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Parasite infection but not chronic microplastic exposure reduces the feeding rate in a freshwater fish☆

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ABSTRACT

Microplastics (plastics <5 mm) are an environmental contaminant that can negatively impact the behaviour and physiology of aquatic biota. Although parasite infection can also alter the behaviour and physiology of their hosts, few studies have investigated how microplastic and parasite exposure interact to affect hosts. Accordingly, an interaction experiment tested how exposure to environmentally relevant microplastic concentrations and the trophically transmitted parasite *Pomphorhynchus tereticollis* affected the parasite load, condition metrics and feeding rate of the freshwater fish final host chub *Squalius cephalus*. Microplastic exposure was predicted to increase infection susceptibility, resulting in increased parasite loads, whereas parasite and microplastic exposure were expected to synergistically and negatively impact condition indices and feeding rates. Following chronic (≈170 day) dietary microplastic exposure, fish were exposed to a given number of gammarids (4/8/12/16/20), with half of the fish presented with parasite infected individuals, before a comparative functional response experiment tested differences in feeding rates on different live prey densities. Contrary to predictions, dietary microplastic exposure did not affect parasite abundance at different levels of parasite exposure, specific growth rate was the only condition index that was lower for exposed but unexposed fish, with no single or interactive effects of microplastic exposure detected. However, parasite infected fish had significantly lower feeding rates than unexposed fish in the functional response experiment, with exposed but unexposed fish also showing an intermediate decrease in feeding rates. Thus, the effects of parasitism on individuals were considerably stronger than microplastic exposure, with no evidence of interactive effects. Impacts of environmentally relevant microplastic levels might thus be relatively minor versus other stressors, with their interactive effects difficult to predict based on their single effects.

1. Introduction

Aquatic ecosystems are simultaneously threatened by increasing levels of stressors such as environmental contaminants, climate change, parasites and infectious diseases (Crutzen and Stoermer, 2000; Zalasiewicz et al., 2011). Microplastic (plastics <5 mm in size) contamination is a topical stressor within freshwater systems that can induce a range of lethal and sublethal effects in exposed animal populations, alter food web structure, and cause direct and indirect effects on ecosystem structure, function and services (Eerkes-Medrano et al., 2015; Li et al., 2020; Li et al., 2018). While typically produced on land from the degradation of larger plastics, microplastics are then dispersed into aquatic ecosystems via water and wind, with particles then ingested by resident biota (Collard et al., 2019; Eerkes-Medrano et al., 2015; Windsor et al., 2019).

The almost ubiquitous recovery of microplastics from the gastrointestinal tract, skin and gills of wild freshwater fishes, combined with the negative behavioural, physiological and ecological effects after experimental microplastic exposure, raise major concerns over the detrimental impacts microplastics could be exerting, especially if exposure increases the susceptibility to additional stressors, such as parasite infection (Collard et al., 2019; Parker et al., 2021). While the increased stress, immune and metabolic costs resulting from microplastic exposure has been shown to reduce feeding and morphometrics such as condition, growth and organ indices (Foley et al., 2018; Parker et al., 2021; Salerno et al., 2021), with suggested negative consequences for ecological interactions (Parker et al., 2021; Wootton et al., 2021), the potential interactive effects of chronic plastic exposure (e.g. > 90 days) with

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additional stressors remains poorly understood.

Parasite infections can act as considerable stressors to animal populations through their substantial physiological and ecological host consequences (Barber et al., 2000; Lafferty, 2008; Slavík et al., 2017). Parasite infections can negatively impact fitness and population dynamics of hosts, alter the symmetry of competition between infected and uninfected individuals, and modify host phenotypes through differences in the expression of life history traits, behaviours and habitat utilisation (Barber et al., 2000; Hatcher et al., 2006, 2012). Individual host responses to infections include altering their life-history traits prior to maturity when individuals allocate more resources to gonadal development than growth and survival to ensure reproduction before resource depletion and/or castration (Agnew et al., 2000; Michalakis and Hochberg, 1994). Where the parasite has a complex lifecycle involving trophic transmission then the behavioural modification of infected intermediate hosts can increase the probability of their consumption by final hosts (Barber and Huntingford, 1995; Barber et al., 2004; Lagrue et al., 2007). Microplastic exposure has been posited to alter investment in the host immune system, with the increased immune cost and any subsequent compensatory changes to foraging likely to impact both the encounter and susceptibility to parasites and therefore patterns of trophic transmission (Parker et al., 2021).

Increased parasite transmission and abundance has often resulted from other environmental contaminants, for example trace metals and oils, where exposure can suppress host immune responses and/or alter parasite pathogenicity (Khan and Thulin, 1991; Lafferty and Kuris, 1999; Tort, 2011). In zebrafish (*Danio rerio*) and rainbow trout (*Oncorhynchus mykiss*), microplastic exposure has altered the regulation of gene expression and immune cells (Limonta et al., 2019; Zwollo et al., 2021), although studies assessing the relationships between microplastic loads and parasite infection levels in wild populations remain inconclusive (Alves et al., 2016; Parker et al., 2022a). While the exposure to both microplastics and pathogenic microorganisms in controlled conditions resulted in synergistic effects in the clinical parameters of rainbow trout (Banihashemi et al., 2021), the exposure of zebrafish to microplastics did not significantly alter their bacterial infections or mortality rates (Ding et al., 2022).

