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Research Paper

Treatment-level impacts of microplastic exposure may be confounded by variation in individual-level responses in juvenile fish

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ABSTRACT

Microplastic (MP) pollution is a key global environmental issue and laboratory exposure studies on aquatic biota are proliferating at an exponential rate. However, most research is limited to treatment-level effects, ignoring that there may be substantial within-population variation in responses to anthropogenic stressors. MP exposure experiments often reveal considerable, yet largely overlooked, inter-individual variation in particle uptake within concentration treatments. Here, we investigated to what degree treatment-level responses to MP exposure may be affected by variation in MP ingestion rates in the early life stages of a marine fish, the Gilt-head seabream, *Sparus aurata*. First, we tested whether MP ingestion variation is repeatable. Second, we assessed to what degree this variation may determine individual-level effects of MP exposure on fitness-related behavioural performance (i.e., escape response). We found that consistent inter-individual variation in MP ingestion was prevalent and led to differential impacts within exposure treatments. Individuals with high MP ingestion rates exhibited markedly inferior escape responses, a result that was partially concealed in treatment-level analyses. Our findings show that the measured response of populations to environmental perturbations could be confounded by variation in individual-level responses and that the explicit integration of MP ingestion variation can reveal cryptic patterns during exposure experiments.

1. Introduction

Natural populations consist of individuals that exhibit diverse levels of variation in phenotypic traits. This variation is the raw material of natural selection and has central consequences for the eco-evolutionary dynamics of populations and communities (Bolnick et al., 2011; Violle et al., 2012). In times of rapid environmental change, a better understanding of individual-level variation in the response to anthropogenic stressors will thus be important for efficient conservation strategies (Sih, 2013). However, empirical studies on such responses typically report impacts as a treatment-level effect, thereby implicitly assuming that conspecifics are ecologically equivalent. This approach may misrepresent the impact of the examined stressor, as a lack of treatment-level impacts may be masking more subtle yet important individual-level effects (Harding et al., 2019).

One of the most prominent emergent contributors to global environmental change is the contamination of natural systems with plastic litter (Thompson and Napper, 2018). Plastic waste is being generated at an exponential rate (Jambeck et al., 2015), which has led to a significant increase of plastic debris in marine ecosystems over recent decades (Ostle et al., 2019). Microplastics (MPs), most recently defined as particles of 1 to 1000 μm in size (Hartmann et al., 2019), have been found at varying concentrations in virtually all marine environments, ranging from surface waters to the deep sea and from coral reefs to the Arctic ice sheet (Auta et al., 2017; Rezania et al., 2018). The abundance of MPs in natural systems is believed to increase exponentially with decreasing particle size (Cózar et al., 2014; Erni-Cassola et al., 2017) and emerging sampling approaches revealed seawater concentrations of small MPs (< 333 μm) orders of magnitude higher than previously reported (Brandon et al., 2020). Even if all input of plastic waste into the oceans stopped

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today, the fragmentation of debris already in the system would result in a continued increase in MP abundance (Thompson and Napper, 2018). The weight concentration of pelagic MPs has been predicted to double by 2030 and quadruple by 2060 (Isobe et al., 2019).

Their pervasive nature and small size make MPs available for ingestion by a wide range of aquatic organisms (Wright et al., 2013), and gut content analyses confirm uptake of small plastics by nearly all investigated species, including annelids, bivalves, molluscs, echinoderms, cnidaria, crustaceans, zooplankton, fishes, reptiles, birds and mammals (Egbeocha et al., 2018; Markic et al., 2019; Rezania et al., 2018; Wesch et al., 2016). Potential adverse effects of MP ingestion have been suggested to arise from direct (inherent chemicals) and/or indirect (adhered chemicals or microbes) toxicity, or physical effects such as gut inflammation or blockage (Jovanović, 2017; Wright et al., 2013). Moreover, ingestion of indigestible particles may impose energetic costs, potentially increasing vulnerability to predation by forcing individuals to increase their foraging activity to compensate for reduced energy efficiency (Watts et al., 2016; Wen et al., 2018; Yin et al., 2018).

In fishes, uptake of MPs chiefly occurs in three ways: (i) inadvertently or passively during foraging, drinking or respiration (most relevant for very small particles and/or large fishes), (ii) via trophic transfer, or (iii) deliberately by mistaking plastic particles for food (Roch et al., 2020). Visual similarity to prey items has been shown to drive MP ingestion in several fish species (Mizraji et al., 2017; Ory et al., 2018, 2017) and likely represents an important pathway of ingestion for micro- and mesoplastics (Lusher et al., 2017).

Uptake of MP particles, however, is not uniform across individuals. Controlled MP exposure experiments in fishes often yield high inter-individual variation in MP ingestion even within the same particle concentrations (Table 1). Albeit largely overlooked, this variation may represent more than sampling noise (Harding et al., 2019). Recent research indicates that inter-individual MP ingestion variation is not random within populations but may covary with phenotypic traits in juvenile anemonefish (Nanninga et al., 2020). Moreover, zebrafish were shown to recognise MPs as non-edible and actively avoid ingestion or spit out captured particles in some instances (Kim et al., 2019). If such individual-level MP preference and/or avoidance patterns are consistent over time, they may exert strong selective pressures on feeding behaviour by co-determining vulnerability to plastic pollution.

Phenotype-dependent variation in MP ingestion may thus have severe consequences for natural populations. Two fish larvae encountering the same concentration of MPs in the wild may be affected in very different ways, depending on whether one ingests a much larger quantity than the other. On the population level, these differences may translate into non-random effects of MP pollution, where certain phenotypes are consistently more affected than others due to inherent differences in (MP) ingestion behaviour.

Ignoring inter-individual variation in MP ingestion during experiments may lead to inaccurate conclusions about the consequences of MP exposure. Exposure studies typically report mean-level effects of different MP treatments (e.g. control vs. different MP exposure concentrations) on a specific toxicity endpoint (e.g. performance, growth). Past studies have shown that MP ingestion can be unrelated to particle exposure concentrations in certain marine and freshwater fish species (Critchell and Hoogenboom, 2018; Mbedzi et al., 2020) and that increasing MP concentrations can correlate with an increasing coefficient of variation in ingestion rates (Nanninga et al., 2020; Roch et al., 2020); i.e., high concentration treatments still contain individuals with very low ingestion rates. Thus, if only a certain proportion of the population actually ingests notable amounts of MPs, within-treatment effects may vary significantly across individuals. In a situation of prolonged MP exposure, whether in an experimental or natural context, this effect will be magnified if ingestion variation is consistent within individuals, rather than entirely stochastic.

Here we assessed the potential individual-level effects of MP exposure on behavioural performance in early life stages of Gilt-head bream, *Sparus aurata*. The aims of this study were two-fold: Firstly, we used repeated measures of MP ingestion to assess the consistency of inter-individual variation. In other words, we aimed to test whether ingestion variation is random (variable between and within individuals) or stable over time (variable between but consistent within individuals). Secondly, we compared the treatment- and individual-level effects of MP exposure on escape performance by measuring startle response parameters in combination with MP gut contents. If MP ingestion varies non-randomly and consistently between individuals, actual exposure and associated impacts may not be homogenous within treatments, highlighting the potential for ecological and analytical consequences of MP ingestion variation.

Table 1

Within-treatment variation in microplastic (MP) ingestion. The table presents a list of values extracted from published exposure studies on marine and freshwater fishes identified by a non-exhaustive literature review. Calculated coefficients of variation (CV) are compared to expected CVs relative to mean ingestion, assuming a Poisson distribution (expected CV = 1/sqrt(mean)).

