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Death by geochemistry: sudden mortality among larvae of Korean Clawed Salamander, *Onychodactylus koreanus* Min et al., 2012, in Hwanseon Cave, South Korea

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Subterranean environments, including caves, typically offer unique habitats with less exposure to climatic fluctuations compared to terrestrial environments (Kováč, 2018; Moldovan et al., 2018). Numerous ecological explanations have been proposed for why certain species use subterranean cave habitats for part, or all of their life cycles (Culver and Pipan, 2019). This includes avoiding extreme terrestrial conditions, escaping predators or competitors, and approaching unique resources. Among vertebrates, amphibians, particularly Caudata, are noted for their use of subterranean caves (Hutchison, 1958; Lanza et al., 1995; Manenti et al., 2017). The Korean Clawed Salamander, *Onychodactylus koreanus* Min et al., 2012, a hynobiid species endemic to the Korean Peninsula, breeds inside the Hwanseon Cave in South Korea, making it the only known accessible breeding site for the species (Park, 2005). This semi-aquatic species inhabits montane valleys and larvae spend 2−3 years in streams before metamorphosing and moving to the streamside. Specifically, they rely on cutaneous respiration throughout juvenile and adult stages due to degenerated lungs (Kuzmin, 1995). *Onychodactylus koreanus* has been predicted to experience substantial habitat decrease due to climate change, which increases the frequency of extreme weather conditions (Lee et al., 2021; Shin et al., 2021). Additionally, ranavirus has recently been reported in wild populations of this species in Korea (Kim et al., 2024), which is a new threat for the species because it is a well-known agent of mass mortality in amphibians (Price et al., 2014; Brunner et al., 2021). Herein, we report an acute mortality event of larval *O. koreanus* in the Hwanseon Cave, and investigate potential causes of this event.

Materials and Methods

Study area. This event occurred in Hwanseon Cave (37.3319°N, 129.0594°E, elevation ca. 500 m; Fig. 1), situated in Samcheok, Gangwon Province, South Korea. Hwanseon Cave is a limestone cave approximately 8500 m in length, with a linear distance of approximately 2000 m from the entrance to the deepest point. The cave portions have been developed for tourism since 1996 (Lee, 2008). Inside the cave, various-sized pools and streams are present, and a constant water flow exits through the channels year-round (Park et al., 2024). Also, many water courses from rock wall cracks flow into the main stream. To date, 75 animal species representing 70 genera, 54 families, 29 orders, 13 classes, and 4 phyla have been identified within the cave (Park et al., 2024). Additionally, it is the only known site where *O. koreanus* has been directly observed breeding underground (Park, 2005).

Field observations. Population surveys of larval *O. koreanus* were conducted at four sites in the cave twice a week, beginning 21 April 2024, to determine its breeding phenology. On 21 January 2025, 16 dead individuals (12.3% of 130 larvae) were found at one site. At other sites, larval mortality was not observed on that day. This site is located 120 m from the cave entrance, where a small amount of cave water outflows from crack walls and several small pools are present (Fig. 2). The water flow finally joins the main stream of the cave. Dead and live larvae were observed in shallow water (< 0.5 cm deep), small pools, or on nearby dried-out rocks (Fig. 2). No littering was detected at or upstream of the site, despite the site lying within 2 m of a tourist pathway. A follow-up survey two weeks later showed no additional mortalities.

Data analysis. Five dead larvae in relatively good

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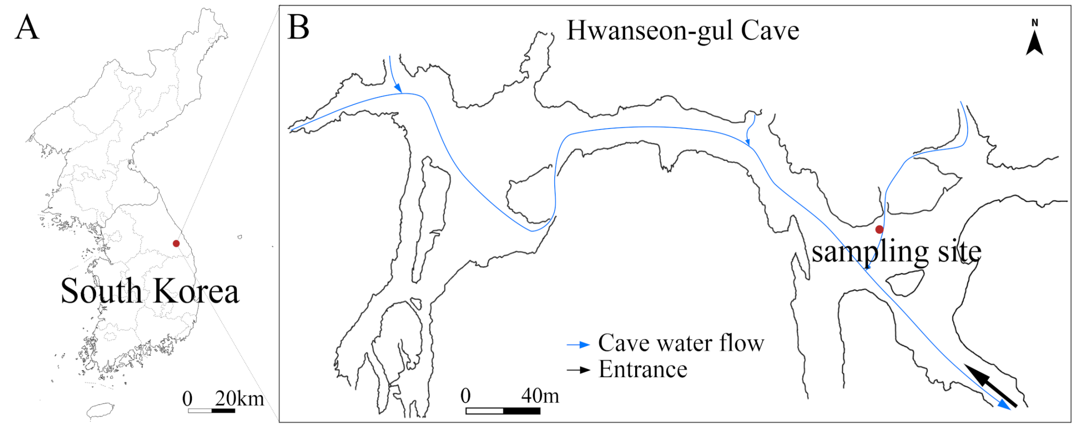

