

Plastic particles as a food source and their incorporation in tests of large benthic foraminifera

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Large benthic foraminifera (LBF) are essential components of tropical coral reef communities and key carbonate-producing organisms. LBF can be utilized as indicators of pollution and environmental change. Marine litter, particularly plastic debris, presents a largely unquantified stress on foraminifera. Most studies on plastic pollution have focused on physiological responses of few organism groups (e.g., fishes, corals). Previous studies showed negative effects of micro- and nanoplastics on organismal physiology and ecosystem functioning. The potential responses of foraminifera remain widely unknown.

We present some of the first feeding choice experiments on LBF, comparing plastics with common food choices. Initially, we document the impact of microplastics (150-300 μm) on heterotrophic feeding behavior of *Amphistegina gibbosa* incubated either with *Artemia sp. nauplii* only, with pristine microplastic particles only or with a choice of nauplii and pristine microplastic. In a duplicate experiment, we compared the effect of pristine microplastic vs. microplastic that was pre-conditioned in artificial seawater. Our results indicate a strong feeding selection against pristine microplastic, suggesting a selective ability of the foraminifera to discern between potential food sources. The presence of pre-conditioned microplastic caused similar feeding interaction rates as with the natural food source *Artemia*. This suggests that feeding behaviour (and subsequently energy resources) of LBF may be more severely impacted by microplastics with longer residence times in marine environments.

In a subsequent long-term study, we exposed *A. lobifera* and *A. gibbosa* to nanoplastic particles ($\sim 1 \mu\text{m}$) and sterilized *Nannochloropsis* algae cells as a natural food source within the same size range. Here, observed the uptake of polymer nanoparticles deep into the foraminiferal test and the incorporation of plastic particles into the outer calcite walls of the tests. Despite the high degree of specialization regarding skeletal formation of LBF, in this study, abundant cases of nanoplastic encrustation into the calcite tests were observed. Nanoplastic incorporation into the test was associated with LBF growth by formation of new chambers, in conjunction with continuous nanoplastic ingestion and subsequent incomplete egestion. Microalgae presence in nanoplastic treatments significantly increased the initial feeding response after 1 day, but regardless of microalgae presence, nanoplastic ingestion was similar after 6 weeks of chronic exposure. While $\sim 40\%$ of ingesting LBF expelled all nanoplastic from their cytoplasm, nanoplastic was still attached to the outer test surface and subsequently encrusted by calcite. These findings highlight the need for further investigation regarding plastic pollution impacts on foraminifera, such as their function as potential plastic sinks or plastic pollution indicators, as well as the effects of alterations in the structural integrity of foraminiferal tests. Large-scale incorporation of nanoplastic into LBF tests as well as potential consequences (e.g., test instability, toxicity) could impact ecosystem functions related to LBF, such as carbonate sediment generation on coral reefs.