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Chapter

Micro and nanoplastics in the aquatic environment with special reference to synthetic fibers

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9.1 Introduction

Plastics are polymers produced from synthetic solid petroleum- or bio-based materials and often mixed with chemical additives. The annual global production of these polymers has exponentially increased over the past decades, reaching 348 million tons in 2017, and is expected to double by 2050 [1, 2]. Such an increase is related with an important production of waste, of which a fraction will eventually end up in the environment due to poor waste management or inappropriate disposal. Plastics are insoluble in water and have (extremely) slow degradation rates after being discarded. Global leakage of persistent plastic waste flowing into rivers and waterways, and ultimately into the oceans, was estimated in the order of 10 million tonnes per year (Mty^{-1}) [3], resulting in a widespread occurrence and accumulation of microplastic pollution.

Plastic particles in the micrometer-size range have been detected worldwide in virtually all aquatic compartments, such as surface waters, water column, sea floor, coastlines, polar ice, rivers, small waterways and lakes, as well as in a wide range of species (e.g., [4–9]. This raises scientific and public concern on their possible impact on aquatic populations, food webs, and food production for humans. The debate is fuelled by the persistence of the plastics and a projected future increase in microplastic pollution levels [10, 11]. The term microplastics (MPs), whether originating from intentional production (primary microplastics) or fragmentation of larger plastics (secondary microplastic), was coined by Thompson and co-workers in 2004 to describe the widespread occurrence of microscopic plastic particles and fibers in the oceans [12]. Since then, microplastic research has attracted increasing attention in environmental sciences, interlinking them between the fields of macro-plastic pollution and the field of nanoparticle toxicology [13].

Microplastic debris is heterogeneous in nature with a large variety of shapes, from spherical particles to angular fragments or films and long fibers [14]. All plastic particles or fibers in the size range $1-5000 \mu m$ (in their largest dimension) are referred to as microplastics (MPs) and those in the size range $< 1 \mu m$ (in their largest dimension) as nanoplastics (NPs) [14, 15]. Microplastic fibers are a specific type of microplastics defined as any artificial fibrous materials (e.g., acrylic, polyesters, nylon) of threadlike structure with a diameter less than 50 μm , length ranging from 1 to 5000 μm , and length to diameter ratio greater than 100 [16]. Some studies have used different morphological criteria to confirm a fibrous particle, for example "a length to diameter ratio of > 3" [17] or "a length substantially longer than its width" [14].

Field studies to date have mostly focused on plastic particles and fibers in the micro-size range. Due to analytical limitations, nano-sized plastic particles and fibers are not considered in field studies, but they have been commonly used in laboratory studies to understand the behavior and toxic effects of micro- and nanoplastics (MNPs) in aquatic organisms. The bulk of field and laboratory studies concerning environmental concentrations and effects to date have been performed for marine environments, but the impact of MNPs may be equally serious for freshwater and terrestrial environments. However, the occurrence, fate and ecological effects of this type of pollution are still poorly understood. In the past decade, a rapidly growing body of empirical research on aquatic microplastics has aimed at elucidating and understanding the ecological impact of MNPs to provide evidence to inform and support policy makers remediating plastic pollution (e.g., [4, 18–31].

This chapter aims to present a summary of what is currently known about MNP pollution in aquatic ecosystems. Special attention will be given to synthetic fibers, which are a predominant type of MNP pollution. Factors influencing MNP uptake and absorption, their bioaccumulation and ecological effects will be considered as well. Case studies on phytoplankton and bivalves will be highlighted. We conclude by discussing important knowledge gaps that need to be addressed in future work.

9.2 Sources, pathways and sinks

9.2.1 Major sources of MNPs

MNPs enter the aquatic environment from a variety of sources. The weathering and fragmentation of large plastic pieces, such as containers, packaging materials, bottles, and ropes are the most important source of secondary MNPs throughout the world ocean (e.g., [22, 32, 33]). It is estimated that between 1.15 and 2.41 million tons of both macro-and micro-plastic debris enter the ocean every year from rivers, with the top 20 polluting rivers, mostly located in Asia, accounting for 67 per cent of the global total [34]. Wastewater treatment plants are significant sources of microplastics in river catchments with concentrations reaching up to 125 particles per liter [35]. In addition to this, sea-based sources of plastic litter (beach littering, shipping and fisheries) and atmospheric transport and deposition of MNPs, may also be important pathways [16, 22, 36].

Not all MNPs originate from the breakdown of larger plastic items or particles. MNPs can also be generated by erosion and abrasion during the production, maintenance, and use of plastic products. In this case, tyre wear and tear during driving and shredding of synthetic fibers from textiles during washing are relevant and major sources [3, 37]. In their review on wear and tear of tyres, Kole and co-workers estimated that the per capita emission ranges from 0.23 to 4.7 kg y⁻¹, with a global average of 0.81 kg y⁻¹ [38]. These estimates indicate that car wear and tear, consisting largely of elongated particles of rubber polymers [39], significantly contribute to the flow of (micro)plastics into the aquatic environment with a relative contribution of 5–10% of total global oceanic plastic.

Some portion of MNPs are manufactured as micro or nano-sized particles and directly released to the environment, such as pellets, powders, or microbeads in personal care products (PCP) [22]. However, these primary particles are probably only a small fraction of the total amount of MNPs in the aquatic environment [3].

Well-known are plastic preproduction resin pellets, usually 1 to 5 mm in size and of variable composition. They are found in aquatic samples and on beaches all over the world and their presence is often linked to spillage during transportation or industrial effluents. Other major, but lesser known sources of plastic particles and fibers emitting to the environment are abrasion of polymeric paints from shipping, water works, city dust, and household dust [3, 40]. Microplastic pollution by smaller size micropellets has also been observed. For example, micro-debris detected in European river (Rhine) sediments close to polymer plants contained overall 18% PS pellets (100–200 µm) showing visual and spectroscopic resemblance to primary preproduction pellets/powder [41]. Another very recent example is the shipping accident of the MSC Zoe in 2019 during a storm in the North Sea. The loss of 342 containers of goods, several of which contained 5 mm plastic preproduction pellets (polystyrene and HPPE), resulted in a wide spread

microplastic contamination of Dutch and German islands in the Wadden Sea (a protected area under European Natura 2000 law).

9.2.2 Sources of fibrous MNPs

Synthetic microfibers in the aquatic environment originate from primary textile microfibers that are used in the textile and clothing industry, and the fragmentation of larger fabrics originates during textile production, use, and from discarded textiles [16, 42, 43]. Other sources of fibrous microplastics are fishing nets, ropes, and certain polymeric paints and coatings. Microfibers are easily shed from clothes and household textiles with loose structures, such as fleece sweaters, during daily use [44]. Boucher and Friot [3] estimated that approximately 35% of microplastics in the world's oceans arise from laundry of synthetic textiles. Some estimates are lower, but even a value of 20% for 2014 as reported by Eunomia [45] means that the equivalent to 0.19 million tons of textile microfibers from the production and normal use of synthetic textiles, particularly household washing of clothing, enters the marine environment alone annually (see Henry et al. [43]). Browne et al. [46] found that a single piece of clothing can produce > 1900 synthetic microfibers per wash, while in a recent study by Napper and Thompson [44], it was estimated that the release of synthetic fibers from polyester, polyester-cotton blend and acrylic fabrics into waste water during each use of a washing machine could be as high as 700,000 microscopic fibers. Microplastics can be found in wastewater where approximately 35% of microplastics are thought to be fibers from synthetic clothes [47]. Although treated effluents only contain few microplastics per liter, the high volume of effluents constantly released constitutes a considerable source of microplastic contamination of aquatic systems. However, it is likely that globally over 80% of wastewater is released to the environment without treatment, resulting in an even much greater emission of synthetic fibers [48].

9.2.3 Pathways and sinks

Relevant pathways of MNPs into the aquatic environment are through littering of larger plastic items, road and land runoff, storm water, flooding, soil amendments and irrigation, wind transfer, and atmospheric outfall [3, 26, 49, 50]. Sewage Treatment Plants (STPs) are considered important pathways of MNPs to surface waters [4], especially in parts of the world where sewage water is treated less rigorously. Plastic particles and fibers contained in personal care products (PCPs) and those originating from laundry, tyre wear, etc., will at least partly end up in the STP effluents [6, 35, 51], and thus get released into receiving surface waters. Atmospheric deposition of suspended atmospheric microplastics, especially textile fibers, has been recently recognized as an important input pathway [16]. Marine systems, especially sediments, still appear to be the ultimate sink for microplastics in the environment, but freshwater systems such as estuaries and lakes are also important recipients and reservoirs of microplastics pollution (e.g., [6, 52, 53]. Relatively high levels of MPs can be found in oceanic gyres and enclosed sea water regions, remote mountain lakes, industrial effluents, sites near factories, estuaries, seabed and urban sediments, filter feeding biota, and household dust [24, 52–60]. Generally, aquatic biota, such as filter and deposition feeders may represent relevant reservoirs, as they will temporarily retain plastic particles from other environmental compartments [61].

