

# Contaminant Mixtures and Warming: Bioaccumulation and Organismal Stress in an Invaded Freshwater Trophic Chain

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## Introduction & Aim

The global increase in plastic production has led to widespread microplastic (MP) pollution, with MPs now detected even in remote habitats [1]. In freshwater ecosystems, MPs can co-occur with other emerging contaminants, such as silver nanoparticles (AgNPs), and may be ingested by organisms, causing adverse effects [2-4]. Climate change may further modify contaminant behaviour, organism metabolism, feeding activity, and exposure pathways [5-6]. At the same time, non-native species can act as additional stressors by altering trophic interactions and accumulating contaminants that may be transferred to native predators [7]. Critically, the combined effects of pollution, warming, and non-native species remain largely unexplored [7-9].

We aimed to evaluate whether different climate scenarios can enhance the accumulation of MPs and AgNPs in a simple three-level trophic chain, composed of algae and two non-native species in Portugal, the snail, *Physa acuta*, and the signal crayfish, *Pacifastacus leniusculus*, with potential implications for contaminant transfer to native predators.

## Methods

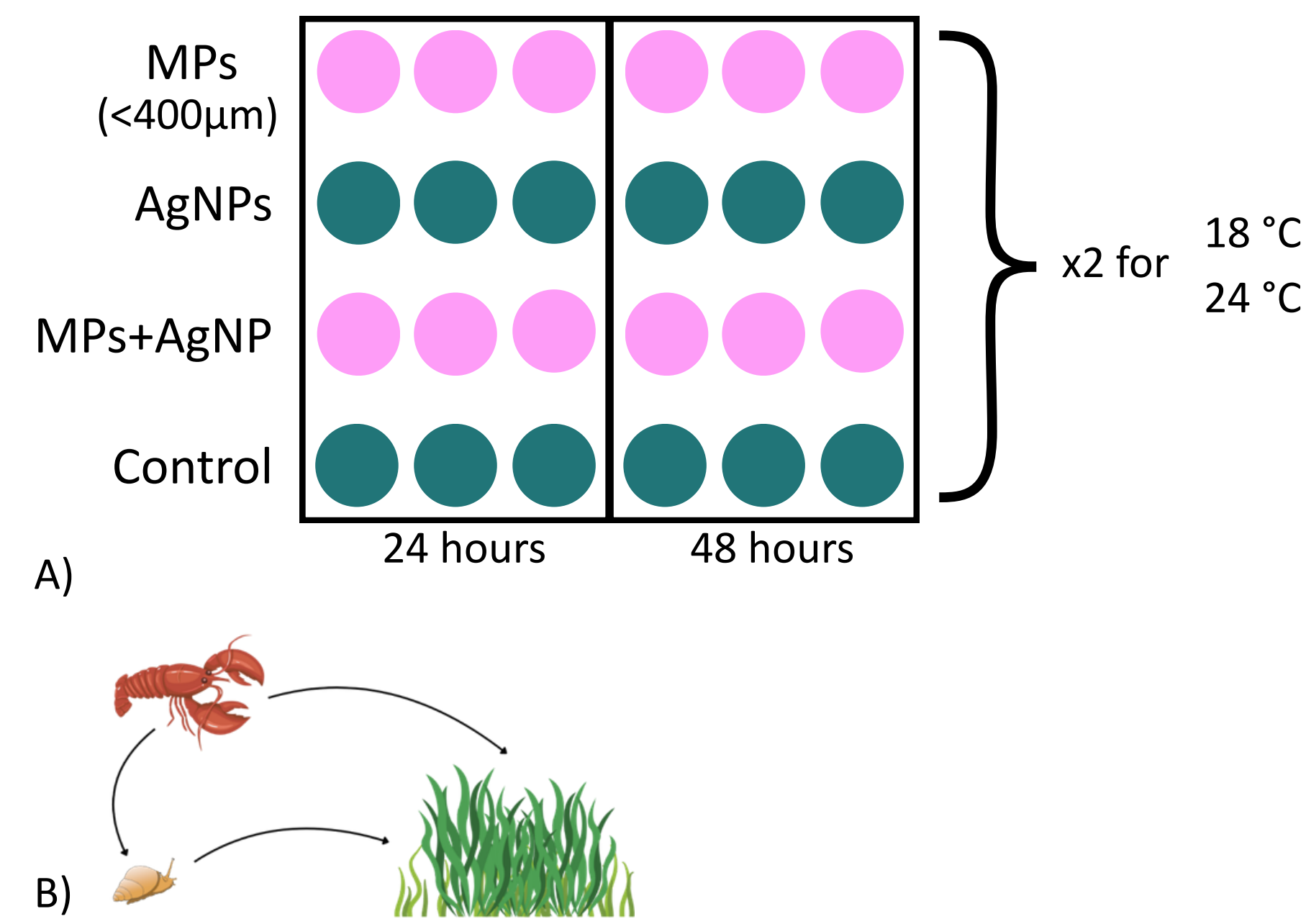


Fig. 1 - Diagram of the experimental design. (A) Outline of the experimental setup. (B) Trophic chain used in the experiment.

