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Short Communication

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Short Communication

No Effect of Realistic Microplastic Exposure on Growth and Development of Wild-caught Culex (Diptera: Culicidae) Mosquitoes

Markus Thormeyer[1,3] and Michelle Tseng[2]

[1]Department of Zoology, Biodiversity Research Centre, University of British Columbia, 4200-6270 University Boulevard, Vancouver, BC, V6T 1Z4, Canada, [2]Departments of Botany and Zoology, Biodiversity Research Centre, University of British Columbia, 3156-6720 University Boulevard, Vancouver, BC, V6T 1Z4, Canada, and [3]Corresponding author, e-mail: thormeyer@zoology.ubc.ca

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Abstract

Microplastic (MP) pollution is a threat to environments around the world and mosquitoes are particularly affected because of their high chance of encountering MP as larvae. Mosquitoes have been shown to readily consume microplastics and they have a significant impact on health in society, yet we have limited knowledge on the effects of MP exposure on fitness-related traits. Additionally, the data we do have come primarily from studies that have used unrealistically high microplastic concentrations, or unrealistic methods of exposure. Here we exposed wild-type first instar *Culex pipiens* and *Culex tarsalis* larvae to two 4.8–5.8 μm polystyrene microplastic concentrations (0 particles/ml, 200 particles/ml, 20,000 particles/ml) to evaluate the effect of MP exposure on body size, development, and growth rate. We found no effect of microplastics on any of the traits in either species. These results indicate microplastic exposures comparable to levels found in nature have minimal effects on these fitness-related traits. Future directions for this work include examining whether the effects of MP exposure are exacerbated when evaluated in combination with other common stressors, such as warming temperatures, pesticides, and food limitation.

Key words: Culex, Culicidae, urban pest, toxicology

Microplastics (MPs) are classified as plastic particles smaller than 5 mm in diameter, are potentially toxic, and are omnipresent throughout natural and urban systems (Silva et al. 2018, Corradini et al. 2019, Dris et al. 2016, Eriksen 2013, Lin 2021). Most of the work on MP done in the last few decades has focused on characterizing the quality and quantity of MP in the environment, while more recent work has focused on the consequences of MP ingestion; mainly physiological effects, (Muhammad et al. 2021, Fudlosid et al. 2022), accumulation in tissues (McIlwraith et al. 2021, Wang et al. 2021a, b), and trophic effects (Al-Jaibachi et al. 2018). Particular effects of MPs depend on the type of MP type used (i.e., polystyrene, polyethylene, polypropylene, polyester), the size of the MP, the shape of the MP (i.e., fiber, sphere, crushed particles), and the organism ingesting the microplastic (Sanchez-Hernandez 2021). Most studies to date have focused on MPs in marine environments, however MPs are also prevalent in freshwater and terrestrial environments (Eerkes-Medrano et al. 2015).

In lakes and ponds, MPs can remain buoyant in the aquatic environment or they can sink to the bottom (Semcesen and Wells 2021). Consequently, filter-feeding organisms such as mosquitoes and zooplankton have a high probability of encountering and ingesting MPs. Mosquitoes in particular are likely to ingest MPs because they can feed in the water column as well as graze off various surfaces. Despite their importance for public health, we currently have a limited understanding of the effects of MP exposure on mosquito fitness. Studies on laboratory colonies of third instar *Culex pipiens* L. demonstrated that MP doses of up to 200 MPs/ml did not affect adult weight (Al-Jaibachi et al. 2019). In the same study system at higher doses (8×10^5 MPs/ml), MP accumulated in the gut and Malpighian tubules of larvae, and were also found in the Malpighian tubules post-metamorphosis (Al-Jaibachi et al. 2018). Other experiments have demonstrated that the presence of MPs did not affect oviposition behavior of C. *pipiens* (Cuthbert et al. 2019) and finally, exposure of fourth instar *Culex quinquesfasciatus* Say to

MP (4.2×10^6/L) delayed development and altered their biochemical profiles (Malafaia et al. 2020).

