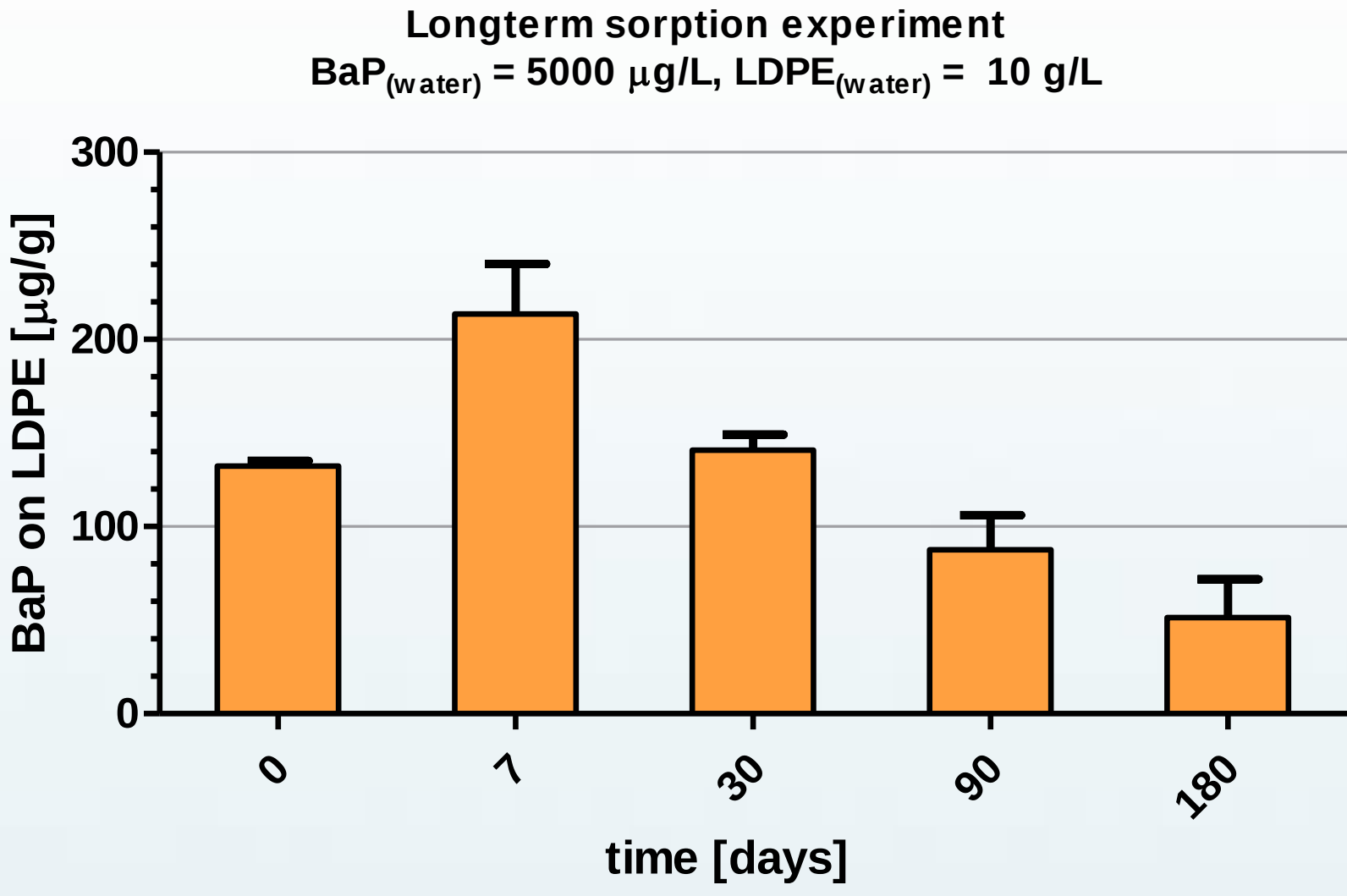
