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„Uptake and effect of micro-plastic particles on the symbiont-bearing foraminifera *Heterostegina depressa* and *Amphistegina lobifera*"

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Abstract

Foraminifera are unicellular marine protists inhabiting the oceans worldwide. The presented study investigates the uptake of polystyrene (PS) micro-plastic particles of the two symbiont-bearing large benthic foraminifera (LBF) species *Heterostegina depressa* and *Amphistegina lobifera* over a period of four weeks. The effect of those particles on the organism's health was studied by using imaging PAM analysis to assess the photosynthetic efficiency of the symbiotic diatoms as well as stable isotope analysis of ^{13}C and ^{15}N to assess the metabolic activity of the foraminifera. Also, the impact of food availability on the micro-plastic uptake was investigated. *Heterostegina depressa* – a species that is not taking up extracellular food but is completely nourished by their phototrophic symbionts – took up less micro-plastic particles compared to *A. lobifera* that will actively feed on external particles in addition to consuming its symbiont's photosynthesis products. Also, the location of incorporated particles differed between the two species. While most particles got stuck on the foraminifera's pseudopods of both species, only *A. lobifera* transported them significantly further into their cytoplasm. The photosynthetic efficiency in both species was not significantly affected by the presence of plastic, suggesting that the diatom symbionts did not suffer from the incorporated particles. The uptake of nitrogen was not affected either, but a difference in carbon uptake was noticed. While *A. lobifera* decreased their carbon uptake, *H. depressa* increased it, suggesting different strategies of dealing with the pollutant by the foraminifera species. Finally, the presence of *Nannochloropsis salina* algae impacted neither the uptake of plastic particles nor the photosynthetic efficiency and isotope uptake, suggesting that food availability and plastic incorporation are not linked. While the pathway and number of plastic particles taken up depended on the foraminifera's metabolic lifestyle, both species seem to be relatively resistant to the micro-plastic pollutant.

Zusammenfassung

Foraminiferen sind einzellige, marine Organismen, die in den Meeren weltweit vorkommen. Diese Studie untersucht die Aufnahme von Polystyrol (PS) Mikroplastik-Partikeln durch die beiden Symbionten-tragenden, benthischen Großforaminiferen (LBF) *Heterostegina depressa* und *Amphistegina lobifera* über einen Zeitraum von vier Wochen. Um die Auswirkungen dieser Partikel auf die Gesundheit der Organismen feststellen zu können, wurde einerseits die photosynthetische Effizienz ihrer symbiotischen Diatomeen mittels imaging PAM Analyse untersucht und andererseits der Metabolismus der Foraminiferen durch Isotopen-Analyse der stabilen Isotope ^{13}C und ^{15}N. Ebenso wurde untersucht, ob die Verfügbarkeit von Nahrung einen Einfluss auf die Mikroplastik Aufnahme hat. *Heterostegina depressa* – eine Art, die sich vollständig von den Photosyntheseprodukten ihrer Symbionten ernährt – nahm weniger Plastik auf als *A. lobifera,* die zusätzlich aktiv Nahrung aufnimmt. Auch der Ort, an dem die Partikel gefunden wurde, unterschied sich. Obwohl bei beiden Arten das meiste Plastik an den Pseudopodien hängenblieb, transportierte nur *A. lobifera* die Partikel maßgeblich weiter in das Zytoplasma hinein. Die photosynthetische Effizienz blieb bei beiden Arten gleich, was darauf hinweist, dass die symbiontischen Diatomeen nicht unter dem aufgenommenen Plastik leiden. Auch bei der Aufnahme von Stickstoff konnte kein Unterschied festgestellt werden. Die Aufnahme von Kohlenstoff durch *H. depressa* stieg unter dem Einfluss von Mikroplastik an, während die Aufnahme durch *A. lobifera* sank, was darauf hindeutet, dass die beiden Organismen unterschiedlich reagieren, um mit der Plastikverschmutzung umzugehen. Auch die Anwesenheit der Alge *Nannochloropsis salina* hatte weder eine Auswirkung auf die Menge an aufgenommenem Plastik durch die beiden Arten noch auf die photosynthetische Effizienz oder die Isotopenaufnahme. Es kann daher davon ausgegangen werden, dass die Verfügbarkeit von Nahrung keinen Einfluss auf die Auswirkungen von Mikroplastik hat. Zwar konnte gezeigt werden, dass das Nahrungsaufnahme-Verhalten der Foraminiferen einen Einfluss darauf hat, wie viel Mikroplastik sie aus der Umgebung aufnehmen, sie scheinen aber im Großen und Ganzen relativ resistent gegen die Verschmutzung durch Polystyrolpartikel zu sein.

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1. Introduction

Foraminifera are a group of marine micro-organisms that are usually unknown to the general public. However, despite not getting media attention like corals do, they are widely recognized in the scientific community and often compared to corals in terms of their importance for marine ecosystems, calcification potential, primary production (through symbionts) and the global carbon cycle. Also, they are highly responsive to environmental changes and pollution, making them an important object to studies on the impact of human activities on marine ecosystems. Their disappearance could have serious detrimental effects on their surroundings. For that reason, multiple studies have been conducted analysing the impact of a variety of different environmental parameters and toxic substances on those organisms.

The presented study aims to investigate one of the biggest talking points when it comes to the pollution of the oceans: plastic. It is probably the pollutant that gathers the biggest media coverage and is recognised as a problem that we will have to deal with rather quickly. Since the mechanical and chemical forces present in the ocean break down plastic into micro-plastic particles, it is obvious that even small organisms like foraminifera might be at risk of taking up those particles. Studying the effect of micro-plastic on foraminifera is interesting because of multiple reasons. A harmful effect of plastic uptake would not only endanger the health of the foraminifera itself, but also that of the corresponding eco-system. Furthermore, since foraminifera stand relatively low on the food chain, an accumulation of plastic particles in them could act as a gateway for the plastic to get into bigger organisms and finally even us.

Experiments and different analyses have been conducted in this study with two species of large benthic foraminifera – *Amphistegina lobifera* and *Heterostegina depressa* – to examine whether they take up micro-plastic particles and if so, how it affects their metabolism and photosynthetic efficiency. Whether the availability of food and the different feeding habits of said species have an effect on the uptake of plastic has also been investigated.

1.1. Foraminifera

Foraminifera are single-celled organisms that belong to the group of protists (Erez, 2003), which makes them eucaryotic organisms that are neither animals nor plants nor fungi. Within the Infrakingdom of Rhizaria they are located in the Phylum of Retaria (Margulis and Schwartz, 1998). Foraminifera are quite similar to amoeba but differ in that they possess reticulopodia which are a special kind of pseudopodia (Hausmann et al., 2003) that they use for attachment, locomotion and feeding (Jones, 2014). A second very important feature of foraminifera is the formation of a test. These shells are composed of (in rare cases) resistant protein, calcite, high-Mg calcite (miliolid forms) or agglutinated substrate (Gross, 2005). Additionally, some do not build such a solid test, but a soft wall out of organic material instead. These species are often connected to freshwater habitats (Pawlowski, 2003), which can be considered another exception since for a long time foraminifera were thought to be strictly marine or brackish water organisms (Holzmann and Pawlowski, 2002).

Since their mineralized test cannot be expanded, many foraminiferal species will enlarge their shells when growing by adding bigger and bigger chambers; the most recent chamber has a mouth opening called aperture. This aperture remains when a new chamber is built and will then connect the compartments (Gross, 2005; Murray, 1991). This aperture also gives foraminifera their name – coming from the latin word “foramen” meaning “hole” and being introduced 1826 by Alcide Dessalines d’ Orbigny (BouDagher-Fadel, 2008). Through this opening and small pores in the test (mainly in calcareous forms) the foraminifera can stretch out their pseudopodia (ibid.).

Different modes of life have been explored by foraminifera – most species live benthic on the seafloor, but there are also many planktonic open water species and even some parasitic (Erez, 2003) whereas the benthic mode is the oldest one with the planktonic mode following in the Jurassic era (Oxford et al., 2002). Just as diverse as their habitat is their reproduction; some species alternate between sexual and asexual reproduction, some procreate mainly asexually, some only sexually (Jones, 2014). The feeding habits of foraminifera are equally varied and include detritus and suspension feeding, parasitism and digestion of photosynthetic products of symbionts or in some cases even kleptoplasts (Murray, 1991).

Photo-symbiosis is a common strategy in foraminifera and probably evolved multiple times independently (Fay, 2010). Most common partners are diatoms and dinoflagellates (Lee et al., 1995); less common are red algae and green algae (Fay, 2010). These partners are specific to the respective foraminifera species and hosted in the endoplasm. Symbiotic diatoms appear "naked" without their frustules (Murray, 1991 and Schmidt, 2018). Usually, the foraminifera will use the photosynthetic products as supplementary feeding strategy while still actively feeding by using their pseudopods (Toler, 2002) although there are species (e.g. *Heterostegina depressa*) that rely solely on the photosynthesis of their symbionts (Röttger, 1972). But this is not the only benefit of having symbionts – the chemical reaction induced by photosynthesis facilitates calcification and therefore helps in building the test (Erez 2003). Apart from the protection that the foraminifera's test provides (Lee and Hallock, 1987) this symbiosis however is heavily in the foraminifera's favour and sometimes the symbionts will even be digested before sexual reproduction to generate additional energy (Toler, 2002).

Foraminifera are a very old group of organisms with fossil records dating back to the Proterozoic 560 million years ago, while some molecular-genetic research suggests an even older origin between about 690 and 1 150 million years back (Pawlowski et al., 2003). While we know of over 4 000 living species, more than 40 000 species have a fossil record showing a relatively rapid evolution (Erez, 2003). This means that many distinct species of foraminifera only lived for a short period of time. Also, they often occur in masses and their shells form sediment that withstands the test of time, which results in them being one of the most commonly found fossil (Gross, 2005). These facts combined lead to foraminifera being an important index fossil.

1.1.1. Larger Benthic Foraminifera

Usually, foraminifera are separated into two major groups – benthic and planktonic ones. Within the benthic group there is an important subgroup that is referred to as "larger benthic foraminifera" (LBF for short). The term "larger" does not necessarily refer to their morphological size, but the complex internal structure of their tests (BouDagher-Fadel, 2008). However, this usually leads to a bigger size anyways (from 500 µm up to several cm).

While benthic foraminifera in general inhabit substrate at all water depths, LBF occur abundantly mainly in shelf regions of tropical or subtropical shallow oceans in warm temperatures and carbonate-rich environments (Beck Eichler and Barker, 2020). This makes them an important inhabitant of coral reefs in those regions.

The first mention of larger benthic foraminifera dates back to ancient Greece and the Histories of geographer and historian Herodotus from the 5th century BC, who mentioned structures in the limestone the Egyptian pyramids were built from. Those were later identified as foraminifera from the Nummulites-group (Rawlinson et al., 1862; BouDagher-Fadel, 2008). The group of LBF itself exists since the early Silurian and throughout its over 440 million years of existence saw two major evolutionary developments: the biomineralization of $CaCO_3$ tests and the acquisition of photosymbionts (Ross, 1974). The development of high-MG calcite Miliolida occurred during the early Carboniferous; hyaline calcite species followed later in the Permian (Reymond, 2022). The first instance of photosymbiosis probably appeared in the late Silurian (Lee and Hallock, 1987).

There are multiple factors that make this group of foraminifera especially interesting and are reason for them to be addressed as their own subgroup. Firstly, they are widely recognised as important index fossils, keystone species and bioindicators (Nagy and Alve, 1987; Raymond et al., 2022). Their abundant occurrence makes them sediment-forming and their susceptibility to environmental changes leads to rapid evolution and frequent extinction. The fossil record of LBF is rich in individual fossils and enables a horizontal as well as vertical variation to be studied in the stratigraphic record (BouDagher-Fadel, 2008).

Secondly, their size and preference for steady conditions makes LBF suitable organisms for scientific experiments. Since they tend to react to even small changes in their environment, the effect of various parameters and pollutants such as temperature, salinity, alkalinity, organic waste, heavy metals, pesticides, oil and many more can be tested on foraminifera (Grefstad, 2019) to reach an understanding of how those changes could affect the habitat of LBF.

The third reason is the impact of LBF on the carbon cycle and with that their place in recent environmental discussion. The reef regions (while only making up about 0.17% of oceanic surface) are responsible for a sixth of the ocean's carbonate production and

although corals and their symbionts are responsible for most of that, about 4.8% of the reefs carbonate production (about 43 million tons annually) is contributed by foraminifera and 80% of that by the group of LBF (Langer et al., 1997). This is relevant in the context of global climate change discussion. About 30% of the atmospheric CO_2 produced by humans is estimated to having been absorbed by the world's oceans over the last 200 years (Pettit, 2015) and foraminifera herby make an important contribution. Although the biomineralization process of $CaCO_3$ by foraminifera initially leads to the release of CO_2 (Langer et al., 1997), the carbon then stays bound in the shells, which will be buried in the sediment for a very long time (Erez, 2003).

Larger benthic foraminifera can reach sizes of multiple millimetres or in extreme cases even over 15 cm while still being a single-cell organism (Binczewska et al., 2015). Such sizes are only advantageous under stable environmental conditions and in habitats with limited food resources (Hallock, 1985). There are different considerations that could explain this evolutionary trend. One is the hosting of photosymbionts. These allow the foraminifera to thrive in oligotrophic settings such as coral reefs (BouDagher-Fadel, 2008). Their complex internal structure also makes it easier to house symbionts (Hallock, 1985). Another explanation is that when environmental conditions are stable and the supply of nutrients is secured, one of the biggest factors left that has to be dealt with is high juvenile mortality (ibid.). Hallock showed that bigger sizes lead to better chance at survival for juvenile individuals since usually the adult organism dies when reproducing and divides its cytoplasm amongst its offspring (BouDagher-Fadel, 2008). Also, the LBF's reproductive cycle alternates between microsperic Agamonts (2n) and megalospheric Gamonts (n). However, a third form has been described in many LBF: megalospheric Schizonts that are produced asexually but in contrast to the zygote start their life as large, symbiont-bearing cells (Dettmering et al., 1998). This trimorphic life cycle leads to a relative abundance of megalospheric individuals in the population which adds to the perception of there being many large individuals.

1.1.2. Foraminifera in this Study

In the presented study, two species of LBF were used: *Heterostegina depressa* and *Amphistegina lobifera*. Both host symbionts and are abundant in shallow, warmer, marine shelf habitats.

Heterostegina depressa

The genus *Heterostegina* originated in the late Eocene and belongs to the family of Nummulitidae (BouDagher-Fadel, 2008). The species *H. depressa* was first described in 1826 by Alcide Dessalines d' Orbigny (Orbigny, 1826).

It is distributed throughout tropical shelf areas around the world in shallow water and prefers living on hard substates (Reiss and Hottinger, 1984). Nowadays, it is also mentioned as alien species (originally not native) in the Mediterranean Sea and spreading from the eastern part to the middle of the Mediterranean Sea (Stulpinaite et al., 2020) since it is better adapted to increasing temperatures compared to other foraminifera there (Schmidt et al., 2014; Lintner et al. 2022).

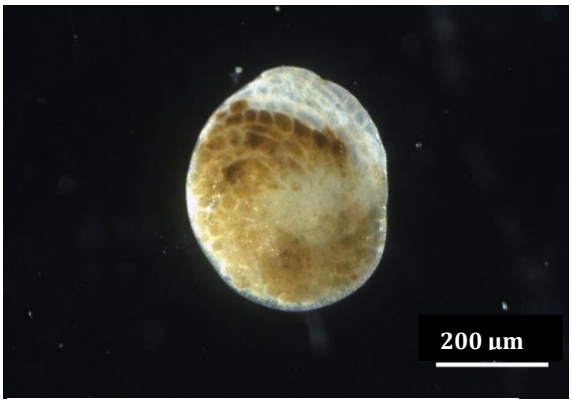

Fig. 1: Light microscope image of *Heterostegina depressa.*

Like most LBF species H*. depressa* hosts obligatory photosymbionts in the form of diatoms without frustules. This makes it very difficult to determine the diatom species, however Lee and McEnery successfully let them rebuild their frustules after having isolated them from their foraminifera host (Lee and McEnery, 1979). This demonstrated that the missing shell is an adaptation to the symbiosis and also made identification of the symbionts on species level possible. They found that LBF can not only host one single symbiont but two, sometimes up to three different diatom species, whereby *Nitzschia panduriformis* was found in all individuals of *H. depressa* and most of *Amphistegina lobifera* they analysed (ibid.) which probably makes them the most important symbiosis partner for these two foraminifera. Concerning the distribution of symbionts, it was discovered that in *H. depressa* they were most densely packed in the intermediate and outer chambers of the organism but rare in the first whorl (McEnery and Lee, 1981).

