# La difficile évaluation de la toxicité des microplastiques

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EPHEMARE
ECOTOXICOLOGICAL EFFECTS OF MICROPLASTICS IN MARINE ECOSYSTEMS

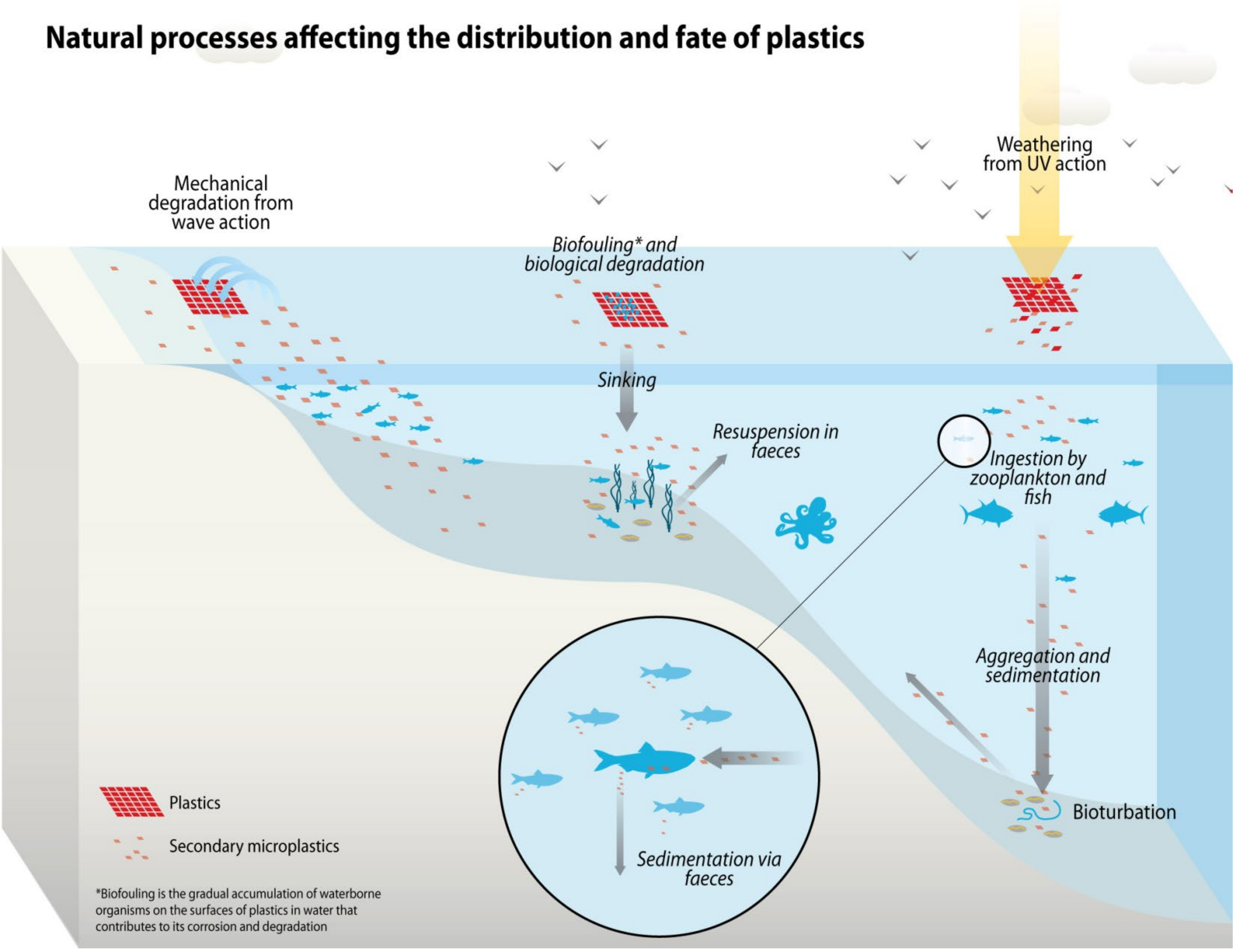
# Microplastics in the environment

- 1 nm < nano < 1 µm < microplastic < 5 mm < macro



- MPs are very diverse
  - shapes (particles, beads, fibres…)
  - plastic types (HDPE, LDPE, PVC, PS…) + biodegradables
  - additives (flame retardant, dye…)

