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Do microplastics affect the zoanthid *Zoanthus sociatus?*

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Abstract

Microplastics (1 μm – 5 mm), a ubiquitous and persistent marine pollutant, pose a severe threat to coral reefs when recently associated with physiological distress and increased diseases on corals. Studies conducted so far have only reported effects on scleractinian species. Knowledge about its effects on other corals (e.g. Order Zoantharia) remains uncovered, and responses at biochemical levels remain poorly documented. This study aimed to assess the potential effects induced by the presence of microplastics (1 and 10 mg L^{-1} low-density polyethylene, LDPE MP, or polyvinyl chloride, PVC MP) in the tropical and subtropical cosmopolitan species *Zoanthus sociatus* (order Zoantharia. Anthozoa: Hexacorallia), at organism level (survival and behaviour), endosymbionts (photosynthetic efficiency) and the cellular level (oxidative stress, detoxification capacity and energy metabolism). In a short-term exposure (96h), this species was more sensitive to PVC MP. The presence of this polymer at a concentration of 10 mg L^{-1} caused a ten-fold higher adhesion to the coral epidermis, increased photosynthetic efficiency, lipid peroxidation, and antioxidant defences; without, however, inducing energetic costs. Although the observed physiological and biochemical effects did not compromise *Z. sociatus* survival in the short term, it does not rule out potential long-term (cumulative) effects that could endanger this and other physiologically similar species that underlie coral reefs.

1.1. Introduction

Coral reefs harbour the highest biodiversity among marine ecosystems and provide critical natural resources for Humans, particularly in developing countries (Wilkinson 2008; Carpenter et al. 2008). However, both local (e.g., overfishing, pollution, intentional capture for ornamental aquaria, pharmaceutical or biotechnological purposes) and global stressors (e.g., ocean acidification and global warming, pollution) have been causing severe damage to coral reef ecosystems over the last decades (Hughes et al. 2018). Recently, the accumulation of plastic debris, especially microplastics (size ranging from 1 μm to 5 mm, Frias and Nash 2019), in shallow water reefs has been raising particular concern when associated with increased susceptibility to diseases on corals (Lamb et al. 2018). Moreover, the proportion of microplastics in reef sediments and corals epidermis have been higher than those observed in local beach sediments (Cheang et al., 2018), highlighting coral reefs as a potential sink for microplastics from inland activities (Martin et al., 2019).

Acute and chronic exposures of corals to microplastics have only considered scleractinian species (also known as stony corals or hard corals; Anthozoa: Hexacorallia: Scleractinia), with data showing stress responses at different physiological levels. For example, corals from the Merulinidae, Pocilloporidae and Faviidae families were able to ingest microplastics; a behaviour possibly drove by chemoreception mechanisms (Allen et al., 2017; Hall et al., 2015; Reichert et al., 2018). Such ingestion seemed closely related with a long-term decrease in the uptake of natural preys, calcification, zooxanthellae density and chlorophyll content, growth and fecundity (Sussarellu et al. 2016; Hankins et al., 2018; Lo and Chan, 2018; Murphy and Quinn, 2018; Tang et al., 2018). Corals from Acroporidae and Carophylliidae family revealed an unbalance of microbial symbionts (Chapron et al., 2018; Meistertzheim et al., 2016), reduced healing processes (Mydlarz et al., 2006), bleaching and tissue necrosis (Syakti et al., 2019). Corals from the Merulinidae, Acroporidae and Pocillopora families also seemed to be affected by microplastics adhered to their epidermis, with increased mucus production (Reichert et al., 2018) and altered photobiology (Okubo et al., 2018;

Reichert et al., 2019; Syakti et al., 2019). Such deleterious effects can, however, be energetically costly, concomitantly interfering with antioxidant and detoxifying response mechanisms (Tang et al., 2018). Nevertheless, such cellular responses remain, so far, poorly uncovered.

Zoanthids, a group of marine benthic cnidarians (commonly known as "button polyps"; Anthozoa: Hexacorallia: Zoantharia), are the third-largest order of Hexacorallia, with a cosmopolitan distribution from temperate to tropics, from intertidal areas or shallow reef waters to deep-sea (below 5000 m) (e.g., Rabelo et al., 2015; Kumari et al., 2016). Similar to their scleractinian counterparts, many zoanthids host symbiotic dinoflagellates (zooxanthellae) inside their tissues, performing essential roles in reefs' structure and functions (Santos et al., 2016; Kumari et al., 2017). Zoanthids are also recognised for their high phenotypic plasticity, adaptive and resilience capacities to extreme stressors (Reimer et al., 2007; Belford and Philip, 2012; Rosa et al., 2016; Leal et al., 2016), which give them high scientific relevance for the assessment of the potential effect of anthropogenic stressors, such as microplastics. To provide the first evidence on the effect of microplastics on zoanthids, we exposed *Zoanthus sociatus,* a cosmopolitan species that inhabits reefs and intertidal zones of tropical and sub-tropical areas, to two polymers commonly found in sediment and corals epidermis (low-density polyethylene – LDPE MP, and polyvinyl chloride – PVC MP; Cheang et al., 2018) for 96 h. The hypothesis of this study assumes that the microplastics (through ingestion and/or surface adhesion) can affect *Z. sociatus* photosynthetic efficiency, energy consumption, antioxidant and detoxification capacities. Thus, this study assessed the number of microplastics adhered to- and ingested by- corals, photosynthetic efficiency, energy metabolism, antioxidant defences (catalase and glutathione *S*-transferase activities) and oxidative damage (lipid peroxidation).

