Reproductive output of the brown frog *Rana kukunoris* at high altitude of the Tibetan plateau

Wei Chen[1,*,#], Li Zhao[1,#], Ying Wang[2], Hua Li[3], Dujun He[2], Lina Ren[2], Zhonghai Tang[2], Xin Lu[4]

[1]*Ecological Security and Protection Key laboratory of Sichuan Province, Mianyang Normal University, Mianyang, 621000, China. *Corresponding author.* E-mail: wchen1949@gmail.com. [#]*Co-first authors*
[2]*College of Life Science and Biotechnology, Mianyang Normal University, Mianyang, 621000, China*
[3]*Management Bureau of Zoige Wetland National Nature Reserve, Zoige 624500, China*
[4]*Department of Zoology, College of Life Sciences, Wuhan University, Wuhan, 430072, China*

Submitted on 2013, 24th April; revised on 2013, 31st October; accepted on 2013, 31st October.

Abstract. *Rana kukunoris* is endemic to the eastern Tibetan plateau and its breeding ecology was not known so far. In this study, we investigated the reproductive output patterns of the species during the breeding periods of 2012 and 2013. Our results showed that clutch size and total clutch volume increased with maternal body size. However, no significant correlation between egg number and egg size was observed, which suggested that the tradeoff between offspring number and size in this species was lack.

Keywords. Clutch size, egg size, clutch volume, *Rana kukunoris*, trade-off, high altitude.

Offspring number and size can determine simultaneously the fitness of animals (Bernardo, 1996; Räsänen et al., 2005; Marangoni and Tejedo, 2008; Marangoni et al., 2008). Accordingly, female amphibians usually show a trade-off between offspring number and size when resources available for reproductive production are limited (Jørgensen, 1981; Berven, 1988; Sinervo and Licht, 1991; Lips, 2001; Lüddecke, 2002). Given limited clutch holding capacity (Jørgensen, 1981; Berven, 1982; Cummins, 1986; Ryser, 1988; Elmberg, 1991; Donnelly, 1999; Lüddecke, 2002; Castellano et al., 2004), and fluctuating environments between years, female reproductive output is usually changeable (Wells, 2007). Suprisingly, studies on anurans reproductive output have focused on low-altitude native species (Morrison and Hero, 2003; Wells, 2007), virtually no data exist on the trade-off pattern of reproductive output in anurans from high-altitude zone, especially from Tibetan plateau.

Rana kukunoris is endemic to the eastern Tibetan plateau (Chen et al., 2011), and the species is an explosive breeder with a pure capital breeding strategy, allocating to reproduction the reserves stored in previous years (Lu et al., 2008; Chen and Lu, 2011; Chen et al., 2011). Female fecundity is known to be positively related to body size (Lu et al., 2008) and males prefer to mate with larger females (Chen and Lu, 2011). The breeding biology of *R. kukunoris* has been generally described in some studies concerning the population age structure (Li and Li, 1991; Chen et al., 2011), hibernation habitat use (Liu and Shi, 2000), seasonal patterns of body condition (Dai et al., 2004), post-breeding habitat selection (Dai et al., 2005) and prehibernation energy storage (Chen et al., 2013b). Little is known about *R. kukunoris* maternal investment, although the species seems to be a good model to study the trade-offs in reproductive output of females at high altitudes due to large clutch sizes and no parental care (Wells, 2007). In this study, we investigated in detail the breeding ecology of *R. kukunoris*, with the aim to investigate its maternal investment, such as the trade-off between egg number and egg size, and the influence of body size on clutch parameters.

ISSN 1827-9635 (print)
ISSN 1827-9643 (online)

Fieldwork was conducted in Zoige county, Sichuan from March to April 2012 and 2013. Being *R. kukunoris* a capital breeder, the climatic patterns of the year preceding the reproduction are important, so we collected the climatic data of 2011 and 2012 at study site located 20 km away from city of Zoige. Annual average temperature of 2011 and 2012 at this site was 2.3°C and 2.5°C, respectively, and annual total precipitation was 702.8 mm and 686.6 mm (data from the weather station of Zoige county, Fig. 1). The study site (located at 103.004°E and 33.557°N; 3500 m a.s.l.) was a spawning pond with an area of 60 m^2 and water depth of 10-40 cm. Vegetation covering the pond consisted of short terrestrial and aquatic grasses.