Table 1 - Depiction of the tissues and methods used for the MP, AgNPs and biomarkers analyses.

	Tissue			Quantification Method
MPs	gills + digestive tract	whole body	whole tissue	Fluorescence microscopy
AgNPs	abdominal muscle	whole body	whole tissue	Mass spectrometry
Biomarkers (CAT, GSTs, AChE)	gills (CAT & GSTs) + eyes (AChE)	whole body	-	Quantification of activity

## Results

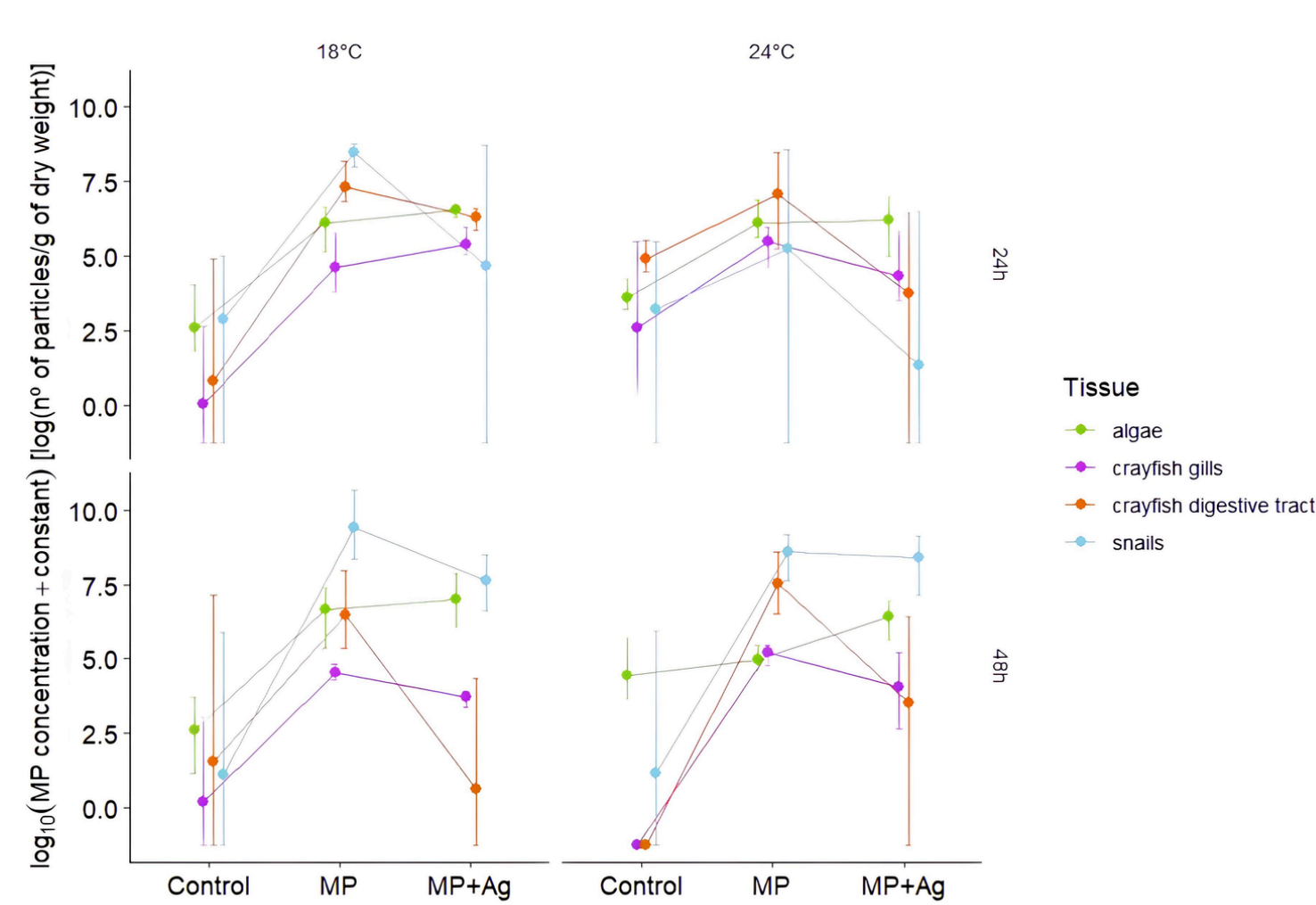


Fig. 2 - Interaction plot of mean MP concentrations (± bootstrapped confidence intervals), as log<sub>10</sub>(x+constant), across the different tissues and by experimental conditions.

