

## Complex responses in complex scenarios: the use of biomarkers in *Chironomus riparius* larvae in a polluted river

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At present ecotoxicologists have a vast array of techniques and biomarkers available to test the effects of multiple stressors in model organisms. However, most of these laboratory-tested protocols have been scarcely used in natural scenarios, partly because of the complex mixtures of factors that might have an effect on those organisms. The objective of the present work has been to gain a deeper knowledge on the physiology of the larvae of the model organism *Chironomus riparius* by exploring cellular stress, endocrine functioning, detoxification response and energy metabolism. The novelty of our approach is that these aspects have been investigated in a complex scenario, using individuals from a chronically-polluted river.

Larvae of *Chironomus riparius* were collected at five sampling stations in a 14 km-long stretch of river Sar (NW Spain). Sampling stations were selected to obtain a diverse array of stress conditions, mainly those possibly associated to the presence of important facilities and infrastructures. Stress responses were investigated through changes in 1) expression of genes related to cryoprotection (*hsp70* and *hsc70*); hormonal signalling (*EcR*); and xenobiotic detoxification pathways (*cyp4g*), 2) growth and metabolic rates indicators (RNA:DNA ratio, total protein and glycogen content), and 3) developmental sublethal effects (mouthpart deformities). Results show that several of these biomarkers (*hsp70*, *EcR*, *GAPDH*, total protein content) present a higher basal activity in field conditions. Also, differences in some biomarkers (*hsp70*, *EcR*, glycogen content, DNA:RNA) were detected among sampling points, which arises the question of the possible influence of chemical characteristics of the sediments at those sites. Opposite, *hsc70* and *cyp4g* appeared to be stable. Interestingly, the *hsp70* gene seems to be particularly sensitive to conditions of pollutant exposure, while its constitutive counterpart *hsc70* showed invariable expression, reinforcing the *hsc70/hsp70* ratio as a potential indicator of polluted environments.

Although these differential responses should be further investigated, they open a new window to explore present conditions organisms are facing in natural ecosystems, which complexity clearly exceeds that experienced in laboratory tests.

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