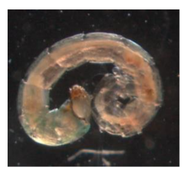
Ingestion of microplastics by the chironomid *Chironomus sancticaroli* and effects on the microbiome in the presence of PBDEs

Alice A. Horton[1], Angela M. Palacio-Cortés[2], Elma Lahive[1], Lindsay Newbold[1], M. Gloria Pereira[3], Rodrigo G. Disner[2], Mário A. Navarro-Silva[2], Marco Tadeu Grassi[2], David J. Spurgeon[1]

[1] *Centre for Ecology and Hydrology, Maclean Building, Benson Lane, Crowmarsh Gifford, Oxfordshire, OX10 8BB, UK.*
[2] *Departamento de Zoologia, Setor de Ciências Biológicas, Universidade Federal do Paraná. Caixa Postal 19020, 81531-980 Curitiba, PR, Brazil.*
[3] *Centre for Ecology and Hydrology, Library Avenue, Lancaster Environment Centre, Lancaster, Bailrigg LA1 4AP, UK.*

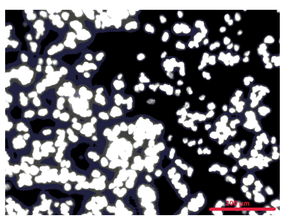
Background

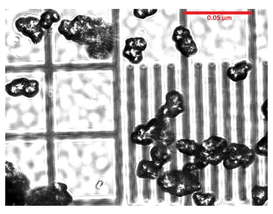


Microplastic particles in the environment can associate with persistent organic pollutants (POPs) due to the hydrophobic nature of plastics and organic chemicals. PBDEs (polybrominated diphenyl ethers) are widely used as flame-retardants in products such as textiles and soft furnishings, with the potential to leach into the environment and be associated with microplastics. If ingested, the gut environment of an organism may favour desorption of adsorbed chemicals due to gut condition. Therefore the ingestion of microplastic particles has implications for uptake and bioaccumulation of these chemicals. Furthermore the presence of microplastics and chemicals in the gut of an organism can also influence the gut environment itself. Gut microbiomes are known to hold a vital role in host metabolism, nutrition and immunity and as such understanding the influence of chemicals and microplastics on the gut microbiota is key.

The aims of this study were to investigate:

1. The ingestion of microplastics by the chironomid (*Chironomus sancticaroli*)
2. The effect of microplastics in the presence or absence of PBDEs on chironomids and their microbiome.



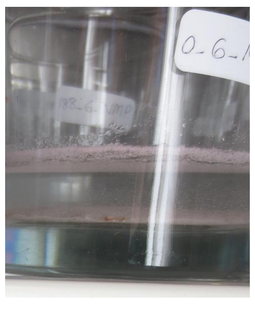


Experimental approach

Nylon ingestion by chironomids

- Nylon particles (< 50µm, dyed with nile red for fluorescence) were mixed dry with sand to achieve 1% plastic in the sand.
- 10 chironomids per replicate were exposed for 48 hours in total. An individual was removed from each replicate at 6, 24 and 48 hours and fluorescence measured.
- After 48 hours remaining individuals were transferred to clean sand with no microplastics and elimination of plastics was checked after 6, 24 and 48 hours.

Chironomid exposure to nylon and PBDEs



- Nylon particles were mixed dry with sand to reach achieve 1% plastic in the sand.
- PBDEs were spiked into the sand/nylon mix using solvent to create a concentrations series: 93.8, 187, 375, 750, 1500, 3000 µg/kg each in 6 replicates.
- Chironomids (15 individuals per replicate) were exposed to PBDEs alone and with plastics present.
- Controls were also included with no plastics or PBDES added to the sand.
- Exposure was for 96 hours. Survival was checked daily.
- At the end of exposure organisms were removed and rinsed. Chironomids from 3 replicates were weighed and freeze-dried for tissue chemical analysis and 3 replicates were stored in RNAlater for microbiome analysis.
- Chironomid gut microbiome DNA was extracted using the DNeasy blood and tissue kit (Qiagen). A library targeting the 16S small subunit ribosomal RNA gene was sequenced at a concentration of 6 pM with a 0.6 pM addition of an Illumina generated PhiX control library. Sequencing runs, generating 2 x 300 bp, reads were performed on an Illumina MiSeq using V3 chemistry.
- The sequences were clustered into operational taxonomic units (OTUs) with UCLUST) and representative sequences were selected. The taxonomy of representatives was determined by QIIME's UCLUST consensus taxonomy assigner (assign_taxonomy.py, QIIME) using the Greengenes database release 13_2.

