REVIEW ARTICLE



Medical and Veterinary

Microplastics (plastic particles <5 mm) permeate aquatic and terrestrial ecosystems and

constitute a hazard to animal life. Although much research has been conducted on the

effects of microplastics on marine and benthic organisms, less consideration has been

given to insects, especially those adapted to urban environments. Here, we provide a

perspective on the potential consequences of exposure to microplastics within typical

larval habitat on mosquito biology. Mosquitoes represent an ideal organism in which to explore the biological effects of microplastics on terrestrial insects, not least because of

their importance as an infectious disease vector. Drawing on evidence from other organ-

isms and knowledge of the mosquito life cycle, we summarise some of the more plausi-

ble impacts of microplastics including physiological, ecotoxicological and immunological responses. We conclude that although there remains little experimental evidence dem-

onstrating any adverse effect on mosquito biology or pathogen transmission, significant

knowledge gaps remain, and there is now a need to quantify the effects that microplastic

pollution could have on such an important disease vector.

microbiome, microplastic, mosquito, vector

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A perspective on the impacts of microplastics on mosquito biology and their vectorial capacity

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Abstract

KEYWORDS

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INTRODUCTION

Humans have shaped mosquito biology and demography for centuries. One of the most clear and recent examples is the evolution of insecticide resistance and behavioural shifts in response to the massive upscale of insecticide-based vector control interventions at the turn of the 21st century (Sanou et al., 2021). Mosquitoes have simultaneously co-evolved and adapted to urbanisation (Krystosik et al., 2020), agricultural expansion (Chan et al., 2022) and global

transport (Ahn et al., 2023), each of which plays a role in defining mosquito-human interactions. During the last few decades, plastics and plastic waste have become ubiquitous in the environment, which represents another opportunity for anthropogenic activity to affect mosquito biology and the pathogens they transmit. The concept of environmental and household waste (e.g., tyres and plant pots) as receptacles for mosquito oviposition is a well-established adaptation of urban mosquitoes and has been reviewed elsewhere (Krystosik et al., 2020; Maquart et al., 2022). However, given the broad spectrum

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of larval habitats colonised by mosquitoes, there is the potential for larvae to ingest much smaller (i.e., less than 5 mm) plastic fragments, fibres and debris (microplastics [MP]). Recent experimental work has shown that mosquitoes can ingest MPs, but the consequences of this on physiology, development and vector competence remains speculative.

There is increasing scientific interest on the impact of MP pollution on invertebrates, and mosquitoes represent an ideal organism to understand the effect of MPs on dipteran species. First, given their medical relevance, there is a wealth of biological and genomic resources available and second, many of the most important urban vector species such as *Aedes aegypti* (Diptera: Culicidae) (Linnaeus) and *Ae. albopictus* (*Skuse*) lay their eggs within, and are adapted to, highly plasticised environments (Maquart et al., 2022). Additionally, the larvae of *Anopheles stephensi* (*Liston*), the main malaria vector in urban settings, thrive in discarded tyres and plastic containers in parts of Africa where they have recently spread (Mnzava et al., 2022). Here, we critically examine the evidence on whether mosquito larvae are exposed to meaningful concentrations of MPs in typical larval habitat, evaluate their capacity to ingest MPs and explore the possible implications of the exposure.

EXPOSURE OF MOSQUITOES TO MPs IN THE ENVIRONMENT

MPs are spheres, fibres or fragments <5 mm diameter and are continuously released into the environment either directly (e.g., in wastewater) or from the fragmentation of larger macroplastics via physical, photo- or biodegradation processes (Wagner et al., 2014). The persistence and accumulation of plastic polymers in the environment has led to an intensive research focus on the harmful or toxic effects they can cause across a range of organisms. Initially, most of this research focused on marine organisms (Wright et al., 2013), although increasingly, laboratory and field studies have investigated the impact of MPs on freshwater (Triebskorn et al., 2019) and terrestrial ecosystems (Rillig & Lehmann, 2020).

The toxicity of MPs depends on the exposure (time, concentration), the polymer (type, size, shape) and the bioavailability of the particles with respect to the behaviour of the species. Determining the effects on a single species is highly complex, made even more difficult by the lack of accurate measurements of typical concentrations, size distributions and particle types in natural settings, especially for smaller particles (<80 μm) (Eerkes-Medrano et al., 2015). Controlled laboratory experiments, exposing groups of organisms or individuals to measured volumes of MPs and quantifying either a physiological or a behavioural response against a non-exposed control, is the most common form of experiment to identify a toxic or sub-lethal effect of MP exposure. Even with such experiments, toxicity varies considerably between and within species, and extrapolating the results to the natural environment is difficult given the coexistence of other pollutants and numerous interdependent biotic and abiotic factors that will further influence MP exposure (Weber et al., 2018).

To date, most research on the effects of MPs on invertebrates has focussed on planktonic and benthic organisms with the majority of studies using model organisms (e.g., *Daphnia*; Ogonowski et al., 2016); in contrast, a relatively small number of studies have been conducted on insects. Insects that complete part or all of their life cycle within the aquatic environment are susceptible to MPs given that (i) they colonise freshwater habitats prone to MP pollution and (ii) they often encounter and ingest inorganic matter within the water column and the sediment. Typical sources of MP pollution come from rivers, drainage systems, agricultural run-off, wastewater effluent, flooding events and atmospheric deposition (Gündoğdu et al., 2018; Li et al., 2018; Triebskorn et al., 2019; Villafañe et al., 2023).

Environmental sampling of MPs from freshwater ecosystems shows an exponential size distribution for particles <20 um in diameter (Triebskorn et al., 2019), with >90% of MPs from water treatment plants being 1-10 µm in size (Pivokonsky et al., 2018). MP pollution in freshwater mainly consists of polypropylene, polyethylene, polystyrene and polyethylene terephthalate, with fibres and fragments being the major morphological types (Li et al., 2018). Estimations of MP concentrations from freshwater bodies are difficult to compare (see Triebskorn et al., 2019 for a summary), but in water bodies where aquatic insects are typically found (urban canal, reservoir, river), and for particles less than 20 μ m, consistent estimates of 10⁴-10⁵ particles/m³ are observed, with greater abundance and diversity of MPs in urban areas (Laju et al., 2023). A general consensus is that most experimental exposure studies use unrealistically high concentrations of MPs (Lenz et al., 2016), meaning environmentally relevant data on the effect of MPs on invertebrates are lacking.

