

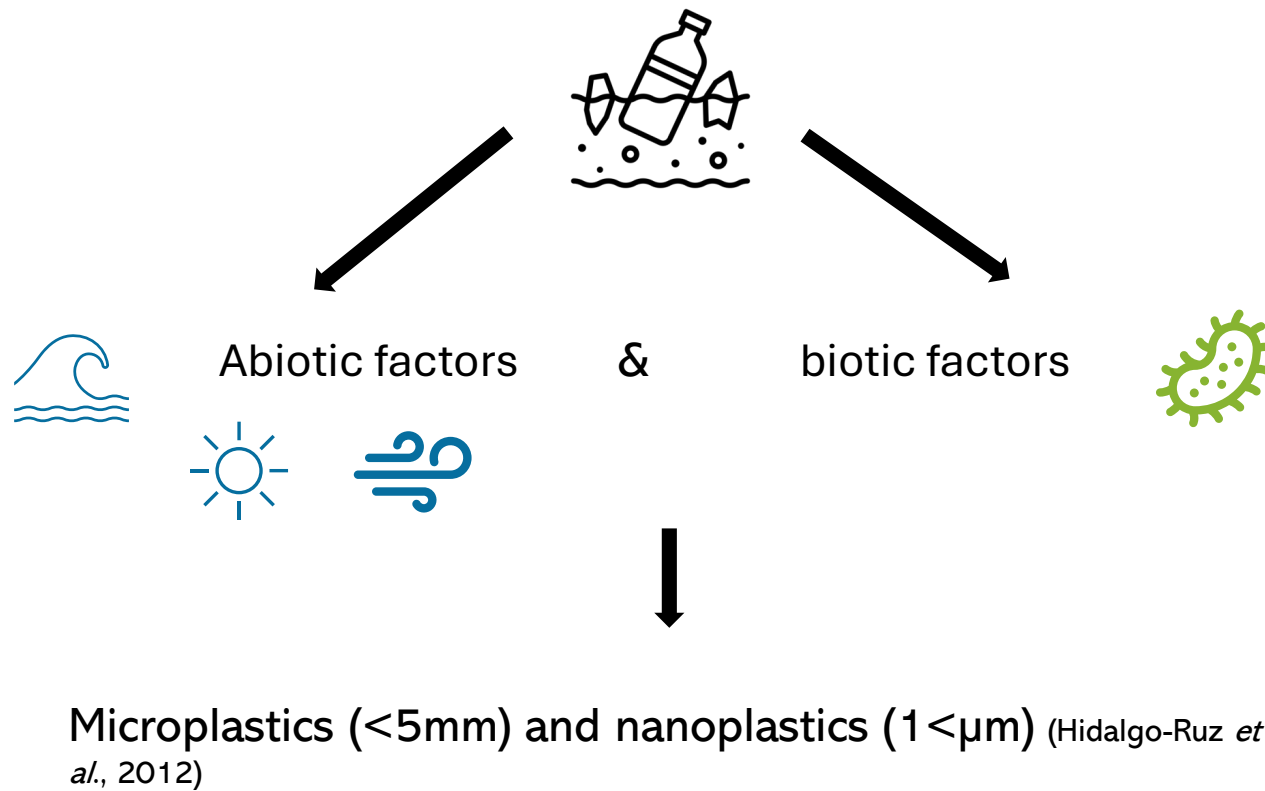


# Microplastics on the menu; exploring interactions between two mosquitoes species and microplastics

Léa Poirier

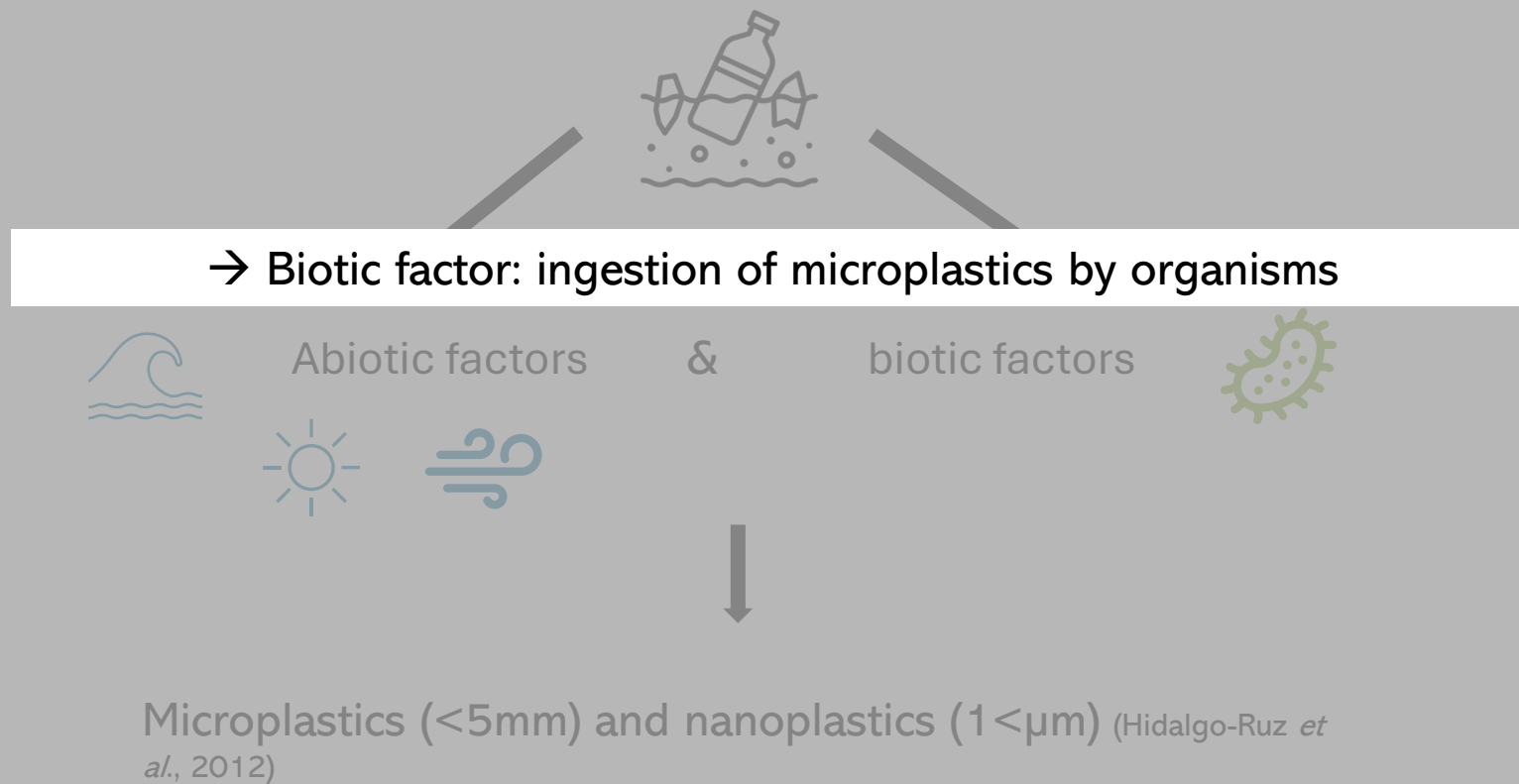
# Introduction

- Plastics: a global threat (Geyer *et al.*, 2017)
- Plastics in the environment come in all shapes and sizes (Andrady., 2011):



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Impact of microplastics on organisms:

**Ingestion**

Impact of organisms on microplastics :

