called “epoch 0”), and after the stress (i.e. “epoch 1”). This approach requires the definition of a reference system unique and stable over time (Valenca et al., 2012; Abate, 2019; Laporte-Fauret et al., 2019). In underwater applications, it is complex to guarantee a unique reference system, and there are few applications to this end (see Nocerino et al., 2020; Rossi et al., 2020). In this study, the comparison of the two resulting point clouds was performed after a co-registration of models based on unchanged portions (the support on which the

organisms were fixed). 3D deformations were then evaluated through meshes comparison.
The consecutive acquisition of fluorescence images was performed in the same conditions: stable relative positioning of the camera, illuminator, and sample. The multitemporal analysis was based on investigations of pixels showing a fluorescence value above a pre-defined threshold: numerosity, average intensity, distribution on the image space. A threshold value of 15 was empirically chosen.

## 3. RESULTS

The acquired photogrammetric datasets led to the creation of 3D models with a sub-millimetre resolution (0.1 mm) and accuracy on GCPs just below the millimetre (see Fig. 3A). The obtained results were suitable to identify single polyps and potentially monitor their changes after exposure to stressful conditions. Figure 3A reports a view of the generated 3D model in solid and textured aspects.

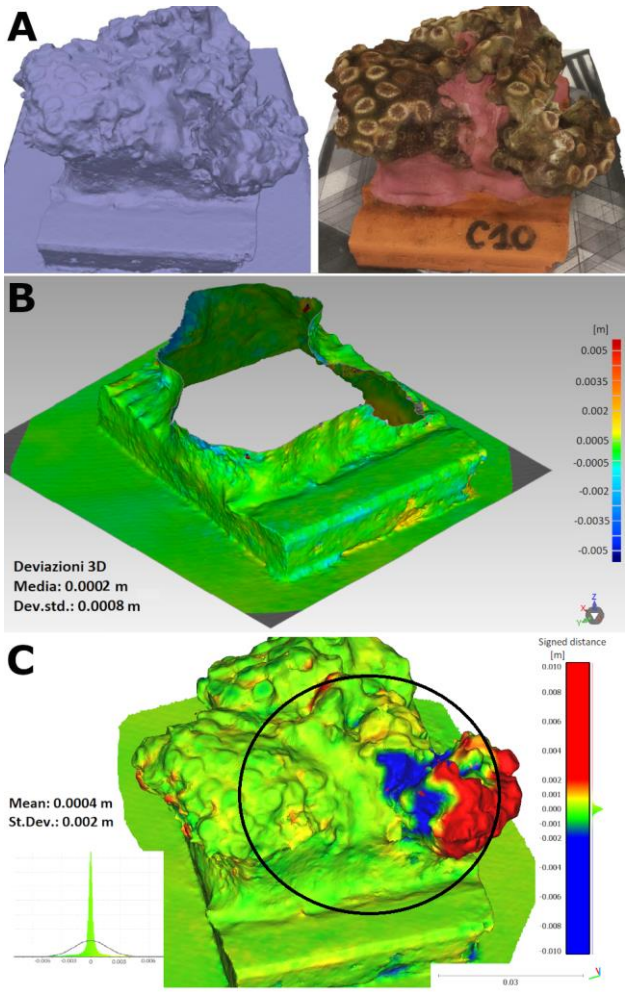


**Figure 3**. A- Views of photogrammetric 3D model. The colony was 6-7 cm in length. B-Portion used for the co-registration of subsequent epochs, deviation analysis. C- Results of multitemporal comparison, calculated distances, and statistics; black circle points out the impact of the anchor.

Regarding the monitoring of geometric deformations, a co-registration and then a comparison of 3D models were performed since the application of a common reference system for subsequent epochs reconstructions was not possible. Figure 3B shows the areas used for registering the models: a portion of the calibration frame, the bases on which the sample was fixed, and unchanged lower portions. Co-registration provided a mean distance between the unchanged portions of 0.2 mm and a st. dev. of 0.8 mm (Fig 3B).
The geometric deformation was defined through a comparison of the two 3D models: the mean distance was 0.4 mm with a st. dev of 2 mm (Fig 3C). the applied mechanical stress caused a break in the sample. Specifically, some polyps were damaged and a whole region fall apart (red and blue part in Fig.3C).

