

RESEARCH REVIEW

Microplastics and the functional traits of fishes: A global meta-analysis

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Abstract

Over the years, concern about the effects of microplastics has grown. Here, we answered the main question “What are the impacts of microplastics on the functional traits of fish species?” through a meta-analysis. The general impact of microplastic exposure on the functional traits of fishes and specifically on eight variables, namely, behaviour, development, fecundity, feeding, growth, health, hatching and survival was explored. Subgroup analyses were performed to detect correlations between the impact of microplastics and the following factors: species, life stage, habitat, water column habitat, day of exposure to microplastics and microplastic size, type and shape. A meta-regression analysis allowed understanding the correlation between the impact of microplastics and the size of organisms. Generally, microplastics have a negative effect on the functional traits of fishes. Feeding and behaviour, followed by growth showed the greatest impact. Among the subgroup analysis, four of the eight variables considered showed a significant difference between groups: species, life stage, microplastic shape and days of exposure to microplastics. Depending on their life stage, organisms may be more sensitive to microplastic pollution. Changes in growth rates, development of early life stage and behavioural patterns in fishes may have a negative effect on the structure and functions of aquatic ecosystem in the long term and consequently affect the ability of aquatic ecosystems to provide ecosystem services and sustain human communities.

KEYWORDS

effects size, fishes, functional traits, meta-analysis, microplastics

1 | INTRODUCTION

The accumulation and fragmentation of plastics represent one of the most recent, ubiquitous, long-lasting and warning signals for our planet. Within just a few decades, since mass production of plastic products, plastic debris have accumulated, from terrestrial environments, in the open oceans and even the most remote environments such as the deep-sea floor (Galgani et al., 1996; Kane et al., 2020). Microplastics are typically defined as plastic pieces of <5 mm in size

primarily derived from larger plastic items that are crumbled in the environment as a consequence of prolonged exposure to UV light and physical abrasion (Andrady, 2003; Thompson et al., 2004). Microplastics have been documented in all aquatic systems from both marine and freshwater habitats (Barnes et al., 2009), on beaches (Browne et al., 2011), sediments (Claessens et al., 2011), and ubiquitously along the water columns (Eriksen et al., 2013). The sources of microplastics found across all ecosystems vary and include food or drink containers, packaging, fibres from synthetic clothing, industrial waste and microbeads (components of some beauty products; Biginagwa et al., 2016; Kershaw & Rochman, 2015).

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Over the years, plastic pollution has become a growing concern. Jambeck et al. (2015) calculated that 275 million metric tons (MT) of plastic waste was generated in 192 coastal countries (93% of the global population) in 2010, with 4.8–12.7 million MT entering the ocean. A part of these plastic debris floats on the sea surface. Eriksen et al. (2014) estimated that at least 5.25 trillion plastic particles weighing 26,894 tons were floating in the world's oceans and 3554 tons of these were microplastics, whose early detection through spectral characterisation is promising (Corbari et al., 2020). The vast majority of plastic ends up in the deep sea. The seafloor can be considered as a hotspot of microplastic pollution, exhibiting the highest densities in the order of up to 1.9 million particles per m^2 (e.g. Tyrrhenian Sea), which is driven by near-bed thermohaline currents (bottom currents), assembling extensive seafloor sediment accumulations (Kane et al., 2020). These currents supply oxygen and nutrients to deep-sea benthos, suggesting that deep-sea biodiversity hotspots are also likely to be microplastic hotspots (Azpiroz-Zabala et al., 2017; Davies et al., 2009). Based on these data, it could be assumed that the highest amount of plastic per individual may well be found in benthopelagic fish.

The number of studies examining the potential impacts of microplastics on aquatic ecosystems, marine species and food webs has increased exponentially (Bucci et al., 2020; Lusher et al., 2017). Microplastics have been found in the digestive tracts of both farmed and wild-caught fish (Foekema et al., 2013; Ma et al., 2020; Phillips & Bonner, 2015) and aquatic invertebrates (Cole et al., 2011). Organisms may actively ingest microplastic particles, confusing them with prey (Savoca et al., 2017), or passively during particle filtration (Collignon et al., 2014). Azevedo-Santos et al. (2019) reported plastic ingestion by 427 fish species (mostly marine species) from different regions, ecosystems and guilds.

After ingestion, microplastics can remain in the digestive tracts of organisms from days to weeks (Batel et al., 2016; Browne et al., 2008). This uptake/ingestion, retention and egestion/elimination of microplastics—kinetic processes and exposure to microplastics from both diet and water—could adversely affect the health of organisms (e.g. gastrointestinal tract). Yin et al. (2019) found that microplastic exposure altered behavioural traits, energy reserves and the nutritional quality of demersal black rockfish. However, some studies have concluded that microplastics have no effect on organisms, unless associated with organic contaminants (Le Bihanic et al., 2020; Schmiege et al., 2020).