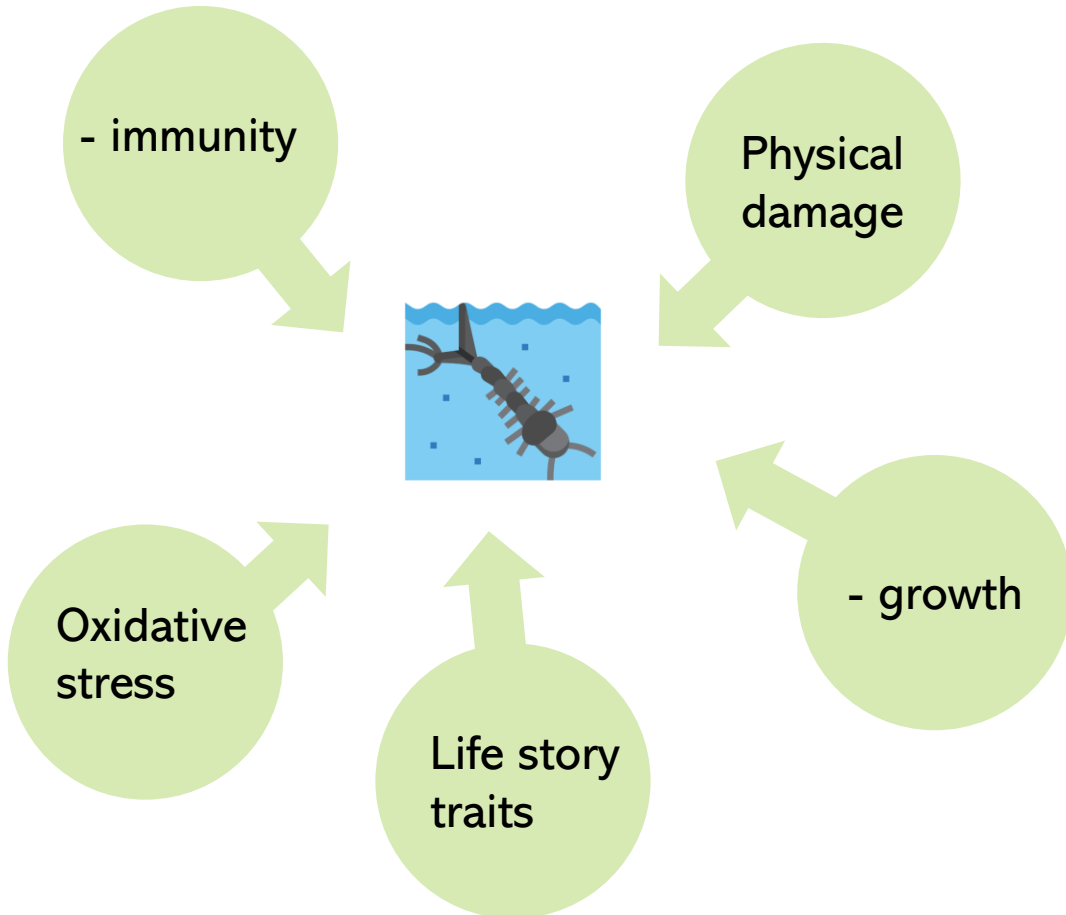


# Introduction

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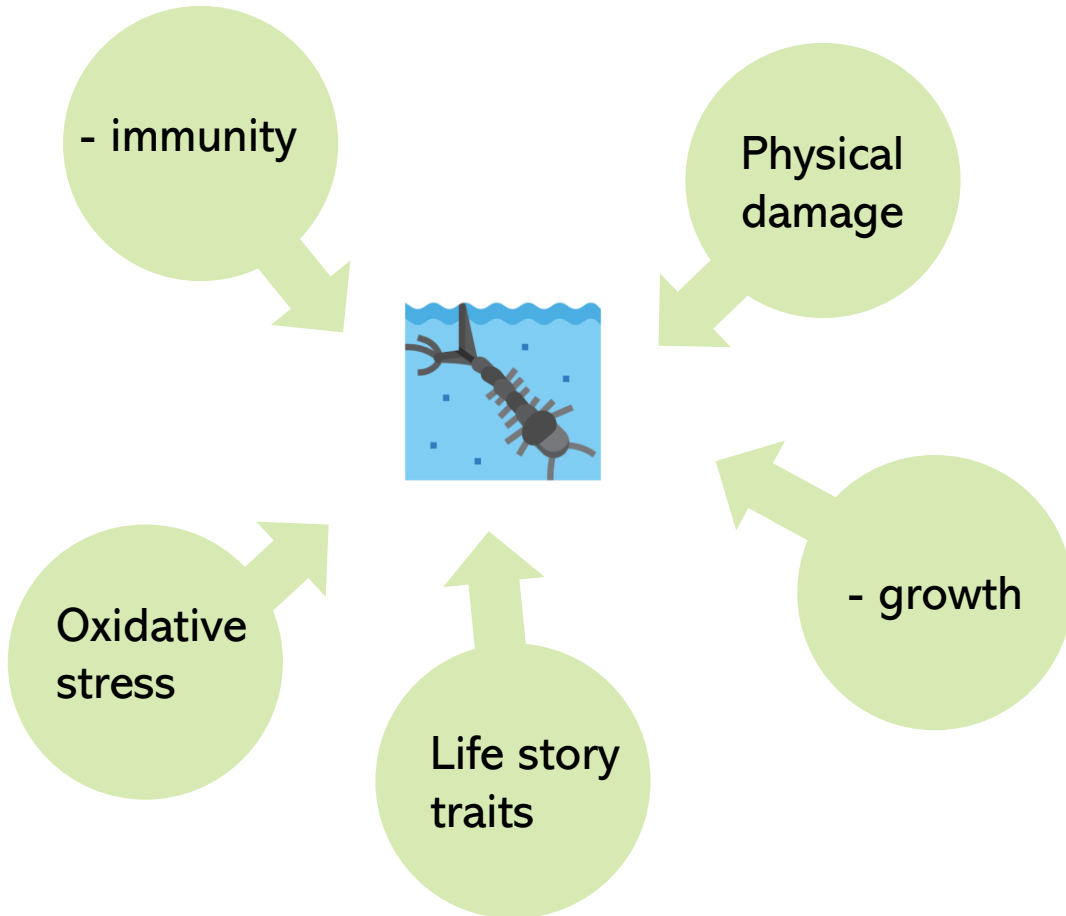
**Ingestion**

Impact of organisms on microplastics :



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Impact of microplastics on organisms:



## Ingestion

Impact of organisms on microplastics :



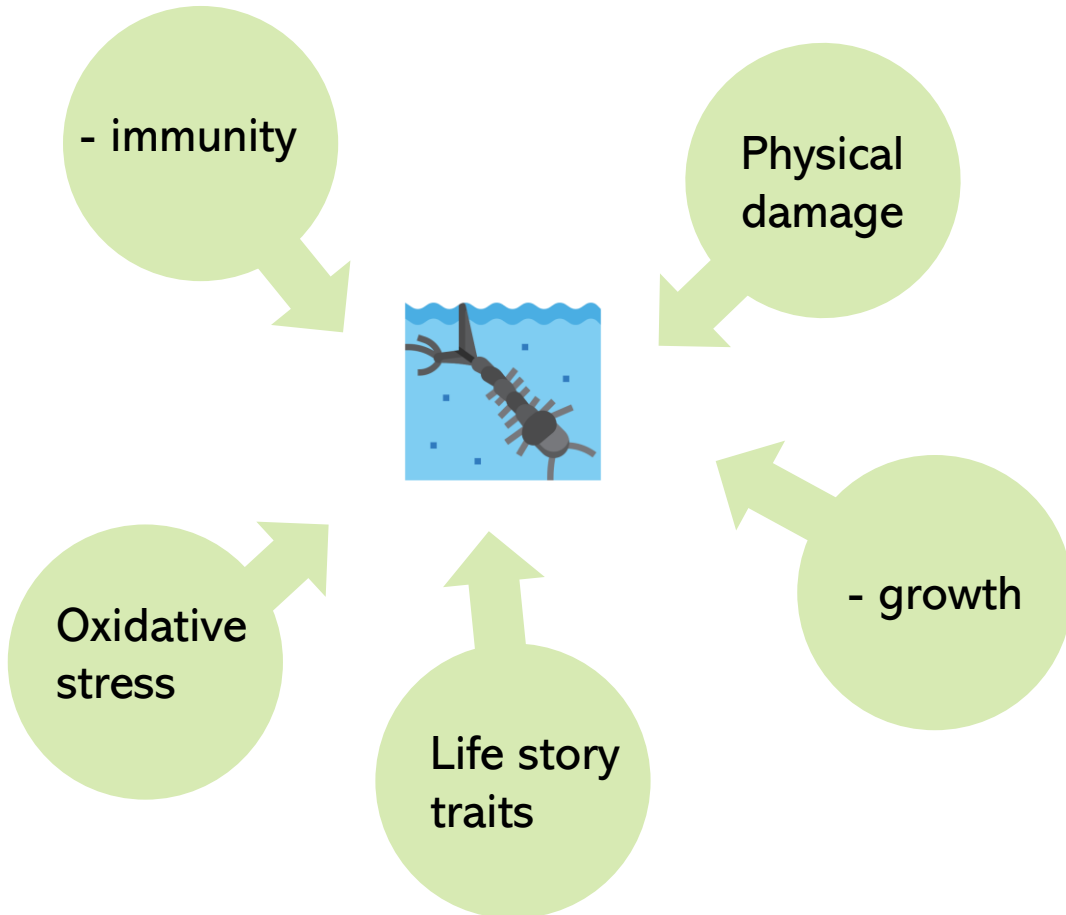
- Biofragmentation by macroinvertebrates (gammarids, krill, chironomids)
- Creation of nanoscopic fragments
- 2 alterations: chemical or mechanical
- But little information on the role of macroinvertebrates

(Dawson et al., 2018)

(Sanchez-Hernandez,. 2021)

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- Creation of nanoscopic fragments
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- But little information on the role of macroinvertebrates

(Dawson et al., 2018)

→ **What about mosquitoes?**

# Introduction

Arthropods, disease vectors

Females lay eggs on surface water (Yee *et al.*, 2004)



*Anopheles gambiae*



*Aedes albopictus*



# Introduction

Arthropods, disease vectors

Females lay eggs on surface water (Yee *et al.*, 2004)

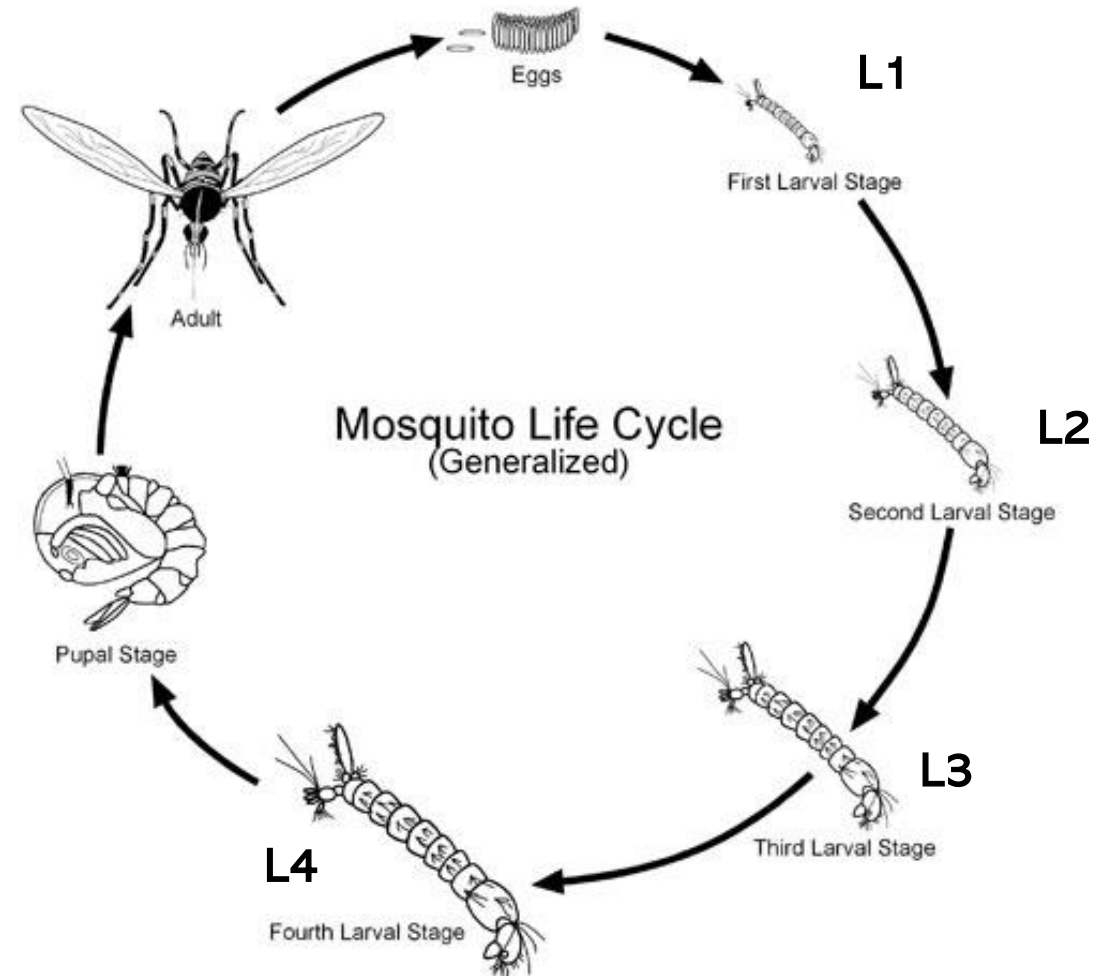
Development: 4 larval instars and a pupal instar



*Anopheles gambiae*



*Aedes albopictus*



(Illustration by: Scott Charlesworth, Purdue University)

# Introduction

Arthropods, disease vectors

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Development: 4 larval instars and a pupal instar

- → Surface filter feeders: *An. gambiae*
- → Filter feeders & grazers: *Ae. albopictus*



*Anopheles gambiae*



*Aedes albopictus*



Surface water

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Arthropods, disease vectors

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Development: 4 larval instars and a pupal instar

