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Edoardo Zelli, Gaëlle Quéré, Nicola Lago, Giulia Di Franco, Federica Costantini, et al.. Settlement dynamics and recruitment responses of Mediterranean gorgonians larvae to different crustose coralline algae species. Journal of Experimental Marine Biology and Ecology, 2020, 10.1016/j.jembe.2020.151427 . hal-02927655

HAL Id: hal-02927655

https://hal.sorbonne-universite.fr/hal-02927655

Submitted on 24 Nov 2020

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Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: http://ees.elsevier.com

Settlement dynamics and recruitment responses of Mediterranean gorgonians larvae to different crustose coralline algae species

Edoardo Zelli [a,b,f], Gaëlle Quéré [a], Nicola Lago [a,c], Giulia Di Franco [a,d], Federica Costantini [b,e], Sergio Rossi [c,d,e], Lorenzo Bramanti [a,*]

[a] CNRS-Sorbonne Université, Laboratoire d'Ecogéochimie des Environnements Benthiques, LECOB, Observatoire Océanologique de Banyuls sur Mer, 1 avenue Pierre Fabre, 66650 Banyuls sur Mer, France
[b] Dipartimento di Scienze Biologiche, Geologiche ed Ambientali (BiGeA) & Centro Interdipartimentale di Ricerca per le Scienze Ambientali (CIRSA), University of Bologna, Via S. Alberto 163, I-48123 Ravenna, Italy
[c] Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali, Università del Salento, 73100 Lecce, Italy
[d] Dipartimento di Scienze della Terra e del Mare (DiSTeM), Università di Palermo, Palermo, Italy
[e] CoNISMa, Consorzio Nazionale Interuniversitario per le Scienze del Mare, Rome, Italy
[f] Universidade Federal do Ceará, Instituto de Ciências do Mar (Labomar), 8, Fortaleza, Ceará, Brazil

ABSTRACT

Sessile marine species such as Anthozoans act as ecosystem engineers due to their three-dimensional structure. Gorgonians, in particular, can form dense underwater forests that give shelter to other species increasing local biodiversity. In the last decades, several Mediterranean gorgonians populations have been affected by natural and anthropogenic impacts which drastically reduced their size. However, some species showed unexpected resilience, mainly due to the supply of new individuals. To understand the mechanisms underlying recovery processes, studies on the first life history stages (i.e. larval dispersal, settlement and recruitment) are needed. In tropical coral reefs, crustose coralline algae (CCA) are known to influence coral larvae habitat selection and settlement. This capacity however is not ubiquitous among CCA species and larvae of different coral species may have different preferences. The present work focuses on three Mediterranean gorgonians (*Eunicella singularis, Paramuricea clavata* and *Corallium rubrum*) with the objective of quantifying settlement and recruitment in presence of two common CCA species (*Litophyllum styctaeforme* and *Litophyllum incrustans*). Results showed that the presence of CCA activates earlier settlement in *E. singularis* and increase the density of recruits, with different trends for the three species. Our results suggest that CCA should be taken into account in the implementation of conservation strategies. Moreover, a deeper comprehension of settlement mechanisms could help improving restoration techniques based on sexual reproduction.

1. Introduction

Gorgonians are among the most prominent three-dimensional species in Mediterranean coralligenous habitat and the most representative in the coastal benthic environment (Gili and Ros, 1985; Gili and Coma, 1998). Similarly to other ecosystem engineer species (sensu Jones et al., 1994), gorgonians provide structural complexity (Rossi et al., 2017), forming habitats which species richness can be compared with tropical coral reefs (Ballesteros, 2006; Boudouresque et al., 2017, and references therein). Anthropogenic and natural disturbances (e.g. overfishing and climate change) may pose a threat to gorgonian populations (Garrabou et al., 2009; Tsounis et al., 2013). For example, in shallow areas of the Mediterranean Sea (10–30 m depth), in the last decades, several gorgonian populations have been hit by mass mortalities linked to acute thermal anomalies (Cerrano et al., 2000; Garrabou et al., 2009; Bramanti et al., 2005; Linares et al., 2008a, 2008b; Turicchia et al., 2018). For some gorgonian species, recruitment may be impacted by prolonged high temperature (Rossi et al., 2019); nevertheless, some gorgonian populations have been able to recover, probably due to the increased recruitment after disturbance (Cupido et al., 2008, 2012; Padrón et al., 2018). Understanding the processes driving settlement and recruitment is thus fundamental to shed light on recovery potential of gorgonian populations and help developing proper management and conservation plan at local level (Whalan et al., 2012).

* Corresponding author.
E-mail address: philebo@gmail.com (L. Bramanti)

https://doi.org/10.1016/j.jembe.2020.151427
Received 7 April 2020; Received in revised form 12 June 2020; Accepted 14 June 2020
Available online xxx
0022-0981/© 2019.

