MULTISYSTEMIC EMPHYSEMA (GAS BUBBLE DISEASE)-ASSOCIATED ACUTE MASS MORTALITY IN A FREE-RANGING POPULATION OF COMMON FROG (RANA TEMPORARIA) IN SWITZERLAND

Authors: Borel, Stéphanie, and Origgi, Francesco

Source: Journal of Wildlife Diseases, 59(3) : 442-452

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/JWD-D-22-00147

DOI: 10.7589/JWD-D-22-00147

Journal of Wildlife Diseases, 59(3), 2023, pp. 442–452

MULTISYSTEMIC EMPHYSEMA (GAS BUBBLE DISEASE)-ASSOCIATED ACUTE MASS MORTALITY IN A FREE-RANGING POPULATION OF COMMON FROG (*RANA TEMPORARIA*) IN SWITZERLAND

Stéphanie Borel[1] and Francesco Origgi[1,2,3]

[1] Institute for Fish and Wildlife Health (FIWI), Department of Infectious Diseases and Pathobiology, Vetsuisse Faculty, University of Bern, Länggassstrasse 122, 3012 Bern, Switzerland
[2] Institute of Infectious Diseases, College of Veterinary Medicine, University of Messina, Piazza Pugliatti 1, 98122 Messina, Italy
[3] Corresponding author (email: francesco.origgi@unibe.ch)

ABSTRACT: In April 2020, nearly 5,000 free-ranging common frogs (*Rana temporaria*) were found dead on the surface of the water in a retention pond in the Swiss Alps. Macroscopic and microscopic lesions revealed multisystem emphysema, affecting multiple organs. The most severe lesions were seen in the skin, eyes, and blood vessels of internal organs and were secondary to the sudden massive distension of the skin and other affected organs. All frogs had similar lesions consistent with those described associated with gas bubble disease. No obvious pre-existing conditions potentially priming the occurrence of the observed lesions could be detected. All the examined frogs were negative by PCR for *Batrachochytrium dendrobatidis*, *Ranavirus* and *Ranid Herpesvirus 3* (now *Batravirus ranidallo 3*). The proposed etiology is considered to be an undetermined physical event, leading to an abrupt change in the molecular or physical characteristics of the water (namely pressure and oxygen or other gas supersaturation), resulting in the occurrence of the observed lesions in the frogs. No obvious pumping system malfunction was recorded in the Mägisalp ponds before the mass mortality, but a sudden and temporary undetected change in the water flow, which then quickly rebalanced, cannot be excluded. Other hypotheses include weather conditions, such as lightning strikes in the water, or a device detonating in the water.

Key words: Anurans, gas bubble disease, gas supersaturated water, oxygen supersaturated water, wildlife.

INTRODUCTION

Gas bubble disease (GBD) is a recognized noninfectious disease entity (syndrome) occurring in aquatic species when the water is supersaturated with atmospheric gases (Colt et al. 1984a). The maximum volume of gas that can be dissolved in a liter of liquid at a specific pressure and temperature is defined as the gas solubility. The resulting solution is then saturated. A supersaturation of total dissolved gas (TDG) occurs when the volume of dissolved gas exceeds the gas solubility (Lu et al. 2019).

The first description of GBD was in the 19th century in aquarium fish (Weitkamp and Katz 1980). Several studies have since shown that TDG supersaturation was associated with fish mortality (Dawley and Ebel 1975; Weitkamp and Katz 1980; Bentley and Dawley 1981; Kulshrestha and Mandal 1982; Speare 1990; Geist et al. 2013; Machova et al. 2017). Nitrogen and, in smaller amounts, argon are the main gases involved (Colt et al. 1984b). As oxygen is assimilated by the animal's metabolism, it is less likely to cause persistent bubble formation, but it can be dangerous at very high concentrations (Noga 2010).

The main cause of death of the affected animals is anoxia, secondary to blood stasis caused by emboli (Stroud et al. 1975; Weitkamp and Katz 1980; Colt et al. 1984b; Machado et al. 1987). Level of supersaturation greater than 115% may be lethal to fish (Dawley and Ebel 1975; Huang et al. 2010; Geist et al. 2013). Lesion severity depends on the degree of supersaturation, the duration of exposure, and the age and species of the affected animals (Lutz 1993; Roberts 2012; Lu et al. 2019). Thus, supersaturation can also cause chronic pathologies in aquaculture (Colt 1986; Tsai et al. 2017). This syndrome has been reported at all life stages of fish (Roberts 2012), although earlier stages of development are less severely affected than more advanced stages (Geist et al. 2013).

Gas bubble disease remains a common problem in fish aquaculture; it can also affect other invertebrate and vertebrate species such as molluscs (Malouf et al. 1972; Elston 1983; Bisker and Castagna 1987; Ross et al. 2018), crustaceans (Johnson 1976; McDonough and Hemmingsen 1984), and anuran species (Colt et al. 1984a; Mutschmann 2005; Tsai et al. 2017). In amphibians, this disease has been mainly described in captive individuals. The effects of water supersaturation have been experimentally studied in various *Rana* spp. (Egusa 1954). Only scarce information is available for free-ranging amphibians, such as what occurred in Barton Spring salamander (*Eurycea sosorum*) in 2002 in the USA (U.S. Fish and Wildlife Service 2005).

We report a massive acute mortality event with findings consistent with GBD in a free-ranging population of common frog (*Rana temporaria*) in Switzerland.