2. Material and methods

2.1. Test species: culture conditions and fragmentation

Zoanthus sociatus (Anthozoa: Hexacorallia) was used as test species. These organisms have phenotypic plasticity, endosymbiosis with zooxanthellae, and quick regeneration. Three colonies of *Z. sociatus*, collected in Batam – Indonesia, were purchased from a marine ornamentals wholesale company. Colonies were selected under similar conditions (light and depth) and stocked for one week for acclimation to laboratory conditions (water and light test conditions). During this period, organisms were carefully monitored for any evidence of disease.

The acclimation modular system, based on Rocha et al. (2015), consisted in a glass tank (1.48 m x 32.5 m x 48.5 m; 235 L), connected to a 100 L filter tank equipped with a protein skimmer (Deltec SC 500), a calcium hydroxide reactor (Deltec KM 500S), a refrigerator (Hailea HC-500A) and two heaters (Eheim Jäger 300 W) to maintain water temperature at 25±1 °C, one UV filter (55W UV-c), one biological filter (5 L of bio balls), one chemical filter (1 L of activated charcoal), one osmoregulator (Deltec aquastat 1001) to automatically compensate water loss by evaporation by adding freshwater purified by reverse osmosis (V2 Pure 360 Reverse Osmosis System). Two water pumps (EHEIM universal 1200 and EHEIM universal 3400) were used to pump water sequentially through the refrigerator and UV system and to pump the water from the filter tank to the acclimation tank (approximately 2500 $L.h^{-1}$), respectively. Additionally, acclimation tank was equipped with two circulation pumps (Tunze, Turbelle nanostream-6040) with a turbelle controller providing a wave simulation with oscillation flow (200 – 4500 $L.h^{-1}$). Illumination was provided by four 80 W t5 fluorescent lamps (REEF-SPEC™, Red Sea) with a Photosynthetically Active Radiation (PAR) of 70 ± 10 µmol quanta $m^{-2}s^{-1}$, under a 12:12 photoperiod (light: dark, hours). Acclimation system operated with synthetic saltwater, salinity 35, obtained by mixing Red Sea Coral Pro Salt with reverse osmosis water.

After one week of acclimatization, each colony was fragmented with a sterilized scalpel, originating 125 mini colonies, with three polyps each. Each coral fragment was glued (3M Vetbond, n-Butyl cyanoacrylate) to a ceramic substrate (4 x 4 cm) thoroughly washed prior use. Mini colonies were allowed to recover, for two weeks, in the acclimation tank used for their mother colonies, kept under the same conditions.

2.2. Experimental setup

Commercially available low-density polyethylene (LDPE MP, CAS 9002-88-4, irregularly shaped, maximum size 125 µm) and polyvinyl chloride (PVC MP, 671-973-36, irregularly shaped, maximum size 250 µm) were purchased from Sigma-Aldrich and Goodfellow suppliers, respectively. Prior to use, LDPE and PVC MP particles were sieved (vibratory sieve shaking, mesh pore-sizes: 500, 250, 125, 63 and 32 µm) and the size fraction of 63-125 µm was selected for testing, in two concentrations: 1 and 10 mg L^{-1} (corresponding to ~0.5 x 10^5 - 4 x 10^5 or ~0.7 x 10^5 - 1.5 x 10^5 particles L^{-1} of LDPE and PVC, respectively). A total of four treatments plus a control, in triplicate, was applied (see Fig. S1, as supplementary data). Microplastics from each treatment were allowed to age in 1 L of artificial seawater for two weeks before experiments. Each replicate (aquarium) consisted of five colonies of three polyps distributed centrally to receive the same PAR intensity from full-spectrum fluorescent lamps (70 ± 10 µmol quanta m^{-2} s^{-1}, under a 12:12 photoperiod), in glass aquaria containing 38 L of synthetic seawater. Each aquarium contained a heater (Eheim Jäger 50 W) and a water circulation pump (Tunze, Turbelle nanostream-6015). The test endured 96 h at 25 ± 1 °C, pH~8, salinity 35. No food was supplied during the exposure period. Water parameters and microplastics in test medium were monitored every two days.

2.3. *In vivo* chlorophyll fluorescence

Chlorophyll fluorescence was measured *in vivo* using a pulse amplitude modulation (PAM) fluorometer, at the beginning (T_0) and the end of the exposure (T_{96h}) as described elsewhere (Rocha et al., 2013a, Rocha et al., 2014). Minimum (F_o), and maximal (F_m) fluorescence were measured in

dark-adapted samples (for 20 min), with Junior PAM and WinControl3 software (Walz™). Saturating light pulses (450 nm) were performed perpendicularly in each sample, with a 1.5 mm fibre optic. The maximum quantum yield (F_v/F_m) of photosystem II was calculated as $F_v/F_m = (F_m - F_0)$.

