

A Comparative Approach for Modeling the Toxicity of Metal Mixtures in Two Ecologically Related Three-Spined (*Gasterosteus aculeatus* L.) And Nine-Spined (*Pungitius pungitius* L.) Sticklebacks

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Abstract : Sticklebacks (*Gasterosteiformes*) are increasingly used in ecological and evolutionary research and become well-established role as model species for biologists. However, ecotoxicology studies concerning behavioural effects in sticklebacks regarding stress responses, mainly induced by chemical mixtures, have hardly been addressed. Moreover, although many authors in their studies emphasised the similarity between three-spined and nine-spined stickleback in morphological, neuroanatomical and behavioural adaptations to environmental changes, several comparative studies have revealed considerable differences between these species in and their susceptibility and resistance to various stressors in laboratory experiments. The hypothesis of this study was that three-spined and nine-spined stickleback species will demonstrate apparent differences in response patterns and sensitivity to metal-based chemicals stimuli. For this purpose, we investigated the swimming behaviour (including mortality rate based on 96-h LC50 values) of two ecologically similar three-spined (*Gasterosteus aculeatus*) and nine-spined sticklebacks (*Pungitius pungitius*) to short-term (up to 24 h) metal mixture (MIX) exposure. We evaluated the relevance and efficacy of behavioural responses of test species in the early toxicity assessment of chemical mixtures. Fish exposed to six (Zn, Pb, Cd, Cu, Ni and Cr) metals in the mixture were either singled out by the Water Framework Directive as priority or as relevant substances in surface water, which was prepared according to the environmental quality standards (EQSs) of these metals set for inland waters in the European Union (EU) (Directive 2013/39/EU). Based on acute toxicity results, *G. aculeatus* found to be slightly (1.4-fold) more tolerant of MIX impact than those of *P. pungitius* specimens. The performed behavioural analysis showed the main effect on the interaction between time, species and treatment variables. Although both species exposed to MIX revealed a decreasing tendency in swimming activity, these species' responsiveness to MIX was somewhat different. Substantial changes in the activity of *G. aculeatus* were established after 3-h exposure to MIX solutions, which was 1.43-fold lower, while in the case of *P. pungitius*, 1.96-fold higher than established 96-h LC50 values for each species. This study demonstrated species-specific differences in response sensitivity to metal-based water pollution, indicating behavioural insensitivity of *P. pungitius* compared to *G. aculeatus*. While many studies highlight the usefulness and suitability of nine-spined sticklebacks for evolutionary and ecological research, attested by their increasing popularity in these fields, great caution must be exercised when using them as model species in ecotoxicological research to probe metal contamination. Meanwhile, *G. aculeatus* showed to be a promising bioindicator species in the environmental ecotoxicology field.

Keywords : acute toxicity, comparative behaviour, metal mixture, swimming activity

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