Before the start of breeding season, we randomly hand-collected 50 frog pairs (26 pairs in 2012 and 24 pairs in 2013, Table 1) at the spawning pond of *R. kukunoris*. Almost 1,000 frogs come at this breeding site every year, therefore the probability of recapturing the same individuals in two consecutive years was very low. After capture, individuals were temporarily held in pails (40 cm in width × 60 cm in height) containing pond water and transported to the field laboratory (50 m away from the study site). After finishing their egg-laying, we measured snout-vent length of females with a ruler to the nearest 0.1cm. In addition, we also counted egg number and photographed the eggs (10-50 eggs per female) with a scale ruler using a canon camera with macro lens (EOS 550) in order to measure egg size. All frogs and clutches were then released again into the spawning pond.

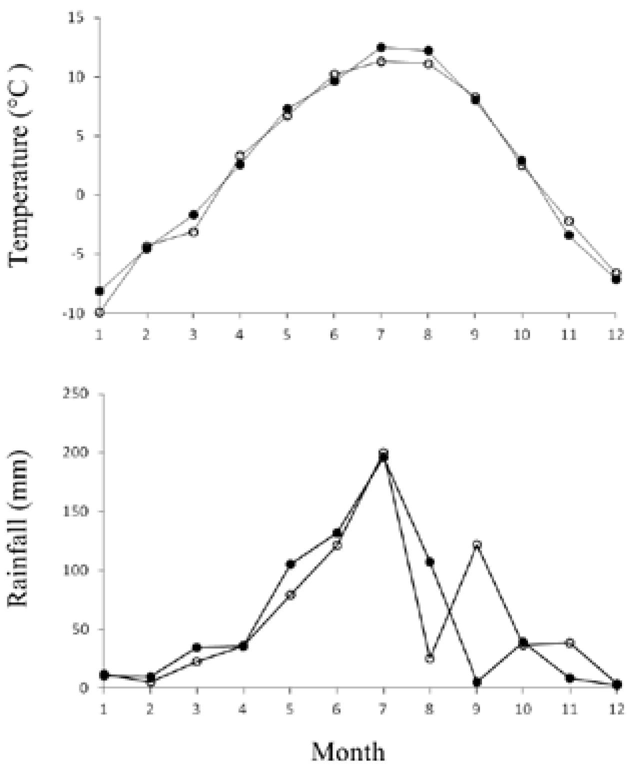

Fig. 1. Monthly mean air temperatures and monthly mean rainfall of 2011 (hollow point) and 2012 (solid point) at the study site from which *Rana kukunoris* were collected (data are from the weather station of Zoige county).

In the laboratory, egg size (10-30 eggs randomly selected from the pictures) was measured to the nearest 0.01 mm, and averaged (minimum and maximum diameter for each egg) to obtain mean egg diameter. Egg size was always measured at Gosner stage 9-10 (Gosner, 1960) and all measurements were obtained by the same person. Clutch volume was defined by the cube of egg dimensions.

Pooled data from both years were analyzed by Pearson correlation to explore the relationship between clutch size and egg size. To control for the effect of body size, partial correlation was performed with clutch size and egg size as dependent variables and body size as controlling variable. We then investigated the yearly variation in clutch parameters (clutch size, egg size and clutch volume) and the relationship between these parameters and body size by using general linear models (GLMs), with clutch parameter (clutch size, egg size or clutch volume) as dependent variable, year as fixed factor and body size as covariate. All variables were log10-transformed to approximate normality and enhance homogeneity of variances before the analysis. All statistical tests were performed with SPSS 16.0 statistical software.

R. kukunoris breeding began when average daily temperature was above 0°C, and amplexing females began to lay egg on April 4, 2012 and March 17, 2013. The breeding period lasted about four weeks with a peak period of seven days. The number of eggs per clutch ranged from 160 to 1285 ($n = 50$), average egg diameters of different clutches from 1.72 mm to 1.88 mm ($n = 50$) and total clutch volume from 2516.1 mm^3 to 4086.5 mm^3 (Table 1).

There was no significant correlation between clutch size and egg size ($r = -0.14$, $n = 50$, $P = 0.332$, Fig. 2). This held true even when the effect of body size was removed (Partial correlation: $r = -0.061$, $n = 47$, $P = 0.675$). We found significant correlations between body size and clutch size as well as between body size and total clutch volume, but not for body size and egg size (Table 2). Interannual variation in the clutch parameters was low and did not show significant differences. No significant interactive effects between year and body size on parameters were found (Table 2).

Generally, our results showed that large *R. kukunoris* lay larger clutches than smaller individuals, but egg size only exhibited little variation among the female individuals with different sizes. We also found low variation in clutch size, egg size and clutch volume between 2012 and 2013.

