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Micro- and mesoplastics in the northern Baltic Sea: their fate in the seafloor and effects on benthic fauna

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MICRO- AND MESOPLASTICS IN THE NORTHERN BALTIC SEA: THEIR FATE IN THE SEAFLOOR AND EFFECTS ON BENTHIC FAUNA

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Marine litter, especially microplastics (plastic fragments < 5 mm), has been a subject of increasing interest in recent decade, due to its ubiquitous distribution in the marine environment. Most marine litter will eventually sink to the seafloor, and many field studies to date have confirmed the accumulation of microplastics in fine-grained soft sediments. The numbers of microplastics in the environment are expected yet to increase; thus, the seafloor sediments represent both current and future hotspots for microplastic pollution, making it important to investigate the fate and potential impacts of plastic litter in these habitats.

In this thesis, the interactions between microplastics, the benthic invertebrate community and harmful contaminants were examined in four different mesocosm studies that together shed light on how the size, properties (polymer type and associated contaminants) and vertical distribution of plastics on the seafloor may affect the benthic fauna. The most common benthic invertebrates in the northern Baltic Sea, the Baltic clam *Limecola balthica*, polychaete *Marenzelleri*a spp. and amphipod *Monoporeia affinis*, were selected for the experiments that investigated how the activities of the benthic community shape the vertical distribution of microplastics in the sediment. A follow-up study further examined the bacterial communities developing on the surface of different biodegradable (cellulose acetate, poly-L-lactic acid) and conventional (polyamide, polystyrene) mesoplastics together with the capacity of plastics to sorb polycyclic aromatic hydrocarbons (PAHs) from the sediment. Lastly, the effects of acute (5 days) and chronic (29 days) exposure to tyre rubber fragments on *L. balthica* were examined, using a suite of biomarkers and cell ultrastructural examination of clam tissues.

The results demonstrate that bioturbation by common benthic fauna buried microplastics in the sediment up to a depth of 5 cm and at the same time reduced their bioavailability to the invertebrates feeding from the sediment surface. In the experiments, 25% of the exposed clams ingested microplastics from the sediment surface, but the availability of microplastics decreased with depth; only 1% of the clams were found to ingest microplastics that were placed at depths of 2–5 cm in the sediment. In addition to the location of the microplastics, their bioavailability was also governed by the species-specific particle-size range for ingestion. Furthermore, the redistribution of buried microplastics at the sediment surface by bioturbation was

negligible, supporting the hypothesis of seafloors acting as a final sink for microplastics.

When incubated in the sediments, the bacterial communities developed on biodegradable cellulose acetate diverged from the other polymer types examined and harboured potentially biodegrading bacteria. The results also showed that all the polymer types examined sorbed PAHs from the sediments, but had varying PAH sorption capacities, indicating that if ingested, the microplastics' role as PAH vectors is dependent on the polymer type. However, comparison of the PAH concentrations in plastics and in the sediment also suggested that the ingestion of plastics is not likely to increase the PAH burden of deposit-feeders. In contrast, the contaminants already present in microplastics may pose elevated risk for benthic fauna, as was found in the study carried out with tyre rubber. Both PAHs and trace metals were quantified from the tyre rubber, and the clams exposed to an environmentally relevant concentration of tyre rubber fragments exhibited multiple sublethal responses, indicating oxidative stress and damage to vital cellular structures.

In essence, this thesis provides novel information that contributes to fulfilling the current knowledge gaps regarding the fate and impacts of microplastics on the seafloor, and will further aid in assessing the potential risks microplastics pose to the benthic fauna, especially in the study area of the northern Baltic Sea. It remains unclear whether the impacts of microplastics could span from the individual to the population dynamics and ecosystem functioning, but the results obtained call for further research on the complex interactions taking place in the seafloor to better understand the impacts of microplastics on the marine environment.

LIST OF ABBREVIATIONS

1-MNP	1-methylnapthalene
2-MNP	2-methylnapthalene
2,3,5-TMN	2,3,5-trimethylnapthalene
2,6-DMN	2,6-dimethylnapthalene
ABS	acrylonitrile butadiene styrene
ACE	acenaphthene
AChE	acetylcholinesterase
ACY	acenaphthylene
ADS	antioxidant defence system
ANOVA	analysis of variance
ANT	anthracene
ASV	amplicon sequence variant
BaA	benzo[a]anthracene
BaP	benzo[a]pyrene
BbF	benzo[b]fluoranthene
BeP	benzo[e]pyrene
BghiP	benzo[g,h,i]perylene
BkF	benzo[k]fluoranthene
BL	blebbed nuclei
BN	binucleated cells
BNb	binucleated cells with nucleoplasmic bridges
CA	cellulose acetate
CAT	catalase
CHR	chrysene
CI	condition index
DahA	dibenzo[a,h]anthracene
DG	digestive gland
dw	dry weight
EPS	expanded polystyrene
FLA	fluoranthene
FLU	fluorene
GC	gas chromatography
GPx	glutathione peroxidase
GR	glutathione reductase
GSH	glutathione
GSSG	glutathione disulphide
HDPE	high-density polyethylene
HMW	high molecular weight
HOC	hydrophobic organic compound
IcdP	indeno[c,d]pyrene
ICP-MS	inductively coupled plasma-mass spectrometry
LDPE	low-density polyethylene
LMS	lysosomal membrane stability
LMW	low molecular weight
LOQ	limit of quantification
LPO	lipid peroxidation
MN	micronuclei
MS	mass spectrometry
MSFD	Marine Strategy Framework Directive
MW	molecular weight
NB	nuclear buds
NBf	nuclear buds on filament
NP	naphthalene
PA	polyamide
PAH	polycyclic aromatic hydrocarbon
PBDE	polybrominated diphenylether
PCB	polychlorinated biphenyl
PCR	polymerase chain reaction
PER	perylene
PET	polyethylene terephthalate
PHA	polyhydroxyalkanoate
PHE	phenanthrene
PLLA	poly-L-lactic acid
PP	polypropylene
PS	polystyrene
PVC	polyvinyl chloride
PYR	pyrene
ROS	reactive oxygen species
SBR	styrene-butadiene rubber
SOD	superoxide dismutase
TRI	triphenylene
TXRF	total-reflection X-ray fluorescence spectrometry
TZS	Tvärminne Zoological Station
VKL	Vanhankaupunginlahti
WH	West Harbour
ww	wet weight

CONTENTS

1 INTRODUCTION

1.1 Marine litter – a global concern

Marine litter, defined as "*any persistent, manufactured or processed solid material discarded, disposed of or abandoned in the marine and coastal environment*" (UNEP 2009), is a ubiquitous threat to the environment that also affects the economy, health and society. It consists of a variety of different materials, such as plastic, metal, glass, paper, cardboard, wood, ceramics, rubber and textiles (UNEP 2009). Marine litter has raised wide concern, due to its deleterious effects on the environment, especially wildlife. To date, marine litter is known to affect over 800 species among many groups of wildlife, and the number is predicted to rise (Kühn et al. 2015, CBD 2016). Not only does marine litter threaten wildlife and disturb ecological processes, it can also cause economic and societal losses. The direct economic costs of marine litter are related, e.g. to its removal from the environment and repairing the damage it causes to the fishing industry, marine traffic or human health (Newman et al. 2015). Some of the impacts are harder to assess in economic terms; these include loss of revenue, recreation values, biodiversity and important ecosystem services (McIlgorm et al. 2011, Newman et al. 2015).

While the harm caused by visible macrolitter has been recognized and on stage since the 1970s (reviewed by Ryan 2015), microlitter rose into the limelight only after Thompson described tiny pieces of plastics found in the marine waters and sediments of the Northeast Atlantic (Thompson et al. 2004, Frias & Nash 2019). At the same time, finding the "Great Pacific Garbage Patch" in the North Pacific Ocean (Moore et al. 2001) raised interest in the abundance and impacts of marine litter in our oceans (Ryan 2015). Shortly after, in 2008, the European Union Marine Strategy Framework Directive (MSFD; Directive 2008/56/EC) included microlitter in one of its 11 qualitative descriptors. The MSFD strives to achieve Good Environmental Status in EU marine waters, requiring the member states to monitor both macro- and microlitter and ensure that litter does not cause harm to the coastal and marine environment. This, at the latest, gave rise to a completely new field of study – microlitter and microplastic research.

1.2 From nano to mega – definitions and properties of plastic litter

The concerns related to marine litter revolve around plastics, since they form most of all marine litter (Pham et al. 2014, Galgani et al. 2015, Agamuthu et al. 2019). Their predominance as a material among other types is a consequence of many factors: wide application in society, the ever-growing production volumes of plastics, irresponsible consumer behaviour, lack of proper waste-management practices and resistance to degradation (Andrady 2015, PlasticsEurope 2020). The production

of plastics has grown exponentially since their invention: while in 1950 the global plastic production was 1.7 million tonnes, in 2019 the production volume had reached 368 million tonnes (PlasticsEurope 2013, 2020). The amount of plastic litter in the environment goes hand in hand with its production volumes and is expected to still rise in future decades (Law & Thompson 2014, Koelmans et al. 2016).

Most plastics are produced from petroleum, and currently approximately 6% of the world's oil production is exploited for their production (Ellen MacArthur Foundation et al. 2016). The percentage of bioplastics (i.e. plastics produced from biological, renewable sources) in the global market is still less than 1%, but their production is expected to increase and diversify over the forthcoming years (Verbeek & Uitto 2017, European Bioplastics 2020). The renewable resources in bioplastic production include e.g. starch, cellulose, plant oils and polyhydroxyalkanoates (PHAs) produced by bacteria (Chen 2010, Iwata 2015), but they are mostly a solution to the imminent crude oil depletion, not directly to marine plastic pollution. Despite being made of renewable sources, the properties of the final plastic product are not influenced by which raw material is utilized (Sudesh & Iwata 2008). To mitigate the accumulation of plastic litter in the environment, the material must be fully biodegradable under the conditions in which it ends up; as such, it can be converted to carbon dioxide, water and biomass by microorganisms (Song et al. 2009).

In general, plastics are synthetic or semisynthetic organic compounds that consist of polymers and different additives (Lithner et al. 2011). The polymers, in turn, consist of monomers that form polymer chains or three-dimensional networks in a process called polymerization. The most common monomers include ethylene, propylene, vinyl chloride and styrene, each having its own unique chemical structures and characteristics (Lithner et al. 2011, PlasticsEurope 2020) (Table 1). The specific gravity of different polymers is one of the most important factors affecting their distribution in the marine environment. Approximately 60% of the plastic produced has a lower density than seawater (1.025 g/cm^3), and hence tends to float on the sea surface or is carried to shore, whereas denser plastics are suspended in the water column or sink to the seafloor (Andrady 2011). During manufacturing of plastics, additives are mixed with the polymer to modify the properties of the final product. Thousands of different additives are used in plastic compounding, and include fillers, plasticizers, flame retardants, colorants, ultraviolet (UV) stabilizers, thermal stabilizers and processing aids (Lithner et al. 2012, Andrady & Rajapakse 2019)

While a systematic, universal framework for defining and categorizing marine plastic litter is lacking, according to a recent proposal by Hartmann et al. (2019) plastic litter should be defined as "*objects consisting of synthetic or heavily*

modified natural polymers as an essential ingredient that, when present in natural environments without fulfilling an intended function, are solid and insoluble at 20 °C". This definition comprises a wide spectrum of artificial or modified polymer materials that may have similar environmental behaviour, such as slow degradation, or similar hazardous properties or biological effects in the environment. However, sometimes this definition conflicts with the classical definitions of polymer science that do not consider e.g. elastomeric materials to be plastics (ISO 472:2013). Since a universal definition is yet to be agreed on, in this thesis the definition by Hartmann et al. (2019) is adopted due to its simplicity and usefulness in describing marine plastic litter; it also enables fragments of tyre rubber and polymer-containing paints to be included under the plastic litter umbrella. This approach is also taken by the European Chemical Agency (ECHA) in defining microplastics for regulatory purposes (ECHA 2019).

Further classification of plastic litter usually takes into account the size, shape, structure and colour of the items and, if possible, the purpose of use (e.g. fishing-related debris). Furthermore, in the case of microplastics, they are typically categorized into primary and secondary, based on their origin; primary microplastics are intentionally manufactured to be of small size, whereas secondary microplastics are formed by fragmenting from larger plastic items (GESAMP 2015). Size is an environmentally relevant parameter because it considerably affects the particle's environmental fate (e.g. sinking behaviour; Chubarenko et al. 2016) and interaction with biota, but currently there is no clear consensus on size categories (Hartmann et al. 2019). In this thesis, a traditional division stretching from nano- (< 1 µm), micro- (1 µm – 5 mm), meso- (5 mm – 25 mm), macro- (25 mm – 1 m) to megaplastics (> 1 m) is used (HELCOM 2015, GESAMP 2019).

Table 1. Some common polymer types found in the marine environment, the specific gravities (g/cm^3) of the virgin resins and their use in various products (Andrady 2011, Claessens et al. 2013, GESAMP 2015, Wypych 2016, PlasticsEurope 2020).

Polymer type	Abbreviation	Specific gravity	Purpose
Polyethylene (low-density)	LDPE	0.91–0.93	Bags, trays and containers, agricultural film, food packaging
Polyethylene (high-density)	HDPE	0.94	Toys, milk and shampoo bottles, pipes, houseware
Polypropylene	PP	0.85–0.83	Food packaging, microwave containers, pipes, ropes
Polystyrene	PS	1.04–1.09	Food packaging, building insulation, electrical equipment
Polystyrene (expanded)	EPS	0.01–1.05	Floats, foam cups
Polyamide/nylon	PA	1.13–1.15	Fishing nets, lines and traps, ropes
Polyethylene terephthalate	PET	1.34–1.39	Beverage bottles
Polyvinyl chloride	PVC	1.16–1.42	Window frames, floor and wall covering, cable insulation
Acrylonitrile butadiene styrene	ABS	1.03–1.09	Hub caps
Cellulose acetate	CA	1.22–1.24	Cigarette filters

1.3 Sources and pathways of plastic litter

Annually 4.8–12.7 million tonnes of plastic waste are estimated to enter the marine environment, and without improvements in waste-management infrastructures, the quantity will continue to increase (Jambeck et al. 2015). In general, most of the plastic litter (~80%) is derived from land-based sources, such as illegal dumping and inadequate waste management, coastal tourism as well as packaging, agriculture, construction and plastic recycling sectors (UNEP 2005, 2016, Galgani et al. 2015, Sebille et al. 2016). The rest originates from sea-based sources including fisheries, aquaculture, commercial shipping and offshore industries, maritime-based tourism and other recreational activities (UNEP 2005, 2016, Loulad et al. 2017, Lebreton et al. 2018).

The sources of microplastics compare partly with the sources of macroplastics, since secondary microplastics are derived from large litter items, due either to fragmentation in the environment, or because of the wear and tear of plastic items and plastic-containing products (Andrady 2017, Hann et al. 2018). Fragmentation is a result of plastic degradation, which is defined as being any change in the physical or chemical properties of plastic that is induced by chemical, physicochemical (photodegradation, thermal degradation, mechanical degradation) or biological processes (Fotopoulou & Karapanagioti 2019). Fragmentation starts typically by photodegradation (Fotopoulou & Karapanagioti 2019), in which UV radiation initiates changes e.g. in the surface properties (micro-cracking) and decreases the average molecular weight (MW) of the plastic (Andrady 2015). Photodegradation together with thermal and mechanical degradation result in the weathering and embrittlement of the material, leading ultimately to the generation of microplastics (Andrady 2015, 2017). One of the single largest sources of microplastics is estimated to be road traffic, which generates fragments from tyres undergoing friction on the road (Kole et al. 2017, Hann et al. 2018, Wagner et al. 2018). The sources of primary microplastics, in turn, are related to the plastic production sector (unintentional leakages of pellets and powders from industrial processes) and also come from products where they are intentionally added (e.g. cosmetics and personal-care products, paints, 3D printing powders) (UNEP 2016, Boucher & Friot 2017).

Once the litter enters the ocean, its ownership is lost; thus, plastic pollution represents yet another example of “the tragedy of the commons”, in which the responsibility of the problem becomes clouded. Although many sources of marine litter have been recognized to date, their emission volumes are extremely difficult to assess. This is particularly a problem for microplastics, whose source becomes practically untraceable as soon as they enter the environment. Moreover, the relative importance of different sources is not clear and is likely to vary, depending on the location. One important factor

determining the significance of different sources in littering is the pathway by which the litter from the source is transported to the sea. The pathways of plastic litter include rivers, stormwaters, direct release and dumping, winds, surface runoff, snow dumping and municipal wastewaters (Jambeck et al. 2015, Talvitie et al. 2017, Schmidt et al. 2017, Setälä & Suikkanen 2020, Ziajahromi et al. 2020). As with the relative importance of different sources, the relative importance of different pathways also varies geographically, further hampering the assessment of emissions.

1.4 Seafloors as pollution hotspots

Since most plastics are lightweight, they have long residence times in surface waters and can be transported far from their source (Ryan et al. 2009, Schernewski et al. 2020). Plastic litter has been found at the water surface and in sea ice, the water column, seafloor and at beaches (Thompson et al. 2004, Vianello et al. 2013, Stolte et al. 2015, Peeken et al. 2018, Zobkov et al. 2019). The physical environment governing the distribution of plastics include winds, waves, currents and density-stratification of the water column (Chubarenko et al. 2016, Kane et al. 2020, Uurasjärvi et al. 2021), but biological processes are also involved. For example, the density of a litter item can change as a result of biofilm formation (i.e. organisms colonizing the surface of a plastic), subsequently leading to sinking (Lobelle & Cunliffe 2011, Kooi et al. 2017). In the case of microplastics, their transport to the seafloor can also occur inside faecal pellets of animals ingesting plastics (Cole et al. 2013, Katija et al. 2017) or when incorporated into descending phytoplankton aggregates (Long et al. 2015).

Seafloors are proposed to act as sinks for marine plastic debris (Barnes et al. 2009, Eriksen et al. 2014, Cózar et al. 2014, Woodall et al. 2014). The abundance and composition of macroscopic plastic litter on the seafloor varies spatially and is dependent e.g. on the water depth, strengths of the bottom currents, wave action, seabed structure and proximity to different sources (UNEP 2016). In coastal areas, litter densities (including plastic) range from 0 to >7700 items per km^2 (Galgani et al. 2015, Loulad et al. 2017, Maes et al. 2018). Benthic litter commonly accumulates in areas of low circulation (Ioakeimidis et al. 2014) and, similarly, microplastics are often also most abundant in areas characterized by weak water flow and fine-grained sediments (Strand et al. 2013, Vianello et al. 2013, Maes et al. 2017, Willis et al. 2017, Enders et al. 2019, Sun et al. 2021). Environmental sampling has confirmed that microplastics found on the seafloor include plastic types that typically are positively buoyant in seawater (Claessens et al. 2011, Vianello et al. 2013, Sun et al. 2021), supporting the hypothesis of the seafloor as an ultimate sink for marine microplastics (Woodall et al. 2014).

The microplastics found in marine sediments vary with size, shape, polymer type, and origin, but most are

secondary fragments derived from larger plastic items (Shim et al. 2018). Similar to larger plastic litter, microplastics are spread from shallow coastal areas (Thompson et al. 2004, Claessens et al. 2011, Vianello et al. 2013) to deep-sea floors (Van Cauwenberghe et al. 2013, Woodall et al. 2014, Fischer et al. 2015, Bergmann et al. 2017, Courtene-Jones et al. 2020). Their concentrations are spatially highly variable, even at adjacent sampling sites (Tirroniemi 2019, Barrett et al. 2020, Pagter et al. 2020), which potentially reflects the heterogenous mosaiclike patterns of these habitats that also shape the spatial structuring of benthic communities (Kraan et al. 2009). However, in general the concentrations are quite high: 42–6595 microplastics per kg sediment (dw; dry weight) have been observed in the Arctic Ocean (Bergmann et al. 2017), and even 12 000–200 000 microplastics per kg sediment (dw) in an urban fjord in Norway (Haave et al. 2019). Concentrations of similar magnitude also seem to be common in other sea areas (Barrett et al. 2020, Abel et al. 2021), and typically microplastic abundance in sediments is inversely related to their size (Bergmann et al. 2017, Tirroniemi 2019, Haave et al. 2019, Courtene-Jones et al. 2020).

Not only microplastics but also various anthropogenic contaminants accumulate in seafloor sediments (HELCOM 2010a). These include both older types of contaminants, such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), heavy metals (e.g. Hg, Pb) and pesticides (e.g. dichlorodiphenyltrichloroethane [DDT]) as well as newer modern contaminants, such as triclosan, organotin compounds and polybrominated diphenylethers (PBDEs) (Heim & Schwarzbauer 2013). Since many of these substances are hydrophobic, they associate strongly and descend to the seafloor with the settling organic matter (Hedman et al. 2008). Similar to microplastics, they tend to accumulate in depositional low-energy environments characterized by weak current and wave action, which raises questions about the potential interactions of microplastics and various contaminants. For example, it is known that hydrophobic organic compounds (HOCs) present in the water have high affinity for the amorphous regions of organic matter, similar to the amorphous regions in plastics (Teuten et al. 2009, Rochman et al. 2013b), and in seawater the sorption of various HOCs to different plastic types has been shown in both field and experimental studies (e.g. Endo et al. 2005; Mato et al. 2001; Rochman et al. 2013a). A field adsorption experiment with plastic pellets revealed significantly higher concentrations of PCBs and dichlorodiphenyldichloroethylene (DDE) in microplastics than in the ambient seawater (Mato et al. 2001). This has led to suggestions that plastics could act as vectors for HOCs (Teuten et al. 2009, Hartmann et al. 2017).

In the sediment, the concentrations of HOCs typically exceed the concentrations in seawater, and while

the use of many older contaminants has declined or been completely prohibited in recent decades, the remnants of past pollution have not yet disappeared; they are still accumulated and preserved in the sediment records (HELCOM 2010a, Heim & Schwarzbauer 2013). It is unknown how the increasing plastic loads in marine waters and sediments interact with the historical and current pools of HOCs. If plastics act as their adsorption surfaces, the sorption of HOCs to plastic litter may shape their availability in the environment and transport to biota. Furthermore, many additives present in the polymer matrix, such as bisphenol-A (BPA) and PBDEs, are known to be harmful to the environment and can desorb from plastics (Teuten et al. 2009, Lithner et al. 2011, 2012).

1.5 Processes affecting the fate of plastics on the seafloor

When microplastics sink to the seafloor, they are often subject to various activities performed by the benthic fauna. The animals in and on the seafloor continuously modify the physical, chemical and biological properties of their surroundings in a process called bioturbation (Rhoads 1974, Aller 1982, Lohrer et al. 2004). Bioturbation covers all activities of benthic fauna, such as burrowing, feeding, defecation and ventilation, that directly or indirectly affect the sediment structure by transporting solutes or particles in the sediment-water interface (Kristensen et al. 2012). Bioturbation plays a vital role, e.g. in carbon and nutrient cycling, in increasing oxygen penetration into the sediment, as well as in the metabolism, dispersion and burial of marine pollutants (Rhoads 1974, Snelgrove 1998). This reworking is most intensive within the upper parts of the sediment surface (Rhoads 1974) and can hence act as an important process affecting the small-scale spatial distribution of microplastics in the sediment.

The ability of the benthic macrofauna to affect particle mixing within the sediment is dependent on various species-specific characteristics, such as their mobility, depth of residence and feeding behaviour (particularly feeding mode and particle selection) (Rhoads 1974, François et al. 1997, Gerino et al. 2007, Kristensen et al. 2012). It is generally believed that the smaller the particles are, the more easily they are moved by the benthic fauna, either via ingestion or other activities, such as locomotion or tube building (Rhoads 1974, Wheatcroft 1992). The species involved in bioturbation processes can be classified into five broad categories called functional groups: biodiffusers, upward conveyors, downward conveyors, gallery-diffusers and regenerators (François et al. 1997; Michaud et al. 2006; see Fig. 1). Biodiffusers are often clams that move sediment particles randomly over short distances (François et al. 1997). Polychaetes are typically upward or downward conveyors: the upward conveyors occupy the sediment head down and transport sediment from the deeper layers to the sediment surface by ingesting sediment; the downward

conveyers transport particulate material in the opposite direction (François et al. 1997). Many polychaetes create complex burrow networks in the surficial sediment layer and cause diffusive local mixing; thus, they are called gallery-diffusers (Kristensen et al. 2012). The regenerators are typically crabs that release sediment to the overlying water, and their abandoned burrows are filled with surface sediment (Kristensen et al. 2012). These functional groups affect the direction, distance and extent of particle transport, and since the benthic community composition varies in different sea areas, benthic animals likely also have varying impact on the vertical distribution of microplastics on the seafloor.

Bioturbation also modifies the chemical and microbiological environment of the plastics. Abiotic conditions, such as the presence of oxygen, affect the fate of plastic litter, especially their degradation potential (Andrady 2015). Although the current abiotic conditions can either accelerate or decelerate the degradation processes, the roles played by various processes affecting plastic litter degradation in different marine environments are still poorly understood. It is generally believed that plastic degradation predominantly

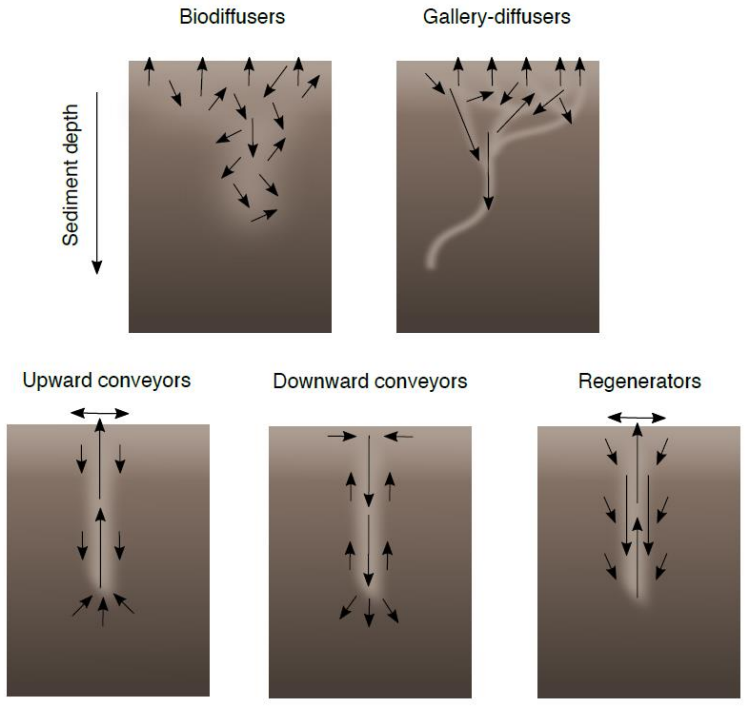


Figure 1. Effects of different functional groups on sediment reworking. The arrows represent the movement of particles. Redrawn and modified from François et al. (1997) and Michaud et al. (2005).

occurs at the sea surface and on the beaches, where exposure to UV radiation and temperature changes are higher. Therefore, as the plastic reaches the seafloor, the dark, cold and sometimes oxygen-deprived conditions prevailing at depths efficiently slow down many degradation processes (e.g. photodegradation, thermal degradation), resulting in slow accumulation of plastic on the seafloor (Andrady 2011, 2015).

Biodegradation is part of the degradation process and typically follows the aforementioned abiotic degradation (Jacquin et al. 2019). In biodegradation, the organic carbon of plastic is converted into biogas and biomass by microorganisms (bacteria, fungi) colonizing the plastic surface (Shah et al. 2008). Biodegradation is a stepwise process consisting of four phases: biodeterioration, which modifies the structure of the polymer matrix; biofragmentation of the polymer chain by the enzymes secreted by the microorganisms; assimilation, in which small oligomers are taken up by the cells to be used as a carbon source and mineralization, in which completely oxidized metabolites (e.g. H_2O, CO^2, CH^4) are excreted (Lucas et al. 2008, Fotopoulou & Karapanagioti 2019).

The polymer type and other properties of plastic litter (e.g. surface properties) influence their colonization and subsequent breakdown by microorganisms (Artham et al. 2009, Oberbeckmann & Labrenz 2020). Whereas in some cases certain biodegradable plastics do show signs of degradation in the benthic environment (Eich et al. 2015), other studies have failed to find differences between the degradation of conventional and biodegradable plastics in marine sediments (Nauendorf et al. 2016). Although potentially plastic-degrading microorganisms exist in the oceans (Wright et al. 2020b), the rate of degradation is expected to be too slow to remove plastics and prevent them from accumulating in the environment (Andrady 2015, Oberbeckmann & Labrenz 2020). In the absence of degrading forces, the plastics may be preserved on the seafloor for decades or even centuries (Goldberg 1997), causing long-lasting harm to benthic ecosystems. Due to their ubiquitous presence and persistence in seafloor deposits, they have even been proposed for use as stratigraphic indicators for the Anthropocene – a geological epoch characterized by human influence on the environment (Zalasiewicz et al. 2016).

