

# **Abstract**

 Seafloor macrolitter is ubiquitous in world's oceans; still, huge knowledge gaps exist on its interactions with benthic biota. We report here the colonization of plastic substrates by the Mediterranean red coral *Corallium rubrum* (L. 1758), occurring both in controlled conditions and in the wild at ca. 85 meters depth in the Western Mediterranean Sea. Juveniles settled on seafloor macro-litter, with either arborescent or encrusting morphology, ranged from 0.6 to 3.5 mm in basal diameter and 0.2 - 7.1 years of age, also including a fraction (20%) of potentially sexually mature individuals. In controlled conditions, larvae settled and survived on plastic substrates for >60 days. Our insights show that marine plastic debris can provide favourable substrate for *C. rubrum* settlement either in controlled conditions or in the wild, suggesting their possible use in restoration activities. However, we pinpoint here that this potential benefit could result in adverse effects on population dynamics.

**Keywords:** *Corallium rubrum*; Marine plastic debris; Litter-fauna interactions;

Mediterranean Sea; restoration;

 Plastic is the main component of seafloor litter (Canals et al., 2021; Pham et al., 2014; Worm et al., 2017) and its presence has been documented across all geographic and bathymetric boundaries (Bergmann and Klages, 2012; Cau et al., 2018a; Chiba et al., 2018). Its impact on marine ecosystems has become a matter of great concern for scientists, conservationists and policy makers. One of the reasons for such attention is linked to the wide spectrum of direct interactions occuring between seafloor litter and marine biota (e.g., Romera-Castillo et al., 2018). Among these interactions, the most emblematic examples refer to the accidental ingestion and retention /fragmentation of plastic by organisms associated with the seabed (Cau et al., 2020, 2019; Courtene-Jones et al., 2017) and the entanglement of Derelict Fishing Gears (DFGs) with structuring fauna, including corals (Angiolillo et al., 2015; Angiolillo and Fortibuoni, 2020; Consoli et al., 2019).

 Beside the abovementioned inteactions, as per any kind of 'new' surface that enters the marine environment, plastic can be rapidly colonized by organisms that accumulate over time (Wright et al., 2020a, 2020b). The organisms that colonize the hydrophobic surface of plastic can span from microbes and fungi to macro-invertebrates such as bivalves, barnacles, gastropods, polychaetes, bryozoans, hydrozoan colonies and anthozoan corals (e.g., Battaglia et al., 2019; Santín et al., 2020). In the case of floating plastic, the colonizing fauna can be transported for very long distances (Hoeksema et al., 2012) and thus, plastic surfaces can become potential vectors for their dispersion (Barnes, 2002; Barnes and Milner, 2005). For instance, recent studies highlighted the possible role of plastic surfaces in facilitating the dispersion of invasive alien species, which could often be more successful than endemic species in the competition for space and resources (Barnes, 2002; Barnes et al., 2009; Li et al., 2016). While the role of floating plastic items has been repeatedly documented, the role of seafloor litter as an artificial substrate for encrusting and sessile fauna remains still largely unexplored (Galgani, 2015; Katsanevakis et al., 2007). This aspect could be of particular relevance as seafloor litter can enhance the overall complexity of the habitat, particularly in those otherwise flat and sandy bottoms, paradoxically enhancing local biodiversity (Katsanevakis et al., 2007; Song et al., 2021).



 polyethylene; Fig. 2A); a plastic tape fragment (BT, polyvinyl chloride; Fig. 2B) and two ropes (R, polypropylene; Fig. 2C).

 The synthetic nature of collected items was confirmed by attenuated total reflectance (ATR) Fourier transform infrared spectroscopy (FTIR), using a Spectrum Two spectrometer (PerkinElmer) equipped with the Universal ATR accessory and operating with Spectrum 10 Software. The IR spectra 113 were acquired with a resolution of 4 cm<sup>-1</sup> in the Middle Infrared region (wavenumber range  $=$  4000- 600 cm<sup>-1</sup>) with 4 scans after a background scan. The identification of polymers was performed by comparison with both commercial libraries of standard spectra (PerkinElmer®) and custom-made libraries. Polymers matching with reference spectra for more than 70% were validated (Supplementary Figure 1). Additionally, we observed few cases of *C. rubrum* recruitment on plastic substrates (polypropylene) during a settlement experiment carried out at the Observatoire Oceanologique in

 Banyuls sur Mer (France). Approximately 660 larvae were maintained in closed circuit, oxygenated and temperature-controlled aquaria, to test for substratum preferences (see Zelli et al., 2020 for details). Under these conditions, 5% of the larvae (n=33) settled on plastic substrates (Fig. 3A) and survived for at least 60 days. Production of sclerites was observed in all newly settled individuals (Fig. 3B) and, for some of them, a second polyp was observed next to the primary one, suggesting a good health status of the young settlers.