Although microplastic and parasite exposure can thus individually elicit considerable physiological and immunological responses in fish, the extent to which this exposure alters the outcomes of parasite exposure, and how the interaction of parasite and microplastic exposure affects the performance of individual fishes (e.g. in foraging) remains highly uncertain. The use of morphometric indices such as condition factor, and the relative spleen, liver and gonad weights, could provide useful information about the differential impacts of microplastic exposure on the general health, immune response, metabolic function and reproductive investment of fishes (Chenet et al., 2021; Mancia et al., 2020). The relative size of the spleen as a proxy of immune activity, as well as the general body condition, might be particularly responsive to microplastic and parasite exposure if impacted hosts increase investment in the immune system relative to feeding and growth (Parker et al., 2021), although this mechanism has yet to be demonstrated experimentally. To overcome this knowledge gap, the interactive effects of chronic microplastic contamination and exposure to different numbers of a trophically transmitted parasite were tested experimentally to assess the consequences for parasite loadings, fish morphometric indices and feeding rates. We test the hypotheses that, relative to the control diet: (1) feeding on microplastics increases fish parasite loads across a range of different parasite exposure levels (2) microplastic exposure increases spleen size while reducing fish growth and condition, and (3) the interaction of exposure to microplastics and parasites has negative synergistic effects on fish feeding rates (indicated by altered comparative functional response metrics).

2. Materials and methods

2.1. Experimental animals and husbandry

A total of 150 juvenile chub (*Squalius cephalus* L.) were used as the model host species. To minimise variability in their starting lengths and mass, and to use fish that had not been exposed previously to the acanthocephalan parasite, the fish were sourced from a local hatchery (Sheath et al., 2018). These fish had been pond-reared with diets that were only partially supplemented by formulated feeds and thus had experience of feeding on natural prey. Their mean starting standard length and wet weight (±SE) was 6.43 ± 0.02 cm and 4.26 ± 0.05 g. To acclimatise fish to the laboratory environment, they were held in relatively large groups (≈30) for 10 days in 100 L aquaria at 17 °C under a 16:8 h light-dark regime, with water quality maintained on a flow-through system. Concomitantly, 15 of the fish were selected at random and tested for the presence of both microplastics and intestinal parasites. This involved their euthanasia (overdose of tricaine methanesulfonate, MS222), followed by dissection of the intestinal tract and its screening using a glass compressorium (Hauptner) under a stereomicroscope (BMDZ, Brunel Microscopes Ltd.). No parasites or microplastics were detected in these fish. At the end of the acclimation period, the fish were measured, weighed and transferred into individual experimental tanks (Exo Terra Standard Faunarium Medium: PT2260, L x W x H: 30 × 19.3 × 20.6 cm, Supplementary material: Fig. S1), with each fitted with a small corner filter with filter medium (Xin You XY-2008) and a plastic PVC pipe tunnel (D x L:7 × 9 cm). All fish were fed a control diet for 7 days before changing to their experimental diet.

2.2. Experimental procedure

The experimental design involved a three-step process: (1) chronic exposure to microplastics; (2) exposure to the acanthocephalan parasite; and (3) the functional response experiment (Fig. 1).

2.2.1. Chronic exposure to microplastics

Following their random allocation to the tanks, fish chronic microplastic exposure was mediated through their diet, where three diet conditions were used (n = 50 per diet condition): (1) control (C; no microplastic exposure) (2) environmental exposure (E; 0.5 microplastic particles d^{-1}) and (3) twice the environmental exposure (2E, 1 microplastic particle d^{-1} based on E) through feeding with control and microplastic-spiked pellets. These exposure levels were largely based on the mean loadings and features of microplastic particles recovered from the gastrointestinal tracts of wild chub in two water courses within southern England (Parker et al., 2022a, 2022b): the Bourne Stream 0.63 ± 0.22 and Dorset Stour 0.69 ± 0.19 particles, where ≈ 70% of all recovered particles were <1 mm in size and predominately polyolefins, such as polyethylene. Wild chub from both study systems were assumed to trophically ingest the microplastics directly and/or indirectly via contaminated prey items (Parker et al., 2022a, 2022b), thus spiked food pellets were considered as the most appropriate microplastic exposure method, based on Coppens' 2 mm diameter Premium Select Carp Pellets. As the feed pellets potentially already contained microplastic particles (de Carvalho et al., 2021), 100 pellets were randomly selected and processed to confirm that no microplastics were present.

