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Contribution to the Special Issue: Marine Animal Forest of the World (MAF WORLD)

Polyp number estimation through photogrammetry: a proof of concept using the example of *Corallium rubrum*

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Abstract

The Mediterranean red coral (*Corallium rubrum)* is a gonochoric octocoral facing population decline due to overexploitation. Effective management and restocking of this species require accurate estimates of reproductive potential, such as fecundity, which depends on the number of polyps. In the present study, we propose a novel method for estimating the number of polyps in *C. rubrum* colonies based on polyp density and colony surface. The latter can be determined using existing structure from motion (SfM) techniques. Here, we describe a protocol developed to produce reliable estimates of polyp density, based on counting the number of polyps on branch sections with known surface areas. The mean polyp density, determined on colonies collected in the NW Mediterranean at 25-30 m depth, was 16.9 ± 4.5 polyps cm^{-2}. Hence, the total number of polyps in a colony can be calculated by multiplying this density with the colony's total surface area. Method accuracy was assessed by manually counting the exact number of polyps on small-sized colonies and comparing the result to estimates obtained from both our new method and an existing approach based on colony basal diameter. While the latter incurred a mean estimation error of 29%, our new approach yielded a considerably improved accuracy with a mean estimation error of 9%. Further research is needed to validate this new approach across different red coral populations and potentially other coral species.

Keywords: Ocotcoral; Mediterranean Red Coral; Fecundity; Polyps; Structure from Motion.

Introduction

Mediterranean red coral (*Corallium rubrum*) is an octocoral belonging to the family of Corallidae, the so-called precious corals (Tsounis *et al.*, 2010). It is an iconic species endemic to the Mediterranean Sea and adjacent Atlantic rocky shores, whose bathymetric distribution spans depths ranging from 10 to 1000 m (Zibrowius *et al.*, 1984; Knittweis *et al.*, 2016). Red coral is a key component of Mediterranean marine ecosystems where it forms Marine Animal Forest (MAF, *sensu* Rossi *et al.*, 2017). Furthermore, it adds a three-dimensional complexity to the substrate and serves as both a refuge and nursery ground for several species (Maggioni & Bramanti, 2022).

Due to the use of its red calcareous skeleton in the manufacture of jewelry, this species has long been subjected to intensive exploitation (Tsounis *et al.*, 2013; Santangelo & Bramanti, 2010). In recent years, the combined effects of marine heatwaves and overfishing have led to the endangerment of shallow populations (10-50 m depth; Santangelo *et al.*, 2007; Viladrich *et al.*, 2022; Garrabou *et al.*, 2022). Despite the implementation of various management and conservation measures, the species' slow growth rate and reproductive strategy require more active interventions such as local restocking, particularly for populations that have experienced significant local depletion.

Effective restoration and restocking plans depend on a comprehensive understanding of the life-history characteristics of the target species as well as knowledge of the reproductive potential. For modular organisms, such as corals, the reproductive potential is intrinsically linked to the number of reproductive modules within colonies, i.e., the polyps (Sakai, 1998). Therefore, methods capable

of delivering accurate polyp counts are needed to obtain reliable estimates of reproductive potential (Beiring & Lasker, 2000). In *C. rubrum*, polyps are not evenly distributed along a colony but exhibit higher densities in primary branches and lower densities in the basal section (Benedetti *et al.*, 2020). For some time, a common approach to estimate the total number of polyps in *C. rubrum* was based on the relationship between colony basal diameter and the number of polyps (Santangelo *et al.*, 2007; Bramanti *et al.*, 2009; Bramanti *et al.*, 2015). This method may not accurately estimate the total number of polyps as it relies on an indirect relationship linking basal diameter to colony size. A better approach would be to use colony surface area as a proxy for the number of polyps, as both are directly correlated. Moreover, the previous methods do not account for the sex of the colonies, neglecting any differences in polyp distributions between males and females.