After chlorophyll fluorescence measurement at 96 h exposure, mini colonies were split for microplastics counting (one polyp of each mini colony) and biochemical analysis (remaining two polyps of each mini colony). Polyps used for microplastics counting were put in 2 mL microtubes and immediately frozen at -20 °C. Polyps used for biochemical analysis were put into 2 mL microtubes, snapped-frozen in liquid nitrogen and stored at –80 °C until further analysis.

2.4. Microplastics quantification in water and biological samples

Considering that all aquaria contained plastic material (e.g., water circulation pumps and heaters) that could retain or contribute with microplastics, the number of particles in the water column was monitored every two days. For this purpose, per treatment, three replicates of 50 mL of the water column were collected. Water samples were vacuum-filtered into black polycarbonate filters (PCTE, 0.2 μm, ∅ 47cm, GE Healthcare Whatman, U.S.), stained with Nile Red (Sigma Aldrich, U.S.A.; concentration: 100 μg mL^{-1} ETHO), photographed (Canon IXUS 240HS) under visible light (blue light, 540 nm, SPEX Forensics, U.S.A) and UV light (254 nm, 230V, Uvitec, UK), and quantified with the Microplastic Visual Analysis Tool (MP-VAT) developed by Prata et al. (2019), with the freely available software Image J (https://imagej.nih.gov/). Only particles revealing red fluorescence (under UV and blue light) were considered (LDPE or PVC) microplastic.

The number of microplastics adhered to corals epidermis was firstly quantified by visual inspection under a stereomicroscope (stereoscopic zoom microscope — SMZ 1500, Nikon Corporation) associated to NIS-Elements D 3.2 microscope imaging software. Several photos of each polyp were taken, in all angles, and quantified on Image J. Next, polyps were gently cleaned with a soft brush and ultra-pure water and put into glass vials for further digestion with 10% KOH, for 2h, at 50 °C,

followed at room temperature for the next 48h. After digestion, samples were diluted with ultra-pure water (10 ×), vacuum-filtered into black polycarbonate filters, and microplastics quantification was performed as described above. In doubt, a visual inspection under a stereomicroscope was performed, and microplastics were quantified with the hot needle test (De Witte et al., 2014).

Preliminary tests ensured that the selected alkaline digestion did not affect the tested polymers. Nevertheless, sample digestion processes were accompanied by three replicates containing only LDPE MP or PVC MP particles. Contamination control measures included the use of 100 % cotton clothing. Glassware was washed with acid and pre-cleaned with ultrapure water before use. Whenever possible, samples were covered with aluminium foil, and work was performed in a clean air cabinet (Bassaire 03VB, BS EN ISO14644, class 5, with additional cover). Air-borne contamination was assessed by applying laboratory blanks composed of cleaned Petri dishes filled with ultrapure water in each laboratory room used for the analysis. Air-borne contamination was then discarded and not quantified as synthetic plastic debris present in the biological tissues.

2.5. Assessment of energy consumption, oxidative damage, antioxidant and detoxification capacities.

For biochemical analysis, frozen corals were smashed with a glass pestle. Then, samples were homogenized (pulsed mode of 10 % for 30 s, 250 Sonifier, Branson Ultrasonics) with 1000 µL of ultra-pure water. From the homogenate, a 300 µL aliquot was taken for the energy consumption analysis - measured by estimating the electron transport system (ETS) activity; and a 200 µL aliquot was taken for the determination of lipid peroxidation (LPO), to which 4 µL of 4% BHT (2,6-Di-tert-butyl4-methylphenol) in methanol was added. The remaining homogenate was then diluted in 500 µL of 0.2M K-phosphate buffer, pH 7.4, centrifuged for 20 min at 9000 × g (4 °C) and the post-mitochondrial supernatant (PMS) was used to assess catalase (CAT) and glutathione *S*-transferase (GST) activities, and protein levels.

The energy consumption (via ETS activity) measurement followed the method of De Coen and Janssen (1997) with slight modifications for microplate reading Rodrigues et al. (2015). Briefly, 150 µL of homogenization buffer (0.3 M Tris base; 0.45% (w/v) Poly Vinyl Pyrrolidone; 459 mM MgSO4; 0.6% (v/v) Triton X-100 at a pH of 8.5) was added to the 300 µL aliquot of homogenate and centrifuged (1000 × g, 10 min, 4 °C). To 50 µL of supernatant was added 150 µL of buffered solution (0.13 M Tris base containing 0.27% (v/v) Triton X-100; 1.7 mM NADH; 274 mM NADPH) and 100 µL of INT solution (p-iodonitrotetrazolium; 8 mM). The absorbance was read at 490 nm over 3 min. The energy consumption rate was calculated using the stoichiometric relationship (2 mmol of INT-formazan formed 1 mmol of oxygen consumed) and using the formula of Lambert-Beer using a ε = 15.900 M^{-1} cm^{-1} for INT-formazan.