Control fish received 4 normal feed pellets every day, corresponding to 1% of the starting mean body mass, where E fish received 1 spiked pellet and 3 normal feed pellets or the control diet on alternating days (for a mean exposure of 0.5 microplastic particles d^{-1}) and 2E fish received 2 spiked pellets and 2 normal feed pellets or the control diet on alternating days (for a mean exposure of 1 microplastic particle d^{-1}). Irregular shaped microplastics were produced from blue polyethylene sheets (PE8, Lows of Dundee) through the repeated cutting and sieving of particles 0.1–1 mm in size. Spiked pellets were made by individually embedding single microplastics into wetted pellets, reforming them and

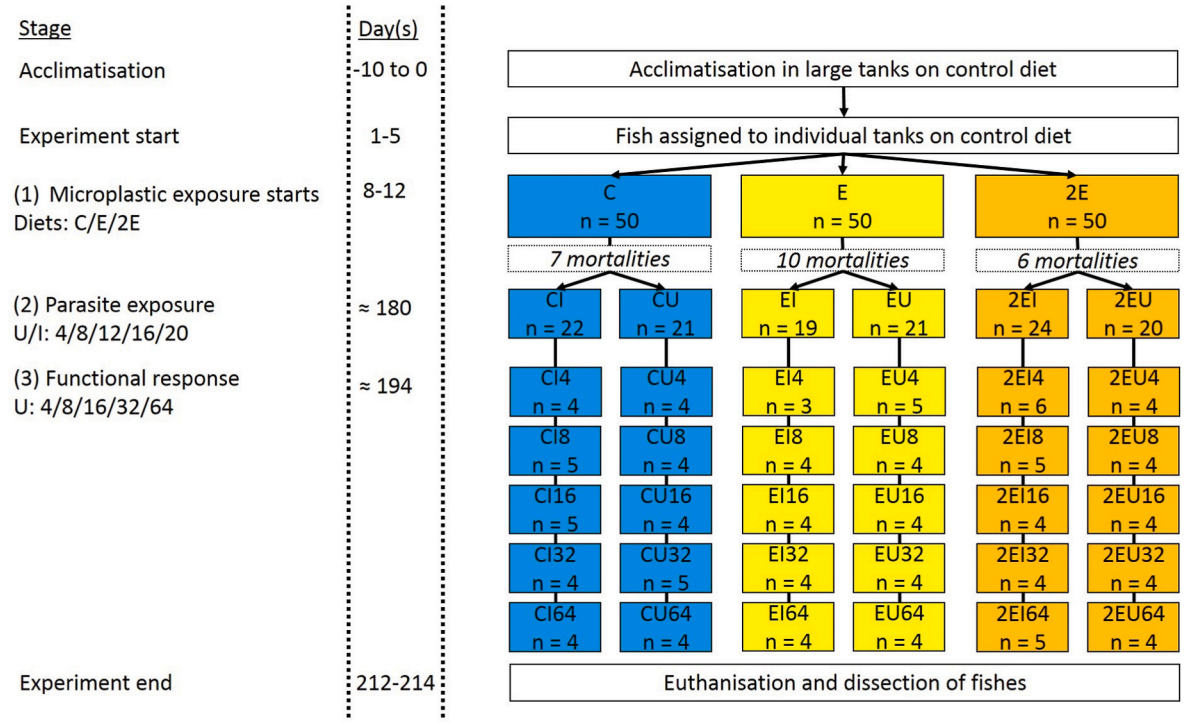

Fig. 1. Experimental design with timings and replication overview. Replication numbers (n = x) are given for all combinations of diet: E = mean environmental and 2E = twice mean environmental microplastic exposure; parasite exposure: I = infected and U = unexposed gammarids; prey density = 4/8/16/32/64 uninfected prey. Within diet parasite exposure conditions, fish were randomly exposed to 4/8/12/16 or 20 infected or uninfected gammarids.

allowing them to dry overnight at 50 °C. Pre-experiment trials using non-experimental fish indicated that the spiked pellets sank and retained their shape in the water, and were then consumed whole rapidly by fish. Approximately 50% of the microplastic particles were then recoverable from the gastrointestinal tracts of the fish the day after feeding, suggesting the likelihood of plastic accumulation was low (data not presented). The size range of selected particles (0.1–1 mm) was also deliberately selected to exceed those that may translocate the gastrointestinal barrier and reach other parts of the body such as the liver, brain and muscle (Parker et al., 2021).

The fish were initially exposed to the microplastics for ≈170 days, during which water chemistry was monitored to ensure it remained within safe limits (NH_3: <0.2, NO_2: <0.5, NO_3: <70 mg L^{-1}) for the species with 50% water changes used where necessary. In the initial 50 days of the experiment, 23 of the fish died, but mortality was not significantly related to the experimental diet (C: 7, E: 10, 2E: 6; Pearson's Chi-squared test: $\chi^2 = 6$, df = 4, p = 0.20). These fish were removed from all subsequent data analyses (Fig. 1) and no further mortality was observed after this initial period.

