Morphological insights into the 3-Dimensional Complexity of Rhodolith Beds

Andrea Cabrito Rubau

cabrito@icm.csic.es

ICM: Institut de Ciencies del Mar  https://orcid.org/0000-0002-3429-5201

Silvia de Juan
Institut Mediterrani d'Estudis Avançats: Institut Mediterrani d'Estudis Avancats

Hilmar Hinz
Institut Mediterrani d'Estudis Avançats: Institut Mediterrani d'Estudis Avancats

Francesc Maynou
Institut de Ciències del Mar: Institut de Ciencies del Mar

Research Article

Keywords: rhodophyta, red calcareous algae, biodiversity, morphometrics, Mediterranean Sea

Posted Date: December 11th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3669592/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Version of Record: A version of this preprint was published at Marine Biology on May 3rd, 2024. See the published version at https://doi.org/10.1007/s00227-024-04437-y.
Morphological insights into the 3-Dimensional Complexity of Rhodolith Beds.

Andrea Cabrito\textsuperscript{1}, Silvia de Juan\textsuperscript{2}, Hilmar Hinz\textsuperscript{2} and Francesc Maynou\textsuperscript{1}

Abstract

The ecological importance of rhodolith beds lies in the fact that they are a structurally complex three-dimensional habitat formed by free-living red calcareous algae. The habitat complexity increases the ecological niches for cryptofauna and provide refuge for a high number of organisms, which is why they are considered biodiversity hotspots. Their structural importance is due to their great variety of complex and branching morphologies that create interstitial spaces between the nodules and increase their surface area. In this work, we study a rhodolith bed located in the Menorca Channel, formed by several species of red calcareous algae, that exhibit a great variety of morphologies that form an extensive and heterogeneous habitat. This study further explores the morphological diversity of this rhodolith bed, comparing the ‘core habitat’ (the best-preserved area) with the boundaries or ‘adjacent area’ where rhodolith density is lower. Our results suggest that all growth forms of rhodoliths (branched, pralines and boxwork) in the core habitat have higher interstitial space and are larger than the ones from adjacent zones. Moreover, we explore three-dimensional techniques to study morphological metrics that have historically been studied in two dimensions. The results of this study reinforce the use of three-dimensional measurements to provide more realistic data of their forms and the use of the interstitial space (IS) of branched rhodoliths as a proxy for habitat complexity. This study contributes to the knowledge of morphological diversity in deep well preserved rhodoliths beds from the North-Western Mediterranean Sea.

Key words: rhodophyta, red calcareous algae, biodiversity, morphometrics, Mediterranean Sea.
Rhodoliths (also known as maërl beds) are long-lived and slow-growing non-geniculate calcareous algae (Rhodophyta: Corallinaceae). They thrive in subtidal areas characterized by coarse, detritic sediments consisting of gravels and clean sands (Illa-López et al. 2023). They are distributed globally (Riosmena-Rodríguez et al. 2017) in environments where currents prevent their burial by finer sediment, and where irradiance is low but still adequate for photosynthesis (Peña et al. 2014; Carvalho et al. 2020). In consequence, the depth limit of rhodolith beds is determined by the optical properties of the water body (transparency, turbidity, sediment load), affecting light quality and intensity (Basso et al. 2017). In Mediterranean islands, rhodoliths thrive at great depths, reaching 100 m in the Mediterranean western basin (Ballesteros 1988; Barberá et al. 2012).

Rhodolith beds build hard, three-dimensional complex structures that provide habitat for other algae and invertebrates (Bordehore et al. 2003; Teichert et al. 2014). Therefore, these organisms are often referred to as ecosystem engineers (Nelson 2009). Rhodoliths can acquire various complex morphologies, from highly branched to massive round thalli (Bosence 1983) by transforming 2-dimensional homogeneous landscapes into more complex 3-dimensional ones (Foster et al. 2007). Therefore, they provide a greater array of microhabitats at different scales, from the interstitial spaces between branches that provide biogenic substrate for a variety of organisms (Bernard et al. 2019; Fredericq et al. 2019; Boyé et al. 2019), to the seascape level (Amado Filho et al. 2017). In consequence, the accumulation of rhodoliths over sandy sediments contributes to increase the local and regional biodiversity (Bernard et al. 2019; de Juan et al. 2023).