Species	Polymer	Concentration [MPs/L]	MP size range [μm]	Sample size	Mean MP ingestion [MPs/fish]	MP ingestion range	SD	CV	Expected CV	Study
Amphiprion ocellaris	PE	500	180–212	10	110	304	104	0.95	0.10	1
		100		10	79	231	75	0.95	0.11	
		50		10	37	109	43	1.16	0.16	
		10		10	10	25	8	0.80	0.32	
Danio rerio	PE	2300	250–300	14	180	580	150	0.83	0.07	2
		920		15	72	125	67	0.93	0.12	
		2760		5	5	15	6	1.20	0.45	
Diplodus sargus	PS	4.2	500–1000	151	9	78	19	2.11	0.33	3
		4.2		152	4	20	6	1.50	0.50	
Mugil cephalus	PS	2500	100–1000	10	90	n/a	126	1.40	0.11	4
Myoxocephalus brandti	PE	138,000	27–32	5	63	n/a	99	1.57	0.13	5
		13,800		5	28	n/a	40	1.43	0.19	
Oncorhynchus mykiss	Mix	9.1	1000–2000	50	6	n/a	13	2.17	0.41	6
Pomacentrus amboinensis	PS	50	200–300	60	4.4	33	5.7	1.30	0.48	7
Thymallus thymallus	Mix	9.1	1000–2000	50	5	n/a	13	2.60	0.45	6
Tilapia sparrmanii	PE	77	125	5	21	28	11	0.52	0.22	8

Polymer: PE = Polyethylene, PS = Polystyrene; SD = standard deviation; CV = coefficient of variation; Studies: 1 = (Nanninga et al., 2020), 2 = (Kim et al., 2019), 3 = (Müller et al., 2020), 4 = (Avio et al., 2015), 5 = (Hasegawa and Nakaoka, n.d.), 6 = (Roch et al., 2020), 7 = (McCormick et al., 2020), 8 = (Mbedzi et al., 2020).

2. Methods

2.1. Study species

Gilthead seabream larvae, *Sparus aurata* (Linnaeus, 1758), used in this study were provided by a commercial mariculture facility (ARDAG Red Sea Mariculture Ltd., Israel), and were the progeny of an adult breeding stock of 87 females and 43 males (mean weight: 1.71 kg, females; 1.00 kg, males). The breeding stock is from a self-source (i.e., fish bred by the facility). They are kept at 23–26 °C. The hatchery has several breeding schools which are used interchangeably. Usually a school will be used for 6–10 weeks before rotating out for ~3 months. *Sparus aurata* is a euryhaline marine fish with a broad geographic distribution. Reproduction occurs in the open sea where larvae develop for 50–60 days before they migrate to coastal lagoons and estuaries as juveniles (Tandler et al., 1995). *Sparus aurata* is an important aquaculture species and thus commonly used as model organism in feeding experiments (e.g., Sommerfeld and Holzman, 2019 and references therein).

After yolk-sac absorption, larvae were fed with a mixture of live Rotifers and Artemia until 38 days post-hatching (dph); the diet then switched to commercial dry food (< 300 μm). At day 45 dph, approximately 300 individuals were collected and transported to the Inter-University Institute, Eilat. All experiments were conducted in a temperature-controlled room (22 °C) with an automated light regime of 12 light:12 dark.

2.2. Characterisation of employed particles

Fluorescent orange Polyethylene microspheres were purchased from Cospheric (Santa Barbara, US, UVPMS-BO-1.03). These particles have a narrow size distribution (180–212 μm), a uniform spherical shape and a density of 1.03 g cm^{-3}. According to the supplier, no solvents are used during the manufacturing processes and all ingredients are inert. Particles consist of pure Polyethylene with an encapsulated fluorophore (excitation and emission wavelengths of 606 nm and 577 nm, respectively). Polyethylene is one of the most commonly found polymers in marine environmental samples (Shahul Hamid et al., 2018) and yellow to brown make up the second most commonly found colour range after white-transparent (Martí et al., 2020). These particles also were chosen because of their (a) fluorescence, facilitating quantification in the water and inside the exposed fish, (b) size range, resembling that of the fishes' natural zooplankton prey, and (c) near neutral buoyancy, facilitating random and long-lasting distribution of particles in the water column through turbulence created by an air stone. Particles were supplied to treatments from a stock solution of 6.15×10^6 particles l^{-1} (25 g l^{-1}), prepared in 0.1% Tween-20 surfactant (Cospheric) to avoid aggregation of particles. In the fast start experiment (point 2.4), tween was also added to the control to account for any potential effects it might have on fish behaviour; the final concentrations of Tween in the water were approximately 1.7×10^{-5} %.

2.3. Repeatability of microplastic ingestion variation

Based on existing reports of rapid gut clearance times of spherical MP particles in marine and freshwater fishes (Hoang and Felix-Kim, 2020; Huuskonen et al., 2020; Kim et al., 2019; Mazurais et al., 2015), we devised an approach to assess the repeatability of individual-level MP ingestion by quantifying particle egestion after exposure. Prior to the actual experiment, we tested the method during a pilot trial (see Supplemental Material (SM)).

First, 40 fish (size: 22 mm ± 1.53 SD) were isolated in individual 0.75-L glass holding jars filled with 0.5 L of filtered seawater at 49 dph and acclimated for 48 h prior to first exposure (temperature: 22 °C, salinity: 40‰). During this time, fish were fed once per day with 5 mg L^{-1} of commercial dry food (the same food as they had previously received at the hatchery). After the acclimation period, individual-level plastic ingestion was assessed repeatedly during five consecutive days.

Between 10 and 11 am each day, approximately 500 MP particles were added to each jar from the stock solution (equating to 10^3 particles L^{-1} or 4.1 mg L^{-1}). This particle concentration was chosen to avoid a limit to the possible number of particles each individual could consume while remaining ecologically relevant (see SM for further discussion). The fish were left with the particles for 2 h. Particles were slightly negatively buoyant and were kept in suspension by an air stone, set to equal bubbling levels in each jar.

After the exposure period, each fish was removed from its jar with a small hand net and placed in a 4 cm Petri dish filled with fresh, filtered seawater. Any MP particles inadvertently transferred with the fish were removed using a pipette. Identification of particles was facilitated by a UV-torch illuminating the fluorescent spheres. Fish were then placed into a new jar with fresh, filtered seawater with constant aeration, fed with 5 mg L^{-1} of dry food and left overnight (~ 21 h).

In the morning of the following day, the bottom of each jar was checked for excreted microspheres using a UV-torch. Recent research in fathead minnow larvae showed that excreted MPs are coated with intestinal fluid that is denser than water, resulting in aggregation and sinking of the particles (Hoang and Felix-Kim, 2020), and our pilot experiment showed that there is little re-ingestion of excreted particles (see SM). If particles were present at the bottom of the jar, they were removed with a pipette and counted under a dissection microscope (Fig. S1). Particles were then added again to each jar for 2 h. This procedure was repeated five times. After the fifth trial (i.e., in the morning of the sixth day), all fish were euthanized via cold shock, measured to the nearest millimetre and dissected to quantify any particles left in the digestive tract.

2.4. Startle response trials

From the same cohort of *S. aurata* larvae as used in the repeatability experiment, 180 and 42 randomly chosen individuals were placed into an 80-L and a 20-L aquarium, respectively, at 49 dph and left to acclimate for 48 h. We then added MP particles from stock solution at a concentration of approximately 10^3 particles L^{-1} (4.1 mg L^{-1}) to the larger aquarium (i.e., the plastic treatment), resembling the concentration of the repeatability experiment. Particles were held in suspension by two large air stones positioned at two opposing corners of the tank. The smaller aquarium acted as control (0 mg L^{-1}).

The differential sizes of the two treatment groups were based on the outcome of the repeatability pilot experiment, showing that only a small fraction of individuals could be expected to ingest relatively large amounts of particles (i.e., ~20%; see SI). The sample size of 180 individuals was thus employed with the aim to end up with a sub-group of individuals from the plastic treatment with relatively high MP ingestion that would be roughly comparable in size to the control group (i.e., 20% of 180 = 36).

Both treatment groups were fed once per day with commercial dry food at 5 mg L^{-1}. Each morning starting on the second day, 2/3 of the water in both tanks was replaced. Air stones were removed 1 h in advance to allow waste (and MP particles in the treatment tank) to settle. Wastes (and microspheres) were then siphoned out into a bucket and fresh, filtered seawater was added to refill the tanks. The concentration of remaining microspheres in the treatment tank was assessed by removing 3 × 10 ml of water from the centre of the tank with a pipette and counting particles under a dissection microscope. New microspheres were then added to achieve a concentration of 10^3 particles L^{-1} again.

