Prodiamesa olivacea, a potential sentinel organism for ecotoxicity studies in natural scenarios

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Toxicological studies on non-model organisms complement and provide powerful information regarding natural ecosystems and along with traditional approaches, they confer a breakthrough in ecotoxicology. *Chironomus* (Diptera) has four OECD standardized tests that assess classical toxicity parameters for evaluating water and sediment toxicity (survival, immobilization, reproduction and development). *Prodiamesa olivacea* (Diptera) is a non-model aquatic insect species not used in toxicity tests and that frequently shares habitat with *C. riparius*, although it requires higher oxygen levels and less extreme conditions. Regarding this, it is of special interest to study the possible differences in the response of both species to pollutant exposure.

This work characterizes and tests the effectiveness of early effect biomarkers related to environmental pollution in water on natural populations of *P. olivacea* larvae under different stress conditions. This will contribute to increasing the limited knowledge about xenobiotics effects on benthic aquatic invertebrates, one of the most sensitive group. The *de novo* RNAseq transcriptome was obtained from *P. olivacea* and used to identify and characterize genes related to stress and immune system responses such as *Hsp60, Hsp70, PGRP, Toll* or *JAK/hopscotch* among others. Quantitative real-time PCR was used to evaluate the expression of selected genes. In this study, the toxicity of benzyl butyl phthalate (BBP; CAS No. 85-68-7) was elucidated in *P. olivacea* and *C. riparius* species from a polluted river (Sar) in Galicia (Spain). Transcriptional effects of acute BBP 4-h and 24-h exposures were evaluated, and the results revealed species-dependent gene responses. *P. olivacea* showed a higher sensitivity to BBP than *C. riparius*, as more severe effects were observed for all the analyzed biomarkers.

This research provides new tools for assessing and monitoring water quality and it highlights the importance of a multi-organism ecotoxicological approach to deep into BBP toxicity. It is essential to assess the tolerance / sensitivity of not only natural populations of model organisms but also non-model insect species chronically exposed to complex mixtures of pollutants. This approach will avoid drawing incomplete conclusions in the light of highly tolerant model organisms. This approach will allow us to have a broader view of the risk associated with pollutant's presence in ecosystems in the short, medium, and long term.