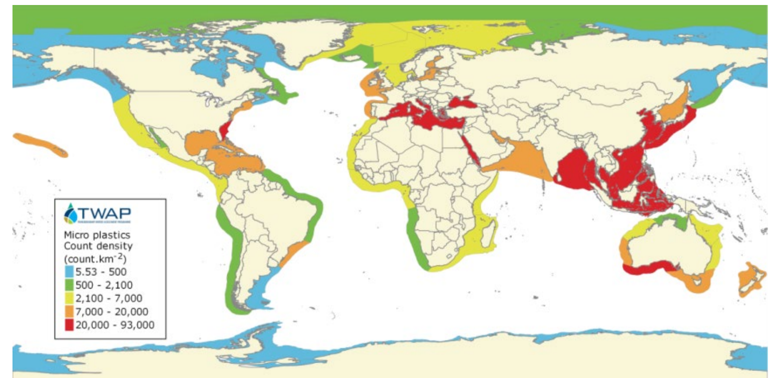
# Microplastics in the environment

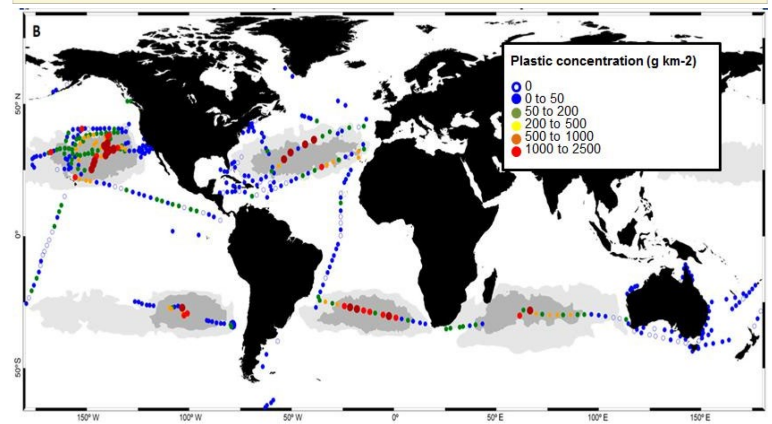



https://www.grida.no/resources/6911

# Microplastics in the environment

- Multiple sources
- Ubiquitous distribution in aquatic environment
- Most studies are focused on water compartment of marine environment but the same issues occurred in freshwater
- Sediment is an underestimated compartment



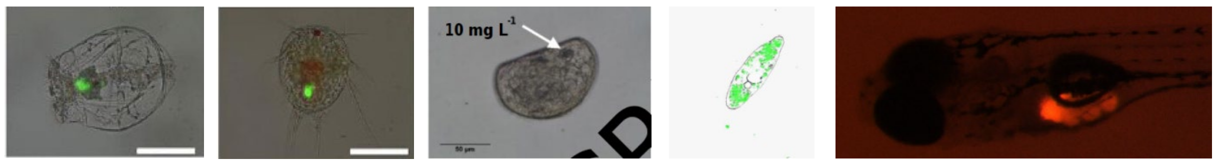





TWAP Large Marine Ecosystems Indicators (2015)
Cozar et al. (2014) *PNAS* **111**, 10239–10244
Browne et al. (2011) *Environ Sci Technol* **45**, 9175–9179

# Direct uptake of MPs

- All tested organisms were able to ingest MPs




  - 10-20 µm seems to be the upper limit for plankton
- No internalisation for cells in culture or microalgae
  - In the micrometer range
- Size, shape, weathering seem to influence uptake
- Egestion is often fast (within hours) and evidences for translocation are debated
  - Translocation seems to be limited to some organisms
  - Translocation seems limited to the smallest MPs or NPs

# Importance/relevance of trophic transfer for MPs uptake?

- Preys can act as a funnel delivering high amounts of MPs
- and transport across compartments act as a new source of MPs (e.g. sediment → plankton → pelagic)
- May be important for some organisms or life stages (e.g. fish larvae)

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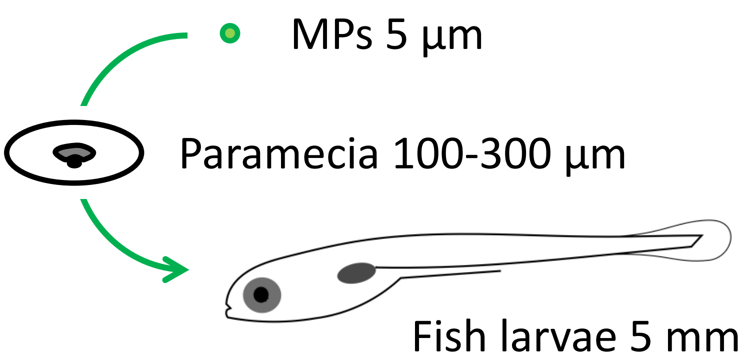
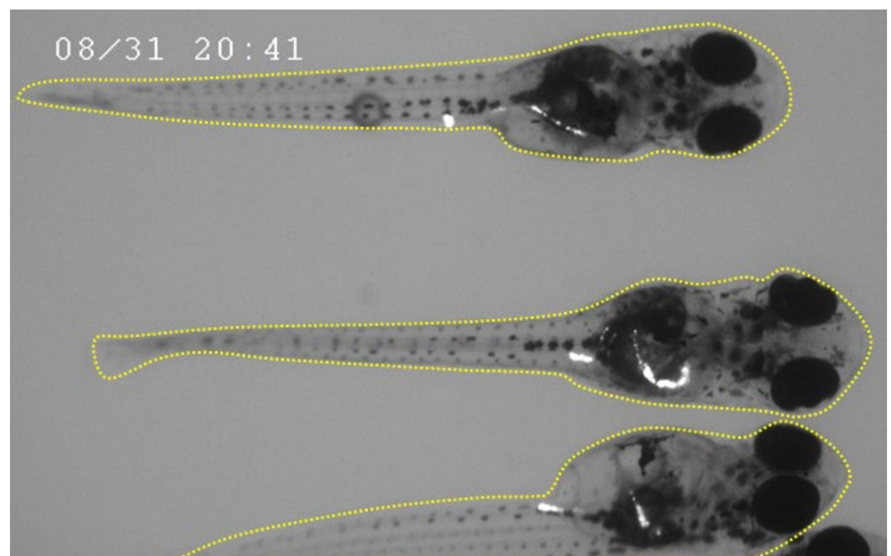
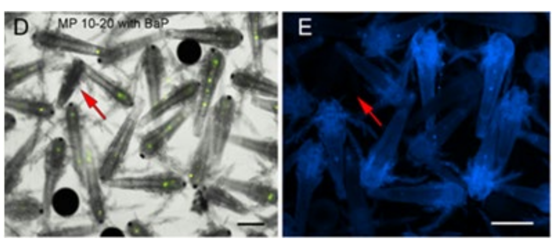


