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Microplastics in freshwater fishes: Occurrence, impacts and future perspectives

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Abstract

Microplastics (MPs) are small, plastic particles of various shapes, sizes and polymers. Although well studied in marine systems, their roles and importance in freshwater environments remain uncertain. Nevertheless, the restricted ranges and variable traits of freshwater fishes result in their communities being important receptors and strong bioindicators of MP pollution. Here, the current knowledge on MPs in freshwater fishes is synthesized, along with the development of recommendations for future research and sample processing. MPs are commonly ingested and passively taken up by numerous freshwater fishes, with ingestion patterns often related to individual traits (e.g. body size, trophic level) and environmental factors (e.g. local urbanization, habitat features). Controlled MP exposure studies highlight various effects on fish physiology, biochemistry and behaviour that are often complex, unpredictable, species-specific and nonlinear in respect of dose–response relationships. Egestion is typically rapid and effective, although particles of a particular shape and/or size may remain, or translocate across the intestinal wall to other organs via the blood. Regarding future studies, there is a need to understand the interactions of MP pollution with other anthropogenic stressors (e.g. warming, eutrophication), with a concomitant requirement to increase the complexity of studies to enable impact assessment at population, community and ecosystem levels, and to determine whether there are consequences for processes, such as parasite transmission, where MPs could vector parasites or increase infection susceptibility. This knowledge will determine the extent to which MP pollution can be considered a major anthropogenic stressor of freshwaters in this era of global environmental change.

KEYWORDS

anthropogenic stressors, bioindicators, effects, egestion, exposure, ingestion

1 | INTRODUCTION

1.1 | Microplastics in the environment

The ubiquity of microplastics (MPs), small plastics particles <5 mm in diameter (Barnes et al., 2009), has recently developed into an environmental issue of high societal concern, especially as MP pollution is intricately linked to the use of plastics in everyday life (Rodrigues et al., 2019). Primary MPs are deliberately manufactured within this general size range for use in industry or various cosmetic products (Godoy et al., 2019; Guerranti et al., 2019; Yurtsever, 2019), whereas secondary MPs form from the breakdown of larger plastics

through physical, chemical and biological degradation (Kundungal et al., 2019; Raddadi & Fava, 2019; Sánchez, 2019; Winkler et al., 2019).

Microplastics are highly diverse and vary in size, shape, colour, polymer type and their constituent chemicals that all affect how they behave in the environment (e.g. their transport, degradation, adsorption capacity and ultimate fate). Nevertheless, variants of polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), polystyrene (PS) and polyvinyl chloride (PVC) account for 90% of all plastic polymers used (Andrady & Neal, 2009) and, therefore, most MPs also. All MPs can be considered as dynamic, being continually modified and degraded over time to produce ever smaller particles, eventually forming nanoplastics (NPs) <1 μm (Gigault et al., 2018).

Microplastics were identified within the marine environment in the 1970s (Carpenter et al., 1972; Carpenter & Smith, 1972), though the term was introduced later by Thompson et al. (2004), with many studies subsequently identifying MPs in freshwater and terrestrial systems, where both water (Akdogan & Guven, 2019; Bank & Hansson, 2019; Wu et al., 2019) and wind (Chen et al., 2020; Huang, Qing, et al., 2020; Zhang et al., 2019) are major transport mechanisms. Several studies have also highlighted that waste plastics, including MPs, in aquatic systems typically originate from the land, demonstrating the interconnectedness of aquatic and terrestrial systems (Malizia & Monmany-Garzia, 2019; de Souza Machado et al., 2018).

Plastic particles move throughout aquatic systems and float, sink or settle depending on particle properties (density, shape etc.), environmental features (water density, salinity, flow rate etc.) and aquatic processes (e.g. water currents and storm events) (Bondelind et al., 2020; Li, Zhang, et al., 2020). The ultimate fate of MPs is to accumulate in sinks, such as river sediments (Simon-Sánchez et al., 2019; de Villiers, 2019). MPs may also become temporarily or permanently trapped within algal mats (Feng et al., 2020) or by physical barriers such as dams (Watkins et al., 2019). Environmental perturbations, such as flooding, weather events and habitat alterations, can then free previously trapped or sunk MPs into the environment, which then gradually pass through aquatic systems and biota (von Friesen et al., 2020; O'Connor et al., 2019; Ockelford et al., 2020).

Studies on ingestion reveal that environmental MPs are consumed by a range of different taxa across varying trophic positions (TPs), feeding types and habitats (Gouin, 2020; Ribeiro et al., 2019). Many of these studies have focused on marine organisms, especially taxa of ecological, economic or conservation interest (Casabianca et al., 2019; Katyal et al., 2020; Setälä et al., 2014; Ward et al., 2019). A secondary goal is often trying to understand the potential human exposure via ingestion of contaminated fauna (Oliveira et al., 2019; Prata et al., 2020; Rainieri & Barranco, 2019; Walkinshaw et al., 2020). Complementary studies have focused on developing understandings of how MP exposure affects animal physiology, population dynamics, ecology and behaviour (Anbumani & Kakkar, 2018; Franzellitti et al., 2019; Guzzetti et al., 2018; Prokić et al., 2019). Although the main research focus has been on marine species and systems, there is increasing knowledge on how MPs behave and their consequent effects in freshwater (Li et al., 2018; Strungaru et al., 2019; Triebskorn et al., 2019).

1.2 | Issues of MPs in freshwaters and freshwater fishes

Though covering only a relatively small proportion of the surface of the earth (<0.01%), freshwaters are highly biodiverse and support a wide range of key ecosystem services (Dodds et al., 2013). They are also already at high risk from multiple anthropogenic stressors, including nutrient pollution, habitat loss, biological invasions and climate change (Jackson et al., 2016; Ormerod et al., 2010; Reid et al., 2019). Consequently, freshwater MPs potentially represent an additional stressor, with freshwater environments also representing a critical target habitat for future MP remediation and mitigation strategies (Karbalaei et al., 2018; Wong, Lee, et al., 2020). As much as 80% of aquatic plastic waste originates from terrestrial sources (Andrady, 2011) and often reaches marine environments via connecting freshwaters (Galloway et al., 2017).

Freshwater fishes comprise a highly diverse taxonomic group, covering a range of TPs, ecological guilds and life history strategies (Noble et al., 2007). With the exception of diadromous fishes, they spend their lives within a limited area, where the presence of anthropogenic barriers may further limit

their range (Grill et al., 2019). Thus, freshwater fish populations and communities may be continuously exposed to a range of MPs throughout their lives and must adapt to, or tolerate all changes within their local environment, particularly where their movement is restricted. Freshwater fish are, therefore, a key receptor and bioindicator of MP pollution and so represent strong model taxa for developing knowledge on how MPs affect the ecology and behaviour of animals, from individuals through to community levels. Consequently, in this review, we synthesize the issues and knowledge gaps relating to MPs in freshwater fishes and suggest future research directions and approaches. The objectives of this knowledge synthesis are to: (a) summarize the major sources of and transport of MPs into freshwaters; (b) outline the major ingestion–egestion and processing pathways within freshwater fish; (c) detail the principal impacts of MPs on freshwater fish; and (d) outline a series of future perspectives on research priorities and approaches. Figure 1, below, summarizes the sources, transport, processes and pathways relating to MPs in freshwater fish discussed in this review.

