Age estimates for a population of the Indian Tree Frog *Polypedates maculatus* (Gray, 1833) (Anura: Rhacophoridae)

Altersschätzungen an einer Population des Gefleckten Ruderfrosches *Polypedates maculatus* (Gray, 1833) (Anura: Rhacophoridae)

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KURZFASSUNG

Die Arbeit berichtet über die skelettochronologische Abschätzung von Alter und Lebenserwartung sowie des Alters bei Eintritt der Geschlechtsreife bzw. Fortpflanzung bei *Polypedates maculatus* (Gray, 1833). Die Aufsammlung der Frösche (n = 76) erfolgte von Juni bis September in den Jahren 2005 und 2006. Bei jedem Tier wurden die Kopf-Rumpf-Länge gemessen und die 4. Zehe des linken Hinterbeines unter lokaler Betäubung abgetrennt und in 70 %igem Äthanol fixiert. Acht Tiere aus unterschiedlichen Größenklassen wurden getötet und dienten zur Bestimmung des Reifegrades der Gonaden; an ihnen wurde auch die Histologie der Phalangen- und Langknochen Humerus und Femur untersucht. Nach äußeren und gonadalen Merkmalen wurden drei Gruppen von Fröschen unterschieden - unreife Jungtiere (Gruppe I), geschlechtsreife Männchen und Weibchen (Gruppe II) und Pärchen in Umklammerung (Gruppe III) Die gewonnenen Phalangen- und Langknochen wurden in 10 %iger EDTA entkalkt und zur Anfertigung histologischer Schnitte weiterverarbeitet. Ein bis sechs Wachstumsringe, jeder aus einer Wachstumszone und einer Zone verminderten Wachstums (LAG) bestehend, waren bei geschlechtsreifen Fröschen ausgebildet. Da jedes Jahr ein Wachstumsring hinzukommt, ergibt sich bei dieser Froschart in natürlichen Populationen ein Höchstalter von sieben Jahren . Unreife Jungtiere zeigten keine LAGs. Bei Pärchen in Umklammerung hatten die Individuen eine bis fünf LAGs. Dabei waren die Weibchen immer größer (Kopf-Rumpf-Länge) als ihre Partner, die ihrerseits entweder ebensoalt wie oder jünger als die zugehörigen Weibchen waren.

ABSTRACT

Skeletochronological estimation of age, longevity, age at sexual maturity and breeding of the Indian Tree Frog *Polypedates maculatus* (Gray, 1833) is described in this paper. The frogs (n=76) were collectedduring the months of June – September in the years 2005 – 2006. The snout-vent length of each frog was recorded. The 4th toe of the left hind limb of each frog was clipped under anesthesia and fixed in 70% ethanol. Eight frogs belonging to different size groups were sacrificed to determine the gonadal status. The same frogs were utilized to study the histology of both long (humerus and femur) and phalangeal bones. Based on the external morphology and gonadal status, the frogs were divided into three groups i.e. the immatures (Group-I), mature males and females (Group-II) and amplecting pairs (Group-III). Subsequently, phalanges and long bones were decalcified in 10% EDTA and processed for histology. One to six growth rings consisting of growth zones and lines of arrested growths (LAGs) were observed in mature frogs. As a single growth ring is added every year, maximum age of this species is considered to be seven years in natural populations. Immature frogs did not show any LAG. The amplecting pairs showed one to five LAGs and the females were always larger in size (snout-vent length) than the corresponding male partners. Among the amplecting pairs, males were either of the same age group or younger than their female partners.

KEYWORDS

Amphibia: Anura: Rhacophoridae: *Polypedates maculatus*, lines of arrested growth, age, growth pattern, growth rings, longevity, population ecology, India

INTRODUCTION

Skeletochronology is considered to be the most reliable and powerful tool to estimate the age and longevity of amphibians (Castanet & Smirina 1990; Smirina 1994; Kumbar & Pancharatna 2001a). In long bones as well as phalanges, amphibians and reptiles develop growth marks in which broader zones correspond to periods of fast

Table 1: Snout-vent length (SVL, mm) and number of LAGs in different groups of *Polypedates maculatus* (Gray, 1833). n – number of frogs; SD – Standard deviation.

Tab. 1: Kopf-Rumpf-Länge (SVL, mm) und Anzahl der Linien verminderten Wachstums (LAGs) in verschiedenen Altersgruppen von *Polypedates maculatus* (Gray, 1833). n – Stichprobengröße; SD – Standardabweichung; Female – Weibchen; Male – Männchen.

