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Review article

Microplastics as an emerging threat to amphibians: Current status and future perspectives

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ABSTRACT

Given their pervasiveness in the environment, particularly in aquatic ecosystems, plastics are posing a growing concern worldwide. Many vertebrates and invertebrates in marine, freshwater, and terrestrial ecosystems exhibit microplastic (MP) uptake and accumulation. Some studies have indicated the fatal impacts of MPs on animals and their possible transfer through food chains. Thus, it is crucial to study MP pollution and its impacts on environment-sensitive and globally threatened animal groups, such as amphibians, which also play an important role in the energy transfer between ecosystems. Unfortunately, research in this field is lacking and sources of organized information are also scarce. Hence, we systematically reviewed published literature on MPs in amphibians to fill the existing knowledge gap. Our review revealed that most of the previous studies have focused on MP bioaccumulation in amphibians, whereas, only a few research highlighted its impacts. We found that more than 80% of the studied species exhibited MP accumulation. MPs were reported to persist in different organs for a long time and get transferred to other trophic levels. They can also exhibit cytotoxic and mutagenic effects and may have fatal impacts. Moreover, they can increase the disease susceptibility of amphibians. Our study concludes the MPs as a potential threat to amphibians and urges increasing the scope and frequency of research on MP pollution and its impacts on this vulnerable animal group. We also provide a generalized method for studying MPs in amphibians with future perspectives and research directions. Our study is significant for extending the knowledge of MPs and their impacts on amphibians and guiding prospective research.

1. Introduction

Given their pervasiveness in the environment, particularly in aquatic ecosystems, plastics are posing a growing concern worldwide [1,2,3,]. Since the 1950s, the annual global production of plastics has increased dramatically (from 1.5 Mt in 1950 to 391 Mt in 2021; [4]). Recent research has suggested that only negligible parts of plastics (less than 10%) get recycled, while almost the entire plastic waste produced worldwide gets accumulated in the environment [4–6]. Most of this plastic waste does not biodegrade; instead, it photodegrades, i.e., slowly breaks down into smaller fragments [7]. Plastic particles sized less than 5 mm, also known as microplastics

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(MPs), account for a significant portion of the plastics found in the environment, including freshwater ecosystems [8]. Therefore, studies on the bioaccumulation and effects of MPs on species, communities, and ecosystems are exponentially increasing [9–12].

Studies already reported that most turtles and many other vertebrates and invertebrates in marine ecosystems are suffering from MP uptake and accumulation [13,14,]. However, in addition to oceanic ecosystems [15,16], recent studies across the globe have indicated the vulnerability of inland animal species [3,17,18], including humans [14,19–21]. In particular, wetland and water habitat-dependent animals, such as amphibians, have been found to exhibit MP accumulation [22–25]. Furthermore, several studies have suggested that MPs can be transported from aquatic ecosystems to terrestrial ecosystems through the food chain [26–28]. Experimental studies and some in-the-wild studies discovered that MPs were deposited within MP-exposed individuals until they were eaten by predators. Subsequently, they transferred to the next trophic level of the food chain or treated for identifying MP accumulation [26,29–31]. Moreover, MPs could induce neurobehavioral disorders and impair the survival, body condition, and function of many animals, including amphibians [32,33].

Despite the recent increase in the number of novel species descriptions because of integrated taxonomic approaches [34–37], amphibians are considered one of the most vulnerable vertebrate groups worldwide [38,39]. Although the research and data availability are increasing rapidly [40,41], researchers assume that the amphibian diversities in many regions are underestimated [23,36,40,42,43] and the extinction crisis is still an emergency [44]. Extinction risks are resulting from various factors, such as infectious diseases [45–49], climate change [46,50,51], habitat destruction [52–54], invasive species [55,56], and their synergistic impacts [57,58]. Anthropogenic interferences and subsequent environmental pollution also may hamper their ecology and behavior and make them more vulnerable [59–61]. Mainly the unique characteristics, which benefit amphibians to adapt to a biphasic (aquatic larval and

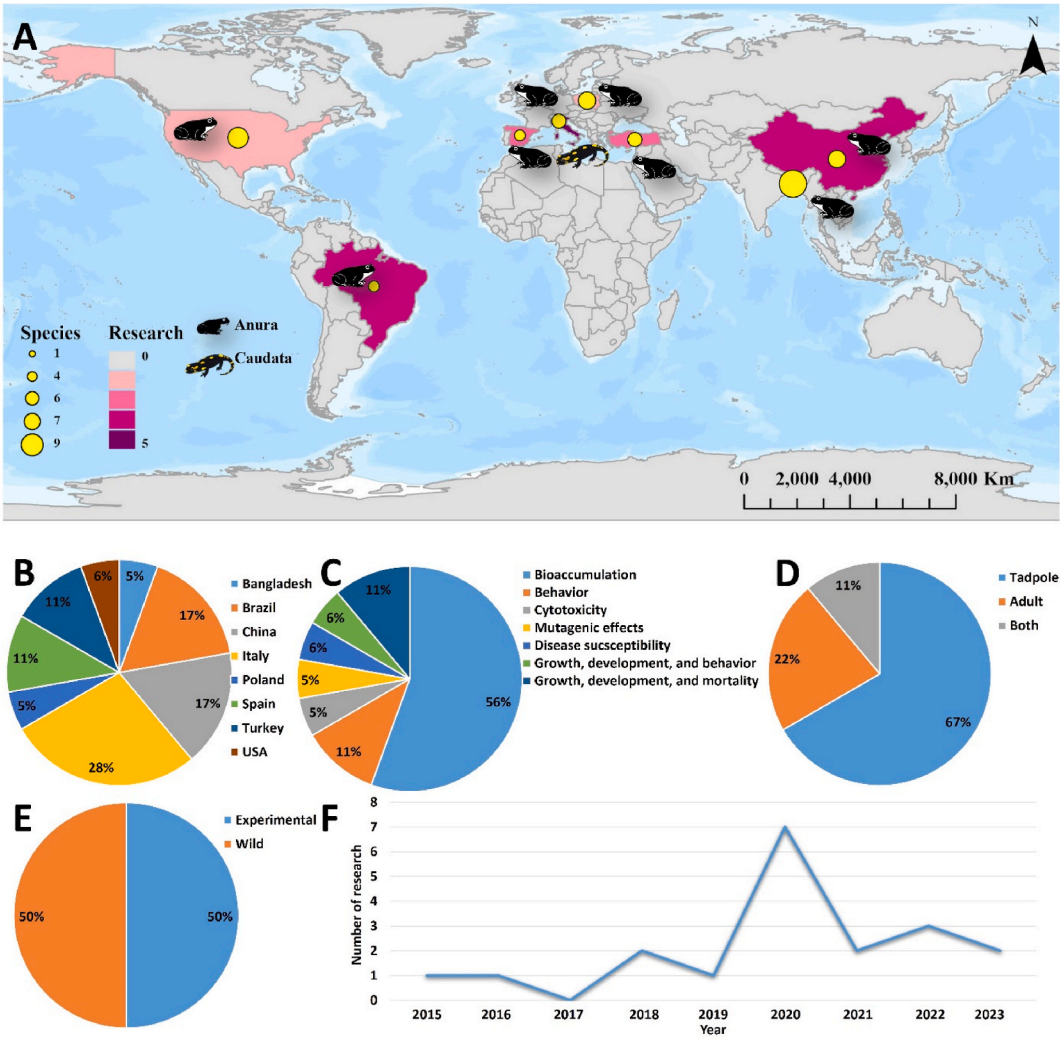

Fig. 1. Current status of studies on microplastics in amphibians. A. Contributing countries with research intensity and explored species. B. Proportion of contribution from contributing countries. C. Proportion of research focuses. D. Proportion of use of life stages in microplastic studies on amphibians. E. Proportion of in-the-wild and experimental studies. F. Research trends of microplastic studies on amphibians.