2 | OCCURRENCE OF MPs IN FRESHWATER FISHES: FROM SOURCES TO EGESTION

2.1 | Sources of freshwater MPs

Most freshwater MPs originate from terrestrial systems (Andrady, 2011), with the extent of local urbanization being a strong predictor of MP loadings in nearby water bodies, which are dependent on, and also a proxy of, local plastic usage and disposal (Kataoka et al., 2019; Tibbetts et al., 2018). The breakdown of larger plastic materials (including paints, tyres and litter) by physical, chemical and biological processes is an important source of secondary MPs to freshwaters (Fadare et al., 2020; Horton et al., 2017; Karbalaei et al., 2018; Knight

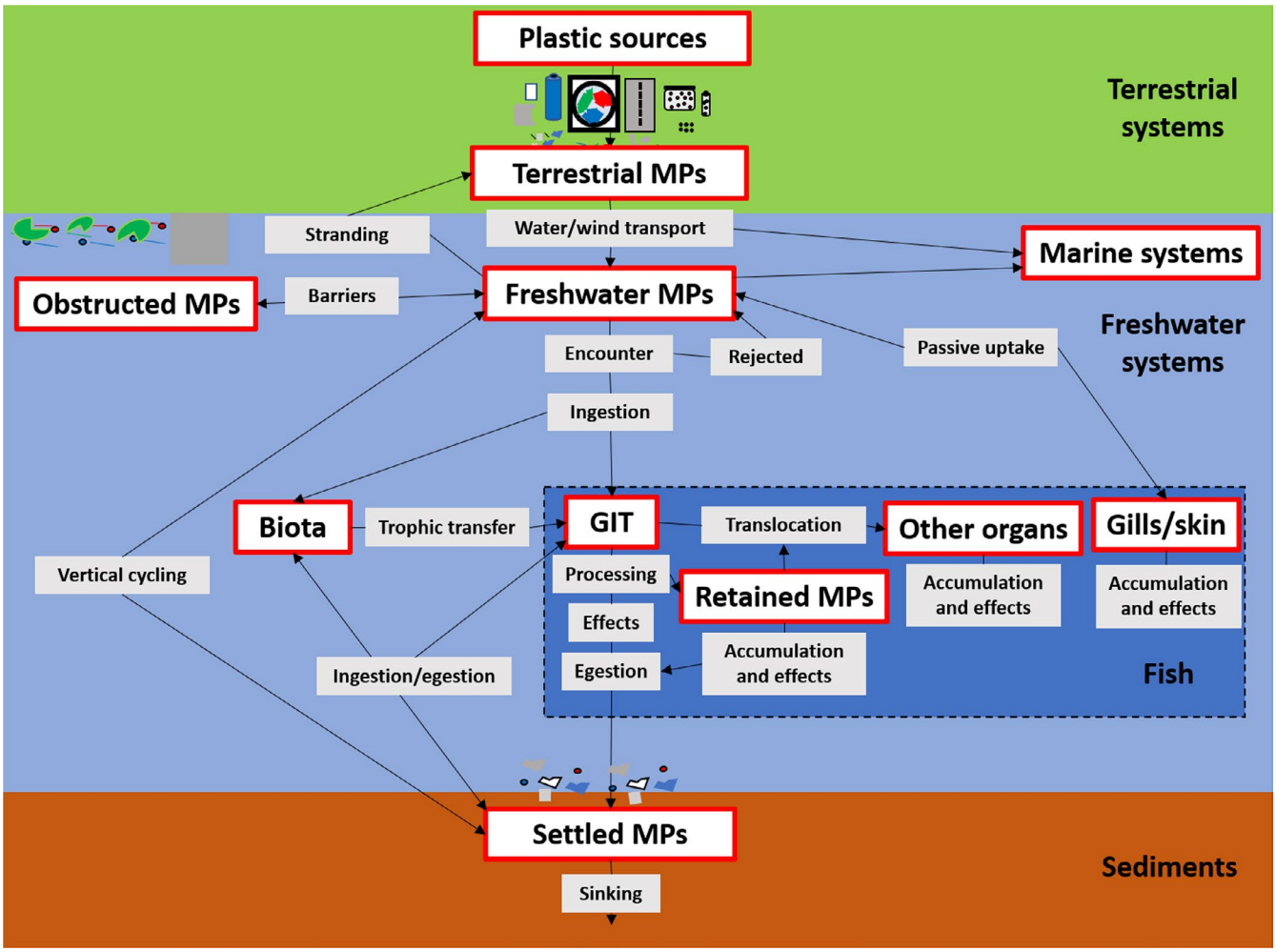

FIGURE 1 Generalized overview of microplastics in freshwater fishes including sources, transport and transfer between different biota and systems. Grey boxes indicate processes, red outlined boxes indicate abiotic and biotic compartments and arrows indicate the directionality of microplastic transfer. "MPs" is used as an abbreviation for microplastics and "GIT" for the gastrointestinal tract. [Colour figure can be viewed at wileyonlinelibrary.com]

et al., 2020). Recreational freshwater activities such as boating and angling may also contribute MPs or larger plastic waste directly into rivers and lakes through the degradation of plastic lines, nets, boats, waders etc.; however, these potential sources have yet to be investigated and quantified. MPs, along with larger litter, accumulate on impermeable surfaces or in dusts, and are washed into storm drains then water courses after sufficient rain (Liu, Li, et al., 2019; Roychand & Pramanik, 2020; Yukioka et al., 2019). Some MPs and smaller litter may also be transported by the wind and deposited in various urbanized or remote environments, where dispersal tends to be higher for smaller and lighter particles, particularly fibres (Chen et al., 2020; Huang, Qing, et al., 2020; Y. Zhang et al., 2019; Zhang et al., 2020).

The textile industry is another source of MP pollution as synthetic polymers such as polyester are commonly used to make clothing. The different synthetic materials used as well as the particular manufacturing process may dictate the sustainability and lifespan of the item of clothing (Janaina et al., 2020) and how many secondary MPs, typically fibres, are shed when the item is worn or washed (Belzagui et al., 2019; Henry et al., 2019; Hernandez et al., 2017; Napper & Thompson, 2016). Aspects of the wash cycle, the machine and the washed load may also impact both the number and type of fibres released (Cai et al., 2020; De Falco et al., 2019; Yang et al., 2019). Synthetic fibres from clothes washing, together with rinsed cosmetics and other flushable plastics (Guerranti et al., 2019; Morritt et al., 2014), then navigate the sewage system within wastewater.

This wastewater eventually passes through the sewage system to wastewater treatment plants (WWTPs), which treat domestic and industrial waste before release into the natural environment (often rivers and estuaries) (Ngo et al., 2019). Waste may undergo biological, chemical and physical processing to remove large debris, neutralize harmful chemicals and degrade biological materials, with these processes simultaneously degrading and modifying MPs throughout treatment (Enfrin et al., 2019; Li, Mei, et al., 2019). Furthermore, there is evidence that MPs may reduce the efficiency of the treatment processes if they contain harmful internal or bound chemicals that can inhibit biological processing stages (Zhang & Chen, 2019). While WWTPs vary in their treatment processes, even highly efficient WWTPs that approach 98% MP removal/exclusion still allow the daily discharge of substantial numbers of MPs in treated sewage effluents (Conley et al., 2019; Hidayaturrahman & Lee, 2019; Hyesung Lee & Kim, 2018). Waste sludge may accumulate up to 98% of MPs from the treated water (Gies et al., 2018; Nizzetto et al., 2016), which can account for up to 3% of biowaste by weight (Mohajerani & Karabatak, 2020), and is often modified for use as fertilizer if it is not incinerated or disposed at landfill (Edo et al., 2020; Rolsky et al., 2020). Agricultural application thereby provides a secondary opportunity for these MPs to directly reach water courses through runoff, wind dispersal and deposition, together with any MP-bound or internalized chemicals.