2.2.2. Parasite exposure

The acanthocephalan parasite used in the experiment was *Pomphorhynchus tereticollis* (Rudolphi, 1809), which has a complex lifecycle involving a freshwater amphipod (*Gammarus* spp.) intermediate host and a fish final host, usually chub in Southwest England (Andreou et al., 2020; Hine and Kennedy, 1974a, 1974b; Kennedy, 2006). Parasite infected gammarids are easily identifiable by the presence of an orange spot observable through the body (Hine and Kennedy, 1974a, 1974b; Kennedy, 2006). To test how different levels of parasite exposure interacted with microplastic exposure, at the end of the microplastic exposure period, all fish were randomly assigned within diets and exposed to one of a pre-determined gammarid abundance groups (n = 4, 8, 12, 16 or 20). Half of the surviving fish were exposed to infected gammarids and the remainder to uninfected ones. All gammarids were collected from the River Avon, Hampshire (50.8864, −1.788279), by kick sampling with a 1 mm mesh net. Exposure was always on the day of gammarid collection. Initial screening of 50 gammarids revealed no microplastics were present and a further 40 were examined to confirm that the parasite was at the particular life stage and size infective to fish. Prior to gammarid exposure, the fish were starved for 24 h and the PVC tunnel and corner filter temporarily removed and replaced with an air stone to continue aeration but prevent gammarids seeking refuge behind the corner filters. Following the addition of the pre-determined number and infection status of gammarids, fish were left to consume them for 24 h. At the end of this period, any remaining gammarids were siphoned out and counted, the corner filters and tunnels were then added back to tanks, and fish resumed their experimental diet of pelleted food the following day. Surviving gammarids were not reused.

2.2.3. Functional response experiment

Fourteen days after parasite exposure (a time sufficient for attachment and infection within the gastrointestinal tract (Hine and Kennedy, 1974a, 1974b; Kennedy, 2006)), all fish were used in a comparative functional response experiment, where the prey were all live uninfected gammarids (collected from the field site). Fish were subject to a 24 h starvation period prior to the trial to standardise hunger levels. Tunnels and corner filters were then removed from tanks and a specific number (4, 8, 16, 32, 64) of gammarids randomly assigned within diet-parasite exposure combinations (Fig. 1). Fish were allowed to feed without disturbance for 1 h before the remaining gammarids were recovered and counted by siphoning through a sieve. Corner filters and tunnels were then added back to the tank and the fish returned to their experimental diet. Individual fish were exposed to a single prey density and surviving gammarids were not reused.

2.3. Experiment conclusion and data collection

Following the functional response experiment, the fish were fed their experimental diet for six more days before being euthanised (MS222 overdose), re-measured, weighed and then dissected, with removal of

the gastrointestinal tract and spleen. Gastrointestinal tracts were then pressed to 1 mm thickness using a glass compressorium (Hauptner) and screened under stereomicroscope (BMDZ, Brunel Microscopes Ltd.) for counting the number of microplastics and parasites present. Screenings were performed blind to the microplastic and parasite exposure, and any parasites were removed and weighed to more accurately determine the total end fish body weight.

Several morphometric indices relating to body condition, growth rate and immune activity were calculated for all individuals surviving until the experiment end:

$$Fulton^{'}s\ condition\ factor\ (K) = 100 \times \frac{W}{SL^3}$$

$$Specific\ growth\ rate\ (SGR) = \frac{100 \times (ln(W_E) - ln(W_S))}{\Delta t}$$

$$Splenosomatic\ index\ (SSI) = 100 \times \frac{SW}{W}$$

where W is total body weight (excluding the weight of all parasites), SL standard length, W_E and W_S are the end and start weights, respectively, Δt the change in time (days) between measurements, and SW the end spleen weight.

To test the effects of parasite exposure in the morphometric and functional response analyses, fish were assigned to three different parasite exposure categories depending on the status of the presented gammarids and the end parasite load (identified during dissection): (i) unexposed (fish fed with uninfected gammarids that were thus uninfected; (ii) exposed fish (fish presented with infected gammarids but were uninfected on dissection), and (iii) infected (fish presented with infected gammarids and that had parasites within the gastrointestinal tract). The data for 12 individuals (4 for each diet) were excluded from the analyses as no prey items were consumed which was assumed to be an unnatural behaviour. A single parasite was recovered from an unexposed fish, assumed to have resulted through the accidental addition of an infected gammarid, and was subsequently excluded from the analyses. After assigning to parasite exposure categories, 61 fish were unexposed, 33 were exposed, and 32 were infected (n = 67 parasites recovered, mean ± SE: 2.09 ± 0.25 parasites per infected fish).

2.4. Statistical analyses

All data analyses were carried out in RStudio version 3.5.1 (RStudio: Integrated Development for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com). Within each analysis, the model selection process first compared between a pair of beyond optimal models with all fixed effects and their interactions: a linear mixed effects model (LMEM) containing batch and rack as random effects, with a simpler general linear model (GLM) on the basis of Akaike Information Criterion (AIC). The simpler model was selected where the AIC value was two points lower. The progressing model was then subject to a top-down approach, as outlined by Zuur et al. (2009), working backwards and sequentially removing the least significant term in each iteration until an optimal model was reached (where all remaining variables were significant or all remaining variables were non-significant). The optimal model was then checked for overdispersion using the ratio between the residual variance and degrees of freedom (Zuur et al., 2009) where ratios <1 indicate no overdispersion. Overdispersion was not identified in any of the analyses.

For infected fish, a Poisson model examined if parasite loads were related to the interaction of microplastic exposure and the number of infected gammarids consumed (indicating parasite exposure) to examine if infection was higher for fish exposed to microplastics. Separate Gaussian models were then performed to test the interaction of microplastic exposure and parasite exposure categories on change in Fulton's condition factor (ΔK), SGR and SSI as indices of general health, growth throughout the experimental period and immune investment, respectively.

