

Conservation implications of the age/size distribution of Giant Bullfrogs (*Pyxicephalus adspersus*) at three peri-urban breeding sites

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Nothing is known about the age of wild Giant Bullfrogs (*Pyxicephalus adspersus*); yet this information has important conservation implications for this regionally threatened species. We quantified and compared the age, body size and body condition of adult male and female *P. adspersus* caught during spawning events at peri-urban breeding sites in Diepsloot and at Glen Austin and Bullfrog pans in Gauteng Province, South Africa. Age was estimated from lines of arrested growth (LAG) counted in cross-sections of animal phalanges. Males and females from all three sites possessed 6 ± 2 (max. 16) and 4 ± 1 (max. 11) LAG, respectively, suggesting shorter female longevity. Individuals with < 3 LAG were not encountered at the breeding sites, implying that newly metamorphosed *P. adspersus* require at least three years to reach sexual maturity. There was no significant difference in the LAG counts of same-sex animals between the three sites. However, mean male snout-vent length, mass, and body condition was greatest at Glen Austin Pan, and lowest at Bullfrog Pan. The latter is possibly explained by chemical contamination of Bullfrog Pan from an adjacent disused landfill. At Glen Austin Pan males and females sampled in 2004–06 for this study were significantly shorter than those sampled at the same site in 1992–93 for a different study. Our results suggest that male *P. adspersus* may live for 20 years or more in the wild, but at some peri-urban breeding sites adult life expectancy is declining. Juvenile *P. adspersus* are most threatened by terrestrial habitat transformation because they take ≥ 3 years to mature, during which period they may move great distances from their natal site. Differences in the size and condition of *P. adspersus* between the study sites suggests that the species requires site-specific management in addition to conservation at larger spatial scales.

Key words: anuran, pollution, population, skeletochronology, South Africa

INTRODUCTION

In temperate, mid-latitude environments anurans require from several months to three years to reach sexual maturity (e.g. Zug & Zug, 1979; Tsiora & Kyriakopoulou-Sklavounou, 2002) while at high latitudes or altitudes anurans may take four or more years to mature (e.g. Metter, 1967; Matthews & Maud, 2007). Many anurans live less than 10 years (e.g. Measey, 2001; Esteban et al., 2004; Guarino & Erismis, 2008), but larger species and/or captive individuals can exceed this, e.g. wild and captive *Rana catesbeiana* reached 10 and 16 years, respectively (Oliver, 1955; Goin & Goin, 1962) and wild and captive *Bufo marinus* reached 15 and 40 years, respectively (Tyler, 1975). Age at maturity and longevity have important conservation implications for threatened anurans. As age at maturation increases, generation time increases and population resilience decreases (Duellman & Trueb, 1994). Older females are generally larger than younger females, and therefore produce larger and more clutches during a breeding season (Howard, 1978; Reading & Clarke, 1995; Reading, 2007). Although there

have been numerous studies on anuran age (see Tables 2–9 in Duellman & Trueb, 1994; Table 1 in Monnet & Cherry, 2002), very limited data have been obtained for mainland Africa species, e.g. *Amietophrynus pardalis* (Cherry & Françillon-Vieillot, 1992), *R. saharica* (Esteban et al., 1999; Meddeb et al., 2007) and *Xenopus laevis* (Measey, 2001).

The Giant Bullfrog (*Pyxicephalus adspersus*) is one of the largest extant anurans (Du Preez & Cook, 2004) and is exceptional in that males (~400–1000 g) can weigh up to ten times more than females (~90–300 g; Cook, 1996). The species is widely distributed across the grassland and savanna regions of southern Africa (Channing, 2001) but is regarded as Near-Threatened in South Africa due to habitat loss and other factors (Harrison et al., 2001; Minter et al., 2004). Due to the very brief and unpredictable appearance of *P. adspersus* above ground during summer (Yetman & Ferguson, 2011a), little systematic field-based research and monitoring has been performed on this species (Jacobsen, 1989; Kok et al., 1989; Van Wyk et al., 1992; Cook, 1996). The resulting lack of demographic information has impeded

the assessment of the conservation status of *P. adspersus*, and provides a weak baseline for evaluation of efforts to protect threatened populations. Captive *P. adspersus* have reportedly reached an estimated 45 years of age (Channing, 2001) but nothing is known about the age of wild specimens. Juvenile *P. adspersus* are expected to require several years to mature given the large body size of adults (Peters, 1983); therefore populations may have slow generation turnover and low resilience to perturbations in density.

