Science of the Total Environment 651 (2019) 871–876

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Examining effects of ontogenic microplastic transference on *Culex* mosquito mortality and adult weight

Rana Al-Jaibachi [a], Ross N. Cuthbert [a,b], Amanda Callaghan [a,*]

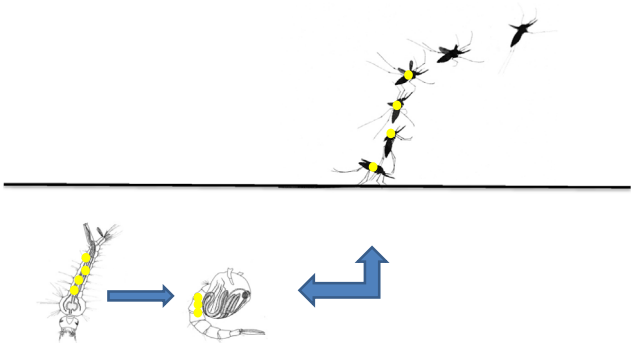
[a] Ecology and Evolutionary Biology, School of Biological Sciences, University of Reading, Harborne Building, Reading RG6 6AS, England, United Kingdom of Great Britain and Northern Ireland
[b] Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, Medical Biology Centre, Belfast BT9 7BL, Northern Ireland, United Kingdom of Great Britain and Northern Ireland

HIGHLIGHTS

- *Culex* mosquito larvae readily eat microplastics.
- Microplastics are transferred from feeding to non-feeding life stages during metamorphosis.
- Adult mosquitoes that have eaten microplastics as larvae emerge into the terrestrial environment with them in their guts.
- Microplastic ontogenic transference is dependent on particle size.
- Microplastics had no impact on the growth or mortality of the organism (here a mosquito).

GRAPHICAL ABSTRACT

Polystyrene MP beads (yellow dots) are ingested by *Culex* mosquito larvae and transferred to the non-feeding pupal stage during metamorphosis. When adults emerge they carry the MPs in their abdomen from the freshwater up into the terrestrial environment. The amount of plastic transferred reduces with each stage but has no impact on growth or mortality.



ARTICLE INFO

Article history:
Received 13 August 2018
Received in revised form 17 September 2018
Accepted 18 September 2018
Available online 19 September 2018

Editor: D. Barcelo

Keywords:
Microplastics
Mosquitoes
Ontogenic transference
Habitat transference

ABSTRACT

Microplastics (MPs) continue to proliferate and pollute aquatic and terrestrial environments globally. The impacts of MP pollution on ecosystems and their functioning remain poorly quantified, with most research hitherto focusing on marine ecosystems. There is a paucity of information on the impacts of MPs in freshwater ecosystems, despite the broad range of pathways through which MPs can proliferate and the extensive range of species which actively ingest MPs in these systems. Of particular interest are organisms that bridge aquatic and terrestrial habitats. The present study thus examines the uptake, ontogenic transference and effect of different concentrations (0, 50, 100 and 200 MPs mL^{-1}) and sizes (2 and 15 μm) of polystyrene MPs between aquatic and terrestrial life stages of *Culex pipiens* complex mosquitoes. Both 2 and 15 μm MPs transferred from the aquatic larval to terrestrial adult stage of *Culex* mosquitoes, and uptake correlated tightly with initial exposure concentration. However, neither concentration nor size of MPs significantly influenced mortality rates between the aquatic larval and terrestrial adult stage. There was also no impact of MPs on the weight of emerging mosquito adults. We thus demonstrate that MPs can be transferred ontogenically through organisms with complex life histories, presenting a potential pathway for dispersal of MPs into terrestrial environments. We also show that MPs exposure does not affect mortality rates between life stages of freshwater *Culex* populations. This suggests that MPs do not impact nutritional uptakes,

* Corresponding author.
E-mail address: a.callaghan@reading.ac.uk (A. Callaghan).

https://doi.org/10.1016/j.scitotenv.2018.09.236

with unhampered development to adulthood facilitating subsequent dispersal of MPs aerially and between freshwater and terrestrial habitats.

1. Introduction

Microplastics (MPs) continue to proliferate in marine, freshwater and terrestrial ecosystems, with biotic impacts frequently unknown (Sighicelli et al., 2018). Microplastic pollution has been detected from the poles to the deep ocean, and more recently in bottled drinking water (Mason et al., 2018; Wagner and Lambert, 2018). Whilst there is little doubt over the enormity of plastic and MP pollution in scale, the vast majority of research has concentrated on marine environments whilst neglecting other ecosystems (Redondo-Hasselerharm et al., 2018; Wagner and Lambert, 2018). To date there is a paucity of information on the impacts of MPs in freshwater ecosystems, despite the broad range of pathways through which MPs can proliferate (Mason et al., 2016), and the extensive range of species which actively ingest MPs in these systems (Canniff and Hoang, 2018; Imhof et al., 2016, 2013; Nel et al., 2018; Qu et al., 2018).

