

ALTITUDINAL VARIATION IN THE AGE AT MATURITY, LONGEVITY, AND REPRODUCTIVE LIFESPAN OF ANURANS IN SUBTROPICAL QUEENSLAND

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ABSTRACT: Skeletochronology has been widely and successfully used to age temperate amphibians, enabling geographic comparisons of longevity and the age at maturity. To date, however, there have been very few similar studies conducted using skeletochronology in tropical or sub-tropical amphibians. In this study, we examined the applicability of skeletochronology for aging four sub-tropical anuran species (*Litoria chloris*, *L. lesueuri*, *L. pearsoniana*, and *Mixophyes fleayi*) that occur across a range of altitudes in southeast Queensland, Australia. We then used reliable estimates to examine altitudinal variation in longevity, age at maturity (AM), and potential reproductive lifespan (PRLS) for each species. Skeletochronology was successful in three of the four species. The age of *L. lesueuri* individuals from low altitude sites could not be reliably estimated due to extended activity seasons. On average, females were older than males in *L. chloris*, *L. pearsoniana*, and *M. fleayi* and were also older when breeding for the first time. There was, however, no significant difference in the PRLS between males and females within any of the three species. There were trends towards greater longevity and older AM in high altitude populations of all three species; however, there was no significant altitudinal variation in PRLS in any of the species. Our results suggest little intraspecific variation in the number of years that individuals of the four species are capable of breeding, regardless of gender, geographic location, longevity, and AM.

Key words: Altitude; Body size; Delayed reproduction; Frogs; LAGs; Precocity; Skeletochronology

THE LENGTH of time required from birth or hatching to sexual maturity is an important aspect of any species population dynamics. The age at maturity (AM) is pivotal because fitness is often more sensitive to changes in this trait than to changes in any others (Stearns, 1992). The AM is linked with factors including length of the growing or activity season, level of metabolism, and difference in size between adults and juveniles (Fitch, 1970) and is usually determined by the size at maturation and juvenile growth (Ryser, 1996). As reproductive maturity is dependent on body size, species or individuals with faster growth rates will reach the minimum size required for reproduction sooner than those with slower growth rates and, thus, will be able to begin breeding at a younger age.

The AM is a compromise among other life history variables. Maturing and reproducing early is one method of increasing lifetime fitness. However, small body size reduces the

number and/or size of offspring, and smaller adults tend to experience higher levels of predation than larger adults (Zug, 1993). The benefits of delayed reproduction are less obvious and generally relate to advantages associated with large body size. As growth rates of ectotherms decrease greatly after the attainment of sexual maturity (Hemelaar, 1988), organisms that delay reproduction are able to achieve larger body sizes than precocious individuals. This larger body size results not only in increased success at holding breeding territories (Tinkle, 1969), but also in greater survivorship of parents and production of larger clutches (Gibbons and McCarthy, 1986) and/or larger offspring that may have higher survival rates than smaller offspring (Begon et al., 1990; Berven, 1982; Stearns, 1992).

The AM is proportional to longevity (Houck, 1982; Miaud et al., 2000; Tilley, 1980). Animals with shorter lifespans start reproducing at a younger age to compensate for their short breeding lives, while those with longer lifespans are older when breeding for the first time (Caetano and Castanet, 1993; Zug, 1993). Longevity or length of life is not as essential in

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its own right as it is in determining the reproductive lifespan of an individual (Begon et al., 1990). How long an individual lives is not as important to a population's demography and survival as the number of years it reproduces.

Due to restricted growing seasons, high altitude temperate amphibians exhibit slower growth and development rates through metamorphosis and juvenile stages and, consequently, tend to live longer and mature later than their lowland counterparts (Joly and Grolet, 1996; Miaud et al., 1999, 2000; Ryser, 1996; Schabetsberger and Goldschmid, 1994; Smirina and Rocek, 1976). This delay in maturation results in larger body sizes, greater fecundity, and higher offspring survival in high altitude populations (Joly and Grolet, 1996; Miaud et al., 2000; Ryser, 1996; Sagor et al., 1998). Although trends appear strong in temperate species, to date no studies have examined altitudinal variation in longevity and AM in tropical or subtropical amphibian species.

