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Caulerpa cylindracea Spread on Deep Rhodolith Beds Can Be Influenced by the Morphostructural Composition of the Bed

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Abstract: The green alga *Caulerpa cylindracea* Sonder (Chlorophyta; Bryopsidales) is one of the most invasive alien macroalgae in the Mediterranean Sea, where it is also spreading on rhodolith beds, an important biogenic assemblage typical of deep substrates. Despite the importance of rhodoliths, data on the competitive interactions with *C. cylindracea* are still scarce. To deepen the knowledge on the topic, *C. cylindracea* occurrence on the rhodolith bed of Capo Carbonara Marine Protected Area (Italy) was explored. Quantitative analyses of videoframes obtained from Remote Operated Vehicle records in three different MPA sites, Is Piscadeddus, Santa Caterina, and Serpentara, allow for estimates of both the cover of rhodoliths (considering the main morphotypes) and of *C. cylindracea*, as well as their competition. All sites showed a well-developed rhodolith bed, although some differences were highlighted in their composition in terms of morphotype, shape, and dimension of rhodoliths, as well as in the *C. cylindracea* cover. In particular, Santa Caterina appeared to be the site with the highest mean total cover of rhodoliths (68%), and of *C. cylindracea* (25%). The obtained results suggest that different competitive interactions occur between *C. cylindracea* and rhodolith beds, in relation to the morphostructural composition of the latter and in response to environmental conditions that affect rhodolith bed composition.

Keywords: temperate mesophotic ecosystem; rhodoliths; *Caulerpacee*; competitive interactions; Capo Carbonara MPA

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1. Introduction

The genus *Caulerpa* (Chlorophyta: Bryopsidales) encompasses a group of marine green siphonous algae widely distributed in tropical and subtropical regions [1], some of which have recently seriously invaded the Mediterranean Sea [2]. This genus includes about 104 species [3], and it is considered one of the most widespread, conspicuous, and abundant groups of seaweeds worldwide [4]. In the last few decades, *Caulerpa* species have attracted considerable research interest due to the presence of some particularly invasive algae among them. In particular, *Caulerpa taxifolia* (Vahl), C. Agardh, and *Caulerpa cylindracea* Sonder have significantly expanded their range of distribution in the Mediterranean Sea, and, currently, *C. cylindracea* is counted among the most invasive alien macroalgae in the basin [2,5,6]. This species was initially considered a Lessepsian migrant, but genetic analysis has recently proven its southwestern Australian origin [4]. *C. cylindracea* is found on all kinds of soft and hard substrates at depths ranging from 0 to 70 m, where it currently represents one of the most relevant threats to biodiversity conservation [7], actively competing with several native species [8] and, therefore, significantly modifying benthic assemblages [9]. In SW Australia, *C. cylindracea* mainly occurs intermixed with other algae without forming monospecific meadows [4]. Instead, it is present in the Mediterranean Sea with continuous and quite dense meadows in different photophilic and sciaphilic benthic assemblages, including rhodoliths [2], which are currently considered priority marine benthic habitats of high conservation interest for the European Community [9]. Certainly, they represent important biodiversity hotspots in the basin and play a key role in the carbonate budget, epitomizing a non-renewable resource because of the slow growth rate and related carbonate deposition [10,11]. For these reasons, they are protected within the framework of the United Nations Program's Mediterranean Action Plan and included in the monitoring program of the Marine Strategy Framework Directive 2008/56/EC of the European Community. Particularly, two of the main species structuring this habitat in the Mediterranean, *Phymatolithon calcareum* (Pallas) Areschoug and *Lithothamnion corallioides* (P. Crouan and H. Crouan), are included in the European Community Habitat and Species Directive 92/43/EEC (Annex V) (12).

Rhodoliths are unattached nodules, mostly consisting of non-articulated coralline algae [11,12], slow-growing and long-lived organisms that act as ecosystem engineers by secreting high-Mg carbonate, forming both mobile (i.e., rhodoliths) and stable, hard substrates (i.e., coralligenous algal reefs). Living rhodolith beds are formed by the aggregation of living (>10%) rhodoliths [12–14]. From an ecological point of view, they significantly increase the structural complexity of the seabed in comparison with soft sediments, thus providing more space, nurseries, refuges, and resources for cryptofauna, macroalgae, and fishes, and thus enhancing species richness and abundance [13–15].

