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Do whitefish (*Coregonus lavaretus*) larvae show adaptive variation in the avoidance of microplastic ingestion?☆

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ABSTRACT

The presence of microplastics in aquatic ecosystems has recently received increased attention. Small plastic particles may resemble natural food items of larval fish and other aquatic organisms, and create strong selective pressures on the feeding traits in exposed populations. Here, we examined if larval ingestion of 90 μm polystyrene microspheres, in the presence of zooplankton (*Artemia* nauplii, mean length = 433 μm), shows adaptive variation in the European whitefish (*Coregonus lavaretus*). A full-factorial experimental breeding design allowed us to estimate the relative contributions of male (sire) and female (dam) parents and full-sib family variance in early feeding traits, and also genetic (co)variation between these traits. We also monitored the magnitude of intake and elimination of microplastics from the alimentary tracts of the larvae. In general, larval whitefish ingested small numbers of microplastics (mean = 1.8, range = 0–26 particles per larva), but ingestion was marginally affected by the dam, and more strongly by the full-sib family variation. Microsphere ingestion showed no statistically significant additive genetic variation, and thus, no heritability. Moreover, microsphere ingestion rate covaried positively with the ingestion of *Artemia*, further suggesting that larvae cannot adaptively avoid microsphere ingestion. Together with the detected strong genetic correlation between food intake and microplastic intake, the results suggest that larval fish do not readily possess additive genetic variation that would help them to adapt to the increasing pollution by microplastics. The conflict between feeding on natural food and avoiding microplastics deserves further attention.

1. Introduction

Accumulation of plastic litter in aquatic environments is an increasing concern across the globe requiring urgent research and mitigation activities (Law and Thompson, 2014; Jambeck et al., 2015). While enormous quantities of macroscopic plastic waste are mostly observed in certain oceanic gyres, smaller microscopic plastic particles, i.e. microplastics with particle size under 5 mm, are nearly ubiquitously present in both marine and freshwater environments (Law and Thompson, 2014; Eerkes-Medrano et al., 2015; de Sá et al. 2018; Rochman, 2018), including even remote lakes (Free et al., 2014). Correspondingly, research on the abundance and effects of microplastics in freshwater systems has rapidly increased (Horton et al., 2017; Scherer et al., 2018). Microplastics can resemble planktonic organisms in size and shape, and they can thus be unintentionally ingested as food items by many planktivores, such as fish larvae. Ingestion of microplastics can be active or passive, when plastic particles are taken along with natural food items (Auta et al., 2017; Steer et al., 2017; Sun et al., 2017). By being indigestible and potentially harmful, microplastics pose a significant novel ecological and evolutionary factor that living organisms have to cope with. However, virtually nothing is known about possible avoidance and selective feeding of microplastics by actively foraging larval fish. If some individuals were systematically more likely to ingest microplastics than others, fish could adapt to microplastic pollution through rapid evolution, leading to reduced microplastic intake, just like fish in heavily polluted sites have become tolerant for other anthropogenic exposures (e.g. Reid et al., 2016). Thus far only a few studies have focused on the susceptibility

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of fish larvae to microplastic intake (Malinich et al., 2018), although larval and juvenile fish may be more vulnerable to the negative effects of microplastics than adult fish (Foley et al., 2018).

Both field observations and experimental studies have confirmed that zooplankton and fishes ingest plastic debris (e.g. Setälä et al., 2014; Neves et al., 2015; Lu et al., 2016; de Sá et al. 2018) such as fibers, fragments, beads, films and foams (Free et al., 2014). Microplastics can also be transferred through aquatic food webs (Setälä et al., 2014; Matsson et al. 2015). The adverse effects of microplastics have been anticipated to arise from direct (Lu et al., 2016) and/or indirect toxicity due to adhered chemicals (Cole et al., 2013; Wardrop et al., 2016) or physical effects such as gut-blockage (e.g. Cole et al., 2013; Wright et al., 2013). Furthermore, ingestion of any indigestible particles imposes fitness costs through energetic costs, time costs, and potentially increased vulnerability to predation as individuals are forced to increase their activity and feeding rate to compensate for decreased energy efficiency of food intake (Matsson et al., 2015). The harmfulness of microplastic ingestion likely depends on the elimination speed of plastic particles, but retention times of microplastics have rarely been studied in fish. In adult goldfish (*Carassius auratus*), elimination speed did not differ from normal egestion time of gut contents, suggesting that accumulation would be unlikely (Grigorakis et al., 2017). Egestion potential of small (10–45 μm) polyethylene microbeads was high also in European sea bass (*Dicentrarchus labrax*) larvae (Mazurais et al., 2015). Aquatic food webs are strongly size-dependent, and particle size is relevant for the exposure of differently sized fish to microplastics (Law and Thompson, 2014). Further, particles of various size can have different effects in organisms: 20 μm polystyrene beads accumulated in the gills and gut of zebrafish (*Danio rerio*) whereas smaller 5 μm and 70 nm beads were also found in liver causing oxidative stress, inflammation, and accumulation of lipids (Lu et al., 2016).