Although skeletochronology is now widely used to age amphibians, very few skeletochronological studies have been carried out on amphibians from tropical or sub-tropical regions (Halliday and Verrell, 1988; Khonsue et al., 2000). Most previous studies used temperate species, e.g., *Bufo bufo* (Hemelaar, 1985, 1988), *B. calamita* (Denton and Beebee, 1996), *Rana sylvatica* (Bastien and Leclair, 1992; Sagor et al., 1998), *R. temporaria* (Ryser, 1996), and *R. sakuraii* (Kusano et al., 1995), to name a few. Fewer studies have been carried out on desert species, including *B. pentoni* (Barbault et al., 1979), *Scaphiopus couchii* (Tinsley and Tocque, 1995), and *B. raddei* (Kuzmin and Ischenko, 1997). To date, only four studies have been carried out on tropical species, *Mantidactylus microtympaanun* in Madagascar (Guarino et al., 1998), *R. nigrovittata* in Thailand (Khonsue et al., 2000), *R. cyanophlyctis* in India (Pancharatna et al., 2000), and three other anuran species found in India (Kumbar and Pancharatna, 2001). Consequently, the first aim of our study was to examine the applicability of the skeletochronological method for anurans from sub-tropical Australia.

We selected four anuran species, *Litoria chloris*, *L. lesueuri*, *L. pearsoniana* (Anura: Hylidae), and *Mixophyes fleayi* (Anura: Myo-

batrachidae), from a range of altitudes in the southeast Queensland region for study. These species are associated with stream habitats (mainly in rainforest), and *L. lesueuri* and *M. fleayi* have fairly protracted breeding/activity seasons (up to 8 mo), while the activity season length (ASL) in *L. chloris* and *L. pearsoniana* is comparatively restricted (approximately 4 mo) (Morrison, 2001).

If skeletochronology was found to be applicable, the primary aim of our study was to determine whether life history patterns observed in sub-tropical anuran species were similar to those previously reported for temperate anuran species. Specifically, our aims were (1) to determine the average longevity, AM, and PRLS for each population, (2) to determine whether ASL has an influence on LAG formation, (3) to determine whether females lived longer, matured at an older age, and had greater PRLS than males, (4) to determine whether average longevity, AM, and PRLS increased with altitude, and (5) to determine whether body size could be used to estimate age accurately and reliably in these species.

MATERIALS AND METHODS

Field Methods

We collected samples from thirteen 200-m long transects at a range of altitudes (100–800 m) in southeast Queensland, Australia. Ten of these sites were repeatedly surveyed over three field seasons from September 1997–May 2000 to also determine ASL for each population (see Table 1 for site details). Samples were collected once a year from the remaining three sites. The snout-urostyle length (SUL) of each individual captured was measured to the nearest 0.1 mm using vernier calipers, and then each individual was marked with a unique toe-clip code. Clipped toes were stored in either formaldehyde acetic acid calcium chloride (FAACC) or 70% alcohol until they were ready for processing.

Laboratory Methods: Skeletochronology

The toes were rinsed in tap water, then decalcified in 10% nitric acid for approximately 24–48 h. Once decalcification was complete, the toes were rinsed three times in distilled water, left overnight in distilled water, and then

TABLE 1.—Study site descriptions for *Litoria chloris*, *L. lesueuri*, *L. pearsoniana*, and *Mixophyes fleayi*. * indicates sites that were repeatedly surveyed over 3 yr.

Site	Lat/Long	Altitude (m)	Species
Austinville*	28° 10' 46" S/153° 18' 16" E	100	<i>L. chloris</i> , <i>L. pearsoniana</i>
Waterfall Creek	28° 09' 02" S/153° 14' 43" E	110	<i>L. lesueuri</i>
Tallebudgera*	28° 13' 05" S/153° 19' 05" E	120	<i>L. lesueuri</i>
Currumbin*	28° 14' 36" S/153° 21' 06" E	160	<i>L. chloris</i> , <i>L. pearsoniana</i> , <i>L. lesueuri</i> , <i>M. fleayi</i>
Cedar Creek*	27° 53' 40" S/153° 11' 16" E	250	<i>L. lesueuri</i>
Natural Bridge*	28° 13' 58" S/153° 14' 36" E	250	<i>L. chloris</i> , <i>L. pearsoniana</i> , <i>L. lesueuri</i> , <i>M. fleayi</i>
Canungra Creek*	28° 12' 40" S/153° 09' 50" E	500	<i>M. fleayi</i>
Bundoomba Creek*	28° 13' 08" S/153° 08' 21" E	520	<i>M. fleayi</i>
Stockyard Creek	28° 12' 56" S/153° 06' 58" E	550	<i>M. fleayi</i>
Purling Brook*	28° 11' 30" S/153° 16' 12" E	600	<i>L. lesueuri</i>
Pyramid Creek	28° 10' 50" S/153° 09' 20" E	640	<i>M. fleayi</i>
Bouloomba Creek*	26° 42' 57" S/152° 34' 40" E	700	<i>M. fleayi</i>
Mundora*	28° 13' 28" S/153° 17' 02" E	800	<i>L. chloris</i> , <i>L. pearsoniana</i>

