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Catch-up growth during juvenile life can compensate for the small metamorphic size in *Euphlyctis cyanophlyctis*

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Metamorphic size in amphibians with a biphasic mode of life is known to affect adult fitness. To test whether this is true of tropical anurans, influence of metamorphic size on future survival, growth rate, age and size at sexual maturation were studied in a continuously breeding tropical frog, *Euphlyctis cyanophlyctis*. In nature, intra-population size at metamorphosis (when the tail completely disappears; Gosner stage 46) varies significantly (22–30 mm in snout-vent length (SVL)). Analysis of growth rate and determination of age at first reproduction in a group of frogs reared in the outdoor enclosures revealed that rate of increase in SVL differed between small and large frogs only initially but comparable subsequently. Rate of increase in body mass on the other hand, was comparable between the two groups from the first month onwards until the termination of the experiment. Mortality was also comparable among the two groups. There was no difference in the size and age of individuals at first reproduction. In females, the fecundity (number of eggs laid at first reproduction) was comparable between the smaller and larger group frogs. The present study revealed no significant influence of metamorphic size on adult traits in the tropical year-round breeding frog, *E. cyanophlyctis* and that the juveniles compensate for their smaller size at metamorphosis during their terrestrial life.

Keywords: Anura, *Euphlyctis cyanophlyctis*, post-metamorphic growth, sexual maturity.

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IN amphibians with a complex life cycle, the aquatic larval stage is an adaptation for exploiting rich resources of temporary water bodies that are generally devoid of permanent predators including the fish and ensure rapid growth before embarking on to the terrestrial mode of life[1–3]. Rapid growth as larvae is believed to result in a larger size at metamorphosis which, in turn, might influence post-metamorphic survival, foraging efficiency, growth rate and adult fecundity in many anuran amphibians[4–10]. Also, large size at metamorphosis may result in large size at first reproduction in addition to aiding early attainment of sexual maturity[6]. It is well known that fecundity and female body size are correlated in anurans[11,12]. Theoretically, optimal size at metamorphosis should maximize fitness. However, inter- and intra-population variations in the larval period and size at metamorphosis are widespread in anurans due to various factors including phenotypic plasticity and prevailing ecological conditions[1,3,13]. For instance, factors such as temperature, larval density, resource availability, pond duration, kinship environment and predator pressure are known to affect larval period and size of metamorphs in anurans[14–16].

Although both field and laboratory studies have shown the variation in larval period, growth, development and size at metamorphosis for anurans[4,13,17,18], the adaptive significance of this variation in the metamorphic traits is demonstrated only in a few temperate anurans[5,19]. Moreover, it is not clear whether metamorphic size directly affects the post-metamorphic growth, juvenile mortality and timing of sexual maturation or indirectly through altered efficiency in foraging, survival and predator avoidance. What influence does the size of metamorphs have on the age and size at sexual maturity is not known especially for continuously breeding tropical anurans. Hence, the present study addresses the question whether size at metamorphosis in the Indian skipper frog, *Euphlyctis cyanophlyctis* affects juvenile survival, post-metamorphic growth and, size and age at maturity when the food is abundant and predator pressure is absent.

E. cyanophlyctis is a medium-sized multi-clutched aquatic frog distributed throughout India. It inhabits temporary as well as the permanent water bodies and breeds throughout the year. The tadpoles complete their metamorphosis within 45–60 days post-fertilization, and inter- and intra-clutch variations in growth and development of larvae leading to variations in the metamorphic size are common[20]. The average size of metamorphs is 25 mm ± 1 in nature. However, metamorphs as small as 22 mm snout-vent length (SVL) weighing 0.88 g and as large as 30 mm SVL weighing 2.35 g are observed in different natural populations (pers. obs.).

