Determination of microplastics in reared black soldier fly larvae (Hermetia illucens) and yellow mealworms (Tenebrio molitor) using polarized light optical microscopy

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Introduction

- Novel protein source for food and feed
- Valorisation of waste materials (e.g. former foodstuffs)
- Chemical & physical hazards
- Packaging materials: microplastics





Introduction

- Microplastics: < 5 mm</p>
- Insufficient separation of packaging materials from biomass
- Risks for conventional livestock, but also for insects?
 - Suffocation
 - Transfer
- Presence of MPs in BSFL and YMW





Experimental setup

	Black soldier fly		Yellow mealworm	
	Study 1	Study 2		
Plastics	PET, LDPE-300, LDPE-125, LDPE-film		PET, LDPE-300, LDPE-125, BLμP 53-63, BLμP 90-106, GRμP 90-106, PA, PTFE, PS, PP	
Exposure	1, 3, 10%	0.15, 3%	1%	
Total treatments	12	8	10	
Replicates per treatment	3	3	3	
Negative control	n=9	n=9	n=9	
After harvesting	No washing	Washing	Washing	



Experimental setup

Larval survival & growth

Black soldier fly			Yellow mealworm
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Experimental setup

Detection of particles

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Method







Storage at -20 °C



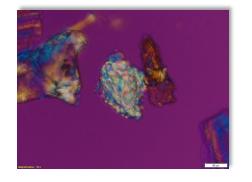
Digestion





Filtering





Microscopy with polarized light

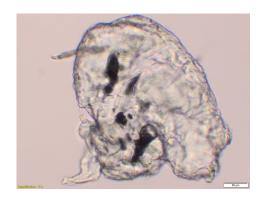




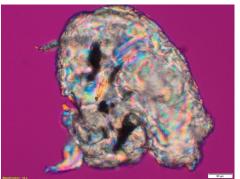


Microscopy: why polarized light?

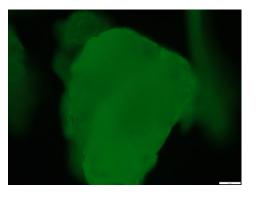
- Nile red
 - Fluorescent dye
 - Likes everything that is very apolar



Normal light



Polarized light



Stained with Nile Red



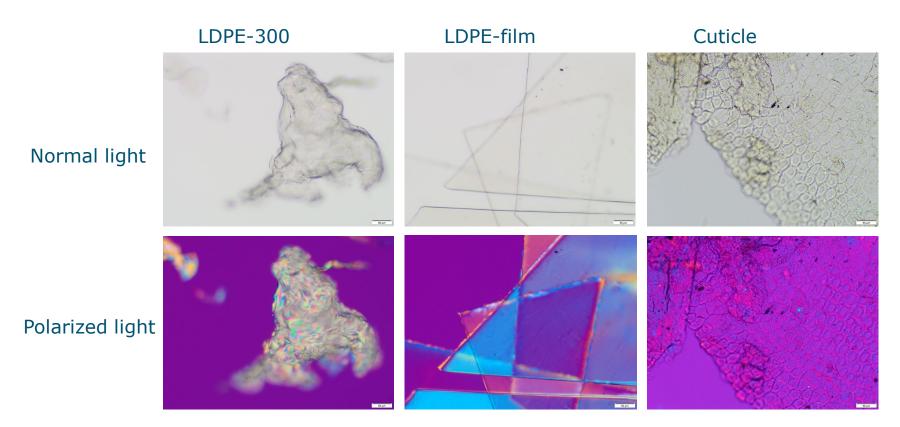


Microscopy: why polarized light?

	Nile Red	Polarized light
Pros	Fluorescence easily visible against dark background	No staining procedure necessary Unique pattern observed for certain microplastics
Cons	Possible co-staining of matrix Possible autofluorescence of matrix	Specific microscopic skills necessary



Microscopy: why polarized light?





Results: survival & yield

No significant difference between groups

BSF study 1 YMW

Tested plastics have no influence on survival and growth





Results: particles found

	Bla	ck so	oldier f	ily	
	Stud 1%	y 1 3%	10%	Study 2 0.15%	3%
PET	-	-	-	± 10% of all particles, plastic	> 50% of all particles, plastic
LDPE-300	-	-	-	Not present	Trace
LDPE-125	-	-	-	Not present	Trace
LDPE-film	-	-	-	Not present	Trace

Not washing = no particle detection!



Discussion

Black soldier fly

- More plastic particles found in PET treatment compared to LDPE treatment (Both 300 µm size & powder)
- At 3%, more plastic particles than at 0.15%
 - Ingestion of MPs depends on initial particle load (Lievens et al. 2023)
- Possibly chemical structure plays a role

Results: particles found

Yellow mealworm	
Significantly different	Not significantly different
PET, LDPE-300, GRµP 90-106	LDPE-125, BLµP 53-63, BLµP 90-106, PA, PTFE, PS, PP



Discussion

Yellow mealworm

- Significantly more plastic particles in: PET, LDPE-300, GRµP 90-106
 - PET & LDPE-300 both 300 μm size
- Many factors could play a role for consumption
 - Size
- Shape

- Chemical structure
- Density



Conclusions & further steps

- Presence of microplastics in insect feed did not affect survival & growth of YMW and BSFL
- **BSFL**: More plastic particles were found in substrate spiked with PET, with more particles present at a higher concentration substrate spiked with LDPE showed no or trace amounts of plastic particles
- YMW: significantly more plastic particles found in substrate spiked with PET, LDPE-300 and GRµP 90-106
- Future research to focus on more types of plastics, shapes, densities, sizes



Acknowledgements

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