A special property of *H. depressa* is that unlike most symbiont-bearing LBF, it does not actively feed but lives solely off of its symbionts photosynthesis products (Röttger, 1976; Lee et al., 1988; BouDagher-Fadel, 2008). This might also be an explanation for the thin disk-like shape of this species.

Heterostegina depressa has yet another special property that differentiates it from other LBF and (most importantly for this study) from *A. lobifera*. Calcareous LBF usually have a canal system and pores that allow cytoplasmic flow between the chambers and communication of cytoplasm between the outside environment and the intra-shell endoplasm (Erez, 2003). These canals flow into pores in the outside of the test and are plugged by an organic lining on the inside of the test-wall (ibid. and Toler, 2002). But while most foraminifera prefer to stretch out their pseudopods from the aperture, *H. depressa* can be observed quite often to extrude its pseudopods from any point of this canal system (Murray, 1991).

Amphistegina lobifera

The genus *Amphistegina* belongs to the family of Rotalioidea and emerged in the Eocene with fossil records reaching back 50 million years (Loeblich and Tappan, 1988). It was also first described by Alcide Dessalines d' Orbigny in 1826 (Orbigny, 1826).

Amphistegina lobifera inhabits similar habitats to *H. depressa* and prefers warm, shallow water (between 15 and 40 m) near the coastline (Schmidt et al., 2011). There it is most commonly found living epiphytic on algae or algal-coated rubble (BouDagher-Fadel, 2008). Coming from the Red Sea it is considered an alien species in the Mediterranean Sea and because of its impact on the ecosystems it moves to, is classified as invasive (Stulpinaite et al., 2020).

Fig. 2: Light microscope image of *Amphistegina lobifera.*

Amphistegina lobifera builds a noticeable thicker test and is much rounder compared to *H. depressa*. It was discovered though that with increasing water depth its morphology changes to having a thinner and more flat test (Hottinger, 2000) which allows more light to reach its symbionts. Its durable test and geotactic sense allows it to inhabit areas with more turbulent water and return to the surface when buried in sediment (Toler, 2002).

Although they too live in an obligatory symbiosis with diatoms (*Fragilario shiloi* seems to be this group's most important diatom symbiont) (Lee et al., 1979) and need them for

calcification, in contrast to *H. depressa*, individuals of *A. lobifera* are not completely reliant on their photosymbionts when it comes to nutrients and will actively feed (Toler, 2002). *Amphistegina lobifera* has a perforate test with pores but will extend its pseudopodia from its slit-shaped aperture (ibid.).

The genus *Amphistegina* was recognised for being able to survive extended periods of time without any light and appears to be able to go into dormancy as a survival response to environmental hazards such as absence of light, anoxia or adverse temperatures (Ross and Hallock, 2019).

1.2. Plastic Pollution of the Oceans

Plastics are synthetic organic polymers produced by polymerisation of monomers extracted from oil (Cole et al., 2011; Grefstad, 2019). It is no secret that because of their versatile areas of application they are produced in masses and used practically everywhere today. The production of those materials started around the 1930s and quickly picked up from there (Jambeck et al., 2015). In 2021 an estimate of 390.7 million metric tons of plastics was produced worldwide (Statista Research Department, 2023). Despite the efforts to recycle plastic a huge portion will end up as waste. In the year 2010 about 270 MMT were produced (ibid.) but an estimated 275 MMT of plastic waste was generated and approximately 4.8 to 12.7 MMT of this waste ended up in the oceans (Jambeck et al., 2015). Looking at a longer period of time an estimated 10-11% of produced plastics will find its way into the ocean (Jambeck et al., 2015; Borrelle et al., 2020). About 80% of plastics found in the ocean have a terrestrial source and get transported into the sea by rivers (Andrady, 2011; Cole 2011). Eriksen et al. (2014) estimated how much plastic in total is in the ocean and while they came to a result of 5.25 trillion particles with a combined weight of over 250 000 MT in 2014, in 2023 they upped that to an estimate of 82-358 trillion particles with a weight between 1.1 and 4.9 MMT (Eriksen et al., 2023). Because of their durability and buoyancy those particles spread easily and are globally distributed across all oceans (Eriksen et al., 2014). Some plastics however are denser than water and sink which makes them also omnipresent in seafloor sediments and even in great depths (Barnes et al., 2009; Gohla et al., 2021).

Plastic that stays in the ocean will over time be mechanically and chemically broken down by the water and sunlight into smaller pieces (Thompson et al. 2004; Cole et al., 2011;

Eriksen et al., 2013). This is problematic because smaller particles are easier distributed, are more likely to be taken up by marine organisms and are much harder to detect and collect (Andrady, 2017). Plastic particles are divided by size but different authors defined different sizes and names which makes the term "microplastic" used relatively loosely (Cole et al., 2011; Cauwenberghe et al., 2013; Andrady, 2017). However, the most commonly used and widely accepted classification is: macroplastics (>5mm), microplastics (<5mm) and nanoplastics (<1 μm) (Andrady, 2017). Therefore, the term "microplastic" includes all particles between 1 μm and 5mm which is quite a big range and leads to many studies being difficult to compare. Further a distinction is made between primary microplastics – plastics that are industrially manufactured as microbeads – and secondary microplastics that derive from fragmentation of bigger debris as mentioned (Fendall and Sewell, 2009; Andrady, 2017). Because of the long persistence of those materials – ranging from decades to hundreds of years – they accumulate in the ocean (Birarda et al., 2021) and by now make up the most parts of plastic debris. More than 92% of all plastic waste in the ocean consists of particles smaller than 5mm (Eriksen et al., 2014) and particles smaller than 1mm make up 65% of all debris (Browe et al., 2010). Because of the fragmentation, even if no additional plastic would enter the ocean, the amount of microplastic would still increase over time.

The most common plastics found in the ocean are polyethylene (PE), polypropylene (PP) and polystyrene (PS) (Hidalgo-Ruz et al., 2012; Andrady, 2017). Technically all of those materials are biochemically inert and none of them is inherently toxic (Grefstad, 2019). Still micro-plastic particles pose multiple potentially harmful implications for basically all organisms for various reasons:

A) Microplastic particles can absorb and carry so called persistent organic pollutants (POP) which can be toxic (Barnes et al., 2009; Andray, 2017).
B) In a similar way the particles can host and transport non-indigenous species to new locations (Barnes et al., 2009).
C) While polymers are usually biochemically inert the same does not account for the monomers they are made of. PE and PP do not carry residual monomers but other plastics like PS do (Andray, 2017).
D) Plastics often include additives – chemicals intentionally added to achieve certain properties. Those include colouring pigments, flame retardants, stabilizers, antimicrobial agents and plasticisers (Andray, 2017 and Grefstad, 2019).

The additives are usually the most problematic aspect since they can be used in high concentrations, are often easily released from the polymers and for the most part are lipophilic which enables them to penetrate cell membranes (Hammer et al., 2012 and Andray, 2017). Some plasticisers have even been shown to affect the hormone systems of various fish and invertebrates (Oehlmann et al., 2009).

Whether plastic particles will be taken up by marine organisms has been tested in many different studies in the past decades. Bhattacharya et al. (2010) for example observed the uptake of PS beads in the two algae species *Chlorella sp.* and *Scenedesmus sp.* (Bhattacharya et al., 2010). In 2008 Browne et al. (2008) showed the accumulation of plastic beads in the mussel *Mytilus edulis* (Browne et al., 2008). Ingestion has also been proven in seabirds (Avery-Gomm et al., 2012), sea turtles (Bugoni et al., 2001), whales (Jacobsen et al., 2010) and fish (Carson, 2013; Romeo et al., 2015). Those are just a view examples. It has also been proven in various studies, that ingested plastic can be transferred through the food web and from organism to organism (e. g.: Murray and Cowie, 2011; Farrell and Nelson, 2013; Setälä et al., 2014).

It is undeniable at this point, that plastic can be encountered by virtually any marine organism regardless of habitat and size and that it can have potentially harmful effects on them.

1.3. Pollution in Foraminifera

Since the importance of foraminifera as bioindicators has been discussed already, it is no surprise that the effect of different pollutants on those organisms has already been subject of various studies in the past. When treated with pollutants the foraminifera often showed deformation of their test, bleaching (photosymbionts dying off) or died completely (e.g.: van Dam et al. 2012; Boehnert et al., 2020; Caridi et al., 2020; Lintner et al. 2022).

Bubl (2022) investigated the effects of four heavy metals that are often connected to anthropogenic pollution (Cu, Fe, Pb, Zn) on LBF *H. depressa* and found that especially copper and zinc in higher concentration were toxic for the organisms. For lead no negative effect was noticed which correlates with studies from Boehnert et al. (2020) and Lintner et al. (2021). The herbicide diuron that was known to be detrimental to corals was tested on thirteen different tropical foraminifera species by van Dam et al. in 2012 and proved

to lead to destruction of the photosystem II in their symbionts (van Dam et al., 2012). Lintner et al. (2022) tested different sunscreens and Ensulizole (a common agent in sunscreens) on *H. depressa* and noticed a strong negative effect on the symbiont's photosynthetic performance. Especially "ecofriendly" sunscreens had a stronger negative effect which was suspected to be caused by titanium dioxide (TiO_2) and zinc dioxide (ZnO_2) used in those sunscreens (ibid.). Negative effects of titanium dioxide and silicon dioxide (SiO_2) nano-particles could also be shown by Ciacci et al. (2019). In 2020 Caride et al. exposed multiple LBF species to cigarette butts and found them to be highly toxic to all of them.

Those examples show that a multitude of different pollutants have been tested on foraminifera with most of the studies finding detrimental effects on the organisms.

1.3.1. Plastics in Foraminifera

Although there are many studies on pollutants in foraminifera, compared to the magnitude of plastic pollution present in the oceans, research on the effect of plastics on foraminifera is relatively scarce. However, a view studies on the matter have been conducted in the past view years.

Ciacci at al. (2019) investigated the uptake and effect of PS nanoparticles on *Ammonia parkinsoniana.* After an incubation period of 24 hours, they were able to locate particles in the foraminifera and noticed that the mitochondria of treated individuals were swollen and degenerated and the production of reactive oxygen species (ROS) was increased. They assumed that the most likely route of the particles entering the organism was through uptake together with larger particles such as food or detritus (Ciacci et al., 2019).

The uptake of microplastic particles by foraminifera was studied by Grefstad (2019). She mixed fluorescent PS-beads of different sizes (0.5 µm, 1 µm, 6 µm) into collected sediment and after a period of six hours and four weeks identified all present living foraminifera and checked whether plastic could be found inside them or not. In total she identified 41 species, 17 of which took up plastic after six hours and 21 after four weeks (Grefstad, 2019). She noticed that most foraminifera did not differentiate between microplastic size. However, the exact amount of particles in the organisms was not determined. Also, neither *H. depressa* nor *A. lobifera* were found in the samples and the plastic concentration was quite high (over 100 million particles per ml sediment for the 1 µm beads). She could

however prove, that many foraminifera species will indeed incorporate plastic after a very short amount of time.

In 2021 Birarda et al. used FTIR microscopy to identify plastic particles in foraminifera collected from a plastic bag on the sea floor they had been attached to and found that they did incorporate parts of the plastic they lived on. They also did in vitro experiments on *Rosalina globularis* treated with DEHP (a commonly used plasticiser) and confirmed that the foraminifera did incorporate it in their cytoplasm (Birarde et al., 2021). Even though DEHP at room temperature is in a liquid state and therefore not comparable to solid plastic beads, those results are very interesting for the fact that this means that foraminifera could potentially be harmed by substances released from plastic waste even if the plastic itself is not taken up.

Langlet et al. (2020) tested PP leachates on *Haynesina germanica* at environmentally realistic and chronic concentrations to see whether it would have an effect on the locomotion or metabolism of those organisms. They however were not able to detect any effect, suggesting that benthic foraminifera might be more resistant to some pollutants than marine metazoans.

The question, if foraminifera are able to differentiate between plastic and food particles was recently investigated by Joppien et al. (2022). They compared the number of interactions by *Amphistegina gibbose* with PE particles to the number of interactions with nauplii larvae of *Artemia sp.* and found that the foraminifera had a strong preference for the larvae (Joppien et al, 2022). The plastic particles used had a similar size to the nauplii larvae and therefor were quite big (150-300 µm) which means that the foraminifera probably were not able the incorporate them but the experiment still shows that in principle they are able to differentiate between plastic and food.

1.4. Goals and Hypotheses

The presented study aims to research the uptake of plastic particles by the two LBF species *Heterostegina depressa* and *Amphistegina lobifera,* as well as the effect of those particles on the health of the foraminifera. There are three main questions that shall be answered here:

Uptake of plastic particles

The fact that foraminifera can take up microplastic beads was proven in the past. However past research also suggested that those particles mainly enter the organism together with other food or substrate particles. *Heterostegina depressa* is known to not actively feed while *Amphistegina lobifera* uses a combination of photosymbiosis and feeding for its energy and nutrient uptake. Also, it might be possible that microplastic particles can enter the foraminifera through their pores. The exact function of those pores is still not completely clear although it is assumed that they are mainly used for respiration (Rathburn et al., 2018). Those pores can measure up to 10 µm in diameter and are closed by an organic lining (Erez, 2003). The pores of *A. lobifera* specifically have been measured to have an average diameter of 5.1 µm (Stuhr et al., 2019) which would make the 2 µm PS beads used in this study easily fit through. While the organic plug definitely lets through some particles (Glock, 2011), it is unclear if that will be the case for PS beads. *Heterostegina depressa* will stretch out cytoplasm from its pores but selection of trapped particles is possible (Murray, 1991).

Comparing the plastic uptake of those two species, as well as the amount and location of incorporated beads, will have interesting implications for the relationship between feeding habits and microplastic uptake.

Hypotheses I: Based on the different metabolic lifestyle, *A. lobifera* will take up more plastic than *H. depressa* as long as they will be incubated under the same environmental conditions.

Effect of plastic on foraminifera

Whether plastic particles pose a threat to the foraminifera's health is one of the most pressing questions and at the same time not easy to answer. Since health and wellbeing cannot be measured easily, they have to be assessed secondarily through other factors. In the presented study two indicators will be considered:

- The photosynthetic efficiency of the symbionts as well as the photoactive surface will be measured using imagingPAM-microscopy. Higher values here mean healthy symbionts and therefore healthy hosts.

- The metabolism of the foraminifera will be measured by assessment of isotope uptake from the surrounding seawater using stable isotope analysis. A higher metabolism rate also indicates a healthier organism.

Hypotheses II: The uptake and accumulation of microplastic particles will have negative effects on the photosynthetic efficiency as well as the metabolism rate of the studied foraminifera species.

Effect of food availability

Past studies have shown that foraminifera are able to differentiate between food and non-edible particles. The third question the presented study tries to answer is whether the availability of food has an impact on the amount of plastic incorporated. Since *H. depressa* is not actively feeding the presence of food should not have a big impact on its behaviour. For *A. lobifera* however it is possible that the presence of food is an incentive for the foraminifera to start interacting more with particles around it and therefore to take up more plastic. It is also possible that the availability of food makes *A. lobifera* ignore plastic particles it would otherwise have inspected for a lack of better options.

Hypotheses III: The presence of food does not change the amount of plastic particles incorporated by *H. depressa* but does have an effect on the amount taken up by *A. lobifera.*

2. Material and Methods

The uptake and effect of plastic was tested multiple times over a period of four weeks to investigate short- to long-term effects.

