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Chronic effects of exposure to polyethylene microplastics may be mitigated at the expense of growth and photosynthesis in reef-building corals

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ABSTRACT

The causes of the physiological effects of microplastic pollution, potentially harming reef-building corals, are unclear. Reasons might include increased energy demands for handling particles and immune reactions. This study is among the first assessing the effects of long-term microplastic exposure on coral physiology at realistic concentrations (200 polyethylene particles L^{-1}). The coral species *Acropora muricata*, *Pocillopora verrucosa*, *Porites lutea*, and *Heliopora coerulea* were exposed to microplastics for 11 months, and energy reserves, metabolites, growth, and photosymbiont state were analyzed. Results showed an overall low impact on coral physiology, yet species-specific effects occurred. Specifically, *H. coerulea* exhibited reduced growth, *P. lutea* and *A. muricata* showed changes in photosynthetic efficiency, and *A. muricata* variations in taurine levels. These findings suggest that corals may possess compensatory mechanisms mitigating the effects of microplastics. However, realistic microplastic concentrations only occasionally affected corals. Yet, corals exposed to increasing pollution scenarios will likely experience more negative impacts.

1. Introduction

Reef-building corals are key ecosystem engineers and form one of the most biodiverse ecosystems on the planet (Bowen et al., 2013). However, they are increasingly exposed to environmental stressors such as ocean warming (Hughes et al., 2018a, 2018b), acidification (Cornwall et al., 2021), sedimentation (Tuttle and Donahue, 2022), eutrophication (Lesser, 2021), and pollution (Wear and Thurber, 2015). An emerging marine pollutant is microplastics, which are ubiquitous in the oceans in varying concentrations (Amelia et al., 2021; Ding et al., 2019; Yusof et al., 2023) and expected to increase 3–50 fold by 2100 (Everaert et al., 2018; Koelmans et al., 2017). The definition of microplastics is still controversial, and they are defined as particles <1 (Andrady, 2015; Browne et al., 2007; Hartmann et al., 2019) or <5 mm (Frias and Nash, 2019).

Corals may respond to microplastic exposure in several ways, such as ingestion (Allen et al., 2017; Hall et al., 2015; Reichert et al., 2018), overgrowth (Reichert et al., 2018), and skeletal incorporation (Hierl et al., 2021; Reichert et al., 2022). These interactions are often associated with negative effects on coral growth (Chapron et al., 2018; Hankins et al., 2021; Reichert et al., 2019), changes in feeding activity (Corinaldesi et al., 2021; Rotjan et al., 2019; Savinelli et al., 2020), altered photosynthesis of their symbionts (Mendrik et al., 2021; Reichert et al., 2019), and impacts on the immune system (Liao et al., 2021; Tang et al., 2024, 2018; Xiao et al., 2021). Further, corals showed limitations in developing adaptive behavior to chronic microplastic exposure (Rades et al., 2022).

Several mechanisms have been identified that might affect coral health: i) Microplastics may be mistaken for food, and ingestions might lead to false satiety or gastric blockage (Corinaldesi et al., 2021; Rotjan et al., 2019; Savinelli et al., 2020). ii) Pathogens or toxins adhering to microplastics (Bowley et al., 2021) may be transferred to the coral (Rotjan et al., 2019; Saliu et al., 2019). iii) Prolonged handling of microplastic particles and increased immune activity (Bove et al., 2023) may lead to higher energy requirements, to which the coral holobiont might respond with compensatory mechanisms such as increased heterotrophic feeding (Chapron et al., 2018; Chen et al., 2022b; Reichert et al., 2019) or altered photosynthesis (Lanctôt et al., 2020; Reichert et al., 2019).

Evidence for the potential impacts of microplastics on corals has been controversial. While Zhou et al. (2023) found that increased energy reserves were positively correlated with microplastic concentration,

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other studies found no effects on energy reserves (i.e., in lipids (Boodraj and Glassom, 2022; Mouchi et al., 2019) and proteins (Lanctôt et al., 2020; Rocha et al., 2020)) and behavior (i.e., heterotrophy and defensive reactions (Bejarano et al., 2022)). Regarding the effects of microplastics on photosynthesis, some authors found an increased photosynthetic efficiency (Lanctôt et al., 2020; Reichert et al., 2019), higher chlorophyll content (Jiang et al., 2021; Xiao et al., 2021), or decreased symbiont density (Chen et al., 2022a; Zhou et al., 2023), indicating the presence of a compensatory mechanism by upregulation of photosynthesis (Lanctôt et al., 2020; Reichert et al., 2019). However, other studies have reported no effect on parameters of the photosynthesis of coral symbionts (Boodraj and Glassom, 2022; Plafcan and Stallings, 2022; Reichert et al., 2019; Tang et al., 2018). This heterogeneous picture may be due in part to species-specificity (Mendrik et al., 2021; Mouchi et al., 2019; Reichert et al., 2019), experimental microplastic concentration (Plafcan and Stallings, 2022; Xiao et al., 2021), and too short exposure durations (Chen et al., 2022b; Lanctôt et al., 2020). The observed differences in the effects of microplastics could also be partly due to differences in study design, such as the polymer type of microplastics used (Mendrik et al., 2021; Reichert et al., 2024b).

When comparing short-term studies, there is often a high variability in results. Therefore, our study aimed to assess, for the first time, the chronic effects of microplastic exposure on a wide range of physiological parameters related to energy reserves and photosynthesis in a long-term, multi-species experiment. We used a realistic microplastic concentration (i.e., a high-pollution scenario of 200 particles L^{-1}) because similar concentrations (i.e., 200–717 particles L^{-1}) have been found in highly polluted reef areas (Patterson et al., 2020; Yusof et al., 2023). Specifically, we determined the effects of microplastics on i) host energy reserves (i.e., lipid, carbohydrate, and protein contents), ii) host metabolites (i.e., amino acid levels), iii) coral growth parameters (i.e., surface, volume, and calcification), and iv) photosymbiont state (i.e., density, chlorophyll content, and photosynthetic efficiency). To this end, we exposed four common and widespread coral species (i.e., *Acropora muricata* (Linnaeus, 1758), *Pocillopora verrucosa* (Ellis & Solander, 1786), *Porites lutea* Milne-Edwards & Haime, 1851, and *Heliopora coerulea* (Pallas, 1766)) for 11 months in a controlled microcosm experiment to microplastics.

