



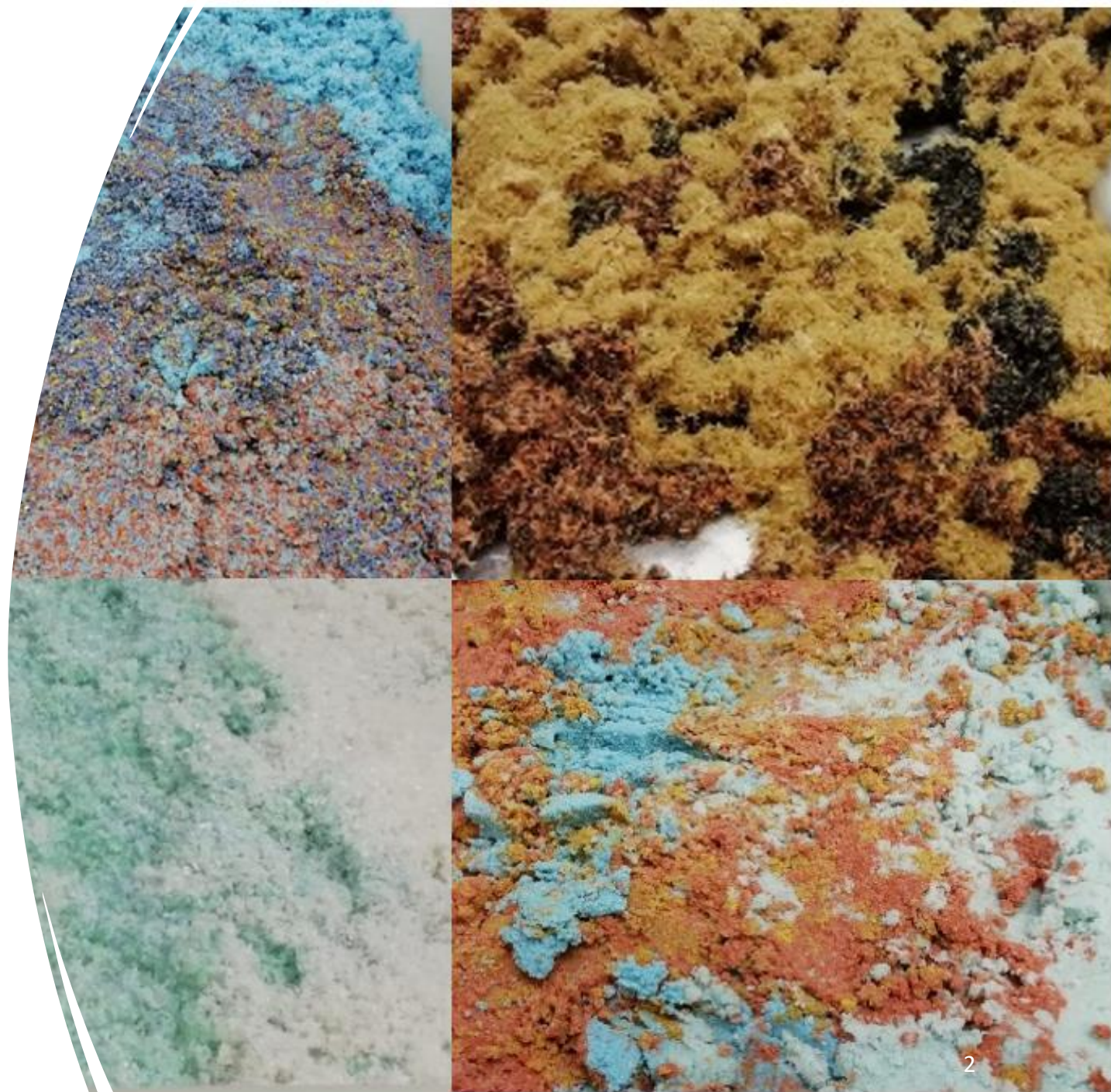
Unveiling the effects of microplastics: QA/QC, effect thresholds and effect mechanisms

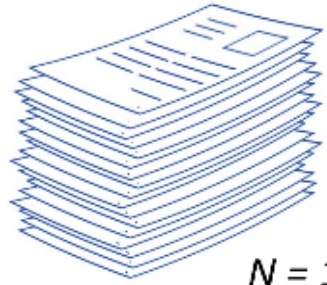
ECO49 – MICROPLASTIC EFFECT THRESHOLDS FOR AQUATIC SPECIES (METAS)

Vera de Ruijter, Martine van den Heuvel-Greve, Ivo Roessink, Todd Gouin, Paula Redondo Hasselerharm and Bart Koelmans

Content

1. Review paper QA/QC
2. Effect studies Marine and freshwater
3. Factors affecting effects *Lumbriculus variegatus*





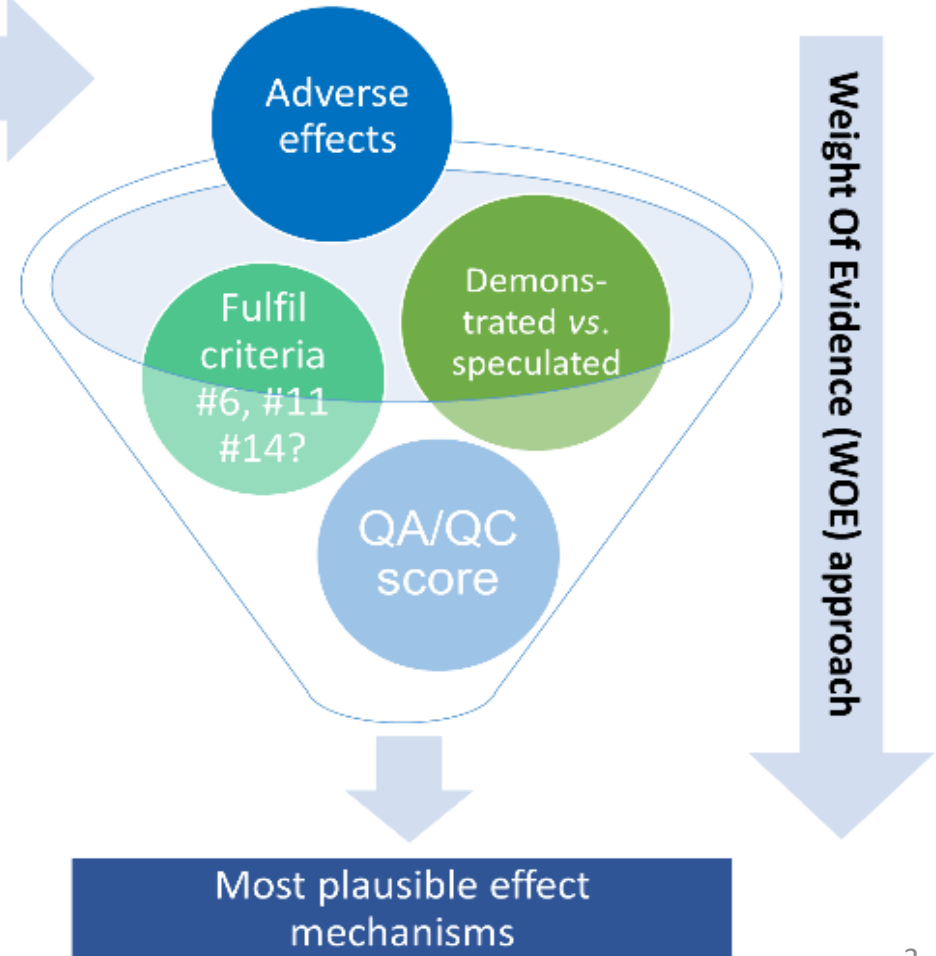
N = 105

Study characteristics

- Size shape and polymer type
- Ecosystem, class, species
- Exposure duration, endpoints and effect thresholds