Scientific studies have identified a relationship between rhodolith morphological complexity and the associated biodiversity, evidencing that the diversity of cryptofauna is related to thallus size, growth form and structural complexity (Kamenos et al. 2004; Steller et al. 2003; Figueiredo et al. 2007). In particular, it has been demonstrated that higher cryptofauna diversity is associated with highly branched forms of rhodoliths (Steller et al. 2003; Foster et al. 2007; Meihoub-Berlandi et al. 2012; Méndez-Trejo et al. 2021). Additionally, it has been observed that high-density of rhodoliths harbor higher epibenthic and infaunal richness (Veras et al. 2020), while larger sizes of rhodoliths seem to facilitate reproduction and feeding in fishes and invertebrates (Steller et al. 2003; Gagnon et al. 2012). Therefore, the three-dimensional structure of these habitats potentially increases biodiversity and ecosystem functions in subtidal environments.

The influence of physical processes such as sedimentation or turbulence can affect rhodolith morphology and, therefore, their associated communities (Pérez-Peris et al. 2023). Trawl or dredge fishing contribute to increased sedimentation rates (Puig et al. 2015), and to physical damage of the rhodolith (e.g., breaking or chipping) caused by the fishing gear (Cabanellas-Reboredo et al. 2017; Gabara et al. 2018). Therefore, trawling activities potentially modify the three-dimensional structure of rhodoliths with impacts on the associated biodiversity (Illa-López et al. 2023). Rhodolith beds have a high conservation value; they are included in the European “Habitats Directive” (Council Directive 92/43/EEC). However, in the Mediterranean, they continue to face threats from trawling activities (de Juan et al. 2013) and current protection measures are often inadequate. Due to their slow growth (of the order of 1 mm yr−1).
1, Blake et al. 2007) rhodoliths exhibit exceptionally low renewal rates, which can result in slow recovery trajectories (Tauran et al. 2020). However, some studies show evidence of recovery after relatively short periods of protection (3 to 6 years), while in other studies the benefits of protection may be inconclusive (Barberà et al. 2017; Farriols et al. 2022).

In this study, we analysed the morphology of rhodoliths from a well preserved rhodolith bed in the Menorca Channel (Western Mediterranean, 3°45' E, 39°75' N) (Barberà et al. 2012). The main objective of the study is to assess the morphology of rhodoliths, as a proxy for habitat structural complexity. By analyzing samples from the well-preserved rhodolith bed this study (i) analyses the diversity of rhodolith morphologies; (ii) determines the allometric growth curve for each of the major morphologies; (iii) produces a discriminant rule to classify rhodoliths based on few morphometric measurements; (iv) compares 2D with 3D measurements of branched rhodoliths to examine whether simple, relatively easy to measure 2-D metrics would be sufficient to describe the morphometry of a naturally 3-D object. The understanding of the structural complexity of rhodolith beds, achieved by exploring objectives i to iv, is essential for deducing their functional importance (Stelzer et al. 2021) and to improve their protection and preservation status globally (del Río et al. 2022).

METHODOLOGY

Study area

The study area is located in the Menorca Channel between 45 and 80 m depth (Fig. 1). For this study, 5 sampling stations were selected based on previous studies (Moranta et al. 2014) that identified a well-preserved rhodolith bed that alternates with detritic bottoms and other biogenic species of algae (de Juan et al. 2023). The Menorca Channel was declared a European Site of Community Importance (LICESZZ16002) in 2016, including a trawling ban in most of the channel. Moreover, the presence of underwater communication cables crossing the channel delimits an exclusion area that has been protected from trawling for more than 4 decades.

The presence of the cables exclusion zone, in conjunction with the gently sloping bottom geomorphology (reaching a maximum depth of 100 meters) and the prevalence of strong bottom currents, creates optimal conditions for the development of rhodoliths. In consequence, the study area includes the largest identified rhodolith bed in the area, which is considered a high diversity biogenic habitat that alternates with sandy sediments (de Juan et al. 2023). As the distance from this well-preserved bed increases, the slope and currents also increase towards the east, the depth decreases towards the coast, and the north-west area is probably influenced by the sandy platform of the bay of Alcudia (Fig.1). Overlapping these changing environmental factors, the density of rhodoliths decreases as the distance from the center of the rhodolith bed increases, hereafter core habitat (Fig.1).

In this study we assess the variability of rhodolith morphology in relation to the distance to the core habitat. Sampling stations were thus classified into: (i) "Core Habitat", including samples within the cable zone (S13) and in the trawl exclusion area close to the cable zone (S15), where rhodolith cover is typically > 50%, and (ii) "Adjacent Habitat",
including samples far from the cables zone (S18 and L1) or outside the reserve area (S05), where rhodolith cover is less than 50% (Fig. 1).

Fig. 1. Map of the study area in the Menorca Channel, showing the positions of the dredges. Area limited by the cables zone (“Cables zone” in the figure) and the “Trawl Exclusion Reserve” (declared 2016, green cross-hatch).