2. Materials and methods

2.1. Experimental design and replication

The response of reef-building corals to microplastics (polyethylene (PE); ~200 particles L^{-1}) was studied over 11 months in a controlled aquarium experiment. Four widespread Indo-Pacific coral species were used: *Acropora muricata* (Linnaeus, 1758), *Pocillopora verrucosa* (Ellis & Solander, 1786), *Porites lutea* Milne-Edwards & Haime, 1851, and *Heliopora coerulea* (Pallas, 1766). These coral species share similar polyp sizes (1–2 mm in diameter) but differ in morphology (i.e., branching, massive, and columnar), growth rates (i.e., 1.2–7.5 cm $year^{-1}$), and life history traits (e.g., more heterotrophic vs. more autotrophic). Coral fragments were generated from different mother colonies to account for genetic variability (three origin colonies for *A. muricata*, *P. verrucosa*, and *Porites lutea*, and one origin colony for *H. coerulea* (for details on colony origins and fragmentation see Table S1 and Supplementary Text)). Due to the mortality of fragments from one colony of *P. verrucosa*, this resulted in different numbers of replicates per species and treatment (i.e., $n_{A.\ muricata} = 18$, $n_{P.\ verrucosa} = 12$, $n_{P.\ lutea} = 18$, and $n_{H.\ coerulea} = 6$). Corals were kept under laboratory conditions in the Ocean2100 facility of the Justus Liebig University for at least six months prior to the long-term experiment (temperature: 26 ± 0.5 °C (mean ± SD); light intensity: 200 μmol photons m^{-2} s^{-1} at a photoperiod of 10:14 dark:light).

2.2. Microplastic characteristics and long-term exposure

Coral fragments were exposed to black, irregularly-shaped polyethylene microplastics (PE; diameter: 175.5 ± 73.5 μm (mean ± SD), more details see Fig. S1 in Reichert et al. (2019); density: 0.95 g cm^{-3}; Novoplastik, Germany; for FTIR chart, see Supplements Fig. S1) or control conditions without microplastics for 11 months (i.e., 324 days). The PE particles represent a common polymer type in reef waters (Patterson et al., 2020; Saliu et al., 2018) and correspond to the size of the natural coral plankton diet (Houlbrèque and Ferrier-Pagès, 2009). The experiment was conducted in six 80 L tanks ($n = 3$ microplastic treatment and control tanks each, see Fig. S2) connected to an artificial reef mesocosm system (~4000 L) with an exchange rate of 120 L day^{-1} (≙ 150 % of tank volume). Coral fragments were distributed among the tanks (one fragment per colony per species per tank) three weeks before the start of the long-term exposure and were randomly positioned within the tanks at a distance of ≥ 5 cm between fragments. Filters (65 μm mesh size) on the outflows retained microplastic particles inside their tanks. Controlled conditions were maintained during the long-term experiment (temperature: 26 ± 0.2 °C (mean ± SD); light intensity: 135 μmol photons m^{-2} s^{-1} at a photoperiod of 10:14 dark:light). Corals received nutrition indirectly from frozen food (i.e., copepods and *Mysis* spp.) supplied to the connected seawater aquarium system.

Prior to the experiment, PE particles were sterilized in ethanol (70 % abv; 24 h) and then rinsed with deionized water. 2.5 mg L^{-1} of the prepared microplastics were added per microplastic exposure tank. After 48 h, approximately 10 % of the particles were distributed in the water column, resulting in a concentration of ~200 particles L^{-1} or 0.25 mg L^{-1}. The other ~90 % of the microplastic particles adhered to the glass or floated on the surface and were flushed back into the tank every other day to prevent excessive accumulation. Microplastic concentrations were monitored every two months, with more frequent intervals at the beginning until stable concentrations were established. For this purpose, 50 mL water samples were taken from the water column between the corals and filtered onto a gauze filter (65 μm mesh size; 3–5 replicates per tank), and the particles were counted under a stereo microscope to extrapolate the concentration per liter. A constant concentration was aimed for (201 ± 67 particles L^{-1} (mean ± SD), $n = 518$ measurements at 125 time points, Fig. S3), which was achieved by adding new particles as needed. The microplastic concentrations did not differ between the treatment tanks (see Fig. S3, Kruskal–Wallis test, $\chi^2 = 1.27$, d.f. = 2, $n_{obs} = 105$, $p = 0.53$). This study is a continuation of a 6-month experiment (Reichert et al., 2019) extending the physiological monitoring and adding analyses of coral energetics for a subset of samples (see Table S2 and Supplemental Text for further information on the setup).

2.3. Coral sample preparation

After 11 months, corals were snap-frozen in liquid nitrogen and stored frozen (−80 °C) upon further analysis (one fragment per colony per species per tank). Coral tissue was removed from the skeleton using an airbrush (Starter Class, Revell, Germany) and ultra-pure water at a working temperature of 4 °C. After collecting the tissue slurry in a plastic bag, the slurry was transferred to a 50 mL centrifuge tube. Then, the tissue slurry was homogenized (duration: 60 s; Homogenizer 150, Thermo Fisher Scientific, USA) and subsequently centrifuged to separate coral host material and symbiont cells (1000 g, 5 min at 4 °C, Labofuge 400R, Heraeus, Germany). The supernatant, containing the coral host material, was transferred to a pre-weighed container, and the symbiont pellet was resuspended with pre-chilled ultra-pure water (15–30 mL, depending on fragment size, 4 °C). Aliquots of the symbiont resuspension were prepared for chlorophyll content determination (2 mL) and symbiont cell counts (500 μL). The supernatant was freeze-dried for 72 h. Tissue dry weight (DW) was determined (Quintix 224, Sartorius, Germany), and four aliquots were prepared (i.e., for lipid, protein, and carbohydrate analysis: 15 mg DW and for amino acid analysis: 25 mg

DW). The aliquots were stored at −80 °C until further analysis.

2.4. Analysis of energy reserves of coral host

2.4.1. Lipid content

The lipid content of the coral host samples was determined using the colorimetric sulfo-phospho-vanillin (SPV) method (modified after Zöllner and Kirsch, 1962) with Menhaden fish oil (CAS 8002-50-4, Sigma-Aldrich, USA) as standard. Briefly, 2 mL concentrated sulfuric acid was added, and the sample was then heated to 105 °C for 10 min. After cooling to room temperature, 1 mL of phospho-vanillin reagent was added to a 50 μL aliquot, and the sample was incubated at room temperature for 40 min. Then, absorbance was measured at 530 nm with a spectrophotometer (Varian Cary 50, Agilent Technologies, USA). Lipids were calculated using a calibration curve generated with the standard.

