Receding water levels hasten metamorphosis in the frog, *Sphaerotheca breviceps* (Schneider, 1799): a laboratory study

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Gosner stage 19 tadpoles of *Sphaerotheca breviceps* were exposed to constant or progressively decreasing water levels until metamorphosis with abundant food supply. The tadpoles experiencing constant water levels (column height of 40 mm) reached metamorphic climax (MC) in 35.07 ± 0.44 days and metamorphosed at 39.00 ± 0.43 days at a mean body mass of 409 ± 9.0 mg and 14.64 ± 0.08 mm snout-vent length. In contrast, the larvae experiencing decreasing water levels (from 40 to 12 mm column height) reached MC in 30.93 ± 0.35 days and metamorphosed at 34.73 ± 0.35 days at a significantly smaller body mass and size compared to those reared in constant water levels. In both the treatments survival of tadpoles was 100%. The study reveals that *S. breviceps* tadpoles are capable of developmental plasticity and with progressive decrease in water levels, the trade-off between growth and development is in favour of development, resulting in early metamorphosis at a small size.

Keywords: Metamorphosis, receding water levels, *Sphaerotheca breviceps*, tadpoles.

AMPHIBIANS generally have a complex life cycle that involves an aquatic larval stage. Further, the larval stage is critical as the larvae have to complete development and attain a threshold size before metamorphosis and emergence on land. The important metamorphic traits in anurans are larval period and size at metamorphosis[1,2]. The aquatic stage is designed to exploit the aquatic medium for growth and therefore larval duration has an impact on the size at metamorphosis. The size at metamorphosis, therefore, depends upon the developmental and growth rates during the larval stage. Most anuran amphibians opportunistically breed in temporary water bodies and face many challenges such as crowding, competition for food and space (resources), predator pressure (generally aquatic insects and carnivorous tadpoles of conspecifics and heterospecifics, etc.) and importantly, pond desiccation. All of these necessitate evolution of appropriate strategies for successful completion of metamorphosis and emergence on land[3]. Indeed, several empirical studies on anurans have shown that factors such as kinship and density[4–8], predator pressure[4,9–11], tempera-

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ture variation[12–17], resource availability and pond desiccation[10,12,15,18–21] affect the metamorphic traits.

In and around Dharwad, several species of anurans, including *Sphaerotheca breviceps* breed in rain-filled puddles formed after pre-monsoon showers. Such water bodies are shallow and often do not last even for a fortnight in the absence of intermittent showers. Therefore, *S. breviceps* tadpoles provide a good model to study adaptive plasticity in larval development when they are exposed to receding water levels. In nature, in addition to hydroperiod, several other factors outlined above also influence developmental rate in tandem and thus each factor may contribute to phenotypic plasticity exhibited by individuals in a population. Our earlier study has shown that *S. breviceps* tadpoles reared in crowded condition in a laboratory set-up metamorphose later at a smaller size than those reared in lesser density[7]. The present study was designed to determine the influence of gradual depletion of water (a factor operating in water bodies experiencing desiccation) in a laboratory set-up where these tadpoles are not influenced by confounding factors such as predator pressure, food scarcity or temperature variation.

Eggs from four clutches of *S. breviceps* were collected from rain-filled puddles on the Karnatak University Campus in July 2010 and placed separately in glass aquaria ($75 \times 45 \times 15$ cm) containing 10 l aged (dechlorinated) tap water. All the eggs hatched almost synchronously at Gosner stage 19 on the next day[22]. Immediately after hatching they were mixed and used for experiment. The experiment consisted of two groups. Two hundred tadpoles were arbitrarily picked up from the mixed stock. Then, 20 tadpoles each were stocked per plastic tub (16 cm height; dia 38 cm at the top and tapering to 26 cm at the bottom) with five replicates per group. The tadpoles of the first group were reared in a constant volume of 3 l water. Tadpoles of the second group were reared in 3 l water for the first 6 days and then subjected to progressive decrease in water; 0.5 l water was reduced at 6-day intervals. The water column thus decreased progressively from the initial 40 mm to a final height of 12 mm on day 30 of the experiment. The water level was then maintained at 12 mm height in this group until the tadpoles reached metamorphic climax (MC, Gosner stage 42). A minimum of 12 mm water column was necessary to avoid tadpole desiccation and mortality.