One hundred tadpoles of *E. cyanophlyctis* at Gosner stage 43–44 (angle of the mouth is increasing while length of the tail is decreasing)[21] were collected from a pond in the vicinity of Karnatak University, Dharwad, Karnataka (15°17′N and 75°3′E) in the first week of July and transported to the laboratory. They were maintained in a large cement cistern (300 × 250 × 60 cm) until metamorphosis. The tadpoles metamorphosed within three days of their collection. The body parameters like SVL and body mass were recorded at metamorphosis when the tail is completely lost (stage 46). SVL was recorded using a plastic ruler (accuracy ± 1 mm). Measurements were taken in triplicate to reduce measurement error. Body mass was recorded using an electronic balance (±1 mg accuracy). After metamorphosis (Gosner stage 46; the tail is completely lost), the frogs were reared in outdoor enclosures (300 × 100 × 100 cm) each housing 20 frogs. The assignment of frogs to each terrarium was random. The enclosures situated in the open were exposed to natural photoperiod and temperature. Each terrarium was provided with sand mixed soil, small herbs, grass and a small pool of water on one side (100 × 100 × 10 cm). The metamorphs were marked by a unique combination of toe-clipping but first fingers were kept intact. No more than two toes of any frog were clipped. The wounds were cleaned with dettol and antiseptic powder was applied. Toe-clipping did not affect any of the activities of the frogs. The froglets were fed a variety of food items such as small insects and their larvae, grasshoppers, small guppies *ad-libitum* daily between 1600 and 1700 h. Sexing was done in retrospect when the frogs matured. Development of secondary sexual characters, the vocal sacs and nuptial excrescences indicated sexual maturity of males. The remaining frogs were considered as females. Spawning confirmed maturity of the female frogs. One female spawned spontaneously based on which others were induced to spawn by injecting (ip) progesterone (2 mg/ml). Number of eggs spawned was considered as the fecundity.

All the metamorphs were reared in groups randomly without taking into account their initial size at metamorphosis. For the analysis of growth, the metamorphs were arbitrarily divided into two groups depending on their size. The metamorphs measuring <25 mm SVL on an average were considered as 'small', and those measuring >25 mm SVL on an average as 'large'. SVL and body mass were recorded for individual frogs at monthly intervals until the termination of the experiment, a year later. Of the hundred frogs, 22 died (14 large and 8 small) during the course of the experiment due to unknown reasons.

Size at metamorphosis, growth rate, size and age at sexual maturity attained by the 'small' and 'large' metamorphs were compared using independent sample *t*-test. Growth rate of these two groups was analysed using repeated measures of ANOVA. The association between SVL and body mass was analysed using Pearson correlation coefficient analysis. Size-at-maturity of the two groups ('small' and 'large' metamorphs) was analysed using Mann–Whitney *U*-test. Mortality was analysed using χ^2 and fecundity of females of the two groups was analysed by Mann–Whitney *U*-test. All the statistical analyses were carried out using SPSS.

Mean SVL and body mass of 'large' sized metamorphs were 26.42 mm ± 0.28 (range: 25–30 mm; Table 1) and 1.42 g ± 0.05 (range: 0.98–2.350 g; Table 1) respectively. Likewise, SVL and body mass of 'small' metamorphs were 23.43 mm ± 0.10 (range: 21–24 mm) and 1.15 g ± 0.03 (range: 0.88–1.86 g) respectively. A positive correlation was observed between SVL and body mass in metamorphs, irrespective of whether they belonged to small ($r = 0.44$, $P < 0.05$) or large groups ($r = 0.57$, $P < 0.05$). The small and large metamorphs (newly metamorphosed) differed significantly in their SVL ($t = 10.08$, $df = 76$, $P < 0.0005$) as well as body mass ($t = 4.54$, $df = 76$, $P < 0.0001$). Comparison of SVL between smaller and larger individuals in subsequent months revealed that SVL of the large-size group was significantly greater than the small group ($t = 2.46$, $df = 76$, $P < 0.05$) at the end of the 1st month (Table 1). But, body mass was comparable ($t = 0.71$, $df = 76$, $P > 0.05$) throughout. Growth rate (SVL) of smaller metamorphs was significantly higher than the larger metamorphs in the first two months ($F > 5.00$, $P < 0.05$; Figure 1 *a*). Likewise, during the first month, increase in the rate of body mass of smaller frogs was significantly higher in comparison to the larger frogs ($F = 5.67$, $P < 0.05$; Figure 1 *b*). Males of both the groups attained sexual maturity at comparable SVL ($U = 12$, $df = 1$, $P > 0.05$) and body mass ($U = 11$, $df = 1$, $P > 0.05$). In females, mean SVL and body mass of larger frogs at maturity was 57 mm ± 0.931 and 15.24 g ± 0.920 ($N = 6$) respectively. Mean number of eggs produced by these females was 551.0 ± 29.24 ($N = 6$). Likewise, mean SVL of smaller metamorphs was 56.8 mm ± 0.92 and body mass was 15.40 g ± 0.635 ($N = 5$). Mean number of eggs produced by small group females was 568 ± 30.20. In females, at maturity neither SVL ($U = 14$, $df = 1$, $P > 0.05$) nor body mass ($U = 13.0$, $df = 1$, $P > 0.05$) differed significantly between individuals

