Long-term effects of microplastics on the behaviour and physiology of reef-building corals

DISSERTATION

zur Erlangung des akademischen Grades

Doktor der Naturwissenschaften

— Dr. rer. nat. —

angefertigt am Institut für

Allgemeine und Spezielle Zoologie

am Fachbereich 08 – Biologie und Chemie

der Justus-Liebig-Universität Gießen

vorgelegt von

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Gießen, 2024

JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN

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Eingereicht am | *Submitted on*: 07.10.2024
Disputation am | *Disputation on*: 09.12.2024
Deutscher Titel | *German title*: Langzeiteffekte von Mikroplastik auf das Verhalten und die Physiologie riffbildender Korallen

Zitiervorschlag | *Please cite as*

Marvin Rades (2024), Long-term effects of microplastics on the behaviour and physiology of reef-building corals, Dissertation zur Erlangung des akademischen Grades „Doktor der Naturwissenschaften" des naturwissenschaftlichen Fachbereichs 08 – Biologie und Chemie der Justus-Liebig-Universität Gießen, Deutschland

The great tragedy of science—the slaying of a beautiful hypothesis by an ugly fact.

—Thomas Henry Huxley (1870)

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1 Synthesis

1.1 Zusammenfassung

Anthropogene Einflüsse dezimieren Korallenriffe, wobei die Rolle der allgegenwärtigen Verschmutzung durch Mikroplastik unklar ist. Mikroplastik beeinträchtigt riffbildende Korallen vor allem bei hohen Konzentrationen. Meist liegen jedoch niedrigere und damit relevantere Konzentrationen vor, die oft geringere Auswirkungen haben. Der Mangel an Daten zur Langzeitexposition erschwert jedoch die Bewertung von Mikroplastikstress, seiner Ursachen und der verhaltensbiologischen und physiologischen Akklimation von Korallen im Laufe der Zeit. Ziel dieser Thesis war es daher, aus den Langzeiteffekten von Mikroplastik auf das Verhalten und die Physiologie von Korallen Rückschlüsse auf Akklimationsmechanismen zu ziehen. Dazu wurden die vier häufig vorkommenden riffbildenden Korallenarten *Acropora muricata*, *Pocillopora verrucosa*, *Porites lutea* und *Heliopora coerulea* in einem kontrollierten Langzeit-Aquarienexperiment einer realistischen Mikroplastikkonzentration von 200 Polyethylenpartikeln L^{-1} ausgesetzt. Nach 11 Monaten wurden die Energiereserven, Stoffwechselprodukte, das Wachstum und der Zustand der Photosymbionten analysiert. Nach 15 Monaten wurden die Aufnahmeraten von Mikroplastik und natürlicher Nahrung (d. h. Zysten von *Artemia* sp.), die Nahrungsunterscheidung (d. h. das Aufnahmeverhältnis von Mikroplastik zu natürlicher Nahrung) und die Reaktionen auf beides in einer 24-stündigen Impuls-Exposition ermittelt. Obwohl die Korallen langfristig Mikroplastik ausgesetzt waren, zeigte sich, dass sie ihr Fress-, Unterscheidungs- und Abwehrverhalten nicht änderten, um die Aufnahme von Mikroplastik zu reduzieren. Daher wird angenommen, dass Korallen keine Mechanismen zur Verhaltensanpassung, wie heterotrophe Plastizität, nutzen oder besitzen, um den langfristigen Mikroplastikstress zu reduzieren. Trotz dieser fehlenden Verhaltensakklimation war die Physiologie der Korallen nur geringfügig beeinträchtigt, da gelegentliche artspezifische Effekte auf physiologische Akklimationsmechanismen hindeuten: Bei Korallen könnten erhöhte Taurinwerte, verringertes Wachstum und eine veränderte photosynthetische Effizienz den Mikroplastikstress bei umweltrelevanten Konzentrationen, bei denen die Photosymbiose der Korallen intakt bleibt, wahrscheinlich abmildern. Unter Szenarien mit prognostizierten Anstiegen der Mikroplastikkonzentration und anderen kumulativen anthropogenen Stressfaktoren könnte die Akklimation durch Kompensationsmechanismen jedoch nur

begrenzt möglich sein, was die negativen Auswirkungen auf die Gesundheit der Korallen wahrscheinlich noch verstärken dürfte. Insgesamt hat diese Arbeit neue Einblicke in die langfristigen Akklimationsmechanismen geliefert, die Korallen helfen können, mit relevanten Mikroplastikkonzentrationen umzugehen, und hat ferner zu einem umfassenderen Verständnis der Empfindlichkeit von Korallen gegenüber Stressoren beigetragen, das notwendig ist, um Schutzbemühungen zur Eindämmung des Rückgangs der Korallenriffe zu steuern. Allerdings könnten diese Akklimationsmechanismen durch kumulative Auswirkungen von multipolymerem Mikroplastik und anderen anthropogenen Stressoren an ihre Grenzen stoßen – eine offene Frage, für die diese Arbeit einen Ausgangspunkt für zukünftige Forschung bietet.

1.2 Abstract

Coral reefs are declining because of anthropogenic pressures, but the role of ubiquitous microplastic pollution is unclear. Microplastics affect reef-building corals mostly at high concentrations, while effects are often attenuated at lower environmentally relevant concentrations. However, the scarcity of long-term exposure data hinders the assessment of microplastic stress, its causes, and coral behavioural and physiological acclimation over time. Therefore, this thesis aimed to infer acclimation mechanisms from long-term effects of microplastics on coral behaviour and physiology. For this, the four common reef-building coral species *Acropora muricata*, *Pocillopora verrucosa*, *Porites lutea*, and *Heliopora coerulea* were exposed to a realistic microplastic concentration (i.e., 200 polyethylene particles L^{-1}) in a controlled long-term aquarium experiment. After 11 months, energy reserves, metabolites, growth, and photosymbiont state were analysed. After 15 months, feeding rates on microplastics and natural food (i.e., *Artemia* sp. cysts), feeding discrimination (i.e., ratio of feeding on microplastics and natural food), and reactions to both were determined in a 24-hour pulse exposure. Although long-term exposed to microplastics, results showed that corals did not change their feeding, discrimination, and defence behaviour to reduce microplastic uptake. Therefore, it is assumed that the corals did not use, or lacked, behavioural acclimation mechanisms, such as heterotrophic plasticity, to mitigate long-term microplastic stress. Despite this absence of behavioural acclimation, coral physiology was only marginally affected, as occasional species-specific effects may indicate physiological acclimation mechanisms. Increased taurine levels, reduced growth, and altered photosynthetic efficiency in corals are likely to mitigate microplastic stress at environmentally relevant concentrations where coral photosymbiosis remains intact. However, under scenarios with predicted increases in microplastic concentration and other cumulative anthropogenic stressors, acclimation through compensatory mechanisms may be limited, likely exacerbating adverse effects on coral health. Overall, this thesis has provided unprecedented insights into long-term acclimation mechanisms that may help corals cope with relevant microplastic concentrations and has contributed to a more holistic understanding of coral stressor susceptibility, which is needed to guide conservation efforts to curb coral reef decline. However, these coral acclimation mechanisms may be challenged by the combined effects of multi-polymer microplastics and other anthropogenic stressors, an open question for which this work provides a starting point for future research.

1.3 Introduction

1.3.1 Rationale

The Anthropocene, regardless of the controversy over the designation as an epoch or event, is undoubtedly hallmarked by humans shaping influence on Earth's realms, accompanied by loss of habitat and biodiversity, and fuelling climate change (Boivin et al., 2024). Ecosystems are under pressure worldwide, and tropical coral reefs are no exception; in fact, they are severely affected in many ways, including that the renowned trump of their specialised keystone species may turn out to be their Achilles heel (Chan et al., 2019; Hughes et al., 2017; J. Jiang et al., 2021; Putnam et al., 2017). The key taxa of reefs, hermatypic scleractinian corals (Anthozoa, Cnidaria), live in symbiosis with unicellular, photosynthetic dinoflagellates (family Symbiodiniaceae), to which reef growth is mainly due. However, this productive yet tenuous and intricate symbiosis is vulnerable to rapid environmental changes, such as global warming, which has already caused massive bleaching events and loss of coral cover (Hughes et al., 2018a). Additionally, corals face anthropogenic disturbances, such as sedimentation, overfishing, and pollution (Erftemeijer et al., 2012; Zaneveld et al., 2016). The latter includes eutrophication, chemicals, and plastics (Lamb et al., 2018; Pinheiro et al., 2023). Microplastics (particles < 1 mm) are ubiquitous in reef waters and are ingested by corals, making them a potential stressor (Hall et al., 2015). However, there is a lack of knowledge on the effects of long-term exposure to environmentally relevant microplastic concentrations, which is urgently needed to understand the impact of microplastic particles on these key reef-builders. Using four widespread coral species under long-term exposure to microplastics, behavioural (i.e., changes in feeding behaviour, particle discrimination, and frequency of defence reactions) and physiological responses (i.e., surface area, volume, weight gain, energy reserves, metabolite profiles, symbiont density, and chlorophyll content) of the corals and their symbiotic dinoflagellates were assessed to provide a comprehensive picture of the effects of microplastics on hermatypic corals.

1.3.2 Coral reef ecosystems

Coral reefs represent the Earth's largest biogenic structures by binding carbon and calcium to a gross production of about 300–2,500 tonnes of calcium carbonate ($CaCO_3$, (Hart and Kench, 2007; Hubbard and Miller, 1990; Spalding and Grenfell, 1997))—year after year for over

200 million years (Stolarski et al., 2011). However, in an ever-changing world, this process is not a one-way street to unlimited growth: throughout Earth's history, geological and climate events have also led to cutbacks in coral cover, e.g., because of changes in sea level, temperatures, and ocean acidification, and the emergence and disappearance of coastlines (Hönisch et al., 2012; Pandolfi, 2011; Renema et al., 2016). Limiting factors such as light availability, temperature, and salinity restrict tropical coral reefs to only ~0.07% of the global ocean area, yet they harbour marines' most tremendous diversity with an estimated 1 million (Fisher et al., 2015; Spalding and Brown, 2015), if not 10 million species (Knowlton et al., 2010). Although corals can be found worldwide, coral reefs usually grow only within the 20 °C surface isotherm in shallow waters (Figure 1) and down to depths of 50 metres because of light limitation (Spalding et al., 2001). As their upward growth is only limited by air exposure during low tide, they act as massive breakwaters that protect islands or the mainland from erosion (Hubbard et al., 2016). These complex three-dimensional frameworks provide various habitats, contributing to a high biodiversity (Messmer et al., 2011).

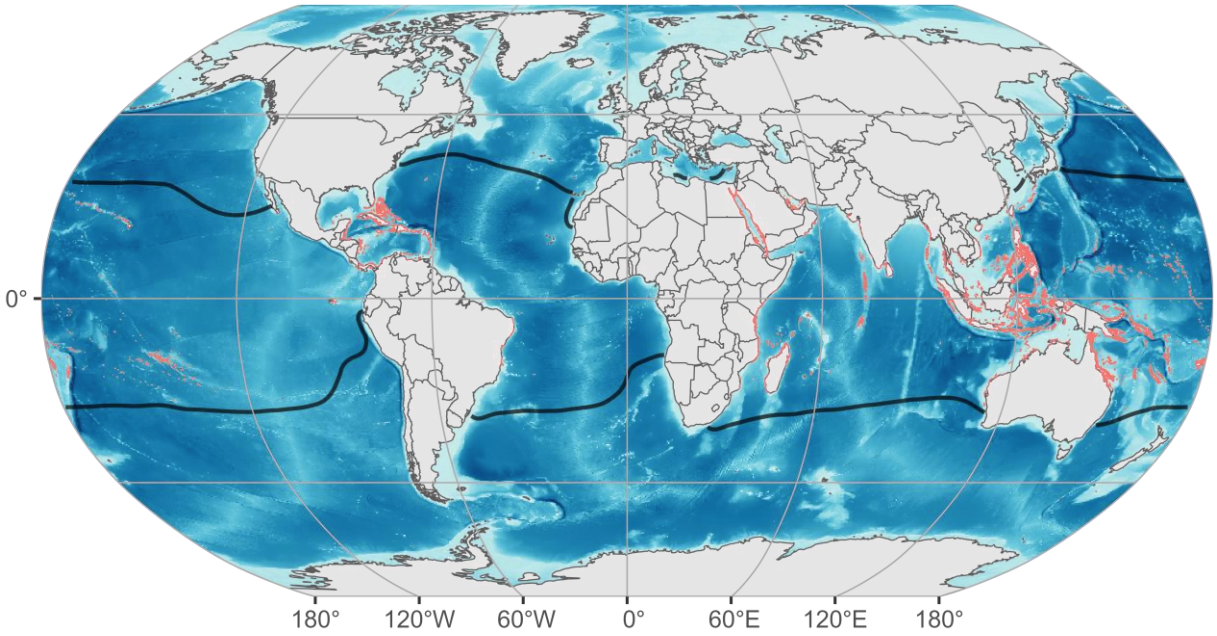

Figure 1: World map (Robinson projection) showing the distribution of tropical coral reefs (red, UNEP-WCMC et al., 2021), the two 20 °C isothermal lines (black), and bathymetric information (NOAA, 2022) on the topography of the sea floor (light blue = coastal shallow water, dark blue = deep sea). Graphic created with `R` (R Core Team, 2022) and the packages `ggplot2` (Wickham, 2016), `tidyterra` (Hernangómez, 2023), and `marmap` (Pante et al., 2023).

Within tropical marine waters that are oligotrophic and exhibit very low productivity (18–50 g C m^{-2} y^{-1}), coral reefs stand out as patches of high productivity

(1,825–7,300 g C m^{-2} y^{-1}) (Atkinson, 1992; Sorokin, 1995a). This phenomenon can partly be explained by reefs serving as sinks for nutrients brought in from outside, as they have also been referred to as "walls of mouths" (Fabricius and Metzner, 2004). However, the high productivity of reefs mainly originates from high primary productivity enabled by symbiosis (Birkeland, 2015a). Possibly the most important and best known is the symbiosis in reef-building (i.e., hermatypic) corals, which harbour unicellular, photosynthetic dinoflagellates ("zooxanthellae", family Symbiodiniaceae (LaJeunesse et al., 2018)) that enhance coral growth by transferring nutrients (e.g., carbons) to the coral host (Goldberg, 2018; Wall et al., 2021), a process referred to as the "engine of the reef" (Roth, 2014). Hermatypic corals, therefore, have a decisive advantage over their ahermatypic counterparts, which usually lack Symbiodiniaceae and hardly ever form reefs (Martin-Garin and Montaggioni, 2023; Schuhmacher and Zibrowius, 1985). In exchange, Symbiodiniaceae receive protection as well as access to CO_2 and other metabolic by-products from the coral (Roberty et al., 2020), which they can recycle into organic form and return to the coral host. Close coupling of reef consumers and producers, such as symbiotic photosynthetic organisms with their hosts (e.g., corals, sponges, and clams) (Cui et al., 2023), makes nutrients directly available, minimises losses, and helps to bind nutrients to the reefs (Uthicke, 2001).

1.3.3 Hermatypic corals

Among the primary reef builders are scleractinian stony corals (class Hexacorallia, subphylum Anthozoa), although blue corals (genus *Heliopora* de Blainville, 1830, class Octocorallia, subphylum Anthozoa) and fire corals (genus *Millepora* Linnaeus, 1758, class Hydrozoa, subphylum Medusozoa) can also have a substantial local share (Andréfouët et al., 2014; Atrigenio et al., 2020; Lewis, 2006; Taninaka et al., 2021). Beneath their polyps, these cnidarians produce an exoskeleton of aragonite, a crystalline calcium carbonate ($CaCO_3$) (Von Euw et al., 2017). Almost all hermatypic corals are colonial, i.e., many interconnected polyps form a thin layer of tissue that spans a massive colonial skeleton called a corallum. The joint tissue enables the exchange of nutrients and the linking of the nervous system (Bouderlique et al., 2022). In anthozoans, a colony originates from a single polyp founded by a settled planktonic planula larva after metamorphosis. Subsequent growth occurs by "budding", an asexual form of reproduction by polyp division (Combosch and Vollmer, 2013; Harrison, 2011; Sentoku and Ezaki, 2012). Thus, a coral colony comprises many polyps, which are genetically

identical clones. This feature proves very useful in experiments, as small sections can be cut or sawed from the "mother colony" to obtain several new small colonies (called nubbins) that share the same DNA, which is analogous to the natural process of fragmentation (Shafir et al., 2006, 2003). Each coral colony is unique, shaped not only by its genetic blueprint but also by its environment. Species affiliation presets principal morphologies, such as branching, massive, columnar, foliaceous, plate-like, or encrusting (Pratchett et al., 2015; Zawada et al., 2019). However, the individual shape of this genetically predetermined form is influenced by water flow, light availability, sedimentation, and competing neighbouring corals (Osinga et al., 2011; Todd, 2008).

Anthozoan corals, as used in this thesis, are bilateral, diploblastic animals with an outer ectoderm, a fibrous mesoglea, and an internal endoderm (McFadden et al., 2021; Swain et al., 2017; Technau, 2020) that harbours the endosymbiotic dinoflagellates. The polyps are housed in cup-shaped recesses of the skeleton, called corallites, into which they can retract for protection. A ring of tentacles on top of each polyp surrounds the mouth, the only opening of the gastric cavity (Figure 2). Corals use their tentacles to actively engage with their environment, as they are equipped with sensory receptors and stinging capsules (called nematocysts (Reft and Daly, 2012)) used to capture (Houlbrèque et al., 2015; Muscatine, 1973) and pass on zooplankton (Price and Patterson, 2023) and for competition (Yosef et al., 2020). Ciliary beating can also help to deliver captured prey to the mouth and establish microcurrents for nutrient and oxygen exchange (Pacherres et al., 2022; Shapiro et al., 2014). Corals can also protrude mesenterial filaments, long coiled strands attached to the walls of the gastric cavity, which can secrete digestive enzymes for extracoelenteric digestion (Raz-Bahat et al., 2017). Some coral species specialise in catching food with mucus sheets (Bythell and Wild, 2011).

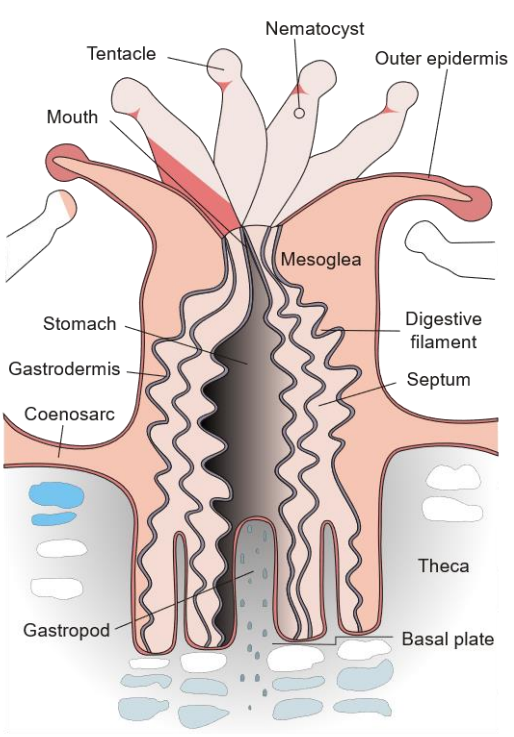

Figure 2: Schematic representation of the anatomy (cross-section) of a coral polyp. NOAA (2014), ® Public Domain

Coral colonies provide a refuge for various microorganisms (e.g., bacteria, archaea, fungi, endoliths, viruses, and protists) that inhabit the

coral tissue, mucus, and skeleton (Peixoto et al., 2021). These microbiota are vital for the functioning of the coral, and together, they form a holobiont metaorganism (Puntin et al., 2022; van Oppen and Raina, 2023).

Regarding nutritional needs, Porter (1976) hypothesised that corals with smaller polyps and shorter tentacles rely more on their symbiotic partners rather than being carnivorous and nourish heterotrophically. However, it is also species strategies, such as the type of hunting (tentacles vs. mucus) and diurnal activity (day vs. night), and habitat conditions, such as light availability, that tip the scales in favour of a more heterotrophic or autotrophic energy source (Einbinder et al., 2009; Sorokin, 1995b). As mixotrophs, symbiotic corals draw energy from both sources to varying degrees, known as trophic plasticity (Fox et al., 2019; Sturaro et al., 2021). Symbiodiniaceae are nitrogen-limited *in hospite* and cannot utilise much of the photosynthetically fixed carbon for their growth (Krueger et al., 2020). The density of Symbiodiniaceae in gastrodermal cells varies interspecifically and intraspecifically, but usually because of the nutritional state of the coral (Cui et al., 2023, 2022; McIlroy et al., 2022). The hermatypic coral hosts not only benefit from the photosynthates obtained to the extent that they can cover most (up to 100%) of their daily energy requirements (Grottoli et al., 2006; Muscatine et al., 1981), but also as they feed on excess symbiont cells (Wiedenmann et al., 2023).

The functioning of an intact symbiosis between corals and Symbiodiniaceae is limited by the water temperature, with corals from warmer locations having higher upper thermal limits than corals from colder latitudes (Evensen et al., 2021; Grottoli et al., 2017; Krueger et al., 2017). Excessively high temperatures disrupt the symbiosis, which becomes parasitic (Baker et al., 2018; Morris et al., 2019; Rädecker et al., 2021). Corals then expel their dinoflagellates and turn white, known as coral bleaching (Boilard et al., 2020; Suggett and Smith, 2020). This process can be reversible (Puntin et al., 2023; Scharfenstein et al., 2022), and hermatypic corals can temporarily survive bleaching, but if temperatures do not drop in time, they starve to death (Allen-Waller and Barott, 2023; Matsuda et al., 2020; Plass-Johnson et al., 2015).

1.3.4 Anthropogenic pressures on corals

The sharp increase in pressures on coral reefs goes along with a growing human population and its steeply rising CO_2 footprint. Almost all pressures coral reefs face are of human origin

and can be categorised as local or global stressors. Globally, coral reefs are confronted with climate change, entailing rising temperatures (Hughes et al., 2018b; McManus et al., 2020), ocean acidification (Comeau et al., 2019; Hill and Hoogenboom, 2022; Martins et al., 2022), increased frequencies and intensities of catastrophic events such as storms (Baird et al., 2018), other weather anomalies (e.g., El Niño and localised heat waves) (Leggat et al., 2019; McClanahan et al., 2019), and rising sea levels are yet to come (Perry et al., 2018). At the local level, pressures result from human activities in or near the reef. In varying intensities and combinations, those local pressures are unsustainable fishing (e.g., overfishing and dynamite fishing) (Hampton-Smith et al., 2021; Loh et al., 2015), pollution (e.g., eutrophication and litter) (Duprey et al., 2016; Guan et al., 2020; Mulochau et al., 2020), increased sedimentation (e.g., riverine inputs and dredging) (Bartley et al., 2014; Erftemeijer et al., 2012; Tuttle and Donahue, 2022), and introduced species (Anton et al., 2019; Creed et al., 2020). Outbreaks of corallivorous crown-of-thorns starfish (*Acanthaster planci*) are natural events *per se* but may be fostered by climate change (Fabricius et al., 2010; Uthicke et al., 2015).

Besides mass coral bleaching and mortality due to these pressures, weakened corals are more susceptible to diseases (Howells et al., 2020; Maynard et al., 2015; Miller and Richardson, 2015). Also, sublethally affected corals often exhibit lower growth and energy reserves (Kornder et al., 2018; Leinbach et al., 2021). For example, heat stress, accompanied by a decrease in symbionts, can change the ratio of coral energy reserves (Leinbach et al., 2021; Schoepf et al., 2015; Wall et al., 2019).

Resilient corals may have been exposed to anthropogenic stressors for decades and may have had sufficient time and capacity to acclimatise or adapt to the changed environmental conditions (e.g., increased heatwaves (Brown et al., 2023; Fox et al., 2021; Hughes et al., 2021)), considering corals' general ability for plasticity (Coles et al., 2018; Edmunds and Gates, 2008). However, the number of reef-building coral species that might avoid their predicted demise in this way is unclear and might be small given the unprecedented pace of current change (Hoegh-Guldberg, 2014). Anthropogenic stressors aside, corals exhibit a certain phenotypic plasticity depending on their local environmental conditions (e.g., temperature, depth, salinity, or water flows), which may be reflected in morphological and physiological variations (e.g., growth rate and form, calcification, heterotrophic feeding rates, and respiration) (Hoogenboom et al., 2008; Smith et al., 2007; Todd, 2008). While the term acclimation is reserved for phenotypic plasticity to new experimental conditions, acclimatisation refers to phenotypic plasticity to new environmental conditions *in situ*, and

adaptation describes genetically manifested phenotypic changes across generations (Hackerott et al., 2021; Putnam, 2021). Indeed, rapid environmental change may not be a good fit for long-lived coral hosts, but their short-lived symbionts may be better candidates for real-time plasticity in the holobiont (Chakravarti et al., 2017; Putnam, 2021; Putnam et al., 2017). Acclimatisation may be controlled by modulated gene expression (e.g., epigenetic gene regulation) (Hackerott et al., 2023; Kenkel and Matz, 2016; Rivera et al., 2021). Examples of rapid coral acclimatisation may be turbidity, where it became apparent in just four weeks (Padilla-Gamiño et al., 2012), and non-native reef conditions, where corals acclimatised after transplantation in three to eighteen months (Barott et al., 2021; Baumann et al., 2021; Marhoefer et al., 2021). Acclimatisation to heat stress is considered limited, as tropical corals already live at their upper thermal limit, but prior heat exposure may initiate "environmental memory" for subsequent stress mitigation (Drury et al., 2022; Hackerott et al., 2021). As initially mentioned, resilience may result from acclimatisation or even adaptation to stressors, as corals from fished reefs may be less susceptible to bleaching events, albeit at the cost of lower coral cover and diversity (Darling et al., 2013). Trophic plasticity (as described in Chapter 1.3.3), triggered by acclimation or acclimatisation to stressors such as heat, sedimentation, and ocean acidification, might dampen these pressures to some extent (Anthony and Fabricius, 2000; Grottoli et al., 2006; Towle et al., 2015).

Certain traits may predict the persistence of coral species, making them "winners" or "losers" of environmental degradation when a modified version of Grime's CSR theory is applied (Darling et al., 2012; Grime and Pierce, 2012). Therein, four major life history strategies of reef corals were identified: "competitive", "weedy", "generalist", and "stress-tolerant". Competitive coral species (e.g., many Acroporidae and Pocilloporidae) are often dominant in cover but susceptible to stress and may decline upon climate change (Birkeland, 2015b). Massive growth forms (e.g., many Poritidae) and "weedy" species are typically stress-tolerant and might increase in cover (e.g., *Porites astreoides*, *Porites lobata*, *Dipsastraea favus*, and *Tubastraea coccinea*), while others (e.g., *Platygyra lamellina*) might decline (Darling et al., 2013; Levas et al., 2013; Luz et al., 2020; Shlesinger and van Woesik, 2021). Currently, there is no trait-based classification of *H. coerulea* or species of the family Helioporidae, but the tolerance of *H. coerulea* and its relatives to stress (Courtney et al., 2021; Reimer et al., 2021; Reverter et al., 2021) suggests affiliation with the stress-tolerant category.

Although the aspects mentioned above of climate change are unequally severe for coral communities, the simultaneous occurrence of most of the stressors poses the main threat to

the survival of tropical coral reefs (Cornwall et al., 2021; Ellis et al., 2019; Klein et al., 2022). Multiple stressors can act cumulatively, increasing the likelihood that physiological thresholds will be exceeded, beyond which corals cannot survive or recover, leaving remnants of former reef stocks (Ortiz et al., 2018; Setter et al., 2022) in remaining refugia (McClanahan et al., 2024; Winslow et al., 2024). To cope with this predicted future scenario, scientists are developing measures for viable coral reefs (DeFilippo et al., 2022; Voolstra et al., 2021). These include approaches such as restoration efforts (Shaver et al., 2022; van Oppen et al., 2017) and high-temperature resistant so-called "super corals" (Darling and Côté, 2018), which are naturally selected or artificially manipulated corals (i.e., by selective coral breeding (Humanes et al., 2021; Quigley, 2024) or inoculation with heat-resistant Symbiodiniaceae (Buerger et al., 2020)). Identifying measures for coral reef protection can be complicated because the best measure may depend on the presence or absence of rapid coral acclimatisation or adaptation (Walsworth et al., 2019).

1.3.5 Plastic pollution in coral reefs

Ocean pollution with insoluble solids can be mainly attributed to plastics and microplastics (Morales-Caselles et al., 2021). Because of their floatability, the most common plastics are ubiquitous, including in remote reefs where they can pose a threat (Lamb et al., 2018; Pinheiro et al., 2023). Macroplastics (plastics > 1 cm (Hartmann et al., 2019)) can cover coral colonies (Chapron et al., 2018; Mouchi et al., 2019) or, paradoxically, corals may benefit from the new floating growth substrate by drifting as stowaways (Bergami et al., 2021; Carugati et al., 2021), including, unfortunately, also invasive species (Mantelatto et al., 2020; Soares et al., 2023a). Lost fishing gear (i.e., synthetic nets and lines) can be a typical macroplastic in coral reefs (Mulochau et al., 2020), affecting reef corals by shading or breaking off branches (Mueller and Schupp, 2020; Valderrama Ballesteros et al., 2018; Ying et al., 2021).

Microplastics are plastic particles between 1 µm and < 1 mm (Andrady, 2015; Browne et al., 2007; Hartmann et al., 2019) or up to 5 mm, according to another definition (Frias and Nash, 2019; Moore, 2008). The controversy over the size definition is problematic as it may affect comparability between studies and is more relevant for the next larger category of mesoplastics (1 to < 10 mm), as the upper limit of 5 mm classifies most mesoplastic particles as microplastics. Microplastic particles are found in different concentrations in reef waters (< 0.01–717 particles L^{-1} (references in Table 1), mean ± SD: ~24 ± 78 particles L^{-1} (calculated

from data in Table 1)), but also in reef sediments (Patti et al., 2020; Soares et al., 2023b; Utami et al., 2021). Outside the reef, but in the immediate vicinity, microplastic concentration can be up to 76,000 particles L^{-1} (Badylak et al., 2021). The considerable variation in the concentrations of microplastic particles may not only result from sampling sites distributed around the world but may also be amplified by different sampling methods (e.g., net vs. grab samples; see Table 1) (Watkins et al., 2021). Especially in net samplings, mesh sizes were often used that cannot represent the entire size spectrum of microplastic particles, which probably led to underestimated concentrations (Conkle et al., 2018; Koelmans et al., 2020; Lindeque et al., 2020), as microplastics with a typical size median of 20 µm are well below commonly used mesh sizes of ~300 µm (Everaert et al., 2018; Koelmans et al., 2022; Mintenig et al., 2018). Koelmans et al. (2020) recently provided correction factors to scale concentrations from studies with different upper or lower particle size limits to the full microplastic size range. Unlike net sampling, grab sampling only collects small volumes of water, so large microplastic particles may be underestimated because of their low abundance (Tamminga et al., 2019). However, underestimation of larger particles is likely to be less of an issue due to the small number of particles in the upper size range of microplastics compared to the much larger number in the lower size range (Kooi and Koelmans, 2019). Although current microplastic loads in some reefs do not reach these peak levels (see Table 1), increasing concentrations are predicted (i.e., 48- to 54-fold by 2100; (Everaert et al., 2018; Koelmans et al., 2017a, 2017b)), although this has yet to be confirmed *in situ* (Galgani et al., 2021).

Various additives may be added during plastic production to improve physical properties (Fink, 2010), but these additives can leach into the environment and cause harm (Andrade et al., 2021; Hermabessiere et al., 2017). Plastic additives were found in reef sediments (Vered and Shenkar, 2023), waters (Ranjbar Jafarabadi et al., 2021b), and organisms (Giametti and Finelli, 2022; Montano et al., 2020; Saliu et al., 2019), which may be correlated to the environmental plastic concentration (Saliu et al., 2019; Vencato et al., 2024).

The polymer types found in marine waters are blends of the thermoplastics polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyamide (PA), and polyester (PEST) (including polyethylene terephthalate (PET)), but also artificially regenerated cellulose (e.g., rayon and cellophane) (Miller et al., 2023, 2020). PP and PE usually comprise the largest share (Erni-Cassola et al., 2019; Garcés-Ordóñez et al., 2021; Nunes et al., 2023), including in reef waters (Jeyasanta et al., 2020; Patterson et al., 2022, 2020; Saliu et al., 2018; Zhang et al., 2020). There, microplastics can be commonly found as spheric or irregular

particles, fibres, and films. Animals at the lower levels of the ecological pyramid (i.e., primary and secondary consumers) may be particularly susceptible to the ingestion of passively floating small-sized microplastics (Miller et al., 2023; Porter et al., 2023b; Walkinshaw et al., 2020). Therefore, it has been hypothesised that trophic transfer might pass microplastics through the trophic levels (Carbery et al., 2018). This trophic transfer has been shown in other species (Farrell and Nelson, 2013; Hasegawa and Nakaoka, 2021; Setälä et al., 2014), but direct evidence for corals has yet to be provided, and biomagnification in biota beyond technically possible trophic transfer is also still controversial (Gouin, 2020; Miller et al., 2020).

