

# Prehibernation Energy Storage in Heilongjiang Brown Frogs (*Rana amurensis*) from Five Populations in North China

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**Abstract** Energy storage is an important component in the life history of species that directly influences survival and reproduction. The energetic demands of amphibian reproduction can differ between the sexes, with environmental conditions, reproductive pattern or process of the species, and depending upon the timing of breeding, and the reproductive season for a species. Surprisingly, comparative studies of pre-hibernation energy storage for anuran populations from different latitudes are relatively few in Asia, especially in China. Here we investigated the patterns of pre-hibernation energy storage of Heilongjiang brown frogs *Rana amurensis*, based on five populations along a finely latitudinal gradient in north China (40.7–43.7°N). We found that pre-hibernation energy storage of the frogs did not show a clear latitudinal cline, but differed strongly between the sexes, with males depositing more energy reserves into the muscle and liver, whereas females accumulate more energy in the gonads. The sexual differences in energy storage may result from differential timing of energy allocation for reproduction.

**Keywords** energy storage, pre-hibernation, *Rana amurensis*, sexual differences

## 1. Introduction

Energy storage is crucial for species survival during winter that they live in temperate and cold zone and plays an important role in shaping the life-history strategy for a species living in temperate and cold zone (Roff, 2002; Wells, 2007; Jönsson *et al.*, 2009). Animals must trade off their energy allocation for tissue maintenance, growth and reproduction as energy resources available may be limited in nature. In general, however, the amount of body energy storage can influence the survival time and the strategy of energy investment into fitness traits (*e.g.* egg number or the ability to secure more mates), so energy storage is viewed to strongly influence individual fitness in amphibians (Komorowski *et al.*, 1998; Lu *et al.*, 2008).

Anurans deposit energy in the form of triglycerides and glycogen and in different organs [*e.g.*, liver, abdominal fat bodies (Fitzpatrick, 1976), gonadal tissues (Villemo *et al.*, 1999), and muscle tissue (Donohoe *et al.*, 1998)], and these stores are used for overwintering (Boutilier, 2001; Pope and Matthews, 2002), gamete production (Girish and Saidapur, 2000), and breeding activities (Pope and Matthews, 2002; Jackson and Ultsch, 2010). Because these activities are tightly linked to environmental conditions and resource availability, environmental factors can influence energy storage of species and the relationship between environments and energy storage has been a pivotal point in our understanding of life-history strategy (Elmberg, 1991; Wells, 2007; Jönsson *et al.*, 2009; Chen *et al.*, 2011, 2013).

The reproductive behavior of anurans can be divided into two basic patterns which apply to temperate species of anurans: income breeding or capital breeding (Wells, 2007). Income breeders feed after emergence from winter dormancy and before breeding, whereas capital breeders begin feeding only after breeding (Drent and Daan, 1980;

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Jönsson, 1997; Jönsson *et al.*, 2009). In anuran income breeders, males and females invest main energy into breeding after overwintering, however, in the anurans with capital breeding strategies, males invest energy into breeding after overwintering, whereas females finish investing the main energy component into egg growth prior to overwintering (Lu *et al.*, 2008; Jönsson *et al.*, 2009; Chen *et al.*, 2011). Thus, sex differences in the timing of energy allocation to breeding are common in the anurans species with capital breeding strategies, and the differences results in life history variation across populations and species (Jönsson *et al.*, 2009; Chen *et al.*, 2011, 2013).

Heilongjiang brown frogs *Rana amurensis* are distributed in north China, Mongolia and Russia (Kuzmin, 1999; Fei *et al.*, 2010). Individuals of this species overwinter under the water in shallow ponds, and after emerging the following spring breed prior to feeding (Solomonova *et al.*, 2011). Little is known about prehibernation energy storage of this capital breeder (Solomonova *et al.*, 2011), with females larger in body size than males (Chen and Lu, 2011) and larger females producing more eggs (Solomonova *et al.*, 2011). We investigated the prehibernation energy storage of five populations of this species in north China distributed along a finely latitudinal gradient. Our aims were to explore whether there were sex differences in the patterns of body energy storage and investigate whether there was a latitudinal cline of energy storage along a finely latitudinal gradient.

## 2. Materials and Methods

In total, 89 *Rana amurensis* (43 males and 46 females)

were opportunistically captured by hand in the water from five overwintering sites (Lindong, Gongzhuling, Yitong, Siping, Meihekou and Tonghua) along a latitudinal gradient in north China (40.7–43.7°N, Table 1), when the mean diurnal temperature was around 10°C at each site. Captured frogs were maintained in pond water for 24 hours at 15°C in lab to allow full hydration before being euthanized with an overdose of MS-222 (hydration status can influence animal body mass; Ladyman and Bradshaw, 2003). We measured snout-vent length of the frogs with flattened bodies (SVL; to 0.1 mm), and then dissected out and weighed the liver, fat bodies, gonads and remaining carcass (0.001 g with an electronic balance) after placing the organs on water-absorbing paper for five minutes (Lu, 2004).

