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Author for correspondence:
Amanda Callaghan
e-mail: a.callaghan@reading.ac.uk

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Community ecology

Up and away: ontogenic transference as a pathway for aerial dispersal of microplastics

Rana Al-Jaibachi[1], Ross N. Cuthbert[1,2] and Amanda Callaghan[1]

[1]Ecology and Evolutionary Biology, School of Biological Sciences, University of Reading, Harborne Building, Reading RG6 6AS, UK
[2]Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, Medical Biology Centre, Belfast BT9 7BL, UK

RNC, 0000-0003-2770-254X; AC, 0000-0002-2731-3352

Microplastics (MPs) are ubiquitous pollutants found in marine, freshwater and terrestrial ecosystems. With so many MPs in aquatic systems, it is inevitable that they will be ingested by aquatic organisms and be transferred up through the food chain. However, to date, no study has considered whether MPs can be transmitted by means of ontogenic transference, i.e. between life stages that use different habitats. Here, we determine whether fluorescent polystyrene beads could transfer between *Culex* mosquito life stages and, particularly, could move into the flying adult stage. We show for the first time that MPs can be transferred ontogenically from a feeding (larva) into a non-feeding (pupa) life stage and subsequently into the adult terrestrial life stage. However, transference is dependent on particle size, with smaller 2 µm MPs transferring readily into pupae and adult stages, while 15 µm MPs transferred at a significantly reduced rate. MPs appear to accumulate in the Malpighian tubule renal excretion system. The transfer of MPs to the adults represents a potential aerial pathway to contamination of new environments. Thus, any organism that feeds on terrestrial life phases of freshwater insects could be impacted by MPs found in aquatic ecosystems.

1. Introduction

Microplastics (MPs) are ubiquitous pollutants found in marine, freshwater and terrestrial ecosystems [1–3]. There is little doubt that plastic and MP pollution is a major environmental concern globally. Despite this, there is relatively little research into the impact of MPs on freshwater ecosystems, with most research concentrating on marine systems and organisms [2]. MPs have been defined as plastic particles smaller than 5 mm in size [4,5]. However, this simple description covers a wide range of types, including, among others, polypropylene, polyethylene and polystyrene MPs entering the environment in different shapes and sizes, including fibres, pellets and cosmetic beads [6,7]. MPs are categorized based on their origin as primary or secondary types, depending on whether they were released into the environment as MPs (primary) or have degraded to that size in the environment (secondary) [8,9]. MPs pass through terrestrial environments in household wastewater [2,10]. Rivers can subsequently deliver MPs into the sea and lakes, where they can be found in high concentrations [11–13].

MPs are ingested by aquatic organisms and can be transferred through the food chain in both freshwater and marine environments [14–18]. However, to date, no study has considered whether MPs can be transmitted by means of ontogenic transference, i.e. between life stages that use different habitats. Freshwater environments are inhabited by insects that spend their juvenile stages in water but their adult stages in the terrestrial environment. Such