Skeletochronology is a commonly-used technique for aging anurans (Halliday & Verrell, 1988) and has been applied to a wide variety of species from arid (Rogers & Harvey, 1994; Tessa et al., 2007), temperate (Miaud et al., 1999; Guarino et al., 2003), subtropical and even tropical environments (Morrison et al., 2004; Lai et al., 2005). The technique involves analysis of concentric lines of arrested growth (LAG) visible in cross-sections of animal long bones and phalanges (Castanet & Smirina, 1990). LAG are deposited during periods of animal inactivity and are therefore pronounced in species with highly seasonal activity. Adult *P. adspersus* are active after heavy rainfall for cumulatively less than a month between October and March (Yetman & Ferguson, 2011a), and were therefore considered appropriate subjects for phalangeal skeletochronology, which precludes the need to sacrifice whole animals. Using this method we aimed to estimate the age of wild, adult *P. adspersus* to provide important life history and demographic information for improved conservation management of this species. In particular we wanted to determine whether *P. adspersus* at different breeding sites differed significantly in age, body size or body condition, and therefore, require site-specific conservation management. Specific objectives of this study were to (i) estimate the age range of wild, breeding *P. adspersus* males and females using skeletochronology, (ii) examine the relationship between body size and age in adult *P. adspersus*, (iii) compare the age, body size and body condition of adult *P. adspersus* at different breeding sites, and (iv) infer consequences of the results on the conservation management of *P. adspersus*.

MATERIALS AND METHODS

Study sites

Adult *P. adspersus* were caught by hand or with a hand-held net at spawning events between 1 October and 31 March in the 2003–2004, 2004–2005 and 2005–2006 summer seasons at three peri-urban, seasonal breeding sites in Gauteng Province, South Africa. At the Diepsloot site (25°56'23.59"S, 28°01'21.88"E) *P. adspersus* bred in three small (~0.2–0.9 ha) artificial dams where there was a relatively high level of human activity. Glen Austin Pan (25°58'36.00"S, 28°09'58.33"E; ~9 ha) and Bullfrog Pan (26°08'22.35"S, 28°18'51.10"E; ~81 ha) were proclaimed bird sanctuaries that gave limited protection for the historically large resident populations of *P. adspersus* (Cook, 1996; Slater-Jones, 1996). The three sites (separated by 15–36 km) had very similar climates and remaining natural grassland (Mucina et al., 2005) relative to the variety of habitats that *P.*

adspersus inhabits in southern Africa. The agricultural small-holdings surrounding each site were becoming increasingly urbanized.

Field work

Study animals were handled in a manner complying with the "Guidelines for Use of Live Amphibians and Reptiles in Field Research" (Society for the Study of Amphibians and Reptiles, the American Society of Ichthyologists and Herpetologists, and The Herpetologists' League). We used an electronic balance (accurate to 1 gram) and steel tape-measure (accurate to 1 millimeter) to measure each animal's body mass and snout-vent length (SVL), respectively. Animal body condition was estimated as mass/SVL (Schulte-Hostedde et al., 2005). A sterilized bone cutter or wire clipper was used to clip the two most distal phalanges from the second (i.e. longest) toe on the right hind limb of each animal. Toe clips were stored in separate vials containing 70% ethanol, and animals were released near their point of capture.

Histology

To analyze animal body size in relation to age, we selected toe clips of five small (90 mm < SVL ≤ 110 mm) and five large (110 mm < SVL ≤ 130 mm) females, as well as five small (130 mm < SVL ≤ 150 mm), five medium (150 mm < SVL ≤ 170 mm) and five large (SVL > 170 mm) males from each site except Bullfrog Pan, where only three large males were caught. Toe clips of the three largest males caught during the study (which included two males from Diepsloot and one from Glen Austin Pan with a SVL ≥ 190 mm) were added to the largest size category. Data for the size-age analysis were therefore obtained from 76 animals, including 30 females and 46 males (hereon referred to as the "size selected" animals). To compare the age of same-sex animals between the three locations we used the complete sample set again to randomly select toe clips of 10 females and 15 males from each site. Hence data for comparisons of animal age between the populations were obtained for 75 animals, including 30 females and 45 males (hereon referred to as the "randomly selected" animals).

Toe clips were decalcified in 8% formic acid for 48 hours, and embedded in paraffin wax. A rotary microtome was used to cut 6 μm thick sections which were then stained with Ehrlich's haematoxylin. This method was adapted from the method used by Castanet & Smirina (1990), which involved nitric acid for decalcification, and a freezing microtome. We examined mounted sections under a standard light microscope at 4x, 10x or 20x magnification, and selected for each sampled animal one or two sections with the smallest medullar cavity. We counted lines of arrested growth (LAG) from the endosteal bone outwards and treated the perimeter of the periosteal bone as a LAG if the animal had been sampled during the first spawning event of a season. False and double LAG were respectively, distinguished as incomplete or complete feint LAG within bands of summertime bone growth (Hemelaar & Van Gelder, 1980; Sagor et al., 1998; Cvetković et al., 2005).