wax embedded. Toes were processed for paraffin wax embedding on a Tissue Tek VIP Processor and then sectioned at 10 μ m using an AO-820 Spencer microtome. Sections were stained for 40 min with Ehrlich's haematoxylin, then rinsed twice in tap water. Stained sections were coverslipped and sealed with Depex mountant. Care was taken to ensure that sections never dried out during the staining process. All sections were then dried at 60 C overnight before growth rings were assessed. Sections were examined under a Nikon SE light microscope at 100 \times magnification. The haematoxylin stained the growth rings pink to light purple and the lines of arrested growth (LAGs) dark purple. Good sections for each individual were photographed using a Spot Camera (Diagnostics Instruments Inc., Model No. 1.4.0) and viewed using the computer package Image Pro Plus.

Validation.—To ensure that LAGs were produced annually and represented the actual age of the individual, the number of LAGs in toes collected from the same individual in subsequent years was compared in all species except *L. pearsoniana* (no individuals recaptured in subsequent years). In some cases, toes were collected from individuals after a period of 2 yr.

Age determination.—The LAGs occur in periosteal bone and can sometimes be obscured in older individuals by the replacement of periosteal bone with endosteal bone, which originates at the perimeter of the marrow cavity and progresses toward the bone perim-

eter (Hemelaar, 1985). This endosteal resorption can completely destroy LAGs, leading to errors in skeletochronological age estimations. For example, if LAG 1 (formed during the first post-metamorphic hibernation) has been completely destroyed by endosteal resorption, the innermost visible LAG is, in fact, LAG 2 not LAG 1. If endosteal resorption was not identified, this would result in an age estimate 1 yr less than the actual age.

Identification of endosteal resorption was carried out as per Sagor et al. (1998). The longest and shortest perpendicular axes of each LAG in each section examined were measured. Axis measurements were multiplied together and the square root of the product calculated (average diameter of each LAG). This procedure was done for the LAGs in each of three diaphyseal sections per specimen. To identify cases of LAG resorption, the diameters of the innermost and second visible LAGs (based on average of three sections per specimen) were plotted on a frequency distribution (Fig. 1). If diameters for the innermost visible LAG were >2 SD greater than the group mean, they were interpreted as cases of LAG 1 resorption, where the innermost LAG was actually LAG 2 and not LAG 1 (Sagor et al., 1998).

Once all specimens had been checked for endosteal resorption, the LAGs were counted in each specimen on two separate occasions, always blind to specimen identity (see Trenham et al., 2000).

Effect of activity season length (ASL) on LAG formation.—The LAGs are formed when

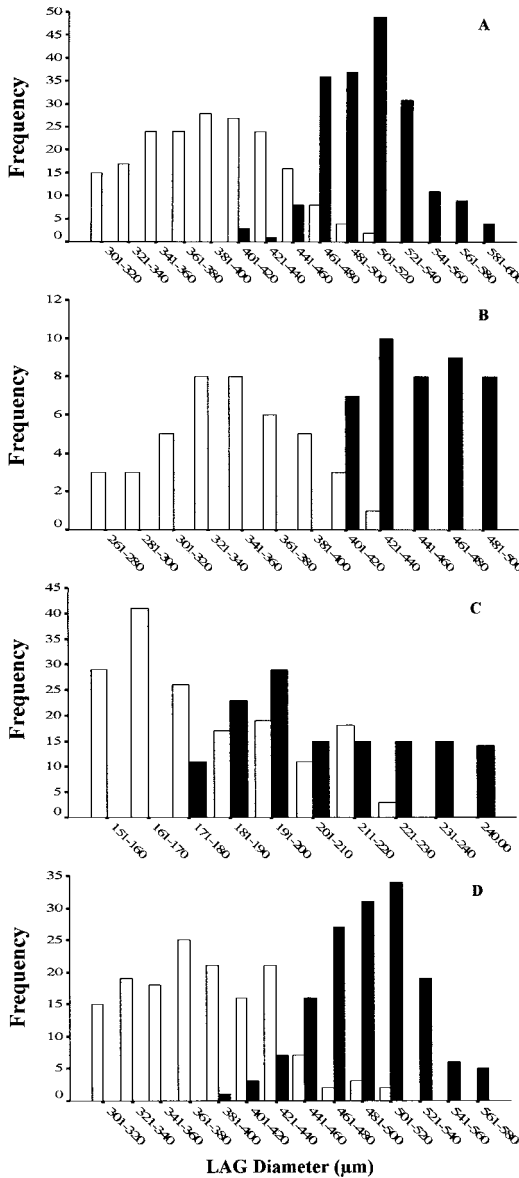


FIG. 1.—The distribution of diameters of the first or innermost (open bars) and the second (closed bars) visible lines of arrested growth (LAG) in phalangeal sections of (A) *Litoria chloris*, (B) *L. lesueuri*, (C) *L. pearsoniana*, and (D) *Mixophyes fleayi*.

