Effects of *in vitro* and *in vivo* exposure of polystyrene nanoplastics in the marine mussel *Mytilus galloprovincialis*

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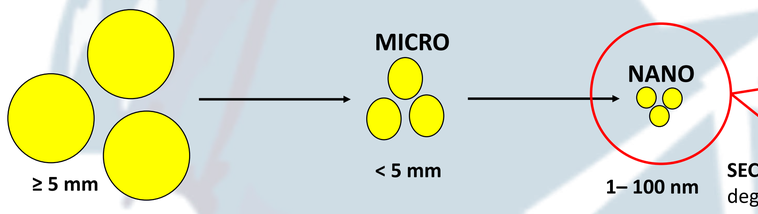
Introduction

A WORLDWIDE CONCERN → PLASTIC POLLUTION IN THE MARINE ENVIRONMENT INCREASED IN THE LAST DECADE

ONLY 6-26% OF PLASTIC USED IS RECYCLED



PRIMARY → enter marine environment in original small size

SECONDARY → macro/microplastic degradation

- **Polystyrene** (PS) is the **4th** most abundant plastic

Travel faster and further within the environment ← **NPs** → Increasing ability to cross cellular boundaries & accumulate within organisms → Easily entering food web & possibly move up trophic levels

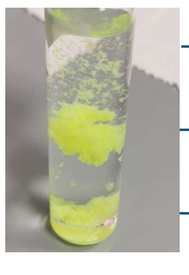
Large surface areas → facilitating sorption of pollutants & release of sorbates and constituent chemicals

Objective:

Evaluate the effects 50 nm polystyrene nanoplastics (nPS, 10 µg/L) have on the marine mussel *Mytilus galloprovincialis* under an *in vitro* (24 h) and *in vivo* (21 days) exposure.

Methods

Polystyrene Nanoplastics (nPS) Characterization:



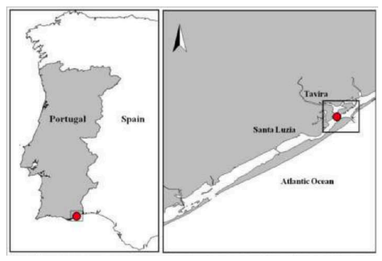
Fluoresbrite® YG Microspheres – 50 nm (CAS: 17149-10) aquired from Polysciences Europe GmbH

DLS particle sizer (ZetaSizer Nano ZS90, Malvern Inc.) used to measure the hydrodynamic diameter of nPS in freshwater and sea water. ζ-potential was also determined.

The sedimentation rate measured by the change in turbidity with time.

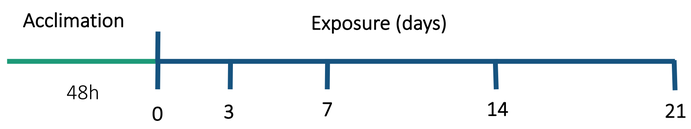
Mussel collection:

M. galloprovincialis (60 ± 5 mm shell length) collected in the Ria Formosa Lagoon, Southeast of Portugal (37°06'59.4"N, 7°37'45.0"W)



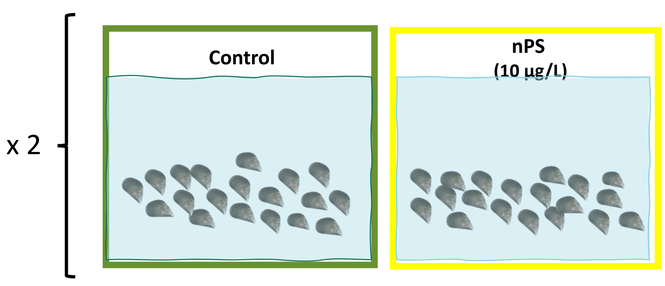
In vitro:

In vivo:



Experimental Design:

Duplicate design → 15 L tanks, 10 L seawater → 2.0 mussels L^{-1}



Parameters Analysed

→Genotoxicity:
Komet Assay
→Antioxidant enzyme activities:
Superoxide dismutase (SOD),
Catalase (CAT),
Glutathione peroxidase (GPx)
→Biotransformation enzyme activities:
Glutathione-S-transferase (GST)
→Oxidative damage:
Lipid peroxidation (LPO)

Results

nPS Characterization:

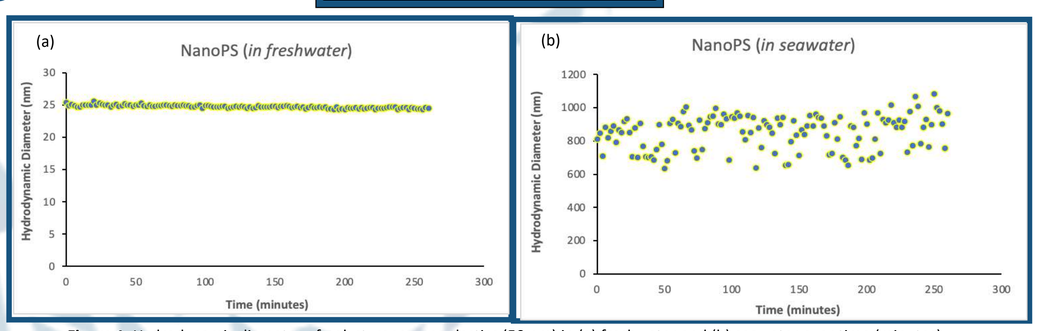


Figure 1. Hydrodynamic diameter of polystyrene nanoplastics (50 nm) in (a) freshwater and (b) seawater over time (minutes).

nPS in freshwater:
- Average hydrodynamic = 25 nm
- ζ-potential of -68.4 mV = no aggregation

nPS in seawater:
- Hydrodynamic diameter **INCREASES**
- .ζ-potential of 0.068 mV = in these conditions

In vitro:

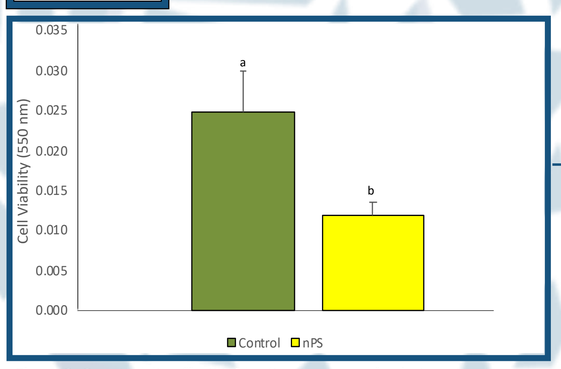


Figure 2. Genotoxicity effects of *in vitro* exposure of polystyrene nanoplastics in the haemolymph of *M. galloprovincialis.* Different lower case letters indicate significant differences between treatments (p<0.05)

Genotoxicity occurred in the haemolymph of nPS exposed mussels.

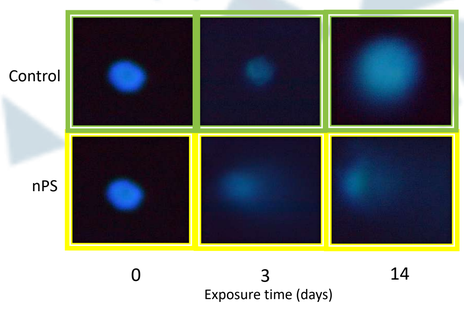


Figure 4. Examples of comet assay images of Control *M. galloprovincialis* haemocytes and those exposed to polystyrene nanoplastics.

nPS lead to genotoxicity in the haemolymph of exposed mussels.

In vivo:

Genotoxicity:

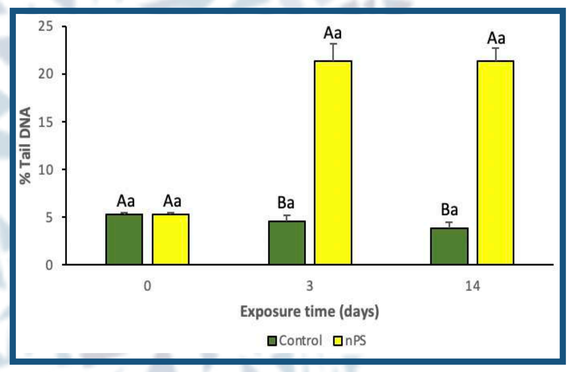


Figure 3. Genotoxicity effects of *in vivo* exposure of polystyrene nanoplastics in the haemolymph of *M. galloprovincialis.* Different upper and lower case letters indicate significant differences between treatments for the same time, and between time for the same treatments, respectively (p<0.05).

Oxidative stress in gills:

→Enzymatic activity decreased after 3 days of exposure to nPS in comparisson to unexposed.