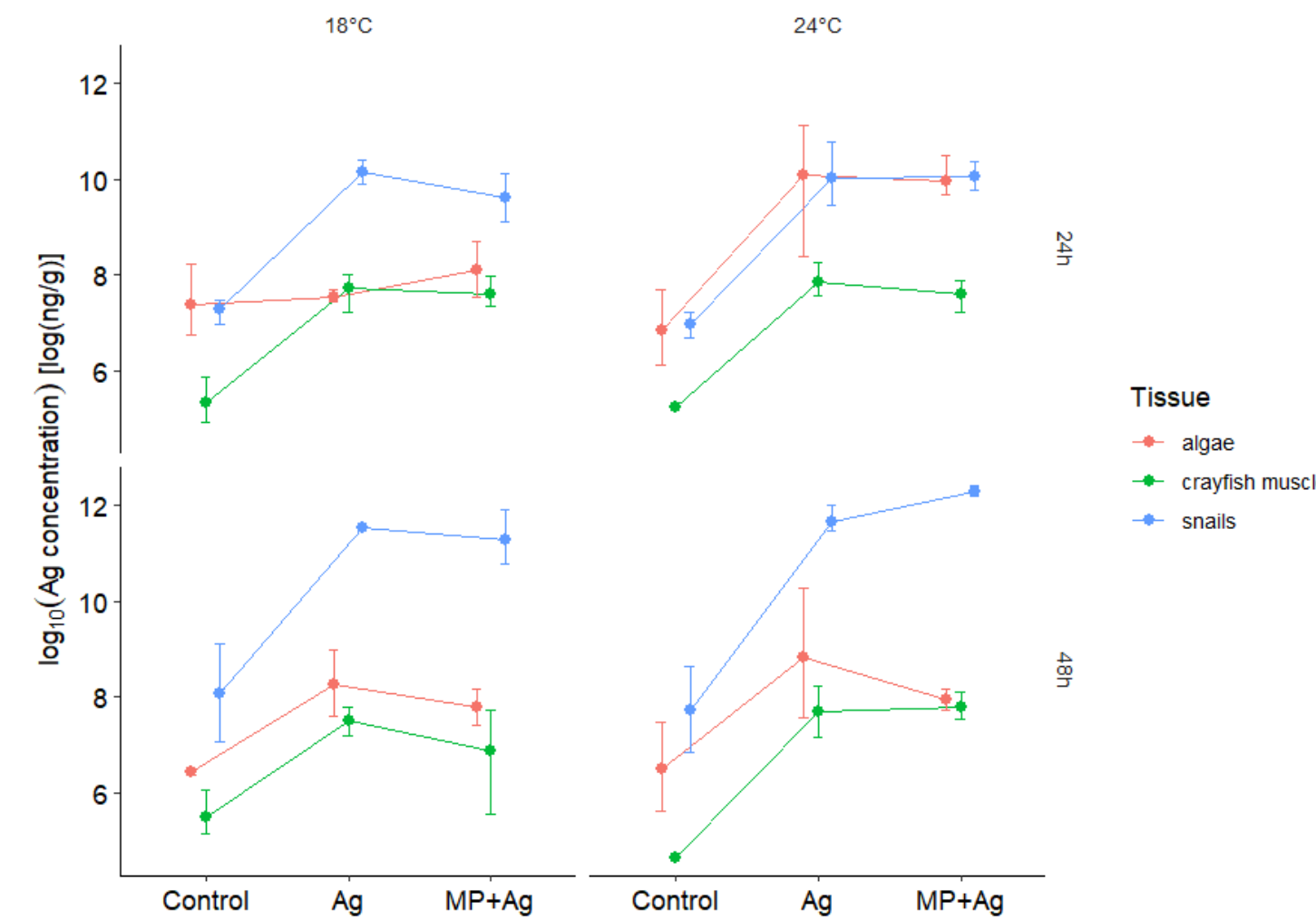


Fig. 3 - Interaction plot of mean Ag concentrations (± bootstrapped confidence intervals), as log<sub>10</sub>, across the different tissues and by experimental conditions.