Long-term persistence of animal populations is dependent on their resilience to environmental changes and their capacity to adapt to these changes (Hendry et al., 2018). Ingested microplastics may exert strong selection on larval feeding traits, but whether the intake of potentially harmful particles shows additive genetic variation, i.e. heritability, is not known. However, in one-summer-old European whitefish (*Coregonus lavaretus*), daily feed intake has been shown to exhibit moderate heritability (Quinton et al., 2007). Further, in fruit flies (*Drosophila melanogaster*), the larval feeding rate was estimated to have a realized heritability of 11–21% (Sewell et al., 1975), but no studies to date have analyzed the heritability of larval ingestion of microplastics or other artificial items in aquatic animals. To evaluate the evolvability of larval feeding traits, quantitative genetic parameter estimates of microplastic intake are thus needed.

Here, we studied parental and family effects on the polystyrene microsphere ingestion by European whitefish larvae in the presence of live zooplankton. In order to estimate heritabilities of foraging traits, we used families that were produced using a full-factorial breeding design and reared in replicated common-garden conditions. To assess potential harmfulness of microplastics, we monitored the magnitude and dynamics of microplastic intake and elimination from the alimentary tract. We hypothesized that: 1) whitefish larvae ingest microspheres because they have a natural tendency to feed on small particles; 2) larvae show family-specific variation in microsphere intake in the presence of natural food; 3) individual ingestion rate of microspheres shows biologically significant additive genetic variance i.e. narrow-sense heritability; and 4) both microsphere intake and elimination follow similar patterns as those of zooplankton.

2. Material and methods

2.1. Parental fish and gamete collection

Parental fish originated from the River Kokemäenjoki (Finland) anadromous whitefish population and represented the third selectively bred and pedigreed hatchery generation (year class 2014) maintained at the Tervo Fish Farm of the Natural Resource Institute Finland (Luke; national Finnish breeding programme of the European whitefish; Kause et al., 2011). The fish were reared together in an outdoor raceway and the sexes were kept separated for ten days prior to gamete collection. Gametes from ten males (fresh mass: 879.2 ± 109.3 g; mean ± SD) and five females (fresh mass: 1142.8 ± 123.2 g) were stripped on 9 November 2017. For both sexes, individuals were haphazardly selected from different families. Stripped eggs were kept in 0.9 l plastic boxes and milt in air-filled zipper bags (Minigrip®, Georgia, USA) on ice until fertilization later the same day at the University of Eastern Finland, Joensuu.

