ANNUAL CYCLE OF NUTRITIONAL ORGAN MASS IN A TEMPERATE-ZONE ANURAN, *RANA CHENSINENSIS*, FROM NORTHERN CHINA

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Body reserves of temperate anurans go through an annual cycle in response to highly seasonal environments. Here I describe how changes in relative mass of storage organs of *Rana chensinensis* occurring in northern China contributed to this cycle. Body reserves of both sexes dropped to their lowest levels after hibernation, and experienced a resting period of five months, then the reserves started increasing and attained peaks shortly before hibernating. During hibernation, the frogs' ovaries kept growing and liver and fat bodies declined accordingly. Based on comparable data in other studies, two distinct models of ovarian development in temperate anurans, hibernation-growth and non-hibernation-growth, are suggested. I also show a decreased relative ovarian mass with increased climate harshness.

Key words: body reserves, ecological energetics, environmental physiology, Ranidae

INTRODUCTION

Temporal organization of metabolic activities is a crucial life history trait in temperate-zone organisms. For anurans, liver, fat bodies and gonads (especially of females) are major organs of energy storage, and their relative mass provides a convenient indication of individual nutritional status (Pasanen & Koskela, 1974; Jørgensen *et al.*, 1979; Morton, 1981; Elmberg, 1991; Das, 1996; Tsiora & Kyriakopoulou-Sklavounou, 2001). In response to highly seasonal environments, northern frogs must go through a pronounced annual cycle in the masses of these organs. The pattern of such seasonal changes is a result of species' adaptation to local ecological conditions, and thus shows interspecific and inter-population variation (Pasanen & Koskela, 1974; Jørgensen *et al.*, 1979).

Rana chensinensis was once considered a subspecies of *R. temporaria* (Pope & Boring, 1940), and more recently an independent species endemic to China (Xie *et al.*, 2000). The frogs occur commonly in mountain rivers across northern China (Liu & Hu, 1961). In the present paper, I investigate annual dynamics of storage organ mass of the frogs in a typically seasonal environment, aimed at assessing nutritional strategies of this species in comparison with other temperate-zone anurans.

MATERIALS AND METHODS

Frogs were collected between March 1993 and February 1994, from Jie-xiu (37°04'N, 112°03'E) in Shanxi province, northern China. Climate of the surveyed area is typically continental (Fig. 1a). The frogs lived in two small streams surrounded by loess ravine with shrub and farmland. The spawning period was between mid-February and late March, and the hibernation period extended from early November to mid-February (Fig.

1a). I caught the frogs by hand in streams, ponds or springs (hibernation sites, see Lu, 2001) around the middle of each month. The specimens were brought to the laboratory immediately and were humanely killed. For each frog, after measuring snout-vent length (SVL, to the nearest 0.1 mm) and body mass (to nearest 0.1 g), I opened the body cavity and removed the liver, fat bodies, ovaries and oviducts. These organs were placed on water-absorbing paper for about five minutes and then weighed (to 0.001g). The testis was not estimated because of its small size and less pronounced seasonal changes (e.g. Jørgensen et al., 1979; Geng et al. 1986). I calculated the relative organ mass (percentage of organ mass accounting for body mass) in order to examine the nutritional status of each frog. A frog was classified as a sexually-mature male if it displayed nuptial pads, and as a sexually-mature female if it had developed oocytes (September to pre-spawning) or was over 35 mm SVL (the minimum size of females in amplexus; Lu, 1994, 2001). Most (about 95%) young of the year showed sexual characteristics before their first hibernation and a few (about 5%) did not. Here, I focus solely on the seasonal change of storage organs of sexually mature individuals. In total, 410 (219 males, 191 females) specimens were examined.

I used Pearson's or Spearman's correlation coefficient to evaluate relationships between variables, and Student's *t* test to estimate significance of slopes of regression lines, and analyses of covariance (ANCOVA, with SVL as the covariate to adjust body size of compared specimens to the same level) to compare differences in storage organ mass between sexes or between different stages of the frog's life cycle. Prior to analysis, percentage variables were arcsin transformed and SVL ln-transformed to improve normality.

