

Microplastic ingestion from contaminated prey in the bearded fireworm *Hermodice carunculata* (Pallas, 1766): evidence for rapid excretion and low degradation

Valentina Ferrari ^{1,2}, Roberto Simonini ¹, Carola Murano ^{3,4}, Daniela Prevedelli ¹, and Elisa Bergami ^{1,4,*}

- ¹ Department of Life Sciences, University of Modena and Reggio Emilia, Via Campi 213/D, Modena, Italy
 - ² Department of Chemical and Geological Sciences, University of Modena and Reggio Emilia, Via Campi 103, Modena, Italy
 - ³ Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Brindisi Marine Centre, 72100 Brindisi, Italy
 - ⁴ NBFC, National Biodiversity Future Center, Palermo 90133, Italy
- * Correspondence: elisa.bergami@unimore.it; Tel.: + 39 0592058263

2. Materials and methods

2.3. Experimental set-up

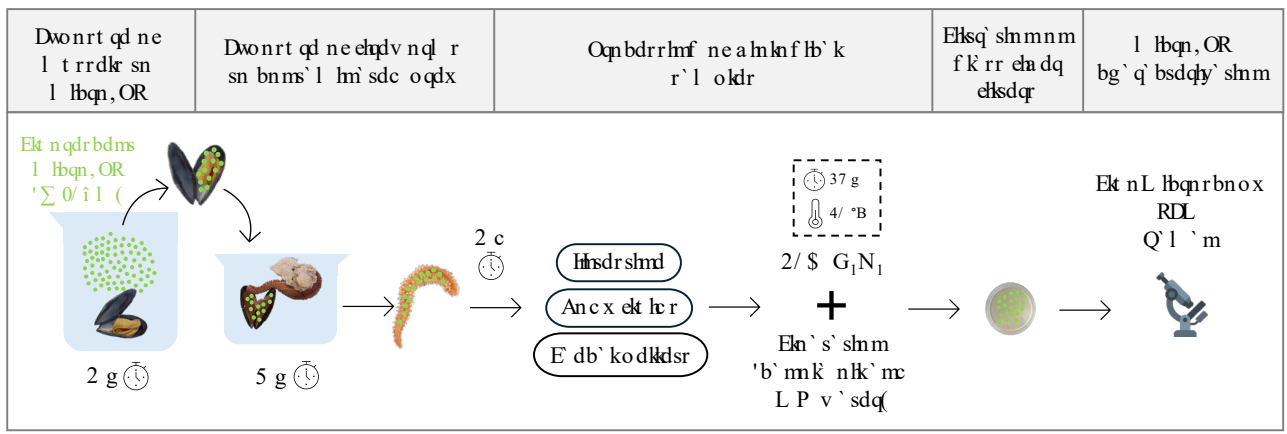


Figure S1. Schematic representation of the two-level experimental set-up, sample processing, and analysis. Abbreviations: micro-PS = polystyrene microspheres, MQ water = ultrapure Milli-Q water, H₂O₂ = hydrogen peroxide, and SEM = Scanning Electron Microscopy.

2.3.1. Exposure of mussels to micro-PS

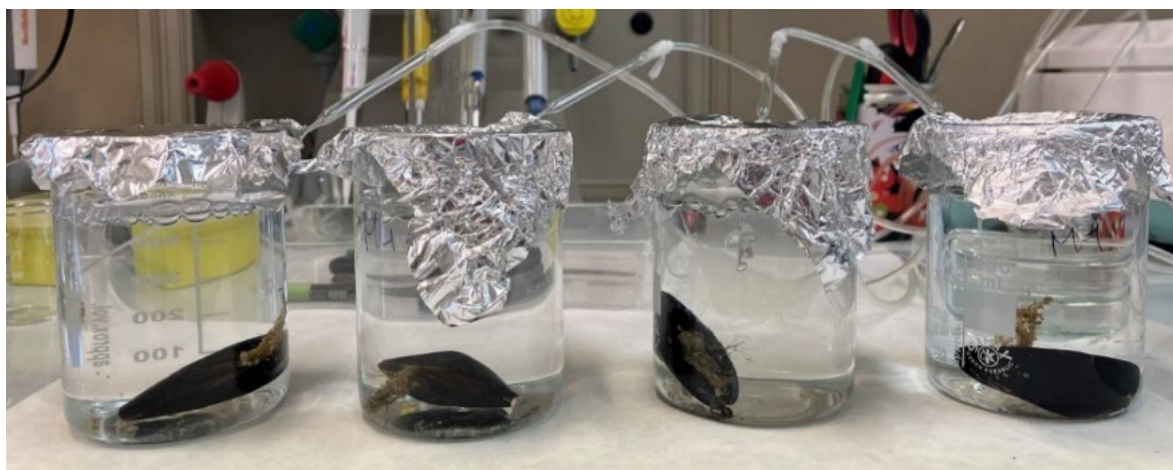


Figure S2. Preliminary experiment with individuals of the Mediterranean mussel (*Mytilus galloprovincialis*) exposed to suspensions of polystyrene microspheres (micro-PS) in aerated seawater.