2.4.2. Carbohydrate content

Carbohydrates of the coral host samples were determined by the colorimetric phenol-sulfuric acid method (modified after Albalasmeh et al., 2013) using D-glucose (CAS: 50-99-7) as standard. Briefly, the sample material (diluted 1:10) was treated with 3 mL concentrated sulfuric acid (> 95 %, purity grade: p.a.). The samples were whirled (10 s), shaken (3 min), and cooled down to room temperature (30 min). Finally, the samples were measured in a spectrophotometer (Varian Cary 50, Agilent Technologies, USA) at a wavelength of 315 nm. Carbohydrates were calculated using a calibration curve generated with the standard.

2.4.3. Protein content

Proteins of the coral host samples were analyzed using the bicinchoninic acid (BCA) assay with bovine serum albumin (BSA) as a standard (Smith et al., 1985). A test kit was used (Uptima BCA test kit, Interchim, France). Briefly, saline (0.9 %, 250 μL) was added to the sample material. Of this, 25 μL was transferred to a microtiter plate. Then, 200 μL BCA reagent was added, and the samples were incubated (30 min at 37 °C) and then cooled to room temperature. Protein content was determined colorimetrically in a microplate reader (Infinite M200, Tecan Group, Switzerland) at 562 nm wavelength. Proteins were calculated using a calibration curve generated with the standard.

2.4.4. Total energy

Total energy was calculated from the sum of the lipid, carbohydrate, and protein contents after conversion into joules using the enthalpies of combustion (Gnaiger and Bitterlich (1984); 39.5 J mg^{-1}, 17.5 J mg^{-1}, and 23.9 J mg^{-1}, respectively).

2.5. Metabolites of the coral host

A set of 20 metabolites (i.e., amino acids) was analyzed for a subset of samples from the coral host material (due to limited sample material: $n = 54$, see Table S3) using Ultra Performance Liquid Chromatography (UPLC) coupled with fluorescence detection. Samples were extracted with 0.1 M HCl and derivatized with AccQ Tag (Waters Corporation, USA). Norleucine was used as an internal standard for normalization. Determination of proteinogenic amino acid levels was performed as described in Weger et al. (2016). Analyses were performed at the Metabolomics Core Technology Platform (MCTP) in Heidelberg, Germany.

2.6. Documentation of coral growth and mortality

Coral growth in surface area, volume, and calcification rate was studied over the 11 months of the experiment. For this, all fragments were 3D scanned, and surface area and volume values were assessed at the beginning and the end of the exposure experiment using a handheld 3D scanner (Artec Spider with Artec Studio 10, Artec 3D, Luxembourg), following established procedures (see Reichert et al., 2016 for details, and Supplementary Text and Table S4). In addition, the weight of the coral fragments was determined at the same time points using the buoyant weight method (Jokiel et al., 1978). For this, the corals were weighed in artificial seawater (salinity: 34, temperature: 26 °C) using a fine balance (KB 360-3N, Kern & Sohn, Germany; accuracy: 0.001 g). Coral growth in tissue surface area (relative difference between start and end) and volume (absolute change in mm^3 per cm^2 per month between start and end) was calculated from the scans. Coral calcification (absolute change in mg per cm^2 per month) was calculated from buoyant weight and surface area data. Seawater density was calculated in R using the rho function of the seacarb package (Gattuso et al., 2024). The tissue surface area at the end of the experiment (t_7) was used to standardize measurements of energy (lipids, proteins, carbohydrates, and total energy), amino acids, and symbiont data (symbiont densities and chlorophyll content). Coral mortality, defined here as >50 % coral tissue loss or tissue bleaching, was documented and analyzed using 3D scanning at 8 time points (i.e., after 6, 12, 18, 24, 30, 40, and 46 weeks) over the course of the long-term experiment.

2.7. Analysis of coral photosymbionts

2.7.1. Symbiont densities

To fix the symbiont cells, 20 μL of 1 % glutardialdehyde (Carl Roth, Germany) was added to the 500 μL aliquot (2.3 Sample preparation). Symbiodiniaceae densities were determined via hemocytometer counts (Thoma, Paul Marienfeld, Germany), with $n = 10$ replications per sample. For this, the hemocytometer counting chambers were photographed under a digital microscope (VHX-2000 equipped with the VH-Z250R lens, Keyence, Japan), and symbiont cells were counted using ImageJ (v1.53t, Rueden et al., 2017).

2.7.2. Chlorophyll content

The 2 mL aliquot was centrifuged (3110 *g*, 10 min, at 4 °C, Labofuge 400R, Heraeus, Germany), and the supernatant was discarded. 2 mL pure acetone (≥ 99.5 %, Carl Roth, Germany) was added, and the sample was dark incubated for 24 h on ice and shaken continuously (90 rpm, HS 501 digital, IKA-Werke, Germany). The sample was then centrifuged again (1600 *g*, 10 min, at 4 °C), and the supernatant was measured with a spectrophotometer (calibrated with pure acetone; Biomate 3, Thermo Electron, USA) at three wavelengths (i.e., 630, 663, and 750 nm).

2.7.3. Photosynthetic efficiency

The photosynthetic efficiency of the photosymbionts was assessed by pulse amplitude modulated (PAM) fluorometry at the end of the experiment. We used a PAM-2500 fluorometer (Walz, Germany) equipped with a fiber-optic probe with 6 mm diameter and spacer (5 mm distance and 60° angle to the tissue). Effective ($\Delta F/F'_m$) and maximum (F_v/F_m) photochemical efficiencies were measured at three positions per coral fragment. $\Delta F/F'_m$ was assessed during daytime (after 3 h of light exposure), and F_v/F_m was measured after 40 min of darkness following 3 h of light exposure at daytime. In addition, rapid light curves (RLC) were generated. RLCs were generated under ambient light with intensity increasing in 10 steps (0, 1, 30, 100, 197, 362, 618, 980, 1385, and 2014 μmol photons m^{-2} s^{-1}). Hyperbolic tangent functions were fitted to each RLC, and the efficiency of light capture (α), maximum relative electron transport rate ($rETR_{max}$), and the minimum saturating irradiance (E_k) were calculated using the equations of Platt et al. (1980).