Data analysis

Statistical tests were performed in Statistica 7.0 (© StatSoft, Inc. 1984–2004 Tulsa). We used least squares regression analyses to examine relationships between the body mass, SVL and LAG counts of size selected males or females from the three sites combined. We used ANOVA to compare LAG counts between small and large females, and between small, medium and large males. The SVL, body mass, body condition, or LAG counts between randomly selected males and females from the three sites treated separately, or combined, were compared using *t*-tests (when $n < 30$) or ANOVA (when $n > 30$). We used ANOVA to compare the SVL, body mass, body condition or LAG counts of randomly selected same-sex animals between the three sites. In addition, we calculated the two-sample *z*- and Smirnov *d*-statistics to, respectively, compare the mean values and cumulative frequency distributions of the SVL or body mass of all males or females sampled during spawning events at Glen Austin Pan in 2004–06 (for this study) and in 1992–93 (for a separate study; Cook, 1996). The data presented are mean \pm standard deviation (SD) and the level of significance for all tests was $p = 0.05$. We applied sequential Bonferroni corrections to comparisons of SVL, body mass, body condition, or LAG counts between randomly selected males and females from the same site or same-sex animals from different sites, or all same-sex animals sampled at Glen Austin Pan for the two separate studies.

RESULTS

Interpretation of sections

The most hematoxylinophilic LAG observed in the sections (Fig. 1) were arranged in a consistent geometric pattern among the samples (e.g. Fig. 1a and b in Bastien & Leclair, 1992; Fig. 1c and d in Guarino et al., 2003; Fig. 1 in Tessa et al., 2007). We assumed that each of these LAG was deposited while an animal experienced torpor underground during a six to eight-month winter period (Loveridge & Withers, 1981). Animals experienced the most bone growth during their second summer. Growth declined slightly during the third summer and decreased dramatically thereafter. The number of complete and partially remaining LAG prior to this transition in bone growth revealed that complete or partial resorption of the first LAG and partial resorption of the second LAG was common among the samples. Within bands of summertime bone growth multiple false and double LAG were observed, which did not exhibit any consistent pattern among the samples.

Body size and age of size-selected animals

The females ($n = 30$) weighed 99–285 g, measured 92–136 mm in SVL and possessed 3–11 LAG. The males ($n = 46$) weighed 213–872 g, measured 130–198 mm in SVL and possessed 3–16 LAG. No animal possessed < 3 LAG. The oldest female (with 11 LAG) weighed 285 g, measured 136 mm in SVL and was sampled at Bullfrog Pan. The oldest male (with 16 LAG) weighed 704 g, measured 178 mm and was sampled at Glen Austin Pan. The three males with a $SVL \geq 190$ mm possessed 7 or 8 LAG each.



Fig. 1. Black arrows indicate eight lines of arrested growth (LAG) visible in a phalangeal cross-section of an adult *Pyxicephalus adspersus*. These LAG, we assumed, were each deposited during 6–8 months of winter torpor and are numbered consecutively from the endosteal bone outwards. The first LAG has been partially resorbed. White arrows point to comparatively faint, incomplete LAG within bands of summer bone growth. These LAG were likely deposited during extended periods of rest in summer between bouts of animal activity following rainfall.

Body mass increased exponentially with SVL in both males ($y = 42.10 x^{2.465}$, $t = 11.7$, $p < 0.001$, $n = 46$) and females ($y = 26.05 x^{2.317}$, $t = 8.0$, $p < 0.001$, $n = 30$; Fig. 2A). There was a weak linear increase in body mass with LAG counts in both males ($y = 29.92x + 289.63$, $r^2 = 0.2$, $p < 0.001$, $n = 46$) and females ($y = 18.52x + 17.63$, $r^2 = 0.6$, $p < 0.001$, $n = 30$). SVL increased logarithmically with LAG counts in males ($y = 31.96 \ln(x) + 103.27$, $r^2 = 0.3$, $p < 0.001$, $n = 46$) and linearly with LAG counts in females ($y = 4.09x + 90.36$, $r^2 = 0.5$, $p < 0.001$, $n = 30$; Fig. 2B). LAG counts differed significantly between small, medium and large males ($F_{2, 43} = 7.0$, $p = 0.002$, $n = 46$; Fig. 2C), and between small and large females ($F_{1, 28} = 6.9$, $p = 0.01$, $n = 30$; Fig. 2D). The lack of a strong sigmoid relationship between LAG counts and SVL precluded growth analysis to estimate maximal body size of males and females.