Rhodoliths can develop without any type of nucleus or around skeletal or non-skeletal nuclei, usually producing a quite concentric arrangement of their thalli [10,16]. Rhodoliths show a wide variety of shapes that can range among ellipsoidal, discoidal, and spheroidal [17]. Their 3D structure serves as a habitat for a diverse associated community and as a local hotspot of biodiversity, providing a suite of ecosystem goods and services [18]. They are distributed worldwide, particularly in the Mediterranean Sea [12]. Although some rhodolith makers [e.g., *Lithophyllum dentatum* (Kützing) Foslie and *Lithophyllum racemus* (Lamarck) Foslie] can thrive in shallow waters, rhodolith beds typically occur within the range of 30–75 m of water depth, generally located around islands and capes, on flat or gently sloping areas [12,14]. According to [19], the cover of live rhodoliths in a bed may affect the diversity and abundance of associated species; In terms of overgrowth, fleshy algae can pose a significant threat to the conservation of rhodolith beds. Interactions between fleshy and coralline algae can seriously influence the dynamics of marine ecosystems, playing a role in nutrient availability and habitat characteristics for higher trophic levels [20]. Indeed, such interactions affect rhodoliths by reducing water flow and light conditions, increasing sedimentation, and reducing gas exchange, all of which can slow rhodolith growth and enhance further macroalgal overgrowth. The interactions between rhodoliths and *Caulerpa sertularioides* Gmelin were analyzed by [21], another invasive *Caulerpa* species that has invaded deep substrates in the last decade, competing directly with native algal flora along Costa Rica's coasts. According to [22], studying competitive interactions can be particularly useful in invasion biology and ecology [23], as they represent a major driver of ecosystem dynamics. Specifically, considering the role of species interactions in invasion dynamics, two main hypotheses are currently taken into account: the enemy release hypothesis (according to which exotic species spread because they lack enemies in their invasion ranges [24,25]) and the biotic resistance hypothesis (according to which introduced species are limited in their spread by strong interactions with native species [23]).

With regard to *C. cylindracea*, a recent local heavy overgrowth of this species on rhodolith beds was reported for other Mediterranean areas, and it appeared to be facilitated by anthropogenic impacts that could directly affect the rhodolith assemblage [26]. Anyway, the data available in the literature regarding the competitive interactions between *C. cylindracea* and rhodoliths in the Mediterranean Sea are still very scarce [2]. The aim of this research project is to try to fill in this gap of knowledge by focusing on the interactions between a heterogeneous and relatively deep rhodolith bed [16] in Capo Carbonara MPA and *C. cylindracea*, the presence of which was first signalled [16] but never deeply investigated in the area.

2. Materials and Methods

2.1. Study Area

The study was conducted in the Marine Protected Area "Capo Carbonara" along the Southern Sardinian Coasts (Italy) (Figure 1). The protected area extends from Capo Boi (W) to the area in front of Serpentara Island (E) on the whole continental shelf (maximum average water depth of 125 m), where rhodoliths are indicated to be particularly abundant [16,27]. The MPA is characterized by areas with a different level of protection (total reserve zone, general reserve zone, and partial reserve zone, with, respectively, a decreasing level of protection). In the area, three different sites were identified in the general reserve zone of the MPA, at a distance of >200 m (Figure 1): the Is Piscadeddus area (from here on Is Piscadeddus) towards W (45 m of depth), the Santa Caterina shoal (from here on Santa Caterina) in the middle (40 m of depth), and the Serpentara Island (from here on Serpentara) towards E (49 m of depth) (Figure 1).