2.3.2. Exposure of fireworms to contaminated prey



Figure S3. Positioning of a mussel with open valves in the tank containing an individual of the bearded fireworm *Hermodice carunculata* (left); predation of a bearded fireworm during incubation.



Figure S4. Representative faecal pellets of the bearded fireworm. Scale bar: 1 cm.

2.4. Processing of biological samples

Dissection was performed according to [1] by placing a single anaesthetised worm specimen in a glass Petri dish kept on ice to minimise tissue degradation. A first incision was made at the 12th chaetiger, followed by a longitudinal cut along the cephalic region. Using forceps, the dorsal body wall was grasped at the decapitation cut and laterally cut on both sides down to the last 10–15 chaetigers. The pharynx, in continuity with the intestine, was grasped with forceps, and mesenteries were cut to extract the intestine, which was stored in Falcon tubes at -20°C . Likewise, all body fluids leaking during dissection, potentially containing micro-PS, were collected in Falcon tubes and frozen.

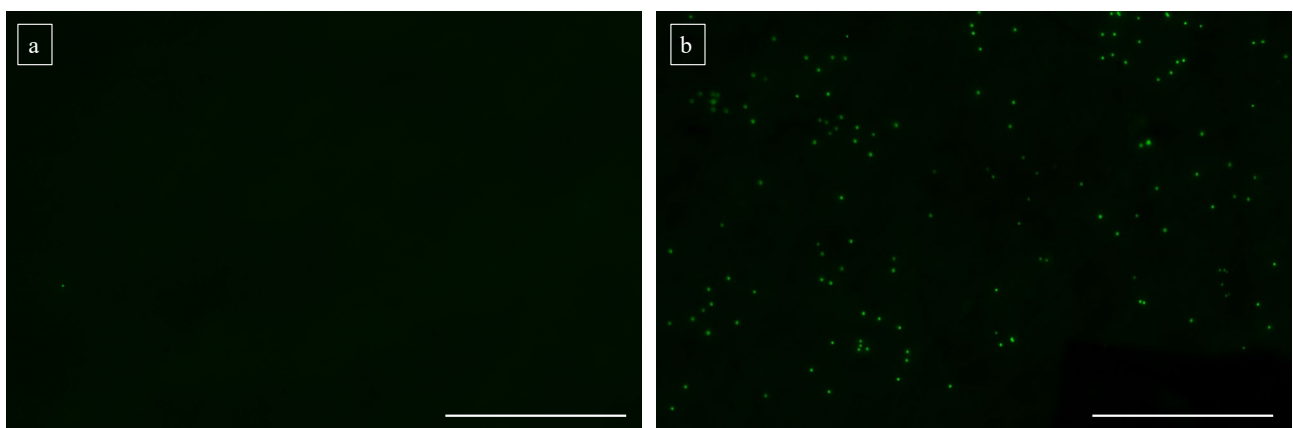


Figure S5. Optical fluorescent images of Yellow-Green-labelled polystyrene microspheres (micro-PS) extracted from the bearded fireworm intestine (a) and faecal pellets (b) and deposited on filters. Scale bar: 1 mm.

3. Results and discussion

The preliminary test was conducted using Mediterranean mussels with an average wet weight of 18 ± 3 g, soon after their purchase, with no laboratory acclimation. This led to the release of sand during incubation with micro-PS, affecting filtration and hampering accurate detection of the particles under fluorescence microscopy. Moreover, three out of four bearded fireworms regurgitated prey within a few days after ingestion, suggesting that such large mussels highly stressed their predators.

For the two-level experiment, optimal conditions were set by selecting smaller mussels (Table S1), which were acclimated in the laboratory at $19 \pm 1^\circ\text{C}$ for at least 1-2 weeks before conducting the experiments. As a result, bearded fireworms displayed an active behaviour and did not regurgitate prey during incubation.

Table S1. Morphometric traits of mussels contaminated with polystyrene microspheres (micro-PS) and offered to fireworms: wet weight (WW, including shell), shell dry weight (DW), length, and width.

Mussel	WW (g)	Shell DW (g)	Shell length (cm)	Shell width (cm)
M1	9.24	1.5	4.5	2
M2	10.61	1.5	4.7	2.1
M3	11.41	1.4	4.7	2.2
M4	13.01	1.9	4.9	2.3
M5	16.86	2.9	5.2	2.5
M6	10.82	1.8	4.5	2.2
M7	14.14	2.0	5	2.0
Mean	12.30	1.85	4.79	2.19
SD	2.58	0.53	0.26	0.17

For each bearded fireworm specimen, total length and chaetiger count were recorded from photographs using the open-source software ImageJ (v.1.53e, National Institutes of Health, Bethesda, MD, USA). Chaetigers were easily identified by a distinct dorsal yellow intersegmental line. Body weight was estimated from the chaetiger number using equations from [1]. Morphometric traits are reported in Table S2.

