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Higher pre-hibernation energy storage in anurans from cold environments: a case study on a temperate frog *Rana chensinensis* along broad latitudinal and altitudinal gradients

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Temperate amphibians in colder regions are expected to store more energy prior to hibernation for successful overwintering and subsequent spring breeding. We tested this prediction on a capital breeding species — *Rana chensinensis* — using samples collected from 27 populations across 1200-km latitudinal (33.6–44.2°N) and 1768-m (112–1880 m) altitudinal gradient in northern China. Our data showed that frogs from colder regions (high latitude or altitude) had relatively heavier liver and fat bodies than those from warmer regions, but that the weight of carcasses tended to become smaller. The greater pre-hibernation energy reserves in colder regions could be an adaptive response to the longer and colder winter period, whereby meeting the energy demands for overwintering, and the subsequent energy requirements of reproduction in the spring.

Introduction

Energy storage plays an important role in the life history of temperate-zone anurans that hibernate in winter on land or under water and do not feed until spring (Koskela & Pasanen 1974, Bradford 1983). Income breeding anurans feed after emergence and before breeding (Willis *et al*. 1956, Hulse *et al*. 2001). In contrast, pure capital breeders do not feed until after breeding in the spring (Pasanen & Koskela 1974). Thus, anurans with a pure capital breeding strategy must enter hibernation with sufficient energy reserves not only for overwintering (Long 1987, Donohoe *et al*. 1998, Boutilier 2001, Pope & Matthews 2002, Jackson & Ultsch 2010) but also for subsequent spring reproduction.

Although anurans can deposit energy in the form of lipids, proteins or carbohydrates, lipids may be the most important energy source (Smith 1950, Bush 1963, Fitzpatrick 1976, Jackson & Ultsch 2010). Lipids are mainly deposited in the liver (Fitzpatrick 1976), fat bodies (Brown 1964, Fitzpatrick 1976), and somatic tissue (Seymour 1973, Fitzpatrick 1976, Whitford & Meltzer 1976, Morton 1981, Fournier & Guderly 1993, Donohoe *et al*. 1998). By investigating the weights of these organs, researchers have found seasonal (Mizell 1965, Morton 1981, Lu 2004), altitudinal (Elmberg 1991, Elmberg & Lundberg

1991, Lu *et al*. 2008) and latitudinal variation in energy storage patterns (Pasanen & Koskela 1974, Jönsson *et al*. 2009).

Considering the wide altitudinal and latitudinal range (equaling to a wide temperature range) of habitats where temperate-zone amphibians occur, one would expect large variation in energy storage patterns both within and among species. Despite the importance of energy storage in life histories of temperate anurans, however, to date only a few studies on this topic, mostly restricted to narrow geographic gradients (Elmberg 1991, Elmberg & Lundberg 1991), have been made. A large-scale study of *Rana temporaria* conducted shortly after hibernation, suggested that animals should acquire more energy towards the north as a risk-averse strategy when facing harsh and unpredictable environments (Jönsson *et al*. 2009). However, large-scale studies on pre-hibernation energy stores in anurans are still lacking.

The aim of this study was to investigate the pre-hibernation energy stores of a widely distributed temperate-zone anuran, *Rana chensinensis*, in environmentally different populations. We compared the weights of the (i) liver, (ii) fat bodies, and (iii) carcass relative to body length in 27 populations over a 1200-km latitudinal (33.6–44.2°N) and 1768-m altitudinal gradient across northern China. We predicted that in thermally harsher environments with prolonged hibernation periods, energy stores will be higher.

Material and methods

Study species and sampling

Rana chensinensis is a medium-sized frog endemic to, and widely distributed in northern China (Liu & Hu 1961, Tanaka-Ueno *et al*. 1999, Xie *et al*. 2000, Lu *et al*. 2008). The frogs have a variable hibernation-period length (3–5 months) and a short breeding period (3–4 weeks; Lu 2004). They are not active during winter and do not feed after emergence and before breeding; the species accordingly is viewed as an explosive (Wells 1977) and capital breeder (Jönsson *et al*. 2009). The frogs begin building their body reserves shortly after breeding, peaking in the late autumn before winter hibernation (Lu 2004). This storage may function primarily to sustain individuals through the winter and enhance subsequent reproductive performance in the spring (Lu *et al*. 2008).

