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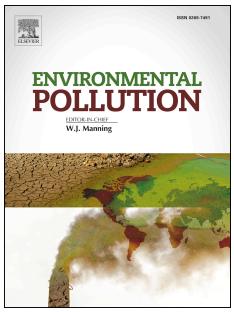

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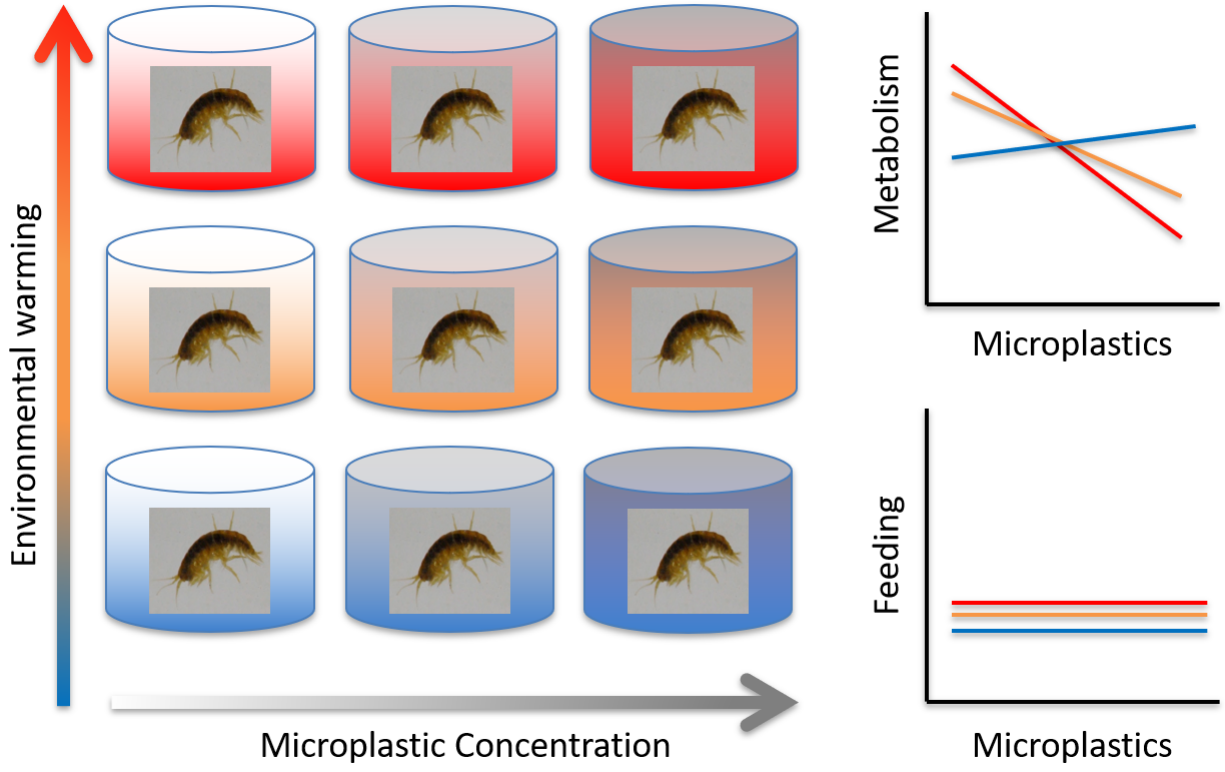

Interactive effects of warming and microplastics on metabolism but not feeding rates of a key freshwater detritivore

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ABSTRACT

Microplastics are an emerging pollutant of high concern, with their prevalence in the environment linked to adverse impacts on aquatic organisms. However, our knowledge of these impacts on freshwater species is rudimentary, and there is almost no research directly testing how these effects can change under ongoing and future climate warming. Given the potential for multiple stressors to interact in nature, research on the combined impacts of microplastics and environmental temperature requires urgent attention. Thus, we experimentally manipulated environmentally realistic concentrations of microplastics and temperature to partition their independent and combined impacts on metabolic and feeding rates of a model freshwater detritivore. There was a significant increase in metabolic and feeding rates with increasing body mass and temperature, in line with metabolic and foraging theory. Experimental warming altered the effect of microplastics on metabolic rate, which increased with microplastic concentration at the lowest temperature, but decreased at the higher temperatures. The microplastics had no effect on the amount of litter consumed by the detritivores, therefore, did not result in altered feeding rates. These results show that the metabolism of important freshwater detritivores could be altered by short-term exposure to microplastics, with greater inhibition of metabolic rates at higher temperatures. The consequences of these metabolic changes may take longer to manifest than the duration of our experiments, requiring further investigation. Our results suggest little short-term impact of microplastics on litter breakdown by gammarid amphipods and highlight the importance of environmental context for a better understanding of microplastic pollution in freshwater ecosystems.

Keywords: Climate warming, leaf litter breakdown, multiple stressors, oxygen consumption, pollution, shredder.

RESULTS SUMMARY:

Warming alters the effect of microplastics on metabolic rates. Increased microplastic concentrations only inhibited metabolism at the highest temperatures.

1. INTRODUCTION

Human societies rely on freshwaters for vital ecosystem services, including food and water provision, climate regulation, and recreation (MEA, 2005). With the human population projected to reach 8.4 – 10.9 billion by 2050, demand on these ecosystem services will further increase (Hall, 2015). Freshwater ecosystems are also faced with unprecedented environmental changes, such as climate warming (IPCC, 2014) and pollution (MEA, 2005; Dudgeon et al., 2006). Given the prevalence of anthropogenic development near these ecosystems, freshwaters are particularly susceptible to the combination of these global and local environmental pressures (Dudgeon et al., 2006; Ormerod et al., 2010).

Plastics have become an integral part of modern life since the 1950s, resulting in a global demand of 348 million tonnes in 2017 (Plastics Europe, 2018). The increasing rate of plastic production combined with dispersal from landfills, sewer overflow, and agricultural runoff have resulted in unprecedented amounts of this material in the environment (Dris et al., 2015; Browne et al., 2011). Plastic pollutants are categorised into three size classes: macro- (>5 mm), micro- (1 μm – 5 mm), and nano- (<1 μm) plastics. Microplastics can result from the fragmentation of macroplastics through abrasion, wave action, collisions, saltation, and traction (Dris et al., 2015), or can be produced in micro sizes (Fendall and Sewell, 2009). Due to the varying densities of plastic polymers, microplastics are located throughout the water column from surface to sediment making them easily ingestible by species of variable sizes and feeding modes (Wright et al., 2013). Although microplastics have been detected in over 200 species (Teuten et al., 2007) most research efforts have focused on their impacts in marine environments, and our understanding of the biological effects of microplastics on freshwater species remains rudimentary (Dris et al., 2015; Horton et al., 2017).