For the number of uninfected gammarids consumed within the functional response experiment, first a Poisson GLM, Poisson LMEM and a negative binomial LMEM were compared on the basis of AIC to determine the best model fit. The progressing model tested the number of uninfected prey items consumed based on the interaction of the parasite and microplastic exposure categories. Comparative functional response curves were then determined for significant effects only using functions from the package "FRAIR" (Pritchard et al., 2017). Attack rate (a) and handling rate (h) were calculated for data aggregated by each of the above parasite exposure categories (excluding those individuals where 0 gammarids were consumed) using "frair_fit" (based on a Type II response, a = 1.2, h = 0.015) and "frair_boot" (200 iterations), before carrying out pairwise comparisons between parasite exposure categories using the "frair_compare" function. Attack rate is the rate at which an organism encounters prey items at a particular density, whereas the handling rate defines the time taken to process a prey item.

3. Results

3.1. Microplastic and parasite exposure impacts on parasite load and fish morphometrics

No fish accumulated microplastics during the experiment, with dissections at its conclusion revealing no remaining plastics in the gastrointestinal tracts, either from the experimental treatments or other sources. The best fitting model for the parasite load data was the general linear model structure (Poisson GLM AIC = 113, Poisson LMEM AIC = 117). The resulting optimal parasite load model indicated that parasite load increased with the number of parasites ingested only (Poisson GLM: $\chi^2 = 4.06$, df = 1, $p < 0.05$, Fig. 2, S1 models), with microplastic exposure having non-significant single and interactive effects ($p > 0.05$, S1 models)..

The best fitting models for all morphometric data were GLM rather than LMEM variants (Change in condition: Gaussian GLM AIC = 5,

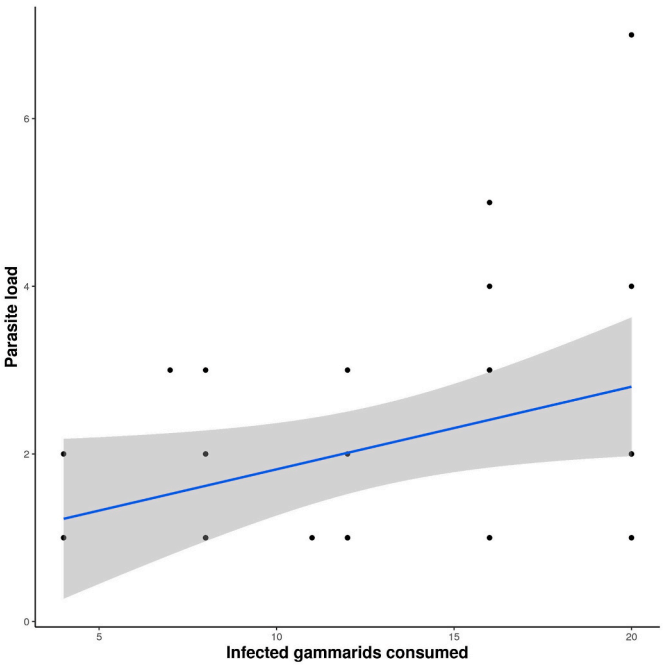

Fig. 2. The relationship between parasite load and the number of infected gammarids consumed. End parasite loads and the number of parasites ingested (via infected gammarid intermediate hosts) are given for the 32 infected fish. The model fitted line is plotted along with the standard error border margins.

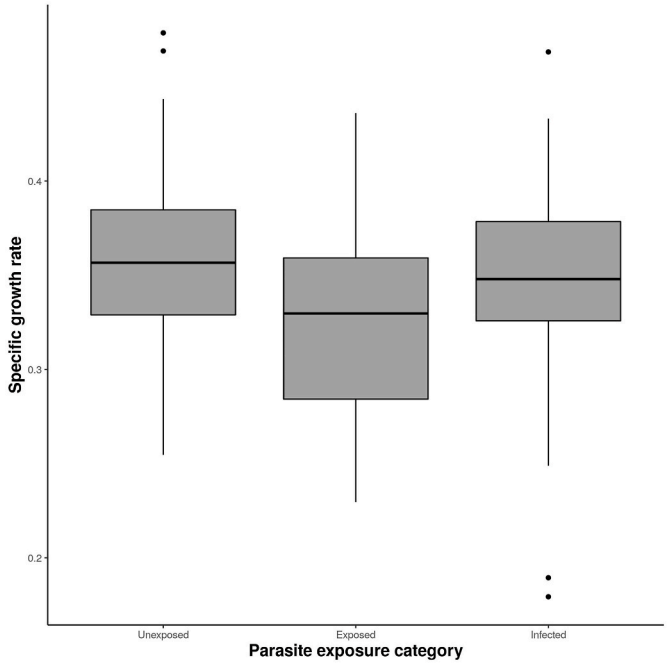

Fig. 3. Specific growth rates between the different parasite exposure categories. Boxplots show the distribution of specific growth rate values for all fishes aggregated within the Unexposed (n = 61), Exposed (n = 33) and Infected (n = 32) categories.