Figure 1. Location of the study site, Hwanseon Cave, Korea (A), and the specific site within the cave where dead larvae were observed (B).

condition and five randomly selected live larvae were collected on the day and analysed for snout-vent length (SVL) and developmental stage to compare the morphological characteristics between dead and live individuals (Fig. 2). The SVL was measured using ImageJ (https://imagej.net/ij/download.html), and the developmental stages were determined according to the 72-stage system for *O. japonicus* (Iwasawa and Kera, 1980).

We examined water quality and presence of diseases to investigate the potential causes of the mortality event. For assessing water quality, two litres of water were separately collected from the site where the mortality occurred and a site within the main stream. Samples were analysed at the Gangwon Institute of Health and Environment for the following parameters: pH, dissolved oxygen (DO), biochemical oxygen demand (BOD), total organic carbon (TOC), electrical conductivity, total nitrogen (T-N), total phosphorus (T-P), suspended solids (SS), and aluminum concentration (Al).

Meteorological data were obtained from the Korea Meteorological Administration (KMA), using records

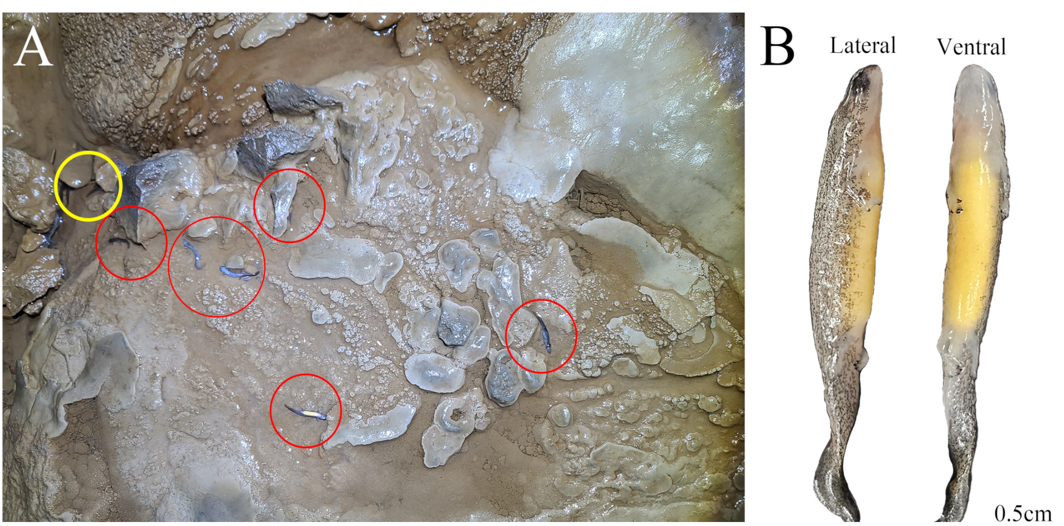

Figure 2. Photographs of the mortality site (A) and a dead individual (B) observed in the Hwanseon Cave, Korea. Red and yellow circles indicate dead and living larvae, respectively. Photos by Jaejin Park.

from the Singi Automatic Weather Station (AWS), situated 5.9 km apart from the study site. We analysed the daily maximum and minimum temperatures, total precipitation, and the number of days with precipitation for the 30 days prior to the mortality event based on a previous study (Park et al., 2024).