Although we now have some knowledge of how MP exposure may affect growth and development in mosquitoes, several gaps remain. For example, some experiments in this area have used MP doses that are orders of magnitude higher than concentrations found in the environment (Al-Jaibachi et al. 2018, Koelmans et al. 2019, Bucci et al. 2020, Stanton et al. 2020), and thus the relevance of these studies to natural ecosystems may be limited. Additionally, the few studies that have examined the effects of MP on mosquitoes have used third or fourth instar larvae (Al-Jaibachi et al. 2019, Malafaia et al. 2020). Exposing organisms to MPs only after certain developmental stages can give misleading insights into the effects of MP ingestion in nature, as they are likely to be exposed to MPs continuously immediately after hatching. MP toxicity experiments that use concentrations and exposure durations comparable to those found in natural settings are necessary to better understand the ecological effects of these pollutants.

Here, we exposed wild-caught first instar Cx *pipiens* and *Culex tarsalis* Coquillett larvae to two polystyrene microplastic concentrations. The objectives of this experiment were to determine if mosquito growth and development were affected by MP doses that are more similar to those observed in nature, and to assess the effects of MPs when larvae are continuously exposed immediately after hatching. We used zero, 200 particles/ml, and 20,000 particles/ml of 4.8–5.8 µm polystyrene beads for our MP treatments. About 200 particles/ml represents the highest values of MP seen in urban waters to date (e.g., in waste water treatment plants) but is also below concentrations of MP found in river, pond, and lake sediment (Koelmans et al. 2019), (sediment refs: Turner et al 2019, Yang et al 2021, Castañeda et al 2014, Duis and Coors 2016). Because mosquitoes both filter water in the water column and graze on sediment and detritus (REF), we believe 200 particles/ml is a reasonable approximation for quantities of MP that may be encountered by *Culex* larvae in urban environments. We used 20,000 particles/ml because many other studies have used this concentration and thus we could compare our results to existing studies. We chose *Culex* species because of their wide-ranging global distribution and public health importance. Given the results of similar studies, we predicted that a MP dose of 20,000 particles per ml would result in minor decreases in mosquito growth rate but that the dose of 200 MP/ml would not affect mosquito growth or development.

Methods

Microplastic Solutions and Experimental Design

MP solution was made by mixing 4.8–5.8 µm polystyrene spherical beads (more information in the supplementary materials) with 1 liters of reverse osmosis (RO) water. The three experimental treatments were control (0 particles/ml), low (200 particles/ml), and high (20,000 particles/ml). These concentrations were made by calculating the mass per particle and weighing out the appropriate amounts, following the equations in (Connors et al. 2017). These equations are made available in the Supplementary Materials (S1).

Culex pipiens and Cx *tarsalis* egg rafts were collected from artificial containers on the University of British Columbia campus in July 2021. Egg rafts were separated and kept in reverse osmosis (RO) water. The two egg rafts (per species) that hatched soonest after collection were used for the experiment, and first instar larvae were transferred into respective treatments one day after hatching. Each level in the MP treatment (0, 200 MP/ml, 20,000 MP/ml) contained 20 individually-reared Cx *pipiens* and 20 individually-reared Cx *tarsalis*, and each egg raft contributed 10 mosquitoes to each treatment, for a total of 120 mosquitoes across all treatments. Individual mosquitoes were reared singly in 50 ml beakers, and water or MP solution in each beaker was replaced every 7 d. In the treatments with microplastics, larvae were thus exposed microplastics from one day posthatching until they emerged as adults. To ensure unlimited food, larvae were fed 0.8 mg ground TetraMin tropical flake fish food daily (Reiskind and Wilson 2008). The experiment was conducted in Panasonic MIR-254 incubators on a 16/8 h light-dark photoperiod and 25/18°C day/night temperature regime.

Data Collected

Adult emergence was dates recorded and development time was defined as the number of days from hatching to adult. Upon emergence, adult mosquitoes were immediately sexed and frozen at −18°C. We used right wing length as a proxy of body size (Petersen et al. 2016). Right wings were removed and photographed under a dissecting microscope at 2.0× magnification (Zeiss Stemi 508). We used ImageJ (Rasband 1997–2018) to measure the length of the wing from the alular notch to wing tip. We calculated growth rate by dividing wing length by development time.

Analysis

All statistical analyses were done in R version 4.1.1. For both species, we created three linear mixed-effects models with wing length, development time, and growth rate as the dependant variables. For each model sex, MP treatment, and an interaction between sex and MP treatment were included as the explanatory variables. Egg raft ID was included as a random effect.

Results

Survival rates in this experiment were high for both species in all treatments. Cx *pipiens* survival was 100%, 95%, and 95% for the 0, 200, and 20,000 particles/ml treatments respectively. Cx *tarsalis* survival rates were 75%, 90%, and 90% in the 0, 200, and 20,000 particles/ml treatments respectively.

