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Nanoplastics and biota behaviour: Known effects, environmental relevance, and research needs

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ABSTRACT

Behaviour is increasingly recognized as a sensitive screening tool to detect the effects of environmental disturbances on biota, resulting from molecular, biochemical, and physiological processes. In this sense, several (eco)toxicological studies have been assessing and reporting the behavioural effects of xenobiotics, even at very low doses. Different behavioural endpoints may help to estimate more accurately the impact of nanoplastics (NPLs) in fitness-related behaviours and, therefore, should be included in environmentally relevant exposure scenarios. This paper presents a critical review of current scientific knowledge regarding tested behavioural endpoints on freshwater organisms exposed to NPLs, findings' environmental relevance and research needs. Overall, the limited number of studies addressed only 3 types of polymers, with spherical polystyrene (PS) being the most studied, but all identified behaviour as a sensitive endpoint to NPLs exposure, with potential effects on populations. However, applied methodologies differ making comparison of effects between different sizes or polymers difficult.

1. Linking nanoplastics (NPLs) research and behavioural ecotoxicology

Plastic contamination is currently one of the most prominent subjects in ecotoxicology and a side effect of the considerable plastic demand and consumption, that results from millions of tonnes of plastic waste generated annually [1]. Despite the efforts to recycle and convert plastic waste into energy, a considerable portion of is still improperly disposed of in the environment. Over time, plastic waste is subjected to fragmentation processes, through biotic and abiotic action, resulting in smaller particles that can reach the nanometer size. The definition of "nanoplastics" (NPLs) is still subject to controversy [2], with some authors setting the upper size at 1000 nm (e.g. Ref. [3], and others at 100 nm (e.g., Ref. [4]. For this discussion, NPLs refer to plastic particles smaller than 100 nm, following the European Commission's definition of nanomaterials [5].

NPLs can enter the environment not only through waste disposal but also as a result of their inclusion in products such as coatings, drug delivery systems and medical diagnostics [6,7]. This global-scale plastic challenge has raised concerns in the scientific community regarding the effects on biota and prompted research to understand the potential impacts of NPLs on the environment. Due to their smaller size, high specific surface area and free energy [2], NPLs are anticipated to be more reactive and more likely to be incorporated by smaller organisms and even cells, affecting cellular components and mechanisms, which may translate into unpredictable outcomes.

In the last years, the number of studies addressing the presence, accumulation, and effects of small plastic particles on aquatic organisms has grown considerably, but the knowledge regarding the effects of NPLs can be considered limited [6]. Particular focus must be given to environmentally relevant conditions that include most commonly polymers in the environment, concentrations, shape, and surface characteristics associated with ageing as well as co-exposure with other environmental contaminants. Additionally, potential exposure routes such as waterborne, foodborne or their combination should be considered (especially when dealing with organisms from upper trophic levels, like fish, given the emerging evidence of NPLs accumulation in organism tissues and trophic transfer). The challenges associated with sampling, sample treatment for isolation and polymer identification, as well as particle characterization in terms of size, shape/format, surface properties, and chemical added/sorbed substances may explain the limited available information regarding the risks of NPLs (e.g., Refs. [1,8–10].

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Studies addressing the effects of environmentally relevant concentrations of NPLs are mainly based on estimates, that comprise a high degree of uncertainty. Those studies reported limited lethality of NPLs (e.g. Refs. [11–14], a result also obtained when unrealistic concentrations were tested to attain some type of effect to allow comparison between types of polymers and sizes [15]. As a complement to standard guidelines and protocols, some studies have considered sublethal endpoints, such as behavioural changes, that may provide information on effects that may be translated at higher levels (e.g., population). Behavioural ecotoxicology (a broad term that includes a wide range of different behaviours displayed by organisms [16,17]) is not yet included in regulatory frameworks for plastics (in general), unlike for pesticides or metals. Nonetheless, some of these endpoints may be a very useful tool to detect subtle changes that may reflect effects on organisms' fitness (individual behaviours), providing information on potential increased susceptibility to predators or environmental conditions as well as potential effects at a population level (social behaviour) (Fig. 1).

Considering that NPLs are expected to persist in the aquatic environment for several years without causing immediate lethality or significant sublethal effects at low concentrations, behavioural ecotoxicology becomes particularly relevant. Behaviour is the result of many biological processes that arise from interactions between organisms and the surrounding environment [18] and thus changes in behaviour can provide environmentally relevant information on the potential consequences of sublethal exposure. Thus, behavioural ecotoxicology may be interpreted as an upstream approach compared to standard guidelines focused on mortality and/or morphological abnormalities, providing relevant information for regulatory purposes that can also promote the reduction of plastic consumption and/or disposal.

2. Behaviour: bridging individual and population-level effects of NPLs

Environmental risk assessment frameworks that integrate behavioural parameters may be considered an improved approach given that behavioural changes can be considered as the ultimate

Fig. 1. Linkage of different behavioural endpoints exhibited at the individual level and their potential translation to lower (sub-individual) and higher (population) levels of biological organization.

result of multilevel biological effects, and a sensitive early warning tool of contamination [19]. In this sense, the study of behavioural changes in organisms, at a laboratory scale, allows the prediction of potential alterations in animals' fitness upon exposure to environmental stressors (Fig. 1), with potential repercussions at higher levels of biological organization, allowing to prediction potential consequences at the populational level. For instance, impairment of normal behavioural responses may change demographic parameters as already reported for a salmonid species when exposed to an anxiolytic drug [20].

Fish erratic swimming patterns, for example, may be the result of contaminant-induced disturbances in neurotransmission and fleeing/avoidance behaviours indication of recognized unfavourable conditions [21–23]. Thus, based on the environmental relevance of behaviour alterations and the increasing reports of its sensitivity and early warning nature compared to other classical endpoints (e.g. Refs. [22,24,25], it is highly relevant to understand what is known for NPLs.

To date, most reviews addressing the topic of NPLs have primarily focused on issues such as its presence and distribution, fate and behaviour in aquatic environments, and analytical techniques used for its detection, with emphasis on research needs and harmonization of methodologies [1,10,26,27]. In terms of biological effects, most studies have failed to provide lethal and sublethal benchmarks following NPL exposure (compiled and discussed elsewhere [1,28–30], and to the best of our knowledge, no review article has specifically addressed the behavioural ecotoxicology of NPLs and how it may improve NPLs risk assessment. Attending to this notable gap in the current literature, this article aimed to review the available studies addressing the behavioural effects of NPLs on freshwater organisms, highlighting relevant effects and sensitive endpoints. To achieve this goal, different behavioural parameters were discussed, highlighting the relevance of each specific behaviour to organisms' fitness, NPLs (polymer type, shape, size), methodological approaches used, main results, and the relevance of an integrative and holistic approach in NPLs risk assessment.

3. Methodological approach

A literature review in Scopus and Google Scholar databases was performed between December 21st 2022 and January 24th 2023 to extract all the available studies addressing the effects of NPLs (<100 nm) on freshwater organisms, that have included behavioural parameters assessment (Fig. 2). The search assembled with the combination of the keywords "nanoplastic*", "behavi*" and "freshwater" revealed a total of 45 studies. The search was then refined by adding the following keywords: "Avoidance" or "Preference" or "Swimming" or "Feeding" or "Foraging" or "Mating" or "Capture" or "Escape" or "Hide" or "Burrowing" or "Anxiety-like" or "Learning" or "Memory" or "Predation" or "Social" or "Shoaling". The articles were then analysed by carefully reading the abstract to confirm if they were within the scope of the study. From those, thirteen studies provided information on the behaviour of NPLs which was not in the scope of the present work, and the other five were reviews comprising information and analysis on particle behaviour in different test media as well; ten papers appeared in duplicate; all these papers were excluded. Conference papers, notes, and book chapters were also excluded. The papers included in this review and their main characteristics are presented in Table 1.