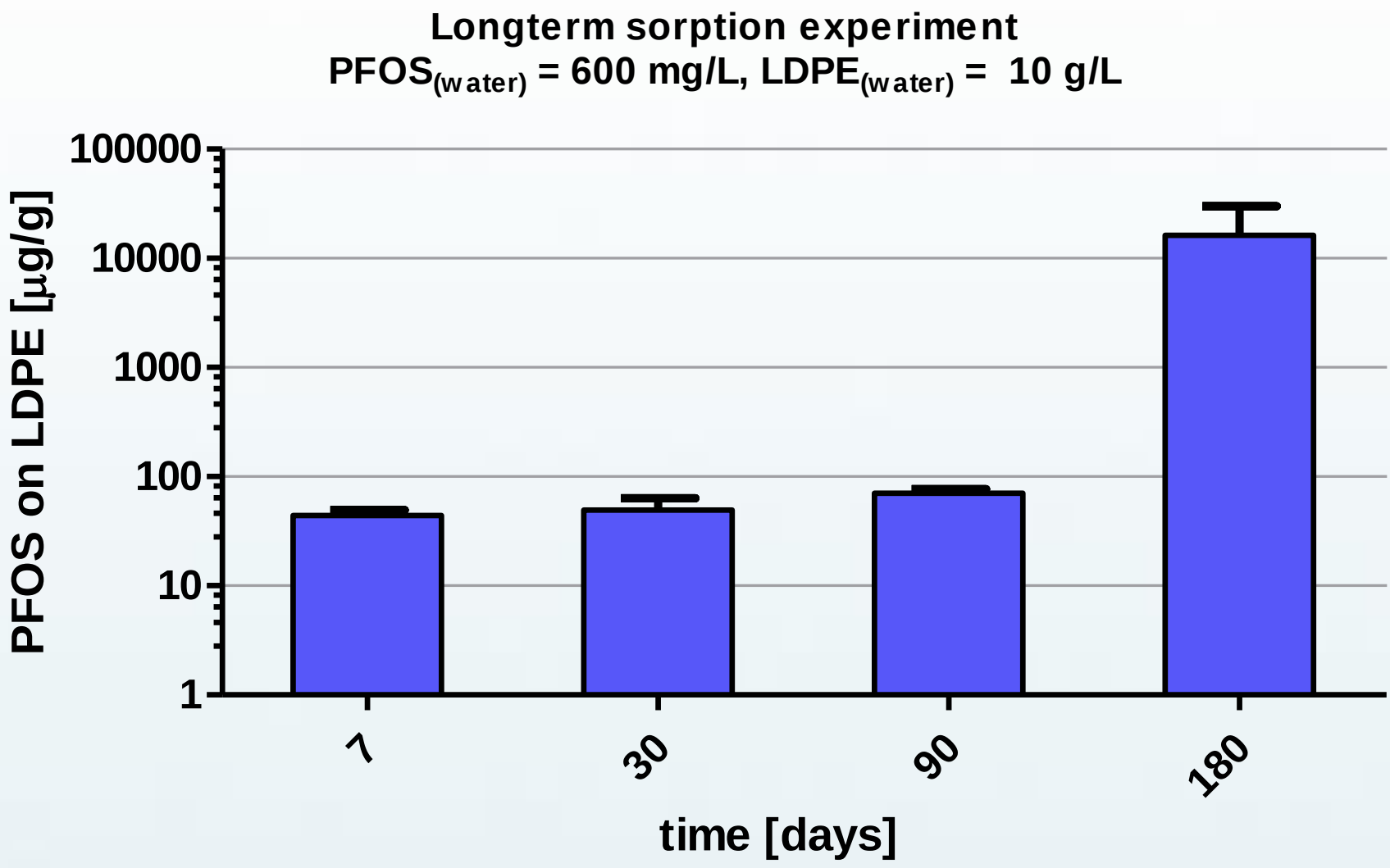