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In gorgonians, as in most marine sessile species with a larval phase the success of early life-history stages (e.g. larval dispersal, settlement and recruitment) is critical for the persistence and recovery of populations (Mumby and Steneck, 2008; Connolly and Baird, 2010; Adjeroud et al., 2016). In particular, larval settlement is a crucial phase during which pelagic larvae select a suitable substrate on which they will attach, metamorphose and grow (Harrison and Wallace, 1990). The choice of a suitable surface often relies on external cues, and it can influence post-settlement survival and recruitment (Harrington et al., 2004) driving the distribution, abundance and structure of the adult coral community (Babcock and Mundy, 1996; Chanmethakul et al., 2010; Price, 2010; Rocha et al., 2013; Vermeij and Sandin, 2008). For example, planulae of different scleractinian and octocoral species can settle and metamorphose in response to a wide range of cues, from abiotic factors such as light (Grossowicz and Benayahu, 2015; Mundy and Babcock, 1998), color (Mason et al., 2011) or sound (Vermeij et al., 2010; Lillis et al., 2018) to biotic factors such as the presence of bacteria (Baird and Morse, 2004; Golbuu and Richmond, 2007; Negri et al., 2001), microbial biofilmed surfaces (Webster et al., 2004; Erwin et al., 2008), carbonate skeletons (Golbuu and Richmond, 2007; Benedetti et al., 2011), calcified green algae (Nugues and Szmant, 2006) and calcified red algae (Morse et al., 1988, 1996; Heyward and Negri, 1999; Negri et al., 2001; Harrington et al., 2004; Ritson-Williams et al., 2010). Several species of crustose coralline algae (CCA) have been shown to play a fundamental role in triggering the settlement process of many corals (Harrington et al., 2004; Ritson-Williams et al., 2009). The positive effect of CCA on larval settlement is the result of an intricate interaction between CCA-chemical compounds (i.e. cell wall bound morphogens; Morse et al., 1996; Tebben et al., 2015) and CCA-surface bacteria (i.e. CCA-associated biofilms) (Gómez-Lemos et al., 2018). The cues required to transition into a sessile polyp have been far less investigated in octocorals than in scleractinians. Sebens (1983) observed that CCA can activate settlement and metamorphosis in a temperate soft-coral and Lasker and Kim (1996) reported an increase in settlement and survival of tropical gorgonian recruits in the presence of CCA. Despite those observations, no studies have investigated the interactions between CCA and gorgonian larvae in temperate marine ecosystems, these algae being an essential part of the structure of many benthic habitats (Boudouresque et al., 2017).

CCA are widely distributed among many marine habitats (Morse et al., 1988; Baird and Morse, 2004; Harrington et al., 2004; Webster et al., 2004; Ritson-Williams et al., 2010) and they are important calcifying species that greatly contribute to accretion and stabilization of the calcareous substrates in coral reefs (Littler and Littler, 2013). In the Mediterranean Sea, CCA are the foundations of the three-dimensional complexity of coralligenous framework, offering a compact substrate potentially suitable for settlement of several species such as gorgonians (Ballesteros, 2006; Gibson et al., 2006; Boudouresque et al., 2017). Gorgonians and crustose coralline algae are both key organisms that contribute to the three-dimensional complexity of this habitat and intricate relationships exist between them. It has been shown for instance that the presence of gorgonians can increase the abundance of CCA, while their disappearance can foster a shift from CCA-dominated to filamentous algae-dominated assemblages (Ponti et al., 2014). The objective of the present study is to test the effect of two common Mediterranean CCA species *Lithphyllum stictaeforme* and *Lithphyllum incrustans* on the settlement and recruitment processes of the larvae of three gorgonian species *Eunicella singularis*, *Paramuricea clavata* and *Corallium rubrum* (the most representative gorgonians of the Mediterranean coralligenous) in order to better understand the distribution patterns and recovery capabilities of their populations. To this aim we tested the following hypotheses: (1) the presence of CCA fosters settlement and increases recruitment, and (2) the effect of CCA is species-specific.

2. Materials and methods

The three gorgonians (*E. singularis*, *P. clavata* and *C. rubrum)* and the two crustose coralline algae species *(*L. *stictaeforme* and *L. incrustans)* selected for the present experiment are common in the hard-bottom substrates of the north-western Mediterranean Sea (Ballesteros, 2006). All the species have been collected by SCUBA-diving between 15 and 30 m depth on coralligenous outcrops in the area adjacent to the Marine Reserve Cerbere/Banyuls (North Western Mediterranean Sea, France).