terrestrial adult) life cycle, attributed to make them sensitive to environmental changes [62,63]. Considering the amphibian sensitivity and susceptibility is biotic and abiotic factor dependent and evidenced by a manifold increase even with the presence of other species [64], pollutants like MPs may pose an additional threat to their conservation. It is already reported that MP uptake and bio-accumulation is common in amphibians, which is evident to have negative impact on their growth, development, body condition, and survivability through histopathological and mutagenic effects and disease susceptibility [33,65–67]. However, historically MPs in amphibians did not get much attention from the scientific community. While MPs were found in marine herpetofauna as early as the 2000s [68], a review of the literature showed that research on MPs in amphibians was first reported in 2015 [18]. Moreover, given the existing reports on using them as a model group to study the status and impacts of many pollutants [69–71], amphibians can be used as ecological indicators in MP-polluted environment. Thus, amphibians' unique characteristics, vulnerability, and ability to transfer pollutants between aquatic and terrestrial ecosystems [26] demand intensified research on MP accumulation and its subsequent effects on them and more attention from the scientific community.

Although research on MPs in amphibians is crucial, negligible research has been conducted so far worldwide [18]. Reviews on the current research status on MPs in amphibians and future perspectives and directions are also limited. Moreover, there are no well-established common methods to study MPs in amphibians. This study aimed to compile current research information on MPs in amphibians, formulate a simplified method, measure and predict the plausible impacts of MPs on amphibians, identify the limitations of existing knowledge, and provide future directions. To this end, we searched articles published up to April 2023 in the ISI Web of Science, ScienceDirect, and Scopus databases. We combined the words "amphibian," "tadpole," "frog," "toad," "salamander," and "caecilians" in both singular and plural forms with the phrase "microplastics" during the search. We excluded studies that were

Table 1
List of amphibian species included in the studies on microplastics (MPs).

Species name	Family	IUCN status	Habitat	Life stage	Study type	Accumulation status	MPs/individual	References
Alytes obstetricans	Alytidae	LC	G	Tadpoles	E	Y	ND	[9,14]
Bufo bufo	Bufonidae	LC	G	Tadpoles	W	Y	0–3	[8]
Bufotes viridis	Bufonidae	LC	G	Tadpoles	W	Y	0–1	[8]
Bufo gargarizans	Bufonidae	LC	G	Tadpoles	W	Y	0–2	[4]
Anaxyrus americanus	Bufonidae	LC	G	Adults	W	N	0	[6]
Duttaphrynus melanostictus	Bufonidae	LC	G	Adults	W	Y	19	[18]
Duttaphrynus himalayanus	Bufonidae	LC	G	Adults	W	Y	19	[18]
Fejervarya limnocharis (Rana limnocharis)	Dicroglossidae	LC	G	Tadpoles	W	Y	3–19	[4,15,18]
Euphlyctis cyanophlyctis	Dicroglossidae	LC	G	Adults	W	Y	19	[18]
Fejervarya moodiei	Dicroglossidae	LC	Gr/Wl/T/A	Adults	W	Y	19	[18]
Fejervarya cancrivora	Dicroglossidae	LC	F/Wl/A	Adults	W	Y	19	[18]
Hoplobatrachus tigerinus	Dicroglossidae	LC	G	Adults	W	Y	19	[18]
Hyla arborea	Hylidae	LC	G	Tadpoles	W	Y	0–2	[8]
Physalaemus cuvieri	Leptodactylidae	LC	G	Tadpoles	E	Y	ND	[7,10]
Microhyla ornata	Microhylidae	LC	G	Tadpoles	W	Y	1–3	[4,15]
Microhyla heymonsi	Microhylidae	LC	G	Tadpoles	W	Y	1–3	[15]
Pelobates fuscus	Pelobatidae	LC	G	Tadpoles	W	Y	0–1	[8]
Xenopus tropicalis	Pipidae	LC	G	Tadpoles	E	Y	ND	[2]
Xenopus laevis	Pipidae	LC	G	Tadpoles	E	Y	ND	[1,5]
Rana temporaria	Ranidae	LC	G	Tadpoles	W	Y	0–3	[8,16]
Pelophylax esculentus complex	Ranidae	NE	ND	Tadpoles	W	Y	0–3	[8]
*Pelophylax nigromaculatus**	Ranidae	NT	G	Tadpoles	W	Y	1–19	[4,18]
Pelophylax ridibundus	Ranidae	LC	G	Tadpoles/Adults	W	Y	307	[12,17]
Rana macrocnemis	Ranidae	LC	G	Tadpoles	W	Y	303	[12]
Rana latastei	Ranidae	VU	F/Wl/T	Tadpoles	E	Y	ND	[3]
Pelophylax bedriagae	Ranidae	LC	G	Adults	W	Y	5	[17]
Lithobates septentrionalis	Ranidae	LC	Wl	Adults	W	N	N	[6]
Lithobates catesbeianus	Ranidae	LC	Wl/A	Adults/Unknown	W	N	N	[6]
Lithobates pipiens	Ranidae	LC	G	Adults/Unknown	W	N	N	[6]
Rana clamitans	Ranidae	LC	F/Wl	Adults/Juveniles	W	N	N	[6]
Lithobates palustris	Ranidae	LC	G	Adults	W	N	N	[6]
Polypedates leucomystax	Rhacophoridae	LC	G	Adults	W	Y	19	[18]
Triturus carnifex	Salamandridae	LC	G	Adults	W	Y	6	[13]
Unknown	Unknown	ND	ND	Juveniles	W	N	N	[6]

*Erroneously stated from its known geographical distribution range; IUCN status and habitats as per the IUCN 2022: LC = least concern, NE = not evaluated, NT = near threatened, VU = vulnerable; habitat: G = habitat generalist, Gr = grassland, Wl = wetland, T = terrestrial, A = aquatic, F = forest; study type: E = Experimental, W = in-the-wild; accumulation status: Y = MPs present, N = MPs absent; MPs/individual: ND = not defined; references: numbers correspond to the IDs provided in Table 2 and Appendix 1; species names are arranged according to families.

