Mikroplastik in limnischen Ökosystemen

Vorkommen, Interaktion mit Biota und Toxizität

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“The human race is challenged more than ever before to demonstrate our mastery, not over nature but of ourselves.”

(Rachel Carson, 1907-1964)

Abstract

Since the 1950s, plastic has become an indispensable resource in everyday human life. As a negative consequence of this boom, an increasing pollution of aquatic ecosystems with plastic waste and its degradation products, so-called “microplastics” (MP, < 5 mm) or “nanoplastics” (NP, < 1 µm), has been observed throughout the last decades. The aim of this work was to investigate the occurrence of MP in freshwater ecosystems, to analyse the interaction between MP and freshwater invertebrate species and its subsequent toxicity as well as to provide a first risk assessment.

The occurrence of MP in freshwater ecosystems was examined based on the example of the Elbe, a large German river. By evaluating eleven sample sites along the course of the Middle and Lower Elbe, it was shown that mean MP concentrations in the sediment ($2.26x10^4$–$2.27x10^7$ P m^{-3}) were about 150,000 times higher than in the water phase (0.88–13.24 P m^{-3}). Sediments, thus, represent a sink for MP. The polymer type composition as well as the particle shape of the detected MP particles indicated that the MP particles origin from both diffuse and point sources (e.g. industrial waste water). In a global context, MP concentrations in German rivers are currently rather average compared to other rivers worldwide. However, published environmental MP concentrations possibly underestimate actual concentrations. The Elbe study could show that the fine sediment fraction <100 µm contained a significant proportion of polymer materials. Most of the previous studies on MP-concentrations in rivers, however, have not considered particles <100 µm in their analysis so far.

The interaction of MP with freshwater biota was investigated in detail using the species groups of mussels (Bivalvia), snails (Gastropoda) and crustaceans (Crustacea). Interaction intensity mostly depends on the MP uptake by the different species. On the basis of numerous uptake studies with various freshwater invertebrate species, including the mussels *Dreissena polymorpha*, *Sinanodonta woodiana* and *Anodonta anatina*, the pulmonate *Lymnaea stagnalis* and the amphipod *Gammarus pulex*, it could be shown that the MP uptake depends on the properties of the exposed species/individuals, on the MP characteristics as well as on the exposure conditions. The mussel experiments highlighted the rapid uptake and excretion of MP particles within few hours. In all three groups of organisms, the uptake was concentration-dependent with increasing uptake with rising MP level. However, a simultaneous exposure to other particles (e.g. food) reduced the MP abundance in the test organism. Further, the size of the test organism influenced the overall MP uptake: considering mussels and crustaceans, smaller individuals (and in case of mussels also smaller species) ingested relatively (per body mass) more MP particles than larger individuals/species. Finally, for all three groups of organisms it was shown that MP size affects the quantity of ingested MP particles.

When comparing the results for MP ingestion between the three examined groups of organisms, mussels (filter-feeders) ingested more MP than amphipods (shredders) and snails (grazers) at same exposure concentrations. In contrast, in the environment crustaceans and snails use the transition zone between the water and sediment phase as habitat and foraging ground. Hence, these two invertebrate groups may be exposed to higher MP concentrations in the environment (due to the higher MP concentrations in sediments) compared to mussels which acquire their food from the water phase. However, extrapolations of the experimental results from the uptake studies as well as a comparison of this data with published environmental data suggest that MP abundance in individuals of all three species groups is limited to few MP particles per individual so far. Pronounced differences between the species groups have not yet been identified.

MP toxicity studies with *G. pulex*, *L. stagnalis* as well as *D. polymorpha* revealed hardly any MP-related toxicity although the experiments considered a large number of endpoints (mortality, reproduction, feeding, oxidative stress, energy reserves, immune cell activity) and involved also very high MP concentrations which exceed current environmental concentrations by far. The few observed effects included increasing filtration activity of *D. polymorpha* as well as a change in the hemolymph cell immune functioning in *L. stagnalis*.

For a further MP risk assessment, these study results were combined with published data on marine and freshwater mussels and crustaceans in two species sensitivity distributions (SSD). A SSD analysis only for freshwater species is currently not possible due to the limited amount of available data. The resulting SSD highlight that current environmental MP concentrations in extremely MP-polluted freshwater ecosystems may already be high enough to trigger effects in particularly sensitive mussel and crustacean species. Despite the great uncertainties associated with the creation of the SSD, MP-induced effects, especially at a subindividual level, can currently not be ruled out. The SSD also indicate that MP toxicity may differ between groups of organism and effects in mussels may be more pronounced than in other groups.

Furthermore, laboratory-based toxicity studies with MP as a single stressor do not take into account that freshwater species in the environment are exposed to a large number of stressors. Accordingly, MP toxicity in combination with a second stressor was investigated in two multi-stressor experiments with *L. stagnalis* (second stressor: copper ions) and *D. polymorpha* (heat stress). In contrast to the second stressor, MP had few or no effects in the two effect studies. However, other previously published multi-stressor studies with marine and freshwater mussel and snail species suggest that MP in combination with another (chemical) stressor can induce relevant MP toxicity (additive, synergistic, antagonistic). Eventually, comprehensive conclusions on MP toxicity in a multiple-stressor-environment will require additional research.

In the future, MP pollution in the aquatic ecosystems will continue to increase due to the on-going input of plastic debris into the environment. As a consequence, rising environmental MP concentrations will lead to a more pronounced interaction of MP with biota, making toxic effects increasingly likely. To prevent a further increase of MP pollution in the environment and resulting negative impacts, our society must adapt its plastic consumption behavior and find new ways to use this resource more sustainably.

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1. Einleitung

1.1 Plastikkonsum und Eintrag von Plastikabfällen in die Umwelt

Plastik hat unser gesellschaftliches Leben in nahezu all seinen Bestandteilen revolutioniert – ob im Beruf oder in der Freizeit: Ein Alltag ohne Plastikprodukte würde sich wesentlich von unserem heutigen Leben unterscheiden. Daher wird Plastik auch als ein Indikator für das aktuelle Zeitalter des Anthropozän angesehen (Zalasiewicz et al. 2016).

Plastik zeichnet sich durch seine chemische Zusammensetzung aus synthetischen oder semi-synthetischen Polymeren, durch einen festen Aggregatzustand bei Temperaturen < 20 °C sowie durch eine geringe Wasserlöslichkeit (< 1 mg L^{-1} bei 20 °C) aus (Hartmann et al. 2019). Über 80 % der Plastikproduktion gehen dabei auf nur sechs Polymertypen zurück: Polyethylen (PE, high density: PE-HD, low density: PE-LD), Polypropylen (PP), Polystyren (PS), Polyethylenterephthalat (PET), Polyurethan (PUR) sowie Polyvinylchlorid (PVC) (PlasticsEurope 2018). Diese sechs Polymertypen können in vielfältiger Form eingesetzt werden, u.a. als Verpackung, im Hausbau, in der Automobil- und Elektronikbranche sowie im Haushalt und der Landwirtschaft. Auf Grund der hohen Nachfrage nach Plastikprodukten ist die globale Produktion seit den 1950er Jahren exponentiell angestiegen (von 1.7 Megatonnen (Mt) in 1950 auf 348 Mt in 2017). Im globalen Vergleich ist die Plastiknachfrage in Europa und Deutschland besonders hoch: 2017 betrug sie in Europa 14,7 % sowie in Deutschland 3,6 % der weltweiten Plastikproduktion bei einem relativen Weltbevölkerungsanteil von 9,8 % bzw. 1,1 % (PlasticsEurope 2018, United Nations 2017). Plastik ist für den Menschen daher zu einer unverzichtbaren Ressource des Alltags geworden.

Die zunehmende Verwendung von Plastikprodukten ist jedoch mit negativen Auswirkungen auf die aquatische Umwelt verbunden. Bis 2015 hat die Menschheit insgesamt 6.300 Mt Plastikmüll produziert, von denen ungefähr 79 % auf Mülldeponien oder in der Umwelt abgelagert worden sind. Wenn die derzeitigen Bewirtschaftungsstrategien für Plastikabfälle fortgesetzt werden, könnte sich die Menge des Plastikabfalls in der Umwelt bis 2050 weiter verdoppeln (Geyer et al. 2017).

Aquatische Ökosysteme sind eine wesentliche Senke für Plastikmüll in der Umwelt. Jedes Jahr werden sowohl durch Gewässernutzungsformen wie Schifffahrt und Fischerei wie auch durch Landquellen (z. B. durch Industrie, illegale Deponierung oder durch Abwässer) große Mengen an Plastikabfällen in aquatische Lebensräume eingetragen (Li et al. 2016c). Im Jahr 2012 gelangten weltweit etwa 4,8 bis 12,7 Mt Plastikmüll von Landflächen in die Ozeane, und es wird erwartet, dass die eingetragenen Mengen in Zukunft noch weiter ansteigen werden (Jambeck et al. 2015). Flüsse sind einer der wesentlichen Eintragspfade, durch die Plastikmüll vom Land in die Ozeane gelangen kann. Der weltweite Plastikeintrag von Flüssen in die Ozeane beträgt jährlich ca. 0,4 bis 4 Mt (Lebreton et al. 2017, Schmidt et al. 2017). Daher sind nicht nur marine, sondern auch limnische Ökosysteme (Süßwasserökosysteme) vorübergehende oder möglicherweise sogar langfristige Senken für Plastik in der Umwelt.

1.2 Mikroplastik in aquatischen Ökosystemen

Der überwiegende Anteil der Plastikprodukte ist unter Umweltbedingungen kaum biologisch abbaubar (Urbanek et al. 2018). So verbleiben angesammelte Plastikabfälle über Monate bis Jahre in aquatischen Ökosystemen (Fotopoulou & Karapanagioti 2019), bevor sie aufgrund mechanischer, chemischer und biologischer Verwitterung allmählich fragmentieren (Browne et al. 2007, Potthoff et al. 2017). Ein Schlüsselauslöser für Plastikfragmentierung ist Sonnenlicht. Ultraviolette (UV) Strahlung induziert Oxidationsreaktionen in der Polymermatrix, die zu Bindungs- und Strangbrüchen in der Matrix führen. Die reduzierte Stabilität der Polymermatrix führt zudem zum vermehrten Herauslösen von Additiven (chemische Substanzen mit niedrigem Molekulargewicht, die dem Plastik zugesetzt werden, um dessen Verarbeitung zu erleichtern bzw. dessen Eigenschaften oder Leistungsfähigkeit zu verbessern, Hartmann et al. 2019). Durch den Verlust der Additive können die Plastikprodukte wiederum auf Grund mechanischer (z. B. Wasserbewegungen) oder biologischer Prozesse (z. B. Fraß durch aquatische Organismen) noch leichter fragmentieren. Ein langfristiger Verbleib der Plastikabfälle in der Umwelt führt somit zur Bildung großer Mengen mikroskopisch kleiner Plastikpartikel, sogenanntem „Mikroplastik" (MP, Cole et al. 2011).

Während der Fragmentierung entstehen nicht nur Partikel mit einer Größe im Mikro- sondern auch im Nanobereich (< 1 µm, „Nanoplastik", NP, Lambert & Wagner 2016). NP hat eine so geringe Größe, dass es biologische Barrieren überwinden und so Gewebe und Organe infiltrieren kann (Mattsson et al. 2017). Die aktuell verfügbaren Mess- und Detektionstechniken sind jedoch nicht ausreichend, um NP in Umwelt- oder Gewebeproben nachweisen zu können (Mattsson et al. 2018, Schwaferts et al. 2019). Es ist daher aktuell unklar, inwieweit NP bereits in der Umwelt und in Organismen verbreitet ist.

MP-Partikel entstammen jedoch nicht nur Fragmentierungsprozessen, sondern werden häufig auch gezielt für Industrie- und Haushaltsanwendungen produziert. So werden MP-Partikel beispielsweise als Schleifmittel in Kosmetikprodukten (z. B. in Hautpflegemitteln, Cheung & Fok 2017, Zitko & Hanlon 1991), in Druckluftstrahlern zur Reinigung von Maschinen oder Booten (Gregory 1996) sowie in der Forschung zur gezielten Pharmakotherapie (gezielte Wirkstofffreisetzung am Zielort, Dalmo et al. 1995 Soppimath et al. 2001) eingesetzt. Diese Plastikpartikel werden oft als "primäres Mikroplastik" bezeichnet, da diese Partikel direkt in mikroskopischer Größe hergestellt werden. Dementsprechend wird MP, das durch Fragmentierung entsteht, unter dem Begriff „sekundäres Mikroplastik" zusammengefasst. Primäres MP kann durch häusliche und industrielle Abwässer, Regenwasser oder unangemessene Abfallentsorgung in die Umwelt gelangen und, sofern es nicht von Kläranlagen zurückgehalten wird, in Flüsse und Ozeane gelangen (Browne 2015).

Polymertextilien sind eine weitere wichtige MP-Quelle. Beim Tragen sowie Waschen von synthetischer Kleidung kommt es zum Abrieb mikroskopisch kleiner, synthetischer Fasern, welche durch die Luft sowie durch Ab- oder Regenwasser in die Umwelt gelangen (Browne 2015, Hartline et al. 2016, Hernandez et al. 2017).

Ebenso wie die ursprünglichen Plastikprodukte kann auch das aus ihnen entstehende MP stark variierende Eigenschaften besitzen. So variieren in der Umwelt nachgewiesene MP-Partikel in Form (z. B. Kugeln, Fragmente, Fasern, Filme), Größe, Farbe und Polymertyp (Eerkes-Medrano & Thompson 2018, Eriksen et al. 2014, Lusher 2015, Mani et al. 2015). Diese Vielzahl von Partikeleigenschaften erschwert aktuell die allgemeine Klassifizierung und Definition von MP-Partikeln.

1.3 Mikroplastik definieren

Erste Nachweise für mikroskopisch kleine Plastikpartikel in der Umwelt wurden in den 1970er (Carpenter & Smith 1972, Carpenter et al. 1972) und 1980er Jahren (Harper & Fowler 1987) erbracht. Eine gezielte Erforschung der Quellen, Verbreitung und Auswirkungen dieser Partikel begann jedoch erst zu Beginn des 21. Jahrhunderts und wurde insbesondere durch die Studie „Lost at sea: Where is all the plastic?" von Thompson et al. (2004) angeregt. Die Autoren berichteten über eine weiträumige Verbreitung und ein kontinuierlich zunehmendes Vorkommen von Plastikpartikeln in mikroskopischer Größe im Nordostatlantik. Thompson et al. (2004) bezeichneten diese Partikel als „Mikroplastik" – ein Begriff, der sich seitdem in der Forschung sowie im gesellschaftlichen Kontext zunehmend etabliert hat.

Eine offizielle Definition von „Mikroplastik" wurde bis heute jedoch nicht abschließend festgelegt. Im Jahr 2008 definierten Mitglieder der National Oceanic and Atmospheric Administration in einem Treffen zum Thema „Mikroplastik in den Meeren" MP als Plastikpartikel < 5 mm (Arthur et al. 2009). Die getroffene Definition ist jedoch keinesfalls abschließend, da ihr Angaben zu einer Größenuntergrenze sowie Angaben zu physikochemischen Partikeleigenschaften fehlen. Daher wurden seit 2008 mehrfach weiterführende Vorschläge erarbeitet, um Makro-, Meso-, Mikro- und Nanoplastikpartikel in einer Definition voneinander abzugrenzen (Hartmann et al. 2019). Bisher konnte auf internationaler Ebene jedoch kein Konsens zu einer einheitlichen Definition gefunden werden.

2019 hat sich daher eine Gruppe anerkannter Experten in der MP-Forschung zusammengeschlossen, um in einem neuen Versuch eine gemeinsame Definition für MP-Partikel zu erarbeiten. Hartmann et al. (2019) definieren MP als synthetische oder stark modifizierte natürliche Polymere (unabhängig von ihrem Additivgehalt) in einem Größenbereich von 1 bis 1000 µm (der größte Durchmesser bestimmt die Partikelgröße), die bei 20 °C Umgebungstemperatur einen festen sowie wasserunlöslichen Zustand besitzen. Partikel < 1 µm und > 1 mm werden als Nano- bzw. Mesoplastik eingestuft. Da diese Definition derzeit die umfangreichste ist und von einer großen Anzahl an Forschenden anerkannt wird, orientiert sich die folgende Thesis an dieser Definition (falls nicht explizit anders angegeben).

1.4 Vorkommen und Verteilung von Mikroplastik in der aquatischen Umwelt

MP konnte bereits in nahezu allen Lebensräumen der Erde nachgewiesen werden: von den Tropen bis zu den Polarregionen, an der Wasseroberfläche sowie in tieferen Wasserschichten, im Eis, in der Atmosphäre sowie in Biota (Eerkes-Medrano & Thompson 2018, Lusher 2015). Globale Meeresströmungen sowie atmosphärische Transportvorgänge sind Schlüsselwege für die globale Verbreitung von MP, wodurch diese Partikel auch in extrem abgelegene Regionen der Erde gelangen können (Free et al. 2014, Lavers & Bond 2017, Waller et al. 2017). Als weltweit wichtige Senken für MP gelten insbesondere die fünf subtropischen Wirbel (große geschlossene Systeme mit zirkulärer Meeresströmung, Eriksen et al. 2014) sowie die Tiefsee (Peng et al. 2018b, Woodall et al. 2014).

Frühere Forschungsanstrengungen zur globalen Häufigkeit und Verteilung von MP konzentrierten sich hauptsächlich auf die marinen Ökosysteme, während die Erforschung von MP in limnischen Systemen erst in den letzten zehn Jahren stärker in den Fokus gerückt ist (Li 2018, Wagner et al.

2014). Frühere limnische Studien haben insbesondere das Vorkommen von MP in Seen und Flüssen in Nordamerika, Europa und Asien untersucht (Li 2018). In Europa konzentrierte sich die Forschung insbesondere auf Flüsse in Westeuropa. Dabei wurden sowohl kleine als auch große (z. B. Rhein, Donau, Weser, Seine, Rhône) Flusssysteme in Deutschland, Frankreich, Portugal, den Niederlanden, dem Vereinigten Königreich, der Schweiz und Österreich näher analysiert (Blair et al. 2019, Dris et al. 2015, Faure et al. 2015, Heß et al. 2018, Lechner et al. 2014, Leslie et al. 2017, Klein et al. 2015, Mani et al. 2015, 2019, Rodrigues et al. 2018). MP-Forschung zu europäischen Seenlandschaften sind hingegen auf Studien aus den Alpenregionen (Gardasee sowie zahlreiche Seen in der Schweiz, Faure et al. 2015, Imhof et al. 2013), aus Zentralitalien (Lake Bolsena, Lake Chiusi, Fischer et al. 2016) sowie aus dem Vereinigten Königreich beschränkt (Vaughan et al. 2017).

In Flüssen wurde MP bereits sowohl in der Wasser- als auch der Sedimentphase quantifiziert. Die nachgewiesenen MP-Partikel in beiden Umweltkompartimenten besaßen dabei eine Vielzahl unterschiedlicher Formen (Kugeln, Fragmente, Fasern, Folien) sowie Polymertypen (z. B. PE, PP, PS, Baldwin et al. 2016, Klein et al. 2015, Mani et al. 2015, Peng et al. 2018a).

Bisher ermittelte MP-Konzentrationen in der Wasserphase von Flüssen (Stand: Mai 2019) schwanken zwischen $2{,}25 \times 10^{-2}$ (Minimum, Baldwin et al. 2016) und $5{,}19 \times 10^{5}$ Partikeln m^{-3} (P m^{-3}, Lahens et al. 2018). Ein Vergleich dieser Konzentrationsspanne mit MP-Konzentrationen in Flusssedimenten ist nur begrenzt möglich, da frühere Studien zur MP-Abundanz in Sedimenten meist abweichende Konzentrationsangaben (P m^{-2}, P m^{-3}, P (kg Sediment)$^{-1}$) verwendeten. Bisher publizierte MP-Konzentrationen für Flusssedimente schwanken danach zwischen 1 (Nel et al. 2018) und 7.48×10^{4} P (kg Sediment)$^{-1}$ (Wang et al. 2018). Zusätzlich wird ein Vergleich des MP-Vorkommens in der Wasser- und Sedimentphase auch dadurch erschwert, dass Studien meist jeweils nur eines der beiden Kompartimente untersucht haben. Bisher haben lediglich Leslie et al. (2017) sowie Rodrigues et al. (2018) das Vorkommen von MP parallel in der Wasser- und Sedimentphase von Flusssystemen betrachtet. Die MP-Konzentrationen in den beiden untersuchten Umweltkompartimenten waren jedoch auf Grund abweichender Konzentrationsangaben wiederum nicht vergleichbar. Rückschlüsse auf die Verteilung von MP in Flüssen sind daher bisher nur begrenzt möglich.

Aktuelle Herausforderungen bei der Analyse der MP-Häufigkeit in limnischen Ökosystemen ergeben sich auch aus den angewendeten Methoden für die Probenahme und -verarbeitung sowie die MP-Identifizierung. Als gängige Methodik zur Beprobung der Wasserphase in marinen und limnischen Ökosystemen werden Manta-Schleppnetze oder Neuston-Netze eingesetzt. Diese beiden Techniken ermöglichen die Beprobung großer, repräsentativer Wassermengen (Löder & Gerdts 2015), sind jedoch auf einen Partikelgrößenbereich > 100 µm (Nettoporengröße) beschränkt. Werden stattdessen vollständige Wasserproben genommen und untersucht, ist die Analyse meist auf geringe Volumina beschränkt, da der hohe Organikgehalt in den Wasserproben zu einem raschen Verschluss der Membranfilter beim Filtrieren der Proben führt (Leslie et al. 2017). Ein kleines analysiertes Wasservolumen begrenzt jedoch die Repräsentativität der Stichprobe. Hohe Organikanteile in Wasserproben sind daher eine zentrale Einschränkung für die Probenahme und -verarbeitung von Umweltproben für die MP-Analyse.

Ähnlich herausfordernd ist die Analyse von MP-Vorkommen in Sedimenten. Um MP-Partikel aus der Vielzahl an organischen und anorganischen Partikeln in Sedimentproben abzutrennen, wurden in den vergangenen Jahren verschiedene Strategien zur Extraktion und Probenaufreinigung getestet. Für die MP-Extraktion wenden fast alle Studien mindestens eine der folgenden Extraktionstechniken an:

Sieben zur Trennung von Partikeln in verschiedene Größenklassen (Rocha-Santos & Duarte 2015), Ölextraktion unter Verwendung der oleophilen Eigenschaften von MP (z. B. Rapsöl, Crichton et al. 2017) oder Dichteseparation. Die letztere Methode basiert auf der unterschiedlichen Dichte von Kunststoffpolymeren (0,8 (Silikon)–1,4 g cm^{-3} (PET, PVC)) und der übrigen Matrix der Umweltproben (z. B. Sedimente, 2,65 g cm^{-3}, Löder & Gerdts 2015). Durch Suspension der Umweltprobe in einer Trennflüssigkeit mit „mittlerer Dichte“ (z. B. Zinkchloridlösung (1,5–1,7 g cm^{-3}), gesättigte Natriumchloridlösung (1,2 g cm^{-3})) sammelt sich das MP auf Grund seines Auftriebs an der Oberfläche und kann entsprechend abgetrennt werden (Löder & Gerdts 2015). Neuere Studien haben darüber hinaus Extraktionsmethoden entwickelt, die auf einer elektrostatischen (Felsing et al. 2018) oder einer magnetischen Trennung basieren (letzteres durch die Verwendung hydrophober Eisennanopartikel, die an MP-Partikeln adsorbieren, Grbic et al. 2019). MP-Extraktionstechniken werden häufig mit weiteren Verfahren zur Probenaufreinigung kombiniert. Diese Reinigungsschritte umfassen die Inkubation der Proben mit Säuren, Basen, Oxidationsmitteln (z. B. Wasserstoffperoxid) oder Enzymen, um den organischen Gehalt in den Umweltproben zu reduzieren (z. B. Claessens et al. 2013, Cole et al. 2014, Courtene-Jones et al. 2017b). Sowohl die MP-Extraktion als auch die Probenaufreinigung sind zeitlich sehr aufwendig. Da derzeit keine schnelleren Alternativen verfügbar sind, sind Studien zum MP-Vorkommen in der Umwelt häufig in ihrer Stichprobengröße beschränkt.

In Bezug auf die Identifikation von MP in Umweltproben ist die manuelle Sortierung und visuelle Analyse von MP-Partikeln immer noch die am häufigsten eingesetzte Detektionstechnik. Trotz der Aufstellung wesentlicher Kriterien zur visuellen Identifikation von MP (Norén 2007) sind die Ergebnisse dieser Analysemethode stark von den Forschenden, der technischen Ausrüstung (z. B. Vergrößerung des Mikroskops) sowie der Hintergrundmatrix und der Menge des verbliebenen organischen Materials auf den Membranfiltern beeinflusst (Löder & Gerdts 2015). Dadurch kann es sowohl zur Über- (Löder & Gerdts 2015) als auch zur Unterschätzung (Nel et al. 2019) der tatsächlichen MP-Konzentrationen kommen. In den vergangenen Jahren haben fortschrittliche Analysemethoden (u.a. µ-Fourier-Transformations-Infrarotspektroskopie (µ-FTIR), Raman-Spektroskopie, thermische Extraktionsdesorptionsgaschromatographie-Massenspektrometrie (TED-GC-MS), Pyrolyse-GC-MS (Pyro-GC-MS) oder Rasterelektronenmikroskopie (REM), Elert et al. 2017, Löder & Gerdts 2015, Rocha-Santos & Duarte 2015) die Identifikation von MP und somit die Aussagekraft der Ergebnisse verbessert. Während TED-GC-MS und Pyro-GC-MS nur die Bestimmung der Gesamtmasse der verschiedenen Plastikpolymertypen in einer Probe ermöglichen, kann durch µ-FTIR bzw. Raman-Spektroskopie die Gesamtanzahl an MP-Partikeln sowie der Polymertyp separat für jeden Partikel ermittelt werden. Ähnlich wie bei der MP-Extraktion und Probenverarbeitung sind die vorgestellten Detektionstechniken jedoch zeit- und kostenaufwendig und begrenzen daher meist die untersuchbare Probenanzahl.

Hervorzuheben ist hier eine umfangreiche Studie von Heß et al. (2018), die die Abundanz von MP in der Wasserphase von Fließgewässern an 52 Untersuchungsstellen in Süd- und Westdeutschland untersucht haben. In der Studie wurde für die MP-Identifikation auf eine visuelle Analyse verzichtet und stattdessen eine umfassende Analyse mittels FT-IR durchgeführt. Solche Studien stellen bis heute jedoch eher eine Ausnahme dar. Derzeit ist daher nur schwer abzuschätzen, wie repräsentativ visuelle Analysedaten zur Häufigkeit von MP in aquatischen Ökosystemen tatsächlich sind und ob eine Vergleichbarkeit zu Ergebnissen mit fortgeschrittenen Analysetechniken besteht.

1.5 Exposition und Toxizität in aquatischen Organismen

Die globale Verteilung von MP in der Umwelt ermöglicht die Interaktion von MP mit einer Vielzahl von Organismen. Frühere Forschungsarbeiten konnten bereits die Aufnahme von MP durch Fische, Vögel, Reptilien, Säugetiere und verschiedene Gruppen von Wirbellosen (Invertebraten, z. B. Muscheln, Krebstiere, Anneliden, Nesseltiere sowie Stachelhäuter, GESAMP 2016, Lusher 2015, Taylor et al. 2016) nachweisen. Die meisten Studien haben sich dabei auf die Exposition von marinen Fisch- und Muschelarten beschränkt, während andere Tiergruppen nur wenig untersucht worden sind (Sá et al. 2018).

Für die MP-Interaktion mit Tieren sind drei verschiedene Expositionswege denkbar: dermale Exposition (Hautexposition), Inhalation/Kiemenexposition und Ingestion. Im Folgenden wird ein Konzept zur möglichen Beziehung zwischen den Expositionspfaden, der daraus resultierenden Interaktion zwischen MP und Organismus sowie der induzierten Toxizität vorgestellt (Abb. 1). Jeder der drei Expositionspfade ist mit mindestens zwei Interaktionsformen (physische Interaktion, chemische Interaktion, Reduktion des zur Verdauung zur Verfügung stehenden Darmvolumens) verbunden, die wiederum mit den weiterführenden nachgeschalteten möglichen Effekten verbunden sind.

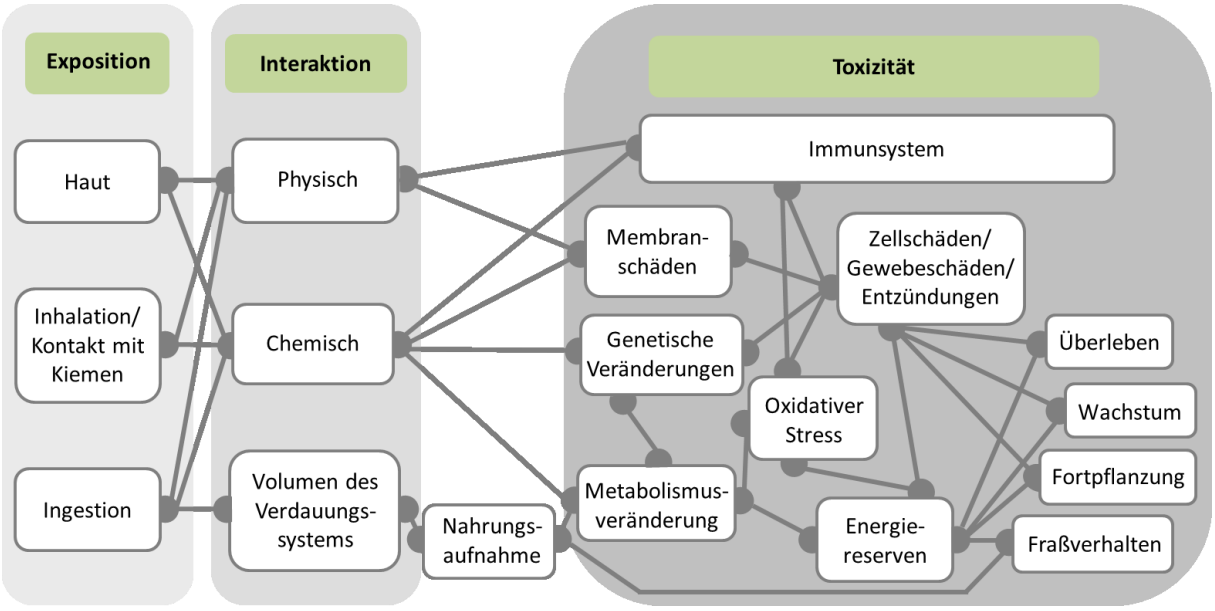

Abb. 1: Konzept zur Beziehung zwischen Hautexposition, Inhalation und Ingestion von Mikroplastik (MP), den damit verbundenen Möglichkeiten der Interaktion zwischen MP und dem Organismus sowie der mit der Exposition verbundenen möglichen Toxizität in aquatischen Organismen.

1.5.1 Exposition und Interaktion von aquatischen Organismen mit Mikroplastik

Physische Interaktion

Eine physische Interaktion zwischen MP und dem Körpergewebe von Biota kann über dermale Exposition oder durch Inhalation sowie Ingestion stattfinden. Die Partikel können dabei mit den Oberflächenepithelien (Haut, Kiemen-/Lungenepithel, Darmepithel) interagieren, möglicherweise in

diese eindringen bzw. diese durchdringen und ggf. sogar in angrenzende Membranen, Gewebe und Organe des Organismus übergehen. Dadurch können ggf. physische Schäden an den Zellen und Geweben, aber auch eine Aktivierung des Immunsystems eintreten, welche Entzündungsreaktionen hervorrufen können. Diese Veränderungen können möglicherweise zudem Folgereaktionen in den Organismen auslösen: So ist es denkbar, dass als Reaktion auf das Eindringen von MP der oxidative Stress in den Organismen ansteigt und (auf Grund einer gesteigerten Metabolismusrate) verstärkt Energiereserven verbraucht werden. Dies wiederum kann das Überleben, das Wachstum, das Fraßverhalten sowie die Reproduktion der jeweiligen Spezies negativ beeinflussen (Abb. 1).

Die physische Interaktion von MP mit Hautgeweben wurde bisher hauptsächlich in der Humantoxikologie untersucht. Studien an menschlicher Haut sowie an der Haut von Wirbeltier-Modellorganismen haben gezeigt, dass nur NP, nicht jedoch MP-Partikel, durch Phagozytose in Epithelzellen (z. B. Keratinozyten oder Langerhans-Zellen) oder durch Eindringen in Haarfollikel in die Hautepithelien eindringen können. Von den Haarfollikeln aus ist eine Übertragung in das Lymphsystem möglich. Im Gegensatz dazu werden von Keratinozyten aufgenommene NP-Partikel aufgrund der Keratinisierung der Zellen und der anschließenden Zellablösung wieder aus der Epidermis eliminiert, wodurch ein Übertritt in das Lymphsystem verhindert wird (Kato et al. 2003, Kohli & Alpar 2004, Latkovic & Nilsson 1979, Mahe et al. 2009, Triebskorn et al. 2019, Vogt et al. 2006, Wolff & Konrad 1971).

Im Gegensatz zur dermalen Exposition wurde bei Krebstieren, Muscheln und Fischen bereits eine physische Interaktion durch Inhalation oder Kiemenexposition nachgewiesen. So wurden die Kiemen der Gemeinen Strandkrabbe *Carcinus maenas* über einen Zeitraum von bis zu 21 Tagen (d) nach Ende einer Exposition mit PS-Sphären (8–10 µm) auf verbliebenes MP untersucht. Hierbei konnte gezeigt werden, dass auch 21 d nach Ende der Exposition noch PS-Sphären auf der Außenfläche der Kiemen nachweisbar waren. Die PS-Sphären drangen jedoch nicht in das Kiemengewebe ein (Watts et al. 2014). Ähnliche Beobachtungen wurden in Studien mit Muscheln gemacht, wobei in diesen teilweise auch ein Eindringen der MP-Partikel (< 100 µm) in das Kiemengewebe beobachtet werden konnte (Avio et al. 2015, Guilhermino et al. 2018, Moos et al. 2012). Moos et al. (2012) vermuteten, dass die Partikel möglicherweise durch Endozytose in die Zellen des Kiemengewebes aufgenommen worden sein könnten. In Fischen konnte bisher nur eine vorübergehende Adsorption von NP und MP auf der Kiemenoberfläche beobachtet werden (Ding et al. 2018a, Lu et al. 2016).
Bei einer MP-Exposition über die Kiemen muss jedoch berücksichtigt werden, dass die Kiemen von Fischen bzw. zahlreichen Wirbellosen von einer Schleimschicht überzogen ist, die die Kiemen schützt und teilweise für die Nahrungsaufnahme (z.B. bei Muscheln) relevant ist. Durch regelmäßige Erneuerung dieser Schleimschicht werden adsorbierte Partikel von der Kiemenoberfläche entfernt (Batel et al. 2018, Jørgensen 1955). Es muss daher davon ausgegangen werden, dass die Interaktion von MP mit dem Kiemengewebe aquatischer Organismen meist zeitlich begrenzt ist, außer die MP-Partikel dringen in das Kiemengewebe der Organismen ein.

In den meisten Tiergruppen ist die Aufnahme durch Ingestion vermutlich jedoch der häufigste Expositionsweg (GESAMP 2015, Ding et al. 2018a, Lu et al. 2016). Frühere Studien konnten bereits in zahlreichen limnischen und marinen Tierarten MP im Magendarmtrakt nachweisen, insbesondere in Krebstieren (z. B. Au et al. 2015, Cole et al. 2013, Farrell & Nelson 2013, Imhof et al. 2013, Watts et al. 2014), Muscheln (z. B. Browne et al. 2008, Guilhermino et al. 2018), Schnecken (e.g. Imhof et al. 2013), Fischen (z. B. Boerger et al. 2010, Chae et al. 2018, Ding et al. 2018a), Vögeln (z. B. Reynolds &

Ryan 2018, Terepocki et al. 2017), Insekten (e.g. Al-Jaibachi et al. 2019, Kim et al. 2018), Stachelhäutern (z. B. Graham & Thompson 2009) und Anneliden (z. B. Imhof et al. 2013, Jang et al. 2018).

Die aufgenommenen MP-Partikel verbleiben jedoch nicht nur im Verdauungssystem, sondern können durch aktive Phagozytose oder durch parazelluläre Penetration (Persorption, Wright & Kelly 2017) in das Darmepithel eindringen. Entsprechende Partikelverlagerungen konnten in Muscheln bereits für PE- und PS-MP (< 100 µm) nachgewiesen werden (Avio et al. 2015, Pittura et al. 2018). Darüber hinaus konnte gezeigt werden, dass MP-Partikel ≤ 10 µm zudem auch in die Hämolymphe der Muscheln eindringen können (Avio et al. 2015, Browne et al. 2008, Guilhermino et al. 2018, Magni et al. 2018). Auch in Fischen konnte bereits nachgewiesen werden, dass 5 und 20 µm PS-Sphären das Darmepithel durchdringen und in das Fischgewebe eintreten (Lu et al. 2016, Qiao et al. 2019).

2019 stellten Triebskorn et al. fest, dass 22 von 31 verfügbaren Forschungsstudien eine Translokation von MP und NP-Partikeln in das Gewebe von aquatischen Organismen beobachtet hatten. Während die Phagozytose oder Persorption von Partikeln in Nanogröße möglich erscheint, betonen die Autoren, dass insbesondere die Translokation größerer MP-Partikel in Mikrogröße jedoch fraglich ist. Viele Organismen besitzen Anpassungen (z. B. Darmmembranen oder spezifische Filtersysteme, Triebskorn et al. 2019, Weber et al. 2018) die im Regelfall die Translokation von Partikeln (zumindest mit einer Größe im Mikrobereich) in darmassoziierte Organe verhindern. Ferner kann der Eindruck einer Translokation von Partikelgewebe auch leicht irrtümlich durch eine Kontamination der Probe, durch histologische Artefakte oder durch Auslaugen des fluoreszierenden Farbstoffs aus den NP- und MP-Partikeln entstehen (Schür et al. 2019, Triebskorn et al. 2019). Daher sollten Daten aus Experimenten zur Partikeltranslokation sorgfältig geprüft und ihre methodischen Einschränkungen berücksichtigt werden.

Zusammenfassend ist die physische Interaktion von MP mit Biota durch dermale Exposition, Inhalation sowie Ingestion relevant. Das Ausmaß der Exposition hängt jedoch von verschiedenen Faktoren ab. So können spezies- und individuenspezifische Merkmale (z. B. Morphologie, Verhalten, Größe), MP-Partikeleigenschaften (z. B. Größe, Form, Polymertyp) sowie die Expositionsdauer die Interaktion zwischen MP-Partikeln und Organismen maßgeblich beeinflussen. Die Relevanz dieser Faktoren für die Interaktion von MP mit den verschiedenen Gruppen an Wasserorganismen ist derzeit jedoch nur wenig verstanden. Zwar wurden bereits Studien zum Einfluss der benannten Faktoren auf die MP-Interaktion mit verschiedenen Organismen publiziert, jedoch wurden diesen meist stark voneinander abweichende Studiendesigns zu Grunde gelegt, und es wurden nur einzelne Einflussfaktoren auf die MP-Exposition untersucht. Entsprechend ist die Vergleichbarkeit der Ergebnisse bzw. eine Ableitung möglicher Schlussfolgerungen zur Relevanz der verschiedenen Expositionsfaktoren aktuell stark eingeschränkt.

Chemische Interaktion

Die chemische Interaktion von MP mit Körpergeweben ist der zweite relevante Mechanismus, durch den MP-induzierte Toxizität in aquatischen Organismen hervorgerufen werden kann. Die chemische Zusammensetzung von MP umfasst das Kunststoffpolymer selbst, aber auch die in die Polymermatrix integrierten Chemikalien sowie die adsorbierten Substanzen auf der MP-Oberfläche. Bei einem

physischen Kontakt zwischen MP und Körpergewebe können Chemikalien direkt vom Partikel in das Körpergewebe des Wasserorganismus übertragen werden. Ferner können Chemikalien auch in das umgebende Wasser oder in die Sekrete/Schleimschicht auf/in den Atem- bzw. Verdauungsorganen (z. B. Magensekret) übergehen und aus diesen über Haut-, Kiemen- oder Darmepithelien in das Körpergewebe aufgenommen werden (Hartmann et al. 2017, Koelmans et al. 2014).

Eine der möglichen, aus der Polymermatrix austretenden Substanzen sind Kunststoffmonomere. Diese werden üblicherweise während der Kunststoffherstellung durch Polymerisation zu langen Polymerketten verbunden. Jedoch läuft der Polymerisationsprozess nicht bei allen Kunststoffarten immer vollständig ab: Während in PE- und PP-Produkten nur äußerst selten Monomere (Ethylen, Propen) verbleiben, kann PS bis zu 0,6 % (Gew./Gew.) Styrolmonomere und -oligomere enthalten (Andrady 2017, Garrigós et al. 2004). Somit hat PS ein erhöhtes Potential zum Auslaugen von Monomeren. Bisher sind noch keine Publikationen verfügbar, die ein direktes Auslaugen von Styrenen in das Gewebe von Wasserorganismus nachgewiesen hätten. Daten aus der Humantoxikologie zeigen jedoch, dass erhöhte Styrolspiegel in menschlichen Fettgeweben nach regelmäßiger Nutzung von Plastikprodukten auftreten (Pierce & Tozer 1992). Dies legt nahe, dass das Auslaugen von PS-Monomeren ein relevanter Expositionsweg auch in aquatischen Ökosystemen sein könnte.

Neben verbliebenen Kunststoffmonomeren sind Additive eine zweite Quelle für mögliche Substanzen, die aus MP-Partikel austreten können. Plastikprodukte werden durch Additive modifiziert, um ihre Eigenschaften zu optimieren und ihre Leistungsfähigkeit entsprechend zu verbessern (Hartmann et al. 2019). Additive können gemäß ihres Verwendungszwecks kategorisiert werden, z. B. als funktionelle Additive (Licht- und Wärmestabilisatoren, Säurefänger, Schmiermittel, Antioxidationsmittel, Vernetzungsmittel, Haftvermittler, Antistatika, Flammschutzmittel, Weichmacher), als Farben (Pigmente), als Füllstoffe (z. B. Ton) und als Strukturstabilisierer (z. B. Glas und Kohlenstofffasern, Bolgar et al. 2016, Hahladakis et al. 2018). Zu den häufig in Kunststoffen nachweisbaren Additiven gehören Phthalate, UV-Stabilisatoren (u.a. Benzophenone sowie Benzotriazole und deren Derivate), bromierte Flammschutzmittel, Antioxidationsmittel (Phenolderivate und Organophosphorverbindungen), Bisphenol A (BPA) und Nonylphenole (Rani et al. 2015). BPA stellt dabei eine Besonderheit dar, da es in PP, PE und PVC als Additiv eingesetzt wird, während es in Polycarbonat (PC) und Epoxidharzen zugleich das Monomer bildet (Crain et al. 2007). Für einige der benannten Additive wurde bereits nachgewiesen, dass diese eine Gefährdung für Mensch und Natur darstellen (Hermabessiere et al. 2017). Zudem muss berücksichtigt werden, dass der relative Anteil und die Additivzusammensetzung zwischen den verschiedenen Polymertypen erheblich variieren kann. Beispielsweise enthalten PVC-Produkte meist einen höheren Additivgehalt als Polyolefine (PE, PP) oder Polystyrole (Murphy 2001).

Da Additive üblicherweise Substanzen mit niedrigem Molekulargewicht sind und eine begrenzte Bindung an die Polymermatrix besitzen, können sie leicht aus der Polymermatrix entweichen und in Körpergewebe übergehen (Lithner et al. 2011). Das Auslaugen der Additive wird im Fall der Verwitterung/Alterung der MP-Partikel und der damit verbundenen Destabilisierung der Polymermatrix und der Zunahme der Partikeloberfläche noch weiter verstärkt (Bandow et al. 2017, Luo et al. 2020). Frühere Laborstudien haben das Auslaugen von Additiven aus MP in das Darmgewebe von Wasserorganismen bestätigt. So haben Rochman et al. (2013b) den Transfer von polyzyklischen aromatischen Kohlenwasserstoffen (PAK) von LD-PE-Partikeln in das Gewebe von Japanischen Reisfischen (*Oryzias latipes*) nachgewiesen. In Vögeln konnten Tanaka et al. (2015)

außerdem zeigen, dass die Zusammensetzung der Verdauungsflüssigkeiten (Magenöl) das Auslaugen von Additiven nach MP-Ingestion sogar noch beschleunigen kann.

Zudem können Kunststoffe auch unbeabsichtigt hinzugefügte Chemikalien (non-intentionally added substances, NIAS) enthalten. NIAS können Degradationsprodukte der Kunststoffpolymere bzw. -additive, Verunreinigungen und Kontaminationen im Kunststoff sowie Reaktionsnebenprodukte sein (Nerin et al. 2013). Ebenso wie Additive, die dem Kunststoff absichtlich zugeführt worden sind, können auch NIAS aus Kunststoffen auslaugen (He et al. 2021) und möglicherweise in aquatische Organismen übergehen.

Neben dem Auslaugen von Monomeren, Additiven bzw. NIAS kann MP auch ein Vektor für Umweltschadstoffe sein. So können Chemikalien wie Spurenmetalle (Turner & Holmes 2015), Chlorkohlenwasserstoffe (z. B. polychlorierte Biphenyle (PCB, Velzeboer et al. 2014)), Dichlordiphenyltrichlorethan (DDT, Bakir et al. 2014), per- und polyfluorierte Alkylverbindungen (PFAS, Llorca et al. 2018), PAK (z. B. Phenanthren, Bakir et al. 2014) und Antibiotika (Li et al. 2018b) auf der Oberfläche von MP adsorbieren. Treten Organismen nun in Interaktion mit MP-Partikeln, so können diese adsorbierten Umweltschadstoffe in die Körpergewebe der Organismen übergehen. Eine entsprechende Übertragung wurde in Laborexperimenten mit Krebstieren, Fischen, Vögeln und Würmern bereits nachgewiesen (Lohmann 2017). Die Zusammensetzung der „Schadstoffcorona“ auf den MP-Partikeln hängt jedoch stark von den Eigenschaften der Umweltschadstoffe, deren Konzentration in der Umwelt sowie vom umgebenden Milieu ab (Mato et al. 2001, Rochman et al. 2013a). Unter unterschiedlichen Umweltbedingungen können Art und Menge der Schadstoffe, die an MP adsorbieren, daher stark variieren. Dies erschwert allgemeine Schlussfolgerungen zur Schadstoffadsorption an Umwelt-MP und zur anschließenden Translokation der Schadstoffe in Wasserorganismen.

Interaktion mit dem Volumen des Verdauungssystems

Neben der physischen und chemischen Interaktion kann MP den Organismus auch indirekt durch Ausfüllen des Magendarmraums beeinflussen. Die Aufnahme großer Mengen an MP-Partikeln verringert das verbleibende Verdauungsvolumen für die Nahrungsaufnahme. Choi et al. (2018) beobachteten bei Fischlarven (*Cyprinodon variegatus*), dass sich durch die Aufnahme von sphärischem sowie fragmentiertem MP eine Überdehnung des Darms einstellt. Ferner konnte für die Gemeine Strandkrabbe (*C. maenas*) bereits gezeigt werden, dass die chronische Exposition gegenüber MP-Fasern zu einer Verringerung der Nahrungsaufnahme führt (Watts et al. 2015).

1.5.2 Toxizität von Mikro- und Nanoplastik

Aufgrund der Vielzahl von Expositions- und Interaktionswegen kann eine MP-induzierte Toxizität in zahlreichen Erscheinungsformen sowie unterschiedlichen Intensitäten auftreten. Es ergibt sich dadurch ein komplexes Zusammenspiel von Effekten, deren Wirkung sich in verschiedenen Zielorganen (Haut, Kiemen, Verdauungssystem) sowie auf verschiedenen Organisationsebenen (Molekül, Zelle, Gewebe, Individuum, Abb. 1) entfaltet. Entsprechend groß ist die Vielfalt möglicher Effekte, die durch eine MP-Exposition hervorgerufen werden können. Am Beispiel von Muscheln konnte gezeigt werden, dass u.a. Zell- und Gewebeschäden (Bråte et al. 2018),

Entzündungsreaktionen (Paul-Pont et al. 2016), oxidative Stressreaktionen (Magara et al. 2018, O'Donovan et al. 2018), eine Veränderung der Energiereserven (Gardon et al. 2018), Neurotoxizität (Magni et al. 2018), Einflüsse auf Wachstum und Entwicklung (Balbi et al. 2017, González-Soto et al. 2019), eine veränderte Nahrungsaufnahme (Oliveira et al. 2018) sowie eine veränderte Fortpflanzung (Gardon et al. 2018) mögliche Auswirkungen einer MP-Exposition sein können.

Toxizität durch physische Interaktion

Durch die physische Interaktion von MP mit Organismen kann eine Schädigung von Zellmembranen, Zellen, interzellulären Verbindungen oder Geweben auftreten. Zusätzlich kann es zu einer Aktivierung des Immunsystems kommen, sowohl durch eine Anwesenheit der Partikel im Magendarmraum (insbesondere in Wirbeltieren kann das Immunsystem Fremdpartikel im Magendarmraum detektieren und ggf. eine Immunantwort auslösen, Hart et al. 1988, Ratcliffe 1985) als auch durch ein Eindringen in assoziierte Gewebe oder das Blut- und Lymph- bzw. Hämolymphsystem. In der Folge ist es möglich, dass durch die Schädigung der Zellen oxidativer Stress und entzündliche Prozesse hervorgerufen werden, welche bei starker Ausprägung wiederum negative Auswirkungen auf die Energiereserven, das Fraßverhalten, das Wachstum, die Fortpflanzung sowie das Überleben der MP-exponierten Individuen haben könnten (Abb. 1).

In Bezug auf die dermale Exposition liegen derzeit keine experimentellen Toxizitätsdaten für aquatische Organismen vor. Daten zur dermalen Toxizität von MP im Menschen legen stattdessen nahe, dass eine Exposition mit 50 nm PS-Sphären keine erhöhte Phototoxizität, Hautreizung oder Hautsensibilisierung induziert (Park et al. 2011).

Im Gegensatz dazu ist die Toxizität von MP nach Inhalation bzw. Kiemenexposition bereits in einigen Studien mit aquatischen Organismen untersucht worden, mit zum Teil sehr unterschiedlichen Ergebnissen. Watts et al. (2016) beobachteten einen reduzierten Sauerstoffverbrauch und eine Veränderung des zellulären Ionenaustauschs in der Gemeinen Strandkrabbe (*C. maenas*) nach einer Exposition gegenüber 8 µm COOH- und NH_2-PS-Sphären. In Fischlarven verursachte eine Exposition gegenüber 1–5 µm MP-Sphären Lipidperoxidation in den Kiemen sowie eine Aktivierung des antioxidativen Abwehrsystems, um den oxidativen Stresseffekten entgegenzuwirken (Barboza et al. 2018). Im Gegensatz dazu induzierte die Exposition der limnischen Fischart *Danio rerio* gegenüber MP-Fragmenten (Größe: etwa 70 µm) aus verschiedenen Polymertypen sowie 0,1, 1 und 5 µm PS-NP- und MP-Sphären keine histologischen Veränderungen in den Kiemen (Lei et al. 2018b).

Auch in Bezug auf die Toxizität von MP-Partikeln nach deren Ingestion variieren die bisher publizierten Ergebnisse stark. Im Großen Wasserfloh (*D. magna*) verursachten 51 nm PS-Sphären eine Verformung bzw. ein Ausreißen von Mikrovilli im Verdauungstrakt (Chae et al. 2018). In Zebrabärblingen (*D. rerio*) bewirkte die Aufnahme von 0,5 bis 50 µm PS-Sphären eine Veränderung des Darmschleimvolumens sowie oxidativen Stress und entzündliche Prozesse (Jin et al. 2018, Qiao et al. 2019). Im Gegensatz dazu führte eine chronische Exposition der Fischart *Sparus aurata* gegenüber MP (23–112 µm) zu keinen Stressreaktionen oder pathologischen Veränderungen. Ebenso konnten Bussolaro et al. (2019) keine zytotoxischen oder genotoxischen In-vitro-Effekte von 220 nm PS-Sphären auf die Darmepithelzellen der Regenbogenforelle *Oncorhynchus mykiss* beobachten.

MP-induzierte Effekte auf das Immunsystem wurden bereits für marine Muscheln (*Mytilus* spp.) nachgewiesen. In diesen bewirkte die Exposition mit PE- und PS-Fragmenten (< 100 µm) eine Veränderung der relativen Zusammensetzung der Immunzelltypen im Körper, eine Verringerung der Stabilität der lysosomalen Membran innerhalb der Immunzellen (Hämozyten) sowie DNA-Veränderungen (Avio et al. 2015). Zudem beobachteten Paul-Pont et al. (2016) eine erhöhte phagozytische Aktivität von *Mytilus*-Hämozyten nach einer chronischen Exposition der Muscheln gegenüber 2,6 µm PS-Sphären. Dies deutet auf eine MP-induzierte Aktivierung des Immunabwehrsystems hin.

Die Auswirkungen der physischen Interaktion von MP mit Körpergeweben sind vermutlich stark mit den physikalischen Partikeleigenschaften (z. B. Partikelform und -größe, Polymertyp, Oberflächenbeschaffenheit) verknüpft. So wird vermutet, dass MP-Partikel mit einer fragmentierten Oberfläche im Vergleich zu kugelförmigen Partikeln verstärkt Effekte in Organismen hervorrufen können, da ihre vergrößerte und rauere Oberfläche die physische Interaktion mit Zellen und Membranen verstärkt. So zeigten Lei et al. (2018b), dass eine Exposition mit Polyamid (PA)-, PE-, PP- und PVC-Fragmenten (etwa 70 µm) intensive Darmschäden (Zottenrissbildung, Spaltung von Enterozyten) in Zebrabärblingen (*D. rerio*) auslöste, während eine Exposition gegenüber PS-Sphären (0,1–5,0 µm) keine morphologischen Veränderungen verursachte. Welchen Einfluss die Partikelform auf die Toxizität genommen hat, lässt sich in dieser Studie jedoch nicht abschließend benennen, da sich die zwei untersuchten Partikeltypen sowohl in ihrer Form als auch in ihrem Polymertyp und in ihrer Größe unterschieden haben. In einer anderen Studie konnten Choi et al. (2018) beobachten, dass abweichende Partikelformen das Schwimmverhalten von Fischen beeinflussen können. So verstärkten PE-Fragmente (6–350 µm) das Schwimmverhalten der Fischart *Cyprinodon variegatus* im Vergleich zu einer Exposition gegenüber PE-Sphären (150–180 µm).

Neben der Partikelform wird auch die Größe der MP-Partikel als determinierender Faktor für die Toxizität diskutiert. Die Größe der Partikel, mit denen die Organismen in Kontakt treten, ist zum einen von der Partikelgrößenverteilung von MP in der Umwelt, zum anderen aber auch von den morphologischen Eigenschaften des Mund- und Verdauungsapparats der aquatischen Organismen abhängig (Burns 1968). Bisherige Laborstudien haben jedoch gezeigt, dass insbesondere Partikel mit einer Größe im Submikron- bzw. im Nanobereich mit toxischen Effekten in Verbindung gebracht werden (Lambert et al. 2017). So beobachteten Lee et al. (2013) bei einer Exposition des Ruderfußkrebses *Tigriopus japonicus* gegenüber 50 nm, 500 nm bzw. 6 µm PS-Sphären, dass die Mortalität der Testorganismen mit sinkender Partikelgröße zunahm. Vergleichbare Beobachtungen wurden für das Rädertierchen *Brachionus koreanus* im Hinblick auf subletale Endpunkte gemacht (Jeong et al. 2016). Im Gegensatz dazu verursachten bei einer Exposition des Fadenwurms *Caenorhabditis elegans* gegenüber 0,1, 0,5, 1, 2 und 5 µm PS-Sphären die 1 µm großen Sphären die stärksten Effekte im Hinblick auf Mortalität, Wachstumshemmung, Verhaltensänderungen, Neurotoxizität und Schäden durch oxidativen Stress (Lei et al. 2018a).
Bei diesen Studien muss jedoch jeweils berücksichtigt werden, dass in den Expositionen von jeder Partikelsorte die gleiche Partikelmasse eingesetzt worden ist. Auf Grund der unterschiedlichen Partikelgröße entsprechen die gleichen Massen jedoch nicht vergleichbaren Partikelkonzentrationen. Expositionen mit NP-Partikeln enthielten somit mehr Partikel als solche mit MP-Partikeln. Es bleibt also unklar, ob die beobachteten Effekte tatsächlich auf die Partikelgröße oder doch eher auf die unterschiedlichen Partikelexpositionskonzentrationen zurückzuführen sind.

Wenig Beachtung fand in der bisherigen MP-Forschung der Umstand, dass aquatische Organismen in der Umwelt nicht nur gegenüber MP, sondern auch gegenüber einer Vielzahl anderer natürlicher Partikel exponiert sind. Für natürliche Partikel konnte bereits gezeigt werden, dass diese die Mortalität, die Fraßaktivität, die Fortpflanzung und das Populationswachstum von Wasserorganismen beeinflussen können (Scherer et al. 2017). Trotz der Ähnlichkeiten zwischen MP und natürlichen Partikeln haben bisher jedoch nur sehr wenige Studien die Toxizität dieser beiden Partikeltypen direkt miteinander verglichen. Ogonowski et al. (2016) berichteten, dass eine Exposition mit PE-Fragmenten (Durchmesser (Ø): 2,6 µm) die Mortalität und Reproduktion des Großen Wasserflohs *Daphnia magna* erhöhen, während Kaolin (ein Äquivalent für natürliche Partikel, Ø: 4,4 µm) und MP-Sphären (1–5 µm) keine derartigen Effekte auslösten. Diese Studie legt nahe, dass sich Toxizitätsdaten für natürliche Partikel möglicherweise nicht direkt übertragen lassen. Nichtsdestotrotz ist zukünftig ein tiefergehendes Verständnis der Wirkungsprozesse von natürlichen Partikeln sowie ein direkter Vergleich zwischen den Effekten von MP und natürlichen Partikeln wesentlich, um langfristig zu verstehen, ob die durch natürliche Partikel und MP ausgelösten Prozesse untereinander vergleichbar oder voneinander abweichend sind.

Wie in der vorangegangenen Diskussion dargestellt, konnten bereits eine Reihe an Experimentalstudien toxische Effekte auf Grund einer MP-Exposition nachweisen. In ihrer Gesamtheit sind die Daten zur Toxizität von MP in aquatischen Organismen jedoch keinesfalls konsistent. Einige Studien weisen signifikante MP-Effekte nach, während in vergleichbaren Studien keine Auswirkungen erkennbar waren (Weber et al. 2021a). So berichteten beispielsweise Xu et al. (2017) über eine deutliche Verringerung der Nahrungsaufnahme für die Muschelart *Atactodea striata* nach einer Exposition gegenüber PS-Fragmenten, wohingegen Rochman et al. (2017) während einer Exposition der limnischen Muschelart *Corbicula fluminea* gegenüber ähnlich großen PS-Fragmenten keine entsprechenden Auswirkungen beobachten konnten. Es ist denkbar, dass diese komplexen Effektmuster eine Folge von Unterschieden in Bezug auf die verwendeten Expositionsszenarien (z. B. Expositionszeit, MP-Konzentration), der MP-Partikeleigenschaften (z. B. Form, Größe, Polymertyp) sowie der Empfindlichkeit der getesteten Arten bzw. Endpunkte sind. Dementsprechend wäre es voreilig, auf Basis der darstellten Ergebnisse bereits eine abschließende Schlussfolgerung zur Toxizität von MP zu treffen. Zusätzlich wird eine Bewertung des Risikos von MP für aquatische Organismen dadurch erschwert, dass viele der bisher publizierten MP-Laborstudien Expositionskonzentrationen verwendet haben, die die derzeitigen MP-Konzentration in der Umwelt um ein Vielfaches überschreiten. Eine Extrapolation der in den einzelnen Studien beobachteten Effekte auf die realen MP-Konzentrationen ist somit nur begrenzt möglich.

Zusammengefasst besteht somit Bedarf für eine bessere Vergleichbarkeit zwischen den Ergebnissen bereits publizierter MP-Toxizitätsstudien sowie eine Anpassung der Expositionskonzentrationen an aktuelle bzw. zukünftig mögliche Umweltkonzentrationen. Zudem sollten in weiterführenden Studien stets die Effekte durch MP mit solchen durch natürliche Partikel verglichen werden, um eine bessere Abschätzung der Umweltrelevanz der möglichen, beobachteten MP-Effekte zu erhalten.

Toxizität durch chemische Interaktion

Toxizität durch MP-Partikel kann nicht nur durch physische, sondern auch durch chemische Interaktion, insbesondere durch chemische Komponenten, die in die Polymermatrix eingebettet (Monomere, Additive, NIAS) oder an dieser adsorbiert sind (Umweltschadstoffe, siehe Kapitel 1.5.1),

ausgelöst werden. Die chemische Toxizität von MP hängt stark von der beteiligten chemischen Substanz sowie ihrer Kinetik und Dynamik im exponierten Individuum ab. Ebenso können die Chemikalien auf vielfältigen Ebenen ihre Wirkung entfalten, beispielsweise durch Eingriff in metabolische Zellprozesse, durch Veränderung des Erbguts, durch Schädigung der Zellmembran oder durch Modulation des Immunsystems. Ähnlich wie im Fall der physischen Interaktion können diese Veränderungen möglicherweise weitere nachgeschaltete Auswirkungen auf die Integrität von Zellen, Geweben und Organen, auf oxidative Stresslevel und Energiereserven sowie auf das Überleben, das Wachstum, das Fraßverhalten und die Fortpflanzung des Organismus hervorrufen (Abb. 1).

Frühere Studien konnten die Toxizität von Chemikalien in Kunststoffen für aquatische Organismen bereits nachweisen. So beeinflussten diese die Mortalität und Reproduktion von Muscheln und Krebstieren, wobei ausgelaugte Chemikalien aus plastifiziertem PVC und PUR besonders toxisch für die Testorganismen waren (Bejgarn et al. 2015, Gandara e Silva et al. 2016, Li et al. 2016a, Lithner et al. 2009, 2012). Diese Studien untersuchten jedoch lediglich die Migrate von Kunststoffen (also solche Chemikalien, die durch Inkubation in Wasser aus dem Kunststoffpolymer in die Wasserphase übertreten). Es bleibt daher unklar, ob vergleichbare Effekte eintreten, wenn Muscheln und Krebse die Partikel in ihren Verdauungstrakt aufnehmen und die Chemikalien durch den Gewebekontakt direkt in das Körpergewebe übergehen können. Dieser Aufnahmewege könnte insbesondere bei schlecht wasserlöslichen Substanzen relevant sein, da diese in Migraten kaum enthalten sind.

Zur Aufklärung dieser Fragestellung verglichen Zimmermann et al. (2020) anhand des Großen Wasserflohs *D. magna* die Reproduktionstoxizität von MP-Partikeln (PVC, Polymilchsäure (PLA), PUR) nach deren Ingestion mit der Toxizität von Extrakten (Extraktion mit Methanol) bzw. Migraten (Extraktion mit Wasser) dieser MP-Partikel. Zusätzlich überprüften die Autoren auch die Toxizität der Partikel, aus denen die extrahierbaren Kunststoffchemikalien entfernt wurden, um zwischen partikulärer Toxizität und Chemikalientoxizität unterscheiden zu können. Die Ergebnisse der Studie zeigten, dass im Fall der PVC-Partikel zwar die Extrakte, nicht jedoch die Migrate oder die unbehandelten/extrahierten MP-Partikel eine Veränderung der Reproduktion auslösten. Bei der Exposition mit PUR- und PLA-Partikel trat ebenfalls Toxizität auf, welche jedoch eher auf die partikulären MP-Eigenschaften und nicht auf die in den Kunststoffen enthaltenen Chemikalien zurückzuführen war. Unter Umweltbedingungen ist eine Exposition gegenüber Kunststoffchemikalien (in Form von Migraten bzw. solchen, die in MP-Partikeln enthalten sind) daher möglicherweise weniger relevant als die partikulären Effekte selbst.

Eine große Herausforderung bei einer umfassenden Abschätzung des Beitrags von Plastikchemikalien zur MP-Toxizität ist jedoch, dass Kunststoffpolymere eine Mischung aus zahlreichen verschiedenen Chemikalien enthalten können, deren Zusammensetzung nicht veröffentlicht oder identifizierbar ist (Zimmermann et al. 2019). Aus früheren Toxizitätsstudien ist bereits bekannt, dass einige der häufig in Kunststoffen nachweisbaren Chemikalien (z. B. bromierte Flammschutzmittel, Phthalate, Nonylphenol, BPA) ein endokrines Wirkpotential aufweisen und daher hormonähnliche Wirkungen auslösen können (Hermabessiere et al. 2017). Die Bewertung der Toxizität von Chemikalienmischungen ist hingegen deutlich komplexer. Eine abschließende Klärung, welchen Beitrag Chemikalien in Kunststoffen an der MP-Toxizität in aquatischen Organismen haben, wenn diese mit den MP-Partikeln in direkte Interaktion treten, ist daher bisher nur begrenzt möglich.

Von ähnlicher Komplexität ist die Bewertung der Toxizität von Umweltschadstoffen, die auf der Oberfläche von MP adsorbieren und durch Interaktion der Organismen mit MP in deren Gewebe

übertreten können (Vektoreffekt). Wie in Kapitel 1.5.1 erläutert, hängt die Adsorption von Umweltschadstoffen auf MP-Partikeln sowohl von deren Umweltkonzentration als auch von deren chemischen Eigenschaften ab. Auf Grund der hohen Anzahl an Schadstoffen in der Umwelt sowie ihren vielfältigen Eigenschaften ist eine allgemeine Abschätzung der Toxizität, die durch den Vektoreffekt verursacht wird, schwierig. Jüngste Studien haben jedoch diskutiert, dass die Bedeutung von MP als Vektor für Umweltkontaminanten unter Umweltbedingungen möglicherweise vernachlässigt werden kann, da MP-Konzentrationen in der Umwelt derzeit noch vergleichsweise niedrig sind. Andere natürliche Expositionswege für Umweltschadstoffe, beispielsweise über die Nahrung, über natürliche Partikel/Schwebstoffe oder über das Wasser, erscheinen für aquatische Organismen somit wesentlich relevanter (Bakir et al. 2016, Beckingham & Ghosh 2017, Hartmann et al. 2017, Koelmans et al. 2016, Lohmann 2017).

Zusammengefasst leisten chemische Substanzen somit vermutlich einen Beitrag zur Gesamttoxizität von MP – ihr Beitrag zur Toxizität ist im Vergleich zu den Auswirkungen durch die physische Interaktion von MP mit Organismen (partikuläre Toxizität) jedoch möglicherweise geringer.

Toxizität durch Verkleinerung des Verdauungsvolumens

Als dritte Form der Interaktion zwischen MP und Organismus wird angenommen, dass eine Aufnahme von MP das Magendarmvolumen und somit auch eine mögliche Nahrungsaufnahme verringert. Wenn das reduzierte Verdauungsvolumen nicht durch einen höheren Nahrungsdurchsatz oder durch eine verbesserte Verwertung der aufgenommenen Nahrung kompensiert wird, könnte die verringerte Energieaufnahme zu Stoffwechselveränderungen auf Zell- und Gewebeebene führen, die wiederum möglicherweise oxidativen Stress bzw. einen Verbrauch der Energiereserven auslösen (Abb. 1). Erste Daten aus einer Studie mit der Gemeinen Strandkrabbe (*C. maenas*) stützen dieses Konzept: Durch eine chronische Exposition gegenüber MP-Fasern reduzierten sich die Nahrungsaufnahme sowie die Energiereserven von *C. maenas* (Watts et al. 2015).

1.6 Mikroplastik in der Umwelt mit multiplen Stressoren

Eine Gesamtabschätzung der Toxizität von MP in der Umwelt ist somit nur möglich, wenn der Beitrag der partikulären sowie chemischen Toxizität von MP sowie die Auswirkungen durch die Reduktion des Verdauungsvolumens nach MP-Ingestion genauer abschätzen werden kann. Aber selbst bei genauer Kenntnis der Interaktion und Toxizität von MP als Einzelstressor bleibt eine Bewertung der möglichen Auswirkungen in der Umwelt komplex, da Laborexperimente nur selten berücksichtigen, dass außer MP noch eine Vielzahl anderer Stressoren auf aquatische Organismen in ihrer natürlichen Umwelt einwirken. Kramm et al. (2018) argumentieren, dass dieser „reduktionistische“ Ansatz nicht ausreicht, um die tatsächlichen Umweltauswirkungen von MP zu analysieren. Die Testung von MP als Einzelstressor muss daher mit Studien zu multiplen Stressor-Effekten kombiniert werden, um eine realistischere Abschätzung der Toxizität in der Umwelt zu erhalten.

1.7 Beitrag dieser Dissertation zum aktuellen Stand der Forschung

In den vergangenen Jahren wurden große Fortschritte bei der Aufklärung des Vorkommens, der Verteilung und der Toxizität von MP in der Umwelt erzielt. Trotz des starken Anstiegs der Veröffentlichungen zu MP in der Umwelt in den letzten 10–15 Jahren (Barboza & Gimenez 2015) liegt

der Schwerpunkt der Forschung nach wie vor auf den marinen Ökosystemen. Limnische Ökosysteme wurden im Gegensatz dazu bisher weit weniger untersucht. Dieser Trend wurde insbesondere zu Beginn dieser Arbeit (2016) beobachtet (Wagner et al. 2014), trifft jedoch auch bis heute noch weiterhin zu (PubMed-Suche, Stand: März 2020, Suchbefehle: „microplastic*“: 2156 Publikationen, „microplastic* marine“: 1203, „microplastic* freshwater“: 433).

Wesentliches Ziel dieser Dissertation war es daher, das bestehende Wissen zu MP in limnischen Ökosystemen, insbesondere in Bezug auf dessen Verteilung und Häufigkeit sowie auf die Aufnahme und Toxizität in limnischen Organismen, zu erweitern und zu vertiefen. Die Forschungsarbeiten wurden durch das Kooperationsprojekt „Vorkommen und biologische Wirkungen von Mikroplastik in großen Flüssen“ (gefördert durch das Bundesministerium für Verkehr und digitale Infrastruktur) der Abteilung Aquatische Ökotoxikologie der Goethe-Universität Frankfurt und der Bundesanstalt für Gewässerkunde (BfG) ermöglicht.

Das Kooperationsprojekt hatte zum Ziel, die Häufigkeit und die biologischen Auswirkungen von MP in großen deutschen Flüssen zu analysieren und dadurch den benötigten wissenschaftlichen Hintergrund zu erarbeiten, der für die Bewertung, Überwachung und Regulierung von MP in Gewässern langfristig erforderlich ist. Das Projekt umfasste insgesamt vier Forschungsbereiche, in denen die Forschenden des Projekts als interdisziplinäres Team zusammenarbeiteten:

Forschungsbereich 1: Häufigkeit von MP in Sedimenten und Schwebestoffen großer Flüsse
Forschungsbereich 2: Aufnahme von MP durch limnische Organismen
Forschungsbereich 3: Biologische Wirkungen von MP auf limnische Organismen
Forschungsbereich 4: MP-Risikobewertung

Diese Dissertation setzt sich aus insgesamt fünf Studien zusammen, die zu den Forschungsfeldern 1 (Studie 1) sowie 2 und 3 (Studie 2–5) des Kooperationsprojektes beitragen. Zur Bestimmung der Abundanz und Verteilung von MP in deutschen Fließgewässern wurde im Rahmen von **Studie 1** (Scherer et al. 2020, Anhang A1, „Comparative assessment of microplastics in water and sediment of a large European river”) das Vorkommen von MP im Sediment sowie in der Wasserphase exemplarisch für den Fluss Elbe bestimmt. **Studie 1** ist dabei die erste Publikation, die Daten zum Vorkommen von MP in der Elbe veröffentlicht hat. Die Häufigkeit von MP sowie dessen Eigenschaften (Form, Größe, Polymertyp) wurde an elf Probenahmestellen entlang der Mittel- und Unterelbe bis zur ihrer Mündung bestimmt. Hauptziel der Studie war es, das Vorkommen von MP in deutschen Fließgewässern besser abschätzen sowie Schlussfolgerungen in Bezug auf die Verteilung von MP im Flussverlauf bzw. zwischen Sediment und Wasserphase ziehen zu können. Darüber hinaus ist diese Studie eine der ersten, die die Ergebnisse der visuellen MP-Identifizierung mit Daten aus fortgeschrittenen Techniken (Pyro-GC-MS, Kapitel 1.4) vergleicht.

In Bezug auf die Aufnahme und Toxizität von MP durch limnische Organismen lagen bis 2016 noch sehr wenige publizierte Forschungsergebnisse vor. Die **Studien 2** (Weber et al. 2021a, Anhang A2, „Ingestion and toxicity of polystyrene microplastics in freshwater bivalves”), **3** (Weber et al. 2018, Anhang A3, „PET microplastics do not negatively affect the survival, development, metabolism and feeding activity of the freshwater invertebrate *Gammarus pulex”*) und **4** (Weber et al. 2021b, Anhang A4, „Ingestion and toxicity of microplastics in the freshwater gastropod *Lymnaea stagnalis*: No microplastic-induced effects alone or in combination with copper”) hatten daher zum Ziel, die MP-Aufnahme und MP-Toxizität in limnischen Muscheln (Studie 2), Krebstieren (Studie 3) und Gastropoden (Studie 4) näher aufzuklären. Krebstiere, Muscheln und Gastropoden wurden als

Modellorganismen ausgewählt, da diese einige der wesentlichen Gruppierungen innerhalb der limnischen Wirbellosen darstellen (Engelhardt et al. 2015). Darüber hinaus repräsentieren diese drei Artengruppen die drei wesentlichen Gilden (Arten mit ähnlicher Ernährungsstrategie) in limnischen Gewässern (Vannote et al. 1980): Zerkleinerer/Schredder (Krebstiere), Filtrierer (Muscheln) und Weidegänger (Gastropoden). Grundlegend für die Ausgestaltung der Studien 2–4 war die Annahme, dass verschiedene Ernährungsstrategien eine unterschiedlich starke Aufnahme von MP-Partikeln bewirken und sich dadurch auch die Toxizität in den verschiedenen Tiergruppen unterscheidet.

Die MP-Aufnahme hängt jedoch nicht nur von der Ernährungsstrategie der Organismen ab. Zusätzlich können Faktoren wie die MP-Partikeleigenschaften, das verwendete Expositionsszenario sowie art- bzw. individuenspezifische Unterschiede die Eigenschaften der exponierten Individuen maßgeblich beeinflussen. Im Rahmen der Studien 2–4 wurde die Relevanz dieser verschiedenen Faktoren für die MP-Aufnahme durch limnische Organismen eingehend untersucht. Die untersuchten Faktoren umfassten die Größe (Studie 2 und 4) von MP-Partikeln, deren Konzentration (2, 3), die Expositionszeit (2, 4), den Weg der Partikelapplikation (2) sowie die Größe bzw. Masse der exponierten Individuen (2, 3).

Darüber hinaus untersuchten die Studien 2–4 auch die Toxizität von MP auf limnische Tierarten. Toxizität kann durch verschiedene Expositions- und Interaktionswege der MP-Partikel mit den Organismen ausgelöst werden (Kapitel 1.5). Da eine umfassende Überprüfung aller Expositions- und Interaktionswege den Rahmen der Dissertation überschritten hätte, wurde auf die Untersuchung chemischer Toxizität im Rahmen der Studien 2–4 verzichtet. Vielmehr stand die partikelinduzierte Toxizität sowie die Toxizität durch eine Verringerung des Verdauungsvolumens im Vordergrund der Studien. Chemische Toxizität wurde weitestgehend dadurch ausgeschlossen, dass nur Polymere mit einem geringen Additivgehalt für die Untersuchungen eingesetzt wurden.

Partikuläre Toxizität kann sich in limnischen Organismen auf sehr unterschiedlichen Organisationsebenen entfalten, u.a. auf molekularer und zellulärer Ebene, aber auch im Gewebe sowie auf Individualebene (Kapitel 1.5.2). In den Studien 2–4 wurde die MP-Toxizität, verursacht durch eine akute (2) oder chronische (2–4) Exposition, daher anhand einer Vielzahl unterschiedlicher Endpunkte untersucht, einschließlich Mortalität und Energiereserven (2–4), oxidativem Stress (2, 4), immunologischen Veränderungen (4), Veränderungen der Nahrungsaufnahme (2, 3) und Reproduktion (4).

Als Teil dieses Rahmentextes sollen die MP-Aufnahme und Toxizität in den drei getesteten Tiergruppen (Muscheln, Krebstiere, Schnecken) untereinander sowie mit weiteren, verfügbaren Daten für limnische und marine Arten verglichen werden. Insbesondere soll durch diesen Vergleich ermittelt werden, ob in Bezug auf die MP-Aufnahme und -Toxizität Unterschiede zwischen den drei getesteten Tiergruppen bestehen. Zusätzlich wird auf Basis dieses Vergleichs im Kapitel 2.5 eine erste Abschätzung zum Risiko von MP-Partikeln für die aquatische Umwelt getroffen.

Die Toxizitätsanalyse durch Laborexposition von Organismen gegenüber MP-Partikeln ist ein erster Schritt in Richtung einer übergreifenden Risikobewertung für MP-Partikel in der Umwelt. Eine Einzelstressor-Exposition mit MP als alleinigem Stressor spiegelt jedoch keinesfalls die realen Umweltbedingungen wider, da Organismen in der Umwelt meist gegenüber mehreren Stressoren gleichermaßen exponiert sind (Kapitel 1.6). In **Studie 4** (Weber et al. 2021b) und **Studie 5** (Weber et al. 2020, Anhang A5, „Combined effects of polystyrene microplastics and thermal stress on the

freshwater mussel *Dreissena polymorpha"*) wurden daher die Effekte von MP in einem Multi-Stressor-Szenario getestet. Dabei wurde zum einen ein chemischer Stressor (Exposition mit Kupfer, Studie 4 mit *Lymnaea stagnalis*) und zum anderen ein physikalischer Stressor (Hitzestress, Studie 5 mit *Dreissena polymorpha*) in einer Co-Exposition mit MP eingesetzt.

In Kapitel 3 werden schließlich die Informationen aus der ersten MP-Risikoabschätzung und den Studien mit mehreren Stressoren abschließend zusammengeführt.

2. Diskussion

2.1 Vorkommen von Mikroplastik in Süßgewässern

2.1.1 Wichtige Forschungsergebnisse aus Studie 1 „Comparative assessment of microplastics in water and sediment of a large European river“ (Scherer et al. 2020, Anhang A1)

Weltweit wurde MP bereits in zahlreichen aquatischen Lebensräumen nachgewiesen, wobei die marinen Lebensräume im Vergleich zu Süßgewässern bisher deutlich intensiver untersucht worden sind (Kapitel 1.4). 2015 wurde daher Studie 1 zum MP-Vorkommen in der Elbe begonnen, um Wissenslücken in Bezug auf die Häufigkeit und Verteilung von MP in großen Fließgewässern in Deutschland zu schließen. Folgende neue Erkenntnisse wurden durch Studie 1 erlangt:

- MP_{vis}-Konzentrationen in der Elbe (MP_{vis} = visuell als MP identifizierte Partikel):
  - Wasserphase (150–5,000 µm): 0,88–13,24 P m^{-3}
  - Sedimente (125–5,000 µm): 2,26x10^4–2,27x10^7 P m^{-3}
- Im Mittel waren die MP_{vis}-Konzentrationen im Sediment fast 150.000-fach höher als in der Wasserphase (7,6×10^5 P m^{-3} vs. 5,11 P m^{-3}).
- Die MP_{vis}-Konzentrationen, insbesondere in den Sedimenten, nahmen im Verlauf der Elbe ab. Ein möglicher Grund könnte das Stauwehr bei Geesthacht sein, das die Elbe in einen Abschnitt mit und ohne Tideeinfluss unterteilt.
- Die Elbesedimente enthielten im Durchschnitt kleinere Partikel als die Wasserphase.
- In der Wasserphase traten Fasern am häufigsten auf, während in den Sedimenten Sphären und Fragmente dominierten.
- Nahe Dessau wurde ein außergewöhnlich hohes Vorkommen an Sphären aus PS-Divinylbenzol (PS-DVB) entdeckt, die möglicherweise aus einer industriellen Emissionsquelle stammen.
- In der Wasserphase bestanden die MP_{vis}-Partikel überwiegend aus PE und PP, während im Sediment eine deutlich vielfältigere Polymerzusammensetzung beobachtet wurde.
- Die Ergebnisse der visuellen MP-Identifikation stimmen sehr gut mit den Ergebnissen aus der Pyro-GC-MS-Analyse überein.

2.1.2 Mikroplastikvorkommen und -verteilung in Flüssen

Die Verbreitung von MP in Flüssen ist in den vergangenen Jahren zunehmend in den Fokus der Forschung gerückt, da Flüsse einen möglichen Eintragspfad für MP in die Meere darstellen (Lebreton et al. 2017). Zudem sind Flüsse ein wichtiger Lebensraum für zahlreiche limnische Arten, die ebenso wie marine Arten von einer MP-Exposition betroffen sein könnten (Wagner et al. 2014). Zusätzliche Studien für limnische Ökosysteme sollen dabei helfen, das Wissen zur globalen Verteilung von MP in Flüssen und Seen zu erweitern. Studie 1 enthält einen umfassenden Überblick über die bisher publizierten MP-Konzentrationen in 51 früheren Studien zum MP-Vorkommen in Flüssen (Abb. 2). Danach variieren die bisher ermittelten MP-Konzentrationen zwischen 0,17 (Minimum, Rodrigues et al. 2019) und 5,19x10^5 P m^{-3} (Maximum, Lahens et al. 2018) in der Wasserphase bzw. 1,00x10^{-2} (Minimum, Castañeda et al. 2014) und 1,62x10^8 P m^{-3} (Maximum, Wang et al. 2018) in den Flusssedimenten. Hohe MP-Konzentrationen wurden bisher insbesondere in asiatischen, aber auch in

einigen europäischen Flusssystemen beobachtet. Hohe MP-Vorkommen in der Umwelt sind somit nicht kontinentspezifisch, sondern eine Herausforderung mit globalem Maßstab.

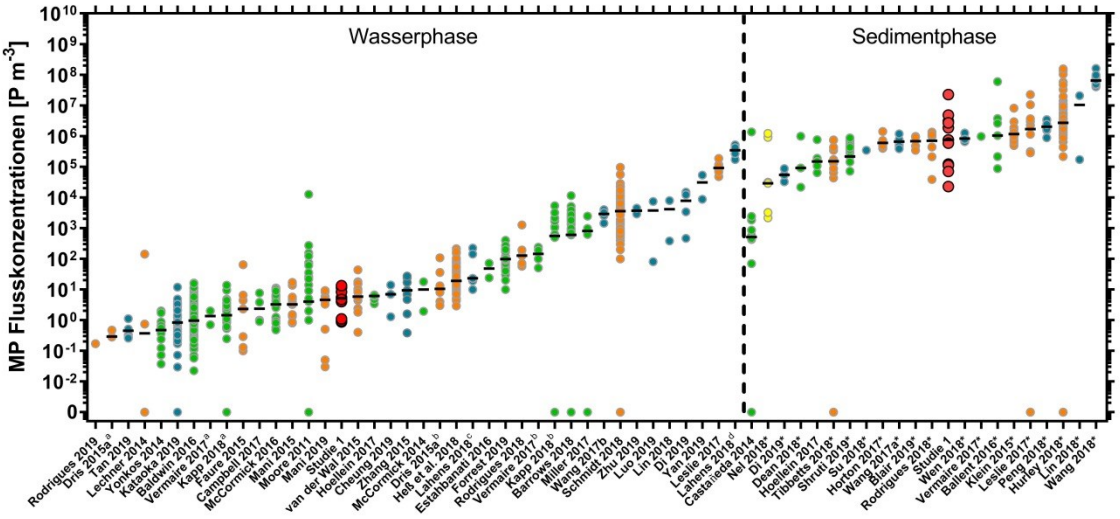

Abb. 2 (verändert nach Scherer et al. 2020): Überblick über die bereits publizierten Mikroplastik (MP)-Konzentrationen in Flüssen (sortiert nach dem Median der publizierten MP-Konzentrationen der jeweiligen Studie) in Europa (orange, Studie 1 (rot)), Nord- und Südamerika (grün), Asien (blau) und Afrika (gelb). [a,b]Für Studien, die das MP-Vorkommen mit zwei verschiedenen Beprobungsmethoden untersucht haben, werden die Ergebnisse getrennt für beide Beprobungsmethoden aufgeführt. [c,d]Lahens et al. (2018) bestimmte die MP-Konzentrationen getrennt für Fragmente und Fasern. * = In diesen Studien wurden die MP-Konzentrationen in der Einheit P (kg Sediment)$^{-1}$ angegeben. Diese Konzentrationen wurden unter Berücksichtigung einer Sedimentdichte von 2,17 kg m^{-3} (durchschnittliche Dichte der elf Elbeprobestellen in Studie 1) in die Einheit P m^{-3} umgerechnet. Aus Vereinfachungsgründen wird jeweils nur der Erstautor genannt; eine ausführliche Liste der Autoren ist in den Supplementary Data zur Studie 1 (Anhang A1) enthalten.

Alle untersuchten Elbeproben, sowohl aus der Wasser- als auch der Sedimentphase, enthielten MP. Die gemessenen MP-Konzentrationen entsprachen dabei in beiden Fällen dem mittleren Drittel der Spannbreite globaler MP-Konzentrationen (Abb. 2). Ähnliches gilt für einen Vergleich der Elbedaten mit Studien aus dem europäischen Raum. In einigen europäischen Flüssen wurden im Vergleich zur Elbe höhere, in anderen niedrigere MP-Konzentrationen ermittelt: Beispielsweise wiesen Faure et al. (2015) für die Wasserphase in Schweizer Flüssen eine niedrigere mittlere MP-Konzentration (2,30 P m^{-3}, Median) nach, während die Konzentrationen im Niederländischen Flussdelta (9,1x10^{4} P m^{-3}) die Elbekonzentrationen (5,11 P m^{-3}) um ein Vielfaches überschritten (Leslie et al. 2017). Gleiches gilt für die Sedimente, wobei die Konzentrationsunterschiede geringere Schwankungen aufwiesen: So ermittelten Tibbetts et al. (2018) für die Themse und ihre Zuflüsse eine niedrigere (1,52x10^{5} P m^{-3}) und Hurley et al. (2018) für 40 Flüsse im Nordwesten Englands eine höhere mittlere MP-Sedimentkonzentration (2,71x10^{6} P m^{-3}) im Vergleich zur Elbe (7,57x10^{5} P m^{-3}).

Im deutschen Vergleich sind die in der Elbe gemessenen MP-Konzentrationen hingegen sehr gut mit den Daten aus anderen großen deutschen Flüssen vergleichbar: In Bezug auf die Wasserphase stimmen die MP-Konzentrationen aus der Elbe gut mit den Ergebnissen aus Rhein, Donau und Weser überein (3,27–19,10 P m^{-3}, Heß et al. 2018, Mani et al. 2015). Ebenso sind die Daten für die Sedimente weitestgehend mit den Daten aus Rhein und Main vergleichbar (1,16×10^{6} P m^{-3}, Klein et al. 2015). Die Elbe bzw. die großen Flüsse Deutschlands sind somit aktuell noch von einem im

globalen Vergleich eher mäßig hohen MP-Vorkommen betroffen. Nichtsdestotrotz verdeutlicht die bereits zu beobachtende, großflächige Verbreitung von MP in deutschen Flüssen, dass MP in deutschen Flusssystemen allgegenwärtig ist und mit zukünftig steigenden Umweltkonzentrationen als Stressor stärker an Bedeutung gewinnen könnte.

Das Auffinden großer Ansammlungen von Plastikpartikeln in Meeren (Eriksen et al. 2013b) sowie Befunde, dass durch Flüsse erhebliche MP-Mengen in die Meere eingetragen werden (Lebreton et al. 2017), verdeutlichen, dass die Weltmeere ein „Sammelpunkt“ für Plastikmüll und dessen Degradationsprodukte sind. Dass es jedoch auch in Flüssen zur Ansammlung von MP kommen kann, wurde in den vergangenen Jahren erst durch die vermehrte Publikation von Daten aus Süßgewässern deutlich. Ein Vergleich der MP-Konzentrationen in der Wasserphase von Flüssen und Meeren (Abb. 3a) zeigt, dass die aktuell messbaren MP-Konzentrationen in Flüssen im Mittel sogar höher sind als in den Meeren. Auf Basis der ermittelten kumulativen Verteilung beträgt das MP-Vorkommen an 50 % der Probestellen in Flüssen ≥ 23 P m^{-3}. In den Meeren trifft dies nur auf 19 % der Probestellen zu. Lediglich an den am stärksten kontaminierten Standorten sind die MP-Konzentrationen aktuell vergleichbar. Dies unterstreicht, dass der Eintrag von MP keinesfalls nur marine Lebensräume, sondern auch Süßgewässer betrifft. Zudem ist die Belastung in Flüssen möglicherweise sogar höher als in marinen Lebensräumen, weshalb die Auswirkungen insbesondere auf limnische Organismen genauer untersucht werden müssen.

Neben dem Gesamtvorkommen von MP in der Elbe untersuchte Studie 1 zudem auch die Verteilung der Partikel zwischen der Wasser- und der Sedimentphase. Als wesentliches Ergebnis konnte Studie 1 zeigen, dass Sedimente eine Senke für MP-Partikel darstellen. Diese Schlussfolgerung ist eine wesentliche neue Erkenntnis in der limnischen MP-Forschung, da frühere Studien entweder nur die Wasser- oder die Sedimentphase von Süßgewässern beprobt oder anderenfalls ihre Konzentrationsangaben für die beiden Kompartimente in abweichenden Einheiten dargestellt haben (Kapitel 1.4). Schlussfolgerungen zur MP-Verteilung in Flüssen waren somit kaum möglich. Durch eine Vereinheitlichung der Einheiten, sowohl für die Elbeergebnisse wie auch für die bisher publizierten MP-Konzentrationen, wurden die verfügbaren Daten im Rahmen von Studie 1 erstmalig vergleichbar gemacht. Dadurch konnte gezeigt werden, dass die gemessenen MP-Konzentrationen im Sediment der Elbe im Mittel 150.000-fach höher waren als in der Wasserphase. Auch im globalen Vergleich wurden für Flusssedimente vielfach höhere MP-Konzentrationen publiziert (Abb. 3b). Dies deutet darauf hin, dass insbesondere Sedimentbewohner bzw. am Flussboden lebende Organismen vermehrt gegenüber MP exponiert sind und daher stärker von möglichen toxischen Auswirkungen betroffen sein könnten.

