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Growth and ageing of feral *Xenopus laevis* (Daudin) in South Wales, U.K.

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Abstract

A feral population of African clawed frogs *Xenopus laevis* from a small pond in South Wales was sampled continuously for 2 years to assess morphometric growth. Toe-clips taken at intervals over a 4-year period were found to contain lines of arrested growth (LAG) which corresponded to each consecutive winter. The first toe-clips revealed a population structure within the pond consisting of a dominant cohort of frogs with one LAG that metamorphosed in 1993, and a few older individuals. Subsequent toe-clips in 1996 and 1998 gave two and four more LAG, respectively. Morphometric growth was found to be restricted to a short growing season, significant differences in the growth rates of males and females being first detected in their third growth season. Reproductively active frogs were still within their initial period of growth, suggesting that *Xenopus laevis* does not conform to the standard energy resource allocation mechanisms of typical ectotherms.

Key words: African clawed frogs, *Xenopus laevis*, ecology, growth, invasive amphibians, skeletochronolgy

INTRODUCTION

Characteristic growth patterns of ectotherms are typified by a sinusoidal curve. The change from the steep parabola to the later asymptotic phase is often ascribed to a change in energy resource allocation; a shift from premature growth to mature breeding (Charnov, 1993). Among ectotherms, studies of growth in post-metamorphic amphibians are rare in contrast with the wealth of literature available for fish (e.g. Dutta, 1994 and references therein). This is probably a result of the economic importance of the latter group. In comparison, investigations into growth of anurans often seem inadequate, frequently making only one annual morphological measurement and then making assumptions about age and cohort membership (e.g. Reading, 1991). The importance of individual age in the study of growth of individuals within a population is crucial, and in this way the study of growth and age are inextricably linked.

Ageing in amphibians previously relied on morphometric studies, measuring especially snout–vent length (SVL) and mass of individuals. Assumptions that body size and age correlate were reviewed by Halliday & Verrell (1988) who showed that such techniques are inappropriate for sexually mature adults. Skeletochronology, the study of ageing individual organisms based on evidence found in bone tissue, has now been widely accepted as a reliable and efficient method of ageing amphibians (see Smirina, 1994). Skeletochronology has also been used to demonstrate annual growth patterns of individual amphibians (e.g. Hemelaar, 1986). However, this method is not without its problems and assumptions. The material required often relies upon the destruction of large numbers of individuals (see Gittins, Kennedy & Williams, 1985), although the use of phalanges can alleviate this problem (Hemelaar, 1981; Marnell, 1997). Resorption of periosteal bone in long bones can erase growth rings and hence hamper age estimates (Smirina, 1972; Leclair & Castanet, 1987), although calculations to take account of this have been made in some studies (e.g. see Hemelaar, 1985). An assumption that lines of arrested growth (LAG) represent annual events has been made by many authors, but double LAG (Acker, Kruse & Krehbiel, 1986; Forester & Lykens, 1991), faint or false LAG (Smirina, 1994) and double annual LAG (Caetano, Castanet & Francillon, 1985) are all known to occur. Few authors have attempted to validate this method (Halliday & Verrell, 1988) despite a need for unambiguous interpretation of skeletochronological preparations, for studies on both individual species and their habitats.

Many skeletochronological studies of anurans have

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used single (Gittins *et al*., 1982; Acker *et al*., 1986; Platz & Lathrop, 1993; Kellner & Green, 1995; Ryser, 1996) or annual (Gibbons & McCarthy, 1983; Gittins *et al*., 1985; Kusano, Fukuyama & Miyashita, 1995) captures of individuals to collect material, presumably linked to annual breeding patterns when collections may easily be made. Mark–recapture methods have been used on such occasions to determine population size and toe-clipping has provided a convenient means of both marking and obtaining bone tissue for skeletochronology. 'Ideally, a cohort of animals of known age are individually marked and later recaptured at regular time intervals.' (Halliday & Verrell, 1988). Undoubtedly, the combined use of mark–recapture with skeletochronology and morphometric data would give information needed to make unambiguous age analysis as well as linking proportions of growth rings to individual changes in size. The difficulties of regular and repeated captures of most anuran species are that populations are terrestrial for most of the year with wide home ranges (e.g. see Licht, 1974). Although skeletochronological and morphometric growth data have been previously combined in studies (e.g. Leclair & Castanet, 1987) these have been confounded by the lack of large numbers of individuals from a single cohort.