Table 1. Size distribution of metamorphs and post-metamorphic growth rate in *Euphlyctis cyanophlyctis*

	Large group				Small group			
Month	SVL (mm ± SE)	Growth rate of SVL	Body mass (g ± SE)	Growth rate of body mass	SVL (mm ± SE)	Growth rate of SVL	Body mass (g ± SE)	Growth rate of body mass
Meta	26.42 ± 0.28*	–	1.42 ± 0.05*		23.43 ± 0.10		1.15 ± 0.03	
1	33.06 ± 0.28	0.224 ± 0.01	3.03 ± 0.15	0.740 ± 0.050	32.24 ± 0.42	0.320 ± 0.014	2.88 ± 0.13	0.888 ± 0.048
2	39.47 ± 0.50	0.177 ± 0.007	5.40 ± 0.24	0.585 ± 0.030	39.10 ± 0.41	0.194 ± 0.014	5.15 ± 0.19	0.594 ± 0.054
3	41.47 ± 0.49	0.049 ± 0.006	6.43 ± 0.24	0.185 ± 0.028	41.10 ± 0.35	0.051 ± 0.007	6.17 ± 0.20	0.191 ± 0.028
4	42.31 ± 0.42	0.021 ± 0.006	6.57 ± 0.22	0.024 ± 0.021	42.02 ± 0.33	0.023 ± 0.061	6.50 ± 0.21	0.054 ± 0.028
5	42.92 ± 0.38	0.015 ± 0.004	6.88 ± 0.24	0.046 ± 0.012	42.33 ± 0.34	0.007 ± 0.004	6.64 ± 0.22	0.021 ± 0.012
6	43.67 ± 0.44	0.017 ± 0.005	7.12 ± 0.24	0.035 ± 0.018	43.02 ± 0.39	0.016 ± 0.005	7.03 ± 0.26	0.530 ± 0.022
7	45.42 ± 0.52	0.039 ± 0.006	7.94 ± 0.05	0.103 ± 0.016	44.90 ± 0.44	0.041 ± 0.004	7.62 ± 0.29	0.078 ± 0.013
8	46.72 ± 0.54	0.028 ± 0.004	9.25 ± 0.38	0.150 ± 0.016	46.12 ± 0.41	0.027 ± 0.004	8.71 ± 0.28	0.141 ± 0.017
9	48.00 ± 0.59	0.027 ± 0.004	10.23 ± 0.40	0.104 ± 0.016	46.98 ± 0.46	0.018 ± 0.004	9.18 ± 0.30	0.051 ± 0.012
10	48.25 ± 0.62	0.050 ± 0.003	10.49 ± 0.39	0.027 ± 0.009	47.36 ± 0.50	0.008 ± 0.004	9.50 ± 0.30	0.035 ± 0.014
11	48.81 ± 0.69	0.011 ± 0.003	10.63 ± 0.43	0.009 ± 0.009	47.95 ± 0.56	0.012 ± 0.002	9.65 ± 0.36	0.011 ± 0.009
12	49.19 ± 0.69	0.008 ± 0.002	10.90 ± 0.48	0.022 ± 0.009	48.40 ± 0.63	0.009 ± 0.002	9.98 ± 0.42	0.028 ± 0.012

*Indicates significant difference between large and small frogs.