2.1. Larvae collection

Forty-eight sexually mature colonies (> 20 cm height; Ribes et al., 2007) of *E. singularis* were collected in late June 2018 at 15–20 depth, and 45 sexually mature *C. rubrum* colonies (> 7 mm basal diameter; Santangelo et al., 2003) were collected in July 2018 at 25–30 m depth. Colonies were kept in open circuit aquaria with air bubbling system and maintained at 16–18 °C based on the temperature measured in situ. To determine the sex of each colony, small fragments (2–5 cm) of the apical branches were inspected under a stereomicroscope for the presence of oocytes or spermatic sacks inside the gastrovascular cavity of the polyps (Santangelo et al., 2003; Cupido et al., 2012). Colonies were then re-arranged in the aquaria in order to have females and males in each aquarium and ensure oocytes fertilization. Aquaria were filled with seawater, 90% of which was changed every 4 days in order to remove accumulated debris and mucus. On June 26, 2018, oocytes from the external surface of female colonies of *P. clavata*, located at 20 m depth, were collected underwater using 60-mL syringes. Collected oocytes were maintained in open circuit aquaria with air bubbling system at 16–18 °C (temperature measured in situ). All the aquaria were inspected daily for larval release (*E. singularis* and *C. rubrum*) or larval development (*P. clavata*), which started on June 22nd, June 27th and August 4th for *E. singularis, P. clavata*, and *C. rubrum* respectively. Larvae of each species were transferred in small containers with 0.7-μm-filtered seawater (FSW-Millepore) and maintained until the beginning of the experiments. Aquaria were subjected to two LED white light (LUMIVIE 50 cm, 9 W. Aquavie) under a cycle of 12-h light-12-h dark with irradiation values of 300–500 μE m^{-2} s^{-1}. After larval release, *E. singularis* and *C. rubrum* colonies were successfully transplanted in Banyuls-sur-Mer public aquarium.

2.2. CCA collection

Fragments of L. *stictaeforme* and L. *incrustans* were collected using hammer and chisel at the depth of 20 m. After collection, CCA fragments were kept in open circuit aquaria, with air bubbling system and maintained at 16–18 °C for one week prior to experiments. Pieces of approximately equal sizes were used for the experiments and the surface of each piece was cleaned from sediment and epiphytic organisms using tweezers. One piece of each CCA species was kept for taxonomic identification and checked under a dissecting microscope using reproductive and morphological features as diagnostic characters (Steneck, 1986).

2.3. Experimental design

The experiments were carried out at constant ambient temperature (16–18 °C), under a cycle of 12-h light-12-h dark with irradia-

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tion values of 300–500 μE m^{-2} s^{-1} and performed in closed-circuit aquaria filled with filtered sea water (0.7 μm). Every 3 days, 75% of the water was changed to prevent bacterial growth.

Three replicates aquaria were used for each treatment, each one equipped with air bubbling system. Larvae were exposed to the following treatments: (1) a fragment of L. *incrustans*, (2) a fragment of L. *stictaeforme* and (3) a fragment of bare rock collected at the same sites of CCA, as control. In each treatment a fragment of bare rock was added in order to provide substrate for settlement. Two fragments of rock were thus present in the control treatment (Fig. 1A). In order to avoid any biological cue other than the two CCA species, each fragment of rock was previously put in the oven at 450 °C for 4 h.

In total, 990 (2–3 days old), 2700 (2–3 days old) and 900 (1–17 days old) larvae of *E. singularis*, *P. clavata* and *C. rubrum* respectively were collected. Larvae of each species were then evenly distributed among the 9 experimental tanks (n = 27: 3 replicate tanks for each treatment for each species), with 110, 350 and 100 larvae $tank^{-1}$ for *E. singularis*, *P. clavata* and *C. rubrum* respectively. For each species, experiment started as soon as at least 900 larvae per species were obtained (i.e. 100 larvae in each replicate tank) (Fig. 1A).

The number of settlers and new polyps was recorded every two days until no more swimming larvae were observed in the aquaria. In the framework of this study, we defined larval release as the day in which larvae were released from the mother colonies and we considered settlement when the larva stops the swimming activity to cling onto the substrate. The pre-competence period (PCP) was defined as the number of days between larval release and larval settlement, and metamorphosis when larvae undergo physiological and morphological differentiation that turns them into a polyp (see Weinberg, 1979, and Linares et al., 2008c for a description of the process). Finally, we considered as recruit a polyp that survived until the end of the experiment.

2.4. Data analysis

Recruits density (polyps cm^{-2}) was calculated counting the number of polyps over the available substrate which area was measured using NIH ImageJ software (Abràmoff et al., 2004). Overall, difference in surface areas among treatments were neglectable (<7% differences). For *E. singularis* and *C. rubrum*, as no settlement was observed on the surface of the aquarium tank, this substrate was not considered in the calculation of density.