From the information above, it can be concluded that sources of aquatic MNPs are diverse. A major source of plastic fibers originates from wear, tear, and maintenance of textile and apparel. In addition to direct emissions into surface waters through effluents and raw untreated wastewater, atmospheric transport and deposition is likely to play a role in the distribution of MNPs, notably fibers.

9.3 Composition of aquatic micro- and nanoplastic debris

MNPs in the aquatic environment are highly complex cocktails of contaminants, including polymeric materials, chemical additives, residual monomers, ambient chemical substances that sorb to plastic, and proteins (ecocorona) [62]. Furthermore, the plastic debris serves as a novel substrate for microbial community structures (biofilms) on

their surfaces. The combinations of polymeric materials and associated chemical and biological contaminants are countless making each plastic particle or fiber having its own unique properties [63]. The polymeric, chemical, and biological components of plastic debris, in particular MNPs, are further described below.

9.3.1 Debris polymers

The distribution and abundance of MNPs is inseparably linked to macroplastic debris, largely via the degradation of bulk plastic into smaller particles down to the nanoscale [64]. Aquatic debris polymers, which are most commonly found, are mostly high production volume polymers (or mixtures thereof), such as polyethylene (as both high-density, HDPE, and low density, LDPE), polypropylene (PP), polystyrene (PS), polyvinylchloride (PVC), polyamide (nylon) (PA), polyurethane (PUR), polyethylene terephthalate (PET), polyester resin and glass fiber, and cellulose acetate (CA). Most debris-prone polymers contain a variety of chemical additives (chemicals which are used to polymerize, process or to modify end use properties of plastics), unpolymerized monomer residues and impurities, depending on polymer surface structure and affinity [23, 65, 66]. Less commonly reported debris polymers include polyvinyl alcohol (PVA), acrylic (AC), poly-methylmethacrylate (PMMA), alkyd (AKD), polyurethane (PU), styrene butadiene rubber (SBR) [41, 67, 68]. Polyamide 66 (nylon66), PVA, polyester, and PP are produced as synthetic monofilaments and used in fibrous materials in textiles and apparel [43], and for fishing lines and fishing nets [23]. Considering that overall 8000 different plastic grades are currently in the market, it is likely that the total number of debris polymers might add up to several hundreds (https://www.campusplastics.com).

9.3.2 Chemical additives

Besides synthetic polymers or mixtures thereof, a wide range of chemicals are present in plastic as a result of manufacturing specifications. Chemical additives in polymeric and plastic packaging materials include monomers (e.g., styrene), intermediates, solvents, surfactants, plasticizers (bisphenol-A and phthalates), stabilizers, biocides, flame retardants, accelerators, and colorants [69]. Also, the polymerization process may leave trace quantities of residual monomer or low-molecular-mass polymers in the plastic, especially in primary MNPs. Furthermore, upon aging/weathering, polymer degradation products are likely to be formed, for example, as result of UV-exposure. Many of these substances are not exclusively used by the plastic industry and have applications elsewhere as well, for example in textiles and food technology industry [70]. A considerable number of plastic additives have been listed as chemicals of very high concern because of their endocrine disrupting or carcinogenic properties and other toxicological hazards [71, 72]. Once in the water, hazardous additives might rapidly leach out of the plastics to the surroundings and into the food chain, as it degrades. As such, debris plastics, especially macro-sized objects, significantly contribute to chemical contamination of aquatic systems [73–75].

9.3.3 Adsorption of chemical contaminants

MNPs have a hydrophobic nature (i.e., low polarity) with relatively large ratio of surface to volume with a significant ability to highly accumulate a wide range of chemicals [76] either directly from the environment or as a result of biofouling. Under laboratory conditions, PE, PVC, PP, and PS display high sorption capacity for polycyclic aromatic hydrocarbons (PAHs), dichlorodiphenyltrichloroethane (DDT), hexachlorocyclohexanes, chlorinated benzenes, musks, pharmaceuticals, and personal care products [77–82]. Many of the contaminants adsorbed from the surrounding water have toxic, endocrine disrupting and/or immunomodulating properties [23, 83]. Examples are metals [84] and hydrophobic contaminants such as PCBs, PAHs and PBDEs [23, 85] and emerging contaminants such as PFAS [86] and pharmaceuticals [87]. Compared to microplastics, nanoplastics exhibit a large surface area with increased sorption capacity and are able to bind increased levels of organic contaminants [88] and metals [89]. The contaminants present in MNPs may migrate from the particles into the organism by equilibrium partitioning [88] and can cause chemical toxicity [71, 76]. Contaminant transfer is bi-directional and can either increase or decrease contaminant body burden

depending on polymer type, environmental conditions, and chemical fugacity/transport gradients [88, 90]. The above studies show that MNPs could be significant vectors of both organic and inorganic substances, potentially affecting bioavailability and chemical toxicity.

9.3.4 Eco-corona, biofilm and biofouling

In addition to polymers and chemical additives and adsorbed chemicals, MNPs can bind organic matter, nutrients, and also macromolecules (e.g., lipids, proteins, polysaccharides) and may serve as substrates for microbial biofilms, forming a so called eco-corona around the MNPs [62]. Subsequently, MNPs can be colonized by a distinct fouling community of aquatic microorganisms and small invertebrates, such as diatoms, bacteria, cyanobacteria, fungi, unicellular algae, ciliates, dinoflagellates, bryozoans, crustaceans, worms, and marine insect eggs [91–93].

Attracted by nutrients and depending on the polymer type, MNPs develop unique microbial community structures compared to their surrounding environment [93]. Main bacterial colonies found on MPs appear to be established by their adherence properties, such as hydrophobicity and surface roughness, rather than by the type of polymer itself [94], although species able to degrade the specific polymeric material or chemical additives usually also occur in the biofilm communities [95–97]. Thus, additional to weathering processes, some bacteria may also act as plastic degraders and accelerate MP degradation processes [98], although biodegradation in aquatic habitats is recognized to be low [97]. On the other hand, biofilm micro-organisms may produce exudates acting as infochemicals of food occurrence, potentially increasing the ingestion rate of MNPs [62].

An increasing number of studies on (micro)plastic-microbiological research indicate that aquatic (micro)plastics represent a distinct environmental niche that can potentially harbor and disperse pathogenic bacteria [92, 99–103], antibiotic resistant micro-organisms [104, 105], toxic dinoflagellates [106], toxic cyanobacteria [97], thus posing novel health risks to fish and other aquatic organisms and potentially humans as well [92, 99, 100, 103–105]. The long decomposition time coupled with lower density of MNPs favor their dispersal in aquatic systems compared to non-plastic particles [107].

It is becoming clear that eco-corona and biofilm formation and subsequent biofouling and plastic biodegradation may change the identity and density of MNPs, and could influence their behavior, fate, bioavailability, and effects [62, 108–112]. Overall, these interactions between MNPs and microbial ecology are far from being understood and require further research.

9.4 Factors that control degradation and fate of polymeric material

Physical and chemical processes, such as exposure to UV light (photodegradation), oxidation, leaching of additives, biofilm growth, and mechanical abrasion (with rocky shores and sandy beaches), initiate plastic degradation and fragmentation, consequently releasing numerous plastic particles from the surface of larger items or fragments [66, 113, 114]. The fragmentation process primarily depends on the type of plastic, the concentrations of antioxidants and stabilizers used as additives [115] and the physical-chemical environmental conditions including contact by biota [116]. Eco-corona and biofilm formation on plastic particles surfaces could prevent the plastic from photo-catalysis, either directly or via decreased buoyancy, thereby promoting microplastic sedimentation. Over time, the polymeric material itself degrades very slowly and consequently, MNPs will persist for decades and even centuries [113, 115, 117]. Decreasing particle size as a consequence of fragmentation will further result in a higher particle abundance and increase encounter probability with biota [23]. Further, nanoplastics can be generated from microplastics through digestive fragmentation by organisms such as Antarctic krill [118]. These effects have potential consequences as the number of particles that are sufficiently small for translocation into the body, but it introduces a new role for biota in the fate of plastic and nutrient cycling and biogeochemical processes. In fact, the likelihood that marine and freshwater biota has not been exposed to MNPs from any source is very low.

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The fate of microplastics in the aquatic environment primarily depends on polymer density, particle size and shape which influences buoyancy, and residence time (biofilm development and the potential interaction with detritus, living organisms, and inorganic matter) [18, 119]. Further, the fate of MNPs in the water column is highly affected by the aggregation of nano and microplastic with suspended solids [120]. The densities of the most encountered plastic materials, such as polyethylene, polypropylene, nylon, and polyester typically range between 0.9 and 1.4 gcm^{-3} . Polymers less dense than (sea)water (e.g., PE and PP) will tend to float on or within the water surface layers, while those with higher density (e.g., PVC, polyester, acrylics, and polyamide) will sink. Settling/resuspension behavior of MPs is highly dependent on the particle shape (i.e., flat fragments may stay motionless and flexible threads and fibers can be easily captured by the flow) [121]. The transport and distribution of fibrous MNPs are intrinsically differently due to the low sinking rate, delayed settling and low re-suspension threshold compared to non-fibrous plastic particles [122]. Biofilm formation and the settlement of organisms on the plastic surface increase the weight of particles, thus increase their sinking velocity [123–125], with small-sized particles losing buoyancy much faster than larger ones, depending on the shape and especially specific density [126]. Other studies have shown that MPs flocculate and adhere to sticky exo-polymers [119] or be incorporated into faecal pellets [127], influencing their buoyancy and position in the water column. On the other hand, evidence indicates that aggregates in marine waters are involved in removing MPs from the ocean surface and facilitating their transfer to marine food webs [128].