bone growth slows down during hibernation or aestivation. Populations or individuals with extended activity or growing seasons may not produce LAGs (or distinct LAGs) due to their limited hibernation or aestivation periods, thereby making age estimation using skeletal-

chronology very difficult. By comparing ASL with the proportion of individuals within the population with clear LAGs, we were able to determine the effect of ASL on LAG formation.

Average Longevity, AM, and PRLS

The average population longevity was calculated as being the average age of all the individuals captured in the population. The AM was the youngest age of all reproductive individuals captured for the population/species. The PRLS was calculated for each population by subtracting the AM from the age of the oldest individual captured. This calculation provides an estimate of the number of years that an individual is expected to breed.

Statistical Analyses

All statistical analyses were executed with SPSS Version 10 for Windows. All response variables were tested for normality using either the Shapiro-Wilk Statistic or Levene's Test. Variables not conforming to assumptions for parametric analyses (ASL, proportion of aged individuals, body size) were analyzed by non-parametric methods. Students *t*-tests were used to compare average longevity, AM, and PRLS in males and females of each species. Spearman's rank correlations were used to determine whether there was a relationship between ASL and the proportion of individuals in the population with clear LAGs.

Simple linear regressions were used to determine the influence of altitude on average longevity, AM, and PLRS for each sex/species. In the cases of individuals being recaptured in more than 1 yr, the oldest recorded age was used in the analyses. Altitude was used in these analyses instead of the two critical climatic variables, rainfall (no significant difference in rainfall between the different sites; Morrison, 2001) and mean minimum monthly temperature (significant negative correlation with altitude; Morrison, 2001). The relationship between body size and age for each sex/species was examined using Spearman's Rank correlations. In the case of individuals being recaptured more than once, data from the last capture was used to avoid pseudoreplication. A 5% type one error was used in all analyses.

RESULTS

Applicability of Skeletochronology and Validation

Diameters of LAGs for all species increased with the age at which they were deposited. The diameter values for the first (innermost) and second LAGs exhibited approximately normal distributions (Fig. 1), and no instances of resorption were identified.

In all individuals recaptured in one or more subsequent years (with the exception of *L. lesueuri*), the number of additional LAGs corresponded directly to the number of years between recaptures (i.e., if recaptured 1 yr apart, there was one extra LAG; if recaptured 2 yr apart, there were two extra LAGs). The direct correspondence between the number of additional LAGs and the number of years between recaptures supports the hypothesis that LAGs are laid down on an annual basis and can be used reliably to age individuals of these species. In the case of *L. lesueuri*, LAGs were only present in individuals from the highest altitude and were absent in low altitude individuals known to be at least 2–3 yr of age (based on mark and recapture results). Consequently, skeletochronology was not considered applicable for *L. lesueuri*.

Effect of ASL on LAG formation.—We were able to estimate reliably the age of captured frogs for 93% (174 of 187) of *L. pearsoniana*, 81% (149 of 184) of *M. fleayi*, 74% (179 of 241) of *L. chloris*, and 10% (42 of 421) of *L. lesueuri* using skeletochronology. The remaining individuals could not be reliably aged due to indistinct resting lines. There was a negative correlation between ASL and the proportion of individuals in a population with distinct LAGs in *L. lesueuri* (Spearman's Rank correlation $r = -0.857$, $P = 0.029$, Table 2). Similar trends were also found in *L. chloris* (Spearman's Rank correlation $r = -0.800$, $P = 0.200$), *L. pearsoniana* (Spearman's Rank correlation $r = -0.949$, $P = 0.058$), and *M. fleayi* (Spearman's Rank correlation $r = -0.872$, $P = 0.054$).

Average Longevity, AM, and PRLS

Litoria pearsoniana.—The vast majority of individuals of both sexes were 2 yr of age. However, small number were 1-yr-old males (4 in total) and 3-yr-old females (3 in total)

(Table 3). The average AM, as well as the average longevity, for most populations was 2 yr of age for both males and females. The 1-yr-old males were only found at the very end of the breeding season (at low altitudes only) and did not have pigmented nuptial pads; consequently, they are most likely to have been juvenile males that were not actually reproductively active that season. The 3-yr-old females found at Mundora Creek may have been females that started breeding at 2 yr of age and survived to breed a second year, or, alternatively, they may have delayed reproduction until they were 3 yr of age. As almost all individuals were 2 yr of age, overall (all years and sites combined) and within-site comparisons did not reveal significant differences in the average longevity between males and females (Students t -test: $t_{122} = 1.145$, $P = 0.861$). Lack of variation in AM and PRLS also meant that there was also no difference between males and females in these characters.

