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## LETTER

**Microplastics alter feeding strategies of a coral reef organism**Marlena Joppien <sup>1,2,3\*</sup> Hildegard Westphal <sup>1,2,3</sup> Marleen Stuhr <sup>1,4</sup> Steve S. Doo <sup>1,3</sup><sup>1</sup>Geoecology and Carbonate Sedimentology Group, Leibniz Centre for Tropical Marine Research (ZMT), Bremen, Germany;<sup>2</sup>Department of Geosciences, University of Bremen, Bremen, Germany; <sup>3</sup>King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia; <sup>4</sup>Inter-University Institute for Marine Sciences (IUI), Eilat, Israel**Scientific Significance Statement**

Large benthic foraminifera (LBF) are key carbonate-producing organisms on coral reefs, as well as indicators of environmental change and pollution. Marine pollution, particularly plastic debris, presents a novel, yet largely unquantified stress on LBF. Here, we document one of the first feeding choice experiments on LBF, comparing microplastics with common food choices. There was a strong LBF feeding selection against pristine microplastic, suggesting a selective ability to discern between potential food sources. However, this selectivity of food choice disappeared when conditioned microplastics were used, suggesting feeding behavior (and subsequently energy resources) of LBF may be impacted by microplastics with longer residence times in the water. These results have significant implications on ecosystem processes, such as carbonate production rates on coral reefs.

**Abstract**

Increasing marine microplastic pollution has detrimentally impacted organismal physiology and ecosystem functioning. While previous studies document negative effects of microplastics on coral reef animals, the potential responses of organisms such as large benthic foraminifera (LBF) are largely unknown. Here, we document the impact of microplastics on heterotrophic feeding behavior of LBF. Specimens of *Amphistegina gibbosa* were incubated in three experimental treatments: (1) *Artemia* sp. nauplii only; (2) pristine microplastic particles only; and (3) choice of nauplii and pristine microplastic. Feeding responses were evaluated 24 h after initiation of treatments. A separate experiment was conducted to compare the effect of conditioned vs. pristine microplastic. Our results indicate that *A. gibbosa* is able to selectively feed on *Artemia*, avoiding interactions with pristine microplastic. However, the presence of conditioned microplastic causes similar feeding interaction rates as with *Artemia*. This suggests that microplastics with longer residence times may have a larger impact on facultative detritivores.

Human impacts on aquatic ecosystems include the increasing deposition of plastic waste into the marine environment (Thompson et al. 2009; SAPEA 2019). Since first

documented in the 1970s (Carpenter et al. 1972), plastic pollution has become an increasing concern, as by now plastics have been documented in all marine environments (Fischer

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**Associate editor:** Elise Granek**Author Contribution Statement:** MJ, SSD, and HW conceptualized the study. MJ and SSD designed the study approach and experimental setup. MJ conducted the food choice experiments and analyzed the results. All authors contributed to data interpretation, editing, and writing of the manuscript.**Data Availability Statement:** Data and metadata are available in the Dryad data repository at 10.5061/dryad.12jm63z0j.

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et al. 2015). Recently, microplastic particles, a subgroup of plastics generally defined as particles and fragments in the size range of 1  $\mu\text{m}$  to 5 mm (Gigault et al. 2018), and the potential hazards they pose to ecosystems (e.g., ingestion, contamination through leachates) have received more attention (GESAMP 2015; SAPEA 2019). Microplastics are of particular concern in aquatic environments because they are relatively inert and highly resistant to biological degradation (Rios et al. 2007; Cole et al. 2011). These properties and their increasing accumulation in the marine environment highlight the need for assessments of how marine biota will be impacted.

Observed interactions between microplastic and marine biota have led to distinct findings, implying physiological consequences when organisms confuse microplastic particles with food. Previous studies have found that uptake of microplastics can potentially lead to depleted energy reserves as a result of intestinal blockages, egestion efforts or false satiation in fish, crabs and worms (Wright et al. 2013; Watts et al. 2015; Müller et al. 2020). Furthermore, microplastic particles permeate marine food webs, magnifying potential impacts across multiple trophic levels (Andrady 2011; Lusher 2015). Additionally, the increased affinity for sorption of contaminants and heavy metals to their surface, and the potential for some microplastics to incorporate toxic components is a cause for further concern (Rios et al. 2007; Teuten et al. 2007).