A one-way ANOVA was applied to test the differences in recruit's density on the final day between treatments for each gorgonian species, with treatment as fixed factor (three levels: CCA1, CCA2 and Control) and aquaria as replicates (n = 3). When a significant effect of treatment was found (p < .05), individual post-hoc tests (Tukey test with Bonferroni correction) were run to detect which treatments were responsible for significant differences. Analyses were performed in RStudio software, package 'stats'.

3. Results

During the pre-competence period (PCP), larvae of *E. singularis* and *P. clavata* (Fig. 2 A, B) were observed either continuously changing their position alternating upward and downward through the water column swimming or crawling on the bottom of the aquaria, a behavior probably linked to the search of a suitable substrate (Weinberg and Weinberg, 1979; Linares et al., 2008c). In both *E. singularis* and *P. clavata,* once larvae were ready to settle, they approached the substrate, became shorter and thicker and assumed a pear-shaped form (Fig. 2A, B). Subsequently the larvae metamorphosed in primary polyps (Fig. 3A, B). *C. rubrum* larvae

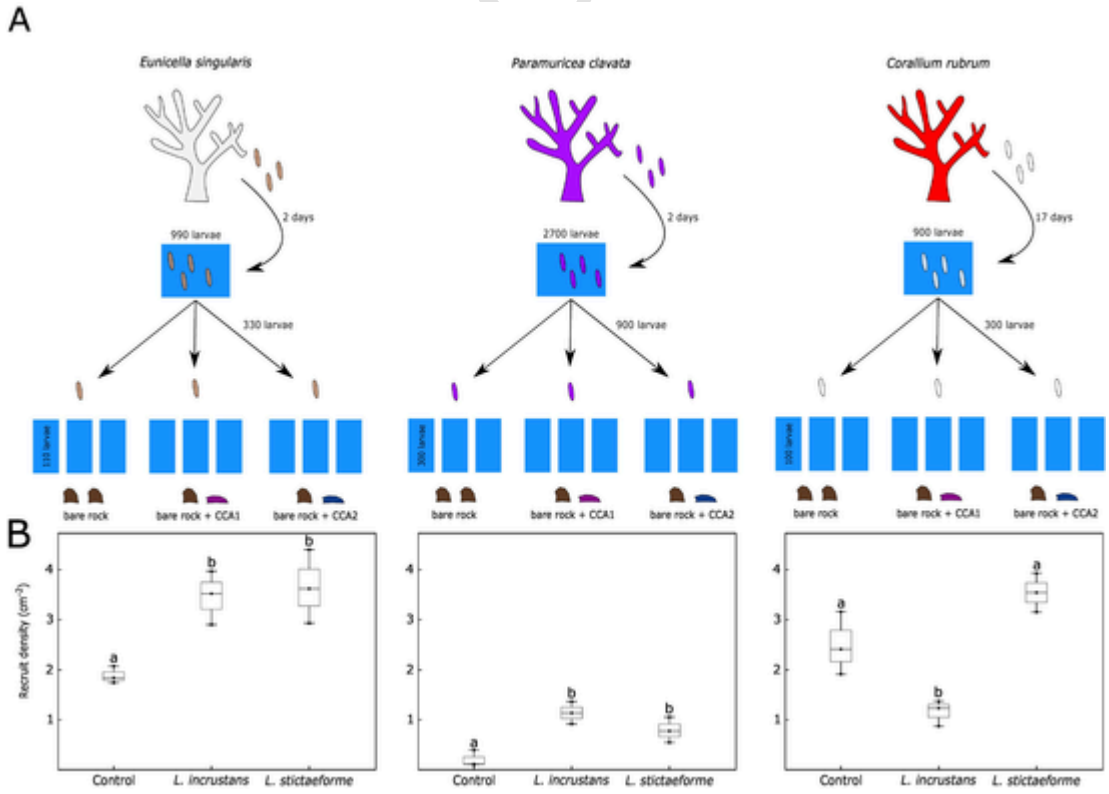

Fig. 1. Schematic diagram of the experimental design: (A) after release, larvae were collected and then distributed among the three replicates of each treatment for each gorgonian species; (B) boxplot reporting recruit density (cm^{-2}) at the end of the experiment for the three species in the different treatments. Letters (a and b) indicate statistical difference at p < .05.