MATERIAL AND METHODS

Field data

In April 2020, during a field survey, a collaborator of the Department for Nature Promotion (*Abteilung Naturförderung*) of the canton of Bern, Switzerland found about 5,000 dead common frogs floating in a mountain retention pond in Mägisalp, Bern, Switzerland at about 1,700 m above sea level (Fig. 1). The frogs floated on the surface of the water, most on their backs, although some on their bellies, with fore and hind limbs extended. These ponds offer a natural spot for reproduction of the common frog. This species is widespread across Europe and the most common frog species in Switzerland (Lüscher et al. 2016), with distribution extending up to 2,800 m in altitude (Speybroeck et al. 2021).

A pump from a village farther down the valley fills the retention pond automatically (P. Michel pers. comm. 2022). The water comes from the overflow of the Mägisalp drinking water supply. There is no automatic draining; only a vertical pipe in the lake regulates the overflow (P. Michel pers. comm. 2022). A second smaller independent pond not connected to the previous one is located about 100 m from the affected retention pond.

Animals and pathology investigation

Eight dead adult common frogs, two females and six males, were submitted to the Institute of Fish and Wildlife Health (FIWI) at the University of Bern for a full postmortem examination, including macroscopic, histopathologic, and microbiologic investigation. All organs were collected, fixed in 10% buffered formalin, processed, and embedded in paraffin and stained with H&E according to the standard protocols carried out at the Institute of Animal Pathology of the University of Bern, Bern, Switzerland. Additional samples of skin and liver were frozen at –80 C for further analysis, including detection of *Batrachochytrium dendrobatidis* (*Bd*), *Ranavirus*, and *Ranid Herpesvirus 3* (now *Batravirus ranidallo 3*) as previously described (Origgi et al. 2021).

RESULTS

Field data

The day before this mass mortality, no abnormalities had been observed in the pond and its surroundings. In addition to the dead frogs in the pond, overtly unaffected living frogs were observed on the banks of the pond (alive and apparently healthy; they were not subjected to necropsy). Although this pond is also home to the common toad (*Bufo bufo*) and alpine newt (*Ichthyosaura alpestris*) during the summer, neither live nor dead individuals of these species were observed. Their absence is consistent with the fact that their migration had not yet occurred at the time of the event. No abnormalities were recorded in the second smaller independent

FIGURE 1. Retention pond at Mägisalp, Bern, Switzerland. The pipe in the foreground of (B) leads from the pump, which comes from a village located farther down the valley, and which fills the retention pond autonomously.

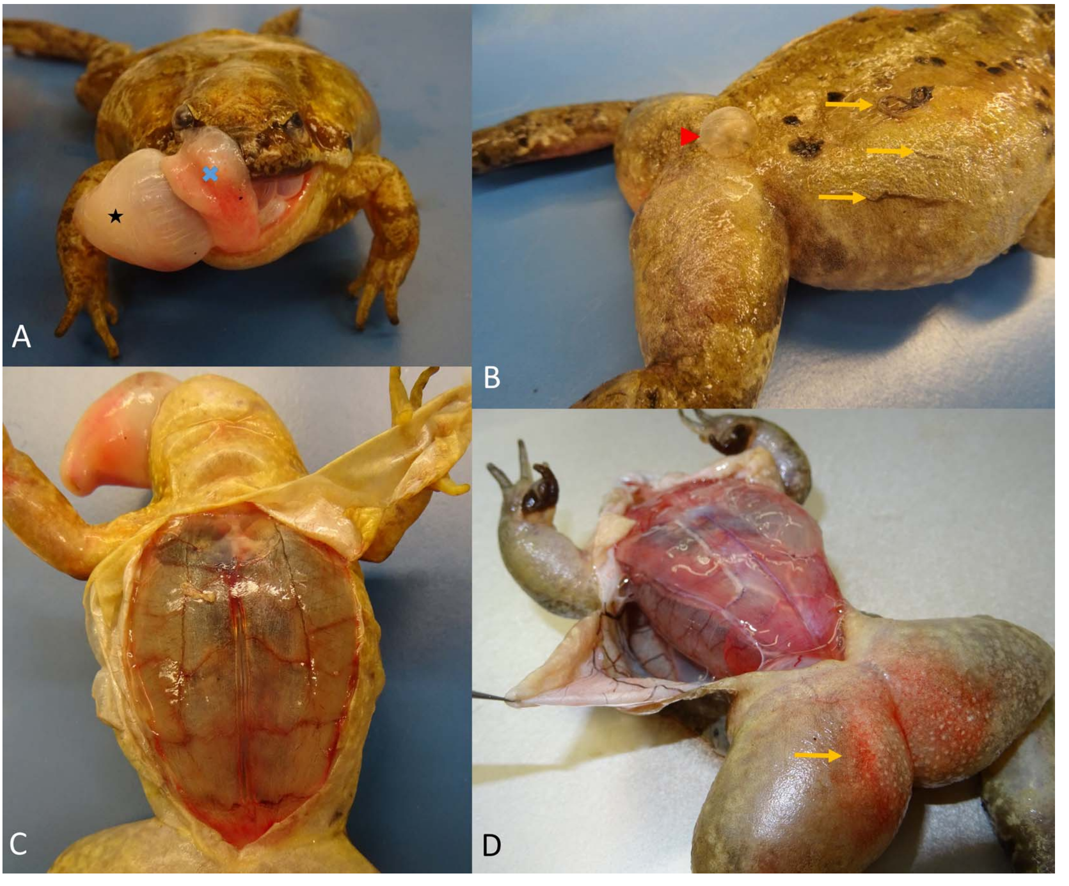

FIGURE 2. Gross lesions seen in free-ranging common frogs (*Rana temporaria*) found dead on the surface of the water in a retention pond at Mägisalp, Bern, Switzerland, April 2020. (A) Protrusion of the tongue (blue cross) and prolapse of the stomach (black star). (B) Multifocal large-scale skin detachment on the flank and the back (orange arrows) and intracutaneous bulla (red arrowhead). (C) Same animal as in (A). Bloating of the coelomic cavity (pneumocoelom). (D) Subcutaneous emphysema and hyperemia on the caudoventral region of the thighs (orange arrow).

pond. On the night preceding the discovery, a severe thunderstorm had occurred in the area, though unfortunately more information about the weather was not recorded.

