Age and body size of *Rana amurensis* from northeastern China

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**Abstract** Age and body size are two important demographic traits that determine the life history strategies of populations and species. We measured these two parameters of *Rana amurensis*, at a 900 m and a 500 m altitude site in northeastern China. At the two sites, age at first reproduction was 2 years for males and 3 years for females. The maximum age of males and females at the high-altitude site was 6 and 7 years, and 5 and 7 years at the low-altitude population, respectively. Females were significantly larger than males in both populations, due to greater age in both the high- and low-altitude sites. Body size of either males or females did not differ significantly between populations; only males showed increased body size at the high-altitude site when age effect was statistically controlled for. The increased cline of male body size may be attributable to delayed maturation of the sex due to a shorter growing season at high altitudes [Current Zoology 57 (6): 781–784, 2011].

**Keywords** Age structure, Body size; life history; *Rana amurensis*

Age and body size are two important life history traits, and they often vary along environmental gradients. Altitude is among environmental gradients known to influence demographic traits of organisms including amphibians (Ryser, 1996; Morrison and Hero, 2003). Its effect mainly results from differences in temperature and the length of activity seasons between lower and higher altitudes. This is particularly true for ectotherms, including amphibians (Walters and Hassall, 2006). Many comparative studies reveal that due to weak migrating abilities of amphibian species and differences in habitat quality, amphibians are highly sensitive to environmental gradients in terms of life history traits, even when the gradients are fine. Moreover, sexual differences can arise in response to environmental changes (Lu et al., 2006; Sinsch et al. 2007; Liao et al., 2010; Chen et al., 2011).

*Rana amurensis* is a common amphibian species widely distributed in temperate areas, including north China, Mongolia and Russia, with streams surrounded by forests as their favorable habitats (Kuzmin, 1999; Fei et al., 2010). Here we investigated the age structure and body size of two populations in northern China. Our objective was to explore patterns of covariation between these two demographic parameters and altitude.

1 Materials and Methods

We conducted field work at two localities with different altitudes in Aershan city, inner-Mongolia, northeastern China. The high-altitude site (900 m a.s.l.) was a pond near Yiershi town (47°17'N, 119°48'E), and the low-altitude site (500 m a.s.l.) was a stream near Minshuihe town (46°43'N, 120°49'E). The mean annual temperature of the high- and low-altitude sites was −5.9 °C and −3.5 °C, respectively. In this area, the frogs reproduce in early May and commence hibernating in October, at the bottom of lakes and rivers and usually in groups (Solomonova et al., 2011). Their growth period was considered to be 130 days at the high-altitude site, and 110 days at the low-altitude one.

In total, 155 adult individuals (76 females and 79 males; Table 1) were randomly captured by hand in the water at their winter hibernation habitats in October 2009. Sexes were recognized by secondary sex characteristics (nuptial pads on the foredigits for male; easily visible oocytes through the skin of the abdomen for female). Body size (snout-vent length, SVL) of each individual was measured to the nearest 0.1 mm using a vernier caliper. The longest phalange of the left hindlimb was clipped and reserved in 10% formalin for histological analysis. All frogs were then released at the site of capture.

For age determination, histological sections of the frog phalanges were produced following Ma and Lu (2009). As reported by other authors (Morrison et al. 2004; Lai et al. 2005), we assumed that the number of lines of arrested growth (LAG) corresponded to the age of an individual (Castanet and Smirina, 1990),
since the frogs’ life goes through a strong seasonal cycle, as do other temperate amphibians (Lu 2004). The age of each frog was established by two people who independently counted the number of LAGs present in the bone sections.

All variables were first tested for normality using the one-sample Kolmogorov-Smirnov procedure (Age: $P < 0.001$; Body size: $P > 0.05$). Mann-Whitney $U$ tests were performed to compare the mean age between populations and sexes, and Chi-square tests to compare differences in age distributions between populations and sexes. Student’s $t$-tests were used to compare the mean body size between populations and sexes. In order to remove age effects, ANCOVA were conducted to explore the difference in body size between populations with body size as a dependent variable, population as a factor and age as a covariate. All statistical analyses were performed with SPSS 13.0 (SPSS Inc., Chicago, USA). Probabilities are two-tailed and all values given as the mean ± standard deviation ($SD$).

2 Results

The histological sections of the longest phalange showed a series of narrow concentric haematoxylinophilic rings or lines that can be used to count age of the frogs (Fig. 1). In both populations, the minimum age at first reproduction was 2 years for males and 3 years for females. The maximum age of males was 6 and 5 years in high and low altitudes, respectively, and the value of females in each population was 7 years (Fig. 2). Average age between the sexes at each site differed significantly (Mann-Whitney $U$ test, low altitude: $z = 3.18$, $P = 0.001$; high altitude: $z = 4.11$, $P < 0.001$). Age distribution between sexes was different at each site (high altitude: $\chi^2 = 32.57$, $df = 5$, $P < 0.001$; low altitude: $\chi^2 = 60.90$, $df = 5$, $P < 0.001$; Fig. 2). There was no significant difference in the average age of males and females between the two populations (male: $z = 0.16$, $P = 0.87$; female: $z = 1.71$, $P = 0.09$). The age distributions of each population were different (male: $\chi^2 = 29.66$, $df = 4$, $P < 0.001$; female: $\chi^2 = 51.95$, $df = 4$, $P < 0.001$).