2.2. Artificial fertilization and incubation of the eggs

Ten males and five females were crossed in all possible combinations (N = 50 full-sib families in two replicates, North Carolina II design: Lynch and Walsh, 1998). The utilized full-factorial experimental design was chosen as it has been demonstrated to provide sufficient statistical power to detect biologically meaningful variances and high-level interaction effects between input variables (Kekäläinen et al., 2018). The average genetic relationship (a) among males and females was 0.025 (±0.033 SD; min: 0.000, max: 0.125, N = 50 male-female combinations). Correspondingly, the average a among males was 0.018 (±0.033; min: 0.000, max: 0.125, N = 10) and among females 0.028 (±0.045; min: 0.000, max: 0.125, N = 5). Approximately 150 eggs (mean = 154.4 ± 14.6, SD) from each female were distributed into 20 Petri dishes and fertilized with sperm of all ten males (two replicate fertilizations per male). In order to equalize sperm numbers across all fertilizations we measured spermatocrit (relative volume of spermatozoa) for all the males by centrifuging the sperm samples for 6 min (11 000 rpm) in a micro-hematocrit centrifuge. Sperm volumes were then equalized by using the highest male-specific spermatocrit (24%) as a reference value. The final sperm volume in all fertilizations was 2.4 μl of pure spermatozoa (equivalent to 10 μl of milt with 24% spermatocrit). Immediately after injecting the sperm on the eggs with a micropipette, 50 ml of 4.5 °C natural water, transported from Tervo Fish Farm, was poured on the Petri dish and each dish was gently shaken for 3 s to allow the eggs to be fertilized. Fertilized eggs were then randomly divided into individual incubating containers (two replicate containers per family) in two 600 l water tanks filled with 4 °C non-chlorinated tap water, where they were incubated until all the eggs were hatched in March 2018. Water temperature was gradually raised to 6.0 °C during 16–19 February 2018, to imitate arrival of spring and to facilitate hatching. Dead embryos were counted and removed weekly throughout the incubation period.

2.3. Feeding experiment

After hatching, 15 haphazardly selected food-naïve whitefish larvae from each incubation container (50 families, two replicates per family) were transferred directly into 300 ml plastic containers with 75 ml of water for a feeding trial at 6 °C. All the family replicates were tested within four days over 6–9 March 2018 using 25 identical containers with 15 larvae each. As the study aimed to focus on microplastic ingestion in the presence of real food, the

containers were supplied with newly-hatched *Artemia* nauplii (Sanders®, Great Salt Lake Artemia, USA) and polystyrene microspheres with concentrations of 7.5 and 30 pcs ml^{-1}, respectively (Fig. S1). Standard 90 μm (mean diameter of 91.6 μm, SD ± 3.53 μm) polystyrene microspheres (Polybead®, Polysciences Europe GmbH, Germany) were used in the exposure. Mean length of *Artemia* nauplii was 433.1 μm (SD ± 20.1 μm, N = 10). We estimated that the volume of one nauplius corresponded to approximately four microspheres, offering the fish larvae a clear size difference between food items and microspheres. Larvae were allowed to feed for 6 h while the containers were continuously agitated by a platform shaker at 20 rpm to imitate wave action and to keep the *Artemia* and microspheres suspended in the water column. In addition, the containers were manually shaken after 3 h to induce complete resuspension of the particles. After the exposure, larvae were euthanized via overdose of tricaine methanesulfonate (Tricaine-S, Western Chemical Inc., USA) and preserved in water-filled Eppendorf tubes at −20 °C until processing. After thawing, the larvae were first measured for total length under a dissecting microscope, and *Artemia* fullness of the alimentary tract was scored on a scale of 0–4, where 0 = empty, 1 < 25%, 2 = 25–50%, 3 = 50–75%, and 4 > 75% of the alimentary tract volume filled with *Artemia* (Fig. S2). Due to a rapid digestion rate of *Artemia* nauplii, their individual counting was not possible. The alimentary tracts were then dissected using a pair of fine tip forceps and the number of ingested microspheres was counted. Larval mortality during the feeding experiment was 0.3%.

2.4. Microplastic accumulation and elimination

To separately quantify intake and depuration dynamics of microspheres, we carried out two experiments. First, whitefish larvae in 12 containers (300 ml) were exposed to identical *Artemia*/microsphere suspension (see above) for 24 h to estimate the microsphere accumulation, and three replicate containers with 15 larvae were sampled at intervals of 2, 6, 12, and 24 h. These larvae represented 15 different families (one haphazardly selected larva per family in each container). The experimental procedure, preservation of the larvae, and analysis of alimentary tract followed the feeding experiment (see above) with the exception that the remaining containers were manually shaken at intervals of 2, 6, and 12 h. There was no larval mortality during the accumulation experiment.

Second, whitefish larvae in 24 containers were exposed to *Artemia*/microsphere suspension for 6 h and then followed for the elimination dynamics by transferring the larvae into new containers with or without continued feeding. Half of the larvae (12 containers) were kept unfed in clean water, whereas the other half (12 containers) were allowed to continue feeding on pure *Artemia* at a concentration of 7.5 nauplii ml^{-1}. Three replicate containers with 15 larvae were again sampled 2, 6, 12, and 24 h after the transfer from the initial feeding regime. The larvae represented 15 different families (one haphazardly selected larva per family in each container). The experimental procedure, preservation of the larvae and analysis of alimentary tract were the same as above. Larval mortality during the elimination experiment was 1.7%.