Gross and microscopic examination

The frogs were necropsied the day after the event, approximately 24 h after death. At this altitude, the temperature is cool overnight (about 5–10 C) and autolysis of tissues was minimal at the time of necropsy.

Males weighed between 36 and 47 g (mean 41.8 g); the two females weighed 38 and 60 g respectively. Macroscopically, all examined frogs were severely and diffusely bloated, with open mouth, either with a distended tongue projected outside (n=6) or with a prolapsed stomach (n=4; Fig. 2) or both. The epidermis was characterized by multifocal large-scale detachment on both flanks of all frogs, and intracutaneous bulla (Fig. 2). Skin hyperemia was common: six individuals showed obvious hyperemia diffusely on the caudoventral region of the thighs (colloquially known as drink patches; Fig. 2), extending along the ventral side of the hind legs (n=5), as well as on the back skin (n=2). Three of eight frogs had transparent, light-red-colored, serosanguineous fluid under the skin in the subcutaneous lymphatic sac. The skeletal muscles

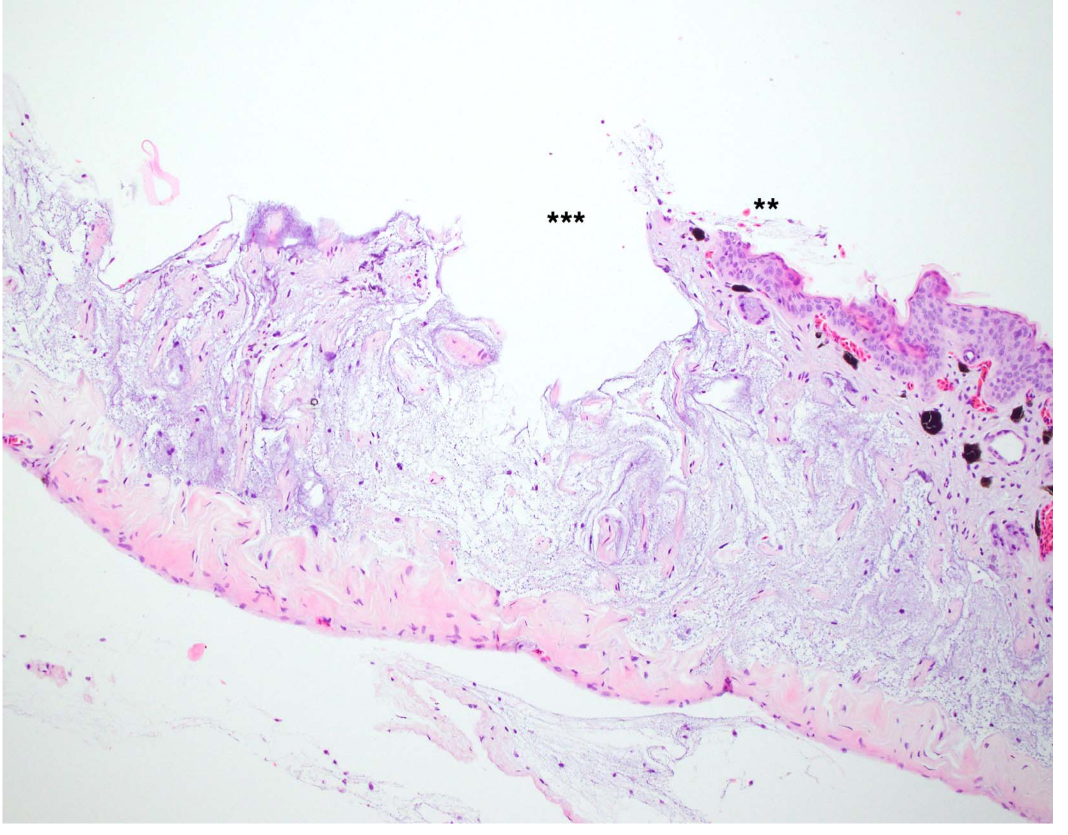

FIGURE 3. Histopathologic lesions seen in free-ranging common frogs (*Rana temporaria*) found dead on the surface of the water in a retention pond at Mägisalp, Bern, Switzerland, April 2020. Locally extensive epidermal ulceration (three asterisks) with hemorrhages (two asterisks). H&E.

forming the ventral part of the coelomic wall (*Musculus cutaneous pectoralis*, *M. rectus abdominus*, *M. pectoralis major*, and *M. external oblique*) were distended and increased in size (Fig. 2). The epimysium was lifted from the underlying muscle bundles. The coelomic cavity of all the frogs was severely distended (pneumocoelom), with the organs displaced cranially and the lung dark-red colored. The two females had few eggs left in the terminal portion of the oviduct.