Females had longer wings than males (Fig. 1) in both Cx *pipiens* ($F_{(1,52)} = 242.86, p < 0.001$) and Cx *tarsalis* ($F_{(1,44)} = 55.12, p < 0.001$). Variation in wing length was not explained by MP treatment for Cx *pipiens* ($F_{(2,52)} = 2.211, p = 0.120$) or Cx *tarsalis* ($F_{(2,44)} = 1.625, p = 0.209$). The interaction term between sex and MP treatment was also not significant: Cx *pipiens* ($F_{(2,52)} = 0.466, p = 0.630$) and Cx *tarsalis* ($F_{(2,44)} = 0.955, p = 0.393$).

Females took longer to develop than males did (Fig. 1) for both Cx *pipiens* ($F_{(1,51)} = 19.421, p < 0.001$), and Cx *tarsalis* ($F_{(1, 44)} = 5.853, p = 0.012$). There was no effect MP treatment on mosquito development time for Cx *pipiens* ($F_{(2,51)} = 0.944, p = 0.396$), or Cx *tarsalis* ($F_{(2, 44)} = 0.444, p = 0.644$). The interaction between sex and MP treatment was also not significant: Cx *pipiens* ($F_{(2,51)} = 0.808, p = 0.451$) and Cx *tarsalis* ($F_{(2,44)} = 0.519, p = 0.599$).

Growth rate was faster in females (Fig. 1) than males for both Cx *pipiens* ($F_{(1, 52)}= 242.86, p < 0.001$), and Cx *tarsalis* ($F_{(1,44)} = 55.12, p < 0.001$). There were no effects of MP treatment on the growth rate of Cx *pipiens* ($F_{(2,52)} = 2.212, p = 0.120$), or Cx *tarsalis* ($F_{(2, 44)} = 1.625, p = 0.209$). The interaction between sex and MP was also not significant for Cx *pipiens* ($F_{(2,52)} = 0.4657, p = 0.630$), or Cx *tarsalis* ($F_{(2, 44)} = 0.955, p = 0.392$).

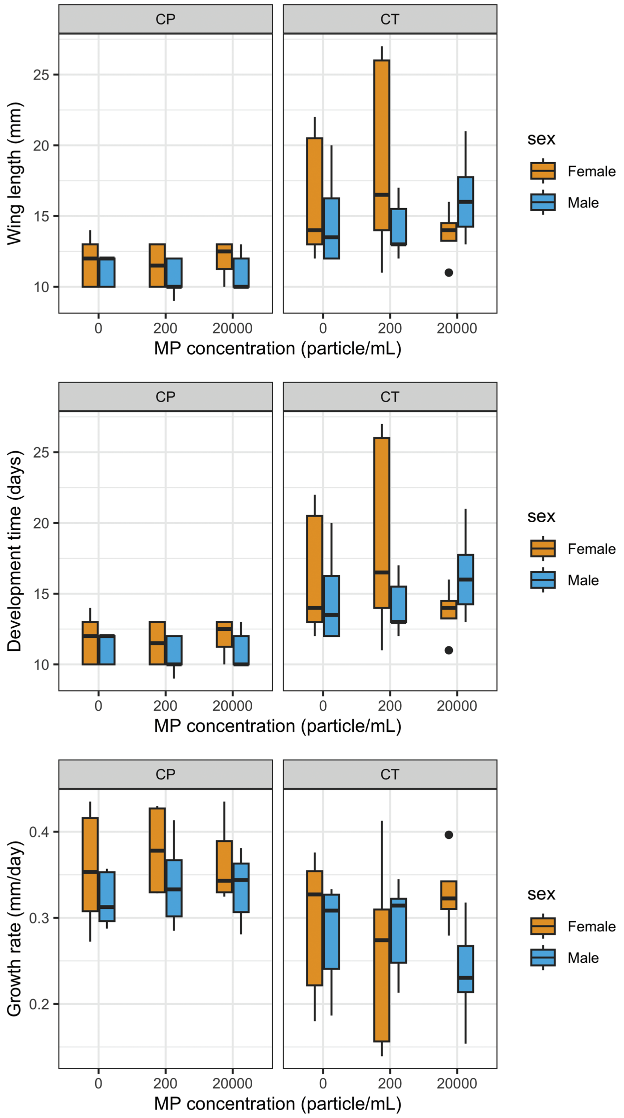

Fig. 1. The effect of MP exposure on *Culex pipiens* (control *n* = 20; 200, 20,000 particles/ml *n* = 19) and *Culex tarsalis* (control *n* = 15; 200, 20,000 particles/ml *n* = 18) (a) wing length (b) development time, and (c) growth rate. The three MP concentrations are 0 particles/ml, 200 particles/ml, and 20,000 particles/ml. Within each treatment sex is differentiated by color, with the female data on the left and male data on the right.