There are approximately 330 mosquito disease vectors out of a total of \sim 3500 species (Yee et al., 2022). The breadth of mosquito adaptation to the environment is vast, exemplified by the wide range of aquatic habitats in which the adult female lays her eggs and immature development from larva to pupa progresses. The nature of these habitats varies from temporary to permanent, natural to human made and urban to rural (Fillinger et al., 2004; Maguart et al., 2022). Although the type of habitat colonised is species-specific, some generalisations can be made to identify which of the most important vectors are exposed to MP pollution. For example, exposure to MPs in urban environments is more likely given the proximity to human activity especially in fast-growing and unplanned towns and cities with poor sanitation and drainage. Some of the most important urban vectors which may encounter higher MP concentrations include (i) An. stephensi, an urban malaria vector, adapted to water storage tanks, containers and tyres, which is currently spreading from its native range on the Indian sub-continent into Africa (Sinka et al., 2020), (ii) Culex quinquefasciatus (Say), a West Nile Virus vector which breeds in highly organic water bodies (Calhoun et al., 2007) and (iii) the globally distributed urban Aedes spp., including Ae. aegypti and Ae. albopictus, which lay eggs in a wide variety of human-made plastic containers and tyres and are vectors of dengue, chikungunya, yellow fever and Zika virus (Maquart et al., 2022).

Certain urban environments may facilitate MP exposure. A large body of evidence shows that *Cx. quinquefasciatus* is abundant in

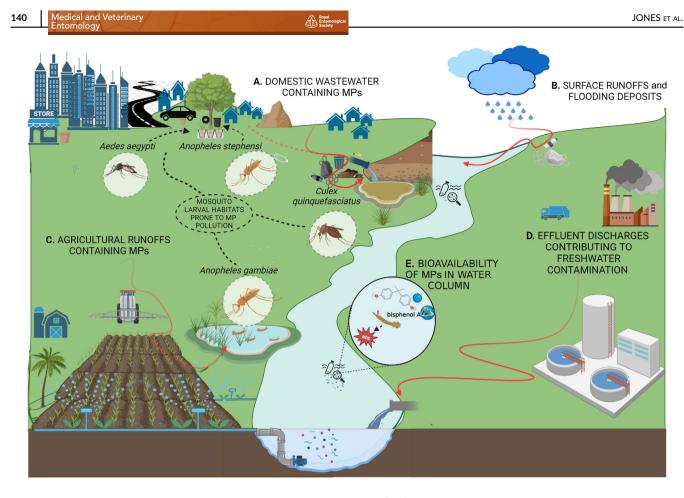


FIGURE 1 A schematic of the most likely routes of exposure to microplastic (MP) pollution for mosquito vectors.

combined sewage overflows (combined storm and waste systems) (Calhoun et al., 2007; Chaves et al., 2009). The effluence from wastewater treatment works is a major source of MPs (even after secondary water treatment) (Murphy et al., 2016), and so it is reasonable to expect that this vector will be exposed to MP pollution in some form, depending on the treatment facility and water flow. Another likely route of exposure comes from larval development in discarded tyres. Tyre wear particles contribute to MP pollution (Wagner et al., 2018) with the invasive Ae. albopictus and Ae. aegypti disseminated through the tyre trade. Another route of entry for MPs into the terrestrial ecosystem is through agricultural activities including the application of sewage sludge and biosolids as fertiliser (Wong et al., 2020) or plastic mulching of soil used for growing crops (Corradini et al., 2019) in both rural and urban settings (Figure 1). Many Anopheles species are adapted to agricultural land due to the presence of irrigated surface water (Chan et al., 2022; Frake et al., 2020; Jones et al., 2023) and so depending on local farming practices, will become exposed to MPs in agroecosystems.

The above examples are not exhaustive and remain largely conjectural in terms of the concentrations of MP that mosquitoes are exposed to. Although there are several estimates of the concentrations, polymers and size distributions of MPs from typical mosquito habitat (Triebskorn et al., 2019), no accurate measurements have been taken from water bodies containing free-living mosquito populations. To date, there are no studies demonstrating a direct interaction between MPs and mosquitoes in any natural habitat. Estimating meaningful exposures across a range of natural breeding habitats for different vector species needs addressing to ultimately determine whether mosquito biology is significantly impacted by MP exposure.

THE CAPACITY OF MOSQUITOES TO INGEST MPs

The impact of MPs on mosquito physiology will be influenced by the ability of larvae to ingest MP particles in their native larval habitat. Mosquitoes in the genera *Anopheles*, *Culex* and *Aedes* are generally considered 'collecting-filtering' feeders (Merritt et al., 1992). Broadly speaking, this mode of feeding behaviour utilises mouth brushes (lateral palatal brushes) extending from the larval head to create a water flow or current, which entraps fine organic particulate matter. All species that use this method, feed from the water column either passively (using surrounding water currents) or actively (expending energy), at depths ranging from the air-water interface (e.g., most *Anopheles* and some *Aedes* species) to greater depths within the water column (e.g., *Culex*) (Merritt et al., 1992). Among the different freshwater invertebrate feeding groups, filter feeders are particularly susceptible to ingesting MPs suspended in water, with evidence of a linear