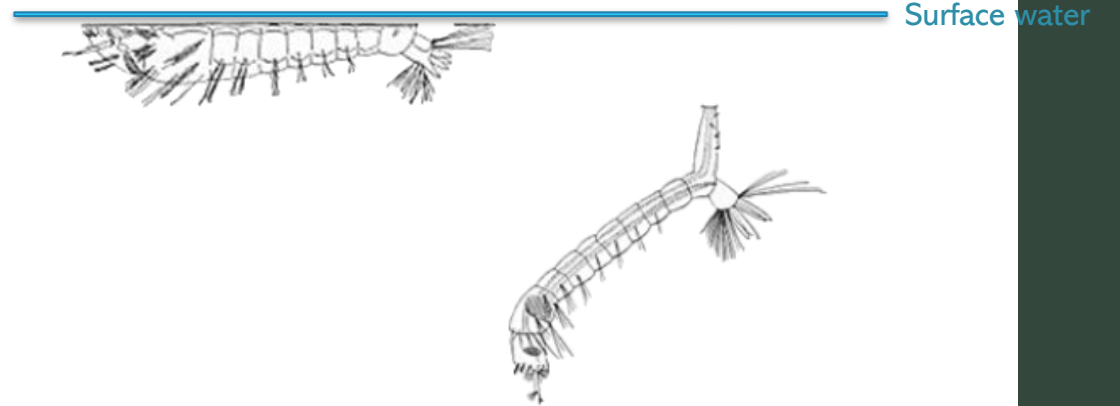
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*Anopheles gambiae*



*Aedes albopictus*



Ingestion of microplastics by mosquito larvae:

Larvae able to ingest microplastics

Effects still poorly understood

(Griffin *et al.*, 2021)

# Problems

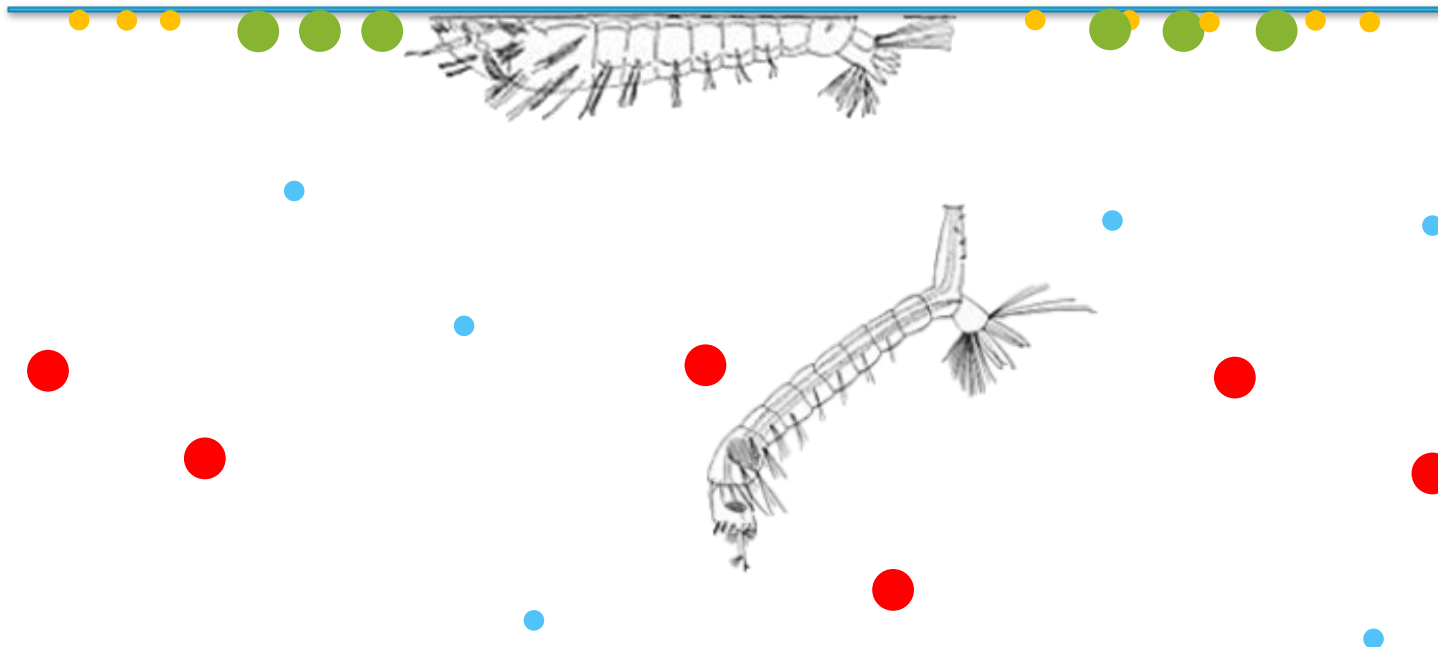
## Experiment 1: Who eats what?

Axis 1

Species with different feeding behavior are exposed to the same risk of ingestion?

Axis 2

Impacts of microplastics on life-history traits (survival, sex ratio and size)



# Problems

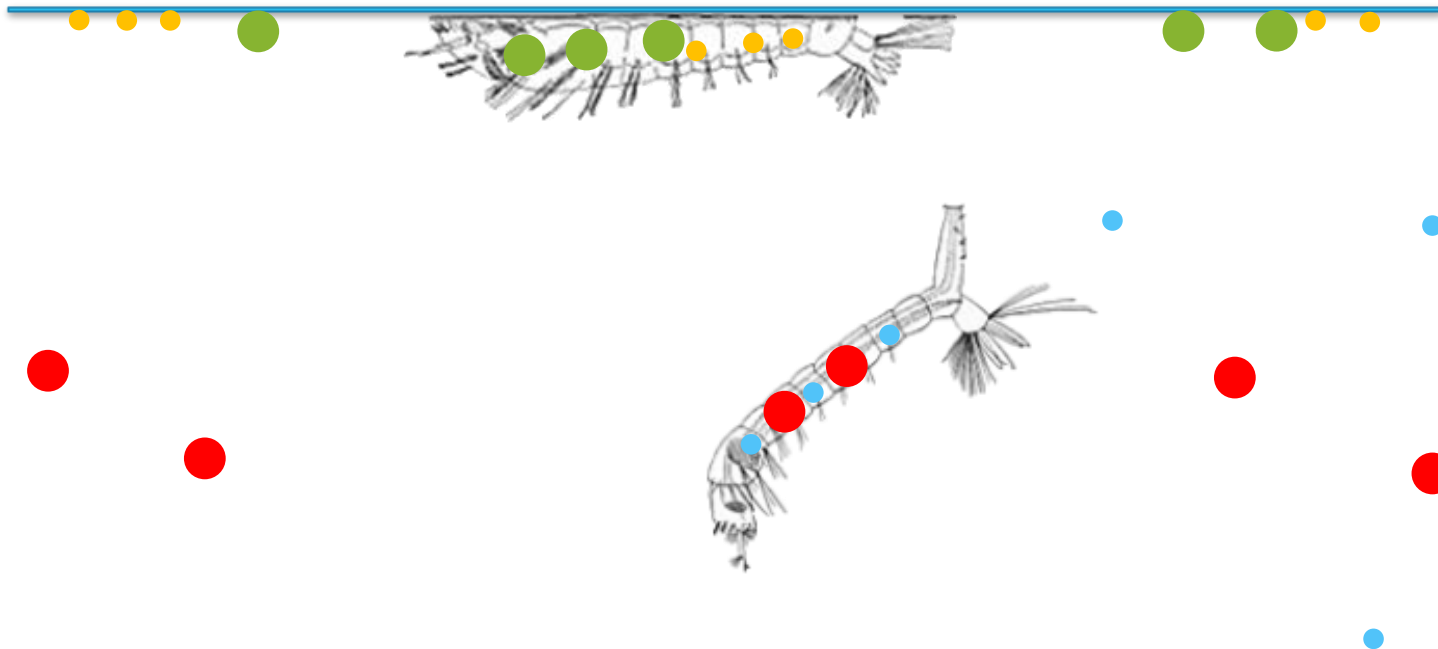
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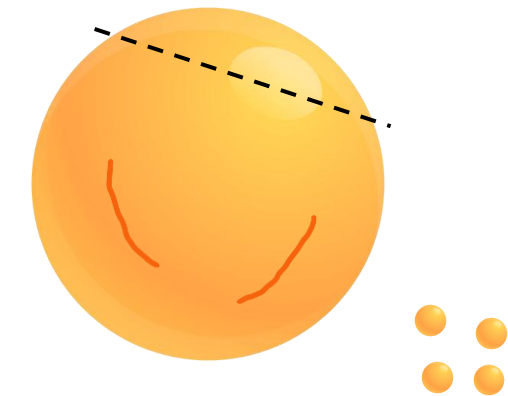
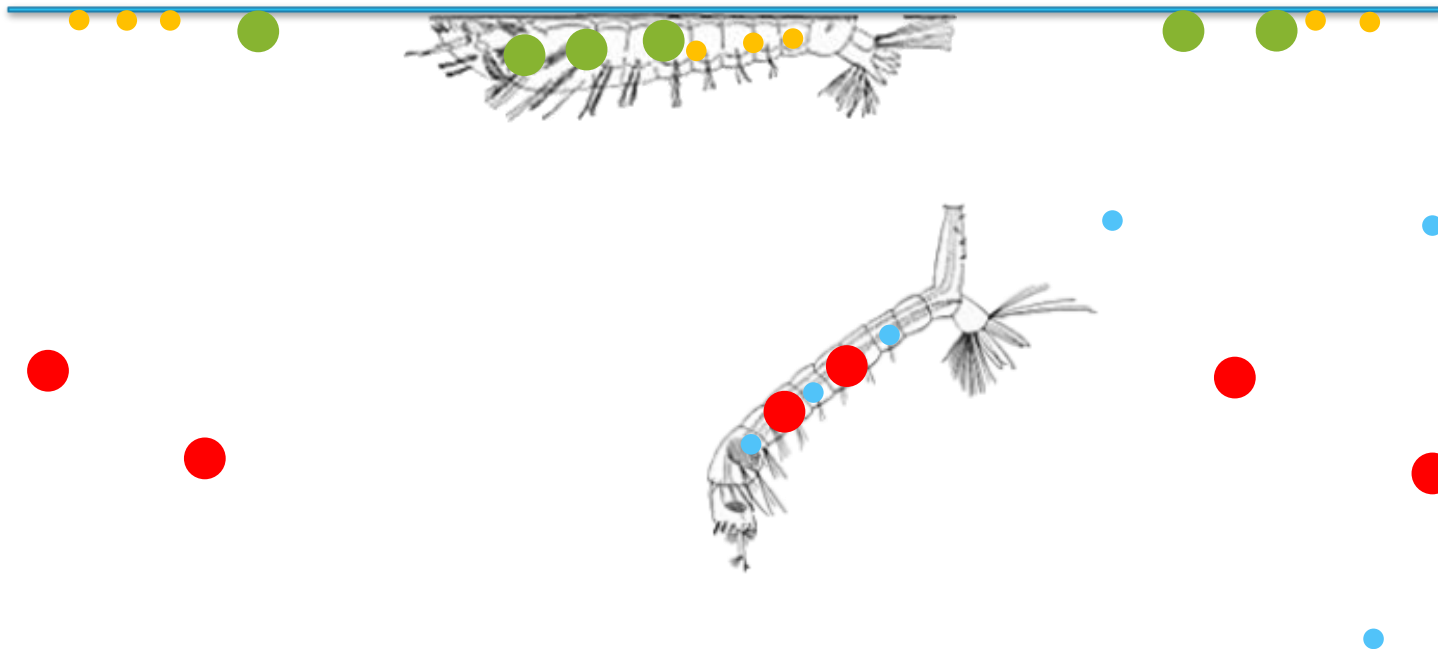
### Axis 2

Impacts of microplastics on life-history traits (survival, sex ratio and size)

## Experiment 2: digestion

### Axis 3

Biofragmentation of microplastics by mosquito larvae?