After five days, all individuals were tested in a vibration-based startle response set-up to assess individual-level escape performance. Individual fish were removed from the treatment tanks with a small hand net and placed into a 9 cm Petri dish. Water depth was set to 2 cm to limit vertical movements. Six dishes holding one fish each were then placed onto the platform of the startle set-up. The set-up consisted of an illuminated bottom plate on which the dishes were positioned in a circular pattern around a tapping device driven by an electromagnet (Fig. S2). The tapping stimulus could be triggered remotely and was synchronized with an LED to signal the exact moment of the vibration stimulus. The entire set-up was covered on all sides with Styrofoam to avoid visual disturbance during the test. After an acclimation period of 2 min, a fast start was evoked by triggering the vibration stimulus. The reactions of the six fish were filmed from above at 350 frames per second using a high-speed video camera (1024 × 1024 pixels resolution; Photron SA3 120K, Photron, Japan). After the experiment, all individuals were euthanized via cold shock, measured to the nearest millimetre and dissected to quantify particle ingestion (Fig. S1).

2.5. *Video analysis*

Videos were analysed using a custom algorithm in MATLAB (see SM for a full explanation). The following two kinematic variables of the fast start were extracted:

Response latency: the time interval (in milliseconds) between the stimulus onset and the first movement leading to the escape. The temporal resolution of this variable was 2.86 ms, imposed by the employed frame rate.

Response strength: a standardised measure of the intensity of the reaction to the stimulus computed as the total number of pixels in the video frame that changed due to the fish's movement in a predefined period of time (37 ms), chosen to incorporate stages 1 and 2 of the fast start (average duration = 31.4 ms). Stages 1 & 2 represent the first two axial bends as defined by Domenici and Blake (1997), which is considered to be the key period for avoiding ambush predator attacks (Webb, 1976) (please refer to SM for a more thorough discussion of this variable).

Conceivably, larger individuals yielded a higher relative change in pixels when exhibiting similar levels of response strength than smaller conspecifics. Response strength scores were hence scaled according to fish length (in mm) by the inverse of the linear relationship between raw response strength and fish length ($y = 262.2x + 2820$; scaled response strength $= y + 2820 / 262.2x$). Both response latency and strength were only measured when fish performed a C-start (commencement of fast start that results in the individual forming a C-shape (Domenici and Blake, 1997)).

The distance of the fish from the wall of the Petri dish can affect the nature of the startle response (Domenici and Blake, 1997). To account for potential bias introduced by varying positions of individuals within the dish, the shortest distance (in mm) of the centre of mass of each fish to the dish wall was measured from a still image at the onset of the stimulus. This variable was later included as a covariate in the model selection process described below.

2.6. *Natural food ingestion*

We also explored existing data on individual-level ingestion rates of natural food items in *S. aurata* larvae. This data had been collected in 2012–2013 on fish sourced from the same hatchery as the ones used in the current experiments (China and Holzman, 2014). Larvae of ages 8, 13 and 23 dph ($n = 122$, 84 and 81, respectively) were offered rotifers at a concentration of approximately 100 individuals ml^{-1} for 30 min and were then euthanised and dissected to quantify ingestion rates. Ingestion of individual rotifers was assessed by counting indigestible mouthparts (i.e., mastax) under a dissection microscope. For simplicity, ingestion data of the three age classes were combined into one data set of rotifer ingestion.

This data was used to compare the shape of the density distribution of food ingestion rates across individuals with that obtained from the MP exposure experiment ($n = 180$). The differences in age and size distributions between the data sets precluded a precise comparison but should allow us to gauge general trends in food vs. MP ingestion; e.g. to show whether low (and zero) ingestion rates of MPs are due to an aversion of plastics or reflective of natural variation in ingestion rates (and vice versa).

2.7. *Statistical analysis*

2.7.1. *Repeatability*

All analyses were conducted in R (version 4.0.0). Visual inspection of the log-transformed frequency distribution of mean ingested particles per individual across the five trials indicated a strong dichotomy in the data (Fig. S3). We therefore employed univariate K-means clustering using the package Ckmeans.1d.dp (Wang and Song, 2011) to detect potential partitions in mean MP ingestion among individuals. The algorithm yielded two clusters, the first representing individuals with relatively low ($n = 32$) and the other with high mean ingestion ($n = 8$), from here on referred to as low- and high-MP-ingestion group, respectively.

Adjusted repeatability (R) of microplastic ingestion variation across the five trials was assessed using the package rptR (Stoffel et al., 2017) by fitting a Generalized Linear Mixed-effects Model (GLMM) with fish ID as random factor, a Poisson error distribution and sqrt link function (Nakagawa and Schielzeth, 2010). To control for the dichotomy of the low- vs. high-MP-ingestion group, we included a fixed factor *group* specifying the association of each individual to either cluster. Significance of parameter estimates was assessed by 1000 permutations and 95% confidence intervals (CIs) were generated using 1000 bootstraps.

We assessed the potential relationship of particle ingestion with fish size in the full data set as well as within the high-MP-ingestion group only using a Generalized Linear Model (GLM) with a quasipoisson error distribution to account for overdispersion of MP counts.

2.7.2. *Startle response*

Nine individuals (4.1% of 222) showed no reaction to the startle stimulus and were removed from further analyses (see Results), leading to a sample size for startle response parameters of 41 and 172 in the control and MP treatment, respectively. We analysed the effects of MP exposure on startle response parameters (latency and strength) at three different levels of resolution regarding information on MP ingestion (Table 2). First, we tested for treatment-level effects, representing the basic level of analysis that most MP exposure studies pursue. The fixed factor *treatment* had two levels, control ($n = 41$) and plastic exposure ($n = 172$), which contained no information on MP ingestion. Second, we incorporated information on MP ingestion by grouping individuals within the plastic treatment according to MP gut content into a low-MP-ingestion (individuals with < 30 particles in their gastrointestinal tract; $n = 144$) and a high-MP-ingestion group (> 30 particles; $n = 28$). The threshold of 30 particles was chosen according to the identified split in the repeatability data set; tests of other thresholds (i.e., 50, 75, 100 particles) yielded the same overall conclusions (data not shown). The fixed factor *group* thus had three levels (control, low- and high-MP-ingestion). Third, we assessed the linear relationship of the two response variables (latency and strength) with individual-level MP gut

Table 2
Levels of analysis to assess the effects of microplastic (MP) on startle response activity and latency in larval *Sparus aurata*. For each dependent variable, three models were run, testing the effect of exposure (Level 1: control vs. treatment), ingestion group (Level 2: control vs. low- vs. high-MP-ingestion) and individual-level ingestion (Level 3: MP gut content), respectively.

Level of analysis	Fixed effect	Type	Levels		
Treatment	*treatment*	categorical	1. control ($n = 41$)	2. plastic ($n = 172$)	
Sub-sample	*group*	categorical	1. control ($n = 41$)	2. low-MP-ingestion ($n = 144$)	3. high-MP-ingestion ($n = 28$)
Individual	*mp_count*	continuous		1. plastic ($n = 172$)	

content within the total plastic treatment ($n = 172$; continuous fixed factor *mp_count*).

We used information-theoretic model selection in the package MuMIn package (Barton and Barton, 2019) to identify the most parsimonious model out of six full models (two dependent variables × three levels of analysis) containing the following fixed effects: the above-described levels of analysis (*treatment*, *group* or *mp_count*), *fish_size* (continuous, in mm) and *wall-distance* (continuous, in mm), as well as their interaction terms. Given that during each trial, one testing plate containing six fish in individual Petri dishes was assessed, all models contained a random effect for 'well location' (position on the testing plate, 1–6) nested in 'testing plate ID'. Models with ΔAICc > 2 from the best model were considered as not being supported by the data (Burnham and Anderson, 2003).

3. Results

3.1. Repeatability

Overall, there was high inter-individual variation in MP ingestion across the 40 fish (mean: 21.7 ± 54.5 SD; range: 0–290; CV = 2.51; Fig. 1A). Individuals either never ingested more than a few particles or they consistently ingested relatively large quantities (Fig. S3 and S4). Accordingly, cluster analysis yielded two clusters: a low-MP-ingestion ($n = 32$; overall mean across individual across five trials: 0.98 ± 0.99 SD; mean range per individual: 0–4.4;) and a high-MP-ingestion group ($n = 8$; overall mean: 104.8 ± 57; mean range: 30.6–198.2; Table S1). In only four instances did individuals from the high-MP-ingestion group consume less than 30 particles and no individuals from the low-MP-ingestion group ever consumed more than a maximum of 13 particles.