1.6 Impacts of plastics on the benthos

The accumulation of plastic litter on the seafloor has raised concerns for the possible impacts of plastics within these environments. Globally, most of the seafloor consists of fine-grained, soft sediments (Rhoads 1974), and benthic animals living in these habitats form the largest faunal assemblage on the planet by areal coverage (Snelgrove 1998). It is therefore possible that benthic communities will encounter and become exposed to high quantities of plastics during their

lifetime (Van Cauwenberghe et al. 2015). In addition, the biodiversity hotspots on deep-sea floors may even be regarded as microplastic hotspots, due to the currents supplying oxygen, nutrients – and microplastics – to these areas (Kane et al. 2020).

Plastic litter alters benthic environments and, hence, the living conditions of benthic fauna, e.g. by smothering benthic fauna and the seafloor (Mordecai et al. 2011, Green et al. 2015), inhibiting gas exchange in the sediment-water interphase (Suhrhoff & Scholz-Böttcher 2016), altering nitrogen-cycling processes in sediments (Seeley et al. 2020) and leaching out harmful substances (Suhrhoff & Scholz-Böttcher 2016). They can also directly entangle marine organisms (Kühn et al. 2015). Of particular concern is the ingestion of microplastics. Most animals living in muddy sediments are deposit-feeders (Shull 2009), which may be particularly vulnerable to microplastic pollution (Depledge et al. 2013). The surface sediments may even be passed through them multiple times per year (Rhoads 1974), and therefore, it is not surprising that evidence for microplastics invading benthic food webs already exists (Van Cauwenberghe et al. 2015, Taylor et al. 2016). In addition to deposit-feeders, many other taxa living in the benthic habitat and utilizing different feeding modes (e.g. suspension-feeders) ingest microplastics (Setälä et al. 2016b). Ingestion of microplastics in the environment has been observed in benthic fauna, including cnidarians (Iliff et al. 2020), bivalves (Davidson & Dudas 2016), gastropods (Courtene-Jones et al. 2017), annelids (Van Cauwenberghe et al. 2015), crustaceans (Hara et al. 2020), echinoderms (Courtene-Jones et al. 2017) and demersal fish (Lusher et al. 2013). Laboratory experiments indicate that microplastics can also move up from one trophic level to another via predator-prey interactions (Farrell & Nelson 2013).

When ingested, microplastics may cause internal physical damage, such as abrasions or blockages in the gastrointestinal tract (Wright et al. 2013) or decrease the food assimilation efficiency (Blarer & Burkhardt-Holm 2016). It has also been suggested that microplastics could induce damage caused by the harmful ingredients and substances in the plastic material. These harmful substances can be divided into ingredients of the plastic material (e.g. residual monomers, different additives), by-products of manufacturing (e.g. solvents, initiators, catalysts and other polymerization additives), and chemicals sorbed from the environment (e.g. PAHs, PCBs) (Andrady 2011, Rochman 2015) that are not covalently bound and thus have the capacity to desorb or leach out from the polymer matrix (Lithner et al. 2011, 2012, Hartmann et al. 2017). Toxicological responses caused by plastics may be related to some of or a combination of all these chemicals (de Ruijter et al. 2020), and the exposure can be either direct (i.e. when the organism is in contact with the particle) or indirect (i.e. through the aqueous phase containing desorbed contaminants) (Hartmann et al. 2017).

The impacts of microplastics are commonly investigated at the organismal level or below (e.g. tissues, cells, molecules). Acute mortality in response to microplastic exposure is seldom reported, but various sublethal effects have been detected (reviewed by de Sá et al. 2018). Sublethal effects can be assessed, using biomarkers, i.e. changes in the biological responses of an organism that are related to exposure or toxic effects of environmental chemicals (Peakall 1994). In general, biomarkers can be visible behavioural reactions (e.g. reduced burrowing; Archambault et al. 2013), physiological responses (e.g. increased oxygen consumption; Martins et al. 2007) or cellular and subcellular responses (e.g. changes in enzyme activity; Valavanidis et al. 2006). Multiple biomarkers representing different functions and levels of organization are commonly examined concurrently to evaluate the response of the organism to a certain contaminant.

The animal's exposure to contaminants increases the formation of reactive oxygen species (ROS) that can cause oxidative damage to proteins, lipids and DNA in the cells (Livingstone et al. 1990, Valavanidis et al. 2006). The cells are protected by a specific antioxidant defence system (ADS) that tries to compensate for the harmful effects by scavenging ROS; if this neutralization is incomplete, the animal experiences oxidative stress (Davies 1995). Therefore, changes in the activity of the ADS enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR), are often examined as an indication of oxidative stress. Fighting to counteract the destructive effects of contaminants reduces cellular energy stores and may eventually lead to less energy for growth and reproduction (Trestrail et al. 2020). Since biomarker responses can be detected relatively soon after exposure, they act as early warning signs that may signal potential impacts at higher organizational levels (population, community, ecosystem) that would require longer periods of time to observe (Fig. 2).

The biomarker approach has been taken in many studies investigating the impacts of microplastics on different organisms (e.g. Avio et al. 2015; O'Donovan et al. 2018; Oliveira et al. 2013; Ribeiro et al. 2017; Santana et al. 2018; von Moos et al. 2012). The detected effects may include oxidative damage in the form of increased lipid peroxidation (LPO), DNA strand breaks (genotoxicity) and changes in ADS (especially SOD, CAT and GPx activities) (reviewed by Prokić et al. 2019). Furthermore, microplastics can alter energy metabolism by inhibiting the activity of enzymes participating in the citric-acid cycle, produce neurotoxic effects by decreasing acetylcholinesterase (AChE) activity, and cause histological changes and subsequent inflammatory effects (von Moos et al. 2012, Prokić et al. 2019).

However, the results gained from different studies are sometimes contradictory and are highly dependent on the polymer type, particle size, concentration and associated contaminants (Prokić et al.

2019). Currently, the weight of evidence for many environmental impacts of microplastics is limited, partly due to the discrepancy in particle type, size, shape or concentration in comparison to environmental conditions (de Ruijter et al. 2020). Moreover, ecotoxicological studies tend to focus on certain model species and rely on short exposure time, leaving the long-term environmental effects for less attention (SAPEA 2019).

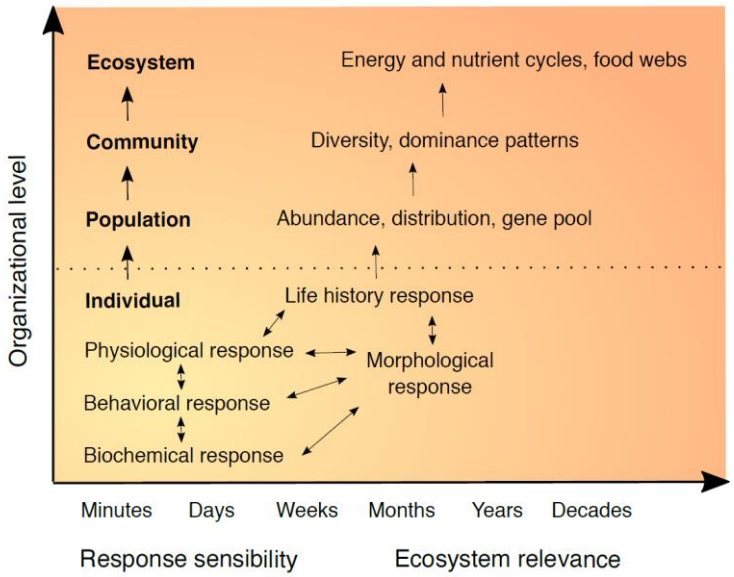


Figure 2. Biological responses to a contaminant at different biological organization levels plotted against time scale for response times. Redrawn and modified from Gerhardt (2007).

2 AIMS OF THE THESIS

There are currently many knowledge gaps regarding the fate and impacts of microplastics in the marine environment. Due to the pressing need to quantify and assess the risks related to microplastic pollution, my research is directed to the identified pollution hotspot – the seafloor. While field surveys are practical when investigating the presence, abundance and distribution of microplastic pollution in the marine environment, understanding the biological processes shaping their distribution patterns or studying their effects on organisms is more feasible in controlled laboratory settings, at least in the beginning when knowledge of the problem is still scarce. Hence, my thesis is based on mesocosm experiments mimicking the natural conditions of the northern Baltic Sea. Special focus was directed towards microplastics, but mesoplastics were also examined.

The general aim of my thesis was to examine what happens to plastics when they reach the seafloor. How do benthic macroinvertebrate communities shape the distribution of microplastics in the sediment? Do different types of plastics have the capability to sorb sedimented PAHs and potentially act as their vectors to biota? Do the sediments harbour bacteria capable of plastic biodegradation? Does the exposure to microplastics and their cocontaminants negatively impact the benthic fauna? These questions were answered by investigating the interactions between plastics, benthic communities and harmful substances in the northern Baltic Sea in four different studies (I–IV) that together paint a picture of how the size, properties (polymer type, cocontaminants) and vertical distribution of plastic litter on the seafloor affect the exposure and risks of plastic to benthic fauna in the northern Baltic Sea ecosystem. The studies focus on the most common benthic invertebrates in the northern Baltic Sea: the Baltic clam *Limecola balthica* (earlier known as *Macoma balthica*), the polychaete *Marenzelleria* spp. and the amphipod *Monoporeia affinis*.

Specific aims of studies I–IV:

I

Bioturbation transports secondary microplastics to the deeper layers in soft marine sediments of the northern Baltic Sea

The aim here was to explore whether bioturbation by the benthic community is able to bury microplastics that are settled on the sediment surface. The bioavailability of differently sized microplastics for the study species (*L. balthica*, *Marenzelleria* spp., *M. affinis*) was also investigated.

II

Seafloor sediments as microplastic sinks in the northern Baltic Sea – negligible upward transport of buried microplastics by bioturbation

As a follow-up to the previous study, this experiment aimed at investigating whether the buried microplastics remain in the sediment records or the benthic fauna are able to redistribute them back to the sediment surface. Two size classes and burial depths of microplastics were used to examine the impact of depth and particle size on their potential upward transport. The ingestion of microplastics was again inspected to assess their availability to the study species (*L. balthica*, *Marenzelleria* spp., *M. affinis*).

III

Polycyclic aromatic hydrocarbon sorption and bacterial community composition of biodegradable and conventional plastics incubated in coastal sediments

This study aimed at comparing the sorption of PAHs on two biodegradable and two conventional plastic types in coastal sediments collected from different sites subjected to various anthropogenic pressures. In addition, the impact of polymer type on the bacterial community composition of plastics was inspected to indicate the potential degradation of plastics on the seafloor.

IV

Tyre rubber exposure causes oxidative stress and intracellular damage in the Baltic clam (Limecola balthica)

The aim of this study was to investigate the impacts of one apparently abundant type of microplastics, tyre rubber, on the Baltic clam *L. balthica* in acute and chronic exposure scenarios. Both direct (ingestion, physical contact) and indirect (no physical contact) exposure pathways were examined, and the sublethal effects were studied, using biomarkers and examination of cell ultrastructure.

3 MATERIALS AND METHODS

3.1 Study area and species

The Baltic Sea is the largest brackish body of water on Earth and often referred to as the most polluted sea in the world (HELCOM 2010a). Its susceptibility to various anthropogenic pressures stems from its natural features, such as shallowness (average depth 54 m), long water-residence time (approx. 30 years) and large catchment area. More than 85 million people live in the catchment area, which is also characterized by intensive agriculture, industrial activities and traffic (HELCOM 2010a). Furthermore, the Baltic Sea is one of the most active sea areas for maritime traffic (Rytkönen et al. 2002). The unique fauna of the brackish Baltic Sea consists of a mixture of marine and freshwater species distributed along the geographical salinity gradient. Many of them live at the limit of their physiological tolerance, which makes them vulnerable to various disturbances (e.g. eutrophication, toxic algal blooms, hazardous substances, hypoxia, alien species, noise and marine litter) (Tedengren et al. 1988, Westerbom et al. 2002, HELCOM 2013). One of the most impacted areas in the Baltic Sea is the Gulf of Finland, which according to the Baltic Sea Pressure Index suffers especially from the input of nutrients, organic matter and heavy metals (HELCOM 2010b).

Since adopting the MSFD, efforts have been made to investigate and monitor the abundance, distribution and impacts of marine macro- and microlitter in the Baltic Sea. In general, the average concentration of microplastics (> 333 µm) in the water surface in the northern Baltic Sea is < 1 particle per m^3, and still < 10 particles per m^3 at highest (Setälä et al. 2016a, Gewert et al. 2017). Water samples taken from the water column have shown higher microplastic concentrations, with averages ranging from 32 (> 174 µm) to 440 (> 50 µm) microplastics per m^3 (Zobkov et al. 2019, Uurasjärvi et al. 2021). Similar to other sea areas, the highest concentrations of microplastics are found in the coastal sediments: the highest detected concentration was over 24 000 microplastics (> 25 µm) per kg sediment (dw), but the concentrations were also highly variable (mean: 6230 microplastics per kg sediment (dw)) (Tirroniemi 2019).

The first indication of the exposure of benthic macroinvertebrates to microplastics in the northern Baltic Sea was obtained from a study that found microplastics in the Baltic clam *Limecola balthica* collected near Stockholm on the coast of Sweden (Bråte et al. 2020). Another field campaign in the Gulf of Finland revealed that microplastics are found in many benthic deposit- and suspension- feeding invertebrates: *L. balthica*, the polychaete *Marenzelleria* spp. and the larvae of *Chironomus* spp. (Mustonen 2020). These species, along with the amphipod *Monoporeia affinis*, are the most common species in the benthic communities of the northern Baltic

(Gogina et al. 2016). They are all small-sized, mainly shallow-burrowing surface deposit-feeders and biodiffusors, being responsible for many important ecosystem processes (Lopez & Elmgren 1989, Lin & Hines 1994, Michaud et al. 2005, Hedman et al. 2008, Norkko et al. 2012, Renz & Forster 2013) and serving, in turn, as prey for various invertebrate and vertebrate predators (Aarnio & Bonsdorff 1993, Bonsdorff et al. 1995). Especially, the Baltic clam, predominates in terms of biomass, inhabiting sediments from the shallow shores to 200 m in depth (Nikula et al. 2008). The impacts of increasing levels of plastic pollution and potential combined effects with other anthropogenic stressors to these communities are hard to predict.

3.2 Mesocosm experiments

3.2.1 Preparation of plastics for the experiments

All plastic types for the experiments were selected, based on their specific gravity (i.e. tendency to sink in the brackish Baltic Sea water [specific gravity 0.005 g/cm^3; Leppäranta & Myrberg 2009], and ability to be extracted with density separation; I, II) and potential to be found in sediments. Secondary microplastics were produced from fishing line (I), children's toy bricks (II) and car tyres (IV). Fishing line made of PA (Trilene sensation, Berkley, diameter 200 µm; Berkley Fishing, Spirit Lake, IA, USA) was cut into 50-, 150- and 300-µm-long pieces with a McIlwain™ Tissue Chopper (Ted Pella Inc., Redding, CA, USA) (I). Each size class was cut from a different coloured line to aid their identification. Four colours of children's toy bricks made of acrylonitrile butadiene styrene (ABS) were ground with a kitchen grater and sieved to produce two colours of > 500-µm fragments and two colours of 100–300-µm fragments (II). Irregular fragments (2–190 µm, median 22 µm) of styrene-butadiene rubber (SBR) were obtained from a recycling company that had separated the microrubber from recycled tyres by cryogenic grinding (IV). Mesoplastics were produced by cutting transparent films (Goodfellow Cambridge Ltd., Huntingdon, England) over a plotting paper into same-sized, but differently shaped pieces (1 cm^2) (III). The films represented both conventional plastic types (polystyrene PS; polyamide PA), and bio-based, biodegradable plastic types (cellulose acetate CA; poly-L-lactic acid PLLA) with a similar thickness (0.06 mm for PA; 0.05 mm for other types).

3.2.2 Field collections

Sediment and animals for experiments I, II and IV were collected onboard R/V Saduria from the vicinity of the Tvärminne Zoological Station (TZS; University of Helsinki) using a van Veen sediment grab and a small bottom trawl. Sediment and water for experiment III were collected as part of City of Helsinki's routine monitoring on the coast of Helsinki (West Harbour [WH] and

Vanhankaupunginlahti [VKL]), using a GEMAX corer for the sediment and Ruttner-type water sampler for the near-bottom water.

The collected sediment was sieved through a 1-mm sieve to remove all the resident macrofauna (I, II, IV), and in some cases an additional 0.5-mm sieve was used (II). Macrofauna (clam *Limecola balthica*, polychaete *Marenzelleria* spp., amphipod *Monoporeia affinis*) retained on sieves were used in the experiments and transported to a temperature-controlled room in TZS for acclimation in ambient seawater (for acclimatization periods, see Table 2). Sediment that passed through the sieve was let to settle in buckets, the cleared water was removed and sediment from different buckets homogenized before adding it to the experimental units. The sediments collected from Helsinki were not sieved to preserve their polycyclic aromatic hydrocarbon (PAH) concentrations, but all animals (*L. balthica*) visible in the sediment were removed with tweezers (III).

3.2.3 Experimental setup

Since all four experiments focused on resolving different questions, the experimental conditions varied considerably (see Table 2. for summary). The bioturbation experiments (I, II) were performed in transparent cylindrical units (height 20 cm, Ø 14 cm). The first experiment (I), examining microplastic burial by the benthic fauna, consisted of 30 cylinders; 15 of which contained a macrofaunal community (*Limecola balthica*, *Marenzelleria* spp., *Monoporeia affinis*) in their natural densities (Rousi et al. 2013), and 15 acted as controls without animals. The cylinders received approximately 490 pieces (50 μm), 880 pieces (150 μm) and 390 pieces (300 μm) of fishing line, which sank to the sediment surface and corresponded to a concentration of 1790 pieces per kg of dry sediment. This concentration was chosen according to the results of Vianello et al. (2013), who found up to 2175 microplastics per kg (dw) from the Lagoon of Venice sediments. To follow the effect of time on the distribution of microplastics in the sediment, the incubations lasted 1, 2 or 3 weeks.

In the second bioturbation experiment (II), the microplastics were spiked at specific depths in the sediment column in 16 transparent cylinders to follow their redistribution by bioturbation. Frozen sediment layers including microplastics were prepared from the same homogenous sediment pool that was also used to fill the cylinders. Two types of frozen sediment layers were prepared: the lower layer contained 150 green (> 500 μm) and on average 161 red (100–300 μm) microplastics, while the upper layer contained 150 pink (> 500 μm) and on average 163 yellow (100–300 μm) microplastics. The frozen sediment layers were stacked, alternating with the fresh sediment. After the sediment compacted, the microplastics incorporated in the frozen sediment layers ended up at depths of approximately 2 cm (pink > 500 μm, yellow 100–300 μm) and 5 cm (green > 500 μm, red 100–

300 µm). The macrofaunal community (*Limecola balthica*, *Marenzelleria* spp., *Monoporeia affinis*) was added to eight of the cylinders, while the rest acted as controls.

The third experiment (III) focused on comparing the PAH sorption and bacterial community composition on different plastic types. The study was assembled, using 12 glass cylinders (height 21 cm, Ø 16.5 cm). From both sites (WH and VKL), six cylinders were filled: three with water and 400 ml of homogenized sediment and three with only water (total volume of water 2.5 l in both types of cylinders). In all, 80 plastic pieces (20 of each polymer type) were added to each cylinder, avoiding overlap. The pieces placed on the sediment surface were gently buried a few millimetres below the surface, and in the water cylinders they were placed on the bottom.

The fourth experiment (IV) was a mesocosm study investigating the biomarker responses and ultrastructural changes of *Limecola balthica* cells following tyre rubber exposure. The experiment was conducted in six large glass aquaria (length: 60 cm, width: 50 cm, height: 50 cm), and each received 35 l of homogenized sediment, 60 l of seawater and 100 clams. The experiment was divided into acute (5 days) and chronic exposures (29 days), and both had one control aquarium, one leachate aquarium and one particle aquarium. In the leachate aquaria, 100 g of tyre rubber particles were enclosed in a 1-µm nylon mesh bag to simulate indirect exposure to tyre rubber, whereas in the particle aquaria the particles were let to sink freely onto the sediment surface. Similar bags without the tyre rubber particles were also prepared for the control and particle aquaria and placed in the middle of the aquaria in a glass jar (height 16 cm, Ø 14 cm). For the particle aquaria, 100 g of tyre rubber particles were premixed with 300 ml of seawater and 180 µl of surfactant (Tween20; Sigma-Aldrich, now MilliporeSigma [Merck KGaA, Darmstadt, Germany]) before adding them to the aquaria to prevent agglomeration of the particles. All other aquaria also received 180 µl of Tween20, whose concentration fell below the no observed effect concentration (NOEC; 7 µl/l) (Beiras et al. 2018). The various aquaria are referred to with the following abbreviations: AC = acute control, AL = acute leachate, AP = acute particle, CC = chronic control, CL = chronic leachate, CP = chronic particle.

Table 2. Summary of the experimental setups of the studies in this thesis (PA = polyamide, ABS = acrylonitrile butadiene styrene, PS = polystyrene, PLLA = poly-L-lactic acid, CA = cellulose acetate, SBR = styrene-butadiene rubber, WH = West Harbour, VKL = Vanhankaupunginlahti, dw = dry weight).

	I	II	III	IV
Number of treatments	6	2	2	6
Number of replicate tanks	5	8	3	1
Plastic type	PA	ABS	PS, PA, PLLA, CA	SBR
Plastic concentration (pieces per kg dw)	1790	1219	418 (WH), 885 (VKL)	
Plastic concentration (mg per kg dw)	15.4	35.7	2580 (WH), 5480 (VKL)	5700
Plastic shape	cylindrical	irregular fragment	film	irregular fragment
Plastic size	50 x 200 µm, 150 x 200 µm, 300 x 200 µm	100–300 µm, > 500 µm	1 cm2	2–190 µm
Plastic location	sediment surface	sediment column	just below the sediment surface	sediment surface
Density of the macrofauna (m2)				
Limecola balthica	1038	908		333
Marenzelleria spp.	519	452		
Monoporeia affinis	195	390		
Sediment depth	10 cm	9.5 cm		11 cm
Grain size (dominant fractions)	51% fine and very fine sand (250–63 µm), 42% silt and clay (< 63 µm)	74% silt and clay (< 63 µm), 21% fine and very fine sand (250–63 µm)		
Acclimatization period	9 weeks	4 days		4 days
Temperature in the room	10 °C	4 °C	8 °C	10 °C
Dark:light cycle		12:12	dark	14:10
Aeration	overflow	overflow	air pump	air pump
Length of the experiment	1/2/3 weeks	10 weeks	14 weeks	5 days/29 days
Sampling	at the end	at the end	at the end	at the start, middle and end

3.2.4 Maintenance and sampling during the experiments

All experiments were conducted in temperature-controlled rooms at TZS. Oxygenation of the experimental units was organized via constant water circulation and overflow (I, II) or aeration with air pumps and syringes (III, IV). The escape of animals or microplastics from the overflow units was prevented, using 500-µm steel-mesh lids above the units. Although the animals received some food through the ambient seawater dripping into the units (I, II), they were also fed once per week with live *Nannochloropsis* algal concentrate (PhytoMaxx, NYOS® Aquatics, Korntal-Münchingen, Baden-Württemberg, Germany) (II). The algal concentrate was also provided for the clams in experiment IV after every water renewal, which was done every other day to prevent the accumulation of ammonia and other metabolites in the aquaria. Approximately half of the water volume was renewed at one time, and the microrubber concentration was maintained by filtering the exiting water through a 20-µm plankton net, which was then rinsed back into the aquarium (IV).

The water in the aquaria was sampled on experiment days 1, 3, 5, 17 and 29 for elemental analysis (10 ml) and on days 3, 5 and 29 for the PAH analysis (500 ml) (IV). In addition, the seawater control was taken for elemental analysis on the same sampling days to monitor the background contamination. On day 3, water was sampled both before and after the water renewal to determine how much the concentrations of metals and PAHs became diluted. This sampling approach was selected to cover the assumed variation in metal and PAH concentrations: the highest concentration was expected on day 3 before the first water renewal, and the lowest concentrations at the end of the experiment (day 5 for the acute experiment and day 29 for the chronic experiment). Water taken for elemental analysis was fixed immediately with 65% nitric acid (Suprapur, Merck) and stored in +4 °C until analysis. Water sampled for PAH analysis was stored in coolers and transported immediately to analysis.

During the experiments, the temperature and dissolved oxygen were determined weekly (I, II, III) or every other day from the units (IV) (YSI Environmental ProODO™; YSI Inc., Yellow Springs, OH, USA, now Xylem Inc., Rye Brook, NY, USA). In addition, the pH was determined every other day and ammonia once per week (IV).

3.2.5 Ending the experiments

In the bioturbation experiments, the experimental cylinders had movable bottoms that allowed cutting horizontal slices of the sediment, using a HAPS corer sample-ejection aggregate equipped with a cutting plate on top (I, II). The sediment column was sliced into six 1.7-cm-thick slices (I), or five layers with varying thicknesses; from top to bottom, the thicknesses of the layers were 1, 2, 1, 2 and 3.5 cm (II). The

sediment slices, still containing the benthic invertebrates, were frozen at -20 °C.

For the bacterial community analysis, 0.5 l of water was taken from each unit and filtered on a 0.2-µm sterile mixed-cellulose ester filter (III). The sediment community was sampled by pipetting the surficial sediment. The incubated mesoplastics were retrieved by pouring the contents of the units through a metal sieve with a mesh size of 4 mm. Ten plastic pieces of each type were collected in glass vials and stored at -20 °C for further PAH analysis, and the remaining mesoplastics were stored in plastic tubes for bacterial community analysis.

At the end of experiment IV, the clams were picked out from the sediment and their mortality was recorded. All live clams from each aquarium were randomized into groups as follows: 3 clams for the cell ultrastructure analyses, 40 clams for biomarker analyses, 25 clams for the burrowing experiment (of which 15 were used later to calculate their condition index (CI) and to determine their metal concentration), and all the remaining 27–31 clams for PAH analysis.

3.3 Laboratory processes

3.3.1 Extracting microplastics from the sediments

Extracting the microplastics from the sediments (I, II) was done, using density separation (Thompson et al. 2004, Hidalgo-Ruz et al. 2012). This method is based on the density differences of the sediment grains and plastics; denser sediment grains (density 2.65 g/cm^3) will readily sink in the solution, whereas the lighter plastics would float. Prior to density separation, the sediments used in the experiments were mixed with solid NaCl crystals for 20 min to compensate for the dilution caused by the wet sediment sample. Saturated NaCl (density: 1.2 g/cm^3) was then added and the sample was stirred for 1 min and let to settle for 8 min. The supernatant was led to the 100-µm nylon mesh filter, and the mixing with NaCl was repeated twice. To quantify the numbers of extracted microplastics, the filters were examined with a stereomicroscope (Leica MZ 7.5 and Leica CLS 150 XE, magnification 0.63–5.0 ×).

3.3.2 Quantifying microplastic ingestion

The benthic invertebrates used in the experiments were retrieved from the frozen sediment samples before density separation (I, II). The animals were carefully rinsed prior to analysis to remove microplastics attached to their surfaces. To examine the ingested nylon pieces, the animals were dissected (I). *Marenzelleria* spp., *Monoporeia affinis* and the gills of *Limecola balthica* were placed on an object glass, and the body of *L. balthica* was placed on an Utermöhl base plate for examination with an epifluorescence microscope (Leica DMI 3000 B, Leica I3 filter cube, magnification 0.4–40 ×). In

experiment II, an enzymatic digestion protocol developed by Railo et al. (2018) was applied to disintegrate the animal tissues. The animals were incubated 48 h at 37.5 °C in a solution consisting of 50% sodium dodecyl sulphate (SDS) (5 g/l, Sigma-Aldrich), 25% of Biozym F and 25% of Biozym SE (Spinnrad GmbH, Bad Segeberg, Germany). The disintegrated samples were filtered through 100-µm nylon mesh filters and the ingested ABS fragments were quantified, using a Leica MZ 7.5 stereomicroscope (magnification 0.63–5.0 ×) (Leica Camera AG, Wetzlar, Germany).

3.3.3 Sediment characterization

Sediment grain-size analysis was done in the sediments used in the bioturbation experiments (I, II). Wet sediment samples were covered with 6% H_2O_2 for 48 h and stirred two times per day to digest the organic material. The samples were sieved with water through a stack of sieves having mesh sizes of 500, 250 and 63 µm, and each size fraction was separately dried in preweighed containers at 60 °C. The water and the < 63-µm size fraction was let to settle for 2–3 days, after which the water was carefully removed and the remaining sediment dried at 60 °C in a preweighed container and weighed when dry (Sartorius LG620 Masterpro; Sartorius AG, Göttingen, Germany).

3.3.4 PAH analyses

The PAH analyses (III, IV) were performed by two laboratories: the Laboratory Centre of the Finnish Environment Institute SYKE and Metropolilab Oy Ab, both of which are accredited by the Finnish Accreditation Service (FINAS). In total, 26 PAH congeners were analysed from four matrices: plastics and sediment (III) and seawater and clam tissues (IV) (Table 3).