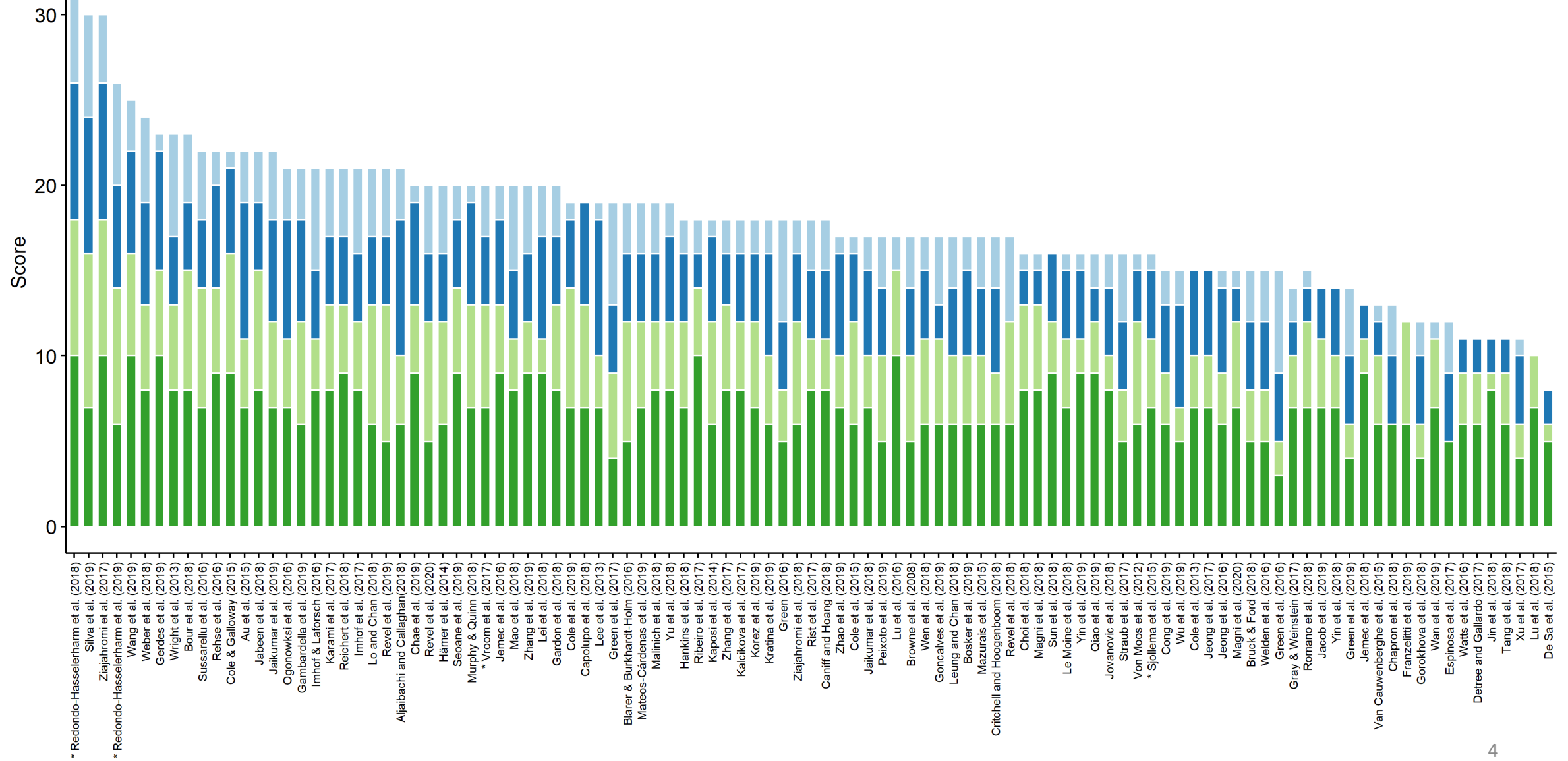
Research knowledge gaps

Quality Assurance/Quality Control (QA/QC) scoring system



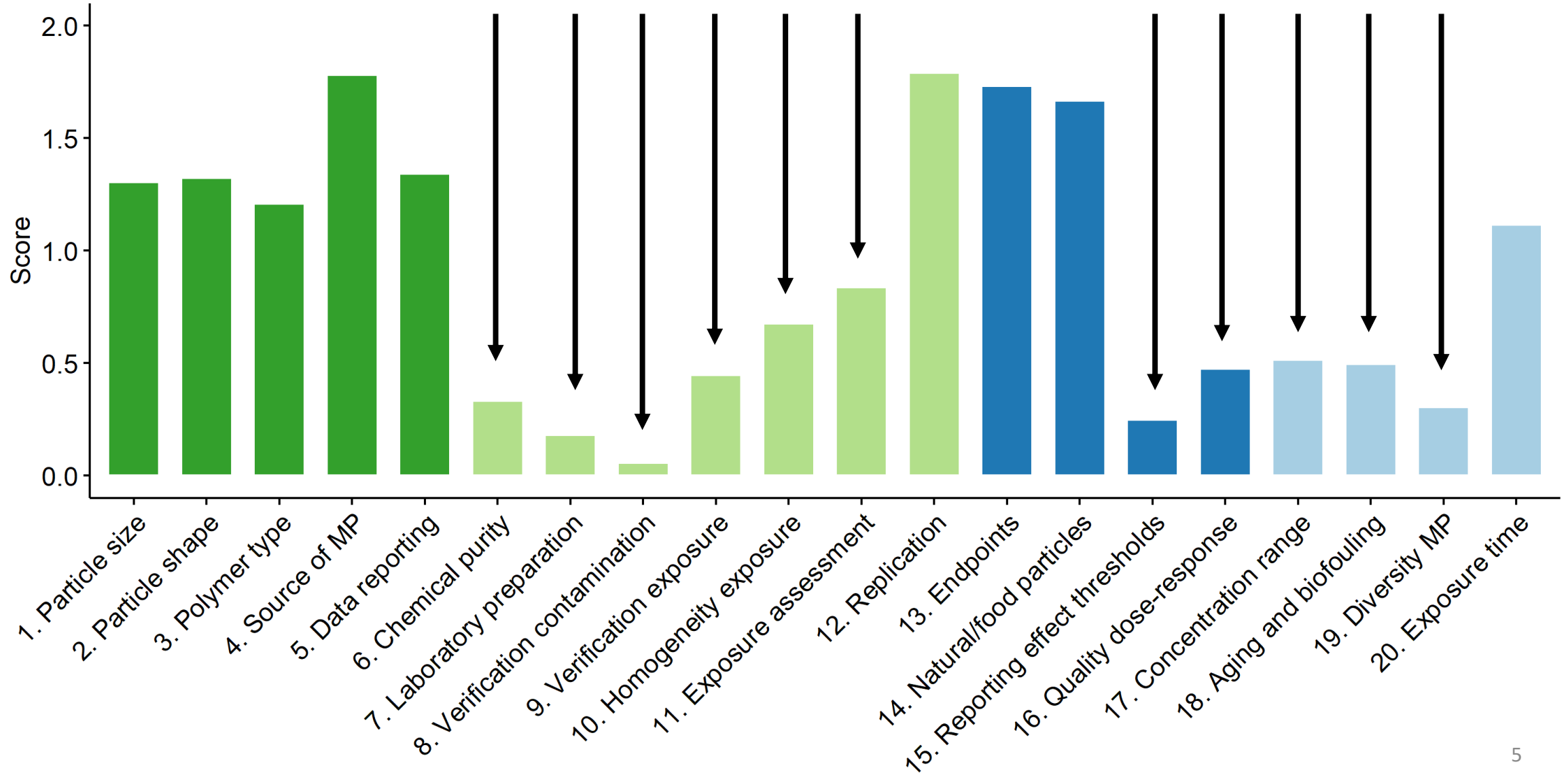
Score per study, n=105

■ Particle characterization
 ■ Experimental design
 ■ Applicable for RA
 ■ Ecological relevance

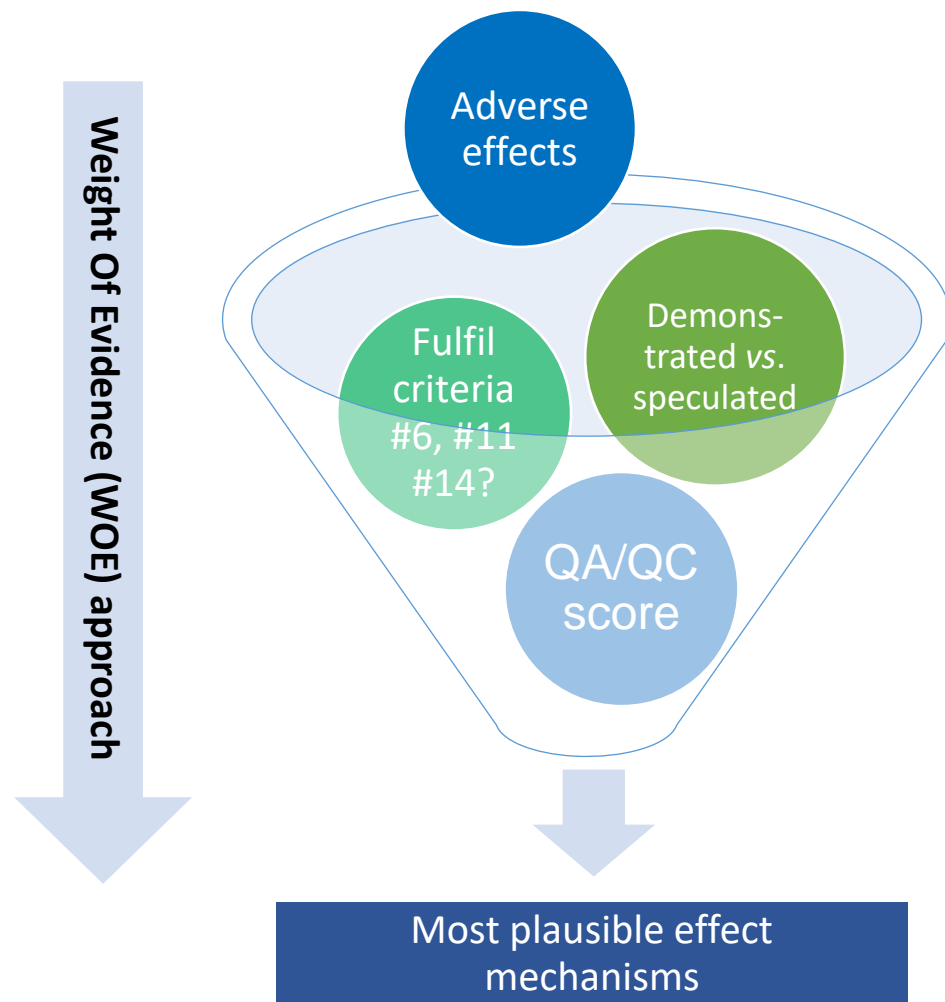


Average score per criteria, n=105

■ Particle characterization ■ Experimental design ■ Applicable for RA ■ Ecological relevance



