**Constraints and priorities for conducting experimental exposures of marine organisms to microplastics**

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## Supplementary files

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**Supplementary file 1**: Overview of recent works on translocation of micro- and nanoplastics (MP/NP) in aquatic organisms.

**Supplementary file 1**

# MP Translocation : research without success

| References | Experimental (mode) or Environmental | Model(s) | Exposition | Methodology/ Results (*text extract*) |
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| (Sussarellu et al., 2016) | Experimental (balneation) | *Crassostrea gigas* | 2 and 6 µm PS beads fluorescent / 2 months (0.023 $mg \cdot L^{-1}$) | Histology<br>*"Here no evidence of micro-PS transfer from the digestive tract to the circulatory system and other tissues was detected on the histological slides."* |
| (Cole et al., 2013) | Experimental (balneation) | Zooplankton (13 taxa) | 1.7 – 30.6 µm polystyrene beads/ 635 to 3000 beads/mL/ 1h to 24h | Epifluorescent Microscopy<br>*"However, CARS (coherent anti-Stokes Raman scattering microscopy) provided no evidence of microplastic translocation."* |
| (Watts et al., 2014) | Experimental (« ventilation » / feeding mussels) | Crabs *Carcinus maenas* | 10 µm fluorescent beads/ 24 h and 21 days / between 9.4 $x10^5$ and 4.0$x10^4$ microspheres $L^{-1}$ or 4.0$x10^3$ microspheres $g^{-1}$ | Fluorescent Microscopy :<br>*"In this study, no microspheres were found in the crab's haemolymph at any of the sampling points for either the ventilatory or food exposure routes. This suggests that no translocation of plastic particles of this particular size occurred from either the foregut or the gills into the haemolymph."* |
| (Kaposi et al., 2014) | Experimental (balneation) | larvae of the sea urchin *Tripneustes gratilla*. | 10 – 45 µm diameter fluorescent green polyethylene microspheres at 1, 10, 100 and 300 spheres.$mL^{-1}$ / max 5 days | Fluorescent Microscopy<br>*"T. gratilla egested all microplastics from their digestive system within 420 min of ingesting them…,"* |

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| (Grigorakis et al., 2017) | Experimental (in food pellet) | goldfish (*Carassius auratus*) | Microfiber/ microplastic beads/ ad libitum | Digestion / stereomicroscope<br>*"Microplastics were also examined in carcass samples of treatment fish but were not observed apart from the gut tissue and gut contents analyzed separately"* |
| (Au et al., 2015) | Experimental | freshwater amphipod, *Hyalella azteca* | 10-d to 42-d exposure / fluorescent blue polyethylene 10 µm to 27 µm / > 0-100,000 part/mL | Confocal microscope<br>*"There was also no evidence of microplastic translocation out of the gut."* |
| (Bruck and Ford, 2018) | Experimental (algal feed speaked) | amphipod *Echinogammarus marinus* | gelatinous algal feed spiked with microbeads (8 µm Fluoro-Max Red fluorescent polystyrene microspheres) / 0.9, 9 and 99 microplastics/g 35 days | Fluorescent microscope<br>*" However, we observed no evidence of any gastrointestinal accumulation of microplastics or translocation…"* |
| (Katzenberger, 2015) | Experimental (trophic transfer) | *Artemia* sp. / three-spined sticklebacks *Gasterosteus aculeatus* | *Artemia* exposed to 1 or 9.9 µm fluorescent PS/ 0.106 mg microplastic $mL^{-1}$). fish fed 7 days | Blood smears / histological section analysis under a fluorescent microscope.<br>*"There was also no evidence for translocation of the plastics from the gastrointestinal tract to the circulatory system"* |

## MP Translocation : Research with positive results but raising doubts, or translocation expressed as an hypothesis among others

| References | Experimental (mode) or Environmental | Model(s) | Exposition | Methodology/ Results (*text extract*) |
|---|---|---|---|---|
| (Ribeiro et al., 2017) | Experimental (balneation) | Clam *Scrobicularia plana* | 1 mg $L^{-1}$ (PS 20 µm) for 14 days, | Optical microscopy analysis of the haemolymph<br>*"But, the question is if the translocation really occurred or was it a contamination by microplastic while sampling haemolymph? …microplastics tend to translocate in the circulatory system but this needs to be confirmed in future studies."* |
| (Karami et al., 2018) | Environmental (canned fish) | sardines and sprats | - | Eyes selection + Raman<br>*"The presence of micro- and mesoplastics in the canned sardines and sprats might be due to the translocation of these particles into the edible tissues, improper gutting, or the result of contamination from the canneries."* |

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| (Van Cauwenberghe and Janssen, 2014) | Environmental | Mussel *Mytilus edulis* / oyster *Crassostrea gigas* | - | Whole flesh, acid digestion, filtration, count of microplastic.<br>*"The specific removal of larger microplastics as a result of gut depuration might be an indication that the remaining, smaller, particles may have translocated through the gut wall and are subsequently retained in the tissues and circulatory system."* |

## MP Translocation : Research with positive results and showing a size limit

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| (Lusher et al., 2017) | Critical review | *"Microplastics larger than this (0.5 mm) do not readily pass through the gut wall without pre-existing damage, and the likelihood of translocation into tissues is too low to warrant regular investigation."* |