9.5 Physical and chemical quantification and characterization of MNPs

9.5.1 Analysis of microplastics in aquatic matrices

There is currently no ideal analytic method to quantify and chemically characterize plastic particles in aquatic samples (< 5 mm). The major issues are the complexity of environmental samples – to adequately remove the organic fraction (e.g., biological tissues) – and the lower detection limit for particle size. Often one or more extraction and cleaning steps are needed to isolate the microplastics from the surrounding matrix prior to analysis. These extractions and cleaning techniques include visual sorting, density gradient-based separation, filtration, acid and alkaline cleaning, and enzymatic destruction [14, 129–131].

Microplastic measurement results rely strongly on good sampling and sample purification. Therefore, the combination of a separating and qualifying method has been widely used [132]. Visual sorting can lead to large error values (e.g., [67, 133, 134]) in determining the concentration of MPs. Other techniques reportedly generate much higher recovery and identification rates for MPs, although these rates generally decrease as the particle size decreases [135].

Analysis techniques commonly used for the identification of (micro)plastics include Fourier transform infrared microscopy (FTIR), Raman spectroscopy, scanning electron microscopy with energy dispersive x-ray spectroscopy (SEM-EDS), and pyrolysis-based gas chromatography mass spectrometry (GC-MS) [129, 130, 132, 133]. The spectroscopic FTIR and Raman techniques are currently best suited for the detection of plastic particles larger than about 10 and 1 µm respectively, depending on the equipment used [67]. Stimulated Raman Scattering (SRS) microscopy is a powerful spectroscopic imaging technique based on the synchronized action of two pulsed laser beams with a specific photon energy difference. Compared to conventional Raman spectroscopy, it allows a thousand-fold faster mapping of, for instance, particles on a filter surface. A multispectral SRS-approach of this method was recently developed for MPs in the low-micrometer size range from harbour sediment, although this technique needs improvement to be used for small sized particles in a high throughput mode [136].

The advantages and limitations of the various methods are discussed in detail elsewhere [14, 132, 137]. A major limitation in the analysis of MPs in complex environmental (organic rich and weathered plastics) matrices is the lack of standard operating procedures for detecting and quantifying microplastics. Clearly, there is a need for harmonized protocols for sampling, analysis, and identification of microplastic particles and fibers. Further research is required to determine whether any of the currently available analytical techniques can be developed to the extent where they can be routinely used for MNP exposure assessment the entire range of sizes. Most suitable methods for analyzing

microplastics reported in aquatic matrices have been reviewed and recommended by GESAMP [14].

Field studies on microplastic abundance reported in the literature contain mostly particle counts and less often quantitative data on polymer types. Environmental concentrations are usually expressed in particle number per unit (volume, surface area, mass) and rarely in mass per unit. The physico-chemical heterogeneity (i.e., in polymer type, size, shape) of MPs found in field samples implies that the conversion between particle and mass-based concentrations is fraught with imprecision. On the other hand, mass-based concentrations are commonly reported in laboratory studies using test MNPs, exhibiting more homogenous characteristics. It would be preferable, when possible, to record both number and mass (e.g., using pyrolysis GC-MS) to facilitate comparisons of quantities between micro-sized (high mass) and nano-sized (high numbers) debris. From a toxicological perspective, particle numerical concentrations are preferred, because these represent the number of MNPs biota are exposed to. For further discussion, see Skåre et al. [31].

9.5.2 Analysis of microplastic fibers and nanoplastics

Fibers may even be more difficult to count and qualify than non-fibrous particles and some recent reviews have questioned the effectiveness of microfiber identification [138–140]. There are several issues with the accurate identification of synthetic microfibers. Natural (e.g., cotton, flax, wool, linen, silk, and hair) and artificial (regenerated) cellulosic microfibers (e.g., viscose (or rayon), bamboo and acetate fibers) have similar morphological properties, but are not plastic [141]. For example, the difficulty of separating natural and synthetic microfibers following visual inspections may result in misidentification of synthetic microfibers (e.g., viscose) [138]. Spectroscopic analysis is essential to ascertain the plastic nature and differentiate the different types of fibers. On the other hand, fibers are often too thin and their abundance in water samples taken with bigger net mesh sizes is therefore often likely to be underestimated [41]. A simple and effective step before spectroscopic analysis seems to be the use of Nile Red (9-diethylamino-5H-benzo[a]phenoxazine-5-one), offering the possibility of selective identification of plastics, based on surface polarity characteristics of plastic particles, including small MPs (< 100 μ m) [142, 143]. For recommendations on how to accurately differentiate types of fibers, the reader is referred to the work of Woodall et al. [144] and Comnea-Stancu et al. [138].

Due to methodological challenges, the smaller sized MNPs, such as sub-micron or nano-sized plastics, are yet to be detected in environmental field samples or in tissues of field-collected organisms [14, 145–147]. Advanced separation techniques, such as asymmetric flow-field-flow fractionation (AF4), might also be applicable to nano-sized plastic particles and fibers. Similarly, electron microscopy-based analytical techniques might also be helpful in visualizing nano-sized particles and fibers, although this technique probably would have to be combined with other analytical techniques, such as pyrolysis GC-MS, for sub-micron-sized particle identification. Possible routes for developing adequate sampling preparation and analytical techniques for the detection of the yet unanalyzed MNPs could come from the field of engineered nanoparticles and fiber toxicology.

9.5.3 Uncertainties in aquatic MNP measurements

The reliability of some early microplastic results is questioned [134]. This is because cross-contamination is likely to occur at each stage of sampling, handling, and analysing microplastics samples, as demonstrated by various studies (e.g., [134, 144]). In most instances, investigations pinpoint that microplastics were most likely acquired from worker clothing, unwashed lab equipment, and general contamination from airborne microplastics in the laboratory. Recommendations on avoiding contamination and applying quality criteria for the analysis of microplastics in biota, including the use of procedural blank analysis and clean air devices, have been described by [134].

9.6 Occurrence of microplastics in aquatic systems

9.6.1 Microplastics in abiotic matrices

Concentrations reported in water vary from a few particles up to thousands of particles m^{-3} [26, 148]. Microplastic concentrations in suspended matter and sediment can reach several thousand particles kg⁻¹ of dry sediment [148–151]. In a study in Dutch coastal and inland waters, microplastic analysis (particle sizes between 10–5000 µm) in major rivers Rhine and Meuse and urban water in and around Amsterdam in 2014, showed concentrations between 100 and 3600 particles kg⁻¹ of dry sediment along the Dutch North Sea coast and between 68 and 10,500 MPs kg⁻¹ in urban canal sediments. Amsterdam canal water samples contained microplastic concentrations (48–187 MPs1⁻¹) similar to those observed in treated wastewater effluent in the area. These data show that MPs do settle in urban waters, meaning that at least a part of the MPs is retained in freshwater systems. On the other hand, microplastic concentrations in riverine suspended particulate matter appearing to be high (1400–4900 MPs kg⁻¹ dry weight (dw)) [6], substantiating previous studies that rivers are a key factor for microplastics to move towards the ocean [26, 152].

In aquatic samples, PE, PP and PS are the most frequently found polymers [24], but also a range of other polymer types have been occasionally identified, for example PET, nylon, polyether urethane, styrene acrylate, alkyd, styrene butadiene rubber, and polymethyl methacrylate [41, 136, 153]. Haave et al. [60] identified twenty different polymer types, at concentrations from 12,000 to 200,000 MPskg⁻¹ dw. Over 95% of the MPs were smaller than 100 μ m and commonly consisted of polyurethane acrylate resins, while the larger MPs consisted mostly of polyamide fibers.

Several studies indicate fibers to be a most common type [23]. However, this may be partly due to cross contamination of samples as discussed above. Burns et al. [27] reported that the most abundant shape type in water and sediment is fibers (48.5%), followed by fragments (31%), spherical beads (6.5%), films (5.5%), and foam (3.5%). However, till date, published microplastic data for aquatic matrices are only partly complete and as previously explained, limited to the upper end of the particle size spectrum. In addition to this, published field and monitoring studies often do not mention any specific aspect ratio to distinguish fibrous from non-fibrous particles (e.g., irregular shaped thin fragments or films). As microplastic measurements can be fraught with uncertainties, the representativeness and reliability of obtained data can be compromised. Moreover, existing microplastic data collected from aquatic systems may vary considerably largely because different methods and level of QA have been applied [67], hampering useful comparison. Generally, methods that can sample or detect smaller particles, such as mesh size effects on sample composition, will result in higher concentrations [65, 154]. These studies indicate the significance of the unanalyzed MNP fraction and the need for improved methods for MNP sampling and analysis. To illustrate, microplastic measurements in marine and coastal surface waters have often been limited to manta trawl or neuston samples using nets with > 0.3 mm mesh, underestimating the actual concentrations of microplastics that are likely to accumulate at the sea surface.

