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Soft corals and microplastics interaction: first evidence in the alcyonacean species *Coelogorgia palmosa*

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ABSTRACT: Microplastics pollution differentially impacts coral reef systems, by threatening corals physically, through physiological distress and by increasing diseases. However, most of the studies to date have focused on scleractinian corals. The present work reports for the first time the patterns of microplastic ingestion and adhesion by the alcyonacean *Coelogorgia palmosa*. Feeding and adhesion tests were carried out with various concentrations of polyethylene microbeads. Results showed a wide range of surface adhesion, ranging from 3 to 1573 microbeads per coral fragment, suggesting that adhesion driven by mucus is the main mechanism of microplastic trapping. Polyethylene was ingested by 60% of coral fragments, and the average number of ingested microbeads was much lower compared to scleractinian corals. Considering the ecological importance of soft corals in coral reef ecosystems, specific attention regarding microplastic pollution effects on this taxon is recommended.

KEY WORDS: Corals · Polyethylene · Microplastics · Pollution

1. INTRODUCTION

Plastic accounts for 80% of all accumulated ocean litter, with an estimated global emission to the oceans in 2010 of 8 million metric tons (Jambeck et al. 2015), an amount that has likely exponentially increased since then (Borrelle et al. 2020). Lamb et al. (2018) reported that 11.1 billion plastic items were entangled on coral reefs across the Asia-Pacific, estimating that this number will likely increase by 40% by 2025.

Plastic wastes gradually break into microscopic fragments (Huang et al. 2021), known as microplastics (<5 mm in size). Recently, microplastic ingestion by scleractinian corals has been demonstrated, and several studies have documented their negative effects on coral health (e.g. Allen et al. 2017, Reichert et al. 2018). The interaction between microplastics and coral involves ingestion (Allen et al. 2017, Axworthy & Padilla-Gamiño 2019), egestion (Reichert et al. 2018) and surface adhesion (Martin et al. 2019). Laboratory studies have demonstrated that microplastic exposure might adversely influence growth rate, health status and physiology of corals, with consequences for feeding behaviour, photosynthetic performance, skeletal calcification, tissue bleaching and necrosis (reviewed in Huang et al. 2021).

Corals respond differently to microplastic stress, depending on the species (Reichert et al. 2018), the plastic size (Syakti et al. 2019) and the presence of microbial biofilm on the plastic (Allen et al. 2017).

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However, most such studies to date have focused on scleractinian species, while non-scleractinian anthozoans have been neglected. Currently, studies on the interaction between non-scleractinian anthozoans and microplastics are circumscribed to the zoanthids, known as 'button polyps' (Anthozoa: Hexacorallia: Zoantharia). In Rocha et al. (2020), *Zoanthus sociatus* showed an elevated sensitivity to polyvinylchloride (PVC) microplastics, which caused high levels of epidermis adhesion, lipid peroxidation and antioxidant defences. Moreover, PVC, polyethylene (PE) and polymethylmethacrylate (PMMA) microplastic adhesion and ingestion caused mucus secretion and bleaching in *Protopalythoa* sp. (Anthozoa: Hexacorallia: Zoantharia) (Jiang et al. 2021).

Alcyonaceans (Anthozoa: Octocorallia: Alcyonacea) represent a diverse component of coral reef communities and comprise the second most common group of benthic animals on shallow reefs (Norström et al. 2009). They are fundamental in coral reef communities since they provide food, suitable habitat, shelter for reef dwellers and other services that underpin ecosystem biodiversity (Steinberg et al. 2020).

Coelogorgia palmosa Milne-Edwards & Haime, 1857 (Order: Alcyonacea), a common soft coral in different countries of the Indian and West Pacific Oceans, was chosen to explore for the first time the interaction mechanisms between microplastics and alcyonaceans in order to investigate their responses to the presence of microplastics. In this study, microplastic ingestion and adhesion were measured at 2 different microplastic concentrations, and the alcyonacean health status was evaluated by monitoring abnormal mucus production and polyp extension.