Typical microplastic polymers, such as the polyolefins PE and PP (density ρ: 915–960 and 900–915 kg m^{-3} (Lechner, 2005)), have a lower density than tropical seawater (density ρ: 1023 ± 1 kg m^{-3}, calculated with the `swRho` function of the `R` package `oce` (Kelley and Richards, 2023)), so their buoyancy allows them to float. Over time, a biofilm forms on the surface of the particles, which can increase their weight, allowing them to float to greater depths or eventually sink to the seabed (Coyle et al., 2020; Li et al., 2023; Mendrik et al., 2023). By then, microplastics are usually drifted by ocean currents and thus reach even the most remote areas (Wichmann et al., 2019). However, long-distance horizontal transport for polymers with a higher density than seawater (e.g., PS, PVC, most PAs, and PET) remains largely unresolved. Those negatively buoyant polymer types in remote marine areas may be caused by floating macroplastics, such as bottles or other air-filled hollow objects that break down locally into microplastics after drifting. Andrady (2022) instead surmised that weathering at the water surface may be too low for macroplastics to break down into microplastics and suggested beaches as a site of weathering-induced degradation from where microplastics might be washed into the sea. In long-term *ex situ* weathering experiments simulating beach conditions, only fragmentation into very small microplastics (< 2 µm) (Reineccius et al., 2023) and primarily small microplastics (PE ~86%: < 100 µm) occurred (Song et al., 2017). Degradation rates of up to ~470 µm $year^{-1}$ were estimated for PVC during 12 months of *in situ* weathering in seawater that exceed previously published rates of 4.5–22 µm $year^{-1}$ for high-density PE (Chamas et al., 2020), although only nanoplastics (< 1 µm) formed (Carbery et al., 2023). However, our understanding of the formation of larger microplastics through these processes is still in its infancy.

The effects of microplastics on coral reefs are still largely obscure. Microplastics in reef sediments may be partially taken up by sand-digging animals (Porter et al., 2023a) or may be overgrown by reef-building species (Reichert et al., 2018). Microplastics suspended in reef

Table 1: Microplastic concentrations in shallow coral reef waters. Values were rounded if necessary. NP = not provided in the reference. grab = water sampling with catch tank.

Microplastic particles (n L^{-1})			Method	Mesh size (µm)	Nearest country	Reference
Min	Max	Mean				
0.00001	0.0002	0.00006	net	80	Colombia	(Portz et al., 2020)
0.00001	0.0002	0.00006	net	333	Philippines	(Tan et al., 2020)
0.00007	0.0002	0.0001	net	> 200	Bangladesh	(Al Nahian et al., 2022)
0.00004	0.0005	NP	net	355	Australia	(Jensen et al., 2019)
0.0003	0.0005	NP	net	80	Sri Lanka	(Sevwandi Dharmadasa et al., 2021)
0.00001	0.0010	0.0001	net	355	Australia	(Miller et al., 2022)
0.00009	0.0002	0.0001	net	125	Fiji	(Ferreira et al., 2020)
0.0001	0.0004	0.0002	net	300 & 50	Australia	(Carbery et al., 2022)
0.00009	0.0006	0.0003	net	335	Vanuatu & Solomon Islands	(Bakir et al., 2020)
0.00002	0.0005	0.0003	net	200	Maldives	(Saliu et al., 2018)
0.00015	0.0008	0.0005	net	160	Philippines	(Wang et al., 2019)
0.00025	0.0008	0.0005	net	100	Israel & Jordan	(Vered and Shenkar, 2023)
0.00004	0.0031	0.0011	net	100	Tonga	(Markic et al., 2022)
0.00147	0.0127	0.0047	net	75	Australia	(Miller et al., 2023)
NP	NP	0.007	net	160	China	(Liang et al., 2023)
0.00238	0.0205	0.0098	net	300	Sri Lanka	(Hansani et al., 2023)
0.01	0.09	0.05	net	100	Palau	(Béraud et al., 2022)
0	0.16	NP	grab	20	China	(Liu et al., 2022)
0.2	0.6	NP	grab	50	China	(Zhang et al., 2020)
1.25	3.2	1.7	grab	48	Philippines	(Nie et al., 2019)
1.4	8.1	4.9	grab	50	Philippines	(Huang et al., 2019)
3	7	5	grab	20	India	(Ragesh et al., 2024)
1	12.2	6.1	grab	0.45	China	(Ding et al., 2019)
6.2	14.6	9.5	grab	0.45	China	(Zhou et al., 2022b)
3.3	46.6	NP	grab	0.45	China	(Zhou et al., 2023)
1	96	10.0	grab	125	Thailand	(Vibhatabandhu and Srithongouthai, 2022)
15.5	22.14	18.4	grab	0.45	China	(Lei et al., 2021)
NP	NP	57.2	grab	1.6	USA	(Wightman and Renegar, 2023)
NP	NP	96.0	net	333	India	(Jeyasanta et al., 2020)
28.4	126.6	NP	net	333	India	(Patterson et al., 2022)
60	126.6	NP	net	100	India	(Patterson et al., 2020)
180	717	389.3	net	330	Malaysia	(Yusof et al., 2023)

waters, either during their sinking or floating, may be available to a broader range of reef-associated species. Possibly also for this reason, the uptake of microplastics or their leachates and, in a few cases, subsequent adverse effects have been found in several reef-associated species: from small foraminifera (Joppien et al., 2022; Zientek et al., 2024) over porifera (Fallon and Freeman, 2021), gastropods (Seuront, 2018), echinoderms (Rahmawati et al., 2023), mussels (Aranda et al., 2022; Arossa et al., 2019; Zhou et al., 2022a), alcyonarian corals (Montalbetti et al., 2022; Vencato et al., 2021), and crustaceans (de Lemos Santana et al., 2022; Miller et al., 2023), to fish (Huang et al., 2023; Nie et al., 2019; Santana et al., 2021) and sea turtles (Caron et al., 2018; Duncan et al., 2019). However, given the species richness of coral reefs, only a few species and even fewer families and orders have been tested. The next chapter (1.3.6) focuses on the effects of microplastics on reef-building corals.

1.3.6 Corals in the face of microplastic pollution

Because of the presence of microplastics and corals' innate striving for planktonic food, encounters with microplastics have been observed to occur due to confusion with food (i.e., by capture with their tentacles or mucus sheets (Hall et al., 2015)) but also adhesion to the coral surface (Corona et al., 2020; Martin et al., 2019; Rocha et al., 2020). Corals can suffer the aftermath of such exposure in several ways: Ingestion (Allen et al., 2017; Axworthy and Padilla-Gamiño, 2019; Hall et al., 2015), which is facilitated by particle sizes that often fit the calyxes of coral polyps (Hankins et al., 2022), and lower feeding rates (Corinaldesi et al., 2021; Rotjan et al., 2019; Savinelli et al., 2020) can lead to reduced growth (Chapron et al., 2018; Hankins et al., 2021; Reichert et al., 2019) and symbiont's photosynthesis (Lanctôt et al., 2020; Mendrik et al., 2021; Reichert et al., 2019), and a weakened immune system (Liao et al., 2021; Tang et al., 2024, 2018; Xiao et al., 2021). In addition, particle overgrowth (Reichert et al., 2018) and skeletal incorporation (Hierl et al., 2021; Krishnakumar et al., 2021; Reichert et al., 2022) can occur, which may decrease skeletal hardness (Matijaković Mlinarić et al., 2024). Skeletal incorporation also led to considering corals as biological sinks (Reichert et al., 2022) that complement abiotic sinks (de Smit et al., 2021). Sometimes, conspicuous signs of coral health impairment, namely bleaching and necrosis, have been observed (Reichert et al., 2018; Syakti et al., 2019).

These adverse effects are thought to result from feigned satiety or blockage of the gastrovascular cavity (Corinaldesi et al., 2021; Rotjan et al., 2019; Savinelli et al., 2020). Other

explanations include the transfer of pathogens from the microplastic biofilm, also called "plastisphere" (Amaral-Zettler et al., 2020; Bowley et al., 2021; Zhou et al., 2024), to the coral (Rotjan et al., 2019). Further explanatory approaches are toxic chemicals such as plasticizers (Montano et al., 2020; Ranjbar Jafarabadi et al., 2021a), flame retardants (van der Schyff et al., 2021), and heavy metals (Patterson et al., 2020) leaching from plastic (Saliu et al., 2019). Thereby, the transfer and thus the effect may increase with prolonged handling times (C.-Y. Chen et al., 2022), which might meanwhile impede foraging on nutritious prey (Reichert et al., 2019). The most likely cause of the adverse effects may be a combination of these mechanisms.

The range of coral impairment can be wide, ranging from unaffected (Bejarano et al., 2022; Boodraj and Glassom, 2022; Plafcan and Stallings, 2022) over minor species-specific changes all the way to mortality (Reichert et al., 2018; Syakti et al., 2019). Remarkably, adverse effects appear to be related not only to species characteristics (Mendrik et al., 2021; Mouchi et al., 2019; Reichert et al., 2019) but also to the particle concentration (Plafcan and Stallings, 2022; Xiao et al., 2021) and the duration of exposure (C.-Y. Chen et al., 2022; Lanctôt et al., 2020).

The duration of exposure is an essential part of the experiment, as the ability of corals to adapt their behaviour to long-term exposure to microplastics and other stressors is virtually unknown. Acclimation in the form of increased feeding rates was reported after two months of sedimentation (Anthony and Fabricius, 2000) and ocean acidification (Towle et al., 2015). In contrast, Mouchi et al. (2019) reported impaired prey capture in the azooxanthellate *Lophelia pertusa* after five months of microplastic exposure, suggesting a lack of behavioural acclimation. However, it is unknown whether corals adapt their feeding rates on microplastics or natural food after long-term exposure.

Little is known about corals' strategies to counteract or mitigate microplastics. An indirect countermeasure would be increased heterotrophy to compensate for potential energy losses, as Zhou et al. (2023) explained. However, this may then again inadvertently foster microplastic particle capture. Direct countermeasures must intervene at the first point: with detection and defence on contact. As an example of natural particles, sessile suspension feeders come into contact with floating plankton and sediments, whereby the latter are usually not ingested but rejected (Duckworth et al., 2017), as corals can recognise nutritious prey (see Chapter 1.3.3). To free themselves from unwanted particulate matter, corals can use cilia beat, mucus production, tissue pulsing, and extrusion of mesenterial filaments (Erftemeijer et al., 2012; Jones et al., 2016). Some corals react similarly when encountering

microplastics (Lanctôt et al., 2020; Martin et al., 2019; Mouchi et al., 2019; Reichert et al., 2018). In pure sediments, feeding triggers released by prey when struck by fired nematocysts (see Chapter 1.3.3) are absent, and it is unclear why microplastics can trigger feeding reactions. One assumption is that either chemicals leached from microplastics act as triggers (Allen et al., 2017) or that the trigger originates from the biofilmed particle surface, as biofilmed particles are sometimes preferred (Corona et al., 2020; Reichert et al., 2024a; Weideman et al., 2020). Also unclear is corals' "preference" when comparing prey vs. microplastic ingestions. The decisive factor could be species specificity or concurrent food supply, as a higher degree of heterotrophy *per se* and the presence of natural food may increase microplastic ingestion (Axworthy and Padilla-Gamiño, 2019; Reichert et al., 2024a; Savinelli et al., 2020). If identified as errant capture, microplastics are tried to get repelled or egested (Allen et al., 2017; Hankins et al., 2018; Reichert et al., 2018). However, prior handling times can last several hours, and microplastics' nutritional yield may be negligible (Murphy and Quinn, 2018; Reichert et al., 2019, 2018).

This potential nutrition deficiency led to the suggestion of reduced coral energy budgets (Chapron et al., 2018; Mouchi et al., 2019; Reichert et al., 2019), although this does not appear to be the case for lipids (Boodraj and Glassom, 2022; Mouchi et al., 2019) and proteins (Lanctôt et al., 2020; Rocha et al., 2020), and data on carbohydrates is lacking.

In contrast to the adverse effects on various aspects of coral physiology and behaviour described earlier in this chapter, several studies have found no changes in indicators of the state of the coral symbiosis, including symbiont density (Boodraj and Glassom, 2022; Lanctôt et al., 2020; Ng and Todd, 2023; Plafcan and Stallings, 2022; Reichert et al., 2019; Tang et al., 2021, 2018) and chlorophyll content (Boodraj and Glassom, 2022; Isa et al., 2024; Lanctôt et al., 2020; Tang et al., 2021). In addition, unchanged enzyme activity was found (Grillo et al., 2021), as well as unchanged coral growth (Boodraj and Glassom, 2022; Lanctôt et al., 2020), behaviour (Isa et al., 2024), and necrosis (Reichert et al., 2024b). Furthermore, one study reported decreasing effects over time (Reichert et al., 2019), and some even reported mitigating (Reichert et al., 2021) and positive effects (i.e., volume growth (Reichert et al., 2024b)). These sometimes conflicting and ambiguous findings highlight a complex situation that coral research on microplastics must now address.

In general, the effects of stressors and their impact on organisms inevitably depend on the study design, as the exposure conditions are likely to influence the outcome strongly. For example, the choice of species and stressors, their concentration and duration of exposure,

and a sensible choice of biological parameters to be tested are essential. Different scientific approaches exist to assess the effects of microplastics on corals, which can be, *inter alia*, categorised according to the duration (i.e., short-term vs. long-term), the number of stressors (i.e., single vs. multiple), and the number of concentrations used (i.e., single vs. multiple). Ideally, all combinations should be tested, but practical limitations (e.g., laboratory capacity) force scientists to limit the experiments sensibly. In addition, the duration of the experiment must be adapted to the species traits to be measured. In corals, for example, growth parameters cannot be meaningfully determined in short-term experiments because of their relatively slow growth. Furthermore, keeping corals in captivity (i.e., in aquaria) can be delicate, so long-term *ex situ* experiments with corals carry the risk of partial or complete failure of the study system and its corals. Accordingly, only four laboratory studies by one research group (i.e., the Systematics & Biodiversity Lab, JLU Giessen) have conducted long-term experiments on corals and microplastics, two of which are presented in this thesis. All other long-term studies to date have used corals already exposed to microplastics *in situ*.

Currently, no universally accepted classification of the specific duration of short-term, intermediate, and long-term exposures exists. Therefore, durations are defined here as follows: < 1 month = short-term, 1–5 months = intermediate, and ≥ 6 months = long-term. Microplastic exposure in the ocean can be assumed to be permanent; thus, *in situ* studies using already present microplastics are referred to as long-term exposure. This classification is applied to the published studies, as shown in Figure 3. Short-term studies are overrepresented within the three categories of study duration, as the other two categories only reach a similar total when combined (Figure 3A). Over the years, there has been an increase in the share of long-term experiments, which started as non-existent and now account for almost half of the annual publications. Overall, *ex situ* studies predominate the study locations (Figure 3B), whereas *in situ* studies outnumber laboratory studies when only long-term experiments are considered (Figure 3C). This latter pattern is expected because of the higher expenditures and risks associated with long-term *ex situ* studies. However, these long-term *ex situ* studies also have advantages such as a negative control group, controlled conditions (e.g., microplastic concentration), and a more feasible before-and-after comparison. Herein, these advantages were combined with long-term exposure to provide a holistic understanding of the effects of microplastic stress on reef-building corals.

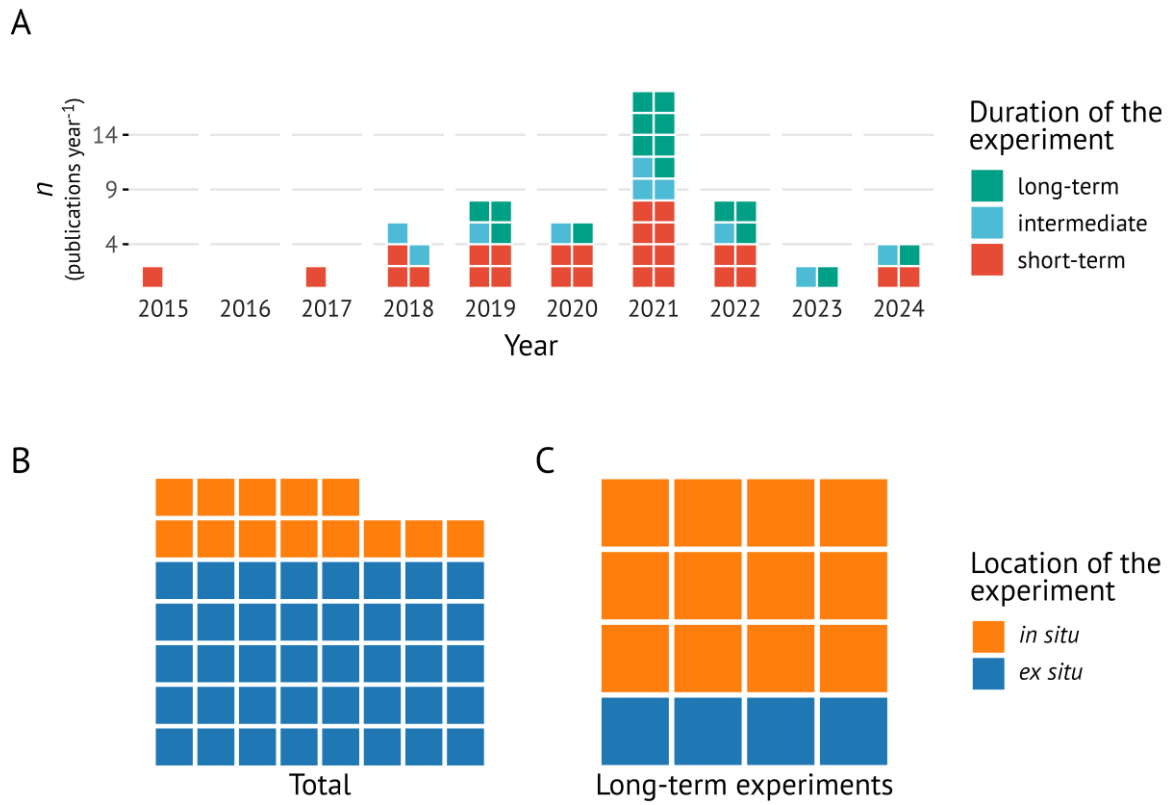

Figure 3: Studies on corals exposed to microplastics, shown as coloured squares. **A** The colour scheme shows the duration of microplastic exposure as defined above in the main text, and studies are ordered by year of publication. **B** All studies, colour-coded by location (i.e., *in situ* and *ex situ*). **C** Only the long-term studies are shown with a colour code according to their location. For the underlying data set, see Chapter 3.2. Graphic created with `R` (R Core Team, 2022) and the packages `ggplot2` (Wickham, 2016) and `waffle` (Rudis and Gandy, 2023).

1.3.7 Scope and aims

Based on the current state of research and its knowledge gaps, this doctoral thesis aims to understand better how microplastics at realistic concentrations affect coral behaviour and physiology in the long term. Only sound knowledge of the role of microplastics as a coral stressor grants the ability to prioritise tailored conservation measures for global reefs, which are already at a crossroads. Instead, our understanding is hampered by unknown coral behavioural adaptions to avoid microplastics in the long term. Therefore, the first part of the thesis (Chapter 2.1) had the general **aim**

(I) to assess whether corals change their behaviour after long-term microplastic exposure and, if so, to determine the mechanisms involved.

Specifically, changes in feeding behaviour (i.e., heterotrophic plasticity), particle discrimination (i.e., ingestion of microplastic particles per natural food particle), and frequency of defence reactions (i.e., presence of mucus production and mesenterial filaments) were determined. To this end, coral behaviour and mechanisms were tested in a pulse exposure experiment following long-term exposure to microplastics in a controlled aquarium experiment and compared to a control group.

The complex situation of current knowledge, which shows a mixed picture of the effects of microplastics on corals, needs to be addressed to deepen the understanding of how microplastics affect coral physiology in the long term. In addition, the absence of acclimation in coral behaviour suggested physiological implications. Therefore, the second part of the thesis (Chapter 2.2) had the general **aim**

(II) to assess the chronic effects of microplastic exposure by determining changes in vital parameters of coral physiology using different species long-term exposed to microplastics.

Specifically, changes in coral energy reserves (i.e., lipid, carbohydrate, and protein levels), metabolites (i.e., amino acid levels), growth (i.e., in surface area, volume, and calcification), and photosymbiont state (i.e., density, chlorophyll content, and photosynthetic efficiency) were determined. For this, coral growth parameters were determined first, and then tissues were separated from their skeletons after long-term exposure to microplastics. The coral host and Symbiodiniaceae material were analysed individually for a wide range of key physiological parameters. Parameters of long-term exposed corals were compared with those of a control group.

1.4 Publications outlined

This cumulative dissertation is based on two scientific research articles published or accepted for publication in renowned peer-reviewed scientific journals. The topic of the dissertation was taken up in the first article with studies on behavioural reaction mechanisms to microplastics and—building on these first findings—continued in the second article with studies on the physiological effects of microplastics. The first article focuses on behavioural changes in corals after long-term exposure to microplastics (**Aim I**) and the second on the effects of chronic microplastic exposure on coral physiological parameters to address the mixed picture of microplastic effects on coral physiology (**Aim II**).

—Research article I—

Rades M, Schubert P, Wilke T and Reichert J (2022) Reef-Building Corals Do Not Develop Adaptive Mechanisms to Better Cope With Microplastics. Front. Mar. Sci. 9:863187. DOI: 10.3389/fmars.2022.863187

The full article can be found in Chapter 2.1.

Contributions of the author of the dissertation:

Formal Analysis	Statistical analysis
Investigation	Data collection
Methodology	Contributed to development of methodology
Software	Programming, implementation of the computer code
Visualization	Data presentation
Writing – Original Draft	Writing initial draft

Outline—The first paper explores potential long-term effects on reef-building corals' feeding and defence behaviour to test whether they show acclimation mechanisms to cope with current microplastic exposure. Four common reef-building coral species (*Acropora muricata*, *Porites lutea*, *Pocillopora verrucosa*, and *Heliopora coerulea*) were divided into two groups exposed to microplastics or control conditions for 15 months in a controlled aquarium experiment. After exposure, coral feeding rates on microplastics and natural food (*Artemia* sp. cysts), particle feeding discrimination, and coral reactions were assessed during a 24-hour pulse exposure experiment. Thereby, a novel method was successfully implemented that enabled the consistent provision of microplastics and natural food to corals. Feeding rates were determined by comparing particle numbers in the feeding chambers before and after. Thereto, a code-based method for automatically counting large numbers of microplastic particles was developed and successfully applied. Particle discrimination was calculated from feeding rates on microplastics and natural food. Coral reactions were recorded visually at the end of the pulse exposure. Coral tissue surface areas were precisely measured using the latest 3D scanning techniques to compare corals of different sizes.

The results showed unaltered coral feeding rates despite long-term exposure to microplastics. Likewise, coral feeding discrimination between microplastics and natural food (i.e., fed microplastic particle per fed natural particle) was unchanged, too. Also, corals' defensive behaviour (i.e., mucus production and extrusion of mesenterial filaments) did not

change despite long-term exposure to microplastics. Overall, the lack of adaptive behavioural mechanisms against microplastics suggested adverse effects on corals, calling for research on the physiological effects of microplastics on corals.

—Research article II—

Rades M, Poschet G, Gegner H, Wilke T, Reichert J (2024) Chronic effects of exposure to polyethylene microplastics may be mitigated at the expense of growth and photosynthesis in reef-building corals. Mar. Pollut. Bull. 205:116631. DOI: 10.1016/j.marpolbul.2024.116631
The full article can be found in Chapter 2.2.
Contributions of the author of the dissertation:

Formal Analysis	Statistical analysis
Investigation	Contributed to data collection
Methodology	Contributed to development of methodology
Project administration	Contributed to research activity planning and execution
Resources	Contributed to specific materials
Visualization	Data presentation
Writing – Original Draft	Writing initial draft

Outline—The second article builds on the first by focusing on whether realistic microplastic concentrations affect vital parameters of coral physiology, as corals have not developed behavioural mechanisms against microplastics. It also addresses the contradictory picture of microplastic effects on corals, ranging from detrimental to no effects. After eleven months of long-term exposure to microplastics, corals of four common species (*Acropora muricata*, *Porites lutea*, *Pocillopora verrucosa*, and *Heliopora coerulea*) were removed from the experiment and tested for several key physiological parameters in the most comprehensive way to date. Specifically, coral growth, energy reserves, metabolites, and photosymbiont parameters (i.e., symbiont density, chlorophyll content, and photosynthetic performance) were analysed and compared to their counterparts from microplastic-free control conditions. Coral growth was determined by reference class 3D scanning and buoyant weighing. After snap-freezing, the thin tissue from the small coral fragments was separated (by airbrushing) and specially processed to allow all the analyses to be carried out despite the minute amount of material per coral fragment. Coral energy reserves were analysed using spectrophotometric methods, and metabolite data (i.e., a set of amino acids) on microplastic exposure were collected for the first time in relation to microplastic exposure using Ultra

Performance Liquid Chromatography (UPLC). Separated photosymbionts were analysed for density (by counting chamber), chlorophyll content (by spectrophotometer), and photosynthetic performance (by pulse amplitude modulated fluorometry).

These analyses showed that coral energy reserves, symbiont density, and symbiont chlorophyll content did not change after long-term microplastic exposure. Coral growth, the metabolite taurine, and symbionts photosynthetic efficiency showed occasional species-specific changes. In conclusion, long-term microplastic exposure at realistic concentration hardly seems to affect coral physiology, as they may compensate for adverse effects through effective mitigation (i.e., increased taurine levels, reduced growth, or increased photosynthetic efficiency). Therefore, microplastics alone are unlikely to threaten reef-building corals under most current conditions. However, microplastics might contribute to the multiple, mutually aggravating anthropogenic stressors by reducing the intensity of individual stressors required to affect corals.

1.5 Discussion

Corals appeared to be sensitive to microplastics in some, mostly short-term and high-dose exposure experiments (see Chapter 1.3.6), but the long-term consequences of the sometimes adverse physiological and behavioural changes were unclear, especially at realistic concentrations. This knowledge gap hampered the assessment of microplastic stress and led to calls for insight into the long-term effects of realistically dosed microplastics on coral physiology and behaviour. Long-term exposure combined with realistic concentrations fills this gap, ensures the transferability of results to *in situ* conditions, and identifies future research priorities. Accordingly, as part of this doctoral thesis on assessing the long-term effects of microplastic exposure on corals, two complementary studies have been published that provide unprecedented insights into the behaviour, physiology, and acclimation mechanisms of corals long-term exposed to microplastics.

In the first part, behavioural responses to microplastic exposure were identified and assessed by testing corals' capacity for adaptive feeding plasticity, particle discrimination, and defence (**Aim I**). In the second part, as the corals did not show such mitigating mechanisms in their behaviour—suggesting reduced energy reserves—I took the next logical step and analysed key aspects of coral physiology after long-term microplastic exposure to identify chronic effects (**Aim II**). As it turned out, coral physiology was largely unaffected,

although occasional species-specific changes occurred in a few parameters that may indicate physiological mitigation mechanisms (i.e., increased taurine levels, reduced growth, and altered photosynthetic efficiency). Altogether, both studies converge in their overriding aim to deepen the understanding of microplastics' long-term effects on corals and critically extend scientific knowledge on long-term acclimation in corals to microplastics at realistic concentrations. This section synthesises the findings of both publications by integrating their discussion with the latest advances in microplastics and coral research.

1.5.1 Absence of behavioural avoidance despite long-term exposure

Analysis of coral behaviour after long-term exposure to microplastics (**Aim I**) revealed that despite chronic microplastic exposure, corals did not reduce microplastic uptake. Corals failed to acclimate their behaviour in the long term, as microplastic uptake could not be avoided any better. Accordingly, corals did not show mechanisms of heterotrophic plasticity (i.e., up- or downregulation of heterotrophic feeding) in their feeding behaviour, which can help corals under stress to maintain a stable energy balance (see Chapter 1.3.3). This limited behavioural plasticity suggests that as microplastic concentrations increase, ingestion of microplastics may also increase, similar to the ingestion of suspended particulate matter (Anthony, 1999; Anthony and Fabricius, 2000) and sediments (Anthony, 2000). In line with this, Zhou et al. (2023) found a positive correlation between the concentration of microplastics in seawater and the proportion of heterotrophically derived energy in their *in situ* study on *Porites pukoensis*. They attribute this positive correlation to heterotrophic plasticity, which shifts the energy supply towards heterotrophy because of reduced symbiont density. However, maintaining their energy balance in this way may be difficult for corals under future scenarios of increased microplastic pollution, where a shift to heterotrophy might do more harm than good, as increased heterotrophic feeding may be accompanied by increased microplastic ingestion. Nevertheless, their results are consistent with those presented here, as corals do not appear to activate heterotrophic plasticity mechanisms when chronically exposed to microplastics as long as their symbiosis with Symbiodiniaceae is intact.

With or without long-term exposure to microplastics, corals showed higher feeding rates on natural food—a general preference previously confirmed by Axworthy and Padilla-Gamiño (2019), Bejarano et al. (2022), Savinelli et al. (2020), and later by Reichert et al. (2024a,

2024b), where corals were more likely to respond to and ingest copepods or *Artemia* and showed egestion only with microplastic particles. In particular, Reichert et al. (2024a) found in their study on feeding responses that corals do not discriminate on first contact but after handling, which usually leads to rejection with microplastics, in contrast to ingestion with natural food. However, the success of this discrimination process may decline when feeding triggers are present (i.e., natural food or a biofilm on the microplastic particles), and it may be generally lower in species that exhibit high feeding rates on natural food (Reichert et al., 2024a). Nevertheless, coral uptake of microplastics in the absence of additional feeding triggers such as natural food is now confirmed by this study as well as previous and subsequent studies (Corona et al., 2020; Reichert et al., 2024a).

Corals' preference for natural food may be based on successful particle discrimination. However, long-term microplastic exposure did not improve corals' particle discrimination abilities. Discrimination is essential for avoidance and foraging behaviour, but corals chronically exposed to microplastics did not adapt their discrimination process. Discrimination requires sensory cues, i.e., chemical stimuli that trigger feeding or, conversely, their absence, such as in pristine microplastics and sediments. The assumption that their biofilm disguises weathered microplastics is probably why they are still more likely to be ingested than pristine microplastics and sediments (Corona et al., 2020; Reichert et al., 2024a; Weideman et al., 2020), as biofilm may contain small amounts of chemical feeding stimuli. This disguising effect is supported by the longer handling times of biofilmed microplastics compared to natural food, however, most of which result in microplastic rejection rather than ingestion (Reichert et al., 2024a). The capacity for discrimination of microplastics from natural food is reported to be species-specific (Allen et al., 2017; Reichert et al., 2024a; Rotjan et al., 2019). It is likely that those species that rely more heavily on heterotrophic feeding and, therefore, generally have higher feeding rates are less discriminating in their capture and ingestion of microplastics, making them particularly susceptible (Rades et al., 2024; Reichert et al., 2024a).

Defence is an essential part of the behavioural repertoire of corals and is linked to other behaviours, in particular the corals' ability to discriminate, as defensive behaviour depends on successfully identifying threats. This link might explain why defensive reactions (i.e., mucus production and mesenterial filament protrusion) were also observed to be likewise species-specific and more frequent under microplastic exposure in just those species that had the lowest microplastic ingestion rates and were the only ones to exhibit defensive reactions

(i.e., *A. muricata* and *P. lutea*). The observed defensive reactions confirmed previous studies that reported such defensive behaviour under microplastic exposure (S. Jiang et al., 2021; Martin et al., 2019; Mouchi et al., 2019; Reichert et al., 2018). However, corals appear to lack acclimation in their defensive behaviour to chronic microplastic exposure, as they did not change the intensity of their defence (i.e., they did not increase mucus production). One explanation is that active defence may be a double-edged sword, as successful defence is costly and risky because it requires energy expenditure and may hinder the uptake of natural food.

In conclusion, although long-term exposure to microplastics is considered harmful, the corals tested do not use or lack behavioural acclimation (i.e., microplastic avoidance through feeding, discrimination, or defence mechanisms). For reefs, where corals are exposed to microplastics *in situ*, this finding implies that corals may need to find other ways to cope with the stress of microplastic contact and ingestion. Furthermore, the ubiquitous presence of microplastics in reef waters, although often at lower concentrations than in the long-term exposure study presented here, is predicted to increase (see Chapter 1.3.5). Thus, corals are likely to be repeatedly exposed to microplastics and—as we now know—do not adapt their feeding and defensive behaviour to reduce the adverse effects of ingesting microplastics. This new baseline led to the hypothesis that chronic exposure to microplastics may affect coral physiology, which was investigated in a subsequent study as the second part of this dissertation, discussed in the next chapter.

1.5.2 Physiological mitigation mechanisms may emerge in the long term

As behavioural acclimation to chronic exposure to microplastics ceased to be a compensatory mechanism, it was suggested that coral physiology may be affected in the long term. Therefore, I focused on analysing coral physiology after long-term exposure to microplastics (**Aim II**) and found that chronic exposure had little overall effect on coral physiology. However, occasional and species-specific effects may be associated with mitigation of microplastic impacts, as changes in an amino acid, growth, and photosynthetic efficiency may act as compensatory mechanisms.

Coral energy reserves (i.e., lipid, protein, and carbohydrate contents) were unaffected by chronic microplastic exposure, suggesting that realistic microplastic concentrations may not cause an energy deficiency in the long term. This finding is consistent with previous studies

on corals with short-term and intermediate exposure durations that reported unaffected lipid (Boodraj and Glassom, 2022; Mouchi et al., 2019) and protein contents (Lanctôt et al., 2020; Rocha et al., 2020). Energy reserves might be stable because of low microplastic ingestion (see Chapter 2.1 and Reichert et al., 2024a) or avoidance of detrimental microplastic adherence through active particle removal (Corona et al., 2020; Reichert et al., 2018). Other explanations for the unchanged energy reserves of corals might be that a stable energy balance has been achieved at the expense of indirect negative effects such as reduced fertility or immune resistance and that the concentrations of microplastics used might be below the threshold of detectable impairment of energy reserves (C.-Y. Chen et al., 2022). Another essential prerequisite for effective stress mitigation is an intact symbiosis with Symbiodiniaceae, on which reef-building corals are highly dependent (Grottoli et al., 2006; Roth, 2014). When realistic microplastic concentrations cause only minor stress to the coral host (e.g., through handling, adhesion, and ingestion) and its photosymbionts, the then intact symbiosis may have the capacity for upregulation, likely compensating for any adverse effects. However, this picture may change under predicted scenarios of higher microplastic concentrations or under mixtures of different polymers and forms already present.