Five of the ten collected dead larvae were selected, individually conserved with ice packs, and transported to the laboratory at Kangwon National University to be screened for ranavirus infection. DNA was extracted from tissue samples using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) and stored at -80 °C until quantitative polymerase chain reaction (qPCR) analysis. Ranavirus infection was tested using the RVMCPKim3_F and RVMCPKim3_R primers (Kimble et al., 2015). We did not analyse the possibility of chytrid fungus infection since it does not induce mortality of Korean amphibians (Bataille et al., 2013; Fong et al., 2015; Lee et al., 2025). Each qPCR reaction consisted of 10 µL Power SYBR Green PCR Master Mix (Applied Biosystems, Waltham, MA, USA), 0.5 µL of each primer, and 4 ng of DNA, with the final volume adjusted to 20 µL using molecular biology grade water (Cytiva, Marlborough, MA, USA). Subsequently, qPCR was performed on a QuantStudio 1 thermocycler (Applied Biosystems, Waltham, MA, USA) under the following conditions: 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 62.5 °C for 20 s. Each sample was tested in triplicates, along with a negative (molecular biology grade water) and a positive control (gBlock).

Results

The mean SVL of the examined dead larvae was 1.63 ± 0.05 cm (n = 5), and their developmental stages were either 65 or 66. The living larvae's average SVL was 1.69 ± 0.06 cm (n = 5), with developmental stages ranging from 66 to 67. Water quality analysis at the mortality site showed the following values: pH 8.0, DO 10.9 mg/L, BOD 0.7 mg/L, TOC 0.8 mg/L, conductivity 359 µS/cm, T-N 4.247 mg/L, T-P 0.003 mg/L, SS 0.3 mg/L, and Al 0.07 mg/L. The values at the main stream were as follows: pH 7.9, DO 11.5 mg/L, BOD 0.1 mg/L, TOC 0.4 mg/L, conductivity 279 µS/cm, T-N 3.33 mg/L, T-P 0.008 mg/L, SS 0.2 mg/L, and Al 0.06 mg/L. Meteorological data for the 30 days prior to the mortality event revealed a mean daily maximum temperature of 6.41 ± 3.37 °C and a mean daily minimum temperature of -3.70 ± 2.55 °C at local areas. Total precipitation during the 30 days prior to the mortality event was 0.5 mm, with just one day of localised rainfall. None of the dead larvae samples tested positive for ranavirus infection.

Discussion

We believe the observed mortality of the larval *O. koreanus* reported on here can be assigned to changes in aquatic environmental conditions. Several lines of evidence support such a summary: (1) larvae lacked any clinical and serological evidence for disease, (2) dead larvae were only found at one site out of the four survey sites, (3) the site of the incidence is apparently vulnerable to the geo-chemical effects of reduced water flows, and (4) preceding the event precipitation was at an extreme low in the local area.

Abrupt environmental shifts such as droughts, infectious diseases, or ultraviolet radiation typically trigger mortality events in salamanders (Worthylake and Hovingh, 1989; Blaustein et al., 1995; Rohr et al., 2004; Gray et al., 2015). In this case, water samples collected at the mortality site demonstrated higher BOD, TOC, conductivity, and T-N compared to the main stream. This implied lower water quality at the mortality site than in the main stream. Considering the shallow water flow and small pools, which are formed by waters draining from rock wall cracks, the mortality site might be regularly facing reduced water flows and associated changes in geo-chemical parameters. In another natural habitat of *O. koreanus* larvae, Hong (2017) reported a DO value of 43.2 mg/L (range 40–55 mg/L), which is much higher than that recorded at our study site. Additionally, Park et al. (2024) reported that the mortality site had relatively high chloride, nitrate, and sulphate ion concentrations compared to other locations in Hwanseon Cave. Furthermore, the exceptionally low rainfall during 30 days prior to the event may have further reduced the water level, thereby abruptly increasing the ion concentration. Indeed, a reduction in water quantity can result in a decline in water quality (Mosley, 2015). The cave water in the Hwanseon Cave is mainly derived from the precipitation in the Guinemi village, which is located 500 m higher than the cave (Park et al., 2024). Therefore, low winter rainfall in the area will have resulted in a decrease in the water volume within the cave, probably resulting in worsened geo-chemical conditions of the water and increased local desiccation at the site. These changes may have ultimately become noxious and deadly for early larval stages of *O. koreanus*, leading to acute mortality (Walls et al., 2013). This incident exemplifies how climate change can disrupt microhabitats, even within subterranean cave ecosystems, and may pose a threat to organisms that are otherwise thought to be sheltered from terrestrial fluctuations (Mammola et al., 2019). While de-icing salts, which have been shown to negatively affect congeneric species (Terui et al., 2018), are occasionally used in the cave, we, however, believe their contribution would have been minimal as salts would have been deployed downstream of the mortatlity site.