Oxidative damage was inferred by lipid peroxidation levels (LPO), by measuring thiobarbituric acid-reactive substances (TBARS) at 535 nm, using 1.56 x 105 M^{-1} cm^{-1} as molar extinction coefficient, according to Bird and Draper (1984). Antioxidant and detoxification capacities were inferred by catalase (CAT) and glutathione *S*-transferase (GST) activities. The CAT activity was determined by measuring the decomposition of the substrate H_2O_2, at 240 nm, using 40 M^{-1} cm^{-1} as molar extinction coefficient, according to Claiborne (1985). GST activity was determined by assessing GSH conjugation with 1-chloro-2,4-dinitrobenzene, at 340 nm, for 5 min using 9.6 x 103 M^{-1} cm^{-1} as molar extinction coefficient, according to Habig et al. (1974). Protein levels in the homogenate and PMS were determined according to the Bradford method (Bradford, 1976), in a 96 well flat bottom plate, using bovine γ-globulin as a standard.

2.6. Statistical analysis

All data were presented as means ± standard errors. Comparisons between sampling points (on water samples) were performed using one-way analysis of variance (ANOVA), followed by Dunnetts' test ($p < 0.05$). Comparisons between biological samples, within each polymer type, were performed with *t*-test (1 mg L^{-1} *vs* 10 mg L^{-1}). Linear mixed-effects models (LMM) were performed to investigate

the effect of PVC MP or LDPE MP on the photochemical and biochemical endpoints. In the mixed models, the concentration of the toxic was considered as a fixed effect. To account for the repeated measures, individual aquarium was included in the model as a random effect. The outliers were excluded before statistical analysis, being the outliers calculated as the mean ± 2*standard error. The analysis was computed using statistical R software (Lmer package; Bates et al., 2015).

3. Results and Discussion

The water quality parameters (pH, salinity, oxygen) remained stable during exposure period (sampling at 3, 24, 48, 72, 96h): pH: 8.2 ± 0.04; temperature: 24.4 ± 1.05 °C; salinity: 34.94 ± 0.21; dissolved oxygen saturation: 70 % – 90 %.

All corals survived to the experimental conditions.

3.1. Concentration of microplastics during the test

The number of LDPE MPs in the water column decrease with time, particularly at the lowest concentration (Fig. 1A; one-way ANOVA, Dunnett test, $F_{(2, 15)} = 5.07$, $p = 0.02$). On the other hand, in PVC MP treatments, the number of microplastics remained stable (Fig. 1B; one-way ANOVA, Dunnett test, 1 mg L^{-1}: $F_{(2, 13)} = 0.96$; 10 mg L^{-1}: $F_{(2, 13)} = 1.29$; p values > 0.05). The low-density of PE contributed to free distribution of such particles in the water column and surface, which could have contributed to their retention by, for instance, the water circulation pumps. The high density of PVC particles allowed them to settle in the bottom, and likewise less-prompt to be collected and retained by water circulation pumps. No other than the tested particles (white colour, irregularly ball-shaped) were observed in the water samples, which indicate absent contamination by the plastic equipment. The ratio between the lowest and the highest tested concentrations, particularly in the LDPE MP treatments, remained similar within each treatment.

3.2. Microplastics adhered to- and ingested by *Zoanthus sociatus*

The number of microplastics adhered to *Z. sociatus* epidermis after 96h of exposure increased with concentration for both polymers (Table 1, Fig. S2 as supplementary information; *t*-test, $p < 0.05$). Such microplastic adherence was, however, higher for PVC MP treatments (6 to 10 times). This adhesion was an expectable result considering the PVC MP negative floatability. Similar findings were observed in scleractinian corals from the Acroporidae, Pocilloporidae and Merulinidae families, with high-density particles covering higher coral surface area than low-density microplastics (Hall et al., 2015; Hankins et al., 2018; Tang et al., 2018; Martin et al., 2019; Reichert et al., 2018, Reichert et al., 2019; Syakti et al., 2019). However, in these previous studies, the adherence was positively correlated with the number of ingested particles. On this study, the number of the microplastics in corals' gut remained similar between concentrations and polymer type (Table 1, *t*-test, all *p* values > 0.05, Fig. S3, supplementary data). Such low levels of microplastics in *Z. sociatus'* gut suggest low ingestion or retention of these particles, possibly due to a potential low heterotrophy need of *Z. sociatus* in such short-term exposure (Leal et al., 2017). As zooxanthellate zoanthids, the tested species can remain without feeding for long periods, particularly during stress episodes, obtaining their energy and carbon source from zooxanthellae photosynthesis (Reimer et al., 2006; Rabelo et al., 2014, Leal et al., 2017). Other possible explanations might be related with a lower tentacle cleaning rate (Stafford-Smith, 1993), or mucus production that has been proposed as a trap for particles (although also a carrier of such particles to sediments) in reefs ecosystems (Wild et al., 2004), and may have the same role for microplastics. The increment in mucus production has also been recognized as a defence mechanism in corals, triggered by the presence of extraneous particles (Martin et al., 2019). An example, *Acropora hemprichii* presented higher production of mucus compared with *Goniastrea retiformis* and *Pocillopora verrucosa*, which also revealed a higher microplastics removal capacity (Martin et al., 2019). In the present study, no visible alteration in the mucus production was found after exposure to microplastics, suggesting that this mechanism may not be significantly relevant for eliminating