Histologically, the most severe lesions were mainly observed in the skin and the eyes. However, changes occurred also in almost all examined organs. The areas of skin detachments corresponded to regions of epidermal ulceration in five cases (Figs. 3, 4). Several epithelial cells showed vacuolization of the cytoplasm, and occasionally ruptured (n=3). Hemorrhages were observed within the affected skin areas of five individuals. Diffuse severe emphysema was observed in the dermis and epidermis (n=8; Figs. 5, 6). Eye changes included retinal detachment with mild to moderate tombstoning of the pigmented retinal epithelium (n=8), mild hemorrhages (n=3), and uveal detachment (n=2). In the eight frogs, neither bacteria nor fungi were observed histologically.

Lesions of internal organs (lung, heart, liver, kidney, spleen, pancreas [five of eight available samples], stomach, small and large intestine) were similar, comprising mainly multifocal mild to severe congestion, vascular ectasia, and hemorrhage (Fig. 7). A thrombus

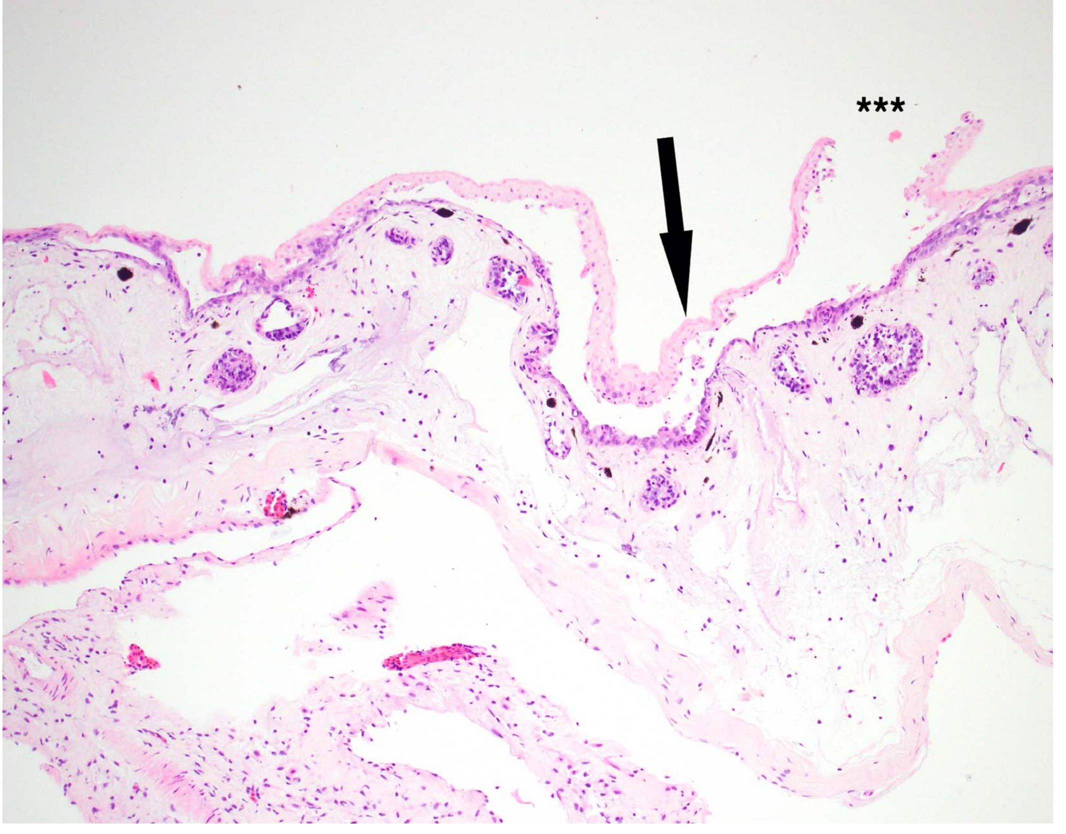

FIGURE 4. Histopathologic lesions seen in free-ranging common frogs (*Rana temporaria*) found dead on the surface of the water in a retention pond at Mägisalp, Bern, Switzerland, April 2020. The epidermal layer is fissured (black arrow) and ruptured (three asterisks). The devitalized hypereosinophilic upper layer is separated by clear space from the lower, still-viable layer. H&E.

was observed in a large blood vessel proximal to the heart of one of eight examined frogs. The frogs with stomach prolapse (n=4) showed an extroflected mucosa and an introflected serosa. Lungworms (consistent with *Rhabdias* sp.) and gastrointestinal nematodes were commonly observed in five and four frogs respectively, without associated inflammation. The brain and the intestine of all eight examined frogs showed no abnormalities.

Molecular investigation

The PCR analyses for the detection of *Bd*, *Ranavirus*, and *Ranid Herpesvirus 3* (now *Batravirus ranidallo 3*) were all negative. No additional ancillary investigation (bacteriologic, viral, or toxicologic) was performed.

DISCUSSION

Macro- and microscopic lesions revealed multisystemic emphysema affecting almost all organs (except brain and intestine). The severity of lesions often varied, depending on the individual and the organ affected. The most severe lesions were observed in the skin, eyes, and blood vessels of the internal organs and were presumably secondary to the massive and sudden distension that the skin and other affected organs underwent. Blood vessels showed serious and potentially life-threatening changes, such as dilation (ectasia) and rupture. It is therefore very likely that hypoxia might have been a significant contributor to the death of these frogs. The observed lesions were consistent with those

