CHAPTER II

Pattern of Post-metamorphic Growth and Age at Sexual Maturity in *Rana tigrina* and *Rana curtipes*
INTRODUCTION

Populations are made up of conspecific individuals of different sizes, sexes and age groups. Even within an age class the size of the members may vary due to the inherent growth property of the individuals. The ultimate measure of an organism's evolutionary success is its ability to pass on its genes successfully to the next generation through reproduction for which it has to survive, grow and attain sexual maturity (Jørgensen 1995). A major life-history problem an animal faces pertains to the attainment of a proper body size, age and timing of first reproduction (Daan and Tinbergen, 1997). The longer the animal delays its maturation the lower is the probability of reaching the reproductive stage while, an early attainment of sexual maturity enhances chances of passing on its genes to the next generation through reproduction. Generally, there is a positive correlation between fecundity of the animal and the body size leading to a trade-off between energy allocation for body growth and reproduction (Daan and Tinbergen, 1997).

Most amphibians exhibit a complex life history pattern that normally includes an aquatic larval form followed by metamorphosis into a juvenile–adult form. Considerable attention has been paid to examine the effects of environmental factors on different stages of larval and adult life (Wilber and Collins, 1973; Gould, 1977; Werner, 1986; Petranka et al., 1987; Semlitsch et al., 1988). After metamorphosis, growth in the terrestrial mode of life is closely linked with longevity, and age or size at sexual maturity; features that depend on the habitat structure, resource availability, predation risk and competition. It is
generally believed that a relatively sedentary aquatic larval stage is dedicated to
growth and a more mobile terrestrial stage to dispersal (Wilber, 1980). However,
the argument tends to overlook the importance of post-metamorphic growth in
terrestrial phase.

Amphibian growth and adult body size are characteristically plastic and
indeterminate (Jørgensen, 1995). Therefore, they are greatly influenced by the
external environmental factors that affect the genetically fixed pattern of growth.
Most important factors include temperature, water and food availability etc.
However, studies on the larval growth and development, size at metamorphosis,
the pattern of post-metamorphic growth, growth rate, and age at first
reproduction are known only for a few anurans that too from temperate regions
(Turner, 1960; Porter, 1972; Duellman and Trueb, 1986; McDiarmid and Altig,
1999). Corresponding studies on tropical amphibians are virtually lacking.

The Indian bullfrogs *Rana tigrina* breed seasonally soon after monsoon
showers. Their larval duration is short (≈ 2 months). The bicoloured frog, *Rana
curtipes* is endemic to Western Ghats of southern India. In this species larval
duration ranges from 8–12 months and in some cases upto 20 months (my
unpublished observations). The present study was undertaken to elucidate the
patterns of post-metamorphic growth, monthly growth rate and age at sexual
maturity in *R. tigrina* and *R. curtipes*, which exhibit short and long larval lives,
and large and small adult sizes respectively.
MATERIALS AND METHODS

1. *Rana tigrina*

The tadpoles of *R. tigrina* between stages 40–42 (Gosner, 1960) were collected from a temporary pond (n = 50) near Karnataka University Campus, (15°17’N and 75°3’E) during the second week of June 1998 and reared in outdoor cement cisterns (6.5’x 8.5’x 1.5’). They were provided with insect larvae, water insects and tadpoles of other species (especially those of *Bufo melanostictus*) as food, *ad libitum*.

The bullfrog tadpoles metamorphosed within 15 days of their collection (in the first week of July). The newly metamorphosed individuals were maintained initially for 3 months in outdoor cement cisterns (4’ x 4’x 2.5’). Later, they were transferred to two separate outdoor terraria each housing 20 frogs. Each terrarium (9’x 4’x 2.5’) was provided with sand mixed soil bed with small plants, shrubs, hiding places and water in a 3.5’ x 4’ trough with 3” water column. The juvenile frogs were fed with termites and other small insects during the first 3 months. Subsequently, they were fed with a variety of foods like fishes, grasshoppers, roaches, silk-moths and insect larvae. In the first 6 months following metamorphosis, out of 40 frogs, 10 individuals died.

Four frogs developed vocal sacs and thumb pads at the end of first year, distinguishing themselves as males while, the sex of the remaining six males was identified in retrospect when they developed vocal sacs and thumb pads, in April 2000 i.e. 22 months after metamorphosis. A biopsy of gonads was done in two
individuals of each sex to judge the condition of gonadal development by the routine histological procedures.

The frogs were marked by clipping the toes when they were one year old to follow changes in SVL and body mass of individual specimens, during the second year. In June 2000, two years after their metamorphosis, 7 amplexed pairs were observed and one female spawned. Since the others did not spawn a week following amplexus, the females were given progesterone (2mg /ml saline) injection (IP) to induce ovulation and spawning. The number of eggs spawned was counted to estimate fecundity.

2. *Rana curtipes*

The tadpoles of *R. curtipes* at Gosner stage 39–41 were collected from a stream (n = 150) in Western Ghat area near Anamod village (15°4' N 74°33' E) during the first week of March 1999 and were maintained in a large cement cistern (6.5'x 8.5'x 1.5'). They were fed on boiled spinach. The tadpoles metamorphosed within a month of their collection (during the first week of April) The newly metamorphosed frogs were then maintained in a large outdoor terrarium (6.5'x 8.5'x 1.5') with access to water.

The juvenile frogs were fed with small insects, insect larvae and grasshoppers during their early post-transformation period and later with large insects, insect larvae, grasshoppers and fishes. Two months following metamorphosis gonadal biopsy was done in 4 males and 5 females to histologically assess the gonadal condition.
Six months after metamorphosis 4 pairs of frogs showed amplexus and a frog spawned in captivity indicating the attainment of sexual maturity. Hence, the experiment was terminated 6 months after metamorphosis. Fifteen females at termination of the experiment were autopsied to assess the fecundity by counting the ovulatory sized eggs in the ovaries.