microplastics from the external surface of *Z. sociatus*, or that the presence of microplastics (composition and tested concentrations) does not trigger a need for cleaning in this species. A longer-term exposure could provide an essential insight into the role of ciliary movements of epidermic cells and mucus production on microplastics ingestion and/or removal by *Z. sociatus*. Notwithstanding, coral species present different physiologies and behaviour. Thus, the ingestion of microplastics, adherence, and cleaning processes are likely to be species-specific and potentially driven by polyp's chemoreception properties, as underlined by previous investigations (Allen et al., 2017; Reichert et al., 2018, Reichert et al., 2019).

3.3. Photosynthetic efficiency

The short-term exposure to LDPE MP did not alter photochemical efficiency in *Z. sociatus* (Fig. 2, Table S2 as supplementary data; LLM-ANOVA, factor: concentration, all p values > 0.05). However, 10 mg L^{-1} PVC MPs exposure induced significant alterations on the photochemical efficiency, with an increment of approximately 6 % in the photosynthetic yield (F_v/F_m) (Fig. 2; LLM-ANOVA, factor: concentration; F = 4.87; $p < 0.05$). The difference in photochemical efficiency between *Z. sociatus* exposed to LDPE MP and PVC MP (at 10 mg L^{-1}) is likely related with the number of adhered particles, ca. 10 times higher in PVC MP treatment (Table 1). Such high adhesion of PVC MPs on *Z. sociatus* surface may have decreased the light reaching the endosymbiotic zooxanthellae, that probably contributed to the increase in F_v/F_m. Increasing photosynthetic efficiency with decreasing PAR intensity has already been described for other photosynthetic corals grown under low PAR conditions (Rocha et al., 2013b). Similar results were also observed in scleractinian corals from the Aroporidae, Pocilloporidae and Merulinidae families, that revealed a relationship between the surface area covered by microplastics and photosynthetic efficiency (e.g., Tang et al., 2018; Martin et al., 2019; Syakti et al., 2019). A similar trend has also been observed for sediment settling particles on corals surface, under a turbidity gradient (e.g. Junjie et al., 2014).

Corals photosynthetic efficiency has also been positively correlated with the symbiont density and chlorophyll content (e.g., Tang et al., 2018). Although these endpoints in *Z. sociatus* were not assessed in this study, it may be hypothesized that the increased photosynthetic efficiency could have resulted from increased chlorophylls levels in the photosynthetic apparatus rather than an increased number of the zooxanthellae.

3.4. Energy consumption and oxidative stress responses mechanisms

Z. sociatus short-term exposure to LDPE MP did not affect energy consumption (ETS), levels of antioxidant defences (CAT and GST) nor oxidative damage (LPO) in (Fig. 3; LLM-ANOVA, factor: concentration, all p values > 0.05, Table S2 as supplementary data). However, 10 mg L^{-1} of PVC MPs induced an increase of CAT activity (LLM-ANOVA, factor: concentration; F = 3.73; p = 0.03) and peroxidative damage – LPO (LLM-ANOVA, factor: concentration; F = 5.61; p = 0.04). No significant effects on energy consumption (ETS) and detoxification capacity (GST) were detected after PVC exposure (Fig. 3; LLM-ANOVA, factor: concentration, both p values > 0.05, Table S2 as supplementary data). The increase in CAT activity and lipid damage on *Z. sociatus* exposed to the highest PVC MP concentration can be related with an increment on reactive oxygen species (ROS), particularly of H_2O_2 and OH^-, which may increase CAT activity and induce the propagation of lipid peroxyl radicals. ROS production could be a result of different processes, such as: i) PVC MP ingestion, which could have induced an inflammatory/ immune response by the potential abrasions in mesenterial tissues within the coral gut cavity ii) PVC plasticisers (e.g., phthalates, that are normally absent in pristine LDPE particles; Andrady, 2017) that could have leachate during exposure and could consequently altered the digestion/egestion processes; and iii) limited cellular gas exchange and unbalanced photosynthetic capacity caused by the high number of microplastics adhered to corals epidermis. The latter hypothesis is partially supported by transcriptomic results on *Pocillopora damicornis* (Family: Pocilloporidae) exposed to other highly dense polystyrene microplastic, which showed an increment in CAT activity, along with 134 up-regulated genes mostly

related to oxidative stress response, zymogen granule, and JNK signal pathway, along with 12 genes related with photosynthate translocation transporters (Tang et al., 2018). It also revealed a decrease in GST levels but at 50 mg L^{-1}, a concentration five times higher than the highest concentrations tested in our study.