Plastic pieces of the same polymer type in each experimental cylinder were pooled into one sample (III). This resulted in three replicates of each plastic type (PS, PA, PLLA, CA) incubated in the sediment and three replicates incubated in water at both sites (WH and VKL). The PAHs were identified and quantified by gas chromatography –tandem mass spectrometry (GC-MS/MS) (Trace 1310 GC Ultra gas chromatograph [Thermo Fisher Scientific, San Jose, CA, USA]; TSQ Quantum XLS ultra mass spectrometer [Thermo Fisher Scientific]; TriPlus RSH autosampler [Thermo Fisher Scientific, Rodano, Milan, Italy]).

One pooled sediment sample from each site (WH and VKL) was used for analysis (III). The PAHs were extracted and analysed according to SFS-ISO 18287:2007, using gas chromatography–mass spectrometry (GC-MS) (Agilent 6890 GC [Agilent Technologies, Beijing, China] - 5973N MSD [Agilent Technologies, Santa Clara, CA, USA]). The PAH concentrations were normalized, using a Dutch standard of 10% organic matter and expressed as µg/kg sediment dw.

Table 3. Polycyclic aromatic hydrocarbon (PAH) congeners analysed from different sample matrices in experiments III and IV. The PAHs from plastics and clam tissues were analysed by SYKE's Laboratory Centre, whereas the PAHs from sediment and water were analysed by Metropolilab Oy Ab.

		Article III		Article IV	
PAH	**Abbreviation**	**plastic**	**sediment**	**clam tissues**	**water**
Napthalene	NP	x	x	x	x
2-Methylnapthalene	2-MNP	x	x	x	x
1-Methylnapthalene	1-MNP	x	x	x	x
Biphenyl	BP		x		x
2,6-Dimetylnapthalene	2,6-DMN		x		x
Acenapthylene	ACY	x	x	x	x
Acenapthene	ACE	x	x	x	x
2,3,5-Trimetylnapthalene	2,3,5-TMN		x		x
Fluorene	FLU	x	x	x	x
Dibenzothiophene	DBD			x	
Phenanthrene	PHE	x	x	x	x
Anthracene	ANT	x	x	x	x
1-Metylphenanthrene	1-MPH		x		x
Fluoranthene	FLA	x	x	x	x
Pyrene	PYR	x	x	x	x
Benzo[a]anthracene	BaA	x	x	x	x
Chrysene	CHR	x	x	x	x
Triphenylene	TRI	x		x	
Benzo[b]fluoranthene	BbF	x	x	x	x
Benzo[k]fluoranthene	BkF	x	x	x	x
Benzo[e]pyrene	BeP	x	x	x	x
Benzo[a]pyrene	BaP	x	x	x	x
Perylene	PER	x	x	x	x
Indeno[1,2,3-cd]pyrene	IcdP	x	x	x	x
Dibenzo[a,h]anthracene	DahA	x	x	x	x
Benzo[ghi]perylene	BghiP	x	x	x	x

The PAHs from the water samples (IV) were analysed according to ISO/TS 28581:2012, 2012, using GC-MS: 7890A GC (Agilent Technologies USA) - 7000B Triple Quadrupole (Agilent Technologies USA); analytical column Agilent HP5-MSUI (30 m x 0.25 mm x 0.25 um).

In all, 27–31 clams from experiment IV were pooled to form two or three replicates from each aquarium to ensure adequate biomass for the analysis (two replicates from treatments CC, CL, CP; three replicates from treatments AC, AL, AP). The samples were stored at -20 °C until analysis, then homogenized, using an IKA Ultra-turrax homogenizer T18 (Staufen, Germany), and the chemical analysis was performed using a GC–MS/MS; Thermo Scientific Trace 1310 GC system [Milan, Italy] coupled to a

Thermo Scientific TSQ Ultra mass spectrometer [San Jose, CA, USA]. The PAHs were extracted and analysed from the water samples according to ISO/TS 28581:2012, 2012, using GC-MS: 7890A GC (Agilent Technologies USA) - 7000B Triple Quadrupole (Agilent Technologies USA); analytical column Agilent HP5-MSUI (30 m x 0.25 mm x 0.25 µm).

3.3.5 Elemental analyses

The concentrations of trace metals and other elements from the experiment water (IV) were determined with inductively coupled plasma-mass spectrometry (ICP-MS; NeXION 350D instrument [PerkinElmer Inc. Waltham, MA, USA] equipped with an ESI PrepFAST autosampler [Elemental Scientific Inc., Omaha, NE, USA]). The data were processed, using PerkinElmer Syngistix Data-Analysis Software™. The concentrations of S, Cl and Br were measured by total-reflection X-ray fluorescence spectrometry (TXRF; S2 Picofox TXRF instrument [Bruker Corp., Karlsruhe, Germany] with Spectra software for data analysis).

The freeze-dried clams used in determining the CI were divided into two replicates from each treatment, from which the following trace metals were analysed: As, Cd, Co, Cr, Cu, Fe, Ni, Pb, SE, U and Zn. The samples were acid-digested with 30% H_2O_2 in a single reaction chamber microwave digestion unit (Ultrawave, Milestone S.r.l., Sorisole, Italy). Prior to elemental analysis, the samples were diluted with deionized water. Elemental analysis utilized Sc, Ga, Rh and Ir as internal standards and was performed using ICP-MS (Thermo iCAP Q, Thermo Fisher Scientific, Bremen, Germany).

3.3.6 Bacterial community analyses

The bacterial community composition from plastics, water and sediment was analysed, based on 16S ribosomal RNA gene region V3-V4 (III). To monitor possible contamination, negative controls without samples were also extracted. After DNA extraction, this region was amplified with a two-step polymerase chain reaction (PCR), and Illumina MiSeq (Illumina Inc, San Diego, CA, USA) was used for paired-end multiplex sequencing at the Institute of Biotechnology, University of Helsinki (Aho et al. 2019). The primers were removed from the raw reads (Martin 2011), and the reads were merged and processed according to the DADA2 pipeline (Callahan et al. 2016). A total of 4.2 million nonchimeric sequences were used for further analyses, and taxonomic classification of the amplicon sequence variants (ASVs) was done (Quast et al. 2013, Callahan 2018). Before performing statistical analyses, the chloroplast and mitochondrial sequences were removed, resulting in 7357 ASVs.

3.3.7 Cell ultrastructure analyses

For the cell ultrastructure analyses (IV), the gills, foot muscle and digestive gland (DG) were detached from three clams from each aquarium. Small (1×1 mm) pieces of tissues were cut inside fixative droplets (3% glutaraldehyde in 0.1 M sodium cacodylate buffer) and stored in the same fixative at 4 °C. The samples were then postfixated and embedded in epoxy resin. Initially, 1-µm-thin sections were cut with an ultramicrotome and stained with 1% toluidine blue before examination with light microscopy (Zeiss AxioImager M2, Carl Zeiss Microscopy GmbH, Jena, Germany). For electron microscopy analysis, representative sites were chosen with light microscopy: ultrathin sections were cut with an ultramicrotome, stained and then inspected as a blinded examination at a voltage of 200 kV with a JEOL JEM-2100F transmission electron microscope (Jeol Ltd, Tokyo, Japan) equipped with a digital camera (Olympus-SIS; Olympus Corp., Münster, Germany).

3.3.8 Biomarkers

In all, 40 clams were dissected to retain their gills, foot and DG for the biomarker analysis (IV). The gills and foot from two individuals were pooled together to obtain adequate mass for the analysis, whereas the DGs were stored individually. The foot and DG samples were frozen in liquid nitrogen and stored at -80 °C, and the gills were tapped for 1 min with a mixture of acetic acid and ethanol (1:3) on an object glass to detach cells from the gill tissue. The detached cells were fixed to the glass with methanol and stored at room temperature for further analyses.

A set of nine biochemical biomarkers was analysed, following previously validated and published methods (Table 4). DG samples of 20 clams from each treatment were used to measure the CAT, glutathione-S-transferase (GST), GR, GPx and SOD activities. Another 20 individuals were used to measure the level of LPO, the ratio of reduced to oxidized glutathione (GSH/GSSG) and the oxygen-radical antioxidant capacity (ORAC) from their DGs. For AChE, 20 foot samples (pooled from two individuals) were used. All tissues were individually homogenized with assay-specific instructions, and the supernatants were stored at −80°C. The CAT, GST, GR, GPx, SOD and AChE activities as well as the homogenate protein concentrations and LPO were measured in 96-well half-area plates, using a microplate reader (Infinite 200, TECAN; Tecan Co., Tokyo, Japan) and analysed with Magellan software (TECAN). The reaction rate was evaluated according to the best linearity range of the curves. The protein concentrations of the samples were determined, using the Bradford (1976) method and a bovine serum albumin standard, and utilized to adjust the enzyme activities. The ORAC and GSH/GSSG ratio were measured in 384-well plates with a TECAN Spark spectrophotometer.

All samples were measured in triplicate or quadruplicate.

The geno- and cytotoxicity parameters were analysed from the gill samples, following the methods described in Baršienė et al. (2006, 2004), using bright-field Olympus BX51 microscopes (Tokyo, Japan) with an immersion objective (1000×). From each clam, 2000 cells with intact cellular and nuclear membranes were evaluated, using blind scoring. The results were expressed as the mean value (‰) of the sums of the analysed individual lesions scored in 1000 cells per individual sampled from every treatment. Induction of micronuclei (MN), nuclear buds on filament (NBf), nuclear buds (NB), blebbed nuclei (BL) and binucleated cells with nucleoplasmic bridges (BNb) were assessed as genotoxicity endpoints, and induction of fragmented apoptotic (FA), binucleated (BN), and 8-shaped nuclei cells as cytotoxicity endpoints. The total genotoxicity (MN + NBf + NB + BL + BNb), and total cytotoxicity (8-shaped nuclei + BN) endpoints were summed to express the total cytogenetic damage. Nuclear abnormalities were identified, using predefined criteria (Heddle et al. 1991, Fenech et al. 2003, Baršienė et al. 2014).

The CI was calculated from 15 individuals from each treatment according to Bonsdorff and Wenne (1989). The clams were removed from their shells, rinsed carefully with clean seawater, freeze-dried 24 h at −60 °C and in 10^{-1} atm (SuperModulyo freeze dryer, Thermo Electron Corporation, Waltham, MA, USA), and weighed (Mettler AT250; Mettler Toledo, Columbus, OH, USA). The shells were measured with callipers from the largest dimension.

Table 4. Summary of the methods used in this thesis. The methods are described in detail in papers I−IV (PAHs = polycyclic aromatic hydrocarbons, AChE = acetylcholinesterase, CAT = catalase, GPx = glutathione peroxidase, GR = glutathione reductase, SOD = superoxide dismutase, ORAC = oxygen-radical antioxidant capacity, GSH/GSSG = glutathione/glutathione disulphide ratio, GST = glutathione-S-transferase, LPO = lipid peroxidase, CI = condition index, FA = fragmented apoptotic cells, BN = bi-nucleated cells, MN = micronuclei, NBf = nuclear buds on filament, NB = nuclear buds, BL = blebbed nuclei, BNb = bi-nucleated cells with nucleoplasmic bridges).

Category	Variable	Method description	Reference	Paper
Physical and chemical variables	Temperature and dissolved oxygen	Optical-based sensor (YSI Environmental ProODO™)		I, II, III, IV
	pH	PMU 6100 Multiparameter		IV
	NH4-4	Manual spectrometric method	ISO 7150/1-1984	IV
	PAHs	Gas chromatography–mass spectrometry, gas chromatography–tandem mass spectrometry	SFS-ISO 18287: 2007 ISO/TS 28581:2012	III, IV
	Trace elements	Inductively coupled plasma mass-spectrometry, X-ray fluorescence spectrometry		IV
	Sediment grain size	Determining size fractions by wet sieving	Blott and Pye 2001; Joensuu et al. 2018	I, II
	Number of microplastics extracted from sediments	Density separation, stereomicroscopy	Thompson et al. 2004	I, II
Bacteria	Bacterial community composition	DNA extraction (DNeasy Power Soil kit (Qiagen)), Sequencing (PCR, Illumina MiSeq), Bioinformatics (primer removal, read merging and processing, taxonomic classification)	Kit spesific instructions, Martin 2011, Quast et al. 2013, Callahan et al. 2016	III
Demography	Mortality rate	Number of dead clams at the end of the experiment		IV
	Number of ingested microplastics	Disintegration of animal tissues, epifluorescence microscopy, stereomicroscopy	Railo et al. 2018	I, II
Cell ultrastructure	Changes in cell ultrastructure	Transmission electron microscope	Korkalainen et al. 2017	IV
Biomarkers	AChE	Hydrolysis rate of acetylcholine	Bocquené and Galgani 1998	IV
	CAT	Degradation rate of hydrogen peroxide (H2O2)	Claiborne 1985, Vuori et al. 2015	IV
	GPx	Decrease in NADPH absorbance during the oxidation of GSH to GSSG	Vuori et al. 2015	IV
	GR	Consumption rate of NADPH in the reduction of GSSG to GSH	Vuori et al. 2015	IV
	SOD	Inhibition rate of cytochrome C reduction (Merck 19160)	Kit spesific instructions	IV
	ORAC	OxiSelect Oxygen Radical Antioxidant Capacity Activity Assay (Cell Biolabs STA-345)	Kit spesific instructions	IV
	GSH/GSSG	Arbor Assays Detect X Glutathione Fluorescent Detection Kit, Catalog No K006-F5	Kit spesific instructions	IV
	GST	Formation rate of the GSH conjugated substrate	Habig et al. 1974	IV
	LPO	Amount of thiobarbituric acid reactive substances (TBARS)	Ohkawa et al. 1979	IV
	Genotoxicity	Induction of FA, BN and 8-shaped nuclei cells	Baršienė et al. 2004; 2006	IV
	Cytotoxicity	Induction of MN, NBf, NB, BL and BNb	Baršienė et al. 2006	IV
	CI	Tissue dry weight (mg)/shell length^3 × 100 (mm)	Bonsdorff and Wenne 1989	IV
	Burrowing rate	Number burrowed clams at certain time points	Sokolowski et al. 1999	*

* Side experiment, not included in the paper IV. Shortly: 25 individuals from each aquarium were randomly selected and placed in separate aquaria filled with clean sediment and fresh seawater (8.7–8.8 °C, dissolved oxygen 11.2–11.4 mg/L, and pH 7.96–8.02). The number of burrowed individuals was monitored every half an hour during a 90-minute time period.

3.4 Data handling and statistical analyses

Statistical analyses were carried out, using SPSS (version 23) (I, II) or R (v. 4.0.3; R Core Team 2020), supplemented with the following packages: car (v3.0-8; Fox & Weisberg 2019), PMCMRplus (v. 1.6.1; Pohlert 2020), and DESeq2 (v. 1.28.1; Love et al. 2014) (III, IV). A significance level of 0.05 was used in all analyses.

In the bioturbation experiments (I, II) the variously sized microplastics showed varying recovery rates. Therefore, instead of actual numbers, the percentages of microplastics found in different sediment layers were compared between treatments. An arcsine transformation was made to the total numbers of retrieved microplastics to ensure normality of the residuals, followed by one-way analysis of variance (one-way ANOVA) (I). The nonparametric Kruskal-Wallis test for independent samples and Mann-Whitney U test were used if the normality was not achieved by transformations, and were used to examine the number of ingested microplastics and the distribution of variously sized microplastics in different sediment layers (I). In the second bioturbation experiment, a nonparametric Mann-Whitney U test for independent samples was applied to compare the percentages of extracted microplastics per layer with the animal and control cylinders (II).

For PAH concentrations falling below the limit of quantification (LOQ), the concentrations were assumed to be at the LOQ; thus, the used values represent maximum estimates of concentrations (III, IV). To compare the PAH concentrations of different plastic types (III) and to test for differences in biomarker responses between treatments (IV), one-way ANOVA with Tukey's test was used. Differences between matrices (sediment/water) were explored with the t-test for independent samples (III). The homogeneity of variances was verified, using Levene's test, and normality was confirmed, using the Shapiro-Wilk test. The Welch ANOVA with Games-Howell post hoc test was used if the assumptions of equal variances were not fulfilled, and deviations from normality were resolved, using log10 transformation. The Kruskal-Wallis test followed by Dunn's test with Bonferroni adjustment (III) or the pairwise Wilcoxon rank-sum test with BH adjustment (IV), or the Mann-Whitney U test was used when normality issues could not be corrected (III, IV). The DESeq2 package with default parameters was used to analyse the differential abundant taxa between bacterial communities on PA and CA (III).

In addition to data presented in articles I–IV, supplementary calculations for this thesis were done from the previously unpublished data of the first experiment to inspect the number of ingested microplastics by the clams retrieved from different sediment layers (I), and from a small burrowing experiment conducted adjacent to experiment IV. Figures 3 and 7 were drawn specifically for this

thesis using R (v. 4.0.3; R Core Team 2020) and the packages rcartocolor (v.1.0.0.; Nowosand 2018), RColorBrewer (v.1.1-2; Neuwirth 2014), ggforce (v.0.3.2; Lin Pedersen 2020). Inkscape (v. 1.0) was used for the illustrations (Fig. 1 and 2).

4 RESULTS AND DISCUSSION

4.1 Overview of the results

Here, we investigated the interactions between plastics, benthic communities and harmful substances in northern Baltic Sea sediments. The studies included in this thesis examined some of the important processes impacting the fate of plastic litter on the seafloor, such as bioturbation, bacterial colonization and sorption of HOCs, and explored the potential impacts of microplastics on benthic communities. The results yielded novel information on the importance of bioturbation in distributing microplastics vertically in soft sediments (I, II) and the differing colonization of biodegradable and conventional plastics by bacterial communities (III). The results further demonstrate that the risks of microplastics for the common benthic fauna of the northern Baltic Sea are likely to depend on the size (I, II) and polymer type (III, IV) of the particles, as well as their residence depth in the sediment (I, II). The presence of harmful chemicals in plastics was verified (III, IV), and exposure to tyre rubber caused oxidative stress and intracellular damage in *Limecola balthica* (IV). In the following chapters, these results and perspectives will be further elaborated and discussed.

4.2 The availability of microplastics to the benthic fauna is dependent on microplastic size and location in the sediment

4.2.1 Microplastic concentration and size affect their probability of becoming ingested

The microplastics were ingested in the experiments only by *Limecola balthica* (I, II). In examining the size distribution of the ingested microplastics, the clams seemed not to prefer certain size classes over others (I): in total, 12 pieces of the smallest (50 × 200 µm), 25 pieces of the medium-sized (150 × 200 µm) and 24 pieces of the largest (300 × 200 µm) microplastics were ingested. While the smallest microplastics were less abundant in clams than the larger plastics, the numbers of all variously sized ingested microplastics were relative to their numbers extracted from sediments. Since *L. balthica* is known to be a nonselective feeder (Self & Jumars 1988), the disproportion of the sizes of the ingested particles may be an artefact from difficulties in detecting the small-sized, translucent particles by visual inspection in the clam and sediment samples. This observation on the relative abundances of ingested and available microplastics is in accordance with the laboratory study showing that the number of ingested microplastics by *L. balthica* is related to the available concentration (Setälä et al. 2016b). The microplastic concentration in the surrounding sediment also positively correlated with the number of microplastics in

the gut contents of another deposit-feeder, the lugworm *Arenicola marina*, that was exposed to PS spheres in a laboratory (Besseling et al. 2013).

The sizes of the ingested microplastics were all below 300 µm (I, II), corresponding to the dimensions of natural particles ingested by the Baltic clams (Gilbert 1977). In the environment, the mean (± SD) size of the ingested microplastics by *L. balthica* was found to be 109 ± 110 µm (Mustonen 2020). Since the numbers of the ingested microplastics did not increase with the experimental time (I; Table 5), it can be concluded that they seem not to accumulate inside the clams, but rather are egested among other nondigestible material. Since the Baltic clams are facultative deposit-feeders, they are most likely accustomed to handling foreign undigestible particles in their digestive tracts. The same likely applies to other deposit-feeding animals as well: for example, Besseling et al. (2013) demonstrated that the microplastics ingested by *Arenicola marina* were not accumulated in their guts, but rather were egested. However, in these studies quite compact particles were examined, and fibrous material may possibly have caused further difficulty, as shown by Murray and Cowie (2011).

The two other species examined, *Monoporeia affinis* and *Marenzelleria* spp., did not ingest any microplastics (I, II), because the spiked microplastics were likely too large for them. The maximum prey size for *M. affinis* is approximately 60 µm (Ankar 1977), whereas *Marenzelleria* spp. are known to ingest particles as large as 250 µm (Bock & Miller 1999). Even the smaller particles may have been outside the size range available for ingestion, since the *Marenzelleria* spp. individuals used in the study were smaller than in the study by Bock and Miller (1999), and in the field the mean (± SD) size of the ingested microplastics by *Marenzelleria* spp. in the northern Baltic Sea was only 72 ± 40 µm (Mustonen 2020). In addition, *Marenzelleria* spp. prefer smaller particles over larger ones (Bock & Miller 1999), and since most of the sediment consisted of silt and clay and was thus smaller than the offered microplastics, the polychaetes may have selected sediment particles instead of microplastics. In a previous study, the *Marenzelleria* spp. collected from the same area as in this study readily ingested PS beads of 10 µm from the sediment (Setälä et al. 2016b).

4.2.2 Location and feeding types of the species

The Baltic clam *Limecola balthica*, polychaete *Marenzelleria* spp. and the amphipod *Monoporeia affinis* are all small, relatively shallow-burrowing species, although the burrows of *Marenzelleria* spp. can reach to a depth of 30 cm (Hedman et al. 2008, Norkko et al. 2012). In both bioturbation experiments (I, II), their distribution throughout the sediment cores followed their species-specific preferences; *M. affinis* individuals inhabited the sediment surface, *L.*

balthica resided mainly in the uppermost 3−4 cm of the sediment and *Marenzelleria* spp. preferred the deeper layers (Fig. 3).

The distribution of the study species relatively near the sediment surface corresponds to their feeding types, since they are all classified primarily as surface deposit-feeders (Lopez & Elmgren 1989, Lin & Hines 1994, Hedman 2008). *Limecola balthica* is a facultative deposit- and suspension-feeder that vacuums material from the sediment surface or filters the overlying water with its incurrent siphon (Lin & Hines 1994). When microplastics were initially distributed on the sediment surface, 25% of the clams ingested them (I). The mean (± SD) number of ingested microplastics was 1.22 ± 1.06 per clam, but the individual microplastic load was highly variable, since the highest observed concentration in one clam was 15 microplastics. The size of the clam did not explain the differences in numbers of ingested microplastics ($p > 0.05$). The variable numbers likely resulted at least partly from the patchy distribution of microplastics in the experimental cylinders, since in the water they were forming aggregates before settling to the sediment surface.

When the microplastics were spiked into deeper layers of the sediment, only 1% of the clams ingested them (II). Ingestion was verified for only one individual out of

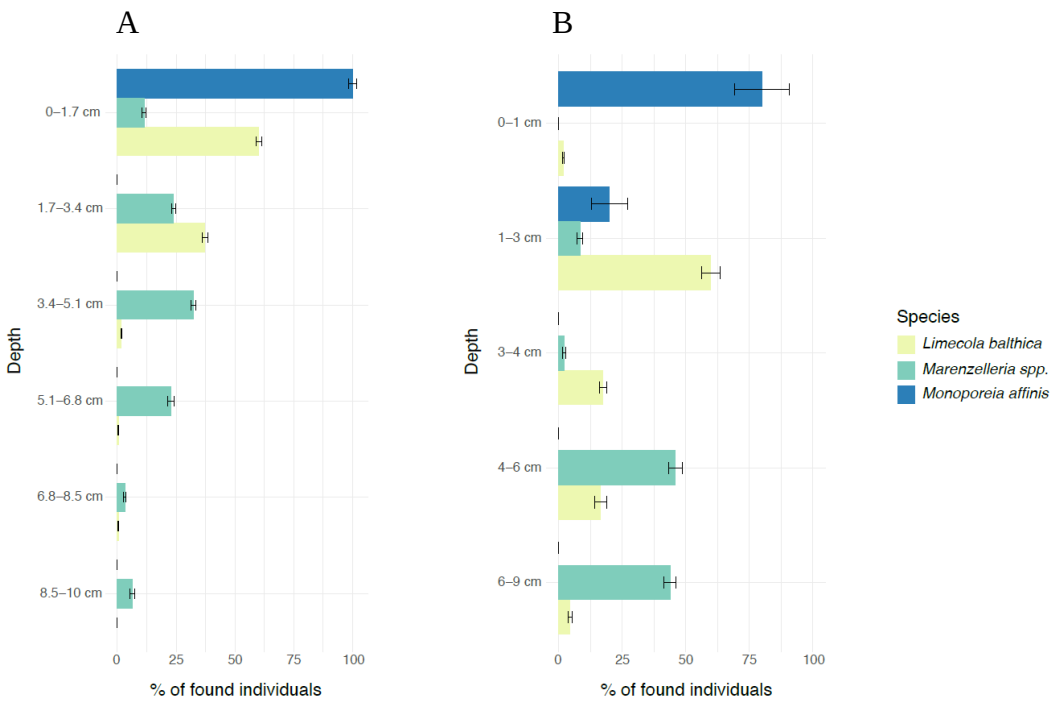


Figure 3. Vertical distributions of all study animals found in the sediment cores at the end of the experiment in both bioturbation studies (A = experiment I; B = experiment II).

112 inspected clams; the clam was found from the depth of 1−3 cm in the sediment, and the microplastic that was ingested was initially placed at 2 cm.

Since *L. balthica* feeds at the surface, its exposure to buried microplastics when feeding seems negligible. The results from these studies (I, II) together indicate that the bioavailability of microplastics to *L. balthica* decreases rapidly as the microplastics are buried in the sediment. This is also supported by the data of microplastic ingestion by clams at different time points (I). Out of a total of 61 ingested particles, 39 were found in clams after the first week, resulting in an average of 1.90 ± 1.16 pieces per individual, 9 after the second week (0.45 ± 0.25 pieces per individual) and 13 after the third week (1.30 ± 1.27 pieces per individual). Although these differences were not significant ($p > 0.05$), they potentially also suggest that the burial of particles during the experiment made them less available to the clams.

Additional calculations from the data (I) revealed that the clams occupying the uppermost sediment layer (0–1.7 cm) had on average ingested twice as many microplastics as clams residing in the second layer (1.7–3.4 cm) (Table 5). While it is expected that all clams fed at the sediment surface, those residing at shallower depths can cover a larger surface area with their siphons (Zwarts et al. 1994), and hence potentially collect a higher number of microplastics. It has been shown that scarcity of suspended food particulates pressures *L. balthica* to move closer to the sediment surface to enable it to change from suspension-feeding to deposit-feeding, and to enlarge the feeding area (Lin & Hines 1994). Similarly, *L. balthica* also decreases its burial depth in response to hypoxic conditions (Tallqvist 2001, Long et al. 2008, Villnäs et al. 2019), making it at the same time more vulnerable to lethal predation and nonlethal siphon cropping by epibenthic predators (Lin & Hines 1994). This behavioural response to unfavourable environmental conditions may render clams not only more susceptible to predation, but also to microplastic ingestion.

Table 5. Numbers of microplastics ingested by *Limecola balthica* residing in different depth layers (I).

	1st week	2nd week	3rd week	total
1st layer (0-1.7 cm)				
total number of ingested microplastics	27	7	13	47
microplastics/clam	1.8	0.4	0.8	3.0
number of inspected clams	15	19	16	50
2nd layer (1.7-3.4 cm)				
total number of ingested microplastics	12	2	0	14
microplastics/clam	1.2	0.3	0	1.5
number of inspected clams	10	7	9	26

4.3 Role of bioturbation in distributing microplastics in sediments

4.3.1 Bioturbation buries microplastics

Microplastics that were initially placed at the sediment surface were distributed throughout the sediment cores in both control cylinders and animal-containing aquaria and presented a clear vertical gradient in their distribution (I). Over 90% of microplastics were located in the top layer of the sediment core (depth 0–1.7 cm), and their abundance decreased gradually towards the deeper parts of the core. Differences between the control and animal cylinders were observed in the uppermost 5 cm of the sediment (layers 1–3). The animals significantly decreased the abundance of microplastics in the topmost layer (depth 0–1.7 cm). The animal cylinders contained fewer microplastics (92.0 ± 2.7%) than did the control cylinders (96.5 ± 1.2%) ($p = 0.000$), whereas the microplastics were more abundant in the animal cylinders in the second (depth 1.7–3.4 cm) and third layers (depth 3.4–5.1 cm) than in the control aquaria (2nd layer $p = 0.000$; 3rd layer $p = 0.010$).

These results demonstrate that bioturbation by the common benthic invertebrates of the northern Baltic Sea buries microplastics up to depths of 5 cm in the sediment, with mixing being most prominent near the surface (I). This observation corresponds to estimations that the depth of the bioturbated layer in the Baltic Sea is typically less than 5 cm (Hedman 2008). However, throughout the entire temperate North Atlantic realm, the Baltic Sea has the lowest recorded mean (± SD) sediment-mixing depth (0.9 ± 0.7 cm) (reviewed by Teal et al. 2008). Hence, while the burial of microplastics by bioturbation is verified, the results of this study are not directly applicable to other sea areas. Since the worldwide mean (± SD) of the mixed-layer depth is estimated to be 5.75 ± 5.67 cm (Teal et al. 2008), it can be expected that globally the burial depth of microplastics is likely to exceed the depths observed in this study (I). The sediment-reworking activities, and thus the extent to which the benthic fauna transports particles, is affected by the environmental conditions (e.g. dissolved oxygen, temperature, food availability) and the ecology of the local community (e.g. density, functional traits of the species), which vary geographically (Kearns et al. 1996).