Plastic particles

The plastic particles chosen were pure synthesized polystyrene (PS) beads with a diameter of 2 µm (Sigma-Aldrich©, L4530). The reason for this is that PS is one of the most commonly used plastics and therefore also one of the most common plastics in the ocean. The size was chosen because it fits within the particle size range most common in the ocean and also is small enough to be reasonably taken up by organisms in the size range

of foraminifera. It is also in the size range of algae preyed upon by *A. lobifera.* Another benefit of PS is its density. Most common plastic polymers have a density between 0,9 and 1.5 g/cm^3 while seawater has a density of around 1.02-1.03 g/cm^3 (depending on salt content) (Gohla et al., 2021). Polystyrene with its density of 1.05 g/cm^3 therefore is just slightly denser than seawater. This means that over time it will sink to the ground to end up in the sediment where LBF live. But since the difference is so small that means even small turbulences in the water can disperse it which explains how it gets distributed so far. Those properties of PS make it a substance that LBF are very likely to encounter in their natural habitat.

Organisms

The foraminifera for all experiments were taken from their respective main culture at the Department of Palaeontology of the University of Vienna.

The main culture of *H. depressa* came from a shark tank from the "Haus der Natur" in Salzburg in Austria and is cultivated in the Department of Palaeontology since 2015. They are held at a temperature of 25°C and a salinity of 35 PSU with an eight-hour light period per 24 hours from 8-16h.

The main culture of *A. lobifera* was brought to the Department of Palaeontology in 2019. The original organisms were collected in the bay of Agia Pelagia, a small town around the middle of the north coast of Crete. They are cultivated at a temperature of 25°C and a salinity of 38 PSU with an eight-hour light period per 24 hours from 8-16h.

Only adult individuals containing brownish cytoplasm, which covered the whole test and with approximately the same size were used for the experiments.

2.1. Experimental Setup

The whole experimental setup was done twice – once for each foraminifera species. Each setup was split into three groups, one for the fluorescence microscopy, one for PAM-microscopy and one for isotope analysis. For all three methods measurements were conducted at the start of the test series and then after 1, 7, 14, 21 and 28 days. Throughout the experiment there were three different cultures in every group with six replicates each:

- Culture C → Control group, individuals were incubated in clean artificial sea water with a salinity of 35 PSU for *H. depressa* and 38 PSU for *A. lobifera.* The artificial sea water was made from filtered, pure water and artificial reef salt (Aquaforest®)
- Culture P → Plastic group, individuals were incubated in artificial sea water with added micro plastic particles. Water was made as described for (C) with plastic beads then being added. The used particles were yellow-green-fluorescent polystyrene beads with a diameter of 2 µm (Sigma-Aldrich©). The final concentration was 52,5 mg plastic per litre of artificial sea water which is equivalent to approximately 100.000 particles per ml water.
- Culture PA → Algae group, individuals were incubated in artificial sea water with added micro plastic particles as well as algae. The water was prepared like described in (P) before having added live microalgae of the species *Nannochloropsis salina.* This species was chosen because of its worldwide occurrence – which means that both foraminifera species can reasonably come across this alga in nature – and its size of around 2 µm in diameter which is consonant with the size of the plastic particles used.

Fig. 3: Cultures incubated in the Hettich PRC.

All cultures were incubated at 25°C and a 12:12 hour day-night-cycle in a Hettich PRC 1200 SL cabinet.

Fluorescence Analysis

Six individuals were investigated in the beginning of the experiments to ensure that no fluorescence signal was observable besides the PS beats. Then the cultures for each time of measurement (1d, 7d, 14d, 21d, 28d) were prepared separately. A crystallisation dish was filled with eighter 200 ml of artificial sea water (C), 200 ml of sea water treated with plastic particles (P) or 200 ml of sea water with plastic and algae (PA). Six

replicates/foraminifera were placed in every crystallisation dish. This way for every time of measurement six individuals could be analysed in each of the cultures (C, P, PA) – finally 5x3 (time x culture type) dishes with 6 individuals each. The crystallisation dishes were sealed with parafilm to avoid evaporation and incubated at 25°C for certain time.

PAM Fluorescence

Since the PAM-analysis does not harm the foraminifera, less individuals were needed in this group, because specimens were used for repeated measurement. After six individuals were measured to assess the start values, three crystallisation dishes were prepared like described above – one for each culture (C, P, PA). Six individuals were placed in each one of them. The same individuals were used throughout the whole experiment and placed back in their respective dish after each measurement and the dishes were sealed again.

Isotope Analysis

The experimental setup for the isotope analysis was identical to the setup for the fluorescence analysis with the only difference that the stable isotopes ^{13}C and ^{15}N were added to the culture medium. Isotopes were added in the form of $NaH^{13}CO_3$ and $^{15}NH_4Cl$ to a final concentration of 0.235 mmol ^{13}C/l and 0.220 mmol ^{15}N/l.

2.2. Analysis

The data were collected in two test series in the time period between November 2022 and February 2023 and later analysed.

2.2.1. Fluorescence Analysis

The following process was repeated at every time of measurement with all three cultures: The individual foraminifera were picked from their crystallisation dish with a fine brush and then cleaned three times in clear artificial sea water with a clean brush to get rid of any PS particles that might stick to the test. After that the cleaned individual were transferred to a glass slide. The calcareous test was then dissolved using 5% hydrochloric

acid (HCl). This step was necessary since the fluorescence signal could not be picked up well through the test (especially with *A. lobifera*s thicker test). The low concentration of HCl resulted in less aggressive formation of gas bubbles and therefore the organic lining of the organism was preserved quite well.

The samples were then observed under an Axio-Imager.M1 fluorescence microscope (ZEISS®) at the University of Vienna Biology Building (Department of Functional and Evolutionary Ecology) using a 10 AF 488 filter (ZEISS®). This filter made the plastic particles appear in a bright green colour but did not pick up the fluorescence signal of the symbiotic diatoms. Pictures were taken of every individual with the fluorescence microscopy settings as well as with normal light microscopy settings using the ZEISS ZEN software (v 1.0.1.0). This way the two pictures could then be compared to determine the exact location of PS particles within the organism.

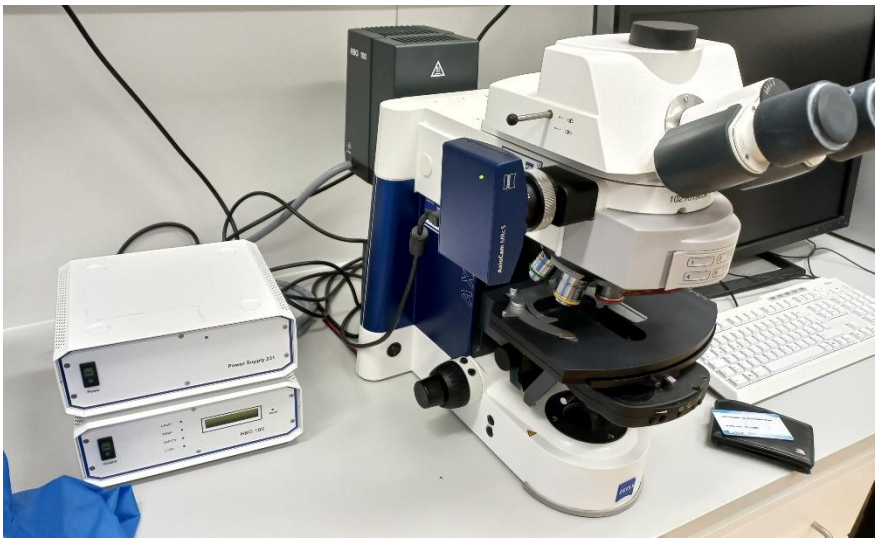

Fig. 4: Axio-Imager.M1 – the fluorescence microscope used to detect the PS beads.

Besides the location of the particles, the amount of incorporated particles was determined. This was done using the open-source image processing software "ImageJ" (v 1.53t) by Wayne Rasband. First it was analysed how many pixels a single PS plastic bead made up at a certain magnification. Then the software was used to count all pixels of the corresponding colour spectrum. This way the number of plastic beads in the picture could be calculated.

2.2.2. ImagingPAM Analysis

The PAM-analysis was conducted at the University of Vienna Biology Building (Department of Functional and Evolutionary Ecology) using the MICROSCOPY-Version of the M-Series (Hein Walz GmbH) in combination with the ImagingWin software (v 2.56p). The settings used remained constant over all measurements (Int.: 3, Frequency: 8, Gain: 3, Damping: 3)

This system allows to not only measure the quantum yield of the symbionts but also visualises the fluorescence which makes it possible to see where exactly photosynthesis is happening and therefore how much surface is covered with symbionts. The name "ImagingPAM" stems from this feature.

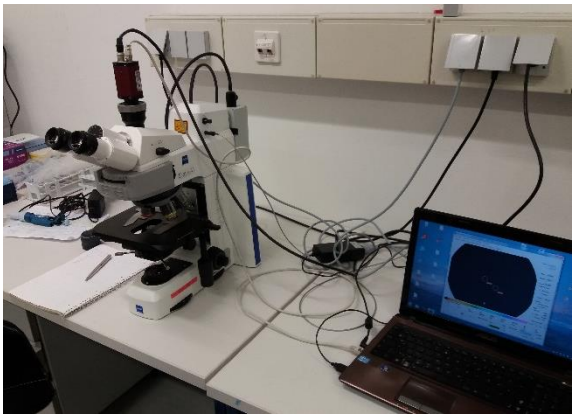

Fig. 5: MICROSCOPY-Version, M-Series – the imagingPAM-microscope used for PAM analysis.

Quantum yield is the ratio between the variable fluorescence (F_v) and the maximum fluorescence (F_m) and represents the efficiency of the Photosystem II. During photosynthesis the light energy can meet three possible fates: transformation into chemical energy, heat radiation and fluorescence (Schreiber, 2004; Kalaji et al., 2017). The lower the percentage of heat and fluorescence, the more efficient is the energy transformation. Two separate measurements are taken. The first is done in darkness when all reaction centres (P680) are open and electron acceptors (Q_A) are oxidised, here the ground fluorescence (F_0) is measured. The second measurement is taken directly after a light impulse that closes the reaction centres and when electron acceptors are deoxidised (F_m). The difference between those two is the variable fluorescence ($F_v = F_m - F_0$). The quantum yield is then calculated as follows:

$$\frac{Fv}{Fm} \text{ or } \frac{(Fm-F0)}{Fm}$$

The calculated value between 0 and 1 represents the photosynthetic efficiency (the higher the more efficient). Those two separate measurements are necessary in order to factor out the heat radiation (Schreiber, 2004; Kalaji et al., 2017).

To measure the foraminifera the individuals were picked from the crystallisation dish with a fine brush and placed on a glass slide with a small chamber that offered enough space for the organism and some water so it would not fall dry. Then the mean quantum yield of the whole foraminifera was measured and a picture of the imaging depiction was taken. The image was later analysed as described for the fluorescence analysis using ImageJ to calculate the total photoactive area.

2.2.3. Isotope Analysis

For the isotope analysis the individuals were picked from their crystallisation dish, dried and placed into pre-weighted clean tin capsules (Sn 99.9%, IVA Analysentechnik GmbH & Co. KG). Afterwards the foraminifera were decarbonised using 12.5 µl of 5% hydrochloric acid and placed in a drying oven at 50°C for three days to remove all moisture and were weighted to an accuracy of one thousandth milligrams. The samples were then stored in a desiccator until isotope measurements.

The measurements were carried out at the Stable Isotope Laboratory for Environmental Research (SILVER) at the University of Vienna using an isotope ratio mass spectrometer (IRMS, Delta^{PLUS} with a ConFlo III interface to an elemental analyser EA 1110, Thermo Finnigan). The ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ were determined and then used to calculate the amount of absorbed C and N as follows.

Calculation after Lintner et al. (2020 and 2021):

The atomic percentage of heavy isotopes (al. % ^{13}C and al. % ^{15}N) was calculated according to

$$\text{al. \%} = \frac{100 \times R_{standard} \times \left(\frac{\delta X_{sample}}{1000} + 1\right)}{1 + R_{standard} \times \left(\frac{\delta X_{sample}}{1000} + 1\right)}$$

Hereby $R_{standard}$ depicts the ratio of heavy isotopes to light isotopes after the international standards for C (Vienna PeeDee Belemnite R_{VPDB} = 0.0112372) and N (atmospheric nitrogen R_{atmN} = 0.0036765). The variable X stands for eighter C or N and δX is calculated after the measurement according to

$$\delta X = (R_{standard} / R_{standard} - 1) \times 1000$$

After having calculated the al. % *X,* it has to be corrected for the al. % *X* present in the natural environment since both isotopes (^{13}C and ^{15}N) occur naturally. This was done by determining the isotope excess (E) after Middelburg et al. (2000):

$$E = \frac{atomX_{sample} - atomX_{background}}{100}$$

After that the absorbed amount of isotopes can be determined according to

$$I_{iso}[\mu g\, mg^{-1}] = E \times C(N)[\mu g\, mg^{-1}]$$

The atomic percentage of C and N is thereby transferred into the amount of isotopes incorporated (I_{iso}) in µg per mg of cytoplasmic mass.

The more isotopes were taken up the higher was the foraminifers metabolic activity.

2.2.4. Evaluation, Statistics and Presentation

For all data sets (amount of incorporated plastic particles, mean quantum yield, photoactive surface and isotope incorporation for C and N) a two-way variance test (ANOVA) was applied for time and culture (C, P, PA).

The collected data was evaluated as follows.

Plastic uptake: The number of incorporated particles was determined in every individual at every time of measurement. Then the average of the six replicates was calculated and used to plot the change over time as well as differences between the different cultures (C, P, PA) and between species.

PAM analysis: The mean quantum yield of the fluorescence signal was measured for every individual at every time of measurement and the photoactive surface was calculated as described above. The average yield and average surface of the six replicates was calculated and then used further. Before the results were plotted against change over time and difference between cultures and species the values were put into relation to the starting values from day 0. This was necessary because not all individuals had the same size or same photosynthetic efficiency to start with. So instead of using the raw values, the percentage of mean yield and surface in relation to the starting value was used to account for different pre-conditions. A loss or gain in surface or yield was therefore shown as

percentage loss or gain. Also, the percentages of yield and surface were combined by averaging them to get a more accurate depiction of total photosynthetic efficiency.

Isotope analysis: The amount of incorporated C and N isotopes (in ng/mg) was calculated for every individual and then averaged between the six replicates. The results were plotted against time and difference between cultures and species.

3. Results

Statistically relevant data will be discussed here, all results and measurements will be presented in tables found in the appendix.

3.1. Micro Plastic Uptake

Statistical analysis (two-way-ANOVA) showed that for *H. depressa* the amount of plastic incorporated differs significantly ($p=0.028$) between cultures (C, P, PA) but not significantly ($p=0.132$) with time. A post-hoc test on the different groups revealed that group C differs significantly ($p=0.026$) from group P but not ($p=0.149$) from group PA. The difference between P and PA is also not significant ($p=0.721$). For *A. lobifera* the statistical analysis showed a significant difference ($p=0.031$) with time as well as between groups ($p=<0.001$). The post-hoc test confirmed that group C differs significantly ($p=<0.001$ for both) from groups P and PA whereas group P and PA do not differ significantly (0.940) from each other. A one-way ANOVA was performed to test the different behaviour between the two species and showed that they differ significantly ($p=<0.001$) in the amount of plastic incorporated.

The control group did not show any fluorescence signal on d0 for both species so pre-contamination can be ruled out. On d1 *H. depressa* showed minimal amounts of contamination for individuals C1, C2 and C3 (max. 14 particles). Since those were the very first measurements after cultivation this was likely due to not cleaning the brush thoroughly enough. The cleaning procedure was adjusted and no particles were found in the control group for the rest of the experiment in both species.

The number of incorporated plastic particles in group P and PA over time and in comparison between the two species is shown in figure 6.

Both species did take up plastic particles. *Amphistegina lobifera* overall took up more plastic with an individual peak of 16 602 particles (P2, d14) and an average peak of 5 164 particles (group PA, d14) whereas *H. depressa* had an individual peak of 12 184 particles (PA2, d28) and an average peak of 2 528 particles (group P, d28). The difference is more apparent in the average comparison since the uptake varies greatly between individuals.