Data acquisition

In each station, we collected between three and four dredge samples using a “Naturalist” Dredge (frame 45 x 18.5 cm, fitted to a 1 mm mesh bag). The sampling was carried out in summer 2022, with a total of 19 valid dredges recovered (See an example on Supplementary material – Fig. S1). For each dredge, a volume of approximately 7,000 cm$^3$ of the content was systematically collected in a tray. The tray was photographed in order to visually assess the proportion of live and dead rhodoliths. All the rhodoliths were then classified into nine morphotypes described following Barberà et al. (2017) (Table 1). Each morphotype was photographed and stored dry for further processing in the laboratory. Finally, a sample of sediment was stored for granulometry and carbon content analysis. The remaining content of the dredges was returned to the sea.

In the laboratory, the rhodolith morphotypes were merged into broader categories or growth forms: “Branched” (Br), “Praline” (Pr) and “Boxwork” (Bo), following Basso et al. (2016). The number and frequency of morphotype (A to I)
and category or growth forms (Br, Pr and Bo) in each dredge and station was estimated from the photographs where a total of 3877 rhodoliths were counted (see Supplementary Material Table-S1).

The granulometry of the sediment was analysed using a laser diffraction particle size analyser (HORIBA LA-950V2) after disaggregation and removal of the biogenic fraction (10% H$_2$O$_2$ solution) and dispersion in ultrasonic bath. The carbonate contents were determined by measuring the CO$_2$ volume displaced by a sample of sediment attacked with 20% HCl compared with de CO$_2$ volume displaced by a standard 100% CaCO$_3$ reference sample.

Table 1. Rhodolith growth forms (top) and description of morphotypes defined in the finer classification (A to I). Images taken from the MaCoBioS fieldwork and putative species (ongoing sequencing DNA study) to which they belong to.

<table>
<thead>
<tr>
<th>MORPHOTYPES</th>
<th>BRANCHED</th>
<th>PRALINES</th>
<th>BOXWORK</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Large size with wide branching</td>
<td>Rough, bumpy and quite irregular</td>
<td>Rounded and incrusted in other substratum</td>
</tr>
<tr>
<td></td>
<td>Small size, less and short branching</td>
<td>Rounded with small projections or short branches</td>
<td>Rounded and incrusted in other substratum</td>
</tr>
<tr>
<td></td>
<td>Small size with slender branches</td>
<td>Many short branches, spherical</td>
<td>Irregular form, incrusted in other substratum</td>
</tr>
<tr>
<td>A</td>
<td>Lithohammer sp.</td>
<td>E</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Lopholithamnus sp.</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Polyphyllia sp.</td>
<td>G</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Lopholithamnus sp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>Many short branches, spherical</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i. 2D - Measurements</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Five rhodoliths (when possible) were selected for each morphotype at each station, resulting in a total of 189 individual rhodoliths belonging to the 9 morphotypes (Supplementary material Table-S2). These 189 rhodoliths were photographed to obtain 2D morphological metrics, air-dried, weighed and stored individually for future molecular studies. For the 189 rhodoliths, a total of 15 metrics were measured. The maximum and minimum axis of the fitted ellipse (Amax and Amin, mm), surface area (A, mm$^2$), ConvexHull area (CHA, mm$^2$), shape perimeter (P, mm), the angle (a, °), the Feret length (Lmax, mm) and Feret's angle (Fa, °) were obtained measuring the rhodolith shape and making the convex hull of the shape. All of them were measured using ImageJ software (Schneider et al. 2012).

In addition, the dimensionless metrics circularity (C), roundness (R), aspect ratio (AR) and solidity (S) were determined automatically by ImageJ program according to the following formulas:

\[ C = 4\pi \frac{A}{P^2} \]

\[ R = 4 \frac{A}{\pi A_{max}^2} \]
Finally, three additional metrics were obtained:

(i) the interstitial space (IS, mm$^2$) as the missing space (area) between the convex hull area and the rhodolith surface area

$$IS = CHA - A$$

(ii) the individual dry weight (W, g) and

(iii) the shape coefficient (D, Kooijman 2010) extracted from:

$$D = \sqrt[3]{\frac{W}{2.71L_{max}^3}}$$

being $d = 2.71$ g/cm$^3$ the density of calcite.