Although we were interested in the sexual effects on energy storage, we incorporated altitude, latitude and their interaction into our statistical models to help explain more variance in energy storage among populations. Generalized linear mixed models (GLMMs) were performed to investigate the relationship sex and organ weight as well as between latitude and organ weight, using population as a random variable, sex, SVL, latitude and altitude as covariates, and organ weight as the dependent variable. One-way ANOVAs then were used to investigate whether males and females differ in their pattern of energy storage, using relative organ weight (predicted from GLMMs) as the dependent variable. We log-transformed all variables prior to analysis to match the requirements of GLMMs and ANOVAs. All statistical tests were performed with SPSS software (Version 20.0). All probabilities were two tailed and summary statistics are presented as mean  $\pm$  standard deviation (SD). All field and laboratory work was performed under permission

**Table 1** Organ mass for female and male *Rana amurensis* from five populations in north China, showing the latitude, altitude, sample size (*n*) and organ mass (mean  $\pm$  standard deviation).

| Sex    | Latitude (°) | Altitude (m) | <i>n</i> | Body length (cm) | Carcass (g)      | Liver (g)       | Fat body (g)    | Gonads (g)      |
|--------|--------------|--------------|----------|------------------|------------------|-----------------|-----------------|-----------------|
| Female | 41.73        | 610          | 6        | 6.35 $\pm$ 0.56  | 19.64 $\pm$ 5.58 | 0.48 $\pm$ 0.34 | 0.03 $\pm$ 0.04 | 3.47 $\pm$ 1.26 |
|        | 42.37        | 363          | 8        | 6.16 $\pm$ 0.69  | 11.22 $\pm$ 2.48 | 0.59 $\pm$ 0.25 | 0.03 $\pm$ 0.05 | 2.36 $\pm$ 0.60 |
|        | 43.34        | 186          | 8        | 5.22 $\pm$ 0.48  | 9.37 $\pm$ 2.34  | 0.64 $\pm$ 0.21 | 0.01 $\pm$ 0.01 | 2.19 $\pm$ 0.64 |
|        | 43.59        | 186          | 13       | 4.70 $\pm$ 0.43  | 6.26 $\pm$ 2.61  | 0.73 $\pm$ 0.28 | 0.02 $\pm$ 0.03 | 1.53 $\pm$ 0.62 |
|        | 43.67        | 145          | 11       | 4.91 $\pm$ 0.36  | 7.60 $\pm$ 2.20  | 0.39 $\pm$ 0.10 | 0.07 $\pm$ 0.08 | 1.94 $\pm$ 0.76 |
| Male   | 41.73        | 610          | 9        | 5.86 $\pm$ 0.49  | 17.25 $\pm$ 5.45 | 0.55 $\pm$ 0.26 | 0.01 $\pm$ 0.02 | 0.02 $\pm$ 0.01 |
|        | 42.37        | 363          | 6        | 5.61 $\pm$ 0.41  | 12.95 $\pm$ 3.21 | 0.91 $\pm$ 0.29 | 0.02 $\pm$ 0.01 | 0.02 $\pm$ 0.01 |
|        | 43.34        | 186          | 6        | 5.31 $\pm$ 0.33  | 13.63 $\pm$ 3.58 | 0.96 $\pm$ 0.15 | 0.02 $\pm$ 0.02 | 0.02 $\pm$ 0.01 |
|        | 43.59        | 186          | 11       | 4.80 $\pm$ 0.49  | 10.00 $\pm$ 3.69 | 1.30 $\pm$ 0.46 | 0.02 $\pm$ 0.02 | 0.02 $\pm$ 0.01 |
|        | 43.67        | 145          | 11       | 4.63 $\pm$ 0.28  | 7.91 $\pm$ 2.21  | 0.45 $\pm$ 0.17 | 0.07 $\pm$ 0.04 | 0.01 $\pm$ 0.01 |

from the Wildlife Protection Law of China.