**Study of Growth Patterns**

Snout vent length (SVL, cm) and body mass (g) for both *R. tigrina* and *R. curtipes* were recorded at monthly interval from metamorphosis onwards until termination of the experiments. The SVL was recorded using a thread nearest to 0.1 cm by gently pressing the body of the frogs against a flat surface. Body mass was recorded after emptying the bladder.

Specimens of *R. tigrina* (sub-adults and adults) collected locally were used to determine the relationship between body mass and SVL (in nature) using curvilinear regression and the frogs were later released in the capture site. The relationship could be described by the power equation \( W_g = 0.0944 \times \text{SVL (cm)}^{2.973} \) where \( W_g \) is the mass of the individuals. The value of the exponent was close to 3 indicating that the post-metamorphic growth in *R. tigrina* is isometric. We assumed that the post-metamorphic growth in *R. curtipes* to be also approximately isometric. Changes in the condition index (CI) of both species of frogs was determined throughout the experiment at monthly intervals using the formula \( (CI) = 1000 \times W_g \times \text{SVL (cm)}^{-3} \) (Jørgensen, 1983). The condition index of both species at metamorphosis was considered as the standard, to further
measure changes in the nutritional status of the individuals throughout the experiment.

**Statistical analysis**

**R. tigrina:** The growth curves were constructed based on the data on SVL, body mass and their ratio using a modified logarithmic function \( \log Y = b_0 + b_1 \times \log(t) \), where \( Y \) = growth parameter, \( b_0 \) = slope of the curve, \( b_1 \) = intercept and \( \log(t) \) = time in months. Monthly growth rate was calculated as either \( GR = \log \left( \frac{W_t}{W_0} \right) \) or \( GR = \ln \left( \frac{L_t}{L_0} \right) \) where, \( W_t \) is the weight of the individuals at the end of the month, and \( W_0 \) is the weight at the beginning of the month, \( L_t \) is the SVL at the end of the month and \( L_0 \), at the beginning. The data on growth rates for the first and second years were separately analyzed. Data on SVL and body mass of the first year frogs were analyzed by multivariate analysis of variance (ANOVA) followed by Bonferroni (multiple comparison test) accepting the level of significance at 0.05. Growth rate for each individual and each sex (for the second year frogs) was analyzed by repeated measures of ANOVA. Size (SVL) difference between sexes was analyzed using Mann-Whitney U test for one-year and two-year old frogs. Residuals for body mass were generated by regressing the body mass on SVL. Residual body mass between the sexes was compared using Mann-Whitney U test.

**R. curtipes:** For *R. curtipes* growth curves were separately constructed for males and females (as both differed in size at metamorphosis) using the exponential function \( Y = b_0 \times \exp(b_1 t) \) where \( Y \) = size, \( b_0 \) = slope of the curve, \( b_1 \) = intercept and \( t \) = time. Growth rates were calculated as described before. Data
pertaining to SVL and body mass for males and females were separately analyzed by MANOVA. Growth rates of males and females were compared using student's t test. Mann-Whitney U test was used for the analysis of size difference between males and females as in *R. tigrina*.

The condition index (CI) in both the species was compared different months using Mann-Whitney U test.

**Fecundity**

The relationship between SVL and fecundity and, body mass and fecundity in females of both the species was analyzed by linear regression using log-transformed data.

**RESULTS**

1. *Rana tigrina*

The pattern of post-metamorphic growth of SVL and body mass from July 1998 (metamorphosis) to September 2000 is shown in Fig. 2.1B along with the data on the ambient temperature (Fig. 2.1A). After a brief period of rapid growth following metamorphosis from July to October 1998, growth almost ceased between November 1998 to February 1999 (Fig. 2.1B). From March to September 1999 again there was a rapid growth followed by an arrest of growth from October 1999 to March 2000. Growth pattern from March onwards, exhibited a steady increase upto September 2000.

The values of CI index over a period of 28 months are represented in the Fig 2.2A along with the growth rates during the first year in Fig. 2.2B. The comparison of monthly CI showed a significant variation during the first year. The
Cl values were significantly high during 2nd and 3rd months following metamorphosis as compared to that at metamorphosis (U = 0.0, P < 0.05; U = 0.0, P < 0.05). During December 1998 (7th month), the Cl increased significantly (U = 12.0, P < 0.05) but dropped in subsequent months till June 1999 (Fig. 2.2A). The values of Cl did not change much during the second year when compared to that at metamorphosis.

Post-metamorphic Growth Rate

Data on age, SVL, body mass of frogs and monthly growth rates of SVL and body mass during the first year are presented in Table 2.1 and Fig. 2.2B.

There was a significant variation in the length specific growth rate (LSGR) with the advancement of age of individuals during the first year from July 1998 to June 1999 (F = 6.164, P < 0.0001). Further, LSGR significantly increased between July–September followed by no significant variation between October–November. There was a significant decrease in the LSGR during November–February. From March onwards until June no significant change occurred in LSGR.