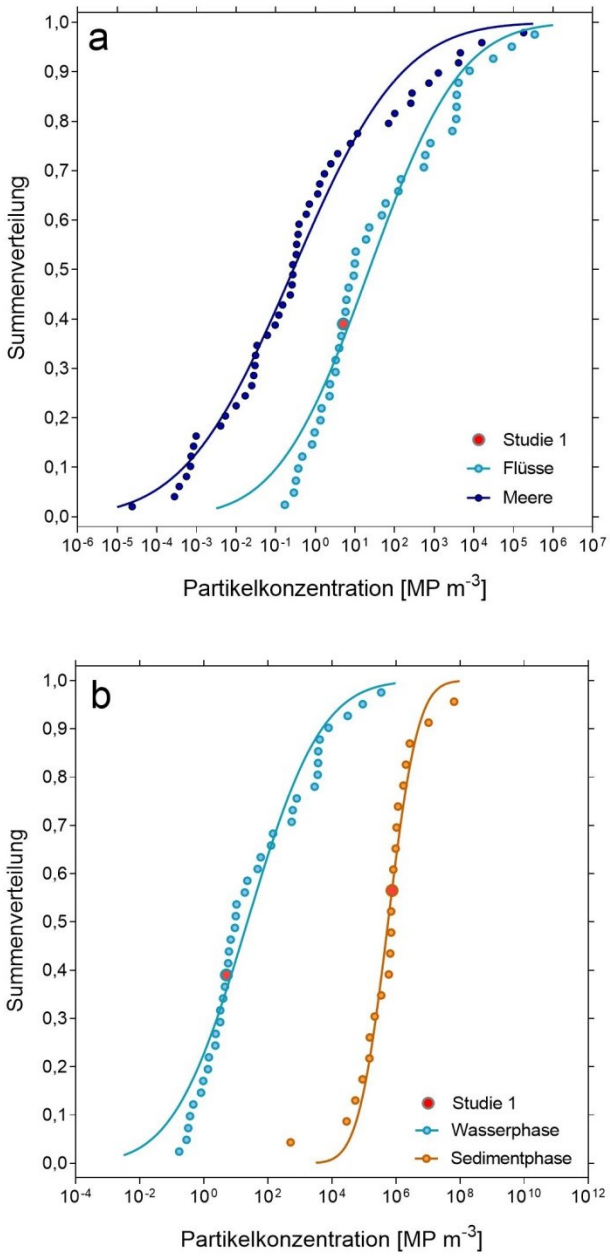

Abb. 3: Kumulative Verteilung der Mikroplastik (MP)-Konzentrationen [P m^{-3}] (a) in der Wasserphase von Flüssen und Meeren weltweit sowie (b) in der Wasser- und Sedimentphase in Flüssen weltweit. Die Daten zur Wasser- und Sedimentphase entstammen dem Überblick über die weltweiten MP-Konzentrationen aus Studie 1 (Abb. 2, hier dargestellt sind die Medianwerte der einzelnen Studien). Die marinen Daten wurden aus drei Reviews zum Vorkommen von MP in marinen Ökosystemen (Lusher 2015, Li 2018, Rezania et al. 2018) zusammengestellt. Alle der Abbildung zu Grunde liegenden Daten sind in Tab. A1 und A2 in Anhang A6 aufgeführt.

Als Ursprung von MP-Partikeln in der Umwelt kommen vielfältige Quellen bzw. Eintragspfade in Betracht. So werden insbesondere urbane Zentren als Ursprung für hohe MP-Vorkommen in europäischen Flüssen diskutiert. Mit steigender Populationsdichte in Flussnähe steigen häufig auch die MP-Konzentrationen in der Umwelt an (Eerkes-Medrano et al. 2015). Die gesteigerten Einträge in

urbanen Regionen können zum einen mit diffusen Quellen (z. B. MP-kontaminierter Straßenstaub (Yukioka et al. 2020), der durch Oberflächenabflüsse in Flüsse gelangt), zum anderen aber auch mit punktuellen Einträgen, beispielsweise durch Haushalts- und Industrieabwässer (Mani et al. 2015, Schmidt et al. 2018, Tibbetts et al. 2018), zusammenhängen. Haushaltsabwässer können beispielsweise synthetische Textilfasern (Kleidungsabrieb während des Waschens, Falco et al. 2019) oder MP aus Körperpflegeprodukten enthalten (Lei et al. 2017). Auch industrielle Abwässer enthalten regelmäßig MP-Partikel, beispielsweise Plastikrohgranulat aus der kunststoffverarbeitenden Industrie oder Ionenaustauscherharze (Lechner et al. 2014, Mani et al. 2019). Kläranlagen können einen großen Anteil dieses MP-Eintrags abfangen, ein Resteintrag (< 16 %) in die angrenzenden Flüsse bleibt jedoch weiterhin bestehen (Magni et al. 2019a, Murphy et al. 2016).

Studie 1 konnte den Ursprung bzw. die Eintragungspfade für MP in der Elbe nicht abschließend klären. Die Polymerzusammensetzung der Partikel deutet auf eine Vielzahl unterschiedlicher Quellen hin. So wurden in der Wasserphase insbesondere PE und PP, in den Sedimenten darüber hinaus auch PS/PS-DVB, PVC, Acrylnitril-Butadien-Styrol (ABS), PA, PET und PMMA als Polymertyp nachgewiesen. Die letztgenannten Polymere besitzen dabei eine höhere Dichte als Wasser und können dadurch verstärkt sedimentieren und sich im Sediment anreichern (Horton et al. 2017). Weiterhin spiegeln die nachgewiesenen Polymertypen, insbesondere die hohen Anteile an PE und PP (Wasserphase: 92,5 %, Sedimente: 46,9 %), die europäische Nachfrage nach Plastikpolymeren wider (PE: 29,8 %, PP: 19,3 %, Rest: 50,9 %, PlasticsEurope 2018). Dies unterstützt die Vermutung, dass die MP-Partikel in der Elbe aus einer Vielzahl an unterschiedlichen Quellen stammen. Andererseits konnte Studie 1 als Besonderheit in der Nähe von Dessau eine sehr hohe Anzahl an gleichförmigen Sphären, bestehend aus PS-DVB, nachweisen. Sphärisches PS-DVB wird in der Industrie u.a. als Ionenaustauscher eingesetzt (Mani et al. 2019). Als möglicher Ursprungsort der Sphären in der Elbe wird ein Industriegebiet nahe Dessau angenommen, die tatsächliche Quelle konnte jedoch nicht abschließend bestimmt werden. Zusammengefasst stammt das MP-Vorkommen in der Elbe somit vermutlich aus einer Vielzahl an Quellen und wird sowohl punktuell wie auch diffus in die Elbe eingetragen.

Neben der Konzentration ist auch die Größe von MP-Partikeln für deren Verteilung in der Umwelt entscheidend. Sowohl die Mobilität der Partikel im Gewässer als auch ihre Bioverfügbarkeit für die verschiedenen Tiergruppen wird maßgeblich durch die Partikelgröße beeinflusst. Je kleiner die Partikel sind, desto leichter können sie bei Wasserbewegungen remobilisiert (Nizzetto et al. 2016) bzw. durch Organismen niedrigerer trophischer Ebene aufgenommen werden (Wright et al. 2013). In der Elbe stieg im Partikelgrößenbereich zwischen 125/150 µm und 2.000 µm sowohl für die Wasser- als auch die Sedimentphase die MP-Anzahl mit sinkender Partikelgröße exponentiell an. Ein ähnlicher exponentieller Anstieg der Umweltkonzentration mit sinkender Partikelgröße wurde bereits von Eo et al. (2019) und Imhof et al. (2016) in limnischen Systemen beobachtet. Eine sehr wahrscheinliche Erklärung dafür ist, dass die in der Umwelt akkumulierten MP-Partikel fragmentieren und sich dadurch der Anteil kleiner Partikel zunehmend erhöht.

Interessanterweise enthielten die Elbe-Sedimente im Mittel jedoch kleinere Partikel als die Wasserphase. Eine Ursache hierfür könnte die Fragmentierung von Plastikpartikeln in der Sedimentphase, aber auch ein erhöhter Anteil an Fasern in der Wasserphase sein. Während in der Wasserphase insbesondere Fasern als Partikelform dominierten, wurden in den Sedimenten überwiegend Sphären und Fragmente nachgewiesen. Fasern können auf Grund ihrer geringeren Sinkgeschwindigkeit länger in der Wasserphase verbleiben (Waldschläger & Schüttrumpf 2019) und da bei Fasern stets die Gesamtlänge als Partikellänge zu Grunde gelegt wird, kann ein hoher

Faseranteil die Partikelverteilung in der Wasserphase hin zu höheren durchschnittlichen Partikelgrößen verschieben. Somit geht die erhöhte Anzahl an kleineren MP-Partikeln in den Sedimenten nicht zwangsläufig nur auf Fragmentierungsprozesse zurück.

Um die Abundanz kleiner MP-Partikel in Flüssen besser abschätzen zu können, wurde in der Elbe neben der Partikelgrößenfraktion 125–2.000 µm auch die Sedimentfeinfraktion (20–125 µm) untersucht. Geht man von einem kontinuierlichen Fragmentierungsprozess der MP-Partikel in den Flusssedimenten aus, so müsste auch in der Feinfraktion ein exponentieller Anstieg des MP-Vorkommens mit sinkender Partikelgröße zu beobachten sein (Koelmans et al. 2020). Eine Identifikation von MP-Partikeln < 100 µm ist jedoch auf Grund der geringen Größe visuell nicht mehr möglich, sondern erfordert (nach einer sehr aufwendigen Probenaufbereitung) eine Analyse mittels µ-FTIR- oder Raman-Spektroskopie. Da diese Techniken für Studie 1 nicht zur Verfügung standen, wurde alternativ mittels Pyro-GC-MS die Gesamtmasse an PE und PP in den Feinsedimenten bestimmt (eine zusätzliche Analyse von PS führte zu inkonsistenten Ergebnissen). Die durchschnittliche Polymermasse in den Feinsedimenten betrug 65,6 g m^{-3} mit einem Anteil von 80,9 % PE und 19,1 % PP. Rechnet man diese Masse auf eine theoretische Anzahl an 20 µm bzw. 125 µm Sphären (mit einer durchschnittlichen Partikeldichte von 0,93 g cm^{-3}) um, so enthielten die Feinfraktionen durchschnittlich $1{,}68 \times 10^{10}$ (20 µm) bzw. $6{,}90 \times 10^{7}$ P m^{-3} (125 µm). Die Partikelkonzentrationen waren in der Feinfraktion (20–125 µm) somit um das 21- bis 5.014-fache höher als in der 125–2.000 µm Sedimentfraktion. Daraus kann geschlossen werden, dass die auf Basis des Größenbereich 125–2.000 µm für die Elbe ermittelten MP-Konzentrationen die tatsächliche Umweltkonzentrationen in der Elbe stark unterschätzen.

Eine Unterschätzung der tatsächlichen MP-Partikelkonzentrationen liegt vermutlich bei einem Großteil der bisher publizierten Studien zum MP-Vorkommen in Flüssen vor. Ein Grund hierfür ist, dass MP in bisherigen Studien meist durch visuelle Analyse identifiziert wurde. Diese Methode ist zwar kostengünstig und erfordert einen geringen technischen Aufwand, ist jedoch auf den Größenbereich > 100 µm beschränkt. Zudem besteht bei Wasserproben auch die Herausforderung, dass bei einer Beprobung mittels Manta- oder Planktonnetz die Netzmaschenweite meist ≥ 100 µm beträgt, um ein schnelles Verschließen der Maschen zu verhindern (Löder & Gerdts 2015, Kapitel 1.4). Auf Grund dieser experimentellen Einschränkungen haben bisher nur sehr wenige Studien bei der Bestimmung ihrer publizierten MP-Konzentrationen die Partikelgrößenfraktion < 100 µm mitberücksichtigt.

Es kann daher vermutet werden, dass auch die in Abb. 2 dargestellten MP-Konzentrationen das tatsächliche Vorkommen von MP in Süßgewässern unterschätzen. In einer kürzlich veröffentlichten Studie extrapolierten Koelmans et al. (2020) publizierte MP-Partikelkonzentrationen auf den gesamten MP-Größenbereich (1–5.000 µm). Während in den ursprünglichen Studien MP-Konzentrationen von 10^{-5}–200 P L^{-1} angegeben waren, ergab sich durch die Extrapolationsansätze ein Konzentrationsbereich von 10^{-3}–800 P L^{-1}. Diese Ergebnisse unterstreichen die gegenwärtig meist unvollständige Erfassung von MP-Partikelkonzentration in Flüssen und die daraus resultierende Unterschätzung der MP-Umweltkonzentrationen.

Dieser Argumentation könnte jedoch entgegengesetzt werden, dass auch die visuelle Analyse fehlerbehaftet ist und es durch diese möglicherweise zu einer Überschätzung der MP-Konzentrationen kommen kann. Im Fall von MP-Partikeln > 500 µm aus der Elbe konnten 5,0 % der visuell als MP identifizierten Partikel (MP_{vis}) aus der Wasserphase sowie 29,3 % der MP_{vis}-Partikel aus

der Sedimentphase durch FTIR-Analyse nicht eindeutig einer Kunststoffpolymerart zugeordnet werden. Bei diesen Partikeln kann es sich daher durchaus um fehlerhaft als MP identifizierte Partikel gehandelt haben. Selbst eine Überschätzung der MP-Konzentrationen um bis zu 30 % auf Grund visueller Analyse kann jedoch eine Unterschätzung der MP-Konzentrationen durch Vernachlässigung der Fraktion < 100 µm (mit Konzentrationen, die im Fall der Elbe bis zu 5.000-fach höher waren als in der Fraktion > 100 µm) keinesfalls aufwiegen. Somit ist aktuell davon auszugehen, dass die publizierten MP-Konzentrationen für Flüsse das tatsächliche Vorkommen eher unter- als überschätzen und zumindest die Flusssedimente bereits jetzt einen hohen Anteil an MP-Partikeln < 100 µm beinhalten. Diese Schlussfolgerung ist insbesondere im Hinblick auf die Bewertung eines möglichen Risikos von MP für aquatische Organismen wesentlich, denn mit sinkender Größe werden die Partikel für eine zunehmende Anzahl von limnischen Organismen, insbesondere kleine Wirbellosenarten, bioverfügbar und können zu einem relevanten Stressor werden.

2.1.3 Kernerkenntnisse aus Kapitel 2.1

- MP ist in Flüssen weltweit nachweisbar. Im globalen Vergleich sind die MP-Konzentrationen in deutschen Flüssen durchschnittlich.
- Flüsse enthalten ähnliche bzw. zum Teil höhere MP-Konzentrationen als die Weltmeere (bezogen auf die Wasserphase).
- Flusssedimente sind (im Vergleich zur Wasserphase) Senken für MP.
- Je kleiner die Partikel, desto größer ihr Vorkommen in Flüssen. Zwischen der Partikelgröße und dem relativen Vorkommen besteht wahrscheinlich ein exponentieller Zusammenhang.
- Viele Abundanzstudien unterschätzen voraussichtlich das MP-Vorkommen in Flüssen, sofern sie die Größenfraktion < 100 µm unberücksichtigt lassen.

2.2 Interaktion von Mikroplastik mit limnischen Muschelarten

2.2.1 Wichtige Forschungsergebnisse aus Studie 2 „Ingestion and toxicity of polystyrene microplastics in freshwater bivalves“ (Weber et al. 2021a, Anhang A2)

Auf Grund der weiträumigen Verbreitung in Süßgewässern kann MP mit einer Vielzahl von limnischen Organismen interagieren. Entsprechend untersuchte Studie 2 die Aufnahme und Toxizität von MP in limnischen Muscheln anhand von drei verschiedenen Arten (*Dreissena polymorpha*, *Anodonta anatina*, *Sinanodonta woodiana*). Im Hinblick auf die MP-Aufnahme war das wesentliche Ziel der Studie, ein tiefgreifendes Verständnis der Faktoren zu erwerben, die die MP-Aufnahme modulieren (z. B. MP-Eigenschaften, Expositionsbedingungen und individuen- bzw. artspezifische Merkmale, Kapitel 1.7). Darüber hinaus enthält Studie 2 neue wertvolle Erkenntnisse zur MP-Toxizität in limnischen Muschelarten. Folgende neue Erkenntnisse wurden durch Studie 2 gewonnen:

I.) Mikroplastikaufnahme:

- **Faktor 1: Expositionszeit**
  - Limnische Muscheln (*S. woodiana, D. polymorpha*) können innerhalb weniger Stunden (1–6 h) eine große Anzahl (hunderte bis tausende) an MP-Partikeln aufnehmen.
  - Ein Großteil der aufgenommenen Partikel wird innerhalb von 72 h wieder ausgeschieden. Kleinere Muscheln (*D. polymorpha*) scheiden die Partikel dabei schneller aus als größere Muscheln (*S. woodiana*).
  - Nach 7 d sind mehr als 95 % (*S. woodiana*) bzw. 99 % (*D. polymorpha*) der MP-Partikel, die während einer 12-stündigen Exposition aufgenommen worden sind, wieder ausgeschieden worden.
- **Faktor 2: Körpergröße**
  - Bezogen auf die absolute Aufnahme (pro Individuum) nehmen große Muscheln (*A. anatina*, *S. woodiana*) mehr MP-Partikel auf als kleine Muscheln (*D. polymorpha*). Artspezifische Unterschiede zwischen Individuen gleicher Größe konnten nicht beobachtet werden.
  - In Bezug auf die relative Aufnahme (pro Körpergewicht) nahmen kleinere Muscheln relativ mehr MP-Partikel auf als größere Muscheln. Zusätzlich wurden artspezifische Unterschiede bei Individuen gleicher Größe beobachtet.
- **Faktor 3: Nahrung**
  - Eine steigende Algenmenge in der Wasserphase reduziert die MP-Aufnahme (*D. polymorpha*).
- **Faktor 4: MP-Konzentration**
  - Steigende MP-Konzentrationen erhöhen die MP-Aufnahme durch die Muscheln (*D. polymorpha*), der Anstieg ist jedoch nicht linear.
- **Faktor 5: MP-Partikelgröße**
  - Kleinere Arten bzw. kleinere Individuen einer Art nehmen durchschnittlich kleinere Partikel auf als größere Arten bzw. Individuen einer Art (*D. polymorpha*, *A. anatina*, *S. woodiana*).

II.) Mikroplastiktoxizität:

- In *D. polymorpha* führte eine akute (1, 3, 7 d) bzw. chronische (42 d) Exposition gegenüber PS-Fragmenten (6,4 (umweltähnliche Konzentration)–100.000 P mL^{-1}) zu einer signifikanten Steigerung der Fraßaktivität, während sie zu keinen signifikanten Veränderungen der Mortalität, der Energiereserven oder des oxidativen Stresses führte.

2.2.2 Mikroplastikaufnahme durch limnische Muscheln

Muscheln sind als Filtrierer auf die Aufnahme und Selektion großer Partikelmengen aus der Wasserphase (Jørgensen 1990) spezialisiert. Zu diesem Zweck besitzen Muscheln ein mehrstufiges Filtrationssystem, das die gezielte Auswahl spezifischer suspendierter Partikel aus der Wasserphase ermöglicht. Dabei werden die Partikel mit Hilfe eines internen Wasserstroms aufgenommen und an den Kiemenfilamenten entlang geführt. Durch Wimpernhaare auf den Filamenten werden die Partikel aus dem Wasserstrom selektiert und durch Furchen und über die Labialpalpen zum Verdauungssystem weitergeleitet (Silverman et al. 1999, Ward & Shumway 2004). Dieser Transport beinhaltet weitere Selektionsschritte, wobei nicht verdauliche Partikel als Pseudofäzes wieder ausgeschieden werden (Vaughn et al. 2008). Zusätzlich findet auch im Magen eine weitere Sortierung der Partikel statt, wobei verdauliche Stoffe in die Mitteldarmdrüse weitertransportiert und nicht verdauliche Stoffe direkt wieder ausgeschieden werden.

Der hochspezialisierte Filtrationsapparat scheint jedoch unverdauliche MP-Partikel nicht vollständig herausselektieren zu können (Ward & Shumway 2004). So konnte Studie 2 nachweisen, dass trotz dieser etablierten Selektionsmechanismen eine hohe Anzahl an unverdaulichen MP-Partikeln in das Verdauungssystem von limnischen Muscheln gelangen kann. In früheren Studien wurde gezeigt, dass MP-Partikel nach ihrer Aufnahme insbesondere im Magen, in der Mitteldarmdrüse und dessen Tubuli sowie im Hämolymphsystem von Muscheln nachweisbar sind (Gonçalves et al. 2019, Guilhermino et al. 2018, Magni et al. 2019b, Ribeiro et al. 2017, Pittura et al. 2018).

Es bestehen somit bereits ausreichend Nachweise dafür, dass Muscheln MP aufnehmen können. Unzureichend verstanden ist hingegen die Kinetik dieser Aufnahme. Diese kann von verschiedensten Faktoren abhängen, darunter den physikalisch-chemischen Eigenschaften der MP-Partikel (z. B. Größe, Form, Polymertyp), den Expositionsbedingungen (z. B. MP-Konzentration, Konzentration an anderen suspendierten Partikeln, Expositionsdauer) sowie individuen- bzw. artspezifischen Unterschieden. Hauptziel von Studie 2 war es daher, die MP-Aufnahme in Abhängigkeit dieser Faktoren in limnischen Muscheln aufzuklären. Im Folgenden werden die wichtigsten Schlussfolgerungen kurz zusammengefasst und Implikationen für Muschelpopulationen in der Umwelt hervorgehoben.

Faktor *Expositions- und Ausscheidungszeit*: Die MP-Aufnahme durch Muscheln wird maßgeblich durch die Expositionszeit beeinflusst. Studie 2 zeigte für die Arten *D. polymorpha* und *S. woodiana* eine rasche Aufnahme innerhalb weniger Stunden mit maximalen MP-Belastung im Körper nach 1 h (*D. polymorpha*) bzw. 6 h (*S. woodiana*). Wurden die Muscheln jedoch in eine MP-freie Umgebung überführt, so schieden die beiden Muschelarten die MP-Partikel innerhalb weniger Tage (< 7 d) bis auf geringe Restmengen wieder aus. Pulsartige Expositionen mit einer hohen MP-Konzentration führen somit zwar zu einer hohen Aufnahme, durch die kontinuierliche Ausscheidung ist die Interaktion zwischen Muschel und MP jedoch zeitlich begrenzt.

Pulsartige Einträge hoher MP-Mengen sind in der Umwelt eher durch Einträge aus Punktquellen (z. B. stark MP-kontaminierte Industrieabwässer) zu erwarten und daher regional begrenzt. Bei diffusen Quellen kann hingegen von einem kontinuierlichen Eintrag ausgegangen werden. Trotz einer begrenzten Aufenthaltszeit der MP-Partikel in den Muscheln kann es durch diffuse Einträge (im Fall einer konstanten Bioverfügbarkeit der MP-Partikel für limnische Muscheln) somit zu einer chronischen Exposition der Tiere kommen.

Trotz des ausgeprägten Ausscheidungsverhaltens wird innerhalb von 7 d keine vollständige Exkretion der MP-Partikel erreicht. Studie 2 zeigte, dass kleine MP-Mengen (< 5 % der ursprünglich aufgenommenen Partikelzahl) auch noch bis zu einer Woche nach Beendigung der MP-Exposition in den getesteten limnischen Muschelarten nachweisbar waren. Frühere Studien von Gonçalves et al. (2019), Paul-Pont et al. (2016) sowie Ribeiro et al. (2017) zeigten vergleichbare Ergebnisse mit marinen Muscheln, wobei die Autoren noch eine Woche nach Ende einer MP-Exposition entsprechende Partikel im Magen, im Darm, in der Verdauungsdrüse sowie in den Kiemen nachweisen konnten. Zudem zeigten Browne et al. (2008), dass MP-Partikel bis zu 48 d nach Expositionsende im Hämolymphsystem nachweisbar waren. Dies deutet an, dass eine kontinuierliche Exposition der Muscheln mit MP nicht nur durch eine konstante Bioverfügbarkeit in der Umwelt, sondern auch durch einen Übergang von MP-Partikel in das Gewebe bzw. die Hämolymphe der Muscheln (mit anschließendem Verbleib der Partikel im Körper) der Muscheln eintreten kann.

Faktor *Körpergröße*: Neben der Expositionsdauer hängt die MP-Aufnahme der Muscheln auch mit deren Körpergröße zusammen. In Studie 2 nahmen im Hinblick auf die absolute Aufnahme größere Muscheln mehr MP-Partikel auf als kleine Muscheln. Dieser Trend kehrte sich jedoch um, wenn man die relative Aufnahme (Anzahl aufgenommener Partikel pro Körpergewicht) betrachtete. Der gleiche Zusammenhang traf zudem auch auf unterschiedlich große Individuen derselben Muschelart zu. Dieser Unterschied geht vermutlich darauf zurück, dass kleinere Muscheln (Arten bzw. Individuen innerhalb einer Art) im Vergleich zu größeren Muscheln eine relativ höhere Filtrationsrate besitzen (Jones et al. 1992, Kryger & Riisgård 1988). Im Hinblick auf Freilandpopulationen deutet dies an, dass auf Grund ihrer höheren relativen Aufnahme kleine Muschelarten bzw. kleinere Individuen einer Art stärker exponiert sind und diese Tiere stärker durch eine Interaktion mit MP betroffen sein könnten.

Faktor *MP-Partikelgröße*: Zusätzlich besteht auch zwischen der Größe der Partikel und der Körpergröße ein Zusammenhang. So nahmen kleinere Muschelarten bzw. kleine Individuen innerhalb einer Art im Mittel kleinere Partikel auf. Ursache hierfür könnten insbesondere morphologische Unterschiede sein, die die Funktionsweise des Filtrations- und Partikelselektionsmechanismus beeinflussen. Beispielsweise kann *D. polymorpha* mit ihren laterofrontalen Cirri auf den Kiemen Partikel mit einer Größe ≥ 1.5 µm aus dem Wasserstrom herausfiltern, während bei der größeren Süßwassermuschel *Anodonta cygnea* Partikel mit einer Größe von 4 µm zwischen den Cirri hindurchtreten (Kapitel 4.2 in Studie 2 (Anhang A2) bzw. Jones et al. 1992, Jørgensen 1990, Kiørboe & Møhlenberg 1981, Ward & Shumway 2004). Berücksichtigt man, dass das relative Vorkommen von MP-Partikeln in der Umwelt mit abnehmender Partikelgröße exponentiell ansteigt (Kapitel 2.1.2), so sind kleinere Muschelarten bzw. kleinere Individuen einer Muschelart in Freilandpopulationen möglicherweise vermehrt gegenüber MP exponiert, wodurch sich die Interaktion zwischen MP und den Individuen verstärken kann. Berücksichtigt man zudem die relativ höhere Gesamtaufnahme, so kann vermutet werden, dass in Folge gesteigerter Exposition kleinere Muschelarten bzw. -individuen auch stärker durch mögliche toxische Effekte betroffen sein könnten. Dies würde wiederum bedeuten, dass sich eine Exposition mit MP u.a. verstärkt auf juvenile Individuen in

Muschelpopulationen auswirkt, was im Fall ausgeprägter Toxizität langfristig zu einer Beeinträchtigung der Gesamtpopulation führen könnte.

Faktor *Abundanz von MP und weiteren Partikeln*: Ein weiterer wesentlicher Faktor, der die MP-Aufnahme in Muscheln beeinflusst, ist die Konzentration an MP-Partikeln bzw. die Anwesenheit zusätzlicher Partikel (z. B. Algenzellen). Mit zunehmendem MP-Vorkommen stieg auch die MP-Aufnahme durch die Muscheln in Studie 2 an. Bei einer steigenden Abundanz anderer Partikel (in Studie 2: Algenzellen) wiederum nahm die MP-Aufnahme ab. Insbesondere letzterer Zusammenhang könnte die MP-Aufnahme in Freilandpopulationen von limnischen Muschelarten erheblich beeinflussen. In Flüssen mit hohem Schwebstoffanteil bzw. im Fall einer gesteigerten Nahrungsverfügbarkeit (z. B. durch eine Algenblüte in oligotrophen Gewässern) könnte die MP-Aufnahme im Vergleich zu einem partikelarmen Süßgewässer reduziert sein.

Faktor *MP-Verteilung zwischen Wasser- und Sedimentphase*: Darüber hinaus ist auch die Verteilung zwischen der Wasser- und Sedimentphase (und damit der Weg der Partikelapplikation im Laborexperiment) entscheidend für die Aufnahme durch Muscheln, da diese die Bioverfügbarkeit der MP-Partikel beeinflusst. Auf Grund ihrer filtrierenden Lebensweise nehmen Muscheln ihre Partikel primär aus der Wasserphase auf. In geringem Umfang ist zusätzlich jedoch auch eine Aufnahme aus dem Sediment denkbar (Kapitel S6 in Studie 2, Anhang A2). Für die Nahrungsaufnahme erzeugen Muscheln einen internen Wasserstrom, durch den Wasser und in der Wasserphase gelöste Partikel durch den Inhalationssiphon in die Mantelhöhle transportiert werden. Neben einer Aufnahme durch den Siphon werden Wasser und Partikel (in kleineren Mengen) zusätzlich auch entlang des gesamten Randes der Muschelschale aufgenommen (Nichols et al. 2005). Wenn sich sedimentbewohnende Muschelarten, beispielsweise *S. woodiana* oder *A. anatina*, mit ihrem Fuß teilweise eingegraben haben, ist eine MP-Aufnahme somit auch aus dem Sediment durch Transport entlang des Fußes und in die Mantelhöhle denkbar. Studie 2 konnte nachweisen, dass der Aufnahmeweg von MP über das Sediment im Vergleich zu einer Aufnahme aus der Wasserphase jedoch geringere Bedeutung hat.

Wie in Kapitel 2.1.2 beschrieben, sind die MP-Konzentrationen in der Wasserphase von Flüssen um ein Vielfaches geringer als im Sediment. Die höheren Konzentrationen im Sediment sind somit nur für Muschelarten relevant, die sich im Sediment vergraben (z. B. *A. cygnea*, Hinz & Scheil 1972) und mit ihrem Sipho Wasser direkt oberhalb der Sedimentoberfläche aufnehmen bzw. durch ihre Grabbewegungen die Partikel aus dem Sediment resuspendieren. Sessile Arten, die sich über ihre Byssusfäden an Hartsubstrat befestigen (z. B. *D. polymorpha,* Stewart et al. 1998), haben hingegen meist geringeren Kontakt zum Sediment und sind daher hauptsächlich durch eine Exposition über die Wasserphase betroffen. Für Muscheln, die nicht in Sedimentnähe filtrieren, könnte MP somit nur begrenzt bioverfügbar sein, wodurch die Interaktion zwischen MP und diesen Muschelarten sowie daraus folgende toxische Effekte limitiert sein könnten.

Bisher sind nur sehr wenige wissenschaftliche Daten zum Vorkommen von MP in limnischen Muschelarten verfügbar. Schessl et al. (2019) konnten in den limnischen Muschelarten *D. polymorpha* sowie *D. bugensis* kein MP nachweisen (Abb. 4). Domogalla-Urbansky et al. (2019) zeigten für die limnische Muschelart *Unio pictorum* eine durchschnittliche Abundanz von 1,14 P Individuum^{-1}. Vergleichsweise höhere MP-Konzentrationen wurden in Individuen der Süßwassermuschel *A. anatina* in einer Freilandpopulation in Südschweden nachgewiesen (29,2 P Individuum^{-1}, Berglund et al. 2019). In Bezug auf ihr Körpergewicht enthielten die Individuen

einer Freilandpopulation von *C. fluminea* durchschnittlich 2,6 P (g Körpergewicht (feucht))$^{-1}$ (Su et al. 2018).

Auf Grund der begrenzten Verfügbarkeit von Umweltdaten werden hier zusätzlich die Daten aus Studie 2 herangezogen und extrapoliert. Im Experiment nahm *D. polymorpha* über 12 h bei einer Gesamtexpositionskonzentration von 0,9 P mL^{-1} (0,9x10^{6} P m^{-3}, jeweils 0,3 P mL^{-1} jedes Partikeltyps (5, 10, 45 µm PS-Sphären)) durchschnittlich 30,7 P Individuum^{-1} bzw. 12,8 P (g Körpergewicht (feucht, mit Schale))$^{-1}$ auf. Im Vergleich dazu betrug die Aufnahme bei einer zehnfach höheren Gesamtexpositionskonzentration (9 P mL^{-1}) 156,9 P Individuum^{-1} bzw. 72,8 P (g Körpergewicht (feucht, mit Schale))$^{-1}$.

Eine vergleichbare Untersuchung wurde auch mit der Muschelart *A. anatina* durchgeführt (nicht in Studie 2 publiziert; Methodik wie für *D. polymorpha* im Kapitel „Material und Methoden", Abschnitt „Microplastic concentration (experiment 4)", in Studie 2 (Anhang A2) beschrieben). Innerhalb einer 12-stündigen Exposition gegenüber 5, 10 und 45 µm PS-Sphären (jeweils 0,3 P mL^{-1}) nahm *A. anatina* durchschnittlich 270,7 P Individuum^{-1} und 6,01 P (g Körpergewicht)$^{-1}$ auf. Bei einer Gesamtexpositionskonzentration von 9 P mL^{-1} betrug die Aufnahme 2.294 P Individuum^{-1} bzw. 90,4 P (g Körpergewicht)$^{-1}$.

Legt man nun die aktuelle MP-Abundanz in der Wasserphase von Flüssen (0,17 (Rodrigues et al. 2019)–5,19x10^{5} P m^{-3} (Lahens et al. 2018), Kapitel 2.1.2) und eine Potenzfunktion[1] als Näherung für die Abhängigkeit zwischen MP-Expositionskonzentration und Aufnahme zu Grunde, so würde das MP-Vorkommen in *D. polymorpha* und *A. anatina* für aktuelle Umweltkonzentrationen in folgenden Konzentrationsbereichen liegen:

- *D. polymorpha:* 5,24x10^{-4}–20,8 P Individuum^{-1}/1,10x10^{-4}–8,48 P (g Körpergewicht (feucht))$^{-1}$
- *A. anatina*: 1,56x10^{-4}–162,4 P Individuum^{-1}/7,32x10^{-8}–3,14 P (g Körpergewicht (feucht))$^{-1}$

Bei benthischen Muscheln, wie z.B. *A. anatina*, ist es jedoch auch möglich, dass diese MP-Partikel nicht nur aus der Wasserphase, sondern auch aus dem Sediment aufnehmen können - wenn auch in reduzierter Anzahl (s.o.). Daher wurden die Aufnahmeergebnisse für *A. anatina* zusätzlich auch in Bezug auf die aktuelle MP-Abundanz in Flusssedimenten (1,00x10^{-2} (Minimum, Castañeda et al. 2014) und 1,62x10^{8} P m^{-3} (Maximum, Wang et al. 2018)) extrapoliert:

- *A. anatina*: 1,12x10^{-5}–33.539 P Individuum^{-1}/2,61x10^{-9}–2.714 P (g Körpergewicht (feucht))$^{-1}$

Insbesondere die Abschätzungen auf Basis aktueller MP-Konzentrationen in der Wasserphase von Flüssen zeigen eine hohe Übereinstimmung mit bisher publizierten Daten für limnische (s.o. sowie Abb. 4) und marine Muschelarten (Abb. 4). So wurden in marinen Arten bisher MP-Abundanzen von bis zu 126,5 P Individuum^{-1} (*Mytilus edulis*, Mathalon & Hill 2014) bzw. 20,0 P (g Körpergewicht)$^{-1}$ (*Amiantis umbonella*, Naji et al. 2018) nachgewiesen. In über 80 % der Studien betrug das MP-Vorkommen in den Muscheln jedoch ≤ 8 P Individuum^{-1} bzw. ≤ 3,0 P (g Körpergewicht)$^{-1}$. Diese MP-

[1] Studie 2 legt nahe, dass steigende MP-Konzentrationen die MP-Aufnahme durch Süßwassermuscheln erhöhen - der Anstieg ist jedoch nicht linear (Kapitel 2.2.1). Entsprechend wurde die Abhängigkeit zwischen MP-Aufnahme und MP-Konzentration hier durch Potenzfunktionen angenähert. Die zu Grunde gelegten Potenzfunktionen wurden aus der Aufnahme von *D. polymorpha* bzw. *A. anatina* bei einer Expositionskonzentration von 9x10^{5} bzw. 9x10^{6} P m^{-3} abgeleitet (s.o.).

Mengen sind vergleichbar mit dem oberen Ende der auf Basis der MP-Konzentrationen in Flüssen (Wasserphase) sowie den Aufnahmedaten aus Studie 2 extrapolierten Konzentrationsspannen.

Auf Grundlage dieser Vergleichsdaten kann vermutet werden, dass limnische und marine Muschelpopulationen in der Umwelt aktuell nur geringe MP-Mengen (mit wenigen Partikeln pro Individuum) enthalten. Eine Interaktion von MP und Muscheln in der Umwelt ist somit vorhanden, ihre Intensität ist vermutlich jedoch eher gering.

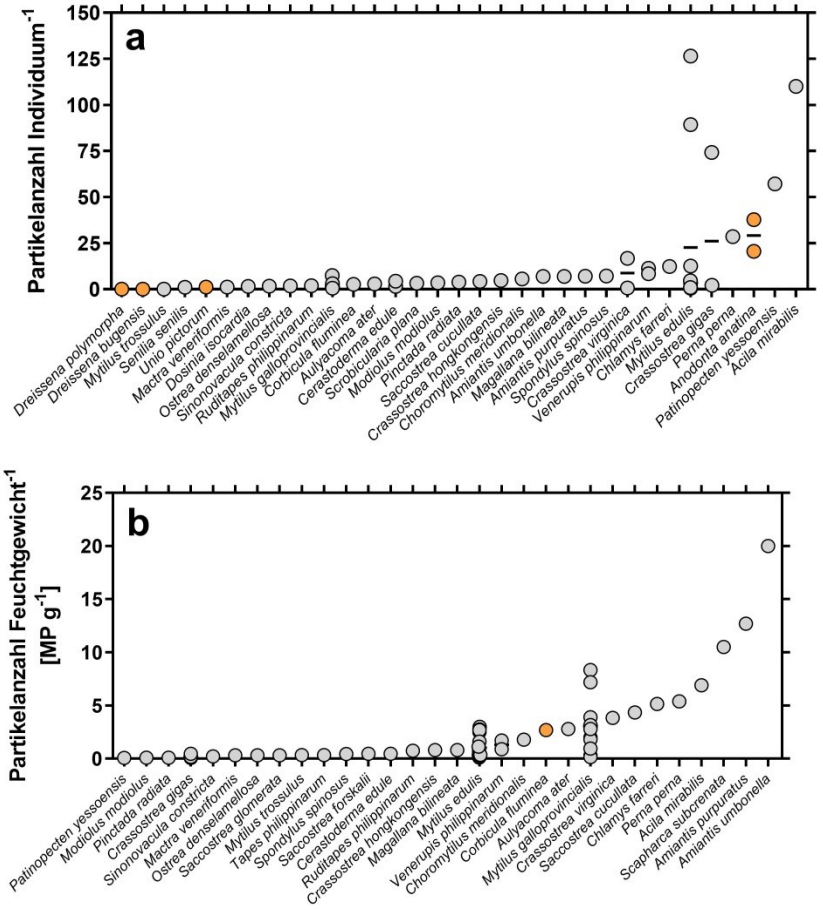

Abb. 4: (a) Absolute und (b) relative Abundanz (Partikel pro Feuchtgewicht) an Mikroplastik (MP)-Partikeln in Muscheln aus Freilandpopulationen. Orange = limnische Arten, grau = marine Arten. Die Daten wurden aufsteigend nach der durchschnittlichen absoluten bzw. relativen MP-Abundanz in den verschiedenen Muschelarten sortiert (Linie = Durchschnitt verschiedener Studien mit der gleichen Muschelart). Die der Abbildung zu Grunde liegenden Daten und Referenzen sind in Tab. A3 in Anhang A6 aufgeführt.

2.2.3 Mikroplastiktoxizität in limnischen Muscheln

Trotz eines breiten Spektrums getesteter Endpunkte, von der Molekularebene bis zum Individuum, konnte Studie 2 lediglich in Bezug auf die Filtrationsrate signifikante toxische Effekte auf die limnische Muschelart *D. polymorpha* nachweisen. Die Mortalität, Energiereserven bzw. der oxidative Stress blieben hingegen unbeeinflusst. Eine Erklärung für die begrenzten MP-Auswirkungen auf *D. polymorpha* könnte sein, dass die beobachtete Steigerung der Filtrationsrate möglicherweise eine Anpassung zur Kompensation von MP-Effekten (u.a. einer reduzierten Aufnahme von verdaulichen

Nahrungsbestandteilen durch die zusätzliche Aufnahme von MP-Partikeln) darstellt (siehe Diskussion in Studie 2, Abschnitt „Microplastic toxicity in *D. polymorpha*“, Anhang A2). Die begrenzten Effekte sind bemerkenswert, da die in Studie 2 eingesetzten MP-Konzentrationen oberhalb aktuell publizierter Umweltkonzentrationen liegen (Studie 2: $6{,}40 \times 10^{6}$–$1{,}0 \times 10^{11}$ P m^{-3}; höchste bisher publizierte MP-Konzentration in der Wasserphase von Flüssen: $5{,}19 \times 10^{5}$ P m^{-3} (Lahens et al. 2018), Abb. 2). Die Ergebnisse aus Studie 2 legen somit nahe, dass aktuelle PS-MP-Umweltkonzentrationen nur begrenzte Auswirkungen auf *D. polymorpha* haben. MP in der Umwelt ist für diese Muschelart daher möglicherweise bisher ein kaum relevanter Stressor.

Betrachtet man jedoch die weiteren bereits publizierten Studien zur MP-Toxizität in limnischen Muschelarten, lässt sich kein einheitliches Bild ableiten. Binelli et al. (2020), Guilhermino et al. (2018), Magni et al. (2018, 2019b, 2020) und Oliveira et al. (2018) wiesen anhand der limnischen Muschelarten *D. polymorpha* und *C. fluminea* MP-induzierte Effekte auf einige (jedoch meist nicht alle) getesteten Endpunkte nach. Die nachgewiesenen toxischen Effekte umfassten dabei Zelltoxizität sowie negative Einflüsse auf das oxidative Stresslevel, das Nervensystem, die Nahrungsaufnahme sowie die Entwicklung von Muschelembryonen und -larven. Hingegen konnten Rochman et al. (2017) und Baudrimont et al. (2020) bei einer Exposition der limnischen Muschelart *C. fluminea* mit MP-Fragmenten aus PET, PE, PVC und PS keine Auswirkungen auf die Filtrationsaktivität, Nahrungsaufnahme, Entgiftungsmechanismen (Cytochrom P450 (CYP450)) oder das endokrine System (Vitellogenin) feststellen. Zusammengefasst sind limnische Muschelarten in Laborstudien bisher somit von sehr unterschiedlichen Effekten mit stark schwankender Intensität betroffen gewesen.

Diese komplexen Toxizitätsmuster können verschiedene Ursachen haben, beispielsweise Unterschiede in der art- und individuenspezifischen Sensitivität, den Expositionsbedingungen (u.a. MP-Eigenschaften, Weg der Partikelapplikation, Expositionsdauer) oder der Sensitivität der getesteten Endpunkte (siehe Diskussion in Studie 2, Abschnitt „Microplastic toxicity in *D. polymorpha*“, Anhang A2). Möglicherweise kann die Effektintensität auch durch Anpassungen von Muscheln an hohe Schwebstoffmengen im Wasser beeinflusst werden. So können sich Muscheln beispielsweise durch Verhaltensänderungen (z. B. Anpassung der Filtrationsrate und Nahrungsaufnahme) bzw. physiologische (z. B. Kiemenoberfläche) sowie metabolische (z. B. Sauerstoffverbrauch) Veränderungen (Aldridge 1987, Summers et al. 1996) an hohe Konzentrationen suspendierter Partikel in der Wasserphase von Süßgewässern anpassen. Wie in Studie 2 diskutiert (Diskussion in Studie 2, Abschnitt „Microplastic toxicity in *D. polymorpha*“, Anhang A2), führen die beschriebenen Verhaltensanpassungen jedoch eher zu einer Verringerung als zu einer Steigerung der Filtrationsrate (wie im Fall der MP-Exposition beobachtet). Bisher ist somit unklar, inwieweit Anpassungsmechanismen gegenüber erhöhter Turbidität auch bei einer Exposition mit MP aktiviert werden. Zusätzlich muss beachtet werden, dass auch das Maß der Anpassung sowohl inter- als auch intraspezifisch variieren kann (Aldridge 1987, Payne et al. 1995). Ein umfassenderes Verständnis von MP-Toxizität in limnischen Muscheln kann daher nur erreicht werden, wenn Anpassungsmechanismen an hohe Partikelkonzentrationen in Süßgewässern verstanden und MP-Effekte anhand eines breiten Spektrums an Muschelarten unter vergleichbaren experimentellen Bedingungen untersucht werden.

Insbesondere die Vergleichbarkeit von Toxizitätsstudien stellt bisher jedoch eine große Hürde in der MP-Forschung dar. Bisherige Studien haben eine Vielzahl unterschiedlicher experimenteller Ansätze zur Untersuchung von MP-Toxizität auf Muscheln verwendet – ein direkter Vergleich einzelner

Studien war daher bisher nur selten möglich. Trotz der begrenzten Vergleichbarkeit wird in der MP-Forschung zunehmend das Instrument der Species Sensitivity Distribution (SSD) eingesetzt, durch das die Toxizitätsdaten mehrerer Arten zusammengefasst und verglichen werden können. In früheren Publikationen (z. B. Adam et al. 2019, Besseling et al. 2018, VKM et al. 2019) wurde das SSD-Konzept bereits auf MP-Toxizitätsdaten angewendet, jedoch immer artgruppenübergreifend. Diese Dissertation zeigt hingegen erstmals eine SSD spezifisch für die Artgruppen der Muscheln (Bivalvia) und Krebstiere (Crustacea, Kapitel 2.3.3).

In Bezug auf limnische Muschelarten ist die Menge der veröffentlichten Daten derzeit noch zu gering, um eine eigene SSD erstellen zu können. Daher wurden in diesem Rahmentext stattdessen Toxizitätsdaten von marinen und limnischen Muschelarten kombiniert, um die bisher verfügbaren Ergebnisse in einer gemeinsamen SSD zu visualisieren. Grundlage der SSD-Erstellung war eine systematische Literaturrecherche (PubMed-Suche: „microplastic“, „microplastics“, „nanoplastic“ oder „nanoplastics“ in Kombination mit „bivalve“ oder „mussel“) im April 2020. Insgesamt wurden 54 Studien zur MP- und NP-Toxizität in Muscheln identifiziert (Tab. A4 in Anhang A6, aus Vereinfachungsgründen wird trotz Einbeziehung von Studien zur NP-Toxizität im Folgenden der Begriff „MP-Toxizität“ verwendet). 14 Studien konnten auf Grund unzureichender Angaben nicht für die SSD berücksichtigt werden (Tab. A4). Die verbliebenen 40 Studien lieferten Daten zur MP-Toxizität in zwei limnischen (*D. polymorpha*, *C. fluminea*) und 15 marinen Arten (Abb. 5). Aus jeder der verfügbaren Studien wurde die LOEC („Niedrigste beobachtete Effektkonzentration“), die NOEC („Höchste Expositionskonzentration, bei der kein Effekt beobachtet wurde“) oder die LC_{50}/EC_{50} („mittlere letale Konzentration“/„mittlere Effektkonzentration“) ermittelt. Falls in einer Studie keine MP-induzierte Toxizität auftrat, wurde die höchste Expositionskonzentration als NOEC angenommen. Weiterhin wurden folgende Annahmen bzw. Datentransformationen vorgenommen:

- Wenn Studien Angaben sowohl zur akuten (< 21 d) als auch zur chronischen (≥ 21 d) Toxizität[2] enthielten, wurden die Ergebnisse der chronischen Expositionen priorisiert.

- Unterschiede zwischen Studien in Bezug auf die MP-Eigenschaften (Größe, Form, Polymerart), das Entwicklungsstadium der Individuen (Juvenile, Adulte) sowie die untersuchten Endpunkte (molekulare Ebene bis zum Individuum) blieben unberücksichtigt.

- In Studien, in denen die Expositionskonzentration nur als Massenkonzentration ((g Polymer) m^{-3}) angegeben war, wurde diese auf Basis der angegebenen Informationen zum Polymertyp, der durchschnittlichen Partikelgröße sowie unter Annahme einer kugelförmigen Partikelform in eine Partikelkonzentration (P m^{-3}) umgerechnet.

- Um akute und chronische Toxizitätsdaten zu homogenisieren bzw. LOEC, LC_{50} bzw. EC_{50} in NOEC umzuwandeln, wurden die Effektkonzentrationen mit Bewertungsfaktoren (Assessment Factors, AF) gemäß Adam et al. (2019) verrechnet. Die akuten Toxizitätskonzentrationen wurden durch einen AF von 10 dividiert, während die chronischen Daten unverändert blieben. Die LOEC, LC_{50} und EC_{50} wurden durch einen AF von 2 dividiert; die NOEC blieben unverändert.

[2] In der Ökotoxikologie gelten standardmäßig Expositionszeiten bis zu 96 h als akute, längere als chronische Exposition (Barron et al. 2008). Die hier zu Grunde gelegte, abweichende Unterteilung zwischen akuten und chronischen Expositionen bei einer Grenze von 21 d wurde, ebenso wie die Regeln für die Datentransformation, von Adam et al. (2019) übernommen, um eine Vergleichbarkeit zwischen der hier erstellten sowie der von Adam et al. (2019) berechneten SSD herzustellen. Die von Adam et al. (2019) gewählte Grenze von 21 d leiteten die Autoren aus dem OECD-Test Nr. 211 (*Daphnia magna* Reproduktionstest, OECD 2012) ab.

Die angepassten Daten wurden anschließend in eine kumulative Wahrscheinlichkeitsverteilung umgewandelt und die Schwellenkonzentration, oberhalb der bei 5 % der Arten ein Auftreten von MP-Toxizität zu erwarten ist (HC_5 + 95 % Konfidenzintervall (CI)), berechnet.

Die erstellte SSD (Abb. 5) spiegelt die großen Spanne der NOEC (10^3–10^{14} P m^{-3}) wider, die auf Basis der bisherigen Publikationen für MP-Toxizität in Muscheln ermittelt werden konnten. Basierend auf den derzeit veröffentlichten Daten sind *Mytilus coruscus* und *Ruditapes philippinarum* die am empfindlichsten auf eine MP-Exposition reagierenden Arten (Parolini et al. 2020, Wang et al. 2020a).

In *M. coruscus* verursachte eine bis zu 14-tägige Exposition gegenüber 2 µm PS-Sphären (10^7 P m^3) Veränderungen des oxidativen Stresslevels und der Aktivität der Verdauungsenzyme (Wang et al. 2020a). Die nächstniedrigere MP-Expositionskonzentration von 10^4 P m^{-3} verursachte hingegen keine derartigen Effekte. Unter Berücksichtigung des AF von 10 für das akute Expositionsszenario ergab sich eine NOEC von 10^3 P m^{-3}. Bei der Beurteilung dieser NOEC sollte jedoch berücksichtigt werden, dass sich die LOEC und NOEC um einen Faktor 1.000 unterschieden haben. Im Fall einer niedrigeren Konzentrationsspanne im Experiment hätte ggf. eine höhere NOEC beobachtet werden können.

R. philippinarum wurde über einen Zeitraum von 7 d gegenüber zwei Konzentrationen ($1{,}25 \times 10^5$ und $1{,}25 \times 10^7$ µg m^{-3}) an PET-Fragmenten (8–1.054 µm, Ø: 220 µm) exponiert (Parolini et al. 2020). Die höhere (LOEC), nicht jedoch die niedrigere (NOEC) der beiden Expositionskonzentrationen verursachte oxidativen Stress in den Muschelkiemen. Da Parolini et al. in ihrer Studie jedoch nur Massenkonzentrationen angegeben haben, mussten diese zur Erstellung der SSD in Partikelkonzentrationen ($1{,}62 \times 10^4$ und $1{,}62 \times 10^6$ P m^{-3}) umgerechnet werden. Für die Umrechnung wurde der durchschnittliche Partikeldurchmesser zu Grunde gelegt, welcher auf Grund des breiten Größenbereichs (s.o.) jedoch nur eine grobe Näherung darstellt. Zudem wurde in der Studie (ähnlich wie bei Wang et al. 2020a) erneut eine große Konzentrationsspanne (100x) eingesetzt, die ebenfalls zu einer sehr ungenauen Abschätzung der NOEC führt. Dies zeigt, dass die Studien zu *R. philippinarum* bzw. *M. coruscus* mit hohen Unsicherheiten behaftet sind. Insbesondere der linke Abschnitt der SSD, der die sensitivsten Arten abbildet, sollte daher nur als sehr grobe Abschätzung der tatsächlichen Toxizität der sensitivsten Arten interpretiert werden.

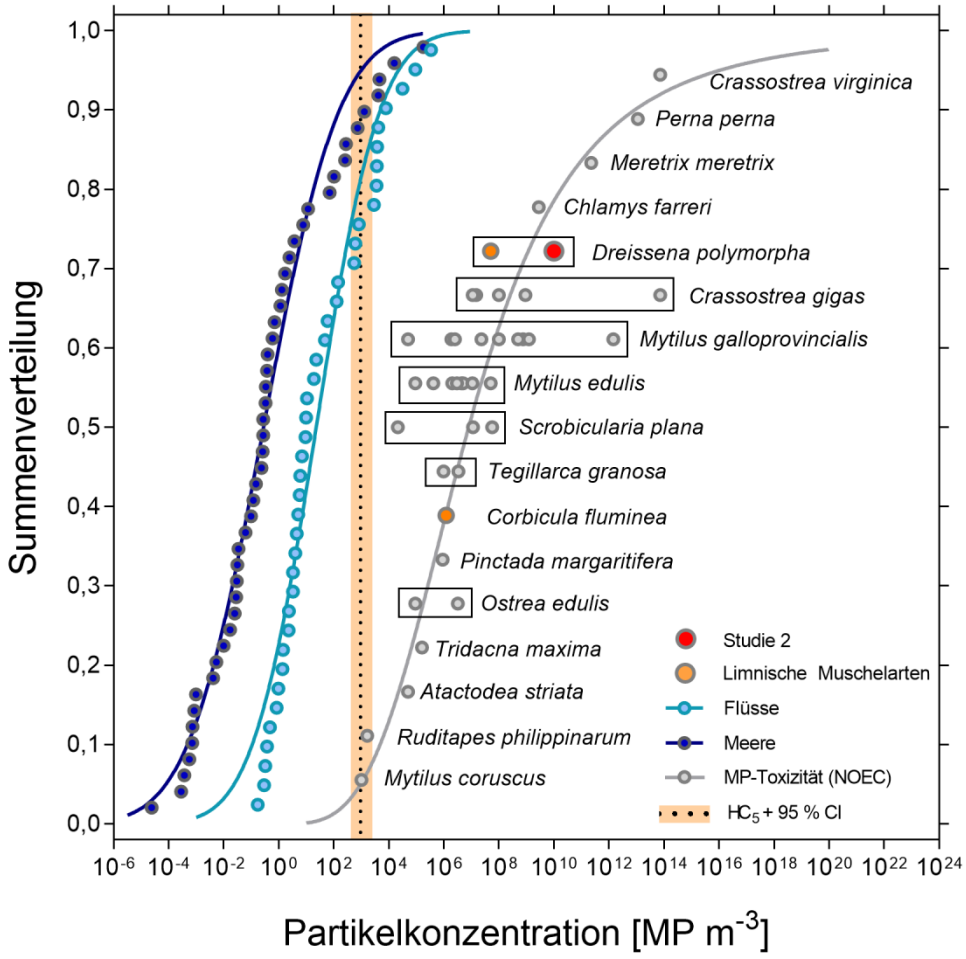

Abb. 5: Species sensitivity distribution (SSD) für die Toxizität von Mikroplastik (MP) in zwei limnischen (orange Punkte) und 15 marinen (graue Punkte) Muschelarten. Zur Erstellung der SSD wurde für jede Art der Durchschnitt der logarithmierten NOEC bestimmt, die Arten entsprechend dieser Durchschnittswerte ansteigend sortiert und anschließend gefittet. Kurvenfit (graue Line): Agonist vs. response - Variable Slope (four parameters, GraphPad Prism, Version 8.4.2). Gepunktete Linie (+oranges Band): HC_5 + 95 % Konfidenzintervall (CI). Die Angaben zum MP-Vorkommen in der Wasserphase von Flüssen (hellblau) und Meeren (dunkelblau) wurden aus Abb. 3a reproduziert.

In Studie 2 wurde mittels two-way ANOVA ein signifikanter MP-Effekt auf die Filtrationsaktivität von *D. polymorpha* nachwiesen. Die weiterführende Analyse mit einem Tukey post-hoc Test zeigte allerdings, dass die Filtrationsrate in der Testgruppe mit der höchsten MP-Konzentration ($1{,}0\text{x}10^{11}$ P m^3) zwar im Vergleich zur $6{,}4\text{x}10^6$-Exposition, nicht jedoch gegenüber der Kontrolle, signifikant erhöht war. Auf Grund der fehlenden Signifikanz im Vergleich zur Kontrolle wird die höchste MP-Konzentration (10^{11} P m^{-3}) daher als NOEC betrachtet. Diese NOEC liegt im oberen Drittel der SSD und ist somit 10^6–10^7-fach höher als die beobachteten NOEC für *M. coruscus* und *R. philippinarum*. Auch in Bezug auf die bisher bekannten Toxizitätsdaten für limnische Muscheln übersteigt die NOEC aus Studie 2 die NOEC für *C. fluminea* (Rochman et al. 2017, $1{,}2\text{x}10^6$ P m^{-3}) sowie *D. polymorpha* (Magni et al. 2018, $1{,}0\text{x}10^9$ P m^{-3}) um das etwa 10^5- bzw. 10^3-fache.

Über die Ursachen der unterschiedlichen MP-Toxizität in Muscheln kann zum jetzigen Zeitpunkt nur spekuliert werden. Gemäß Abb. 5 waren die NOEC weder für Arten aus einem ähnlichen Lebensraum

(z. B. *D. polymorpha* vs. *C. fluminea*, *M. edulis* vs. *Ostrea edulis* oder *Meretrix meretrix* vs. *R. philippinarum*) noch für taxonomisch eng verwandte Arten (*M. coruscus*, *M. edulis*, *Mytilus galloprovincialis*) vergleichbar. Dies impliziert, dass zwischen den MP-Auswirkungen und den Habitatansprüchen der getesteten Muschelarten bzw. deren Taxonomie kaum eine Verbindung besteht, sondern dass Anpassungen vielmehr art- oder sogar populationsspezifisch sein könnten. Darüber hinaus ist es aber auch möglich, dass die Unterschiede insbesondere auf die Vielzahl unterschiedlicher Expositionsszenarien, die in den MP-Toxizitätsstudien eingesetzt wurden (z. B. unterschiedliche Partikeleigenschaften, Expositionsdauern bzw. Endpunkte mit unterschiedlicher Sensitivität), zurückzuführen sind. Somit können sowohl experimentelle als auch ökologische Unterschiede eine denkbare Ursache für das komplexe Toxizitätsmuster in Muscheln sein.

Um die Relevanz der MP-Toxizität für Muschelpopulationen in der Umwelt näher abschätzen zu können, kann die aus der SSD abgeleitete HC_5 für die MP-Toxizität in Muscheln (924 P m^{-3}, 95 % CI: 423–2.624 P m^{-3}) mit der Häufigkeitsverteilung für das MP-Vorkommen in Flüssen und Meeren verglichen werden (Abb. 5). Basierend auf den bisher verfügbaren Daten zum MP-Vorkommen in der Umwelt wird an 5,1 % der Meeres- und 18,7 % der Flussprobestellen der Schwellenwert (HC_5) von 924 P m^{-3} bereits überschritten. Dies deutet darauf hin, dass zumindest ein kleiner Teil der marinen und limnischen Lebensräume derzeit bereits MP-Konzentrationen enthält, die bei den empfindlichsten 5 % der Muschelarten eine relevante Toxizität hervorrufen könnten.

Diese Schlussfolgerung unterliegt jedoch erheblichen Annahmen und Verallgemeinerungen, die bei der Diskussion stets mitberücksichtigt werden müssen. Zum einen wird MP für die Erstellung der SSD als einheitlicher Stressor betrachtet. Dadurch bleiben partikuläre Unterschiede, beispielsweise in Bezug auf Form, Größe bzw. Zusammensetzung, vollständig unberücksichtigt. Zum anderen wurde nicht zwischen verschiedenen Entwicklungsstadien oder betroffenen Endpunkten unterschieden. Somit trugen die Auswirkungen auf Moleküle, Zellen, Organe bzw. auf das gesamte Individuum gleichermaßen zur SSD bei. Im Umweltkontext haben molekulare und zelluläre Veränderungen jedoch meist eine geringere Reichweite als Veränderungen, die Organe oder das gesamte Individuum betreffen. Für eine separate Auswertung der Effekte nach betroffener Organisationsebene liegen bisher jedoch noch nicht ausreichend Ergebnisse aus früheren Studien mit limnischen oder marinen Muschelarten vor. Weiterhin basiert die SSD primär auf Daten für marine Muschelarten. Für limnische Arten ist die aktuelle Datenverfügbarkeit immer noch zu gering, um aussagekräftige Schlussfolgerungen zu ziehen. Nicht zuletzt sollte zudem beachtet werden, dass insbesondere der Bereich niedriger MP-Konzentrationen in der SSD (Bereich der sensitivsten Arten) mit großen Unsicherheiten verbunden ist und daher die abgeleitete HC_5 nur als grobe Näherung betrachtet werden sollte.

Basierend auf den bisher verfügbaren Daten für limnische Muscheln (Abb. 5 bzw. Studie 2, Rochman et al. 2017, Magni et al. 2018) übersteigen die ermittelten NOEC in allen Studien die bisher in der Umwelt beobachteten MP-Konzentration in der Wasserphase von Flüssen weltweit. Daher deuten die bisher verfügbaren Daten nicht darauf hin, dass die aktuellen MP-Vorkommen in limnischen Muscheln signifikante Toxizität auslösen.

Bei den bisher untersuchten limnischen Arten (*D. polymorpha*, *C. fluminea*) handelt es sich jedoch um invasive Arten (Quinn et al. 2014, Sousa et al. 2008), die ein hohes Ausbreitungspotential besitzen. Anhand invasiver Pflanzenarten konnte bereits gezeigt werden, dass invasive Arten eine höhere phänotypische Plastizität besitzen (Davidson et al. 2011). Diese erleichtert es diesen Arten, sich an

neue Umweltbedingungen bzw. Stressoren anzupassen. Es ist somit denkbar, dass sich invasive Muschelarten besser an MP als Stressor anpassen können als einheimische Muschelarten, die möglicherweise sensitiver auf eine MP-Exposition reagieren. Eine Überprüfung von MP-Toxizität in einheimischen europäischen Muschelarten ist jedoch schwierig, da Naturentnahmen auf Grund ihres Schutzstatus meist nicht gestattet sind und nur sehr selten Zuchten für die entsprechenden Arten bestehen. Trotz dieser Herausforderungen wären Toxizitätsdaten zu heimischen Muschelarten langfristig entscheidend, um einen Vergleich zu den Ergebnissen für invasive Muschelarten herstellen zu können.

Zusammenfassend kann daher nicht ausgeschlossen werden, dass besonders sensitive Muschelarten in Gewässern mit sehr hohen MP-Vorkommen bereits jetzt von MP-Toxizität betroffen sind. Ob dies auch spezifisch auf Muscheln in limnischen Lebensräumen zutrifft, kann auf Grund der geringen Anzahl verfügbarer Toxizitätsstudien aktuell nicht abschließend geklärt werden.

2.2.4 Kernerkenntnisse aus Kapitel 2.2

- Der Prozess von der Aufnahme bis zur Ausscheidung von MP-Partikeln läuft in limnischen Muscheln innerhalb von Stunden bis zu wenigen Tagen ab. In Studie 2 verblieben weniger als 5 % der aufgenommenen Partikel für sieben Tage oder länger in den Muscheln. Daraus kann geschlussfolgert werden, dass eine kontinuierliche (intraorganismische) Exposition von Muscheln eine dauerhafte Bioverfügbarkeit von MP und/oder einen Übergang von MP-Partikeln in das Gewebe bzw. die Hämolymphe der Muscheln (mit anschließendem Verbleib der Partikel im Körper) voraussetzt.
- Im Vergleich von großen und kleinen limnischen Muschelarten bzw. –individuen nahmen große Arten absolut, kleine Arten relativ (bezogen auf das Körpergewicht) mehr MP-Partikel auf. Daher könnten ggf. kleinere Muschelarten bzw. Juvenile einer Art im Besonderen von möglichen MP-Auswirkungen betroffen sein.
- Kleinere Muschelarten bzw. kleinere Individuen einer Art nahmen im Mittel kleinere MP-Partikel auf. Auf Grund der hohen Abundanz von MP-Partikeln (< 100 µm, Kapitel 2.1.3) könnten daher insbesondere kleinere Muscheln von MP-Aufnahme und –Toxizität betroffen sein.
- Steigende MP-Konzentrationen führen zu einer höheren MP-Aufnahme durch Muscheln, wobei der Zusammenhang zwischen MP-Konzentrationen und –Aufnahme nicht linear ist. Zukünftig steigende MP-Konzentrationen in der Umwelt werden daher zu einer wachsenden Exposition von limnischen Muscheln gegenüber MP führen, wobei sich die weitere Zunahme der MP-Aufnahme mit steigenden Konzentrationen abschwächen könnte.
- Eine gesteigerte Nahrungsverfügbarkeit (Algen) senkte in Studie 2 die MP-Aufnahme von Muscheln. Insbesondere in limnischen Gewässern mit hoher Primärproduktion bzw. in Jahreszeiten mit verstärktem Algenwachstum könnte die MP-Aufnahme daher ggf. reduziert sein.
- Limnische Muscheln nehmen MP-Partikel überwiegend aus der Wasserphase auf (Studie 2). Hohe MP-Abundanzen in der Sedimentphase (Kapitel 2.1.3) sind daher für Muscheln möglicherweise nur von begrenzter Relevanz.

- Aktuell in limnischen und marinen Muscheln nachweisbare MP-Abundanzen sind auf wenige MP-Partikel pro Individuum begrenzt (80 % der ausgewerteten Studien wiesen Abundanzen von < 8 Partikeln pro Individuum nach). Dies deutet an, dass Freilandpopulationen bisher möglicherweise nur begrenzt gegenüber MP exponiert sind.

- In Studie 2 führte eine Exposition von *D. polymorpha* mit PS-Fragmenten (≤ 63 µm) über bis zu 42 d bei einer MP-Konzentration von bis zu 100.000 P mL^{-1} zu einer signifikanten Steigerung der Fraßaktivität, während die MP-Exposition keine Veränderung der Mortalität, Energiereserven sowie des oxidativen Stresslevels verursachte. Die Süßwassermuschel *D. polymorpha* scheint somit nur begrenzt von PS-MP-Effekten betroffen zu sein.

- Publizierte MP- bzw. NP-Konzentrationen aus der Wasserphase der weltweit am stärksten mit MP kontaminierten aquatischen Lebensräume übersteigen bereits zum jetzigen Zeitpunkt die NOEC für einige (marine) Muschelarten. Daher kann nicht ausgeschlossen werden, dass besonders sensitive (marine) Muschelarten aktuell bereits von MP-Toxizität betroffen sind. Eine ganzheitliche Aussage zur Toxizität in limnischen Muscheln kann auf Grund der begrenzten Datenlage bis jetzt nicht getroffen werden.

2.3 Interaktion von Mikroplastik mit limnischen Krebstieren

2.3.1 Wichtige Forschungsergebnisse aus Studie 3 „PET microplastics do not negatively affect the survival, development, metabolism and feeding activity of the freshwater invertebrate *Gammarus pulex*“ (Weber et al. 2018, Anhang A3)[3]

Ähnlich wie die Tiergruppe der Muscheln steht auch die Gruppe der Krebstiere seit vielen Jahren im Fokus der MP-Forschung (Tab. A5 in Anhang A6). Wiederum lag der Fokus jedoch bisher überwiegend auf den marinen Arten, während sich die Erforschung von limnischen Crustaceen weitestgehend auf den Gemeinen und den Großen Wasserfloh (*Daphnia pulex* bzw. *D. magna*), zwei sehr beliebte Modellorganismen der aquatischen Ökotoxikologie (Seda & Petrusek 2011), beschränkte. In Studie 3 haben wir daher MP-Wechselwirkungen in einer bisher nicht berücksichtigten Art, dem Gewöhnlichen Flohkrebs (*Gammarus pulex*), untersucht. Die Wahl des Testorganismus fiel auch auf Grund der Ernährungsweise auf *G. pulex*, da diese Art kein Filtrierer (wie z.B. Daphnien, Kmeť & Straškraba 2004), sondern ein „Schredder“ (Zerkleinerer) ist. Diese zeichnen sich dadurch aus, dass sie grobes organisches Material, insbesondere Falllaub, in feine Partikel zerkleinern (Schlief & Mutz 2006). Wesentliches Ziel der Studie 3 war entsprechend herauszufinden, wie *G. pulex* MP (PET-Fragmente, 10–150 µm) größen- und konzentrationsspezifisch aufnimmt und welche Auswirkungen eine chronische Exposition auf die Mortalität, die Reproduktion, den Metabolismus sowie die Fraßaktivität von *G. pulex* hat. Folgende wesentliche Erkenntnisse wurden in Studie 3 gewonnen:

- Mit steigenden MP-Konzentrationen nahm *G. pulex* steigende MP-Mengen auf.
- Bei gleicher MP-Konzentration nahmen Juvenile absolut mehr MP-Partikel auf als adulte Individuen.
- Die MP-Partikelaufnahme war größenselektiv mit einer erhöhten Aufnahme von Partikeln < 53 µm.
- Eine chronische Exposition (48 d) gegenüber 0,8–3.530 P mL^{-1} ($8{,}0\times10^{5}$–$3{,}53\times10^{9}$ P m^{-3}) PET-Fragmenten verursachte keine signifikanten Effekte auf die Mortalität, die Fraßaktivität, die Energiereserven bzw. die Entwicklung von *G. pulex*.

2.3.2 Mikroplastikaufnahme durch limnische Krebstiere

Krebstiere bilden ein sehr großes Taxon, dessen Vielfalt sich u.a. in der Vielzahl an Ernährungsstrategien widerspiegelt. Diese reichen von Suspensions-, Substrat- und Detritusfressern bis hin zu Weidegängern (Thiel & Watling 2015). *G. pulex*, der Testorganismus für Studie 3, gehört zu den limnischen Amphipoden und ernährt sich als Zerkleinerer. Diese zerteilen grobes organisches Material durch Fraß bzw. Verdauung in feine Partikel und extrahieren dabei verdauliches Material (MacNeil et al. 2011). Amphipoden ernähren sich von einer großen Vielfalt toten organischen Materials, aber auch von Algen oder assoziierten Bakterien (Hargrave 1970). Während der Nahrungsaufnahme nehmen Amphipoden zudem einen hohen Anteil unverdaulicher Sedimente auf (Fenchel et al. 1975, Hargrave 1970). Als Ausgangspunkt für Studie 3 nahmen wir daher an, dass Amphipoden wie *G. pulex* auch unverdauliche MP-Partikel aufnehmen können. Entsprechend

[3] Studie 3 wurde nur zu Teilen im Rahmen dieser Dissertation erstellt (vgl. Anhang A3, „Erklärung zum Beitrag der Studie 3 zu dieser Dissertation“)

untersuchten wir die Aufnahme von PET-Fragmenten (10–150 µm) durch juvenile und adulte *G. pulex*-Individuen und analysierten deren Abhängigkeit von der MP-Expositionskonzentration.

Die Aufnahme von MP durch *G. pulex* war konzentrationsabhängig mit maximalen MP-Häufigkeiten von bis zu 13.600 P Individuum^{-1} bei einer Expositionskonzentration von 4.000 P mL^{-1} (4×10^{9} P m^{-3}). Bei umweltnäheren MP-Konzentrationen (0,8 P mL^{-1}/0,8×10^{6} P m^{-3} bzw. 40 P mL^{-1}/4×10^{7} P m^{-3}) wurden in adulten *G. pulex*-Individuen durchschnittlich 8,8 bzw. 771 P Individuum^{-1} und in juvenilen 28,0 bzw. 1.254 P Individuum^{-1} nachgewiesen.

Extrapoliert man die Aufnahmeergebnisse aus Studie 3[4] auf die aktuellen Umweltkonzentrationen in der Wasserphase von Flüssen (0,17 (Rodrigues et al. 2019)–5,19x10^{5} P m^{-3} (Lahens et al. 2018), Abb. 1), ergibt sich näherungsweise eine MP-Abundanz von 2,07x10^{-7}–5,37 P Individuum^{-1} in adulten und 9,17x10^{-6}–18,4 P Individuum^{-1} in juvenilen Individuen. Legt man hingegen die aktuellen Umweltkonzentrationen für die Sedimentphase (1,00x10^{-2} (Castañeda et al. 2014)–1,62x10^{8} P m^{-3} (Wang et al. 2018)) zu Grunde (benthische Krebsarten sind nicht nur gegenüber den MP-Partikeln in der Wasserphase, sondern auch gegenüber solchen in den Flusssedimenten exponiert), so ergibt sich eine Abundanz von 8,10x10^{-9}–3.816 P Individuum^{-1} in adulten und 5,84x10^{-7}–4.883 P Individuum^{-1} in juvenilen Individuen.

Bereits publizierte Daten zum MP-Vorkommen in aquatischen Krebstierpopulationen legen jedoch nahe, dass das MP-Vorkommen in Freilandpopulationen z.Z. eher auf wenige Partikel pro Individuum begrenzt ist (Abb. 6). Die limnische Garnelenart *Paratya australiensis*, für die Exemplare aus verschiedenen Populationen aus Bächen und Feuchtgebieten Südaustraliens untersucht wurden, enthielt durchschnittlich 0,52 P Individuum^{-1} (Nan et al. 2020). In marinen Krebstieren wurden im Vergleich zu *P. australiensis* höhere MP-Abundanzen festgestellt, die von 0,63 bis 79,5 P Individuum^{-1} variierten, wobei der überwiegende Anteil der Studien Abundanzen von < 8 P Individuum^{-1} nahelegt (Abb. 6). In Bezug auf die relative Aufnahme pro Körpergewicht wurden in Freilandpopulationen bisher 0,25–26,8 P (g Körpergewicht)$^{-1}$ in marinen Krebstieren (Tab. A5) bzw. 2,4 P (g Körpergewicht)$^{-1}$ in limnischen Krebsarten (*P. australiensis*, Nan et al. 2020) nachgewiesen.

[4] Vgl. Kapitel 2.2.2. Die Potenzfunktionen wurden aus der Aufnahme von adulten bzw. juvenilen *G. pulex*-Individuen bei einer Expositionskonzentration von 8x10^{5} bzw. 4x10^{7} P m^{-3} abgeleitet (vgl. Studie 3).

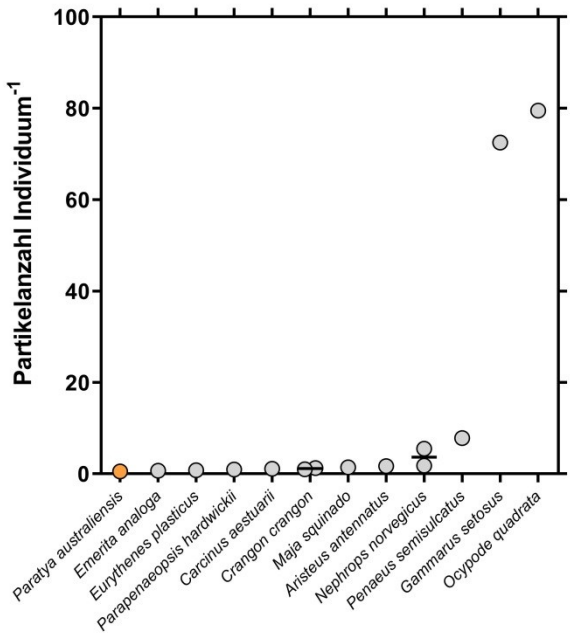

Abb. 6: Abundanz von Mikroplastik (MP)-Partikeln in Freilandpopulationen von marinen und limnischen Krebsarten. Orange = Limnische Arten, grau = marine Arten. Die Daten wurden aufsteigend nach der durchschnittlichen absoluten MP-Abundanz in den verschiedenen Krebsarten sortiert (Linie = Durchschnitt verschiedener Studien mit der gleichen Art). Die der Abbildung zu Grunde liegenden Daten und Referenzen sind in Tab. A5 in Anhang A6 aufgeführt.

Diese Daten liefern erste Hinweise darauf, dass sowohl limnische als auch marine Krebspopulationen in der Umwelt gegenüber MP exponiert sind. Das gegenwärtige MP-Vorkommen in Krebstieren scheint jedoch auf wenige Partikel pro Individuum begrenzt zu sein. Hierbei sollte allerdings berücksichtigt werden, dass in Studie 3 *G. pulex*-Jungtiere absolut mehr Partikel aufnahmen als adulte Individuen. Auch einige wenige Partikel könnten in juvenilen Tieren (auf Grund ihres geringeren Körpergewichts) größere Bedeutung als in adulten Individuen haben und für das Auslösen möglicher toxischer Effekte relevant sein.

2.3.3 Mikroplastiktoxizität in limnischen Krebstieren

In Studie 3 konnte trotz hoher Expositionskonzentrationen von bis zu 3.530 P mL^{-1} ($3{,}53 \times 10^{9}$ P m^{-3}) keine chronische Toxizität von PET-Fragmenten auf *G. pulex* festgestellt werden. Weder die Mortalität, die Entwicklung, der Metabolismus noch die Fraßaktivität wurden durch die MP-Exposition signifikant verändert. Diese Ergebnisse stehen im Widerspruch zu Ergebnissen aus früheren Studien mit limnischen Amphipoden (*Gammarus fossarum*, *Hyalella azteca*). Im Fall der Art *G. fossarum* führte eine 28-tägige Exposition gegenüber PMMA-Fragmenten ($3{,}33 \times 10^{8}$ P m^{-3}, 32–250 µm) bzw. PA-Fasern (500×20 µm, $2{,}84 \times 10^{8}$ P m^{-3}) zu einer (vorübergehenden) Verringerung der Fraßaktivität, der Assimilationseffizienz sowie der Gewichtszunahme (Blarer & Burkhardt-Holm 2016, Straub et al. 2017). Die gleiche Exposition gegenüber Polyhydroxybutyrat (PHB)-Fragmenten ($3{,}33 \times 10^{8}$ P m^{-3}, 32–250 µm) bzw. PS-Sphären ($1{,}25 \times 10^{10}$ P m^{-3}, 1,6 µm) bewirkte hingegen keine derartigen Effekte. In *H. azteca* verursachte eine 10-tägige Exposition sowohl gegenüber PP-Fasern (> $4{,}50 \times 10^{7}$ P m^{-3}, 20–75×20 µm) als auch gegenüber PE-Sphären (≥ $1{,}0 \times 10^{10}$ P m^{-3}, 10–27 µm) eine

erhöhte Mortalität. Zusätzlich führte eine chronische Exposition mit den PE-Sphären über 42 d bei einer Expositionskonzentration ≥ $0{,}5\times10^{10}$ P m^{-3} zu einer Veränderung des Wachstums und der Reproduktion. Somit variieren die bisher publizierten Ergebnisse zur MP-Toxizität in limnischen Krebsarten sowohl intra- als auch interspezifisch. Es bleibt allerdings unklar, wie diese Unterschiede in der Toxizität zustande kommen. Intraspezifische Unterschiede können beispielsweise auf Einflüsse des Testdesigns bzw. der MP-Eigenschaften, aber auch auf populationsbezogene Anpassungsunterschiede hindeuten. Interspezifische Unterschiede legen hingegen auch artspezifische Varietäten nahe. Wie bei limnischen Muscheln bleiben die Ursachen für das komplexe Muster unterschiedlicher Toxizitätslevel in MP-Studien bis jetzt jedoch ungeklärt.

Um trotzdem zumindest einen besseren Vergleich früherer Studienergebnisse zur MP-Toxizität in Krebsen zu erhalten, wurde (wie in Kapitel 2.2.3 für Muscheln beschrieben) eine systematische Literaturrecherche zur MP-Toxizität in marinen und limnischen Krebstieren durchgeführt (PubMed-Suche (April 2020): „microplastic", „microplastics", „nanoplastic" oder „nanoplastics" und „daphnia", „copepod", „crab", „crustacea", „prawn", „artemia", „isopod", „amphipod", „hyalella", „krill", „shrimp" oder „zooplankton") und eine SSD erstellt. Studien zur Toxizität von NP-Partikeln (< 1 µm) wurden ebenfalls einbezogen (aus Vereinfachungsgründen wird im Folgenden jedoch wieder allgemein von „MP-Toxizität" gesprochen).

Im Vergleich zu Muscheln liegt für Krebstiere eine deutlich höhere Anzahl an publizierten Studien vor. Insgesamt wurden 136 Studien (inklusive Studie 3) zur MP- und NP-Toxizität in Krebsarten identifiziert, wobei diese Ergebnisse zu sieben limnischen und 21 marinen Arten enthielten (einige der Studien enthielten Daten zu mehr als einer Art, Tab. A6 in Anhang A6). 26 Studien mussten auf Grund unzureichender Angaben (u.a. zu eingesetzten Partikelkonzentrationen oder zur Statistik) ausgeschlossen werden (Tab. A6). Die getroffenen Annahmen bzw. durchgeführten Datentransformationen waren die gleichen wie in Kapitel 2.2.3.

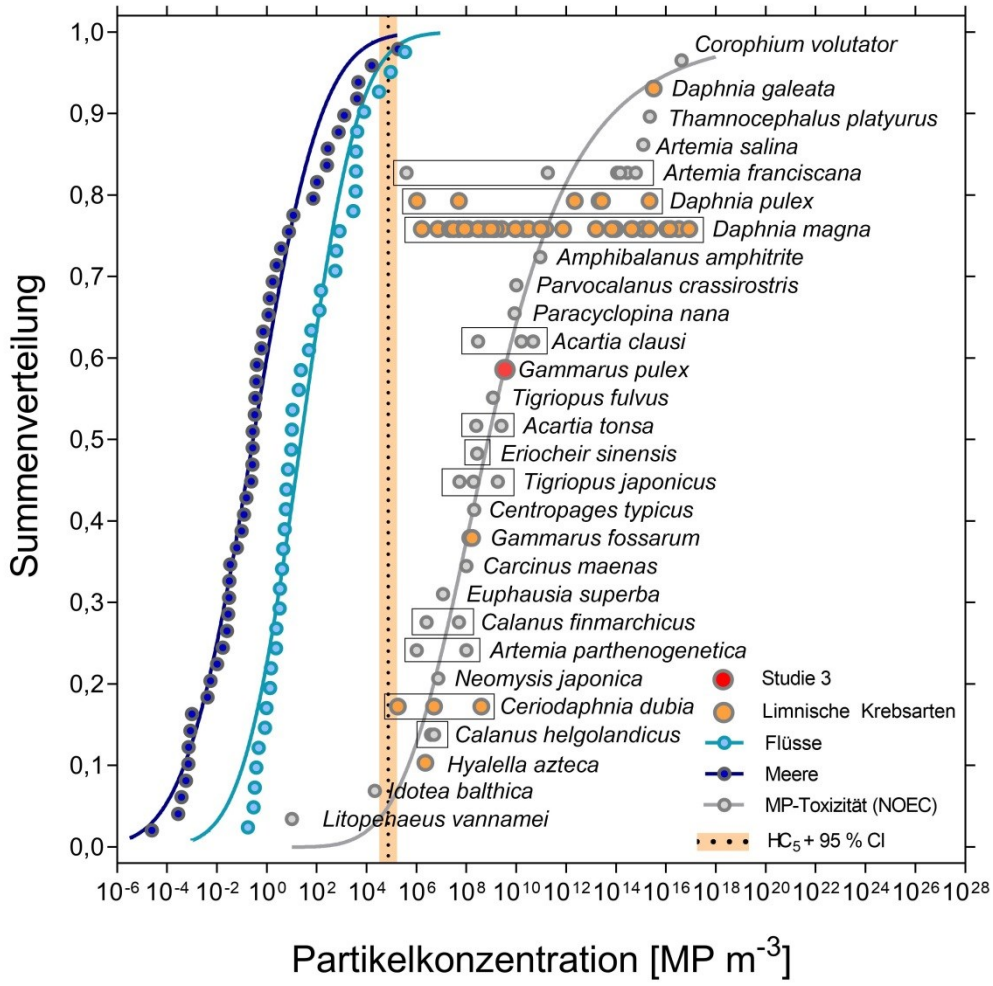

Abb. 7: Species sensitivity distribution (SSD) für die Toxizität von Mikroplastik (MP) in sieben limnischen (orange Punkte) und 21 marinen (graue Punkte) Krebsarten. Zur Erstellung der SSD wurde für jede Art der Durchschnitt der logarithmierten NOEC bestimmt, die Arten entsprechend dieser Durchschnittswerte ansteigend sortiert und anschließend gefittet. Kurvenfit (graue Line): Agonist vs. response - Variable Slope (four parameters, GraphPad Prism, Version 8.4.2). Gepunktete Linie (+oranges Band): HC_5 + 95 % Konfidenzintervall (CI). Die Angaben zum MP-Vorkommen in der Wasserphase von Flüssen (hellblau) und Meeren (dunkelblau) wurden aus Abb. 3a reproduziert.

Ähnlich wie bereits für Muscheln gezeigt, besteht auch bei Krebstieren eine große Spanne der aus früheren Studien abgeleiteten NOEC für die Toxizität von MP (9,95–4,14x10^{16} P m^{-3}), wobei *Litopenaeus vannamei* (Maharana et al. 2020) und *Idotea balthica* (Green 2016) die höchste und *Daphnia galeata* (Cui et al. 2017) bzw. *Corophium volutator* (Booth et al. 2016) die niedrigste Sensitivität gegenüber MP-Partikeln aufwiesen.

In *L. vannamei* verursachte eine 72-stündige Exposition gegenüber PE-Sphären (1 mm) bei einer Expositionskonzentration von 0,15 mg L^{-1} (≈ 99,5 P m^{-3}) einen Anstieg des oxidativen Stresslevels, während eine Exposition bei 0,05 mg L^{-1} keine vergleichbare Toxizität auslöste (Maharana et al. 2020). Unter Berücksichtigung des AF für akute Expositionen (Kapitel 2.2.3) ergab sich somit eine NOEC von 9,95 P m^{-3}.

Darüber hinaus wurde auch für die Art *I. balthica* eine vergleichsweise niedrige NOEC bestimmt. Eine 60-tägige Mesokosmenexposition von juvenilen *I. balthica* gegenüber HD-PE-Sphären (0,48–316 µm, Ø: 102,6 µm) führte bei einer Konzentration von $2{,}11\times10^{6}$ P m^{-3} zu einer signifikanten Veränderung der Individuenanzahl. Eine 100-fach niedrigere Expositionskonzentration führte hingegen zu keinen entsprechenden Veränderungen (NOEC: $2{,}11 \times 10^{4}$ P m^{-3}, Green 2016).

Für beide abgeleiteten NOEC mussten die MP-Partikelkonzentrationen aus den publizierten Massenkonzentrationen berechnet werden. Aufgrund der großen durchschnittlichen Partikelgröße (1 mm (Maharana et al. 2020) bzw. 102,6 µm (Green 2016)) waren die ermittelten Partikelkonzentrationen jedoch entsprechend niedrig. Im Fall der Studie von Green (2016) besaßen die Partikel zudem eine besonders heterogene Größenverteilung, weshalb eine Berechnung der MP-Partikelkonzentrationen auf Basis einer durchschnittlichen Partikelgröße von 102,6 µm nur eine sehr grobe Abschätzung der tatsächlichen Partikelkonzentrationen darstellt. Darüber hinaus setzte Green (2016) eine hohe Konzentrationsspanne (100-fach) ein. Im Fall einer niedrigeren Konzentrationsspanne hätte die ermittelte NOEC ggf. einer höheren MP-Konzentration entsprochen. Somit sind die ermittelten NOEC für die zwei sensitivsten Arten, ähnlich wie im Fall der SSD für Muscheln (Kapitel 2.2.3), mit großen Unsicherheiten behaftet und stellen daher nur eine grobe Abschätzung der tatsächliche Toxizität in den beiden Arten dar.

Im Vergleich zu *L. vannamei* und *I. balthica* wurde in Studie 3 keine chronische MP-Toxizität auf den limnischen Krebs *G. pulex* festgestellt, obwohl die eingesetzten MP-Konzentrationen von bis zu $3{,}5\text{x}10^{9}$ P m^{-3} um mindestens das 10^{5}-fach höher lagen als die NOEC für die zwei empfindlichsten Arten. Im Vergleich nimmt die in Studie 3 ermittelte NOEC ($3{,}5\text{x}10^{9}$ P m^{-3}) eine Position im mittleren bis oberen Bereich der SSD-Verteilung ein (Abb. 7) und liegt somit niedriger als die durchschnittliche NOEC für die beiden Wasserfloharten *D. magna* und *D. pulex* (*D. magna*: $1{,}8\text{x}10^{11}$ P m^{-3} , *D. pulex*: $8{,}4\text{x}10^{11}$ P m^{-3}) sowie höher als die NOEC für die limnischen Amphipodenarten *G. fossarum* und *H. azteca* (*G. fossarum*: $1{,}5\times10^{8}$ P m^{-3}, *H. azteca*: $2{,}2\times10^{6}$ P m^{-3}).

Ähnlich wie für Muscheln sind auch bei den Krebstieren zahlreiche Ursachen für die großen Unterschiede in der MP-Toxizität denkbar, darunter art- bzw. populationsbezogene Unterschiede in der Sensitivität, der Einsatz abweichender Expositionsszenarien bzw. die Analyse von Endpunkten mit unterschiedlicher Sensitivität. Die Ergebnisse aus 41 bzw. 9 Studien zur MP-Toxizität in *D. magna* bzw. *D. pulex* stützen insbesondere die letztere Ursache: Die große Spanne der ermittelten NOEC für *D. magna* ($1{,}6\text{x}10^{6}$–$8{,}5\text{x}10^{16}$ P m^{-3}) sowie *D. pulex* ($1{,}0\text{x}10^{6}$–$2{,}2\text{x}10^{15}$ P m^{-3}) deutet auf einen starken Einfluss der Expositionsbedingungen auf die Toxizitätsergebnisse hin. Aber auch populationsabhängige Ursachen sind denkbar. So ist bekannt, dass verschiedene Klone einer Daphnienart unterschiedliche Sensitivitäten aufweisen können (Sadler et al. 2019). Es bestehen somit mehrere denkbare Ursachen für die großen Unterschiede in der MP-Toxizität auf Krebstiere, deren individueller Beitrag zukünftig noch genauer bestimmt werden muss.