Age group / Altersgruppe	n	SVL (mm) Mean ± SD (Range) Mittelwert ± SD (Spannweite)	Number of LAGs / Anzahl LAGs Median / Mode (Range) Median / Modalwert (Spannweite)
Immature / Jungtiere	16	33.03 ± 9.19 (15.0 – 50.0)	0 / 0 (0 – 0)
Mature / Geschlechtsreife	Female 26	61.32 ± 10.67 (41.0 – 83.0)	3 / 1 (1 – 6)
	Male 24	44.61 ± 3.79 (35.0 – 53.0)	2 / 2 (1 – 4)
Amplecting Pairs / Verpaarte	Female 5	59.9 ± 3.87 (56 – 65.5)	4 / 5 (1 – 5)
	Male 5	45.1 ± 2.45 (42 – 47.5)	3 / 3 (1 – 3)

growth and lines of arrested growth (LAGs) to resting periods (Castanet & Smirina 1990; Smirina 1994; Kumbar & Pancharatna 2001a). So, one broad zone (growth ring) plus its enveloping LAG represents one year of growth, which corresponds to the age of the individual (Halliday & Verrell 1988; Castanet & Smirina 1990; Smirina 1994). There are few reports on skeletochronological estimation of age in some south Indian anurans (Kumbar & Pancharatna 2001a, 2001b, 2002; Pancharatna & Despande 2003). Skeletochronological estimation of age of only two anurans inhabiting eastern India has been reported (Nayak et al. 2007, 2008). So, in the present study an attempt has been made to investigate the age, longevity, age at sexual maturity and breeding in a natural population of the Indian rhacophorid tree frog, *Polypedates maculatus* (Gray, 1833) inhabiting Bhubaneswar (20°18’ N, 85°50’E), eastern India.

MATERIALS AND METHODS

During the course of study (June 2005 to September 2006), 76 specimens of *Polypedates maculatus* (Gray, 1833) measuring 15.0 to 83.0 mm in snout-vent length (SVL) were collected from various localities of Bhubaneswar city, Orissa, eastern India. Following capture, the frogs were anaesthetized with ether and the SVL was recorded. The 4th toe of the left hind limb was cut off from each frog and fixed in 70% ethanol. To study the gonadal status, eight frogs of different size groups were sacrificed. The same specimens were fixed in 70% ethanol for study of histology of both long (femur and humerus) and phalangeal bones. The remaining 68 frogs were released to the locality of their capture. Based on the size, secondary sex characters and gonadal status, the specimens were divided into three groups, i.e. immature males and females (Group I, n = 16), mature males and females (Group II, n = 26 + 24) and amplecting pairs (Group III, n = 5 + 5), respectively. Both long bones and toes were water rinsed for one hour and decalcified in 10% EDTA (ethylene diamine tetra-acetic acid) solution for 24-48 h, depending on the size of the bone. After decalcification, the bones were rinsed thoroughly for 24 h in running tap water. Following rinsing, the bones were dehydrated and processed for paraffin block preparation. Serial sections ranging from 8 to 10 µm were cut using a rotary microtome. The sections were stained in Delafield’s haematoxylin and eosin and examined under a compound microscope. Photographs were obtained from appropriate sections using an Olympus® OIC 04194 microscope and Pentax® K1000 camera. The relationship between body size (SVL) and age was assessed by drawing scattered plots and calculating correlation coefficients (Pearson’s *r*’) (Steel & Torrie 1980).

Table 2: Snout-vent length (SVL, mm) and number of LAGs in 10 individuals of five amplecting pairs of *Polypedates maculatus* (Gray, 1833). F – Female; M – Male.

Tab. 2: Kopf-Rumpf-Länge (SVL, mm) und Anzahl der Linien verminderten Wachstums (LAGs) bei fünf Pärchen (10 Individuen) von *Polypedates maculatus* (Gray, 1833). F – Weibchen; M – Männchen.

Amplecting pair # Pärchen Nr.	SVL (mm) F / M	Number of LAGs / Anzahl LAGs F / M
1	62.0 / 43.0	1 / 1
2	56.0 / 47.0	2 / 2
3	59.0 / 46.0	5 / 3
4	57.0 / 42.0	5 / 3
5	65.5 / 47.5	4 / 3

Growth pattern in body size (SVL) was analyzed by fitting the logistic model $L = a / (1 + be^{ct})$, where 'L' is the average SVL at a given age t, 'a' is the asymptotic SVL; 'e' is the base of natural logarithms, 'b' is the growth rate, 'c' is the constant and 't' is the time (age) in years. We used the software package SPSS® to compute and visualize the logistic regression function.