The mass specific growth rate (MSGR) also followed a similar pattern as the length specific growth rate. There was a significant variation in the MSGR from metamorphosis in July 1998 to one year following metamorphosis (June 1999; F = 6.107, P < 0.0001), and also between July and September 1998. No significant variation was observed during August and October 1998. A significant decrease in the MSGR was observed between November 1998 and February 1999 followed by a slight increase in MSGR between March and June 1999.
Pattern of growth in male and female *R. tigrina* during the second year is represented in Figs. 2.3A and 2.3B. Although males appeared to grow faster, repeated measures of ANOVA (for both SVL and body mass) showed no significant difference in the growth rate between males and females ($F = 0.05, P > 0.05$). Also, there was no significant difference in the body size (SVL and body mass) between males and females of one ($U = 84, P > 0.05; U = 75, P > 0.05$) and two year old frogs ($U = 72.5, P > 0.05; U = 86, P > 0.05$).

*Relationship between Body size and Age*

Results of regression analysis of SVL, body mass and the ratio of body mass/SVL are presented in Table 2.2. Growth curves showed that SVL ($R^2 = 0.89$, $F = 6722.26$, $P < 0.001$), body mass ($R^2 = 0.88$, $F = 5841.39$, $P < 0.001$) and the ratio of body mass to SVL ($R^2 = 0.89$, $F = 4432.94$, $P < 0.001$) were closely related to the age of the individuals (Figs. 2.4, 2.5 and 2.6). With the advancement of age there was an increase in SVL and body mass. However there was a variation in the size of the individuals of same age group irrespective of the sex of the individuals.

*Age and Size at Sexual Maturity*

Out of 10 males 4 developed vocal sacs and thumb pads in the first year. Histology of the testis showed formation of spermatozoa in these frogs (Fig. 2.7). The males that attained maturity in the first year measured 10.33cm ± 0.23 and weighed 85.47g ± 4.57 ($n = 4$). The remaining 6 males developed vocal sacs and thumb pads in the second year i.e. 22 months following metamorphosis. Mean SVL of these frogs was 12.03cm ± 0.33 and mean body mass was 128g ± 12.82.
However, at the end of second year, SVL and body mass of the individuals that attained maturity in the first year were 12.23 cm ± 0.48 and 137.50 g ± 19.84 respectively. In the year old females, the ovaries exhibited first growth phase (FGP) oocytes of 354 μm ± 5.40 diameter (Fig. 2.8). The females of 20–24 month old measured 12.15 cm ± 0.21 (SVL, range 11.5–14.2 cm) and weighed 134.06 g ± 7.99 (n=16). These females possessed large ovulatory sized follicles in their ovaries. The mean number of eggs was 4743.64 ± 542.90 with a range of 2589–7549. The number of eggs of ovulatory sizes was correlated positively with both SVL ($R^2 = 0.82$, $P < 0.001$) and body mass ($R^2 = 0.75$, $P < 0.001$).

2. *Rana curtipes*

The pattern of post-metamorphic growth from first week of April (metamorphosis) to October is shown in figure 2.9 (A, B, C). The body size of males and females was different at metamorphosis. The SVL of males measured 42.21 mm ± 0.47 and they weighed 4.95 g ± 0.23 at metamorphosis. The SVL and body mass of females at metamorphosis was 50.64 mm ± 0.43 (SVL) and 8.23 g ± 0.27 respectively. Between the sexes there was a variation in the growth pattern during the first six months following metamorphosis. Males attained 50.60 mm ± 0.79 (SVL) and 7.29 g ± 0.54 weight while females measured 62.27 mm ± 0.71 in SVL and weighed 15.03 g ± 0.72 in body mass at the end of six months. The pattern of changes in Cl of male and female *R. curtipes* is shown in the fig. 2.9C. Monthly analysis of Cl using Mann-Whitney test revealed that in males the values are significantly high only during the first month ($U = 169$, $P < 0.05$). The Cl values varied during subsequent months. Cl declining to significant
levels During July–September (U = 168, P < 0.05; U = 78, P < 0.05; U = 69, P < 0.05). The Cl values for females were high only during the first month following metamorphosis (U = 159, P < 0.05) and low during June (U = 362, P < 0.05) and September (U = 145, P < 0.05).

Post–metamorphic Growth Rate

Growth rates for males and females of *R. curtipes* are presented in table 2.3. Analysis of LSGR showed that in males there was a significant difference in the LSGR between months (F= 5.49, P < 0.05). The LSGR increased significantly in April (1\textsuperscript{st} month) following metamorphosis while, it dropped significantly in May (2\textsuperscript{nd} month; Table 2.3). There was again a significant rise in the LSGR in June (3\textsuperscript{rd} month; Table 2.3) but it declined subsequently in August (Table 2.3).

In male *R. curtipes* MSGR varied significantly between months (F= 5.23, P < 0.05). It increased slightly during April (1\textsuperscript{st} month), but showed a significant decline in May (2\textsuperscript{nd} month; Table 2.3). In June (3\textsuperscript{rd} month) MSGR increased significantly. In subsequent months (July–September) there was no significant variation in the MSGR (Table 2.3).

In *R. curtipes* females, LSGR varied significantly between months (F = 3.92, P < 0.05). At the end of April (1\textsuperscript{st} month), the increase in LSGR was significant but, a further increase during May (2\textsuperscript{nd} month) was marginal (Table 2.3). During June and July (3\textsuperscript{rd} and 4\textsuperscript{th} months) there was a significant decrease in the LSGR followed by insignificant fluctuations during subsequent months (Table 2.3). As in the males, there was also a significant variation in the overall
MSGR for females between months (F = 5.98, P < 0.05). The MSGR significantly increased during first month after metamorphosis. However, MSGR declined during May (2nd month) followed by a slight increase during June and July (Table 2.3). A significant decrease in MSGR was evident during August (5th month). During September though there was a slight increase in the MSGR, it was not significant (Table 2.3).

