

1 **Gathering at the top? Environmental controls of microplastic uptake and**
2 **biomagnification in freshwater food webs**

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30 **Abstract**

31 Microplastics are ubiquitous in the environment, with high concentrations being detected now
32 also in river corridors and sediments globally. Whilst there has been increasing field evidence
33 of microplastics accumulation in the guts and tissues of freshwater and marine aquatic species,
34 the uptake mechanisms of microplastics into freshwater food webs, and the physical and
35 geological controls on pathway-specific exposures to microplastics, are not well understood.
36 This knowledge gap is hampering the assessment of exposure risks, and potential
37 ecotoxicological and public health impacts from microplastics.

38 This review provides a comprehensive synthesis of key research challenges in analysing the
39 environmental fate and transport of microplastics in freshwater ecosystems, including the
40 identification of hydrological, sedimentological and particle property controls on microplastic
41 accumulation in aquatic ecosystems. This mechanistic analysis outlines the dominant pathways
42 for exposure to microplastics in freshwater ecosystems and identifies potentially critical uptake
43 mechanisms and entry pathways for microplastics and associated contaminants into aquatic
44 food webs as well as their risk to accumulate and biomagnify.

45 We identify seven key research challenges that, if overcome, will permit the advancement
46 beyond current conceptual limitations and provide the mechanistic process understanding
47 required to assess microplastic exposure, uptake, hazard, and overall risk to aquatic systems
48 and humans, and provide key insights into the priority impact pathways in freshwater
49 ecosystems to support environmental management decision making.

50

51 **1. Introduction**

52 Besides the many technological benefits of living in the “plastic age” (Thompson, 2009), the
53 sheer plethora of plastic products, their unsustainable use and disposal combined with their
54 high durability in the environment cause pollution risks with widespread environmental and
55 public health concerns (Andrady, 2009; Koelmans, 2015). A substantial increase in the
56 worldwide production of plastics, from 2 million tons per year in 1950 to more than 335 million
57 tons in 2016 have led to high global levels of plastic pollution (Bergmann, 2015; Dris, 2015;
58 Eriksen, 2014). There is growing concern that waste management is largely incapable of
59 handling the volumes of plastics being produced among the myriad disposal pathways
60 (Koelmans, 2015), resulting in the release of large amounts of plastic waste into the
61 environment. Up to 12.7 million tons of mismanaged plastic entered the world’s oceans in 2010

62 alone, and is predicted to increase by an order of magnitude by 2025 if no action is taken
63 (Jambeck, 2015). Given that these estimates have arisen from consideration of a 50 km coastal
64 margin only and thus, excluded riverine plastic contributions from the wider river basins, the
65 real numbers of plastic inputs into the oceans are likely to be even higher.

66 While larger plastic items present a visible environmental risk, and a concern following
67 ingestion by aquatic animals and birds (Besseling, 2015; Foekema, 2013), there is an increasing
68 awareness and concern about the environmental and public health impacts of smaller
69 microplastic (MP) particles of less than 5 mm size, originating from a variety of sources with
70 different chemical properties (Avio, 2015; Koelmans, 2015). MPs, and their even smaller
71 counterparts nanoplastics (i.e. particles < 100 nm) (Mattsson, 2015), are composed of a wide
72 variety of polymer types resulting in distinct physical properties (i.e., in their shape, size,
73 buoyancy). The variations in their physical properties ultimately influence particle transport
74 and retention dynamics, their deposition, and bioavailability in the environment (Dris, 2015).
75 The chemical and physical properties of MPs found in the environment are, in the first instance,
76 controlled by their source. These can be classified as primary (manufactured) particles such as
77 microbeads used in cosmetics and human care products, or secondary MPs, which result from
78 the breakdown of larger plastic items (Figure 1). Fragmentation commonly results from
79 industrial abrasive processes (GESAMP, 2016), or degradation via micro-cracking and
80 embrittlement as a result of exposure to salt, wave action (Zbyszewski, 2014) and/or UV light
81 and freezing and thawing cycles (Da Costa, 2018).

82 MP pollution is recognised as a global environmental threat with MPs being widely abundant
83 in marine ecosystems, e.g. in the 5 ocean gyres, and stretching to the deep sea and remote arctic
84 areas (Foley, 2018). While the transport, fate and behaviour of MPs have been studied
85 predominantly in marine ecosystems, significant knowledge gaps remain in freshwater and
86 terrestrial ecosystems (Hurley, 2018; Klein, 2015; Wagner, 2014; Windsor, 2019). MPs have
87 been detected at alarming concentrations in river corridors and sediments (Horton, 2017;
88 Rochman, 2018; Tibbetts, 2018). There is growing consensus that rivers are retention sites for
89 MPs and serve as conduits for their downstream transport to the oceans (Fig. 1) (Ballent, 2016;
90 Brennecke, 2016; Duis, 2016; Hoellein, 2019; Hurley, 2018; Klein, 2015; Lebreton, 2017;
91 Thompson, 2004; Wolff, 2019).

92 Due to their unavoidable interactions with food webs they have been found in a wide range of
93 marine organisms from phytoplankton to fishes, sea birds and even filtering cetaceans (Fossi,
94 2016; Setälä, 2014). Recent meta-analyses point towards highly heterogeneous responses to