Structure from motion (SfM) is a photogrammetric technique that uses 2D images to calculate accurate dimensional data allowing 3D reconstructions of objects (Aston *et al.*, 2022). It has emerged as a promising tool that allows the precise determination of coral surface areas, both in field and aquarium settings, without having to destroy the coral (Million *et al.*, 2021; Lange & Perry, 2020; Conley & Hollander, 2021). Other methodologies such as paraffin dipping with double wax (Stimson & Kenzie, 1991) or single wax (Veal *et al.*, 2010) typically involve the removal of live tissue, which is a destructive process that can produce irreversible damage or alterations to the coral skeleton or even lead to the death of the entire organism. In comparison, SfM offers several advantages, particularly due to its non-invasive nature. By capturing multiple overlapping images from different angles, the photogrammetric technique enables the creation of detailed 3D reconstructions of coral colonies. Moreover, it provides a fast, easy, and inexpensive method (Lange & Perry, 2020) for obtaining precise measurements of coral surface area across a wide range of morphologies (Ferrari *et al.*, 2017).

In the present paper, we test a non-destructive method for estimating the number of polyps in *C. rubrum* colonies. The approach uses photogrammetric measurements of colony surface area and manual counts of the number of polys in a representative area. By multiplying the resulting polyp density with the surface area, the total number of polyps in a colony can be estimated. In combination with knowledge of their fecundity (number of larvae released per polyp) this yields precise estimates of their reproductive potential. Due to its non-destructive nature, this technique could be applied at the population level, providing a fundamental parameter for population and meta-population dynamics studies (Bramanti *et al.*, 2009; Guizien & Bramanti, 2014), which can in turn inform conservation and restoration programs.

Material and Methods

Experimental design

C. rubrum colonies were collected by scuba diving in two locations (Banyuls-sur-Mer, France; Cap de Creus, Spain) from depths between 25 and 35 m during the summers of 2021 and 2022. For all individuals, sex was determined both morphologically (following Santangelo *et al*., 2003) and genetically (following Pratlong *et al*., 2017). We selected 28 colonies of both sexes with morphologies that included several branching orders to test for differences in polyp density between 1) sexes and 2) branching orders.

3-D coral reconstruction and surface measurements

After sample collection, each live colony was placed at the center of a black circular tank filled with seawater and screwed onto a cubic support with a visible size scale. Photographs of each coral colony were taken under uniform lightening from two angles (90° and 45°) using an Olympus Tough TG-6, maintaining roughly 80% overlap between 2 consecutive images. Approximately 110-150 photos were necessary to construct an accurate 3D model of a colony using Agisoft Metashape software (Fig. 1A). This 3D model generation involves the following steps: 1) Alignment: align pictures to determine camera positions and orientations; 2) Point cloud model: generate a sparse point cloud model to capture the basic structure of the scene; 3) Dense cloud model: produce a dense point cloud model with more detailed geometry; 4) Mesh model: reconstruct a polygonal mesh representing the object; 5) Textured model: map textures onto the mesh to enhance visual quality. Then, we used Meshlab v2016.12, an open-source software for processing and editing 3D meshes, to calculate the surface areas of the 3D models. Models were cleaned using the "Select Vertices" tool in Meshlab to remove the background, non-coral elements (e.g., PVC support), and any portions of coral that appeared dead or damaged. Gaps that resulted from the removal of dead portions or overgrowth were left uncorrected to avoid adding to the surface area. Finally, the surface area was determined using Filter > Quality Measures and Computations > Computer Geometric Measures (Fig. 1B).

Polyp Density Estimation

Polyp density was determined by randomly selecting between 2 and 4 fragments from each of the 28 coral colonies (106 fragments in total) and measuring their surface area with MeshLab, as previously described. Then, we counted the number of polyps on the selected branches