2.5. Statistical analyses

The independent effects of sire and dam (additive male and female parent effects), and interaction effects of full sibs (accounting for environmental family variance and genetic dominance effects) on the microsphere ingestion were quantified by a generalized linear mixed-effects model (GLMM) with Poisson error distribution and log link function. In the model, testing day and egg incubation tank were used as fixed factors and sire, dam, full-sib family (sire-dam combination), and additional container term as random factors. The analysis was conducted using the *fullfact* package (Houde and Pitcher, 2016) in R (version 3.5.1). All reported P-values are from two-tailed tests with $\alpha = 0.05$. Genetic and phenotypic variances for microsphere ingestion were further estimated using a univariate GLMM animal model in DMU 6.0 software applying Poisson variance function and identity link function (Madsen and Jensen, 2008). In this model, fixed effects were the same as given above, whereas random terms included the genetic animal effect associated with the full pedigree, the common container effect (without a link to the pedigree), and the residual error term. The container term is assumed to capture environmental effects shared by full sibs in the experiment and parts of other non-additive sources of variation (i.e., dominance and maternal effects). Heritability of a trait (h^2) was calculated as the proportion of additive genetic variance of the total phenotypic variance. Correspondingly, common environment effect (c^2) was calculated as the proportion of phenotypic variance explained by the common environment variance.

In addition, to estimate genetic and phenotypic correlation between *Artemia* and microsphere ingestion, we binary-coded both ingestion traits on the basis whether larvae had fed them or not (1/0) and fitted a genetic bivariate GLMM using *Artemia* and microsphere ingestion as dependent binomial variables. In addition to the original scale, heritabilities of the binary traits were calculated on the underlying normally distributed liability scale following Dempster and Lerner (1950). This approach functions as a general normalizing transformation for strongly non-normal distributions (Roff, 2001). GraphPad Prism 5 software was used to plot the results.

3. Results

3.1. Parental effects and heritability of microplastic ingestion

There was high family-specific variation in mean number of ingested microspheres (range 0.4–4.3 microspheres per larva, Fig. 1). The range of ingested number of microspheres was 0–26 in individual larvae, and 25 larvae (1.7% of all) ingested at least 10 microspheres. Total length of the larvae was 12.1 ± 0.5 mm (mean ± SD). However, the length was measured from the full sibs of the experimental fish (N = 300), because the experimental fish experienced unexpected shrinkage during the preservation. The number of ingested microspheres was marginally affected by the dam ($P = 0.05$) and more strongly by the sire-dam interaction (i.e. non-additive full-sib family effect; $P < 0.01$), whereas the variance due to sires was statistically non-significant (Table 1). Based on these results, the microsphere ingestion involved some non-additive (but not additive) genetic and environmental variance shared by the full-sib groups. Pedigree-based animal models confirmed non-existing additive genetic variance (and thus absence of heritability) and presence of common environment variance of full sibs (involving non-additive genetic variation) in microsphere ingestion (Tables 2 and 3).

A bivariate genetic model showed that heritabilities of binomial *Artemia* and microsphere ingestion were low and statistically non-significant (Table 3). The liability scale heritability estimates (±SE) for *Artemia* and microsphere ingestion were 0.107 ± 0.089 and 0.008 ± 0.010, respectively. The genetic correlation between the two ingestion traits was, however, 0.888 (±0.382 SE) and thus statistically significantly different from zero. The corresponding phenotypic correlation was 0.692.

