



Do anurans living in higher altitudes have higher pre-hibernation energy storage? Investigations from a high-altitude frog

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The temporal dynamics of energy storage is an important life history aspect of temperate anurans, but comparative studies of pre-hibernation energy storage of anuran populations from different altitudes are scarce. We investigated energy storage patterns for three *Rana kukunoris* populations living at different altitudes in the Tibetan plateau (2300 m, 2900 m and 3500 m a.s.l.). Analyses of relative energy organ weights and relative carcass weights revealed that pre-hibernation energy stores decreased with increasing altitude, and that males deposit larger energy reserves in fat bodies and liver, while females have larger energy storage in gonads. Lower pre-hibernation energy reserves with increasing altitude may be an adaptive response to a shorter activity period and lower food availability at higher altitudes, while the observed sex difference may result from differential timing of energy allocation for reproduction.

Key words: energy storage, pre-hibernation, *Rana kukunoris*, Tibetan plateau

INTRODUCTION

Energy storage plays an important role in the life histories of anurans inhabiting temperate areas (Wells, 2007). Given a limited amount of available energy, anurans trade off the investment of energy among maintenance, growth and reproduction. Accordingly, this allocation results in different life history strategies (Roff, 2002; Jönsson et al., 2009), and the amount of energy stored in the body is suggested to strongly influence individual fitness (Komoroski et al., 1998; Lu et al., 2008).

In anurans, stored energy in the form of triglyceride and glycogen is deposited in the liver (Fitzpatrick, 1976), abdominal fat bodies (Fitzpatrick, 1976), gonadal tissues (Villemo et al., 1999) and muscle tissue (Donohoe et al., 1998). These stores are used for overwintering (Boutillier, 2001; Pope & Matthews, 2002), gamete production (Girish & Saidapur, 2000), and breeding activities (Pope & Matthews, 2002; Jackson & Ultsch 2010).

Studies show that energy storage of anurans can be affected by the environment (Jönsson et al., 2009; Chen et al., 2011b). Anurans living at higher latitudes or higher elevations are expected to store more energy in order to survive prolonged, colder winters during which foraging is not possible (Irwin & Lee, 2003). Anurans living at high altitude are also challenged by having less food available outside the overwintering period, higher heat loss and lower metabolic rates (Morrison & Hero, 2003).

Anurans are classified as either income breeders or capital breeders, with income breeding anurans feeding after emergence and before breeding, whereas capital breeders refrain from feeding until after breeding (Drent & Daan, 1980; Jönsson, 1997; Jönsson et al., 2009). Sex differences in the time of energy allocation towards breeding are common in capital breeders (Jönsson et al., 2009; Chen et al., 2011b). Males invest energy into breeding activities after overwintering, while females finish their main energy investment into egg growth before overwintering (Lu et al., 2008; Jönsson et al., 2009). These two energy storage strategies suggest that i) frogs living at higher altitudes do not have greater energy storage before overwintering than those from lower altitudes because there is less food available, higher heat loss and lower metabolic rates at higher altitudes, and ii) there are sex differences in pre-hibernation energy storage of anurans with the capital breeding strategy, due to differences in the timing of breeding investment between the sexes. To test these two hypotheses, we selected *Rana kukunoris* as a model species to study pre-hibernation energy storage along an altitudinal gradient.

MATERIALS AND METHODS

Rana kukunoris inhabits open alpine marshes 2200–4400 m in elevation, and is endemic to the eastern Tibetan plateau (Xie et al., 2000). The species is an explosive