Table 2
Methods used for studying microplastics in amphibians.

ID	Study	Country	Study focus	Study type	Body part	Digestion	Identification	MP	Size	MP concentration	Exposure/ Study period
1	[72]	Italy	Growth, development, and mortality	E	ND	ND	ND	PS	50 nm	1.05 g/cm^3	Stage 2–46
2	[73]	China	Accumulation	E	Tadpole and feces	30% H_2O_2	Fluorescence microscopy	PS	1 and 10 μm	10, 103, and 105 particles mL^{-1} (1 mm) and 0.1, 10, and 103 particles mL^{-1} (10 mm)	48 h
3	[22]	China	Accumulation	W	Whole tadpole	30% H_2O_2	μFTIR and SEM	PES, PP, PE, and PS		0–168.48 items/g	7 months
4	[74]	Italy	Growth, development, and behavior	E	Whole tadpole	Dehydrated in ethanol (EtOH) up to 70%	Electron microscopy	PS	3 μm	0.125, 1.25, and 12.5 μg/mL	Tadpole stage 36–46
5	[75]	USA	Accumulation	W	Digestive tract	Dissection	Dissecting microscope, 2 ×	No MPs	No MPs	No MPs	2 months
6	[76]	Brazil	Behavior	E	Whole tadpole	65% HNO_3	Fluorescence microscopy	PE	35.46 μm	60 mg/L	7 days
7	[77]	Poland	Accumulation	W	Whole tadpole	30% H_2O_2	IR–ATR spectroscopy	PA, PU, and PBD	Up to 5 mm	0–3 items/individual	4 months
8	[33]	Spain	Growth, development, and mortality	E	Whole tadpole	30% H_2O_2	Fluorescence microscopy	PS	10 μm	0, 18, 180, and 1800 parts/mL	14 days
9	[65]	Brazil	Hepatotoxicity and mutagenic effects	E	Gills, tail muscle tissues, gastrointestinal tube, and liver of tadpoles	65% HNO_3	Fluorescence microscopy	PE	35.46 μm	60 mg/L	7 days
10	[66]	Brazil	Health status and cytotoxicity	E	Liver	65% HNO_3	Photomicrography	PE	35.46 μm	60 mg/L	7 days
11	[78]	Turkey	Accumulation	W	Whole tadpole	30% H_2O_2	FTIR	EVA, PA, PE, PE-Cl, PET, PAC, PMMA–PVC, PP, PVP, and PVA	ND	302.62–306.69 items/g	2 months
12	[79]	Italy	Accumulation	W	Stomach content	ND	ATR–FTIR	PE	1.2–3.7 mm	6 items/individual	20 months
13	[67]	Spain	Bd susceptibility	E	Whole individual	30% H_2O_2	Fluorescence microscopy	PS	10 μm	0, 18, 180, and 1800 parts/mL	229 days
14	[23]	China	Accumulation	W	Whole tadpole	30% H_2O_2	Raman microscopy	PES, PP, and PE	<0.5–5 mm	4.61–63 items/ individual	8 days
15	[25]	Italy	Accumulation	W	Whole tadpoles and digestive tracts of adult frogs	CREON enzyme	μFTIR	PA, PET, and PE	550.91–2355.51 μm	one item/individual	1 day
16	[80]	Turkey	Accumulation	W	Gastrointestinal tract	30% H_2O_2	FTIR	CT, EVA, PA, PBT, PCT, PE, PET, PS, and PVS	81–1223 μm	4.62 MPs/individual	ND
17	[81]	Italy	Behavior	E	ND	ND	ND	HPDE, PVC, PS, and PES	700 μm	1, 7, and 50 mg/L	2 weeks
18	[82]	Bangladesh	Accumulation	W	Digestive tract	30% H_2O_2	FTIR	PS, PA, EVA, and ABS	96% particles ranged between <0.5 and 5 μm	19 MPs/individual; 0.75 MP/g body weight	1 month

Batrachochytrium dendrobatidis (Bd); not defined (ND); experimental study (E), wild study (W); plastic types: chlorinated polyethylene (PE-Cl), cellulose triacetate (CT), ethylene–vinyl acetate (EVA), polyacrylic (PAC), polyamide (PA; nylon), polybutadiene (PBD), polybutylene terephthalate (PBT), polycyclohexylenedimethylene terephthalate (PCT), polyethylene (PE), polyester (PES), polyethylene terephthalate (PET), polymethyl methacrylate–polyvinyl chloride (PMMA–PVC), acrylonitrile butadiene styrene (ABS), polypropylene (PP), polystyrene (PS), polyurethane (PU), polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP), polyvinyl stearate (PVS).

inappropriate for the goal of our review, e.g., academic theses and dissertations, technical reports, summaries of scientific events, review articles, and duplicate publications, from our analysis and data compilation. Following this, we chose titles and abstracts (or, in some cases, the complete text) by individual reading, ensuring that the outcomes were pertinent to the topic of our review. Our review provides a generalized method for studying MPs in amphibians. Our review is significant for extending the knowledge of MPs and their impacts on amphibians and guiding future research.

2. Research trends and major types of MPs in amphibians

Although research on MP accumulation and its impacts on animals and ecosystems, especially the marine environment, is increasing worldwide, amphibians remain understudied (Fig. 1A). The maximum research on MPs in amphibians has been conducted in Italy (28%), followed by Brazil (17%) and China (17%; Fig. 1B). Spain and Poland have also contributed significantly (11%). One study each has been conducted in the USA, Bangladesh, and Turkey. However, these studies on MPs in amphibians broadly focused on bioaccumulation, behavior, cytotoxicity, mutagenic effects, disease susceptibility, and growth and development. In a few cases, researchers combined the effects of MPs on amphibian behavior and mortality rates with their effects on growth and development. While