## MP Translocation : Research with positive results

| References | Experimental (mode) or Environmental | Model(s) | Exposition | Methodology/ Results (*text extract*) |
|---|---|---|---|---|
| (Kashiwada, 2006) | Experimental (balneation) | Eggs of Fish medaka (*Oryzias latipes*) | Eggs : (~1 mm diam): 39.4-nm, 474-nm, 932-nm, 18,600-nm or 42,000-nm PS particles/ 1 mg/L | Microscopy epifluorescence (eggs, larvae, tissue) ; Blood : fluorescence microplate reader : *"Particles 39.4–42,000 nm in diameter were adsorbed to the chorion of medaka eggs and accumulated in the oil droplets;"* |
| (Browne et al., 2008) | Experimental (balneation) | Mussel *Mytilus edulis* | 4.3 $10^4$/L of 3 or 9.36 µm fluorescent PS beads / 3 hours | Hemolymph bleeding, fixation (methanol) / fluorescent microscopy *"Particles translocated from the gut to the circulatory system within 3 days and persisted for over 48 days*." |
| (Darmody et al., 2015) | Experimental (balneation) | Oyster *Ostrea edulis* | Fluorescent styrene-maleic acid (SMA) microbeads (1–2 µm in size) encapsulated in alginate/ 100 mg/L / 7 days | Heart smear, Histology, fluorescent microscopy : *"The SMA (Fluorescent styrene-maleic acid) microbeads successfully cross the digestive epithelium and disperse into the surrounding tissue. The microbeads were also actively taken up by the oyster hemocytes."* |

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| (Collard et al., 2017) | Environmental | Anchovies, pilchard, herring, *Engraulis encrasicolus, Clupea harengus Sardina pilchardus* | - | Liver digestion, MP isolation / histology (cryosection)<br>*"Both methods separately revealed that MPs, mainly polyethylene (PE), were translocated into the livers of the three clupeid species."* |
| (von Moos et al., 2012) | Experimental (balneation) | Mussel *Mytilus edulis* | 0-80 µm polyethylene / 96 hours / 2.5 g/L | polarized light microscopy<br>*"HDPE particles were taken up into the stomach and transported into the digestive gland where they accumulated in the lysosomal system after 3 h of exposure."* |
| (Farrell and Nelson, 2013) | Experimental (trophic transfer) | *Mytilus edulis* (L.) to *Carcinus maenas* (L.) | Mussels : 0.5µm fluorescent PS beads/ 1.02 $10^6$ $mL^{-1}$ | Hemolymph stomach, hepatopancreas, ovary and gill under a fluorescence microscope<br>*"This study is the first to show 'natural' trophic transfer of microplastic, and its translocation to haemolymph and tissues of a crab".* |
| (Brennecke et al., 2015) | Experimental (trophic transfer from sediment) | crab *Uca rapax* | Aged Polystyrene pellets 180-250µm/ 2 months/ 100 and 1000 mg fragments/kg dry sediment | Gills, stomach and hepatopancreas : stereomicroscope<br>*"Our observation that microsized particles can be translocated to the hepatopancreas of U. rapax corroborates a recent finding…"* |
| (Lu et al., 2016) | Experimental (in food pellet ?) | Zebrafish (*Danio rerio*) | 70 nm / 5 and 20 µm PS/ 7 days/ dose ? | Fluorescent microscopy<br>*"5 µm diameter MPs accumulated in fish gills, liver, and gut, while 20 µm diameter MPs accumulated only in fish gills and gut."* |

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| (Watts et al., 2014) | Experimental (trophic transfer or direct exposure : ventilation) | *Mytilus edulis* (L.) to *Carcinus maenas* (L.) | Mussel : 1000 spheres/mL of 10 µm fluorescent polystyrene microspheres / 4 hours | Fluorescent microscopy *"Unlike other studies (Farrell and Nelson, 2013) the microspheres in this study were very rarely seen in the haemolymph"* |
| (Akhbarizadeh et al., 2018) | Environmental | fish muscles from northeast of Persian Gulf | - | Dissection muscle, digestion KOH, optical analysis. *"MPs with a wide variety of colors, shapes and size were detected in all investigated fish muscle samples"* |
| (Jeong et al., 2017) | Experimental (balneation) | Copepod *Paracyclopina nana* | Fluorescent 0.05 µm for nano-sized microplastics and 0.5 and 6 µm for micro-sized microplastics/ 10 µg/mL / 24h | Fluorescent microscopy of the all copepod *"The dispersed fluorescence observed in 0.05-µm microbead-exposed P. nana could be explained by translocation of polystyrene microbeads across the cellular membranes through the digestive organs of P. nana."* |

## NP Translocation : indirect evidences

| References | Experimental (mode) or Environmental | Model(s) | Exposition | Methodology/ Results (*text extract*) |
|---|---|---|---|---|
| (Ward and Kach, 2009) | Experimental (NP in marine aggregates) | mussels, *Mytilus edulis*; oysters, *Crassostrea virginica* | Aggregates : Polystyrene NPs (red fluorescent, 100 nm; Thermo Fisher) in seawater at a concentration of ca.1.3 $10^4$ particles $ml^{-1}$ | Calculation of the number of NPs ingested and egested<br>*"the longer gut retention time suggests that most NPs were directed into the tubules of the digestive glands and potentially taken up by the digestive cells via endocytosis."* |

## NP Translocation : direct evidences

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| (Mattsson et al., 2017) | Experimental (food chain : algae, copepods) | Crucian carp (*Carassius carassius*) | Polystyrene nanoparticles, 53 nm and 180 nm | CytoViva Hyperspectral Imaging System<br>*"Polystyrene was detected in the brains from all analyzed fish fed with polystyrene nanoparticles, whereas no polystyrene was detected in brains from control fish."* |
| (Kashiwada, 2006) | Experimental (balneation) | Fish medaka (*Oryzias latipes*) | 3 days adult fish : 39.4-nm fluorescent particles at 10 mg/L, for 7 day | Microscopy epifluorescence (tissue); fluorescence microplate reader (Blood)<br>*"39.4 nm Nanoparticles were also detected in the brain, testis, liver, and blood."* |