Concentrations of MPs in marine and coastal sediments that reach from zero to several thousands of particles kg⁻¹ (dw), show generally higher concentrations at more inland sites. For example, the average amount found across the SW North Sea and Channel region in 2014 ranged between 200 and approximately 700 particles kg⁻¹ (dw), with levels up to 3,146 particles kg⁻¹ in sediment in harbor areas (particle sizes between 10 and 5000 μ m). In North Sea sediments, mainly fibers and spheres were found, whereas at the sea surface, fragments were dominant. Several studies have shown that the concentrations of microplastics at the sea surface are lower and more variable than in sediments, indicating that most microplastics including fibers eventually do sink to the seabed supporting the hypothesis that the seabed may act as an important long-term sink [36, 142].

Gago et al. [155] reviewed the presence of microfibers in marine water and sediments and concluded that their occurrence is worldwide and represent a dominant fraction of the total microplastics, in some samples up to 100%. Most common colors were blue (seawater and sediments), transparent and black (seawater), and black (sediments); most common polymers were Polypropylene (water and sediments), Polyethylene (water), Polyester (water and sediments), HD/LD polyethylene and cellophane (only in waters), and rayon (sediments). Concentrations ranged from

0 to 459681 items km⁻² in sea water and from 12 to 160 items kg⁻¹ (dw) in sediments, with blue and polypropylene microfibers as the most abundant microfiber type.

Several studies indicate that microfibers have reached the deep waters of the Mediterranean Sea (3500-meter-deep) and Atlantic (up to 4844-meter-deep) [156], and Indian and West Pacific Ocean (5768 meters deep) [157], where they can be found in similar concentrations as in coastal sediments [158], most likely via the supply of organic detritus "marine snow" containing these particles [55]. Long range transport of synthetic fibers from southern European coastal waters to open deep sea was investigated by Sanchez-Vidal et al. [33]. The measured abundances and composition of microfibers in sediments suggested a downslope transport from coastal seas via submarine canyons to deep open sea floor. They found abundances of 10–70 microfibers in 50 ml of sediment, including both natural and regenerated cellulose, and synthetic fibers; the plastic microfibers consisted of polyester, acrylic, polyamide, polyethylene, and polypropylene.

It can be concluded that microplastics, notably fibers, and also fragments, films and pellets, or granules, are found widespread in all abiotic compartments (surface water, water column, suspended matter, sediments) of marine and freshwater systems. Microplastic concentrations reported for fresh water systems and estuaries are mostly largely similar to those reported for marine and coastal environments, depending on the location, while in some deep-sea sediments microplastics have already reached concentrations similar to those reported for intertidal and shallow sub-tidal sediments [55, 57].

9.6.2 Microplastics in aquatic biota

The occurrence of MNPs in marine biota and commercial marine species have been well documented. Numerous studies show that MPs are ingested by an increasing number of marine species, including invertebrates (protists, small and large crustaceans, mollusks, annelids, echinoderms, cnidarian, and tunicates), pelagic and demersal fish [159], turtles [160] and large top predating marine fish, mammals [161], and penguins [162]. For more comprehensive reviews, the reader is referred to Kershaw et al., Wesch et al., Lusher et al., O'Connor et al. [23, 140, 163, 164].

Taylor et al. [158] provided convincing evidence that microplastics are present within deep-water organisms, and thus have become infiltrated into deep ocean ecosystems. Screening for microplastics and applying major quality control measures, they found 15 rather different plastic fibers ingested or internalized by diverse organisms with different feeding mechanisms. The fibers were made of modified acrylic, polypropylene, viscose, polyester, and acrylic. An increasing number of studies also reported MP consumption by freshwater species such as duck mussel [165], tadpoles [166], wild gudgeons [167], fish eating birds such and cormorants [168]. Overall, reported microplastic data indicate that almost all aquatic species and phyla analyzed to date show plastic particles in their digestive system or tissues. Further, it clearly shows that MNPs have infiltrated aquatic food chains through various pathways, ingestion, and trophic transfer [169].

The concentrations of microplastics in biota generally depend on the habitat and feeding modes [140, 163], but one study on fresh water invertebrates found MPs ubiquitous across different taxa and sites independent of feeding guild and biological traits [170]. Only few studies did not encounter any microplastics in a particular fish or invertebrate species [41, 158, 171].

| Table 9.1.: Percentage of fibro | ous and other and ne | ot specified | shapes in | measurements | of marine | shellfish | and fish |
|---------------------------------|-----------------------|---------------|-----------|--------------|-----------|-----------|----------|
| (based on data from | n Barboza et al. [172 | 2], Table 2). | | | | | |

| Marine food | No of species (No of measurements) | % Fiber/ filament | Other shapes | % Not specified |
|-------------------|---------------------------------------|-------------------|--------------|-----------------|
| Shellfish (whole) | 23 (25) | 87.0 | 4.3 | 8.7 |
| Fish (stomach) | 69 (90) | 78.8 | 19.1 | 2.1 |

Analysis of large number of marine shellfish (whole organisms) and fish species (stomach content) showed the contribution of ingested microplastic fibers to total plastic to be 87% and 78.8% respectively (Table 9.1). A similar conclusion is reached for MP burden in freshwater biota where available field studies report microplastic fibers ranging from 46.6% to 100% (for references, see O'Connor et al. [164].

Most species analyzed for microplastics contained plastic particles or fibers in their stomach content or tissues. For example, field analysis revealed that 8 out of 9 tested invertebrate species from five different phyla from the North Sea and 68% of analyzed individuals of brown trout (Salmo trutta) from the Swedish West Coast had microplastics in them (particle sizes between $10-5000 \,\mu\text{m}$). Despite the poor analytical recovery of the used method, several trends emerged from these data. Among invertebrate species, highest concentrations were found in suspension and filter feeders, such as brittle star, sponges, and mollusks. Grazers analyzed for microplastic content, such as isopods, Patella vulgate, and Littorina littorea, and also secondary consumers (Hemigrapsus sanguineus), showed consistently lower concentrations, and in one species (Patella sp.), no microplastics were found. These findings compare well with those reported in other studies. Biota subsamples analyzed with FTIR confirmed various polymer types including PS, Alkyd resin, poly (methyl methacrylate) [41]. Other studies reported variable MP contents in various fish species, such as 36.5% of specimens of pelagic and demersal fish species in the English Channel [173]. Analyzing sprat from the Belgium coast, it was found that an average of 39% of individuals had ingested microplastic (78% synthetic fibers) (Figure 9.1). Individual whole shrimps from the North Sea contained 63% ingested or internalized microplastics (96.5% synthetic fibers) (Figure 9.1). In shrimps, blue fibers were found that could be related to shrimp trawl nets These values are difficult to compare as they might be biased and use different identification and qualification methods. However, they support claims that in marine biota, fibers are an important and not rarely most common contaminant.



Shrimp (whole organism)
63% of individuals ingested micro-sized plastic (10-1000μm)
96.5% synthetic fibers; 3.5% films and granules
Average: 1.25 MPs / individual

Sprat (stomach content) 39% of individuals ingested plastic (200-1000μm) 78% synthetic fibers; 13 % films; 9% granules Average: 0.5 MPs / individual

Figure 9.1.: Microplastic (MP) contamination in shrimp (*Crangon crangon*) and sprat (*Sprattus sprattus*) from the North Sea. Based on data from Devriese et al., Zoeter Vanpoucke [174, 175]. Photos ©ILVO.

Microplastics were analyzed in the freshwater duck mussel *Anodonta anatina* from a Swedish river by Berglund et al. [165]. All analyzed mussels contained both fibrous and nonfibrous microplastics. Higher abundances of microplastics were observed in larger mussels indicating a size-related uptake effect. The authors reported higher

concentrations of microplastics downstream urban areas with wastewater treatment plants and anthropogenic activities. Comparing the levels of MPs along a salinity gradient in Dutch waters, the MP concentrations from the North Sea coast to Lake IJssel was similar to the MP concentrations going inland wards. MP levels are approximately similar in freshwater mussels compared to marine mussels (Figure 9.2). In the Dutch study performed by [41], whole body concentrations reported in North Sea mussel ranged from 5–37 MPs per gram (dw) (particle sizes between 10–5000 µm), and 50% of the MPs were fibers. Based on the number of MPs per Kg (dw), the MP concentrations were approximately a factor 1000 higher than in surrounding surface water and sediment, with water, sediment and biota containing 51, 34 and 25% fibers respectively [41]. Several studies have indicated the spatial association in coastal and fresh waters and human activities [4, 5, 142, 148, 165, 176, 177]. A study on riverine macroinvertebrates (*Baetidae*, *Heptageniidae*, and *Hydropsychidae*) found that any increase in MP abundance in biota downstream of sewage treatment works, is possibly due to river flow dilution at the time of sampling [170].