Xenopus laevis is principally confined to aquatic habitats, although able to move between water bodies (Measey & Tinsley, 1998). These frogs can be captured easily by means of a simple funnel trap, and marked individually with a freeze-branding technique. This makes *X. laevis* a good subject for repeated measures of morphometric data throughout the year. Growth of a feral population of *X. laevis* in California has been studied by McCoid & Fritts (1989); however, frogs were not individually marked or aged.

The detrimental effects of introduced pipids have been documented in the Santa Clara River, California, U.S.A. (Lafferty & Page, 1997) and in the western area of Lake Valencia, Venezuela (Royero & Hernandez, 1996). A feral population of *Xenopus laevis* has been known from South Wales since 1979 (Tinsley & McCoid, 1996). The population was the focus for an intensive 2 year ecological study (see Measey, 1998; Measey & Tinsley, 1998). The aim of the present study is to test the accuracy of skeletochronology as a method to age *X. laevis* in South Wales, and to combine age data with morphometric data of individually marked animals to reconstruct individual growth histories. This account concentrates on a dominant cohort of post-metamorphic *X. laevis* in a small, densely populated pond in South Wales over a 4-year period.

STUDY SITE

African clawed frogs *Xenopus laevis* were obtained from 1 pond (maximum volume 77.5 m^3 and surface area 144 m^2) situated on top of a hill between 2 pasture fields (40 m a.s.l.). A small river flows intermittently 0.5 km from the pond, at the bottom of the hill. Precipitation is thought to be the only source of water to the pond, with very little resulting from run-off. Climatic conditions in this area are heavily influenced by its proximity to the Bristol Channel (4 km). For comprehensive details of the study site see Measey (1997).

METHODS

Growth

Post-metamorphic *Xenopus laevis* were caught using funnel traps in the pond fortnightly from October 1994 to August 1996, and again in March 1998. All frogs were sexed on the basis of their secondary sexual characteristics: protruding labial lobes in females and formation of nuptial pads on the fore arms of males. Animals were placed on a piece of dry paper towel whilst even pressure was applied with a clear plastic ruler to measure SVL to the nearest mm. Each animal was then weighed to the nearest 0.1 g, on a Sartorius (BA 310P) electronic balance. Preliminary studies showed these measurements were repeatable (see Measey, 1997).

Marking of *X. laevis*

Xenopus laevis caught on 24 October 1994 were dye-marked using a Panjet (Wright Health Group Ltd, Dundee) (Wisniewski *et al*., 1979) with 2 marks on the ventral surface of the right thigh. All other *X. laevis* caught subsequently were freeze-branded (Daugherty, 1976) with 3-digit numbers. Pieces of copper wire (1.3 mm thick) bent into numbers were placed in liquid nitrogen until it stopped boiling. The brand was then placed for 1.5 s on the dried ventral surface of an *X. laevis*.

Skeletochronology

Three sets of toes were taken for skeletochronology, the first 60 in October 1994, and the second 60 in June 1996. Between these dates, toes and femurs were removed from 10 dead animals discovered at the pond. In March 1998, 30 animals were caught and 28 animals were toe-clipped, the remaining 2 having already been clipped twice.

Two phalanges of the fifth toe of the right foot were removed with a razor blade, in a single cut, through the joint with the third phalange. This toe was then immediately placed into 4 ml of 10% formalin for fixation and storage. The phalanges were decalcified in 2.5% nitric acid for 10 h, dehydrated and embedded in wax in a vacuum oven at 60 °C. Transverse serial sections of 15 μm were cut through each phalange. All preparations were stained with Harris' haematoxylin, counter stained with eosin, and mounted in DPX (BDH).

Sections with the smallest outer diameter were

selected for examination using a Leica DMRB light microscope. An image of the specimen was obtained by a 3-colour chip video camera (3CCD Sony) mounted on the microscope and displayed on a video monitor. Movement of a cursor over a digitizing bit-pad (CalComp/Osteometrics Inc., Atlanta, Georgia, U.S.A.) produced a line tracing on the image of the specimen on the video screen. The length of the line tracing and area enclosed were calculated from the cursor movement on the bit-pad using Osteomeasure software (Osteometrics Inc., Atlanta, Georgia, U.S.A.). Using this method and apparatus, measurements of growth ring thickness (R_n) were made. Photomicrographs were taken with a Nikon Optiphot compound microscope at × 40 magnification.

Data analysis

One-way analysis of variance (ANOVA), *t*-tests, regression and Pearson's correlation coefficient, used on morphometric and bone data, were all calculated using MINITAB software. Non-linear regression was calculated using an algorithm for least squares estimation of non-linear parameters (Marquardt, 1963) in STATGRAPHICS software.