Microplastics were also found below 5.1 cm (layers 4–6) in the sediment, but their distribution was similar in all treatments ($p > 0.05$). The small numbers of various tracer particles found in the deeper layers of the control cores are commonly observed in bioturbation studies and may stem from meiofaunal activities, pore-water movement resulting from sediment compaction or sediment smearing upon slicing (e.g. Gebhardt & Forster 2018; Josefsson 2011; Quintana et al. 2007). In the environmental samples collected from the deep-sea sediments of the Rockall Trough, North Atlantic, Courtene-

Jones et al. (2020) found microplastics in sediment layers dated far beyond the plastic era. A positive relationship between sediment porosity and microplastic abundance in the sediment layers was found, suggesting that pore-water burial may also play a role in the transport of microplastics (Courtene-Jones et al. 2020).

4.3.2 Bioturbation rarely redistributes microplastics back to the sediment surface

While our results (I) together with other studies on bioturbation-induced burial of microplastics in the sediments (Gebhardt & Forster 2018, Coppock et al. 2021) support the hypothesis of seafloors being the sink for microplastics (Woodall et al. 2014), the stability of these reservoirs remains unknown. Despite burying, benthic invertebrates can transport particles to other directions as well (Wheatcroft et al. 1990), and thus in some cases, bioturbation may also promote the return of buried microplastics closer to the sediment-water interface, further facilitating their resuspension into the water column. However, only a few microplastics placed at deeper layers in the sediment were distributed to the sediment surface, following a 10-week experiment (II). When all the microplastics were examined and compared, significantly more (mean ± SD) were found in the surface layer (0−1 cm) of the animal cylinders ($1.0 \pm 1.0\%$) than in the control cylinders ($0.2 \pm 0.5\%$) ($p = 0.028$), indicating that the animal activities had enhanced their redistribution. The particles found in this layer were mainly those that were placed a short distance away from the surface. On average, $2.1 \pm 2.5\%$ of the microplastics initially placed at the 2-cm depth were found in the surface layer of the animal cylinders, whereas in the control cylinders only $0.5 \pm 1.1\%$ of the microplastics ended up in this layer. In contrast, microplastics placed at 5-cm depths were completely absent from the surface layer in the control cylinder and only one microplastic from this layer was found at the surface of the animal cylinder.

Our results (II) show that the benthic invertebrate community can facilitate the transport of buried microplastics to the sediment surface; however, this transport seems to be negligible and mainly applies to particles that are already close to the sediment surface (depth of 2 cm). Although the experiments were not designed to assess the transfer fluxes of microplastics in the sediments, our results broadly suggest that the net transfer of particles in the northern Baltic Sea is towards the deeper sediment layers (I, II). Once microplastics are buried below the sediment surface, they tend to stay there, at least when the seafloor communities are dominated by *Limecola balthica*, increasing the residence time of microplastics and reducing their bioavailability to species feeding on the sediment surface. Similar to our results, slight upward transport of microplastics in the uppermost 1 cm of the sediment core has also been detected with

freshwater oligochaete tubificid worms redistributing PS beads (Ø 100 μm) during a 2-month experiment (Kearns et al. 1996). Also, the polychaete worms *Cistenides gouldii* (now *Pectinaria gouldii*) and *Clymenella torquata* promoted the upward translocation of buried copepod resting eggs and plastic beads (Ø 80–125 μm) that were placed at a depth of 3–4 cm in the sediment (Marcus & Schmidt-Gengenbach 1986). In this case, the upward transfer of microplastics from the deeper layers was attributed to the conveyor-belt feeding mode of *C. gouldii* and *C. torquata*, highlighting the need to understand the role of species' functional characteristics in explaining particle translocation (Marcus & Schmidt-Gengenbach 1986).

4.3.3 Influence of functional traits of the fauna on microplastic transport

The burial of microplastics by bioturbation can be attributed to the movement of animals in the sediment matrix or the ingestion and subsequent egestion of the sediment. According to Jumars and Wheatcroft (1989), deposit-feeding is the most important animal activity in displacing particles. Although all the study animals are classified as surface deposit-feeders (Lopez & Elmgren 1989, Lin & Hines 1994, Hedman 2008), the microplastics were only available to *Limecola balthica*, due to the relatively large size of the particles. If deposit-feeding were the primary process for particle translocation, the role of *L. balthica* should have been pronounced in our results. In previous studies, *L. balthica* more efficiently displaced particles vertically in the sediment than did *Monoporeia affinis* and *Marenzelleria* spp. (Viitasalo 2007, Viitasalo-Frösén et al. 2009) and, indeed, in our study (I) the location of *L. balthica* individuals at the end of the experiment also explained well the vertical distribution of microplastics in the sediment ($p = 0.000$, $R2 = 0.81$). No significant effect was observed in the vertical distribution of microplastics and the location of *Marenzelleria* spp. ($p > 0.05$, $R2 = 0.03$). Since all *M. affinis* individuals were found in the topmost sediment layer, their influence on microplastic distribution was not tested (Fig. 3 A).

Animals transported microplastics similarly, regardless of their size in both experiments (I, II: $p > 0.05$). Based on the numbers of microplastics ingested by *L. balthica* (I), the passing of microplastics through the gut supposedly occurred. However, *L. balthica* deposits its faecal pellets on the sediment surface (Black 1980, Henriksen et al. 1983), resulting theoretically in the circling of ingested microplastics back to the surface. Thus, the correlation between microplastic and *L. balthica* distribution (I) is likely explained by microplastics fallen into the voids created around the clams when they moved inside the sediment rather than ingestion, a process described by (Viitasalo 2007, Hedman et al. 2008). Although no relationship between *Marenzelleria* spp. and microplastic distribution was found, this does not mean that they did not contribute to

the burial of microplastics. For example, the feeding activities of *Marenzelleria viridis* resulted in burial of relatively large PA particles (diameter 1.33 mm, length 3.14 mm), because its faecal pellets are deposited on the sediment surface (Delefosse & Kristensen 2012). It remains unknown which species in our study contributed to the upward transport of microplastics (II), but based on the distribution of the animals (Fig. 3 B), *L. balthica* and *Monoporeia affinis* were residing near the surface and are thus likely candidates for transporters.

All the species used in our experiments (I, II) were classified as biodiffusers or gallery-diffusers that transport particles randomly over short distances (Michaud et al. 2005, Hedman et al. 2008, Renz & Forster 2013). Hence, their effect on particle redistribution was expectedly relatively small and followed the typical pattern of biodiffusive mixing, in which tracer distribution shows maximum concentration at the surface and then an exponential decrease with depth (Wheatcroft et al. 1990, Kristensen et al. 2012). In other sea areas, the patterns of microplastic translocation may be very different, depending on the resident fauna. For example, in the western Baltic Sea, the lugworm *Arenicola marina* buried microplastics (PS Ø 1 mm; PA Ø 500 µm) deposited on the sediment surface down to a depth of 20 cm in the sediment (Gebhardt & Forster 2018). Similarly, in the Danish Straits, *A. marina* effectively buried PA particles (length ~3 mm), mimicking eelgrass *Zostera marina* seeds, to deeper layers of the sediment (Valdemarsen et al. 2011, Delefosse & Kristensen 2012). *Arenicola marina* is a subsurface deposit-feeder, which in these studies buried particles by depositing faecal casts on the surface, resulting in surface-layer subduction, and by individual particle transport through its feeding funnel. As a conveyor-belt bioturbator, *A. marina* promotes both upward and downward transport of particles, but this transport is dependent on the particle size (Gebhardt & Forster 2018). Burial by depositing of faecal casts, or through the feeding funnel, was expected to be similar for all sizes of particles. However, the upward transport only applied to particles below the upper size limit of ingestion (typically less than 1 mm), which resulted in circulating smaller, once-buried particles from its feeding layer back to the sediment surface (Gebhardt & Forster 2018).

Based on our studies (I, II) and the evidence collected from other research (e.g. Gebhardt & Forster 2018), the microplastics at different sediment depths seem to be subject to varying bioturbation pressures arising from the residence depth of benthic species, functional group, as well as their species-specific characteristics (e.g. size range of food particles). To date, the effects of bioturbation on microplastic distribution in sediments have been mainly studied in laboratory-based experiments, but recent research has also confirmed the process in the environment. Functional trait-based diversity analysis of field samples collected from the southwestern coast of England revealed that the abundance

of microplastics in the sediment cores matched the presence of upward and downward conveyors (Coppock et al. 2021). Hence, understanding the characteristics of the local benthic fauna is central to assessing the fate and dynamics of microplastics in seafloor sediments in the northern Baltic Sea, and based on the presented results we conclude that the dominant macrofauna primarily facilitate microplastic burial up to a depth of 5 cm.

4.4 Bacterial community composition varies on different polymer types

4.4.1 Bacterial community composition on plastics

The plastics ending up in the marine environment represent a new surface for microbial colonization and biofilm formation (Zettler et al. 2013, Oberbeckmann et al. 2014). In our study (III), the bacterial communities on plastics resembled the community composition of the matrix they were incubated in (Fig. 4). Congruently with previous studies (e.g. Kirstein et al. 2016; Oberbeckmann et al. 2016, 2014; Zettler et al. 2013), the communities present in the water were distinct from those in the sediment and on plastics. This was expected, since particle-associated communities usually differ from the water phase hosting free-living bacteria (Rieck et al. 2015). The similarity of the bacterial communities on plastics and sediments also follows the view that most taxa do not differentiate between natural and artificial surfaces (reviewed by Oberbeckmann & Labrenz 2020; Wright et al. 2020b). Even though differences have been observed for the early colonizers of plastics and other surfaces, the similarity of the community composition increases with the maturation of the biofilm (Pinto et al. 2019, Erni-Cassola et al. 2020). Since mature plastisphere communities can be developed in a single week (Erni-Cassola et al. 2020), it is not surprising that the bacterial community composition in our 14-week study was similar on plastics and the surrounding sediment.

Despite the above-mentioned similarities, the bacterial community on CA differed from that of the other polymer types examined in the sediment incubations and was predominated by the class Bacteroidia at both sites (WH and VKL) (Fig. 4). In comparing biodegradable CA to conventional PA, the classes Bacteroidia and Spirochaetia were especially enriched on CA. These same classes have also been detected on plastics in previous studies (e.g. Oberbeckmann et al. 2016, 2014). Differences between bioplastics and conventional plastics have also been detected by Pinnell and Turner (2019), who found distinct microbial communities on PHA compared with polyethylene terephthalate (PET) incubated in coastal marine sediments, and by Dussud et al. (2018), who detected higher colonization of active and specific bacteria on biodegradable poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) than on PE in seawater. The surface properties of the plastic particles, such as

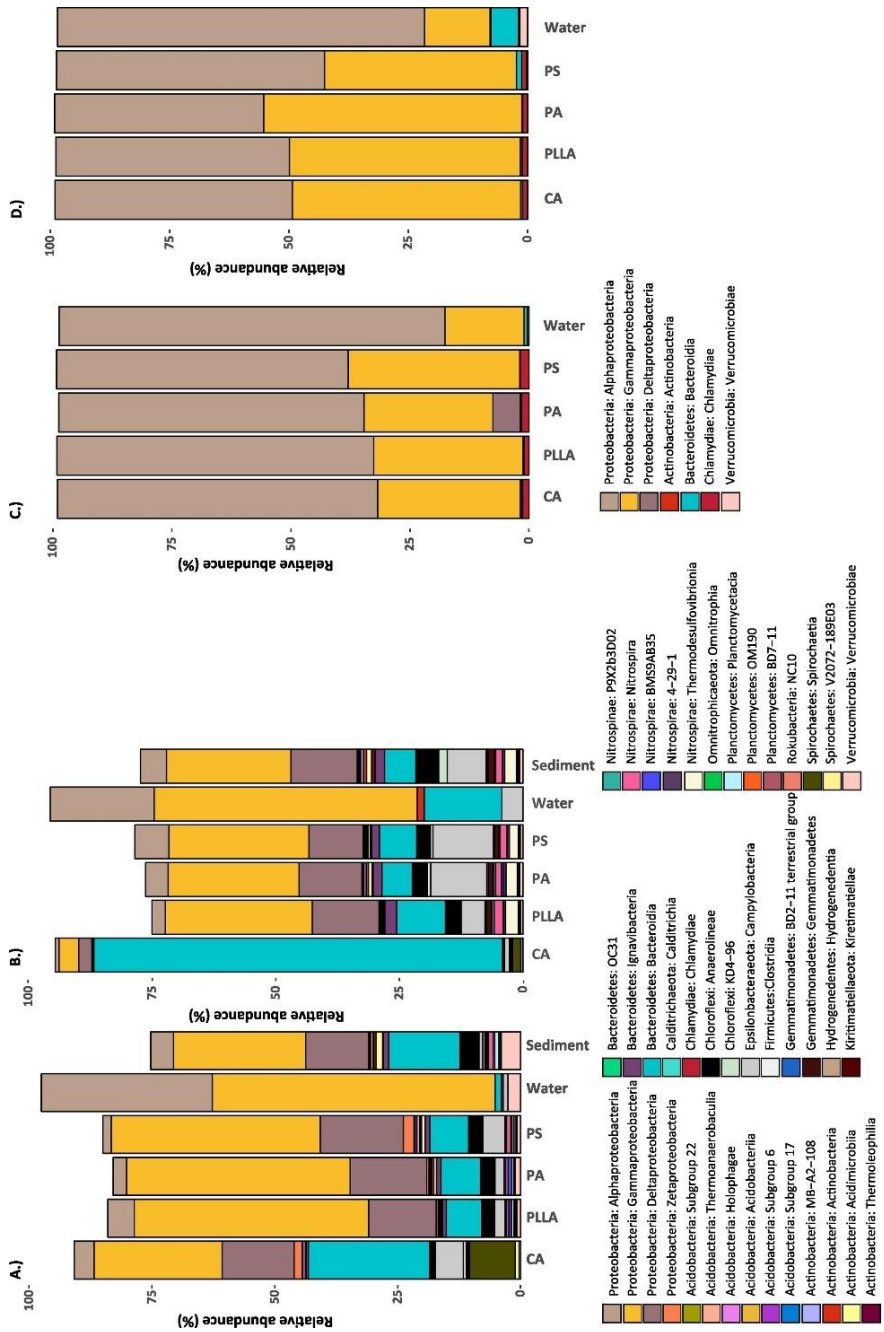


Figure 4. Class-level bacterial diversity of 16S ribosomal RNA gene sequences (~450 base pairs) representing > 0.1% of all amplicon sequence variants (ASVs) on different plastic types (CA = cellulose acetate, PLLA = poly-L-lactic acid, PA = polyamide, PS = polystyrene) and in sediment and water (A = West Harbour sediment, B = Vanhankaupunginlahti sediment, C = West Harbour water, D = Vanhankaupunginlahti water).

hydrophobicity, roughness or electric charge, may have influenced the formation of biofilm, resulting in some of the observed differences between plastic types (Artham et al. 2009, Oberbeckmann & Labrenz 2020).

The overall close resemblance of biofilms detected on plastics and in the sediment in most cases (III) may hinder some organisms' abilities to discriminate plastics from their natural food sources. Especially at risk may be animals that use chemosensory cues in foraging; for example, in pelagic environments the biofouling of microplastics promotes their ingestion by marine copepods (Vroom et al. 2017). Similarly, Botterell et al. (2020) demonstrated increased ingestion rates by marine copepods and European lobster larvae (*Homarus gammarus*) on microplastics infused in dimethyl sulphide (DMS), an infochemical secreted by marine phytoplankton.

4.4.2 Role of biofilm in plastic degradation

While plastic biofilms included hydrocarbonoclastic bacteria (e.g. *Arcobacter* and taxa in class Bacteroidia) specialized in degrading complex carbon substrates, no apparent degradation of any of the plastic types was observed in microscopic examination after the incubations (III). Many of the bacteria especially enriched on CA in the sediment at both sites (WH and VKL), such as Bacteroidia, *Spirochaeta* and Clostridia, are potentially cellulolytic, i.e. capable of decomposing cellulose (Das et al. 2006). In addition, *Pseudorhodobacter* (Alphaproteobacteria) was enriched on CA in the WH sediment. Both *Spirochaeta* and *Pseudorhodobacter* may also possess beta-galactosidase genes that encode enzymes capable of breaking bonds in natural polymers, including starch and other polysaccharides (Li et al. 2016, Chen et al. 2019). The enrichment of potentially cellulolytic bacteria on CA indicates that this bacterial community may have been selected due to their CA-degrading capability (III). While this may indicate potential biodegradation with time, it can only be speculated and would require further information on the specific properties of the used material, because the biodegradation potential of CA is inversely related to the degree of acetylation (Puls et al. 2011). However, since no visible degradation of CA was detected and we did not measure bacterial production or activity, the potential degradation of CA in marine sediments is yet to be confirmed in future investigations.

The observation that potential plastic-degrading communities developed on CA only in the sediments, and not in the water (III), highlights the need to determine where the plastic litter accumulates to be able to assess their persistence and degradation potential in the marine environment. Moreover, the results indicate that not all bioplastics behave similarly in the marine environment, since there were differences between the two bio-based, biodegradable polymer types examined (CA and

PLLA). Of these, CA seemed to be better at recruiting potentially plastic-degrading bacterial communities, and hence may have a higher potential for biodegradation than PLLA, which seemed as inert as the conventional polymer types (PA, PS) in the study. However, since biogeography, environmental conditions and the season may affect the bacterial community composition, the degradation potential of different polymer types may also vary, depending on these factors (Oberbeckmann et al. 2014, 2016, Amaral-Zettler et al. 2015).

To date, the biodegradation of microplastics has not been verified in the marine environment (reviewed by Oberbeckmann & Labrenz 2020). The conclusions of potential biodegradation of plastics have been made, based on weight loss of the material, sometimes coupled with biofilm growth (e.g. Artham et al. 2009). However, this approach has been criticized as not being able to differentiate between the biodegradation of the polymer matrix and the biodegradation of the additives or residual monomers present in the material, hence leading possibly to inaccurate interpretation of the results (Oberbeckmann & Labrenz 2020, Wright et al. 2020a). Wright et al. (2020a) even hypothesized that plastics could act as hotspots of pollutant degradation, because they both concentrate HOCs from the environment and may stimulate the development of specialized biodegrading microbes. In addition to environmentally derived HOCs, plastic additives and weathering subproducts can be present in the plastics and may be more likely degraded by microorganisms, due to their lability, than the polymer matrix (Wright et al. 2020b).

4.5 PAH sorption on plastic is influenced by the polymer type and the surrounding media

4.5.1 PS consistently sorbs more PAHs than the other plastics examined

Plastics are known to sorb various HOCs, including PAHs, from their surrounding environment (e.g. Rochman et al. 2013a, b, Sørensen et al. 2020), and likewise, during the 3-month incubation in PAH-containing sediments, both conventional and biodegradable plastics sorbed PAHs in our study (III). The total concentration of PAHs varied between the plastic types examined at both study sites and matrices: WH sediment ($p = 0.020$), WH water ($p = 0.003$), VKL sediment ($p = 0.034$) and VKL water ($p = 0.040$). In all cases, the highest concentrations of PAHs were observed in PS (Fig. 5). The sum of the PAHs was significantly higher in PS than in CA ($p = 0.032$) and PLLA ($p = 0.024$) in WH sediment, while in WH water it was higher in PS than in PA ($p = 0.009$) and PLLA ($p = 0.004$). In the VKL sediment, a higher sum of PAHs was observed in PS than in PLLA ($p = 0.008$), and a similar pattern was also seen in VKL water: PS showed higher concentrations of PAHs than did PLLA ($p = 0.046$).

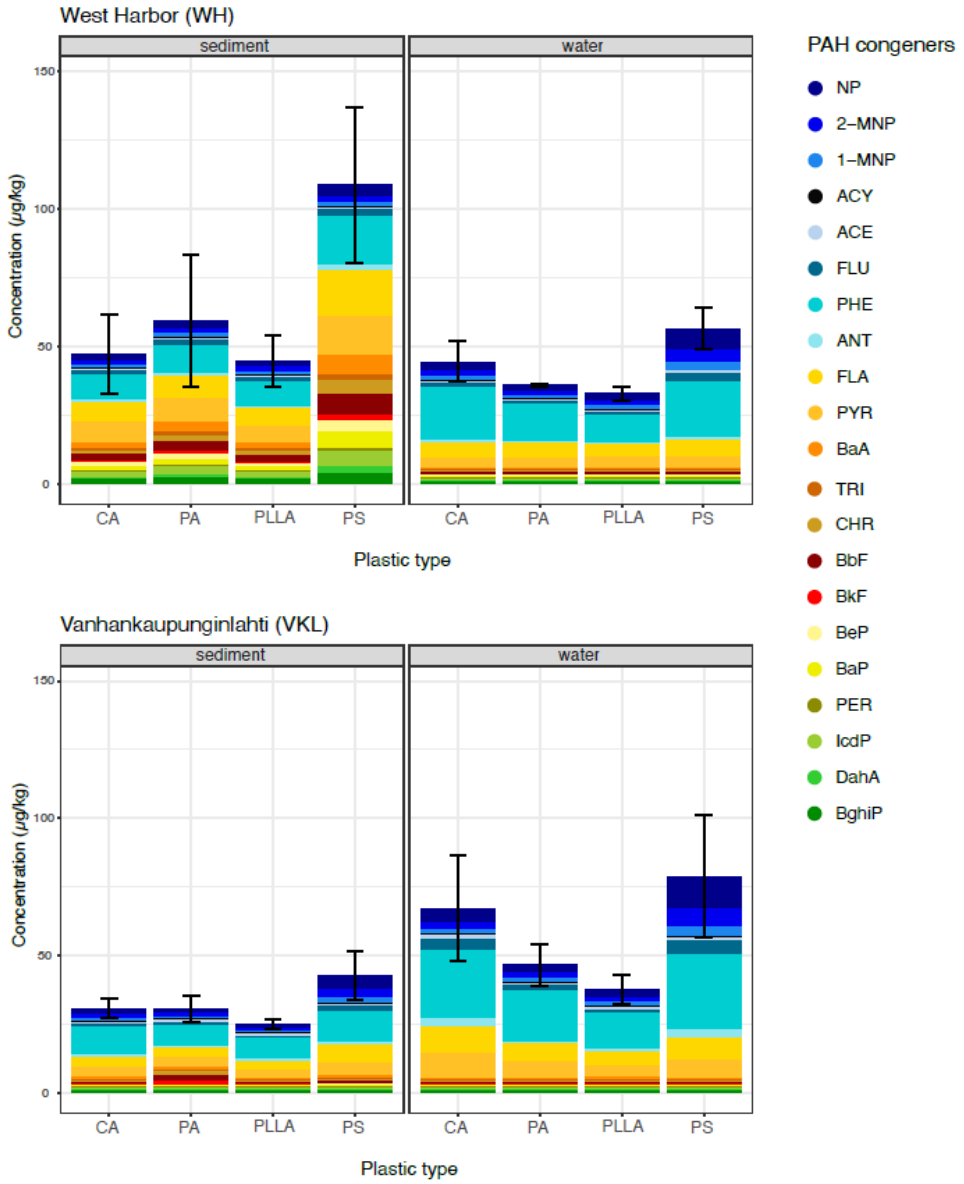


Figure 5. Average sum of polycyclic aromatic hydrocarbon (PAH) congeners and total PAHs (mean ± SD) in the plastic types examined (CA = cellulose acetate, PLLA = poly-L-lactic acid, PA = polyamide, PS = polystyrene) incubated in West Harbour (WH) and Vanhankaupunginlahti (VKL) sediments and water. The low-molecular-weight (LMW) PAHs are visualized, using blue shades and high-molecular-weight (HMW) PAHs yellow, red and green shades.

In the PAH sorption to plastics, the hydrophobic interactions are deemed one of the predominant mechanisms of sorption, since both PAHs and plastics are hydrophobic (Tourinho et al. 2019). The hydrophobicity of polymer types varies: for example, PS is classified as highly hydrophobic, and its aromaticity further enables efficient PAH adsorption, due to noncovalent π–π interactions between the aromatic groups (e.g. Claessens & Stoddart 1997). Another factor explaining the high sorption capacity of PS may lie in its amorphous structure (Rochman et al. 2013b, Velzeboer et al. 2014). Sorption occurs primarily in amorphous domains, and polymers contain different ratios of amorphous and crystalline contents. In crystalline regions the molecules of the polymer show an ordered structure, in which the positions and motions of atoms are restricted. In the amorphous regions, however, the orientation of the polymer chains is more random, and the molecular segments can move more freely, which creates space for sorption (Endo & Koelmans 2019). In the case of PS, each of the styrene monomers contains aromatic rings that are randomly distributed on both sides of the polymer chain, generating a space between adjacent polymer chains where chemicals can diffuse (Pascall et al. 2005).

These characteristics, crystallinity and nonaromaticity, likely may partly explain the observed differences in PAH sorption between polymer types (III). CA is amorphous like PS, but not aromatic, and the other polymer types used in the experiment are all less amorphous than PS; the PA used is a semicrystalline polymer, whereas PLLA has high crystalline content (60–70%). In accordance with its high crystallinity, the concentrations of PAHs measured in PLLA were lower, although not significantly, than in other plastic types.

Our results support previous observations showing that sorption capacity of PAHs is dependent on the plastic polymer type (Rochman et al. 2013a, b, Sørensen et al. 2020). Furthermore, the proposal by Rochman et al. (2013b) that PS may pose a greater risk for the marine environment because of its high sorption capacity and potential to act as an efficient vector for sorbed contaminants is also supported. While for this reason PS, and also PE (Sørensen et al. 2020), have been recognized as materials of elevated risk, it is not fully known how environmental factors alter the sorptive capacities of different polymer types. The state of the plastic changes over time and place, and it has been assumed that the concentration of HOCs may be a function of the age of the plastic, because the surface area available for sorption increases as a result of weathering (Mato et al. 2001, Rios et al. 2007, Liu et al. 2020). In addition to weathering (Karapanagioti & Klontza 2008), biofilm formation and microbial degradation (Johansen et al. 2019), and competitive sorption can affect the sorptive capacities of different polymer types (Bakir et al.

2012, Endo & Koelmans 2019), as well as environmental conditions such as water temperature (Sørensen et al. 2020).

4.5.2 The surrounding matrix and properties of PAH congeners affect the sorption

Since the concentrations of PAHs in the plastics varied, depending on whether they were incubated in the sediment or in the water (Fig. 5), it is evident that the surrounding matrix also affects the sorption (III). In general, in WH the sum of the PAHs was higher in plastics incubated in sediment, whereas in VKL it was higher in water-incubated plastics. The PAH congeners and their concentrations detected on plastics probably reflect the pool of PAHs available in different matrices, as well as the other qualities of the matrices that can further affect sorption (e.g. amount of dissolved and particulate organic matter, clay content, pH; Amelia et al. 2021).

It is known that sorption is partly governed by the MW of the PAHs (Rochman et al. 2013a), since the hydrophobicity of the congener generally increases with the number of aromatic rings (Achten & Andersson 2015). Hence, the concentrations of lower-molecular-weight (LMW; 2–3 aromatic rings) and higher-molecular-weight (HMW; 4–6 aromatic rings) PAHs were investigated separately (III). More LMW PAHs sorbed to plastics from water than from the sediment at both sites, being in line with a study in which LMW PAHs accounted for most of the PAHs in microplastics collected from the sea surface (Mai et al. 2018). Even though the initial PAH concentrations in water were not quantified in the present study (III), in general the concentrations of LMW and HMW PAHs on plastics at WH seemed to reflect the environmental observations on the predominance of LMW PAHs in the water phase and HMW PAHs in the sediment (Witt 1995). However, a similar pattern was not found at VKL, where the LMW PAHs dominated the PAH profile of plastics incubated in the sediment. This may have been due to both the sediment quantity and quality. The sediment samples from VKL showed a higher water content and lower organic matter content than the sediments from WH, which likely restricted the amount of particle-bound HMW PAHs available for partitioning to plastics. The seawater from the VKL site also contained more clay particles than did the WH water. The clay particles may have carried more PAHs to the water units and could explain the high concentrations of PAHs in plastics incubated in the VKL water.

Even in the same matrix, the sorption patterns may differ between polymer types and PAH congeners. Previously, faster saturation was achieved in LMW PAHs than in HMW PAHs when various polymer types (low-density polyethylene [LDPE], high-density polyethylene [HDPE], polypropylene [PP], PET, polyvinyl chloride [PVC]) were studied in seawater, but in a similar study using PS, no clear differences in sorption patterns of LMW and HMW

PAHs were detected (Rochman et al. 2013a, b). While the concentrations of PAHs in PS, HDPE and LDPE were similar after 6 months of incubation, the predicted equilibrium for PS was achieved much faster (Rochman et al. 2013a, b). Since the time to reach equilibrium is not only dependent on the congener and polymer properties, but also on the volume of the surrounding matrix (Koelmans et al. 2016), it could be speculated that our 3-month incubation under the stable conditions of the small experimental aquaria could have been long enough to at least approach equilibrium (III). In the field, equilibrium times as short as one month have been verified for polyoxomethylene (POM) and polydimethylsiloxane (PDMS) films used as passive samplers for PAHs in the sediment (Cornelissen et al. 2008). However, since the sorption kinetics were not followed in our study, it cannot be concluded whether equilibrium between the plastics and the surrounding media was achieved, although it may have been for some polymer types, or for only certain congeners.