RESULTS

Cumulative data on size (SVL) and number of LAGs present in different groups is given in Table 1. The matured males were comparatively smaller in size than the females in the natural population. Similarly, among the amplecting pairs the SVLs of males were found to be less than in the corresponding amplecting females (Table 2).

The histological structure of both long bones and the phalanges showed a central marrow cavity (mc) lined by an endosteal layer (e) (Figs. 1A, 1B). The middle layer (cortex, c) consisted of a matrix and evenly scattered osteocytes (o). The cortex was covered by an outer periosteal layer (p). In the mature frogs, a series of concentric darkly stained chromophilous lines were observed. These lines were distinct and separated from each other by a wide lighter ring or band of osseous tissue. The darker lines were interpreted as lines of arrested growth (LAGs) and the broader light colored rings as growth rings. So, each growth ring along with its LAG was considered as an 'annual ring' or growth mark (Figs. 1C, 1D and 2A - 2D). The osteocytes were sparsely distributed in the growth rings while the LAGs were devoid of osteocytes.

In group I (immature), the bone histology of frogs (SVL 15.0 – 50.0 mm) showed no LAG (Figs. 1A, 1B). The cross section of femur of the smallest frog (SVL 15.0 mm) showed a thin cortex (Fig. 1A). The endosteal and periosteal layers were indistinct. The marrow cavity was filled with vacuolated cells and there was no LAG in the matrix. The cross section of the femur bone of another immature frog (SVL 42.0 mm) showed a comparatively broader cortex than the smallest captured frog, with evenly scattered osteocytes (Fig. 1B) and showed no LAG in the cortex.

In group II (matured) frogs, one to four LAGs were observed in males (Table 1 and Figs. 1C, 2B, 2C), whereas one to six LAGs were found in females (Figs. 1D and 2A, 2D). The youngest matured female (SVL 41 mm) showed one LAG (Fig. 1D) in the femur bone, indicating the age of the specimen to be more than one year or it was in the second year of growth after metamorphosis. A female with SVL 68.0 mm showed six LAGs (Fig. 2D). Similar six rings were observed in female frogs with SVL 58.0 and 65.0 mm, respectively. However, the cross sections of the femur bone of the largest female frog captured in the present study (SVL 83.0 mm) showed only three LAGs (Fig. 3).

The smallest matured male (SVL 35.0 mm) showed one LAG (Fig. 1C) whereas the largest male frog (SVL 53.0 mm)