2.2 | Transport of MPs in freshwater

Microplastics in freshwaters tend to move and behave according to plastic particle properties such as size, shape or polymer density as well as features and processes within the system (Bondelind et al., 2020; Figure 1). MPs may be transported floating, in the water column or carried along the bottom sediments and may have variable residence times in the environment, depending on whether MPs are ingested, impeded or sink and settle onto or into bottom sediments (Daily & Hoffman, 2020; Hoellein et al., 2019; Song, Jongmans-Hochschulz, et al., 2020). Flow conditions and sediment type can often favour MP accumulation within the sediments, even for particles with relatively low polymer densities, resulting in concentrations often exceeding those of the overlaying surface waters (Frei et al., 2019; Simon-Sánchez et al., 2019; de Villiers, 2019).

Microplastics trapped within sediments may eventually permeate into groundwaters or aquifers before re-joining the water cycle (O'Connor et al., 2019; Re, 2019), or may be freed by storm and rain events that may resuspend trapped MPs and introduce them back into aquatic systems (Bondelind et al., 2020; Ockelford et al., 2020; Piñon-Colin et al., 2020). Since freshwater and climatic factors may vary seasonally, there can also be some temporal variation, with environmental MP loadings typically being higher during wet seasons (Campanale et al., 2019; Eo et al., 2019; Kurniawan & Imron, 2019; Weideman et al., 2019; Yuan et al., 2019). Meandering and differences in flow and sediment profiles within systems can also produce spatial differences in MP concentrations between the littoral and mid-channel areas of rivers (Wong, Löwemark, et al., 2020). The same processes also largely affect lakes and ponds, though MPs may persist in static water bodies for longer (Daily & Hoffman, 2020).

The high surface area to volume ratio, the degree of hydrophobicity and the surface structure of MPs may promote the colonization of plastic particles by various microorganisms within natural aquatic systems, altering particle density and interactions with biota and other surfaces (Caruso, 2019; Shen et al., 2019). Degradation and modification of MPs in aquatic systems may also favour the release of internal chemicals (e.g. additives, plasticizers) and/or the binding of various organic and inorganic chemicals present in the environment (e.g. metals, pharmaceuticals, fungicides) depending on environmental conditions, local concentration and MP properties (Caruso, 2019; Godoy et al., 2020; Liu, Zhu, et al., 2019; Magadini et al., 2020; Wang, Yang, et al., 2019). The modification of and binding by MPs in turn impact their transport, density and effects.

2.3 | MP encounter rates in freshwater fishes

As ectotherms, fish activities and feeding rates are intrinsically linked to the environmental temperature, but will also vary according to the size, sex and metabolic activity of individuals, as well as the abundance, nutritional quality and processing time of their prey resources (Jobling, 1981). Consequently, these same factors may also govern the encounter and ingestion rates of MPs by fish. Although MP encounter by fish is assumed to occur mainly during active feeding, there is increasing evidence of MPs being encountered via the gills and/or epidermis of wild freshwater fish (Abbasi et al., 2018; Hurt et al., 2020; Park et al., 2020). Experimental studies have also

demonstrated MP accumulation on the gills (Mak et al., 2019; Roch et al., 2020). Passive uptake of MPs is thus an additional source of MPs following environmental exposure during swimming and respiration. Collard et al. (2017) quantified the ingestion of anthropogenic particles in several marine fishes and found that ingestion was highest in the species with the most efficient filtration apparatus (high filtration area and small gill raker spacing). In a similar way, fish features such as gill surface area, gill structure, habitat etc. may correlate with numbers of MPs on the gills suggesting passive accumulation.

The foraging habitats of freshwater fish should also impact MP encounter rates, given MP distributions and loadings differ, with typically higher loadings in sediments compared to overlaying surface waters (Bondelind et al., 2020; Boucher et al., 2019; Li, Geng, et al., 2019). Consequently, within a given location, pelagic species ought to encounter fewer floating MPs (e.g. less dense fibres and beads) than benthivores, with the latter then potentially encountering higher concentrations of sunk and settled MPs (e.g. denser fragments and films), provided there is also a relatively higher benthic MP concentration. Correspondingly, the trophic level of a fish and feeding guild can also affect MP exposure levels, with obligate piscivores potentially only encountering MPs passively or indirectly via ingested prey, whereas species in other feeding guilds are more likely to directly encounter MPs associated with vegetation or detritus (Hoang & Felix-Kim, 2020; Kalčíková, 2020; Ribeiro et al., 2019).

2.4 | MP ingestion by freshwater fish

The ingestion patterns of MPs in freshwater fishes have been well documented (Table 1) and may vary depending on the encounter rate, MP characteristics and whether the particle is externally identified as a nonresource item (Collard et al., 2019; Markic et al., 2020). Gape size constrains the prey and MP sizes a fish can ingest, with larger fish generally having larger maximum particle sizes and/or MP loadings (Dantas et al., 2012; Pegado et al., 2018; Ramos et al., 2012; Ryan et al., 2019), although there are exceptions (McNeish et al., 2018; Slootmaekers et al., 2019; Vendel et al., 2017). A general review of plastic ingestion in animals suggests that body size alone can explain as much as 42% of variations in plastics consumed by animals and that the ratio of the maximum ingestible plastic size for an animal relative to its body size is approximately 1:20 (Jâms et al., 2020). Sex has also been implicated as a possible explanation for differences in freshwater fish MP patterns (Horton et al., 2018; Su, Nan, et al., 2019), although this might be an artefact of body size, given patterns of sexual dimorphism in fish (Parker, 1992).

The issue of body size is, however, complicated at the individual level, given that ontogenetic dietary shifts facilitate changes in prey items and sizes, which then result in changes in TP (Campbell et al., 2017; Hurt et al., 2020; Khan et al., 2020; McNeish et al., 2018; Roch et al., 2019). With their increased gape, body size, and TP, MPs are potentially accumulated in the gastrointestinal tract (GIT) of apex predators, as demonstrated in populations of largemouth bass (*Micropterus salmoides*, Centrarchidae) (Hurt et al., 2020) and northern pike (*Esox lucius*, Esocidae) (Campbell et al., 2017), which both had higher GIT MP levels compared to fishes of lower TP from the same system. However, pike have also been found to have fewer MPs than other fishes (Roch et al., 2019), suggesting some complexity in the transfer of MPs within food chains and the limitations of current "snapshot" MP screening methods. Moreover, a recent general review of plastic ingestion in animals suggested no evidence of bioaccumulation or biomagnification (Gouin, 2020) where MPs cross the intestinal barrier then translocate into and accumulate within tissues, respectively. It should however be noted that ingestion research to date is heavily biased towards MP accumulation in the GIT which, while inside the body, is considered external as it is continuous with the environment at the mouth and anus, though several studies have identified MP bioaccumulation within the liver, brain and muscle of freshwater fish (Abbasi et al., 2018; Batel et al., 2016; Ding et al., 2020; Su, Nan, et al., 2019). The reported mean MP incidence of 20%, a mean of four particles per individual and a general range of up to 10 pieces per individual (Gouin, 2020) is largely consistent with ingestion studies in freshwater fishes (Table 1). The trophic transfer of MPs has been demonstrated experimentally from water fleas (*Daphnia magna*, Daphniidae) to fathead minnows (*Pimephales promelas*, Cyprinidae) (Elizalde-Velázquez et al., 2020), brine shrimp (*Artemia* sp., Artemiidae) to zebrafish (*Danio rerio*, Cyprinidae) (Batel et al., 2016) and from tintinnid (*Favella* sp., Ptychocyclidae) to inland silversides (*Menidia beryllina*, Atherinopsidae) (Athey et al., 2020). Thus, ingestion of MPs via resource items is a viable transfer mechanism in freshwater fish.