Microplastics have been shown to reduce feeding rates in shore crabs (*Carcinus maenas*; Watts et al., 2015), Asian green mussels (*Perna viridis*; Rist et al., 2016), copepods

(*Calanus helgolandicus*; Cole et al., 2015), and water fleas (*Daphnia magna*; Ogonowski, Schür et al., 2016). The most likely mechanism is a physical blockage of the gut passage or behavioural avoidance of non-nutritious food contaminated by microplastic particles (Wright et al., 2013; Cole et al., 2015; Galloway et al., 2017). Such sub-lethal effects may be contingent on the taxonomic group, since feeding rates were unaffected or enhanced by microplastics in Pacific oysters (*Crassostrea gigas*; Cole and Galloway, 2015, Sussarellu et al., 2016), freshwater amphipods (*Gammarus fossarum*; Blarer and Burkhardt-Holm, 2016), and marine isopods (*Idotea emarginata*; Hämer et al., 2014). Microplastics can also negatively affect metabolic rates due to impairment of oxygen uptake (Rist et al., 2016) or altered enzyme activity (Wen et al., 2018), although variable effects have been reported (Cole et al., 2015; Green 2016; Green et al., 2016). Changes in energy demand (metabolism) and energy intake (feeding) could ultimately alter community structure and ecosystem function (Ward et al., 2016). Whilst it is important to understand the impacts of microplastics under realistic environmental conditions, most of the studies to date have used microplastic exposures between two and seven orders of magnitude higher than any concentration found in natural ecosystems (Lenz et al., 016). Therefore, our understanding of the effects from environmentally realistic microplastic exposures remains limited (Horton et al., 2017).

Ecological communities are also under increasing pressure from global warming, with a doubling in the frequency of heatwaves over the past 40 years (Fröhlicher et al., 2018) and a projected increase in mean annual temperature of at least 1.5 °C by the end of the century (IPCC, 2014). Increasing temperature places a fundamental biological constraint on metabolic and cellular processes of all ectothermic organisms (Gillooly et al., 2001; Ohlberger 2013). Warming increases metabolic rate up to the thermal optimum of an organism, which can increase individual feeding rates and alter consumer-resource interactions (Brown et al., 2004; Rall et al., 2012; Ohlberger 2013). Temperature is also

likely to interact with other stressors to either compound or mitigate their effects on ecological communities (Crain et al., 2008; Kratina et al., 012; Piggott et al., 2015). Since environmental temperature and microplastic pollution generally have the opposite effects on metabolism and feeding, the combined effect of these two stressors is likely to be antagonistic (i.e. less then the sum of the individual impacts). However, only two studies have analysed the combined effects of warming and microplastics on feeding rates (of common gobies) and found no significant interaction between the stressors (Ferreira et al., 2016; Fonte et al., 2016). Despite the increasing importance of both stressors, there is lack of research about the interactive effects of warming and microplastics on metabolic rates (but see Wen et al., 2018). This uncertainty about the potential for environmental temperature to modify the impact of microplastics requires urgent attention if we are to fully understand the current and future risks of microplastic pollution and successfully manage freshwater ecosystems.

To address this critical gap in microplastic research, we experimentally tested the independent and combined impacts of microplastics and warming on the energy demand (metabolism) and energy intake (feeding) of an important and widely distributed freshwater detritivore – the amphipod, *Gammarus pulex*. We hypothesised that there would be: (1) an increase in metabolic and feeding rates with increasing temperature; (2) a reduction in metabolic and feeding rates with increasing microplastic concentration; and (3) weaker effects of microplastics on metabolic and feeding rates at higher temperatures.

2. MATERIALS AND METHODS

2.1. Model species collection and maintenance

Gammarus pulex is a ubiquitous benthic shredder in European running waters that breaks down coarse particulate organic matter, channelling the associated energy to predators such

as fish. By converting terrestrial litter inputs into the fine particulate and dissolved organic matter, these shredders also convey these resources to other invertebrates, especially in upland streams (Wallace and Webster 1996). The species is commonly used as a model organism for assessing the effects of pollutants under laboratory conditions (Miller et al., 2016; Henry et al., 2017; Weber et al., 2018). We collected approximately 400 *G. pulex* by kick sampling the River Cray (Bexley, South-East London, UK, 51°25'59.0" N 0°08'16.4" E) in summer 2017. These amphipods were stored in a temperature-controlled room (15 ˚C, 12h:12h light:dark photoperiod) in two aerated glass aquaria (45 x 25 x 30 cm), each containing 5 L of river water. They were visually inspected, removing any individuals that were smaller than 1cm, bearing eggs, or infected with Acanthocephalan parasites, which can alter amphipod behaviour (Tain et al., 2006; Labaude et al., 2015). The remaining individuals were rinsed with synthetic freshwater (SFW) and transferred to a new glass aquarium with 5 L of aerated SFW in the same temperature- and light-controlled room for acclimatisation, for a minimum of 7 days prior to any experimentation. The SFW used for stock maintenance and experimentation was prepared according to the US Environmental Protection Agency (Weber, 1991), from 1.92 g of $NaHCO_3$, 1.2 g $MgSO_4$, 1.2 g $CaSO_4$, and 0.08 g of KCl dissolved in 20 L of deionised water. The same protocol was used to house *G. pulex* in several other toxicological studies (Miller et al., 2016; Henry et al., 2017). During this maintenance phase, *G. pulex* were fed *ad libitum* with alder-leaves (*Alnus glutinosa*) and coarse pebbles were provided for shelter.

Prior to all experiments, *G. pulex* were moved in groups of six into smaller glass microcosms with 200 mL of aerated SFW, for one-week acclimation. They were fed *ad libitum* with alder leaf disks. These amphipods were transferred from the 15 °C room to temperature-controlled incubators (Stuart SI500, Orbital), where the temperature was changed gradually (+ or – 1 °C h^{-1}) until the three targeted experimental temperatures were

reached (9, 15, and 19 °C). This range of temperatures is commonly experienced by amphipods in UK rivers over their annual life cycle, while maximum temperatures are expected to increase in magnitude and frequency under future climate change scenarios (Hannah and Garner, 2015). Wild populations of G. pulex are known to adapt to changes of 6 °C per day (Maazouzi et al., 2011), making the gradual change in temperature within the tolerance limits of the species. Amphipods remained at the experimental temperature for 1.5 days before being starved for 24 hours to ensure a standardised satiation level among all individuals. During this time, SFW was changed daily to ensure dissolved oxygen levels were sufficient and did not exert any additional stress on the amphipods.

2.2. Microplastics exposure

For microplastics exposure, we used commercially produced polymethyl methacrylate (PMMA) spheres with a diameter of 40.2 μm (Spherotech: FPMA-40056-5, lot number 501), which is within the size range of plastic that can be ingested and egested by *G. pulex* (Imhof et al., 2013). These transparent PMMA spheres have a density (1.19 g cm^{-3}) greater than that of water, allowing them to sink through the water column to the substratum where they become biologically available for amphipods feeding on leaf litter. PMMA is a common microplastic used in personal care and cosmetic products along with polyethylene, nylon, polypropylene, and polyethylene terephthalate. Other uses include facial fillers, patio roofs, conservatories, light guide panels for LCD display screens, lenses for mobile phones, touch screens, street lighting, and many uses within the automobile industry (Plastics Europe, 2018). We used glass material for handling, storage, and exposure experiments to minimise contamination and loss of particles due to adhesion onto plastic materials.

We searched empirical literature reporting sediment microplastic concentrations in freshwater ecosystems to identify realistic concentrations for use in our experiments. We

found that natural concentrations ranged between 0 and 51.70 microplastic particles cm^{-2} (Zbyszewski et al., 2014; Hurley et al., 2018). Our experimental design included this range and also double the maximum natural concentration reported in the literature, to simulate both present and potential future effects (de Sá et al., 2018). For all exposure experiments, experimental glass microcosms were filled with 200 mL of aerated SFW, then one leaf disk of known weight was placed at the bottom of each microcosm. Because we carefully measured the experimental concentrations of PMMA beads and introduced them into the glass microcosms, these represent accurate microplastic concentrations in the experimental environment (i.e. media). After the introduction, we briefly stirred the solution and the microcosms were left to rest for one hour to allow all PMMA spheres to sink. This resulted in a relatively equal distribution of microplastics across the bottom of each microcosm, simulating different intensities of microplastic pollution. A single starved amphipod was introduced into each of the microcosms to initiate the experiment. Finally, lids were placed on all microcosms to prevent water loss and contamination.