Along with oxidative stress, an increment on energy expenditure would be expected. An ingestion and subsequent egestion of food items or suspended particles (e.g., microplastics) usually involve the energetically costly movement of polyps' tentacles, along with tissue contraction (Stafford-Smith and Ormond, 1992). Likewise, when facing high adherence of particles to their epidermis, corals tend to actively remove them through the production of mucus or by active ciliary action (Abdel-Salam et al., 1988). These processes can, therefore, be energetically expensive and lead to increases in energy consumption. However, this was not the case for *Z. sociatus* under the tested conditions, even at the highest PVC MP tested concentrations where a high number of adhered microplastics was observed. Changes on energy consumption may, however, occur in longer-term exposures.

4. Final Considerations

Overall, this study provides new insights into the potential mechanistic responses of corals induced by the presence of microplastics under acute exposure and deliver the first data in zoanthids. Briefly, the presence of highly dense microplastics (PVC MP) at a concentration of 10 mg L^{-1} altered *Z. sociatus* endosymbionts photosynthetic efficiency and induced oxidative stress after four days of exposure, while the presence of less dense microplastics (LDPE MP) caused no effect. However, in aquatic natural environments, processes like aggregation, interaction with suspended particles and biofouling (e.g., microalgae, bacteria, and fungi) may lead to an increased density of LDPE MPs and subsequent settling in sediment (Galloway et al., 2017; Lagarde et al., 2016), which may result in higher bioavailability and palatability to corals and promote toxic effects of these particles.

The available levels of microplastics in coastal waters suggest that currently these organisms will not likely be exposed to these levels of microplastics. However, the continuous fragmentation of larger plastic particles, will most likely lead to a considerable increase in the number of microplastics in the environment (Araujo et al., 2018). Recent studies corroborate this trend, reporting increasing concentrations of microplastics with decreasing size (< 100 mm), suggesting that actual concentrations in the environment could be higher than those reported to date (Brandon et al., 2019; Conkle et al., 2018).

The presence of microplastics in coral reefs are a reality (Cheang et al. 2018). Thus, it is crucial to evaluate their effects in the long run. Long-term studies addressing energy budgets, growth rates, survival, and reproduction of the coral holobiont might help to answer open questions regarding coral-microplastic interactions, particularly in soft corals, which remained poorly documented. Corals are also particularly vulnerable to other stressors, such as increasing water temperatures, high UV light, carbonation, ocean acidification (Anthony and Kerswell, 2007; Putnam et al., 2017). In natural reef systems, microplastic pollution may add up to existing stressors and amplify the corals' susceptibility to bleaching and diseases. The presence of microplastics combined with other natural or anthropogenic stressors might drive corals further towards a critical tipping point and foster community shifts in coral reef assemblages. Therefore, future studies should consider the combined effect of microplastics with other (natural or anthropogenic) stressors, for a proper risk assessment.

Author contributions statement

ALPS, RR, and MO conceived the ideas and designed methodology; LHC, ACMR, DC, APLC, DS ran the experiment and collected the data; ALPS, RR, ACMR, and DC analysed the data and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Conflict of interests

All authors declared no conflict of interests.

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Appendix A. Supplementary data

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Table caption:

Table 1. Number of microplastics adhered to, or ingested by *Zoanthus sociatus*, after 96h exposure to 1 and 10 mg L^{-1} of low-density polyethylene (LDPE MP) or polyvinyl chloride (PVC MP). Data are presented as mean ± standard error (*N*=15). (*)denote significant differences between concentrations, withing polymer type (t-test, $p < 0.05$).

Figure captions

Fig. 1: Number of microplastics in the aquaria throughout time (3, 48 and 96h). A) Low-density polyethylene (LDPE MP); B) Polyvinyl chloride microplastics (PVC MP). Data are presented as means ± standard errors (*n*=3, per sampling time). (*) denote significant differences between groups (Variable: concentration; One-Way NOVA, Dunnetts' test, $p < 0.05$)

Fig. 2: Effect of 1 and 10 mg L^{-1} of low-density polyethylene (LDPE MP) or polyvinyl chloride microplastics (PVC MP) in the photochemical efficiency (F_v/F_m) of *Zoanthus sociatus*, in the beginning (A) and after exposure (B). Data presented as means ± standard errors. (*) denote significant differences compared to control group (Fixed effect: concentration; LLM model, intercept: 0 mg L^{-1}, $p < 0.05$).

Fig. 3: Effect of a short-term exposure (96h) to 1 and 10 mg L^{-1} of low-density polyethylene (LDPE MP) or polyvinyl chloride microplastics (PVC MP) on lipid peroxidation (LPO), catalase activity (CAT), Glutathione *S*-transferase levels (GST), and energy consumption (*via* electron-transference-system, ETS) of *Zoanthus sociatus*. Data presented as mean ± standard error. (*) denote significant differences compared to control group (Fixed effect: concentration; LLM model, intercept: 0 mg L^{-1}, $p < 0.05$).

Table 1. Number of microplastics adhered to, or ingested by *Zoanthus sociatus*, after 96h exposure to 1 and 10 mg L^{-1} of low-density polyethylene (LDPE MP) or polyvinyl chloride (PVC MP). Data are presented as mean ± standard error (*N*=15). (*)denote significant differences between concentrations, withing polymer type (t-test, $p < 0.05$).