WP1 Adsorption and equilibrium partition of persistent pollutants to microplastics

Model target contaminants were selected from a group of candidates initially proposed by all partners. The selection of the two target concentrations (high and low) for each compound was based on data found in the literature. Chemical pollutants selected for the EPHEMARE project are: perfluorooctane sulfonate (PFOS), benzo(a)pyrene (BaP), and oxybenzone (Benzophenone 3, BP3). Initially, cadmium was selected as representative heavy metal; however, pre-tests showed it would not be possible to adsorb cadmium on LDPE particles within a reasonable time. LDPE and PVC microplastic particles of different sizes have been prepared with the three different compounds in two concentrations and distributed to all partners in the project for ecotoxicological testing.

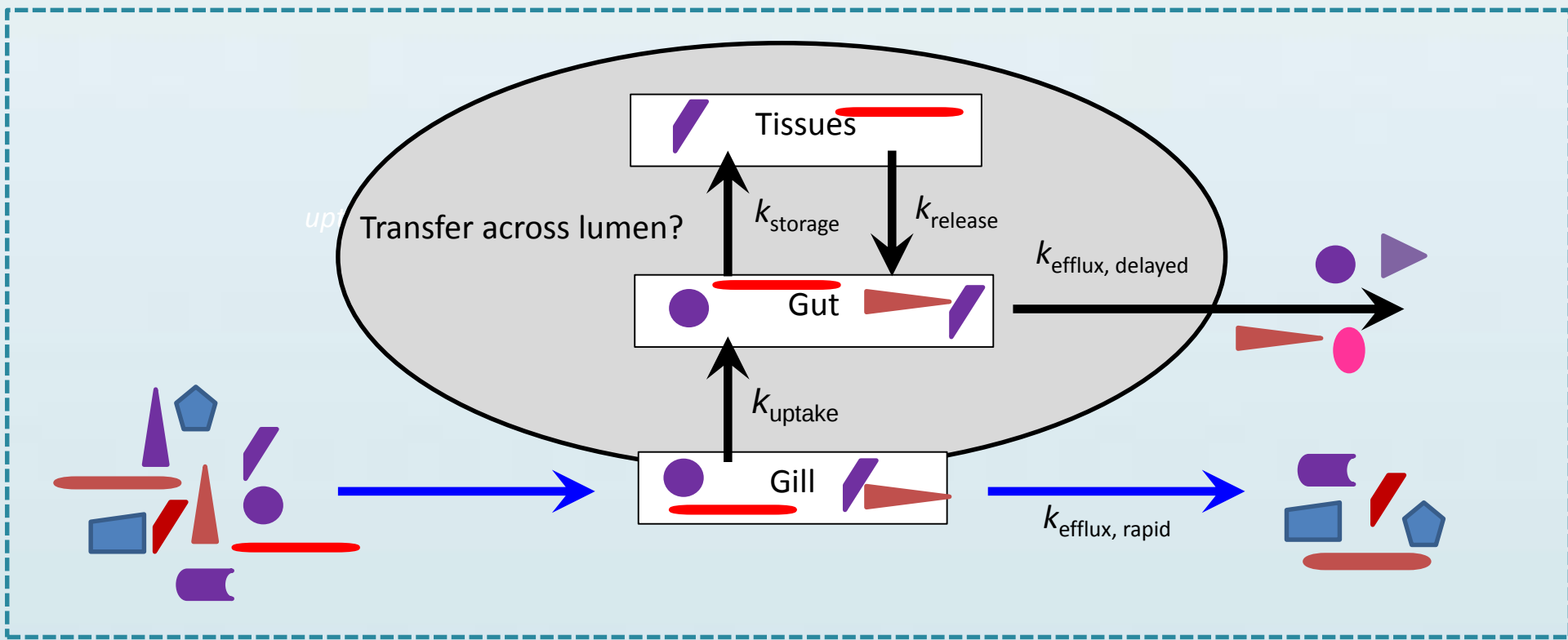
		perfluorooctane sulfonate		benzo(a)pyrene		oxybenzone	
		high	low	high	low	high	low
LDPE	500-125 µm	27.1 - 56.5 µg/g	180.9 ng/g	14.1 µg/g	378.17 pg/g	115 ng/g	1.1 ng/g
	25-20 µm	46.1 µg/g	61.2 ng/g	15.1 µg/g	384.19 pg/g	62 ng/g	0.15 ng/g
	13-11 µm	70.2 µg/g	9.2 ng/g	16.9 µg/g	364.34 pg/g	82 ng/g	< 0.05 ng/g
	6-4 µm	55.7 µg/g	118.2 ng/g	16.6 µg/g	4990.84 pg/g	24 ng/g	0.14 ng/g
PVC	250-100 µm	159.5 µg/g	1603.2 ng/g	11.5 µg/g	102.2 pg/g	66 ng/g	0.13 ng/g