2.3. Quantifying metabolic rates

We measured respiration rates as a proxy for metabolic rate, following a similar protocol to Broderson et al. (2008). Oxygen consumption rates of amphipods were measured following 24 hours of exposure to experimental microplastics concentrations (0.52, 26.12, and 104.48 cm^{-2}) plus a control (0 cm^{-2}) at each of three experimental temperatures (9, 15, and 19 °C). For each treatment combination, we measured respiration rates of 3-5 individuals, for a total of 43 measurements. Individual amphipods were transferred to SFW-filled 2 mL glass chambers fitted with a magnetic stirrer to prevent stratification, which was separated from the organism by a mesh screen. Oxygen concentration was measured every second during three periods of 10-15 seconds each using an oxygen microelectrode (MicroResp, Unisense,

Denmark) fitted through a capillary in the gas-tight stopper of each chamber. An animal-free chamber containing only SFW, a magnetic stirrer, and a mesh screen was used to measure the background oxygen consumption or production by microbes or autotrophs present in the experimental water. Metabolic rates (µmol O_2 h^{-1}) were calculated from the least squares linear regression fitted through all data points measured in each chamber, corrected for background rates in the animal-free chamber and slight differences in chamber volumes. After each experiment, amphipods were preserved in 1 mL of 70% ethanol and their body length was measured from the rostrum to the base of the telson. Length was converted into dry body weight using an established length-weight relationship for *G. pulex* from Gee (1988): $y = 0.0058x^{3.015}$, where y is body mass in mg and x is body length in mm.

2.4. Quantifying feeding rates

For the feeding rate experiments, we exposed amphipods to ten concentrations of microplastics (0.05, 0.26, 0.52, 2.61, 5.22, 15.67, 26.12, 36.57, 52.24, 104.48 cm^{-2}) plus a control (0 cm^{-2}) at each of three experimental temperatures (9, 15, and 19 °C). There were 3-7 replicates of each treatment combination, each containing one individual amphipod. Note that feeding trials, where amphipods shed their skin or died, were not included in the analysis. Amphipods were offered a leaf disk as a food source. To standardize leaf biomass across all experimental treatment combinations, whole alder leaves were soaked in SFW for 10 minutes before 15mm leaf disks were cut out, using a cork borer, avoiding the main vein. Leaf disks were rinsed of any residual silt or substrate, wrapped individually in foil and dried at 60 ˚C for 24 hours before being weighed on an ultra-micro balance to the nearest 0.01 mg (UMX2, Switzerland). Leaf disks were then re-soaked for two days prior to experimental exposures, to prevent them floating to the surface during experiments and ensuring their availability to the amphipods. We also established seven animal-free microcosms at each temperature,

containing only a leaf disk of a known weight and 200 mL of SFW, to account for microbial decomposition. After 24 hours of experimental exposure, amphipods were preserved in 1 mL of 70% ethanol and their body mass was estimated, as for the respiration experiments. All leaf disks were collected, thoroughly rinsed to remove any microplastics or faeces, wrapped individually in foil, dried at 60 ˚C for 23 hours, and then weighed on an ultra-micro balance to the nearest 0.01mg (UMX2, Switzerland). Feeding rate was defined as the amount of ingested leaf mass per day (*i.e.* the initial minus final dry weight of the leaf disks), corrected for microbial decomposition (*i.e.* subtracting the mean loss of leaf dry weight in the animal-free microcosms at the corresponding temperature).

2.5. Statistical analyses

Our response variables (R), metabolic rate (μmol O_2 h^{-1}) and feeding rate (mg day^{-1}), depend on both temperature and body mass according to the Metabolic Theory of Ecology (Brown et al., 2004) and a meta-analysis of feeding experiments (Rall *et al*., 2012) as follows:

$$R = R_0 M^{b_R} e^{E_R \frac{T_R - T_0}{k T_R T_0}} \quad (1)$$

Here, R_0 is the metabolic or feeding rate at T_0, M is dry body mass (mg), b_R is an allometric exponent, E_R is the activation energy of the biochemical reactions underpinning R (eV), k is the Boltzmann constant (8.618×10^{-5} eV K^{-1}), T_R is the absolute experimental temperature (K), and T_0 is 287.15 K (*i.e.* 14 °C, the midpoint of the range of temperatures used in the experiments). We performed a multiple linear regression on the natural logarithm of Equation 1, exploring the main effects of temperature and body mass on metabolic or feeding rate. We then mass-corrected the response variables by dividing metabolic or feeding rate by M^{bR}.

To determine the effect of microplastics on our mass-corrected response variables (R_M), we first calculated the change in metabolic or feeding rate (ΔR_M) relative to the microplastic-free control treatment. We subtracted the mean mass-corrected metabolic or

feeding rate in the control at each temperature from the individual replicate measurements containing microplastics at the corresponding temperature. A positive value of ΔR_M indicates an increase, while a negative value of ΔR_M indicates a decrease in metabolic or feeding rate. We performed a multiple linear regression exploring the main and interactive effects of temperature and microplastic concentration on ΔR_M. Here, a significant intercept or main effect of microplastic concentration would mean that microplastics changed the response variable, irrespective or depending on the concentration, respectively. A significant main effect of temperature or interactive effect of microplastic concentration and temperature would mean that temperature altered the effect of microplastics on the response variable, irrespective or depending on the concentration, respectively. All statistical analyses were carried out in R 3.5.1.

