Plastic and natural inorganic microparticles do not differ in their effects on adult mussels (Mytilidae) from different geographic regions

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HIGHLIGHTS

- First study to compare microplastic effects over a wide biogeographical range
- Comparison between natural inorganic microparticles and plastic microparticles
- Significant effects on byssus production, respiration and clearance rates, but small effect sizes
- No ecologically relevant difference between impact of plastic and natural inorganic microparticles on Mytilidae

GRAPHICAL ABSTRACT
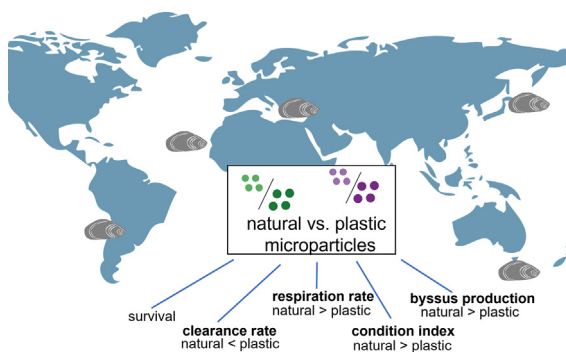
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ABSTRACT

Microplastics are ubiquitous in the marine environment and studies on their effects on benthic filter feeders at least partly revealed a negative influence. However, it is still unclear whether the effects of microplastics differ from those of natural suspended microparticles, which constitute a common stressor in many coastal environments. We present a series of experiments that compared the effects of six-week exposures of marine mussels to two types of natural particles (red clay and diatom shells) to two types of plastic particles (Polymethyl Methacrylate and Polyvinyl Chloride). Mussels of the family Mytilidae from temperate regions (Japan, Chile, Tasmania) through subtropical (Israel) to tropical environments (Cabo Verde) were exposed to concentrations of 1.5 mg/L, 15 mg/L and 150 mg/L of the respective microparticles. At the end of this period, we found significant effects of suspended particles on

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respiration rate, byssus production and condition index of the animals. There was no significant effect on clearance rate and survival. Surprisingly, we observed only small differences between the effects of the different types of particles, which suggests that the mussels were generally equally robust towards exposure to variable concentrations of suspended solids regardless of whether they were natural or plastic. We conclude, that microplastics and suspended solids elicit similar effects on the tested response variables, and that both types of microparticles mainly cause acute responses rather than more persistent carry-over effects.

1. Introduction

Microplastic particles are smaller than 5 mm and ubiquitous in the marine environment today (Eriksen et al., 2014; Wang and Wang, 2018). They occur from urbanized and polluted coastal areas (Zhao, 2015) to remote and pristine ecosystems (Horton and Barnes, 2020) including the deep sea (Cunningham et al., 2020) and polar regions (Waller et al., 2017).

In recent years, evidence has accumulated that microplastics are a threat to a wide range of marine organisms including benthic invertebrates (reviews by Anbumani and Kakkar, 2018; Foley et al., 2018; Haegerbaeumer et al., 2019). Benthic filter-feeding mussels, in particular, are prone to ingesting microplastics because of their feeding technique (Gonçalves et al., 2019), with negative effects such as the inflammation of epithelia, decreased feeding rates, oxidative stress and impaired larval development commonly reported (Balbi et al., 2017; González-Soto et al., 2019; Green et al., 2017; von Moos et al., 2012; Woods et al., 2018). Microplastic particles have also been shown to alter the uptake of phytoplankton by mussels (Chae and An, 2020) as well as the number and the strength of their byssal threads (Green et al., 2019; Rist et al., 2016). Often, effects caused by microplastics were concentration dependent, with higher concentrations generating stronger effects (Rist et al., 2016). Conversely, there are also studies that did not establish negative effects of microplastics on mussels (Browne et al., 2008; Santana et al., 2018; Van Cauwenberghe et al., 2015). These conflicting findings suggest that the effects of microplastics on filter-feeding mussels and therefore their relevance as an environmental pollutant is still not well understood and may be highly context dependent.

In all cases, information about the impact of microplastics on mussels have been obtained from laboratory exposure experiments, in which the test animals were usually exposed to defined concentrations of monospecific, very often spherical microplastic particles (Balbi et al., 2017; Browne et al., 2008; Haegerbaeumer et al., 2019; Ogonowski et al., 2016; Paul-Pont et al., 2016). These particles are also often applied in very elevated concentrations that are environmentally unrealistic (Gonçalves et al., 2019; Lenz et al., 2016; Rist et al., 2016) although an increasing number of recent studies have exposed test organisms to environmentally more realistic particle concentrations (Al-Sid-Cheikh et al., 2018; Hamm and Lenz, 2021). Despite differences in particle concentrations, there is one aspect that almost all of these studies have in common: most laboratory experiments test for the presence of microplastic effects by comparing the performance of a group of test individuals kept in a microplastic-free environment (i.e. the control group) with the performance of conspecifics exposed to microplastics. What these studies lack is a procedural control, in which test individuals are exposed to a natural suspended solid that has comparable bioavailability for the mussels due to similar properties (size, shape and density) as the microplastics under investigation. The non-fulfilment to include this additional treatment group means the observed effects are interpreted as the specific consequences of exposure to microplastics, when they could have been stimulated by any other suspended solid. Indeed, it is known that natural suspended solids, e.g. clay particles, can exert stress and induce effects of the same kind and strength that are reportedly provoked by suspended microplastics on marine filter feeders (Kiørboe et al., 1980; Prins et al., 1991). Therefore, it is important to compare the effects of microplastics to those of natural inorganic particles to identify microplastic effects unambiguously (Ogonowski, 2018; Triebskorn et al., 2019). Furthermore, the ubiquity of microplastics throughout the world's oceans calls for an approach in which the effects of microplastics and natural suspended solids are compared across a variety of closely related species at a global scale following a standardized experimental protocol.

A group of benthic filter feeders suitable for such an approach are mussels of the family Mytilidae since these mussels are widely distributed in shallow coastal waters and intertidal zones worldwide (Gardner, 2002; Gosling, 1992). Mytilidae can form extensive beds making them important habitat engineers in shallow coastal waters (Bouma et al., 2009; Ysebaert et al., 2019). Furthermore, they constitute a food source for a wide range of predators, provide a refuge for many other species (Carranza et al., 2009) and filter the water column, facilitating the flux of matter from the pelagic to the benthic environment (Asmus et al., 1992; Fréchette and Bourget, 1985). These environments commonly exhibit high concentrations of suspended solids for example during times when rivers transport sediment loads from erosion areas towards the oceans (Robinson et al., 2010). Solids suspended in the water column are commonly referred to as seston and include inorganic components such as clay or silt but also organic components such as detritus and phytoplankton (Huguet, 2017).

