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Evaluation of the suitability of sinkhole ponds and springs from two Portuguese karst massifs for amphibian early life stage development

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Abstract

Amphibians in Portugal are commonly found in karst ecosystems. Nonetheless, these ecosystems are threatened by activities such as agricultural and industrial activities and mining, which contaminate both surface water and groundwater. Due to the high permeability of the karst systems, the existence of water on the surface is scarce, with sinkhole ponds and springs representing essential water sources for amphibians, especially for their reproduction. Furthermore, water from springs may reflect the effects of surface contamination on groundwater. Considering the relevance of these water sources, specially to amphibians, which are considered highly sensitive to a widerange of contaminants, our study aimed at assessing the suitability of water from springs and sinkholes from two Portuguese karst massifs, for the development of the early lifestages of *Hyla arborea*. To achieve this, eggs from *H. arborea* were exposed to water from seven springs and four sinkholes, from the Estremenho and Sicó karst massifs, anda control (FETAX). The exposure was carried out with five replicates per condition, each with 20 tadpoles, and ended once the 25th Gosner stage was reached. The parameters evaluated were growth, antioxidant enzyme activity (Glutathione STransferases, Glutathione

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Peroxidase Se-dependent, total-Glutathione Peroxidase and Glutathione Reductase) and lipid peroxidation (LPO). Also, water samples were screened for the presence of six commonly used pesticides (Terbuthylazine, Tebuconazole, Deltamethrin, Dimethomorph, Chlorpyrifos and Glyphosate). The results revealed the presence of glyphosate in three of the sampled water bodies. Concurrently, in one of the sites presenting detectable glyphosate concentrations, tadpoles presented smaller body lengths, and mortality was also observed. In the remaining sites, despite the absence of the screened pesticides, there were some alterations of the biochemical parameters assessed, namely LPO. These results raised some concerns regarding the impact of human activities in surface and groundwater in the Sicó and Estremenho karst massifs.

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