Results

Chironomid uptake and elimination of nylon

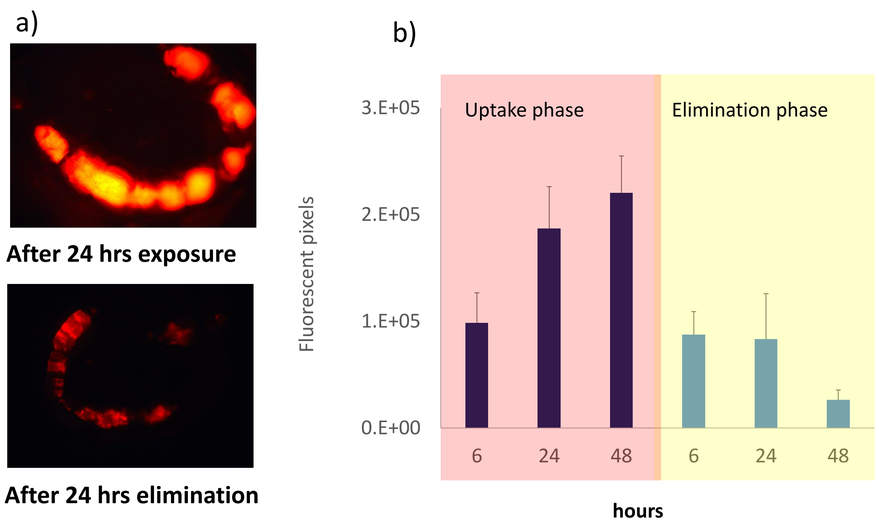


Fig 1: (a) Images (left) of chironomid taken under a fluorescence microscope after 24 hours exposure (top) to 1% nylon in sand and following 24 hours elimination (bottom) in clean sand. **(b)** The uptake and elimination over the two 48 hour periods

Chironomid survival

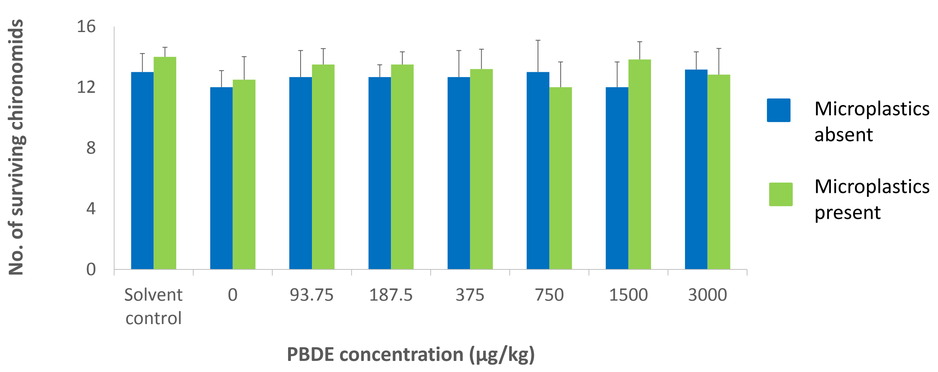


Fig 2. Survival of chironomids after 96 hours exposure to PBDEs in the presence and absence of nylon microparticles.