The rate of growth of SVL varied between males and females. In April (1st month), males grew faster (t = 2.40, P < 0.05) while, females grew faster during May and July (t = 4.64, P < 0.05; t = 2.18, P < 0.05). However, between August–October (4th, 5th and 6th months), there was no difference in the LSGR between the sexes (t = 0.93, P > 0.05; t = 1.30, P > 0.05; t = 0.41, P > 0.05). There was no difference in the MSGR also between the sexes.

**Relation between Age and Size**

Table 2.4 shows different body parameter estimates and the multiple regression analysis carried out for male and female *R. curtipes*. The growth curves fitted to body parameters for males showed a positive and increasing trend with the age (F = 224.45, P < 0.001; F = 58.86, P < 0.001; F = 20, P < 0.001) of the individuals (Fig. 2.10. A, B, C), but the association between body parameters (SVL, body mass and their ratio) and age (R² = 0.56, R²= 0.25 and R² = 0.10 respectively) was not very close.

In female *R. curtipes* also, the curves fitted to body parameters showed an increasing positive trend with the age of the individuals (F = 357.03, P < 0.001; F = 168.35, P < 0.001 and F = 92.99, P < 0.001). But the relationship was
somewhat better when compared to that of the males ($R^2 = 0.60$, $R^2 = 0.41$, and $R^2 = 0.28$; Fig. 2.11 A, B, C).

**Age at Sexual Maturity and Fecundity**

At metamorphosis, the males had the testes with meiotic spermatocytes (Fig. 2.12). They attained maturity in about 2–3 months following metamorphosis as indicated by the presence of spermatocytes and spermatids in the testes (Fig. 2.13). On the other hand, at metamorphosis, females had only small FGPOocytes (Fig. 2.14). The females attained maturity 5–6 months after metamorphosis, and one frog even spawned the others exhibited ovulatory size eggs in their ovaries (Fig. 2.15). The mean SVL and body mass of females at maturity were $62.27mm \pm 0.71$ and $15.03g \pm 0.72$ respectively. The male counterparts, six months following metamorphosis, measured $50.60mm \pm 0.79$ in SVL and weighed $7.29g \pm 0.54$.

In females, mean number of eggs was $924.60 \pm 217.92$ with a range of 601–1286. The fecundity was positively correlated (Fig. 2.16 A and B) with both SVL and body mass ($F = 10.14$, $P < 0.01$ and $F = 359.33$, $P < 0.001$ respectively). Yet, correlation of fecundity vis-à-vis number of eggs produced was more closely related to the SVL body mass ($R^2 = 0.97$, $P < 0.01$) than with the SVL ($R^2 = 0.44$, $P < 0.01$).

**Body size Dimorphism**

There was a marked difference in the body size of males and females.
Mann-Whitney analysis showed that the two sexes differed in size (SVL) at metamorphosis ($U = 3.5, P < 0.001$) as well as at maturity. ($U = 0, P < 0.001$).

The males were always smaller than the females of comparable age.

**DISCUSSION**

Anurans are known to metamorphose at a wide range (7–60 mm SVL) of body sizes (Werner, 1986; Collins, 1979). Whether the size at metamorphosis has any bearing on larval habitat has been a subject of debate. The adaptive significance of larval anurans lies in exploiting the transient opportunities for rapid growth in temporary ponds (Wassersug, 1975; Wilber, 1980). This benefit possibly outweighs the risk of predation and pond desiccation in temporary ponds. Also, the larval life is generally considered as a stage in life history dedicated for growth, and more mobile terrestrial stage to dispersal (Wilber, 1980). However, Werner (1986) rightly pointed out that such arguments undermine the growth attained in the terrestrial phase in several ranids. For instance, newly metamorphosed frog *R. catesbeiana* weigh less than 4% of mean adult size. On the other hand, the mink frog, *Rana septentrionalis* metamorphoses at the largest fraction, amounting to about 20% of its mean adult size. In contrast, the toad, *Bufo marinus* at transformation is < 0.1% of its mean adult mass.

The two species of frogs used in the present study live different types of habitats; *R. tigrina* tadpoles are found in temporary water bodies while those of *R. curtipes* in semipermanent water bodies (gently flowing streams) and also they exhibit different patterns of larval growth. *R. tigrina* metamorphoses at 19%
of its mean adult SVL and at 0.8% of its mean adult mass (2 1/2 years old) while, \( R. \ curtipes \) tadpoles metamorphose at a much larger size around 75% of its mean adult size (SVL) and about 67% of its mean adult mass. Females metamorphose at about 90% of their mean adult SVL and at 57% of their mean adult mass. The apparent difference in growth during metamorphosis and terrestrial life in these two species seems to be related to the larval habitat and duration of metamorphosis. \( R. \ tigrina \) metamorphoses in about 2 months time (SVL ~ 23 mm), while \( R. \ curtipes \) metamorphoses in about 20 months at a size of ~ 45 mm (SVL), twice as large as that of \( R. \ tigrina \). However, post-metamorphic growth (SVL and body mass) is much higher in \( R. \ tigrina \) than in \( R. \ curtipes \).