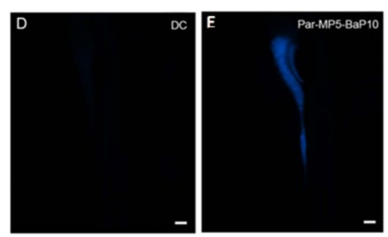



# Can MPs act as vector for pollutants?

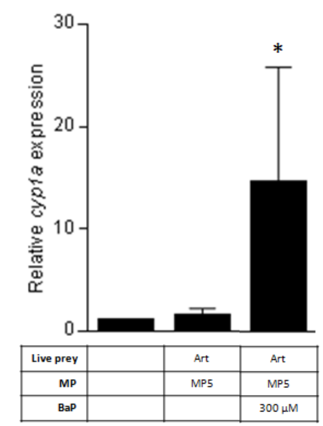
- Beyond additives, many chemicals can adsorb to MPs
- Mechanisms underlying adsorption and desorption inside organisms remain largely unknown
- Chemicals can be delivered to organisms by MPs
- Chemicals may be "pre"-desorbed in preys




BaP desorption in prey




BaP transfer to larvae through food chain




BaP is bioavailable in fish larvae

Batel et al. (2016) *Environ Toxicol Chem* **35**,1656-66.
Cousin et al. (2020) *Mar Environ Res* **161**, 105126

# Regulatory (acute) toxicity tests

| Organisms | | Methods | Size (µm) | LOEC (mg/L) |
|---|---|---|---|---|
| Rotifer | *Brachionus plicatilis* | ASTM | 1-4 | 1 |
| | | | 4-6 | >10* |
| Copepod | *Tigriopus fulvus* | ISO 14669 | 1-4 | 1 |
| | | | 4-6 | >10* |
| Copepod | *Acartia clausi* | ISO 14669 | 4-6 | >30* |
| Mussel | *Mytilus galloprovincialis* | ISO 17244 | 1-4, 4-6, 6-8.5, 11-13 | >100* |
| | | | <63 | >100* |
| Sea urchin | *Paracentrotus lividus* | | 4-6, 11-15 | 100* |
| | | | <40 | >100* |
| Fish | *Oryzias melastigma* | OECD 212 | 4-6 | >10* |

* Maximum tested concentration

→No toxicity using acute tests
→No toxicity at environmental concentration

Beiras et al., (2018) *J Hazard Mat* **360**, 452-460

# Evaluation of chronic toxicity

- Industrial microplastics
  - LDPE 2-10 µm (Micropowders Inc.)
  - PVC 80-200 µm (Fainplast Srl.)

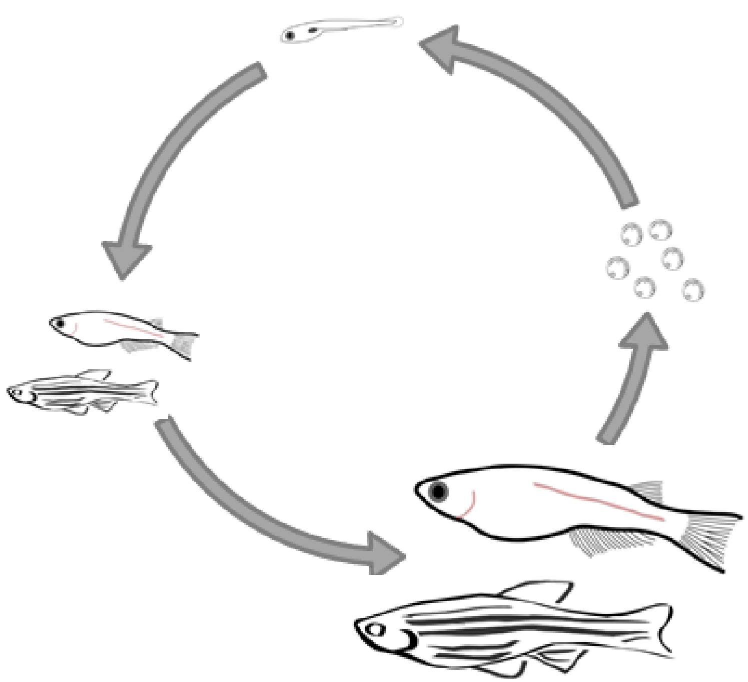
| Plastic types | Control | +perfluorooctane sulfonic acid | +benzo[a]pyrene | + benzophenone-3 |
|---|---|---|---|---|
| PE | PE-MP | PE-PFOS | PE-BaP | PE-BP3 |
| PVC | PVC-MP | PVC-PFOS | PVC-BaP | PVC-BP3 |

- A total of 8 combinations

Cormier et al., (2021) *J Hazard Mat* **415**, 125626.