Diameter reduction?  
Fragments?  
Marks?

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## Experiment 1: Who eats what?

### Axis 1

Species with different feeding behavior are exposed to the same risk of ingestion?

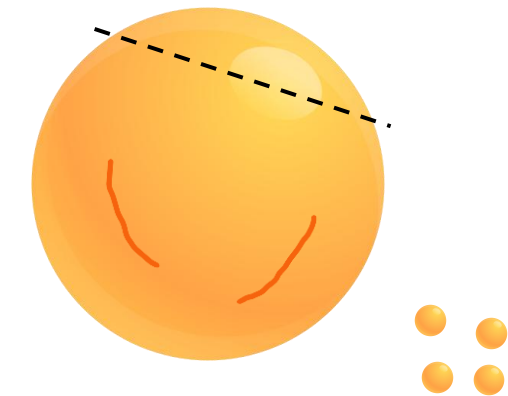
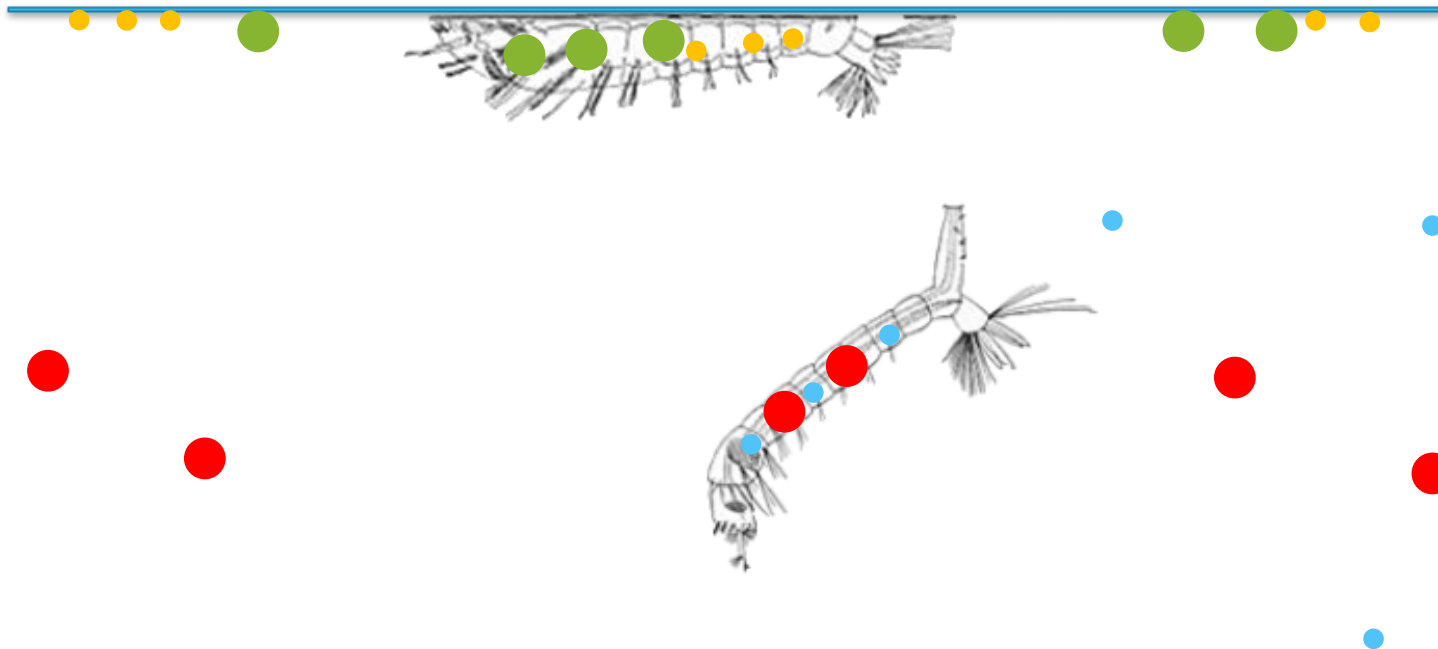
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Fragments?  
Marks?

# Materials and Methods

## Experiment 1: Who eats what?

## Experiment 2: digestion

2 species:

Surface filter feeders: *An. gambiae*

Filter feeders & grazers: *Ae. albopictus*

Polystyrene (PS)

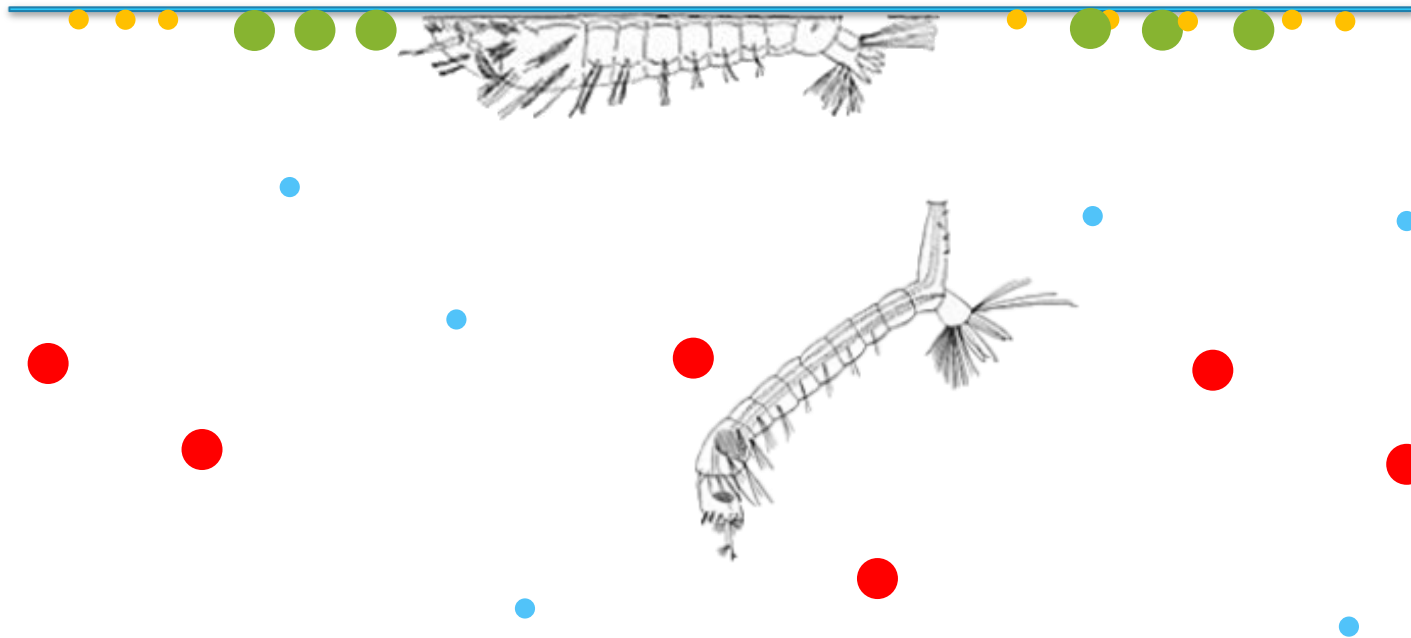
● Polystyrene 50μm  
(PS50)

● Polystyrene 15μm  
(PS15)

Polyethylen (PE)

● Polyethylene 50μm  
(PE50)

● Polyethylene 10μm  
(PE10)

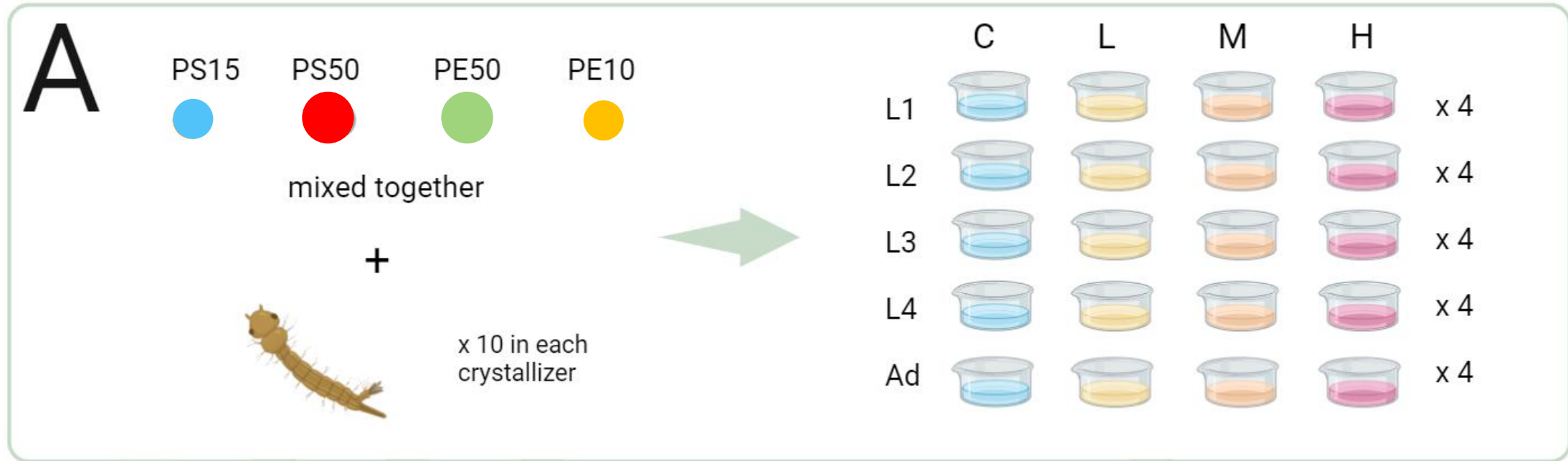




# Materials and Methods

## Experiment 1: Who eats what?

Species with different feeding behavior are exposed to the same risk of ingestion?



→ Mixed microplastics

→ Different concentrations (high (H), medium (M) and low (L) + control (C))

→ 10 larvae/ crystallizer from hatching

→ Exposure: hatching to larval instar of interest or until emergence

→ 4 replicates/ concentration

# Materials and Methods

## Experiment 1: Who eats what?

Species with different feeding behavior are exposed to the same risk of ingestion?