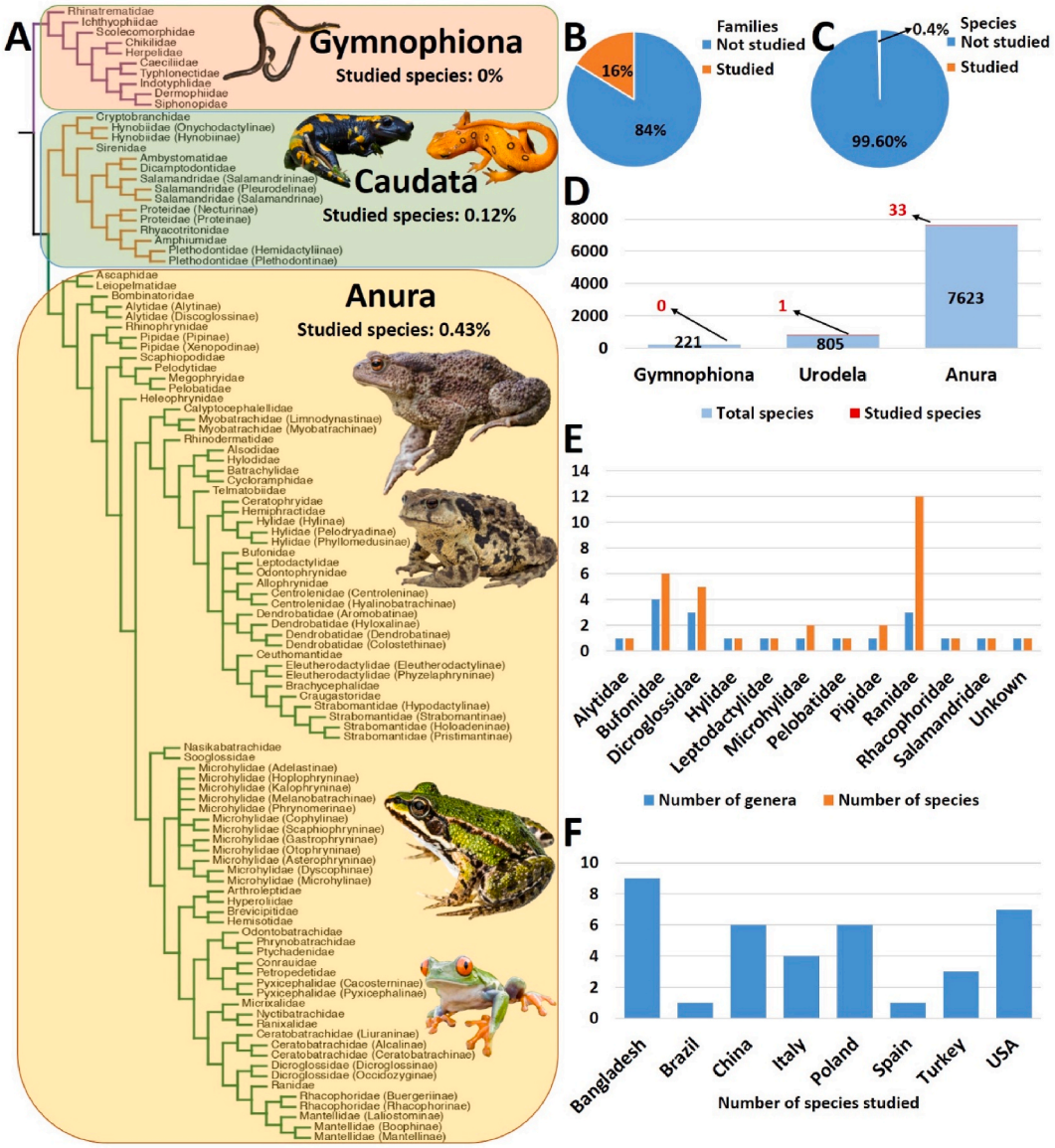

Fig. 2. Taxonomic analysis of studies on microplastics in amphibians. A. Phylogenetic tree with the explored proportion of taxonomic groups (adopted from [83]). B. Proportion of studied families. C. Proportion of studied species. D. Proportion of studied species in each amphibian order. E. Number of genera and species in studied amphibian families. F. Number of species studied in contributing countries.

most studies (56%) focused on the bioaccumulation of MPs in amphibians, 17% of the studies assessed the impacts of MPs on growth and development (Fig. 1C). Regarding the life stages, tadpoles were prioritized over adults [18]. However, a few studies included both tadpoles and adults of the target species to assess the MP bioaccumulation and its impacts. In total, 67% of the studies used tadpoles to observe the impacts of MPs on amphibians (Table 1; Fig. 1D). Half of the studies were experimental, and the rest were based on observations in the wild (Table 2; Fig. 1E).

The major MP types reported from amphibians in-the-wild studies included chlorinated polyethylene (PE-Cl), cellulose triacetate (CT), ethylene–vinyl acetate (EVA), polyacrylic (PAC), polyamide (PA; nylon), polybutadiene (PBD), polybutylene terephthalate (PBT), polycyclohexylenedimethylene terephthalate (PCT), polyethylene (PE), polyester (PES), polyethylene terephthalate (PET), polymethyl methacrylate–polyvinyl chloride (PMMA–PVC), acrylonitrile butadiene styrene (ABS), polypropylene (PP), polystyrene (PS), polyurethane (PU), polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP), and polyvinyl stearate (PVS) [22,23,25,75,77,79,80,82]. However, PE, PES, PET, and PS were the most common MP types in amphibians in-the-wild studies (Table 2). Unlike MP diversities in wild amphibians, researchers used fewer types of MPs to evaluate their impacts in experimental studies. The available experimental studies mainly used PE and PS MPs [33,35,65,67,72–74,76,81]. High-density polyethylene (HDPE) and polyvinyl chloride (PVC) were also used in experimental studies on MPs in amphibians ([81]; Table 2). However, research on the correlation between diversity of microplastics detected in amphibians and potential sources and impacts remained unstudied.

Although MPs were detected in marine herpetofauna in the early 2000s [68], literature review revealed that studies on MPs in amphibians began only in 2015 [18]. Moreover, the research trends were inconsistent. The highest number of research outcomes was obtained in 2020, whereas no research was conducted in 2017 (Fig. 1F). Only a small proportion of the total amphibian species was included in the existing MP studies (Fig. 2A, B, C, D). No species belonging to Gymnophiona were studied, while only 0.12% of Caudata species and 0.43% of Anura species were studied to determine the presence of MPs (Fig. 2A–D). A total of 34 species, which accounted for only 0.4% of the total amphibian species and belonged to 16% of the existing amphibian families, were assessed in the studies on MPs in amphibians (Fig. 2B and C). Although most species were tested in single studies, *Fejervarya limnocharis* was tested in three studies. Moreover, *Alytes obstetricans*, *Microhyla ornata*, *Pelophylax nigromaculatus*, *Physalaemus cuvieri*, *Rana temporaria*, and *Xenopus laevis* were tested in two studies each (Table 1). Although the number of species studied was negligible compared to the total number of species belonging to each family, Ranidae (12 species) was the most studied amphibian family, followed by Bufonidae (six species) and Dicroglossidae (five species). More than one species from Microhylidae and Pipidae were also studied (Table 1; Fig. 2E). MP accumulation and its impacts were ubiquitously evident in the studied species. However, scientific communities worldwide have not focused enough on this potential issue for amphibian conservation ([18]; Fig. 1A and B). The number of species explored to study MPs in amphibians also varied across studies and was negligible (Fig. 2F).

3. Methods of MP isolation and identification in amphibians

The methods used to observe MPs in amphibians varied across studies. These ranged from the simplest to the most complex methods and from requiring no chemicals and relatively simple equipment to requiring sophisticated chemicals and modern equipment. The simplest method involved no precautionary measures during sample collection [75]. In this method, researchers used plastic bags to carry and store the samples. After dissection, they digested the digestive tracts of the frogs naturally and kept them for 2.5 weeks at room temperature, without using any chemical, oven, and/or oscillating incubator. They then observed MPs under a dissecting microscope at 2 × magnification [75]. However, they did not detect MPs in any of the investigated species (Table 2).