Coral reefs are highly biodiverse habitats which provide important services to local communities, including coastal protection, fisheries, and tourism resources, all relying on reef- and sediment-building organisms (e.g., scleractinian corals, calcifying algae, benthic foraminifera) to create habitats and contribute to carbonate production (Chave et al. 1972). However, coral reefs are generally located in areas that accumulate large amounts of plastic waste because of a combination of high population densities, lack of developed waste management (Morrison and Munro 1999; GESAMP 2015), and ocean currents (Berloff et al. 2002; Connors 2017). The responses of reef-building scleractinian corals to microplastic exposure include microplastic ingestion (Hall et al. 2015; Hankins et al. 2018), changes in feeding behavior (Allen et al. 2017; Rotjan et al. 2019; Savinelli et al. 2020), and incorporation of microplastic into the skeleton (Hierl et al. 2021). Furthermore, visible stress reactions (e.g., polyp retraction and increased mucus production), which potentially deplete energy reserves of the coral, and increased disease likelihood have been documented when corals are in contact with plastics (Lamb et al. 2018; Reichert et al. 2018).

While the effects of microplastics on corals are increasingly being documented, the effects on other calcifying organisms are equally important to assess. One group relevant for carbonate production in tropical shallow water settings are photosymbiotic large benthic foraminifera (LBF; Narayan et al. 2021). LBF are essential components of tropical coral reef communities,

contributing annually up to 43 million tons of  $\text{CaCO}_3$  through carbonate production with a turnover of rate  $86 \times 10^{15}$  individuals per year (Langer 2008). This accounts for approximately 5% of the worldwide shallow water carbonate production (Langer 2008; Doo et al. 2017). Although corals are more prominent carbonate producers (Montaggioni and Braithwaite 2009), the vast number of LBF individuals and locally high densities ( $> 1 \text{ kg m}^{-2}$   $\text{CaCO}_3$  biomass; Doo et al. 2017) constitute their ecological importance. LBF evolve rapidly and fill ecological niches, adapting their morphology and symbiotic relationships to environmental conditions, therefore representing valuable tools for paleoenvironmental interpretations (Zabihi Zoeram et al. 2015; Boudagher-Fadel 2018), and bioindicators for environmental assessment and monitoring (Hallock et al. 2003; Martinez-Colon et al. 2009).

Contrary to corals, only few studies have addressed the responses of LBF to microplastic exposure. Recently, nano-sized plastic particles were shown to cause physiological stress in the LBF *Ammonia parkinsoniana*, indicated by the accumulation of neutral lipids and enhanced reactive oxygen species production (Ciacci et al. 2019). However, leachates from seawater-soaked polypropylene microplastic had no significant effect on the locomotion and metabolism of the benthic foraminifer *Haynesina germanica* (Langlet et al. 2020). Several species of aposymbiotic benthic foraminifera showed varied responses to polystyrene (PS) particles (0.5–6  $\mu\text{m}$  in diameter) ingestion, related to skeleton type (agglutinating vs. calcifying), and food preference (Grefstad et al. 2019). However, the still small number of studies and the novelty of this topic demands further investigations.

The LBF *Amphistegina gibbosa* which harbors endosymbiotic diatoms is ubiquitous on coral reefs, and used often in laboratory experiments (Williams and Hallock 2003; Stuhr et al. 2017). *A. gibbosa* are known to switch between autotrophy and heterotrophy (Hallock 1981; Stuhr et al. 2018a), potentially to compensate for altered symbiont density (e.g., due to changing light levels; Williams and Hallock 2003). In general, LBF are thought to have a relatively high flexibility in associating with diatom symbionts (Lee et al. 1997; Prazeres and Renema 2019), and exhibit a facultative heterotrophic lifestyle where the majority of energy is acquired through autotrophic means. For heterotrophic feeding, *A. gibbosa* moves portions of organic matter with its pseudopodia (a temporary extension of eukaryotic ectoplasm), into the inner cell body, where digestion occurs (Bowser et al. 1985). Therefore, the interaction with microplastic particles in size ranges of natural LBF food sources and the impact on heterotrophic feeding are of potentially high relevance to ecosystem processes such as carbonate production. As such, to gain insight into the feeding behavior mechanisms of LBF, the present study documents the choice selection of LBF between microplastic particles (pristine and biofilm-coated) vs. a natural occurring food source (larvae of aquatic crustaceans). For this, specimens of *A. gibbosa* were presented with one of the three food choices: (1) microplastic particles only, (2) *Artemia*

sp. nauplii only, and (3) a 1 : 1 mixture of both, microplastic and *Artemia* sp. nauplii.