95 MP ingestion in different groups of aquatic organisms. For some taxa negative effects were
96 found in terms of impacts on consumption (and feeding), growth, reproduction, and survival
97 of fish and aquatic invertebrates (Foley, 2018; Wright, 2013). Besides the physical impacts on
98 animals (e.g. blocking of digestive pathways, choking), MPs may be potentially toxic to
99 animals, either through the leaching of additives (Dris, 2015; Eerkes-Medrano, 2015; Wagner,
100 2014), or by acting as vectors for pathogens, bacteria, viruses (Lamb, 2018) and contaminants
101 that adsorb to their surface (Hartmann, 2017; Lamb, 2018). Ingested MPs may cause numerous
102 adverse impacts on aquatic organisms. Even pristine plastics can cause acute immobilization,
103 depletion of energy reserves (Wright, 2013), a decrease in predatory performance or hepatic
104 inflammation, while MPs associated with persistent organic pollutants have been shown to
105 cause acute or chronic toxic effects (Rehse, 2016; Rochman, 2013; Van Cauwenberghe, 2014).
106 Critically, additives (e.g. Bisphenol A – BPA, an endocrine disrupting substance) or flame
107 retardants such as Poly-brominated Diphenyl Ethers (PBDEs) have been shown to have
108 increased toxicity effects on organisms when associated with MPs than when exposed in
109 solution (Planelló, 2008; Wardrop, 2016). The reason for this is ‘Trojan horse vector-effects’
110 whereby MPs carry toxic additives directly into the animal’s bodies (de Sá, 2018; Luís, 2015;
111 Oliveira, 2013). With recent evidence of MPs entering the human food chain (Deng, 2017; Li,
112 2018; Wright, 2017), and nanoplastics having the potential to penetrate cell walls of organisms
113 (Kashiwada, 2006; Rosenkranz, 2009), regulators, water and chemical industries globally are
114 concerned about the environmental and public health impacts of additives such as BPA and
115 other endocrine disrupting substances such as Nonylphenol (NP) leaching from MPs (Dris,
116 2015; Eerkes-Medrano, 2015; Wagner, 2014). However, some studies have found that MP
117 presence reduced the short-term effects of BPA on freshwater zooplankton (Rehse, 2018), with
118 long-term effects still being unknown.

119 Despite some recent studies of MP occurrence in rivers and lakes (Ballent, 2016; Besseling,
120 2015; Estahbanati, 2016; Foley, 2018; Klein, 2015), and their potential effects on a variety of
121 different groups of organisms (Rehse, 2016; Rochman, 2013; Van Cauwenberghe, 2015), there
122 remains a critical lack of understanding regarding the fate and transport mechanisms of MPs
123 and how these affect their uptake and propagation in freshwater food webs (Eerkes-Medrano,
124 2015; Foley, 2018). While there is initial evidence of trophic transfer in simple food chains
125 (Chae, 2018), major knowledge gaps remain on the direct and indirect uptake and transfer of
126 MPs in the environment, including their capacity for biomagnification while moving through
127 complex freshwater food webs.

128 Assessing the risk of MP uptake and propagation in aquatic food webs requires the
129 development of detailed understanding of potential direct and indirect MP entry points and
130 mechanistic pathways in food webs. In addition, understanding of how MP fate and transport
131 processes control their accumulation in freshwater habitats, and of the magnitude of site-
132 specific exposures, is needed to enable prediction of MP bioaccumulation and biomagnification
133 and develop appropriate mitigation measures. Here, we present here a comprehensive review
134 and synthesis of key research challenges in:

- 135 (i) Characterising particle properties and their ageing, along with freshwater properties
136 such as hydrodynamic and sediment conditions control the environmental fate,
137 transport and development of accumulation hotspots of MPs in freshwater
138 ecosystems;
- 139 (ii) Analysing how physical controls on MP presence in freshwater environments
140 determine the environmental exposures and accessibility of MPs in freshwater
141 ecosystems
- 142 (iii) Identifying potential uptake mechanisms and entry pathways of MPs and associated
143 contaminants into aquatic food webs, and their capacity to accumulate and
144 biomagnify following initial entry.

145 We identify 7 key research challenges (Table 1) that represent milestones in understanding the
146 controls of exposure, uptake and propagation of MPs in aquatic food webs, and discuss these
147 in further detail below.

148

149 **2. Fate and transport mechanisms control microplastic exposure**

150 The exposure and potential entry points of MPs into aquatic food webs are determined by the
151 spatial distributions and time scales of mechanisms that control the input, transport and
152 transformation as well as potential accumulation of different types of MPs in the environment.
153 An assessment of MP uptake and potential propagation through aquatic food webs therefore
154 requires detailed understanding of MP sources and their activation, as well as of the
155 environmental fate and transport mechanisms of MPs (Fig 1).

156 MPs enter aquatic environments through a wide range of pathways, including runoff from land
157 surfaces and roads, river bank waste disposal, atmospheric deposition and outfall of wastewater
158 treatment plants (Fig 1) (Dris, 2016; GESAMP, 2016; Horton, 2017; Horton, 2018b; Wagner,
159 2014). While it has been shown that wastewater treatment plants can retain significant amounts

160 of MPs and are efficient to filter out some types of primary MP particles (Murphy, 2016; Prata,
161 2018; Ziajahromi, 2016), there is evidence that they are less effective in the retention of
162 synthetic microfibers and nanoplastics (Rochman, 2015). Other MP entry pathways into
163 terrestrial and aquatic ecosystems include agricultural sources including organic fertilizer
164 products (Weithmann, 2018), plastic in potting soil, such as tree ties in forestry and plastic
165 tarps on agricultural fields (Fig 1). Recognition of the specific entry pathways and related
166 differences in transport mechanisms is crucial since the environmental fate of highly
167 concentrated point source inputs (e.g. from wastewater treatment plants) is likely to differ
168 significantly from lower concentration diffuse inputs (e.g. atmospheric fallout, road runoff or
169 agriculture) (Fig 1).