Both species already incorporated plastic particles after one day of cultivation. However, *A. lobifera* took up particles more quickly with an average of 2 390 (P) or 1 077 (PA) particles while *H. depressa* only had taken up an average of 349 (P) or 351 (PA) by d1. The number of incorporated particles only rose slightly in *H. depressa* in group P (1 391 at d14) and stayed relatively stagnant in group PA till d21. Only in the last week of the experiment the amount showed a significant gain. In *A. lobifera* the amount quickly rose from d0 to d1 and then again from d7 to d14 reaching a peak at d14 before dropping off again significantly towards d21.

Although throughout the first two weeks *A. lobifera* consistently had more plastic incorporated, towards the end that drops off again and at the final time of measurement after four weeks the average amount of the two species is quite close to each other (for P: 2 528 in *H. depressa* and 3 149 in *A. lobifera*; for PA: 2 465 in *H. depressa* and 1 988 in *A. lobifera*)

The difference between the group cultivated without and the group with algae is shown for each species respectively in figure 7.

Concerning *H. depressa* the number of incorporated plastic particles stayed very similar for the first week with 349 (P) and 351 (PA) on d1 and 477 (P) and 561 (PA) on d7. After that the amount rose in group P to 1 391 (d14) and 1 362 (d21) while it stayed relatively stagnant in group PA. In the last week the amount of plastic showed a strong increase to almost the same averaged number by d28 (2 528 for P and 2 465 for PA).

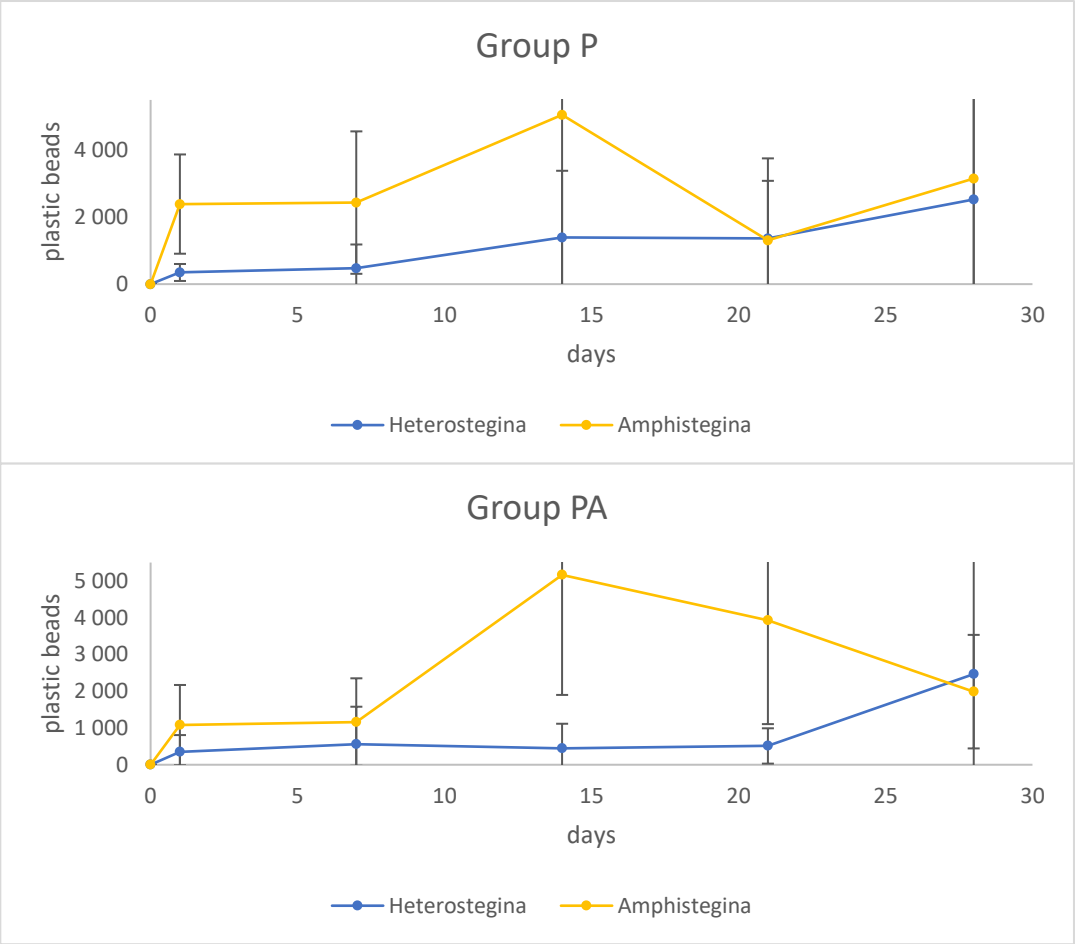

Fig. 6: Average number of PS beads taken up at the times of measurement by individuals of the groups P and PA, showing the difference between species *H. depressa* and *A. lobifera.*

For *A. lobifera* group P saw a strong increase on d1 (2 390) compared to PA (1 077). Those values stayed stagnant in d7 and then increased to an almost equal peak of 5 055 (P) and 5 164 (PA) at d14. After that group P had a very strong decline at d21 (1 310) before rising to 3 149 again at the last measurement while group PA showed a less severe decline at d21 (3 930) but kept declining towards d28 (1 988).

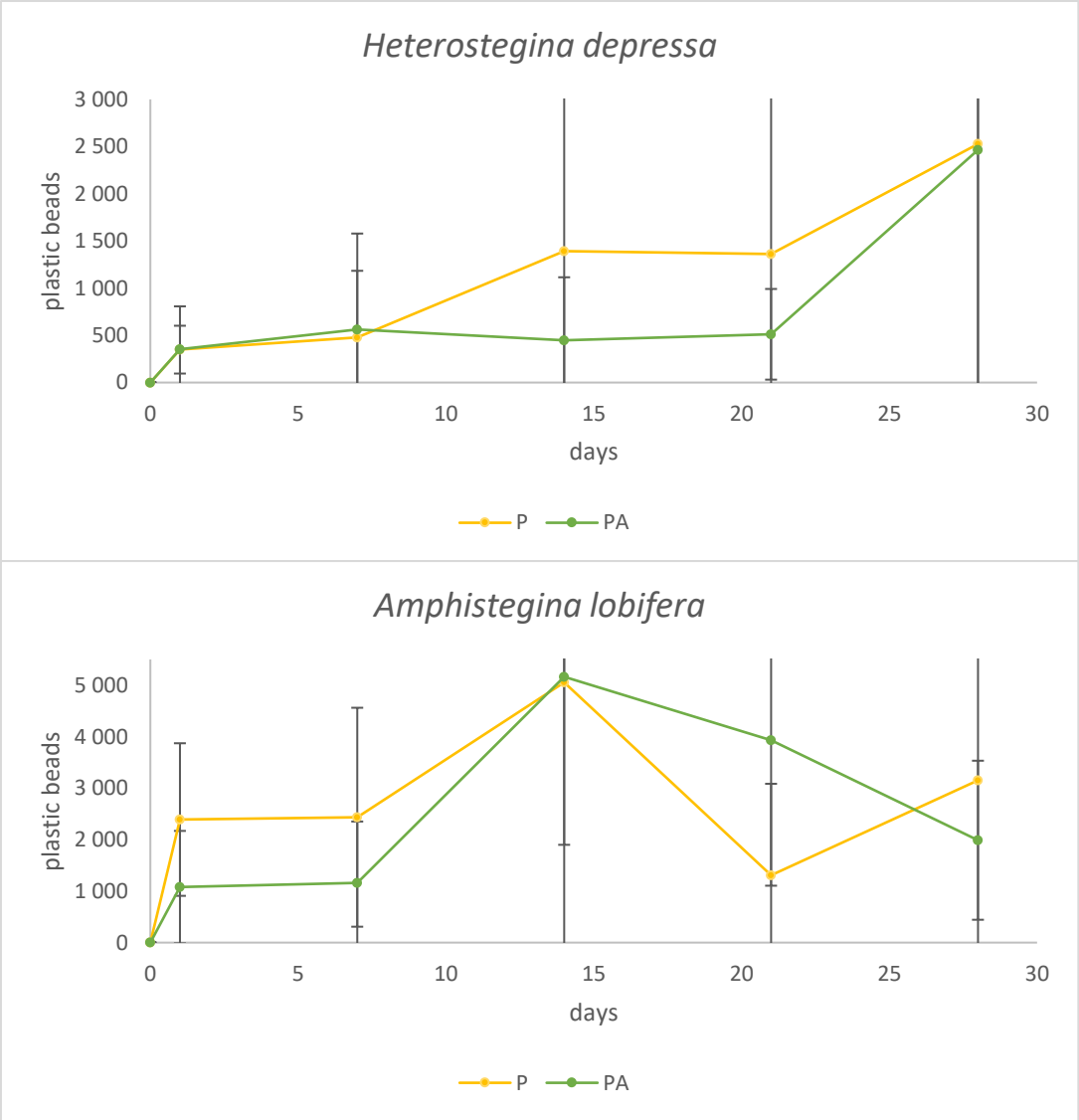

Fig. 7: Average number of PS beads taken up at the times of measurement by individuals of *H. depressa* and *A. lobifera,* showing the difference between group P (without algae) and group PA (with algae).

The location of PS beads could not be quantified but showed very different results comparing the two species. In *H. depressa* the particles were predominantly concentrated around the outer edge of the organism and on the pseudopods (see figure 8). Sometimes the PS beads were only found in the cytoplasm outside of the test or caught in the nets formed and none in the inside (see figure 8 D) while sometimes they seemed to enter the cytoplasm inside of the test in areas where pseudopods originate (see figure 8 B, C). However, the particles seemed not to concentrate around the aperture of the organisms.

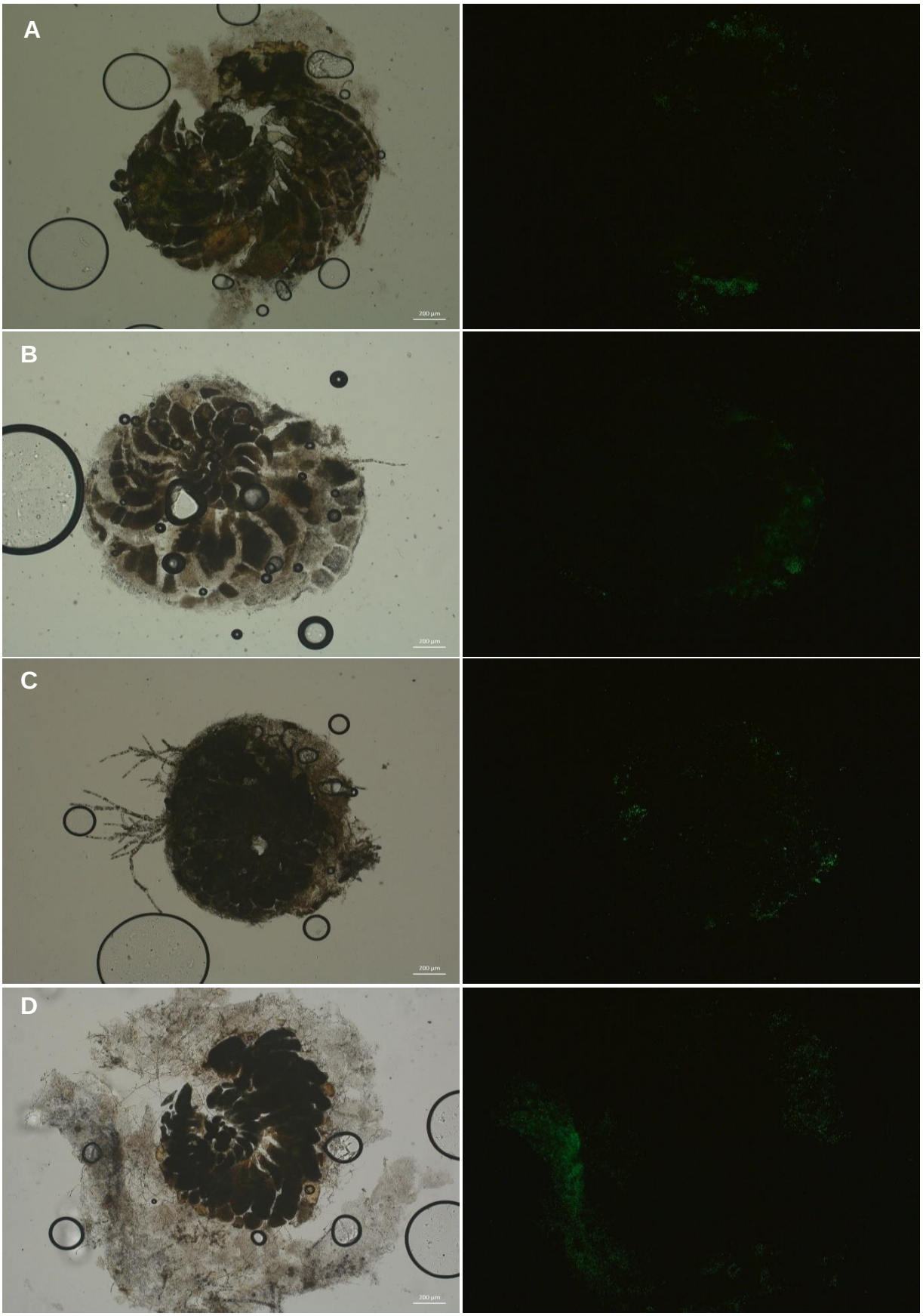

Fig. 8: Light microscope (l) and fluorescence microscope (r) images of *Heterostegina depressa.* The images show the following individuals: **A**: P6 at d14; **B**: P1 at d28; **C**: PA2 at d1; **D**: P6 at d28

In *A. lobifera* the location of PS beads was different. While *A. lobifera* too had micro-plastic particles stuck to their pseudopods the particles were heavily concentrated around the aperture of the individuals and got less concentrated in a gradient towards the older chambers of the foraminifera (see figure 9).

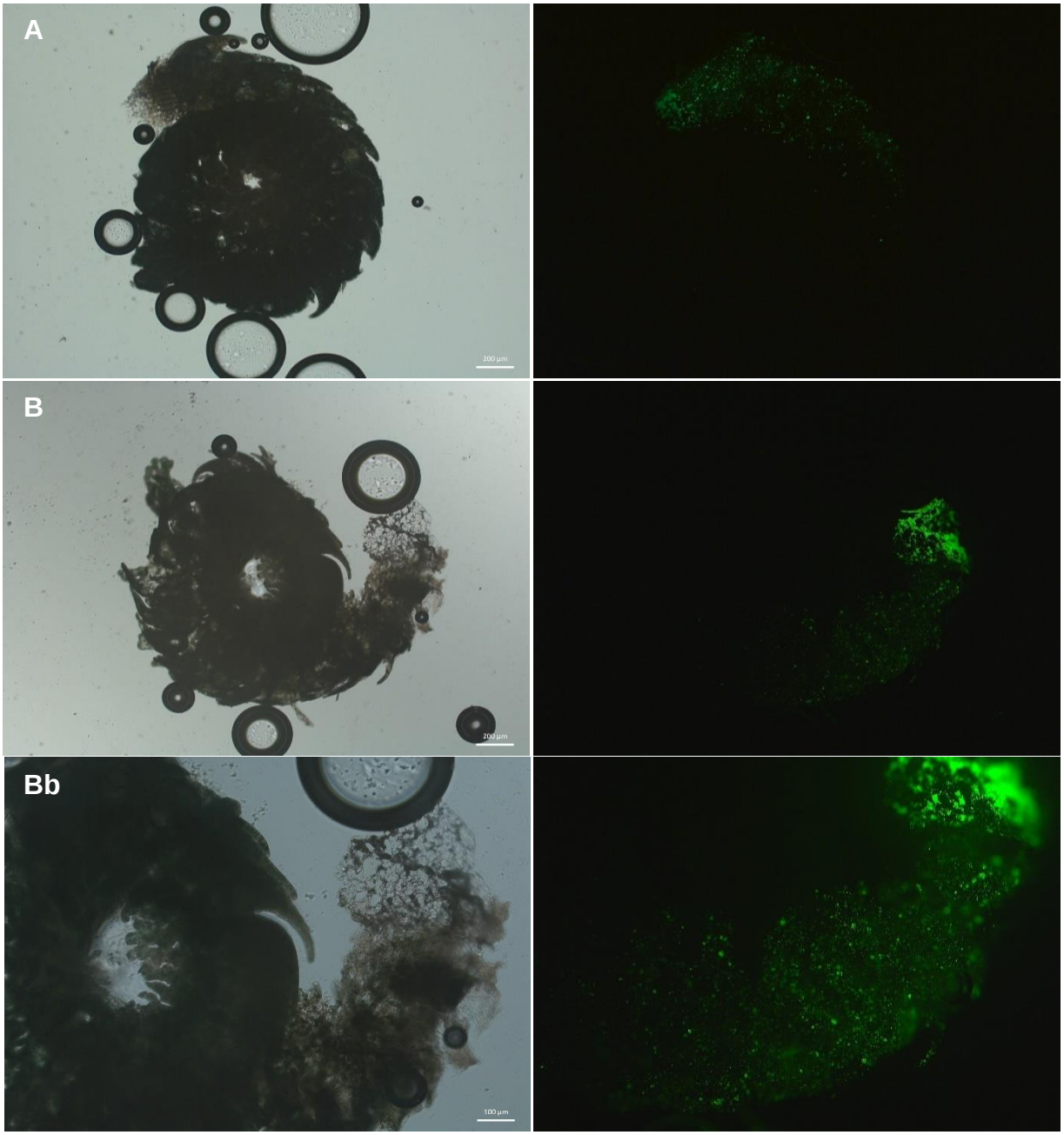

Fig. 9: Light microscope (l) and fluorescence microscope (r) images of *Amphistegina lobifera.* The images show the following individuals: **A**: PA5 at d28; **B**: PA4 at d21. Image **Bb** shows the same individual as B but with double magnification.