## Methods

### Collection and aquaria maintenance

Specimens of *A. gibbosa* were collected from 18 m depth at Tennessee Reef, Florida Keys (24°45'8.33"N, 80°45'26.33"W), in June 2015 by SCUBA divers (Stuhr et al. 2017, 2018b). They were transported to the Marine Experimental Ecology facility (MAREE) at the Leibniz Centre for Tropical Marine Research (ZMT) in Bremen, Germany, for culture establishment.

LBF were kept in 500 mL containers filled with artificial seawater made from Red Sea Salt (*Red Sea*), and carbonate substrata (coral skeletons) at ~24°C bubbled with air to keep the water oxygenated and provide moderate flow. In weekly water changes, ~30% of the water within the culture vessel was replaced with new seawater, keeping the salinity at ~35 PSU. The PAR light sensor measurements show light conditions of 15  $\mu\text{mol m}^{-2} \text{s}^{-1}$  inside the culture vessel (supplied by using a JBL Solar Ultra MARINE Day 15000K fluorescent light). The culture was maintained in these conditions for 5 yrs, and the feeding experiment was carried out under the same conditions in October and November 2020. It is assumed that none of the *A. gibbosa* used in this study were part of the original culture and are clonal progeny from the original cohort. Prior to the start of this experiment, the LBF used in this study were never provided with additional food (e.g., nauplii).

### Experimental setup

#### Food choices

To understand the effect of microplastic pollution on heterotrophic feeding, two food choices were used in this study. The first was microplastic consisting of negatively buoyant polyethylene terephthalate particles (opaque, white color, angular shape, 150–300  $\mu\text{m}$ , Goodfellow Cambridge Ltd.) that are negatively buoyant in seawater such that they sink to the ground. The second food choice was 1-day-old *Artemia* sp. nauplii (Ocean Nutrition, model V154019), here further referred to as nauplii. As *A. gibbosa* generally do not capture actively moving prey and instead mostly feed on detritus, the nauplii were frozen, and kept in –20°C. The microplastic particles and nauplii were approximately in the same size range (180–400  $\mu\text{m}$ ).

#### Feeding experiment

All experimental trials were performed in 12-well PS plates (6.5 mL volume per well, CELLSTAR). Three food choice treatment groups were defined: (1) *Artemia* sp. nauplii only ( $n = 10$  nauplii in each replicate); (2) microplastic particles only ( $n = 10$  microplastic particles in each replicate); and (3) evenly split food choice of *Artemia* sp. nauplii and microplastic ( $n = 5$  each). A total of 12 replicates of each treatment group were set up, and a randomized design was created to assign treatments to wells. Microplastic particles and nauplii were

manually pipetted under a binocular microscope and transferred into wells with ~5 mL of seawater.

Subsequent to establishing the feeding treatment groups, a total of five specimens of *A. gibbosa* (750–1250  $\mu\text{m}$  diameter) were placed into each well, keeping a distance to the microplastic/nauplii particles to ensure that there was no forced interaction prior to the start of the experiment. Approximately 24 h after the initiation of the experiment, feeding activity of *A. gibbosa* on nauplii and microplastic was assessed visually under a *Leica* binocular microscope, by counting the number of remaining nauplii and feeding attempts on microplastic particles and nauplii. For this experiment, feeding on microplastic is defined as any physical interaction with the LBFs' pseudopodia. Two trials were conducted, with a total of 24 replicates (12 per trial) in each treatment. None of the specimens were used in both trials. To ensure counting accuracy, an additional four counting controls per treatment were established, in which no LBF were placed in the well. These treatments showed no change in nauplii/microplastic during the feeding experiment, proving the accuracy in identifying and counting these particles in this setup. While the number of particles of either microplastic or nauplii in the mixed treatment was different compared to single choice treatments, we ensured that enough food resources were present for satiation (i.e., all replicates still contained nauplii at the end of the experimental period).

#### Influence of seawater-soaking on feeding rates

As *A. gibbosa* can also ingest algal food sources, a separate set of experiments was established to assess the feeding potential for microplastic soaked in seawater which potentially leads to toxin leachates but may also allow for biofilm layers to establish on the surface. Soaking took place for 3–5 weeks, using the same seawater as the active *A. gibbosa* culture but keeping the microplastic in a separate water container without organisms. The experiment was repeated using the same setup as above but using seawater-soaked microplastic particles instead of pristine microplastic particles.