Table S2. Morphometric traits of the bearded fireworm specimens used in trophic transfer experiments as total length, number of chaetigers, and wet weight (WW). Morphometric traits of the bearded fireworm specimens used in trophic transfer experiments.

Fireworm	Total length (cm)	Chaetiger (N)	WW (g)
V1	16.71	82	9.31
V2	14.95	79	8.43
V3	16.42	85	10.25
V4	16.35	88	11.24
V5	13.84	78	8.15
V6	15.70	74	7.08
V7	14.05	79	8.43
Mean	15.43	81	8.98
SD	1.17	5	1.40

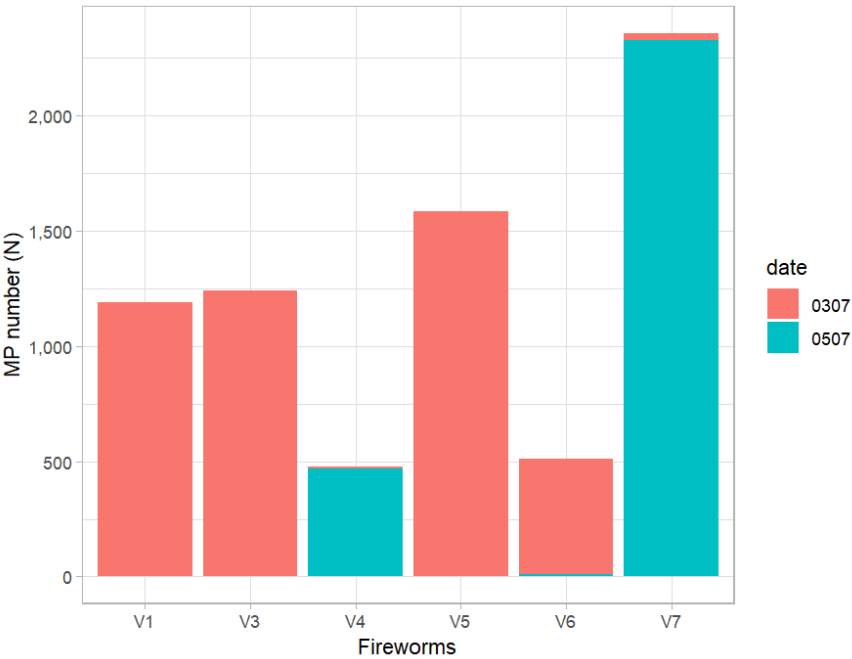


Figure S6. Number of polystyrene microspheres (micro-PS) retrieved in faecal pellets of bearded fireworm specimens (*V-vermis*) (FP) released on different days post-feeding.

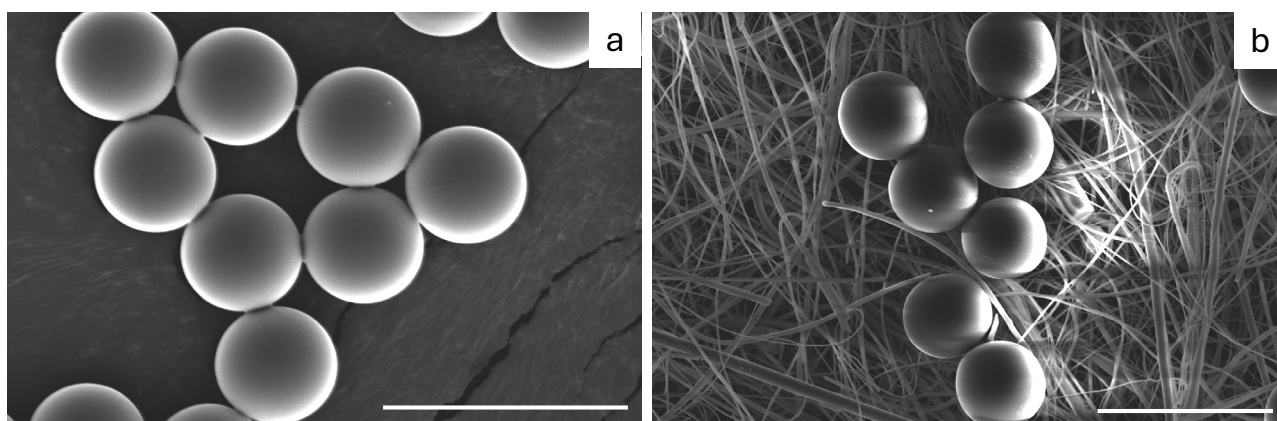


Figure S7. Scanning Electron Microscopy (SEM) images of polystyrene microspheres (micro-PS) as received from the supplier (a) and after oxidative treatment (30% hydrogen peroxide). Scale bar: 10 μm .

References

- [1] Righi, S.; Forti, L.; Simonini, R.; Ferrari, V.; Prevedelli, D.; Mucci, A. Novel Natural Novel natural compounds and their anatomical distribution in the stinging fireworm *Hermodice carunculata* (Annelida). *Marine Drugs* **2022**, *20*(9), 585, doi:10.3390/md20090585.