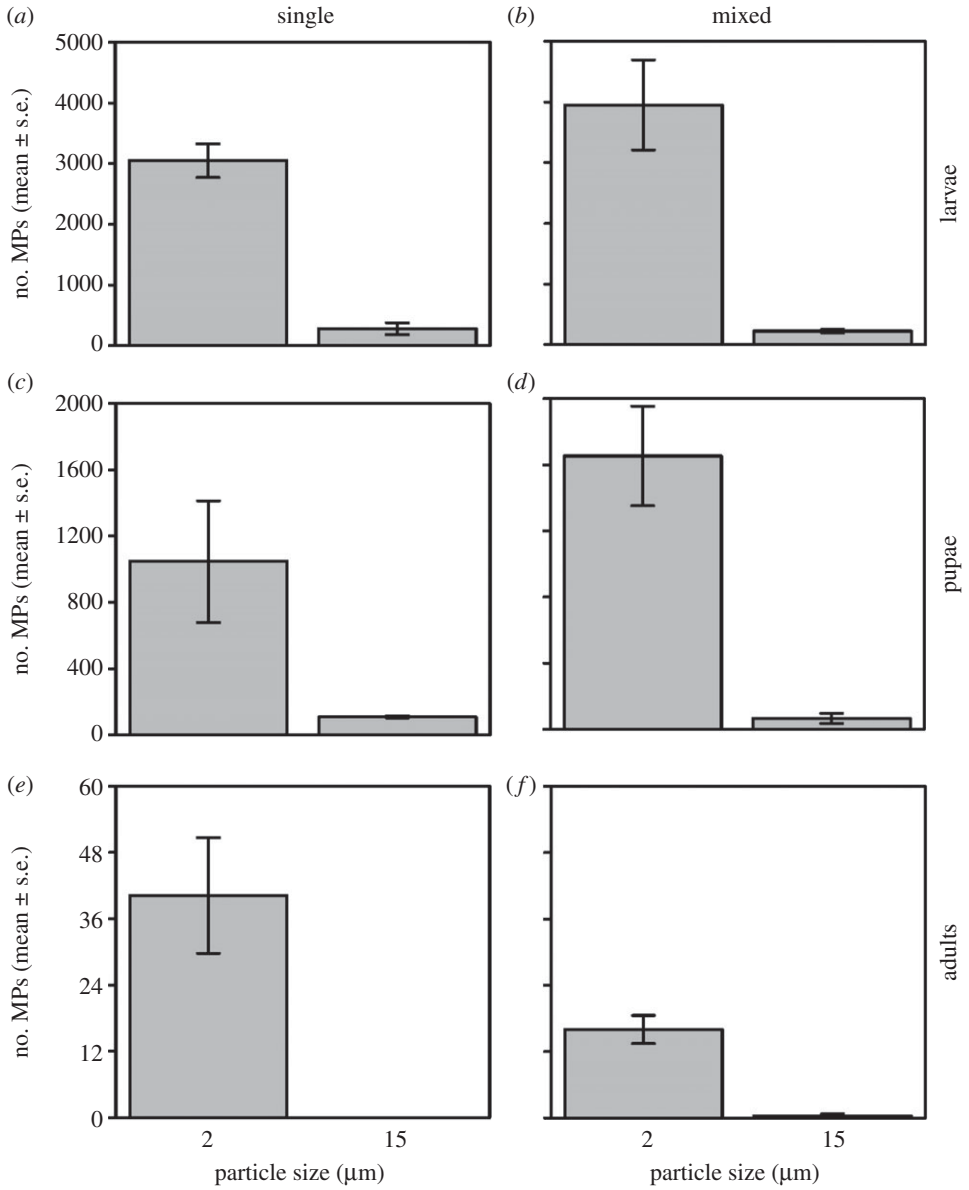

Figure 1. Uptake counts of MPs across larval (*a*,*b*), pupal (*c*,*d*) and adult (*e*,*f*) *Culex* mosquito stages following single (*a*,*c*,*e*) and mixed (*b*,*d*,*f*) exposures to 2 and 15 µm beads. Means are ±s.e. ($n = 5$ per experimental group).

insects include mayflies, dragonflies, midges and mosquitoes, most of which are eaten by terrestrial vertebrates. This raises the potential for MPs to enter terrestrial ecosystems from freshwater habitats aerially via transference to adult invertebrate life stages. Here, we thus determine whether 2 and 15 µm fluorescent polystyrene beads could transfer between insect life stages and, particularly, could move into the flying adult stage. Fluorescent beads were selected to enable MPs to be easily detected in the non-feeding stages and also to allow an investigation of location within the body during metamorphosis. The *Culex pipiens* mosquito complex was selected as a model for this study given their worldwide distribution and broad habitat preference [19]. Mosquitoes develop through four feeding larval instars and a non-feeding pupal stage, and finally emerge into a flying adult.

2. Material and methods

For additional details of all methods and analyses, see the electronic supplementary material.