2. MATERIALS AND METHODS

At the Genoa Aquarium, 13 *Coelogorgia palmosa* fragments of ~10 cm (mean ± SD number of polyps in each coral fragment = 190 ± 4.5) were collected with pliers from 6 different random colonies raised in the aquarium tanks. The fragments were promptly fixed on supports made by 2-component epoxy resin. Subsequently, they were transported in the experimental tank for 48 h of acclimation. After the first 24 h of acclimation, each fragment was transferred into an individual interaction chamber, consisting of a 2 l-capacity glass beaker, filled with 1.5 l of filtered seawater from the aquarium water system. Each interaction chamber was equipped with an air pump, to allow the circulation of microplastics and to imitate the motion of particles as occurring in nature (Martin et al. 2019). Chambers were allocated in a water bath aquarium's tank to maintain the temperature of 25°C. Fragments were randomly assigned to 2 treatments with different concentrations of fluorescent PE microbeads (0.98 g cm^{-3}), with a size range of 180 to 212 µm (Cospheric). This microplastics size range was chosen because it is similar to the dimension of the zooplankton provided to the corals by the Genoa Aquarium (nauplii of *Artemia salina* and *Brachionus rotundiformis*) and it is a size range of microplastics common in other coral–microplastics exposure studies (Huang et al. 2021). PE was chosen since it is one of the most common types of plastic present in the marine environment (Steinberg et al. 2020) and is one of the polymer types most used in similar studies (de Ruijter et al. 2020, Huang et al. 2021). In the first treatment (T1), 0.013 g of microplastics was added to each chamber, corresponding to a concentration of 0.01 g l^{-1}. In the second treatment (T2), 0.1 g of microplastics was added to each chamber, corresponding to a concentration of 0.07 g l^{-1}. Since no reference studies were present for alcyonaceans, PE concentrations were chosen based on previous experiments on scleractinian and button corals (Hall et al. 2015, Jiang et al. 2021).

For each treatment, 5 *C. palmosa* fragments were exposed in single chambers to microplastics for 48 h. In addition, for each treatment, one chamber with the air pump and the support but without the coral (blank) was set up to evaluate the loss of microplastics in the system. Moreover, 3 chambers, each one with a coral fragment but without PE, were used as controls to check the coral health status under the experimental conditions (Martin et al. 2019).

Three water aliquots of 2 ml were collected from each chamber at the beginning of the treatments (0 h) and after 2, 4, 6, 12, 24 and 48 h, in order to evaluate the variation of microplastic concentration through time. Subsequently, they were filtered using a 100 µm nylon mesh and the microbeads were counted under a Paralux stereomicroscope, equipped with a stereo microscope fluorescence adapter with a UV light head (NIGHTSEA) kit. At 0, 2, 4, 6, 12, 24 and 48 h, abnormal mucus produced by each fragment and the degree of polyp extension were noted through visual inspection.

Abnormal mucus production was classified as the presence of mucus strings streaming off the alcyonaceans, while the surface mucus layer was considered as normal mucus.

The degree of polyp extension was classified as completely introflected (State 1), extroflected with closed tentacles (State 2) and extroflected with open tentacles (State 3). After 48 h of treatment, microbead adhesion and ingestion were assessed. Specifically, coral fragments were removed from their chambers and thoroughly rinsed with salt water to count the number of microbeads adhered. Moreover, fragments were inspected under a stereomicroscope (Paralux) integrated with a UV light kit (NIGHTSEA) to ensure the absence of beads strongly attached to the coral surface. Finally, each coral fragment, controls included, was placed in a Petri dish and dissolved in sodium hypochlorite for 2 h, to allow for the complete digestion of the coral tissue (Martin et al. 2019). We considered microbeads 'adhered' when they were found attached to the coral surface, outside the polyps' mouths. We considered microbeads 'ingested' when, once inspected under stereomicroscope, they were found inside the polyps' mouths or observed in the Petri dishes after the complete dissolution of each *C. palmosa* fragment. Subsequently, the solution was observed under a stereomicroscope equipped with a UV light and a yellow filter to count all microplastics ingested.

The Mann-Whitney test was used to evaluate significant differences in microplastic ingestion and adhesion. The chi-squared test of homogeneity was performed to evaluate differences in abnormal mucus production among treatments, while the chi-squared test of independence was performed to evaluate the difference in polyp status between treatments. A Kendall's tau-b non-parametric correlation test was performed to investigate associations between mucus presence and microplastic adhesion. All statistical analyses were performed in IBM SPSS 27.0 software.