2.8. Statistics

Data processing and visualization were done using R (4.1.3, R Core Team, 2022) via the RStudio interface (2023.12.0.369, RStudio Team, 2023). Data were tested for normality (i.e., Shapiro-Wilk test) and homogeneity of variances (i.e., Levene's test), and statistical tests were

chosen accordingly. Statistical tests tested for differences between treatments (i.e., control vs. microplastics). Data on coral energy content were tested using Student's *t*-tests (lipids and proteins) and Wilcoxon tests (carbohydrates and total energy). Differences in mortality between the treatments were tested using Gray's tests (package: tidycmprsk 1.0). The growth data were tested using Student's *t*-tests (surface change and calcification) and Wilcoxon tests (volume change). Differences in the amino acid profiles were tested using Wilcoxon tests. Symbiont data were analyzed using Welch's *t*-test for symbiont densities, Student's *t*-test for chlorophyll content normalized to tissue surface, and Yuen's *t*-test (package: WRS2, 1.1–6) for chlorophyll content normalized to symbiont cell. Photosynthetic activity data were analyzed using Student's *t*-tests (F_v/F_m), Welch's *t*-tests ($\Delta F/F'_m$, $rETR_{max}$), and Wilcoxon tests (E_k and α). RLC of the PAM data were fitted using the fitPGH function from the package phytotools (1.0). Principal component analysis (PCA) based on a correlation matrix (prcomp function; package stats 4.1.3) was used to analyze the following groups: energy reserves (i.e., lipids, proteins, and carbohydrates), metabolites (i.e., 20 amino acids), growth (i.e., change in surface area, volume, and weight), and photosymbionts (i.e., symbiont densities, chlorophyll *a* and c_2 content per cell, and dark- and light-adapted yield). Corresponding *p*-values were derived from permutational multivariate analyses of variance (PERMANOVA) (package: vegan, 2.6–4) and effect sizes (partial ω^2) using the package MicEco (0.9.19) that were interpreted according to Field (2013), $\omega^2 < 0.01$ - very small, $0.01 \leq \omega^2 < 0.06$ - small, $0.06 \leq \omega^2 < 0.14$ - medium, $\omega^2 \geq 0.14$ - large). Differences in the dispersion within the PCA were analyzed using the function betadisper (package: vegan) and tested with subsequent ANOVA. Unless otherwise stated, tests were performed with the rstatix package (0.7.2, Kassambara, 2023). Plots were generated using the package ggplot2 (3.4.4, Wickham, 2016).

3. Results

3.1. Energy reserves of the coral host

Coral energy reserves (i.e., lipids, proteins, carbohydrates, and total energy) did not differ significantly between the control group and microplastic exposed corals in any of the species tested (Student's *t*-tests

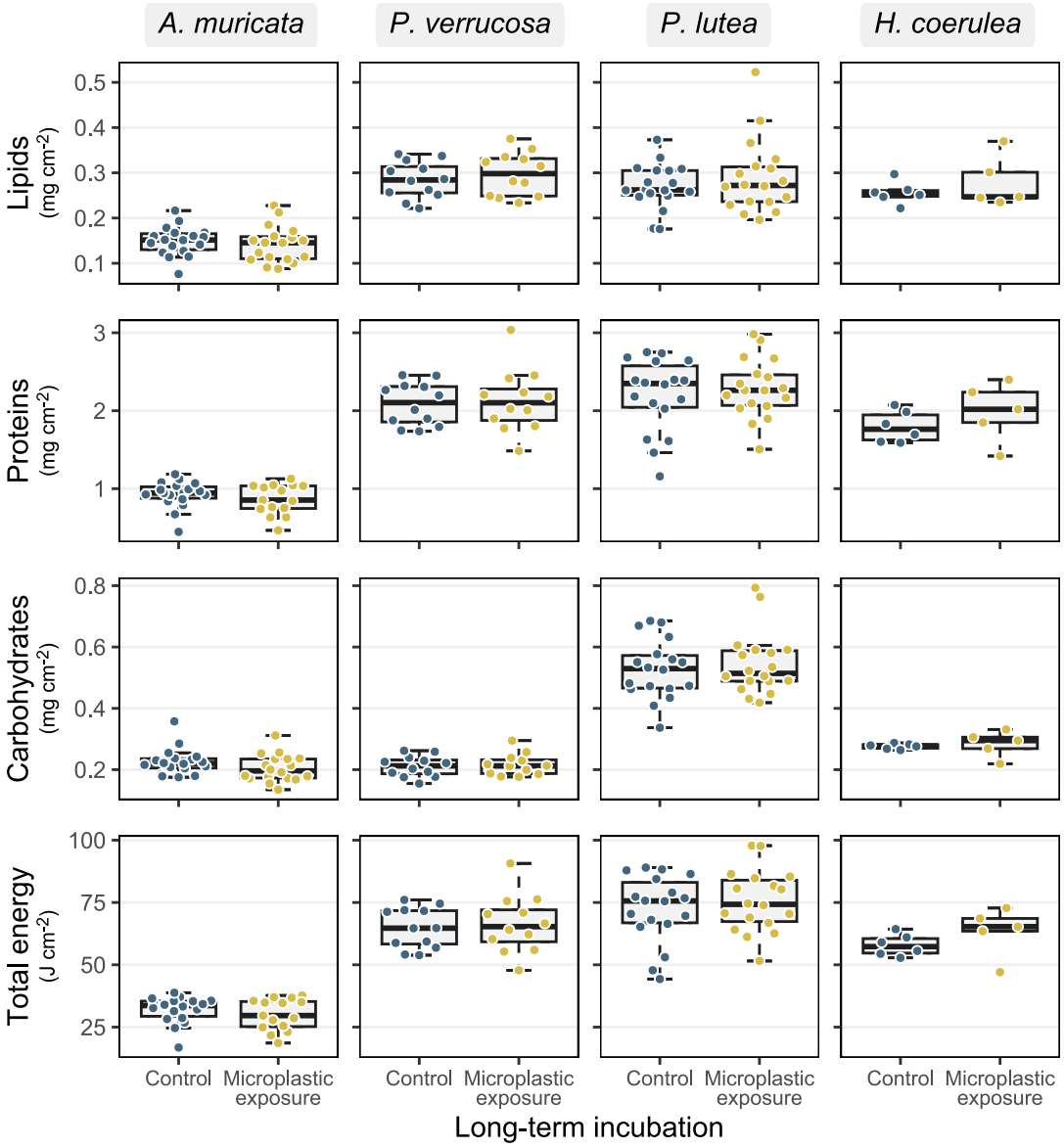

Fig. 1. Energy reserves (i.e., lipids, proteins, and carbohydrates in mg cm^{-2}, total energy in J cm^{-2}) in the four tested coral species (i.e., *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*) exposed to control conditions (blue) and microplastics (yellow). Data are displayed as box-and-whisker plots with raw data points. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(lipids and proteins) and Wilcoxon tests (carbohydrates and total energy), $p > 0.05$, Fig. 1 and Tables S5, S6, S7, and S8). The overall analyses of energy reserves showed a similar picture: PCAs (variables: lipids, proteins, and carbohydrates) did not show significant differences between the control and the microplastic treatment (PERMANOVA, $p > 0.05$, Fig. 3A and Table S17; ANOVA, $p > 0.05$, Table S21).