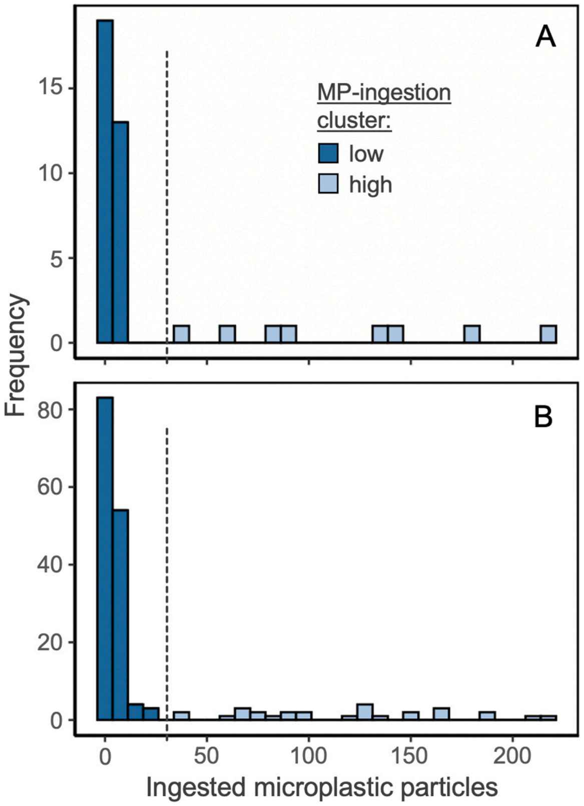

Fig. 1. Frequency distributions of inter-individual microplastic ingestion variation (A) during the repeatability experiment (mean ingestion across five repeated trials, $n = 40$), and (B) after the startle response experiment within the plastic exposure treatment ($n = 180$). K-means analysis split individuals in the repeatability experiment (A) in two clusters (i.e., low- [$n = 32$] and high-MP-ingestion individuals [$n = 8$]); for parts of the analysis, we split the plastic exposure treatment (B) accordingly (low- [$n = 150$] and high-MP-ingestion [$n = 30$]; see Fig. 2); dashed lines indicate the cluster separation threshold.

Inter-individual variation in MP ingestion was significantly repeatable across the five trials when accounting for the dichotomy of low- and high-MP-ingestion individuals ($R = 0.324 \pm 0.086$ SE; CI = 0.006; 0.364; $p < 0.001$; Fig. S4). There was no difference in fish size between the low- and high-MP-ingestion groups (Welch Two Sample *t*-test: $t_{10} = -0.737$, $p = 0.48$). Mean particle ingestion across the five trials was not related to fish size in either the full data set ($F_{1,38} = 1.868$, $p = 0.18$) or within the high-MP-ingestion group ($F_{1,6} = 1.024$, $p = 0.351$). Ten individuals had MP particles remaining in their gut when they were dissected (mean: 4.8; range: 1–12).

3.2. Startle response

Particle ingestion in the MP treatment group was highly variable between individuals (mean = 20.1 ± 46.3; range: 0–218; CV = 2.4). The frequency distribution of MP ingestion was markedly right skewed (Fig. 1B); out of 180 individuals, 87 (48.3%) had ingested zero particles at the time of euthanasia, 63 (35%) had ingested 1–22 particles and 30 (16.7%) had ingested more than 30 particles (16 out of which with over 100 particles). Fish size ranged from 16 to 29 mm (mean = 21.4 ± 1.85).

All but nine individuals showed a startle response reaction to the vibration stimulus (96% of 222). Of the nine that did not, one was from the control (2.4% of 42) and eight were from the plastic treatment (4.4% of 180). Of those eight individuals, four had ingested MP particles (mean: 41.5 ± 58.3, range: 1–125).

At each level of analysis (Table 2), the best-fit models identified by AIC model selection for both response strength and latency contained either the MP-related factor only (i.e., *treatment*, *group* or *mp_count*), or were the null model (Table 3). At the treatment level (control vs. exposure), the best-fit model for response strength was the null model, carrying 87% of the cumulative model weight (CMW). At the MP-ingestion group level, the best-fit model contained *group* as the sole fixed factor (83% CMW). The inclusion of information about MP-ingestion thus revealed patterns not discerned by mere treatment-level analysis; within the MP treatment, high-MP-ingestion individuals showed lower response strengths than individuals with low-MP-ingestion (Fig. 2A+B). At the individual-level, the best-fit model contained *mp_count* as the sole predictor (66% CMW), with the null model as a close second (ΔAICc < 2) (Fig. 2C).

At the treatment- and cluster-level, the best-fit models for response latency contained *treatment* and *group* as the sole predictors, respectively (69% and 72% CMW). The effect of *treatment* was clearly driven by longer latencies in the high-MP-ingestion group (Fig. 2D+E).

At the individual level, the best fit model for response latency was

Table 3

Model selection summary for response activity and latency, based on the three levels of analysis for microplastic (MP) ingestion (see Table 2). Each full model contained fixed effects for MP exposure/ingestion, wall distance, fish size and their interaction terms, as well as a random effect for testing-well nested in test-plate-ID (see main text for further explanation). Only the best models and models with ΔAICc > 2 are shown.

Dependent variable	Level of analysis	Model	LogLik	DF	AICc	Weight
Response activity	Treatment	*NULL*	96.957	4	-185.7	0.868
	Sub-sample	*group*	101.11	6	-189.8	0.829
	Individual	*mp_count*	98.832	5	-187.4	0.657
		NULL	96.957	4	-185.7	0.288
Response latency	Treatment	*treatment*	-588.091	5	1186	0.688
	Sub-sample	*group*	-586.451	6	1185.3	0.715
	Individual	*NULL*	-590.811	4	1189.8	0.846