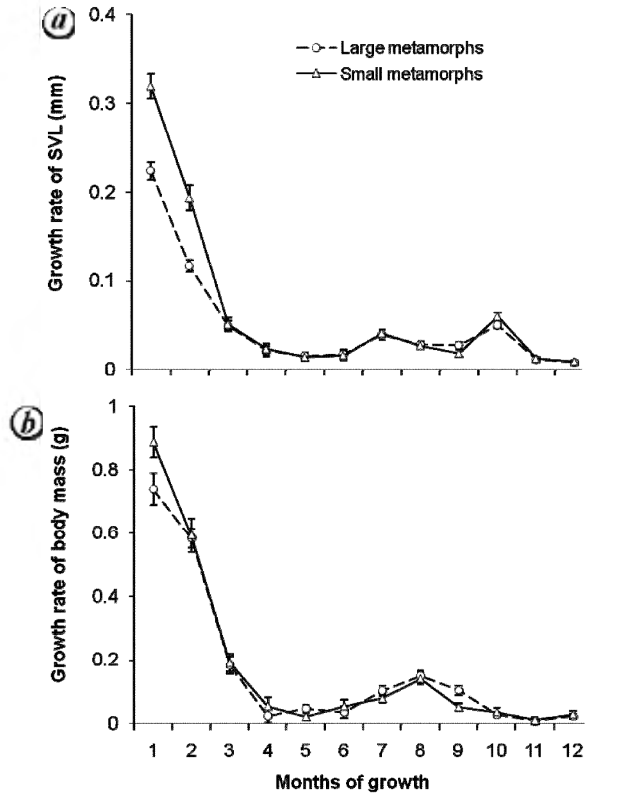

Figure 1. Depicts the growth rate of SVL (***a***) and body mass (***b***) of large and small metamorphs of *Euphlyctis cyanophlyctis*.

arising from smaller or larger metamorphs. Also, there was no significant difference in the fecundity of frogs between the two groups ($U = 13.0$, $df = 1$, $P > 0.05$). There was no significant difference in the mortality of the individuals with respect to small or large metamorphs during the course of the experiment ($\chi_1^2 = 1.6364$, $P > 0.05$).

Metamorphic size is considered as an important life-history component in amphibians that live in temporary unpredictable environments. Larger size of metamorphs is believed to affect post-metamorphic survival, growth, age and size at maturation, and adult fecundity[6,7,19]. In temperate regions, size at metamorphosis is reported to affect adult fitness in natural populations[4,6–10,19]. However, initial disadvantages of small metamorphic size may be overcome before sexual maturity if the environmental conditions are optimal for growth[22]. In *E. cyanophlyctis*, a variation in the size of metamorphs in natural condition might be due to any one of the several factors like density, pond drying, temperature, etc. or it might simply be due to phenotypic plasticity. In laboratory conditions, the size at metamorphosis did not affect the juvenile survival, growth and attainment of sexual maturity. Growth rate of body mass and SVL of the smaller and larger metamorphs differed for the first 1–2 months respectively, but became comparable between the groups in subsequent months. This suggests that if the terrestrial environment is favourable, individuals, irrespective of their initial body size can compensate for their smaller size by utilizing the opportunity for catch-up growth during terrestrial life[22]. The metamorphic size did not affect growth rate and over-winter survival in *Rana calmitans*, *R. sphenocephala*, *R. blairi* and *Bufo woodhousii* when reared in outer enclosures although a trend of larger metamorphs surviving to the following spring was observed[23]. The anuran tadpoles that live in unpredictable environments have evolved phenotypic plasticity so that they can modulate their growth and development vis-à-vis size at transformation in accordance with the habitat structure[14]. They try to emerge as small as possible if the aquatic habitat is deteriorating so that if the resources are adequate in the terrestrial habitat, they can still make up and compensate for their smaller size at metamorphosis[22,23]. The present study shows that this is indeed true for *E. cyanophlyctis*. The food availability and predator pressures in the early juvenile life are also very important in ensuring post-metamorphic survival and growth. In fact, size at metamorphosis may indirectly affect juvenile survival by making smaller individuals more vulnerable to scarcity of food and predation[24], as they cannot jump longer distances or for longer period of time to disperse to safe places [25]. But, once they makeup for their smaller size during the early part of juvenile life by utilizing the resources available on land, there would be no constraint for their growth and sexual maturity. A second possible explanation could be that when the tadpoles metamorphose in large numbers (which is the case in most of the amphibians), they will face severe inter- and intra-specific competition for food and shelter. In such situations, the larger ones will have an edge over the smaller individuals, as they are better equipped with their larger bodies to compete for the available food and space. Moreover, even if the food is not available immediately for a few days, they can still survive by utilizing the available energy in the form of abdominal fat bodies deposited during the larval period[9,26]. In contrast, smaller individuals may not sustain the pressure from larger conspecifics or heterospecifics for the limited food available and therefore have high mortality[9,22,27]. Metamorphic size may influence the future survival, growth and sexual maturity in species that emerge at a smaller proportion of their mean adult size and undergo maximum post-metamorphic growth. *E. cyanophlyctis* generally metamorphoses at a large proportion of its adult mean size and undergo moderate post-metamorphic growth before attaining maturity[28]. It is also reasonable to assume that metamorphic size affects future survival, growth and maturity in temperate species as their growth season is very much restricted by low winter temperatures and low survival rate[22]. But *E. cyanophlyctis* inhabits tropical climates that provide year-round growth opportunities. Therefore, in such species small metamorphic size may not have a large impact on the future survival, growth and maturity of the individuals.

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