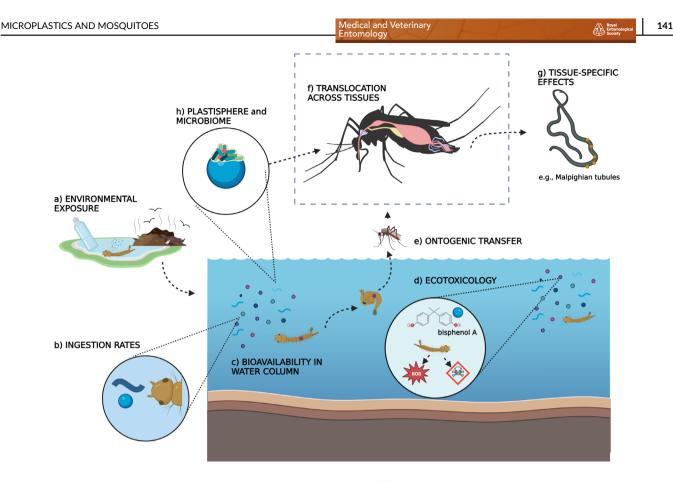


FIGURE 2 Hypothesised interactions between microplastics and mosquitoes. (a) Environmental exposure is determined by the presence of plastic waste and microplastics in aquatic breeding sites. (b) Ingestion of microplastic particles will be limited by feeding strategy of mosquito species and (c) size, shape and availability of microplastics in the water column. (d) Plastic additives such as bisphenol A leach into the environment and can cause oxidative damage (e.g. increase in reactive oxygen species (ROS)) with possible lethal effects. (e) Once ingested, microplastics persist through pupal and adult development (i.e., ontogenic transfer). (f) The ability of microplastic particles to persist and translocate across mosquito tissues is relatively unknown, but (g) there is evidence that the renal excretory organ, the Malpighian tubules, is susceptible. (h) Finally, microplastics in the environment are colonised by microbial biofilm (known as the plastisphere comprised of distinct communities of microorganisms compared to the surrounding environment), which once ingested could disrupt the gut microbiome. Created with BioRender.com.

relationship between MP concentration and ingestion rates (Scherer et al., 2017), although this is likely limited by particle size. Larvae may also selectively reject non-living particulate matter that does not contribute nutrition to their diet. Sedimentation rates, based on the size and density of the MP particle, will also affect availability in the water column (Kowalski et al., 2016). There is evidence that particle size is a limitation for the uptake of MPs for a range of aquatic invertebrates, but other factors such as the density, texture and shape of the MP, together with feeding mode, are just as important (Scherer et al., 2017). The upper limit of particulate matter ingested by mosquito larvae is approximately \sim 50 μ m (Merritt et al., 1992), and large particles that cannot be masticated by larvae are discarded. In most cases, however, a much greater percentage of smaller sized particles are ingested by insects (Weber et al., 2018). In mosquitoes, high concentrations of 2-µm spherical MP particles were found in Culex larvae compared with relatively few 15-µm particles following exposure to extremely high (800-800,000 MP/mL) concentrations of MPs (Al-Jaibachi et al., 2018).

Like many filter feeders, mosquito larvae are not selective in ingestion of organic versus inorganic matter. Non-living and nondigestible material is naturally present in all ecosystems, and so it would not be surprising if MPs were ingested alongside the more essential constituents of a mosquito diet such as microorganisms, algae and metazoans. However, when presented with other organic matter in the laboratory, freshwater invertebrates consumed less MPs (Kowalski et al., 2016; Scherer et al., 2017). Despite the uncertainty about the dynamics of exposure of mosquito larvae to MPs, it is likely that at least some species of mosquito larvae will ingest MP particles; however, whether plastic polymers have any sub-lethal or lethal effects is not clear (see below). MP-mediated adverse or sub-lethal effects in mosquitoes could occur in one of four ways: (i) physiological or behavioural (e.g., feeding rates, movement) (ii) chemical (e.g., leaching or adsorption of toxic compounds), (iii) translocation across tissues and cells or (iv) disruption of the microbiome (Figure 2). Here, we critically discuss the potential for each of these four processes with regards to mosquito larvae.

POSSIBLE EFFECTS OF MPs ON MOSQUITO BIOLOGY AND DEVELOPMENT

Physiological and behavioural

Previous experimental studies conducted on mosquitoes have described the exposure of different larval instar stages to varying concentrations of MPs and subsequently measured physiological and/or behavioural end points (Table 1). From the studies conducted so far, there is little consensus on the physiological or behavioural impacts of MPs. Exposure of newly hatched larvae to 200 or 20,000 polystyrene particles per mL (4.8–5.8 µm) had no effect on body size, growth rate or development of Cx. pipiens or Culex tarsalis (Coquillett) (Thormeyer & Tseng, 2023). Similarly, exposure of Ae. aegypti and Ae. albopictus larvae to 100-100.000 1 um polystyrene MPs had little effect on adult emergence rates (Edwards et al., 2023). On exposure to mixed size classes (1–53 μ m) of polyethylene MPs (60 MP mL⁻¹), mortality was observed for Ae. albopictus but not Cx. quinquefasciatus (Griffin et al., 2023). Although there is some evidence for reduced feeding rate (Cole et al., 2015), reduced weight (Besseling et al., 2013) and increased mortality (Lee et al., 2013) in planktonic and benthic worms, a meta-analysis of freshwater fish and aquatic invertebrates found few, or negligible, effects of ingesting MPs (Foley et al., 2018). Furthermore, only a handful of studies have exposed invertebrates to environmentally realistic MP concentrations, with just a few examples demonstrating any significant effect on development and growth (e.g., non-biting midge, Chironomus tepperi (Skuse); Ziajahromi et al., 2018).

Despite the lack of evidence for any developmental effects, it is clear that once MPs are ingested by mosquito larvae, a small proportion can be vertically transmitted into the emerging adults. Following exposure of mosquito larvae to very high concentrations (800,000 MP/mL) of 2-µm MP particles, approximately 0.01% persisted into adulthood (Al-Jaibachi et al., 2018) with the MP particles accumulating in the Malpighian tubules-five tubule structures connected to the midgut and hindgut which are critical for excreting nitrogenous waste, osmoregulation and detoxification (Piermarini et al., 2017). The Malpighian tubules remain intact during metamorphosis explaining why MPs may persist inside this tissue into adulthood, although whether MPs perturb the function of Malpighian tubules is unknown. MPs were observed in the adult gut of Ae. aegypti and Ae. albopictus following exposure to 1-µm fluorescent polystyrene beads with MPs excreted in the frass of sugar-fed adult Aedes mosquitoes (Edwards et al., 2023). By contrast, no ontogenic transfer of polyethylene MPs to pupae or adults was observed following exposure of first instar larvae (Griffin et al., 2023). Differences in the density between MP type (polyethylene vs. polystyrene) or ability to clear the gastrointestinal tract prior to moulting could explain these contrasting findings.