Microbiome analysis

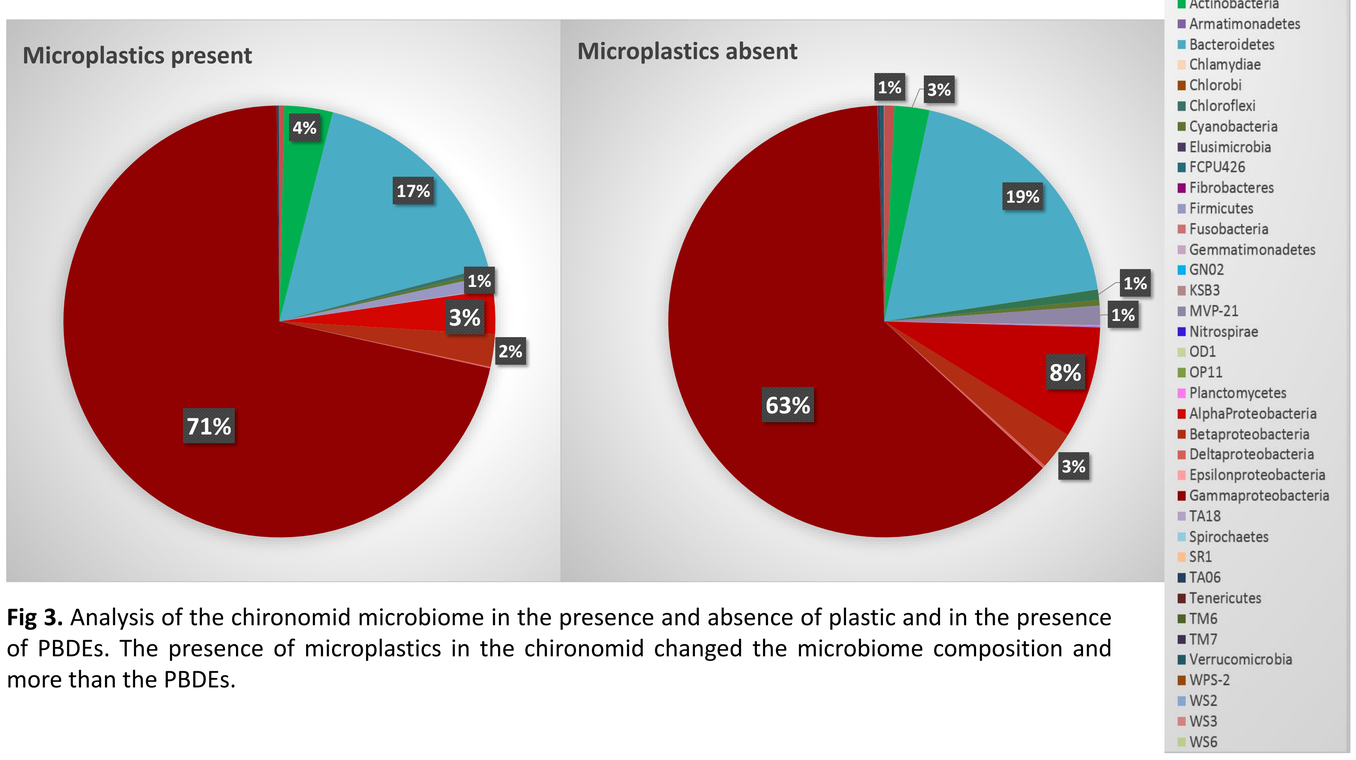


Fig 3. Analysis of the chironomid microbiome in the presence and absence of plastic and in the presence of PBDEs. The presence of microplastics in the chironomid changed the microbiome composition and more than the PBDEs.

Conclusions

- Chironomids ingested nylon microparticles and did not fully eliminate them in the depuration period (48 hrs).
- Nylon (1%) and PBDEs (up to 3000 µg/kg) did not affect chironomid survival.
- The microbiome of the chironomids was affected significantly by the presence of microplastics.

Acknowledgements: The authors wish to thank Marta Margarete Cestari (UFPR) for their assistance in completing this work. This work was funded by **RCUK, Newton Foundation, Fundação Araucaria (**45191.460.30251.29012015)