3.2. Metabolites of the coral host

Coral metabolites, including a set of 20 amino acids, remained largely stable and did not significantly differ between the control and the microplastic exposed group in three of the four species tested (i.e., *P. verrucosa*, *P. lutea,* and *H. coerulea*, Wilcoxon tests, $p > 0.05$, Figs. S4, S5, S6, S7, and Table S9). Only in *A. muricata* taurine was significantly higher in coral fragments exposed to microplastics (Wilcoxon test, $p = 0.011$, Fig. S4 and Table S9), but no significant differences were detected for the other metabolites (Wilcoxon tests, $p > 0.05$, Figs. S5–S7 and Table S9). Overall analyses of metabolites through PCAs (the set of 20 metabolites used as variables) did not show a difference between the control and the microplastic group (PERMANOVA, $p > 0.05$, Fig. 3B and Table S18; ANOVA, $p > 0.05$, Table S21).

3.3. Coral growth and mortality

For the species *A. muricata*, *P. verrucosa*, and *P. lutea*, coral growth (i.e., the change in tissue surface area, volume, and calcification rate) did not differ significantly between control and microplastic exposure (Wilcoxon tests (volume), Student's *t*-tests (surface area), and Welch's *t*-tests (calcification), $p > 0.05$, Fig. S8 and Tables S10, S11, and S12). However, in *H. coerulea*, growth in surface area and calcification rate were significantly lower under microplastic exposure (Student's *t*-test, $p = 0.027$ and Welch's *t*-test, $p = 0.038$). The overall analyses confirmed the individual test results for growth parameters. PCAs (difference in surface area, volume, and calcification as variables) showed no significant differences between control and microplastic exposed treatments in the species *A. muricata*, *P. verrucosa*, and *P. lutea* (PERMANOVA, $p > 0.05$, Fig. 3C and Table S19). In *H. coerulea,* coral fragments exposed to microplastics separated significantly from their control counterparts (PERMANOVA, $p = 0.025$, Fig. 3C and Table S19; ANOVA, $p > 0.05$, Table S21). Coral mortality did not differ significantly between controls and microplastic exposure (Gray's test, in all species $p > 0.05$, Fig. 2 and Table S22).

3.4. Photosymbionts

The photosymbiont state (i.e., symbiont density, the content of chlorophylls *a* and c_2, and the photosynthetic efficiency) did not differ significantly between control and microplastic exposure in most cases (Welch's *t*-tests (light-adapted yield ($\Delta F/F'_m$), $rETR_{max}$, and symbiont density), Student's *t*-tests (chlorophyll, dark-adapted yield (F_v/F_m)), and Wilcoxon tests (α and E_k), $p > 0.05$, Figs. S9, S10, S11, and S12, and Tables S13, S14, S15, and S16). *A. muricata* showed altered chlorophyll *a* and total chlorophyll content, when normalized to symbiont cell. Specifically, the chlorophyll content in *A. muricata* was higher in corals from the microplastic exposure (Yuen's *t*-test, $p = 0.02$ and $p = 0.039$, Fig. S11 and Table S15). Additionally, *A. muricata* exhibited a higher efficiency of light capture (α) in corals from the microplastic exposure (Wilcoxon test, $p = 0.022$, Fig. S12 and Table S16). *P. lutea* showed lower light-adapted yield ($\Delta F/F'_m$) in corals from the microplastic exposure (Welch's *t*-test, $p = 0.024$, Fig. S12 and Table S16). Overall analyses through PCAs (variables: symbiont density, chlorophylls *a* and c_2, and light- and dark-adapted yield) revealed no differences between control and microplastic exposure (PERMANOVA $p > 0.05$, Fig. 3D and Table S20; ANOVA, $p > 0.05$, Table S21), except for *A. muricata*, where microplastics had a significant impact on photosynthetic parameters (i.e., PCA variables, PERMANOVA, $p = 0.031$, Table S20).

4. Discussion

Our study aimed to evaluate the chronic effects of microplastic exposure on physiological parameters related to energy reserves, metabolites, growth, and photosynthesis in an 11-month, multi-species experiment. A physiological effect of microplastic exposure on host energy reserves was absent, while effects on host metabolites, coral growth parameters, and photosymbiont state (i.e., density, chlorophyll content, and most photosynthetic efficiency parameters) occurred only occasionally. Species-specific effects were observed as reduced growth in *H. coerulea*, altered photosynthetic efficiency in *P. lutea* and *A. muricata*, and variations in the taurine levels in *A. muricata*.

4.1. Microplastics did not significantly affect energy reserves of the coral host

Our results suggest that long-term exposure to a realistic microplastic concentration of 200 particles L^{-1} (≙ 0.25 mg L^{-1}) does not affect coral energy reserves, as lipid, protein, and carbohydrate concentrations as well as the total energy did not differ significantly between microplastic exposure and control (see Figs. 1 and 3A and Tables S5, S6, S7, S8, and S17). These results are consistent with some previous studies that found no effects on lipid (Boodraj and Glassom, 2022; Mouchi et al., 2019) and protein contents (Lanctôt et al., 2020; Rocha et al., 2020). In contrast, other studies did reveal effects of microplastics on corals. However, the concentrations used in the latter studies were often several orders of magnitude higher than those used in the present study: 2500 particles L^{-1} (Reichert et al., 2021), 50,000 particles L^{-1} (Lanctôt et al., 2020), 10 mg L^{-1} (Rocha et al., 2020), 50 mg L^{-1} (Jiang et al., 2021; Syakti

