



Effect of Habitat Drying on Age and Size at Metamorphosis in Indian Painted Frog, *Uperodon taprobanicus* (Anura: Microhylidae)

Santosh M. Mogali*, Bhagyashri A. Shanbhag & Srinivas K. Saidapur

Department of Zoology, Karnatak University, Dharwad 580 003, Karnataka State, India; santoshmogali@rediffmail.com

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Abstract: Anurans have complex life cycles. The key metamorphic traits in anurans are larval duration and size at metamorphosis. In most of the anurans, these two metamorphic traits are largely influenced by several biotic and abiotic factors but mainly by habitat drying. We studied the influence of habitat drying on larval duration and size at emergence in *Uperodon taprobanicus* under laboratory conditions. Freshly hatched tadpoles (Gosner stage 19) were exposed to decreasing water levels (gradually or rapidly) up to the beginning of metamorphic climax stage (MC, Gosner stage 42; emergence of forelimbs). The control group was reared in constant water levels. Tadpoles reared in decreasing water levels (gradually or rapidly) reached MC and completed larval duration earlier and metamorphosed at a smaller size than those reared in constant water levels. Further, tadpoles experiencing rapid depletion of water reached MC and completed larval duration earlier and also metamorphosed at smaller size than those experiencing gradual depletion in water levels. Thus, tadpoles of *U. taprobanicus* showed adaptive plasticity in metamorphosis to habitat drying. The study shows that *U. taprobanicus* tadpoles are capable of developmental plasticity and exposed to decrease in water levels, the trade-off between growth and development is in favour of development, resulting in early metamorphosis at a smaller size.

Key words: Anurans, desiccation threat, development, growth, metamorphic traits, tadpole

Introduction

A prime goal of life-history theory is to expect patterns in the age and size of organisms at ontogenetic switch points, for example hatching, metamorphosis and reproductive maturity (Wilbur & Collins 1973, Stearns & Koella 1986, Rowe & Ludwig 1991). In anurans, phenotypic plasticity is a ubiquitous characteristic affecting many life-history traits,

especially those related to reproduction and larval development (Miner et al. 2005, Wells 2010, Richter-Boix et al. 2011, Urban et al. 2014). Optimal time of metamorphosis is determined by a trade-off between opportunities for growth and risks of mortality in aquatic and terrestrial habitats (Wilbur & Collins 1973, Werner 1986, Rudolf & Rödel 2007, Mogali et al. 2011a). In most anurans, the time and size at metamorphosis are extremely plastic and

*Corresponding author: santoshmogali@rediffmail.com

both depend on a several factors such as water level (Loman 1999, Mogali et al. 2017, Székely et al. 2017), larval density (Newman 1987, Mogali et al. 2016), predators (Relyea 2007, Mogali et al. 2011b, 2016, Mogali 2018), temperature (Gómez-Mestre & Buchholz 2006, Maciel & Juncá 2009), food availability (Newman 1998, Morey & Reznick 2000, Enriquez-Urzelai et al. 2013) and the complex interaction between them (Grözinger et al. 2014, Mogali et al. 2016).

In ephemeral ponds, desiccation is a key threat and completion of metamorphosis before the ponds dry is obligatory (Newman 1992). Slow growth rates and prolonged larval durations in unpredictable hydroperiods of the water bodies are sure to decrease the chances of tadpoles completing metamorphosis before the water bodies dry (Newman 1992, Altwegg & Reyer 2003, Johansson et al. 2005). On the contrary, a hastened larval development can lower larval mortality, but it is invariably at the cost of growth resulting in a smaller body size at metamorphosis and that may affect their later survival as well as their reproductive success (Reques & Tejedo 1997, Morey & Reznick 2000, Altwegg & Reyer 2003). However, when larval mortality risk increases due to pond desiccation, an early metamorphosis may be favoured despite the costs associated with a smaller size (Newman 1992). Hence, phenotypic plasticity involving the trade-off between certain life history traits (e.g., larval growth, age and size at transformation) is a useful strategy. The original Wilbur and Collins' (1973) model of amphibian metamorphosis predicts that in aquatic systems when conditions are favourable for larval growth, tadpoles should postpone metamorphosis and transform at a larger body size. But when conditions of the temporary ponds become unstable, a strategy to adjust the developmental processes so as to metamorphose early and emerge on land is useful. A developmental strategy of phenotypic plasticity can decrease the exposition to risky conditions and, thereby, enhance the survival rate.

