Pre-hibernation energy reserves in a temperate anuran, *Rana chensinensis,* along a relatively fine elevational gradient

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Temperate anurans have energy substrates in the liver, fat bodies, carcass and gonads; these stores provide support for metabolism and egg production during hibernation, and for breeding activities in spring. This paper compares the energy budget shortly before hibernation among *Rana chensinensis* populations at elevations of 1400, 1700 and 2000 m along a river in northern China. The larger frogs, regardless of elevation, had relatively heavy storage organs and the masses of nearly all these organs were positively correlated with each other. After controlling for the effect of body size, we found no significant difference in energetic organ mass among different age classes for each of the three populations. There were sexual differences in energy strategy. Males in all populations accumulated greater reserves in liver, fat bodies and carcass than did females. In contrast, females put more energy into their ovaries and oviducts. Frogs from higher elevations tended to have heavier organs than those from lower elevations; however, the pattern did not vary systematically along fine environmental gradients. Mid-elevation *R. chensinensis* built up significantly more reserves than low-elevation individuals, but were similar to their highland conspecifics. Males from higher elevations tended to have heavier liver and fat bodies; females were similar in liver and ovary mass across all elevations, but formed heavier fat bodies, oviducts and somatic tissue at higher elevation sites.

Key words: elevation, life history, sex differences, storage organs

INTRODUCTION

Body reserves of anurans living at high latitudes or high elevations go through a pronounced annual cycle. This cycle begins after reproduction, reaches a maximum prior to the onset of winter, and then drops to its lowest level at spring emergence the next year (Fitzpatrick, 1976; Jørgensen, 1992; Tsiora & Kyriakopoulou-Sklavounou, 2001a, b). Energy in the form of fat or glycogen stores is deposited in the liver (van Beurden, 1980), abdominal fat bodies (Brown, 1964) and skeletal muscle or somatic tissue (Seymour, 1973; Whitford & Meltzer, 1976; Morton, 1981; Fournier & Guderly, 1993; Donohoe et al., 1998), as well as in female gonadal tissue (Long, 1987; Grafe et al., 1992; Villecco et al., 1999). These stores are used for maintenance during winter dormancy (Donohoe et al., 1998; Boutilier, 2001), gamete production (Fitzpatrick, 1976; Girish & Saidapur, 2000) and breeding activities (Docherty et al., 1995; Pope & Matthews, 2002). The ability to develop body reserves strongly influences individual fitness (Komoroski et al., 1998; McCauley et al., 2000).

The amount of energy needed to survive the winter depends on the duration and severity of the winter. Anurans living at higher latitudes or higher elevations are expected to develop more reserves in order to survive prolonged, cold winter periods when foraging is not possible (Pasanen & Koskela, 1974; Irwin & Lee, 2003). Reproductive success is also influenced by available body reserves for both sexes, and this is often size-de-pendent. Larger males tend to call more often and mate more frequently (Arak, 1988; Tejedo, 1992; Marquez, 1993). Large females make a greater investment in egg production (Jørgensen, 1981; Gibbons & McCarthy, 1986) than small females, which devote more energy to growth (Halliday & Tejedo, 1995). Moreover, patterns of resource allocation between growth and reproduction during either a single season or a lifetime may be sex-specific because of the low cost of spermatogenesis relative to vitellogenesis (Halliday & Verrell, 1988; Merkle, 1989; Jørgensen, 1992). Female reproductive outputs, which are closely related to pre-hibernation energy reserves, are often ecologically constrained. Reduced energy requirements in eggs at higher latitudes (Koskela & Pasanen, 1975; Jørgensen et al., 1979; Miaud et al., 1999; Lu, 2004) or elevations (Kozłowska, 1971; Elmberg, 1991) have been observed in temperate anurans. Despite the importance of variations in the pattern of energy allocation in the evolution of life histories of anurans, research on this subject is limited and the proximate causes of the variation remain unclear.