3.2. Photosynthetic Efficiency

Statistical analysis in form of a two-way-ANOVA showed that for both, *H. depressa* and *A. lobifera,* the difference in yield as well as area is significant (p=<0.001 for both species) with time but not significant (p=0.912 for *H. depressa* and p=0.972 for *A. lobifera*) between types of incubation (C, P, PA). Also, there is no significant difference regarding yield (p=0.934) or area (p=0.295) between the different species.

The average starting values of mean quantum yield at d0 were slightly higher in *H. depressa* than those of *A. lobifera* where *H. depressa* started with values of 0.642 (C), 0.671 (P) and 0.660 (PA) and *A. lobifera* started out at 0.614 (C) and 0.618 (P and PA). Nevertheless, all foraminifera were in good condition, which was indicated by a yield above 0.6.

For *H. depressa* the yield values of group C stayed relatively constant for the first two measurements before dropping to 0.379 at d14 and then recovering to 0.467 on d28. Group P also had a noticeable drop-off on d14 (from 0.622 on d7 to 0.495) but could not recover ending with 0.103 on d28. Group PA stayed very constant till d21 (0.649) before losing a lot in the last week and having a value of 0.360 on d28. In relation to the start-values of quantum yield this meant a 27.25% decrease for the control group, an 84.59% decrease for group P and a 45.36% decrease for group PA. The mean quantum yield values of *H. depressa* plotted against time is shown in figure 10.

The control group of *A. lobifera* showed a relatively constant decline, dropping under 0.5 at d14 (0.438) and ending on a value of 0.315 on d28. Group P had a slight increase on d1 (0.635) compared to the start-values and then saw a small but constant decrease over the next times of measurement but having higher values than group C till d21 (0.440) before sinking to 0.113 in the last week. Group PA showed a very similar trend as group P with a slight increase on d1 (0.621) and a constant decrease afterwards, landing on 0.464 on d21. However, group PA did not suffer the same severe decrease in the last week and ended on a value of 0.471. In comparison to the start-values this meant a 48.70% decrease for the control group, an 81.70% decrease for group P and a 23.85% decrease for group PA over the course of the experiment. The whole development over time is shown in figure 11.

The absolute values of average photoactive surface area of *H. depressa* and *A. lobifera* cannot be compared like the quantum yield since the two species do not have the same average size and therefore the size comparison has to be made in percentual values.

The control group of *H. depressa* had an average area of 25 611 pixels in the PAM-image on d0. This went down to 14 509 on d1 and stayed in this range before recovering to 26 776 on d14. From there a decrease was measured again ending with a surface area of 12 912 pixels on d28. Group P had a much bigger start area with an average of 67 926 pixels. From there it dropped rapidly to 28 153 on d1 and had another noticeable decrease on d14 (14 541). After a slight increase on d21 (18 727) it went down again and ended on a value of 7 191. Group PA had a similar start value as group P with 61 153 but a less severe decrease after one day (48 910). On d7 however it reached a comparable area to group P and stayed at similar values for the rest of the test series ending on 7 471. This meant percent decrease for the control group of 49.58%, a decrease of 89.41% for group P and a 87.78% decrease in photoactive area for group PA. The whole development over time is shown in figure 10.

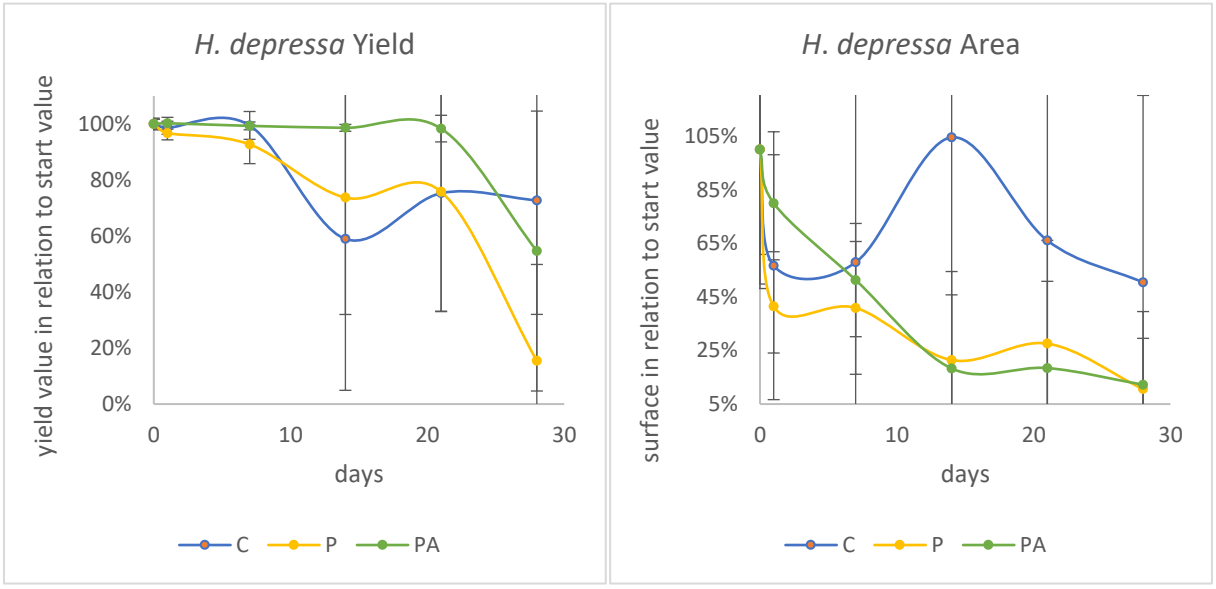

Fig. 10: Mean quantum yield and photoactive area of *Heterostegina depressa* at pulse intensity 3.

A. lobifera group C started with an average area of 109 307 pixels on d0. From there a decrease was measured on d1 (85 049) with a recovery on d7 (100 929). The group then saw a stronger decrease on d14 (64 562) and stayed around that value till the end of the test series (58 117 on d28). Group P had a smaller start area with 79 453 but showed a very similar trend in percentual changes as the control group till d14 with a decrease on d1 (66 411) followed by a recovery on d7 (83 082). From d14 onwards however, the percentual decrease was more severe in this group, dropping to 27 252 on d21 and 3 412 on d28. Group PA followed a different trend. Starting with an area of 67 645 the area got bigger on d1 (76 540) and continued increasing (93 472 on d7). Only after the first week the area started do get smaller constantly, reaching a value close to the starting one on d14 (65 945) and ending on 37 083. This resulted in an overall decrease of 46.83% for the control group, 95.70% for group P and for group PA a very similar value as the control group with 45.18% decrease. The whole development in percentual changes is shown in figure 11.

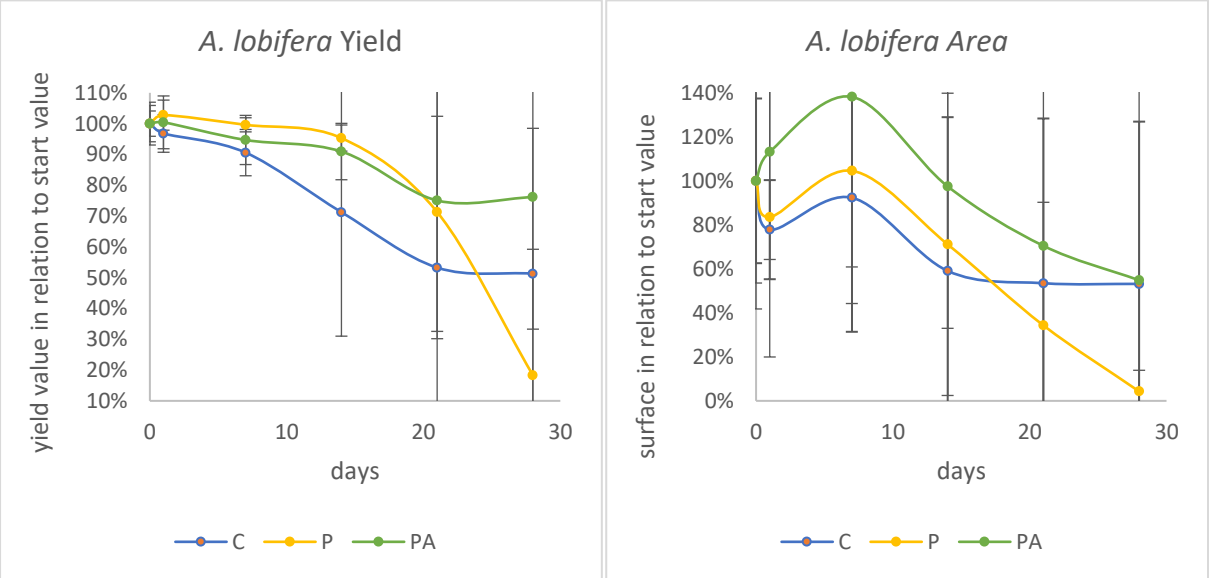

Fig. 11: Mean quantum yield and photoactive area of *Amphistegina lobifera* at pulse intensity 3.

The combined photosynthetic efficiency of the foraminifera's symbionts was calculated as the average of percentual value of quantum yield in comparison to start value and the percentual value of the photoactive area in comparison to starting size. A comparison between the groups (C, P, PA) over time in *H. depressa* and *A. lobifera* are shown in figures 12 and 13.

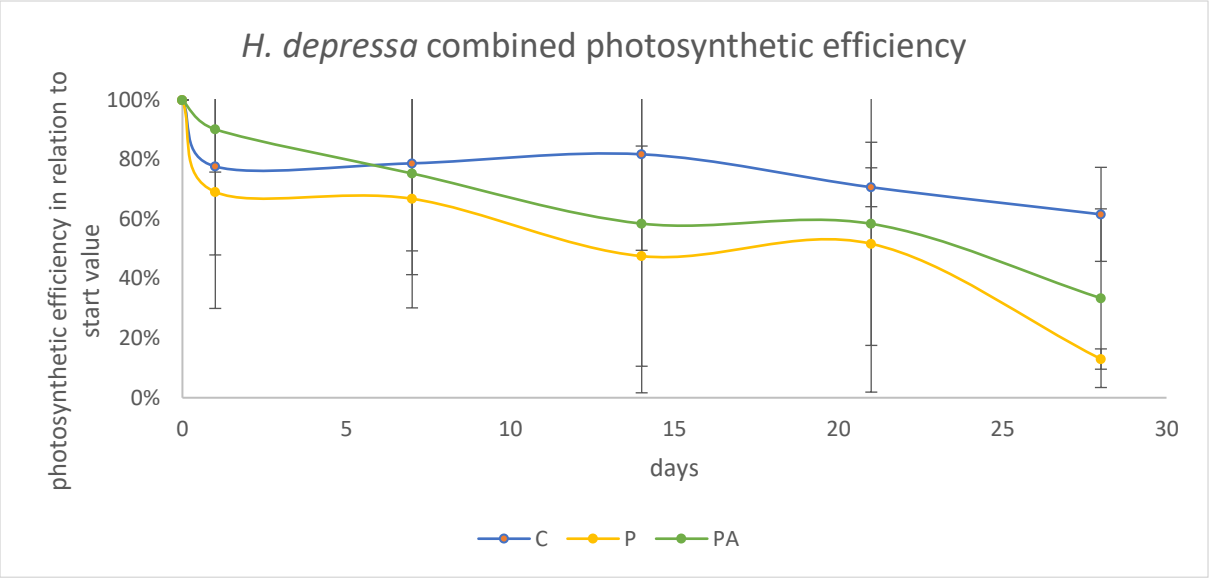

Fig. 12: Average between the percentual quantum yield in relation to start value and the percentual photoactive surface of *Heterostegina depressa* at pulse intensity 3.

The control group of *H. depressa* had a decrease in efficiency on d1 to 77.66% of the start value and then stayed relatively stagnant, ending with 61.58% of initial efficiency. Group P dropped to 69.10% of initial efficiency on d1, saw another noticeable drop on d14 to 47.55% and severe decrease between d21 and d28, ending with just 13.00% of initial efficiency. Group PA had the smallest loss in efficiency after one day (90.13%) and constantly decreased from there, reaching 58.47% efficiency on d14 and having a bigger loss on d28 reaching 33.43% of initial efficiency.

Group C of *A. lobifera* after an initial decrease on d1 to 87.31% efficiency recovered on d7 (91.46%). From there it gradually lost efficiency, reaching 53.36% an d21 and then stagnating at this value till the end of the test series (52.24%). Group P showed a very similar trend in the beginning, even reaching 102.09% of initial efficiency on d7 and 52.79% on d21. However, in the last week this group suffered a severe loss in efficiency ending on only 11.30%. Group PA performed best, seeing an increase of efficiency from d1 (106.78%) and reaching its peak on d7 with 116.44% efficiency compared to start values. From there it gradually dropped off, ending 65.48% efficiency left on d28 and with that outperforming the control group.

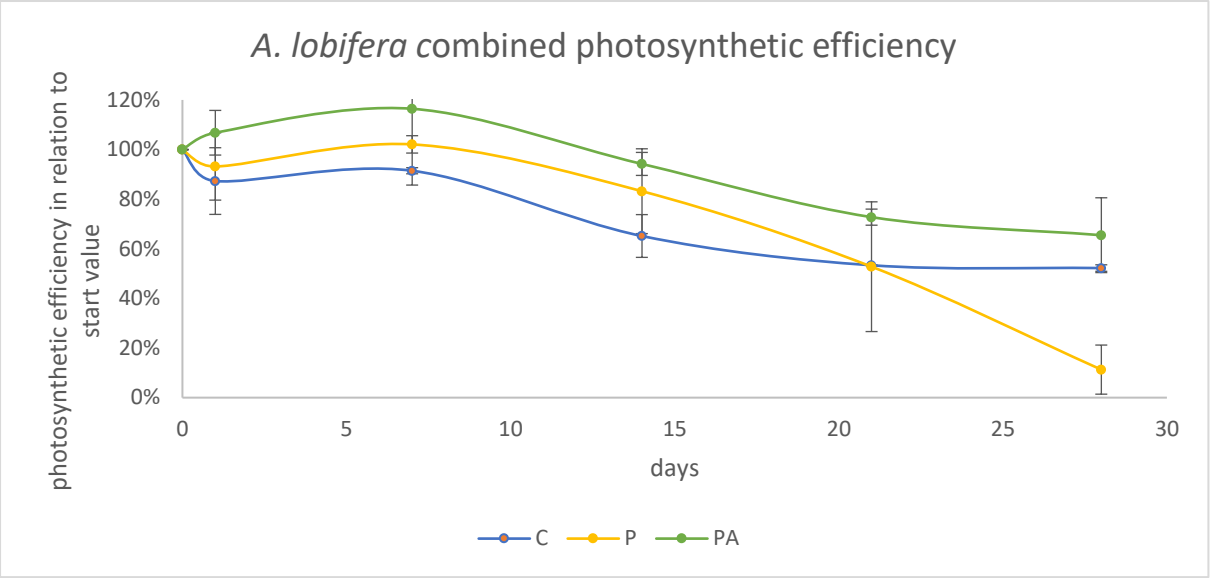

Fig. 13: Average between the percentual quantum yield in relation to start value and the percentual photoactive surface of *Amphistegina lobifera* at pulse intensity 3.