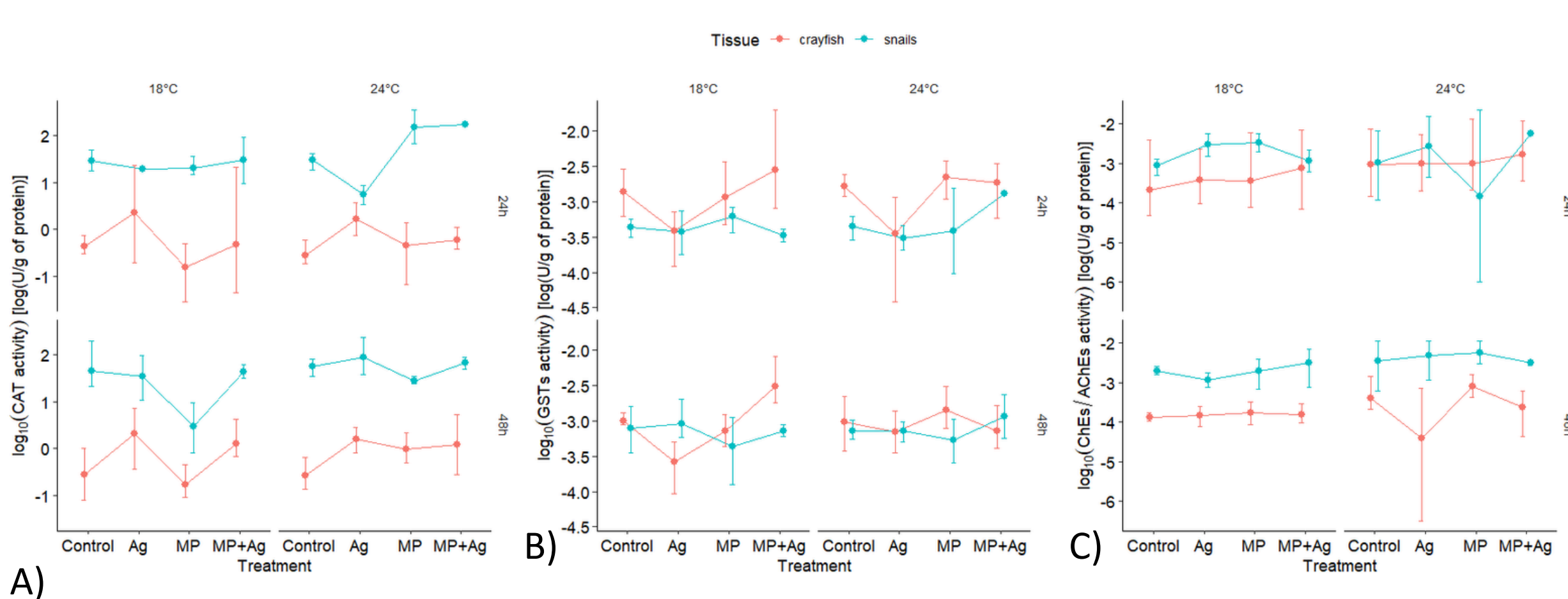


Fig. 4 - Interaction plot of mean (A) CAT, (B) GSTs, and (C) AChE activities (± bootstrapped confidence intervals), as log<sub>10</sub>, across the different tissues and by experimental conditions.

Biomarker activities (CAT, GST, ChEs/AChEs) varied mainly by tissue, with no robust treatment effects, suggesting that the exposure levels and duration of this experiment may have been insufficient to elicit stress responses.

MPs and AgNPs accumulate across every trophic level studied

For MPs, we detected no consistent trends between tissues and no effects of temperature or exposure time.

For AgNPs, we observed a tissue-structured distribution, with snails accumulating the most silver, followed by algae, and then crayfish.

For AgNPs, we also found an effect of exposure duration on the accumulation in snails. Longer exposure resulted in higher accumulation.