Zur Bewertung der aktuellen Umweltrelevanz von MP-Effekten können (ebenso wie für Muscheln) die im Rahmen der SSD-Erstellung ermittelten NOEC bzw. die abgeleitete HC_5 mit den bisher publizierten MP-Konzentrationen für die Wasserphase von Flüssen und Meeren verglichen werden (Abb. 7). Im Hinblick auf marine Ökosysteme wurde bisher nur für die marinen Arten *L. vannamei* (9,95 P m^{-3}, Maharana et al. 2020) und *I. balthica* ($2{,}11\text{x}10^{4}$ P m^{-3}, Green 2016) eine NOEC bestimmt, die im Bereich aktueller mariner MP-Konzentrationen liegt ($2{,}4\text{x}10^{-5}$ (Colton et al. 1974)–$1{,}82\text{x}10^{5}$ P m^{-3} (Song et al. 2015), Tabelle A2 in Anhang A6). Für alle weiteren Arten übertreffen die

ermittelten NOEC die aktuellen Umweltkonzentrationen in marinen Systemen. Ähnliches gilt auch für limnische Krebsarten. Lediglich auf Basis der Studie von Ziajahromi et al. (2017) mit dem Wasserfloh *Ceriodaphnia dubia* konnte eine NOEC ($1{,}8x10^5$ P m^{-3}) ermittelt werden, die im Bereich aktueller limnischer MP-Umweltkonzentrationen ($0{,}17–5{,}19x10^5$ P m^{-3}, Kapitel 2.1.2) liegt. Betrachtet man die aus der SSD ermittelte HC_5 für die Toxizität von MP in Krebstieren ($6{,}66x10^4$ P m^{-3}, 95 % CI: $3{,}93x10^4$–$1{,}21x10^5$ P m^{-3}), so übersteigen die gemessenen MP-Konzentrationen an 3,7 % der Flussprobestellen sowie 0,6 % der marinen Probestellen die ermittelte HC_5. Dies deutet an, dass bereits eine (wenn auch kleine) Schnittmenge zwischen den aktuell messbaren MP-Umweltkonzentrationen und den aus bisher publizierten Studien abgeleiteten NOEC besteht.

Bei der Interpretation der SSD zur MP-Toxizität in Krebstieren müssen jedoch wiederum die bereits in Kapitel 2.2.3 beschriebenen Annahmen und Limitationen berücksichtigt werden. Auf Grund der fehlenden Differenzierung zwischen verschiedenen MP-Eigenschaften, Organisationsebenen der untersuchten Endpunkte bzw. Entwicklungsstadien der Testorganismen sowie auf Grund der großen Unsicherheiten bei der Auflösung der SSD im Bereich der sensitivsten Arten stellt die SSD vermutlich nur eine grobe Näherung der tatsächlichen Sensitivitätsverteilung dar.

Andererseits wurden zur Bewertung der Umweltrelevanz die bisher gemessenen MP-Konzentrationen aus der Wasserphase von limnischen und marinen Ökosystemen herangezogen. Vielfach sind Krebstiere jedoch benthische Organismen, die im Grenzbereich zwischen der Wasser- und der Sedimentphase leben. Da MP-Konzentrationen im Sediment (zumindest in Flüssen, Kapitel 2.1.2) jedoch um ein Vielfaches höher sind als in der Wasserphase, könnte die bisher ermittelte Schnittmenge zwischen aktuellen Umweltkonzentrationen und NOEC bereits deutlich größer sein als hier zunächst abgeschätzt wurde. Auch wenn aktuell noch große Unsicherheiten in Bezug auf die Umweltrelevanz von MP-Toxizität bestehen, so ist es doch zumindest möglich, dass sensitive Krebsarten in limnischen und marinen Lebensräumen mit hohen MP-Vorkommen bereits jetzt von MP-Toxizität betroffen sein könnten.

2.3.4 Kernerkenntnisse aus Kapitel 2.3

- *Gammarus pulex* nahm in Studie 3 MP konzentrationsabhängig auf, wobei Juvenile absolut mehr Partikel aufnahmen als adulte Individuen. Auf Grund der höheren Aufnahme könnten juvenile Süßwasseramphipoden daher ggf. verstärkt von möglichen MP-Auswirkungen betroffen sein.

- Aktuell in limnischen und marinen Krebstieren nachweisbare MP-Abundanzen sind zumeist auf < 8 Partikel pro Individuum begrenzt. Dies deutet an, dass Freilandpopulationen bisher möglicherweise kaum gegenüber MP exponiert sind.

- Studie 3 zeigte, dass eine chronische Exposition (48 d) von *G. pulex* gegenüber PET-Fragmenten (10–150 µm) bei einer Konzentration von 0,8–3.530 P mL^{-1} zu keinen signifikanten Veränderungen der Mortalität, Fraßaktivität, Energiereserven sowie Entwicklung führte. Der Süßwasseramphipod *G. pulex* scheint somit nicht von PET-MP-Effekten betroffen zu sein.

- Publizierte MP- bzw. NP-Konzentrationen in der Wasserphase der weltweit am stärksten mit MP kontaminierten aquatischen Lebensräumen übersteigen bereits zum jetzigen Zeitpunkt die NOEC für einige wenige marine bzw. limnische Krebsarten. Daher kann nicht ausgeschlossen werden, dass besonders sensitive Krebsarten aktuell bereits von MP-Toxizität betroffen sind. Die getroffene Abschätzung anhand von MP-Konzentrationen in der Wasserphase könnte Toxizitäten zudem unterschätzen, da Krebse als Habitat z. T. den Grenzbereich zwischen Sediment und Wasserphase nutzen und insbesondere Sedimente höhere MP-Konzentrationen enthalten.

2.4 Interaktion von Mikroplastik mit limnischen Schnecken

2.4.1 Wichtige Forschungsergebnisse aus Studie 4„Ingestion and toxicity of microplastics in the freshwater gastropod *Lymnaea stagnalis*: No microplastic-induced effects alone or in combination with copper“ (Weber et al. 2021b, Anhang A4)

Die bisher publizierte Literatur zur Aufnahme und Toxizität von MP in aquatischen Organismen enthält nur sehr wenig Daten über das Taxon der Schnecken (Gastropoden, Sá et al. 2018). In Studie 4 untersuchten wir daher die MP-Aufnahme und die damit verbundene Toxizität auf limnische Schnecken, um neue Einblicke in die Interaktion zwischen MP und limnischen Gastropoden zu erhalten. Als Testorganismus wurde die limnische Lungenschnecke *Lymnaea stagnalis* gewählt, da sich diese Art überwiegend als Weidegänger (Amorim et al. 2019) und damit anders als die zuvor untersuchten Tiergruppen (Muscheln (Filtrierer) bzw. Süßwasseramphipoden wie *G. pulex* (Zerkleinerer)) ernährt.

Wesentliche Merkmale der Studie 4 waren die Untersuchung der größen- und zeitabhängigen Aufnahme und Ausscheidung von MP durch *L. stagnalis* sowie mögliche toxische Effekte, die durch eine chronische MP-Exposition ausgelöst werden. Durch Studie 4 konnten folgende neue Erkenntnisse gewonnen werden:

- *L. stagnalis* nahm MP während einer Exposition gegenüber PS-Sphären (5–45 µm, 36 P mL^{-1}) innerhalb eines Tages auf, konnte die aufgenommenen Partikel in einem vergleichbaren Zeitraum aber wieder ausscheiden.
- 5 µm PS-Sphären wurden (im Vergleich zu 10 und 45 µm Sphären) vermehrt aufgenommen.
- Eine chronische Exposition (28 d) von *L. stagnalis* gegenüber PS-Fragmenten (≤ 63 µm, 6,4–100.000 P mL^{-1}) verursachte geringfügige Veränderungen der Phagozytoseaktivität der Immunzellen der Schnecken bei einer niedrigen (6,4 P mL^{-1}), jedoch nicht bei hohen MP-Expositionskonzentrationen, während keine signifikanten Effekte auf das Überleben, die Reproduktion, die Energiereserven sowie das oxidative Stresslevel ausgelöst wurden.

2.4.2 Mikroplastikaufnahme durch limnische Schnecken

Aquatische Schneckenarten zeigen eine Vielzahl unterschiedlicher Ernährungsweisen, beispielsweise Beweidung, die Aufnahme suspendierter Nahrungspartikel oder Prädation (Saleuddin & Wilbur 1983). *L. stagnalis*, eine häufige Lungenschnecke (Pulmonata) in stehenden Gewässern (Glöer & Meier-Brook 2003), die bereits vielfach in ökotoxikologischen Studien eingesetzt worden ist (Amorim et al. 2019), ist ein Beispiel für einen typischen Weidegänger. *L. stagnalis* bewohnt die flachen Ränder stehender Gewässer, die über eine vielfältige Vegetation (und somit ein umfangreiches Nahrungsangebot) verfügen. *L. stagnalis* ernährt sich insbesondere vom Aufwuchs (Periphyton) auf biotischen und abiotischen Oberflächen, nutzt jedoch auch Makrophyten sowie filamentöse Algen als Nahrungsangebot (Bovbjerg 1968, Pieczyńska 2003). Das Abweiden von Oberflächen ist normalerweise mit der Aufnahme eines breiten Spektrums unterschiedlich großer Partikel verbunden. Die aufgenommenen Nahrungspartikel werden in einem spezialisierten Muskelmagen weiter zermahlen, bis sie eine für die Verdauung geeignete Größe besitzen (Carriker 1946). Darüber hinaus nehmen Lungenschnecken jedoch auch unverdauliche Partikel (insbesondere Sandkörner) auf

(Thomas 2001). Zu Beginn von Studie 4 bestand daher die Erwartung, dass *L. stagnalis* auch unverdauliche MP-Partikel aufnehmen kann.

Die Ergebnisse von Studie 4 bestätigten diese Vermutung: *L. stagnalis* kann MP mit unterschiedlicher Form (Sphären, Fragmente) sowie mit stark unterschiedlicher Größe (5–90 µm) aufnehmen, wobei kleine im Vergleich zu größeren MP-Partikeln vermehrt aufgenommen wurden. Die Gründe für die größenspezifische Aufnahme lassen sich vermutlich entweder auf eine artspezifische Präferenz für spezifische Partikelgrößen oder Unterschiede in der Bioverfügbarkeit der MP-Partikel (z. B. Partikelsedimentation, Adsorption an Nahrungsquellen) zurückführen. Die tatsächliche Ursache konnte in Studie 4 jedoch nicht abschließend bestimmt werden (Kapitel 4.1 in Studie 4, Anhang A4). Zusätzlich konnte Studie 4 zeigen, dass *L. stagnalis* fähig ist, bei entsprechender MP-Verfügbarkeit innerhalb von 24 h mehrere tausend MP-Partikel aufzunehmen. Ein Großteil dieser aufgenommenen Partikel kann jedoch innerhalb eines vergleichbaren Zeitraums auch wieder ausgeschieden werden – die Verweildauer von MP in *L. stagnalis* ist somit eher begrenzt. Nichtsdestotrotz könnte bei einer kontinuierlichen Exposition von *L. stagnalis* in der Umwelt eine fortlaufende Aufnahme und Ausscheidung von MP relevant sein.

Bisher ist das tatsächliche MP-Expositionsniveau in Freilandpopulationen limnischer Schneckenarten unbekannt. Daher kann die Abundanz in Freilandpopulationen aktuell nur auf Basis einer Extrapolation aus den Ergebnissen der Aufnahme- und Ausscheidungsexperimente in Studie 4 abgeschätzt werden. Nach einer 6–96 h Exposition gegenüber $3{,}60\times10^{7}$ P m^{-3} (5–45 µm PS-Sphären) enthielten *L. stagnalis*-Individuen im Mittel 966,6 P $Individuum^{-1}$ bzw. 7.425 P $(g\ Trockengewicht)^{-1}$. Unter Annahme einer linearen Beziehung[5] zwischen MP-Aufnahme und Expositionskonzentrationen würde man auf Basis der aktuellen MP-Umweltkonzentrationen (Wasserphase von Flüssen: 0,17 (Rodrigues et al. 2019)–$5{,}19\times10^{5}$ P m^{-3} (Lahens et al. 2018), Kapitel 2.1.2) in *L. stagnalis*-Freilandpopulationen MP-Konzentrationen von $4{,}56\times10^{-6}$–13,9 P $Individuum^{-1}$ oder $3{,}51\times10^{-5}$–107,0 P $(g\ Trockengewicht)^{-1}$ erwarten.

Neben MP in der Wasserphase ist für *L. stagnalis* als Weidegänger jedoch auch das MP-Vorkommen in den Flusssedimenten entscheidend. Extrapoliert man entsprechend die Daten zur MP-Aufnahme aus Studie 4 auf die aktuellen MP-Konzentrationen in den Sedimenten von Flüssen ($1{,}00\times10^{-2}$ (Castañeda et al. 2014)–$1{,}62\times10^{8}$ P m^{-3} (Wang et al. 2018)), so ergeben sich Konzentrationsspannen von $2{,}69\times10^{-7}$–4.350 P $Individuum^{-1}$ bzw. $2{,}06\times10^{-6}$–33.413 P $(g\ Trockengewicht)^{-1}$.

Insbesondere die auf Basis der MP-Konzentrationen in der Wasserphase extrapolierten Näherungswerte für *L. stagnalis* stimmen sehr gut mit bereits verfügbaren Daten zur MP-Abundanz in marinen Schnecken überein. In einer Population der Meeresschnecke *Littorina littorea* in der Bucht von Galway an der Westküste Irlands enthielten 64 % der getesteten Individuen MP-Partikel, wobei die ermittelten MP-Abundanzen an den verschiedenen Probestellen zwischen 0,59 und 2,40 P $Individuum^{-1}$ variierten (Doyle et al. 2019). In einer zweiten Studie wurde für eine *L. littorea*-Population an der Südwestküste der Niederlande eine durchschnittliche Abundanz von 20 P $(g\ Trockengewicht)^{-1}$ bestimmt (Leslie et al. 2017). Die gute Übereinstimmung zwischen den

[5] Im Gegensatz zu Studie 2 mit Süßwassermuscheln (Kapitel 2.2.2) bzw. Studie 3 mit Süßwasseramphipoden (Kapitel 2.3.2) wurde in Studie 4 die MP-Aufnahme von *L. stagnalis* nur für eine MP-Expositionskonzentration ($3{,}60\times10^{7}$ P m^{-3}) bestimmt, weshalb keine Potenzfunktion als Näherung für die Abhängigkeit der MP-Expositionskonzentration und der MP-Aufnahme bestimmt werden konnte. Stattdessen wurde die MP-Abundanz in aktuellen Freilandpopulationen auf Basis eines linearen Zusammenhangs extrapoliert.

extrapolierten Daten für *L. stagnalis* und den aktuellen MP-Expositionsniveaus in den Freilandpopulationen mariner Schnecken lässt vermuten, dass auch in limnischen Freilandpopulationen das aktuelle MP-Vorkommen auf einige wenige MP-Partikel begrenzt ist.

2.4.3 Mikroplastiktoxizität in limnischen Schnecken

Die Toxizität von MP in limnischen Schnecken ist, ebenso wie die Aufnahme, zum jetzigen Zeitpunkt nur wenig erforscht. Allerdings konnte bereits gezeigt werden, dass sich die Exposition gegenüber hohen Mengen an unverdaulichen Partikeln (hohe Turbidität im Gewässer) durchaus negativ auf limnische Schnecken auswirken kann (Harrison & Farina 1965). Bisher liegen jedoch nur Daten aus insgesamt sechs Studien (zwei zu marinen und vier zu limnischen Arten (inklusive Studie 4)) zur MP- und NP-Toxizität in Schnecken vor. Diese Datenmenge reicht bisher nicht aus, um eine aussagekräftige SSD zu erstellen. Folglich bleibt eine Diskussion der bisherigen Erkenntnisse auf einen qualitativen Vergleich beschränkt.

Nur in zwei der insgesamt vier Studien mit limnischen Schnecken konnte bisher MP-Toxizität (bzw. NP-Toxizität) nachgewiesen werden. In Studie 4 löste eine 28-tägige Exposition von *L. stagnalis* gegenüber PS-Fragmenten (≤ 63 µm) bei einer niedrigen (6,4 P mL^{-1}), nicht jedoch bei höheren MP-Konzentrationen eine Steigerung der Phagozytoseaktivität der Immunzellen aus. Die fehlenden Veränderungen bei höheren MP-Konzentrationen deuten an, dass die signifikanten Effekte möglicherweise nicht in Zusammenhang mit der MP-Exposition stehen. In der Süßwasserschnecke *Cipangopaludina cathayensis* lösten 0,7 µm PS-Sphären während einer 96 h-Exposition eine signifikant erhöhte Filtrationsaktivität aus (Qu et al. 2020). Die für die Experimente gewählte Expositionskonzentration von 20 mg L^{-1} (entspricht 1,1x10^{14} P m^{-3}) erscheinen im Vergleich zu bisher bestimmten MP-Konzentration im Wasser bzw. Sediment von Flüssen (Kapitel 2.1.2) jedoch zunächst vergleichsweise hoch. Da auf Grund der fehlenden technischen Möglichkeiten bisher jedoch keine Daten zu NP-Konzentrationen in der Umwelt vorliegen (Mattsson et al. 2018, Schwaferts et al. 2019), können entsprechend hohe Expositionskonzentrationen aktuell nicht ausgeschlossen werden.

Im Gegensatz dazu verursachte eine 96 h-Exposition von *L. stagnalis* gegenüber Nylon-6-Fragmenten (13–19 µm) keine Veränderung des Feuchtgewichts oder des Mikrobioms der getesteten Individuen (Horton et al. 2020), obwohl die Studie MP-Expositionskonzentrationen einsetzte, die die aktuellen Umweltkonzentrationen um ein Vielfaches überschritten (4,1x10^{9} P (kg Sediment)$^{-1}$; höchste Abundanz in Flusssedimenten: 7,5x10^{4} P (kg Sediment)$^{-1}$, Wang et al. 2018). Ebenso hatte auch eine 28-tägige Exposition der Süßwasserschnecke *Potamopyrgus antipodarum* gegenüber einer Mischung aus PA, PET, PC, PS und PVC keine Auswirkungen auf die Embryogenese oder die Juvenilentwicklung dieser Art (Imhof & Laforsch 2016).

Im Hinblick auf marine Schneckenarten konnten nur in einer der zwei bisher durchgeführten Studien signifikante MP-Effekte nachgewiesen werden. Während die Exposition einer *L. littorea*-Population gegenüber MP keinen Einfluss auf das Verhalten der Schnecken (Prädationsvermeidung) hatte (Doyle et al. 2020), konnten Lo & Chan (2018) signifikante Effekte von 2–5 µm PS-Sphären auf die Larvenentwicklung der Pantoffelschnecke *Crepidula onyx* beobachten. Diese Effekte traten jedoch nur bei Konzentrationen (6,0x10^{10}–1,4x10^{11} P m^{-3}) auf, die oberhalb aktueller mariner Umweltkonzentrationen lagen (≤ 1,82x10^{5} P m^{-3}, Song et al. 2015).

Folglich gibt es bisher nur sehr begrenzte Hinweise auf MP-Toxizität in limnischen oder marinen Schneckenarten bei den aktuell in der Umwelt bestehenden MP-Konzentrationen. Möglicherweise wird (wie in Kapitel 4.2 der Studie 4 (Anhang A4) ausführlich diskutiert) die MP-Toxizität in Schnecken durch Verhaltens- bzw. Stoffwechselanpassungen (z. B. verbesserte Nahrungsaufnahme und Verdauungseffizienz, Calow 1975) oder eine effiziente Selektion und Ausscheidung unverdaulicher Stoffe im Verdauungssystem begrenzt (Veldhuijzen 1974). Auf Grund der sehr begrenzten Datenlage kann aktuell jedoch nicht mit Sicherheit ausgeschlossen werden, dass empfindliche Schneckenarten durch MP-Toxizität betroffen sein könnten. Für die Organismengruppe der Schnecken sind daher weiterführende Untersuchungen erforderlich, um eine robuste Bewertung der MP-Toxizität in Gastropoden ausarbeiten zu können.

2.4.4 Kernerkenntnisse aus Kapitel 2.4

- *L. stagnalis* kann MP mit unterschiedlicher Form sowie Größe aufnehmen, wobei die MP-Aufnahme in Studie 4 größenabhängig war.
- *L. stagnalis* hat die Kapazität mehrere tausend MP-Partikel (≤ 90 µm) aufzunehmen. Extrapoliert man die Ergebnisse aus Studie 4 zur MP-Aufnahme auf aktuelle Umweltkonzentrationen in der Wasserphase von Flüssen, so beträgt die MP-Abundanz in Süßwasserschnecken vermutlich nur wenige MP-Partikel pro Individuum (≤ 13 Partikel). Diese Ergebnisse sind mit Abundanzdaten für Meeresschnecken vergleichbar. Für *L. stagnalis* als Weidegänger ist jedoch auch das MP-Vorkommen in den Flusssedimenten relevant. Bei hohen MP-Vorkommen in den Sedimenten könnte die MP-Aufnahme durch *L. stagnalis* entsprechend höher sein.
- Studie 4 mit *L. stagnalis* als Testorganismus zeigte, dass PS-MP (≤ 63 µm, 6,4–100.000 P mL^{-1}) während einer 28-tägigen Exposition eine geringfügige Veränderung der Phagozytoseaktivität der Immunzellen bei niedrigen, jedoch nicht bei hohen MP-Konzentrationen auslöste. Die Exposition verursachte keine signifikanten Effekte auf die Mortalität, die Reproduktion, die Energiereserven sowie das oxidative Stresslevel. Die Süßwasserschnecke *L. stagnalis* ist somit nur begrenzt von PS-MP-Effekten betroffen.
- Bisher sind nur sechs Studien zur MP-Toxizität in marinen und limnischen Schnecken verfügbar, von denen drei Studien signifikante Effekte nachwiesen (jedoch z. T. bei vergleichsweise hohen Expositionskonzentrationen). MP- und NP-Toxizität in Süßwasserschnecken kann daher aktuell nicht ausgeschlossen werden. Für eine genauere Abschätzung sind jedoch weiterführende Forschungsarbeiten notwendig.

2.5 Risikoabschätzung für Mikroplastik als Einzelstressor

Stellt MP somit nun ein „Risiko“ für die Umwelt dar? Um sich einer Antwort auf diese Frage nähern zu können, muss zunächst der Begriff „Risiko“ definiert werden. Je nach Forschungsfeld wird durch den Begriff „Risiko“ ein ganz unterschiedlicher Inhalt definiert. In der Entscheidungstheorie versteht man unter einer „Entscheidung unter Risiko“ eine Situation, bei der eine Person zwischen verschiedenen möglichen Umweltzuständen mit bekannter Eintrittswahrscheinlichkeit auswählt (Kahneman & Tversky 1979). Aus soziologischer Perspektive ist ein „Risiko“ eine Entscheidung unter Wissen der möglichen Folgen. Bei Gefahren liegt das Eintreten einer Folge hingegen nicht unter der Kontrolle des Entscheiders (Mensching 2018). In den Umweltwissenschaften wiederum wird ein „Risiko“ als die Wahrscheinlichkeit des Eintritts einer schädlichen Folge auf Grund einer Handlung oder eines Zustands verstanden. Das Risiko setzt sich dabei aus der Exposition (exposure) sowie dem Gefährdungspotential (hazard) zusammen (Muralikrishna & Manickam 2017).

Das Risiko von MP als Schadstoff für limnische Ökosysteme soll im Folgenden auf Grundlage der umweltwissenschaftlichen Definition abgeschätzt werden. Zur Bestimmung der „Exposition“ werden in Kapitel 2.5.1 die Daten zur Aufnahme von MP durch Muscheln, Schnecken und Krebstiere aus den Kapiteln 2.2.2, 2.3.2 sowie 2.4.2 zusammengetragen und übergreifend diskutiert. Das Gefährdungspotential, im Fall von MP also dessen Toxizität, wird in vergleichbarer Weise in Kapitel 2.5.2 (auf Basis der Ergebnisse aus den Kapiteln 2.2.3, 2.3.3 sowie 2.4.3) diskutiert.

2.5.1 Exposition aquatischer Invertebraten gegenüber Mikroplastik

MP-Partikel können durch eine Vielzahl an aquatischen Tiergruppen aufgenommen werden (GESAMP 2016). In den Studien 2 bis 4 konnte dies beispielsweise für die limnischen Tiergruppen der Muscheln, Schnecken und Krebstiere gezeigt werden. Für eine Einschätzung des MP-Risikos für limnische Ökosysteme ist im Folgenden nun wichtig, wie intensiv die einzelnen aquatischen Organismengruppen in der Umwelt gegenüber MP exponiert sind.

Die Exposition setzt sich hierbei zum einen aus dem Vorkommen von MP im Lebensraum der Organismengruppen und zum anderen aus der Aufnahme der Partikel durch die Organismen zusammen. In Kapitel 2.1.2 konnte gezeigt werden, dass in Flüssen insbesondere das Sediment eine Senke für MP ist. Somit könnten vor allem Benthosorganismen, wie z. B. Süßwasseramphipoden, einige Muschelarten sowie Schnecken, von MP-Partikeln umgeben und so verstärkt von einer Interaktion von MP mit den Oberflächenepithelien (Haut, Kiemen) betroffen sein (Kapitel 1.5). Wie in den Studien 2–4 für Muscheln, Krebstiere und Schnecken gezeigt (Kapitel 2.2.4 und 2.3.4, Anhang A4), nimmt mit zunehmender Expositionskonzentration auch die Aufnahme von MP-Partikeln zu. Extrapoliert man die MP-Abundanz in diesen Arten auf Basis aktueller MP-Konzentrationen in den Flusssedimenten, könnte man eine MP-Abundanz in diesen benthischen Arten von bis zu mehreren tausend MP-Partikel erwarten (Kapitel 2.2.2, 2.3.2, 2.4.2). Bei einer entsprechend gesteigerten Ingestion könnte es vermehrt zur MP-Interaktion mit den Darmepithelien und in begrenztem Maß auch zur Translokation der Partikel in die Körpergewebe kommen (Kapitel 2.2.2). Somit könnte MP in der Umwelt insbesondere für limnische Benthosorganismen ein relevanter Stressor sein.

Hierbei sollte jedoch berücksichtigt werden, dass nicht alle Benthosorganismen gleichermaßen gegenüber MP exponiert sind. Beispielsweise graben sich sedimentbewohnende Muschelarten zwar im Sediment ein, nehmen ihre Nahrung jedoch überwiegend aus der Wasserphase auf (Kapitel 2.2.4).

Krebse und Schnecken hingegen nutzen die Grenzfläche zwischen Wasserphase und Sediment bzw. die Oberfläche der Vegetation als Raum für ihre Nahrungssuche, wodurch diese Organismengruppen ggf. vermehrt gegenüber MP-Partikeln exponiert sind (Kapitel 2.3.4 bzw. Kapitel 2.4.2).

Neben der Bioverfügbarkeit beeinflusst möglicherweise aber auch die Ernährungsstrategie der getesteten Organismen (z. B. Filtrierer, Zerkleinerer, Weidegänger) die MP-Aufnahme. In einer Mesokosmenstudie von Setälä et al. (2016) mit marinen Benthosarten nahmen Muscheln (Filtrierer) im Vergleich zu Flohkrebsen (Amphipoden, Zerkleinerer) absolut höhere Mengen an MP auf. Ebenso hatten weidende Gastropoden in einer Aufnahmestudie von Scherer et al. (2017) eine höhere absolute Aufnahmerate als der Süßwasseramphipod *G. pulex*. Sowohl die Studie von Scherer et al. (2017) als auch von Setälä et al. (2016) konnten darüber hinaus aber auch zeigen, dass freischwimmende Krebsarten höhere absolute Mengen an MP aufnahmen als sedimentassoziierte Krebsarten – womöglich auf Grund der filtrierenden Ernährungsweise der getesteten freischwimmenden Krebsarten, durch die eine große Menge suspendierter Partikel aus der Wasserphase aufgenommen werden konnte. Dieser im Experiment beobachtete Effekt hat in der Umwelt möglicherweise aber weniger Relevanz, da die Bioverfügbarkeit von MP in der Wasserphase (zumindest in Flüssen) um ein Vielfaches geringer ist als in der Sedimentphase (Kapitel 2.1.2).

Diese publizierten Daten können nun mit Inhalten aus der Diskussion in Kapitel 2.2.2, 2.3.2 sowie 2.4.2 in Verbindung gesetzt werden. In diesen Kapiteln wurde auf Basis der Aufnahmeergebnisse in den Studien 2–4 eine Extrapolation der möglichen MP-Abundanz in Muscheln, Schnecken und Krebstieren bei aktuellen MP-Umweltkonzentrationen (Wasserphase) vorgenommen. Auf Basis der aktuellen MP-Konzentrationen in der Wasserphase von Flüssen weltweit (0,17 (Rodrigues et al. 2019)–5,19x10^5 P m^{-3} (Lahens et al. 2018), Kapitel 2.1.2) konnten für die einzelnen Arten folgende Spannen für das absolute MP-Vorkommen in Freilandpopulationen extrapoliert werden:

- *D. polymorpha* (2,5–3,0 cm; 2,0–3,5 g Feuchtgewicht)*:* 5,24x10^{-4}–20,8 P Individuum^{-1}
- *A. anatina* (6,0–9,0 cm; 16–40 g Feuchtgewicht): 1,56x10^{-4}–162,4 P Individuum^{-1}
- *G. pulex* (12,5–17,0 mm; 13,0–44,3 mg Feuchtgewicht): 2,07x10^{-7}–5,37 P Individuum^{-1}
- *G. pulex* (6,0–9,0 mm; 2,0–9,8 mg Feuchtgewicht): 9,17x10^{-6}–18,4 P Individuum^{-1}
- *L. stagnalis* (2,5–3,0 cm): 4,56x10^{-6}–13,9 P Individuum^{-1}

Die höchste MP-Abundanz kann somit in Individuen der Muschelart *A. anatina* erwartet werden, die jedoch im Vergleich zu den anderen getesteten Organismen auch das höchste Körpergewicht besitzen. Die absolute Aufnahme von *D. polymorpha*, *G. pulex* sowie *L. stagnalis* ist hingegen weitestgehend vergleichbar und deutet keine Unterschiede zwischen den verschiedenen Organismengruppen an.

Im Hinblick auf die relative Aufnahme ändert sich dies jedoch: Da die getesteten Individuen des Süßwasseramphipoden *G. pulex* ein vielfach geringeres Feuchtgewicht als *L. stagnalis* und *D. polymorpha* besitzen, ist die relative MP-Aufnahme in *G. pulex* entsprechend höher als in den anderen Organismengruppen. Eine vergleichbare Tendenz konnten wir bereits in Studie 2 (Kapitel 2.2.2.) im Rahmen eines Vergleichs der MP-Aufnahme durch unterschiedlich große Muschelarten bzw. Individuen einer Art nachweisen. Dies deutet an: Je kleiner die Organismen sind, desto höher ist möglicherweise die relative MP-Aufnahme. Es kann somit vermutet werden, dass eine Exposition mit MP insbesondere für Organismengruppen mit geringer Körpergröße relevant ist.

Zur Überprüfung der oben genannten Annahmen können bereits publizierte Literaturdaten herangezogen werden, wobei jedoch bisher nur sehr wenige Studien zum MP-Vorkommen in limnischen Organismen in der Umwelt zur Verfügung stehen. Diese verfügbaren Studien legen nahe, dass das MP-Vorkommen in limnischen Arten bisher noch sehr begrenzt ist. In Individuen der Muschelart *Unio pictorum* wurden durchschnittlich 1,14 P Individuum^{-1} (Domogalla-Urbansky et al. 2019) nachgewiesen. In der australischen Krebsart *P. australiensis* betrug das durchschnittliche MP-Vorkommen 0,52 P Individuum^{-1} (Nan et al. 2020). Das höchste MP-Vorkommen in einer Freilandpopulation einer limnischen Wirbellosenart wurde bisher in der Süßwassermuschel *A. anatina* beobachtet (29,2 P Individuum^{-1}, Berglund et al. 2019).

Auch im marinen Bereich wurden in der überwiegenden Anzahl der Studien in Muscheln, Krebsen und Schnecken nur einige wenige MP-Partikel pro Individuum nachgewiesen (Kapitel 2.2.2., 2.3.2, 2.4.2). Ausnahmsweise wurden jedoch schon Belastungen von mehr als 150 P Individuum^{-1} in Krebsen (*Ocypode quadrata*, Costa et al. 2019) bzw. Muscheln (*M. edulis*, Mathalon & Hill 2014) beobachtet. Es kann daher nicht ausgeschlossen werden, dass in Regionen mit hohen MP-Umweltkonzentrationen auch limnische Wirbellose MP in größeren Mengen aufnehmen.

Selbst im Fall einer verstärkten MP-Aufnahme wird das langfristige Vorkommen von MP in den Individuen jedoch stark von der Dauer der Exposition abhängen. Wie in Studie 2 und 4 gezeigt werden konnte (Kapitel 2.2.2, 2.4.2), sind die Retentionszeiten für MP im Verdauungstrakt von Muscheln und Schnecken sehr begrenzt. Innerhalb weniger Stunden bis Tage konnte jeweils ein großer Anteil der aufgenommenen Partikel durch die Organismen wieder ausgeschieden werden. Eine Akkumulation im Gewebe der Organismen scheint darüber hinaus möglich, aber begrenzt zu sein (Kapitel 1.5.1, 2.2.2). Hohe MP-Abundanzen in Organismen setzen somit eine kontinuierliche oder zumindest regelmäßige, pulsartige MP-Exposition voraus. Ohne eine kontinuierliche Exposition verbleibt die MP-Abundanz in den Organismen hingegen dauerhaft auf einem eher niedrigen Niveau.

Doch auch wenige MP-Partikel können durch Interaktion mit aquatischen Organismen möglicherweise bereits Toxizität in diesen auslösen. Im nächsten Kapitel sollen daher die Ergebnisse aus Kapitel 2.2.3, 2.3.3 und 2.4.3 zur Toxizität von MP auf limnische Organismengruppen noch einmal verglichen bzw. näher diskutiert werden.

2.5.2 Mikroplastiktoxizität in aquatischen Invertebraten

Die Toxizität von MP auf aquatische Organismen wurde im Rahmen dieser Dissertation anhand von drei Süßwasserinvertebraten (Studie 2–4) ermittelt: der Muschelart *D. polymorpha*, der Amphipodenart *G. pulex* sowie der Schneckenart *L. stagnalis*. Obwohl die Untersuchungen eine große Anzahl unterschiedlicher Endpunkte (Mortalität, Reproduktion, Nahrungsaufnahme, oxidativer Stress, Energiereserven, Immunzellaktivität) umfassten, konnten nur sehr wenige MP-induzierte Effekte nachgewiesen werden: So verursachte die Exposition mit MP-Partikeln eine Steigerung der Filtrationsaktivität von *D. polymorpha* und eine Veränderung der Phagozytoseaktivität der Immunzellen von *L. stagnalis*. Die begrenzte Anzahl von beobachten Effekten ist bemerkenswert, da in allen drei Studien MP-Konzentrationen eingesetzt worden sind, die publizierte Umweltkonzentrationen um ein Vielfaches übersteigen.

Für eine weiterführende Bewertung der Toxizität von MP auf limnische Organismen wurden die Ergebnisse aus Studie 2–4 im nächsten Schritt mit bereits publizierten Literaturdaten

zusammengeführt. Als geeignete Methode für eine entsprechende Aggregation bestehender Daten hat sich in den vergangenen Jahren das Konzept der SSD durchgesetzt. Auf Basis einer systematischen Literaturrecherche wurde in den Kapiteln 2.2.3 sowie 2.3.3 entsprechend jeweils eine SSD für die MP-Toxizität für Muscheln und Krebstiere erstellt. Dies ist eine Neuheit, da die bisher publizierten SSD in allen Fällen taxaübergreifend ausgestaltet waren, während die hier erstellten SSD das Ziel haben, eine konkrete Toxizitätsabschätzung für ein einzelnes Taxon zu ermöglichen (Tab. 1).

Die aus den SSD abgeleiteten HC_5 entsprachen dabei 924 P m^{-3} für Muscheln (95 % CI: 423–2.624 P m^{-3}) sowie 66.600 P m^{-3} für Krebstiere (95 % CI: 39.300–121.000 P m^{-3}) und unterschreiten somit in beiden Fällen die bisher höchsten, publizierten MP-Umweltkonzentrationen für die Wasserphase in Flüssen (519.000 P m^{-3} (Lahens et al. 2018), 187.000 P m^{-3} (Leslie et al. 2017)). Dies deutet an, dass die aktuellen Umweltkonzentrationen in den am stärksten mit MP belasteten Regionen bereits ausreichend wären, um toxische Effekte in den sensitivsten 5 % der Muschel- und Krebsarten auszulösen. Aktuelle MP-Umweltkonzentrationen im Sediment sind sogar noch höher, weshalb der überlappende Bereich zwischen aktuellen MP-Umweltkonzentrationen und der SSD für Muscheln bzw. Krebstiere ggf. noch größer sein könnte.

Neben den beiden SSD für Muscheln und Krebstiere ergaben vier der sieben bisher publizierten SSD (Besseling et al. 2018, Everaert et al. 2018, VKM et al. 2019, Zhang et al. 2020c) ebenfalls bereits HC_5, die niedriger als die von Leslie et al. (2017) bzw. Lahens et al. (2018) publizierten MP-Umweltkonzentrationen sind (Tab. 1).

Tab. 1: HC_5 zur Toxizität von Mikroplastik (MP) aus Kapitel 2.2.3 (für Muscheln) bzw. Kapitel 2.3.3 (für Krebse) im Vergleich zu Werten aus früheren Publikationen. 95% CI = 95 %-Konfidenzintervall, Q25 = 25 %-Quartil, Q75 = 75 %-Quartil.

	Autoren	**HC_5**	**Studien-anzahl für SSD**	**Effekte (Organisations-ebene)**	**Berücksichtigte Taxa**
1	Weber 2021 (Kapitel 2.2.3) (MP & NP)	924 P m^{-3} (95 % CI: 423–2.624 P m^{-3})	40 Studien	Molekül, Zelle, Organ, Organismus	Limnische/marine Arten (Mollusca)
2	Weber 2021 (Kapitel 2.3.3) (MP & NP)	66.600 P m^{-3} (95 % CI: 39.300–121.000 P m^{-3})	110 Studien	Molekül, Zelle, Organ, Organismus	Limnische/marine Arten (Arthropoda (Crustacea))
3	Zhang et al. 2020c (MP & NP)	24.600 P m^{-3}	11 Studien	Molekül, Zelle, Organ, Organismus	Limnische Arten (Chlorophyta, Crustacea, Cnidaria, Mollusca, Chordata)
4	Everaert et al. 2018 (MP & NP)	33.300 P m^{-3} (95 % CI: 360–13.943.000 P m^{-3})	14 Studien	Molekül, Zelle, Organismus	Marine Arten (Ochrophyta, Mollusca, Arthropoda, Echinodermata)
5	VKM et al. 2019 (MP & NP)	71.600 P m^{-3} (95% CI: 3.450–1.991.000 P m^{-3})	122 Studien	Molekül, Zelle, Organ, Organismus, Population	Limnische /marine Arten
6	Besseling et al. 2018 (MP & NP (≥ 0,1 µm)	113.000 P m^{-3} (95% CI: 13.000 – 1.000.000 P m^{-3})	10 Studien	Organismus	Limnische/marine Arten (Ochrophyta, Mollusca, Arthropoda, Echinodermata, Rotifera)
7	Koelmans et al. 2020	251.000 P m^{-3} (traditionelle SSD); 75.600 P m^{-3} (95% CI: 11.000 –521.000 P m^{-3}, korrigierte SSD)	16 Studien	Primär: Organismus, z.T. zudem: Molekül, Zelle, Organ	Limnische Arten (Arthropoda, Spermatophytina, Cyanobacteria, Chordata)
8	Adam et al. 2019 (MP & NP)	740.000 P m^{-3} (Q25: 610.000 P m^{-3}, Q75: 1.300.000 P m^{-3})	27 Studien	Organismus	Limnische Arten (Chlorophyta, Spermatophytina, Arthropoda, Mollusca, Chordata)
9	Burns & Boxall 2018	64.000.000 P m^{-3}	17 Studien	Organismus	Limnische/marine Arten (Chlorophyta, Mollusca, Arthropoda, Echinodermata, Rotifera, Chordata)

Ein Vergleich der ermittelten HC_5 verdeutlicht, dass die HC_5 erwartungsgemäß niedriger ausfällt, wenn für die SSD neben Endpunkten auf der Organismenebene auch suborganismische Endpunkte berücksichtigt werden. Abgesehen von den Ergebnissen von Besseling et al. (2018) unterschreiten bisher nur HC_5 von SSD, die Endpunkte auf Ebene des Moleküls bis hin zum Individuum gleichermaßen erfassen, die aktuellen Umweltkonzentrationen. Im Hinblick auf die Relevanz für Freilandpopulationen bedeutet dies, dass sich in stark mit MP belasteten Gewässern induzierte Effekte möglicherweise eher auf niedriger Organisationsebene (molekular, zellulär) äußern und in der Umwelt dadurch weniger deutlich in Erscheinung treten, als wenn sich die Effekte auf Individualebene darstellen würden.

Der nähere Vergleich der HC_5 zeigt jedoch auch, dass die HC_5 für Muscheln um das mehr als 25-fache kleiner als die bisher publizierten HC_5 anderer SSD ist. Dies könnte andeuten, dass unterschiedliche

Taxa verschieden stark durch MP-Toxizität betroffen sind. Muscheln könnten dabei eine entsprechend sensitive Organismengruppe darstellen.

Alle benannten Schlussfolgerungen unterliegen jedoch den erheblichen Unsicherheiten, die auf Grund der Annahmen und Verallgemeinerungen bei der Erstellung der SSD sowie beim Abgleich mit den Umweltkonzentrationen eingegangen wurden. Die Unsicherheiten aus der SSD-Erstellung wurden bereits in Kapitel 2.2.3 benannt: Zusammengefasst entstehen bei der SSD-Erstellung erhebliche Unsicherheiten durch die Betrachtung von Plastikpartikeln als einheitlicher Stressor (partikuläre Unterschiede (Form, Größe, Oberfläche) sowie die chemischen Zusammensetzung bleiben unberücksichtigt), durch eine fehlende Unterscheidung zwischen verschiedenen Entwicklungsstadien (wodurch sensitive Stadien unidentifiziert bleiben), durch Annahmen und Näherungen bei der Bestimmung der NOEC sowie in einigen SSD die fehlende Unterscheidung zwischen Endpunkten auf verschiedenen Organisationsebenen (Molekül, Zelle, Organ, Organismus). Darüber hinaus blieben Effekte auf Populationsebene bzw. Ökosystemebene bei der SSD-Erstellung bisher weitestgehend unberücksichtigt (abgesehen von VKM et al. 2019). Insbesondere Erkenntnisse zu Effekten auf die Populationen verschiedener aquatischer Organismen bzw. auf die unterschiedlichen aquatischen Ökosysteme wären jedoch langfristig notwendig, um die Relevanz einer MP-Belastung auf die Umwelt und die Auswirkungen auf die verschiedenen Organismengruppen abschließend bewerten zu können. In Zukunft sollten sich die Forschungsansätze daher verstärkt an populations- bzw. ökosystemspezifischen Ansätzen orientieren.

Für die Interpretation der bisher ermittelten HC_5 wurden diese mit publizierten Daten aus der Wasserphase von Flüssen weltweit abgeglichen. Auch diese Näherung ist mit Unsicherheiten verbunden, da sich die ermittelten HC_5 nicht nur auf limnische, sondern auch auf marine Arten beziehen (Tab. 1). Ein wesentlicher Grund hierfür ist die aktuell immer noch relativ niedrige Anzahl an Toxizitätsstudien mit limnischen Organismen. Eine wesentlich größere Unsicherheit entsteht jedoch durch die Unterschiede der betrachteten Partikelgrößen: Die bisher erstellten SSD bezogen meist sowohl MP- als auch NP-Toxizität ein. Die Umweltkonzentrationen, mit denen sie verglichen wurden, beziehen sich jedoch nur auf MP-Partikel, wobei der Größenbereich < 100 µm vielfach unterschätzt wird (Kapitel 2.1.2). Die tatsächlichen Umweltkonzentrationen könnten somit sogar deutlich höher sein als bisher angenommen, wodurch aktuelle toxische Effekte in der Umwelt unterschätzt werden würden.

Nicht zuletzt ergeben sich auch aus der Datengrundlage der erstellten SSD erhebliche Unsicherheiten. Laborexperimente können jeweils nur in begrenztem Maße die tatsächlichen Bedingungen in der Umwelt abbilden. Einer der wesentlichsten Unterschiede besteht in der Expositionszeit: Während Organismen in der Umwelt möglicherweise über viele Monate, Jahre, lebenslang oder sogar über viele Generationen gegenüber teils stark schwankenden MP-Konzentrationen exponiert sind, wurde ein überwiegender Anteil der bisher durchgeführten Studien als Akutexpositionen mit gleichbleibenden MP-Konzentrationen durchgeführt. Mehrmonatige Studien wie von Welden & Cowie (2016, 8-monatige MP-Exposition der Krebsart *Nephrops norvegicus*) sind hingegen rar. Bei der Erstellung der SSD können diese durch die verkürzte Expositionszeit entstehenden Unsicherheiten daher nur mit Hilfe eines Bewertungsfaktors (AF) „abgepuffert“ werden (Kapitel 2.2.3), wobei unklar bleibt, ob dieser Faktor tatsächlich ausreicht, um eine adäquate Umrechnung für eine chronische bzw. lebenslange Exposition zu erreichen.

Zusätzlich unterscheiden sich vielfach auch die eingesetzten MP-Partikel. Während Laborstudien MP-Toxizität vielfach mit sphärischen Partikeln testen, sind in der Umwelt neben Sphären auch Fragmente, Fasern und Folien vertreten (Kapitel 2.1.2). Mögliche von der MP-Form abhängige Effekte, beispielsweise durch eine unregelmäßige Oberfläche, die ggf. zu einer verstärkten Interaktion zwischen MP-Partikel und Organismus führt (Kapitel 1.5.2), bleiben dadurch unerkannt. Die Anwendung monodisperser Sphären hat zudem den Nachteil der einheitlichen Partikelgröße. Im Experiment wird meist eine Größe gewählt, die für den zu testenden Organismus bioverfügbar ist. In der Umwelt ist das Größenspektrum der vorhandenen MP-Partikel um ein Vielfaches größer, wobei für die einzelnen Arten bzw. Artgruppen meist jeweils nur ein Ausschnitt des Gesamtspektrums relevant ist.

Diese Problematik wird auch im Zusammenhang mit der Erstellung von SSD durch Koelmans et al. (2020) diskutiert. Die Autoren kritisieren, dass bei der Erstellung der meisten SSD MP-Partikel unterschiedlicher Größe, Form und Dichte als einheitlicher Stressor angesehen werden und dass unberücksichtigt bleibt, dass bestimmte Organismengruppen auf Grund ihrer Physiologie nur einen Teil der in der Umwelt verfügbaren MP-Partikel überhaupt aufnehmen können. Koelmans et al. argumentierten, dass die „klassischen SSD“ und die daraus abgeleiteten HC_5 „fundamental fehlerhaft und weitestgehend bedeutungslos“ sind, da die berücksichtigten Studien keine Vergleichbarkeit untereinander besitzen. Diese Schwachpunkte behoben die Autoren in ihrer Studie durch Korrekturen, indem sie die Effektkonzentrationen aus den publizierten Studien, die anhand von MP-Sphären bestimmt wurden, in „umweltrelevante“ Effektkonzentrationen umrechneten. Die Korrekturen berücksichtigten dabei die Größenlimitation der Aufnahme in verschiedenen Arten sowie die dadurch begrenzte Bioverfügbarkeit der MP-Partikel in der Umwelt. Ein Vergleich der „klassischen“ und der „verfeinerten“ SSD zeigte, dass der HC_5 für die „verfeinerte“ SSD um das Dreifache niedriger lag als für die „klassische“ SSD (Tab. 1). Dies verdeutlicht, dass die „klassische“ Ableitung von SSD durchaus mit größeren Unsicherheiten behaftet ist. Allerdings liegen beide von Koelmans et al. abgeleiteten HC_5 in der Spanne bisher publizierter HC_5 (Tab. 1). Es kann Koelman et al (2020) daher widersprochen werden, dass „klassische SSD“ „weitestgehend bedeutungslos sind“. Trotz der ausgeprägteren Unsicherheiten, die mit ihnen verbunden sind, führen sie trotzdem zu einer Risikoabschätzung mit gewisser Aussagekraft.

Alle bisher diskutierten Ansätze zur Bewertung der MP-Toxizität auf die Umwelt, unabhängig ob „klassisch“ oder „verfeinert“, vernachlässigen jedoch einen wesentlichen Aspekt: Die Umwelt ist im Regelfall eine Umgebung mit multiplen Stressoren. Sowohl physische (z. B. Hitze, Kälte, Stürme) als auch chemische (z. B. Umweltschadstoffe) Stressoren können gemeinsam mit MP auf aquatische Organismen einwirken. Bereits bei zwei Stressoren, die gleichzeitig auf einen Organismus einwirken, sind vielfältige Effektmuster denkbar: Neben einer unabhängigen Einwirkung der beiden Einzelstressoren auf den Organismus sind auch interaktive Effekte denkbar, bei denen sich die Effekte der beiden Stressoren gegenseitig verstärken oder abschwächen. Um die Auswirkungen von MP in einer Umgebung mit multiplen Stressoren genauer verstehen zu können, wurden im Rahmen der Studien 4 und 6 zwei Multi-Stressor-Experimente durchgeführt. Deren Ergebnisse werden im Kapitel 2.6 näher beschrieben und diskutiert.

2.5.3 Kernerkenntnisse aus Kapitel 2.5

- Auf Grund ihrer Ernährungsweise als Filtrierer nahmen Muscheln in Laboruntersuchungen bei gleicher Expositionskonzentration mehr MP-Partikel auf als Krebstiere (Zerkleinerer) bzw. Schnecken (Weidegänger).
- Im Gegensatz zu Muscheln nutzen Krebstiere und Schnecken allerdings die Grenzschicht zwischen der Wasser- und der Sedimentphase als Suchraum für ihre Nahrung und sind in der Umwelt somit möglicherweise gegenüber höheren MP-Konzentrationen exponiert.
- Labor- bzw. Umweltdaten legen nahe, dass sich die MP-Abundanz in Muscheln, Krebstieren und Schnecken (gleicher Größe) in der Umwelt bisher nur wenig unterscheidet und diese auf wenige Partikel pro Individuum begrenzt ist. In Bezug auf die relative Aufnahme (pro Körpergewicht) nehmen jedoch kleine Arten mehr MP auf als größere.
- Zur Abschätzung der MP-Toxizität auf Organismen im Freiland werden aktuell überwiegend Species Sensitivity Distributions (SSD) eingesetzt. Auf Basis von sechs der neun bisher verfügbaren SSD wurden HC_5 (Schwellenkonzentrationen, oberhalb der bei 5 % der Arten ein Auftreten von MP-Toxizität zu erwarten ist) abgeleitet, die die aktuellen Umweltkonzentrationen in stark mit MP belasteten Gewässern unterschreiten. Es kann daher nicht ausgeschlossen werden, dass MP aktuell bereits toxische Auswirkungen auf (zumindest einige) aquatische Arten hat.
- Auf Grund der begrenzten Datenlage werden zur SSD-Erstellung die Toxizitätsdaten für marine und limnische Arten meist vereint, wodurch eine alleinige Risikoabschätzung für limnische Ökosysteme erschwert wird.
- Die HC_5 unterschreiten insbesondere dann aktuelle Umweltkonzentrationen, wenn neben organismischen auch zelluläre und molekulare Endpunkte berücksichtigt werden. MP-Toxizität in der Umwelt äußert sich daher aktuell möglicherweise überwiegend auf suborganismischer Ebene.
- Eine besonders niedrige HC_5 für Muscheln deutet an, dass Muscheln im Vergleich zu anderen Artgruppen möglicherweise verstärkt von MP-Toxizität betroffen sind.
- Die getroffenen Schlussfolgerungen unterliegen allerdings erheblichen Unsicherheiten, die sich aus der begrenzten Datenverfügbarkeit für die SSD ergeben. Die abgeleiteten SSD bzw. HC_5 stellen somit eher eine Näherung und keinesfalls eine abschließende Bewertung der MP-Toxizität für aquatische Organismen dar.

2.6 Mikroplastik im Umfeld multipler Stressoren

2.6.1 Wichtige Forschungsergebnisse aus Studie 4 und 5

In einer Welt, die sich im konstanten Wandel befindet, werden Organismen kontinuierlich sich verändernden Umweltbedingungen und somit auch variablen Stressoren ausgesetzt. Im Unterschied dazu finden Laborstudien unter kontrollierten Bedingungen statt, und in ihnen wird meist nur ein einzelner Parameter variiert. Dadurch sind Laborstudien kaum in der Lage, die Bedingungen einer Welt mit sich kontinuierlich verändernden Umweltstressoren nachzubilden (Gunderson et al. 2016). Um realistischere Bedingungen im Experiment abzubilden, werden daher zunehmend Laborstudien unter Einbindung von mindestens zwei Variablen durchgeführt (Multi-Stressor-Studien).

Um ein besseres Verständnis der MP-Toxizität in einer Multi-Stressor-Umgebung zu gewinnen, wurden im Rahmen der Studien 4 und 5 zwei Multi-Stressor-Studien durchgeführt. In Studie 4 (Weber et al. 2021b, Anhang A4) wurde die Süßwasserschnecke *L. stagnalis* gleichzeitig gegenüber PS-Fragmenten (≤ 63 µm, 6,4–100.000 P mL^{-1}) und Kupfer-Ionen (Cu^{2+}, 7,5 µg L^{-1}), einem chemischen Stressor, exponiert. Im Gegensatz dazu wurde in Studie 5 (Weber et al. 2020, Anhang A5) ein physikalischer Stressor untersucht: Dafür wurde die Süßwassermuschel *D. polymorpha* gegenüber thermischem Stress (14, 23 und 27 °C Wassertemperatur) sowie PS-Fragmenten (≤ 63 µm, 6,4–100.000 P mL^{-1}) exponiert. Folgende wertvolle Erkenntnisse konnten durch die Studien 4 und 5 gewonnen werden:

- **Studie 4**: Die 28-tägige Exposition von *L. stagnalis* gegenüber Kupfer verursachte Reproduktionstoxizität. Eine zusätzliche Exposition gegenüber MP führte zu keiner signifikanten Veränderung der durch Kupfer induzierten Effekte.

- **Studie 5**: Während einer 14-tägigen Exposition verursachte thermischer Stress signifikante Auswirkungen auf die Aktivität, die Energiereserven, den oxidativen Stress sowie die Immunfunktion von *D. polymorpha*. Im Unterschied dazu waren die MP-induzierten Effekte auf eine Veränderung der antioxidativen Kapazität beschränkt. Interaktive Effekte zwischen den beiden Stressoren wurden nicht beobachtet.

2.6.2 Mikroplastiktoxizität in einer multiplen Stressorenumgebung

Die Ergebnisse aus den Multi-Stressor-Studien in Studie 4 und 5 zeigten, dass MP im Vergleich zum zweiten Stressor (thermischer Stress, Kupfer) nur wenige oder gar keine Effekte auslöste. In den gewählten Expositionsszenarien hatte eine Exposition mit PS-Fragmenten somit keine oder nur eine sehr begrenzte Relevanz für die Süßwassermuschel *D. polymorpha* bzw. die Süßwasserschnecke *L. stagnalis*, während die zusätzlichen Stressoren deutliche Auswirkungen auf die Testorganismen hatten.

Wirft man einen Blick auf weitere bereits publizierte Multi-Stressor-Studien mit marinen sowie limnischen Muschel- (9 Studien, Tab. A7 in Anhang A6) und Schneckenarten (2 Studien, Tab. A7), so ergibt sich ein sehr diverses Bild multipler Stressor-Effekte. Alle in Tab. A7 dargestellten Studien untersuchten multiple Effekte von MP in Kombination mit einer Chemikalie. Dabei zeigte sich ein sehr variables Zusammenwirken beider Stressoren:

In einigen Fällen hatten die Stressoren weder als einzelne Stressoren noch in Kombination einen signifikanten Effekt im Vergleich zur Kontrolle (Nr. ① in Tab. A7). In den anderen Fällen bewirkte mindestens ein Stressor einen signifikanten Effekt in einer Einzelexposition, jedoch unterschieden sich die Ergebnisse aus der anschließenden gemeinsamen Exposition: In einigen Fällen addierte sich der Effekt der beiden Stressoren („additiver Effekt", keine Interaktion zwischen den Stressoren, Todgham & Stillman 2013, Nr. ③ in Tab. A7). In anderen Fällen war der ausgelöste Effekt in einer Co-Exposition entweder geringer (bzw. sogar umgekehrt, „antagonistischer Effekt", Todgham & Stillman 2013, Nr. ② in Tab. A7) oder höher („synergistischer Effekt", Todgham & Stillman 2013, Nr. ④ in Tab. A7) als die Summe der Einzeleffekte der Stressoren.

Aus den in Tab. A7 dargestellten Multi-Stressor-Studien lässt sich bisher kein klarer Trend erkennen, ob antagonistische, additive oder sogar synergistische Effekte in einer multiplen Exposition mit MP und einer zusätzlichen Chemikalie dominieren. Eine entsprechende Metaanalyse zur Beantwortung dieser Frage wird zukünftig eine größere Anzahl an Multi-Stressor-Studien sowie eine höhere Vielfalt an getesteten Stressoren (nicht nur chemisch, sondern auch physikalisch) erfordern.

Wie wichtig die Durchführung von Multi-Stressor-Experimenten und eine Metaanalyse dieser Daten ist, wird durch Studien von Gunderson et al. (2016), Przeslawski et al. (2015) sowie Crain et al. (2008) deutlich. Die Autoren konnten in mehreren Metaanalysen zu Multi-Stressor-Effekten in marinen Lebensräumen zeigen, dass interaktive Effekte zwischen Stressoren häufig sind und diese insbesondere synergistisch ausgeprägt waren. Eine Abschätzung der Toxizität eines Stressors in der Umwelt auf Basis von Einzelstressor-Experimenten spiegelt daher nur in ungenügender Weise die tatsächlichen Effekte, die dieser Stressor in der Umwelt auslöst, wider. Im Hinblick auf eine adäquate Risikoabschätzung für MP in der Umwelt sollten Multi-Stressor-Studien daher zukünftig größeres Gewicht in der MP-Forschung finden, um auf Basis von Metaanalysen Interaktionen mit weiteren Umweltstressoren genauer abschätzen zu können.

2.6.3 Kernerkenntnisse aus Kapitel 2.6

- In den im Rahmen von Studie 4 und 5 durchgeführten Multi-Stressor-Studien mit *L. stagnalis* und *D. polymorpha* löste MP (im Gegensatz zum zweiten Stressor) wenige oder gar keine Effekte aus.
- Aus den bisher verfügbaren Multi-Stressor-Studien mit marinen bzw. limnischen Muschel- und Schneckenarten ist aktuell kein Trend zu erkennen, inwieweit MP-Toxizität in Multi-Stressor-Studien auftritt und wie die Toxizität bei einer gemeinsamen Exposition mit einem weiteren Stressor variiert (additive/antagonistische/synergistische Effekte).

3. Zusammenfassung

Seit Beginn der Industrialisierung im 19. Jahrhundert befindet sich die Erde in einem Prozess des intensiven Wandels, in welchem der Mensch bereits in einem erheblichen Ausmaß Einfluss auf die Umwelt genommen hat. Zunehmende industrielle Produktionen führten in den vergangenen 200 Jahren zu einer bedeutenden Steigerung der Lebensqualität, jedoch auch zu einer wachsenden Belastung von natürlichen Lebensräumen (Grübler 1998).

Ein Sinnbild für diese Entwicklung ist das steigende Vorkommen von Plastik in der Umwelt. Plastik wird seit den 1950er Jahren in großen Mengen produziert (PlasticsEurope 2012); die möglichen Auswirkungen auf die Umwelt werden jedoch erst seit einigen Jahren zunehmend deutlich. Plastik und seine Fragmentierungsprodukte (Mikro- (MP) und Nanoplastik (NP)) lassen sich mittlerweile in nahezu allen Lebensräumen der Erde nachweisen (Eerkes-Medrano & Thompson 2018, Lusher 2015). In den vergangenen zehn Jahren wurden nun große Anstrengungen unternommen, das Vorkommen von MP und dessen Verteilung in der Umwelt sowie die Auswirkungen auf Lebewesen besser zu verstehen. Im Rahmen dieser Dissertation wurden insgesamt fünf Studien angefertigt, durch die insbesondere das Verständnis zum MP-Vorkommen und dessen Auswirkungen auf limnische Lebensräume vertieft werden sollte.

Durch die Studie „Comparative assessment of microplastics in water and sediment of a large European river“ (Scherer et al. 2020) wurde das Vorkommen von MP (150/125–5.000 µm) in der Wasserphase sowie im Sediment der Elbe untersucht. Die Konzentrationen an MP-ähnlichen Partikeln variierten in den Untersuchungen zwischen 0,88–13,24 P m^{-3} in der Wasserphase sowie $2{,}26 \times 10^{4}$–$2{,}27 \times 10^{7}$ P m^{-3} in der Sedimentphase und sind somit mit Konzentrationen in anderen deutschen Flüssen vergleichbar. Der Ursprung der MP-Partikel liegt vermutlich sowohl in diffusen Quellen (z. B. MP in Oberflächenabflüssen von Straßen und Agrarflächen) als auch Punktquellen (z. B. Haushalts- und Industrieabwässer). Im globalen Vergleich sind die bisher publizierten MP-Konzentrationen hingegen bedeutend variabler und reichen von 0,17 (Minimum, Rodrigues et al. 2019) bis $5{,}19 \times 10^{5}$ P m^{-3} (Maximum, Lahens et al. 2018) in der Wasserphase bzw. von $1{,}00 \times 10^{-2}$ (Minimum, Castañeda et al. 2014) bis $1{,}62 \times 10^{8}$ P m^{-3} (Maximum, Wang et al. 2018) in der Sedimentphase von Flüssen weltweit. Auf Basis der bisher verfügbaren Daten weisen limnische Gewässer in Deutschland im globalen Vergleich somit eine mäßig hohe MP-Belastung auf.

Durch drei weitere Studien („Ingestion and toxicity of polystyrene microplastics in freshwater bivalves” (Weber et al. 2021a), „PET microplastics do not negatively affect the survival, development, metabolism and feeding activity of the freshwater invertebrate *Gammarus pulex”* (Weber et al. 2018) sowie „Ingestion and toxicity of microplastics in the freshwater gastropod *Lymnaea stagnalis*: No microplastic-induced effects alone or in combination with copper” (Weber et al. 2021b)) wurde darüber hinaus die Aufnahme und Toxizität in drei wesentlichen limnischen Organismengruppen, Muscheln (Bivalvia, am Beispiel der Arten *Dreissena polymorpha*, *Anodonta anatina*, *Sinanodonta woodiana*), Krebstiere (Crustacea, am Beispiel der Art *Gammarus pulex*) und Schnecken (Gastropoda, am Beispiel der Art *Lymnaea stagnalis*), untersucht. Die getesteten Arten sind fähig, MP-Partikel mit unterschiedlicher Größe (meist zwischen 5 und 45 µm, teilweise bis zu 90 µm) sowie Form (Sphären, Fragmente) aufzunehmen. Das absolute Vorkommen an MP-Partikeln in den Organismen hängt dabei stark von der Expositionszeit, der Größe und Konzentration an MP-Partikeln sowie vom zusätzlichen

Nahrungsangebot ab. Betrachtet man das relative Vorkommen, so wurde deutlich, dass die Aufnahme zusätzlich auch von der Körpergröße abhängt. Kleinere Individuen bzw. kleinere Arten nehmen relativ (in Bezug auf ihre Körpergröße) mehr MP-Partikel auf, wodurch eine stärkere Interaktion von MP mit den Organismen möglich ist und toxische Effekte somit ggf. stärker ausgeprägt sind.

Eine Extrapolation der Ergebnisse aus den Aufnahmestudien in Bezug auf aktuelle limnische Umweltkonzentrationen ergab, dass die MP-Abundanz in Freilandpopulationen der getesteten Arten zum jetzigen Zeitpunkt vermutlich nur wenige Partikel pro Individuum beträgt. Publizierte Ergebnisse zur MP-Abundanz in limnischen und marinen Muscheln und Krebstieren aus Freilandpopulationen unterstützen diese Ergebnisse – nur wenige Freilandpopulationen wiesen bisher MP-Abundanzen von > 8 Partikeln pro Individuum auf.

Die Studien zur Toxizität von MP auf *D. polymorpha*, *G. pulex* sowie *L. stagnalis* konnten nur sehr wenige signifikante Effekte nachweisen. Im Fall von *D. polymorpha* (1-, 3-, 7- und 42-tägige Exposition) bzw. *L. stagnalis* (28-tägige Exposition) bewirkten PS-Fragmente (≤ 63 µm) eine Veränderung der Nahrungsaufnahme bzw. der Immunfunktion von Hämolymphzellen. Darüber hinaus konnten bei keiner der getesteten Arten MP-induzierte Auswirkungen auf die Mortalität, Energiereserven, das oxidative Stresslevel oder die Reproduktion festgestellt werden. In allen Toxizitätsstudien wurden dabei sowohl umweltähnliche (*D. polymorpha*/*L. stagnalis*: $6{,}4 \times 10^{6}$ P m^{-3}, *G. pulex*: $0{,}8 \times 10^{6}$ P m^{-3}) wie auch um ein Vielfaches höhere MP-Konzentrationen eingesetzt.

In den einzelnen Studien wurden diese Ergebnisse jeweils mit den Ergebnissen zuvor publizierter Studien verglichen. Dabei zeigte sich stets die große Variabilität in der Intensität der beobachteten MP-Toxizität. Die Spanne erstreckte sich dabei von Experimenten ohne jegliche Toxizität bis hin zu Studien, die ausgeprägte MP-Auswirkungen nachweisen konnten. Auf Grund der stark unterschiedlichen Expositionsszenarien, die in bisherigen MP-Studien eingesetzt wurden, ist eine Rückführung dieser Unterschiede auf spezifische Ursachen bisher kaum möglich gewesen. Es kann jedoch vermutet werden, dass die unterschiedlich ausgeprägte Toxizität mit den variablen Eigenschaften des Stressors MP (u.a. Form, Größe, chemische Zusammensetzung), der exponierten Art (u.a. Größe, Entwicklungszustand, Sensitivität bzw. Anpassungsfähigkeit gegenüber Stressoren) sowie dem gewählten Expositionsszenario (u.a. Dauer, MP-Konzentration, Nahrungsangebot) in Verbindung steht.

Um trotzdem einen gewissen Vergleich zwischen den bisher publizierten Studien zur MP- und NP-Toxizität in Muscheln und Krebsen herstellen zu können, wurde auf Basis einer systematischen Literaturrecherche eine Artenempfindlichkeitsverteilung (Species Sensitivity Distribution) für Muscheln bzw. Krebstiere (marin und limnisch) ermittelt. Anhand der Verteilung konnte wiederum die MP-Konzentration bestimmt werden, oberhalb der in den empfindlichsten 5 % der Arten toxische MP-Effekte (auf Molekül-, Zell-, Organ- oder Organismenebene) hervorgerufen werden (HC_5). Die ermittelten HC_5 betrugen für Muscheln 924 P m^{-3} und für Krebstiere 66.600 P m^{-3} und unterschreiten somit bereits jetzt die höchste publizierte Konzentration für MP in der Wasserphase von Flüssen ($5{,}19 \times 10^{5}$ P m^{-3}, Lahens et al. 2018). Vergleichbare Ergebnisse wurden in vier der sieben weiteren, bisher publizierten SSD beobachtet. Diese vier SSD bezogen zumeist (ebenso wie die SSD für Muscheln und Krebse) Daten für Endpunkte auf Molekül-, Zell-, Organ- sowie Organismenebene ein, während aus SSD, die nur toxische Effekte auf Organismenebene einbezogen, bisher überwiegend HC_5 oberhalb aktueller Umweltkonzentrationen abgeleitet wurden. Dies deutet an, dass toxische

Effekte, zumindest auf suborganismischer Organisationsebene, in stark mit MP belasteten Lebensräumen bereits zum jetzigen Zeitpunkt nicht ausgeschlossen werden können. Abweichende HC_5 zwischen Muscheln und Krebsen zeigen zudem, dass möglicherweise Unterschiede in der MP-Toxizität auf verschiedene Organismengruppen bestehen. MP besitzt somit möglicherweise zumindest für einige aquatische Organismengruppen bereits jetzt eine gewisse Relevanz als Stressor.

Kaum verstanden ist bisher, inwieweit MP-Toxizität durch die Anwesenheit zusätzlicher Stressoren moduliert wird. In den vergangenen drei Jahren wurden daher zunehmend Multi-Stressor-Studien publiziert, die die gemeinsamen Auswirkungen von MP und einem weiteren physikalischen oder chemischen Stressor untersuchten. Als Teil der Studie „Ingestion and toxicity of microplastics in the freshwater gastropod *Lymnaea stagnalis*: No microplastic-induced effects alone or in combination with copper" (Weber et al. 2021b) wurden in einer chronischen Exposition die Multi-Stressor-Effekte von PS-Fragmenten (≤ 63 µm, $6{,}4x10^{6}$–$1{,}0x10^{11}$ P m^{-3}) und Kupfer-Ionen (7,5 µg L^{-1}) auf die aquatische Lungenschnecke *L. stagnalis* überprüft. In der Studie „Combined effects of polystyrene microplastics and thermal stress on the freshwater mussel *Dreissena polymorpha*" (Weber et al. 2020) wurden zudem die multiplen Effekte von thermischem Stress als physikalischem Stressor (16, 23, 27 °C) und PS-Fragmenten (≤ 63 µm, $6{,}4x10^{6}$–$1{,}0x10^{11}$ P m^{-3}) ermittelt. Beide Studien zeigten, dass die MP-Partikel nur sehr begrenzte oder gar keine Auswirkungen auf die Testorganismen hatten, während Hitzestress bzw. Kupfer relevante Stressoren für die Organismen darstellten. Ein Vergleich mit weiteren publizierten Multi-Stressor-Studien zeigte jedoch, dass es in einigen Studien durchaus zu interaktiven Effekten zwischen den Stressoren kam, wobei diese antagonistisch, additiv oder sogar synergistisch ausgeprägt waren. Auf Grund der begrenzten Anzahl an Multi-Stressor-Studien ist eine Metaanalyse multipler Stressoreneffekte für MP bisher kaum durchführbar. Durch einen zukünftigen Fokus auf entsprechende Studiendesigns wird es langfristig hoffentlich aber möglich sein, in Metaanalysen die möglichen Interaktionen zwischen den Effekten von MP und weiteren Umweltstressoren genauer verstehen und durch diese Erkenntnisse die Risikoabschätzung für MP zunehmend verfeinern zu können.

Zusammengefasst kann somit nicht ausgeschlossen werden, dass einige aquatische Organismen bereits zum jetzigen Zeitpunkt von MP-Toxizität betroffen sind. Mit zukünftig steigenden Plastik- bzw. MP-Mengen in der Umwelt (Jambeck et al. 2015, van Wijnen et al. 2019) können sich die toxischen Effekte von MP auf aquatische Lebewesen möglicherweise noch verstärken. Im Sinne des Vorsorgeprinzips sollte daher vermieden werden, dass die Belastung mit MP und NP in der Umwelt zukünftig weiter ansteigt. Die Tragweite dieser Herausforderung wird jedoch erst deutlich, wenn man mathematische Extrapolationen für die zukünftige Entwicklung des Plastikeintrags in die Umwelt betrachtet. 2015 betrug der Plastikeintrag in die Umwelt schätzungsweise 60–99 Millionen Tonnen. Im Falle eines gleichbleibenden Konsumverhaltens für Plastik wird sich dieser Eintrag bis zum Jahr 2060 voraussichtlich auf 155 bis 265 Millionen Tonnen verdreifachen (Lebreton & Andrady 2019). Um die Belastung der Umwelt mit Plastikmüll bzw. MP und NP auf das aktuelle Niveau zu begrenzen, müsste der Plastikeintrag in die Umwelt sofort gestoppt werden – ein aus jetziger Sicht nicht realistisches Szenario. Selbst eine Reduktion des jährlichen Plastikeintrags wird nur dann möglich sein, wenn wir gesellschaftlich und wirtschaftlich einen vollständigen Systemwechsel umsetzen (Lau et al. 2020). Hierzu zählen die Reduktion der Plastiknachfrage, die Substitution von Plastikprodukten durch alternative Materialien sowie ein effizientes Recycling von Plastikmüll. Auch wenn das Bewusstsein für die Umweltprobleme durch Plastikmüll in der Gesellschaft zunehmend vorhanden ist und ein Teil der Gesellschaft sogar zur Veränderung der eigenen Lebensweise bereit ist (Soares et al.

2021), wird ein vollständiger Systemwandel nur möglich sein, wenn die gesamtwirtschaftlichen Material- und Produktionsströme angepasst und zu einer zirkulären Ökonomie (Crippa et al. 2019) weiterentwickelt werden.

Letztendlich ist das Vorkommen von MP in der Umwelt somit nur ein Sinnbild für die unnachhaltige Nutzung von Ressourcen durch den Menschen. Ebenso wie viele andere vom Menschen genutzte Ressourcen, beispielsweise fossile Energieträger, Rohstoffe oder Landfläche, wurden Ressourcen in den vergangenen Jahrzehnten verschwenderisch und mit wenig Weitsicht ausgenutzt. Zukünftig wird der Mensch zügig neue Wege finden müssen, die ihm zur Verfügung stehenden Ressourcen nachhaltiger einzusetzen, damit diese auch nachfolgenden Generationen noch zur Verfügung stehen und um letztendlich Auswirkungen auf die Natur, die Grundlage des menschlichen Lebens, zu begrenzen.

4. Literaturverzeichnis

Das folgende Literaturverzeichnis umfasst die Literaturquellen aus den Kapiteln 1–3 sowie aus Anhang A6.

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5. Abbildungsverzeichnis

6. Abkürzungsverzeichnis

Abkürzung	Begriff
ABS	Acrylnitril-Butadien-Styrol
AF	Assessment Factor (Bewertungsfaktor)
BfG	Bundesanstalt für Gewässerkunde
BPA	Bisphenol A
CYP450	Cytochrom P450
DDT	Dichlordiphenyltrichlorethan
µ-FTIR	µ-Fourier-Transformations-Infrarotspektroskopie
HC_5	Schwellenkonzentration, oberhalb der bei 5 % der Arten Toxizität auftritt
LC_{50}/EC_{50}	Mittlere letale Konzentration/mittlere Effektkonzentration
LOEC	Niedrigste beobachtete Effektkonzentration
MP	Mikroplastik
MP_{vis}	Visuell als MP identifizierte Partikel
Mt	Megatonne
NIAS	Unbeabsichtigt hinzugefügte Chemikalien (non-intentionally added substances)
NOEC	Höchste Expositionskonzentration, bei der kein signifikanter Effekt beobachtet wurde
NP	Nanoplastik
PA	Polyamid
PAK	Polyzyklische aromatische Kohlenwasserstoffe
PBDE	Polybromierte Diphenylether
PC	Polycarbonat
PCB	Polychlorierte Biphenyle
PE-HD	Polyethylen (hohe Dichte)
PE-LD	Polyethylen (niedrige Dichte)
PES	Polyester
PET	Polyethylenterephthalat
PFAS	Per- und polyfluorierte Alkylverbindungen
PHB	Polyhydroxybutyrat/ Polyhydroxybuttersäure
PLA	Polymilchsäure
P m^{-3}	Partikel m^{-3}
PMMA	Polymethylmethacrylat
PP	Polypropylen
PS	Polystyrol
PS-DVB	Polystyrol-Divinylbenzol
PUR	Polyurethan
PVC	Polyvinylchlorid
Pyro-GC-MS	Pyrolyse-GC-MS
REM	Rasterelektronenmikroskopie
SSD	Species Sensitivity Distribution (Artenempfindlichkeitsverteilung)
TED-GC-MS	Thermische Extraktionsdesorptionsgaschromatographie-Massenspektrometrie
UV	Ultraviolett

Anhang

A1. Comparative assessment of microplastics in water and sediment of a large European river

Studie 1

Publikation im peer-reviewed Journal *Science of the Total Environment*:

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Erklärung zu den Autorenanteilen an der Publikation A1:

Entwicklung und Planung												
Autoren	CS	**AW**	FS	SV	HE	CK	NA	CF	GD	MW	NB	GR
%	60	**-**	-	-	-	-	-	-	-	20	10	10
Durchführung der einzelnen Untersuchungen und Experimente												
Autoren	CS	**AW**	FS	SV	HE	CK	NA	CF	GD	MW	NB	GR
%	30	**5**	5	15	15	10	10	5	5	-	-	-
Erstellung der Datensammlungen und Abbildungen												
Autoren	CS	**AW**	FS	SV	HE	CK	NA	CF	GD	MW	NB	GR
%	20	**40**	20	4	4	4	4	2	2	-	-	-
Analyse und Interpretation der Daten												
Autoren	CS	**AW**	FS	SV	HE	CK	NA	CF	GD	MW	NB	GR
%	30	**35**	30	-	-	-	-	-	-	5	-	-
Verfassung des Manuskripts												
Autoren	CS	**AW**	FS	SV	HE	CK	NA	CF	GD	MW	NB	GR
%	30	**30**	30	-	-	-	-	-	-	10	-	-

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Comparative assessment of microplastics in water and sediment of a large European river

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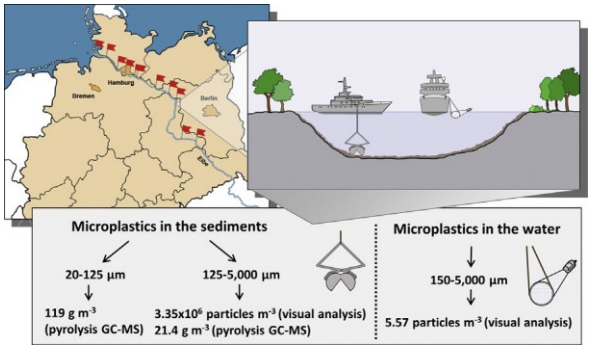
HIGHLIGHTS

- Low to medium microplastic (MP) levels in the river Elbe
- Much higher abundance in sediments compared to the water phase
- Decreasing levels of MP in sediments over the course of the river
- Higher polymer diversity in sediments compared to the water phase
- Industrial emissions possibly caused MP hotspots

GRAPHICAL ABSTRACT



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ABSTRACT

Aquatic ecosystems are globally contaminated with microplastics (MP). However, comparative data on MP levels in freshwater systems is still scarce. Therefore, the aim of this study is to quantify MP abundance in water and sediment of the German river Elbe using visual, spectroscopic (Fourier-transform infrared spectroscopy) and thermoanalytical (pyrolysis gas chromatography mass spectrometry) methods. Samples from eleven German sites along the German part of the Elbe were collected, both in the water and sediment phase, in order to better understand MP sinks and transport mechanisms. MP concentrations differed between the water and sediment phase. Sediment concentrations (mean: 3,350,000 particles m^{-3}, 125–5000 µm MP) were in average 600,000-fold higher than water concentrations (mean: 5.57 particles m^{-3}, 150–5000 µm MP). The abundance varied between the sampling sites: In sediments, the abundance decreased in the course of the river while in water samples no such clear trend was observed. This may be explained by a barrage retaining sediments and limiting tidal influence in the upstream parts of the river. Particle shape differed site-specifically with one site having exceptionally high quantities of spheres, most probably due to industrial emissions of PS-DVB resin beads. Suspended MP consisted predominantly of polyethylene and polypropylene whereas sediments contained a higher diversity of polymer types. Determined MP concentrations correspond well to previous results from other European rivers. In a global context, MP levels in the Elbe relate to the lower (water) to middle

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section (sediment) of the global range of MP concentrations determined for rivers worldwide. This highlights that elevated MP levels are not only found in single countries or continents, but that MP pollution is an issue of global concern.

1. Introduction

Microplastics (MPs) have been investigated for over 45 years especially in the marine environment (Bergmann et al., 2015; Carpenter et al., 1972; Cole et al., 2011), but only in recent years research has also started to focus on freshwater environments (Dris et al., 2015b; Wagner and Lambert, 2018). With regard to European rivers, previous studies have investigated MPs in the catchments of the rivers Rhine (Heß et al., 2018; Klein et al., 2015; Leslie et al., 2017; Mani et al., 2015, 2019), Danube (Heß et al., 2018; Lechner et al., 2014), Weser (Heß et al., 2018), Antuã (Rodrigues et al., 2018), Meuse (Leslie et al., 2017), Seine (Dris et al., 2015a), Rhône (Faure et al., 2015) as well as smaller rivers and tributaries in the United Kingdom (Blair et al., 2019; Horton et al., 2017; Hurley et al., 2018; Tibbetts et al., 2018). Furthermore, Leslie et al. (2017) and Schmidt et al. (2018) published first data on MP contamination in canals of large European cities (Amsterdam, Berlin).

In recent publications, reported MP concentrations in European river water varied distinctively ranging from 0.03 (Mani et al., 2019) to 187,000 particles (p) m^{-3} (Leslie et al., 2017). MPs in river water also obtained a broad range of shapes including spheres, fibres, fragments and foils with varying relative abundances. In the Rhine and the Danube, Heß et al. (2018) mostly detected fibres and fragments in the water phase, while Mani et al. (2015, 2019) and Lechner et al. (2014) predominantly found MP spheres. In regard to MP polymer types, polyethylene (PE), polypropylene (PP) and polystyrene (PS) predominated in the river water (Heß et al., 2018; Mani et al., 2015, 2019; Schmidt et al., 2018).

In European river sediments, reported MP concentrations ranged between 18 (Rodrigues et al., 2018) and 72,400 p kg^{-1} sediment (Hurley et al., 2018) with fragments, fibres and spheres being most abundant (Blair et al., 2019; Horton et al., 2017; Hurley et al., 2018; Klein et al., 2015; Leslie et al., 2017; Rodrigues et al., 2018; Tibbetts et al., 2018). Polymer type composition in the sediments was more diverse than in the water comprising polymer types such PE, PP and PS, but also polyvinyl chloride (PVC), polymethyl methacrylate (PMMA) and dye particles (Horton et al., 2017; Klein et al., 2015; Tibbetts et al., 2018).

MP pollution, both in the water and sediments of European rivers, has been related to multiple pollution sources. Urbanization has been discussed as one major cause of MP pollution in European rivers (Mani et al., 2015; Schmidt et al., 2018; Tibbetts et al., 2018), including pollution both from industry (Mani et al., 2015) as well as land run-offs (Horton et al., 2017). Further, Heß et al. (2018) pointed out that small and medium-sized rather than large rivers obtain high MP levels. Inflowing tributaries and rivers could thus be a relevant MP source for larger rivers (Klein et al., 2015). Wastewater treatment plants (WWTP) are another relevant MP source, although the extent of their importance needs further clarification (Mani et al., 2015; Leslie et al., 2017; Schmidt et al., 2018). Finally, also meteorological and hydrodynamic events may strongly impact MP levels in European river systems (Hurley et al., 2018; Schmidt et al., 2018).

These previous results demonstrate well that MP is an abundant pollutant in freshwater systems across Europe. However, despite the relatively large number of publications, it remains difficult to draw conclusions on the MP distribution in European rivers as most studies investigated MPs only in one specific compartment, that is the riverbed, the water phase or the shoreline. Only two studies included data on MP concentrations both in the water and the sediment phase (Leslie et al., 2017; Rodrigues et al., 2018). However, in these studies MP concentrations were reported using incomparable units that hamper a direct comparison of MP levels in riverine water and sediment. Further, none of the two studies reported compartment-specific data on MP polymer type distributions.

We approached this knowledge gap by analysing the spatial distribution of MPs in the water and sediment phase in the German part of the large European river Elbe. We chose to study the Elbe as it is an important German waterway with industrial zones and large cities such as Hamburg. Our data, thus, adds to the knowledge on MP concentrations in large European rivers. Water and sediment samples were taken at eleven sites along the river course from the Middle Elbe to its estuary. We analysed MPs in the water (150–5000 µm) and the sediment phase (20–5000 µm) using visual identification as well as analytical verification with pyrolysis gas chromatography coupled to mass spectrometry (pyr-GC–MS) and attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR). To enable a comparison between compartments and with previous studies, we provided results in several units.

2. Materials and Methods

2.1 Study site

The Elbe is a 1091 km long river with its source in the Giant Mountains in the Czech Republic. From there, it passes through eastern and northern Germany, before it discharges into the North Sea. The Elbe obtains a total catchment area of 148,268 km^2 with 96,932 km^2 located on German state territory. The Elbe is separated into the Upper Elbe (source to Castle Hirschstein, river km 369.92–96 (km 369.92–0 being located on Czech state territory, km 0–96 km on German state territory), the Middle Elbe (Castle Hirschstein to barrage at Geesthacht, river km 96–585.9) and the Lower Elbe (barrage at Geesthacht to the border of the North Sea at Cuxhaven-Kugelbake, river km 585.9–727.7, Naumann et al., 2003). The most important tributaries of the Elbe river are the Moldau (Czech Republic), Saale, Havel and Mulde (Germany). Saale and Havel contribute a water volume of in average 115 $m^3 s^{-1}$ and the Mulde of 73 $m^3 s^{-1}$ to the mean discharge of the Elbe. In total, eleven sites along the German part of the Elbe were sampled (Table 1, Fig. 1). The sampling sites Wittenberg, Dessau, Havelberg, Wittenberge and Dömitz stretch along the Middle Elbe from km 216 to 516. At Geesthacht (km 585.9), a barrage separates the Middle from the Lower Elbe (the sampling site Geesthacht is

Table 1: Geographical information about the Elbe sampling sites.

Site (abbreviation)	Elbe km	Geo-Longitude	Geo-Latitude
Wittenberg (WB)	216.4	12.6151 E	51.8629 N
Dessau (DS)	261.4	12.2191 E	51.8563 N
Havelberg (HB)	422	12.0766 E	52.8234 N
Wittenberge (WE)	454.9	11.7456 E	52.9904 N
Dömitz (DM)	507	11.2091 E	53.1443 N
Geesthacht (GH)	584.5	10.3586 E	53.4297 N
Elbstorf (ET)	589	10.2909 E	53.4261 N
Hafenstraße (HS)	623.5	10.0391 E	53.5290 N
Lühemündung (LM)	645.5	9.6350 E	53.5727 N
Hollerwettern (HW)	681.4	9.3487 E	53.8270 N
Vogelsand (VS)	746.3	8.4876 E	53.9670 N

located upstream of the barrage). Further downstream, Elbstorf, Hafenstraße, Lühemündung and Hollerwettern are located from Elbe km 589 to 681. Hafenstraße was the most urban sampling site being located in the centre of Hamburg harbour. The site Vogelsand is part of the Outer Elbe, the continuation of the estuary formed by the North Sea.

2.2 Sampling

The sampling was conducted during a routine monitoring of the Elbe by the German Federal Institute of Hydrology (Middle Elbe: 20/07–23/07/2015, Lower and Outer Elbe: 03/08–06/08/2015). Mean flow rates at the sites Wittenberg (km 214) and Wittenberge (km 453) during sampling were relatively low equalling 119–128 $m^3\ s^{-1}$ and 247–260 $m^3\ s^{-1}$, respectively. All samples in the Middle and Lower Elbe were taken either at the entrance of harbours or at the edge of the river where fine-grained sediment accumulates. Ten water samples (one sample per site, except for site Elbstorf where only sediment was collected) were retrieved with an Apstein plankton net (opening: 0.022 m^2, Ø 17 cm, length: 110 cm, mesh size: 150 µm) fixed on the side of a research vessel. The plankton net was placed directly below the water surface. The river surface was sampled over a distance of approximately 1 km at a speed of about 6–7 km h^{-1}. The filtered water volume was calculated using a manual flowmeter (fixed on the plankton net opening) and following formula: Filtered water volume = number of flowmeter revolutions × 0.3 m $revolution^{-1}$ × opening of the net [m^2] × 1000. Depending on the flow velocity, 3.2–32.7 m^3 of water were filtered during 5–10 min (site-specific results in Table S1). The water samples were transferred from the cod end of the net into glass jars via flushing with ultrapure water. After each sampling, the nets were cleaned with ultrapure water to prevent contamination of the subsequent sample.

At the river bed margins, sediment samples were taken with a Van-Veen-grab sampler (2–4 kg per sample). The sediments were stored in closed polypropylene buckets to protect them from external particle contamination.

2.3 Sample preparation

2.3.1 MP extraction from water samples

Water samples were transferred to the Federal Institute of Hydrology and processed as described by Ehlers et al. (2019). In brief, organic matter was digested by adding 5–15 mL of a 1:1 mixture of 10 M potassium hydroxide solution and hydrogen peroxide (30%) to each sample. After agitation for 3–4 days (d), the samples were neutralized with formic acid. Then, the MP particles were isolated from the remaining matrix in a separating funnel by adding 3.61 g potassium formate powder ml^{-1} sample (density: 1.6 g mL^{-1}). After 3–4 d, the uppermost layer of the water phase was separated and pressure-filtrated on anopore inorganic membrane filters (GE Healthcare Life Sciences, Whatman, Anodisc Cat. No. 514–0518, diameter: 47 mm, pore size: 0.2 µm). The filters were closed, air-dried at 40 °C and stored in an aluminium jar. Later, filters were visually inspected and all particles >500 µm as well as selected smaller ones were analysed by means of ATR-FTIR (see Sections 2.4.1, 2.4.2).