In this study, we exposed individual mussels from five species collected in five different locations, including temperate (Japan, Chile, Tasmania), subtropical (Israel) and tropical environments (Cabo Verde) to three concentrations of four different microparticles. We tested the response of mussels to two kinds of microplastics and two types of natural inorganic microparticles and focused on long-term effects rather than acute responses to loads of microparticles, which is why response variables were measured mainly in particle-free laboratory assays. We hypothesize that mussels react differently to plastic microparticles compared to natural inorganic particles and that the difference is dependent on the concentration. After six weeks of exposure (with the exception of the PVC and clay treatments in Tasmania, which lasted only for five weeks), we recorded survival, byssus production, respiration rates, clearance rates and the condition index of the mussels.

2. Material and methods

2.1. Animal collection

Adult mussels of the family Mytilidae in a size range of 15–30 mm were collected at each of the five study sites (Table 1) between May 29th and July 15th 2019, and were brought to the nearby laboratories of the research institutions that participate in the international research and student training program GAME (Global Approach by Modular Experiments, https://www.geomar.de/en/game). The data set presented in Yap et al. (2020) is a part of the global study presented here and it comprises 10% of the data shown in this contribution. We used species of the genera *Brachidontes*, *Mytilus* and *Semimytilus* that all belong to the family Mytilidae. *Mytilus galloprovincialis* and *M. trossulus* constitute a species complex with *M. edulis* in the North Atlantic and can form hybrids that produce fertile offspring, which shows how closely these species are related (Väinölä and Hvilsom, 1991). In addition to the North Atlantic, *M. trossulus* also appears in the North Pacific, while *M. galloprovincialis* occurs in the Mediterranean and *M. edulis* in the Baltic Sea (Riginos and Cunningham, 2004). *Semimytilus* is a genus with only one species, *S. algosus*, which is native to the Chilean coast (Tokeshi and Romero, 1995), where it occurs in dense beds in the lower intertidal zone (Tokeshi and Romero, 1995). As an invasive species, *S. algosus* is also found on the west coast of southern Africa (de Greef et al., 2013). The native range of *Brachidontes pharaonis* is the Indian

Table 1
Overview of the experiments that are presented in this study. Test individuals belonged to different species of the family Mytilidae and were collected at five sites worldwide. The lab temperature reflects the prevailing temperature in the mussels' habitats at the time of collection. Marine bioregions are named after (Spalding et al., 2007).

Species	Country	Marine bioregion	Longitude	Latitude	Sampling location	Replicates	Food algae (cells/mussel/day)	Lab temp. (°C)	Acclimation time (days)[a]	Start date of experiment	CI	Respiration	Clearance rate	Byssus production
Brachidontes puniceus	Cabo Verde	Tropical Atlantic	24°54'22"W	16°54'11"N	Baja das Gatas, São Vicente	15	4200 cells/mL *Thalassiosira weissflogii*	28	10	28.06.2019	X	X	X	
Semimytilus algosus	Chile	Temperate South America	71°21'11"W	29°57'59"S	Punta de Choros	10	20,000 cells/mL *Isochrysis galbana*	15	23	29.05.2019		X	X	X
Brachidontes pharaonis	Israel	Temperate Northern Atlantic	34°55'13"E	32°37'49"N	Dor HaBonim Natural Reserve	12	10,000 cells/mL *Dunaliella salina*	25	10	30.05.2019		X	X	
Mytilus trossulus	Japan	Temperate Northern Pacific	144°49'55"E	43°00'52"N	Akkeshi Bay	10	5000–10,000 cells/mL *Chaetoceros gracilis*	18	9	08.06.2019	X	X	X	X
Mytilus galloprovincialis	Tasmania	Temperate Australasia	147°58'09"E	42°31'18"S	Spring Bay Seafoods hatchery, Oakhampton Bay	10	4000 cells/mL *Terraselmis suecica*	13	10–14	20.06.2019	X	X		X

[a] In Chile, a second fieldtrip was necessary to collect a sufficient number of animals and consequently acclimation time increased.

Ocean including the Red Sea and it is invasive in the Mediterranean Sea (Rilov et al., 2004; Sará et al., 2008), while *B. puniceus* is native to the west African coast including the Cabo Verde archipelago (Morton, 2012). Exact information on sampling locations for all mussels is provided in Table 1 and an overview about the different species is presented in Table S1. All mussels were acclimated to laboratory conditions for 9 to 23 days before the exposure to microparticles began (Table 1); this duration is typically used in laboratory experiments with mytilids (Lenz et al., 2011; Navarro and Contreras, 2010; Nunes et al., 2020) and usually considered sufficient for studies like ours (Liutkus et al., 2012; Woods et al., 2018).

2.2. Microplastics and natural inorganic microparticles

At all study sites, mussels were exposed to four particle suspensions with three different concentrations each. Two of these contained microplastics, made from either Polymethyl methcrylate (PMMA) or Polyvinylchloride (PVC), while the other two were made with particles that resemble natural suspended solids (diatom shells, red clay). The plastic microparticles were additive-free according to their distributing companies, since the PVC particles are used for combustion in fireworks, while the PMMA is a pre-production granulate. The diatom shells and red clay were similar to the microplastics with regard to their size distribution and shape (Table 2, Fig. S2). The best matches for a comparison between natural and plastic suspended solids, according to particle characteristics, were between PMMA ("acrylic superfines", Kunststoff- und Farben GmbH) and diatom shells ("Celite®" Kieselgur 545, eydam) as well as between PVC (www.pyropowders.de) and red clay ("Moroccan red clay powder", Now Solutions®) (Table 2). All four particle types cover a range of sizes which reflects the situation in the environment. The three concentrations chosen were 1.5 mg/L, 15 mg/L and 150 mg/L for each microparticle type. We did not aim to mimic the concentrations at which microplastics currently occur in marine environments (Lindeque et al., 2020), but used particle loads that mussels experience in their natural habitats.

