MICRO 2022
ONLINE ATLAS EDITION

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Neurotoxicity of polystyrene nanoplastics and their ingestion in the marine mussel *Mytilus galloprovincialis*

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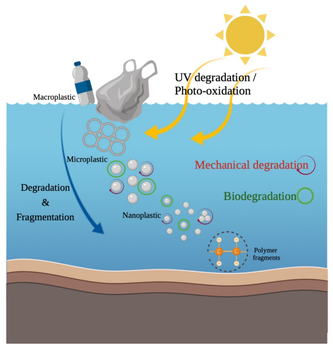
Introduction

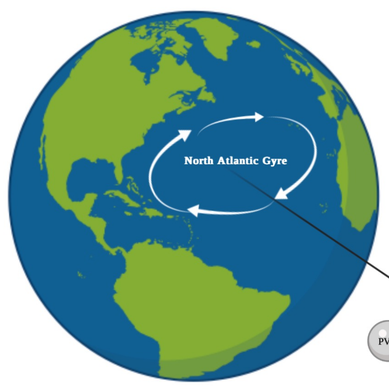
PLASTIC POLLUTION

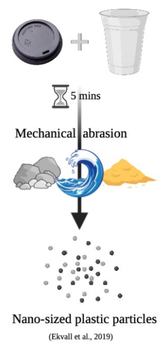
WORLD PLASTIC POLLUTION = **367 MT** *(Europe = 15%)*

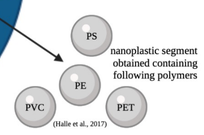
Polystyrene (PS)
is the **4th most** abundant plastic **used worldwide**



1. Hydrolysis
2. Mechanical/Physical degradation
3. Thermal oxidative degradation
4. Photo-degradation
5. Biodegradation







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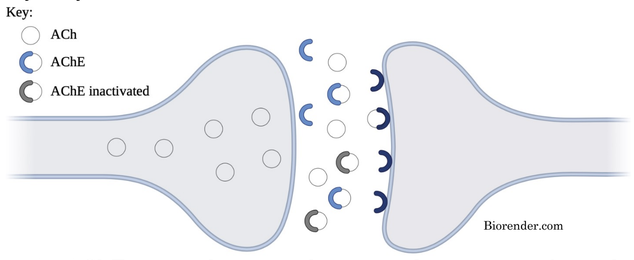
NEUROTOXICITY ACETYLCHOLINESTERASE (AChE)

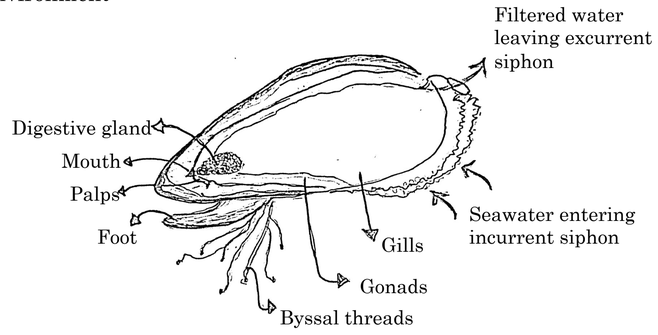
- AChE is a serine protease, with a main function of inactivating acetylcholine (ACh) after its nerve signal transfer function by cleaving ACh molecules into acetate and choline, through hydrolysis .



- As AChE passes between the pre and post synapsis, and the Ach binds to the cholinergic receptors on the post-synapse and stimulates a transmission of impulses.
- Exposure to plastic particles can cause change in levels of neurotransmitters, as these cause an inhibition of AChE, consequently altering the behaviour of some aquatic invertebrates (Yong et al., 2020).

INGESTION & ACCUMULATION OF PLASTIC PARTICLES

- Filter-feeding organisms, such as mussels, can accumulate plastic particles as well as other contaminants in their surrounding environment



- Mussels have shown that they retain MPs in their digestive tract, but also that they ingest and egest these particles and that this is dependent on size (Kinjo et al., 2019)
- What about nanoplastics?

