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Correspondence

Conspecific oophagy by an unhatched larva of the Tsushima Salamander, *Hynobius tsuensis* (Caudata: Hynobiidae), within an egg-sac

Keita Niwa

Akita Prefectural Office, Sanno 4-1-1, Akita, Japan

e-mail: tsushimanda48@gmail.com

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Some arboreal frogs provide unfertilized eggs to growing tadpoles as food, e.g., *Kurixalus eiffingeri* (Ueda 1986) and *Oophaga pumilio* (Brust 1993). Conspecific oophagy is known in several Anura taxa, e.g., *Frankixalus* (Biju et al. 2016), *Gracixalus* (Wassersug et al. 1981), *Kurixalus* (Ueda 1986), and *Oophaga* (Brust 1993), whereas in Caudata, conspecific oophagy is known only from three viviparous salamanders in the Salamandridae: *Salamandra salamandra* (Wourms 1981, Joly 1986, Dopazo & Alberch 1994, Greven 1998, Buckley et al. 2007), *Salamandra atra* (Wake 1993, Guex & Greven 1994, Greven 1998, 2003), and *Lyciasalamandra luschani* (as *Mertensiella luschani antalyana*; Özeti 1979). These larvae feed on intra-uterine eggs and one or two fully metamorphosed young are born (Greven 2003, Özeti 1979). Conspecific oophagy by larvae before hatching from the egg-sac has not been reported in oviparous salamanders.

The Tsushima Salamander, *Hynobius tsuensis* Abe, 1922, is endemic to Tsushima Island, Japan, where it breeds in mountain streams in spring (Sato 1943). A female produces a pair of egg-sacs containing 27 to 75 eggs (Sato 1943) in late March to mid-April (Niwa et al. 2021). This salamander has a low fertilization rate in nature (Oyama 1930, Kuramoto 1972) (see Fig. 1). Kuramoto (1972) reported that more than 60% of eggs were unfertilized in five of eight populations examined (i.e., the fertilization rate was below 40% in these five populations), fertilization rates in aquaria were as low as in the field, and that fertilized eggs packed in an egg-sac with unfertilized eggs developed normally. He inferred that low fertility was inherent in *H. tsuensis* and not due to external influences, such as water temperature (Kuramoto 1972). However, the reason for the low fertilization rate in the species is unclear. Based on my field observations, there were no unfertilized eggs within the egg-sacs when the larvae were just about to emerge from them, and the larvae had bulging bellies (Fig. 2). In this study, I report that a hatched larva of *H. tsuensis*, inside the egg-sac, fed on an unfertilized egg.

On 2 April 2021, S. Ochi collected 12 *H. tsuensis* egg-sacs from a stream in Tsutsu, Tsushima Island, Nagasaki Prefecture, Japan (34°07' N, 129°12' E). Only *H. tsuensis* occurs in this area, whereas *Hynobius nebulosus* is absent from it (Niwa et al. 2022). Air and water temperatures in the field were not recorded. The 12 egg-sacs were each kept in separate cylindrical plastic cups (120 mm diameter, 44 mm

Figure 1. An egg-sac of *H. tsuensis* (KUHE 59002), containing seven larvae and 16 unfertilized eggs. Scale bar = 10 mm. This egg-sac was not used in the present study.

height) in a refrigerator (air temperature ca. 6°C, water temperature 8.1°C). On 24 April, 2021, I examined one of the egg-sacs; it contained 11 larvae (limb bud stage; Stages 40–44 as per Iwasawa & Yamashita [1991]) and 25 undeveloped eggs (no cleavage furrows) (Fig. 3). I treated these undeveloped eggs as "unfertilized eggs" following Kuramoto (1972). The egg-sac formed a C-shape with a distinct whiptail structure at the free end (Fig. 3A). The maximum and minimum egg-sac lengths (excluding the whiptail structure), egg-sac width and height at the middle portion, and length of the whiptail structure at the free end were 141, 58, 15, 13, and 13 mm, respectively. One larva was observed feeding on an unfertilized egg within the egg-sac (Fig. 4), Sometimes shaking its head and swimming about with the unfertilized egg in its mouth (Supplementary Videos S1 and S2). During the observation, the larva held the egg in its mouth for at least 1 minute. The air and water temperatures at the time were 20.6 and 13.0°C, respectively.The egg-sac was subsequently fixed in 5% formalin on 29 April 2021, and deposited at the Graduate School of Human and Environmental Studies, Kyoto University, Kyoto, Japan (catalogue No. KUHE 64924). The 11 larvae and 24 unfertilized eggs (one of the 25 unfertilized eggs was broken) it contained were measured using dial callipers. The mean ± SD snout–vent, total, and head lengths, and the head widths of 10 larvae, stages 43–44 of Iwasawa & Yamashita (1991) were 13.1 ± 0.5 (range 12.3–13.8), 23.2 ± 0.6 (22.3–24.2), 5.3 ± 0.2 (5.0–5.8), and 4.4 ± 0.2 (4.0–4.6), respectively. Those of the remaining larva (stage 40) were 9.0, 14.7, 2.9, and 2.5 mm, respectively. All of the 11 larvae had grown a pair of forelimb buds, balancers, and external gills, but were still with no or only rudimentary hindlimb buds (Figs 3B, C). Of these, seven larvae had bulging bellies (Fig. 3C). The unfertilized eggs ranged from 3.4–3.8 mm (3.6 ± 0.1; n = 24) in diameter. Interestingly, all 24 unfertilized eggs were hemi- to three-quarters spherical in shape, not perfectly spherical.