Chronic exposure to realistic concentrations of microplastics did not affect most of the coral metabolites tested, with only taurine showing a species-specific change, increasing in *A. muricata*. Taurine, as a non-essential and non-proteinogenic amino sulfonic acid, is thought to act as a host release factor in Symbiodiniaceae, initiating the translocation of fixed carbons to the coral host (Huang et al., 2022; Wang and Douglas, 1997). Elevated taurine levels may alter these metabolic processes, allowing the photosymbionts to translocate more energy-rich compounds to their coral host. This potential modification, in turn, suggests that *A. muricata* might require more photosynthetically fixed carbon when chronically exposed to microplastics. However, the biological role of taurine is not yet fully understood. In corals, taurine is also a known osmolyte (Yancey et al., 2010) and is thought to be involved in sulphur recycling (Robbins et al., 2019). Nevertheless, it seems unlikely that microplastics affect the osmoregulation in corals, as the levels of the osmolyte glycine were unchanged.

Chronic exposure to realistic concentrations of microplastics largely unaffected coral growth, although some species-specific changes occurred with reduced surface area growth and calcification in *H. coerulea*. We now know that the energy reserves in *H. coerulea* were unaffected. Thus, the reason for the adverse effects on its growth may lie in its feeding behaviour, and Reichert et al. (2024a) reported that *H. coerulea* is more heterotrophic than the

other species tested and frequently handles microplastics, occasionally leading to ingestion. Therefore, it is likely that *H. coerulea* mitigates the adverse effects of microplastic exposure at the expense of reduced growth. In line with this, Mouchi et al. (2019) reported reduced growth with unchanged lipid content after microplastic exposure in cold-water corals. Furthermore, adverse effects of microplastics on coral growth parameters have also been observed in other corals, such as on the surface area of *Lophelia pertusa* and *Acropora muricata* (Chapron et al., 2018; Reichert et al., 2019), on the surface area and calcification of *Pseudodiploria clivosa* and *Acropora cervicornis* (Hankins et al., 2021), and on the calcification of *Heliopora coerulea* (Reichert et al., 2019). In contrast, other studies reported that microplastic exposure did not affect coral growth (Boodraj and Glassom, 2022; Lanctôt et al., 2020), which is consistent with most of the species tested here, suggesting a species-specific susceptibility to microplastics. Coral mortality was unaffected in any species, but *P. verrucosa* tended to show higher mortality when exposed to microplastics, consistent with previous results (Reichert et al., 2019). This trend suggests that *P. verrucosa* may be particularly sensitive to microplastics and unable to compensate effectively, consistent with previous results (Reichert et al., 2024a, 2024b, 2019, 2018).

The state of coral photosymbionts was largely unaffected by long-term exposure in most parameters (i.e., symbiont density, chlorophyll concentration per tissue surface area, and three out of five photosynthetic efficiency parameters). This result is consistent with other studies that found unchanged symbiont densities (Boodraj and Glassom, 2022; Ng and Todd, 2023; Plafcan and Stallings, 2022; Tang et al., 2021) and chlorophyll concentrations (Boodraj and Glassom, 2022; Lanctôt et al., 2020; Ng and Todd, 2023; Tang et al., 2021). However, other studies found reduced symbiont densities, albeit at much higher microplastic concentrations (i.e., our study: 0.25 vs. 50 mg L^{-1} in S. Jiang et al. (2021) and Xiao et al. (2021)). Microplastic exposure occasionally affected photosynthetic parameters in some species tested. *P. lutea* showed a decrease in light-adapted photosynthetic efficiency ($\Delta F/F'_m$), whereas *A. muricata* showed an increase in the efficiency of light capture (α). A reduced $\Delta F/F'_m$ may lead to a reduction in photosynthetic efficiency in *P. lutea*, as this pattern has been observed in other species as a result of exposure to sediments (Junjie et al., 2014; Philipp and Fabricius, 2003; Piniak, 2007) and microplastics (Mendrik et al., 2021). Finally, reduced photosynthetic efficiency can lead to reduced carbon fixation (Cantin et al., 2007), but in our case, any reductions and their consequences were probably too small and isolated to translate into reduced energy reserves. The increase in α in *A. muricata* may be reflected in increased

photosynthetic efficiency in the light-limited range (*sensu* Ralph et al., 2005; Ralph and Gademann, 2005) and is accompanied by an increase in further photosymbiont parameters (i.e., chlorophyll *a* and total chlorophyll per symbiont cell), and dark-adapted photosynthetic efficiency (F_v/F_m) as well as $\Delta F/F'_m$ tended to be upregulated. All these individual increases resulted in a significant difference between *A. muricata* fragments from microplastic exposure and control conditions at the overall level (i.e., PCA). This overall difference indicates a general change in the photosymbiont status of *A. muricata*, presumably representing a compensatory mechanism, as previously proposed (Bove et al., 2023; Lanctôt et al., 2020; Reichert et al., 2019). As energy reserves were maintained, this compensatory mechanism in *A. muricata* appears to be effective against microplastics.

In conclusion, long-term microplastic exposure only had a minor overall effect on coral physiology despite the previously discovered absence of long-term behavioural acclimation to microplastics. The occasional and species-specific effects that yet occurred at the physiological level provided insights into possible compensatory mechanisms. These mechanisms are likely to help corals alleviate microplastic stress through photosymbiotic upregulations (i.e., of photosynthetic parameters and the taurine level to increase carbon translocation to the coral host) but also cutbacks in coral growth to preserve energy reserves. However, the minor overall effect and effective mitigation depend on current realistic microplastic concentrations (i.e., ≤ 200 particles L^{-1}) at which coral photosymbiosis remains intact. Therefore, adverse effects are likely to be exacerbated under future scenarios of increasing microplastic pollution and other cumulative anthropogenic stressors.

1.5.3 Challenges and prospects of microplastic studies on corals

Microplastic pollution has received considerable scientific attention over the past decade, resulting in a plethora of scientific publications. As a relatively new and interdisciplinary field of research, and because of the complexity of microplastics (i.e., different polymers, sizes, colours, shapes, additives, and degrees of weathering), studies have often used new and different tools, methods, units, and definitions. This lack of standardisation across microplastic studies has led to calls for improved harmonisation and quality assurance (Connors et al., 2017; Mitrano et al., 2023; Provencher et al., 2020). Proposals for standardisation and quality assurance have sought to improve not only the validity of studies but also the comparability between studies and the transferability of results to *in situ*

conditions and processes (de Ruijter et al., 2023, 2020; Koelmans et al., 2020; Kokalj et al., 2021; Kotta et al., 2022). The proposed criteria relate to the specification of particle properties and suggest experimental design and reporting of methods. Although the long-term experiment (see Chapter 2 for details) was planned and carried out before these proposals, its design and the resulting publications were largely in line with the recommendations: in addition to fully specified particle properties and a sophisticated experimental design, the applicability of the risk assessment and the ecological relevance (e.g., particle concentration) were considered.

In microplastic studies, particle concentration, or concentration range, is a key factor, as effects are strongly related to particle concentration. Toxicological studies report hazard concentrations for 5% of the species (HC_5) derived from the species sensitivity distribution (SSD) being at a median of 76 particles L^{-1} for aquatic species (Koelmans et al., 2022) or ~16.7 mg L^{-1} for marine species with microplastics > 100 µm (Beiras and Schönemann, 2020). Specifically for corals, the half maximal effective concentration (EC_{50}) for compromised health was at 430 mg L^{-1}, and the benchmark concentration causing a 10% effect (BMC_{10}) was at 10–20 mg L^{-1} (C.-Y. Chen et al., 2022). These figures are consistent with the small overall effect found here (at a concentration of 200 particles L^{-1} ≈ 0.25 mg L^{-1}) and confirm that realistic concentrations of microplastics typically cause marginal adverse effects and that a large increase in concentration is required to cause severe damage to coral health. The problem with increasing concentrations to levels that cause detectable or even lethal effects is that these are, at best, concentrations from some future scenarios or regionally limited pollution hotspots. To avoid this dilemma and still be able to detect effects, the measurement technique and the parameters to be analysed must be carefully selected to ensure the necessary sensitivity for effects at realistic microplastic concentrations (e.g., 3D scanning, buoyant weighing, and photosynthetic efficiency measurements). Furthermore, coral health should be considered more polyvalent than the mere absence of bleaching and necrosis, but at least as a complex mosaic of photosynthetic efficiency, energy reserves, growth, and metabolites. However, unrealistically high concentrations that exceed current and most predicted pollution scenarios should be transparently declared and reserved for studies that establish toxicological effect thresholds.

1.5.4 Acclimatisation: microplastics versus other anthropogenic stressors

There is a consensus that corals can respond to environmental change through acclimatisation, but success may depend on the pace of change (i.e., an initial decline is followed by a new, lower baseline or recovery to the previous physiological state) (Fox et al., 2021; van Oppen et al., 2017). However, short-term experiments are too brief to assess whether acclimation, if present, is actually beneficial in the long term (Edmunds, 2014).

Anthropogenic stressors affect corals to varying degrees, most notably heat stress from ocean warming, as excessive heat disrupts coral photosymbiosis, leading to coral bleaching. Other anthropogenic stressors, such as ocean acidification, do not primarily affect coral photosymbiosis but growth and skeletal density (Hill and Hoogenboom, 2022; Kline et al., 2019; Leung et al., 2022; van der Zande et al., 2020). Similar to ocean acidification and at realistic concentrations of ≤ 200 particles L^{-1}, microplastics also appear less harmful than heat stress (Isa et al., 2024; Plafcan and Stallings, 2022; Reichert et al., 2021), affect coral growth (Chapron et al., 2018; Hankins et al., 2021; Mouchi et al., 2019; Rades et al., 2024; Reichert et al., 2019), and overall do not primarily affect photosymbiosis, although this is less clear: among reports of no primary effect on photosymbiosis (Hankins et al., 2021), there are reports of detrimental effects (Mendrik et al., 2021), no effect at all (Ng and Todd, 2023; Plafcan and Stallings, 2022), and even increased photosynthetic efficiency (Lanctôt et al., 2020; Mendrik et al., 2021; Rades et al., 2024; Reichert et al., 2024b; Rocha et al., 2020).

At sufficiently low intensities (i.e., at the microplastic concentrations mentioned above and at low (RCP 2.6) to intermediate (RCP 4.5) greenhouse gas emission scenarios (Klein et al., 2022)), corals typically show sublethal effects to microplastics and acidification, presumably allowing acclimatisation. In natural extreme reef environments, such as vents, sites of upwelling or fluctuating pH (i.e., acidified conditions), and the Red Sea or the Persian Gulf (i.e., high water temperatures), corals have had sufficient time to adapt to low (Fabricius et al., 2011; Fantazzini et al., 2015; Griffiths et al., 2019; Prada et al., 2023; Schoepf et al., 2017; Scucchia et al., 2021) and fluctuating pH (Brown et al., 2022; Cornwall et al., 2018; Georgiou et al., 2015; Tanvet et al., 2023) or high (Evensen et al., 2021; Howells et al., 2016; Krueger et al., 2017) and fluctuating temperatures (Barshis et al., 2018).

However, when forced to adapt rapidly, corals are more challenged because they can only use their inherent genetic and behavioural tools. For example, under heat stress, corals often acclimatise by switching to a more heat-tolerant genus of the Symbiodiniaceae family (Claar

et al., 2020; Palacio-Castro et al., 2023) or by upregulating photosynthesis (Hoadley et al., 2016; Roik et al., 2024). These mechanisms increase the thermal tolerance of the holobiont as a trade-off for a decrease in other parameters (e.g., growth; (Cunning and Baker, 2020; Roik et al., 2024)). Upregulation of photosynthesis was again found to be a response to microplastic stress (see above and Chapter 2.2) and may represent a common stress compensation mechanism in corals. Another parallel between heat- and microplastic-induced stress responses in corals is reduced growth, likely due to the aforementioned trade-off at the expense of growth during acclimatisation. In addition, stressed corals appear to prioritise maintaining an energetic status quo over undiminished growth.

In corals, sublethal heat stress patterns (i.e., mild, prolonged, or successive) may lead to environmental memory (reviewed in Brown and Barott, 2022 and Hackerott et al., 2021) that promotes acclimatisation to subsequent heat stress events (DeMerlis et al., 2022; Drury et al., 2022; Marzonie et al., 2023; Schoepf et al., 2022). However, the rapid onset of intense heat often exceeds the acclimatisation capacity of corals, whereas less lethal ocean acidification may allow more species to acclimatise. Although some studies report limited acclimatisation potential to acidification (Camp et al., 2016; Comeau et al., 2019), others report effective acclimation or acclimatisation processes in tropical (Guillermic et al., 2021; Liew et al., 2018; Towle et al., 2015; Wall et al., 2016) and cold-water corals (Carreiro-Silva et al., 2014; Form and Riebesell, 2012; Glazier et al., 2020). These processes include compensatory mechanisms such as upregulation of the calcifying fluid pH (Guillermic et al., 2021; McCulloch et al., 2012), feeding rates (Towle et al., 2015), expression of specific genes (Glazier et al., 2020), as well as epigenetic modification through DNA methylation (Liew et al., 2018). While these mechanisms against acidification are functionally distinct from those identified as mitigating microplastic stress, they have similar efficacy against the respective anthropogenic stressor, averting mortality under most current and projected intensities (i.e., microplastics ≤ 200 particles L^{-1} and low to intermediate greenhouse gas emission scenarios).

1.6 Conclusions and outlook

This thesis synthesises findings on the long-term effects of microplastics on the behaviour and physiology of common reef-building corals and whether they acclimate to long-term microplastic exposure. Despite absent behavioural acclimation, coral physiology showed only a minor overall effect of chronic microplastic exposure. Occasional species-specific

effects provided insight into possible compensatory mechanisms of coral physiology that may mitigate microplastic stress through adjustments in photosymbiosis and growth. These mechanisms may indicate long-term acclimation of coral physiology to environmentally relevant microplastic concentrations. However, pollution scenarios predict increasing microplastic concentrations, under which acclimation through compensatory mechanisms may be less effective. Other, also increasing, anthropogenic stressors may act cumulatively to limit or prevent acclimation, thereby exacerbating adverse effects on coral health. Therefore, future research should focus on so far understudied aspects, such as multi-polymer microplastics combined with multiple stressors, to investigate cumulative effects on corals, as sound knowledge of coral stressor susceptibility may help guide conservation efforts to curb coral reef decline.

1.7 References

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2 Publications

2.1 Microplastic avoidance mechanisms in corals

Reef-building corals do not develop adaptive mechanisms to better cope with microplastics

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Frontiers in Marine Science 9 (2022): 863187

DOI: 10.3389/fmars.2022.863187

*Corresponding author

Published online on 29 June 2022.

Running title: Microplastic avoidance mechanisms in corals

No. of peer-reviewed citations on the date of submission of the dissertation: 10

Impact factor of the journal at the time of publication: 3.7

Contributions of the author of the dissertation:

Formal Analysis	Statistical analysis
Investigation	Data collection
Methodology	Contributed to development of methodology
Software	Programming, implementation of the computer code
Visualization	Data presentation
Writing – Original Draft	Writing initial draft

frontiers | Frontiers in Marine Science

ORIGINAL RESEARCH
published: 29 June 2022
doi: 10.3389/fmars.2022.863187

Reef-Building Corals Do Not Develop Adaptive Mechanisms to Better Cope With Microplastics

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Microplastics are omnipresent in the oceans and threaten marine animals through physical contact or ingestion. Short-term studies have already shown that reef-building stony corals respond differently to microplastics than natural food. However, it remains unknown whether corals exhibit acclimation mechanisms to combat the effects of microplastic exposure. Specifically, the long-term effects of microplastics on the feeding and defense behavior of reef-building corals remain unexplored. Therefore, the goal of this study was to infer potential acclimation mechanisms in the behavior of the corals. For this, four reef-building species (*Acropora muricata*, *Porites lutea*, *Pocillopora verrucosa*, and *Heliopora coerulea*) were exposed in a long-term experiment to microplastics for 15 months. Subsequently, coral feeding rates on microplastics and natural food (*Artemia* sp. cysts), feeding discrimination, and reactions to both were assessed in a 24 h pulse exposure experiment. The results showed that corals' feeding rates did not decrease after long-term exposure to microplastics. Similarly, the feeding discrimination (i.e., ratio of feeding on microplastics and natural food) did not differ after long-term exposure to microplastics. Moreover, corals showed no changes in defense behavior (i.e., mucus production or extrusion of mesenterial filaments) against microplastics. These findings suggest that symbiotic, reef-building corals do not develop mechanisms to adapt to long-term microplastic exposure. Thus, microplastic pollution might constitute a constant stressor for coral organisms, likely leading to sustained energy expenditures and impaired health.

Keywords: coral reefs, feeding rate, ingestion, long-term exposure, acclimation

OPEN ACCESS

Edited by:
Youji Wang,
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Reviewed by:
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Specialty section:
This article was submitted to Marine Pollution, a section of the journal Frontiers in Marine Science

Received: *26 January 2022*
Accepted: *25 May 2022*
Published: *29 June 2022*

Citation:
Rades M, Schubert P, Wilke T and Reichert J (2022) Reef-Building Corals Do Not Develop Adaptive Mechanisms to Better Cope With Microplastics. Front. Mar. Sci. 9:863187. doi: 10.3389/fmars.2022.863187

INTRODUCTION

Microplastic pollution poses an increasing threat to marine ecosystems (Law and Thompson, 2014; Auta et al., 2017; Galloway et al., 2017), including coral reefs (Lamb et al., 2018; de Oliveira Soares et al., 2020; Huang et al., 2021). Microplastics are defined as polymer particles between 1 µm and 1 mm in size and can occur in different shapes, structures, and densities (Hartmann et al., 2019). They may cause harm to marine organisms as they often harbor toxins that can be transferred to the organisms (Saliu et al., 2019; Patterson et al., 2020). In addition, microplastics also develop a distinct biofilm on the surface, called the "plastisphere" (Zettler et al., 2013; Amaral-Zettler et al., 2020), which may contain potential coral pathogens (e.g., *Vibrio* spp. strains; Franco et al., 2020; Kesy et al.,

2020) or foster confusion with prey (Hall et al., 2015). Ultimately, microplastics may also lead to compromised health through increased feeding efforts, reduced food uptake, and subsequent energy losses (Wright et al., 2013; Yin et al., 2018). In reef-building corals, exposure to microplastics is associated with reduced growth (Chapron et al., 2018; Hankins et al., 2021), lower feeding rates (Savinelli et al., 2020; Corinaldesi et al., 2021), changes in photosynthetic efficiency (Lanctôt et al., 2020), a compromised immune system (Tang et al., 2018), and an overall deterioration in health (Reichert et al., 2019). These adverse effects are often attributed to direct contact with microplastic particles, regularly followed by ingestions. Feeding on microplastics is suspected of causing increased energy demand through energetically costly microplastic handling (i.e., repeated particle captures, ingestion, and egestion) (Chapron et al., 2018; Mouchi et al., 2019; Reichert et al., 2019). Although microplastic particles are typically covered with a biofilm, ingestion does not likely result in a considerable nutritional return (Murphy and Quinn, 2018; Reichert et al., 2018; Reichert et al., 2019). Even though previous studies showed that corals typically egest indigestible material within 24 h (Mills and Sebens, 2004; Allen et al., 2017; Reichert et al., 2018), the gastric space is occupied by the indigestible and nutrient-deficient material, which prevents the digestion of food (Murphy and Quinn, 2018; Rotjan et al., 2019). Furthermore, recurrent ingestions and blockages of the digestive tract (Hankins et al., 2018) might cause incorporation into the coral skeleton (Hierl et al., 2021; Krishnakumar et al., 2021; Reichert et al., 2021a). Yet not all coral species ingest microplastics in the same amounts and might thus be unequally threatened. Some coral species, such as *Dipsastraea pallida*, ingest as much microplastics as natural food (Hall et al., 2015). Other species even appear to prefer plastic particles over their natural food, such as *Astrangia poculata* (Rotjan et al., 2019). Still other coral species appear to recognize plastic particles as indigestible or ingest them to a lesser extent compared to natural food, e.g., *Danafungia scruposa* (Corona et al., 2020), *Goniastrea retiformis* (Martin et al., 2019), and *Astroides calycularis* (Savinelli et al., 2020). However, methods vary between studies, limiting comparisons.

Corals can also actively repel unwanted, indigestible particles. General defense mechanisms include active cleaning, i.e., polyp and ciliary movements, mucus production, mesenterial filaments extrusion, and tissue contraction and expansion to remove sediments from their surface (Stafford-Smith and Ormond, 1992; Stafford-Smith, 1993; Erftemeijer et al., 2012). Several coral species showed similar defensive reactions (i.e., mucus production in *Porites* spp. or extrusion of mesenterial filaments in *Pocillopora* spp.) when they encounter microplastics (Reichert et al., 2018; Martin et al., 2019; Mouchi et al., 2019; Lanctôt et al., 2020).

The numerous adverse effects following contact with microplastics suggest that microplastics are a stressor for reef-building corals (Reichert et al., 2019; Hankins et al., 2021). Corals possess various mechanisms to cope with environmental stressors (Dallmeyer et al., 1982; Brown, 1997; Gates and Edmunds, 1999). For example, corals respond with altered feeding behavior to better cope with elevated temperatures, sedimentation, and ocean acidification (Anthony and Fabricius, 2000; Grottoli et al., 2006; Towle et al., 2015). This phenomenon, known as heterotrophic plasticity, helps corals improve their energy budget and maintain their physiological status under extended periods of stress (Fox et al., 2018; Sturaro et al., 2021). However, it is still unclear whether reef-building corals can also adapt their behavior to microplastics and thus avoid microplastic ingestion in the future.

Therefore, the general goal of our study was to assess changes in coral behavior after long-term exposure to microplastic particles. Specifically, we determined whether corals adapt to long-term microplastic exposure through (I) heterotrophic plasticity (i.e., no. of ingested microplastic particles), (II) improved discrimination (i.e., no. of ingested microplastics per natural food particle), or (III) increased defense mechanisms (i.e., the occurrence of mucus production or extrusion of mesenterial filaments). To this end, we conducted a controlled long-term microcosm experiment in which the feeding and defense behavior of four common reef-building coral species exposed to polyethylene (PE) microplastics over 15 months was compared to a microplastic-free control group. The findings may help to better understand the adaptive response of vital reef-building organisms to microplastic pollution.

MATERIALS AND METHODS

Studied Coral Species

Four common Indo-Pacific coral species, *Acropora muricata* (Linnaeus, 1758), *Pocillopora verrucosa* (Ellis & Solander, 1786), *Porites lutea* Milne-Edwards & Haime, 1851, and *Heliopora coerulea* (Pallas, 1766), were used for the controlled microcosm experiment, representing similar polyp sizes (1–2 mm diameter) but different morphologies (i.e., branching, massive, and columnar). For *A. muricata, P. verrucosa, and P. lutea*, 18 fragments per species were included, derived from three origin colonies. For *H. coerulea*, six fragments were included, derived from one origin colony. Corals were sampled in the Red Sea and off Bali between 2013 and 2015 or derived from a zoological garden (for details on colony origin and CITES numbers see **Supplementary Table S1**), and kept under laboratory conditions in the Ocean2100 facility for at least six months before the start of the long-term experiment (temperature: 26 ± 0.5°C; light intensity: 200 µmol photons m^{-2} s^{-1} at a photoperiod of 10:14 dark:light).

Experimental Design

Coral fragments were exposed to polyethylene (PE) microplastics (~200 particles L^{-1}) or microplastic-free control conditions for 15 months, and feeding and defense behavior was studied (**Figure 1**). Feeding rates and defensive reactions were quantified in individual incubations *via* the number of particles left after 24 h of pulse exposure. Coral feeding rates were assessed by exposure to control and microplastic particles: First, a control quantification of feeding activity was conducted with *Artemia* sp.

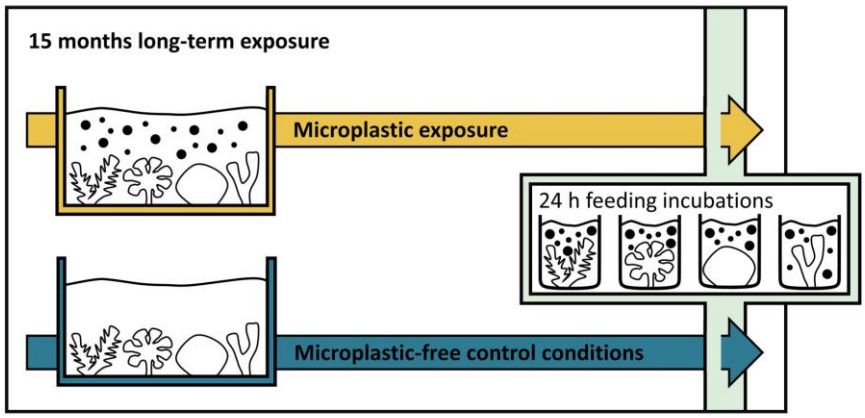

FIGURE 1 | The timeline of the experiment displays coral fragments from 15-month long-term microplastic exposure (in yellow; top) and from long-term control conditions (in blue; bottom). Corals' feeding and defense behavior on *Artemia* sp. cysts and microplastics were assessed in separate, consecutive 24 h pulse exposures at the end of the experiment.

cysts (Kellogg, 1906) to avoid potential effects of microplastic feeding on the control feed. Then the coral feeding rates on microplastics were assessed. Defense behavior was observed at the end of the 24 h exposure.

Long-Term Microplastic Exposure

The long-term exposure was conducted in six 80 L tanks (n = 3 per treatment). The tanks were connected to a reef mesocosm system (~4,000 L artificial seawater) to create near-natural conditions with an exchange rate of 120 L per day (≙ 150% of the tank volume). Three weeks prior to the start, coral fragments were distributed to the tanks (one fragment per colony per species per tank) and randomly allocated within the tanks with a min. distance of 5 cm to avoid interferences. Tank outflows were equipped with filters (65 µm mesh size) to retain particles inside the experimental tanks allowing a biofilm to form on the submerged particles. Water parameters were maintained under controlled conditions during the long-term experiment (temperature: 26 ± 0.2°C; light intensity: 135 µmol photons m^{-2} s^{-1} at a photoperiod of 10:14 dark:light), and corals were fed indirectly through the connected mesocosm system, which was supplied with frozen food (i.e., copepods and *Mysis* spp.) daily. For additional information on the technical setup see **Supplementary Methods S1.1**.

Microplastic Characteristics and Treatment

Irregular black PE microplastic particles (density: 0.95 g cm^{-3}; for FTIR chart see **Figure S1**; Novoplastik, Germany) were used, as it is one of the most common polymer types in reef waters (Saliu et al., 2018; Patterson et al., 2020). The size of the PE particles (diameter: 184 ± 95 µm (mean ± *SD*), for details, see **Figure S2** and **Supplementary Methods S1.2**) corresponds to the size of the plankton diet of corals (Houlbrèque and Ferrier-Pagès, 2009). Pristine particles were sterilized in ethanol (70% abv; 24 h) and then rinsed with deionized (DI) water. 2.5 mg L^{-1} sterilized microplastics were added to the tanks resulting in a concentration of ~ 200 particles L^{-1} or 0.25 mg L^{-1} after 48 h in the long-term experimental tank setup. The biofilm growth on the microplastics reduced the buoyancy to about the same density as seawater, making them available to the corals. Microplastic concentration in the water surrounding the corals was monitored every two months, with higher number of measurements at the beginning of the experiment until concentrations stabilized. For this, 50 mL tank water was filtered onto a 65 µm gauze filter (3–5 replicate measurements per tank) and the number of particles were counted under a stereo microscope to extrapolate the concentration per L. Concentrations were maintained at ~200 particles L^{-1} (201 ± 67 particles L^{-1}, n = 518 measurements at 125 timepoints) over the course of the experiment by the addition of fresh particles when necessary.

Pulse Exposure to Natural Feed and Microplastic

Artemia sp. Cysts Fed as Natural Feed Control

Decapsulated brine shrimp eggs (*Artemia* cf. *franciscana* (Kellogg, 1906); INVE Technologies, Belgium) were used as control feed in the 24 h pulse exposure because they are a standard feed for corals in aquaculture (Helmuth and Sebens, 1993; Sebens et al., 1998). The size of the *Artemia* sp. cysts (diameter: 175 ± 16 (mean ± *SD*) was comparable to the size of the microplastic particles used (**Figure S2**).

Biofouled Microplastics

The same PE particles were used in the long-term and the 24 h pulse exposure. To mimic near-natural conditions in the pulse exposure, biofouled particles were generated. For this, pristine microplastics were sterilized as described above. Then microplastics were incubated for 16 days in 50 L of 35 µm filtrated artificial seawater from the reef mesocosm system to allow for a biofilm to form [26°C, salinity: 34 ± 2 ‰, two 80 W fluorescent lamps (Aquablue Special and Blue Plus, ATI, Germany), two pumps (SW-4, Jebao, China)].

Incubations

Feeding and defense behavior were assessed in incubations where corals were provided with *Artemia* sp. cysts (control feed) or microplastics for 24 h of pulse exposure. The number of particles added to the incubation was chosen to match the number of polyps per coral fragment to ensure the availability of particles. For the *Artemia* sp. cyst feeding, 5 ± 0.008 mg cysts, equivalent to ~1,760 ± 66 particles L^{-1} (weighed with analytical balance (accuracy: 0.001 mg, CPA2P, Sartorius, Germany)) were suspended in 0.8 L of 0.22 µm filtrated seawater (Express Plus Membrane, Millipore, USA). For the microplastic feeding, 270 ml of a microplastic-seawater suspension (see section biofouled microplastics) was added to 530 ml of 0.22 µm filtrated seawater, equivalent to 1,850 ± 230 particles L^{-1}. The coral fragments were hung upside down in the beakers and secured with fishing line (d = 0.12 mm, Spiderwire stealth code red braid, Pure Fishing, USA). This positioning was chosen to avoid particle accumulation at the base of the concrete socket. Particles were uniformly distributed within the water column by air supply (two 228 mm glass pipettes) and a stir bar (20 mm × 8 mm ($l \times d$) rotating at ~500 rpm). Ten pulse exposure incubations were performed simultaneously in one run on a multipoint stirring incubator (Rades et al. 2022). For each run, coral fragments were randomly chosen from the microcosm setup (n = 7), and their feeding behavior was assessed simultaneously with coral-free control incubations (n = 3, equipped with pipettes, stir bars, and bare fishing lines only). A total of eight runs per treatment (each lasting for 24 h, for both microplastics and natural food) were conducted, with a mean time interval of 2–3 days between each run. Temperatures were kept at 26 ± 0.3°C through a water bath.

Assessment of Feeding Rates

Particle Recovery, Documentation, and Quantification

After the 24 h pulse exposure, particles were retrieved from each incubation and quantified. For this purpose, all equipment was removed from the incubation chambers. Fragments were gently shaken in the water to free adhering particles and placed back in the microcosm setup. All equipment and fragments were carefully rinsed with 0.22 µm filtered seawater between all steps to retain all adherent particles and avoid contamination. The seawater particle suspension was vacuum filtrated onto a cellulose nitrate filter (8 µm pore size, Sartorius, Germany). The filter was documented under a digital microscope (VHX-2000, Keyence, Japan) using the automatic 2D image stitching mode with the VH-Z20W lens at 50x magnification and a mounted polarizing filter. Pictures were saved as TIFF. Microplastic particle numbers were automatically determined using ImageJ (v1.51s; Rueden et al., 2017). For a detailed description of image processing and counting, see **Table S2**.

Determination of Coral Surface Area

Coral tissue surface area was used to standardize feeding rates per cm^2. The surface area was quantified *via* 3D scanning, following established procedures (Reichert et al., 2016; Reichert et al., 2018; Reichert et al., 2019). Briefly, 3D models of coral fragments were created after the pulse exposure using a hand-held 3D scanner (Artec Spider with Artec Studio 10, Artec 3D, Luxembourg) and coral tissue surface area derived (for details, see **Table S3** and **Supplementary Methods S1.3**).

Calculation of Feeding Rates

Feeding rates (in number of particles per cm^2 per 24 h) were assessed as the difference between the residual particles of the incubations with corals and the control incubations, based on established procedures (Hii et al., 2009). For this, the count of remaining particles in the coral feeding chamber (number of $particles_{coral}$) was subtracted from the mean number of particles found in the control chambers (mean number of $particles_{control}$) of the respective run (Equation 1).

$$Feeding\ rate\ \left(number\ of\ particles\ cm^{-2}\ 24\ h^{-1}\right) = \frac{mean\ number\ of\ particles_{control} - number\ of\ particles_{coral}}{surface\ area} \quad (1)$$

Calculated feeding rates were standardized to the surface area of the coral and expressed as the number of particles ingested per cm^2 coral surface area per 24 h.

Quantifying the Ability of Corals to Discriminate Between Microplastics and Natural Food

The number of fed microplastic particles per fed *Artemia* sp. cyst was determined to assess the ability of corals to discriminate microplastics from natural food. This discrimination ability value was calculated per coral fragment. These values were then compared between long-term exposure conditions (microplastic vs. microplastic-free).

Reactions of Corals to Microplastic Pulse Exposure

Visible physiological reactions of corals to the microplastic pulse exposure (i.e., mucus production, extruded mesenterial filaments) were documented at the end of the 24 h incubation for each fragment. Because of a possible simultaneous occurrence of both reactions, observations were broadly classified as (1) reaction or (2) no reaction. Reactions during the control feeding with *Artemia* sp. cysts were assessed similarly to distinguish between reactions to the feeding procedure itself and the microplastic exposure.