It is unlikely that the presence of MPs in adult mosquitoes will have any negative impact on behaviours such as flight performance, host-seeking or nectar feeding, although there is a significant lack of data to support this. However, even at the upper estimates of the number of MP particles persisting in adults following high exposure, the mass of these MPs would still be a very small fraction of the mass of an adult female (\sim 2.0 mg), so it is unlikely to impact flight activity. The presence of MPs in adults does, however, make mosquitoes a potential aerial vector of plastic polymers, facilitating the transfer of MPs into new environments and between trophic levels (Al-Jaibachi et al., 2018).

Chemical

A potentially more impactful effect of exposure to MPs on mosquitoes are via the chemical additives which give plastic polymers flexibility and strength, as well as the other environmental pollutants that can adsorb to plastic particles. Plastic additives (plasticisers) are weakly bonded with the polymer and can easily leach into the environment. For this reason, their impact across a range of organisms has been studied widely (Hermabessiere et al., 2017). The two plasticisers given the most attention are bisphenol A (BPA) and the phthalates, and both are considered endocrine disruptors, even at low concentrations (Oehlmann et al., 2009). Contamination by these compounds can occur via natural processes (waterborne) or indirectly through MP ingestion. The main question concerning mosquito exposure is whether individuals are exposed to toxic concentrations of plasticiser in the larval habitat or on ingested MP particles. BPA concentrations of $\sim 1 \text{ mg/L}$ were estimated from plastic-derived stagnant water in which Cx. quinquefasciatus were known to breed (Valsala & Asirvadam, 2022). At this concentration, BPA shortened the time of larval instar development by up to 25% with coincident surges of 20-hydroxy ecdysone, a hormone which controls larval moulting in insects. This agonistic effect, however, is in direct contrast to observations in houseflies where BPA delayed development (Izumi et al., 2008).

Tissue translocation

The translocation of MPs from the external environment into tissues or cells is a pre-requisite for MP-mediated effects such as inflammation or necrosis. Although many studies have reported tissue translocation in freshwater invertebrates, some of the reports are questionable due to the size of the particle and issues concerning the method of detection (Schür et al., 2019; Triebskorn et al., 2019). To penetrate cell epithelia, particles must either be small enough to cross membranes passively or they must cross actively via endocytosis. For mosquitoes and other invertebrates, a further barrier is the peritrophic membrane, a chitinous and semi-permeable barrier limiting the interaction between particles and epithelial cells (Lehane, 1997). At present, there is little evidence demonstrating the tissue translocation of MPs into insect or mosquito tissues and further experimental work is needed. That said, once particles have entered the cell, plastics and their additives can induce oxidative stress responses in humans and wildlife (Pérez-Albaladejo et al., 2020). The consequences of this metabolic disruption are cellular and macromolecule (e.g., lipid)

Microplastic parameters							
Plastic type	Size (µm)	Concentration (MPs/mL)	Method of detection and enumeration	Species	Life stage (of exposure to MPs)	End points	Reference
Carboxylate-modified fluorescent polystyrene	L	100 10,000 100,000	Microscopy	Aedes albopictus Ae. aegypti	First instar	MPs were found in the gut of all treated larvae and adults. MPs remained in adults for up to 4 days. In the gut, increased proportion of <i>Elizabethkingia</i> amplicon sequence variants were found in MP treatments than in controls, and generally, microbial diversity was lower in treatment groups.	Edwards et al., 2023
Carboxylate-modified fluorescent polystyrene Fluorescent polystyrene	15	8×10^{5} 8×10^{2}	Microscopy	Culex pipiens	Third instar	Ontogenetic transfer of MPs occurred with significant loss from larval to adult stages. MPs were located in Malpighian tubules of adults. Uptake of MPs was significantly greater in 2 μm and mixed 2 μm and 15 μm treatment groups.	Al-Jaibachi et al., 2018
Carboxylate-modified fluorescent polystyrene	0	100	Microscopy	Cx. pipiens	Larvae, 0.15-0.2 cm	MPs were detected in exposed mosquito larvae and in non-biting midge larvae (<i>Chaoborus flavicans</i>), a predator of <i>Cx. pipiens,</i> after predation. MP exposure did not affect predation by <i>Cx. flavicans</i> or oviposition by adult mosquitoes.	Cuthbert et al., 2019
Polystyrene	4.8-5.8	200 20,000	N/A	Cx. pipiens Cx. tarsalis	First instar	MP exposure did not affect survival, development time or growth rate.	Thormeyer & Tseng, 2023
Polyethylene	1–53 mixed	60 600 6000	Microscopy	Ae. albopictus Cx. quinquefasciatus	First instar	Species-specific mortality in L1; no effect on development time in survivors; no evidence of ontogenic transfer to adults	Griffin et al., 2023
Carboxylate-modified fluorescent polystyrene	2 15	50 100 200	Microscopy	Cx. pipiens	Third instar	Significantly more 2-µm MPs found in larvae, pupae and adults than 15 µm. MP exposure did not affect survival or weight of adults.	Al-Jaibachi et al., 2019
Abbreviation: MPs, microplastics.	Š						

TABLE 1 An overview of experimental studies exposing mosquitoes to microplastics and a summary of responses to exposure.

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damage; however, in invertebrates, most studies have quantified this using in vitro model systems (Imhof et al., 2017; Ogonowski et al., 2016). Due to high exposure to insecticides applied in vector control and the evolution of metabolic resistance (Ingham et al., 2018), the metabolome of mosquitoes is one the most studied among insects. To determine whether environmentally realistic concentrations of MPs or plasticisers induce oxidative stress pathways, mosquitoes are an ideal candidate organism, but demonstrating translocation of different types and size of MPs across tissue cells should be the first goal.