In other studies, researchers used glass jars to carry the samples from the collection sites [22,23,25,75,77,79,80,78]. The use of plastic equipment was avoided in experimental studies [33,35,65,67,72–74,76,81]. In most studies, researchers used MS-222 for euthanizing the individuals (adults) and then examined their digestive tracts. In the case of tadpoles, they used whole individuals to isolate MPs (Table 2).

MPs were also isolated from stomach contents [79]. In experimental studies, only tadpoles were used, and MPs were isolated from the organs and body parts of interest (Table 2). Although whole tadpoles were treated to isolate MPs in most experimental studies [33,67,73,74,76], feces [73], liver [35,65], gastrointestinal tubes, gills, and tail muscles [66], were also used in some research. Researchers mainly used 30% H_2O_2 to digest and purify the aforementioned body parts (Table 2). Additionally, CREON enzyme [25] and 65% HNO_3 [35,65,76] were used to digest the samples in many studies. Most studies used ovens and oscillating incubators to facilitate thorough digestion of the samples, while others depended on incubation at room temperature. Although a study recommended not to use sonication [72], others sonicated the samples for 2–60 min [25,33,66,67,73].

After digestion, most researchers used cellulose nitrate grid membrane filters to isolate MPs [22,23,35,65,67,73,76]. A few studies also used glass fiber filter papers [33,80,82,78]. The pore sizes of the filter papers ranged from 0.45 to 6 μm, and their diameter ranged from 25 to 47 mm. A few experimental studies did not isolate MPs [72,81], whereas a few others used fluorescence methods and directly examined the samples under fluorescence and electron microscopes [33,74]. The authors either used fluorescent MPs or followed additional steps when MP observations were based on fluorescence. The additional steps included dyeing and further filtration after the first filtration. The Nile red fluorescent dye and rose Bengal dye were mainly used to stain MPs [25,35,65,76]. The filtration step was followed by the observation and identification of MPs. The most commonly used methods for identifying and quantifying MPs were μFTIR microscopy, scanning electron microscopy (SEM), fluorescence microscopy, and Raman microscopy (Table 2). The experimental studies mainly used fluorescence microscopy, whereas the in-the-wild studies used μFTIR microscopy [22,23,25,33,35,65,67,77,79,80,73,74,76,78].

However, in these studies, researchers modified the methods used in previous literature and studies on other taxa. Hence, a generalized method for studying MPs in amphibians is lacking. Given the importance of research on MPs in amphibians, we propose a

simplified and generalized method as a guideline for future research (Box 1).

4. MP uptake and bioaccumulation in amphibians

More than 50% of the existing studies on MPs in amphibians primarily focused on uptake and bioaccumulation (Fig. 1C). Hu et al. [73] first examined the possibility of the uptake, accumulation, and elimination of MPs by amphibians. They found that *X. tropicalis* tadpoles could uptake MPs in both fed and unfed conditions; however, unfed tadpoles could ingest more MPs than fed tadpoles. Moreover, MP elimination occurred in tadpoles, mainly in fed ones, but bioaccumulation for a longer period was also possible [73]. De Felice et al. [74] confirmed the ingestion of MPs by *X. laevis* tadpoles. Moreover, da Costa Araújo et al. [66] assessed the ingestion and bioaccumulation of MPs in *P. cuvieri* tadpoles. Furthermore, Boyero et al. [33] observed the ingestion, egestion, accumulation, and subsequent impact of MPs in another amphibian species, *A. obstetricans*. In addition to experimental studies, many species of frogs, toads, and salamanders were found to exhibit MP accumulation in the natural environment worldwide [22,23,25,77,79,80,78]. MPs were detected in amphibians in different locations and habitat types, including inland small water bodies [22,77,80,78], lakes [22], rivers [22], functional zones in coastal areas [23], and isolated mountain ponds and ditches [25,79]. Moreover, MP accumulation was reported in both tadpole and adult life stages [22,23,25,77,79,80,78]. However, the mean number of MPs per individual ranged from 0 to 18 and 2 to 307 items/g sample weight in adult and tadpole respectively (Table 2). Although being aquatic, the presence of MPs in tadpoles is easily understandable, reports on MPs in adults are also not surprising. They may get it through food, mud, and water. It is also possible to accumulate MPs that were acquired during the tadpole stages.

All studied species exhibited the presence of MPs, except for a few species studied by Schessl et al. [75] (Tables 1 and 2). Thus, MP uptake and accumulation were ubiquitous in the studied amphibians. In terms of ecosystem diversity, species from all ecosystems, including terrestrial (e.g., Bufonidae), grassland (e.g., Dicroglossidae), aquatic (e.g., Pipidae), and arboreal (e.g., Hylidae), exhibited MP accumulation (Table 1; Fig. 1A–E). It indicates that MP pollution is widespread in all ecosystems. In terms of taxonomic groups, MPs were detected in 34 species from 11 families belonging to the order Anura and one family belonging to Caudata (Table 1; Fig. 1A–E). The taxonomy and proper identification of the species studied for MP accumulation were unclear in a few studies. For instance, Schessl et al. [75] failed to identify some individuals, whereas Shetu et al. [82] included a species (*P. nigromaculatus*) from a locality (Bangladesh) far from its known geographical distribution range (East Asia; [38]). This species was not included in any of the country's lists (e.g., [84]), including the latest checklist formulated by Rahman et al. [53]. Moreover, Shetu et al. [82] used photographs of species with apparently erroneous taxonomic names, including a tree frog (possibly *Polypedates leucomystax* complex belonging to Rhacophoridae) with the name *F. cancrivora* (Dicroglossidae). Many studies on MPs in amphibians also did not mention a proper and clear method for species identification.

5. Impacts of MPs on amphibians

5.1. Effects on growth, development, body condition, and survivability

Amphibians are highly sensitive to environmental changes and chemical exposure [85]. Boyero et al. [33] reported that 10 μm yellow–green fluorescent polystyrene microspheres at 1,800 particles mL^{-1} were detrimental to *A. obstetricans* tadpoles at stages 25–28. In addition, they observed a decline in the body condition, feeding, and growth of the tadpoles with an increase in MP concentration. The impacts intensified with an increase in exposure time (14 days in their experiment); however, constant MP ingestion and egestion occurred on continuous exposure for longer durations [33]. Moreover, Tussellino et al. [72] reported the negative impacts

Box 1
Recommended procedure for studying microplastics in amphibians.