Table 1. Year, Samples size (n), Means, Standard deviations (SD) and Minimum and Maximum of body size, clutch size, egg size and clutch volume of *Rana kukunoris* in a population from Tibetan plateau.

Varables	Year	n	Mean	SD	Minimum	Maximum
Body size (cm)	2012	26	4.5	0.6	3.6	5.8
	2013	24	5.0	0.5	4.0	5.9
Clutch size	2012	26	481.4	179	160	870
	2013	24	641.9	268	265	1285
egg size (mm)	2012	26	1.85	0.04	1.72	1.88
	2013	24	1.85	0.03	1.79	1.88
Clutch volume (mm^3)	2012	26	1580.6	569.4	530.4	2516.1
	2013	24	2118.6	857.8	862.8	4086.5

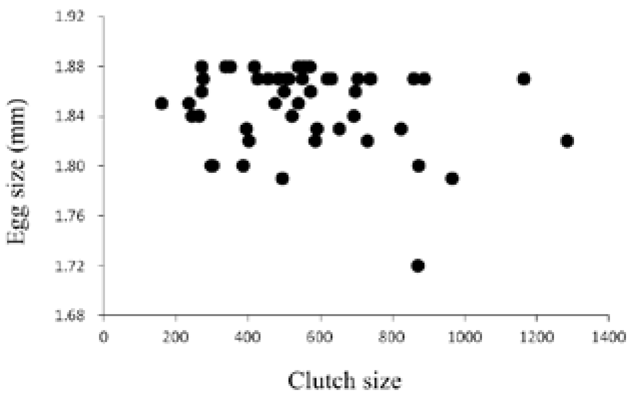

Fig. 2. Scatter plot of clutch size and egg size in *Rana kukunoris* in a population from Tibetan plateau.

Life history theory emphasizes that animals should optimally partition limited resources between growth and reproduction throughout life (Roff, 2002). So larger female amphibians have large clutch size and high clutch volume (Duellman and Trueb, 1986; Roff, 1992; Castellano et al., 2004; Dziminski and Alford, 2005). Similar to the growth pattern of most amphibians, growth of female *R. kukunoris* fitted the von Bertalanffy's model. There was a rapid somatic growth for the earlier time and slower after sexual maturity (Chen et al., 2011). This implied that more energy would be devoted to reproduction with increasing maternal age (Jørgensen, 1992; Czarnoleski and Kozlowski, 1998), ultimately resulting in the body size-specific reproductive output. This also explains the significant differences in clutch size.

Large females often invest more energy into egg growth, so it is common pattern that there is a positive but weak correlation between egg size and body size of female in many amphibians (Jørgensen, 1981; Deullman and Tureb, 1986; Roff, 1992; Castellano et al., 2004; Dziminski and Alford, 2005). In contradiction with this pattern, in *R. kukunoris*, large females did not lay larger eggs. Our result was similar to those from several other investigators (Tejado, 1992; Castellano et al., 2004; Dziminski and Alford, 2005; Chen et al., 2013a), who found that larger female allocated their main investment in larger clutch sizes rather than larger eggs (Jørgensen, 1981).

Table 2. GLMs showing the effect of year, body size and interaction between year and body size on clutch size, egg size and clutch volume of *Rana kukunoris* in a population from Tibetan plateau.

Parameter	df	F	P
Clutch size (n = 50)			
Intercept	1	19.328	< 0.001
Year	1	0.961	0.332
Body size	1	28.420	< 0.001
Year×body size	1	0.912	0.345
Egg size (n = 50)			
Intercept	1	365.640	< 0.001
Year	1	3.208	0.080
Body size	1	2.999	0.090
Year×body size	1	2.976	0.091
Clutch volume (n = 50)			
Intercept	1	43.206	< 0.001
Year	1	1.618	0.210
Body size	1	25.653	< 0.001
Year×body size	1	1.526	0.223

Usually, a negative correlation between clutch size and egg size is a common pattern observed in amphibians (Duellman and Trueb, 1986), although this trade-off may exhibit considerable variation among different species (positive correlation: *Rana ridibunda* Kyriakopoulou-Sklavounou and Loumbourdis, 1990; negative: *Rana temporaria* Cummins, 1986; *Rana sylvatica* Berven, 1988; *Hyla labialis* Lüddecke, 2002, *Rana dalmatina* and *R. temporaria* Weddeling et al., 2005 and no correlation: *Bufo calamita* Tejado, 1992 ; *Bufo viridis* Castellano et al., 2004). Our result showed there was no correlation between these two life-history traits of *R. kukunoris*. This can be explained by the fact that limited physical clutch holding capacity can't increase simultaneously the clutch size and egg size (Jørgensen, 1981) or that the frogs in the study population were below maximum physical constraint and egg size was mainly determined by factors such as energy availability during vitellogenesis (Kaplan and Salthe, 1979).