Mixophyes fleayi.—Males ranged between 2–6 yr and females 3–8 yr of age (Table 4). With the exception of Bundoomba, most males and females were 4 yr of age and tended to mature between 3–4 yr of age. The majority of males and females at Bundoomba were 5 yr old, and this site is also where the oldest individual was found (an 8-yr-old female). Two calling males at Currumbin were found to be 2 yr of age; however, despite calling, they did not have strongly pigmented nuptial pads and were small in size compared to the other males and, consequently, were considered immature males. As their inclusion in the data sets did not alter the results of initial analyses, they were retained in all analyses. Overall (all years and sites combined), females were significantly older than males (Students t -test: $t_{154} = -3.209$, $P = 0.002$). Males on average did not mature significantly earlier than females (Students t -test: $t_8 = -1.633$, $P = 0.141$) nor was there a significant difference in the PRLS of males and females (Students t -test: $t_8 = -0.953$, $P = 0.368$).

Litoria chloris.—Males in general ranged between 2–5 yr and females 3–6 yr of age (Table 5). The majority of males and females were between 3–4 yr of age in all populations and appeared to be 3 yr of age when breeding for the first time. Three males were 2 yr old

TABLE 2.—Average active season length (mo \pm 1 SD) and proportion of individuals aged (%) for *Litoria pearsoniana*, *L. chloris*, *L. lesueuri*, and *M. fleayi* in sites repeatedly surveyed over 3 yr. — indicates that the species does not occur at that site.

Site	<i>L. pearsoniana</i>	<i>M. fleayi</i>	<i>L. chloris</i>	<i>L. lesueuri</i>
Austinville	8.0 \pm 0.0 (88%)	—	7.0 \pm 0.0 (77%)	8.0 \pm 0.0 (0%)
Tallebudgera	—	—	—	7.0 \pm 0.0 (0%)
Curumbin	7.0 \pm 1.0 (93%)	7.3 \pm 0.6 (85%)	6.0 \pm 0.0 (72%)	7.3 \pm 1.2 (0%)
Cedar Creek	—	—	—	6.7 \pm 0.6 (2%)
Natural Bridge	7.0 \pm 0.0 (94%)	5.0 \pm 0.0 (90%)	5.7 \pm 0.6 (78%)	7.0 \pm 0.0 (0%)
Canungra Creek	—	6.3 \pm 0.6 (94%)	—	—
Bundoomba Creek*	—	7.0 \pm 0.0 (85%)	—	—
Purling Brook	—	—	—	5.0 \pm 0.0 (74%)
Boulomba Creek	—	6.5 \pm 0.7 (88%)	—	—
Mundora	5.3 \pm 0.6 (96%)	—	4.7 \pm 0.6 (88%)	—

when found at a chorus at Austinville; although they were calling, they had unpigmented nuptial pads and, therefore, may have been immature males. Though the reproductive status of these three individuals remains unclear, they have been retained in all analyses. Overall (all years and sites combined), females were significantly older than males (Students *t*-test: $t_{177} = -2.926$, $P = 0.004$). Males on average did not mature significantly earlier than females (Students *t*-test: $t_8 = -1.414$, $P = 0.195$) nor was there a significant difference in the PRLS of males and females (Students *t*-test: $t_7 = 0.158$, $P = 0.879$).

Litoria lesueuri.—Skeletochronology was largely unsuccessful for *L. lesueuri* (10% aged successfully). Males ranged from 2–4 yr of age and females 3–5 yr (Table 6). The majority of males were 3 yr old and females 4 yr old. Age at maturity appeared to be 2 yr for males and 3 yr for females. Females were significantly older than males at Purling Brook (Students *t*-test: $t_{39} = -3.025$, $P = 0.003$) for all years combined. As ages were only obtained from one population, we could not statistically examine differences in AM. However, it appears that males mature 1 yr earlier than females at this site.

Effects of Altitude on Average Longevity, AM, and PRLS

Litoria pearsoniana.—Almost all individuals were 2 yr of age regardless of gender or site, suggesting that altitude had no influence on average longevity, AM, or PRLS in either sex (Table 3).