The tadpoles from their feeding stage were provided boiled spinach as food *ad libitum*. The food was always in excess. Water was changed on alternate days and fresh food was added to the water. The positions of rearing tubs were randomized on alternate days to avoid the effect of position, if any. When the tadpoles reached MC, they were transferred to small plastic tubs placed inclined with little water to provide semi-terrestrial area and facilitate metamorphosis. The dates of their reaching MC were recorded. At metamorphosis snout-vent length (SVL), body mass and larval duration (from the date of collection of the eggs till metamorphosis; Gosner stage 46) were recorded. Data on SVL, body mass, days required to reach MC and larval duration of the two groups were analysed by Mann–Whitney *U* test. Data for each parameter were organized into frequency distribution tables to know the percentage of individuals falling within a particular dataset.

The tadpoles of *S. breviceps* ($n = 100$) reared in constant water levels metamorphosed in 39.0 ± 0.4 days and attained a body mass of 409 ± 9.0 mg and their SVL measured 14.6 ± 0.1 mm. In contrast, the tadpoles ($n = 100$) experiencing receding water levels reached MC in 30.9 ± 0.4 days (Figure 1 *a*) and completed their larval period in 34.7 ± 0.4 days (Figure 1 *b*) at a significantly smaller body mass (253 ± 5.4 mg; Figure 1 *c*) and size (12.8 ± 0.1 mm; Figure 1 *d*) compared to those reared in constant volume of water. However, tadpole survival was 100% in both the treatments. The frequency distribution analysis showed that 76% of individuals reared in constant water metamorphosed at >14.0 mm SVL, whereas only 5% of the individuals subjected to decreasing water levels metamorphosed at a similar size. In contrast, in receding water level treatment, 85% of individuals metamorphosed at smaller body mass (< 250 mg) and only 12% of those reared in constant water level group metamorphosed at comparable mass. The data on MC showed that 99% of individuals in decreasing water level treatment took <36 days to reach MC, whereas only 57% of individuals reared in constant water levels attained MC by the same time. Further, 89% of the individuals from reducing water level treatment and 48% of the individuals from the constant water volume treatment took <39 day for metamorphosis.

Studies on the response of anuran tadpoles to pond desiccation reveal diversity in the adaptive plasticity in the metamorphic traits depending upon the species. For example, in anticipation of pond drying, species like *Pleurodema diplolister*, *Rhinella granulosa*[15], *Scaphiopus hammondii*[18], *Pelodytes punctatus*[20], *Rhinella spinulosa*[21] and *Rana sylvatica*[23] metamorphose earlier and at a small body size. Whereas, under similar ecological conditions, tadpoles of *Bufo bufo*, *Rana temporaria* and *Bufo calamita* metamorphose at a smaller size, but the timing of metamorphosis is unaffected[14]. Loman[12] reported that *R. temporaria* tadpoles accelerate their development in response to habitat desiccation, reaching metamorphosis at an earlier age and at a similar size compared to those exposed to constant water. In desert ponds, desiccation is the major cause of mortality; in low-density ponds, tadpoles of *Scaphiopus couchii* develop quickly and metamorphose, whereas in high-density ponds under desiccation they rarely complete their larval development[4]. Thus, depending upon the species, we come across an early or late metamorphosis accompanied by normal, small or large body mass at emergence. When the larval

