



## Investigating the effects of PLA microplastics on *Pocillopora damicornis* (cnidaria, scleractinia)

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### ABSTRACT

While the harmful effects of synthetic microplastics on reef-building corals are well documented, the impacts of their bio-based counterparts remain largely understudied. In this study, we investigate the chemical and physical properties of mechanically grounded polylactic acid microplastics and assess their short-term effects on the physiology and cellular oxidative state of the scleractinian coral *Pocillopora damicornis*. The microplastics, obtained by mechanical grinding, exhibit a wide size distribution, with 90% of particles  $\leq 370 \mu\text{m}$  and 50%  $\leq 192 \mu\text{m}$ . They display irregular size and rough surface, along with reduced crystallinity and molecular weight compared to the original pellets. Coral colonies were exposed to three microplastic concentrations (5 mg/L, 15 mg/L, and 50 mg/L) for 72 h, and no mortality or signs of bleaching were observed in all cases. Although colonies exposed to the higher concentration exhibited an increase in the activity of the antioxidant enzyme glutathione reductase, no significant cellular oxidative damage was caused by the microplastics, as the lipid peroxidation analysis indicated. This study provides a preliminary assessment of the physiological effects of polylactic acid microplastics on stony corals, emphasizing the need for further research on bio-based contaminants and their impact on marine benthic organisms.

### 1. Introduction

Since the early 20th century, advances in polymer science have driven major societal changes with global plastic production continuously increasing and reaching 413.8 million tons in 2023 (Barnes et al., 2009; Statista, 2018). Although plastic has made human life more convenient, it has also contributed to the accumulation of waste in the environment, with estimates indicating that approximately 70% of global debris is composed of plastic (Avio et al., 2017; Conkle et al., 2017). A significant portion of this waste ultimately enters the marine environment where biotic and abiotic degradation processes break down plastic into microplastics (MPs; particles smaller than 5 mm) and nanoplastics (NPs; fragments smaller than 100 nm) (Andrady, 2011;

Arthur et al., 2009; Gewert et al., 2015). Their widespread occurrence is now a significant environmental concern due to their high bioavailability and the largely unknown effects they may have on living organisms (Wright et al., 2013).

Among them, sessile benthic organisms are at great risk of harmful interactions with MPs, as plastic particles tend to sink over time (Kowalski et al., 2016; Wang et al., 2016), because their surface is subjected to microbial colonization, which reduces their buoyancy and leads to their eventual deposition on the sea floor (Wright et al., 2013; Zettler et al., 2013; Kaiser et al., 2017). In fact, MPs significantly impact marine sessile benthic organisms, by disrupting various key physiological functions, and affecting their overall physiological homeostasis (Mkuye et al., 2022). Specifically, exposure to MPs can impair immune

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system functionality, leading to a reduction in hemocyte count, disturbances in oxidative balance, altered respiration, and increased energy consumption due to MP and associated chemical additives ingestion (Mkuye et al., 2022).

These findings highlight the complex and multifaceted impact of MPs on marine sessile benthic organisms, emphasizing the need for further research on their ecological consequences. So far most of the studies have been focused on MPs of synthetic polymer nature (Kushwaha et al., 2024). However, in recent years, bioplastics are continuously gaining space in the commercial field, as a promising alternative to conventional plastic, with polylactic acid (PLA) to be a key thermoplastic biopolymer. Although PLA is considered a biodegradable polymer under aerobic conditions in industrial composting systems, its degradation rate is markedly reduced in natural environments (Song et al., 2025; Li et al., 2022). Therefore, the presence of PLA-derived MPs and NPs into the environment is likely to increase in the following years (Ainali et al., 2022). Current research suggests that, owing to its biodegradable nature, PLA may exhibit distinct environmental behaviors and impacts compared to conventional petroleum-based MPs. However, despite a growing body of research, important gaps remain in understanding its fate and ecological risks (Teng et al., 2025).

Coral reefs contribute significantly to the global economy by providing coastal protection, food resources, and tourism opportunities (Costanza et al., 2014; Spalding et al., 2017). Central to these ecosystems are scleractinia, or hard corals, which form three-dimensional structures that support a wide variety of reef species underscoring the role of coral reefs as biodiversity hotspots (Graham and Nash, 2012). Soft corals are also essential components of coral reef benthic communities, representing the second most abundant group after hard corals and playing a significant role in supporting the high biodiversity characteristic of these ecosystems (Hutchings, 2019; Garra et al., 2020).

While research has highlighted the potential negative impacts of MPs on corals, the precise mechanisms driving these effects remain unclear. Specifically, toxicological studies on petroleum-based MPs, ranging in size from a few micrometers to hundreds of micrometers, have revealed significant biological effects. MPs composed of high-density polyethylene (HDPE) (MPs size 65  $\mu\text{m}$ –410  $\mu\text{m}$ ) (Reichert et al., 2019), polyethylene (PE) fluorescent microbeads (size of 53–500  $\mu\text{m}$ ) (Martin et al., 2019), and polystyrene (PS) (1  $\mu\text{m}$ ) (Tang et al., 2018) have been shown to impair growth, elevate antioxidant enzyme activity, and stimulate mucus production. Furthermore, Liao et al. (Liao et al., 2021) investigated the effects of polyvinyl chloride (PVC), PE, polyethylene terephthalate (PET), and polyamide 66 (PA66) MPs (1–10  $\mu\text{m}$ ) after 24 h of exposure on the coral *Tubastraea aurea*, reporting decreased antioxidant capacity, impaired immune function, reduced calcification, and lower energy consumption. Similarly, *Acropora formosa* exposed to LDPE MPs of different sizes (<100  $\mu\text{m}$ , 100–200  $\mu\text{m}$ , and 200–500  $\mu\text{m}$ ) at concentrations of 0.05, 0.10, and 0.15 g/L for 14 days showed bleaching and mortality caused by increasing MPs concentrations, especially when exposed to MPs sizes <100  $\mu\text{m}$  (Syakti et al., 2019).

Corinaldesi et al. (Corinaldesi et al., 2021) examined the effects of polypropylene (PP), PS, and PE particles (20–100  $\mu\text{m}$ ; 94 particles/L) on *Corallium rubrum* over a 20-day exposure, finding a feeding preference for PP, which led to impaired trophic capacity, mucus accumulation, and gene transcription alterations. Montalbetti et al. (Montalbetti et al., 2022) conducted multivariate analyses on the soft coral *Coelogorgia palmosa* that revealed significant differences in the responses of corals to varying concentrations of PE MPs of sizes ranging from 180 to 212  $\mu\text{m}$ . Corals exposed to high concentrations (50 mg/L and 70 mg/L) showed notable changes in superoxide dismutase (SOD), catalase (CAT), and lipid peroxidation (LPO) when compared to the lower concentration (10 mg/L) and control groups, revealing that oxidative stress is caused by the presence of these particles. Vencato et al. (Vencato et al., 2021) reported that *Coelogorgia palmosa* can interact with PE MPs (180–212  $\mu\text{m}$ ) through both ingestion and adhesion. Additionally, an overproduction of mucus was observed, suggesting that mucus-mediated

adhesion is the primary mechanism for trapping MPs.