### Larvae dissection



Larvae washed 2 times in reverse osmosis water before dissection



x 30 larvae dissected/  
concentration/ treatment

→ Rinsing larvae

→ Dissection on slide

### Microscopy

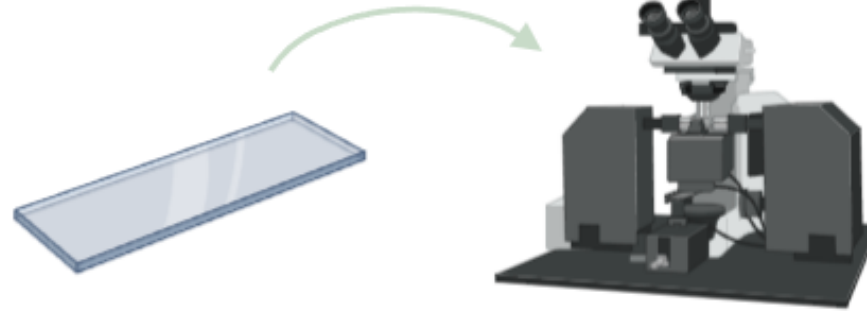


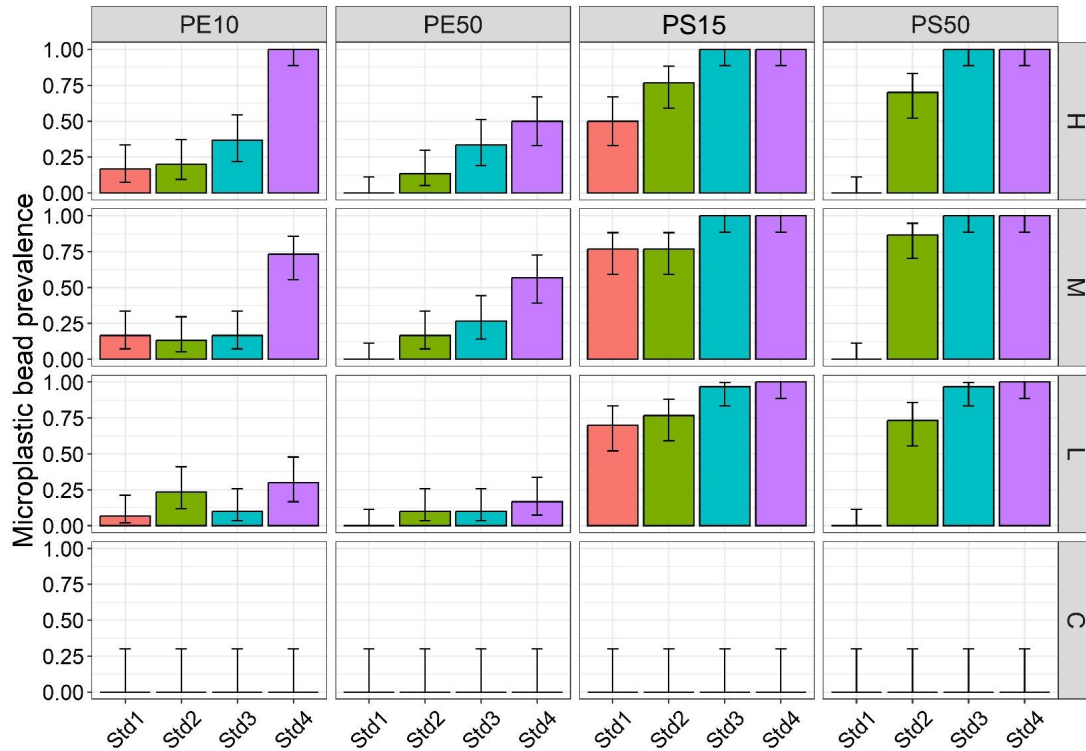
Photo of MPs by using  
Zeiss Axiozoom V.16

→ Microplastic counting

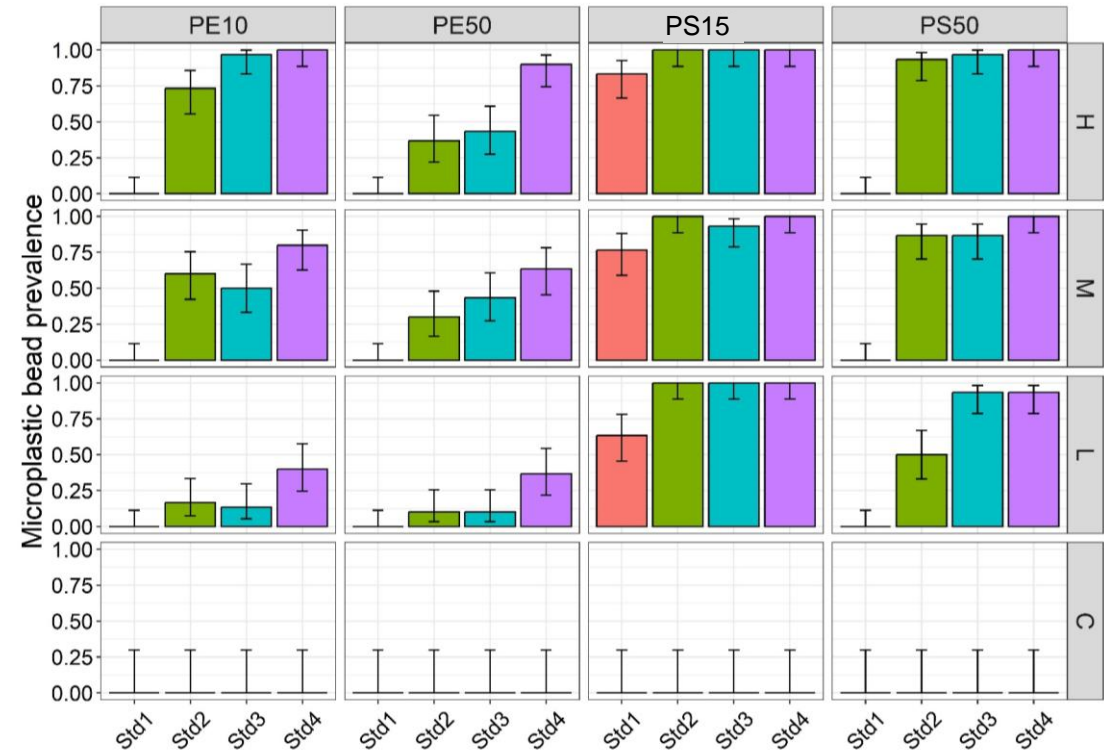
# Results/ Discussion

## Experiment 1: Who eats what?

A) *Anopheles gambiae*



B) *Aedes albopictus*



→ Results were the same for both species

# Results/ Discussion

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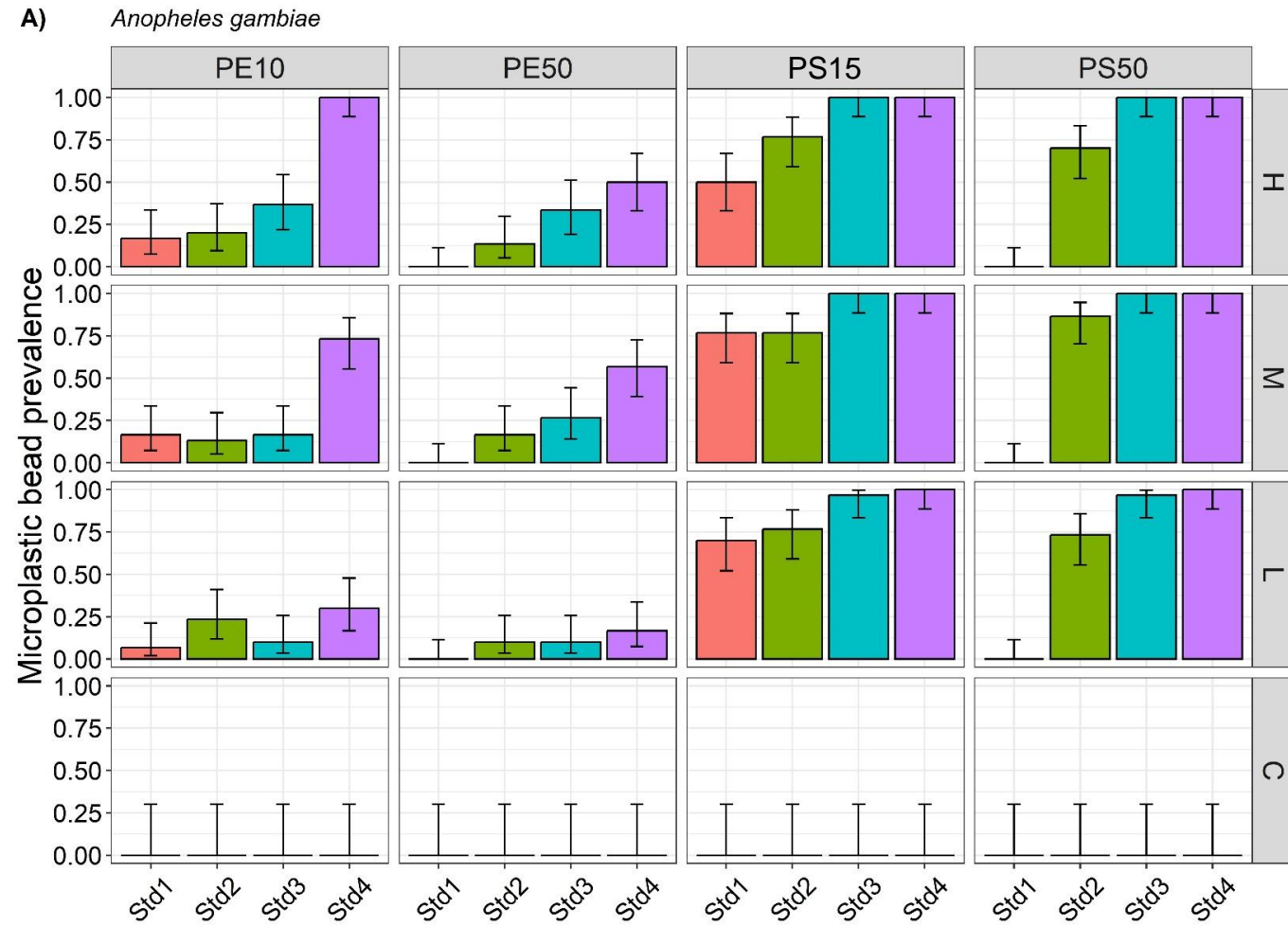
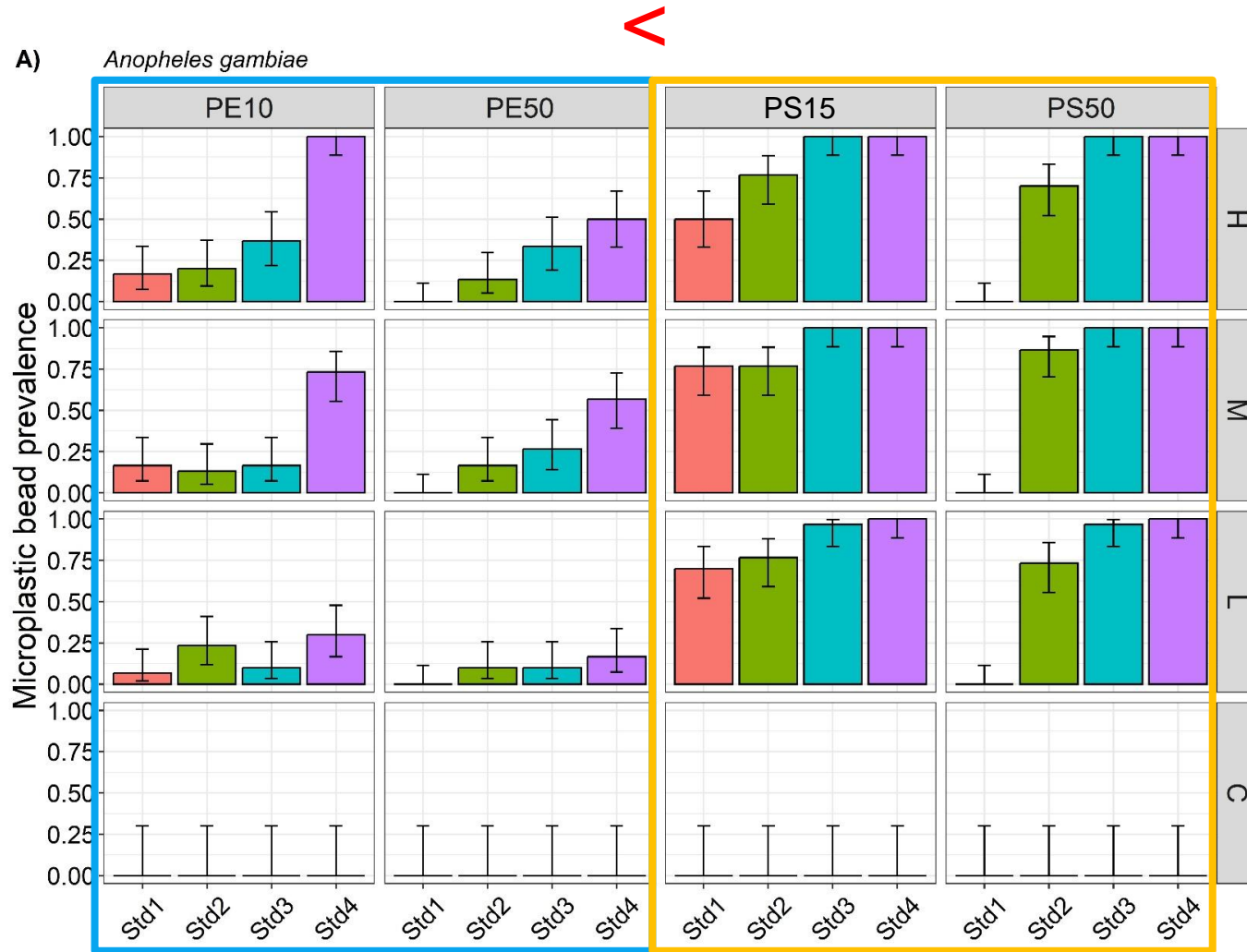