ii. 2D/3D Measurements in branched morphotype A

Due to the structural complexity of the three-dimensional growth of rhodoliths, it is possible that 2D metrics are not sufficiently precise or accurate to provide descriptors for shape or interstitial space, properties which are directly related to the ecological functions provided by rhodoliths, which are naturally 3D objects. Because it is likely that the difference between 2D and 3D metrics is more apparent in the branched growth form, rather than the more rounded praline or boxwork, we sub-sampled (from the 3,877 stored rhodoliths) a new set of 30 rhodoliths classified as branched (morphotype A) from stations S13 and S15 to take 2D and 3D measurements (Supplementary material Fig. S2). Morphotype A was chosen for this analysis as it is the most structurally complex of all the branched species. The 30 rhodoliths were photographed and dry weighed individually. Then, the images were processed through ImageJ in the same way that in the previous section. In this case, and in order to compare the 2D and 3D information extracted, the measurements assessed are subscripted “2D”: the maximum ellipse axis ($A_{max2D}$), the Maximum Feret diameter ($L_{max2D}$), the perimeter ($P_{2D}$), the surface area ($A_{2D}$), the convex hull area ($CHA_{2D}$), the roundness ($R_{2D}$), the solidity ($S_{2D}$) and the interstitial space ($IS_{2D}$).

For 3D measurements, digital twins of selected rhodoliths were created using high-precision blue light 3D scanner (Revopoint MINI) with a precision of up to 0.02 mm and the associated programs Revoscan and Revostudio (www.revopoint3d.com). For each 3D model twin, the surface area ($A_{3D}$, mm$^2$), the maximum length from the center of mass ($L_{3D}$, mm), the volume ($V_{3D}$, mm$^3$) and the convex hull volume ($CHV_{3D}$, mm$^3$) were calculated using an ad-
hoc R script based on libraries Rvcg, geometry and MeshesTools, while the maximum Feret diameter (Lmax3D) and the roundness (R3D) were obtained using Slicer program v. 5.2.2 (Fedorov et al. 2012). With these measurements the 3D interstitial space (IS3D, mm³) was calculated as the difference between the volume of the convex hull and the volume of the rhodolith:

\[ IS_{3D} = CHV_{3D} - V_{3D} \]

and the solidity in 3D (S3D) was calculated with the relation between the 3D rhodolith volume and the 3D convex hull volume:

\[ S_{3D} = \frac{V_{3D}}{CHV_{3D}} \]

Finally, we obtain a matrix of 17 metrics measured (8 from 2D, 8 from 3D and the weight) for the 30 rhodolith samples (see Supplementary material Table S3).

Statistical Analysis

i. Morphological rhodolith composition of the study area

Differences in the proportion of growth forms (Br, Pr and Bo) between Core Habitat and Adjacent Habitat stations were analysed by means of PERMANOVA, with a model design including “Station” nested within distance from Core Habitat. The proportions were arcsin transformed for this analysis. The routine adonis2 of R package vegan was used (Oksanen et al. 2022).

ii. 2D - General Measurements analysis

After removing nine outliers (determined through exploratory data analysis with the aid of boxplots), 180 measured rhodoliths were used to fit several models. Firstly, a regression model was performed to assess the W/L relation, with L corresponding to the Feret’s diameter, by linearizing the allometric growth equation:

\[ W = aL_{max}^{b} \]

This was done for all 180 samples together and for each category or growth form (Br, Bo, Pr) separately. Then, to evaluate the significance in \( a \) and \( b \) parameter of this relation between the three growth forms, we performed a generalized linear model (GLM) from package stats (R Corer Team, 2022) fitted with a Gaussian distribution with logarithm link. To examine the possibility of overdispersion in the data, we performed a dispersion test from the package AER (Kleiber, C., 2022).

iii. 2D - Multivariate analyses

In order to visualize the measured variables and to identify if they can explain the variance between rhodolith morphologies, a correlation Principal Components Analysis (PCA) from R package stats was performed. To do that, we took the same 180 rhodoliths as in the previous section and we standardized all 16 morphometric variables.
After that, we selected the less correlated variables based on the Pearson correlation test from package \textit{corrplot} (Wei and Simko, 2021) to perform a Linear Discriminant Analysis (LDA) from package \textit{MASS} (Venables and Ripley, 2002) to examine how these variables perform as morphology classifiers. The numeric variables used in the model were: Maximum Feret diameter ($L_{\text{max}}$), Roundness ($R$) and Solidity ($S$) and the classification factor was the Growth form (Br, Pr and Bo). To fit the model, the variable $L_{\text{max}}$ was log-transformed and Solidity and Roundness were arcsin-transformed. With these transformations, the assumptions for LDA of multivariate normality and homoscedasticity between groups could not be rejected. The latter assumption was met with p-value = 0.099 or higher in Box's M-test for Homogeneity of the biotools package (da Silva, 2021). The matrix of 180 individuals was randomly re-sampled to split the data into a training data matrix (70%) to train the model and a test data matrix (30%) to test the model. To achieve better prediction accuracy, this process was repeated a total of 100 times.