4. Effects on behaviour

4.1. Feeding

Feeding is among the most studied behavioural endpoints, probably associated with its vital role in an organism's fitness, as the ability to acquire food to obtain nutrients required for anabolic processes, energy production, and metabolism is essential for survival and reproduction. Therefore, it may be assumed that organisms exposed to environmental stressors with compromised

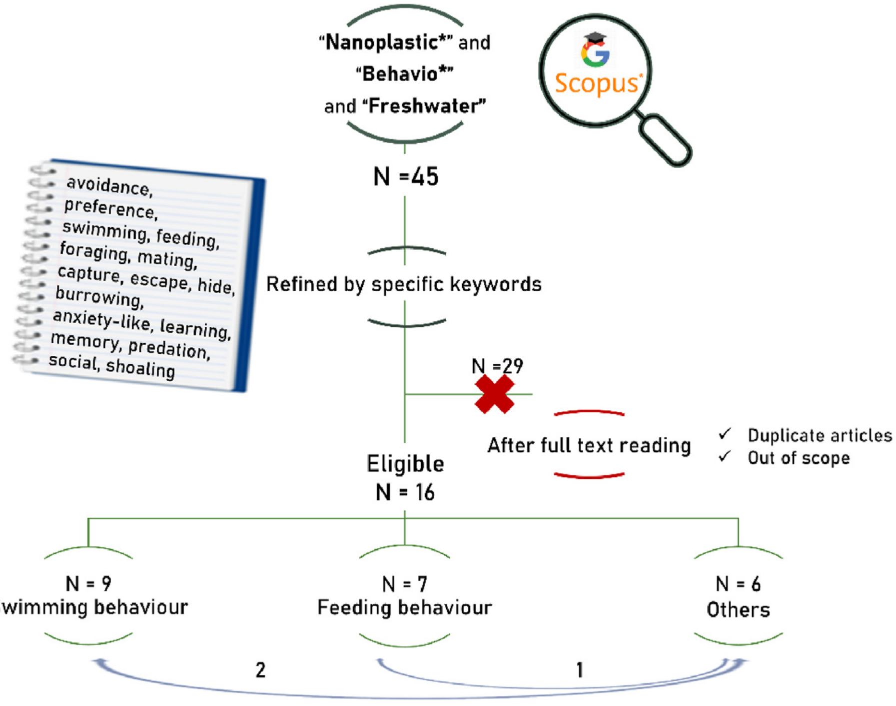

Fig. 2. Schematic representation of literature search methodology, inclusion and exclusion criteria, and obtained results.

Table 1
Studies with freshwater organisms focusing on different behavioural endpoints, assessed during and/or after exposure to nanosized (<100 nm) plastic particles (NPLs). Abbreviations stand for (in alphabetical order): DLS – dynamic light scattering; FTIR - Fourier-transform infrared spectroscopy; hpf – hours post-fertilization; NTA – nanoparticle tracking analysis; orgs – organisms; PMMA – polymethylmethacrylate; PS – polystyrene; SEM – scanning electron microscopy; TEM – transmission electron microscopy. Symbology meaning as follows: ↑ increase; ↓ decrease; ≈ similar.

Reference	Species Filo Order	Polymer (Type, size, and shape)	Origin	Functional group/ labelling	Characterization (Technic, matrices)	Replicates Orgs/ replicate	Age/Animal Developmental stage	Exposure duration/ Medium Renewal	Concentrations	Assessed Endpoints	Major Findings
FEEDING BEHAVIOUR											
[31]	*Carassius carassius* Chordata Cypriniformes	PS 24 nm Spherical	Commercial source	No	DLS in water	4 4 orgs/ replicate	No indication	30 days (five monitoring periods) No indication of medium change routine	100 mg/L to contaminate algae, to fed daphnias, to fed fish	**Feeding time** (time to consume 95% of the contaminated daphnias)	At day 30: **control** fish took on average **6.0** ± 0.7 **min** to fed, and those **exposed to NPLs through food** took on average **16.6** ± 2.7 **min**
[32]	*C. carassius* Chordata Cypriniformes	PS 24 and 27 nm Spherical	Commercial source	No	DLS, NTA Fish water	12 4 orgs/ replicate	Size class 8.2–9.6 cm and weight class 7.5–13.7 g	61 days No indication of medium change routine	1×10^{13} particles (130 mg particles per feeding)	**Feeding rate** (number of ingested daphnia), **Feeding time** (time to consume 92-95% of daphnias), Swimming exploration	Similar activity between non-NPLs-fed (NNF) and NPLs-fed group (NF) Day 61: **NF took longer to fed**, fed less
Rist et al., 2019	*Daphnia magna* Arthropoda Branchiopoda	PS 100 nm Spherical	Commercial source	No Fluorescent (Excitation: 440 nm, Emission: 486 nm)	DLS Suspensions made in test media	3 5 orgs/ replicate	One-week old	24h No	1 mg/L	**Feeding behaviour** (consider also the term ingestion); on *Raphidocelis subcapitata*	**Feeding rates ↓ in 21%** in 100 nm PS exposure
[15]	*Hydra viridissima* Cnidaria Anthoathecata	PMMA 40 nm Spherical	Team synthesized	No	DLS Milli Q water and hydra culture medium	6 1 org/ replicate	Non-budding adults	96h No	1, 5, 10, 20, 40, 80, 160, 320, 640 mg/L	Mortality, Morphological abnormalities, **Post-exposure feeding**	Mortality assay: ↑ **prey** consumption at **20 mg/L**, and ↓ **at 5 mg/L** Regeneration assay: higher prey consumption at 5 mg/L
[33]	*Gammarus roeseli* Arthropoda Amphipoda	PS 100, and 30 nm Spherical	Commercial source	No	No	6 3 orgs/ replicate	Size class 8.3 ± 1.8 mm	2 weeks Once a week	4.31 ng (low dose) 431 ng (high dose)	**Feeding behaviour** on cellulose-phyll tabs loaded with NPLs	**No effect on feeding rates** No changes on lipid, glycogen, or glucose levels NPLs body burden dose-dependent
[34]	*Echinogammarus meridionalis* Arthropoda Amphipoda	PS 100, nm Spherical	Commercial source	No	SEM, DLS, FT-IR Characterization of the stock solution (100 mg/L) performed in stream water	3 1 org/ replicate	Size class of 7.4 ± 3.6 mm	1.5 days No	0.25, 2.5, 25 μg/L	Leaf litter **feeding rates**	**Feeding rate ↓ by 50%** at 25 μg/L of 100 nm PS, though not significantly
[14]	*Xenopus laevis* Chordata Anura	PMMA 40 nm Spherical	Team synthesized	No	DLS Milli Q water and FETAX medium	4 4 orgs/ replicate	Nieuwkoop and Faber stage 45, independent feeding	48h No	1, 100, and 1000 μg/L	**Feeding rates** (ingested algae cells/day) Weight, Length	**No alterations on feeding rates** At 1000 μg/L: severe malformations, retarded growth At 1 μg/L: heavier tadpoles than in control group
SWIMMING BEHAVIOUR											
[11]	*Danio rerio* Chordata Cypriniformes	PS 51 nm Spherical	Commercial source	No Fluorescent (Excitation: 480 nm, Emission: 520 nm)	DLS Test media	80 orgs/ replicate 3 independent assays	Embryos at 6hpf	120h No medium renewal	0.1, 1 and 10 mg/L	Mortality, Hatching rate, Heart rate, Developmental abnormalities, Tissue accumulation, **Swimming behaviour**	Dose-dependent ↓ heart rate for all concentrations Gastrointestinal tract and pancreas accumulation at 1 and 10 mg/L, even after depuration **↓ Larval locomotor activity at 1 mg/L** upon dark conditions.
[35]		PS 50 and	Commercial source	No Fluorescent;	Fluorescent microscopy	5 18 orgs/	Larvae with 6 hpf	114h 10% test	10, 100, 1000, 10000 ppb	Mortality, Hatching rates, Developmental	Dose-dependent accumulation of NPLs