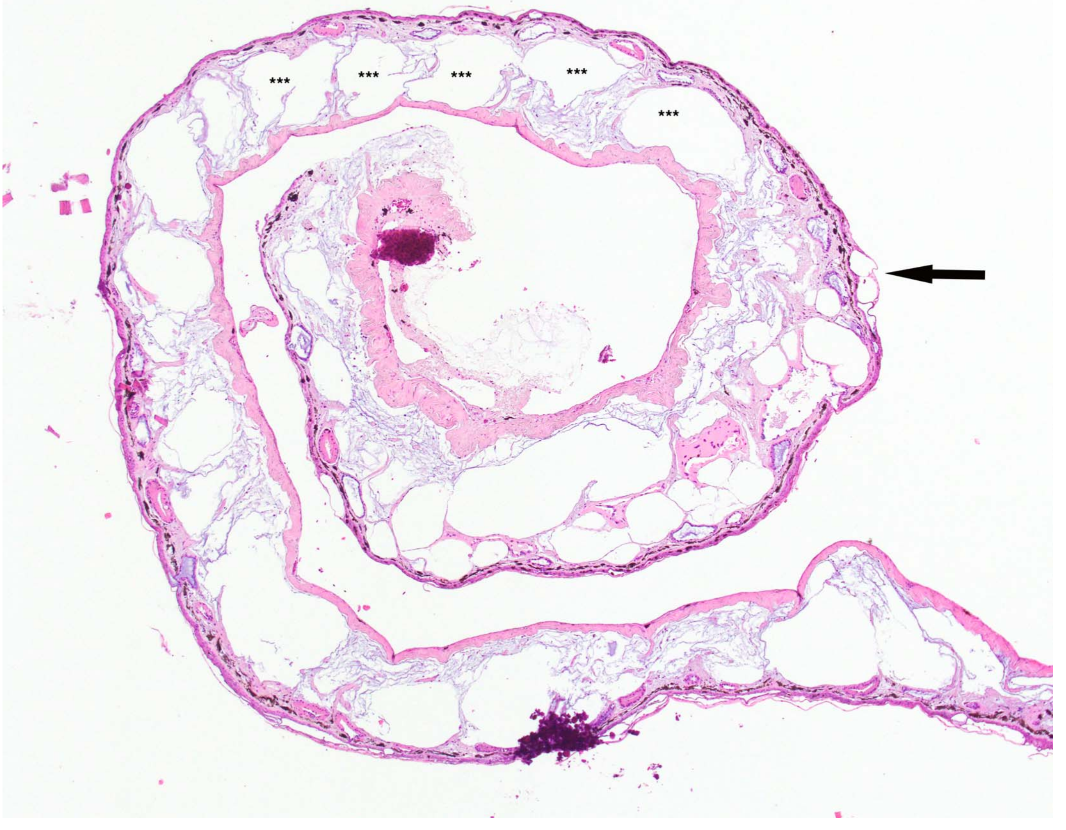

FIGURE 5. Diffusely visible clear spaces (presumptive air collections) in the dermis (three asterisks) and in the epidermis (black arrow) of free-ranging common frogs (*Rana temporaria*) found dead on the surface of the water in a retention pond at Mägisalp, Bern, Switzerland, April 2020. H&E.

described in the literature for GBD in fish and amphibians. However, some common lesions reported in fish, such as exophthalmia (Smith 1988; Bohl 1997) or skin distension by discrete and well-detectable gas bubbles (Machova et al. 2017), were not observed in these frogs. This could be secondary to the anatomy of anurans characterized by a relatively loose connection between the skin and the underlying tissues (Duellman and Trueb 1986), which might favor a solid and diffuse separation of the skin by the infiltrating gas without allowing it to accumulate in discrete regions, different from what would occur in fish (Alibardi 2003).

Although no bacteriologic or virologic tests were performed, no microorganisms and no inflammation were observed microscopically on the histologic sections. We could not find any obvious abnormality in any tissue suggesting an infectious etiology. The only exception was an evident hyperemia of the caudoventral region of the thighs; this might have been caused by distension of the skin and consequent local compression of the numerous blood vessels in that area and consequent passive congestion. Moreover, given the extent and severity of the lesions, it can be argued that deaths may have happened very quickly.

Given that the presence of pre-existing conditions (infectious and degenerative) could be at least histologically ruled out, a physical event is high in the differential among the possible etiologies: in particular, an event that might have led to an abrupt change in the molecular or physical characteristics of the water, resulting in the occurrence of the