RESULTS

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After spawning, liver (male: *r*=-0.38, *n*=69, *P*=0.002; female: *r*=-0.49, *n*=68, *P*<0.001) and fat bodies (male, *r*=0.48, *P*<0.001; female, *r*=-0.39, *P*=0.003) declined at

a low rate until August. The regressed period of storage organs was called the resting period. From late August onwards, both organs (liver: male, r=0.61, n=52, P < 0.001: female, r = 0.71, n = 32, P < 0.001; fat body: male, r=-0.48, P=0.001; female, r=0.56, P=0.002) started growing rapidly and attained their maximum size just before hibernation: October in male and September in female (Fig. 1b,c: the accumulating period). After the peaks, they decreased in size rapidly through the winter until emergence (liver: male, r=-0.41, n=126, P < 0.001; female, r=-0.71, n=123, P < 0.001; fat body: male, r=-0.43, P<0.001; female, r=-0.36, P<0.001), compared to the rates during the resting period (comparison of slopes of regression lines, male, March-August vs October-February, liver: t=1.65, df=193, P=0.04; fat body: t=3.32, P<0.001; female, March-August vs September-February, liver: t=5.30, df=189, P<0.001; fat body: t=0.94, P=0.25). In spite of parallel change in the organ mass of both sexes, males had significantly heavier organs than females during November-February (Fig. 1). ANCOVA, liver: all P<0.003; fat body: all P<0.02, except for January P=0.12). However, there was no significant sexual difference in organ size between February and September (ANCOVA, liver: P=0.09-0.78; fat body: P=0.10-0.82).

From spawning (all eggs were released in one spawning) to pre-hibernation, mass of female reproductive organs varied with a similar profile to those of liver and fat bodies (Fig. 1d). However, the ovaries continued to grow through hibernation and reached the maximum size before spawning (r=0.74, n=150, P<0.001), whereas the oviducts peaked by mid-hibernation (r=0.60, n=93, P<0.001) and then remained relatively stable until pre-spawning (r=-0.06, n=30, P=0.76).

DISCUSSION

The present results followed a general trend: energy reserves of temperate-zone anurans reach their lowest point at or shortly after spawning, remain low through a resting period, and then initiate a new growth cycle, with a peak in late autumn from which, before hibernation, the reserves begin to decrease (Jørgensen *et al.*, 1979).

Metabolism should be synchronized with local environmental factors. Northern *R. temporaria* began establishing body reserves immediately after breeding (Pasanen & Koskela, 1974), whereas southern population of this species exhibited a delayed accumulation (Hong *et al.*, 1968). Similarly, for development of female reproductive organs of temperate-zone anurans, the studies on *R. temporaria* by Koskela & Pasanen (1975) showed that there is a reduced ovarian resting period with increased latitude. Jørgensen *et al.* (1979) concluded that a longer ovarian resting period is correlated with earlier occurrence of spawning. In the present study, *R. chensinensis* had a longer post-reproduction resting period (5 months), compared to that (3 months) of a population further north (ovulation timing late



FIG. 1. Relative organ mass of R. chensinensis in relation to the month of year. (a), annual change of monthly average air temperature in the study area. S, R, A and H at the top of the figure represent spawning, resting, accumulating and hibernating periods, respectively; (b), liver; (c), fat body (filled circles represent males, open circles females); (d) female reproductive organs (filled circles represent ovary, open circles oviduct). The vertical bars are 1 SE.

April; Table 1). These observations suggest that higher seasonal constraints due to harsh climatic conditions result in a tight schedule of energy storage for the northern animals. A question arises as to why those frogs experiencing a long resting period do not save any nutrition in their storage organs. Based on an experiment showing that newly spawned *B. bufo* that are well fed may begin a new ovarian cycle immediately, Jørgensen (1973) argued that nutritional constraints upon females are responsible for a delayed onset of vitellogenetic growth. For *R. chensinensis*, however, 1 found that the proportion (89.2%) of individuals with food-filled stomachs during the resting period did not significantly differ from

TABLE 1. Mean relative ovarian mass of pre-hibernation and pre-ovulation in several temperate-zone anuran species and populations.