### 3. Results

We observed significant differences between the sexes with respect to relative weight of the carcass (GLMM:  $F_{1,82.001} = 25.444$ ,  $P < 0.001$ ), liver ( $F_{1,82.001} = 47.077$ ,  $P < 0.001$ ) and gonads ( $F_{1,83} = 314.608$ ,  $P < 0.001$ ), but not for the fat bodies ( $F_{1,82} = 0.147$ ,  $P = 0.703$ ; Figure 1). Generally, males had significantly heavier carcasses (One-way ANOVA:  $F_{1,87} = 5.723$ ,  $P = 0.019$ ) and livers ( $F_{1,87} = 18.912$ ,  $P < 0.001$ ) than females, whereas females had significantly heavier gonads than males ( $F_{1,87} = 497.673$ ,  $P < 0.001$ ; Figure 1; Table 1).

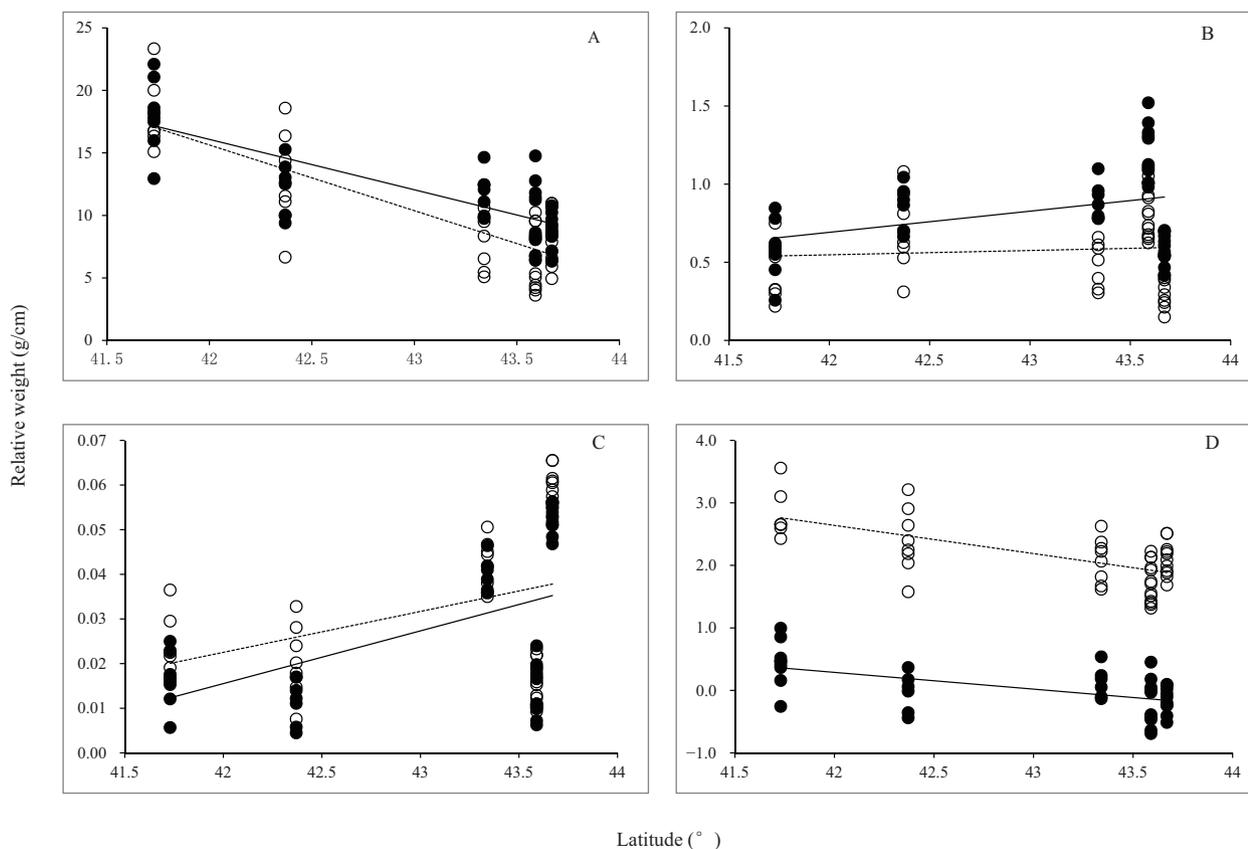
We found that energy storage was associated with body size, with larger individuals of both sexes having significantly higher energy storage than smaller individuals for the carcass (GLMM:  $F_{1,82.276} = 90.057$ ,  $P < 0.001$ ), liver ( $F_{1,40.952} = 40.952$ ,  $P < 0.001$ ), and gonads ( $F_{1,83} = 37.6$ ,  $P < 0.001$ ), but not for the fat bodies ( $F_{1,82.191} = 1.72$ ,  $P = 0.193$ ; Table 2).