Another study conducted by Isa et al. (Isa et al., 2024) suggested also that PE MPs (180–212  $\mu\text{m}$ ) could exacerbate thermal stress effects on coral cellular homeostasis, even at environmentally relevant concentration (1 mg/L). This study indicated that the synergistic interaction between elevated ocean temperatures and PE MPs led to significant disruptions in cellular homeostasis in *P. damicornis* also modifying the cell oxidative status. Previous studies have also demonstrated that petroleum-based MPs exposure induces the production of reactive oxygen species (ROS) at the cellular level in various benthic organisms (Jeong et al., 2017; Sutton et al., 2016; Rocha et al., 2020), including corals (Tang et al., 2018; Soriano-Santiago et al., 2013). These studies clearly show that oxidative stress is one of the main effects triggered in corals by exposure to plastic fragments. However, despite extensive research on the impact of synthetic materials and MPs on corals, the potential effects of bio-based polymer fragments are still largely unknown.

Herein, we focus on the development of PLA MPs, and on the assessment of their potential impact on coral physiology and health. Since MPs ranging from 150 to 500  $\mu\text{m}$  are among the most predominant in the environment (Alfaro-Núñez et al., 2021), it is expected a high possibility of interaction of MPs of such size range with living organisms. Therefore, to study the impact of the PLA MPs on corals, polymeric fragments were produced by mechanically grinding PLA pellets, resulting in particles with a size distribution showing  $D_{90} \leq 370 \mu\text{m}$  and  $D_{50} \leq 192 \mu\text{m}$ . These MPs were then chemically and physically characterized, to highlight differences with the original material. To create more ecologically relevant exposure conditions, the MPs were pre-treated in *Artemia salina* enriched seawater before coral exposure. The reef-building coral *P. damicornis*, a widespread and ecologically significant species, was then exposed to three MPs concentrations (5 mg/L, 15 mg/L, and 50 mg/L) to evaluate potential physiological stress and disruptions of oxidative homeostasis. Exposure concentrations included an environmentally relevant level, selected within the upper range of values reported in natural environments (5 mg/L) (Burns and Boxall, 2018; Cunningham and Sigwart, 2019; Opitz et al., 2021), as well as higher concentrations to simulate high-pollution scenarios and enhance the detectability of PLA effects.

To assess oxidative stress, the activity of key antioxidant enzymes involved in ROS detoxification and neutralization, such as SOD, CAT, glutathione reductase (GR), and glutathione S-transferase (GST), was measured. Additionally, LPO was analyzed to determine cellular oxidative damage. Coral bleaching and the general photophysiological response of corals was evaluated by quantifying chlorophyll *a* and *c2* concentrations within coral tissues. This research provides a preliminary evaluation of the effects of PLA MPs on a specific hard coral species, providing valuable insights into the physiological and ecological consequences of biobased plastic exposure. By examining the interactions between PLA MPs and corals, this study establishes a foundation for future investigations into the broader impacts of bioplastics in marine ecosystems.

## 2. Materials and methods

### 2.1. Materials

Semi-crystalline PLA in the form of pellets with 4 mm average size was supplied by NatureWorks LLC (U.S.A) (commercial name: PLA4043D,  $M_n = 160.000 \text{ g/mol}$ , density:  $1.24 \text{ g/cm}^3$ ). *Artemia salina* water medium was collected from the Genoa Aquarium in June 2024. Upon collection, the medium, containing an average concentration of 250 individuals/mL was filtered (5  $\mu\text{m}$  nylon sieve) to remove the living organisms. All reagents and chemicals used were purchased from Sigma-Aldrich. Milli-Q water was obtained from Milli-Q Advantage A10 purification system.

## 2.2. MPs production

PLA MPs were produced by using Dry mill – IKA – PILOTINA MC and an electrical sieve (Endecotts Minor 200 sieve (Lombardy, Italy) equipped with VWR Test Sieves (200 × 50 mm, mesh size 300 and 150 μm). Initially, the 4 mm pellets were reduced to grains with size lower than 1 mm by the dry mill operating at 1500 rpm, equipped with a 1 mm sieve. The obtained powder was further processed through a finer sieve to obtain powder grain size lower than 750 μm. To isolate particles mostly in the 150–300 μm size range, the powder was subsequently sieved using the electrical sieve with a sieve of 300 μm mesh size, followed by a second sieving step with a 150 μm mesh size. Particles that did not pass through the final sieve were collected for further use.

## 2.3. MPs characterization

The physicochemical properties of the PLA MPs before and after exposure to the *Artemia salina* water medium (1 h), followed by rinsing with Milli-Q water and drying under ambient conditions, were investigated using the following analyses.

Particle size and shape analysis was performed through laser diffraction and dynamic image analysis using the Particle Analyzer SYNC (wet operation Flowsync, Microtrac Retsch GmbH) assuming spherical geometry of the particles (Supporting Information Section S1 for further information). Measurements were conducted at room temperature, and the sample was analyzed three consecutive times. Granulometric analysis allowed us to quantify the percentage distribution of particles across different size classes. Approximately 48% of particles were within the target range, 33% were smaller than 150 μm, and 19% exceeded 300 μm.

Morphological analysis of the MPs surface was conducted by scanning electron microscopy (SEM) (JEOL JSM-6490LA) using the secondary electrons detector with a 10 kV accelerating voltage and a load current of 78 μA. The MPs were attached to aluminum stubs by using carbon tape and coated with a 10 nm gold layer through a high-resolution sputter coater (Cressington 208 HR).

X-ray diffraction analyses were carried out on a Malvern-PANalytical 3rd generation Empyrean X-ray powder diffractometer. Details on the experimental process as well as on the calculation of the degree of crystallinity are provided at the Supporting Information, Section S1.

Gel permeation chromatography (GPC) was performed using an integrated OMNISEC system (Malvern Panalytical Ltd., UK) equipped with a PLgel 5 μm MIXED-D column in series with a PLgel 3 μm MIXED-E operating online at 35 °C and connected to a refractive index and light scattering detectors (OmniseC reveal, Malvern, UK). Details on the experimental procedure are supplied at the Supporting Information Section S1.

Infrared spectra of the PLA pellets and MPs were acquired with a Fourier Transform Infrared (FTIR) spectrometer (Vertex 70v FT-IR, Bruker) coupled to a single-reflection attenuated total reflection (ATR) accessory (MIRacle ATR, PIKE Technologies) (ATR-FTIR). All the spectra presented were the average of 32 repetitive scans in the range of 4000–600 cm<sup>-1</sup> at a resolution of 2 cm<sup>-1</sup>. The changes in the carbonyl band, characteristic of the degradation of the PLA polymer chain, were explored through the calculation of the carbonyl index (CI) (Gomes et al., 2024), i.e. the ratio between the intensity of the carbonyl (C=O) peak at 1750 cm<sup>-1</sup> and that of the methylene (C–H) peak at 1452 cm<sup>-1</sup>.

Raman spectra were collected using Renishaw in Via confocal Raman microscope with 785 nm excitation wavelength. The spectral measurements were conducted with 10 s exposure time, laser power of 100% and 50× of magnification. The spectrometer provides Raman spectra in the range of 200 to 3600 cm<sup>-1</sup>. All spectra represented in this study were baseline corrected.

Surface chemical composition of the samples was investigated by means of X-ray Photoelectron spectroscopy (XPS, Kratos, Axis UltraDL). Details on the experimental process are given at the

Supporting Information Section S1.