iv. 2D/3D-Morphotype A measurements analysis

With the matrix of 17 morphological variables (8 in 2D, 8 in 3D and the weight) a Pearson correlation test was performed from package \textit{corrplot} (Wei and Simko, 2021) to see if the measurements of both dimensions present a high correlation between them and if it can be extrapolated from a 2D measurement to a 3D measurement. Furthermore, considering the total available surface area and the volume as morphological measurements of high biological interest for metabolic or growth studies, linear models from package `stats` were fitted between the surface area measured in 2D ($A_{2D}$) and in 3D ($A_{3D}$) and between the surface area measured in 2D ($A_{2D}$) and the total volume measured in 3D ($V_{3D}$).

All statistical analyses were computed in R (R Development Core Team 2020) under version 3.6.3.

RESULTS

i. Morphological rhodolith composition of the study area

A total of 3877 rhodoliths were recovered from 19 dredges at 5 stations. The proportion of dead (bleached) rhodoliths was generally low (30% or less), but there were significant differences between stations ($p(F)=0.01$). The only dredges with no bleached (dead) rhodoliths were three dredges from station L1, and 2 dredges from both stations S5 and S13. The highest % of dead rhodoliths was found in three dredges from S18, as one of them (dredge 4) reached 50% coverage of dead rhodoliths (Supplementary material Fig. S3).

The relative proportions of growth forms branched (Br), praline (Pr) and boxwork (Bo) are shown in Fig. 2 (left panel). Heterogeneity in the proportions of growth forms is observed among dredges within the same station (Fig. 2, left). Branched forms predominate in all dredges (between 50-90%), with lower proportions of pralines (5-40%) and boxwork (0-30%). Station L1 produced a highly heterogeneous sample, specifically in dredge 4 where boxwork forms predominate (85%) with respect to the rest. Another feature to note is that in S5, the proportion of boxwork is very low, being 0% in dredge number 3. Stations S15, S18 and S13 are more similar to each other, with S13 having more
similar proportions of boxwork and pralines between samples than S15 and S18, where there is a higher percentage of pralines than boxwork.

![Fig.2: (right) Ternary plot of sediment composition and (left) Ternary plot of rhodolith growth forms composition. Each dredge represented and color-coded following the stations sampled.](image)

As for the composition of the sediment, all the dredges have a similar composition (gravel contents 85% or higher) (Fig. 2 left). S15 and S18 are characterised by the absence of silt and clay, although they have varying proportions of sand. The dredges of station L1, together with those of S5 and S13, do present silt and clay but never exceed 15% of the total composition that is mainly sand (60-90%). The proportion of calcium carbonate measured in the dredges (Supplementary Material Table S4) was very high (over 90%) and similar among all dredges and stations.

Overall, of the 3877 rhodoliths, 69.2% had a branched growth form, 19.1% were pralines and 11.8% were boxwork. The most abundant morphotypes in general were C (37.53%), B (14.70%), E (14.24%) and A (10.14%) (Fig. 3) (see Table 1 for reference to the morphotypes). The proportion of branched rhodoliths was generally higher than 50% in all dredge samples, except in 3 of the 19 dredges.

The proportion of growth forms differed significantly by the distance to core habitat (PERMANOVA p(F) = 0.002). The difference was mainly due to the relative importance of non-branched forms (30% or higher) in dredges from the adjacent habitat L1 and S18 stations. The interaction between distance to core habitat and station was also significant (p(F) = 0.001); denoting heterogeneity within the stations.

The branched morphotype A was dominant (between 32 and 39% of abundance) in station S13 located in the core rhodolith bed and this site had also a high proportion of branched morphotype B (12 to 27%). The morphotype A decreases from S13 to S15 and to L1, being absent in S18. The branched Morphotype C has, in general, a high
representation in all stations, being the most abundant in S18. The branched morphotype D was mostly represented in S5, appearing almost exclusively in this station.

The non-branched morphotypes were never dominant, but appeared relatively frequently in dredges from stations L1, S15 and S18 (both within core and adjacent habitats). The praline morphotype E is represented equally in all stations, except for S5 where F was the more predominant morphotype. The praline morphotype G only occurred in 1-2 dredges from S13, S15 and S18. The boxwork growth forms (morphotypes H and I) were proportionally represented in S13, S18 and L1, while morphotype H was predominant in S15 (Fig.3).

![Rhodolith morphotypes proportions](image)

Fig.3: Percentage of morphotypes (A to I) coverage in each station (left). Total proportions of each growth form (Br, Pr and Bo) considering all stations together (right).