2.3. Exposure to microparticles

After acclimation to lab conditions, mussels were placed individually in beverage bottles from which the bottom part was cut off and which were then turned upside down. All bottles were PET and safe for human water consumption, they were e.g. by the trademark Schweppes (Israel) or a local water bottling plant: Leben® (Chile). They had a volume of 1 to 2 L and were bubbled with pressurized air from the bottom to ensure oxygen supply and constant resuspension of the negatively buoyant microparticles. The uptake and ingestions of the different microparticles by the test organisms was verified in pilot studies by inspecting the feces for microparticles under a stereomicroscope. The water inside the containers was 0.22–10 μm filtered natural seawater and was exchanged once a week completely. During the six other days of the week the water was exchanged partially or completely (Chile). These partial or complete water changes removed all particles from the system and the particle loads were re-established immediately afterwards. Three particle stock suspensions were prepared in a way so that 10 mL of each suspension were necessary to adjust one of the three target concentrations in the water body of the experimental units. For the highest concentration (150 mg/L), 15 g of microparticles were added to 990 mL distilled water and 10 mL of a 1% Tween20 solution. The suspension was stirred for homogenisation. The second concentration (15 mg/L) was achieved by diluting the first stock suspension 1:10 and the third concentration (1.5 mg/L) by diluting the second stock suspension in the same way. Tween20 was also added to the experimental units of a group of mussels that were kept in the absence of particles (i.e. the control group) to achieve a final concentration of 0.0001% Tween20 in all experimental units. Mussels were fed with a microalgal suspension once a day at varying concentrations depending on the used algal species (Table 1). Exposure lasted for six weeks except in the treatment combinations of PVC and clay in Tasmania, which lasted five weeks. After exposure to the different particle types and particle loads, various response variables were measured in

the absence of the particles to assess the health status of the mussels (overview in Fig. S1).

2.4. Response variables

Not all response variables were measured at all locations due to technical differences between the laboratories. At all study sites, respiration rates were assessed as the decline of oxygen over time per mussel after six weeks of exposure to the microparticles and five weeks in case of Tasmania's combination of PVC and clay (exposure time for PMMA and diatoms in Tasmania was six weeks as well). For this, mussels were transferred from their experimental units to sealed, microparticle-free respiration chambers (50–240 mL) that were filled with filtered (0.22–2 μm) and oxygen saturated seawater. During handling and measurements, mussels had a chance to recover from microparticle exposure for 1–48 h. The oxygen concentration was measured at the start and either again one hour later or five times every 15 min with either optical sensors (Presens Precision Sensing Fibox 4) or a handheld oximeter (WTW MultiLine® Multi 3510 IDS or LAQUAact OM-71). The change in the oxygen concentration in a container without a mussel was measured as a blank after every 5th, 10th or 14th replicate to assess the bacterial respiration. Respiration rates were calculated using the formula by Lampert (1984) and standardized to the individual shell length.

$$Rr = ((Ci_1 - Cf_1)/Ta - (Ci_0 - Cf_0)/Tc) * V$$

Ci_1: initial O_2 concentration in the container with mussel (mg/L)
Cf_1: final O_2 concentration in the container with mussel (mg/L)
Ta: incubation time for the container with mussel (h)
Ci_0: initial O_2 concentration for the blank (mg/L)
Cf_0: final O_2 concentration for the blank (mg/L)
Tc: incubation time for the blank (h)
V = volume of container (L)

In Israel, Japan and Chile, clearance rates were measured by assessing the number of algal cells that were cleared from the water body by the mussels per unit time. Clearance rates were standardized by shell length and are therefore indicated as mL/min/cm. Prior to assessing the filtration rates, individual mussels were transferred to containers filled with clean seawater as the microparticles in the experimental units would have interfered with the measurements. Mussels were therefore, apart from food particles, in microparticle-free water for 1–7 h during handling and measurements. Then, the experimenters adjusted a concentration of 2500–50,000 cells/mL inside the containers. For this, the same species of microalgae that was also used for feeding the mussels during the exposure period (Table 1) was applied, except for the experiment in Chile, where *Phaedactylum tricornutum* was used for clearance rate measurements and *Isochrysis galbana* for feeding. Microalgal suspensions were free of microplastics in all cases. The decline in the concentration of algal cells in the water body inside the containers was then recorded over time by taking water samples. Depending on the respective laboratory, samples were sometimes fixated or analysed directly with either a counting chamber (Fuchs-Rosenthal or Neubauer), a flow-cytometer or a Coulter Counter.

Table 2
Particles that were used for the exposure experiments with five mussel species and their characteristics.

Particle type	Size range (μm)	Mean size (μm) +/− SD	Colour	Density (g/cm³)
PVC	0.1–100	12.1	White	1.38–1.42
Red clay	0.1–100	13.8	Red-brown	2–3
PMMA	10–400	120.3 +/− 69.9	White	1.18
Diatom shells	4–400	86.9 +/− 90.4	White	2.65

Another important trait that also ensures the survival of mussels is their capacity to form byssal threads. Byssus production per individual mussel per day was measured in Tasmania, Japan and Chile. For this, the mussels were transferred from their experimental units to containers filled with microparticle-free seawater where they attached themselves to the walls or the bottom. After 22–24 h without microparticles, the mussels were carefully removed, and the byssus threads were counted. Respiration rate, clearance rate and byssus production were measured in the absence of particles to measure carry-over effects rather than acute responses to the applied microparticles. Test organisms therefore had a short recovery phase before the response variable was measured. After all measurements had been accomplished, the mussels of the teams in Tasmania, Cabo Verde and Japan were frozen at −20 °C for 24 h, thawed again, cut open and their soft tissue was scraped out of the shells with a scalpel. Both, the tissue and the empty shells, were then dried for 48 h at 60 °C and weighed. The condition index (CI) was calculated as tissue dry weight [mg]/shell length³ [cm³] after (Riisgård et al., 2014).

At all study sites, the survival of the mussels was recorded daily or on six days every week. Mussels that were wide open and did not react to a stimulus by closing their valves were considered dead.

2.5. Statistical analyses

Statistical analyses were done with R (version 3.6.3) using the packages "ggplot2" for graphics and "lme4" for the calculation of mixed effects models. Respiration rates, clearance rates, byssus production rates and the CI were modelled as a function of the fixed factors "particle type" and "particle concentration" with GLMMs that included "study site" as a random factor. "Particle type" had four levels (diatom, red clay, PMMA, PVC), while "particle concentration" had three levels (1.5, 15 and 150 mg/L). Homogeneity of variances was verified with plots of the residuals that were depicted as a function of the fitted values and with the Fligner-Killeen test. Normality of errors was checked with histograms of the residuals. Survival rates were modelled in a factorial Cox-regression that also had the factors "particle type" and "particle concentration". To test for net effect between plastic and natural inorganic particles, all response variables were additionally pooled across the concentration levels and study sites and differences between plastic and natural inorganic microparticles were tested with single-degree-of-freedom contrasts (Table S2). Furthermore, difference between the group not exposed to any particles, i.e. the control group, and the different particle types pooled across concentration again, as there were only minimal differences between the different concentrations, were tested with single-degree-of-freedom contrasts. The results were Bonferroni corrected with p < 0.013 as threshold for statistical significance (Table S3).