## 2.4. PLA MPs effects on corals: oxidative stress and bleaching assessment

### 2.4.1. Experimental setup

At the Genoa Aquarium, 24 nubbins (8–10 cm in length) of *P. damicornis* were collected from six different donor colonies. Nubbins were fixed on supports made of epoxy resin and transferred to two 50 L experimental flow-through tanks for 7 days of acclimation at the temperature of 25 °C, with a light/dark cycle of 11/13 h respectively. The irradiation was equal to 250 PAR (μmol photons m<sup>-2</sup> s<sup>-1</sup>). After acclimation, each nubbin was placed in 2 L glass beaker with an air pump used in each chamber to keep the MPs in constant motion, simulating the water turbulence typically experienced in natural reef environments (Martin et al., 2019) following the procedure previously adopted (Isa et al., 2024). Nubbins (*n* = 6 per treatment) were randomly assigned to one of four treatments, consisting of the three different experimental concentrations of MPs and one control group (without MPs). The PLA MPs were dry-weighed in amounts calculated to obtain the desired final concentrations (5 mg/L, 15 mg/L, and 50 mg/L) for the three treatments. The weighed particles were then transferred into 15 mL Falcon tubes, which were filled to volume with *Artemia* water. After 1 h of incubation, the entire content of each tube was poured into the corresponding beakers for the exposure experiments.

The exposure experiments were performed at 25 °C for 72 h (Fig. S1, Supporting Information). At the conclusion of the experiment, coral fragments from each of the four previously described conditions were collected and immediately stored at –80 °C for subsequent analysis.

### 2.4.2. Protein extraction

The extraction of the total protein content for the enzymatic assays was performed as previously reported (Montalbetti et al., 2023; Montalbetti et al., 2021). Specifically, coral nubbins were ground using a pre-chilled mortar and pestle and homogenized in 750 μL lysis buffer (Tris-HCl 50 mM, pH 7.4, NaCl 150 mM, glycerol 10%, NP40 detergent 1%, EDTA 5 mM) containing 1 mM phenylmethylsulfonyl fluoride. After the first centrifugation step (5 min, 3000 rpm) to remove skeletal components, cells were sonicated (6 × 10 s pulse on ice, amplitude 10 μm, Soniprep 150, Sanyo). Samples were then subjected to a second centrifugation step (15 min, 14,000 rpm, 4 °C), and the supernatant was immediately frozen (–80 °C) until subsequent assays. The total protein content of each sample was determined through the Bradford method using bovine serum albumin (BSA) as a calibration curve.

### 2.4.3. Superoxide dismutase (SOD) activity assay

SOD activity was assessed according to Vance et al. (Vance et al., 1972). As SOD competes with ferricytochrome c for oxygen radicals, the enzyme activity was detected as the ability to inhibit the reduction of ferricytochrome c by O<sub>2</sub> generated from the xanthine/xanthine oxidase system. For the reaction, the following reagents were utilized in a final volume of 1 mL: ferricytochrome c 0.01 mM, EDTA 0.1 mM, xanthine 0.01 mM, and xanthine oxidase 0.0061 U. Different volumes of each sample were tested and added to the reaction mix to determine the 50% inhibition of the reaction rate. The ferricytochrome c reduction rate was followed spectrophotometrically at 550 nm, 25 °C, through a Varian Cary 50 Scan Spectrophotometer (Agilent Technologies). Under the above conditions, one unit of SOD was defined as the amount of enzyme inhibiting the reduction of ferricytochrome c by 50%. Results are expressed as units (U) of enzyme per mg of proteins.

### 2.4.4. Catalase (CAT) activity assay

CAT activity was assessed by considering the peroxidative function of the enzyme based on the degradation of hydrogen peroxide. The method, described by Bergmeyer and Grassl (Bergmeyer and Grassl, 1983), relies on the degradation of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) by the enzyme. The reaction mix contained 50 mM sodium phosphate buffer

pH 7.5 and 12 mM H<sub>2</sub>O<sub>2</sub>) and 12 mM H<sub>2</sub>O<sub>2</sub>. This solution was mixed in a 1 mL cuvette with different volumes, and the decrease of H<sub>2</sub>O<sub>2</sub> was measured spectrophotometrically at 240 nm. Results are expressed as units (U) of enzyme per mg of proteins, and in this case, U refers to k, the first-order kinetic constant (min<sup>-1</sup>), as previously described (Aebi, 1984).

#### 2.4.5. Glutathione reductase (GR) activity assay

The enzymatic assay of GR was analyzed following the procedure described by Wang et al. (Wang et al., 2001). The activity of GR was assessed through the spectrophotometric detection of the absorbance at 340 nm of NADPH oxidation to NADP<sup>+</sup> reaction, which occurs in conjunction with the glutathione reduction and is proportional to the decrease in absorbance over time. NADPH reaction was first measured in the reaction mix (containing 0.1 M potassium phosphate buffer pH 7.6, 0.16 mM NADPH, 1 mg mL<sup>-1</sup> BSA, and 4.6 mM oxidized glutathione) and then adding different sample volumes. GR activity was obtained from the difference of the two absorbance values. One unit of GR activity is defined as the oxidation of 1 nmol NADPH/min at 25 °C. Results are expressed as units (U) of enzyme per mg of proteins.

#### 2.4.6. Glutathione S-transferase (GST) activity assay

GST activity was measured by considering the reaction of the enzyme with the 1-Chloro-2,4-dinitrobenzene (CDNB) substrate, according to Hayes and Strange (2000). The solution (containing 200 mM potassium phosphate buffer pH 6.5, 20 mM CDNB dissolved in 95% ethanol, and 20 mM reduced glutathione) was mixed in a 1 mL cuvette with different volumes of samples. The formation of CDNB-oxidized glutathione conjugate was followed spectrophotometrically at 340 nm. GST activity is expressed as units (U) of enzyme per mg of protein and is proportional to the increase in absorbance caused by conjugated product formation.

#### 2.4.7. Lipid peroxidation (LPO)

Lipid peroxidation levels were assessed by measuring malondialdehyde (MDA) content using an MDA assay kit (Bioxytech LPO-586, Oxis International, USA). This method involves a reaction between a chromogenic reagent, *N*-methyl-2-phenylindole, and MDA at 45 °C. To prepare the samples, frozen coral apexes (approximately 1 g each) were ground using a pre-chilled mortar and pestle and homogenized in 1 mL of 20 mM phosphate buffer at pH 7.4. To prevent oxidation of the samples, 10 µL of 0.5 M butylated hydroxytoluene in acetonitrile was added to the 1 mL tissue homogenate. Afterwards, the samples were centrifuged at 3000 ×g for 10 min at 4 °C, and an aliquot of the supernatant was taken for protein determination using the Bradford method. The subsequent assay (using the hydrochloric acid solvent procedure) was performed following the manufacturer's instructions. The resulting blue product was quantified by measuring absorbance at 586 nm (Gerard-Monnier et al., 1998). The results are expressed as µmol of MDA per µg of protein.