3.3. Isotope Uptake

Statistical analysis showed that for *H. depressa* the amount of incorporated carbon changes significantly (p=<0.001) with time and with group (C, P, PA) (p=<0.001). In contrast the amount of incorporated nitrogen changes significantly with time (p=<0.001) but not with group (p=0.154). The same accounts for *A. lobifera*: the uptake of carbon changes significantly with time (p=<0.001) and group (p=<0.001) whereas the amount of nitrogen only differs significantly with time (p=<0.001) but not group (p=0.401).

Both species have a very similar mass. The average mass of all individuals of *H. depressa* in this experiment was 1.33 mg and the average mass of all *A. lobifera* 1.25 mg. Still, the amount of incorporated isotopes varied greatly between the two species. While the highest amount of incorporated isotopes of a single individual during the whole test series in *H. depressa* was 0.0656 µg/mg (IN, P1, d28), the highest amount taken up by a single individual of *A. lobifera* was 0.1537 µg/mg (IC, C1, d21).

The carbon uptake of *H. depressa* control group stayed relatively stagnant with 1.85 ng/mg incorporated at d1 and a peak value of 5.043 ng/mg at d7. The values from d14 to d28 stay between 3.441 ng/mg (d14) and 2.497 ng/mg (d21). Group P shows a strong increase of incorporated carbon till d14, starting with 4.001 ng/mg at d1 and reaching a peak of 12.864 ng/mg at d14. On the last two times of measurement values were lower

with 6.509 ng/mg (d21) and 7.134 ng/mg (d28). Group PA started with almost the same amount of incorporated carbon at d1 as the control group (1.838 ng/mg). From there however the uptake increased to 8.538 ng/mg at d7. From there the amount decreased to 4.747 at d21 and stayed stagnant till the end of the test series (4.877 at d28).

The nitrogen uptake of *H. depressa* control group started out at d1 with 3.058 ng/mg and increased to 10.417 ng/mg at d7 before dropping to 5.449 at d14. It reached its peak at d21 (17.168 ng/mg) and decreased again towards the end of the test series (13.555 ng/mg at d28). Group P started with a noticeably higher amount by d1 with 8.836 ng/mg and increased slightly till d21 with 13.283 ng/mg incorporated before seeing a strong increase to 29.812 ng/mg at d28. Group PA showed a very strong increase of incorporated nitrogen from d1 (3.33 ng/mg) to d7 (19.629 ng/mg). From there the amount slowly decreased and ended on 11.396 ng/mg nitrogen incorporated.

In general *H. depressa* took up about three time as much nitrogen as it took up carbon. The whole development of IC and IN over times is shown in figure 14. A two-way ANOVA analysis showed that the amount of carbon changes significantly with time and with group (C, P, PA) while the amount of nitrogen is significantly different over time but not significantly different between groups.

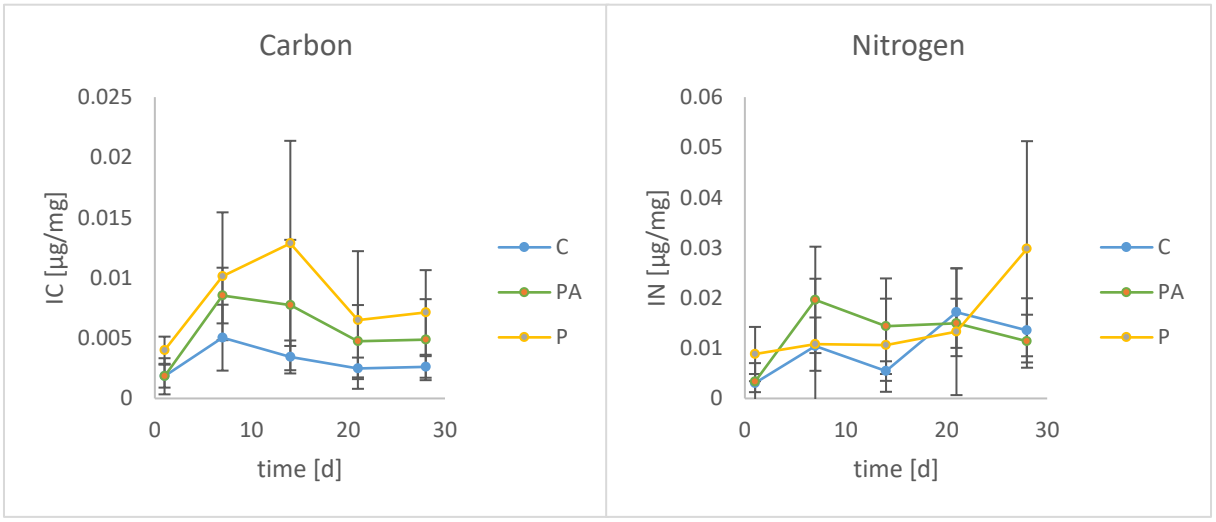

Fig. 14: Amount of incorporated carbon and nitrogen isotopes by *Heterostegina depressa*.

The control group of *A. lobifera* had incorporated 11.576 ng/mg of carbon isotopes at d1 and showed a steady increase till d21, reaching 53.024 ng/mg on d14 and a peak of 95.158 ng/mg on d21 before having a small decrease in the last week and ending on 88.921 ng/mg incorporated carbon isotopes at d28. Group P saw a steady but clearly smaller

increase than group C for the first 21 days, starting with 7.815 ng/mg at d1 and reaching 36.516 ng/mg at d21 before dropping down to 23.724 ng/mg at d28. The development of group PA was very similar to group P till d21, starting with 4.307 ng/mg at d1 and reaching 28.807 ng/mg at d21. Instead of a drop off however, group PA had a strong increase in incorporated carbon in the last week ending with 55.094 ng/mg at d28.

Concerning nitrogen uptake, the control group of *A. lobifera* saw a rapid increase in the first two weeks, starting with 6.017 ng/mg at d1 and reaching an amount of 52.337 ng/mg at d14. After that it kept increasing although much slower, ending on 60.361 ng/mg. Group P had a very similar development with just slightly lower values, starting with 8.84 ng/mg at d1, increasing slowly at first (9.659 ng/mg at d7) and then strongly to 43.26 ng/mg at d14. From there it increased slowly to 56.42 ng/mg at d28. Group PA started with 4.839 ng/mg at d1 and increased steadily till d14 (34.608 ng/mg) before dropping to 25.361 ng/mg at d21 and then seeing a strong increase in the last week ending on the highest amount of incorporated nitrogen of the three groups with 64.888 ng/mg at d28.

In contrast to *H. depressa* the amount of carbon and nitrogen taken up by *A. lobifera* was quite similar. The whole development of IC and IN in *A. lobifera* is shown in figure 15. The significance of the data was again tested using a two-way ANOVA. The amount of incorporated carbon changed significantly with time and group (C, P, PA). The amount of nitrogen taken up in contrast changes significantly with time but not between different groups.

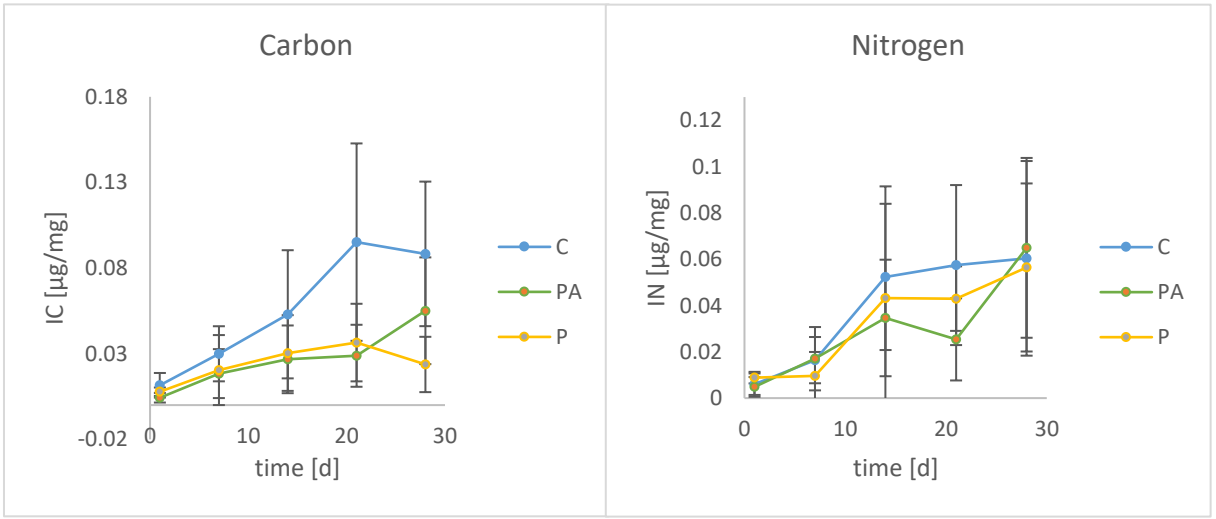

Fig. 15: Amount of incorporated carbon and nitrogen isotopes by *Amphistegina lobifera*.

4. Discussion

In general, the results show that there are often noticeable differences on an individual level which results in a high standard deviation and can make it difficult to interpret data. Statistical analysis is critical to ensure a correct interpretation. Detailed results of the statistical analysis can be found in the appendix.

4.1. Uptake of Micro Plastic

The results show that both species will incorporate PS micro beads. This accords with other studies presented in the past (Grefstad, 2019; Ciacci et al., 2019; Birarda et al. 2021). It is assumed that the main pathway on which plastic particles enter foraminifera is together with other particles like food that the organisms want to take up (Ciacci et al., 2019). However, *H. depressa* does not actually feed and should not be interested in incorporating any particles from its surroundings. Therefore, it can be assumed that the uptake of plastic by *H. depressa* does not happen out of confusing the PS beads with food particles but instead involuntarily and passive. *Amphistegina lobifera* on the contrary tries to actively catch and ingest food particles. It was shown by Joppien et al. (2022) that *Amphistegina gibbose* is able to differentiate between plastic and food particles so uptake because of confusion with food by *A. lobifera* too is unlikely. However, while the uptake by *H. depressa* is probably just passive, for *A. lobifera* it is likely that while feeding plastic particles will unwillingly but actively be incorporated. Looking at the images from the fluorescence analysis, the distribution of micro-plastic particles in both organisms aligns with that assumption.

Amphistegina lobifera stretches out its pseudopodia mainly from its aperture (Toler, 2002) and uses those to catch food particles before transporting them to the aperture for ingestion. Plastic particles that get stuck on the pseudopodia will be transported to the aperture likewise which can explain the heavy concentration of PS beads around the aperture. From there it seems like the particles will be ingested and slowly transported through the cytoplasm which can be seen in form of a gradient of fluorescence along the coiling of the foraminifer.

Heterostegina depressa on the contrary shows the highest concentration of plastic particles around its edges. This might be due to the organism's habit of not extending its

pseudopodia only from the aperture but from all around its test through the pores (Murray, 1991). Particles stuck on the pseudopods will eventually end up at the origination point of the pseudopods. Another explanation for the location of PS beads in *H. depressa* is the organic sheath. A mannerism of this species is the formation of an organic lining or hull around its calcareous test. This sheath is secreted by the ectoplasm that fills the canal system and also forms the pseudopods (Röttger, 1973). Plastic beads will probably get stuck on this ectoplasm sheath and accumulate.

However, before being observed under the fluorescence microscope the foraminifera were cleaned and in doing so the ectoplasm sheath was largely removed. It is possible though that the plastic particles entered the pores of the foraminifera's test. This might happen through eighter pseudopod activity or through the sea water the organism takes up to supply its symbionts. If the PS beads had been stuck in the pores, when dissolving the test, they would have settled on the organic lining around the organism.

Fluorescence images of *A. lobifera* – that does not have such an organic sheath – have been taken before dissolving the test to see if any PS beads would stick to the calcareous material and withstand the cleaning procedure. This however was not the case (see figure 16). The fluorescence signal of the micro-plastic particles is blurred because of the light having to travel through the test; accumulation around the aperture is apparent.

Although the experiment is not able to exactly explain how the plastic particles entered the foraminifera it can show that there must be pathways other than uptake together with food. Whether the pores play a role here or not would have to be investigated in other studies.

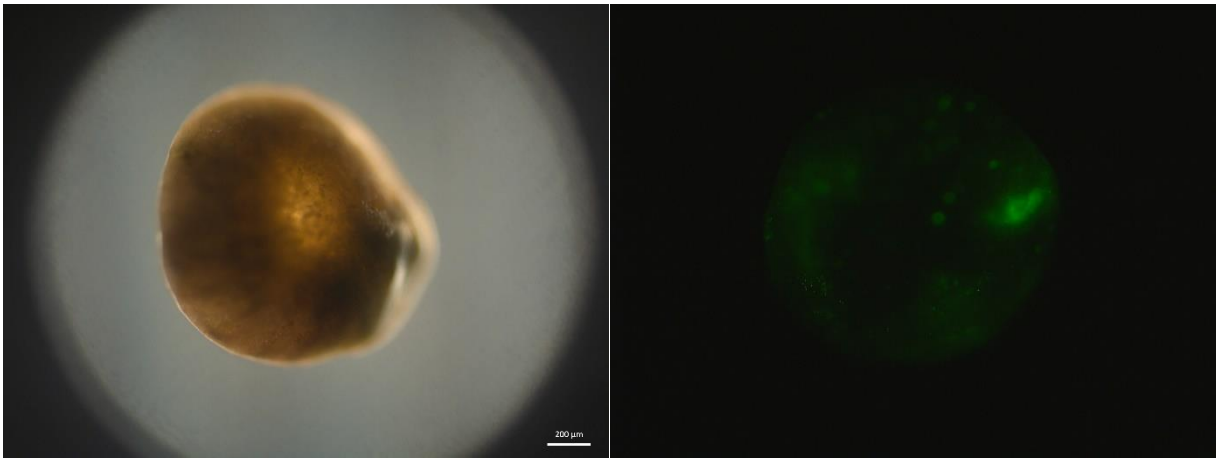

Fig. 16: Light microscope (l) and fluorescence microscope (r) images of individual PA3 at d14 of *Amphistegina lobifera.* The fluorescence signal can be picked up through the test but therefore is blurry.

Statistical analysis of the amount of incorporated plastic particles by *H. depressa* revealed that the change over time is not significant. This further strengthens the assumption that this species does not actively try to ingest the particles. After the initial accumulation of plastic at d1 the number of beads does not increase over the next weeks in group PA. This might be also due to the organisms' sessile mode of life. Usually, *H. depressa* will attach to the substrate using its pseudopods after having found a spot it wants to stay in (Röttger, 1973). Since there were no currents or noticeable water movement in the experimental series that would mean that after having accumulated the particles around them the foraminifera would not come into contact with a noticeable number of new particles. Group P shows an increase in plastic uptake but not of statistical significance. This might change in experiments with a longer incubation time.

However, the uptake of plastic by group P of *H. depressa* still is significant compared to the control group while there is no significant difference between group P and PA which indicates that the presence of algae does not affect the micro-plastic uptake of the foraminifera. Interestingly the difference between the control group and group PA is not statistically significant. Seeing an increase of incorporated particles towards the end of the experiment this however might also change over longer periods of time.