Effect mechanisms explaining adverse effect



Crucial criteria effect mechanism:

#6 = Chemical purity

#11 = Exposure assessment of organism

#14 = Natural/food particles

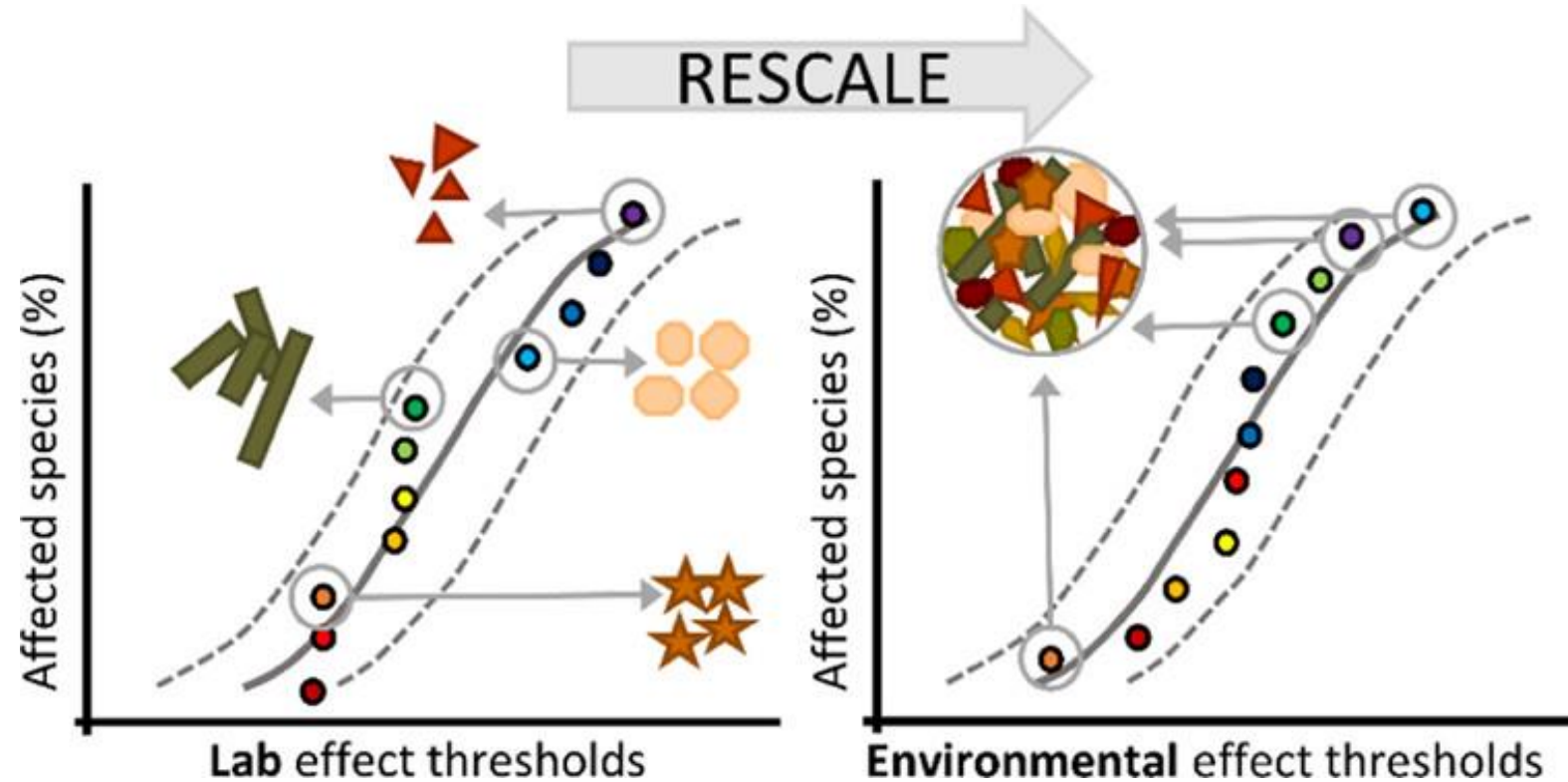
1. Decreased nutritional value/ "food dilution"
2. Internal physical damage
3. External physical damage



2. Effect studies Marine and Freshwater Benthic Macroinvertebrates

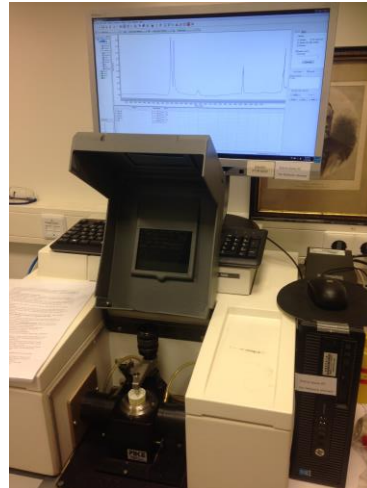
From monodisperse to polydisperse

- Only few studies have tested microplastics in its diversity (de Ruijter *et al.* 2020)
- Mostly monodisperse
- Ideally use shape, size, polymer type exactly as one would encounter in environment

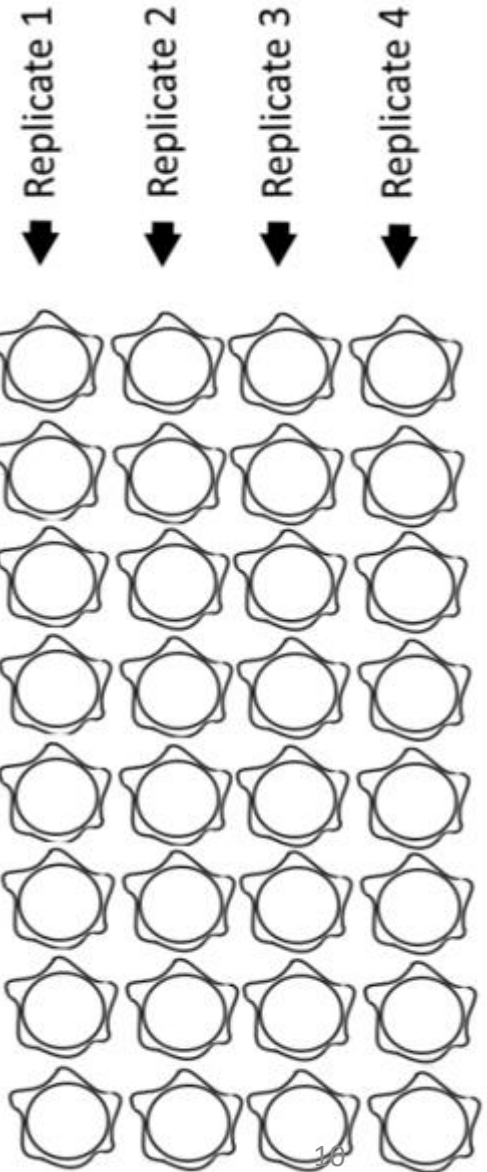
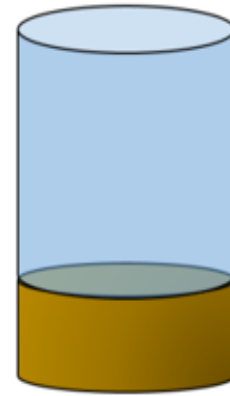


Koelmans *et al.* (2020)

Environmentally relevant microplastic (ERMP)



Experimental design



Freshwater benthic macroinvertebrates

- Tubifex* spp. (worm)
- Lumbriculus variegatus* (worm)
- Potamopyrgus Antipodarum* (mud snail)
- Asellus aquaticus* (water louse)
- Corbicula fluminalis* (clam)
- Hyalella azteca* (amphipod)
- Gammarus pulex* (amphipod)
- Sphaerium corneum* (clam)
- Chironomus riparius* (midge)



Marine benthic macroinvertebrates

Alitta virens (worm)

Arenicola marina (worm)

Porcellana platycheles (crab)

Mytilus edulis (clam)

Cerastoderma edule (clam)

Alitta virens (worm)