In Southern India, many anuran species, including the Indian painted frog *Uperodon taprobanicus* (Parker 1934; family: Microhylidae), reproduce in ephemeral ponds formed during the south-west monsoon. Such ponds are usually small and often do not last even for a fortnight in the absence of intermittent showers (Mogali et al. 2011a, 2017, 2023a). Thus, tadpoles of *U. taprobanicus* provide an excellent model to study adaptive plasticity in larval development when they are exposed to varying degrees of dropping water levels (desiccation threat). The present study explored the influence of gradual

or rapid water depletion on the two major metamorphic traits, larval period and size at emergence, in a laboratory set up. We hypothesised that those tadpoles facing either low or high desiccation threat would metamorphose earlier and at a smaller size than those developing in constant water levels (no desiccation threat). Second, we hypothesised that those tadpoles facing the rapid depletion of water (high desiccation threat) would metamorphose earliest and at smaller size as compared to those developing in conditions of a gradual decline in water levels (low desiccation threat). Importantly, the experimental design permitted us to exclude the influence of confounding factors such as food scarcity, crowding and predator pressure which generally interfere with the growth and development of tadpoles in nature.

Materials and Methods

Fresh eggs of *Uperodon taprobanicus* belonging to three different parental lines (< Gosner stage 10, Gosner 1960) were collected in the early monsoon season from ephemeral ponds in the Karnatak University Campus (15.440407° N, 74.985246° E, altitude 750 m a.s.l.) Dharwad, Karnataka State, India. Soon after collection they were brought to the laboratory and were placed in a plastic tub (42 cm diameter and 16 cm deep) with 5 dm³ of aged tap water. All eggs hatched (Gosner stage 19, Gosner 1960) almost synchronously on the next day. Tadpoles (Gosner stage 19) were picked randomly and were reared in the plastic tubs (32 cm diameter and 14 cm deep) with 0.5 dm³ – 3 dm³ of aged tap water until the onset of metamorphic climax stage (Gosner stage 42; Gosner 1960). Fifteen such tubs with 10 tadpoles in each were maintained (in total 150 tadpoles, i.e., 50 tadpoles in each group). The experimental groups were as follows (see also methodology of Székely et al. 2010):

Group I. Constant water: Tadpoles were reared in constant water levels (3 dm³).

Group II. Gradual desiccation: Tadpoles were reared in 3 dm³ of water for the first 4 days and then subjected to a 0.5 dm³ decrease in water at 4-day intervals.

Group III. Rapid desiccation: Tadpoles were reared in 3 dm³ of water for a day and from the second day onwards 0.25 dm³ of water was removed each day.

In desiccating water groups when water reached 0.5 dm³ (after day 20 in group II and after day 10 in group III) no further reduction was made. The groups II and III thus simulated low and high

desiccation threat. Tadpoles of all groups (i.e., I, II and III) were fed on boiled spinach ad libitum. Water was changed on alternate days and fresh food was provided. The rearing tubs were placed on a flat surface in a room with a natural photoperiod (12 h light: 12 h dark) and temperature. The positions of tubs were randomised on an alternate day to avoid possible effects of position if any. The water temperature (°C) in tubs was recorded twice, daily at 10:00 h and 15:00 h. Following the onset of metamorphic climax (MC, emergence of forelimbs, Gosner stage 42), the subjects were transferred to small plastic tubs (19 cm diameter and 7 cm deep) covered with fine nylon mesh with a little water and placed inclined thus mimicking the semi-terrestrial environment to facilitate emergence. The days to reach MC stage and larval duration were noted for each individual. After completion of metamorphosis (Gosner stage 46), snout-vent length (SVL in mm; measured using digital calliper, accuracy 0.01 mm) and body mass (in mg; measured using an electronic balance, accuracy 0.001 g; KAB7L, Wensar Company, India) were recorded. Not a single tadpole died during the course of the experiment. After completion of experiments, the froglets were released near natural water bodies. Data on days to reach MC stage, larval duration, SVL, body mass of froglets and water temperature were analysed using a one-way ANOVA followed by the Tukey's post-hoc test. All statistical tests were performed using SPSS ver. 16.0.

Results

The days required to reach metamorphic climax stage ($F_{2,147} = 137.852$, $P < 0.05$), complete larval duration ($F_{2,147} = 160.722$, $P < 0.05$), snout-vent

length ($F_{2,147} = 505.364$, $P < 0.05$) and body mass ($F_{2,147} = 394.791$, $P < 0.05$) differed significantly among different treatment groups (Table 1). Tadpoles which were reared in treatments with declining water levels (i.e., group II and III) reached MC and completed larval duration earlier ($P < 0.05$, Table 1) and also metamorphosed at a smaller size (as quantified through SVL and body mass) ($P < 0.05$, Table 1) than those tadpoles which are reared in conditions with constant water levels (i.e., group I, Table 1). Further, tadpoles experiencing rapid depletion of water (group III) reached MC and completed larval duration earlier ($P < 0.05$) and also metamorphosed at smaller size ($P < 0.05$) than those experiencing gradual depletion in water levels (group II, Table 1).