Figure 9.2.: Microplastic particle concentrations in water (MPs1⁻¹) and mussel (MPskg⁻¹ dw) along a salinity gradient. Microplastics contained 25% synthetic fibers. (Based on data from Karlsson [41, 178].

The relatively high concentrations of MPs found in commercial marine species such as fish and shellfish have raised important questions about potential risks in higher trophic level species and human food safety [28, 172, 179–182]. Commercial mussels and oysters especially appear to contain high levels of microplastics, and estimates indicate that, for example, European consumers of shellfish ingest 1800 - 11,000 microplastic particles (size range 5–1000 μ m) per year [183]. Therefore, oral exposure to MNPs is believed to occur through the consumption of sea products, but may also occur through the consumption of livestock fed fishmeal [23], tap water [184], commercial salts across the world, and in several terrestrial foodstuffs such as honey, beer, and sugar (reviewed by Barboza et al., Yang et al., Joon [172, 185, 186] as well as inhalation of air-born MNPs [187]. It was recently demonstrated that the risk of plastic ingestion

via mussel consumption is minimal compared to the fiber exposure during a meal via indoor dust fallout [188]. On basis of currently available data, the risk of plastic ingestion via seafood consumption is relatively small, and at these levels, it is unlikely that MP exposure will represent a significant risk to human health.

To conclude, the current data clearly show that MPs are widespread among aquatic populations and have penetrated aquatic food chains and potentially, humans. Overall, fibers are the most prevalent microplastic type found in aquatic biota.

9.7 Uptake and effects of MNPs on aquatic biota

9.7.1 Uptake

The ingestion of MNPs by aquatic biota can occur either by directly ingesting the particles from the water or from the sediment or indirectly through trophic transfer. Not readily visible plastic particles or fibers are of special concern, because they can be ingested by biota more readily than larger particles. Organisms can either preferentially feed on MPs or can accidentally ingest them. Predators, detritivores, and scavengers may indirectly ingest plastic while consuming prey or scavenging detrital matter (e.g., marine snows) containing microplastic [128, 163]. Microplastics occupy the same size fraction as some planktonic organisms and may be ingested by a wide range of organisms as outlined above. There is evidence for both size-selective [189, 190] and non-selective ingestion [191], as well as avoidance of microplastics by different species [192]. Plastic particles may also adhere on gills, external appendages or stick to the outside of the body, as on algae and deep-sea coral.

Numerous laboratory studies show that particle size is key, but particle numbers, particle shape, charge, hydrophobicity, composition, and other factors such as aggregation of particles and presence of eco-corona may play an important role in causing effects [193, 194]. Once ingested and/or internalized, plastic particles and fibers, especially the nano-size fractions, can cross cell membranes and enter the circulation where they may reach tissues and cells and induce harm, i.e., particle toxicity, including cytotoxic, oxidative stress, DNA damage, and inflammatory responses. Major sites of entry upon ingestion of MNPs include the Peyer's patches in the intestine [195]. Some studies focused on the potential mechanism of uptake of MNPs. Phagocytosis or endocytosis is possible, whereas the paracellular transport can be excluded given the size of MNPs. Based on what we know from mammalian and aquatic model studies, it can be expected that micro-sized plastic particles, owning to their size, will largely remain in the intestinal tract and excreted through faeces, while smaller microplastics ($< 20 \,\mu m$) and submicro-sized particles can be absorbed by the gut and retain in circularly fluids; the very small particles (e.g., < 100 nm are readily absorbed by the gut and are capable to penetrate cells [195–197]). Once within the organism, MNPs interact with serum soluble components, forming a "protein corona", which may confer a biological identity to MNPs and affects their interactions with target cells [198, 199]. There is only limited evidence that MNPs can transfer from the gut into the circulation [200–202], cells [201], organs [203], and brain [203, 204]. Unfortunately, the size-fraction of MNPs capable of being internalized within fluids and tissues of aquatic organisms largely corresponds with the unanalysed fraction of these particles in field studies, thus seriously hampering risk assessment. Generally, smallest diameter particles have prolonged retention times and higher bioavailability [205]. Smaller MNPs, including nano-sized particles, which have the capability to be internalized by organisms, will have a higher probability of ingesting and biomagnifying up the food chain [169]. It is possible that larger fibrous MPs that have a higher risk of being entangled in the gut and intestines may result in a higher probability for trophic transfer than non-fibrous particles of similar size, but this requires further research. Ultimately, this implies that each plastic particle type exhibits different gut uptake rates and pathways.

Several studies provided insights into the biokinetics of MNPs. A study by Sendra and co-workers with Mediterranean mussels (*M. galloprovincialis*) showed size dependent accumulation of PS NPs (50 nm, 100 nm and 1 μ m) in tissues and provided evidence for the translocation of these particles into the hemolymph [202]. The smallest PS NPs tested were detected in the digestive gland and muscle. A fast and size-dependent internalization of particles to the hemolymph was recorded after 3 h of exposure, and this process seemed to be controlled by different endocytic pathways (i.e., caveolae and clathrin mediated endocytosis and phagocytosis). Al-Sid-Cheikh et al. [206] provided evidence that particle size influences the biokinetics of nanoplastics in a mollusk species. In their experiment the uptake, whole body distribution, and depuration were studied in *Pecten maximus* at environmentally realistic concentrations (< 15 μ g MPs1⁻¹) using pulse exposures of ¹⁴C-radiolabeled nanopolystyrene (24 and 250 nm. The results suggest relatively rapid uptake of both particle sizes after 6 h, with 250 nm particles present in intestine and 24 nm particles dispersing throughout the whole body, indicating possible translocation across epithelial membranes. Model extrapolations indicated that it could take 300 days to reach equilibrium in scallop body tissues, although the MP concentrations would still be below 2.7 mg g^{-1} . A comparison with a previous work, in which scallops were exposed to nonplastic (silver) nanomaterials of similar size (20 nm, suggests that nanoparticle composition may also influence the uptake. There are some further examples in the literature on how particle surface properties may influence uptake and associated effects. Proteins secreted by *Daphnia magna* around polystyrene nanoplastics with charged and modified surface created an eco-corona which increased the NP uptake, ecotoxicity, and caused a longer gut retention time with effects on feeding rate [111]. However, the environmental relevance of these surface modifications is questionable, since such modifications are not expected to be part of the natural weathering process of MNPs. Fotopoulou et al. [207] observed significant alteration of functional groups on the surface of eroded PE pellets, whereby the altered surface acquired a negative charge due to ketone groups. Particles of weathered PP, on the other hand, were reported by these authors to remain neutral. Severe toxicity was observed in sea urchin embryos (Paracentrotus lividus) after incubation with amine (PS-NH₂) polystyrene, and in a lesser extent with carboxylated (PS-COOH) polystyrene. Both nanoparticle types, notably PS-COOH, aggregated partially with time and only PS-COOH accumulated inside embryo's digestive tract [208]. However, when these NPs encountered biological fluids (i.e., coelomic), they became covered by a protein-corona, which conferred identical colloidal features to PS-NH₂ and PS-COOH particles, both acquiring a monodisperse size distribution and a negative potential in the coelomic fluid [209]. It has been also evidenced that NPs translocated to mussel hemolymph after ingestion may specifically bind to plasma proteins, forming a protein corona that can affect particle uptake and toxicity in target cells [198] (see above).

Overall, the extent to which MNP particle translocation occurs, the uptake mechanism, tissue distribution, and potential accumulation of particles are still poorly understood and represents a major obstacle in assessing their risk in aquatic organisms. The associated effects following intake of MNPs will be discussed further below.