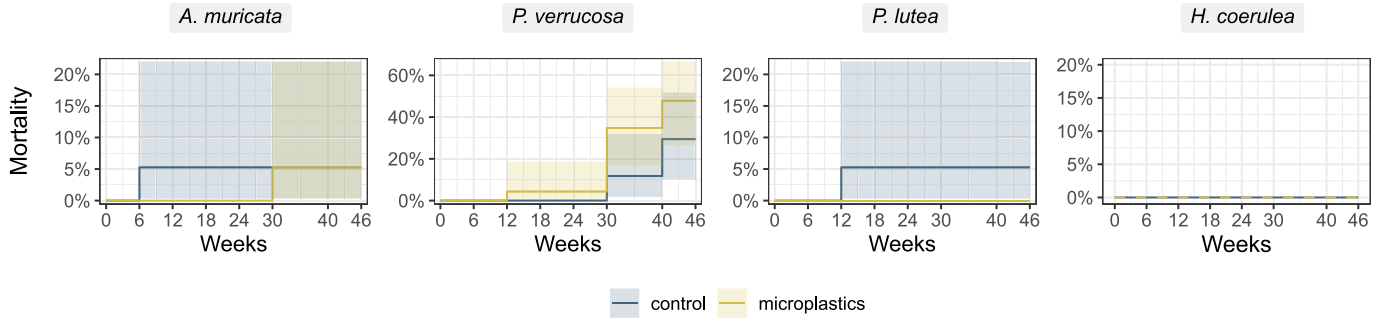

Fig. 2. Mortality (% of corals with >50 % bleached or necrotic tissue) of the four coral species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea* under microplastic exposure (yellow) and control conditions (blue) over the course of the long-term experiment. Mortality was recorded at the 8 time points over the course of the experiment (i.e., after 6, 12, 18, 24, 30, 40, and 46 weeks). Lines depict mortality in %, and shaded areas represent 95 % confidence intervals derived from Gray's tests. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

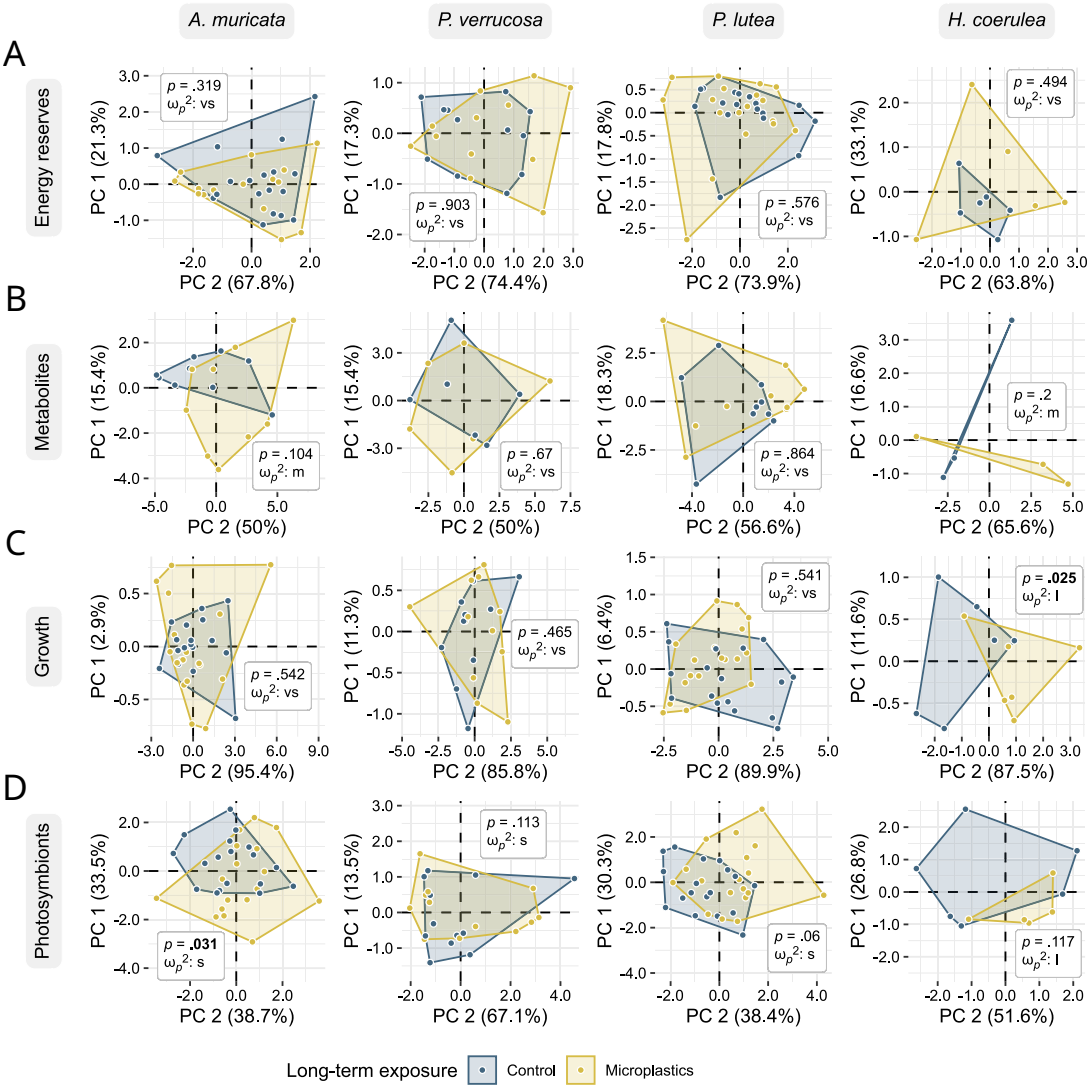

Fig. 3. Differences between control (blue) and microplastic exposure conditions (yellow) in the grouped physiological parameters (rows A–D) and the four coral species tested (columns). Row A: Energy reserves (variables: lipids, proteins, and carbohydrates). Row B: Metabolites (variables: set of 20 metabolites). Row C: Growth (variables: surface area, volume, and calcification). Row D: Photosymbionts (variables: symbiont density, chlorophylls *a* and c_2, and light- and dark-adapted yield). Data are displayed as PCAs; *p*-values were derived from PERMANOVAs, and effect sizes (partial ω^2) are abbreviated (vs = very small, s = small, m = medium, l = large). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

et al., 2019; Xiao et al., 2021), and peaking at 300 mg L^{-1} (Chen et al., 2022a). These very high particle concentrations far exceed current (Jeyasanta et al., 2020; Patterson et al., 2020, 2022; Wightman and Renegar, 2023) and predicted future concentrations in the oceans (Everaert et al., 2018; Koelmans et al., 2017) and may have caused, at least in part, the effects in these studies.