Figure 2. Egg-sac of *Hynobius tsuensis*, containing several larvae that are about to emerge from it. It did not appear to contain unfertilized eggs at the time . This egg-sac was not used in the present study.

This observation suggested conspecific oophagy by unhatched larvae within egg-sacs in *H. tsuensis*, although the observation was made in an aquarium, and is the first such report on oviparous salamanders. The natural fertilization

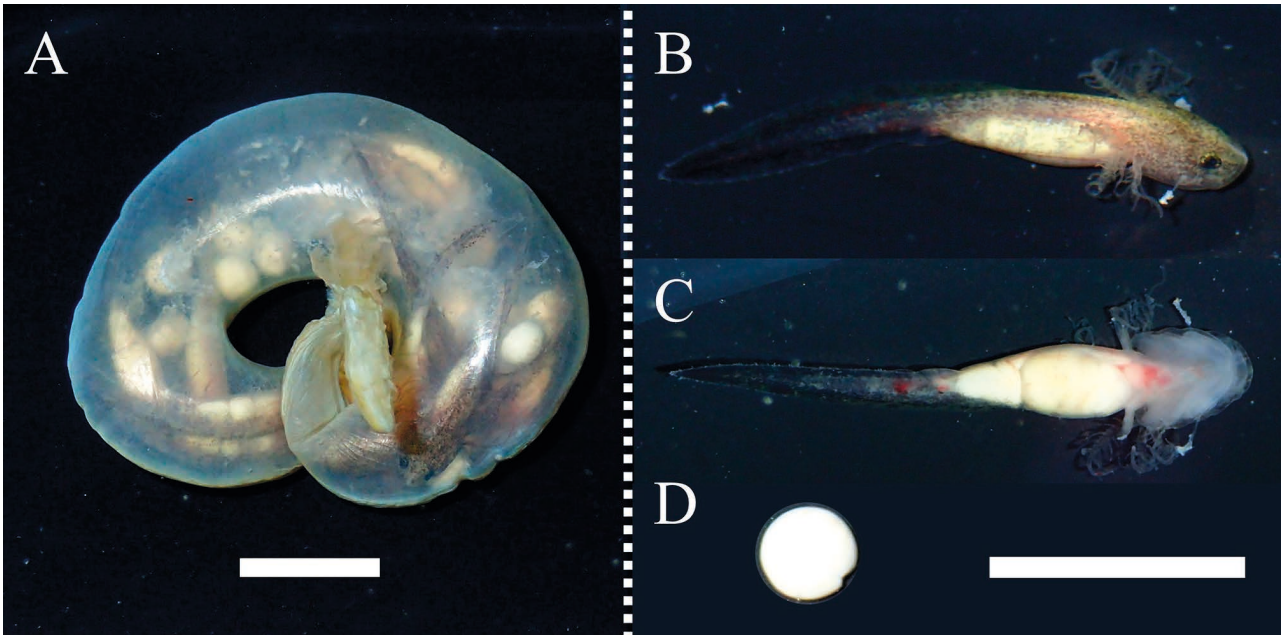

Figure 3. An egg-sac (KUHE 64924) in which a larva fed an unfertilized egg (A). Lateral (B) and ventral (C) views of a larva (limb bud stage; Stage 43), and an unfertilized egg (D) within the egg-sac. Scale bars = 10 mm.

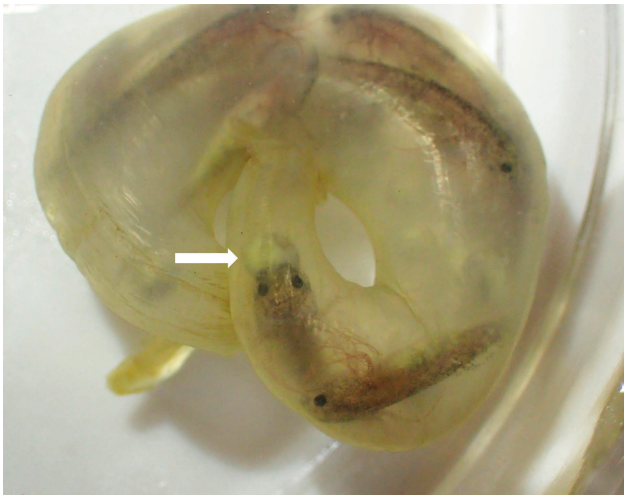

Figure 4. An egg-sac of *H. tsuensis* (KUHE 64924) within which a larva feeds on an unfertilized egg (white arrow).