RESULTS

Over 48 sampling occasions, from November 1994 to March 1998, 3388 captures were made of 913 individually marked *Xenopus laevis*. Of these, 95% of captures were found to be individuals from the same cohort, as were 85% of toe-clips. Any individuals found to be older than the large cohort ($n = 23$) (referred to hereafter as old) were analysed separately.

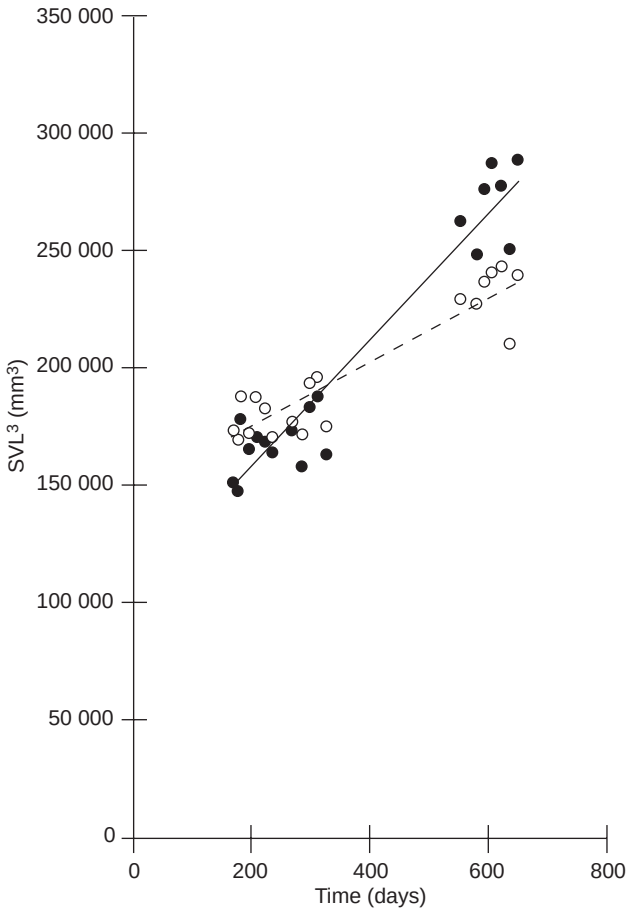

Fig. 1. Mean snout–vent length (SVL) cubed for male and female *Xenopus laevis* in a pond in South Wales from November 1994 to August 1996 on trapping occasions where the standard error of the mean SVL was > 1. Females (closed circles, continuous line), $y = 266.22x + 104256$ ($r^2 = 0.9232$). Males (open circles, dashed line), $y = 135.74x + 147405$ ($r^2 = 0.8506$).

Growth

Using secondary sexual features, sex could be determined for only 12% of individuals caught in November 1994. Mean SVL was 51 mm for juveniles and 53 mm for those identified as females and males (see Table 1). By July 1996, sex could be determined externally for all individuals, and measurements of SVL (mean difference 4 mm) and mass (mean difference 2.3 g) were significantly greater for females than for males (SVL $F_{1,347} = 79.72$, $P < 0.001$; mass $F_{1,347} = 29.96$, $P < 0.001$) (also see Table 1). Figure 1 shows the divergence in differences in SVL with time for males and females (with sex of juveniles retrospectively determined).

The relationship between SVL and total body mass is described significantly by the power (curvilinear) relation $W \sim L^3$ ($r^2 = 0.914$, $P < 0.001$) and also Pough's (1980) equation, $W = 0.006L^{3.24}$ ($r^2 = 0.975$, $P < 0.001$). The expression $W\,L^{-3}$ was used in further analysis of condition. Figure 2 shows the conditions for males and females from the dominant cohort over time. It seems that overall, males do not change in condition with time, whereas the condition of females decreases over time (i.e. lose mass relative to SVL). However, when the rate of change in condition was calculated for individuals ($W\,L^{-3}$ day^{-1}), males and females were not found to be significantly different in 1995 ($F_{1,279} = 3.88$, $P = 0.89$) or in 1996 ($F_{1,169} = 3.90$, $P = 0.78$). Condition rate from 1995 (mean = 9.54 10^{-5} g mm^{-3} day^{-1}, $n = 281$) to 1996 (mean = -1.53 10^{-8} g mm^{-3} day^{-1}, $n = 172$) were found to be significantly different ($F_{1,451} = 3.86$, $P < 0.001$).