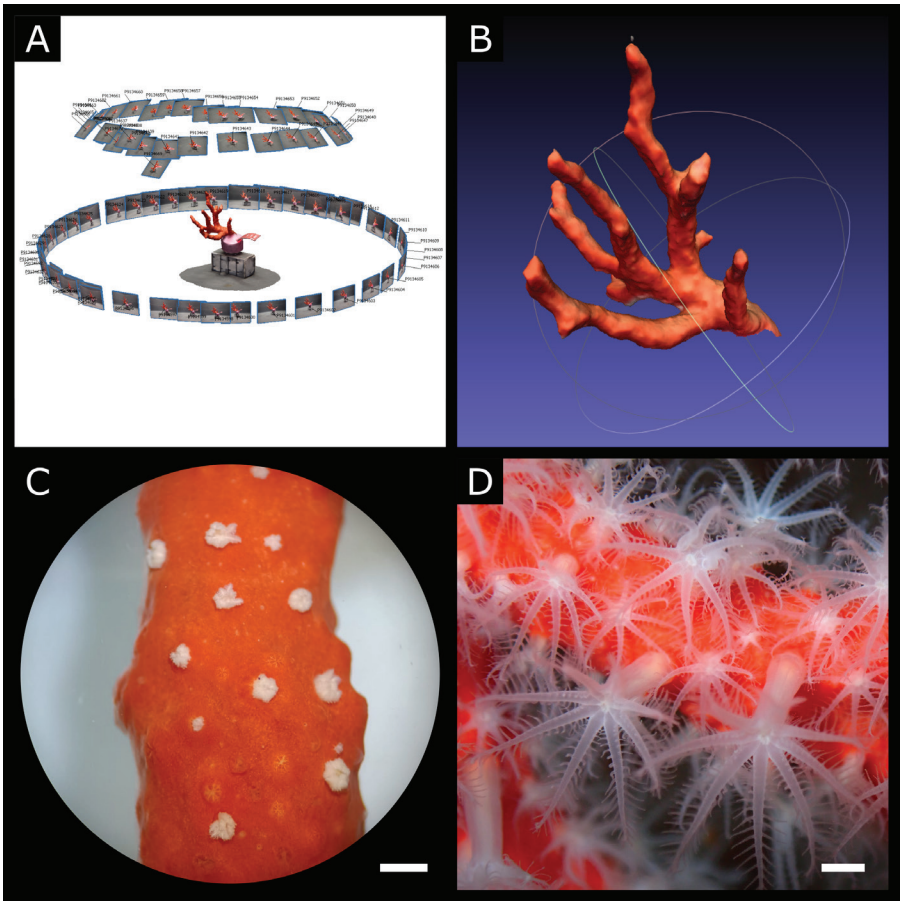

Fig. 1: The figure shows the steps to estimate the polyps' density. A) Screenshot of the 3D model reconstruction through Agisoft Metashape. It shows how the software aligns pictures to determine camera positions and orientations. B) Screenshot of a 3D model imported in MeshLab, showing a refined model of the actual living surface of the coral. C) View of a coral branch with closed polyps at the stereomicroscope (scale bar 1 mm). D) Macro of a coral branch with open polyps (scale bar 1 mm).

using a stereomicroscope (Fig. 1C, D). Polyp density then simply is the number of counted polyps divided by the measured surface area. Polyp counts for each branch were performed in triplicate by two different observers.

According to Benedetti *et al.* (2020), polyp density decreases with branch order, with densities being higher on apical branches. For this reason, we considered three orders of branching: 1st to 3rd, counting from the apex toward the base. To test for statistically significant differences in polyp density between different branch orders, we used a one-way ANOVA (R "stats" package), using the branching level (1st to 3rd) as fixed factor. When a significant effect of branching was found ($p < 0.05$), pairwise post-hoc tests were run (Tukey test with Bonferroni correction). Moreover, we performed independent t-tests to test for differences in polyp density between male and female colonies and to check for the existence of an observer effect in the polyp counts.

The weighted average polyp density across the three branching orders was computed using the proportions of surfaces areas occupied by the three branch types with respect to the total colony surface area (all determined using MeshLab, see above) for 17 colonies out of a total of 28. These proportions were used as weights to calculate the mean density:

$$N_{polyps} = d_{tot} \, x \, S_{tot} =$$

$$S_1 \, x \, d_1 + S_2 \, x \, d_2 + S_3 x \, d_3 =$$

$$S_{tot} \, (w_1 \, x \, d_1 + w_2 x \, d_2 + w_3 \, x \, d_3)$$

$$d_{tot} = (w_1 \, x \, d_1 + w_2 x \, d_2 + w_3 \, x \, d_3)$$

where d_1, d_2, and d_3 are the respective densities of the first, second, and third branching order while d_{tot} is the overall density. w_1, w_2, w_3, are the weights calculated based on the proportion of the are surface area of branches in each order with respect to the total surface area S_{tot}. $S_{S1/2/3}$ are the respective surface areas of the 1st/2nd/3rd branch order.