4.6 Plastics as vectors for PAHs and other contaminants

4.6.1 Plastics may contribute to the downward flux of PAHs and alter their bioavailability in sediments

The PAHs sorbed to plastics in water incubations (III) suggest that floating plastic litter may serve as additional sorptive surfaces for newly emitted PAHs. Due to the particles' tendency to settle, microplastics may then participate in the downward flux of PAHs, similar to the settling phytoplankton and faecal pellets of copepods (Prahl & Carpenter 1979, Witt 2002), but unlike these natural particles, buoyant microplastics may persist in the surface waters for longer periods of time. Previous field studies have detected PAH concentrations up to 1200 µg/kg in plastic pellets and postconsumer plastic fragments (Rios et al. 2007), and even 119 000 µg/kg in surface-water microplastics (Mai et al. 2018). Since polymers vary in their sorptive capacities and specific gravities, plastics are expected to have varying PAH concentrations and residence times at the water surface. Especially buoyant plastics with high sorptive capacities may thus carry HOCs both horizontally and vertically in the marine environment.

When in sediment, the same PAH congeners were quantified from the plastics and the sediment at WH, but the concentrations of PAHs were considerably lower in plastics (III). While the maximum PAH concentration based on LOQs was 2670 µg/kg in the sediment, in plastics it varied between 45 and 109 µg/kg, depending on the polymer type, and even the highest concentration quantified in one PS replicate (139 µg/kg) represented only a fraction of the bulk sediment concentration. It appears that plastics in the sediment aquaria acted as passive samplers, sorbing dissolved PAHs from the sediment pore water. Passive sampling is a common tool for studying dissolved PAH concentrations (Lang et al. 2015), and

rather than the bulk sediment concentrations, those quantified in passive samplers are believed to represent the bioavailable concentration of PAHs and other HOCs in the sediment (Maruya et al. 2009, Beckingham & Ghosh 2013). This dissolved, bioavailable fraction of HOCs is crucial for marine invertebrates, since the major route of HOC exposure to them is estimated to be bioconcentration, i.e. occurring passively via the body surface or respiratory organs (Gray 2002, Teuten et al. 2009), although ingestion of contaminant-loaded sediment (i.e. bioaccumulation) may also play a role for deposit-feeders (Leppänen 1995). Hence, if plastics sorb dissolved PAHs, it could be argued that plastics in the seafloor may reduce the uptake of the bioavailable fraction of PAHs. Indications of this has been received from an experiment in which the presence of PE and PS microplastics in seawater reduced both the concentration of dissolved fluoranthene (FLA) and phenanthrene (PHE), as well as the body burden of these congeners in the copepod *Calanus finmarchicus* during a 96-h exposure (Sørensen et al. 2020). Similarly, a coexposure to FLA and PE microplastics decreased the concentration of FLA in *Mytilus edulis* tissues compared with FLA exposure only (Magara et al. 2018). It is thus possible that the PAHs sorbed to microplastics do not significantly contribute to the body burden of animals under conditions in which the same congeners are present dissolved and taken up from the water phase.

The quantity of plastic that would be needed to significantly modulate the bioavailability of PAHs in sediments is unclear. In the Baltic Sea, macrolitter abundance on the seafloor varies. Kammann et al. (2018) estimated an average of 5.07 items per km^2 (of which 66% were plastics), whereas the data collected in the Baltic International Trawl Surveys (BITS) suggest an average of 58.9 (± 20.9) items per km^2 (of which 30.6% were plastics) (HELCOM 2018). However, the litter is not uniformly distributed on the seafloor, and the present hydrodynamics, geomorphology and human influence (Pham et al. 2014, Galgani et al. 2015) may locally accumulate large aggregations of plastics that may cause small-scale alterations in the benthic habitat. In addition to macroplastics, the microplastic (> 20 μm) abundance in the coastal sediments of the northern Baltic Sea can reach over 20 000 pieces per kg dw (Tirroniemi 2019), and because of their high surface-to-volume ratio they are believed to be efficient in adsorbing PAHs and other HOCs. They may thus also participate in modulating the PAH bioavailability in sediments, but unlike larger plastic litter, microplastics are more prone to being ingested. However, in general, the importance of microplastics as HOC vectors to fauna is assumed to be rather low at current exposure levels compared with other pathways (Gouin et al. 2011, Koelmans et al. 2016, Bakir et al. 2016). The PAH concentrations on plastics and in the sediment (III) indicate that the ingestion of plastics is not likely to increase the PAH burden of deposit-feeding animals. Even if the

equilibrium between plastic and the surrounding media were achieved, the quantity of HOCs in the plastics currently in the ocean is estimated to represent only a tiny fraction of the HOC mass that is held by other media (Koelmans et al. 2016).

4.6.2 Microplastics as sources of contaminants

While acting as a 'sink' for various HOCs in the environment, plastics can also be a source for some contaminants. Rochman et al. (2013b) measured several PAH congeners from virgin PS, probably originating from the plastic-manufacturing process. Similarly, a variety of organic compounds related to plastic manufacturing, such as butylated hydroxytoluene (BHT), acetyltributylcitrate and octabenzone, used respectively as antioxidants, plasticizers and stabilizers, have been detected when screening plastic litter collected from the marine environment (Gauquie et al. 2015). In addition, many heavy metals, such as Pb used as a catalyst and Cr, Br and Cd that are ingredients of colorants and flame retardants, occur in plastic litter (Turner & Lau 2016, Turner 2017). Although the impact of environmentally derived HOCs sorbed to plastics may not pose elevated risk for animals, the vector-effect may be more pronounced for the chemicals that originate from the plastic itself, especially when the plastic is newly emitted to the environment (Koelmans et al. 2016).

One potentially harmful type of microplastics is car tyre fragments, which have only recently gained attention as widespread environmental pollutants (Kole et al. 2017, Wagner et al. 2018). They comprise a mixture of natural and synthetic rubber polymers, and numerous additives such as carbon blacks, stabilizers, process oils, fillers and pigments (Halsband et al. 2020). Several harmful substances have been detected from tyre rubber and its leachates, the most abundant being heavy metals, benzothiazole, PAHs and phenolic compounds (Councell et al. 2004, Celeiro et al. 2014, Halsband et al. 2020, Capolupo et al. 2020). Recently, Tian et al. (2020) also discovered a highly toxic quinone transformation product of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) derived from tyre rubber, where it is used as an antiozonant.

Congruently with earlier studies (Councell et al. 2004, Celeiro et al. 2014, Halsband et al. 2020, Capolupo et al. 2020), the tyre rubber used in experiment IV contained metals and PAHs, which showed the highest concentrations in the experimental waters of both particle treatments AP and CP at the start of the experiment (Day 3). Of the metals, Zn, Co and Cu were especially abundant, and of the PAH congeners, most notably pyrene (PYR), FLA and acenaphthylene (ACY) showed elevated concentrations (Table 6). Similar observations have also been made in other studies that examined the contaminants in tyre rubber and its leachates (Celeiro et al. 2014, Halsband et al. 2020, Capolupo et al.

2020), and as in the present study (IV), the water samples were analysed unfiltered, the measured concentrations representing both the contaminants present in the rubber and the ones that may have leached to the water. The controls and leachate treatments did not markedly differ from each other, indicating that the mesh bags in the AL and CL treatments possibly prevented efficient leaching.

In general, the most abundant PAH congeners and metals measured in the experiment waters were also most abundant in clam tissues (IV). The sum of the PAHs was highest in clams in the particle treatments AP (1029 µg/kg wet weight [ww]) and CP (1033 µg/kg ww), compared with AC (265 µg/kg ww), AL (230 µg/kg ww), CC (116 µg/kg ww) and CL (117 µg/kg ww). The mean concentrations of Zn and Co were also elevated in the clams in particle treatments AP and CP (Zn: 1549–1555 mg/kg dw; Co: 10.6–12 mg/kg dw) compared with leachate treatments AL and CL (Zn: 1047–1154 mg/kg dw; Co: 4.8 mg/kg dw) and controls AC and CC (Zn: 961–1188 mg/kg dw; Co: 4.5–6.2 mg/kg dw). This was expected, since tyre rubber ingestion by *Limecola balthica* in the particle treatments was verified visually during the experiment. The similarity between the contaminant profiles in the water samples and clam tissues in the particle treatments is most likely due to them both containing tyre rubber particles – with the approach used, it is impossible to separate the contaminant load of the particles from that of the potential bioaccumulation of these contaminants in the tissues. Due to the ingestion, however, it can be concluded that the tyre rubber can act as a contaminant vector to clams.

Table 6. Concentrations of some metals and polycyclic aromatic hydrocarbons (PAHs) quantified from water samples on the third experimental day before the water renewal (IV). The contaminants showing the clearest differences between treatments were selected for the table, and the concentrations represent the maximum concentrations based on the limits of quantification (LOQs) (AC = acute control, AL = acute leachate, AP = acute particle, CC = chronic control, CL = chronic leachate, CP = chronic particle, Cu = copper, Co = cobalt, Zn = zinc, ACY = acenaphthylene, PHE = phenanthrene, FLA = fluoranthene, PYR = pyrene, BghiP = benzo[g,h,i]perylene).

	Metals in water samples			PAHs in water samples					
	Cu µg/L	Co µg/L	Zn µg/L	ACY µg/L	PHE µg/L	FLA µg/L	PYR µg/L	BghiP µg/L	sum of PAHs µg/L
AC	2.1	0.5	6.5	0.010	0.020	0.020	0.010	0.007	0.32
AL	1.7	0.5	17.5	0.010	0.020	0.020	0.010	0.005	0.36
AP	5.0	3.0	692.0	0.100	0.042	0.087	0.220	0.031	0.79
CC	2.1	0.4	2.6	0.010	0.020	0.020	0.010	0.002	0.31
CL	2.2	0.6	7.3	0.010	0.020	0.020	0.010	0.007	0.32
CP	6.8	2.7	706.0	0.081	0.059	0.120	0.300	0.042	0.92

4.7 Effects of tyre rubber fragments on the Baltic clam

4.7.1 Exposure to tyre rubber impacts the ADS and causes oxidative stress

Many contaminants present in tyre rubber, such as the heavy metals and PAHs quantified in this study (IV), are well-known inducers of oxidative stress in aquatic organisms (Lushchak 2011), and microplastic particles can also cause similar effects in various invertebrate groups (reviewed by Trestrail et al. 2020). The toxicity studies conducted with tyre rubber particles are still scarce (reviewed by Halle et al. 2020), but their leachates cause toxic effects for many species, including water fleas *Daphnia magna* and *Ceriodaphnia dubia* (Wik et al. 2009), and the Mediterranean mussel *Mytilus galloprovincialis* (Capolupo et al. 2020, 2021).

In the present experiment (IV), no neurotoxic effects were observed (as shown by the AChE activity, Fig. 6), which corresponds to the results obtained by Capolupo et al. (2021), who exposed *M. galloprovincialis* to tyre rubber leachates for a 7-day period. However, the ADS showed variable response patterns in both the acute and chronic experiments, indicating that the clams experienced oxidative stress following the tyre rubber exposure. Notable differences were seen in GPx, ORAC, SOD, GSH/GSSG and GST, whereas CAT, GR, GSH and GSSG showed no differences between treatments (IV).

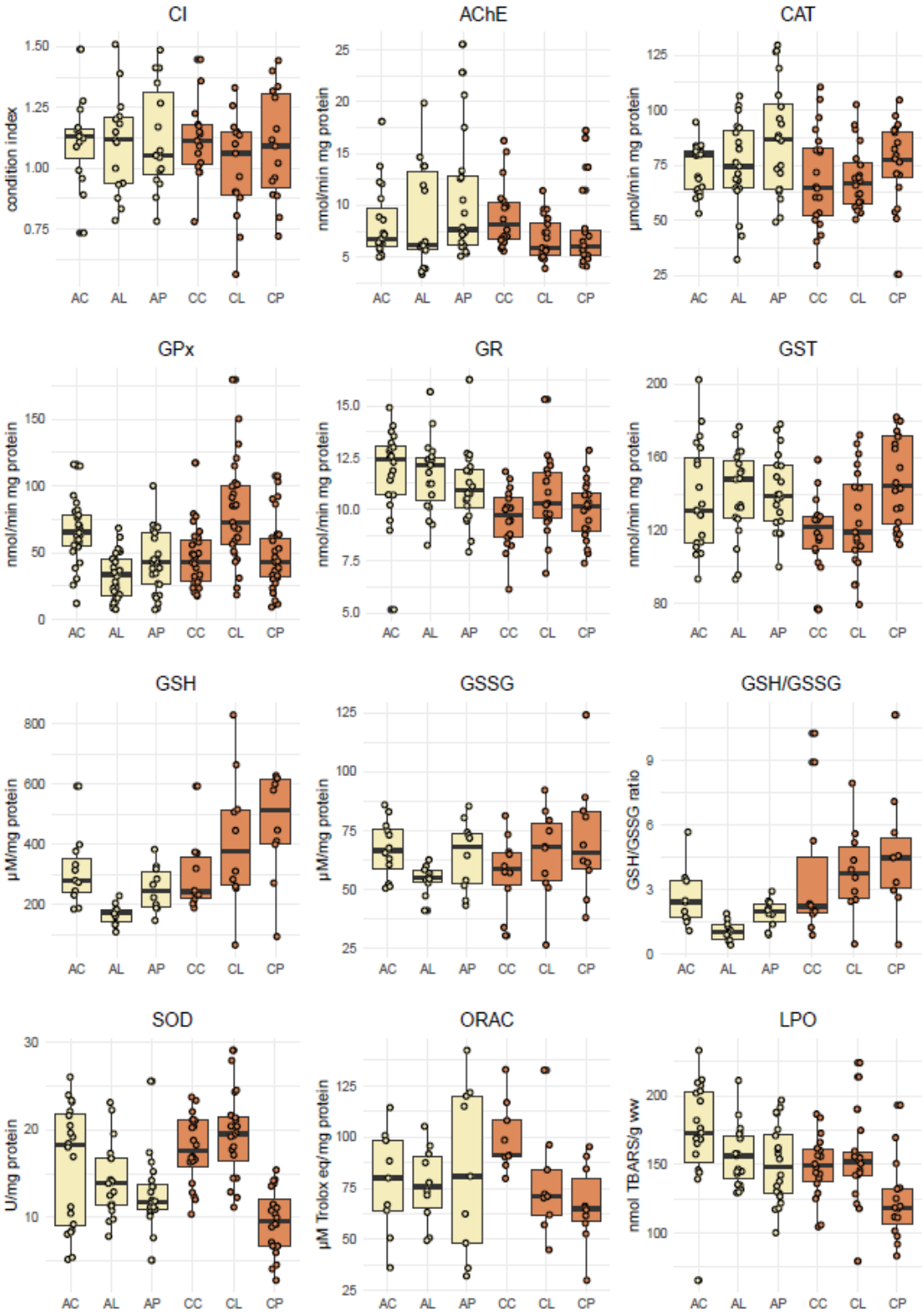
In the acute exposure experiment, the GSH/GSSG ratio was lower in AL than in AC ($p = 0.039$) (Fig. 6), which indicates increased GSH utilization and depletion of the GSH pool in the AL treatment. GSH is a nonenzymatic antioxidant agent that is considered one of the most important ROS scavengers, and it also participates in the detoxification of environmental contaminants (including PAHs) by conjugation (Cheung et al. 2001). The GPx activity was also lower in the AL ($p = 0.000$) and AP ($p = 0.003$) treatments than in the control, signalling potentially compromised protection against hydrogen peroxide. The activity of SOD, the first-line antioxidant defence enzyme, was reduced in the AL and AP treatments compared with AC, albeit not significantly ($p > 0.05$). However, the reduced SOD and GPx activities indicate increased stress as a response to tyre rubber exposure, further supported by the wide-ranging individual variation in ORAC in the AP-treated clams (Fig. 6).

In comparing the responses of the clams in the acute and chronic exposures, the changes in the ADS response patterns in the chronically exposed clams indicate adaptive metabolic arrangements, because neither the GSH/GSSG ratio nor the activity of GR was affected in the treatments containing tyre rubber (Fig. 6). However, the activity of GST was significantly higher in CP than in CC ($p = 0.004$), signifying increased detoxification, and the SOD activity was significantly lower in the CP treatment than in both the CC and CL treatments ($p < 0.000$). The lowered SOD activity in the CP treatment suggests a bell-shaped response in this enzyme; as a result of a month-long

exposure, the physiological capacity of the clams was compromised, leading to the reduction in SOD expression and subsequently to the measured low activity. Similar to the acute experiment, the ORAC also showed changes: the ORAC levels were considerably lower in the CP treatment than in CC ($p = 0.012$), and also lower in the CL treatment, although not significantly ($p > 0.05$). The reduction in the capacity to battle against the increased levels of ROS in the chronically exposed clams likely leads to macromolecular damage and subsequently to various types of health effects and reduced fitness in case the exposure continues.

Interestingly, even though the PAH and metal analyses from the water and clam tissues did not show marked differences in the concentrations of these contaminants between the control and leachate treatments, the biomarkers were able to reveal that the clams in the leachate treatments suffered from elevated stress levels compared with the control clams. For example, in the chronic experiment the GPx shows markedly increased activity in the CL treatment compared with both the CC ($p < 0.000$) and CP treatments ($p = 0.001$). This is a good example of the bell-shaped pattern of response, in which moderate exposure may cause the highest activities of the enzymes while severe stress leads to reduced activity (Dagnino et al. 2007). This pattern observed in the chronic experiment may have been due to slightly elevated concentrations of some metals in the CL treatment at the end of the experiment, but can also indicate the presence of some other leached chemicals that were not quantified in the present study, or reflect the observation by Khan et al. (2019), who found indication that the acute toxicity of tyre rubber particles is distinct from that of its leachates. In their experiment, at low concentrations the tyre rubber leachate was more toxic, but in high concentrations the particles had greater toxic effects on the amphipod *Hyalella azteca*. All in all, since only a few studies to date have examined the effects of tyre wear particles on aquatic organisms (reviewed by Halle et al. 2020), these results call upon further research for quantifying the underlying mechanisms causing the observed toxicity.

Figure 6. Biomarkers measured in clams in different treatments at the end of the experiment (AC = acute control, AL = acute leachate, AP = acute particle, CC = chronic control, CL = chronic leachate, CP = chronic particle, CI = condition index, AChE = acetylcholinesterase, CAT = catalase, GPx = glutathione peroxidase, GR = glutathione reductase, GST = glutathione-S-transferase, GSH = glutathione, GSSG = glutathione disulphide, GSH/GSSG = glutathione/glutathione disulphide ratio, SOD = superoxide dismutase, ORAC = oxygen-radical antioxidant capacity, LPO = lipid peroxidase).


CI
condition index
AChE
nmol/min mg protein
CAT
µmol/min mg protein
GPx
nmol/min mg protein
GR
nmol/min mg protein
GST
nmol/min mg protein
GSH
µM/mg protein
GSSG
µM/mg protein
GSH/GSSG
GSH/GSSG ratio
SOD
U/mg protein
ORAC
µM Trolox eq/mg protein
LPO
nmol TBARS/g ww
AC AL AP CC CL CP

4.7.2 Abnormalities in vital cellular structures of the clams as signs of oxidative damage

When the ADS fails to compensate for the harmful effects of ROS, increased oxidative damage to macromolecules, such as DNA, lipids and proteins, can occur (Livingstone 2001). Surprisingly, even though the LPO levels of clams were lower in CP than in CL and CC (Fig. 6), no increased degradation of lipids was detected between treatments ($p > 0.05$). However, since the level of cytogenetic damage was elevated in the CL and CP treatments compared with the CC, albeit not significantly ($p = 0.056$ in both cases), the DNA and cell viability were impacted (IV). Most of the damage was observed in the genotoxic endpoints, indicating DNA damage that can arise via multiple routes. The contaminant may directly damage DNA or produce damaging metabolites, increase the generation of ROS or hamper DNA synthesis and repair (Lee & Steinert 2003). Although many PAHs and their metabolites are known to have toxic, mutagenic and carcinogenic properties (Baršienė et al. 2012), the level and profiles of cytogenetic damage in the CL and CP treatments were very similar, suggesting that possibly some other components of the tyre rubber may have caused the effects. While research on the genotoxic effects of tyre rubber fragments in aquatic organisms is still lacking, several *in vitro* studies have demonstrated their effects e.g. on the human lung cell line A549 (Gualtieri et al. 2005, Karlsson et al. 2006). Due to the limited knowledge on the underlying mechanisms of tyre rubber toxicity, further research is needed.

In addition to the above-mentioned biomarkers, the stress response to environmental pollution can also be expressed as abnormalities in cell ultrastructure. Qualitative investigation of the gills, DG and foot of the clams revealed disruptions, especially in the mitochondria and lysosomes (IV). Most abnormalities in cell ultrastructure were detected in the AP, CL and CP treatments, giving further indication of the harmful effects of tyre rubber exposure. In the chronic experiment, swollen mitochondria were found in the gills and DG cells of the clams in the CL and CP treatments. Mitochondrial swelling is a typical response to the exposure to various contaminants, such as tributyl chloride and zinc oxide, in aquatic organisms (Tiano et al. 2003, Trevisan et al. 2014), and is induced by ruptures that increased ROS production causes in the outer membranes of the mitochondria, leading to disruption of their volume homoeostasis (Ott et al. 2007, Chatterjee et al. 2009, Slimen et al. 2014). Since mitochondria play a vital role in cellular respiration, severe impairment of their functions may have serious effects on cell viability (Ott et al. 2007, Slimen et al. 2014).

Enlarged lysosomes and increase in their numbers were observed in the gills of the AP and CL clams, while in the foot the number of lysosomes was increased, and they contained dark material not only in the AP and CP clams, but also in the AC clams. These responses have also been recorded,

following exposure to various pollutants, especially in the DG cells of mussels (Marigómez et al. 1996, Marigómez & Baybay-Villacorta 2003). Since lysosomes are the main sites for metal and organic pollution sequestration and detoxification in the cells, metals accumulating in the lysosomes can disturb normal functioning and damage the lysosomal membrane (Viarengo et al. 1987, Moore et al. 2004). This may lead to a reduction in lysosomal membrane stability (LMS), which in turn is often coupled with lysosome enlargement or swelling (Marigómez & Baybay-Villacorta 2003, Moore et al. 2008). For example, heavy-metal or petroleum hydrocarbon exposure can lead to the enlargement of lysosomes in digestive cells of blue mussels (genus *Mytilus*) (Regoli 1992, Cajaraville et al. 1995, Marigómez et al. 2005). Furthermore, previous studies by Capolupo et al. (2020, 2021) demonstrated a reduction in LMS in *M. galloprovincialis* haemocytes as a response to tyre rubber leachates, giving further evidence for the deleterious impacts of tyre rubber on bivalve lysosomes. In one of these studies, an increase in the lysosome-to-cytoplasm volume ratio was also observed concurrently with the reduction in LMS, as well as increased lipofuscin content of lysosomes (Capolupo et al. 2021). Lipofuscins are the endpoints of the peroxidation of autophagocytosed cellular components and indicate autophagy (i.e. degradation of cellular components in lysosomes). While the ADS enzymes form the first tier of defence against oxidative damage, lysosomal autophagy forms a second line of defence, e.g. by removing oxidatively damaged organelles and macromolecules (Moore et al. 2006, 2008). The dark material inside the lysosomes in our study likely also indicates increased autophagy.

Since multiple changes in vital cellular structures, such as DNA, mitochondria and lysosomes, were observed in clam tissues following tyre rubber exposure (IV), it can be concluded that the ADS did not adequately protect the cell from ROS-induced damage in the clams in the AP, CL and CP treatments. The second line of defence (autophagy) was potentially triggered by the ineffective coping with the increased levels of ROS and damage affecting the cell macromolecules. Oxidative stress, DNA damage and mitochondrial dysfunction can all activate autophagy (Pesonen & Vähäkangas 2019). Autophagy is an adaptive process that protects cells (Silvestre 2020), but if autophagic capabilities fail to restore the homoeostasis, autophagy may also serve as a third tier of defence triggering cell death (Moore et al. 2006). Since the experiment did not last any longer, the impacts of prolonged exposure on the clams were left undefined.

4.8 Links between intracellular stress, individual fitness and ecosystem functioning

Biomarkers can be used to link molecular and cellular stress and damage to higher levels of biological

organization. Individuals have limited energy resources, and when they are exposed to contaminants, more energy is allocated to the ADS and other mechanisms fighting to prevent the damage caused by increased production of ROS, restricting the availability of energy for growth or reproduction (Trestrail et al. 2020). This trade-off in energy allocation is common in many invertebrates exposed to environmental contaminants (Petes et al. 2008, Lister et al. 2016) and raises concerns for the impacts of microplastics and associated contaminants in the marine environment. So far, only limited evidence for the effects of microplastics or their leachates on energy allocation has been provided. Some studies have demonstrated a reduction in regeneration rates of the polychaete *Perinereis aibuhitensis* (Leung & Chan 2018), a delay in metamorphosis of *Chironomus tepperi* (Ziajahromi et al. 2018), a decrease in weight and energy reserves of *Arenicola marina* (Wright et al. 2013, Besseling et al. 2013), and depletion of energy reserves in the sediment-dwelling clams *Abra nitida* and *Ennucula tenuis* (Bour et al. 2018). In the Pacific oyster *Crassostrea gigas*, exposure to PS spheres impacted not only energy uptake and allocation, but also reproduction and offspring performance (Sussarellu et al. 2016). However, in many studies no effects on somatic growth have been found (e.g. Bruck & Ford 2018; Redondo-Hasselerharm et al. 2018), showing that the effects of exposure are highly variable and are dependent on e.g. the study species, stage of the life cycle, polymer type and exposure concentration.

Despite the oxidative stress and damage in our study (IV), no indication was found that tyre rubber exposure affected the CI of the clams ($p = 0.642$), albeit higher variability between individuals was observed in tyre rubber-exposed clams than in the controls (Fig. 6). The lack of differences is not surprising, since differences in the CI commonly are not found in bivalves experimentally exposed to microplastics (Sussarellu et al. 2016, Ribeiro et al. 2017, O'Donovan et al. 2018, Bour et al. 2018, Bråte et al. 2018), and in our study the clams were fed on a regular basis, which maintained their nutritional status.

A small side experiment (briefly described in Table 4) was also conducted to investigate whether the reburial activity of the clams was reduced following tyre rubber exposure, since previous studies have demonstrated that the exposure to oil and various metals, including Cd, Cu, Co, Hg and Ni, commonly causes a decrease in *Limecola balthica* burrowing activity (McGreer 1979, Eldon et al. 1980, Stekoll et al. 1980, Sokolowski et al. 1999). In the side experiment, the number of clams that buried themselves in the clean sediment during the 90-min period varied between 14 and 22 clams (56–88%) out of the 25 individuals tested in each treatment (Fig. 7). The burial rate during the first 30 min was fastest in clams taken from the CP and AP treatments, and slowest in the AC clams. After 90 min, most of the clams in the AP and CC treatments had

buried themselves (88% and 84%, respectively), and the lowest reburial was exhibited by clams taken from the AC and CL treatments (56% both). No clear effect of the treatment was seemingly detected, indicating that the clams sustained their burrowing performance despite the tyre rubber exposure. Similar experimental approaches using 30-min observation intervals were used, e.g. by Sokolowski et al. (1999), who concluded that the clams burrowed more slowly after being exposed to high Cu concentrations (37.5–75 µg/l). In contrast, Bour et al. (2018) found no alterations in the burrowing behaviour of *Abra nitida* and *Ennucula tenuis* after exposure to PE microplastics of varying sizes and concentrations, although decreases in energy reserves were detected. Despite the observed intracellular damage in the present study (IV), the exposure concentrations may have been too low to cause clear behavioural alterations.

However, the decreased energy reserves caused by microplastic exposure has been proposed to lead to reduced sediment-reworking activities by benthic invertebrates (Wright et al. 2013). Following studies have since shown that reworking activity can indeed change as a result of microplastic exposure. For example, the burrowing activity of the brittle stars *Amphiura filiformis* was modified when exposed to PA microfibres (Coppock et al. 2021), and the turnover rate of sediment by *Arenicola marina* decreased in sediments containing microplastics (Green et al. 2016). The decreased burrowing by *A. marina* could have led to the reduced surface area available for sediment-water exchange, which in turn would have resulted in reduction in inorganic nutrient release (Green et al. 2016).