Here, we classified a multitude of studies and different related outputs by extracting and synthesising data available in the literature. In order to reply to the main question: "What are the impacts of microplastics on functional traits of fish species?" we conducted a meta-analysis examining the impacts of microplastic exposure on the functional traits of fishes. We focused on the functional traits because most prominent ecological research conducted in the last two decades definitively assigns a primary role to functional traits as the shaping forces of population dynamics and ultimately ecosystem functioning (Sibly et al., 2012). By functional, here we refer

to "morpho-physio-phenological traits which impact fitness indirectly via their effects on growth, reproduction and survival, the three components of individual performance" (Violle et al., 2007, p. 882). Performance traits have been included in our meta-analysis (Arnold, 1983). In fishes—the main target of this study constituting the vast majority of organism biomass in aquatic habitats and food items largely included in human consumption—these traits usually include tolerance and sensitivity to environmental conditions (e.g. Kearney & Porter, 2009). These latter limit the ability of each species to maintain its metabolic machinery (Sarà et al., 2014; Sokolova et al., 2012), to obtain energy from food, and all other behavioural traits such as swimming behaviour, habitat use, the mating system and morphological (e.g. shape) traits (Schoener, 1986), allowing optimisation of energetic income (Krebs & Davies, 1987). Thus, to gain insight on the effects of any potential disturbance factor, such as microplastics on the functional traits of fishes, it is crucial to increase our understanding of how microplastics can impair basic functions (as expressed by organismal functional traits; Violle et al., 2007) of ecological functioning. Several perspective and opinion papers have tried to shed light on the effects of microplastic pollution on the biotic components of aquatic systems and attempted to summarise the accumulated knowledge (Al-Thawadi, 2020; Pirsahab et al., 2020). So far, however, efforts to synthesise available data through meta-analytical quantitative approaches are limited (Foley et al., 2018). Nonetheless, meta-analyses allow quantitative assessment of the effect of a given treatment (in this case, exposure to microplastics) over multiple studies conducted using different experimental procedures, on different species, and by different research groups and to summarise and understand the broader potential impacts of the treatment in question. Foley et al. (2018) examined the impacts of the exposure of fish and aquatic invertebrates to microplastics, considering four responses: consumption and feeding, growth, reproduction and survival. They assessed whether the effects are consistent across different taxonomic groups or plastic shapes, and examined whether the size of the effect varies with experimental conditions. By including only experimental and manipulative studies, we wanted to reach beyond the common aims found across the current literature, which evaluate the general impact of microplastic exposure on the functional traits of all fishes and to increase the number of variables examined by Foley et al. (2018), investigating: four functional traits—behaviour, development, feeding, hatching—and three performance traits—fecundity, growth, survival (as from Arnold's, 1983 framework revisited by Violle et al., 2007). An extra category named "Health" was created to group variables commonly found out into the retrieved literature assessing fish health status (Table 1). Through subgroup analyses, we explored whether there is a correlation between the impact of microplastics and many other factors (e.g. species, life stage, habitat, water column habitat, microplastic size, type, shape and days of exposure). Moreover, given that body size is the major trait driving individual fitness (Barneche et al., 2018), we explored the correlation between the impact of plastic and the body size of fishes.

TABLE 1 List of the eight response categories examined in our meta-analysis, the associated description/quantification and the measured variables used (keywords) in the selected studies. Specifically, the four functional traits—behaviour, development, feeding, hatching—the three performance traits—fecundity, growth, survival—as from Arnold's (1983) framework revisited by Violle et al. (2007), and a last category dealing with 'health', commonly found in the retrieved literature. Regarding the quantification of variables, we followed the quantification reported by the authors, according to the methods applied by them

Trait	Description/Quantification	Measured variables (keywords)
Functional traits		
Behaviour	Organisms' behavioural changes reported after ingestion or exposure to microplastics in comparison to an unexposed control group	Activity rate, locomotor activity, mobility, swimming velocity, maximum velocity, distance travelled, spontaneous movement, turning behaviour, inactivity
Development	Capability to grow specific part of the organisms or to complete transformation stages after exposure to microplastics and in comparison to an unexposed control group	Biometric measurements, head length, head height, head depth, liver weight, gill weight, gonad weight, swim bladder area, optic vesicle area, pericardium area, angle between myosepts, distance between myosepts, interocular distance
Feeding	Feeding activity of organisms in the presence of microplastics and in comparison to an unexposed control group	Predatory performance, feeding success, foraging time, number of ingested prey
Hatching	Variation in the egg hatching phase after exposure to microplastics and in comparison to an unexposed control group	Hatching per cent, hatching success, hatching rate, hatching time
Performance traits		
Fecundity	Variation in egg production and fertilisation after exposure to microplastics and in comparison to an unexposed control group	Egg production, fertilisation rate
Growth	Variation in organismal size in the unit of time measured after exposure to microplastics and in comparison to an unexposed control group	Body weight, body length, standard length, total length, weight gain rate, body mass, changes in body mass, length-weight ratio
Survival	Survival rate of organisms exposed to microplastics and in comparison to an unexposed control group	Survival rate, survival percentage ^a
Health	Changes in general organism health state after exposure to microplastics and in comparison to an unexposed control group	Condition factor, hepatosomatic index, gonadosomatic index, heartbeat, mucus secretion, oxygen consumption

^aFor those studies reporting mortality percentage, where possible, these measures have been converted into survival percentage, so that the values reported for this category were exclusively survival measures.