#### 2.4.8. Quantification of chlorophyll *a* and *c2*

The coral bleaching status and the general photophysiological response of corals was assessed through analysis of the concentration of chlorophyll (Chl) *a* and *c2*. Coral tissue was removed from frozen coral fragments by using airflow from a 1000 µL pipette tip, which was connected via a rubber hose to a benchtop filtered air pressure valve. This process was carried out using 5 mL of ice-cold phosphate-buffered saline (PBS) (Voolstra et al., 2020). The resulting tissue slurry was homogenized and then centrifuged at 3600g for 4 min. After centrifugation, the supernatant was discarded, and the remaining pellet was incubated in 100% acetone for 24 h in the dark at 4 °C. Following this extraction, the sample underwent re-centrifugation at 3600g for another 4 min. The supernatant obtained was used to measure concentrations of Chl *a* and *c2* using fluorescence readings at 630, 663, and 750 nm, applying dinoflagellate-specific equations and normalizing the results to the coral surface area (Jeffrey and Humphrey, 1975). The remaining coral

skeletons were soaked in 10% bleach and allowed to dry for 48 h. The surface area of each fragment was measured using the paraffin wax dipping method (Veal et al., 2010). The change in weight due to the addition of wax was compared against a standard curve of dipped clay cylinders with known surface areas to calculate the skeletal surface area of each coral fragment.

### 2.5. Data analysis

The normality of all data obtained was assessed using the Shapiro-Wilk test. When the assumptions of normality were not met, data transformations were applied. To evaluate significant differences in antioxidant enzyme activities, malondialdehyde (MDA) levels, and concentrations of chlorophyll *a* and *c2* at various MP concentrations, we conducted separate one-way ANOVAs followed by Tukey's HSD post hoc tests. The analyses were performed using SPSS version 29 (IBM). Statistical significance was set at  $p < 0.05$ , and all data are presented as arithmetic means ± standard error (SE) with  $n = 5$  for each biomarker analyzed, unless otherwise noted. Effect sizes ( $\eta^2$ ) were calculated to complement  $p$ -values and better describe the magnitude of treatment effects.

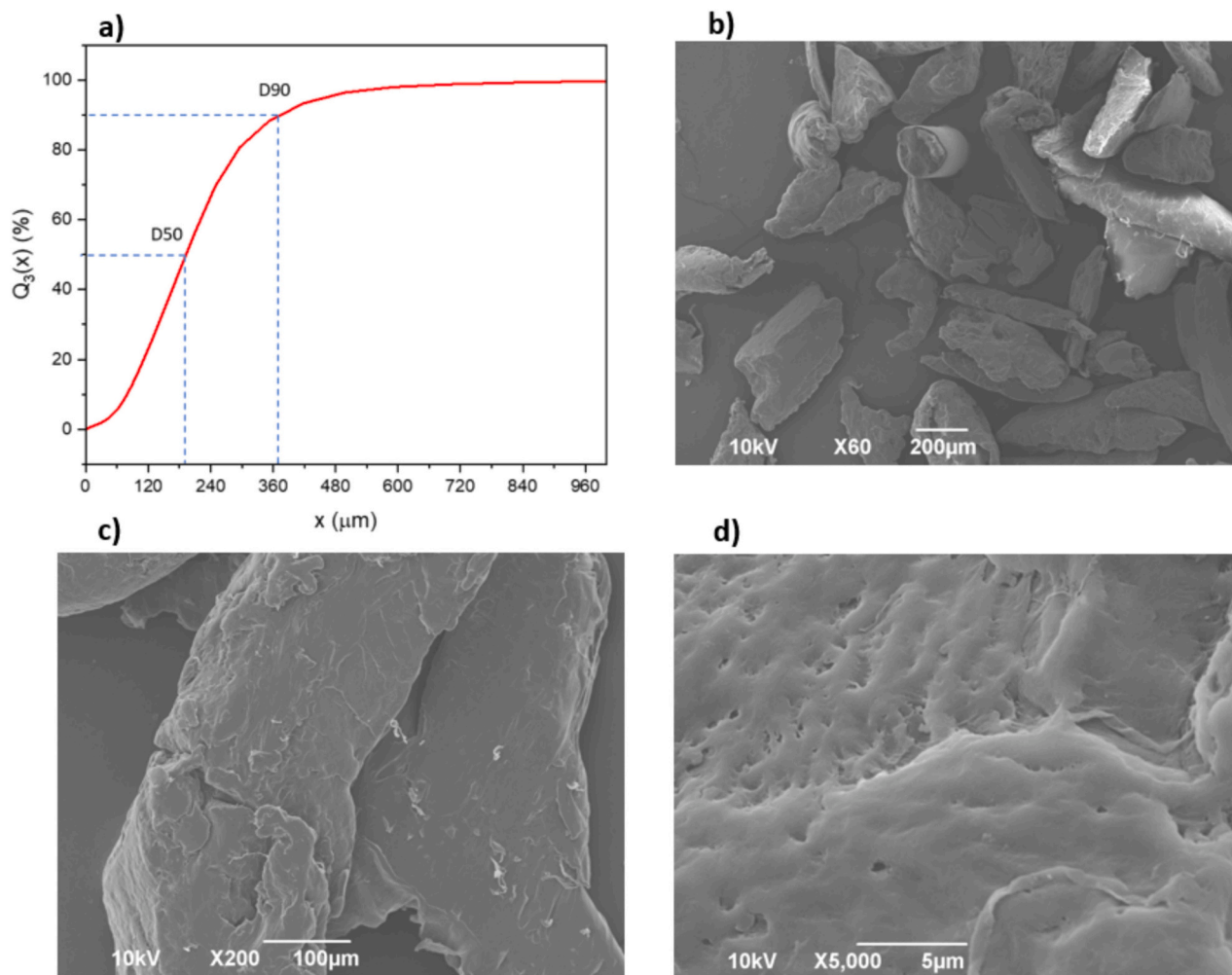
## 3. Results and discussion

### 3.1. PLA MPs properties

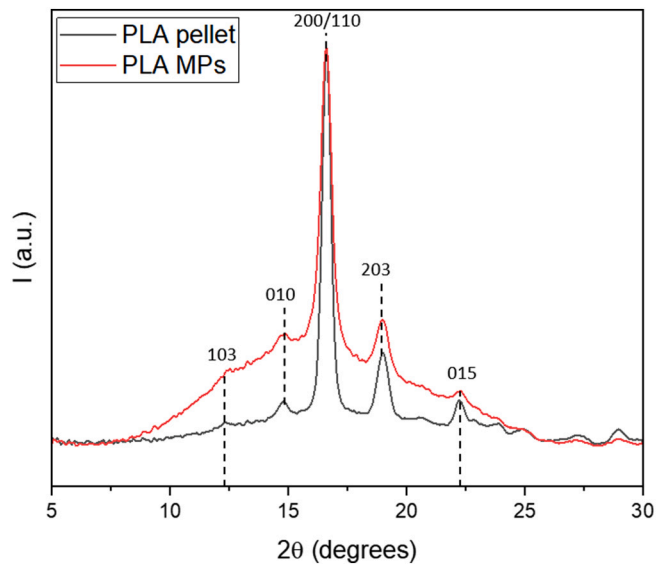
After the grinding process, MPs of specific size range were formed as shown in the granulometry analysis provided in Fig. 1a. In particular, the fabricated MPs have  $D_{90} \leq 370$  µm and  $D_{50} \leq 192$  µm, with approximately 48% of particles between 150 and 300 µm, 33% below 150 µm and 19% above 300 µm. The shape analysis (Supporting information Table S1) indicates that the particles have an irregular shape with a mean sphericity of 0.73. SEM analysis confirms these observations, as evident in Fig. 1 (b–d), with particles exhibiting irregular morphology and broad size distribution, as well as rough surface, in agreement with the morphological characteristics of environmental MPs samples (Kefer et al., 2021).