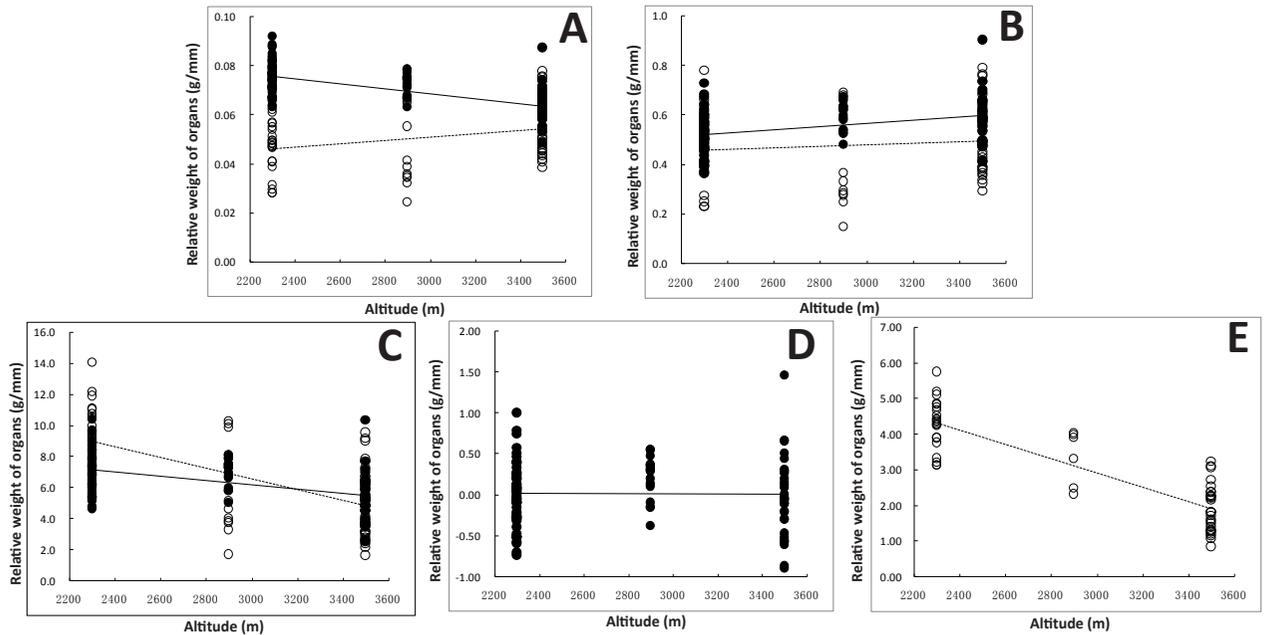


Fig. 1. Relative energy organ weights of *Rana kukunoris* from three populations on the Tibetan plateau; (A) Fat body, (B) Liver, (C) Carcass, (D) Male gonads, (E) Female gonads. Graphs were produced using the predicted values from the general linear models (black circles for males, white circles for females).

breeder with a capital breeding strategy (Lu et al., 2008). Female fecundity is positively related to body size (Lu et al., 2008) and males prefer to mate with larger females (Chen & Lu, 2011). With increasing altitude, body length of males and females decreases, with females generally being larger than males (Chen et al., 2011a).

In total, 104 males and 63 females were opportunistically captured by hand in three overwintering sites with different altitudes (Table 1), when the mean diurnal temperature was around 6°C. All frogs were taken to the lab and kept for at least 1 day in water to attain comparable weight measurements before being killed with an overdose of MS-222.

Snout-vent length (SVL) was measured to the nearest 0.1 mm before dissection. The liver, fat bodies and gonads were removed and weighed to the nearest 0.001 g with an electronic balance after placement on water-absorbing paper for around five minutes (Lu, 2004); the remaining carcass was then weighed, though measurements of dry weight were impossible to obtain due to logistical constraints.

General linear models (GLMs) were used to investigate the relationship between altitude and organ weight with sex as a fixed factor, altitude and SVL as covariates and relative organ weight as the dependent variable. To further investigate the relationship between relative organ weight (the predictive organ weight from GLMs)

and sex, one-way ANOVAs were performed. All variables were log-transformed to improve normality of variances before analysis. All statistical tests were performed with SPSS software (v16.0) and all probabilities were two-tailed with $\alpha=0.05$.

RESULTS

Relative carcass weight ($F_{1,167}=30.39, p<0.001$) and gonad weight ($F_{1,167}=9.18, p=0.003$) decreased with altitude, whereas liver weight ($F_{1,167}=12.14, p=0.001$) increased with altitude; fat body weight did not differ between sites ($F_{1,167}=0.643, p=0.424$; Table 2). We observed significant differences between the sexes with respect to relative weight of gonads ($F_{1,167}=47.026, p<0.001$) and fat bodies ($F_{1,167}=6.13, p=0.014$). Relative carcass and gonad weight decreased differentially with increasing altitude in males and females, resulting in a significant interaction between altitude and sex (carcass: $F_{1,167}=4.23, p=0.041$ and gonad: $F_{1,167}=20.68, p<0.001$; Fig. 1). Generally, larger males and females had higher energy storage (carcass: $F_{167,1}=496.94, p<0.001$; liver: $F_{167,1}=35.06, p<0.001$; fat bodies: $F_{167,1}=7.37, p=0.007$ and gonads: $F_{167,1}=32.67, p<0.001$. Males had heavier livers ($F_{166,1}=16.31, p<0.001$), fat bodies ($F_{166,1}=145.34, p<0.001$), and females had heavier gonads ($F_{166,1}=442.74, p<0.001$).

Table 1. Characteristics of the sample-collecting sites in the Tibetan plateau. Activity period is the number of days with a mean diurnal temperature above 6°C.

Location	Altitude(m)	Mean annual temperature(°C)	Activity period (days)	No. of Females	No. of Males	Total sample size
Jiuzhaigou	2300	7.8	180	22	53	75
Zhuoni	2900	4.6	160	11	16	27
Zoige	3500	2	130	30	35	65
Total	-	-	-	63	104	167

Table 2. Parameter estimates from general linear model analyses of variables for three *Rana kukunoris* populations in the Tibetan plateau.