Mixophyes fleayi.—Older males and females tended to be more common at higher

altitudes than at lower sites; however, these trends were not significant (Linear Regression, males: $R^2 = 0.009$, $F_{130} = 1.142$, $P = 0.287$; females: $R^2 = 0.088$, $F_{24} = 2.215$, $P = 0.150$, Table 4). Altitude was not significantly associated with AM for either males (Linear regression: $R^2 = 0.112$, $F_6 = 0.994$, $P = 0.367$) or females (Linear regression: $R^2 = 0.031$, $F_2 = 0.889$, $P = 0.558$), nor was it significantly associated with the PRLS of individuals in either sex (Linear regression males: $R^2 = 0.148$, $F_6 = 0.872$, $P = 0.393$; females: $R^2 = 0.107$, $F_2 = 0.120$, $P = 0.788$).

Litoria chloris.—Males on average were significantly older at high altitudes than males collected at lower altitudes (Linear regression: $R^2 = 0.174$, $F_{150} = 31.390$, $P < 0.0001$; Table 5). The average longevity of females was not significantly associated with altitude (Linear regression: $R^2 = 0.107$, $F_{27} = 3.103$, $P = 0.090$). Altitude was not significantly associated with AM of males or females (Linear regression, males: $R^2 = 0.239$, $F_4 = 1.046$, $P = 0.382$; females: $R^2 = 0.596$, $F_4 = 4.427$, $P = 0.126$) nor the PRLS of either sex (Linear regression males: $R^2 = 0.173$, $F_4 = 0.627$, $P = 0.486$; females: $R^2 = 0.212$, $F_4 = 0.538$, $P = 0.540$).

Litoria lesueuri.—Because age estimates were only obtained for individuals from Purling Brook, we could not determine the influence of altitude on longevity, AM, and PRLS (Table 6).

Relationship Between Body Size and Age of Reproductive Adults

With the exception of female *M. fleayi*, body size was significantly correlated with age in both males (Spearman's Rank correlation: *L. chloris*, $R = 0.365$, $n = 151$, $P < 0.0001$;

TABLE 3.—Mean (± 1 SE), range, and sample size (n) for mean longevity (ML), age at maturity (AM), and reproductive lifespan (PRLS) in *Litoria pearsoniana* at each site, all years combined.

Site (Altitude)	Sex	ML	Mode	AM	PRLS
Austinville (110 m)	Male (36)	1.92 \pm 0.05 1–2	2.00	2.00	1.00
	Female (15)	2.00 \pm 0.00 2	2.00	2.00	1.00
Currumbin (160 m)	Male (23)	2.00 \pm 0.00 2	2.00	2.00	1.00
	Female (10)	2.00 \pm 0.00 2	2.00	2.00	1.00
N. Bridge (250 m)	Male (31)	2.00 \pm 0.00 2	2.00	2.00	1.00
	Female (8)	2.00 \pm 0.00 2	2.00	2.00	1.00
Mundora (800 m)	Male (39)	2.00 \pm 0.00 2	2.00	2.00	1.00
	Female (11)	2.27 \pm 0.14 2–3	2.00	2.00	1.00

L. pearsoniana, $R = 0.283$, $n = 118$, $P = 0.002$; *L. lesueuri*, $R = 0.697$, $n = 23$, $P = 0.002$; *M. fleayi*, $R = 0.264$, $n = 131$, $P = 0.02$) and females (Spearman's Rank Correlation: *L. chloris*, $R = 0.475$, $n = 28$, $P = 0.011$; *L. pearsoniana*, $R = 0.390$, $n = 43$, $P = 0.010$; *L. lesueuri*, $R = 0.515$, $n = 11$, $P = 0.046$) for all species. Despite these significant correlations however, the regression coefficients were small and variation in body size at comparable ages was large. Consequently, it would not be possible to estimate reliably ages of breeding adults of these species based on body size.

DISCUSSION

Applicability of Skeletochronology

Skeletochronology was largely applicable for age determination in all species except *L. lesueuri*. Many of the *L. lesueuri* specimens (90%) did not have LAGs present in cross sections (particularly lowland populations). There are three possible explanations for these results: (1) there was a problem with the skeletochronological technique, (2) individuals are young, hence no LAGs have been formed, and (3) frogs are active throughout most of the year and LAGs do not form.

Previous studies have reported an absence of LAGs due to a number of processing problems or problems with specimens. For example, LAGs go undetected because of their poor affinity to haematoxylin (Castanet et al.,

1996; Esteban et al., 1996; Hemelaar, 1985) and/or endosteal resorption (Acker et al., 1986; Kuzmin and Ischenko, 1997). The absence of LAGs due to processing problems is unlikely to have occurred in our study because specimens of all species were processed the same way and LAGs were found in all species. Additionally, no endosteal resorption was identified. The second hypothesis is not supported as some specimens that did not display LAGs (low altitude sites) were known to be at least 2 or 3 yr old (based on recaptures in subsequent years). The third explanation is currently the most plausible. Low altitude populations of *L. lesueuri* are active for at least 8 mo of the year (Morrison, 2001), potentially leaving insufficient time for LAGs to form. This hypothesis is supported by the significant negative correlations between ASL and the proportion of individuals with clear LAGs in each *L. lesueuri* population (and similar negative trends in the other species).