**Pattern of post-metamorphic growth rate**

Studies on natural populations have revealed a seasonal pattern in the growth rate in some species of frogs. Both \( Rana \ clamitans \) and \( Rana \ virgatipes \) (Martof, 1956; Standaert, 1967) inhabiting temperate regions hibernate and therefore do not feed during winter months. In these frogs growth rate seems to be related to seasonal changes in temperature cycle. Blair (1953) has also reported that \( Bufo \ valliceps \) in Texas grows slowly during winter months. In contrast, growth rate is not affected by season in \( Scaphiopus \ holbrooki \) (Pearson, 1955) as they seem to come out of hiding places for feeding throughout the year with equally spaced intervals between each feeding (Turner, 1960). In \( Bufowoodhousei \ fowleri \) Clarke (1974) has reported a minimal effect of season on growth rate. In the present study, the laboratory reared \( R. \ tigrina \) showed a
cyclic pattern of growth rate. The growth rate was high and rapid during summer months and very low or nil during winter months (November–January) in both the years of study. The captive frogs did not hibernate although they were provided sand bed and hiding places. However, they ate very little during winter months. In Dharwad, winter is not as severe as the temperate regions. Therefore, a low feeding may account for low growth rate in *R. tigrina*. Possibly there exists an endogenous rhythm of feeding cycle associated with seasonal changes in temperature cycle as suggested for *B. viridis* reared in captivity (Jørgensen, 1983).

The earlier studies on laboratory reared anurans are of very short (< 6 months) duration. (references in Jørgensen, 1995). Yet, they reveal species specific variation in the growth rates following metamorphosis. For instance, in *R. catesbeiana* (Modzelewski and Culley, 1974 cited in Jørgensen, 1995) the maximum growth rate (27mm) was seen in the first month of metamorphosis. In *Bufo houstonensis* (Quinn and Mengden, 1984 cited in Jørgensen, 1995) also, growth rate was maximum (19mm) in the first month and decreased gradually with the advancement in age. In this species lowest growth rate of 3mm was recorded between 4–5 months. The present study on *R. tigrina*, is the longest study (2¼ years) dealing with the study of growth rates among anurans. The study clearly shows that overall growth rate is high during the first year of life in both the sexes (8.03 cm SVL and 88.57g body mass) and low during second and subsequent years. Second year growth of these frogs accounted for only 1.77cm in comparison to 8cm growth in the first year.
In *R. curtipes* growth rate was high during the first four months after metamorphosis and decreased slowly thereafter. In this species, growth rate was recorded for the first six months by which time they attained sexual maturity. Unfortunately, the captive frogs died subsequently. Hence, it is not possible to say whether or not a cyclic pattern of seasonal growth is exhibited by the species.

Condition index is known to reflect the nutritional status of the individuals in a population. It also reflects partitioning of energy between linear growth (generally represented by SVL) and body fattening. A high CI associated with low linear growth period and vice versa is reported in captive *B. viridis* (Jørgensen, 1983). Thus in *B. viridis* there is a differential partitioning of energy towards linear growth and fattening during different periods of growth. In the present study on *R. tigrina*, post-metamorphic CI was very high in the first month but declined in the second month. Though the CI values seem low from 3rd month onwards, they were comparable to the values at metamorphosis. The low CI values associated with rapid growth in SVL in the first year (2 months following metamorphosis) suggests the diversion of energy mainly towards linear growth. In the second year, however, both CI and growth rates did not fluctuate much suggesting equal partitioning of energy for linear growth and fattening. In *R. curtipes* also, the pattern of energy partitioning was similar to that of *B. viridis* (Jørgensen, 1983). The CI in *R. curtipes* decreased with increase in the growth rate.
Relationship between age and size

It is widely accepted that age and body size are correlated in amphibians which exhibit indeterminate growth pattern (Duellman and Trueb, 1986; Kusano et al, 1995). However, Halliday and Verrell (1988) argued that the size is not an accurate indicator of individual’s age, because of high variance in growth rates in the first year, variations between individuals and decline in growth rate after maturity. In addition, a variation in age-size relationship between the sexes is also reported. For instance, the size of female *Bufo bufo* is positively correlated with the age while there is no relationship between the age and size of males (Hoglund and Saterberg, 1989). Likewise, size in male *Rana pipiens* (Leclair and Castanet, 1987) and *Pseudacris crucifer* (Lykens and Forester, 1987) is positively correlated with the age of the individuals while there was no relation between body size and age in females. In contrast, in *P. maculata* no relation between the size and the age of individuals is observed in both the sexes (Platz and Lathrop, 1992). In *R. tigrina* (the present study), the body size (SVL) was closely correlated with the age of the frogs suggesting that SVL is a good indicator of individual’s age. In *R. curtipes*, though SVL is positively correlated to the age in both the sexes, the association was weak. Therefore, in *R. curtipes*, SVL may not be a good indicator of individual’s age. Thus, the present study also reveals the existence of a species-specific difference in the relation between age and body size in frogs.
Body size Dimorphism

Majority of the amphibians exhibit size dimorphism between males and females. Also, in many species the growth rate of females is faster than the males which renders them a larger size (Shine, 1979; Howard, 1981; Woolbright, 1989; Hayes and Licht, 1992). For instance, in many species of *Rana, Bufo* and a few species of *Hyla* (Fitch, 1956; Turner, 1960; Standaert, 1967; Briggs and Storm, 1970; Brown and Alcala, 1970; Clarke, 1974; Rittschof, 1975; Gittins et al., 1982; Hemelaar, 1983; Gibbons and McCarthy, 1984; Francillon et al., 1984; Acker, et al., 1986; Lykens and Forester, 1987) females are bigger than the males. This is due to faster rate of growth or longer period of growth by females when compared to males. Also the growth rate patterns of sexes may differ depending upon the geographic distribution. For example, in Scandinavian population of *Rana temporaria* (Loman, 1978) growth rate between sexes is equal while, in Ireland, females grow faster than males (Gibbons and McCarthy, 1984).