3. RESULTS

3.1. Microplastic surface adhesion and ingestion

At the end of the treatments, all *Coleogorgia palmosa* fragments showed microbeads stuck to their surface (Fig. 1A) and trapped by the produced mucus (Fig. 1B). The highest adhesion value of PE beads per coral fragment was observed in T2, with

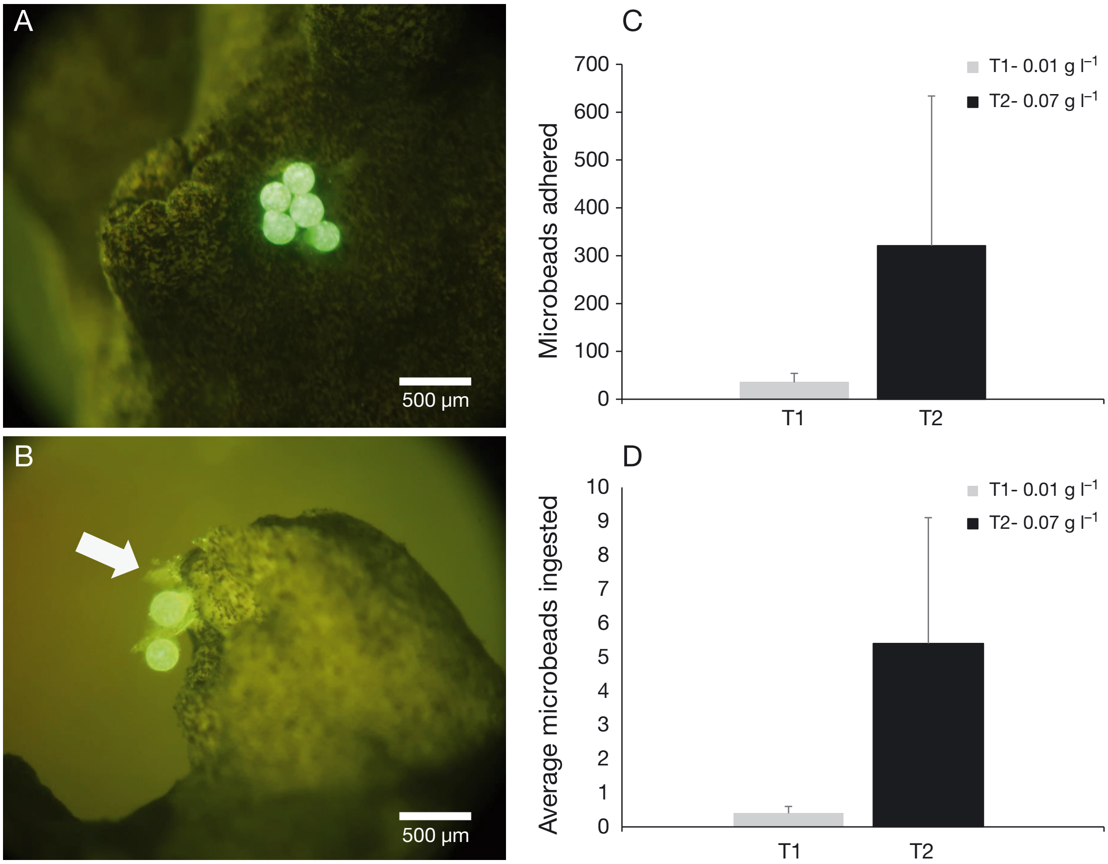

Fig. 1. Microplastic interactions with *Coelogorgia palmosa*. (A) Adhered polyethylene (PE) beads on the coral surface next to a polyp mouth. (B) PE beads trapped by coral mucus; the white arrow shows abnormal mucus coming from a coral polyp. (C) Adhered plastic (average number of PE beads per coral fragment) in corals exposed to treatments T1 and T2. (D) Ingested plastic (average number of PE beads per coral fragment) in corals in T1 and T2. In C and D, bars indicate SEM

an average value 9 times higher than that in T1 (Fig. 1C). Differences in microplastic adhesion between different PE concentrations were not statistically significant ($U = 10$, $z = 0.584$, $p = 0.686$). By contrast, both T1 and T2 showed a statistically significant strong positive correlation between abnormal mucus presence and adhered microplastic number (Kendall's tau-b correlation test, $\tau_b = 0.550$, $p = 0.016$).