	D. rerio Chordata Cypriniformes	nm Spherical		Excitation: 660 nm, Emission: 690 nm		replicate 3 independent assays		media renewal	(0.01, 0.1, 1, 10 mg/L)	abnormalities, **Swimming behaviour**, Tissue accumulation	No effects on the total swimming distance; Accumulation at 1 and 10 mg/L of 50 nm PS. Nervous system-associated gene expression ↑ at 0.1 of 50 nm PS
[36]	*D. rerio* Chordata Cypriniformes	PS 70 nm Spherical	Commercial source	No Fluorescent (Excitation: 485 nm, Emission: 590 nm)	TEM	20 orgs/ treatment	Adult (six-to seven-months-old)	7, 30 days and 7 weeks Test media renewed every two days	0.5, 1.5, 5 mg/L	Tissue accumulation, Stress and genotoxicity biomarkers, Neurotransmitters, **Swimming activity**	**↑ Average speed** **↓ Freezing time movement ratio at 1.5 mg/L** **Alterations in exploratory behaviour at 5 mg/L** (after 7 days-exposure) Accumulation in nervous, digestive, and reproductive tissues ↓ AChE at 1.5 mg/L ↓ Dopamine, melatonin, γ-aminobutyric acid, serotonin, vasopressin, kisspeptin, oxytocin ↑ ROS tissue, DNA damage, cortisol level (liver), ↓ ATP at 5 mg/L
[37]	*Ctenopharyngodon idella* Chordata Cypriniformes	PS range from 20 to 26 nm Spherical	Commercial source	No Fluorescent (Excitation: 470 nm, Emission: 505 nm)	TEM, SEM	32 orgs/ treatment	Adult (six-to seven-months-old)	72h No medium renewal	760 μg/L	**Locomotor behaviour,** Anxiety-like behaviour, Neurotoxicity, Genotoxicity, Oxidative stress	**≈ Swimming speed and distance** and anxiety index ↑Oxidative stress, AChE activity and genotoxicity
[38]	*C. idella* Chordata Cypriniformes	PS 20-26 nm Spherical	Commercial source	No Fluorescent (Excitation: 470 nm, Emission: 505 nm)	TEM, SEM	36 orgs/ treatment	Juveniles	20 days Complete renewal every two days	0.04, 34 and 34000 ng/L	Biometric parameters, **Locomotor behaviour**, Anxiety-like behaviour, Antipredator defensive-response, Neurotoxicity and oxidative stress biomarkers	Non-concentration-dependent effects on biometric parameters (body biomass, standard and total length, peduncle and head height) **No effects on locomotor activity** nor **anxiety-like behaviour** **Alterations in anti-predatory defensive response**
[39]	*Branchinecta gaini* Arthropoda Anostraca	PS 50 and 60 nm Spherical	Commercial source	Carboxylated (-COOH) and amino $(-NH_2)$-modified; Fluorescent (Excitation: 480 nm, Emission: 520 nm)	DLS at 0, 24 and 48h Freshwater	3 12 orgs/ replicate	Adults	48h No medium renewal	1 and 5 μg/mL	Mortality, **Swimming activity**, Moulting, Histology, Gene expression	↑ Moulting, although not significant irrespective of PS NPLs functionalization and surface charge **↓ Swimming capacity upon exposure to PS-NH2**; ↑ Gene expression Histological alterations in the gut epithelial tissues
[40]	*D. magna* Arthropoda Branchiopoda	PS 50 nm Spherical	Commercial source	No Green fluorescent	TEM	6 3 orgs/ replicate	Newborns	48h; 7, 14 and 21 days Acute: no renewal Chronic: daily renewal	Acute: 0.05, 0.1, 0.5, 1, 2, 5, 7, 10 and 15 μg/mL Chronic: 0.05 and 0.5 μg/mL	Immobilization at 24 and 48 h after exposure, Oxidative stress and energetic biomarkers, **Swimming activity** Tissue accumulation	**No effects on swimming activity;** No effects on oxidative stress condition ↑ Energy biomarkers at 0.05 and 0.5 μg/mL of 50 nm PS, after 21 days exposure
[4]	*D. rerio* Chordata Cypriniformes	PS (22 nm) PMMA (32 nm) Spherical	Team synthesized	No	DLS, SEM	20 orgs/ replicate	Embryos/ Larvae	96h	0.001, 0.01, 0.1, 1, 10 and 100 mg/L	Mortality, Hatching, Morphological features, Neurotransmission, Antioxidant status, Oxidative damage, Energy metabolism, **Swimming behaviour**	Delayed hatching at 72h, for 100 mg/L of PS-NPLs and at 48h for all PMMA-NPLs tested concentrations; ↑ **Total distance at 0.001, 1 and 100 mg/L of PS-NPLs**, upon light conditions

(*continued on next page*)

Table 1 (*continued*)

Reference	Species Filo Order	Polymer (Type, size, and shape)	Origin	Functional group/ labelling	Characterization (Technic, matrices)	Replicates Orgs/ replicate	Age/Animal Developmental stage	Exposure duration/ Medium Renewal	Concentrations	Assessed Endpoints	Major Findings
[41]	*D. rerio* Chordata Cypriniformes	PS 44 nm Spherical	Commercial source	No	DLS at 0 and 96h Fish water	4 4 orgs/ replicate	Embryos/ Larvae	120h No medium renewal	0.015, 0.15, 1.5, 15 and 150 mg/ L	Mortality, Heartbeat, Hatching rate, Developmental abnormalities, **Locomotor behaviour,** Oxidative stress, Neurotransmission, Energy budget	↑ **Total distance moved at 0.001, 0.01, 0.1 and 10 mg/L of PMMA-NPLs**, upon light and dark conditions ↑ **swimming distance at 0.015 mg/L** and in catalase activity
SHOALING BEHAVIOUR											
[32]	*C. carassius* Chordata Cypriniformes	PS 24 and 27 nm Spherical	Commercial source	No	DLS, NTA Fish water	12 4 orgs/ replicate	Size class 8.2–9.6 cm and weight class 7.5–13.7 g	61 days No information on medium change routine	1×10^{13} particles (130 mg particles per feeding)	**Shoaling,** Feeding	**Day 61**: less individual action with fish **beavering more as a group.**
[36]	*D. rerio* Chordata Cypriniformes	PS 70 nm Spherical	Commercial source	No Fluorescent (Excitation: 485 nm, Emission: 590 nm)	TEM	20 orgs/ treatment	Adult (six-to seven-months-old)	7, 30 days and 7 weeks Renewal every two days	0.5, 1.5 and 5 mg/L	**Shoaling**	↑ **Shoaling at 0.5 and 1.5 mg/L** (after 7 days-exposure) ≈ **Conspecific social behaviour**
PREDATION-RELATED BEHAVIOURS (foraging for food or escaping larger predators)											
[32]	*C. carassius* Chordata Cypriniformes	PS 24 and 27 nm Spherical	Commercial source	No	DLS, NTA Fish water	12 4 orgs/ replicate	Size class 8.2–9.6 cm and weight class 7.5–13.7 g	61 days No indications on medium change routine	1×10^{13} particles (130 mg particles per feeding)	**Foraging for food/hunting**	**Day 61**: fish less prone for exploring the tanks, **hunting less**
[36]	*D. rerio* Chordata Cypriniformes	PS 70 nm Spherical	Commercial source	No Fluorescent (Excitation: 485 nm, Emission: 590 nm)	TEM	20 org/ treatment	Adult (six-to seven-months-old)	7, 30 days and 7 weeks Test solution refreshed every two days	0.5, 1.5 and 5 mg/L	**Predator avoidance**	↓ **Predator avoidance behaviour at 1.5 mg/L** (after 7-days exposure)
[38]	*C. idella* Chordata Cypriniformes	PS 20-26 nm Spherical	Commercial source	Fluorescent Excitation: 470 nm, Emission: 505 nm	TEM, SEM	36 org./ treatment	Juveniles	20 days Medium fully renewed every two days	0.04, 34 and 34000 ng/L	**Antipredator behaviour**	**Lack of cohesive shoaling facing a predator cue**
AGGRESSIVENESS											
[36]	*D. rerio* Chordata Cypriniformes	PS 70 nm Spherical	Commercial source	No Fluorescent (Excitation: 485 nm, Emission: 590 nm)	TEM	20 orgs/ treatment	Adult (six-to seven-months-old)	7, 30 days and 7 weeks Every two days	0.5, 1.5 and 5 mg/L	**Aggressiveness**	↓**Aggressiveness at 5 mg/L** (after 7 days-exposure)

feeding activity are less likely to survive and reproduce than unaffected organisms [17]. However, feeding behaviour is often associated with other fitness-behavioural endpoints. For instance, in fish, it is closely related to swimming behaviour, as these organisms usually manoeuvre their fins in the water while pursuing food, which also modulates their escaping/avoidance behaviour when facing predators (a well-nourished fish may be in better physical condition to flee), and can shape populational-level behaviours (for instance, triggering higher aggressiveness in social interactions between conspecifics) [17,42]. Nonetheless, the literature search revealed limited research on the interactions between different behavioural endpoints. Feeding behaviour also plays an important role in the incorporation of environmental contaminants, particularly those that are transferred through the food web (e.g., plastics). For instance, Ref. [42] reported that 6-day old *Danio rerio* larvae displayed decreased exploratory behaviour and higher anxiety-like states upon foodborne exposure to quinpirole.