Gaussian LMEM AIC = 36; Specific growth rate: Gaussian GLM AIC = −366, Gaussian LMEM AIC = −315; Splenosomatic index: Gaussian GLM AIC = −183, Gaussian LMEM AIC = −136). Microplastic exposure, parasite exposure and their interaction had no effect on change in condition (all factors $p > 0.05$, S2 models). Specific growth rate varied between parasite exposure categories (Gaussian GLM: $\chi^2 = 8.14$, df = 2, $p < 0.05$) and was lower for exposed relative to unexposed fish (Fig. 3, S3 models, Table S1). Splenosomatic index did not vary between microplastic, parasite exposure categories, or their interaction (all factors $p > 0.05$, S4 models).

3.2. Comparative functional responses

The best fitting model for the number of gammarids consumed was a negative binomial LMEM variant (Poisson GLM AIC = 1572, Poisson LMEM AIC = 1541, Negative binomial LMEM AIC = 837). The optimal model indicated that the number of gammarids consumed differed between parasite exposure categories (Negative binomial LMEM: $\chi^2 = 20.14$, df = 2, $p < 0.001$, S5 models). Correspondingly, the functional response data were grouped by the parasite exposure categories, revealing that fish infected with parasites had significantly lower attack rates but higher handling times than unexposed fishes (Table 1, Fig. 4). Additionally, infected fish had significantly lower attack rates than exposed fish, whereas unexposed individuals had lower handling rates compared to fish exposed to, but not infected by, the parasite (Table 1, Fig. 4).

4. Discussion

This study is the first to investigate the potential interactive effects of environmentally relevant microplastic and parasite exposures on the parasite load, morphometrics and feeding of a freshwater fish. The results revealed that the effects of parasite exposure and infection on functional response parameters were substantially stronger than chronic microplastic exposure, with no significant interactive effects. Within infected fish, diet did not impact parasite load and microplastic exposure had no effect on fish morphometrics; however, specific growth rate was lower in exposed relative to unexposed fish. Significantly lower feeding rates were observed in infected relative to unexposed fish. Additionally, infected fish had a lower attack rate and unexposed fish a lower handling rate relative to exposed individuals. This is a highly important result since the fish were chronically exposed to environmentally relevant microplastic exposure levels to determine if their effects on host-parasite dynamics are similar to those of other environmental contaminants (Khan and Thulin, 1991; Lafferty and Kuris, 1999; Tort, 2011).

Table 1
(A) Functional response coefficient estimates for aggregated parasite exposure categories. Attack (a) and handling rates (h) for all parasite exposure categories are reported after excluding fish where no gammarids were consumed. (B) Outputs of pairwise functional response coefficient tests. Tests compare differences in attack (Da) and handling rate (Dh) between all parasite exposure categories, excluding fish where no gammarids were consumed. SE refers to standard error and significance levels are denoted by "*".

(A)

Factor level	Attack rate (a)	Handling rate (h)
Unexposed	3.27	0.02
Exposed	3.11	0.06
Infected	1.67	0.07

(B)

Comparison	Coefficient	Estimate ± SE	z value	p value
Unexposed-Exposed	Da	0.16 ± 0.55	0.29	0.77
	Dh	−0.04 ± 0.00	−8.25	<0.001 ***
Unexposed-Infected	Da	1.60 ± 0.39	4.06	<0.001 ***
	Dh	−0.05 ± 0.01	−6.67	<0.001 ***
Exposed-Infected	Da	1.44 ± 0.56	2.59	<0.01 **
	Dh	−0.01 ± 0.01	−1.64	0.10

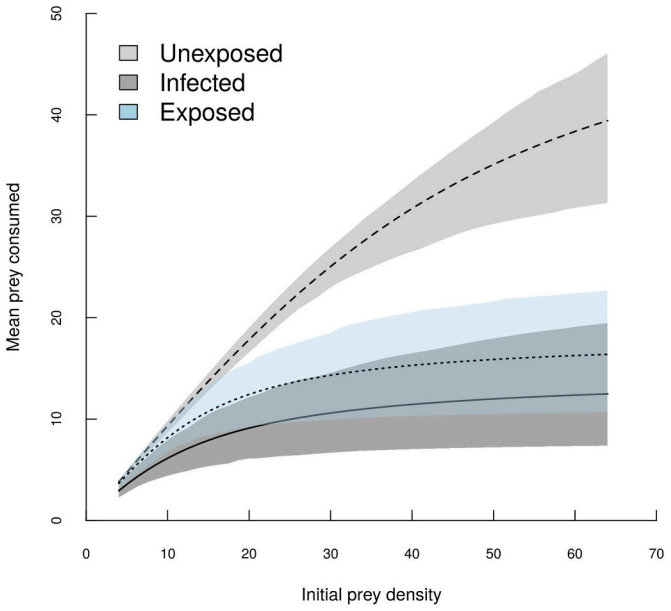

Fig. 4. Functional response curves by parasite exposure category. Curves with confidence intervals are produced using data aggregated by parasite exposure status, excluding fish where no gammarids were consumed. Parasite exposure categories: Unexposed (dashed line, light grey) = fish exposed to but not infected by the parasite, Infected (solid line, dark grey) = fish exposed to and infected by the parasite and Exposed (dotted line, light blue) = individuals exposed to but not infected by the parasite. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4.1. Parasite load and morphometric indices