Latitude and altitude were not significantly associated with the energy storage in any of the organs that we

measured due to the narrow geographic bounds (latitude: carcass:  $F_{1,1.024} = 1.462$ ,  $P = 0.436$ ; liver:  $F_{1,1.054} = 7.990$ ,  $P = 0.206$ ; fat bodies:  $F_{1,1.016} = 0.011$ ,  $P = 0.934$ ; gonads:  $F_{1,83} = 2.451$ ,  $P = 0.121$ ; altitude: carcass:  $F_{1,0.918} = 0.258$ ,  $P = 0.707$ ; liver:  $F_{1,0.822} = 17.498$ ,  $P = 0.19$ ; fat bodies:  $F_{1,0.944} = 0.972$ ,  $P = 0.512$ ; gonads:  $F_{1,83} = 3.476$ ,  $P = 0.066$ ; for all interactions between latitude and altitude,  $P > 0.07$ , Table 2).

### 4. Discussion

Patterns of energy storage in anurans can be constrained by environmental conditions and differ between the sexes, which often differ in the timing of energy allocation towards reproduction (Jönsson *et al.*, 2009; Chen *et al.*, 2011; Chen *et al.*, 2013). In colder environments, anurans are expected to accumulate more energy prior to the onset of winter (Irwin and Lee, 2003; Lu *et al.*, 2008; Chen *et al.*, 2011, 2013). In our study, however, we did not detect a clear latitudinal pattern of energy storage in either male or female *R. amurensis* due to limited geographic bounds. Instead, we found clear sex differences in pre-hibernation energy storage patterns, where males allocate more



**Figure 1** Organ mass for female and male *Rana amurensis* from five populations in north China. (A) Carcass, (B) Liver, (C) Fat bodies, (D) Gonads. Graphs show the predicted values from generalized linear mixed models (open circles for females, filled circles for males).

**Table 2** Parameters estimated from a generalized linear mixed model analyses comparing organ mass among five *Rana amurensis* populations in north China.

| Parameters          | Estimate | <i>t</i> | <i>P</i> | 95% Confidence Interval |          |
|---------------------|----------|----------|----------|-------------------------|----------|
|                     |          |          |          | Lower                   | Upper    |
| <b>Carcass</b>      |          |          |          |                         |          |
| Intercept           | -276.230 | -1.246   | 0.426    | -2919.558               | 2367.098 |
| Sex                 | -2.697   | -5.044   | < 0.001  | -3.760                  | -1.633   |
| Body length         | 5.455    | 9.490    | < 0.001  | 4.312                   | 6.599    |
| Latitude            | 5.944    | 1.209    | 0.436    | -53.113                 | 65.000   |
| Altitude            | 0.449    | 0.508    | 0.707    | -13.441                 | 14.338   |
| Latitude × altitude | -0.010   | -0.469   | 0.726    | -0.352                  | 0.332    |
| <b>Liver</b>        |          |          |          |                         |          |
| Intercept           | -42.889  | -2.981   | 0.194    | -202.580                | 116.802  |
| Sex                 | -0.351   | -6.861   | < 0.001  | -0.453                  | -0.249   |
| Body length         | 0.352    | 6.399    | < 0.001  | 0.243                   | 0.461    |
| Latitude            | 0.901    | 2.827    | 0.206    | -2.692                  | 4.493    |
| Altitude            | -0.232   | -4.183   | 0.190    | -1.420                  | 0.957    |
| Latitude × altitude | 0.006    | 4.191    | 0.190    | -0.024                  | 0.035    |
| <b>Fat bodies</b>   |          |          |          |                         |          |
| Intercept           | 0.580    | 0.141    | 0.911    | -49.569                 | 50.728   |
| Sex                 | 0.003    | 0.383    | 0.703    | -0.013                  | 0.019    |
| Body length         | 0.012    | 1.311    | 0.193    | -0.006                  | 0.029    |
| Latitude            | -0.009   | -0.103   | 0.934    | -1.128                  | 1.109    |
| Altitude            | 0.016    | 0.986    | 0.512    | -0.226                  | 0.259    |
| Latitude × altitude | 0.000    | -0.985   | 0.513    | -0.006                  | 0.006    |
| <b>Gonads</b>       |          |          |          |                         |          |
| Intercept           | -30.782  | -1.665   | 0.100    | -67.547                 | 5.984    |
| Sex                 | 2.021    | 17.737   | < 0.001  | 1.795                   | 2.248    |
| Body length         | 0.747    | 6.132    | < 0.001  | 0.505                   | 0.990    |
| Latitude            | 0.639    | 1.566    | 0.121    | -0.173                  | 1.451    |
| Altitude            | 0.113    | 1.864    | 0.066    | -0.008                  | 0.233    |
| Latitude × altitude | -0.003   | -1.818   | 0.073    | -0.006                  | < 0.001  |

energy to the carcass and females allocate more energy into the gonads.