To evaluate the changes in PLA MPs following the grinding process and provide valuable insights into potential polymer degradation both crystallinity and molecular weight variations were assessed. The crystal structure was examined using XRD and, as shown in Fig. 2, both unprocessed pellets and MPs present typical semi-crystalline diffraction patterns with amorphous halo and crystalline peaks. The prominent diffraction peaks at  $2\theta \sim 16.6^\circ$  (200/110 plane) and  $\sim 18.9^\circ$  (203 plane) as well as the smaller peaks at  $2\theta \sim 14.8^\circ$  (010) and  $\sim 22.2^\circ$  (015) indicate the characteristic orientation of the  $\alpha$ -phase orthorhombic crystals of the PLA structure (Pan et al., 2007) as indexed by the ICDD 00-064-1624 database (Babichuk et al., 2022). The  $X_{C_{XRD}}$  of the samples were calculated following Eq. 1, indicating that PLA pellets exhibit a value of 44.7% while the  $X_{C_{XRD}}$  of the MPs decreases to 20.6% (Table S3, Supporting Information). This is in agreement with a recent study on semicrystalline MPs which proved that prolonged marine weathering results in the decrease of their crystallinity (Maddison et al., 2023).

The chemical changes induced upon the mechanical processing of the PLA pellets were analyzed by using ATR-FTIR spectroscopy (Fig. 3). As shown, both spectra of the pellets and MPs present peaks characteristic of the PLA, indicating that the grinding process did not alter significantly the chemical structure of the polymer (Table S2, Supporting Information). However, variations in the intensity of the peaks characteristic of the polymer's crystallinity are observed. Specifically, the contribution of the absorption band at 921 cm<sup>-1</sup>, associated with the rocking vibration of the CH<sub>3</sub> group [rCH<sub>3</sub>] of the  $\alpha$  crystal form, becomes weaker at the MPs spectrum, while the contribution of the peak at 956 cm<sup>-1</sup>, assigned to the stretching vibration of the C<sub>α</sub>–C backbone [ $\nu$  (C<sub>α</sub>–C)] of the amorphous phase, (Zhu et al., 2022) remains unchanged. Moreover, the distinct peak observed at 1210 cm<sup>-1</sup> in the



**Fig. 1.** (a) Particle size distribution of the MPs measured by granulometry. The plot represents the cumulative volumetric percentage  $Q_3(x)$  (%) of particles as a function of their diameter  $x$  ( $\mu\text{m}$ ). The red curve represents the average of three replicates of the same sample. (b–d) SEM images showing the irregular morphology and surface details of the MPs at different magnifications. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



**Fig. 2.** Representative XRD diffractograms of pellets and MPs.

pellet—associated with the ester C—O stretching vibration and characteristic of the crystalline domains of PLA (Meaurio et al., 2006) indicates a higher degree of crystallinity compared to the MPs, in which this feature appears only as a small shoulder. Such variations confirm that the grinding process causes a decrease of the crystalline phase of the PLA polymer in agreement with the XRD observations.

In the present study, changes in crystallinity may indicate a mechanical degradation process of the PLA molecular chain structure during the formation of the MPs. In fact, the mechanical stress applied during grinding disrupts the crystalline regions of PLA, enhancing its amorphous character (Cha et al., 2024). Such degradation is attributed to the action of shear forces that can lead to the rupture of bonds along the polymer backbone, resulting in polymer chain scission and, consequently, in a molecular weight reduction (Lucas et al., 2008; Niaounakis, 2015). This is also supported by the CI, calculated before and after the grinding process, with an increase of approximately 54% after the mechanical fragmentation (Fig. S2), likely reflecting the onset of polymer degradation induced by the MPs production process. The overall degradation and macromolecular chain scission during the mechanical processing of the pellets is further supported by the molecular weight evaluation of the polymer before and after the mechanical processing. As shown in Table S3 of the Supporting Information, in the case of MPs both the number (Mn) and weight-average molecular weights (Mw) decreased up to 18% for Mw, confirming the PLA degradation

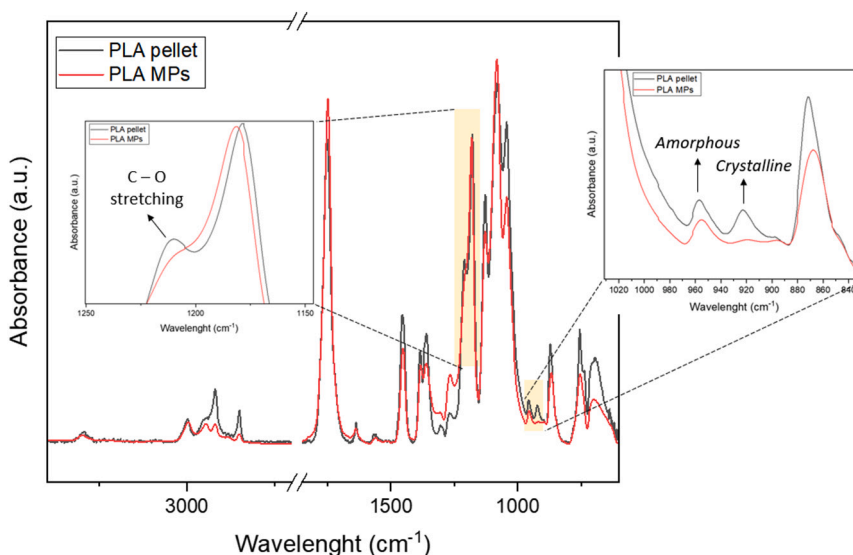


Fig. 3. Representative FTIR spectra of pellets and MPs. Inset: magnified region of a specific wavenumber range.

specifically triggered by mechanical stress induced during the grinding process.

Based on the so far presented results, we may conclude that although environmental occurring MPs have been subjected to natural weathering/aging which is an extremely complicated process, the herein developed MPs may be considered as a valuable environmental relevant model in terms of morphology, structure and chemistry, to be used for further studies.

### 3.2. PLA MPs and *Artemia*'s exposure

PLA MPs were mixed for 1 h with *Artemia salina* water medium, to promote initial biofilm formation through early organic matter adsorption, that typically occurs when plastic debris enters the marine environment (Lazzeroni et al., 2026; Ramsperger et al., 2020; Sucharitakul et al., 2020), while also providing a standardized laboratory prey model for coral feeding experiments.

In the exposure medium various organic compounds released by *Artemia salina* are expected to be present but not the living organisms. This can alter both the physical and chemical surface properties of the MPs, making them more attractive to marine filter feeders such as corals (Feng et al., 2020). This strategy also allows for the standardization of a realistic yet controlled system in the laboratory, avoiding the use of raw seawater, which is more variable and difficult to reproduce consistently across experiments.