3. RESULTS

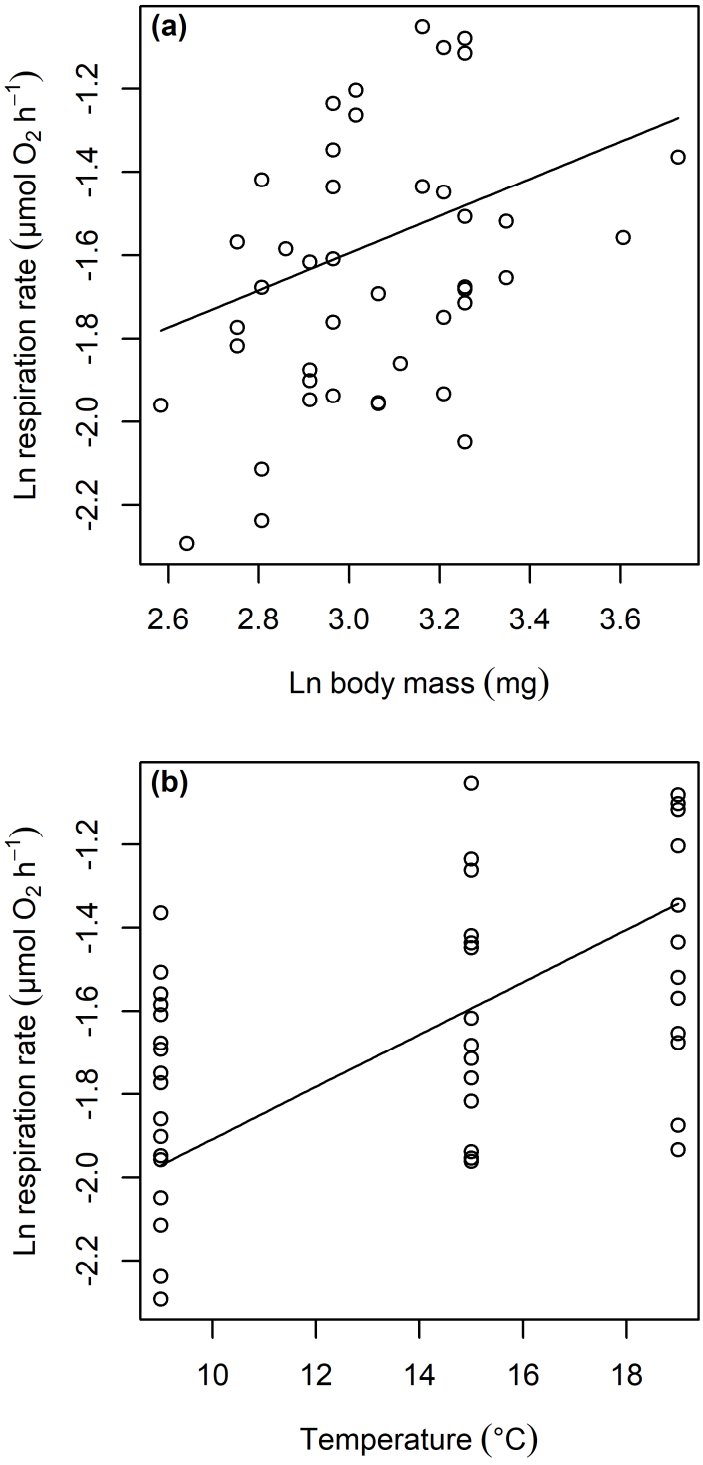
3.1. Metabolic rates

There was a significant log-linear increase in respiration rate with both body mass and temperature ($F_{2,40}$ = 10.64; p = 0.001; r^2 = 0.31; Table 1), supporting our first hypothesis. The respiration rate of *G. pulex* increased with body mass with an allometric exponent of 0.45 ± 0.33 (mean ± 95% CI; Figure 1a) and with temperature with an activation energy of 0.23 ± 0.13 eV (mean ± 95% CI; Figure 1b). There was a significant main effect of microplastic concentration on respiration rate (Table 2), with a reduction in respiration rate relative to the control as microplastic concentration increased (Figure 1), supporting our second hypothesis. There was also an interactive effect of temperature and microplastic concentration on the change in respiration rate relative to the microplastic-free controls ($F_{3,29}$ = 5.73; p = 0.003; r^2 = 0.31; Table 2). Here, there was an increase in respiration rate relative to the controls at the coolest temperature, but a decrease in respiration rate relative to the controls at both 15 and

19 °C as microplastic concentration increased (Figure 2). In contrast to our third hypothesis, this suggests that higher temperatures strengthened the negative effect of microplastics on respiration rates.

Table 1. Parameter estimates with associated standard errors (SE), *t*-values, and *p*-values for the ln-linear models describing the main effects of body mass and temperature on metabolic and feeding rates of amphipods. Parameters correspond to those listed in Equation 1, where R_0 is ln-metabolic rate or ln-feeding rate at T_0, b_R is the allometric exponent, and E_R is the activation energy.

Response variable	Parameter	Estimate	SE	*t*-value	*p*-value
Metabolic rate	R_0	-2.998	0.5141	-5.831	<0.001
	b_R	0.4466	0.1677	2.663	0.011
	E_R	0.2333	0.0682	3.423	0.001
Feeding rate	R_0	-2.800	0.9966	-2.809	0.006
	b_R	0.7159	0.3493	2.049	0.043
	E_R	0.5674	0.1240	4.578	<0.001



285

286 **Figure 1.** Body mass and temperature dependence of amphipod metabolic rates. Ln
287 respiration rate increased significantly with both (a) body mass and (b) temperature (see
288 Table 1). Note that the lines of best fit for the explanatory variables in panels (a) and (b) are
289 shown after setting the other explanatory variable to its median value.

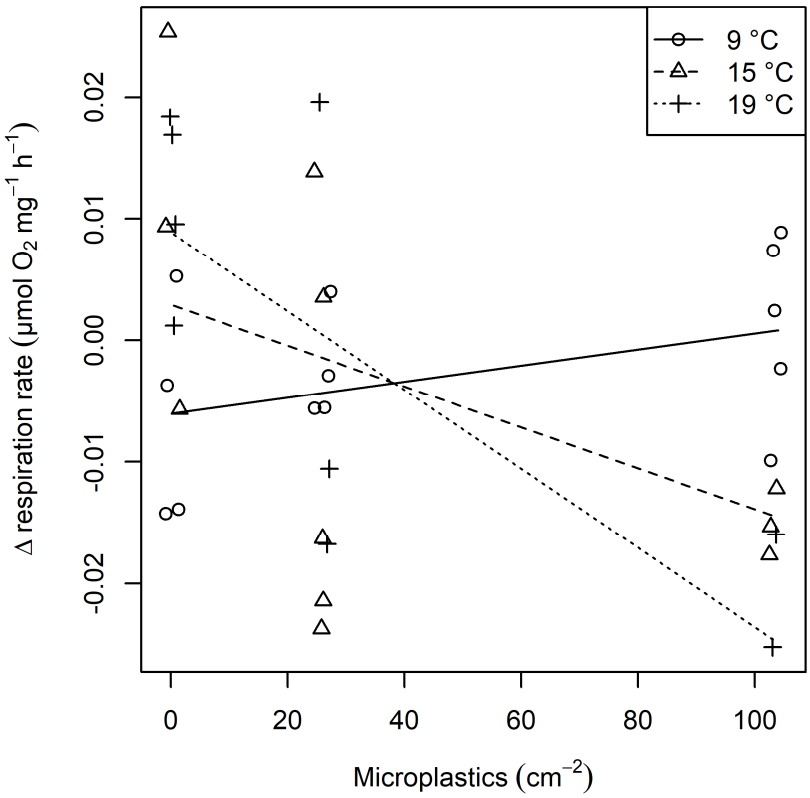


Figure 2. The interactive effect of experimental temperature and microplastic concentrations on the change in amphipod metabolic rates relative to microplastic-free control (see Table 2). The lines of best fit show the effect of microplastic concentration on the response variable at each of the three temperatures.

3.2. Feeding rates

There was a significant log-linear increase in feeding rate with both body mass and temperature ($F_{2,120}$ = 11.89; p < 0.001; r^2 = 0.15; Table 1), supporting our first hypothesis. The feeding rate of *G. pulex* on leaf litter increased with body mass with an allometric exponent of 0.72 ± 0.70 (mean ± 95% CI; Figure 3a) and with temperature with an activation energy of 0.57 ± 0.25 eV (mean ± 95% CI; Figure 3b). There was no significant main effect of microplastic concentration, or interactive effect with temperature, on the change in feeding rate relative to the microplastic-free controls ($F_{3,107}$ = 0.756; p = 0.521; Table 2; Figure 4), in

304 contrast to our second and third hypotheses. Note that there were still no significant effects of
305 temperature or microplastic concentration on the change in feeding rate relative to the
306 microplastic-free controls after analysing only the subset of microplastic concentrations
307 corresponding to the respiration experiments ($F_{3,35}$ = 0.117; p = 0.949; Table 2).

308

309 **Table 2.** Parameter estimates with associated standard errors (SE), *t*-values, and *p*-values for
310 the linear models describing the main and interactive effects of temperature (temp) and
311 microplastic concentration (MPC) on mass-corrected metabolic and feeding rates of
312 amphipods. Note that parameters and summary statistics are also shown for a subset of the
313 feeding rate data with MPCs corresponding to those used in the respiration rate experiment.