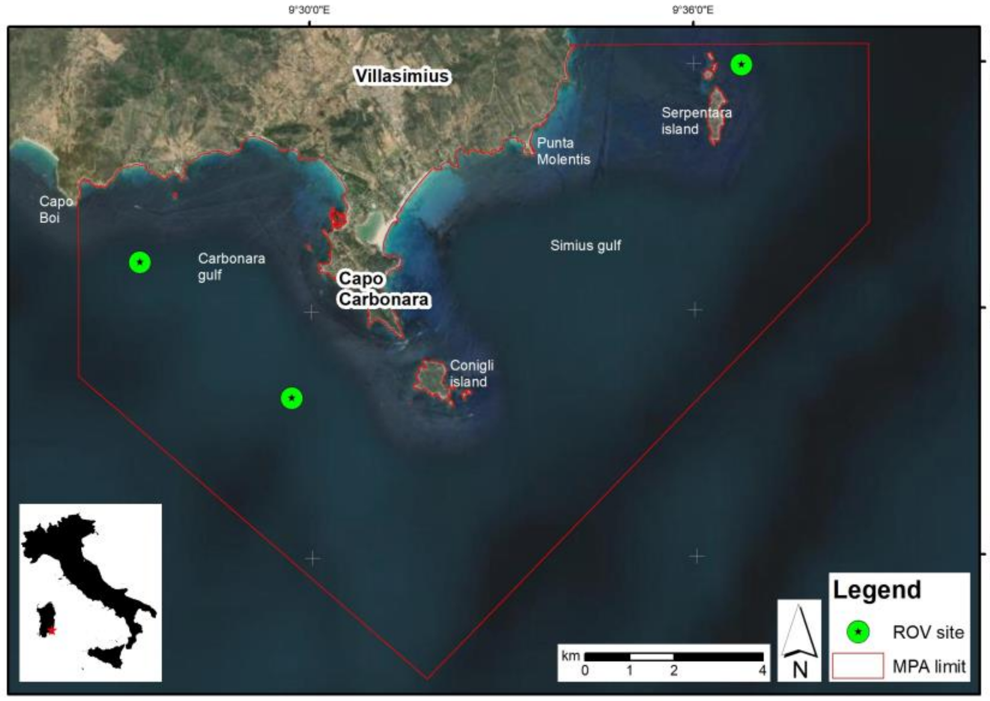

Figure 1. The Marine Protected Area "Capo Carbonara" (limit in red) is located in the southern part of Sardinia Island (Italy), with ROV sites (green dots). Service Layer Credits: Esri Digital Globe, GeoEye, EarthStar Geographic, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community.

2.2. Image Collection and Analysis

Each site was investigated by a Remotely Operated Vehicle (ROV, Steelhead Seamore, owned by the University of Milano-Bicocca, Milan, Italy), equipped with two different video cameras. ROV videos were collected between 40 and 60 m of water depth. At each site, three transects of 200 m were considered. Quantitative image analysis (according to the sub-squared method, [28]) on photoframes randomly extracted from the ROV videos was performed. In detail, ~6 m^2 of substratum for each photoframe was considered (n° = 20 *per* transect) to evaluate the abundance of both the rhodoliths and the invasive seaweed along each transect (Figure 2).

Figure 2. Examples of the photoframes extracted from videos. Red bars indicate the scale for each image.

On each frame, a subsampling of ~1 m^2 of area was selected, on which a grid of twenty-five sub-quadrats (5 × 5) was superimposed using ImageJ software [29]. Each sub-quadrat has been scored, simultaneously estimating the abundance of *C. cylindracea* and of living/dead rhodoliths (considering their colour, as suggested by [30]). Moreover, among living rhodoliths, each sub-quadrat was scored again based on the apparent morphotypes (free-living branches, pralines, or boxwork *sensu* [14,31]). According to [31], boxworks (Br) are mostly irregular multispecific nodules with internal macroscopic voids filled with sediment, due to periods of growth interrupted by episodes of partial burial and/or overturning. The nucleus of a Bw consists of biogenic remains or of a small pebble. Pralines (Pr) are mono/oligo-specific compact nodules with a biogenic or lithic nucleus bearing variously developed protuberances at their surface. Finally, branches are monospecific rhodoliths lacking a macroscopic nucleus and possibly characterized by a high protuberance degree [32].

For the analysis, the attributed scores were between 0 and 4. Scores have been attributed in this way: 0 = absence; 1 = 25%; 2 = 50%; 3 = 75%; and 4 = 100% of cover. The total % cover for both *C. cylindracea* and rhodoliths (also considering the three main morphotypes) values for each frame were then obtained by adding up the 25 resulting values recorded for each sub-quadrat [28].