 Maximum height (cm) and basal diameter (mm) of wild samples were measured and colonies were divided in two morphological categories: branched (B) and non-branched (NB). Basal diameter was used to estimate the age of the samples on the basis of the relationsip between age and basal diameter (Priori et al 2013).

 Overall, 20 juvenile colonies were observed on the different plastic items from the wild, all alive at the moment of collection. Six out of the 20 colonies were classified as branched (Tab. 2). The highest density was observed on the plastic bag (PB) fragment where 4 branched colonies 133 were found, along with 4 unbranched ones, over a surface of ca.  $25 \text{ cm}^2$  (density 0.32 col cm<sup>-2</sup>; Fig. 4). The basal diameter of colonies settled on PB ranged from 0.6 to 3.5 mm, with an average value of

135 2.31  $\pm$  1.02 mm (average  $\pm$  SD; Tab. 2), while the average maximum height was 1.41  $\pm$  0.98 cm, ranging from 0.4 to 3.4 cm.

 The two ropes hosted both branched and unbranched colonies with an encrusting growth pattern of the base (see Fig. 5A,B) and an average basal diameter smaller than that of colonies settled 139 on PB. Colonies settled on the two ropes showed an average basal diameter of  $1.87 \pm 0.87$  mm (min. 1) 140 to 3.5 mm; Tab. 2) and  $1.95 \pm 0.60$  mm (min. 1.2 to 2.6 mm; Tab. 2) for R1 and R2, respectively. The maximum height of juveniles settled on ropes ranged from 0.4 to 5.9 cm, with an average value of 1.32  $\pm$  1.09 cm for R1 and 1.93  $\pm$  2.66 cm for R2.

 The estimated age of colonies retrieved from the wild varied between 0.2 and 7.1 years (Tab. 144 2), with an average of  $2.9 \pm 2.2$  years and modal value of 3.6 years. Despite the uncertainty in age estimation, our results indicate that most of the colonies from the wild may have an age of several years. Our results, while confirming the very long persistence of small plastic debris once they reach the sea bottom, pose the question on their mobility once deposited on the seafloor (*e.g.*, BT and PB samples). Seafloor litter can get partially buried due to sedimentation and remain blocked on the substrate, which could explain how *C. rubrum* larvae could have settled and survive several years on small plastic items.

 According to the few studies available on the reproductive features of deep-dwelling populations (Porcu et al., 2017; Priori et al., 2013), a fraction of colonies (*i.e.*, those with a basal diameter >2.8 mm; 20% of the total) described in the present study are likely to be already sexually mature. The relationship between basal diameter and age (Priori et al., 2013) suggests that age of colonies is comprised between 0.2 and 7 years (Tab. 2), further corroborating this hypothesis since the minimum age at the first reproduction of *C. rubrum* can vary between 6 and 10 years (*i.e.*, 20% of the colonies here described), according to recent age estimation based on the observation of annual growth rings (Gallmetzer et al., 2010; Torrents et al., 2005).The fact that plastic surface can represent a suitable substrate for fouling and epibionts is not a novelty and, very recently, also deep coral settlement was documented to occur on floats from DFGs (Battaglia et al., 2019). Evidences are also building up on the fact that seafloor litter, in certain circumstances, may occasionally become a surface suitable to host benthic species of high conservation concern (Santín et al., 2020). Our results,

 providing the first evidence of precious red coral settlement and growth on plastic debris, add a species of high conservation concern to the list of those capable of colonizing plastic surfaces.

 From an ecological perspective, beside the renown deleterious effects of plastic and microplastic contamination of marine biota (Angiolillo and Fortibuoni, 2020; de Oliveira Soares et al., 2020), the paradox of the "plastic benefits" gained attention at the time when seafloor waste was proved to favour the settlement of non-indigenous species (Mordecai et al., 2011), which can use litter objects as a transport vehicle for their dispersal (Kiessling et al., 2015). This paradox, however holds true also for indigenous benthic species which can use the available surfaces provided by seafloor litter as stepping stones for their dispersal and for enhancing connectivity between populations (Sammarco et al., 2012). This could even have effects at the community level, as larvae of several taxa preferentially settle on non-natural substrates rather than natural ones (Li et al., 2016; Pinochet et al., 2020).