Statistics

Statistical analyzes were conducted with "R" (v4.1.2, R Core Team, 2021) using the "RStudio" interface (v2021.9.0.351, RStudio Team, 2021). Unless otherwise stated, tests from the "rstatix" package (v0.7.0, Kassambara, 2021) were used. Data were tested for normality using the histogram, Shapiro-Wilk test, and Q-Q and P-P plots (Almeida et al., 2019) and found to be non-normally distributed. Therefore, non-parametric tests were used further on. Kruskal-Wallis with Dunn's *post hoc* tests examined interspecific differences in microplastic and *Artemia* sp. cyst feeding rates. Wilcoxon tests were used to evaluate intraspecific differences in feeding rates between particle types (microplastics vs. *Artemia* sp. cysts). Wilcoxon tests were also used to compare the impacts of the two long-term exposure treatments on feeding rates. The effect of long-term exposure on the ability of corals to discriminate between microplastics and natural food (*Artemia* sp. cysts) was evaluated using Wilcoxon tests, with one extreme value ($> Q_3 + 3 \times IQR$) removed beforehand. Kruskal-Wallis with Dunn's *post hoc* tests examined interspecific differences in the ability of discrimination. The impact of long-term exposure to microplastics on coral reactions was assessed using Fisher's exact tests. Pearson's χ^2 tests were used to compare coral reactions between microplastics and *Artemia* sp. cysts. Effect sizes for Fisher's exact test and the χ^2 test were obtained using the "effectsize" package (v0.5, Ben-Shachar et al., 2020).

RESULTS

Coral Feeding Rates on Microplastics After Long-Term Exposure

Long-term microplastic exposure did not affect coral feeding rates on microplastic particles in any species (Wilcoxon tests, $p > 0.05$; **Figure 2A** and **Table S4**). Similarly, the exposure did not affect feeding rates on the control feed (*Artemia* sp. cysts), (Wilcoxon tests, $p > 0.05$; **Figure S4A** and **Table S5**). In general, corals showed lower feeding rates on microplastics (1.06 ± 2.08; mean ± *SD*) than on *Artemia* sp. cysts (7.02 ± 8.18; mean ± *SD*). Specifically, *A. muricata* (Wilcoxon test, $p < 0.001$) and *P. verrucosa* (Wilcoxon test, $p < 0.001$) fed significantly fewer microplastics (**Figure S5** and **Table S6**). However, there were no interspecific differences in feeding rates for either microplastics or *Artemia* sp. cysts (Kruskal-Wallis and Dunn's tests, $p > 0.05$; **Figure S6** and **Table S7 and S8**).

Coral Ability to Discriminate Between Microplastics and Natural Food After Long-Term Exposure

Discrimination ability (no. of microplastic particles ingested per *Artemia* sp. cyst ingested) did not differ between the two long-term conditions (microplastic exposure: 0.34 ± 0.76 microplastic particles per *Artemia* cyst; mean ± *SD* vs. microplastic free: -0.06 ± 1.24 microplastic particles per *Artemia* cyst; mean ± *SD*) in any species (Wilcoxon tests, $p > 0.05$, **Figure 2B** and **Table S8**). In addition, no interspecific differences were found (Kruskal-Wallis and Dunn's tests, $p > 0.05$, **Figure S7** and **Table S10 and S11**).

Coral Defense Reactions After Long-Term Exposure

A. muricata and *P. lutea* reacted with mucus release and extrusion of mesenterial filaments to the 24 h pulse exposures. *P. verrucosa* and *H. coerulea* did not show physiological reactions. Although the reactions occurred more frequently after long-term microplastic exposure, the effect was not statistically significant (Fisher's exact tests, $p > 0.05$; **Figure 2C** and **Table S12**). Similarly, the long-term microplastic exposure did not affect the frequency of reactions when corals were fed *Artemia* sp. cysts (Fisher's exact tests, $p > 0.05$; **Figure S4B** and **Table S12**). In general, reactions occurred more often when corals were fed with microplastics than with *Artemia* sp. cysts (significant for *A. muricata*; Chi-squared test, $p = 0.013$; **Figure S8** and **Table S13**). Specifically, corals fed less when they showed defense reactions (significant for *A. muricata*; Wilcoxon test, $p = 0.048$; **Figure S9** and **Table S14**).

DISCUSSION

Corals Do Not Adapt Feeding Mechanisms to Decrease Microplastic Uptake

Our results indicate that corals do not exhibit heterotrophic plasticity in response to long-term microplastic exposure as feeding rates remained unaltered after long-term microplastic exposure (research question I). To avoid stress potentially caused by long-term microplastic exposure, corals would need to either reduce their feeding rates in general or increase their feeding selectivity. However, unchanged feeding rates suggest that the corals studied do not possess or do not activate mechanisms of heterotrophic plasticity to reduce microplastic uptake in response to long-term exposure to microplastics.

Heterotrophic feeding activity has been found to shift (Hughes and Grottoli, 2013; Fox et al., 2019) under certain environmental stresses (e.g., heat stress, turbidity, or ocean acidification; Anthony and Fabricius, 2000; Bessell-Browne et al., 2014; Towle et al., 2015; Pupier et al., 2021). However, microplastics apparently do not trigger mechanisms of heterotrophic plasticity to reduce microplastic uptake. This suggestion is in line with Axworthy and Padilla-Gamiño (2019), who showed that microplastic feeding rates did not change in response to temperature stress. Furthermore, the findings of the short-term study by Chapron et al. (2018), in which *Artemia* capture rates remained unchanged after microplastic exposure conditions, are confirmed by our findings. A lack of adaptation to changing environmental conditions suggests that as concentrations increase, microplastic particle uptake might also increase linearly, possibly as seen for suspended particulate matter (Anthony, 1999; Anthony and Fabricius, 2000) or suspended sediments (Anthony, 2000).

After offering both types of particles independently, we found that the corals ingested the natural food at a higher rate than

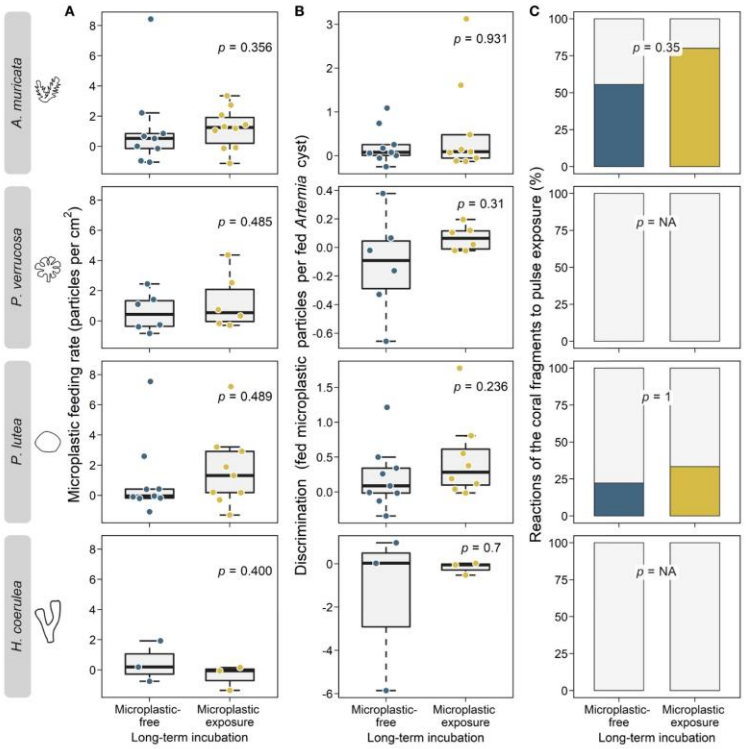

FIGURE 2 | Impacts of long-term exposure to microplastics (in yellow) and microplastic-free control conditions (in blue) on coral microplastic feeding and defense behavior of the four coral species *A muricata*, *P. verrucosa*, *P. lutea*, and *H coerulea*. **(A)**: Coral feeding rates on microplastics (particles per cm^2 coral tissue per 24 h) after long-term exposure to microplastics and microplastic-free control conditions. Data is displayed as box-and-whisker plots with raw data points. *P*-values are derived from Wilcoxon tests. **(B)**: Corals' discrimination ability (no. of fed microplastic particles per fed particle of natural food (*Artemia* sp. cysts)) after long-term exposure to microplastics and microplastic-free control conditions. Data is displayed as box-and-whisker plots with raw data points. *P*-values are derived from Wilcoxon tests. **(C)**: Corals' defense reactions to microplastic (% of fragments that show reactions) after long-term exposure to microplastic and microplastic-free control conditions Data is displayed as percent stacked bar charts and p-values are derived from Fisher's exact tests. NA, not available.

microplastics. In general, these findings are consistent with previous studies (Axworthy and Padilla-Gamiño, 2019; Savinelli et al., 2020), although there is also a counterexample (Rotjan et al., 2019). Yet, particle numbers deviated (*SD*) in average by 38 microplastic particles and 141 *Artemia* sp. cysts in the pulse exposures. Thus, the sometimes even negative feeding rates are a result of the mathematical approach used to quantify the feeding rates, and indicate low or no the feeding during the pulse exposure.

Long-Term Exposure Does Not Lead to Better Discrimination Between Microplastics and Natural Food

Our results also suggest that corals do not better discriminate between microplastics and natural food (research question II). The number of microplastics fed per *Artemia* cyst fed remained unchanged for all species. This result indicates that corals were not more effective in avoiding microplastic ingestion after long-term exposure. Although corals generally fed fewer microplastics than natural food, discriminatory ability did not improve further. The basic discriminatory ability of corals leading to higher feeding rates on natural food has been previously shown for reef-building corals (Hall et al., 2015; Martin et al., 2019) but seems to be species-specific as indicated by other studies (Allen et al., 2017; Rotjan et al., 2019). Chemical stimuli might mediate the discrimination process (Houlbrèque and Ferrier-Pagès, 2009). Microplastic feeding may be triggered by both biofilm- or plastic-related stimulants (Allen et al., 2017; Diana et al., 2020). It can be assumed that the biofilm on the particle mainly drives microplastic uptake, as most studies indicate that particles

covered with a biofilm are more likely to be ingested than pristine particles (Corona et al., 2020; Weideman et al., 2020). A comparison with sediments supports this concept: particles that are perceived as a source of nutrients due to microbiota colonization are more likely to be taken up by corals than sediments that are poor in nutrients (Mills et al., 2004).

Corals Do Not Increase Defense Mechanisms to Reduce Microplastic Uptake

Our results indicate that corals do not change the frequency of defense reactions to adapt to long-term microplastic exposure (research question 3) and do not appear to develop adaptive defense mechanisms to reduce the impacts of microplastic exposure. On the one hand, an increase in defense reactions could reduce the number of ingested particles. However, such defense behavior may also hinder feeding on natural food (Hughes, 1980; Anthony, 2000) and would thus be unfavorable in the long term. On the other hand, it could also have been assumed that defense reactions would be reduced in response to long-term microplastic exposure to avoid continuous energy expenditures. However, none were detected here, suggesting that microplastic exposure does not alter these reactions.

As an aside, our results confirm that defensive reactions like mucus production and extrusion of mesenterial filaments are typical reactions of *A. muricata* and *P. lutea* to microplastics (Reichert et al., 2018; Martin et al., 2019; Mouchi et al., 2019; Jiang et al., 2021). Reactions were observed when corals were exposed to microplastics and less frequently when corals were exposed to natural food. The observed reactions can be interpreted as either defense or feeding reactions, which explains their designation as so-called "multitools" (Brown and Howard, 1985; Brown and Bythell, 2005). However, the observed reactions seem to be defensive, as corals ingested fewer microplastics when reactions were shown.

Our results further indicate that corals apparently ingest microplastics even in the absence of natural food (*sensu* Corona et al., 2020). Yet, coral microplastic feeding rates might be even higher in the presence of natural food, which triggers feeding through inherent chemical stimulants (Helland et al., 2000; Houlbrèque and Ferrier-Pagès, 2009). Additionally, different polymer types might cause different reactions because of their physical and chemical properties (e.g., shape, size, additives). Further, the influence of microplastics may vary over time as the particles' properties can be altered through environmental conditions and absorb locally present pollutants. Therefore, further studies should also consider using other microplastics and combinations (i.e., different shapes, polymer types, concentrations, and mix with natural feed) to better resemble *in situ* conditions.

CONCLUSIONS

In summary, our findings suggest that reef-building corals lack acclimation mechanisms (i.e., feeding, avoidance, and defense mechanisms) to reduce the effects of microplastic exposure over longer periods of time. A lack of acclimation implies that microplastics will constitute a permanent stressor in coral reefs. However, it is still unclear to what degree corals will be cumulatively affected by repeated microplastic contacts during their lifetime. Given the projected increase in microplastic concentrations (Everaert et al., 2018; Isobe et al., 2019) and the lack of adaptive mechanisms (i.e., foraging and defensive), microplastic pollution might be of increasing concern. In combination with other stressors (e.g., global warming and ocean acidification; Hoegh-Guldberg et al., 2007; Conti-Jerpe et al., 2020; Reichert et al., 2021b), microplastics are likely to contribute to shifts in coral reef communities worldwide. Therefore, immediate action is needed to curb microplastic and plastic pollution, in close coordination with protective measures against global warming and ocean acidification, to preserve the remaining coral reefs.

DATA AVAILABILITY STATEMENT

The datasets presented in this study are publicly available at figshare: https://doi.org/10.6084/m9.figshare.19073312.

AUTHOR CONTRIBUTIONS

MR: Methodology, Software, Formal Analysis, Investigation, Writing – Original Draft, Visualization. JR: Conceptualization, Methodology, Formal Analysis, Resources, Data Curation, Writing – Review and Editing, Project Administration, Supervision. PS: Resources, Writing – Review and Editing. TW: Conceptualization, Writing – Review and Editing.

FUNDING

This study was conducted as part of the 'Ocean 2100' global change simulation project of the Colombian-German Center of Excellence in Marine Sciences (CEMarin) funded by the German Academic Exchange Service (DAAD, project number 57480468).

ACKNOWLEDGMENTS

We are thankful for Angelina Arnold's contribution to the 15-month long-term experiment and Maren Ziegler's helpful comments on the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.863187/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary material

S 1 Supplementary methods

S 1.1 Details on the experimental setup of long-term microplastic exposure

Coral colonies were reared under laboratory conditions in the 'Ocean2100' facility at Justus Liebig University Giessen, Germany (10:14 light:dark photoperiod, light intensity (PAR) 200 µmol photons m^{-2} s^{-1}, and temperature 26 ± 0.5 °C) for at least six months before the experiment (see details on coral colonies in Table S1) in accordance with the institutional animals' care guidelines. Corals were fragmented with a small angle grinder to ~3.5 cm branches for *A. muricata*, *P. verrucosa*, and *H. coerulea* and ~1 cm cubes for *P. lutea*. Fragments were glued to self-made concrete bases using a two-component glue (CoraFix SuperFast, Grotech, Germany) to ease the handling.

A total of 90 nubbins were prepared for *Acropora*, *Pocillopora*, and *Porites*, cut equally from three original colonies. For *Heliopora,* 30 nubbins were cut from a single colony due to the lack of replicate colonies. The coral fragments were allocated equally to the tanks. Corals were randomly distributed within the tanks and shuffled once a week to avoid position effects. As one colony of *P. verrucosa* experienced a high mortality rate during the experiment, it was excluded from the analyses. A subset of coral fragments was analyzed in this study (one fragment per species per colony per tank). This resulted in a total of $n = 27$ fragments studied in each, the control and the long-term microplastic exposure treatment (*Acropora*: $n = 9$, *Pocillopora*: $n = 6$, *Porites*: $n = 9$, *Heliopora*: $n = 3$), with each fragment treated as replicate. The physiological responses of the full set of coral fragments were examined in a separate study (Reichert et al. 2019).

The six experimental tanks were equipped with a flow pump for horizontal water movement (RW-8, Jebao, China; 700 L h^{-1}) and a feed pump (S 400, Resun, China; 400 L h^{-1}) for a vertical water circulation that re-immersed floating microplastic particles. A UV clarifier (RWUVC/78/4000, RuWal Aquatech, Italy; 33000 mWs^{-1} cm^{-2} at 4000 L h^{-1}) was upstream of the inlet of the six experimental tanks to reduce pathogens. On the outflow side, a fleece membrane was installed downstream of the 65 µm filters to retain even smaller plastic particles that might have been generated by fragmentation over time.

Small gastropods (*Nassarius* spp., *Euplica* spp., *Turbo* spp., and *Stomatella auricula*) were used to limit algae growth. If necessary, coral nubbins were inspected daily and cleaned from algae and detritus. The connection to a reef mesocosm system included a large 'buffer' tank, harboring corals, fish, and a deep sand bed, together with a protein skimmer and a calcium reactor (pH 6.2–6.4, coral rubble) and provided near-natural water conditions. The system was set up with artificial seawater (Coral ocean plus, ATI, Germany), and water parameters were checked once a week (alkalinity: 2.52 mmol L^{-1}, Ca^{2+}: 410 mg L^{-1}, Mg^{2+}: 1230 mg L^{-1}, PO_4^{3-}: < 0.03 mg L^{-1}, NO_3^-: < 0.02 mg L^{-1}, NO_2^-: < 0.01 mg L^{-1}, NH_4^+ < 0,025 mg L^{-1}, salinity: 34).

After six months of long-term exposure, several of the coral fragments were snap-frozen in liquid nitrogen, as described in Reichert et al. (2019). The remaining coral fragments were further kept under the same experimental conditions to a total long-term exposure period of 15 months, except for omitted periodical quantification of photosynthetic activity, determination of calcification, and growth assessment.

S 1.2 Microplastic particles

The size of microplastic particles used (184 ± 95 µm (diameter: mean ± SD)) is similar to natural marine conditions where small microplastics (< 1 mm) dominate the total microplastic concentration (Hartmann et al. 2019; Koelmans et al. 2020). Accordingly, reef microplastics are often present in sizes (< 500 µm (Saliu et al. 2018; Ding et al. 2019; Huang et al. 2019)) similar to the plankton diet of corals (Palardy et al. 2005; Houlbrèque and Ferrier-Pagès 2009). A concentration of ~200 microplastic particles L^{-1} (≈ 0.25 mg L^{-1}) was chosen for the long-term exposure as this concentration is close to natural conditions anticipated for the years 2030 to 2060 (Isobe et al. 2019).

S 1.3 Determination of corals' surface area

3D models of the coral fragments were constructed in the Artec Studio 11 software (Artec 3D, Luxembourg). Coral fragments were scanned directly after the feeding incubation. Fragments were placed on a motorized turntable within a lightbox and scanned within ~90 s from 45- and 90-degree angles. From the calculated 3D model, the socket, and necrotic and bleached tissue, were removed with the "Eraser" tool, resulting in the living coral tissue only. The final

3D models were saved as OBJ files, and surface area values were determined ("compute geometric measures" command) in MeshLab (v1.3.4 beta; Cignoni et al., 2008).

S 2 Supplementary Figures and Tables

S 2.1 Supplementary Figures

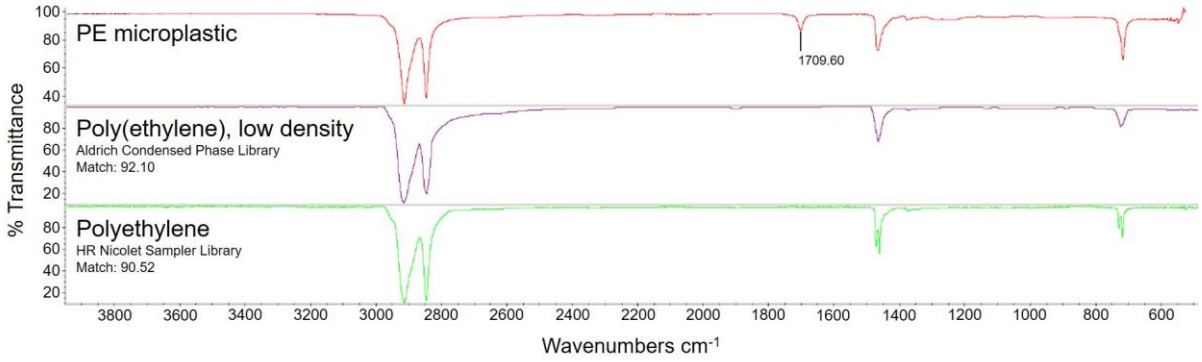


Figure S1: FTIR spectrum of the polyethylene (PE) microplastic particles used in the experiment (top, red), compared to reference spectra of low-density polyethylene (middle, purple and bottom, green). The PE microplastics has a distinct peak at 1709.60 cm^{-1}, indicating the C=O stretching of the polymer. Image source: (Reichert et al. 2022)

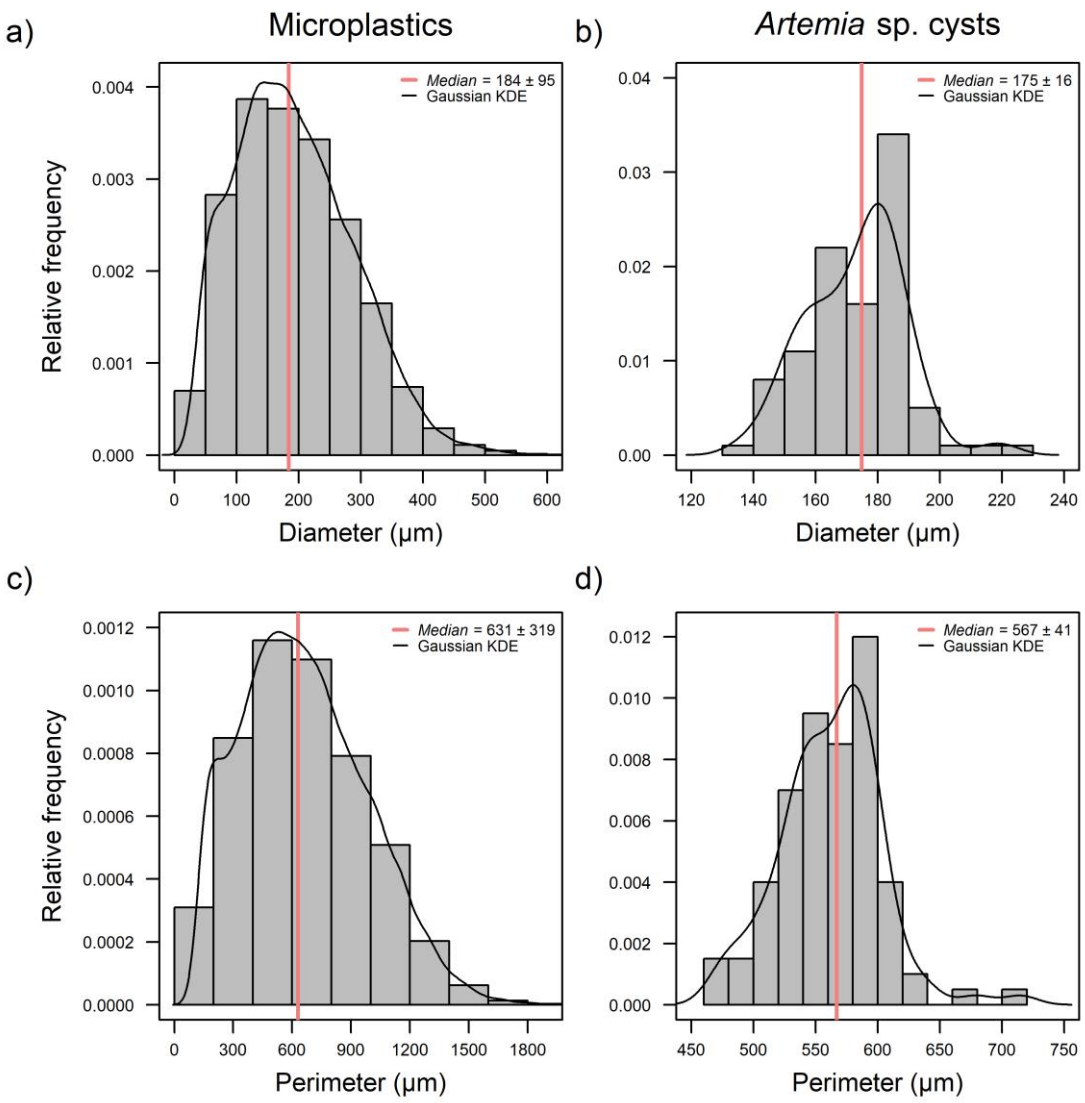


Figure S2: Particle characteristics. Comparison of size distributions of microplastics (a, c) and *Artemia* sp. cysts (b, d) depicted as histograms of diameters (a, b) and perimeters (c, d) with relative frequency values. KDE = kernel density estimation. Statistical values (median ± SD in µm) based on $n = 20474$ (microplastics) and $n = 100$ (*Artemia* sp. cysts).

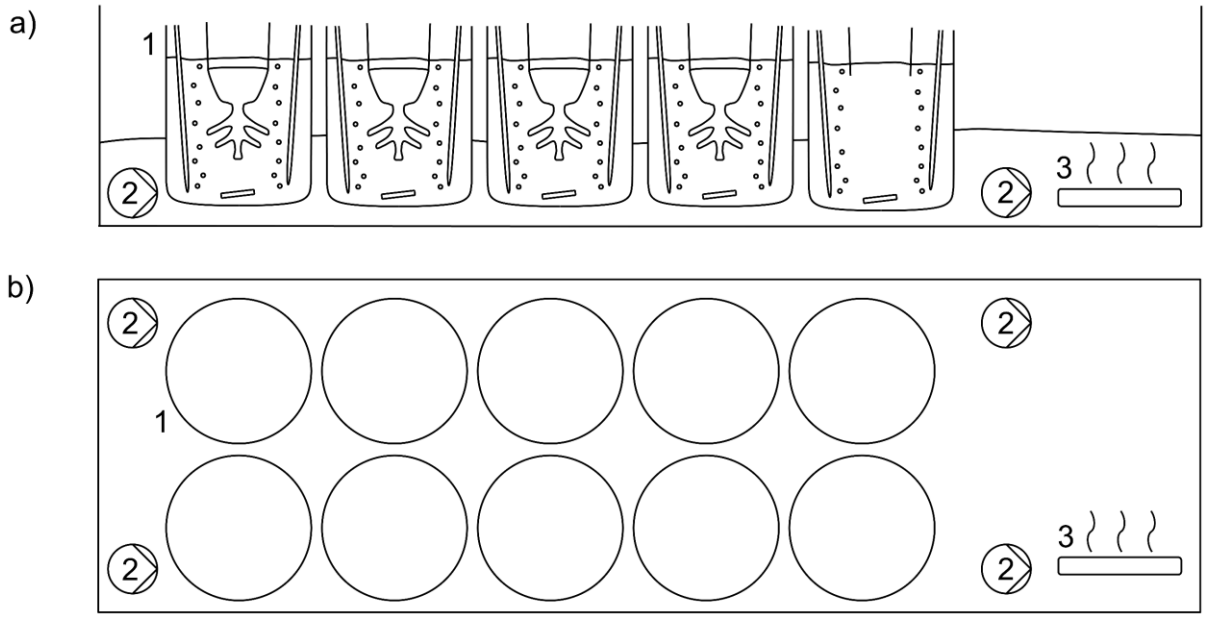


Figure S3: Schematic drawing of 24 h pulse exposure setup in side view (a) and top view (b). Two consecutive rows of five feeding chambers (1) are located in a water bath. Four pumps (2) are located in the corners of the water bath for circulation of the water tempered by a heating rod (3). The feeding chambers are equipped with aeration, a stir bar, and a coral fragment. Control chambers were equipped with fishing lines only and lack the fragment.

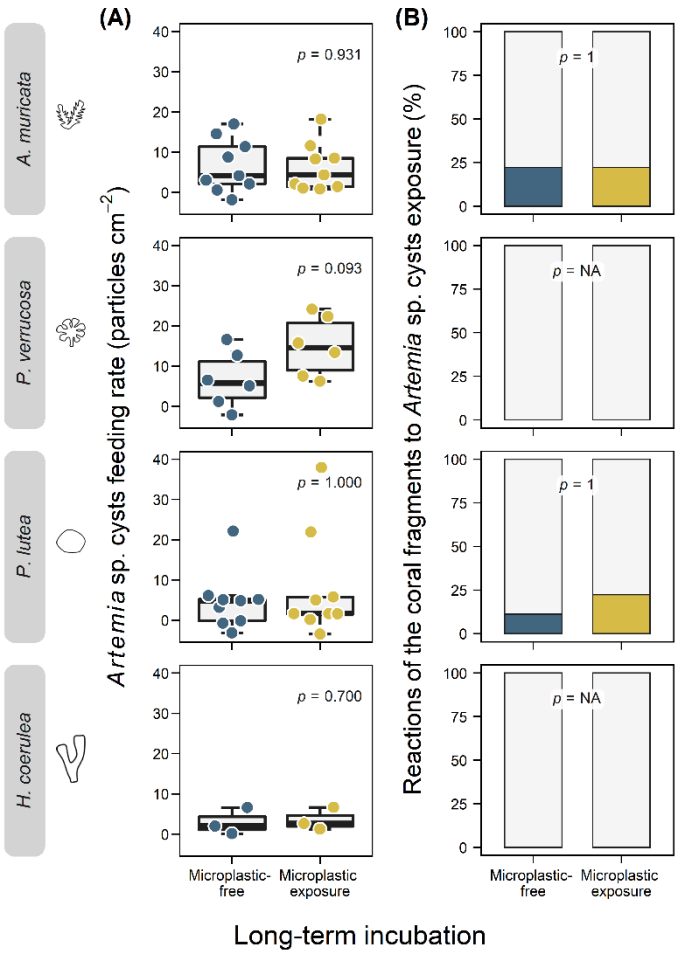


Figure S4: Impacts of long-term exposure to microplastics (in yellow) and microplastic-free control conditions (in blue) on coral feeding on control feed and defense behavior of the four coral species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*. A: Coral feeding rates on *Artemia* sp. cysts (particles cm^{-2}) after long-term exposure to microplastics and microplastic-free control conditions. Data is displayed as box-and-whisker plots with raw data points. *P*-values are derived from Wilcoxon tests. Detailed statistical results are given in Table S5. B: Corals' defense reactions to control feed (% of fragments that show reactions) after long-term exposure to microplastic and microplastic-free control conditions. Data is displayed as percent stacked bar charts, and *p*-values are derived from Fisher's exact tests. Detailed statistical results are given in Table S12.

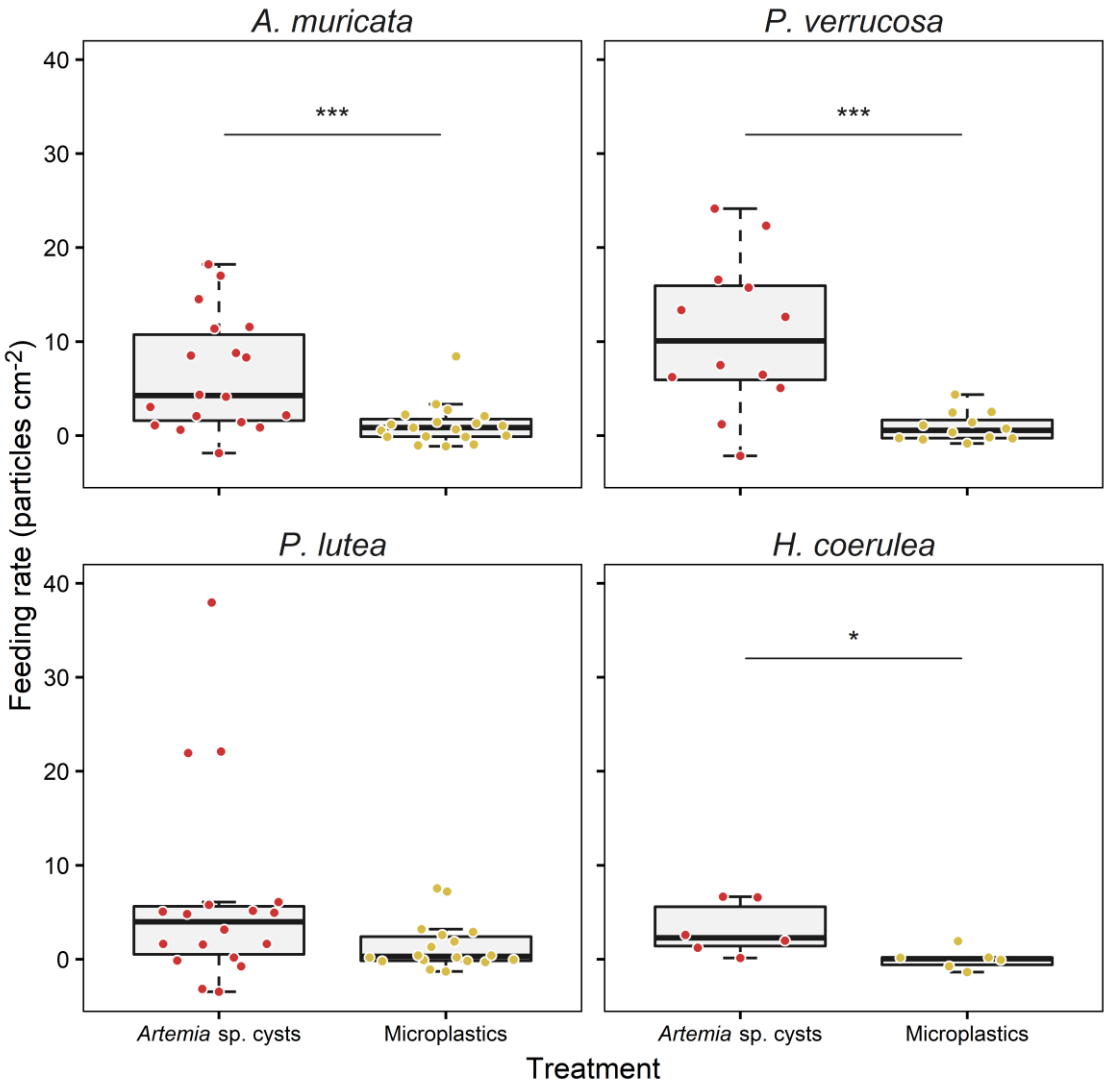


Figure S5: Coral feeding rates on *Artemia* sp. cysts (orange, control) and microplastics (yellow, treatment) for the four coral species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*. Data is displayed as box-and-whisker plots with raw data points. The *p*-values are derived from Wilcoxon tests, and the asterisks indicate significance levels ($p = .05$: *, $p = .01$: **, $p = .001$: ***). Detailed statistical results are given in Table S6.