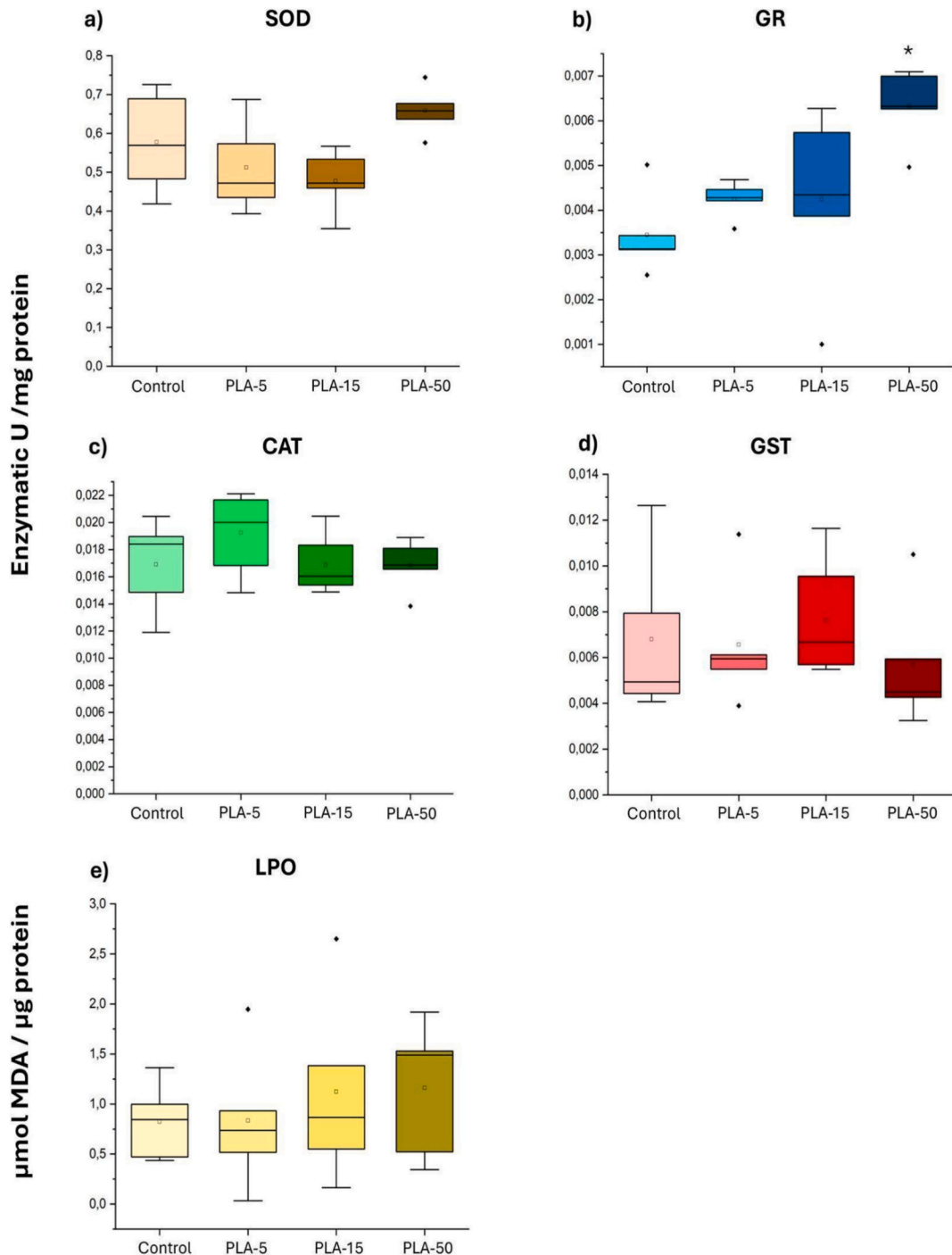
Based on SEM analysis, the exposure to filtered *Artemia salina* water medium did not result in significant alterations to the surface morphology of PLA MPs. In fact, as shown in Fig. S3, the surface of the exposed MPs before and after being thoroughly rinsed with deionized water to remove any non-adhered matter, did not present any noticeable modifications compared to the pristine MPs. This type of pre-treatment did not also evidently affect the chemical properties of the MPs, as the Raman analysis presented in Fig. S4 indicates. Therefore, it can be concluded that exposure to the *Artemia salina* water medium did not affect the overall chemical structure of PLA MPs.

Nonetheless, a closer look to the surface chemistry by XPS analysis of these MPs indicates modifications in their surface chemistry. As shown in the wide scan spectra, the MPs present the main O and C elements representative of the organic nature of the polymer, and small traces of other elements such as In, representative of the substrate, and N possibly attributed to adsorbed contaminants. After exposure to the *Artemia salina* water medium, additional elemental traces were detected on the surface of the MPs attributed to seawater salts and other components

adhered to the MP surface, (Fig. S5) together with the O/C ratio increase. Although a major part of the inorganic components was removed after rinsing the samples with deionized water, XPS analysis still revealed the presence of some additional elements (Table S4) such as sulfur and nitrogen, indicating the occurrence of organic material, together with an O/C ratio higher than that of the pristine counterpart. Specifically, the O/C ratio increased from 0.10 to 0.13 (~30%) in MPs exposed to the *Artemia salina* water medium and subsequently washed after incubation (Fig. S5), indicating the persistence of oxygen-containing species on their surface. Overall, these findings suggest that the rise in the O/C ratio after incubation results from the adsorption of external species, which alters the atomic balance and the distribution of oxygen components. MPs have the potential to act as carriers for a variety of organic substances (Hartmann et al., 2017). When a material is introduced into an aqueous environment, its surface rapidly interacts with organic components initiating the formation of a conditioning film, formed through the adsorption of dissolved and suspended molecules (Lawrence et al., 2015; Loeb and Neihof, 1975; Rittle et al., 1990). Such films can comprise diverse organic constituents, including glycoproteins (Baier, 1980), lipids, nucleic acids, ions, polysaccharides, and proteins (Baier, 1980; Bakker et al., 2004; Rittle et al., 1990) which largely originate from the metabolic by-products of aquatic organisms. Despite this, detailed knowledge of how plastics interact at the microscale with the surrounding chemical milieu in marine systems—particularly how exposure to seawater alters their surface properties—remains limited (Bhagwat et al., 2021). In the present context, it is reasonable to hypothesize that the observed increase in the O/C ratio of PLA MPs may stem from their interaction with oxygen-rich organic matter released by *Artemia* species, leading to surface chemical modifications that render these particles more comparable to MPs naturally present in marine environments.

### 3.3. Oxidative stress

The antioxidant activities of SOD, CAT, GR and GST were analyzed in *P. damicornis* samples exposed to varying concentrations of PLA MPs to assess their effects on the coral cellular oxidative homeostasis. Notably, no partial or complete mortality was observed in any coral nubbin across all treatments. Furthermore, neither mucus production nor polyp retraction was observed during the exposure period. Fig. 4 indicates that the only antioxidant enzyme that displayed a variation in activity in corals exposed to MPs was GR. After 72 h of exposure to PLA MPs, significant differences in GR activity and a large effect size were observed



**Fig. 4.** Enzymatic activities in *P. damicornis* after 72 h exposure to PLA MPs. Activities of (a) SOD, (b) GR, (c) GST, and (d) CAT are expressed as U/mg protein ( $n = 5$ ). (e) LPO levels, expressed as  $\mu\text{mol MDA}/\mu\text{g protein}$  ( $n = 5$ ). Corals were exposed to 5, 15, and 50 mg/L PLA MPs (PLA-5, PLA-15, and PLA-50, respectively) and compared to control conditions (Control). Asterisks indicate statistically significant differences among treatments ( $p < 0.05$ ).

across treatments (one-way ANOVA,  $F(3, 16) = 5.156$ ,  $p = 0.011$ ,  $\eta^2 = 0.49$ ). Specifically, *P. damicornis* nubbins exposed to 50 mg/L of MPs exhibited significantly higher GR activity compared to those in the control tank ( $p = 0.008$ ) and those exposed to 15 and 5 mg/L concentrations ( $p = 0.039$  and  $p = 0.030$ , respectively). GR plays a key role in maintaining and restoring the redox balance between oxidized and reduced glutathione, a tripeptide that interacts with different ROS (Krueger et al., 2015; Lesser, 2006).