314

Response variable	Parameter	Estimate	SE	*t*-value	*p*-value
Metabolic rate	intercept	-1.95×10^{-2}	9.65×10^{-3}	-2.017	0.053
	temp	1.49×10^{-3}	6.55×10^{-4}	2.280	0.030
	MPC	4.17×10^{-4}	1.55×10^{-4}	2.700	0.011
	temp:MPC	-3.90×10^{-5}	1.11×10^{-5}	-3.516	0.001
Feeding rate	Intercept	-1.86×10^{-2}	2.91×10^{-2}	-0.638	0.525
	temp	2.20×10^{-3}	1.86×10^{-3}	1.181	0.240
	MPC	-6.61×10^{-7}	6.82×10^{-4}	-0.001	0.999
	temp:MPC	-7.09×10^{-6}	4.26×10^{-5}	-0.166	0.868
Feeding rate (subset)	intercept	-1.15×10^{-2}	5.02×10^{-2}	-0.230	0.819
	temp	8.87×10^{-4}	3.14×10^{-3}	0.283	0.779
	MPC	-8.82×10^{-5}	7.99×10^{-4}	-0.110	0.913
	temp:MPC	-7.43×10^{-6}	4.96×10^{-5}	0.150	0.882

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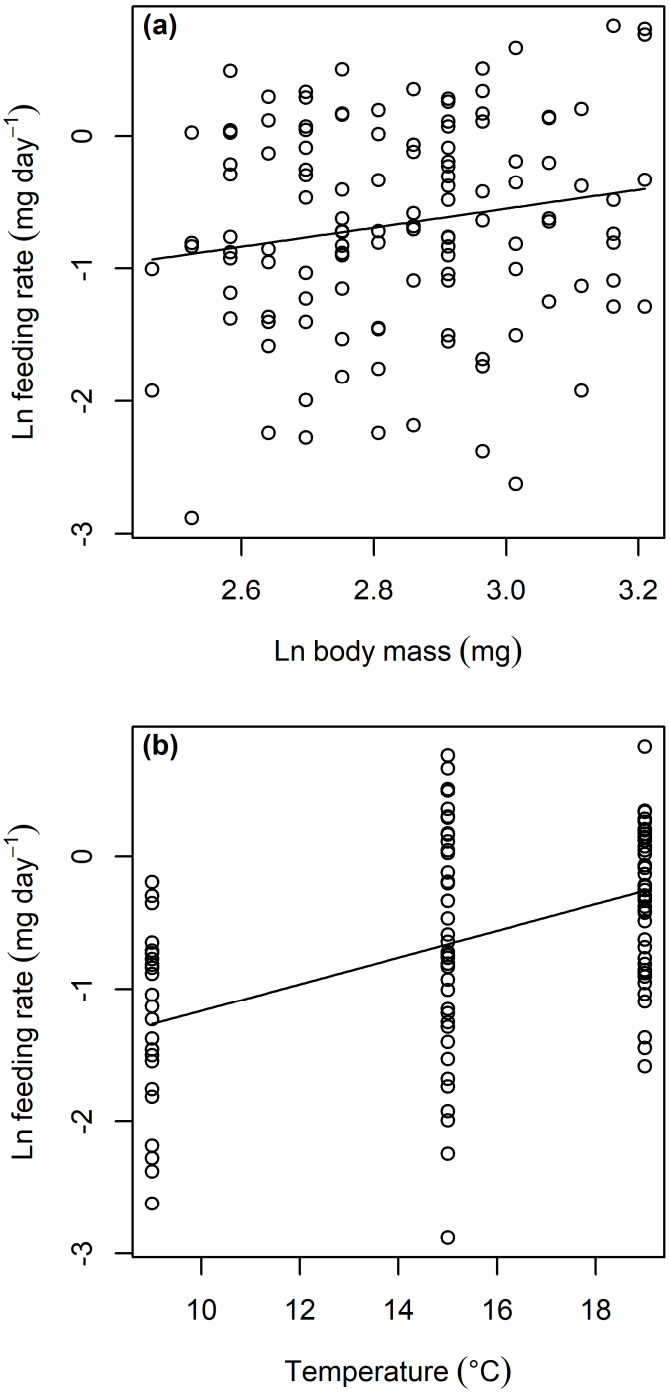

317

318 **Figure 3.** Body mass and temperature dependence of amphipod feeding rates. Ln feeding rate
319 increased significantly with both (a) body mass and (b) temperature (see Table 1). Note that
320 the lines of best fit for the explanatory variables in panels (a) and (b) are shown after setting
321 the other explanatory variable to its median value.

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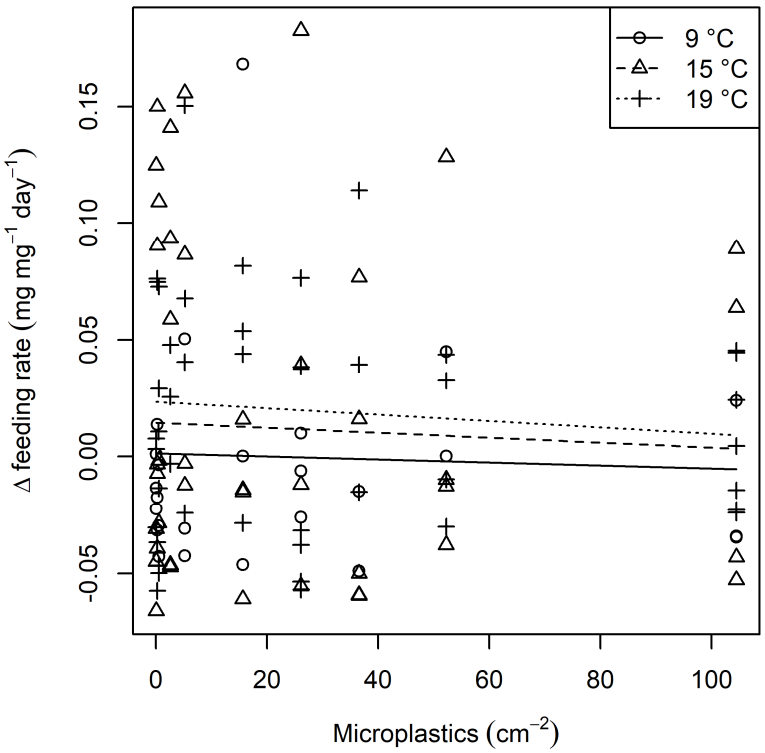

Figure 4. Experimental warming and microplastic concentrations had no effect on the change in amphipod feeding rates relative to microplastic-free control (see Table 2). The lines of best fit show the effect of microplastic concentration on the response variable at each of the three temperatures.

4. DISCUSSION

This study demonstrates how environmental temperature can alter the impact of microplastics on the metabolism, though not feeding rate, of aquatic organisms. Both metabolic and feeding rates of *G. pulex* increased with temperature and body mass, as predicted by the Metabolic Theory of Ecology (Gillooly et al., 2001; Brown et al., 2004) and shown in a meta-analysis of functional response experiments (Rall et al., 2012). The activation energy of metabolic rate was much weaker than expected, with an upper 95% CI (0.36 eV) that did not fall within the expected range of 0.6–0.7 eV (based on the average of observed metabolic rates; Brown et

al., 2004). This may have been driven by metabolic rate levelling off at the highest temperature, with deviations from the Boltzmann-Arrhenius model accounting for a large amount of variability in the thermal sensitivity of biological rates (Pawar et al., 2016). This suggests that this population of *G. pulex* was approaching its thermal optimum for metabolic rate at 19 °C, with further warming likely to induce a decline in metabolic performance (Pawar et al., 2016).