2.3.2 MP extraction from sediment samples

Sediment samples were transferred to the Goethe University Frankfurt am Main. For each sampling site, we homogenised the sediment sample first and weighed in up to 2.5 kg of sediment wet weight (ww) afterwards. The dry weight of each sediment sample was determined by drying and weighing a subsample of 200 g ww for 5–7 d at 45 °C (results in Table S1). The sediments (≤ 2.5 kg) were wet-sieved into three size fractions (20–125 µm, 125–1000 µm, >1000 µm). The >1000 µm fractions were directly visually sorted and particles with a size of 1000–5000 µm were stored for further visual and ATR-FTIR analysis. The 20–125 µm sediment fractions were dried at 45–55 °C for 5–7 d to determine their dry weight (results in Table S1) and afterwards stored in glass jars for pyr-GC–MS analysis. For the sampling sites Dessau, Geesthacht and Elbstorf, we had to wet-sieve two subsamples each, because we lost the 20–125 µm sediment fractions in the first wet-sieving run and could thus only isolate the 125–5000 µm fraction. We therefore performed a second wet-sieving run to also obtain the 20–125 µm sediment fraction from both sites (see Table S1). The 125–1000 µm sediment fractions were processed as following. First, density separation was performed in a custom-made replica of the Munich Plastic Sediment Separator (MPSS, Imhof et al., 2012, details in S.1.2) filled with $ZnCl_2$ (ρ = 1.6–1.8 g cm^{-3}) as separation solution. A 24 h treatment in the MPSS enabled the isolation of particles with ρ < 1.6 g cm^{-3}. Secondly, the organic content in the isolated particle fraction was further reduced by wet peroxidation (10:1 mixture of 30% H_2O_2 and 10% H_2SO_4, 5 d, 55 °C, details in S1.2). Finally, the suspensions were filtered on glass microfiber filters (GE Healthcare Life Sciences, Whatman, GF/D, Cat. No. 1823-047, diameter: 47 mm, pore size: 2.7 µm) for visual and ATR-FTIR analysis.

2.4. Identification of MPs in water and sediment samples

2.4.1 Visual identification

Tentative MPs on the filters were visually inspected with a (stereo) microscope with attached digital camera (for water samples: Keyence VHX2000, for sediment samples: Olympus SZ-40 and camera (JVC, KY-F75U, imaging software: Discus, version 4.80.8238)). For particle identification, we followed the established criteria by Norén (2007). Tentative MP particles were characterised with regard to their colour (black, white, transparent, grey, silver, brown, purple, blue, turquoise, green, yellow, orange, pink, red), size (longest particle diameter) and shape (fragment, sphere, fibre, foils). Based on the mesh size of the plankton net as well as the sieves used for the wet-sieving of the sediments, 150–5000 µm (water samples) and 125–5000 µm (sediment samples) particles were analysed on the filters.

Total particle concentrations in the water phase of each site were calculated based on the filtered water volume and reported as MP number m^{-3} water. Concentrations in sediments are given as MP number m^{-3} dry sediment to allow comparison between water and sediment samples. Sediment volumes were calculated from sediment dry weights and corresponding sediment densities (details in Table S1 and S1.3). We added results on MP concentrations in sediments based on total sediment mass (MP kg^{-1} dry weight) in chapter S2.2.1.

2.4.2 ATR-FTIR analysis

A subsample of the tentative MPs was manually isolated and analysed by ATR-FTIR (Perkin Elmer, Spectrum Two) to determine the polymer type. The majority of the analysed particles were >500 µm. Due to lower MP abundance in the water phase, we could analyse all MP particles >500 µm in the water samples, while for the sediment samples only a subsample could be processed. Particle spectra were compared to a self-established plastic polymer data bank with reference spectra for the most common polymer types (PE, PP, PS, PVC, PMMA, polyethylene terephthalate (PET), acrylonitrile butadiene styrene (ABS), polyamide (PA), polyurethane (PU)) and categorised as "MP" (match >80% and/or clear match of characteristic peaks) or "unknown" (correlation <80% and no match of characteristic peaks). For details of ATR-FTIR analysis see S1.4.

2.4.3 Pyrolysis GC–MS analysis

After the removal of large tentative MPs from the filters for ATR-FTIR analysis, we determined the remaining PE, PP and PS content on the filters (originating from 125 to 5000 µm particles) as well as in the fine sediment fraction (20–125 µm) from the sediment samples via pyr-GC-MS.

We also analysed the filters of the water samples. However, we observed strong interferences in the mass spectra caused by the anodisc filters and did not proceed with the analysis.

For pyr-GC–MS, the glass microfibre filters and the fine sediment fractions were separately ground in a laboratory ball mill. However, the powders of the ground filters were too acidic (due to previous wet peroxidation throughout the extraction of the 125–5000 µm sediment fraction, see Section 2.3.2) to be directly used for pyr-GC–MS analysis. Thus, we resuspended the powder in water, allowed particles to settle (to impede rapid filter blocking) and filtered the majority of the supernatant volume through a glass fibre filter (pore size: 2.7 µm), before adding new water to the ground filter powders. This "washing process" was repeated at least twice (until the washing water had pH 7). Finally, the suspended filter powder was completely transferred on the new filter and dried for 7 d at 55 °C, before milling in a ball mill again.

Polymer extraction and pyr-GC–MS analyses were performed as previously described (Dierkes et al., 2019). In brief, the ground filters with the 125–5000 µm MP particles as well as the fine sediments were pre-extracted with methanol followed by an extraction with tetrahydrofuran using 10 mL extraction cells and an ASE-350 (Dionex, Sunnyvale, CA, USA). The extracts were transferred on calcined silica gel. After the addition of an internal standard (polystyrene-d_5), the calcined silica gel with the adsorbed extracts were analysed by pyr-GC–MS analysis. We monitored for PE, PP and PS based on characteristic pyrolysis products. Polymer abundance was calculated by comparison of the peak intensity of the characteristic pyrolysis products with the results of a calibration standard curve (for further details on pyr-GC–MS analysis, see S1.5).

2.5 *Validation and quality control*

2.5.1 Validation of MP extraction from water samples

We analysed potential effects of chemical digestion on MP integrity by exposing PE, PET, PP, PS und PVC MPs for 24 h to H_2O_2 (30%) and KOH (10 M). All particles were visually inspected afterwards. MP particles showed almost no signs of degradation.

2.5.2 Validation of MP extraction from sediment samples

The methodology for wet-sieving, density separation and acid digestion (125–1000 µm sediment fraction) was pre-validated threefold using artificial sediments spiked with MP. 1500 g of quartz sand were spiked with 125 MP particles (125–1000 µm) made of PE, PP, PS, PMMA and PVC (25 particles each). The total recovery rate for the whole process equals 87.2 ± 4.5% (mean ± SD). The reported MP concentrations may therefore be underestimates of actual concentrations in the sediment samples.

Further, we tested the impact of acid digestion on MP integrity. For this, MPs (473–1385 µm) made of PE, PS, PP, PA and PVC (ten particles each) were incubated in 33 mL of the 10:1 mixture of 30% H_2O_2 and 10% H_2SO_4 for 5 d at 55 °C. Recovery rates were determined both with regard to particle abundance and total surface area. Throughout the acid digestion, no particles were lost and changes in MP particle surface area were minor (≤ 4.4% surface area reduction) proving limited impacts of the acid digestion methodology on particle integrity.

2.5.3 Controls for water samples

For the extraction process, three "processing blanks" were run by digesting and filtrating 10 mL of distilled water in the same way as the water samples. For quantification of atmospheric fallout, we placed aluminium oxide filters ("sorting blanks") next to the digital microscope for 12 h (time needed for visual analysis of filters of one sampling site). MPs on the processing and sorting filters were characterised visually. Total MP concentrations in the water samples were corrected for the average particle number on the processing and the sorting blanks, respectively (results in S2.1.1).

2.5.4 Controls for the sediment samples

As for water samples, we included blanks for the extraction process ("processing blanks") and the visual sorting ("sorting blanks"). Contamination throughout the extraction process was quantified thrice by processing 500 mL distilled water in the same way as the 125–1000 µm sediment fraction (Section 2.3.2). During visual analysis of the samples, we placed empty glass fibre filters next to the stereo microscope to account for atmospheric fallout (one "sorting blank" per sampling site). MPs on the blanks were characterised visually and total MP numbers in the sediment samples were corrected for the average particle number on the processing blank as well as the sample site-specific sorting blank filters (results in S2.1.2 and Table S3).

2.5.5 Controls for the pyrolysis GC–MS

Controls for the pyr-GC–MS methodology were performed multifold by running the pre-extraction and the pyr-GC–MS analysis procedure (see Section 2.4.3) with tempered sea sand (ChemSolute, No. 804.9025) which had been calcined at 600 °C. None of the control runs detected any of the monitored pyrolysis products. In regard to the filters from the extraction of the 125–5000 µm sediment fraction, we further corrected the pyr-GC–MS results for the contamination throughout the extraction ("processing blanks") and visual sorting ("sorting blanks", see Section 2.5.4) of the 125–5000 µm sediment fraction. For this, we determined PE, PP and PS polymer mass concentrations on the "processing" and "sorting blank" filters via pyr-GC–MS (using the same methodology as described in Section 2.4.3) and corrected the mass concentrations of the different sampling sites for the mass concentrations on the blank filters, respectively.

2.6 Statistics

Results were analysed and plotted with GraphPad Prism 7.04 (GraphPad Software Inc., USA). Tentative MP concentrations in water and sediments from the Middle and Lower Elbe were compared with a non-paired, two-tailed *t*-test. Particle size distributions were fitted with a One-phase decay function and a bin width of 200 µm (first bin centre: 250 µm). Further, results from visual MP analysis and pyr-GC–MS were compared with a Spearman correlation.

3. Results

3.1 Numerical concentrations of MPs in Elbe water and sediments

According to the visual analysis of the 125–5000 µm particle fraction, Elbe water samples contained on average 5.57 ± 4.33 (SD) tentative MP particles m^{-3} (p m^{-3}, median: 5.11 p m^{-3}) with concentrations ranging from 0.88 (Geesthacht) to 13.24 p m^{-3} (Dömitz, Fig. 1). The concentration of tentative MPs in sediments was on average 600,000-fold higher (when referring to the same volume). Elbe sediments contained on average $3.35 \times 10^6 \pm 6.60 \times 10^6$ p m^{-3} sediment (median concentration: 7.6×10^5 p m^{-3}; $2.08 \times 10^3 \pm 4.67 \times 10^3$ p kg^{-1} sediment) and ranged from 2.26×10^4 (Vogelsand) to 2.27×10^7 p m^{-3} (Dessau, Fig. 1). In regard to MPs in the water phase in the Middle Elbe, highest MP concentrations were recorded at the Dessau site (11.56 p m^{-3}) and at Dömitz (13.24 p m^{-3}). The mean MP concentrations in the water phase in the Lower and Outer Elbe (Hafenstraße to Vogelsand, 3.07 ± 2.39 p m^{-3}) were lower compared to the Middle Elbe (Wittenberg to Geesthacht, 7.24 ± 4.68 p m^{-3}). However, the difference was not statistically significant (non-paired, two-tailed t-test, $p > 0.05$). Interestingly, MP concentration at Hafenstraße (4.73 p m^{-3}), the site directly located in the city of Hamburg, was lower compared to almost all sites in the Middle Elbe (except for Havelberg), while it was the second highest along the course of the Lower Elbe. Besides Dessau, sediments from Dömitz contained the second highest MP concentrations (4.87×10^6 p m^{-3}). Similar to the water

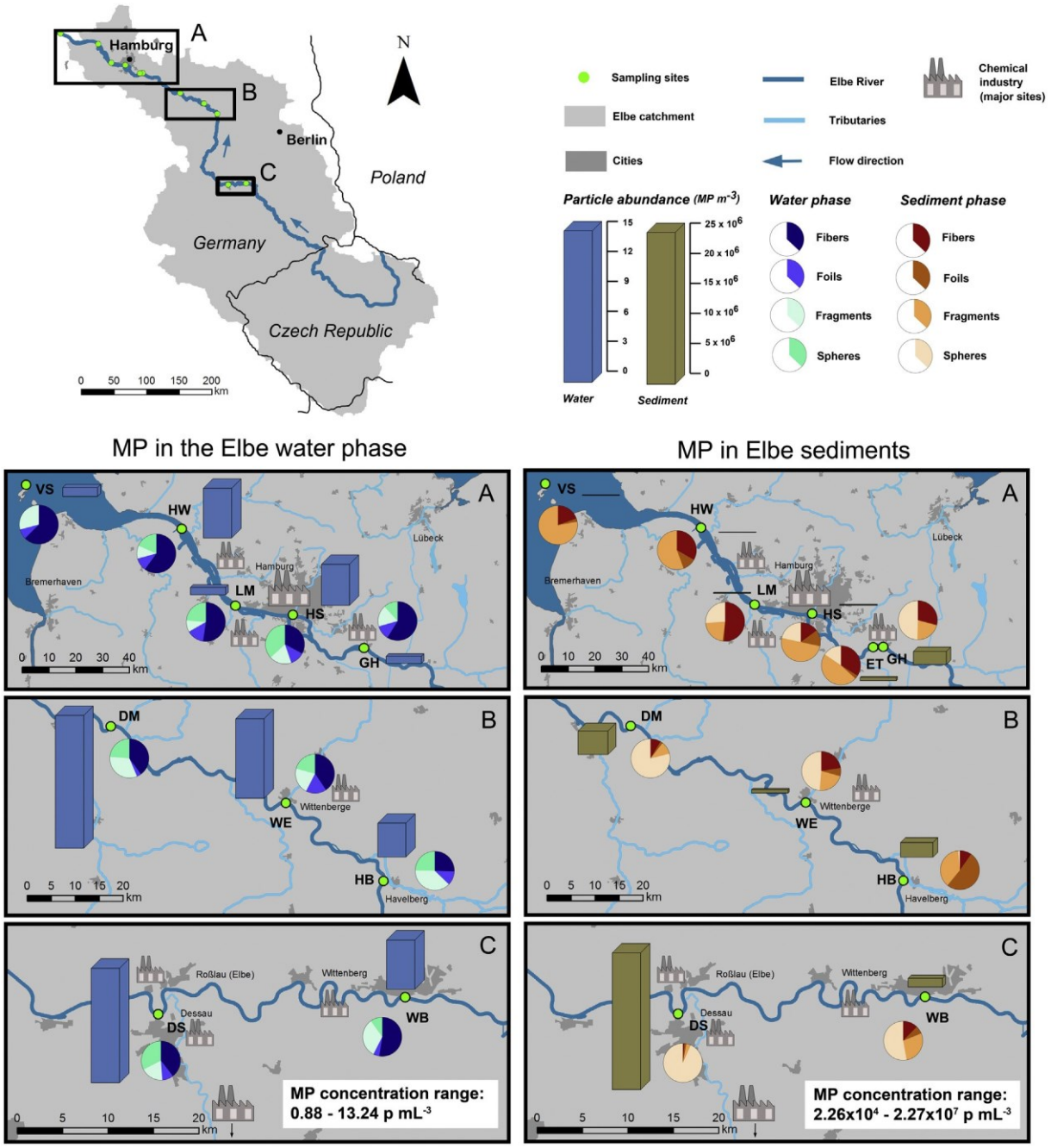

Fig. 1. Concentrations and shape of tentative MPs in water (150–5000 µm particle fraction) and sediment samples (125–5000 µm particle fraction) from eleven sampling sites along the Elbe river. Bars (water: blue, sediment: brown) indicate concentrations (p m^{-3} water/sediment, note the different scales) and pie charts indicate the relative particle shape distribution (fibres, fragments, spheres, foils). Site abbreviations: WB: Wittenberg, DS: Dessau, HB: Havelberg, WE: Wittenberge, DM: Dömitz, GH: Geesthacht, ET: Elbstorf, HS: Hafenstraße, LM: Lühemündung, HW: Hollerwettern, VS: Vogelsand. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

samples, concentrations in the Lower and Outer Elbe (Elbstorf to Vogelsand, $1.84 \times 10^5 \pm 2.32 \times 10^5$ p m^{-3}) were lower compared to the Middle Elbe (Wittenberg to Geesthacht, $6.0 \times 10^6 \pm 8.29 \times 10^6$ p m^{-3}), but these differences were not significant ($p>0.05$). Detailed results on numerical concentrations are provided in Table S3.

All four particle shapes (fibre, fragment, sphere, foil) were found both in water and sediment samples (Fig. 1, Fig. S3). On average, water samples mostly contained fibres (46.5%), while fragments (22.9%), spheres (20.1%) and foils (10.6%) were less abundant. In contrast, sediments contained predominantly spheres (35.5%) and fragments (34.2%), whereas only 21.5% and 9.1% of the particles where fibres and foils, respectively. While the particle shape distribution does not follow a clear trend along the course of the river, notable results were observed at specific sampling sites. At the Dessau site in the Middle Elbe, 93.4% of the MP particles in the sediment were spheres. In the water phase, spheres were less abundant (32.6%) but still the second most common shape following fibres (39.5%). At the river mouth (Hollerwettern, Vogelsand), fibres and fragments were the most abundant MP shapes both in the water and the sediment samples (details in Table S3).

The size distribution of tentative MPs (size range: 125/150–5000 µm) in the water and sediment phase increased exponentially with decreasing particle size (Fig. 2). However, Elbe sediments included a higher proportion of MPs <416 µm than the samples from the

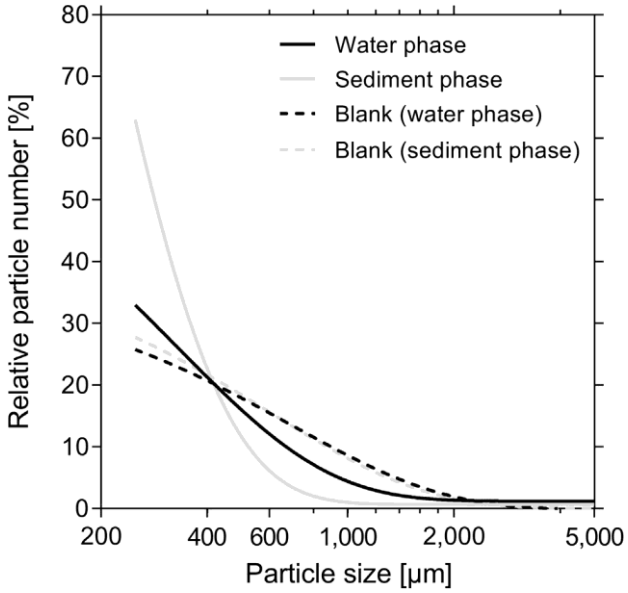

Fig. 2. Average size distributions of tentative MPs in the water and sediment samples from the Elbe river and the corresponding blanks. For better comparability, only particles with a size of 150–5000 µm were included.

water phase (details in Fig. S4). Blanks contained a higher proportion of particles between 500 and 2000 µm.

Tentative MPs in water and sediment samples were mostly transparent (water: 26.0%, sediment: 28.8%), blue (water: 19.7%, sediment: 15.2%) or white (water: 15.0%, sediment: 16.2%, Fig. S5). Furthermore, sediments also contained 10.7% of red MPs. The proportion of transparent and white tentative MP particles in the sediments decreased along the course of the river. At the Dessau site, the high proportion of transparent and white particles (95.5%) is linked to the high concentration of spheres at this sampling site.

3.2 Polymer types of tentative MPs

The polymer type of visually identified MPs in the water and sediment samples was determined by ATR-FTIR (see Section 2.4.2). 41 out of 584 tentative MPs (7.0%) from the water phase and 269 out of 4965 tentative MPs (5.4%) from sediments were analysed. In the water phase, most particles were made of PE (47.5%) and PP (45.0%), while in the sediments a more diverse set of polymer types was detected (Fig. 3). Besides PE (34.4%) and PP (12.5%), MPs in the sediments were also made of PS (18.5%) as well as ABS, PA, PET and PMMA (in total 2.0%). MP spheres found in very high numbers at the Dessau site were characterised as PS (determined by analysing a subsample of all Dessau spheres; later pyr-GC–MS results indicate that spheres may have actually been made of polystyrene-divinylbenzene (PS-DVB), see Section 4.5). Moreover, the proportion of particles that did not match a plastic type was larger for sediment samples (29.3%) compared to water samples (5.0%).

3.3. Mass-based concentrations of PE, PP and PS in Elbe sediments

3.3.1 Polymer content in the 125–5000 µm fraction of sediments

The mass concentrations of PE, PP and PS in the 125–5000µm fractions (excluding the MP particles analysed via ATR-FTIR) of the sediment samples were determined by pyr-GC–MS (Fig. 4a, Table S4). In average, Elbe sediments contained 21.4 ± 19.2 g polymer m^{-3} when summing up the PE, PP, PS content that contributed with 75.0, 16.8 and 8.2%, respectively. A marked decrease in concentrations was observed from the sites Wittenberg to Elbstorf compared to the downstream sites Hafenstraße to Vogelsand. Between Wittenberg and Elbstorf, mass concentrations varied intensively with lowest concentrations at the Dessau site (13.7 g m^{-3}) and highest concentrations at Havelberg (49.2 g m^{-3}). From Hafenstraße to the Elbe estuary, the polymer concentrations were much lower, ranging from 1.37 (Vogelsand) to 0.20 g m^{-3} (Lühemündung). When comparing the numerical and mass-based MP concentrations (Fig. 4b), results of both methodologies correlated significantly (Spearman correlation, $p < 0.05$).

3.3.2 Polymer content in the 20–125 µm fraction of sediments

We also determined the PE, PP and PS content in the fine sediment fraction (20–125 µm particles) by pyr-GC–MS and calculated the resulting mass-based polymer concentrations (Fig. 5). On average, sediments contained a total of 119 ± 149 g m^{-3} MPs (PE, PP and PS combined). The highest MP concentrations were found at Elbstorf (482 g m^{-3}) and Geesthacht (317 g m^{-3}). In comparison, the sampling sites Lühemündung (10.6 g m^{-3}), Hollerwettern (15.4 g m^{-3}) and Vogelsand (10.6 g m^{-3}) at the mouth of the Elbe had much lower

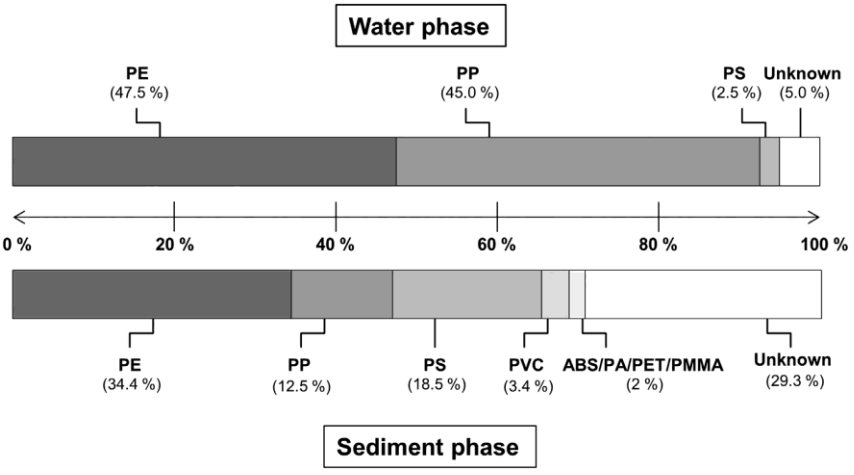

Fig. 3. Average composition of MP polymer types determined by ATR-FTIR analysis in the water and sediment samples from the Elbe river.

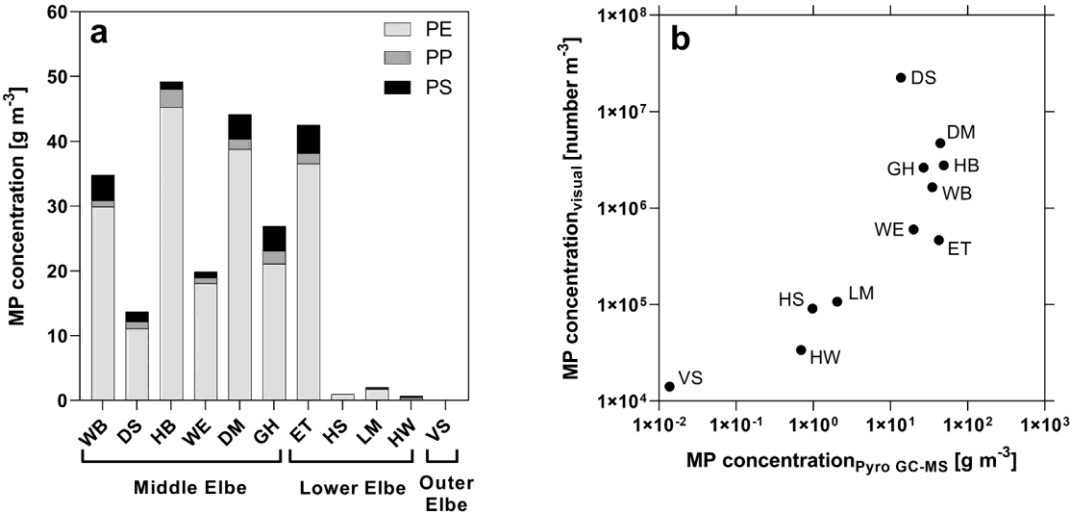

Fig. 4. Mass-based concentrations of the 125–5000 μm fraction of Elbe sediments determined by pyr-GC–MS. (a) Concentrations based on PE, PP and PS content. (b) Comparison of numerical and mass-based MP concentrations. Tentative MP concentrations were corrected for the number of removed particle for ATR-FTIR-analysis as those particles did not contribute to pyr-GC–MS results. For site abbreviations see Fig. 1.

concentrations. Further details on mass concentrations per volume and mass of sediments are provided in Table S5.

PE was the most abundant polymer in the fine sediment fractions at all sites except for Vogelsand. At Elbstorf and Geesthacht, samples contained much higher quantities of PS than PE. When excluding PS, the mean MP concentration was 65.6 ± 46.4 g m^{-3}, with PE (density: ~0.94 g cm^{-3}) contributing in average 80.94% and PP (density: ~0.91 g cm^{-3}) 19.06% to the polymer mass. Further, mean numerical concentrations were recalculated from the average mass-based concentration. Assuming that all particles are spherical and have an average density of 0.93 g cm^{-3}, MP concentrations range between 8.62 × 10^3 p m^{-3} (only 125 μm spheres) and 2.10 × 10^6 p m^{-3} (only 20 μm spheres).

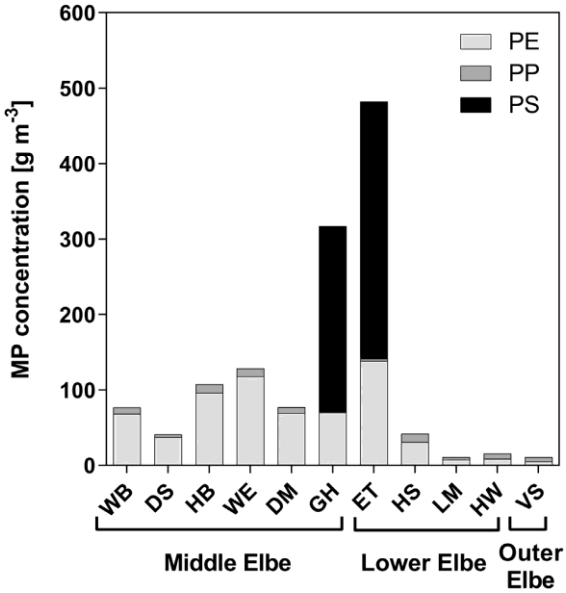

Fig. 5. Mass-based concentrations of the 20–125 μm fraction of Elbe sediments determined by pyr-GC–MS. For site abbreviations see Fig. 1.

4. Discussion

4.1 Riverine sediments are sinks for MPs

MP concentrations (125–5000 μm fraction) in sediments of the river Elbe (2.26 × 10^4 to 2.27 × 10^7 p m^{-3}) were in average 600,000-fold higher compared to the water phase (0.88 to 13.24 p m^{-3}). The same tendency is evident on a global scale (Fig. 6, see Section 4.6). In addition, we detected theoretically buoyant PE and PP in the sediments. This is in accordance with previous literature (Klein et al., 2015; Lin et al., 2018; Rodrigues et al., 2018) as weathering and biofouling, inclusions of substances during production, the formation of hetero-aggregates or sorption of biomolecules can increase particle density (Chubarenko et al., 2016; Corcoran, 2015; Moret-Ferguson et al., 2010). River sediments are, therefore, a key sink of MPs.

4.2 MP concentrations tend to decrease in the course of the river

We observed higher MP concentrations in sediments of the Middle Elbe compared to the Lower and Outer Elbe. The sudden decrease in MP concentrations at the sites Elbstorf and Hafenstraße are probably caused by a barrage at Geesthacht which separates the sampling site Geesthacht (Middle Elbe) from the sites in the Lower Elbe (Elbstorf to Hollerwettern). Thus, the tide affects the Outer and Lower Elbe up to the city of Hamburg (Hafenstraße) and to a lower extend up to the site Elbstorf, but not the sites further upstream. Tidal activity leads to a constant exchange of water bodies which may increase the transport of MPs into the North Sea and limit an accumulation of MPs in sediments. Each year, about 625,000 t of fine-grained sediments enter the estuary from the catchment area of the Elbe (IKSE, 2014; Schwartz et al., 2015). The tides also move marine sediments from the North Sea into the estuary (Schwartz et al., 2015) leading to sediment mixture and a further dilution of MP levels. Interestingly, our observations contrast previous publications that reported higher MP concentrations in the estuary compared to the river in the Yangtze and Yellow river (Xiong et al., 2019; Han et al., 2019). Differing results are potentially caused by river morphology and tidal activity as different tidal currents, circulation and geometry of each estuary strongly impact MP transport in lower rivers and estuaries (Wolanski and Elliott, 2015). In the water phase, MP concentrations were highest in the Middle Elbe and, similar to the sediment phase, also tended to decrease in the course of the river. But in contrast to sediments, concentrations

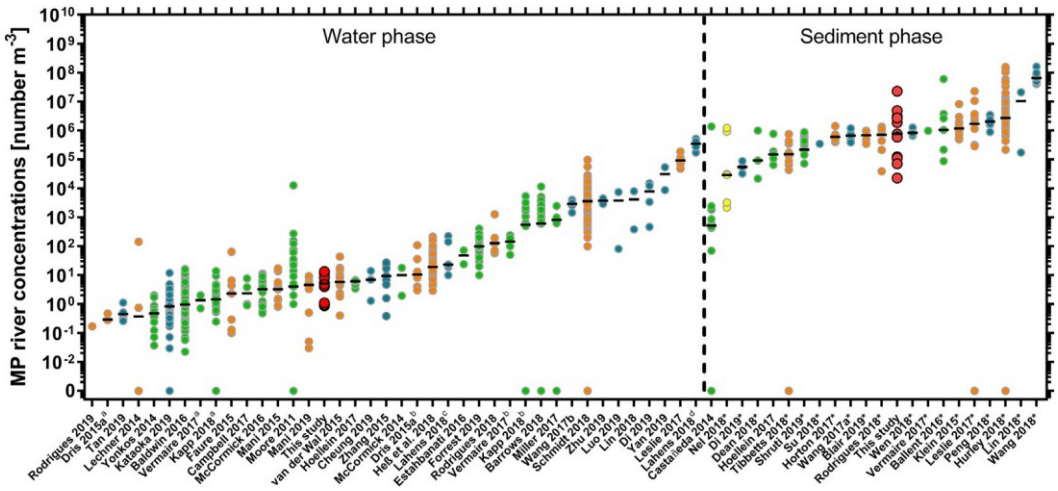

Fig. 6. Summary of published MP concentrations (water and sediment phase, sorted according to median) in rivers in Europe (orange, this study (red)), North- and South America (green), Asia (blue) and Africa (yellow). [a,b]For studies using two sampling techniques, we present MP concentrations separately. [c,d]Lahens et al. (2018) determined MP concentrations separately for fragments and fibres. *Studies reported MP concentrations as p kg^{-1} sediment. Concentrations were recalculated to p m^{-3} sediment based on a density of 2.17 kg dm^{-3} (average den- sity of the eleven Elbe sediments) (e.g., analyzed references are listed in S4). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

varied more intensively as higher MP concentrations were also detected in the Lower Elbe, especially at the sites Hafenstraße and Hollerwettern. Thus, tidal activity does not seem to affect MP concentrations in water in the same way as in sediments. Reduced tidal impacts may be explained by the constant drift of MPs downstream (if particles do not settle). Low retention times may lead to higher (short-term) fluctuation in MP concentrations, while tidal impact, instead, remains rather low.

4.3 MP hotspots highlight the relevance of industrial emissions

Very high MP concentrations were determined in the sediments at the Dessau sampling site. Spherical plastic beads, potentially made of PS-DVB (see Section 4.5, Fig. S6, Table S6), contributed most. This observation coincides with findings in the Lower Rhine. Here, Mani et al. (2019) detected PS-DVB spheres which had most probably been used as ion-exchange resin beads (Mani et al., 2019). The spheres collected at the site Dessau potentially originate from industrial areas of Dessau or Bitterfeld close to the sampling site in which plastic-processing industry has been established (Fig. 1). PS-DVB spheres were probably transported via the Mulde river into the Elbe with its confluence being located close to Dessau. Thus, industrial emissions may play a major role for local MP hotspots. In contrast to primary MPs, the exact origin and entry pathway for secondary MPs remain unknown. For instance, the composition of sampled MPs is heterogeneous and varies without clear trends (Fig. 1, Table S3). However, it is already known that WWTPs contribute to the MP pollution in rivers (Mani et al., 2015; Leslie et al., 2017). In the catchment of the Elbe, >2000 public waste water treatment plants (WWTPs) exist which clean >1.40 × 10^9 m^3 water $year^{-1}$. These are accompanied by industrial WWTPs which clean an additional water volume of 1.42 × 10^8 m^3 water $year^{-1}$ (FGG Elbe, 2014). High cleaning and, thus, discharge rates of WWTPs into the Elbe suggest that WWTPs may be an important source of MP pollution in the Elbe. Determining their relative contribution to the detected MP composition at the examined sampling sites, however, lies beyond the scope of this study. In general, highly populated urban areas are commonly discussed as a key source of MPs (Duis and Coors, 2016; Horton et al., 2017; Rodrigues et al., 2019). For the Elbe catchment, this could not be verified as water and sediment samples from the harbour area of Hamburg (Hafenstraße) contained lower MP concentrations compared to other more rural areas (e.g., Dömitz). Low levels may be explained by the constant dredging of the harbour areas to remove deposited sediments. Moreover, as discussed above, tidal influence may have transported sediments from Hamburg further downstream into the North Sea, thereby, reducing MP levels in the harbour sediments.

4.4 MP composition in the water and sediment differs

Sediments contained higher proportions of smaller particles compared to the water phase. Furthermore, sediments contained mainly spheres (due to high proportions (93.4%) at Dessau) and fragments, while fibres dominated the water phase. Reduced particle size and high levels of fragments in sediments point towards MP settlement and fragmentation in the sediments. Similarly, Lin et al. (2018) also observed a high proportion of fragments in sediments of the Pearl River (China), most probably due to a lower surface to volume ratio followed by sedimentation of these fragments (Wang et al., 2017b). Thus, fragments seem to be a relevant MP shape in river sediments.

The larger mean particle size of suspended MPs may possibly be caused by the enhanced proportion of fibres. Waldschläger and Schuettrumpf (2019) as well as Khatmullina and Isachenko (2017) confirmed that fibres obtain a relatively low settling velocity in freshwater experiments. The same is true for marine conditions (Bagaev et al., 2017). Low settling velocities and a constant water stream along the river thus possibly prevents fibres from sinking causing enhanced abundances in the river water phase and reduced accumulation in the sediments. The polymer distribution differed between the water and sediment phase. In the water samples, MP particles were mostly made of PE and PP (0.85–0.92 g cm^{-3}). This is in accordance with previous literature. For instance, PE and PP fragments also dominated in Antuã River (Portugal) as well as in a tributary of the Thames River (Rodrigues et al., 2018; Horton et al., 2017). In the sediments, polymer distribution was more diverse and also included PS (and PS-DVB, see Section 4.3), PVC, ABS, PA, PET and PMMA. On the one hand, the distribution pattern reflects the high production volumes of PE and PP (~50% of total demand, PlasticsEurope, 2017). On the other hand, an increased polymer diversity in the sediments is probably related to polymers with higher density that sink and deposit more easily in sediments (Horton et al., 2017).

4.5 Comparison of MP identification methodologies

In this study, visual identification, pyr-GC–MS and ATR-FTIR methods have been used for analysing and identifying MPs. Analysis by ATR-FTIR was mostly limited to particles >500 µm, while smaller particles were mainly analysed visually and by pyr-GC–MS. Visual analysis, although still the most commonly applied methodology for MP identification, is often criticised as inaccurate and subjective with a high potential to misestimate actual MP concentrations (Hidalgo-Ruz et al., 2012; Loeder and Gerdts, 2015). In comparison, pyr-GC–MS analysis is often considered advantageous compared to visual analysis due to higher analytical sensitivity (Fischer and Scholz-Böttcher, 2019). To evaluate the quality of both methodologies in regard to MP quantification, we correlated the results for visual and pyr-GC–MS analysis for 125–5000 µm particles in the Elbe sediments. For the correlation, we assumed that the particle size distribution and the polymer distribution were comparable in the different sediment samples. At least for the particle size distribution, this can mostly be confirmed (Fig. S4). A significant correlation of the numerical (visual) and mass-based concentrations (pyr-GC–MS) suggests that methodologies provide consistent results. Thus, relative proportions between MP concentrations at the different sampling sites are sufficiently determined by both methodologies.

However, it remains unclear whether the results from both methods are also comparable in terms of absolute MP levels at the different sampling sites. For instance, at the site Dessau, visual analysis indicated very high MP numbers (especially spheres, see Section 4.3). ATR-FTIR results confirmed that the spheres were mostly made of PS. Pyr-GC–MS analysis, however, could not find high PS mass in the Dessau sediment sample (Fig. 4). A possible explanation for this analytical discrepancy was potentially found in a follow-up sampling at the site Dessau in 2019: Throughout the new sampling, we detected spherical MPs which resembled the spheres detected as part of this study in 2015. A closer comparison of pyr- GC–MS results of the newly sampled MP spheres with PS reference materials revealed that the PS was crosslinked with DVB (Fig. S6, Table S6). The ATR-FTIR analysis performed throughout this study was not able to highlight this difference as the ATR-FTIR reference data base did not include a PS-DVB reference spectrum. PS-DVB particles are commonly used as ion exchange resins (Brady et al., 2017). The cross-linkage of PS with DVB may have thus affected the styrene detection by pyr-GC–MS. As such, the high number of detected PS spheres may not correlate with the PS mass measured in the pyr-GC–MS. A second explanation for low detected PS mass at Dessau may be related to a potential macroporous structure of the spheres. Macroporosity lowers the relative PS mass per particle volume, and thus the detectable PS mass.

In opposite to the 125–5000 µm sediment fraction, high PS contents in the fine sediments (20–125 µm) were only observed at the sampling sites Geesthacht and Elbstorf, but at none of the other sites. Increased PS abundances may originate from enhanced PS polymer fragmentation, but also from styrol-butadien-rubber (SBR) car tyre abrasion or from other polymers containing styrene (e.g., ABS, Eisentraut et al., 2018, Unice et al., 2012). However, the actual source currently remains unknown.

4.6 MP abundance in a global context

Overall, our results coincide well with previously published data from 51 studies on MP concentrations in rivers worldwide (Fig. 6). Here, median global MP concentrations in the water phase and sediments ranged between 0.17 (Rodrigues et al., 2019) and 3.45×10^5 p m^{-3} (Lahens et al., 2018) and 5.15×10^2 (Castañeda et al., 2014) and 6.49×10^7 p m^{-3} (Wang et al., 2018), respectively (Fig. 6). For water, MP levels in the Elbe (median: 5.11 p m^{-3}) are lower compared to other river systems (Fig. 6). In the context of German rivers, instead, MP concentrations in the Elbe are comparable to those reported for the Rhine, Danube and Weser (median: 3.27–19.10 p m^{-3}, Heß et al., 2018, Mani et al., 2015). Higher concentrations (median: 3.60×10^3 p m^{-3}) were only found by Schmidt et al. (2018) who sampled an urban canal in Berlin that may not be representative for MP pollution in large rivers. For sediments, the median MP concentration in the Elbe (7.57×10^5 p m^{-3}) corresponds to the middle section of global distribution (Fig. 6) and are two orders of magnitude lower than in the most contaminated river. In regard to German rivers, Elbe sediment concentrations matched well with concentrations in the rivers Main and Rhine (1.16×10^6 p m^{-3}, Klein et al., 2015). We, thus, assume that our measured MP concentrations and compositions are quite representative for German rivers.

However, such comparisons are hampered by the diversity of analysis and methods used in monitoring studies (Mai et al., 2018; Prata et al., 2019). For instance, MP concentrations are based either on numbers or weight and thus have a different size limit (125 vs. 20 µm) leading to different results. Moreover, different units for suspended (numbers per water volume) and settled MPs (numbers per sediment mass) impede direct comparisons. In this study, we overcome this limitation by recalculated MP sediment concentration (from this as well as previous publications, see Fig. 6) based on the average sediment density found in the Elbe. Although this approach is also associated with uncertainties, it is a relevant new approach to allow a broader comparison of global trends (Fig. 6).

Previous studies on global plastic pollution by Lebreton et al. (2017), Adam et al. (2018) and Besseling et al. (2018) pointed towards Asia as a key source of plastic pollution in aquatic ecosystems. In fact, many of the highest observed MP concentrations in water and sediment samples originate from Asian sampling sites. However, European sites (including the Elbe, especially in regard to its MP sediment concentrations) reach almost comparable levels (Fig. 6). High MP pollution is thus not exclusively restricted to the Asian countries or a single continent but is of global relevance.

5. Conclusion

This study provides for the first time data on MP pollution in the large European river Elbe and focused on the comparison of MPs in the water and sediment phase. MP concentrations were in average 600,000-fold higher in sediments (mean: 3.35×10^6 p m^{-3}) compared to the water phase (mean: 5.57 p m^{-3}). PE and PP were the most common polymer types in the water phase, while in the sediments a more diverse polymer distribution was observed. Riverine sediments are, therefore, a key sink of MP pollution.

Besides differences between the water and sediment, MP concentrations varied also over the course of the river. Decreasing downstream concentrations, especially in the Elbe sediments, can be explained by a barrage dividing the Elbe in a section without (Middle Elbe) and with (Lower and Outer Elbe) tidal influence. Limited tidal activity above the barrage leads to the retention of the sediments in the Middle Elbe causing elevated MP concentrations.

In regard to MP sources, industrial activities seem to be of special relevance for the river Elbe. Industrial emissions near the city of Dessau probably cause local pollution hotspots with PS-DVB spheres. In contrary, no distinctive relation between urban surrounding and MP levels was found.

In a global context, MP concentrations in the Elbe water and sediments range in the lower (water) and medium (sediment) section of the global MP concentration range for river water and sediments. Especially elevated MP concentrations in sediments at some of the Elbe sampling sites illustrate well that MP pollution is not restricted to specific countries or continent, but it must be considered a global issue we need to approach.

CrediT authorship contribution statement

Christian Scherer: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft, Writing - review & editing, Visualization, Validation. **Annkatrin Weber**: Investigation, Formal analysis, Writing - original draft, Writing - review & editing, Visualization. **Friederike Stock**: Investigation, Formal analysis, Writing - original draft, Writing – review & editing. **Sebastijan Vurusci**: Methodology, Investigation, Validation. **Harun Egerci**: Investigation, Validation. **Christian Kochleus**: Methodology, Investigation, Validation. **Niklas Arendt**: Investigation. **Corinna Foeldi**: Methodology, Investigation, Formal Analysis, Writing- original draft, Writing - review & editing, Validation. **Georg Dierkes:** Methodology, Investigation, Formal Analysis, Writing - original draft, Writing - review & editing, Validation. **Martin Wagner:** Conceptualization, Writing - review & editing, Supervision. **Nicole Brennholt:** Conceptualization, Writing - review & editing, Funding acquisition, Project administration. **Georg Reifferscheid:** Conceptualization, Writing - review &editing, Supervision.

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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Appendix A. Supplementary data

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Comparative assessment of microplastics in water and sediment of a large European river

- Supplementary data –

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S1 Supplementary Materials and methods

S.1.1 Sampling sites: Background information on collected samples

Tab. S1: Analysed volume/mass of water and sediments (wet and dry weight) and sediment densities for each sampling site along the Elbe river

Site (abbreviation)	Water samples	Sediment samples				
	Filtered water volume [m³]	Wet weight of total sample [g]	Dry weight of total sample[g]	Dry weight of sediment fraction 20–125 µm [g]	Sediment density [g cm^{-3}]	Volume of total (dry) sample [m³]
Wittenberg (WB)	6.2	1,200	576	63.4	2.16	0.000267
Dessau (DS)	3.2	213 (2,500)*	85.2 (1,000)*	n.a. (97.6)*	1.42 1.42	0.000704 0.000060
Havelberg (HB)	32.7	1,100	385	125.9	1.82	0.000212
Wittenberge (WE)	12.1	1,000	520	33.3	2.34	0.000222
Dömitz (DM)	3.7	1,500	570	152.2	2.14	0.000266
Geesthacht (GH)	12.5	1,066.7 (803)*	326.4 (266)*	n.a. (62.0)*	1.97 1.97	0.000166 0.000135
Elbstorf (ET)	-	623.5 (364)*	257.5 (161)*	n.a. (41.0)*	2.18 2.18	0.000118 0.0000739
Hafenstraße (HS)	14.8	1,128.9	949.4	54.6	2.54	0.000374
Lühemündung (LM)	15.8	2,641	1,357.5	709.9	2.24	0.000606
Hollerwettern (HW)	10.7	1,000	780	14.1	2.63	0.000297
Vogelsand (VS)	16.4	1,020	847.62	7.9	2.39	0.000355

* The sediment fraction 20–125 µm from Dessau, Geesthacht and Elbstorf were lost in the first wet-sieving process and therefore wet-sieving was repeated with a second sample to obtain the 20–125 µm fraction. Numbers in brackets refer to wet and dry weights from the second wet sieving. Wet and dry weights without brackets instead refer to the first wet-sieving from which the sediment fraction 125–5,000 µm was obtained and further processed. n.a. = not analyzed

S1.2 MP extraction from sediment samples

Microplastic (MP) extraction from the Elbe sediments included four processing steps: (I) Dry weight analysis, (II) Wet sieving, (III) Density separation and (IV) Acid digestion. The extracted MPs were identified and characterised (V) via visual sorting, Attenuated total teflection-Fourier-transform infrared spectroscopy (ATR-FTIR) and pyrolysis GC-MS (pyr-GC-MS, Fig. S1).

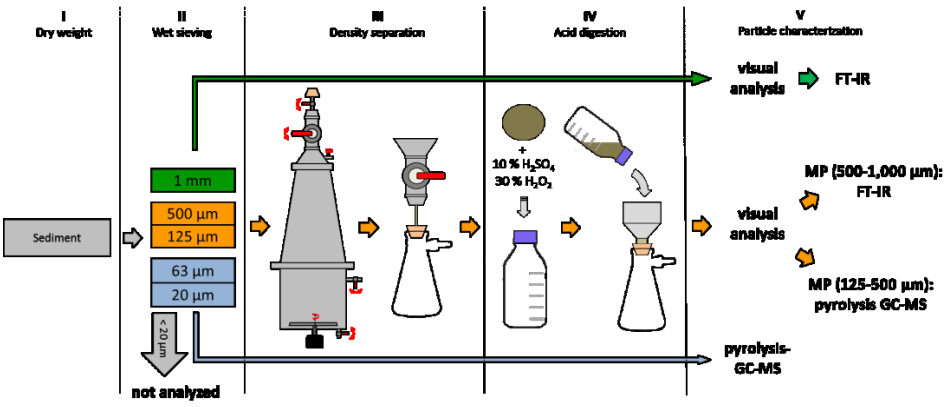

Fig. S1: Overview of the MP extraction methodology

(I) Dry weight determination

Dry weight content was quantified for each sediment sample by drying a subsample of 200 g (WW) over five days (d) at 55 °C and weighing the samples afterwards again. The ratio of dry weight to wet weight equalled the dry weight content [%].

(II) Wet sieving

MP extraction was facilitated by separating the sediment samples into following size classes using wet-sieving: < 20 µm, 20–63 µm, 63–125 µm, 125–500 µm, 500–1,000 µm and > 1,000 µm. The very fine particle fraction (< 20 µm) was discarded. For wet-sieving, we used a vibratory sieve shaker (Retsch Technology, AS 200 basic, vibration intensity: 80 %) with five stacked sieves (Retsch Technology, Test Sieve ISO 3310-1, ø: 200 mm, height: 50 mm, pore size: 20, 63, 125, 500, 1,000 µm). Throughout the sieving process, the sample was washed with distilled water until the run-off water appeared to be particle-free. Depending on the percentage of tone and silt, wet-sieving time ranged from 20 to 80 min.

The resulting sieve retentions were rinsed off with distilled water into 500 mL beakers and united in following three fractions: 20–125 µm, 125–1,000 µm and > 1,000 µm. The sediments > 1,000 µm were directly transferred on glass microfibre filters (GE Healthcare Life Sciences, Whatman, GF/D Cat. No. 1823-047, diameter: 47 mm, pore size: 2.7 µm), while all other fractions were stored in beakers at 4°C in the dark until further processing. Particles > 5,000 µm were not included.

(III) Density separation with the Munich Plastic Sediment Separator

The volume of the 125–1,000 µm sediment fraction was further reduced by $ZnCl_2$ density separation in a replicate of the stainless-steel Munich Plastic Sediment Separator (MPSS, Fig. S2, Imhof et al. 2012).

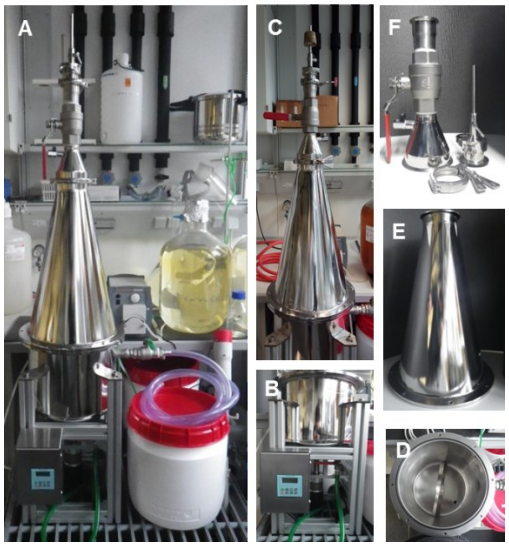

Fig. S2: Replica of the Munich Plastic Sediment Separator (MPSS) originally published by Imhof et al. (2012). Overview of the MPSS (A), sediment chamber (B), dividing chamber and filtration unit attached to the standpipe (C), interior of the sediment chamber (D), standpipe (E), dividing chamber (left) and filtration unit (right) which are connected by a bayonet joint (front, F).

For density separation, the sediment chamber (ø: 30 cm, height: 25 cm) of the MPSS was filled with $ZnCl_2$ solution (ρ = 1.6–1.8 g cm^{-3}) and the wet-sieved sediment sample (125–1,000 µm). After attaching the standpipe ($ø_{bottom\ end}$: 30 cm, $ø_{upper\ end}$: 10.4 cm, height: 50.9 cm) as well as the dividing chamber (height: 23.1 cm), the system was further filled with $ZnCl_2$, until the sample chamber was almost completely loaded with $ZnCl_2$ (approximately 38 L). Finally, the filtration unit with the filter holder was attached to close-off the system. The filter holder was equipped with a glass microfibre filter (GE Healthcare Life Sciences, Whatman, GF/D Cat. No. 1823-047, diameter: 47 mm, pore size: 2.7 µm) to allow direct and contamination-free filtration of the sample in the sample chamber after density separation.
The solution of $ZnCl_2$ and sediments was stirred for 3 h (14 rpm) by a rotor with an external control unit (Siemens, Sinamics G110) at the bottom of the sediment chamber, followed by a 24 h resting phase. Then, the dividing chamber was closed off, rotated 180° degrees and attached to a vacuum filter flask to filter off the retained $ZnCl_2$ solution. The remaining $ZnCl_2$ in the sediment chamber and standpipe was removed and recycled for reuse by vacuum-filtrating through a glass microfibre filter (VWR, Type 696, Cat. No. 516-0879, size: 125 mm, pore size: 1.5 µm). Due to hydrophobicity, MPs also attached to the interior of the standpipe and the dividing chamber. Therefore, the inner surface of the standpipe and the dividing chamber were rinsed with distilled water and the rinse water was also vacuum filtrated on glass microfibre filters (GE Healthcare Life Sciences, Whatman, GF/D Cat. No. 1823-047, diameter: 47 mm, pore size: 2.7 µm) for further analysis.
Due to a limited sediment quantity for the sampling site DS, density separation was performed in a 2 L separating funnel instead of the MPSS to minimise particle loss. After intensively mixing the sediment with $ZnCl_2$, the solution remained in the separating funnel for 24 h before the deposited particles were removed and the remaining sample was transferred on glass microfibre filters (GE Healthcare Life Sciences, Whatman, GF/D Cat. No. 1823-047, diameter: 47 mm, pore size: 2.7 µm).

(IV) Acid digestion
After density separation, the organic content in the separated samples was further reduced by acid digestion. Samples were transferred from the glass microfibre filters into 500 mL Schott bottles with 50–200 mL of a 10:1 mixture of 30 % H_2O_2 and 10 % H_2SO_4. The acid solutions were incubated for 5 d at 55 °C on an orbital shaker and afterwards filtered on glass microfibre filters (GE Healthcare Life Sciences, Whatman, GF/D Cat. No. 1823-047, diameter: 47 mm, pore size: 2.7 µm). Filters were stored in covered Petri dishes at 4 °C until being analysed.

S1.3 Determination of Elbe sediment densities

The volume of the sediment samples was calculated based on its mass and densities. Sample densities were determined by weighing in up to 90 g of dried sediment into volumetric flasks (100–500 mL) and filling up the remaining volume with ultrapure water. We allowed sediments to settle and air bubbles to escape before we filled up the flask to the gauge mark. Densities were calculated as:

Density [kg/m³] = (Sediment mass [kg]) / (Flask volume [m³]–Volume of ultrapure water [m³])

S1.4 Details on ATR-FTIR analysis

We identified a subsample of the visually identified tentative MPs by ATR-FTIR spectroscopy (Spectrum 2 with software: v10.03.09, Perkin Elmer, Waltham, MA, USA). Spectra were acquired with

the range set to 450–4,000 cm^{-1} (resolution: 4 cm^{-1}, total number of scans: 4, peaks referring to CO_2 and H_2O were suppressed). Resulting spectra were compared to a self-prepared reference data base which included spectra of the most common polymer types (PE-LD, PE-HD, PE-X, PP, PS, PVC, PET, PMMA, ABS, PA, PU).

Acid digestion is able to change the chemical surface of MPs. Therefore, we could not exclude the possibility that acid digestion had changed the surface characteristics of the tentative MP particles in our sediment samples. Therefore, we added also spectra of the "digested form" of the reference polymers to the spectra data base. Digestion was performed in an equal way compared to the sediment samples (10:1 mixture of 30 % H_2O_2 and 10 % H_2SO4, 5 d, 55 °C; compare S1.2).

S1.5 Details on pyrolysis GC-MS methodology

S1.5.1 Pyrolysis GC-MS analysis of the MP content in the Elbe sediments

For quantification of the polymers, characteristic pyrolysis products were monitored. These indicator compounds were specific for the certain polymer (Tab. S2). For the coarser sediment fraction (ground filters with the 125–1,000 µm sediment fraction), 1 g of sediment sample was weighed and for the fine sediment fraction (20–125 µm), the entire sample was used. Extraction was done using 10 mL extraction cells and an ASE-350 (Dionex, Sunnyvale, CA, USA). After a pre-extraction with methanol, the polymers were extracted using tetrahydrofuran at 185 °C and 100 bar (Dierkes et al. 2019). Extracts were collected in 60 mL vials containing 200 mg calcined silica gel (600 °C, 2 h). After extraction, 10 µL of Polystyrene-d_5 (270 µg mL^{-1} in dichloromethane) were spiked as internal standard and the solvent was subsequently evaporated. The silica gel was ground and 20 mg weighed into a pyrolysis cup. Pyr-GC-MS analysis was performed using a Multi-Shot Pyrolyzer (Frontier Laboratories, Saikon, Japan) and an Auto-Shot Sampler (Frontier Laboratories, Saikon, Japan) at 600 °C. The pyrolyzer was attached to an Agilent 7890B gas chromatograph (Santa Clara, CA, USA) equipped with an Ultra ALLOY UA-5(MS/HT) metal capillary separation column (Frontier Laboratories, Saikon, Japan). Column dimensions were 30 m length, 250 µm inner diameter and 0.25 µm film thicknesses. Chromatographic separation was performed by the following temperature program: hold at 40 °C for 2 min, increased with 20 °C min^{-1} to 320 °C and hold for 13 min. For detection, an Agilent MSD 5977B in scheduled selected ion monitoring (SIM) modus was used.

Tab. S2: Indicator compounds and selected ions ([a] = used for quantification)

Polymer	Pyrolysis product	Indicator ion	t_R [min]
Polypropylene	2,4-dimethylhept-1-ene	126[a]	4.89
		70	
Polyethylene	1,14-Pentadecadiene	81[a]	10.29
Polyethylene	1-Pentadecene	97[a]	10.32
Polystyrene	Styrene	104[a]	5.54
		91	
Polystyrene d_5	styrene-d_5	109[a]	5.50

A calibration standard was produced for each analysed polymer type (PE, PP, PS) by diluting the polymers in calcined sea sand (600 °C, 1 h) as inert matrix. For that, the polymers were ground in a cryomill (Retsch, Haan, Germany) into a fine powder. Approximately 30 mg of each polymer were exactly weighed in and the mixture of polymers was hence mixed with 10 g calcined sea sand (ChemSolute, No. 804.9025). This polymer/sand mixture was homogenised in a planet mill (Fritsch, Idar-Oberstein, Germany). To obtain calibration curves, the stock mixture was serial diluted in sand by mixing 1–2 g mixture and 8–9 g sand, respectively. The mixture was homogenised in a planet mill (Fritsch, Idar-Oberstein, Germany) after each dilution step. Calibration ranged from 0.005 to 10 mg polymer g^{-1} sand. Calibration samples were analysed in the same way as the other samples. Calibration curves were fitted by the Mass Hunter Quantitative Analysis tool (Agilent, Santa Clara, CA, USA) using 1/x weighting. Pyr-GC-MS results on the MP content in the Elbe sediments are summarized in chapter S2.3.

S1.5.2 Pyrolysis GC-MS analysis of the MP spheres collected at Dessau

Selected spheres from the water phase of a sampling campaign in Dessau (2019) were placed into a cup and flash pyrolyzed at 600°C using the same method as described in S1.5.1. Mass spectrometer was operated in full-scan mode (45–500 amu). Results for the pyr-GC-MS analysis of the MP spheres collected at the site Dessau are included in chapter S2.4.

Supplementary Results

S2.1 Quality controls

S.2.1.1 Quality controls for the visual MP analysis of the water samples

The three processing blanks from the water sample analysis contained in average 3.67 (~4) tentative MP particles of which 81.82 % were fibres and 18.18 % fragments. The majority of the particles were black (45.45 %) and blue (36.36 %). The corresponding sorting blanks included in average 2.33 (~2) MP particles (only black (71.43 %) and blue (28.57 %) fibres).

S.2.1.2 Quality controls for the visual MP analysis of the sediment samples

The three processing blanks for the sediment samples obtained in average 23.33 (~23) tentative MP particles (80.77 % fibres, 17.95 % fragments and 1.28 % foils). The most dominant colours were blue (48.72 %) and grey (21.79 %). MP abundance on sorting blanks for the sediment samples varied between 0 and 13 tentative MP particles (detailed results in Tab. S3). Particles on the sediment sorting blanks were mostly fibres (94.59 %; fragments: 4.05 %, foils: 1.35 %). The most common MP colours on the sorting blanks were blue (51.35 %), grey (18.92 %), transparent (9.46 %), purple and red (both 5.41 %).

S.2.2 Tentative MP in the water and sediment samples (visual analysis)

S.2.2.1 Summary of tentative MP abundances, particles shapes and size distributions

Tab. S3: Abundance and shape of tentative MP (water: 150–5,000 µm, sediment: 125–5,000 µm) in the water and sediment samples of the Elbe, n.a. = not analysed. For site abbreviations, see Tab. S1.

		WB	DS	HB	WE	DM	GH	ET	HS	LM	HW	VS
Water phase	Total counts	40	43	137	106	55	17	n.a.	76	21	65	24
	Processing blank	4	4	4	4	4	4	n.a.	4	4	4	4
	Sorting blank	2	2	2	2	2	2	n.a.	2	2	2	2
	Blank-corrected counts	34	37	131	100	49	11	n.a.	70	15	59	18
	Concentration [MP m^{-3} water]	5.48	11.56	4.01	8.26	13.24	0.88	n.a.	4.73	0.95	5.51	1.10
	Fibres [%]	52.50	39.53	25.55	40.57	40.00	58.82	n.a.	32.89	52.38	60.00	62.50
	Fragments [%]	32.50	18.60	37.96	21.70	32.73	17.65	n.a.	18.42	9.52	10.77	29.17
	Spheres [%]	10.00	32.56	24.82	20.75	23.64	11.76	n.a.	36.84	23.81	18.46	0.00
	Foils [%]	5.00	9.30	11.68	16.98	3.64	11.76	n.a.	11.84	14.29	10.77	8.33
Sediment phase	Total counts	535	1389	678	191	1330	488	103	74	96	45	36
	Processing blank	23	23	23	23	23	23	23	23	23	23	23
	Sorting blank	12	6	9	0	7	13	10	5	5	2	5
	Blank-corrected counts	500	1360	646	168	1300	452	70	46	68	20	8
	Concentration [MP m^{-3} sediment]	1,873,737	22,684,527	3,059,633	757,284	4,872,632	2,723,849	592,578	123,018	111,962	70,917	22,557
	Concentration [MP kg^{-1} sediment]	868	15,962	1,678	323	2,281	1,385	272	48	50	27	9
	Fibres [%]	12.34	1.22	9.73	22.51	8.57	28.89	33.98	14.86	51.04	33.33	19.44
	Fragments [%]	28.22	3.74	37.76	21.99	10.53	21.11	47.57	50.00	21.88	55.56	77.78
	Spheres [%]	52.90	93.38	1.33	48.69	78.87	49.59	15.53	21.62	26.04	0.00	0.00
	Foils [%]	6.54	1.66	51.18	6.81	2.03	0.41	2.91	13.51	1.04	11.11	2.78

S.2.2.2 Tentative MP particle shapes

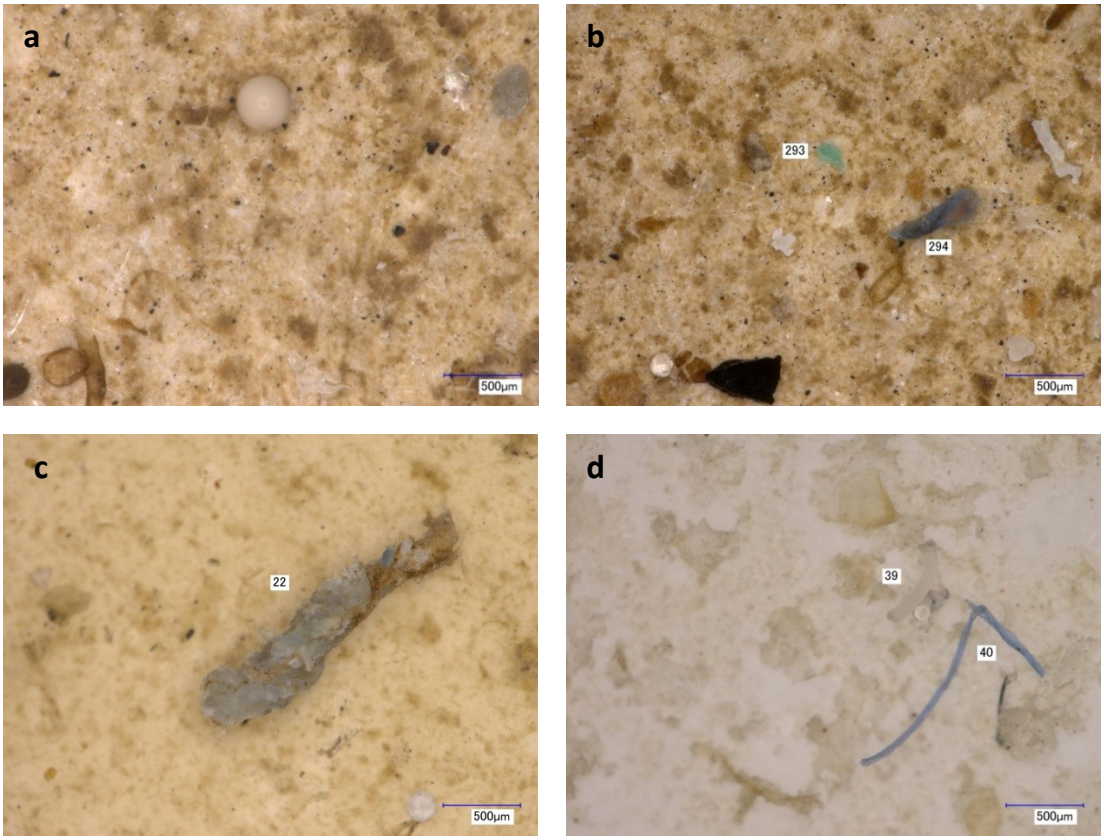

Fig. S3: Particle shape classification. Examples for (a) spheres (sampling site: Dömitz), (b) fragments (sampling site: Dömitz), (c) foils (sampling site: Hafenstraße) and (d) fibres (sampling site: Geesthacht).

S.2.2.2 Tentative MP size distribution at the separate sampling sites

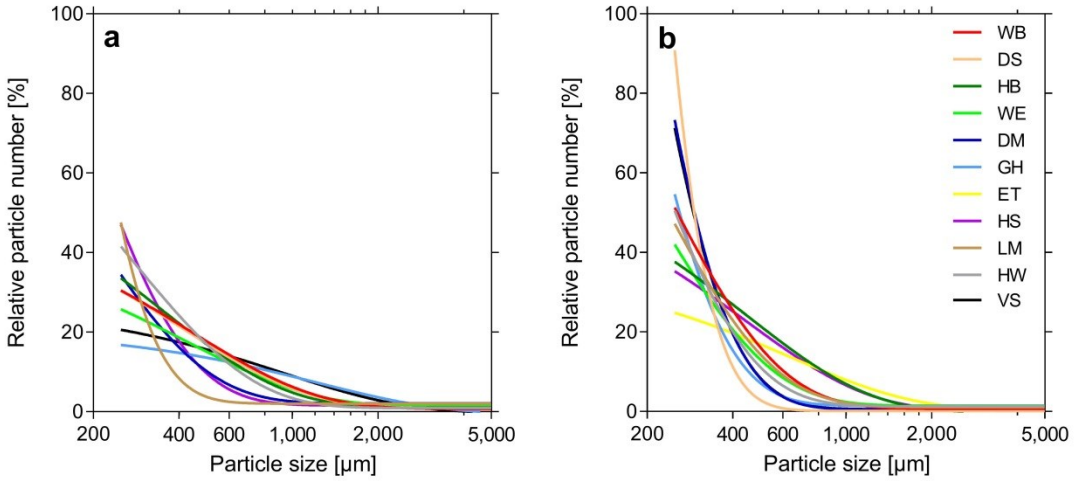

Fig. S4: Size distribution of tentative MP particles (visual analysis) in the water (a) and sediment (b) samples of the eleven sampling sites along the Elbe river. Particle size data was fitted with GraphPad Prism® (fit: One-phase decay). For comparability reason, only particles with a size of 150–5,000 µm were included in the size distribution analysis (leaving out 125–150 µm MPs from the sediments). Bin width: 200 µm, first bin centre: 250 µm. For site abbreviations, see Tab. S1.

S.2.2.3 Coloration of tentative MPs

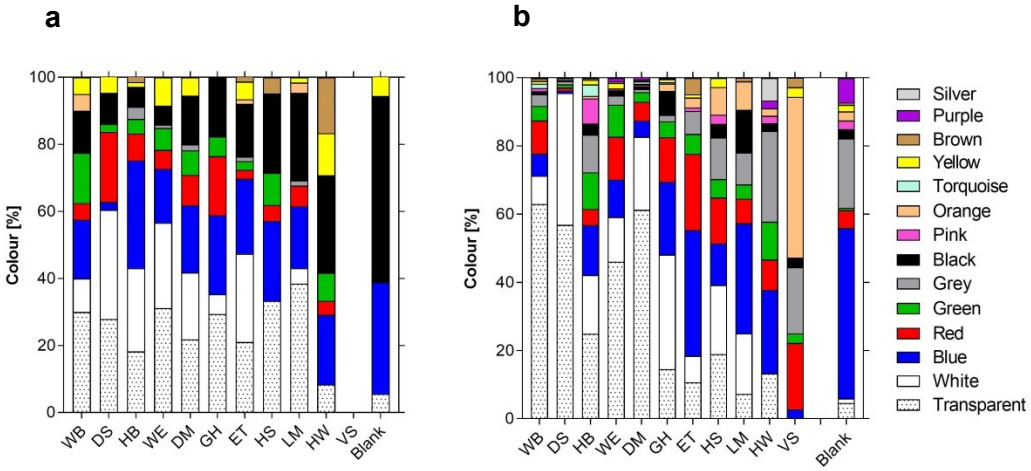

Fig. S5: Colour distribution of tentative MP particles (visual analysis) in the water (a) and sediment (b) samples of the eleven sampling sites along the Elbe river. For site abbreviations, see Tab. S1.

S.2.3 Mass concentration in water and sediment samples (pyrolysis GC-MS)

S2.3.1 Polymer content (originating from 125–5,000 µm MPs) in the sediment samples

Tab. S4: Polyethylene (PE), polypropylene (PP) and polystyrene (PS) content in the sediment samples [mg MP (125–5,000 µm particles) m^{-3} or kg^{-1} sediment (dry weight)] from the river Elbe. For details on the analysed volume/mass, see Tab. S1 (ratios were calculated based on the volume/mass of the total sediment sample, not just of the 125–5,000 µm sediment fraction). For site abbreviations, see Tab. S1.

	Concentration [mg MP m^{-3} sediment]				Concentration [mg MP kg^{-1} sediment]			
Sites	**PE**	**PP**	**PS**	**Total**	**PE**	**PP**	**PS**	**Total**
WB	2.99×10^{4}	9.43×10^{2}	4.03×10^{3}	3.48×10^{4}	13.83	0.44	1.86	16.13
DS	1.11×10^{4}	1.07×10^{3}	1.51×10^{3}	1.37×10^{4}	7.81	0.75	1.06	9.62
HB	4.53×10^{4}	2.75×10^{3}	1.20×10^{3}	4.92×10^{4}	24.87	1.51	0.66	27.04
WE	1.81×10^{4}	8.49×10^{2}	9.98×10^{2}	1.99×10^{4}	7.72	0.36	0.43	8.51
DM	3.88×10^{4}	1.53×10^{3}	3.87×10^{3}	4.42×10^{4}	18.12	0.72	1.81	20.65
GH	2.11×10^{4}	1.96×10^{3}	3.86×10^{3}	2.69×10^{4}	10.70	1.00	1.96	13.66
ET	3.65×10^{4}	1.62×10^{3}	4.36×10^{3}	4.25×10^{4}	16.77	0.75	2.00	19.51
HS	9.31×10^{2}	43.22	0	9.75×10^{2}	0.37	0.02	0	0.38
LM	1.66×10^{3}	2.14×10^{2}	1.62×10^{2}	2.03×10^{3}	0.74	0.10	0.07	0.91
HW	3.21×10^{2}	2.41×10^{2}	1.29×10^{2}	6.92×10^{2}	0.12	0.09	0.05	0.26
VS	0	13.66	0	13.66	0	0.006	0	0.006

S2.3.2 Polymer content (originating from 20–125 µm MPs) in the sediment samples

Tab. S5: Polyethylene (PE), polypropylene (PP) and polystyrene (PS) content in the sediment samples [mg MP (20–125 µm particles) m^{-3} or kg^{-1} sediment (dry weight)] from the river Elbe. For details on the analysed volume/mass, see Tab. S1 (ratios were calculated based on the volume/mass of the total sediment sample, not just of the 20–125 µm sediment fraction). For site abbreviations, see Tab. S1.

	Concentration [mg MP m^{-3} sediment]				Concentration [mg MP kg^{-1} sediment]			
Sites	**PE**	**PP**	**PS**	**Total**	**PE**	**PP**	**PS**	**Total**
WB	6.86×10^{4}	8.08×10^{3}	0	7.67×10^{4}	31.75	3.74	0	35.49
DS	3.72×10^{4}	3.51×10^{3}	0	4.07×10^{4}	26.21	2.47	0	28.68
HB	9.60×10^{4}	1.14×10^{4}	0	1.07×10^{5}	52.76	6.26	0	59.02
WE	1.18×10^{5}	1.03×10^{4}	0	1.28×10^{5}	50.46	4.39	0	54.85
DM	6.94×10^{4}	7.73×10^{3}	0	7.71×10^{4}	32.41	3.61	0	36.03
GH	6.99×10^{4}	1.41×10^{3}	2.46×10^{5}	3.17×10^{5}	35.50	0.71	124.72	160.93
ET	1.38×10^{5}	3.43×10^{3}	3.41×10^{5}	4.82×10^{5}	63.38	1.57	156.29	221.24
HS	3.07×10^{4}	1.10×10^{4}	84.78	4.18×10^{4}	12.08	4.32	0.03	16.44
LM	7.27×10^{3}	3.29×10^{3}	0	1.06×10^{4}	3.24	1.47	0	4.72
HW	8.55×10^{3}	6.84×10^{3}	0	1.54×10^{4}	3.25	2.60	0	5.85
VS	4.74×10^{3}	5.90×10^{3}	0	1.06×10^{4}	1.98	2.47	0	4.45

S2.4 Pyrolysis GC-MS analysis of the polymer spheres at the sampling site Dessau

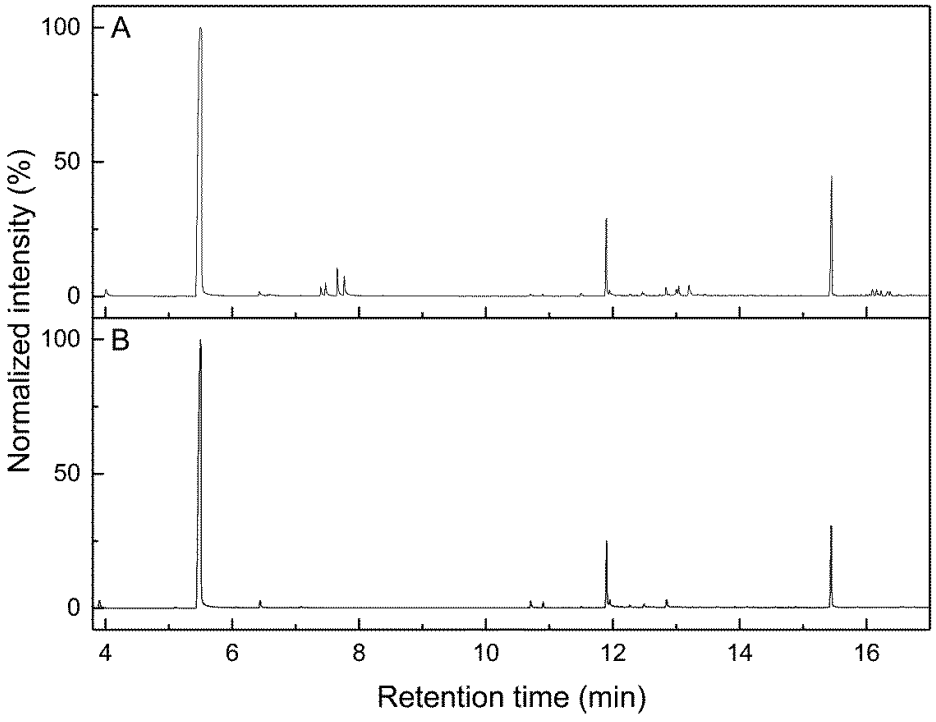

Fig. S6: Chromatogram of PS-DVB spheres at the sampling site Dessau (A) and a PS reference material (B).

Tab. S6: Peak list of PS-DVB and PS for measurements of Fig. S6.

Peak list PS-DVB		**Peak list PS**	
Retention time	**Substance**	**Retention time**	**Substance**
4.00	Toluene	3.90	Toluene
5.50	Styrene	5.50	Styrene
6.43	Alpha-methylstyrene	6.44	Alpha-methylstyrene
7.40	m-ethylstyrene		
7.47	p-ethylstyrene		
7.65	m-divinylbenzene		
7.76	p-divinylbenzene		
10.70	Bibenzyl	10.71	Bibenzyl
10.89	Benzene, 1,1'-(1-methyl-1,2-ethanediyl)bis-	10.90	Benzene, 1,1'-(1-methyl-1,2-ethanediyl)bis-
11.50	Benzene, 1,1'-(1,3-propanediyl)bis-	11.50	Benzene, 1,1'-(1,3-propanediyl)bis-
11.90	3-butene-1,3-diyldi-benzene (styrene dimer)	11.90	3-butene-1,3-diyldibenzene (styrene dimer)
12.84	2,5-diphenyl-1,5-hexadiene	12.85	2,5-diphenyl-1,5-hexadiene
13.00	Hybrid dimer of styrene and divinylbenzene		
13.04	Hybrid dimer of styrene and divinylbenzene		
15.45	5-hexene-1,3,5-triylbenene (styrene trimer)	15.45	5-hexene-1,3,5-triylbenene (styrene trimer)

S3. Supplementary literature

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A2. Ingestion and toxicity of polystyrene microplastics in freshwater bivalves

Studie 2

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%	**50**	35	10	5	-	-	-
Erstellung der Datensammlungen und Abbildungen							
Autoren	**AW**	NJ	CW	SU	NB	GR	MW
%	**60**	20	10	10	-	-	-
Analyse und Interpretation der Daten							
Autoren	**AW**	NJ	CW	SU	NB	GR	MW
%	**60**	30	-	-	-	-	10
Verfassung des Manuskripts							
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Ingestion and Toxicity of Polystyrene Microplastics in Freshwater Bivalves

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Abstract: The ubiquity of microplastics in aquatic ecosystems has raised concerns over their interaction with biota. However, microplastics research on freshwater species, especially mollusks, is still scarce. We, therefore, investigated the factors affecting microplastics ingestion in the freshwater mussel *Dreissena polymorpha*. Using polystyrene spheres (5, 10, 45, 90 µm), we determined the body burden of microplastics in the mussels in relation to 1) exposure and depuration time, 2) body size, 3) food abundance, and 4) microplastic concentrations. *D. polymorpha* rapidly ingested microplastics and excreted most particles within 12 h. A few microplastics were retained for up to 1 wk. Smaller individuals had a higher relative body burden of microplastics than larger individuals. The uptake of microplastics was concentration-dependent, whereas an additional food supply (algae) reduced it. We also compared the ingestion of microplastics by *D. polymorpha* with 2 other freshwater species (*Anodonta anatina*, *Sinanodonta woodiana*), highlighting that absolute and relative uptake depends on the species and the size of the mussels. In addition, we determined toxicity of polystyrene fragments (≤63 µm, 6.4-100 000 p mL^{-1}) and diatomite (natural particle, 100 000 p mL^{-1}) in *D. polymorpha* after 1, 3, 7 and 42 d of exposure, investigating clearance rate, energy reserves, and oxidative stress. Despite ingesting large quantities, exposure to polystyrene fragments only affected the clearance rate of *D. polymorpha*. Further, results of the microplastic and diatomite exposure did not differ significantly. Therefore, *D. polymorpha* is unaffected by or can compensate for polystyrene fragment toxicity even at concentrations above current environmental levels. *Environ Toxicol Chem* 2021;00:1–14. © 2021 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Microplastics; Toxic effects; Mollusk toxicity

INTRODUCTION

Plastics are part of almost every aspect of modern human life. However, the rising global plastic production (PlasticsEurope 2018) coincides with plastic pollution in nature. In particular, the fragmentation of plastic debris results in a global distribution of micrometer-sized plastic particles, so-called microplastics (1–1000 µm; Hartmann et al. 2019), in the aquatic environment. Accordingly, there is emerging concern over the potential environmental impacts of microplastics.

In recent years, research on microplastic exposure and toxicity has especially focused on marine bivalves. Their high filtration activity results in a higher ingestion of microplastics compared to other taxa (Setälä et al. 2016), rendering bivalves especially susceptible to microplastic exposure. Uptake of microplastics has repeatedly been demonstrated in wild and cultured marine bivalves (Li J et al. 2019). The current microplastic body burden varies intensively from ≤1 (Railo et al. 2018) up to several hundred particles per individual (Mathalon and Hill 2014). Numerous experimental studies further confirm that bivalves ingest microplastics of different size, shape, and polymer type (see Brillant and MacDonald 2000; Bråte et al. 2018; Li L et al. 2019).

While there is sufficient evidence demonstrating that bivalves ingest microplastics, less is known regarding the ingestion kinetics. In theory, microplastic ingestion will depend on multiple factors, including the physicochemical properties of microplastics (e.g., size, shape, polymer type), the exposure and depuration time of the individuals, biological traits of

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species (e.g., feeding type), microplastic bioavailability, and the presence of other particulate matter. Previous publications investigated some of these relevant factors in mussels (Brillant and MacDonald 2000; Capolupo et al. 2018; Woods et al. 2018; Fernández and Albentosa 2019; Gonçalves et al. 2019; Rist et al. 2019a, 2019b). However, the use of different experimental designs (e.g., different types of microplastics and exposure periods) limits the comparability of the existing data and prevents a general assessment of microplastic uptake kinetics. Furthermore, understanding better the mussel microplastic ingestion kinetics will improve our understanding of a potential microplastic uptake by humans through mussels as a food source (Mercogliano et al. 2020). Yet, understanding the factors driving microplastic ingestion by bivalves is important to identify species that are particularly susceptible to microplastic exposure and to design adequate toxicity experiments.

To address this knowledge gap, we studied the ingestion and egestion of microplastics in the freshwater species *Dreissena polymorpha* in 4 experiments with similar design. For this, we exposed *D. polymorpha* to a mixture of 5-, 10-, 45-, and 90-µm polystyrene (PS) spheres to analyze how the factors exposure and depuration time (experiment 1), body size (experiment 2), food abundance (experiment 3), and microplastic concentration (experiment 4) affect the body burden of microplastics in *D. polymorpha*.

Dreissenids are a well-established test organism in freshwater ecotoxicology (Péden et al. 2019), but because of their small size and invasive character, they may not be representative of larger species (e.g., Unionidae in European freshwater systems). We, therefore, repeated ingestion experiments 1 and 2 with the 2 larger freshwater species *Anodonta anatina* and *Sinanodonta woodiana* to evaluate species- and sizespecific variations.

In addition to microplastic ingestion, further data are needed regarding the toxicity of microplastics in freshwater bivalves. Previous research on *Corbicula fluminea* (Rochman et al. 2017; Guilhermino et al. 2018; Oliveira et al. 2018; Baudrimont et al. 2020) and *D. polymorpha* (Magni et al. 2018, 2019, 2020; Binelli et al. 2020) provided contradicting results. Although some of these studies identified significant microplastic-induced neurotoxicity, oxidative stress, and a change in feeding behavior, others did not observe such effects. Thus, we exposed *D. polymorpha* over 1, 3, 7, and 42 d to 6.4 to 100 000 particles (p) mL^{-1} using PS fragments (≤63 µm) and analyzed effects on the clearance rate as well as energy reserves (protein, glycogen, lipid content) and oxidative stress levels in the midgut gland (synonyms "digestive gland," "hepatopancreas") of *D. polymorpha*. In addition, we exposed the mussels to diatomite (100 000 p mL^{-1}) to compare the toxicity of microplastics and naturally occurring particles.

MATERIALS AND METHODS

Mussel culture

Dreissena polymorpha were collected from Oberwald Lake in Mörfelden-Walldorf, Germany (49°59′0.242″N, 8°35′48.666″E). *Sinanodonta woodiana* and *A. anatina* were purchased from local fish shops and cultures. In the laboratory, all bivalve species were cultured in aerated Organisation for Economic Cooperation and Development (OECD) medium (Organisation for Economic Co-operation and Development 2016) at 14 °C water temperature and a 16:8-h light:dark cycle. *Sinanodonta woodiana* and *A. anatina* were kept in 150-L tanks with up to 30 individuals $tank^{-1}$. *Dreissena polymorpha* was cultured in a 50-L tank with approximately 200 individuals $tank^{-1}$. Mussels used in the ingestion experiments were allowed to acclimatize for at least 1 wk, whereas those used in the toxicity study (*D. polymorpha*) were cultured for at least 4 wk prior to the experiments. Twice a week, at least half of the medium was renewed. Mussels were fed with algae (*D. subspicatus*) ad libitum at least thrice a week.

Particle characterization

For the ingestion experiments, we used 5-, 10-, 45, and 90-µm plain, fluorescent PS spheres. We used spheres with a homogenous size because this allows for investigation of the size dependency of microplastic ingestion and depuration. The 10-, 45-, and 90-µm PS spheres were purchased from PolyScience (Fluoresbrite YG microspheres; excitation 441 nm, emission 486 nm). The 5-µm spheres were obtained from MagSphere (excitation 538 nm, emission 584 nm). We suspended the PS spheres in ultrapure water (microplastic stock suspensions) and determined the particle concentration and size distribution in the stock suspensions with a Coulter counter (Multisizer 3; Beckman Coulter; details in Supplemental Data, S1).

For the toxicity study, we used PS fragments (≤63 µm) because of the higher environmental relevance of fragments compared to spheres (De Sá et al. 2018) as well as diatomite (Sigma-Aldrich). The PS fragments were prepared from orange fluorescent drinking cups (excitation 360–370 nm) by cryomilling, followed by sieving with a 63-µm sieve. The diatomite particles were sieved in the same way to obtain the size fraction ≤63 µm. In our previous work, we confirmed that the cups were made of PS and had low but detectable concentrations of chemicals which could not be matched to substances commonly used in plastics (see Weber et al. [2020] for details). Further, scanning electron microscopic images of PS fragments as well as diatomite particles are published in Weber et al. (2021). These images indicate that both microplastic and diatomite powder included nano-sized particles. As stated in Weber et al. (2021), nanoparticle abundance was not quantified because micro-sized particles would have blocked the nanoparticle tracking analysis instrument.

We determined the particle number per powder mass (number mg^{-1}) and the particle size distribution with a Coulter counter (size range 2–60 µm). The microplastic and the diatomite powder contained 287 526 and 4 632 990 p mg^{-1}, respectively. Particle size distribution of the PS and the diatomite suspensions increased exponentially with decreasing particle size; 90% of the microplastic and the diatomite particles were smaller than 12.4 and 11.8 µm, respectively (for detailed methods and results see Weber et al. [2020]).

Polystyrene microplastics in freshwater bivalves — *Environmental Toxicology and Chemistry*, 2021;00:1–14

Relevant factors affecting microplastic ingestion and depuration by D. polymorpha

Basic exposure scenario. All ingestion experiments with *D. polymorpha* were performed using the same basic exposure scenario (see Supplemental Data, S2.1). Ten-liter glass tanks (19 × 29.3 × 19 cm) were filled with 7.5 L aerated OECD medium and quartz sand (5 cm layer, previously annealed at 200 °C for 24 h). Each tank was equipped with a pump (Tetra IN400plus; Tetra) set to the lowest rate to create a constant circular water flow and to keep the particles suspended in the water column. The extent to which the particles remained in the water phase was characterized in an extra experiment. Despite a constant water flow, PS spheres settled, with smaller microplastics remaining longer in the water column. From 6 h onward, only 5- and 10-µm PS spheres were present in the water phase; and after 12 h, 50.2% of the 5-µm spheres and 77.3% of the 10-µm spheres had cleared from the water phase (for details on methods and results, see Supplemental Data, S2.2).

Twelve hours prior to the start of the experiments, 6 mussels were transferred to each tank. Algae (*D. subspicatus*) were added 1 h before the start of the experiment to stimulate filtration behavior. Each experiment started when microplastics were added to the tanks by pipetting the microplastic stock suspensions directly below the water surface into the flow of the pump. Because of the short exposure times (1–48 h), the water was not exchanged during the ingestion experiments. Throughout each experiment, we visually monitored each mussel hourly and recorded whether its valves were opened or closed. Only mussels which were open at least at 50% of the monitored time points (e.g., ≥6 time points throughout a 12-h exposure period) were classified as "active mussels" and further analyzed. In the following sections, we used this basic exposure scenario to examine the impact of the factors exposure and depuration time, body size, food abundance, and exposure concentration on microplastic ingestion by *D. polymorpha*.

Exposure and depuration time (experiment 1). The impact of the exposure time on microplastic ingestion was analyzed by exposing *D. polymorpha* (1.8–2.2 cm maximal shell length) for 1, 3, 6, 12, 24, and 48 h to a mixture of 5-, 10-, and 45-µm PS spheres at 3 p mL^{-1} each. Further, we added 90-µm PS spheres (0.1 pmL^{-1}) to examine whether *D. polymorpha* is able to ingest also larger microplastic particles. We applied these at lower concentrations because of high material costs, and the respective data were, thus, analyzed separately. We used one separate tank with 6 individuals for each time point. Algae were added to each tank at a concentration of 1 mg L^{-1} total organic carbon (TOC). Four "active" individuals from each tank were analyzed for their microplastic body burden.

For analysis of the impact of depuration time on the body burden of microplastics in *D. polymorpha*, we exposed 30 mussels (5 tanks with 6 individuals each) in the presence of algae (1 mg L^{-1}) to PS spheres (sizes and concentrations as stated in the previous paragraph) for 12 h. After the exposure, 28 active mussels were randomly selected and transferred into tanks filled with microplastic-free OECD medium. Individual D. polymorpha were held there for 1, 3, 6, 12, 24, 72, and 168 h (one tank per depuration time point with 4 individuals each). Directly after the transfer and subsequently every 24 h afterward, mussels were fed 1 mg L^{-1} TOC algae. Because mussels are able to reingest excreted microplastic, we transferred the mussels into new tanks with fresh medium after 24, 72, and 120 h to minimize microplastic reuptake.

Body size (experiment 2). In the second experiment, we evaluated the relationship between body size and microplastics in *D. polymorpha*. For this, *D. polymorpha* individuals from 3 different size classes (1.0–1.5, 1.8–2.2, and 2.5–3.0 cm) were exposed in the presence of algae (1 mg L^{-1} TOC) to PS spheres (sizes and concentrations as above, see section Exposure and depuration time [experiment 1]) for 12 h. For each size class, 2 tanks were set up with 6 individuals each. After the exposure, microplastic body burden was analyzed in 8 active out of the 12 exposed individuals per size class.

Food abundance (experiment 3). We evaluated how algae abundance affects the microplastic body burden by exposing *D. polymorpha* (1.8–2.2 cm) for 12 h to PS spheres (sizes and concentrations as above, see section Exposure and depuration time [experiment 1]) in the presence of 3 algae concentrations (0.2, 1, or 5 mg L^{-1} TOC algae). The microplastic to algae ratios (based on particle numbers) in the 3 exposures were 1:5589, 1:27 798, and 1:135 477, respectively, based on the sum concentration of 5-, 10-, 45-, and 90-µm spheres and of algae cells in each tank. Algae were added 1 h before the start of the experiment to stimulate filtration activity. For each algae concentration, 2 tanks with 6 mussels each were established and 8 active individuals per treatment analyzed.