Males versus females

The SVL, body mass and body condition of all sampled *P. adspersus*, and LAG counts of the 45 males and 30 females whose toe clips were randomly selected for sectioning are shown in Table 1. At each of the three sites males were significantly longer and heavier than the females (Table 1). These differences, and the difference in LAG counts between males and females from the Diepsloot dams, or from all three sites combined, remained significant following Bonferroni corrections. The frequency distribution of LAG counts of the randomly selected males and females from the three sites combined is shown in Fig. 3. Eighty percent of the males possessed 4–7 LAG,

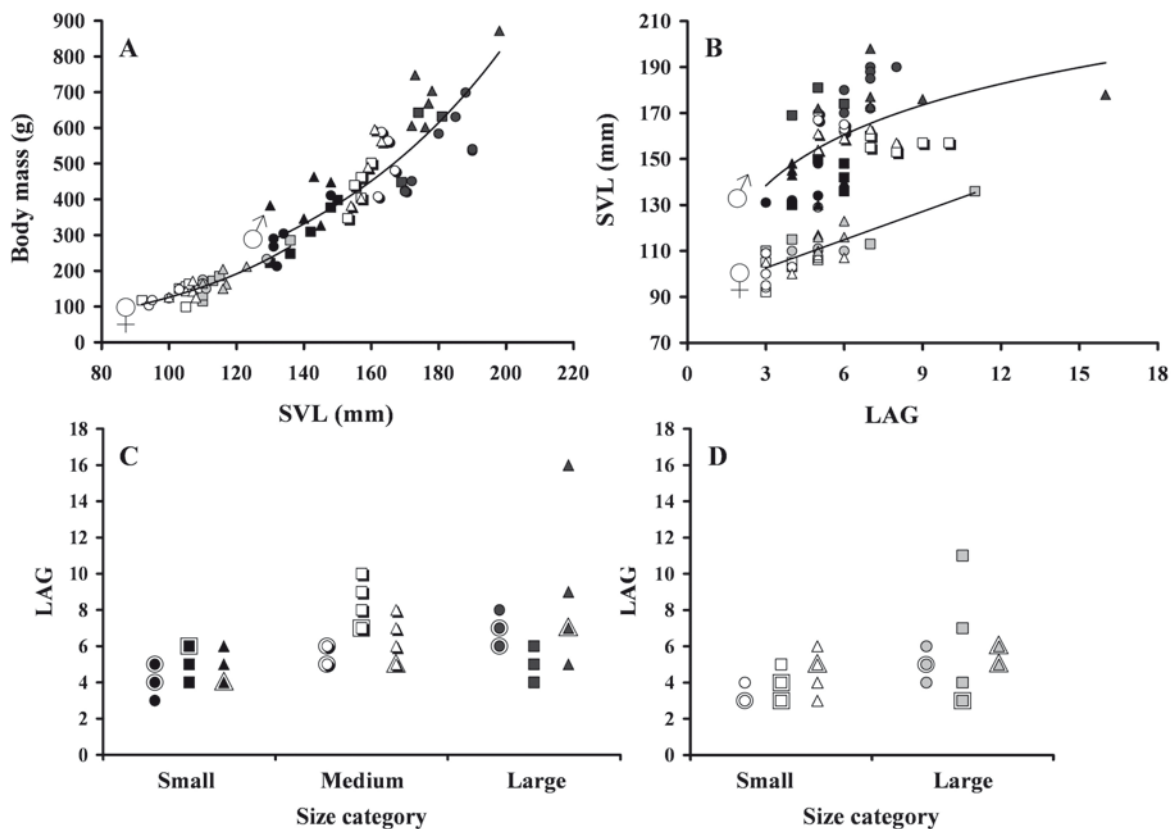


Fig. 2. Scatterplots showing the relationship between (A) snout-vent length (SVL) and body mass, (B) the estimated age, and SVL; or the size category, and estimated age of (C) male ($n=46$), and (D) female ($n=30$) adult *Pyxicephalus adspersus* from three peri-urban breeding sites. Age was estimated as the number of lines of arrested growth (LAG) counted in cross-sections of animal phalanges. Circular, triangular or square data points represent animals sampled at Diepsloot, Glen Austin or Bullfrog Pan, respectively. White or light grey data points represent small or large females, respectively. Black, white with a shadow, or dark grey data points represent small, medium, or large males, respectively. Data points with an extra outline represent more than one individual. Solid regression lines pertain to males (♂) or females (♀) from all three sites combined.

and 83% of the females possessed 3–5 LAG. Only 13% of the males and 17% of the females possessed 8–11 LAG and 6–7 LAG, respectively.

Study site comparisons

There was no significant difference in the LAG counts of randomly selected same-sex animals between the three sites (Table 1). However, mean male SVL, body mass and condition was greatest at Glen Austin Pan and lowest at Bullfrog Pan. Mean female SVL was longest at Glen Austin Pan and shortest at Diepsloot.