3. Results

3.1. Respiration rates

In general, median respiration rates of all experimental groups in which mussels were exposed to suspended particles were similar to the median oxygen consumption of the individuals in the control group (Fig. 1) and contrasts between the control group and the different particle types were not significant (Table S3). Respiration rates of all mussels that were exposed to natural microparticles were 9% higher than of those individuals that were kept in the presence of plastic microparticles (data pooled across experimental groups in both cases) (Fig. 2). Respiration rates were significantly different between the groups of mussels that received different particle types, while the particle concentration had no significant effect on the oxygen consumption. Furthermore, no interaction between the two factors was observed (Table 3). Median respiration rates of mussels that were exposed to 1.5 mg/L of PMMA and 15 mg/L of diatom particles were the highest, while it was the lowest when mussels were exposed to 15 mg/L PMMA and 150 mg/L PVC (Fig. 1). Pooled over concentrations, the respiration rates were the same when mussels were exposed to PVC
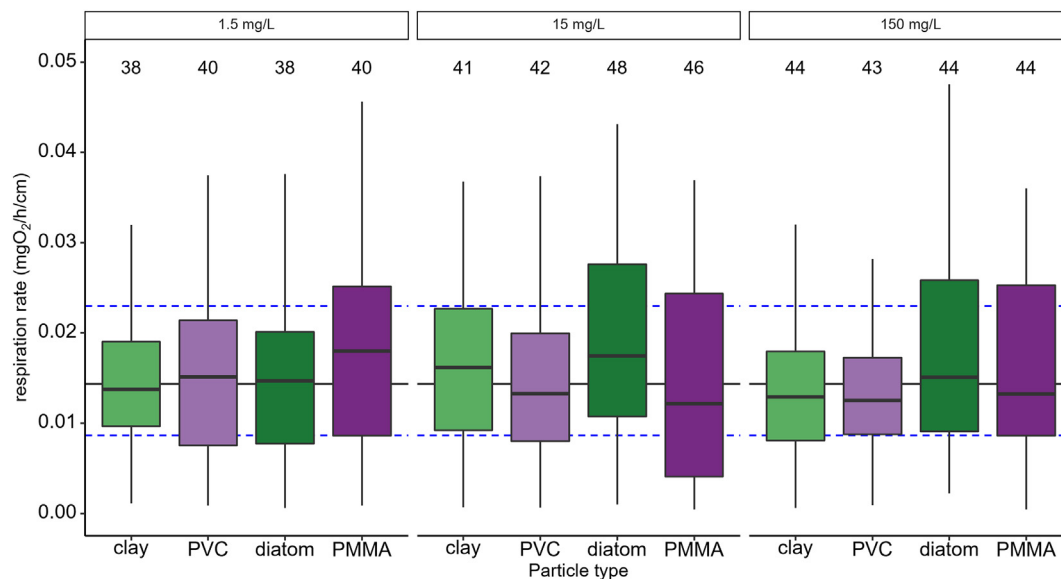
Fig. 1. Respiration rates of individuals from different mussel species after six weeks of exposure (exceptions were PVC and clay treatments in Tasmania, which lasted five weeks) to two types of plastic microparticles (PVC, PMMA; violet boxes) and two natural inorganic microparticles (clay, diatom; green boxes) that were applied at three concentrations (1.5, 15 and 150 mg/L). Boxplots show the interquartile range, the median and the non-outlier range. Numbers above the boxes denote replicate numbers. The solid and dashed horizontal lines extending across the whole graph represent the median, and the lower and upper quartiles, respectively, of the group of mussels that were not exposed to any particles (control group; n = 97, pooled across locations). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

compared to clay, while PMMA elicited 15% lower respiration rates compared with diatoms. However, this difference was not statistically significant (Table S2). An overview of the data per country is in Fig. S3.

3.2. Clearance rates

Clearance rates were not significantly different between the experimental groups and, hence, neither particle type nor particle concentration had an influence on the filtration capacity of mussels after exposure to suspended particles. A significant interaction between particle type and particle concentration was observed (Table 3). There was also no significant difference between the control group and the different particle types (Table S3). The lowest clearance rates were exhibited by the group of mussels that were exposed to 150 mg/L PVC and 150 mg/L clay (Fig. 3), while mussels exposed to 1.5 mg/L of PVC and PMMA performed best. Clearance rate was 12,5% higher when exposed to PMMA compared to diatoms and had again no difference when mussels were exposed to PVC compared to clay (data pooled across concentrations in both cases) and 11% higher for mussels exposed to plastic compared to natural inorganic microparticles (pooled across concentration and particle type) (Fig. 2). However, this difference was also not statistically significant (Table S2). An overview of the data per country is in Fig. S4.

3.3. Byssus production

The effect of particle type on byssus production was significant (Table 3), as well as concentration and the interaction between particle type and concentration (Fig. 4). Highest byssus production was achieved in mussels that were exposed to 1.5 mg/L and 15 mg/L diatoms, while mussels exposed to 15 mg/L and 150 mg/L PVC showed lowest byssus production. Pooled over concentration, byssus production was 6% lower when mussels were exposed to PMMA compared to diatoms and 34% lower when exposed to PVC compared to clay. The contrast between mussel individuals that were exposed to natural inorganic particles and those that experienced plastic particles (data pooled across experimental groups in both cases, Fig. 2) revealed no significant difference (Table S2) and neither did the contrasts between the control group and the different particle types (Table S3). Furthermore, byssus production varied between mussel

individuals but was consistently higher in the groups that were exposed to larger particles, diatoms and PMMA, than in those that were exposed to smaller particles, clay and PVC (Fig. 4). An overview of the data per country is in Fig. S5.