The amount of incorporated plastic in *A. lobifera* on the contrary does significantly change over time, which indicates a constant and active uptake. What is interesting here is that both groups that were incubated with plastic particles show a decrease over the last two weeks. Two explanations seem possible. One reason could be that due to the conditions during the experiment the foraminifera perform worse the longer they are subject to those unfavourable conditions and therefore have a decreased metabolic activity, hence lower uptake. Another reason could be that the foraminifera find a way to excrete the plastic particles after identifying them as not digestible. This however would have to be tested in a separate study.

Post-hoc test of the statistical analysis of *A. lobifera* revealed that there is a significant difference in plastic uptake between the control group and both groups that were subjected to plastic particles. Group P and PA however do not differ significantly. This indicates that the presence of algae does not have an effect on the micro-plastic uptake of *A. lobifera* (see chapter 4.3.).

Finally, the uptake of micro-plastic particles differs significantly between the two species with *A. lobifera* having incorporated more than ten times the amount of *H. depressa* at some points of measurement which probably is due to their different feeding behaviour.

4.2. Effects of Plastic Particles

To assess the effect of micro-plastic particles on the health of the foraminifera one of the used methods was PAM imaging. This method had been used in the past to study the effects of many different pollutants and parameters like heavy metals, toxic substances, temperature or alkalinity (e.g. Schmidt et al., 2014; Bubl, 2022; Lintner et al., 2022). However, technically it primarily indicates the condition of the photoactive organisms, hence the diatom symbionts. For that reason, even if the foraminifera's wellbeing and that of their symbionts is linked, it is helpful to combine the results with other methods.

For *H. depressa* the statistical analysis showed that the mean quantum yield values changed significantly over time. This is not unusual since the experimental conditions are not optimal for the organisms (for example no movement in the water via pump like in the aquarium of the main culture and therefore reduced supply of oxygen) so a decrease in photosynthetic efficiency – even in the control group – can be expected. The difference between groups however was statistically not significant. This means that it seems like the plastic particles do not influence the diatom's quantum yield. A reason for this however could be that because of the location of the particles most of the diatoms did not come into direct contact with the plastic.

A similar outcome can be seen for the photoactive surface area of *H. depressa.* Here too the values do not change significantly with type of incubation while the change over time is significant. Apparently, the loss in photoactive efficiency in *H. depressa* is due to prolonged periods of incubation under experimental conditions and not due to plastic exposure.

The statistical analysis of the *A. lobifera* data draws similar conclusions. While the graphs (see figure 11) show that group P performed worst in quantum yield as well as photoactive surface, because of the high standard deviation the results are not statistically significant. Therefore *A. lobifera* too did not show significant differences concerning type

of incubation in eighter yield or surface. However, both factors changed significantly over time.

There was also no significant difference between the two species in eighter yield or surface. This can be explained due to the fact that PAM-analysis mainly represents the symbiont's efficiency and *H. depressa* and *A. lobifera* in many cases host very similar diatom communities with the same species of diatoms (Lee and McEnery, 1979).

It must be noticed though that in all comparisons group P performed the worst and in the combined photoactive efficiency (see figures 12 and 13) the standard deviation gets smaller; especially in *A. lobifera.* This means that with a bigger sample size or over a longer test period this trend might get statistically significant.

The isotope analysis of carbon and nitrogen uptake was used to complement the data from the PAM-analysis. The PAM analysis could not detect a significant influence of the plastic beads. Also, a past study done by Langlet et al. (2020) looking at the respiration of *Haynesina germanica* could not find any effects of PP particles. The isotope analysis however did find significant changes. The carbon uptake in both species was significantly influenced by the presence of PS beads while the nitrogen uptake was not. To understand the reasons for this difference further research would need to be done looking at the individual fate of different elements taken up by the foraminifera. The results however indicate that the incorporation of plastic can have effects on some health parameters while being irrelevant for others. This means that on one hand foraminifera seem to be relatively tolerant towards plastic pollution but on the other hand means that the effect on important vital functions might be overlooked while just looking at other parameters.

Finding an effect in the foraminifera's metabolism but not in photosynthetic efficiency suggests that the PS micro-plastic beads have an effect on the foraminifera but not directly on the diatoms they are hosting. This might be because of the size proportions of plastic particles and organisms. Looking at the development of the quantum yield values of the group P in both species, a severe drop is noticeable in the last week of the experiment. This could mean that the diatoms do not directly suffer from the plastic but that the foraminifera do and the foraminifera's condition shows a delayed effect on their symbionts because it takes a while for the diatoms to feel the consequences of a stressed host.

Another interesting result is that the control group of *H. depressa* had a relative stagnant amount of incorporated carbon isotopes. Since the isotopes should theoretically accumulate over time, stagnation technically could mean a decrease in metabolism. Another explanation however could be the further use of carbon by the organism since the ^{13}C isotopes can be used in the same way as the ^{12}C ones and possibly be excreted again.

It is also noticeable that group P and PA of *H. depressa* outperform group C in carbon uptake while in *A. lobifera* group C had a noticeable higher carbon uptake than groups P and PA. A possible explanation for this could be that the plastic particles clog the foraminifera's pores and aperture and thereby hinder the flow rate of water through the organisms. Because of their different feeding habits, the foraminifera's response to that problem could be different. *H. depressa* fully depend on their symbionts which might lead to them trying to keep up their supply by actively increasing the flow rate and therefore taking up more carbon isotopes in the process. *A. lobifera* in the contrary could change its behaviour to focus more on feeding to compensate for the loss caused by less active symbionts.

4.3. Impact of Food Availability

Since *H. depressa* does not feed it was not expected that the presence of algae would have an impact on the uptake of plastic particles by this organism. Although it looks like the group PA would take up less particles than group P (see figure 6) this is not the case by the end of the experiment. Also, statistical analysis found the difference between those two groups to not be significant. Same goes for the photosynthetic efficiency – there is no noticeable or statistically significant difference between group P and PA. The only significant difference between the two groups was in carbon isotope uptake where group P took up more carbon than group PA. A possible explanation for this is that the algae might have been in competition with the foraminifera over certain nutrients. This could have slowed down the foraminifera's metabolism without impacting the number of plastic particles getting stuck on the cytoplasm of *H. depressa.*

For *A. lobifera* an influence of the availability of food was expected since studies in the past showed that foraminifera are not only able to differentiate between plastic and food (Joppien et al., 2022) but also are able to selectively feed on phytodetritus (Suhr et al.,

2003). This could have two effects: eighter *A. lobifera* recognizes the PS beads as non-edible and will take up more in group PA involuntarily by feeding on the algae or it will try to ingest the PS beads in absence of an alternative and take up more in group P while selectively feeding on *Nannochloropsis salina* in group PA. Interestingly however, neither of those two expected outcomes was the case. The number of incorporated plastic particles did not differ significantly. Contrary to previous findings this would suggest that *A. lobifera* is not able to selectively choose not to ingest the micro-plastic particles. A possible explanation for this discrepancy is the size of the particles. The particles and pray organisms used by Joppien et al. (2022) had size between 150-300 µm and with that were much bigger than the PS beats used in the presented study (2 µm).

Also, the groups P and PA of *A. lobifera* did not show significant differences in photoactive efficiency, carbon uptake or nitrogen uptake. The availability of food seems to not have any impact on the effects of micro-plastic.

5. Conclusiones

Over a period of four weeks the uptake of PS micro-plastic particles by LBF *Heterostegina depressa* and *Amphistegina lobifera* and the effect of those particles on the foraminifera's photoactive efficiency and metabolism have been studied.

The number of incorporated beads was assessed using fluorescence microscopy. The results showed a difference in the plastic uptake and location of plastic particles between species. *Heterostegina depressa* accumulated particles slower and mainly on its pseudopodia and ectodermic sheath around the organism; only few particles reached inner areas of the organism's cytoplasm. *Amphistegina lobifera* took up plastic particles faster and mainly over its pseudopods through the aperture. From there the particles were transported along the foraminifera's coiling towards the inner chambers. However, the number of particles in *A. lobifera* decreased again towards the end of the test series suggesting that the organisms might be able to excrete the micro-plastic again. For a period of four weeks though Hypotheses I appears to be true: The reason for the difference in uptake is assumed to be the different metabolic lifestyles.

Comparing the groups C, P and PA it turned out that the presence of plastic particles does not have an impact on the mean quantum yield or the photoactive area, neither in *H.*

depressa nor in *A. lobifera.* The isotope analysis showed that both species did not show differences in nitrogen uptake between the groups but did show differences in the carbon uptake whereby the plastic particles had an increasing effect on the carbon uptake of *H. depressa* while they had a decreasing effect on *A. lobifera.* This might be due to different ways of dealing with the problem presented by non-usable particles by the two species. Hypotheses II therefore could not be confirmed. The micro-plastic seems not to have any negative effects on the symbiotic diatoms and while it has an effect on the foraminifera's carbon metabolism, the rate was boosted for one of them. Also, it is not clear whether the particles do have to be ingested or if the presence alone alters the foraminifera's metabolism.

Hypotheses III turned out to be partly true. The presence of algae did not affect *H. depressa.* The uptake of plastic particles, the quantum yield and photoactive area and the isotope uptake were not significantly different between groups P and PA. This was expected because of the foraminifera's behaviour of not feeding. However, the contrary was expected of *A. lobifera* but could not be confirmed in this study. *Amphistegina lobifera* too showed no significant difference between groups P and PA suggesting that even for foraminifera that are feeding on algae the micro-plastic uptake is not affected by this behaviour.

In general, the implications of these results are partially reassuring, partly worrying. On one hand, it seems like PS micro-beads do not have severe implications for foraminifera and they might be able to deal with such a pollutant fairly well. On the other hand, it is apparent that even organisms that do not mistakenly ingest plastic particles because of confusing them with food will accumulate them quickly. This is potentially problematic because of three main reasons. Firstly, only a very limited number of plastics and limited number of factors they might influence have been tested so far. It is very much possible that micro-plastic has a negative effect on foraminifera that just has not been taken into account. Secondly, foraminifera are on the lower end of the marine tropic chain and fall prey to a multitude of different predators (Toler, 2002; Lei et al., 2019). Even if the foraminifera are not affected by the plastic they can act as a gateway for the particles into other species that might suffer from them. Finally, the PS beads in this study were manufactured for scientific purpose and therefore free from any kind of pollutant or additive besides the fluorescent coating. This is usually not the case with PS beads in the ocean. So even though polystyrene itself seems to be relatively unproblematic for

foraminifera, this might not be the case for additives commonly used together with this plastic.

6. Outlook

Although plastic pollution of the oceans is an increasingly pressing issue research on its effect on marine micro-organisms and especially foraminifera has just been picking up over the last few years. There are still many things unknown about the impact that plastic pollution and especially micro-plastic pollution has on marine life. Besides the many other interactions between different plastics, additives and organisms there is a multitude of questions that the presented study raised.

Experiments over a longer period of time could show whether foraminifera are able to excrete micro-plastic particles again. Long time experiments could also descry whether the trends in photosynthetic efficiency that were not significant in this study would continue or not.

Another interesting topic that the presented study was not able to answer is how far the selective capabilities of foraminifera go. While it seems like they are able to selectively incorporate particles to a certain extent, this could not be confirmed for the particle size and material used in this test series.

Finally, tracking the pathway of plastic particles, not only into foraminifera but also from foraminifera into other predator species higher up in the trophic chain promises very interesting implications.

The number of studies from the last few years concerning this topic shows that the potential and necessity of those questions has been recognized in the scientific space and many important findings and insights can be expected in the years to come.

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Images, Figures, Graphs:

All figures and graphs in this study were made by the author using Microsoft Excel.

All images were taken by the author. Fluorescence images were taken using an Axio-Imager.M1 fluorescence microscope (ZEISS®); imaging PAM images were taken using the MICROSCOPY-Version of the M-Series (Hein Walz GmbH).

Appendix

Tables:

- Tab. 1a: Calculated number of micro-plastic beads taken up by *Heterostegina depressa.*

	D0	D1	D7	D14	D21	D28
C1	0	14	0	0	0	0
C2	0	2	0	0	0	0
C3	0	4	0	0	0	0
C4	0	0	0	0	0	0
C5	0	0	0	0	0	0
C6	0	0	0	0	0	0
P1	0	318	45	355	165	3 620
P2	0	42	68	121	20	271
P3	0	612	58	1 040	6 218	653
P4	0	100	568	5 377	485	1 575
P5	0	363	1 861	346	737	49
P6	0	656	264	1 108	550	8 999
PA1	0	134	35	6	163	34
PA2	0	1 277	2 567	1 241	3	12 184
PA3	0	129	689	36	150	2 320
PA4	0	192	51	1 375	966	194
PA5	0	133	4	13	623	29
PA6	0	243	19	10	1 163	29

- Tab. 1b: Statistical analysis of Tab 1a. Statistically significant values are marked.

TWO-WAY-ANOVA *Heterostegina depressa*

	Sum of sqrs	df	Mean square	F	p (same)
TYPE:	2.37E+07	2	11 833 000	3.743	**0.028**
Time:	2.31E+07	4	5 784 070	1.830	0.132
Interaction:	1.46E+07	8	1 823 510	0.577	0.794
Within:	2.37E+08	75	3 161 010		
TOTAL:	2.98E+08	89			

POST-HOC TEST *Heterostegina depressa*

	C	P	PA
C		**0.02562**	0.149
P	**0.02562**		0.721
PA	0.1494	0.7213	

- Tab. 2a: Calculated number of micro-plastic beads taken up by *Amphistegina lobifera.*

	D0	D1	D7	D14	D21	D28
C1	0	0	0	0	0	0
C2	0	0	0	0	0	0
C3	0	0	0	0	0	0
C4	0	0	0	0	0	0
C5	0	0	0	0	0	0
C6	0	0	0	0	0	0
P1	0	5 011	1 157	3 932	323	238
P2	0	1 608	6 401	16 602	4 804	9 561
P3	0	2 675	2 470	1 923	753	4 317
P4	0	607	960	4 593	552	1 741
P5	0	2 523	2 882	151	16	477
P6	0	1 917	740	3 127	1 410	2 562
PA1	0	3 226	207	1	2 159	233
PA2	0	577	291	2 476	2 077	2 027
PA3	0	255	3 190	7 382	6 819	608
PA4	0	1 022	1 886	6 515	8 077	1 445
PA5	0	442	288	5 984	3 210	3 877
PA6	0	942	1 110	8 624	1 236	3 742

- Tab. 2b: Statistical analysis of Tab 2a. Statistically significant values are marked.

TWO-WAY-ANOVA *Amphistegina lobifera*

	Sum of sqrs	df	Mean square	F	p (same)
TYPE:	1.54E+08	2	7.69E+07	14.41	**5.05E-06**
Time:	6.03E+07	4	1.51E+07	2.826	**0.03058**
Interaction:	6.41E+07	8	8.02E+06	1.503	0.1706
Within:	4.00E+08	75	5.33E+06		
TOTAL:	6.78E+08	89			

POST-HOC TEST *Amphistegina lobifera*

	C	P	PA
C		**2.26E-05**	**7.96E-05**
P	**2.26E-05**		0.9396
PA	**7.96E-05**	0.9396	

- Tab. 3: Statistical analysis of Tab. 1a and Tab. 2a tested for differences between species.

ONE-WAY-ANOVA *Heterostegina* vs. *Amphistegina*

	Sum of sqrs	df	Mean square	F	p (same)
SPECIES:	5.94E+07	1	5.94E+07	12.92	**0.000**

- Tab. 4a: Measured mean quantum yield value for *Heterostegina depressa.* To lower the impact of dead individuals the lowest value was ignored when calculating averages.

	D0	d1	d7	d14	d21	d28
C1	0.634	0.625	0.646	0.639	0.605	0.588
C2	0.605	0.000	0.614	0.000	0.000	0.000
C3	0.657	0.617	0.000	0.000	0.592	0.568
C4	0.648	0.628	0.660	0.000	0.000	0.000
C5	0.623	0.653	0.599	0.672	0.625	0.607
C6	0.649	0.645	0.676	0.582	0.596	0.573
P1	0.654	0.645	0.652	0.000	0.591	0.000
P2	0.675	0.677	0.658	0.621	0.670	0.000
P3	0.667	0.647	0.620	0.682	0.000	0.000
P4	0.639	0.561	0.544	0.560	0.601	0.517
P5	0.674	0.634	0.000	0.000	0.000	0.000
P6	0.686	0.644	0.638	0.610	0.682	0.000
PA1	0.647	0.643	0.639	0.654	0.647	0.000
PA2	0.675	0.675	0.664	0.662	0.000	0.000
PA3	0.666	0.651	0.665	0.640	0.638	0.000
PA4	0.658	0.673	0.644	0.633	0.628	0.570
PA5	0.640	0.641	0.649	0.649	0.628	0.603
PA6	0.652	0.665	0.654	0.648	0.703	0.629

- Tab. 4b: Statistical analysis of Tab 4a. Statistically significant values are marked.