Recent research on the effects of NPLs has led to a heightened understanding of their potential vertical transfer, which refers to the movement of NPLs between different trophic levels in the food chain. Thus, NPLs waterborne exposure and foodborne exposure should be considered ecologically relevant scenarios [31,32]. The literature search with the criteria mentioned above, retrieved 7 publications that included feeding, for which three scenarios of delivery of NPLs could be accounted and explored according to the following: first, it is necessary to consider NPLs-waterborne exposure that may subsequently induce changes in feeding patterns of organisms (e.g., post-exposure feeding [15]; second, the assessment of feeding patterns during NPLs exposure [14,43]; and lastly, foodborne exposure (or food preconditioning), i.e. the assessment of the potential effects of NPLs through food products [31–34]. The diversity of methodologies used in feeding assays is most probably related to the methodologies considered most appropriate given the diversity of functional groups used: shredders [33,34], grazers (water column – Rist et al., 2017, or bottom – Venâncio et al., 2022), or secondary consumers (invertebrate – Venâncio et al., 2021, or vertebrate – Cedervall et al., 2012; [32]. Like most research on microplastics, it is difficult to use data from different studies to establish a comparison of sensitivity. Although not straightforward, attempts can be made with *Echinogammarus meridionalis* [34] and *Gammarus roeseli* [33]. [34] opted for conditioning alder leaves (a less artificial food source) with PS-NPLs (100 and 1000 nm) at concentrations ranging from 0 to 25 μg/L, whereas [33] opted to use agar and cellulose-pill tabs loaded with PS-NPLs (sizes of 1000, 500, 100, and 30 nm) at two doses (high load – 431 ng, and low – 4.31 ng). Despite the differences in exposure duration, assay design in terms of replicates, and number of organisms per replicate (1.5 days, 3 replicates, 1 individual/replicate vs. 2 weeks, six replicates, 3 organisms/replicate for [33,34]; respectively), the outcome was similar, with no significant effects on feeding rates reported [33]. found that the lack of effects on feeding was associated with no changes in organisms' energy assimilation (lipid, glycogen, and glucose levels). However, the results of the two available studies with crucian carp (*Carassius carassius*) fed with 24 and 27 nm PS-NPLs-contaminated daphnias, showed that NPLs foodborne-exposed fish presented lower predatory behaviour (thus, decreased feeding) by the end of 30 and 61-days assays [31,32]. In addition to altered feeding rates, both studies reported lower exploratory behaviour, highlighting the transversality of the effects between different behaviours [31,32]. Using another vertebrate species (*Xenopus laevis*) and focusing on the effects of waterborne exposure to 40 nm PMMA-NPLs on the feeding rates [15], found no altered feeding behaviour, although organisms exposed to 1000 μg/L grew less and a high percentage of organisms presented exteriorization of the gut [15].

All seven publications addressing feeding, tested spherical pristine particles. This brings significant challenges when attempting to translate to realistic environmental scenarios as plastic degradation in the environment is expected to lead to dissimilar/irregular NPLs shapes, with surface characteristics altered by ageing processes, that may alter polymer toxicity [44,45]. Most of the seven previous studies mentioned tried to address environmental relevance. In some cases, exposure to concentrations considered by the authors as of environmental relevance did not induce mortality and/or morphological changes, but altered organisms feeding behaviour, especially in studies with fish [31,32], while for other organisms (such as detritivores or grazers), environmentally relevant concentrations seem to induce no effect on this parameter. However, the differences in the observed effects of NPLs may be associated with differences in the duration of the experiments conducted with different species. For instance, studies with fish typically spanned one to two months, while experiments involving other organisms were conducted over shorter periods, ranging from hours to a couple of days. Additionally, the feeding strategies of these organisms could influence their sensitivity to NPLs. It is crucial to acknowledge that the restricted number of studies conducted limits the conclusions that can be drawn. Nevertheless, these preliminary findings can provide valuable insights and lessons for future research, particularly in terms of understanding the potential implications of NPLs on human health. For instance Refs. [31,32], studies that lasted 30 and 61 days, respectively, have shown that organisms at the apex of the trophic chain may be affected by preys' previous exposure (in this case, from algae to daphnids to fish), which is currently a human health concern [46,47].

4.2. Swimming

Swimming is vital for many aquatic organisms as it plays a key role in many fitness-related behaviours [48]. In organisms like invertebrates and fish, alterations in swimming activity may impact their ability to feed (and therefore their growth), as well as predator escaping and/or stressful environments avoidance, exploration of new environments and resources and even reproduction [17,23]. These changes in swimming behaviour patterns, in addition to the direct impact on individual fitness, may also be translated into direct links to population and community level, due to the potential disruption of interspecific and intraspecific social dynamics. Thus, its inclusion in (eco)toxicological studies to assess the effects of waterborne contaminants has been increasing, based on its reported sensitivity and recognized ability to provide early warning signs with a non-invasive and environmentally relevant character [19,49]. Thus, depending on the species used as a biological model, different swimming-associated parameters can be measured, such as speed, distance swam, time spent in inactivity/freezing, swimming (turning) angles, the tendency to swim near the boundaries of a test area and tendency to swim/explore in different layers of the water column. An alteration in such parameters, when compared to control organisms, provides relevant information on a potential disruption of the normal swimming pattern which, based on the endpoint, may have different consequences on animals' fitness.

Nonetheless, after a literature revision, only 9 of the studies addressing the effects of NPLs on freshwater organisms assessed swimming behaviour, reporting alterations in total swimming distance (5 studies), speed (2 studies), types of movements (1 study) and time of inactivity (1 study). In 9 out of the 10 studies, PS nanospheres (sizes ranging from 20 to 100 nm; concentrations between 0.04 ng/L and 10000 mg/L) were tested. A single study used a polymer other than PS - polymethylmethacrylate (PMMA) – which highlights the need for more information regarding effects of polymers of environmental concern (e.g., polypropylene (PP), polyethylene (PE), or polyethylene terephthalate (PET)). Most studies addressed the effects of short-term exposures (8 out of the 10 studies) which may not provide relevant information on the long-term effects, a more environmentally realistic scenario. According to the literature review, 20 days of exposure to PS NPLs (20-26 nm) from 0.04 ng/L up to 34 μg/L induced no effect on *Ctenopharyngodon idella* juvenile's locomotor behaviour [38]. A similar result was reported in a study with *Daphnia magna* exposed for 21 days to 0.05 and 0.5 mg/L PS NPLs (50 nm) that displayed no significant changes in swimming activity [40]. The lowest tested concentrations eliciting alterations in swimming behaviour were in the range of 0.001–0.015 mg/L for PS NPLs (22 nm and 44 nm, respectively) [4,41] and within 0.001 and 0.1 mg/L for PMMA (32 nm) [4]. Both studies assessed the effects of NPLs on the embryonic/larval stage of *D. rerio* (zebrafish), reporting a significant increase in larvae swimming distance which can be suggestive of hyperactive behaviour. Zebrafish larvae exposure for 120h to ~50 nm PS NPLs (44 and 51 nm, respectively) induced increased swimming activity at 0.015 mg/L [41], whereas exposure to 1 mg/L decreased larval locomotor activity [11], which may suggest a non-monotonic response [50] and highlights concentration as an important variable that, modulating nanoparticles behaviour, (e.g. aggregation/agglomeration) [51], will modulate its bioavailability and effects.