Finally, the above approach was validated by manually counting the total number of polyps (Npolyps in the above equations) in a total of 6 colonies and comparing the result to the estimates obtained from the weighted model. For comparison, we also estimated Npolyps using the Santangelo *et al.* (2003) method which is based on the colony basal diameter, and calculated the mean error between the estimated (*est*) and measured (*obs*) values of Npolyps for both methods as $(N_{obs}\text{-}N_{est})/ N_{obs}$. This validation was used to test the robustness of our methodology.

Results

Mean polyp density was 20.0 ± 4.30, 14.4 ± 5.07, and 11 ± 3.97 polyps cm^{-2} (mean ± SD) for the 1st, 2nd, and 3rd branching order, respectively. ANOVA results showed that these differences are statistically significant (Fig. 2; Table 1, F = 41.45; p = 1.94e^{-14}). The post-hoc Tukey test highlighted significant differences between each pair of branches (Table 1).

Results of the t-tests showed that polyp density did not differ significantly between male and female colonies (Fig. 2), with 17.1 ± 6.0 and 16.4 ± 4.6 polyps cm^{-2}, respectively (t = 0.703, df = 53.4, p = 0.48). Both human observers obtained similar polyp density estimates (21.0 ± 3.5 and 21.5 ± 4.6 polyps cm^{-2}, respectively), with no significant differences detected by the t-test (t = 1.61, df = 61.2, p = 0.112).

The 1st, 2nd, and 3rd order branches represent on average 54%, 30%, and 16% of the total surface area of a red coral colony, respectively. This resulted in a mean overall density of:

$(w_1 x\, d_1 + w_2 x\, d_2 + w_3 x\, d_3)$ = 16.9 ± 4.5 polyps cm^{-2}

The mean error was 9% for our new method, while the Santangelo *et al.* (2003) approach produced estimates with a mean error of 29%. This indicates a systematic underestimation by both methods, although the error is considerably improved with the new approach.

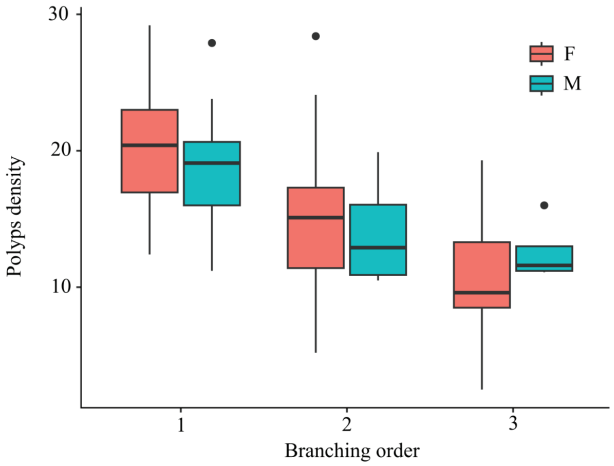

Fig. 2: Boxplot showing the mean polyps' density per branching order and sex. M= males, F=females.

Discussion

In the present study, we tested a novel method for estimating the number of polyps in *C. rubrum* colonies based on generating 3D models of the colonies from hundreds of photographs to accurately determine the colony's surface area.

To be able to estimate the total number of polyps, the overall polyp density must be known, which we determined through manual counts, yielding a mean overall polyp density across all samples of 16.9 ± 4.5 polyps cm^{-2}. Since Benedetti *et al.* (2020) found that polyp density varied with branch order, we determined the polyp density for the top three branches (1st to 3rd counted from the apex toward the base), which yielded statistically significant differences with densities decreasing with increasing branch order (i.e., 1st order > 2nd order > 3rd order). While this trend is in agreement with observations by Bendetti *et al.* (2020), the absolute values differ. For the first and third order branches, our approach yielded polyp densities of 20.0 ± 4.30 and 11 ± 3.97 polyps cm^{-2}, respectively, while the corresponding values of Benedetti *et al.* (2020) are 16.1 ± 1.7 and 8.0 ± 1.2 polyps cm^{-2}, which are likely underestimations due to the approximation of the branch shape to a truncated cone. These polyp densities were used to calculate the weighted average stated above. In contrast, no significant sex-specific differences in polyp densities were found, suggesting that polyp density estimates can be performed on randomly selected *C. rubrum* colonies without accounting for their sex.