170 Understanding of the chemical and physical properties of MPs, as well as how the
171 hydrodynamic and substrate conditions at the sediment-water interface control MP transport in
172 lakes and rivers is still very limited. Recent studies have hinted that MPs are omnipresent in
173 freshwater ecosystems, and in particular at sediment-water interfaces (Besseling, 2015, 2018;
174 Foley, 2018; Hurley, 2018), indicating that benthic and hyporheic zones of rivers may represent
175 MP accumulation hotspots (Drummond et al., 2020; Frei et al., 2019) (Fig 1). Depending on
176 their density and buoyancy, MPs of different origin are likely to differ in their transport
177 properties, their probability of burial in riverbed sediments and thus, the types of interactions
178 with different trophic levels of aquatic organisms. For instance, in contrast to the high-density
179 polymers found in benthic and hyporheic accumulation zones in many rivers (Besseling, 2018;
180 Foley, 2018; Hurley, 2018), most of the MPs polluting the water column of the Canadian Great
181 Lakes were found to be low density synthetic polymers (polyethylene, polyester) (Ballent,
182 2016). Similar results were found within large river systems such as the Danube and Rhine
183 rivers (Lechner, 2014; Mani, 2015). Yet, the fact that low density MPs have also been found
184 in streambed sediments highlights the fact that the mechanisms which control deposition and
185 entrapment (Hoellein, 2019), including potential interactions of the bed substrate and its
186 associated benthic flora, remain to be quantified. While there is increasing understanding and
187 insight into the relevance of the specific mechanisms controlling MP transport in freshwater
188 ecosystems such as hyporheic exchange (Drummond et al., 2020; Frei et al., 2019) or
189 gravitational settling, including governing equations and quantitative models (Box 1),
190 identifying the required parameters across the relevant spatial and temporal scales remains
191 challenging.

192 Furthermore, the spatial patterns and temporal dynamics of proportional contributions of
193 different types and sources of MPs still need to be determined, in order to parameterise
194 transport and exposure models. The vast majority of freshwater MP studies to date have been
195 conducted under steady flow conditions, and little attention has been paid to analysing the
196 impact of hydrodynamic conditions at sampling sites (Fig 2). We have only just started to
197 explore how the temporal dynamics of MP fragmentation, deposition, degradation and
198 eventually removal are controlled by hydrodynamic variability (Drummond et al., 2020;
199 Hurley, 2018), with the balance between these processes being defined by the local exposure
200 time. It will be critical to identify how the hydrodynamic forcing and the variability in physical
201 and chemical properties which affect MPs (Box 1) may change with dynamic flow conditions
202 (e.g. temperature, nutrient content, UV exposure). It has yet to be established how this variation
203 in flow may affect the deposition (Tibbetts, 2018) and transformation of MPs, such as their
204 breakdown and decomposition or release of additives such as BPA and NP (Fig 2). This
205 includes the distribution of MPs within the floodplains of rivers during high flow events as
206 well as their behaviour under drastically different environmental conditions (i.e., when exposed
207 to air during dry conditions in ephemeral streams or on elevated river banks (Fig 2)). Indeed,
208 with globally increasing frequency and severity of droughts (Hoellein, 2019; Naumann, 2018),
209 intermittency and flashiness of streams, understanding of the impact of stream drying and
210 increased UV exposure on MP degradation will become increasingly important, as will
211 understanding the disturbances to sediment-entrained MPs due to turbulent mixing as streams
212 re-wet.

213 The transport mechanisms and accumulation of MPs (Box 1) are furthermore affected by
214 biological processes, including MP particle ageing and decay as well as biomolecule adsorption
215 and biofilm colonization (i.e. accumulation of micro-organisms on wetted MP surfaces)
216 (Harrison, 2018; Nasser, 2016), and entrapment in biofilms at aquatic-terrestrial interfaces (Fig
217 2) (de Sá, 2015; Duis, 2016; Rummel, 2017). Biofilms growing on the surfaces of ageing MPs
218 can change their hydrodynamic and buoyancy properties, potentially affecting their transport,
219 fate, accumulation and uptake by organisms (Corcoran, 2015). The magnitude of such
220 biological interactions and their dependency on environmental conditions such as temperature,
221 light exposure and nutrient concentrations have yet to be established. Additionally, the
222 formation of homo-aggregations (microfiber clumps) and hetero-aggregations (similar to
223 marine snow) may also have an effect on the fate and transport of MPs (Alimi, 2018). Important
224 lessons can be learned here for instance from recent advances in understanding MP settling

225 velocity in marine environments (Khatmullina, 2017), or using mechanistic behaviour of
226 naturally occurring allochthonous particles as analogues (Hoellein, 2019). However, the
227 stability of MPs and their likelihood to form aggregates may be different in freshwater versus
228 saline sea water and will depend on the polymer type (Lagarde, 2016; Michels, 2018).

229 Addressing the interdisciplinary challenges of establishing how the fate and transport of MPs
230 control their entry into freshwater food webs will require:

- 231 - The characterisation of spatially heterogeneous MP sources of different plastic types
232 and how dynamic source activation controls the way MPs are released into the
233 environment [*Challenge 1*] as well as,
- 234 - The identification of how MP properties, and freshwater hydrodynamic and biological
235 processes interact in affecting MP fate and transport, including their degradation,
236 ageing, break down and the release of toxins [*Challenge 2*] (Table 1).

237 For these challenges to be addressed successfully, scientific advances across traditional
238 disciplinary boundaries are necessary that require to leave our current disciplinary ‘comfort
239 zones’ in the material sciences and analytical chemistry, ecohydrology, environmental
240 chemistry and biology (Table 1).

241

242 **3. Microplastics entry points into freshwater food webs**

243 Once they have entered freshwater environments, there is a risk that MPs may be taken up into
244 freshwater food webs. The location and mechanism for MP entry into freshwater food webs is
245 determined by site- and material-specific conditions. Recent investigations have focussed on
246 the discrimination between various routes of entry according to whether the MP particles were
247 primary or secondary MPs (Ballent, 2016; Dris, 2015; Duis, 2016; Weithmann, 2018).