Bone growth dynamic and skeletochronology

Preparations of ten bones from the 1994 collection were either too poor or contained too many deformities to be measured accurately using the imaging system and were thus discarded from further analysis. The pink staining area (mean = 0.055 ± 0.018 mm^2 cortex subtracted) of bone (R_n) or zones (*sensu* Peabody, 1961) were composed of opaque layers of woven fibred periosteal bone

Table 1. Snout–vent lengths (mm) and mass (g) of *Xenopus laevis* at 6-monthly intervals ± SE. Sex was retrospectively determined where possible. One-way ANOVA tests for differences between sexes. *, Significant differences

	Nov 1994	May 1995	Nov 1995	May 1996
Snout–vent lengths				
Juveniles	50.8 ± 0.85 (n = 24)	52 (n = 1)	(n = 0)	(n = 0)
Males	53.2 ± 0.51 (n = 72)	54.5 ± 0.63 (n = 59)	61.7 ± 0.92 (n = 27)	59.9 ± 0.50 (n = 51)
Females	52.9 ± 0.75 (n = 58)	54.2 ± 0.44 (n = 116)	59.6 ± 0.77 (n = 22)	63.3 ± 0.73 (n = 64)
ANOVA F-statistic	3.915	3.896	4.047	3.925
P	0.750	0.703	0.093	< 0.000*
Mass				
Juveniles	12.7 ± 0.66 (n = 24)	13.1 (n = 1)	(n = 0)	(n = 0)
Males	14.5 ± 0.38 (n = 72)	15.3 ± 0.56 (n = 59)	19.8 ± 0.81 (n = 27)	19.6 ± 0.48 (n = 51)
Females	14.7 ± 0.62 (n = 58)	15.3 ± 0.37 (n = 116)	21.2 ± 0.92 (n = 22)	22.2 ± 0.70 (n = 64)
ANOVA F-statistic	3.915	3.896	4.047	3.925
P	0.757	0.960	0.271	0.004*

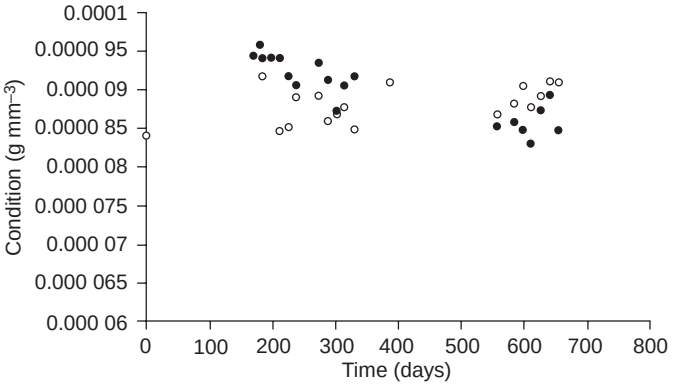

Fig. 2. The change in condition over time for male (open circles) and female (closed circles) *Xenopus laevis* in a pond in South Wales from November 1994 to August 1996 on trapping occasions where the standard error of the mean SVL was > 1.

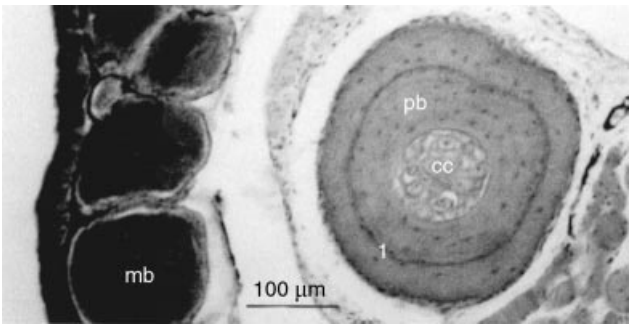

Fig. 3. Transverse section through the diaphysis of a 68 mm SVL female *X. laevis* toe, clipped on 26 October 1994 at the pond, South Wales. cc, central cavity; pb, periosteal bone; mb, muscle blocks; 1, single LAG.

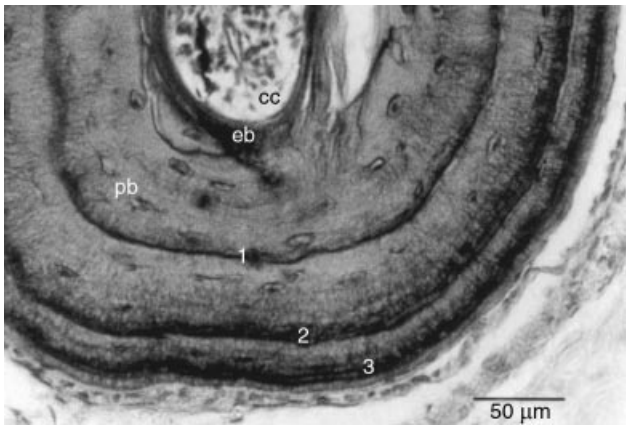

Fig. 4. Transverse section through the diaphysis of a 62 mm SVL male *X. laevis* toe, clipped on 18 June 1996 at the pond, South Wales. cc, central cavity; pb, periosteal bone; eb, endosteal bone; numbers, LAG.