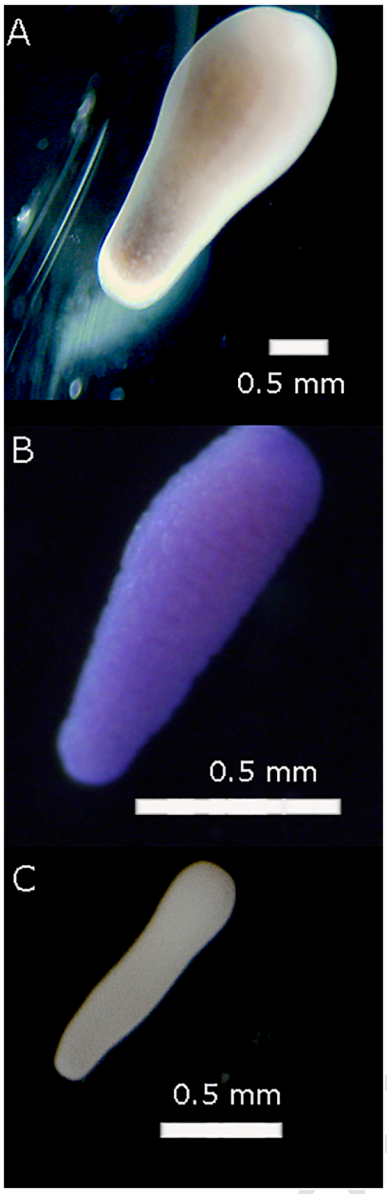

Fig. 2. Larvae of the three gorgonian species: (A) *Eunicella singularis*, (B) *Paramuricea clavata*, (C) *Corallium rubrum*.

(Fig. 2C) spent most of the time swimming up actively in the water column. Before metamorphosing in primary polyps (Fig. 3C) they passed through a transition phase (*spat* phase, Fig. 3D), during which the formation of sclerites was observed. No differences were observed in the behavior of larvae in the different treatments for the three species.

3.1. Settlement trends

The first *E. singularis* primary polyp was observed after 8 days in CCA treatments, and after 13 days in the control treatments, whereas *P. clavata* polyps occurred between 11 and 12 days in all treatments (CCA and controls) (Fig. 4A, B). The first *C. rubrum* larvae settled after 27 days in all treatments (CCA and controls). However, soon after settlement and right before metamorphosis, *C. rubrum* larvae, in all treatments, spent approximately 7 days in form of spat (Fig. 3D; Fig. 4C). Spat phase was observed only in *C. rubrum* (Fig. 3D). The duration of the experiment corresponds to the maximum PLD in absence of predation. For *E. singularis* larvae, maximum PLD differed between treatments: no larvae were present in the water column after 41, 57 and 79 days in the L. *stictaeforme*, *L. incrustans* and control treatments respectively. For *P. clavata* and *C. rubrum* no larvae were found in the water column after 59 and 49 days respectively, independently from the treatment (Fig. 4A, B, C; Table 1).

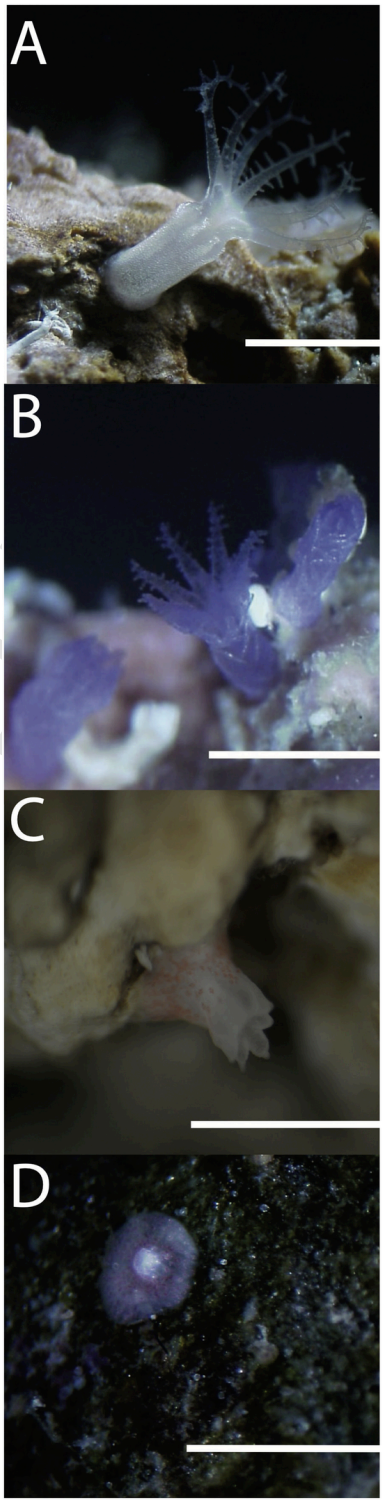

Fig. 3. Primary polyps of the three gorgonian species: (A) polyp of *Eunicella singularis*, (B) polyp of *Paramuricea clavata*, (C) polyp of *Corallium rubrum,* (D) spat of *Corallium rubrum.*