The analysis of the endpoints used to assess the effects of NPLs on swimming behaviour revealed that total swimming distance was the most commonly used parameter (in 7 out of the 10 studies). Alterations in the total distance travelled provide relevant information on the organism's activity level (hyper or hypoactivity) which in turn may adversely impact different behaviours such as feeding (that can affect individual fitness), predator avoidance, social interaction and even reproductive success. Of these 8 studies addressing swimming distance, 4 reported significant alterations in swimming distance [4,11,39,41] highlighting the sensitivity of this endpoint. Within these 4 studies, the results are not consensual, with 2 reporting increases in the distance swam [4,41] and 2 decreased swimming distance [11,39], highlighting the need for a proper characterization of the NPLs particles as it plays a key role on their biological bioavailability and reactivity. Nonetheless, a more integrative approach should be adopted, with the inclusion of different endpoints to allow a better understanding of the behavioural change. For instance, in fish, the inclusion of swimming angles, time spent in swimming or inactivity, swimming speed, and types of movements, may provide a better understanding of the dimension of NPLs-induced impact. For example, a higher distance travelled may be associated with an anxiety increase. A single study included more than 2 swimming-associated parameters [41]. Moreover, as virtually all aquatic organisms are equipped with an endogenous circadian clock and the ability to detect light shifts, assessing swimming behaviour under both light and dark conditions assumes high relevance. Only 3 studies addressed the effects of NPLs on swimming behaviour considering the presence or absence of light. In the particular case of fish, it is also well established that abrupt light transitions can be used as a startle inducer to study contaminants' effects on stress response and recovery response since fish tend to quickly resume swimming activity to the level they had before stress. Contaminant-induced alterations on stress response and learning (stress adaptation response) may directly interfere with fish's ability to optimize their response (e.g., predator escape/avoidance) according to perceived predation risk. However, none of the studies considered eligible for this review, addressed this question when assessing the effects of NPLs exposure on fish locomotor behaviour.

Overall, the reported alterations in fish swimming pattern after exposure to PS and PMMA NPLs highlight the ability of NPLs to interfere with the organism's movement which may have significant ecological implications. Deficits in swimming performance will directly impact other ecologically important behaviours at the individual level but also with later repercussions at the population and community level. Swimming behaviour is a determinant that directly influences the ability to escape predators or to avoid them (antipredation) and to capture prey (feeding) [52,53]. Therefore, activity patterns may influence predator-prey interactions (e.g., to eat or to be eaten) that are important in structuring aquatic communities [54]. Moreover, alterations in swimming performance may adversely affect dispersal and migration-related behaviours that are of vital importance for population survival, especially when facing significant environmental challenges [52,53].

4.3. *Avoidance/escaping, preference, foraging, mating, capture, hide, burrowing, anxiety-like, learning, memory, predation, social, shoaling*

This section includes other types of behavioural endpoints. This decision is based not on the fact that they are considered similar concepts, but rather on the lack of studies that do not allow a structured analysis for each endpoint. Many of these behavioural parameters are species-dependent (they may or may not be measurable to the desired endpoint). For instance, when considering cladocerans, one is not expecting them to display learning or capturing/predation behaviours, while for fish, those endpoints make sense. Thus, the available data on a particular behaviour will ultimately be modulated by the class of organisms. The studies explored were performed on vertebrate species.

Schooling behaviour, the tendency of social fish to group in schools, may be disrupted. This is an important behaviour in social species, where the individual can not only take advantage of the safety of the group to explore territory and feed but also to protect against predators. Nonetheless, only two studies addressed group cohesion upon NPLs exposure, both reporting a similar pattern. A study by Ref. [32] showed that *C. carassius* juveniles, exposed for 61-days to 24-27 nm PS-NPLs (provided through food items such as daphnias), made fewer individual forays into aquaria, exploring fewer areas, conducting more as a group, leading to safety in groups. In another species [36], found that 0.5 and 1.5 mg/L 70 nm PS-NPLs promoted more cohesive groups of adult *D. rerio* after 7 days of exposure [36]. correlated this behaviour with the relationship between conspecific individuals reporting decreased aggression within group organisms at 5 ppm PS-NPLs. The group/shoal response when confronted with a predatory cue was also assessed by Refs. [36,38] that showed that NPLs can make fish less able to respond to predatory threats. This highly relevant result suggests that fish exposed to NPLs may be more vulnerable to predation, with potential consequences for the population. These alterations in individual behaviour may affect interactions between individuals and the ability to recognize and avoid potential threats, especially in vertebrate species, which may provide indications for potential endpoints to be considered in humans. It should be noted that although only two studies have evaluated social cohesion, the studies involving organisms with gregarious behaviour have considered group exposure (e.g., Refs. [15,32]. Future studies should take this into account as well since organisms with high social cohesion can synchronize their behaviours and follow group decisions. The presence of NPLs and the resulting behavioural changes in some individuals may influence the information available to other group members, triggering adaptive or maladaptive responses within the group, and potentially affecting overall behaviour and responses to NPLs.

4.4. NPLs improved risk assessment: the link between different behavioural traits

Considering the overall analysis of the different behavioural parameters, the available data show a clear bias considering the type and shape of the tested polymers, with spherical polystyrene (PS) taking the leading role. This is likely because PS is one of the most abundant and consumed plastic polymers [55], and its micro(nano)particles are widely marketed for research. While attention has been drawn to the urgent need to generate data for underrepresented polymers (e.g. Refs. [6,56], as well as shapes, no data exist on other widely consumed polymers such as polypropylene, polyethylene, or polyethylene terephthalate (PP, PE, or PET, respectively). The lack of reported effects is in this case associated with a lack of studies and not a lack of toxicity [6,56]. In addition, it is also important to understand if the currently available studies focusing on behavioural traits, try to associate the different behaviours with others (i.e. if the studies include only one or several types of behavioural analysis), and if so, if there is an attempt to understand how they affect each other. At the same time, many of these studies have recognized the importance of assessing behavioural traits with the accumulation of NPLs in tissues/organs [11,35,36]. An attempt to understand the link between NPLs tissue accumulation and behaviour should be a priority to allow a better understanding of the mechanisms of action involved. To this end, future studies should consider the conjugation of behaviour endpoints with biomarkers related to neurotoxicity, endocrine disruption, and immune response, among others, [36]. In Ref. [31] study, fish fed for 61 days with NPLs-contaminated food, in addition to a lower feeding rate, presented a decreased/slower swimming activity, exploring less area than control fish. This effect on the ability to explore the surroundings and actively search for food was also observed by Ref. [32] in fish with a similar NPLs exposure condition (PS-NPLs contaminated daphnia), with authors reporting higher fish group cohesion and decreased individualistic behaviour [32]. Despite the higher cohesion of groups upon exposure to NPLs, their response to other changes may be somehow impaired as shown by Refs. [36,38]. Exposure to NPLs increased schooling, but the fish were less able to confront and avoid predatory threats. For social species, for instance, organisms may rely on the group to feed, explore, or fight predators, and these behavioural traits must be envisioned as not mutually exclusive, and therefore there is a need to explore them in combinations whenever possible. These results pinpoint the need to include combined behavioural studies as supportive evidence tools to increase environmental pertinence [16] since behavioural traits analysis is linked to populational levels and therefore may be correlated with ecosystem-level processes (Fig. 3). In summary, the approaches employed above seem to be species-dependent, with the few available studies, focusing on fish, providing the widest analysis on behavioural endpoints, frequently relating fish feeding rates with exploratory/swimming behaviour and/or social interaction with their peers (Fig. 3).