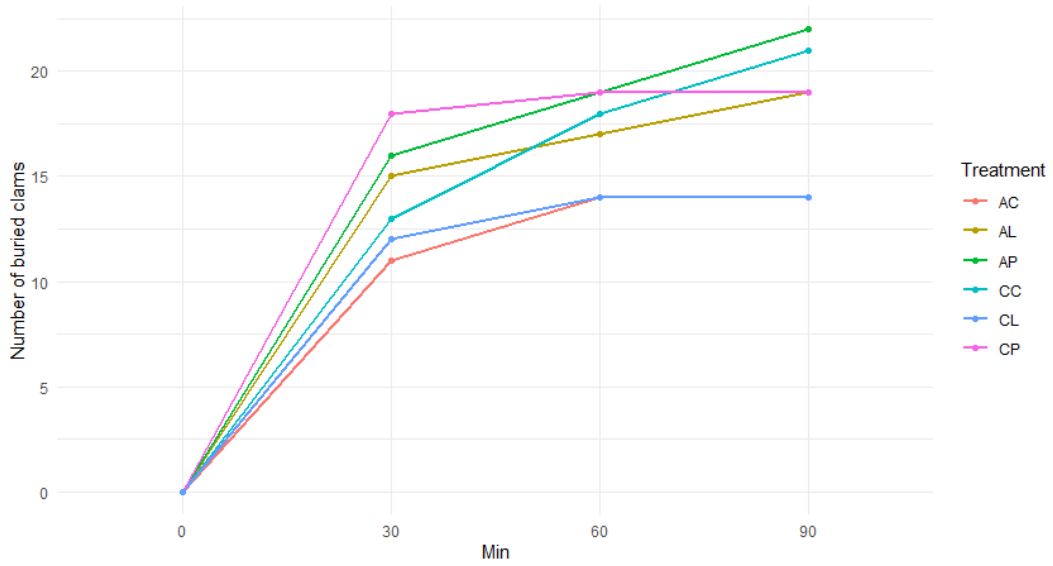


Figure 7. Number of clams buried in the clean sediment at 30-min intervals after ending experiment IV (AC = acute control, AL = acute leachate, AP = acute particle, CC = chronic control, CL = chronic leachate and CP = chronic particle).

Galloway et al. (2017) also stressed the importance of bioturbation as a process that forms a link between individual performance and ecosystem functioning in respect to the impacts that microplastics may have on the marine environment. To date, however, the impacts of microplastic exposure on bioturbation processes (particle and solute transfer) have been only sporadically examined, despite the vital role bioturbation plays in carbon and nutrient cycling, oxygen penetration into the sediment, as well as in the metabolism, dispersion and burial of marine pollutants (Rhoads 1974, Snelgrove 1998). Although the effects of microplastics on bioturbation were not quantified in this thesis, the biochemical and intracellular stress experienced by *Limecola balthica* may have far-reaching consequences, e.g. if it leads to fitness reduction and hampers the growth of individuals. For example, the body size of *L. balthica* is an important factor impacting oxygen and nutrient fluxes, and hence large individuals are vital for ecosystem functioning (Norkko et al. 2013). Since indications of the ingestion of tyre rubber and other microplastics by *L. balthica* in the northern Baltic Sea already exist (Bråte et al. 2020, Mustonen 2020), further studies are needed to assess whether the exposure to microplastics and associated contaminants can affect the individuals in a way that may lead to perturbations in the structure of the populations, benthic communities and eventually seafloor habitats.

5 MICROPLASTICS ON THE SEAFLOOR – CONCLUSIONS AND FUTURE RESEARCH NEEDS

Here, we examined the interactions between plastics, benthic communities and harmful substances in the northern Baltic Sea, shedding light on how the size, properties (polymer type and associated contaminants) and vertical distributions of plastic litter on the seafloor affect their bioavailability and risks to benthic fauna. The results demonstrate that the bioavailability of microplastics is governed by species-specific upper size limits of ingestion, which in the species examined (*Limecola balthica*, *Monoporeia affinis* and *Marenzelleria* spp.) was relatively small (~ 300 µm at the largest). Thus, to assess the risks microplastics pose to benthic fauna, the focus should be on the smallest size fractions that also comprise the majority of microplastics in sediments, as demonstrated e.g. by Bergmann et al. (2017). Furthermore, since these species mainly feed on the sediment surface, the bioavailability of newly settled microplastics is highest and decreases with their burial depth within the sediment.

Bioturbation plays an important role in the vertical distribution of microplastics in sediments: the biodiffusers *Limecola balthica* and *Monoporeia affinis* and gallery-diffusers *Marenzelleria* spp. facilitated the burial of microplastics, while at the same time reducing the bioavailability of these particles and promoting their preservation in the seafloor. Since the redistribution of once-buried microplastics back to the sediment surface by bioturbation was negligible, these results support the hypothesis of the Baltic Sea sediments acting as microplastic sinks. The seafloors represent both current and future hotspots for microplastic pollution, and if loading continues at the current level, the benthic fauna feeding on surficial sediments are likely to be continuously exposed to microplastics during their lifetime.

Monitoring of microplastics on the seafloor is required in the MSFD (Directive 2008/56/EC), and sampling is recommended to cover the uppermost 5 cm of the sediment (Galgani et al. 2013). This sampling approach is suitable for the northern Baltic Sea, since it corresponds to the bioturbated depth observed in this thesis. However, the patterns of bioturbation vary geographically, depending on the fauna present in the sediments, and thus spatial discrepancies in the vertical distribution of microplastics are likely to occur even between sites under similar levels of microplastic loading. To obtain a more comprehensive picture of the microplastic reservoirs in the seafloor, sampling of deeper sediment layers would be reasonable, depending on the structure of the local benthic communities. Furthermore, the microplastic abundance may appear very different between sites, depending on the presence or absence of benthic macrofauna, because the lack of bioturbation likely results in the aggregation of microplastics in the surficial sediment. Taking this into account would be crucial for making correct interpretations of the collected

data. In the Baltic Sea, for example, the scarcity or the complete lack of macroorganisms and thus bioturbation is spread over vast areas. In the Baltic Proper, including the Gulf of Finland and the Gulf of Riga, approximately 24% of the bottoms were estimated to suffer from anoxic conditions and 33% from hypoxia in 2018 (Hansson et al. 2019). In these areas, the sinks can become sources if the microplastics on the sediment surface are more prone to resuspension and subsequent horizontal transport by hydrological or anthropogenic disturbances.

The results obtained here also indicate that after plastics end up in the sediments, the polymer type can influence their fate and risks they pose to the benthic habitat. When plastics of different polymer types were incubated in the sediments, the bacterial communities developed on biodegradable CA diverged from the other polymer types examined and harboured potentially biodegrading bacteria. Thus, the longevity of plastics – and hence the timespan for their harmful effects in the marine environment – can be dependent on the polymer type. However, the differences between the bacterial communities associated with CA and PLLA highlight the need to properly investigate the biodegradation potential of each material, because the behaviour of these two materials labelled as biodegradable may be very different in the marine environment. Although potentially biodegrading bacterial communities were detected, the plastics did not show any visible signs of degradation; thus, further research is needed to verify whether biodegradation of plastics in the sea can truly occur. The needed environmental conditions, specific microorganisms and timelines for the degradation of bio-based, biodegradable materials should be assessed carefully before they are extensively adopted in applications with high risk of ending up in the marine environment.

Overall, the bacterial communities on plastics resembled those in the surrounding sediment, which may hamper some organisms' abilities to discriminate plastics from their natural food sources and lead to unintentional plastic ingestion, as demonstrated by Vroom et al. (2017). Our results also showed that all the polymer types examined sorbed PAHs from the sediments, but had varying PAH sorption capacities, indicating that if ingested, the microplastics' roles as PAH vectors can depend on the polymer type. Of the polymer types examined, PS consistently sorbed the highest concentrations of PAHs, making it potentially more harmful to biota. However, the lower PAH concentrations sorbed on the plastics than those present in the sediment also suggested that the ingestion of plastics is not likely to increase the PAH burden and pose an elevated risk for deposit-feeding animals. Moreover, the quantity of HOCs in the plastics currently residing in the ocean may represent only a tiny fraction of the HOC mass that is held by other media; thus, other exposure pathways are expected to play a larger role than plastic ingestion (Koelmans et al. 2016).

One specific microplastic type that may be particularly abundant and harmful in the environment is the microrubber originating from car tyres (Kole et al. 2017, Wagner et al. 2018). In this thesis, many PAHs and trace metals were quantified from tyre rubber, and since the tyre rubber particles were ingested by *Limecola balthica*, they are able to carry these contaminants to the clams. The environmentally relevant chronic exposure to rubber fragments did not increase the mortality in *L. balthica*, but the clams exhibited multiple sublethal responses, indicating oxidative stress. Due to the reduced ROS-scavenging capacity of the ADS, multiple signs of oxidative damage were observed, e.g. in DNA, mitochondria and lysosomes. This condition could ultimately result in reduced fitness when prolonged, but it remains unclear whether these effects could further be projected beyond the individual level and expand to include population dynamics and ecosystem functioning.

Although tyre rubber has also been recognized as a major emission source of microplastics in Finland (Setälä & Suikkanen 2020), actual data on its abundance in the northern Baltic Sea environment are still needed to evaluate whether the exposure conditions used in the study could induce similar effects in *Limecola balthica* living in the natural environment. Since members of the benthic sedentary fauna that inhabit coastal waters are in close proximity to highly urbanized areas, their exposure to tyre rubber and its associated contaminants is highly likely. When newly emitted tyre rubber particles settle on the sediment surface, they may pose an additional threat to the benthic fauna if they are more readily ingested and still saturated with harmful chemicals present in the material. The recently discovered highly toxic substance 6PPD-quinone originating from car tyres (Tian et al. 2020) demonstrates that many aspects of microplastic pollution are still obscure. However, our results show here the potential of tyre rubber fragments to negatively affect the clams and emphasize the importance of tyre rubber as a yet understudied environmental contaminant.

While this thesis provided novel information contributing to knowledge of the fate of plastics in the seafloor and on the interactions between benthic fauna and microplastics, many knowledge gaps remain. To begin with, the mechanisms causing toxic effects for organisms in microplastic exposures should be further explored. It is unknown whether the impacts are caused by the physical particles, associated contaminants or both, and interestingly, recent research indicates that the particles and leachates may have different toxicity profiles (Khan et al. 2019). Future efforts should also be directed towards conducting more environmentally relevant exposure studies and linking the parameters examined with higher biological levels of organization to gain a better understanding of the potential effects that microplastics may have on individuals, communities and whole-ecosystem functioning. During the last 10 years, discussions on the

impacts of microplastics have revolved around the effects of their ingestion by macroorganisms, but increased focus should be on the indirect impacts of plastics and plastic-associated contaminants on the environment. Since a recent study by Seeley et al. (2020) observed that microplastics can alter the sediment microbial community composition and nitrogen-cycling processes, gaining further insight of the means by how plastics can modify the environment and important biogeochemical processes would broaden our understanding of the complex interactions taking place on the seafloor and aid in assessing the impacts of microplastics on the marine environment.

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7 REFERENCES

Aarnio, K. & Bonsdorff, E. 1993: Seasonal variation in abundance and diet of the sand goby *Pomatoschistus minutus* (Pallas) in a northern Baltic archipelago. — Ophelia 37: 19–30.

Abel, S.M., Primpke, S., Int-Veen, I., Brandt, A. & Gerdts, G. 2021: Systematic identification of microplastics in abyssal and hadal sediments of the Kuril Kamchatka trench. — Environmental Pollution 269: 116095.

Achten, C. & Andersson, J.T. 2015: Overview of Polycyclic Aromatic Compounds (PAC). — Polycyclic Aromatic Compounds 35: 177–186.

Agamuthu, P., Mehran, S., Norkhairah, A. & Norkhairiyah, A. 2019: Marine debris: A review of impacts and global initiatives. — Waste Management & Research 37: 987–1002.

Aho, V.T.E., Pereira, P.A.B., Voutilainen, S., Paulin, L., Pekkonen, E., Auvinen, P. & Scheperjans, F. 2019: Gut microbiota in Parkinson's disease: Temporal stability and relations to disease progression. — EBioMedicine 44: 691–707.

Aller, R.C. 1982: The Effects of Macrobenthos on Chemical Properties of Marine Sediment and Overlying Water. — In: McCall, P. L. & Tevesz, M. J. S. (eds.). Animal-Sediment Relations: The Biogenic Alteration of Sediments, 53–102. Springer. Boston, MA, US.

Amaral-Zettler, L.A., Zettler, E.R., Slikas, B., Boyd, G.D., Melvin, D.W., Morrall, C.E., Proskurowski, G. & Mincer, T.J. 2015: The biogeography of the Plastisphere: implications for policy. — Frontiers in Ecology and the Environment 13: 541–546.

Amelia, T.S.M., Khalik, W.M.A.W.M., Ong, M.C., Shao, Y.T., Pan, H.-J. & Bhubalan, K. 2021: Marine microplastics as vectors of major ocean pollutants and its hazards to the marine ecosystem and humans. — Progress in Earth and Planetary Science 8: 12.

Andrady, A.L. 2011: Microplastics in the marine environment. — Marine Pollution Bulletin 62: 1596–1605.

Andrady, A.L. 2015: Persistence of plastic litter in the oceans. — In: Bergmann, M., Gutow, L. & Klages, M. (eds.). Marine Anthropogenic Litter, 57–72. Springer. Berlin.

Andrady, A.L. 2017: The plastic in microplastics: A review. — Marine Pollution Bulletin 119: 12–22.

Andrady, A.L. & Rajapakse, N. 2019: Additives and Chemicals in Plastics. — In: Takada, H. & Karapanagioti, H. K. (eds.). Hazardous Chemicals Associated with Plastics in the Marine Environment, 1–17. Springer International Publishing. Cham.

Ankar, S. 1977: The soft bottom ecosystem of the northern Baltic proper with special reference to the macrofauna. Ph.D. Dissertation. Contributions from the Askö Laboratory, University of Stockholm, Sweden.

Archambault, J.M., Cope, W.G. & Kwak, T.J. 2013: Burrowing, byssus, and biomarkers: behavioral and physiological indicators of sublethal thermal stress in freshwater mussels (Unionidae). — Marine and Freshwater Behaviour and Physiology 46: 229–250.

Artham, T., Sudhakar, M., Venkatesan, R., Madhavan Nair, C., Murty, K.V.G.K. & Doble, M. 2009: Biofouling and stability of synthetic polymers in sea water. — International Biodeterioration & Biodegradation 63: 884–890.

Avio, C.G., Gorbi, S., Milan, M., Benedetti, M., Fattorini, D., d'Errico, G., Pauletto, M., Bargelloni, L.& Regoli, F. 2015: Pollutants bioavailability and toxicological risk from microplastics to marine mussels. — Environmental Pollution 198: 211–222.

Bakir, A., O'Connor, I.A., Rowland, S.J., Hendriks, A.J. & Thompson, R.C. 2016: Relative importance of microplastics as a pathway for the transfer of hydrophobic organic chemicals to marine life. — Environmental Pollution 219: 56–65.

Bakir, A., Rowland, S.J. & Thompson, R.C. 2012: Competitive sorption of persistent organic pollutants onto microplastics in the marine environment. — Marine Pollution Bulletin 64: 2782–2789.

Barnes, D.K.A., Galgani, F., Thompson, R.C. & Barlaz, M. 2009: Accumulation and fragmentation of plastic debris in global environments. — Philosophical Transactions of the Royal Society of London B: Biological Sciences 364: 1985–1998.

Barrett, J., Chase, Z., Zhang, J., Holl, M.M.B., Willis, K., Williams, A., Hardesty, B.D. & Wilcox, C. 2020: Microplastic Pollution in Deep-Sea Sediments From the Great Australian Bight. — Frontiers in Marine Science 7: 576170.

Baršienė, J., Butrimavičienė, L., Grygiel, W., Lang, T., Michailovas, A. & Jackūnas, T. 2014: Environmental genotoxicity and cytotoxicity in flounder (*Platichthys flesus*), herring (*Clupea harengus*) and Atlantic cod (*Gadus morhua*) from chemical munitions dumping zones in the southern Baltic Sea. — Marine Environmental Research 96: 56–67.

Baršienė, J., Lazutka, J., Šyvokienė, J., Dedonytė, V., Rybakovas, A., Bagdonas, E., Bjornstad, A. & Andersen, O.K. 2004: Analysis of micronuclei in blue mussels and fish from the Baltic and North Seas. — Environmental Toxicology 19: 365–371.

Baršienė, J., Lehtonen, K.K., Koehler, A., Broeg, K., Vuorinen, P.J., Lang, T., Pempkowiak, J., Šyvokienė, J., Dedonyte, V., Rybakovas, A., Repečka, R., Vuontisjärvi, H. & Kopecka, J. 2006: Biomarker responses in flounder (*Platichthys flesus*) and mussel (*Mytilus edulis*) in the Klaipėda-Būtingė area (Baltic Sea). — Marine Pollution Bulletin 53: 422–436.

Baršienė, J., Rybakovas, A., Garnaga, G. & Andreikėnaitė, L. 2012: Environmental genotoxicity and cytotoxicity studies in mussels before and after an oil spill at the marine oil terminal in the Baltic Sea. — Environmental Monitoring and Assessment 184: 2067–2078.

Beckingham, B. & Ghosh, U. 2013: Polyoxymethylene passive samplers to monitor changes in bioavailability and flux of PCBs after activated carbon amendment to sediment in the field. — Chemosphere 91: 1401–1407.

Beiras, R., Bellas, J., Cachot, J., Cormier, B., Cousin, X., Engwall, M., Gambardella, C., Garaventa, F.,

Keiter, S., Le Bihanic, F., López-Ibáñez, S., Piazza, V., Rial, D., Tato, T. & Vidal-Liñán, L. 2018: Ingestion and contact with polyethylene microplastics does not cause acute toxicity on marine zooplankton. — Journal of Hazardous Materials 360: 452–460.

Bergmann, M., Wirzberger, V., Krumpen, T., Lorenz, C., Primpke, S., Tekman, M.B. & Gerdts, G. 2017: High Quantities of Microplastic in Arctic Deep-Sea Sediments from the HAUSGARTEN Observatory. — Environmental Science & Technology 510: 11000–11010.

Besseling, E., Wegner, A., Foekema, E.M., van den Heuvel-Greve, M.J. & Koelmans, A.A. 2013: Effects of Microplastic on Fitness and PCB Bioaccumulation by the Lugworm *Arenicola marina* (L.). — Environmental Science & Technology 47: 593–600.

Black, L.F. 1980: The biodeposition cycle of a surface deposit-feeding bivalve, *Macoma balthica* (L.). — In: Kennedy, V. S. (ed.). Estuarine Perspectives, 389–402. Academic Press Inc. London.

Blarer, P. & Burkhardt-Holm, P. 2016: Microplastics affect assimilation efficiency in the freshwater amphipod *Gammarus fossarum*. — Environmental Science and Pollution Research 23: 23522–23532.

Bock, M.J. & Miller, D.C. 1999: Particle selectivity, gut volume, and the response to a step change in diet for deposit-feeding polychaetes. — Limnology and Oceanography 44: 1132–1138.

Bonsdorff, E., Norkko, A. & Sandberg, E. 1995: Structuring zoobenthos: the importance of predation, siphon cropping and physical disturbance. — Journal of Experimental Marine Biology and Ecology 192: 125–144.

Bonsdorff, E. & Wenne, R. 1989: A comparison of condition indices of *Macoma balthica* (L.) from the northern and southern baltic sea. — Netherlands Journal of Sea Research 23: 45–55.

Botterell, Z.L.R., Beaumont, N., Cole, M., Hopkins, F.E., Steinke, M., Thompson, R.C. & Lindeque, P.K. 2020: Bioavailability of Microplastics to Marine Zooplankton: Effect of Shape and Infochemicals. — Environmental Science & Technology 54: 12024–12033.

Boucher, J. & Friot, D. 2017: Primary microplastics in the oceans: A global evaluation of sources. — IUCN International Union for Conservation of Nature. Gland, Switzerland.

Bour, A., Haarr, A., Keiter, S. & Hylland, K. 2018: Environmentally relevant microplastic exposure affects sediment-dwelling bivalves. — Environmental Pollution 236: 652–660.

Bradford, M.M. 1976: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. — Analytical Biochemistry 72: 248–254.

Bråte, I.L.N., Blázquez, M., Brooks, S.J. & Thomas, K.V. 2018: Weathering impacts the uptake of polyethylene microparticles from toothpaste in Mediterranean mussels (*M. galloprovincialis*). — Science of The Total Environment 626: 1310–1318.

Bråte, I.L.N., Hurley, R.R., Lusher, A.L., Buenaventura, N., Hultman, M.,

Halsband, C. & Green, N. 2020: Microplastics in marine bivalves from the Nordic environment. — Norwegian Environment Agency publication series M-1629 / 2020. Nordic Council of Ministers, TemaNord report TN2020:504.

Bruck, S. & Ford, A.T. 2018: Chronic ingestion of polystyrene microparticles in low doses has no effect on food consumption and growth to the intertidal amphipod *Echinogammarus marinus*? — Environmental Pollution 233: 1125–1130.

Cajaraville, M.P., Abascal, I., Etxeberria, M. & Marigómez, I. 1995: Lysosomes as cellular markers of environmental pollution: Time- and dose-dependent responses of the digestive lysosomal system of mussels after petroleum hydrocarbon exposure. — Environmental Toxicology and Water Quality 10: 1–8.

Callahan, B. 2018: Silva taxonomic training data formatted for DADA2 (Silva version 132).

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A. & Holmes, S.P. 2016: DADA2: High-resolution sample inference from Illumina amplicon data. — Nature Methods 13: 581–583.

Capolupo, M., Gunaalan, K., Booth, A.M., Sørensen, L., Valbonesi, P. & Fabbri, E. 2021: The sub-lethal impact of plastic and tire rubber leachates on the Mediterranean mussel *Mytilus galloprovincialis*. — Environmental Pollution 283: 117081.

Capolupo, M., Sørensen, L., Jayasena, K.D.R., Booth, A.M. & Fabbri, E. 2020: Chemical composition and ecotoxicity of plastic and car tire rubber leachates to aquatic organisms. — Water Research 169: 115270.

CBD 2016: Marine Debris: Understanding, Preventing and Mitigating the Significant Adverse Impacts on Marine and Coastal Biodiversity. — Secretariat of the Convention on Biological Diversity. Montreal.

Celeiro, M., Lamas, J.P., Garcia-Jares, C., Dagnac, T., Ramos, L. & Llompart, M. 2014: Investigation of PAH and other hazardous contaminant occurrence in recycled tyre rubber surfaces. Case-study: restaurant playground in an indoor shopping centre. — International Journal of Environmental Analytical Chemistry 94: 1264–1271.

Chatterjee, S., Kundu, S., Sengupta, S. & Bhattacharyya, A. 2009: Divergence to apoptosis from ROS induced cell cycle arrest: Effect of cadmium. — Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 663: 22–31.

Chen, G.-Q. 2010: Plastics Completely Synthesized by Bacteria: Polyhydroxyalkanoates. — In: Chen, G. G.-Q. (ed.). Plastics from Bacteria: Natural Functions and Applications, Microbiology Monographs, 17–37. Springer. Berlin, Heidelberg.

Chen, I.-M.A., Chu, K., Palaniappan, K., Pillay, M., Ratner, A., Huang, J., Huntemann, M., Varghese, N., White, J.R., Seshadri, R., Smirnova, T., Kirton, E., Jungbluth, S.P., Woyke, T., Eloe-Fadrosh, E.A., Ivanova, N.N. & Kyrpides, N.C. 2019: IMG/M v.5.0: an integrated data management and comparative analysis system for microbial genomes and microbiomes.

— Nucleic Acids Research 47: D666–D677.

Cheung, C.C.C., Zheng, G.J., Li, A.M.Y., Richardson, B.J. & Lam, P.K.S. 2001: Relationships between tissue concentrations of polycyclic aromatic hydrocarbons and antioxidative responses of marine mussels, *Perna viridis*. — Aquatic Toxicology 52: 189–203.

Chubarenko, I., Bagaev, A., Zobkov, M. & Esiukova, E. 2016: On some physical and dynamical properties of microplastic particles in marine environment. — Marine Pollution Bulletin 108: 105–112.

Claessens, C.G. & Stoddart, J.F. 1997: π–π interactions in self-assembly. — Journal of Physical Organic Chemistry 10: 254–272.

Claessens, M., Meester, S.D., Landuyt, L.V., Clerck, K.D. & Janssen, C.R. 2011: Occurrence and distribution of microplastics in marine sediments along the Belgian coast. — Marine Pollution Bulletin 62: 2199–2204.

Claessens, M., Van Cauwenberghe, L., Vandegehuchte, M.B. & Janssen, C.R. 2013: New techniques for the detection of microplastics in sediments and field collected organisms. — Marine Pollution Bulletin 70: 227–233.

Cole, M., Lindeque, P., Fileman, E., Halsband, C., Goodhead, R., Moger, J. & Galloway, T.S. 2013: Microplastic ingestion by zooplankton. — Environmental Science & Technology 47: 6646–6655.

Coppock, R.L., Lindeque, P.K., Cole, M., Galloway, T.S., Näkki, P., Birgani, H., Richards, S. & Queirós, A.M. 2021: Benthic fauna contribute to microplastic sequestration in coastal sediments. — Journal of Hazardous Materials 415: 125583.

Cornelissen, G., Pettersen, A., Broman, D., Mayer, P. & Breedveld, G.D. 2008: Field testing of equilibrium passive samplers to determine freely dissolved native polycyclic aromatic hydrocarbon concentrations.: Environmental Toxicology and Chemistry 27: 499–508.

Councell, T.B., Duckenfield, K.U., Landa, E.R. & Callender, E. 2004: Tire-Wear Particles as a Source of Zinc to the Environment. — Environmental Science & Technology 38: 4206–4214.

Courtene-Jones, W., Quinn, B., Ewins, C., Gary, S.F. & Narayanaswamy, B.E. 2020: Microplastic accumulation in deep-sea sediments from the Rockall Trough. — Marine Pollution Bulletin 154: 111092.

Courtene-Jones, W., Quinn, B., Gary, S.F., Mogg, A.O.M. & Narayanaswamy, B.E. 2017: Microplastic pollution identified in deep-sea water and ingested by benthic invertebrates in the Rockall Trough, North Atlantic Ocean. — Environmental Pollution 231: 271–280.

Cózar, A., Echevarría, F., González-Gordillo, J.I., Irigoien, X., Úbeda, B., Hernández-León, S., Palma, Á.T., Navarro, S., García-de-Lomas, J., Ruiz, A., Fernández-de-Puelles, M.L. & Duarte, C.M. 2014: Plastic debris in the open ocean. — Proceedings of the National Academy of Sciences 111: 10239–10244.

Dagnino, A., Allen, J.I., Moore, M.N., Broeg, K., Canesi, L. & Viarengo, A. 2007: Development of an expert system for the integration of

biomarker responses in mussels into an animal health index. — Biomarkers 12: 155–172.

Das, S., Lyla, P.S. & Khan, S.A. 2006: Marine microbial diversity and ecology: importance and future perspectives. — Current Science 90: 1325–1335.

Davidson, K. & Dudas, S.E. 2016: Microplastic Ingestion by Wild and Cultured Manila Clams (*Venerupis philippinarum*) from Baynes Sound, British Columbia. — Archives of Environmental Contamination and Toxicology 71: 147–156.

Davies, K.J.A. 1995: Oxidative stress: the paradox of aerobic life. — In: Rice-Evans, C., Halliwell, B., Lunt, G. G. (eds.). Biochemical Society Symposia 61, 1–31.

Delefosse, M. & Kristensen, E. 2012: Burial of *Zostera marina* seeds in sediment inhabited by three polychaetes: Laboratory and field studies. — Journal of Sea Research 71: 41–49.

Depledge, M.H., Galgani, F., Panti, C., Caliani, I., Casini, S. & Fossi, M.C. 2013: Plastic litter in the sea. — Marine Environmental Research 92: 279–281.

Dussud, C., Hudec, C., George, M., Fabre, P., Higgs, P., Bruzaud, S., Delort, A.-M., Eyheraguibel, B., Meistertzheim, A.-L., Jacquin, J., Cheng, J., Callac, N., Odobel, C., Rabouille, S. & Ghiglione, J.-F. 2018: Colonization of Non-biodegradable and Biodegradable Plastics by Marine Microorganisms. — Frontiers in Microbiology 9: 1571.

ECHA 2019: ANNEX XV RESTRICTION REPORT: Proposal for a restriction: Substance Name(s): intentionally added microplastics. — European Chemicals Agency.

Eich, A., Mildenberger, T., Laforsch, C. & Weber, M. 2015: Biofilm and Diatom Succession on Polyethylene (PE) and Biodegradable Plastic Bags in Two Marine Habitats: Early Signs of Degradation in the Pelagic and Benthic Zone? — PLoS ONE 10(9): e0137201.

Eldon, J., Pekkarinen, M. & Kristoffersson, R. 1980: Effects of low concentrations of heavy metals on the bivalve *Macoma balthica*. — Annales Zoologici Fennici 17: 233–242.

Ellen MacArthur Foundation, World Economic Forum & MacKinsey & Company (2016) The New Plastics Economy – Rethinking the future of plastics.

Enders, K., Käppler, A., Biniasch, O., Feldens, P., Stollberg, N., Lange, X., Fischer, D., Eichhorn, K.-J., Pollehne, F., Oberbeckmann, S. & Labrenz, M. 2019: Tracing microplastics in aquatic environments based on sediment analogies. — Scientific Reports 9: 15207.