The preparation of the MP particles showed that may be longer exposure time would change the sorption rate of the compounds to the particles. Therefore, 10 g/L LDPE MP particles (500-125 µm) has been exposed to either PFOS (600 mg/L) or BaP (5000 µg/L) for 180 days. Exposure vessels has been constantly shaken and samples has been taken at 7, 30, 90 and 180 days. The results for PFOS shows that until 90 days there is no change of the sorption rate; however, after 180 days the sorption rate of PFOS was more than 200 times higher than before. For BaP during the first 7 days the sorption was increasing but for the subsequent time points the BaP concentration adsorbed on the MP particles was constantly declining.

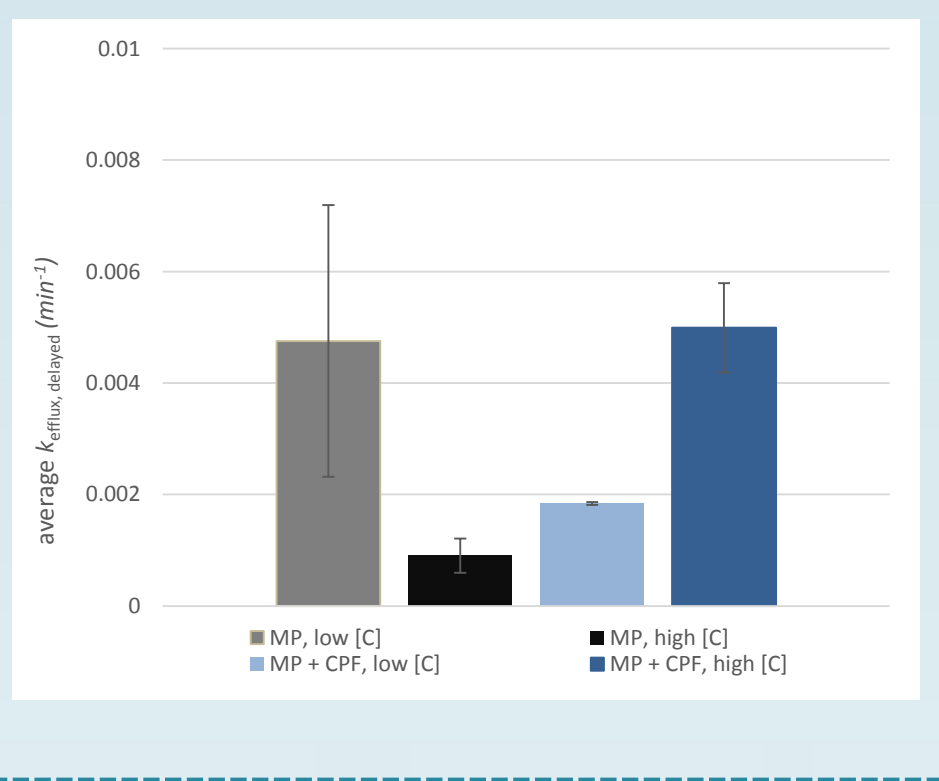
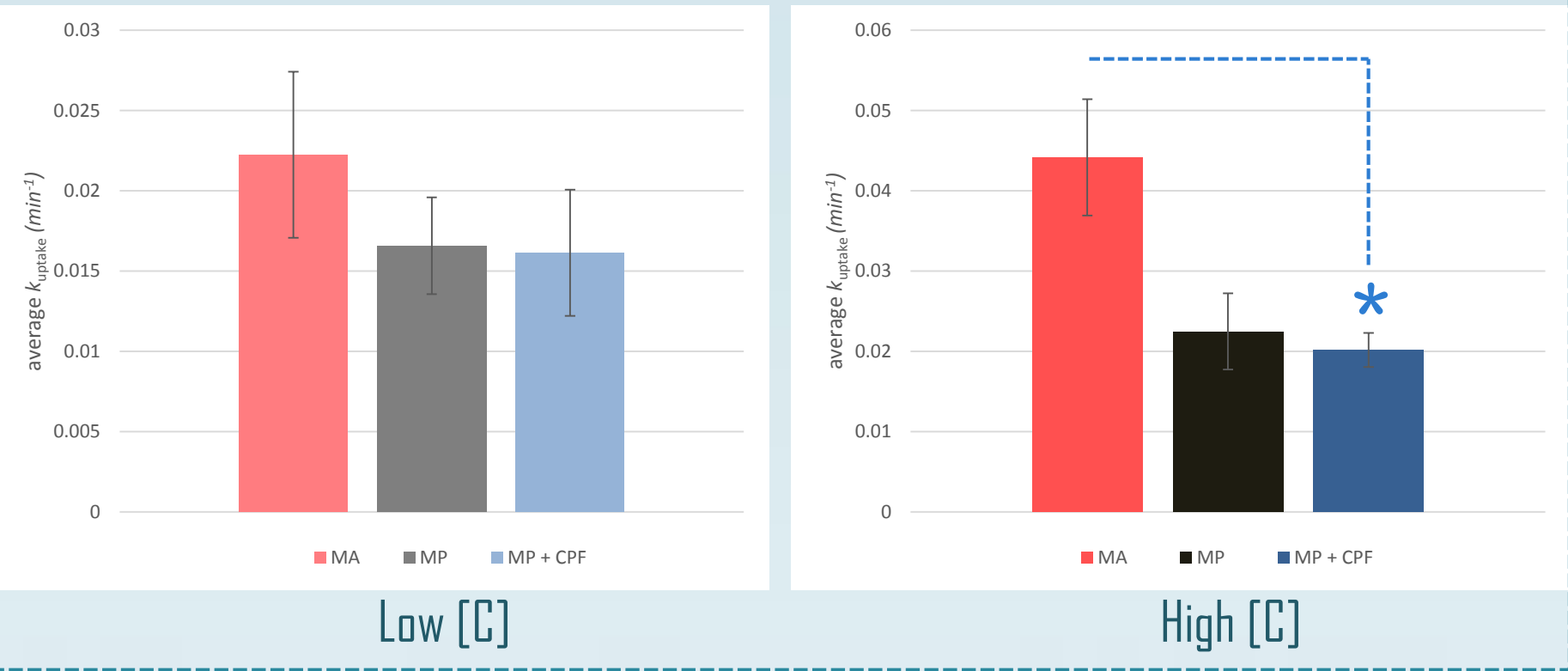
WP2 Developing an uptake/excretion model in the Mediterranean mussel (*M. galloprovincialis*)