The relationship between fish consumption rates and MP exposure concentration followed a Type II functional response curve in banded tilapia (*Tilapia sparrmanii*, Cichlidae), with consumption of MPs even at low concentrations (Mbedzi et al., 2019). Experiments have also suggested that the probability of ingestion is elevated where MPs have similar characteristics (appearance, smell and/or taste) to common prey resources (Roch et al., 2020; de Sá et al., 2015). The ingestion of a red MP fibre by a sight-feeding fish may occur, perhaps due to its similarity to chironomid larvae, whereas the binding or leaching of info-chemicals, such as dimethyl sulphide, can induce ingestion by taste-feeding marine copepods (Procter et al., 2019) and fish (Savoca et al., 2017). While dimethyl sulphide is not present in freshwaters, most likely through an absence of the microorganisms that produce it (Zink & Pyle, 2019), similar freshwater info-chemicals might be discovered.

The increased ingestion rates of MPs when coupled with a feeding cue is supported by experimental evidence that many fish will readily reject MPs except when they are presented in combination with food items (Kim et al., 2019; de Sá et al., 2015; Xiong et al., 2019). It could also explain why older MPs with altered structures and chemistry might be more likely to be consumed than virgin MPs, given that older MPs tend to degrade, develop biofilms or bind other chemicals over time (Chen, Xiong, et al., 2019; Song, Hou, et al., 2020). The adsorption and uptake of MPs by aquatic plants is another understudied concentrating mechanism that has the potential

TABLE 1 Overview of studies accessible before the 15th May 2020 investigating microplastic ingestion in at least one wild freshwater fish (including estuarine/temporarily freshwater fish)

Species	Country	*N*	FO	Mean	Polymer(s)	References
3	Brazil	182	23	–	(PA)[a]	Possatto et al. (2011)
2	Brazil	569	7.9	–	(PA)[a]	Dantas et al. (2012)
3	Brazil	425	13.4	–	(PA)[a]	Ramos et al. (2012)
1	France	186	12	–	–	Sanchez et al. (2014)
4	Switzerland	40	7.5	–	–	Faure et al. (2015)
44	USA	419	8.2	–	PP, PES, AC, PS	Phillips and Bonner (2015)
2	Tanzania	40	20	–	PE, PU, PET	Biginagwa et al. (2016)
2	USA	436	45	0.8	–	Peters and Bratton (2016)
1	South Africa	70	73	3.8	–	Naidoo et al. (2016)
1	Brazil	530	64.2	–	–	Ferreira et al. (2016)
2	UK	76	66	0.5	PES, PA, AC, PET	McGoran et al. (2017)
6	China	–	95.7	2.4	CE, PET, PES	Jabeen et al. (2017)
1	Brazil	48	83	3.6	–	Silva-Cavalcanti et al. (2017)
2	Switzerland	25	24	1.15	–	Roch and Brinker (2017)
69	Brazil	2,233	9	1.06	–	Vendel et al. (2017)
13	China	35	25.7	0.86	PE, PA	K. Zhang et al. (2017)
5	Canada	181	73.5	3.28	–	Campbell et al. (2017)
11	Argentina	87	100	19.2	–	Pazos et al. (2017)
4	South Africa	36	100	–	–	Naidoo et al. (2017)
3	Portugal	120	38	1.67	PE, PP, PET, PA, RAY	Bessa et al. (2018)
3	Australia	93	–	1.37	PET, RAY	Halstead et al. (2018)
1	China	30	60	4.3	PP, PE	Cheung et al. (2018)
1	UK	64	32.8	0.69	PE, PP, PET	Horton et al. (2018)
11	USA	74	85	–	–	McNeish et al. (2018)
46	Brazil	189	13.7	1.2	PA, RAY, PE	Pegado et al. (2018)
2	Brazil	125	–	–	–	Silva et al. (2018)
1	France	60	15	0.15	PET, PP, PAN, PEVA	Collard et al. (2018)
21	UK	876	32	–	PET, PA, PP	McGoran et al. (2018)
16	Brazil	172	26.7	0.56	PE, PVC, PP, PA, PMMA	Andrade et al. (2019)
1	Canada	74	59	1.15	–	Collicutt et al. (2019)
1	Belgium	78	9	0.1	PET, EVA, PVC, PP, PVA, PA, CE	Slootmaekers et al. (2019)
2	China	–	–	1.7	PE, PP	Lv et al. (2019)
13	China	217	–	–	PET, PP, PE	Su, Deng, et al. (2019)
2	Brazil	529	> 50	1.4/1.5	–	Ferreira, Barletta, et al. (2019)
9	China	279	50	7	PE, PP, PET	Zheng et al. (2019)
1	China	11	91	7.64	PE, PP	Yuan et al. (2019)
1	Australia	180	19.4	0.6	PET, RAY, PA, PP	Su, Nan, et al. (2019)
3	Brazil	529	58	1.46	–	Ferreira, Barletta, Lima, Morley, et al., 2019
1	USA	44	100	9	–	Ryan et al. (2019)
22	Germany	1167	18.8	0.2	–	Roch et al. (2019)
1	Argentina	21	100	9.9	–	Blettler et al. (2019)
2	USA	96	100	–	–	Hurt et al. (2020)
2	Poland	389	54.5	1.16	–	Kuśmierek and Popiołek (2020)
4	South Africa	174	52	0.79	RAY, PET, PA, PVC	Naidoo et al. (2020)

(Continues)

TABLE 1 (Continued)

Species	Country	*N*	FO	Mean	Polymer(s)	References
8	Thailand	107	72.9	1.76	—	Kasamesiri and Thaimuangphol (2020)
32	China	120	30/47	2.83	PE, PET, PP, PS	Huang, Koongolla, et al. (2020)
6	South Korea	6	100	22	PFTE, PE, RAY	Park et al. (2020)
2	Egypt	43	76.7	4.91	PE, PET, PP	Khan et al. (2020)
22	Colombia	302	7	0.23	PA, EVA, Latex	Garcés-Ordóñez et al. (2020)
4	China	126	—	—	—	Li, Su, et al. (2020)

Note: Studies are presented in chronological order. "Species" denotes the number of species studied, "*N*" the sample size, "FO" the percentage frequency of microplastic occurrence, "Mean" is the mean number of microplastics per individual and "Polymer" lists the main polymers confirmed through chemical techniques.

Alphabetized polymer abbreviations: AC, acrylate; CE, cellophane; EVA, ethylene vinyl acetate; PA, polyamide; PAN, polyacrylonitrile; PE, polyethylene (high or low density); PES, polyethersulphone; PET, polyethylene terephthalate/polyester; PEVA, polyethylene vinyl acetate; PTFE, polytetrafluoroethylene; PMMA, polymethyl methacrylate; PP, polypropylene; PS, polystyrene; PU, polyurethane; PVA, polyvinyl alcohol; PVC, polyvinyl chloride; RAY, rayon.

[a]Denotes studies in which the polymer was reported without chemical confirmation.

to increase the ingestion probability of associated MPs by herbivorous fish (Kalčíková, 2020). Nevertheless, debate remains over important questions such as whether fish are able to distinguish MPs from prey resources and to assess the suitability of MPs as a food source prior to ingestion, if fish learn to avoid or ingest MPs, whether ingestion is deliberate and under which conditions does MP ingestion increase (Huuskonen et al., 2020; Li, Su, et al., 2020; Peters & Bratton, 2016; Ramos et al., 2012; Ryan et al., 2019).

2.5 | MP processing and egestion

Following ingestion, the morphology of both the GIT and ingested MPs may affect their passage through the fish (Jabeen et al., 2017) and whether they temporarily or permanently lodge on to GIT structures, such as coils or projections. Differences in GIT structure have been suggested as a driver of species-specific differences in MP levels (Jabeen et al., 2017), although uncertainty remains over how particular MP morphometrics and physicochemical properties affect their passage rates through fishes and whether there are systematic biases in the MP screening of fish GITs as a consequence.