248 Potential MP entry points through direct uptake into aquatic food webs exist at almost every
249 trophic level and may involve suspended MPs that are filtered from the open water column or
250 MPs accumulated in the benthos at the sediment-water interface (Fig 3). Phytoplankton can
251 directly adsorb nanoplastics through cell-walls and zooplankton such as daphnia and fish can
252 ingest nanoplastics and MPs directly from the water column (Costa, 2016). Lacustrine
253 invertebrates such as oligochaete worms, amphipods, and freshwater snails can ingest
254 artificially manufactured plastic microspheres under laboratory conditions (Imhof, 2013).
255 However, so far only limited empirical evidence of MP uptake mechanisms and trophic transfer
256 by freshwater fauna exists, as only isolated cases of direct ingestion under laboratory

257 conditions, or detection in the wild (without knowledge of the specific plastic sources) have
258 been reported (Imhof, 2013; Pazos, 2017).

259 It is plausible that benthic fauna, especially filter feeders, like zebra and quagga mussels
260 (*Dreissena sp.*) will ingest MPs suspended in the water column, with the risk of transferring
261 them to the benthic food chain via coating in faeces or pseudofaeces (Lederer, 2006) (Fig 3).
262 Pseudofaeces of *Dreissena* and other common freshwater bivalves (*Corbicula sp.* and
263 Unionidae mussels) are rejected organic and inorganic particles covered by mucus in the mantle
264 cavity of the mussel, and expelled outside (Dermott, 2005). It is well established that many
265 benthic animals prefer to feed on *Dreissena* pseudofaeces because of their high nutritional
266 values (Stewart, 1998). Due to their high biomass, filter feeding benthic macroinvertebrates
267 such as bivalves may therefore be responsible for significant transfer of MP from the water
268 column to benthic food webs.

269 A further preferential pathway of MPs to enter freshwater food webs is through their
270 entrapment in benthic or hyporheic biofilms (Sgier, 2016), where they accumulate and are
271 potentially made more readily available to other organisms. Here they can pose a critical risk
272 for preferential direct MP uptake and subsequent ingestion by biofilm grazers (Fig 3)
273 (McCormick, 2016). In marine environments, organic aggregates (so called marine snow) can
274 trap MPs and transport the normally buoyant plastics to the deep ocean, ultimately increasing
275 their bioavailability to deep-sea fauna (Porter, 2018).

276 So-called Trojan horse vector-effects may potentially increase the indirect uptake of
277 xenobiotics from MPs, for instance when additives such as Bisphenol-A (BPA) leach from the
278 biofilm after ingestion by biofilm grazers (Fig 3). Furthermore, biofilms on MP surfaces may
279 create shortcuts for MPs to enter food webs at several trophic levels (Rummel, 2017). In addition
280 to Trojan horse effects, MPs may affect the structure and metabolic functionality of biofilms
281 (Harrison, 2018; Rummel, 2017). Since biofilms carry out a number of crucial ecosystem
282 services in rivers, such as respiration, nutrient uptake and attenuation of pollutants, any MP
283 induced damage to biofilms bears the risk of impacting ecosystem functioning at the river scale
284 (Besseling, 2018; Harrison, 2018).

285 Beyond physical controls on MP fate and transport, it is important to advance the understanding
286 of how animal behaviour and feeding types affect MP uptake and ingestion, which may vary
287 by development stage and morphological characteristics (Scherer, 2017). For example, De Sá
288 (2015), showed that the common goby (*Pomatoschistus microps*) ingests MP particles (420–

289 500 µm) of different colours together with natural prey (*Artemia salina*) (de Sá, 2015). Here,
290 the selectivity of MP uptake was dependent on the particle colour, with similar behaviour found
291 for sunfish (Peters, 2016). Similarly, benthic sea turtles showed strong selectivity for soft,
292 clear plastic, suggesting that sea turtles ingested plastic because it resembled natural prey such
293 as jellyfish (Schuyler, 2012). Pelagic turtles however were found to be less selective in their
294 feeding, though they showed a trend towards selectivity for rubber items such as balloons
295 (Schuyler, 2012).

296 In order to assess the entry point specific risks of MP uptake and their dependence on
297 environmental exposure conditions, MP exposure hotspots (e.g. through filter feeding or
298 biofilm entrapment) need to be identified and mechanisms of potential uptake pathways
299 characterised and their relative contributions to total organism body-burdens quantified
300 **[Challenge 3]** (Table 1). This includes uptake by phytoplankton as well as MP entrapment in
301 aquatic biofilms, presenting a potential pathway for increased MP uptake by biofilm grazers
302 and entry into freshwater food webs. The quantification of potential environmental and health
303 consequences will require:

- 304 - Determination of the potential for Trojan Horse effects or shortcuts for xenobiotics into
305 aquatic food webs **[Challenge 4]** and
- 306 - Quantification of the importance of ecological behavioural aspects (from colour
307 preferences to bioturbation) that affect MP abundance and uptake **[Challenge 5]** (Table 1).

308

309 **4. Migration and potential biomagnification of microplastics in freshwater food webs**

310 Once MPs and nanoplastics enter freshwater food webs, there is a risk of biomagnification into
311 higher trophic levels (Sanchez, 2014). The potential for biomagnification is generally not yet
312 well established. Previous studies on MPs in freshwater food webs have been either limited to
313 a single species (Rehse, 2016; Van Cauwenberghe, 2015), or trophic transfer in simple food
314 chains (Chae, 2018; Rochman, 2017). Possible transfers across more complex aquatic food
315 webs have not yet been assessed.