Apart from GR, no other significant changes were observed in SOD and CAT activities, together with a low or negligible effect size of

treatments, indicating that MP exposure did not affect their activity (one-way ANOVA,  $F(3, 16) = 1.430$ ,  $p = 0.271$ ,  $\eta^2 = 0.16$  and  $F(3, 16) = 0.104$ ,  $p = 0.950$ ,  $\eta^2 = 0.02$  respectively), (Fig. 4 a–d). SOD and CAT are key enzymes in the direct ROS detoxification, working synergistically to neutralize superoxide radicals ( $\cdot\text{O}_2^-$ ) and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), thereby preventing the formation of highly reactive hydroxyl radicals ( $\cdot\text{OH}$ ) and subsequent cellular damage (Vance et al., 1972; Bergmeyer and Grassl, 1983; Fridovich, 2022). SOD, a critical antioxidant in coral physiology, acts as the first line of defense against ROS by catalyzing the disproportionation of  $\text{O}_2^-$  into  $\text{H}_2\text{O}_2$  and molecular oxygen

(O<sub>2</sub>) (Gardner et al., 2016), and is found in various cellular compartments (Verma et al., 2019). CAT, another key antioxidant in the coral redox system, converts H<sub>2</sub>O<sub>2</sub> into H<sub>2</sub>O and O<sub>2</sub> (Levy et al., 2006).

This is also the case for the GST activity, as no significant changes and a low effect size were observed after exposure to PLA MPs (one-way ANOVA,  $F(3, 15) = 1.625, p = 0.226, \eta^2 = 0.18$ ), (Fig. 4 a–d). GST is central to Phase II biotransformation, facilitating the conjugation of intermediary metabolites produced during xenobiotic exposure with glutathione (Hayes and McLellan, 1999; Limón-Pacheco and Gonsebatt, 2009). The GST antioxidant enzyme system also aids in the conversion of peroxides and hydroxyl radicals into water, mitigating intracellular damage from ROS (Doyotte et al., 1997; Jiang et al., 2017).

Taken together, our results show that exposure to mechanically ground PLA MPs does not induce mortality and evident antioxidant response in *P. damicornis*. In fact, although a significant increase in GR activity was observed at the highest concentration (50 mg/L), we hypothesized that this response could be part of an adaptive antioxidant mechanism rather than a sign of overt oxidative damage. Indeed, GR, while not a classical antioxidant enzyme, plays an essential role in maintaining the proper ratio between GSH and its oxidized form, glutathione disulfide (GSSG), and thereby preserving the intracellular redox status in marine organisms (Regoli and Giuliani, 2014), fundamental for their health status. These outcomes contrast with previous findings on synthetic polymers/MPs, which often report oxidative stress responses in corals. For instance, Tang et al. (Tang et al., 2018) reported that *P. damicornis* exposed to 1 µm PS MPs (50 mg/L, 24 h) led to a significant increase in the SOD and CAT activity and reduced GST activity. Similarly, exposure to PVC MPs in *Zoanthus sociatus* (size range 63–125 µm, 10 mg/L, 96 h) and *Tubastraea aurea* (size range 5–10 µm, 30 mg/L, 72 h) led to a marked increase in CAT activity (Rocha et al., 2020; Xiao et al., 2021) and LPO (Rocha et al., 2020), indicating oxidative stress.

Chen et al. (Chen et al., 2022) reported a significant increase in antioxidant enzymes in *Goniopora columna* exposed to PE MPs (40–48 µm) across various concentrations. Specifically, they observed Xiao et al. (Xiao et al., 2021) observed an increase of SOD and GSH in response to PET, PA66, and PET MPs (10–40 µm, 96 h, 50 mgL<sup>-1</sup>). Likewise the soft coral *Protopalycha* sp. exposed to 1–20 µm MPs (PE, PVC, PMMA) (50 mg/L, 96 h) showed increased antioxidant enzyme activity, particularly SOD increased following exposure to PVC MPs, while both GST and GSH increase in response to all tested MPs types (Jiang et al., 2021). Compared to these findings, PLA MPs appear to induce milder effects, suggesting lower short-term toxicity.

These comparisons suggest that PLA, a bio-based polymer, may elicit a less severe oxidative response in coral species compared to more persistent synthetic plastics such as PVC, PS or PE.

This was confirmed also by the analysis of the LPO levels which are indicative of the oxidative damage generated by PLA MPs in corals. In fact, although a slight, non-significant, increase was observed in corals exposed to PLA concentrations of 15 and 50 mg/L compared to controls (Fig. 4e), this was not statistically significant and in general, no significant changes were observed in LPO levels between all treatments (one-way ANOVA,  $F(3, 16) = 0.323, p = 0.809, \eta^2 = 0.08$ ).

In contrast, several studies report that increased lipid peroxidation (LPO) following exposure to synthetic plastics. Rocha et al. (Rocha et al., 2020) observed a significant increase in peroxidative damage in *Zoanthus sociatus* after 96 h exposure to PVC MPs (63–125 µm, 10 mg/L). Similarly, *Protopalycha* sp. showed increased LPO after 96 h exposure to PVC, PE, and PMMA MPs (1–20 µm, 50 mg/L) (Jiang et al., 2021). *Goniopora columna* also exhibited elevated LPO after 7 days of exposure to various concentrations of PE MPs (40–48 µm) (Chen et al., 2022). Based on the results obtained and comparison with similar studies, it can be inferred that PLA MPs induce a lower oxidative stress response compared to their synthetic counterparts. This suggests that biopolymers such as PLA may pose a reduced risk in terms of oxidative damage in marine invertebrates, although further studies are needed to

fully assess their long-term biological effects and environmental persistence. Similarly, it should be noted that the origin of each nubbin relative to its donor colony was not tracked during the experiment. Consequently, potential colony- or genotype-specific differences in responses to the treatments could not be assessed, representing a limitation that should be addressed in future investigation. Nevertheless, the limited variability observed among fragments within each treatment may suggest that differences among donor colonies were likely small.