	LDPE MP		PVC MP	
	1 mg L^{-1}	10 mg L^{-1}	1 mg L^{-1}	10 mg L^{-1}
Adhered to epidermis	1.2 ± 0.6	17.7 ± 5.7*	7.0 ± 1.6	167± 28.2 *
Gut cavity	0.8 ± 0.4	1.0 ± 0.8	0.5 ± 0.4	0.3 ± 0.2

Graphical abstract

Highlights

Microplastics (MPs) may threaten coral reefs due to physiological distress

Zoanthus sociatus polyps exposed to 1 and 10 mg L^{-1} LDPE and PVC particles 63-125 μm

PVC MPs were more harmful to *Zoanthus sociatus* than LDPE MPs

PVC MPs altered photobiology and induced oxidative stress on polyps only at 10 mg L^{-1}

MPs at concentrations below 1 mg L^{-1} might do not pose a risk to *Z. sociatus*

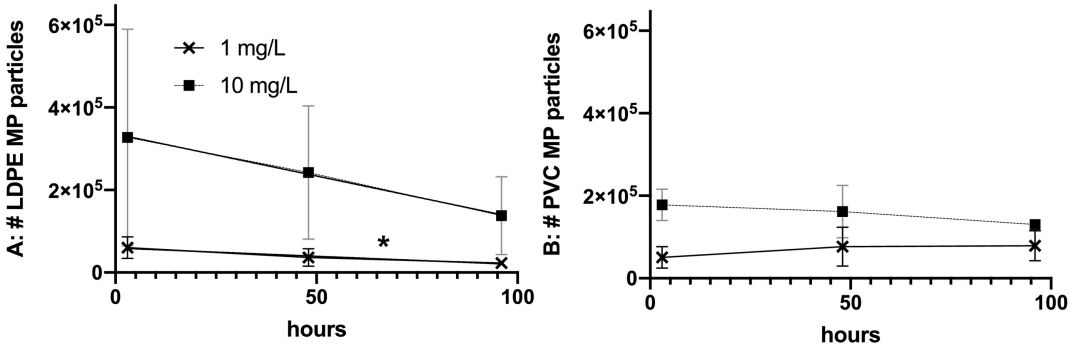
A: # LDPE MP particles
6×10^5
4×10^5
2×10^5
0
1 mg/L
10 mg/L
*
0
50
100
hours
B: # PVC MP particles
6×10^5
4×10^5
2×10^5
0
50
100
hours

Figure 1

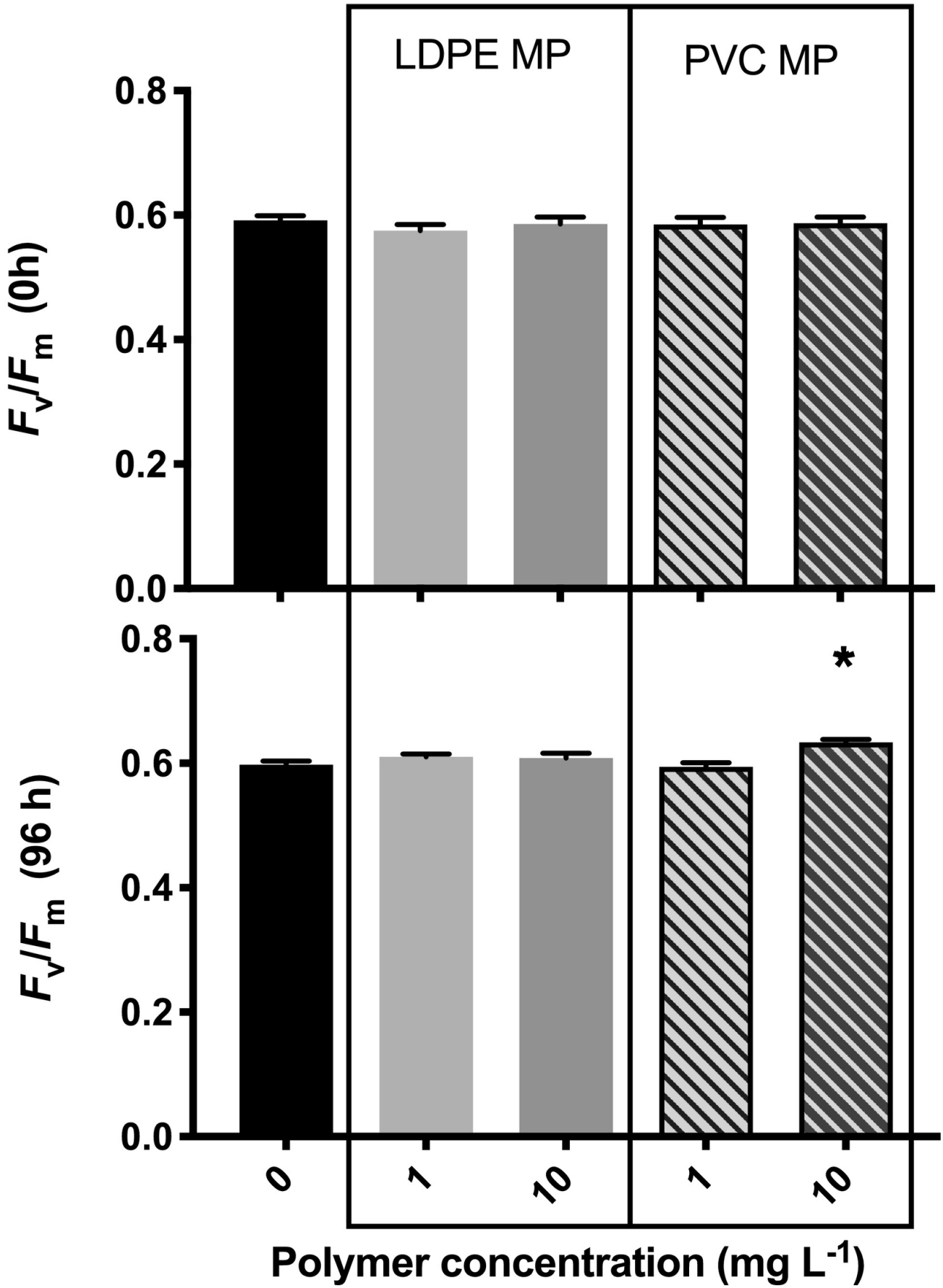
LDPE MP
PVC MP
0.8
0.6
0.4
0.2
0.0
F_v/F_m (0h)
0.8
0.6
0.4
0.2
0.0
F_v/F_m (96 h)
*
0
1
10
1
10
Polymer concentration (mg L^{-1})