Mussels were exposed to microalgae (MA), polyethylene microplastic (MP), and pollutant-spiked MP (MP+CPF) diets, to study the amount of MPs ingested and excreted and develop a mathematical model. Initially, just two parameters were considered: k_{uptake} and $k_{\text{efflux, delayed}}$.



Uptake

Delayed efflux



→ It appears exposure to MPs in mussels decreases uptake of food particles, and increases excretion, particularly with contaminated MPs.

WP5 MP uptake and transfer in simplified food chains: Establishment of food chains



- Analyses of uptake rates, transfer and accumulation pattern in all trophic organisms
- Histological analyses and fluorescence microscopy
- Transfer kinetics

- After successful establishment of food chain, feeding of POP-spiked MPs (self-spiked or from WP1)



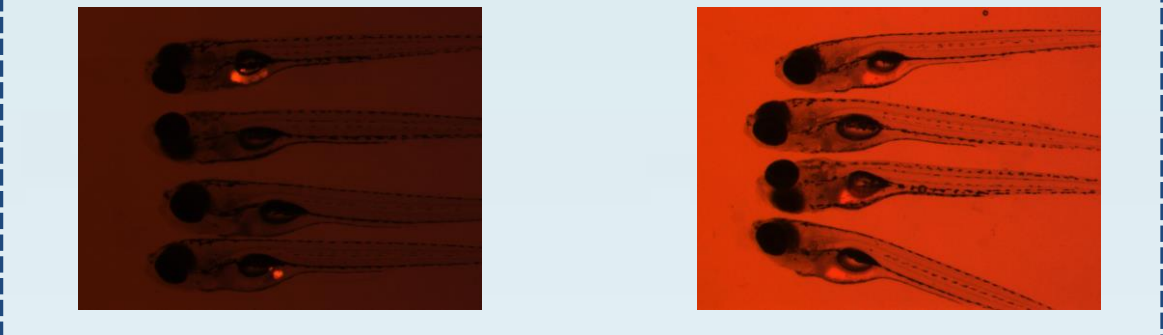
Nile Red dyeing as a novel approach to monitor microplastic ingestion *in vivo*