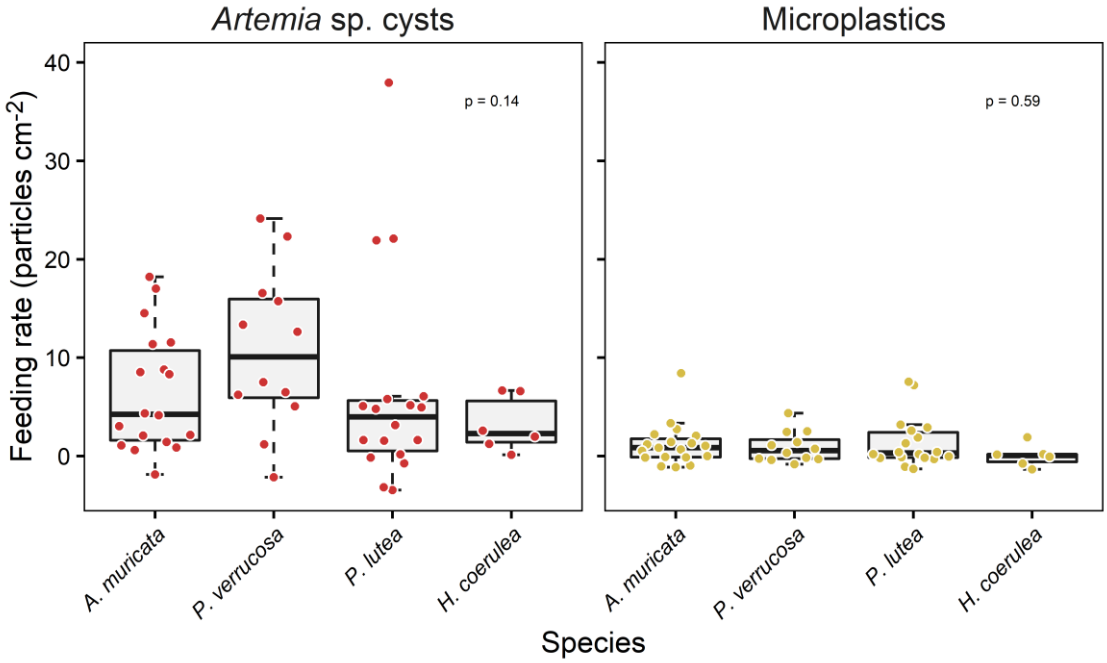


Figure S6: Interspecific differences in feeding rates for the four coral species, *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*. Rates are given for feeding on *Artemia* sp. cysts (left, orange) and microplastics (right, yellow). Data is displayed as box-and-whisker plots with raw data points. The *p*-values are derived from Kruskal-Wallis tests followed by Dunn post hoc tests. Detailed statistical results are given in Table S7 and 8.

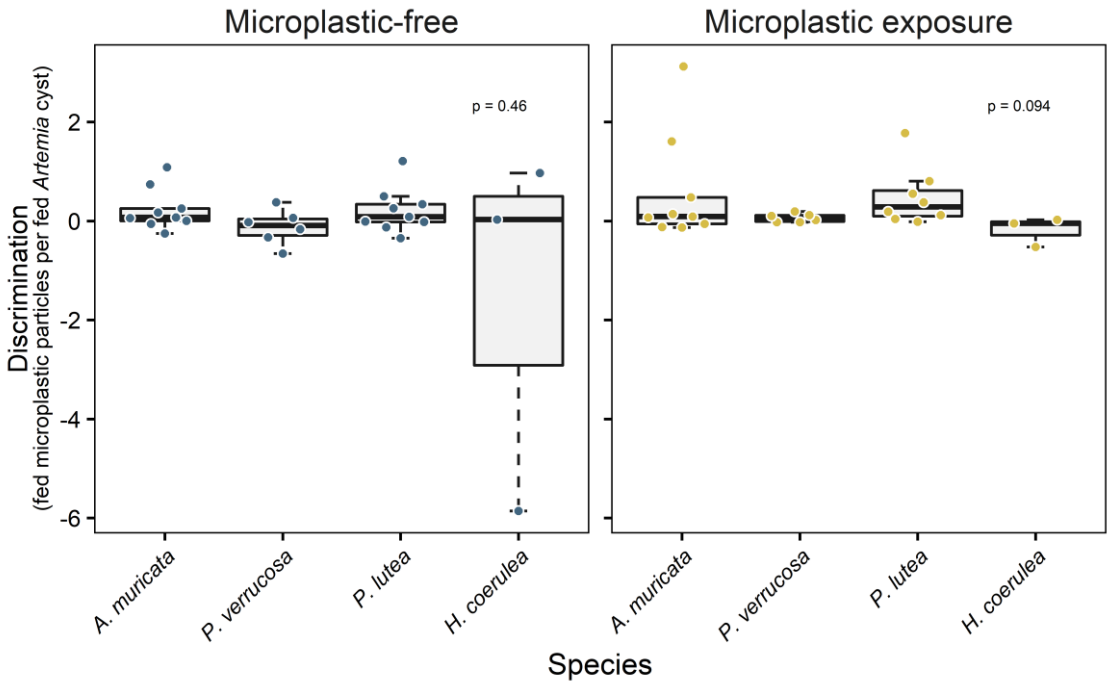


Figure S7: Interspecific differences in the ability to discriminate microplastics from natural food for the four coral species, *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea,* in the two long-term conditions (microplastic-free and microplastic exposure). Data is displayed as box-and-whisker plots with raw data points, and the *p*-values are derived from Kruskal-Wallis tests followed by Dunn post hoc tests. Detailed statistical results are given in Table S10 and 11.

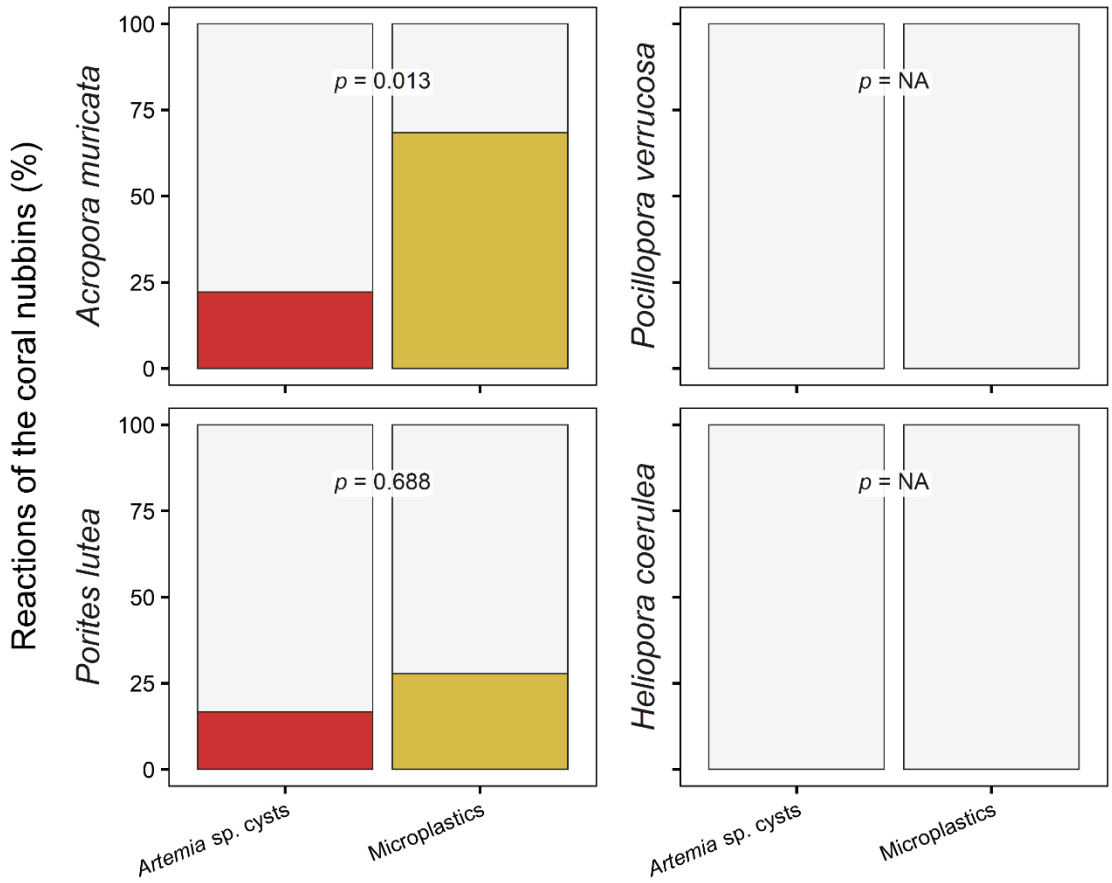


Figure S8: Corals' defense reactions (% of fragments that show reactions) to control feed (*Artemia* sp. cysts, left, orange) and microplastics (right, yellow) of the four coral species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*. Data is displayed as percent stacked bar charts, and *p*-values are derived from chi-squared tests. Detailed statistical results are given in Table S13.

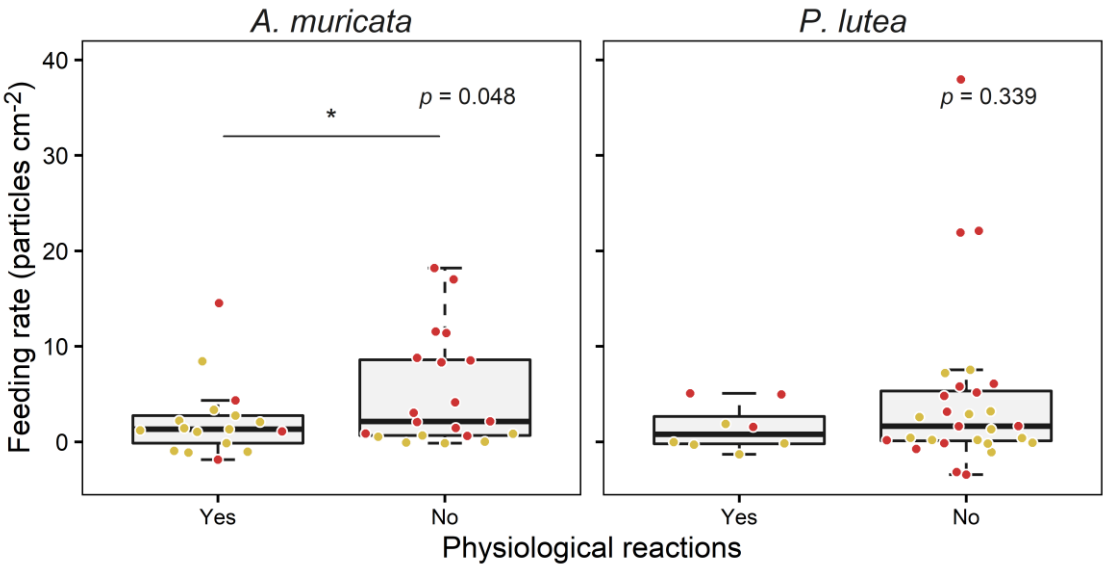


Figure S9: Coral feeding rates on *Artemia* sp. cysts (orange) and on microplastics (yellow) separated for the occurrence of defense reactions of the coral species *A. muricata* and *P. lutea*. *P. verrucosa* and *H. coerulea* showed no defense reactions. Data is displayed as box-and-whisker plots with raw data points, and *p*-values are derived from Wilcoxon tests. Asterisks indicate significance levels ($p = .05$: *, $p = .01$: **, $p = .001$: ***). Detailed statistical results are given in Table S14.

S 2.2 Supplementary Tables

Table S1: CITES numbers and origin of coral colonies studied. Origin, dates of collections, CITES numbers, and arrival date at the aquarium facilities at Justus Liebig University are given for the colonies studied. * details on colonies are not available due to collections prior to the implementation of the CITES regulations.

Species	Colony	Origin	Collection	Arrival	CITES number
Acropora muricata	A	Indonesia	12/2007	12/2007	14846/IV/SATS-LN/2007
Acropora muricata	B*	Zoo Frankfurt, Germany	NA	05/2015	NA
Acropora muricata	C	Indonesia	12/2007	12/2007	14846/IV/SATS-LN/2007
Pocillopora verrucosa	A	Saudi Arabia	05/2015	06/2015	15-SA-000882-PD
Pocillopora verrucosa	B	Indonesia	04/2014	05/2014	14NL214371/11
Pocillopora verrucosa	C	Indonesia	12/2007	12/2007	14846/IV/SATS-LN/2007
Porites lutea	A	Indonesia	05/2014	05/2014	14-NL-216270-11
Porites lutea	B	Indonesia	05/2014	05/2014	14-NL-216270-11
Porites lutea	C	Indonesia	05/2014	05/2014	14-NL-216270-11
Heliopora coerulea	A*	Zoo Frankfurt, Germany	NA	05/2015	NA

Table S2: Working steps for particle counting. The steps from image acquisition to image processing to automatic particle counting are presented with an example image, the goal of each step, the tools used and their settings.

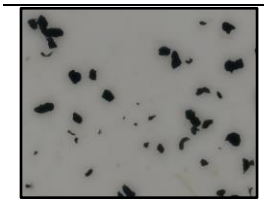
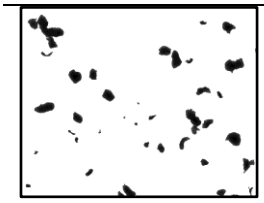
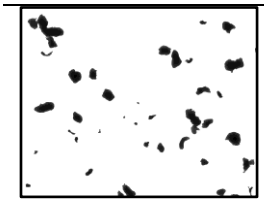
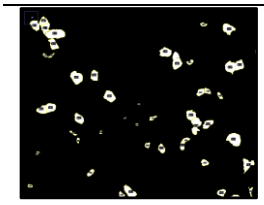
Example image	Aim	Software	Tool	Settings
	Documentation of particles	Keyence Terminal Software, Keyence, Japan	Keyence VHX-2000 digital microscope	Magnification: 50x Lens: VH-Z20W 2D stitching mode Manually specify area Autofocus: off Mount Polarizer Safe Images as TIFF
	Adjust white balance	RawTherapee Image Manipulation Program, https://rawtherapee.com	Adjust white balance	Manual selection of white background
	Adjust brightness	ImageJ Fiji, https://fiji.sc	Stack Deflicker	-1
	Noise reduction		Non Local Means Denoise	Sigma = 12 Smoothing factor = 1
			Thresholded Blur	Radius = 3 Threshold = 11 Softness = 0.10 Strength = 1
	Background removal	ImageJ Fiji	Color Thresholder	Pass: Y = 0-83 U = 119-133 V = 121-134 Color space: YUV
	Enhance contrast		Enhance Local Contrast	Deselect: fast
	Noise reduction	ImageJ Fiji	Thresholded Blur	Radius = 3 Threshold = 11 Softness = 0.10 Strength = 1
			Bi-Exponential Edge-Preserving Smoother	Range filter = gauss Photometric SD = 4.0 Spatial decay = 0.01 Iteration = 1
	Separate aggregated particles	ImageJ Fiji	Greylevel Watershed	Watershed = '0 1 0 95 0 0' Display = '0'
			Watershed Irregular Features	Erosion = 1 Convexity threshold = 0 Separator size = 27-300
	Count particles		Extended Particle Analyzer	Area = 60-19150 Perimeter = 20-590 Circularity = 0.29-1.00 Roundness = 0.2-1.00 Solidity = 0.59-0.985 Aspect ratio = 1.045-Infinity

Table S3: 3D scanning and post-processing settings for calculating 3D models of the four coral species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea* in Artec Studio 11.

Parameter	*A. muricata*	*P. lutea*	*P. verrucosa*	*H. coerulea*
Scan sensitivity	4	4	5	4
Fine registration (FR)	Texture and geometry			
FR: Refine serial	on			
FR: Loop closure	off			
Global registration (GR)	Texture and geometry			
GR: Min. distance	10			
GR: Iterations	$1 \cdot 10^5$			
Outlier removal: Std. Dev.	3	5	NA	5
Outlier removal: Resolution	0.2	0.2	NA	0.2
Sharp fusion: Resolution	0.2	0.2	0.2	0.3
Sharp fusion: Fill holes	By radius			
Sharp fusion: Max. hole	3			
Sharp fusion: Remove targets	off			
Small object filter mode	Leave biggest object			
Generate texture atlas	on			
Inpaint missing texture	off			
Remove targets	off			
Output texture size	4096 • 4096			

Table S4: Comparison of feeding rates on microplastics between both long-term conditions (microplastic-free control vs. microplastic exposure) for the species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*. Values of Wilcoxon test statistics (alternative hypothesis: two sided) and effect sizes rounded to three decimal places. n_{obs} = number of observations; CI = 95% confidence interval.

Species	Comparison					t-value	p-value	CI low	CI high	Effect size (r-value)	Magnitude of effect size
	Long-term conditions	(n_{obs})	vs.	Long-term conditions	(n_{obs})						
A. muricata	control	(9)	–	microplastics	(10)	57	0.356	-0.978	2.146	0.225	small
P. verrucosa	control	(9)	–	microplastics	(9)	49	0.485	-1.58	2.951	0.231	small
P. lutea	control	(6)	–	microplastics	(6)	23	0.489	-1.13	2.963	0.177	small
H. coerulea	control	(3)	–	microplastics	(3)	2	0.4	-3.274	0.911	0.445	moderate
Overall	control	(27)	–	microplastics	(28)	442	0.288	-0.306	1.241	0.145	small

Table S5: Comparison of feeding rates on *Artemia* sp. cysts between both long-term conditions (microplastic-free control vs. microplastic exposure) for the species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*. Values of Wilcoxon test statistics (alternative hypothesis: two sided) and effect sizes rounded to three decimal places. n_{obs} = number of observations; CI = 95% confidence interval.

Species	Comparison					t-value	p-value	CI low	CI high	Effect size (r-value)	Magnitude of effect size
	Long-term conditions	(n_{obs})	vs.	Long-term conditions	(n_{obs})						
A. muricata	control	(9)	–	microplastics	(9)	39	0.931	-7.692	6.194	0.031	small
P. verrucosa	control	(9)	–	microplastics	(9)	41	0.093	-0.827	17.896	0.508	large
P. lutea	control	(6)	–	microplastics	(6)	29	1	-4.453	8.961	0.010	small
H. coerulea	control	(3)	–	microplastics	(3)	6	0.7	-5.362	6.536	0.267	small
Overall	control	(27)	–	microplastics	(27)	423	0.318	-1.603	5.164	0.138	small

Table S6: The two pulse exposure conditions (microplastics vs. *Artemia* sp. cysts) were compared for each of the four coral species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*. Values of Wilcoxon test statistics (alternative hypothesis: two sided) and effect sizes rounded to three decimal places. Bold numbers indicate significant differences ($p \leq 0.05$). n_{obs} = number of observations; CI = 95% confidence interval.

	Comparison									
Species	Pulse exposure condition	(n_{obs})	vs.	Pulse exposure condition	(n_{obs})	*t*-value	*p*-value	CI low	CI high	Effect size (*r*-value)	Magnitude of effect size
A. muricata	microplastics	(19)	–	*Artemia* cysts	(18)	65	<0.001	-8.512	-1.177	0.53	large
P. verrucosa	microplastics	(18)	–	*Artemia* cysts	(18)	16	<0.001	-15.409	-4.731	0.66	large
P. lutea	microplastics	(12)	–	*Artemia* cysts	(12)	109	0.1	-5.004	0.31	0.279	small
H. coerulea	microplastics	(6)	–	*Artemia* cysts	(6)	4	0.03	-6.654	-0.187	0.647	large

Table S7: Feeding rates during the two pulse exposure conditions (microplastics and *Artemia* sp. cysts) were compared separately among the four coral species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*. Values of Kruskal-Wallis test statistics and effect sizes rounded to three decimal places. n_{obs} = number of observations; η^2_H = eta-squared based on the Kruskal-Wallis *H* test.

Pulse exposure condition	n_{obs}	**Statistic (χ^2)**	**Degrees of freedom**	***p*-value**	**Effect size (η^2_H)**	**Magnitude of effect size**
microplastics	55	1.929	3	0.587	-0.021	small
***Artemia* sp. cysts**	54	5.497	3	0.139	0.05	small

Table S8: Feeding rates during the two pulse exposure conditions (microplastics and *Artemia* sp. cysts) were compared pairwise among the four coral species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*. Values of the Dunn post hoc test statistics rounded to three decimal places, and *p*-values were adjusted according to the Benjamini-Hochberg method (1995). n_{obs} = number of observations.

	Comparison							
Pulse exposure condition	Species	(n_{obs})	vs.	Species	(n_{obs})	*z*-value	*p*-value	Adjusted *p*-value
***Artemia* sp. cysts**	*A. muricata*	(18)	–	*P. verrucosa*	(12)	1.402	0.161	0.322
***Artemia* sp. cysts**	*A. muricata*	(18)	–	*P. lutea*	(18)	-0.816	0.415	0.498
***Artemia* sp. cysts**	*A. muricata*	(18)	–	*H. coerulea*	(6)	-0.824	0.41	0.498
***Artemia* sp. cysts**	*P. verrucosa*	(12)	–	*P. lutea*	(18)	-2.132	0.033	0.198
***Artemia* sp. cysts**	*P. verrucosa*	(12)	–	*H. coerulea*	(6)	-1.822	0.068	0.205
***Artemia* sp. cysts**	*P. lutea*	(18)	–	*H. coerulea*	(6)	-0.247	0.805	0.805
Microplastics	*A. muricata*	(19)	–	*P. verrucosa*	(12)	-0.423	0.672	0.841
Microplastics	*A. muricata*	(19)	–	*P. lutea*	(18)	-0.2	0.841	0.841
Microplastics	*A. muricata*	(19)	–	*H. coerulea*	(6)	-1.355	0.175	0.677
Microplastics	*P. verrucosa*	(12)	–	*P. lutea*	(18)	0.242	0.809	0.841
Microplastics	*P. verrucosa*	(12)	–	*H. coerulea*	(6)	-0.957	0.339	0.677
Microplastics	*P. lutea*	(18)	–	*H. coerulea*	(6)	-1.206	0.228	0.677

Table S9: Comparison of ratios (no. of fed microplastic particles per fed *Artemia* sp. cyst) between the two long-term conditions (microplastic-free control vs. microplastic exposure) for the four coral species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*. Values of Wilcoxon tests (alternative hypothesis: two sided) are rounded to three decimal places. n_{obs} = number of observations; CI = 95% confidence interval.

Species	Comparison					*t*-value	*p*-value	CI low	CI high	Effect size (*r*-value)	Magnitude of effect size
	Long-term conditions	(n_{obs})	vs.	Long-term conditions	(n_{obs})						
A. muricata	control	(9)	–	microplastics	(9)	39	0.931	-0.729	0.291	0.031	small
P. verrucosa	control	(6)	–	microplastics	(6)	11	0.31	-0.634	0.182	0.324	moderate
P. lutea	control	(9)	–	microplastics	(8)	23	0.236	-0.68	0.22	0.303	moderate
H. coerulea	control	(3)	–	microplastics	(3)	6	0.7	-5.882	1.493	0.267	small
Overall	control	(27)	–	microplastics	(26)	301	0.382	-0.283	0.097	0.122	small

Table S10: Differences in the ability to discriminate between microplastics and natural food were compared among the four coral species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*. Values of Kruskal-Wallis tests and effect sizes rounded to three decimal places. n_{obs} = number of observations; CI = 95% confidence interval; η^2_H = eta-squared based on the Kruskal-Wallis *H* test.

Long-term conditions	n_{obs}	Statistic (χ^2)	Degrees of freedom	*p*-value	Effect size (η^2_H)	Magnitude of effect size
Microplastic-free	27	2.586	3	0.46	-0.018	small
Microplastic exposure	26	6.383	3	0.094	0.154	large

Table S11: Differences in the ability to discriminate between microplastics and natural food were compared pairwise among the four coral species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*. Dunn's post hoc test results rounded to three decimal places, and *p*-values were adjusted according to the Benjamini-Hochberg method (1995). n_{obs} = number of observations.

Long-term conditions	Comparison					*z*-value	*p*-value	Adjusted *p*-value
	Species	(n_{obs})	vs.	Species	(n_{obs})			
microplastic-free	*A. muricata*	(9)	–	*P. verrucosa*	(6)	-1.461	0.144	0.502
microplastic-free	*A. muricata*	(9)	–	*P. lutea*	(9)	-0.089	0.929	0.929
microplastic-free	*A. muricata*	(9)	–	*H. coerulea*	(3)	-0.525	0.6	0.773
microplastic-free	*P. verrucosa*	(6)	–	*P. lutea*	(9)	1.381	0.167	0.502
microplastic-free	*P. verrucosa*	(6)	–	*H. coerulea*	(3)	0.594	0.553	0.773
microplastic-free	*P. lutea*	(9)	–	*H. coerulea*	(3)	-0.462	0.644	0.773
microplastics	*A. muricata*	(9)	–	*P. verrucosa*	(6)	-0.469	0.639	0.639
microplastics	*A. muricata*	(9)	–	*P. lutea*	(8)	1.162	0.245	0.294
microplastics	*A. muricata*	(9)	–	*H. coerulea*	(3)	-1.613	0.107	0.266
microplastics	*P. verrucosa*	(6)	–	*P. lutea*	(8)	1.503	0.133	0.266
microplastics	*P. verrucosa*	(6)	–	*H. coerulea*	(3)	-1.171	0.242	0.294
microplastics	*P. lutea*	(8)	–	*H. coerulea*	(3)	-2.422	0.015	0.093

Table S12: Differences in the occurrence of reactions to the two long-term exposure conditions (microplastic-free control and microplastic exposure) for the two coral species *A. muricata* and *P. lutea*, separated by pulse exposure condition. *P. verrucosa* and *H. coerulea* did not show physiological reactions and were not tested. Values of Fisher's exact test statistics (alternative hypothesis: two sided) and effect sizes (odds ratios with CI's) rounded to three decimal places. n_{obs} = number of observations; CI = 95% confidence interval; OR = odds ratio.

Species	Pulse exposure condition	n_{obs}	*p*-value	CI low	CI high	OR	OR CI low	OR CI high
A. muricata	microplastics	19	0.35	0.296	45.353	3.2	0.419	24.417
P. lutea	microplastics	18	1	0.141	26.987	1.75	0.215	14.224
A. muricata	*Artemia* sp. cysts	18	1	0.057	17.581	1	0.108	9.229
P. lutea	*Artemia* sp. cysts	18	1	0.094	151.255	2.286	0.169	30.959

Table S13: The occurrence of reactions in the two coral species *A. muricata* and *P. lutea* were compared between the two pulse exposure treatments (microplastics vs. *Artemia* sp. cysts). *P. verrucosa* and *H. coerulea* did not show physiological reactions at all. Values of Chi-squared test statistics and effect sizes (odds ratios with CI's) rounded to three decimal places. Bold numbers indicate significant differences ($p \leq 0.05$). n_{obs} = number of observations; CI = 95% confidence interval; OR = odds ratio.

Species	n_{obs}	Statistic (χ^2)	Degrees of freedom	*p*-value	OR	OR CI low	OR CI high
A. muricata	37	6.19	1	**0.013**	7.583	1.738	33.089
P. lutea	36	0.161	1	0.688	1.923	0.383	9.646

Table S14: The feeding rates of the two coral species *A. muricata* and *P. lutea* were compared between the two possible states of reactions (yes vs. no). *P. verrucosa* and *H. coerulea* did not show physiological reactions at all. Values of Wilcoxon test statistics (alternative hypothesis: two sided) and effect sizes rounded to three decimal places. Bold numbers indicate significant differences ($p \leq 0.05$). yes = reactions are present; no = no reactions present; n_{obs} = number of observations; CI = 95% confidence interval.

Species	Comparison					*z*-value	*p*-value	CI low	CI high	Effect size (*r*-value)	Magnitude of effect size
	Reactions	(n_{obs})	vs.	Reactions	(n_{obs})						
A. muricata	yes	(17)	–	no	(20)	105	**0.048**	-0.401	-0.001	0.326	moderate
P. lutea	yes	(8)	–	no	(28)	86	0.339	-0.35	0.099	0.165	small

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2.2 Reef-building corals may mitigate microplastic effects

> # 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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Marine Pollution Bulletin 205 (2024): 116631

DOI: 10.1016/j.marpolbul.2024.116631

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Published online on 24 June 2024.

Running title: Reef-building corals may mitigate microplastic effects

No. of peer-reviewed citations on the date of submission of the dissertation: 1

Impact factor of the journal at the time of publication: 5.8

Contributions of the author of the dissertation:

Formal Analysis	Statistical analysis
Investigation	Contributed to data collection
Methodology	Contributed to development of methodology
Project administration	Contributed to research activity planning and execution
Resources	Contributed to specific materials
Visualization	Data presentation
Writing – Original Draft	Writing initial draft

Marine Pollution Bulletin 205 (2024) 116631

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

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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ARTICLE INFO

Keywords:
Plastic
Stony corals
Physiology
Long-term exposure
Photosynthesis
Energy reserves

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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https://doi.org/10.1016/j.marpolbul.2024.116631
Received 29 March 2024; Received in revised form 13 June 2024; Accepted 17 June 2024
Available online 24 June 2024

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

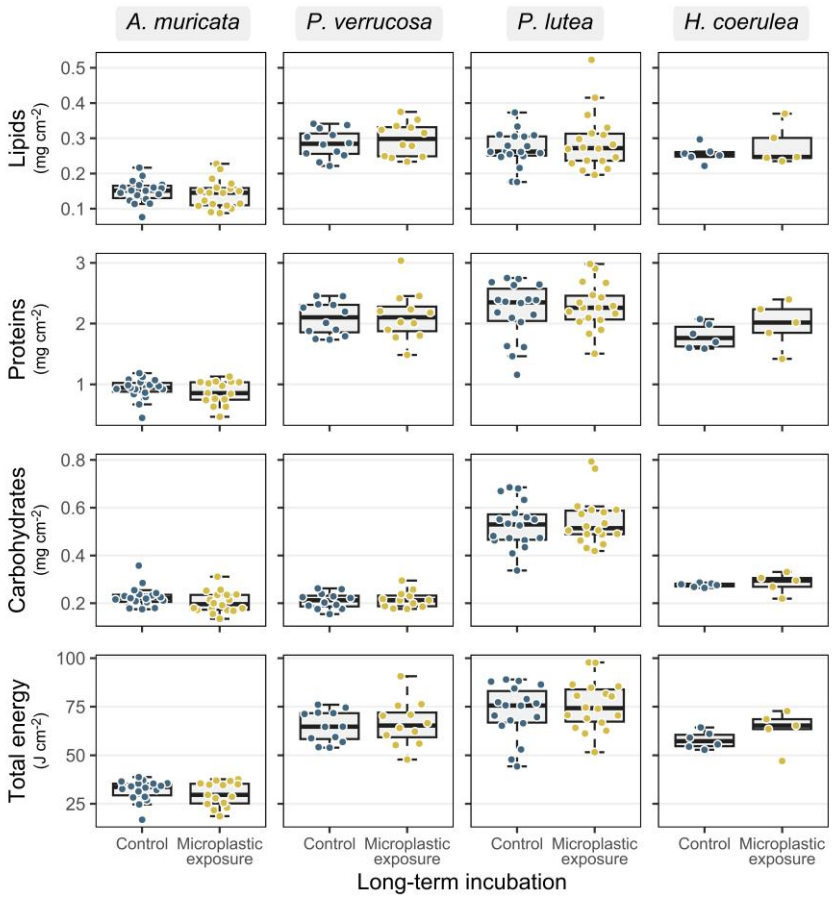

Fig. 1. Energy reserves (i.e., lipids, proteins, and carbohydrates in mg cm^{-2}, total energy in J cm^{-2}) in the four tested coral species (i.e., *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*) exposed to control conditions (blue) and microplastics (yellow). Data are displayed as box-and-whisker 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

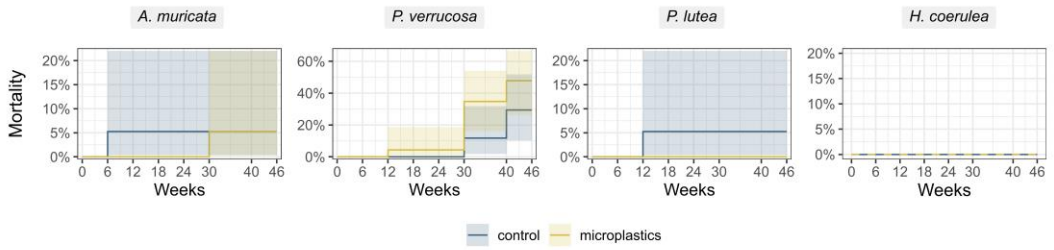

Fig. 2. Mortality (% of corals with >50 % bleached or necrotic tissue) of the four coral species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea* under microplastic exposure (yellow) and control conditions (blue) over the course of the long-term experiment. Mortality was recorded at the 8 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.)