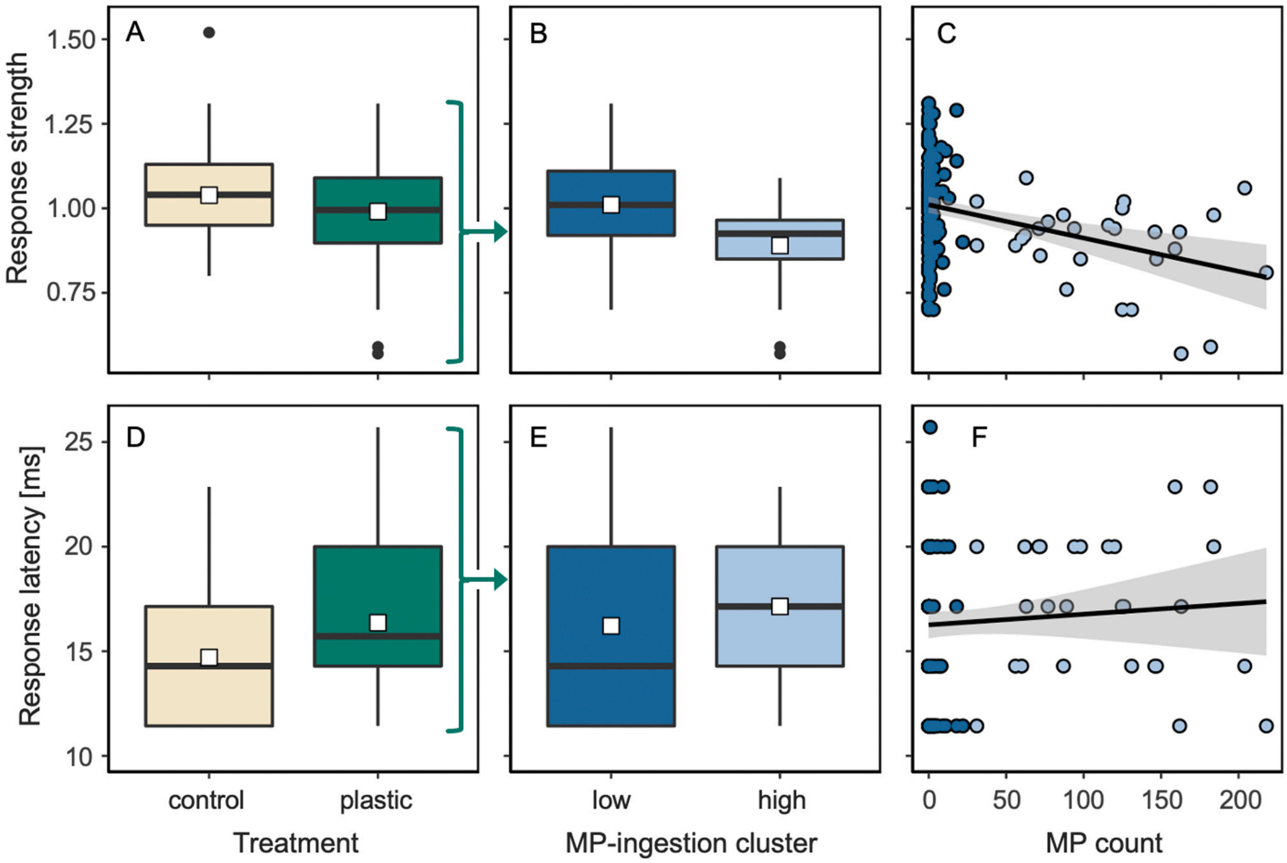

Fig. 2. Effects of MP exposure on scaled startle response strength scores (A, B, C) and response latency (D, E, F) at different levels of analysis (Table 2). A + D treatment-level comparisons (control, $n = 41$ vs. plastic, $n = 172$); B + E cluster-level comparisons of MP-ingestion clusters within the treatment: (1) low- (< 30 ingested particles; $n = 144$) and (2) high-MP-ingestion individuals (> 30 particles; $n = 28$); E + F individual-level analysis of MP-counts in fish gut contents. Cluster- (B) and individual-level (C) analyses revealed marked impacts on response strength in high-MP-ingestion individuals that was not discernible at the treatment level (A). Treatment-level impacts on response latency (D) were driven by markedly slower reactions in the high-MP-ingestion cluster (E).

the null model (85% CMW).

3.3. Food vs. MP ingestion

To compare the overall shape of inter-individual variation in ingestion rates of MPs with that of live food, we included an existing data set of rotifer ingestion in larval *S. aurata* across three age classes (8, 13, 23 dph; $n = 287$). Rotifer ingestion was highly variable between individuals (mean = 7.2 ± 13.5; range: 0–123; CV = 1.9). Density distributions of rotifer and MP ingestion were remarkably similar in that they both formed a strongly leptokurtic, right-skewed distribution with a long, flat tail (Fig. 3). In other words, the majority of individuals ingested little to no food and/or MPs, while a few individuals ingested variable but large quantities. This overlap in density distributions of ingestion rates between rotifers and MPs was observed despite a 100-fold difference in exposure concentrations and a 4-fold difference in exposure time (MPs: 1 ml^{-1} for 2 h; rotifers: 100 ml^{-1} for 0.5 h).

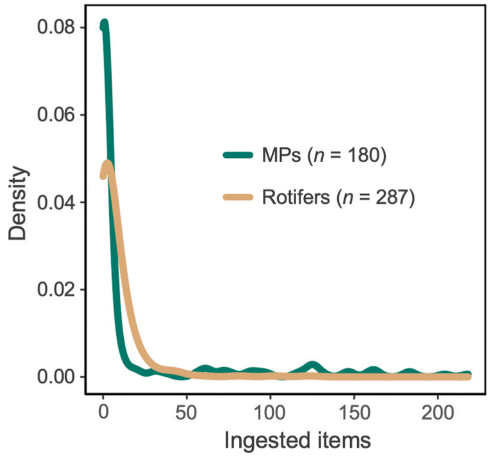

Fig. 3. Congruence of density distributions of individual food and microplastic (MP) ingestion rates. Lines represent the 180 *Sparus aurata* individuals exposed to 1000 MPs l^{-1} prior to the startle response experiment and a combined group of larvae of different age classes (8, 13, 23 days post hatching) fed with rotifers in a previous study (China and Holzman, 2014).

4. Discussion

Our findings demonstrate that inter-individual MP ingestion variation is high and repeatable in settlement-stage *Sparus aurata*, and that its incorporation into exposure experiments can reveal hidden impacts within a given exposure level. While mere comparisons of control vs. exposure treatment did not detect any impact on startle response strength, the integration of individual-level gut content revealed a marked decrease in individuals with high MP ingestion rates (Fig. 2), presumably because MP exposure did not affect all individuals equally. MP exposure had a detectable effect on startle response latency at the treatment-level. Yet importantly, the inclusion of information on MP-ingestion revealed that this pattern was driven by markedly longer latencies in the high-MP-ingestion group. Collectively, our findings indicate that consistent inter-individual variation in MP ingestion may have important consequences for juvenile fish.

4.1. *Potential mechanisms of MP impacts*

While the underlying mechanisms of the observed MP impacts have not explicitly been investigated here, we can infer potential pathways from previous research. The observed behavioural impacts in high-MP-ingestion individuals may in part be driven by decreased energy efficiency imposed by the ingestion indigestible particles (Watts et al., 2016; Wen et al., 2018; Yin et al., 2018). Initial MP exposure could thus lead to positive feedback loops, where a higher demand for nutritional resources leads to a further increase in MP uptake (Montiglio and Royauté, 2014). The sheer amount particles ingested by some individuals (Fig. S1) may also have affected mobility by simply overburdening the fish.

Alternatively, or additionally, behavioural impacts may stem from leachates of toxic compounds from the MP particles. Similar Polyethylene particles to the ones used in this study were shown to leach trace amounts of bisphenol and phthalates (Chae et al., 2019). Phthalates were shown to decrease the function of acetylcholine esterase (AChE) activity in muscle tissue in freshwater fish (Ghorpade et al., 2002), which could explain the poorer escape responses observed in high-MP-ingestion individuals. Regardless of the specific mechanisms behind the observed effects, inter-individual variation in MP ingestion may have far reaching ecological consequences.

4.2. *Effects of inter-individual MP ingestion variation on natural systems*

Food limitation and predation are considered to be key factors affecting survival in the early life stages of marine fishes (Almany and Webster, 2006; Peck and Hufnagl, 2012). The ‘bigger-is-better’ hypothesis stipulates links between high ingestion rates, increased larval growth and enhanced survival through reduced vulnerability to predation (Meekan et al., 2006; Miller et al., 1988). Our findings indicate that this fundamental framework may be disrupted by MP contamination.

In line with other recent MP exposure studies (Table 1), we found that MP ingestion is highly variable among individuals of the same cohort. Importantly, this variation was significantly repeatable, indicating that a certain proportion of the population (here approximately 20–25%) will consistently be more affected by plastic pollution than others because of inherent differences in MP ingestion rates.

Intake rates of natural food items have long been known to vary among individuals (MacKenzie et al., 1990) and high ingestion rates would typically denote a trait of selective advantage (Houde and Schekter, 1980). Our data indicates that inter-individual variability in ingestion rates of natural food items and MP particles follow similar trends (Fig. 3), with the majority of individuals exhibiting relatively low uptake. In systems with high MP pollution, high ingestion rates may thus entail a fitness trade-off, and individuals that would normally be most likely to constitute successful recruits (bigger-is-better) may be the ones most affected by adverse effects of MP ingestion.

Because of their small size and limited ability to regulate their internal environment, early life stages of marine organisms are particularly vulnerable to pollution (Sussarellu et al., 2016; Weis and Weis, 1989). At the same time, pelagic larvae may be more exposed to MPs than adult stages because they tend to aggregate in oceanographic features, such as surface slicks and eddies, which also accumulate high concentrations of plastic particles (Gove et al., 2019; Markic et al., 2018). Inherent and consistent inter-individual variation in the proneness to ingest MP particles during the larval stage may thus affect larval condition directly, but also entail carry-over effects for subsequent life stages.

During the settlement process, predation constitutes a major selective pressure (Almany and Webster, 2006) and reactivity to a predator strike is a key factor affecting an individual’s likelihood of survival (McCormick et al., 2018). Here, we show that both the overall strength of the escape response and response latency are markedly reduced in individuals with high MP intake rates (Fig. 2). Collectively, these impacts may directly translate to increased vulnerability to predation (Domenici, 2010; McCormick et al., 2018; Scharf et al., 2003). Our results corroborate recent findings of increased predation-related mortality in MP exposed individuals of juvenile damselfish, *Pomacentrus amboinensis*, on artificial patch reefs (McCormick et al., 2020). We should note that *S. aurata* is a social species that typically lives in shoals after settlement. The group dynamics that drive escape performance in shoals varies from that of more solitary species, such as *P. amboinensis*.