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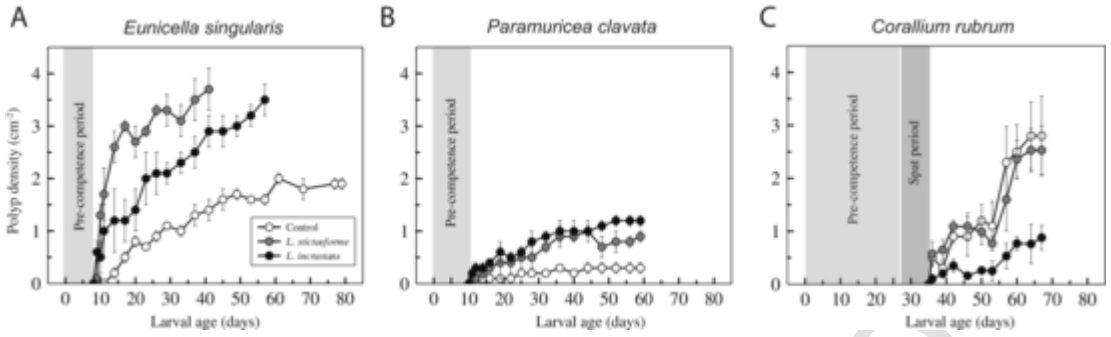

Fig. 4. Settlement trends: density of polyps (cm^{-2}) for the three species in the different treatments. (A) *Eunicella singularis*. (B) *Paramuricea clavata*. (C) *Corallium rubrum*. Pre-competence period (light grey band) is the number of days between larval release and first settlement. Spat period (dark grey band) is the time interval between settlement and metamorphosis in primary polyp and it has been observed only for *C. rubrum*. Larval age is the number of days since larval release. *E. singularis* and *P. clavata* larvae have been released between day 0 and day 2. *C. rubrum* larvae have been released between day 0 and day17.

Table 1
Number of days marking the start and the end of the experiments for each gorgonian species and treatments as well as recruit density at the end of the experiment. Polyp density = number of polyps cm^{-2}.

	E. singularis			*P. clavata*			*C. rubrum*		
Treatment	Day of first settlement	Day of experiment end	Polyp density at the end	Day of first settlement	Day of experiment end	Polyp density at the end	Day of first settlement	Day of experiment end	Polyp density at the end
L. stictaeforme	8	41	3.65 (± 0.42 SE)	11	59	0.89 (± 0.15 SE)	27	49	3.68 (± 0.22 SE)
L. incrustans	8	57	3.46 (± 0.31SE)	11	59	1.25 (± 0.13 SE)	27	49	1.30 (± 0.14 SE)
Control	13	79	1.88 (± 0.10 SE)	11	59	0.31 (± 0.10 SE)	27	49	2.64 (± 0.36 SE)

3.2. Recruitment density

The average density of *E. singularis* recruits was 3.65 ± 0.42 (average ± SE), 3.46 ± 0.31 (average ± SE) and 1.88 ± 0.10 recruits cm^{-2} (average ± SE) in L. *stictaeforme*, *L. incrustans* and control treatments respectively (Table 1). ANOVA results showed that recruitment density was significantly different among treatments Table 2A; $F_{2,6} = 9.88$; $p = .013$). Post hoc Tukey HSD test highlighted significant differences between L. *stictaeforme* and the control ($p = .016$) and between L. *incrustans* and the control ($p = .026$) while no significant differences were found between L. *incrustans* and L. *stictaeforme* ($p = .906$) (Table 2B). The average density of *P. clavata* recruits was 0.89 ± 0.15recruits cm^{-2} (average ± SE) in *L. stictaeforme* treatments, 1.25 ± 0.13 recruits cm^{-2} (average ± SE) in L. *incrustans* treatments and 0.31 ± 0.10 recruits cm^{-2} (average ± SE) in control treatment (Table 1). ANOVA results showed that recruitment density was significantly different among treatments (Table 2A; $F_{2,6} = 14.01$; $p = .005$). In particular, post hoc comparisons underlined significant differences between the control and L. *stictaeforme* ($p = .038$), significant differences between the control and L. *incrustans* (p = .005), and no significant differences between L. *stictaeforme* and *L. incrustans* ($p = .207$) (Table 2b). The average density of *C. rubrum* recruits was 3.68 recruits cm^{-2} (SE ± 0.22) in *L. stictaeforme* treatments, 1.30 recruits cm^{-2} (SE ± 0.14) in *L. incrustans* treatments and 2.64 recruits cm^{-2} (SE ± 0.36) in the control treatments (Table 1). ANOVA results showed that *C. rubrum* recruitment density was significantly different among treatments (Table 2A; $F_{2,6} = 20.90$; $p = .002$). Post-hoc test

Table 2
(A) Results of the one-way ANOVA test comparing the recruit density on the final day between treatments for each gorgonian species. df = degrees of freedom; SS = Sums of squares value; MS = mean square value; F═F ratio; * indicate p < .05; ** indicate $p < .01$. (B) Tukey post hoc comparisons for each gorgonian species. Diff = Difference in density; lwr and upr = lower and upper bounds of 95% confidence interval; p adj = p adjusted for multiple comparisons.

