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Size-dependent uptake of micro- and nanoplastics in *Daphnia magna*

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Introduction and aim

The number of species, for which active and passive ingestion of microplastics has been observed, is steadily increasing. However, most studies so far focused on marine biota and knowledge on freshwater species is limited. Additionally, current approaches are mostly qualitative, since quantitative measures of ingested particles are analytically challenging.

Aims of this study:

- Use a quantitative approach for determining the micro- and nanoplastic body burden of the freshwater crustacean *Daphnia magna*
- Analyse the impact of particle size on particle uptake
- Investigate how feeding affects depuration of ingested micro- and nanoplastics

Methodology

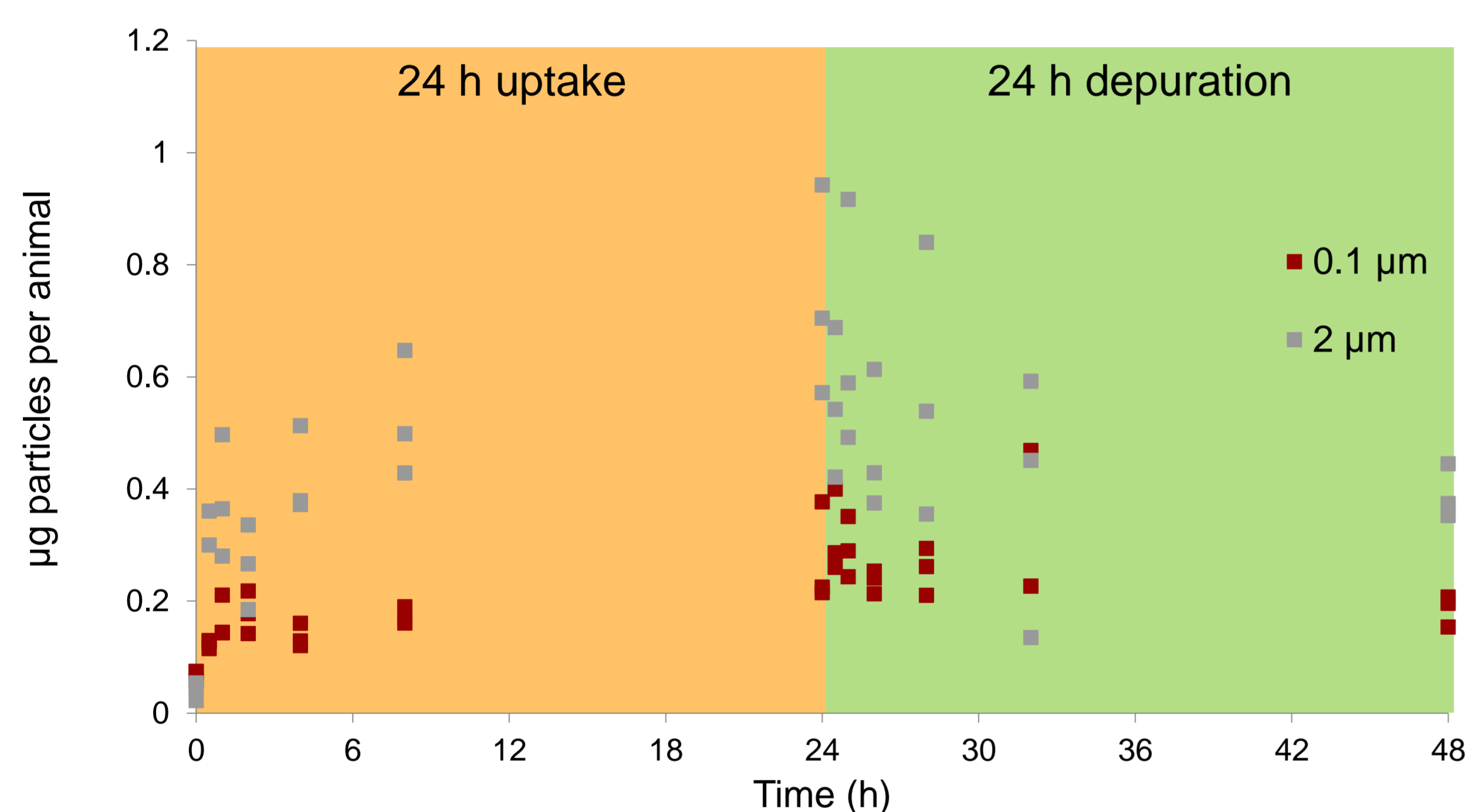
Uptake and depuration of micro- and nanoplastics:

- 24 h uptake phase: *D. magna* (1 week old) exposed to fluorescently labelled polystyrene beads of 0.1 μm or 2 μm (exposure concentration: 2 mg/l)
- 24 h depuration phase: animals transferred to clean medium
- During uptake and depuration: animals sampled at 0.5 h, 1 h, 2 h, 4 h, 8 h and 24 h, respectively (n=3)
- Enzymatic digestion of tissues (proteinase K protocol)
- Fluorescence measurement to determine particle load of the animals

Effect of food on depuration:

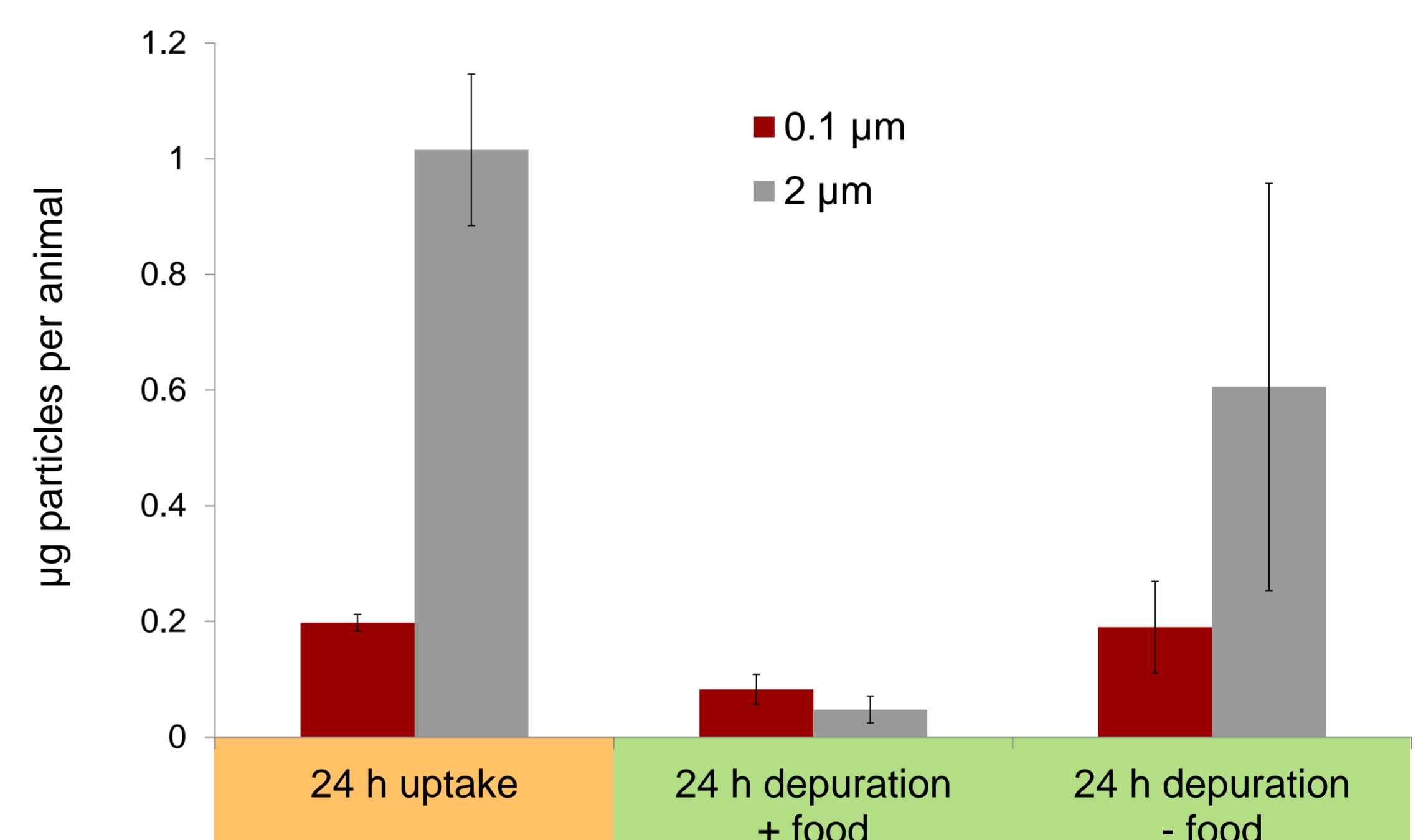
- Feeding test: animals fed with 10×10^6 algal cells (*Pseudokirchneriella subcapitata*) per test beaker at the beginning of the depuration phase
- Particle load was measured after 24 h of depuration

Uptake and depuration of 0.1 μm and 2 μm beads



Particle mass (μg) per animal of 3 replicates each at 6 different time points of uptake and depuration (0.5 h, 1 h, 2 h, 4 h, 8 h, 24 h respectively).

Effect of food on depuration of particles



Mean particle mass (μg) per animal with the standard deviation, measured after 24 h exposure to 2 mg/l particle solution and after 24 h depuration with or without the addition of algal cells.

Results

- Particle body burdens increased with exposure time up to a maximum after 24 h uptake.
- During 24 h depuration, particle body burdens decreased, but showed a high variability between replicates.
- After 24 h depuration, the particle body burdens were still 50-60% of the maximum load (reached after 24 h uptake) for both particle sizes.
- Particle body burden expressed as mass per animal was higher for the 2 μm beads than the 0.1 μm beads at all time points. However, in terms of particle number per animal the opposite was observed.
- The addition of food increased the depuration of both particle types, but to a larger extent for the 2 μm beads.

Conclusions

- The quantity of ingested micro- and nanoplastic particles depends on the particle size. In terms of mass the 2 μm particles are taken up in higher quantities than the 0.1 μm particles, while the picture reverses for particle number.
- Those differences could be a result of different uptake mechanisms, where the 2 μm beads are ingested actively, since they are in the size range of the food algae, while the 0.1 μm beads could be ingested passively with respiration and/or drinking.
- In the presence of food, the animals clear their guts faster, which enhances the egestion of particles. The results indicate, however, that the egestion is less efficient for the 0.1 μm particles. Nanoplastics might therefore remain in the gut for a longer period.