For the remaining species, skeletochronology based on the phalanges from toe-clips appears to be an appropriate method for aging individuals without recourse to mark and recapture techniques. Furthermore, sampling is non-destructive, an important consideration when conserving small populations.

Average Longevity, AM, and PRLS

Overall, average longevity and AM were greater for females than for males in most

TABLE 4.—Mean (± 1 SE), range, and sample size (n) for mean longevity (ML), age at maturity (AM), and reproductive lifespan (PRLS) in *Mixophyes fleayi* at each site, all years combined.

Site (Altitude)	Sex	ML	Mode	AM	PRLS
Currumbin (160 m)	Male (40)	3.95 \pm 0.13 2–6	4.00	2.00	4.00
	Female (9)	4.33 \pm 0.29 3–6	4.00	3.00	3.00
N. Bridge (250 m)	Male (5)	3.6 \pm 0.24 3–4	4.00	3.00	1.00
Canungra (500 m)	Male (18)	4.28 \pm 0.14 3–5	4.00	3.00	2.00
	Female	4.00	—	—	—
Bundoomba (520 m)	Male (38)	4.42 \pm 0.12 3–6	4.00	3.00	3.00
	Female (4)	4.75 \pm 0.48 4–6	4.00	4.00	2.00
Pyramid (650 m)	Male (15)	4.53 \pm 0.17 4–6	4.00	4.00	2.00
Bouloomba (700 m)	Male (7)	4.57 \pm 0.20 4–5	5.00	4.00	1.00
	Female (10)	5.30 \pm 0.37 4–8	5.00	4.00	4.00
Stockyard (700 m)	Male (9)	4.20 \pm 0.49 3–6	4.00	3.00	3.00
	Female	5.00	—	—	—

species. Similar results have been reported in many other amphibian species, including *T. alpestris* (Joly and Grolet, 1996; Miaud et al., 2000; Schabetsberger and Goldschmid, 1994), *T. marmoratus* (Caetano and Castanet, 1993), *T. boscai* (Caetano and LeClair, 1999), *B. bufo* (Gittins et al., 1982; Hemelaar, 1985, 1988), *R. sylvatica* (Sagor et al., 1998), *R. temporaria* (Ryser, 1996), *R. perezii* (Paton et al., 1991), *R. subaquavocalis* (Platz et al., 1997), and *Pelobates fuscus* (Eggert and Guyétant, 1999).

The difference between the sexes in AM in our study may be attributed to intersexual differences in the minimum size at maturity and differences in the growth coefficient (k). Because females are usually larger at maturity and have lower growth coefficients than males (Morrison, 2001), they take longer to reach the minimum size needed to mature and, hence, are older when breeding for the first time. As AM is proportional to longevity (Houck, 1982; Miaud et al., 2000; Tilley, 1980), females that are older than males when breeding for the first time also tend to live longer than males (Miaud et al., 2000; Tilley, 1980). However, on average, despite maturing at different ages (or showing trends towards doing so) and being older, females did not have longer PRLS than

males. Thus, regardless of when they started breeding, males and females breed for approximately the same number of years.

Influence of Altitude on Average Longevity, AM, and PRLS

Altitude did not influence AM in most of the species but, on average, produced a trend toward greater longevity in high altitude populations. The small sample size associated with some of our study populations may have influenced results. Alternatively, the small altitudinal gradient involved in our study (compared to previous studies), along with less extreme climatic differences associated with sub-tropical regions, may not influence post-metamorphic growth and development enough to produce significant differences in the time taken to reach maturity and, subsequently, longevity.

The trends produced in our study follow those reported in many previous temperate studies, whereby the cold temperatures at high altitude delay both growth and development, producing cohorts that are larger and/or older upon attainment of normal development stages than conspecifics under milder climatic conditions (Sagor et al., 1998). Increasing AM and

TABLE 5.—Mean (± 1 SE), range, and sample size (n) for mean longevity (ML), age at maturity (AM), and reproductive lifespan (PRLS) in *Litoria chloris* at each site, all years combined.