with numerous round osteocytes (Fig. 3). Toward the centre, 19% were found to have endosteal bone, although no resorption of periosteal bone could be determined. Endosteal bone was easily distinguished as a darkly staining lamellar bone often with darker staining rings. LAG were preceded by distinct annuli (*sensu* Peabody, 1961) corresponding to periods of slow osteogenesis. There was no significant difference in area between R_1 and R_2 in bones which had one LAG ($F_{1,84} = 3.955$, $P = 0.716$).

Of *X. laevis* toe-clipped in June 1996, 97% had three LAG (Fig. 4) and the old individuals five and six LAG (Fig. 5; Table 2). Endosteal bone was found in 15% of bones with three LAG, although no periosteal resorption could be seen. The total area (mean = 0.076 ± 0.012 mm^2 cortex and endosteal subtracted) of bone was found to be significantly greater than that of the 1994 bones ($F_{1,99} = 3.937$, $P < 0.001$), but the area of R_1 was not found to be significantly different ($F_{1,99} = 3.937$, $P = 0.591$).

Xenopus laevis caught in March 1998 showed a continuation of the above pattern, with two additional LAG (Table 2), although new periosteal bone had not been laid over LAG_5. Of the 29 frogs from the same cohort, 45% were found to have endosteal bone, but periosteal bone showed no signs of being resorbed. The total area (mean = 0.112 ± 0.013 mm^2 cortex and

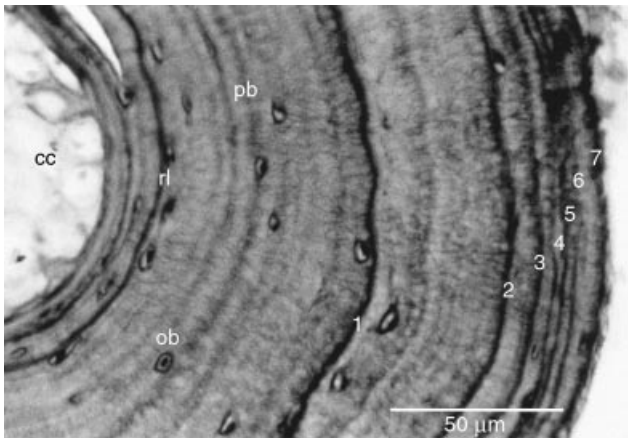

Fig. 5. Transverse section through the diaphysis of a 101 mm SVL female *X. laevis* toe, clipped on 18th June 1996 at the pond, South Wales. cc, central cavity; pb, periosteal bone; ob, osteoblasts; rl, reversal line; numbers, LAG.

Table 2. LAG frequency in toes clipped from *Xenopus laevis* from a pond in South Wales

LAG	October 1994	June 1996	March 1998
1	43		
2			
3		57	
4			
5	4		29
6	2		
7	1	1	
8		1	1
9		1	

endosteal subtracted) of bone was found to be significantly greater than that of the 1996 bones ($F_{1,85} = 167.485$, $P < 0.001$). The area of R_1 was not found to be significantly different from those caught in 1994 ($F_{1,72} = 3.974$, $P = 0.321$). Moreover, R_3 was not found to be significantly different from animals caught in 1996 ($F_{1,87} = 3.951$, $P = 0.352$).

Table 3 shows the difference in cumulative area of bone between males and females from pooled 1996 and 1998 toe-clips. The area of cortex (including endosteal where present) was found to be significantly greater in females; therefore calculations for LAG area between males and females were made with cortex and endosteal subtracted. However, the area of endosteal alone was not found to be significantly different between males and females. Males generally have a larger bone area than females, from LAG_3 onwards, but none of these differences were found to be significant.

Femurs from six of 10 animals that were found dead in the pond were found to have the first LAG partially resorbed and consequently R_1 was significantly smaller than that seen in phalanges (paired-$t_6 = 2.83$; $P = 0.018$). Resorption in femurs was characteristically from one side, while endosteal bone formed on the opposing side. Numbers of LAG counted in femurs and phalanges were identical. All *X. laevis* found dead in 1995 had two LAG ($n = 5$), whilst those recovered in 1996 had three.

