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The translocation of nano- and microplastics to lipid droplets of *Daphnia magna* is an artefact

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The last decade has seen a surge in research investigating various aspects of micro- and nanoplastics, including occurrence, uptake, and potential effects in biota. Our knowledge about biota-particle-interactions is still limited and often based on early studies that – due to the infancy of the field – may have deficiencies in the experimental design and quality controls. One such example relates to the potential of plastic particles to cross the epithelium of the digestive tract and translocate to other tissues. This has been reported in the literature for the freshwater cladoceran *Daphnia magna* and – if true – is toxicologically relevant. The aim of our study was to replicate previous findings while covering additional experimental scenarios.

We exposed 205 starved *Daphnia magna* neonates to two particle types (fluorescent polystyrene spheres of 20 nm and 1000 nm diameter) at two concentrations (2 µg/L and 2 mg/L) for two time periods (4 h and 24 h). Additionally, we adapted a tissue clearing method to the use with *Daphnia* to improve visibility of particles inside the specimen. We used confocal laser scanning microscopy to investigate the translocation of particles from the gut to other tissues, including lipid storage droplets. This is supplemented by experiments regarding the leaching of the dye from the particles.

The 1000 nm particles can be imaged individually inside the specimen. At the higher particle concentration we detected fluorescence in the lipid droplets that for the 1000 nm particles was clearly separate from particles and faded within minutes of observation. Our findings indicate that the tissue translocation to the lipid storage droplets reported previously is probably an experimental artefact caused by the leaching of the dye used in commercially available plastic beads. This underlines the importance of scepticism and replication in our field, especially if a plausible underlying biological mechanism is lacking.