Male ($n=510$) and female ($n=204$) *P. adspersus* sampled by Cook (1996) during 1992 or 1993 at Glen Austin Pan had a body mass of 561 ± 87 g (range: 320–970 g) and 173 ± 51 g (range: 60–400 g), and a SVL of 184 ± 13 mm (range: 137–227 mm) and 116 ± 10 mm (range: 95–141 mm), respectively. The mean SVL of males and females sampled at this site in 2004–06 for our study measured, respectively, 19 mm ($z=15.9$, $p<0.001$) and 3 mm ($z=2.1$, $p=0.02$) less than in 1992–93. These differences in mean SVL remained significant following sequential Bonferroni corrections. Male and female mean body mass measured 6 g ($z=0.5$) and 4 g ($z=0.4$) less, respectively, in 2004–06 than in 1992–93, but these differences in mean body mass

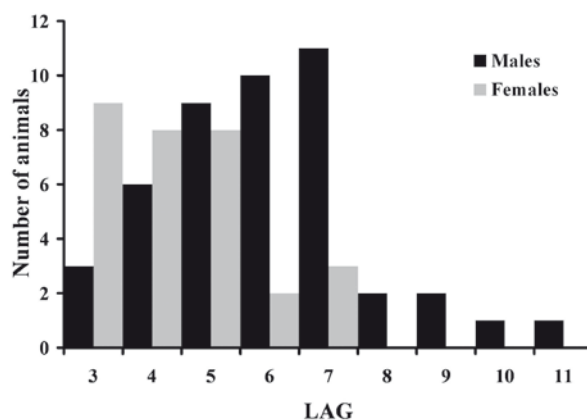


Fig. 3. Frequency distribution of the estimated age of randomly selected adult male ($n=45$) and female ($n=30$) *Pyxicephalus adspersus* from three peri-urban breeding sites. Age was estimated as the number of annual lines of arrested growth (LAG) counted in cross-sections of animal phalanges.

Table 1. Mean±standard deviation, and range, of the snout-vent length (SVL), body mass, body condition or age (estimated from LAG = lines of arrested growth in phalanges) of male or female *Pyxicephalus adspersus* from three peri-urban breeding sites. ANOVA *F*, *t*, or *z*-test, and *p* values (***)*p*<0.001) pertain to comparisons of variables for same-sex animals between the three sites (column 3) or between males and females from the three sites combined (column 4) or treated separately (columns 5, 6 and 7). Values of *p*>0.01 were non-significant following sequential Bonferroni correction.

Column	2	3	4	5	6	7
	Sex	<i>F</i> , <i>p</i>	All three sites	Diepsloot dams	Bullfrog Pan	Glen Austin Pan
SVL (mm)	♂	$F_{2,208}=8.5$ ***	163±13 130–198 <i>n</i> =211	160±14 131–190 <i>n</i> =56	155±12 130–181 <i>n</i> =23	165±12 130–198 <i>n</i> =132
	♀	$F_{2,65}=5.8$ <i>p</i> =0.004	109±9 92–136 <i>n</i> =68	105±8 93–129 <i>n</i> =30	110±12 92–136 <i>n</i> =16	113±6 100–123 <i>n</i> =22
Body mass (g)	♂	$F_{2,208}=25.2$ ***	512±131 204–872 <i>n</i> =211	454±108 204–699 <i>n</i> =56	405±102 222–643 <i>n</i> =23	555±124 239–872 <i>n</i> =132
	♀	$F_{2,65}=3.4$ <i>p</i> =0.04	152±43 90–294 <i>n</i> =68	139±33 95–233 <i>n</i> =30	154±53 90–285 <i>n</i> =16	169±42 117–294 <i>n</i> =22
Body condition	♂	$F_{2,208}=30.2$ ***	3.1±0.6 1.5–4.4 <i>n</i> =211	2.8±0.5 1.5–3.7 <i>n</i> =56	2.6±0.5 1.7–3.7 <i>n</i> =23	3.3±0.6 1.8–4.4 <i>n</i> =132
	♀	$F_{2,65}=2.5$ <i>p</i> =0.09	1.4±0.3 0.9–2.5 <i>n</i> =68	1.3±0.2 1.0–1.8 <i>n</i> =30	1.4±0.3 0.9–2.1 <i>n</i> =16	1.5±0.3 1.0–2.5 <i>n</i> =22
LAG	♂	$F_{2,42}=1.3$ <i>p</i> =0.3	6±2 3–11 <i>n</i> =45	6±1 3–7 <i>n</i> =15	6±1 3–8 <i>n</i> =15	7±2 3–11 <i>n</i> =15
	♀	$F_{2,27}=1.2$ <i>p</i> =0.3	4±1 3–7 <i>n</i> =30	4±1 3–5 <i>n</i> =10	5±2 3–7 <i>n</i> =10	5±1 3–6 <i>n</i> =10
			$F_{1,277}=978.2$ ***	$F_{1,84}=376.0$ ***	<i>t</i> =-11.6***	$F_{1,152}=386.5$ ***
			$F_{1,77}=498.7$ ***	$F_{1,84}=242.1$ ***	<i>t</i> =-9.0***	$F_{1,152}=209.2$ ***
			$F_{1,277}=504.4$ ***	$F_{1,84}=242.9$ ***	<i>t</i> =-8.9***	$F_{1,152}=218.6$ ***
			$F_{1,73}=18.2$ ***	<i>t</i> =-3.7, <i>p</i> =0.001	<i>t</i> =-1.6, <i>p</i> =0.1	<i>t</i> =-2.5, <i>p</i> =0.02

were non-significant (*p*=0.3 for both tests). Frequency distributions of the SVL or body mass of all male or female *P. adspersus* sampled in 1992–93 or 2004–06, are shown in Figures 4A–D. The cumulative frequency distributions of SVL and body mass differed significantly between the two studies for males (Smirnov *d*=0.41 and 0.18, respectively, *p*<0.05), but not for females (*d*=0.20 and 0.24, respectively).