Here, we may actually have underestimated the effects of MP ingestion on both response variables because some high-MP-ingestion individuals may have cleared their gut contents right before the experiment, leading to their erroneous classification as low-MP-ingestion individuals upon dissection. This could also explain the overlap of startle response values between the two groups; i.e., why we may have observed very low response strengths and high latencies in the low-MP-ingestion group (Fig. 2B+E). However, this is hypothesis is speculative.

Overall, our findings indicate that consistent inter-individual variation in MP ingestion may affect key behavioural capacities during a critical life history transition. Microplastic pollution may thus alter patterns of replenishment in marine fish populations by disproportionately affecting high-(MP)-ingestion phenotypes.

4.3. *Impact of inter-individual MP ingestion variation on exposure studies*

Microplastic exposure studies have been proliferating at an exponential rate over the past decade. Despite frequently using exceedingly high exposure concentrations, these studies often yield inconsistent results regarding potential adverse effects. Some studies report significant reductions in feeding rates, growth, body condition, swimming speeds, as well as altered behaviours and differential gene expression in exposed fishes (Barboza et al., 2018a, 2018b; Choi et al., 2018; de Sá et al., 2015; Mazurais et al., 2015; Naidoo and Glassom, 2019; Yin et al., 2018). In contrast, other research found no or limited evidence for adverse effects on comparable endpoints (Critchell and Hoogenboom, 2018; Jacob et al., 2019; Mazurais et al., 2015; Tosetto et al., 2017). Similar inconsistencies prevail across taxa and may stem from differences in experimental design (e.g. exposure concentration or duration, particle type or polymer) or possible inter-species variation in MP tolerances (Foley et al., 2018; Phuong et al., 2016).

Our findings indicate that inconsistencies may also arise from a lack of consideration for individual-level effects in the majority of past exposure studies. In our analysis, effects of MP exposure on escape strength were masked by the large proportion of individuals in the sample (75–80%) that ingested limited numbers of particles. Only the incorporation of individual-level MP ingestion data revealed the existence of significant behavioural effects.

4.4. *Mechanisms of inter-individual MP ingestion variation*

Field surveys of microplastic ingestion patterns in marine organisms have been a major research focus over recent years. These studies typically yield high within-species variation in the number of ingested particles (Egbeocha et al., 2018; Markic et al., 2019), yet the underlying mechanisms of MP ingestion variation have only recently become the focus of increased research interest.

Several past studies have focused on possible external drivers of MP ingestion and found that particle characteristics may affect the likelihood of ingestion, including size (Hoang and Felix-Kim, 2020), colour (Mizraji et al., 2017; Ory et al., 2018), presence of biofilms (Müller et al., 2020; Peterson et al., 2017; Procter et al., 2019; Vroom et al., 2017), appearance (food-like vs. non-food-like) and density (Roch et al., 2020). Ingestion of MPs was also shown to be affected by temperature (Wen et al., 2018) and the presence/absence of genuine food (Kim et al., 2019; Roch et al., 2020).

Few studies to date have looked into intrinsic factors driving inter-individual MP ingestion variation. Across species, feeding mode was shown to be an important factor, involving higher MP ingestion in species with visual compared to chemosensory foraging modes (Roch et al., 2020) and in generalist compared to specialist feeders (Markic et al., 2019; Vroom et al., 2017; but see Bråte et al., 2016). Within species, gut-fullness and fish size have been implied as potential correlates of MP ingestion, but results are inconsistent across studies. Field surveys have reported both existing (Bråte et al., 2016) and non-existing (de Vries et al., 2020) relationships of organic stomach content with MP particle ingestion. In our repeatability experiment, we have attempted to standardise gut-contents by isolating individuals and feeding them exactly the same amount of food before and during the experiment. While it is difficult to eliminate this effect entirely, any influence of hunger level on MP ingestion variation should thus have been minimal.

Inconsistent results have also been reported for fish size. While Roch et al. (2020), for instance, report a significant positive relationship of fish length with MP ingestion, other studies found no or little correlation (de Vries et al., 2020; Nanninga et al., 2020). Here we found no relationship of ingestion rates with fish size in either experiment (i.e., repeatability and escape response), further pointing towards a limited role of body size as a predictor for MP ingestion.

Recent evidence suggests that MP ingestion may instead be phenotype-dependent in marine fishes. Nanninga et al. (2020) examined individual-level MP ingestion rates in conjunction with behavioural profiles in juvenile anemonefish, *Amphiprion ocellaris*, and found that ingestion variation had a significant relationship with repeatable activity levels. Based on these findings and the results of this study, we may hence expect differential MP exposure along the pace-of-life continuum (Réale et al., 2010), where individuals at the higher end of the spectrum (i.e., characterised by high metabolism, activity and ingestion rates) consistently consume higher quantities of MPs than individuals at the lower end of the spectrum.

4.5. Conclusions

Our findings corroborate recent calls for a more frequent integration of individual-level analyses into impact studies of anthropogenic contaminants. Exploring and accounting for inter-individual variation might be especially relevant for the emergent field of MP ecotoxicology. Most anthropogenic stressors (e.g., temperature rise, ocean acidification, pesticide or heavy metal pollution) typically affect individuals passively and more or less uniformly upon exposure. In contrast, plastic particles are likely to induce adverse effects only upon ingestion (depending on 'particle size to fish size' ratios). Consistent differences in the propensity to ingest MP particles may thus lead to uneven exposure within treatments and/or populations. Individuals that would naturally gain selective benefits via high food ingestion rates may be the ones most affected by MP pollution. Overall, we advocate the incorporation of individual-level MP ingestion data into future exposure studies of microplastics.

Ethics statement

All research was conducted in accordance with the Hebrew University's Animal Ethics guidelines with approval from their Animal Ethics Committee (NIH approval OPRR-A01-5011; HUJI approval NS-20-16302).

Data accessibility

All raw data and the video analysis Matlab code used during this study have been included as supplementary files (Appendices A and B, respectively). Startle response videos are available from the corresponding author.

CRediT authorship contribution statement

Conceptualization and methodology: GBN, MK, RH and AM; Investigation: GBN and IP; Software: AP; Formal analysis: GBN and MK; Resources: RH; Writing - original draft: GBN; Writing - review & editing: MK, RH and AM; Visualization: GBN; Supervision: AM, MK and RH; Project administration: GBN; Funding acquisition: GBN.

Declaration of Competing Interest

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

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2021.126059.

References

Almany, G.R., Webster, M.S., 2006. The predation gauntlet: early post-settlement mortality in reef fishes. Coral Reefs 25, 19–22. https://doi.org/10.1007/s00338-005-0044-y.

Auta, H.S., Emenike, C.U., Fauziah, S.H., 2017. Distribution and importance of microplastics in the marine environment: a review of the sources, fate, effects, and potential solutions. Environ. Int. 102, 165–176.

Avio, C.G., Gorbi, S., Regoli, F., 2015. Experimental development of a new protocol for extraction and characterization of microplastics in fish tissues: first observations in commercial species from Adriatic Sea. Mar. Environ. Res. 111, 18–26.

Barboza, L.G.A., Vieira, L.R., Branco, V., Figueiredo, N., Carvalho, F., Carvalho, C., Guilhermino, L., 2018a. Microplastics cause neurotoxicity, oxidative damage and energy-related changes and interact with the bioaccumulation of mercury in the European seabass, Dicentrarchus labrax (Linnaeus, 1758). Aquat. Toxicol. 195, 49–57.

Barboza, L.G.A., Vieira, L.R., Guilhermino, L., 2018b. Single and combined effects of microplastics and mercury on juveniles of the European seabass (Dicentrarchus labrax): changes in behavioural responses and reduction of swimming velocity and resistance time. Environ. Pollut. 236, 1014–1019.

Barton, K., Barton, M.K., 2019. Package 'MuMIn.' Multi-model inference. version 1.

Bolnick, D.I., Amarasekare, P., Araújo, M.S., Bürger, R., Levine, J.M., Novak, M., Rudolf, V.H.W., Schreiber, S.J., Urban, M.C., Vasseur, D.A., 2011. Why intraspecific trait variation matters in community ecology. Trends Ecol. Evol. 26, 183–192. https://doi.org/10.1016/j.tree.2011.01.009.