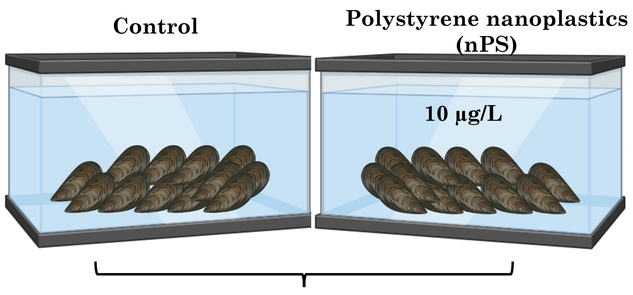
Objective: Evaluate neurotoxicity in mussels after exposure to 10 µg/L of polystyrene nanoplastics (50 nm; nPS) and the ingestion of nPS in the gonads of *M. galloprovincialis*.

Methods

Mussel collection

Mytilus 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 vivo exposure



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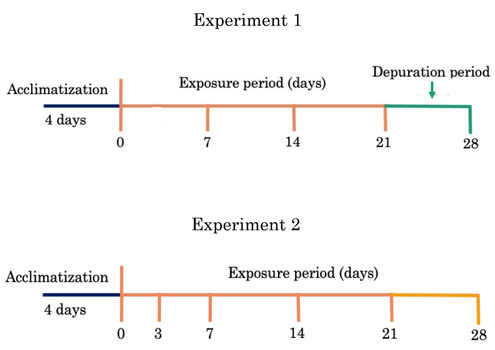
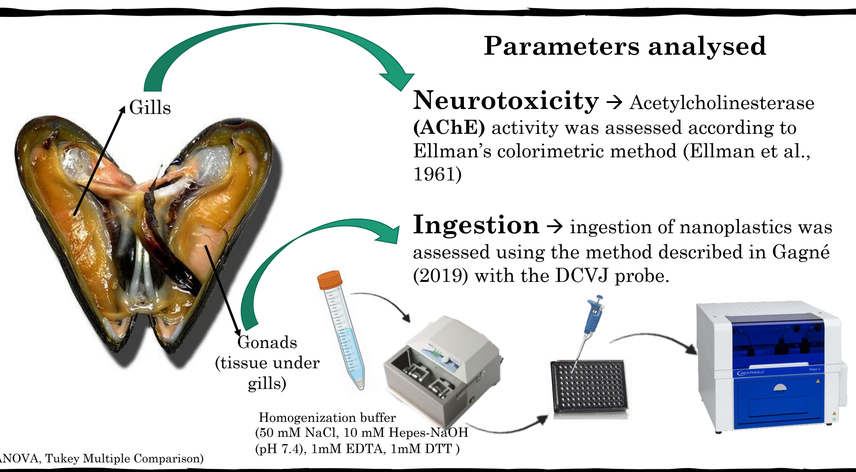


All statistical analysis were performed on Graphpad Prism 9.4.2. (Shapiro-Wilk, ANOVA, Tukey Multiple Comparison)

Parameters analysed



Neurotoxicity → Acetylcholinesterase **(AChE)** activity was assessed according to Ellman's colorimetric method (Ellman et al., 1961)

Ingestion → ingestion of nanoplastics was assessed using the method described in Gagné (2019) with the DCVJ probe.

Results

AChE activity in GILLS of mussels

Experiment 1

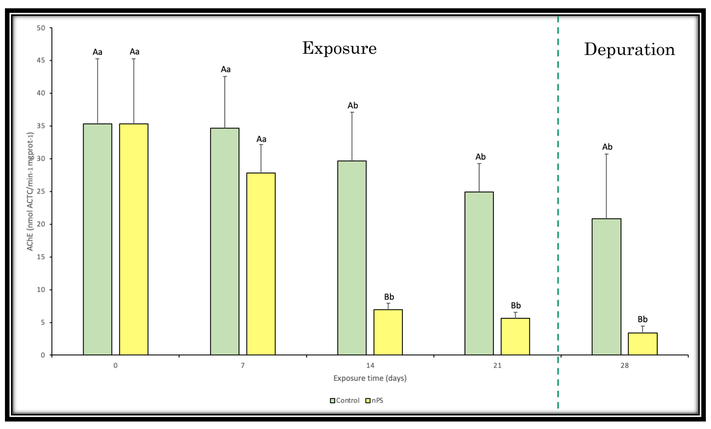


Figure 1. AChE activity (mean ± sd) in the gills of *M. galloprovincialis* after a 21-day exposure to 10 µg/L of polystyrene nanoplastics (50 nm) and 7-day depuration period. *Capital and lower-case letters represent significant differences between treatments at the same time and between times for the same treatment, respectively ($p < 0.05$).*