### ii. 2D- Morphological measurements

By analysing the set of 180 rhodoliths for which morphometric measures were obtained, a PCA helped reveal the main morphometric variables describing the 2D rhodolith shape. These results showed that 46.7% of the variance could be explained by the first dimension while the second dimension explained a 25.2% of the variance. Fig. 4 shows that the most differentiated group is the one formed by the branched forms. Praline and boxwork rhodoliths overlapped in the biplot.
Fig. 4: Principal components analysis (PCA) of individual rhodolith metrics from the Menorca Channel. A: area; a: angle; Amax: Maximum Ellipse Axis; Amin: Minimum Ellipse Axis; AR: Aspect Roundness; C: Circularity; CHA: Convex Hull Area; D: Shape coefficient; Fa: Feret angle; IS: Interstitial Space; Lmax: Maximum Feret diameter; P: Perimeter; R: Roundness; S: Solidity; W: Weight.

With the variables represented in the PCA, the Maximum Feret diameter (Lmax), both area (A) and convex hull area (CHA), weight (W) and the maximum and minimum ellipse axis (Amax and Amin) were highly correlated with each other and defined the first dimension in the X-axis. Shape coefficient (D), Circularity (C), Solidity (S) and Roundness (R) appeared correlated among them and defined the second dimension in Y-axis Aspect Ratio (AR) and Roundness (R) were negatively correlated, indicating that branched forms have lower roundness (and higher aspect ratio) than pralines and boxwork rhodoliths.

Because of the high correlation between several shape measurements, only a representative subset (Lmax, IS, W, A, D and P) was used to examine differences between samples recovered from the core habitat and the adjacent habitat. We show in the boxplots from Figure 5 that boxwork growth forms tend to be bigger, larger and heavier than praline and these than branched forms. There is a clear effect of the core habitat in terms of interstitial space, maximum Feret diameter and area being all of them higher in all growth forms in the sites within the core habitat compared to the sites within the adjacent habitat.
Figure 5: Boxplots for a subset of six morphological variables both in core habitat and in adjacent habitat for the three growth forms of rhodoliths. From left to right at the top: Interstitial space (IS), Maximum Feret diameter (Lmax) and Perimeter (P). From left to right at the bottom: Area (A), Sphericity coefficient (D) and Weight (W). All variables were log transformed.

As a result of the linear models fitted for the relationship between weight and the Lmax, the allometric growth curves \( W = a L^b \) for each rhodolith growth form were estimated (Fig. 6). The parameter \( a \) was significantly different between the Boxwork and Branched curves (p value < 0.001), but not between Praline and Branched curves (p value = 0.097). The parameter \( b \) showed no significant differences between growth forms. Therefore, the three groups show a similar allometric growth rate, despite the groups having significant different weights for the same length. The test for overdispersion was significant (p-value < 0.001), but with a low value of dispersion 1.80.
Fig. 6: Rhodolith allometric growth curves, representing the relation between $L_{\text{max}}$ and $W$ for each morphotype or growth form.

### iii. Discriminant analysis

A linear discriminant analysis was used to build a classifier to predict growth forms from a set of relatively easy to measure variables. The results of the LDA showed that, although the three groups had a large degree of overlap, they can be distinguished statistically. The variables with the greatest weight in defining the first dimension of the LDA were the *Roundness* ($R$) and the *Solidity* ($S$) while the *Maximum Feret diameter* ($L_{\text{max}}$) has the greatest weight in the second dimension. The first LDA axis explains 96.6% of the variance. This first direction separates the rhodoliths with greatest solidity (less branching), greatest roundness and relatively large Feret’s diameter in the positive axis from the ones with less solidity (more branching) and less roundness in the negative axis. The second LDA dimension explains the rest (3.37%) of the variance and is determined for the *Maximum Feret’s diameter* with longer rhodoliths in the positive axis and shorter in the negative one. Thus, the first dimension separates more clearly the bigger, the most rounded and the most solid rhodoliths (both pralines and boxwork growth forms) from the Branched forms. (Fig. 7). The classification equations based on *Maximum Feret diameter*, *Roundness* and *Solidity* are:

\[
\text{LDA1: } 1.996 \times L_{\text{max}} + 0.252 \times R + 0.274 \times S
\]

\[
\text{LDA2: } 21.232 \times L_{\text{max}} + 0.123 \times R + 0.147 \times S
\]
Applying the estimation model to the data test, we found that the classification rate was correct in 63.15% of the cases. Finally, after reproducing the analysis 100 times and running the predictive model, the classification rate for a new rhodolith was 69.5%, being the percentages of success for each growth form different (Table 2). The best classified form is always the Branched form with 88.13% of success. The more common misclassification is when classifying a Boxwork as a Praline (40.87% of times) or a Praline as a Boxwork (30.73% of times).