Figure 1: Microplastic bead prevalence, expressed as the proportion of larvae with a least one ingested bead, for each type of microplastic (PE10, PE50, PS10, PS50), concentration (H: high, M: medium, L: low, C: control) and larval stage (L1, L2, L3 and L4) of *Anopheles gambiae*.

# Results/ Discussion

## Experiment 1: Who eats what?



- Lower prevalence for PE50 > PE10 > PS50 > PS15

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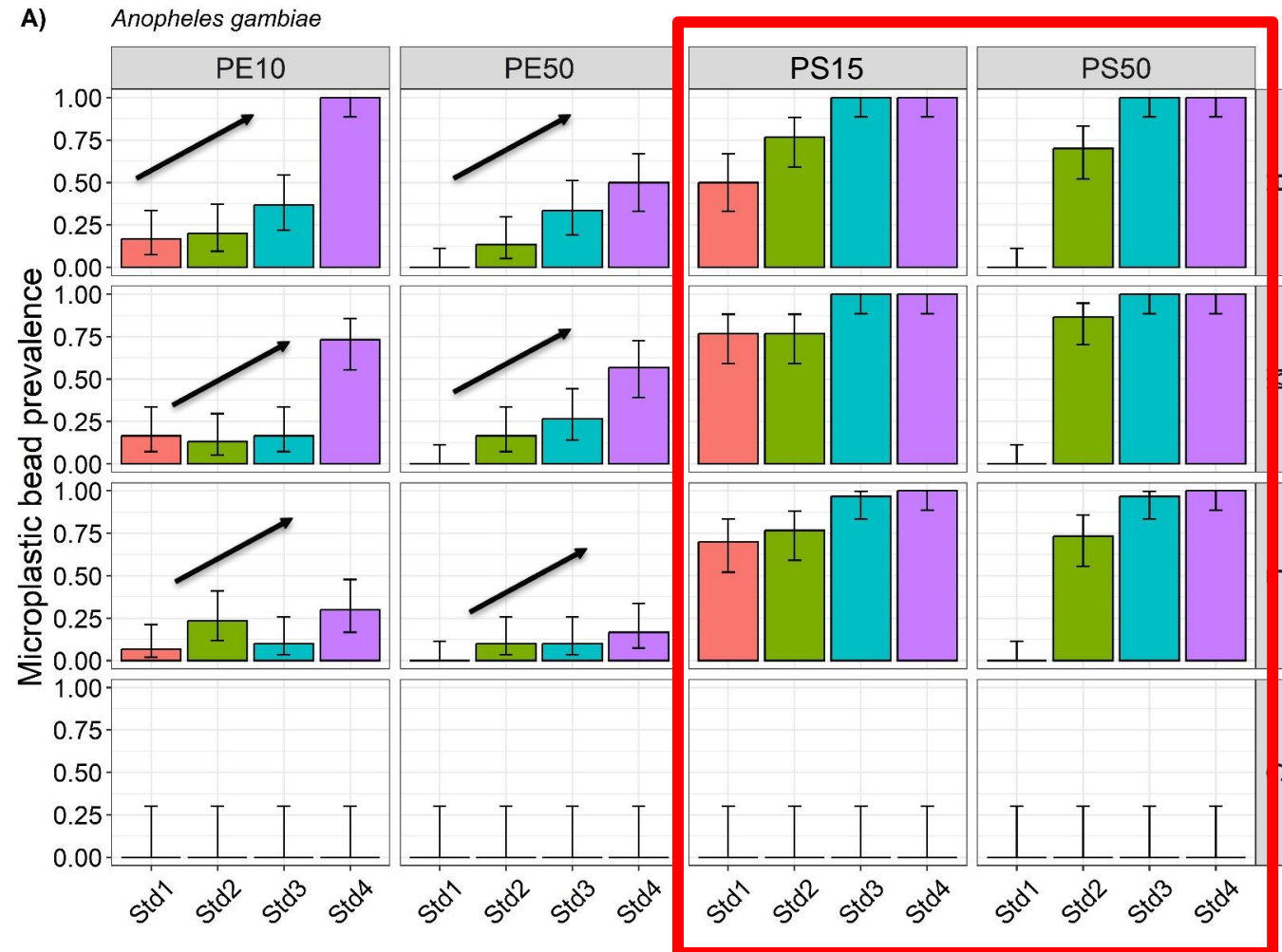


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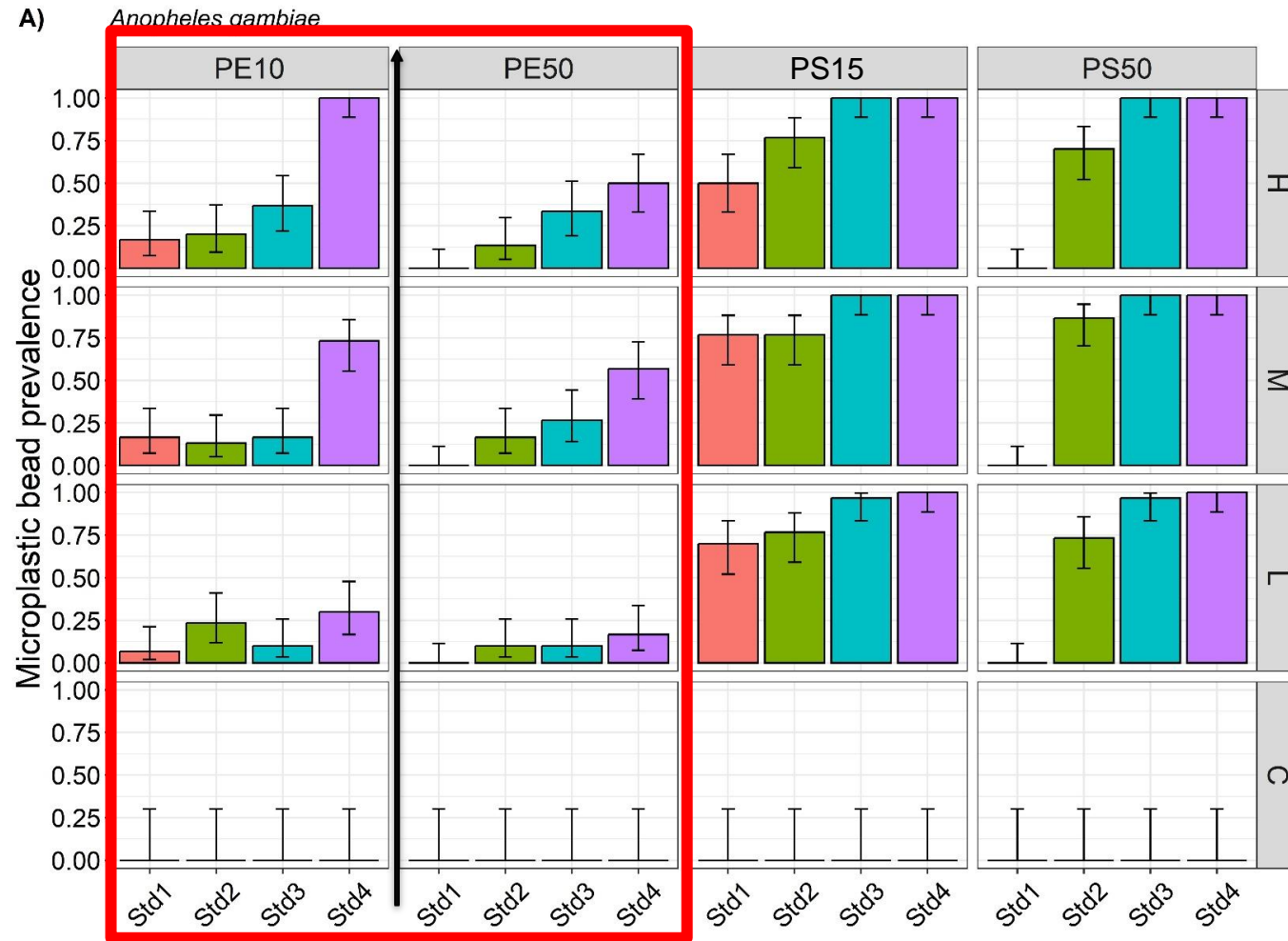