2 | MATERIALS AND METHODS

2.1 | Literature search and data collection

The scientific papers included in our meta-analysis were collected by performing a literature search (Moher et al., 2009; Pullin & Stewart, 2006) aimed at answering our main question. A complex search string was used on the two main literature databases, ISI Web of Knowledge (Web of Science Core Collection package, Clarivate Analytics, 2019) and Scopus, with no temporal scale restriction. The search string was:

("microplastic" OR "micro plastic*" OR "micro-plastic*") AND ("trait*" OR "function*" OR "response*" OR "measure*" OR "rate*" OR "Behaviour" OR "Feeding" OR "Growth" OR "Health" OR "Hatching" OR "Survival") AND ("laboratory" OR "mesocosm*" OR "experiment*" OR "treatment*") AND ("fish*")*

The search string was created to include four main elements of our primary question (linked by the Boolean operator 'AND'; Moher et al., 2009; Pullin & Stewart, 2006), respectively: the exposure (i.e. microplastic and related keywords), the measured outcomes (i.e.

traits and related keywords), the target population (or subject of the search, i.e. fish) and the observation type (e.g. laboratory and related terms). This latter group of keywords was added to avoid the inclusion of the large number of studies reporting only the microplastic occurrence in the field with not associated measured effects reported (details on the search string creation strategy are reported in Table S1). The measured outcomes, list of traits taken into exam, an associated description/quantification and the measured variables used (keywords) in the selected studies are in Table 1. All the synonymous were linked by the Boolean operator 'OR'. By running the search string (final search date 20 May 2020; Table S1) and after checking for duplicates (Figure S1), we obtained 208 scientific peer-reviewed papers. Spurious results were removed, for example studies concerning environmental microplastic pollution, description of microplastic extraction methods and microplastic effects on several aquatic organisms other than fish, from zooplankton to benthic organisms (Table S2). Given that our study aimed at testing the global effects of microplastics on fish species, we considered species from all the water realms (i.e. freshwater, marine and estuarine) and fish specimens at any life stage (i.e. from the embryo stage to adults).

We selected those studies that clearly compared experimental treatment groups against one or more controls (i.e. group of organisms exposed to microplastics tested against untreated organisms) and those studies that reported the mean values of the measured functional trait variables, the number of replicates and a measure of the variability around the mean (Table S3). We selected those studies focusing on the effects of microplastics on the functional traits of fishes, measurable at individual level, and variables that had direct effect at population level. The functional traits that we focused on included morphological (e.g. body length), physiological (e.g. hepatosomatic index, respiration) and behavioural (e.g. swimming activity) traits (Arnold, 1983; Violle et al., 2007). These traits are usually involved in the optimisation of individual fitness and have effects at the population level, such as growth and mortality response. Thus, we excluded all observational studies, that is studies assessing the presence and concentration of ingested microplastic polymers or those using pollutants or other chemical compounds (e.g. antibiotics) added to microplastics, and those focusing on the effects at the sub-organismal level (e.g. studying cellular and subcellular variables such as oxidative stress, gene expression, immunological responses, etc.). Reviews, analyses on the effect of nanoplastics and papers not related to the functional trait of fish or with insufficient data were also excluded (see the list of excluded studies in Table S2). By applying such selection criteria, we selected only those with a clear description of experimental design, such as comparisons of experimental treatment groups with one or more control groups (i.e. a group of organisms exposed—'treated'—to microplastics tested against not exposed organisms, 'untreated'). Our final datasets included 43 scientific papers that were considered suitable for our analysis (Table S3; Figure S1 reporting the adapted PRISMA flow diagram; Moher et al., 2009).

2.2 | Calculation and analysis of effects

Due to the different variables and approaches adopted in the selected scientific papers, we used the Hedges' *g* statistic, which is the bias-corrected standardised mean difference between the treatment and control groups, divided by the pooled standard deviation (Hedges, 1981; Sarà, 2007). The Hedges' *g* value and its variance were calculated for each case study ($k = 754$ total case studies within our dataset) in order to estimate the difference in the effects of microplastics between an experimental treated group and a control group. Hedges' *g* weighs cases by their sample size and the inverse of their variance (Borenstein et al., 2011). The value of Hedges' *g* ranges from $-\infty$ to $+\infty$ and can be interpreted as follows (sensu Koricheva et al., 2013): $|g| \leq 0.2$ considered a small effect; $0.2 \leq |g| \leq 0.5$ a medium effect; $0.5 \leq |g| \leq 0.8$ a large effect; and $|g| \geq 0.8$ a very large effect. The effect size Hedges' *g* was calculated as follows (Borenstein et al., 2011):

$$\text{Hedges' } g = \frac{\bar{Y}_t - \bar{Y}_c}{\text{standard deviation pooled}} \times J,$$

where \bar{Y}_c and \bar{Y}_t are the mean of the control and experimental treatment groups respectively.

The correction for bias attributed to different sample sizes, represented by *J*, was estimated through differential weighting as follows:

$$J = 1 - \frac{3}{4(N_t + N_c - 2) - 1}.$$

The following formula was used to calculate the pooled standard deviation (standard deviation pooled):

$$\text{Standard deviation pooled} = \sqrt{\frac{(N_t - 1) \times SD_t^2 + (N_c - 1) \times SD_c^2}{N_t + N_c - 2}},$$

where *N* is the sample size and SD is the standard deviation of the treated or control group. In order to account for inequality in study variance, effect sizes have been weighted using the inverse of the sampling variance, therefore calculating variance for each effect size (V_g) as follows (Koricheva et al., 2013):

$$V_g = \frac{N_t + N_c}{n_t n_c} + \frac{g^2}{2(n_t n_c)}.$$

As the sign of Hedges' *g* tells the direction of the effect, a negative value of Hedges' *g* indicates that microplastics have a higher effect on impairing that specific analysed response.