Starvation or changes in food availability are unlikely causes, given that larvae at this stage mainly rely on yolk for energy (Landberg, 2014). We did not find any differences in SVL or developmental stage between the dead and living larvae. Furthermore, ranavirus was not detected in any of the tested samples. No external symptoms, such as excessive skin shedding and skin erythema or discolouration, were observed (Voyles et al., 2009; Van Rooij et al., 2015; Kim et al., 2024), which is observed with chytrid fungus and ranavirus infection. These results remove the possibility of the mortality by malnutrition or disease infection.

In conclusion, the mortality of *O. koreanus* larvae in the Hwanseon Cave could have been caused by water geo-chemical changes following extreme winter droughts. With the intensification of winter droughts due to climate change (Chang et al., 2024), the frequency of such mortality events may further increase. Thus, long-term monitoring of the *O. koreanus* population in the Hwanseon Cave is crucial to track population trends and mortality events. Such efforts will help identify and mitigate future mortality, thereby strengthening conservation strategies for cave-dwelling salamanders facing climate change.

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References

Bataille, A., Fong, J.J., Cha, M., Wogan, G.O., Baek, H.J., Lee, H., Min, M.S., Waldman, B. (2013): Genetic evidence for a high diversity and wide distribution of endemic strains of the pathogenic chytrid fungus *Batrachochytrium dendrobatidis* in wild Asian amphibians. Molecular ecology **22**(16): 4196–4209.

Blaustein, A.R., Edmond, B., Kiesecker, J.M., Beatty, J.J., Hokit, D.G. (1995): Ambient ultraviolet radiation causes mortality in salamander eggs. Ecological Applications **5**(3): 740–743.

Brunner, J.L., Olson, D.H., Gray, M.J., Miller, D.L., Duffus, A.L. (2021): Global patterns of ranavirus detections. Facets **6**(1): 912–924.

Chang, D.Y., Jeong, S., Park, C.E., Park, H., Shin, J., Bae, Y., Park, H., Park, C.R. (2024): Unprecedented wildfires in Korea: historical evidence of increasing wildfire activity due to climate change. Agricultural and Forest Meteorology **348**: 109920.

Culver, D.C., Pipan, T. (2019): The Biology of Caves and Other Subterranean Habitats. Oxford, UK, Oxford University Press.

Fong, J.J., Cheng, T.L., Bataille, A., Pessier, A.P., Waldman, B., Vredenburg, V.T. (2015): Early 1900s detection of *Batrachochytrium dendrobatidis* in Korean amphibians. PLoS ONE **10**(3): e0115656.

Gray, M.J., Lewis, J.P., Nanjappa, P., Klocke, B., Pasmans, F., Martel, A., et al. (2015): *Batrachochytrium salamandrivorans*: the North American response and a call for action. PLoS Pathogens **11**(12): e1005251.

Hong, N.R. (2017): Habitat Environmental Characteristics of Korean Clawed Salamander (*Onychodactylus koreanus*) at Mt. Baegun in Guangyang, Jeonnam Province. Unpublished MSc Thesis, Seoul National University, Seoul, Korea.

Hutchison, V.H. (1958): The distribution and ecology of the cave salamander, *Eurycea lucifuga*. Ecological Monographs **28**(1): 2–20.

Iwasawa, H., Kera, Y. (1980): Normal stages of development of the Japanese lungless salamander, *Onychodactylus japonicus* (Houttuyn). Japanese Journal of Herpetology **8**: 73–89.

Kim, J., Sung, H.W., Jung, T.S., Park, J., Park, D. (2024): First report of endemic frog virus 3 (FV3)-like ranaviruses in the Korean clawed salamander (*Onychodactylus koreanus*) in Asia. Viruses **16**(5): 675.

Kimble, S.J.A., Karna, A.K., Johnson, A.J., Hoverman, J.T., Williams, R.N. (2015): Mosquitoes as a potential vector of ranavirus transmission in terrestrial turtles. EcoHealth **12**: 334–338.

Kováč, Ľ. (2018): Caves as Oligotrophic Ecosystems. In: Cave Ecology, p. 297–307. Moldovan, O., Kováč, Ľ., Halse, S. Eds., New York, USA, Springer Press.

Kuzmin, S.L. (1995): The Clawed Salamanders of Asia. Magdeburg, Gemany, Westarp Wissenschaften.