DISCUSSION

Interpretation of sections

The reliability of skeletochronological age estimates can be affected by endosteal resorption of periosteal bone, deposition of false and double LAG and rapprochement of LAG near the perimeter of the periosteal bone (Hemelaar & Van Gelder, 1980; Castanet & Smirina, 1990). Skeletochronological age estimates are also less

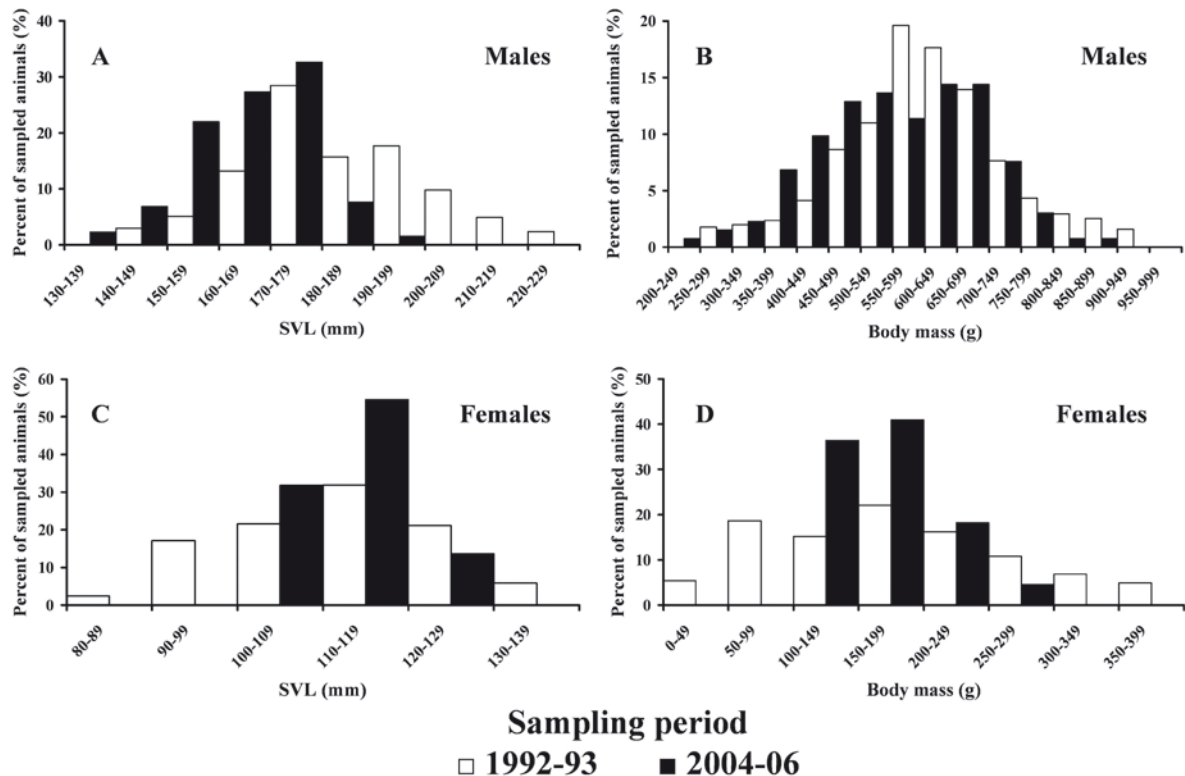


Fig. 4. Frequency distribution of the snout-vent length (SVL) or body mass of male or female *Pyxicephalus adspersus* caught during spawning events at Glen Austin Pan in 1992-93 by Cook (1996) or in 2004-06 for this study.

reliable when phalanges (not long bones) are used or study species are long-lived (Wagner et al., 2011). Therefore the application of skeletochronology ideally requires reference to LAG in bones of known-age individuals. Known-age, wild *P. adspersus* were not available; therefore we attempted to sample captive specimens. However, most owners would not allow toe-clipping, accurate age records did not exist and many specimens were fed and remained active throughout the year (CAY, pers. obs.).

Nevertheless, the progressive increase in numbers of LAG with the body size of sampled *P. adspersus* (Fig. 2B-D) indicates that the use of LAG was effective. The geometric pattern of LAG deposition and the complete or partial resorption of the first and/or second LAG observed in the *P. adspersus* sections is also common among anurans (Smirina, 1972; Hemelaar, 1985; Patón et al., 1991). False and double LAG were however, unusually abundant among the sampled *P. adspersus* (Fig. 1). False LAG have been associated with periods of drought during the active season of other anurans (e.g. Rogers & Harvey, 1994), and were probably deposited when sampled *P. adspersus* remained inactive for extended periods during summer between activity episodes following heavy rainfall. This was evident at the Diepsloot site where radio tracked *P. adspersus* were buried for approximately 22-24 weeks cumulatively during the six month (~26 week) summer period (Yetman & Ferguson, 2011a).