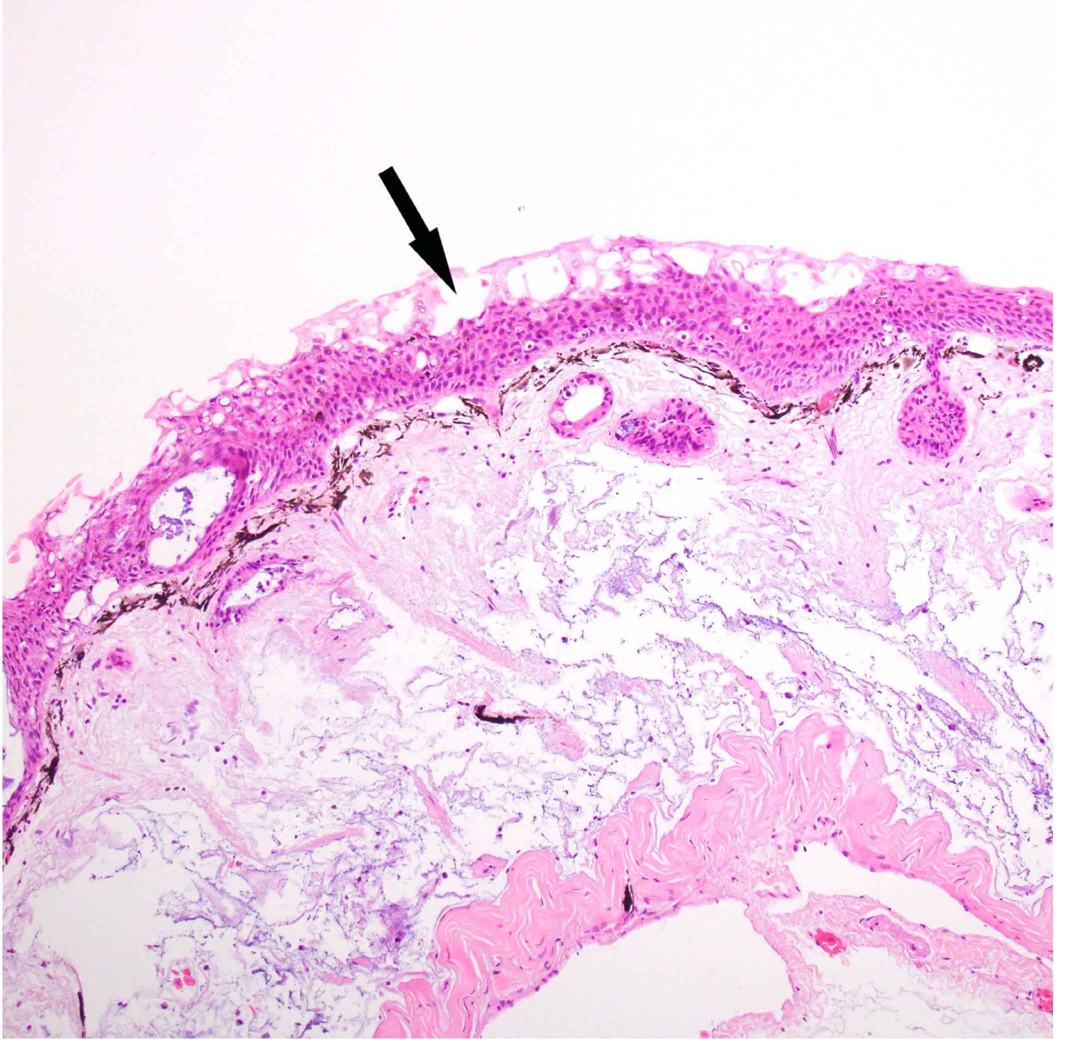

FIGURE 6. Multiple, variably extensive clear spaces (presumptively air collections) are present in the epidermal layer (black arrow) of free-ranging common frogs (*Rana temporaria*) found dead on the surface of the water in a retention pond at Mägisalp, Bern, Switzerland, April 2020. H&E.

observed lesions in the frogs. Five main mechanisms are known to cause supersaturation of water in nature (Colt et al. 1984b; Bossy and Surbeck 2004): 1) bubble formation (e.g., in pumping systems, dams, and waterfalls) followed by a quick increase of the hydrostatic pressure; 2) increase of the water temperature; 3) changes in atmospheric pressure; 4) photosynthesis (production by aquatic organisms of more oxygen than can be diffused in the water; Ignjatovic 1968); and 5) high bacterial activity. Qu et al. (2011) carried out numerous experiments on gas supersaturation related to dams and concluded that the concentration of TDG could also be positively influenced by the pressure of water, aeration, and turbulence intensity. If water is in contact with air, the two media will eventually equilibrate. However, if the change is sudden, organisms in the water may be affected before this balance can be reached. Most fish species may experience, for example, the abrupt increase in TDG concentration downstream of hydraulic structures, such as spillways of dams (Orlins and Gulliver