316 Direct ingestion and trophic transfer of MPs have been confirmed in several field and
317 laboratory experiments (Kim, 2018; Ziccardi, 2016) and repeated occurrence of MP particles
318 in fish have been explained by both direct ingestion and trophic transfer (Phillips, 2015).
319 Indeed, fish have been most widely studied to date, with findings ranging from an absence of
320 MPs (Lake Geneva) (Faure, 2012), to 8% of the freshwater fish and 10% of marine fish in the

321 Gulf of Mexico (Phillips, 2015), 12% of adult gudgeons (*Gobio gobio*) in 11 French streams
322 (Sanchez, 2014), 45% of all sunfish (*Lepomis gibbosus*) specimens in the Brazzos river (Texas,
323 USA) (Peters, 2016) and 55% of Nile perch (*Lates niloticus*) and Nile tilapia (*Oreochromis*
324 *niloticus*) from Lake Victoria (Biginagwa, 2016). Despite the growing number of case studies
325 evidencing MP presence in fish (Collard, 2018; Horton, 2018a), there is little understanding of
326 the mechanisms and drivers of their uptake and propagation in aquatic food webs. It has been
327 suggested that the feeding behaviour of different species appears to significantly affect their
328 risk of MPs ingestion. Given high benthic concentrations of MPs relative to water column
329 concentrations, benthivore fish may have a greater MP exposure than planktivorous fish (Fig
330 3) (McNeish, 2018). The absence of MPs in the fish studied in Lake Geneva as compared to
331 gudgeons in French streams may be explained by a difference in the feeding preferences of the
332 different species. Gudgeons are primarily benthivores, thus might be exposed to a higher risk
333 of MP ingestion than carnivorous, planktivorous or herbivorous fish (Sanchez, 2014).

334 Simplified food chain studies have demonstrated that when MPs are abundant, they can be
335 readily transferred along trophic levels (Ivar do Sul, 2014). Trophic transfer of fluorescent
336 plastic microspheres from *Artemia nauplii* to zebrafish (*Danio rerio*) (Batel, 2016) and Spined
337 Stickleback (*Gasterosteus aculeatus*) was shown in laboratory studies (Katzenberger, 2015)
338 where fish were fed with MP-exposed Nauplius (crustaceous larvae). Both, MPs and persistent
339 organic pollutants associated with MPs such as BPA, have been shown to migrate to fish tissue
340 (Batel, 2016), highlighting the risks of Trojan Horse effects. Furthermore, direct trophic
341 transfer from mussels (*Mytilus edulis*) to crabs (*Carcinus maenus*) has been observed under
342 laboratory conditions, with plastic microspheres being transferred into the crab's haemolymph,
343 stomach, hepatopancreas, ovary and gills (Farrell, 2013).

344 Despite the mounting evidence of MPs within all levels of freshwater food webs described
345 above, evidence for the transfer among different components of food webs is limited. It will be
346 crucial to identify and understand the mechanisms of MP uptake and pathways in more
347 complex food webs than previously studied in order to understand what risk potential
348 biomagnification presents for human consumption [**Challenge 6**] (Table 1). In this respect it
349 will be essential to understand how MPs are retained in tissues over long time scales, or if they
350 are largely expelled during egestion, growth, or metamorphosis. For example, Al-Jaibachi et
351 al. (2018) showed that fluorescently stained MP beads taken up by mosquito larvae remained
352 after metamorphosis in the winged adults. This suggests that long term retention of MPs in

353 tissues is possible, and that insects with aquatic larval stages and terrestrial adult stages may
354 represent an underappreciated flux of MPs among ecosystems (Al-Jaibachi, 2018).

355

356 **5. The ecological and public health impacts - Microplastic effects on aquatic** 357 **ecosystems functioning**

358 The effects of MPs on freshwater ecosystem functioning remains largely unknown (Eerkes-
359 Medrano, 2015; Lambert, 2016). Laboratory studies confirmed that water fleas (*Daphnia*
360 *magna*), freshwater invertebrates and several fish species actively feed on MP particles (1-100
361 μm), and that uptake of MP particles caused acute immobilisation of these animals (Besseling,
362 2018; Oliveira, 2013; Rehse, 2016), and affected predator–prey relationships (Rochman,
363 2017). However, some studies also found MP impacts on ecosystem processes to be negligible
364 (Canniff, 2018; Malinich, 2018; Redondo-Hasselerharm, 2018; Weber, 2018), complicating
365 predictions of ecosystem level implications. Furthermore, transgenerational effects on *Daphnia*
366 *magna* indicated only negligible effects on the first generation but when neonates were exposed
367 to same concentration of MPs they went extinct after 2 generations (Martins, 2018). This
368 highlights the need to expand testing further than the first generation. While it is reasonable to
369 expect that some ecosystem effects occur at longer time scales or larger spatial scales, their
370 specific relevance has yet to be established.

371 Many benthic consumers in freshwaters (e.g., Chironomidae larvae, Oligochaeta worms,
372 gammaridae, amphipods) are ecosystem engineers and as such are highly exposed to MPs, their
373 chemical additives, sorbed contaminants, and potential microbial pathogens (Frère, 2018;
374 McCormick, 2014), resulting in a critical risk of wide ranging impacts in particular on benthic
375 ecosystems functioning (Izvekova, 1972; Ward, 2007). For example, lugworms (*Arenicola*
376 *marina*) that ingested MPs showed decreased bioturbation (Wright, 2013), which lowered the
377 primary productivity of bioturbated sediment and altered lugworm respiration (Green, 2016).
378 Similar impacts on key bioturbators of freshwater ecosystems could have significant
379 consequences at the ecosystem level. In shallow lakes, filter-feeding benthic species such as
380 *Chironomus plumosus* larvae filter large volumes of water, importing oxygen and particulate
381 matter down to 20 cm into the sediment and regulate lake's phosphorus concentrations and
382 trophic state (Hölker, 2015; Lewandowski, 2007; Roskosch, 2011). Thus, any reduction of the
383 activity of *C. plumosus* larvae due to MPs might severely impact on the entire lake ecosystem.

384 In addition to identifying pathways and mechanisms of how MPs enter and propagate within
385 aquatic food webs, future research should establish how freshwater MPs impact the behaviour
386 and performance of organisms and key ecosystem processes. We suggest new research to move
387 beyond the assessment of individual species interactions with MPs. The next frontier in this
388 research will measure MP effects on food webs and ecosystem functioning, as well as
389 quantifying the potential combined impacts of MPs and their associated additives, sorbed
390 contaminants and pathogens [*Challenge 7*] (Table 1).