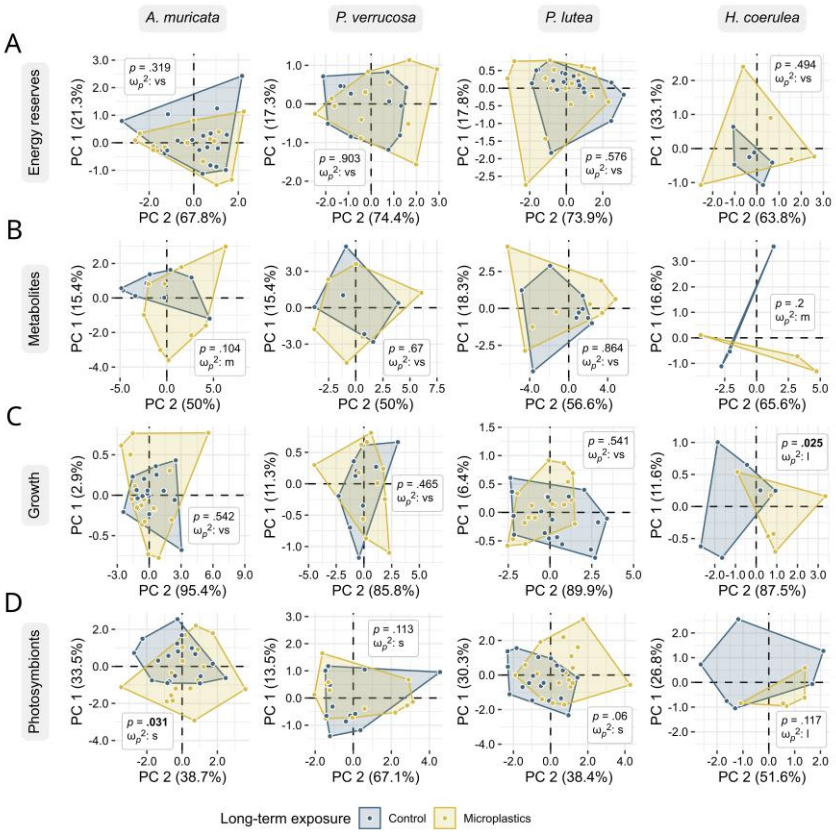

Fig. 3. Differences between control (blue) and microplastic exposure conditions (yellow) in the grouped physiological parameters (rows A–D) and the four coral species tested (columns). Row A: Energy reserves (variables: lipids, proteins, and carbohydrates). Row B: Metabolites (variables: set of 20 metabolites). 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.

Funding

This study was part of the 'Ocean 2100' global change simulation project of the Colombian-German Center of Excellence in Marine Sciences (CEMarin) funded by the German Academic Exchange Service (DAAD, project number 57480468).

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.

Acknowledgments

We thank Angelina Arnold and Patrick Schubert for their contribution to the long-term experiment. We would like to thank the Metabolomics Core Technology Platform of the Excellence Cluster "CellNetworks" (University of Heidelberg; ZUK49/2) for support with the metabolite analyses. We thank Erika Most, Klaus Eder, and the team of the Institute of Animal Nutrition and Nutrition Physiology at the Justus Liebig University Giessen for their help with the energy reserves analyses.

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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Supplementary Materials for

Title

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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Supplementary material and methods

Detailed description of the experimental setup

Corals were fragmented with a compact angle grinder (Dremel 3000, DREMEL Europe, the Netherlands) to ~2–4 cm long terminal branches for *A. muricata*, *P. verrucosa*, and *H. coerulea* and cubes with a surface area of 2 cm × 2 cm for *P. lutea*. Fragments were glued onto self-made concrete bases using a two-component glue (CoraFix SuperFast, Grotech, Germany) to ease the handling. Ninety nubbins were prepared for *Acropora*, *Pocillopora*, and *Porites*, cut equally from three original colonies. For *Heliopora,* 30 nubbins were cut from a single colony due to the lack of replicate colonies. Coral fragments were distributed evenly across the tanks. The corals were randomly distributed within the tanks (n_{total} = 50 tank^{-1}) and shuffled once a week to avoid positional effects. As one colony of *P. verrucosa* experienced high mortality during the experiment, it was excluded from the analyses. This study analyzed a subset of coral fragments.

The six experimental tanks were equipped with a flow pump for horizontal water movement (RW-8, Jebao, China; 700 L h^{-1}) and a feed pump (S 400, Resun, China; 400 L h^{-1}) for a vertical water circulation that re-immersed floating microplastic particles. A UV clarifier (RWUVC/78/4000, RuWal Aquatech, Italy; 33000 mWs^{-1} cm^{-2} at 4000 L h^{-1}) was upstream of the inlet of the six experimental tanks to reduce pathogens. On the outflow side, a fleece membrane was installed downstream of the 65 µm filters to retain smaller plastic particles that fragmentation might have generated over time. Small gastropods ($n \approx 50$ tank^{-1}; *Nassarius* spp., *Euplica* spp., *Turbo* spp., and *Stomatella auricula*) were used to limit algae growth. Coral nubbins were inspected daily and cleaned from algae and detritus if necessary. The connection to a reef mesocosm system included a large "buffer" tank harboring corals, fish, and a deep sand bed, together with a protein skimmer and a calcium reactor (pH 6.2–6.4, coral rubble) and provided near-natural water conditions. The system was set up with artificial seawater (Coral Ocean plus, ATI, Germany), and water parameters were checked once a week (alkalinity: 2.52 mmol L^{-1}, Ca^{2+}: 410 mg L^{-1}, Mg^{2+}: 1230 mg L^{-1}, PO_4^{3-}: < 0.03 mg L^{-1}, NO_3^-: < 0.02 mg L^{-1}, NO_2^-: < 0.01 mg L^{-1}, NH_4^+: < 0.025 mg L^{-1}, salinity: 34). Coral fragments were inspected daily and algae were removed if necessary. Detritus was removed from the tanks by siphoning twice a week. After six months of long-term exposure, several of the coral fragments were snap-frozen in liquid nitrogen, as described in Reichert et al. (2019). The remaining coral fragments were kept under the same experimental conditions for a total

long-term exposure period of 11 months, except for omitted periodical quantification of photosynthetic activity, determination of calcification, and growth assessment.

Documentation of corals' surface area and volume

3D models of the coral fragments were constructed using the Artec Studio 11 software (Artec 3D, Luxembourg). First, fragments were placed on a motorized turntable within a lightbox and scanned within ~90 s from 45- and 90-degree angles. For each fragment, the 3D models of both timepoints (t_0 = start and t_7 = end) were aligned and horizontally trimmed, thus resulting in the concrete base with the coral nubbin. To determine tissue surface area, the models were cropped at the edge of the tissue using the "Eraser" tool, whereby necrotic and bleached areas were also cut away so that only the living coral tissue remained. The final 3D models were saved as OBJ files, and surface area and volume were determined ("compute geometric measures" command) in MeshLab (1.3.4 beta; Cignoni et al., 2008).

Supplementary figures

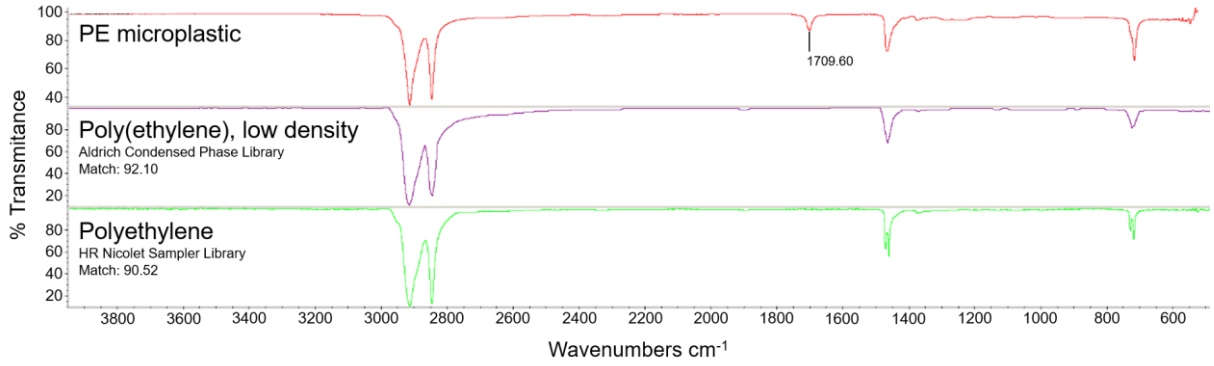


Figure S1: The FTIR spectrum of the polyethylene (PE) microplastics used in the experiment (top, red) was compared to the reference spectra of low-density polyethylene (center in purple and bottom in green). The PE microplastic shows a clear peak at 1709.60 cm^{-1}, which indicates the C=O stretching of the polymer.

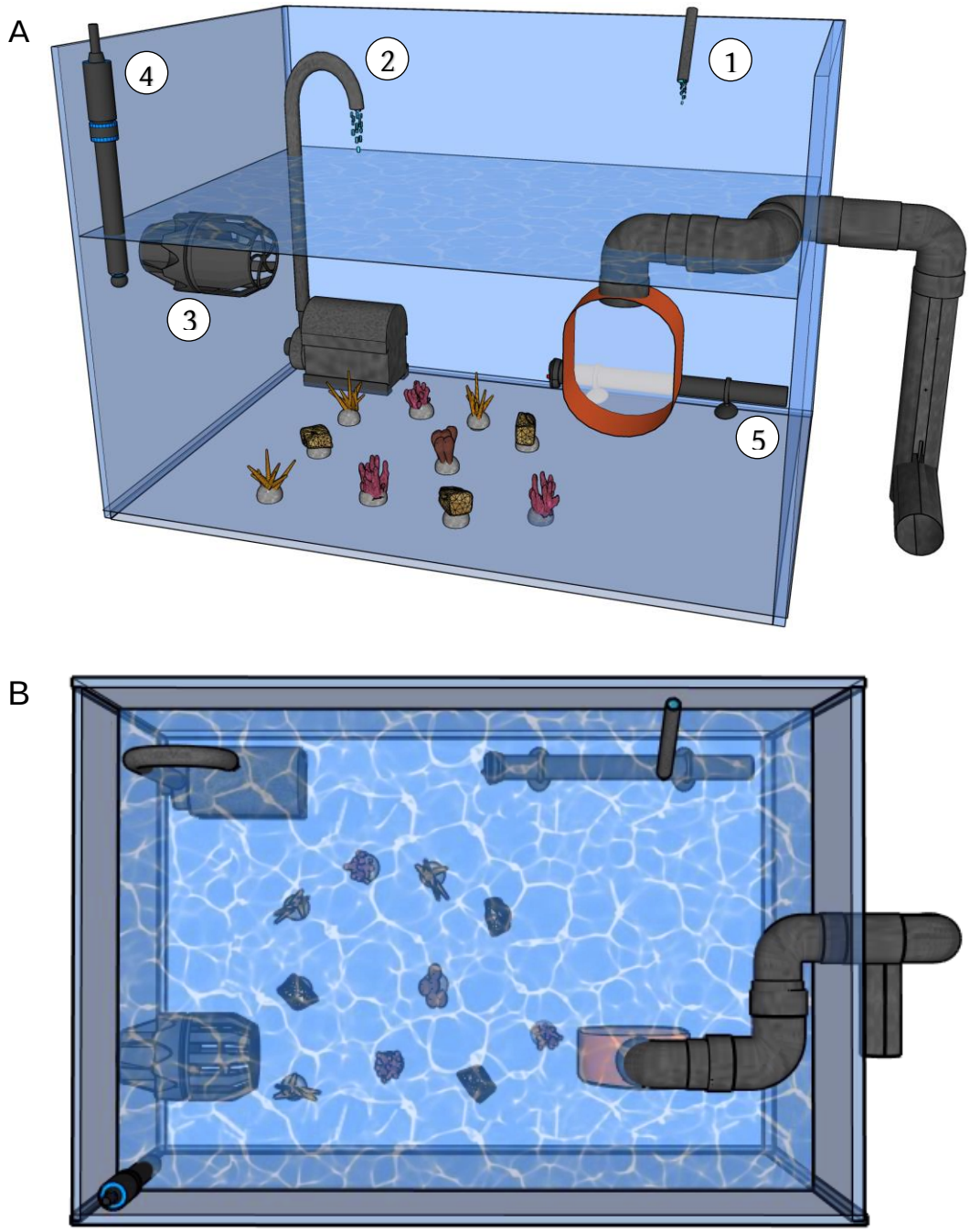


Figure S2: Experimental setup with six tanks (three each for control and microplastic treatment) in side (A) and top view (B). Each tank, as exemplary depicted in the figure for one tank, was equipped with an inlet (1), an outlet with a 65 µm filter (orange frame), a pump for vertical water transport above the water level to submerge floating microplastics (2), a horizontally aligned flow pump (3), and a temperature probe (4). A heating rod was used to temper the water (5). The coral fragments were placed centrally on the bottom of the tanks, growing on their concrete bases. Two lamps with fluorescent tubes (2 × 80 W Aquablue Special and 2 × 80 W Blue Plus, ATI, Germany) covered three tanks each (not shown in the figure).

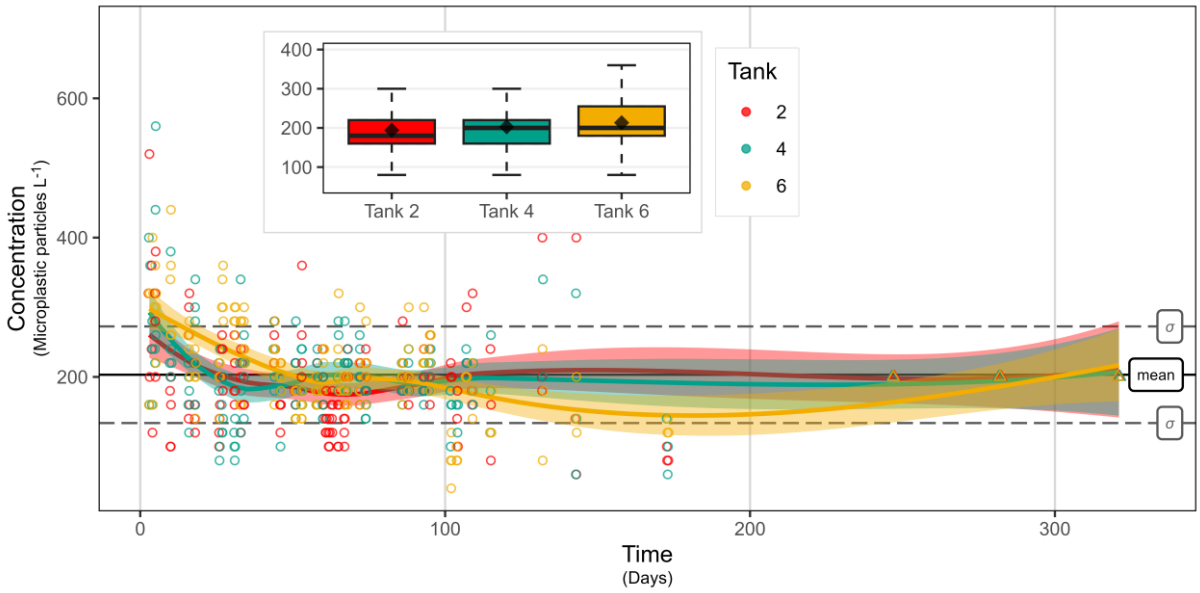


Figure S3: Microplastic concentration in the three tanks with the microplastic particle treatment shown during the experiment. Concentrations are presented as raw data points (colored circles; with jitter to aid visualization of overlapping circles), the overall mean (black solid line) and its standard deviation (σ, gray dashed lines), and local polynomial regression fittings (colored lines) with their 95% confidence intervals (colored shading). The inset figure shows the microplastic concentrations per tank as boxplots and the respective mean values (black diamonds). Microplastic concentrations were monitored at least weekly for the first four months of the experiment until concentrations stabilized. Concentrations were then measured less frequently and later verified to be within a range of 200 ± 50 particles L^{-1}, but not recorded.

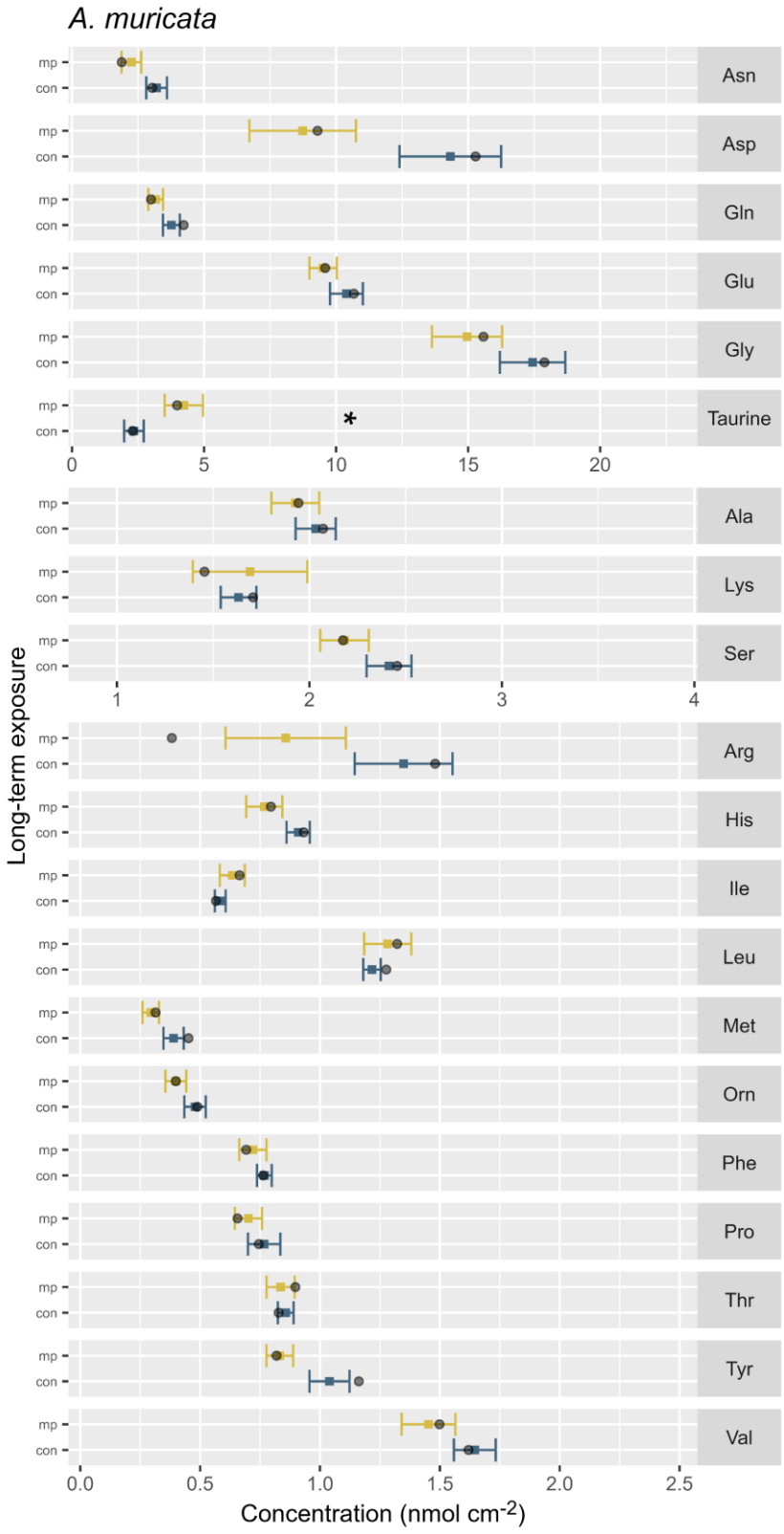


Figure S4: Concentration of selected amino acids in *A. muricata*. Per metabolite, concentrations are compared between microplastic exposure conditions (yellow) and control conditions (blue). Data is shown as mean (box) with SEM (line). The black dots represent the medians. The asterisk highlights that taurine was significantly higher under microplastic exposure. Statistical details are presented in Table S9.

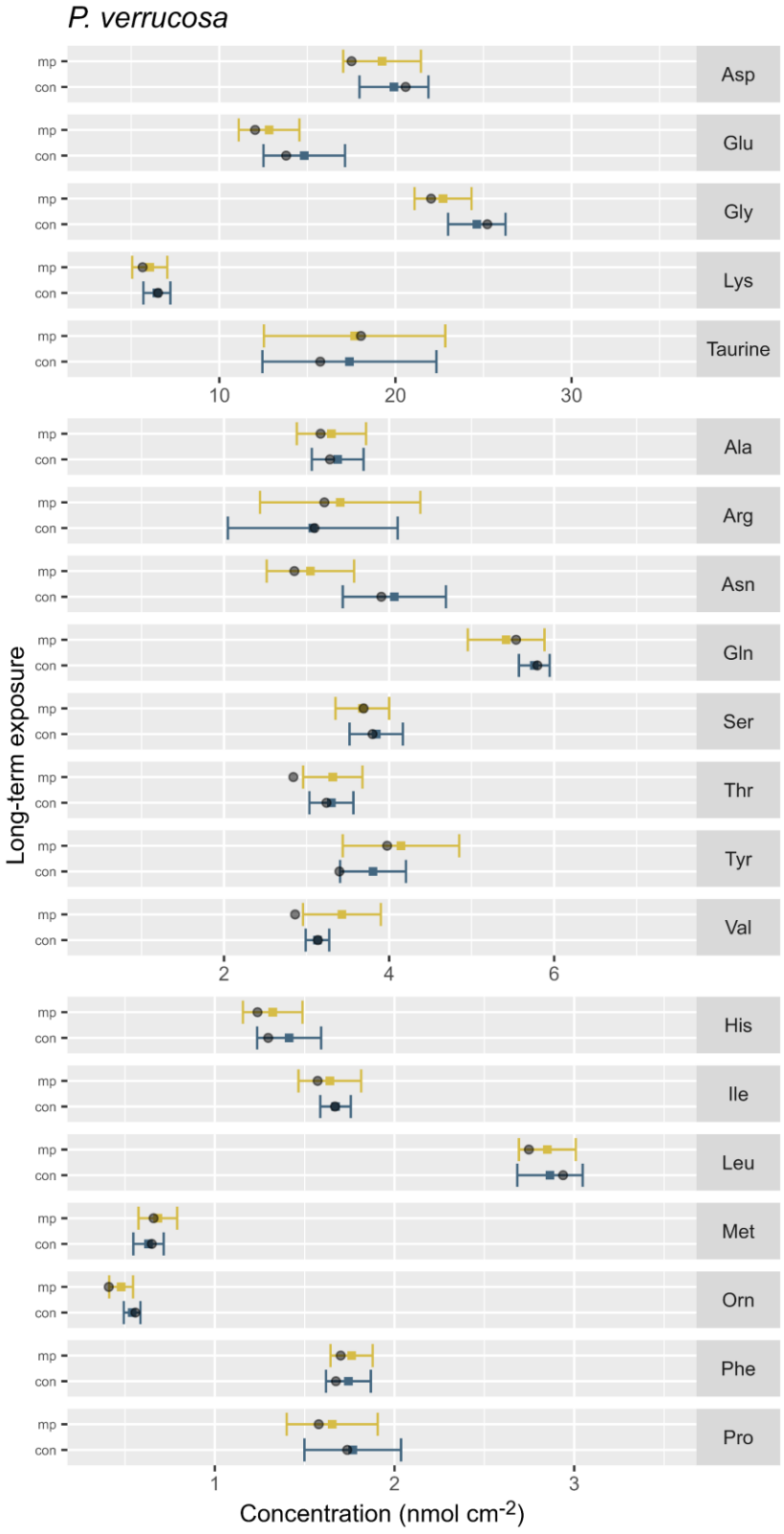


Figure S5: Concentration of selected amino acids in *P. verrucosa*. Per metabolite, concentrations are compared between microplastic exposure conditions (yellow) and control conditions (blue). Data is shown as mean (box) with SEM (line). The black dots represent the medians. Statistical details are presented in Table S9.

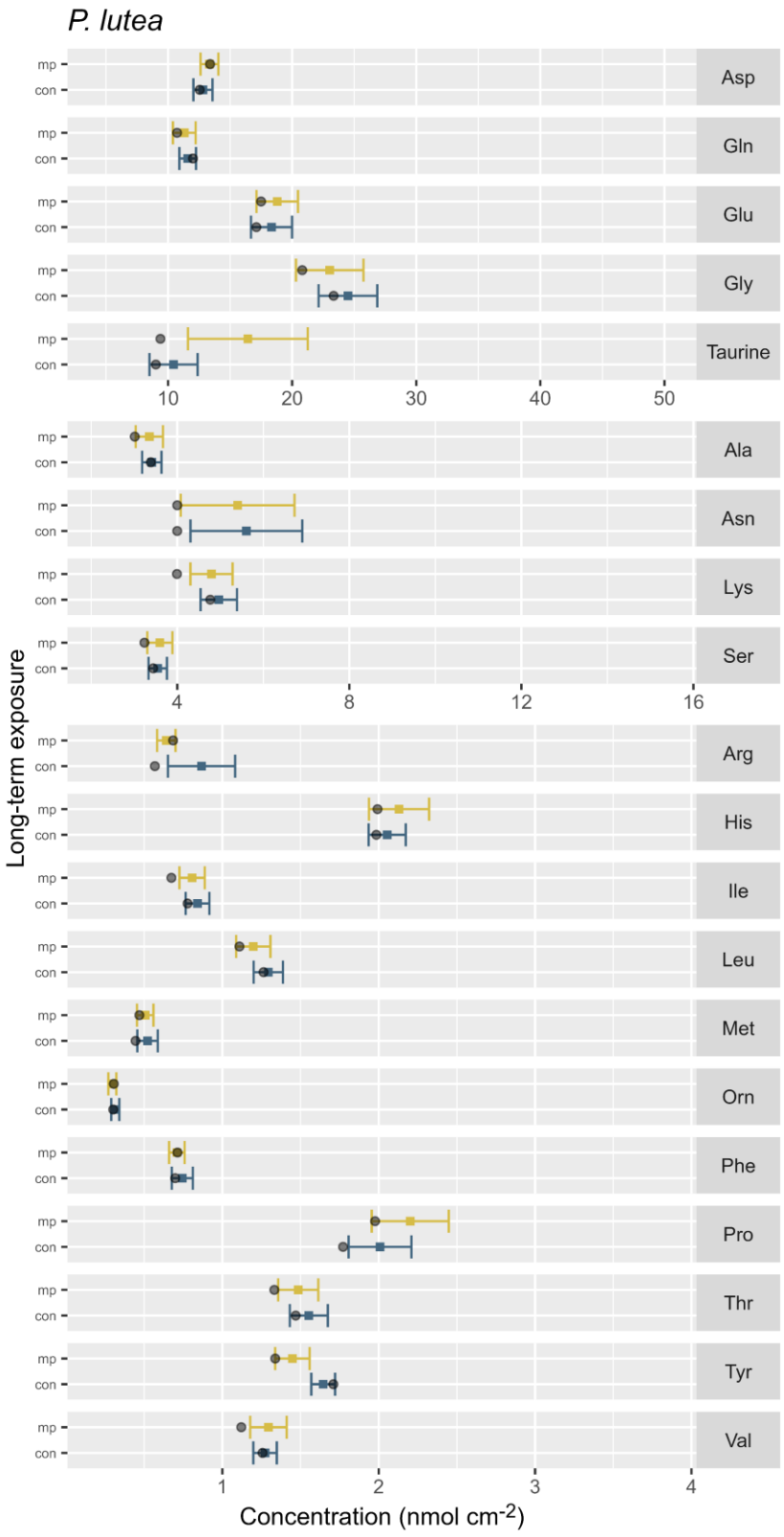


Figure S6: Concentration of selected amino acids in *P. lutea*. Per metabolite, concentrations are compared between microplastic exposure conditions yellow) and control conditions (blue). Data is shown as mean (box) with SEM (line). The black dots represent the medians. Statistical details are presented in Table S9.

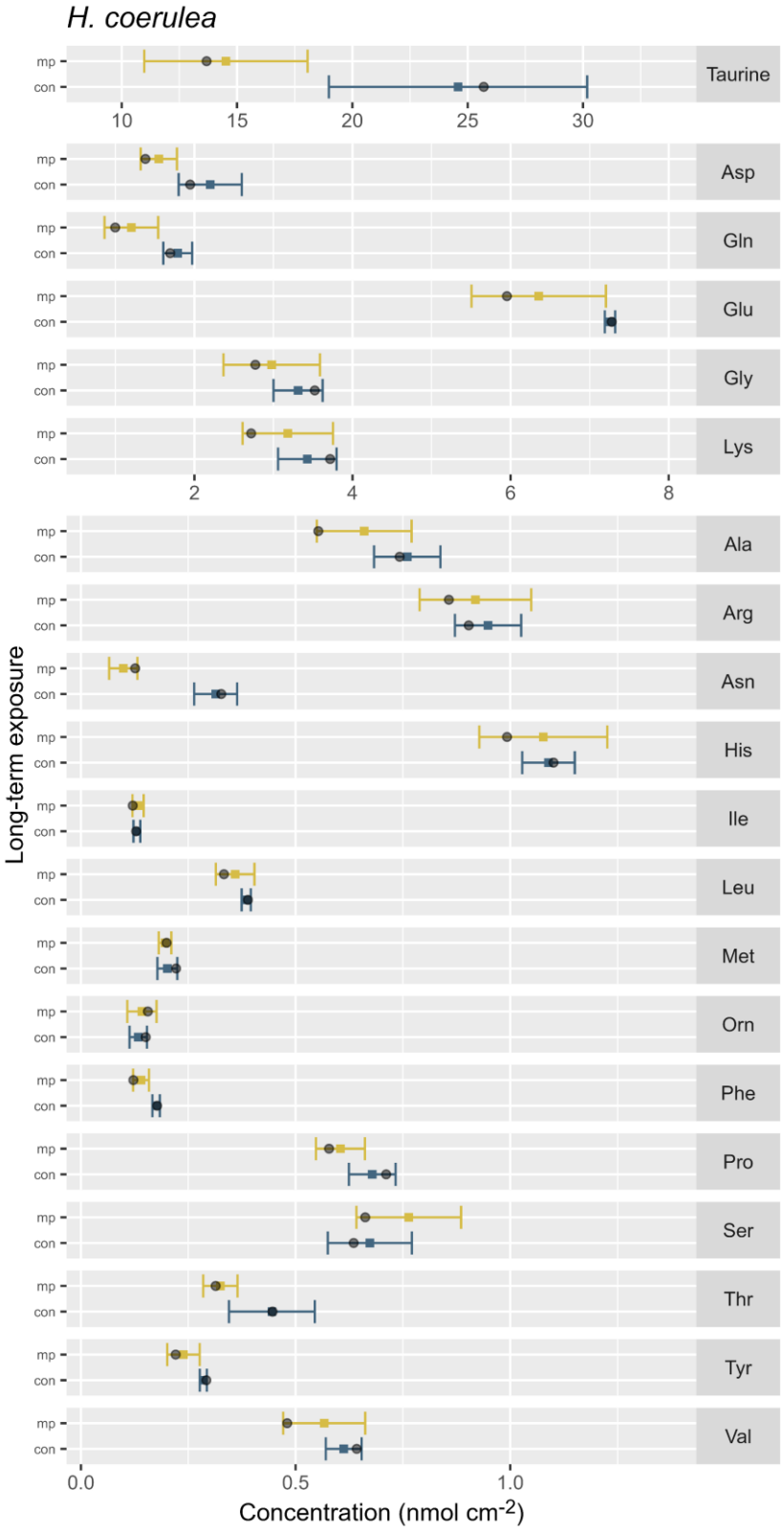


Figure S7: Concentration of selected amino acids in *H. coerulea*. Per metabolite, concentrations are compared between microplastic exposure conditions (yellow) and control conditions (blue). Data is shown as mean (box) with SEM (line); *n* = 3 per group per amino acid. The black dots represent the medians. Statistical details are presented in Table S9.

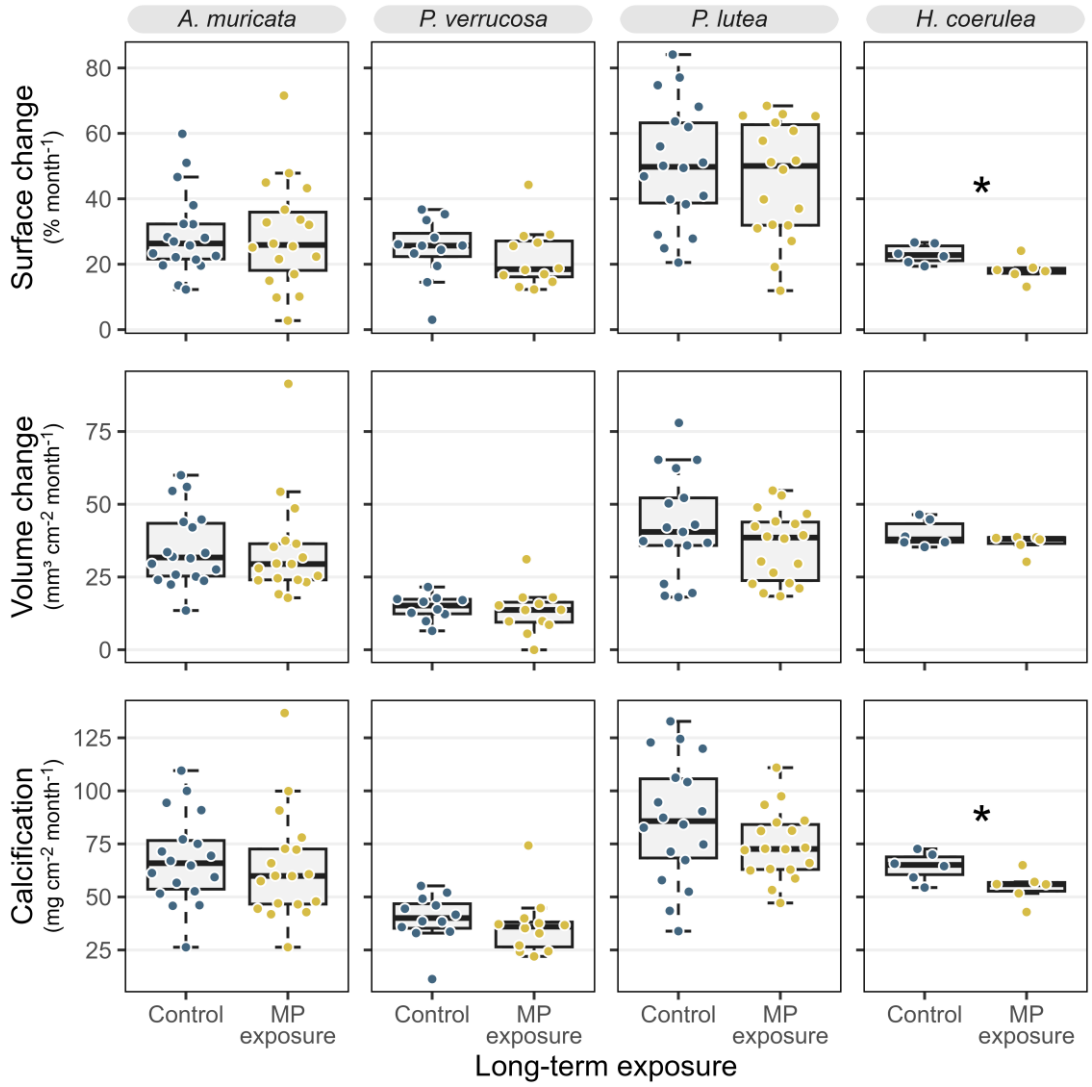


Figure S8: Coral growth parameters (i.e., change in surface area, volume, and weight) are shown for the coral species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*. Per parameter and species, raw data points of individual coral fragments are shown with a box-and-whisker plot per treatment: Control (blue) compared to microplastic exposure (yellow). Asterisks highlight significantly lower changes in surface area and weight in *H. coerulea*. t_0 = start, t_7 = end of the experiment. Statistical details are presented in Tables S10, S11, and S12.