→At day 7 and 14, a significant inhibition is observed in all enzymatic activities

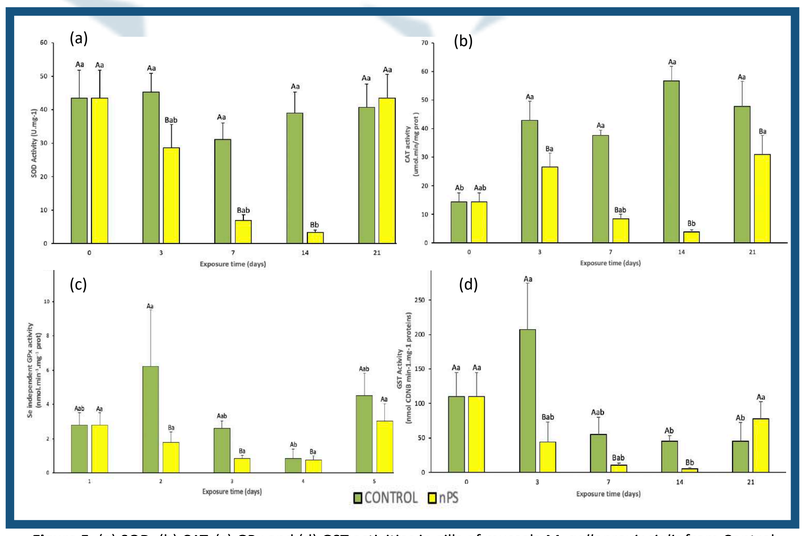


Figure 5. (a) SOD, (b) CAT, (c) GPx and (d) GST activities in gills of mussels *M. galloprovincialis* from Control and exposed to 10µg/L polystyrene nanoplastics (nPS) for 21 days (mean ± std). Different upper and lower case letters indicate significant differences between treatments for the same time, and between time for the same treatments, respectively (p<0.05).

Oxidative damage in gills:

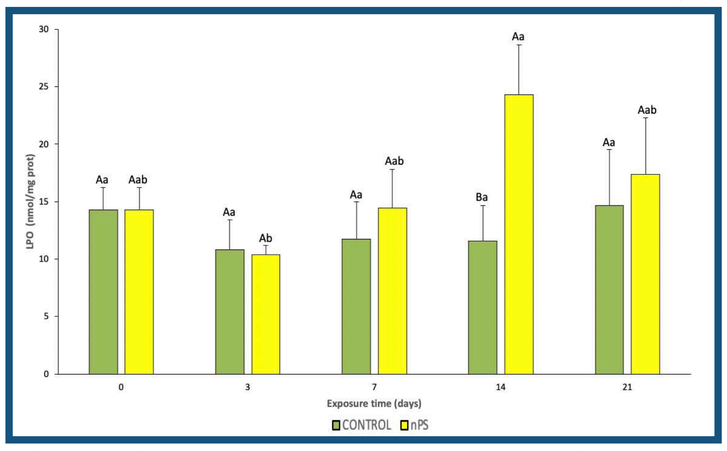


Figure 6. Lipid Peroxidation (LPO) in gills of mussels *M. galloprovincialis* from Control and exposed to 10µg/L polystyrene nanoplastics (nPS) for 21 days (mean ± std). Different upper and lower case letters indicate significant differences between treatments for the same time, and between time for the same treatments, respectively (p<0.05).

→Significant oxidative damage observed in mussels after 14 days of exposure to nPS in comparison to the 3rd day.

→At the 14th day, enzymatic activity at its lowest and significant, therefore ROS overwhelmed antioxidant defence mechanisms leading to oxidative damage.

Conclusion

- Both biotic and abiotic **characteristics of seawater** lead to an **increase** in the **hydrodynamic diameter** of nPS particles as well as in the **aggregation** of **nPS**.
- In *M. galloprovincialis*, 10µg/L of nPS (50 nm) caused **genotoxicity**, overwhelmed antioxidant defences and lead to **oxidative damage**.
- **Inhibition of antioxidant defences** may be a result of an increase in ROS production by nanoplastics.
- After **21** days, an **adaptive response** of exposure period leads to the **activation of repair mechanisms**.

Future Perspectives

- Important to compreehend the behaviour and toxicity of nPS towards marine biota, as particles interaction differs when compared to freshwater.
- Further analysis on a longer-term exposure assay should be carried out to comprehend how mussels respond to nanoplastic exposure post 21-days.

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