SVL was significantly different between sexes in each age class (Table 1). Females are larger than males at each population (Student’s $t$-test: high altitude, $t = 6.50$, $df = 77$, $P < 0.001$; low altitude, $t = 6.42$, $df = 71$, $P < 0.001$). For each sex, the average body size between the two populations did not differ significantly (male: $t = 0.79$, $df = 74$, $P = 0.433$; female: $t = 0.42$, $df = 77$, $P = 0.673$). When the effect of age was removed the difference in body size in females between high and low altitudes remained non-significant, whereas a significant difference in body size in males was found (Table 2).

3 Discussion

Skeletochronology has been widely adopted to evaluate age in amphibians (Khonsue et al., 2000; Miaud et al., 2007; Guarino and Erismis, 2008), including the closely related species of $R$. amurensis ($R$. temporaria, Miaud et al., 1999; $R$. chensinensis, Lu et al., 2006; $R$. kukunoris, Chen et al., 2011). In this study, we obtained clear LAGs in phalangeal cross sections of

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**Fig. 1** Phalangeal cross-sections (14 μm thick, stained by Ehrlich’s hematoxylin, × 400) of adult $R$. amurensis (a three-year-old male from 500 m a.s.l. site) Arrows indicate the lines of arrested growth (LAG). KL represents the Kastschenko line, the interface between the endosteal and periosteal zones.

**Fig. 2** Age distribution of adult $R$. amurensis (males: black bars; females: white bars)
Table 1  Average body size (mm) ± SD according to age in \textit{R. amurensis}

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>900-m site</th>
<th>500-m site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male ((n=40))</td>
<td>Female ((n=39))</td>
</tr>
<tr>
<td>2</td>
<td>34.0 ± 1.0 (12, 33.2-36.3)</td>
<td>−</td>
</tr>
<tr>
<td>3</td>
<td>46.8 ± 4.1 (14, 35.4-49.5)</td>
<td>48.3 ± 1.2 (9, 41.4-52.4)</td>
</tr>
<tr>
<td>4</td>
<td>55.5 ± 1.0 (9, 53.3-56.4)</td>
<td>59.8 ± 3.3 (16, 53.0-64.0)</td>
</tr>
<tr>
<td>5</td>
<td>57.1 ± 0.4 (3, 56.7-57.5)</td>
<td>65.0 ± 0.6 (8, 64.3-66.3)</td>
</tr>
<tr>
<td>6</td>
<td>66.0 ± 1.7 (2, 64.8-67.2)</td>
<td>71.4 ± 2.3 (5, 69.2-74.3)</td>
</tr>
<tr>
<td>7</td>
<td>−</td>
<td>76.2 (1, −)</td>
</tr>
</tbody>
</table>

Sample size and range are given in parentheses.

Table 2  Results of ANCOVA for body size of \textit{R. amurensis}

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>(F)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>1</td>
<td>397.94</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Population</td>
<td>1</td>
<td>5.19</td>
<td>0.026</td>
</tr>
<tr>
<td>Age</td>
<td>1</td>
<td>527.86</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Error</td>
<td>73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>1</td>
<td>182.17</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Population</td>
<td>1</td>
<td>3.65</td>
<td>0.06</td>
</tr>
<tr>
<td>Age</td>
<td>1</td>
<td>182.90</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Error</td>
<td>76</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textit{R. amurensis}. Evidence shows that endosteal resorption in amphibians can destroy a certain number of LAGs by widening the marrow cavity and thus affect the accuracy of counting LAGs (Leclair, 1990; Guarino et al., 1995). Our results showed that in \textit{R. amurensis}, the LAG resorption only partially reached the innermost LAGs, so it was easily distinguishable and would not affect our age estimations.

Many anurans tend to show greater longevity and attain larger body sizes in high latitudes and altitudes where climates are cold and seasonal time constrains strong (reviewed by Morrison and Hero, 2003). For these species, longevity and body size increases as environments become colder and the shorter seasonal time available for growth in colder regions may limit annual growth of animals so that they mature later, get older and thus reach a larger body size (Miaud et al., 1999; Liao and Lu, 2010). In our study, however, only male \textit{R. amurensis} followed this pattern, as observed in several anuran species (Ryser, 1996; Morrison and Hero, 2002; Lu et al., 2006). The sex-specific response in life-history strategy to altitude shift remains to be explored.

At the two sites, females on average had significantly longer longevity and larger body size than males as reported by Solomonova et al. (2011). This is a widespread pattern seen in amphibians (Shine, 1979; Monnet and Cherry, 2002). Longer longevity in females may result from a high male-biased mortality rate (Shirose et al., 1993), and/or from delayed maturation in the sex (Czarnoleski and Kozlowski, 1998; Kozlowski et al., 2004). Sexual size dimorphism may also be due to sexual differences in growth rates (Howard, 1981) and/or age structure (Monnet and Cherry, 2002). In amphibians, females have slower growth than males (Morrison and Hero, 2003), so we speculate that age might be a major contributor to sexual difference in body size observed in \textit{R. amurensis}. This idea is also consistent with previous work for numerous anuran species (e.g. Khonsue et al., 2001; Eaton et al., 2005).

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References  

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