3.4. Condition index

We observed a significant effect of particle type on the condition index of the mussels, while the influence of particle concentration and the interaction between both factors was not significant (Table 3, Fig. 5). Pooled over concentration, CI was 5% higher for mussels exposed to PMMA compared to diatoms and 2% higher for PVC compared to clay. Pooled across concentration and particle type (natural or plastic), CI was 1% higher for mussels exposed to plastic compared to natural inorganic microparticles, this contrast was also not significant (Fig. 2, Table S2). Highest values were achieved by the control group and mussels exposed to 1.5 mg/L PMMA, while mussels exposed to 1.5 mg/L clay and 15 mg/L PVC showed lowest CI. The contrasts between the control group and the different particle types (Table S3) were also insignificant. An overview of the data per country is in Fig. S6.

3.5. Survival

A total of 93 individuals of the 795 experimental mussels died during the course of the exposure to suspended microparticles, but there was no significant effect of any of the factors on the mussel's survival (Fig. S7, Table 3).

4. Discussion

We compared the effects of plastic and natural inorganic microparticles on various traits and functions of mussels. The experiments were conducted simultaneously using comparable methods in five biogeographic regions in both hemispheres, resulting in two experiments happening during austral winter (Tasmania, Chile) and three experiments in boreal summer (Israel, Cabo Verde, Japan). Differences between the treatment groups were very small, and hence, the statistical significance that was revealed by the
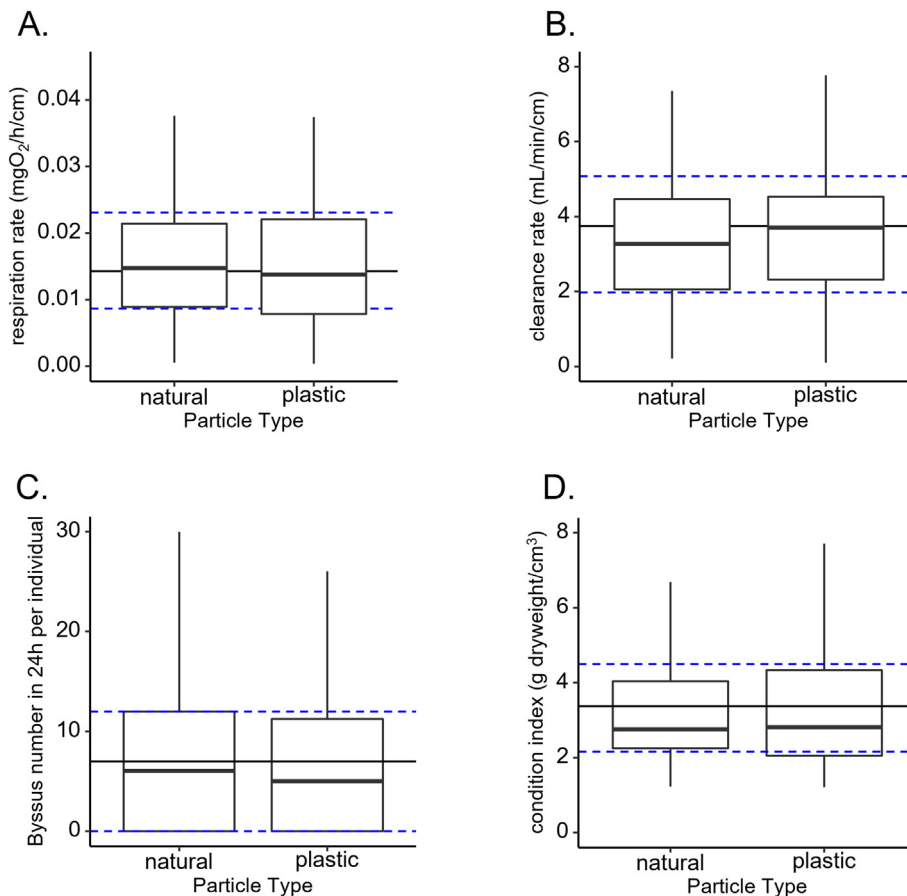
Fig. 2. Effect of natural (clay and diatom shells) and plastic (PVC and PMMA) microparticles on different response variables that were collected from mussel of five different species after six weeks of exposure (exceptions were PVC and clay treatments in Tasmania, which lasted five weeks). Data were pooled across all concentrations within the natural inorganic and plastic treatments. A. respiration rate, B. clearance rate, C. byssus number per mussel individual in 24 h and D. condition index. Boxplots show the interquartile range, the median and the non-outlier range. The solid and dashed lines represent the median, and the lower and upper quartile, respectively, of the group of mussels that was not exposed to any microparticles (control group).

modelling of the data is likely attributed to the large sample sizes that we had in this modular study and does not reflect a biologically relevant difference in the influence of the various particle types. Survival was not affected since even high seston loads only lead to sub-lethal effects (Incze et al., 1980; Widdows et al., 1979).

The small effect sizes of microparticle suspensions in all but byssus production was surprising, because we applied the microparticles at increasing concentrations along a logarithmic scale (1.5 mg/L, 15 mg/L, 150 mg/L). The concentrations chosen covered a wide range of environmental scenarios that mussels can encounter in, for instance, embayments (Puls et al., 1997). Under calm conditions, seston concentrations range between 0.3 mg/L and 50 mg/L, which are considered moderate levels (Puls et al., 1997; Widdows et al., 1979). During storm events or periods of enhanced river run-off, however, they can be 450 mg/L (Kiørboe et al., 1980) and even reach 1000 mg/L (Fahey and Coker, 1992). The experimental concentrations herein were chosen to test whether a potential difference in the effects of natural inorganic vs. plastic suspended solids increases when microparticle concentrations increase. However, in almost all cases, mussel species appeared to be robust towards the stress that was exerted by the microparticles regardless of their nature over the course of six weeks. Their performance was commonly in the same range as that of the control and this was true for all concentration levels. Hence, no interaction between microparticle concentration and particle type was observed except for byssus thread production and clearance rate.