TWO-WAY ANOVA – YIELD *Heterostegina depressa*

	Sum of sqrs	df	Mean square	F	p (same)
TYPE:	0.241473	2	0.120737	0.09203	0.9123
Time:	1.3899	5	0.27798	8.674	**1.12E-06**
A X B:	0.389322	10	0.038932	0.8444	0.5866
A X Subj:	44.6039	34	1.31188		
B X SUBJ:	2.72393	85	0.032046		
A X B X SUB:	7.83835	170	0.046108		
TOTAL:	17.1391	107			

- Tab. 5a: Measured mean quantum yield value for *Amphistegina lobifera.* To lower the impact of dead individuals the lowest value was ignored when calculating averages.

	D0	d1	d7	d14	d21	d28
C1	0.540	0.535	0.582	0.534	0.000	0.000
C2	0.575	0.580	0.000	0.000	0.000	0.000
C3	0.613	0.559	0.588	0.551	0.550	0.546
C4	0.593	0.571	0.521	0.503	0.482	0.469
C5	0.671	0.652	0.598	0.601	0.604	0.561
C6	0.620	0.612	0.494	0.000	0.000	0.000
P1	0.586	0.569	0.576	0.548	0.000	0.000
P2	0.610	0.589	0.617	0.619	0.000	0.000
P3	0.634	0.659	0.608	0.583	0.622	0.000
P4	0.604	0.623	0.626	0.599	0.500	0.565
P5	0.588	0.663	0.595	0.594	0.601	0.000
P6	0.652	0.639	0.630	0.547	0.478	0.000
PA1	0.625	0.614	0.531	0.479	0.000	0.000
PA2	0.552	0.547	0.608	0.556	0.606	0.574
PA3	0.489	0.649	0.572	0.570	0.559	0.617
PA4	0.603	0.588	0.557	0.567	0.523	0.544
PA5	0.657	0.694	0.658	0.638	0.631	0.618
PA6	0.653	0.558	0.000	0.000	0.000	0.000

- Tab. 5b: Statistical analysis of Tab 5a. Statistically significant values are marked.

TWO-WAY ANOVA – YIELD *Amphistegina lobifera*

	Sum of sqrs	df	Mean square	F	p (same)
FACTOR A:	0.07184	2.000	0.03592	0.028	0.9729
Factor B:	1.63429	5	0.326858	13.45	**1.17E-09**
A X B:	0.387023	10.000	0.038702	0.938	0.4997
A X Subj:	44.3706	34	1.30502		
B X SUBJ:	2.06578	85	0.024303		
A X B X SUB:	7.01311	170	0.041254		
TOTAL:	16.26	107			

- Tab. 6a: Measured photoactive area in pixels for *Heterostegina depressa.* To lower the impact of dead individuals the lowest value was ignored when calculating averages.

	d0	d1	d7	d14	d21	d28
C1	42 466	26 358	55 675	117 533	74 644	43 471
C2	112	0	13	0	0	0
C3	26 855	4 884	17	0	355	2 650
C4	15 847	5 988	0	0	0	0
C5	9 391	4 806	1 456	6 012	4 035	4 447
C6	33 498	30 513	17 018	10 335	5 574	13 995
P1	34 301	26 852	20 784	0	1	0
P2	52 778	29 940	26 483	5 207	92	0
P3	46 710	16 096	5 364	50	0	0
P4	88 939	20 870	35 767	53 394	39 066	28 766
P5	16 071	433	0	0	0	0
P6	116 902	47 007	50 524	14 057	54 477	0
PA1	38 677	26 707	7 554	9 786	18	0
PA2	35 183	41 675	30 714	39	0	0
PA3	92 020	66 708	53 222	385	222	0
PA4	42 591	39 398	28 009	4 462	10 347	5
PA5	51 433	52 292	19 316	40 482	45 798	37 348
PA6	81 046	44 480	25 544	840	7	6

- Tab. 6b: Statistical analysis of Tab 6a. Statistically significant values are marked.

TWO-WAY ANOVA – AREA *Heterostegina depressa*

	Sum of sqrs	df	Mean square	F	p (same)
TYPE:	0.444035	2	0.222018	0.228	0.7975
Time:	1.64695	5	0.329389	6.018	**8.15E-05**
A X B:	0.739844	10	0.073984	1.061	0.3954
A X Subj:	33.1322	34	0.974477		
B X SUBJ:	4.65202	85	0.05473		
A X B X SUB:	11.8581	170	0.069753		
TOTAL:	46.8789	107.000			

- Tab. 7a: Measured photoactive area in pixels for *Amphistegina lobifera.* To lower the impact of dead individuals the lowest value was ignored when calculating averages.

	d0	d1	d7	d14	d21	d28
C1	35 455	8 906	25 104	1 138	0	0
C2	70 164	64 011	0	0	0	0
C3	68 294	61 437	59 400	27 605	609	912
C4	165 515	113 634	184 624	156 222	171 824	123 373
C5	117 304	77 639	154 539	137 847	119 776	166 300
C6	125 260	108 527	80 980	0	0	0
P1	123 760	114 808	131 669	136 537	0	0
P2	52 430	4 932	650	75	0	0
P3	58 092	75 492	92 438	64 196	95	0
P4	29 515	23 604	61 874	2 472	104 005	17 064
P5	2 195	34	11 716	8 357	4 201	0
P6	133 468	113 220	117 715	71 023	27 961	0
PA1	28 617	38 116	112 977	71 031	0	0
PA2	101 652	111 195	1 657	15 482	411	15 239
PA3	76	46 098	126 353	89 630	68 325	8 717
PA4	89 561	84 030	124 867	122 890	149 877	158 841
PA5	77 182	103 263	101 508	30 696	19 721	2 622
PA6	41 216	10	0	0	0	0

- Tab. 7b: Statistical analysis of Tab 7a. Statistically significant values are marked.

TWO-WAY ANOVA – AREA *Heterostegina depressa*

	Sum of sqrs	df	Mean square	F	p (same)
FACTOR A:	0.384403	2	0.192202	0.1393	0.8704
Factor B:	1.75601	5	0.351203	5.179	**0.000339**
A X B:	0.296358	10	0.029636	0.3417	0.9684
A X Subj:	46.8972	34	1.37933		
B X SUBJ:	5.76372	85	0.067808		
A X B X SUB:	14.7431	170	0.086724		
TOTAL:	47.0223	107			

- Tab. 8: Statistical analysis of Tab. 4a, 5a, 6a and 7a tested for differences between species.

ONE-WAY ANOVA – YIELD *Heterostegina* vs. *Amphistegina*

	Sum of sqrs	df	Mean square	F	p (same)
FACTOR A:	0.001884	1	0.001884	0.007144	0.9336

ONE-WAY ANOVA – AREA *Heterostegina* vs. *Amphistegina*

	Sum of sqrs	df	Mean square	F	p (same)
FACTOR A:	2.17382	1	2.17382	1.167	0.2951

- Tab. 9a: Amount of incorporated carbon isotopes (IC) and incorporated nitrogen isotopes (IN) by *Heterostegina depressa* in µg/mg.

IC	d1	d7	d14	d21	d28
C1	0.00266783	0.00549985	0.00540549	0.00149759	0.00317824
C2	0.00309326	0.00403554	0.00255857	0.00322935	0.00181654
C3	0.0021665	0.0015977	0.00445429	0.00223646	0.00223883
C4	0.00132964	0.00443973	0.00224281	0.00300302	0.0041789
C5	0.00058411	0.00476049	0.00202737	0.00353876	0.00212355
C6	0.00125958	0.00992549	0.00395615	0.00147664	0.00213902
P1	0.00469884	0.00973094	0.00440995	0.00389619	0.01298442
P2	0.0037009	0.00785334	0.00733311	0.00366535	0.0027642
P3	0.00410508	0.00697164	0.01933679	0.01604546	0.00796233
P4	0.00189891	0.00453836	0.02028473	0.00359129	0.00666082
P5	0.00462477	0.01962517	0.00388225	0.01082856	0.00452198
P6	0.00497886	0.01209928	0.0219397	0.00102914	0.00790784
PA1	0.0010593	0.00610016	0.00682783	0.00503329	0.00284089
PA2	0.00465731	0.00762038	0.01757314	0.00662192	0.00396982
PA3	0.0010737	0.00609669	0.00932512	0.00240356	0.01146574
PA4	0.00243264	0.01057836	0.0017596	0.00370939	0.0047703
PA5	0.0008451	0.00925307	0.00501109	0.00122582	0.00215708
PA6	0.00096185	0.0115769	0.00599921	0.00948915	0.00405771

IN	d1	d7	d14	d21	d28
C1	0.00475096	0.00459086	0.00644027	0.01318982	0.00624291
C2	0.00496914	0.00440474	0.00704816	0.01178782	0.01937372
C3	0.00223818	0.00228069	0.00796514	0.01719187	0.01884623
C4	0.00256175	0.01012867	0.00363048	0.01359062	0.01821935
C5	0.00014581	0.00383396	0.00424073	0.01263023	0.00536335
C6	0.00368505	0.03726524	0.00336712	0.03461701	0.01328631
P1	0.01472617	0.00563594	0.00415753	0.03372651	0.06564655
P2	0.00806117	0.00543972	0.0039952	0.0085268	0.0047022
P3	0.00699732	0.00791963	0.021082	0.00650822	0.01956429
P4	0.00035493	0.0151308	0.00730047	0.00286544	0.01723851
P5	0.01475267	0.01248917	0.00334941	0.02405094	0.03126059
P6	0.0081228	0.01825727	0.02369674	0.00402119	0.04046267
PA1	0.00088449	0.0073144	0.01524702	0.01462808	0.01008899
PA2	0.00893543	0.00711996	0.03020193	0.01777322	0.00804357
PA3	0.00094953	0.01888204	0.01795941	0.00800628	0.01943123
PA4	0.00707429	0.02905195	0.00349742	0.01612281	0.00728979
PA5	0.00183052	0.02363616	0.00604607	0.01131056	0.00704293
PA6	0.00030865	0.03177044	0.0133345	0.02195104	0.01647794

- Tab. 9b: Statistical analysis of Tab. 9a. Statistically significant values are marked.

TWO-WAY ANOVA for IC *Heterostegina depressa*

	Sum of sqrs	df	Mean square	F	p (same)
TIME:	0.000395	4	9.87E-05	6.865	**9.17E-05**
Type:	3.81E-04	2	1.91E-04	13.25	**1.18E-05**
Interaction:	9.58E-05	8	1.20E-05	0.8327	0.5767
Within:	1.08E-03	75	1.44E-05		
TOTAL:	0.001951	89			

TWO-WAY ANOVA for IN Heterostegina depressa

	Sum of sqrs	df	Mean square	F	p (same)
TIME:	0.001836	4	0.000459	5.161	**0.000998**
Type:	0.000341	2	0.00017	1.915	0.1544
Interaction:	0.001615	8	0.000202	2.27	**0.03122**
Within:	0.006671	75	8.89E-05		
TOTAL:	0.010463	89			

- Tab. 9a: Amount of incorporated carbon isotopes (IC) and incorporated nitrogen isotopes (IN) by *Amphistegina lobifera* in µg/mg.

IC	D1	D7	D14	D21	D28
C1	0.01052682	0.00293264	0.01090019	0.15370838	0.01296615
C2	0.01602382	0.02606329	0.00795975	0.03810427	0.12974888
C3	0.0011579	0.02341297	0.05940264	0.11880933	0.10051774
C4	0.0053692	0.03899943	0.07175609	0.00893471	0.12386255
C5	0.01927649	0.04113078	0.10499463	0.13122267	0.08237017
C6	0.0171046	0.04722097	0.06312889	0.12017012	0.08028078
P1	0.00915983	0.00691357	0.00629339	0.0619022	0.04638963
P2	0.00452329	0.00158821	0.017949	0.03475111	0.03310614
P3	0.00873972	0.05346475	0.06790261	0.00597941	0.00556574
P4	0.0099858	0.02924928	0.03204971	0.05188612	0.03313274
P5	0.00487464	0.02875695	0.04130501	0.05116748	0.0145319
P6	0.00960907	0.00278163	0.01700227	0.01340956	0.00961718
PA1	0.0071244	0.01003928	0.005683	0.05635616	0.03567138
PA2	0.0077025	0.01548031	0.04823357	0.01847057	0.04218101
PA3	0.0011111	0.00449298	0.04357251	0.04025521	0.09530089
PA4	0.00125427	0.01017049	0.00483133	0.00399153	0.08009603
PA5	0.00469135	0.04301302	0.04074494	0.02987038	0.06627987
PA6	0.00395761	0.02713222	0.01728309	0.02389901	0.01103187

IN	D1	D7	D14	D21	D28
C1	0.00940437	0.00061839	0.00991551	0.09973076	0.0151332
C2	0.00403351	0.01374166	0.03932997	0.02784944	0.10260048
C3	0.00016966	0.01047608	0.08464382	0.06373307	0.02028777
C4	0.0031737	0.02197853	0.0314595	0.00899541	0.09564286
C5	0.00623233	0.02609999	0.09121675	0.05671145	0.0968104
C6	0.01309049	0.025476	0.05745826	0.08770028	0.03169413
P1	0.00834832	0.00165479	0.00468413	0.03513842	0.10446655
P2	0.00557766	0.00045846	0.02765354	0.04043301	0.06915484
P3	0.01192318	0.01696859	0.11519835	0.04417368	0.01396114
P4	0.00712619	0.01112718	0.01310192	0.07014659	0.06023577
P5	0.01160526	0.02586734	0.0925521	0.03327513	0.07737601
P6	0.00845924	0.00187709	0.0063686	0.03462998	0.01332785
PA1	0.01118689	0.0097353	0.00472451	0.05511563	0.03795193
PA2	0.00720901	0.00916676	0.0392629	0.01656959	0.02179971
PA3	0.00291943	0.01144665	0.07962169	0.03351868	0.10224672
PA4	4.934E-05	0.00316089	0.02135304	0.00707072	0.11445613
PA5	0.00671339	0.0337096	0.03497816	0.01132138	0.07834249
PA6	0.0009564	0.03487666	0.02770901	0.0285688	0.03453209

- Tab. 9b: Statistical analysis of Tab. 9a. Statistically significant values are marked.

TWO-WAY ANOVA for IC *Amphistegina lobifera*

	Sum of sqrs	df	Mean square	F	p (same)
TIME:	0.029753	4	7.44E-03	10.73	**6.26E-07**
Type:	1.86E-02	2	9.29E-03	13.4	**1.06E-05**
Interaction:	1.28E-02	8	1.60E-03	2.304	**0.02885**
Within:	5.20E-02	75	6.93E-04		
TOTAL:	0.113109	89			

TWO-WAY ANOVA for IN *Amphistegina lobifera*

	Sum of sqrs	df	Mean square	F	p (same)
TIME:	0.035873	4	0.008968	12.59	**6.89E-08**
Type:	0.001319	2	0.00066	0.9263	0.4005
Interaction:	0.003189	8	0.000399	0.5598	0.8073
Within:	0.053411	75	7.12E-04		
TOTAL:	0.093792	89			

Eidesstaatliche Erklärung:

Ich erkläre eidesstattlich, dass ich die Arbeit selbständig angefertigt habe. Es wurden keine anderen als die angegebenen Hilfsmittel benutzt. Die aus fremden Quellen direkt oder indirekt übernommenen Formulierungen und Gedanken sind als solche kenntlich gemacht. Diese schriftliche Arbeit wurde noch an keiner Stelle vorgelegt.

Wien am 16.8.2023 Alexander Zientek