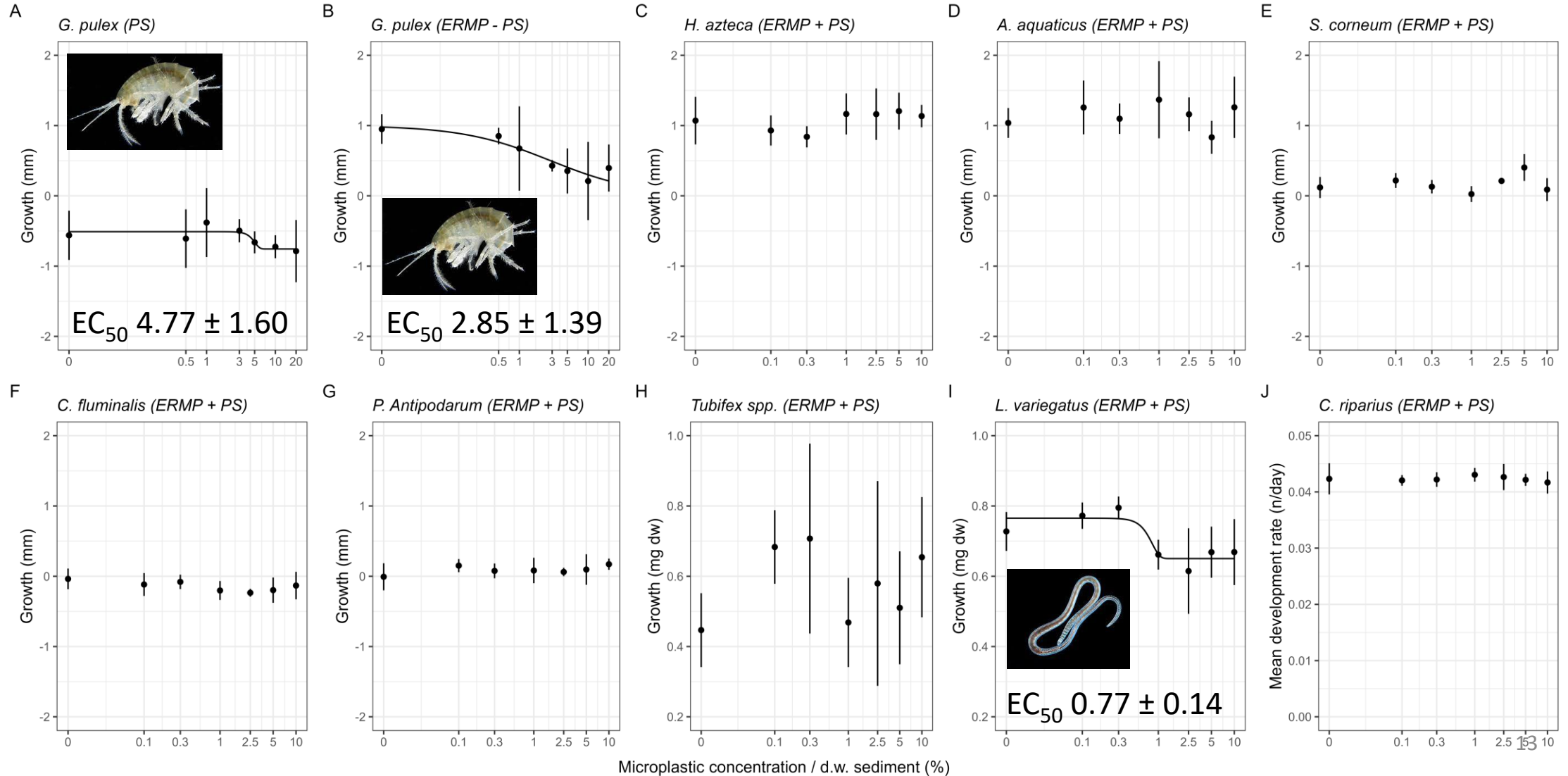
Cerastoderma edule (clam)

Corophium volutator (amphipod)

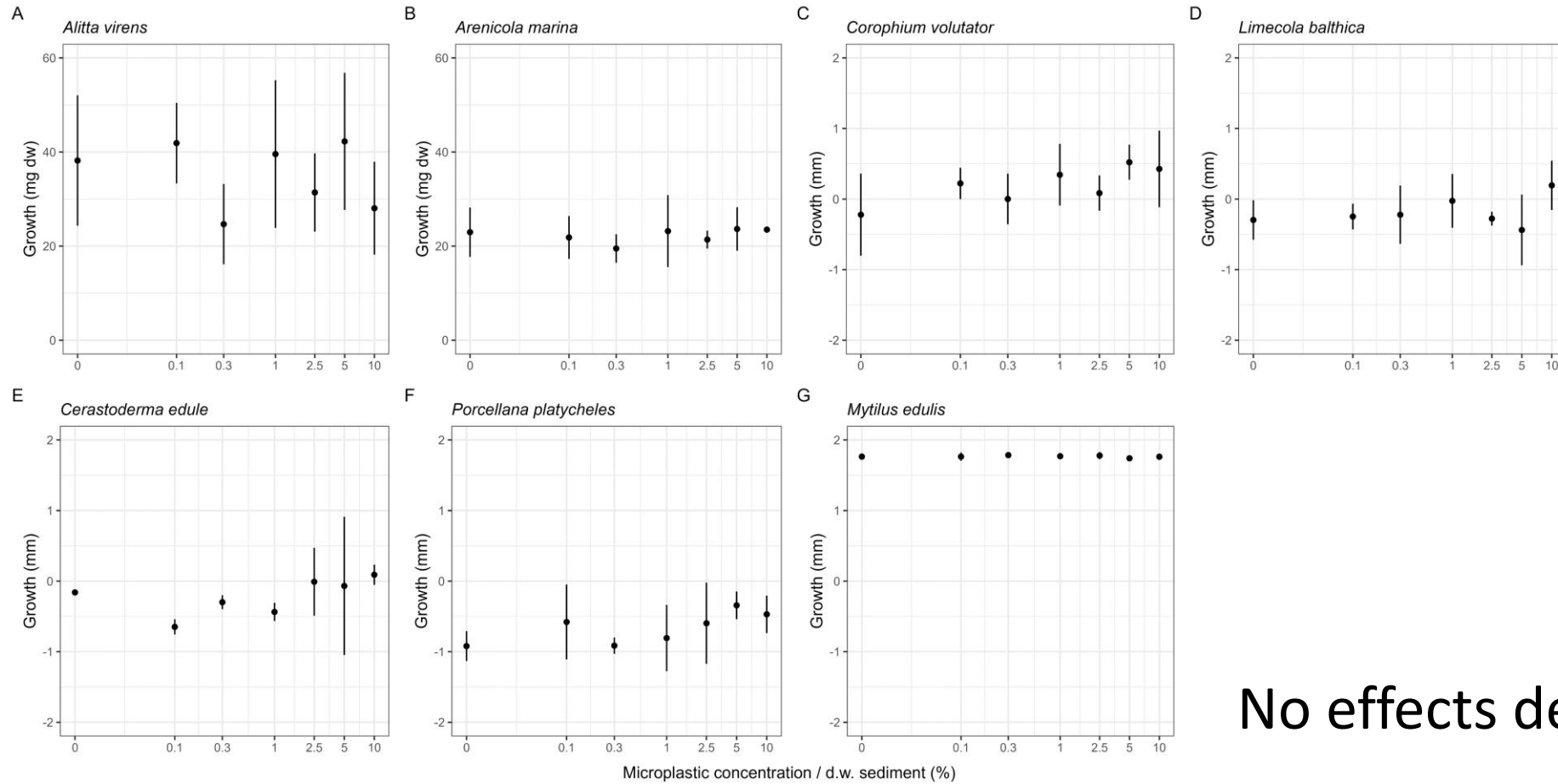
Limecola balthica (clam)



