ARE *CAULERPA* SPECIES ABLE TO SETTLE AND DEVELOP ON RHODOLITH BEDS? THE CASE STUDY OF MARINE PROTECTED AREA "CAPO CARBONARA"

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Abstract – The two green algae *Caulerpa taxifolia* (M.Vahl) C. Agardh and *Caulerpa cylindracea* Sonder, (Chlorophyta; Bryopsidales) are currently considered among the most invasive alien macroalgae in the Mediterranean Sea, where they have spread very quickly in the last three decades, with severe effects on biodiversity conservation (Streftaris and Zenetos, 2006). Currently both *C. taxifolia* and *C. cylindracea* as well as their congeneric *Caulerpa prolifera* (Forsskäl) J.V. Lamouroux, that is considered as native in the Mediterranean Sea, appear to be particularly abundant all over the basin. Nevertheless, little is still known about the competitive relationships that are established among them and with other organisms, such as rhodoliths. The aim of this study is to investigate the presence and abundance of *C. prolifera*, *C. taxifolia* and *C. cylindracaea* on the relatively deep rhodolith bed of the Capo Carbonara Marine Protected Area (MPA), along the Southern Sardinian Coasts (Italy).
The percent cover of the substratum by the three species was estimated by image analysis on frames extracted from videos in three different sites of the MPA (Is Piscadeddus, Santa Caterina and Serpentara, three transects of 200 m in each). The obtained results were analysed by means of univariate statistical analysis techniques (ANOVA and SNK test), considering as factors both the site and the transect.
Significant differences in the percent cover of the substratum by the three species were highlighted among sampling sites and species. *C. cylindracea* seems the most abundant species, especially in Santa Caterina, where it reached the 24 %. Significantly lower percent covers were instead recorded for the other two species in all sites. Anyway, the presence of the three species in the study area proved that they are all able to settle also in deep and particular habitats such as rhodolith beds, representing a possible threat for their conservation. However, only *C. cylindracea* seems to find there the right conditions to diffuse significantly and, thus, must be monitored with particular attention, due to its high invasive potential.

Introduction

Rhodoliths are unattached nodules, mostly consisting of Crustose Coralline Algae (CCA) [1], slow-growing and long-lived organisms that act as ecosystem engineers secreting high-Mg carbonate, forming both mobile (i.e., rhodoliths) and stable substrates (i.e., algal reefs).

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Usually, rhodoliths are in the form of a single or multiple coralline algal species, frequently overgrowing one over the other, and with a wide variety of growth forms, from foliose to fruticose to lumpy [2]. Rhodolith beds are formed by aggregation of free-living (>10 %) rhodoliths [3,4] and they are considered as an important biogenic marine habitat between mobile and stable substrates. 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 [5].

They are distributed worldwide, and they generally develop on float or gently sloping seabed [4] from the intertidal down to 270 m depth [6]. In the Mediterranean Sea, they occur typically within 30 and 75 m depth [7] and they are considered of high conservation interest and they are protected within the framework of the United Nations Program's Mediterranean Action Plan. Moreover, they are included in the monitoring program in the Marine Strategy Framework Directive 2008/56/EC of the European Community.

According to [8], rhodoliths survival depends on the possibility to continue to overturn, avoiding in particular overgrowing by other organisms and smothering by burial [8,9]. With regard to overgrowing, flashy algae such as some invasive *Caulerpaceae*, able to form dense carpets on the substratum of different environments can represent a significant stressor for rhodoliths, as described by [10], analysing the interactions between rhodoliths and *Caulerpa sertularioides* Gmelin Howe. No data are instead available on the presence and abundance of other *Caulerpa* species, potentially invasive in such habitats. In particular, the two green algae *Caulerpa taxifolia* (M.Vahl) C. Agardh and *Caulerpa cylindracea* Sonder, (Chlorophyta; Bryopsidales) are currently considered among the most invasive alien macroalgae in the Mediterranean Sea, where they have spread very quickly in the last three decades, with severe effects on biodiversity conservation and could represent a threat also for rhodoliths conservation [11].