Endo, S. & Koelmans, A.A. 2019: Sorption of Hydrophobic Organic Compounds to Plastics in the Marine Environment: Equilibrium. — In: Takada, H., Karapanagioti & H. K. (eds.). Hazardous Chemicals Associated with Plastics in the Marine Environment, 185–204. Springer International Publishing. Cham.

Endo, S., Takizawa, R., Okuda, K., Takada, H., Chiba, K., Kanehiro, H., Ogi, H., Yamashita, R. & Date, T. 2005: Concentration of polychlorinated biphenyls (PCBs) in beached resin pellets: Variability among individual particles and

regional differences. — Marine Pollution Bulletin 50: 1103–1114.

Eriksen, M., Lebreton, L.C.M., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., Galgani, F., Ryan, P.G. & Reisser, J. 2014: Plastic Pollution in the World's Oceans: More than 5 Trillion Plastic Pieces Weighing over 250,000 Tons Afloat at Sea. — PLoS ONE 9: e111913.

Erni-Cassola, G., Wright, R.J., Gibson, M.I. & Christie-Oleza, J.A. 2020: Early Colonization of Weathered Polyethylene by Distinct Bacteria in Marine Coastal Seawater. — Microbial Ecology 79: 517–526.

European Bioplastics 2020: Bioplastics Market Update 2020. — European Bioplastics.

Farrell, P. & Nelson, K. 2013: Trophic level transfer of microplastic: *Mytilus edulis* (L.) to *Carcinus maenas* (L.). — Environmental Pollution 177: 1–3.

Fenech, M., Chang, W.P., Kirsch-Volders, M., Holland, N., Bonassi, S. & Zeiger, E. 2003: HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures. — Mutation Research/Genetic Toxicology and Environmental Mutagenesis 534: 65–75.

Fischer, V., Elsner, N.O., Brenke, N., Schwabe, E. & Brandt, A. 2015: Plastic pollution of the Kuril–Kamchatka Trench area (NW Pacific). — Deep Sea Research Part II: Topical Studies in Oceanography 111: 399–405.

Fotopoulou, K.N. & Karapanagioti, H.K. 2019: Degradation of Various Plastics in the Environment. — In: Takada, H. & Karapanagioti, H. K. (eds.). Hazardous Chemicals Associated with Plastics in the Marine Environment, 71–92. Springer International Publishing. Cham.

Fox, J. & Weisberg, S. 2019: An R Companion to Applied Regression (Third edition). Sage. Thousand Oaks CA.

François, F., Poggiale, J.-C., Durbec, J.-P. & Stora, G. 1997: A New Approach for the Modelling of Sediment Reworking Induced by a Macrobenthic Community. — Acta Biotheoretica 45: 295–319.

Frias, J.P.G.L. & Nash, R. 2019: Microplastics: Finding a consensus on the definition. — Marine Pollution Bulletin 138: 145–147.

Galgani, F., Hanke, G. & Maes, T. 2015: Global Distribution, Composition and Abundance of Marine Litter. — In: Bergmann, M., Gutow, L. & Klages, M. (eds.). Marine Anthropogenic Litter, 29–56. Springer International Publishing. Cham.

Galgani, F., Hanke, G., Werner, S., Oosterbaan, L., Nilsson, P., Fleet, D., Kinsey, S., Thompson, R.C., Van Franeker, J.A., Vlachogianni, T., Scoullos, M., Veiga, J.M., Palatinus, A., Matiddi, M., Maes, T., Korpinen, S., Budziak, A., Leslie, H., Gago, J.& Liebezeit, G. 2013: Guidance on Monitoring of Marine Litter in European Seas. — JRC Scientific and Policy Reports. Publications Office of the European Union. Luxembourg.

Galloway, T.S., Cole, M. & Lewis, C. 2017: Interactions of microplastic debris throughout the marine ecosystem. — Nature Ecology & Evolution 1: 1–8.

Gauquie, J., Devriese, L., Robbens, J. & De Witte, B. 2015: A qualitative

screening and quantitative measurement of organic contaminants on different types of marine plastic debris. — Chemosphere 138: 348–356.

Gebhardt, C. & Forster, S. 2018: Size-selective feeding of *Arenicola marina* promotes long-term burial of microplastic particles in marine sediments. — Environmental Pollution 242: 1777–1786.

Gerhardt, A. 2007: Aquatic Behavioral Ecotoxicology—Prospects and Limitations. — Human and Ecological Risk Assessment: An International Journal 13: 481–491.

Gerino, M., Frignani, M., Mugnai, C., Bellucci, L.G., Prevedelli, D., Valentini, A., Castelli, A., Delmotte, S. & Sauvage, S. 2007: Bioturbation in the Venice Lagoon: Rates and relationship to organisms. — Acta Oecologica 32: 14–25.

GESAMP 2019: Guidelines or the monitoring and assessment of plastic litter and microplastics in the ocean. — Reports and Studies No. 99. IMO/FAO/UNESCO-IOC/UNIDO/ WMO/IAEA/UN/UNEP/UNDP/ISA Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection.

GESAMP 2015: Sources, fate and effects of microplastics in the marine environment: a global assessment. — Reports and Studies No. 90. IMO/FAO/UNESCOIOC/UNIDO/ WMO/IAEA/UN/UNEP/UNDP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection.

Gewert, B., Ogonowski, M., Barth, A. & MacLeod, M. 2017: Abundance and composition of near surface microplastics and plastic debris in the Stockholm Archipelago, Baltic Sea. — Marine Pollution Bulletin 120: 292–302.

Gilbert, M.A. 1977: The behaviour and functional morphology of deposit feeding in *Macoma balthica* (Linne, 1758), in New England. — Journal of Molluscan Studies 43: 18–27.

Gogina, M., Nygård, H., Blomqvist, M., Daunys, D., Josefson, A.B., Kotta, J., Maximov, A., Warzocha, J., Yermakov, V., Gräwe, U. & Zettler, M.L. 2016: The Baltic Sea scale inventory of benthic faunal communities. — ICES Journal of Marine Science 73: 1196–1213.

Goldberg, E.D. 1997: Plasticizing the Seafloor: An Overview. — Environmental Technology 18: 195–201.

Gouin, T., Roche, N., Lohmann, R. & Hodges, G. 2011: A Thermodynamic Approach for Assessing the Environmental Exposure of Chemicals Absorbed to Microplastic. — Environmental Science & Technology 45: 1466–1472.

Gray, J.S. 2002: Biomagnification in marine systems: the perspective of an ecologist. — Marine Pollution Bulletin 45: 46–52.

Green, D.S., Boots, B., Blockley, D.J., Rocha, C. & Thompson, R. 2015: Impacts of Discarded Plastic Bags on Marine Assemblages and Ecosystem Functioning. — Environmental Science & Technology 49: 5380–5389.

Green, D.S., Boots, B., Sigwart, J., Jiang, S. & Rocha, C. 2016: Effects of conventional and biodegradable microplastics on a marine ecosystem engineer (*Arenicola marina*) and

sediment nutrient cycling. — Environmental Pollution 208: Part B, 426–434.

Gualtieri, M., Andrioletti, M., Mantecca, P., Vismara, C. & Camatini, M. 2005: Impact of tire debris on in vitro and in vivo systems. — Particle and Fibre Toxicology 2: 1.

Haave, M., Lorenz, C., Primpke, S. & Gerdts, G. 2019: Different stories told by small and large microplastics in sediment - first report of microplastic concentrations in an urban recipient in Norway. — Marine Pollution Bulletin 141: 501–513.

Halle, L.L., Palmqvist, A., Kampmann, K. & Khan, F.R. 2020: Ecotoxicology of micronized tire rubber: Past, present and future considerations. — Science of The Total Environment 706: 135694.

Halsband, C., Sørensen, L., Booth, A.M. & Herzke, D. 2020: Car Tire Crumb Rubber: Does Leaching Produce a Toxic Chemical Cocktail in Coastal Marine Systems? — Frontiers in Environmental Science 8: 125.

Hann, S., Sherrington, C., Jamieson, O., Hickman, M., Kershaw, P.J., Babasola, A. & Cole, G. 2018: Investigating options for reducing releases in the aquatic environment of microplastics emitted by (but not intentionally added in) products. — Eunomia Research & Consulting and ICF.

Hansson, M., Viktorsson, L. & Andersson, L. 2019: Oxygen Survey in the Baltic Sea 2019 – Extent of Anoxia and Hypoxia, 1960–2019. Report Oceanography No. 67. — Swedish Meteorological and Hydrological Institute. Göteborg, Sweden.

Hara, J., Frias, J. & Nash, R. 2020: Quantification of microplastic ingestion by the decapod crustacean *Nephrops norvegicus* from Irish waters. — Marine Pollution Bulletin 152: 110905.

Hartmann, N.B., Hüffer, T., Thompson, R.C., Hassellöv, M., Verschoor, A., Daugaard, A.E., Rist, S., Karlsson, T., Brennholt, N., Cole, M., Herrling, M.P., Hess, M.C., Ivleva, N.P., Lusher, A.L. & Wagner, M. 2019: Are We Speaking the Same Language? Recommendations for a Definition and Categorization Framework for Plastic Debris. — Environmental Science & Technology 53: 1039–1047.

Hartmann, N.B., Rist, S., Bodin, J., Jensen, L.H., Schmidt, S.N., Mayer, P., Meibom, A. & Baun, A. 2017: Microplastics as vectors for environmental contaminants: Exploring sorption, desorption, and transfer to biota: Microplastics as Contaminant Vectors: Exploring the Processes. — Integrated Environmental Assessment and Management 13: 488–493.

Heddle, J.A., Cimino, M.C., Hayashi, M., Romagna, F., Shelby, M.D., Tucker, J.D., Vanparys, P. & MacGregor, J.T. 1991: Micronuclei as an index of cytogenetic damage: Past, present, and future. — Environmental and Molecular Mutagenesis 18: 277–291.

Hedman, J. 2008: Fate of contaminants in Baltic Sea sediment ecosystems: the role of bioturbation. Ph.D. Dissertation. Stockholm, Department of Systems Ecology, Stockholm University.

Hedman, J., Bradshaw, C., Thorsson, M., Gilek, M. & Gunnarsson, J. 2008: Fate

of contaminants in Baltic Sea sediments: role of bioturbation and settling organic matter. — Marine Ecology Progress Series 356: 25–38.

Heim, S. & Schwarzbauer, J. 2013: Pollution history revealed by sedimentary records: a review. — Environmental Chemistry Letters 11: 255–270.

HELCOM 2010a: Hazardous substances in the Baltic Sea – An integrated thematic assessment of hazardous substances in the Baltic Sea. — Baltic Sea Environment Proceedings No. 120B. Baltic Marine Environment Protection Commission.

HELCOM 2010b: Towards a tool for quantifying anthropogenic pressures and potential impacts on the Baltic Sea marine environment: A background document on the method, data and testing of the Baltic Sea Pressure and Impact Indices. — Baltic Sea Environment Proceedings No. 125. Baltic Marine Environment Protection Commission.

HELCOM 2013: HELCOM Red List of Baltic Sea species in danger of becoming extinct. — Baltic Sea Environment Proceedings No. 140. Baltic Marine Environment Protection Commission.

HELCOM 2015: Regional Action Plan for Marine Litter in the Baltic Sea. — Baltic Marine Environment Protection Commission.

HELCOM 2018: SPICE report: Task 2.1.2 Development of baselines of marine litter – Litter on the seafloor in the HELCOM area- analyses of data from BITS trawling hauls 2012–2016. — Baltic Marine Environment Protection Commission.

Henriksen, K., Rasmussen, M.B. & Jensen, A. 1983: Effect of Bioturbation on Microbial Nitrogen Transformations in the Sediment and Fluxes of Ammonium and Nitrate to the Overlaying Water. — Ecological Bulletins: 193–205.

Hidalgo-Ruz, V., Gutow, L., Thompson, R.C. & Thiel, M. 2012: Microplastics in the Marine Environment: A Review of the Methods Used for Identification and Quantification. — Environmental Science & Technology 46: 3060–3075.

Iliff, S.M., Wilczek, E.R., Harris, R.J., Bouldin, R. & Stoner, E.W. 2020: Evidence of microplastics from benthic jellyfish (*Cassiopea xamachana*) in Florida estuaries. — Marine Pollution Bulletin 159: 111521.

Ioakeimidis, C., Zeri, C., Kaberi, H., Galatchi, M., Antoniadis, K., Streftaris, N., Galgani, F., Papathanassiou, E. & Papatheodorou, G. 2014: A comparative study of marine litter on the seafloor of coastal areas in the Eastern Mediterranean and Black Seas. — Marine Pollution Bulletin 89: 296–304.

ISO 472:2013 2013: Plastics — Vocabulary (ISO Standard No. 472:2013).

ISO/TS 28581:2012 2012: Water quality — Determination of selected non-polar substances — Method using gas chromatography with mass spectrometric detection (GC-MS).

Iwata, T. 2015: Biodegradable and Bio-Based Polymers: Future Prospects of Eco-Friendly Plastics. — Angewandte Chemie International Edition 54: 3210–3215.

Jacquin, J., Cheng, J., Odobel, C., Pandin, C., Conan, P., Pujo-Pay, M., Barbe, V., Meistertzheim, A.-L. & Ghiglione, J.-F. 2019: Microbial Ecotoxicology of Marine Plastic Debris: A Review on Colonization and Biodegradation by the "Plastisphere.". — Frontiers in Microbiology 10: 865.

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R. & Law, K.L. 2015: Plastic waste inputs from land into the ocean. — Science 347: 768.

Johansen, M.P., Cresswell, T., Davis, J., Howard, D.L., Howell, N.R. & Prentice, E. 2019: Biofilm-enhanced adsorption of strong and weak cations onto different microplastic sample types: Use of spectroscopy, microscopy and radiotracer methods. — Water Research 158: 392–400.

Josefsson, S. 2011: Fate and transport of POPs in the aquatic environment with focus on contaminated sediments. PhD Thesis. Umeå universitet, Kemiska institutionen, Umeå.

Jumars, P.A. & Wheatcroft, R.A. 1989: Responses of benthos to changing food quality and quantity, with a focus on deposit feeding and bioturbation. — In: Berger, W. H., Smetacek, V. S. & Wefer, G. (eds.). Productivity of the Ocean: Present and Past, 235–253. John Wiley & Sons Limited.

Kammann, U., Aust, M.-O., Bahl, H. & Lang, T. 2018: Marine litter at the seafloor – Abundance and composition in the North Sea and the Baltic Sea. — Marine Pollution Bulletin 127: 774–780.

Kane, I.A., Clare, M.A., Miramontes, E., Wogelius, R., Rothwell, J.J., Garreau, P. & Pohl, F. 2020: Seafloor microplastic hotspots controlled by deep-sea circulation. — Science 368: 1140–1145.

Karapanagioti, H.K. & Klontza, I. 2008: Testing phenanthrene distribution properties of virgin plastic pellets and plastic eroded pellets found on Lesvos island beaches (Greece). — Marine Environmental Research 65: 283–290.

Karlsson, H.L., Ljungman, A.G., Lindbom, J. & Möller, L. 2006: Comparison of genotoxic and inflammatory effects of particles generated by wood combustion, a road simulator and collected from street and subway. — Toxicology Letters 165: 203–211.

Katija, K., Choy, C.A., Sherlock, R.E., Sherman, A.D. & Robison, B.H. 2017: From the surface to the seafloor: How giant larvaceans transport microplastics into the deep sea. — Science Advances 3: e1700715.

Kearns, C.M., Hairston, N.G. & Kesler, D.H. 1996: Particle transport by benthic invertebrates: its role in egg bank dynamics. — Hydrobiologia 332: 63–70.

Khan, F.R., Halle, L.L. & Palmqvist, A. 2019: Acute and long-term toxicity of micronized car tire wear particles to *Hyalella azteca*. — Aquatic Toxicology 213: 105216.

Kirstein, I.V., Kirmizi, S., Wichels, A., Garin-Fernandez, A., Erler, R., Löder, M. & Gerdts, G. 2016: Dangerous hitchhikers? Evidence for potentially pathogenic *Vibrio* spp. on microplastic particles. — Marine Environmental Research 120: 1–8.

Koelmans, A.A., Bakir, A., Burton, G.A. & Janssen, C.R. 2016: Microplastic as a Vector for Chemicals in the Aquatic Environment: Critical Review and Model-Supported Reinterpretation of

Empirical Studies. — Environmental Science & Technology 50: 3315–3326.

Kole, P.J., Löhr, A.J., Van Belleghem, F.G.A.J. & Ragas, A.M.J. 2017: Wear and Tear of Tyres: A Stealthy Source of Microplastics in the Environment. — International Journal of Environmental Research and Public Health 14: 1265.

Kooi, M., Nes, E.H. van, Scheffer, M. & Koelmans, A.A. 2017: Ups and Downs in the Ocean: Effects of Biofouling on Vertical Transport of Microplastics. — Environmental Science & Technology 51: 7963–7971.

Kraan, C., Meer, J. van der, Dekinga, A. & Piersma, T. 2009: Patchiness of macrobenthic invertebrates in homogenized intertidal habitats: hidden spatial structure at a landscape scale. — Marine Ecology Progress Series 383: 211–224.

Kristensen, E., Penha-Lopes, G., Delefosse, M., Valdemarsen, T., Quintana, C. & Banta, G. 2012: What is bioturbation? The need for a precise definition for fauna in aquatic sciences. — Marine Ecology Progress Series 446: 285–302.

Kühn, S., Bravo Rebolledo, E.L. & van Franeker, J.A. 2015: Deleterious Effects of Litter on Marine Life. — In: Bergmann, M., Gutow, L. & Klages, M. (eds.). Marine Anthropogenic Litter, 75–116. Springer International Publishing. Cham.

Lang, S.-C., Hursthouse, A., Mayer, P., Kötke, D., Hand, I., Schulz-Bull, D. & Witt, G. 2015: Equilibrium passive sampling as a tool to study polycyclic aromatic hydrocarbons in Baltic Sea sediment pore-water systems. — Marine Pollution Bulletin 101: 296–303.

Law, K.L. & Thompson, R.C. 2014: Microplastics in the seas. — Science 345: 144–145.

Lebreton, L., Slat, B., Ferrari, F., Sainte-Rose, B., Aitken, J., Marthouse, R., Hajbane, S., Cunsolo, S., Schwarz, A., Levivier, A., Noble, K., Debeljak, P., Maral, H., Schoeneich-Argent, R., Brambini, R. & Reisser, J. 2018: Evidence that the Great Pacific Garbage Patch is rapidly accumulating plastic. — Scientific Reports 8: 4666.

Lee, R.F. & Steinert, S. 2003: Use of the single cell gel electrophoresis/comet assay for detecting DNA damage in aquatic (marine and freshwater) animals. — Mutation Research/Reviews in Mutation Research 544: 43–64.

Leppänen, M. 1995: The role of feeding behaviour in bioaccumulation of organic chemicals in benthic organisms. — Annales Zoologici Fennici 32: 247–255.

Leppäranta, M. & Myrberg, K. 2009: Physical Oceanography of the Baltic Sea. Springer-Verlag Berlin Heidelberg.

Leung, J. & Chan, K.Y.K. 2018: Microplastics reduced posterior segment regeneration rate of the polychaete *Perinereis aibuhitensis*. — Marine Pollution Bulletin 129: 782–786.

Li, A.-H., Liu, H.-C., Hou, W.-G. & Zhou, Y.-G. 2016: *Pseudorhodobacter sinensis* sp. nov. and *Pseudorhodobacter aquaticus* sp. nov., isolated from crater lakes. — International Journal of Systematic and Evolutionary Microbiology 66: 2819–2824.

Lin, J. & Hines, A.H. 1994: Effects of Suspended Food Availability on the Feeding Mode and Burial Depth of the Baltic Clam, *Macoma balthica*. — Oikos 69: 28.

Lin Pedersen, T. 2020: ggforce: Accelerating "ggplot2."

Lister, K.N., Lamare, M.D. & Burritt, D.J. 2016: Dietary pollutants induce oxidative stress, altering maternal antioxidant provisioning and reproductive output in the temperate sea urchin *Evechinus chloroticus*. — Aquatic Toxicology 177: 106–115.

Lithner, D., Larsson, Å. & Dave, G. 2011: Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. — Science of The Total Environment 409: 3309–3324.

Lithner, D., Nordensvan, I. & Dave, G. 2012: Comparative acute toxicity of leachates from plastic products made of polypropylene, polyethylene, PVC, acrylonitrile–butadiene–styrene, and epoxy to *Daphnia magna*. — Environmental Science and Pollution Research 19: 1763–1772.

Liu, P., Zhan, X., Wu, X., Li, J., Wang, H. & Gao, S. 2020: Effect of weathering on environmental behavior of microplastics: Properties, sorption and potential risks. — Chemosphere 242: 125193.

Livingstone, D. 2001: Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. — Marine pollution bulletin 42: 656–666.

Livingstone, D.R., Martinez, P.G., Michel, X., Narbonne, J.F., O'Hara, S., Ribera, D. & Winston, G.W. 1990: Oxyradical Production as a Pollution-Mediated Mechanism of Toxicity in the Common Mussel, *Mytilus edulis* L., and Other Molluscs. — Functional Ecology 4: 415–424.

Lobelle, D. & Cunliffe, M. 2011: Early microbial biofilm formation on marine plastic debris. — Marine Pollution Bulletin 62: 197–200.

Lohrer, A.M., Thrush, S.F. & Gibbs, M.M. 2004: Bioturbators enhance ecosystem function through complex biogeochemical interactions. — Nature 431: 1092–1095.

Long, M., Moriceau, B., Gallinari, M., Lambert, C., Huvet, A., Raffray, J. & Soudant, P. 2015: Interactions between microplastics and phytoplankton aggregates: Impact on their respective fates. — Marine Chemistry 175: 39–46.

Long, W.C., Brylawski, B.J. & Seitz, R.D. 2008: Behavioral effects of low dissolved oxygen on the bivalve *Macoma balthica*. — Journal of Experimental Marine Biology and Ecology 359: 34–39.

Lopez, G. & Elmgren, R. 1989: Feeding depths and organic absorption for the deposit feeding benthic amphipods *Pontoporera affinis* and *Pontoporeia femorata*: Pontoporeia feeding. — Limnology and Oceanography 34: 982–991.

Loulad, S., Houssa, R., Rhinane, H., Boumaaz, A. & Benazzouz, A. 2017: Spatial distribution of marine debris on the seafloor of Moroccan waters. — Marine Pollution Bulletin 124: 303–313.

Love, M., Ahlmann-Eltze, C., Forbes, K., Anders, S. & Huber, W. 2014: Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. — Genome Biology 15: 550.

Lucas, N., Bienaime, C., Belloy, C., Queneudec, M., Silvestre, F. & Nava-Saucedo, J.-E. 2008: Polymer biodegradation: Mechanisms and estimation techniques – A review. — Chemosphere 73: 429–442.

Lushchak, V.I. 2011: Environmentally induced oxidative stress in aquatic animals. — Aquatic Toxicology 101: 13–30.

Lusher, A.L., McHugh, M. & Thompson, R.C. 2013: Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. — Marine Pollution Bulletin 67: 94–99.

Maes, T., Barry, J., Leslie, H.A., Vethaak, A.D., Nicolaus, E.E.M., Law, R.J., Lyons, B.P., Martinez, R., Harley, B. & Thain, J.E. 2018: Below the surface: Twenty-five years of seafloor litter monitoring in coastal seas of North West Europe (1992–2017). — Science of The Total Environment 630: 790–798.

Maes, T., Van der Meulen, M.D., Devriese, L.I., Leslie, H.A., Huvet, A., Frère, L., Robbens, J. & Vethaak, A.D. 2017: Microplastics Baseline Surveys at the Water Surface and in Sediments of the North-East Atlantic. — Frontiers in Marine Science 4: 135.

Magara, G., Elia, A.C., Syberg, K. & Khan, F.R. 2018: Single contaminant and combined exposures of polyethylene microplastics and fluoranthene: accumulation and oxidative stress response in the blue mussel, *Mytilus edulis*. — Journal of Toxicology and Environmental Health, Part A 81: 761–773.

Mai, L., Bao, L.-J., Shi, L., Liu, L.-Y. & Zeng, E.Y. 2018: Polycyclic aromatic hydrocarbons affiliated with microplastics in surface waters of Bohai and Huanghai Seas, China. — Environmental Pollution 241: 834–840.

Marcus, N.H. & Schmidt-Gengenbach, J. 1986: Recruitment of individuals into the plankton: The importance of bioturbation1. — Limnology and Oceanography 31: 206–210.

Marigómez, I. & Baybay-Villacorta, L. 2003: Pollutant-specific and general lysosomal responses in digestive cells of mussels exposed to model organic chemicals. — Aquatic Toxicology 64: 235–257.

Marigómez, I., Lekube, X., Cajaraville, M.P., Domouhtsidou, G. & Dimitriadis, V. 2005: Comparison of cytochemical procedures to estimate lysosomal biomarkers in mussel digestive cells. — Aquatic Toxicology 75: 86–95.

Marigómez, I., Orbea, A., Olabarrieta, I., Etxeberria, M. & Cajaraville, M.P. 1996: Structural changes in the digestive lysosomal system of sentinel mussels as biomarkers of environmental stress in mussel-watch programmes. — Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology 113: 291–297.

Martin, M. 2011: Cutadapt removes adapter sequences from high-throughput sequencing reads. — EMBnet.journal 17: 10–12.

Martins, J.C., Saker, M.L., Teles, L.F.O. & Vasconcelos, V.M. 2007: Oxygen consumption by Daphnia magna straus as a marker of chemical stress in the aquatic environment. — Environmental Toxicology and Chemistry 26: 1987–1991.

Maruya, K.A., Zeng, E.Y., Tsukada, D. & Bay, S.M. 2009: A passive sampler based on solid-phase microextraction for quantifying hydrophobic organic contaminants in sediment pore water. — Environmental Toxicology and Chemistry 28: 733–740.

Mato, Y., Isobe, T., Takada, H., Kanehiro, H., Ohtake, C. & Kaminuma, T. 2001: Plastic Resin Pellets as a Transport Medium for Toxic Chemicals in the Marine Environment. — Environmental Science & Technology 35: 318–324.

McGreer, E.R. 1979: Sublethal effects of heavy metal contaminated sediments on the bivalve *Macoma balthica* (L.). — Marine Pollution Bulletin 10: 259–262.

McIlgorm, A., Campbell, H.F. & Rule, M.J. 2011: The economic cost and control of marine debris damage in the Asia-Pacific region. — Ocean & Coastal Management 54: 643–651.

Michaud, E., Desrosiers, G., Mermillod-Blondin, F., Sundby, B. & Stora, G. 2006: The functional group approach to bioturbation: II. The effects of the *Macoma balthica* community on fluxes of nutrients and dissolved organic carbon across the sediment–water interface. — Journal of Experimental Marine Biology and Ecology 337: 178–189.

Michaud, E., Desrosiers, G., Mermillod-Blondin, F., Sundby, B. & Stora, G. 2005: The functional group approach to bioturbation: The effects of biodiffusers and gallery-diffusers of the *Macoma balthica* community on sediment oxygen uptake. — Journal of Experimental Marine Biology and Ecology 326: 77–88.

Moore, C.J., Moore, S.L., Leecaster, M.K. & Weisberg, S.B. 2001: A Comparison of Plastic and Plankton in the North Pacific Central Gyre. — Marine Pollution Bulletin 42: 1297–1300.

Moore, M., Koehler, A., Lowe, D., Viarengo, A. & Klionsky, D.J. 2008: Lysosomes and Autophagy in Aquatic Animals. — In: Klionsky, D. J. (ed.). Methods in Enzymology 451: 581–620. Academic Press. Burlington.

Moore, M.N., Allen, J.I. & Somerfield, P.J. 2006: Autophagy: role in surviving environmental stress. — Marine Environmental Research 62: Supplement 1, S420–425.

Moore, M.N., Depledge, M.H., Readman, J.W. & Paul Leonard, D.R. 2004: An integrated biomarker-based strategy for ecotoxicological evaluation of risk in environmental management. — Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 552: 247–268.

von Moos, N., Burkhardt-Holm, P. & Köhler, A. 2012: Uptake and Effects of Microplastics on Cells and Tissue of the Blue Mussel *Mytilus edulis* L. after an Experimental Exposure. — Environmental Science & Technology 46: 11327–11335.

Mordecai, G., Tyler, P.A., Masson, D.G. & Huvenne, V.A.I. 2011: Litter in submarine canyons off the west coast of Portugal.: Deep Sea Research Part II. — Topical Studies in Oceanography 58: 2489–2496.

Murray, F. & Cowie, P.R. 2011: Plastic contamination in the decapod crustacean *Nephrops norvegicus* (Linnaeus, 1758). — Marine Pollution Bulletin 62: 1207–1217.

Mustonen, A.-R. 2020: Microplastics in benthic invertebrates in the Gulf of Finland. M.Sc. Thesis. Faculty of Biological and Environmental Sciences, University of Helsinki.

Nauendorf, A., Krause, S., Bigalke, N.K., Gorb, E.V., Gorb, S.N., Haeckel, M., Wahl, M. & Treude, T. 2016: Microbial colonization and degradation of polyethylene and biodegradable plastic bags in temperate fine-grained organic-rich marine sediments. — Marine Pollution Bulletin 103: 168–178.