Freshwater species: Growth

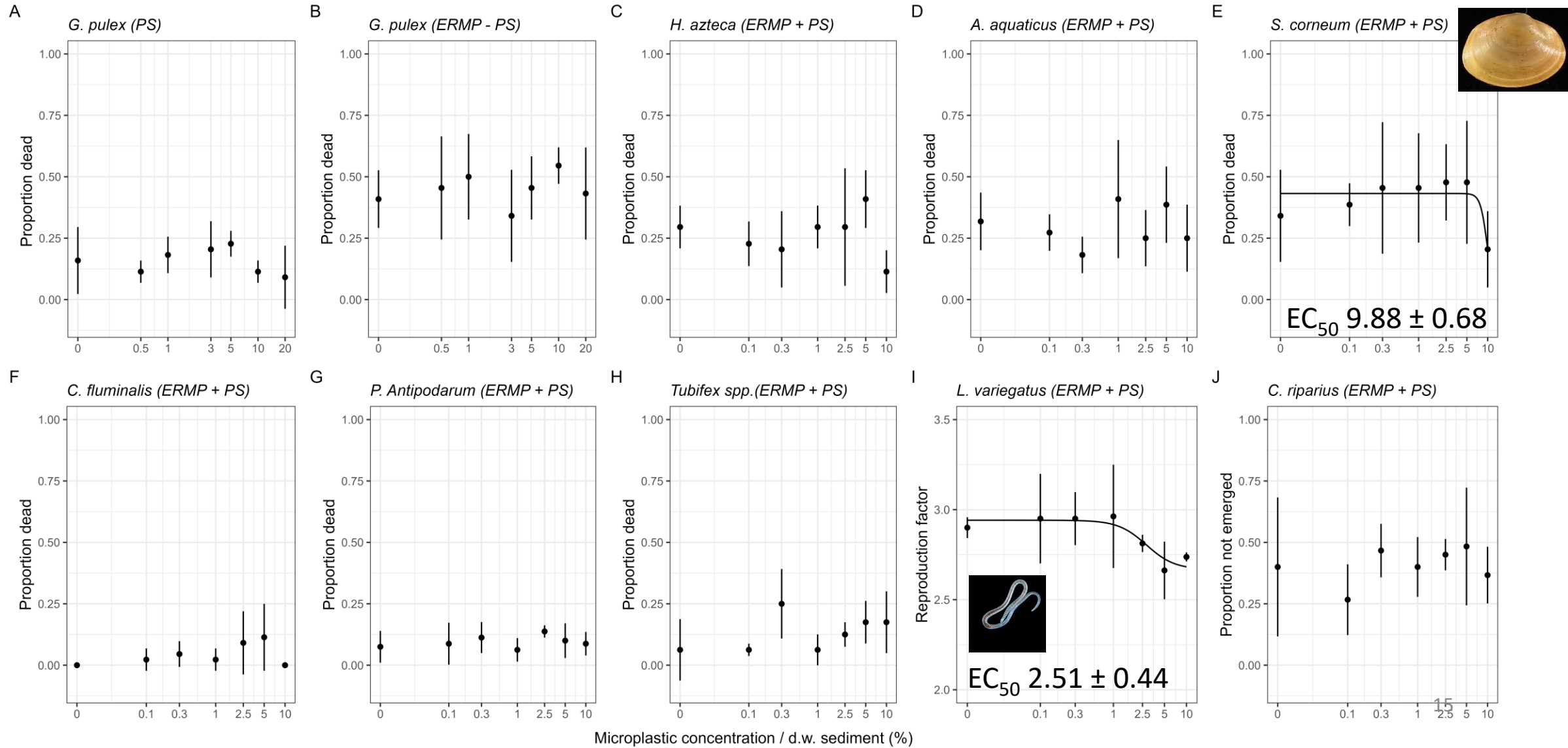


Marine species: Growth

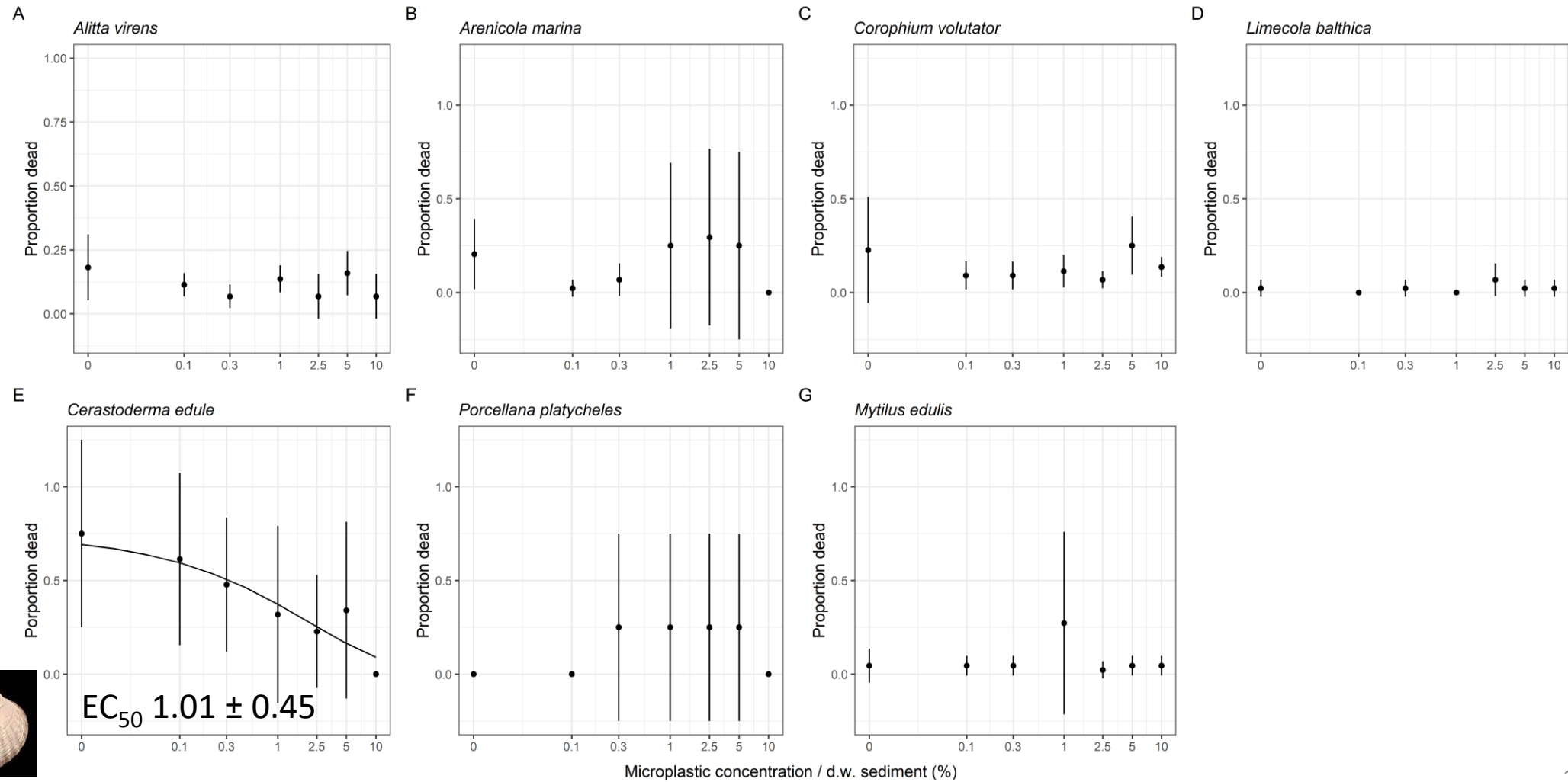


No effects detected

Freshwater species: Mortality, reproduction and emergence

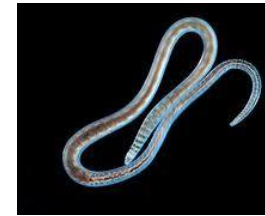
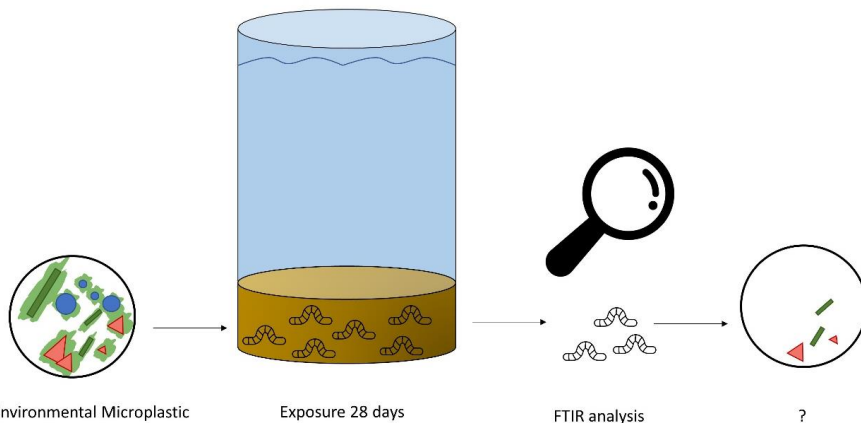


Marine species: Mortality



Ongoing work: Exposure assessment

- Dissection/ digestion
- Analysis FTIR → siMPLe
- Preference MP?
- Retention and egestion?
- Food dilution?



Treatments	Annelida	Insecta/Crustacea	Molluscs/Bivalves
10-15 ml H ₂ O ₂	24h, 37°C	24h, 37°C	24h, 37°C
1 ml Chitinase + 20 ml NaOAc buffer	-	5 days, 37°C	-
5 ml Protease + 25 ml Tris HCL buffer	24h, 50°C	24h, 50°C	24h, 50°C
1 ml Lipase + 20 ml Tris HCL buffer			24h, 40°C
10-15 ml H ₂ O ₂	24h, 37°C	24 h days, 37°C	24h, 37°C