Coral polyps ingested microplastics in both treatments. *Coleogorgia palmosa* in T2 reported the highest values of ingested PE beads per coral fragment (Fig. 1D) but no statistically significant differences in microplastic ingestion between the treatments were detected ($U = 4.5$, $z = -1.433$, $p = 0.190$). Under the fluorescent stereomicroscope, most of the ingested microplastics were found inside the polyps' mouths (Fig. 2), while others entered the coral tissue. No leaching of the fluorescent dye was noted. No PE microbeads were found in the control fragments.

3.2. Mucus production and polyp extension

In both treatments, *C. palmosa* fragments showed evidence of stress, with abnormal mucus production and the shrinkage of tentacles. During the experiment, 57% of the treated fragments had polyps in State 2, while most of control fragments (64%) presented polyps in the healthier State 3 and never presented polyps in State 1 (Fig. 3A). After 2 h of exposure to microplastics, 40% of fragments already presented extra mucus filaments that remained throughout the entire exposure time (Fig. 3B). At the beginning, there was a strong impact of the microplastics on the coral fragments in both treatments, followed by a slight decline within the next 6 h and a peak in abnormal mucus presence (60% of coral fragments in both T1 and T2) around 12 h after the start of the experiment (Fig. 3B). By contrast, control fragments did not show any abnormal mucus production. No statistically significant differences in abnormal mucus occurrence between treatments were observed ($\chi^2_1 = 0.583$, $p > 0.05$, $N = 70$). However, when comparing T1 and T2 with the control, statistically significant differences in abnormal mucus presence were found ($\chi^2_1 = 9.234$, $p < 0.05$, $N = 91$). The post hoc test (z-test of 2 proportions) confirmed significant differences between each treatment and the control ($\chi^2_1 = 9.234$, $p < 0.05$, $N = 91$). Regarding the differences in polyp status, the chi-squared test of independence showed no statistically significant differences between T1 and T2, nor between either treatment and the control ($\chi^2_4 = 0.230$, $p > 0.05$, $N = 91$).

4. DISCUSSION

We report for the first time the microplastic ingestion and adhesion patterns of an alcyonacean. During the experiment, *Coleogorgia palmosa* control fragments did not exhibit signs of stress, whereas fragments exposed to microplastics showed a quick abnormal mucus production that generally persisted

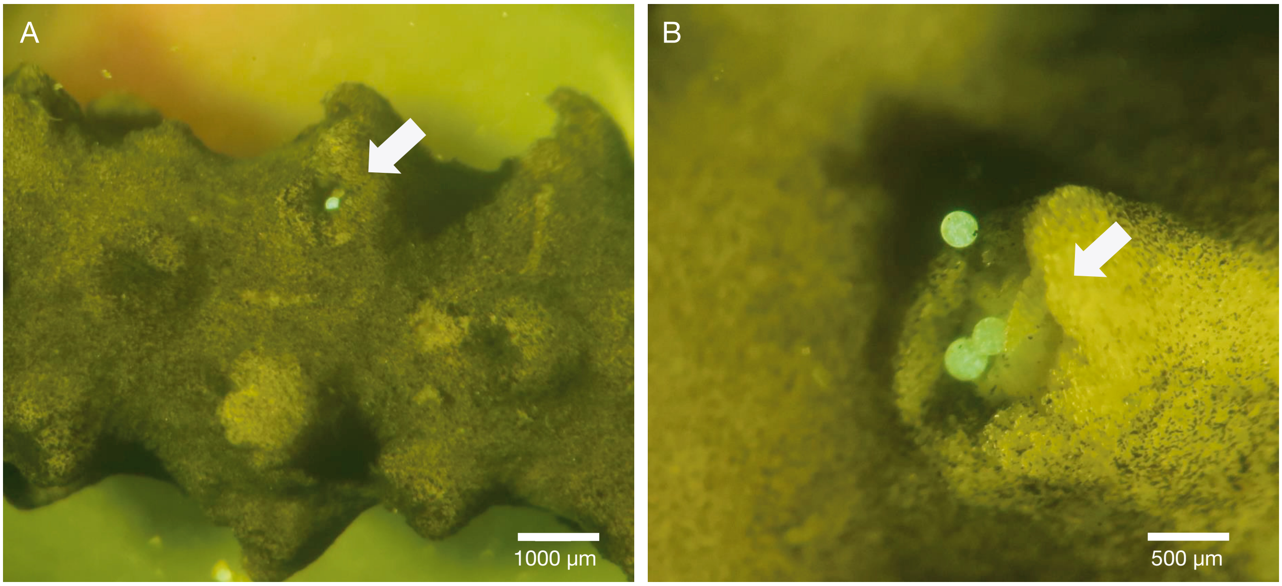

Fig. 2. Polyethylene (PE) ingestion by *Coelogorgia palmosa*. (A) A PE bead inside a polyp's mouth. (B) Beads trapped inside a polyp; the white arrow shows a polyp tentacle interacting with a bead