# Model species

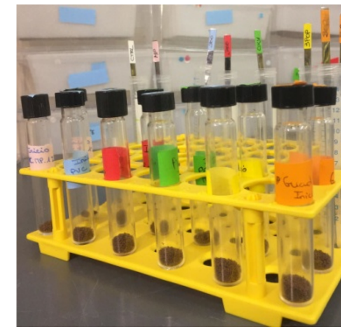
- Fish species
  - Zebrafish (*Danio rerio*) short lifecycle freshwater fish
  - Marine medaka (*Oryzias melastigma*) short lifecycle estuarine fish

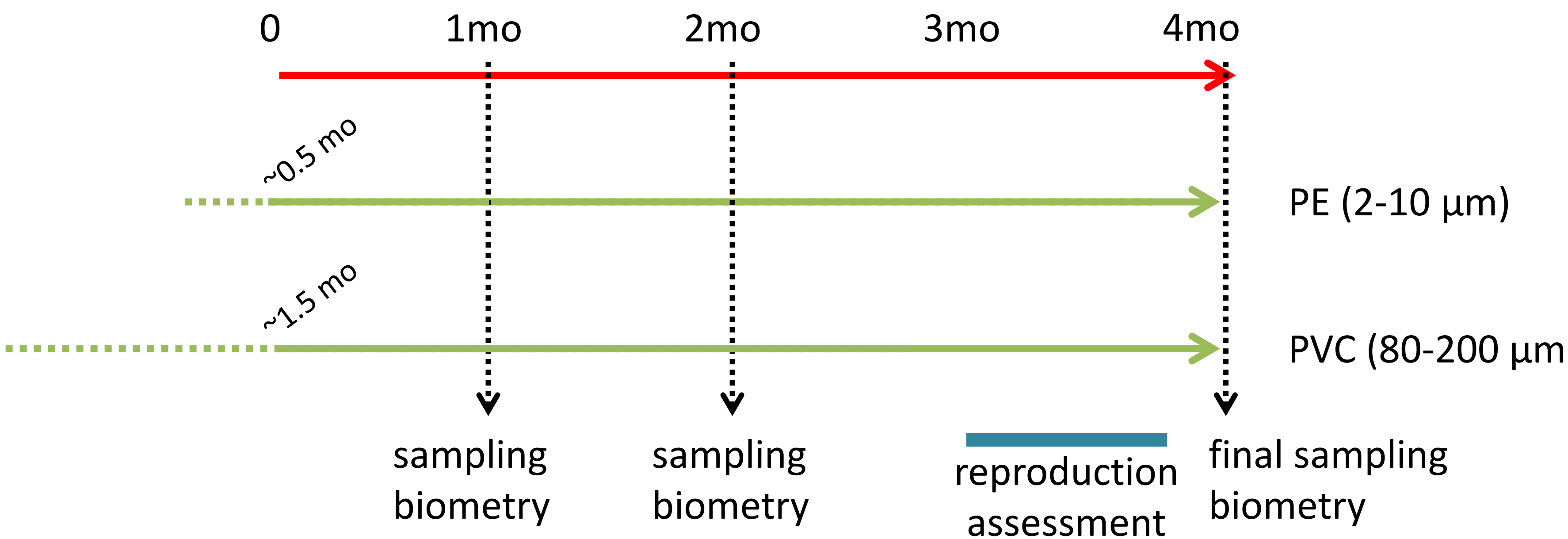


→ From egg to reproducing adult in ~3 months

# Long-term exposure

- Dietary exposure over 4 months
  - Spiking of diet (food pellets 100-400 µm) at 1% w/w
  - Exposure schedule according to MPs size





# Long-term exposure

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This means:

- **1.5-3.5 particles** of 330x330x330 µm

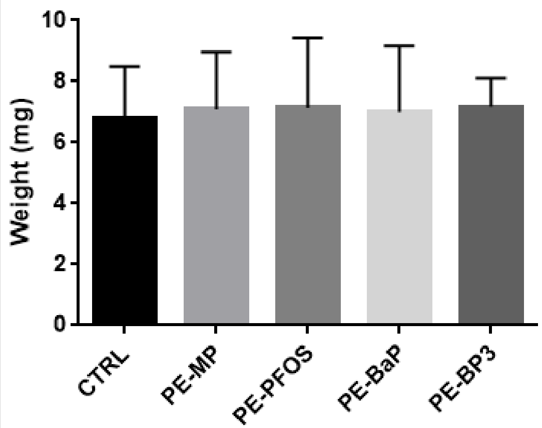
per individual per day for adults

Automated flow-through system with 30% of daily water renewal

# No or weak early disruption of growth

| →1 month of exposure | →2 months of exposure |
|---|---|

→ medaka /PE

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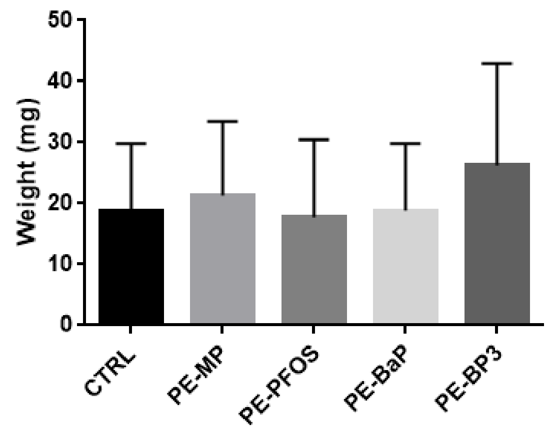
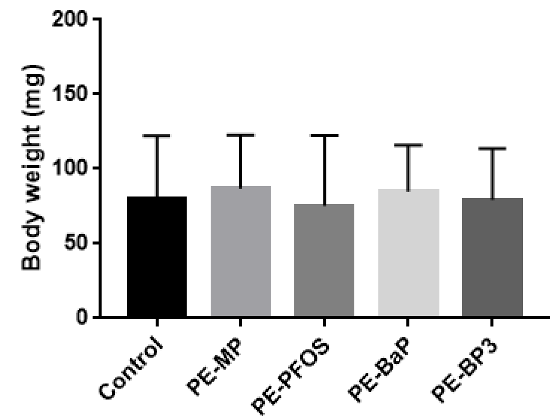
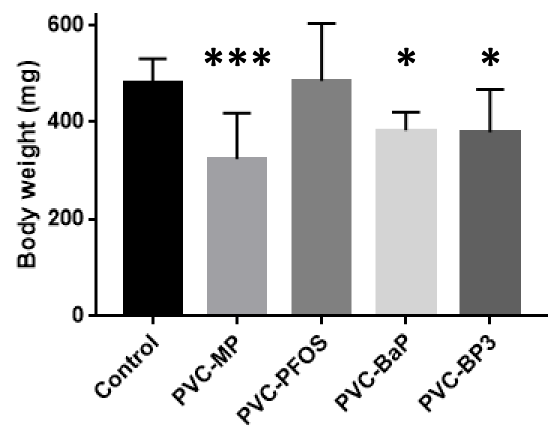


