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Effect of hypoxia on the embryonic and larval development of the Nagaland montane torrent toad *Duttaphrynus chandai* in India

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Oxygen availability is a critical factor in freshwater ecosystems (Maitland, 1978); the concentration of oxygen in water is low relative to air and varies according to biological oxygen demand (BOD), oxygen production by photosynthesis, and water temperature (Dejours, 1981; Ginot & Herve, 1994; Kumaroto, 1975). The oxygen available to amphibian embryos fluctuates widely and is often low enough to affect developmental rate and hatching (Mills & Barnhart, 1999) although relatively few studies have addressed the effects of hypoxia on amphibian embryos (review by Seymour & Bradford, 1995). Kuramoto (1975) suggested that hatched embryos of those frog species that breed in summer or warm waters are smaller and consume less oxygen than those that breed in winter or cool waters. More recently, studies using acute exposures to anoxia (no oxygen) and severe hypoxia (partial lack of oxygen) indicated that, although amphibian embryos are very tolerant of short periods of hypoxia, exposure to acute anoxia inhibits metabolism and can cause death (Weigmann & Altig, 1975; Adolph, 1979; Bradford & Seymour, 1988; Seymour & Roberts, 1991; Seymour et al., 1995). In the case of the salamander *Ambystoma maculatum*, ephemeral ponds with hypoxic conditions had consistent hatching failure and exposure to dissolved oxygen (DO) at < 4.0 mg/L resulted in the death of all embryos before hatching (Sacerdote & King, 2009).

The Nagaland montane torrent toad *Duttaphrynus chandai* is endemic to north-east India described from Nagaland by Das et al. (2013) and was later reported from Mizoram, Bhutan and Manipur (Lalremsanga et al., 2020; Wangyal et al., 2020; Decemson et al., 2021). Herein we report the effects of hypoxia during the embryonic and larval development of *D. chandai*. While studying the breeding and developmental biology of *D. chandai*, we observed breeding sites from three perennial streams surrounding Murlen National Park, Champhai district, Mizoram, namely Tuithing (23° 38’36.39” N, 93° 17’51.70” E; 1597 m a.s.l.), Tuithoh (23° 3915.17” N, 93° 19’02.58” E; 1129 m a.s.l.) and Kelchi (23° 3 9’51.55” N, 93° 16’40.19” E; 1122 m a.s.l.) for 22 days from 24 February 2022 to 17 March 2022. However, recordings were taken from only one stream at Kelchi for a period of 10 days where deposition of eggs had taken place in a stagnant pool. Water quality tests for dissolved oxygen (DO) and pH were made daily between 18:00 h – 20:00 h using an oxygen meter (Lutron DO-5510) and pH meter (Hanna HI 991003). Embryos were monitored in the natural habitat while some larvae along with their natural water were placed in three plastic trays (25 cm × 19 cm × 13 cm) filled with 8 cm depth of water (approx. 4 litres). There were 20 individuals per tray which were observed in the laboratory where the DO level was measured daily. The water temperature fluctuated between 16–18 °C while air temperature was maintained at 16 °C in the laboratory. Water was changed every 24 h with fresh water collected from the microhabitat. The laboratory observations were made under permit no. MZU-IEAC/2018/12 issued by Institutional Animal Ethics Committee, Mizoram University.

The study reveals that *D. chandai* can be found at altitudes greater than 1000 m a.s.l. and breeds in pools with running water (Figs. 1 A & B) at DO concentration of 6.5–7.2 mg/L (mean ± sd, 6.67±0.23, n=10) and pH 6.5–6.9. During the investigation, water temperature in both running and still water of the stream ranged from 11.1–14.3 °C. However, we observed one egg clutch (Fig. 1C) out of more than 15 clutches in an isolated pool with stagnant water (Fig. 1D) having initial DO level of 6.0 mg/L. The DO of the isolated stagnant pool declined from 6.0 mg/L to 2.7 mg/L within 10 days (Fig. 2) while the pH remained relatively stable at 6.6–6.9. We recorded the highest DO level on the fifth day (28 February 2022) (7.2 mg/L) in running waters (Fig. 2) apparently due to a slight rainfall in the area. In the stagnant pool, we observed that embryos at Gosner stage 12 (late gastrula) gradually became pale in colour (Fig. 3B) compared with the normal dark blackish coloration (Fig. 3A); the entire egg clutch died when reaching a DO level of 3 mg/L and below. Within 9–11 days, DO of water in the rearing tray drops to 2.5–3.0 mg/L, larvae at hindlimb bud development (Gosner stage 26) bulged in a star-like shape (Fig. 3D) so that body width was greater by 1.41 mm and tail shorter by 1.76 mm within 2 days compared to normally developing tadpole (Fig. 3C).

This is the first study on the effect of declining DO level on the embryonic development of *Duttaphrynus* toads under natural conditions. It was found that embryos of *D. chandai* are susceptible to minor change in the environmental factors during Gosner stage 10 (dorsal lip) onwards. During the study, the pH of the running water and of the stagnant pool was very similar but the DO levels in the stagnant pool gradually declined from 6 mg/L to 2.7 mg/L within 10 days and the embryos start decaying on the ninth day of monitoring. In addition to this, tadpoles reared in trays tend to show a sign

