Saxitoxin effects on *Daphnia magna*

An attempt at linking epigenetic response with phenotypic effects

Albano Pinto[1,2] ✉ albanopinto@ua.pt, Inês P. E. Macário[1,2], Sérgio M. Marques[1,2], Joana Lourenço[1,2], Inês Domingues[1,2], Jana Asselman[3], **Patrícia Pereira**[1,2] and Joana Luísa Pereira[1,2]

[1] CESAM – Centre for Environmental and Marine Studies, Department of Biology, University of Aveiro, Portugal
[2] Department of Biology, University of Aveiro, Aveiro, Portugal
[3] Blue Growth Research Lab, Ghent University, Bluebridge Building, Ostend Science Park 1, 8400, Ostend, Belgium

CONTEXT

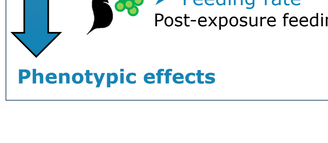
- **Saxitoxin (STX)** and its derivatives are potent natural aquatic neurotoxins produced by freshwater and marine phytoplankton species during harmful bloom events.
- Due to climate change, the occurrence and severity of harmful bloom events and related toxins are predicted to increase.
- STX human health adverse impacts are well known. The World Health Organization established a safety guideline for these toxins in recreational freshwaters of 30 µg L^{-1} [1]. However, **STX effects on freshwater biota remain largely unexplored**.

Aim

- Assess the effects of an **environmentally-plausible** pulse exposure (24 h) to 30 µg L^{-1} of STX on the freshwater model species *Daphnia magna*.

Methods

1 Exposure



- *Daphnia magna* juveniles (4 days old)
- 24-hour exposure
- 5 replicates per condition

2 Biomarkers analysis

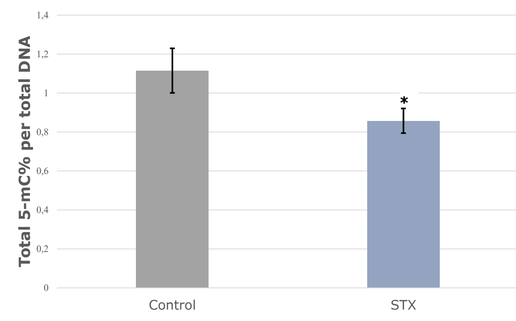
Epigenetic response

- Total DNA methyltransferases (DNMTs) activity
  EpigenTek™ EpiQuik DNMT Activity/Inhibition ELISA Kit
- Total 5-methylcytosine (5-mC) DNA methylation levels
  EpigenTek™ MethylFlash Global DNA Methylation ELISA kit
- Antioxidant enzymes activity
  Colorimetric assay
- Lipid peroxidation (TBARS)
  Colorimetric assay
- DNA damage levels
  Alkaline comet assay
- Total cholinesterases (ChEs) activity
  Colorimetric assay
- Behavioral patterns
  Viewpoint ZebraBox analysis
- Heart rate
  Visual counting using slow motion videos
- Feeding rate
  Post-exposure feeding inhibition assay

