Age and body size of *Rana kukunoris,* a high-elevation frog native to the Tibetan plateau

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We compared age and body size of breeding individuals between two populations of *Rana kukunoris*, a species endemic to the eastern Tibetan plateau, at different altitudes (3400 m and 3100 m). Minimum age at maturity was three years for males and four years for females at 3400 m, and three years in both sexes at 3100 m. The oldest males were seven (3400 m) and six (3400 m) years old, and the maximum age in females was eight years in both populations; there was no significant difference in age structure between the two populations. Frogs at 3400 m had an average body length of 47.7 mm (±0.18) in males and 54.8 mm (±0.27) in females, significantly shorter than the 49.5 mm (±0.60) and 59.7 mm (±0.40) in males and females at 3100 m, respectively. At both sites, females were significantly larger than males. The reduced body size might be attributable to the shorter growing season at the higher site, and the sex difference in body size could be associated with earlier maturity in males than in females.

Key words: body length, elevation, skeletochronology

Body size and age at maturity are important demographic traits. Knowledge concerning these traits is key to understanding the life history of a species. In amphibians, for which the duration of the growth period and temperature are two important factors determining body size and age (Miaud et al., 1999; Morrison & Hero, 2003), this type of data can be obtained through skeletochronology (Miaud et al., 1999; Lu et al., 2006; Ma et al., 2009).

Rana kukunoris is a frog endemic to the eastern Tibetan plateau (29–41ºN, 93–104ºE). It inhabits open alpine marshes from 2000 to 4400 m elevation, and is one of the highest living amphibians globally. In earlier taxonomic revisions it was considered a subspecies of *R. temporaria*, and later a subspecies of *R. chensinensis*, a species widespread across northern China (Fei et al., 2010). Its status as a valid species was established based on morphology (Xie et al., 2000) and mitochondrial DNA sequences (Jiang et al., 2002). In this study, we document age and body size of breeding *R. kukunoris* in two populations at different altitudes. Our major objectives are to 1) provide basic demographic information about this little-known frog, and 2) investigate how age and body size are related to local climatic conditions.

All specimens were collected throughout the breeding seasons of 2008 and 2009 from two localities (3100 m and 3400 m elevation; Table 1). Frogs were randomly caught by hand in their spawning habitats. About forty percent of the frogs were sampled at each site. We identified a frog as a sexually mature male if it displayed nuptial pads on the foredigits, and as a sexually mature female if it had well-developed oocytes (visible through the skin). We measured snout–vent length (SVL) to the nearest 0.1 cm using calipers. We clipped the longest phalange of the left hindlimb and preserved it in 10% formalin for histological analysis (total sample: 277 frogs, Table 1). All frogs were released at the site of capture.

For age determination, histological sections of phalanges were produced following Ma & Lu (2009). Since the frogs live in an area with distinct seasonality, we assumed that the number of LAGs (lines of arrested growth; Castanet & Smirina, 1990) correspond to the age of an individual. LAGs were counted and independently checked by both authors. For statistical analysis, Student's *t* tests were used to test for differences in mean age and mean body size, and chi-square tests were used to compare differences in age distributions between populations and sexes. Spearman's rank correlation was used to assess the relationship between body size and age. All statistical analyses were performed with SPSS 13.0 (SPSS Inc., 2004). Probabilities were two-tailed and all values are given as the mean ± standard deviation (SD).

Table 2 showed the minimum and maximum breeding age of males and females. For neither sex did mean age differ significantly between populations (Student's *t*-test: males, $t_{182}= -1.32$, $P=0.19$; females $t_{91}= -0.98$, $P=0.33$), despite significant differences in the distribution of age

Table 1. Characteristics of the sites where frogs were collected.

Characteristics	3400 m site	3100 m site
Geographical location	Gahai	Wanmao
Latitude and longitude (N,E)	34°15′, 102°27′	34°46′, 103°14′
Elevation (m)	3400	3100
Duration of active season (days)	200	230
Mean temperature of active season (°C)	8.2	9.8
Frog habitat	Alpine meadow marsh	Alpine meadow marsh
Spawning season	Early April–early May	Late March–late April
Total numbers of specimens examined	Male: 293; female: 215	Male: 156; female: 51
Numbers of aged specimens	Male: 63; female: 42	Male: 121; female: 51

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Table 2. Body size and age of breeding adult *Rana kukunoris* at two localities on the Tibetan plateau.

		Age (year)			Body size (mm)		
Site	Sex	Mean ± SD	*n*	Range	Mean ± SD	*n*	Range
3400 m site	Male	4.16±0.09	63	3–7	47.7±0.18	293	35.7–57.9
	Female	4.76±0.16	42	4–8	54.8±0.27	215	41.7–66.6
3100 m site	Male	4.29±0.05	121	3–6	49.5±0.60	156	38.5–58.8
	Female	4.96±0.13	51	3–8	59.7±0.40	51	50.9–68.2

classes (males, χ^2=12.07, df=4, P=0.017; females, χ^2=18.30, df=5, P=0.003; Fig. 1). Males were significantly younger than females at both sites (Student's *t*-test: 3400 m: t_{84}=4.01, P<0.001; 3100 m: t_{154}=5.87, P<0.001). The age distribution did not differ significantly between the sexes at 3400 m (χ^2= 12.75, df =5, P=0.26), whereas the difference was significant at 3100 m (χ^2=45.36, df =5, P<0.001).