The daily water temperature of various rearing tubs fluctuated between 22.5-23.0 °C and as such did not differ significantly throughout the course of the experiments (morning: $F_{2,147} = 1.951$, $P = 0.146$; and afternoon hours: $F_{2,147} = 0.662$, $P = 0.517$).

The frequency distribution data showed that all individuals (100%) from the rapid desiccation group (group III) metamorphosed at < 8.00 mm SVL, but none of the individuals subjected to gradual desiccation (group II) or constant water level (group I) metamorphosed at comparable SVL (Figure 1). Further, all individuals (100%) from the rapid desiccation group (group III) and 38% individuals from the gradual desiccation group (group II) metamorphosed at a smaller body mass (< 75 mg) while all of the individuals subjected to constant water levels (group I) metamorphosed at different body masses (Figure 2). The data on days needed to reach MC showed that all individuals (100%) reared in under conditions of rapid desiccation (group III), also 72% individuals from the gradual desiccation

Table 1. Snout-vent length (SVL), body mass of metamorphs, days required to reach metamorphic climax stage (Gosner stage 42) and length of larval duration (days, Gosner stage 46) in *Uperodon taprobanicus* reared in waters with different levels of desiccation. Data represent mean \pm SD (minimum-maximum) and analysed by one-way ANOVA followed by Tukey's' HSD post-hoc test. N = 50 tadpoles for each group (150 tadpoles in total); dissimilar superscripts (a, b, c) indicate significant difference between the groups in the same column; significance level was set to 0.05.

Rearing groups	SVL (mm)	Body mass (mg)	Days required to reach MC	Larval duration (days)
I. Constant water	10.01 \pm 0.49 ^a (9.15-10.90)	121.54 \pm 16.20 ^a (100-149)	26.96 \pm 1.29 ^a (25-31)	29.68 \pm 1.36 ^a (27-33)
II. Gradual desiccation	8.86 \pm 0.35 ^b (8.45-9.98)	80.78 \pm 10.76 ^b (67-113)	24.93 \pm 0.94 ^b (23-27)	27.56 \pm 1.12 ^b (25-30)
III. Rapid desiccation	7.60 \pm 0.26 ^c (7.09-7.99)	57.32 \pm 4.74 ^c (50-65)	23.38 \pm 0.96 ^c (20-25)	25.48 \pm 1.00 ^c (22-27)
F and P values	$F_{2,147} = 505.364$, $P < 0.05$	$F_{2,147} = 394.791$, $P < 0.05$	$F_{2,147} = 137.852$, $P < 0.05$	$F_{2,147} = 160.722$, $P < 0.05$

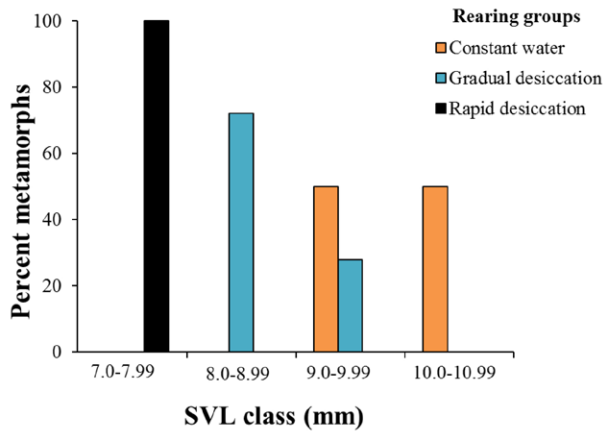


Fig. 1. Percent metamorphs of *Uperodon taprobanicus* per snout-vent length class (mm) in different rearing groups

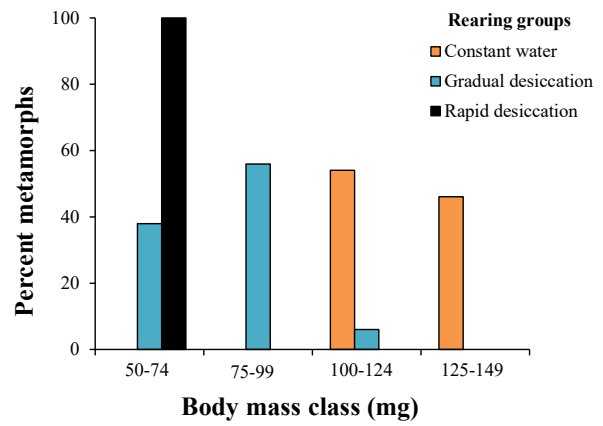


Fig. 2. Percent metamorphs of *Uperodon taprobanicus* per body mass class (mg) in different rearing groups