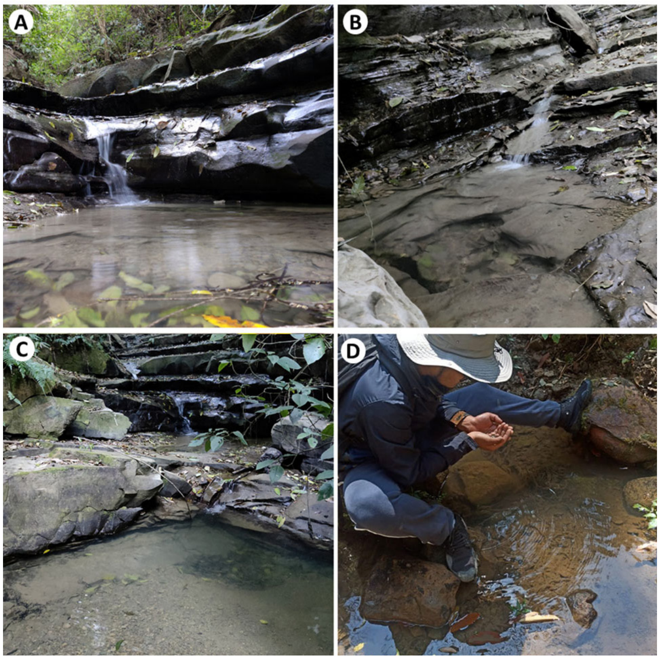

Figure 1. Water bodies in Murlen National Park, Champhai district, Mizoram, India, that support egg clutches and tadpoles of *Duttaphrynus chandai* - **A.** & **B.** Pools with running water, **C.** Egg clutch of *D. chandai* in a stagnant pool (**D.**)

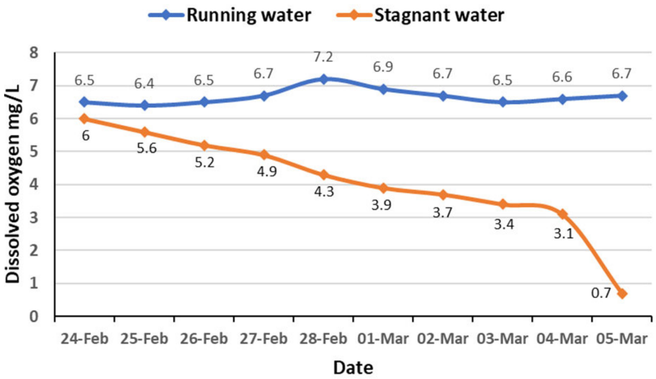

Figure 2. Dissolved oxygen values in running water and a stagnant pool in Murlen National Park, Champhai district, Mizoram

of stress, swimming vigorously, when DO level dropped lower than 3 mg/L, while tadpoles above 3 mg/L remain docile and calm at the bottom of the tray. Kumaroto (1975) showed that DO consumption by embryos of anurans differs species to species and stated that, all else being equal, embryos of species adapted to lower temperatures where higher DO prevails consume more DO as a result of higher metabolic rate. Since our studied species is a winter breeder (February–March), we conclude that these toad embryos and larva need higher concentration of DO for normal development and survival although other toad species such as *D. melanostictus* can thrive in a wider range of DO (4.9–9.6 mg/L) (Shangphliang et al., 2016). This might be one of the reasons why *D. melanostictus* is widely distributed across south and south-east Asia, while the less tolerant sister species, *D. chandai*

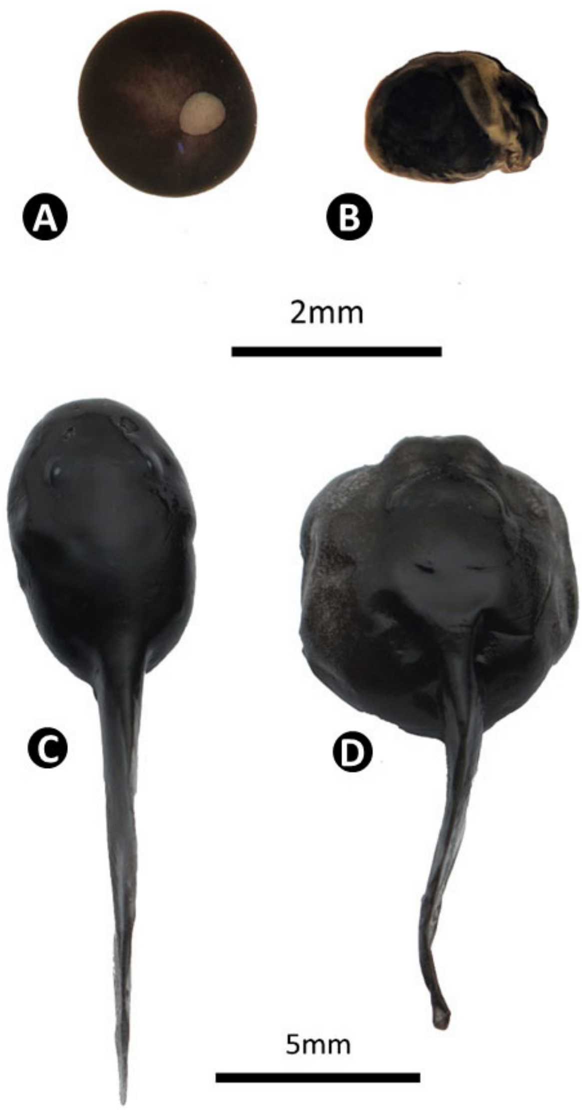

Figure 3. Embryos and tadpoles of *Duttaphrynus chandai* subject to normal and hypoxic conditions - **A.** Normal embryo at Gosner stage 12, **B.** Defected embryo at Gosner stage 12, **C.** Normal larva at Gosner stage 26, and **D.** Abnormal larva with star-like shape body and reduced tail at Gosner stage 26

inhabits limited and selected microhabitats at higher altitude. Should ex situ conservation be required *D. chandai* then it would appear that aeration of eggs and larvae is probably required to rear this species successfully in captivity.

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