Microplastic concentration (experiment 4). We investigated the relationship of microplastic concentration and microplastic body burden in *D. polymorpha* (2.5–3.0 cm) by exposing the mussels in the presence of algae (1 mg L^{-1} TOC) for 12 h to 5-, 10-, and 45-µm PS spheres at either 0.3 or 3 p mL^{-1} each. Again, we also added 90-µm PS spheres (0.01 or 0.1 p mL^{-1}, respectively). The 10-fold lower concentration of 0.3 p mL^{-1} was chosen to resemble environmental concentrations already reported for freshwater systems (Leslie et al. 2017; Lahens et al. 2018). For both concentrations, 2 exposure tanks were prepared (6 mussels per tank) and 8 active mussels were analyzed per concentration.

Analysis of microplastic body burden in D. polymorpha. After each experiment, *D. polymorpha* individuals were thoroughly rinsed with tap water and frozen at –80 °C. After defrosting, the shells were removed. All tissues were lyophilized to determine the total dry weight of each mussel. Afterward, tissues were lysed in 20 to 40mL 10% potassium hydroxide solution at 55 °C for 24 to 48 h. The lysate was filtered on glass fiber filters (pore size 1.25 µm; VWR). Each filter was analyzed visually with a fluorescence microscope (BX50, ×40 magnification;

Olympus), and the number of fluorescent spheres on the whole filter was determined for each microplastic type (for details see Supplemental Data, S2.2). The body burden in the mussels was characterized both separately for each microplastic type and as the total number of ingested microplastics (total body burden). The latter corresponds to the sum of 5-, 10-, and 45-µm PS spheres. Because of the divergent exposure concentrations (see section Exposure and depuration time [experiment 1]), the results for the 90-µm spheres are not included in the total microplastic body burden and are presented separately.

Quality assurance. The background contamination with fluorescent particles in *D. polymorpha* tissues was determined by analyzing mussels (1.0–1.5, 1.8–2.2, 2.5–3.0 cm) from the culture which had not been exposed to microplastics. We lysed 3 *D. polymorpha* individuals from each size class, as described in Analysis of microplastics body burden in *D. polymorpha*, and corrected all data from the ingestion experiments for the microplastic body burden in those control mussels (see Supplemental Data, S3.2) by subtracting the average number of microplastic-resembling particles per control mussel from the microplastic body burden in exposed individuals (separate data correction for each particle type).

Comparison of microplastic ingestion between freshwater mussel species

We further compared microplastic ingestion by *D. polymorpha* with other freshwater mussel species. Originally, we intended to repeat the experiments just described with the native species *A. anatina*. However, the number of available *A. anatina* specimens was too low. Therefore, we used a second species (*S. woodiana*) with similar morphology and ecology and limited the comparative studies to experiments 1 and 2.

We repeated experiment 1 with *S. woodiana* (9.5–12.0 cm). For the depuration experiment, we exposed 24 mussels (4 tanks with 6 individuals each). After the exposure, 16 "active" *S. woodiana* (for definition, see section Basic exposure scenario) were randomly selected and transferred into tanks filled with microplastic-free OECD medium. We limited the number of depuration time points to 4 (12, 24, 72, and 168 h) because of the limited number of mussels available.

We repeated experiment 2 with *S. woodiana* and *A. anatina* (2 size classes each: 6.0–8.0, 9.5–12.0 cm). For each species and size class, 2 tanks were set up with 6 individuals each. After the exposure, microplastic body burden was analyzed in 8 active out of the 12 exposed individuals from each treatment. Because of the mussels' inactivity in some treatments, the experiment was repeated with a third tank to obtain 8 active individuals per treatment.

For analysis of the microplastic body burden in *S. woodiana* and *A. anatina*, we did not analyze the whole body as we did for *D. polymorpha* because of the large body size and, thus, insufficient tissue lysis. Instead, we removed the mantle, gills, and foot. In a prior experiment, we demonstrated that microplastic levels in the mantle, gills, and foot were low compared to the other tissues; and we, thus, consider the number of microplastics in the removed tissues negligible (for details see Supplemental Data, S3.1). After dissection, we lyophilized both the mantle, gills, and foot and the remaining tissues to determine the total dry weight of each mussel. The mantle, gills, and foot tissue was discarded afterward, whereas the remaining tissue was analyzed for the microplastic body burden as described in Analysis of microplastics body burden in *D. polymorpha*. Again, we determined background contamination by lysing control mussels and correcting the results from the ingestion experiments accordingly (for detailed results, see Supplemental Data, S3.2).

Microplastic toxicity in D. polymorpha

Exposure scenario. *Dreissena polymorpha* were exposed to either 6.4, 160, 4000, or 100 000 p mL^{-1} PS fragments (≤63 µm) or 100 000 p mL^{-1} diatomite for 1, 3, 7 (acute exposure), and 42 d (chronic exposure). We also included a negative control without microplastic/diatomite. Each particle concentration was tested in a separate glass tank (14 × 20 × 20 cm) with 3 L of OECD medium and 40 mussels (2.0–2.3 cm). To keep the experiments manageable, we set up one set of 6 glass tanks (one tank per treatment) for the acute exposures (1, 3, and 7 d; for all 3 time points mussels were sampled from the same tank), whereas for the chronic exposure we set up a separate set of 6 glass tanks (42 d; scheme of the experimental design in Supplemental Data, Figure S2).

The required masses of microplastic and diatomite were weighed for each treatment and added directly to the medium in the aquaria. For the 6.4 p mL^{-1} treatment, we used a 100-fold diluted stock suspension in OECD medium, which was applied to the respective tank. Each tank was constantly aerated through 2 glass pipettes to enhance particle dispersion in the water phase. The mussels were fed with algae (*D. subspicatus*, 0.25 mg TOC $individual^{-1}$) daily. In the chronic exposure experiment, the medium was completely renewed every 7 d by transferring the mussels to new tanks prepared as described.

Mortality was recorded daily, and dead individuals were removed. After 1, 3, 7, and 42 d, the clearance rate of 10 individuals per treatment was determined. In case of acute exposures, individuals were reintroduced into their corresponding tanks afterward. Further, at each time point, 10 individuals were frozen in liquid nitrogen and stored at –80 °C for energy reserve and stress metabolite analysis.

Clearance rate, energy reserves, and stress metabolites. We quantified the clearance rate of *D. polymorpha* by placing 10 mussels per treatment and time point individually in an algae suspension and determining algae concentrations in the medium as chlorophyll fluorescence (in relative fluorescence units [RFUs]) prior to and after 45 min (Tecan; GENios; excitation 440 nm, emission 680 nm). The starting concentration was 4000 ± 360 RFU (±standard deviation), representing 1.21 × 107 ± 1.08 × 106 algae cells mL^{-1}. The clearance rate is the difference in RFUs before and after 45 min. We used *Raphidocelis subcapitata* (formerly *Pseudokirchneriella*

subcapitata) instead of *D. subspicatus* because it is unicellular, allowing for more accurate fluorescence analyses.

As biochemical endpoints, we analyzed the energy content as well as oxidative stress markers in the midgut gland of *D. polymorpha*. The midgut glands from 10 individuals per treatment were dissected, wet-weighed, homogenized, and frozen at –80 °C. Energy reserves in the midgut gland homogenates were measured as the protein content according to Bradford (1976) and as the glycogen content (anthrone assay) and the total lipid content (sulfo-phospho-vanillin assay) according to Benedict (2014). Oxidative stress was quantified as the malondialdehyde (MDA) content (an important biomarker for lipid peroxidation) as well as the remaining antioxidant capacity. Concentrations of MDA were measured using the thiobarbituric acid reactive substances (TBARS) assay (Hodges et al. 1999; Furuhagen et al. 2014). The remaining antioxidant capacity in the midgut gland was measured with the oxygen radical absorbance capacity (ORAC) assay (Ou et al. 2001; Furuhagen et al. 2014). Further methodological details are provided in Weber et al. (2020). Because of highly divergent results for midgut gland with a wet weight < 5 mg compared to midgut gland > 5 mg, we excluded results on energy reserves and oxidative stress from individuals with an midgut gland < 5 mg (n = 7–10).

Statistics

All statistical analyses were performed with IBM SPSS Statistics (Ver 25) using one-way or 2-way analysis of variances (ANOVAs). Prior to each analysis, we tested for normality (Shapiro-Wilks test), variance homogeneity (Levene test), and heteroscedasticity (F test). Very few treatments violated the normality criteria. In this case, we reperformed the statistical analysis after outlier exclusion but did not observe changes in the results. All data were visualized using GraphPad Prism 8.4.3 (GraphPad Software).

Statistics for the ingestion experiments with D. polymorpha. We analyzed the effects of the factors exposure time and depuration time (experiment 1), individual size (experiment 2), food abundance (experiment 3), and microplastic concentration (experiment 4) on the microplastic body burden by applying separate one-way ANOVAs for each factor (dependent variable, absolute or relative microplastic body burden; fixed variables, factors listed). In addition, we performed Tukey's posttests to analyze differences between the treatments of each experiment (full-factorial comparison of all treatments). Data for total microplastic body burden were logtransformed (depuration time, individual size, microplastic concentration), square root–transformed (food abundance), or not transformed (exposure time) for the statistical analysis.

Statistics for the comparison of microplastic ingestion between species. We analyzed the effects of the variable species in combination with the variable exposure time, depuration time (experiment 1), or individual size (experiment 2) as well as their interaction on the total microplastic body burden (absolute or relative) in the analyzed freshwater mussels with 2-way ANOVAs. Both variables were integrated as fixed variables, whereas the total microplastic body burden was used as a dependent variable. Further, for the results of experiment 2 we performed Tukey's posttest to determine homogenous subgroups in regard to the different species (*D. polymorpha*, *S. woodiana*, *A. anatina*) as well as size classes (1.0–1.5, 1.8–2.2, 2.5–3.0, 6.0–8.0, 9.5–12.0 cm). Data for total microplastic body burden were log-transformed (depuration time, individual size) or square root–transformed (exposure time) for the statistical analysis.

Statistics for the microplastic toxicity study with D. polymorpha. In the toxicity study, the effects of microplastic concentration and exposure time (both fixed variables) as well as their interaction (microplastic concentration × exposure time) were determined with 2-way ANOVAs for each endpoint (clearance rate, protein, glycogen, total lipids, MDA, antioxidative capacity) as a dependent variable. Data for the dependent variable were integrated as either log- (glycogen, lipids, MDA [TBARS], Trolox equivalents [ORAC]), square root– (protein), or third root–transformed (RFU [clearance rate]).

Statistical comparison of the microplastic and the diatomite exposure (both 100 000 p mL^{-1}) with 2-way ANOVAs was performed as described for the toxicity study but with the variable particle type instead of microplastic concentration. Data for the dependent variable were transformed as described in the previous paragraph.

RESULTS

Factors affecting microplastic ingestion and depuration by D. polymorpha

Exposure and depuration time (experiment 1). Exposure time significantly affects total microplastic number in *D. polymorpha* ($p < 0.01$). The total microplastic body burden (5-, 10-, 45-µm PS spheres) in *D. polymorpha* was highest after 1 h and decreased afterward, with another peak after 12 h (Figure 1A). These 2 peaks were significantly higher compared to 48 h of exposure ($p < 0.05$). The 10-µm PS spheres were found in highest quantities except after 12-h exposure, when the quantity of 5-µm PS spheres exceeded that for 10-µm spheres.

Similarly, the depuration time had a significant effect on microplastic number ($p < 0.001$). The total microplastic body burden significantly decreased from 1 h of depuration onward compared to the 12-h exposure without any depuration phase (12(+0) h). Further, the total body burden after 1 h of depuration was still significantly higher compared to 3, 24, 72, and 168 h of depuration ($p < 0.05$). The decrease was most distinct for 5- and 10-µm PS spheres (Figure 1B). After 7 d, microplastic numbers had decreased to 0.3% (0.5 p $individuals^{-1}$ [median]) of the original body burden after 12(+0) h (151.0 p $individuals^{-1}$). We did not detect 90-µm spheres in *D. polymorpha* tissues in experiment 1 or in any of the following experiments.

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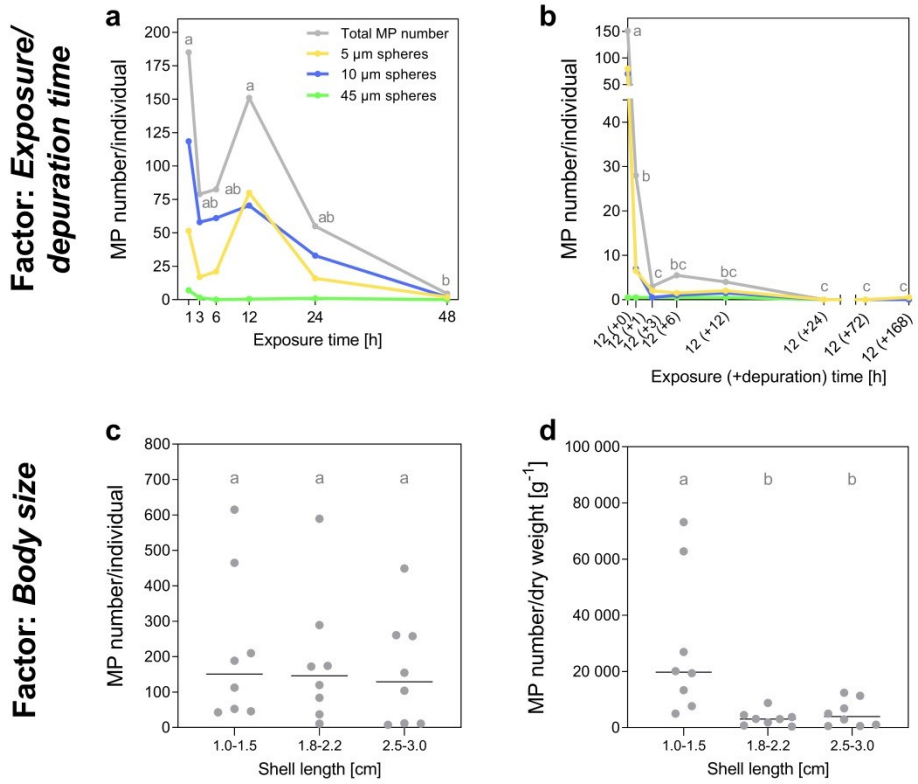

FIGURE 1: Impact of exposure and depuration time (**A**, **B**) as well as body size (**C**, **D**) on microplastic (MP) ingestion by *Dreissena polymorpha*. Mussels were (**A**) exposed to microplastic (5-, 10-, and 45-µm polystyrene [PS] spheres, 3 p mL^{-1} each; 90-µm PS spheres, 0.1 p mL^{-1}) for 1 to 48 h or (**B**) exposed for 12 h (12 (+0)) and then transferred to microplastic-free medium for up to 168 h. n = 4 for each time point. Data points indicate median values. (**C**, **D**) Mussels from 3 size classes were exposed to microplastic for 12 h, and the (**C**) absolute total body burden (sum of 5-, 10-, and 45-µm spheres) as well as the (**D**) relative total body burden (per dry wt) were determined (n = 8). Lines indicate the median. Statistics: one-way analysis of variance with Tukey's posttest; different letters indicate significant differences between the treatments. No 90-µm spheres were detected in *D. polymorpha* in any of the exposure experiments.

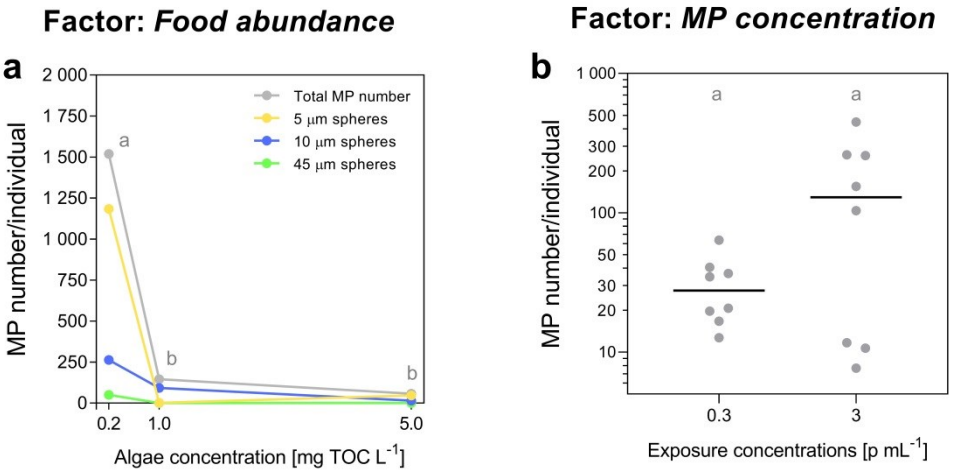

FIGURE 2: Impact of food abundance (**A**) and microplastic (MP) concentration (**B**) on microplastic ingestion by *Dreissena polymorpha*. (**A**) Mussels were exposed to microplastic (5-, 10-, and 45-µm polystyrene [PS] spheres, 3 p mL^{-1} each; 90-µm PS spheres, 0.1 p mL^{-1}) for 12 h in the presence of 3 algae concentrations (n = 8, data points indicate median). (**B**) Mussels were exposed to microplastic (5-, 10-, 45-, and 90-µm PS spheres) for 12 h at concentrations of 0.3 and 3 p mL^{-1} each (90-µm spheres, 0.01 and 0.1 p mL^{-1}) for 12 h. Data points represent the total body burden (sum of 5-, 10-, and 45-µm PS spheres) per individual. The line indicates the median. Statistics: one-way analysis of variance with Tukey's posttest; different letters indicate significant differences between the treatments. No 90-µm spheres were detected in *D. polymorpha* in any of the exposure experiments. TOC = total organic carbon.

Body size (experiment 2). Ingestion of microplastics by *D. polymorpha* varied considerably within the 3 tested size classes. The absolute microplastic body burden in individuals of the 3 size classes did not differ significantly (Figure 1C; $p > 0.05$). When considering the relative body burden (microplastic number per dry wt), the smallest individuals had significantly higher microplastic numbers compared to larger individuals (Figure 1D; $p < 0.01$).

Food abundance (experiment 3). *Dreissena polymorpha* ingested a lower amount of microplastic when more food was available. The decrease of the total body burden was, however, not linear but most pronounced between 0.2 and 1 mg TOC L^{-1} algae (equivalent to 5.08×10^4 and 2.53×10^5 algae cells L^{-1}; Figure 2A). The mussels did not take up 45-µm spheres when fed 5 mg TOC L^{-1} algae.

Microplastic concentration (experiment 4). In *D. polymorpha*, the total body burden increased when exposing the mussels to a 10-fold higher microplastic concentration, the increase was, however, not significant ($p > 0.05$; Figure 2B). Further, the increase was not proportional: a 10-fold increase in microplastic concentrations resulted only in a 5.1-fold (30.7 vs 156.9 microplastic individual^{-1}) higher microplastic burden.

Comparison of microplastic ingestion between freshwater mussel species

We reperformed experiment 1 (factors exposure and depuration time) with *S. woodiana* as a second species and compared these data with the results for *D. polymorpha* to determine species-specific differences (Figure 3A and B). Considering the microplastic ingestion after various exposure periods, total body burden (sum of 5-, 10-, 45-µm PS spheres) differed significantly between *S. woodiana* and *D. polymorpha* ($p < 0.001$), with *S. woodiana* ingesting much more microplastic than *D. polymorpha* (Figure 3A). In *S. woodiana*, the body burden peaked after 3 and 6 h, whereas in *D. polymorpha* microplastic levels peaked after 1 and 12 h. Consequently, no significant effect of exposure time was observed ($p > 0.05$). The same applies to the interaction of both factors ($p > 0.05$).

In the depuration experiments, both the variables species ($p < 0.001$) and depuration time ($p < 0.001$) as well as their interaction ($p < 0.01$) significantly affected microplastic body burden in the mussels. In both species, microplastic numbers reduced with increasing depuration time (Figure 3B). However, microplastic depuration was faster in *D. polymorpha* with almost complete microplastic clearance within 12 h, whereas the lowest body burden in *S. woodiana* was reached after 72 h. After 7 d of depuration, microplastic clearance in both species was similar (*D. polymorpha*, 99.7%; *S. woodiana*, 95.8%). However, whereas in *D. polymorpha* only few microplastic particles remained in the mussel tissues, we detected > 100 microplastics in *S. woodiana*.

When comparing absolute body burdens in *D. polymorpha*, *A. anatina*, and *S. woodiana* of various sizes (experiment 2; Figure 3C), neither body size nor species nor their interaction had a significant effect. The Tukey's posttest, however, showed that *D. polymorpha* as well as the 3 smaller size classes (1.0–1.5, 1.8–2.2, and 2.5–3.0 cm) form homogenous subgroups which differ from the remaining subgroups consisting of *A. anatina* and *S. woodiana* as well as the 2 larger size classes (6.0–8.0 and 9.5–12.0 cm). Absolute microplastic ingestion by *D. polymorpha* was, therefore, lower compared to the 2 larger freshwater species.

With regard to relative body burden in the freshwater mussels (Figure 3D), the variables individual size ($p < 0.001$) and species ($p < 0.05$) had a significant effect, although no interaction was observed ($p > 0.05$). For the variable species, all 3 species formed separate subgroups (Tukey's posttest), with *D. polymorpha* having the highest and *A. anatina* the lowest microplastic number in its tissues. With regard to individual size, 3 subgroups were identified (1.0–1.5 cm; 1.8–2.2, 2.5–3.0, and 6.0–8.0 cm; and 6.0–8.0 and 9.5–12.0 cm), which indicates that the relative body burden increases with decreasing individual size.

All 3 species ingested high proportions of 10-µm spheres (Figure 3E). Also, *D. polymorpha* (especially 1.0–1.5 cm) ingested large quantities of 5-µm spheres, whereas in the larger species, *S. woodiana* and *A. anatina*, 45-µm spheres were more abundant. Thus, smaller mussel species seem to ingest smaller microplastics. The same trend applied to differently sized individuals within the 3 species (except for *D. polymorpha*, 1.8–2.2 cm). The burden of smaller microplastics (5 and 10 µm) increased with smaller body size. In contrast to *D. polymorpha*, the 2 larger species also ingested 90-µm PS spheres. After a 12-h exposure, we detected up to 37 (6.0–8.0 cm) and 49 (9.5–12.0 cm) 90-µm PS spheres in *A. anatina* as well as up to 47 (6.0–8.0 cm) and 56 (9.5–12.0 cm) 90-µm spheres in *S. woodiana*.

Microplastic toxicity in D. polymorpha

No mortality occurred in any treatment during the acute exposure (1, 3, and 7 d) of *D. polymorpha* to PS fragments or diatomite. In the chronic exposure (42 d), mortality remained low, with 0% in the 100 000 p mL^{-1} microplastic treatment, with 10% in the control, 160 and 4000 pmL^{-1} microplastic treatment, as well as with 12.5% in the 6.4 pmL^{-1} microplastic and 100 000 p mL^{-1} diatomite treatment.

The exposure of *D. polymorpha* to microplastics (6.4–100 000 p mL^{-1}) for 1, 3, 7, and 42 d did not induce significant effects ($p > 0.05$) on the energy reserves (proteins, glycogen, lipids) or oxidative stress levels (MDA content, remaining antioxidant capacity) in the midgut gland of the analyzed individuals (Figure 4 and Table 1). However, we observed significant microplastic effects on the clearance rate of *D. polymorpha* ($p < 0.05$; Table 1), with a pronounced increase in the 100 000 p mL^{-1} treatment after 7 and 42 d of microplastic exposure (Figure 4F). In contrast, the exposure time significantly affected the energy reserves and oxidative stress level in D. polymorpha ($p < 0.01$; Table 1). However, there was no clear linear trend between the exposure time and the endpoints except for the remaining antioxidative capacity, which decreased with increasing exposure time (Figure 4E).

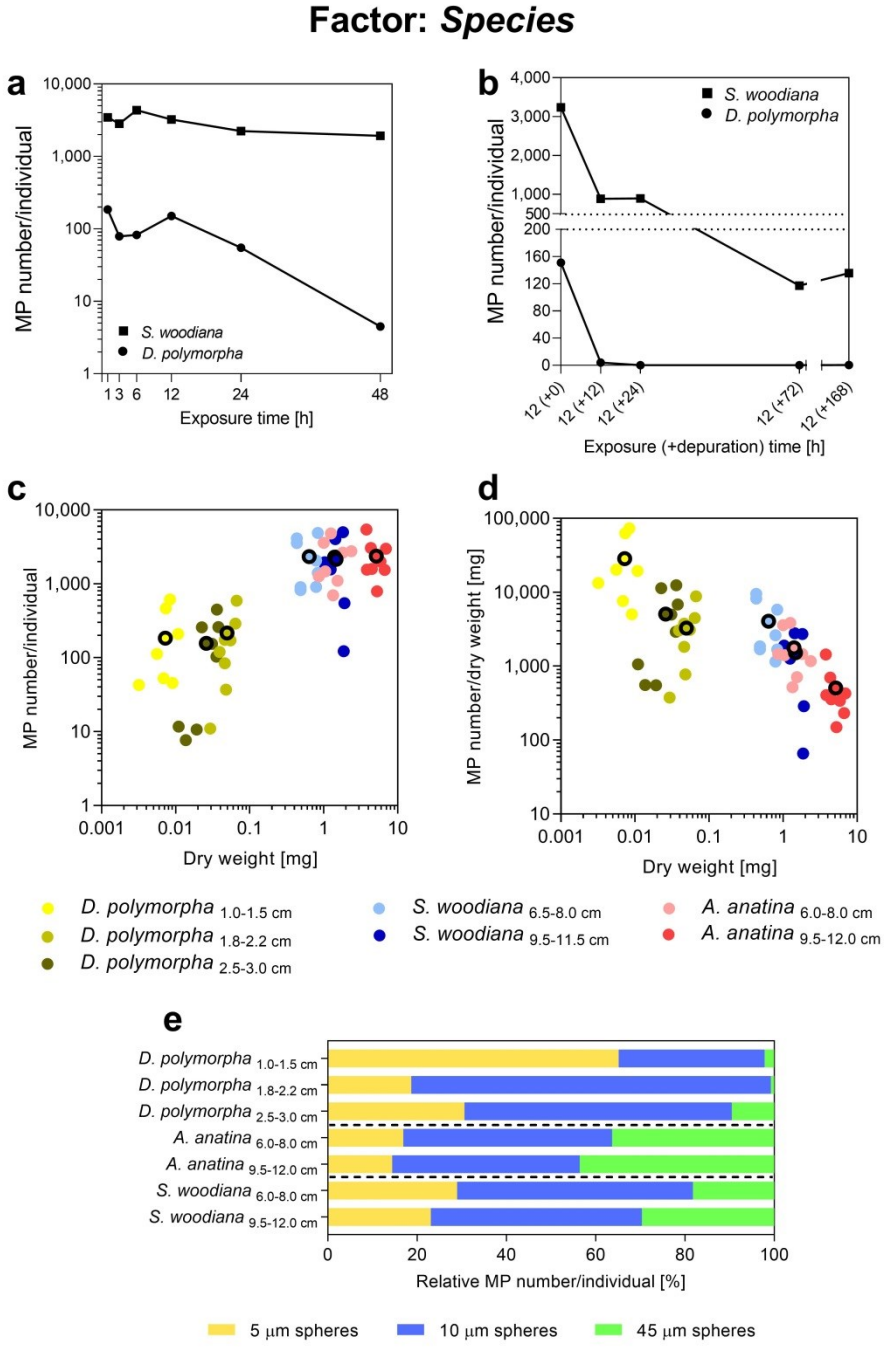

FIGURE 3: Comparison of the total body burden of microplastics (MP) in *Sinanodonta woodiana* and *Dreissena polymorpha* after different exposure (**A**) or depuration (**B**) time periods as well as comparison of the (**C**) absolute (per individual) and (**D**) relative body burden (total microplastic number per dry wt) in *D. polymorpha*, *S. woodiana*, and *Anodonta anatina* of various body sizes (12-h exposure). (**E**) Ratios of the microplastic types in mussels of different size classes (12-h exposure). (**A**, **B**) *Sinanodonta woodiana* and *D. polymorpha* were exposed as described in Figure 1A and B (n = 4, data points indicate median). (**C**–**E**) *Sinanodonta woodiana*, *A. anatina*, and *D. polymorpha* were exposed as described in Figure 1C and D (n = 8, black circles indicate geometric mean).

In addition, we statistically compared the 100 000 p mL^{-1} microplastic and diatomite exposures for each endpoint considering the variables particle type and exposure time as well as their interaction. Significant differences were only observed for the exposure time ($p < 0.05$; Supplemental Data, Table S4) regarding the protein content, MDA (TBARS), and the antioxidative capacity (ORAC). The particle type did not cause significant effects ($p > 0.05$). Particle effects due to an microplastic and diatomite exposure did not, therefore, differ in *D. polymorpha*.

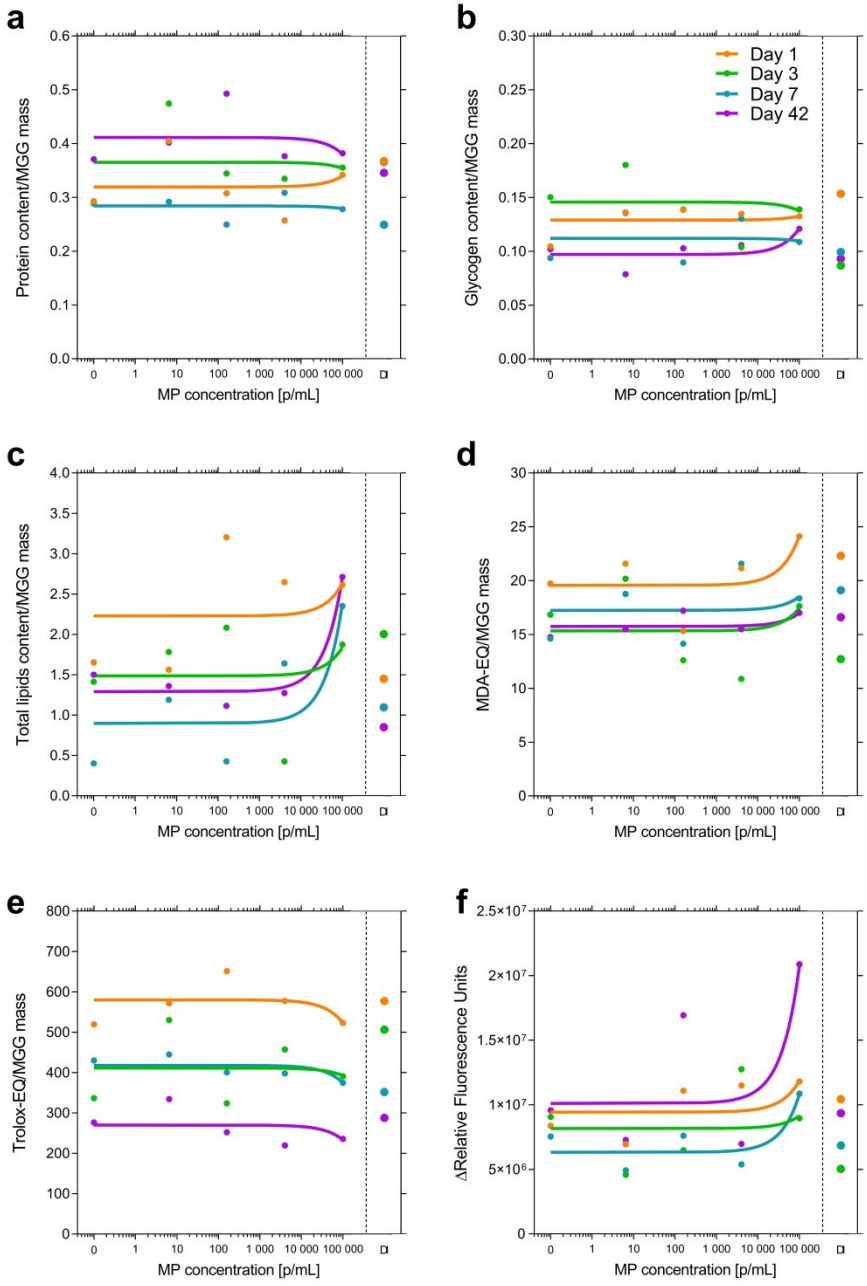

FIGURE 4: Chronic toxicity of polystyrene microplastic fragments (≤63 µm, 6.4–100 000 p mL^{-1}) and diatomite (≤63 µm, 100 000 p mL^{-1}) in *Dreissena polymorpha* exposed for 1, 3, 7, and 42 d. Endpoints were (**A**) protein, (**B**) glycogen, (**C**) total lipids, (**D**) malondialdehyde content, and (**E**) the remaining antioxidant capacity (Trolox equivalents) in the midgut gland, as well as (**F**) clearance rate. Dots indicate mean of each treatment; lines indicate linear regressions (separate regression for each exposure duration). (**A-E**) n = 7–10, (**F**) n = 10. MGG= midgut gland. MDA = malondialdehyde; EQ = equivalent.

DISCUSSION

Factors affecting microplastic ingestion by D. polymorpha

Bivalves selectively filter-feed on particles from the water column. The mussels' ciliated gills as well as ciliated labial palps separate digestible food particles from the remaining particle load by transporting the former to the digestive system and rejecting the latter as pseudofeces (Ward and Shumway 2004; Vaughn et al. 2008; Tuttle-Raycraft and Ackerman 2019). This selection mechanism is, however, inefficient for microplastics because we detected plastic particles in almost every individual after a respective exposure. Accordingly, more knowledge on the factors affecting the ingestion is required to better understand the mussels' exposure to microplastics.

Exposure and depuration time. *Dreissena polymorpha* rapidly ingested microplastic, with a maximum body burden reached after 1 and 12 h (experiment 1). The excretion of microplastic was similarly fast in *D. polymorpha*, with a significant

TABLE 1: Two-way analysis of variance results for the effects of the variables "microplastic concentration" and "exposure time" and their interaction on the clearance rate, energy reserves (proteins, glycogen, total lipids), and oxidative stress (TBARS, ORAC) of *Dreissena polymorpha*

Variable		Clearance rate	Proteins	Glycogen	Total lipids	TBARS	ORAC
Microplastic concentration	*df*	4	4	4	4	4	4
	F	3.205	2.099	0.483	0.918	2.132	1.545
	p	**0.014**	0.083	0.748	0.455	0.079	0.192
Exposure time	*df*	3	3	3	3	3	3
	F	0.861	7.671	5.320	6.348	4.670	11.254
	p	0.463	**<0.001**	**0.002**	**<0.001**	**0.004**	**<0.001**
Microplastic concentration × exposure time	*df*	12	12	12	12	12	12
	F	0.990	1.117	1.680	1.356	1.133	0.910
	p	0.460	0.350	0.075	0.192	0.337	0.538

Bold indicates significance.
TBARS = thiobarbituric acid reactive substances; ORAC = oxygen radical absorbance capacity; MP = microplastic; DI = diatomite.

reduction of microplastic body burden after 1 h of depuration and a reduction to fewer than 10 particles from 3 h onward. These results agree well with data on marine bivalves; for example, *Mytilus edulis* ingests polyethylene terephthalate fibers with a maximum body burden after 6 h (Woods et al. 2018), and *Geukensia demissa* excretes ingested polyethylene spheres within 12 h (Khan and Prezant 2018). This confirms that maximal microplastic burden in mussels is reached already after a few hours of exposure. Furthermore, decreasing microplastic burdens following peak levels highlight the fast egestion of microplastic particles by mussels but possibly also indicate decreasing exposure concentrations throughout prolonged exposure periods.

Despite fast excretion, few microplastics remained in *D. polymorpha* after 7 d of depuration. Therefore, mussel clearance mechanisms do not eliminate all internalized microplastics within 1 wk after exposure. The residual particles may have been either retained in the digestive tract or translocated into tissues or the circulatory system. In previous research, microplastics (≤25 µm) were detected not only in the stomach and intestine but also in the associated midgut gland ducts and diverticula (blind-ending tubules [Owen 1974]) and the circulatory system of bivalves (Von Moos et al. 2012; Guilhermino et al. 2018; Magni et al. 2018; Pittura et al. 2018; Gonçalves et al. 2019). These translocation processes may prevent the removal of the microplastic particles from the body and, thus, cause prolonged retention in mussels such as *D. polymorpha*.

When considering microplastic ingestion after extended time periods (>12 h), we observed a constant decrease of the microplastic body burden without reaching a steady state. This indicates that the microplastic exposure or bioavailability decreased during the experiment, possibly because the microplastic sedimented or the mussels had taken up the available microplastic and excreted it as (pseudo)feces. We did not examine feces production and deposition in our study and, therefore, cannot quantify its contribution to bioavailability reduction. Nonetheless, we investigated the settlement of particles in an additional experiment (Supplemental Data, S2.2) and observed that from 6 h onward, only 5- and 10-µm spheres remained in the water phase and that after 12 h only approximately half the 5-µm spheres and one-third of the 10-µm spheres remained in the water phase. This indicates that from 12 h onward, microplastic uptake by *D. polymorpha* was possible lower over time, whereas a constant depuration resulted in an overall decreasing microplastic body burden. From this, we conclude that stable microplastic burden in mussels from wild populations are only possible in case of continuous microplastic bioavailability and ingestion by the mussels.

Body size. The absolute microplastic ingestion (per individual) varied distinctively within each size class of *D. polymorpha*. Accordingly, no significant differences between the 3 size classes were observed. This indicates that the interindividual differences in absolute microplastic burden in mussels may be unrelated to body size. Considering the relative body burden (microplastic per dry wt), instead, microplastic ingestion was significantly higher in smaller individuals. Hence, smaller mussels (e.g., juveniles) in the environment may be exposed to relatively higher microplastic levels. This is possibly related to higher relative feeding activity. In *Mytilus* spp., the weight-specific pumping rate increases exponentially with decreasing body size. The same is true for the weight-specific gill area (Jones et al. 1992; Duinker et al. 2007). Higher relative pumping rates and larger relative gill areas allow smaller mussels to take up more microplastics per body mass.

Food abundance. Providing algae as food caused a significant, dose-dependent decrease in microplastic ingestion in *D. polymorpha* (experiment 3). Rist et al. (2019a) observed the same trend for *M. edulis* larvae, which were exposed to 2-µm PS spheres in the absence and presence of algae. In adult *M. edulis* instead, higher algae concentrations increased the ingestion of 30-nm PS spheres (Wegner et al. 2012). Wegner et al. (2012) discuss that nanospheres may adsorb on the algae as a potential reason for their findings. Because we used microplastics with a similar or larger size than *D. subspicatus* cells (8 × 5 µm; Hessen and Van Donk 1993), indirect microplastic ingestion through adsorption on algae was probably not a relevant pathway. Considering environmental conditions, these results indicate that high food abundance may reduce overall microplastic ingestion by bivalves. Especially in freshwater systems with high primary production or in seasons with elevated food availability (e.g., algae bloom in spring),

mussels may take up fewer microplastics than in oligotrophic systems.

Microplastic concentration. *Dreissena polymorpha* ingested microplastics in a concentration-dependent manner (experiment 4). The increase in microplastic uptake was, however, not linear such that animals exposed to a 10-fold higher concentration had 10-fold higher body burdens. Therefore, microplastic body burdens in mussels may behave rather logarithmically, suggesting the existence of a maximal level. A logarithmic relation between microplastic water concentrations and body burdens has previously been reported for *M. galloprovincialis* larvae (Capolupo et al. 2018) and *M. edulis* (Woods et al. 2018). Limited microplastic uptake at very high concentrations in the water phase is mostly related to pseudofeces production. When the gill cilia which sort particles prior to ingestion (see section Ingestion of microplastics in 3 mussel species) are overloaded, excess particles are gathered, embedded into pseudofeces, and rejected (Ward et al. 1993). However, overloading of the bivalves' sorting mechanism with microplastics is possibly not very relevant in nature because the highest reported environmental concentrations of 0.2 to 0.5 p mL^{-1} (Leslie et al. 2017; Lahens et al. 2018) are much lower than the concentrations used in the laboratory studies mentioned above (Capolupo et al. 2018 [50–10 000 p mL^{-1}]; Woods et al. 2018 [3–30 p mL^{-1}]).

Ingestion of microplastics in 3 mussel species

The ingestion experiments with *D. polymorpha* pointed out that numerous factors affect microplastic ingestion in this species, including exposure and depuration time, body size, food abundance, and microplastic concentration. Species-specific differences (e.g., size, morphology) may, however, be another relevant factor influencing microplastic ingestion by freshwater mussels. In light of a broader assessment of microplastic ingestion in freshwater species, we repeated the experiments for the factors exposure and depuration time (experiment 1) as well as body size (experiment 2) with the 2 larger freshwater mussel species, *A. anatina* and *S. woodiana*.

Distinct differences between the 3 freshwater species were seen with regard to the absolute microplastic ingestion. The smaller *D. polymorpha* ingested fewer microplastics than the 2 larger species (experiment 1) when considering the absolute body burden. Between the 2 larger species instead, absolute microplastic ingestion did not differ distinctively. Higher microplastic ingestion in larger species may be caused by either higher absolute filtration rates of larger mussels as reported by Kryger and Riisgård (1988) or a higher bioavailability of microplastics to the larger, sand-dwelling unionids. The latter would be true if the sand-dwelling species are able to take up additional microplastics from the sediment. Based on an additional experiment (see Supplemental Data, S6), we did, however, show that microplastic uptake by unionids from the sediment phase is rather limited and that the filtration rate is, thus, the determining factor for absolute microplastic ingestion. Accordingly, body size seems to be a relevant indicator for microplastic body burden, with larger species ingesting higher microplastic quantities. Differences between species of similar size, instead, seem to be of limited relevance for absolute microplastic ingestion.

In contrast, the relative microplastic body burden (per dry wt) was higher in smaller specimens, with both species and individual size having a significant effect. Variations in relative microplastic ingestion are possibly caused by differences in relative filtration rate between mussels of different species and size. Kryger and Riisgård (1988) observed that smaller bivalves (e.g., *D. polymorpha*) often have a higher relative filtration rate (per mussel dry wt) compared to larger species (e.g., Unionidae) but that filtration rates may also vary between species of similar size (e.g., *Sphaerum corneum* vs *D. polymorpha*). This suggests that smaller species may be more susceptible to microplastic ingestion than larger ones but that species-specific variations may also affect the overall microplastic exposure in mussel individuals.

Further, we compared the ingestion and depuration behavior of *D. polymorpha* and *S. woodiana* over various exposure and depuration time periods. Ingestion behavior over a period of up to 12 h was rather similar because both species reached peak levels within 12 h. Depuration, instead, differed in both species, with *D. polymorpha* excreting microplastics faster than *S. woodiana*. *Dreissena polymorpha* removed ingested PS spheres almost completely within 12 h, whereas microplastic levels in *S. woodiana* decreased further until 72 h of depuration. Because we compared 2 species with different size, it remains unclear whether the difference is species- or rather size-specific. The latter has already been shown for *Mytilus chilensis*, in which smaller individuals had a higher weight-specific excretion rate compared to larger ones (Navarro and Winter 1982). Hence, smaller mussels have a higher relative microplastic body burden but also excrete microplastics faster than larger mussels. In smaller mussels, higher relative uptake may, therefore, be compensated for by a higher microplastic depuration.

Interestingly, the 3 freshwater species ingested microplastic size-dependently. Although 10-µm PS spheres were most abundant in all species, *D. polymorpha* also ingested large quantities of 5-µm spheres, whereas the larger species tended to ingest larger particles (45- and 90-µm spheres). Variations in feeding size selectivity are possibly due to differences in the particle selection mechanism and, more specifically, to morphological variations of the lamellated gills (Ward and Shumway 2004; Rosa et al. 2018), as well as the selection mechanism in the stomach. Lamellated gills are composed of numerous filaments with associated laterofrontal cirri (bundle of cilia) with which inhaled particles are selected from the inhaled water current (Ward and Shumway 2004; Silverman et al. 1999). Jørgensen et al. (1984) have shown that the retention potential of the laterofrontal cirri is species-dependent. Although *D. polymorpha* efficiently retains particles as small as 1.5 µm with the laterofrontal cirri, in *Anodonta cygnea* 4-µm particles pass through the cirri. The difference in laterofrontal cirri morphology may, thus, be a reason for higher ingestion of 5-µm spheres in *D. polymorpha*. Following the lamellated gills, particle selection involves further sorting steps on the labial palps as well as within the

digestive system. As a final step, particles are separated in specialized grooves in the stomach and either redirected into the digestive tubules of the midgut gland or into the intestine for direct excretion (Ward and Shumway 2004). Ten Winkel and Davids (1982) reported that the stomach of *D. polymorpha* preferentially selects for algae with a size between 10 and 50 µm, with a maximal preference for 20-µm algae cells. This size preference was also seen in our ingestion experiments because all tested freshwater species mostly ingested 10-µm spheres. Still, a high burden of 5-µm PS spheres, especially in *D. polymorpha*, indicates that stomach sorting is not exclusively limited to 10- to 50-µm particles.

Besides interspecies differences, we also observed that the size selectivity varied in each species. For all 3 tested species, smaller individuals ingested larger quantities of 5- and 10-µm spheres compared to larger individuals (experiment 2). Again, this is probably related to morphological changes once a bivalve grows: for *M. edulis*, the gill and labial palp area as well as the distance between gill filaments enlarge with increasing body length (Kiørboe and Møhlenberg 1981; Jones et al. 1992), and larger interfilamentary spaces will reduce the retention of small particles. This points toward a higher relevance of an ingestion of small microplastics for smaller individuals (e.g., juveniles).

Microplastic toxicity in D. polymorpha

The exposure of *D. polymorpha* to PS fragments (≤63 µm) for 1 to 42 d at concentrations up to 100 000 p mL^{-1} caused a significant increase in clearance rate, whereas mortality, metabolic endpoints (energy reserves), and oxidative stress were not altered. Hence, the increase in clearance rate, especially in the 100 000 p mL^{-1} microplastic treatment, may be an adaptation mechanism to compensate for potential microplastic-related effects (e.g., reduced food uptake). Considering the exposure time, we observed a significant general change in energy allocation as well as basic stress levels in *D. polymorpha* throughout the 42-d exposure. Because *D. polymorpha* individuals were accustomed to the medium as well as the food source as early as 4 wk prior to the toxicity study, we assume that changes in endpoints over the exposure time were not caused by the exposure design itself but may rather be related to stress reactions caused by the water changes and the transfer of the mussels into new exposure tanks. Such a stress reaction could have, thus, also caused the observed decrease of the antioxidative capacity with increasing exposure time. The definite reason, however, remains unclear.

Interestingly, we also did not observe significant differences between the effects of the microplastic and diatomite treatments. This suggests that *D. polymorpha* is rather insensitive to an exposure to high concentrations of microplastic and natural particles alike. These results contrast with those of earlier studies in which mussels reduced their clearance rate with increasing turbidity, resulting in a lower food uptake (Aldridge et al. 1987; Tuttle-Raycraft and Ackerman 2019). Mussels are able to compensate for such reduced feeding by increasing the particle selection efficiency via morphological adaptations (Payne et al. 1995; Tuttle-Raycraft and Ackerman 2019). Hence, compensation mechanisms to microplastic exposure (increase in clearance rate) in our study seem to differ from those for high turbidity. It will, however, require additional studies to elucidate whether these differences are stressor-dependent (microplastic vs natural particle turbidity) or a result of different adaptation periods (42-d microplastic exposure vs lifelong exposure in turbid environment). Notwithstanding, compensation mechanisms seemed to be efficient enough to protect *D. polymorpha* from microplastic effects even at concentrations far higher than currently reported for freshwater environments.

The absence of negative effects in our study only partially agrees with previous research. In a systematic literature search in May 2020 (PubMed, search "microplastics" OR "microplastic" AND "mussel" OR "bivalve," studies evaluating toxicity after depuration excluded), we identified 8 toxicity studies with freshwater mussels (*D. polymorpha* and *C. fluminea*; see Supplemental Data, Table S5). Two studies did not report any microplastic-induced effects, whereas one study observed effects on all and 5 studies on some of the analyzed endpoints. Although the majority of the endpoints remained unaffected (Supplemental Data, Table S5), these reports show that freshwater bivalves may be, at least to some extent, susceptible to microplastic exposure. However, applied microplastic concentrations in the toxicity studies with *D. polymorpha* and *C. fluminea* exceeded those currently reported for freshwater ecosystems (0.52 p mL^{-1} [Lahens et al. 2018]). It, thus, remains unknown whether the observed effects are relevant in the environmental context.

Interestingly, effects on feeding, histology, neurology, and oxidative stress metabolites varied intensively, with similar endpoints being affected in some but not in other studies. Effects may be species-specific; however, even when comparing the same species (*D. polymorpha*), marked differences were observed (e.g., effects on catalase and glutathione peroxidase in Magni et al. [2018, 2020]). We, therefore, believe that complex effect patterns are also a consequence of differences in exposure scenarios (especially exposure time and concentration), microplastic properties (e.g., polymer type, size, shape), and the sensitivity of endpoints. Accordingly, drawing general conclusions on microplastic risks for bivalves may be overly simplistic. An identification of the most sensitive species and their respective traits provides a way forward. Beyond that, a better understanding of the biological mechanisms protecting mussels from high loads of suspended particles as well as of the mechanism of microplastic toxicity in mussels is required. This knowledge is fundamental to determining whether microplastics can indeed bypass defense mechanisms in bivalves and, thus, represents a risk to wild mussel populations.

CONCLUSION

We investigated the kinetics of microplastic ingestion and egestion in *D. polymorpha*. Comparing multiple relevant factors, we demonstrate that exposure and depuration time, body size, food abundance, and microplastic concentration affect overall microplastic ingestion. *Dreissena polymorpha* rapidly ingested microplastics and excreted the majority of the particles within

12 h, with only a few particles being retained for more than 1 wk. Smaller individuals ingested more microplastics compared to larger individuals relative to their body size. An additional supply of food reduced the uptake of microplastics. Further, we compared microplastic ingestion in *D. polymorpha* with 2 larger unionid species (*A. anatina*, *S. woodiana*). Absolute microplastic ingestion was higher in the 2 unionid species but did not differ between the unionids. Relative microplastic ingestion, instead, differed significantly with regard to both the species and the body size of the individuals, with smaller mussels ingesting more microplastics. With regard to microplastic size, smaller species as well as smaller individuals within each species also ingested smaller microplastics. We also analyzed the toxicity of PS fragments (≤63 µm, 6.4–100 000 p mL^{-1}) in *D. polymorpha*. Exposure for up to 42 d caused a significant increase in the clearance rate but did not affect energy reserves or oxidative stress. Further, no significant difference between the effects of microplastics and natural particles (diatomite) was observed. Enhanced filtration rate may be a compensatory mechanism rendering *D. polymorpha* rather insensitive to microplastic exposure even at very high concentrations. Taking into account previous research on microplastic toxicity in bivalves, this does not imply that mussels in general are not susceptible to microplastic exposures. Divergent toxicity data probably originate from species-specific differences or variations in experimental design. A better understanding of the traits and mechanisms rendering some species and endpoints more sensitive than others is needed to prioritize species potentially at risk.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at https://doi.org/10.1002/etc.5076.

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Data Availability Statement— Data, associated metadata, and calculation tools are available from the corresponding author (martin.wagner@ntnu.no).

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Ingestion and toxicity of polystyrene microplastics in freshwater bivalves

Supplementary Information

S1 Particle concentrations in the MP stock suspensions for the ingestion experiments

Particle concentrations in the microplastic (MP) stock suspensions were determined using a Coulter Counter (Beckman Coulter, Multisizer 3, Krefeld, Germany). For the analysis of the 5, 10 and 45 µm polystyrene (PS) sphere stock suspensions, we mixed 5–10 µL of each stock suspension with 100–150 mL of electrolyte solution (0.98 % NaCl solution, < 0.2 µm sterile-filtered). The resulting suspension was constantly stirred and directly measured with a 100 µm capillary (Beckman Coulter, Krefeld, Germany, detection range: 2–60 µm, current: -1,600, gain: 2, analytical volume: 0.5–1 mL). Particle concentrations in the stock suspension of the 90 µm spheres were determined likewise but with a 400 µm capillary (Beckman Coulter, Krefeld, Germany, detection range: 8–240 µm, current: -1,600, gain: 2, average analytical volume: 7.7 mL). Additionally, control measurements with the pure electrolyte solution were performed to quantify background particle concentrations. The determined MP concentrations were corrected for the background particle concentrations accordingly. Average particle concentrations in the PS sphere stock suspensions were 2.1×10^{8} p mL^{-1} (5 µm), 4.6×10^{7} p mL^{-1} (10 µm), 585,000 p mL^{-1} (45 µm) and 37,500 p mL^{-1} (90 µm).

S2 Basic exposure scenario and particle fate in the exposure scenario

S2.1 Exposure scenario setup

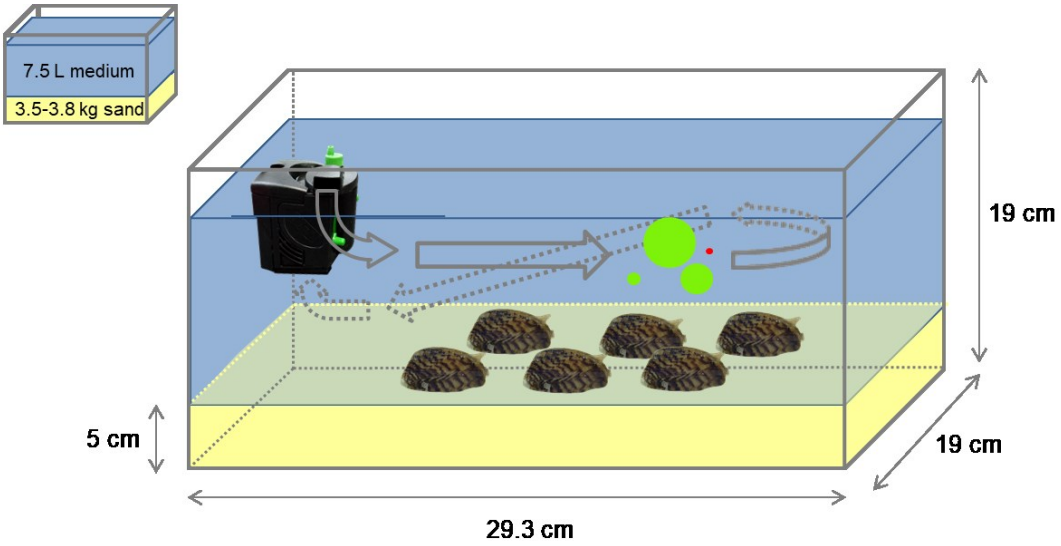

Fig. S1: Basic exposure scenario for the ingestion experiments.

S2.2 Particle fate in the exposure scenario

We determined particle fate in the exposure tanks in a pilot experiment over 1-48 h. One tank was set up as described in 2.3.1, except that no mussels were introduced and glass beads (MeinPool24.de, Bindlach, Germany; 0.4–0.8 µm) were used instead of sand to facilitate the MP sampling of the sediment surface. For sediment sampling, we buried seven small glass vials (ø: 2.2 cm, height: 4 cm) within the glass particles prior to the start of the experiment with the vials being in an upright position and the top edge of their lids being covered by a thin layer of glass bedding, respectively.

1 h prior to the experiment, 7.5 mg TOC of algae (1 mg L^{-1} TOC) were added into the tank. At the beginning of the experiment, 5, 10, 45 (3 p mL^{-1}) and 90 µm (0.1 p mL^{-1}) PS spheres were introduced simultaneously. We sampled both the water column and the sediment surface at 1, 3, 6, 12, 24 and 48 h after the experimental start. For sampling of the water phase, we removed 20 mL of medium directly from below the water surface as well as from the lower third of the water column and filtered it using glass fiber filters (class 696 filters, product no.: 516-0879, VWR, Darmstadt, Germany, pore size: 1.25 µm).

For sediment sampling, we used a plastic tube with a closed top end to prevent water intrusion while introducing the tube into the tank. The tube was placed vertically into the tank

and its lower end was pressed on top of the lid of one of the buried glass vials. As the plastic tube and the glass vials stuck together tightly (only possible due to the coarseness of the glass particles, but not possible with quartz sand), we could remove both the plastic tube and the glass vial from the tank without mixing of the surrounding (MP-containing) medium.

The sediment surface entrapped in the glass vial represented a surface area of 3.46 cm² (0.62 % of the total sediment surface in the tank). The sampled sediment was thoroughly rinsed and the rising water was filtered on glass fiber filters (see above).

Particle abundance was determined visually by examining the total filter surface with a fluorescence microscope (Olympus, BX50, Hamburg, Germany, NV (400–410 nm excitation, used for 10, 45 and 90 µm PS spheres) and NG (530–550 nm excitation, used for 5 µm PS spheres) filter cubes, 40× magnification). Particle quantities were extrapolated to the total water volume or the total sediment surface in the tank, respectively, and relative abundance was determined in comparison to total particle number introduced into the tank.

The particle fate experiment indicates that smaller spheres sedimented more slowly compared to larger particles and, thus, remained longer in the water phase (Tab. S1). From 6 h onwards, only 5 and 10 µm spheres remained in the water phase. The MP concentrations in the water phase decreased further with time and very few MP was detected after 48 h. Likewise, the MP number on the sediment surface increased over time with major portions of the MP having settled after 48 h. Interestingly, we did not recover all the MP from the water and sediment compared to the number of particles originally spiked to the tank. This indicates that some particles may have adsorbed to the tank walls and/or the pump.

Tab. S1: Particle fate in experimental setup over 1-48 h. The relative particle abundance is presented as ratio of MP (5, 10, 45, 90 µm PS spheres) detected compared to the total MP numbers introduced into the tank [%].

Size of	**Water phase**						**Sediment surface**					
PS spheres	**1 h**	**3 h**	**6 h**	**12 h**	**24 h**	**48 h**	**1 h**	**3 h**	**6 h**	**12 h**	**24 h**	**48 h**
5 µm	83.3	52.8	42.1	49.8	22.8	10.2	32.9	5.0	4.3	5.1	5.1	13.1
10 µm	67.5	50.2	41.9	22.7	6.7	1.7	0.7	4.3	6.5	5.8	15.2	14.5
45 µm	15.8	0.8	0.0	0.0	0.0	0.0	7.9	22.2	25.1	20.1	35.9	25.9
90 µm	0.0	0.0	0.0	0.0	0.0	0.0	21.4	42.9	64.6	43.2	43.3	43.5

S3 Analysis of MP in *D. polymorpha*, *A. anatina* and *S. woodiana*

S3.1 Particle loss due to the removal of the mantle, gills and foot

We determined the distribution of MP between the mantle, gills and foot compared to the other tissues of *Sinanodonta woodiana* and *Anodonta anatina* by exposing three *S. woodiana* individuals (9.4–9.9 cm) to 5, 10 and 45 µm PS spheres at 3 particles mL^{-1} (p mL^{-1}) as well as to 90 µm PS spheres at 0.1 p mL^{-1} and 1 mg L^{-1} TOC of algae for 12 h. Following the exposure, individuals were frozen at -80 °C and dissected after defrosting. The mantle, gills and foot (MGF) were separated from the remaining tissues and pooled separately for each individual. Both MGF and the remaining tissue were lysed and analyzed for PS particles as stated in chapter 2.3.6. We determined the fraction of particles in the MGF tissues compared to the total body burden separately for the different MP types.

During the experiment, individual 1 was inactive and, thus, ingested very little MP overall. In the other two mussels, ≤ 1.8 % of 5, 10 and 45 µm PS spheres were present in the MGF compared to the total body burden. The number of ingested 90 µm PS spheres was very low both in the MGF and the remaining body tissues (max. 1 sphere $individual^{-1}$). Therefore, despite the close interaction of the gills (as key organ for particle sorting, see 4.2) and mantle with ingested MP particles, particle adsorption on the MGF tissues seems to be limited and the removal of the MGF tissues following the ingestion experiments has, therefore, little effect on the overall results in case mussels ingest a high number of MP.

Tab. S2: Fraction of MP (5, 10, 45, 90 µm PS) in the mantle, gills and foot (MGF) compared to the total MP body burden in *S. woodiana* (MGF plus other tissues).

	5 µm PS spheres		**10 µm PS spheres**		**45 µm PS spheres**		**90 µm PS spheres**	
	Total counts	**MGF fraction**	**Total counts**	**MGF fraction**	**Total counts**	**MGF fraction**	**Total counts**	**MGF fraction**
Individual 1	25	72.0 %	21	95.2 %	7	85.7 %	1	100 %
Individual 2	3505	0.7 %	1243	0.6 %	72	1.4 %	0	-
Individual 3	2220	1.2 %	612	1.8 %	393	0.3 %	1	0 %

S3.2 Quality assurance

Tab. S3: Background contamination by particles resembling PS spheres in *D. polymorpha, A. anatina* and *S. woodiana.* We analyzed three individuals per species and size and determined the mean particle number individual^{-1}.

Species	**Size [cm]**	**Number of particles resembling:**			
		5 µm PS spheres	10 µm PS spheres	45 µm PS spheres	90 µm PS spheres
D. polymorpha	1.0–1.5	0	0.33	0	0
D. polymorpha	1.8–2.2	0	0	0	0
D. polymorpha	2.5–3.0	0.33	0	0	0
A. anatina	6.0–8.0	5.00	0.67	0	0
A. anatina	9.5–12.0	4.33	0.33	0	0
S. woodiana	6.0–8.0	3.33	1.66	0.33	0
S. woodiana	9.5–12.0	2.33	1	0	0

S4 Microplastics toxicity study

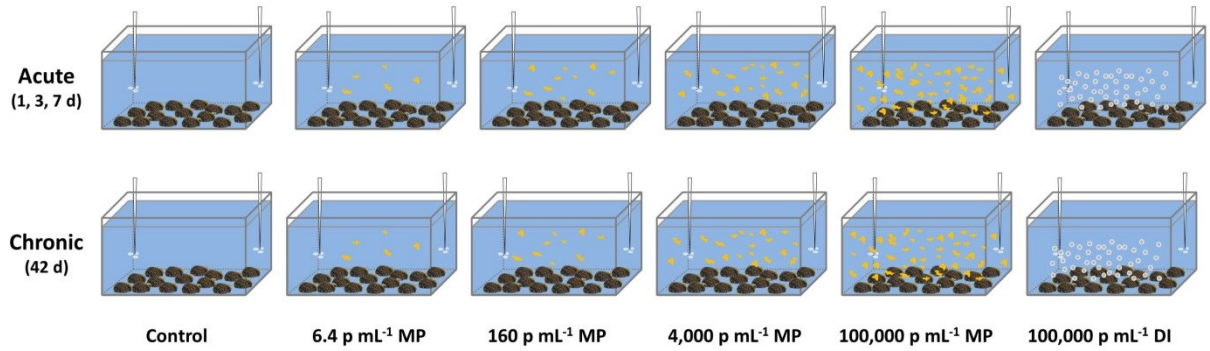

Fig. S2: Experimental setup for the microplastic (MP) toxicity study with *D. polymorpha*. DI = diatomite

S5 Statistical comparison of MP and DI toxicity

Tab. S4: Results of the general linear models (two-way ANOVA) analyzing the effects of particle type (microplastic vs. diatomite) and exposure time (1, 3, 7, 42 days) as well as their interaction on mussel clearance rate as well as energy reserves (total energy, proteins, glycogen, total lipids) and oxidative stress (malondialdehyde (TBARS), Trolox-equivalents (ORAC)) in the midgut gland of *D. polymorpha*.

Variable		**Clearance rate**	**Proteins**	**Glycogen**	**Total lipids**	**TBARS**	**ORAC**
Particle type	*df*	1	1	1	1	1	1
	F	2.693	0.260	1.716	1.410	0.980	0.087
	p	0.105	0.612	0.195	0.239	0.326	0.769
Exposure time	*df*	3	3	3	3	3	3
	F	1.320	3.033	2.698	0.838	3.235	5.117
	p	0.275	**0.035**	0.053	0.478	**0.028**	**0.003**
Particle type × Exposure time	*df*	3	3	3	3	3	3
	F	1.404	0.225	1.755	0.671	0.718	0.233
	p	0.249	0.879	0.165	0.573	0.545	0.873

S6 MP ingestion: Application pathway

We compared the impact of the particle application route on MP ingestion by exposing *A. anatina* (6.0–8.0 cm) to PS spheres (5, 10, 45 µm (3 p mL^{-1}, each), 90 µm (0.1 p mL^{-1})) for 12 h (experimental setup as described in 2.3.1). Particles were either added to the sediment or to the water phase. For the particle application via sediment, we filled the tanks with sediment and 1 L of OECD medium first, added the particle suspensions and stirred the sediment for homogenous particle distribution. Then, we carefully added the remaining 6.5 L OECD medium and allowed the particles to settle for 24 h (pump turned off). At the beginning of the experiment, the mussels were introduced into the tanks and the pump was turned on. For the experiment with the waterborne particle application, mussels and MP were introduced to the tanks as described in 2.3.1. Both application routes were tested in two tanks with six mussels each. Eight active individuals per treatment were analyzed.

Experiment results show that MP body burden was significantly higher in mussels exposed via the water phase compared to a sediment-bound exposure (Fig. S3). Consequently, the water phase seems to be the more relevant source for MP exposure compared to the sediments. Considering that MP levels in the environment are higher in sediments, probably only a small fraction of sediment-borne MP may be bioavailable to mussel populations.

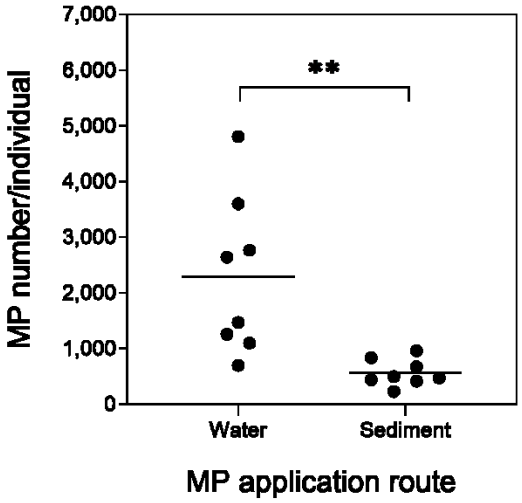

Fig. S3: Total MP body burden (comprised of 5, 10 and 45 µm PS spheres) in *A. anatina* exposed via the water phase or the sediment. Statistics: Unpaired t-test, ★★ $p < 0.01$. The line indicates the mean.

S7 Summary of literature data on the MP toxicity in freshwater bivalves

Tab. S5: Summary on toxicity studies with microplastics and freshwater bivalves. The summary includes details on species, particle characteristics, exposure concentration and time as well as endpoints. red = MP-induced toxicity, green = no MP-induced toxicity, ↑ = increase, ↓ = decrease, ↑↓ = change. n.s. = not stated, MGG = midgut gland, PE = polyethylene, PP = polypropylene, PS = polystyrene, PET = polyethylene terephthalate, PVC = polyvinyl chloride. * = Equivalent particle concentrations were calculated based on the stated polymer type and mean particle size assuming spherical particle shape. Full literature references are included in the reference section of the main manuscript.

	Authors	Species	Polymer	MP size	MP shape	MP concentration	Exposure time	MP effect
1	Baudrimont et al. 2020	*Corbicula fluminea*	PE	350 µm	Spheres	1, 10, 100, 1000, 10,000 µg/L (equivalent to: 0.046-460 p/L) *	48 h	**No effects:** Feces and pseudofeces production Filtration activity
2	Binelli et al. 2020	*Dreissena polymorpha*	Environmental MP (mostly PE and PP)	0.063–5 mm	Mostly fibers and fragments	n.s.	7 days	**Effects:** Glutathione peroxidase ↓ Glutathione S-transferase ↓↑ Apoptosis ↑ Protein carbonylation content ↑ Cell viability ↓ **No effects:** Superoxide dismutase Catalase Reactive oxygen species release Necrosis Micronuclei
3	Guilhermino et al. 2018	*Corbicula fluminea*	n.s.	1–5 µm	Spheres	200, 700 µg/L	4 days	**Effects:** Cholinesterase activity (adductor muscle) ↓ **No effects:** Histopathological alterations Feeding activity Lipid peroxidation (adductor muscle, foot, MGG) Isocitrate dehydrogenase (foot) Octopine dehydrogenase (foot) Glutathione S-transferase (gills, MGG) Glutathione reductase (gills, MGG) Glutathione peroxidase (gills) Catalase (gills, MGG)
4	Magni et al. 2018	*Dreissena polymorpha*	PS	1, 10 µm	Spheres	500,000; 2,000,000 p/L (for each particle type)	6 days	**Effects:** Catalase (whole body tissue) ↑ Glutathione peroxidase (whole body tissue) ↓ Dopamine ↑ **No effects:** Hemocyte viability

Tab. S5 (continued)

	Authors	Species	Polymer	MP size	MP shape	MP concen-tration	Exposure time	MP effect
4 (con.)								Glutathione S-transferase Superoxide dismutase Lipid peroxidation Protein carbonyl content Serotonin Glutamate Acetylcholinesterase Monoamine oxidase Micronuclei frequency
5	Magni et al. 2019	*Dreissena polymorpha*	PS	1, 10 µm	Spheres	500,000; 2,000,000 p/L (for each particle type)	6 days	**Effects:** Proteomics ↓↑
6	Magni et al. 2020	*Dreissena polymorpha*	PVC (1.38 g/cm³), Mater-Bi® (1.28 g/cm³)	56 ± 35 µm (PVC) 41 ± 36 µm (Mater-Bi®)	Fragments	1 mg/L (equivalent to: PVC: 7,880 p/L, Mater-Bi®: 21,649 p/L) *	6, 14 days	**Effects:** Glutathione S-transferase **No effects:** Superoxide dismutase Catalase Glutathione peroxidase Reactive oxygen species production Propionyl-CoA-Carboxylase Lipid peroxidation Apoptosis (hemocytes) Necrosis (hemocytes) Micronuclei (hemocytes)
7	Oliveira et al. 2018	*Corbicula fluminea*	n.s.	1–5 µm	Spheres	130 µg/L	8 days	**Effects:** Filtration rate ↓ Lipid peroxidation (gills) ↑ Cholinesterase (adductor muscle) ↓ **No effects:** Mortality Isocitrate dehydrogenase (foot) Octopine dehydrogenase (foot) Catalase (gills) Glutathione reductase (gills) Glutathione peroxidase (gills) Glutathione S-transferase (gills)
8	Rochman et al. 2017	*Corbicula fluminea*	PET, PE, PVC, PS	PET: 12-704 µm (mean: 98 µm), PE: 14-704 µm (mean: 209 µm), PVC: 80-704 µm (mean: 169 µm), PS: 68- 704 µm (mean: 179 µm)	Fragments	4.1 mg/L (PET), 2.8 mg/L (PE), 4.2 mg/L (PVC), 3.2 mg/L (PS) (equivalent to: 731 p/L (PET), 604 p/L (PE), 1,204 p/L (PVC), 1,015 p/L (PS)) *	28 days	**No effects:** CYP450 expression Vitellogenin expression Clearance rate

A3. PET microplastics do not negatively affect the survival, development, metabolism and feeding activity of the freshwater invertebrate *Gammarus pulex*

Studie 3

Publikation im peer-reviewed Journal *Environmental Pollution*:

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Erklärung zu den Autorenanteilen an der Publikation A3:*:

Entwicklung und Planung					
Authors	**AW**	CS	NB	GR	MW
%	**60**	25	-	-	15
Durchführung der einzelnen Untersuchungen und Experimente					
Authors	**AW**	CS	NB	GR	MW
%	**100**	-	-	-	-
Erstellung der Datensammlungen und Abbildungen					
Authors	**AW**	CS	NB	GR	MW
%	**90**	-	-	-	10
Analyse und Interpretation der Daten					
Authors	**AW**	CS	NB	GR	MW
%	**70**	15	-	-	15
Verfassung des Manuskripts					
Authors	**AW**	CS	NB	GR	MW
%	**70**	5	0.5	0.5	24

***Erklärung zum Beitrag der Studie 3 zu dieser Dissertation**:

Die in Studie 3 vorgestellten Experimente zur Mikroplastikaufnahme und –toxizität im Süßwasseramphipoden *Gammarus pulex* wurden bereits im Rahmen meiner Staatsexamensarbeit als Teil meines Gymnasiallehramtsstudiums (Biologie, Chemie) durchgeführt. Später wurden im Rahmen dieser Dissertation die gewonnenen Rohdaten mit einer verbesserten Analysemethode erneut ausgewertet und auf Basis dieser Neuauswertung das Manuskript zur Studie 3 erstellt und publiziert. Somit können nur die Kategorien „Erstellung der Datensammlungen und Abbildungen“, „Analyse und Interpretation der Daten“ sowie „Verfassung des Manuskripts“ als Beitrag zu dieser Dissertation gewertet werden. Obwohl Studie 3 nur in Teilen im Rahmen dieser Dissertation entstanden ist, sind ihre Ergebnisse ein wesentlicher Baustein im Gesamtkontext der Dissertation und für diese Arbeit daher unverzichtbar.

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PET microplastics do not negatively affect the survival, development, metabolism and feeding activity of the freshwater invertebrate *Gammarus pulex*

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ABSTRACT

Over the past decade, microscopic plastic debris, known as microplastics, emerged as a contaminant of concern in marine and freshwater ecosystems. Although regularly detected in aquatic environments, the toxicity of those synthetic particles is not well understood. To address this, we investigated whether the exposure to microplastics adversely affects the amphipod *Gammarus pulex*, a key freshwater invertebrate. Juvenile (6-9 mm) and adult (12-17 mm) individuals were exposed to irregular, fluorescent polyethylene terephthalate fragments (PET, 10-150 mm; 0.8-4,000 particles mL^{-1}) for 24 h. Results show that body burden after 24 h depends on the dose and age of *G. pulex* with juveniles ingesting more microplastics than adults. After chronic exposure over 48 d, microplastics did not significantly affect survival, development (molting), metabolism (glycogen, lipid storage) and feeding activity of *G. pulex*.

This demonstrates that even high concentrations of PET particles did not negatively interfere with the analyzed endpoints. These results contradict previous research on marine crustaceans. Differences may result from variations in the exposure regimes (e.g., duration, particle concentrations), plastic characteristics (e.g., type, size, shape, additives) as well as the species-specific morphological, physiological and behavioral traits. As a detritivorous shredder *G. pulex* is adapted to feed on non-digestible materials and might, therefore, be less sensitive towards exposure to synthetic particles. Accordingly, we argue that the autecology needs to be taken into account and that research should focus on identifying traits that render species susceptible to microplastic exposure.

1. Introduction

Plastic debris, especially its microscopic form termed microplastics (MP, commonly defined as < 5 mm in diameter), accumulates in aquatic ecosystems and is of concern due to their persistence, mobility and increasing abundance in marine and freshwater waterbodies (Auta et al., 2017; Eerkes-Medrano et al., 2015; Thompson et al., 2004). Due to their capacity to interact with or to be ingested by aquatic organisms, MP might be able to negatively affect ecosystem (reviewed by Cole et al., 2011; Eerkes-Medrano et al., 2015). So far, laboratory and field studies reported that over 160 different marine species ingest MP, including invertebrates, reptiles, fish, birds and mammals (reviewed by Lusher, 2015). In contrast, MP ingestion was confirmed in 39 freshwater species only, including crustaceans, annelids, insects, mollusks and fish (Scherer et al., 2017).

Ingested MP can trigger molecular, cellular or physiological effects (Browne et al., 2015). First investigations in freshwater amphipods and fish point towards particle-induced alterations of behavior, physiology and reproduction (Au et al., 2015; Carlos de Sá et al., 2015; Mattsson et al., 2015). Still, knowledge on the biological impact of MP is limited and sometimes conflicting, preventing a science-based risk assessment of synthetic polymers in freshwater systems (Wagner et al., 2014). To address this gap of knowledge, we investigated the uptake (i.e., body burden) as well as the effects of MP in the freshwater amphipod *Gammarus pulex*. The species is

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0269-7491/

vastly distributed throughout European rivers and lakes (Engelhardt et al., 2015; Hynes, 1955) and widely used in ecotoxicological studies (De Lange et al., 2006; McCahon and Pascoe, 1988).

Laboratory studies have mainly analyzed the uptake and toxicity of uniform, spherical MP made of polyethylene (PE) or polystyrene (PS, Lusher, 2015). While PE and PS are among the most abundant polymers in the environment (Wagner et al., 2014), the impacts of other plastic types remain mostly unstudied. The same is true for other particle shapes: Because fragments, foams and pellets are more abundant in freshwater ecosystems than plastic spheres (Klein et al., 2015; Mani et al., 2015; Moore et al., 2011), we used irregular polyethylene terephthalate (PET) particles in this study. PET is predominantly used as packaging material and makes up to 7.1% of the total European plastic consumption (Plastics Europe, 2014). Studies by Klein et al. (2015) and Gasperi et al. (2014) highlight that PET MP, though not as dominant as the polymer materials PE and polypropylene (PP), make up an important part of the overall MP load in large European river systems. Furthermore, PET MP have been detected in several lake systems worldwide (Corcoran et al., 2015; Imhof et al., 2016; Zbyszewski and Corcoran, 2011; Zhang et al., 2016). Due to its density >1 g cm^{-3}, PET sinks rapidly and is especially available for benthic species such as *G. pulex*.

We investigated the ingestion of MP by the shredder *G. pulex* over a period of 24 h.We hypothesized that (1) *G. pulex* ingests MP and that (2) the body burden after 24 h of exposure depends on the particle concentration (2a) as well as the age (2b). Chronic toxicity was evaluated over 48 d. Based on the results of previous studies (Cole et al., 2015; Hämer et al., 2014; Lee et al., 2013; Rosenkranz et al., 2009) we hypothesized that (3) MP uptake affects the feeding activity which subsequently alters the metabolism (energy reserves) and development (molting) of *G. pulex*, ultimately resulting in a lower survival.

2. Materials and methods

2.1. Microplastics preparation

The MP particles were prepared from green fluorescent soft drink bottles made of PET (fluorescence excitation at 465-495 nm). The polymer type of the bottles was confirmed by ATR-FTIR spectroscopy (PerkinElmer, Spectrum Two, Waltham, Massachusetts). In brief, 1 g bottle material was snap frozen in liquid nitrogen and ground in a swing mill to produce irregular MP particles with a size of ≤ 150 µm in the largest diameter (for details see 2.1 in the Supplementary data (SD)). The surface structure of the particles was analyzed qualitatively by scanning electron microscopy (SEM, Hitachi, S4500, Krefeld, Germany).

2.2. Preparation of stock suspensions

A 40,000 particles mL^{-1} (p mL^{-1}) MP stock suspension was prepared by suspending 1.59 g MP in 1.5 L ISO medium (ISO, 1996). 300 mg L^{-1} cetyl alcohol pellets were added as water-insoluble surfactant to facilitate homogenous particle distribution. Further suspensions with nominal concentrations of 4, 40, 400 and 4,000 p mL^{-1} were prepared by serial dilution. Particle concentrations in the 40,000 and 4,000 p mL^{-1} suspensions as well as total particle volume in the 40,000 p mL^{-1} suspension were measured with a Coulter Counter (Beckman Coulter, Multisizer 3, Krefeld, Germany; software version 3.53, see 2.2 in SD). For the 4, 40 and 400 p mL^{-1} suspensions, actual particle concentrations were determined by filtering 1 mL on Metricel Black PES membrane filters (Ø 25 mm, Pall Corporation, Dreieich, Germany; pore size: 0.8 µm) and counting particles under a fluorescent microscope. Particle concentrations refer to MP in a size range between 10 and 150 µm. Particles < 10 µm in the stock suspensions were not further examined due to analytical limitations of the fluorescence microscope methodology (see 2.3.1 in SD).

2.3. Uptake of microplastics

The uptake study included the test variables particle concentration (0.4, 40 and 4,000 p mL^{-1}) and age with two classes: juveniles (6-9 mm length) and adults (12-17 mm length, length measured from rostrum to telson).We here defined the smaller size class as "juveniles" although some of the larger individuals in this class may already be sexually mature (Welton and Clarke, 1980). Testing each exposure concentration with both adults and juveniles resulted in a total of six treatment groups with ten replicates of one individual each.

G. pulex was collected on May 1st, 2015 in the Urselbach near Frankfurt/Main, Germany (N 50° 10.2490 E 8°37.1000) and kept for 7 d in a 20 L aerated aquarium with ISO medium at a temperature of 16 ± 1 °C and a 16:8 h light-dark cycle. Collected oak leaves (N 50° 12.7970 E 8° 30.8190) were cleaned twice with distilled water and provided as food source ad libitum.

24 h before the experiment *G. pulex* individuals were transferred to a separate 20 L aquarium with ISO medium to allow gut clearance from remaining food particles. 200 mL screw top glasses were filled with 45 mL ISO medium and 5 mL MP suspension each resulting in nominal exposure concentrations of 0.4, 40 and 4,000 p mL^{-1}. *G. pulex* individuals were exposed for 24 h at a temperature of 16 ± 1 °C, a 16:8 h light-dark cycle and constant aeration via glass pipettes. Aeration intensity was adjusted to a level that allowed PET particles to settle at the bottom of the glass vessel becoming available for *G. pulex*. Afterwards, individuals were removed from the vessels, cleaned from attached MP in 100 mL ISO medium and directly frozen at -80 °C. We determined the abundance of MP in the digestive tract of *G. pulex* qualitatively by direct examination under the fluorescent microscope (Olympus, BX50, Hamburg, Germany, Narrow Band (NB) filter, 100x magnification) as well as quantitatively by lysing the individuals enzymatically (for methodology details see 2.3.1 in SD). The lysates were filtered on black PES membrane filters (see 2.2), fixed on microscope slides and analyzed under a fluorescent microscope with the image analyzer software ImageJ (National Institute of Health, version: 1.46r, Rockville Pike, Maryland, USA). We examined both the total particle number on the filter surface as well as the size of the particles. Due to high concentrations of heterogeneously distributed particles overlapping each other on the filter surface, we could not accurately determine particle abundance in six individuals with the analyzer software. These replicates were consequently excluded from analysis (one adult in the 0.4 p mL^{-1} treatment, three juveniles in the 440 p mL^{-1} treatment, two adults in the 4,000 p mL^{-1} treatment, compare Table S1).

In the same way, we lysed four *G. pulex* individuals which were not previously exposed to PET particles to determine background contamination caused by lysis and analysis. In average, four green fluorescent particles per individual were observed in unexposed animals. Subsequently, all particle abundance results in the study were corrected by this blank value. In addition, the size distribution of particles in the 40,000 p mL^{-1} stock suspension was examined using the same analytical method as for lysates (see 2.3.1 in SD).

2.4. Effects of chronic microplastic exposure

In the effect study we tested the same variables as in the uptake study (particle concentration, age), but we used five MP concentrations (0.4, 4, 40, 400 and 4,000 p mL^{-1}) as well as a negative and

a solvent (cetyl alcohol) control. Cetyl alcohol (1-hexadecanol) is almost insoluble in water (maximum of 40 mg L^{-1} at 25 °C; Yalkowsky et al., 2010) but reduces the surface tension (Boyd and Schubert, 1957). It is frequently used in acute and chronic ecotoxicology tests with *Daphnia* spp. (Ashford and Yan, 2008). Its use was required in the highly concentrated MP stock suspension to facilitate homogenous particle distribution, to reduce particle accumulation at the surface and therefore to improve the preparation of serial dilutions. Due to limited data on cetyl alcohol effects on *G. pulex*, we included a solvent control. Despite the extremely low solubility of cetyl alcohol, we cannot exclude the adsorption of a small cetyl alcohol fraction to the MP particles.

Testing each exposure concentration with both juveniles and adults resulted in 14 treatment groups with ten replicates of one individual each. Test conditions in the effect study were identical to those in the ingestion study described in section 2.3. Test vessels for the negative control (NC) contained 50 mL ISO medium. In the solvent control (SC) 5 mL of the 50 mL ISO medium were substituted by ISO medium which had contained 300 mg L^{-1} cetyl alcohol for 24 h.

G. pulex were exposed individually over 48 d. Every 4 d the individuals were transferred to fresh ISO medium and MP suspension. Leaf circles (Ø 40 mm; mass: 60-100 mg) were produced from oak leaves (*Quercus petraea*) decayed over the winter. One circle per individual was provided as food source and renewed every 8 d. After 4 d, leaf circles were carefully rinsed with distilled water to remove attached MP particles before being reintroduced into the experiment. Before and after exposure, leaf circles were dried at 40 °C and weighed to determine mass loss as indicator for absolute feeding activity (mg leaf material consumed). After 48 d, the average mass loss of the six measurements and the wet weight of the individuals were used to determine the relative daily feeding activity (mg leaf material consumed per mg body mass per day). The daily physical leaf abrasion was evaluated in blank treatments without *G. pulex* individuals (see 2.4.1 in SD). Subsequently, absolute daily feeding rates of *G. pulex* were first corrected by the average weight loss of blank leaf circles, before we determined the relative daily feeding activity.

Throughout the experiment, all animals were examined daily for molt activity and mortality. Dead individuals were removed from the test system. After 48 d, all individuals were frozen and stored at -80 C. After defrosting, *G. pulex* individuals were rinsed with distilled water to remove MP attached to the carapace which might have interfered with the spectrometric determination of the energy reserves and the carapace was dried from attached moisture. Sex, wet weight and energy reserves (glycogen and lipid content) were determined as described in sections 2.4.2 and 2.4.3 in the SD (for sex distribution results see section 3.4 in SD). The intermolt period in *G. pulex* was calculated as the average time difference between two molts. Only individuals that molted at least twice over the course of the experiment were evaluated (for numbers of individuals see Table S1).

2.5. Statistical analysis

All statistical analyses were performed with the software GraphPad Prism (GraphPad Software, version: 5.04, La Jolla, California, USA) and $p < 0.05$ was considered significant.

For the statistical evaluation of the uptake experiments, we compared the body burden in *G. pulex* from the different MP exposure groups with each other separately for adults and juveniles using non-parametric Kruskal-Wallis tests with Dunn's post hoc test. In addition, differences between particle abundances in juveniles and adults exposed to the same MP concentrations were analyzed using non-parametric Mann-Whitney tests. The size distributions of ingested particles and the size distribution in the stock suspension were plotted as relative distributions and fitted using a One-phase decay model. For the chronic toxicity experiment, results from negative and solvent control were pooled as these two treatments did not differ significantly for any of the analyzed endpoints (Mann-Whitney tests). For each endpoint (daily feeding activity, molting, energy reserves), results of the different MP exposure groups were compared to the control using non-parametric Kruskal-Wallis tests with Dunn's hoc test (analyzed separately for adults and juveniles). Mortality rates in the solvent control as well as the MP exposure groups were compared to the control with Fisher's exact tests with Bonferroni correction (analyzed separately for adults and juveniles).

Finally, we analyzed age-related differences in each endpoint (feeding activity, molting, energy reserves) by pooling data from all MP exposure groups and the controls for adults respectively juveniles. We compared the resulting two pools (juveniles vs. adults) with a non-parametric Mann-Whitney test.

3. Results

3.1. Particle shape, size, concentration and volume

SEM imaging of the ground MP revealed a highly irregular surface shape with fractures and smaller fragments adsorbed to larger particles (Fig. 1A, Fig. S1). Actual MP concentrations (determined according to 2.2 in SD, $n = 3$) of the ten-fold concentrated stock suspensions were in accordance with nominal concentrations for the 400 (405 ± 33), 4,000 (4,250 ± 600) and 40,000 (35,300 ± 4,200) particles per mL (p mL^{-1}) suspensions. In the 4 (8.33 ± 0.88) and 40 (67.0 ± 17.5) p mL^{-1} suspensions, the actual particle concentrations were twice the nominal concentration resulting in final actual concentrations of 0.8 and 7 p mL^{-1} in the treatments, respectively. To highlight this, we use actual concentrations (0.8, 7, 40, 400 and 4,000 p mL^{-1}) in the following text. With regard to particle volume, we estimated that the 40,000 p mL^{-1} suspension contained 494 mm^3 L^{-1} MP. Particle number increased exponentially with decreasing particle size (Fig. 1B).

3.2. Uptake of PET microplastics by *G. pulex*

No mortality occurred throughout the 24 h MP exposure. Depending on the concentration, *G. pulex* contained up to several thousand particles after 24 h (Fig. 1C). In the 0.8 and 4,000 p mL^{-1} treatments the body burden was significantly higher in juveniles (28.0 ± 6.9 and 6,505 ± 1,247 particles) compared to adults (8.8 ± 2.8 and 3,287 ± 1,923 particles, $p < 0.05$), while in the 40 p mL^{-1} treatment no significant difference was observed (juveniles: 1,254 ± 421 and adults: 771 ± 262 particles, $p > 0.05$).

The MP body burden in *G. pulex* after 24 h of exposure were dose-dependent (Fig. 1C). An exposure to 4,000 p mL^{-1} resulted in a significantly higher body burden ($p < 0.001$) both in juveniles and adults compared to the 0.8 p mL^{-1} treatment. In addition, the body burden for adults differed significantly in the 0.8 compared to the 40 p mL^{-1} exposure group ($p < 0.01$).