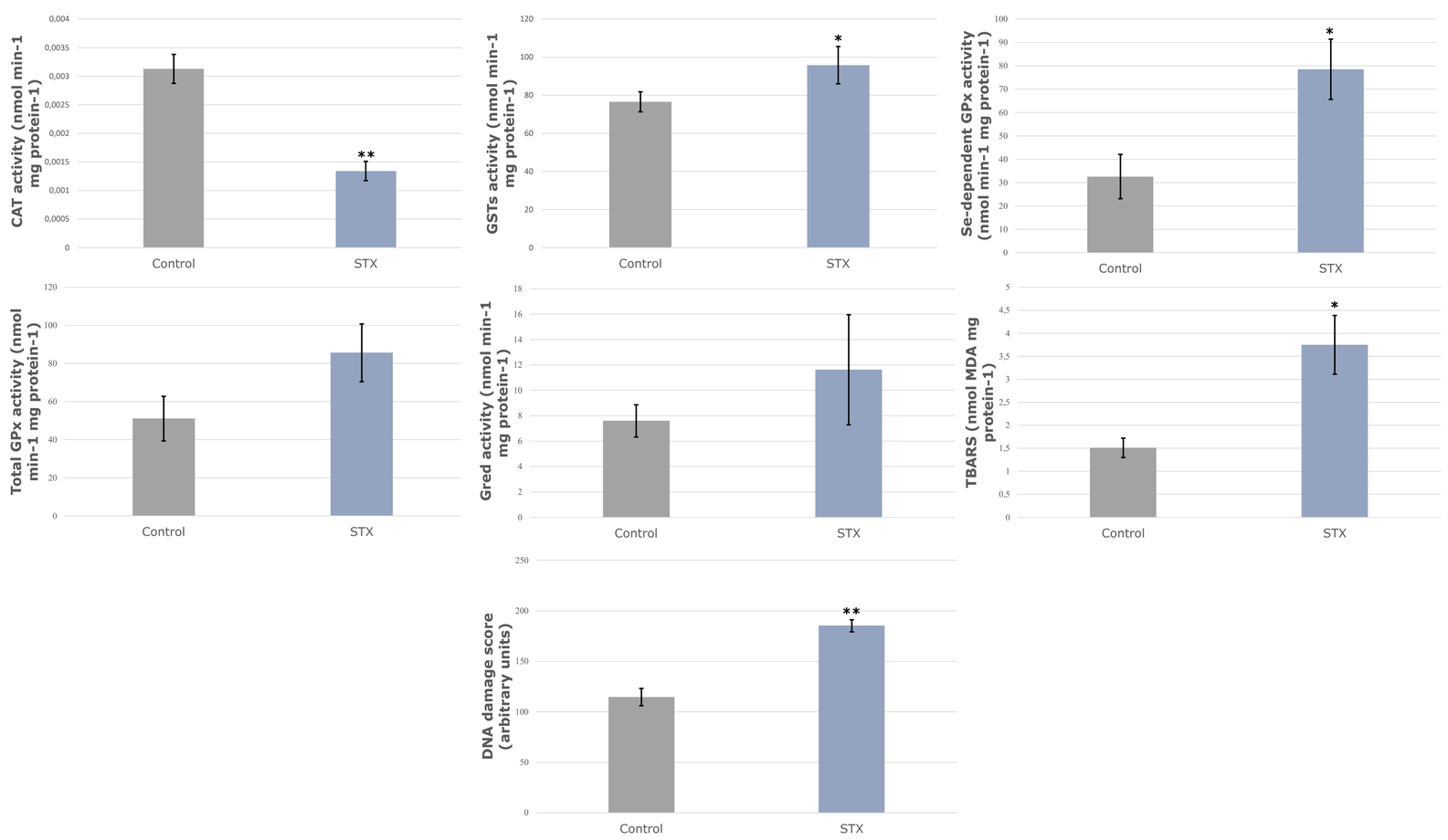
Phenotypic effects

Check out the full results at

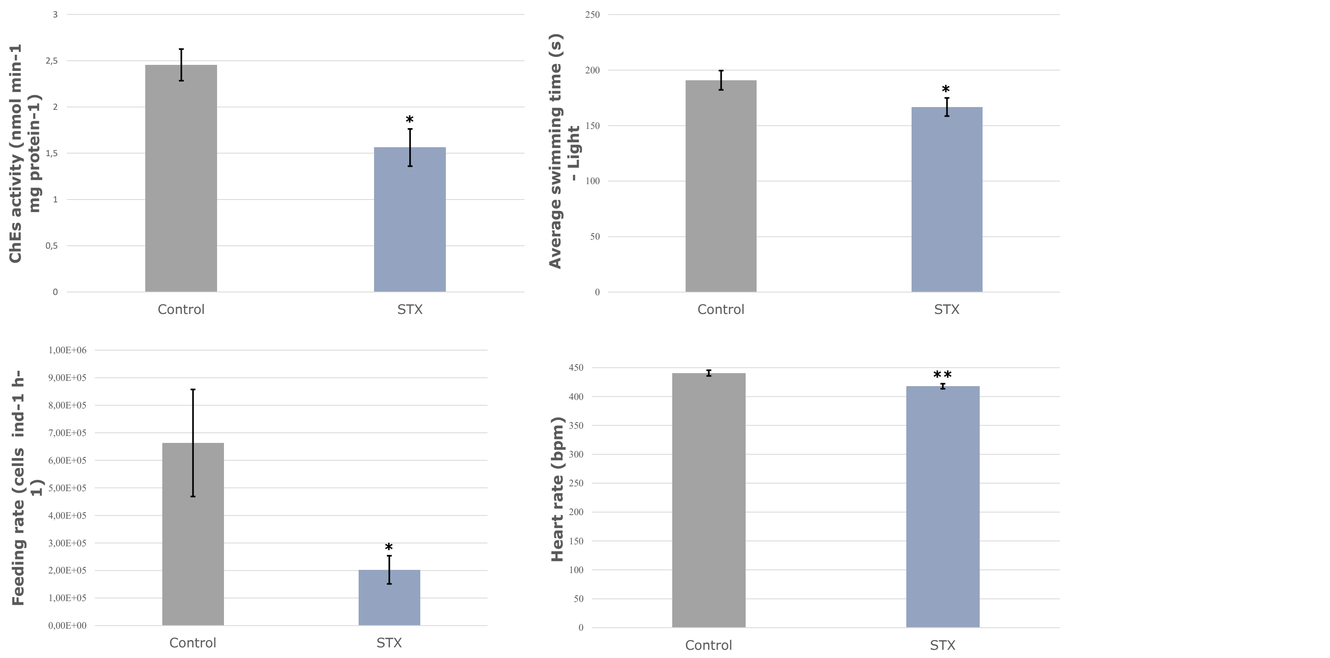
RESULTS



- STX exposure causes a decreased total DNA 5-mC levels, likely through an interaction with key epigenetic players, e.g., DNA methyltransferases expression/enzymatic activity inhibition
- The observed reduction in total 5-mC DNA methylation levels probably elicits a short-term gene expression modulation, which may correlate with the responses found for the other biomarkers used in the present work, thus functioning as the molecular initiating event



- STX exposure inhibited CAT activity, possibly leaving organisms with an impaired antioxidant metabolism
- The glutathione detoxification system (particularly GSTs and Se-dependent GPx) seems to play a crucial role in STX detoxification, but the increase in their activity was not sufficient to prevent oxidative stress-related lipid peroxidation and DNA damage



- STX lead to the oxidation and inhibition of ChEs activity, which can explain the reduced locomotion and the reduced feeding rate
- STX impairs *D. magna* heart rate, potentially compromising exposed organisms' overall fitness and leaving them more vulnerable to predators

Conclusions

- STX exposure induces important alterations in *D. magna*, from the epigenetic landscape up to physiological function.
- This study highlights the toxicity of STX towards cladocerans and reinforces the need to address the current knowledge gaps concerning the effects of STX on freshwater aquatic biota.

References
[1] World Health Organization (2020) Cyanobacterial toxins: saxitoxins. World Health Organization. Available at: https://iris.who.int/bitstream/handle/10665/338069/WHO-HEP-ECH-WSH-2020.8-eng.pdf

Acknowledgements
This work was developed under the specific scope of the project EPIBOOST, funded by the European Union through the Grant 101078991; and by and by the PARC partnership funded by the European Union through Grant 101057014. AP is grateful to FCT for the awarded individual PhD grant with the reference 2022.10817.BD. PP is funded by FCT under a researcher contract (CEECIND/01144/2017).