Rana chensinensis is a frog native to northern China (Tanaka-Ueno et al., 1999). This frog is found across a broad latitudinal (31–42ºN) and elevational (300–3100 m) range. Within this range, *R. chensinensis* is restricted to montane rivers where it is the most abundant amphibian. The species has an extended period of inactivity (3–5 months) and a short breeding period (3–4 weeks). The frogs start to build up energy reserves shortly after breeding, reaching a peak by late autumn (Lu, 2004), prior to the

Table 1. A comparison of body size (SVL, mm), age and dry masses (mg) of storage organs in *Rana chensinensis* from three elevations (1400, 1700 and 2000 m) along a river in northern China. Values are given as mean ± SE, with range in parentheses.

	Low elevation	Mid-elevation	High elevation
Male			
n	26	22	24
SVL	39.7±0.5 (35.1–43.7)	43.0±0.6 (38.7–49.1)	43.7±0.9 (34.0–52.3)
Age	1.9±0.1 (1–2)	2.1±0.4 (1–3)	2.5±0.2 (1–4)
Liver	85.0±5.3 (41–142)	153.0±10.4 (87–235)	148.3±12.0 (63–337)
Fat body	22.5±3.0 (5–52)	61.1±5.8 (8–126)	75.1±7.9 (18–151)
Carcass	1034.3±55.5 (619–1754)	1399.3±66.7 (932–2021)	1567.2±121.3 (643–3125)
Female			
n	21	20	20
SVL	44.8±1.1 (36.5–54.5)	43.6±1.3 (33.1–55.9)	50.7±0.9 (43.5–60.7)
Age	2.7±0.2 (1–5)	2.6±0.2 (1–6)	3.9±0.3 (2–7)
Liver	100.0±7.7 (40–185)	112.8±12.4 (40–229)	156.0±12.5 (94–335)
Fat body	15.7±2.8 (7–52)	34.8±4.3 (13–87)	50.8±4.2 (24–84)
Ovary	560.3±65.9 (126–1320)	511.2±65.1 (184–1124)	959.6±87.2 (214–1614)
Oviduct	138.1±19.5 (34–362)	190.8±29.1 (47–449)	338.5±30.0 (35–628)
Carcass	1157.5±101.0 (550–2196)	1250.9±107.5 (710–2426)	2078.4±131.7 (1289–3121)

period of winter inactivity. Larger females are more fecund, and females lay all their eggs in a single clutch (Lu, 1994). In a mating system with a male-biased sex ratio, larger males would have competitive advantages for larger females, and are more likely to have multiple matings (Halliday & Tejedo, 1995). In a comparative demographic study of *R. chensinensis* populations along a montane river, we have shown that frogs from higher elevations are characterized by delayed maturity, larger size at maturity and slower growth rates, compared with populations at lower elevations (Lu et al., 2006). There were exceptions to this pattern between some neighbouring populations, and the differences were sex specific. This study was designed to examine whether energy reserves of *R. chensinensis* vary along elevational gradients, and whether there are different patterns in males and females. Our aims were to understand how energy budgets may be linked to the life history traits of this species and more generally, temperate anurans.

MATERIALS AND METHODS

The Ximafang River runs from an altitude of 2200 m down to 1380 m in the Luya Mountains (39°40'N, 112°20'E) of Shanxi Province, China. Mean annual air temperature at the 1400 m site was 6.3 °C. We used a regression model developed for montane areas of northern China (Li & Huang, 2004) to estimate that mean annual air temperatures at 1700 and 2000 m would be 5.0 and 4.1 °C, respectively. Frogs were collected during late autumn of 2002 from sites at altitudes of 1400, 1700 and 2000 m along 11.3 km of the Ximafang River.

The frogs hibernated (aggregately submerged and inactive) beneath stones in the river, in hollows under the riverbank, or at the bottom of deep ponds. We caught frogs by hand at their hibernation habitats. At each elevational site, we attempted to capture sexually mature frogs over a period of one or two consecutive days.

Sexual maturity was determined by the presence of nuptial pads on the thumbs of male frogs and by fully developed oocytes visible through the skin of females (Lu, 2004). Sexually mature frogs were killed and preserved in 10% formalin, while juvenile frogs were released at the point of capture. A total of 133 adult frogs were captured. This included 26 males and 21 females at 1400 m, 22 and 20 at 1700 m, and 24 and 20 at 2000 m.