Internal environments within fish will differ in temperature and pH which may modify MPs and promote the release of certain harmful MP-associated chemicals (Coffin et al., 2019; Khan et al., 2017; Lee et al., 2019; Wu et al., 2020). These in vitro studies suggest that a variety of chemical and physiological cues in different species and sections of the GIT may change the absorption profile of MPs to leach out chemicals internalized within the particle as well as those bound to the external surface of the particle. Chemicals within MPs often include additives such as flame retardants and bisphenols which are added to plastics to achieve certain properties (Chen, Allgeier, et al., 2019; Gunaalan et al., 2020; Sun, Nan, et al., 2019), while externally bound chemicals may include a variety of pharmaceuticals, fertilizers, pesticides and heavy metals encountered and bound in the freshwater environment (Atugoda et al., 2020; Bradney et al., 2019; Caruso, 2019; Guan et al., 2020). MPs may therefore vector or leach a range of different chemicals into fish and other biota that then produce effects according to factors such as the type of chemical, concentration, where the chemical is released within the GIT and whether the chemicals are taken up across the intestinal barrier (Bradney et al., 2019; Gunaalan et al., 2020).

Microplastics size and shape are also important features affecting the processing of ingested MPs, particularly as small particles, typically <10 μm, may translocate across the intestinal barrier, reaching the blood and eventually the rest of the body (Ribeiro et al., 2019). The range of particles that may cross the intestinal barrier does, however, vary with species and only particles of a particular shape, size and chemistry may pass (Ribeiro et al., 2019). The MPs ingested by fish may already be capable of translocation; however, MPs may also be modified and degraded internally throughout the GIT by processes such as maceration, digestion etc. which may produce particles capable of translocation within fish, though this has yet to be investigated.

Within fish, translocated MPs, NPs and their associated contaminants have been recovered from regions such as the liver, muscle and brain (e.g. Abbasi et al., 2018; Batel et al., 2016; Ding et al., 2020; Su, Nan, et al., 2019), suggesting some risk of MP trophic transfer to piscivorous fauna. The MPs present in a fish at the point of capture should thus be considered a snapshot representing those currently trapped, as well as those yet to be egested or translocated. The varying individual diets, rates of internal physiological processes and time since egestion once caught/euthanized will, therefore, provide varying GIT MP loadings, even if the fish are of the same species, size and sex, and from the same local environment. Additionally, processing tissues of the same fish may provide a greater level of data on fish MP exposure over a slightly longer time period. Tissue MP levels could be correlated to both gut concentrations and factors such as body condition which may depend more on translocated tissue MP concentrations than those accumulated, temporarily or permanently, in the gut.

Laboratory experiments have demonstrated that goldfish (*Carassius auratus*, Cyprinidae) may clear 50% of MPs within 10 hr and 90% within 33 hr of ingestion (Grigorakis et al., 2017), although egestion rates vary within and between species, depending on the MP and GIT structure, food availability and stomach fullness (Elizalde-Velázquez et al., 2020; Gouin, 2020; Hoang & Felix-Kim, 2020; Xiong et al., 2019). For example, fibres tend to accumulate at higher levels relative to fragments and pellets, as they are harder to egest (Qiao et al., 2019). Faecal pellets may act as a concentrated source of MPs and organic material which may be utilized by a range of pelagic or benthic organisms as it sinks and settles, transferring MPs and material between biota (Hoang & Felix-Kim, 2020; Ribeiro et al., 2019).

3 | IMPACTS OF INGESTED MPs ON FRESHWATER FISHES

Following ingestion, MPs can affect fish via three broad, nonmutually exclusive ways: (a) through physical effects of the MP itself (e.g. blocking the GIT or causing false satiation); (b) the leaching of plasticizers, additives and other harmful chemicals from within the MPs; and (c) by the desorption of harmful pollutants bound to the MPs (Strungaru et al., 2019). The effects of MP exposure on freshwater fish are thus highly variable (Table 2; Figure 2), depending on the interaction of the MP exposure (e.g. concentration, size, polymer and shape) and the ecology of the species. Effects vary from no effect to measurable changes in feeding rates, movement, gene expression, physiology, development, and/or survival (Jovanović, 2017; Wang, Ge, et al., 2019).

In general, much of the experimental MP literature for freshwater fish has focussed on using various life stages of cyprinids (Table 2), especially zebrafish, as the model species to test the effects of MP exposure, most likely due to their extensive use in toxicology research (Dai et al., 2014). The broad diet and different feeding behaviours of zebrafish (Froese & Pauly, 2019) allow several MP exposure methods, including presented paired with food items (Batel et al., 2018; Lei et al., 2018; Lu et al., 2016), within feed (Mak et al., 2019) and/or via contaminated prey resources (Batel et al., 2016, 2020).

Within controlled experimental studies, freshwater fish MP exposure levels have been highly variable, based on the type, size and shape of MPs, and, taking weight alone, span at least five orders of magnitude from 10 μg/L (Qiao et al., 2019) up to 6 g/L (LaPlaca & van den Hurk, 2020). While these exposure levels are largely within the variation of recorded freshwater environmental loads (e.g. Li, Busquets, et al., 2020; Li et al., 2018; Tibbetts et al., 2018), some higher exposure levels can be considered ecologically irrelevant based on these current freshwater data (e.g. LaPlaca & van den Hurk, 2020; Mazurais et al., 2015). Several studies have replicated specific environmental MP loadings appropriate to the life stage and habitat of the model fish (e.g. Mazurais et al., 2015; Rochman et al., 2014, 2017), with Naidoo and Glassom (2019) additionally also replicating the types and shapes of MPs in the exposure regime.

Several studies have demonstrated dose-dependent impacts of MP exposure on freshwater fish, though these effects may only occur at a particular MP concentration, suggesting MP thresholds for impact, with the relationship between exposure and impact thus being more complex than a simple linear dose–effect relationship (Lei et al., 2018; Mazurais et al., 2015; Qu et al., 2019; Zhao et al., 2020). MP impacts are often exaggerated when the fish is coexposed to an additional chemical (Banaee et al., 2019; Qiao et al., 2019; Roda et al., 2020), although antagonistic interactions can also occur where MPs modulate the harmful effects of another chemical in the fish, or may also have no interaction at all (Hatami et al., 2019; Oliveira et al., 2013; Wen, Jin, et al., 2018). MP effects may also be life stage-specific, and are occasionally more detrimental to larval than adult fish, especially when MP exposure affects development (Pannetier et al., 2020).

3.1 | Physiological impacts

The most common impacts of MP exposure have been recorded at the level of the individual fish, or lower, with impacts most typically including modified patterns of expression and/or protein activity (Figure 2; Table 2). Chemicals within or bound to the MP may also be released within the fish, causing a range of impacts including altered immune activity, expression or blood biochemistry in response to the foreign MP and any associated chemicals (Table 2). GIT oxidative stress and histological damage are common impacts at the organ/tissue level (Figure 2), where the processing of MPs and/or any associated chemicals induce immune response, resulting in localized cell damage and altered morphology of physiological structures (Lu et al., 2016; Yu et al., 2020). Changes in GIT morphology may also alter the types and activity of symbiotic microorganisms, resulting in gut dysbiosis and altered metabolism (Ding et al., 2020; Jabeen et al., 2018; Qiao et al., 2019; Xia et al., 2020; Zhao et al., 2020). Typically, sufficient cellular level effects scale up to tissue/organ then individual-level impacts etc. as different numbers and types of cells are affected by MP exposure (Figure 2).