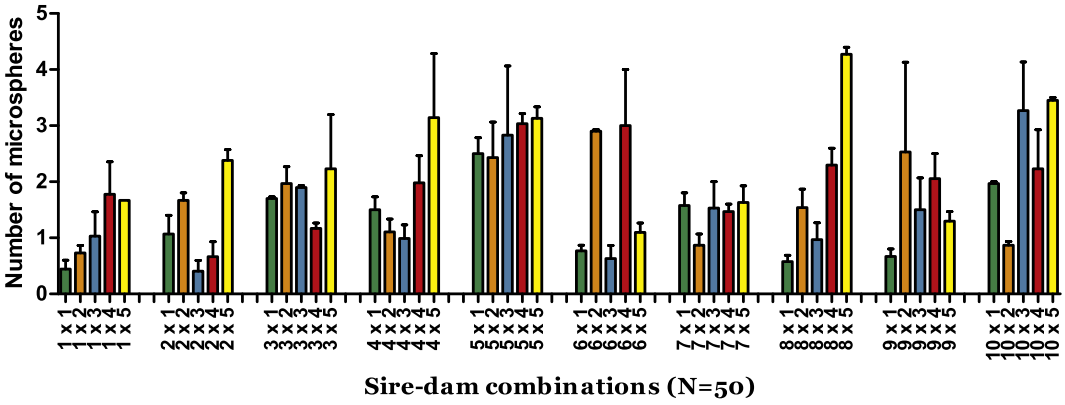

Fig. 1. Number of ingested microspheres per larva (mean ± SE) in different families (sire × dam).

Table 1
Generalized linear mixed model (GLMM) statistics for microsphere ingestion. The percentages of variance explained by the random and fixed factors are also given. Statistically significant *P*-values (based on likelihood ratio test) are indicated in bold.

Effects	Variance	χ^2	*P*	% variance
Random effects				
Sire	0.04	2.26	0.13	4.9
Dam	0.04	3.83	**0.05**	5.1
Full-sib family (Sire × Dam)	0.13	12.07	**< 0.01**	15.4
Container	0.08	52.26	**< 0.01**	9.8
Fixed effects	0.02			2.8
Testing day		11.21	**< 0.01**	
Incubation tank		0.61	0.44	
Residual	0.51			61.9

Table 2
Variance components and variance ratios for microsphere ingestion estimated by a GLMM animal model.

Parameter[1]	Estimate (±SE)
V_g	2.42×10^{-8} (0.11)
V_c	0.39 (0.11)
V_e	3.08 (0.13)
V_p	3.47
h^2	0.00 (0.03)
c^2	0.11 (0.03)

[1]Variance components: V_g = additive genetic variance; V_c = common environment variance of full sibs; V_e = residual variance; V_p = phenotypic variance; h^2 − heritability = V_g/V_p; c^2 − common environment effect ratio = V_c/V_p.

3.2. Microplastic accumulation and elimination

In the accumulation experiment, larvae started to feed on *Artemia* rapidly, and after 2 h mean *Artemia* index exceeded 1.5 (Fig. 2). The index peaked at 6 h, and decreased gradually after that, probably reflecting declining availability of the nauplii. The dynamics of the microsphere accumulation in the alimentary tract of whitefish larvae were almost identical to that of *Artemia* (Fig. 2). In general, only few microspheres were found in the larvae (range 0–6 particles per larva).

In the elimination experiment, the number of microspheres in the alimentary tracts decreased gradually with time (Fig. 3). Larvae fed during the depuration period had higher number of microspheres at the beginning of the experiment, but after 24 h both fed and unfed larvae contained similar numbers. The depuration efficiency was 64% in the fed larvae and 51% in the unfed larvae. The range of retained microspheres was 0–12 particles per larva during the elimination experiment.

4. Discussion

Microsphere ingestion by newly-hatched whitefish larvae did not exhibit statistically significant additive genetic variance, suggesting that feeding traits related to the intake of novel particles would not rapidly respond to environmentally-induced directional selection on these traits. Instead, the positive relationship between *Artemia* and microsphere ingestion observed both at the genetic and phenotypic level, as well as the similarity of *Artemia* and microsphere accumulation curves, suggested that any change in the feeding of microplastics is strongly dependent on the change in natural feeding rate. However, our study alludes to the presence of non-additive genetic variation in the feeding traits and thus indicates that evolutionary responses to some types of microplastics are possible in nature. How non-additive genetic variance and covariation between feeding on natural and anthropogenic particles would reflect to the adaptation of larval fish to microplastic pollution remain to be demonstrated in the future.

While any less than maximal feeding rates might appear disadvantageous for larvae (e.g. Miller et al., 1988; Houde, 2008), earlier research has typically found moderate heritability estimates

Table 3
Variance components, heritability (h^2, in original scale), and common environment effect ratio (c^2) for binomial *Artemia* and microsphere ingestion (fed or not) estimated using a bivariate genetic model.