391 **6. Concluding remarks and a look forward**

392 Increasing evidence and rising public awareness of the omnipresence of MPs in understudied
393 freshwater ecosystems and within humans at the end of the food chain (e.g. identification of
394 MPs in human stool samples (Parker, 2018)), highlight that scientific interactions and
395 knowledge exchange across disciplinary boundaries are critical for evaluating the
396 environmental and public health risks of MPs. There are increasing concerns of MP uptake
397 through food and bottled drinking water or by inhalation of microfibers from clothing or soft
398 furnishings (Catarino, 2018), in particular with the proven transport of microplastics in the
399 atmosphere along large distances (Allen, 2019). The challenges we present are interlinked and
400 require interdisciplinary collaboration among aquatic ecologists, (eco)hydrologists, fluvial
401 geomorphologists, biogeochemists, analytical chemists and microbiologists, cell biologists and
402 physicians. The seven major challenges outlined here will help interdisciplinary researchers to
403 facilitate collective goals and direct future systematic analyses of MP fate and transport
404 controls on potential entry points and uptake mechanisms into aquatic food webs. The resulting
405 knowledge will enable confirmation of the major propagation and biomagnification routes and
406 subsequent organismal and ecosystem impacts of MPs in freshwater ecosystems and
407 determination of the relative risks to human health, allowing targeted mitigation strategies with
408 maximum impact to be developed.

409

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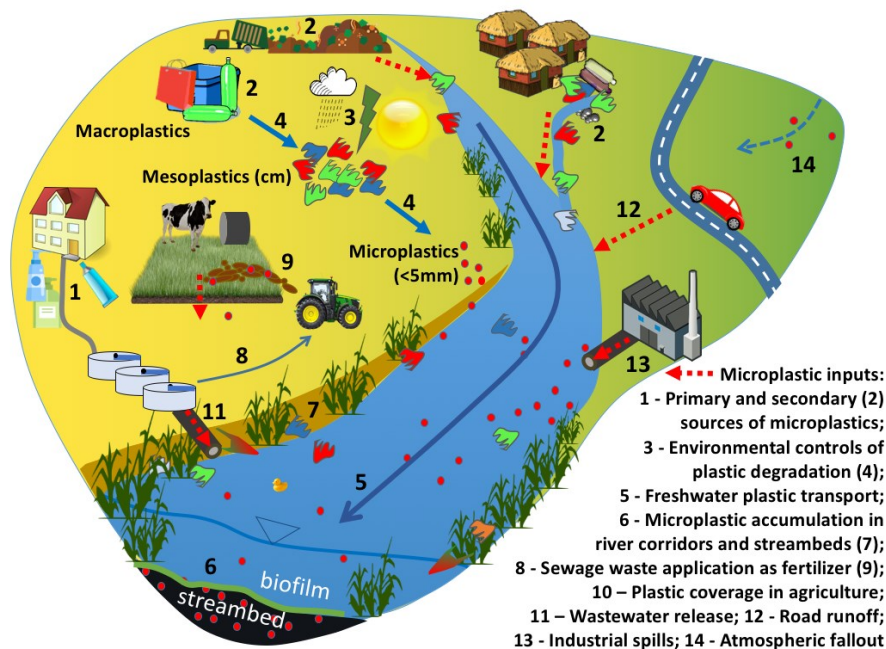
417 **Author contributions**

418 All co-authors contributed to the drafting of the manuscript, and all have approved the final
419 version.

420

421 **Figures:**

422 Figure 1 Mechanisms of plastic fate and transport processes across the river basin
423 continuum, highlighting sources, input pathways and accumulation and breakdown
424 mechanisms.