Of the 23 *X. laevis* in the pond considered to be older than the dominant cohort, 14 were toe-clipped during the study. No measurements could be taken from four histological preparations of these toes because of gross distortion of the diaphysis, and therefore they were not included in further analysis. As with younger animals, no resorption of LAG in phalanges was noted in these bones, although the shape of the cross-sectional area of the diaphysis was found to be ovoid. Endosteal bone

Table 3. Mean and standard deviation of area beneath cumulative successive LAG (with cortex and endosteal subtracted) for males and females for toe-clips from 1996 and 1998. One-way ANOVA tests for differences between sexes. *, Significant differences

	No. of LAG										
	Cortex (including endosteal)	Endosteal	1	2	3	4	5	6	7	8	9
Males											
n	28	13	28	28	28	5	5	3	3	3	1
x ± SD (mm^2)	0.0067 ± 0.0024	0.0064 ± 0.0027	0.0202 ± 0.0094	0.0478 ± 0.0159	0.0654 ± 0.0169	0.0940 ± 0.0228	0.1097 ± 0.0213	0.1314 ± 0.0169	0.1367 ± 0.0204	0.1443 ± 0.0206	0.1709
Range	0.0018 – 0.0100	0.0034 – 0.0122	0.0066 – 0.0398	0.0240 – 0.0767	0.0336 – 0.0964	0.0646 – 0.1214	0.0858 – 0.1405	0.1157 – 0.1493	0.1197 – 0.1593	0.1267 – 0.1670	0.1709
Females											
n	62	27	62	62	62	26	26	1	1	0	0
x ± SD (mm^2)	0.0109 ± 0.0108	0.0066 ± 0.0029	0.0219 ± 0.0120	0.0460 ± 0.0139	0.0625 ± 0.0145	0.0823 ± 0.0124	0.1063 ± 0.0109	0.1304	0.1375		
Range	0.0054 – 0.0162	0.0015 – 0.0121	0.0100 – 0.0433	0.0256 – 0.0728	0.0375 – 0.0887	0.0607 – 0.1120	0.0780 – 0.1252	0.1304	0.1375		
ANOVA											
F-statistic	3.949	0.016	3.949	3.949	3.949	4.183	4.183				
P	0.047*	0.898	0.516	0.596	0.410	0.103	0.600				

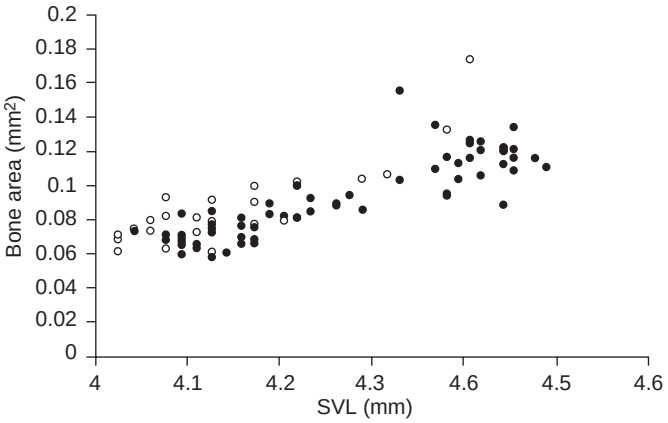

Fig. 6. The relationship between snout–vent length (SVL) and bone area (R_T) for male (open) and female (solid) *Xenopus laevis* from a pond in South Wales.

was present in all of the older bones examined and growth rings were found to decrease in width toward the edge of the bone (see Fig. 5).

Combined morphometric and skeletochronology data

The relationship between SVL (ln mm) and total bone area (R_T mm^2) was determined by linear regression (males $\ln \text{SVL} = 3.82 + 3.76R_T$, $r^2 = 0.744$, $n = 27$; females $\ln \text{SVL} = 3.80 + 5.11R_T$, $r^2 = 0.734$, $n = 62$). Figure 6 shows that whilst SVL and total bone area are significantly correlated for both males and females, there is no clear sexual dimorphism in this relationship. Both outliers were old animals, no. 167 a nine-LAG male and no. 523 a seven-LAG female, both caught in June 1996.

A female *X. laevis* (no. 222) was one of 5.3% ($n = 47$) of frogs caught 10 or more times in the pond. This individual was one of two animals toe-clipped in June 1996 and March 1998. In June 1996, no. 222 was noted to be in breeding condition (with swollen pink labial lobes) at the same time that males were heard calling in the pond. If LAG area is plotted at points in mid-winter (1 January), the pattern of bone growth from the two toes clipped from no. 222 match exactly (see Fig. 7a). The change in SVL and mass with time (Fig. 7b), can be seen to mirror the change in phalange diameter with time.