Discussion

Overall, our results showed that doses of 200 or 20,000 particles per ml of 4.8–5.8 µm polystyrene beads had no effect on the body size, development, or growth rate of Cx *pipiens* or Cx *tarsalis*. Our study is unique in that we exposed mosquitoes to microplastics immediately after hatching, we used wild-caught species instead of laboratory colonies, and we used MP doses similar to that found in polluted waters in nature. We also exposed Cx *tarsalis* to MPs, the first experiment to our knowledge to do so. These data suggest that under ideal growing conditions (unlimited food, no competition) MP exposure had minimal effects on mosquito growth and development.

The lack of response of mosquitoes to the 200 MP/ml treatment matched our predictions and was similar to the response of Cx *pipiens* body weight reported in Al-Jaibachi et al. (2019). Together these studies suggest that at this low dose, whether larvae are exposed at first (this experiment) or third instar (Al-Jaibachi et al. 2019 study) makes little difference to the overall outcome. However, our results for the 20,000 MP/ml treatment differed from those found by Malafaia et al. (2020). In that study, fourth instar Cx *quinquefasciatus* that were exposed to similar doses of larger, irregularly shaped particles (17–53 µm) exhibited delayed growth. It is possible that the particle type and size used in Malafaia et al. (2020) had greater negative effects on mosquito development than smaller-size polystyrene particles used here, or that Cx *quinquefasciatus* is inherently more sensitive to MP ingestion compared to Cx *pipiens* or Cx *tarsalis*. MP particle size affected organism morbidity in zooplankton (Liu et al. 2022) and earthworms (Xiao et al. 2022) but in both cases, smaller-sized particles caused more damage than larger ones. Finally, it is also possible we did not see effects of microplastics on mosquito life history traits because mosquitoes actively avoided ingesting them. We do not believe this to be the case because previous research has shown that third and fourth Cx. *pipiens* readily ingest microspheres up to 100 microns in diameter and that first and second instars can ingest spheres up to approximately 60 um in size (Dadd 1971). Additionally, studies testing the effects of similar-sized MP and concentrations on closely related mosquito species have all reported consumption of the particles by mosquito larvae (Al-Jaibachi et al. 2018, Malafaia et al. 2020).

Our study examined the effects of MP exposure on mosquito life history traits because of their clear link to overall mosquito population growth and fitness (Ruybal et al. 2016). In other insects, MP exposure is speculated to reduce total body size by affecting the ability of insects to properly assimilate nutrients, either by blocking the digestive tract, damaging intestinal tissue, or by acting as a poor quality dietary addition and diluting ingested nutrients (Silva et al. 2018, Malafaia et al. 2020, Sanchez-Hernandez 2021, Fudlosid et al. 2022). A limitation of investigating life history traits is that the effects of MPs may be more apparent at the physiological level. For example, the silk moth *Bombyx mori* L. exhibited clear immune responses when exposed to a 5–5.9 µm 9.78×10^4 MPs/ml dosage in food (Muhammad et al. 2021). Recent studies on honeybees found that exposure to MPs increased gene regulation associated with detoxification and immune responses (Deng et al. 2021) and altered the gut microbiome (Wang et al. 2021a, b). The up or down regulation of immune function by MP could have significant implications for disease spread by mosquitoes (Bartholomay and Michel 2018). An additional limitation to this study is that it was conducted in controlled laboratory conditions and thus whether these doses of MP also have no effect on mosquitoes in the wild, where they are exposed to other environmental factors, such as increased temperatures, heavy metals, or pesticides (Mao et al. 2022), is unknown.

In conclusion, here we have documented that relatively realistic doses of MP have no effects on three life history traits in two species of *Culex* mosquitoes. Our study provides some balance to the many existing experiments that have subjected study organisms to doses of MP that far exceed what is commonly found in nature (Bucci et al. 2020). We echo the call made by others (e.g., Bucci et al. 2020) that more ecologically and environmentally relevant studies are needed to better understand the effects of this ubiquitous pollutant in natural systems.