C. cylindracea was initially considered as a Lessepsian migrant in the basin but, recently, its south-western Australian origin has been proved [12]. *C. taxifolia*, instead, is native to the Caribbean Sea and was accidentally released from the Oceanographic Museum of Monaco into the Mediterranean Sea [13]. Both the species are able to colonize any type of substratum at depths ranging from the intertidal zone down to almost 90 m in particular conditions and to actively compete with the endemic seagrass *Posidonia oceanica* (L.) Delile [14]. Besides *C. taxifolia* and *C. cylindracea*, also their congeneric *Caulerpa prolifera* (Forsskäl) J.V. Lamouroux, that is currently considered as native in the Mediterranean Sea, appears to be particularly abundant all over the basin. Although numerous studies have been conducted in the last decades on the three species, little is still known about the competitive relationships that are established between them and on their presence in relatively deep environments.

In the framework of an agreement between the Marine Protected Area (MPA) "Capo Carbonara" and the University of Milano-Bicocca for the realization of the monitoring project for the Italian Marine Strategy Framework Program, we firstly described a wide heterogeneous and relatively deep rhodolith bed (41.08 km^2, 40-60 m water depth [15]). In the same sites, now we study the presence and abundance of *C. prolifera*, *C. taxifolia* and *C. cylindracaea*, which occurrence was firstly signalled but never deeply investigated, by using a quantitative approach, define which species are present and their local distribution and abundance.

Materials and Methods

Study area

The study was conducted in the MPA "Capo Carbonara", along the Southern Sardinian Coasts (Italy) in the summer of 2017 (Fig. 1). The MPA was established by the Ministry of 'Environment and of Land and Sea (currently Ministry of Ecological Transition), by Ministerial Decree of 15 September 1998, modified in 1999 and totally replaced by Ministerial Decree of 7 February 2012 (GU No. 113 of 16 May 2012).