Plastisphere interactions with the mosquito microbiome

MPs in the environment are guickly colonised by biofilms with a distinct microbial signature to that of the surrounding environment. This so-called 'plastisphere' can support complex microbial communities, including viruses, prokaryotes and eukaryotes (Amaral-Zettler et al., 2020; Moresco et al., 2021; Ormsby et al., 2023). As such, ingestion of MPs offers a potential route for microbial infection to the mosquito gut. However, it is unknown whether these microbes could effectively colonise the gut, are transient or fail to infect due to colonisation resistance by native symbionts. Regardless, these microbes will likely interact with the host pathways, stimulating host immunity, and other microbes, but potentially altering microbiome homeostasis. For example, it is known that microbe-microbe interactions can dictate bacterial gut composition, and these interactions subsequently affect host phenotypes (Kozlova et al., 2021). Furthermore, bacterial infection of larvae has carryover effects for vector competence to arboviral pathogens in the adult (Dickson et al., 2017), so bacteria capable of gaining access to the larval mosquito gut can have significant phenotypic ramifications for the host and could alter vectorial capacity (VC) (Cansado-Utrilla et al., 2021). From what we understand about the accumulation of MPs in the adult Malpighian tubules, this might allow plastisphere bacteria to hitchhike and thereby infect new tissues within the insect. As mosquitoes are holometabolous insects, gut-associated bacteria naturally infect these tissues as part of a transstadial transmission route (Chavshin et al., 2015), so these transient MP-mediated infections could have the potential to infect multiple life stages from an initial larval infection in a similar manner to native gut microbiota.

Evidence from a range of aquatic and terrestrial fauna indicate MPs disturb the gut microbiome (Fackelmann & Sommer, 2019). In the honeybee, both nanoplastics and MPs cause microbiome dysbiosis and altered intestinal immunity (Wang et al., 2022). Given the interplay between immunity and the microbiome, it may be challenging to devise directionality of these interactions, but nevertheless, these processes could be influenced by plastics. The interaction with host immunity is not surprising, and various forms of MPs (sephadex, polystyrene and latex beads) have been used experimentally to deplete haemocytes and alter the immune system in a variety of vector (Barreaux et al., 2016; Borges et al., 2008) and non-vector species

(Silva et al., 2021). Disruption of microbiome homeostasis has also been seen in Drosophila, with MPs causing physical damage to the gut epithelium (Zhang et al., 2020). Larval ingestion of increasing concentrations of polystyrene MPs (1 µm) in Ae. aegypti and Ae. albopictus perturbed the gut microbiome and mycobiome (Edwards et al., 2023). This lends support to the hypothesis that MP consumption causes damage to the gut epithelial tissue in mosquitoes, potentially allowing for systemic infection of gut bacteria in other mosquito tissues, although it is not clear whether there are carryover effects from larvae to adults. This would have a detrimental effect on the host larvae as evidenced by the translocation of Serratia from the gut to the haemocoel, causing lethality in Anopheles mosquitoes (Wei et al., 2017). Additionally, epithelium damage in the adult gut could facilitate pathogen infection, potentially increasing or decreasing the vector competence of mosquitoes. Intriguingly, in some insect systems, microbiota provide protection or tolerance to the lethal effects of MPs (Wang et al., 2021). Taken together, evidence from other vertebrate and invertebrate systems indicates that MPs affect the microbiome, which will likely impact host biology, and thus, further investigations are warranted to investigate these interactions in mosquitoes.

IMPLICATIONS OF MP EXPOSURE ON VC

The ability of mosquitoes to transmit pathogens is described by the VC equation, an adaptation of the basic reproduction number (R0) that considers the most important elements of a mosquito's life history that matter for transmission (Brady et al., 2016).

Determining the effect of biotic and abiotic stressors on individual components of the VC is the subject of much research (e.g., Cansado-Utrilla et al., 2021; Paaijmans et al., 2011), particularly to improve our understanding of disease transmission within different environmental context and to optimise vector control efforts. For MPs to have an epidemiological impact, they must modulate individual components of VC, and from the evidence above, there is little direct evidence to date that MPs can significantly affect transmission. Mosquito density (m) is determined not only by developmental and growth rates but also by reproductive output and the ability to colonise habitat. Using 200 particles per millilitre as an environmentally relevant concentration for MPs in an urban habitat, exposure did not adversely affect development or growth rate in two Culex species (Thormeyer & Tseng, 2023). No impact on emergence rates were observed in Aedes sp. exposed to 100-100,000 MPs/mL (Edwards et al., 2023) and water treated with 100 MP/mL did not affect Culex pipiens (Linnaeus) oviposition (Cuthbert et al., 2019). In similar experiments, no effect on survival rate (p) were observed in Culex sp. (Al-Jaibachi et al., 2019; Thormeyer & Tseng, 2023). The biting rate (a) is determined by the ability of the mosquito to seek and take a blood meal from a host and so MPs would have to impact either mosquito flight or the olfactoryvisual system, of which there is no evidence currently. The most likely-albeit untested-route of MPs altering the VC, is the modulation of vector competence (b) via alterations to the mosquito microbiome, altering insect immunity (as described above) or directly

impacting the pathogen itself. When considering each of the VC parameters together, we conclude that there is little current evidence that MPs impact pathogen transmission in mosquitoes. Additional studies are needed, particularly concerning mosquito behaviour and pathogen-microbiome-MP interactions to understand the effect of MP ingestion on VC.

CONCLUSION

Filter-feeding aquatic insects, including mosquitoes, are susceptible to the ingestion of MPs. Of the roughly 3500 mosquito species, an estimated 331 transmit infectious pathogens (Yee et al., 2022), colonising a diverse range of aquatic habitats from urban containers, river and lake edges and tree holes. The rate of MP ingestion is determined in part by the bioavailability of MPs in the water column and although we may expect this to be highest for vectors living in highly urbanised or plasticised areas, the ubiquity of plastic waste across environments means that at least some mosquito species (e.g. Cx. quinquefasciatus, An. stephensi, Aedes sp.) are susceptible to a certain degree. The true impact of environmental plastics on animal life is still not well defined, and from our scoping review, we find little current evidence that MPs significantly affect mosquito biology. That said, there are still plenty of knowledge gaps concerning environmentally relevant exposures, impacts on mosquito behaviour and interactions with the microbiome. Mosquitoes represent an ideal organism in which to test hypotheses concerning the effect of MPs on individual components of the VC and disease transmission, not least because MPs can have a cascading effect on host-parasite-vector interactions in other systems (Schampera et al., 2021).