Adult frogs (380 males, 306 females) were captured in the water, by hand, during daytime in their hibernation habitats from September to November 2009 according to the phenology of local populations to allow comparisons. These sampling sites (n = 27) covered a ca. 1200-km latitudinal (33.6–44.2°N) and 1768-m altitudinal (112–1880 m) gradient (Table 1). Average annual air temperatures of these localities were obtained from local weather stations

All frogs were killed by an overdose of MS-222. Before dissection to obtain liver and fat bodies, snout–vent length (SVL) of each frog was measured to the nearest 0.1 mm. The remaining carcasses and each of the organs were weighed separately to the nearest 0.001 g with an electronic balance after being placed on water-absorbing paper for about 5 minutes (Lu 2004). The ages of adult frogs were determined by skeletochronology (Lu *et al*. 2006, Ma *et al*. 2009, Chen *et al*. 2011).

All field and laboratory work was done under a license from the Wildlife Protection Law of China.

Statistical analyses

Differences in weights of organs among populations along mean annual temperature gradients were tested using linear mixed models (LMM) with sex, SVL, age and mean annual temperature included as fixed effects, and population as a random effect. Organ variables were ln-transformed to better attain normality. All statistical tests were performed with the SPSS software (ver. 16.0) and all probabilities were two-tailed with α = 0.05; values presented are means ± standard deviations (SD).

Results

Descriptive statistics for the analyzed variables are shown in Table 1. In both sexes, liver and fat-body masses were higher at lower ambient tem-

Table 1. Means ± SDs and sample sizes (*n*) for the analyzed variables of *Rana chensinensis* populations in northern China. Mean temp. = mean annual temperature, SVL = snout-vent length, CC = carcass mass, LV = liver mass, FB = mass of fat bodies. * Mean ages were not available for this population.