In contrast to absolute MP numbers, the relative body burden (ratio of MP in *G. pulex* after 24 h to total available MP in the exposure vessels) decreased with increasing particle concentrations (Fig. 1D) except for the 0.8 and 40 p mL^{-1} exposure of adult individuals (21.9 vs. 38.6% of total available MP). In the lowest concentration *G. pulex* juveniles contained in average 70% of the provided particles. Compared to this, the relative body burden after exposure to 4,000 p mL^{-1} declined to 3.3% for juveniles ($p < 0.01$) and 1.6% for adults ($p > 0.05$). In addition, significant differences were determined for juveniles ($p < 0.01$) and adults ($p < 0.01$)

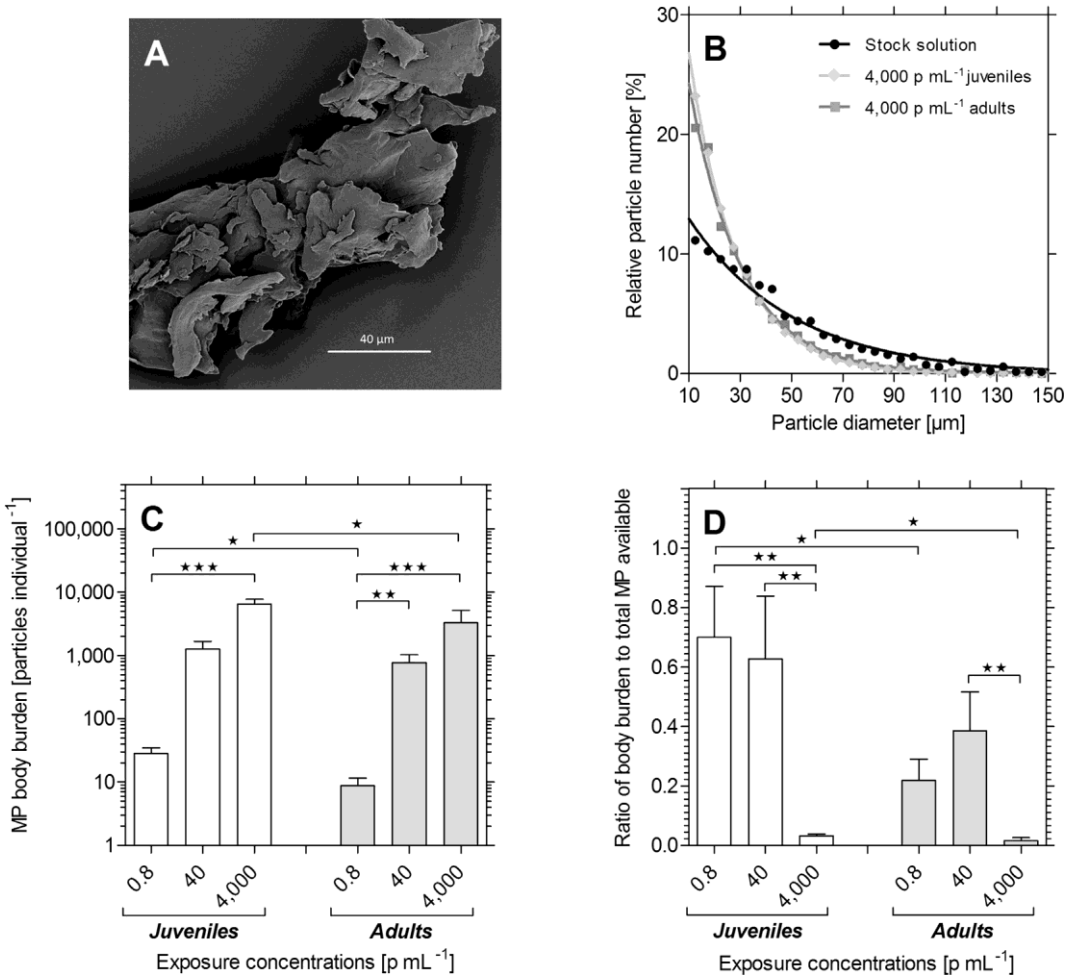

Fig. 1. Body burden of PET microplastics (MP) in *Gammarus pulex* after 24 h. (A) Scanning electron microscopy image of MP used in the study. (B) Particle size distributions in the 40,000 p mL^{-1} stock suspension (black) and of particles present in *G. pulex* after 24 h (grey, 4,000 p mL^{-1} exposure), One-phase decay model. (C) Total and (D) relative body burden (ratio of particles present in *G. pulex* to total available MP) of juveniles (white, n = 7-10) and adults (grey, n = 8-10). Mean + standard error. Statistical differences within adult or juvenile exposures were determined by Kruskal-Wallis tests. The body burden of adults and juveniles at same exposure concentrations were compared by Mann-Whitney tests. ★ $p < 0.05$, ★★ $p < 0.01$, ★★★ $p < 0.001$. For details on sample sizes and size distributions see Table S1 and Fig. S3.

between the test groups 40 p mL^{-1} and 4,000 p mL^{-1}.

Interestingly, we observed large accumulations of PET particles in the cephalic, thoracic or pleonic section of the gut in five out of 60 individuals (Fig. S2) comparable in shape to typical fecal pellets of *Gammarus* (Lautenschlager et al., 1978). However, this observation was unrelated to age or exposure concentration.

In addition, we compared the particle size distribution in the stock suspension and the particles present in *G. pulex* after 24 h to assess a potential size-selective particle uptake/retention in *G. pulex* in the size range of 10-150 µm. The relative particle concentration in *G. pulex* after 24 h increased exponentially with decreasing particle size for individuals from all treatments. Compared to the stock suspension, *G. pulex* lysates contained more particles < 40 µm, while the relative amount of particles > 40 µm was reduced (Fig. 1B, Fig. S3). Although larger particles were observed, on average 90% of the particles present in *G. pulex* after 24 h were < 53 µm.

3.3. Toxicity after chronic MP exposure

After 48 d of exposure, we did not observe any significant effects of MP exposure on the feeding activity, energy reserves and molt periods (Fig. 2A-D). The slight variations observed for each endpoint were neither significantly different compared to the control nor related to the MP dose. In the 7 p mL^{-1} exposure group, juvenile feeding activity and energy reserves were constantly lower compared to the other treatments. This results is not an effect of MP exposure but caused by a higher average wet weight (6.73 vs. 4.39-5.84 mg) in this exposure group.

While we did not observe MP-related toxicity, all endpoints were significantly different when comparing pooled data of adults with those of juveniles ($p < 0.001$). Both the feeding activity (Fig. 2A) (juveniles: 0.080 ± 0.005 mg d^{-1} mg body mass^{-1}, adults: 0.024 ± 0.001 mg d^{-1} mg body mass^{-1}) and the energy budgets (Fig. 2C and D) were significantly higher in juvenile *G. pulex* than in adults. The lipid content in juveniles was 3.04 ± 0.21 J mg body weight^{-1} compared to 0.55 ± 0.04 J mg body weight^{-1} in adults. The glycogen content was 0.14 ± 0.01 J mg body weight^{-1} in juveniles compared to 0.049 ± 0.003 J mg body weight^{-1} in adults. The duration between two molts (Fig. 2B) ranged from 14.7 to 17.6 d (15.8 ± 0.5 d) for juveniles and was significantly shorter than for adults for which molt periods ranged from 21.5 to 33.7 d (27.0 ± 2.0 d).

Mortality in the exposure groups varied from 0 to 20% for juveniles and 10-40% for adults (Table S2). While mortality rates in the different MP treatments did not differ significantly from the

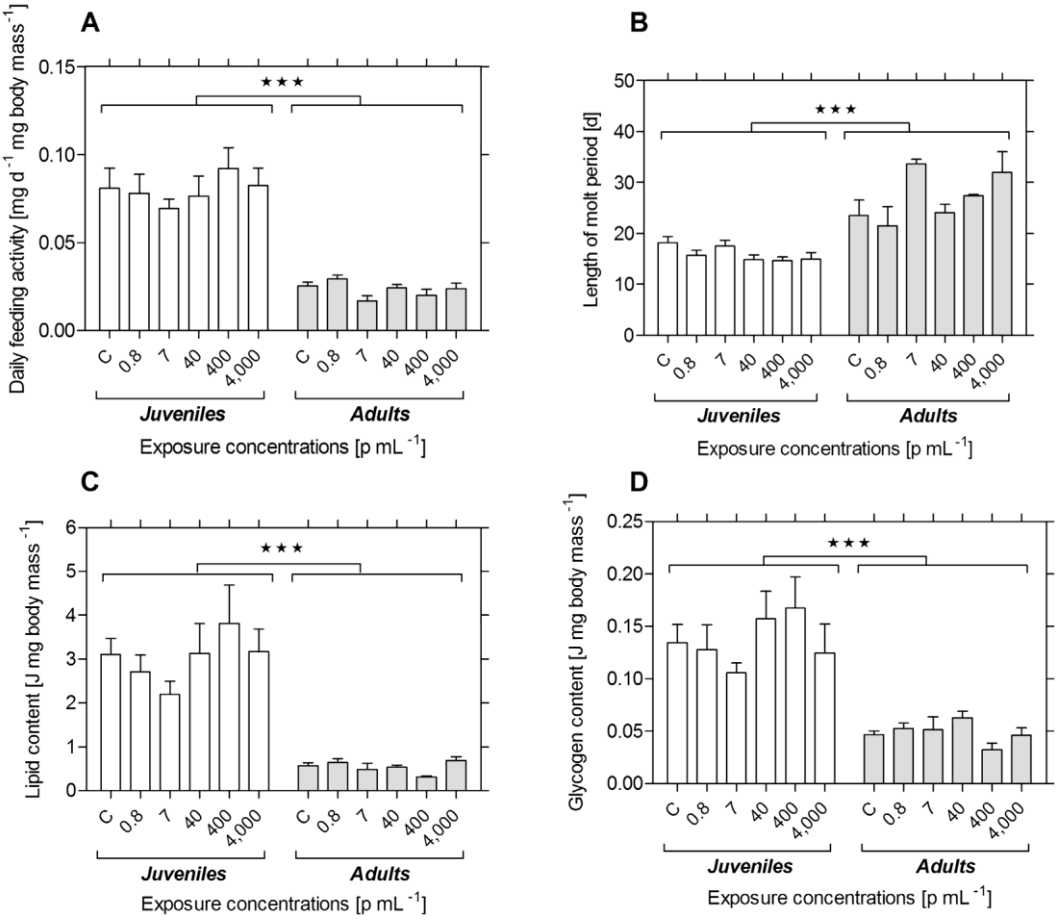

Fig. 2. Effects of chronic microplastics exposure (48 d) on *Gammarus pulex*. (A) Relative feeding activity (juveniles: n = 8-18, adults: n = 6-17), (B) molt periods (juveniles: n = 7-19, adults: n = 3-10), (C) lipid content (juveniles: n = 8-18, adults: n = 6-17) and (D) glycogen content (juveniles: n = 8-18, adults: n = 6-17) of juveniles (white) and adults (grey). Results of the control and the five MP treatments were compared separately for adults and juveniles with a Kruskal-Wallis test ($p > 0.05$) to analyze MP-related effects. To compare adults and juveniles, data from all treatments for each age (juveniles, adults) were pooled for each endpoint and compared with a Mann-Whitney test (★★★ $p < 0.001$). Mean + standard error. Details on sample sizes can be found in Table S1.

control group for juveniles, in adults it was significantly increased in the 7 p mL^{-1} and 400 p mL^{-1} treatments (40% mortality) compared to the control (20% mortality, $p < 0.008$). However, this was not dose-dependent.

4. Discussion

4.1. Experimental microplastics vs. current environmental concentrations

Due to methodological limitations with regard to the sampling and identification of very small MP (Eerkes-Medrano et al., 2015; Hidalgo-Ruz et al., 2012), comprehensive data on the environmental concentrations of MP < 80 µm is scarce. Current concentration estimates for MP > 80 µm in river water vary between 2.8x10^{7} and 0.013 p mL^{-1} (Dris et al., 2015; Lechner et al., 2014; Moore et al., 2011; Sadri and Thompson, 2014). A pilot study on MP in the surface water of Lake Zurich found concentrations of 1.6 p mL^{-1} in the size range of 5-10 µm (Witzig et al., 2017). Therefore, our lowest exposure concentration can be considered realistic for some aquatic systems.

Regarding the size distribution of environmental MP, the study by Mintenig et al. (2017) on waste water treatment plant effluents indicated an exponential size distribution of MP in a size range of 50-250 µm and 500-2,500 µm, respectively, with a maximum abundance of 50-100 µm particles. Imhof et al. (2016) have recently observed a similar pattern for paint particles in lake sediments. Given the lack of knowledge regarding the environmental concentrations of very small MP, it remains uncertain whether the same exponential size distribution also applies to environmental MP < 80 µm. However, because environmental plastics constantly fragment, a higher abundance of smaller MP appears realistic (see section 4.1 in the SD for in-depth discussion).

4.2. *Gammarus pulex* readily ingests microplastics

Throughout the exposure period of 24 h, all *G. pulex* individuals ingested PET particles (hypothesis 1). Based on body burden, *G. pulex* dose-dependently ingested and/or retained MP, with particles <53 mm being especially abundant. *G. pulex* individuals contained up to 6,500 particles (Fig. 1C). Compared to this, only 1 p individual^{-1} was found in field populations of the marine shrimp *Crangon crangon* (Devriese et al., 2015). A lower exposure concentration and the presence of natural organic matter are potential reason for lower MP body burden in field populations. MP particle uptake depends on the availability of the MP particles. We intentionally chose PET for the *G. pulex* study as denser polymers (> 1 g cm^{-3}) rapidly sink in thewater column and become

especially available for benthic species. Compared to that, pelagic species are more vulnerable to less dense polymers (e.g., PE, PP, PS) which remain in the water column over an extended period of time. Due to these differences in availability, a comparison of MP ingestion between benthic and pelagic species is difficult and becomes even more complex in case of different feeding strategies. In their mesocosm study Setälä et al. (2016) reported that filter feeders (e.g., bivalves) ingest more MP compared to sediment-based herbivorous crustaceans.

Notwithstanding the differences in habitat and plastic type used, we observed the same relationship between feeding type and particle ingestion: In a laboratory study with a similar exposure scenario (75 p mL^{-1}, 24 h, 20 µm PS microbeads) to ours (40 p mL^{-1}, 24 h, PET fragments), MP ingestion of the marine copepod *Calanus helgolandicus* was higher (3,280 p d^{-1}, Cole et al., 2015) than body burden in *G. pulex* adults (771 p d^{-1}) and juveniles (1,254 p d^{-1}) (Fig. 1C). This may be caused by methodological differences as Cole et al. (2015) determined total particle ingestion over 24 h while we analyzed the body burden after 24 h. Still, there might also be biological reason: *G. pulex* as shredder mainly feeds on decomposing leaves (Graça et al., 1993) while *C. helgolandicus* selectively filters phytoplankton (Cole et al., 2015). Thus, filtrating crustacean may be more susceptible to MP ingestion compared to ones with other feeding strategies.

MP body burden in *G. pulex* depend on both the exposure concentration (hypothesis 2a) as well as age of the exposed individuals (hypothesis 2b). Similar to the freshwater amphipod *Hyalella azteca* (Au et al., 2015) gammarids contained higher quantities of MP with increasing exposure concentrations (Fig. 1C). Interestingly, the relative particle concentrations in *G. pulex* after 24 h (compared to the total number of particles available) decreased with increasing MP concentrations suggesting that there is a maximum MP body burden (Fig. 1D). This has already been observed for several pelagic crustacean species fed with different algae concentrations (DeMott,1982; Kiørboe et al., 1982). A key limiting factor for particle ingestion could be the feeding speed of *G. pulex* as well as the volume of the digestive tract which restricts further particle uptake (see 4.4 for discussion on gut obliteration).

In terms of age, juveniles contained more MP after 24 h than adults (hypothesis 2b) and are therefore conceivably more prone to MP exposure than adults (Fig. 1C). This is somewhat counterintuitive as we assumed the opposite based on higher absolute feeding mass of adult *G. pulex* (Sutcliffe et al., 1981). This indicates that total feeding mass does not necessarily correlate with total particle burden. Instead, increased body burden could either result from differences in gut retention times of juveniles and adults (depending on the experimental conditions, Willoughby and Earnshaw, 1982) or juveniles selectively feeding on MP.

In addition, the particle size distribution of MP found in *G. pulex* after 24 h indicates a size-selectivity (Fig. 1B, Fig. S3). Compared to the size distribution in the particle stock, we observed a higher number of particles < 40 µm while larger particles were less abundant. One reason could be a longer retention time for smaller particles while larger MP pass the gut system rather quickly. However, we cannot exclude that *G. pulex* also ingested larger agglomerates of PET particles which disintegrated during sample processing resulting in an increased abundance of smaller particles. Alternatively, this pattern may also be feeding-associated and small particle may become especially available if they preferably adsorb to the leaf material on which *G. pulex* feeds. Moreover, the gammarids may be able to differentiate between plastic and food particles and selectively reject the ingestion of particles > 40 µm. Particle size may have also been reduced throughout the gut passage in *G. pulex*. The process of particle fragmentation in the crustacean gut has already been shown by Watts et al. (2015) for PP microfibers in the gut of the crab *Carcinus maenas*. While the exact reason remains to be elucidated, our results demonstrate that very small MP may be more relevant for amphipods than larger MP usually investigated in monitoring studies.

4.3. Toxicological endpoints are age-dependent

Although not the main aim of our study, we observed significant differences in development and metabolism of juveniles and adults throughout the chronic MP study (Fig. 2). *G. pulex* juveniles had a significantly higher relative feeding activity (Fig. 2A) as well as energy reserves (Fig. 2C and D) and shortened molt period intervals (Fig. 2B) compared to adults. This may be related to the accelerated growth of juveniles. A higher feeding activity will enhance the glycogen and lipid metabolism as well as somatic growth, which in turn reduces the molt period intervals. These differences between adults and juveniles have already been observed in previous studies, especially in regard to molt and feeding activities: Compared to 15-18 d for juveniles in this study, molt periods of *G. pulex* were 14 d in a study by Willoughby and Sutcliffe (1976) at similar culturing conditions. Longer molt periods of adults (21-34 d) are in accordance to results of 19-29 d by Ducruet (1975). Regarding feeding activity, Agatz and Brown (2014) reported increased relative feeding of juveniles (mg food d^{-1} mg body $weight^{-1}$) which is comparable to the results from our study.

The comparison of physiological endpoints with regard to age was not the main purpose of this study. However, our results demonstrate that age is highly relevant when investigating the toxicity of MP and should be considered accordingly.

4.4. Microplastics do not affect the survival, feeding activity, energy reserves and molting of *G. pulex*

Based on the high body burden after 24 h of exposure, we expected MP to affect the feeding activity due to an obstruction of the gut resulting in a subsequent depletion of energy reserves, longer molt periods and increased mortality. Indeed, Blarer and Burkhardt-Holm (2016) investigated the toxicity of PS microbeads and polyamide (PA) fibers over a 28 d exposure in Gammarus fossarum. While observing no effects of PS, PA slightly reduced the food assimilation efficiency after 14 d.

Similar effects were especially expected for juveniles at the highest exposure concentration as the total particle volume (2.5 mm^3) was comparable to the volume of consumed elm leaves in a full gut of juvenile *G. pulex* (1.5-3.2 mm^3 per 2-10 mg body wet weight, Sutcliffe et al., 1981). This MP volume should be sufficient to completely block the gut in case all available particles were taken up at once. Counter this expectation, we did not observe significant changes in feeding activity (Fig. 2A), metabolism (energy reserves, Fig. 2C and D) and development (molting, Fig. 2B) (falsification of hypothesis 3). In addition, we did not observe a relation between mortality and increasing MP concentrations (Table S1). Instead, mortality rates are in line with previous feeding studies (with decayed oak leaves as food source) on *G. pulex* (Sutcliffe et al., 1981; Willoughby and Sutcliffe, 1976).

After 24 h exposure, we observed PET particles in the feces of several gammarids (Fig. S2). Although we have not specifically examined the egestion process, we hypothesize that PET particles have rather low retention times in the digestive system of *G. pulex*, which may limit the interactions of MP with the epithelium. In the marine amphipod *Allorchestes compressa* irregular PE particles (11-700 µm) passed the gut system within 36 h (Chua et al., 2014). In *G. fossarum*, the excretion of PA fibers was even faster with almost all MP being cleared from the gut system after 4 h (Blarer and Burkhardt-Holm, 2016). In contrast, Au et al. (2015) reported

that plastic fibers reside longer in the gut of *H. azteca* than PE fragments (10-27 μm) and induce greater toxicity. In case of the study with *H. azteca*, fibers and - to a lower extend - particles might have affected food processing resulting in a decreased growth, reproduction and mortality. These observations are in accordance with results from Ogonowski et al. (2016) who analyzed the effects of regular vs. irregular MP. They reported an increased mortality and prolonged inter-brood periods as well as a decreased reproduction for *Daphnia magna* in treatment groups with irregular PE fragments (~2.6 mm), while spherical plastics of equal size had no extensive toxic effects.

Compared to that, Hämer et al. (2014) did not observe any gut blockage and impacts on the feeding and molt activity of the marine isopod *Idotea emarginata*, neither for PS beads (1-100 μm) nor for polyacrylic fibers (20-2,500 μm). The authors explain this with a limited MP retention, especially because no particles translocated into the midgut gland, the principle site of particle absorbance. Several crustacean orders (Decapoda, Isopoda, Amphipoda, including *Gammarus* spp.) have a specific filtering system that prevents the passage of particles > 1 mm into the tubules of the midgut gland/hepatopancreas (Agrawal, 1965; Felgenhauer, 1992; Schmitz, 1967; Strus and Storch, 2004; von Moos et al., 2012; Wood and Griffiths, 1988). These morphological adaptations may protect *G. pulex* against MP-induced physical injuries, gut blockage and tissue translocation, thus, limiting more downstream effects on metabolism and development.

Another potential explanation for the null effects of MP observed in this study is related to the crustacean gut morphology. As detritivorous shredders, amphipods are evolutionary adapted to process non-digestible food components: The chitinous peritrophic membrane is secreted in the midgut where it encloses the food to protect the digestive system against particle-induced injuries (Forster, 1953; Lautenschlager et al., 1978). In the digestive tract of *G. pulex* PET MP particles are densely packaged (Fig. S2) implying that they are covered in the peritrophic membrane and processed like other non-digestible particles.

While the copepod species *C. helgolandicus* and *Tigriopus japonicus* do possess a peritrophic membrane (Nott et al., 1985; Yoshikoshi and Kô, 1988) that prevents injuries and tissue translocation, studies by Cole et al. (2015) and Lee et al. (2013) demonstrated MP effects on feeding, mortality and fecundity. Both studies were carried out with spherical MP beads for which effects were rather limited in other crustacean species (see above). This illustrates that even in presence of a peritrophic membrane MP effects are highly variable and other parameters such as feeding strategy and particle shape might be at least similarly important. For example, both copepod species are filtration feeders rendering them more sensitive due to an increased MP ingestion and a reduced food intake.

Extended exposure durations can also enhance MP effects as shown by Welden and Cowie (2016) who exposed *Nephrops norvegicus* to 3-5 mm PP fibers for eight months and observed a decrease of feeding rate, body mass, metabolic activity and energy reserves (possibly due to reduced nutrient availability). Accordingly, we cannot exclude MP toxicity in *G. pulex* at an exposure length > 48 d.

Finally, MP toxicity may depend on the polymer types used for exposure. Interestingly, in contrast to our study with *G. pulex*, Au et al., 2015 observed toxic effects of PE particles on growth, reproduction and mortality in a chronic study with *H. azteca*. Both studies (*Gammarus* vs. *Hyalella*) included sediment-based amphipods with the same feeding strategy (shredders), similar MP concentrations (4,000 p mL^{-1} vs. 5,000-10,000 p mL^{-1}), MP size (10-150 μm vs.10-27 μm) and exposure durations (48 d vs. 42 d). The major difference being the polymer type (PET vs. PE) and species indicates that MP toxicity is possibly also highly dependent on the polymer type as well as organism. In summary, the toxicity of MP varies considerably within same classes, orders and even within same species. This diverse pattern is possibly the result of the complex composite of exposure scenarios and biological traits. Here, the toxicity will depend on the characteristics of the exposure (e.g., time and concentration), the stressor (e.g., MP type, size, shape and associated chemicals) and the biological receptor (e.g., autecology, behavior and morphology). Therefore, we cannot exclude that MP induce toxicity in *G. pulex* in a different exposure scenario. As effects are mainly tested in single species, the extrapolation of results to the actual environmental situation is even more difficult: Stressor-biota interactions within ecosystems are highly variable and complex involving numerous interdependent abiotic and biotic factors. Thus, the evaluation of risk MP pose to aquatic ecosystems remains a complexity challenge, especially since information on MP effects is still limited.

5. Conclusions

So far, about 20 studies, mostly focusing on *Daphnia magna*, have examined MP effects in freshwater species. Our study demonstrates that an exposure of the freshwater amphipod *Gammarus pulex* to PET MP over 24 h resulted in high body burden predominantly consisting of particles < 53 mm. However, a long-term exposure over 48 d did not significantly affect feeding activity, energy reserves, molt or mortality. This null effect contrasts studies in other crustaceans but may be explained by differences in the exposure scenarios, feeding strategy and morphological adaptations. Obviously, detritivorous shredders are adapted to feed on non-digestible material and, thus, might be less susceptible to MP exposure. This highlights that the autecology of aquatic species needs to be taken into account in toxicity studies with microplastics. Accordingly, future research should focus on identifying traits that render taxa susceptible to MP exposures.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2017.11.014.

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PET microplastics do not negatively affect the survival, development, metabolism and feeding activity of the freshwater invertebrate *Gammarus pulex*

- Supplementary data -

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2. Supplementary materials and methods

2.1 Microplastics preparation

For microplastics (MP) preparation, 1 g of 0.15 cm² pieces of a green fluorescent PET plastic bottle was cooled with liquid nitrogen for 2 min and ground with a swing mill (Retsch, MM400, Haan, Germany) at 30 Hz using a Ø 25 mm stainless steel ball. Milling time varied between 5 and 24 min (allowing the production of both smaller and larger plastic fragments) and fractions were combined to produce a MP sample with a particle size range of ≤ 150 µm.

The particle shape was analyzed qualitatively using a scanning electron microscope (SEM, Hitachi, S4500, Krefeld, Germany). For microscopic examination, particles were fixed on aluminium discs with coal glue and coated with a gold monolayer (Agar Scientific, Sputter Coater, Stansted, United Kingdom).

2.2 Preparation of stock suspensions

In the 40,000 particles mL^{-1} (p mL^{-1}) suspension, the particle concentration and total volume were measured in technical triplicates with a Coulter Counter (Beckman Coulter, Multisizer 3, Krefeld, Germany; software version 3.53) in 1,000-fold diluted suspensions with a 100 µm capillary (measuring range: 10–23 µm; analytical volume: 5 mL) and in 10-fold dilutions in a 400 µm capillary (measuring range: 23–150 µm; analytical volume: 13.7 mL). Dilutions were prepared with Isoton II solution (0.9% NaCl solution), which was filtered sterile using 0.45 µm PES membrane filters (Thermo Scientific, Nalgene Rapid-Flow, Braunschweig, Germany) to minimize contamination by particles and bacteria. For the 4,000 p mL^{-1} suspension, the measurement procedure was similar to the 40,000 p mL^{-1} suspension, except that the 100 µm capillary analyses were performed with a 100-fold dilution.

2.3 Uptake of microplastics

2.3.1 Quantitative analysis of microplastics uptake after 24 h

For determination of quantity and size of particles found in *G. pulex* after 24 h (body burden), individuals were enzymatically lysed in 6 mL (adults) or 2 mL (juveniles) citrate phosphate buffer (pH 5.7) with chitinase (2 mL, ASA Spezialenzyme, No. 2620, Wolfenbüttel, Germany) and lipase (2 mL, ASA Spezialenzyme, No. 2425) for 13 d (adults) or 10 d (juveniles) at 40 °C and orbital shaking at 450 rpm. Afterwards, 5 mL protease (ASA Spezialenzyme, No. 3610) was added and solutions were incubated at 45 °C and 80 rpm for another 7 d. The lysis was improved by removing the antennae and extremities of the individuals before the enzymatic digestion. Additionally, the lysates were mixed using an electrical pistil (VWR, Darmstadt, Germany) to accelerate the digestion.

The lysates were transferred to Metricel Black PES membrane filters (Ø 25 mm, pore size: 0.8 µm; Pall Corporation, Dreieich, Germany) and analyzed under a fluorescence microscope (Olympus, BX50, Hamburg, Germany, Narrow Band (NB) filter, 100× magnification). For individuals from the 0.4 p mL^{-1} treatment, all particles were evaluated. Otherwise, due to the high particle concentrations, only 10.6% of the 2.5 mm² filter surface area was analyzed. MP counts were extrapolated to the overall filter surface. The size and abundance of MP were determined with ImageJ (National Institute of Health, version 1.46r, Rockville Pike, Maryland, USA). ImageJ identified fluorescent particles on images of the filter surface according to their color contrast to the grey background. Due to a limited

resolution of these images, only particles in a size range between 10 µm and 150 µm were analyzed. Besides the total number of particles, the size of the particles was determined as Feret's diameter.

The size distribution of MP in the stock suspension was determined by filtering 1 mL of the 40,000 p mL^{-1} stock suspension on Metricel Black PES membrane filters. Particle counts and sizes were determined as described above.

2.4 Effects of chronic microplastic exposure

2.4.1 Determination of physical leaf abrasion

Ten screw top glasses each filled with 50 mL ISO-medium and a leaf circle were aerated for 8 d in the absence of *G. pulex*. The weight difference before and after the experiment was determined as measure of the leaf abrasion caused by the aeration system. The average daily leaf abrasion was 0.35 (± 0.07) mg d^{-1}.

2.4.2 Determination of sex and body weight

The body weight was determined as wet weight of each *G. pulex* individual after defrosting and removal of attached moisture. The sex was determined according to the existence of penis papillae or a brood pouch (Bulnheim 1965) at the end of the experiment.

2.4.3 Determination of energy reserves in **Gammarus pulex**

The spectrometric determination of energy reserves was modified according to Benedict (2014). The individuals were separately transferred into a 2 mL Eppendorf tube with 300 µL of 2% (w/w) $NaSO_4$ solution and one Ø 5 mm stainless steel ball. The organisms were homogenized in the swing mill for 3 min at 30 Hz. 100 µL homogenate was mixed with 1.6 mL of a 1:1 chloroform:methanol solution and centrifuged for 2 min at 3,000 rpm (1,400× g; Eppendorf, Centrifuge 5702, Wesseling-Berzdorf, Germany). The resulting lipid fraction in the supernatant was separated from the glycogen fraction (pellet) and transferred into a tube containing 0.6 mL demineralized water. Centrifugation for 2 min at 3,000 rpm produced two phases of which the upper one was discarded, while the denser phase contained the lipid fraction.

For glycogen analysis, the pellet was dissolved in 5 mL anthrone reagent (750 mg anthrone (Merck, Darmstadt, Germany), 150 mL demineralized water, 385 mL 98% H_2SO_4) and incubated at 95 °C for 17 min. For calibration, 0, 25, 50, 100, 150 and 200 µL of a 0.1% (w/w) glucose solution was mixed with anthrone reagent, which was added to a final volume of 5 mL, and incubated like the samples. Optical density was determined spectrometrically (Eppendorf, BioSpectrometer kinetic, Wesseling-Berzdorf, Germany) in 3 mL cuvettes at a wavelength of 625 nm.

For lipid analysis, the phase with the lipid fraction was reduced to a volume of 100–200 µL by evaporation in a water bath at 70 °C. After the addition of 200 µL of 98% H_2SO_4 the solution was incubated for 13 min at 70 °C. For calibration, 0, 50, 100, 200 and 400 µL of a 0.1% rape oil solution (100 mg oil dissolved in 100 mL chloroform) was also incubated with sulfuric acid. After the samples cooled down to room temperature, vanillin reagent (600 mg Vanillin, 100 mL demineralized water, 400 mL 85% H_3PO_4) was added up to the 5 mL level and mixed. The solutions were incubated for 5 min at room temperature and optical density was determined at 625 nm.

The optical densities of the reference sugar and lipid solutions were plotted as a function of its energy content, which was determined according to the total glycogen and lipid content [µg] and the energy values of 16 J mg^{-1} for glucose (Hornback 2006) as well as 37 J mg^{-1} for lipids (REWE Group, product information). The energy content of the *G. pulex* samples was interpolated from a linear (glucose) and a quadratic (lipid) regression of these calibration points.

3. Supplementary results

3.1 Particle shape, size, concentration and volume

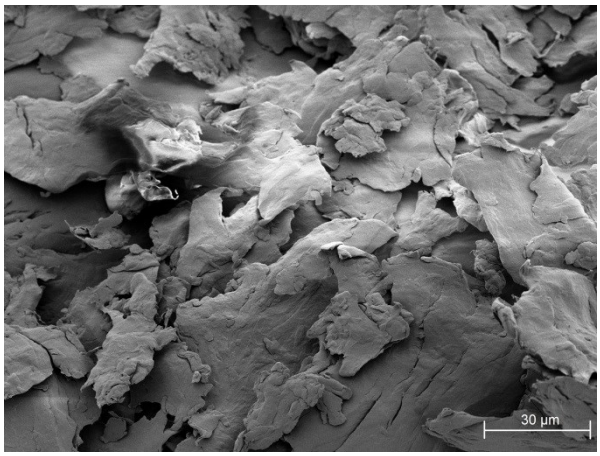

Figure S1: SEM images of the MP particles prepared from PET bottles ground by cryogenic milling.

3.2 Abundance of PET microplastics in G. pulex

3.2.1 PET agglomeration in the gut of Gammarus pulex

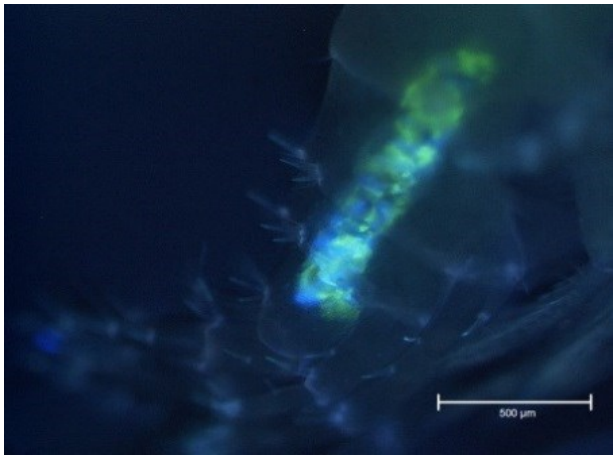

Figure S2: Green fluorescent MP aggregates in the gut of *Gammarus pulex*. The autofluorescence of the amphipod carapace appears blue.

3.2.2 Particle size distribution of particles found in G. pulex *after 24 h and the stock suspension*

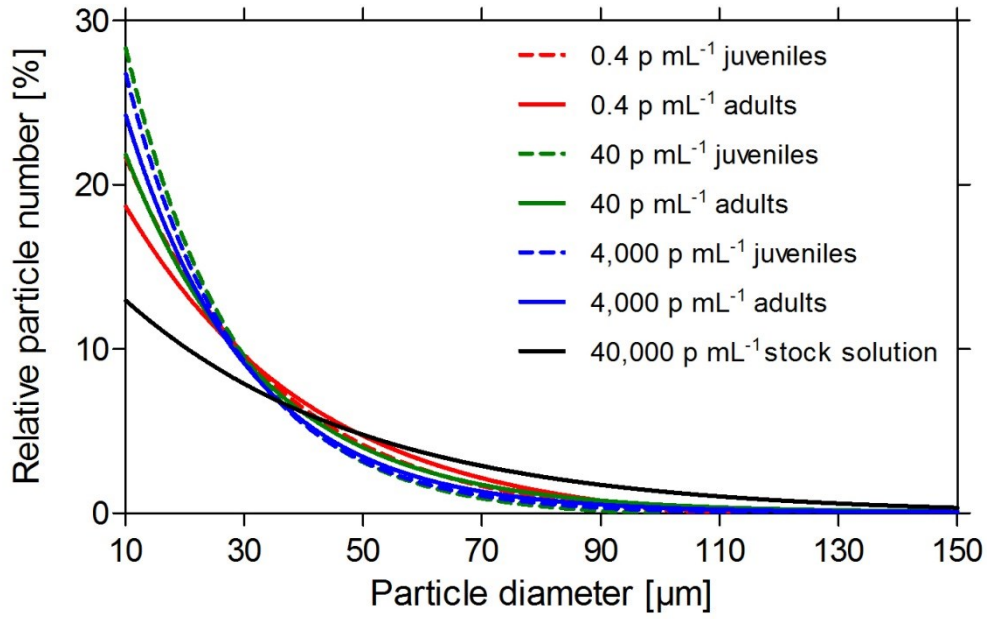

Figure S3: Size distribution (One phase decay) of MP particles found in *Gammarus pulex* adults and juveniles after a 24 h exposure to PET MP (0.8, 40 and 4,000 p mL^{-1}, colored) in comparison to the PET particle size distribution in the 40,000 p mL^{-1} stock suspension (black).

3.2.3 Sample sizes of the uptake and effect study

Table S1: Number of *Gammarus pulex* individuals (biological replicates) investigated for specified endpoints in the short-term (uptake study) and chronic exposure (effect study). Control = negative + solvent control. n.a. = not analyzed

Endpoint	Figure	Age	MP concentration [p mL^{-1}]					
			Control	0.8	7	40	400	4,000
Particle abundance (24 h)	Fig. 1	Juveniles	n.a.	10	n.a.	7	n.a.	10
		Adults	n.a.	9	n.a.	10	n.a.	8
Feeding activity and	Fig. 2A,	Juveniles	18	8	8	8	8	8
energy reserves (48 d)	C, D	Adults	17	7	6	7	6	7
Molting (48 d)	Fig. 2B	Juveniles	19	10	7	8	9	8
		Adults	10	3	3	6	3	3

3.3 Results of toxicological endpoints in the chronic MP study

3.3.1 Mortality throughout 48 day long-term exposure

Table S2: Mortality rates of *Gammarus pulex* in the effect study (n = 10).

	NC	SC	0.8 p mL^{-1}	7 p mL^{-1}	40 p mL^{-1}	400 p mL^{-1}	4,000 p mL^{-1}
Juveniles	20%	0%	20%	20%	20%	20%	20%
Adults	20%	10%	30%	40%	30%	40%	30%

3.4 Sex distribution

Post-experimental evaluation of individuals' sex indicated equal distribution (1:1) in four and almost equal size distribution (2:3 or 3:2) in seven out of 14 test groups. The three remaining test groups consisted of seven males and three females each. Data evaluation according to sex revealed no distinct difference to results from the combined data (data not shown).

Table S3: Sex distribution of *Gammarus pulex* in the effect study (n = 10).

	NC	SC	0.8 p mL^{-1}	7 p mL^{-1}	40 p mL^{-1}	400 p mL^{-1}	4,000 p mL^{-1}
Juveniles							
Males	50%	40%	50% *	70%	60%	60%	40%
Females	50%	60%	40% *	30%	40%	40%	60%
Adults							
Males	70%	50%	50%	60%	40%	70%	50%
Females	30%	50%	50%	40%	60%	30%	50%

* sex of one individual (10%) could not be identified due to decomposition of the body tissues

4.1 Experimental microplastics vs. current environmental concentrations

The cryogenically ground PET particles, we used in our study, had a highly irregular and fractured surface shape (Fig. 1A, Fig. S1). A visual comparison of the PET particle surface with images of weathered beach plastic particles (Cooper and Corcoran 2010) indicates a similar brittle and fractured surface structure. Accordingly, the use of irregular particle in ecotoxicological studies represents a more environmentally realistic alternative compared to uniform shaped microbeads commonly used in toxicological studies.

Currently, an exponential size distribution (increasing particle abundance with decreasing particle diameter) has only been shown in two different studies (Imhof et al. 2016, Mintenig et al. 2017) with only one focusing on MP particles. Still, an exponential distribution for particles size can also be inferred from the following simplistic thought experiment for particle fragmentation processes: We consider a certain amount of equally sized MP. Each of these particles fragments in smaller, identical particles. If we assume that each fragmentation process (in two, three, four particles, etc.) is equiprobable, the resulting size distribution will follow an exponential function with an increase of particle numbers for decreasing particle diameters. This theoretical approach is supported by the particle size distribution of our artificially produced MP.

We are aware, however, that this simulated degradation process may not mimic long-term leaching and photo-oxidation processes of environmental plastics and further studies are needed to confirm MP degradation for particles < 80 µm according to the thought experiment.

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A4. Ingestion and toxicity of microplastics in the freshwater gastropod *Lymnaea stagnalis*: No microplastic-induced effects alone or in combination with copper

Studie 4

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Ingestion and toxicity of microplastics in the freshwater gastropod *Lymnaea stagnalis*: No microplastic-induced effects alone or in combination with copper

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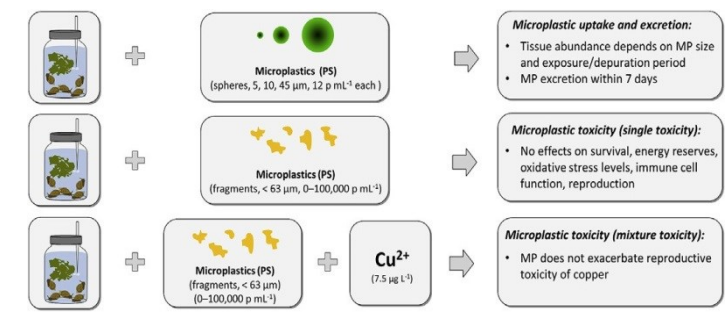
HIGHLIGHTS

- *Lymnaea stagnalis* readily ingests and egests PS microplastics (5-90 µm).
- Ingestion and excretion of spherical PS (5-45 µm) is size- and time-dependent.
- Almost complete depuration of ingested spherical PS within 7 days.
- No significant chronic toxicity of irregular PS (≤63 µm).
- In mixture, irregular MP does not exacerbate the reproductive toxicity of copper.

GRAPHICAL ABSTRACT



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ABSTRACT

The interaction of microplastics with freshwater biota and their interaction with other stressors is still not very well understood. Therefore, we investigated the ingestion, excretion and toxicity of microplastics in the freshwater gastropod *Lymnaea stagnalis*.

MP ingestion was analyzed as tissues levels in *L. stagnalis* after 6-96 h of exposure to 5-90 µm spherical polystyrene (PS) microplastics. To understand the excretion, tissue levels were determined after 24 h of exposure followed by a 12 h-7 d depuration period. To assess the toxicity, snails were exposed for 28 d to irregular PS microplastics (<63 µm, 6.4-100,000 particles mL^{-1}), both alone and in combination with copper as additional stressor. To compare the toxicity of natural and synthetic particles, we also included diatomite particles. Microplastics ingestion and excretion significantly depended on the particle size and the exposure/depuration duration. An exposure to irregular PS had no effect on survival, reproduction, energy reserves and oxidative stress. However, we observed slight effects on immune cell phagocytosis. Exposure to microplastics did not exacerbate the reproductive toxicity of copper. In addition, there was no pronounced difference between the effects of microplastics and diatomite. The tolerance towards microplastics may originate from an adaptation of *L. stagnalis* to particle-rich environments or a general stress resilience. In conclusion, despite high uptake rates, PS fragments do

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not appear to be a relevant stressor for stress tolerant freshwater gastropods considering current environmental levels of microplastics.

1. Introduction

The realization that plastic pollution is ubiquitous in aquatic ecosystems worldwide has raised concern on the possible consequences for aquatic life (SAPEA, 2019). Previous research has focused on microplastics (MP, 1-1000 µm, Hartmann et al., 2019) due to their global distribution (Rezania et al., 2018). While the ingestion of MP has been demonstrated in field studies for numerous taxa (Eerkes-Medrano et al., 2015; Lusher, 2015), the implications of MP ingestion are still unclear. In previous toxicity studies, MP concentrations inducing adverse effects varied by orders of magnitude (reviewed by Anbumani and Kakkar, 2018; Strungaru et al., 2019; Triebskorn et al., 2019) leading to sometimes divergent interpretations whether MP pose a current and future risk for the aquatic environment (Adam et al., 2018; Burns and Boxall, 2018; Guzzetti et al., 2018).

Previous toxicity studies have mostly focused on the MP effects on fish, bivalves and crustaceans (de Sa et al., 2018). In contrast, effects on gastropods are rarely studied and, therefore, underrepresented in current risk assessments. MP ingestion by gastropods has already been demonstrated both in the field and laboratory (Courtene-Jones et al., 2017; Gutow et al., 2016, 2019; Karlsson et al., 2017; Watermann et al., 2017). Gastropods ingest spherical and irregular particles of various polymer types.

Knowledge on MP toxicity in aquatic gastropods, however, is currently limited to five peer-reviewed studies. In the marine slipper snail *Crepidula onyx*, 2-5 µm polystyrene (PS) spheres reduced settling and growth of juveniles at high (60,000 and 140,000 particles mL^{-1} (p mL^{-1})) but not at low (10 p mL^{-1}) concentrations (Lo and Chan, 2018). Qu et al. (2020) reported significantly enhanced filtration rates in the freshwater gastropod *Cipangopaludina cathayensis* after exposure to 0.7 µm PS MP (20 mg L^{-1}). In the freshwater mudsnail *Potamopyrgus antipodarum*, high MP concentrations in food (250 mg MP and 100 mg food in agar) did not cause any toxicity on growth, development, reproduction and survival (Imhof and Laforsch, 2016). In the pond snail *Lymnaea stagnalis*, nylon particles (1% w/w in sediments) caused no changes in wet weight or microbiome (Horton et al., 2020). Finally, Doyle et al. (2020) did not observe a correlation between environmental MP levels and the individual emergence behaviour in *Littorina littorea* populations.

Given the scarcity of data on gastropods, the aim of this study was to investigate the ingestion and excretion of MP (5-90 µm PS spheres) as well as its effects (irregular PS, ≤63 µm, 6.4-100,000 p mL^{-1}) on adult freshwater pond snail *L. stagnalis*. Effects were determined after 28 d of exposure with energy reserves, oxidative stress, immune cell phagocytosis activity and reproduction as endpoints.

Further, we compared the toxicity of MP to that of natural particles. High particle loads in aquatic ecosystems reduce the food supply and quality of benthic invertebrates (Camargo and Alonso, 2017). As a consequence, snails can adapt their feeding strategy (Calow, 1975) but may also adapt evolutionary to high loads of suspended particulate matter. Therefore, the toxicity of MP needs to be benchmarked against that of natural materials (Scherer et al., 2018). Here, we used diatomite (DI, 100,000 p mL^{-1}) to compare the effect of synthetic and natural particles.

In the real world, MP will be just one amongst many stressors (Backhaus and Wagner, 2019) and mixture toxicity studies can be an important second step towards more environmentally realistic study scenarios (Syberg et al., 2015). Inorganic copper (Cu) is a relevant stressor for freshwater organism with *L. stagnalis* being one of the most sensitive species (Brix et al., 2001; ECI, 2008). Cu concentrations as low as 2-10 mg L^{-1} affect survival, growth, metabolism and reproduction in *L. stagnalis* (Atli and Grosell, 2016; Brix et al., 2011; Das and Khangarot, 2011; Ng et al., 2011). Accordingly, we studied the joint effects of Cu^{2+} and MP on *L. stagnalis*. We exposed the snails to 7.5 µg L^{-1} Cu^{2+} and either MP (6.4-100,000 particles mL^{-1}) or DI (100,000 p mL^{-1}) using the energy reserves, oxidative stress and reproduction as endpoints.

2. Materials and methods

2.1 Lymnaea stagnalis culture

L. stagnalis individuals were obtained from an in-house culture at Goethe University and raised in aerated 50 L tank with 100-200 individuals per tank (maximal density according to OECD (2016): 5 individuals L^{-1}) at 20 °C water temperature (optimal water temperature for *L. stagnalis* growth and development: 16-20 °C, Van der Schalie and Berry, 1973). Tanks were filled with ISO medium prepared in accordance with OECD guideline no. 243 (OECD, 2016, *Lymnaea stagnalis* Reproduction Test). The ISO medium was composed of 294 mg L^{-1} $CaCl_2 \cdot 2H_2O$, 123 mg L^{-1} $MgSO_4 \cdot 7H_2O$, 64.7 g L^{-1} $NaHCO_3$ and 5.75 mg L^{-1} KCl. Once to twice a week, 50% of the water volume in each tank was renewed. *L. stagnalis* was fed three times a week with both butterhead lettuce (*Lactuca sativa* var. *capitata*, organic quality according to EU-Eco-regulation or stricter) and Tetramin (Tetra GmbH, Melle, Germany) *ad libitum*.

1.1.

2.2 Particle preparation

For the MP ingestion and excretion study, non-functionalized 10, 45 and 90 µm PS spheres (density: 1.05 g cm^{-3}) were purchased from Polysciences Europe (Fluoresbrite YG microspheres, Hirschberg an der Bergstraße, Germany, excitation: 441 nm, emission: 486 nm). Non-functionalized 5 mm PS spheres were obtained from Magsphere (Pasadena, CA, USA, excitation: 538 nm, emission: 584 nm).

For the toxicity studies, we produced irregular MP with a broad particle size range from yellow-orange fluorescent PS drinking cups (excitation: 360-370 nm) by cryomilling. DI particles were purchased from Sigma-Aldrich (Taufkirchen, Germany). From both the MP and DI, we isolated the particle fraction ≤63 µm by sieving. The polymer type and chemical content of the drinking cups was determined by Attenuated Total Reflection-Fourier Transform Infrared-spectroscopy (ATR-FTIR-spectroscopy) and pyrolysis-GC-MS. A low level of chemicals was detected in the PS cups but the compounds could not be matched to common substances from polymer industry (further details on particle preparation and polymer analysis in Weber et al. (2020)).

The purchased PS sphere stock suspensions for the ingestion and excretion study were diluted in ultrapure water and their numerical particle concentrations were determined with a Coulter

Counter (Multisizer 3; Beckman Coulter, USA). For the toxicity studies, we determined particle concentrations (number per mg powder) and size distribution in the ≤63 µm MP and DI powder using the Coulter Counter (size range: 2-60 µm). For both the MP and DI powder, particle concentrations increased exponentially with decreasing particle size. Within the 2-60 µm size range, 95% of the MP and the DI particles were smaller than 9.7 and 13.6 µm, respectively (details in S1.1 and S2.1). For qualitative assessment of particles <2 µm, MP and DI powder were imaged with a scanning electron microscope (SEM, details in S1.2). SEM imaging confirms that nanoparticles are present in the MP and DI powder (Fig. S5) but a quantitative analysis (e.g., by nanoparticle tracking analysis) was not possible due to the abundance of large particles that would have blocked the instrument.

1.2.

2.3 Ingestion and excretion study

We analyzed the MP ingestion by exposing *L. stagnalis* simultaneously to a mixture of 5, 10 and 45 µm fluorescent PS spheres (12 p mL^{-1} each) over an exposure period of 6, 12, 24, 48 or 96 h. In addition, we added 90 µm PS spheres (2 p mL^{-1}) to test whether the snails are also able to ingest larger MP. We used PS spheres with homogenous sizes instead of polydisperse fragments to investigate the size-dependency of MP ingestion and excretion.

For each exposure period, we filled one glass jar (diameter: 10 cm, height: 18 cm) with 1 L ISO medium and added five snails (2.5-3.0 cm, apex to basal lip) and 10 g lettuce as food source (see 2.1). The jars were filled with ISO medium first before the pre-diluted PS sphere stock suspensions with known particle concentrations (see 2.2) were added to the ISO medium. Stock suspensions were stirred thoroughly prior to pipetting to allow homogenous particle distribution. Throughout the experiment, the jars were gently aerated using glass pipettes and water loss (due to evaporation, up to approximately 20 mL d^{-1}) was adjusted daily by adding ultrapure water if necessary.

In previous experiments, we observed that (despite a constant water movement through aeration) the 5, 10, 45 and 90 µm PS spheres as well as the PS fragments used in the toxicity experiments sedimented within few hours to days, with smaller spheres staying longer in the water phase compared to larger spheres (unpublished data). Since *L. stagnalis* not only grazes on surfaces but also floats freely in the water column and on the water surface (OECD, 2016), both suspended and settled particles were bioavailable throughout the experiments. For the excretion study, snails were exposed for 24 h as described above (five jars with five individuals each), then transferred into glass jars with aerated, MP-free ISO medium and kept in those for another 12, 24, 48, 96 h or 7 d (one jar with five individuals per depuration period). The water in the jars was exchanged daily to remove excreted particles and minimize re-uptake. Directly after the transfer of *L. stagnalis* into the MP-free medium as well as after each water exchange, snails were fed with 0.5 g lettuce $individual^{-1}$ d^{-1}.

After the exposure, the shell and soft tissue of all individuals were thoroughly rinsed with tap water to remove attached MP and directly frozen at 80 °C. After defrosting, the shell of each individual was removed, and the soft tissue of each gastropod was lysed individually in 20-40 mL 10% potassium hydroxide solution at 55 °C for 24-48 h. The resulting lysate was filtered on glass fiber filters (VWR, Darmstadt, Germany, pore size: 1.25 µm). The total number of 5, 10, 45 and 90 µm PS spheres on each filter was determined visually using a fluorescence microscope (BX50, 40 magnification, Olympus, Hamburg, Germany).

For quality assurance, another five individuals were exposed for 48 h as described above but without added MP to determine the background contamination throughout the exposure and lysis. We found one particle each in two out of five snails resembling 5 µm PS spheres but none with the color and shape of 10, 45 or 90 µm spheres. Thus, we corrected the ingestion and excretion rates of 5 µm PS spheres for this background contamination (0.4 particles per individual).

2.4 Microplastics toxicity study

Toxic effects of MP were analyzed by exposing *L. stagnalis* over 28 d to irregular PS MP (<63 mm) at concentrations of 6.4, 160, 4000 and 100,000 p mL^{-1} ($MP_{6.4}$, MP_{160}, $MP_{4,000}$, $MP_{100,000}$; concentrations correspond to the size range 2-60 µm). In addition, we included a control without MP (control) and a treatment with DI (100,000 p mL^{-1}, $DI_{100,000}$, for test design see Fig. S1). The study was designed according to OECD guideline no. 243 (OECD, 2016). For each of the six treatments, six glass jars were filled with 1 L ISO medium (gentle aeration through glass pipettes), the corresponding particles and five snails (2.5-3.0 cm; 30 individuals per treatment). For the $MP_{4,000}$, $MP_{100,000}$ and $DI_{100,000}$ exposures, particles were weighed in and added to the 1 L ISO medium present in each jar (based on the particle number per plastic powder mass, see 2.2 and S2.1). For the two lower MP concentrations ($MP_{6.4}$, MP_{160}), we prepared 100-fold concentrated suspensions which were then diluted in the exposure vessels as MP mass was too low to weigh it in. Prior and throughout the dilution, the suspensions were stirred intensively to minimize particle agglomeration and maximize homogenization. As we did not use any surfactant, we cannot exclude that MP particles (especially PS fragments) aggregated throughout the exposures causing heterogenous MP distribution in the exposure vessels. Due to mobility of *L. stagnalis* (see 2.3), the effect of a heterogenous particle distribution on bioavailability, however, was probably small.

All snails were exposed at 20 ± 1 °C and a 16:8 h day-night-cycle. For water exchange, the experimental set-up was completely renewed every 3-4 d and gastropods were transferred into new jars (filled as described above). Egg clutches in the former jars were removed and analyzed to quantify reproductive output (see 2.7). Feces was collected from each vessel and analyzed qualitatively for MP presence with a fluorescent microscope (Olympus, BX50, Hamburg, Germany). In addition, we analyzed the water quality (water temperature, oxygen content, pH, conductivity, water hardness) in two out of six jars per treatment (one jar for water hardness). After the transfer of the snails into the new jars, they were fed with lettuce (0.5 g $individual^{-1}$ d^{-1}). Each jar was checked daily for mortality and dead individuals (motionlessness after touching, abnormal foot position and decomposed tissues) were removed. Based on this, mortality rates and time of survival were calculated (2.6). Throughout the toxicity study, the OECD validity criteria (mortality and reproduction in the control, water parameters) were fulfilled excepted for slightly deviations of the average exposure temperature (18.8 °C compared to 20 ± 1 °C) as well as water conductivity and hardness (detailed results in S2.4). The removal of individuals from the exposures may have affected particle uptake by the remaining individuals in the low (6.4 p mL^{-1}) but not in the higher MP treatments, as the total MP number (1.6×10^5 p jar^{-1} in the exposure vessels with 160 p mL^{-1}) by far exceeded the particle numbers measured in *L. stagnalis* (maximal ingestion of 2,235 p $individual^{-1}$ at an exposure concentrations of 36 p mL^{-1}, see 3.1).

Three days prior to the 28 d exposure, individuals were acclimatized to the test conditions. The experiment was started after this period as in more than half of the jars an egg clutch had been laid (OECD guideline prerequisite). After 28 d of exposure, we sampled hemolymph from 6 to 7 individuals per treatment by

irritating the foot with a plastic pipette tip (careful rubbing of the foot with the tip), collected the released hemolymph and directly analyzed the phagocytic activity of the hemocytes (see 2.9). The remaining individuals were directly frozen in liquid nitrogen and stored at -80 °C for analyses of energy reserves and oxidative stress in the midgut gland (MGG, see 2.8).

2.5 Mixture toxicity study with copper and microplastics

In a second toxicity experiment, we analyzed whether MP modulates the toxicity of Cu as primary stressor. The experiment included six treatment groups with 7.5 µg L^{-1} Cu (actual concentration according to chemical analytics, spiked as Cu^{2+} from $CuCl_2$) and 0, 6.4, 160, 4,000 and 100,000 p mL^{-1} irregular PS MP (Cu, Cu+$MP_{6.4}$/MP_{160}/$MP_{4,000}$/$MP_{100,000}$) or 100,000 p mL^{-1} DI (Cu + $DI_{100,000}$). In addition, we included a control treatment without Cu and MP particles to verify test validity (for test design see Fig. S2). For each of the seven treatments, seven glass jars with five snails (2.5-3.0 cm, 35 individuals per treatment) each were used. To avoid Cu leaching from the jars or the glass pipettes throughout the experiment, all glass ware was placed in 70 °C pre- subboiled (DST-4000, Savillex, Minnesota, USA), distilled HNO_3 solution (1.3% (v/v)) for at least 7 d and afterwards cleaned several times with ethanol and ultrapure water. The jars were filled with MP and medium first (as described in 2.4) before we spiked the Cu using a 100-fold concentrated stock solution. The snails were introduced 1 h later to allow for an equilibration of the MP and Cu distribution in the jars. The following experiment was performed as described in 2.4. Again, reproduction as well as the energy reserves and oxidative stress in the MGG were analyzed as endpoints. Hemocyte phagocytosis analysis could not be performed due to technical failure. OECD test validity criteria were fulfilled excepted for slight deviations of water hardness, pH and oxygen content in very few treatments (results in S2.4).

With each water exchange every 3-4 d (complete renewal of the set-up), water samples for Cu analysis were taken. One sample (49 mL) of the unspiked ISO medium as well as one sample (49 mL) of the ISO medium spiked with 7.5 µg L^{-1} Cu^{2+} (sampling 1 h after Cu spiking to allow for Cu distribution in the jar) were taken from freshly prepared new exposures (0 d). Further, after the 3-4 d exposure, we pooled the water of all jars from the same treatment and took one sample (49 mL) from each treatment (throughout each water exchange). Each water sample was aspired with a new syringe (B. Braun, Omnifix Luer Lock, latex-free, 20 mL), directly sterile-filtered (Spartan syringe filters, regenerated cellulose, pore size: 0.45 µm, Whatman, Sigma-Aldrich, Taufkirchen, Germany) into pre-leached DigiTubes (cleaned with HNO_3 as described above, DigiTubes: 010-500-264, SCP Science, Quebec, Canada), preserved with 1 mL subboiled HNO_3 (65%) and stored at 5 °C in the dark until being analyzed. Filtration of the water samples ensured the removal of all particles >0.45 mm (e.g., MP or food) from the water samples which could have affected the Cu concentrations in the water phase. However, a potential influence of particles <0.45 µm on the Cu analysis cannot be ruled out. Total Cu concentrations (all copper species) in the water samples were measured with ICP-QQQ-MS (details in S1.6).

After the 28 d exposure, four snails per treatment were transferred to pure ISO medium (with 0.5 g lettuce $individual^{-1}$ d^{-1}) for another 3 d to allow the excretion of Cu-contaminated remains in the digestive system. Afterwards, individuals were frozen, their shell was removed and wet and dry weight (after freeze-drying) was determined. Individuals from each treatment were pooled and tissue Cu concentrations (total concentration of all copper species) were measured using an MLS turboWAVE system and ICP-QQQ-MS (see S1.6). In addition, background Cu tissue concentrations were analyzed in a pool of four *L. stagnalis* individuals from the culture frozen prior to the experiment.

To understand the distribution of Cu in the exposure vessels, we further performed a separate Cu distribution study (details in S1.5). For this, both unspiked and Cu-spiked (7.5 µg L^{-1}, spiked as Cu^{2+} from $CuCl_2$) ISO medium was incubated (without snails and lettuce) for 3 d in presence and absence of PS MP (6.4, 160, 4000 or 100,000 p mL^{-1}) or DI (100,000 p mL^{-1}) to evaluate potential impacts on Cu water concentrations. Further, Cu-spiked ISO medium was also incubated with 1.5, 4.5 and 7.5 g lettuce to determine Cu adsorption on the food. Cu concentrations in the water (sample taken as described above) as well as on the lettuce were measured both before the start of the Cu distribution study as well as 3 d after. Cu concentrations on MP were not quantified because currently no appropriate reference material (MP with adsorbed metals) is available for ICP-QQQ-MS analysis. For snail tissues and lettuce, we used standard mussel tissue and standard white cabbage as reference materials (S1.6).

2.6 Mortality and time of survival

Total mortality in each treatment was determined as ratio of dead individuals compared to the original number of snails in each treatment. The time of survival reported as "total survival days" was determined for each jar as the sum of days which the five individuals per jar survived (maximum: 140 d). In case of mortality, survival days of the dead individual were calculated as $((t+1)+t)/2$ with t = last day on which the individual was alive (OECD, 2016).

2.7 Reproduction

The reproductive output of *L. stagnalis* was quantified by determining the total number of laid eggs and egg clutches in each jar over a period of 28 d. The number of eggs in each clutch was determined using a stereo microscope. As endpoints, we calculated the number of eggs per egg clutch and the number of eggs or egg clutches per survival days (2.6) in each of the six (MP toxicity experiment) or seven (mixture toxicity experiment) jars per treatment.

2.8 Energy content and oxidative stress in the midgut gland

MGGs from ten individuals per treatment (at least one individual from each jar per treatment) were dissected, weighed and homogenized (S1.7) to analyze the energy content and oxidative stress. Homogenates were stored at 80 °C until being analyzed. In brief, energy reserves in the MGG homogenates were measured as protein (Bradford, 1976), glycose (anthrone assay, Benedict, 2014) and total lipid content (sulfo-phospho-vanillin assay, Benedict, 2014). Oxidative stress was determined as malondialdehyde content (MDA, biomarker for lipid peroxidation) based on Furuhagen et al. (2014) and the remaining antioxidant capacity in the MGG was measured with the ORAC assay (Ou et al., 2001; Furuhagen et al., 2014). The antioxidant capacity is expressed as Trolox equivalents (reference antioxidant) per MGG mass [mmol mg^{-1}]. Further details on energy and oxidative stress assays are reported in Weber et al. (2020).

2.9 Hemocyte phagocytosis activity

The immune function of *L. stagnalis* was assessed through the phagocytic activity of the hemocytes in response to foreign stimuli. The methodology was applied with some modifications as described by Weber et al. (2020, methods and modifications are summarized in S1.8 and S2.6). In brief, hemocytes of seven snails

per treatment (at least one individual from each jar per treatment) were exposed individually to 1 µm PS microspheres (25 spheres cell^{-1}, Fluoresbrite YG, PolyScience, Hirschberg an der Bergstraße, Germany) for 3 h and the number of hemocytes with 3 phagocytized microspheres was compared to the total number of living cells with a BD FACSVerse (BD Biosciences, Heidelberg, Germany, see S2.6). For some of the samples, the minimal number of live cells (>5000) required for the FACS analysis was not reached resulting in 5-7 remaining replicates per treatment.

2.10 Data analyses

MP ingestion and excretion were statistically analyzed using a general linear model (GLM, IBM SPSS Statistics, version 25) with the two fixed factors "MP particle size" and "exposure period" (ingestion study) or "depuration period" (excretion study) as well as their interaction term (MP size exposure period or MP size depuration period). The number of MP in the *L. stagnalis* individuals was used as dependent variable and was square root transformed (ingestion study) or log (x-0.5)-transformed (excretion study) to maximize variance homogeneity. Prior to each GLM, normality (Shapiro-Wilks test) and variance homogeneity (White test) requirements were tested. Requirements were fulfilled except for the treatment with 45 µm spheres for 24 h, (ingestion study, $p = 0.04$) and with 5 µm spheres for 168 h depuration (excretion study, $p = 0.03$). As we exposed the snails to a mixture of MP with different sizes, the analysis is based on the assumption that these do not interact, that is, affected the ingestion of the other MP. For the toxicity experiments and the Cu distribution study, statistical differences between the treatments were determined using either a one-way ANOVA with Sidak's post-test (in case of normal data distribution and variance homogeneity) or a Kruskal-Wallis test with Dunn's post-test (in case of non-normal data distribution or variance heterogeneity). Normality was checked using Shapiro-Wilk tests. To test for variance homogeneity Bartlett tests were used. In the two toxicity studies, for each endpoint a separate analysis was performed (details in Figs. 2 and 3, S11). Replicates numbers per treatment were n = 10 for energy reserves and oxidative stress (see 2.8), n = 5-7 for immune cell phagocytosis (see 2.9) and n = 6 (MP toxicity study) or n = 7 (Mixture toxicity study, see 2.7) for reproduction. In the Cu distribution study, every test was performed with 3 jars each (see 2.5, S1.5).

3. Results

3.1 Microplastics ingestion and excretion

MP abundance in the tissues of *L. stagnalis* after an exposure to a mixture of 5, 10 and 45 µm PS spheres over 6-96 h significantly depended on the "particle size" (GLM, df = 2, $p < 0.001$), the "exposure period" (df = 4, $p < 0.001$) and interaction of both factors (df = 8, $p < 0.01$). With regard to MP size, 5 µm spheres were more abundant (range: 59 ± 44 (±SD to 1572 ± 683 individual^{-1}) in *L. stagnalis* compared to 10 µm (range: 61 ± 66 to 328 ± 178 p individual^{-1}) and 45 µm spheres (range: 110 ± 107 to 353 ± 453, Fig. 1a). The snails ingested also 90 µm PS spheres (Fig. S6) but we did not compare the uptake quantitatively due to the lower exposure concentration. Considering the exposure period, MP levels markedly increased during the first hours of exposure (mostly due to enhanced ingestion of 5 µm PS spheres) with a peak at 24 h and a subsequent decrease afterwards.

Similarly, the MP excretion depended both on the factors "particle size" (df = 2, $p < 0.001$) and "depuration period" (df = 5, $p < 0.001$), while no significant interaction of both factors was observed (df = 10, $p > 0.05$). The total particle numbers in *L. stagnalis* decreased most distinctively within the first 12 h of depuration (Fig. 1b, Fig. S7). However, the excretion was not continuous over time as MP levels were lower after 12 h compared to 24 h depuration. After that, the tissue levels decreased exponentially.

Overall, snails excreted smaller MP faster than larger MP (Fig. S7). For instance, after 48 h of depuration, the tissue levels had decreased by 95.7% (5 µm PS spheres), 91.0% (10 µm) and 87.5% (45 µm) compared to tissue levels after 24 h MP exposure without subsequent depuration period (Fig. S7). After 7 d of depuration, the mean tissue levels of all MP types had decreased by more than 99%.

3.2 Microplastics toxicity study

Throughout the MP toxicity study, feces of *L. stagnalis* contained MP which confirms ingestion of the PS fragments by the snails (Fig. S8). Qualitative visual analysis suggested a higher MP ingestion with increasing particle concentrations. Despite the high ingestion of PS fragments, MP exposure did not significantly affect energy reserves (Fig. 2a-c), oxidative stress (Fig. 2d and e) and reproduction (Fig. 2g-i) in *L. stagnalis* (Kruskal-Wallis tests with

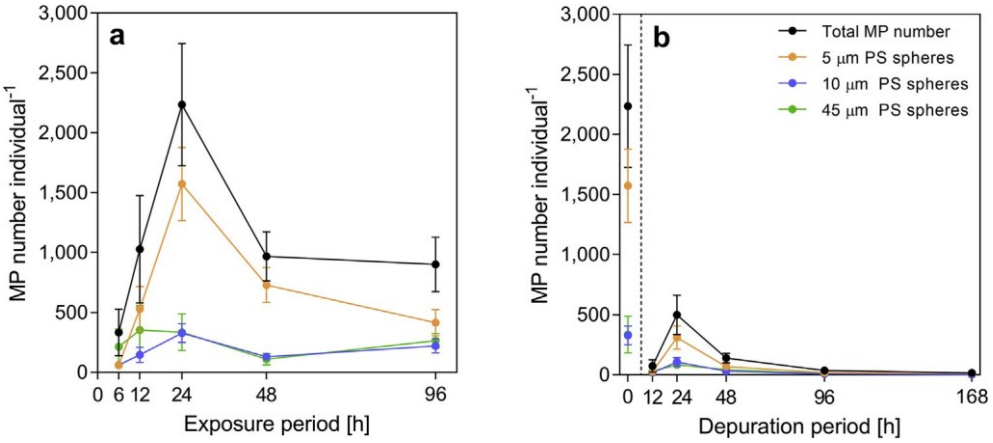

Fig. 1. Ingestion and excretion of microplastics (MP) by *L. stagnalis*. (a) Mean (±standard error) number of MP per individual after 6-96 h of exposure to a mixture of 5, 10, and 45 µm PS spheres (12 p mL^{-1} each, n = 5 per exposure period). (b) Mean (±standard error) number of MP per individual after 24 h of exposure to 5, 10 and 45 µm PS spheres (12 p mL^{-1} each, 0 h = 24 h in a) followed by a 12-168 h depuration period in MP-free medium (n = 5 per depuration period).

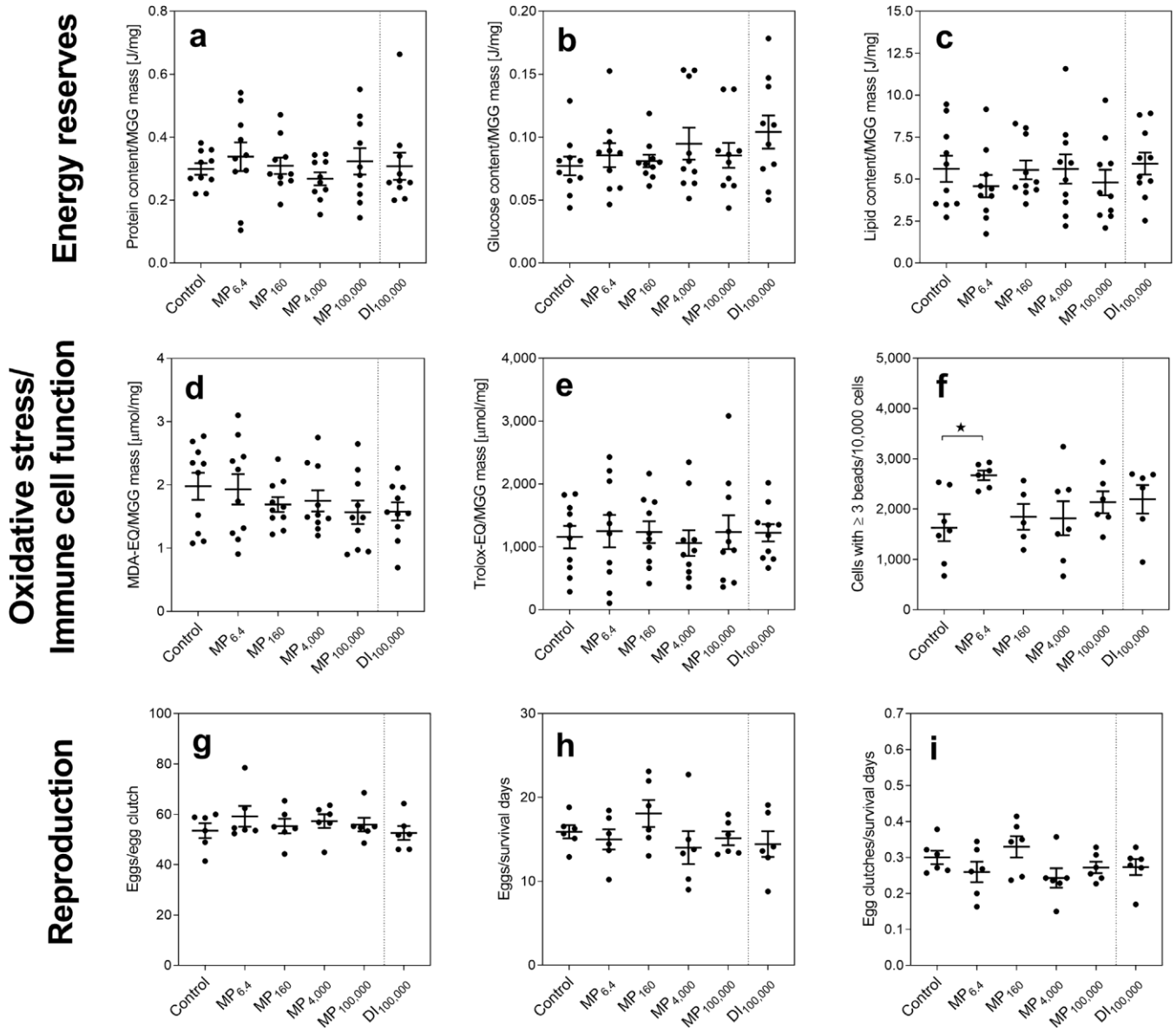

Fig. 2. Effects of irregular PS microplastics (MP, ≤ 63 µm, 6.4-100,000 p mL^{-1}) and diatomite (DI, 100,000 p mL^{-1}) on the energy reserves ((a) protein, (b) glycose and (c) lipid content in the midgut gland (MGG)), oxidative stress ((d) MDA content and (e) remaining antioxidant capacity in the MGG), immune cell phagocytosis ((f) hemocyte phagocytosis activity) and on the reproduction ((g) average clutch size, (h) number of eggs, (i) number of egg clutches) of *L. stagnalis*. (a-e): n = 10, (f): n = 5-7, (g-i): n = 6. Statistics: Comparison: Control vs. all MP-treatments + $MP_{100,000}$ vs. $DI_{100,000}$; a, b, c, g: Kruskal-Wallis test with Dunn's post-test; d, e, f, h, i: one-way ANOVA with Sidak's post-test, ★ = $p < 0.05$.

Dunn's post-test/one-way ANOVA with Sidak's post test, $p > 0.05$). The phagocytic activity of hemocytes increased significantly in snails from the $MP_{6.4}$ treatment compared to control (Fig. 2f, one-way ANOVA with Sidak's post-test, df = 5, $p < 0.05$). No difference was observed in the other exposure groups compared to the control. Importantly, there was a high inter-individual variation for almost all endpoints. A statistical comparison of the treatment groups $MP_{100,000}$ and $DI_{100,000}$ did not reveal significant difference for any endpoint.

3.3 Mixture toxicity study with copper and microplastics

3.3.1 Copper analyses

Background contamination in the ISO medium was 7.4 ± 0.2 µg L^{-1} (±SD) and did not change significantly after 3 d of incubation with and without MP (Fig. S10a, Kruskal-Wallis test with Dunn's post-test, $p > 0.05$). However, Cu concentrations tended to be generally lower after 3 d compared to fresh ISO medium (Fig. S10a). Spiking of the ISO medium increased Cu concentrations by 7.9 µg L^{-1} (nominal: 7.5 µg L^{-1}) to 15.4 ± 0.8 µg L^{-1}. A 3 d exposure decreased mean Cu concentrations significantly by 23.8% compared to the freshly spiked ISO medium (Fig. S10c, one-way ANOVA with Sidak's post-test, df = 4, $p < 0.01$).

An additional incubation with MP or lettuce did not significantly affect the Cu concentrations compared to the spiked medium without MP after 3 d (Fig. S10b: Kruskal-Wallis test with Dunn's post-test, $p > 0.05$; Fig. S10c: one-way ANOVA with Sidak's post-test, df = 4, $p > 0.05$). Nevertheless, Cu concentrations on the lettuce significantly increased after 3 d in the Cu treatments (Fig. S10d, one-way ANOVA with Sidak's post-test, df = 3, $p < 0.05$). Here, Cu

concentrations per lettuce wet weight were highest in the treatments with 1.5 g lettuce and decreased with increasing lettuce mass (Fig. S10d). Accordingly, 1.5, 4.5 and 7.5 g lettuce adsorbed in total 4.3, 4.9 and 4.5 µg Cu, respectively, which was equivalent to 28-32% of the overall copper in the water phase. As we did not quantify Cu concentrations on the MP, it can, however, not be discriminated between Cu that directly adsorbed to the lettuce surface and Cu from Cu-contaminated MP which had aggregated on the lettuce surface.

In the mixture toxicity study, control treatments filled with ISO medium contained a mean total Cu concentration of 9.5 ± 1.4 µg L^{-1} in the freshly prepared exposures throughout each water exchange (0 d). Spiking with copper significantly increased Cu levels (at 0 d) to 17.2 ± 1.3 µg L^{-1} (spiked: 7.4 µg L^{-1}, nominal: 7.5 µg L^{-1}; Kruskal- Wallis test with Dunn's post-test, $p < 0.05$, Fig. S11a). Feeding with lettuce introduced an extra 0.49 µg Cu g^{-1} lettuce (wet weight) into each jar. Cu concentrations in the ISO medium decreased after 3-4 d both in the unspiked (-23%) and spiked medium (-41%). However, even after 3-4 d exposure (directly prior to the next water exchange), Cu concentration was still significantly higher in the Cu treatment compared to the control (Kruskal-Wallis test with Dunn's post-test, $p < 0.05$). A co-exposure with MP did not alter Cu water concentrations compared to the Cu exposure without MP (Fig. S11a).

Cu concentrations in *L. stagnalis* tissues increased in all test groups, including the control, throughout the 28 d exposure compared to pre-experimental tissue concentrations (Fig. S11b). In individuals from the Cu+$MP_{6.4}$ and Cu+MP_{160} groups, tissue concentrations were higher (15.1 and 11.3 µg Cu g^{-1} tissue) compared to individuals from the unspiked control (9.2 µg Cu g^{-1} tissue) and the Cu exposure (10.8 µg Cu g^{-1} tissue). In the Cu+$MP_{4,000}$, Cu+$MP_{100,000}$ and Cu+$DI_{100,000}$ groups, instead, average Cu tissue concentrations were lower than in the control and the Cu exposure (7.0-8.6 µg Cu g^{-1} tissue).

3.3.2 Copper toxicity

In the mixture toxicity experiment, exposure to Cu alone affected the reproduction of *L. stagnalis* significantly by reducing the number of eggs per clutch (Fig. 3f, one-way ANOVA with Sidak's post-test, df = 6, $p < 0.01$) but not the total number of eggs or egg clutches per survival days (Fig. 3g and h, one-way ANOVA with Sidak's post-test, df = 6, $p > 0.05$). Further, Cu exposure did not affect the energy reserves (Fig. 3a-c) or induce oxidative stress (Fig. 3d and e).

3.3.3 Effects of the additional MP exposure

The combined exposure to Cu and $MP_{4,000}$ significantly reduced the number of eggs per egg clutch compared to the control (Fig. 3f, one-way ANOVA with Sidak's post-test, df = 6, $p < 0.05$), while no significant differences compared to control were observed for any of the other combined treatments (Fig. 3f, $p > 0.05$). Further, none of the combined treatments affected reproduction (number of laid eggs or egg clutches per survival days, Fig. 3g and h), energy reserves (Fig. 3a-c) or oxidative stress (Fig. 3d and e) in the MGG. Furthermore, the effects of a co-exposure of Cu+$MP_{100,000}$ and Cu+$DI_{100,000}$ did not differ significantly for any endpoint.

4. Discussion

4.1 Microplastics ingestion and excretion

L. stagnalis is a continuous and primarily herbivorous grazer that sometimes also feeds omnivorously (Jager, 1971; Veldhuijzen, 1974). It has a specialized muscular stomach (gizzard) which grinds up large particles into a digestible size (Carriker, 1946) allowing the ingestion of food particles with a very broad size range. In case of PS spheres, *L. stagnalis* was able to ingest MP in the size range between 5 and 90 µm. The ingested quantities differed significantly both in regard to particle size and exposure period. Here, 5 µm spheres were more abundant in the snails compared to larger MP.

One reason for the size-dependent MP uptake may be a size-selectivity in *L. stagnalis* feeding. Usually, *L. stagnalis* is classified as unselective herbivore because its scraping apparatus, the radula, is not able to selectively scrape specific food items from a mixture (e.g., a biofilm, Groendahl and Fink, 2016). This implies that *L. stagnalis* cannot discriminate between different particle sizes. However, because our results demonstrate a significantly higher uptake of smaller MP, *L. stagnalis* individuals may have still either fed size-selective to a certain extend or smaller particles were more bioavailable to the individuals.

The importance of particle bioavailability on particle ingestion has recently been highlighted by Scherer et al. (2017). They observed a higher uptake of 10 µm and 90 µm compared to 1 µm PS spheres in the freshwater snail *Physa acuta* and discuss that larger MP had settled faster and, therefore, had become more bioavailable for the grazing snails. For *L. stagnalis*, particle sedimentation would have had less impact on overall bioavailability as it can both float freely in the water phase and graze on surfaces (see 2.3). Differences in MP availability due to a heterogenous distribution may still have originated from the formation of hetero-aggregates of small and large PS MP as well as adsorption to the lettuce. Here, 5 µm PS spheres may have stayed longer in the water phase (see 2.3) and, thus, could have better adsorbed to the lettuce, increasing their bioavailability compared to larger particles. Indeed, previous research has shown that 1-2 µm PVC particles frequently adsorb on phytoplankton and form hetero-aggregates (Long et al., 2017; Zhang et al., 2017). However, as we did not analyze the size-specific particle distribution in our exposure vessels, its contribution to the observed size-dependent MP ingestion remains unknown.

Regarding particle excretion, *L. stagnalis* can process and egest particles within 4 h after ingestion (Veldhuijzen, 1974). Egestion of MP was similarly rapid with particle abundance in the individuals decreasing mostly within 12 h. While the slightly higher MP concentrations after 24 h of depuration might be due to a re-uptake of MP-containing feces (coprophagy, Noland and Carriker, 1946; Scheerboom and Van Elk, 1978), particle abundance in *L. stagnalis* individuals dropped to below 5% of the initial level after 96 h for all particle types. This implies that long-term retention of MP particles in *L. stagnalis* over several days is rather limited.

The retention of MP by *L. stagnalis* is comparable to other aquatic invertebrates. The freshwater crustacean *Gammarus fossarum* excretes ingested polyhydroxybutyrate and polymethyl methacrylate particles (32-63 µm) almost completely within 64 h (Straub et al., 2017). In the bivalve *Mytilus galloprovincialis*, 90% of 1, 10 and 90 µm PS spheres pass the digestive tract within 12-84 h, with smaller spheres being excreted faster than larger ones (Kinjo et al., 2019). In goldfish, MP retention was modelled to range between 15 h and 6 d (Grigorakis et al., 2016). The fast egestion of MP and the rather constant MP tissue levels of 10 and 45 µm spheres in the ingestion study suggest that *L. stagnalis* has fed on and excreted MP continuously. In contrast, we observed a marked increase in the abundance of 5 µm spheres in the snails after 24 h. This was not due to a prolonged retention in the digestive tract as *L. stagnalis* excreted smaller MP faster than larger ones. A potential explanation is that a change in feeding activities (Ter Maat et al., 2007) in combination with a heterogenous MP distribution (e.g., adsorption of MP to the lettuce) have caused this phenomenon, but the exact reason remains to be investigated.

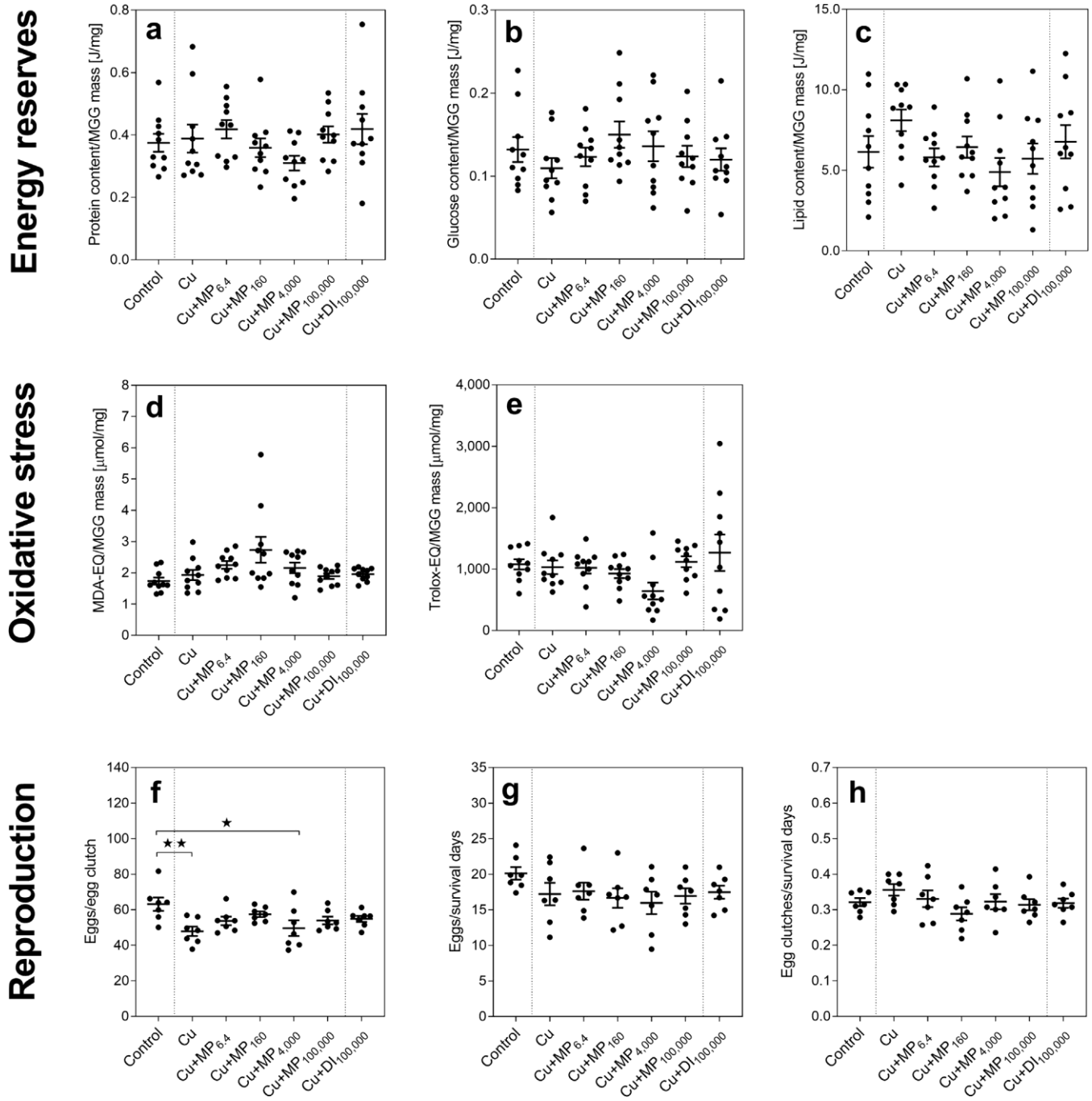

Fig. 3. Effects of copper (Cu, spiked as Cu^{2+}, 7.5 µg L^{-1}) alone or in combination with microplastics (irregular PS MP, ≤ 63 µm, 6.4-100,000 p mL^{-1}) or diatomite (DI, 100,000 p mL^{-1}) on the energy reserves ((a) protein, (b) glycose and (c) lipid content in the midgut gland (MGG)), oxidative stress ((d) MDA content and (e) remaining antioxidant capacity in the MGG) and on the reproduction ((f) average clutch size, (g) number of eggs per survival days, (h) number of egg clutches per survival days) of *L. stagnalis*. Replicate numbers: a-e: n = 10, f-h: n = 7. Statistics: Comparison: Control and Cu vs. all Cu+MP treatments + $Cu+MP_{100,000}$ vs. $Cu+DI_{100,000}$; a, d, e: Kruskal-Wallis test with Dunn's post-test; b, c, f-h: one-way ANOVA with Sidak's post-test, ★ = $p < 0.05$, ★★ = $p < 0.01$.

4.2 Microplastics toxicity

An exposure to PS fragments (≤ 63 µm) did not induce significant effects regarding survival, reproduction, energy reserves or oxidative stress in *L. stagnalis*, neither at environmentally relevant (6.4 p mL^{-1}) nor at very high MP concentrations (100,000 p mL^{-1}). These results contradict data by Lo and Chan (2018) who reported strong MP effects on the veliger larvae of the slipper limpet *C. onyx* exposed to 2-5 µm PS spheres (at 60,000 and 140,000 p mL^{-1}). MP also affected *C. onyx* juveniles after larval settling. These MP effects may be due to the different feeding behavior of the *C. onyx* larvae compared to adult *L. stagnalis*. While the latter are grazers, *C. onyx* veliger larvae are planktotrophic filter feeders (Chiu et al., 2007; Li and Chiu, 2013) which rely on high algae abundance throughout the first days of growth. Starvation during early development reduces larval growth with long-term effects (Chiu et al., 2008).

In addition, varying effect levels may also be related to the different life stages of the snails (juvenile *C. onyx* vs. adult *L. stagnalis*). However, a higher sensitivity of juveniles compared to adults was not observed in *P. antipodarum*, a grazing freshwater snail like *L. stagnalis* (Imhof and Laforsch, 2016). This may indicate that, in some species, the feeding type might be more relevant for MP toxicity than the life stage. Yet, this assumption deserves further investigation in future MP toxicity studies with gastropods tested under comparable exposure conditions.

We observed some effect of MP on immune cell phagocytosis. Here, exposure to 6.4 p mL^{-1} MP significantly increased the phagocytic activity of snail hemocytes. This effect was, however, not observable at higher MP concentrations pointing towards a MP-unrelated cause.

In conclusion, MP exposure had no adverse effects on *L. stagnalis*. As nanoplastics were present in the PS powder we used, this is probably also true for the nanofraction that was part of the polydisperse MP suspension. The lack of effects is especially surprising as the qualitative analysis of MP in the feces suggests high ingestion rates. For example, feces of snails exposed to 100,000 p mL^{-1} consisted almost exclusively of MP. Because the ingestion of high quantities of non-digestible particles will reduce food and energy uptake (Gardon et al., 2018), *L. stagnalis* must have developed mechanisms to compensate changes in food quality and quantity. A previous starvation experiments with freshwater gastropods has shown that gastropods increase their ingestion rate with rising proportion of non-digestible material in the food source (Calow, 1975). Further, *L. stagnalis* can also increase its digestion efficiency by enlarging the gut space, enhancing MGG activity and increasing MGG digestive juice secretion (Calow, 1975). In addition, the presence of MP in the stomach may have facilitated the mechanical gridding of ingested food, enhancing assimilation efficiency and, thus, compensating effects due to reduced food uptake. Increasing food scavenging and/or a more efficient food digestion could have prevented adverse effects from reduced feeding success.

MP effects may have also been limited due to the highly adapted digestive system. As deposit feeder, *L. stagnalis* has a highly specialized selection system in the pylorus (stomach) which separates digestible and non-digestible particles. Digestible particles are transported to the MGG, while non-digestible particles are transferred to the intestine for direct excretion. Only particles <0.4 µm are transported into the digestive gland (Veldhuijzen, 1974). We, therefore, assume that MP >0.4 µm were directly transported from the pylorus into the intestine for egestion without reaching the digestive gland limiting MP-induced effects on metabolism. While smaller MP present in the suspension may have passed into the MGG, they did not induce any effects on the examined endpoints.