Table 2: Table of predictions when classifying rhodoliths according to the variables considered in the LDA.

<table>
<thead>
<tr>
<th>Predicted Class</th>
<th>Branched</th>
<th>Praline</th>
<th>Boxwork</th>
</tr>
</thead>
<tbody>
<tr>
<td>Branched</td>
<td>0.8813</td>
<td>0.096</td>
<td>0.0225</td>
</tr>
<tr>
<td>Praline</td>
<td>0.1364</td>
<td>0.5563</td>
<td>0.3073</td>
</tr>
<tr>
<td>Boxwork</td>
<td>0.0608</td>
<td>0.4087</td>
<td>0.5304</td>
</tr>
</tbody>
</table>

Fig. 7: Representation of the Linear Discriminant Analysis (LDA).

iv. 3D – Morphological measurements in a subset of morphotype A
For each of the 30 rhodoliths selected, a 3D model twin of the complete rhodolith shape (Fig. 8) and its corresponding measurements were obtained. The correlation test showed that both sets of metrics (2D vs. 3D) are highly correlated (Supplementary material Fig. S5) especially those that are the same but in the other dimension (e.g., Area 2D and Area 3D) but also between those in the same dimension (e.g. Perimeter 2D with Area 2D or Volume 3D with Interstitial space in 3D).

The linear models (with intercept at 0) fitted between 2D area and 3D area and between 2D area and the volume, shows that both relations are highly correlated (R=0.99 and R=0.98, respectively) (Fig. 9). The 3D area resulted in 4.27 times the 2D area (Fig. 9a) while the volume is 6.45 times the 2D area (Fig. 9b). These equations can be useful in estimating the ecologically meaningful quantities (area, volume) in 3D from their equivalent quantities in 2D, which are much easier to measure.

Fig. 8: Example of 3D model twin from a morphotype A rhodolith.
**DISCUSSION**

Rhodoliths enhance biodiversity and ecosystem functions in subtidal soft-bottom environments by transforming 2D landscapes into complex 3D structures, with rhodolith morphological complexity influencing the diversity of associated cryptofauna, epibenthic richness and the facilitation of reproduction and feeding of marine organisms.

The present study has characterized the morphological composition of a well-preserved rhodolith bed in the continental shelf of the Menorca Channel (western Mediterranean). This site was selected due to its good conservation status, with a well-developed rhodolith bed (i.e., above 50% rhodolith coverage), and adjacent locations with decreasing rhodolith cover as it alternates with detritic sands. The results have shown that the proportion of dead rhodoliths was generally low (less than 30%) supporting the hypothesis that the rhodolith bed found in the Menorca Channel is well preserved. Although branched forms predominate in all sites, we observed small scale heterogeneity in the proportions of growth forms and even in the proportions of morphotypes. The heterogeneity observed agrees with previous studies in Mediterranean rhodolith beds (i.e, Bracchi et al. 2022) and particularly in the Menorca Channel (Farriols et al. 2022). The present study, together with preliminary results from ongoing molecular analysis, shows that the Menorca Channel rhodolith bed is a multispecific rhodolith bed where we found, at least, 10 species,
for example: Lithothamnion corallioides, Lithothamnion cf. valens, Roseolithon cf. crispatum, Phymatolithon calcarenum, Phymatolithon lusitanicum, Lithophyllum cf. racemus, Spongites cf. fruticulosus and a record of species belonging to Sporolithon and nine types of morphologies (A to I) with some species forming more than one morphology such as Lithothamnion corallioides (forming morphologies C and F) or Phymatolithon calcarenum (morphologies C and D). In general, the proportion of branched growth forms, and particularly the proportion of morphotype A that belongs to Lithothamnion cf. valens, was more abundant in the core habitat. More interestingly, a decrease in morphotype A from S13 (in the cables zone) to the edge of the exclusion zone in S18 was observed suggesting that this species is a potential candidate indicator of the conservation status of the habitat.