Microplastics have been defined as plastic particles of 5 mm or less in size (Imhof et al., 2013; Eriksen et al., 2014). This is a broad definition as MPs manifest in a variety of forms, such as fibres, pellets and cosmetic beads, which all routinely enter the environment (Watts et al., 2014). Microplastics differ in their chemical composition, and can consist of various polymers such as polypropylene, polyethylene and polystyrene (Andrady and Neal, 2009; Rocha-Santos and Duarte, 2014). Furthermore, MPs can either be primary or secondary in origin, with the former released directly into the environment as MPs, and the latter having degraded over time to reach the MP size class (Moore, 2008; Barnes et al., 2009). Despite such inherent variation, there has been little work to compare differential impacts of the varied types of MPs on recipient organisms.

Movement of MPs through terrestrial and aquatic environments has been investigated, and several pathways have been suggested. For example, movement through the air due to wind (Dris et al., 2016) or directly through water courses from wastewater treatment plants into rivers and eventually the marine environment (Mason et al., 2016; Wagner and Lambert, 2018). Rivers can also deliver MPs into lakes, where they can be found in high concentrations and presumably fall into the sediment (Vaughan et al., 2017). In North America the highest MP concentrations were found in Lake Ontario-Erie, with an average range of 90,000–6,700,000 MPs km^{-2} (Fischer et al., 2016). In Europe, Lake Geneva contained the highest MP concentration with a mean of 220,000 (±SD: ±160,000 MPs km^{-2}) (Eriksen et al., 2013), and in Asia, Lake Taihu contained a range of 10,000–6,800,000 MPs km^{-2} (Su et al., 2016).

Given the densities of MPs in freshwater ecosystems, it is likely that they will be ingested by aquatic organisms, and, in turn, probable that they will be transferred up through the food chain (Cole et al., 2013; Sussarellu et al., 2016; Scherer et al., 2017; Redondo-Hasselerharm et al., 2018). Laboratory experiments have demonstrated the uptake of MPs, and it is well established that they are ingested by many invertebrates in both freshwater and marine environments (Imhof et al., 2013; Al-Jaibachi and Callaghan, 2018). However, considerations of whether MPs can be transmitted by means of ontogenic transference, *i.e.* between life stages within an individual, have remained scarce. Insects comprise an important component of freshwater environments and are often highly abundant (Macadam and Stockan, 2015). Many insects have complex life histories, consisting of successive aquatic and terrestrial stages. Examples of such insects are stoneflies, damselflies, midges and mosquitoes, most of which are eaten by birds in their terrestrial stage. We have recently shown that MPs can be transferred into mosquito adults following ingestion as larvae (Al-Jaibachi et al., 2018). Thus, ontogenic transference of MPs presents a further pathway for MPs to enter new ecosystems from aquatic environments, with the potential to enter organisms that do not feed on the aquatic stages of freshwater or marine organisms.

The present study was undertaken to determine whether MPs which transfer between insect life stages of species with complex life histories could affect survival and adult size, which is linked to fecundity (Takken et al., 2013). Mosquitoes (Diptera: Culicidae) are ideal for this study since they go through four feeding larval instars, a non-feeding pupal stage and finally emerge into a flying adult that feeds on nectar and/or vertebrate blood depending on the sex and species. Here, we investigate the ingestion of 2 ± 0.2 and 15 ± 1.1 μm fluorescent polystyrene beads, and whether consumption is concentration-dependent. 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. *Culex pipiens* complex mosquitoes were selected for this study because they exhibit a global distribution and colonise a broad range of aquatic habitats, such as stream pools, lake edges, marshes and shallow permanent ponds, alongside both natural (phytotelmata) and artificial containers (Townroe and Callaghan, 2015). The group is also known to be an important food source for birds and other terrestrial organisms in the adult stage (Dow et al., 1994). We hypothesise that: (1) MPs will move ontogenically from larval to pupal stages, and subsequently into adult mosquito stages, and that transference is both MP concentration- and size-dependent; (2) uptake of MPs will reduce the survivability of larval mosquitoes to the adult stage; (3) exposure to MPs will affect the nutrition and thus development of larval mosquitoes, resulting in smaller-sized adults upon emergence.

2. Materials and methods

2.1. Preparation of microplastics (MPs)

Two types of MPs were used: a 2 ± 0.2 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}, excitation 480 nm, emission 520 nm; Bangs Laboratories Inc., USA).

The 2 μm MPs were stored as a stock suspension (2.5 mg mL^{-1}) in distilled water and mixed using a vortex (Whirlimixer Cyclone, UK) prior to dilutions. The 15 μm MPs were also stored as stock suspension (1% solid) polystyrene microspheres. Particles were washed prior to use by adding 1 mL from the stock solution into a 1.5 mL Eppendorf tube and then centrifuging at 9000 rpm for 10 min. The supernatant was discarded and 1 mL of distilled water was added. The solution was then resuspended by using the vortex and centrifuged again at the same speed and duration. This process was repeated two more times.

2.2. Mosquito colonies

Larvae of the *C. pipiens* mosquito complex were obtained from colonies reared at the University of Reading, UK following the methodology of Cuthbert et al. (2018). This colony originated from individuals collected in Cyprus in 2005 by A. Callaghan and have been reared in laboratory conditions since then. Adult *C. pipiens* were fed overnight twice a week with defibrinated horse blood (TCS Biosciences, UK) using