5. Final considerations

Based on the current knowledge, this review aimed to draw attention to the fact that NPLs may not be a hazard in the sense of inducing high mortality or significant effects on somatic growth and reproduction, but they still may pose a threat due to subtle changes in the behaviour of organisms, that will modulate their interaction with the surrounding environment, individuals of their species and other species. This concern can be highlighted by the reported interaction of NPLs with other environmental contaminants. Managing the nonlethal effects of disturbance on populations has been a long-term goal for biologists and decision-makers, and assessment of such effects is currently required by European Union and United States legislation [57]. The inclusion of behavioural endpoints to assess sublethal effects of xenobiotics exposure is of direct ecological relevance, as animal behaviour underlies many critical ecosystem functions by shaping interactions with conspecifics, other species, and the abiotic environment [17,58]. Moreover, monitoring behaviours that are directly transferable to ecosystem function (e.g., foraging, dispersal,

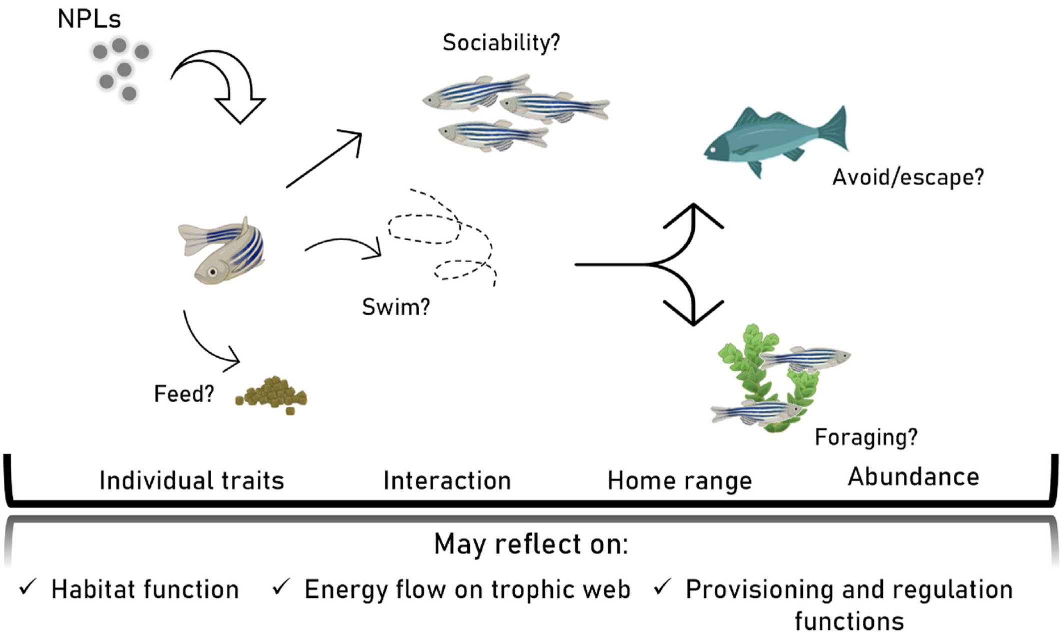

Fig. 3. Illustration linking some of the different behavioural traits to be considered for combined evaluation in future research works, and their potential outcome at the ecosystem level. The example here used was a fish, as it was the biological model to which more studies were available relating several behavioural endpoints, though such a framework is not exclusive to this group.

migration) as opposed to or in addition to those that could have indirect implications (e.g., flight behaviours, which may or may not impact foraging; activity, boldness, exploration, sociality that may affect or not dispersal and migration) will be valuable in anticipating ecosystem impacts.

The limited data available seem to point out different behavioural states as early and more sensitive signs of stress than other previously considered endpoints in NPLs research endowed with higher environmental relevance. Alterations in swimming performance were reported after 96h exposure to PS NPLs (22 nm) at 0.001, 1 and 100 mg/L and to PMMA NPLs (32 nm) at 0.001, 0.01, 0.1 and 10 mg/L, while no effects on biochemical biomarkers were observed for the same concentrations [4]. Exposure to environmentally relevant concentrations of NPLs, are commonly reported to induce low mortality rates or other physiological impairments such as malformations, but there is emerging evidence that NPLs interfere with organism's ability to feed properly, swim efficiently, or even interact with their peers (Fig. 4). These data, although limited, are of great concern. For instance, NPLs-induced alterations in swimming performance may compromise the ability of organisms to escape predators - predator avoidance - and to capture prey-feeding - which are ecologically relevant behaviours as they can be related to growth and survival [52]. Furthermore, it can also influence (fish) shoaling cohesion which can translate into increased vulnerability to predation, compromising survival and population persistence. Alterations in aggressiveness may influence

Fig. 4. Highlights on the major results retrieved from studies relating nanoplastics (NPLs) effects and behavioural alterations. The sizes of the blue boxes are in accordance with the number of studies published; the box in grey represents the gap of knowledge in this topic.

intra and interspecific competition which in turn may have repercussions on individual feeding rate, mating success and parental care, decreasing the survival of organisms and leading to population decline. As such, the different behavioural endpoints are difficult to segregate and rank (i.e., which behaviour determines which), so it is also of added value to include and correlate, whenever possible, different behaviours to increase knowledge, to preview with more accuracy the potential ecological consequences of NPLs exposure.

Therefore, studies monitoring behavioural responses over time would be extremely beneficial to infer the actual ecosystems status in plastic-contaminated environments. Thus, the behavioural repertoire is vital for individual fitness throughout an animal's lifetime [52]. In response to different stimuli, organisms adjust their behaviour to optimize their adaptation to new conditions. Behavioural changes may represent either compensatory, reversible adaptive responses to mitigate potential overt effects (e.g., direct behavioural response after perception of stress) or irreversible effects of a toxicant on a behavioural mechanism or expression after toxicokinetic and toxicodynamic processes have started, such as AChE inhibition exerted by neurotoxins [59,60]. By adultering the contexts in which animals make decisions, human impacts can uncouple formerly reliable environmental cues from actual/expected outcomes. Therefore, future studies should also assess the ability of organisms to revert from NPLs-induced behavioural alterations through behavioural reassessment after a depuration period.

According to the information delivered in this review, there are additional important remarks that should guide future NPLs research: (i) the need to invest in standardization of behavioural analysis (methodological approaches) for target biological models (endpoints, exposure durations, assessment duration, number of replicates); (ii) study particles of widely produced polymers (oil based and biobased), environmentally relevant shapes (e.g., irregular, fibres), different sizes (as size plays a key role in bioavailability and biocompatibility), age and with different environmental background (pristine and environmentally sampled at sites of different contamination profiles as effects may be modulated by adsorbed compounds); (iii) NPLs stability (dispersion or aggregation behaviour) studies on the test media used for the assays (concomitantly with ecotoxicity reporting) are recommended because variations in tested media may influence the final conformations of the particles, determine their fate and/or bioavailability to the organisms (e.g., the single use of SEM or TEM does not relate to the particles' behaviour in test media for instance); (iv) keep also in mind that the choice of the behavioural endpoint to be tested is species-dependent, thus, the lack of studies on some specific trophic groups may persist as some species may lack responsiveness to some behavioural traits (e.g., sessile species lack active avoidance behaviour, or filter-feeders lack predation skills); (v) within the same behavioural endpoint, the lack of commonality on the parameters analysed difficult interpretation, even when comparing similar sized particles and the same polymer type, and may further entangled conclusions to be drawn, being suggested to provide information whenever possible related to endpoints already studied in the literature (for instance, when studying swimming behaviour, distance and time are very common parameters to be analysed); (vi) for some newer, less studied behavioural endpoints, data reported are mostly qualitative, for which computational and tracking/recording equipment are thus fundamental, since the lack of quantifiable parameters to be delivered also makes data comparison difficult.

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.

Data availability

No data was used for the research described in the article.

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References

[1] N. Singh, O.A. Ogunseitan, M.H. Wong, Y. Tang, Sustainable materials alternative to petrochemical plastics pollution: a review analysis, Sustainable horizons 2 (2022), 100016.

[2] J. Gigault, A. Ter Halle, M. Baudrimont, P.Y. Pascal, F. Gauffre, T.L. Phi, H. El Hadri, B. Grassl, S. Reynaud, Current opinion: what is a nanoplastic? Environ. Pollut. 235 (2018) 1030–1034.