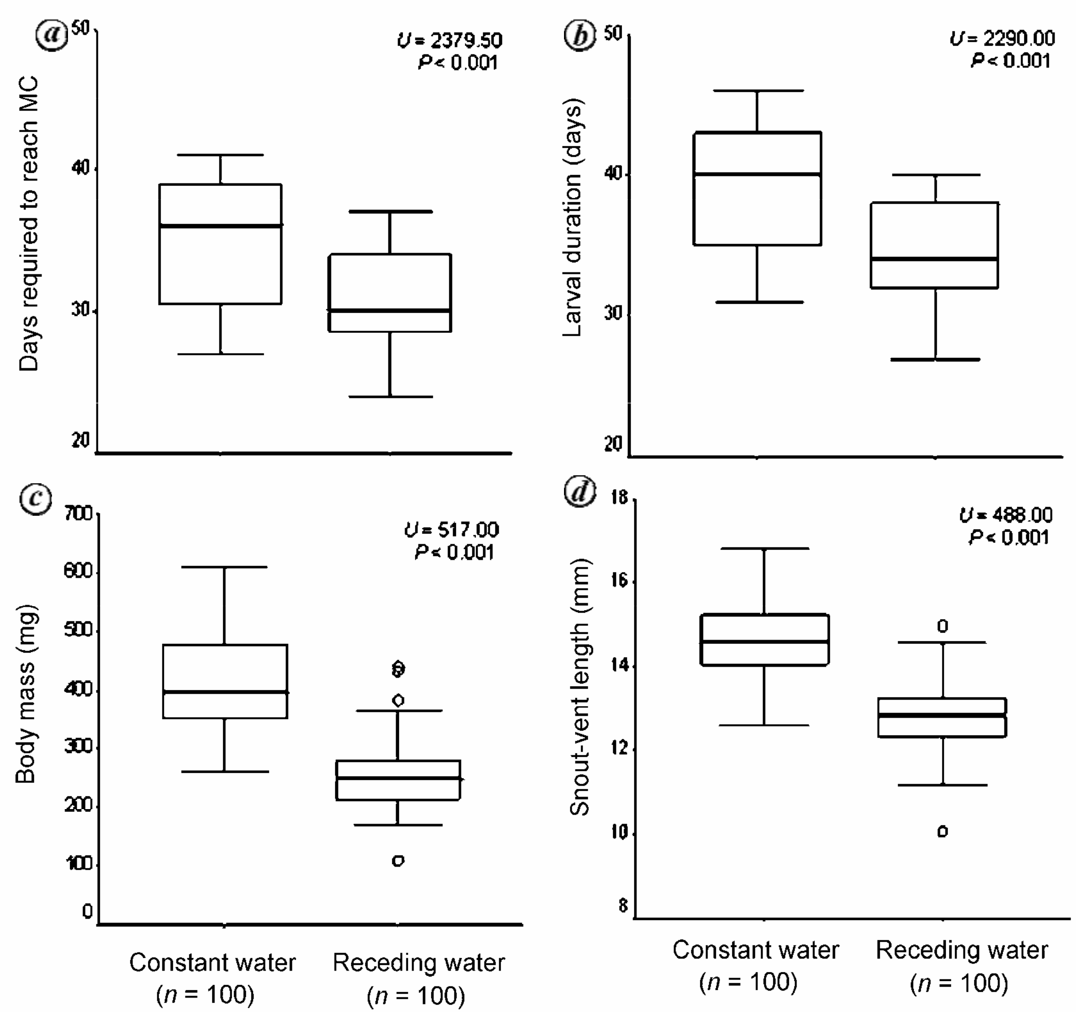

Figure 1. Box whisker plots depicting (***a***) the days required to reach metamorphic climax (MC); (***b***) larval duration, (***c***) body mass and (***d***) snout-vent length of *Sphaerotheca breviceps* whose tadpoles were reared in constant or receding water levels. Boxes represent interquartile ranges. Horizontal bars in the boxes represent medians; whiskers represent farthest points that are not outliers. Open circles represent outliers.

period is short, the size at metamorphosis is usually smaller and a large size at metamorphosis is associated with late metamorphosis[1,24], with a few exceptions. A classic example is that of tadpoles of *R. temporaria*, which when reared in low food level and decreasing water prolonged their larval period, but still metamorphosed at a small size[10]. Therefore, in the case of imminent pond desiccation, trade-off between the two important metamorphic traits, the larval period and size at metamorphosis, may depend upon the species and also other ecological conditions.

In the present study, *S. breviceps* tadpoles reared in progressively receding water levels completed development early at the cost of growth, i.e. 99% of the individuals reached MC in less than 36 days in contrast to only 57% in the constant water group. As a matter of fact, as the water level recedes, these tadpoles also face crowded condition when compared to the tadpoles reared in constant water levels. Yet, they metamorphose early in contrast to our earlier study, where *S. breviceps* tadpoles reared in higher density but constant water levels delayed metamorphosis[7].

The present study thus shows that *S. breviceps* tadpoles perceive receding water levels, and in anticipation of pond drying and to avoid imminent mortality due to desiccation, metamorphose early by accelerating advancement of development, and decelerating their growth to emerge at a small size. As the present study was carried out indoors at ambient temperature, it is unlikely that the temperature was the confounding factor influencing metamorphic traits of *S. breviceps*. Likewise, we rule out the possibility of food as a factor in inducing phenotypic plasticity in metamorphic traits, as it was always in excess in both the groups. There was no mortality and therefore the death of group member per se is also ruled out as a factor in hastening metamorphosis. In conclusion, in the present experimental set-up, receding water levels hasten metamorphosis at the cost of body size in *S. breviceps*.

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