Several exposure studies previously assessed the influence of different concentrations of suspended solids, represented either by natural materials such as silt or by microplastics on respiration rates of mussels. In some

cases, oxygen consumption declined with increasing microparticle loads (Rist et al., 2016) or no effect was recorded (González-Soto et al., 2019; Opitz et al., 2021). In our study, respiration rates were unaffected by microparticle concentrations but differed slightly between particle types, with diatoms eliciting the highest median respiration rates. Another study, which applied microparticles (4,5 μm or 0.5 μm PS spheres) at a concentration (0.058 mg/L) that was three times lower than the lowest concentration in this study, also found no effects on the respiration rates of *M. galloprovincialis* after exposure for 7 and 26 days (González-Soto et al., 2019). Other researchers compared conventional plastic microparticles made from mineral oil to particles derived from the biodegradable plastic Polylactic acid (PLA). The respiration rates of *Ostrea edulis* were not affected after 60 days of repeated exposure to 80 μg/L HDPE microparticles (0.48–316 μm) (Green et al., 2016). However, PLA microparticles (0.6–363 μm) elicited increased respiration rates in *O. edulis*. That study was similar to ours, since it compared two different kinds of microparticles, albeit both were plastic. This is rare, since most studies only examine one particle type (but see Gray and Weinstein, 2017; Hamm and Lenz, 2021; Zimmermann et al., 2020).

In a study with *M. edulis* on the effects of natural microparticles (silt) that were applied in several concentrations of up to 350 mg/L (which was more than two times higher than the highest concentration in our study) (Widdows et al., 1979), mussel respiration rates also did not change with microparticle concentration. However, oxygen extraction efficiency increased since the mussel's gills were ventilated less due to decreased clearance rates and consequently higher respiration was required to compensate for the deficit in oxygen uptake. Combined with data from the
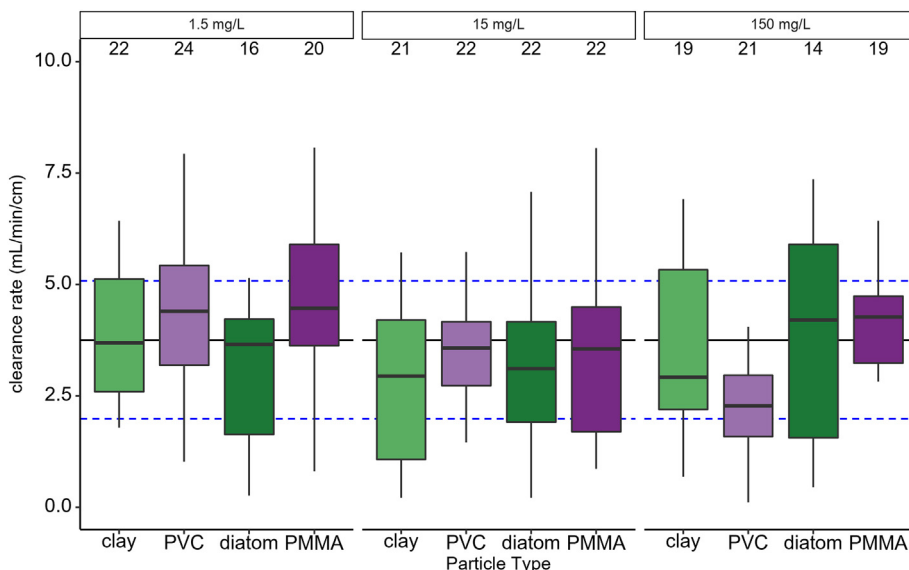
Fig. 3. Clearance rates of individuals from different mussel species after six weeks of exposure to two types of plastic microparticles (PVC, PMMA; violet boxes) and two natural inorganic microparticles (clay, diatom; green boxes) that were applied at three concentrations. Boxplots show the interquartile range, the median and the non-outlier range. Numbers above the boxes denote replicate numbers. The solid and dashed horizontal lines extending across the whole graph represent the median, and the lower and upper quartiles, respectively, of the group of mussels that was not exposed to any particles (control group, n = 63). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

other studies, it seems respiration is not affected by plastic microparticles and therefore seems to be an inferior parameter for measuring negative effects of plastic microparticles.

According to Widdows et al. (1979), clearance rates decreased with increasing silt concentration and reached a minimum at 220 mg/L silt which is 70 mg/L higher than the highest microparticle concentration in our study. In contrast to Widdows et al. (1979), we did not measure clearance rates in the presence of microparticles, but after a short recovery period in a clean environment as carry-over effect. This suggests that microparticles may provoke a change in clearance rates of mussels as a direct reaction to their presence, rather than impairing this performance trait permanently (Riisgård et al., 2003). Any deviation from the normal performance that is

documented after the recovery phase reflects a sustained influence of the previously experienced environmental conditions and is not an acute response. Since the concentrations of suspended solids, both of natural and of plastic microparticles, vary substantially in time in most aquatic environments (Wong et al., 2020), filter feeders, in their natural environment, should experience phases of stress that are followed by recovery periods. Hence, assessing potential carry-over effects, which integrate the impact of suspended solids over longer time spans, is presumably more informative than documenting acute responses to conditions that may prevail only for short time spans.

Clearance rates in *M. trossulus* were reduced after exposure to 1876–2500 particles/mL of fluorescent PE spheres (32–38 μm) and microalgae for one
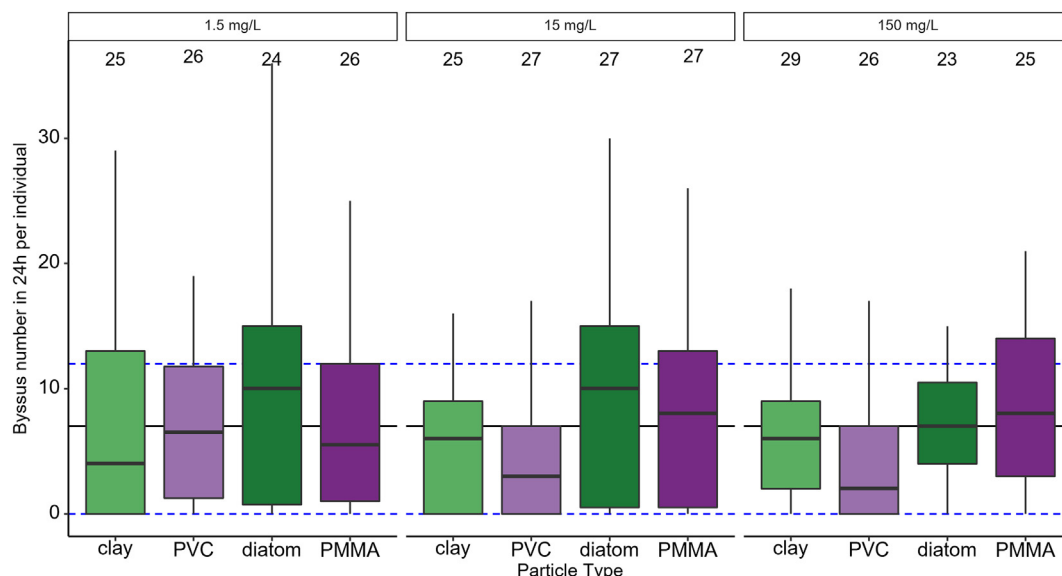