Snout–vent length (SVL) was measured to the nearest 0.1 mm for each frog and age was determined by skeletochronology (Lu et al., 2006). Once frogs were preserved, we removed the liver, fat bodies, ovaries and oviducts. The organs and the remaining carcass (exclusive of all the viscera) were dried to constant mass at 60 °C (Bradford, 1984) and weighed (to 0.001g) using an electronic balance. According to the suggestions of Packard and Boardman (1999), we assessed energy budgets of the frogs using dry mass of each storage organ and the carcass.

We used Pearson's correlation coefficient to evaluate the relationships between different organs, and between organ mass and body size. Differences in average organ mass between either age classes, sexes or elevations were tested using ANCOVAs with SVL as a covariate. If the overall ANCOVA results were significant for elevational comparisons, we did post hoc multiple comparisons (Fischer's LSD) to evaluate differences between pairs of populations. Prior to analysis, SVLs and organ masses were ln-transformed to attain a normal distribution. All probabilities were two-tailed with $\alpha = 0.05$, and values are presented as mean ± standard error (SE).

RESULTS

Chronology and population structure

In 2002, hibernation began in early November at 1400 m, late October at 1700 m, and mid-October at 2000 m. In 2003, breeding occurred in late March, mid-April and late April

Table 2. Regression of liver, fat bodies, ovaries, oviducts and carcass (in mg) against SVL (in mm) for *Rana chensinensis* from three elevations (1400, 1700 and 2000 m) along a river in northern China. Allometric function y = ax^b, where y is somatic index, x is SVL, and a and b are constants. For all the relationships that are significant, r^2>0.37, P<0.003.

	Low elevation	Mid-elevation	High elevation
Male			
Liver	$y = -9.31x^{3.73}$	$y = -5.99x^{2.92}$	$y = -6.02x^{2.90}$
Fat body	NS	NS	$y = -12.40x^{4.39}$
Carcass	$y = -6.04x^{3.52}$	$y = -2.86x^{2.68}$	$y = -5.78x^{3.46}$
Female			
Liver	$y = -5.72x^{2.70}$	$y = -5.14x^{2.59}$	$y = -4.75x^{2.49}$
Fat body	$y = -12.46x^{3.96}$	$y = -5.14x^{2.59}$	NS
Ovary	$y = -9.47x^{4.13}$	$y = -7.02x^{3.48}$	$y = -12.36x^{4.88}$
Oviduct	$y = -13.53x^{4.81}$	$y = 11.87x^{4.49}$	$y = -15.29x^{5.35}$
Carcass	$y = -5.55x^{3.30}$	$y = -0.70x^{2.06}$	$y = -4.04x^{2.97}$

at the three study sites. Males ranged from one to four years of age, with the oldest being two, three and four years at low-, mid-, and high-elevation populations. Similarly, females ranged from one to seven years, with the oldest being five, six and seven years at the three elevations. Adult sizes varied greatly within populations, with a wider size range in females (SVLs of the largest individuals exceeded 40–69% of those of the smallest) than in males (the corresponding value was 25–54%, Table 1).

Correlations between storage organ masses

With the exception of the liver and carcass of frogs at the mid-elevation site ($r=0.21$, $P=0.34$), the masses of all the organs and carcass of male frogs were positively correlated with each other at all three elevations (all $P<0.02$). For females, there was a significant positive relationship in ten, eight and seven of the ten correlations ($P<0.02$) for low-, mid- and high-elevation populations, respectively. The correlation was not significant between liver and ovary mass ($r=0.42$, $P=0.07$) or between fat body and ovary mass ($r=0.42$, $P=0.07$) in mid-elevation populations. In high-elevation females, correlations were not significant for liver and fat body ($r=0.18$, $P=0.44$), fat body and ovary mass ($r=0.27$, $P=0.24$) or fat body and oviduct ($r=0.16$, $P=0.51$).

Storage organ mass in relation to body size

Within each population, larger individuals had greater absolute mass for most, but not all, organs. In males this was true for seven of nine comparisons ($P<0.003$), but not for fat bodies at low ($r=0.37$, $P=0.06$) and mid elevations ($r=0.24$, $P=0.31$). For females, 14 of 15 comparisons were significant ($P<0.002$), but not for fat bodies at high elevation ($r=0.41$, $P=0.07$; Table 2).