2.3. Statistical Analysis

Data regarding the cover of the substratum by *C. cylindracea* and rhodoliths, respectively, were analyzed by means of univariate statistical analysis techniques (ANOVA and SNK test) [33]). In particular, one one-way ANOVA was run for *C. cylindracea*, considering Site (3 levels) as a fixed factor. For rhodoliths, instead, one one-way and two two-way ANOVAs were run to test for differences in the total cover and in the cover in relation to the health status (living vs. dead) and the morphotypes (boxworks vs. branches vs. pralines), respectively. For this purpose, Site (3 levels) and, alternatively, health status (2 levels) and morphotypes (3 levels) were considered fixed, orthogonal factors. Cochran's test was used prior to ANOVA to check for homogeneity of variance, while SNK tests were used for posteriori comparisons of means [33]. All tests were performed using the software GMAV5 [34]. Finally, Spearman's rank-order correlation coefficient (rs) was used to test for a possible correlation between the abundance of *C. cylindracea* and that of total and living rhodoliths, respectively.

3. Results

3.1. *C. cylindracea* Cover

Caulerpa cylindracea was found to be present in all three investigated sites, but some significant differences in its abundance were overall highlighted among them. In particular, the macroalga was significantly more abundant in Santa Caterina, where it reached a mean percent cover of the substratum of about 25%. On the contrary, both in Serpentara and Is Piscadeddus, *C. cylindracea* was recorded with a mean cover that did not exceed 1% of the substratum, and no significant differences were highlighted between these last two sites (ANOVA: $F_{(2,6)} = 48.19$; $P = 0.0002$; SNK: Santa Caterina > Serpentara = Is Piscadeddus) (Figure 3).

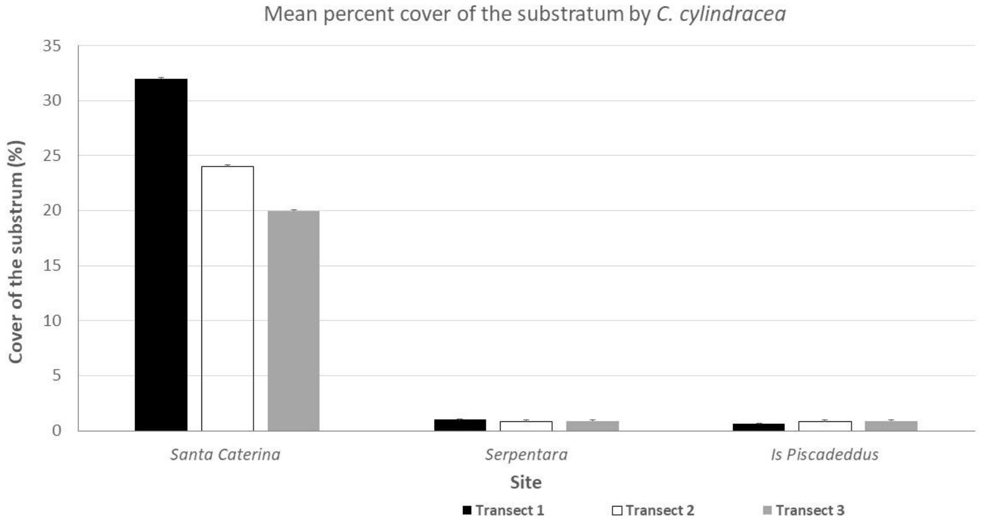

Figure 3. Mean (±SE) percent cover of the substratum by *C. cylindracea* in each of the three transects considered for the three sites.

3.2. Rhodolith Cover

All three sites showed a well-developed rhodolith bed, though there were some relevant differences in terms of cover and morphotypes composition that were observed overall. First, considering both living and dead rhodoliths, Santa Caterina appeared to be the site with the highest cover value (68%), followed by Is Piscadeddus (58%) and Serpentara (51%). However, the ANOVA test did not support statistically these differences in the total percent cover of the substratum (ANOVA: $F_{(2,6)} = 1.16$; $P = 0.3749$). The living percent cover in both Serpentara (47%) and Santa Caterina (49%) appeared to be significantly higher than that of dead rhodoliths (20% and only 1.2%, respectively, in the two above-mentioned sites), while for Is Piscadeddus the difference between living (37%) and dead (20%) rhodoliths was less pronounced (Figure 4a; Table 1). Incidentally, the percent cover of living rhodoliths at the three sites appeared to be similar, whereas the dead cover was significantly lower in Santa Caterina (1.2%) than in the other two sites (20% for both Serpentara and Is Piscadeddus), as confirmed also by the statistical analysis performed (Figure 4a; Table 1). Finally, considering the main morphotypes composing the bed in the three sites, pralines had the highest cover value if compared to boxworks and branches in all of them, although, on the whole, they appeared more abundant in Santa Caterina (78%) and in Serpentara (86%), than in Is Piscadeddus (33%; Figure 4b; Table 1). Both branches and boxworks, instead, have a significantly low cover (<5%). Finally, the cover of branches in Is Piscadeddus, although limited in terms of absolute value (4%), contributed statistically to distinguish this site from the other two, where it does not exceed the value of 2% (Figure 4b; Table 1).