Although previous studies followed different methods to study MPs in amphibians (Table 2), a generalized outline was not available. Considering the importance of studying MPs in amphibians, we present a straightforward method for future research.

i Sample collection: After obtaining the necessary permits (environmental and ethical), capture the specimens (wild/experimental mesocosms) and carry them in glass jars.
ii Dissection: Euthanize the specimens using MS-222 (100–250 mg L^{-1} for tadpoles and 600 mg L^{-1} for adults). Dissect the adults and remove the digestive tract. Dissect the target body parts in the case of organ-specific experiments. Cut the digestive tract/whole tadpole/target organ into small pieces.
iii Digestion: Overflow the samples with either 30% H_2O_2 or 65% HNO_3. Keep the samples in an oscillating incubator at 65 °C and 80 rpm until the solution becomes transparent and free of organic materials.
iv Filtration: Filter the digested solution using a vacuum filter with a 47 mm diameter (the diameter of the filter may depend on the MP identification equipment) or a cellulose nitrate grid membrane filter or GF/C glass fiber filter paper with a pore size less than 6 μm.
v Identification and quantification: Identify and quantify MPs using μFTIR/SEM/fluorescence microscopy/Raman microscopy.

of PS nanoplastics (50 nm) on the development of *X. laevis*. They exposed tadpoles from stage 2, and their experiment lasted until stage 46. They noted growth retardation and organ malformation in the MP-exposed tadpoles. Furthermore, they noted abnormal pigmentation, an immature gut, small eyes, and forked tails in these tadpoles. In addition, da Costa Araújo et al. [66] noted morphological changes in *P. cuvieri* tadpoles at stage 29–30 after being exposed to 60 mg/L PE MPs for 7 days. They also reported abnormal skin pigmentation resulting from exposure to PE MPs in this species. In contrast, De Felice et al. [74] did not detect any impact of PS MPs on *X. laevis* tadpoles. They used 3 μm PS MPs at 0.125, 1.25, and 12.5 μg/mL. Moreover, they used tadpoles at stage 36–37 and exposed them to MPs until stage 46. Similarly, Scribano et al. [81] did not find any correlation between the growth or mortality of *R. latastei* tadpoles at stage 28 and MP concentrations. These results indicate that the tadpole stage, MP size, and exposure time may influence the effects of MPs.

5.2. Histopathological effects

Although the histopathological effects of MPs on amphibians are crucial, only a single study confirmed the direct histopathological toxicity of PE MPs in amphibians. da Costa Araújo et al. [65] exposed *P. cuvieri* tadpoles at stage 28–29 to 60 mg/L PE MPs for 7 days. They calculated the 'Histopathological Index (HI)', as adapted by Bernet et al. [86], and noted a higher HI in the MP-exposed tadpoles. They also noted larger areas with blood vessel dilation, infiltration, congestion, hydropic degeneration, hypertrophy, and hyperplasia in these tadpoles. Physicochemical analyses of the water supplied to the tadpoles (without PE MP group vs. 60 mg/L PE MP group) confirmed the histopathological effects of MPs on *P. cuvieri* [65]. Moreover, da Costa Araújo et al. [66] examined histological images and reported that the order of richness of MPs was as follows: gut > gills/liver > muscle tissues of the tail.

5.3. Cellular and mutagenic effects

In addition to reporting histopathological toxicity, da Costa Araújo et al. [65] reported changes in the size, volume, and shape of nuclei in individuals exposed to PE MPs. In their study, individuals exposed to PE MPs had hepatocyte nuclei had an increased area, perimeter, and volume and longer major and minor axes. Moreover, the nuclei had increased elongation and decreased sphericity. da Costa Araújo et al. [66] also confirmed the mutagenic and cytotoxic potentials of PE MPs. In contrast to hepatocytes, erythrocytes of the PE MP-exposed tadpoles were smaller and had smaller nuclei in terms of area, perimeter, breadth, length, and radius. This evidence supports the cytotoxicity of PE MPs. In that study, PE MPs exhibited a mutagenic effect on tadpoles and caused various nuclear modifications, including the formation of binucleated and notched, blebbed, kidney-shaped, and multilobulated nuclei. The authors argued that MP exposure may affect the survival, reproduction, or genetic heritage of tadpoles through induced mutations. Thus, MPs can also be the precursors of more complex and harmful events in amphibians [66].

5.4. Effects on behavior

Little is known about the impact of MPs on the behavior of amphibians. da Costa Araújo and Malafaia [76] confirmed the behavioral toxicity of MPs in *P. cuvieri* tadpoles at stage 28–29. They conducted open field and antipredator response tests to examine the behavior of tadpoles after being exposed to 35 μm PE MPs for 7 days. They noted a correlation between MP accumulation and reduced locomotion in these tadpoles. In the open field test, the animals exposed to the micropollutant covered a smaller distance and moved more quickly than those not exposed to it. Moreover, these animals had higher anxiety indices than those in the control group, indicating the anxiogenic effects of PE MPs. The authors also found that the thigmotactic behavior displayed by the tadpoles was exacerbated upon exposure to MPs based on the longer time spent by the animals exposed to PE MPs in the perimeter and center of the apparatus [76]. Scribano et al. [81] reported a reduced travel distance in MP-exposed *R. latastei* tadpoles in response to predator cues. However, they did not notice any changes in the response to predators between the treatment and control groups. Similarly, De Felice et al. [74] did not notice any impact of PS MPs on the swimming performance of *X. laevis* tadpoles. Scribano et al. [81] concluded that tadpole size may influence the severity of the effects of MPs, with larger tadpoles being less vulnerable to the harmful effects of MP exposure.

5.5. Effects on disease susceptibility

In their brief study, Bosch et al. [67] highlighted a serious issue connecting two global amphibian conservation concerns: MP pollution and chytridiomycosis. They simulated a natural interaction between a highly vulnerable amphibian species (*A. obstetricans*) and a *Batrachochytrium dendrobatidis* (Bd)-infected reservoir species (*Salamandra salamandra*) in the presence and absence of MPs to determine whether any synergies exist between this infectious disease and MP pollution. They discovered that the burden of Bd infection increased on MP consumption in a concentration-dependent manner. They also noted lesser MP accumulation in Bd-exposed amphibians and assumed that this could be attributed to the damage caused to the tadpoles' mouthparts by Bd [67]. Their findings necessitate further investigation on the possible interaction between these biotic and abiotic risks to amphibians.

6. Limitations of the studies and future directions

The presence of MPs in amphibians and their subsequent impacts is a flourishing research field, and many aspects need to be studied. Although a few studies have reported MP uptake and accumulation in some species, the status of most amphibians worldwide

remains unstudied. Future research should focus on more species from more geographical regions. The relation between MP susceptibility and the phylogenetic position of amphibians is also unreported. Moreover, the method used for species identification in many existing studies (e.g., [82]) is questionable. Hence, we suggest adding a section in the 'Methods' to explain the species identification procedure clearly. Despite admitting a shortage of taxonomists [87], we urge the inclusion of herpetologists in research groups or at least in one step of the publication process (writing, reviewing, or editing stage) when research is conducted on amphibians and/or reptiles.