Adaptations of deposit feeders such as *L. stagnalis* towards the ingestion of high loads of non-digestible particles may, therefore, be a key mechanism limiting MP toxicity. This assumption is also supported by the fact that an exposure to high concentration of natural DI particles did not induce adverse effects. *L. stagnalis*, therefore, seems to react similarly to natural and plastic particles. Accordingly, grazing freshwater gastropods might have a limited sensitivity to MP exposure due to their evolutionary adaption to a particle-rich environment.

This conclusion must, however, be considered in the light of a potential stress tolerance of the *L. stagnalis* culture used in this study (see 4.3.1). Vinebrooke et al. (2004) hypothesized that a tolerance towards one stressor is positively correlated to a co-tolerance towards additional stressors. For instance, in the freshwater snails *Biomphalaria glabrata*, a parental exposure to a predator threat increased the tolerance to cadmium in its offspring (Plautz et al., 2013). As the snails in our laboratory culture were inadvertently exposed to low levels of copper in the ISO medium over several generations, they may have developed a certain stress tolerance towards copper as well as MP. While this could be investigated using snails cultured under especially pristine conditions, other stressors (including metals) are abundant in natural environments. Thus, a hypothetical pre-adaptation to stress might be common in nature. In summary and considering MP concentrations in freshwater systems of up to 0.5 p mL^{-1} (Lahens et al., 2018), the current levels of MP do not seem to pose an overt risk to gastropods. MP toxicity may, however, increase in combination with other environmental stressors. In a second experiment, we therefore co-exposed *L. stagnalis* to a mixture of MP and Cu.

4.3 Mixture toxicity of copper and microplastics

4.3.1 Copper analysis

The unspiked ISO medium contained a background concentration of up to 9.8 µg L^{-1} Cu. Such high concentrations are usually toxic to *L. stagnalis*, especially for juveniles (Atli and Grosell, 2016; Brix et al., 2011; Das and Khangarot, 2011; Ng et al., 2011). The copper contamination in our experiments originated from the in-house distilled water supply. As this contamination probably had already existed for several months, multiple *L. stagnalis* generations in our culture had been raised in this water. We, thus, believe that the specimens used in our study have adapted to elevated Cu levels. Both in the unspiked and Cu spiked medium, copper concentrations decreased in the water phase throughout the 3-4 d exposure suggesting some adsorption of copper on the vessels. A further addition of MP did not cause distinct changes in Cu concentrations in the water phase although we cannot exclude some adsorption of copper to MP (as shown by Holmes et al. (2012) on polyethylene pellets). Further, we observed Cu adsorption to the lettuce. Elevated Cu concentrations on the lettuce may originate from directly adsorbed Cu or (indirectly) from Cu-contaminated MP attached to the lettuce surface. Besides dermal uptake of dissolved Cu through passive diffusion at cell membranes (Grosell et al., 2002), dietary uptake is estimated to be the second relevant exposure pathway for freshwater gastropods (Hoang et al., 2008). Cu adsorption to the lettuce (both directly and indirectly) suggests that, besides dermal uptake, dietary exposure was a second relevant Cu exposure pathway for *L. stagnalis*.

4.3.2 Copper toxicity

The exposure to 7.5 µg L^{-1} Cu significantly reduced the number of eggs per clutch, while all other reproductive endpoints (eggs and clutches survival d^{-1}) remained unaffected. These effects are partially in accordance with a previous study in which exposure to 5.6 and 10 mg L^{-1} Cu significantly reduced the total egg number and the number of eggs per egg clutch, respectively (Das and Khangarot, 2011).

Cu exposure did not induce oxidative stress in *L. stagnalis*. We expected Cu to activate the antioxidant system of *L. stagnalis* as Cu toxicity is mostly caused by the generation of reactive oxygen species (Gaetke and Chow, 2003). High levels of reactive oxygen species usually activate the antioxidant system of mollusks to prevent tissue damage (Manduzio et al., 2005). For instance, exposure of *L. stagnalis* to 2 mg L^{-1} Cu activated the antioxidant system in the hepatopancreas (Atli and Grosell, 2016). However, we did not observe a significant oxidative stress induction, probably because of the previous Cu contamination and corresponding Cu tolerance in the *L. stagnalis* culture (see 4.3.1), such as, the expression of metallothioneins (metal-ion binding proteins) as well as

the formation of metal-rich granulae (Ng et al., 2011). These mechanisms may have limited oxidative stress in *L. stagnalis* tissues. However, these detoxification mechanisms are energetically costly (Moolman et al., 2007). Accordingly, *L. stagnalis* might have allocated less energy to reproduction while maintaining the energy storages (glycogen, lipids) in the MGG.

4.3.3 Effects of the additional MP exposure

In regard to reproduction, the number of eggs per clutch was significantly reduced in the Cu+$MP_{4,000}$ treatment but not the Cu+ $MP_{100,000}$ treatment compared to the control. The energy reserves and oxidative stress were not significantly affected by a treatment with MP/DI and Cu compared to the control. Further, also no difference between the mixed exposure treatments and the Cu treatment were observed for any of the tested endpoints. Accordingly, an additional exposure to MP or natural particles did not exacerbate the limited toxicity Cu induced alone. MP was, therefore, neither a relevant single stressor nor a relevant stressor in combination with Cu for *L. stagnalis*. Again, this conclusion especially refers to our snail population that may be stress tolerant (see 4.2).

So far, knowledge on mixture toxicity of MP with additional chemical stressors is very limited for gastropods. For *L. stagnalis*, Horton et al. (2020) observed that in a 96 h mixed exposure to polybrominated diphenyl ethers (PBDE, 94-3000 ng g^{-1} sediment) and MP (1% nylon w/w in sediments), snails in PBDE-only treatments lost significantly more weight compared to treatments with PBDE and MP. In contrast, a mixed exposure of methamphetamine (0.1-50 mg L^{-1}) and MP (0.7 µm PS, 20 mg L^{-1}) enhanced mortality and filtration rates compared to a methamphetamine-only exposure of the freshwater gastropod *C. cathayensis* (Qu et al., 2020).

With regards to bivalves, previous studies exposed either the freshwater species *Corbicula fluminea* or the marine mussel *Mytilus edulis* to MP in combination with florfenicol, fluoranthene or mercury chloride (Guilhermino et al., 2018; Magara et al., 2018; Oliveira et al., 2018; Paul-Pont et al., 2016). Similar to the gastropods, these studies reported highly divergent results and suggested either no interaction, antagonistic or synergistic effects of MP and a chemical.

A key factor affecting the interaction is the sorption of the chemical to MP which mediates the uptake, but also elimination of the chemical by mollusks. The partition of a chemical between the solid (MP) and the liquid phase, however, depends on the physicochemical characteristics of both the polymer particles and the chemicals. Potential joint effects of MP and chemicals on mollusks in the environment will, therefore, depend on the abundance, type and interaction of MP and chemicals in the surrounding habitat complicating general predictions on mixture toxicity effects in the environment.

5. Conclusion

This study investigated the ingestion and toxicity of PS MP in the freshwater gastropod *L. stagnalis*. MP ingestion and excretion depended both on the particle size as well as the duration of the exposure and depuration period. Here, the snails ingested more 5 µm compared to 10 and 45 µm PS spheres and highest tissue levels were observed after 24 h of exposure. Further, *L. stagnalis* excreted MP within few days with <1% of the original particle abundance remaining after 7 d of depuration.

Despite the high MP ingestion, a 28 d exposure to PS fragments (≤ 63 µm) at concentrations up to 100,000 p mL^{-1} did not affect survival, reproduction, energy reserves and oxidative stress of *L. stagnalis*. This suggests that current MP concentrations in freshwater ecosystems do not represent an overt risk for *L. stagnalis* populations when considering MP as sole stressor. The same was true when we exposed snails to a mixture of Cu and MP. Here, MP did not exacerbate the reproductive effects induced by Cu alone.

CrediT author statement

Conceptualization and Methodology: AW and MW, Investigation: AW, MvR and AV (all experiments); BM, MvA and EF (copper analysis), Formal analysis: AW, MvR and AV, Visualization: AW, Writing - Original Draft: AW, MW, BM, MvA, Writing - Review & Editing: all authors, Supervision and Funding acquisition: MW.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2020.128040.

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Ingestion and toxicity of microplastics in the freshwater gastropod *Lymnaea stagnalis*: No microplastic-induced effects alone or in combination with copper

Supplementary data

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S1 Materials and methods

S1.1 Particle concentration and size distribution

S1.1.1 Numerical particle concentrations in the stock suspensions for the ingestion and excretion study

The numerical particle concentrations in the microplastic (MP) stock suspensions were determined by CoulterCounter (Beckman Coulter, Multisizer™ 3, Krefeld, Germany). Due to high particle concentrations in the stock suspensions, we pre-diluted the stock suspensions in ultrapure water. For analysis of the 5, 10 and 45 µm PS sphere stock suspension, we further mixed 5–10 µL of each pre-diluted stock suspensions with 100–150 mL of electrolytic solution (0.9% NaCl solution, <0.2 µm sterile-filtered). The resulting suspension was constantly stirred and directly measured with a 100 µm capillary (Beckman Coulter, Krefeld, Germany, detection range: 2-60 µm, electric current: -1,600 µA, gain: 2, analytical volume: 0.5-1 mL, n = 2). Particle number concentration in the 90 µm stock suspension were determined likewise, but with a 400 µm capillary (Beckman Coulter, Krefeld, Germany, detection range: 8-240 µm, electric current: -1,600 µA, gain: 2, average analytical volume: 7.7 mL, n = 2).

S1.1.2 Particle number concentration and size distribution in the PS powder for the toxicity study

Details on the methodology for particle number concentration and size distribution analysis for the PS powder used in the toxicity study are included in the Supplementary data (chapter S1.3) by Weber et al. (2020).

Results on particle size distributions from Coulter Counter measurements were averaged and fitted with GraphPad-Prism Software (Version 7.04, San Diego, CA). Relative particle size distributions were approximated with a “One phase decay” fit, while for cumulative particle size distributions we used a “One phase association” (MP particle distribution) and a “Cumulative Gaussian-Percentage” fit (DI particle distribution). From cumulative particle size distributions, we determined the maximum size which in average 50%, 75% 90%, 95% and 99% of the particles obtained.

Results on particle abundance as well as on particle size distribution in the MP and diatomite (DI) powder used in this study are included in chapter S2.1.

S1.2 Scanning electron microscopy of microplastics and diatomite

Shape and size of particles in the MP and DI powder were qualitatively analysed with a scanning electron microscope (SEM, Hitachi, S4500, Krefeld, Germany). For examination, particles were fixed on aluminium discs with coal glue and coated with a gold monolayer (Agar Scientific, Sputter Coater, Stansted, United Kingdom). Resulting SEM images are included in chapter S2.2.

S1.3 Test design of the microplastics toxicity study

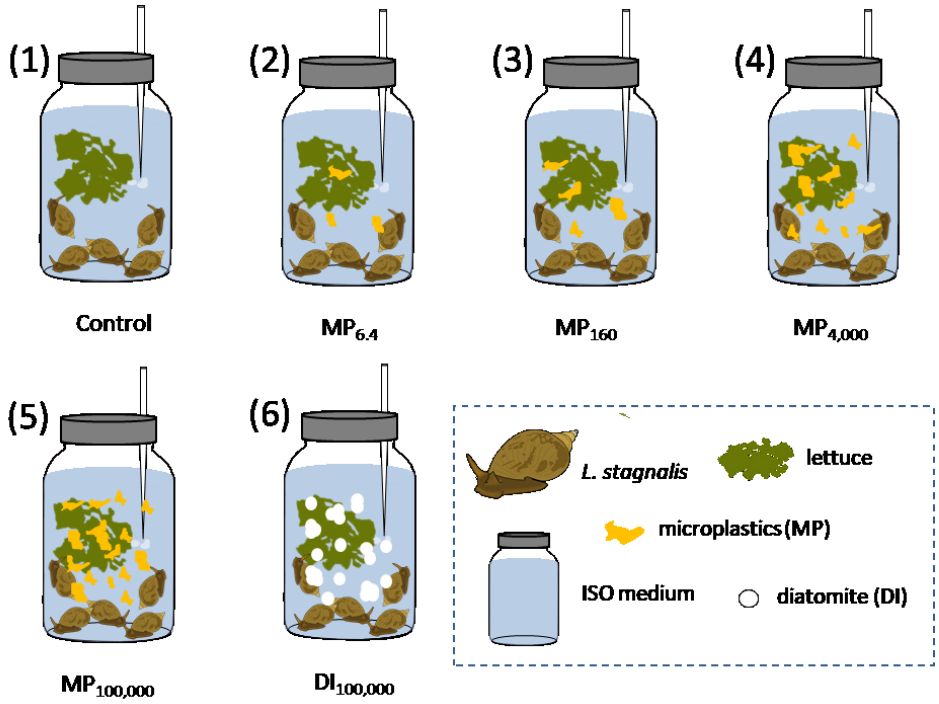

Fig. S1: Study design of the microplastics toxicity study. Subscript numbers indicate the particle number concentrations (particles mL^{-1} (p mL^{-1})). Number of jars per treatment: n=6.

S1.4 Test design of the mixture toxicity study

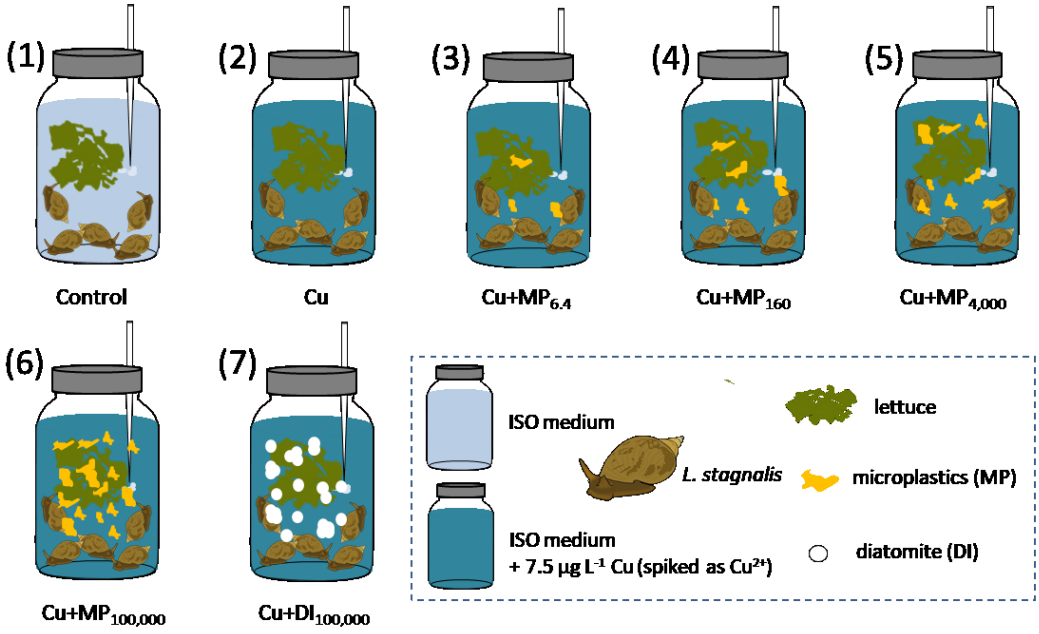

Fig. S2: Test design of the mixture toxicity study with copper and microplastics. Subscript numbers indicate the particle number concentrations (p mL^{-1}). Number of jars per treatment: n=7.

S1.5 Copper distribution analysis

For copper (Cu) distribution analysis (Fig. S3), we established 15 tests (with 3 jars each) using 0 or 7.5 µg L^{-1} Cu (spiked as Cu^{2+}) and varying amounts of MP/DI (6.4–100,000 p mL^{-1}) or lettuce (1.5, 4.5, 7.5 g).

Test 1 examined Cu contamination in the ISO medium prior and after a 3 day (d) incubation of the medium in aerated 1 L glass jars (to address a potential leaching of Cu). Tests 2–6 evaluated a possible Cu contamination originating from the MP and DI particles (samples were taken after a 3 d exposure; Tests 2–6 were prepared from the same ISO medium as Test 1 and therefore we did not take additional samples from Tests 2–6 prior to the experiment).

In Test 7, we analyzed the Cu concentration directly after spiking the ISO medium (we used the same medium as for Tests 1–6) with 7.5 µg L^{-1} Cu (spiked as Cu^{2+}) as well as after 3 d incubation in the 1 L glass jars. Tests 8–12 address the effects of MP or lettuce on Cu water concentrations in spiked ISO medium over 3 d (samples were taken after 3 d; Tests 8–12 were prepared from the same ISO medium and spiked with the same Cu stock solution as Tests 7 and therefore we did not take additional samples from Tests 8–12 prior to the experiment). Water samples were sterile filtered, preserved, stored and analyzed as described in chapters 2.5 and S1.6.

In addition to the Cu concentration in the water phase, we determined Cu concentrations adsorbed to the lettuce (Tests 13–15). For Cu analysis, we wet-weighed the lettuce from the Tests 13–15 as well as a further non-exposed sample of the same lettuce, freeze-dried it and determined the dry weight, before performing Cu analysis (see chapter S1.6).

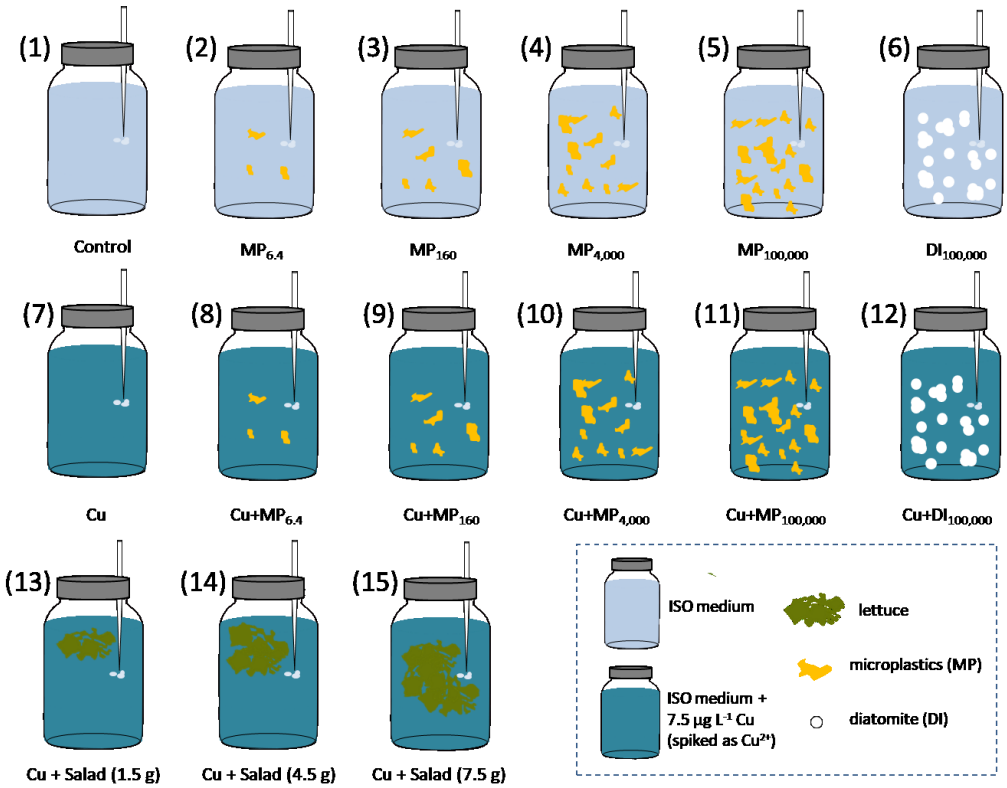

Fig. S3: Test design of the copper distribution study with 15 different tests. Subscript numbers indicate the particle number concentrations (p mL^{-1}). Number of replicate jars for each of the tests (1) to (15): n=3.

S1.6 Copper analysis in the water, tissue and lettuce samples

The snail and lettuce samples were digested using a MLS turboWAVE system (MLS GmbH, Leutkirch im Allgäu, Germany). To do so, approximately 50 mg of the freeze-dried and milled sample were weighted into a PTFE beaker. Afterwards, 1 mL of nitric acid (65%, Emsure, Merck KGaA, Darmstadt, Germany) which had previously been purified via a subboiling procedure (70 °C, DST-4000, Savillex, Minnesota, USA), was added and the beaker was microwaved (heating program: 2.5 min 80 °C (ramp), 8.0 min 160 °C (ramp), 4.0 min 220 °C (ramp), 9.0 min 220 °C (hold)). The resulting solution was diluted to 50 mL in DigiTubes (SCP Science, Quebec, Canada, 1.3% (v/v) subboiled HNO_3) by adding purified water (18.2 MΩ, Satorius AG, Göttingen, Germany).

In order to analyze the water, tissue and lettuce samples, ICP-QQQ-MS (8800 Triple Quadrupole ICP-MS, Agilent Technologies, Inc., Japan) was applied. The KED modus was set to identify the concentration of copper and the isotope ^{63}Cu was detected (plasma parameters: power: 1550 W, sampling depth: 9 mm, carrier gas: 0.82 L min^{-1}, makeup gas: 0.24 L min^{-1}; CRC parameters: helium flow: 5 mL min^{-1}, oct. bias: -18 V, oct. RF 160 V, energy dis.: 3 V, the other lenses were optimized daily). The internal correction was ensured by using a 50 µg L^{-1} rhodium solution, which was added on-line at an ISTD mixing ratio of 1:10. As reference material for the experiments, we used standard mussel tissue (ERMCE278K (elements), Merck KGaA, Darmstadt, Germany) for the snails as well as standard white cabbage (BCR679 (trace elements), Merck KGaA, Darmstadt, Germany). Both reference materials were processed as described for the samples. The ICP-MS analyses were verified in means of the reference materials SPS-SW1 (Spectrapure Standards, Oslo, Norway) and 1640a (NIST, Maryland, USA).

S1.7 Preparation of midgut gland homogenates

Midgut glands (MGG) were mixed with 600 µL of potassium-phosphate-buffer (PPB; 10 mM, pH 7.4) and homogenated with two stainless steel balls (ø 3 mm) in a swing mill for 20 min (10x2 min) at 30 Hz. The tissue samples as well as its homogenates were constantly cooled between the processing steps to avoid degradation.

For the glycogen and lipid assay, 150 µL of MGG homogenate were mixed with 50 µL 2% (m/v) Na_2SO_4 solution (Sigma-Aldrich, Munich, Germany). 25 µL of this mixture were further diluted with 6.25 µL of 2% Na_2SO_4 solution and 18.75 µL of dest. H_2O to obtain the required dilution for the protein assay.

For the thiobarbituric reactive substances assay (TBARS), 100 µL of each tissue homogenate were used directly in the assay. The dilution for the oxygen radical absorbance capacity assay (ORAC) was produced by mixing 10 µL of the TBARS dilution with 90 µL PPB.

S1.8 Hemocyte phagocytosis activity

Hemocyte phagocytic activity was determined according to Weber et al. (2020, Supplementary data, chapter S.1.7) with following modifications:

For the analysis, we extracted 400–500 µL hemolymph from five to seven gastropods per treatment. Hemolymph samples were diluted with *L. stagnalis* serum to a total volume of 600 µL and a concentration of 600,000 cells mL^{-1}. 1 µm PS spheres (Fluoresbrite® YG microspheres, PolyScience, Hirschberg an der Bergstraße, Germany, excitation: 441 nm, emission: 486 nm) suspended in

L. stagnalis serum were added at a ratio of 25 spheres per hemocyte cell (9 µL of a 10^9 spheres mL^{-1} stock solution) to the hemolymph. Each exposure sample was gently vortexed and directly split in two subsamples with 300 µL each, one being incubated at room temperature and the other one on ice.

S2 Results

S2.1 Particle size distribution in the PS powder for the toxicity study

The MP and the DI powder contained 456,704 p mg^{-1} and 4,633,920 p mg^{-1}, respectively. Fig. S4 illustrates the size distributions of particles in the MP and DI powder (2–60 µm). The insets in Fig. S4 further summarize results from the cumulative particle size distributions regarding the maximum size of 50, 75, 90, 95 and 99% of all particles.

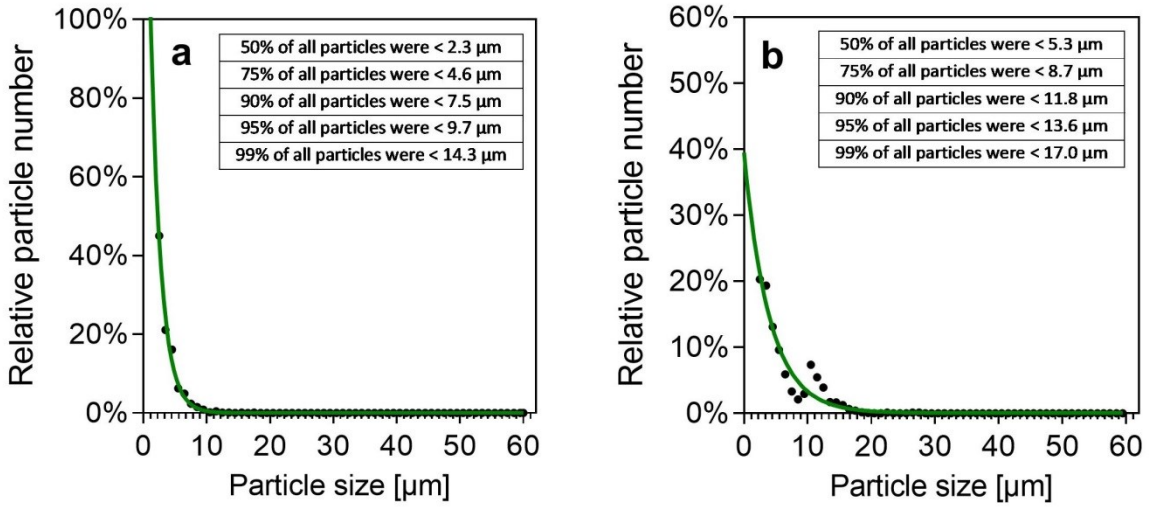

Fig. S4: Relative size distribution of (a) microplastics (MP) and (b) diatomite (DI) particles determined in three (DI) or four (MP) measurements and fitted with a One phase decay model using GraphPad Prism. The insets summarize results from the cumulative particle distributions.

S2.2 Scanning electron microscopy of the microplastics and diatomite

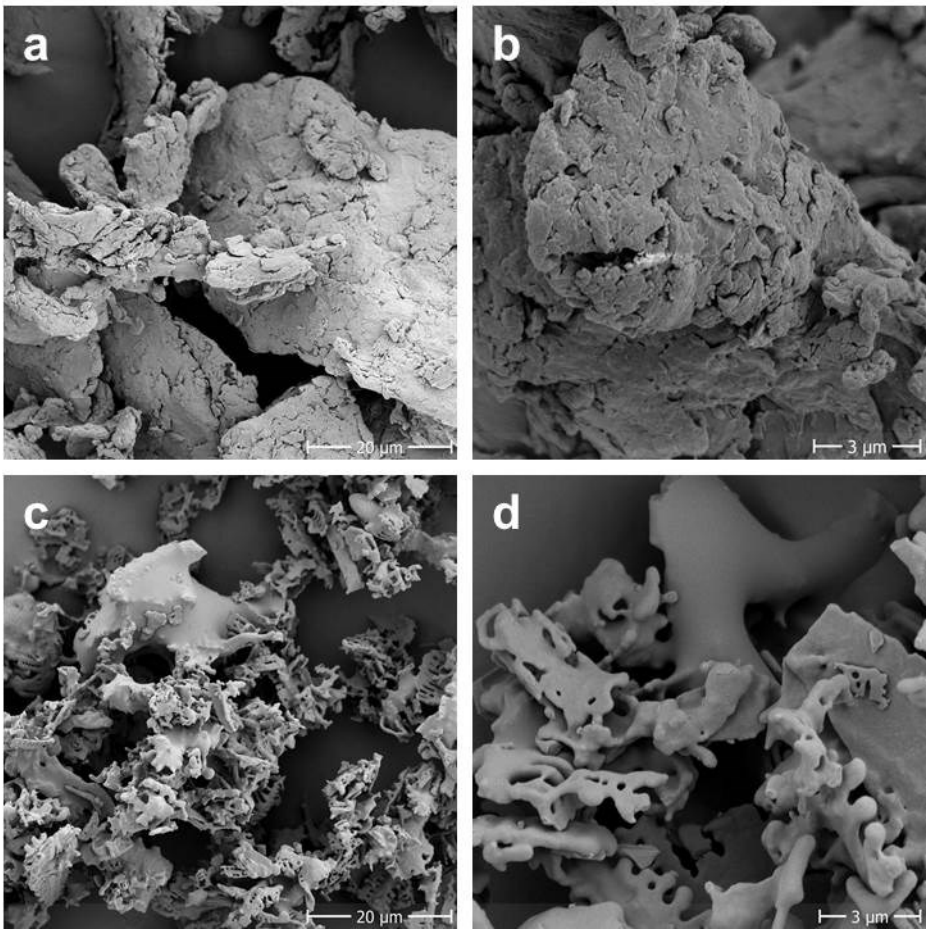

Fig. S5: Scanning electron microscopy images of the (a,b) microplastic and (c,d) diatomite particles used in the toxicity studies. Magnification: 1,500× (a,c) and 7,000× (b,d).

S2.3 Microplastic ingestion and excretion

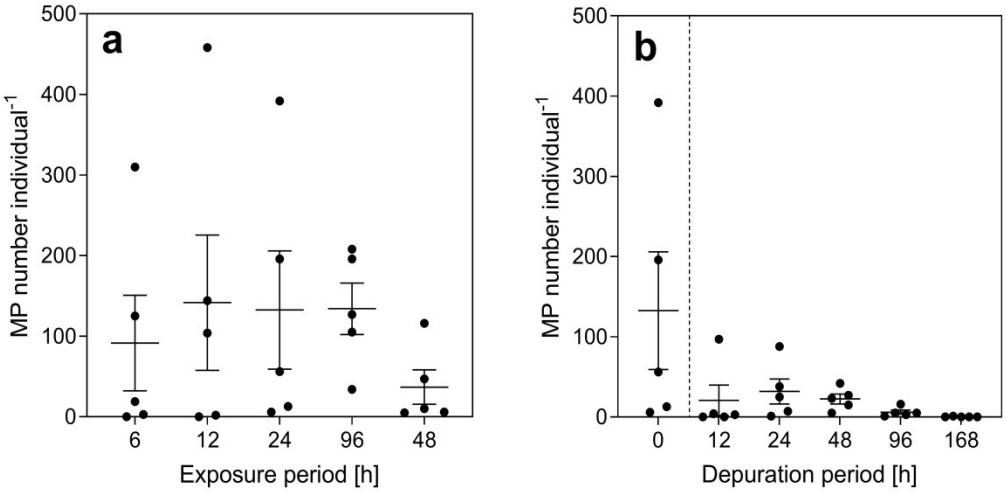

Fig. S6: Ingestion and excretion of 90 µm PS spheres (MP) by *L. stagnalis*. (a) Mean (± standard error) number of spheres per individual after 6–96 h exposure (2 p mL^{-1}, n=5 per exposure period). (b) Mean (± standard error) number of spheres per individual after 24 h of exposure (2 p mL^{-1}, 0 h = 24 h in a) followed by a 12–168 h depuration period in MP-free medium (n=5 per depuration period).

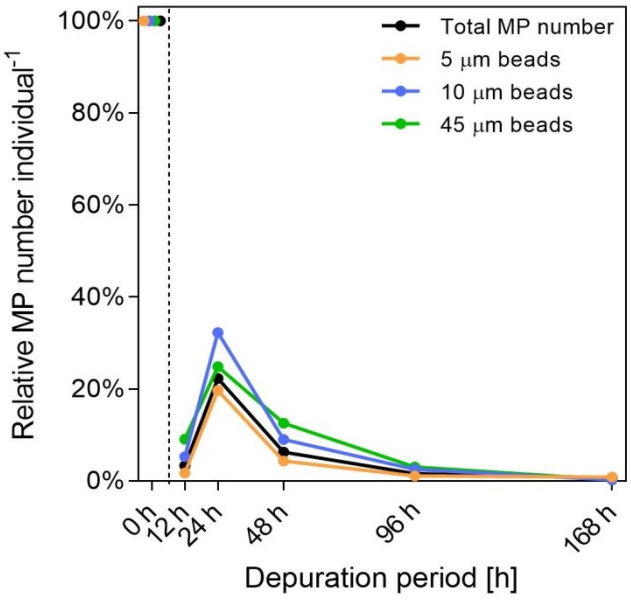

Fig. S7: Mean relative tissue levels of MP in *L. stagnalis* after a 24 h exposure to 5, 10 and 45 µm PS spheres (t = 0) followed by a 12–168 h depuration period in MP-free medium. Relative levels were determined by comparing the number of MP for each particle type after the various depuration periods with the respective number after 24 h exposure without depuration (0 h depuration period, respective data was derived from the ingestion study, see Fig. 1a). n=5 per depuration period.

S2.4 Test validity

The MP toxicity study was valid according to the OECD criteria with a mortality rate of 13.3% and a reproduction of 7.83 egg clutches individual^{-1} in the control (over 28 d exposure) as well as an oxygen content of > 6 mg L^{-1} and pH of 6.5–8.5 in all jars. However, the average water temperature was 18.8 ± 0.4 °C compared to the 20 ± 1 °C foreseen by OECD due to technical difficulties with the air conditioning. In few jars, water conductivity exceeded the suggested maximum of 800 µS cm^{-1}, but average conductivity in the six treatments was still within the reference frame. Water hardness ($CaCO_3$) was on average 265 ± 15 mg L^{-1} and, thus, slightly higher than suggested (250 mg L^{-1}). Detailed results on temperature and water parameters are included in Tab. S1.

In the mixture toxicity study, OECD (2016) validity criteria for mortality (17.1% in the control) and reproduction (8.06 egg clutches individual^{-1} in the control throughout 28 d) were fulfilled. Further, in all jars a constant water temperature of 20 ± 1 °C and a required conductivity of 600 ± 200 µS cm^{-1} were maintained at all times. In very few jars, oxygen content and pH fell below the required minimum (O_2: > 6 mg L^{-1}; pH: 6.5), but average results for the six treatment groups were within the required limits. Similar to the toxicity study, the average water hardness (252 ± 15 mg L^{-1}) was slightly higher than suggested (250 mg L^{-1}, detailed results in Tab. S1).

Tab. S1: Mean water parameter measurements (± standard deviation) from each treatment group in the microplastics and mixture toxicity study.

		Temperature [°C]	Oxygen content [mg L^{-1}]	Conductivity [µS cm^{-1}]	pH	Water hardness [mg L^{-1}]
Microplastics toxicity study	**Control**	**18.78 ± 0.35**	8.24 ± 0.34	735.6 ± 109.0	7.19 ± 0.33	264.4 ± 20.3
	$MP_{6.4}$	**19.08 ± 0.33**	7.74 ± 0.59	706.5 ± 112.3	7.12 ± 0.19	262.5 ± 16.7
	MP_{160}	**18.89 ± 0.39**	8.15 ± 0.36	730.4 ± 118.8	7.18 ± 0.23	264.4 ± 14.5
	$MP_{4,000}$	**18.81 ± 0.53**	7.95 ± 0.74	729.1 ± 110.1	7.24 ± 0.32	268.1 ± 15.1
	$MP_{100,000}$	**18.61 ± 0.44**	8.19 ± 0.23	733.8 ± 111.4	7.23 ± 0.25	267.5 ± 19.1
	$DI_{100,000}$	**18.83 ± 0.24**	7.86 ± 0.57	723.6 ± 111.0	7.29 ± 0.19	264.4 ± 13.5
Mixture toxicity study	**Control**	**19.96 ± 0.36**	7.40 ± 0.83	506.5 ± 30.0	7.29 ± 0.35	246.9 ± 14.9
	Cu	**19.88 ± 0.38**	7.32 ± 0.73	525.7 ± 21.4	7.18 ± 0.43	256.3 ± 11.9
	Cu+$MP_{6.4}$	**19.82 ± 0.25**	7.46 ± 0.69	512.1 ± 26.4	7.18 ± 0.44	250.6 ± 16.1
	Cu+MP_{160}	**19.69 ± 0.17**	6.85 ± 0.88	514.1 ± 29.0	7.14 ± 0.42	254.4 ± 15.0
	Cu+$MP_{4,000}$	**19.75 ± 0.13**	7.12 ± 0.75	508.9 ± 24.3	7.11 ± 0.40	245.6 ± 14.7
	Cu+$MP_{100,000}$	**19.78 ± 0.25**	6.85 ± 1.15	510.1 ± 26.7	7.09 ± 0.33	248.8 ± 13.6
	Cu+$DI_{100,000}$	**19.85 ± 0.28**	6.91 ± 1.00	519.3 ± 22.6	7.11 ± 0.34	250.0 ± 15.8

S2.5 Qualitative analysis of MP in L. stagnalis *feces*

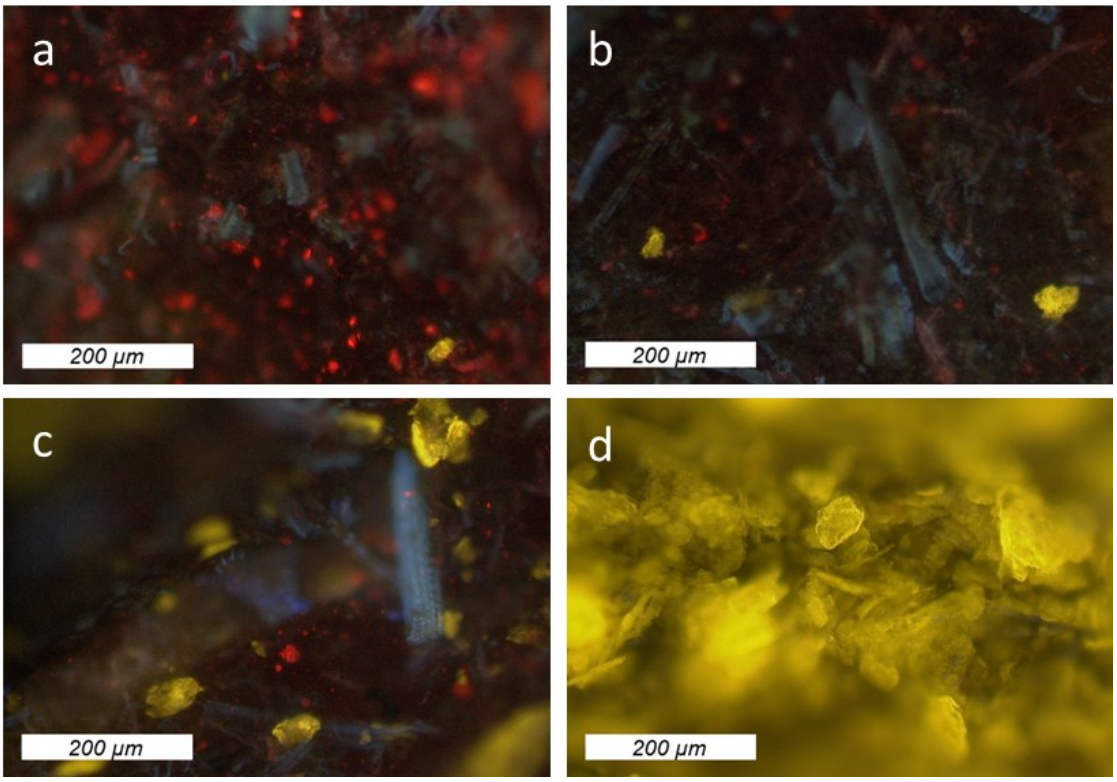

Fig. S8: Microplastics (yellow) in feces of *L. stagnalis* exposed to (a) 6.4, (b) 160, (c) 4,000 and (d) 100,000 p mL^{-1}. No yellow fluorescent particles were observed in feces from control animals. The red fluorescence may originate from chlorophyll in non-digested lettuce.

S2.6 Hemocyte phagocytosis activity

Results from FACS analysis (performed and analyzed as described by Weber et al. (2020)) are summarized in Fig. S9. Dead cells were excluded from the main population by propidium iodide staining (PI, Fig. S9a–b, Gate P2: living hemocytes in the main cell population; Gate P5: dead hemocytes in the main cell population). Gate P6 (Fig. S9c) represents the subpopulation of living hemocytes with ≥ 3 spheres from Gate P2. Only FACS analyses with ≥ 5,000 living cell counts (P2) were used. We extrapolated data from all FACS measurements to 10,000 living cells to allow data comparability between the different samples. Results from the sample exposed at room temperature were corrected for the number of hemocytes with ≥ 3 spheres from sample exposed on ice to account for particles which were adsorbed on the hemocytes cell surface, but not phagocytized. Based on the corrected data, we determined the fraction of living hemocytes with ≥ 3 spheres compared to all analyzed living hemocytes.

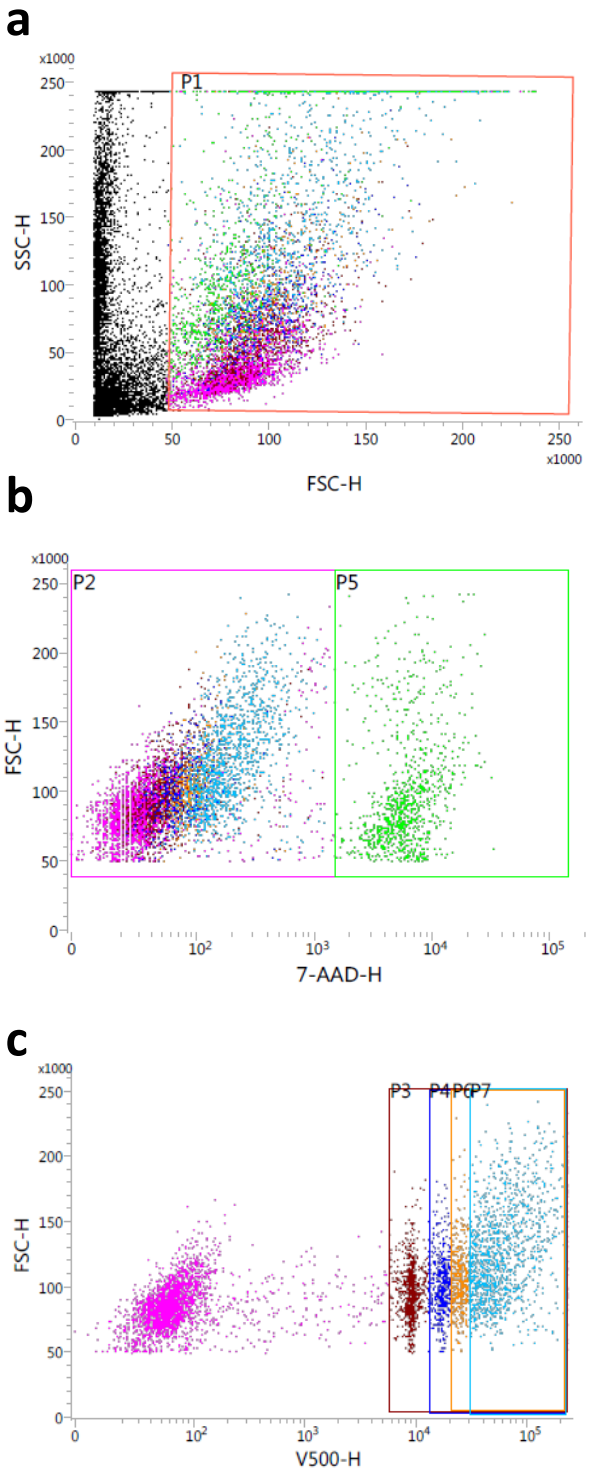

Fig. S9: Characterization of *L. stagnalis* hemocytes exposed to 1 µm PS spheres using a BD FACSVerse. (a) Size (FSC) vs. granularity (SSC) of hemocytes (488 nm laser, SSC filter: 481-496 nm), (b) Gating of living (gate P2) and dead (gate P5) hemocytes due to propidium iodide fluorescence (488 nm laser, 7-AAD (filter: 673-727, mirror: 665 LP)), (c) Gating of living hemocytes (from Gate P2) with ≥ 1 (P3), ≥ 2 (P4), ≥ 3 (P6) or ≥ 4 spheres (P7) (488 nm laser, Alexa 488 (filter: 511-543, mirror: 507 LP)).

S2.7 Copper distribution experiment

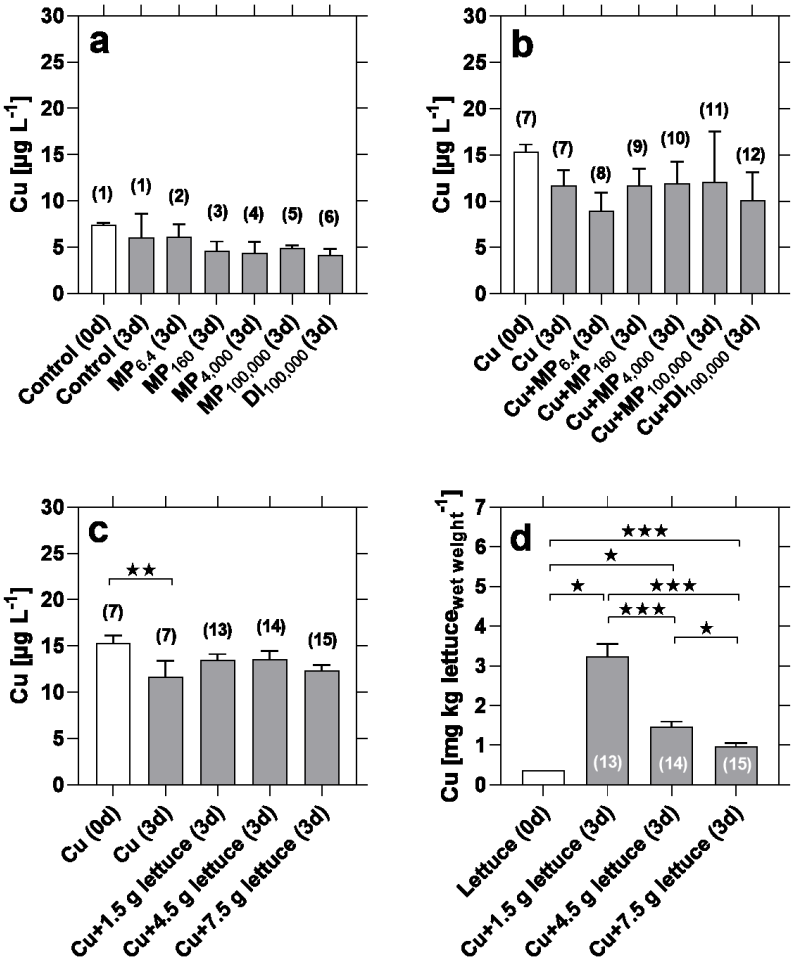

Fig. S10: Copper (Cu) concentrations (total concentration of all copper species) in the Cu distribution study. (a) Water concentrations in the unspiked ISO medium before (0d) and after 3 days (3d) of incubation with and without microplastics (MP, 6.4–100,000 p mL^{-1}) or diatomite (DI, 100,000 p mL^{-1}). (b, c) Water concentrations directly after spiking with 7.5 µg L^{-1} Cu and after 3 d of incubation with (b) MP, DI or (c) lettuce. (d) Cu concentration in the lettuce before and after 3 d of incubation in Cu-spiked ISO medium. The numbers above/in the bars refer to the labelling of the 15 tests as shown in Fig. S3. Subscript numbers indicate particle concentrations (p mL^{-1}). Statistics: (a,b) Kruskal-Wallis test with Dunn's post-test, comparisons: Cu (3d) vs. all other tests; (c) One-way ANOVA with Sidak's post-test, comparisons: Cu (3d) vs. all other tests; (d) One-way ANOVA with Sidak's post-test, comparisons: all possible pairwise combinations. ★ = $p<0.05$, ★★ = $p<0.01$, ★★★ = $p<0.001$. Number of jars per test: n = 3 (except for (d) lettuce (0d): n = 1).

S2.8 Copper concentrations in the mixture toxicity study

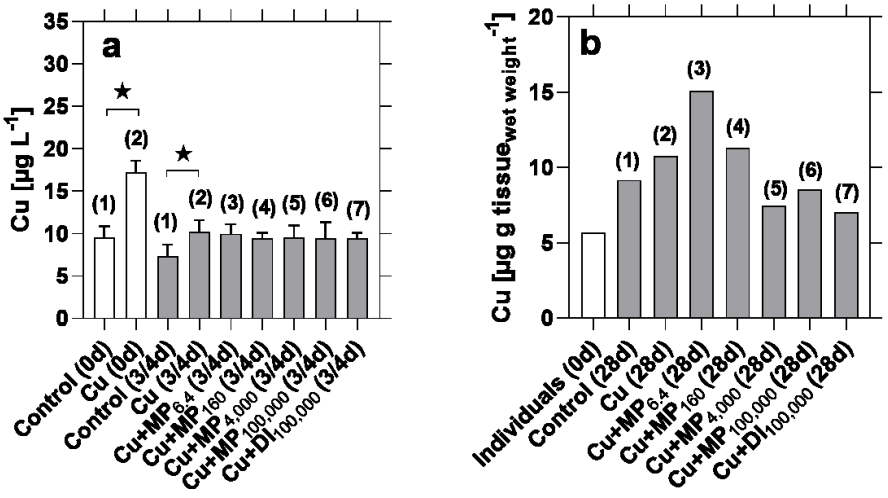

Fig. S11: Copper (Cu) concentrations (total Cu concentration of all copper species) in the mixture toxicity study. (a) Water concentrations in the freshly prepared unspiked and Cu-spiked (7.5 µg L^{-1}) medium before the water exchange (0d) as well as after 3–4 days of exposure (3/4d). (b) Tissue concentrations in *L. stagnalis* individuals (pool of four individuals per treatment group) prior (0d) and at the end (28d) of the mixture toxicity study. The numbers above the bars refer to test numbers in Fig. S2. Subscript numbers indicate particle concentrations (p mL^{-1}). Statistics for (a): Kruskal-Wallis test with Dunn's post-test; comparison: Control (0d) vs. Cu (0d) + Control (0d) vs. Control (3/4d) + Cu (0d) vs. Cu (3/4d) + Control (3/4d) vs. Cu (3/4d) + Cu (3/4d) vs. all Cu+MP treatments and the $Cu+DI_{100,000}$ treatment. ★ = $p<0.05$. Number of tested water samples/tissue samples per treatment (see 2.5 for details): (a) n = 7–8; (b) n = 1.

S3 References

Weber, A., Jeckel, N., Wagner, M., 2020. Combined effects of polystyrene microplastics and thermal stress on the freshwater mussel *Dreissena polymorpha*. *Science of the Total Environment* 718, 137253. DOI: 10.1016/j.scitotenv.2020.137253.

A5. Combined effects of polystyrene microplastics and thermal stress on the freshwater mussel *Dreissena polymorpha*

Studie 5

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Durchführung der einzelnen Untersuchungen und Experimente			
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%	**50**	50	-
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Autoren	**AW**	NJ	MW
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Analyse und Interpretation der Daten			
Autoren	**AW**	NJ	MW
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Combined effects of polystyrene microplastics and thermal stress on the freshwater mussel *Dreissena polymorpha*

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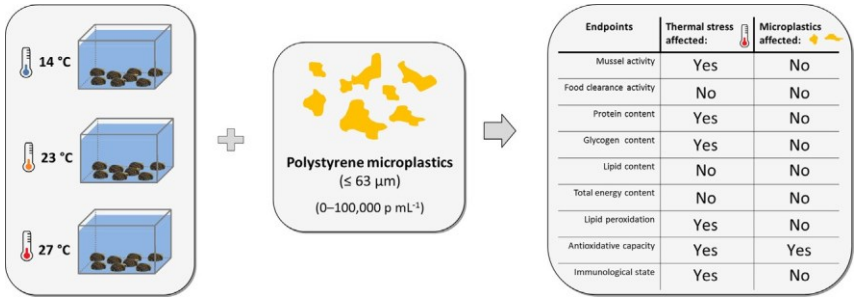
HIGHLIGHTS

- Thermal stress induces stronger effects than microplastics in *Dreissena polymorpha*.
- Thermal stress affects behavior, metabolism and immune function.
- Microplastics only affect the antioxidative capacity.
- Microplastic and diatomite effects only differ for the antioxidative capacity.

GRAPHICAL ABSTRACT



Endpoints	Thermal stress affected:	Microplastics affected:
Mussel activity	Yes	No
Food clearance activity	No	No
Protein content	Yes	No
Glycogen content	Yes	No
Lipid content	No	No
Total energy content	No	No
Lipid peroxidation	Yes	No
Antioxidative capacity	Yes	Yes
Immunological state	Yes	No

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ABSTRACT

Human-induced changes in the environment have increased the number of stressors impacting aquatic organism. In the light of climate change and plastic pollution, thermal stress and microplastics (MP) have become two of the most intensively studied stressors in aquatic ecosystems. Previous studies, however, mostly evaluated the impacts of thermal and MP stress in isolation, thereby neglecting joint effects.

To examine the combined effects of both, we exposed the freshwater mussel *Dreissena polymorpha* to irregular polystyrene MP (6.4, 160, 4000, 100,000 p mL^{-1}) at either 14, 23 or 27 °C for 14 days and analyzed mortality, mussel activity and clearance rate, energy reserves, oxidative stress and the immunological state. Further, we exposed the mussels to diatomite (natural particle equivalent, 100,000 p mL^{-1}) at each of the three water temperatures to compare MP and natural particle toxicity.

An increase in water temperature has a pronounced effect on *D. polymorpha* and significantly affects the activity, energy reserves, oxidative stress and immune function. In contrast, the effects by MP are limited to a change in the antioxidative capacity without any interactive effects between MP and thermal exposure. The comparison of the MP with a diatomite exposure revealed only limited influence of the particle type on the response of *D. polymorpha* to high concentrations of suspended particles.

The results indicate that MPs have minor effects on a freshwater mussel compared to thermal stress, neither alone nor as interactive effect. Limited MP toxicity could be based on adaptation mechanism of dreissenids to suspended solids. Nonetheless, MP may contribute to environmental impacts of multiple anthropogenic stressors, especially if their levels increase in the future. Therefore, we suggest integrating MP into the broader context of multiple stressor studies to understand and assess their joint impacts on freshwater ecosystems.

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1. Introduction

Since the beginning of the Anthropocene, industrialization has enhanced the demand for resources causing vast global changes, including human-induced environmental impacts (Steffen et al., 2007). Anthropogenic impacts on the environment are diverse, range from regional to global scale and they include, amongst others, the emission of pollutants (e.g., plastics), the change in land use, the exploitation of natural resources as well as climate change. In combination with naturally occurring stressors, anthropogenic changes, thus, lead to increasingly complex and challenging living conditions for biota.

Previous meta-analyses for marine, coastal and freshwater ecosystems have shown that relevant aquatic stressors (e.g., temperature or water chemistry change, habitat disturbance, chemical pollution, invasive species) do not only affect aquatic ecosystems individually but also in combination (Crain et al., 2008; Jackson et al., 2016). These joint effects may be additive synergistic or antagonistic. Thus, experiments with multiple stressors are important to understand their joint impact and interaction. This also adds more "realism" in ecotoxicological research (Beyer et al., 2014) and improves our understanding of the impacts of global change on aquatic ecosystems.

Global warming is a highly relevant stressor in aquatic ecosystems (Sommer et al., 2012) with freshwater habitats being particularly susceptible (Williamson et al., 2009). Global mean surface temperatures are projected to increase by 0.9–5.4 °C by 2100 relative to 1850–1900 likely causing an increase in water temperature (IPCC, 2019). In addition to gradually increasing water temperatures, rapid changes will be caused by more frequent and intense acute heatwaves (IPCC, 2019). Since the impacts due to global warming may further be altered due to additional stressor in aquatic ecosystems. Investigating combinatorial effects of climate change-associated factors and anthropogenic stressors is highly relevant (Przeslawki et al., 2015). In this respect, microplastics (MP) might be one of these relevant anthropogenic stressors. As MP are ubiquitous in aquatic ecosystems (Chae and An, 2017), concerns over potential impacts on biota have been raised. First risk assessments concluded that current environmental concentrations of MP are much lower than the ones required to induce adverse effects in aquatic species (Burns and Boxall, 2018; Adam et al., 2018; Besseling et al., 2018). However, Adam et al. (2018) and Besseling et al. (2018) also stated that MP may pose an environmental risk in hotspots of pollution, especially in Asia. Furthermore, these assessments looked at MP as a single stressor and did not consider the interaction with other stressors. As effects of multiple stressors can add up, the risk MP pose to aquatic environments may be underestimated. Previous studies focusing on combinatorial effects of MP and a second stressor mostly investigated the influence of chemical pollutants such as polycyclic aromatic hydrocarbons and polychlorinated biphenyls, pharmaceuticals, pesticides or heavy metals (e.g., Guven et al., 2018; Horton et al., 2018; Lin et al., 2019; Oliveira et al., 2013; Zhang et al., 2019). In comparison, the interaction of MP and thermal stress has been much less studied. Current knowledge is restricted to several studies which investigated thermal stress in combination with polyethylene (PE) and polyvinyl chloride (PVC) particles in fish, daphnids and mollusks (Ferreira et al., 2016; Fonte et al., 2016; Jaikumar et al., 2018; Lenz, 2016; Wen et al., 2018). As with MP research in general (Eerkes-Medrano and Thompson, 2018), little work has been done on freshwater biota.

To fill this knowledge gap, we analyzed the combined effects of thermal stress and MP by exposing the freshwater bivalve *D. polymorpha* at 14, 23 and 27 °C water temperature to irregular polystyrene (PS) MP (≤ 63 µm, 6.4, 160, 4,000 and 100,000 particles mL^{-1} (p mL^{-1})). After 14 d of exposure, we examined the effects on the energy reserves (proteins, glycogen, total lipids), the antioxidant system (lipid peroxidation, remaining antioxidant capacity), the immunological function and the mortality of *D. polymorpha*. During the experiment, we additionally determined the activity of the mussels (individuals with open valves) as well as the clearance rate of *D. polymorpha*. Finally, we analyzed the effects of diatomite (DI) as natural reference particle at the three temperature regimes and compared those to the MP effects.

2. Materials andmethods

2.1 Mussel culture

About 1,000 *D. polymorpha* individuals were collected in October 2017 at the Oberwald Lake in Mörfelden-Walldorf, Germany (49° 59′ 0.242″ N, 8° 35′ 48.666″ E). In the laboratory, bivalves were maintained in a 50 L tank at approximately 14 °C water temperature and a 16:8 h light:dark cycle for 4 weeks prior to the experiments. First, individuals were cultured in aerated lake water which was substituted stepwisely with aerated OECD medium (OECD, 2016, guideline no. 242) within the first week. From week two, 50% of the medium was renewed twice a week. Mussels were fed with algae (*Desmodesmus subspicatus*) ad libitum thrice a week.

2.2 Particle preparation

DI powder was purchased from Sigma-Aldrich (product no: 18514, Taufkirchen, Germany). Irregular MP were prepared from orange fluorescent PS drinking cups by cryomilling (for details see chapter S1.1). The polymer type of the cups was verified by attenuated total reflection-Fourier-transform infrared spectroscopy (ATR-FTIR spectroscopy, Spectrum Two, PerkinElmer, Waltham, MA, USA). Further, we characterized the chemicals in the polymer using pyrolysis-GC–MS analysis with a Multi-Shot Pyrolyzer and Auto-Shot sampler (Frontier- Laboratories, Saikon, Japan) attached to an Agilent 7890B gas chromatograph and an Agilent 5977B Mass Selective Detector (Agilent, Santa Clara, CA, USA). The PS cups had low but detectable levels of chemicals. However, their thermodesorption products could not be matched to compounds commonly used in plastics (for details see Chapter S1.2). The DI and MP fractions ≤ 63 µm were isolated by sieving (see S1.1). Particle abundance and size distribution in the 2–60 µm fraction were determined with a Coulter Counter (Multisizer 3, Beckman Coulter, USA). For both the DI and MP powder, particle abundance increased exponentially with decreasing particle size. 90% of the MP and the DI particles were smaller than 12.4 µm and 11.8 µm, respectively (for details see chapter S1.3).

2.3 Mussel exposure with microplastics and diatomite

We exposed *D. polymorpha* at three temperatures (14, 23 or 27 °C) either to MP (6.4, 160, 4000 or 100,000 p mL^{-1}) or DI (100,000 p mL^{-1}) for 14 d. At each exposure temperature, one additional treatment without particles served as control group. In total, our study design included 18 treatments each consisting of 52 *D. polymorpha* individuals (20–23 mm) kept in a tank (14 × 20 × 20 cm) with 3 L OECD medium. Prior to the exposure, we accustomed mussels to be exposed at elevated temperature (23 and 27 °C) slowly over 1–3 d to the new culturing temperature to prevent high mortality rates due to the sudden increase of water temperature.

We constantly aerated the tanks through glass pipettes to ensure algae and particle distribution in the water column. Because we still observed a partial sedimentation of the particles a few hours after particle addition, we also mixed the water column manually at least once a day with a stainless-steel spoon in each treatment. The top of the tank was covered with aluminum foil to prevent particle loss as well as cross-contamination between the different treatments. Water temperature was monitored in 15 min intervals over the whole exposure period in each treatment using submersed temperature loggers (HOBOWare, Onset Computer Corporation, Bourne, USA). The average temperature (± standard deviation) of the respective treatments were 13.77 ± 0.80, 23.42 ± 0.41 and 26.76 ± 0.47 °C.

The bivalves were fed daily with live algae (*D. subspicatus*) at a concentration of 0.25 mg TOC individual^{-1} (equivalent to 4.3 mg TOC L^{-1}) based on Walz (1978), recited by Clarke (1999), who reported that one to two-year old *D. polymorpha* require up to 2 mg L^{-1} TOC of algae. Mortality was recorded daily and dead individuals were removed from the tanks. On days 5 and 12 of the experiment, we additionally examined the mussels' activity by determining the percentage of individuals with opened valves in each treatment (three separate observations per day). Evaporation from the treatments was checked daily and, if necessary, accounted for by refilling with distilled water. Every 3–4 d, the medium was completely renewed by transferring the mussels to tanks filled with new medium and the corresponding particle concentrations. The required MP and DI mass was weighed in on a precision scale for each treatment, except for the 6.4 p mL^{-1} treatment. For the latter, we prepared a 100-fold concentrated stock solution in OECD medium and substituted 30 mL of the medium in the 3 L tank with stock solution to reach a final concentration of 6.4 p mL^{-1}.

After 14 d, hemolymph from five individuals per treatment (approximately 200–300 µL individual^{-1}) was withdrawn from the posterior adductor muscle with a sterile syringe (0.5 mL, 29Gx1/2 in.) and the phagocytic activity of the hemocytes was determined. The other mussels were directly frozen in liquid nitrogen and stored at −80 °C until examined for energy content and oxidative stress metabolites in the midgut gland (MGG).

2.4 Energy content and oxidative stress in the midgut gland

For energy content and oxidative stress analysis, MGGs from ten individuals per treatment were dissected, weighed (wet weight), homogenized (see S1.4) and frozen at −80 °C until analyzed. The MGG masses varied between the individuals but the mean MGG mass in the treatment groups did not differ significantly (Fig. S3). The protein content in the homogenates was analyzed based on Bradford (1976) using a serial dilution of bovine serum albumin (0.1%, m/v) as standard. The glycogen (anthrone assay) and the total lipid content (sulfo-phospho-vanillin assay) were determined according to Benedict (2014) with slight modifications. For glycogen and lipid analysis, a serial dilution of a glucose solution (0.1%, m/v) and a canola oil-chloroform-mixture (0.1%, *v*/v) were used as standards, respectively. Based on the standards, protein, glycogen and total lipid contents in the homogenates were calculated and are given as energy content per MGG mass (J mg^{-1}). The total energy content in the MGGs (J mg^{-1}) was estimated as the sum of the protein, glycogen and total lipid content.

Lipid peroxidation in the homogenates was assayed using the thiobarbituric reactive substances assay (TBARS), following the protocol from Furuhagen et al. (2014). The TBARS assay quantifies malondialdehyde (MDA, a major decomposition product from lipid peroxidation) through the reaction of MDA with thiobarbituric acid which produces a fluorescent product. Based on a serial dilution of an 80 µM MDA stock solution, MDA concentrations in the homogenates were calculated. Results on the MDA content in the homogenates are expressed as MDA equivalents per MGG mass (µmol mg^{-1}).

The antioxidative capacity in the homogenates was determined by the ORAC assay (oxygen radical absorbance capacity, Ou et al., 2001, Furuhagen et al., 2014). The assay is based on the ability of the antioxidants in the homogenates to delay the degradation of fluorescein in presence of a radical forming chemical. As antioxidant standard, we used a serial dilution of a 200 µM Trolox solution (6-hydroxy-2,5,7,8-tetramethylchroman -2-carboxylic acid, vitamin E derivate). The antioxidative capacity of the homogenates is presented as Trolox equivalents per MGG mass (µmol mg^{-1}). Further details on energy and oxidative stress assays are included in chapters S1.5 and S1.6.

2.5 Phagocytic activity of D. polymorpha *hemocytes*

Effects on the immune function of *D. polymorpha* were assessed through the phagocytic activity of the hemocytes in response to foreign stimuli. Hemocytes (500 µL hemolymph with 150,000 cells) of six mussels per treatment were exposed individually to 1 µm PS microbeads (50 beads per cell, Fluoresbrite YG microspheres, PolyScience, Hirschberg an der Bergstraße, Germany) for 3 h at room temperature. 250 µL of each cell suspension was separated and incubated with microbeads on ice. This control sample was analyzed in the same way as the remaining sample to correct the phagocytosis rates for particles which only adsorbed to the cell surface but were not phagocytized. We excluded dead cells from the FACS analysis by staining with propidium iodide (Sigma-Aldrich, Taufkirchen, Germany, details in S1.7). For all samples, we determined the fraction of hemocytes with ≥3 phagocytized microbeads compared to the total number of living cells with fluorescence-activated cell sorting (FACS) on a BD FACSVerse (BD Biosciences, Heidelberg, Germany). Results of hemocyte samples with <5,000 FACS counts were excluded, for which reason replicate numbers in the different treatments range between 4 and 6.

2.6 Clearance rate of D. polymorpha

To determine the clearance rates, we used three additional tanks (1.5 L OECD medium) with 26 individuals (20–23 mm) for each temperature (14, 23 or 27 °C) under same conditions as described in Section 2.3. One tank per temperature served as a control, while the other two included 100,000 p mL^{-1} MP or DI, respectively. On day 3 and 10 of the exposure, we quantified the clearance rate of ten individuals per tank as the difference in the chlorophyll fluorescence of an algae suspension (*Pseudokirchneriella subcapitata*) in the medium prior to and after an individual exposure of the bivalves to the algae suspension for 45 min (for details see S1.8). After the clearance experiments, individuals were returned into their tanks.

2.7 Statistics

We determined the effects of the factors "temperature", "MP "as well as their interaction (temperature × MP) on each of the endpoints using general linear (mixed) models (GL(M)M, temperature: fixed variable, MP: covariable) with IBM SPSS Statistics (version 25). Data for each endpoint was used as dependent variable and was either integrated untransformed, log-transformed, square root transformed or logit-transformed into the model. Prior to each GLM, normality (Shapiro-Wilks test), variance homogeneity (Levene test) and heteroskedasticity (White test) requirements were tested. Statistical analysis of particle type effects (MP vs. DI) were performed in the same way by running GL(M)M with the variables "temperature", "particle type" (both fixed variables) and its interaction (temperature × particle type).

Data for each endpoint was visualized using GraphPad Prism 7.04 (GraphPad Software Inc., USA) and linear regression between MP concentrations and the dependent variable were calculated for each temperature (for details see S1.9).

3. Results

3.1 Effects of thermal stress

Throughout the 14 d exposure of *D. polymorpha* individuals to 0–100,000 pmL^{-1} irregular PS MP at 14, 23 and 27 °C, mortality only occurred at 27 °C. Here, 21 out of a total of 260 animals died (8.1%) without relationship to particle exposure. Furthermore, thermal stress significantly affected the mussel activity, the protein, glycogen, lipid peroxidation (TBARS assay) and the remaining antioxidant capacity (ORAC assay) in the midgut gland as well as the phagocytic activity of the mussel hemocytes (immune assay, Table 1, Fig. 1, $p < .05$).

In contrast, an increase in temperature did neither affect the clearance rate (Fig. 2) nor the total lipid and energy content in the MGG (Fig. S5).
The mussel activity was highest at 14 °C (average of all MP exposure groups (including the control): 43.1%), decreased to 39.1% at 27 °C and was lowest at 23 °C (30.7%). Mussel activity did therefore not decrease proportionally with increasing exposure temperature, but rather had its minimum at 23 °C (Fig. 1). The same relation was observed for the glycogen and the antioxidant capacity which were highest at 14 °C (glycogen: 0.139 J mg^{-1} MGG, antioxidant capacity: 495 µmol Trolox mg^{-1} MGG) and lowest at 23 °C (glycogen: 0.122 J mg^{-1} MGG, antioxidant capacity: 307 µmol Trolox mg^{-1} MGG). The opposite trend was observed for the protein content being highest in the MGG of mussels exposed at 27 °C (0.465 J mg^{-1} MGG) and lowest in individuals at 23 °C (0.339 J mg^{-1} MGG).
The lipid peroxidation in the MGG and the phagocytic activity of the hemocytes decreased proportionally with increasing exposure temperature. For both endpoints, the strongest effects were observed at 14 °C (MDA content: 14.3 µmol mg^{-1} MGG, phagocytic activity: 10.1%) that decreased at 27 °C to 11.4 µmol mg^{-1} MGG and 5.85%, respectively.

3.2 Effects of microplastics exposure

Exposure to increasing MP concentrations (6.4–100,000 p mL^{-1}) did not result in a significant effect on mortality. Mortality occurred in none of the exposures at 14 and 23 °C. At 27 °C, mortality rates were 9.62% in the control, 13.46% in the 6.4 p mL^{-1} and 5.77% in the 160, 4,000 and 100,000 p mL^{-1} PS exposures. Therefore, mortality was not dose-dependent.
Furthermore, the exposure of *D. polymorpha* to MP had no significant effects on the mussel activity, energy reserves, lipid peroxidation, immune function or the clearance rate (Figs. 1, 2, S4, Table 1, $p > .05$). However, the remaining antioxidant capacity in the MGG was significantly affected by the MP exposure (Table 1, $p < .01$). At the three temperatures, the antioxidant capacity decreased with increased MP concentrations, especially at high MP concentrations (4,000 and 100,000 p mL^{-1}). This effect was more pronounced at 14 °C compared to mussels exposed at 23 and 27 °C.

3.3 Comparison of microplastics and diatomite effects

We additionally compared the effects of MP with DI as a representative of naturally occurring particles. For each temperature, we therefore performed a separate treatment with 100,000 p mL^{-1} DI particles and compared the results to the corresponding MP treatment (100,000 p mL^{-1}).
Mortality in the DI and the MP treatment were identical at 14 °C (0%) and 27 °C (5.77%) and very similar at 23 °C (DI: 1.9%, MP: 0%). There was no significant difference between the effects of DI and MP regarding the mussel activity, energy reserves, lipid peroxidation, immune function and the clearance rate (Figs. 1, 2, S4, Table S2). However, the remaining antioxidant capacity was significantly higher in the DI compared to the PS MP treatments at all temperatures ($p < .05$, Table S2).

3.4 Interaction of temperature and particle exposure

Throughout the exposure of *D. polymorpha* to MP at 14, 23 and 27 °C, no significant interaction between the two stressors (MP, temperature) was observed (Table 1, $p > .05$). Similarly, the statistical comparison of the MP and DI exposure did not indicate any significant interaction between the two variables temperature and particle type (Table S2, $p > .05$).

4. Discussion

4.1 Effects of thermal stress

The exposure of *D. polymorpha* at three different water temperatures (14, 23 and 27 °C) causes major effects on the behavior, metabolism and immune function. 14 °C was chosen as a reference temperature as Walz (1978) reported the highest ingestion rate for *D. polymorpha* at this temperature. *D. polymorpha* individuals are more active at 14 °C compared to animals held at 23 and 27 °C and have the highest glycogen levels, remaining antioxidative capacity and phagocytic activity of the hemocyte cells. These results suggest that 14 °C represents the optimal out of the three temperatures allowing for high activity rates, energy storage and a functional immune response.
In response to increasing water temperatures, *D. polymorpha* changes its behavior and metabolism to enhance its thermotolerance. Previous studies with *D. polymorpha* have shown that these adaptative mechanisms enable the species to survive temperatures of up to 30 °C, while the exposure to higher temperatures over several days often increases mortality (reviewed by Karatayev et al., 1998 and McMahon, 1996). We, therefore, selected 23 and 27 °C as temperatures inducing thermotolerance mechanisms of *D. polymorpha* in a medium (23 °C) and extreme scenario (27 °C). These temperatures are not supposed to fully mimic climate change conditions. Still, water temperature measurements by Quednow and Püttmann (2008) in freshwater systems of southern Hesse (the region from which our dreissenids originated) show that average surface water temperatures can range between 4 °C in winter and > 20 °C in summer. In the light of increasing freshwater temperatures due to climate change (IPCC, 2014) and a predicted strong increase in frequency and intensity of heatwave events (IPCC, 2019), rapidly increasing water temperatures up to 23 °C may be reached in these freshwater systems now or in future.
With regard to behavior, increasing water temperatures in our experiments reduces the valve opening of *D. polymorpha*, especially at 23 °C. Valve closure is a common strategy of mussels to outlive unfavorable conditions for a short time. Over an extended period, however, the

Table 1
Results of the general linear model and general linear mixed model (only between-subject) for the effects of temperature and microplastics exposure on mussel activity, clearance rate (on days 3 and 10), energy reserves (total energy, proteins, glycogen, total lipids) and oxidative stress (TBARS, ORAC) in the midgut gland of *D. polymorpha* as well as the phagocytic activity of hemocytes.

Variable		Mussel activity	Clearance rate (3 d)	Clearance rate (10 d)	Total energy	Proteins	Glycogen	Total lipids	TBARS	ORAC	Phagocytic activity
Temperature	*df*	2	2	2	2	2	2	2	2	2	2
	F	10.852	0.066	2.412	0.275	12.302	5.081	0.049	7.331	11.225	9.553
	p	0.004	0.936	0.099	0.760	b0.001	0.007	0.953	0.001	<0.001	<0.001
Microplastics	*df*	1	1	1	1	1	1	1	1	1	1
	F	0.041	0.055	1.850	0.185	2.349	2.725	0.080	0.005	8.336	0.882
	p	0.844	0.816	0.179	0.668	0.128	0.101	0.778	0.943	0.004	0.351
Temperature × Microplastics	*df*	2	2	2	2	2	—[a]	2	2	2	2
	F	0.140	0.129	1.593	1.455	0.123	—[a]	1.449	0.636	2.203	0.959
	p	0.871	0.879	0.213	0.237	0.884	—[a]	0.238	0.531	0.114	0.388

[a] Interaction term not included in the model.

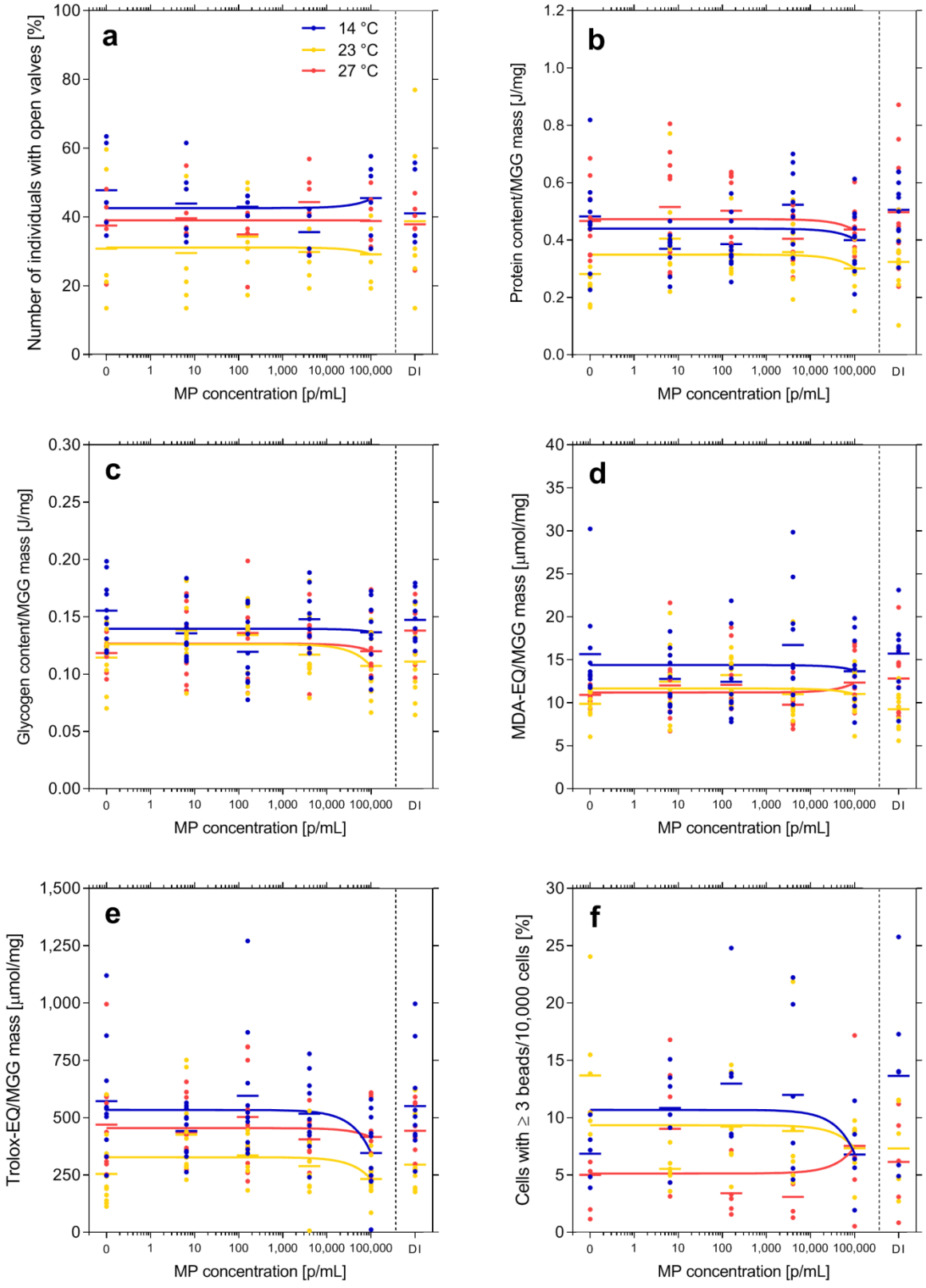

Fig. 1. Effect of polystyrene microplastics (MP, 0–100,000 p mL^{-1}) or diatomite (DI, 100,000 p mL^{-1}) on *D. polymorpha* exposed at 14, 23 and 27 °C for 14 d. (a) Mussel activity, (b) protein content, (c) glycogen content, (d) malondialdehyde content (MDA, TBARS assay) and (e) the remaining antioxidant capacity (Trolox equivalents, ORAC assay) in the midgut gland and (f) the phagocytic activity of hemocytes. Dots = results from the replicates, short lines = mean, regression = linear regression of the results in the microplastics exposures. (a) $n = 6$ (2 d with three observations each), (b–e) $n = 10$ individuals, (f) $n = 4–6$ individuals.

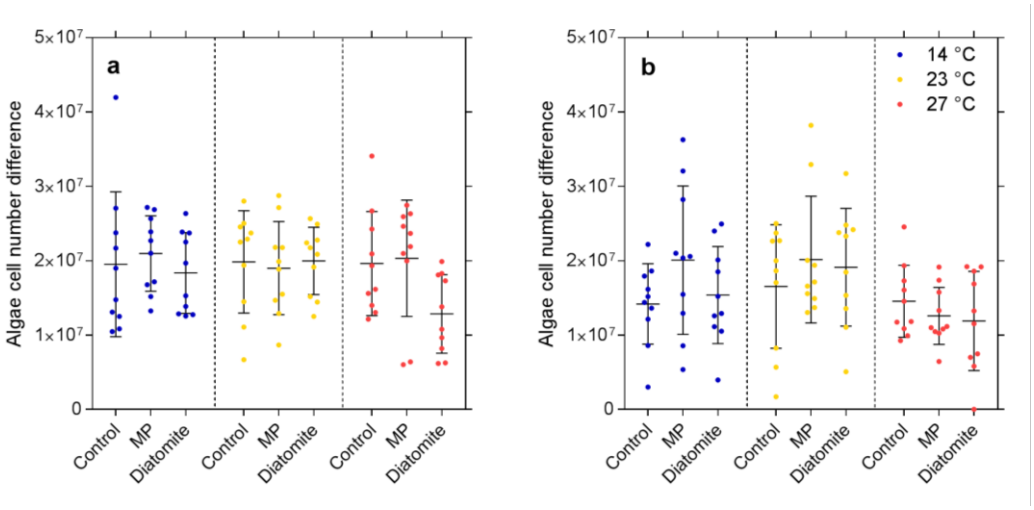

Fig. 2. Clearance rate (mean ± standard deviation) of *D. polymorpha* after an exposure to particle-free medium (Control), polystyrene microplastics (MP, 100,000 p mL⁻¹) or diatomite (100,000 p mL⁻¹) for (a) 3 d and (b) 10 d at 14, 23 and 27 °C. n = 10 for each group.

reduced gas exchange causes metabolic depression, a shift from an aerobic to an anaerobic metabolism and a depletion of the energy reserves (Anestis et al., 2007). Furthermore, elevated water temperatures also enhance the energy metabolic of mussels, while the clearance rate for food particles usually decreases (Anacleto et al., 2014; Anestis et al., 2010; Hornstein et al., 2018; Juárez et al., 2018). Consequently, we expected the heat-stressed mussels to reduce their clearance rate and deplete their energy reserves to maintain their metabolism. We did not observe a reduction of clearance rates in our experiments with increasing water temperatures, probably due to the high interindividual variation. Instead, the glycogen content is reduced in individuals held at 23 and 27 °C, indicating an increased mobilization of energy reserves in these individuals. Under stress conditions, mussels mobilize both lipid and carbohydrate reserves first before they change towards protein catabolism (Aldridge et al., 1995). Surprisingly, however, we did not find a significant temperature effect on the total lipid storage and the total energy storage of *D. polymorpha*. We assume this may be related to the high interindividual variation of the total lipid content in the MGG as well as by the limited sensitivity of our lipid assay.

Besides a change in the metabolism, thermal stress also depleted the antioxidant capacity in *D. polymorpha*. This is an indicator that the mussels experienced oxidative stress; a common response towards chemical or physical stressors (such as thermal stress) that triggers the production of reactive oxygen species (ROS) (reviewed by Verlecar et al., 2007). In case of oxidative stress, mussels express high amounts of antioxidant enzymes which scavenge ROS (Verlecar et al., 2007). The high protein levels in the MGG of animals held at 27 °C may be a consequence of the activated antioxidant system but could have also resulted from a generally enhanced metabolic activity.

Excess ROS can react with biomolecules and cause oxidative damage, for instance lipid peroxidation. A typical reaction production of ROS with polyunsaturated fatty acids is MDA (Vlahogianni et al., 2007). Therefore, based on the reduced antioxidant capacities in mussels held at 23 and 27 °C, we expected MDA concentrations to increase. A temperature-induced MDA increase has already been shown in studies with the freshwater mussel *Anodonta anatina* (Falfushynska et al., 2014) as well as the marine mussels *Mytilus galloprovincialis* (Coppola et al., 2018) and *Perna viridis* (Verlecar et al., 2007) which were exposed to thermal stress for ≥ 14 d. Contrasting our expectation, *D. polymorpha* held at 14 °C water temperature had the highest MDA concentrations. A very similar observation has been made for the marine clam *Chamelea gallina* (Matozzo et al., 2013). The authors assume that the induction of antioxidant enzymes was at least partially responsible for the lower MDA content in the clams under thermal stress. Furthermore, the high MDA levels in the control group may also be a result of the TBARS method which can overestimate MDA levels due to a cross-reaction of thiobarbituric acid with cyclic peroxides, β-unsaturated aldehydes and other contaminants (Oakes and Van Der Kraak, 2003). Nevertheless, we assume that the reduced lipid peroxidation in *D. polymorpha* at higher temperatures is related to an induction of the antioxidant defense system. The depleted antioxidant capacity indicates that the mussels experienced thermal stress. Thermal stress also affects the immune function of *D. polymorpha* by reducing the phagocytic activity of the hemocytes in our study. These results are in accordance with earlier studies that reported a similar depression in immunology and hemocyte phagocytosis due to thermal stress (Hégaret et al., 2003; Hornstein et al., 2018; Monari et al., 2007). A suppression of immune function resulting from thermal stress may render mussel more susceptible to infections. Interestingly, thermal stress has stronger effects on individuals held at 23 °C compared to the more extreme 27 °C, for instance regarding the valve opening rates, glycogen reserves and the antioxidant capacity. Interestingly, mussels held at 23 °C had the lowest protein content compared to the 14 and 27 °C treatments. As described earlier, reduced mussel activity may have led to the depletion of the glycogen reserves and a switch to protein catabolism as well as to enhanced oxidative stress. The reasons for these unexpected effects at the intermediate temperature, however, remain unknown.

Nevertheless, *D. polymorpha* held at 23 and 27 °C show changes in behavior, metabolism and immune function due to thermal stress. In a second step, we were further interested how the combined exposure with MP alters the temperature-induced effects and whether a combination of both stressors enhances the overall toxicity.

4.2 Effects of microplastics exposure

MP cause much lower effects in *D. polymorpha* compared to thermal stress (with no interaction between the two stressors). The antioxidative capacity in the MGG is the only endpoint affected by the additional MP exposure, while no effect was determined on behavior, metabolism or immune function. The depletion of the antioxidant capacity was most prominent in mussels exposed to the highest MP concentration (100,000 p mL⁻¹). Further, the effect is more pronounced at 14 °C water temperature and is rather minor at 23 and 27 °C. This is consistent with the reduced antioxidative capacity in mussels experiencing thermal stress. Here, the additional effect of the MP on an already depleted

antioxidative system will be less pronounced compared to unstressed control individuals. As a consequence, MP effects seem to be more relevant in unstressed mussels, while in combination with more potent stressors, such as higher temperatures, their effects may be less relevant.

Lenz (2016) made a very similar observation when analyzing the combined effects of thermal stress and PVC MP in six marine mussel species. In *Mytilus trossulus* and *Crassostrea gigas*, they observed both thermal and MP-induced effects on the respiration, but, similar to our results, MP effects were only present in the control animals. In a third species, *Perna viridis*, however, MP induced effects at all temperatures (Lenz, 2016). The authors discuss that thermal stress masks other stressors especially in species which are adapted to low and stable water temperatures (e.g., *Mytilus trossulus* and *Crassostrea gigas*). Those species are especially susceptible to thermal stress compared to species which are adapted to temperature variations and occasional thermal stress situations (*Perna viridis*). As discussed in Section 4.1, surface water temperature in Hessian freshwater systems can vary intensively over the course of the year and, thus, *D. polymorpha* is adapted tomarked changes inwater temperature. However, these changes are slow allowing sufficient time for adaptation, while the increase in water temperature in the experiments occurred rapidly within 1–3 d. Such rapid temperature increase may have disrupted an adaptation causing thermal stress and, thus, masked the oxidative stress caused by MP (similar to *M. trossulus* and *C. gigas* in the study by Lenz, 2016). Besides mussels, the joint effects of thermal stress and MP have previously been studied in fish. Ferreira et al. (2016) and Fonte et al. (2016) exposed *Pomatoschistus microps* juveniles to elevated water temperatures, PE MP and a chemical stressor. In both studies, MP effects were limited to a change in lipid peroxidation, while temperature, similarly to mussels, affected the behavior and induced the oxidative stress response. In contrast, Wen et al. (2018) reported greater impacts of PE compared to thermal stress on the predatory performance, digestion and energy production of the freshwater fish *Symphysodon aequifasciatus*. Moreover, PE MP induced mortality in daphnids which were more sensitive at higher water temperatures (Jaikumar et al., 2018). Accordingly, the results of the available studies are inconsistent when taking into account other than mussel species. From this, we hypothesize that the intensity of the applied stressors as well as the adaption mechanism of freshwater species towards the applied stressors may influence the impact of the tested stressors. Hence, in different species or under divergent exposure conditions, MP can have different effects from the ones we observed.

Furthermore, when interpreting multiple stressor toxicity of thermal stress and MP, it has to be considered that most studies applied MP concentrations higher than currently found in the environment. MP concentrations in rivers are still rather low ranging up to 0.5 p mL^{-1} (Lahens et al., 2018). Effects at MP concentrations of up to 100,000 p mL^{-1} therefore seem to be currently not environmentally relevant. In our study, we aimed at including both environmentally relevant concentrations (6.4 p mL^{-1}) which may be reached in the near future due to increasing plastic pollution in aquatic ecosystems (Lebreton et al., 2019) as well as high MP concentrations as a proof-of-principle. Limited MP toxicity even at high MP concentrations, thus, indicates that PS fragments (≤ 63 μm) currently represent a rather low hazard for dreissenids in the environment.

4.3 Comparison to natural particles

Both, in our experiment and in the study by Lenz (2016), MP were a minor stressor compared to changes in water temperature. A reason for why mussels are resistant to MP exposures may be the evolutionary adaption of many filter-feeding organisms to high turbidity and suspended solids. Hence, we compared the effects of natural DI particles and MP and observed very little differences. Significant particle type effects were only detected for the antioxidative capacity in *D. polymorpha*. Further, we observed a non-significantly enhanced phagocytic activity of the hemocytes in the DI compared to the MP exposure at 14 °C. This suggests that the particle type had some, but rather limited influence on the response of *D. polymorpha* to high concentrations of suspended particles and that under the conditions used in this study, MP are not much more toxic than natural particles.

5. Conclusion

Our multiple stressor study illustrates that thermal stress has a stronger impact on *D. polymorpha* compared to PS MP (≤ 63 μm). Increasing the water temperatures from 14 to 23 and 27 °C significantly affects the activity of the mussels, their energy reserves (protein, glycogen content) and oxidative stress markers (lipid peroxidation, antioxidative capacity) as well as the immune function. In contrast, MP exposure to very high concentrations (up to 100,000 p mL^{-1}) only affects the antioxidative capacity without significant interactive effects of MP and thermal stress.

Our results imply that dreissenid mussels are more sensitive to thermal stress than to MP exposure because they are evolutionary adapted to high loads of suspended particles. The latter is supported by the results from an exposure to natural DI particles which resulted in largely similar effects like MP.