[3] A. Ter Halle, L. Jeannau, M. Martignac, E. Jardé, B. Pedrono, L. Brach, J. Gigault, Nanoplastic in the North atlantic subtropical gyre, Environ. Sci. Technol. 51 (23) (2017) 13689–13697.

[4] P. Manuel, M. Almeida, M. Martins, M. Oliveira, Effects of nanoplastics on zebrafish embryo-larval stages: a case study with polystyrene (PS) and polymethylmethacrylate (PMMA) particles, Environ. Res. 213 (2022), 113584.

[5] EU, Commission recommendation of 18 October 2011 on the definition of nanomaterial (2011/696/EU), Official Journal L 275 (2011) 38–40.

[6] I. Ferreira, C. Venâncio, I. Lopes, M. Oliveira, Nanoplastics and marine organisms: what has been studied? Environ. Toxicol. Pharmacol. 67 (2019) 1–7.

[7] T. Atugoda, H. Piyumali, H. Wijesekara, C. Sonne, S.S. Lam, K. Mahatantila, M. Vithanage, Nanoplastic occurrence, transformation and toxicity: a review, Environ. Chem. Lett. (2022) 1–19.

[8] M. Oliveira, M. Almeida, The why and how of micro (nano) plastic research, Trac. Trends Anal. Chem. 114 (2019) 196–201.

[9] J. Gigault, H. El Hadri, B. Nguyen, B. Grassl, L. Rowenczyk, N. Tufenkji, S. Feng, M. Wiesner, Nanoplastics are neither microplastics nor engineered nanoparticles, Nat. Nanotechnol. 16 (5) (2021) 501–507.

[10] P.K. Rai, V. Kumar, C. Sonne, S.S. Lee, R.J. Brown, K.H. Kim, Progress, prospects, and challenges in standardization of sampling and analysis of micro-and nano-plastics in the environment, J. Clean. Prod. 325 (2021), 129321.

[11] J.A. Pitt, J.S. Kozal, N. Jayasundara, A. Massarsky, R. Trevisan, N. Geitner, M. Wiesner, E.D. Levin, R.T. Di Giulio, Uptake, tissue distribution, and toxicity of polystyrene nanoparticles in developing zebrafish (*Danio rerio*), Aquat. Toxicol. 194 (2018) 185–194.

[12] T.F. Lins, A.M. O'Brien, T. Kose, C.M. Rochman, D. Sinton, Toxicity of nanoplastics to zooplankton is influenced by temperature, salinity, and natural particulate matter, Environ. Sci.: Nano 9 (8) (2022) 2678–2690.

[13] O. Pikuda, E.R. Dumont, S. Matthews, E.G. Xu, D. Berk, N. Tufenkji, Sub-lethal effects of nanoplastics upon chronic exposure to Daphnia magna, Journal of Hazardous Materials Advances 7 (2022), 100136.

[14] C. Venâncio, I. Melnic, M. Tamayo-Belda, M. Oliveira, M.A. Martins, I. Lopes, Polymethylmetacrylate nanoplastics can cause developmental malformations in early life stages of, Xenopus laevis. Science of the Total Environment 806 (2022), 150491.

[15] C. Venâncio, A. Savuca, M. Oliveira, M.A. Martins, I. Lopes, Polymethylmethacrylate nanoplastics effects on the freshwater cnidarian *Hydra viridissima*, J. Hazard Mater. (2021) 402.

[16] M. Ågerstrand, K. Arnold, S. Balshine, T. Brodin, B.W. Brooks, G. Maack, E.S. McCallum, G. Pyle, M. Saaristo, A.T. Ford, Emerging investigator series: use of behavioural endpoints in the regulation of chemicals, Environ. Sci.: Process. Impacts 22 (1) (2020) 49–65.

[17] A.T. Ford, M. Ågerstrand, B.W. Brooks, J. Allen, M.G. Bertram, T. Brodin, Z. Dang, S. Duquesne, R. Sahm, F. Hoffmann, H. Hollert, The role of behavioral ecotoxicology in environmental protection, Environ. Sci. Technol. 55 (9) (2021) 5620–5628.

[18] M. Oliveira, D.N. Cardoso, A.M.V.M. Soares, S. Loureiro, Effects of short-term exposure to fluoxetine and carbamazepine to the collembolan *Folsomia candida*, Chemosphere 120 (2015) 86–91.

[19] M. Saaristo, A. Lagesson, M.G. Bertram, J. Fick, J. Klaminder, C.P. Johnstone, B.B.M. Wong, T. Brodin, Behavioural effects of psychoactive pharmaceutical exposure on European perch (*Perca fluviatilis*) in a multi-stressor environment, Sci. Total Environ. 655 (2019) 1311–1320.

[20] G. Hellström, J. Klaminder, F. Finn, L. Persson, A. Alanärä, M. Jonsson, J. Fick, T. Brodin, GABAergic anxiolytic drug in water increases migration behaviour in salmon, Nat. Commun. 7 (1) (2016) 1–7.

[21] M. Sharma, J. Thakur, S. Verma, Behavioural responses in effect to chemical stress in fish: a review, International Journal of Fisheries and Aquatic Studies 7 (1) (2019) 1–5.

[22] C. Venâncio, R. Ribeiro, I. Lopes, Active emigration from climate change-caused seawater intrusion into freshwater habitats, Environ. Pollut. 258 (2020), 113805.

[23] R.A. Moreira, C.V. Araújo, T.J. da Silva Pinto, L.C.M. da Silva, B.V. Goulart, N.P. Viana, C.C. Montagner, M.N. Fernandes, E.L.G. Espindola, Fipronil and 2, 4-D effects on tropical fish: could avoidance response be explained by changes in swimming behavior and neurotransmission impairments? Chemosphere 263 (2021), 127972.

[24] S. Gaaied, M. Oliveira, I. Domingues, M. Banni, 2, 4-Dichlorophenoxyacetic acid herbicide effects on zebrafish larvae: development, neurotransmission and behavior as sensitive endpoints, Environ. Sci. Pollut. Control Ser. 27 (4) (2020) 3686–3696.

[25] M.S.S. Silva, M. Oliveira, P. Valente, E. Figueira, M. Martins, A. Pires, Behavior and biochemical responses of the polychaeta *Hediste diversicolor* to polystyrene nanoplastics, Sci. Total Environ. 707 (2020), 134434.

[26] A.A. Horton, A. Walton, D.J. Spurgeon, E. Lahive, C. Svendsen, Microplastics in freshwater and terrestrial environments: evaluating the current understanding to identify the knowledge gaps and future research priorities, Sci. Total Environ. 586 (2017) 127–141.

[27] V. Kumar, E. Singh, S. Singh, A. Pandey, P.C. Bhargava, Micro-and nano-plastics (MNPs) as emerging pollutant in ground water: environmental impact, potential risks, limitations and way forward towards sustainable management, Chem. Eng. J. 459 (2023), 141568.

[28] Y. Chae, Y.J. An, Effects of micro-and nanoplastics on aquatic ecosystems: current research trends and perspectives, Mar. Pollut. Bull. 124 (2) (2017) 624–632.

[29] S.A. Strungaru, R. Jijie, M. Nicoara, G. Plavan, C. Faggio, Micro-(nano) plastics in freshwater ecosystems: abundance, toxicological impact and quantification methodology, Trac. Trends Anal. Chem. 110 (2019) 116–128.

[30] B. Zhang, J. Chao, L. Chen, L. Liu, X. Yang, Q. Wang, Research progress of nanoplastics in freshwater, Sci. Total Environ. 757 (2021), 143791.

[31] T. Cedervall, L.A. Hansson, M. Lard, B. Frohm, S. Linse, Food chain transport of nanoparticles affects behaviour and fat metabolism in fish, PLoS One 7 (2) (2012), e32254.

[32] K. Mattsson, M.T. Ekvall, L.-A. Hansson, S. Linse, A. Malmendal, T. Cedervall, Altered behavior, physiology, and metabolism in fish exposed to polystyrene nanoparticles, Environ. Sci. Technol. 49 (2015) 553–561.

[33] A. Götz, S. Beggel, J. Geist, Dietary exposure to four sizes of spherical polystyrene, polylactide and silica nanoparticles does not affect mortality, behaviour, feeding and energy assimilation of Gammarus roeseli, Ecotoxicol. Environ. Saf. 238 (2022), 113581.