There was a net negative effect of microplastics on metabolic rate, though not feeding rate of *G. pulex*, offering only partial support for our second hypothesis. Suppression of metabolic rates through exposure to microplastics has been described in other aquatic organisms (Rist et al., 2016; Wen et al., 2018), highlighting the potential for these tiny pollutants to impede physiological performance. Lower metabolism is likely to result in reduced activity and thus a diminished rate of resource acquisition (Cloyed et al., 2019; Brown et al., 2004). It is interesting then that the lower metabolic rates of *G. pulex* did not translate into reduced feeding rates on their preferred leaf litter resources at higher microplastic concentrations in the water. Lowered metabolic rates in response to thermal acclimation also did not immediately lead to reduced feeding rates, suggesting either a delayed response in the latter, or that feeding rate may be more directly influenced by the rate of gastric digestion than oxygen consumption (Wallace 1973). There was also no change in the feeding rate of *G. pulex*, or its congeneric *G. fossarum*, after exposure to microplastics, despite the use of much higher concentrations than in this study (Blarer and Burkhardt-Holm 2016; Weber et al., 2018). While Straub et al., (2017) found an initial depression of feeding rates of *G. fossarum* after one-week exposure to polyhydroxybutyrate and PMMA (333 particles mL^{-1}), this effect disappeared by the second week of their experiment. This evidence generally points to weak short-term effects of microplastics (i.e. <1 week exposure)

on leaf litter breakdown rates in gammarid amphipods, whereas the impacts of sustained microplastic exposure (i.e. weeks to months) remain a promising avenue for further research.

Interestingly, the effect of microplastics on the metabolic rate of our model freshwater detritivore was contingent on environmental temperature. In contrast to our expectations, the reduction in metabolic rate with increasing microplastic concentration only occurred at the highest temperatures in our experiment, with a positive effect of microplastic concentration on metabolic rate at the coolest temperature. Increased metabolic rates in response to high concentrations of microplastics have also been described for the lugworm, *Arenicola marina* (Green et al., 2016) and European flat oyster, *Ostrea edulis* (Green 2016). Note that an increased metabolic rate does not necessarily equate to increased performance and may reflect more rapid breathing due to impaired respiratory function (Hebel et al., 1997). Nevertheless, the mean effect of microplastics on metabolic rate at the coolest temperature was zero, i.e. there was very little change relative to the microplastic-free control (Figure 2). Thus, the negative effects of microplastic concentration on metabolic rate were only manifested at the higher temperatures, highlighting the potential for climate change or even seasonal fluctuations in environmental temperature to alter microplastic effects on organismal physiology. Warming has been shown to increase the accumulation of microplastics in fish, affecting metabolic enzyme activity, which hints at a potential mechanism underpinning the changes observed here (Wen et al., 2018). A more detailed mechanistic understanding of the physiological processes underpinning altered metabolic rates in response to multiple environmental stressors is now required (Jackson et al., 2016).

To date, the only other research testing the impacts of microplastics in the context of environmental warming focused on juvenile marine fish – the common goby, *Pomatoschistus microps*. This work showed that experimental warming (from 20 to 25 °C) did not alter the effects of microplastics on feeding rates or fish health (Ferreira et al., 2016; Fonte et al.,

2016). In the current study, there were also no interactive effects of microplastics and warming on feeding rates despite the ample evidence that climate warming readily interacts with other environmental stressors (Kratina et al., 2012; Piggott et al., 2015; Jackson et al., 2016). It is possible that the feeding behaviour of freshwater amphipods is robust to microplastic pollution. Alternatively, the lack of feeding responses could be due to high variation in individual feeding rates (Scherer et al., 2017) or the short-term duration of experiments, allowing insufficient time for the effects to manifest.

The diameter (40.2 μm) of the PMMA particles used for both the feeding and metabolism experiments was in line with the typical size of microplastics (10-90 μm) that *G. pulex* tend to ingest (Scherer et al., 2017). Larger microplastic particles are likely to be encountered more often by benthic detritivores, due to their heavier weight and rapid sinking rates. Although we were not able to quantify ingested PMMA particles in the guts of *G. pulex*, our preliminary exposures indicate that these particles are being ingested. It is likely that the physical presence of non-nutritious microplastic particles in place of food, can lead to longer gut passage times (Wright et al., 2013) and adverse biological impacts (Galloway et al., 2017). A reduction in metabolism due to a combination of warming and high concentration of microplastics could further reduce the amount of energy assimilated for individual and population growth rates. Two recent studies have shown that energy assimilation decreased in *G. fossarum*, when exposed to microplastics (Blarer and Burkhardt-Holm, 2016; Straub et al., 2017). The changes in respiration rates seen here could help to explain such findings.

The range of microplastic concentrations used in this study covers environmentally relevant concentrations and double the highest concentration that has currently been reported in aquatic sediments. With microplastic concentrations in aquatic ecosystems likely to increase over time, simulating a range of microplastic exposures in experiments enhances our

understanding of both present and potential future effects (de Sá et al., 2018). Our results indicate that negative physiological responses of freshwater shredders to microplastics may become common in the future warmer world, but changes to leaf litter decomposition by amphipods are likely to be weak. These findings are vital for assessing the risk of microplastic damage in freshwater ecosystems, but effects observed at higher concentrations should be interpreted with caution. Future work should seek to replicate the environmentally relevant microplastic exposures used in this study, and further investigate the consequences of changes in respiration rates on populations, trophic interactions, and the structure and dynamics of aquatic ecosystems. Such improved mechanistic understanding of microplastic pollution is essential if we are to mitigate the risk and successfully manage freshwater ecosystems under climate warming.

Declarations of interest

None.

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Data accessibility

Data will be archived in the public archive Dryad (*http://datadryad.org*).

435 **REFERENCES**

436 Blarer, P., Burkhardt-Holm, P., 2016. Microplastics affect assimilation efficiency in the
437 freshwater amphipod *Gammarus fossarum*. *Environ. Sci. Pollut. R.* 23(23), 23522–
438 23532. doi: 10.1007/s11356-016-7584-2

439 Brodersen, K.P., Pedersen, O., Walker, I.R., Jensen, M.T., 2008. Respiration of midges
440 (Diptera; Chironomidae) in British Columbian lakes: oxy-regulation, temperature and
441 their role as palaeo-indicators. *Freshwater Biol.*, 53, 593–602. doi: 10.1111/j.1365-
442 2427.2007.01922.x

443 Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M., West, G.B., 2004. Toward a metabolic
444 theory of ecology. *Ecology*, 85, 1771–1789. doi: 10.1890/03-9000

445 Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway T., et al., 2011.
446 Accumulation of microplastic on shorelines worldwide: Sources and sinks. *Environ. Sci.*
447 *Technol.*, 45, 9175–9179. doi: 10.1021/es201811s

448 Cloyed, C.S., Dell, A.I., Hayes, T., Kordas, R.L., O'Gorman, E.J., 2019. Long□term
449 exposure to higher temperature increases the thermal sensitivity of grazer metabolism
450 and movement. *J. Anim. Ecol*., doi: 10.1111/1365-2656.12976

451 Cole, M.,Galloway, T., 2015. Ingestion of nanoplastics and microplastics by Pacific oyster
452 larvae. *Environ. Sci. Technol.*, 49, 14625–14632. doi: 10.1021/acs.est.5b04099