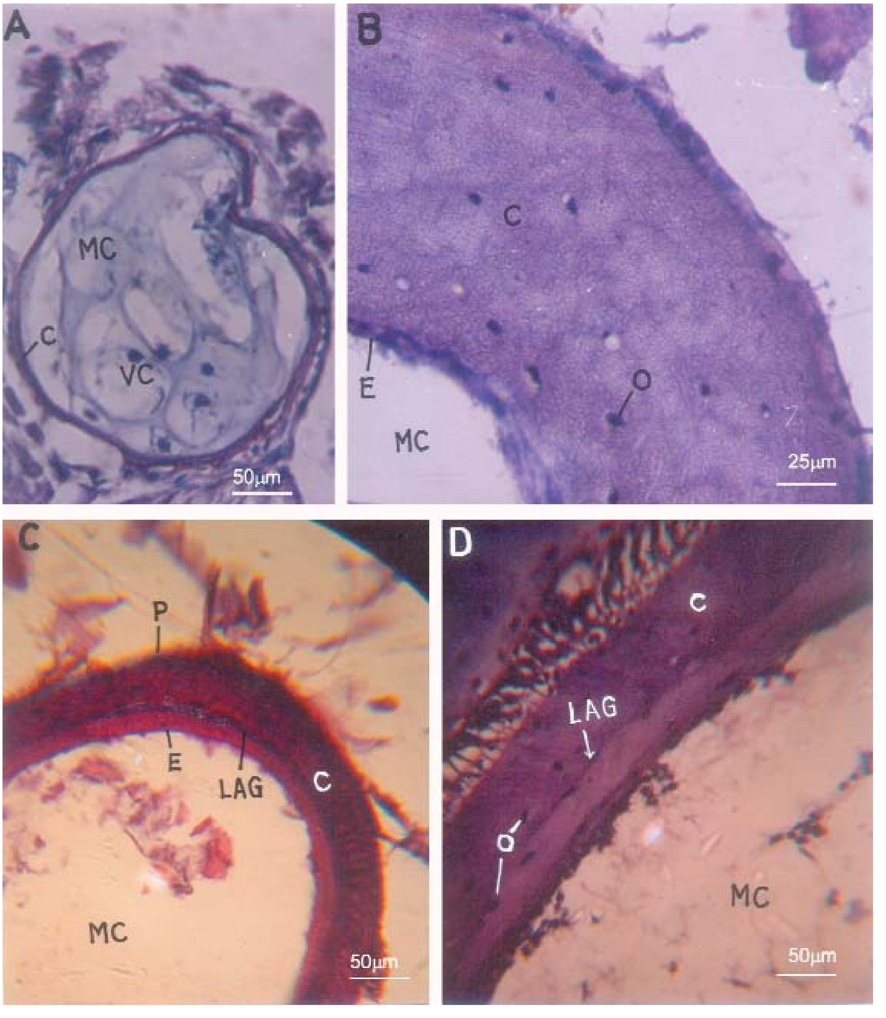

Fig. 1: Cross sections (CS) of femur bones of *Polypedates maculatus* (GRAY, 1833).
Arrows indicate Lines of Arrested Growth (LAGs). SVL - Snout-vent length.

1A - CS of the femur of a metamorphosed frog from Group I (SVL = 15.0 mm) showing the marrow cavity (MC) filled with vacuolated cells (VC), a thin cortex (C) and no LAG. 1B - CS of the femur of an immature frog of Group I (SVL = 42.0 mm) showing the central marrow cavity (MC), a thicker cortex (C) lined by the outer periosteal layer and the inner endosteal layer (E), evenly scattered osteocytes (O) in the cortex (C) and no LAG. 1C - CS of the femur of the smallest mature male frog (SVL=35.0 mm) showing a central marrow cavity (MC) and one LAG in the cortex. 1D - CS of the femur of the smallest matured female (SVL = 41.0 mm) showing a central marrow cavity (MC) and one LAG in the cortex.

Abb. 1: Querschnitte von Oberschenkelknochen bei *Polypedates maculatus* (GRAY, 1833).
Pfeile zeigen auf Linien verminderten Wachstums (LAGs). KRL - Kopf-Rumpf-Länge.

1A - Querschnitt des Femurs eines metamorphosierten Frosches von Gruppe I (KRL = 15,0 mm) mit einer von vakuolisierten Zellen (VC) erfüllten Markhöhle (MC), dünnem Kortex (C) und ohne LAGs. 1B - Querschnitt des Femurs eines Jungtieres von Gruppe I (KRL = 42,0 mm) mit zentraler Markhöhle (MC), dickerem Kortex (C), außen von periostalen, innen von endostalen (E) Zellagen begrenzt, Osteozyten (O) im Kortex (C) regelmäßig verteilt, keine LAGs. 1C - Querschnitt des Femurs des kleinsten geschlechtsreifen Männchens (KRL = 35,0 mm) mit zentraler Markhöhle (MC) und einer LAG im Kortex. 1D - Querschnitt des Femurs des kleinsten geschlechtsreifen Weibchens (KRL = 41,0 mm) mit zentraler Markhöhle (MC) und einer LAG im Kortex.