#### Data analysis

The observed number of feeding attempts (number of pseudopodal interactions and ingested particles) per treatment was used to calculate numerical feeding rates (particles fed upon replicate<sup>-1</sup>). To analyze these data, a one-way mixed-effects ANOVA was conducted for each of the experiments initially, using feeding rate as the response variable, food choice treatment as the fixed factor and trial (1 or 2) as the random factor. In all instances for this study, the trial effect was  $p > 0.25$ , and removed from the model as described by Underwood 1997. The model was then rerun as a one-way ANOVA with food choice as the fixed factor and feeding rate as the response variable. Although the data were not normally distributed, we proceeded with the analysis due to the robust nature of ANOVA tests. The ANOVA analyses were performed in R

**Table 1.** Results of one-way ANOVA analyzing the effect of treatments (single food choice vs. mixed,  $n = 24$ ) with the food choices (A) *Artemia* sp. nauplii, and (B) Pristine microplastic on *A. gibbosa* feeding response.

Source	df	SS	MS	F	p
A. Experiment 1: <i>Artemia</i> sp. nauplii, single choice vs. mixed treatments					
Treatment	1	1.257	1.257	4.788	<b>0.033</b>
Residuals	46	12.075	0.263		
Total	47	13.332			
B. Experiment 1: Pristine microplastic, single choice vs. mixed treatments					
Treatment	1	0.434	0.434	5.022	<b>0.030</b>
Residuals	46	3.976	0.087		
Total	47	4.41			

Bold  $p$ -values signalize statistical significance.

(R Core Team 2020), with software package GAD to incorporate random effects (Sandrini-Neto and Camargo 2014).