9.7.2 Physical effects

Physical effects of MNPs

Although a few laboratory studies report little or no physical harm to aquatic biota [210–213], by far, most laboratory studies reveal adverse effects on marine and freshwater organisms at various levels of biological organization, from changes in gene expression, inflammation, and energy allocation to effects on reproduction, immune system, and central nerve system to death [23, 214, 215] (Table 9.2). In several of these cases, it is not clear if the effect is particle-related or caused by associated toxic co-contaminants or impurities. An example of a typical physical effect is perhaps the study of [18] exposing the polychaete worm, *Arenicola marina* (a deposit feeder) to unplasticised polyvinylchloride (UPVC) hold in sediments spiked at concentrations coinciding with high levels found in the environment. The worms showed reduced feeding activity, increased gut residence time of ingested particles, inflammation and significantly depleted energy stores by up to 50% compared to control. Such energy-reduced effects, in the above case mainly linked to a reduction in lipid reserves, can have an impact on growth, reproduction, and ultimately, survival. Other studies have shown similar effects on energy budget, but negative effects have been reported for micro- and nano-sized plastic particle interactions with marine invertebrates by several research groups, indicating that in marine invertebrates the immune function represents a potential target for plastic (polystyrene) nano-plastics [202, 216].

| Species | Observed effect | Reference |
|---------------------|---|--------------------|
| MARINE BIOTA | | |
| Marine bacteria | Effects on ecological function, oxidative stress, grow inhibition | [217] |
| Microalgae | Reduced chlorophyll content | [210] |
| | Lower feeding rates; negative effects on growth and photosynthesis | [218] |
| | Reduced growth | [219] |
| | Inhibition of photosynthesis and promotion of ROS | [220] |
| Ascidians | Slowed down metamorphosis, growth inhibition | [221] |
| Sea urchin | Developmental defects | [208] |
| Copepod | Reductions in fecundity | [222] |
| | Increased immune response, lower feeding rates | [223] |
| Blue Mussel | Oxidative stress, reduced lysosomal membrane stability, cell damage, inflam- mation, granuloma formation | [201] |
| | Damaged ecophysiological functions | [224] |
| Mediterranean mus- | Alterations of immunological responses, lysosomal compartment, peroxiso- | [225] |
| sel | mal proliferation, antioxidant system perturbations and genotoxicity; altered gene expression | |
| Brown mussel | Impaired larval development | [226] |
| Asian green mussel | Reduced filtration behavior, respiration rate and byssus production | [227] |
| Pacific Oyster | Immune system modulation and alteration of homeostasis | [228] |
| · | Decrease in fertilization and embryogenesis success | [229] |
| Pearl oyster | Impact on assimilation efficiency and energy balance, with negative repercus- sions on reproduction | [230] |
| Lugworm | Effects on fitness and bioaccumulation, reduced feeding capacity | [210] |
| 6 | Reduced feeding, weight loss and oxidative stress | [224] |
| | Reduced growth, inflammation | [18] |
| Oyster, sea urchin, | Decreased fecundity; negative impacts on subsequent generations | [223, 231–233] |
| copepod | II. | |
| FRESHWATER BIOTA | | |
| Microalgae | Growth inhibition (enhanced by enhanced growth), oxidative stress | [234] |
| | Growth inhibition | [219] |
| | Inhibited algal photosynthesis and growth; promotion of ROS indicative of oxidative stress | [220] |
| Polyp | Significant changes in morphology | [235] |
| Copepod | Effects on fecundity, survival and feeding | [236] |
| Nematod | Significant reduction in survival, decreased body length and reproduction impairment | [237] |
| Water flea | Decreased survival | [238] |
| | Lower feeding and reproduction at high microplastic levels | [239] |
| | Inhibited reproduction and induced abnormal embryonic development | [240] |
| Perch | Inhibition of hatching, decreased growth rates, and altered feeding preferences and innate behaviors | [241] |
| Crucian carp | Effects on behavioral and fat metabolism | [108] |
| * | Brain damage, behavioral disorders | [204] |
| Common goby | Decrease in predatory performance | [242] |
| | Cont | inued on next page |

| Table 9.2.: Selected examples of frequently reported effect | s observed in marine and freshwater biota after exposure to |
|---|---|
| MNPs in laboratory studies. | |

| Species | Observed effect | Reference | | | | | |
|-----------|---|-----------|--|--|--|--|--|
| Zebrafish | Increased activity of antioxidant enzymes inflammation and lipid accumula- tion in liver, altered metabolic profile in liver | [243] | | | | | |
| | Intestinal damage, including cracking of villi and splitting of enterocytes | [237] | | | | | |
| | Inhibited larvae locomotion | [244] | | | | | |

Table 9.2 – continued from previous page

More toxic effects of smaller microplastics below 10 µm were reported in aquatic organisms, compared to plastic particles of larger size. The majority of lab exposure studies have used nano-sized plastic particles that appear to be more readily absorbed and accumulated in tissues of aquatic species [204, 245, 246]. Several studies indicate that microplastic fragments or irregular fluffy microplastics were readily ingested by different species [201, 247, 248], and in some cases effects of these irregular shaped microplastics were observed, while by comparison spherical microplastics did not reveal any effect [248], indicating that particle shape, in addition to their separate influence on uptake, can influence the effects of MNPs. In box 9.1 and 9.2, we have summarized laboratory studies of MNPs on phytoplankton and marine mussel respectively, which elucidate insights into possible mechanisms underlying the observed microplastic effects.

Box 9.1: Effects on phytoplankton

The risk that MNPs may affect autotrophic phytoplankton that form the basis of the aquatic food chains and represent the major global source of oxygen to the atmosphere is of particular concern. Several studies have demonstrated negative effects on marine and freshwater phytoplankton species under laboratory conditions. Negatively charged nano-PS can sorb to microalgae, inhibiting microalgal photosynthesis and promoting ROS [220], Furthermore, nano-PS (0.22 and 103 mg l^{-1}) exposure to microalgae affects the growth and photosynthesis in terms of reduced chlorophyll content [210]. No effects of negatively charged and uncharged PS particles (0.05, 0.5 and $6 \mu m$) on microalgal photosynthesis were noticed. However, microalgae growth was negatively affected (up to 45%) by uncharged PS particles, but only at high concentrations (250 mg l^{-1}). These effects were demonstrated to increase with decreasing particle size [219]. In another study a negative effect of PVC particles on growth (up to 39.7%) was seen only at high concentration (50 mg l^{-1}). Toxic effects were likely to be caused by absorption and aggregation [218]. The above findings are not always consistent, for example, Besseling et al. [210] found effects on the growth and photosynthesis, while Sjollema et al. [219] and Lagarde et al. [249] observed no changes in algal growth rate but a significant reduction in photosynthesis. These differences may be due to different experimental test conditions. Lagarde et al. [249] presented the first evidence on microplastic-induced molecular toxicity in freshwater microalgae, Chlamydomonas reinhardtii. Exposure to high density polyethylene (HDPE) and polypropylene (PP) particles induced hetero aggregates consisting of microplastics, microalgae, and exopolysaccharides, eventually leading to vertical pathway transfer of microplastics from surface water to sediment. This was evidenced by elevated expression of genes (UGD and UGE) involved in rhamnose and xylose synthesis representing exopolysaccharide biosynthesis pathway elevated gen-expression [249].

Box 9.2: Effects on marine bivalve mollusks

The biology of filter feeding bivalves such as mussels and oysters makes them particularly suitable models for investigating the fate and effects of MNPs in organisms. Bivalves have a highly developed phagocytosis process for the cellular internalization of particles from 0.1 to 100 μ m to intracellular digestion and cellular immunity [250], and they can select their food particles using various criteria, including size [18, 251]. Internalization of

MPs in bivalves can occur by means of two routes: i) MPs can be trapped by gills through microvilli activity and endocytosis processes, and; ii) they can be taken up via ciliary movement in the digestive system and subsequent translocation to the hemolymph (reviewed by Franzellitti et al. [29]. Bivalve pallial cavity is of vital importance as a portal and barrier to invade microorganism and microparticles, and the mucus layer secreted by pallial organs (gills, mantle, etc.) generally allows to effectively capture particles as small as 3–4 µm and smaller [18]. Therefore, bivalves are effective concentrators of MNPs from the surrounding environment. The ingestion, bioaccumulation, and translocation of plastic microparticles by bivalves, with particular efference to mussels, has been demonstrated in numerous studies [252-255]. Physical blockages and biological changes have been described in Ostrea edulis by ingestion of MPs [256]. Polystyrene microspheres filtered by Mytilus edulis were found to be accumulated in gut and digestive tubules and subsequently translocated into hemolymph and hemocytes [200]. A significant reduction in the filtration rate was observed in mussels exposed to microplastic fibers (459 ± 2.25 (SE) µm), although more than 70% of fiber uptake by mussels were also found to be quickly rejected as pseudofeces, with approximately 9% ingested and < 1% excreted in feces [257]. There are evidences pointing that small MPs in the digestive gland are slower processed and eliminated than larger ones, and that a translocation of small MPs occurs from the digestive system to the gills [197]. Indeed, there is evidence indicating that bivalves are more likely to consume smaller MPs instead of larger microfibers [258]. More recently, microfibers have been observed in foot, mantle, and fused in to the byssus of mussels in experimental exposure experiments, and the adherence and fusion to these and other organs has been proposed as a novel way for mussels to uptake microplastics beyond ingestion, contributing about 50% of the microplastic uptake in mussels [259, 260].

Innate immune defence in mussels is comprised of cell-mediated and humoral mechanisms, in which hemocytes are primarily responsible for defence against parasites and pathogens. Consequently, immune functional parameters of bivalves are used as a model for investigating the major mechanisms involved, modes of actions and effects of MNPs on innate immune responses in organisms. Several *in vivo* studies have found that experimental exposure of *Mytilus galloprovincialis* to virgin MNPs may trigger upregulation of lysosome and immune-related functions, such as changes in hemolymph total cell counts, phagocytosis-related activities, extracellular lysozyme and oxyradical production and cause significant effects on the cellular (lysosomal membrane destabilization, genotoxic effects) and tissue level responses associated with infiltration of immune cells (suggestive of inflammatory processes) besides a modulation of antioxidant defenses and detoxification enzymes [201, 225, 261–263].