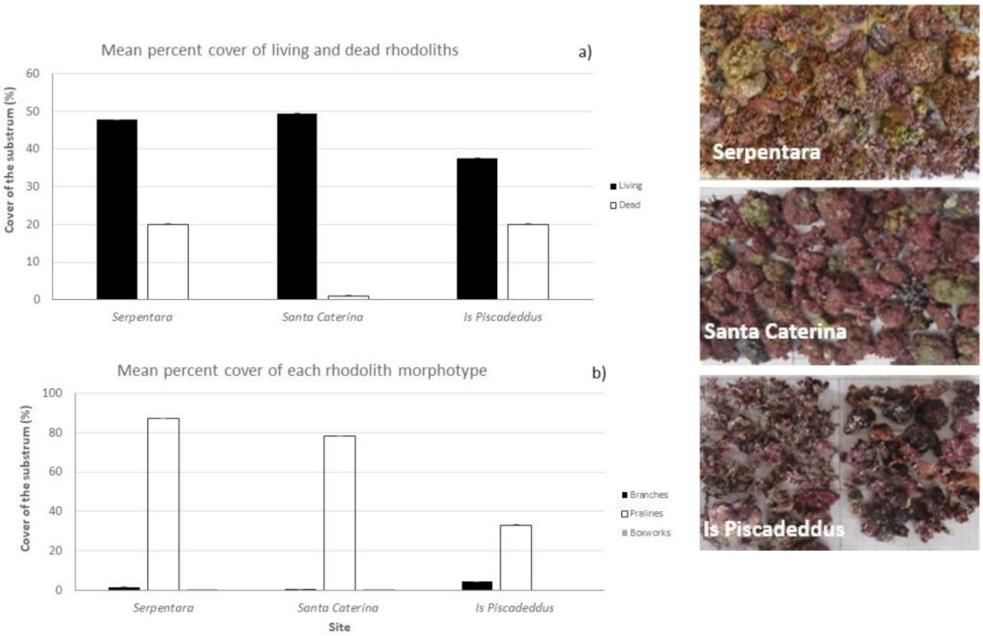

Figure 4. Mean (±SE) percent cover of the substratum by rhodoliths in relation to their health status (**a**) and morphotypes (**b**) in each of the three transects considered for the three sites. On the right, an example of a rhodolith sample for each site is shown, with the different morphotypes clearly visible.

Table 1. Results of ANOVA and SNK were used to test for significant differences in rhodoliths' percent cover of the substratum in the three investigated sites (Sc = Santa Caterina; Se = Serpentara; IP = Is Piscadeddus) in relation to their health status (L = living; D = dead) and present morphotypes (Br = branches; Bw = boxwork; Pr = pralines). Significant results are given in bold. Moreover, the SNK test for the significant interactions between Site × Health Status and Site × Morphotype is also presented.

ANOVA **(a)**							
		Health Status			***Morphotype***		
Source	**df**	**MS**	**F**	**P**	**MS**	**F**	**P**
Site	2	1114.5060	1.20	0.3359	722.5744	22.19	**0.0000**
Status	1	4309.0139	45.02	**0.0000**	12,706.1219	390.26	**0.0000**
Site × Status	2	372.4735	3.89	**0.0498**	870.2610	26.73	**0.0000**
Residual	12	95.7169			32.5582		
Total	17						
		Cochran's Test = 0.7124				**Cochran's Test = 0.7124**	
	SNK ***Site × Status***				**SNK** ***Site × Morphotype*** **(b)**		
Site	**Status**	**Status**	**Site**	**Site**	**Morphotype**	**Morphotype**	**Site**
SC	Liv. > Dead	L	SC = Se = IP	*SC*	Br = Bw < Pr	Br	SC = Se = IP
Se	Liv. > Dead	D	SC = IP > Se	*Se*	Br = Bw < Pr	Pr	SC = Se < IP
IP	Liv. = Dead			*IP*	Br = Bw < Pr	Bw	SC = Se = IP
	E.S. = 0.2345						