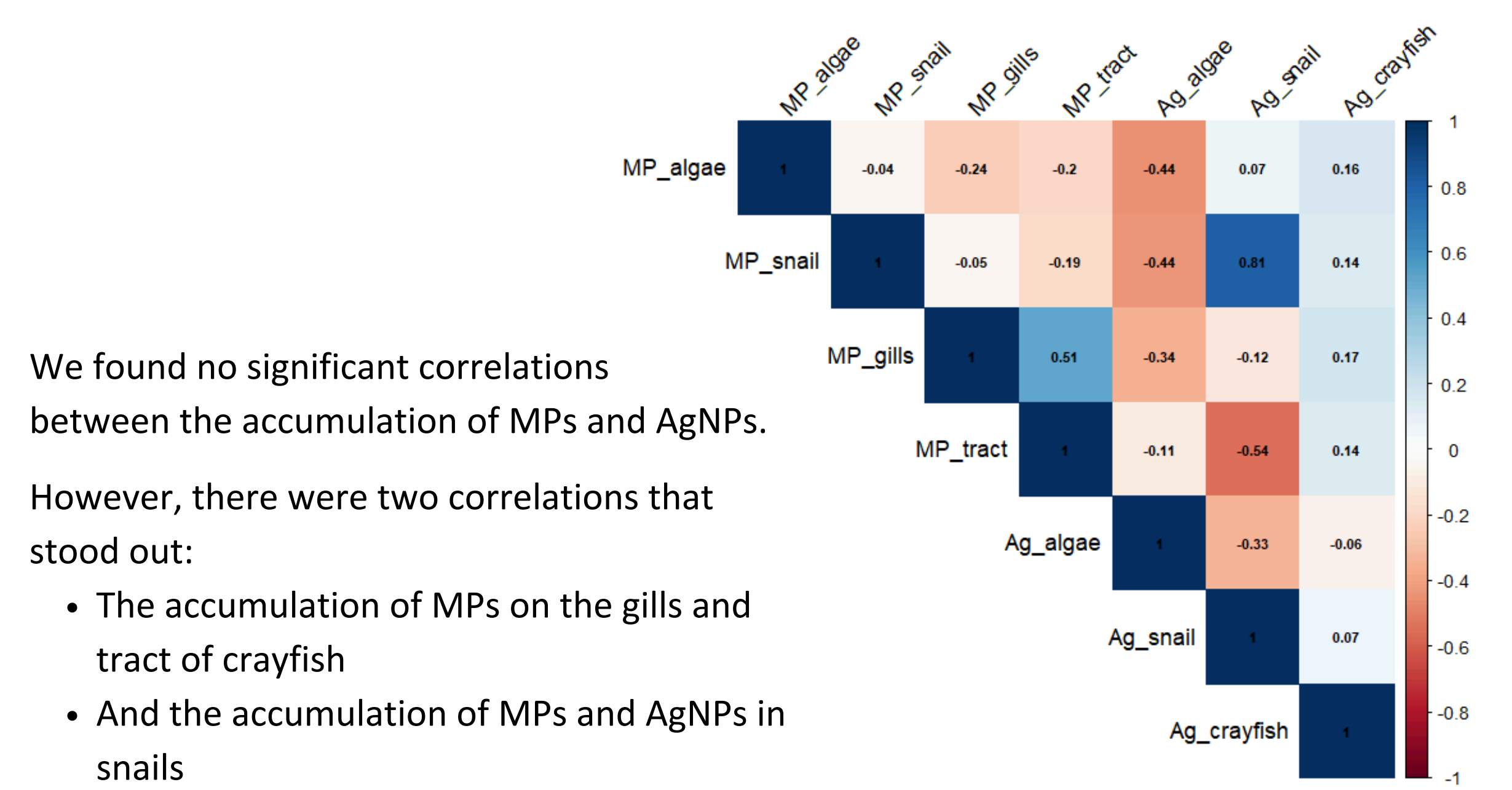


Fig. 5 - Correlation matrix between MP and AgNPs accumulation on the different tissues. Correlation coefficients are also shown.

We found no significant correlations between the accumulation of MPs and AgNPs.

However, there were two correlations that stood out:

- The accumulation of MPs on the gills and tract of crayfish
- And the accumulation of MPs and AgNPs in snails

## Conclusion & Future Perspectives

- This study represents an important first step toward understanding how multiple contaminants and warming interact to affect contaminant accumulation and stress responses across invaded freshwater trophic levels.
- Our results reinforce that the ecological risks posed by these contaminants are complex and cannot be extrapolated by simplified essays.
- Based on our results and limitations found we believe future works should:
  - prioritize the use of realistic contaminant mixtures in ecologically relevant concentrations
  - expand the temperature gradients and evaluate chronic exposure when possible
  - include MPs of different polymers and sizes, as well as conditioned MPs
  - and integrate complex trophic webs and analyze multiple tissues and stress mechanisms, to get a complete picture of effects.

All of this should be done with optimized and standardized protocols.

## Conflict of Interest

The authors declare no conflicts of interest.

## Acknowledgments

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