Location (coordinates)	Altitude (m)	Mean temp. (°C)	Sex	SVL (mm)	CC (g)	LV (g)	FB (g)	Age (years)	*n*
Nanzhao	907	10.7	F	41.1 ± 4.5	4.15 ± 1.14	0.28 ± 0.11	0.05 ± 0.03	3.0 ± 0.7	5
(112.29°E, 33.67°N)			M	41.6 ± 2.2	5.21 ± 1.09	0.25 ± 0.08	0.06 ± 0.02	2.9 ± 0.7	7
Zhenwuding	1041	10.0	F	50.3 ± 6.0	9.01 ± 3.63	0.45 ± 0.18	0.09 ± 0.07	3.6 ± 0.5	9
(112.29°E, 33.68°N)			M	39.3 ± 3.4	4.87 ± 1.29	0.27 ± 0.09	0.06 ± 0.03	2.9 ± 0.7	10
Wulongkou	261	13.6	F	56.0 ± 4.9	10.34 ± 2.83	0.53 ± 0.19	0.08 ± 0.00	3.3 ± 0.5	4
(112.70°E, 35.19°N)			M	48.6 ± 3.3	8.25 ± 1.74	0.53 ± 0.18	0.12 ± 0.27	3.0 ± 0.9	11
Manghe	845	7.7	F	45.8 ± 8.9	5.22 ± 3.06	0.29 ± 0.17	0.05 ± 0.02	3.0 ± 1.0	3
(112.41°E, 35.30°N)			M	44.4 ± 5.7	6.11 ± 2.40	0.40 ± 0.19	0.12 ± 0.04	3.3 ± 0.6	3
Longgang	1169	9.1	F	38.2 ± 6.1	2.97 ± 1.71	0.19 ± 0.09	0.04 ± 0.03	1.8 ± 0.8	13
(112.04°E, 35.73°N)			M	37.9 ± 2.4	3.74 ± 1.04	0.23 ± 0.09	0.06 ± 0.03	2.9 ± 0.8	19
Dashanmen	1172	10.3	F	42.7 ± 8.3	4.92 ± 3.48	0.29 ± 0.13	0.04 ± 0.02	3.0 ± 1.1	10
(108.31°E, 35.86°N)			M	40.4 ± 2.7	4.45 ± 0.92	0.34 ± 0.10	0.05 ± 0.02	2.6 ± 1.1	5
Lianjiabian	1174	9.4	F	42.5 ± 5.8	4.02 ± 1.92	0.24 ± 0.09	0.03 ± 0.02	3.1 ± 1.1	10
(108.55°E, 36.08°N)			M	42.5 ± 3.3	5.14 ± 1.14	0.41 ± 0.06	0.05 ± 0.02	2.4 ± 1.0	7
Laoshan	273	11.0	F	45.3 ± 6.8	5.13 ± 2.42	0.25 ± 0.11	0.04 ± 0.02	2.7 ± 0.8	12
(120.60°E, 36.21°N)			M	44.7 ± 4.3	5.52 ± 1.58	0.30 ± 0.12	0.07 ± 0.12	2.9 ± 0.9	16
Kecheng	1365	6.5	F	42.1 ± 9.0	4.66 ± 2.94	0.41 ± 0.23	0.15 ± 0.22	3.1 ± 0.9	15
(111.24°E, 36.58°N)			M	39.7 ± 5.5	5.03 ± 1.90	0.39 ± 0.18	0.10 ± 0.06	2.8 ± 0.9	25
Huangtu	968	9.5	F	34.9 ± 5.5	2.36 ± 1.25	0.21 ± 0.12	0.02 ± 0.02	2.3 ± 0.7	32
(111.03°E, 36.62°N)			M	35.8 ± 4.2	3.06 ± 1.12	0.14 ± 0.09	0.03 ± 0.02	2.3 ± 0.8	19
Shangzhuang	1177	7.6	F	40.1 ± 4.9	3.73 ± 1.62	0.25 ± 0.12	0.03 ± 0.03	3.0 ± 1.4	2
(111.20°E, 36.64°N)			M	40.1 ± 3.9	4.20 ± 1.40	0.19 ± 0.07	0.03 ± 0.02	3.8 ± 0.4	9
Qili	1026	11.3	F	35.9 ± 3.5	2.29 ± 0.91	0.18 ± 0.05	0.01 ± 0.00	2.3 ± 0.9	13
(110.95°E, 36.75°N)			M	36.3 ± 2.6	3.28 ± 0.79	0.15 ± 0.05	0.03 ± 0.01	2.3 ± 0.9	13
Jiexiu	815	10.2	F	40.2 ± 8.9	4.72 ± 3.18	0.41 ± 0.21	0.05 ± 0.05	*	18
(112.06°E, 37.07°N)			M	39.5 ± 6.4	4.88 ± 2.27	0.42 ± 0.22	0.09 ± 0.07	*	19
Fangshan	1345	7.2	F	41.9 ± 6.6	4.46 ± 1.96	0.33 ± 0.14	0.04 ± 0.02	2.8 ± 0.8	20