3.3. *Correlation Rhodoliths—C. cylindracea Cover and Abundance*

Spearman's rank-order correlation coefficients highlighted the existence of a strong positive correlation (0.9122) between the total cover of rhodoliths and that of *C. cylindracea* in all the considered sites. On the contrary, no clear correlation was underlined by the above-mentioned coefficient (0.5831) when considering only the living portion of rhodoliths. Finally, a remarkably strong negative correlation (-1) was highlighted between dead rhodoliths and *C. cylindracea* abundance on the substratum in this case at all three investigated sites.

4. Discussion

The results of this study are particularly relevant from both an ecological and a management point of view, as they provide some interesting insights on the ability of *C. cylindracea* to settle and develop in quite peculiar habitats, such as rhodolith beds, while also focusing on their competitive interactions.

First, the data here provided represent one of the first reports of *C. cylindracea* in the rhodolith beds of Capo Carbonara MPA. Indeed, despite the fact that *C. cylindracea* has already been observed at similar depths [2], very little information is currently available in the literature on its presence on rhodolith beds [35]. Only very recently, [26] provided some interesting information on the cover of *C. cylindracea* on the rhodolith beds of the Tremiti Islands. There, the macroalgae not only caused a significant shading related to its settling on the rhodoliths but also influenced the quantity and biochemical composition of sedimentary organic matter across the beds.

Even if [36] asserted that where rhodoliths occur, they provide a favourable surface for colonization and other seaweeds may occur, the available data on the presence of *Caulerpa* in oceanic rhodolith beds are mainly referred to other species than *C. cylindracea* (e.g., [21]) and take into account significantly more superficial beds (20 m depth) if compared with the ones considered in this study (40–60 m depth). In some cases, the abundance of the macroalga can also increase significantly in shallow rhodolith beds, generating blooms, while such events are not commonly described for deeper beds. In particular, this is the case described by [37], who observed considerable blooms of *Caulerpa sertularioides* on quite superficial rhodolith beds along the Pacific and Mexican coasts. Such events appeared to be mainly temperature-related, implying that, if increases in seawater temperatures are going to gradually affect deep waters, as stated by [38], because of the ongoing climatic changes, despite the limited light, *C. cylindracea* could become responsible for massive blooms, affecting rhodoliths in the Capo Carbonara MPA. Indeed, according to [39], temperature, along with light, is a key environmental parameter in regulating the distribution and abundance of several alien species of macroalgae, including *C. cylindracea.*

Even though, according to the literature, green algae such as *Caulerpaceae* are expected to be more abundant in shallow waters, they seem to find quite suitable conditions to settle and develop in the mesophotic zone [40]. Capo Carbonara rhodolith beds, indeed, are quite deep, and, therefore, it is reasonable to consider primarily light as a key factor determining seaweed distribution, in particular that of green macroalgae, as they appear to be spectrally limited. Indeed, green seaweeds, having chlorophyll as the main photosynthetic pigment, are presumed to be scarce in deep waters, where only a few species successfully adapted to tolerate dim light conditions can be found [41]. Specifically, [42] observed that chloroplast streaming and rhizoid initiation of other *Caulerpaceae* exhibit marked patterns of light availability, but they were nonetheless able to colonize quite low-light environments, significantly modifying their growth rates.

For this reason, despite the very high invasive potential of *C. cylindracea*, which is currently considered one of the most threatening invaders in the Mediterranean Sea [5,43], it is plausible that specific light penetration conditions occurring at the study sites control *C. cylindracea* abundance in some way. Indeed, low light is among the major factors negatively affecting the canopy development of *C. cylindracea* [36,44]. Actually, *C. cylindracea* appeared to be significantly more abundant in Santa Caterina, which, due to its shallower position (40 m) and seafloor morphology, which is regularly gently inclined, is characterized by clear waters that favour the penetration of light. Light has recently been considered the limiting factor in the invasion of native Mediterranean seagrass meadows by the macroalga [44]. This hypothesis suggests that light is important in determining the competitive interactions of *C. cylindracea* with the other plants that dominate the habitats it invades, namely the rhodolith-forming coralline algae.