Blood biochemistry and immune biomarkers may indicate the absorption and translocation of MPs or associated chemicals across the gut, and are useful indicators for identifying dysfunction and damage in various non-GIT organs and tissues (e.g. Banaee et al., 2019; Hamed et al., 2019; Karami et al., 2016; Qiao et al., 2019; Roda et al., 2020). Changes to blood composition may result in anaemia, altered immune function and nutrient supply throughout the body (e.g. Hatami et al., 2019; Karami et al., 2016; Roda et al., 2020). MPs in the brain may interfere with the endocrine or central nervous system, which can impact individual growth, body condition, behaviour and/or survival (e.g. Athey et al., 2020; LaPlaca & van den Hurk, 2020; Lei et al., 2018; Xia et al., 2020). Functional disruption of key organs, such as the kidneys, liver and brain, can then impact body fluid composition, neurotransmitter and endocrine pathways (Walpitagama et al., 2019; Wang, Wei, et al., 2019), with survival consequences for the individual (Figure 2).

TABLE 2 Overview of studies accessible before the 15th May 2020 investigating impacts of microplastics on freshwater fish (including estuarine/temporarily freshwater fish), grouped by taxa

Taxa	Impact(s)	Source
Acipenser transmontanus, Acipenseridae	Protein levels and feeding behaviour altered (J)	Rochman et al. (2017)[a]
Ambassis dussumieri, Ambassidae	Growth, survival and body condition reduced (J)	Naidoo and Glassom, (2019)
Barbodes gonionotus, Cyprinidae	Increased protein levels; epithelial thickening (J)	Romano et al. (2018)
Carassius auratus, Cyprinidae	Reduced body weight; mouth and GIT damage GIT damage; oxidative stress; altered growth/activity (L)	Jabeen et al. (2018) Yang et al. (2020)
Clarias gariepinus, Clariidae	GIT damage; protein levels/blood chemistry altered (J) Altered protein levels; liver damage; oxidative stress (J)	Karami et al. (2016)[a] Iheanacho and Odo, (2020)
Cyprinus carpio, Cyprinidae	Oxidative stress; protein levels/blood chemistry altered (J) Blood chemistry, protein and immune activity altered (J) Reduced growth; GIT damage; protein levels altered (L)	Hatami et al. (2019)[a] Banaee et al. (2019)[a] Xia et al. (2020)
Danio rerio, Cyprinidae	No impact on protein levels or GIT structure (A) GIT damage; metabolism and protein levels altered (A) Altered protein levels in adults only (A + E) GIT damage leading to some mortality (A) GIT damage; protein levels and gut biota altered (A) Altered protein levels in larvae only (E + L) Metabolism, protein levels and gut biota altered (L) Protein activity and swimming behaviour altered (L) Altered protein levels (E) Altered protein levels GIT damage; protein levels and behaviour altered (A) GIT damage; protein levels and behaviour altered (A) GIT damage; metabolism and gut biota altered Condition, protein levels and metabolism altered (A) No adverse effects (A) Oxidative stress; protein levels altered (A) Protein levels and metabolism altered; delayed hatching (E) Protein activity altered due to lead leaching (L)	Batel et al. (2016)[a] Lu et al. (2016) Batel et al. (2018)[a] Lei et al. (2018) Jin et al. (2018) LeMoine et al. (2018) Wan et al. (2019) Qiang and Cheng, (2019) Cormier et al. (2019)[a] Qiao et al. (2019)[a] Limonta et al. (2019) Mak et al. (2019) Qiao et al. (2019) Zhao et al. (2020) Batel et al. (2020)[a] Yu et al. (2020)[a] Duan et al. (2020) Boyle et al. (2020)
Dicentrarchus labrax, Moronidae	Protein levels altered and some mortality (L)	Mazurais et al. (2015)
Fundulus heteroclitus, Fundulidae	Protein levels altered and some mortality (A)	LaPlaca and van den Hurk (2020)
Lates calcarifer, Latidae	Coexposure impaired feeding and swimming (J)	Guven et al. (2018)[a]
Menidia beryllina, Atherinopsidae	Reduced growth rate (L)	Athey et al. (2020)[a]
Misgurnus anguillicaudatus, Cobitidae	Coexposure induced oxidative stress (A)	Qu et al. (2019)[a]
Oreochromis niloticus, Cichlidae	Blood and body chemistry altered; anaemia induced (J) Altered brain activity and metabolism (A)	Hamed et al. (2019) Ding et al. (2020)
Oryzias latipes, Adrianicthyidae	Protein levels, signalling and germ lines altered (A) Morphology, behaviour and protein levels altered (E + L)	Rochman et al. (2014)[a] Pannetier et al. (2020)
Pimephales promelas, Cyprinidae	No adverse effects (L) Protein levels altered and some mortality (A)	Malinich et al. (2018) LaPlaca and van den Hurk (2020)
Pomatoschistus microps, Gobiidae	Metabolism and neurotransmission altered (J) Predatory efficiency and performance reduced (J) Protein levels and neurotransmission altered (J)	Oliveira et al. (2013)[a] de Sá et al. (2015) Luís et al. (2015)[a]
Prochilodus lineatus, Prochilodontidae	DNA damage; blood, brain and protein activity altered (J)	Roda et al. (2020)[a]
Symphysodon aequifasciatus, Cichlidae	Predatory behaviour, metabolism and signalling altered (J) Oxidative stress; protein levels altered (J)	Wen, Zhang, et al. (2018) Wen, Jin, et al. (2018)[a]

Bracketed letters refer to the life stage(s) of taxa: "A," adult; "E," embryo; "J," juvenile; "L," larvae.
[a]Indicates studies investigating an interaction between MPs and an additional chemical.

3.2 | Biological consequences

Behavioural alterations resulting from MP exposure often occur as MPs and/or their associated chemicals impact cells in the brain or central nervous system which may negatively affect swimming activity and/or survival in freshwater fishes (Limonta et al., 2019; Mak et al., 2019; Qiang & Cheng, 2019; Yang et al., 2020). Impairments to swimming behaviour might be temporary; however, some studies