(111.23°E, 37.18°N)			M	39.3 ± 4.1	4.17 ± 1.15	0.25 ± 0.10	0.05 ± 0.03	2.7 ± 0.7	15
Yantai	112	10.7	F	44.8 ± 7.6	4.41 ± 1.95	0.38 ± 0.23	0.01 ± 0.01	3.6 ± 1.3	10
(121.74°E, 37.30°N)			M	40.7 ± 4.5	4.37 ± 1.76	0.39 ± 0.16	0.01 ± 0.02	2.9 ± 0.7	20
Jiaoshui	770	10.0	F	44.3 ± 7.8	5.44 ± 3.03	0.40 ± 0.21	0.03 ± 0.02	3.0 ± 1.0	3
(112.07°E, 37.51°N)			M	37.2 ± 1.7	3.24 ± 0.94	0.21 ± 0.10	0.03 ± 0.01	2.0 ± 0.0	3
Jiaocheng	1257	3.6	F	41.3 ± 6.1	3.86 ± 2.22	0.29 ± 0.13	0.03 ± 0.02	2.9 ± 0.9	22
(111.79°E, 37.66°N)			M	39.8 ± 3.2	3.89 ± 0.87	0.25 ± 0.09	0.07 ± 0.04	2.7 ± 0.9	27
Pangquangou	1880	4.3	F	51.0 ± 4.1	8.21 ± 1.99	0.71 ± 0.17	0.09 ± 0.04	4.1 ± 0.8	15
(111.48°E, 37.84°N)			M	46.1 ± 4.0	7.33 ± 1.77	0.70 ± 0.17	0.17 ± 0.05	3.3 ± 0.6	18
Fengjiacun	1429	6.7	F	43.8 ± 7.3	5.54 ± 2.91	0.46 ± 0.24	0.07 ± 0.04	3.2 ± 0.7	13
(111.34°E, 37.91°N)			M	45.2 ± 3.3	6.59 ± 1.62	0.48 ± 0.12	0.16 ± 0.08	3.0 ± 0.0	11
Dongzhai	1588	5.5	F	42.1 ± 7.6	4.33 ± 2.55	0.32 ± 0.24	0.11 ± 0.21	3.6 ± 0.8	12
(112.09°E, 38.81°N)			M	39.0 ± 3.1	3.90 ± 0.96	0.33 ± 0.16	0.08 ± 0.03	3.1 ± 0.9	14
Tianchi	1781	4.4	F	42.1 ± 3.5	3.78 ± 1.08	0.25 ± 0.09	0.05 ± 0.02	3.1 ± 0.6	13
(112.20°E, 38.87°N)			M	42.2 ± 3.3	4.40 ± 0.95	0.36 ± 0.14	0.12 ± 0.02	2.9 ± 0.5	11
Xiayuzhuang	1620	5.3	F	43.5 ± 7.3	4.67 ± 3.29	0.30 ± 0.14	0.06 ± 0.06	3.0 ± 1.2	11
(112.22°E, 38.92°N)			M	40.6 ± 3.9	5.49 ± 1.97	0.37 ± 0.12	0.10 ± 0.04	3.1 ± 0.8	8
Huairong	991	5.6	F	44.5 ± 5.5	4.85 ± 2.04	0.28 ± 0.14	0.02 ± 0.01	3.2 ± 0.7	13
(114.51°E, 40.63°N)			M	40.4 ± 4.7	4.16 ± 1.39	0.37 ± 0.18	0.04 ± 0.02	3.1 ± 0.6	20
Meiligeng	1181	5.7	F	48.3 ± 6.6	7.03 ± 2.96	0.42 ± 0.22	0.02 ± 0.02	3.6 ± 1.5	9
(109.44°E, 40.67°N)			M	41.3 ± 5.4	5.25 ± 2.15	0.34 ± 0.17	0.03 ± 0.02	2.7 ± 0.7	9
Jiufengshan	1321	5.6	F	46.8 ± 7.4	6.06 ± 2.80	0.42 ± 0.18	0.04 ± 0.03	3.8 ± 1.4	8
(110.68°E, 40.71°N)			M	38.9 ± 3.4	4.34 ± 1.21	0.38 ± 0.10	0.06 ± 0.03	2.9 ± 0.7	42
Wudan	546	6.3	F	46.1 ± 4.6	5.91 ± 2.16	0.31 ± 0.12	0.01 ± 0.01	3.8 ± 0.8	5
(119.27°E, 43.05°N)			M	41.7 ± 5.2	4.54 ± 1.52	0.27 ± 0.09	0.03 ± 0.02	2.7 ± 0.8	11
Hansaiwula	973	3.3	F	48.5 ± 5.7	6.82 ± 2.33	0.53 ± 0.12	0.03 ± 0.01	3.5 ± 0.5	6
(118.63°E, 44.25°N)			M	40.1 ± 5.6	4.35 ± 1.81	0.37 ± 0.17	0.15 ± 0.05	3.6 ± 0.7	8