Brandon, J.A., Freibott, A., Sala, L.M., 2020. Patterns of suspended and salp-ingested microplastic debris in the North Pacific investigated with epifluorescence microscopy. Limnol. Oceanogr. Lett. 5, 46–53.

Bråte, I.L.N., Eidsvoll, D.P., Steindal, C.C., Thomas, K.V., 2016. Plastic ingestion by Atlantic cod (Gadus morhua) from the Norwegian coast. Mar. Pollut. Bull. 112, 105–110.

Burnham, K.P., Anderson, D.R., 2003. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. Springer Science & Business Media.

Chae, Y., Kim, D., An, Y.-J., 2019. Effects of micro-sized polyethylene spheres on the marine microalga Dunaliella salina: focusing on the algal cell to plastic particle size ratio. Aquat. Toxicol. 216, 105296.

China, V., Holzman, R., 2014. Hydrodynamic starvation in first-feeding larval fishes. Proc. Natl. Acad. Sci. 111, 8083–8088.

Choi, J.S., Jung, Y.-J., Hong, N.-H., Hong, S.H., Park, J.-W., 2018. Toxicological effects of irregularly shaped and spherical microplastics in a marine teleost, the sheepshead minnow (Cyprinodon variegatus). Mar. Pollut. Bull. 129, 231–240.

Cózar, A., Echevarría, F., González-Gordillo, J.I., Irigoien, X., Úbeda, B., Hernández-León, S., Palma, Á.T., Navarro, S., García-de-Lomas, J., Ruiz, A., 2014. Plastic debris in the open ocean. Proc. Natl. Acad. Sci. 111, 10239–10244.

Critchell, K., Hoogenboom, M.O., 2018. Effects of microplastic exposure on the body condition and behaviour of planktivorous reef fish (Acanthochromis polyacanthus). PLoS One 13, 0193308.

Domenici, P., Blake, R., 1997. The kinematics and performance of fish fast-start swimming. J. Exp. Biol. 200, 1165–1178.

Domenici, P. Escape responses in fish: kinematics, performance and behavior Fish. Locomot. eco-ethological Perspect, 2010, 123 170.

Egbeocha, C.O., Malek, S., Emenike, C.U., Milow, P., 2018. Feasting on microplastics: ingestion by and effects on marine organisms. Aquat. Biol. 27, 93–106.

Erni-Cassola, G., Gibson, M.I., Thompson, R.C., Christie-Oleza, J.A., 2017. Lost, but found with Nile Red: a novel method for detecting and quantifying small microplastics (1 mm to 20 µm) in environmental samples. Environ. Sci. Technol. 51, 13641–13648.

Foley, C.J., Feiner, Z.S., Malinich, T.D., Höök, T.O., 2018. A meta-analysis of the effects of exposure to microplastics on fish and aquatic invertebrates. Sci. Total Environ. 631, 550–559.

Ghorpade, N., Mehta, V., Khare, M., Sinkar, P., Krishnan, S., Rao, C.V., 2002. Toxicity study of diethyl phthalate on freshwater fish Cirrhina mrigala. Ecotoxicol. Environ. Saf. 53, 255–258.

Gove, J.M., Whitney, J.L., McManus, M.A., Lecky, J., Carvalho, F.C., Lynch, J.M., Li, J., Neubauer, P., Smith, K.A., Phipps, J.E., 2019. Prey-size plastics are invading larval fish nurseries. Proc. Natl. Acad. Sci. 116, 24143–24149.

Harding, H.R., Gordon, T.A.C., Eastcott, E., Simpson, S.D., Radford, A.N., 2019. Causes and consequences of intraspecific variation in animal responses to anthropogenic noise. Behav. Ecol. 30, 1501–1511.

Hartmann, N.B., Hüffer, T., Thompson, R.C., Hassellöv, M., Verschoor, A., Daugaard, A. E., Rist, S., Karlsson, T., Brennholt, N., Cole, M., 2019. Are we speaking the same language? Recommendations for a definition and categorization framework for plastic debris.

Hasegawa, T., Nakaoka, M., n.d. Trophic transfer of microplastics from mysids to fish greatly exceeds direct ingestion from the water column. Environ. Pollut. 116468.

Hoang, T.C., Felix-Kim, M., 2020. Microplastic consumption and excretion by fathead minnows (Pimephales promelas): Influence of particles size and body shape of fish. Sci. Total Environ. 704, 135433.

Houde, E.D., Schekter, R.C., 1980. Feeding by marine fish larvae: developmental and functional responses. Environ. Biol. Fishes 5, 315–334.

Huuskonen, H., i Folguera, J.S., Kortet, R., Akkanen, J., Vainikka, A., Janhunen, M., Kekäläinen, J., 2020. Do whitefish (Coregonus lavaretus) larvae show adaptive variation in the avoidance of microplastic ingestion? Environ. Pollut. 262, 114353.

Isobe, A., Iwasaki, S., Uchida, K., Tokai, T., 2019. Abundance of non-conservative microplastics in the upper ocean from 1957 to 2066. Nat. Commun. 10, 417.

Jacob, H., Gilson, A., Lanctôt, C., Besson, M., Metian, M., Lecchini, D., 2019. No effect of polystyrene microplastics on foraging activity and survival in a post-larvae coral-reef fish, acanthurus triostegus. Bull. Environ. Contam. Toxicol. 102, 457–461.

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Sciences 347, 768–771, 80-.

Jovanović, B., 2017. Ingestion of microplastics by fish and its potential consequences from a physical perspective. Integr. Environ. Assess. Manag. 13, 510–515.

Kim, S.W., Chae, Y., Kim, D., An, Y.-J., 2019. Zebrafish can recognize microplastics as inedible materials: quantitative evidence of ingestion behavior. Sci. Total Environ. 649, 156–162.

Lusher, A., Hollman, P., Mendoza-Hill, J., 2017. Microplastics in fisheries and aquaculture: status of knowledge on their occurrence and implications for aquatic organisms and food safety. FAO Fish. Aquac. Tech. Pap.

MacKenzie, B.R., Leggett, W.C., Peters, R.H., 1990. Estimating larval fish ingestion rates: can laboratory derived values be reliably extrapolated to the wild? Mar. Ecol. Prog. Ser. 67, 209–225.

Markic, A., Niemand, C., Bridson, J.H., Mazouni-Gaertner, N., Gaertner, J.-C., Eriksen, M., Bowen, M., 2018. Double trouble in the South Pacific subtropical gyre: increased plastic ingestion by fish in the oceanic accumulation zone. Mar. Pollut. Bull. 136, 547–564.

Markic, A., Gaertner, J.-C., Gaertner-Mazouni, N., Koelmans, A.A., 2019. Plastic ingestion by marine fish in the wild. Crit. Rev. Environ. Sci. Technol. 1–41.

Martí, E., Martin, C., Galli, M., Echevarría, F., Duarte, C.M., Cózar, A., 2020. The colors of the ocean plastics. Environ. Sci. Technol. 54, 6594–6601.

Mazurais, D., Ernande, B., Quazuguel, P., Severe, A., Huelvan, C., Madec, L., Mouchel, O., Soudant, P., Robbens, J., Huvet, A., 2015. Evaluation of the impact of polyethylene microbeads ingestion in European sea bass (Dicentrarchus labrax) larvae. Mar. Environ. Res. 112, 78–85.

Mbedzi, R., Dalu, T., Wasserman, R.J., Murungweni, F., Cuthbert, R.N., 2020. Functional response quantifies microplastic uptake by a widespread African fish species. Sci. Total Environ. 700, 134522.

McCormick, M.I., Fakan, E., Allan, B.J.M., 2018. Behavioural measures determine survivorship within the hierarchy of whole-organism phenotypic traits. Funct. Ecol. 32, 958–969.

McCormick, M.I., Chivers, D.P., Ferrari, M.C.O., Blandford, M.I., Nanninga, G.B., Richardson, C., Fakan, E.P., Vamvounis, G., Gulizia, A.M., Allan, B.J.M., 2020. Microplastic exposure interacts with habitat degradation to affect behaviour and survival of juvenile fish in the field. Proc. Biol. Sci. 287, 20201947.

Meekan, M.G., Vigliola, L., Hansen, A., Doherty, P.J., Halford, A., Carleton, J.H., 2006. Bigger is better: size-selective mortality throughout the life history of a fast-growing clupeid, Spratelloides gracilis. Mar. Ecol. Prog. Ser. 317, 237–244.