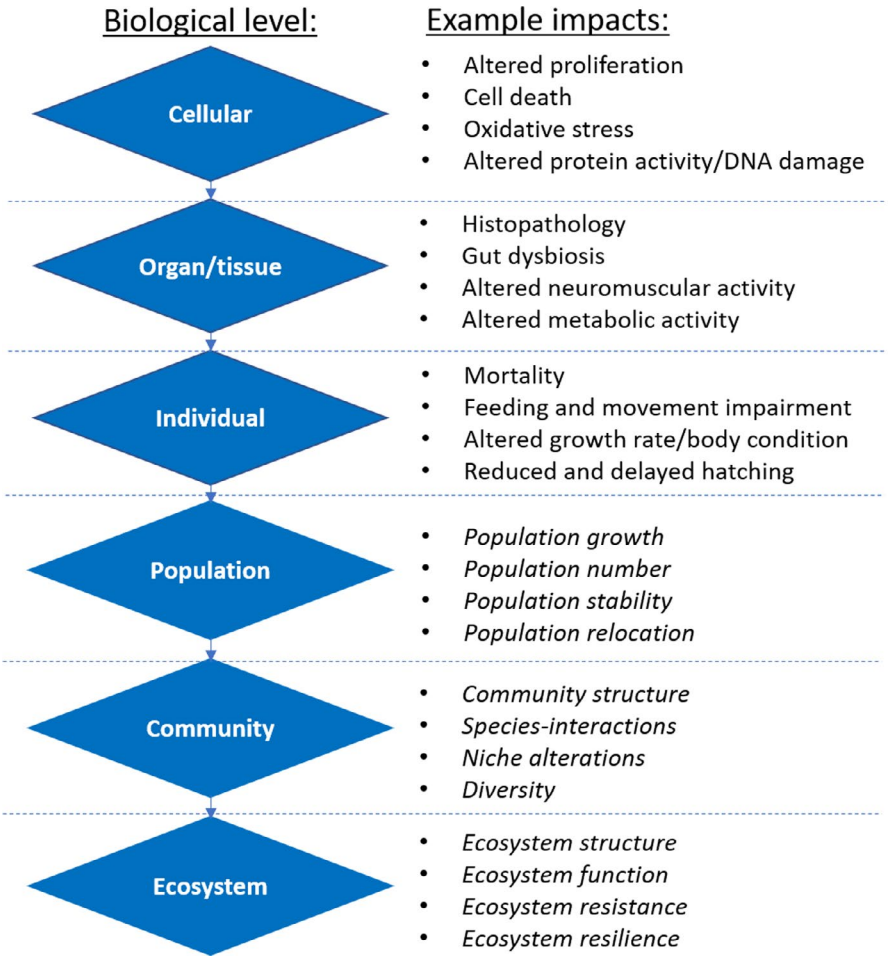

FIGURE 2 Overview of microplastic impacts on freshwater fish at different levels of biological complexity and how impacts can scale up to effects at higher levels of organization. Several common impacts are given for each biological level. Population, community and ecosystem level impacts resulting from freshwater fish exposure are thus far unknown, and so suggested potential impacts are italicized. [Colour figure can be viewed at wileyonlinelibrary.com]

also suggest more damaging effects if MP exposure impacts early development (Duan et al., 2020; Pannetier et al., 2020). Fish eggs can also externally bind MPs and/or uptake smaller NPs that can alter gaseous exchange and delay hatching times (Batel et al., 2018; Duan et al., 2020).

MP-induced reductions in predatory behaviour and efficiency have been demonstrated in juvenile common goby (*Pomatoschistus microps*, Gobiidae), most likely through affecting the discrimination of copresented prey and MP items (de Sá et al., 2015). Changes to predator–prey dynamics may modify food webs and communities through altering interaction strengths (Figure 2) and is particularly relevant since higher trophic levels and predators appear to be more vulnerable to MPs than their prey (Huang, Lin, et al., 2020). While goldfish experimentally rejected MPs that could not be chewed and swallowed, this processing damaged the mouth (Jabeen et al., 2018), potentially affecting their subsequent foraging behaviours and consumption rates.

3.3 | MPs as biological vectors

Microplastics are often implicated to aid in the binding and transport of various pathogens and invasive species (Caruso, 2019; Shen et al., 2019); however, the potential role of MPs in macroparasite transport remains unexplored. As MP uptake in fish occurs predominately through feeding (Gouin, 2020; Ribeiro et al., 2019), then the association of parasites to MPs may potentially benefit trophically transmitted parasites through increasing their transmission probabilities to fish hosts. MPs might also indirectly increase transmission rates and parasite virulence by suppressing the immune response and/or the general condition of the impacted individual (Limonta et al., 2019; Luís et al., 2015). The trophic transmission of parasites can often involve parasite manipulation of the behaviour of the intermediate host to promote their ingestion by a final host (Thomas et al., 2010). For example, infection of three-spined stickleback (*Gasterosteus aculeatus*, Gasterosteidae) by the cestode *Schistocephalus solidus* (Schistocephalidae) results in infected fish foraging on smaller prey items (Barber & Huntingford, 1995) and altering habitat utilization to increase predation encounter and subsequent parasite transfer to a final bird host (Barber et al., 2004). These parasite-mediated behavioural modifications can result in trophic differences between infected and uninfected fish within populations (Britton & Andreou, 2016), potentially also altering their exposure to MPs.

Nevertheless, the consequences of MPs for freshwater fish host–parasite relationships remain uncertain. In marine environments, anecdotal evidence suggests that MPs and parasites accumulate in the same part of the gut in seals (Hernandez-Milian et al., 2019), and fish closer to urban areas have both higher MP and parasite loads (Alves

et al., 2016). Microplastics and trophic (ingested) parasites are also most likely accumulated in the same way, with typically more of both in larger and/or predatory individuals (Lester & McVinish, 2016). Several exposure studies in freshwater fish have also suggested that MP exposure might increase susceptibility to parasites or disease (Limonta et al., 2019; Luís et al., 2015), though this was not studied. In addition, environmental plastics do attract and harbour distinct bacterial and fungal assemblages compared to natural particles, many of which can be pathogenic to fish, and may provide similar mechanisms for transporting parasites (Gong et al., 2019; Munier & Bendell, 2018; Vethaak & Leslie, 2016).

4 | FUTURE PERSPECTIVES

4.1 | Experimental approaches

Microplastics exposure studies have revealed a wide range of effects in freshwater fishes (Table 2), but there remains considerable uncertainty in how these translate into measurable impacts in wild populations, particularly above the individual level (Figure 2). Understanding the impacts of MPs on freshwater fish requires, at least in part, controlled studies that enable the decoupling of the impacts of MPs from other stressors and that use appropriate exposure conditions and model species. Therefore, it is recommended that exposure studies initially test the effects of current (or predicted) ecologically relevant MP concentrations and ensure that the concentrations, sizes, shapes and polymers of the MPs reflect the natural exposure levels of that species and life stage. In controlled studies to date, there has been a bias towards using spherical beads during exposure (e.g. Mazurais et al., 2015; Oliveira et al., 2013; de Sá et al., 2015), so there is also a need to further investigate the impacts on freshwater fish arising from exposure to fragments, fibres and films, especially as these are already known to have different egestion rates in freshwater fish (Qiao et al., 2019). The actual exposure mechanism should also aim to simulate how a particular fish might encounter MPs in the wild, such as sprinkling floating MPs and food for surface filter feeders, but spiking resource items with MPs for species at higher trophic levels.

Most studies have exposed fish to MPs via the water when feeding (e.g. Batel et al., 2018; Lei et al., 2018; Lu et al., 2016), with this an appropriate exposure route for most of the investigated species and life stages to date, typically larvae/juveniles of cyprinid zooplanktivores. However, there have been fewer impact studies using benthic-feeding or piscivorous fishes (e.g. Iheanacho & Odo, 2020; Karami et al., 2016; Oliveira et al., 2013; de Sá et al., 2015), as well as a lack of studies exposing fish via contaminated resources (e.g. Batel et al., 2016, 2020; Rochman et al., 2017), despite this often being a more effective MP transmission route than coupled with commercial fish food (Athey et al., 2020). Consequently, there remains a lack of knowledge over whether benthic fishes, which often tend to ingest more MPs than pelagic feeders within the same environment (McGoran et al., 2017, 2018; McNeish et al., 2018), are similarly disproportionally impacted by MP exposure. Trophic transfer studies could additionally determine if MP transfer efficiencies are similar from prey to predatory fish as between invertebrates and fish, which might explain different MP ingestion patterns in wild fish occupying different niches and trophic levels (Campbell et al., 2017; Hurt et al., 2020; McNeish et al., 2018; Roch et al., 2019).

For scaling up from individual MP impacts to higher levels of biological organization (Figure 2), the controlled conditions provided within mesocosm studies provide a strong experimental framework using fully factorial designs that enable the effects of MPs on community structure and function to be quantified. Such studies enable complexity to be developed and investigated, including how MP impacts are affected by other stressors, such as warming and nutrient enrichment. For example, studies utilizing mesocosms have experimentally demonstrated the trophic transfer of NPs across several trophic levels into top-predator fish (Chae et al., 2018; Mattsson et al., 2015), where quantified impacts included histological damage and alterations to feeding, shoaling behaviours and metabolism.