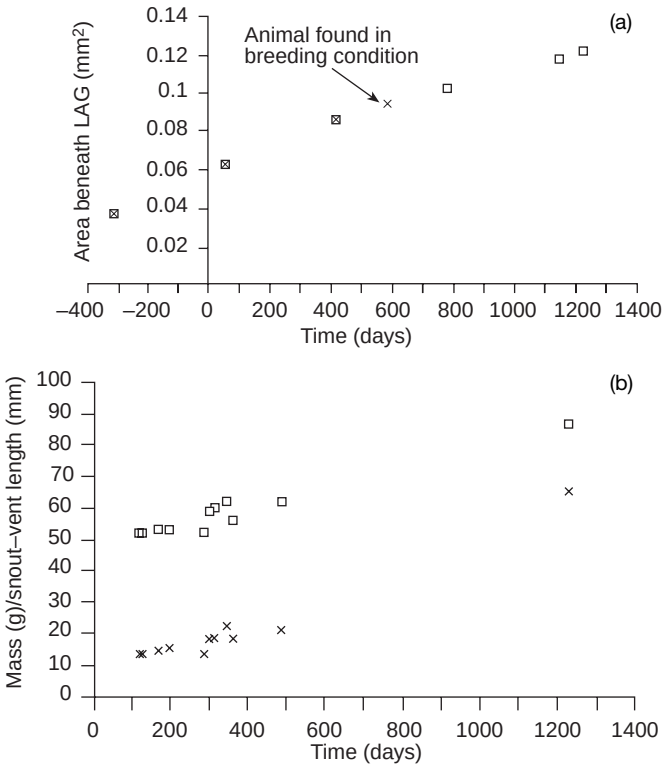

Fig. 7. Case study of *Xenopus laevis* no. 222 caught in a pond in South Wales. (a) Change in bone area (R_T) beneath lines of arrested growth with time (1996 toe-clip, crosses; 1998 toe-clip, squares) from November 1994 to August 1996; (b) change in snout–vent length (squares) and mass (crosses) with time.

DISCUSSION

Skeletochronology, as a technique, lends itself to studies of ecology, demographics and growth. As such, it is now commonplace in herpetological literature as a method for ageing populations of amphibians and reptiles (see Castanet, Francillon-Vieillot & de Ricqlés, 1993). However, evidence has been collected from few taxa to verify that this method is repeatable and that potential major problems, such as the resorption of periosteal bone, do not create discrepancies when interpreting data. Many authors also seem to rely on the *a priori* assumption that LAG are annual. Notable exceptions to this have attempted to use laboratory simulations of resting periods (Measey, 1997; Waye & Gregory, 1998), animals of known age (Schroeder & Baskett, 1968; Gibbons & McCarthy, 1983), and vital fluorescent labelling (Smirina, 1972; Francillon & Castanet, 1985). This long-term field study has enabled the morphological growth of individually marked anurans from a single cohort to be followed, and further, to combine this with areas of periosteal bone laid down in phalanges. Animals from which two phalanges were taken on separate occasions prove beyond doubt the validity of skeletochronology for this study, but by following animals from a known cohort, it has also been possible to combine this information with morphological growth.

None of the problems associated with interpretation of skeletochronological preparations (Halliday & Verrell, 1988) was encountered during this study. Clear unambiguous LAG were found in all *X. laevis* bones sectioned. The presence of two additional LAG in toes clipped in June 1996 (and the single additional LAG of those found dead in 1995), compared to those clipped in 1994, and the further addition of two LAG in animals sampled in 1998, unambiguously demonstrates that LAG are laid down annually by *X. laevis* in South

Wales. It is considered that these concentric haematoxylinophilic lines are formed every year as a consequence of arrested bone growth. Previous workers have linked proportions of bone growth with morphometric growth (Kleinenberg & Smirina, 1969; Augert & Joly, 1993; Ryser, 1996) and have used distances between LAG to infer a population's growth history (Gibbons & McCarthy, 1983; Hemelaar, 1986). Data in this study show the same direct link between morphometric data and total phalangeal bone growth.

Studies of skeletochronology have generally used either the diameter across the bone and distances between LAG or a LAG circumference using a curvimeter on photomicrographs (see Smirina, 1994). Histological preparations used in this study were previously measured using the former method, but problems were encountered because of the assumption that bone sections are circular (Measey, 1997). Diameter measurements were found to be adequate for most analyses, but comparison of bones taken from the same individual at two different times was found to have far more accurate measures when bone areas were calculated from Osteomeasure software (see Fig. 7). Area measures also provided the means of subtracting the area of the bone cortex from total bone area (including endosteal if present), which is significantly different between males and females, so that periosteal bone tissue alone could be used in calculations.