In order to measure the effect size on single response variable and to minimise the high heterogeneity of the dataset, we used behaviour, development, fecundity, feeding, growth, health, hatching and survival as eight response categories (Table 1). Therefore, we run a model estimating overall effect size and 95% CI per category.

To investigate possible differences in the pooled effect size among tested variables related to the biology and ecology of fishes or to the experimental conditions of exposure to microplastics, we performed subgroup analysis. Such an analysis included the following categorical fixed factors as moderators of the mixed-effects model: habitat (freshwater, marine and estuarine), water column habitat (pelagic, benthopelagic and demersal), species (difference in the effects at individual level), life stage of fishes (embryos, larva, juvenile, adult), microplastic type (difference in the effects depending on the use of a mix of microplastics or different types of polymers), microplastic shape (fibres, fragments, spheres), microplastic size (<25, 25–100, 100–500, >2000 μm) and days of exposure (from less than a day to more than 90 days) subgroups.

Finally, to investigate the possible correlation between the dimension of the organisms (total length, mm) on different life stages and the effect of microplastic on traits, we run meta-regression analysis with mixed-effects model including organism size (total fish length expressed in mm) retrieved from the studies included in our meta-analysis as continuous fixed factor.

The meta-analyses were conducted using the metafor package for R (Anton et al., 2019; Viechbauer, 2010). We performed mixed-effects models using the 'rma.mv' function which uses a

Wald-type test to determine statistical significance. We ran the statistical model that included the study identification number (i.e. Id of the study in our dataset) and the response variable (i.e. functional trait categories) as a random factor to account for heterogeneity (Viechtbauer, 2007) and non-independence of results from the same study (Konstantopoulos, 2011).

Effect sizes for the models including categorical fixed factor were considered to be significant if their 95% confidence interval (CI) did not overlap with zero and if their $p \leq 0.05$. For the model with a continuous fixed factor (i.e. length of the organisms, mm), the predictor was considered to be significant at $p \leq 0.05$. Differences between the groups included as moderators in the subgroup analysis were considered to be significant when the p -value of the test for moderators (Q_m) calculated in the mixed-effects model was ≤ 0.05 .

2.3 | Publication bias

Results in meta-analysis might be distorted by publication bias, which is the selective publication of articles finding significant effects over those that find non-significant effects (Koricheva et al., 2013). Our analysis could result in an overestimate of the effects of microplastics on the functional traits due to the publication bias that was evaluated using Egger's regression test (Egger et al., 1997) by running models that included the standard error of the effect sizes (included as the square root of the variance) as a moderator (Habeck & Schultz, 2015). Potential publication bias was determined when the intercept of the model was different from zero at $p \leq 0.05$ (Anton et al., 2019). If potential bias was detected, we examined the data for potential outliers by looking at the effect sizes with standardised residual values exceeding

the absolute value of three (Viechbauer et al., 2010) using the `rstandard` function in R. Potential outliers were removed to adjust for publication bias. Adjusting for publication bias did not change the outcome of the analyses (by comparing fitted random-effects models with and without the influence of the potential outliers), except for the effects of microplastics on growth, freshwater species, benthopelagic and demersal species, embryos and larvae life stage and the species *Dicentrarchus labrax*, *Perca fluviatilis* and *Sebastes schlegelii* (Table S4). We then removed from the dataset the potential outliers detected in the sensitivity analysis and re-ran the mixed-effects models to evaluate the effects of microplastics on the different variable analysed. Otherwise, the sensitivity analyses showed that our results of the models were robust against publication bias (detailed information on sensitivity analysis reported in Table S4).

