POST-METAMORPHIC GROWTH, SEXUAL MATURATION AND BODY SIZE DIMORPHISM IN THE SKIPPER FROG, EUPHLYCTIS CYANOPHLYCTIS (SCHNEIDER)

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Post-metamorphic growth to sexual maturity was studied in a tropical frog Euphlyctis cyanophlyctis that breeds all the year round, in large outdoor terraria. Sexual size dimorphism (SSD) was studied in both a natural population and those reared in outdoor terraria. The growth rate was high in the first 2-3 months in both sexes, but subsequently declined, reaching a plateau following sexual maturation. The growth rate was high during summer and low during winter, and its pattern paralleled the changes in the ambient temperature. The males matured between 3-6 months depending upon their growth rates; at this stage they developed vocal sacs and spermatozoa and engaged in calling. The females matured between 8-11 months of age and responded to injections of progesterone by producing mature eggs. Fecundity was positively correlated to snout-vent length (SVL)/body mass. The males and females attained maturity when they reached a critical minimum SVL of ~42 mm and ~55 mm respectively. Mean adult body size (SVL and body mass) of females collected from nature was 67.0±0.85 mm and 32.8±1.56 g, and that of males was 48.0±0.37 mm and 10.1±0.31 g. Male to female size ratio (F/M) was 1.4 indicating SSD and a larger female size. In laboratory-reared specimens also, SSD was obvious at sexual maturity. A larger body size in females is due to delayed sexual maturity relative to the males. These findings suggest that in E. cyanophlyctis SSD is manifested primarily due to differences in the age at sexual maturity rather than an inherent difference in the post-metamorphic growth rate between the sexes.

Key words: age at maturity, amphibia, Anura, reproduction, sex ratio

INTRODUCTION

In amphibians, regulation of larval period, larval growth, size at metamorphosis, post-metamorphic growth and survival rates may affect the age, body size and timing of sexual maturation. Therefore, pattern of growth is a key aspect in the life history of amphibians. Though post-metamorphic growth and adult body size are under genetic control, several proximate factors such as food, predator pressure and geographic distribution (latitude and longitude) modulate the growth rates and size at maturity within and between species (Hemelaar, 1988; Jørgensen, 1992; Augert & Joly, 1993; Leclair & Laurin, 1996; Claude et al., 1999). Sexual maturation is a key element in the life history of any organism, as the age and size at which it occurs may influence reproductive fitness (Stearns, 1992; Bernardo, 1993; Adolph & Porter, 1996). Several biotic and abiotic factors influence age and size at sexual maturity in several ways (Stearns & Koella, 1986). Also, individuals within a population may differ in their growth patterns/trajectories and mature at different ages.

Age at sexual maturity varies widely among amphibian species, some maturing as early as six months and others as late as 5–6 years (Bastein & Leclair, 1992; Cherry & Francillon, 1992; Jørgensen, 1992, Marquez et al., 1997; Gramapurohit et al., 2004a). Generally, species inhabiting the tropics are known to grow fast and mature earlier than the temperate species since environmental factors do not vary drastically, and are therefore presumed to promote growth year round (Duellman & Trueb, 1986; Jørgensen, 1992). Nevertheless, growth rate and age at sexual maturity are species-specific and there exists a trade-off between growth and sexual maturation.

Studies that deal with post-metamorphic growth, age and size at maturity and sexual size dimorphism (SSD) are mostly on anurans that are seasonal breeders from temperate regions (Woolbright, 1983, 1989; Ryser, 1988; Cherry & Francillon, 1992; Tucker, 1995; Katsikaros & Shine, 1997; Platz et al., 1997; Sagor et al., 1998; Monnet & Cherry, 2002). To the best of our knowledge, there are no such studies on frogs that breed year round. The skipper frog, Euphlyctis cyanophlyctis is distributed throughout India. In southern India it breeds year round with peak breeding activity coinciding with the southwest monsoon. Many aspects of reproduction such as gonadal cycles, kinetics of germ cell production, sites of gonadal steroidogenesis are well known for this species (Saidapur, 1989). However, other aspects of the life history are not known. Therefore, it was of interest to study the growth rate, age at maturity and SSD in E. cyanophlyctis reared in out-door enclosures.