Conclusions

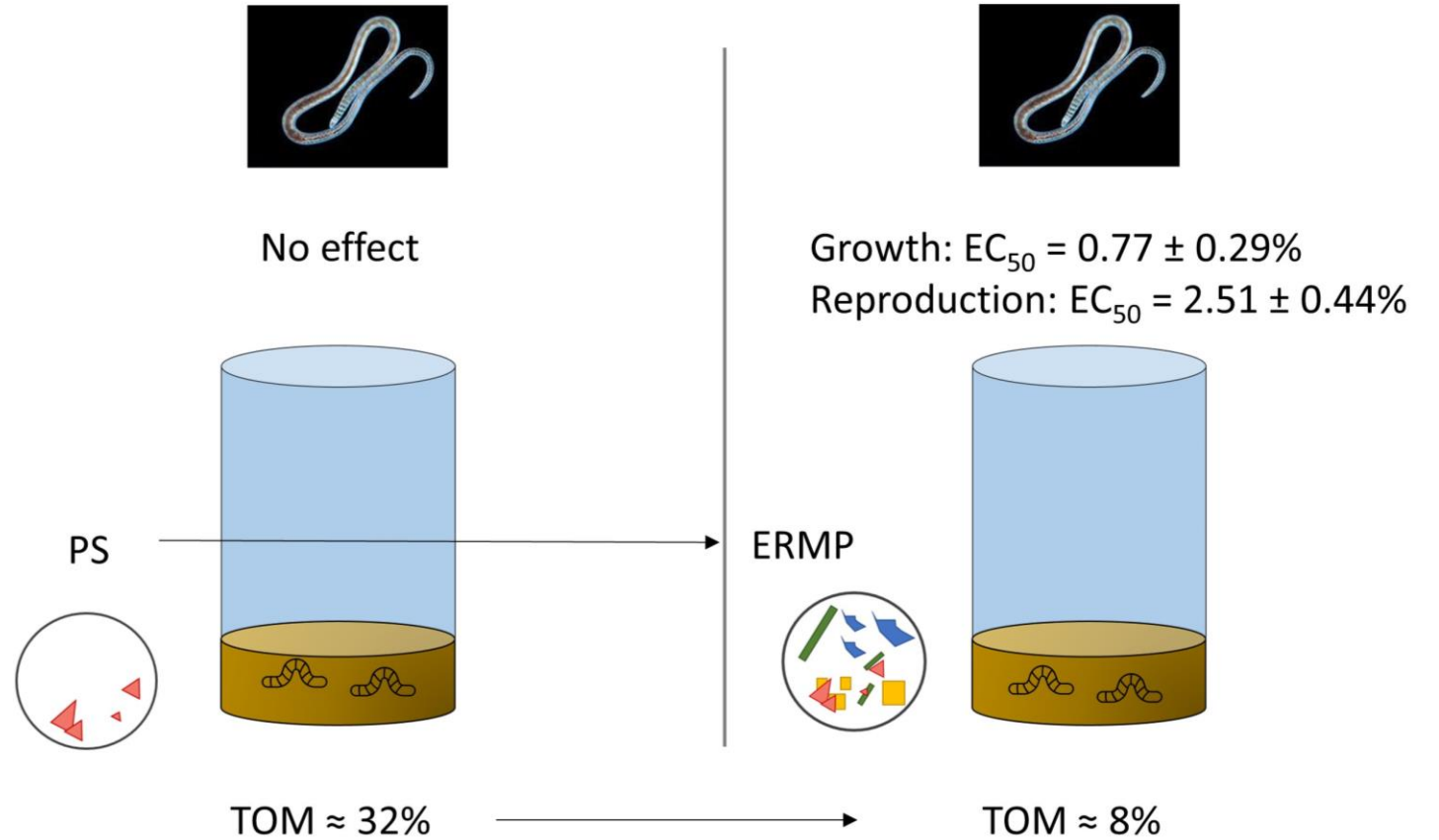
- Mostly no effects detected (28/34)
- Negative effects detected on growth and reproduction freshwater organisms (4/34):
G. pulex (PS), *G. pulex* (EMRP-PS), *L. variegatus*, reproduction and growth
- Positive effects detected on mortality clams (2/34):
S. corneum (freshwater), *C. edule* (marine)
- We demonstrate that high relevance via QA/QC and ERMP is possible → SSD



Lumbricus variegatus

3. Factors affecting effects

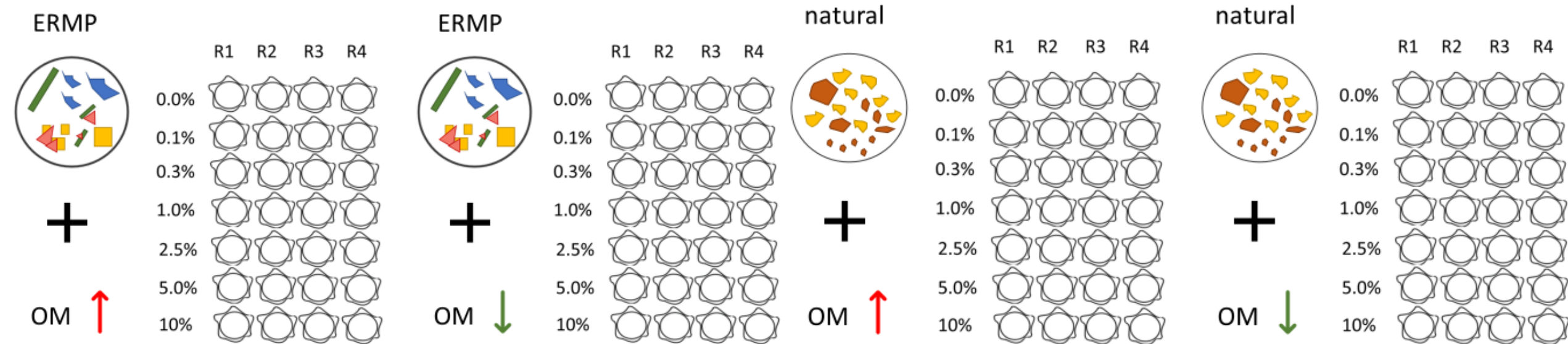
- Possible mechanism → Food dilution
- OM content important factor?



Redondo-Hasselerharm *et al.* (2018)

de Ruijter *et al.* (submitted)

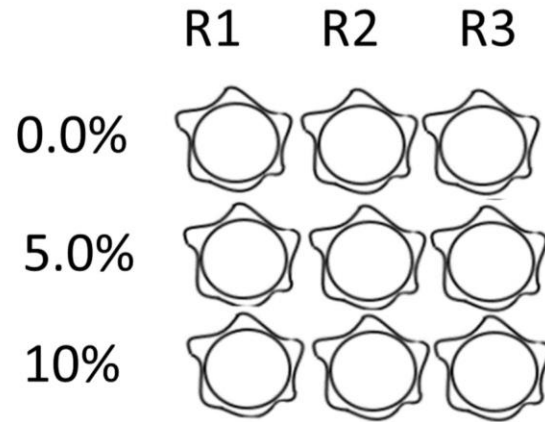
Experimental design



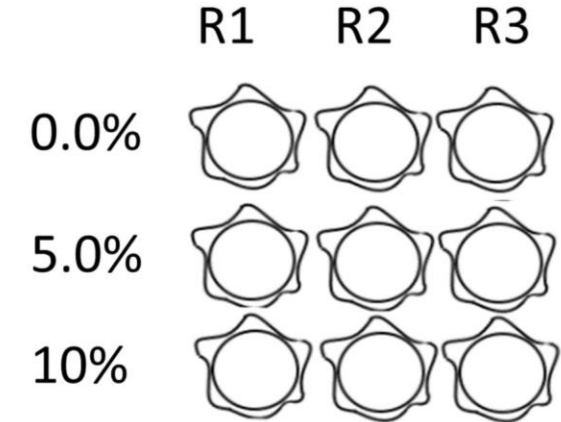
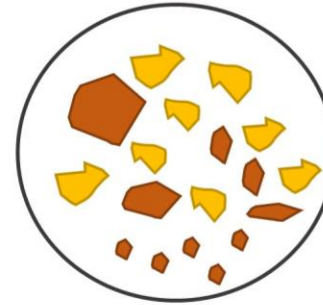
- Endpoints: growth and reproduction

- Exposure time = 28 days

ERMP



natural



Egestion study

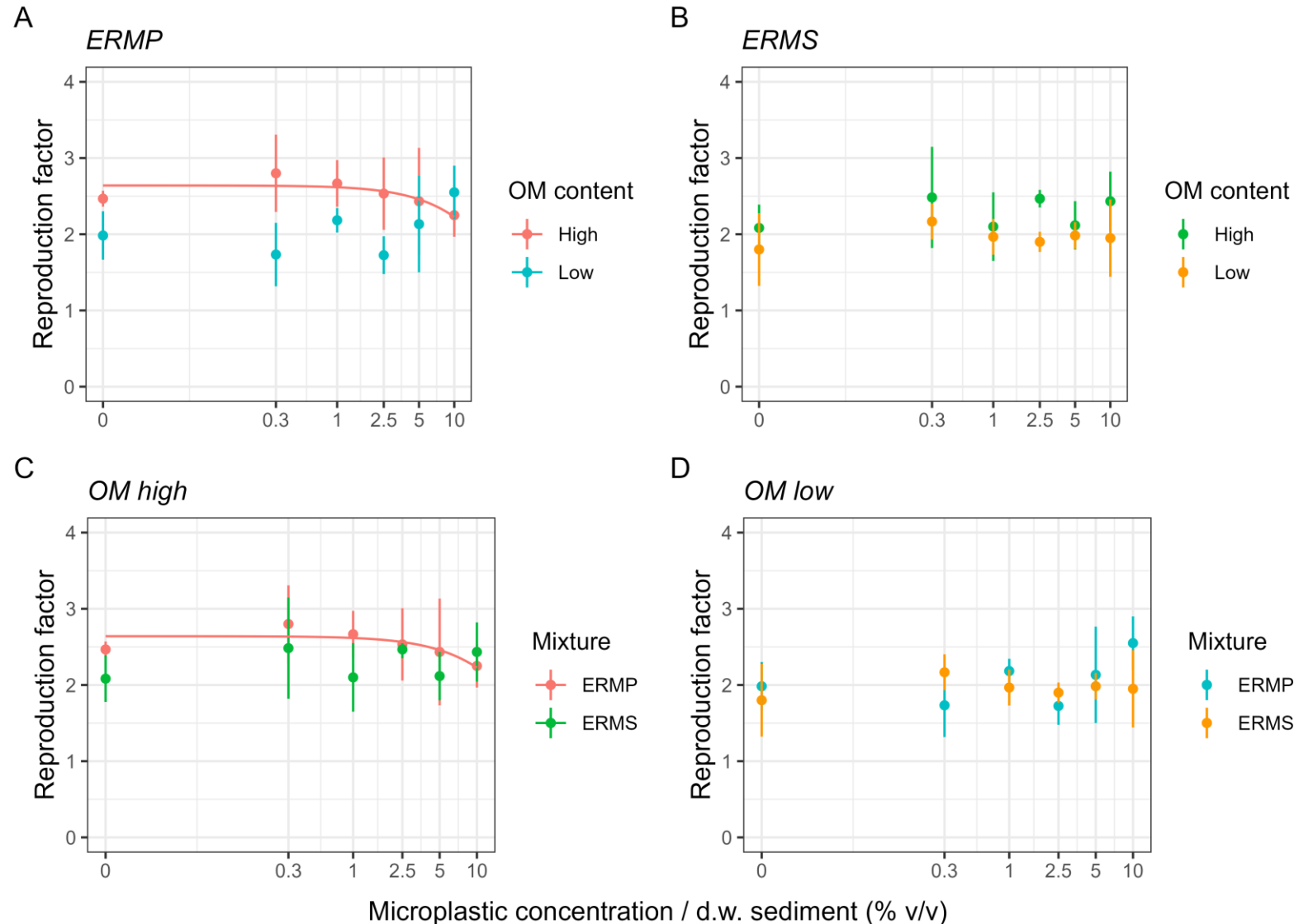
- 14 days, collecting faecal pellets every other day.

