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Microplastic ingestion decreases energy reserves in marine worms

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The indiscriminate disposal of plastic to the environment is of concern. Microscopic plastic litter (<5mm diameter; 'microplastic') is increasing in abundance in the marine environment, originating from the fragmentation of plastic items and from industry and personal-care products [1]. On highly impacted beaches, microplastic concentrations (<1mm) can reach 3% by weight, presenting a global conservation issue [2]. Microplastics are a novel substrate for the adherence of hydrophobic contaminants [1], deposition of eggs [3], and colonization by unique bacterial assemblages [4]. Ingestion by indiscriminate deposit-feeders has been reported, yet physical impacts remain understudied [1]. Here, we show that deposit-feeding marine worms maintained in sediments spiked with microscopic unplasticised polyvinylchloride (UPVC) at concentrations overlapping those in the environment had significantly depleted energy reserves by up to 50% (Figure 1). Our results suggest that depleted energy reserves arise from a combination of reduced feeding activity, longer gut residence times of ingested material and inflammation.

Seabeds worldwide are composed of a range of organic and inorganic sediments that sustain a vast range of marine species. The polychaete worm *Arenicola marina* (lugworm) of the globally distributed family *Arenicolidae* is a keystone species inhabiting intertidal sediments in Northern Europe; it bioturbates and irrigates the sediment and is an important secondary producer, as a prey species for fish and wading birds. Using a laboratory mesocosm, we performed chronic (four weeks) and short-term (48 hours) experiments, exposing *A. marina* to natural sediments containing clean, chemically-inert UPVC ranging from 0–5% by weight. PVC is denser than seawater and sinks out of suspension to sediments; >25% of microplastics sampled from estuarine sediments inhabited by *A. marina* were PVC [5]. Thus, we selected UPVC, mimicking the size and shape of sediment (130 µm mean diameter; Figure 1E). We assessed chronic effects on feeding activity, immunity and energy reserves and made short term observations on gut residence times.

Worms chronically exposed to 5% UPVC by weight displayed significantly reduced feeding activity compared to control and 1% UPVC-exposed worms (Figure 1A), supporting recent findings whereby 7.4% polystyrene by weight inhibited feeding activity in *A. marina* over 10 days [6]. Reduced feeding activity implies that either fewer particles are ingested overall or a lack of a protein coating on the clean UPVC weakens particle adhesion to the worm's feeding apparatus, reducing uptake efficiency. Suppressed feeding activity may decrease energy assimilation, compromising fitness. It could also decrease bioturbation and therefore oxygenation of the sediment, which is crucial for maintaining infaunal diversity.

Chronic UPVC exposure significantly increased the phagocytic activity of *A. marina's* immune cells, although this was not dose-dependent (Figure 1C). Enhanced phagocytic activity is indicative of an inflammatory response, which is a metabolically demanding process. Interestingly, the UPVC powder is classified as an irritant to human health following dermal contact.

The total available energy reserves in worms chronically exposed to 1% and 5% UPVC by weight were significantly reduced compared to pre-exposure and control animals. Worms exposed to 5% UPVC by weight had approximately 50% less total available energy reserves compared to controls (Figure 1D) and all UPVC-exposed animals had significantly lower lipid reserves than controls (Supplemental information). Jonker *et al*. [7] found lipid reserves declined in a freshwater oligochaete worm (*Lumbriculus variegatus*), following chronic exposure to powdered activated carbon, presumably due to reduced feeding activity. In our experiments, depleted energy reserves, which closely followed the trend for lipid reserves, could compromise somatic maintenance and growth, maturity and reproduction.

We determined the time it took ingested material to traverse the gut and found intervals between egestion events were 1.5 times longer (mean 1086 seconds) in animals exposed to 5% UPVC by weight, with an average of 0.33 ± 1 (median ± range) hourly egestion events compared to control worms (1.33 ± 2.33 (median ± range); Figure 1B; Supplemental information). *A. marina's* digestion is characterised by material continuously entering and exiting the digestive tract, with negligible mixing during passage. Prolonged gut residence times imply that microplastics, which are of low nutritional value, are being retained and subjected to extensive digestion, at an energetic cost.

Polychaete worms exhibit positive correlations between organic content and feeding activity [8]. We therefore tested the hypothesis that UPVC reduced the organic content of the sediment to a level at which food concentration becomes a limiting factor. When *A. marina* was exposed to natural sediment of varying organic content — altered by adding clean silica sand — there was no significant reduction in feeding activity (Supplemental information). This suggests that the observed reduction in feeding activity of 5% UPVC-exposed worms is likely attributed to a characteristic of the UPVC and not the secondary effect of decreased food concentration.