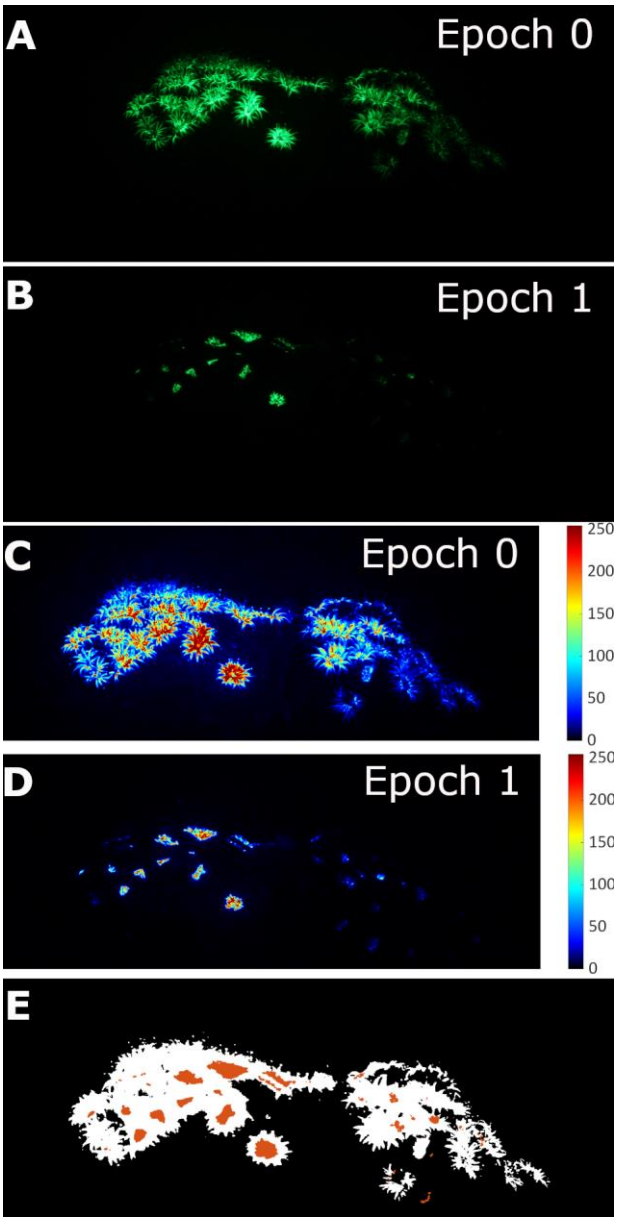


**Figure 4**. A, B- fluorimetry images acquired after and before the event, white circle points out the impact area. C, D- map of fluorescence intensity values. E- representation of relevant pixel before (white) and after (red) the event.

Fluorimetry images were acquired in manual mode and manual focus to exploit repeatability and avoid signal saturation (lens aperture F/20, 1,6 sec shutter speed). Figure 4A and figure 4B show fluorescence images obtained with the illumination source powered on before and after the stress event.

Adopting an acquisition set-up stationary for the whole acquisition process, allowed to capture consecutive images perfectly aligned. Figure 4C and Figure 4D represent the mean pixel intensity calculated on the image datasets before and after the event (ten images). The mean number of pixels above a threshold at epoch 0 was 5.65*10^5 with a st. dev. about 0.5%, the mean pixel intensity was 84.5. At epoch 1, in the first image after the event, the number of pixels above the same threshold was 5.81*10^4 with a mean intensity of 90. The calculation of a mean number of pixels and st. dev values were not significant at epoch 1 because the behaviour of polyps was unstable during the acquisition of the set. In epoch 1, the number of fluorescent polyps dropped because of the stress (Fig. 4 C, D). Only the polyps with a high fluorescence intensity in epoch 0 and large dimensions remained open and continued emitting light (Fig. 4 A-D). Figure 4E shows a direct comparison of fluorimetry images belonging to epoch 0 (white channel) and epoch 1 (red channel); on the right portion of the image, the effects of the broken branch are noticeable. The right portion of Fig. 4E seems to be affected by a misalignment of the two epochs; but actually, the polyps don't overlap before and after the event because of the occurred geometric modification.

## 4. DISCUSSIONS AND CONCLUSIONS

The development of an approach for non-invasive and non-destructive fine-scale accurate underwater data collection is discussed in this paper. RGB and fluorescence imagery provides an effective solution for the analysis and multi-temporal monitoring of habitat-forming anthozoan for management and restoration activities. Proposed results are obtained during laboratory tests (in an aquarium) necessary to test the instrumentations and evaluate their performance in controlled conditions. Investigated organisms are living *C. caespitosa* colonies.