Two types of MPs were used: a 2 µm fluorescent yellow-green carboxylate-modified polystyrene (density 1.050 g cm^{-3}, excitation 470 nm; emission 505 nm, Sigma-Aldrich, UK) and a 15.45 ± 1.1 µm fluorescent dragon green polystyrene (density 1.06 g cm^{-3} (5×10^6 particles ml^{-1}), excitation 480 nm; emission 520 nm, Bangs Laboratories, Inc., USA). Four treatments were used: a control with no MPs, a treatment of 8×10^5 2 µm particles ml^{-1}, a treatment of 8×10^2 15 µm particles ml^{-1} and a 1 : 1 mixture of both treatments. Each replicate (five per treatment) contained 10 third instar *C. pipiens* larvae in a 50 ml glass beaker filled with 50 ml of tap water. The control and all treatments contained 100 mg of pelleted guinea pig food. Treatments were assigned randomly to a position on the laboratory bench to reduce experimental error.

One random individual was removed from each beaker when every mosquito had moulted into the fourth instar, and again when they pupated or emerged as adults. All samples were then placed in separate 1.5 ml Eppendorf tubes and stored at −20°C prior to examination. MPs were extracted from mosquitoes by homogenization and filtration. The filter membrane was examined using an epi-fluorescent microscope (Zeiss Axioskop) under a 20× lens to count the number of fluorescent MPs. Adults were further dissected under a binocular stereo microscope (0.7×–4.5×) to extract

the gut and quantify the numbers of MPs under the epi-fluorescent microscope [20].

All data were analysed using the statistical software R v. 3.4.2 [21]. MP counts were analysed using generalized linear models assuming a quasi-Poisson distribution. Uptake of MPs was examined with respect to 'particle size', 'treatment' and 'life stage'. We performed model simplification via stepwise removal of non-significant effects. Tukey's tests were used post hoc for multiple comparisons.

3. Results

No MPs were found in control groups of any mosquito life stage. Densities of MPs were significantly different between life stages ($F_{2,56} = 160.42$, $p < 0.001$), with MP numbers significantly falling as mosquitoes moved between successive ontogenic levels (all $p < 0.001$) (figure 1; electronic supplementary material, table S1 and S2). MP transference to adults was confirmed by fluorescent microscopy where the beads were detected in the adult abdomen, specifically inside the Malpighian tubules (figure 2).

Significantly more 2 µm particles were found in mosquito life stages than 15 µm particles overall ($F_{1,58} = 303.98$, $p < 0.001$). MPs uptake was also significantly greater overall in mixed exposure treatments ($F_{1,55} = 6.00$, $p = 0.02$). Although 2 µm particles were transferred to adults in all instances, we found no transference of 15 µm particles following single treatment exposures. However, in the mixed MPs treatment, transference to adults of both 2 and 15 µm particles was evidenced (figure 1).

4. Discussion

Here, we show for the first time that MPs can be transferred ontogenically from a feeding (larval) into a non-feeding (pupal) life stage and subsequently into the flying (adult) life stage. Transference through to adults was found in both MP sizes, although the larger 15 µm MPs were not ingested as readily as the 2 µm MPs. Dissection of mosquito adults showed that 2 µm MPs accumulated in the renal excretion system of Malpighian tubules which, unlike the gut, pass from larvae to adult stages without visible reorganization [22]. This has been demonstrated previously to provide a physical transport system between stages during metamorphosis for *Pseudomonas* bacteria and seems to be important for ontogenic transmission from larvae to adults [23].

Few 15 µm MPs were transferred into adults, suggesting that MP size is an important factor in ontogenic transfer which could be related to the transfer and accumulation of MPs in the Malpighian tubes. Although the translocation mechanism of MPs to the Malpighian tubules is unclear in mosquitoes, analysis of fish, fiddler crab and marine mussels has demonstrated that MPs can be translocated from gastrointestinal tracts into other tissues in a wide range of phyla [24–26]. Malpighian tubules have an entry point to the gut between the mid- and hindgut of mosquitoes, but the flow of fluid is from the Malpighian tubules to the hindgut [27]. Diptera are known to produce structures called concretions in the Malpighian tubules which have been shown to sequester heavy metals [28]. However, it is unlikely that this pathway would operate with a solid MP.