Site (Altitude)	Sex	ML	Mode	AM	PRLS
Austinville (110 m)	Male (38)	3.11 \pm 0.07 2–4	3.00	2.00	2.00
	Female (4)	3.50 \pm 0.29 3–4	3.00	3.00	1.00
Currumbin (160 m)	Male (41)	3.22 \pm 0.09 3–4	3.00	3.00	1.00
	Female (9)	3.56 \pm 0.24 3–5	3.00	3.00	2.00
N. Bridge (250 m)	Male (41)	3.29 \pm 0.08 3–5	3.00	3.00	2.00
	Female (10)	3.60 \pm 0.22 3–5	3.00	3.00	2.00
P. Brook (600 m)	Male (9)	3.89 \pm 0.20 3–5	3.00	3.00	2.00
	Female (1)	4.00 \pm 0.00 4	4.00	3.00	—
Mundora (800 m)	Male (22)	3.73 \pm 0.13 3–5	4.00	3.00	2.00
	Female (4)	4.25 \pm 0.63 3–6	4.00	4.00	2.00

greater longevity (due to delayed maturity) with higher altitude have been observed in populations of *T. alpestris* (Joly and Grolet, 1996; Miaud et al., 2000; Schabetsberger and Goldschmid, 1994), *R. temporaria* (Elmberg, 1991; Ryser, 1996), *R. pretiosa* (Licht, 1975), *R. sylvatica* (Berven, 1981), *B. bufo* (Hemelaar, 1988), and *D. ochrophaeus* (Tilley, 1980).

As previously mentioned, AM is a compromise between many selective pressures with the goal of maximizing an individual's contribution to the next generation. Low altitude individuals in our study appear to maximize fitness by maturing and reproducing earlier, while high altitude individuals maximize their fitness by delaying maturity and benefiting from their larger body size (e.g., increased fecundity, higher offspring survival). In both cases, there are disadvantages associated with the timing of maturity. For example, precocious individuals are smaller, produce fewer and smaller offspring, experience higher levels of predation as adults (energy allocated to reproduction rather than growth or survival), while individuals that delay maturity risk the increased chance that they will not survive to reproduce (Begon et al., 1990; Berven, 1982; Stearns, 1992; Zug, 1993).

Despite altitudinal variation in the average longevity and AM of most of the species, there

was no significant altitudinal variation in the PRLS in any of the species. This suggests that, regardless of the AM and average longevity of each population, there was no difference in the number of years in which they were capable of breeding, e.g., individuals in all *L. chloris* populations breed for approximately 2 yr regardless of whether they occur at 110 m or 800 m and mature at 2 or 4 yr of age, respectively.

Relationship Between Body Size and Age

Body size is not an accurate and reliable indicator of the age of reproductive individuals in our study species. Despite significant correlations between body size and age in some of the species, the correlation coefficients were very low and variation in body size at comparable ages was very high (i.e., the size ranges of different age classes overlapped extensively). For example, a male *L. chloris* at Austinville with a SUL of 49.6 mm could be from 2–4 yr of age. This variance suggests that other factors, such as growth rate prior to maturation, are much more significant sources of variation in body size than age (Halliday and Verrell, 1988; Kusano et al., 1995). Similar results have been reported in *R. sylvatica* (Sagor et al., 1998), *R. subaquavocalis* (Platz et al., 1997), *R. sakuraii* (Kusano et al., 1995), *B. bufo* (Gittins et al.,

TABLE 6.—Mean (± 1 SE), range, and sample size (n) for mean longevity (ML), age at maturity (AM), and reproductive lifespan (PRLS) in *Litorialesueuri* at Purling Brook, all years combined.

Sex	ML	Mode	AM	PRLS
Male (32)	3.25 \pm 0.09 2–4	3.00	2.00	2.00
Female (9)	3.89 \pm 0.20 3–5	4.00	3.00	2.00

1982), and *T. vulgaris* and other species in the review by Halliday and Verrell (1988).

As body size and fecundity are often strongly correlated in amphibians, it is important to know whether body size (displaying a large variation) is primarily a function of age or growth early in life since each of these factors will affect lifetime reproductive success in very different ways. If individual body size (relative to the rest of the individuals in the age group) is fixed at time of first breeding and we assume similar annual survival rates, smaller individuals will have a markedly lower average lifetime fecundity than larger individuals (Halliday and Verrell, 1988). In this case, natural selection will tend to act primarily on growth rates early in life. Alternatively, if body size is primarily a function of age, reproductive success will increase markedly with age and selection will act primarily on adult survival (Halliday and Verrell, 1988). In our study, body size does not appear to be primarily a function of age; rather, juvenile growth is a more important factor. In our study species, techniques other than correlations between body size and age (e.g., skeletochronology and mark and recapture studies) will be required to estimate accurately the ages of individuals.

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