While we did not observe an interaction of thermal stress and MP exposures in dreissenid mussels, MP represents just one out of numerous anthropogenic stressors in aquatic ecosystems. They, thus, may contribute to effects in a complex, multi-stressor environment which cannot be predicted based on simplified laboratory experiments. In the light of increasing plastic pollution in aquatic environments, future MP concentrations will likely increase (Lebreton et al., 2019) making joint effects more probable. Therefore, the integration of our data into the broader context of future multi-stressor studies will enable a comprehensive assessment of risks resulting from plastic pollution in a changing aquatic environment.

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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Author contributions

AW and NJ conceived and performed the study and analyzed the data. All authors wrote the manuscript and agreed on the final version.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.137253.

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Combined effects of polystyrene microplastics and thermal stress on the freshwater mussel *Dreissena polymorpha*

Supplementary Information

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S1 Supplementary materials and methods

S1.1 Particle preparation

Microplastics (MP) were produced by cryomilling in a swing mill (Retsch, MM400, Haan, Germany) with a stainless-steel milling chamber (volume: 50 mL, Retsch Technology, Haan, Germany) and a Ø 25 mm stainless steel ball. The polystyrene (PS) cup was crushed into pieces of up to 3 cm length and 5 g of these pieces were ground three times for 4 min at 30 Hz. Before and between the runs, the chamber was cooled with liquid nitrogen for 2 min.
300 g of MP and diatomite (DI) powder were separately sieved through a 63 µm woven wire mesh sieve (Retsch, product no.: 60.131.000063, Haan, Germany) on a sieving tower (Retsch Technology, AS200basic, Haan, Germany) for 4 h (amplitude: 20 Hz).

S1.2 Particle characterization

PS as polymer type was verified by Fourier-transform infrared spectroscopy in Attenuated Total Reflection mode (ATR-FTIR spectroscopy, Spectrum 2, Perkin Elmer, Waltham, MA, USA). The polymer spectrum was measured with the range set to 4,000–450 cm^{-1} (4 scans per wave number, resolution: 4 cm^{-1}, suppression of CO_2 and H_2O peaks). The major peaks in the FTIR spectrum (Fig. S1a) were in accordance with the data published by Jung et al. (2018) and confirmed that the material was PS.
The chemical content in the PS sample was determined by pyrolysis-GC-MS (py-GC-MS, Multi-Shot Pyrolyzer and Auto-Shot Sampler (Frontier Laboratories, Saikon, Japan) attached to an Agilent 7890B gas chromatograph and an Agilent 5977B Mass Selective Detector (Agilent, Santa Clara, CA, USA)). PS powder (100–300 mg per pyrolysis cup) was heated to 280 °C for 5 min. Thermodesorption products were detected for 30 min in selected ion monitoring mode.

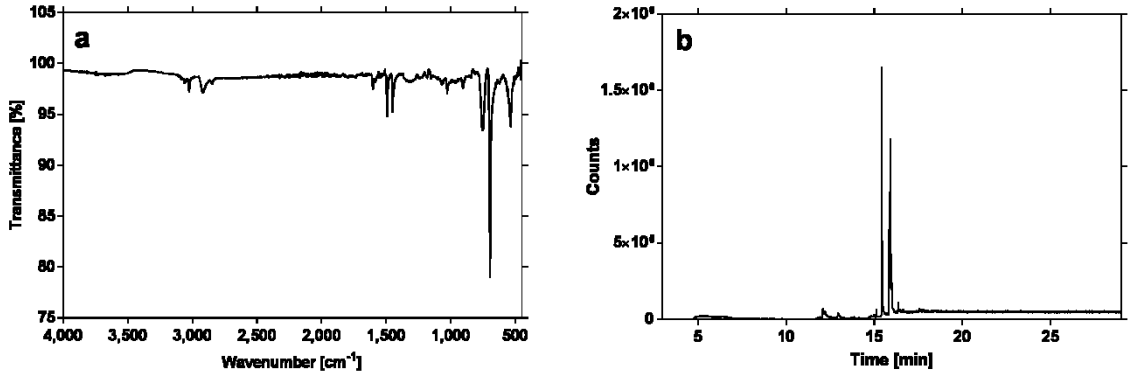

Fig. S1: (a) ATR-FTIR spectrum and (b) py-GC-MS spectrum of the polystyrene drinking cups.

The py-GC-MS spectrum (Fig. S1b) was analyzed with the Agilent MassHunter Workstation Software (version B.05.00, Agilent Technologies, Santa Clara, CA, USA) using “Chromatogram Deconvolution”. We only analyzed peaks with an absolute height of at least 2 % of the highest peak and with a relative height of at least 5,000 counts. Additional settings were: RT window size factor = 100, peak

filter = excluded m/z: 28, extraction window: left m/z delta = 0.3 and right m/z delta = 0.7. The mass spectra of the resulting peaks were compared to the NIST 2011 Mass Spectral Library (National Institute of Standards and Technology, Gaithersburg, MD, USA).

We detected the fluorophore 1,4-diphenyl-,(E,E)-1,3-butadiene, (Tab. S1) as well as m-phenethyl-benzonitrile, and (2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide as thermodesorption products. The fluorophore might originate from the dye of the drinking cup. The other two desorption products, however, are not commonly used in polymers and their origin, therefore, remains unknown.

Tab. S1: Detected peaks in the py-GC-MS mass spectrum and corresponding tentative identification based on the NIST database (highest match scores).

	Polymer	Base Peak	RT	Height	Area	Compound name	Score
1	**PS**	90.99	12.089	21,601	245,656	not identified	none
2	**PS**	104.01	12.223	14,719	85,223	not identified	none
3	**PS**	204.02	12.947	6,699	66,710	not identified	none
4	**PS**	90.99	15.430	622,777	2,067,796	Benzonitrile, m-phenethyl-	57.03
5	**PS**	90.99	15.856	156,943	1,009,078	1,3-Butadiene, 1,4-diphenyl-, (E,E)-	73.09
6	**PS**	90.99	15.904	233,782	1,639,405	(2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide, trans-	75.13
7	**PS**	90.99	15.942	83,831	477,232	(2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide, trans-	59.17
8	**PS**	90.99	15.983	84,599	781,256	(2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide, trans-	67.09
9	**PS**	90.99	16.364	14,536	65,237	not identified	none
10	**PS**	90.99	16.485	6,457	98,021	not identified	none

S1.3 Particle concentrations and size distribution

The concentrations of MP and DI particles (≤ 63 µm) were determined using a Coulter Counter (Beckman Coulter, Multisizer 3, Krefeld, Germany) by suspending 2 mg MP or DI in 50 mL electrolytic solution (0.9 % NaCl solution, < 0.2 µm sterile-filtered) and adding 5 mL of these suspensions to 147 mL electrolytic solution. The suspension was directly measured three times with a 100 µm capillary (Beckman Coulter, Krefeld, Germany, detection range: 2–60 µm, aperture: -1,600, gain: 2, analytical volume: 1 mL). The measurements were repeated three times for both particle types. Additionally, background measurements without MP or DI were performed to quantify contamination in the electrolyte solution. The MP and DI concentrations were corrected, accordingly. The MP and the DI powder contained 287,526 particles per mg^{-1} and 4,632,990 particles mg^{-1}, respectively.

Results on particle size distributions from Coulter Counter measurements were averaged and fitted with GraphPad-Prism Software (Version 7.04, San Diego, CA) using a “One phase decay” function (relative particle size, Fig. S2) or a “One phase association” function (cumulative particle size of MP) and a “Cumulative Gaussian-Percentage” function (cumulative particle size of DI). From the cumulative size distributions, we determined the maximum size of 50, 75, 90, 95 and 99 % of the particles (insets in Fig. S2).

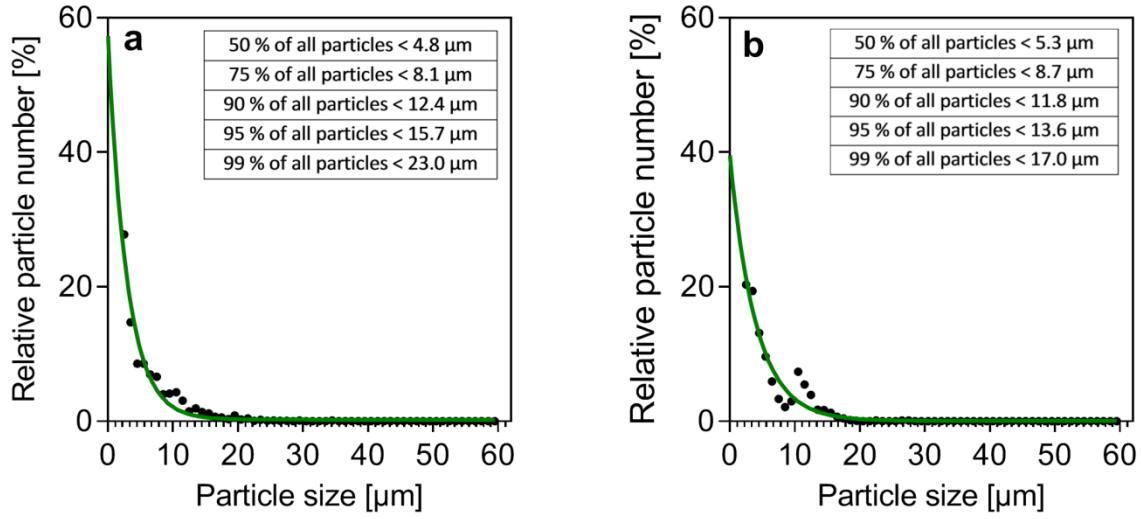

Fig. S2: Relative size distribution of (a) microplastics and (b) diatomite particles. Size distribution was summarized from three separate measurements and approximated with a One phase decay function using GraphPad Prism. The tables in the insets present the results derived from cumulative particle distributions.

S1.4 Preparation of midgut gland homogenates

Midgut glands (MGG) from ten individuals per treatment were dissected and weighed. Fig. S3 indicates that MGG mass varied intensively within each exposure group, while MGG mass did not differ significantly between any of the exposure groups (Kruskal-Wallis test, $p > 0.05$).

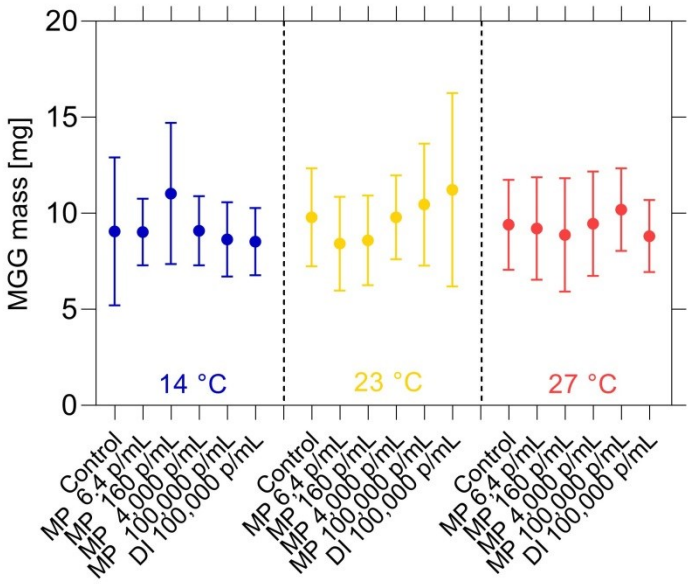

Fig. S3: Migut gland mass (MGG, mean ± standard deviation) of *D. polymorpha* after an exposure to particle-free medium (Control, 0 p mL^{-1}), polystyrene microplastics (MP, 6.4–100,000 p mL^{-1}) or diatomite (DI, 100,000 p mL^{-1}) for 14 d at 14, 23 and 27 °C.

Midgut glands (MGG) were homogenized in 360 µL of potassium phosphate buffer (PPB; 10 mM, pH 7,4) with two stainless steel balls (∅ 2–3 mm) in a swinging mill for 30 min (15×2 min). The tissue as well as its homogenate was constantly placed on ice between the processing steps to avoid degradation.

For the glycogen and total lipid assay, 150 µL of midgut gland homogenate was mixed with 50 µL 2 % (m/v) Na_2SO_4 solution (Sigma-Aldrich, Munich, Germany). 25 µL of this mixture was further diluted with 6.25 µL 2 % Na_2SO_4 solution and 18.75 µL PPB to obtain the required dilution for the protein assay.

For the thiobarbituric reactive substances assay (TBARS), 160 µL of each tissue homogenate were mixed with 160 µL PPB (10 mM, pH 7.4). The dilution for the oxygen radical absorbance capacity assay (ORAC) was produced by mixing 10 µL of the TBARS dilution with 90 µL PPB.

S1.5 Protein, glycogen and total lipid assay

S1.5.1 Protein assay

The protein assay was performed according to Bradford (1976). As standard, 1, 3, 6, 12.5, 25, 37.5 and 50 µL of a 0.1 % (m/v) bovine serum albumin solution (BSA; Sigma-Aldrich, Darmstadt, Germany) were mixed with 2 % Na_2SO_4 solution to obtain a total volume of 50 µL. 50 µL of 2 % Na_2SO_4 solution were used as negative control. The negative control, the standards as well as 50 µL of the homogenate dilution (see S1.4) were mixed with 1.5 mL Bradford reagent (A6932, AppliChem GmbH, Darmstadt, Germany) and incubated for 5 min at room temperature. Subsequently, 2×200 µL were pipetted into transparent 96-well plates and optical density was determined spectrometrically at 595 nm (Spark 10, Tecan, Switzerland).

S1.5.2 Glycogen and total lipid assay

100 µL of homogenate dilution for the glycogen and total lipid analysis (S1.8) were mixed with 1.6 mL of a 1:1 chloroform-methanol solution (chloroform: VWR International, Darmstadt, Germany; methanol: Carl Roth, Karlsruhe, Germany). After 1 h of incubation on ice, homogenates were centrifuged at 845 g for 2 min (Centrifuge 5702, Eppendorf, Hamburg, Germany). The pellet was analysed for its glycogen content, while the supernatant (which includes the lipid fraction) was separated, mixed with 600 µL distilled water and centrifuged at 845 g for 2 min. Subsequently, the resulting upper phase was removed, and the lower phase was used for total lipid analysis.

For glycogen analysis, the pellet was dissolved in 5 mL of anthrone-sulphuric acid reagent (750 mg anthrone, Merck, Darmstadt, Germany; 385 mL 98 % H_2SO_4, VWR, Darmstadt, Germany; 150 mL demineralized water) and incubated in a water bath at 95 °C for 17 min. As standard, 1, 3, 6, 12.5, 25, 50, 100, 200, 400 and 800 µL of a 0.1 % (m/v) D-(+)-glucose solution (VWR, Darmstadt, Germany) was processed in the same way. As negative control, 5 mL of anthrone-sulphuric acid reagent were incubated alone. 2×200 µL from each replicate were pipetted into transparent 96-well plates and optical density was determined spectrometrically at 625 nm.

Lipid fractions were reduced to a volume of approximately 20 µL by evaporation in a water bath at 70 °C. As standard, 1, 3, 6, 12.5, 25, 50, 100, 200, 400 and 800 µL of a canola oil solution (0.1 % v/v in chloroform, REWE, Cologne, Germany) were concentrated in the same way. Then, 200 µL of

sulphuric acid (95–98 %, CAS: 7664-93-9, VWR, Darmstadt, Germany) were added and samples were incubated in the water bath at 70 °C for 13 min. As negative control, 200 µL of sulphuric acid were incubated alone. After the incubation, 5 mL of vanillin-phosphoric acid reagent (600 mg vanillin, Sigma-Aldrich, Munich, Germany; 400 mL 85 % H_3PO_4, VWR, Darmstadt, Germany, 100 mL demineralized water) were added to all samples and incubated at room temperature for 5 min. Finally, 2×200 µL from each tube were transferred into a transparent 96-well plate and the absorbance was recorded at 560 nm using a Tecan Spark 10 photospectrometer.

S1.5.3 Calculation of the energy content

Protein, glycogen and total lipid contents were determined by plotting the optical densities of the BSA, glucose and canola oil standard solutions as a function of its energy content [µg]. Energy values were estimated as 17.2 J mg^{-1} for proteins and glucose (Higgs et al. 1995) as well as 34.04 J mg^{-1} for lipids (REWE, product information) and recalculated for each standard dilution. The energy contents of the MGG samples were interpolated from a quadratic regression of the results from the standard measurements and normalized to the MGG wet weight [J mg^{-1}]. Finally, we calculated individual protein, glycogen and total lipid contents in the MGG as well as the total energy content (= energy content of proteins+glycogen+total lipids).

S1.6 Oxidative stress assays

S1.6.1 Thiobarbituric acid assay (TBARS)

Malondialdehyde (MDA) as standard substance was produced by hydrolysis of 1,1,3,3-Tetramethoxypropane (TMP, Sigma-Aldrich, Munich, Germany). 41.2 µL of TMP were added to 250 mL ultrapure water and 0.25 mL hydrochloric acid (1 M, CAS: 7647-01-0, VWR, Darmstadt, Germany) and incubated at 52–55 °C in a heating cabinet for at least 1 h through which a 1 mM aqueous solution was produced. A serial dilution (80, 40, 20, 10, 5, 2.5 µM) of the MDA solution was prepared as standard. Ultrapure water was used as negative control.

100 µL of the homogenate dilutions (S1.4, two replicates per homogenate), of the negative control (three replicates) as well as of the MDA standard (three replicates per concentration) were mixed with 100 µL of an ice-cold 10 % (m/v) trichloroacetic acid solution and 120 µL of a 2 mM thiobarbituric acid solution (Sigma-Aldrich, Munich, Germany). The mixture was incubated at 95 °C for 1 h and, after cooling down to room temperature, mixed with 180 µL butanol-pyridine-mixture (14:1 ratio; 1-butanol: AppliChem, Darmstadt, Germany; pyridine: Sigma-Aldrich, Munich, Germany). Samples were centrifuged at 5,200 g and 0 °C for 5 min for phase separation. Subsequently, 80 µL of the upper phase were pipetted into black 96-well plates (Nunc F96 MicroWell, Thermo Fisher Scientific, Waltham, USA) and fluorescence was measured (extinction: 540 nm, emission: 590 nm, gain: 48) using a Tecan Spark 10 spectrophotometer. Fluorescence of the replicate samples (see above) was averaged.

We determined lipid peroxidation in the MGG by comparing the fluorescence of samples with the fluorescence of the MDA standard. For this, results of the MDA standard and the negative control were plotted as a quadratic function of the concentration and MDA equivalents in the MGG samples were interpolated from this function. Due to high variability of total lipid contents in the different

treatments, we chose to normalize the resulting MDA equivalents to MGG wet weight (µmol mg^{-1}) and not to the total lipid content.

S1.6.2 Oxygen radical absorbance capacity assay (ORAC)

For each ORAC assay, a standard was produced from a 200 µM 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) solution (Sigma-Aldrich, Munich, Germany) by dilution (200, 100, 50, 25, 12.5, 6.25 µmol). PPB was used as negative control.

20 µL of the control (three replicates), of the homogenate dilutions (S1.4, two replicates per homogenate) as well as of the Trolox standard (three replicates per concentration) were pipetted into a black 96-well plates. 150 µL of a 0.106 µM fluorescein solution (Sigma-Aldrich, Munich, Germany) was added to each well and plates were incubated at 37 °C for 20 min. Finally, 30 µL of a 152.66 mM 2,2'-azobis (2-methylpropionamidine) dihydrochloride solution (AAPH, Sigma-Aldrich, Munich, Germany) were quickly added to each well and fluorescence (extinction: 485 nm, emission: 520 nm, gain: 43) was recorded at 37 °C in 1 min intervals for 12 h on a Tecan Spark 10 instrument.

For data analysis, fluorescence values of the Trolox standard and the analysed samples were plotted as a function of the measurement time (12 h) and the area under each curve (AUC) was determined for each sample. AUC results of replicates were expressed as means. AUC values of the Trolox standard were plotted as a function of the concentration and the antioxidative capacity of the homogenates were interpolated as Trolox equivalents. Correspondingly to the TBARS assay, we normalised the antioxidative capacity to the MGG wet weight (µmol mg^{-1}).

S1.7 Phagocytic activity of D. polymorpha *hemocytes*

For analysis of hemocyte phagocytic activity, we extracted 250–300 µL hemolymph from five mussels per treatment and immediately stored it in separate reaction tubes on ice. Hemocyte concentrations in the hemolymph were determined with a Neubauer improved counting chamber using trypan blue staining. Then, each hemolymph sample was diluted with *D. polymorpha* serum to a total volume of 500 µL and a concentration of 300,000 cells mL^{-1}. *D. polymorpha* serum was produced by pooling hemolymph extracted from non-exposed individuals and directly heating it to 56 °C for at least 30 min. After centrifugation at 21,130 g for 15 min, the supernatant was directly frozen and later used as serum. 1 µm PS spheres (Fluoresbrite YG microspheres, PolyScience, Hirschberg an der Bergstraße, Germany, excitation: 441 nm, emission: 486 nm) suspended in *D. polymorpha* serum were added at a ratio of 50 spheres per hemocyte cell (7.5 µL of a 10^9 spheres mL^{-1} stock solution) to the hemolymph. Each sample was gently vortexed and directly split in two subsamples with 250 µL each, one being incubated at room temperature and the other one on ice.

After 3 h of incubation, 10 µg mL^{-1} propidium iodide (PI, Sigma-Aldrich/Merck, Taufkirchen, Germany, excitation: 482 nm, emission: 608 nm) were added and samples were directly analysed using a BD FACSVerse (BD Biosciences, Heidelberg, Germany). PI penetrates the membrane of dead cells and intercalates with nucleic acids. After excluding dead cells from the main cell population according to PI fluorescence (Fig. S4a–b, Gate P2: living hemocytes in the main cell population; Gate P3: dead hemocytes in the main cell population), we determined the number of living hemocytes with ≥ 3 spheres (Gate P6 in Fig. S4c, Gate P6 is a subpopulation from Gate P2). Only FACS analyses

with ≥ 5,000 living cell counts were used. We extrapolated data from all FACS measurements to 10,000 living cells to allow data comparability between the different samples. Results from the sample exposed at room temperature were corrected for the number of hemocytes with ≥ 3 spheres from sample exposed on ice to account for particles which were adsorbed on the hemocytes cell surface, but not phagocytized. Based on the corrected data, we determined the fraction of living hemocytes with ≥ 3 spheres compared to all analyzed living hemocytes.

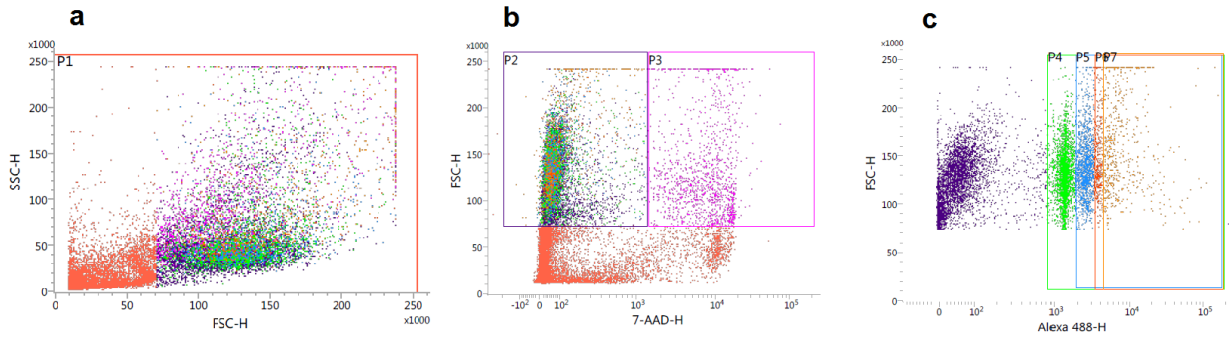

Fig. S4: Characterization of hemocytes exposed to 1 µm polystyrene spheres with FACSVerse. (a) Size (FSC) vs. granularity (SSC) of analyzed hemocytes (488 nm laser, FSC: 254.6 Voltage, SSC (filter: 481-496 nm): 324.5 Voltage), (b) Gating of living (gate P2) and dead (gate P3) hemocytes due to PI fluorescence (488 nm laser, 7-AAD (filter: 673-727, mirror: 665 LP), 300.3 Voltage), (c) Gating of living hemocytes (from Gate 2) with ≥ 1 (P4), ≥ 2 (P5), ≥ 3 (P6) or ≥ 4 spheres (P7) (488 nm laser, Alexa 488 (filter: 511-543, mirror: 507 LP), 304.5 Voltage).

S1.8 Clearance rate of D. polymorpha

Clearance activity of *D. polymorpha* was assessed by exposing mussels individually to an algae suspension (*Pseudokirchneriella subcapitata*, 5,000–5,500 relative fluorescence units (RFUs), approximately 1.48–1.64×10^6 cells mL^{-1}) and measuring chlorophyll fluorescence prior to and after the exposure (emission: 440 nm, extinction: 680 nm, GENios, Tecan, Männedorf, Switzerland). 250 mL glass jars were filled with 50 mL algae suspension and gently stirred with a magnetic stirrer. Ten *D. polymorpha* individuals were randomly selected from each of the treatments and individually exposed to the algae suspension for 45 min (beginning from the time when the mussel opened its valves). Before the introduction of the mussels as well as after 45 min, 3×200 µL of algae suspension were removed from each exposure vessel and transferred into black 96-well plates. Algae concentrations were determined using chlorophyll fluorescence (RFU). OECD medium (OECD 2016, guideline no. 242) was used as blank sample to account for background fluorescence. Replicate results were expressed as means and the clearance rate of each *D. polymorpha* individual was expressed as ΔRFU = RFU (prior to exposure) – RFU (after exposure).

S1.9 Statistics

For clearance rate, energy reserves, oxidative stress and immunological results, we ran separate general linear models (GLM) with IBM SPSS (version 25) to determine the contribution of the

variables “temperature“, “MP “ as well as their interaction on the overall effect. We included temperature (14, 23, 27 °C) as fixed variable and MP (0–100,000 p mL^{-1}) as covariable in the model. In the GLM for the clearance rate, the variable MP obtained only two states (Control, 100,000 p mL^{-1} MP) and MP was, therefore, also considered a fixed variable. For mussel activity, we analyzed the data from the six consecutive observation with a general linear mixed model (GLMM) with “time“ as inner-subject variable and “temperature“ and “MP“ as between-subject variables.

Data for each endpoint was used as dependent variable and was either integrated untransformed (glycogen, clearance rate), log-transformed (protein, TBARS, ORAC), square-root transformed (total energy, total lipids) or logit-transformed (immunity, mussel activity) into the model. Normality (Shapiro-Wilks test), variance homogeneity (Levene test) and heteroskedasticity (White test) requirements were met for most data sets. In case of the GLMs for the ORAC and the immune assay results, outliers caused non-Gausian distribution and heterogenic variances, respectively. In case of the GLM for the ORAC results, outlier removal did not lead to different results indicating robustness of the GLM, while for the immune function GLM the interaction term turned significant. In the results section, however, we present the more conservative results including the outlier to avoid overinterpretation of data.

We initally included the temperature × MP interaction term in all GLMs. In case of a non-significant interaction term, we refined GLMs excluding the interaction terms. Results, however, did not differ and we, therefore, present the final results including the interaction term (except for the glycogen GLM where the interaction term is left out).

Statistic analysis of particle type effects (MP vs. DI) were performed in the same way as for MP effects. We ran GLM and GLMM with the variables “temperature“, “particle type“ (both fixed variables) and its interaction (temperature × particle type) as well as “time“ as inner-subject variable in case of the GLMM for mussel activity. In case of the GLMM, the number of degrees of freedom were too low to integrate the interaction term into the model.

Dependent variable data was included untransformed (total energy, glycogen, total lipids, TBARS, ORAC, clearance rate), square root-transformed (protein) or logit-transformed (immunity, mussel activity) into the separate models. Normality, variance homogeneity and heteroskedasticity criteria were met for all data sets except for the clearance rate at day 3. Data on clearance rates was non-normally distributed according Shapiro-Wilks test, but visual analysis did not indicate a severe violation of normality. A further exclusion of the non-significant interaction term removed a former significant effect of particle type. Again, we here present the more conservative results excluding the interaction term.

2 Results

2.1 Effects on the energy reserves

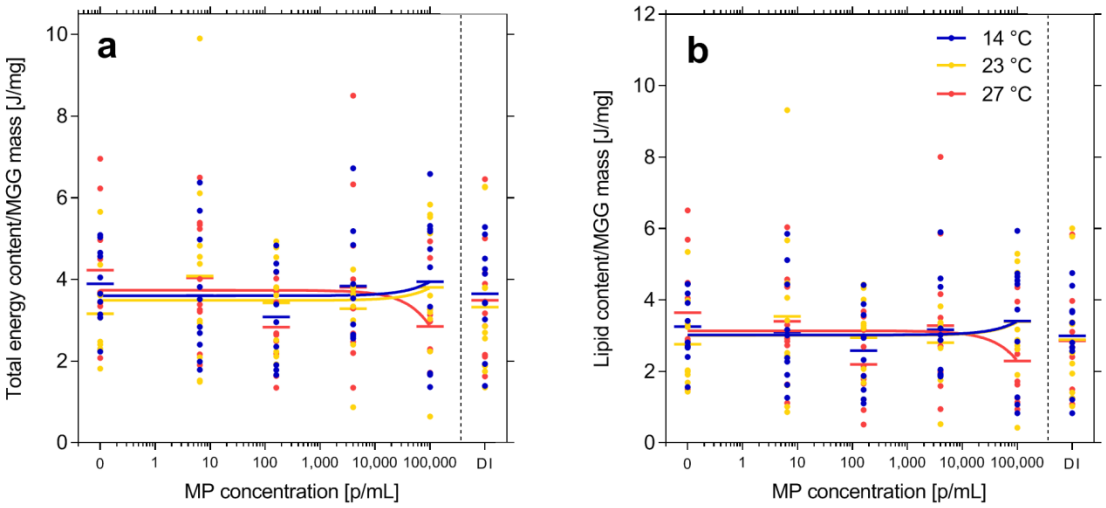

Fig. S5: Relative (a) total energy content and (b) total lipid content in the midgut gland (MGG) of *D. polymorpha* after an exposure to particle-free medium (Control, 0 p mL^{-1}), polystyrene microplastics (MP, 6.4–100,000 p mL^{-1}) or diatomite (DI, 100,000 p mL^{-1}) for 14 d at 14, 23 and 27 °C. Dots = results of each replicate, short lines = mean, regression = linear regression of the results in the MP treatment. n = 10 for each temperature and particle concentration.

2.2 Effects of microplastics versus natural particles

Tab. S2: Results of the general linear model and general linear mixed model (only between-subject results) analyzing the effects of temperature and particle type (microplastics vs. diatomite) on mussel activity, clearance activity (on day 3 and 10), energy reserves (total energy, proteins, glycogen, total lipids) and oxidative stress (TBARS, ORAC) in the midgut gland of *D. polymorpha* as well as the phagocytic activity of hemocytes. * = interaction term not included in the model

Variable		Endpoints									
		Mussel activity	Clearance (3 d)	Clearance (10 d)	Total energy	Proteins	Glycogen	Total lipids	TBARS	ORAC	Phagocytic activity
Temperature	*df*	2	2	2	2	2	2	2	2	2	2
	F	2.165	1.654	5.276	0.839	10.524	7.090	1.027	8.665	8.140	1.337
	p	0.316	0.201	**0.008**	0.438	**< 0.001**	**0.002**	0.365	**0.01**	**0.001**	0.280
Particle type	*df*	1	1	1	1	1	1	1	1	1	1
	F	0.101	3.868	1.221	0.013	3.228	2.279	0.092	0.077	5.688	0.447
	p	0.781	0.054	0.274	0.909	0.078	0.137	0.763	0.782	**0.021**	0.510
Temperature × Particle type	*df*	- *	- *	2	2	2	2	2	2	2	2
	F	- *	- *	0.442	0.758	0.628	0.322	0.751	1.523	1.734	0.820
	p	- *	- *	0.645	0.474	0.537	0.726	0.477	0.227	0.186	0.452

3 References

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A6. Zusätzliche Daten

Tabelle A1: Studien zum Vorkommen von Mikroplastik (MP) in Flüssen weltweit (Abb. 2). Details zu den Literaturangaben sind im Literaturverzeichnis enthalten. * = Die Studien gaben die von ihnen bestimmten MP Konzentrationen in P kg^{-1} Sediment an. Die angegebenen Konzentrationen wurden auf Basis einer Dichte von 2,17 kg m^{-3} (mittlere Dichte der Elbesedimente in Studie 1) in die Einheit P m^{-3} Sediment umgerechnet.

Nr.	Autoren	Studienort	MP-Konzentration [P m^{-3}] (Median)	MP-Konzentration [P kg^{-1}] (Median)
Flüsse (Wasserphase)				
1	Rodrigues et al. 2019	Douro (Portugal)	0,17	-
2	Dris et al. 2015 (Mantanetz)	Seine, Marne (Frankreich)	0,29	-
3	Tan et al. 2019	Bei Jiang (China)	0,33	-
4	Lechner et al. 2014	Donau (Österreich)	0,37	-
5	Yonkos et al. 2014	Patapsco, Magothy River, Rhode River und Corsica River (USA)	0,47	-
6	Kataoka et al. 2019	29 Flüsse in Japan	0,83	-
7	Baldwin et al. 2016	29 Nebenflüsse der Great Lakes (USA)	0,96	-
8	Vermaire et al. 2017 (Mantanetz)	Ottawa River (Kanada)	1,35	-
9	Kapp & Yeatman 2018 (Netz)	Snake River, Unterer Columbia River (USA)	1,44	-
10	Faure et al. 2015	Rhône (Frankreich)	2,30	-
11	Campbell et al. 2017	Prairie Creek (Kanada)	2,35	-
12	McCormick et al. 2016	Fünf Flüsse in Chicago (USA)	3,25	-
13	Mani et al. 2015	Rhein (Schweiz, Deutschland, Niederlande)	3,27	-
14	Moore et al. 2011	Coyote Creek, San Gabriel Creek, Los Angeles River (USA)	4,00	-
15	Mani et al. 2019	Rhein (Deutschland)	4,55	-
16	Studie 1	Elbe	5,11	-
17	van der Wal et al. 2015	Rhein (Niederlande), Dalålven (Schweden), Donau (Rumänien), Po (Italien)	5,80	-
18	Hoellein et al. 2017	Chicago River-Kanal (USA)	6,10	-
19	Cheung et al. 2019	Lam Tsuen (Hongkong, China)	6,97	-
20	Zhang et al. 2015	Yangtze (China)	9,36	-
21	McCormick et al. 2014	Chicago River-Kanal (USA)	9,94	-
22	Dris et al. 2015 (Planktonnetz)	Seine, Marne (Frankreich)	10,5	-
23	Heß et al. 2018	Rhein, Donau, Weser (Deutschland)	19,1	-
24	Lahens et al. 2018 (Fragmente)	Saigon (Vietnam)	23,0	-
25	Estahbanati & Fahrenfeld 2016	Raritan River (USA)	47,9	-
26	Forrest et al. 2019	Ottawa (Kanada)	60,0	-
27	Rodrigues et al. 2018	Rio Antuã (Portugal)	126,0	-
28	Vermaire et al. 2017 (Zufallsprobennehmer)	Ottawa (Kanada)	145,0	-
29	Kapp & Yeatman 2018 (Zufallsprobennehmer)	Snake River, Unterer Columbia River (USA)	552,0	-
30	Barrows et al. 2018	Gallatin River (USA)	600,0	-
31	Miller et al. 2017	Hudson River (USA)	802,5	-
32	Wang et al. 2017b	Hanjiang, Yangtze (China)	2.900	-
33	Schmidt et al. 2018	Teltow-Kanal, Britz-Kanal (Deutschland)	3.600	-
34	Zhu et al. 2019	Zufluss zur Maowei See (China)	3.700	-
35	Luo et al. 2019	Flüsse in Shanghai, Suzhou, Huangpu - Fluss (China)	3.740	-
36	Lin et al. 2018	Perlfluss (China)	4.152	-
37	Di et al. 2019	Han-Fluss, Dan Jiang (China)	7.850	-
38	Yan et al. 2019	Perlfluss (China)	30.988	-
39	Leslie et al. 2017	Rhein, Meuse, Kanäle in Amsterdam (Niederlande)	91.500	-
40	Lahens et al. 2018 (Fasern)	Saigon (Vietnam)	344.500	-
Flüsse (Sedimente)				
1	Castañeda et al. 2014	Sankt-Lorenz-Strom (Kanada)	$5{,}15 \times 10^{2}$	0,24
2	Nel et al. 2018	Bloukrans (Südafrika)	$2{,}88 \times 10^{4}$ *	13,3
3	Di et al. 2019	Han-Fluss, Dan Jiang (China)	$5{,}42 \times 10^{4}$ *	25,0
4	Dean et al. 2018	Nebenflüsse von Lake Erie (Kanada)	$9{,}10 \times 10^{4}$ *	42,0
5	Hoellein et al. 2017	Chicago River-Kanal (USA)	$1{,}48 \times 10^{5}$	68,4

6	Tibbetts et al. 2018	Tame (Vereinigtes Königreich)	$1{,}52x10^{5}$ *	70,0
7	Shruti et al. 2019	Rio Atoyac (Mexiko)	$2{,}17x10^{5}$ *	100,0
8	Su et al. 2018	Yangtze (China)	$3{,}47x10^{5}$ *	160,0
9	Horton et al. 2017	Themse, River Leach, River Lambourn, The Cut (Vereinigtes Königreich)	$5{,}99x10^{5}$ *	276,5
10	Wang et al. 2017a	Beijiang-Fluss (China)	$6{,}62x10^{5}$ *	305,5
11	Blair et al. 2019	Kelvin (Vereinigtes Königreich)	$7{,}06x10^{5}$ *	296,5
12	Rodrigues et al. 2018	Rio Antuã (Portugal)	$7{,}08x10^{5}$ *	327,0
13	Studie 1	Elbe	$7{,}57x10^{5}$ *	323,1
14	Wen et al. 2018	Fluss Jin, Longwanggang, Laodao-Fluss, Liuyang He (China)	$8{,}30x10^{5}$ *	383,3
15	Vermaire et al. 2017	Ottawa, Rideau River, Rideau-Kanal, Brewery Creek (Kanada)	$9{,}75x10^{5}$ *	450,0
16	Ballent et al. 2016	Red Hill Creek, Etobicoke Creek, Humber River, Don River (Kanada)	$1{,}04x10^{6}$ *	480,0
17	Klein et al. 2015	Rhein, Main (Deutschland)	$1{,}16x10^{6}$ *	537,5
18	Leslie et al. 2017	Rhein, Meuse, Kanäle in Amsterdam (Niederlande)	$1{,}71x10^{6}$ *	1.050
19	Peng et al. 2018a	Changjiang Ästuarsystem (China)	$2{,}04x10^{6}$ *	942,5
20	Hurley et al. 2018	Urbane, suburbane und ländliche Flüsse im Nordwesten Englands (Vereinigtes Königreich)	$2{,}71x10^{6}$ *	1.250
21	Lin et al. 2018	Perlfluss (China)	$1{,}05x10^{7}$ *	4.839
22	Wang et al. 2018	Wen-Rui Tang-Fluss (China)	$6{,}49x10^{7}$ *	29.985

Tabelle A2: Studien zum Vorkommen von Mikroplastik (MP) in marinen Ökosystemen weltweit (Abb. 2). Die angegebenen MP-Konzentrationen wurden aus den Reviews von Lusher (2015), Li (2018) und Rezania et al. (2018) entnommen. Bei Angabe mehrerer Konzentrationen wurde jeweils der Median bestimmt. Die Einzelquellnachweise für die angegebenen Literaturquellen sind im Literaturverzeichnis enthalten.

Nr.	Autoren	Studienort	Publizierte MP-Konzentration [P m^{-3}]
1	Colton et al. 1974	Karibik	$2{,}4x10^{-5}$
2	Law et al. 2010	Karibik	$2{,}8x10^{-4}$
3	Morris 1980	Cape Basin (Südatlantik)	$3{,}7x10^{-4}$
4	Carpenter & Smith 1972	Westliche Sargossasee	$7{,}1x10^{-4}$
5	Ryan 1988	Westkap (Südafrika)	$7{,}3x10^{-4}$
6	Reisser et al. 2013	Australische Küste	$8{,}5x10^{-4}$
7	Wilber 1987	Nordwestlicher Atlantik	$9{,}8x10^{-4}$
8	Eriksen et al. 2013a; 2013b	Südpazifischer subtropischer Wirbel	$5{,}4x10^{-3}$
9	Ivar do Sul et al. 2013	St. Peter Archipel, St. Paul Archipel (Brasilien)	$1{,}0x10^{-2}$
10	Carson et al. 2013	Südpazifischer subtropischer Wirbel	$1{,}7x10^{-2}$
11	Thompson et al. 2004	Nordatlantik	$2{,}5x10^{-2}$
12	Frias et al. 2014	Portugiesische Küste	$2{,}8x10^{-2}$
13	Ivar do Sul et al. 2014	Küste Brasiliens	$3{,}0x10^{-2}$
14	Isobe et al. 2017	Antarktis	$3{,}1x10^{-2}$
15	Yamashita & Tanimura 2007	Japanstrom	$3{,}4x10^{-2}$
16	Collignon et al. 2014	Bucht von Calvi, Korsika (Frankreich)	$6{,}2x10^{-2}$
17	Doyle et al. 2011	Beringsee	$9{,}7x10^{-2}$
18	Goldstein et al. 2012	Nordpazifik	0,12
19	Lucia et al. 2014	Golf von Oristano, Sardinien (Italien)	0,15
20	Lima et al. 2014	Goiana Ästuar (Brasilien)	0,26
21	Cole et al. 2014	Englischer Kanal (Vereinigtes Königreich)	0,27
22	Collignon et al. 2012	Nordwesten des Mittelmeeres	0,27
23	Zhang et al. 2017	Golf von Bohai (China)	0,33
24	Lusher et al. 2015	Arktische polare Gewässer	0,34
25	Magnusson 2014	Nordsee (Finnland)	0,37
26	Isobe et al. 2014	Seto-Binnenmeer	0,39
27	Cincinelli et al. 2017	Rossmeer, Antarktis	0,59
28	Castillo et al. 2016	Qatar	0,71
29	Kanhai et al. 2017	Atlantischer Ozean	1,15
30	Carpenter et al. 1972	Neuengland (USA)	1,29
31	Reisser et al. 2015	Nordatlantischer Wirbel	1,70
32	Lusher et al. 2014	Offene See um Irland	2,46
33	Isobe et al. 2015	Südostasiatisches Meer	3,70
34	van der Hal et al. 2017	Mittelmeerküste (Israel)	7,68
35	Di Mauro et al. 2017	Nördlicher Golf von Mexiko	11,6
36	Courtene-Jones et al. 2017a	Nordatlantischer Ozean (Schottland)	70,8
37	Norén & Naustvoll 2010	Skaggerak (Schweden)	102,0
38	Enders et al. 2015	Nordatlantischer Wirbel	257,0
39	Austin & Stoops-Glas 1977	Block Island Sound (USA)	278,5
40	Nel & Froneman 2015	Südostküste von Südafrika	736,5
41	Norén 2007	Westküste von Schweden	1.275,0
42	Zhao et al. 2014	Yangtze Ästuarsystem (China)	4.137,0
43	Desforges et al. 2014	Nordöstlicher Pazifischer Ozean	4.604,0
44	Song et al. 2014	Insel Geoje (Südkorea)	16.000,0
45	Song et al. 2015	Bucht von Jinhae (Südkorea)	182.000,0

Tabelle A3: Studien zum Vorkommen von Mikroplastik (MP) in Muscheln (Abb. 4). Details zu den Literaturangaben sind im Literaturverzeichnis enthalten.

Nr.	Autoren	Muschelarten	Herkunft	MP Anzahl pro Individuum [Anzahl Individuum^{-1}]	MP Anzahl pro Körpergewicht [Anzahl (g Körpergewicht)$^{-1}$]
1	Schessl et al. 2019	*Dreissena polymorpha*	Wildpopulation	0	-
2	Schessl et al. 2019	*Dreissena bugensis*	Wildpopulation	0	-
3	Railo et al. 2018	*Mytilus trossulus*	Wildpopulation	0,07	0,33
4	Lourenço et al. 2017	*Senilia senilis*	Wildpopulation	1,00	-
5	Domogalla-Urbansky et al. 2019	*Unio pictorum*	Wildpopulation	1,14	
6	Ding et al. 2019	*Mactra veneriformis*	Fischmarkt	1,20	0,31
7	Lourenço et al. 2017	*Dosinia isocardia*	Wildpopulation	1,50	-
8	Wu et al. 2020	*Ostrea denselamellosa*	Wildpopulation	1,67	0,31
9	Wu et al. 2020	*Sinonovacula constricta*	Wildpopulation	1,80	0,21
10	Ding et al. 2019	*Ruditapes philippinarum*	Fischmarkt	1,90	0,74
11	Digka et al. 2018	*Mytilus galloprovincialis*	Wildpopulation	0,85	3,90
12	Ding et al. 2019	*M. galloprovincialis*	Fischmarkt	1,60	0,29
13	Renzi et al. 2018	*M. galloprovincialis*	Muschelfarm	7,40	8,33
14	Renzi et al. 2018	*M. galloprovincialis*	Wildpopulation	3,00	7,20
15	Ding et al. 2018b	*M. galloprovincialis*	Fischmarkt	1,90	3,17
16	Ding et al. 2018b	*M. galloprovincialis*	Wildpopulation	0,53	2,00
17	Sparks 2020	*M. galloprovincialis*	Wildpopulation	3,40	2,80
18	Vandermeersch et al. 2015	*M. galloprovincialis*	Supermarkt	-	0,15
19	Gomiero et al. 2019	*M. galloprovincialis*	Wildpopulation (Küste)	-	1,82
20	Gomiero et al. 2019	*M. galloprovincialis*	Wildpopulation (Offene See)	-	0,95
21	Su et al. 2018	*Corbicula fluminea*	Wildpopulation	2,70	2,60
22	Sparks 2020	*Aulyacoma ater*	Wildpopulation	2,90	2,80
23	Hermabessiere et al. 2019	*Cerastoderma edule*	Wildpopulation	1,51	0,47
24	Lourenço et al. 2017	*C. edule*	Wildpopulation	4,30	-
25	Lourenço et al. 2017	*Scrobicularia plana*	Wildpopulation	3,30	-
26	Catarino et al. 2018	*Modiolus modiolus*	Wildpopulation	3,50	0,09
27	Naji et al. 2018	*Pinctada radiata*	Wildpopulation	3,90	0,10
28	Li et al. 2018a	*Saccostrea cucullata*	Wildpopulation	4,20	4,35
29	Zhu et al. 2019	*Crassostrea hongkongensis*	Wildpopulation	4,70	0,80
30	Sparks 2020	*Choromytilus meridionalis*	Wildpopulation	5,60	1,80
31	Naji et al. 2018	*Amiantis umbonella*	Wildpopulation	6,90	20,00
32	Patterson et al. 2019	*Magallana bilineata*	Wildpopulation	6,96	0,81
33	Naji et al. 2018	*Amiantis purpuratus*	Wildpopulation	7,10	12,70
34	Kazour et al. 2019	*Spondylus spinosus*	Wildpopulation	7,20	0,45
35	Waite et al. 2018	*Crassostrea virginica*	Wildpopulation	16,80	3,84
36	Keisling et al. 2020	*C. virginica*	Wildpopulation	0,72	-
37	Davidson & Dudas 2016	*Venerupis philippinarum*	Muschelfarm	11,30	1,70
38	Davidson & Dudas 2016	*V. philippinarum*	Wildpopulation	8,40	0,90
39	Ding et al. 2018b	*Chlamys farreri*	Fischmarkt	12,30	5,15
40	Catarino et al. 2017	*M. edulis*	Wildpopulation	12,60	2,50
41	Catarino et al. 2018	*M. edulis*	Käfigexposition	3,40	0,74
42	Catarino et al. 2018	*M. edulis*	Wildpopulation	3,20	3,00
43	Li et al. 2016b	*M. edulis*	Wildpopulation	4,60	2,70
44	Li et al. 2016b	*M. edulis*	Muschelfarm	3,30	1,60
45	Phuong et al. 2018a	*M. edulis*	Wildpopulation	0,60	0,23
46	Phuong et al. 2018b	*M. edulis*	Wildpopulation	0,93	0,23
47	Hermabessiere et al. 2019	*M. edulis*	Wildpopulation	0,77	0,20
48	Scott et al. 2019	*M. edulis*	Wildpopulation	4,54	-
49	Mathalon & Hill 2014	*M. edulis*	Muschelfarm	126,50	-
50	Mathalon & Hill 2014	*M. edulis*	Wildpopulation	89,30	-

51	Cho et al. 2019	*M. edulis*	Fischmarkt	-	0,12
52	Courtene-Jones et al. 2017b	*M. edulis*	Wildpopulation	-	2,75
53	van Cauwenberghe & Janssen 2014	*M. edulis*	Wildpopulation	-	0,20
54	Vandermeersch et al. 2015	*M. edulis*	Supermarkt	-	0,13
55	van Cauwenberghe & Janssen 2014	*M. edulis*	Muschelfarm	-	0,36
56	Witte et al. 2014	*M. edulis*	Wildpopulation	-	0,39
57	Witte et al. 2014	*M. edulis*	Supermarkt	-	0,35
58	Kazour & Amara 2020	*M. edulis*	Wildpopulation	-	1,14
59	Phuong et al. 2018a	*Crassostrea gigas*	Wildpopulation	2,10	0,18
60	Wang et al. 2019a	*C. gigas*	Wildpopulation	74,30	-
61	Martinelli et al. 2020	*C. gigas*	Wildpopulation	1,77	0,10
62	Cho et al. 2019	*C. gigas*	Fischmarkt	-	0,07
63	van Cauwenberghe & Janssen 2014	*C. gigas*	Supermarkt	-	0,47
64	Birnstiel et al. 2019	*Perna perna*	Wildpopulation	28,60	5,40
65	Berglund et al. 2019	*Anodonta anatina*	Wildpopulation	29,17	-
66	Li et al. 2015	*Patinopecten yessoensis*	Fischmarkt	57,20	-
67	Cho et al. 2019	*P. yessoensis*	Fischmarkt	-	0,08
68	Wang et al. 2019b	*Acila mirabilis*	Wildpopulation	110,00	6,90
69	Thushari et al. 2017	*Saccostrea forskalii*	Wildpopulation	-	0,46
70	Jahan et al. 2019	*Saccostrea glomerata*	Wildpopulation	-	0,32
71	Li et al. 2015	*Scapharca subcrenata*	Fischmarkt	-	10,5
72	Cho et al. 2019	*Tapes philippinarum*	Fischmarkt	-	0,34

Tabelle A4: Studien zur Toxizität von Mikroplastik (MP) in Muscheln. Darstellung der Studien, die zur Erstellung der SSD in Abb. 5 berücksichtig worden sind bzw. unter Angabe einer Begründung ausgeschlossen wurden. * = Die MP-Konzentration wurde auf Basis der publizierten Massenkonzentration sowie den Angaben zu Polymertyp und mittlerer Partikelgröße und unter Annahme einer sphärischen Partikelform berechnet. Ø = Durchschnitt.

Nr.	Autoren	Muschelarten	Polymertyp	MP-Form	MP-Größe/ Länge [µm]	Expositions–zeit	LOEC [P m^{-3}]	NOEC [P m^{-3}]
1	Browne et al. 2008	*Mytilus edulis*	PS	Sphären	3; 9,6	12 h		$4{,}3x10^{7}$
2	Magara et al. 2018	*M. edulis*	PE	Sphären	10-90	96 h	$1{,}0x10^{8}$	
3	Rist et al. 2019	*M. edulis*	PS	Sphären	2; 100	15 d	$9{,}6x10^{7}$	
4	Green et al. 2017	*M. edulis*	PLA/HD-PE	Fragmente	PLA: 0.6-363 (ø: 65,6); HD-PE: 0,48-316 (ø: 102,6)	50 d	$8{,}4x10^{5}$	$9{,}3x10^{4}$
5	Green et al. 2019	*M. edulis*	PLA/HD-PE	Fragmente	Vgl. Green et al. 2017	52 d	$8{,}4x10^{5}$	
6	Li et al. 2020	*M. edulis*	PVC	Fragmente	1-75	7 d		$2{,}0x10^{7}$
7	Magara et al. 2019	*M. edulis*	PE, Polyhydroxybutyrat (PHB)	Sphären	10-90	96 h	$1{,}0x10^{9}$	
8	Woods et al. 2018	*M. edulis*	PET	Fasern	459 ± 2,25	0-72 h		$3{,}0x10^{7}$
9	van Cauwenberghe et al. 2015	*M. edulis*	PS	Sphären	10; 30; 90	14 d		$1{,}1x10^{8}$
10	Balbi et al. 2017	*Mytilus galloprovincialis*	PS-NH_2	Sphären	0,05	48 h	$1{,}5x10^{14}$ *	$1{,}5x10^{13}$ *
11	Bråte et al. 2018	*M. galloprovincialis*	PE (unbehandelt/verwittert)	Fragmente	50-600	21 d		$1{,}9x10^{6}$
12	Capolupo et al. 2018	*M. galloprovincialis*	PS	Sphären	3	48 h	$5{,}0x10^{7}$	
13	Détrée & Gallardo-Escárate 2017	*M. galloprovincialis*	HD-PE	Sphären	1-50	24 h	$1{,}5x10^{10}$	
14	Franzellitti et al. 2019	*M. galloprovincialis*	PS	Sphären	3, 45	48 h – 4d	$1{,}0x10^{6}$	
15	González-Soto et al. 2019	*M. galloprovincialis*	PS	Sphären	0,5;4,5	26 d	$1{,}0x10^{9}$	
16	Gonçalves et al. 2019	*M. galloprovincialis*	PS	Sphären	2; 6; 10	12 – 48 h		$1{,}0x10^{9}$
17	Détrée & Gallardo-Escárate 2018	*M. galloprovincialis*	HD-PE	Sphären	1-50	18 d	$4{,}6x10^{8}$	
18	Pittura et al. 2018	*M. galloprovincialis*	LD-PE	?	20-25	28 d	$2{,}5x10^{9}$ *	
19	Bringer et al. 2020	*Crassostrea gigas*	HD-PE	Sphären	4-6; 11-13; 20-25	24 h	$3{,}0x10^{8}$	
20	Revel et al. 2020	*C. gigas*	PE, PP	Fragmente	0,4- 500	10 d		$1{,}1x10^{8}$
21	Sussarellu et al. 2016	*C. gigas*	PS	Sphären	2; 6	2 Monate	$1{,}8x10^{9}$	
22	Thomas et al. 2020	*C. gigas*	PS	Sphären	6	80 d	$1{,}0x10^{9}$	$1{,}0x10^{8}$
23	Tallec et al. 2018	*C. gigas*	PS, PS-COOH, PS-NH_2	Sphären	0,05; 0,5; 2	1.5 – 24 h	$1{,}5x10^{15}$ *	
24	Magni et al. 2018	*Dreissena polymorpha*	PS	Sphären	1; 10	6 d	$1{,}0x10^{9}$	
25	Studie 2	*D. polymorpha*	PS	Fragmente	< 63	42 d		$1{,}0x10^{11}$
26	Gaspar et al. 2018	*Crassostrea virginica*	PS	Sphären	0,05; 3	48 h		$7{,}3x10^{14}$ *
27	Luan et al. 2019	*Meretrix meretrix*	PS-COOH, PS-NH_2	Sphären	0,1; 0,2	24 h	$4{,}5x10^{12}$ *	
28	O'Donovan et al. 2018	*Scrobicularia plana*	LD-PE	?	11-13	14 d	$1{,}2x10^{9}$ *	

Nr.	Autoren	Muschelarten	Polymertyp	MP-Form	MP-Größe / Länge [µm]	Expositions-zeit	LOEC [P m^{-3}]	NOEC [P m^{-3}]
29	Ribeiro et al. 2017	*Scrobicularia plana*	PS	?	20	14 d	$2{,}3x10^{8}$	
30	Green 2016	*Scrobicularia plana*	PLA/HD-PE	?	Vgl. Green et al. 2017	60 d	$2{,}1x10^{6}$	$2{,}1x10^{4}$
31	Gardon et al. 2018	*Pinctada margaritifera*	PS	Sphären	6; 10	2 Monate	$1{,}8x10^{6}$ *	
32	Santana et al. 2018	*Perna perna*	PVC	?	0,1-10	90 d		$1{,}1x10^{13}$
33	Green 2016	*Ostrea edulis*	PLA/HD-PE	Fragmente	Vgl. Green et al. 2017	60 d		$3{,}1x10^{6}$
34	Green et al. 2017	*Ostrea edulis*	PLA/HD-PE	Fragmente	Vgl. Green et al. 2017	50 d	$1{,}9x10^{5}$	
35	Xu et al. 2017	*Atactodea striata*	PS	Fragmente	63-250	1 – 10 d	$1{,}0x10^{6}$	
36	Rochman et al. 2017	*Corbicula fluminea*	PET, PE, PVC, PS	Fragmente	PET: 12-704 (ø: 198); PE: 14-704 (ø: 209); PVC: 80-704 (ø: 169); PS: 68-704 (ø: 179)	28 d		$1{,}2x10^{6}$ *
37	Arossa et al. 2019	*Tridacna maxima*	PE	Sphären	53-500	12 d		$1{,}6x10^{6}$
38	Parolini et al. 2020	*Ruditapes philippinarum*	PET	Fragmente	8-1,054 (ø: 220)	7 d	$1{,}6x10^{6}$ *	$1{,}6x10^{4}$ *
39	Shi et al. 2020	*Tegillarca granosa*	PS	Sphären	0,5; 30	14 d	$2{,}0x10^{7}$ *	
40	Tang et al. 2020a	*Tegillarca granosa*	PS	Sphären	0,5; 30	4 d	$6{,}7x10^{7}$ *	
41	Wang et al. 2020a	*Mytilus coruscus*	PS	Sphären	2	7, 14 d	$1{,}0x10^{7}$	$1{,}0x10^{4}$
42	Xia et al. 2020	*Chlamys farreri*	PS	Sphären	2	15 d		$2{,}8x10^{10}$

Publikationen, die nicht zur Erstellug der SSD in Abb. 5 berücksichtigt wurden

	Autoren	Muschelarten	Gründe für den Ausschluss der Studien
43	Avio et al. 2015	*M. galloprovincialis*	Umrechnung von Massen- zu Partikelkonzentrationen nicht möglich
44	Baudrimont et al. 2020	*C. fluminea*	Umrechnung von Massen- zu Partikelkonzentrationen nicht möglich
45	Binelli et al. 2020	*D. polymorpha*	Keine Informationen zu MP Expositionskonzentrationen
46	Canesi et al. 2015	*M. galloprovincialis*	Zellexpositionen – keine Exposition von Muschelindividuen
47	González-Fernández et al. 2018	*C. gigas*	Zellexpositionen – keine Exposition von Muschelindividuen
48	Guilhermino et al. 2018	*C. fluminea*	Umrechnung von Massen- zu Partikelkonzentrationen nicht möglich
49	Magni et al. 2019b	*D. polymorpha*	Keine statistischen Ergebnisse in der Studie vorhanden
50	Magni et al. 2020	*D. polymorpha*	Statistik: Two-way ANOVA – keine Information zu NOEC, LOEC, LC_{50}, EC_{50}
51	Oliveira et al. 2018	*C. fluminea*	Umrechnung von Massen- zu Partikelkonzentrationen nicht möglich
52	Paul-Pont et al. 2016	*Mytilus spp.*	Gemischte Exposition von *M. edulis* and *M. galloprovincialis*
53	Revel et al. 2019	*Mytilus spp.*	Gattung jedoch keine Art bestimmt
54	Trestrail et al. 2020	*M. galloprovincialis*	Umrechnung von Massen- zu Partikelkonzentrationen nicht möglich
55	Moos et al. 2012	*M. edulis*	Umrechnung von Massen- zu Partikelkonzentrationen nicht möglich
56	Wegner et al. 2012	*M. edulis*	Keine statistischen Ergebnisse in der Studie vorhanden

Tabelle A4: Fortsetzung

Tabelle A5: Studie zum Vorkommen von Mikroplastik (MP) in Krebstieren (Abb. 6). Details zu den Literaturangaben sind im Literaturverzeichnis enthalten.

Nr.	Autoren	Krebsarten	Herkunft	MP Anzahl pro Individuum [Anzahl Individuum^{-1}]	MP Anzahl pro Körpergewicht [Anzahl (g Körpergewicht)$^{-1}$]
1	Hara et al. 2020	*Nephrops norvegicus*	Wildpopulation	1,75	-
2	Cau et al. 2019	*Nephrops norvegicus*	Wildpopulation	5,50	-
3	Cau et al. 2019	*Aristeus antennatus*	Wildpopulation	1,66	-
4	Devriese et al. 2015	*Crangon crangon*	Wildpopulation	1,23	0,68
5	McGoran et al. 2018	*Crangon crangon*	Wildpopulation	1,00	-
6	Abbasi et al. 2018	*Penaeus semisulcatus*	Wildpopulation	7,80	1,51
7	Horn et al. 2019	*Emerita analoga*	Wildpopulation	0,65	-
8	Costa et al. 2019	*Ocypode quadrata*	Wildpopulation	79,50	-
9	Iannilli et al. 2019	*Gammarus setosus*	Wildpopulation	72,50	-
10	Nan et al. 2020	*Paratya australiensis*	Wildpopulation	0,52	2,40
11	Wu et al. 2020	*Parapenaeopsis hardwickii*	Wildpopulation	0,95	0,25
12	Piarulli et al. 2019	*Carcinus aestuarii*	Wildpopulation	1,10	-
13	Weston et al. 2020	*Eurythenes plasticus*	Wildpopulation	0,75	-
14	Welden et al. 2018	*Maja squinado*	Wildpopulation	1,39	-
15	Wang et al. 2019b	*Cancer gibbosulus*	Wildpopulation	-	19,80
16	Wang et al. 2019b	*Crangon affinis*	Wildpopulation	-	8,60
17	Wang et al. 2019b	*Oregonia gracilis*	Wildpopulation	-	26,75
18	Thushari et al. 2017	*Balanus amphitrite*	Wildpopulation	-	0,33

Tabelle A6: Studien zur Toxizität von Mikroplastik (MP) in Krebstieren. Darstellung der Studien, die zur Erstellung der SSD in Abb. 7 berücksichtig worden sind bzw. unter Angabe einer Begründung ausgeschlossen wurden. * = Die MP-Konzentration wurde auf Basis der publizierten Massenkonzentration sowie den Angaben zu Polymertyp und mittlerer Partikelgröße und unter Annahme einer sphärischen Partikelform berechnet. Ø = Durchschnitt.

Nr.	Autoren	Krebsarten	Polymertyp	MP-Form	MP-Größe / Länge [µm]	Exposi-tionszeit	LC_{50}/EC_{50} [P m^{-3}]	LOEC [P m^{-3}]	NOEC [P m^{-3}]
1	Au et al. 2015	*Hyalella azteca*	PE/PP	PE: Sphären/ PP: Fasern	PE: 10-27 PP: 20-75	10 d		$4{,}5x10^{7}$	$2{,}3x10^{7}$
2	Blarer & Burkhardt-Holm 2016	*Gammarus fossarum*	PS/PA	PS: Sphären / PA: Fasern	PS: 1,6 PA: 500	28 d		$2{,}8x10^{8}$	
3	Straub et al. 2017	*Gammarus fossarum*	PHB/ PMMA	Fragmente	32-64/64-125/125-250	28 d		$3{,}3\ x10^{8}$	
4	Booth et al. 2016	*Corophium volutator*	PMMA	Sphären	0,125	10 d			$4{,}1x10^{17}$ *
5	Booth et al. 2016	*Daphnia magna*	PMMA	Sphären	0,125	24-48 h			$8{,}3x10^{17}$ *
6	Al-Jaibachi et al.	*D. magna*	PS	Sphären	15	84 d		$1{,}0x10^{8}$	
7	Chen et al. 2020	*D. magna*	PS	Sphären	5	24-72 h		$2{,}4x10^{10}$	
8	Eltemsah & Bøhn 2019	*D. magna*	PS	Sphären	6	21 d		$4{,}2x10^{10}$ *	
9	Kelpsiene et al. 2020	*D. magna*	PS-NH_2/ PS-COOH	Sphären	PS-NH_2: 53 PS-COOH: 26; 62	103 d		$2{,}4x10^{15}$ *	
10	Felten et al. 2020	*D. magna*	PE	Sphären	1-4	21 d		$1{,}3x10^{12}$ *	$1{,}3x10^{11}$ *
11	Jaikumar et al. 2018	*D. magna*	?/PE	Sphären/ Fragmente	?: 1-5 PE: 1-10	96 h			$3{,}2x10^{9}$
12	Jaikumar et al. 2019	*D. magna*	?/PE	Sphären/ Fragmente	?: 1-5 PE: 1-10	21 d		$1{,}0x10^{8}$	
13	Kokalj et al. 2019	*D. magna*	PE	Fragmente	Ø: 140	24-96 h			$7{,}3x10^{7}$ *
14	Kalčíková et al.	*D. magna*	PE	Fragmente	Ø: 180,5	48 h			$1{,}6x10^{7}$
15	Vicentini et al. 2019	*D. magna*	PS	Sphären	0,0901	21 d			$1{,}5x10^{16}$ *
16	Felice et al. 2019	*D. magna*	PS	Sphären	1; 10	21 d		$2{,}3x10^{8}$ *	
17	Lin et al. 2019a	*D. magna*	PS/PS-NH_2/ PS-COOH	Sphären	PS: 0,1 PS-NH_2: 0,05; 0,1; 0,11 PS-COOH: 0,3	48 h		$1{,}8x10^{15}$ *	
18	Sadler et al. 2019	*D. magna*	PS-COOH	Sphären	0,5	< 21 d		$1{,}5x10^{10}$	
19	Schrank et al. 2019	*D. magna*	PVC (hart, flexibel)	Fragmente	Hart: 4-141 (ø: 29) Flexibel: 12-276 (ø: 51)	26-31 d		$4{,}3x10^{7}$	
20	Lin et al. 2019b	*D. magna*	PS	Sphären	0,1	48 h	$9{,}1x10^{15}$ *		
21	Zhang et al. 2020a	*D. magna*	PS-NH_2/ PS-COOH	Sphären	1	48 h	$1{,}5x10^{13}$ *		
22	Kokalj et al. 2018	*D. magna*	PE/PET	Sphären/ Fragmente/ Fasern	PE-Sphären: 183; 103; 63; 264/PE- Fragmente: ø: 137/ PET-Fasern: 100-500 (ø: 20)	48 h			$5{,}1x10^{9}$

Tabelle A6: Fortsetzung

Nr.	Autoren	Krebsarten	Polymertyp	MP-Form	MP-Größe /Länge [µm]	Expositions-zeit	LC_{50}/EC_{50} [P m^{-3}]	LOEC [P m^{-3}]	NOEC [P m^{-3}]
23	Imhof et al. 2017	*D. magna*	Mix A: PA, PC, PET, PVC/ Mix B: ABS, Polyoxymethylene (POM), PVC, Styrol-Acrylnitril-Copolymer (SAN)	Fragmente	Mix A: PC: ø:28; PVC: ø:28; PET: ø:39; PA: ø:73/ Mix B: ABS: ø:31; POM: ø:28; PVC: ø:49; SAN: ø:22	22 d		$5,8x10^{8}$	
24	Mattsson et al. 2017	*D. magna*	PS-NH_2/PS-COOH/ PS-SO_3H/PMMA	?	PS-NH_2: 0,052,; 0,053; 0,057,;0,058; 0,12; 0,18; 0,33/PS-COOH: 0,026; 0,06; 0,092; 0,16; 0,19; 0,22/ PS-OSO3H; 0,025; 0,2/ PMMA: 0,068; 0,140	24 h		$6,4x10^{17}$ *	$3,2x10^{17}$ *
25	Ma et al. 2016	*D. magna*	PS	Sphären	0,05; 0,5; 5; 10; 15	48 h	$2,2x10^{17}$ *		
26	Saavedra et al. 2019	*D. magna*	PS-NH_2/PS-COOH	Sphären	0,2	48 h	$8,2x10^{15}$ *		
27	Renzi et al. 2019	*D. magna*	PE/PP/PVC	Fragmente	10-100	96 h		$6,0 x10^{8}$ *	
28	Frydkjær et al. 2017	*D. magna*	PE	Sphären / Fragmente	Sphären: 10-106 Fragmente: 10-75	48 h	$1,7x10^{9}$ *		
29	Rist et al. 2017	*D. magna*	PE	Sphären	0,095; 2,37	24 h		$3,1x10^{14}$	
30	Wu et al. 2019b	*D. magna*	PS/PS-NH_2/PS-COOH	Sphären	PS: 0,1/PS-NH_2: 0,05-0,1; 0,11/ PS-COOH: 0,3	48 h		$1,3x10^{15}$ *	
31	Zhang et al. 2019b	*D. magna*	PS/PS-NH_2/PS-COOH	Sphären	0,05-0,1	24-48 h		$4,3x10^{16}$ *	
32	Zhang et al. 2019c	*D. magna*	PS	Sphären	1; 10	48 h		$1,8x10^{11}$	
33	Rehse et al. 2016	*D. magna*	PE	Sphären	1-4; 90-106	96 h		$2,9x10^{12}$ *	$1,5x10^{12}$
34	Kim et al. 2017	*D. magna*	PS/PS-COOH	Sphären	PS: 0,202/ PS-COOH: 0,191	48 h		$5,2x10^{10}$	$2,6x10^{10}$
35	Al-Jaibachi & Callaghan 2018	*D. magna*	PS-COOH	Sphären	2	21 d		$2,5x10^{9}$ *	
36	Besseling et al. 2014	*D. magna*	PS	Sphären	0,07	21 d		$1,7x10^{17}$ *	
37	Booth et al. 2016	*D. magna*	PMMA	Sphären	0,125	24-48 h			$8,3x10^{17}$ *
38	Canniff & Hoang 2018	*D. magna*	PE	Sphären	63-75	21 d			$7,6x10^{9}$
39	Chae et al. 2018	*D. magna*	PS	Sphären	0,051	48-72 h			$1,4x10^{17}$ *
40	Rehse et al. 2018	*D. magna*	PA	Fragmente	5-50	24-48 h			$7,9x10^{10}$ *
41	Horton et al. 2018	*D. magna*	PS	Sphären	1,2	72 h			$3,0x10^{11}$
42	Tang et al. 2019	*D. magna*	PS	Sphären	1,25	10 d		$1,9x10^{12}$ *	
43	Martins & Guilhermino 2018	*D. magna*	?	Sphären	1-5	4 Generationen		$1,8x10^{10}$	
44	Ogonowski et al. 2016	*D. magna*	?/PE	Sphären / Fragmente	?: 4,1/PE: 2,6	10 d		$3,0x10^{10}$	
45	Pacheco et al. 2018	*D. magna*	?	Sphären	1-5	21 d		$1,9x10^{9}$ *	
46	Green 2016	*Idotea balthica*	HD-PE/PLA	?	HD-PE: 0,48-316 (ø: 102,6)/ PLA: 0,6-363 (ø: 65,6)	48 d		$2,1x10^{6}$	$2,1x10^{4}$
47	Studie 3	*Gammarus pulex*	PET	Fragmente	< 150	60 d			$3,5x10^{10}$

Tabelle A6: Fortsetzung

Nr.	Autoren	Krebsarten	Polymertyp	MP-Form	MP-Größe / Länge [µm]	Exposition-zeit	LC_{50}/EC_{50} [P m^{-3}]	LOEC [P m^{-3}]	NOEC [P m^{-3}]
48	Beiras et al. 2019b	*Acartia clausi*	PVC	Fragmente	ø: 7,9	48 h		$8,6x10^{9}$ *	
49	Beiras et al. 2018	*A. clausi*	PE	?	4-6	48 h			$4,7x10^{11}$ *
50	Beiras et al. 2019a	*A. clausi*	PE	Sphären	4-6	48-96 h			$1,6x10^{11}$ *
51	Yu et al. 2018	*Eriocheir sinensis*	PS	Sphären	5	21 d		$5,4x10^{8}$	
52	Liu et al. 2019a	*E. sinensis*	PS	Sphären	5	21 d		$5,4x10^{8}$	
53	Bergami et al. 2016	*Artemia franciscana*	PS-COOH/PS-NH_2	Sphären	PS-COOH: 0,04/PS-NH_2: 0,05	48 h			$2,8x10^{15}$ *
54	Sendra et al. 2020	*A. franciscana*	PS	Sphären	0,1	3, 5, 24 h			$1,1x10^{15}$ *
55	Kokalj et al. 2018	*A. franciscana*	PE/PET	Sphären / Fragmente/ Fasern	PE-Sphären: 183; 103; 63; 264/ PE-Fragmente: ø: 137/ PET-Fasern: 100-500 (ø: 20)	48 h		$7,8x10^{6}$	
56	Bergami et al. 2017	*A. franciscana*	PS-COOH/PS-NH_2	Sphären	PS-COOH: 0,04/PS-NH_2: 0,05	14 d	$1,2x10^{16}$ *		
57	Varó et al. 2019	*A. franciscana*	PS-NH_2	Sphären	0,05	14 d		$1,5x10^{15}$ *	
58	Gambardella et al. 2017	*A. franciscana*	PS	Sphären	0,1	48 h		$1,8x10^{12}$ *	
59	Gambardella et al. 2017	*Amphibalanus amphitrite*	PS	Sphären	0,1	48 h		$1,8x10^{12}$ *	
60	Jeong et al. 2017	*Paracyclopina nana*	PS	Sphären	0,05; 0,5; 6	24 h		$1,7x10^{11}$ *	
61	Lee et al. 2013	*Tigriopus japonicus*	PS	Sphären	0,05; 0,5; 6	14 d		$1,1x10^{9}$ *	
62	Choi et al. 2019	*T. japonicus*	PS	Sphären	0,05; 10	24-48 h		$3,6x10^{10}$	
63	Zhang et al. 2019a	*T. japonicus*	PS	Sphären	6	2 Generationen		$1,9x10^{9}$	$1,9x10^{8}$
64	Cui et al. 2017	*Daphnia galeata*	PS	Sphären	0,052	5 d		$6,5x10^{16}$ *	
65	Cole et al. 2015	*Calanus helgolandicus*	PS	Sphären	20	9 d		$7,5x10^{7}$	
66	Coppock et al. 2019	*C. helgolandicus*	PE/PA/PET	Sphären / Fragmente/ Fasern	PE-Sphären: 10-27; 20-27; 27- 32/ PA-Fragmente: 20 PA-Fasern: 40; 100 PET-Fasern: 60; 70	24 h		$1,0x10^{8}$	
67	Watts et al. 2016	*Carcinus maenas*	PS/PS-COOH/PS-NH_2	Sphären	8	1-24 h		$1,0x10^{10}$	
68	Dawson et al. 2018	*Euphausia superba*	PE	Sphären	27-32	10 d			$1,2x10^{8}$
69	Liu et al. 2018	*Daphnia pulex*	PS	Sphären	0,075	48 h		$4,3x10^{14}$ *	
70	Liu et al. 2019b	*D. pulex*	PS	Sphären	0,075	21 d		$5,3x10^{13}$	
71	Liu et al. 2020a	*D. pulex*	PS	Sphären	0,075	4 Generationen		$4,3x10^{12}$ *	
72	Liu et al. 2020b	*D. pulex*	PS	Sphären	0,075	21 d		$5,3x10^{13}$	
73	Liu et al. 2020c	*D. pulex*	PS	Sphären	0,075	3 Generationen		$4,3x10^{12}$ *	
74	Wu et al. 2019a	*D. pulex*	PS	Sphären	0,075	21 d		$5,3x10^{13}$	
75	Zhang et al. 2020b	*D. pulex*	?/PE	Sphären/Fragmente	0,075	21 d			

Tabelle A6: Fortsetzung

Nr.	Autoren	Krebsarten	Polymertyp	MP-Form	MP-Größe /Länge [µm]	Expositions-zeit	LC_{50}/EC_{50} [P m^{-3}]	LOEC [P m^{-3}]	NOEC [P m^{-3}]
76	Jaikumar et al. 2019	*D. pulex*	?/PE	Sphären/Fragmente	?: 1-5/PE: 1-10	21 d		$1{,}0x10^{8}$	
77	Jaikumar et al. 2018	*D. pulex*	?/PE	Sphären /Fragmente	?: 1-5/PE: 1-10	96 h			$1{,}0x10^{7}$
78	Ziajahromi et al. 2017	*Ceriodaphnia dubia*	Polyester (PES)/PE	Fasern/Sphären	PES-Fasern: 280/PE-Sphären: 1-4	8 d	$3{,}5x10^{6}$		
79	Jaikumar et al. 2019	*C. dubia*	?/PE	Sphären/Fragmente	?: 1-5/PE: 1-10	7 d		$1{,}0x10^{8}$	
80	Jaikumar et al. 2018	*C. dubia*	?/PE	Sphären /Fragmente	?: 1-5/PE: 1-10	96 h			$4{,}0x10^{9}$
81	Wang et al. 2019d	*Artemia parthenogenetica*	PS	Sphären	10	14 d			$1{,}0x10^{9}$
82	Wang et al. 2019c	*A. parthenogenetica*	PS	Sphären	10	24 h		$1{,}0x10^{8}$	$1{,}0x10^{7}$
83	Heindler et al. 2017	*Parvocalanus crassirostris*	PET	Fragmente	< 11	24 d		$2{,}0x10^{10}$	
84	Beiras et al. 2018	*Tigriopus fulvus*	PE	?	1-4; 4-6	48 h		$1{,}2x10^{11}$ *	$1{,}2x10^{10}$ *
85	Cole et al. 2013	*Centropages typicus*	PS	Sphären	7,3	24 h		$4{,}0x10^{9}$	
86	Syberg et al. 2017	*Acartia tonsa*	PE	Sphären	10-90	48 h			$2{,}5x10^{10}$
87	Bellas & Gil 2020	*A. tonsa*	PE	?	1,4-42 (ø: 7,73)	48 h			$2{,}5x10^{9}$
88	Vroom et al. 2017	*Calanus finmarchicus*	PS	Sphären	15	11 d			$5{,}0x10^{8}$
89	Cole et al. 2019	*C. finmarchicus*	PA	Sphären/Fasern	Sphären: 10-30/Fasern: 30	6 d		$5{,}0x10^{7}$	
90	Mishra et al. 2019	*Artemia salina*	PS	Sphären	0,071; 0,122	24 h	$2{,}5x10^{16}$ *		
91	Maharana et al. 2020	*Litopenaeus vannamei*	PE	Sphären	1.000	72 h		$3{,}0x10^{2}$ *	$9{,}9x10^{1}$ *
92	Saavedra et al. 2019	*Thamnocephalus platyurus*	PS-NH_2, PS-COOH	Sphären	0,2	24 h	$4{,}4x10^{16}$ *		
93	Wang et al. 2020b	*Neomysis japonica*	PS, PS-COOH	Sphären	5	96 h		$1{,}5x10^{8}$ *	

Publikationen, die <u>nicht</u> zur Erstellug der SSD in Abb. 7 berücksichtigt wurden

	Autoren	Krebsarten	Gründe für den Ausschluss der Studien
94	Devriese et al. 2017	*Nephrops norvegicus*	MP Exposition über die Nahrung – eine Bestimmung der MP Konzentration in der Wasserphase ist nicht möglich
95	Watts et al. 2015	*Carcinus maenas*	MP Exposition über die Nahrung – eine Bestimmung der MP Konzentration in der Wasserphase ist nicht möglich
96	Welden & Cowie 2016	*N. norvegicus*	MP Exposition über die Nahrung – eine Bestimmung der MP Konzentration in der Wasserphase ist nicht möglich
97	Panko et al. 2013	*Hyalella azteca*,	MP Exposition über das Sediment – eine Bestimmung der MP Konzentration in der Wasserphase ist nicht möglich
98	Bruck & Ford 2018	*Echinogammarus marinus*	MP Exposition über die Nahrung – eine Bestimmung der MP Konzentration in der Wasserphase ist nicht möglich
99	Chua et al. 2014	*Allorchestes compressa*	Umrechung von Massen- zu Partikelkonzentrationen nicht möglich
100	Hämer et al. 2014	*Idotea emarginata*	MP Exposition über die Nahrung – eine Bestimmung der MP Konzentration in der Wasserphase ist nicht möglich
101	Jemec et al. 2016	*D. magna*	Keine statistischen Ergebnisse in der Studie vorhanden

Tabelle A6: Fortsetzung

Nr.	Autoren	Krebsarten	Gründe für den Ausschluss der Studien
102	Nasser & Lynch 2016	*D. magna*	Keine statistischen Ergebnisse in der Studie vorhanden
103	González-Pleiter et al. 2019	*D. magna*	Umrechung von Massen- zu Partikelkonzentrationen nicht möglich
104	Grav & Weinstein 2017	*Palaemonetes pugio*	Statistik: Generalisiertes lineares Modell; kein statistischer Vergleich zwischen den einzelnen Testgruppen
105	Bosker et al. 2019	*D. magna*	Statistik: Lineares mixed Modell; kein statistischer Vergleich zwischen den einzelnen Testgruppen
106	Galloway et al. 2017	*Thamnocephalus platyurus*	Umrechung von Massen- zu Partikelkonzentrationen nicht möglich
107	Colomer et al. 2019	*D. magna*	Keine statistischen Ergebnisse in der Studie vorhanden
108	Redondo-Hasselerharm et al. 2018a	*Gammarus pulex, Hyalella azteca, Asellus aquaticus*	MP Exposition über das Sediment – eine Bestimmung der MP Konzentration in der Wasserphase ist nicht möglich
109	Redondo-Hasselerharm et al. 2018b	*G. pulex, A. aquaticus*	MP Exposition über das Sediment – eine Bestimmung der MP Konzentration in der Wasserphase ist nicht möglich
110	Peixoto et al. 2019	*Artemia franciscana*	Statistik: Korrelationen; kein statistischer Vergleich zwischen den einzelnen Testgruppen
111	Carrasco et al. 2019	*Orchestoidea tuberculata*	MP Exposition über die Nahrung – eine Bestimmung der MP Konzentration in der Wasserphase ist nicht möglich
112	Chae et al. 2019	*Litopengaeus vannamei*	Indirekte Exposition der Krebse durch den Fraß von MP-kontaminiertem Muschelgewebe
113	Gerdes et al. 2019	*D. magna*	MP-Toxizität in einer Multi-Stressor-Exposition – keine Angabe zur Toxizität von MP als Einzelstressor
114	Korez et al. 2019	*I. emarginata*	MP Exposition über die Nahrung – eine Bestimmung der MP Konzentration in der Wasserphase ist nicht möglich
115	Kratina et al. 2019	*G. pulex*	Statistik: Regressionsanalyse; kein statistischer Vergleich zwischen den einzelnen Testgruppen
116	Mateos-Cárdenas et al. 2019	*Gammarus duebeni*	Indirekte Exposition der Krebse durch den Fraß von MP-kontaminierten Algen (*Lemna* spp.)
117	Sørensen et al. 2020	*G. duebeni*	MP-Toxizität in einer Multi-Stressor-Exposition – keine Angabe zur Toxizität von MP als Einzelstressor
118	Yardy & Callaghan 2020	*Gammarus pulex*	MP Exposition über die Nahrung – eine Bestimmung der MP Konzentration in der Wasserphase ist nicht möglich
119	Zocchi & Sommaruga 2019	*D. magna*	Statistik: Two-way ANOVA; kein direkter statistischer Vergleich zwischen den einzelnen Testgruppen

Tabelle A7: Studien zu Multi-Stressor-Effekten von Mikroplastik (MP) und einem zweiten Stressor bei gleichzeitiger Exposition. Dabei werden die Effekte (sortiert nach Endpunkt) wie folgt kategorisiert: ① Die Stressoren bewirken weder einzeln noch in Kombination einen signifikanten Effekt im Vergleich zur Kontrolle. ② Mindestens ein Stressor bewirkt in der Einzelexposition einen signifkanten Effekt und in einer gemeinsamen Exposition ist der Effekt geringer (oder kehrt sich sogar um) im Vergleich zur Addition beider Effekte. ③ Mindestens ein Stressor bewirkt in der Einzelexposition einen signifikanten Effekt und in einer gemeinsamen Exposition addieren sich die Effekte. ④ Mindestens ein Stressor bewirkt in der Einzelexposition einen Effekt und in einer gemeinsamen Exposition ist der Effekt höher als die Summe der Einzeleffekte. Endpunkte wurden teilweise mehreren Kategorien zugeordnet, sofern die Co-Exposition der Stressoren mehrfach mit verschiedenen Konzentrationen der Stressoren durchgeführt wurde und die jeweiligen beobachteten Effekte voneinander abwichen. Ø = Durchschnitt.

Autoren	Art	Stressor 1	Stressor 2	Expos.-Dauer	Beobachtete Effekte[6]
Muscheln					
Brandts et al. 2018	*Mytilus galloprovin-cialis*	Carbama-zepine ,Cbz, 6,3 µg L^{-1}	PS-Sphären , 106 ± 10 nm, 0.05 mg L^{-1}	96 h	① Endpunkte: TOS (MDD, KM, Häm.), TAC (MDD, KM, Häm.), ChE (MDD, KM), AST (MDD, Häm.), ALT (MDD, Häm.), Glucose (MDD, KM, Häm.), EA (MDD), LPO (MDD, KM) ② Endpunkte: ChE (Häm.), AST (KM), ALT(KM), DNA-Schädigung ③ Endpunkte: EA (KM) ④ Endpunkte: -
Guilhermino et al. 2018	*Corbicula fluminea*	Florfenicol, FL, 1,8 und 7,1 mg L^{-1}	PS-Sphären, 2 µm, 0,2 und 0,7 mg L^{-1})	96 h	① Endpunkte: LPO (AM, Fuß, MDD), ODH (Fuß), GST (KM, MDD), GR (KM, MDD), GPx (MDD) ② Endpunkte: - ③ Endpunkte: ChE-Aktivität, IDH (Fuß) ④ Endpunkte: Fraßaktivität, IDH (Fuß), LPO (Fuß), GST (KM, MDD), GR (MDD)
Li et al. 2020	*Mytilus edulis*	Cd^{2+}, 200 µg L^{-1}	PVC-Fragmente, 1-75 µm, 20 P mL^{-1}	7 Tage	① Endpunkte: - ② Endpunkte: LMS ③ Endpunkte: - ④ Endpunkte: -
Magara et al. 2018	*Mytilus edulis*	Fluoranthen, Flu, 50 und 100 µg L^{-1}	PE-Sphären, 10–90 µm, 100 und 1.000 P mL^{-1}	96 h	① Endpunkte: GR (KM) ② Endpunkte: SOD (KM, MDD), CAT (KM, MDD), GPx (KM, MDD), Se-GPx (KM, MDD), GST (KM, MDD), GR (MDD) ③ Endpunkte: CAT (KM), SeGPx (KM), GR (MDD) ④ Endpunkte: -
Oliveira et al. 2018	*Corbicula fluminea*	Quecksilber-chlorid, Hg^{2+}, 0,13 mg L^{-1}	MP-Sphären, Polymer unbekannt, 1-5 µm, 30 µg L^{-1}	14 Tage	① Endpunkte: - ② Endpunkte: Filtrationsrate, ChE, IDH, GR, GPx, GST, LPO ③ Endpunkte: CAT ④ Endpunkte: -

Tabelle A7: Fortsetzung

Autoren	Art	Stressor 1	Stressor 2	Expos.-Dauer	Beobachtete Effekte[6]
Paul-Pont et al. 2016	*Mytilus spp.*	Fluoranthen, Flu, 30 µg L^{-1}	PS-Sphären, 2 und 6 µm, 2.000 P mL^{-1}	7 Tage	① Endpunkte: Hämozytenkonzentration, Granulozytenkonzentration, GST ② Endpunkte: Hämozytenviabilität, ROS Produktion, Phagozytoseaktivität, GR, LPO ③ Endpunkte: Catalase, SOD ④ Endpunkte: -
Tang et al. 2020b	*Tegillarca granosa*	Bisphenol A, BPA, 10 und 100 ng L^{-1}	PS-Sphären, ø: 490 nm, 1 mg L^{-1}	14 Tage	① Anteil Hyalinozyten ② Hämozyten-Anzahl, Ach (Häm.) ③ Hämozyten-Anzahl, Anteil rote Granulozyten, Hämozytenphagozytose, GABA (Häm.), DOP (Häm.), Ach (Häm.) ④ Anteil basophile Granulozyten
Sikdokur et al. 2020	*Ruditapes philippina-rum*	Hg^{2+}, 10 µg L^{-1}	PE-Sphären, 10-45 µm, 25 µg L^{-1} = 144 P mL^{-1}	7 Tage	① CAT (KM, MDD)/ LPO (KM, MDD)/ GSH (KM, MDD) ② Filtrationsrate, Hämozytenviabilität ③ - ④ -
Xia et al. 2020	*Chlamys farreri*	Decabromo-diphenylether, BDE-209, 10 und 100 µg L^{-1}	PS-COOH-Sphären, 2 µm, 125 µg L^{-1} = 2,84×10^4 P mL^{-1}	15 Tage	① - ② - ③ Hämozytenphagozytose ④ Hämozytenphagozytose, DNA-Schäden
Schnecken					
Horton et al. 2020	*Lymnaea stagnalis*	Polybromierte Diphenylether (PBDE-47/99/ 100/153, 94-3.000 ng g Sediment^{-1})	Polyamid-6-Fragmente, 13-19 µm, 1 %, w/w Sediment	96 h	① Endpunkte: Diversität des Mikrobioms ② Endpunkte: Änderung des Feuchtgewichts ③ Endpunkte: Änderung des Feuchtgewichts ④ Endpunkte: -
Qu et al. 2020	*Cipango-paludian cathayensis*	Methamphe-tamine, rac-M, 0,1-50 mg L^{-1}	PS-Sphären, 20 mg L^{-1}, 700 nm	96 h	① Endpunkte: - ② Endpunkte: Fraßaktivität ③ Endpunkte: - ④ Endpunkte: Caspase-3

[6] Abkürzungen: KM (Kieme), MDD (Mitteldarmdrüse), AD (Adduktormuskel), GPx (Glutathionperoxidase), LPO (Lipidperoxidation), CAT (Katalase), ODH (Octopindehydrogenase), GR (Glutathionreduktase), ChE (Cholinesterase), IDH (Isocitratdehydrogenase), GST (Glutathion-S-transferase), Natriumdehydrogenase (SOD), Se-GPx (Se-Glutathionperoxidase), ROS (Reaktive Sauerstoffspezies), TOS (Totaler Oxidationsstatus), Häm. (Hämolymphe), TAC (Totale antioxidative Kapazität), AST (Aspartataminotransferase), ALT (Alanintransaminase), EA (Esteraseaktivität), GABA (ɣ-Aminobuttersäure), AchE (Acetylcholinesterase), DOP (Dopamin), LMS (Lysosomenmembranstabilität)