Figure 2

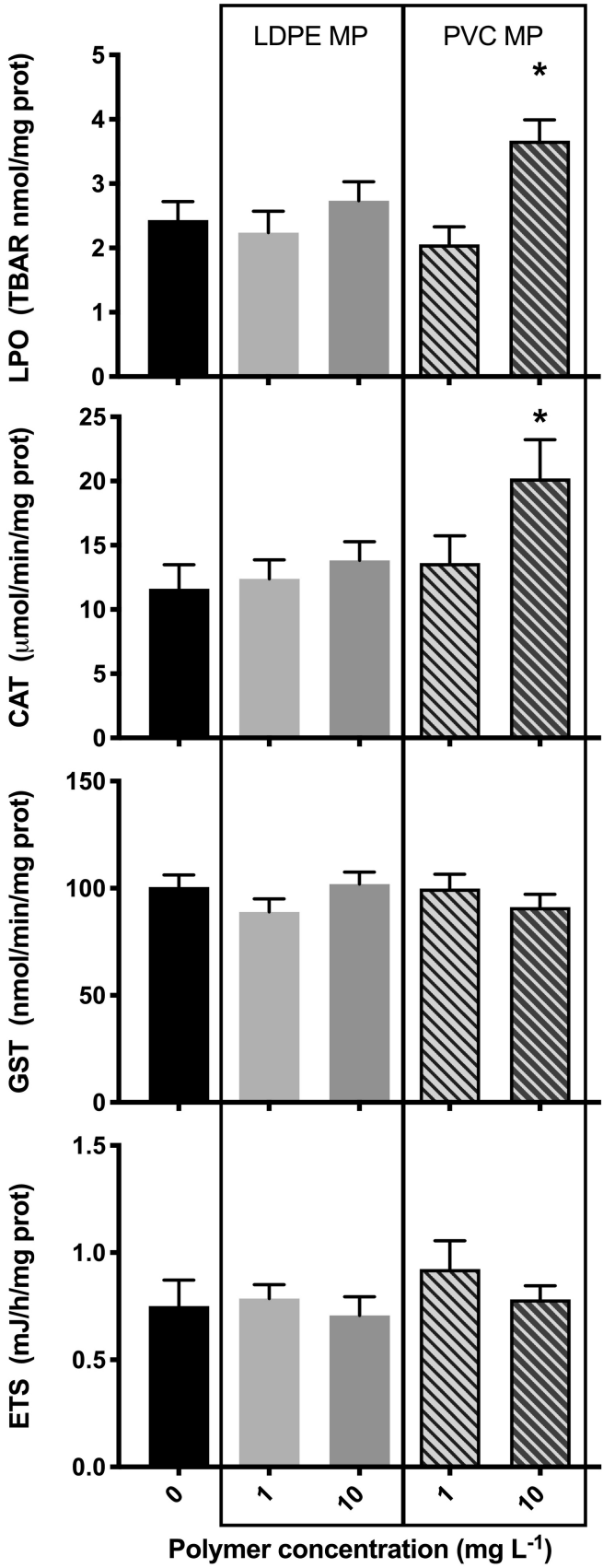
LDPE MP
PVC MP
LPO (TBAR nmol/mg prot)
CAT (µmol/min/mg prot)
GST (nmol/min/mg prot)
ETS (mJ/h/mg prot)
*
*
0
1
10
1
10
Polymer concentration (mg L-1)

Figure 3