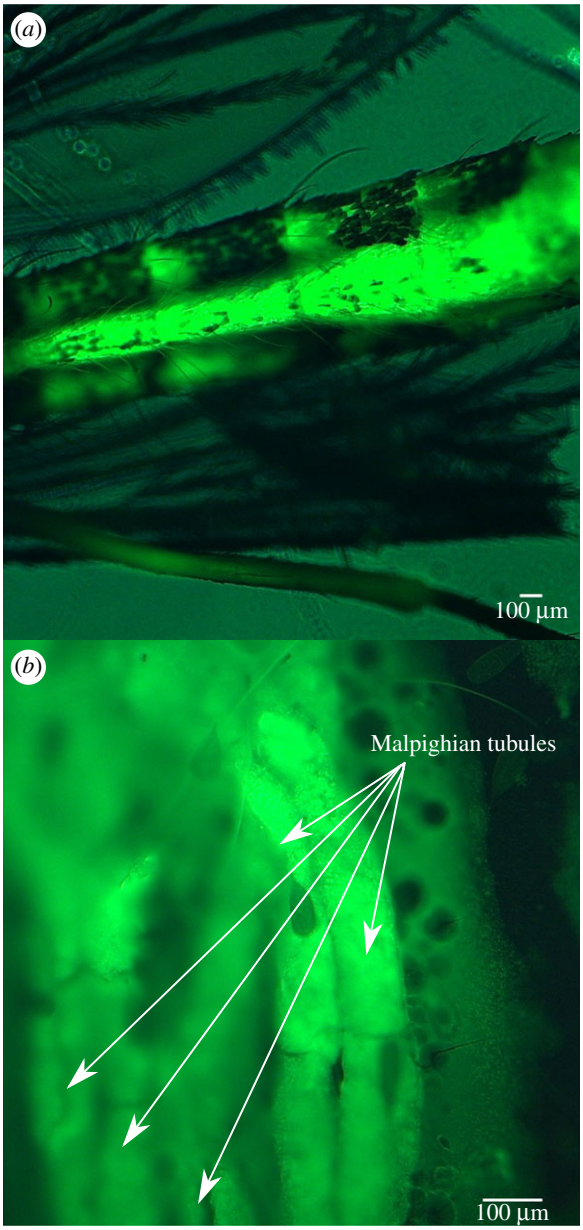

Figure 2. Epi-fluorescent microscope images showing fluorescent MP particles within (*a*) the abdomen of an adult mosquito before dissection and (*b*) the abdominal Malpighian tubules following dissection.

Our results have important implications because any aquatic life stage that is able to consume MPs and transfer them to their terrestrial life stage is a potential vector of MPs onto novel aerial and terrestrial habitats. Ingestion of MP-contaminated organisms by terrestrial organisms is not new [29]. Indeed, the widespread distribution of MPs in marine environments has meant that animals such as fish and shellfish sold for human consumption are contaminated with a range of plastics with a consequent transference of MPs between trophic levels [24]. Unlike MP fibres, which are common in the air and atmosphere, there has been no evidence for MP beads being transported into the air [30]. We have demonstrated here that species with aquatic and terrestrial life stages can harbour MPs through their life history. Adults are predated on emergence by many animals including dipteran flies Empididae and Dolichopodididae, while resting predominantly by spiders and in flight they are the prey of dragonflies, damselflies, birds (such as swallows and swifts) and bats [31]. Where many insects are emerging

from a highly contaminated site, the possibility of contamination of these predators could be high. While mosquitoes were used here as a model organism, any freshwater insect that can ingest MPs will likely equally transmit plastics into a terrestrial adult stage. This has implications for organisms that feed on adult stages with aerial and terrestrial animals accordingly open to MP exposure and transference appearing to occur at a higher rate for smaller MPs.

Data accessibility. Data files are available in the electronic supplementary material.

Authors' contribution. All authors provided substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; were involved in drafting the article or revising it critically for important intellectual content; approved the final version to be published and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Competing interests. We declare we have no competing interests.

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