Frogs of both sexes at 3400 m were significantly smaller than those from 3100 m (Student's *t*-test: males, t_{447}=5.71, P<0.001; females t_{91}=3.29, P<0.001; Table 2). At both sites, males were significantly smaller than females (3400 m: t_{506}=22.67; 3100 m: t_{205}=17.32; P<0.001 in both cases; Table 2). There were positive correlations

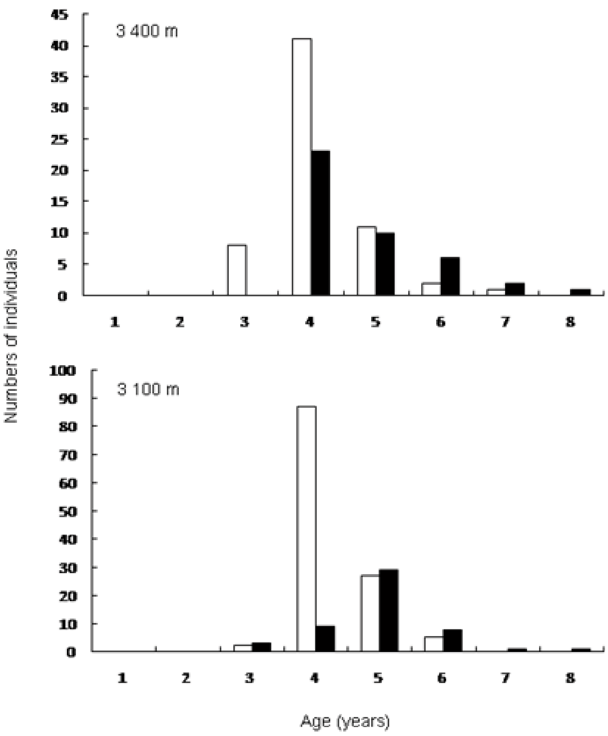

Fig. 1. Individuals per age category (males: white bars; females: black bars) in breeding *Rana kukunoris* from two populations on the Tibetan plateau.

between age and body size at both sexes and sites (3400 m: males, r_s=0.89, n=63, females, r_s=0.90, n=42; 3100 m: males, r_s=0.79, n=121, females, r_s=0.62, n=51; P<0.001 in all cases).

Previous studies indicate that amphibians in colder regions (high latitude or altitude) have larger body size and longer life span than those inhabiting warmer sites (Elmberg, 1991; Miaud et al., 1999; Morrison & Hero, 2003; Lu et al., 2006; Liao & Lu, 2010). Typically, this is thought to be related to a shorter activity period available for growth, which delays the time at which sexual maturity is reached (Morrison & Hero, 2003; Angilletta et al., 2004). Animals from colder environmental conditions therefore reach a larger body size and greater age at sexual maturity (Monnet & Cherry, 2002). In our study, *R. kukunoris* had a smaller body size with decreasing temperatures and a reduced annual activity period. This is contrary to Bergmann's rule (that animals in colder regions have larger body sizes than those in warmer regions) and has previously been observed in other amphibians (Ashton & Feldman, 2003; Angilletta et al., 2004; Ma et al., 2009). Because a shorter growth season would cause smaller size even if growth rates do not change, it is the duration of the growth period that could play a major role in shaping temperature-dependent differences in body size.

In both populations, females were older and larger than males, in accordance with most other studies on anurans (Monnet & Cherry, 2002). This sexual difference can be explained by a trade-off of sex-specific energy allocation between current growth and future reproduction (Williams, 1996). To maximize lifetime reproductive success, females invest more energy into somatic growth, which will allow them to delay sexual maturity and so reach a larger final body size and attain higher fecundity (Miaud et al., 1999; Monnet & Cherry, 2002; Morrison & Hero, 2003). Our previous results (Lu et al., 2008) support this prediction by finding that larger female *R. kukunoris* had higher fecundity than smaller females. Because annual growth rates of females have been reported to be lower than those of males (Miaud et al., 1999; Morrison & Hero, 2003), we speculate that age was the major contributor for sexual difference in body size in *R. kukunoris*, consistent with previous results from other anuran species (*R. nigromaculata*: Khonsue et al., 2001; *R. swinhoana*: Lai et al., 2005; *Hyla annectans chuanxiensis*: Liao & Lu, 2010).

Most previous studies have shown that body size and age are positively but weakly correlated in adult amphibians (Halliday & Verrell, 1988), although sometimes only one sex shows a correlation between age and size (e.g. *R. pipiens*; Kutrup et al., 2005), and no correlation between body size and age has even been found (Wake & Castanet, 1995). Similar to, for example, *H. annectans chuanxiensis* (Liao & Lu, 2010), *R. kukunoris* had a positive correlation between body size and age for both sexes at both study sites. Apart from the duration of the annual period of activity, other environmental and genetic factors on age and body size need to be addressed in future studies.

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