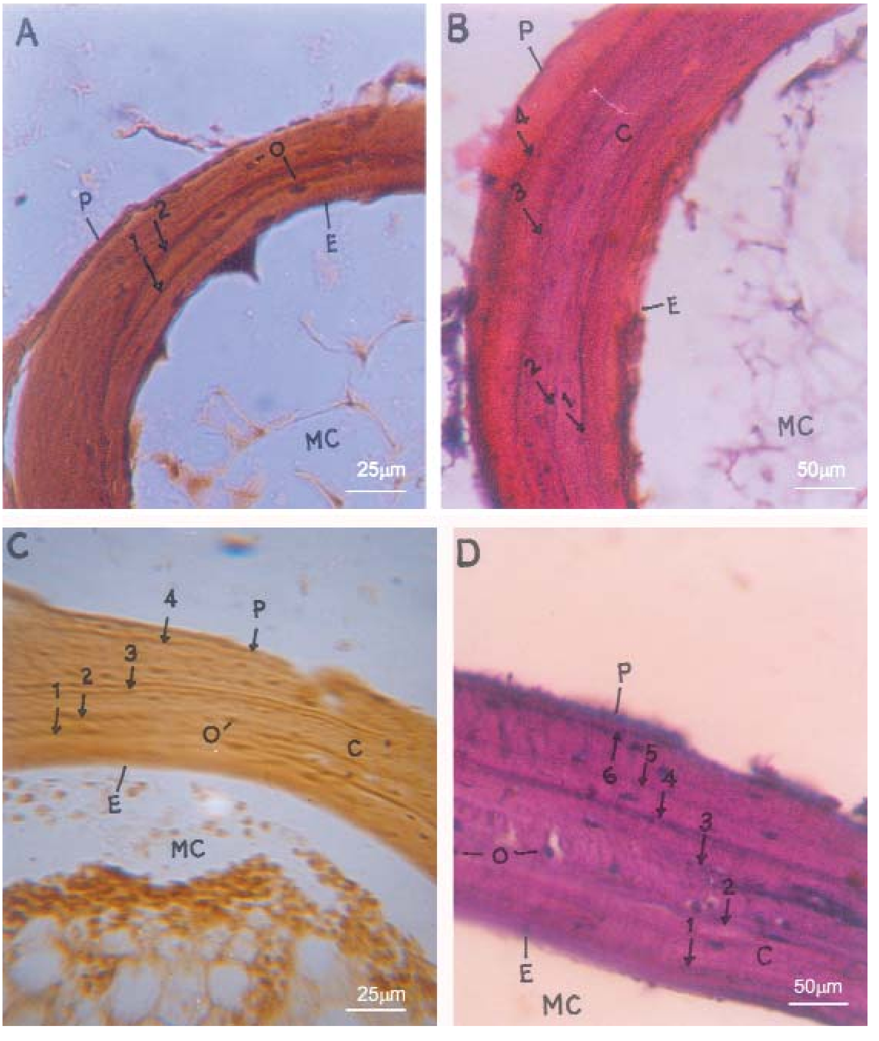

Fig. 2: Cross sections (CS) of femur bones and phalanges of matured *Polypedates maculatus* (GRAY, 1833). Arrows indicate Lines of Arrested Growth (LAGs). SVL - Snout-vent length.

2A - CS of 4th toe of the hind limb of a matured female frog (SVL = 46.5 mm) showing a spacious marrow cavity (MC), and two LAGs (1, 2) in the cortex peripheral from the endosteal layer (E).
2B - CS of the femur of a matured male frog (SVL= 45.0 mm) showing the central marrow cavity (MC) and four LAGs (1-4) in the cortex (C).
2C - CS of the 4th toe of the male frog of Fig. 2B showing four LAGs (1-4) in the cortex (C).
2D - CS of the femur of the oldest female frog (SVL= 68.0) showing six LAGs (1-6) in the cortex (C) peripheral from the endosteal layer (E) and evenly scattered oesteocytes (O) in the cortex (C).

Abb. 2: Querschnitte von Oberschenkel- und Zehenknochen bei *Polypedates maculatus* (GRAY, 1833). Pfeile zeigen auf Linien verminderten Wachstums (LAGs). KRL - Kopf-Rumpf-Länge.

2A - Querschnitt der 4. Hinterbeinzehe eines reifen Weibchens (KRL = 46,5 mm) mit geräumiger Markhöhle (MC) und 2 LAGs (1, 2) im Kortex außerhalb der endostalen Zellage (E).
2B - Querschnitt des Femurs eines reifen Männchens (KRL = 45,0 mm) mit 4 LAGs (1-4) im Kortex (C).
2C - Querschnitt der 4. Hinterbeinzehe des Männchens von Abb. 2B mit 4 LAGs (1-4) im Kortex (C).
2D - Querschnitt des Femurs des ältesten Weibchens (KRL = 68,0 mm) mit 6 LAGs (1-6) im Kortex (C) außerhalb der endostealen Zellage (E) und regelmäßig verteilten Osteozyten (O) im Kortex (C).

showed two LAGs (Fig. 4). The maximum number of growth rings was four in the males of this species and found in one male with SVL 45 mm (Figs. 2B, 2C). Similar numbers of LAGs were observed in two more males with SVL 40.5 and 45.0 mm, respectively. Based on the LAGs, maximum age of males is considered to be 5 years in this species. The largest male frog (SVL 53.0 mm) showed only two LAGs (Fig. 4). So, SVL should not be considered as the sole criterion for age determination in this species.

Five amplecting pairs (group III) were investigated in the present study (Table 2). In the first pair, both the male and the female had 1 LAG each. In the second pair, two LAGs were observed both in the male and the female specimen. However, in both the third and the fourth pair, the females showed 5 LAGs whereas males showed 3 LAGs each. In the fifth pair, the female had developed four LAGs while the male had three LAGs. In all the pairs the males were smaller in size (SVL) than the corresponding females. Males amplexed females which were either of their own age group or older.

