## Poster 41

# **Impact of environmental caffeine contamination in zebrafish: ecotoxicological approach**

I. Pinto <sup>1,2,3,4,\*</sup>, B. S. Diogo <sup>1,2,3</sup>, S. C. Antunes <sup>2,3</sup> and S. Rodrigues <sup>2,3</sup>

<sup>1</sup> ICBAS, Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313, Porto, Portugal

<sup>2</sup> CIMAR/CIIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos, S/N, 4450-208 Matosinhos, Portugal.

<sup>3</sup> FCUP, Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre S/N. 4169-007 Porto, Portugal

<sup>4</sup> UMIB-ICBAS, Unidade Multidisciplinar de Investigação Biomédica - Instituto Ciências Abel Salazar da Universidade do Porto, Rua de Jorge Viterbo Ferreira n.º 228, 4050-313, Porto, Portugal

\* Correspondence: ivofilipemp@fc.up.pt

## Abstract

Background: Caffeine (CAF) is considered a suitable indicator of anthropogenic contamination of aquatic environments, and several pharmacological and biological effects have been observed in target and non-target organisms [1,2]. Objective: Assess the chronic effects of environmental concentrations of CAF in several biomarker responses in Danio rerio. Methods: After chronic exposure to CAF (0.16, 0.42, 1.09, 2.84, 7.40, 19.23, and 50 µg/L) on Danio rerio, several biomarkers of antioxidant defense (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GRed) activities, and glutathione (GSH) content), biotransformation (glutathione S-transferase activity (GSTs)), lipid peroxidation (thiobarbituric acid reactive substances (TBARS) levels), energetic reserves (glycogen (GLY), lipids (LIP), protein (PROT) contents, and lactate dehydrogenase (LDH) activity), and neurotransmission (acetylcholinesterase (AChE) activity) were evaluated. A multi-biomarker approach known as Integrated Biomarker Response (IBR) was also employed to perceive the most sensitive and responsive biomarker, after CAF exposure. Results: Exposure to CAF disrupts several metabolic pathways in D. rerio, such as changes in antioxidant defenses, specifically in the activities of SOD, GRed, and GSH content, which may have led to lipid peroxidation. LDH activity decreased at all tested concentrations, while AChE activity was only affected at the highest concentrations (19.23 and 50  $\mu$ g/L). IBR revealed that the highest stress occurred at a concentration of 50 µg/L and showed clearly that biomarker responses were responsive and coherent to demonstrate the stress caused independently to CAF concentration in fish. Conclusions: These results highlight the urgent need to minimize the increasing load of CAF on the aquatic ecosystems, considering the adverse impacts of CAF pollution in the aquatic environment. These results show that the implementation of scientific programs and monitoring projects are imperative to classify the CAF as a high-priority environmentally hazardous emerging pollutant.

Keywords: Danio rerio; chronic exposure; physiologically-based ecotoxicity; biochemical biomarkers

## Acknowledgments

This research was funded by Foundation for Science and Technology, and by the Strategic Program UIDB/04423/2020 and UIDP/04423/2020. Sara Antunes and Sara Rodrigues are hired through the Regulamento do Emprego Científico e Tecnológico – RJEC from the FCT program (CEECIND/01756/2017 and 2020.00464.CEEC-IND, respectively). Bárbara Diogo and Ivo Pinto were supported by FCT Ph.D. grants (2022.10505.BD and 2022.10194.BD respectively).

## References

1. Abdelkader, T.S., Chang, S.N., Kim, T.H., Song, J., Kim, D.S., Park, J.H., 2013. Exposure time to caffeine affects heartbeat and cell damage-related gene expression of zebrafish Danio rerio embryos at early developmental stages. *J Appl Toxicol* 33, 1277–1283.

2. Ferreira, A.P., de Lourdes, C., da Cunha, N., 2005. Anthropic pollution in aquatic environment: Development of a caffeine indicator. *Int J Environ Health Res* 15, 303-311.



In *Scientific Letters*, works are published under a CC-BY license (Creative Commons Attribution 4.0 International License at <a href="https://creativecommons.org/licenses/by/4.0/">https://creativecommons.org/licenses/by/4.0/</a>), the most open license available. The users can share (copy and redistribute the material in any medium or format) and adapt (remix, transform, and build upon the material for any purpose, even commercially), as long as they give appropriate credit, provide a link to the license, and indicate if changes were made (read the full text of the license terms and conditions of use at <a href="https://creativecommons.org/licenses/by/4.0/legalcode">https://creativecommons.org/licenses/by/4.0/</a>).