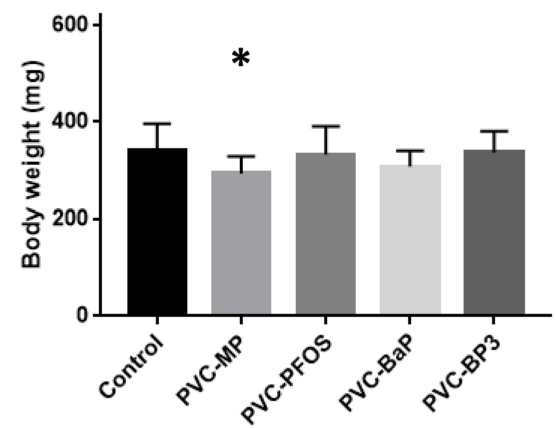

→ zebrafish /PE




→ zebrafish / PVC / female

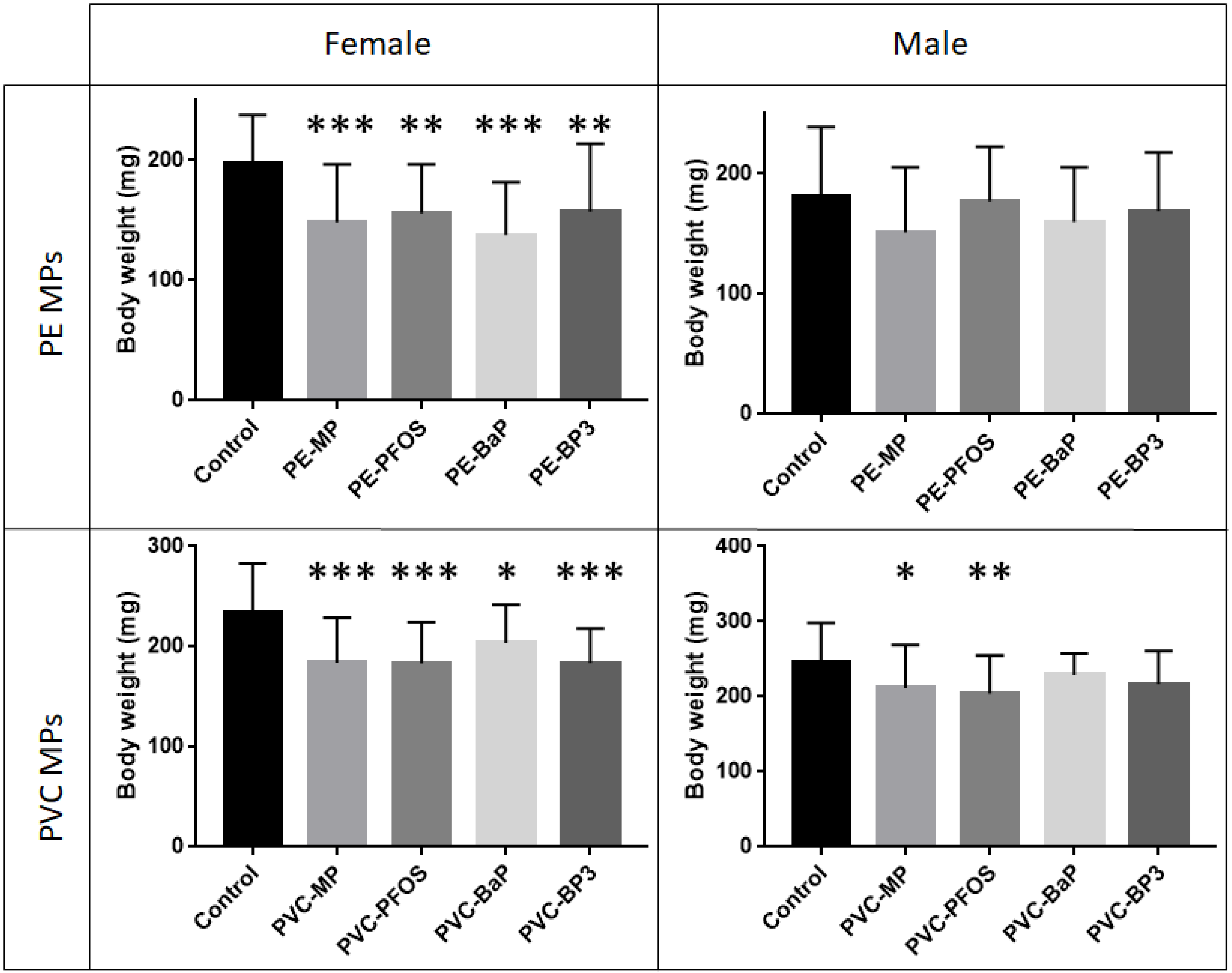



→ zebrafish /PVC / male

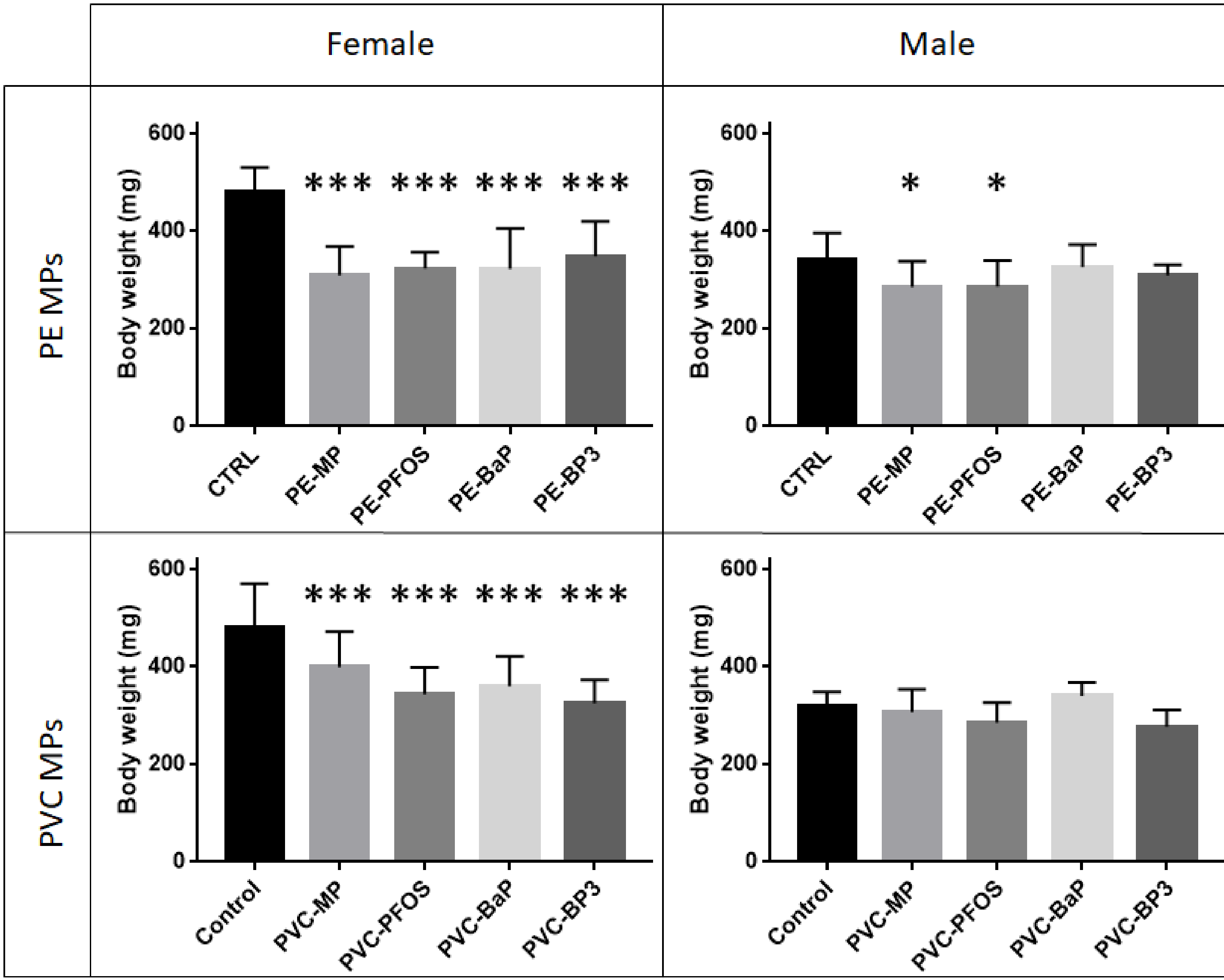



→ No or weak early effects on growth
→ No difference between plastic types
→ No major difference between MP and MP+pollutant