The growth curves of *P. maculatus* based on snout-vent length (SVL) and estimated by the Logistic Model indicated a higher growth rate for both males and females up to the second year (Fig. 5). The curves showed the mean SVL of 20.75 mm for juveniles at '0' age and grew till attainment of a mean SVL of 55.3 mm in females and 41.17 mm in males in the second year, by which time they attained sexual maturity. Beyond this age, growth was slower showing variation in growth rates between the sexes. The growth rate was higher for females (b = 21.85) than for males (b = 14.45). After the second year, the females grew slowly but steadily till a maximum age of seven years attaining a mean SVL of 63.7 mm. Growth was much slower in males reaching up to a mean SVL of 44.5 mm at the maximum age of five years. When the body size (SVL) of the smallest metamorphosed (SVL 15.0 mm) was compared with that of the largest adults, they were larger by 3.53 times in males and by 5.53 times in females. This simple comparison between body sizes of the smallest the largest frogs also clearly indicated a higher growth rate in the females than in males.

The number of LAGs remained the same both in the long as well as phalangeal bones (Figs. 2B, 2C) in this species.

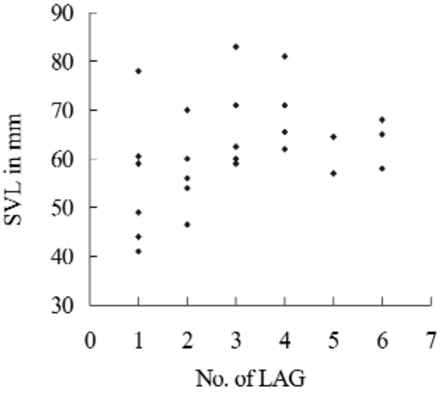

Fig. 3: Correlation between snout-vent length (SVL, mm) and number of LAGs in matured female *Polypedates maculatus* (Gray, 1833) (n = 26).

Abb. 3: Beziehung zwischen Kopf-Rumpf-Länge (SVL, mm) und Anzahl der LAGs bei eifen Weibchen von *Polypedates maculatus* (Gray, 1833) (n = 26).

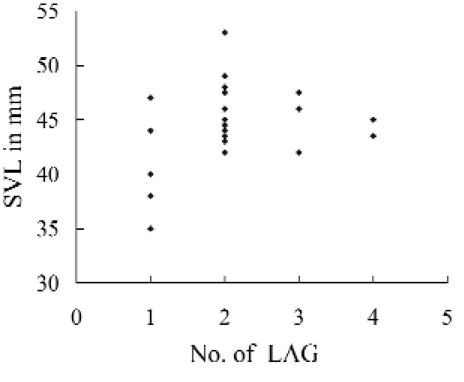

Fig. 4: Correlation between snout-vent length (SVL, mm) and number of LAGs in matured male *Polypedates maculatus* (Gray, 1833) (n = 24).

Abb. 4: Beziehung zwischen Kopf-Rumpf-Länge (SVL, mm) und Anzahl der LAGs bei eifen Männchen von *Polypedates maculatus* (Gray, 1833) (n = 24).

DISCUSSION

Based on the findings of the present investigation, the technique of skeletochronology is suggested to be the most reliable method for age determination in the Indian Tree Frog, *P. maculatus*. In anurans the hatching of tadpoles is accepted as age '0' and a LAG is formed each winter while periosteal growth begins anew each spring (Kalb & Zug 1990). By the end of spring frogs usually mature and become ready for breeding activities in the coming monsoon. So, the age of frogs with one LAG is considered as more than one year as they had completed one year of growth. Absence of growth rings (LAGs) in immature frogs shows that the animals had not gone through the dormant stage of winter when LAGs are formed in the bone (Kalb & Zug 1990; Marnell 1997). The age of these frogs was considered to be within one year. In the present study bone histology has revealed that the cortexes of smaller immature frogs are thinner than of larger immature frogs (Figs.1A and 1B). So, during the process of growth with increase in body size, bones also become thicker. The smallest mature male investigated in the present study showed one LAG, whereas immature males of the same size showed no LAG. Thus, size cannot be considered as one of the characters for estimation of age, i. e., a larger frog is not necessarily older.

It is normally the number of winters an animal has survived that is counted rather than the true age of the animal. So, some researchers interpret the skeletochronological age estimation in amphibians as 1+, 2+ or 3+ years (Kalb & Zug 1990; Marnell 1997) instead of 2, 3 or 4 years, respectively. In the present study, the specimens were collected mostly during the breeding season (June to September). The smallest mature male as well as the female showed one LAG each, indicating their age to be 1+ year or are considered as two years (Figs. 1C and 1D). None of the frogs were found to be mature without LAG and all the frogs having LAGs were matured. Therefore, it is concluded that sexual maturity in this species is attained during the second year of their growth i.e. when they have one LAG. Similar findings have also been reported in other tropical anurans i. e. *Microhyla ornata* (Duméril & Bibron, 1841) (Kumbar & Pancharatna 2001b) and *Euphlyctis cyanophlyctis* (Schneider, 1799) (1996).