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# Results/ Discussion

## Experiment 1: Who eats what?

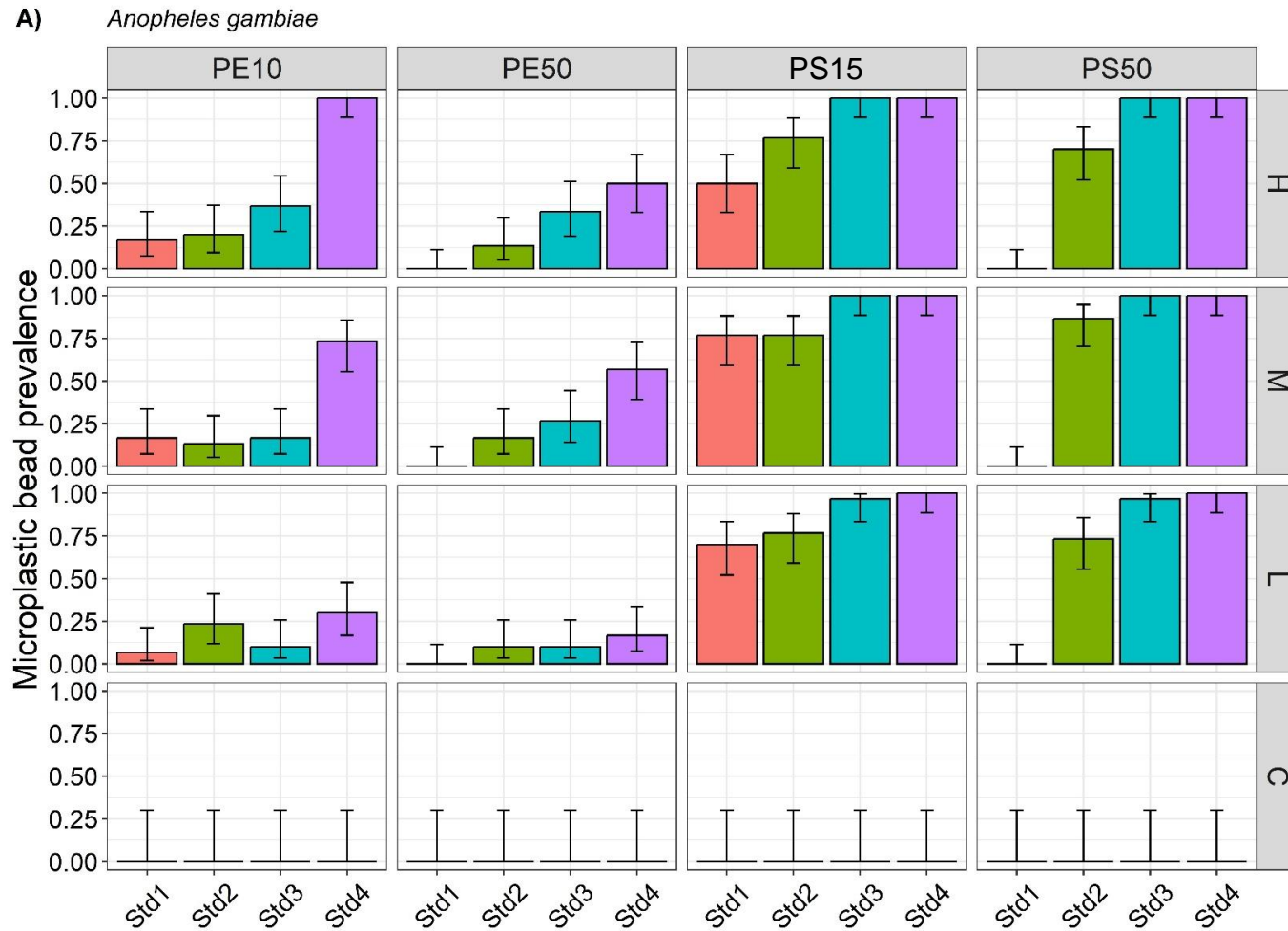


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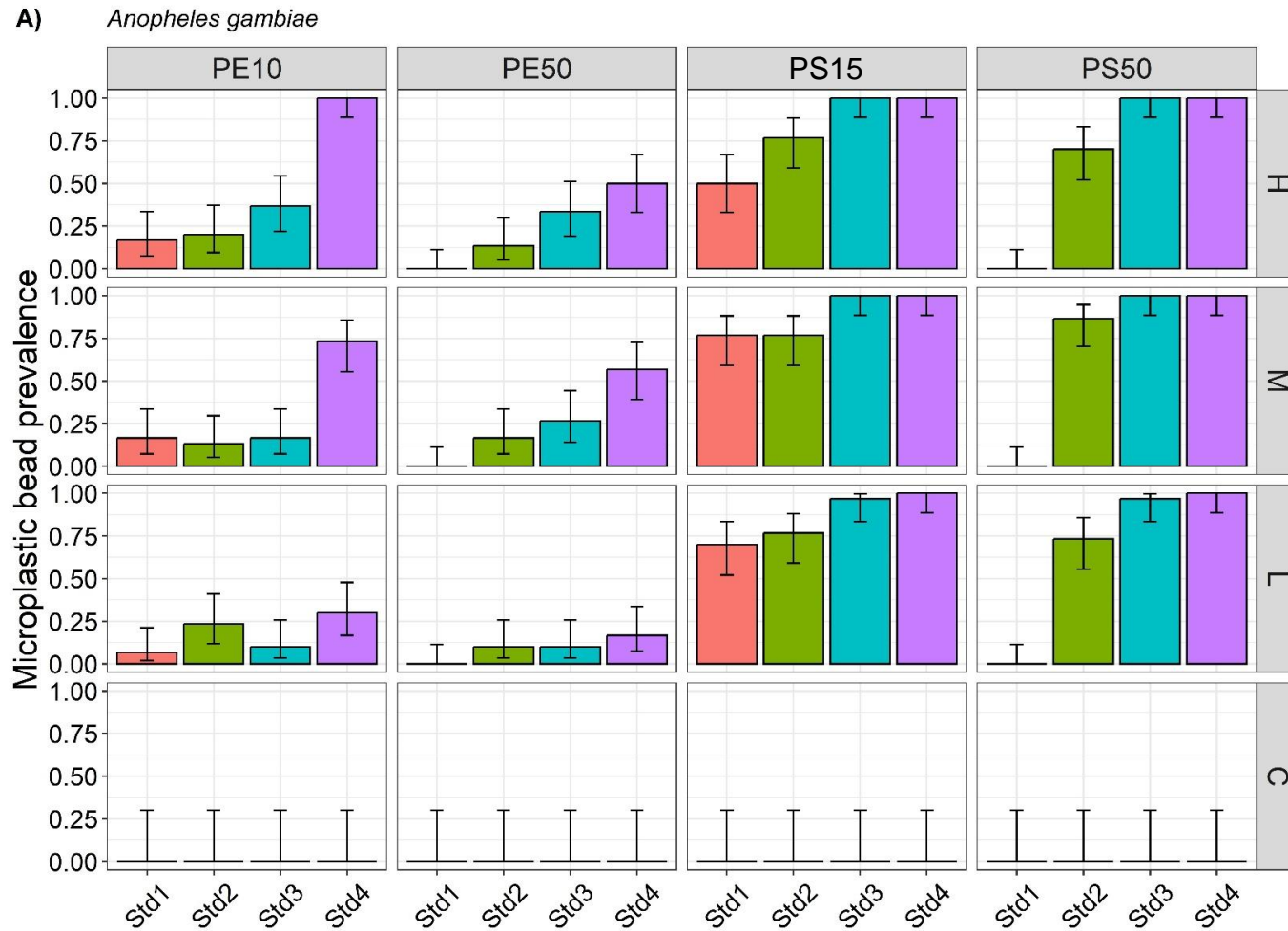


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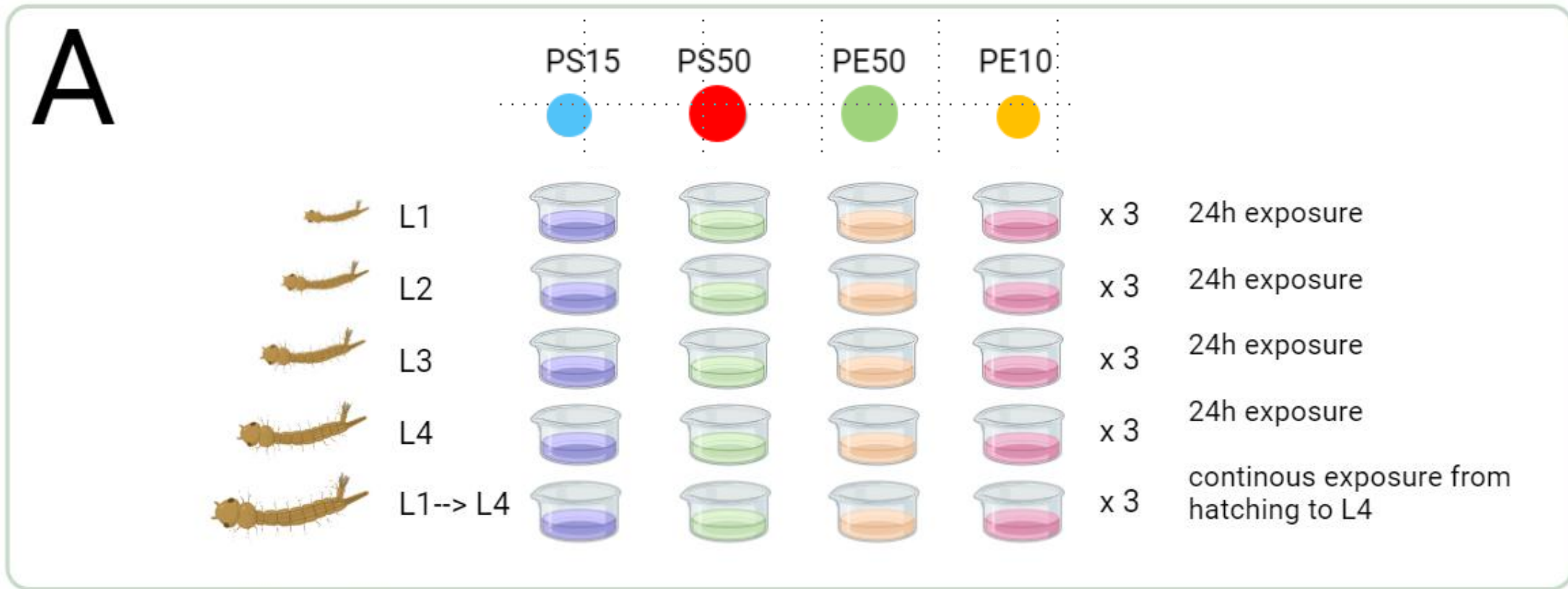
→ polystyrene was more likely to be ingested than polyethylene

→ same pattern of ingestion observed

# Materials and Methods

## Experiment 2: digestion

Biofragmentation of microplastics by mosquito larvae?