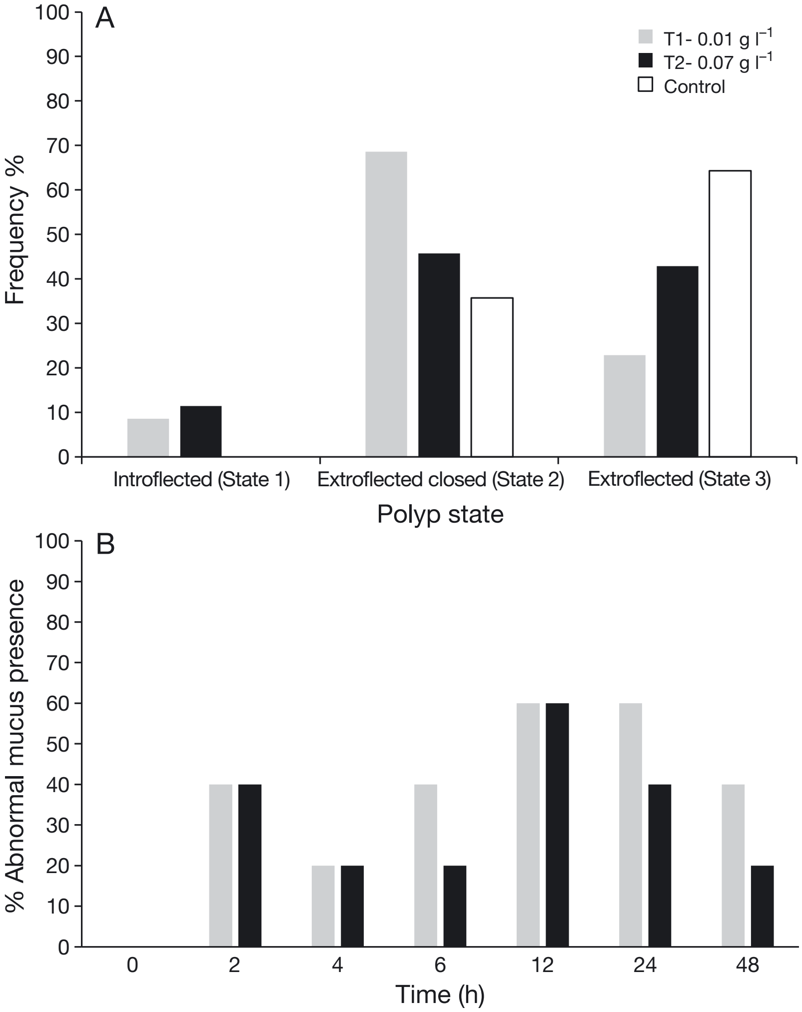

Fig. 3. (A) Frequency of the different *Coelogorgia palmosa* polyp states during treatments T1 and T2, and in control fragments after 48 h of experiment conditions. (B) Frequency of abnormal mucus presence in *C. palmosa* fragments during T1 and T2 and in controls over the duration of the microplastic exposure

during the interaction time in all treatments. Moreover, they displayed polyp contraction, with polyps that mostly remained extroflected but closed. Jiang et al. (2021) reported similar responses for the button coral *Protopalythoa* sp. interacting with microplastics at a concentration of 0.05 mg l^{-1}. Our results showed that alcyonaceans can ingest microplastics, as already observed in scleractinian corals (Hall et al. 2015, Allen et al. 2017) and button corals (Rocha et al. 2020, Jiang et al. 2021). In contrast to other studies (Martin et al. 2019, Jiang et al. 2021), in this work we found a small number of microbeads ingested, and this was not correlated to the microplastic concentration in the water. Similar results for microplastic ingestion were described by Rocha et al. (2020), where the average ingestion was equal to 1.0 ± 0.8 microbeads per coral, at a PE concentration of 10 mg l^{-1}. The authors proposed that the low levels of microplastics observed in *Zoanthus sociatus* gut were due to low ingestion of these particles caused by a potential low heterotrophic requirement of *Z. sociatus* in the short-term exposure. This hypothesis could also be valid for our observations, as *C. palmosa* is a zooxanthellate alcyonacean and it relies on the photosynthesis of zooxanthellae for energy and as a carbon source. However, it is possible that, as already reported by Martin et al. (2019), the mucus occurrence here acted like a microplastic trap. In this case, microplastics on the alcyonacean surface may produce an involucre that might bury the polyps, preventing the extension of the polyps and their ability to capture external particles (Reichert et al. 2018).