Females were comparatively larger in size (SVL) than the males (Table 1). Larger females have also been observed in different other anuran species i.e. *Bufo* (*Amietophrynus*) *pardalis* (Hewitt, 1935) (Cherry & Francillon–Vieillot 1992), *B.* (*Anaxyrus*) *americanus* (Holbrook, 1836) (Kalb & Zug 1990), *E. cyanophlyctis* (Pancharatna et al. 2000), *M. ornata* (Kumbar & Pancharatna 2001b). Analysis of correlation between SVL and LAGs for both the males and females (Figs. 3 and 4), in the present study showed the correlation coefficient '*r*' to be 0.371 for females and 0.257 for males, respectively. In both males and females the correlation was positive but was not significant. A positive correlation has also been reported in *P. maculatus* inhabiting south India (Kumbar & Pancharatna 2001a). However, a small number of adult specimens irrespective of sex were investigated and the maximum numbers of LAGs observed were 3 in contrast to 6 of the present finding.

Comparing the present and earlier studies (Kumbar & Pancharatna 2001a) it is suggested that *P. maculatus* inhabiting eastern India live longer (7 years) than the frogs inhabiting south India (4 years) (Kumbar & Pancharatna 2001a). The difference, is suggested to be related to the geographical position of the two regions. A similar difference in maximum age has also been reported for *B.* (*Duttaphrynus*) *melanostictus* (Schneider, 1799) (Nayak et al. 2007; Kumbar & Pancharatna 2001a). It has been reported earlier that longevity of temperate anurans inhabiting colder regions is usually more than the tropical anurans inhabiting warmer regions (Smirina 1994). The temperate frogs such as *Pelophylax ridibundus* (Pallas, 1771), *P. esculentus* (Linnaeus, 1758), and *Rana temporaria* Linnaeus, 1758 survive for 12, 12 and 14 years, respectively (Smirina 1994). Whereas, longevity of tropical *E. cyanophlyctis* has been reported to be 7 years (Kulkarni & Pancharatna 1996). However, longevi-

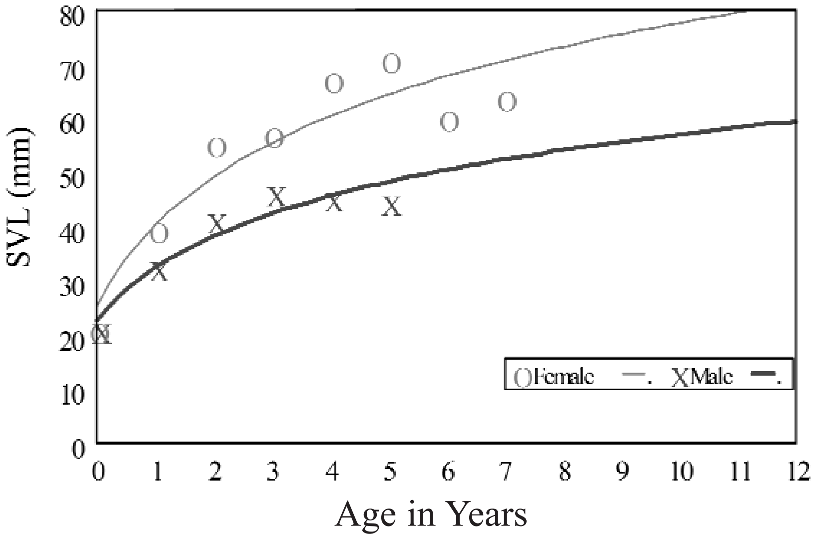

Fig. 5: Growth curves for males and females of *Polypedates maculatus* (GRAY, 1833) as described by the Logistic Model. The logistic functions are $L = 46.0/(1+14.45*e^{22.49\,t})$ for males (X) and $L = 70.8/(1+21.85*e^{25.31\,t})$ for females (O).

Abb. 5: Wachstumskurven für Männchen und Weibchen von *Polypedates maculatus* (GRAY, 1833) wie sie vom Logistischen Modell beschrieben werden. Die logistischen Funktionen sind $L = 46.0/(1+14.45*e^{22.49\,t})$ für Männchen (X) und $L = 70.8/(1+21.85*e^{25.31\,t})$ für Weibchen (O).