Landberg, T. (2014): Embryonic yolk removal affects a suite of larval salamander life history traits. Journal of Experimental Zoology Part B: Molecular and Developmental Evolution **322**(1): 45–53.

Lanza, B., Caputo barucchi, V., Nascetti, G., Bullini, L. (1995): Morphologic and Genetic Studies on the European Plethodontid Salamanders: Taxonomic Inferences (Genus *Hydromantes*). Torino, Italy, Museo Regionale di Scienze Naturali Torino.

Lee, J.E., Park, J.K., Do, Y. (2025): Analysis of reproductive strategies and immunological interactions in *Batrachochytrium dendrobatidis*-resistant Japanese tree frogs. Animals **15**(2): 154.

Lee, S.Y., Choi, S.Y., Bae, Y.S., Suh, J.H., Jang, H.J., Do, M.S. (2021): Distribution prediction of Korean clawed salamander (*Onychodactylus koreanus*) according to the climate change. Korean Journal of Environment and Ecology **35**(5): 480–489.

Lee, Y.H. (2008): Seasonal characteristics of the tourists visiting Hwanseongul (Cave). Journal of the Speleological Society of Korea **89**: 24–26.

Mammola, S., Piano, E., Cardoso, P., Vernon, P., Domínguez-Villar, D., Culver, D.C., Papan, T., Isaia, M. (2019): Climate change going deep: The effects of global climatic alterations on cave ecosystems. Anthropocene Review **6**(1-2): 98–116.

Manenti, R., Lunghi, E., Ficetola, G.F. (2017): Cave exploitation by an usual epigean species: a review on the current knowledge on fire salamander breeding in cave. Biogeographia–The Journal of Integrative Biogeography **32**(1): 31–46.

Moldovan, O., Kováč, Ľ., Halse, S. (2018): Cave Ecology. New York, USA, Springer Press.

Mosley, L.M. (2015): Drought impacts on the water quality of freshwater systems; review and integration. Earth-Science Reviews **140**: 203–214.

Park, D. (2005): The first observation of breeding of the long-tailed clawed salamander, *Onychodactylus fischeri*, in the field. Current Herpetology **24**(1): 7–12.

Park, Y., Kim, L., Lee, J., Jeon, C., Kim, H. (2024): Origin and geochemical characteristics of cave water in Hwanseongul Cave. Journal of Engineering Geology **34**(4): 625–636.

Price, S.J., Garner, T.W., Nichols, R.A., Balloux, F., Ayres, C., de Alba, A.M.C., Bosch, J. (2014): Collapse of amphibian communities due to an introduced ranavirus. Current Biology **24**(21): 2586–2591.

Rohr, J.R., Elskus, A.A., Shepherd, B.S., Crowley, P.H., McCarthy, T.M., Niedzwiecki, J.H., et al. (2004): Multiple stressors and salamanders: Effects of an herbicide, food limitation, and hydroperiod. Ecological Applications **14**(4): 1028–1040.

Shin, Y., Min, M.S., Borzée, A. (2021): Driven to the edge: Species distribution modeling of a clawed salamander (Hynobiidae: *Onychodactylus koreanus*) predicts range shifts and drastic decrease of suitable habitats in response to climate change. Ecology and Evolution **11**(21): 14669–14688.

Terui, S., Ota, H., Ishikawa, H., Goda, T. (2018): Effects of $CaCl_2$ on the hatchability and survival of larvae salamanders (*Hynobius retardatus* and *H. lichenatus*). Japanese Journal of Conservation Ecology **23**(1): 67–73.

Van Rooij, P., Martel, A., Haesebrouck, F., Pasmans, F. (2015): Amphibian chytridiomycosis: a review with focus on fungus-host interactions. Veterinary research **46**: 1–22.

Voyles, J., Young, S., Berger, L., Campbell, C., Voyles, W.F., Dinudom, A., et al. (2009): Pathogenesis of chytridiomycosis, a cause of catastrophic amphibian declines. Science **326**(5952): 582–585.

Walls, S.C., Barichivich, W.J., Brown, M.E. (2013): Drought, deluge and declines: the impact of precipitation extremes on amphibians in a changing climate. Biology **2**(1): 399–418.

Worthylake, K.M., Hovingh, P. (1989): Mass mortality of salamanders (*Ambystoma tigrinum*) by bacteria (Acinetobacter) in an oligotrophic seepage mountain lake. Great Basin Naturalist **49**(3): 364–372.

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