 Anyway, while some fast-growing organisms can be effectively facilitated in their dispersal when settled on litter (Katsanevakis et al., 2007; Zettler et al., 2013), this could not be the case for long- lived benthic species, with lifespan overpassing 100 years, such as *C. rubrum*. Indeed, the small colonies we found (max basal diameter of 3.5 mm), despite being likely sexually mature, host a small number of polyps and this, coupled with the generally low number of fertile polyps of wild colonies (Porcu et al., 2017; Santangelo et al., 2007; Torrents et al., 2005), suggest that those colonies could provide a very small contribution to larvae production. Moreover, plastic substrates like those described in the present study are relatively small and thus their stability on the bottom is unlike; thus, the risk of being moved by near-bottom currents (Kane et al., 2020) and/or being buried into sediments is considerable. Whatever the case, it is likely that it would occur in a time frame that will not allow red coral colonies to reach a highly reproductive size classes, with hundreds of fertile polyps.

 So, while plastic debris in the wild can provide favourable settlement substrates for *C. rubrum* in otherwise unfavourable environments (*e.g.*, a plastic item that lay over soft bottoms or highly silted environments), at the same time it could prevent larvae from settling on neighbouring favourable substrates *e.g.*, rocky surfaces or crustose algae (Zelli et al., 2020). In this latter case, since the

 lifespan of the settled colonies could be reduced, it could potentially affect the local population demographical dynamics, particularly in environments accumulating huge amounts of plastic items, including, for instance submarine canyons (Cau et al., 2017; Dominguez-Carrió et al., 2020). On the other hand, the results obtained from the laboratory experiments (Fig. 3) suggest that in the case of *C. rubrum*, for which the use of artificial non-plastic substrates and transplanting/restoration trials provided scarce or null results, the use of plastic surfaces as a settlement substrate appears a suitable and promising tool for future restoration actions. However, other external cues are known to mediate coral larvae settlement (Heyward and Negri, 1999), including either abiotic variables, such as light, colour, or sound (Lillis et al., 2018; 200 Mason et al., 2011), or biotic ones, such the presence of microbial films,  $CaCO<sub>3</sub>$  skeletons or crustose coralline algae (Golbuu and Richmond, 2007; Negri et al., 2001; Nugues and Szmant, 2006; Webster et al., 2004; Zelli et al., 2020; Jorissen et al 2020). Thus, we foster new experiments on settlement preferences and larval behaviour of red coral and other species of conservation concern, for which restoration measures are needed.

### **Declaration of competing interest**

 The authors declare that they have no known competing financial interests or personal relationships 208 that could have appeared to influence the work here reported.

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## **Figures captions**

- **Figure 1.** Map of the sampling sites along the Northern coast of Sardinia (Western Mediterranean Sea). Sea).
- **Figure 2.** Overview of the *in-situ* samples: a plastic bag fragment (2A); a plastic tape fragment (2B) 227 and two ropes (2C).
- **Figure 3**. Newly settled (45 days old) individuals of *Corallium rubrum* on plastic substrate. At the
- base of the two polyps it is possible to see the sclerites embedded in the coenenchyme. Single polyp
- (A). A second small polyp is originating at the base of the big one (B).
- **Figure 4**. Overview of red coral colonies settled on the plastic bag sample (PB).
- **Figure 5.** Overview and details of red coral colonies settled on the first (A) and second rope (B).
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236 **Table 1.** *In-situ* samples: polymeric composition of *in-situ* samples, sites of collection, geographical 236<br>237<br>238

237 coordinates, depth of collection. PVC = Polyvinyl chloride;  $PE = Polyethylene$ ;  $PP = Polypropylene$ .



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242 **Table 1.** *In-situ* samples: measured colonies code, basal diameter (in millimetres) and maximum 243 height (centimetres), the branching pattern (branched (B) / not-branched (NB) colonies) and the 244 estimated age, according to the growth rates by Priori et al., 2013 (y =  $1.3257 \times x^{0.4947}$ ). NA = not 245 available.

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