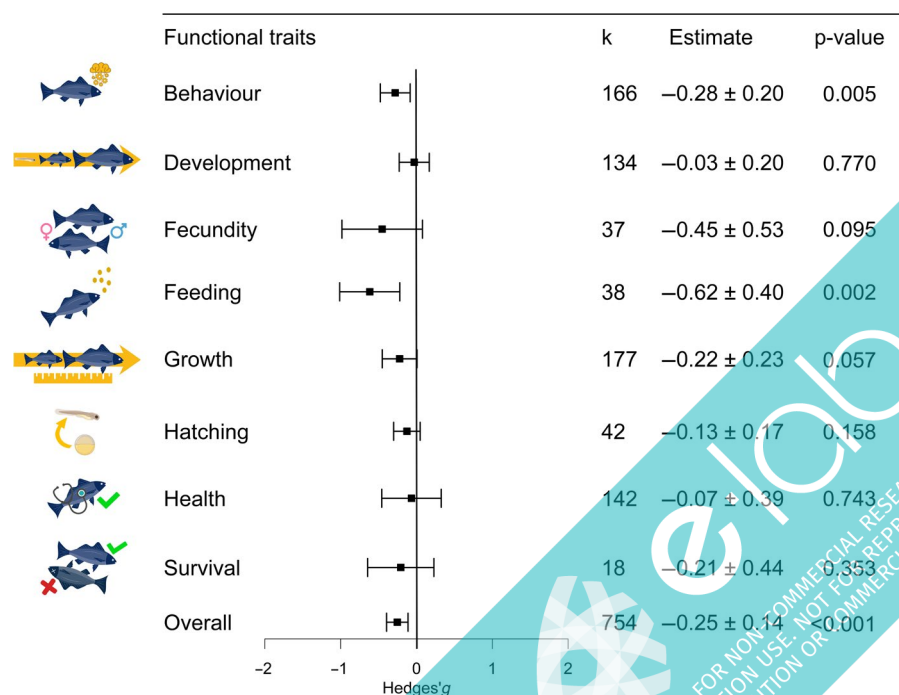
3 | RESULTS

The current analysis included 754 case studies obtained from the 43 selected papers (details of the included studies, that is variables associated with the studies and number of case studies, are reported in Table S3).

The overall analysis conducted by mixed-effects model on the entire database (including all the individual response variables) showed a medium negative effect size ($g = -0.25 \pm 0.14$; $***p < 0.001$; Figure 1). This result shows an overall effect of microplastics on fishes causing a decrease of mean response variable respect to the controls.

Concerning the response variables, three out of eight (specifically behaviour, feeding and growth) showed a significant effect (Figure 1).

FIGURE 1 Forest plot of the overall effect size and individual response variable effect size. Analysis conducted with mixed-effects model, using the `rma.mv` function of the `metaphor` package in R, including study Id and functional trait as random factor. Black boxes represent Hedges' g value and the horizontal lines represent the 95% CI for each g value; Q_m = omnibus test of moderators from the model; k = number of study cases [Colour figure can be viewed at wileyonlinelibrary.com]



3.1 | Subgroup analysis

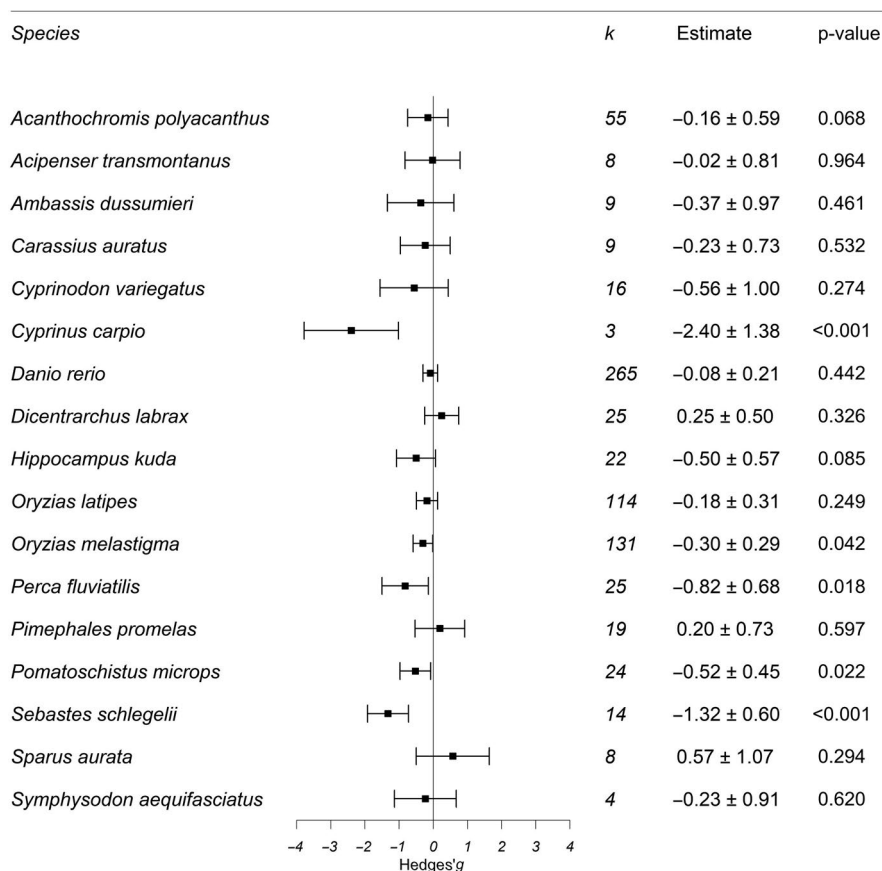
A significant difference between groups was found for four of the eight considered variables, specifically, species, life stage, microplastic shape and days of exposure to microplastic subgroups. For species, the analysis revealed significant differences between species subgroups ($Q_m = 66.04$, $***p < 0.001$) suggesting that the effect of microplastics on an individual's functional traits varies as a function of the species. The mixed-effects model analysis within subgroups revealed significant effect size for six out of 19 species with the effect varying from positive to negative, and small to very large (see Figure 2 for major details).

Embryos, larvae, juvenile and adult groups appeared to be significantly different ($Q_m = 8.52$, $*p = 0.03$). Our mixed-effects analysis within subgroups showed a medium negative effect of microplastics on juveniles ($g = -0.47 \pm 0.24$, $***p < 0.001$). No significant effect was detected within the other life stage subgroups (Figure 3).

An investigation of possible differences according to habitat in general and the water column in particular did not identify significant differences between subgroups (Figure 3).