# After 4 months of exposure in medaka



# After 4 months of exposure in zebrafish



# Long-term exposure disrupted growth

→ Disruption of growth occurred after several months of exposure

→ A long-term effect

→ No difference between plastic types

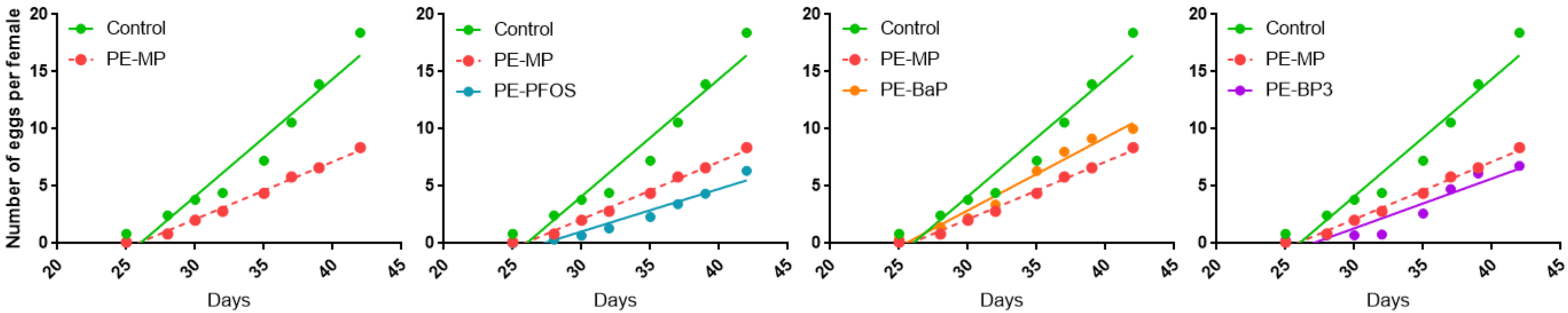
→ No difference between MP and MP+pollutant