Fig. 4. Production of byssus threads per mussel individual in 24 h after six weeks of exposure (exceptions were PVC and clay treatments in Tasmania, which lasted five weeks) to two types of plastic microparticles (PVC, PMMA; violet boxes) and two natural inorganic microparticles (clay, diatoms; green boxes) that were applied at three concentrations. Boxplots show the interquartile range, the median and the non-outlier range. Numbers above the boxes denote replicate numbers. The solid and dashed lines extending across the whole graph represent the median, and the lower and upper quartiles, respectively, of the group of mussels that was not exposed to any particles (control group, n = 54). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
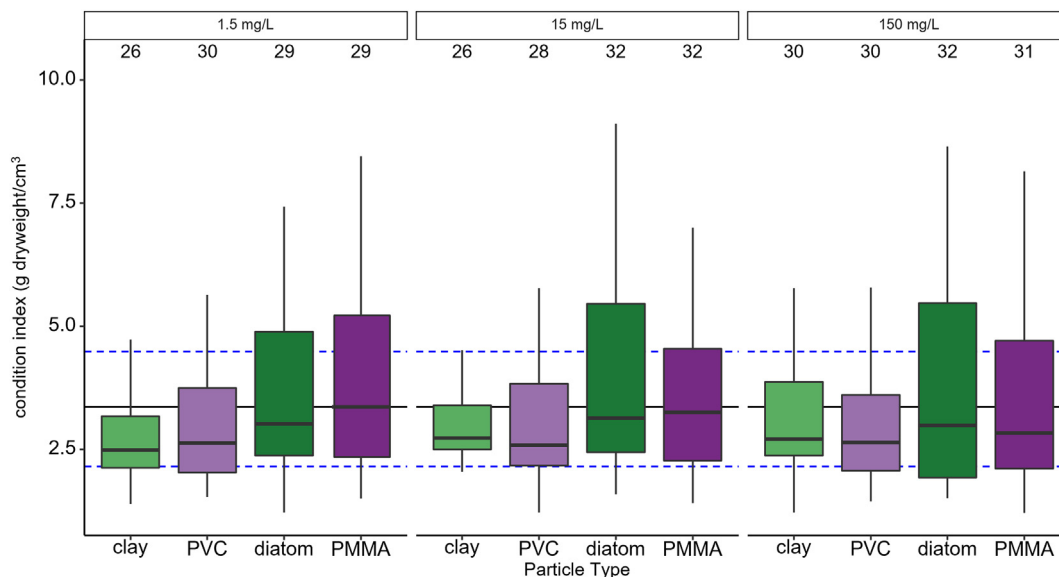
Fig. 5. Condition index of individuals from different mussel species after six weeks of exposure (exceptions were PVC and clay treatments in Tasmania, which lasted five weeks) to two types of plastic microparticles (PVC, PMMA; violet boxes) and two natural inorganic microparticles (clay, diatoms; green boxes) that were applied at three concentrations. Boxplots show the interquartile range, the median and the non-outlier range. Numbers above the boxes denote replicate numbers. The solid and dashed lines represent the median, and the lower and higher quartiles, respectively, of the group of mussels that was not exposed to any particles (control group, n = 69). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

hour, but not when exposed to the same amount of silt (30–37 μm) (Harris and Carrington, 2020). However, the authors also measured clearance rates in the presence of the microparticles and, hence, documented an acute response. This strengthens the argument that mytilid mussels can resume normal filtration as soon as the microparticle stressor is removed.

We observed a lower byssus production in mussels that were exposed to plastic microparticles (Table S2, Figs. 2 and 4). Only a few studies have focused on the effect of plastic microparticles on byssus thread production, but all of them found a negative influence of plastic on the number of byssus threads formed (Green et al., 2019; Rist et al., 2016; Webb et al., 2020). For example, there was a reduction in byssal threads and their tenacity in *M. edulis* after 52 days of repeated 2 h/day exposure to 25 μg/L HDPE

Table 3
Influence of particle type and particle concentration on response variables of mussels after exposure to microplastics and natural inorganic suspended solids. Results from GLMMs with family gamma (respiration, clearance rate, condition index), Poisson (byssus) and Cox regression (survival).

	Chisq	Df	p
Respiration rate			
Particle type	14.01	3	**0.0028**
Concentration	0.22	2	0.89
Particle type:concentration	3.2	6	0.77
Clearance rate			
Particle type	4.77	3	0.18
Concentration	3.49	2	0.17
Particle type:concentration	13.20	6	**0.04**
Byssus			
Particle type	16.52	3	**0.0008**
Concentration	10.77	2	**0.0045**
Particle type:concentration	31.93	6	$\mathbf{1.68 * 10^{-5}}$
Condition Index			
Particle type	20.88	3	**0.0001**
Concentration	4.47	2	0.11
Particle type:concentration	9.02	6	0.17
Survival			
Particle type	2.14	3	0.54
Concentration	0.92	2	0.62

Significant findings are in bold.

microparticles (~0.5–330 μm), but not after exposure to PLA or when the mussels were kept in a clean environment (Green et al., 2019). The number of byssus threads was also reduced in individuals of *Perna canaliculus* (Mytilidae) after they were exposed to 0.5 mg/L PE beads (38–45 μm) for 48 h (Webb et al., 2020). The concentrations of particles in those two studies were by far lower than the lowest concentration used in our study. *Perna viridis* also exhibited decreased byssus production with rising PVC microparticle concentrations (21.6, 216 and 2160 mg/L) (Rist et al., 2016) with significant results for 216 and 2160 mg/L, concentrations a lot higher than the ones we used. We measured the production of byssus threads in a clean environment and not during the presence of microplastics to capture possible carryover effects. The weak effects we recorded further suggest that mussels are more likely to be negatively influenced during acute exposure and do not experience a lasting limitation to their capacity to form byssus. Rist et al. (2016) also measured microplastic carryover effects and they observed significantly reduced byssus production only at concentrations that were substantially higher than our highest concentration.