Miller, T.J., Crowder, L.B., Rice, J.A., Marschall, E.A., 1988. Larval size and recruitment mechanisms in fishes: toward a conceptual framework. Can. J. Fish. Aquat. Sci. 45, 1657–1670. https://doi.org/10.1139/f88-197.

Mizraji, R., Ahrendt, C., Perez-Venegas, D., Vargas, J., Pulgar, J., Aldana, M., Ojeda, F.P., Duarte, C., Galbán-Malagón, C., 2017. Is the feeding type related with the content of microplastics in intertidal fish gut? Mar. Pollut. Bull. 116, 498–500.

Montiglio, P.O., Royauté, R., 2014. Contaminants as a neglected source of behavioural variation. Anim. Behav. 88, 29–35.

Müller, C., Erzini, K., Teodósio, M.A., Pousão-Ferreira, P., Baptista, V., Ekau, W., 2020. Assessing microplastic uptake and impact on omnivorous juvenile white seabream Diplodus sargus (Linnaeus, 1758) under laboratory conditions. Mar. Pollut. Bull. 157, 111162.

Naidoo, T., Glassom, D., 2019. Decreased growth and survival in small juvenile fish, after chronic exposure to environmentally relevant concentrations of microplastic. Mar. Pollut. Bull. 145, 254–259.

Nakagawa, S., Schielzeth, H., 2010. Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. Biol. Rev. 85, 935–956.

Nanninga, G.B., Scott, A., Manica, A., 2020. Microplastic ingestion rates are phenotype-dependent in juvenile anemonefish. Environ. Pollut. 259, 113855.

Ory, N.C., Sobral, P., Ferreira, J.L., Thiel, M., 2017. Amberstripe scad Decapterus muroadsi (Carangidae) fish ingest blue microplastics resembling their copepod prey along the coast of Rapa Nui (Easter Island) in the South Pacific subtropical gyre. Sci. Total Environ. 586, 430–437.

Ory, N.C., Gallardo, C., Lenz, M., Thiel, M., 2018. Capture, swallowing, and egestion of microplastics by a planktivorous juvenile fish. Environ. Pollut. 240, 566–573.

Ostle, C., Thompson, R.C., Broughton, D., Gregory, L., Wootton, M., Johns, D.G., 2019. The rise in ocean plastics evidenced from a 60-year time series. Nat. Commun. 10, 1–6.

Peck, M.A., Hufnagl, M., 2012. Can IBMs tell us why most larvae die in the sea? Model sensitivities and scenarios reveal research needs. J. Mar. Syst. 93, 77–93.

Peterson, E.K., Buchwalter, D.B., Kerby, J.L., LeFauve, M.K., Varian-Ramos, C.W., Swaddle, J.P., 2017. Integrative behavioral ecotoxicology: bringing together fields to establish new insight to behavioral ecology, toxicology, and conservation. Curr. Zool. 63, 185–194.

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

Procter, J., Hopkins, F.E., Fileman, E.S., Lindeque, P.K., 2019. Smells good enough to eat: dimethyl sulfide (DMS) enhances copepod ingestion of microplastics. Mar. Pollut. Bull. 138, 1–6.

Réale, D., Garant, D., Humphries, M.M., Bergeron, P., Careau, V., Montiglio, P.-O., 2010. Personality and the emergence of the pace-of-life syndrome concept at the population level. Philos. Trans. R. Soc. B Biol. Sci. 365, 4051–4063.

Rezania, S., Park, J., Din, M.F.M., Taib, S.M., Talaiekhozani, A., Yadav, K.K., Kamyab, H., 2018. Microplastics pollution in different aquatic environments and biota: a review of recent studies. Mar. Pollut. Bull. 133, 191–208.

Roch, S., Friedrich, C., Brinker, A., 2020. Uptake routes of microplastics in fishes: practical and theoretical approaches to test existing theories. Sci. Rep. 10, 1–12.

de Sá, L.C., Luís, L.G., Guilhermino, L., 2015. Effects of microplastics on juveniles of the common goby (Pomatoschistus microps): confusion with prey, reduction of the predatory performance and efficiency, and possible influence of developmental conditions. Environ. Pollut. 196, 359–362.

Scharf, F.S., Buckel, J.A., McGinn, P.A., Juanes, F., 2003. Vulnerability of marine forage fishes to piscivory: effects of prey behavior on susceptibility to attack and capture. J. Exp. Mar. Biol. Ecol. 294, 41–59.

Shahul Hamid, F., Bhatti, M.S., Anuar, Norkhairiyah, Anuar, Norkhairah, Mohan, P., Periathamby, A., 2018. Worldwide distribution and abundance of microplastic: how dire is the situation? Waste Manag. Res. 36, 873–897.

Sih, A., 2013. Understanding variation in behavioural responses to human-induced rapid environmental change: a conceptual overview. Anim. Behav. 85, 1077–1088.

Sommerfeld, N., Holzman, R., 2019. The interaction between suction feeding performance and prey escape response determines feeding success in larval fish. J. Exp. Biol. 222, 222.

Stoffel, M.A., Nakagawa, S., Schielzeth, H., 2017. rptR: Repeatability estimation and variance decomposition by generalized linear mixed-effects models. Methods Ecol. Evol. 8, 1639–1644.

Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M.E.J., Le Goïc, N., Quillien, V., Mingant, C., Epelboin, Y., 2016. Oyster reproduction is affected by exposure to polystyrene microplastics. Proc. Natl. Acad. Sci. 113, 2430–2435.

Tandler, A., Anav, F.A., Choshniak, I., 1995. The effect of salinity on growth rate, survival and swimbladder inflation in gilthead seabream, Sparus aurata, larvae. Aquaculture 135, 343–353.

Thompson, R.C., Napper, I.E., 2018. Microplastics in the environment. Plast. Environ. 47, 60–81.

Tosetto, L., Williamson, J.E., Brown, C., 2017. Trophic transfer of microplastics does not affect fish personality. Anim. Behav. 123, 159–167.

Violle, C., Enquist, B.J., McGill, B.J., Jiang, L.I.N., Albert, C.H., Hulshof, C., Jung, V., Messier, J., 2012. The return of the variance: intraspecific variability in community ecology. Trends Ecol. Evol. 27, 244–252.

de Vries, A.N., Govoni, D., Árnason, S.H., Carlsson, P., 2020. Microplastic ingestion by fish: body size, condition factor and gut fullness are not related to the amount of plastics consumed. Mar. Pollut. Bull. 151, 110827.

Vroom, R.J.E., Koelmans, A.A., Besseling, E., Halsband, C., 2017. Aging of microplastics promotes their ingestion by marine zooplankton. Environ. Pollut. 231, 987–996.

Wang, H., Song, M., 2011. Ckmeans. 1d. dp: optimal k-means clustering in one dimension by dynamic programming. R. J. 3, 29–33.

Watts, A.J.R., Urbina, M.A., Goodhead, R., Moger, J., Lewis, C., Galloway, T.S., 2016. Effect of microplastic on the gills of the shore crab Carcinus maenas. Environ. Sci. Technol. 50, 5364–5369.

Webb, P.W., 1976. The effect of size on the fast-start performance of rainbow trout Salmo gairdneri, and a consideration of piscivorous predator-prey interactions. J. Exp. Biol. 65, 157–177.

Weis, J.S., Weis, P., 1989. Tolerance and stress in a polluted environment. Bioscience 39, 89–95.

Wen, B., Zhang, N., Jin, S.-R., Chen, Z.-Z., Gao, J.-Z., Liu, Y., Liu, H.-P., Xu, Z., 2018. Microplastics have a more profound impact than elevated temperatures on the predatory performance, digestion and energy metabolism of an Amazonian cichlid. Aquat. Toxicol. 195, 67–76.

Wesch, C., Bredimus, K., Paulus, M., Klein, R., 2016. Towards the suitable monitoring of ingestion of microplastics by marine biota: a review. Environ. Pollut. 218, 1200–1208.

Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: a review. Environ. Pollut. 178, 483–492.

Yin, L., Chen, B., Xia, B., Shi, X., Qu, K., 2018. Polystyrene microplastics alter the behavior, energy reserve and nutritional composition of marine jacopever (Sebastes schlegelii). J. Hazard. Mater. 360, 97–105.