Body size and age

Pyxicephalus adspersus has a voracious appetite (Branch, 1976; Paukstis & Reinbold, 1984) and juveniles grow rapidly post-metamorphosis (Van Wyk et al., 1992; Douglas, 1995). After nine months, which included winter torpor, newly metamorphosed *P. adspersus* kept under semi-natural conditions had grown 1326% heavier and twice as long (Conradie et al., 2010). The consistently widest band of bone growth between the second and third LAG (Fig. 1) among the sections in this study suggests that sampled *P. adspersus* experienced the greatest somatic growth during their second active season. Subsequent reduction in bone growth appeared typical of a shift in resource allocation from growth to reproduction at sexual maturation (Caetano & Castanet, 1993; Smirina, 1994), suggesting that at our study sites, female and at least some male *P. adspersus* attained sexual maturity following their third active season. Other large anurans mature at an earlier or similar age, e.g. *Bufo marinus*, 1-2 years (Easteal, 1982) and *R. catesbeiana*, 1-4 years (Howard, 1981; Harding, 1997).

We suspect, considering that the oldest aged male and female in this study possessed 16 and 11 LAG respectively, that at undisturbed sites male and female *P. adspersus* can reach ≥ 20 and ≥ 15 years of age respectively. Similar ages have been reached by other anurans in the wild, e.g. >20 years for *Bombina variegata* (Plytycz & Bigaj, 1993) and 15 years for *Bufo marinus* (Tyler, 1975). In more arid areas such as the Kalahari, where *P. adspersus* can reportedly

spend several successive years in torpor underground (Du Preez & Carruthers, 2009), individuals are likely to live much longer than 20 years. This is because, across different habitats, *P. adspersus* may have a similar mean physiological longevity calculated as, e.g. mean age in years multiplied by the mean number of summer rainfall days at a specific locality (modified from Bastien & Leclair, 1992).

Males versus females

Male *P. adspersus* fight aggressively during spawning events, and since the largest males secure more matings and perform parental care of offspring (Cook, 1996; Cook et al., 2001), there is probably strong selection for large male body size in *P. adspersus* (Shine, 1979; Arak 1988). At our three study sites male *P. adspersus* were on average approximately three times (360 g) heavier, 1.5 times (54 mm) longer and possessed two more LAG than females suggesting shorter female longevity. There have been few similar studies on other anurans with reversed sexual size dimorphism (e.g. Briggs & Storm, 1970; Sinsch et al., 2001). Although greater body size may be attained through greater longevity of the larger sex in anurans (Monnet & Cherry, 2002), male *P. adspersus* were much larger than females of comparable LAG count (Fig. 2B). This was probably due to slower growth in females compared to males, which was observed in captive *P. edulis* (mistaken as *P. adspersus*, it seems) following the appearance of differentiated gonads in both sexes from approximately 60 days post-metamorphosis (Hayes & Licht, 1992). Gonadectomy of a portion of these animals did not affect their subsequent growth, suggesting that sex hormones are not related to the divergence in the growth rates of male and female *Pyxicephalus*.

Study site comparisons

Differences in the size and condition of animals between the three study sites were not matched by differences in their age (Table 1). This was probably because the aged animals represented a small subset of all the sampled animals ($n=45$ of 211 males; $n=30$ of 68 females). When we compared the mass, SVL or condition of only the aged males or females (not shown here), there was no significant difference in their size or condition between the three sites. Therefore animals at Glen Austin Pan were larger because they were probably also older.

A plausible explanation for the smaller body size and poorer condition of males at Bullfrog Pan is the contamination of this site by toxic leachate from an adjacent disused landfill. Since 1993 the leachate was channelled by a plastic drain to a pump-house; but in 1996 a leak in the drain was discovered. At this time a study (Slater-Jones, 1996) was performed when two newly metamorphosed *P. adspersus* with facial deformities were found in the pan. The study revealed an elevated concentration of lead (6.4 parts per million) in the tissue of newly metamorphosed *P. adspersus*, and a significant reduction in their body size with decreasing distance from the leak. Assuming in our study that males with 7–10 LAG were born between 1994 and 1999, those from Bullfrog Pan were possibly exposed to significant contamination

during metamorphosis, which could have reduced their size and condition as froglets and later as adults. In 1997 the leak was fixed, which could explain why males with <7LAG (born after 1997) were larger than males with ≥ 7 LAG at this site. All but two females at this site possessed <7LAG. The one female with 7 LAG appeared to be small for her age (Fig. 2B). The other female with 11 LAG was large (Fig. 2B), possibly because she was born in 1993 when leachate was successfully diverted from the pan. Deformities and reduced growth, body size, condition and/or longevity of amphibians from chemically contaminated sites have been reported in various studies (e.g. Carey & Bryant 1995; Rowe et al., 2001; Spear et al., 2009; Brodeur et al., 2011).