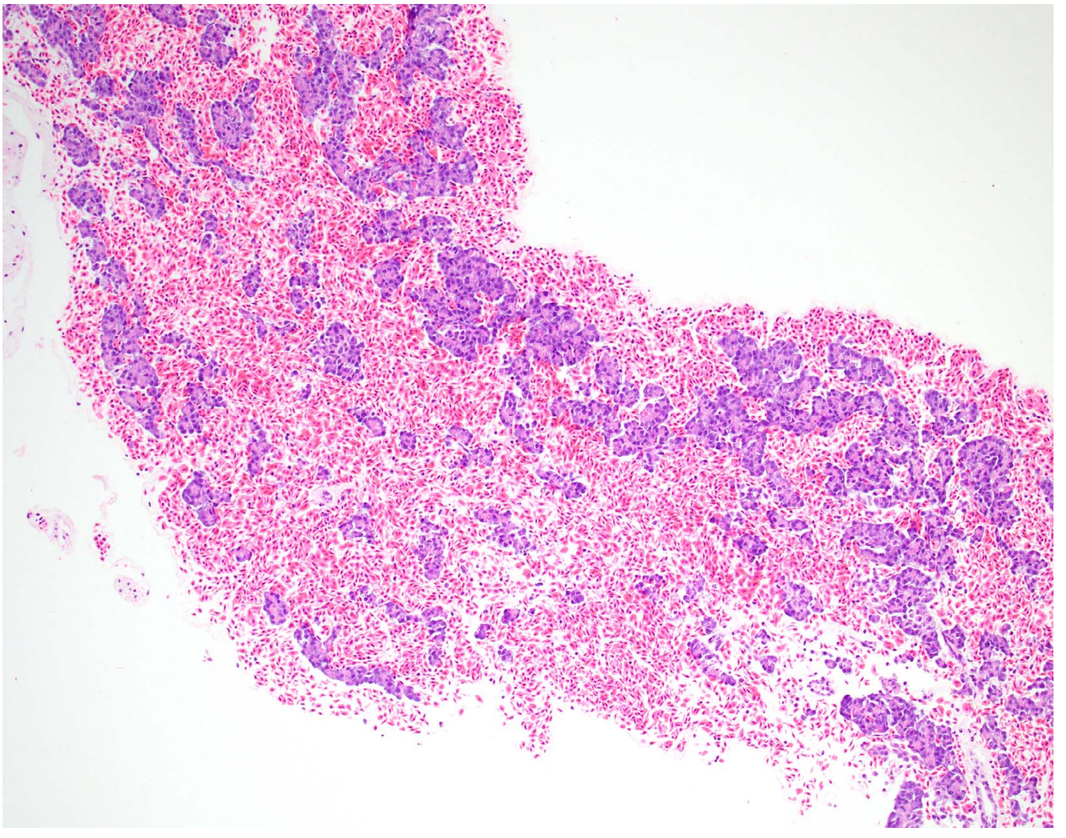

FIGURE 7. Histopathologic lesion seen in free-ranging common frogs (*Rana temporaria*) found dead on the surface of the water in a retention pond at Mägisalp, Bern, Switzerland, April 2020. The exocrine pancreatic parenchyma is diffusely expanded and dissected by a severe hemorrhage. H&E.

2000). Thus, effects of such increases at several hydroelectric dams have been subjected to deeper study on the Columbia and Snake rivers in Washington State because they were suspected of contributing to the mortality of salmonids at different life stages (Orlins and Gulliver 2000). No obvious system malfunction had been recorded at the ponds in Mägisalp before the mass mortality; however, an undetected sudden and temporary change in the water flow, which then might have quickly re-equilibrated, cannot be ruled out. Other hypotheses include weather conditions, including lightning striking the water; however, the presence of a gondola lift nearby and two steel cables of a ski lift that are stretched over the water might have shielded the pond from a lightning strike. No irregularities in the water level were visible (P. Michel, pers. comm. 2022). Finally, even though no suspicious sounds were heard, the pond is in an easily accessible and popular area, and a detonating device (a bomb exploding in water) cannot be totally excluded either. Indeed, such an explosion in the water might also result in multisystemic emphysema. Interestingly, in human medicine, it is described, in the case of an explosion, that the supersonic blast wave compresses gas-filled spaces, which then rapidly re-expand, causing tearing forces and damaging tissue. These blast injuries can result in subcutaneous emphysema (Jorolemon et al. 2022) or systemic air embolism (Madsen 2021). As far as we know, this has never been described for amphibians in the literature.

Although a toxic origin of the die-off could not be totally excluded, the tissue changes are highly indicative more of a strong and hyperacute physical event leading to a sudden gas change, which primed irreversible tissue changes and death. However, the exact cause could not be determined. This event highlights how occurrences traditionally described in captive animals might be relevant also for free-ranging individuals and provide an additional consideration for amphibian mortality events.

ACKNOWLEDGMENTS

We thank the cantonal hunting authorities for their collaboration, R. Wyss for bringing frog carcasses to FIWI, and all the FIWI team for laboratory work and technical assistance. Many thanks also go to P. Michel for providing information on the location and operating system of the pond.

LITERATURE CITED

Alibardi L. 2003. Adaptation to the land: The skin of reptiles in comparison to that of amphibians and endotherm amniotes. *J Exp Zool B Mol Dev Evol* 298: 12–41.

Bentley WW, Dawley EM. 1981. Effects of supersaturated dissolved atmospheric gases on Northern squawfish, *Ptychocheilus oregonensis*. *Northwest Sci* 55:50–61.

Bisker R, Castagna M. 1987. Effect of air-supersaturated seawater on *Argopecten irradians concentricus* (Say) and *Crassostrea virginica* (Gmelin). *J Shellfish Res* 6: 79–83.

Bohl M. 1997. Gasblasenkrankheit der Fische. *Tierärztl Praxis* 25:284–288.

Bossy F, Surbeck H. 2004. La sursaturation gazeuse dans les sources karstiques: Exemples de la source de la Neirivue (Gruyère, Préalpes fribourgeoises): Second Swiss Geoscience Meeting, 19–20 November 2004, Lausanne, Switzerland.

Colt J. 1986. Gas supersaturation—Impact on the design and operation of aquatic systems. *Aquac Eng* 5:49–85.

Colt J, Orwicz K, Brooks D. 1984a. Gas bubble disease in the African clawed frog, *Xenopus laevis*. *J Herpetol* 18:131–137.

Colt J, Orwicz K, Brooks D. 1984b. Effects of gas-supersaturated water on *Rana catesbeiana* tadpoles. *Aquaculture* 38:127–136.

Dawley EM, Ebel WJ. 1975. Effects of various concentrations of dissolved atmospheric gas on juvenile chinook salmon and steelhead trout. *Fish Bull* 73: 787–796.

Duellman WE, Trueb L. 1986. Integumentary, sensory and visceral system. In: *Biology of amphibians*, Duellman WE, Trueb L, editors. MacGraw-Hill, New York, New York, pp. 367–378.

Egusa S. 1954. Morbidity of the frog due to the oversaturated air in the medium water. *Proc Japan Acad (Serie B)* 30:232–235.

Elston R. 1983. Histopathology of oxygen intoxication in the juvenile red abalone, *Haliotis rufescens* Swainson. *J Fish Dis* 6:101–110.

Geist DR, Linley TJ, Cullinan V, Deng Z. 2013. The effects of total dissolved gas on chum salmon fry survival, growth, gas bubble disease, and seawater tolerance. *N Am J Fish Manag* 33:200–215.

Huang X, Li K-F, Du J, Li R. 2010. Effects of gas supersaturation on lethality and avoidance responses in juvenile rock carp (*Procypris rabaudi* Tchang). *J Zhejiang Univ Sci B* 11:806–811.