Nile Red dyeing protocol

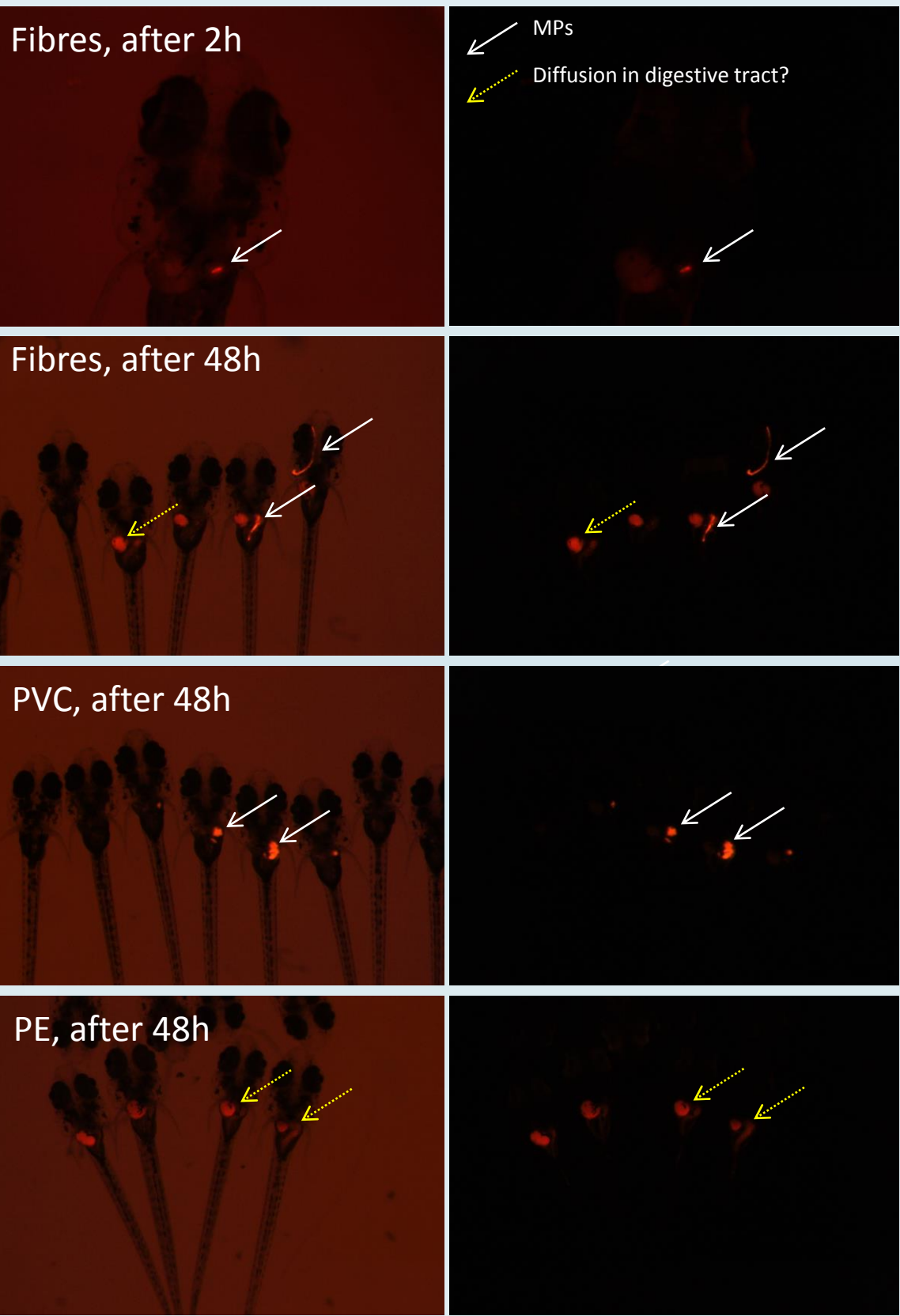
Any microplastic particle, of any shape, can be dyed with Nile Red