The modest decrease in SVL of male and female *P. adspersus* between 1992–93 and 2004–06 at Glen Austin Pan was unlikely due to differences in sampling or measurement because these were performed in the same way (Cook, 1996). Considering the increasing loss of habitat and mortality of *P. adspersus* with urban encroachment in Gauteng (CAY, pers. obs.), the reduction in SVL of adults at Glen Austin Pan possibly indicates that very large males (SVL > 190 mm) and females (SVL > 130 mm) have become increasingly rare. This is suggested by all four graphs in Fig. 4. The significance of the size decrease of *P. adspersus* at Glen Austin Pan is emphasized by considering that the mean SVL and mass of adults at this site during 1992–93 was greater than at any of the three sites during our study. Mean SVL of males at Glen Austin Pan in 1992–93 was also greater than the maximum SVL of males at Bullfrog Pan in 2004–06. Although we cannot completely discount variation in ecological conditions (e.g., rainfall, food availability or predation; Reaser, 2000) as a cause of the temporal or spatial differences in the body size or condition of *P. adspersus* in this study, indirect evidence suggests that large *P. adspersus* have become scarcer at several peri-urban breeding sites.

Conservation implications

Since some anurans take four or more years to mature (e.g. Metter, 1967; Matthews & Miaud, 2007), the generation time of *P. adspersus* is less than may have been predicted from the large adult size of this species (Peters, 1983). Therefore *P. adspersus* populations could have greater resilience than has been assumed. However, population breeding success is likely to fluctuate dramatically because *P. adspersus* spawning and larval survival is strongly related to rainfall which varies greatly between years (Read 1990; Yetman & Ferguson 2001a). In addition, juvenile *P. adspersus* probably experience high mortality prior to their sexual maturation when they spend ≥ 3 years moving large distances overland, as revealed by the philopatric behaviour of adults (Yetman & Ferguson, 2011b) and gene flow between populations ≥ 20 km apart (Yetman & Ferguson, unpubl. data). Terrestrial habitat conservation is therefore critical for *P. adspersus* juvenile survival, dispersal and recruitment, which probably exerts a stronger influence than adult survival on the growth of populations (Conroy & Brook, 2003; Grafe et al., 2004).

The oldest aged animal in this study was less than half the maximum age of 45 years reported for a captive *P. adspersus* (Channing, 2001). Moreover, <20% of males and females in this study had >7 or >5 LAG respectively (Fig. 3). This suggests that the life expectancy of *P. adspersus* at peri-urban sites is low, which would explain why very large males and females appear to have become increasingly rare, such as at Glen Austin and Bullfrog pans. This is of conservation concern because the largest male *P. adspersus* are reproductively most successful, partly because usually only they perform parental care of tadpoles, which are highly vulnerable to desiccation and predation (Cook, 1996; Cook et al., 2001). As in other anurans, the largest female *P. adspersus* probably produce the greatest number of eggs and clutches in a season (Howard, 1978; Reading & Clarke, 1995; Reading, 2007). Therefore efforts to reduce unnatural mortality of *P. adspersus* at threatened sites are strongly recommended. Habitat loss and road traffic generally represent the greatest threats to *P. adspersus* (Du Preez & Cook, 2004). Therefore the destruction of undeveloped terrestrial habitat connected to breeding sites should be prevented (Yetman & Ferguson, 2011b), and safer movement of *P. adspersus* across roads near breeding sites should be ensured (Langton, 1989; Puky, 2005).

Differences in the size and condition of *P. adspersus* at different breeding sites, suggests that the species requires conservation management at site-specific, as well as broader spatial scales (Boyd et al. 2008). An important local concern is the poorer condition of *P. adspersus* at Bullfrog Pan, which is the largest known historical breeding site for this species in Gauteng, and perhaps South Africa. Research is warranted to determine if *P. adspersus* at Bullfrog Pan is being adversely affected by pollution or some other factor. Studies have shown that the body size and/or age of anurans can give useful indications of habitat quality. For example, SVL and age of *R. catesbeiana* was significantly lower at sites with high pesticide contamination (Spear et al., 2009), and age at maturity in males and longevity in females of *Bufo viridis* was negatively related to the intensity of human land use within a 1 km radius of breeding sites (Sinsch et al., 2007). Presuming that the spatio-temporal differences in body size and condition of the sampled *P. adspersus* reflect anthropogenic transformation of habitat, *P. adspersus* could serve as a valuable indicator of degradation of seasonal wetlands and grasslands, which are both highly threatened in South Africa (Low & Rebelo, 1996; Le Roux, 2002).

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