453 Cole, M., Lindeque, P., Fileman, E., Halsband, C.,Galloway, T.S., 2015. The impact of
454 polystyrene microplastics on feeding, function and fecundity in the marine copepod
455 *Calanus helgolandicus*. *Environ. Sci. Technol.*, 49(2), 1130-1137. Doi:
456 10.1021/es504525u

457 Crain, C.M., Kroeker, K., Halpern, B.S., 2008. Interactive and cumulative effects of multiple
458 human stressors in marine systems. *Ecol. Lett.*, 11(12), 1304-1315.
459 doi: 10.1111/j.1461-0248.2008.01253.x

de Sá, L. C., Oliveira, M., Ribeiro, F., Rocha, T.L., Futter, M.N., 2018. Studies of the effects of microplastics on aquatic organisms: What do we know and where should we focus our efforts in the future? *Sci. Total Environ.*, 645, 1029–1039. doi: 10.1016/j.scitotenv.2018.07.207

Dris, R., Gasperi, J., Rocher, V., Saad, M., Renault, N., Tassin, B., 2015. Microplastic contamination in an urban area: A case study in greater Paris. *Environ. Chem.*, 12:592–599. doi: 10.1071/EN14167

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.I., Knowler, D.J., Lévêque, C., Naiman, R.J., Prieur-Richard, A.H., Soto, D., Stiassny, M.L., Sullivan, C.A., 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. *Biol. Rev.*, 81(2), 163–182. doi: 10.1017/S1464793105006950

Fendall, L.S., Sewell, M.A., 2009. Contributing to marine pollution by washing your face: microplastics in facial cleansers. *Mar. Pollut. Bull.*, 58 (8), 1225e1228. doi: 10.1016/j.marpolbul.2009.04.025

Ferreira, P., Fonte, E., Soares, M. E., Carvalho, F., Guilhermino, L., 2016. Effects of multi-stressors on juveniles of the marine fish *Pomatoschistus microps*: Gold nanoparticles, microplastics and temperature. *Aquat. Toxicol.*, 170, 89–103. doi:10.1016/j.aquatox.2015.11.011

Fonte, E., Ferreira, P., Guilhermino, L., 2016. Temperature rise and microplastics interact with the toxicity of the antibiotic cefalexin to juveniles of the common goby (*Pomatoschistus microps*): Post-exposure predatory behaviour, acetylcholinesterase activity and lipid peroxidation. *Aquat. Toxicol.*, 180, 173–185. doi: 10.1016/j.aquatox.2016.09.015

Frölicher, T.L., Fischer, E.M., Gruber, N., 2018. Marine heatwaves under global warming. *Nature*, 560, 360–364. doi: 10.1038/s41586-018-0383-9

Galloway, T.S., Cole, M., Lewis, C., 2017. Interactions of microplastic debris throughout the marine ecosystem. *Nature Ecol. Evol.,* 1(5), 0116. doi: 10.1038/s41559-017-0116

Gee, J.H., 1988. Population dynamics and morphometries of *Gammarus pulex* L.: evidence of seasonal food limitation in a freshwater detritivore. *Freshwater Biol.*, 19(3), 333–343. doi: 10.1111/j.1365-2427.1988.tb00355.x

Gillooly, J.F., Brown, J.H., West, G.B., Savage, V.M., Charnov, E.L., 2001. Effects of size and temperature on metabolic rate. *Science*, 293(5538), 2248–2251. doi: 10.1126/science.1061967

Green, D.S., 2016. Effects of microplastics on European flat oysters, *Ostrea edulis* and their associated benthic communities. *Environ. Pollut.*, 2016, 95–103. doi: 10.1016/j.envpol.2016.05.043

Green, D.S., Boots, B., Sigwart, J., Jiang, S., Rocha, C., 2016. Effects of conventional and biodegradable microplastics on a marine ecosystem engineer (*Arenicola marina*) and sediment nutrient cycling. *Environ. Pollut.*, *208*, 426–434. doi: 10.1016/j.envpol.2015.10.010

Hall, R., 2015. Population and the future. *Geography*, 100(1), 36–44.

Hämer, J., Gutow, L., Köhler, A., Saborowski, R., 2014. Fate of microplastics in the marine isopod *Idotea emarginata*. *Environ. Sci. Technol.*, 48, 13451–13458. doi: 10.1021/es501385y

Hannah, D.M., Garner, G., 2015. River water temperature in the United Kingdom: Changes over the 20th century and possible changes over the 21st century. *Prog. Phys. Geog.*, 39(1), 68–92. https://doi.org/10.1177/0309133314550669

Hebel, D.K., Jones, M.B., Depledge, M.H., 1997. Responses of crustaceans to contaminant exposure: a holistic approach. *Estuar. Coast. Shelf S., 44*, 177–184. doi: 10.1006/ecss.1996.0209

Henry, Y., Piscart, C., Charles, S., Colinet, H., 2017. Combined effect of temperature and ammonia on molecular response and survival of the freshwater crustacean *Gammarus pulex*. *Ecotox. Environ. Safe.*, 137, 42–48. doi: 10.1016/j.ecoenv.2016.11.011

Horton, A.A., Walton, A., Spurgeon, D.J., Lahive, E., Svendsen, C., 2017. Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. *Sci. Total Environ.*, 589, 127–141. doi: 10.1016/j.scitotenv.2017.01.190

Hurley, R., Woodward, J., Rothwell, J.J., 2018. Microplastic contamination of river beds significantly reduced by catchment-wide flooding. *Nat. Geosci.*, 11, 251–258. doi: 10.1038/s41561-018-0080-1

Imhof, H.K., Ivleva, N.P., Schmid, J., Niessner, R., Laforsch, C., 2013. Contamination of beach sediments of a subalpine lake with microplastic particles. *Curr. Biol.*, 23(19), R867–R868. doi: 10.1016/j.cub.2013.09.001

IPCC, 2014. Synthesis report. Contribution of working groups I, II and III to the fifth assessment report of the intergovernmental panel on climate change. Core Writing Team, R.K. Pachauri & L.A. Meyer, eds. Geneva, Switzerland.

Jackson, M.C., Loewen, C.J.G., Vinebrooke, R.D., Chimimba, C.T., 2016. Net effects of multiple stressors in freshwater ecosystems: a meta□analysis. *Glob. Change Biol.*, 22, 180–189. doi: 10.1111/gcb.13028

Kratina, P., Greig, H.S., Thompson, P.L., Carvalho-Pereira, T.S.A., Shurin, J.B., 2012. Warming modifies trophic cascades and eutrophication in experimental freshwater communities. *Ecology*, 93, 1421–1430. doi: 10.2307/23213771

Labaude, S., Cézilly, F., Tercier, X., Rigaud, T., 2015. Influence of host nutritional condition on post-infection traits in the association between the manipulative acanthocephalan

Pomphorhynchus laevis and the amphipod *Gammarus pulex*. *Parasite. Vector.*, 8:403. Dio: 10.1186/s13071-015-1017-9

Lenz, R., Enders, K., Nielsen, T.G., 2016. Microplastic exposure studies should be environmentally realistic. *P. Natl. Acad. Sci. USA*, *113*(29), 943–952. doi: 10.1073/pnas.1606615113

Maazouzi, C., Piscart, C., Legier, F., Hervant, F., 2011. Ecophysiological responses to temperature of the "killer shrimp" *Dikerogammarus villosus*: is the invader really stronger than the native *Gammarus pulex*? Comparative Biochemistry and Physiology Part A: *Ann Ny. Acad. Sci.*, 159(3), 268–274. doi: 10.1016/j.cbpa.2011.03.019

MEA, 2005. Ecosystems and human well-being: wetlands and water. World resources institute, Washington, DC, 5.