Alternative experimental approaches, such as exposure studies translocating organisms within mesh cages, also provide opportunities to identify how different species respond to different MP levels in the environment, as well as whether previous exposure to MPs may impact susceptibility to future exposure. Similar studies have already been completed in marine systems for blue mussel (*Mytilus edulis*, Mytilidae) (Kazour & Amara, 2020) and European flounder (*Platicthys flesus*, Pleuronectidae) (Kazour et al., 2018) and are another promising experimental framework for future MP research in freshwater fishes.

4.2 | Nonfatal field sampling

Current field sampling methods tend to result in fish being euthanized for analyses in the laboratory that provide limited short-term data (Ferreira et al., 2016; Possatto et al., 2011). Correspondingly, future studies should consider using nondestructive sampling methods where feasible. For example, stomach flushing has been used to recover up to 95% of spiked MP samples from juvenile Mexican crocodiles (*Crocodylus moreletii*, Crocodilidae) (Gonzalez-Jauregui et al., 2019), with flushing techniques already used in fish dietary analyses (e.g. Correa & Anderson, 2016; Kamler & Pope, 2001). It should, however, be noted that stomach lavage is only suitable for larger fish and can potentially cause damage, and sometimes fatality, in smaller and/or agastric individuals. The application of nonfatal MP recovery techniques can then be complemented by the ecological application of stable isotope analysis (SIA) that can provide temporal and spatial information on fish diet composition (Grey, 2006). While SIA would be unable to quantify MP loads in individual fish, it does enable information to be developed for populations in relation to their trophic (isotopic) niches (Jackson et al., 2012) and the extent of individual dietary specialization (Araújo et al., 2011), that can be tested against the numbers of MPs recovered from the GIT. This complementary approach could also identify whether differences in MP loads between individuals are a consequence or driver of dietary

specialization (Britton & Andreou, 2016). For MPs encountered passively, such as those on the gills (Hurt et al., 2020) and epidermis (Abbasi et al., 2018), then the use of "skin scrapes" and "gill swabs" (e.g. Roberts et al., 2009; Young et al., 2008) could prove suitable and noninvasive methods to quantify this mechanism of MP uptake.

Research investigating the bioaccumulation of MPs has so far focused almost exclusively on those found in the GIT (Gouin, 2020), despite being continuous with the external environment and smaller MPs being known to translocate to other parts of the body (Ribeiro et al., 2019), including the liver, muscle and brain of freshwater fish (e.g. Abbasi et al., 2018; Batel et al., 2016; Ding et al., 2020; Su, Nan, et al., 2019). The development of nondestructive techniques for tracing MPs in fish organs can potentially utilize MP carbon isotopes, as these have been used experimentally to demonstrate the incorporation of MP materials by microbes and to track the trophic transfer of these particles into animals (Taipale et al., 2019), as fluorescent dyes are often problematic and less reliable (Schür et al., 2019).

4.3 | Laboratory analyses

Reviews of current laboratory processing techniques highlight a range of different methods of digestion and MP analyses (Collard et al., 2019), with particular techniques working better for different species (Bianchi et al., 2020), but that might under- or over-estimate counts for particular polymers (Karami et al., 2017). A single, standardized procedure for all MP processing is, therefore, not possible, although attempts should be made to reduce the signal–noise ratio in samples and to comprehensively outline and critique the processing steps. In a review of plastic ingestion in wild freshwater fish, Collard et al. (2019) made numerous recommendations to standardize fish MP processing protocols, including on sample sizes, MP target size and visualization methods. In addition to these recommendations, where additional density separation of samples is employed, floatation reagents should have a minimum relative density of 1.5 g cm^{-3} to maximize the number of MPs recovered from samples and to reduce systematic underrepresentation of denser plastics (Coppock et al., 2017; Quinn et al., 2017).

Contamination is a recurring problem in MP research and it is important that all reasonable attempts are made to eliminate contamination and to determine the efficacy of implemented contamination control steps by quantifying remaining sources of contamination. Best practice reviews suggest the wearing of nonplastic personal protection equipment, the use of laminar flow cabinets to minimize atmospheric sample exposure, carrying out procedural blanks, and ensuring reagents and equipment are filtered and cleaned prior to use are all effective ways to reduce contamination to acceptable levels (e.g. <10% of sample counts) (e.g. Collard et al., 2019; Dehaut et al., 2019; Gong & Xie, 2020; Lusher et al., 2017; Scopetani et al., 2020). Studies should additionally outline the steps taken to reduce contamination, as well as declare any residual contamination and/or subsequent data correction.

Microplastic visual screening varies with individual experience and the particular shapes, sizes and colours of MP particles (Cadiou et al., 2020). MP screenings under microscopy should aim to be conservative and carried out blind, in a randomized order, with a pre-determined and standardized search time and search criteria to reduce bias and variation in counts. The processing of samples by multiple observers is another effective way of reducing individual bias by assessing interobserver reliability.

Visual screening should also be supplemented by chemical confirmation, through spectroscopy or other techniques, on a subset of samples (e.g. 10%) to confirm the proportion of suspected MPs that are actually plastics and to determine polymer types. Automated techniques, such as image processing software, should also be favoured preferentially to more subjective manual visual processing (Andrade et al., 2020; Dehaut et al., 2019; Renner et al., 2019). Many studies on MPs in freshwater fish have not utilized chemical techniques, indicated by those studies in Table 1 without polymer data, and so for these studies estimates of MP incidence are likely to be unreliable and to include various other particles of nonplastic origin. As an example, Collard et al., (2018) identified approximately a quarter of anthropogenic particles as plastics while Slootmaekers et al., (2019) found only half of suspected MPs were actually plastic. The chemical analysis of suspected MPs is therefore critical to determine reliable MP counts and to assess the actual risks of MP pollution faced by freshwater fish.

4.4 | Scaling up complexity

Much MP research in freshwater fish has been on single species (Table 1) and has focussed on individual-level effects and below (Table 2; Figure 2). Consequently, as knowledge develops, there is a need to fill in the gaps to understand MP impacts at population, community and ecosystem levels (Figure 2), as well as continuing to investigate the relative impacts of body size, sex, trophic level and other traits that affect MP ingestion patterns. Field studies should aim to collect representative communities with sufficient numbers of fish per trophic level and functional group (excluding those of conservation concern), with consideration of sampling across different seasons and under different river flows and levels.

5 | CONCLUSIONS

Both field and laboratory approaches have, to date, contributed knowledge on how particular characteristics of freshwater fish affect the encounter, ingestion, effects and egestion of MPs. Studies highlight considerable variability in ingestion patterns and effects, but that these differences can generally be explained through a combination of traits of the fish (e.g. size, trophic level, habitat), its environment (e.g. the extent of local urbanization) and the MP characteristics (e.g. size, shape, polymer, internal/external chemicals). Future field research should identify MPs in novel fish species and

locations, and develop population level and community-wide assessment approaches using standardized methods that maximize MP recovery while minimizing contamination. Experimental studies should ensure that exposure regimes and routes are environmentally relevant and investigate uptake and effects in a wider range of species, including piscivorous and benthic fishes. The interactive impacts of MPs with other anthropogenic stressors are also required, given that MP pollution is likely acting as a further stressor in environments already exposed to multiple stressors. Data generated through field- and laboratory-based studies can then move towards a more quantitative assessment of the risks faced by particular freshwater fish, with criteria on freshwater MPs able to be incorporated into freshwater monitoring programmes and species action plans.

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DATA AVAILABILITY STATEMENT

Data availability is not applicable to this article as no new data were created or analysed in this study.

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