Species	Locality	Resting period length	Pre-hib- ernation	Pre-ovu- lation	References
R. temporaria	Finland, 64°N, 100 m	0	10.3	10.9	Koskela & Pasanen, 1975
	Sweden, 64°N, 25 m	0	10.0		Elmberg, 1991
	Poland, 52°N		11.4	12.3	Juszcyzk, 1959
	Poland, 50°N, 1000 m	2-3		12.2	Kozlowska, 1971
	Poland, 50°N, 200 m	3-4		14.5	Kozlowska, 1971
	Denmark, 57°N, 100 m		11.7		Jørgensen, 1981
	England, 53°N	3-4	15.0	16.0	Smith, 1950
R. chensinensis	Jilin, China, 43°N, 700 m	3	9.8		Ma, 1982
	Shanxi, China, 38°N, 1650 n	n 3-4	11.2		X. Lu unpubl data
	Shanxi, China, 37°N, 760 m	5	12.5	24.0	This study
R. nigromaculata	Beijing, China, 40°N, 150 m	3-4	10.3	26.7	Wu, 1965
B. bufo	Denmark, 57°N, 100 m	2-3	13.7	18.4	Jørgensen et al., 1979
B. viridis	Denmark, 57°N, 100 m			20.1	Jørgensen, 1981
R. esculenta	Switzerland, 47°N	1-2	15.0	20.0	Cited from Jørgensen et al., 1979
	Poland, 52°N	2	12.0	18.0	Juszcyzyk & Zamachowski, 1973

that (91.8%) during autumn. To assess the mechanism, the underlying metabolism of amphibians during the resting period and to understand its adaptive implication, further quantitative studies on food availability in relation to physiological regulation of metabolic activity should be made.

Using observations on R. temporaria, B. bufo and B. viridis, Jørgensen et al. (1979) and Jørgensen (1981) argued that vitellogenetic growth of oocytes in temperate-zone amphibians has already finished by autumn and thus ovarian mass remains stable throughout the winter. However, significant winter increases in ovarian mass have been detected in R. nigromaculata and several populations of the R. esculenta complex (Table 1). Jørgensen et al. (1979) attributed this increase to accumulation of fluid. In a R. chensinensis population adjacent to the study area, I compared mean ovarian dry mass $(0.53\pm0.05, n=44)$ during pre-hibernation with that (1.46±0.13, n=27) during pre-spawning and found a significant increase (ANCOVA: F_{1.68}=179.67, P<0.001). I therefore hypothesize that there exist two distinct models of ovarian development in temperate-zone anurans: hibernation-growth and non-hibernation-growth.

In amphibians, egg production is energy-consuming and associated with metabolic activities of liver and fat bodies (Krawczyk, 1971; Pasanen & Koskela, 1974; Maruyama, 1979; Elmberg, 1991; Das, 1996). For *R. chensinensis*, females depleted more energy during hibernation than did males and the increase in the size of ovaries (140.6%) was approximately equal to the loss of mass (152.2%) in both liver and fat body (Table 2), suggesting that most expenditure of the stored energy in females was devoted to sustained winter growth of the oocytes.

Metabolic rate depression is a common mechanism for amphibians to cope with environmental stresses in winter, but hibernating animals do not reduce metabolism to zero and thus they must build up body reserves before winter (Pinder et al., 1992). The amount of depleted nutrition during hibernation should depend on the length of winter. Therefore, it might be expected that northern frogs save more energy before hibernation to survive a longer winter (Pasanen & Koskela, 1974). However, relative ovarian mass decreases with increased climate harshness (Jørgensen et al., 1979; Elmberg, 1991; summarized from the data in Table 1 of this study, correlation between length of resting period and relative ovarian mass, R. temporaria: $r_{=}=0.95$, n=5, P=0.01; R. chensinensis: r_s=1.00, n=3, P<0.001), indicating northern female animals have a lower reproduction investment than southern animals. Interestingly, the longer resting period of nutrient organs in

TABLE 2. Change in relative mass of nutritional organs of *R. chensinensis* from pre-hibernation (maximum organ mass, as 100%) to emergence from hibernation.

Nutritional organ	Male (October-February)	Female (September-February)
Liver	-21.6	-59.1
Fat body	-78.0	-93.1
Ovary		+140.6
Oviduct		-6.4
Total energy expenditure	-99.6	-18.0

southern animals due to warmer climates results in a growth period similar in length to that of northern populations. This suggests that despite having similar available foraging time for nutrient acquisition, the animals living in different climate conditions may adopt different strategies of energy allocation.

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