Table 1. (A) Results of the one-way ANOVA test comparing the polyps' density between branching order. df = degrees of freedom; SS = Sums of squares value; MS = mean square value; F = F ratio; * indicate p < .05; ** indicate p < .01; *** p < 0.001. (B) Tukey post hoc pairwise comparisons. Diff = Difference in density; lwr and upr = lower and upper bounds of 95% confidence interval; p adj = p adjusted for multiple comparisons.

A-*One-way ANOVA*	**df**	**SS**	**MS**	**F value**	**p (> F)**
branching	2	1646	823.1	41.45	1.94e-14 ***
Residuals	121	2403	19.9		
B-*Tukey post-hoc*		diff	lwr	lwr	p adj
2nd-1st		5.616875	-7.873213	-3.3605371	0.0000001
3rd-1st		-9.020000	-11.604411	-6.4355888	0.0000000
3rd-2nd		-3.403125	-6.331580	-0.4746696	0.0183324

When comparing our non-destructive method with an approach based on live tissue samples that calculates polyp densities from colony basal diameter (Santangelo *et al.*, 2007; Cánovas-Molina *et al.*, 2009; Bramanti *et al.*, 2015), results showed that the new method performs better with considerably reduced errors of 9% vs 29%. The higher accuracy of the new approach is likely due to the fact that we directly measure the surface area (using photogrammetry and 3D reconstructions) instead of having to infer it from the basal diameter. Nevertheless, the colony basal diameter can be determined fairly quickly which renders this approach a viable option for the analysis of extensive datasets, such as those generated by ROV surveys (Cau *et al.*, 2016; Carugati *et al.*, 2022), for which our more time-consuming photogrammetric method may be impractical. Our method seems more suited to punctual research when more accurate estimations of polyp numbers are needed.

In *C. rubrum*, as in all marine anthozoans, the production of gametes is a function of both module (polyp) fertility and the number of fertile polyps per colony (Sakai, 1998). An accurate determination of the number of polyps is therefore crucial to obtain reliable estimates of key reproductive traits such as fecundity, which is an important parameter in ecosystem models that aim to generate forecast of the population dynamics of modular organisms (Cant *et al.*, 2024; Bramanti *et al.*, 2009; Santangelo *et al.*, 2007). Furthermore, this new approach could be applied to standardize results across several manipulative experiments, such as in ecotoxicological and physiological studies, which depend on normalized polyp numbers rather than nubbins or surface area (e.g., Lange *et al*., 2023). For instance, this method could be used in studies that aim to accurately determine the prey capture rate per polyp (e.g., Chapron *et al*., 2021), which is important to gauge the energy expenditure in feeding. Moreover, studies that assess prey capture by dissecting polyps (e.g., Tsounis *et al*., 2005) could take advantage of our methodology to obtain estimations at the colony level. In general, the present method which was developed for *C. rubrum* can be adapted to other cnidarians, providing a valuable, non-destructive approach for studying coral ecology, conservation, and restoration.

Conclusion

Our study introduced a new method for the accurate estimation of polyp numbers in *C. rubrum* based on 3D photogrammetric reconstructions of live colonies. We found an overall mean polyp density of 16.9 ± 4.5 polyps cm^{-2}, with significant differences between branching orders (i.e., higher densities in 1st-order branches). This approach constitutes an improvement with regard to existing methods as it allows a more precise estimations of polyps number, crucial for understanding reproductive dynamics and informing conservation plans. While our method is more time-consuming than the approach based on basal diameter measurements, its higher accuracy makes it ideal for more focused studies on a limited number of colonies. This method will lead to an improved understanding of *C. rubrum* ecology and offers a valuable tool for broader marine anthozoan research and conservation applications.

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