→ Microplastics exposed individually for 24 hours to each larval instar

→ Microplastics exposed for the entire duration of larval development (hatching → L4)

# B

### Digestion



all larvae  
were washed  
twice



1mL H<sub>2</sub>O<sub>2</sub> 30%

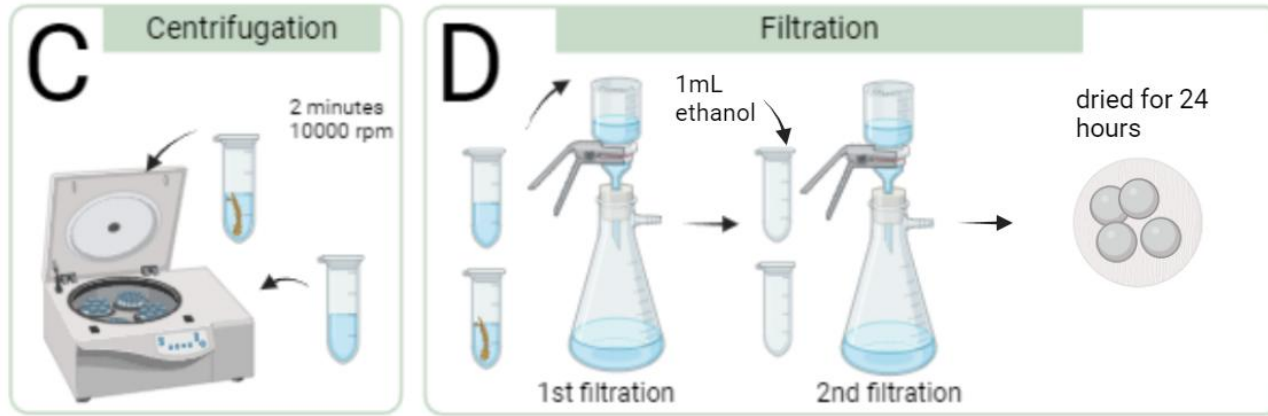
digestion  
conditions: 72h,  
50°C

→ Larvae rinsed and digested in H<sub>2</sub>O<sub>2</sub> to extract microplastics

# Materials and Methods

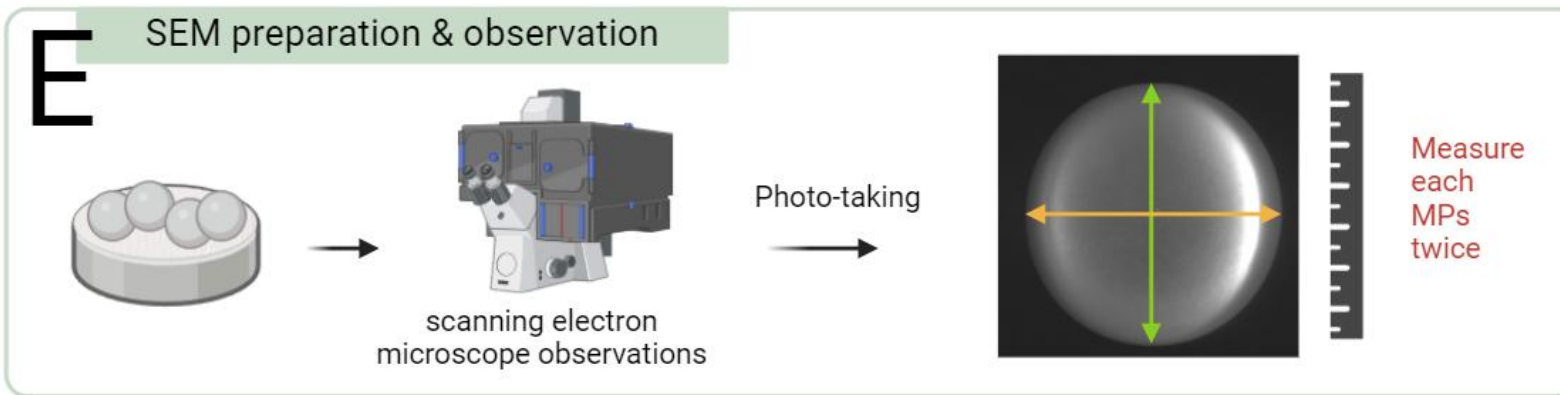
## Experiment 2: digestion

Biofragmentation of microplastics by mosquito larvae?



→ Centrifugation

→ Filtration



→ Observations scanning electron microscope

→ Microplastics measurement

# Results/ Discussion

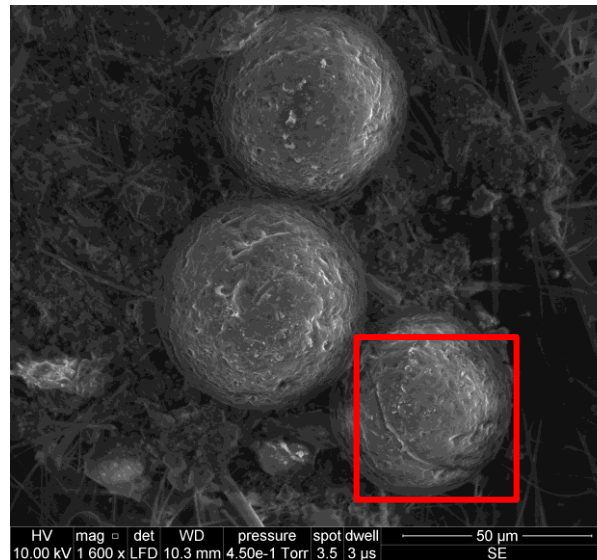
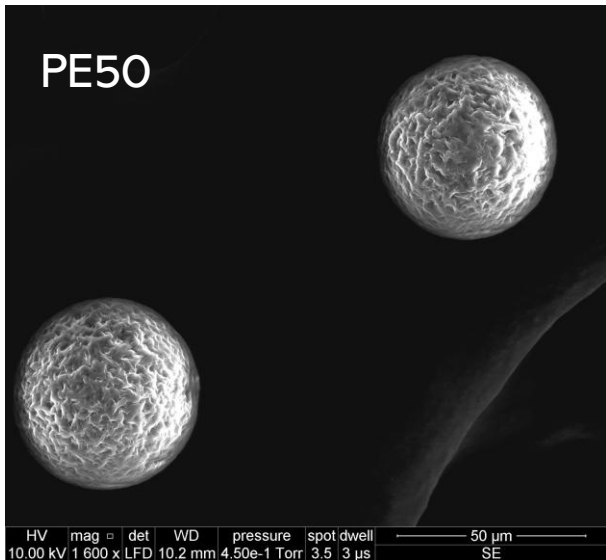
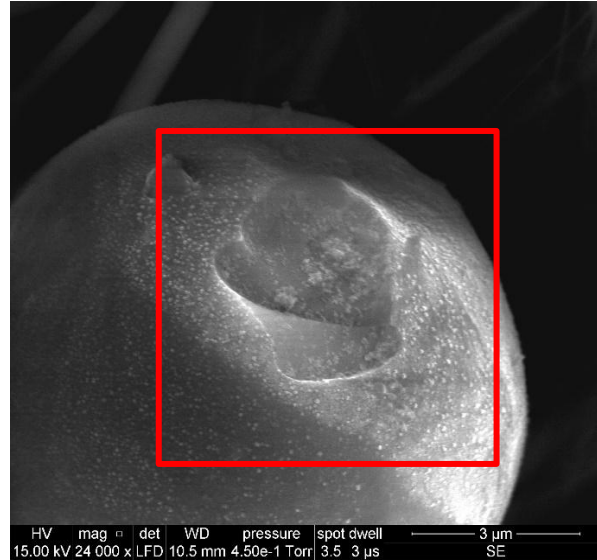
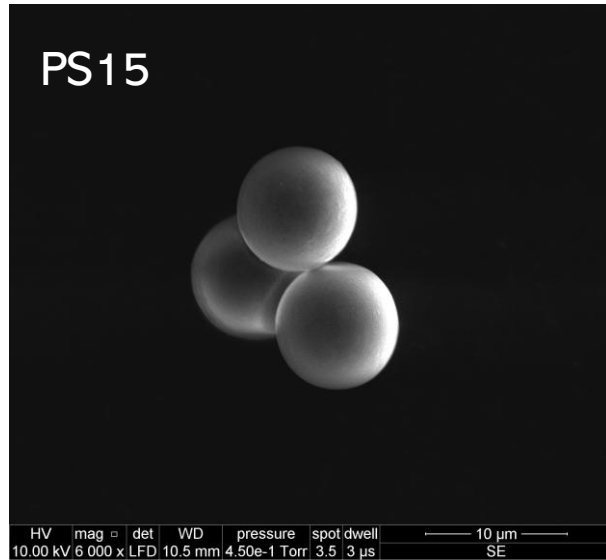
## Experiment 2: digestion

### 1. Chemical digestion :

- For both species: no size reduction observed
- Chemical digestion has no effect on microplastics

# Results/ Discussion

## Experiment 2: digestion



### 2. Mechanical digestion:

No fragments observed

Rare marks :

→ 2 observations out of 229 PE50

→ 1 observation out of 295 PS10

Difficult to attribute to larval action as no regular pattern observed.

### What about other macroinvertebrates?

Krill, gammarids, daphnia and chironomids

→ breaking down microplastics into smaller particles

→ reduce their diameter

(Dawson et al., 2018)

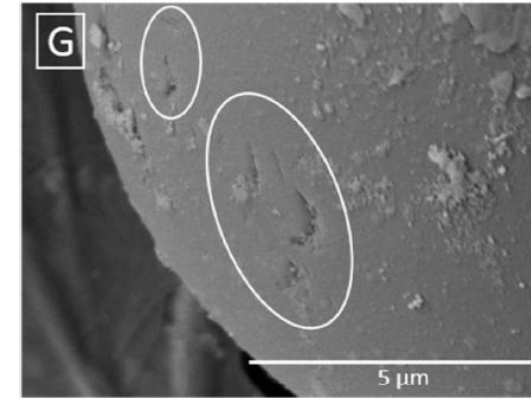
Chironomids mark microplastics on the surface

→ Mosquito larvae have the same chitinized mouthparts as the other macroinvertebrates mentioned.

→ Many similarities between their digestive enzymes

Surprising result ?

Estimate the transit time in the digestive tract



(Queiroz et al., 2024)

PS1

HV mag =  
10.00 kV 6 000 x

PE5

HV mag = det WD pressure spot dwell 50 μm  
10.00 kV 1 600 x LFD 10.2 mm 4.50e-1 Torr 3.5 3 μs SE

HV mag = det WD pressure spot dwell 50 μm  
10.00 kV 1 600 x LFD 10.3 mm 4.50e-1 Torr 3.5 3 μs SE



# Thanks