AUTHOR CONTRIBUTIONS

Christopher M. Jones: Conceptualization; funding acquisition; writing – original draft; writing – review and editing. Grant L. Hughes: Writing – original draft; writing – review and editing. Richard S. Quilliam: Conceptualization; funding acquisition; writing – original draft; writing – review and editing; project administration. Rosie Fellows: Writing – review and editing; visualization. Sylvester Coleman: Writing – review and editing; visualization.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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REFERENCES

- Ahn, J., Sinka, M., Irish, S. & Zohdy, S. (2023) Modeling marine cargo traffic to identify countries in Africa with greatest risk of invasion by Anopheles stephensi. Scientific Reports, 13(1), 876.
- Al-Jaibachi, R., Cuthbert, R.N. & Callaghan, A. (2018) Up and away: ontogenic transference as a pathway for aerial dispersal of microplastics. *Biology Letters*, 14(9), 20180479.
- Al-Jaibachi, R., Cuthbert, R.N. & Callaghan, A. (2019) Examining effects of ontogenic microplastic transference on *Culex* mosquito mortality and adult weight. *Science of the Total Environment*, 651, 871–876.
- Amaral-Zettler, L.A., Zettler, E.R. & Mincer, T.J. (2020) Ecology of the plastisphere. Nature Reviews. Microbiology, 18(3), 139–151.
- Barreaux, A.M.G., Barreaux, P. & Koella, J.C. (2016) Overloading the immunity of the mosquito Anopheles gambiae with multiple immune challenges. Parasites & Vectors, 9, 210.
- Besseling, E., Wegner, A., Foekema, E.M., van den Heuvel-Greve, M.J. & Koelmans, A.A. (2013) Effects of microplastic on fitness and PCB bioaccumulation by the lugworm Arenicola marina (L.). *Environmental Science & Technology*, 47(1), 593–600.
- Borges, A.R., Santos, P.N., Furtado, A.F. & Figueiredo, R.C.B.Q. (2008) Phagocytosis of latex beads and bacteria by hemocytes of the triatomine bug *Rhodnius prolixus* (Hemiptera: Reduvidae). *Micron*, 39(4), 486–494.
- Brady, O.J., Godfray, H.C.J., Tatem, A.J., Gething, P.W., Cohen, J.M., McKenzie, F.E. et al. (2016) Vectorial capacity and vector control: reconsidering sensitivity to parameters for malaria elimination. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 110(2), 107–117.
- Calhoun, L.M., Avery, M., Jones, L., Gunarto, K., King, R., Roberts, J. et al. (2007) Combined sewage overflows (CSO) are major urban breeding sites for *Culex quinquefasciatus* in Atlanta, Georgia. *American Journal* of Tropical Medicine and Hygienes, 77(3), 478–484.
- Cansado-Utrilla, C., Zhao, S.Y., McCall, P.J., Coon, K.L. & Hughes, G.L. (2021) The microbiome and mosquito vectorial capacity: rich potential for discovery and translation. *Microbiome*, 9(1), 111.
- Chan, K., Tusting, L.S., Bottomley, C., Saito, K., Djouaka, R. & Lines, J. (2022) Malaria transmission and prevalence in rice-growing versus non-rice-growing villages in Africa: a systematic review and metaanalysis. *The Lancet Planetary Health*, 6(3), e257–e269.
- Chaves, L.F., Keogh, C.L., Vazquez-Prokopec, G.M. & Kitron, U.D. (2009) Combined sewage overflow enhances oviposition of *Culex quinque fasciatus* (Diptera: Culicidae) in urban areas. *Journal of Medical Entomology*, 46(2), 220–226.
- Chavshin, A.R., Oshaghi, M.A., Vatandoost, H., Yakhchali, B., Zarenejad, F. & Terenius, O. (2015) Malpighian tubules are important determinants of pseudomonas transstadial transmission and longtime persistence in Anopheles stephensi. Parasites & Vectors, 8, 36.
- Cole, M., Lindeque, P., Fileman, E., Halsband, C. & Galloway, T.S. (2015) The impact of polystyrene microplastics on feeding, function and fecundity in the marine copepod *Calanus helgolandicus*. *Environmental Science & Technology*, 49(2), 1130–1137.
- Corradini, F., Meza, P., Eguiluz, R., Casado, F., Huerta-Lwanga, E. & Geissen, V. (2019) Evidence of microplastic accumulation in agricultural soils from sewage sludge disposal. *Science of the Total Environment*, 671, 411–420.