Contrary to our hypotheses, no interactive effects were observed, with diet not impacting parasite load and load only increasing with the number of ingested parasites. This result of microplastic exposure not interactively impacting the susceptibility of organisms to parasite infection is also contrary to assumptions that microplastic exposure may impair immune function (Limonta et al., 2019; Masud et al., 2022; Parker et al., 2021) and additionally suggests that any correlation between microplastic and parasite loads in wild and experimental fish may be coincidental (Alves et al., 2016). Parasite loads were positively related to the number of parasites consumed and high parasite exposures were required to achieve infection, as also found elsewhere (Sheath et al., 2016, 2018). However, some fish in the present study did not become infected, even after consuming >10 parasites, perhaps due to differences in the experimental conditions, host response and/or perhaps differences in parasite infectivity (Hine and Kennedy, 1974a, 1974b; Kennedy, 2006). Fish in the present study were sourced from a local hatchery to reduce variations in size, age and genetic variation, as well as to ensure that fish had no previous exposure to the parasite and microplastic exposure used. As such, a population of wild *S. cephalus* would likely be more resistant to the microplastic and parasite treatments due to prior exposure, reduced stress and/or a higher resistance to parasites (Oliva-Teles, 2012; Wysocki et al., 2007), however individual variation in responses would likely also be much greater due to greater genetic and individual variation (Kohlmann et al., 2007; Norris et al., 1999).

Microplastic exposure had no effect on fish morphometric indices, while parasite exposure only impacted the specific growth rate for exposed but not infected fish. These results were contrary to the hypothesis that condition and specific growth rate will be lower in fish that experienced chronic microplastic and shorter term parasite exposure, since polyethylene ingestion (Hu et al., 2022; Jabeen et al., 2018; Ottová et al., 2005; Šimková et al., 2008; Tarasco et al., 2022) and *P. tereticollis* exposure (Bosi and Dezfuli, 2015; Dezfuli et al., 2002, 2015) can both negatively affect body condition (and other morphometrics) in *S. cephalus* and other cyprinid fishes. Similarly, the splenosomatic index, a proxy of spleen size and immune function, was also predicted to be higher in fish exposed to microplastics as well as parasites, given the metabolic, immunological and pathological costs to microplastic (Chenet et al., 2021; Zhu et al., 2020) and parasite exposure singly (Bosi and Dezfuli, 2015; Dezfuli et al., 2002, 2015). The absence of these relationships could result from the use of environmentally realistic microplastic exposures in the present study, based on two rivers in southern England (Parker et al., 2022a, 2022b), as other studies have demonstrated effects at higher and/or environmentally unrealistic exposures (Hu et al., 2022; Jabeen et al., 2018; Tarasco et al., 2022). Alternatively, it is possible that the experimental exposure was impacting other organs such as the liver and gastrointestinal tract, not investigated here but previously demonstrated to be impacted by microplastic exposure (Foley et al., 2018; Parker et al., 2021; Salerno et al., 2021). Additionally, the period of parasite exposure was relatively short compared to other studies (Sheath et al., 2016, 2018) and may thus exert effects on other morphometrics over longer timescales.

4.2. Comparative functional responses

Microplastic and parasite exposure had no interactive effect on the number of gammarids consumed, with the aggregated functional response curves only identifying reduced feeding in infected - and to a lesser degree exposed - fish versus unexposed fish. This result was in partial support of the hypothesis and is consistent with previous work that identified significantly lower attack rates and higher handling times for fish exposed to this parasite (Sheath et al., 2018). The ingestion of *P. tereticollis* parasites via infected intermediate hosts may result in reduced feeding due to, for example, pseudo-satiation, blockage of the gastrointestinal tract or altered behaviour (Bosi and Dezfuli, 2015; Dezfuli et al., 2002, 2015). *Pomphorhynchus* spp. infection in wild *S. cephalus* has been shown to be associated with several histopathologies of the small intestines, locally to the attachment sites of parasites, as well as increased levels of immune cells (Bosi and Dezfuli, 2015; Dezfuli et al., 2002, 2015), supporting a negative single effect of infection that might also impair feeding. Parasite attachment may depend on factors such as parasite fitness affecting their ability to attach, the immune response mounted by the fish, the condition of the organ surface structure and the remaining space available which likely impacts the efficiency of trophic transfer and the subsequent handling of food items if digestive structures are damaged (Bosi and Dezfuli, 2015; Dezfuli et al., 2002, 2015). The handling rate for exposed fish was significantly higher than for unexposed individuals and was no different than for infected individuals, suggesting that even exposure to the parasite and/or infected prey items may have negatively impacted feeding, perhaps through a short-term physiological or immune response and/or cost (Bosi and Dezfuli, 2015; Dezfuli et al., 2002, 2015). Paired with the lower specific growth rate observed, parasite exposure thus resulted in both a behavioural and physiological change within fish exposed to parasites.