The analysis conducted on the experimental conditions revealed significant differences between subgroups of microplastic shape ($Q_m = 9.31$, $*p = 0.02$) and between subgroups of days of exposure to microplastics ($Q_m = 20.29$, $**p = 0.005$; Figures S2 and S3). The analysis within subgroups showed a medium negative effect size for all three microplastic shape: fibres ($g = -0.53 \pm 0.47$, $*p = 0.02$), fragments ($g = -0.42 \pm 0.30$, $**p = 0.006$) and spheres ($g = -0.30 \pm 0.26$, $*p = 0.02$). Regarding the time of exposure to microplastics, the analysis within subgroups showed: medium negative effect size for 8–14 days of exposure ($g = -0.41 \pm 0.29$, $**p = 0.006$); medium negative effect size for 15–21 days ($g = -0.55 \pm 0.30$, $***p = 0.001$); medium negative effect size for 22–30 days ($g = -0.41 \pm 0.39$, $*p = 0.03$); large negative effect size for 61–90 days ($g = -0.68 \pm 0.46$, $**p = 0.004$). No significant differences were detected between groups of microplastic type or size (Figure S2).

The results of the meta-regression did not indicate any variation of the effect size in correlation with the size of the organisms regardless of the life stage ($p = 0.25$, ns). These results remained unchanged for the juvenile ($p = 0.23$, ns) and adult life stages ($p = 0.11$, ns), while being significant ($**p = 0.007$) for the larval life stage with the effect size becoming more negative as the larval size increased (Figure 4).



Test of moderators (coefficient 2:3):

	QM	df	p -val
Between groups	66.4	18	<0.001

FIGURE 2 Forest plot of the 'Species' subgroup effect size. Analysis conducted with mixed-effects model, using the rma.mv function of the metaphor package in R including study Id and functional trait as random factor. Black boxes represent the Hedges' g value and the horizontal lines represent the 95% CI for each g value; Q_m = omnibus test of moderators from the model; k = number of study cases

FIGURE 3 Forest plot of the (a) 'Habitat', (b) 'Water column habitat' and (c) 'Life stage' subgroups effect size. Analysis conducted with mixed-effects model, using the `rma.mv` function of the `metaphor` package in R, including study Id and functional trait as random factor. Black boxes represent the Hedges' g value and the horizontal lines represent the 95% CI for each g value; Q_m = omnibus test of moderators; k = number of study cases

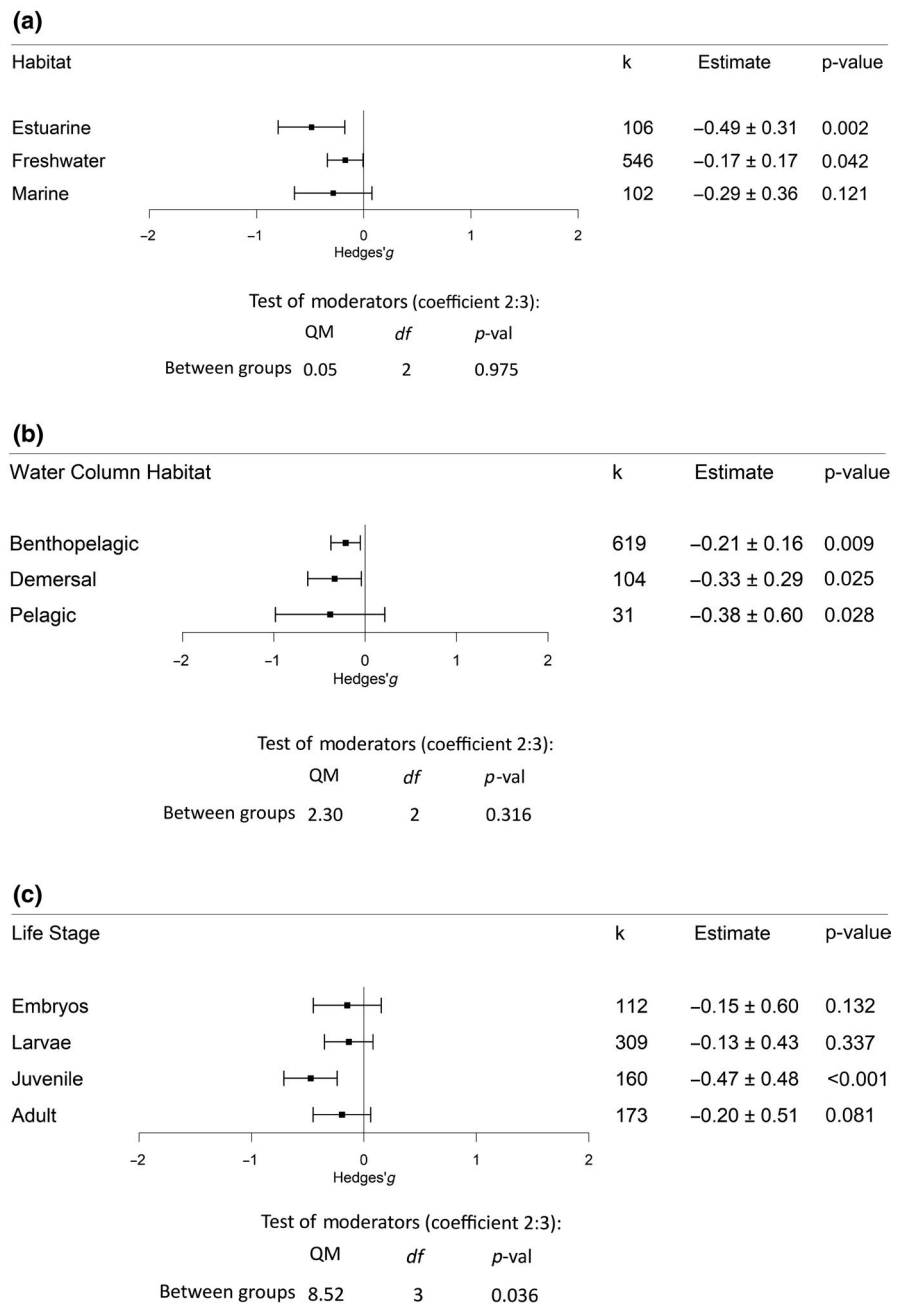
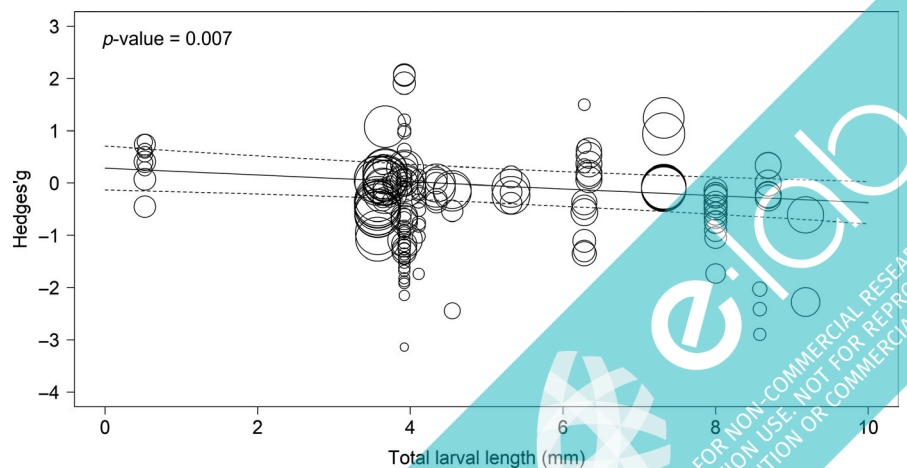