In vitro studies hemocytes of *M. galloprovincialis*, which have also evidenced immunomodulation (including increase in extracellular reactive oxygen species and nitric oxide production) and apoptotic processes induced by cationic polystyrene nanoparticles (PS-NH₂ NPs) [216]. The formation of a protein-corona complex around cationic polystyrene nanoparticles incubated in hemolymph serum has been described [264]. The authors found that cellular/lysosomal damage, ROS production, and p-p38MAPK levels were higher when cells were exposed to nanoplastics in hemolymph serum than in seawater, demonstrating that biological fluids can affect NP impact in marine species [264]. Lastly, it has been found that exposure to virgin micro-PS leads to an imbalance of energy reserves causing feeding alterations and reproductive disruption in oysters *Crassostrea gigas* (significant reductions in oocyte number and decreased sperm velocity) with impacts on offspring (significant reduced larval count and larval development) [231]. However, the ecological relevance of the observed sublethal effects under laboratory conditions is limited or remains unclear for reasons which are later discussed (see paragraph 9.7.5).

Physical effects of synthetic fibers

By far, most lab studies reported in the literature used regular shaped microplastics, i.e., microbeads of spheres, and not microfibers. Laboratory exposure studies using fibers are largely limited mostly to crustaceans, mostly small species (Table 9.3). Reported effects including mortality are limited to crustaceans, in particular amphipods and copepods. The majority of ingested microplastics reported in field studies were fibers, and several studies indicate that this shape of microplastics appear to have a higher potential than other forms of microplastics to enter the food chain (see above). It is unclear whether this shape is more bioavailable and may also influence the severity of resulting biological effects due to increased risk for entanglement and increased gut passage time. Differences in biological response due to the shape of microplastics, especially fibrous shapes, are however not well understood. Compared to other particle shapes, fibers may have (1) a greater potential to entangle the gut contents or externalities; (2) increased gut passage time; (3) increased chemical reactivity (and resulting effects) due to relatively large surface area to volume ratio. More research is needed to confirm whether entanglement of fiber-shaped microplastics could worsen adverse effects [13, 18].

| Species | Exposure conditions | Observed effects | Reference |
|---|---|--|-----------|
| Marine lobster (<i>Nephrops norvegi-</i> <i>cus</i>) | PP fibers (from ropes) 3-5 mm in length and 0.2 mm in diameter; five fibers per feeding; uncertain what pro- portion of ingested plastic is retained in the foregut; fed and starved controls were used. | Reduced body mass and reductions in blood protein and stored lipids. | [265] |
| Marine crab (<i>Carci-</i> nus maenas) | PP fibers (from ropes) (1- 5 mm in length chronic 4 week feeding studies, crabs that ingested food containing microfibers (0.3- 1.0 % plastic by weight). | Effect on food consumption and energy balance (scope for growth). | [266] |
| Marine copepod (<i>Calanus finmarchi-</i> <i>cus</i>) | Mixture of cultured microal- gae (control), $\sim 50 \text{ ml}^{-1}$ of nylon granules (10-30 µm or nylon fibers (10 × 30 µm), similar in shape and size to the microalgal prey. | Nylon fibers: altered prey selectivity ($P < 0.01$) and nonsignificant 40% decrease in algal ingestion rates. Nylon granules and fibers: premature moulting in juvenile copepods. | [267] |
| Freshwater am- phipod (<i>Hyalella</i> <i>Azteca</i>) | PE particles and PP fibers; conducting 10-d and 42-d bioassays using individual 250 ml chambers. | Acute EC50: PE particle and PP fibers 4.64×10^4 and 71.43 particles/fibers per ml respectively. Observed chronic effects: decreased growth and reproduction. | [268] |
| Freshwater amphi- pod (<i>Gammarus</i> fossarum) | PA fibers; 50-200 μ m; expo- sure levels: 100, 540, 2680, 13,380 fibers cm ⁻² base area of glass beakers. | Reduced assimilation efficiency, reduced weigh. Found in gut after 0.5 hr of expo- sure; egestion in 16 h after exposure stop; No effects with PS 1.6 mm beads. | [269] |
| Freshwater crus- tacean (<i>Dapnia</i> <i>magna</i>) | PET textile microfibers (length range: 62-1400 μm, width 31-528 μm, thickness 1-21.5 μm). | Ingestion of very long microfibers (even 1400 µm long); increased mortality, even after 24 h of exposure in MP free medium following 48 h of MP exposure. | [13] |

| Table 93. | Observed | effects in | marine and | freshwater | biota aft | er exposui | e to s | vnthetic | fibers in | 1 laboratory | studies |
|-----------|----------|------------|------------|--------------|-----------|------------|--------|----------|-----------|--------------|----------|
| 10010 7.5 | Observeu | chiecto m | marme and | 110011 water | orota are | ci chposui | 0 10 5 | ynuncuic | moors m | 1 Iuoorator | studies. |

Blarer et al. [269] found reduced assimilation efficiency and weight after ingestion of PA fibers (50-200 μ m) in freshwater amphipod (*Gammarus fossarum*) while no effects were present after ingestion of 1.6 mm PS microbeads. Jemec and Drobne [13] exposed *Daphnia magna* to a size range of PET textile microfibers (62–1400 μ m) and found that while the majority of ingested fibers were around 300 μ m, some very large twisted microfibers even up to 1400 μ m were present inside the gut. As noted above, several studies have shown that the size, shape, and surface physicochemical characteristics of MNPs are essential determinants of their fate and effects. However, this knowledge is mainly based on studies using spherical and other non-fibrous MNPs. Cole et al. [267], exposing marine copepods to nylon fibers vs nylon granules, found that shape and chemical profile of a MP can influence its bioavailability and toxicity. Exposure to nylon fibers caused significant shifts in prey selectivity resulting in a 40% decrease in algal ingestion rates, while nylon granules negatively affected lipid accumulation.

The limited use of plastic microfibers and nanofibers in experimental work has provided a very limited view on their fate and potential effects. In addition to this, there are several limitations related with the experimental design of published laboratory findings that hamper translation of the findings to the field situation in nature, which will be described in 9.7.6.

9.7.3 Chemical-mediated effects of MNPs

With the sorption of chemicals on MNPs, the transfer of harmful substances such as persistent organic pollutants (POPs), can lead to organic bioaccumulation of such contaminants within the upper part of the food chain [210]. Nevertheless, laboratory studies and model estimates indicate that the relative importance of MNPs as a carrier of hydrophobic organic chemicals is low compared to other media (for review, see Koelmans et al. [90, 270]). However, the bioaccumulation can vary depending on polymer nature, chemical type, and fish species traits [88]. Microfibers exhibit a high surface to volume ratio compared to non-fibrous microplastic shapes and thus may exhibit enhanced pollutant-sorption performance and bioavailability of toxic compounds. Thus, the combined effects of microplastics, in particular microfibers, and other pollutants, including persistent organic pollutants (POPs) and heavy metals, through aquatic food chains requires further attention. The real risk for aquatic and human consumers still needs to be elucidated [172, 181].

Mechanisms potentially contributing to chemical impacts of microplastic particles and microfibers on aquatic biota include leaching of plastic additives and transfer of sorbed compounds such as POPs and metals. The chemical effect on organisms mediated via the ingestion of MNPs depends on the amount and nature of both the polymeric materials and sorbed/desorpted hydrophobic pollutants. Uptake and negative effects of additives and sorbed contaminants attributed to leaching from plastics has been documented in several cases [224, 225, 232, 271–275]. Several of these studies showed that microplastics and sorbed chemicals had a greater effect than chemicals alone; other studies did not reveal such effects [276, 277]. This can be largely contributed to the different experimental test conditions used in these studies. The combined effect of microplastics and sorbed co-contaminants in aquatic organisms is still not well understood. Some studies found additive effects of microplastics and sorbed contaminants, others did not find such combined effects. For example, Rainieri et al. [275] exposed zebrafish to feed spiked with 2% microplastics (LD-PE 125–250 µm of diameter) or a sorbed mixture of PCBs, BFRs, PFCs and methylmercury and a combination of both. After 3 weeks of exposure, microplastics alone did reveal no relevant health effects on zebrafish, but the combined effect of microplastics and sorbed contaminants organ toxicity in a greater manner than the contaminants alone.

The above studies suggest that ingestion of microplastics can move additives and sorbed pollutants into the tissues of organisms at concentrations that are sufficient to cause health effects or ecophysical functions (e.g., [83, 224, 278]. However, in most of these cases, it will be difficult to separate physical effects of the polymeric material and chemical effects caused by desorption from the particles.

Several studies indicate that so-called virgin microplastics are toxic to the larval stage of aquatic organisms through the leaching of chemicals, with subsequent consequences for the evaluation of microplastic laboratory experiments.