A higher growth rate in females might reflect the size advantage as fecundity is positively correlated with body size (Clarke, 1974; Davies and Halliday, 1977; Jørgensen, et al., 1979; Banks and Beebee, 1986; Gibbons and McCarthy, 1986; Jørgensen, et al., 1986). On the other hand, *Rana arvalis, R. temporaria, R. catesbeiana* and *R. clamitans*, show equal growth rates between the sexes. In contrast, in *S. holbrooki* and *Pyxicephalus adspersus* growth rate is faster in males than in females (Pearson, 1955; Hayes and Licht, 1992). The present study on *R. tigrina* shows that there is no sexual size dimorphism as the
growth rate between sexes is similar up to maturity. On the other hand, *R. curtipes* exhibit a distinct size dimorphism between sexes since females were larger (SVL) at maturity than the males of same age.

**Age at sexual maturity**

In majority of the amphibians studied so far, males mature earlier than the females but for a few exceptions like *Rana perezi* (Docampo and Milagrosa, 1991) and *Pelobates cultripes* (Talavera, 1989) in which males mature later than the females. A study on *B. bufo* has shown that females attain maturity 1 year later than the males (Hemelaar, 1986, 1988). In *R. catesbeiana* and *R. tigrina* (the present study) males mature earlier than the females (Collins 1975; Howard, 1978; Márquez et al., 1997). However, only 40% of captive *R. tigrina* attained maturity in the first year. These observations suggest that all males in a natural population that metamorphose together may not attain sexual maturity at the same age. The strategy of maturing at different ages may be inherent to the particular individuals. Such a variation in the attainment of sexual maturity among individuals of a sex within a population is also documented in the literature (Ryser, 1988; Jøgensen, 1995). However, all females of *R. tigrina* reached maturity 20–24 months after metamorphosis. In *R. curtipes* also, males matured earlier than the females. Both the sexes in *R. curtipes* matured at much younger age (2–3 months in males and 5–6 months in females) as compared to *R. tigrina*.

Thus, the present study on two species of anurans has revealed a diversity in growth patterns, size at metamorphosis and, age and size at sexual
maturity. *R. tigrina* metamorphosed at a smaller size and grew essentially in the terrestrial phase of life while, the opposite is true for *R. curtipes*. Further *R. tigrina* males matured in the first year while females matured in the second year. On the other hand, both male and female *R. curtipes* matured within six months of metamorphosis.
The present study describes patterns of post-metamorphic growth and age at sexual maturity in *R. tigrina* and *R. curtipes*. In *R. tigrina* has short larval duration, metamorphoses at a small size (2.38 cm) but grows to a size of about 12.18 cm at maturity. Most of the growth in this species occurs at terrestrial stage. On the other hand, in *R. curtipes* with longer larval duration (6–20 months) grows maximum during its larval stages and metamorphose at 42–50 mm size. Relatively Growth on land in this species is minimum. Further *R. tigrina* shows a cyclic pattern of growth even though the variation in the seasonal temperature cycle is not much. Males of both the species attain maturity earlier ages than that of females. In *R. tigrina* some males attained maturity in the first year following metamorphosis while remaining males and females reached maturity during the second year. In *R. curtipes* males matured shortly after metamorphosis (2–3 months) while females matured after 6 months of metamorphosis. The present investigation also revealed that in *R. tigrina* there is no age specific size dimorphism between males and females while, in *R. curtipes* females are much larger than males of comparable age.
Table 2.1: Shows age, body parameters, growth rate and condition Index (Cl) during the first year following metamorphosis (July 1998 – June 1999) in laboratory reared *Rana tigrina*.

<table>
<thead>
<tr>
<th>Age (Months)</th>
<th>Months</th>
<th>SVL (cm±SE)</th>
<th>Length specific Growth Rate (mm±SE)</th>
<th>Body mass (g±SE)</th>
<th>Mass specific Growth Rate (g±SE)</th>
<th>Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>MM</td>
<td>2.34 ± 0.02</td>
<td>---</td>
<td>1.01 ± 0.03</td>
<td>---</td>
<td>78.83</td>
</tr>
<tr>
<td>1</td>
<td>July</td>
<td>2.70 ± 0.06</td>
<td>1.3 ± 0.2</td>
<td>3.57 ± 0.57</td>
<td>0.97 ± 0.12*</td>
<td>181.35*</td>
</tr>
<tr>
<td>2</td>
<td>Aug</td>
<td>4.29 ± 0.21</td>
<td>4.3 ± 0.5*</td>
<td>8.95 ± 2.36</td>
<td>0.63 ± 0.20*</td>
<td>113.36*</td>
</tr>
<tr>
<td>3</td>
<td>Sept</td>
<td>5.24 ± 0.21</td>
<td>2.4 ± 0.2*</td>
<td>11.60 ± 1.68</td>
<td>0.62 ± 0.12*</td>
<td>80.62</td>
</tr>
<tr>
<td>4</td>
<td>Oct</td>
<td>6.03 ± 0.24</td>
<td>1.3 ± 0.2</td>
<td>18.20 ± 2.36</td>
<td>0.49 ± 0.07</td>
<td>83.01</td>
</tr>
<tr>
<td>5</td>
<td>Nov</td>
<td>6.29 ± 0.25</td>
<td>0.4 ± 0.2*</td>
<td>21.34 ± 2.32</td>
<td>0.17 ± 0.06</td>
<td>85.75</td>
</tr>
<tr>
<td>6</td>
<td>Dec</td>
<td>6.36 ± 0.24</td>
<td>0.2 ± 0.1*</td>
<td>24.59 ± 4.67</td>
<td>0.07 ± 0.04*</td>
<td>95.58*</td>
</tr>
<tr>
<td>7</td>
<td>Jan</td>
<td>6.38 ± 0.21</td>
<td>0.0 ± 0.1*</td>
<td>22.05 ± 2.23</td>
<td>-0.01 ± 0.04*</td>
<td>84.91</td>
</tr>
<tr>
<td>8</td>
<td>Feb</td>
<td>6.62 ± 0.20</td>
<td>0.4 ± 0.1*</td>
<td>23.72 ± 2.09</td>
<td>0.12 ± 0.03*</td>
<td>81.76</td>
</tr>
<tr>
<td>9</td>
<td>Mar</td>
<td>7.10 ± 0.17</td>
<td>0.8 ± 0.2</td>
<td>28.65 ± 3.29</td>
<td>0.16 ± 0.07*</td>
<td>80.05</td>
</tr>
<tr>
<td>10</td>
<td>Apr</td>
<td>7.79 ± 0.27</td>
<td>0.9 ± 0.2</td>
<td>41.38 ± 4.2</td>
<td>0.36 ± 0.08</td>
<td>87.53</td>
</tr>
<tr>
<td>11</td>
<td>May</td>
<td>8.97 ± 0.31</td>
<td>1.3 ± 0.2</td>
<td>61.40 ± 6.47</td>
<td>0.37 ± 0.05</td>
<td>85.07</td>
</tr>
<tr>
<td>12</td>
<td>June</td>
<td>9.87 ± 0.30</td>
<td>1.0 ± 0.2</td>
<td>70.02 ± 6.49</td>
<td>0.17 ± 0.06*</td>
<td>72.82</td>
</tr>
</tbody>
</table>