Parameter	<i>B</i>	<i>t</i>	<i>p</i>	95% Confidence Interval	
				Lower	Upper
Fat body					
Intercept	0.016	0.412	0.680	-0.059	0.091
Sex	-0.096	-2.475	0.014	-0.173	-0.019
Altitude	<0.001	-0.832	0.406	<0.001	<0.001
Body length	0.001	2.715	0.007	<0.001	0.003
Sex×Altitude	<0.002	1.869	0.063	<0.001	<0.001
Liver					
Intercept	-0.687	-3.116	0.002	-1.123	-0.252
Sex	-0.324	-1.442	0.151	-0.769	0.119
Altitude	<0.001	2.452	0.015	<0.001	<0.001
Body length	0.021	5.921	<0.001	0.014	0.028
Sex×Altitude	<0.001	0.719	0.473	<0.001	<0.001
Carcass					
Intercept	-6.571	-7.060	<0.001	-8.409	-4.733
Sex	1.124	1.185	0.238	-0.749	2.998
Altitude	<0.001	-3.126	0.002	-0.001	<-0.001
Body length	0.337	22.292	<0.001	0.307	0.367
Sex×Altitude	<-0.001	-2.056	0.041	-0.001	<-0.001
Gonads					
Intercept	-5.061	-4.742	<0.001	-7.169	-2.953
Sex	7.173	6.858	<0.001	5.107	9.239
Altitude	<0.001	1.061	0.290	<0.001	<0.001
Body length	0.102	5.724	<0.001	0.067	0.136
Sex×Altitude	-0.002	-4.548	<0.001	-0.002	<-0.001

DISCUSSION

Both different environmental constraints and sexual differences in the timing of energy investment for reproduction determine the energy storage patterns in pre-hibernating *R. kukunoris*. The relative weight of carcasses and gonads in both sexes decreased with altitude, whereas the weight fat body in males and females was unrelated to altitude. Elmberg (1991) showed that *Rana temporaria* had a higher rate of fat body growth in summer than conspecifics at a lower altitude, and both Lu et al. (2008) and Chen et al. (2011b) found that pre-hibernation energy storage of *R. chensinensis* increased with altitude and latitude. It would be expected that frogs facing longer hibernation periods should deposit relatively larger pre-hibernation energy reserves, but why is the opposite trend the case for *R. kukunoris*? Three possible reasons could explain the phenomenon of lower pre-hibernation energy storage of higher altitude *R. kukunoris*. Firstly, resource levels at higher altitudes might be lower in the Tibetan plateau, in line with lower insect abundance (Yuan & Wang, 1993; Sanchez-Rodrigues & Baz 1995). Secondly, the available foraging time for nutrient acquisition of populations living in higher altitudes becomes shorter (Morrison & Hero, 2003; Lu et al., 2008). Finally, lower oxygen levels

might decrease the ability to effectively convert ingested food into organ growth (as demonstrated for insects, Dillon et al., 2006).

Relative liver weight of *R. kukunoris* tended to increase with altitude. A possible reason is that the energy requirements during winter dormancy are mainly derived from the liver (Díaz-Páez & Ortiz, 2001; Jackson & Ultsch, 2010) with heavier livers being important for individuals with longer hibernation because they must deposit greater pre-hibernation energy stores to satisfy metabolic requirements (Pasanen & Koskela, 1974; Irwin & Lee, 2003; Lu et al., 2008). Previous studies show that liver glycogen is a source of energy during overwintering because the aquatic environment generally becomes hypoxic, and there is a heavier reliance on glycogen stores in the liver to fuel the overwintering period (Jackson & Ultsch, 2010). For anurans, during overwintering liver glycogen content was reduced by 51% in males and 56% in females (Tattersall & Ultsch, 2008). At the same time, in some high-altitude 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). Accordingly, liver weight of *R. kukunoris* in increased high altitudes might be an adaptation to the hypoxic aquatic environment in regions with a prolonged winter.

Similar to observations in *R. temporaria* (Elmberg, 1991) and *R. chensinensis* (Lu, 2004), the relative gonad weight of *R. kukunoris* decreased with altitude. This is consistent with the common pattern that reproductive investment of anurans living in cold regions is lower (Hettyey et al., 2005). A classical explanation for lower reproductive investment by anurans at higher altitudes is that shorter foraging periods and lower food resource levels of higher altitudes limit the investment of energy for reproduction (Elmberg, 1991; Ryser, 1996).

For anurans with capital breeding strategies, females finish their main energy investment in autumn, whereas males do so during the breeding period after winter (Jørgensen, 1981). Similarly to *R. temporaria* (Jönsson et al., 2009), the difference in reproductive investment between male and female *R. kukunoris* leads to differences in the timing of energy allocation. In fact, female *R. kukunoris* finish their main follicular growth before hibernation, and their gonad weight increases before overwintering (personal observation). Typically, fat bodies have a direct relationship to gonadal growth (Delgado et al., 1990; Girish & Saidapur, 2000; Chen et al., 2011b). Accordingly, female *R. kukunoris* had smaller fat bodies but a heavier gonad weight compared to males before overwintering. This pattern of sexual differences in pre-hibernation energy storage is in accordance with results from *R. chensinensis* (Chen et al., 2011b).

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