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is located on the traditional, ancestral, and unceded territory of the Musqueam Nation.

Supplementary Data

Supplementary data are available at *Journal of Medical Entomology* online.

References Cited

Al-Jaibachi, R., R. N. Cuthbert, and A. Callaghan. 2018. Up and away: ontogenic transference as a pathway for aerial dispersal of microplastics. *Biol. Lett.* 14: 20180479.

Al-Jaibachi, R., R. N. Cuthbert, and A. Callaghan. 2019. Examining effects of ontogenic microplastic transference on Culex mosquito mortality and adult weight. *Sci. Total Environ.* 651: 871–876.

Bartholomay, L. C., and K. Michel. 2018. Mosquito immunobiology: the intersection of vector health and vector competence. *Annu. Rev. Entomol.* 63: 145–167.

Bucci, K., M. Tulio, and C. M. Rochman. 2020. What is known and unknown about the effects of plastic pollution: a meta-analysis and systematic review. *Ecol. Appl.* 30: e02044.

Castañeda, R. A., S. Avlijas, M. A. Simard, and A. Ricciardi. 2014. Microplastic pollution in St. Lawrence River sediments. *Can. J. Fish. Aquat. Sci.* 71: 1767–1771.

Connors, K. A., S. D. Dyer, and S. E. Belanger. 2017. Advancing the quality of environmental microplastic research: advancing the quality of environmental microplastic research. *Environ. Toxicol. Chem.* 36: 1697–1703.

Corradini, F., P. Meza, R. Eguiluz, F. Casado, E. Huerta-Lwanga, and V. Geissen. 2019. Evidence of microplastic accumulation in agricultural soils from sewage sludge disposal. *Science of the Total Environment*. 671: 411–420.

Cuthbert, R. N., R. Al-Jaibachi, T. Dalu, J. T. A. Dick, and A. Callaghan. 2019. The influence of microplastics on trophic interaction strengths and oviposition preferences of dipterans. *Sci. Total Environ.* 651: 2420–2423.

Dadd, R. H. 1971. Size Limitations on the Infectibility of Mosquito Larvae by Nematodes during Filter-Feeding. *Journal of Invertebrate Pathology*. 18: 246–251.

Deng, Y., X. Jiang, H. Zhao, S. Yang, J. Gao, Y. Wu, Q. Diao, and C. Hou. 2021. Microplastic polystyrene ingestion promotes the susceptibility of honeybee to viral infection. *Environ. Sci. Technol.* 55: 11680–11692.

Dris, R., J. Gasperi, M. Saad, C. Mirande, and B. Tassin. 2016. Synthetic fibers in atmospheric fallout: A source of microplastics in the environment? *Marine Pollution Bulletin*. 104: 290–293.

Duis, K., and A. Coors. 2016. Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. *Environ Sci Eur.* 28: 2.

Eerkes-Medrano, D., R. C. Thompson, and D. C. Aldridge. 2015. Microplastics in freshwater systems: a review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. *Water Res.* 75: 63–82.

Eriksen, M. 2013. Microplastic pollution in the surface waters of the Laurentian Great Lakes. *Marine Pollution Bulletin*. 6.

Fudlosid, S., M. W. Ritchie, M. J. Muzzatti, J. E. Allison, J. Provencher, and H. A. MacMillan. 2022. Ingestion of microplastic fibres, but not microplastic beads, impacts growth rates in the Tropical House Cricket Gryllodes Sigillatus. *Front. Physiol.* 13: 871149.

Koelmans, A. A., N. H. Mohamed Nor, E. Hermsen, M. Kooi, S. M. Mintenig, and J. De France. 2019. Microplastics in freshwaters and drinking water: critical review and assessment of data quality. *Water Res.* 155: 410–422.

Lin, J., X. Xiao-Ming, Y. Bei-Ying, X. Xiang-Po, L. Jin-Zhong, Z. Qing, and W. Jiang-Hai. 2021. Multidecadal records of microplastic accumulation in the coastal sediments of the East China Sea. 10.

Liu, Q., L. Liu, J. Huang, L. Gu, Y. Sun, L. Zhang, K. Lyu, and Z .Yang. 2022. The response of life history defense of cladocerans under predation risk varies with the size and concentration of microplastics. *J. Hazard. Mater.* 427: 127913.