Experiment 2

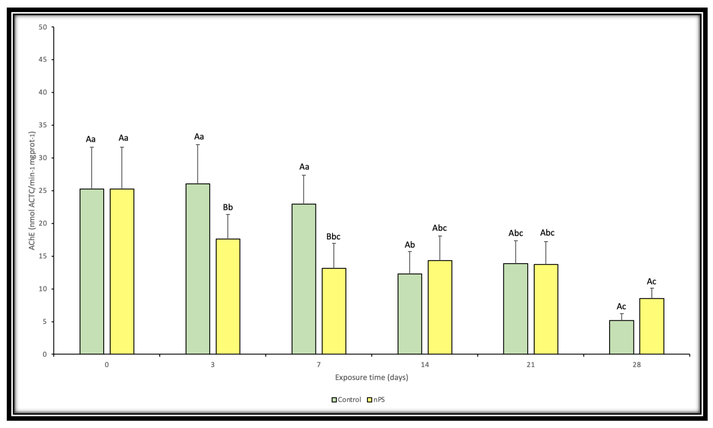


Figure 2. AChE activity (mean ± sd) in the gills of *M. galloprovincialis* after a 28-day exposure to 10 µg/L of polystyrene nanoplastics (50 nm).
Capital and lower-case letters represent significant differences between treatments at the same time and between times for the same treatment, respectively ($p < 0.05$).

- **Experiment 1:**
  - → 4-fold ↓ between 7 and 21 days of exposure to nPS
  - →Significant inhibition of AChE activity observed at 14, 21 and at 28 days (= 7 day depuration period)
  - → Significant ≠ between unexposed mussels at 14, 21 and 28 compared to the beginning and after 3 days.
- **Experiment 2:**
  - → AChE activity ↓ after 3 days of exposure
  - → 2-fold ↓ between 3 and 28 days of exposure
  - → Significant ≠ between unexposed mussels at 14, 21 and 28 compared to the beginning and after 3 and 7 days.

Ingestion of polystyrene nanoplastics (50 nm) in GONADS of mussels

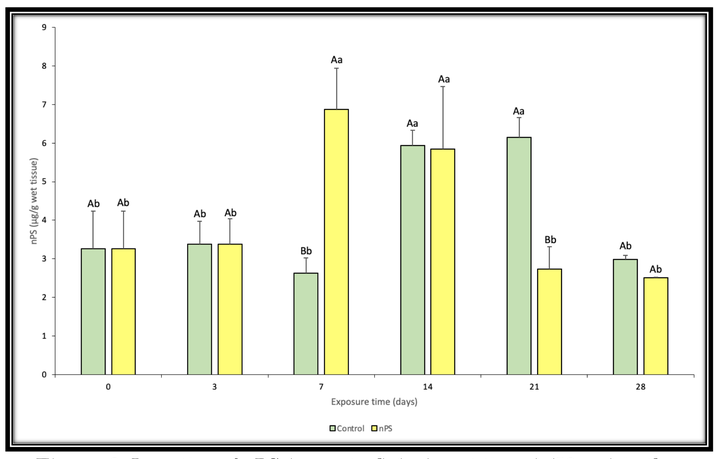


Figure 3. Ingestion of nPS (mean ± sd) (µg/g wet tissue) (50 nm) in the gonads of the mussel *M. galloprovincialis* after a 28-day exposure to 10 µg/L of nPS.
Capital and lower-case letters represent significant differences between treatments at the same time and between times for the same treatment, respectively ($p < 0.05$).