Parameter[1]	*Artemia* ingestion Estimate (±SE)	Microsphere ingestion Estimate (±SE)
V_g	0.008 (0.007)	0.004 (0.007)
V_c	0.005 (0.002)	0.009 (0.004)
V_e	0.140 (0.007)	0.943 (0.040)
V_p	0.153	0.956
h^2	0.052 (0.043)	0.005 (0.007)
c^2	0.030 (0.015)	0.009 (0.004)

[1]For variances, see Table 2.

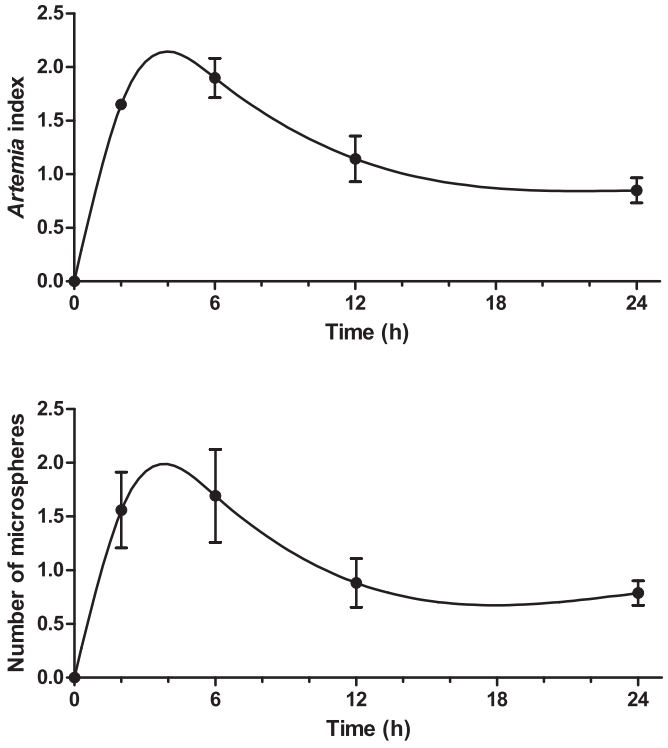

Fig. 2. Mean *Artemia* index (±SE, upper) and number of ingested microspheres (±SE, lower) per whitefish larva after 2, 6, 12, and 24 h of feeding in the accumulation experiment. Smooth piecewise polynomial curves have been fitted to illustrate the dynamics of the microsphere accumulation.

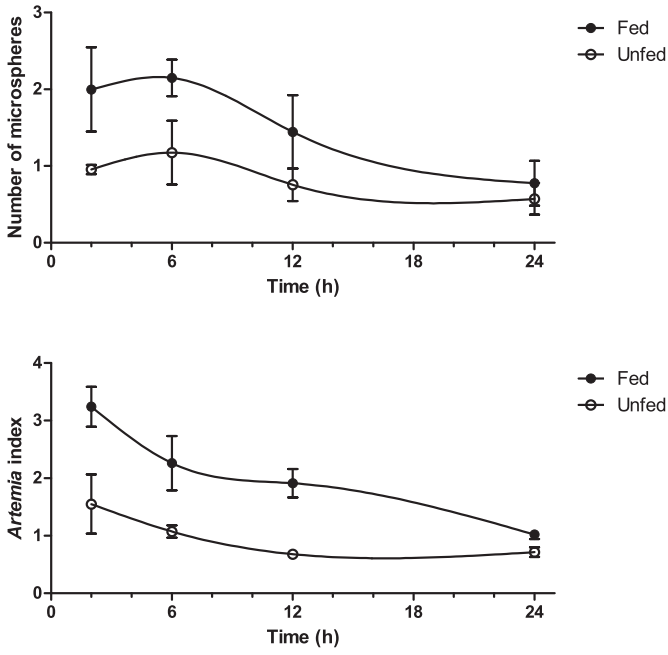

Fig. 3. Mean number of ingested microspheres (±SE, upper) and *Artemia* index (±SE, lower) in fed and unfed whitefish larvae 2, 6, 12, and 24 h after exposure to *Artemia*/microsphere suspension for 6 h in the elimination experiment. Smooth piecewise polynomial curves have been fitted to illustrate the dynamics of the microsphere elimination.