In contrast to the hypothesis, diet did not impact the number of infected parasites consumed. It was posited that microplastic exposure would reduce the feeding of fish through mechanisms such as increased metabolic stress, immune investment and physiological damage to feeding apparatus, as identified in other freshwater cyprinids (Hu et al., 2022; Jabeen et al., 2018; Tarasco et al., 2022), which might induce behavioural and feeding changes. Discrepancies may arise from the different exposure conditions, especially the level and type of microplastics used, as well as the particular organism whereby environmentally irrelevant exposures may produce artificial effects not seen in and relevant to nature. While few studies have directly investigated the impact of microplastic exposure on comparative functional responses, no impact of different types and concentrations of microplastic were detected in European green crab *Carcinus maenas* feeding on blue mussels *Mytilus edulis* (Cunningham et al., 2021). Finally, the greater retention time for smaller particles and/or fibres may mean that the impacts of microplastic exposure may depend as much on the particle features as the concentration and might lead to the systematic over- or under-estimation of the negative impacts of microplastic exposure depending on the particular particles used (Hoang and Felix-Kim, 2020; Kim et al., 2019; Xiong et al., 2019).

4.3. Microplastic exposure

No microplastics, from the experimental diets or introduced throughout, were identified in any of the fish during blind dissections at the end of the experiment, suggesting no contamination and that the desired microplastic exposure levels were achieved in the experiment comparable to the loadings seen in wild *S. cephalus* (Parker et al., 2022a, 2022b). Further, pre-experiment assessments of pellets, source fish and gammarids ensured that the only exposure of the experimental fish to microplastics was through the spiked pellets. The pilot studies indicated approximately 50% of microplastics were retained the day after feeding, which is comparable to levels detected in other cyprinids under controlled conditions (Hoang and Felix-Kim, 2020; Kim et al., 2019; Xiong et al., 2019). However, these studies highlight that particle features, fish body size and feeding rates will impact egestion, therefore pilot egestion studies are crucial to determine particle turnover (Hoang and Felix-Kim, 2020; Kim et al., 2019; Xiong et al., 2019). Additionally, we emphasise that the exact particle retention times at different exposure levels were not determined and thus fish may have differed in their egestion times and therefore actual loadings at any particular time (Hoang and Felix-Kim, 2020; Kim et al., 2019; Xiong et al., 2019). The recovery of microplastics from wild *S. cephalus* and other freshwater fishes (Collard et al., 2018; Parker et al., 2022a, 2022b) demonstrates

not all particles are immediately egested, and that the encounter and ingestion rate might often exceed egestion. While studies have found that polyethylene microplastic ingestion can impact the feeding of freshwater cyprinids by altering the buccal cavity (Jabeen et al., 2018), no such effects were detected here.

5. Conclusions

This experiment represents, to our knowledge, the first interaction experiment to investigate how environmentally relevant microplastic loadings and acanthocephalan parasite exposure affects the host-parasite relationships, morphometrics and feeding ecology in a freshwater fish. Although microplastics are considered an environmental contaminant of high concern, detrimental effects on fish hosts were not evident in the behavioural functional response metrics and morphometric indices, perhaps due to the use of environmentally derived exposure levels. In contrast, both exposure to and infection by parasites increased the handling but decreased the attack rate of foraging fish, whereas the specific growth rate was lower in exposed fish only, indicating a cost of both exposure and infection. It is important to emphasise that reductions in feeding rate and the reduced specific growth rate for exposed fish were detectable only two weeks after parasite exposure, yet no alterations to feeding or morphometrics were observed even after several months of microplastic exposure. The absence of interactive effects between environmentally relevant microplastic and parasite exposures suggests microplastics have minor effects when compared with other stressors, although we suggest additional interaction studies are needed to understand the conditions under which more severe impacts could manifest. Finally, future studies should investigate the potential interactive effects of microplastics with other parasites spanning different costs of infection and mechanisms of action.

Permissions and ethical statement

The described work was carried out under the UK Home Office 1986 Animals (Scientific Procedures) legislation through an approved protocol of work within project licence PA2C7C4E6 and following ethical review. All euthanasia was carried out following humane end points and in line with the project licence (anaesthetic overdose, tricaine methanesulfonate).

Credit roles

Ben Parker: Conceptualization, Methodology, Formal analysis, Investigation, Writing-Original Draft, Writing-Review and Editing; **J. Robert Britton**: Conceptualization, Methodology, Investigation, Writing-Original Draft, Writing-Review and Editing, Supervision; **Iain D. Green**: Conceptualization, Methodology, Investigation, Writing-Original Draft, Writing-Review and Editing, Supervision; **Fátima Amat-Trigo**: Conceptualization, Methodology, Investigation, Writing-Original Draft, Writing-Review and Editing; **Demetra Andreou**; Conceptualization, Methodology, Investigation, Writing-Original Draft, Writing-Review and Editing, Supervision.

Declaration of competing interest

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

Data availability

The data are already accessible at: https://doi.org/10.18746/bmth.data.00000249 The final analyses code will be added also.

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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.2023.121120.

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