Source	*df*	*Eunicella singularis*				*Paramuricea clavata*				*Corallium rubrum*			
		SS	*MS*	*F*	*P(>F)*	*SS*	*MS*	*F*	*P(>F)*	*SS*	*MS*	*F*	*P(>F)*
A. One-way Anova													
Treatment	2	5.652	2.826	9.880	**0.012***	1.336	0.668	14.010	**0.0055****	8.562	4.281	20.900	**0.002****
Residuals	6	1.716	0.286			0.286	0.048			1.229	0.205		
B. Tukey post-hoc		*diff*	*lwr*	*upr*	*p adj*	*diff*	*lwr*	*upr*	*p adj*	*diff*	*lwr*	*upr*	*p adj*
L. incrustans-Control		1.5800	0.2401	2.9199	**0.0259**	0.5867	0.0398	1.1336	**0.0047**	1.3367	0.2029	2.4705	**0.0259**
L. stictaeforme-Control		1.7667	0.4268	3.1066	**0.0159**	0.9333	0.3864	1.4802	**0.0381**	2.3833	1.2495	3.5171	**0.0670**
L. stictaeforme-L. incrustans		0.1867	−1.1532	1.5266	0.9057	0.3467	−0.2002	0.8936	0.2068	1.0467	−0.0871	2.1805	**0.0016**

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highlighted a significant difference between L. *stictaeforme* and *L. incrustans* (p = .002) and between the control and *L. incrustans* (p = .03) while no significant differences were found between the control and *L. stictaeforme* (p = .07) (Table 2B).

4. Discussion

In the present work we tested the effect of two different crustose coralline algae species (*L. incrustans* and *L. stictaeforme*) on the settlement and recruit density of three Mediterranean gorgonian species (*E. singularis, P. clavata* and *C. rubrum*). Our results showed that recruitment is affected by the presence of CCA. In *E. singularis* and *P. clavata,* recruitment density is significantly higher in presence of both CCA species, suggesting that CCA provide positive cues for both species. Conversely, *C. rubrum* recruitment density is lower in presence of L. *incrustans*, suggesting that the latter CCA species provides a negative cue for the settlement of *C. rubrum* larvae. The role of CCA as a suitable substrate or settlement facilitator has been highlighted in previous works (Miller and Mundy, 2003a, 2003b; Birrell et al., 2005; Fabricius, 2011) showing that, at least for several species of scleractinian corals, CCA can positively influence larval settlement (Harrington et al., 2004; Doropoulos et al., 2012; Tebben et al., 2015). In tropical reefs as well as in the Mediterranean coralligenous habitat, CCA cover a relatively vast areas of the substrate with percentage varying according to the place (Fabricius and De'Ath, 2001; Boudouresque et al., 2017). Thus, the abundance and the distribution of CCA can affect the recovery capabilities of certain species. Harrington et al. (2004) showed that the preferred substratum for two scleractinian species in the Great Barrier Reef (GBR) is the CCA *Titanoderma prototypum* that represent only 5% of the algae in the GBR. Moreover, an impairment of the relationships between larvae and CCA due, for example, to ocean acidification (Doropoulos et al., 2012) or disease (Quéré and Nugues, 2015), could drastically change the recruitment capabilities of a set of species. Our results also showed that CCA can have a different effect on different species. *C. rubrum* larvae, in fact, seem to be "choosy" with respect to CCA species, settling at lower rates in presence of L. *incrustans* than in its absence. A negative effect of CCA has been observed in *Orbicella faveolata* and *Diploria labyrinthiformis* settlement (Quéré and Nugues, 2015). In the case of *C. rubrum*, the effect of L. *incrustans* could be one of the mechanisms underlying the observed light dependent distribution of the species, which is more commonly found in low light environment (Rossi et al., 2008). The presence of *C. rubrum* in low light environment, in fact, has always been explained as a result of a supposed negative phototactic behavior of the larvae, which has never been demonstrated (Weinberg, 1979). Our results offer an alternative explanation based on the CCA community present in low light environment. The differential substrate choice could also explain the segregated distribution of different species with similar three-dimensional structures and trophic needs. The association between CCA and gorgonians has been highlighted in previous studies (e.g, Ponti et al., 2014; Ballesteros, 2006) but, to our knowledge, it has never been described at the species level and demonstrated with a specific experimental set up. The difficult in situ taxonomic identification of CCA (Steneck and Adey, 1976) could have discouraged benthic surveys at the species level for this group. However, a few studies on scleractinians (e.g Harrington et al., 2004; Ritson-Williams et al., 2014; Quéré and Nugues, 2015), as well as the present results on octocorals, show a species-specific effect of CCA on coral settlement which should be further investigated.