MATERIALS AND METHODS

Tadpoles of E. cyanophlyctis (n=100) at stage 45 ( Gosner, 1960) with rudimentary tails were collected during monsoon season between 1-5 September 2000.
from a pond on the Karnatak University Campus, Dharwad (15°17' N, 75°3' E) to study post-metamorphic growth and sexual maturity. They were maintained in a cement cistern (100 × 100 × 100 cm) until metamorphosis. The tail resorbed within three days of their collection. Individual frogs were marked at metamorphosis by toe clipping (avoiding clipping of the thumbs so as not to interfere with clasping ability during mating). The metamorphs were then reared in outdoor terraria (300 × 100 × 100 cm) each housing 20 individuals. Each terrarium was provided with a pool of water on one side and some refugia on the other. The frogs were given food ad libitum daily (small fishes, various insects and their larvae).

The snout-vent length (SVL) and body mass for individual frogs were recorded regularly at monthly intervals and each group of frogs was interchanged among terraria to avoid any positional effects. SVL was recorded to the nearest 1 mm using a ruler by gently pressing the frogs against a hard surface. Body mass was recorded on an electronic balance after emptying the bladder. The frogs were gently blotted before weighing. Sex of the frogs was identified in retrospect when they matured. The males have external vocal sacs that are easily seen. Attainment of sexual maturity was ascertained by the presence of sperm in the testes of males, and spawning in the case of females. The females were induced to spawn by injecting with progesterone (2 mg/ml saline/frog). Number of eggs produced by each female indicated fecundity. The air temperature was recorded daily at 1400 hrs during the study period.

One hundred newly metamorphosed frogs from the same pond were also toe-clipped and their monthly growth in nature was recorded for comparison with that of laboratory reared frogs. The pond dried after four months and hence no further recordings could be made. Monthly growth rate for each frog was calculated by ln(SVL/ SVL_o) where SVL_o is the length at the end of a month while SVL_n is length at the beginning and for body mass by ln(W/ W_o) where W_o is the mass at the end of month and W_n at the beginning.

The SVL and body mass were recorded for adult male and female E. cyanophlyctis (n=196) collected at random during the breeding season in July 2000 to determine SSD and male to female ratio (MFR) as per Lovich & Gibbons (1992). The MFR was calculated as mean adult size of the females / mean adult size of the males.

Monthly variation in growth rate of individual frogs was compared using multivariate analysis of variance (MANOVA) followed by a posteriori Bonferroni test. Monthly changes in SVL, body mass and the growth rates between sexes were compared using Mann Whitney U-tests. The relationship between SVL or body mass and fecundity was determined using linear regression. Growth rates of SVL and body mass of males and females in natural population were compared with that of the laboratory reared individuals using t-tests. All statistical tests were performed using SPSS software.

RESULTS

GROWTH PATTERN

Pattern of growth of females and males reared from metamorphosis to 1 yr old (September 2000 to September 2001) is shown in Figs. 1-2. In general, E. cyanophlyctis metamorphosed at 12.6% of adult body mass. At metamorphosis, mean SVL and body mass of males and females was comparable in laboratory and natural populations. During the first three months after metamorphosis, growth was high in males but declined later. The growth curve reached a plateau in males around the 3rd month. In females, the period of growth before sexual maturity was longer. They grew faster than their male counterparts from the 3rd month onwards although growth rate was not significantly different be-

![FIG. 1. Pattern of growth in SVL of E. cyanophlyctis reared in out-door terraria and from natural population (four months). Arrows indicate the onset of sexual maturity in laboratory-reared specimens. Met = metamorphosis](image1)

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![FIG. 2. Pattern of growth in body mass of E. cyanophlyctis reared in out-door terraria and from natural population (four months). Arrows indicate the onset of sexual maturity in laboratory-reared individuals. Met = metamorphosis](image2)
FIG. 3. Monthly variation in growth rate of SVL of *E. cyanophlyctis* in relation to ambient temperature from metamorphosis (September) to one year.

between sexes during some months. Of the 100 frogs reared, 22 died due to unknown causes during the study period.

The growth pattern of frogs in a natural population (studied for four months) was similar to those reared in laboratory (Fig. 1-2). The recovery rate in the natural population became progressively low. At the end of the 4th month only 13 frogs could be recaptured of which eight were males with vocal sacs and five were females (without vocal sacs).

**GROWTH RATE**

Changes in the growth rate (SVL and body mass) in both sexes closely followed the changes in the ambient temperature (Figs. 3-4).

**Males.** The pattern of changes in growth rate of both SVL and body mass was similar (Figs. 3-4). Monthly growth rate of SVL (F<sub>1,112</sub>=262.2, P<0.0001) and body mass (F<sub>1,112</sub>=193.82, P<0.0001) varied significantly. It was highest in the 1st month of metamorphosis (September) but declined significantly in subsequent months up to December-January (Table 1). There was a significant rise in growth rate in March, which peaked in April-May (Table 1). Between June-August, rate of growth declined significantly.