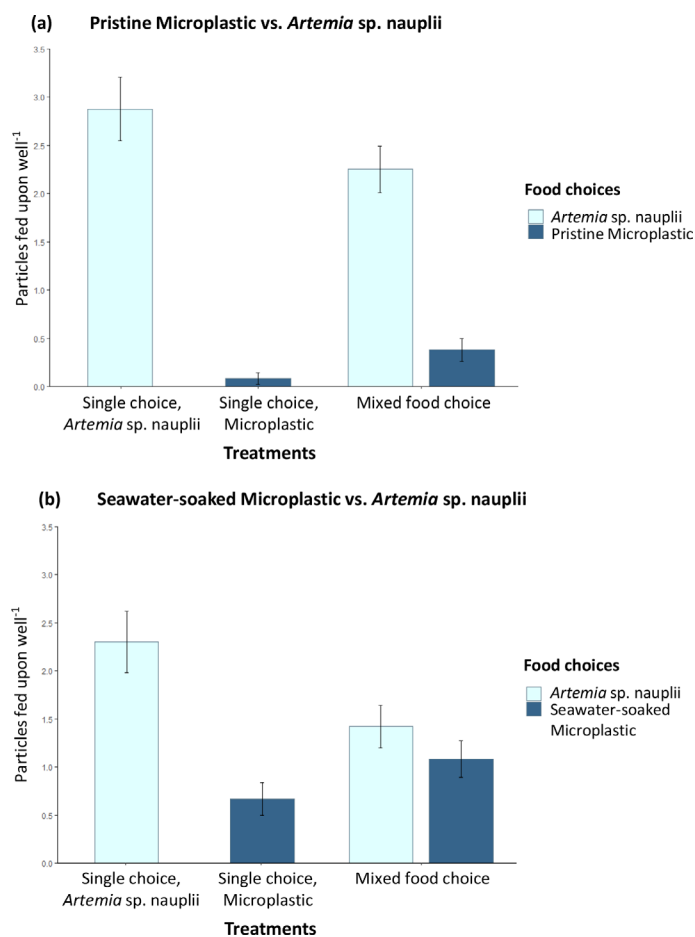
## Results

### Experiment 1: *Artemia* sp. nauplii and pristine microplastic particles

*A. gibbosa* was observed feeding on both *Artemia* sp. nauplii and microplastic. While nauplii were found in different stages of consummation (trapped with pseudopodia to fully consumed), microplastics were moved into proximity to the LBF aperture but could not be ingested in the same manner as nauplii (Fig. 2a,b). Overall, LBF feeding rates on nauplii in mixed treatments were 22% lower than compared to single food choice, thus significantly decreased ( $F_{1,47} = 4.79$ ,  $p = 0.03$ ; Table 1A). In treatments providing nauplii as a single food choice, the mean feeding rate was  $2.88 \pm 0.33$  particles replicate<sup>-1</sup> ( $n = 24$ , mean  $\pm$  SE; Fig. 1a). In mixed treatments, the mean feeding on nauplii decreased to  $2.25 \pm 0.24$  particles replicate<sup>-1</sup> ( $n = 24$ , mean  $\pm$  SE; Fig. 1a). Contrary to nauplii treatments, LBF feeding rates on pristine microplastic increased in mixed food treatments, when compared to single choice treatments ( $F_{1,47} = 5.02$ ,  $p = 0.03$ ; Table 1B). In the pristine microplastic-only treatments, there was minimal feeding of  $0.08 \pm 0.06$  particles replicate<sup>-1</sup> ( $n = 24$ , mean  $\pm$  SE; Fig. 1a), which increased in the mixed treatments to  $0.38 \pm 0.12$  particles replicate<sup>-1</sup> ( $n = 24$ , mean  $\pm$  SE; Fig. 1a).

### Experiment 2: *Artemia* sp. nauplii and seawater-soaked microplastic particles

Feeding rates of *A. gibbosa* on nauplii only treatments were similar across the two experiments. However, feeding on nauplii was significantly decreased by 39% in mixed treatments with seawater soaked microplastics when compared to nauplii only single food choice treatments ( $F_{1,47} = 5.06$ ,  $p = 0.03$ ; Table 2A). In treatments with nauplii only, there was a mean feeding rate of  $2.3 \pm 0.32$  particles replicate<sup>-1</sup> ( $n = 24$ , mean  $\pm$  SE; Fig. 1b). In mixed treatments, feeding on nauplii



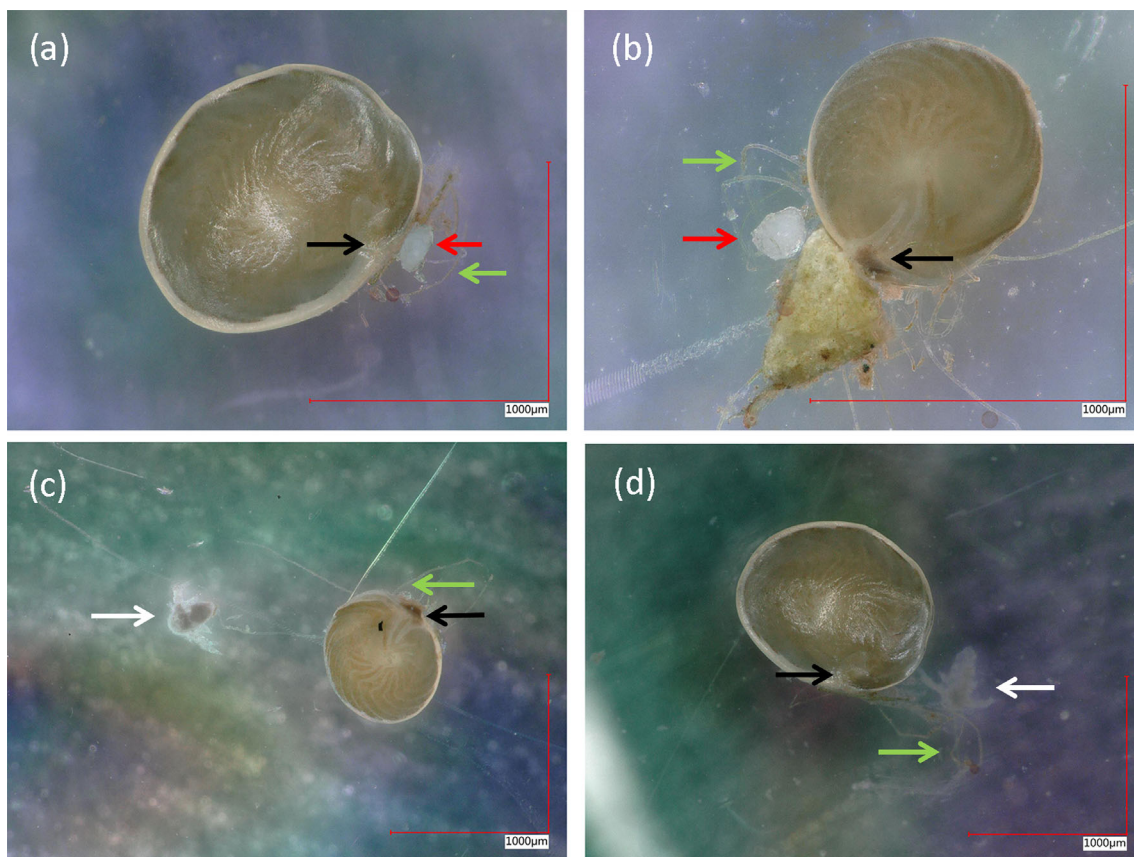
**Fig. 1.** Observed feeding response of *A. gibbosa* on microplastic particles and *Artemia* sp. nauplii after 24 h of exposure. (a) Displays the feeding response in Experiment 1 (pristine microplastic vs. *Artemia* sp. nauplii), (b) displays the feeding response in Experiment 2 (seawater-soaked microplastic vs. *Artemia* sp. nauplii). Bars (microplastic: dark blue; nauplii: light blue) show the mean number of particles fed upon well<sup>-1</sup> in three experimental treatments, with each well containing 5 LBF specimens. Error bars show calculated standard error.