Our study suggests that realistic microplastic concentrations may not have a significant long-term effect on coral energy reserves, indicating the presence of effective strategies to reduce the impact of the exposure. These strategies might be particle avoidance mechanisms, such as shortened particle handling (Savinelli et al., 2020), low ingestion (Reichert et al., 2024a), or active removal from adhesion (Reichert et al., 2018, 2024a) as well as mitigation strategies such as reduced reproduction or immune system resilience. It is possible that the tested corals, as well as corals in situ, use one or more of these strategies to reduce microplastic stress. In addition, the energy supply of reef-building corals is highly dependent on an intact symbiosis with Symbiodiniaceae (Grottoli et al., 2006; Roth, 2014). Energy reserves were probably not affected for several reasons: First, the realistic microplastic concentrations are likely to have caused only minor stress effects through handling, adhesion, and ingestion, and second, the minor effects on the photosymbionts indicate that the symbiosis is still intact and its capacity for upregulation is likely to compensate for any adverse effects. However, this picture may change under predicted higher concentrations or under mixtures of different polymers and forms already present in the oceans.

4.2. *Metabolites remain largely unaffected, except for taurine*

Overall, our study showed that realistic microplastic concentrations have only a limited effect on most coral metabolites. Of the 20 amino acids tested, 19 were unaffected by our long-term exposure experiment (Figs. S4, S5, S6, S7, and Table S9). Only taurine increased in *Acropora muricata* when exposed to microplastics (Fig. S4 and Table S9). Taurine is a non-essential and non-proteinogenic amino sulfonic acid, which is thought to act as a host release factor in Symbiodiniaceae and initiates the translocation of carbon fixation products such as sugars into the coral host (Huang et al., 2022; Wang and Douglas, 1997). Elevated taurine levels may alter metabolic pathways in the photosymbionts, allowing for more energy-rich compounds to be translocated into the

coral host (Huang et al., 2022; Wang and Douglas, 1997). Thus, our results suggest that *A. muricata* requires more photosynthetically fixed carbon from its symbiotic partner when exposed to microplastics.

However, the biological functions of taurine are not fully understood. While we are not aware of any other study that has examined taurine levels in corals in relation to microplastic exposure, a microplastic study in mussels also found elevated taurine levels, possibly indicating impaired osmoregulatory processes (Cappello et al., 2021). Taurine is also a known osmolyte in corals (Yancey et al., 2010) and is thought to play a role in sulfur recycling in coral holobionts (Robbins et al., 2019). However, as the osmolyte glycine remained unchanged in our study (Figs. S4–S7, Table S9), an effect of microplastics on the osmoregulation of corals seems unlikely.

4.3. *Heliopora coerulea* reacts to microplastic exposure with reduced growth

Our study showed that realistic microplastic concentrations may have small species-specific effects on coral growth parameters. The growth parameters of most coral species were not affected in our microplastic exposure experiment, and only *H. coerulea* showed decreased surface area growth and calcification rate (Fig. S8, Tables S10, S11, and S12). This decrease may be related to the coral's feeding behavior, as Reichert et al. (2024a) suggested that *H. coerulea* is more heterotrophic, frequently interacts with microplastics, and occasionally ingests particles. As all other physiological parameters remained unaltered under microplastic exposure, *H. coerulea* likely mitigates the effects of microplastics at the expense of growth. This mechanism was previously proposed for cold-water corals, where growth was reduced, but lipid content remained unchanged (Mouchi et al., 2019). Negative effects of microplastics on growth parameters have also been observed in some other coral species (e.g., surface area of *Lophelia pertusa* in Chapron et al. (2018), surface area of *Acropora muricata* and calcification rate of *Heliopora coerulea* in Reichert et al. (2019), and surface area and calcification rate of *Pseudodiploria clivosa* and *Acropora cervicornis* in Hankins et al. (2021)).

In contrast, other studies showed no effects of microplastics on coral growth parameters (Boodraj and Glassom, 2022; Lanctôt et al., 2020), which is largely consistent with our multi-species study and suggests species-specific differences in susceptibility to microplastics. However, some previous studies that found no effects on coral growth were conducted over a much shorter time period (1–2 days vs. 11 months in the current study (e.g., Hankins et al., 2018; Liao et al., 2021)) and used a less precise method to determine growth parameters (e.g., aluminum foil wrapping vs. high-resolution 3D scanning (Plafcan and Stallings, 2022; Tang et al., 2018; Xiao et al., 2021)). Thus, methodological issues may contribute to the differences between the studies.

While coral mortality was not affected significantly, mortality rates were slightly higher for *P. verrucosa* under microplastic exposure, as seen previously (Reichert et al., 2019). This trend might indicate that *P. verrucosa* might be especially sensitive to microplastic exposure and lacks effective compensation measures, which is consistent with previous studies (Reichert et al., 2018, 2019, 2024a, 2024b).

4.4. Photosymbiont state remains largely unaffected despite subtle species-specific changes

Our long-term microplastic exposure experiment had no effect on most parameters of the corals' photosymbiont state (i.e., symbiont density, chlorophyll concentration per tissue surface area, and three of five photosynthetic efficiency parameters). These findings are widely consistent with other studies that found no changes in symbiont density (Boodraj and Glassom, 2022; Ng and Todd, 2023; Plafcan and Stallings, 2022; Tang et al., 2021) and chlorophyll concentration (Boodraj and Glassom, 2022; Lanctôt et al., 2020; Ng and Todd, 2023; Tang et al., 2021) under microplastic exposure. In contrast, two previous studies concluded that microplastic exposure reduces symbiont density (Jiang et al., 2021; Xiao et al., 2021), although these studies were conducted at much higher microplastic concentrations (i.e., 50 mg L^{-1}).