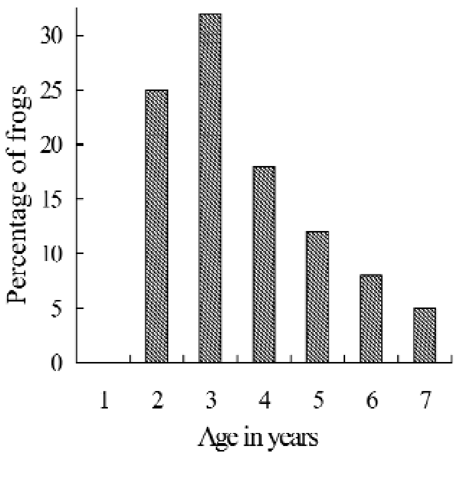

Fig. 6: Frequency distribution (percentage) of age classes among the *Polypedates maculatus* (GRAY, 1833) individuals studied.

Abb. 6: Häufigkeitsverteilung (Prozentsatz) der Altersklassen bei den untersuchten Individuen von *Polypedates maculatus* (GRAY, 1833).

ty of an east Indian ranid, *Euphlyctis hexadactylus* (LESSON, 1834) has been described to be 14 years (NAYAK et al. 2008) which is comparable to the temperate anurans. Longevity of *B. bufo* (LINNAEUS, 1758), *B. pentoni* ANDERSON, 1893 and *B.* (*Rhinella*) *arenarum* (HENSEL, 1867) inhabiting temperate regions has been described to be 12, 6 and 8 years, respectively (HEMELAAR 1983). Longevity of other tropical anurans has been described as 5 years for *M. ornata* (KUMBAR & PANCHARATNA 2001b) and 4 years for *Fejervarya limnocharis* (GRAVENHORST, 1829) (PANCHARATNA & DESPANDE 2003).

Females usually lived longer than the males in the present study. Maximum age of females was found to be 7 years, whereas it was 5 years in males. Longevity of *P. maculatus* inhabiting south India has been described to be 4 years but age of males and females has not been described separately (KUMBAR & PANCHARATNA 2001a). Similar reports describing females to be older than males has been reported for *B. bufo*, *R. tem-*

poraria, P. ridibundus, and *Lithobates pipiens* (Schreber, 1782) (Smirina 1994), *B.* (*A.*) *americanus* (Kalb & Zug 1990) and *F. limnocharis* (Pancharatna & Despande 2003).

Among the amplecting pairs, both the partners were either of the same age group or females were older than the males. In all the amplectant couples the females were larger in size (SVL) than the males, which is in agreement with the earlier reports on other anuran species – *B. bufo*, Hemelaar (1983); *R. temporaria*, Ryser (1988); *B.* (*A.*) *pardalis*, Cherry & Francillon–Vieillot (1992); *B.* (*Epidalea*) *calamita*, Tejedo et al. (1997); *E. cyanophlyctis,* Pancharatna et.al. (2000); *M. ornata*, Kumbar & Pancharatna (2001b); *F. limnocharis*, Pancharatna & Despande (2003).

Analysis of the growth curves based on snout-vent length (SVL) in *P. maculatus* showed a higher rate of growth for both males and females up to the second year of age i.e. until the frogs became sexually mature. After the second year, the growth rate became slower in both the sexes. But in males, the growth rate slowed down more quickly than in the females, which resulted in a higher growth rate (b = 21.85) in females than in males (b = 14.45). Such a difference in growth rates between sexes after sexual maturity was regarded as the main cause of sexual size dimorphism in species (Misawa & Matsui 1999). Similar growth curves have also been reported for several amphibians i.e. *R. temporaria* (Ryser 1988); *Lithobates chiricahuensis* (Platz & Mecham, 1979) (Platz et al. 1997); *Hynobius kimurae* Dunn, 1923 (Misawa & Matsui 1999); and *R. latastei* Boulenger, 1879 (Guarino et al. 2003).

In order to study the longevity of amphibians in a natural population a random collection of large sample size (> 60, consisting of all the possible age groups) is advised for skeletochronology (Smirina 1994). In the present study, 76 frogs belonging to various age groups were taken for skeletochronological analysis. However, 60 matured frogs (31 females and 29 males) were considered for analysis of frequency of frogs belonging to various age groups in the natural population (Fig. 6). The highest number of frogs belonged to the 3-years age group (32%), followed by the 2-years (25%) and 4-years (18%) age group, respectively. There was a gradual decline in the number of frogs with increase in age. Hence, in a natural population, the older frogs are less in number.

The following facts are concluded from the present investigation: (i) female *P. maculatus* are larger in size than the males; (ii) females live longer than males; (iii) both males and females attain sexual maturity during the second year of their life cycle; (iv) maximum age of *P. maculatus* is seven years in natural population; (v) there exists a positive correlation (not significant) between body size and LAGs both for male and female frogs and (vi) the males amplect larger females which are either of their own age group or older.

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