for feeding traits. For example, daily feed intake of one-summer-old whitefish was estimated to have heritability of 0.23 (Quinton et al., 2007). In a review of 93 vertebrate and invertebrate populations, the mean heritability of foraging behavior was 0.29 ± 0.03 (SE) (Stirling et al., 2002). Genetic variation in the feeding traits suggests that these traits may be significant in resolving growth − mortality trade-offs in early life. However, demonstrated negligible additive genetic variation in microplastic ingestion suggests that microplastic intake may not show rapid evolutionary response in the nature. On the other hand, a large proportion of the genetic variation underlying socially affected traits typically remains hidden and is not accounted for by the direct heritability estimates (e.g. Baud et al., 2017; Marjanovic et al., 2018). Behavioral traits are also known to show generally lower heritability than morphological or physiological traits (Meffert et al., 2002), possibly because behaviors are influenced by multiple underlying traits as well as multiple neuronal and hormonal mechanisms (Stirling et al., 2002; Sinn et al., 2006). Similarly, heritability estimates of various traits closely connected with fitness have been observed to be low (Stirling et al., 2002). However, the true fitness effects of microplastic ingestion remain to be explored, and significant mortality might induce changes that cannot be detected by just estimating total microplastic intake at a single occasion. In our short-term study, the mortality during the three experiments was very low (11 larvae in total). However, it is possible that significant selective mortality could occur in longer exposure and produce genetic effects via the non-additive genetic variation in microplastic intake. To evaluate fitness costs related to energetics and feeding activity (Cedervall et al., 2012, Matsson et al. 2015), longer-term or multigenerational studies would be required.

In our experiments, the microsphere concentration was roughly matched to the total volume of *Artemia* nauplii. Despite this, consumed numbers of microspheres were low and suggestive of unintentional intake rather than directed feeding on the microspheres. Whitefish larvae can be classified as cruising zooplanktivores that consume individual prey items by suction (Braum, 1978; Mahjoub et al., 2008). Newly-hatched whitefish larvae feed mostly on calanoid and cyclopoid nauplii as well as cyclopoid copepodids (Sarvala et al., 1988; Sutela and Huusko, 2000). Also rotifers and cladocerans belong to the diet of whitefish larvae. Typical crustacean zooplankton concentrations in the littoral area of oligotrophic Finnish lakes are less than 0.1 pcs ml^{-1} (Rahkola-Sorsa, 2003). Hence, our experimental concentrations of *Artemia* (7.5 pcs ml^{-1}) and microspheres (30 pcs ml^{-1}) were high. Such high concentrations were required to stimulate food-naïve larvae to initiate feeding during the relatively short experimental period and to provide us with sufficient variation in the intake rates. Although the *Artemia* concentration in our relatively small experimental water volume (75 ml) was high, the absolute average number of nauplii per larva (37.5) was not extensive, as a first-feeding whitefish larva is able to ingest up to 24 nauplii in 5 min (Huuskonen et al., 2009). The length range of crustacean prey items is roughly 100–900 μm (Karjalainen, 1992), and the smallest cyclopoid nauplii are smaller than 100 μm in length (J. Karjalainen, University of Jyväskylä, Finland, pers. comm.). Mouth gape size has been found to vary from 640 to 710 μm in 9–10 mm long whitefish larvae (Hartmann and Klein, 1993). Generally, gape size limits the maximum size of the prey items, but large gape size does not necessarily mean a preference for a larger prey. For example, Ponton and Müller (1990) reported that whitefish larvae ingested the most abundant prey size without selecting the largest ones although they would have been able to ingest them. Given their round shape, we considered 90 μm microspheres to be relevantly sized food items for whitefish larvae. However, fish larvae utilize visual and chemical (odor, taste) stimuli to detect suitable food (Rønnestad et al., 2013). Hence, it was not unexpected that whitefish larvae did not appear to actively select more microspheres in the presence of *Artemia*, even though

they were experiencing food particles for the first time. Supporting this view, Kim et al. (2019) reported that adult zebrafish (*Danio rerio*) were able to recognize polyethylene particles as inedible materials and exhibited spitting behavior after ingesting them.