→ A particle effect apparently not potentiated by chemicals

→ No difference between species

→ Major difference between sexes with 20-35% of growth decrease in females

# Consequences on reproduction - medaka

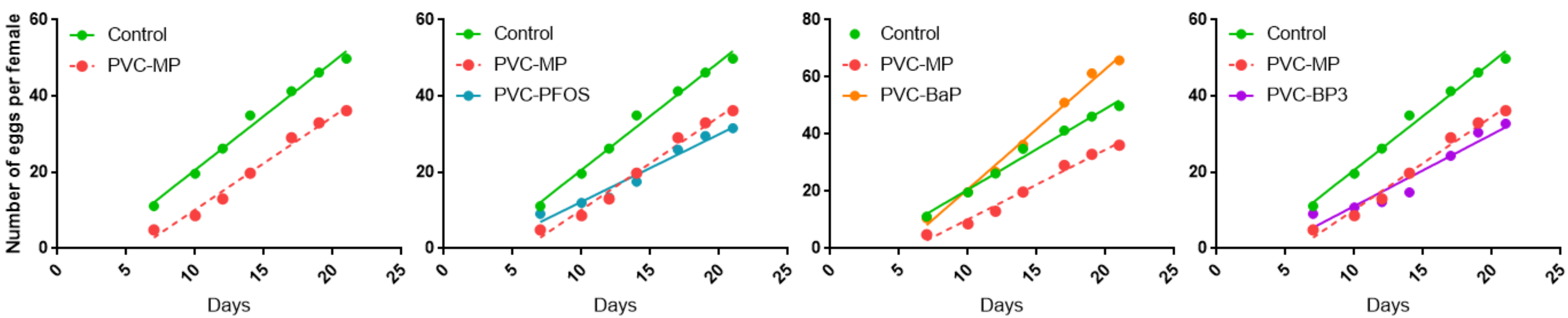



PE-MP ↘↘

PE-MP ↘ ↘
PE-PFOS ↘ ↘ ↘

PE-MP ↘ ↘
PE-BaP ↘

PE-MP ↘ ↘
PE-BP3 ↘ ↘

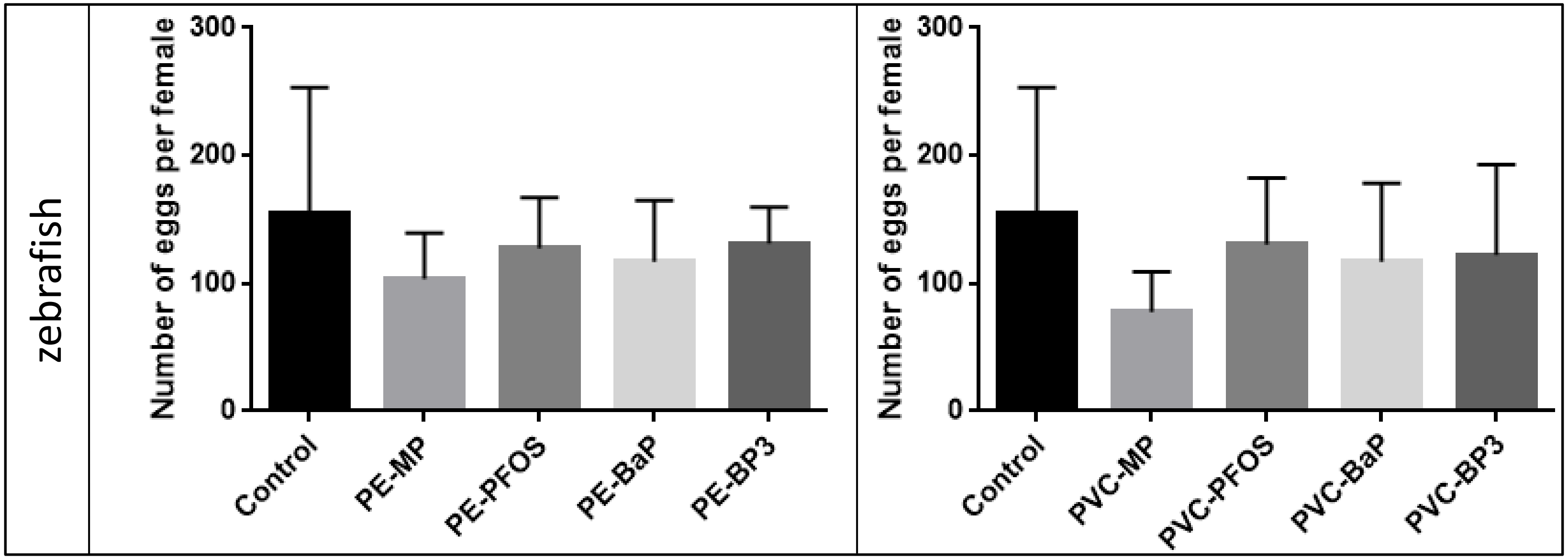



PVC-PFOS ↘

PVC-BaP ↗

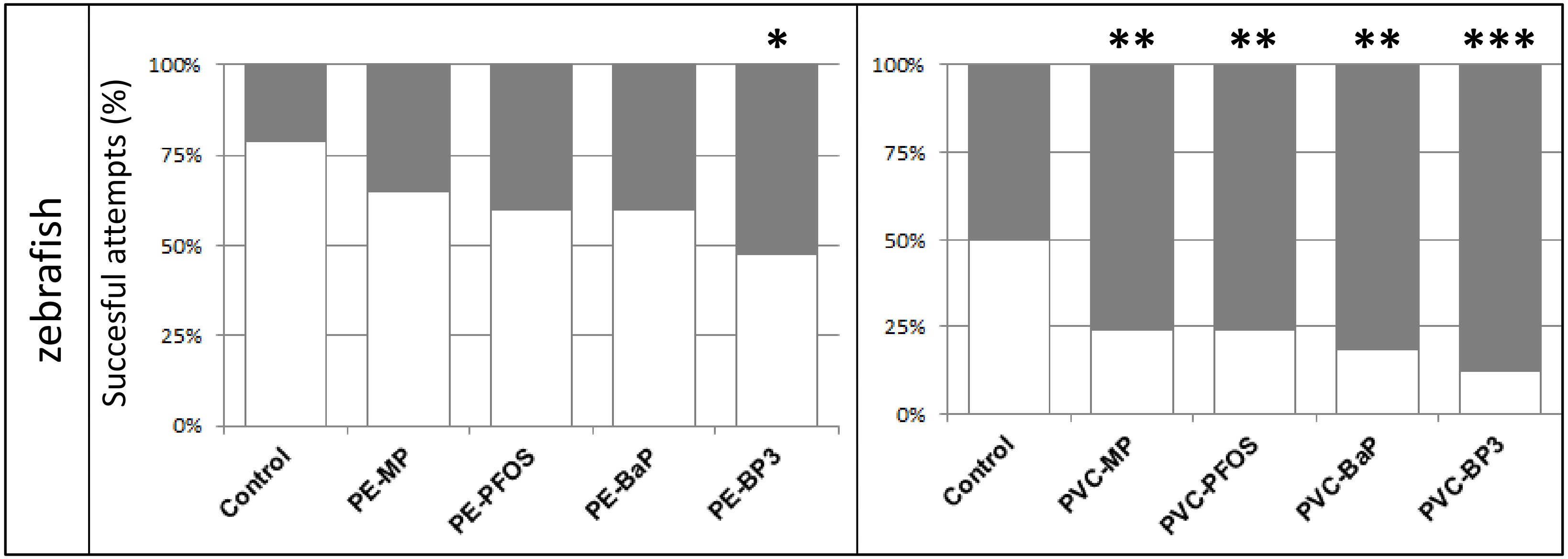
PVC-BP3 ↘

# Consequences on reproduction - zebrafish




→ No change in number of eggs per female = spawn size

# Consequences on reproduction - zebrafish




→ Significant decrease in the number of spawns obtained

→ Difference between plastic types
→ Difference between spiked chemicals

→ In medaka, number integrates spawn size and successful attempts
→ There are however some differences regarding sensitivity which seems to depend on plastic types

# Conclusions on toxicity

- Depends on the way it is assessed
- **Regulatory tests** often **failed** to reveal toxicity
  - Too short, too insensitive and not necessarily suitable for MPs (e.g. buoyancy, aggregation)
- There are evidences of **toxicity after long-term exposure**
  - Growth, reproduction
  - Energy unbalance?
  - Potential consequences at population level
- There are evidences of toxicity due to adsorbed pollutants

# Still needed

- High-throughput but relevant toxicity tests
- Evaluation of smaller sizes
  - Combination with translocation potential
- Evaluation of other plastic types
  - Fibres
  - Polymers including biodegradable ones

- Validation of the energy unbalance hypothesis
- Interference with microbiota
- Evaluation of ecological relevance for chemical transfer

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