It is worth to note that the presence of CCA does not affect only the density of recruits, but also the dynamics of settlement. According to our results, *E. singularis* larvae, differently than *P. clavata* and *C. rubrum* ones, spent more time in the water column in absence of CCA (79 days) than in their presence (41 and 57 days for L. *stictaeforme* and L. *incrustans* respectively) (Fig. 3). Data on the time interval between attachment and completion of metamorphosis for coral larvae under ideal conditions are very scarce (Miller and Mundy, 2003a, 2003b), and almost non-existent for octocorals. In *E. singularis*, recruit density is higher in presence of L. *incrustans* and L. *stictaeforme*, and larvae settle at a faster rate. The faster settlement in the presence of the two CCA species could result in a reduction of pelagic larval duration (PLD). On the one hand, a shorter PLD could result in lower mortality rates: the less time a larva spends in the water column, the lower is the probability of being predated, having more energy available for growth (not spent in searching for a settlement place, Viladrich et al., 2017). This has important consequences not only for the population viability through time, but also for connectivity and metapopulation dynamics (Connolly and Baird, 2010). On the other hand, a shorter PLD could negatively affect potential connectivity: the potential dispersal distance is, at some extent, positively related to the PLD. In general, the longer a larva remains in the water column, the further it is transported by currents (Padrón et al., 2018; Miller and Mundy, 2003a, 2003b). Current regime plays an essential role in the retention/dispersion of larvae (Shanks, 2009; Martínez-Quintana et al., 2015; Padrón et al., 2018) and together with larval behavior and PLD, it should be taken into account in the modeling of larval dispersal. In the case of *E. singularis* and *P. clavata*, however, a shorter PLD would probably not affect dispersal as it has been shown that, at least in the habitat configuration of the Gulf of Lion and Liguria Sea, gene flow among different populations is best explained by PLDs ranging from 7 to 14 days (Padron et al., 2018). In the case of *E. singularis*, the faster and more abundant settlement in presence of CCA could explain the recovery capabilities after a mass mortality event observed in 2018 in the Gulf of Lion (Bramanti, L. data not published).

The frequency of climate change-related disturbances affecting Mediterranean gorgonian populations has been increasing in the last 20 years and for some species, such as *C. rubrum*, mass mortalities are coupled with harvesting pressure (Bramanti et al., 2009; Rossi et al., 2019). Larval settlement is a key bottleneck to the recovery of damaged populations, and it depends, in part on the capacity of larvae to select the optimal substrate (Harrington et al., 2004; Fabricius, 2011). This capacity could be further reduced by the negative effects of warming and acidification on CCA cover (Webster et al., 2011; Ragazzola et al., 2012; Doropoulos and Diaz-Pulido, 2013; Webster et al., 2013), resulting in a reduction of suitable substrate for new recruits (Comeau and Cornwall, 2017). In a warmer and more acidic ocean, the negative effects on gorgonian populations could be amplified by the diminished effectiveness of CCA as settlement inducers. Changes in the type of substrate indirectly affect the population dynamics of benthic species. In the Caribbean, for example, a spread of a peyssonnelid algal crust is changing substrate availability (Bramanti et al., 2017), probably favoring the observed shift between scleractinians and gorgonians (Edmunds et al., 2019; Lenz et al., 2015). The occupation of the substrates by turf algae (Birrell et al., 2005) or the bleaching of CCA (Rindi et al., 2019; Martone et al., 2010), may be disrupting factors for colonization, and this may be a potential scenario also in the Mediterranean Sea (Hereu et al., 2018).

5. Conclusions

Our study does not aim at an exhaustive description of all the cues available for the larvae of the three gorgonian species, but it shows that Mediterranean gorgonians larvae also react to the presence of CCA. Mediterranean gorgonians are key ecosystem engineers and the populations dwelling in the shallower part of their bathymetrical distribution are affected by natural and anthropogenic dis-

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turbances (Garrabou et al., 2009; Cerrano et al., 2000). Conservation of biogenic structures should be a priority, and to this aim, substrate composition and dynamics should be included in management and conservation plans, as the complex equation of population dynamics and maintenance of benthic organisms (including anthozoans) is tightly linked to the species and substrate composition of the seascape.

Declaration of Competing Interest

There is no conflict of interest among all authors.

Acknowledgement

EZ was financially supported by School of Science, University of Bologna. GQ was financially supported by a post-doc contract from the LabEx CORAIL. NL was financially supported by ERASMUS program. The authors gratefully acknowledge G. Vetion and E. Peru for the technical support and the aquarium system set up, B. Hesse and JC Roca and the boat crew for the support during SCUBA diving operations. A special thanks to Diving Center Cadaques and Plongée Bleue for the help in *P. clavata* larval collection and to L. Moirand and L. Guionnet for the help in rearing larvae.

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