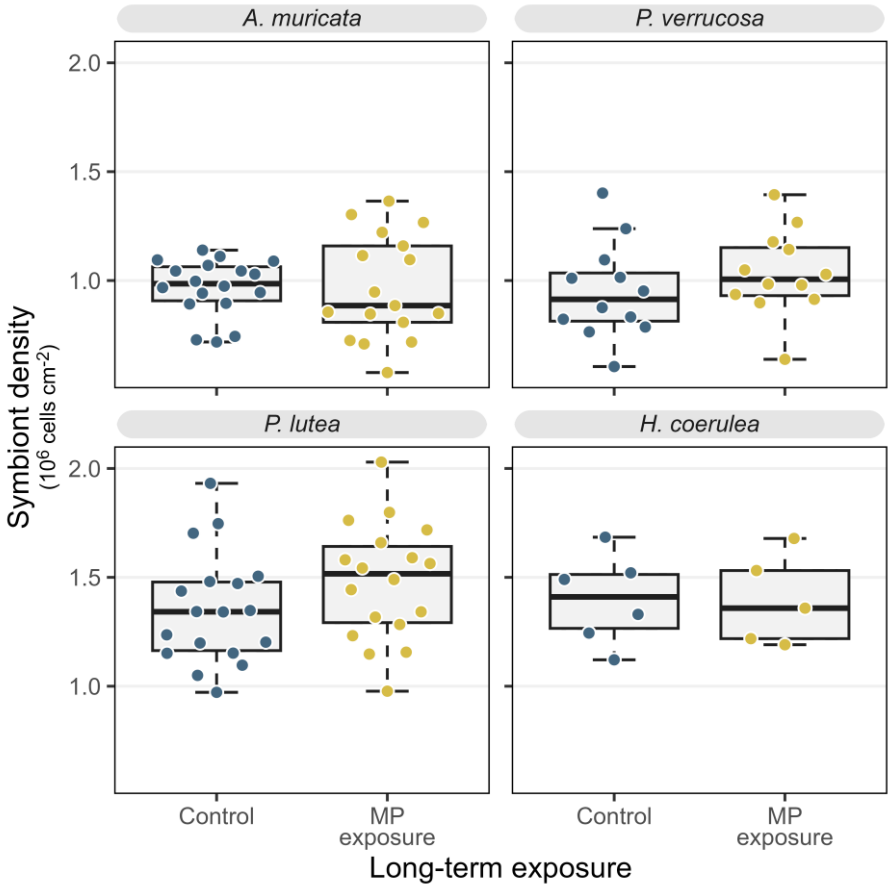


Figure S9: Densities of symbiotic dinoflagellates of the family Symbiodiniaceae in tissues of coral species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*. Per species, raw data points of individual coral fragments are shown with a box-and-whisker plot per treatment: Control (blue) compared to microplastic exposure (yellow). Statistical details are presented in Table S13.

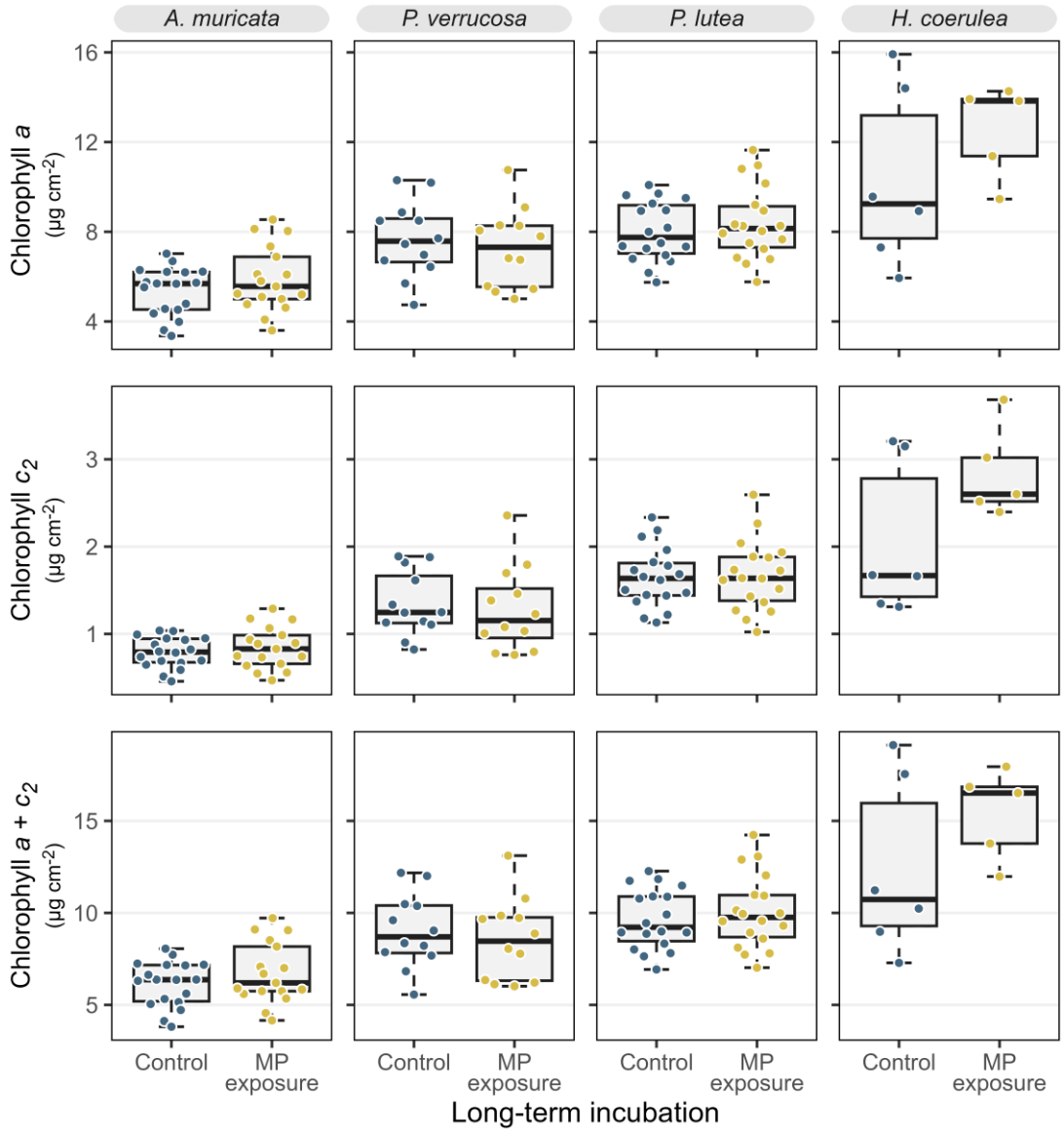


Figure S10: Chlorophyll concentrations (i.e., *a*, c_2, and total chlorophyll) in tissues of the coral species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*. Per parameter and species, raw data points of individual coral fragments are shown with a box-and-whisker plot per treatment: Control (blue) compared to microplastic exposure (yellow). Statistical details are presented in Table S14.

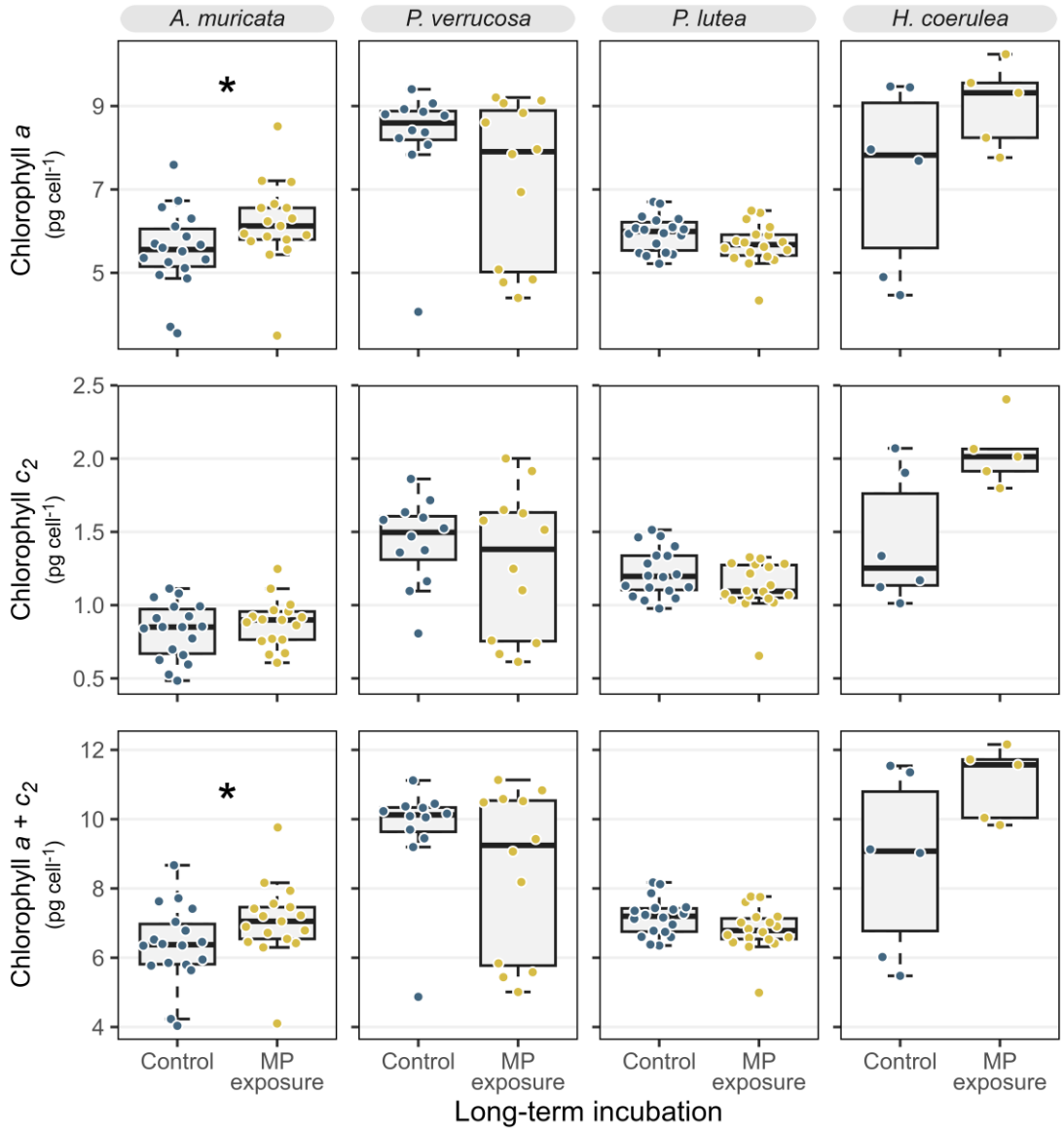


Figure S11: Chlorophyll concentrations (i.e., *a*, c_2, and total chlorophyll) per symbiont cell of the coral species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea.* Per parameter and species, raw data points of individual coral fragments are shown with a box-and-whisker plot per treatment: Control (blue) compared to microplastic exposure (yellow). Asterisks indicate statistically significant differences. Statistical details are presented in Table S15.

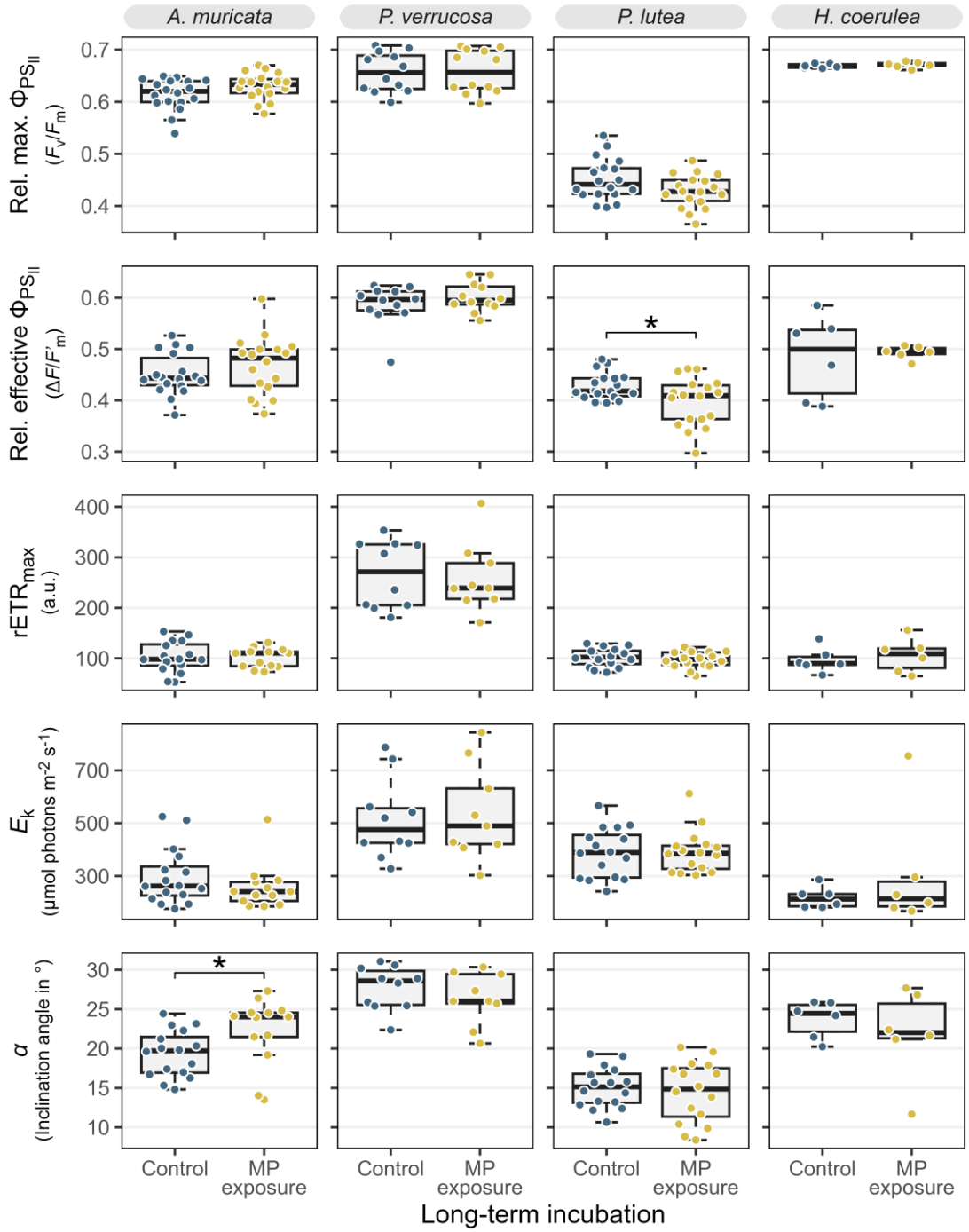


Figure S12: Parameters of photosynthetic efficiency derived from PAM analysis of coral species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*. Per parameter and species, raw data points of individual coral fragments are shown with a box-and-whisker plot per treatment: Control (blue) compared to microplastic exposure (yellow). Asterisks highlight significantly higher light-adapted yield ($\Delta F/F'_m$) in fragments of *P. lutea* exposed to control conditions and a higher α in fragments of *A. muricata* exposed to microplastics. ϕ_{PSII} = quantum yield of photosystem II. a.u. = arbitrary unit. Statistical details are presented in Table S16.

Supplementary tables

Table S1: CITES numbers and origin of coral colonies studied. The origin, dates of collection, CITES numbers, and arrival dates at the aquarium facilities at Justus Liebig University are given for the colonies studied.

Species	Colony	Origin	Collection	Arrival	CITES number
Acropora muricata	A	Indonesia	12/2007	12/2007	14846/IV/SATS-LN/2007
Acropora muricata	B*	Zoo Frankfurt, Germany	NA	05/2015	NA
Acropora muricata	C	Indonesia	12/2007	12/2007	14846/IV/SATS-LN/2007
Pocillopora verrucosa	A	Saudi Arabia	05/2015	06/2015	15-SA-000882-PD
Pocillopora verrucosa	B	Indonesia	04/2014	05/2014	14NL214371/11
Pocillopora verrucosa	C	Indonesia	12/2007	12/2007	14846/IV/SATS-LN/2007
Porites lutea	A	Indonesia	05/2014	05/2014	14-NL-216270-11
Porites lutea	B	Indonesia	05/2014	05/2014	14-NL-216270-11
Porites lutea	C	Indonesia	05/2014	05/2014	14-NL-216270-11
Heliopora coerulea	A*	Zoo Frankfurt, Germany	NA	05/2015	NA

* Details of these colonies are unavailable as they were collected before the CITES regulations became effective.

Table S2: Overview of coral fragments used for the physiological parameters. Individual statistical tests (Tables S4–S18) may differ due to missing data.

species	$n_{fragments}$											
	all	per colony			per tank no.						per treatment	
		A	B	C	1	2	3	4	5	6	con	mp
A. muricata	36	12	12	12	6	6	6	6	6	6	18	18
P. verrucosa	24	0	12	12	4	4	4	4	4	4	12	12
P. lutea	36	12	12	12	6	6	6	6	6	6	18	18
H. coerulea	12	12	0	0	2	2	2	2	2	2	6	6

Table S3: Coral fragments used for metabolite analysis. Number (n_{total} = 54) of fragments for the species *Acropora muricata*, *Pocillopora verrucosa*, *Porites lutea*, and *Heliopora coerulea*, overall, and divided per colony, per tank, and per treatment used for amino acid determination.

species	$n_{fragments}$											
	all	per colony			per tank no.						per treatment	
		A	B	C	1	2	3	4	5	6	con	mp
A. muricata	18	6	5	7	2	3	3	2	4	4	9	9
P. verrucosa	12	0	6	6	2	2	2	2	2	2	6	6
P. lutea	18	6	6	6	3	3	3	3	3	3	9	9
H. coerulea	6	6	0	0	1	1	1	1	1	1	3	3

Table S4: 3D model calculation settings. Settings in Artec Studio 10 used to calculate the 3D models of *Acropora muricata*, *Pocillopora verrucosa*, *Porites lutea*, and *Heliopora coerulea*.

	A. muricata	*P. verrucosa*	*P. lutea*	*H. coerulea*
Fine serial registration	Texture and Geometry, loop closure: off			
Global registration	Texture and Geometry, minimal distance 10, iterations 2000			
Outliers removal	Std Dev 3	Std Dev 5	Std Dev 5	Std Dev 5
	Res 0.2	Res 0.2	Res 0.2	Res 0.2
Sharp fusion	Res 0.2			
	Fill holes by radius, max. hole radius 0.5			
Small objects filter	Leave biggest object			

Table S5: Comparison of lipid content between both long-term conditions (control vs. microplastic exposure) for the species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*. Student's *t*-test results (alternative hypothesis: two-sided) and effect sizes (Cohen's *d*). n_{obs} = number of observations; CI = 95% confidence interval.

	Comparison										
Species	Long-term conditions	(n_{obs})	vs.	Long-term conditions	(n_{obs})	d.f.	*t*-value	*p*-value	CI low	CI high	Effect size (*d*-value)	Magnitude of effect size
A. muricata	control	(18)	–	microplastics	(18)	34	0.618	0.541	-0.017	0.032	0.206	small
P. verrucosa	control	(12)	–	microplastics	(12)	22	-0.713	0.484	-0.050	0.025	-0.291	small
P. lutea	control	(18)	–	microplastics	(18)	34	-0.895	0.377	-0.066	0.026	-0.299	small
H. coerulea	control	(6)	–	microplastics	(5)	9	-0.928	0.378	-0.081	0.034	-0.562	moderate

Table S6: Comparison of protein content between both long-term conditions (control vs. microplastic exposure) for the species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*. Student's *t*-test results (alternative hypothesis: two-sided) and effect sizes (Cohen's *d*). n_{obs} = number of observations; CI = 95% confidence interval.

	Comparison										
Species	Long-term conditions	(n_{obs})	vs.	Long-term conditions	(n_{obs})	d.f.	*t*-value	*p*-value	CI low	CI high	Effect size (*d*-value)	Magnitude of effect size
A. muricata	control	(18)	–	microplastics	(15)	31	1.002	0.324	-0.066	0.194	0.350	small
P. verrucosa	control	(12)	–	microplastics	(12)	22	-0.274	0.787	-0.328	0.252	-0.112	negligible
P. lutea	control	(18)	–	microplastics	(18)	34	-0.574	0.570	-0.368	0.206	-0.191	negligible
H. coerulea	control	(6)	–	microplastics	(5)	9	-1.060	0.317	-0.592	0.214	-0.642	moderate

Table S7: Comparison of carbohydrate content between both long-term conditions (control vs. microplastic exposure) for the species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*. Wilcoxon test results (alternative hypothesis: two-sided) and effect sizes (Pearson's *r*). n_{obs} = number of observations; CI = 95% confidence interval.

	Comparison									
Species	Long-term conditions	(n_{obs})	vs.	Long-term conditions	(n_{obs})	*t*-value	*p*-value	CI low	CI high	Effect size (*r*-value)	Magnitude of effect size
A. muricata	control	(18)	–	microplastics	(18)	210	0.134	-0.006	0.048	0.253	small
P. verrucosa	control	(12)	–	microplastics	(12)	70	0.932	-0.036	0.026	0.024	small
P. lutea	control	(18)	–	microplastics	(18)	151	0.743	-0.071	0.055	0.058	small
H. coerulea	control	(6)	–	microplastics	(5)	10	0.429	-0.052	0.057	0.275	small

Table S8: Comparison of total energy content between both long-term conditions (control vs. microplastic exposure) for the species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*. Wilcoxon test results (alternative hypothesis: two-sided) and effect sizes (Pearson's *r*). n_{obs} = number of observations; CI = 95% confidence interval.

	Comparison									
Species	Long-term conditions	(n_{obs})	vs.	Long-term conditions	(n_{obs})	*t*-value	*p*-value	CI low	CI high	Effect size (*r*-value)	Magnitude of effect size
A. muricata	control	(18)	–	microplastics	(15)	152	0.556	-2.182	6.775	0.107	small
P. verrucosa	control	(12)	–	microplastics	(12)	69	0.887	-10.827	7.917	0.035	small
P. lutea	control	(18)	–	microplastics	(18)	153	0.791	-10.997	6.660	0.048	small
H. coerulea	control	(6)	–	microplastics	(5)	7	0.177	-15.784	8.556	0.440	moderate

Table S9: Comparison of metabolite contents between both long-term conditions (control vs. microplastic exposure) for the species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*. Wilcoxon test results (alternative hypothesis: two-sided) and effect sizes (Pearson's *r*). n_{obs} = number of observations; CI = 95% confidence interval. *Amu* = *A. muricata*, *Pve* = *P. verrucosa*, *Plu* = *P. lutea*, *Hel* = *H. coerulea*.

		Comparison										
Species	*Metabolite*	*Long-term condition*	*(n_{obs})*	*vs.*	*Long-term condition*	*(n_{obs})*	*t-value*	*p-value*	*CI low*	*CI high*	*Effect size (d-value)*	*Magnitude of Effect size*
Amu	*Ala*	*con*	*(9)*	–	*mp*	*(9)*	*49*	*0.489*	*-0.237*	*0.520*	*0.177*	*small*
Amu	*Arg*	*con*	*(9)*	–	*mp*	*(9)*	*59*	*0.113*	*-0.245*	*1.326*	*0.385*	*moderate*
Amu	*Asn*	*con*	*(9)*	–	*mp*	*(9)*	*61*	*0.077*	*-0.414*	*2.093*	*0.427*	*moderate*
Amu	*Asp*	*con*	*(9)*	–	*mp*	*(9)*	*61*	*0.077*	*-1.105*	*12.324*	*0.427*	*moderate*
Amu	*Gln*	*con*	*(9)*	–	*mp*	*(9)*	*53*	*0.297*	*-0.358*	*1.675*	*0.260*	*small*
Amu	*Glu*	*con*	*(9)*	–	*mp*	*(9)*	*53*	*0.297*	*-1.029*	*2.817*	*0.260*	*small*
Amu	*Gly*	*con*	*(9)*	–	*mp*	*(9)*	*59*	*0.113*	*-1.205*	*6.609*	*0.385*	*moderate*
Amu	*His*	*con*	*(9)*	–	*mp*	*(9)*	*54*	*0.258*	*-0.073*	*0.356*	*0.281*	*small*
Amu	*Ile*	*con*	*(9)*	–	*mp*	*(9)*	*26*	*0.222*	*-0.171*	*0.072*	*0.302*	*moderate*
Amu	*Leu*	*con*	*(9)*	–	*mp*	*(9)*	*27*	*0.258*	*-0.288*	*0.086*	*0.281*	*small*
Amu	*Lys*	*con*	*(9)*	–	*mp*	*(9)*	*51*	*0.387*	*-0.368*	*0.540*	*0.219*	*small*
Amu	*Met*	*con*	*(9)*	–	*mp*	*(9)*	*60*	*0.094*	*-0.053*	*0.263*	*0.406*	*moderate*
Amu	*Orn*	*con*	*(9)*	–	*mp*	*(9)*	*55*	*0.222*	*-0.073*	*0.233*	*0.302*	*moderate*
Amu	*Phe*	*con*	*(9)*	–	*mp*	*(9)*	*52*	*0.340*	*-0.084*	*0.182*	*0.239*	*small*
Amu	*Pro*	*con*	*(9)*	–	*mp*	*(9)*	*46*	*0.666*	*-0.147*	*0.236*	*0.114*	*small*
Amu	*Ser*	*con*	*(9)*	–	*mp*	*(9)*	*54*	*0.258*	*-0.144*	*0.680*	*0.281*	*small*
Amu	*Tau*	*con*	*(9)*	–	*mp*	*(9)*	*12*	***0.011***	*-3.191*	*-0.230*	*0.593*	*large*
Amu	*Thr*	*con*	*(9)*	–	*mp*	*(9)*	*43*	*0.863*	*-0.132*	*0.164*	*0.052*	*small*
Amu	*Tyr*	*con*	*(9)*	–	*mp*	*(9)*	*61*	*0.077*	*-0.037*	*0.483*	*0.427*	*moderate*
Amu	*Val*	*con*	*(9)*	–	*mp*	*(9)*	*52*	*0.340*	*-0.133*	*0.504*	*0.239*	*small*
Pve	*Ala*	*con*	*(6)*	–	*mp*	*(6)*	*20*	*0.818*	*-1.363*	*1.326*	*0.092*	*small*
Pve	*Arg*	*con*	*(6)*	–	*mp*	*(6)*	*16*	*0.818*	*-4.170*	*3.640*	*0.092*	*small*
Pve	*Asn*	*con*	*(6)*	–	*mp*	*(6)*	*25*	*0.310*	*-0.584*	*3.066*	*0.324*	*moderate*
Pve	*Asp*	*con*	*(6)*	–	*mp*	*(6)*	*19*	*0.937*	*-6.183*	*8.510*	*0.046*	*small*

Pve	*Gln*	*con*	*(6)*	–	*mp*	*(6)*	*21*	*0.699*	*-0.926*	*1.719*	*0.139*	*small*
Pve	*Glu*	*con*	*(6)*	–	*mp*	*(6)*	*21*	*0.699*	*-4.535*	*9.513*	*0.139*	*small*
Pve	*Gly*	*con*	*(6)*	–	*mp*	*(6)*	*23*	*0.485*	*-3.624*	*6.980*	*0.231*	*small*
Pve	*His*	*con*	*(6)*	–	*mp*	*(6)*	*20*	*0.818*	*-0.428*	*0.633*	*0.092*	*small*
Pve	*Ile*	*con*	*(6)*	–	*mp*	*(6)*	*23*	*0.485*	*-0.455*	*0.445*	*0.231*	*small*
Pve	*Leu*	*con*	*(6)*	–	*mp*	*(6)*	*18*	*1.000*	*-0.483*	*0.628*	*0.000*	*small*
Pve	*Lys*	*con*	*(6)*	–	*mp*	*(6)*	*23*	*0.485*	*-2.359*	*3.671*	*0.231*	*small*
Pve	*Met*	*con*	*(6)*	–	*mp*	*(6)*	*17*	*0.937*	*-0.388*	*0.282*	*0.046*	*small*
Pve	*Orn*	*con*	*(6)*	–	*mp*	*(6)*	*25*	*0.310*	*-0.163*	*0.237*	*0.324*	*moderate*
Pve	*Phe*	*con*	*(6)*	–	*mp*	*(6)*	*15*	*0.699*	*-0.397*	*0.419*	*0.139*	*small*
Pve	*Pro*	*con*	*(6)*	–	*mp*	*(6)*	*19*	*0.937*	*-0.734*	*1.057*	*0.046*	*small*
Pve	*Ser*	*con*	*(6)*	–	*mp*	*(6)*	*21*	*0.699*	*-0.961*	*1.120*	*0.139*	*small*
Pve	*Tau*	*con*	*(6)*	–	*mp*	*(6)*	*18*	*1.000*	*-17.822*	*17.667*	*0.000*	*small*
Pve	*Thr*	*con*	*(6)*	–	*mp*	*(6)*	*21*	*0.699*	*-1.204*	*0.739*	*0.139*	*small*
Pve	*Tyr*	*con*	*(6)*	–	*mp*	*(6)*	*18*	*1.000*	*-2.124*	*1.117*	*0.000*	*small*
Pve	*Val*	*con*	*(6)*	–	*mp*	*(6)*	*21*	*0.699*	*-1.886*	*0.674*	*0.139*	*small*
Plu	*Ala*	*con*	*(9)*	–	*mp*	*(9)*	*47*	*0.605*	*-0.757*	*0.989*	*0.135*	*small*
Plu	*Arg*	*con*	*(9)*	–	*mp*	*(9)*	*39*	*0.931*	*-0.269*	*0.715*	*0.031*	*small*
Plu	*Asn*	*con*	*(9)*	–	*mp*	*(9)*	*40*	*1.000*	*-1.742*	*2.359*	*0.010*	*small*
Plu	*Asp*	*con*	*(9)*	–	*mp*	*(9)*	*33*	*0.546*	*-2.782*	*2.175*	*0.156*	*small*
Plu	*Gln*	*con*	*(9)*	–	*mp*	*(9)*	*44*	*0.796*	*-2.104*	*3.366*	*0.073*	*small*
Plu	*Glu*	*con*	*(9)*	–	*mp*	*(9)*	*37*	*0.796*	*-4.639*	*4.522*	*0.073*	*small*
Plu	*Gly*	*con*	*(9)*	–	*mp*	*(9)*	*48*	*0.546*	*-3.755*	*8.101*	*0.156*	*small*
Plu	*His*	*con*	*(9)*	–	*mp*	*(9)*	*41*	*1.000*	*-0.675*	*0.423*	*0.010*	*small*
Plu	*Ile*	*con*	*(9)*	–	*mp*	*(9)*	*48*	*0.546*	*-0.238*	*0.241*	*0.156*	*small*
Plu	*Leu*	*con*	*(9)*	–	*mp*	*(9)*	*50*	*0.436*	*-0.192*	*0.404*	*0.198*	*small*
Plu	*Lys*	*con*	*(9)*	–	*mp*	*(9)*	*47*	*0.605*	*-1.239*	*1.606*	*0.135*	*small*
Plu	*Met*	*con*	*(9)*	–	*mp*	*(9)*	*41*	*1.000*	*-0.145*	*0.149*	*0.010*	*small*
Plu	*Orn*	*con*	*(9)*	–	*mp*	*(9)*	*43*	*0.863*	*-0.058*	*0.104*	*0.052*	*small*
Plu	*Phe*	*con*	*(9)*	–	*mp*	*(9)*	*43*	*0.863*	*-0.160*	*0.230*	*0.052*	*small*
Plu	*Pro*	*con*	*(9)*	–	*mp*	*(9)*	*34*	*0.605*	*-0.819*	*0.393*	*0.135*	*small*
Plu	*Ser*	*con*	*(9)*	–	*mp*	*(9)*	*40*	*1.000*	*-1.078*	*0.741*	*0.010*	*small*

Plu	*Tau*	*con*	*(9)*	*–*	*mp*	*(9)*	*31*	*0.436*	*-12.533*	*3.786*	*0.198*	*small*
Plu	*Thr*	*con*	*(9)*	*–*	*mp*	*(9)*	*44*	*0.796*	*-0.355*	*0.437*	*0.073*	*small*
Plu	*Tyr*	*con*	*(9)*	*–*	*mp*	*(9)*	*53*	*0.297*	*-0.138*	*0.527*	*0.260*	*small*
Plu	*Val*	*con*	*(9)*	*–*	*mp*	*(9)*	*44*	*0.796*	*-0.400*	*0.268*	*0.073*	*small*
Hel	*Ala*	*con*	*(3)*	*–*	*mp*	*(3)*	*7*	*0.400*	*-0.245*	*0.356*	*0.445*	*moderate*
Hel	*Arg*	*con*	*(3)*	*–*	*mp*	*(3)*	*5*	*1.000*	*-0.326*	*0.368*	*0.089*	*small*
Hel	*Asn*	*con*	*(3)*	*–*	*mp*	*(3)*	*9*	*0.100*	*0.085*	*0.360*	*0.802*	*large*
Hel	*Asp*	*con*	*(3)*	*–*	*mp*	*(3)*	*7*	*0.400*	*-0.333*	*1.718*	*0.445*	*moderate*
Hel	*Gln*	*con*	*(3)*	*–*	*mp*	*(3)*	*7*	*0.400*	*-0.334*	*1.397*	*0.445*	*moderate*
Hel	*Glu*	*con*	*(3)*	*–*	*mp*	*(3)*	*6*	*0.700*	*-0.853*	*2.232*	*0.267*	*small*
Hel	*Gly*	*con*	*(3)*	*–*	*mp*	*(3)*	*5*	*1.000*	*-1.424*	*1.674*	*0.089*	*small*
Hel	*His*	*con*	*(3)*	*–*	*mp*	*(3)*	*5*	*1.000*	*-0.389*	*0.317*	*0.089*	*small*
Hel	*Ile*	*con*	*(3)*	*–*	*mp*	*(3)*	*4*	*1.000*	*-0.042*	*0.026*	*0.089*	*small*
Hel	*Leu*	*con*	*(3)*	*–*	*mp*	*(3)*	*6*	*0.700*	*-0.082*	*0.104*	*0.267*	*small*
Hel	*Lys*	*con*	*(3)*	*–*	*mp*	*(3)*	*5*	*1.000*	*-1.624*	*1.365*	*0.089*	*small*
Hel	*Met*	*con*	*(3)*	*–*	*mp*	*(3)*	*6*	*0.700*	*-0.064*	*0.058*	*0.267*	*small*
Hel	*Orn*	*con*	*(3)*	*–*	*mp*	*(3)*	*4*	*1.000*	*-0.100*	*0.079*	*0.089*	*small*
Hel	*Phe*	*con*	*(3)*	*–*	*mp*	*(3)*	*8*	*0.200*	*-0.018*	*0.068*	*0.624*	*large*
Hel	*Pro*	*con*	*(3)*	*–*	*mp*	*(3)*	*6*	*0.700*	*-0.141*	*0.230*	*0.267*	*small*
Hel	*Ser*	*con*	*(3)*	*–*	*mp*	*(3)*	*3*	*0.700*	*-0.481*	*0.236*	*0.267*	*small*
Hel	*Tau*	*con*	*(3)*	*–*	*mp*	*(3)*	*8*	*0.200*	*-6.650*	*24.827*	*0.624*	*large*
Hel	*Thr*	*con*	*(3)*	*–*	*mp*	*(3)*	*7*	*0.400*	*-0.128*	*0.355*	*0.445*	*moderate*
Hel	*Tyr*	*con*	*(3)*	*–*	*mp*	*(3)*	*6*	*0.700*	*-0.043*	*0.110*	*0.267*	*small*
Hel	*Val*	*con*	*(3)*	*–*	*mp*	*(3)*	*6*	*0.700*	*-0.228*	*0.202*	*0.267*	*small*

Table S10: Comparison of surface area change between both long-term conditions (control vs. microplastic exposure) for the species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*. Student's *t*-test results (alternative hypothesis: two-sided) and effect sizes (Cohen's *d*). n_{obs} = number of observations; CI = 95% confidence interval.