**Table 2.** Results of one-way ANOVA analyzing the effect of treatments (single food choice vs. mixed,  $n = 24$ ) with the food choices (A) *Artemia* sp. nauplii, and (B) conditioned microplastic on *A. gibbosa* feeding response.

Source	df	SS	MS	F	p
A. Experiment 2: <i>Artemia</i> sp. nauplii, single choice vs. mixed treatments					
Treatment	1	2.311	2.312	5.057	<b>0.029</b>
Residuals	46	21.027	0.457		
Total	47	23.338			
B. Experiment 2: Conditioned microplastic, single choice vs. mixed treatments					
Treatment	1	0.528	0.528	2.947	0.093
Residuals	46	35.17	0.765		
Total	47	35.698			

Bold  $p$ -values signalize statistical significance.

**Fig. 2.** *A. gibbosa* interacting with food particles. (a) Microplastic caught by LBF pseudopodia; (b) *A. gibbosa* attached to debris and microplastic; (c, d) *Artemia* sp. nauplii in the process of ingestion by *A. gibbosa*. Elements are highlighted with arrows: microplastic (red arrow), nauplii (white arrow), pseudopodia (green arrow), and feeding aperture (black arrow).

decreased significantly to  $1.4 \pm 0.22$  particles replicate<sup>-1</sup> ( $n = 24$ , mean  $\pm$  SE; Fig. 1b).

In seawater-soaked microplastics treatments, *A. gibbosa* interacted frequently with microplastics when they were provided as a single food choice. There was no significant difference between feeding rates on seawater-soaked microplastic in

the mixed vs. single food choice treatments ( $F_{1,47} = 2.95$ ,  $p = 0.09$ ; Table 2B). In treatments with seawater-soaked microplastic only, mean overall feeding was  $0.67 \pm 0.17$  particles replicate<sup>-1</sup> ( $n = 24$ , mean  $\pm$  SE; Fig. 1b). There were more counted feeding attempts on seawater-soaked microplastic particles in the mixed treatments, resulting in a mean feeding

rate of  $1.08 \pm 0.19$  particles replicate<sup>-1</sup> ( $n = 24$ , mean  $\pm$  SE; Fig. 1b). The conditioned particles were also observed to lump together, forming clusters of up to five particles.

## Discussion

### Food selection and chemotaxis in LBF

Our results suggest that *A. gibbosa* actively chose between food particles and nonfood particles of similar size, as evidenced in the low number of pseudopodal interaction with pristine microplastics (Fig. 1a). Even in mixed treatments feeding on nauplii remained six times higher than on pristine microplastic (Fig. 1a). While this study did not specifically examine chemotaxis effects of feeding in *A. gibbosa*, other LBF species including *Amphistegina* spp. are known to distinguish food sources, reflected in directed movements toward suitable food sources, and selective ingestion (Lee et al. 1988; Langer and Gehring 1993). Studies have also found that varied food choices evoke different feeding responses (Lee et al. 1991; Nomaki et al. 2006).