Miller, T.H., McEneff, G.L., Stott, L.C., Owen, S.F., Bury, N.R., Barron, L.P., 2016. Assessing the reliability of uptake and elimination kinetics modelling approaches for estimating bioconcentration factors in the freshwater invertebrate, *Gammarus pulex*. *Sci. Total. Environ.*, 547, 396–404. doi: 10.1016/j.scitotenv.2015.12.145

Ogonowski, M., Schür, C., Jarsén, Å., Gorokhova, E., 2016. The effects of natural and anthropogenic microparticles on individual fitness in *Daphnia magna*. *PloS one*, 11(5). Doi: 10.1371/journal.pone.0155063

Ohlberger, J., 2013. Climate warming and ectotherm body size from individual physiology to community ecology. *Funct. Ecol.*, 27, 991–1001. doi: 10.1111/1365-2435.12098

Ormerod, S.J., Dobson, M., Hildrew, A.G., Townsend, C.R., 2010. Multiple stressors in freshwater ecosystems. *Freshwater Biol.*, 55(s1), 1–4. doi: 10.1111/j.1365-2427.2009.02395.x

Pawar, S., Dell, A.I., Savage, V.M., Knies, J.L., 2016. Real versus artificial variation in the thermal sensitivity of biological traits. *Am. Nat.*, 187, E41–E52. doi: 10.1086/684590

559 Piggott, J.J., Townsend, C.R., Matthaei, C.D., 2015. Climate warming and agricultural
560 stressors interact to determine stream macroinvertebrate community dynamics. *Glob.*
561 *Change Biol.*, 21, 1887–1906. doi: 10.1111/gcb.12861
562 Plastics Europe. Polymethyl methacrylate (PMMA), 2017. Available at:
563 http://www.plasticseurope.org/what-is-plastic/types-of-plastics-11148/engineering-
564 plastics/pmma.aspx (Accessed: 15th May 2017).
565 Plastics Europe, 2018. Plastics – the Facts 2017. An analysis of European plastics production,
566 demand and waste data. Plastics Europe, Brussels - Belgium.
567 R Development Core Team, 2018. R: A language and environment for statistical computing.
568 R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-
569 project.org/.
570 Rall, B.C., Brose, U., Hartvig, M., Kalinkat, G., Schwarzmüller, F., Vucic-Pestic, O.,
571 Petchey, O.L., 2012. Universal temperature and body-mass scaling of feeding rates.
572 *Philos. T. R. Soc. B.*, 367, 2923–2934. doi: 10.1098/rstb.2012.0242
573 Rist, S.E., Assidqi, K., Zamani, N.P., Appel, D., Perschke, M., Huhn, M., Lenz, M., 2016.
574 Suspended micro-sized PVC particles impair the performance and decrease survival in
575 the Asian green mussel *Perna viridis*. *Mar. Pollut. Bull.*, *111*(1), 213–220. doi:
576 10.1016/j.marpolbul.2016.07.006
577 Scherer, C., Brennholt, N., Reifferscheid, G., Wagner, M., 2017. Feeding type and
578 development drive the ingestion of microplastics by freshwater invertebrates. *Sci. Rep-*
579 *UK*, 7, 17006. doi: 10.1038/s41598-017-17191-7
580 Straub, S., Hirsch, P.E., Burkhardt-Holm, P., 2017. Biodegradable and petroleum-based
581 microplastics do not differ in their ingestion and excretion but in their biological effects
582 in a freshwater invertebrate *Gammarus fossarum*. *Int. J. Env. Res. Pub. He.*, 14(7), 774.
583 doi: 10.3390/ijerph14070774

584 Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M. E.J., Corporeau,
585 C., 2016. Oyster reproduction is affected by exposure to polystyrene microplastics. *P.*
586 *Natl. Acad. Sci. USA*, 113(9), 2430–2435. doi: 10.1073/pnas.1519019113
587 Tain, L., Perrot-Minnot, M., Cézilly, F., 2006. Altered host behaviour and brain serotonergic
588 activity caused by acanthocephalans: evidence for specificity. *P. R. Soc. B.*, 273(1605):
589 3039–3045. doi: 10.1098/rspb.2006.3618
590 Tatar, S., Yildirim, N.C., Serdar, O., Yildirim, N., Ogedey, A., 2018. The using of *Gammarus*
591 *pulex* as a biomonitor in ecological risk assessment of secondary effluent from municipal
592 wastewater treatment plant in Tunceli, Turkey. *Hum. Ecol. Risk Assess.*, 24(3), 819–829,
593 doi: 10.1080/10807039.2017.1400374
594 Teuten, E.L., Rowland, S.J., Galloway, T.S., Thompson, R.C., 2007. Potential for plastics to
595 transport hydrophobic contaminants. *Environ. Sci. Technol.*, 41(22), 7759–7764. doi:
596 10.1021/es071737s
597 Wallace, J.C., 1973. Feeding, starvation and metabolic rate in the shore crab *Carcinus*
598 *maenas. Mar. Biol.*, 20(4), 277–281. doi: 10.1007/BF00354271
599 Wallace, J.B., Webster, J.R., 1996. The role of macroinvertebrates in stream ecosystem
600 function. *Annu. Rev. Entomol.*, 41, 115–139. doi: 10.1146/annurev.en.41.010196.000555
601 Ward, T.D., Algera, D.A., Gallagher, A.J., Hawkins, E., Horodysky, A., Jørgensen, C.,
602 Killen, S.S., McKenzie, D.J., Metcalfe, J.D., Peck, M.A., Vu, M., 2016. Understanding
603 the individual to implement the ecosystem approach to fisheries management. *Conserv.*
604 *Physiol.*, 4(1). doi: 10.1093/conphys/cow005
605 Watts, A.J.R., Urbina, M.A., Corr, S., Lewis, C., Galloway, T.S., 2015. Ingestion of plastic
606 microfibers by the crab *Carcinus maenas* and its effect on food consumption and energy
607 balance. *Environ. Sci. Technol.*, 49, 14597–14604. doi: 10.1021/acs.est.5b04026

Weber, C.I. (Ed.), 1991. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms (p. 197). Environmental Monitoring Systems Laboratory, Office of Research and Development, US Environmental Protection Agency.

Weber, A., Scherer, C., Brennholt, N., Reifferscheid, G., Wagner, M., 2018. PET microplastics do not negatively affect the survival, development, metabolism and feeding activity of the freshwater invertebrate *Gammarus pulex*. *Environ. Pollut.*, 234, 181–189. doi: 10.1016/j.envpol.2017.11.014

Wen, B., Zhang, N., Jin, S., Chen, Z., Gao, J., Liu, Y., Liu, H., 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. doi: 10.1016/j.aquatox.2017.12.010

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. doi: 10.1016/j.envpol.2013.02.031

Zbyszewski, M., Corcoran, P.L., Hockin, A., 2014. Comparison of the distribution and degradation of plastic debris along shorelines of the Great Lakes, North America. *J. Great Lakes Res.*, 40(2), 288–299. doi: 10.1016/j.jglr.2014.02.012

- Metabolic and feeding rates of model detritivores increase with both body mass and temperature
- Microplastics pollution reduces metabolic rates but not feeding rates
- Experimental warming alters the effect of microplastics on metabolic rates
- Increased microplastics concentrations enhance metabolism at the coolest temperature, but inhibit metabolism at the highest temperatures

Declaration of interests

☐ 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. NONE

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Declarations of interest: none