FIGURE 4 Meta-regression showing relation of effect size and organism size (total length expressed in mm). Analysis conducted with mixed-effects model, using the `rma.mv` function of the `metaphor` package in R, including study Id and functional trait as random factor. The size of the point is related to the standard error of the study; dotted lines indicate 95% CI



4 | DISCUSSION

Our results showed that microplastics have a general negative effect on the functional traits of fishes regardless of habitats and species. Regarding the analysis as a whole, which includes all the heterogeneous data, the effect size appears to be statistically significant and negative. Moreover, all the single response variables, that were statistically significant, exhibited a negative effect size. Compared to the Foley et al. (2018) study, our meta-analysis has confirmed the significant impact of exposure to microplastics on feeding and growth traits, and has highlighted a significant impact on fish behaviour and an important relationship between exposure to microplastics, size and life stage of fish.

Among the investigated response variables, the one with the greatest impact is feeding, followed by behaviour and growth. These results could be explained by the fact that the accumulation of ingested microplastics in the digestive tract of fishes may lead to malnutrition and eventual starvation (Boerger et al., 2010), and consequently lower energy intake. This could mean less energy and resources allocated to the fundamental physiological processes of growth and reproduction (Marn et al., 2020). Yin et al. (2019) reported that, in order to maintain normal functions under stressful conditions, black rockfish exposed to polystyrene showed significant reduction of available energy for growth through increased metabolic demands, while a reduction in protein content was observed as well. The presence of plastics produced a negative scope for growth in crabs; ammonia excretion and the metabolic costs of oxygen consumption outweigh the energy obtained from food ingestion (Watts et al., 2015). Moreover, plastics could affect ingestion or cause gastrointestinal blockage (Avery-Gomm et al., 2012; Cole et al., 2015). Organisms need to use internal reserves for their maintenance when plastics are present in the environment, something that may result in impairment of fundamental physiological processes. Fishes that could count on a minor amount of energy may become less reactive and slower at swimming. Moreover, lesions in organs such as the liver and the intestine, due to microplastic ingestion, might result in an abnormal fish swimming pattern (Yin et al., 2019).

Even when not ingested, microplastics can still have a negative impact on fish and their behaviour. For example, adherence of microplastics to gills and skin may change oxygen consumption and ion regulation causing respiratory stress, thus influencing behaviour (Abdel-Tawwab et al., 2019; Watts et al., 2016). Moreover, locomotion impairment can have a significant impact on fish as a predator and as a prey, influencing their survival (higher predation) or growth rate (feeding efficiency) and possibly leading to reduced populations (Little & Finger, 1990). The altered prey-predator relationships constitute an ecological concern, as regards the preservation of the marine food web and community structure. Green et al. (2017) exposed sediment cores containing a benthic community to microplastic particles and found changes in the filtration rates of bivalves and differences in associated infaunal invertebrate assemblages, thus highlighting the potential effect of microplastics on marine ecologic functioning and structure of sedimentary habitat.