Age and sexual variation in organ mass within a population

When controlling for body size using ANCOVAs, age had no effect on mass of the storage organs in any population for either males ($P>0.04$ for 8 of 9 tests, with the exception of liver at the mid-elevation site; $F_{[2, 23]}=17.57$, $P=0.001$, with a Bonferroni correction for the significant multiple comparisons), or females ($P>0.03$ for all 15 tests).

ANCOVAs, which removed the effect of SVL, revealed that males had significantly heavier liver, fat bodies and carcasses than females at all populations (low-elevation site, $F_{[2,44]}>6.40$, $P<0.015$; mid-elevation site, $F_{[2,39]}>9.67$, $P<0.003$; high-elevation site, $F_{[2,41]}>11.76$, $P<0.001$, in all organs; Table 1). However, when analysis was made for the sum of all the storage organs (including ovaries and oviducts of females), we found that females exceeded males at all populations (low elevation $F_{[2,44]}=3.36$, $P=0.074$; mid-elevation $F_{[2,39]}=17.20$, $P<0.001$; high elevation $F_{[2,41]}=17.22$, $P=0.001$).

Elevational variation in storage organ masses

Liver and fat bodies of males were significantly heavier with increasing elevation ($F_{[3,68]}>11.85$, $P<0.001$, Table 1). Pairwise comparisons showed mid-elevation males were significantly greater in both of the organs than low-elevation males ($P<0.001$), but not significantly different from those at high elevation ($P>0.22$). No significant difference in carcass mass was found across elevations ($F_{[3,68]}=1.94$, $P=0.15$).

Female fat body, oviduct and carcass masses differed significantly across elevations ($F_{[3,57]}>9.87$, $P<0.001$), but liver and ovary masses did not ($F_{[3,57]}<1.50$, $P>0.23$, Table 1). Mid-elevation frogs had significantly heavier fat bodies than low-elevation females ($P<0.001$), but they were similar to those from the high-elevation site ($P=0.91$). The oviducts (mid versus low elevation, $P<0.001$; mid versus high elevation, $P=0.57$) and carcass (mid versus low elevation, $P=0.003$; mid versus high elevation, $P=0.065$) followed the same trend.

DISCUSSION

There were strong positive correlations between organ masses in *R. chensinensis*, and larger frogs had greater organ masses. Exponents of the regression of mass and SVL were approximately three in nearly all organs. Because body mass is related to the cube of SVL, we expected energy stores in the organs to vary directly with body mass, as observed in a variety of other vertebrates including anurans (Withers & Hillman, 2001). For male fat

bodies in the high-elevation population, and female ovaries in the low- and high-elevation populations and oviducts in all the populations, the exponents were greater than predicted, indicating that these organs increased in mass at a faster rate than body length.

We found significant sex differences in energy budget, with males storing more in liver, fat bodies and carcass tissues, and females having a higher total condition index, mostly as a result of more high-fat stores in the ovaries and oviducts. Males of many species have higher energetic costs than females for reproductive activities (calling, courtship and aggression: Taigen & Pough, 1985; Prestwich et al., 1989; Wells et al., 1996). This is also likely to be true for male *R. chensinensis* because they engage in competition for mates (Lu, unpubl. data). Males use their energy reserves, especially lipids, for breeding activities rather than spermatogenesis (Halliday & Verrell, 1988; Jørgensen, 1992). In females, reproductive success is influenced by their ability to produce eggs, and thus they put a high proportion of energy reserves into ovaries and oviducts.

As expected, frogs at higher elevations had greater energy reserves, regardless of size or age. Greater stores are an adaptation to the long, cold winters and the associated metabolic demands. Although foraging time at higher elevations is limited, *R. chensinensis* may shorten or skip post-reproductive dormancy in order to obtain enough energy reserves, as do other high-elevation anurans (Pasanen & Koskela, 1974; Irwin & Lee, 2003).