One probable route of microplastics to whitefish larvae in nature will be trophic transfer via ingestion of contaminated zooplankton, as has been observed in marine organisms (Setälä et al., 2014; Welden et al., 2018). In the present study, the microspheres were too large to be ingested by *Artemia* (Dobbeleir et al., 1980). This most likely means that microspheres entered the fish oral cavity either with water during suction feeding, or they were adhered to the surface of ingested *Artemia* (c.f. Cole et al., 2013). Thus, future studies should address the intake of microplastics by the natural diversity of zooplanktonic organisms and by fish larvae in the presence of native zooplankton taxa. It might well be that microplastics eventually modify species composition of zooplankton and fish communities more than directly affect the evolution of any individual species, and these community level ecological effects remain completely unstudied to date.

In the present study, genetic and phenotypic covariation of favorable *Artemia* intake and harmful microplastic ingestion revealed a potential fitness trade-off in larval whitefish. Larvae with high food intake grow rapidly, thus increasing probability of survival (Miller et al., 1988), but they may simultaneously obtain microplastic particles with a higher rate. If this dilemma cannot be solved by developing specificity in feeding, microplastics may modify the growth-mortality trade-off by piling up on natural selection pressures. However, whether feeding rates can evolve in certain directions remains an open question because the family effects observed in microplastic ingestion were largely non-additive, involving both environmental and genetic interaction effects among full-sib groups or parental fish. Non-additive genetic effects are generally strong in all animal behaviors (Meffert et al., 2002), and this source of variation represents a genetic component that may bear a complex genetic architecture, which implies variable vulnerability to microplastics for offspring of different parental combinations. These interactions might thus play an important role in determining whether certain offspring will ingest a high or low number of microplastic particles.

Although the numbers of ingested microspheres were generally small and whitefish larvae were able to eliminate a majority of them in our short-term study, microplastics may represent a greater threat in nature where the range of their sizes, shapes, and chemical compositions is wide (Au et al., 2017; Burns and Boxall, 2018; Scherer et al., 2018). The effects are also likely to vary spatially, as microplastic density has been observed to decrease with the distance from shore in a lake (Free et al., 2014). In many lakes, high densities of microplastics are predicted to occur locally close to littoral zones, where larger plastic debris degrades due to e.g. ultraviolet radiation and mechanical action of waves (cf. marine environment; Browne et al., 2011; Cole et al., 2011), but where European whitefish larvae are also typically found after hatching in spring (Karjalainen et al., 2002).

In the present study, additive genetic variance and the corresponding h^2 estimates were small and associated with relatively large standard errors, and a major part of the phenotypic variation was explained by environmental effects. The large error estimates apparently resulted, to a great extent, from a relatively low number of parental fish used in the experiment. For this reason, point estimate for the genetic variation might have been too low compared to what is actually present in the study population. Moreover, common environmental variance shared by full sibs was likely confounded, to some extent, with additive genetic variance – a typical situation when small data size or number of parents is used (Berg and Henryon, 1998; Martinez et al., 1999). Yet, large family sizes and the use of a full-factorial mating design presumably promoted the estimation and separation of genetic and common environment effects from each other (Sae-Lim et al., 2010).

5. Conclusions

The present study demonstrated that the additive genetic variation in the ingestion of 90 μm microspheres by whitefish larvae is very low at best, and indistinguishable from the ingestion of natural foods. Although the generalizability of our results needs to be confirmed in different fish species and different types of microplastics, the present findings raise the concern that despite the presence of non-additive family variation in microplastic ingestion, fish may not be able to adapt to the presence of plastic particles in their natural environment by starting to avoid them actively. Obviously, more research is required to understand the ecological effects of microplastics on fish larvae and their prey. Meanwhile, all actions to reduce plastic pollution and develop solutions to restore already contaminated reproduction areas of fish are strongly encouraged.

Declaration of competing interest

The authors declare no conflict of interest.

CRediT authorship contribution statement

Hannu Huuskonen: Conceptualization, Investigation, Writing - original draft. **Joan Subiron i Folguera:** Investigation. **Raine Kortet:** Conceptualization, Investigation, Writing - original draft. **Jarkko Akkanen:** Conceptualization, Investigation, Writing - original draft. **Anssi Vainikka:** Conceptualization, Formal analysis, Writing - original draft. **Matti Janhunen:** Formal analysis, Writing - original draft. **Jukka Kekäläinen:** Conceptualization, Investigation, Formal analysis, Writing - original draft.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2020.114353.

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