The Capo Carbonara rhodolith bed has already been described as a heterogeneous bed by [16]. Results from quantitative video analysis confirm that the living cover is higher in both Santa Caterina and Serpentara, whereas in is Piscadeddus [16] observed a lower value. Moreover, the estimate of living cover per morphotype assigns the highest values for praline, whereas boxwork and branches are quite limited. These discrepancies can be explained by a different method of rhodolith cover evaluation based on the analyses of three replicates per site in [16]. As suggested by [14], indeed, to well study a rhodolith bed, it is mandatory to include different techniques that integrate results from different scales of observation. Anyway, these results are in accordance with those of other studies aimed at characterizing Mediterranean deep rhodolith beds. Ref. [45] reported pralines as the predominant morphotype in several deep beds along the coasts of Campania. Moreover, [46] observed a similar situation for beds near Capri Island, where, once again, a significantly higher cover of pralines was recorded. These results might indicate a first step or a steady-state situation in bed development [14], but more details would be needed to verify this hypothesis. Certainly, environmental conditions seem to play an important role not only in defining the distribution of rhodoliths but also their characterization in terms of dominant morphotypes. In particular, water motion is currently considered one of the most important factors in determining their morphology, as already observed by [16] for Capo Carbonara beds. It is generally assumed that without movement, dense aggregations of live rhodoliths will grow together or become bound together via overgrowth [18,31]. In most beds, indeed, cessation of movement would probably lead to rapid burial by sediment [31] or overgrowth by other algae and invertebrates, thus explaining the presence of *Culerpa*.

Instead, ref. [47] described a different situation in which they found a significantly higher percent cover of boxworks than of pralines and branches along the coasts of Spain. However, the data collected during such a study focused on a quite shallow bed. Moreover, ref. [26] also reported a significantly higher abundance of unattached branches if compared to the other two morphotypes, analysing some different Apulian deep beds. In any case, those beds, like those of Capo Carbonara MPA, appeared to be characterized by high heterogeneity. The above-mentioned heterogeneity of rhodoliths observed, especially at the Santa Caterina site, appears to promote *Caulerpa* species development, as highlighted at the end of other studies on the ability of this species to develop on different types of substrates [48]. Moreover, generally, detritic bottoms with a coarse texture, such as Santa Caterina one, seem to facilitate the spread of the algae more than fine sand [48]. Furthermore, this pattern becomes particularly relevant in rhodolith beds because these bottoms provide a complex three-dimensional substrate for stolon attachment [49]. This hypothesis can be useful to explain the strong positive correlation observed between the total cover of rhodoliths and that of *C. cylindracea* during this study. According to the observations in [50], indeed, the presence of an abundant and well-structured rhodolith bed could enhance the settlement and development of *Caulerpa* instead of limiting it, as happens, on the contrary, with seagrasses [51,52]. In fact, the habitat structure is usually considered an important invasion barrier [53].

We can exclude a relationship between the occurrence of *C. cylindracea* and dead rhodoliths. At Santa Caterina, where *C. cylindracea* abundance is high, the dead rhodolith cover is limited, suggesting that despite the remarkable invasive potential of the green macroalga, rhodoliths seem to successfully cope with *C. cylindracea* competition, apparently without any serious damage.

Finally, we can suppose that the geometry and geomorphological constraints of the substrate play a key role in determining the occurrence and spread of *C. cylindracea* in the study area. Santa Caterina, where *C. cylindracea* cover is considerable, is the shallowest and most-exposed site. Moreover, its seafloor is characterized by an open shelf gently inclined towards the open sea, possibly favouring in some way the mechanism of spreading of *C. cylindracea*, which is able to successfully expand on the substrate by vegetative fragmentation [54], especially when no physical barriers are present, as in the case of Santa Caterina. On the contrary, the highest mud percentage in the sediments of Is Piscadeddus probably hampers *C. cylindracea* colonization, whereas the Serpentara seafloor, at 59 m depth, is probably too deep to support or favour *C. cylindracea* spreading.

5. Conclusions

In conclusion, the obtained results suggest that different competitive interactions occur between *C. cylindracea* and rhodolith beds, in relation to the percentage cover and the morpho-structural composition of the latter, and in response to environmental conditions that affect the development ability of *C. cylindracea* on rhodolith beds. In particular, the main forcing parameters for *C. cylindracea* development seem to be the depth, the gentle slope, and the percentage cover of hard substrates.

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