Although some experimental studies have assessed the impacts of MPs on amphibians, the specific threshold for MP pollution has not yet been defined. Moreover, previous studies also did not assess the impacts of MPs together with other pollutants and environmental stressors. It is unrealistic to have a natural environment polluted by MPs alone. Hence, future research should mimic a naturally polluted environment to assess the synergistic effects on amphibians. Furthermore, researchers should consider observing the disease and parasite susceptibility of MP-exposed amphibians. Amphibians may also experience organ deformity and malformation in an MP-polluted environment. Hence, future studies should evaluate the effects of MPs on amphibian eggs and development.

Future research may also highlight the genetic and mutagenic impacts of MPs on amphibians. Although a few studies have separately reported MP accumulation in tadpoles and adults [33,35,65,67,72–74,76,81], its impacts on the metamorphosis and reproduction of amphibians remain unclear. Impacts of MPs on different age groups (possibly through skeletochronology; [88]) of the population also lacking. The long-term persistence of MPs (at least before and after metamorphosis) in amphibian organs and tissues is also understudied. As amphibians are model animals, the possibility of long-term persistence of MPs and the subsequent lethal and sublethal effects on amphibians from tadpole to adult stages can provide a clear idea about the future of MP pollution and its impacts on other animals, including humans. Moreover, large-scale studies on the trophic transfer of MPs through amphibians may help

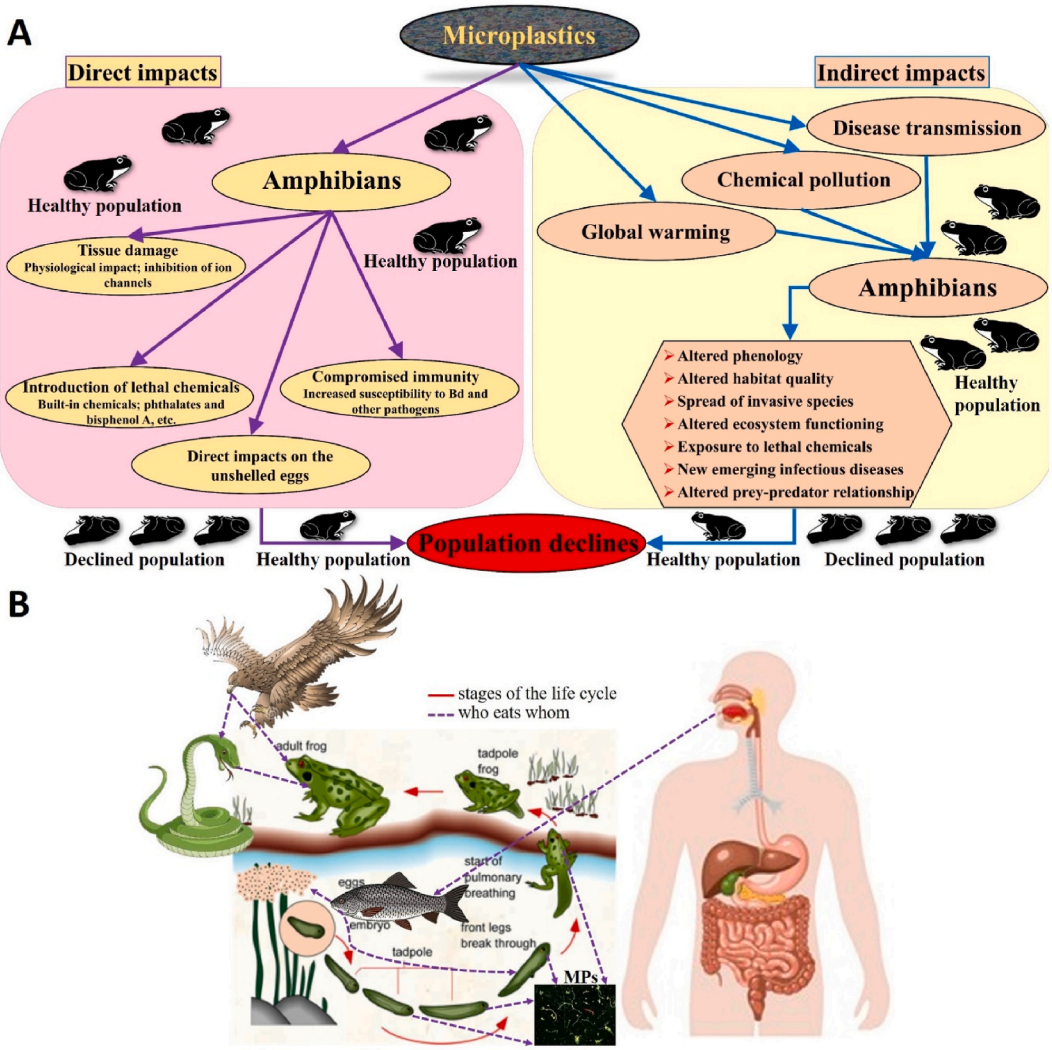

Fig. 3. Plausible impacts of microplastics on amphibians in freshwater and terrestrial habitats. A. Direct and indirect impacts of microplastics on amphibians. B. Ability of amphibians to transport microplastics between aquatic and terrestrial ecosystems.

understand the intensity of its effects on the ecosystem and human health. Finally, experiments have shown that nanoplastics have immense negative impacts on the health and survivability of many animal groups; however, amphibians were included only in one such study. Therefore, future research should also focus on this field.

7. Future perspectives

7.1. Conservation concerns

The potential negative impacts of MP pollution are already evident in almost all life forms. Although initial discussions of MP accumulation and its impacts focused on oceanic ecosystems, recent studies conducted worldwide have also indicated its potential threats to inland vertebrate species ranging from amphibians to humans (e.g., [19,23]). In particular, wetland- and aquatic habitat-dependent animals, such as amphibians and fishes, have been reported to exhibit MP accumulation [23]. Given their susceptibility, mode of life (requiring aquatic and terrestrial environments), and physiological features, amphibians may experience the worst direct and indirect impacts of MP pollution (Fig. 3A). Amphibians play an exceptionally important role in both aquatic and terrestrial ecosystems. They also act as potential carriers between these ecosystems to facilitate the flow of energy and pollutants. Therefore, research focusing on the impacts of MP pollution on amphibians should be highly prioritized. Unfortunately, this animal group has not received adequate attention from the scientific community and little is known about the real risk of MP pollution to these animals. We tried to rationally explain the possible means by which amphibians can get affected by MP pollution and why it can be fatal to them.

Experimental studies have proven that MPs can affect the survival, body condition, and function of amphibians (e.g., [33]). Although researchers did not explain how MPs affect amphibians, previous studies on other amphibian diseases and the impacts of MPs on other animals may provide some clues. MPs can enter animal cells and tissues and significantly damage and impair their functions [89]. In particular, the internal organs, tissues, and cells of several aquatic animals have been reported to exhibit MP accumulation and subsequent reduction of physiological activities [89]. Considering their related habitat types and susceptibilities, amphibians may respond in the same manner to MP accumulation in their gills (for tadpoles), liver, and gut and experience reduced physiological performance of the respective organs.