At a density of 85 individuals per m^2, which is typical of a tidal flat habitat, *A. marina* is estimated to process 400cm^3 of sediment annually [9]. Microplastic debris (<1mm) comprising 3.17% by weight of the sediment has been reported, which when adjusted for density could represent up to 6.34% of the sediment volume at contamination hotspots [2]. Using the Wadden Sea, where *A. marina* is a keystone species, as an example, if contamination accumulated to such levels *in situ*, *A. marina* could consume up to 33m^3 of microplastics annually. We found overall feeding activity reduced by approximately 25% in worms exposed to 5% UPVC by weight for a month. Using the Wadden Sea example, this would result in 130m^3 less sediment being reworked annually. Our current observations indicate that 1% microplastics by weight can reduce total energy reserves by approximately 30%, mainly linked to a reduction in lipid reserves. We propose a conceptual model (Figure 1F), whereby

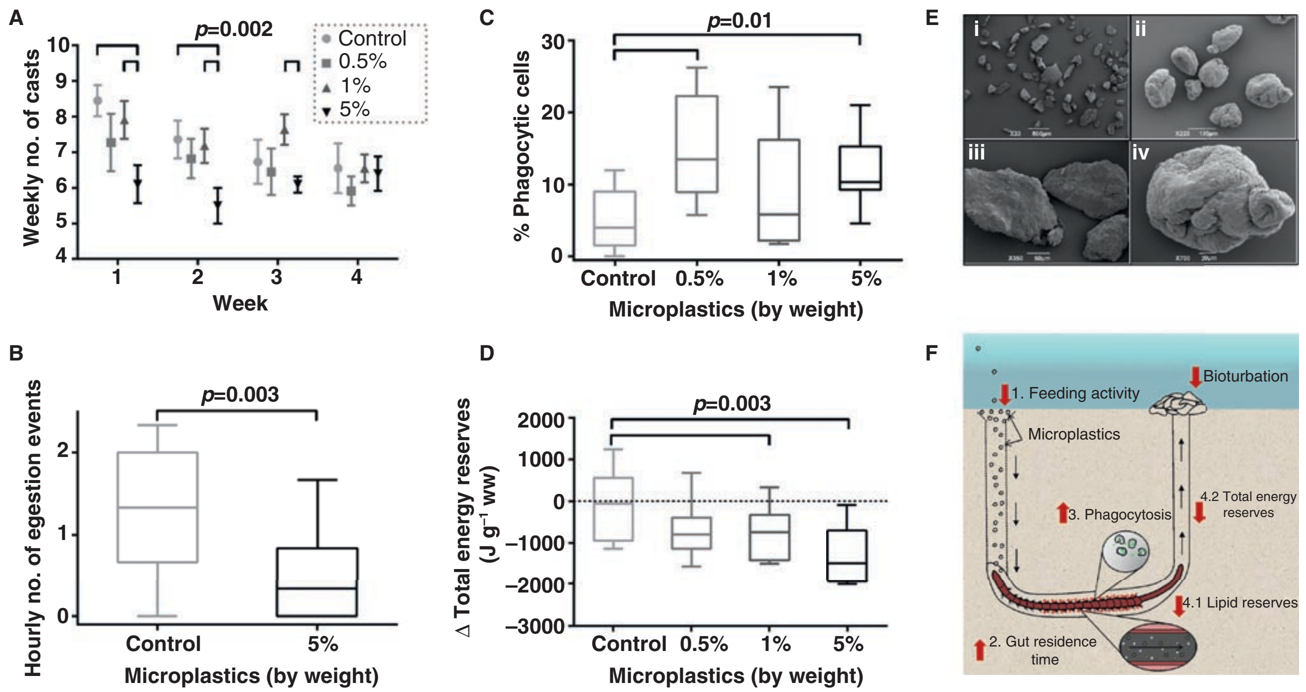

Figure 1. The impacts of microscopic UPVC on *A. marina*.
(A) The effects of UPVC exposure on weekly feeding activity (Generalised Estimating Equation (GEE); p=0.002 for 'time*treatment'). Data are presented as weekly average (mean ± SE) per worm. **(B)** The average (median ± range) hourly number of egestion events following 48 h exposure (Mann Whitney U test, p=0.003). **(C)** Effects of UPVC exposure on phagocytosis (one-way ANOVA, p=0.01), which was enhanced for 0.5% and 5% exposed worms (Fisher's LSD test, p=0.002 and p=0.013 for 0.5% and 5%, respectively). **(D)** The effects of UPVC exposure on total available energy reserves in *A. marina*. Data presented as average (median ± range) compared with pre-exposure baseline (dotted line) (one way ANOVA, p=0.003). For 1% and 5% exposed worms, p=0.036 and 0.001, respectively (Fisher's LSD test). **(E)** Scanning electron micrographs of i) natural sediment (x33, scale bar 500 μm); ii) UPVC (x220, scale bar 100 μm); iii) natural sediment (x350, scale bar 50 μm); iv) UPVC (x700, scale bar 20 μm). **(F)** A conceptual model of the effects of microscopic UPVC on *A. marina*: 1) suppressed feeding activity; 2) prolonged gut residence times; 3) inflammation; and 4) reduced lipid and total available energy reserves. Horizontal bars indicate a significant difference at the 0.05 confidence level. Data for the following can be found in Supplemental Information: statistical output; impacts on average feeding activity, cumulative number of casts, feeding status and egestion time; feeding activity in reduced food concentration; grain size distribution of natural sediment and UPVC; differences in weight (pre and post exposure); impacts on lipid, protein and sugar reserves.

high concentrations of microplastics could induce suppressed feeding activity, prolonged gut residence times, inflammation and reduced energy reserves, impacting on growth, reproduction and ultimately survival. We have shown that microplastics can cause physical harm to an important marine species, emphasizing the need to reconsider how discarded PVC, polystyrene, polyurethane and polycarbonate (30% of global production), are classified in terms of hazard [10].

Supplemental Information

Supplemental Information including experimental procedures and two figures can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.10.068.

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