MANOVA was performed followed by Bonferroni to analyze the growth rates. 'MM' indicates metamorphosis. * indicates a significant difference (P < 0.05) when compared to previous month; * indicates significant difference in Cl compared to that at metamorphosis.
Table 2.3: Shows age, body parameters, growth rate and condition index (CI) till 6 months following metamorphosis in *Rana curtipes*.

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Month</th>
<th>Sex</th>
<th>SVL (mm±SE)</th>
<th>Length specific Growth Rate (mm±SE)</th>
<th>Body mass (g±SE)</th>
<th>Mass specific Growth Rate (g±SE)</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>MM</td>
<td>Male</td>
<td>42.21 ± 0.47</td>
<td>---</td>
<td>4.95 ± 0.23</td>
<td>---</td>
<td>65.87</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>50.64 ± 0.43</td>
<td>---</td>
<td>8.23 ± 0.27</td>
<td>---</td>
<td>63.53</td>
</tr>
<tr>
<td>1</td>
<td>Apr</td>
<td>Male</td>
<td>45.02 ± 0.42</td>
<td>0.06 ± 0.01*</td>
<td>6.44 ± 0.18</td>
<td>0.27 ± 0.05</td>
<td>70.67a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>52.02 ± 0.30</td>
<td>0.03 ± 0.01*</td>
<td>10.63 ± 0.22</td>
<td>0.29 ± 0.04*</td>
<td>75.60b</td>
</tr>
<tr>
<td>2</td>
<td>May</td>
<td>Male</td>
<td>45.20 ± 0.32</td>
<td>-0.01 ± 0.01*</td>
<td>6.28 ± 0.17</td>
<td>-0.04 ± 0.04*</td>
<td>68.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>55.45 ± 0.45</td>
<td>0.07 ± 0.01</td>
<td>10.99 ± 0.29</td>
<td>0.03 ± 0.03*</td>
<td>63.94</td>
</tr>
<tr>
<td>3</td>
<td>June</td>
<td>Male</td>
<td>46.08 ± 0.27</td>
<td>0.02 ± 0.01*</td>
<td>6.66 ± 0.10</td>
<td>0.05 ± 0.04*</td>
<td>68.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>58.56 ± 0.63</td>
<td>0.05 ± 0.01*</td>
<td>11.75 ± 0.42</td>
<td>0.04 ± 0.01</td>
<td>58.39b</td>
</tr>
<tr>
<td>4</td>
<td>July</td>
<td>Male</td>
<td>48.80 ± 0.51</td>
<td>0.06 ± 0.01</td>
<td>6.99 ± 0.26</td>
<td>0.07 ± 0.05</td>
<td>60.15a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>60.26 ± 0.65</td>
<td>0.04 ± 0.01*</td>
<td>13.66 ± 0.54</td>
<td>0.19 ± 0.05</td>
<td>62.30</td>
</tr>
<tr>
<td>5</td>
<td>Aug</td>
<td>Male</td>
<td>50.84 ± 0.64</td>
<td>0.04 ± 0.02*</td>
<td>7.52 ± 0.32</td>
<td>0.09 ± 0.05</td>
<td>57.36a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>62.18 ± 0.60</td>
<td>0.01 ± 0.02</td>
<td>15.03 ± 0.72</td>
<td>0.04 ± 0.07*</td>
<td>63.46</td>
</tr>
<tr>
<td>6</td>
<td>Sept</td>
<td>Male</td>
<td>50.60 ± 0.79</td>
<td>0.00 ± 0.02</td>
<td>7.29 ± 0.34</td>
<td>-0.02 ± 0.07</td>
<td>56.27a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>62.27 ± 0.71</td>
<td>0.02 ± 0.02</td>
<td>14.27 ± 0.79</td>
<td>0.02 ± 0.07</td>
<td>59.01b</td>
</tr>
</tbody>
</table>

**MANOVA** was used followed by Bonferroni to analyze monthly changes in growth rates. 'MM' indicates metamorphosis.

* indicates a significant difference (P < 0.05) when compared to previous month; a and b indicate significant difference in CI compared to that at metamorphosis in males and females respectively.

MANOVA was used followed by Bonferroni to analyze monthly changes in growth rates. 'MM' indicates metamorphosis.