In this study, mating and egg laying by *X. laevis* was observed during June 1996. Males were first heard calling at 21:30 on 2 June 1996 when water temperature was noted as 14 °C. Animals caught at that time were in breeding condition, 14 females with pink swollen papillae and 20 males with dark 'gloves' on their forelimbs (see Berk, Cheetham & Shapiro, 1935). Of these animals, three females and one male were toe-clipped. On 3 June 1996 the first eggs were noted on submerged vegetation in the pond. Small larvae found in July were presumed to be from this mating activity. Larvae were observed for 4 weeks after 14 July 1996, but in small numbers (see Measey, 1997). Whilst direct comparisons between morphological characters of individuals and their bone measurements were limited to a few animals, growth rings in bones and morphometric measurements both suggest that all *X. laevis* in this cohort were reproductively active during their initial growth period (see Fig. 7a). This deviates from the generally accepted amphibian growth patterns reported in the literature where the initial growth period is equated with premature growth (Hota, 1994).

Onset of breeding in Californian populations was also seen when animals were within their initial growth period; animals were recorded to grow and breed year round (McCoid & Fritts, 1995; pers. obs.). Such plasticity in life history has been considered of key importance for successful invasive species (di Castri, 1990). *Xenopus laevis* can be regarded as an invasive species as it has become problematic in several areas of its anthropogenic facilitated distribution (see Tinsley & McCoid, 1996).

The age structure of the population of *X. laevis* in the pond is disjointed, the bulk of the population were animals from a single cohort that metamorphosed in 1993 (see Table 2). Recorded numbers of older animals in this population are deceptive as more old individuals have been found in ponds in the catchment of the river and are known to move between sites (Measey & Tinsley, 1998). Reproductive effort in 1995 and 1996 was observed to be unsuccessful with respect to adult recruitment (see Table 2) probably as a result of cannibalism (see Measey, 1998). This disjointed age structure does not seem to be unusual, even for amphibians in their indiginous habitats. Long term survival of a population relies upon recruitment to the breeding population. A successful reproductive season was observed to occur in summer 1998, leading to a new dominant cohort within the pond (R. C. Tinsley, pers. comm.).

In their study of growth of feral *X. laevis* in California, McCoid & Fritts (1989) also found that the relationship between mass and length was curvilinear. They also looked at the increase in SVL over 2 years, and their data were described by the equations; $y^3 = 0.44x + 58.65$ for male growth, and $y^3 = 0.96x + 18.04$ for female growth (where x is time in days and y is SVL in cm). Figure 1 shows a much smaller overall growth rate for *X. laevis* in this study, as well as males growing at approximately half the rate ($y^3 = 0.14x + 147.41$) of females ($y^3 = 0.27x + 104.26$). Growth rates for *X. laevis* in South Wales may be low because of their shortened growth season, as they are presumably close to the limit of their climatic range.

The small variation in bone width of R_1 (mean = 0.055 ± 0.018 mm^2 for those toe-clipped in 1994) suggests that all of these individuals were the result of a short spawning period. In their native southern Africa, spawning periods are known to be long (Kalk, 1960; pers. obs.), relative to the explosive breeding of some other anurans. In California, breeding has been observed during most of the year (McCoid, 1985), and conditions for breeding are thought to include temperature > 20 °C (McCoid & Fritts, 1989). However, these conditions occur for a short period only in the South Wales pond, although breeding activity (calling) occurred when temperatures were as low as 15 °C (Measey, 1997). The variation that exists in bone width, and size, may solely be a result of the different development rates and hence size at metamorphosis of tadpoles.

The invasive ability of pipids is now well recorded in the literature (Royero & Hernandez, 1996; Tinsley & McCoid, 1996; Measey & Tinsley, 1998). Measey (1998) suggested that food availability for the South Wales population of *X. laevis* was limiting only during periods of severe drought. This growth study of a feral population of southern African anurans in South Wales, U.K., suggests that growth occurs at *c*. one-third of the rate of feral *X. laevis* found in San Diego, California, where conditions enable year-round growth and reproduction. Mediterranean habitats have long been recognized as

being vulnerable to invasion by many species (see Groves & di Castri, 1991). In South Wales, *X. laevis* is limited by a short growing season and not by the availability of food. This may help explain why *X. laevis* has not emerged as a threatening invader of South Wales, U.K.

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