Transparent larvae (such as medaka or zebrafish) are exposed to the dyed particles

Their ingestion can be monitored *in vivo* using a stereoscope with a fluorescent filter (such as the Texas Red): the particles will fluoresce even after ingestion



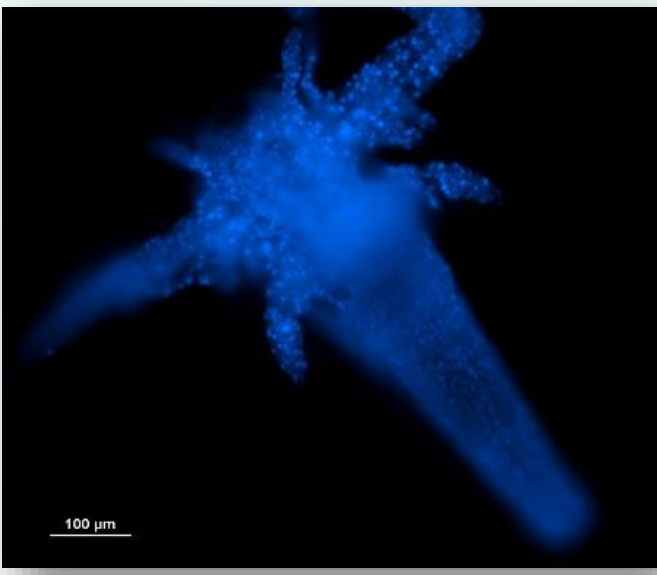
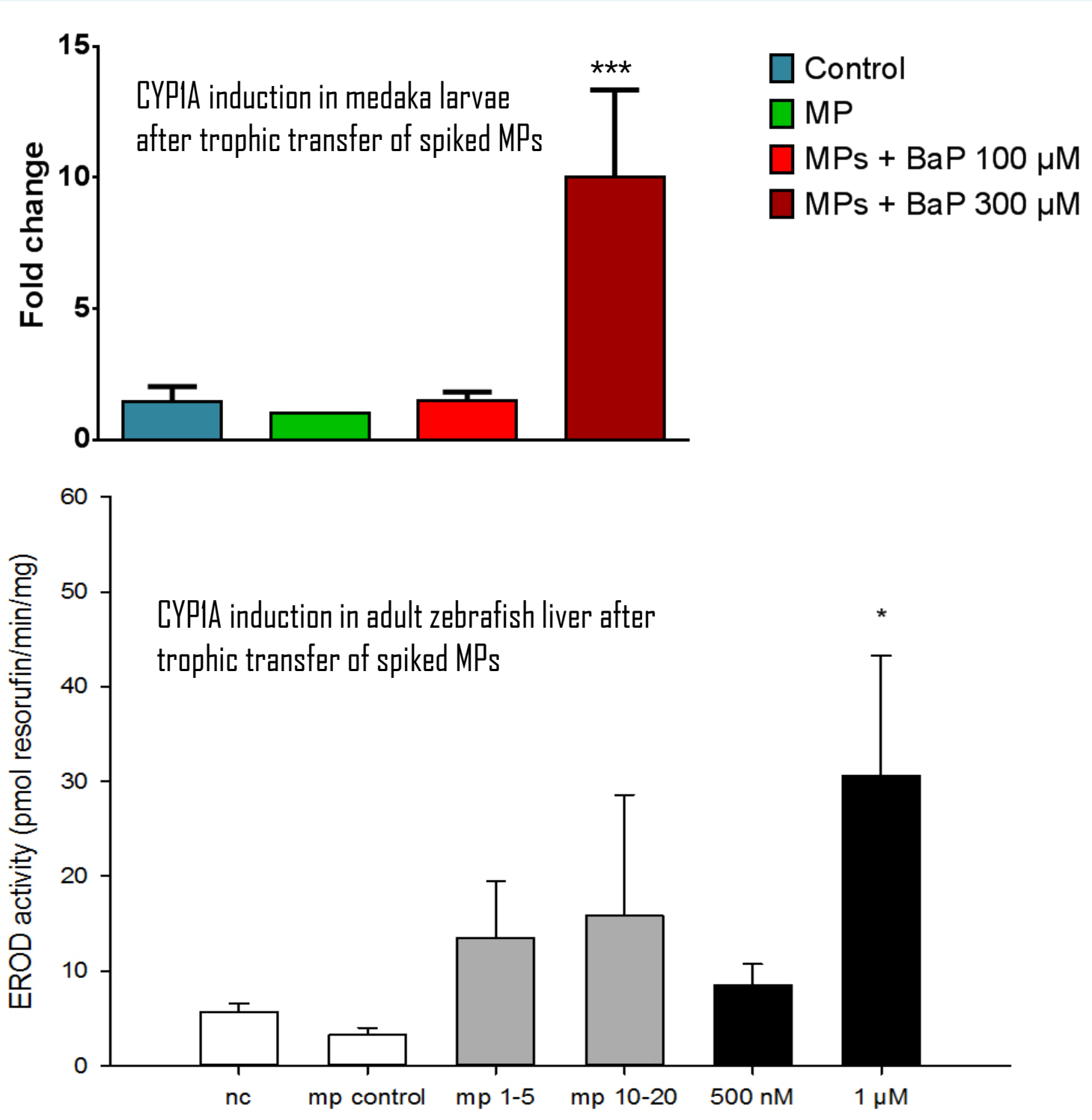
Zebrafish (*D. rerio*) and Medaka (*D. latipes*) exposed to Nile Red-dyed microplastics