A response variable that integrated the influence of the microparticles over the entire exposure period of six weeks was the CI. The range of CI values that we measured were comparable to values documented by other studies that assessed mussels in the field (Riisgård et al., 2014). This indicates that mussels were fed sufficiently at all the experimental sites. Although we found a significant influence of particle type on the CI, all CI values were within the range of variance of the control group. There was also no significant difference between the control and the different particle types (Table S2). This indicates that the ecological relevance of the influence of microparticles on the CI is negligible. Although this difference was not significant, CI values of the control group were always slightly higher than those of mussels that were exposed to plastic or natural inorganic microparticles. This suggests that mussels exposed to microparticles fed less over the experiment than conspecifics not exposed to microparticles. This is plausible since it is known that a common reaction of mussels to high loads of suspended microparticles is to close their valves, reducing the time they spend feeding in order to avoid the uptake of microparticles into their mantle cavity (Widdows et al., 1979). Another reason could be that the digestive system of the mussels was filled with microparticles, thus decreasing filtration due to false satiation (Sorrentino and Senna, 2021). This might have led to a significant decrease of CI if the experiment had run longer.

In our study, exposure to PMMA and diatom shells resulted in a slightly higher median performance for CI and byssus production than when the mussels were kept in the presence of PVC and red clay. These first two types of microparticles were larger (up to 400 μm) compared to PVC and red clay microparticles (up to 100 μm). When confronted with a mix of differently sized microparticles, mussels generally reject a higher proportion of the bigger microparticles (i.e. > 100 μm) than of the smaller ones (Ward et al., 2019). This might lead to weaker negative effects for larger particles as fewer microparticles are actually being ingested and are instead rejected. This effect of microparticles may therefore be a function of size and number and not of the material, since the most obvious difference between the microparticle pairs PVC – clay and PMMA – diatoms is the particle size. This also leads to a difference in numbers, since particle loads were standardized for weight and not number. This resulted in approximately 10 times more particles for PVC and clay compared to PMMA and diatoms. Smaller particles therefore seem to be more detrimental for mussels than larger ones (Kinjo et al., 2019).

Studies that comprise various taxonomically related test species as well as study sites in different biogeographic and climatic regions are a very useful but rarely used tool for assessing the impact of an anthropogenic influence on marine organisms. They allow the distinction between the general signal that is caused by the impact and the context dependency that is associated with the different species and study systems. By this, they facilitate the assessment of the overall ecological significance of an influence, but also allow for detection of differences in tolerance towards the stressor between species. In our case, all the species we tested showed a high level of tolerance towards the presence of suspended solids in their environment, while differences in the effects between natural and plastic microparticles were rather small. Absolute values of parameters varied between countries, but trends were very similar. The only notably different trend was clearance rate recorded for *M. trossulus* in Japan (Fig. S4) where plastic microparticles elicited higher clearance than natural inorganic microparticles. This suggests that this group of mussels is generally robust towards this form of environmental pollution, which can be very different for other groups of marine organisms.

Only a few studies have adopted this approach with a rigorous microparticle control and they all found the plastic microparticles to be more detrimental for the test organisms than the natural microparticles (Casado et al., 2013; Harris and Carrington, 2020; Ogonowski et al., 2016; Scherer, 2020; Schür et al., 2020; Watts et al., 2014; Yap et al., 2020). Our findings from a multi-site experiment with several mussel species do not confirm this picture. However, a comparison between our study and previous studies is difficult, since most of the organisms tested were functionally different, i.e., freshwater crustaceans and insects (*Daphnia magna, Thamnocephalus platyurus, Chironomus riparius*), but also, marine crustacean (*Carcinus maenas*), a microalga (*Pseudokirchneriella subcapitata*) and a virus (*Vibrio fischeri*). One other study that focused on marine mussels with this approach was done by Harris and Carrington, 2020, who showed, that *Mytilus trossulus* exhibits reduced clearance rates when exposed to high concentrations of polyethylene spheres, which was not the case when mussels experienced silt of similar concentrations after 1 h of exposure. The microparticle concentrations used by Harris and Carrington (2020) (1–2500 abiotic particles/mL, 2500 particles/mL corresponding roughly to 0.072 mg/mL) were a lot lower than the ones we used (1.5–150 mg/L) and consisted of spherical polyethylene beads and autoclaved and sieved silty sediment. They also measured their response variables in the presence of particles while the majority of our measurements was in a particle-free environment. This indicates that an important aspect that needs to be considered when comparing exposure studies that dealt with filter feeders is whether the response variable was measured in the presence or absence of the suspended solids. The fact that we did not find such carry-over effects of microparticle exposure when we measured respiration rates, and clearance rates could indicate that all of the tested mussel species have a high plasticity (Chandurvelan et al., 2013) that allowed them to return to their normal performance during the time interval between the end of the exposure and the measurements (i.e., recovery phase). In contrast,

M. galloprovincialis had a slightly reduced condition index when the animals were exposed to PVC in comparison to red clay, indicating lower nutritional status, but no difference in the effects of the two particle types on the other response variables respiration rate, byssus production and survival were observed (Yap et al., 2020).

The absence of specific microplastic effects in our study does not mean that microplastics do not affect mussels at all. We tested adult mussels, but other life history stages such as larvae or juveniles can be affected differently and are often more susceptible than adult animals (Pandori and Sorte, 2019). Microplastics could also cause endocrine disruptions, alter gene expression patterns, change metabolite composition or impair the performance of mussels mainly in combination with pathogens, pollutants or other stressors (Foley et al., 2018). Hence, the choice of response variables might be particularly important for assessing the effects of microplastics and this is also the case when the aim is to assess differences in the mode of action between natural and plastic microparticles. We are convinced that assessing response variables that integrate over larger time scales, such as the condition index, is very suitable when it comes to learning about the influence of plastic microparticles. Nevertheless, to understand the underlying mechanisms better, future research on this topic should also consider cellular mechanisms such as gene expression patterns and immune system responses. These mechanisms are presumably susceptible to very subtle fluctuations in environmental conditions and might therefore be an appropriate tool to identify differences in the mode of action between different types of suspended microparticles.

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CRediT authorship contribution statement

T. H. methodology, formal analysis, writing – original draft, writing – review and editing. M.L. conceptualization, methodology, writing – review and editing, funding acquisition, J. B., A.-L. G, L. L. G., M. G., D. H., U. K., A. S. L., L. N. T., P. V., V. Y.: conceptualization, methodology, investigation, data curation, writing – review and editing. M.T.: conceptualization, methodology, supervision, writing – review and editing. C. A., Z. C., C. L. H., J. L. L., M. N., G. R., J.W.: supervision, writing – review and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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