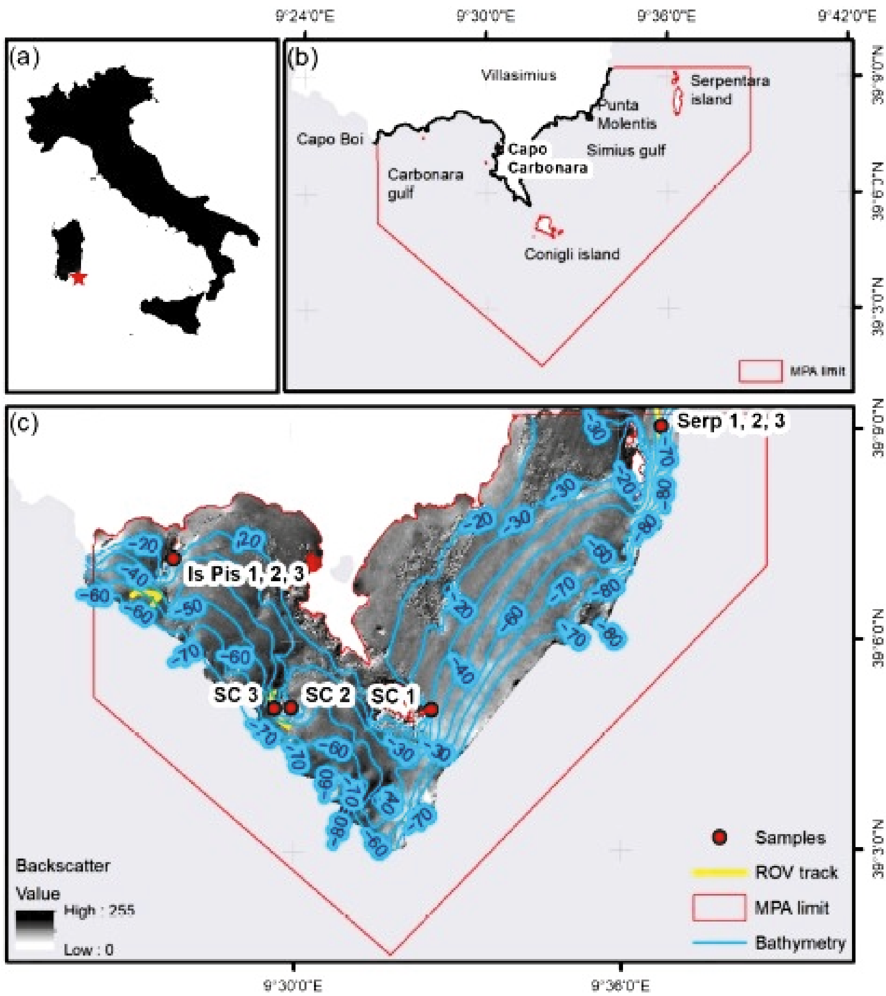

Figure 1 – Backscatter photomosaic of the area (property of the MPA) with the indication of the ROV tracks and sampling location. Is Pis is for Is Piscadeddus, SC is for Santa Caterina, and Serp is for Serpentara.

The territory of the MPA extends from Capo Boi (W) to the area in front of Serpentara Island (E) on the whole continental shelf, down to an average of 125 m water depth (Fig. 1). Rhodoliths are indicated to be particularly abundant in the area [16].

In the area, 3 different sites were identified at a distance of >200 m (Fig. 1): the Is Piscadeddus shoal (from here on Is Piscadeddus) towards W, the Santa Caterina slope (from here on Santa Caterina) in the middle, and the Serpentara Island (from here on Serpentara) towards E (Fig. 1).

Image collection and analysis

Each site was investigated by a remotely operated vehicle (Steelhead Seamore, property of the University of Milano-Bicocca, Milan, Italy), equipped with two video-cameras. In each site, three transects of 200 m were considered (Fig. 1).

To evaluate the abundance of the three seaweeds on the substratum, their percent cover was estimated by image analysis (sub-squared method) on photo-frames extracted from the videos obtained by the ROV at 40-50 m of depth (with a the shooting angle of the ROV camera of about 45°). In particular, from each video, 20 frames (6 m^2 of substratum for each) were obtained. Onto each image, a grid of twenty-five sub-quadrats (1 m^2 of area) was superimposed on a computer screen, and each sub-quadrat scored from 0 to 4 %. The total % cover value was obtained by adding up the 25 resulting values [17] (Fig. 2).

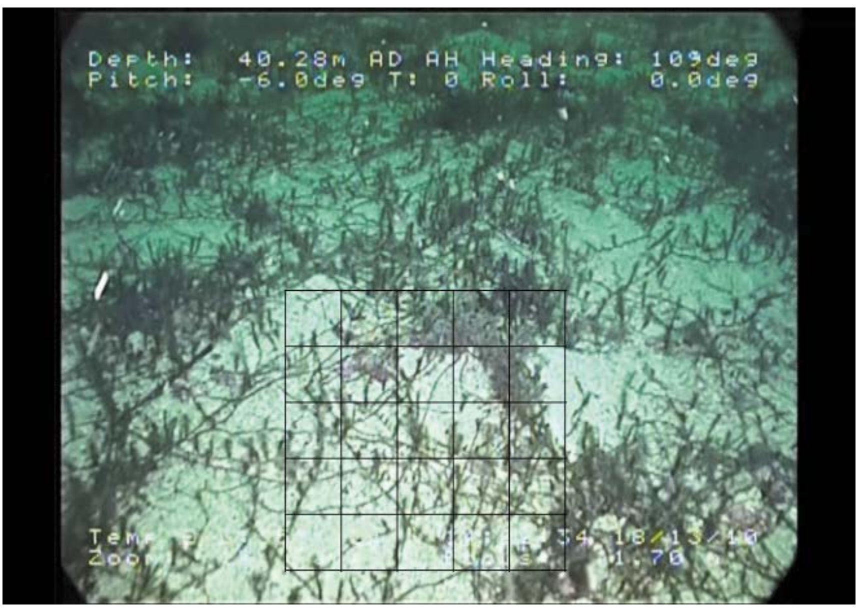

Figure 2 – Example of the analysed frames.