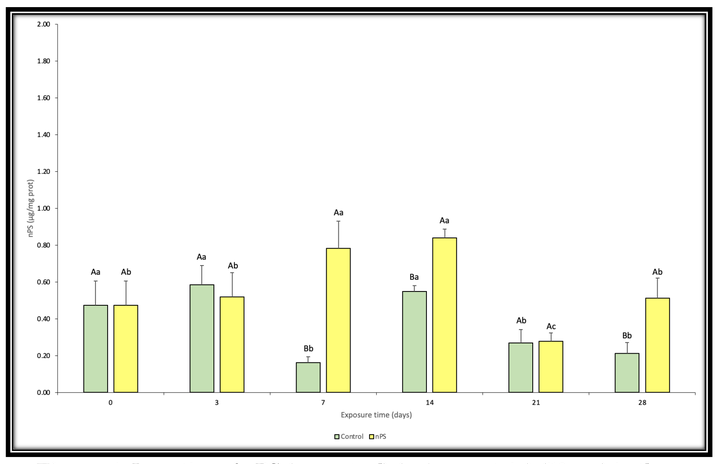


Figure 4. Ingestion of nPS (mean ± sd) (µg/mg protein) (50 nm) in the gonads of the mussel *M. galloprovincialis* after a 28-day exposure to 10 µg/L of nPS.
Capital and lower-case letters represent significant differences between treatments at the same time and between times for the same treatment, respectively ($p < 0.05$).

- **Ingestion results are here presented in two forms:**
  1- Ingested µg of nPS per g of wet tissue weight
  2- Ingested µg of nPS per mg of protein
- **Looking at Fig. 3:**
  - → At day 7 an ↑ in µg of nPS in the gonads of exposed mussels is noteworthy
  - → There is an ↑ of µg of nPS in unexposed mussels at 14 and 21 days. May this be detecting nPS from the environment?
- **Looking at Fig. 4:**
  - → After 7 and 14 days of exposure to nPS there is a significant ↑ in µg of nPS in the gonads, as well as at day 28.
  - → Significant ≠ in unexposed mussels at days 7, 21 and 28 compared to 0, 3 and 14.

Conclusion

- 10 µg/L of nPS exposure leads to neurotoxicity in gills of mussels
- AChE → ≠ between Experiment 1 & 2, although trend is similar.
- Mussel gonads do ingest nPS, but possibly also egest, and/or this may be due to the nanoplastics ability to cross cellular boundaries itself because of it's size range.
- In MPs exposed mussels, the smaller size particles are excreted but can also be retained within the mussel, this may also be true for nanoplastics, however further analysis is necessary to conclude this.

Future Perspectives

- Analyse the ingestion of nanoplastics in other mussel tissues (gills and digestive gland).
- Histopathological analysis of gonads exposed to nanoplastics may enlighten how, if and where these nano-sized particles are retained.
- Analyse the ingestion of nanoplastics in mussel larvae.

References

Gagné, F. (2019). Detection of polystyrene nanoplastics in biological tissues with a fluorescent molecular rotor probe. Journal of Xenobiotics, 9, 7–9. https://doi.org/10.4081/xeno.2019.8147
Kinjo, A., Mizukawa, K., Takada, H., & Inoue, K. (2019). Size-dependent elimination of ingested microplastics in the Mediterranean mussel *Mytilus galloprovincialis*. Marine Pollution Bulletin, 149(April), 110512. https://doi.org/10.1016/j.marpolbul.2019.110512
Yong, C. Q. Y., Valiyaveettil, S., & Tang, B. L. (2020). Toxicity of microplastics and nanoplastics in Mammalian systems. International Journal of Environmental Research and Public Health, 17(5). https://doi.org/10.3390/ijerph17051509

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ARNET AQUATIC RESEARCH NETwork

This research was supported by a PhD grant (UI/ BD/150758/2020), EMERGEMIX (PTDC/BIA-BMA/30922/ 2017) and the JPI Oceans projects RESPONSE (FCT JPIOCEANS/0005/2020), all of which were funded by Fundação para a Ciência e a Tecnologia, I.P., the European Regional Development Fund (ERDF) through Portugal 2020 and Portuguese national funds. We also appreciate the financing provided by FCT, under the project LA/P/0069/2020 granted to the Associate Laboratory ARNET and UID/00350/2020 to CIMA, University of Algarve.

FCT Fundação para a Ciência e a Tecnologia