- Cuthbert, R.N., Al-Jaibachi, R., Dalu, T., Dick, J.T.A. & Callaghan, A. (2019) The influence of microplastics on trophic interaction strengths and oviposition preferences of dipterans. *Science of the Total Environment*, 651, 2420–2423.
- Dickson, L.B., Jiolle, D., Minard, G., Moltini-Conclois, I., Volant, S., Ghozlane, A. et al. (2017) Carryover effects of larval exposure to different environmental bacteria drive adult trait variation in a mosquito vector. *Science Advances*, 3(8), e1700585.
- Edwards, C.C., McConnel, G., Ramos, D., Gurrola-Mares, Y., Dhondiram Arole, K., Green, M.J. et al. (2023) Microplastic ingestion perturbs the microbiome of Aedes albopictus (Diptera: Culicidae) and Aedes aegypti. Journal of Medical Entomology, 60(5), 884–898.
- Eerkes-Medrano, D., Thompson, R.C. & Aldridge, D.C. (2015) Microplastics in freshwater systems: a review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. *Water Research*, 75, 63–82.
- Fackelmann, G. & Sommer, S. (2019) Microplastics and the gut microbiome: how chronically exposed species may suffer from gut dysbiosis. *Marine Pollution Bulletin*, 143, 193–203.
- Fillinger, U., Sonye, G., Killeen, G.F., Knols, B.G.J. & Becker, N. (2004) The practical importance of permanent and semipermanent habitats for controlling aquatic stages of *Anopheles gambiae* sensu lato mosquitoes: operational observations from a rural town in western Kenya. *Tropical Medicine & International Health*, 9(12), 1274–1289.
- Foley, C.J., Feiner, Z.S., Malinich, T.D. & Höök, T.O. (2018) A meta-analysis of the effects of exposure to microplastics on fish and aquatic invertebrates. *Science of the Total Environment*, 631–632, 550–559.
- Frake, A.N., Namaona, W., Walker, E.D. & Messina, J.P. (2020) Estimating spatio-temporal distributions of mosquito breeding pools in irrigated agricultural schemes: a case study at the Bwanje Valley Irrigation Scheme. *Malaria Journal*, 19(1), 38.
- Griffin, C.D., Tominiko, C., Medeiros, M.C.I. & Walguarnery, J.W. (2023) Microplastic pollution differentially affects development of diseasevectoring Aedes and Culex mosquitoes. Ecotoxicology and Environmental Safety, 267, 115639.
- Gündoğdu, S., Çevik, C., Ayat, B., Aydoğan, B. & Karaca, S. (2018) How microplastics quantities increase with flood events? An example from Mersin Bay NE Levantine coast of Turkey. *Environmental Pollution*, 239, 342–350.
- Hermabessiere, L., Dehaut, A., Paul-Pont, I., Lacroix, C., Jezequel, R., Soudant, P. et al. (2017) Occurrence and effects of plastic additives on marine environments and organisms: a review. *Chemosphere*, 182, 781–793.
- Imhof, H.K., Rusek, J., Thiel, M., Wolinska, J. & Laforsch, C. (2017) Do microplastic particles affect *Daphnia magna* at the morphological, life history and molecular level? *PLoS One*, 12(11), e0187590.
- Ingham, V.A., Wagstaff, S. & Ranson, H. (2018) Transcriptomic meta-signatures identified in *Anopheles gambiae* populations reveal previously undetected insecticide resistance mechanisms. *Nature Communications*, 9(1), 5282.
- Izumi, N., Yanagibori, R., Shigeno, S. & Sajiki, J. (2008) Effects of bisphenol a on the development, growth, and sex ratio of the housefly Musca domestica. Environmental Toxicology and Chemistry, 27(6), 1343– 1353.
- Jones, C.M., Wilson, A.L., Stanton, M.C., Stothard, J.R., Guglielmo, F., Chirombo, J. et al. (2023) Integrating vector control within an emerging agricultural system in a region of climate vulnerability in southern Malawi: a focus on malaria, schistosomiasis, and arboviral diseases. Current Research in Parasitology & Vector-Borne Diseases, 4, 100133.
- Kowalski, N., Reichardt, A.M. & Waniek, J.J. (2016) Sinking rates of microplastics and potential implications of their alteration by physical, biological, and chemical factors. *Marine Pollution Bulletin*, 109(1), 310–319.
- Kozlova, E.V., Hegde, S., Roundy, C.M., Golovko, G., Saldaña, M.A., Hart, C.E. et al. (2021) Microbial interactions in the mosquito gut

determine Serratia colonization and blood-feeding propensity. The ISME Journal, 15(1), 93–108.

- Krystosik, A., Njoroge, G., Odhiambo, L., Forsyth, J.E., Mutuku, F. & LaBeaud, A.D. (2020) Solid wastes provide breeding sites, burrows, and food for biological disease vectors, and urban zoonotic reservoirs: a call to action for solutions-based research. *Frontiers in Public Health*, 7, 405. Available from: https://doi.org/10.3389/fpubh.2019. 00405
- Laju, R.L., Jayanthi, M., Jeyasanta, K.I., Patterson, J., Bilgi, D.S., Sathish, N. et al. (2023) Microplastic contamination in Indian rural and urban lacustrine ecosystems. *Science of the Total Environment*, 895, 165146.
- Lee, K.W., Shim, W.J., Kwon, O.Y. & Kang, J.H. (2013) Size-dependent effects of micro polystyrene particles in the marine copepod *Tigriopus japonicus*. *Environmental Science & Technology*, 47(19), 11278– 11283.
- Lehane, M.J. (1997) Peritrophic matrix structure and function. Annual Review of Entomology, 42(1), 525–550.
- Lenz, R., Enders, K. & Nielsen, T.G. (2016) Microplastic exposure studies should be environmentally realistic. National Academy of Sciences of the United States of America, 113(29), E4121–E4122.
- Li, J., Liu, H. & Paul, C.J. (2018) Microplastics in freshwater systems: a review on occurrence, environmental effects, and methods for microplastics detection. *Water Research*, 137, 362–374.
- Maquart, P.O., Froehlich, Y. & Boyer, S. (2022) Plastic pollution and infectious diseases. *The Lancet Planetary Health*, 6(10), e842–e845.
- Merritt, R., Dadd, R., Walker, E., Merritt, R.W., Dadd, R.H. & Walker, E.D. (1992) Feeding behavior, natural food, and nutritional relationships of larval mosquitoes. Annu rev Entomol 37: 349-376. Annual Review of Entomology, 37, 349–376.
- Mnzava, A., Monroe, A.C. & Okumu, F. (2022) Anopheles stephensi in Africa requires a more integrated response. *Malaria Journal*, 21(1), 156.
- Moresco, V., Oliver, D.M., Weidmann, M., Matallana-Surget, S. & Quilliam, R.S. (2021) Survival of human enteric and respiratory viruses on plastics in soil, freshwater, and marine environments. *Envi*ronmental Research, 199, 111367.
- Murphy, F., Ewins, C., Carbonnier, F. & Quinn, B. (2016) Wastewater treatment works (WwTW) as a source of microplastics in the aquatic environment. *Environmental Science & Technology*, 50(11), 5800–5808.
- Oehlmann, J., Schulte-Oehlmann, U., Kloas, W., Jagnytsch, O., Lutz, I., Kusk, K.O. et al. (2009) A critical analysis of the biological impacts of plasticizers on wildlife. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364(1526), 2047–2062.
- 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), e0155063.
- Ormsby, M.J., Akinbobola, A. & Quilliam, R.S. (2023) Plastic pollution and fungal, protozoan, and helminth pathogens—a neglected environmental and public health issue? *Science of the Total Environment*, 882, 163093.
- Paaijmans, K.P., Blanford, S., Chan, B.H.K. & Thomas, M.B. (2011) Warmer temperatures reduce the vectorial capacity of malaria mosquitoes. *Biology Letters*, 8(3), 465–468.
- Pérez-Albaladejo, E., Solé, M. & Porte, C. (2020) Plastics and plastic additives as inducers of oxidative stress. *Current Opinion in Toxicology*, 20-21, 69-76.
- Piermarini, P.M., Esquivel, C.J. & Denton, J.S. (2017) Malpighian tubules as novel targets for mosquito control. *International Journal of Environmental Research and Public Health*, 14(2), 111.
- Pivokonsky, M., Cermakova, L., Novotna, K., Peer, P., Cajthaml, T. & Janda, V. (2018) Occurrence of microplastics in raw and treated drinking water. *Science of the Total Environment*, 643, 1644–1651.
- Rillig, M.C. & Lehmann, A. (2020) Microplastic in terrestrial ecosystems. *Science*, 368(6498), 1430–1431.