**Females.** Monthly variation in the growth rates of SVL (F<sub>1,112</sub>=47.67, P<0.0001) and body mass (F<sub>1,112</sub>=31.43, P=0.05) was significant and followed a similar pattern (Figs. 3-4). Growth rates of SVL and body mass were highest in the first month of metamorphosis (Table 1) following which it declined gradually until December-January. From January-February onwards there was a significant rise in the growth rate until May followed by a steady decline until August.

Except for the first two months, the overall rate of growth of SVL (U=41266, n<sub>1</sub>=67, n<sub>2</sub>=11, P<0.0001) and body mass (U=42944, n<sub>1</sub>=67, n<sub>2</sub>=11, P<0.0001) of laboratory-reared frogs over a period of one year varied significantly between the sexes. The growth rate was generally higher in females than in males from the 3rd month onwards though it was not statistically significant in some months.

Although growth rates were slightly higher in frogs in the natural population than in laboratory-reared frogs, these differences were not significant in either sex. (male SVL: t=0.80, df=1, P>0.05; male body mass: t=0.57, df=1, P>0.05; female SVL: t=0.49, df=1, P>0.05; female body mass: t=0.62, df=1, P>0.05).

**Age and size at sexual maturity.** In the laboratory, males attained sexual maturity between 3-6 months and females between 8-11 months after metamorphosis. Of these, 21 males matured three months after metamorphosis at a mean±SE, SVL of 42.6±0.25 mm, and a mean mass of 6.9±0.17 g. Those that did not mature during the 3rd month (n=46) were significantly smaller (SVL: 40.7±0.32 mm; t=4.81, df=1, P<0.001; body mass: 6.0±0.16 g; t=3.97, df=1, P<0.001) than those that matured. The females that matured at eight months, mean±SE SVL and mass at maturity were 55.0±0.70 mm and 14.8±0.85 g respectively (n=5) while, those that did not (n=6) were smaller in size (SVL: 50.3±1.15 mm; body mass: 12.0±0.51 g). All females matured by 11 months. In general, the fecundity (number of eggs) was positively correlated with both SVL and body mass. Irrespective of their age, the male and female frogs attained maturity when they reached SVL of ~42 mm and ~53 mm (~22 mm) respectively (Fig. 5).

**Sexual size dimorphism.** The size frequency distribution of male and female *E. cyanophlyctis* collected from nature varied (Figs. 6-7). Mean±SE adult SVL and mass of females were 67.2±0.85 mm and 32.8±1.56 g respectively. Mean adult size (SVL and body mass) for males was 48.0±0.37 mm and 10.1±0.31 g. Male to female ratio (MFR: F/M) was 1.4. Among the laboratory reared frogs adult males were also always smaller than adult females of comparable age although they were of comparable size at metamorphosis (SVL: U=328, n<sub>1</sub>=67, n<sub>2</sub>=11, P>0.05; body mass: U=273.5, n<sub>1</sub>=67, n<sub>2</sub>=11,
and also during subsequent two months (Table 1). After attaining maturity in 3rd month, the growth rate of males declined. From then on they remained significantly smaller ($U = 15612, n_1 = 67, n_2 = 11, P < 0.0001$) than females of comparable ages.

### DISCUSSION

The size at metamorphosis is known to vary among different species of anurans. For instance, some bufonids metamorphose at a small size representing <0.1% of the adult body size while some ranids metamorphose at 20% of the adult body size (Werner, 1986). It is also

 compelled that both sexes matured when they reached a critical minimum size regardless of their age. (number in parentheses indicate the sample size).

**FIG. 5.** The size and age at sexual maturity in *E. cyanophlyctis*. Note that both sexes matured when they reached a critical minimum size regardless of their age. (number in parentheses indicate the sample size).

**FIG. 6.** The size (SVL) distribution of male and female *E. cyanophlyctis* in nature.
well known that size at metamorphosis tends to be smaller in species breeding in temporary water bodies compared to those that breed in permanent water bodies (Werner, 1986). Therefore, in most aquatic species, size at metamorphosis tends to be larger than that of the terrestrial species (Werner, 1986). *Euphlyctis cyanophlyctis* is an aquatic species having smaller adult size and breeds in relatively permanent water bodies. *Hoplobatrachus tigerinus* a sympatric ranid is relatively terrestrial and has larger adult size and breeds in temporary ponds. Of these two *E. cyanophlyctis* metamorphosed at a larger size (12.6% of adult body mass) than *H. tigerinus* (0.4% of its mean adult mass; Gramapurohit et al., 2004b). The present findings are in agreement with those reported for other temperate species and they corroborate the views of Werner (1986).