Discordance with the expected model was observed in populations at neighbouring elevations. In both male and female *R. chensinensis*, mid-elevation frogs had energetic organ masses similar to high-elevation frogs, but differed significantly from their low-elevation conspecifics. This was probably because environmental conditions did not vary systematically along the relatively small elevational gradient that we studied. There was, however, a sex-related difference in energy storage along the elevational gradient. Higher-elevation males stored more energy in liver and fat bodies. Higher-elevation females did the same in fat bodies, oviducts and carcass tissues, but females at all elevations put similar reserves into both liver and ovaries. These patterns appear to be associated with sex differences in resource allocation between maintenance and reproduction.

Liver glycogen is a source of energy during hibernation (Jones, 1980). In some high-elevation anurans that have developed the ability to tolerate freezing temperatures, liver glycogen is released into the blood as freezing progresses (Croes & Thomas, 2000; Layne & Jones, 2001). However, we found that the patterns of elevational variation in liver mass differed between sexes, with males having heavier livers with increased elevations while females did not. Elmberg (1991) also found lower liver reserves in high-elevation female *R. temporaria*. An experimental study showed that hibernating *R. temporaria* shift their metabolism from lipid- to carbohydrate-based (Donohoe et al., 1998), or use anaerobic pathways to utilize skeletal muscle glycogen after exhaustion of liver glycogens (Donohoe & Boutilier, 1999). Boutilier & St-Pierre (2002) demonstrated that lipids in the carcass also supply energy for the mitochondrial metabolism of frogs during hibernation. For our species, *R. chensinensis*, because only females at higher elevations had a heavier carcass mass, we assumed that there is a sex difference in energy allocation between liver and carcass.

In *R. chensinensis*, ovary mass was not related to elevation. This is in contrast with observations in other anurans where ovarian mass tends to decrease with decreasing temperatures at high latitude (Pasanen & Koskela, 1974; Jørgensen et al., 1979; review in Lu, 2004) or elevation (Elmberg, 1991). Lower reproductive investment by anurans in colder climates is thought to be due to the shorter time available to accumulate energy for reproduction (Lu, 2004). At high latitudes or elevations, however, animals may shorten the post-oviposition resting period and hence attain growth rates similar to lower latitude or lower elevation congeners (Elmberg, 1991; Lu, 2004). Female *R. chensinensis* at higher elevations may follow this strategy to achieve a reproductive output similar to that of frogs at lower elevations.

Anuran body reserves are believed to play a role in vitellogenesis, serving as sources of energy for follicular growth (Chieffi et al., 1975; Prasadmurthy & Saidapur, 1987; Delgado et al., 1990; Girish & Saidapur, 2000). Vitellogenesis continues through hibernation in some anurans (Rastogi et al., 1983; Delgado et al., 1990; Loumbourdis & Kyriakopoulou-Sklavounou, 1996), including *R. chensinensis* (Lu, 2004). Maruyama (1979) found that with a decrease in total lipids, ovarian lipids of *R. nigromaculata* nearly doubled during hibernation. Moreover, greater fat body reserves are related to larger-sized eggs in a number of anurans (Halliday & Tejedo, 1995; Girish & Saidapur, 2000). We did observe larger egg sizes and relatively heavy clutch masses in *R. chensinensis* populations at higher elevations (Lu, unpubl. data).

Rana chensinensis at higher elevations were both older and larger at sexual maturity. Theoretically, higher elevation frogs should allocate more resources to growth and less to reproduction when they are young, but this pattern changes as frogs became older (Kozłowski, 1992); however, we found no age-related difference in energy budgets for either sex in any population. In addition, the demographic response of *R. chensinensis* to elevation was sex-specific (Lu et al., 2006). Males at the mid-elevation site were closer in body size and age to frogs in the low-elevation population, but different from high-elevation frogs. Females at mid elevation were closer to high-elevation frogs, but differed from low-elevation frogs. This pattern did not parallel changes in energy reserves of either sex along the elevation gradient in this study. We believe that pre-hibernation energy reserves mainly serve proximate needs for hibernation metabolism and subsequent reproduction, while demographic traits are a result of resource allocation between growth and reproduction over a lifetime (Czarnoleski & Kozłowski, 1998; Kozłowski et al., 2004). However, because of the limited sample size and altitudinal range over which the study was done, caution is needed in these interpretations. If a comparison of frogs from a wide altitudinal gradient is conducted, we might have more clear-cut results.

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