rate of *H. tsuensis* is much lower than that of other *Hynobius* species, including its relatives *H. nebulosus* from Iki Island (96.7%) and *Hynobius dunni* from Oita (97.9%) (Kuramoto 1972), and seems to have been low since at least 1930 (Oyama 1930, Kuramoto 1972). Considering these facts, unfertilized eggs of *H. tsuensis* may not occur accidentally, and might be a reproductive strategy that enables the larva to feed on unhatched/ unfertilized larvae, even though the mechanisms are as yet unknown. The observation in the present study possibly constitutes a case of maternal care or a new reproductive strategy in oviparous salamanders.

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References:

Biju, S. D., G. Senevirathne, S. Garg, S. Mahony, R. G. Kamei, A. Thomas, Y. Shouche, C. J. Raxworthy, M. Meegaskumbura & I. V. Bocxlaer (2016): *Frankixalus*, a new rhacophorid genus of tree hole breeding frogs with oophagous tadpoles.– PloS ONE, **11**: e0145727.

Brust, D. G. (1993): Maternal brood care by *Dendrobates pumilio*: A frog that feeds its young. – Journal of Herpetology, **27**: 96–98.

Buckley, D., M. Alcobendas, M. García-París & M. H. Wake (2007): Heterochrony, cannibalism, and the evolution of viviparity in *Salamandra salamandra*. – Evolution & Development, **9**: 105–115.

Dopazo, H. & P. Alberch (1994): Preliminary results on optional viviparity and intrauterine siblicide in *Salamandra salamandra* populations from northern Spain. – Mertensiella, **4**: 125–138.

Guex, G. D. & H. Greven (1994): Structural and physiological aspects of viviparity in *Salamandra atra*. – pp. 161–208 in: Greven, H. & B. Thiesmeier (eds): Biology of *Salamandra* and *Mertensiella*. – DGHT, Bonn, Germany.

Greven, H. (1998): Survey of the oviduct of salamandrids with special reference to the viviparous species. – Journal of Experimental Zoology, **282**: 507–525.

Greven, H. (2003): Larviparity and puerparity. – pp. 447–475 in: Sever, D. M. (ed.): Reproductive Biology and Phylogeny of Urodela. – Science Publisher Inc, Enfield.

Iwasawa, H. & K. Yamashita (1991): Normal stages of development of a hynobiid salamander, *Hynobius nigrescens* Stejneger. – Japanese Journal of Herpetology, **14**: 39–62.

Joly, J. (1986): La reproduction de la salamandre terrestre (*Salamandra salamandra* L.). – pp. 471–486 in: Grasse, P. P. & M. Delsol (eds): Traité de Zoologie. Vol. 14, Amphibiens. – Masson, Paris.

Kuramoto, M. (1972): Low natural fertilization rate in *Hynobius tsuensis* Abe (Amphibia: Urodela). – Herpetologica, **28**: 38–41.

Niwa, K., M. Kuro-o & K. Nishikawa (2021): Discovery of two lineage of *Hynobius tsuensis* (Amphibia, Caudata) endemic to Tsushima Island, Japan. – Zoological Science, **38**: 259–266.

Niwa, K., D. V. Tran & K. Nishikawa (2022): Differentiated historical demography and ecological niche forming present distribution and genetic structure in coexisting two salamanders (Amphibia, Urodela, Hynobiidae) in a small island, Japan. – PeerJ, **10**: e13202.

Oyama, J. (1930): *Hynobius tagoi* and *Hynobius tsuensis*. – Bulletin of the Biogeographical Society of Japan, **2**: 20–35.

Özeti, N. (1979): Reproductive biology of the salamander *Mertensiella luschani antalyana*. – Herpetologica, **35**: 193–197.

Sato, I. (1943): A monograph of the tailed batrachians of Japan. – Nippon Shuppan-sha, Osaka.

Ueda, H. (1986): Reproduction of *Chirixalus eiffingeri* (Beettger). – Scientific Report of the Laboratory for Amphibian Biology, Hiroshima University, **8**: 109–116.

Wake, M. H. (1993): Evolution of oviductal gestation in Amphibians. – Journal of Experimental Zoology, **266**: 394–413.

Wassersug, R. J., K. J. Frogner & R. F. Inger (1981): Adaptations for life in tree holes by rhacophorid tadpoles from Thailand. – Journal of Herpetology, **15**: 41–52.

Wourms, J. P. (1981): Viviparity: the maternal-fetal relationship in fishes. – American Zoologist, **21**: 473–515.

Supplementary data

The following data are available online:

Supplementary Video S1. A larva feeding on an unfertilized egg within an egg-sac, sometimes shaking its head.

Supplementary Video S2. A larva feeding on an unfertilized egg and swimming about with the egg in its mouth within the egg-sac.