High-resolution photogrammetry is employed to generate 3D reconstructions of living colonies and derive measures of fine-scale changes in its structure, aiming at obtaining greater precision and accuracy than existing methodologies. The repeatability and accuracy of the proposed approach for deformation monitoring were tested in this study. High accuracies and resolutions of photogrammetric 3D reconstructions, together with co-registration errors lower than 1 mm, led to accurate detection of occurred changes. Geometric changes slightly greater than 1 mm can be detected with the proposed approach. The colour distortion effects generated by the water medium were not appreciable in this case and the issue is not addressed in this paper. The presence of a colour checker will allow a colour calibration and the repeatability of colorimetric analyses, especially if activities in the open sea are planned.

The use of highly textured support is mandatory to have a good relative orientation of images and enhance an accurate 3D reconstruction. The small size of the concretion and the absence of distinctive texture contents at the polyp's scale, make the use of proper support necessary (a similar solution was adopted by Gutierrez-Heredia, 2016). In addition, the configuration "camera fixed - rotating object" prevents SfM algorithms from searching tie-points in background areas. Preliminary tests showed a growth of up to ten times in the number of detected tie points if the proposed textured support is used. Tie points numerosity is not a big deal in natural conditions (open sea) because the camera "moves" around the investigated object and the natural background is sufficiently textured. The set up of metric references is still required to obtain metric results and compare multitemporal reconstructions.

Multi-temporal analysis of subsequent 3D models is performed in this study through the co-registration (based on unchanged portions) and comparison between meshes. Comparison between point clouds has been long preferred over comparison between meshes because the meshing operation introduces additional uncertainties and approximations in the final model, also accompanied by a loss of high-frequency details (smoothness) and arbitrary hole filling (Nocerino et al., 2020). In this case, the holes were low and tiny. Also, we were looking for the smoothing effect of the meshing process in order to reduce the high-frequency noise generated by residual images misalignments and the movement of polyp's tentacles.

In this study, the ability of photogrammetry and fluorimetry in revealing changes in the investigated concretion after a stress event was evaluated. The applied anthropic pressure, the anchor, changed the morphology and the overall geometry (well detected by photogrammetry, difficult to identify in fluorescence images); it also stressed the corals causing them to re-enter (detectable trough fluorescence analysis, not identifiable in photogrammetric reconstruction). Thus, photogrammetry and fluorimetry are complementary approaches in the early detection of changes in corals' physiological state and the fine-scale measurement of occurring changes.

In future research, our intent is to test the potential of the methodological approaches proposed in this paper, working with scuba divers and a small and highly portable ROV on a real site (the BlueROV2). ROVs are effective for coral investigations and their use is increasing (Price et al 2019; Lim et al 2020). Developing an efficient ROV-based system to work on a real site must face a range of challenges. Effects of light absorption by seawater and acquisition carried out from a moving ROV can prevent measuring correctly the intensity of coral fluorescence (Teague et al 2019). The presence of high currents or turbidity during image acquisition must be considered also to avoid occlusions, blurry effects and collect suitable datasets for 3D reconstructions. Camera axes need to be properly oriented (nadiral, low-oblique imagery), the shooting starts and frequencies need to be triggered from the surface. Concerning fluorescence imagery, a high shooting rate, proper trigger camera-light sources are mandatory to allow the acquisition of blank and fluorescence images in similar conditions and perspective views.

In conclusion, the present experiment strongly supports the potentiality of combining photogrammetry techniques with fluorescence imaging to provide 3D models and fluorimetry information to detect changes in both coral morphology and health state. The acquisition of high-resolution images allowed the fine-scale reconstruction of single corallites with high accuracy. Indeed, the texture mapping and the *C. caespitosa* surface were detailed enough to reveal the rugosity and complexity of the coral structure, despite the small size of the colony (Fig. 3A). The testing approach based on the setup and validation of the measuring system in laboratory conditions will give the possibility of deriving colony biometric traits, such as surface area and volume, from 3D models in a non-intrusive way. Thus, calculations could be performed without relying on dry imaging, which is an important step forward to reduce measuring errors (e.g., those due to overestimations caused by layers of mucus secreted by the corallites out of water; Gutierrez-Heredia et al., 2016). Also, the decrease in fluorescence emission following the stressful event was clearly recorded (Fig. 4A, B). The impact with an anchor resembles the injuries naturally occurring in marine environments, highlighting the potentiality of the approach to provide relevant information from on-site images. Furthermore, combining multitemporal acquisitions and 3D models will provide critical

data to investigate the nature of the changes detected, assessing how and to what degree they affect both the morphology of the colony, and the single polyps over time, which could extend again from corallites after stresses, or face irreversible bleaching phenomena.

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