The specimens from the well-preserved rhodolith bed (Core Habitat) were larger and with more interstitial spaces (even in the praline morphologies). It is likely that rhodoliths at the edge of the core habitat are less well-preserved, due to suboptimal environmental conditions combined with being exposed, directly or indirectly, to trawling. Both natural and anthropogenic processes may be responsible for increased fragmentation of branched growth form, resulting in smaller and less ramified (more solid) branched rhodoliths (Barberá et al. 2012). Even so, for the three growth forms, the value of morphological indicators is higher in the Core Habitat than in the adjacent areas. In particular, branched rhodoliths were more branched and had higher interstitial space (IS) in samples from the Core Habitat, suggesting that good habitat condition contributes to maintain the architectural complexity of this habitat and hence the ecosystem function related to biodiversity is likely to be enhanced. The spaces between branches provide microhabitats for diverse assemblages of other algae, epiphytes and invertebrates including sponges, cnidarians, bryozoans, tunicates, polychaetes and mollusks (Phillips, JA, 2023) (see examples found in Supplementary material – Fig.S05). Thus, our results support the idea proposed by Jardim et al. (2022) of using the rhodolith interstitial space (or Solidity as equivalent indicator) as proxy for habitat complexity.

The high correlation identifies between 2D and 3D metrics for morphotype A demonstrates that 2D metrics may yield useful indicators that are quicker to obtain and potentially effective to be incorporated in routine monitoring of rhodolith beds. 3D measurements resulted to be time-consuming and they can be estimated to a good approximation through 2D metrics if 2D to 3D conversions have been estimated previously. However due to the relation obtained, we strongly recommend using the linear relations between measures as a method to rapidly obtain a total photosynthetic surface. This measure is important for calculating the photosynthesis rates for this morphotype, as well as to obtain the rhodolith volume and quantify the calcium carbonate produced by this morphotype (Dulin et al. 2020). These types of indicators are important as a proxy for measuring the ecosystem function of rhodolith beds (Amado-Filho et al. 2017).

In algal physiology studies, it is common to use dry weight to standardize indicators such as biomass of epiphytes by unit mass of maërl or in the estimates of respiration and production (photosynthesis). However, in multi-specific rhodolith beds, as in our case study, it is often difficult to individualise different morphologies (as shown in the results of the discriminant analysis). Individual weight may vary among morphologies, for the same longitudinal size, and even within individuals of the same morphology because of the weight of organisms forming the nucleus of some of
the rhodoliths (bryozoans, polychaetes, some boring organisms) (see an example in Fig.10) (Pérez-Peris et al. 2023).

For these reasons we propose using the area corresponding to the thallus surface in 3D as a more realistic measurement to determine metabolic rates or to standardize cryptofaunal abundance. Rhodolith volume is important as a measurement related to carbon retention and is a metric that can only be obtained with sufficient precision with 3D measurements.

In conclusion, the intricate morphological diversity observed in rhodolith species, coupled with their significant role as habitat-forming organisms and potential carbon reservoirs, underscores the need for sustained investment in resources and research efforts. By identifying key measures of morphological complexity, as well as assessing parameters such as photosynthetic surface and carbonate volume, we gain valuable indicators of the rhodolith bed's status. This research contributes to our comprehensive understanding of how the structural complexity of rhodoliths enhances ecosystem functioning, emphasizing the ongoing importance of advancing our knowledge and developing additional morphological indicators for these vital species.

**Fig. 10:** (Left) Red calcareous algae growing and completely recovering a bryozoan. (Right) broken branch of a rhodolith from morphology A, with a bryozoan found inside.

**REFERENCES**


ACKNOWLEDGMENTS

The authors are very thankful to Laia Illa-López and Géraldine Pérez for their back up during the material recollection and classification and also to Julia Castro for her support on creating the 3D digital twins of the rhodoliths. This work is developed on the framework of the EU H2020 project “MaCoBioS” (contract nº 869710). This work is contributing to the ICM’s ‘Center of Excellence’ Severo Ochoa (CEX2019-000928-5) and to the ‘Maria de Maetzu’ accredited to the IMEDEA (CEX2021-001198) SdJ was supported by a Ramon y Cajal postdoctoral grant funded by the Ministry of Science and Innovation (Plan Estatal I+D+I, 2017-2020; grant no. RyC2020-029062-l).

FUNDING

The authors acknowledge funding from the EU H2020 project “MaCoBioS” (contract nº 869710).

AUTHOR INFORMATION

Authors and Affiliations

Andrea Cabrito & Francesc Maynou

Institut de Ciencies del Mar (ICM-CSIC), Passeig Marítim de la Barceloneta 37-49, Barcelona, Spain

Institut Mediterrani d’Estudis Avançats (IMEDEA-CSIC), Carrer Miquel Marques 21, Esforles, Spain
Silvia de Juan & Hilmar Hinz

Contributions

AC: Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing, Visualization. SdJ: Conceptualization, Writing – review & editing. HH: Conceptualization, Writing – review & editing. FM: Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing, Funding acquisition.

Corresponding author

Correspondence to Andrea Cabrito, cabrito@icm.csic.es

ETHICS DECLARATIONS

Conflict of interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.
Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterial.docx