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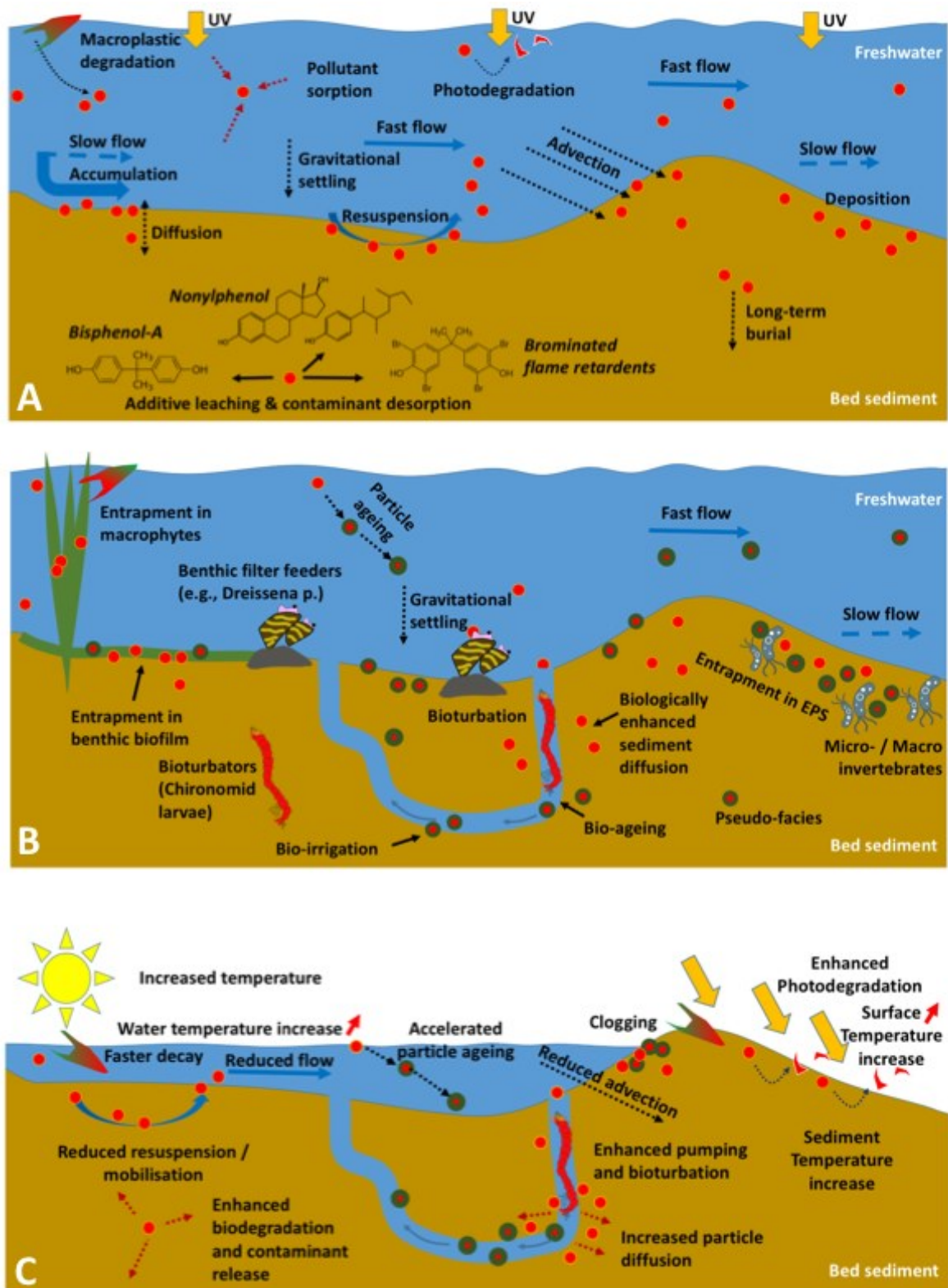
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Box 1 Quantification of key microplastic transport processes in freshwater systems: While many of the processes that control the dynamics of small, light, organic particles in freshwater systems are reasonably well understood, understanding of which specific processes and system conditions dominate the fate of different types of microplastics is still lacking. Consequently, microplastic transport and ecological uptake can only be partially quantified at the present time. The current approaches used in modeling microplastic transport processes are illustrated in Figure 2. Models for *microplastic transport and retention in streams* currently assume that Stokes' settling velocity describes the transport of microplastics to the sediment bed. Stokes' settling velocity depends on particle density, size, and shape (Chubarenko et al., 2016; Dietrich, 1982; Kooi et al., 2017). Hydrodynamic transport – specifically *hyporheic exchange* – also delivers microplastic to the streambed, and this process is generally more important than settling of microplastic, especially for smaller particles (Drummond et al., 2020). Hyporheic exchange is caused by flow interactions with sediment beds and benthic algal mats, and is generally proportional to the square of in-stream velocity (Arnon et al., 2010; Boano et al., 2014; Packman et al., 2000). *Remobilization* of microplastics occurs during both baseflow and stormflow (Drummond et al., 2014; Drummond et al., 2017; Phillips et al., 2019), and remobilization has been modeled as a function of stream power, bed friction factor and particle size (Nizzetto et al., 2016). *Filtration, aggregation and other immobilization processes* within the sediments have been observed in laboratory columns and the processes parameterized in ways that can be incorporated into larger-scale models (Alimi, 2018). *Bioturbation* mixes particles in sediment beds, and models are available to describe both the burial of microplastic and their remobilization to the sediment-water interface (Roche et al., 2016). *Photodegradation* of microplastic has been measured in laboratory experiments and been shown to be both polymer-specific and flow-dependent (Hebner and Maurer-Jones, 2020). Note that episodic burial/remobilization will definitely influence the amount of time microplastics are exposed to light vs. benthic/hyporheic biodegradation; while this is unlikely to have been observed directly *in situ*, the significance of this process is known (Ward et al., 2015). Biochemical pathways of polymer biodegradation by bacteria and fungi are highly complex, and include polymer hydrolysis and oxidative degradation, which can transform both hydrolyzable and nonhydrolyzable polymers (Yuan et al., 2020). *Uptake and bioaccumulation* of microplastics and nanomaterials in organisms is dependent on particle size and species-specific uptake rates that vary based on their developmental stage (Fueser et al., 2019; McNeish et al., 2018; Scherer, 2017; van den Brink et al., 2019). The role of atmospheric transport and disposition as routes of environmental exposure and transport are just beginning to be explored (Lim et al., 2020). The parameterization of the governing equations in aforementioned models for quantifying MP fate, transport, degradation and uptake processes remains a challenge, with further experimental evidence needed to evaluate the range in polymer types and environmental conditions which alter each of the rates quantified for each process.

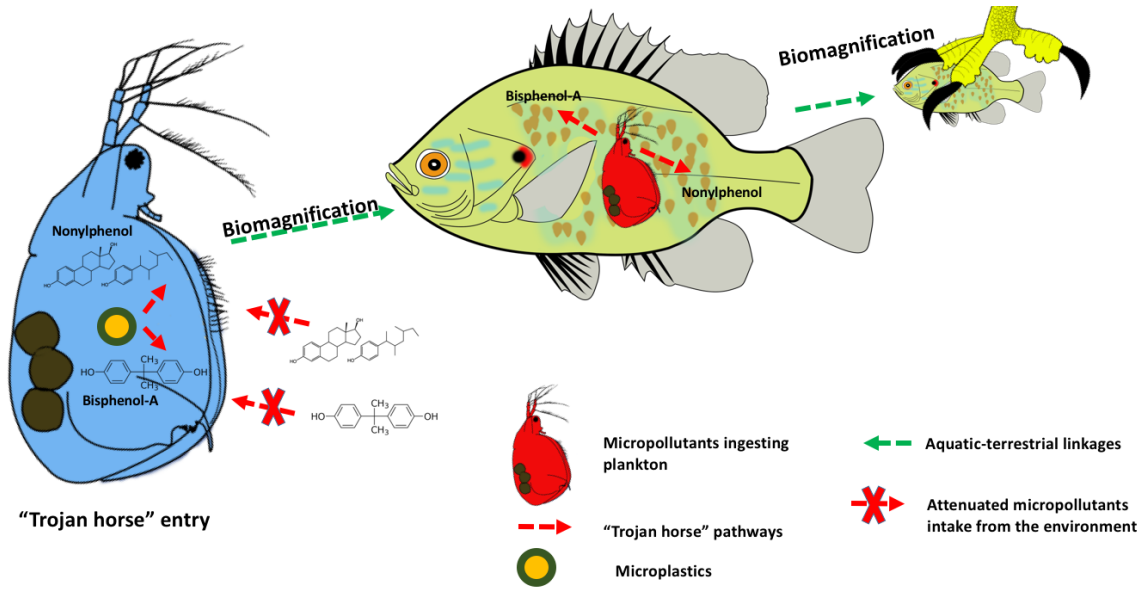
431 Figure 2 Physical (A), and biological (B) controls of microplastic fate and transport in
 432 freshwater ecosystems, including (C) impacts of flow variability and increased temperature
 433 during low-flow conditions and flow intermittence on microplastic accumulation and
 434 breakdown