However, photosynthetic efficiency was affected occasionally in our study. Significant differences occurred between treatment and control groups for relative effective yield of PSII ($\Delta F/F'_m$ = light-adapted photosynthetic efficiency) in *P. lutea* and efficiency of light capture (α) in *A. muricata* (Fig. S12, Table S16). The decrease in $\Delta F/F'_m$ (*P. lutea*) suggests that the microplastic exposure reduces the photosynthetic efficiency (sensu Schreiber, 2004), as previously observed in exposure experiments with sediments (Junjie et al., 2014; Philipp and Fabricius, 2003; Piniak, 2007) and microplastics (Mendrik et al., 2021). This decrease may ultimately lead to reduced carbon fixation (Cantin et al., 2007). However, possible consequences of such reductions (i.e., reduced energy acquisition) were likely too small and isolated to be detected by the energy reserve analysis performed. The increase in α (*A. muricata*) indicates increased photosynthetic efficiency, especially in the light-limited range (sensu Ralph and Gademann, 2005; Ralph et al., 2005). The increased photosynthetic efficiency in *A. muricata* is accompanied by a significant increase in chlorophyll *a* and total chlorophyll content (per symbiont cell, Fig. S11, Table S15). This increase is also reflected as a trend in the upregulation of the relative maximum yield of PSII (F_v/F_m = dark-adapted photosynthetic efficiency) and $\Delta F/F'_m$ in *A. muricata*. The individual changes in photosymbiont parameters are reflected at the overall level (see Fig. 3), indicating a general change in photosymbiont status in *A. muricata*. This increase in photosynthetic efficiency is likely a compensatory mechanism, as previously proposed (Bove et al., 2023; Lanctôt et al., 2020; Reichert et al., 2019). The increase in chlorophyll content and upregulation of photosynthetic efficiency while maintaining energy reserves in *A. muricata* might indicate a successful compensation method against microplastic exposure.

4.5. Translating findings to coral reef ecosystems

To translate our findings to coral reef ecosystems, the exposure conditions need to be discussed in the context of the complex picture of microplastic pollution in coral reefs (Huang et al., 2021). Important factors to consider are exposure concentrations, polymer composition and shape, exposure duration, and representativeness of tested coral species.

Microplastic concentrations in coral reef waters can be as high as ~700 particles L^{-1} at local hotspots (Yusof et al., 2023), although the current in situ concentrations often do not reach this maximum (Jeyasanta et al., 2020; Patterson et al., 2020, 2022; Wightman and Renegar, 2023). However, the 200 particles L^{-1} used here can be found as average concentrations in highly polluted reef sites (Patterson et al., 2020; Yusof et al., 2023) and might become more frequent under expected future scenarios (Everaert et al., 2018; Koelmans et al., 2017). Therefore, we consider the concentrations applied here as a realistic high-pollution scenario that already occurs in nature and is orders of magnitude lower than those applied in other exposure studies (Chen et al., 2022a; Jiang et al., 2021; Lanctôt et al., 2020; Rocha et al., 2020; Syakti et al., 2019; Xiao et al., 2021).

While PE is one of the most common polymer types found in coral reef environments (Jeyasanta et al., 2020; Patterson et al., 2020, 2022; Saliu et al., 2018; Zhang et al., 2020), environmental samples often show a combination of microplastics of different polymers and shapes (Garcés-Ordóñez et al., 2021; Nunes et al., 2023), as well as different additives and absorbed toxins (Andrade et al., 2021; Ranjbar Jafarabadi et al., 2021; Vencato et al., 2024). Multi-polymer studies showed that PE is representative in terms of effects on corals, although different types of microplastics can cause more pronounced effects (Reichert et al., 2024b). Mixtures may generally have the potential to cause stronger effects, as different hazards might occur simultaneously (e.g., entanglement by fibers and ingestion of plastic pellets). Thus, the effects observed here might underestimate those occurring under natural, more

complex, high-pollution conditions, and future studies should also consider polymer mixtures that more closely resemble natural conditions.

In nature, the exposure duration to microplastic pollution is highly variable. To date, data on microplastic pollution over time, particularly within coral reef ecosystems, is lacking from the literature. Due to the high variability of ocean currents, local weather conditions, and pollution events, concentrations might fluctuate strongly, while in other locations, more stable hotspots might form in the long term due to stronger permanent ocean currents or constant pollution inputs from land (Erni-Cassola et al., 2019). Our study aimed to provide a first picture of the long-term effects of microplastic exposure, which goes beyond previously observed acute short-term reactions. Thus, this study is among the first to investigate chronic long-term effects, potentially occurring under environmentally stable high-pollution hotspots, suggesting that these differ strongly and highlighting the importance of potential compensation mechanisms.

Tropical reef-building corals are highly diverse and comprise over 800 species (Dietzel et al., 2021; Veron et al., 2016). We chose representatives of the most common and widely distributed reef-building coral genera to assess a realistic picture of microplastics' impacts on tropical coral reef ecosystems. The diversity of species tested here, together with other studies (e.g., Bejarano et al., 2022; Chen et al., 2022a; Plafcan and Stallings, 2022), provides a comprehensive picture of microplastic effects on corals with different genetic backgrounds, growth forms, and other life history traits. We identify genera that emerge to be more susceptible to microplastics (e.g., *Acropora* spp., *Pocillopora* spp. (Mendrik et al., 2021; Reichert et al., 2019), and *Heliopora* spp. herein) and that might be the target of future studies to better understand the mechanisms of the microplastic stressor and evaluate their use as in situ indicators. Yet, other species that have not been tested to date or are cryptic potentially fulfill different functions in the coral reef ecosystem than the main reef-builders tested so far and might exhibit different physiological responses to microplastics. Thus, these species might also be of interest for future studies.

4.6. Conclusions

While the long-term exposure to microplastics had mostly no effects on coral physiology, species-specific effects on metabolite levels, growth parameters, and photosymbionts were occasionally observed. The increase in the amino acid taurine (*A. muricata*), the reduced growth (*H. coerulea*), the altered photosynthetic efficiency (*A. muricata* and *P. lutea*), and the altered chlorophyll content (*A. muricata*) may be different forms of coral mitigation mechanisms against microplastics, as the overall effects were subtle and limited. Considering the often lower in situ concentrations in coral reef areas, microplastics alone may not be a current threat to global coral reef communities. However, pollution hotspots or local pollution events as well as the constantly increasing microplastic concentrations, might increase the role of microplastics as a local stressor for corals, especially through cumulative effects with other stressors (e.g., rising temperatures and sea levels as well as ocean acidification) predicted to be exacerbated by climate change.

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CRediT authorship contribution statement

Marvin Rades: Writing – original draft, Visualization, Investigation, Formal analysis. **Gernot Poschet:** Writing – review & editing, Investigation. **Hagen Gegner:** Writing – review & editing, Conceptualization. **Thomas Wilke:** Writing – review & editing, Conceptualization. **Jessica Reichert:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, 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.

Data availability

The data presented will be available on GitHub upon publication of the study: https://github.com/MarvinRades/coral-physiology.

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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.2024.116631.

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