A case of amphibians breeding in sulfurous water in Romania

For amphibians, the electrolyte contents and concentrations of the water they live and/or breed in is of the greatest importance. Thus, various studies have highlighted the problems faced by amphibians when dealing with brackish or saline water, which only a few species such as *Bufo viridis* LAURENTI, 1768, *Bufo calamita* LAURENTI, 1768, *Fejervarya cancrivora* (GRAVENHORST, 1829), *Pelophylax ridibundus* (PALLAS, 1771) and *P. perezi* (SEOANE, 1885) can tolerate (see, e.g., SHPUN et al 1992; WELLS 2007; SILLERO & RIBEIRO 2010).

Amphibians also encounter, naturally or as a result of human intervention, waters with a high content of other electrolytes, such as sulfurous waters, rich in hydrogen sulfide (H_2S). Hydrogen sulfide is toxic for most metazoans (see, e.g., GRIESHABER & VÖLKEL 1998) as it blocks respiration, when in high concentration, or, in lower amounts, increases the oxygen needs of the organism (YONG & SEARCY 2001). Amphibians were reported to live in thermal sulfurous waters with high hydrogen sulfide concentration (e.g. ORUÇI, S. 2010), but the impact of hydrogen sulfide upon their biology has not been addressed yet. For Romania there are extensive studies dealing with the amphibians living in thermal waters in Western Romania (e.g. COVACIU-MARCOV et al. 2006; SAS et al. 2010; COVACIU-MARCOV et al. 2011) but these studies deal only with thermal acclimation (for which see also the discussion in DUELLMANN & TRUEB 1994), reproduction through winter and other temperature-related topics, whereas the effects of hydrogen sulfide have rarely been considered (and if so, no hydrogen sulfide contents of these thermal waters were reported).

In the present study, we report a case of several amphibian species breeding in a water body with high hydrogen sulfide content (and generally high mineralization). This situation was encountered in Săcelu, a town in the sub-Carpathian Hills of Oltenia (southwest Romania) where numerous natural sulfurous springs and drillings are found, some being used therapeutically in a small spa resort. These mineral springs are described as sulfurous and saline, being rich in hydrogen sulfide, and in sodium, chlorine, bromide and iodine ions; they are also accompanied by gaseous hydrocarbons such as methane, and at least one has a high concentration of radium, enough for it being unfit for drinking. The total mineralization of these springs varies between ca. 2000 and ca. 80000 mg/l, (i. e. ca. 2-80 g/l) while the H_2S concentration ranges from negligible to ca. 250 mg/l; these values are also variable over time, for each individual spring. The temperature of spring and drill waters varies with the season in the range of 11-14ºC (HUICĂ & ANIȚEI 1997).

We observed amphibian presence and reproduction in April 2011 within the perimeter of Săcelu town, in a pond located at 45°06.621'N, 023°25.543'E and 348 m a.s.l., fed by a drill yielding sulfurous water, identified as Drill no. 50. This water source has a total mineralization varying between 21703.15 and 46422.3 mg/l, and its known H_2S content has varied along time between negligible and 100.2 mg/l. It also contains sodium, chlorine, bromide and iodine ions, as well as methane gas (HUICĂ & ANIȚEI 1997), being a typical Săcelu spring in this respect. At the time of our observations, the water appeared to have a high concentration of hydrogen sulfide, its waters having the typical pungent, rotten-egg odor and appearing milky, because of the colloidal sulfur suspension resulting from the oxidation of H_2S in surface waters (Fig. 1); sulfur was also abundantly deposited upon underwater plants, rocks, etc., these being coated in a whitish layer.

However, amphibians were present and involved in the process of reproduction there, as we observed live specimens of *Triturus cristatus* (LAURENTI, 1768), *Lissotriton vulgaris* (LINNAEUS, 1758), *Pelophylax ridibundus*, as well as spawn of *Bufo bufo* (LINNAEUS, 1758) and *Rana dalmatina* FITZINGER in BONAPARTE, 1839, and also a few dead specimens of *B. bufo* (Fig. 2) and *R. dalmatina*. The spawn appeared viable (most of it was hatching – Fig. 3), and the newts were in breeding condition (Fig. 4), in this sulfide-rich water. However, we could notice that *T. cristatus* specimens

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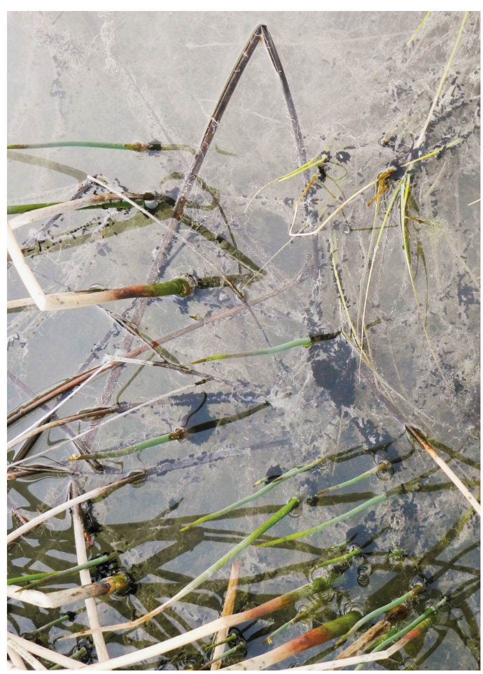

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were gulping air with great frequency, suggesting that the presence of sulfide did, in fact, increase their metabolic oxygen requirement (cf. YONG & SEARCY 2001). Also, the dead toads and frogs could be the victims of sudden increases in the sulfide contents of the water body, due to the observed variations in the sulfide concentration of the feeding drill (however, other mortality causes could not be ruled out). Thus, amphibians can and do exploit this highly mineralized (the total mineralization of this springs occasionally comes close to that of brackish water) and sulfurous water, albeit with possible metabolic and mortality costs. It is probable that there is also a gain for them in being less exposed to pathogens and/or predators during development, but this hypothesis needs to be further checked in the field.

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KEYWORDS: Amphibia, hydrogen sulfide, reproduction, physiology, ecology, toxicology, Săcelu sulfurous springs, Romania

SUBMITTED: November 9, 2011

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Figs. 1-4 (opposite page)

Fig. 1. The studied sulfurous pond in Săcelu, Romania; notice the milky color of water, given by colloidal sulfur (photo: O. IFTIME).

Fig. 2. Dead, bloated *Bufo bufo* (LINNAEUS, 1758) adult in the sulfurous pond, Săcelu, Romania (photo: A. IFTIME).

Fig. 3. Hatching larvae of *Bufo bufo* (LINNAEUS, 1758) on remains of egg strings, coated with sulfur, Săcelu, Romania (photo: A. IFTIME).

Fig. 4: *Lissotriton vulgaris* (LINNAEUS, 1758), adult breeding male in sulfurous water, among sulfur-coated plants, Săcelu, Romania (photo: A. IFTIME).