In addition, amphibians have developed unique morphological, histological, and physiological features to facilitate their special life cycles that require both terrestrial and aquatic habitats. Among other strategies, the acquisition of specialized permeable skin has granted amphibians unique abilities in terms of water intake, respiration, excretion, and ion exchange between the body and the environment. Any anomalies or impairments of skin tissue may lead to death; this may also be responsible for the global mortality of amphibians resulting from chytridiomycosis [90]. Bd can damage skin tissue by inhibiting epithelial ion channels, resulting in the reduction and/or complete seizure of ion exchange. The decrease in Na^+, K^+, and Cl^- ions causes reduces the electrical function of the heart and finally results in cardiac arrest [90]. Given their miniature size, MPs and nanoplastics may use the permeable skin of amphibians as an access point and subsequently clog and damage it. Thus, MPs may directly affect amphibian skin cells and tissues and cause the same symptoms as those caused by Bd. Moreover, MPs may increase the susceptibility of amphibians to Bd and chytridiomycosis [67].

MPs may also act as carriers of pathogens and harmful chemical pollutants, particularly hydrophobic agents that have high affinities to plastics [91]. When amphibians ingest MPs, they also consume chemicals and pathogens that may hamper their health. As mentioned above, amphibians are susceptible to chemical pollutants, including those outside their body, which could be detrimental when ingested. In addition, considering the use of phthalates and bisphenol A in the manufacture of plastics, MPs may act as carriers of these endocrine disruptors. Phthalates and bisphenol A have been proven to affect reproductive success, sexual development, embryonic development, sex ratios, and metamorphosis in many amphibian species [92]. Moreover, MPs may expose amphibians to novel pathogens and may cause a pandemic. MPs may also cause mechanophysical stress to unshelled amphibian eggs and affect hatching rates.

MP pollution-induced climate change and global warming are other issues that may adversely impact amphibians. Plastics contribute to the emission of greenhouse gases throughout their life cycle, including MP stages [93]. Even bio-based plastics can contribute to global warming by stimulating microbial metabolism and the subsequent release of excess CO_2 into the environment. Given the sensitivity of amphibian body temperatures to the environment, global warming can hamper the physiology of amphibians and may cause lethal and sublethal outcomes. The effects of global warming and climate change are already apparent in many amphibian species in terms of a shift in hibernation, breeding, and active seasons. Changes in breeding and active seasons may transform the community structure and ecosystem functioning by establishing interspecific interactions previously separated by spatial or temporal barriers, competition, and prey–predator relationships and reshape breeding success [94]. Global warming may also contribute to tadpole mortality, prolonged metamorphosis, increased release of stress hormones, and accelerated aging [95]. On a larger scale, it may trigger the spread of invasive species [96] and expose susceptible species to emerging infectious diseases [97].

However, the current lack of research on the physiological and ecological impacts of MPs on various amphibian species poses a critical gap in our understanding of the long-term implications of MP pollution on amphibian populations and biodiversity. As amphibians play vital roles in ecosystems, including serving as ecological indicators, the absence of comprehensive studies hinders our ability to assess and mitigate potential threats posed by microplastics. Future research in this area is essential to unravel the intricate connections between MPs exposure, physiological responses in different amphibian species, and the broader ecological consequences for their populations and the biodiversity of aquatic ecosystems. Addressing this research gap is imperative for informed conservation strategies and the sustainable management of amphibian habitats in the face of growing environmental challenges.

7.2. Food chains and dissemination of MPs among ecosystems

The trophic transfer of MPs is already evident in many experimental and natural ecosystems [26,30,98–100]. Although studies on the trophic transfer of MPs mainly focused on arthropods [30,99,100], an experimental study reported the transfer of MPs from tadpoles and fishes to mammals [26]. Thus, even animals with less chance of coming into contact with an MP-polluted environment can no longer be considered to be safe. Because of their biphasic life cycles and their important role in both aquatic and terrestrial ecosystems, amphibians have the potential to transfer MPs to both ecosystems (Fig. 3B). Amphibians form an important part of the food chain and can thus contribute to MP dissemination to higher trophic levels, including humans. The trophic transfer of MPs is possible both from feed to amphibians and from amphibians to top predators. Additionally, the effects of MPs on animals remain unchanged even after being transferred through various trophic levels [26]. This evidence of the trophic transfer of MPs highlights the importance of studying MPs in amphibians further.

8. Conclusion

This review indicates that MP pollution poses potential threats to amphibians and emerges as a significant concern for them. MP pollution is leading to the ongoing global decline of amphibian populations. While our understanding of the specific impacts of MPs is still evolving, several key factors highlight their potential risks. Amphibians, including tadpoles and adults, may inadvertently ingest MPs, which may accumulate in their digestive system, liver, gills, and other organs for long durations. MPs can also be indirectly consumed by amphibians through their prey and similarly get transferred to the next trophic levels. Moreover, they can act as carriers for other pollutants, such as endocrine disruptors or heavy metals, thereby transferring them into amphibian tissues and potentially increasing their toxicity. This can have detrimental effects on the health and survival of amphibians. MPs may also increase the disease susceptibility of amphibians. Furthermore, they can affect the behavior of amphibians, including their feeding, breeding, and movement patterns. Exposure to MPs may have long-term effects on the growth, development, and overall fitness of amphibians. The extent and severity of these impacts can vary depending on various factors, such as the size, type, and concentration of MPs, as well as the specific characteristics and behaviors of different amphibian species. While further research is warranted to gain a comprehensive understanding, it is clear that efforts to reduce and mitigate MP pollution are crucial to safeguard amphibian habitats and promote their conservation. Considering the research gaps, researchers should assess the risk by focusing on the mechanism of action and toxicity of MPs in amphibians. Some strategies, such as reducing plastic waste, improving waste management systems, and raising awareness about the impacts of MPs, can help mitigate this issue. Further studies investigating the ecological effects of MP pollution on amphibians are also essential to implement conservation practices and policy decisions aimed at protecting these vital ecosystem components. Addressing MP pollution can help ensure the long-term health and survival of amphibian populations and preserve the delicate balance of the aquatic ecosystems they inhabit. This review urges implementing comprehensive conservation strategies, including enhanced waste management practices and stringent regulations on plastic use, to mitigate the detrimental impact of microplastics on amphibians, and emphasizing the need for collaborative policy recommendations to safeguard their ecosystems.

Data availability statement

All data are included in the article/supplementary Material or referenced in the article.

CRediT authorship contribution statement

Md Mizanur Rahman: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Eung-Sam Kim:** Writing – review & editing, Validation, Supervision, Funding acquisition. **Ha-Cheol Sung:** Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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