[34] S. Seena, I.B. Gutiérrez, J. Barros, C. Nunes, J.C. Marques, S. Kumar, A.M. Gonçalves, Impacts of low concentrations of nanoplastics on leaf litter decomposition and food quality for detritivores in streams, J. Hazard Mater. 429 (2022), 128320.

[35] A.F. Pedersen, D.N. Meyer, A.-M.V. Petriv, A.L. Soto, J.N. Shields, C. Akemann, B.B. Baker, W.-L. Tsou, Y. Zhang, T.R. Baker, Nanoplastics impact the zebrafish (*Danio rerio*) transcriptome: associated developmental and neurobehavioral consequences, Environ. Pollut. 266 (2) (2020), 115090.

[36] S. Sarasamma, G. Audira, P. Siregar, N. Malhotra, Y.-H. Lai, S.-T. Liang, J.-R. Chen, Kelvin H.-C. Chen, C.-D. Hsiao, Nanoplastics cause neurobehavioral impairments, reproductive and oxidative damages, and biomarker responses in zebrafish: throwing up alarms of wide spread health risk of exposure, Int. J. Mol. Sci. 21 (2020) 1410.

[37] F.N. Estrela, A.T.B. Guimarães, F.G. Silva, T.M. Luz, A.M. Silva, P.S. Pereira, G. Malafaia, Effects of polystyrene nanoplastics on *Ctenopharyngodon idella* (grass carp) after individual and combined exposure with zinc oxide nanoparticles, J. Hazard Mater. 403 (2021), 123879.

[38] A.T.B. Guimarães, F.N. Estrela, A.S.L. Rodrigues, T.Q. Chagas, P.S. Pereira, F.G. Silva, G. Malafaia, Nanopolystyrene particles at environmentally relevant concentrations causes behavioral and biochemical changes in juvenile grass carp (*Ctenopharyngodon idella*), J. Hazard Mater. 403 (2021), 123864.

[39] E. Bergami, A.K. Emerenciano, L.P. Pinto, W.R. Joviano, A. Font, T.A. de Godoy, J.R.M.C. Silva, M. González-Aravena, I. Corsi, Behavioural, physiological and molecular responses of the Antarctic fairy shrimp *Branchinecta gaini* (Daday, 1910) to polystyrene nanoplastics, NanoImpact 28 (2022), 100437.

[40] B. De Felice, M. Sugni, L. Casati, M. Parolini, Molecular, biochemical and behavioral responses of *Daphnia magna* under long-term exposure to polystyrene nanoplastics, Environ. Int. 164 (2022), 107264, 2022.

[41] J. Santos, A. Barreto, E.M.L. Sousa, V. Calisto, M.J.B. Amorim, V.L. Maria, The role of nanoplastics on the toxicity of the herbicide phenmedipham, using *Danio rerio* embryos as model organisms, Environ. Pollut. 303 (2022), 119166.

[42] D.D. Nabinger, S. Altenhofen, J.V. Peixoto, J.M.K. da Silva, R. Gerlai, C.D. Bonan, Feeding status alters exploratory and anxiety-like behaviors in zebrafish larvae exposed to quinpirole, Prog. Neuro Psychopharmacol. Biol. Psychiatr. 108 (2021), 110179.

[43] S. Rist, A. Baun, N.B. Hartmann, Ingestion of micro- and nanoplastics in Daphnia magna – quantification of body burdens and assessment of feeding rates and reproduction, Environ. Pollut. 228 (2017) 398–407.

[44] N.N. Phuong, A. Zalouk-Vergnoux, L. Poirier, A. Kamari, A. Châtel, C. Mouneyrac, F. Lagarde, Is there any consistency between the microplastics found in the field and those used in laboratory experiments? Environ. Pollut. 211 (2016) 111–123.

[45] R. Trevisan, P. Ranasinghe, N. Jayasundara, R.T. Di Giulio, Nanoplastics in aquatic environments: impacts on aquatic species and interactions with environmental factors and pollutants, Toxics 10 (6) (2022) 326.

[46] C.J. Thiele, M.D. Hudson, A.E. Russell, M. Saluveer, G. Sidaoui-Haddad, Microplastics in fish and fishmeal: an emerging environmental challenge? Sci. Rep. 11 (1) (2021) 1–12.

[47] M. Bhuyan, Effects of microplastics on fish and in human health, Front. Environ. Sci. (2022) 250.

[48] C. Cano-Barbacil, J. Radinger, M. Argudo, F. Rubio-Gracia, A. Vila-Gispert, E. García-Berthou, Key factors explaining critical swimming speed in freshwater fish: a review and statistical analysis for Iberian species, Sci. Rep. 10 (1) (2020) 1–12.

[49] J.M. Orozco-Hernandez, L.M. Gomez-Oliván, G.A. Elizalde-Velazquez, K.E. Rosales-Pérez, J.D. Cardoso-Vera, G. Heredia-García, H. Islas-Flores, S. García-Medina, M. Galar-Martínez, Fluoxetine-induced neurotoxicity at environmentally relevant concentrations in adult zebrafish Danio rerio, Neurotoxicology 90 (2022) 121–129.

[50] S. Matthews, E.G. Xu, E.R. Dumont, V. Meola, O. Pikuda, R.S. Cheong, M. Guo, R. Tahara, H.C. Larsson, N. Tufenkji, Polystyrene micro-and nanoplastics affect locomotion and daily activity of Drosophila melanogaster, Environ. Sci.: Nano 8 (1) (2021) 110–121.

[51] A. Barreto, L.G. Luis, A.V. Girão, T. Trindade, A.M. Soares, M. Oliveira, Behavior of colloidal gold nanoparticles in different ionic strength media, J. Nanoparticle Res. 17 (2015) 1–13.

[52] T. Brodin, S. Piovano, J. Fick, J. Klaminder, M. Heynen, M. Jonsson, Ecological effects of pharmaceuticals in aquatic systems—impacts through behavioural alterations, Phil. Trans. R. Soc. B 369 (2014), 20130580.

[53] M. Faimali, C. Gambardella, E. Costa, V. Piazza, S. Morgana, N. Estevez-Calvar, F. Garaventa, Old model organisms and new behavioral endpoints: swimming alteration as an ecotoxicological response, Mar. Environ. Res. 128 (2017) 36–45.

[54] J.S. Weis, G. Smith, T. Zhou, C. Santiago-Bass, P. Weis, Effects of Contaminants on Behavior: biochemical Mechanisms and Ecological Consequences: killifish from a contaminated site are slow to capture prey and escape predators; altered neurotransmitters and thyroid may be responsible for this behavior, which may produce population changes in the fish and their major prey, the grass shrimp, Journal Article. BioScience 51 (3) (2001) 209–217.

[55] Plastic Europe, Plastics - the Facts 2021 an Analysis of European Plastics Production, Demand and Waste Data, 2021, p. 34. https://plasticseurope.org/wp-content/uploads/2021/12/Plastics-the-Facts-2021-web-final.pdf. (Accessed 2 January 2023).

[56] L.C. De Sá, M. Oliveira, F. Ribeiro, T.L. Rocha, M.N. Futter, Studies of the effects of microplastics on aquatic organisms: what do we know and where should we focus our efforts in the future? Sci. Total Environ. 645 (2018) 1029–1039.

[57] S.L. King, R.S. Schick, C. Donovan, C.G. Booth, M. Burgman, L. Thomas, J. Harwood, An interim framework for assessing the population consequences of disturbance, Methods Ecol. Evol. 6 (2015) 1150–1158.

[58] D. Start, B. Gilbert, Predator personality structures prey communities and trophic cascades, Ecol. Lett. 20 (3) (2017) 366–374.

[59] A. Gerhardt, Monitoring behavioural responses to metals in *Gammarus pulex* (L.) (Crustacea) with impedance conversion, Environ. Sci. Pollut. Control Ser. 2 (1995) 15–23.

[60] G. Dell'Omo (Editor), Behavioural Ecotoxicology, John Wiley & Sons, 2002.