	Comparison										
Species	Long-term conditions	(n_{obs})	vs.	Long-term conditions	(n_{obs})	d.f.	*t*-value	*p*-value	CI low	CI high	Effect size (*d*-value)	Magnitude of effect size
A. muricata	control	(18)	–	microplastics	(18)	34	0.062	0.951	-9.661	10.269	0.021	negligible
P. verrucosa	control	(12)	–	microplastics	(12)	22	0.687	0.499	-5.240	10.428	0.280	small
P. lutea	control	(18)	–	microplastics	(18)	34	0.694	0.492	-8.138	16.579	0.231	small
H. coerulea	control	(6)	–	microplastics	(6)	10	2.601	**0.027**	0.703	9.112	1.502	large

Table S11: Comparison of volume change between both long-term conditions (control vs. microplastic exposure) for the species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*. Wilcoxon test results (alternative hypothesis: two-sided) and effect sizes (Pearson's *r*). n_{obs} = number of observations; CI = 95% confidence interval.

	Comparison										
Species	Long-term conditions	(n_{obs})	vs.	Long-term conditions	(n_{obs})	*t*-value	*p*-value	CI low	CI high	Effect size (*r*-value)	Magnitude of effect size
A. muricata	control	(18)	–	microplastics	(17)	172	0.546	-0.005	0.009	0.106	small
P. verrucosa	control	(10)	–	microplastics	(12)	70	0.539	-0.003	0.007	0.141	small
P. lutea	control	(17)	–	microplastics	(18)	176	0.463	-0.004	0.018	0.128	small
H. coerulea	control	(6)	–	microplastics	(6)	23	0.485	-0.002	0.009	0.231	small

Table S12: Comparison of calcification between both long-term conditions (control vs. microplastic exposure) for the species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*. Welch's *t*-test results (alternative hypothesis: two-sided) and effect sizes (Cohen's *d*). n_{obs} = number of observations; CI = 95% confidence interval.

	Comparison										
Species	Long-term conditions	(n_{obs})	vs.	Long-term conditions	(n_{obs})	d.f.	*t*-value	*p*-value	CI low	CI high	Effect size (*d*-value)	Magnitude of effect size
A. muricata	control	(18)	–	microplastics	(18)	32.729	0.483	0.632	-12.203	19.793	0.161	negligible
P. verrucosa	control	(12)	–	microplastics	(12)	21.269	0.686	0.500	-7.242	14.384	0.280	small
P. lutea	control	(18)	–	microplastics	(18)	26.799	1.503	0.144	-4.288	27.752	0.501	moderate
H. coerulea	control	(6)	–	microplastics	(6)	9.946	2.386	**0.038**	0.632	18.655	1.378	large

Table S13: Comparison of symbiont densities between both long-term conditions (control vs. microplastic exposure) for the species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*. Welch's *t*-test results (alternative hypothesis: two-sided) and effect sizes (Cohen's *d*). n_{obs} = number of observations; CI = 95% confidence interval.

	Comparison										
Species	Long-term conditions	(n_{obs})	vs.	Long-term conditions	(n_{obs})	d.f.	*t*-value	*p*-value	CI low	CI high	Effect size (*d*-value)	Magnitude of effect size
A. muricata	control	(18)	–	microplastics	(17)	24.466	0.010	0.992	-0.135	0.136	0.003	negligible
P. verrucosa	control	(12)	–	microplastics	(12)	21.710	-0.990	0.333	-0.261	0.092	-0.404	small
P. lutea	control	(18)	–	microplastics	(18)	33.935	-1.442	0.159	-0.304	0.052	-0.481	small
H. coerulea	control	(6)	–	microplastics	(5)	8.593	0.024	0.981	-0.282	0.288	0.015	negligible

Table S14: Comparison of chlorophyll content per cm^{-2} coral surface between both long-term conditions (control vs. microplastic exposure) for the species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*. Student's *t*-test results (alternative hypothesis: two-sided) and effect sizes (Cohen's *d*). n_{obs} = number of observations; CI = 95% confidence interval.

		Comparison											
Species	Chlorophyll type	Long-term conditions	(n_{obs})	vs.	Long-term conditions	(n_{obs})	d.f.	*t*-value	*p*-value	CI low	CI high	Effect size (*d*-value)	Magnitude of effect size
A. muricata	$a + c_2$	control	(18)	–	microplastics	(17)	33	-1.220	0.231	-1.595	0.399	-0.413	small
A. muricata	a	control	(18)	–	microplastics	(17)	33	-1.264	0.215	-1.419	0.331	-0.428	small
A. muricata	c_2	control	(18)	–	microplastics	(17)	33	-0.770	0.447	-0.198	0.089	-0.260	small
P. verrucosa	$a + c_2$	control	(12)	–	microplastics	(12)	22	0.547	0.590	-1.315	2.256	0.223	small
P. verrucosa	a	control	(12)	–	microplastics	(12)	22	0.578	0.569	-1.053	1.867	0.236	small
P. verrucosa	c_2	control	(12)	–	microplastics	(12)	22	0.362	0.721	-0.301	0.429	0.148	negligible
P. lutea	$a + c_2$	control	(18)	–	microplastics	(18)	34	-0.652	0.519	-1.634	0.840	-0.218	small
P. lutea	a	control	(18)	–	microplastics	(18)	34	-0.766	0.449	-1.387	0.628	-0.255	small
P. lutea	c_2	control	(18)	–	microplastics	(18)	34	-0.139	0.890	-0.270	0.235	-0.047	negligible
H. coerulea	$a + c_2$	control	(6)	–	microplastics	(5)	9	-1.263	0.238	-8.414	2.385	-0.765	moderate
H. coerulea	a	control	(6)	–	microplastics	(5)	9	-1.127	0.289	-6.707	2.248	-0.682	moderate
H. coerulea	c_2	control	(6)	–	microplastics	(5)	9	-1.744	0.115	-1.803	0.233	-1.056	large

Table S15: Comparison of chlorophyll content per symbiont cell^{-1} between both long-term conditions (control vs. microplastic exposure) for the species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*. Yuen's *t*-test results and effect sizes (Algina-Keselman-Penfield's d_R). n_{obs} = number of observations; CI = 95% confidence interval.

		Comparison								
Species	Chlorophyll type	Long-term conditions	(n_{obs})	vs.	Long-term conditions	(n_{obs})	d.f.	*t*-value	*p*-value	Effect size (d_R-value)
A. muricata	$a + c_2$	control	(18)	–	microplastics	(17)	19.843	2.211	**0.039**	-0.634
A. muricata	a	control	(18)	–	microplastics	(17)	7.410	1.330	0.223	2.243
A. muricata	c_2	control	(18)	–	microplastics	(17)	21.547	1.947	0.065	0.593
P. verrucosa	$a + c_2$	control	(12)	–	microplastics	(12)	4.181	1.304	0.259	-0.598
P. verrucosa	a	control	(12)	–	microplastics	(12)	20.212	2.519	**0.020**	-0.736
P. verrucosa	c_2	control	(12)	–	microplastics	(12)	7.549	1.393	0.203	2.038
P. lutea	$a + c_2$	control	(18)	–	microplastics	(18)	21.636	1.664	0.111	0.510
P. lutea	a	control	(18)	–	microplastics	(18)	3.962	1.066	0.347	-0.479
P. lutea	c_2	control	(18)	–	microplastics	(18)	18.493	0.671	0.510	-0.184
H. coerulea	$a + c_2$	control	(6)	–	microplastics	(5)	9.953	0.927	0.376	0.620
H. coerulea	a	control	(6)	–	microplastics	(5)	20.826	1.142	0.267	0.334
H. coerulea	c_2	control	(6)	–	microplastics	(5)	3.386	2.443	0.083	-1.045

Table S16: Comparison of relative maximum ϕ of photosystem II (F_v/F_m), relative effective ϕ of photosystem II ($\Delta F/F'_m$), maximum relative electron transport rate ($rETR_{max}$), minimum saturating irradiance (E_k), and photosynthetic rate in the light-limited region (α) between both long-term conditions (control vs. microplastic exposure) for the species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*. Student's *t*-test results (alternative hypothesis: two-sided), Welch's *t*-test results (alternative hypothesis: two-sided), and Wilcoxon test results (alternative hypothesis: two-sided) together with their effect sizes (all Cohen's *d* except for Pearson's *r* in the Wilcoxon tests). n_{obs} = number of observations; mp = microplastics; CI = 95% confidence interval. If applicable: d.f. = Degrees of freedom, otherwise NA; Effect sizes: n = negligible, s = small, m = moderate, l = large.

	Comparison												
Species	Treatment	(n_{obs})	vs	Treatment	(n_{obs})	d.f.	*t*-value	*p*-value	CI low	CI high	Effect size	Magnitude of effect size	Statistical test	Parameter
A. muricata	control	(18)	–	mp	(18)	34	-1.652	0.108	-0.034	0.004	-0.551	m	Student's *t*-test	F_v/F_m
P. verrucosa	control	(12)	–	mp	(12)	22	-0.072	0.943	-0.035	0.032	-0.030	n	Student's *t*-test	F_v/F_m
P. lutea	control	(18)	–	mp	(18)	34	1.826	0.077	-0.003	0.047	0.609	m	Student's *t*-test	F_v/F_m
H. coerulea	control	(6)	–	mp	(6)	10	-0.679	0.513	-0.009	0.005	-0.392	s	Student's *t*-test	F_v/F_m
A. muricata	control	(18)	–	mp	(18)	30.839	-1.004	0.323	-0.050	0.017	-0.334	s	Welch's *t*-test	$\Delta F/F'_m$
P. verrucosa	control	(12)	–	mp	(12)	19.677	-1.020	0.320	-0.044	0.015	-0.416	s	Welch's *t*-test	$\Delta F/F'_m$
P. lutea	control	(18)	–	mp	(18)	26.737	2.394	**0.024**	0.004	0.056	0.798	m	Welch's *t*-test	$\Delta F/F'_m$
H. coerulea	control	(6)	–	mp	(6)	5.248	-0.266	0.801	-0.094	0.076	-0.153	n	Welch's *t*-test	$\Delta F/F'_m$
A. muricata	control	(16)	–	mp	(13)	25.634	0.154	0.879	-17.734	20.599	0.056	n	Welch's *t*-test	$rETR_{max}$
P. verrucosa	control	(10)	–	mp	(9)	16.679	0.248	0.807	-57.890	73.298	0.114	n	Welch's *t*-test	$rETR_{max}$
P. lutea	control	(16)	–	mp	(16)	29.420	0.759	0.454	-7.744	16.895	0.268	s	Welch's *t*-test	$rETR_{max}$
H. coerulea	control	(6)	–	mp	(6)	9.144	-0.536	0.605	-46.995	28.953	-0.310	s	Welch's *t*-test	$rETR_{max}$
A. muricata	control	(16)	–	mp	(13)	NA	131	0.249	-17.340	90.435	0.220	s	Wilcoxon test	E_k
P. verrucosa	control	(10)	–	mp	(9)	NA	44	0.968	-201.938	128.816	0.019	s	Wilcoxon test	E_k
P. lutea	control	(16)	–	mp	(16)	NA	124	0.897	-64.389	71.262	0.027	s	Wilcoxon test	E_k
H. coerulea	control	(6)	–	mp	(6)	NA	18	1.000	-467.766	57.750	0.000	s	Wilcoxon test	E_k
A. muricata	control	(16)	–	mp	(13)	NA	52	**0.022**	-0.131	-0.020	0.423	m	Wilcoxon test	α
P. verrucosa	control	(10)	–	mp	(9)	NA	53	0.549	-0.034	0.099	0.150	s	Wilcoxon test	α
P. lutea	control	(16)	–	mp	(16)	NA	135	0.809	-0.042	0.060	0.047	s	Wilcoxon test	α
H. coerulea	control	(6)	–	mp	(6)	NA	19	0.937	-0.064	0.162	0.046	s	Wilcoxon test	α

Table S17: Comparison of energy reserves between both long-term conditions (control vs. microplastic exposure) for the species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*. Energy reserves comprise lipid, protein, and carbohydrate content data. PERMANOVA test results (using distance matrices) and effect sizes (partial ω^2). con = control; mp = microplastic exposure.

Species	Term	Sum of squares	d.f.	*t*-value	*p*-value	Effect size (ω^2-value)	Magnitude of effect size
A. muricata	con vs. mp	0.010	1	0.965	0.319	-0.001	very small
A. muricata	Residual	0.330	31				
A. muricata	Total	0.341	32				
P. verrucosa	con vs. mp	0.000	1	0.061	0.903	-0.041	very small
P. verrucosa	Residual	0.128	22				
P. verrucosa	Total	0.128	23				
P. lutea	con vs. mp	0.004	1	0.438	0.576	-0.016	very small
P. lutea	Residual	0.311	34				
P. lutea	Total	0.315	35				
H. coerulea	con vs. mp	0.003	1	0.587	0.494	-0.039	very small
H. coerulea	Residual	0.048	9				
H. coerulea	Total	0.051	10				

Table S18: Comparison of metabolites between both long-term conditions (control vs. microplastic exposure) for the species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*. Metabolites include data on the content of 19 amino acids and taurine. PERMANOVA test results (using distance matrices) and effect sizes (partial ω^2). con = control; mp = microplastic exposure.

Species	Term	Sum of squares	d.f.	*t*-value	*p*-value	Effect size (ω^2-value)	Magnitude of effect size
A. muricata	con vs. mp	0.046	1	2.290	0.104	0.067	medium
A. muricata	Residual	0.319	16				
A. muricata	Total	0.365	17				
P. verrucosa	con vs. mp	0.004	1	0.195	0.670	-0.072	very small
P. verrucosa	Residual	0.221	10				
P. verrucosa	Total	0.225	11				
P. lutea	con vs. mp	0.005	1	0.230	0.864	-0.045	very small
P. lutea	Residual	0.314	16				
P. lutea	Total	0.319	17				
H. coerulea	con vs. mp	0.038	1	1.720	0.200	0.107	medium
H. coerulea	Residual	0.088	4				
H. coerulea	Total	0.125	5				

Table S19: Comparison of growth between both long-term conditions (control vs. microplastic exposure) for the species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*. Growth comprises data on surface, volume, and calcification changes. PERMANOVA test results (using distance matrices) and effect sizes (partial ω^2). con = control; mp = microplastic exposure.

Species	Term	Sum of squares	d.f.	*t*-value	*p*-value	Effect size (ω^2-value)	Magnitude of effect size
A. muricata	con vs. mp	0.028	1	0.533	0.542	-0.014	very small
A. muricata	Residual	1.720	33				
A. muricata	Total	1.747	34				
P. verrucosa	con vs. mp	0.036	1	0.745	0.465	-0.012	very small
P. verrucosa	Residual	0.958	20				
P. verrucosa	Total	0.994	21				
P. lutea	con vs. mp	0.015	1	0.391	0.541	-0.018	very small
P. lutea	Residual	1.230	33				
P. lutea	Total	1.245	34				
H. coerulea	con vs. mp	0.032	1	6.104	**0.025**	0.298	large
H. coerulea	Residual	0.053	10				
H. coerulea	Total	0.085	11				

Table S20: Comparison of photosymbiont state between both long-term conditions (control vs. microplastic exposure) for the species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*. Photosymbiont state comprises data on symbiont densities, chlorophyll content, and light- and dark-adapted ϕ_{PSII} ($\Delta F/F'_m$ and F_v/F_m). PERMANOVA test results (using distance matrices) and effect sizes (partial ω^2). con = control; mp = microplastic exposure.

Species	Term	Sum of squares	d.f.	*t*-value	*p*-value	Effect size (ω^2-value)	Magnitude of effect size
A. muricata	con vs. mp	0.0188	1	3.07	**0.031**	0.056	small
A. muricata	Residual	0.202	33				
A. muricata	Total	0.221	34				
P. verrucosa	con vs. mp	0.023	1	1.87	0.113	0.035	small
P. verrucosa	Residual	0.270	22				
P. verrucosa	Total	0.293	23				
P. lutea	con vs. mp	0.006	1	2.69	0.06	0.045	small
P. lutea	Residual	0.075	34				
P. lutea	Total	0.081	35				
H. coerulea	con vs. mp	0.029	1	2.88	0.117	0.146	large
H. coerulea	Residual	0.09	9				
H. coerulea	Total	0.118	10				

Table S21: Comparison of physiological parameters between both long-term conditions (control vs. microplastic exposure) for the species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*. Physiological parameters comprise data on PCA components (i.e., energy reserves, metabolites, growth, and photosymbionts). Results of ANOVAs that followed an analysis of multivariate homogeneity of group dispersions. SSQ = sum of squares, con = control; mp = microplastic exposure.

Parameter	Species	Term	d.f.	SSQ	Mean SSQ	*f*-value	*p*-value
Energy	*A. muricata*	con vs. mp	1	0.006	0.006	1.429	0.241
Energy	*A. muricata*	Residuals	31	0.124	0.004		
Energy	*P. verrucosa*	con vs. mp	1	0	0	0.318	0.578
Energy	*P. verrucosa*	Residuals	22	0.032	0.001		
Energy	*P. lutea*	con vs. mp	1	0.002	0.002	0.379	0.542
Energy	*P. lutea*	Residuals	34	0.139	0.004		
Energy	*H. coerulea*	con vs. mp	1	0.002	0.002	1.341	0.277
Energy	*H. coerulea*	Residuals	9	0.016	0.002		
Metabolites	*A. muricata*	con vs. mp	1	0.009	0.009	1.976	0.179
Metabolites	*A. muricata*	Residuals	16	0.071	0.004		
Metabolites	*P. verrucosa*	con vs. mp	1	0.002	0.002	0.608	0.454
Metabolites	*P. verrucosa*	Residuals	10	0.028	0.003		
Metabolites	*P. lutea*	con vs. mp	1	0.003	0.003	0.75	0.4
Metabolites	*P. lutea*	Residuals	15	0.061	0.004		
Metabolites	*H. coerulea*	con vs. mp	1	0.001	0.001	0.511	0.514
Metabolites	*H. coerulea*	Residuals	4	0.005	0.001		
Growth	*A. muricata*	con vs. mp	1	0.037	0.037	2.148	0.152
Growth	*A. muricata*	Residuals	33	0.574	0.017		
Growth	*P. verrucosa*	con vs. mp	1	0	0	0.02	0.89
Growth	*P. verrucosa*	Residuals	20	0.287	0.014		
Growth	*P. lutea*	con vs. mp	1	0	0	0.016	0.9
Growth	*P. lutea*	Residuals	33	0.342	0.01		
Growth	*H. coerulea*	con vs. mp	1	0	0	0.191	0.671
Growth	*H. coerulea*	Residuals	10	0.021	0.002		
Photosymbionts	*A. muricata*	con vs. mp	1	0	0	0.015	0.902
Photosymbionts	*A. muricata*	Residuals	33	0.08	0.002		
Photosymbionts	*P. verrucosa*	con vs. mp	1	0.017	0.017	2.8	0.108
Photosymbionts	*P. verrucosa*	Residuals	22	0.136	0.006		
Photosymbionts	*P. lutea*	con vs. mp	1	0	0	0.274	0.604
Photosymbionts	*P. lutea*	Residuals	34	0.017	0.001		
Photosymbionts	*H. coerulea*	con vs. mp	1	0.01	0.01	3.17	0.109
Photosymbionts	*H. coerulea*	Residuals	9	0.027	0.003		

Table S22: Comparison of coral mortality between both long-term conditions (control vs. microplastic exposure) for the species *A. muricata*, *P. verrucosa*, *P. lutea*, and *H. coerulea*. Results of Gray's test (1988), NA = not available, as no fragment of *H. coerulea* died. con = control; mp = microplastic exposure.

Species	Term	d.f.	*z*-value	*p*-value
A. muricata	con vs. mp	1	0	0.985
P. verrucosa	con vs. mp	1	1.71	0.191
P. lutea	con vs. mp	1	0.947	0.330
H. coerulea	con vs. mp	NA	NA	NA

3 Appendix

3.1 Additional publications

3.1.1 Peer-reviewed article

Reichert J, Arnold A L, Hammer N, Miller I B, **Rades M**, Schubert P, Ziegler M, and Wilke T (2022). Reef-building corals act as long-term sink for microplastic. *Global Change Biology*, 28(1): 33–45. DOI: 10.1111/gcb.15920

Published online on 28 October 2021.

No. of peer-reviewed citations on the date of submission of the dissertation: 42

Contributions of the author of the dissertation:

Investigation	Contributed to performing the experiments, data collection
Methodology	Contributed to development of methodology
Software	Contributed to programming, implementation of the computer code
Writing – review & editing	Contributed to critical review, commentary

3.1.2 Technical protocol

Rades M, Schubert P, Ziegler M, Kröckel M, and Reichert J (2022). Building plan for a temperature-controlled multi-point stirring incubator. *Protocols.io*, 06.04.2022. DOI: 10.17504/protocols.io.dm6gpb34dlzp/v1

No. of peer-reviewed citations on the date of submission of the dissertation: 3

Contributions of the author of the dissertation:

Conceptualization	Contributed to ideas and aims
Methodology	Development of methodology
Visualization	Creation of images
Writing – original draft	Writing initial draft

3.1.3 Conference contributions

Rades M, Reichert J, Schubert P, and Wilke T (2018). Uptake of microplastics in hermatypic corals. 5th Young Reef Scientists Meeting, Munich, Germany, **Poster**

Rades M, Reichert J, Schubert P, and Wilke T (2019). A costly decision—picky corals prefer food over microplastics. International Conference for YOUNG Marine Researchers (ICYMARE), Bremen, Germany, **Talk**

Rades M, Reichert J, Schubert P, and Wilke T (2020). Reef-building corals do not acquire avoidance mechanisms against microplastic uptake. International Conference on Marine Science (ICMS): tropical oceans for the future, Colombia, **Poster**

Rades M, Reichert J, Schubert P, and Wilke T (2020). Reef-building corals may not develop mechanisms to prevent microplastic uptake. 13th Annual GGL Conference, Giessen, Germany, **Talk**

Rades M, Reichert J, Schubert P, and Wilke T (2021). Reef-building corals might not develop mechanisms to avoid microplastic ingestion. 14th International Coral Reef Symposium (ICRS), Bremen, Germany, **Talk**

Rades M, Reichert J, and Wilke T (2021). Effects of long-term microplastic exposure on the symbiosis of corals. 14th Annual GGL Conference, Giessen, Germany, **Talk**

Rades M, Reichert J, and Wilke T (2022). Corals mitigate effects of microplastics by maintaining energy reserves and symbiosis at the expense of growth. 15th Annual GGL Conference, Giessen, Germany, **Poster**

Rades M, Wilke T, and Reichert J (2023). Does chronic exposure to microplastics affect the physiology of reef-building corals? International Conference on Marine Science (ICMS): Working today for the ocean of tomorrow, Cartagena de Indias, Colombia, **Poster**

3.2 Data basis for Figure 3

Table 2: Scientific studies on microplastics and corals at the editorial deadline (May 2024) used as data basis for Figure 3. "st" = short-term, "in" = intermediate, "lt" = long-term. NA = No study published in 2016. See the bibliography (Chapter 1.7) for details of references.

Year	Duration	Location	Author
2015	st	*ex situ*	(Hall et al., 2015)
2016	NA	NA	NA
2017	st	*ex situ*	(Allen et al., 2017)
2018	in	*ex situ*	(Reichert et al., 2018)
2018	st	*ex situ*	(Hankins et al., 2018)
2018	in	*ex situ*	(Chapron et al., 2018)
2018	st	*ex situ*	(Tang et al., 2018)
2018	st	*ex situ*	(Murphy and Quinn, 2018)
2019	st	*ex situ*	(Romanó de Orte et al., 2019)
2019	lt	*in situ*	(Saliu et al., 2019)
2019	st	*ex situ*	(Axworthy and Padilla-Gamiño, 2019)
2019	st	*ex situ*	(Syakti et al., 2019)
2019	lt	*ex situ*	(Reichert et al., 2019)
2019	lt	*in situ*	(Rotjan et al., 2019)
2019	st	*ex situ*	(Martin et al., 2019)
2019	in	*ex situ*	(Mouchi et al., 2019)
2020	st	*ex situ*	(Savinelli et al., 2020)
2020	st	*ex situ*	(Rocha et al., 2020)
2020	st	*ex situ*	(Corona et al., 2020)
2020	st	*ex situ*	(Diana et al., 2020)
2020	in	*ex situ*	(Lanctôt et al., 2020)
2020	lt	*in situ*	(Weideman et al., 2020)
2021	st	*ex situ*	(Liao et al., 2021)
2021	st	*ex situ*	(Mendrik et al., 2021)
2021	st	*ex situ*	(Vencato et al., 2021)
2021	st	*ex situ*	(S. Jiang et al., 2021)
2021	lt	*in situ*	(Tang et al., 2021)
2021	in	*ex situ*	(Reichert et al., 2021)
2021	st	*ex situ*	(Xiao et al., 2021)
2021	lt	*in situ*	(Lei et al., 2021)
2021	lt	*in situ*	(Devereux et al., 2021)
2021	st	*in situ*	(de Smit et al., 2021)
2021	in	*ex situ*	(Hierl et al., 2021)
2021	in	*ex situ*	(Hankins et al., 2021)
2021	st	*ex situ*	(Grillo et al., 2021)
2021	lt	*in situ*	(Ranjbar Jafarabadi et al., 2021a)
2021	lt	*in situ*	(Krishnakumar et al., 2021)
2021	st	*ex situ*	(Corinaldesi et al., 2021)
2021	lt	*in situ*	(Carugati et al., 2021)
2021	lt	*in situ*	(van der Schyff et al., 2021)
2022	lt	*in situ*	(Zhou et al., 2022b)
2022	lt	*ex situ*	(Reichert et al., 2022)
2022	st	*ex situ*	(Y.-T. Chen et al., 2022)
2022	in	*ex situ*	(Boodraj and Glassom, 2022)
2022	st	*ex situ*	(Plafcan and Stallings, 2022)
2022	st	*ex situ*	(Bejarano et al., 2022)
2022	lt	*ex situ*	(Rades et al., 2022)
2022	st	*ex situ*	(Hankins et al., 2022)
2023	lt	*in situ*	(Zhou et al., 2023)
2023	in	*ex situ*	(Ng and Todd, 2023)
2024	st	*ex situ*	(Reichert et al., 2024a)
2024	in	*ex situ*	(Reichert et al., 2024b)
2024	st	*ex situ*	(Yen et al., 2024)
2024	lt	*ex situ*	(Rades et al., 2024)

4 Acknowledgements

First and foremost, a dissertation is, in good part, a product of constant support, motivation, and criticism to get the best out of the PhD student and his research. With this, I would like to open the round of thanks to all those who have inspired this achievement and thus made it possible:

My first thanks go to Prof. Dr. Tom Wilke, who not only invited me to do research in his working group but also awarded me the CEMarin doctoral scholarship. This trust laid the foundation for my doctorate, for which I am very grateful.

At least as important has been the many years of great collaboration with my mentor, Dr. Jessica Reichert, who has guided and inspired my scientific career as a mastermind of research on microplastics and corals. I would like to take this opportunity to thank her most sincerely.

I am indebted to the Cooperation Centre of Excellence in Marine Sciences (CEMarin) for financially supporting my doctoral project by granting me a doctoral scholarship.

In this large and diverse working group, exceptionally friendly and helpful support was never far away in the laboratories, during experiments in the aquarium facilities, or in everyday office life, but could be found everywhere. I would therefore like to thank all current and former working group members for their time and support.

Special thanks go to Sylvia Nachtigall for her help in the laboratory and to Dr. Patrick Schubert for his relentless efforts in the aquarium facilities for the well-being of the corals.

The analyses of the energy reserves took place at the Institute of Animal Nutrition and Nutritional Physiology under the direction of Prof. Dr. Klaus Eder, who kindly granted me the use of his laboratories. Under the expert guidance of Dr. Erika Most, I was able to complete the analyses successfully, thanks also to the active and friendly support of Anja Marx and Marco Jäger. A big thank you to Prof. Eder's team.

I would also like to thank my fellow doctoral students, in particular. They have supported me in a wonderful way with technical questions and hurdles and enriched my time as a doctoral student enormously with a lot of humour and amicable conversations. Thank you, Vanessa Tirpitz, Catarina Martins, Mareike Oponczewski, Erik Ferrara, Olivia Metz, and María Antonieta López Hernández!

Outside of the university, I have received crucial support from my partner, Liesa Balles, who has always stood by me and had my back. I am incredibly grateful to her.

5 Selbstständigkeitserklärung | *Declaration of Authorship*

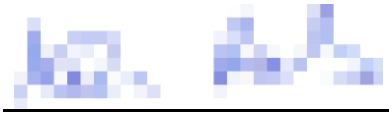
Erklärung gemäß der Promotionsordnung des Fachbereichs 08 vom 21.01.2016 § 17 (2):

Ich erkläre: Ich habe die vorgelegte Dissertation selbstständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht. Ich stimme einer evtl. Überprüfung meiner Dissertation durch eine Antiplagiat-Software zu. Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ niedergelegt sind, eingehalten.

Declaration acc. to the doctoral regulations of Faculty 08 dated 21.01.2016 § 17 (2):

I declare that I have completed this dissertation single-handedly without the unauthorized help of a second party and only with the assistance acknowledged therein. I have appropriately acknowledged and cited all text passages that are derived verbatim from, or are based on, the content of published work of others, and all information relating to verbal communications. I consent to the use of an anti-plagiarism software to check my thesis. I have abided by the principles of good scientific conduct laid down in the charter of the Justus Liebig University Giessen “Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis” in carrying out the investigations described in the dissertation.

7. Oktober 2024



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