Martínez-Gómez et al. [233] evaluated the effects of virgin, weathered, and leachate of PS 6 μ m and HDPE fluff particles < 80 μ m in the sea urchin, *Paracentrotus lividus*. During the 48-h exposure time, fertilization and larval development were significantly impaired. The authors concluded that sea urchin embryotoxicity was attributed to the chemical leachate of the exposed plastic particles. Major identified compounds likely responsible for the observed effects were aliphatic hydrocarbons (C14, C16, C18, C20, and C22) from HDPE and styrene monomers from PS particles [279]. Embryotoxicity and impairment of larval development was also reported after exposure of brown mussel to PP microplastic leachate [226]. This makes the point that previous studies using virgin microplastics may have difficulties of separating between physical and chemical effects, and that weathered microplastics should provide more realistic exposure scenarios. Cleaning the MP particles with organic solvent may be insufficient to stop continuous leakage of chemical co-contaminants or residual monomers, such as styrene, which is known to be toxic to aquatic organisms.

Despite this, there is some evidence for effects of MPs on organisms at environmentally relevant concentrations. Rochman et al. [83] demonstrated that the ingestion of plastic microdebris (PE particles and associated chemicals) by Japanese medaka (*Oryzias latipes*) at environmentally relevant concentrations in sea water is capable of altering endocrine system function. However, a critical review of published studies and empirical evidence by Koelmans et al. [270] concluded that the ingestion of contaminated microplastics was not likely to increase the overall exposure to and risk of hydrophobic organic chemicals in marine organisms, highlighting current uncertainty in impacts. In a study by our group, ingestion of PCB-spiked microplastics by Norway lobster (*Nephros novergicus*) showed limited PCB bioaccumulation for polyethylene and negligible PCB bioaccumulation for polystyrene. No PCB depuration from lobster tissue to microplastics was observed [93]. A similar conclusion was reached independently using a modelling approach. Transfer of sorbed organic contaminants from microplastics was modelled to marine biota using the model OMEGA (Optimal Modelling for Ecotoxicological Applications) with different conditions of temperature, pH and gut surfactants. There was a negligible input from plastic compared to intake from food and water under both relevant and worst-case scenarios [280]. However, given the discrepancies in research results and the many uncertainties in risk assessment of microplastics and sorbed chemicals, more research is needed in this area.

9.7.4 Microbial effects of MNPs

An increasing number of studies provide ample evidence that potential pathogenic bacteria (e.g., Vibrio spp., Aeromonas salmonicida) bind particularly strong to plastic debris [91, 100, 101, 281]. In several studies, for example, the bacterial richness including *Vibrio spp.* was higher on MPs than in the surrounding seawater, [102, 282, 283]. Other studies were unable to confirm an enrichment of *Vibrio spp.* with plastic [282–284]. Gene sequencing analysis has shown that the microbial assemblages associated with marine MPs are very diverse and different between geographical zones, the greater species richness found at lower latitudes [30, 97, 285]. Research on riverine microplastics showed that bacteria assembling differently on riverine microplastics have lower taxa richness, diversity, and evenness on plastic than on non-plastic substrates [286]. Especially, plastic polluted surface waters which directly receive urban storm water or raw sewage effluents may pose direct plastic associated microbial risks, including the spread of potential pathogens and antimicrobial resistance (AMR). It has been shown that microplastic particles and fibers passing through sewage treatment plants may become enriched with pathogens [100] and subsequently may then be dispersed to the marine environment [99]. However, so far there is no evidence that microplastic-associated pathogens, AMR, and biotoxins caused health effects in aquatic organisms. Extensive multidisciplinary research is needed to identify disease risks, ecological impacts and potential implications for human health [99]. A key question concerns whether and how long microbial pathogens may persist on aquatic MPs and whether they pose a potential risk to aquatic ecosystems.

9.7.5 Potential ecological effects of MNPs

Although hypothesized, the ubiquitous, persistent, and anticipated increase of microplastic pollution could in the long-term reach concentrations that physically alter ecologically significant processes. Several studies have proposed potential ecological impacts of microplastics that could provide avenues for future research. For example, the potential of MP pollution to alter ecosystem microbial function [287], whether MP pollution could affect the functioning and structure of sedimentary environments and the structure of benthic assemblages [288, 289], and the potential impact of microplastics on marine pelagic primary producers [290]. The assessment of potential ecological impacts strongly relies on predictions from experimental studies and modelling studies [291] and more thorough research is needed before refined and more reliable assessments can be made.

9.7.6 Field evidence and ecological relevance of laboratory studies

Biological effects of microplastics in the field have not been demonstrated, mainly due to the fact that the environmental concentrations detected are below laboratory effect thresholds and these effects are non-specific and difficult to separate from the effect of associated chemicals [27, 292] and similar effects caused by other multiple natural and anthropogenic stressors acting on aquatic animals and ecosystems. The many uncertainties and knowledge gaps as highlighted throughout this chapter seriously hamper an adequate MNP risk assessment.

In the absence of field evidence for MNPs, one must rely on the available laboratory exposure studies, which for several reasons are difficult to extrapolate meaningfully to effects likely to occur in the field. Most of the studies looking at the effects of microplastics on model organisms are fraught with one or more of the following limitations: (1) conducted at unrealistically high MP concentrations, using only one type of particular polymeric particle, while aquatic organisms in nature are exposed to much lower concentrations and also much larger diversity of plastic particles; (2) lack information on dose-response curves; (3) used particles smaller than those reported from the field; (4) used virgin particles not taking the physical-chemical weathering, eco-corona/microbial biofilm interactions into their effect assessment; (3) lack of reference natural (non-plastic) particles as control treatment; (5) laboratory studies are typically short term (and high concentration), whilst in reality exposure is of a chronic nature and at low concentration (high dose acute exposure versus low dose chronic exposure). Clearly, these experimental limitations hinder translation of the observed effects to the actual field situation. Moreover, not unimportantly, to date, only a handful of experiments with fibrous MNPs, the environmentally most prevalent plastic particle shape, have been conducted. It is evident that the experimental results derived of lab-based MNP studies have only limited ecological relevance and are difficult to translate to the real nature [293].

Adverse effects in biota have rarely been shown experimentally at environmentally relevant concentrations of MNPs. Still, despite their limitations, the majority of reported laboratory studies on MNP health effects are at levels that are sub-organismal or affect individual organisms. That said, it is clear that most of the observed toxicity endpoints, such as survival, growth, and reproduction are of high ecological relevance and can potentially impact ecosystem function [246]. Provisional risk assessments show that current MP levels likely do not pose a widespread ecological risk, except in some coastal locations [28]. However, as said previously, a full characterisation of the risk of MNPs in the aquatic environment is not yet feasible, due to large data gaps. Laboratory and field-derived data to inform more comprehensive ecological risks assessments of MNPs are needed and expected to rapidly grow, as the levels of MNP are expected to increase in the future and the subject will continue to attract significant scientific attention.

9.8 Key conclusions

• Micro and nanoplastics are highly complex mixtures of polymer materials, chemical contaminants, and biological agents. They exhibit variable properties (particle composition, size, density, charge, toxicity, aggregation state, age, associated epibiota), representing a diverse and complex class of environmental contaminants.

- Microplastics, often in the form of microfibers, are common in both marine and freshwater systems from around the world. Current field data generally document only the larger plastic particles and fibers, seriously limiting field assessments. PP, PE, polyester, PET, HDPE, LDPE, PVC, and cellophane are most common polymer types; rayon, polyacrylonitrile, nylon only occasionally reported.
- Synthetic fibers are the most prevalent type of aquatic microplastics, and their occurrence appears to be related to effluents, fishing activity, and atmospheric deposition.
- Heterogeneous sampling methodologies and units are often applied resulting in poor quality data, hampering comparison with other studies. Microfibers are particularly difficult to monitor as they are pervasive throughout environment and laboratory.
- MNP debris is an emerging multi-stressor in the aquatic environment due to the physical hazards of the polymeric material, and the chemical and microbial hazards associated with it.
- There is ample laboratory evidence that exposure to MNPs can impact aquatic species across many taxa at various levels of biological organization, albeit at high concentrations. But there is only limited evidence from nature with many gaps in our understanding. Smaller particles are more harmful than large particles and micro-fibers are more harmful than non-fibrous particles.
- The fate and effects of synthetic and natural microfibers are understudied and poorly understood.

9.9 Key knowledge gaps and research priorities

Our overview of the current state of knowledge on aquatic MNPs displays important research gaps which need to be filled in order to investigate the possible risks posed by MNPs. Major knowledge gaps are summed up below.

- For all aquatic compartments, there is a pressing need for improved characterization and quantification of MNPs and standardized methods for sampling and analysis. Good quality data on microfibers deserves special attention.
- A particular challenge is the development of analytical methods for small microplastics up to several micrometers and down to the nanoscale in aquatic matrices.
- There is a clear need for chronic experiments with environmentally relevant concentrations, particle sizes, shapes and compositions relative to natural particles. Special attention deserves microfiber fate and effects.
- Given the ubiquity of microplastics in our aquatic environments, there is a need to assess population and ecosystem level effects of increasing MNP pollution.
- Another challenge is to better understand how MNPs are cycled through aquatic ecosystems, where they go and how they degrade.
- Future research should elucidate the role of MNPs as a transport vector of pathogens, antibiotic resistance and biotoxins and the potential for dispersing diseases in aquatic systems and humans.

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