Statistical analysis

Data regarding the percent cover of the substratum by each *Caulerpa* species were analysed by means of univariate statistical analysis techniques (ANOVA and SNK test) [18]. In particular, a two-way ANOVA was run when the three species were found on the substratum, considering Species (3 levels: *C. taxifolia* vs *C. cylindracea* vs *C. prolifera*) and Transect (3 levels, t1 *vs* t2 *vs* t3) as fixed factors.

Results

Some significant differences in the distribution (percent cover) by the three species were highlighted among sampling sites. While their presence was significant at Santa Caterina, where their total cover was more than 25 %, they appeared negligible at both Serpentara and Is Piscadeddus (<1 % of mean cover) (Fig. 3).

Considering only Santa Caterina site, some quite interesting differences of cover were observed among the three considered species (Fig. 3; Tab. 1).

C. cylindracea is the most abundant species in all the transects with a mean percent cover of the substratum that reached 25 %. Significantly lower percent covers were instead recorded for both *C. prolifera* and *C. taxifolia* (<1 %) but, anyway some differences were highlighted also in their abundance in relation to the considered transect of the same site (Tab. 1).

While in transects 1 and 2 the mean cover by these two species appeared to be very similar (<0.1 %), a different situation occurred in transect 3, where *C. prolifera* was significantly more abundant (~ 5 %) than *C. taxifolia* (<0.1 %). The latter, in particular, was scarcely present in all the transects with a mean cover of 0.08 % (Fig. 3; Tab. 1). Finally, some significant differences among transects were highlighted also for *C. cylindracea*, for which a significantly higher percent cover of the substratum was observed in transect 2 (Tab. 1).

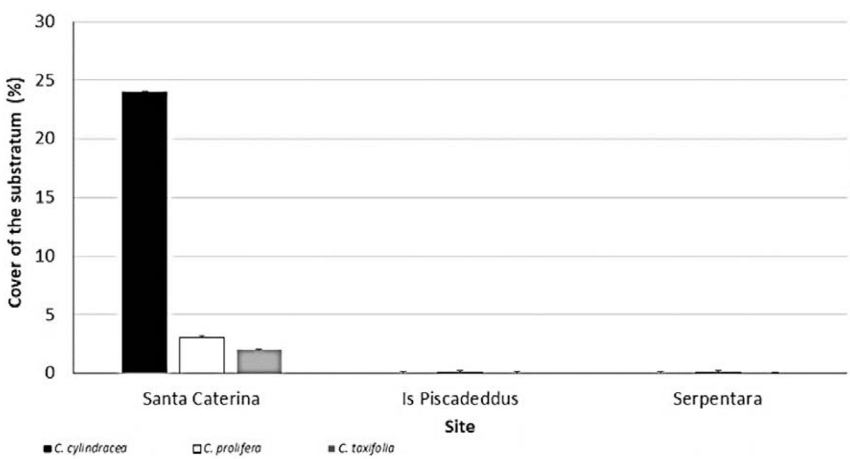

Figure 3 – Mean (SE) percent cover of the substratum of the three species in the three considered sampling sites.

Table 1 – Results of ANOVA and SNK to test for significant differences in the percent cover of the substratum between species and transects for Santa Caterina site. Significant (P < 0.05) results are in bold. The SNK test for the interaction Species*x*Transect is also presented.