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437 Figure 3 Microplastic uptake and propagation in an example aquatic food web,
 438 including leaching of additives and sorbed contaminants as part of Trojan horse effects, and
 439 potential biomagnification pathways

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	Research need	Scientific advances needed to achieve this
Challenge 1:	Characterize the spatially heterogeneous sources of different plastic types and how their activation and contribution to river corridor plastic pollution vary over time.	<p>Agreed and validated methods and protocols for sampling, identification and quantification of MPs of different compositions and shapes</p> <p>Global baseline of MP abundance in freshwater ecosystems and characterisation of MP properties.</p> <p>Temporally and spatially dynamic sampling strategies that support identification of event-based (drought, flooding and re-wetting) inputs of MPs from point versus diffuse sources</p>
Challenge 2:	Establish how microplastic properties, hydrodynamics, and biological processes affect the fate and transport of microplastics, their degradation, ageing, breakdown, and the release of toxins	<p>Analytical tools for assessment of MP ageing, degradation, and determination of the relative contribution of different processes (e.g. abrasion, UV-irradiation) on degradation as a function of MP composition (e.g. shape, density)</p> <p>Lab-assays for assessment of release of additives from MPs under ecologically and biologically relevant conditions (ionic strengths, pH, natural organic matter and abundance of other biomolecules)</p> <p>Flume and field-scale approaches for assessing the impacts of hydrodynamics, geological setting on accumulation of MPs in sediment; agreed sampling methods will be needed here also to allow comparisons of different hydrological conditions</p> <p>Full characterisation of MP ageing of different polymer products under the influence of variable environmental conditions, and re-evaluation using aged MPs where relevant</p>
Challenge 3:	Quantify microplastic exposure hotspots and mechanisms of uptake pathways into food webs	<p>Based on knowledge from Challenges 1 and 2 confirm hotspots of MP accumulation, and characterise conditions for deposition and accumulation; and for MP degradation</p> <p>From these determine organisms at specific risk, and undertake targeted lab and mesocosm studies to determine if these organisms do preferentially accumulate MPs (pristine versus variously aged and bio-fouled)</p> <p>Laboratory experiments of food webs of increasing realism to assess role of amongst others, food, water, natural organic</p>

		matter, pseudofaeces on MP uptake and accumulation in target organisms. Again, comparison of pristine and variously aged and bio-fouled MPs is needed.
Challenge 4:	Identify potential Trojan Horse effects and other shortcuts for xenobiotics into aquatic food webs	<p>Lab-based experiments under realistic exposure conditions (e.g. presence of natural organic matter and other competitive binders) to assess affinity of pollutants for MP surfaces and residence times – comparison of pristine and variously aged and bio-fouled MPs binding affinities, loading capacities and retention times</p> <p>Studies mimicking gut pH of target organisms to assess release of additives from the MPs before / during / following uptake into target organisms, including biofilms again considering pristine and aged/bio-fouled variants</p> <p>Investigation of the role of natural organic matter as a sink / source for additives / xenobiotics and how this influences MP Trojan Horse effects</p>
Challenge 5:	Determine the importance of ecological behavioural aspects (from colour preferences to bioturbation) that affects microplastic abundance and uptake.	<p>Grouping of recognition mechanisms and food preferences of different organisms and determination of correlations with different types / sources of plastics and their resulting MP degradation products</p> <p>Targeted lab-based experiments to confirm if preferential uptake occurs under realistic exposure conditions, i.e. in the presence of equal or larger quantities of test organisms preferred food sources and whether aged or bio-fouled MPs are taken up preferentially</p> <p>Analysis of MP abundance effects and thresholds relative to natural food sources to determine if tipping point effects are likely</p>
Challenge 6:	Identify microplastic propagation pathways in	Mesocosm experiments with complex food-webs and realistic environmental and ecological conditions, including also realistic

	complex food webs and assess risks of potential biomagnification for human health	hydrological conditions (e.g. using recirculating streams) and aged / bio-fouled MPs Comparison / quantification of various uptake routes – direct exposure, indirect exposure via food / pseudofaeces – and their relative contributions to total uptake under food abundant and food scarcity scenarios
Challenge 7:	Assess the impacts of freshwater microplastic accumulation and associated additives, sorbed contaminants, and pathogens on the behaviour and performance of host organisms and key ecosystem functioning	Ecotoxicity tests related to target organisms and under the realistic and competitive exposure conditions outlined above with appropriate controls for the various additives that can potentially be released Spiking experiments for additives to assess co-exposure effects, again under realistic / competitive conditions since the target xenobiotic will not be the only species that binds to the MP surface under environmental conditions Co-investigation of MPs and flow conditions (flooding/drought / riffles for deposition etc.) on bioavailability, uptake and retention of MPs. Assessment of wider ecosystems services, such as water purification by biofilms, bioturbation by benthic species etc.

Table 1 The key research challenges whose resolution will represent milestones in understanding the controls of exposure, uptake and propagation of microplastics in aquatic food webs and selection of scientific advances required to address those key challenges.

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