peratures after controlling for the effects of body size and age (Fig. 1 and Table 2). Carcass weight tended to decrease with reduced ambient temperatures, but the correlation between these two variables was not significant (Fig. 1 and Table 2).

Larger individuals contained more stored energy (Table 2), while age showed no measurable effect on the relative weights of organs (Table 2). Males deposited more energy in fat bodies than did females, and differences in the weights of fat bodies tended to increase as mean annual temperature decreased (Fig. 1 and Table 2).

Discussion

Anurans that hibernate in colder climatic conditions accumulate more energy before winter (Pasanen & Koskela 1974, Pinder *et al*. 1992, Irwin & Lee 2003, Lu *et al*. 2008), and even after emerging and before breeding (Jönsson *et al*. 2009). Our findings supported this prediction: *R. chensinensis* stored more energy in colder regions. These reserves are used for energy requirements during prolonged dormancy and subsequent spring reproduction (Fitzpatrick 1976, Lu 2004, Lu *et al*. 2008, Jönsson *et al*. 2009, Jackson & Ultsch 2010), and for buffering against uncertain environmental conditions (Jönsson *et al*. 2009).

Rana chensinensis had heavier livers in colder regions. Similar results in terms of geographic variation in energetics have been reported by Pasanen and Koskela (1974). They showed that the content of glycogen in the liver of *Rana temporaria* increased in colder environmental conditions (i.e., further north). The aquatic environment generally becomes hypoxic, resulting in a greater reliance on glycogen stores in the liver to fuel the overwintering period (Jackson & Ultsch 2010). During hibernation, the liver glycogen content of anurans has been shown to reduce by 51% in males and 56% in females (Tattersall & Ultsch 2008). Accordingly, in *R. chensinensis* the increase in liver weight with decreasing temperature may be an adaptation to the hypoxic aquatic environment in these regions with a prolonged winter.

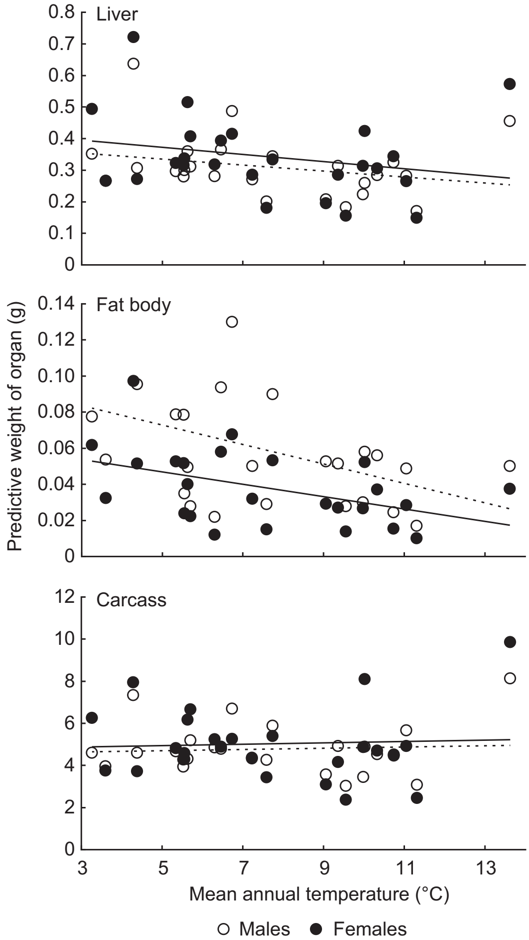

Fig. 1. The effect of annual mean temperature on mean organ mass in males and females from different populations. Solid and dashed lines are fitted lines for females and males, respectively. All variables were ln-transformed and the data are predictive values from the LMMs.

Our results showed that the weight of fat bodies of *R. chensinensis* increased in colder regions, in accordance with findings by Elmberg (1991). Elmberg showed that at higher altitudes, *R. temporaria* displayed a higher rate of fat body growth in the summer than frogs at lower altitudes. This could be explained by the fact that, in anurans, fats are the preferred substrates of aerobic metabolism if oxygen is not limiting, and are the main source of at least 80% of the energy used during hibernation (Tattersall & Ultsch 2008). Accordingly, the weights of fat bodies of *R. chensinensis* increased with decreasing temperature, which could also be an adaptation to prolonged winters in colder regions.