* indicates a significant difference (P < 0.05) when compared to previous month; a and b indicate significant difference in CI compared to that at metamorphosis in males and females respectively.
Table 2.2: Shows the results of multiple regression analysis of growth curves of *Rana tigrina* fitted to SVL, body mass and their ratio.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Estimates</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min.</td>
<td>Max.</td>
</tr>
<tr>
<td>SVL (cm)</td>
<td>2.34</td>
<td>12.76</td>
</tr>
<tr>
<td>Body mass (g)</td>
<td>1.07</td>
<td>143.35</td>
</tr>
<tr>
<td>Mass/Length Ratio</td>
<td>0.432</td>
<td>11.234</td>
</tr>
</tbody>
</table>
Table 2.4: Shows the results of regression analysis of growth curves fitted to the body mass, SVL and their ratio in male and female *Rana curtipes*.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sex</th>
<th>Estimates</th>
<th>Regression analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Min.</td>
<td>Max.</td>
</tr>
<tr>
<td>SVL (mm)</td>
<td>Male</td>
<td>42.21</td>
<td>50.84</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>50.64</td>
<td>62.27</td>
</tr>
<tr>
<td>Body mass (g)</td>
<td>Male</td>
<td>4.95</td>
<td>7.52</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>8.23</td>
<td>15.03</td>
</tr>
<tr>
<td>Mass/SVL Ratio</td>
<td>Male</td>
<td>0.12</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.16</td>
<td>0.23</td>
</tr>
</tbody>
</table>
Fig. 2.1

(A) Temperature

(B) Body mass (g) ± SE and SVL (cm) ± SE

('MM' indicates the size at metamorphosis)
Fig. 2.2

A. 

Condition index

MM A O D F A J A O D F A J A O

B. 

Length specific growth rate (mm ± SE)

Mass specific growth rate (g ± SE)

LSGR

MSGR

0 1 2 3 4 5

7 8 9 10 11 12 13 14 15 16

July Aug Sept Oct Nov Dec Jan Feb Mar Apr May June

1998 1999
Fig. 2.3: Shows the pattern of length specific growth (A) and mass specific growth (B) in male and female *R. tigrina* during the second year following metamorphosis.
Fig. 2.3

A

SVL ± SE (cm)

B

Body mass ± SE (g)

- □ Male
- ● Female

Calendar dates:
- 1999
- 2000
Fig. 2.4: Shows the growth curve fitted to snout-vent length in *R. tigrina*
Fig. 2.4

\[ Y = 0.2434 \ln(x) + 0.3414 \]

\[ R^2 = 0.8944 \]
Fig. 2.5: Shows the growth curve fitted to the body mass in *R. tigrina*.
Fig. 2.5

\[ Y = 0.7108 \ln(x) - 0.048 \]

\[ R^2 = 0.8798 \]

Body mass (g) vs. Age (months)
Fig. 2.6: Shows the growth curves fitted to the ratio of body mass to SVL in *R. tigrina*
Fig. 2.6

Y = 0.5226\ln(x) + 0.4384
R^2 = 0.7675

body mass/SVL ratio vs. Age (months)
Fig. 2.7: Shows the cross section of the testis of one year old male *R. tigrina* Note the presence of spermatogenesis indicating the attainment of sexual maturity in same males.

[Scale line = 50 μm]

Fig. 2.8: Shows the cross section of the ovary of one year old female *R. tigrina*. The ovary contains many FGP oocytes.

[Scale line = 100 μm]
Fig. 2.9: Shows the pattern of growth in male (A), female (B) and condition index (C) in *R. curtipes*. 
Fig. 2.9

A: Male

B: Female

Condition index (± SE)

Body mass (g ± SE)
Fig. 2.10: Shows the growth curves fitted to SVL (A), body mass (B) and their ratio (C) in female *R. curtipes*. 
Fig. 2.10

A

SVL (mm)

$Y = 1.6887e^{0.0101x}$

$R^2 = 0.5989$

B

Body mass (g)

$Y = 0.9121e^{0.0384x}$

$R^2 = 0.3789$

C

Body mass (g)/SVL (mm)

$Y = 0.5404e^{0.0282x}$

$R^2 = 0.3021$
Fig. 2.11: Shows the growth curves fitted to SVL (A), body mass (B) and their ratio (C) in male *R. curtipes*. 
Fig. 2.11

A

\[ Y = 1.6126e^{0.0084x} \]
\[ R^2 = 0.5598 \]

B

\[ Y = 0.708e^{0.0327x} \]
\[ R^2 = 0.2434 \]

C

\[ Y = 0.4376e^{0.0249x} \]
\[ R^2 = 0.187 \]
Fig. 2.12: Shows a cross section of testis of *R. curtipes* at metamorphosis.

[Scale line = 50 μm].

Fig. 2.13: Shows a cross section of ovary of *R. curtipes* at metamorphosis.

[Scale line = 100 μm]

Fig. 2.14: Shows the cross section of the testis of *R. curtipes* two months after metamorphosis, at maturity (arrow shows bundle of spermatids).

[Scale line = 50 μm]

Fig. 2.15: Shows a cross section of the ovary 4 months following metamorphosis in *R. curtipes* showing vitellogenic follicles (V).

[Scale line = 100 μm]
Fig. 2.16: Shows the relationship between SVL and the number of eggs (A) and body mass and the number of eggs (B) in *R. curtipes*.
**Fig. 2.16**

**A**

Number of eggs vs. SVL (mm)

\[ Y = 68.661x - 3469.7 \]

\[ R^2 = 0.3969 \]

**B**

Number of eggs vs. Body mass (g)

\[ Y = 94.167x - 649.37 \]

\[ R^2 = 0.9652 \]