Neuwirth, E. 2014: RColorBrewer: ColorBrewer Palettes.

Newman, S., Watkins, E., Farmer, A., Ten Brink, P. & Schweitzer, J.-P. 2015: The economics of marine litter. — In: Bergmann, M., Gutow, L. & Klages, M. (eds.). Marine Anthropogenic Litter, 367–394. Springer International Publishing. Cham.

Nikula, R., Strelkov, P. & Väinölä, R. 2008: A broad transition zone between an inner Baltic hybrid swarm and a pure North Sea subspecies of *Macoma balthica* (Mollusca, Bivalvia). — Molecular Ecology 17: 1505–1522.

Norkko, A., Villnäs, A., Norkko, J., Valanko, S. & Pilditch, C. 2013: Size matters: implications of the loss of large individuals for ecosystem function. — Scientific Reports 3: 2646.

Norkko, J., Reed, D.C., Timmermann, K., Norkko, A., Gustafsson, B.G., Bonsdorff, E., Slomp, C.P., Carstensen, J. & Conley, D.J. 2012: A welcome can of worms? Hypoxia mitigation by an invasive species. — Global Change Biology 18: 422–434.

Nowosand, J. 2018: "CARTOColors" Palettes. R package version 1.0.0.

Oberbeckmann, S. & Labrenz, M. 2020: Marine Microbial Assemblages on Microplastics: Diversity, Adaptation, and Role in Degradation. — Annual Review of Marine Science 12: 209–232.

Oberbeckmann, S., Loeder, M.G.J., Gerdts, G. & Osborn, A.M. 2014: Spatial and seasonal variation in diversity and structure of microbial biofilms on marine plastics in Northern European waters. — FEMS Microbiology Ecology 90: 478–492.

Oberbeckmann, S., Osborn, A.M. & Duhaime, M.B. 2016: Microbes on a Bottle: Substrate, Season and Geography Influence Community Composition of Microbes Colonizing Marine Plastic Debris. — PLOS ONE 11: e0159289.

O'Donovan, S., Mestre, N.C., Abel, S., Fonseca, T.G., Carteny, C.C., Cormier, B., Keiter, S.H. & Bebianno, M.J. 2018: Ecotoxicological Effects of Chemical Contaminants Adsorbed to Microplastics in the Clam *Scrobicularia plana*. — Frontiers in Marine Science 5: 143.

Oliveira, M., Ribeiro, A., Hylland, K. & Guilhermino, L. 2013: Single and combined effects of microplastics and pyrene on juveniles (0+ group) of the common goby *Pomatoschistus microps* (Teleostei, Gobiidae). — Ecological Indicators 34: 641–647.

Ott, M., Gogvadze, V., Orrenius, S. & Zhivotovsky, B. 2007: Mitochondria, oxidative stress and cell death. — Apoptotis 12, 913–922.

Pagter, E., Frias, J., Kavanagh, F. & Nash, R. 2020: Varying levels of microplastics in benthic sediments

within a shallow coastal embayment. — Estuarine, Coastal and Shelf Science 243: 106915.

Pascall, M.A., Zabik, M.E., Zabik, M.J. & Hernandez, R.J. 2005: Uptake of Polychlorinated Biphenyls (PCBs) from an Aqueous Medium by Polyethylene, Polyvinyl Chloride, and Polystyrene Films. — Journal of Agricultural and Food Chemistry 53: 164–169.

Peakall, D.B. 1994: The role of biomarkers in environmental assessment (1). Introduction. — Ecotoxicology 3: 157–160.

Peeken, I., Primpke, S., Beyer, B., Gütermann, J., Katlein, C., Krumpen, T., Bergmann, M., Hehemann, L. & Gerdts, G. 2018: Arctic sea ice is an important temporal sink and means of transport for microplastic. — Nature Communications 9: 1505.

Pesonen, M. & Vähäkangas, K. 2019: Autophagy in exposure to environmental chemicals. — Toxicology Letters 305: 1–9.

Petes, L.E., Menge, B.A. & Harris, A.L. 2008: Intertidal Mussels Exhibit Energetic Trade-Offs Between Reproduction and Stress Resistance. — Ecological Monographs 78: 387–402.

Pham, C.K., Ramirez-Llodra, E., Alt, C.H.S., Amaro, T., Bergmann, M., Canals, M., Company, J.B., Davies, J., Duineveld, G., Galgani, F., Howell, K.L., Huvenne, V.A.I., Isidro, E., Jones, D.O.B., Lastras, G., Morato, T., Gomes-Pereira, J.N., Purser, A., Stewart, H., Tojeira, I., Tubau, X., Van Rooij, D. & Tyler, P.A. 2014: Marine Litter Distribution and Density in European Seas, from the Shelves to Deep Basins. — PLoS ONE 9: e95839.

Pinnell, L.J. & Turner, J.W. 2019: Shotgun Metagenomics Reveals the Benthic Microbial Community Response to Plastic and Bioplastic in a Coastal Marine Environment. — Frontiers in Microbiology 10: 1252.

Pinto, M., Langer, T.M., Hüffer, T., Hofmann, T. & Herndl, G.J. 2019: The composition of bacterial communities associated with plastic biofilms differs between different polymers and stages of biofilm succession. — PLOS ONE 14: e0217165.

PlasticsEurope 2013: Plastics – the Facts 2013: An analysis of European latest plastics production, demand and waste data. — PlasticsEurope Association of Plastics Manufacturers.

PlasticsEurope 2020: Plastics – the Facts 2020: An analysis of European plastics production, demand and waste data. — PlasticsEurope Association of Plastics Manufacturers.

Pohlert, T. 2020: PMCMRplus: Calculate Pairwise Multiple Comparisons of Mean Rank Sums Extended. R package version v. 1.6.1.

Prahl, F.G. & Carpenter, R. 1979: The role of zooplankton fecal pellets in the sedimentation of polycyclic aromatic hydrocarbons in Dabob Bay, Washington. — Geochimica et Cosmochimica Acta 43: 1959–1972.

Prokić, M.D., Radovanović, T.B., Gavrić, J.P. & Faggio, C. 2019: Ecotoxicological effects of microplastics: Examination of biomarkers, current state and future perspectives. — Trends in Analytical Chemistry 111: 37–46.

Puls, J., Wilson, S.A. & Hölter, D. 2011: Degradation of Cellulose Acetate-Based Materials: A Review. — Journal

of Polymers and the Environment 19: 152–165.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J. & Glöckner, F.O. 2013: The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. — Nucleic Acids Research 41: D590–D596.

Quintana, C.O., Tang, M. & Kristensen, E. 2007: Simultaneous study of particle reworking, irrigation transport and reaction rates in sediment bioturbated by the polychaetes *Heteromastus* and *Marenzelleria*. — Journal of Experimental Marine Biology and Ecology 352: 392–406.

R Core Team 2020: R: A language and environment for statistical computing.

Railo, S., Talvitie, J., Setälä, O., Koistinen, A. & Lehtiniemi, M. 2018: Application of an enzyme digestion method reveals microlitter in *Mytilus trossulus* at a wastewater discharge area. — Marine Pollution Bulletin 130: 206–214.

Redondo-Hasselerharm, P.E., Falahudin, D., Peeters, E.T.H.M. & Koelmans, A.A. 2018: Microplastic Effect Thresholds for Freshwater Benthic Macroinvertebrates. — Environmental Science & Technology 52: 2278–2286.

Regoli, F. 1992: Lysosomal responses as a sensitive stress index in biomonitoring heavy metal pollution. — Marine Ecology Progress Series 84: 63–69.

Renz, J.R. & Forster, S. 2013: Are similar worms different? A comparative tracer study on bioturbation in the three sibling species *Marenzelleria arctia, M. viridis*, and *M. neglecta* from the Baltic Sea. — Limnology and Oceanography 58: 2046–2058.

Rhoads, D.C. 1974: Organism-sediment relations on the muddy sea floor. — Oceanography and Marine Biology: An Annual Review 12: 263–300.

Ribeiro, F., Garcia, A.R., Pereira, B.P., Fonseca, M., Mestre, N.C., Fonseca, T.G., Ilharco, L.M. & Bebianno, M.J. 2017: Microplastics effects in *Scrobicularia plana*. — Marine Pollution Bulletin 122: 379–391.

Rieck, A., Herlemann, D.P.R., Jürgens, K. & Grossart, H.-P. 2015: Particle-Associated Differ from Free-Living Bacteria in Surface Waters of the Baltic Sea. — Frontiers in Microbiology 6: 1297.

Rios, L.M., Moore, C. & Jones, P.R. 2007: Persistent organic pollutants carried by synthetic polymers in the ocean environment. — Marine Pollution Bulletin 54: 1230–1237.

Rochman, C.M. 2015: The Complex Mixture, Fate and Toxicity of Chemicals Associated with Plastic Debris in the Marine Environment. — In: Bergmann, M., Gutow, L. & Klages, M. (eds.). Marine Anthropogenic Litter, 117–140. Springer International Publishing. Cham.

Rochman, C.M., Hoh, E., Hentschel, B.T. & Kaye, S. 2013: Long-Term Field Measurement of Sorption of Organic Contaminants to Five Types of Plastic Pellets: Implications for Plastic Marine Debris. — Environmental Science & Technology 47: 1646–1654.

Rochman, C.M., Manzano, C., Hentschel, B.T., Simonich, S.L.M. & Hoh, E. 2013: Polystyrene Plastic: A Source and Sink for Polycyclic

Aromatic Hydrocarbons in the Marine Environment. — Environmental Science & Technology 47: 13976–13984.

Rousi, H., Laine, A.O., Peltonen, H., Kangas, P., Andersin, A.-B., Rissanen, J., Sandberg-Kilpi, E. & Bonsdorff, E. 2013: Long-term changes in coastal zoobenthos in the northern Baltic Sea: the role of abiotic environmental factors. — ICES Journal of Marine Science 70: 440–451.

de Ruijter, V.N., Redondo-Hasselerharm, P.E., Gouin, T. & Koelmans, A.A. 2020: Quality Criteria for Microplastic Effect Studies in the Context of Risk Assessment: A Critical Review. — Environmental Science & Technology 54: 11692–11705.

Ryan, P.G. 2015: A Brief History of Marine Litter Research. — In: Bergmann, M., Gutow, L. & Klages, M. (eds.). Marine Anthropogenic Litter, 1–25. Springer International Publishing. Cham.

Ryan, P.G., Moore, C.J., van Franeker, J.A. & Moloney, C.L. 2009: Monitoring the abundance of plastic debris in the marine environment. — Philosophical Transactions of the Royal Society B: Biological Sciences 364: 1999–2012.

Rytkönen, J., Siitonen, L., Riipi, T., Sassi, J. & Sukselainen, J. 2002: Statistical Analyses of the Baltic Maritime Traffic. VTT Research Report VAL34-012344. — VTT Technical Research Centre of Finland.

de Sá, L.C., Oliveira, M., Ribeiro, F., Rocha, T.L. & Futter, M.N. 2018: Studies of the effects of microplastics on aquatic organisms: What do we know and where should we focus our efforts in the future? — Science of The Total Environment 645: 1029–1039.

Santana, M.F.M., Moreira, F.T., Pereira, C.D.S., Abessa, D.M.S. & Turra, A. 2018: Continuous Exposure to Microplastics Does Not Cause Physiological Effects in the Cultivated Mussel *Perna perna*. — Archives of Environmental Contamination and Toxicology 74: 594–604.

SAPEA 2019: A Scientific Perspective on Microplastics in Nature and Society. — Science Advice for Policy by European Academies. Berlin.

Schernewski, G., Radtke, H., Hauk, R., Baresel, C., Olshammar, M., Osinski, R. & Oberbeckmann, S. 2020: Transport and Behavior of Microplastics Emissions From Urban Sources in the Baltic Sea. — Frontiers in Environmental Science 8: 579361.

Schmidt, C., Krauth, T. & Wagner, S. 2017: Export of Plastic Debris by Rivers into the Sea. — Environmental Science & Technology 51: 12246–12253.

Sebille, E., Spathi, C. & Gilbert, A. 2016: The ocean plastic pollution challenge: towards solutions in the UK. — Briefing Paper No. 19. Grantham Institute & Imperial College London.

Seeley, M.E., Song, B., Passie, R. & Hale, R.C. 2020: Microplastics affect sedimentary microbial communities and nitrogen cycling. — Nature Communications 11: 2372.

Self, R.F.L.& Jumars, P.A. 1988: Cross-phyletic patterns of particle selection by deposit feeders. — Journal of Marine Research 46: 119–143.

Setälä, O., Magnusson, K., Lehtiniemi & M., Norén, F. 2016: Distribution and abundance of surface water microlitter

in the Baltic Sea: A comparison of two sampling methods. — Marine Pollution Bulletin 110: 177–183.

Setälä, O., Norkko, J. & Lehtiniemi, M. 2016: Feeding type affects microplastic ingestion in a coastal invertebrate community. — Marine Pollution Bulletin 102: 95–101.

Setälä, O. & Suikkanen, S. 2020: Suomen merialueen roskaantumisen lähteet. — Suomen ympäristökeskuksen raportteja 9 / 2020. Finnish Environment Institute (SYKE).

SFS-ISO 18287:2007 2007: Soil quality. Determination of polycyclic aromatic hydrocarbons (PAH) - Gas chromatographic method with mass spectrometric detection (GC-MS).

Shah, A.A., Hasan, F., Hameed, A. & Ahmed, S. 2008: Biological degradation of plastics: A comprehensive review. — Biotechnology Advances 26: 246–265.

Shim, W.J., Hong, S.H. & Eo, S. 2018: Marine Microplastics: Abundance, Distribution, and Composition. — In: Zeng, E. Y. (ed.). Microplastic Contamination in Aquatic Environments, 1–19. Elsevier.

Shull, D.H. 2009: Bioturbation. — In: Steele, J. H. (ed.). Encyclopedia of Ocean Sciences (Second Edition), 395–400. Academic Press. Oxford.

Silvestre, F. 2020: Signaling pathways of oxidative stress in aquatic organisms exposed to xenobiotics. — Journal of Experimental Zoology Part A: Ecological and Integrative Physiology 333: 436–448.

Slimen, I.B., Najar, T., Ghram, A., Dabbebi, H., Mrad, M.B. & Abdrabbah, M. 2014: Reactive oxygen species, heat stress and oxidative-induced mitochondrial damage. A review. — International Journal of Hyperthermia 30: 513–523.

Snelgrove, P.V.R. 1998: The biodiversity of macrofaunal organisms in marine sediments. — Biodiversity and Conservation 7: 1123–1132.

Sokolowski, A., Wolowicz, M., Hummel, H. & Bogaards, R. 1999: Physiological responses of *Macoma balthica* to copper pollution in the Baltic. — Oceanologica Acta 22: 431–439.

Song, J.H., Murphy, R.J., Narayan, R. & Davies, G.B.H. 2009: Biodegradable and compostable alternatives to conventional plastics. — Philosophical Transactions of the Royal Society B: Biological Sciences 364: 2127–2139.

Sørensen, L., Rogers, E., Altin, D., Salaberria, I. & Booth, A.M. 2020: Sorption of PAHs to microplastic and their bioavailability and toxicity to marine copepods under co-exposure conditions. — Environmental Pollution 258: 113844.

Stekoll, M.S., Clement, L.E. & Shaw, D.G. 1980: Sublethal effects of chronic oil exposure on the intertidal clam *Macoma balthica*. — Marine Biology 57: 51–60.

Stolte, A., Forster, S., Gerdts, G. & Schubert, H. 2015: Microplastic concentrations in beach sediments along the German Baltic coast. — Marine Pollution Bulletin 99: 216–229.

Strand, J., Lassen, P., Shashoua, Y. & Andersen, J. 2013: Microplastic particles in sediments from Danish waters. ICES Annual Science Conference (ASC).

Sudesh, K. & Iwata, T. 2008: Sustainability of Biobased and

Biodegradable Plastics. — CLEAN – Soil, Air, Water 36: 433–442.

Suhrhoff, T.J. & Scholz-Böttcher, B.M. 2016: Qualitative impact of salinity, UV radiation and turbulence on leaching of organic plastic additives from four common plastics — A lab experiment. — Marine Pollution Bulletin 102: 84–94.

Sun, X., Wang, T., Chen, B., Booth, A.M., Liu, S., Wang, R., Zhu, L., Zhao, X., Qu, K. & Bin Xia 2021: Factors influencing the occurrence and distribution of microplastics in coastal sediments: From source to sink. — Journal of Hazardous Materials 410: 124982.

Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M.E.J., Le Goïc, N., Quillien, V., Mingant, C., Epelboin, Y., Corporeau, C., Guyomarch, J., Robbens, J., Paul-Pont, I., Soudant, P. & Huvet, A. 2016: Oyster reproduction is affected by exposure to polystyrene microplastics. — Proceedings of the National Academy of Sciences 113: 2430–2435.

Tallqvist, M. 2001: Burrowing behaviour of the Baltic clam *Macoma balthica*: effects of sediment type, hypoxia and predator presence. — Marine Ecology Progress Series 212: 183–191.

Talvitie, J., Mikola, A., Koistinen, A. & Setälä, O. 2017: Solutions to microplastic pollution – Removal of microplastics from wastewater effluent with advanced wastewater treatment technologies. — Water Research 123: 401–407.

Taylor, M.L., Gwinnett, C., Robinson, L.F. & Woodall, L.C. 2016: Plastic microfibre ingestion by deep-sea organisms. — Scientific Reports 6: 33997.

Teal, L., Bulling, M., Parker, E. & Solan, M. 2008: Global patterns of bioturbation intensity and mixed depth of marine soft sediments. — Aquatic Biology 2: 207–218.

Tedengren, M., Arnér, M. & Kautsky, N. 1988: Ecophysiology and stress response of marine and brackish water Gammarus species (Crustacea, Amphipoda) to changes in salinity and exposure to cadmium and diesel-oil. — Marine Ecology Progress Series 47: 107–116.

Teuten, E.L., Saquing, J.M., Knappe, D.R.U., Barlaz, M.A., Jonsson, S., Björn, A., Rowland, S.J., Thompson, R.C., Galloway, T.S., Yamashita, R., Ochi, D., Watanuki, Y., Moore, C., Viet, P.H., Tana, T.S., Prudente, M., Boonyatumanond, R., Zakaria, M.P., Akkhavong, K., Ogata, Y., Hirai, H., Iwasa, S., Mizukawa, K., Hagino, Y., Imamura, A., Saha, M. & Takada, H. 2009: Transport and release of chemicals from plastics to the environment and to wildlife. — Philosophical Transactions of the Royal Society of London B: Biological Sciences 364: 2027–2045.

Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D. & Russell, A.E. 2004: Lost at sea: Where is all the plastic? — Science 304: 838–838.

Tian, Z., Zhao, H., Peter, K.T., Gonzalez, M., Wetzel, J., Wu, C., Hu, X., Prat, J., Mudrock, E., Hettinger, R., Cortina, A.E., Biswas, R.G., Kock, F.V.C., Soong, R., Jenne, A., Du, B., Hou, F., He, H., Lundeen, R., Gilbreath, A., Sutton, R., Scholz, N.L., Davis, J.W., Dodd, M.C., Simpson, A., McIntyre,

J.K. & Kolodziej, E.P. 2020: A ubiquitous tire rubber–derived chemical induces acute mortality in coho salmon. — Science 371: 185–189.

Tiano, L., Fedeli, D., Santoni, G., Davies, I. & Falcioni, G. 2003: Effect of tributyltin on trout blood cells: changes in mitochondrial morphology and functionality. — Biochimica et Biophysica Acta (BBA) - Molecular Cell Research 1640: 105–112.

Tirroniemi, J. 2019: Microplastics in northern Baltic Sea bottom sediments: distribution and method development. M.Sc. Thesis. Faculty of Biological and Environmental Sciences, University of Helsinki.

Tourinho, P.S., Kočí, V., Loureiro, S. & van Gestel, C.A.M. 2019: Partitioning of chemical contaminants to microplastics: Sorption mechanisms, environmental distribution and effects on toxicity and bioaccumulation. — Environmental Pollution 252: 1246–1256.

Trestrail, C., Nugegoda, D. & Shimeta, J. 2020: Invertebrate responses to microplastic ingestion: Reviewing the role of the antioxidant system. — Science of The Total Environment 734: 138559.

Trevisan, R., Delapedra, G., Mello, D.F., Arl, M., Schmidt, É.C., Meder, F., Monopoli, M., Cargnin-Ferreira, E., Bouzon, Z.L., Fisher, A.S., Sheehan, D. & Dafre, A.L. 2014: Gills are an initial target of zinc oxide nanoparticles in oysters Crassostrea gigas, leading to mitochondrial disruption and oxidative stress. — Proceedings from the 17th International Symposium on Pollutant Responses in Marine Organisms (PRIMO17) 153: 27–38.

Turner, A. 2017: Trace elements in fragments of fishing net and other filamentous plastic litter from two beaches in SW England. — Environmental Pollution 224: 722–728.

Turner, A. & Lau, K.S. 2016: Elemental concentrations and bioaccessibilities in beached plastic foam litter, with particular reference to lead in polyurethane. — Marine Pollution Bulletin 112: 265–270.

UNEP 2005: Marine Litter, an analytical overview. — United Nations Environment Programme. Nairobi, Kenya.

UNEP 2009: Marine Litter: A Global Challenge. — United Nations Environment Programme. Nairobi, Kenya.

UNEP 2016: Marine plastic debris and microplastics – Global lessons and research to inspire action and guide policy change. — United Nations Environment Programme. Nairobi, Kenya.

Uurasjärvi, E., Pääkkönen, M., Setälä, O., Koistinen, A. & Lehtiniemi, M. 2021: Microplastics accumulate to thin layers in the stratified Baltic Sea. — Environmental Pollution 268: 115700.

Valavanidis, A., Vlahogianni, T., Dassenakis, M. & Scoullos, M. 2006: Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. — Ecotoxicology and Environmental Safety 64: 178–189.

Valdemarsen, T., Wendelboe, K., Egelund, J.T., Kristensen, E. & Flindt, M.R. 2011: Burial of seeds and seedlings by the lugworm Arenicola

marina hampers eelgrass (Zostera marina) recovery. — Journal of Experimental Marine Biology and Ecology 410: 45–52.

Van Cauwenberghe, L., Claessens, M., Vandegehuchte, M.B. & Janssen, C.R. 2015: Microplastics are taken up by mussels (*Mytilus edulis*) and lugworms (*Arenicola marina*) living in natural habitats. — Environmental Pollution 199: 10–17.

Van Cauwenberghe, L., Claessens, M., Vandegehuchte, M.B., Mees, J. & Janssen, C.R. 2013: Assessment of marine debris on the Belgian Continental Shelf. — Marine Pollution Bulletin 73: 161–169.

Velzeboer, I., Kwadijk, C.J.A.F. & Koelmans, A.A. 2014: Strong Sorption of PCBs to Nanoplastics, Microplastics, Carbon Nanotubes, and Fullerenes. — Environmental Science & Technology 48: 4869–4876.

Verbeek, C.J.R. & Uitto, J.M. 2017: Bioplastics. — In: Encyclopedia of Polymer Science and Technology (Ed.) 1–37. John Wiley & Sons Inc. New York, NY, USA.

Vianello, A., Boldrin, A., Guerriero, P., Moschino, V., Rella, R., Sturaro, A. & Da Ros, L. 2013: Microplastic particles in sediments of Lagoon of Venice, Italy: First observations on occurrence, spatial patterns and identification. — Estuarine, Coastal and Shelf Science 130: 54–61.

Viarengo, A., Moore, M.N., Mancinelli, G., Mazzucotelli, A., Pipe, R.K. & Farrar, S.V. 1987: Metallothioneins and lysosomes in metal toxicity and accumulation in marine mussels: the effect of cadmium in the presence and absence of phenanthrene. — Marine Biology 94: 251–257.

Viitasalo, S. 2007: Effects of bioturbation by three macrozoobenthic species and predation by necto-benthic mysids on cladoceran benthic eggs. — Marine Ecology Progress Series 336: 131–140.

Viitasalo-Frösén, S., Laine, A. & Lehtiniemi, M. 2009: Habitat modification mediated by motile surface stirrers versus semi-motile burrowers: potential for a positive feedback mechanism in a eutrophied ecosystem. — Marine Ecology Progress Series 376: 21–32.

Villnäs, A., Norkko, A. & Lehtonen, K.K. 2019: Multi-level responses of *Macoma balthica* to recurring hypoxic disturbance. — Journal of Experimental Marine Biology and Ecology 510: 64–72.

Vroom, R.J.E., Koelmans, A.A., Besseling, E. & Halsband, C. 2017: Aging of microplastics promotes their ingestion by marine zooplankton. — Environmental Pollution 231: 987–996.

Wagner, S., Hüffer, T., Klöckner, P., Wehrhahn, M., Hofmann, T. & Reemtsma, T. 2018: Tire wear particles in the aquatic environment - A review on generation, analysis, occurrence, fate and effects. — Water Research 139: 83–100.

Westerbom, M., Kilpi, M. & Mustonen, O. 2002: Blue mussels, *Mytilus edulis*, at the edge of the range: population structure, growth and biomass along a salinity gradient in the north-eastern Baltic Sea. — Marine Biology 140: 991–999.

Wheatcroft, R.A. 1992: Experimental tests for particle size-dependent bioturbation in the deep ocean. —

Limnology and Oceanography 37: 90–104.

Wheatcroft, R.A., Jumars, P.A., Smith, C.R. & Nowell, A.R.M. 1990: A mechanistic view of the particulate biodiffusion coefficient: Step lengths, rest periods and transport directions. — Journal of Marine Research 48: 177–207.

Wik, A., Nilsson, E., Källqvist, T., Tobiesen, A. & Dave, G. 2009: Toxicity assessment of sequential leachates of tire powder using a battery of toxicity tests and toxicity identification evaluations. — Chemosphere 77: 922–927.

Willis, K.A., Eriksen, R., Wilcox, C. & Hardesty, B.D. 2017: Microplastic Distribution at Different Sediment Depths in an Urban Estuary. — Frontiers in Marine Science 4: 419.

Witt, G. 2002: Occurrence and transport of polycyclic aromatic hydrocarbons in the water bodies of the Baltic Sea. — Marine Chemistry 79: 49–66.

Witt, G. 1995: Polycyclic aromatic hydrocarbons in water and sediment of the Baltic Sea. — Marine Pollution Bulletin 31: 237–248.

Woodall, L.C., Sanchez-Vidal, A., Canals, M., Paterson, G.L.J., Coppock, R., Sleight, V., Calafat, A., Rogers, A.D., Narayanaswamy B.E. & Thompson, R.C. 2014: The deep sea is a major sink for microplastic debris. — Royal Society Open Science 1: 140317.

Wright, R.J., Erni-Cassola, G., Zadjelovic, V., Latva, M. & Christie-Oleza, J.A. 2020a: Marine Plastic Debris: A New Surface for Microbial Colonization. — Environmental Science & Technology 54: 11657–11672.

Wright, R.J., Langille, M.G.I. & Walker, T.R. 2020b: Food or just a free ride? A meta-analysis reveals the global diversity of the Plastisphere. — The ISME Journal 15: 789–806.

Wright, S.L., Rowe, D., Thompson, R.C. & Galloway, T.S. 2013: Microplastic ingestion decreases energy reserves in marine worms. — Current Biology 23: R1031–R1033.

Wypych, G. 2016: Handbook of Polymers. ChemTech Publishing. Toronto, Ontario, Canada.

Zalasiewicz, J., Waters, C.N., Ivar do Sul, J.A., Corcoran, P.L., Barnosky, A.D., Cearreta, A., Edgeworth, M., Gałuszka, A., Jeandel, C., Leinfelder, R., McNeill, J.R., Steffen, W., Summerhayes, C., Wagreich, M., Williams, M., Wolfe, A.P. & Yonan, Y. 2016: The geological cycle of plastics and their use as a stratigraphic indicator of the Anthropocene. — Anthropocene 13: 4–17.

Zettler, E.R., Mincer, T.J. & Amaral-Zettler, L.A. 2013: Life in the "Plastisphere": Microbial Communities on Plastic Marine Debris. — Environmental Science & Technology 47: 7137–7146.

Ziajahromi, S., Drapper, D., Hornbuckle, A., Rintoul, L. & Leusch, F.D.L. 2020: Microplastic pollution in a stormwater floating treatment wetland: Detection of tyre particles in sediment. — Science of The Total Environment 713: 136356.

Ziajahromi, S., Kumar, A., Neale, P.A. & Leusch, F.D.L. 2018: Environmentally relevant concentrations of polyethylene microplastics negatively impact the survival, growth and emergence of sediment-dwelling invertebrates. —

Environmental Pollution 236: 425–431.

Zobkov, M.B., Esiukova, E.E., Zyubin, A.Y. & Samusev, I.G. 2019: Microplastic content variation in water column: The observations employing a novel sampling tool in stratified Baltic Sea. — Marine Pollution Bulletin 138: 193–205.

Zwarts, L., Blomert, A.-M., Spaak, P. & de Vries, B. 1994: Feeding radius, burying depth and siphon size of *Macoma balthica* and *Scrobicularia plana*. — Journal of Experimental Marine Biology and Ecology 183: 193–212.