Results chronic study: Reproduction

- ERMP high OM:

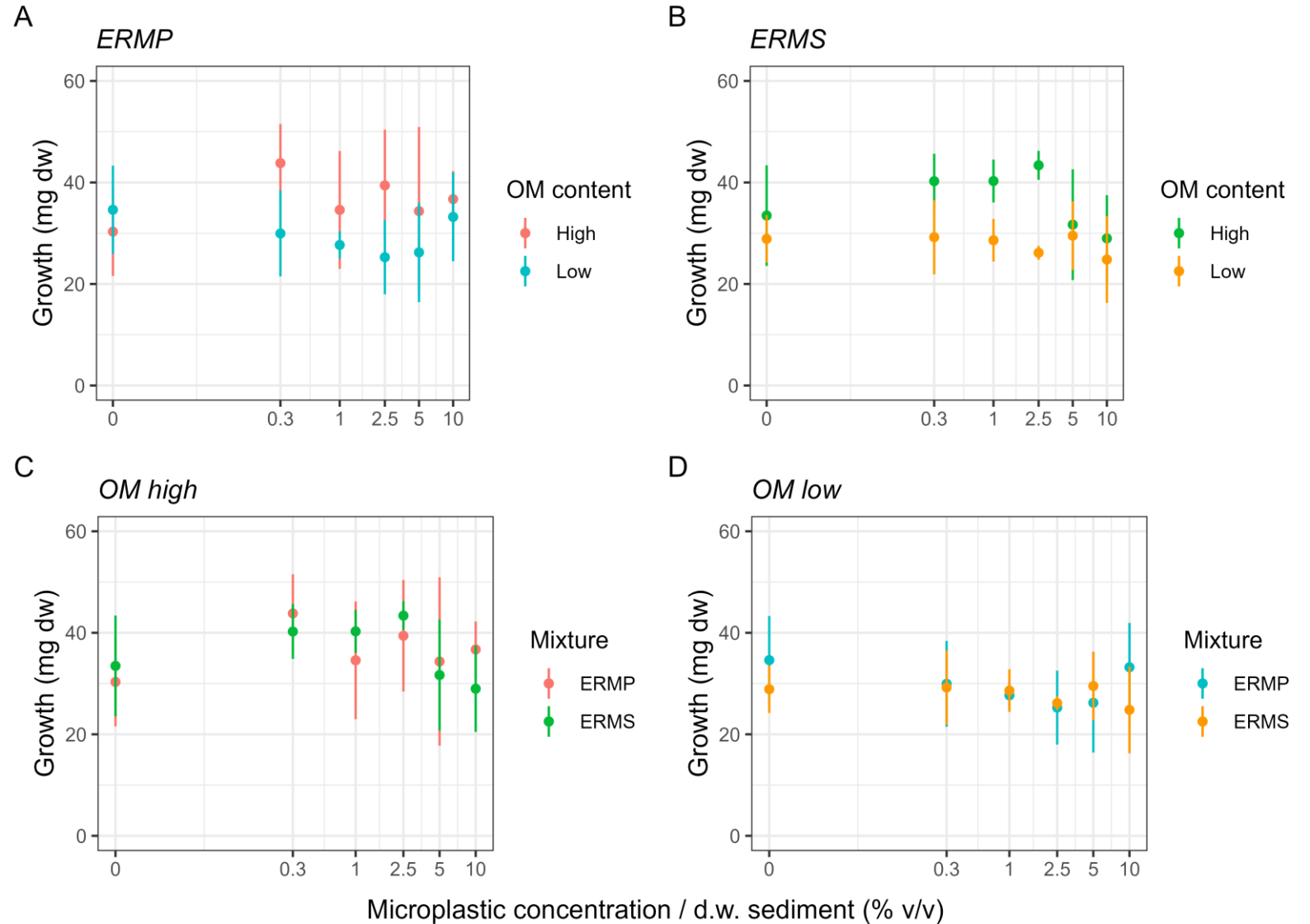
$$EC_{50} = 13.68 \pm 5.54 (\% \text{ v/v})$$

- For other treatments no significant EC_{50} could be derived.



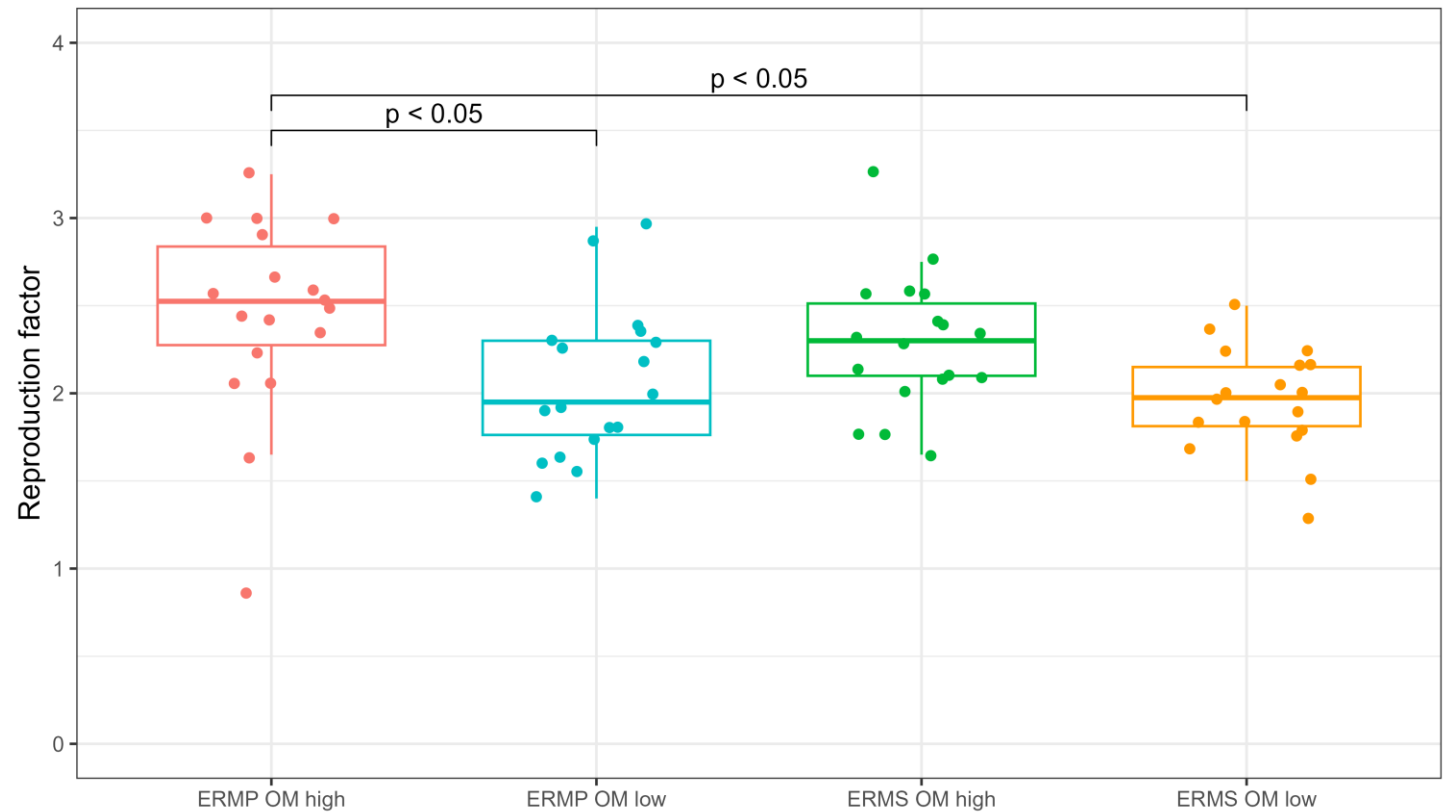
Results chronic study: Growth

- No effect of concentration on growth (dw mg) for the different treatments.