Malafaia, G., T. M. da Luz, A. T. B. Guimarães, and A. P. C. Araújo. 2020. Polyethylene microplastics are ingested and induce biochemical changes in Culex quinquefasciatus (Diptera: Culicidae) freshwater insect larvae. *Ecotoxicol. Environ. Contam.* 15: 79–89.

Mao, X., Y. Xu, Z. Cheng, Y. Yang, Z. Guan, L. Jiang, and K. Tang. 2022. The impact of microplastic pollution on ecological environment: a review. *Front. Biosci.-Landmark*. 27: 046.

McIlwraith, H. K., J. Kim, P. Helm, S. P. Bhavsar, J. S. Metzger, and C. M. Rochman. 2021. Evidence of microplastic translocation in wild-caught fish and implications for microplastic accumulation dynamics in food webs. *Environ. Sci. Technol.* 55: 12372–12382.

Muhammad, A., X. Zhou, J. He, N. Zhang, X. Shen, C. Sun, B. Yan, and Y. Shao. 2021. Toxic effects of acute exposure to polystyrene microplastics and nanoplastics on the model insect, silkworm Bombyx mori. *Environ. Pollut.* 285: 117255.

Petersen, V., M. J. Marchi, D. Natal, M. T. Marrelli, A. C. Barbosa, and L. Suesdek. 2016. Assessment of the correlation between wing size and body weight in captive Culex quinquefasciatus. *Rev. Soc. Bras. Med. Trop.* 49: 508–511.

Rasband, W.S., ImageJ, U. S. **1997–2018**. National Institutes of Health, Bethesda, Maryland, USA. https://imagej.nih.gov/ij/.

Reiskind, M. H., and M. L. Wilson. 2008. Interspecific competition between Larval Culex restuans Theobald and Culex pipiens L.(Diptera: Culicidae) in Michigan. *J. Med. Entomol.* 45: 208–227.

Ruybal, J. E., L. D. Kramer, and A. M. Kilpatrick. 2016. Geographic variation in the response of Culex pipiens life history traits to temperature. *Parasit. Vectors*. 9: 116.

Sanchez-Hernandez, J. C. 2021. A toxicological perspective of plastic biodegradation by insect larvae. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* 248: 109117.

Semcesen, P. O., and M. G. Wells. 2021. Biofilm growth on buoyant microplastics leads to changes in settling rates: implications for microplastic retention in the Great Lakes. *Mar. Pollut. Bull.* 170: 112573.

Silva, A. B., A. S. Bastos, C. I. L. Justino, J. P. da Costa, A. C. Duarte, and T. A. P. Rocha-Santos. 2018. Microplastics in the environment: challenges in analytical chemistry - a review. *Anal. Chim. Acta.* 1017: 1–19.

Stanton, T., M. Johnson, P. Nathanail, W. MacNaughtan, and R. L. Gomes. 2020. Freshwater microplastic concentrations vary through both space and time. *Environ. Pollut.* 263: 114481.

Turner, S., A. A. Horton, N. L. Rose, and C. Hall. 2019. A temporal sediment record of microplastics in an urban lake, London, UK. *J Paleolimnol.* 61: 449–462.

Wang, K., J. Li, L. Zhao, X. Mu, C. Wang, M. Wang, X. Xue, S. Qi, and L. Wu. 2021a. Gut microbiota protects honey bees (Apis mellifera L.) against polystyrene microplastics exposure risks. *J. Hazard. Mater.* 402: 123828.

Wang, T., M. Hu, G. Xu, H. Shi, J. Y. S. Leung, and Y. Wang. 2021b. Microplastic accumulation via trophic transfer: can a predatory crab counter the adverse effects of microplastics by body defence? *Sci. Total Environ.* 754: 142099.

Xiao, X., E. He, X. Jiang, X. Li, W. Yang, J. Ruan, C. Zhao, R. Qiu, and Y. Tang. 2022. Visualizing and assessing the size-dependent oral uptake, tissue distribution, and detrimental effect of polystyrene microplastics in Eisenia fetida. *Environ. Pollut.* 306: 119436.

Yang, L., Y. Zhang, S. Kang, Z. Wang, and C. Wu. 2021. Microplastics in freshwater sediment: A review on methods, occurrence, and sources. *Science of the Total Environment*. 754: 141948.