The fact that the growth rate of *E. cyanophlyctis* was similar in frogs reared in the laboratory and those from nature – at least during the first four months following metamorphosis – suggests near optimal conditions for growth in laboratory outdoor terraria. An initial period of rapid growth followed by a rapid decline as seen in *E. cyanophlyctis* is characteristic of post-metamorphic growth among most anurans (Jørgensen, 1992). In many temperate anurans, seasonal growth is influenced mainly by temperature (Jørgensen, 1992) with a few exceptions (Clarke, 1974). The fact that growth rates (SVL and body mass) in *E. cyanophlyctis* paralleled the ambient temperature (though winter is not severe in southern India) suggests that seasonal growth is not a characteristic feature of only temperate species and that it may be encountered in tropical species as well. Apparently, both internal and environmental factors appear to regulate seasonal growth (Jørgensen, 1992).

There is no general trend with regard to age at maturity between sexes in anurans. In some species both males and females attain sexual maturity at the same age (Francillon et al., 1984; Acker et al., 1986; Esteban, 1990) while in others males attain early maturity (Cherry & Francillon, 1992; Marquez et al., 1997). Interestingly, in *Pelobates cultripes* and *Rana perezi*, males are known to mature later than the females (Talavera, 1989; Docampo & Milagrosa, 1991). Unlike many temperate anurans, both sexes of *E. cyanophlyctis* attain sexual maturity in the first year itself and males attain sexual maturity much earlier than the females. In *Rana curtipes* (endemic to Western Ghats of southern India) males and females also attain sexual maturity in the first year, within six months of metamorphosis (Gramapurohit et al., 2004a). To the best of our knowledge, the present study on *E. cyanophlyctis* is the second report documenting early attainment of sexual maturity following metamorphosis among anurans. In an earlier study, female *E. cyanophlyctis* was reported to attain maturity in the second year based on skeletochronological observations (Kulkarni & Pancharatna, 1996). Attainment of sexual maturity in the first year provides unequivocal proof regarding the age at which females attain sexual maturity in *E. cyanophlyctis*. It also suggests that conclusions based on mere skeletochronological observations may not be reliable. Further, in *E. cyanophlyctis* age at maturity is related to size rather than the age, as in the temperate-zone salamander *Triturus cristatus* (Sinsch et al., 2003). On the other hand, in *H. tigerinus* sexual maturity was related to age (Gramapurohit et al., 2004b) rather than size. Therefore, dependency on age and size for attaining sexual maturity appears to be species-specific and needs to be interpreted with due caution. An inherent variation in growth rate among individuals of male and female *E. cyanophlyctis* population is in agreement with the earlier reports on anurans (Halliday & Verrell, 1988). This is largely because of a variation in the inherent growth potential of different individuals to utilize resources before sexual maturity as suggested by Halliday & Verrell (1988). However, the present study reveals that attaining a critical SVL is crucial in attaining sexual maturity in male and female *E. cyanophlyctis*, a life history trait that might have been fixed in its phylogenetic history.

Most amphibians exhibit SSD with females being larger in about 90% of the species while, in the other 10%, males are either larger or no SSD exists (Shine, 1979, 1989, 1990). However, post-metamorphic growth rate and age at sexual maturity, the two important factors that affect or control SSD have received little attention (Arak, 1988, Monnet and Cherry, 2002). The present study on *E. cyanophlyctis* shows that SSD occurs due to differences in the post-metamorphic growth period between the sexes before sexual maturity. In fact, male and female *E. cyanophlyctis* are of comparable size at metamorphosis but a trade-off between body growth and attainment of sexual maturity is dissociated between the sexes. The males attain maturity as early as three months and all individuals mature by six months depending upon their growth rates. On the other hand, female reproduction necessitates energy storage for production of yolk-laden eggs. Therefore, female *E. cyanophlyctis* grow for a longer period (8-11 months) to
attain a critical minimum size and then attain sexual maturity. Production of ovariolyzized eggs requires 3-4 months (Pancharadina & Saidapur, 1985). Thus, attainment of proper body size and delayed maturity help in regulating fecundity vis-à-vis fitness of female E. cyanophlyctis. Therefore, sexually mature females of E. cyanophlyctis are larger than mature males. Thus, in E. cyanophlyctis, differences in age at sexual maturity are chiefly responsible for SSD rather than differences in post-metamorphic growth rates per se between the sexes.

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