Since abnormal mucus production and polyp status were similar among treatments, this may suggest that the occurrence and intensity of these coral responses do not depend, as expected, on microplastic concentrations. Rather, they might depend on the length of the interaction between *C. palmosa* and microplastics.

Microplastics are ubiquitous in marine environments, yet to date, only limited coral reef regions have been investigated. Microplastic abundance in the surface water of coral reefs generally ranges from zero to tens of thousands of items per cubic meter, while in sediments and corals it is difficult to quantify due to the lack of a relatively standardized unit or enough available data (Huang et al. 2021). At the present environmental microplastic concentrations, it is possible to miss or underestimate an organism response resulting from interaction with microplastics (Cunningham & Sigwart 2019, Opitz et al. 2021). Therefore, when we set our experimental concentrations, we adopted a higher microplastic concentration range with respect to the environmental one. Still, our microplastic concentrations are similar to those used in other microplastic–coral feeding trials; this was done in order to be able to observe reliable responses of these overlooked organisms to microplastic presence, as well as to be able to compare our results with other peer-reviewed results. Moreover, it should be highlighted that temporary very high concentrations of microplastics in seawater have been recorded in the past, especially close to coastal areas, and according to our results, these contaminants can impact benthic fauna even only after a short time exposure (Sun et al. 2018, Everaert et al. 2020).

However, additional studies using realistic environmental microplastic concentrations and long-term exposure are required to obtain a clearer picture of the effects of microplastics on alcyonaceans. Moreover, in order to better evaluate the intensity of the coral stress caused by the interaction with microplastics, it might also be interesting to assess quantitatively the abnormal mucus production.

Recently, adhesion has been recognized as one of the dominant interaction mechanisms between microplastics and scleractinian corals, responsible for removing microplastics from the water column (Martin et al. 2019, Corona et al. 2020). Our results extend this hypothesis to soft corals. At 48 h of exposure, all fragments had similar numbers of PE beads adhered to their surface, regardless of the microplastic concentration. This suggests that, in nature, the adhesion may depend not only on the microplastic concentration, but also on the mucus production. Indeed, PE beads attached to *C. palmosa* were mostly glued to the mucus filaments produced by the stressed polyps. The positive correlation between mucus production and the amount of microplastics adhered highlights the concept that the more mucus *C. palmosa* creates, the more microplastic will stick on its surface. Since corals produce mucus when subjected to stress (Brown & Bythell 2005), factors that induce the production of abnormal mucus may enhance the adhesion of random plastic present in the water column, promoting adhesion and adding plastic pollution to the multitude of other coral stress factors.

5. CONCLUSIONS

Alcyonaceans provide fundamental services to coral ecosystems (Steinberg et al. 2020), and will acquire greater importance in the reefs of the future, since transitions from scleractinian-dominated to non-scleractinian-dominated reefs have been already suggested (Bradbury & Reichelt 1983, Bryce et al. 2018). Although conditions (PE shape and concentrations) and responses described here may not be representative of present natural reef environments, they may become more relevant over time, due to increases in microplastic concentrations in the wild as the result of the ongoing input compounded with the further fragmentation of larger plastic debris (Cunningham & Sigwart 2019). This study reports for the first time that soft corals are able to ingest microplastics, and our results provide an important first demonstration on how microplastics can have negative effects on soft coral species. Moving on from our observations, both laboratory experiments and *in situ* studies could be carried out to assess on the finest scale possible the reaction of soft corals to microplastic interactions. This might expand the research interest on these overlooked organisms, leading to a better understanding of resilience capacities in coral reef ecosystems affected by the increasing plastic pollution in the marine environment.

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