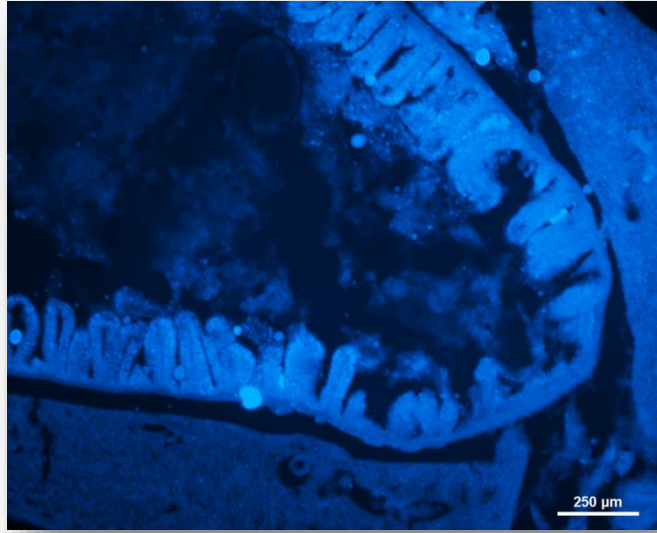
- Zebrafish and medaka larvae (~0-2 days post hatching) were incubated with MPs (PE, PVC, and polyester fibres), for 2 to 48 hours
- Monitoring of ingested MPs was performed after incubation and 2 days after.
- After exposure larvae were transferred in clean water and left undisturbed for 4 additional days
- PVC: 2 out of 14 larvae were still positive
- PE: 17 out of 18 larvae were still positive
- Dye seemed to diffuse out of the polyester fibres, although this hypothesis was invalidated during successive tests.

Persistent organic pollutant (POP) transfer via MP transfer in food chains

- CYP1A induction in adult zebrafish liver (EROD assay) and zebrafish/medaka larvae (qPCR)
- Fluorescence tracking of benzo(a)pyrene
- POP transfer via MPs and trophic food chains shown successfully in different species!



Fluorescence tracking of BaP signal in *Artemia* spec. nauplii



Fluorescence tracking of BaP in intestinal tract and liver of adult zebrafish



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