- Sanou, A., Nelli, L., Guelbéogo, W.M., Cissé, F., Tapsoba, M., Ouédraogo, P. et al. (2021) Insecticide resistance and behavioural adaptation as a response to long-lasting insecticidal net deployment in malaria vectors in the cascades region of Burkina Faso. *Scientific Reports*, 11(1), 17569.
- Schampera, C., Wolinska, J., Bachelier, J.B., de Souza Machado, A.A., Rosal, R., González-Pleiter, M. et al. (2021) Exposure to nanoplastics affects the outcome of infectious disease in phytoplankton. *Environmental Pollution*, 277, 116781.
- Scherer, C., Brennholt, N., Reifferscheid, G. & Wagner, M. (2017) Feeding type and development drive the ingestion of microplastics by freshwater invertebrates. *Scientific Reports*, 7, 17006.
- Schür, C., Rist, S., Baun, A., Mayer, P., Hartmann, N.B. & Wagner, M. (2019) When fluorescence is not a particle: the tissue translocation of microplastics in *Daphnia magna* seems an artifact. *Environmental Toxicology and Chemistry*, 38(7), 1495–1503.
- Silva, C.J.M., Beleza, S., Campos, D., Soares, A.M.V.M., Patrício Silva, A.L., Pestana, J.L.T. et al. (2021) Immune response triggered by the ingestion of polyethylene microplastics in the dipteran larvae Chironomus riparius. Journal of Hazardous Materials, 414, 125401.
- Sinka, M.E., Pironon, S., Massey, N.C., Longbottom, J., Hemingway, J., Moyes, C.L. et al. (2020) A new malaria vector in Africa: predicting the expansion range of Anopheles stephensi and identifying the urban populations at risk. National Academy of Sciences of the United States of America, 117(40), 24900–24908.
- Thormeyer, M. & Tseng, M. (2023) No effect of realistic microplastic exposure on growth and development of wild-caught *Culex* (Diptera: Culicidae) mosquitoes. *Journal of Medical Entomology*, 60, 604–607.
- Triebskorn, R., Braunbeck, T., Grummt, T., Hanslik, L., Huppertsberg, S., Jekel, M. et al. (2019) Relevance of nano- and microplastics for freshwater ecosystems: a critical review. *TrAC Trends in Analytical Chemistry*, 110, 375–392.
- Valsala, A.G.R. & Asirvadam, E.D. (2022) Bisphenol A acts as developmental agonist in Culex quinquefasciatus Say. Environmental Science and Pollution Research, 29(49), 74428–74441.
- Villafañe, A.B., Ronda, A.C., Rodríguez Pirani, L.S., Picone, A.L., Lucchi, L.D., Romano, R.M. et al. (2023) Microplastics and anthropogenic debris in rainwater from Bahia Blanca, Argentina. *Heliyon* [Internet], 9(6), e17028 https://www.cell.com/heliyon/abstract/S2405-8440(23) 04236-6
- Wagner, M., Scherer, C., Alvarez-Muñoz, D., Brennholt, N., Bourrain, X., Buchinger, S. et al. (2014) Microplastics in freshwater ecosystems: what we know and what we need to know. *Environmental Sciences Europe*, 26(1), 12.
- Wagner, S., Hüffer, T., Klöckner, P., Wehrhahn, M., Hofmann, T. & Reemtsma, T. (2018) Tire wear particles in the aquatic environment—

a review on generation, analysis, occurrence, fate and effects. *Water Research*, 139, 83–100.

Wang, K., Li, J., Zhao, L., Mu, X., Wang, C., Wang, M. et al. (2021) Gut microbiota protects honey bees (*Apis mellifera* L.) against polystyrene microplastics exposure risks. *Journal of Hazardous Materials*, 402, 123828.

l and Veterinary

- Wang, K., Zhu, L., Rao, L., Zhao, L., Wang, Y., Wu, X. et al. (2022) Nanoand micro-polystyrene plastics disturb gut microbiota and intestinal immune system in honeybee. *Science of the Total Environment*, 842, 156819.
- 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*. Environmental Pollution, 234, 181–189.
- Wei, G., Lai, Y., Wang, G., Chen, H., Li, F. & Wang, S. (2017) Insect pathogenic fungus interacts with the gut microbiota to accelerate mosquito mortality. *National Academy of Sciences of the United States of America*, 114(23), 5994–5999.
- Wong, J.K.H., Lee, K.K., Tang, K.H.D. & Yap, P.S. (2020) Microplastics in the freshwater and terrestrial environments: prevalence, fates, impacts and sustainable solutions. *Science of the Total Environment*, 719, 137512.
- Wright, S.L., Thompson, R.C. & Galloway, T.S. (2013) The physical impacts of microplastics on marine organisms: a review. *Environmental Pollution*, 178, 483–492.
- Yee, D.A., Dean Bermond, C., Reyes-Torres, L.J., Fijman, N.S., Scavo, N.A., Nelsen, J. et al. (2022) Robust network stability of mosquitoes and human pathogens of medical importance. *Parasites & Vectors*, 15(1), 216.
- Zhang, Y., Wolosker, M.B., Zhao, Y., Ren, H. & Lemos, B. (2020) Exposure to microplastics cause gut damage, locomotor dysfunction, epigenetic silencing, and aggravate cadmium (Cd) toxicity in *Drosophila*. *Science of the Total Environment*, 744, 140979.
- Ziajahromi, S., Kumar, A., Neale, P.A. & Leusch, F.D.L. (2018) Environmentally relevant concentrations of polyethylene microplastics negatively impact the survival, growth and emergence of sediment-dwelling invertebrates. Environmental Pollution, 236, 425–431.

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