Ignjatovic LR. 1968. Effect of photosynthesis on oxygen saturation. *J Water Pollut Control Fed* 40(Suppl): R151–R161.

Johnson PT. 1976. Gas-bubble disease in the blue crab, *Callinectes sapidus*. *J Invertebr Pathol* 27:247–253.

Jorolemon MR, Lopez RA, Krywko DM. 2022. Blast injuries. In: StatPearls [Internet]. http://www.ncbi.nlm.nih.gov/books/NBK430914/. Accessed January 2022.

Kulshrestha AK, Mandal PK. 1982. Pathology of gas bubble disease in two air-breathing catfishes (*Clarias batrachus* Linn. and *Heteropneustes fossilis* Bloch.). *Aquaculture* 27:13–17.

Lu J, Li R, Ma Q, Feng J, Xu W, Zhang F, Tian Z. 2019. Model for total dissolved gas supersaturation from plunging jets in high dams. *J Hydraul Eng* 145: 4018082.

Lüscher B, Beer S, Grossenbacher K. 2016. The altitudinal distribution of the common toad (*Bufo bufo*) in the Bernese Oberland (Switzerland) under changing climatic conditions. *Z Feldherpetol* 23:47–58.

Lutz DS. 1993. *Gas supersaturation and gas bubble trauma in fish downstream from a moderately-sized reservoir*. MS Thesis, Iowa State University, Ames, Iowa, 155 pp.

Machado JP, Garling DL Jr, Kevern NR, Trapp AL, Bell TG. 1987. Histopathology and the pathogenesis of embolism (gas bubble disease) in rainbow trout (*Salmo gairdneri*). *Can J Fish Aquat Sci* 44:1985–1994.

Machova J, Faina R, Randak T, Valentova O, Steibach C, Kocour Kroupova H, Svobodova Z. 2017. Fish death caused by gas bubble disease: A case report. *Vet Med (Praha)* 62:231–237.

Madsen J. 2021. *Explosives and blast injuries—injuries; poisoning*. MSD Manual Professional Edition, https://www.msdmanuals.com/professional/injuries-poisoning/mass-casualty-weapons/explosives-and-blast-injuries. Accessed April 2022.

Malouf R, Keck R, Maurer D, Epifanio C. 1972. Occurrence of gas-bubble disease in three species of bivalve molluscs. *J Fish Res Board Can* 29:588–589.

McDonough PM, Hemmingsen EA. 1984. Bubble formation in crustaceans following decompression from hyperbaric gas exposures. *J Appl Physiol Respir Environ Exerc Physiol* 56:513–519.

Mutschmann F. 2005. Amphibien. In: *Krankheiten der Heimtiere*, 6th Ed, Fehr M, editor. Schlütersche, Hannover, Germany, pp. 849–884.

Noga EJ. 2010. *Fish disease: Diagnosis and treatment*. Blackwell Publishing, Ames, Iowa, 544 pp.

Origgi FC, Otten P, Lohmann P, Sattler U, Wahli T, Lavazza A, Gaschen V, Stoffel MH. 2021. Herpesvirus-associated proliferative skin disease in frogs and toads: Proposed pathogenesis. *Vet Pathol* 58:713–729.

Orlins JJ, Gulliver JS. 2000. Dissolved gas supersaturation downstream of a spillway II: Computational model. *J Hydraul Res* 38:151–159.

Qu L, Li R, Li J, Li KF, Wang L. 2011. Experimental study on total dissolved gas supersaturation in water. *Water Sci Eng* 4:396–404.

Roberts RJ, editor. 2012. *Fish pathology*, 4th Ed., Wiley-Blackwell, Chichester, West Sussex, UK, 592 pp.

Ross PM, Pande A, Jones JB, Cope J, Flowers G. 2018. First detection of gas bubble disease and *Rickettsia*-like organisms in *Paphies ventricosa*, a New Zealand surf clam. *J Fish Dis* 41:187–190.

Smith CE. 1988. Communications: Histopathology of gas bubble disease in juvenile rainbow trout. *Prog Fish-C* 50:98–103.

Speare DJ. 1990. Histopathology and ultrastructure of ocular lesions associated with gas bubble disease in salmonids. *J Comp Pathol* 103:421–432.

Speybroeck J, Beukema W, Bok B, van der Voort J, Velikov I. 2021. *Field guide to the amphibians and reptiles of Britain and Europe*. Bloomsbury Wildlife Guides, London, UK.

Stroud RK, Bougr GR, Nebeker AV. 1975. Pathology of acute and chronic exposure of salmonid fishes to supersaturated water. In: *Chemistry and physics of aqueous gas solutions*. Adams WA, Greer G, Desnoyers JE, Atkinson G, Kell GS, Oldham KB, and Walkley J, editors. The Electrochemical Society, Princeton, New Jersey, pp. 435–449.

Tsai JY, Felt SA, Bouley DM, Green SL. 2017. Acute and chronic outcomes of gas-bubble disease in a colony of African clawed frogs (*Xenopus laevis*). *Comp Med* 67: 4–10.

U.S. Fish and Wildlife Service. 2005. Barton Springs salamander (*Eurycea sosorum*): Recovery Plan, https://www.biologicaldiversity.org/species/amphibians/Barton_Springs_salamander/pdfs/Barton-Springs-Salamander-recovery-plan.pdf. Accessed August 2020.

Weitkamp DE, Katz M. 1980. A review of dissolved gas supersaturation literature. *Trans Am Fish Soc* 109: 659–702.

Submitted for publication 29 September 2022.
Accepted 3 March 2023.