Subgroup analyses revealed different sensitivities of microplastic exposure at the species level. This result was expected to a certain extent. In fact, organisms belonging to different species have physiological, anatomical and behavioural differences that make them suitable for living in their environment but at the same time reacting differently to the same stressor, such as microplastic pollution. This could explain the differences among species responses to microplastics (some have been highly impacted and others less, while some displayed a positive effect size). However, these differences are not found between groups during habitat and water column habitat subgroup analyses; this leads us to think that freshwater, marine or estuarine species, or species adapted to life in the water column or near the bottom, are equally vulnerable to microplastics.

Life stage subgroup analyses highlighted significant differences between development phases, suggesting that, depending on their life stage, organisms may be more sensitive to microplastic pollution. Interestingly, results showed greater sensitivity in juveniles. We infer the size of an organism is a key factor for interpreting these results. It might be easier for juveniles to access a wider range of microplastic sizes through ingestion. During the larval stage, in fact, larvae can eat food (or microplastic) particles that are a bit smaller than their mouth gaps. Slow development of the oral cavity of *Epinephelus coioides* at an early stage created difficulties in feeding the larvae with the right food (Kohno et al., 1997). Azfar Ismail et al. (2019) found that larval mahseer hybrid started exogenous feeding at 3 days after hatching as soon as its mouth began to open and move (average total body length 4.80 mm); the study also indicated that suitable prey size for the first feeding of the mahseer larvae was 111–173 μm in diameter. Thus, it is believed that larvae developing slowly, with too small or not fully developed mouthparts, may be unable to swallow microplastic items that are larger than their mouth gaps and, therefore, are not exposed to specific ranges of microplastic sizes. Based on this idea, we ran a meta-regression analysis, relating the size of the organisms and the effect size. Results showed a significant correlation between larval dimension and effect size; the larger the size, the larger the negative effect size. In line with our initial idea, it appears that, up to the juvenile phase, the impact of microplastics depends on size at larval stage and size of mouthpart, which means that the impact becomes increasingly important with growth. Adults, on the other hand, do not seem to be particularly sensitive to microplastics and this is consistent with the findings of Foley et al. (2018). This main finding is in accordance with differences found when comparing the effect of microplastics exposure on various taxonomic groups, that is juvenile stages of echinoderms and mollusc invertebrates were less impacted compared to juveniles of zooplankton and fishes (Foley et al., 2018).

In the course of our study, we also wondered whether the effects of microplastics on organisms could vary depending on the intrinsic characteristics of the microplastics or the conditions under which fish encounter microplastic particles. Our analysis showed that among the experimental conditions analysed, the time of exposure to microplastics was relevant, suggesting that organisms exposed to microplastics for long periods were the most vulnerable. Among

shapes, fibres and fragment plastic particles were reported as having a greater impact according to Pirsahab et al. (2020), who found that fibres and fragments with a rough surface (sharp edges) caused more physical damage than spherical microplastics. The analysis conducted on microplastic type suggested that there was no specific type of microplastic that affected organisms more than others. Lei et al. (2018) who tested the toxic effects of five different types of microplastics on zebrafish conclude that the toxicity of microplastics is closely dependent on their size and concentration, rather than their composition. Unfortunately, we did not have sufficient case studies to test microplastic concentration, due to the high heterogeneity of methods used to report microplastic concentrations (highly variable information from study to study), precluding a comprehensive analysis of concentration effects. The high heterogeneity of experimental designs and methods used was one of the more relevant issues encountered while performing this meta-analysis, highlighting the need for future standardised methods.

Moreover, some information was missing from several studies such as, for example, the type of error presented in tables or figures, or the sample size which was not always reported. We strongly recommend always including these details as well as the non-significant results, that sometimes were not reported even in the Supporting Information, to fully understand the impact of emerging issues.

5 | CONCLUSIONS

Evidence from our global meta-analysis confirmed that microplastic pollution can compromise the fitness of fishes, which can affect ecosystem functioning. Changes in growth rates, and development of early life stage and behavioural patterns in fishes may have a negative effect on the structure and functions of the aquatic ecosystem in the long term and consequently influence the ability of these to provide ecosystem services and sustain human communities. Fishes represent major sources of protein for entire communities relying exclusively on fish. A decrease in fish populations and the nutritional quality of individuals may have a large impact not only on the human diet (and other predators too), but also influence cultural traditions and the economies (from coastal to global). Thus, we pinpoint that the effects of microplastic exposure represent a major issue for practically all aquatic systems on the planet, and our findings support the scientific and public concern over plastic pollution of aquatic ecosystems. Therefore, we acknowledge the importance of echoing the scientific community's call for more standardised reporting and evaluation methods for microplastic densities and experiments (Bucci et al., 2020; Foley et al., 2018), and recommend evaluation of bioaccumulation (interaction between microplastics and contaminants) and biomagnification (capacity of microplastic particles per se as the toxic micropollutant of interest) that may influence the performance of organisms across the food web (Alava, 2020; Rochman et al., 2013). Finally, our results underline the importance to focus on functional and performance traits to support a trait-based indicator development (Beauchard et al., 2017).

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DATA AVAILABILITY STATEMENT

Data are available on request from the authors.

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SUPPORTING INFORMATION

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