### 3.4. Coral bleaching assessment

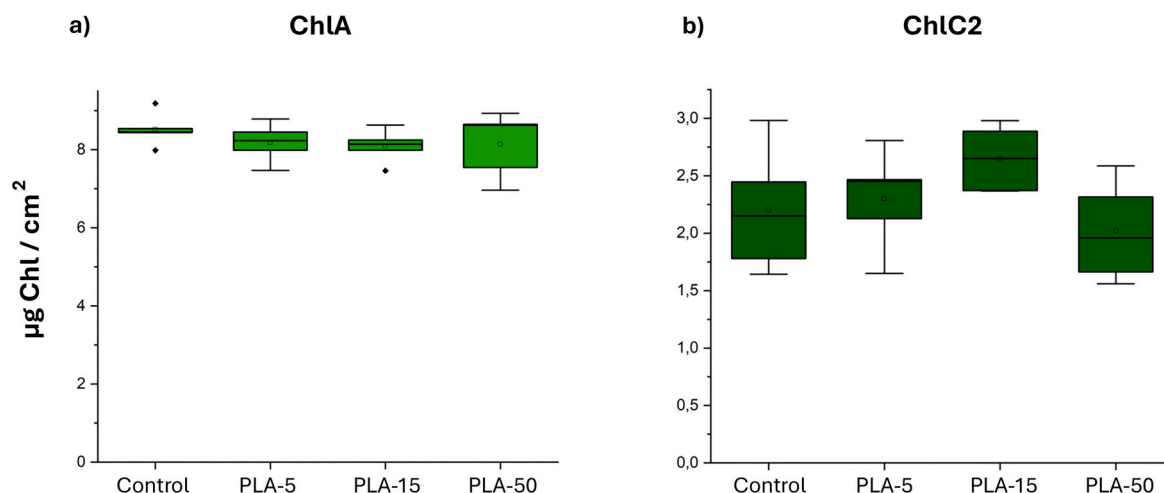
Finally, to assess the possible coral bleaching and the photo-physiological response triggered by the exposition to different concentrations of PLA MPs, the concentration of Chl *a* and *c2* were analyzed. As shown in Fig. 5, Chl *a* and *c2* concentration showed no significant differences among the various treatments, with low effect size observed (one-way ANOVA,  $F(3, 16) = 0.564, p = 0.647, \eta^2 = 0.1$  and  $F(3, 16) = 1.890, p = 0.172, \eta^2 = 0.15$ , respectively). Therefore, no signs of bleaching were found in corals subjected to any concentration of PLA MPs, suggesting that they do not affect the maintenance of the coral symbiosis.

On the contrary, Xiao et al. (Xiao et al., 2021) found that *Acropora* sp. exposed to PET, PA66, and PE MPs (20–40 µm, 50 mg/L, 96 h) showed a significant decrease in chlorophyll content. Conversely *Protopalycha* sp. exposed to PE and PMMA MPs (1–20 µm, 50 mg/L) resulted in a significant increase in chlorophyll (*a* + *c2*) content at the short-term exposure (24 h), and a significant decrease after prolonged exposure (28 days) (Jiang et al., 2021). Similarly Tang et al. (Tang et al., 2018) reported a chlorophyll increase of chlorophyll after 12 h of exposure to PS MPs (1 µm, 50 mg/L).

Table 1 provides a comprehensive and visually accessible summary of the differential effects of synthetic MPs reported in previous studies compared to the effects of the PLA biobased MPs, investigated in the present study. The table highlights variations in antioxidant enzymes, LPO responses, and chlorophyll (*a* + *c2*) content, emphasizing both inter-study differences and the distinctive outcomes observed for PLA MPs. As shown and based on the results obtained from both oxidative stress and bleaching-related biomarkers, it can be suggested that PLA MPs produced through mechanical grinding do not induce detectable cellular or physiological damage in corals at the doses used. Although longer exposures and smaller, more reactive MPs and NPs, could elicit different responses, the present setup allowed us to characterize early physiological effects under controlled and reproducible conditions, providing a valuable baseline for future investigations. In particular, future studies may involve longer exposure times, smaller MPs and NPs with different surface roughness and chemistry, loaded with various types of environmental pollutants, exposures at different environmental conditions, or in the presence of nutrients to name some.

## 4. Conclusions

This study investigates the effects of bio-based MPs, specifically mechanically ground PLA particles, on *Pocillopora damicornis*, addressing a gap in research dominated by synthetic MPs. The PLA MPs treated in *Artemia salina* water medium to mimic environmental conditions, showed reduced crystallinity, lower molecular weight and higher carbonyl index traits resembling weathered marine plastics. After 72 h of exposure to three different MPs concentrations, coral fragments showed no signs of mortality, visible stress responses, or bleaching, contrasting with studies reporting harm from synthetic MPs. Overall, the results suggest that short-term exposure to PLA MPs at the tested concentrations does not cause detectable physiological effects in corals and appears less harmful compared to the synthetic counterparts. Nevertheless, further research is essential to investigate the long-term impacts of biobased MPs, and to develop a more comprehensive, ecologically relevant, understanding of their effects on coral reef ecosystems.



**Fig. 5.** (a) Chlorophyll *a* (ChlA) and (b) Chlorophyll *c2* (ChlC2) concentrations, expressed as  $\mu\text{g}/\text{cm}^2$  ( $n = 5$ ), in *P. damicornis* after 72 h exposure to 5, 15 and 50 mg/L of PLA MPs (PLA-5, PLA-15 and PLA-50, respectively) compared to control conditions (Control).

**Table 1**

Overview of enzymatic and oxidative stress responses induced by PLA MPs in comparison with synthetic MPs from previous studies. Symbols indicate the observed outcome:  $\uparrow$  significant increase;  $\downarrow$  significant decrease;  $-$  no significant change;  $/$  not investigated.

Study	(Tang et al., 2018)	(Rocha et al., 2020)	(Jiang et al., 2021)	(Liao et al., 2021)	(Xiao et al., 2021)	(Chen et al., 2022)	Present study
MPs	PS	PVC, LDPE	PVC, PE, PMMA	PVC	PET, PA66, PE	PE	PLA
Polymer type	Synthetic	Synthetic	Synthetic	Synthetic	Synthetic	Synthetic	Biobased
Size ( $\mu\text{m}$ )	1	63–125	1–20	5–10	10–40	40–48	150–300
Concentration (mg/L)	50	1, 10	50	1, 30	50	5, 10, 50, 100, 300	5, 15, 50
Exposure time (h)	24	96	96	72	96	168	72
Coral species	<i>P. damicornis</i>	<i>Z. sociatus</i>	<i>Protopalythoa</i> sp.	<i>T. aurea</i>	<i>A. pruinosa</i>	<i>G. columna</i>	<i>P. damicornis</i>
SOD	$\uparrow$	$/$	$\uparrow$ (PVC)	$\downarrow$	$\uparrow$	$\uparrow$	$-$
CAT	$\uparrow$	$\uparrow$ (PVC)	$/$	$\uparrow$	$/$	$\uparrow$	$-$
GST	$\downarrow$	$-$	$\uparrow$ (PVC, PE, PMMA)	$/$	$/$	$\uparrow$	$-$
GHS	$/$	$/$	$\uparrow$ (PVC, PE, PMMA)	$\uparrow$	$\uparrow$	$\uparrow$	$/$
GR	$/$	$/$	$/$	$/$	$/$	$/$	$\uparrow$
LPO	$/$	$\uparrow$ (PVC)	$\uparrow$ (PVC, PE, PMMA)	$\downarrow$	$/$	$\uparrow$ (PVC, PE, PMMA)	$-$
Chl ( <i>a</i> + <i>c2</i> )	$\uparrow$	$/$	$\uparrow$	$/$	$\downarrow$	$/$	$-$

### CRedit authorship contribution statement

**Giorgia Ferrari:** Writing – original draft, Methodology, Investigation, Conceptualization. **Enrico Montalbetti:** Writing – review & editing, Methodology, Investigation. **Davide Seveso:** Writing – review & editing, Validation, Methodology, Data curation. **Valerio Isa:** Writing – review & editing, Methodology, Investigation. **Silvia Lavorano:** Resources. **Sergio Marras:** Writing – review & editing, Investigation. **Stefania Sganga:** Investigation. **Riccardo Carzino:** Writing – review & editing, Investigation. **Paolo Galli:** Resources, Funding acquisition. **Athanassia Athanassiou:** Writing – review & editing, Resources, Funding acquisition. **Despina Fragouli:** Writing – review & editing, Supervision, Conceptualization.

### Declaration of competing interest

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

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marpolbul.2026.119685>.

### Data availability

Data will be made available on request.

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