Relative carcass mass decreased towards colder climates for both sexes of *R. chensinensis*, a pattern opposite to that found for the liver and fat bodies. Jänsson *et al*. (2009) also detected a similar pattern for female *R. temporaria*. In *R. chensinensis*, the ovaries continue to develop throughout hibernation (Lu 2004), a pattern also reported for some temperate species (Maruyama 1979, Delgado *et al*. 1990, Loumbourdis & Kyriakopoulou-Sklavounou 1996). Vitellogenesis must, therefore, rely on body reserves (Delgado *et al*. 1990, Girish & Saidapur 2000). For example, Bradford (1983) reported that *R. muscosa* used more than half of its whole-body fat stores (including those in the carcass tissue) for gametogenesis and breeding before feeding, and used the remaining stores for surviving the 8-month-long hibernation period. Therefore, we can assume that more reserves deposited into the carcass tissues by female *R. chensinensis* in warmer regions will be converted into the energy expenditures for egg production than will be by females in colder regions. In contrast, females of some species, including those where a negative relationship between body reserves and female yearly reproductive effort along environmental gradients has been found (e.g. *R. temporaria*), have finished vitellogenesis during hibernation (Lu 2004). For male *R. chensinensis*, the lack of support of a trade-off in life history strategies is likely because male-male competition during the breeding season was not investigated.

Sexual differences in the weights of *R. chensinensis* fat bodies could be explained in terms of the timing of energy investment for reproduction; most of the fat bodies in females are used for ovarian development prior to winter (Rastogi *et al*. 1983), while in males fat reserves are used for spring reproductive behaviors (Tattersall & Ultsch 2008). Typically, there is a direct relationship between fat bodies and gonadal growth in females (Chieffi *et al*. 1975, Rastogi *et al*. 1983, Prasadmurthy & Saidapur 1987, Delgado *et al*. 1990, Girish & Saidapur 2000). For capital breeding anurans, fat bodies in mature females are utilized primarily by providing energy for the developing gonads that occur during the late autumn and winter, whereas in males, the fat bodies are required for early spring calling and breeding activities (Jørgensen *et al*. 1979, Jönsson *et al*. 2009). As such, sex difference in the weights of fat bodies increase with decreasing temperatures.

In general, our results indicate that amphibians inhabiting colder environments adopt a strategy of accumulating relatively larger energy reserves prior to winter, presumably to meet energy demands for overwintering and increase subsequent reproductive performance in the spring. Sex differences may originate from the

Table 2. Fixed effect results from linear mixed model analyses of variables for *Rana chensinensis* populations in northern China. SE = standard error, 95% HPDI = highest posterior density interval.

Variate	Effect	Estimate	SE	95% HPDI	
				Lower	Upper
Liver	Intercept	–10.047	0.500	–11.029	–9.065
	Sex	–0.118	0.027	–0.172	–0.064
	SVL	2.439	0.144	2.157	2.721
	Age	0.004	0.022	–0.039	0.046
	Temperature	–0.031	0.014	–0.061	–0.002
Fat body	Intercept	–10.485	1.067	–12.581	–8.388
	Sex	–0.619	0.057	–0.731	–0.508
	SVL	2.205	0.300	1.616	2.793
	Age	0.013	0.045	–0.075	0.101
	Temperature	–0.097	0.040	–0.181	–0.014
Carcass	Intercept	–8.917	0.230	–9.368	–8.466
	Sex	–0.208	0.013	–0.233	–0.183
	SVL	2.801	0.066	2.671	2.931
	Age	0.014	0.010	–0.006	0.033
	Temperature	0	0.006	–0.012	0.013

asynchrony of the main energetic investment in reproduction between the sexes. Females had already invested most of the energy needed for reproduction before winter, while the main energy investments for males are used for behavioral activities related to attracting mates in the spring. Apart from mean annual temperature, other environmental factors such as winter temperature, the duration of winter, and even summer temperature, which may lead to higher body reserves before winter need to be addressed in future studies.

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