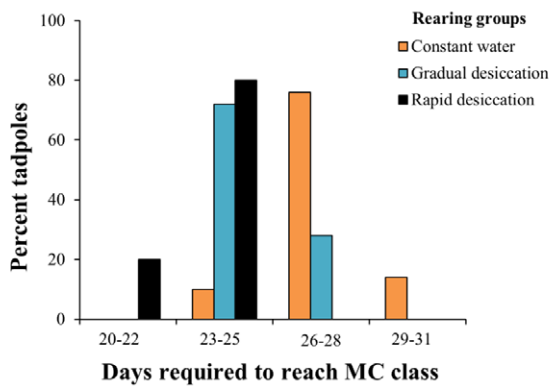


Fig. 3. Percent tadpoles of *Uperodon taprobanicus* per class (days) to reach metamorphic climax (MC, Gosner stage 42) in different rearing groups

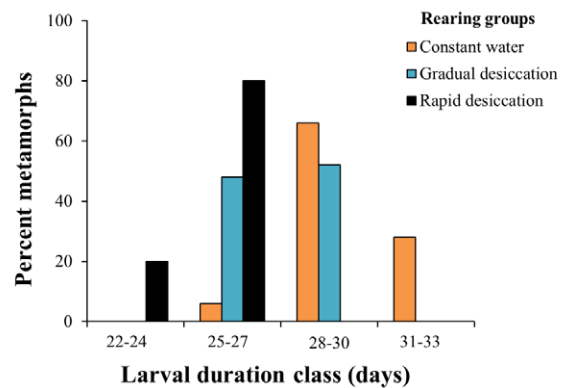


Fig. 4. Percent metamorphs of *Uperodon taprobanicus* per class (days) to complete its larval duration (Gosner stage 46) in different rearing groups

group (group II) but only 10% of the individuals from the constant water-level group (group I) took < 25 days to reach MC (Figure 3). The data on larval duration (days) showed that all individuals (100%) reared under rapid desiccation conditions (group III), also 48% of the individuals from the gradual desiccation group (group II) but only 6% of the individuals from the constant water-level group (group I) took < 27 days to complete larval duration (Figure 4).

Discussion

Our results confirmed our two hypotheses. The laboratory designed study confirms that tadpoles of *U. taprobanicus* show phenotypic plasticity by being able to time their metamorphosis in response to availability of water. Tadpoles raised in constant water levels metamorphosed later and at a larger size

than tadpoles that were raised under conditions of decreasing water levels (gradual or rapid). Tadpoles that were raised in gradual depletion water levels metamorphosed later and at a larger size than those tadpoles from rapid depletion water levels. Hence, tadpoles of *U. taprobanicus* clearly showed adaptive plasticity in metamorphosis to pond drying. Our results are in accordance with Wilbur and Collins' model (1973) for amphibian metamorphosis, which model assumes that tadpoles encountering favourable conditions (e.g. constant water level for this study) postpone metamorphosis, capitalising on the opportunity for additional growth, while tadpoles exposed to hostile conditions (rapid desiccation threat for this study) develop quicker and leave the water body earlier, also supporting earlier studies (Crump 1989, Newman 1989, Denver et al. 1998, Loman 1999, Mogali et al. 2011a, 2017, 2023b Székely et al. 2017).

The mechanisms proposed to explain the acceleration of metamorphosis in anuran tadpoles facing the risk of desiccation differ. Elevated temperature (Newman 1992, Tejedo & Reques 1994) or a decrease in food (Alford & Harris 1988, Newman 1994), mortality of conspecific members has been attributed to lowered growth rate with accelerated developmental rate. Yet, other studies indicated that the temperature has no influence on developmental rate (Loman 1999, Laurila & Kujasalo 1999, Márquez-García et al. 2009, Mogali et al. 2017). In the present study, there was no difference in water temperature (both in the morning and afternoon) of the rearing containers in all treatment groups and food was provided in excess quantity. Also, there was no mortality and, therefore, the death of group members per se could also be ruled out as a factor in speeding up metamorphosis. Hence, the key factor influencing accelerated metamorphosis of tadpoles of *U. taprobanicus* is the desiccation threat. In conclusion, tadpoles of *U. taprobanicus* clearly showed adaptive plasticity in metamorphosis to habitat drying.

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