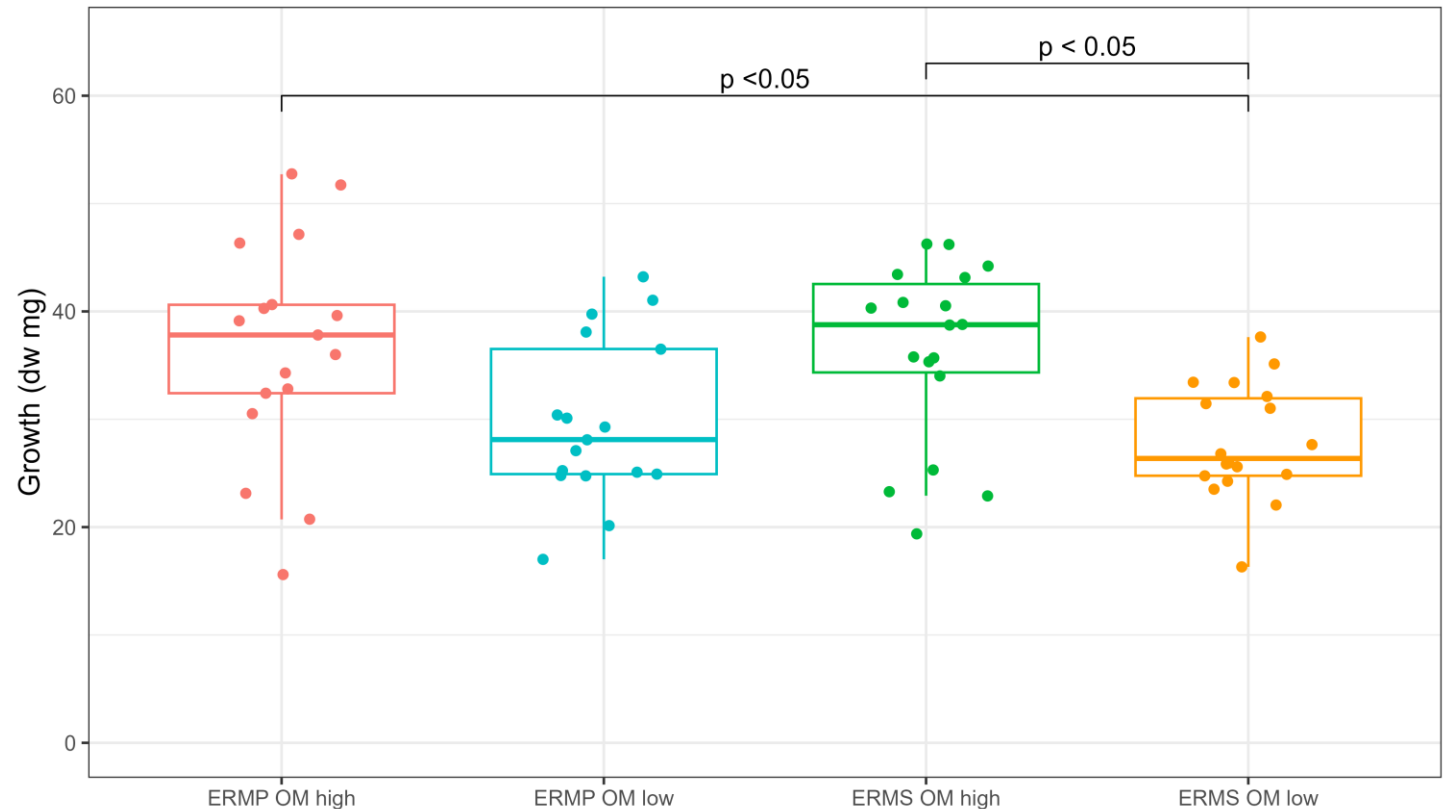
Results chronic study: Reproduction (three-way ANCOVA)

- Mixture: no difference → in line with “food dilution”
- OM content: significant factor explaining reproduction

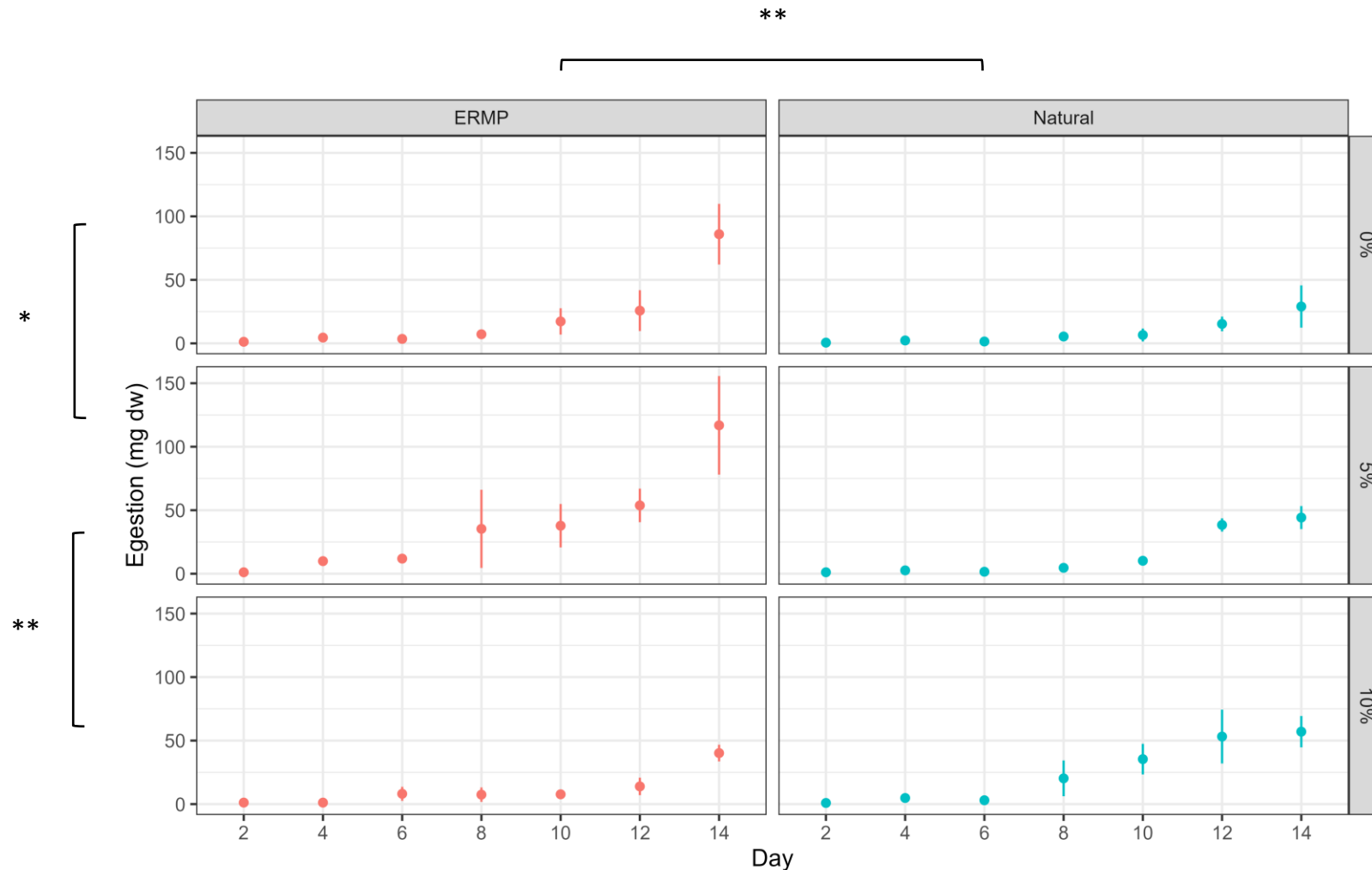


Results chronic study: Growth (three-way ANCOVA)

- Mixture: no difference → in line with “food dilution”
- OM content: significant factor explaining Growth



Results egestion study (three-way Repeated ANOVA)



- Mixture: significant difference → ERMP egest more, more energy
- Natural: Egestion concentration dependent → food dilution
- EMRP: Egestion concentration dependent → gut blockage or not bioavailable?

Conclusions factors affecting effects

Chronic study:

- **No** difference between **Mixture** types → mechanism food dilution
- **OM** has significant effect on Reproduction and Growth
- **ERMP high OM**; $EC_{50} = 13.68 \pm 5.54$ (% v/v)

Egestion study:

- Differences between **Mixture** types. ERMP > Natural
- **Egestion** is **Concentration** dependent → food dilution





Thank you for your attention !



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