ANOVA				
Source	**df**	**MS**	**F**	**P**
Species	2	9903.1722	19.54	**0.0086**
Transect	2	336.0056	6.49	**0.0019**
SpeciesxTransect	4	506.8056	9.79	**0.0000**
Residual	171	51.7485		
Total	179			
Cochran's test = 0.5539				
SNK test (Species x transect)				
Species	**Transect**	**Transect**	**Species**	
C. prolifera (CP)	t1=t2=t3	t1	CT=CP<CC	
C. taxifolia (CT)	t1=t2=t3	t2	CT=CP<CC	
C. cylindracea (CC)	t1=t2<t3	t3	CT<CP<CC	
S.E. =1.6085				

Discussion

These preliminary results appear particularly interesting from both an ecological and a management point of view. They furnish some interesting insights on the ability of the *Caulerpa* species to settle and develop in quite peculiar habitats, such as rhodoliths beds, focusing also on their competitive interactions.

First of all, these results represent one the first report of *Caulerpa* species in the rhodolith bed of the MPA "Capo Carbonara", and in particular the two alien species *C. cylindracea* and *C. taxifolia* as well as of the native one *C. prolifera.* Until today, indeed, some species of the *Caulerpa* genus had been reported in oceanic rhodolith beds (*e. g.* [19,3], which, however, are significantly more superficial (20 m depth) than the ones considered during this study (45 m depth) and in which, therefore, green algae as *Caulerpaceae* are expected to find quite suitable conditions to settle and develop. In particular, while *C. cylindracea* has already been observed at similar depths [20], even if not in rhodoliths beds, both *C. taxifolia* and especially *C. prolifera* are reported to be typical of more superficial waters [21,22] even if other researchers observed the former also at about 50 m of depth [23]. Therefore, the presence of the two latter Caulerpac*e*e in the study area provides the first evidence of their ability to survive also in deep environments, where the environmental conditions, and in particular light, are not usually favourable for green algae colonisation. In such environments, indeed, light that is considered the key variable for seaweed distribution appears to be spectrally limited. According to Ramus et al. [24], sunlight becomes blue-green after passing through several meters of seawater. For this reason, seaweeds living deep beneath the sea receive little light of wavelengths which are effectively absorbed by chlorophyll (violet and red light). Indeed, green seaweeds, having chlorophyll as the main photosynthetic pigment, are normally presumed to be scarce in deep waters, where only few species successfully adapted to tolerate such extreme light conditions can be found [24].

Analysing data regarding the mean percent cover of the substratum by three species, some quite interesting information can be obtained. Indeed, significantly different covers were recorded. In particular, *C, cylindracea* appeared to be more abundant than the other two species, thus suggesting that this species find in rhodolith beds more favourable right conditions to diffuse significantly if compared with the other two species. The above described differences can be partially explained taking into account the very high invasive potential of this macroalga, that is currently considered as one of the most threatening invaders in the Mediterranean Sea [25,14]. Beside its ability to tolerate the quite peculiar environmental conditions typical of high depths habitats, also the type of substratum seems to play a key role in determining *C. cylindracea* presence in the study area. Detritic bottoms with coarse texture, indeed, seem to facilitate the spread of the alga more than fine sand [26] and this pattern becomes particularly relevant in rhodolith beds, because these bottoms provide a complex three-dimensional substrate for stolon attachment [27]. Moreover, due to its high invasive potential, *C. cylindracea* seems to be able to win the competition with the other two species, that also suffer because they are less adapted to the peculiar conditions of the area, as already proved by [28].

Finally, also the significant differences in the percent cover of the substratum by all the considered species among sampling sites and also transects prove that the peculiar geomorphological features of the considered area can play a key role for the successful establishment and expansion of these species. Regarding the sites, in particular, some important cover differences were observed, as the three species were found only in Santa Caterina slope. Indeed, such site, due to its position and substratum morphology, is characterized by clear waters that favour the penetration of light and is interested by currents that avoid the presence of muddy sediments typical of deep environments, that usually don't promote *Caulerpa* species development, as highlighted at the end of other studies on the ability of the above-mentioned species to develop on different types of [29].

Conclusion

The obtained results suggest that *C. cylindracea* is currently the only *Caulerpa* species representing a potential threat for rhodolith beds conservation and, thus, its presence, abundance and expansion trend should be monitored with particular attention in such kind of habitats.

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