Reproductive success of female anurans is determined by their ability to produce eggs, and thus females often deposit a high proportion of energy into the gonads to produce more high-energy eggs (Wells, 2007). By contrast, the reproductive success of male frogs is influenced by their ability to locate and secure potential mates, and thus males use their energy reserves for breeding activities (such as mate attraction through calling) rather than investing into cheaper spermatogenesis (Halliday and Verrell, 1988; Jørgensen, 1992). This sex difference in reproductive investment leads to differences in the timing of energy allocation, as has been shown for other frogs (*R. temporaria*: Jönsson *et al.*, 2009; *R.*

*chensinensis*: Chen *et al.*, 2011; *R. kukunoris*: Chen *et al.*, 2013). For anurans with capital breeding strategies, females finish investing energy in autumn before winter dormancy, whereas males do so during the breeding period of the following spring (Jørgensen, 1981).

In *R. amurensis*, females significantly increase their gonad weight before winter, finishing most follicular growth before hibernation (Chen, pers. observ.). Typically, somatic tissues have a direct relationship to gonadal growth (Delgado *et al.*, 1990; Girish and Saidapur, 2000; Chen *et al.*, 2011), so the energy in somatic tissues has been invested into gonadal growth and converted into gonads. However, in males the energy in somatic tissues remains until it is invested into breeding activities the next spring, e.g. mate attraction or competition (Pope and

Matthews, 2002; Jackson and Ultsch, 2010). Accordingly, female *R. amurensis* had lighter carcasses but heavier gonads when compared to males. This sexual pattern of pre-hibernation energy storage is in accordance with the study results from *R. temporaria* (Jönsson *et al.*, 2009) and *R. chensinensis* (Chen *et al.*, 2011).

The differences in most physiological data results partly from variation in body size of the animals being studied, because the intensity of a physiological process is usually higher in large individuals than in small ones (Packard and Boardman, 1999). In anurans, a common pattern of energy storage is that larger males and females have higher energy storage (Wells, 2007). In *R. amurensis*, larger males and females also accumulate more energy. This body-size-dependent pattern of pre-hibernation energy storage is in accordance with results from other anurans (*e.g.* *R. temporaria*: Jönsson *et al.*, 2009; *R. chensinensis*: Chen *et al.*, 2011; *R. kukunoris*: Chen *et al.*, 2013). A possible reason is that larger individuals have enhanced feeding abilities and/or have optimized foraging strategies (Wells, 2007; Chen *et al.*, 2011).

Frogs living in colder environments are expected to store more energy in order to survive prolonged and colder winters (Lu, 2004; Jönsson *et al.*, 2009; Chen *et al.*, 2011; Chen *et al.*, 2013). For example, the liver plays an important role in energy maintenance requirements during winter dormancy (Pasanen and Koskela, 1974; Díaz-Páez and Ortiz, 2001; Irwin and Lee, 2003; Lu *et al.*, 2008), especially in the aquatic environment which has low oxygen or can be hypoxic (Jackson and Ultsch, 2010). Evidence shows that during overwintering the liver glycogen content was reduced by 51% in males and 56% in females of ranid frogs (Tattersall and Ultsch, 2008). The weight of the liver thus will increase with increasing latitude and altitude before winter to satisfy metabolic requirements during longer and colder winter hibernation (Pasanen and Koskela, 1974; Irwin and Lee, 2003; Lu *et al.*, 2008; Chen *et al.*, 2011). In contrast to previous studies (*R. temporaria*: Jönsson *et al.*, 2009; *R. chensinensis*: Chen *et al.*, 2011; *R. sylvatica*: Costanzo *et al.*, 2013), the energy storage of *R. amurensis* did not increase with increasing latitudes. This could be explained by the fact that the latitudinal range was too limited to detect a clear latitudinal trend, and the environmental differences along the limited latitudinal range that we investigated did not impose strong enough selection to alter energy storage. Further studies should be performed to investigate whether there is a clear latitudinal pattern of energy storage in *R. amurensis*.

Generally, the males of *R. amurensis* deposit more

energy into the carcass and liver, but the females accumulated more energy into the gonads. This sexual difference in energy storage may result from differential timing of energy allocation for reproduction, which is keeping with capital breeding pattern of frogs. However, we did not detect a clear latitudinal cline of energy storage, which is mainly because of limited latitudinal range. If a comparison of the species from a wider latitudinal gradient is conducted and/or of larger sample size, we might gain more clear results.

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