Contrary to pristine microplastic treatments, feeding on seawater-soaked microplastic was similar to feeding on nauplii in mixed treatments (Fig. 1b). The similarity of feeding interactions between nauplii and soaked microplastics suggests that *A. gibbosa* perceived seawater-soaked microplastic as a food source and that particles appeared similarly attractive as nauplii. Allen et al. (2017) propose that seawater-soaking potentially removes phagostimulants from microplastic particles and thereby reduces feeding responses to microplastic in corals. Here, in contrast, the consequential formation of biofilms on particle surfaces might have increased *A. gibbosa*'s feeding response, which is consistent with the findings that several LBF species feed on bacterial biofilms (Bernhard and Bowser 1992; Gradziński et al. 2004). Thus, the biofilm on particle surfaces may have led to increased pseudopodal interaction. This is supported by our study as seen in soaked microplastic eliciting more feeding responses than pristine microplastic particles (Fig. 1a). Chemical energy spent on pseudopod formation, movement (Zhu and Skalak 1988; Bowser et al. 1992), and interaction with seawater-soaked microplastic particles might potentially be compensated by feeding on microbial biofilms. However, benefits of feeding on microbial films presumably depend on bacterial composition on the particle surface, which is determined by the type of microplastic (Kniggendorf et al. 2021), due to the species-specific dietary needs of LBF (Lee et al. 1991; Suhr et al. 2003). Although *A. gibbosa* exhibits a potential resilience of its feeding mechanism to pristine microplastic, our results indicate that the presence of seawater-soaked microplastic did significantly impair the uptake of nauplii by *A. gibbosa*. Consequentially, when heterotrophic nutrient uptake is impaired, LBF holobionts would be reliant on autotrophy. As LBF growth rates and calcification depend on their energy budget

(Hallock 1981) and the ingested material (Lee et al. 1991), LBF carbonate production might be decreased.

### Food web implications

Our results indicate that *A. gibbosa* interacts with both pristine microplastic and seawater-soaked microplastic to varied degrees in all treatments. In mixed treatments, feeding on pristine and conditioned microplastic was significantly higher than in single choice treatments (Fig. 1). This suggests that the presence of a natural occurring food source may stimulate the overall feeding activity of *A. gibbosa*, similarly as documented for scleractinian corals (Axworthy and Padilla-Gamiño 2019; Savinelli et al. 2020). These feeding attempts might result in blockage of the feeding aperture, leading to a decreased uptake of natural food sources. We interpret our observation where microplastic particles were positioned close to the feeding aperture (Fig. 2a,b) as blockages, potentially inhibiting the ingestion of nauplii. We documented decreases in feeding rate on nauplii in the presence of microplastic in both experiments, although to a greater extent in Experiment 2 (Fig. 1). Feeding on nauplii and seawater-soaked microplastic particles in mixed treatments of Experiment 2 was in fact similar (Fig. 1b). The decrease in nauplii uptake might be the consequence of aperture blockages or energy needed to expel microplastic particles prior to feeding on nauplii, this will however need further investigation. Furthermore, our study highlights the difference between interactions of the LBF with pristine and seawater-soaked microplastic particles, demanding for caution when interpreting aquaria experiments with pristine particles. As microplastic in the natural environment is usually seawater-soaked, the effects might be much more severe than deduced from experiments with pristine plastics.

As our study is one of first to document an active choice mechanism for a benthic calcifier, further work is needed to gain understanding on the impact of differently shaped and sized microplastic particles as well as natural abiotic particles (Harris and Carrington 2020). Particles that pass through the LBF aperture may have greater physiological impact. Further work to understand the mechanisms of pseudopodal interaction with microplastic (feeding vs. interaction) is needed to understand the energetic expenditure of such interactions. The role of biofilms, which form on microplastics and potentially increase feeding responses, needs further attention, as well as egestion mechanisms for microplastic and other non-food particles. An additional concern are the effects of microplastic on diatom endosymbionts, as these are vital for LBF survival and calcification and have been shown to be negatively impacted by microplastic exposure (Guo et al. 2020; Wang et al. 2020). Thus, in context of the global degradation of reefs, understanding how reef carbonate production rates will be impacted by microplastic pollution is necessary. For that purpose, the present study provides first insight into potential behavioral responses of LBF, which will in the

future hopefully allow for better assessment of microplastic pollution impacts on LBF as important members of coral reef ecosystems.

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