Previous studies from temperate-zone amphibians with capital breeding strategy showed that they exhibited year to year variation in both clutch size and egg size (Cummins, 1986; Berven, 1988; Kaplan and King, 1997). Dissimilar to this pattern, *R. kukunoris* with capital breeding strategy showed only low yearly variation in these two life-history traits during 2012 and 2013. This may be the result of very similar climate conditions (temperature and rainfall) in the preceding years 2011 and 2012, so food availability and the nutritional condition of females could be more or less similar.

ACKNOWLEDGEMENTS

We thank Dr. David Shackleton for their comments on the early draft of the manuscript and thank Dr. Rocco Tiberti for valuable suggestion to improve the manuscript. Financial support was provided by the Scientific Research Foundation of Mianyang Normal University (No. 2011A17 and No. QD2012A13), Youth Foundation of Sichuan Provincial Department of Education (No. 11ZB138). All field and laboratory work was performed under licenses from the Wildlife Protection Law of China.

REFERENCES

Bernardo, J. (1996): The particular maternal effect of propagule size, especially egg sizes: patterns, models, quality of evidence and interpretations. Am. Zool. **3**: 216-236.

Berven, K.A. (1982): The genetic basis of altitudinal variation in the wood frog *Rana sylvatica*. I. An experimental analysis of life history traits. Evolution **36**: 962-983.

Berven, K.A. (1988): Factors affecting variation in reproductive traits within a population of wood frogs (*Rana sylvatica*). Copeia **1988**: 605-615.

Castellano, S., Cucco, M., Giacoma, C. (2004): Reproductive investment of female green toads (*Bufo viridis*). Copeia **2004**: 659-664.

Chen, W., Lu, X. (2011): Sex recognition and mate choice in male *Rana kukunoris.* Herpetol. J. **21**: 141-144.

Chen, W., Tang, Z.H., Fan, X.G., Wang., Y., Pike, D.A. (2013a): Maternal investment increases with altitude in a frog on the Tibetan Plateau. J. Evol. Biol. **26**: 2710-2715.

Chen, W., Wang, X.Y., Fan, X.G. (2013b): Do anurans living in higher altitudes have higher prehibernation energy storage? Investigations from a high-altitude frog. Herpetol. J. **23**: 45-49.

Chen, W., Yu, T.L., Lu, X. (2011): Age and body size of *Rana kukunoris*, a high-elevation frog native to the Tibetan plateau. Herpetol. J. **21**: 149-151.

Cummins, C.P. (1986): Temporal and spatial variation in egg size and fecundity in *Rana temporaria*. J. Anim. Ecol. **55**: 303-316.

Czarnoleski, M., Kozlowski, J. (1998): Do Bertalanffy's growth curves result from optimal resource allocation. Ecol. Lett. **1**: 5-7.

Dai, Q., Dai J.H., Zhang, M., Zhang, J.D., LI, C., Liu, B., Liu, Z.J., Wang, Y.Z. (2005): Terrestrial habitat selection of three amphibians (*Rana kukunoris*, *Nanorana pleskei* and *Bufo minshanicus*) during summer-autumn in Zoige Wetland National Nature Reserve. Zool. Res. Sinica **26**: 263-271.

Dai, Q., Zhang, M., Li, C., Zhang, J.D., Wang, Y.Z. (2004): Study on relative fatness of *Rana kukunoris* in Zoige wetland in Sichuan. Sichuan J. Zool. **24**: 351-354.

Donnelly, M.A. (1999): Reproductive phenology of *Eleutherodactylus bransfordii* in northeastern Costa Rica. J. Herpetol. **33**: 624-631.

Duellman, W.E., Trueb, D.L. (1986): Biology of Amphibians. McGraw-Hill Inc, New York.

Dziminski, M.A., Alford, R.A. (2005): Patterns and fitness consequences of intraclutch variation in egg provisioning in tropical Australian frogs. Oecologia **146**: 98-109.

Elmberg, J. (1991): Ovarian cyclicity and fecundity in boreal common frogs *Rana temporaria* L. along a climatic gradient. Funct. Ecol. **5**: 340-350.

Gosner, K.L (1960): A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica **16**: 183-190.

Jørgensen, C.B. (1981): Ovarian cycle in a temperate zone frog, *Rana temporaria*, with special reference to factors determining number and size of eggs. J. Zool. **195**: 449-458.

Jørgensen, C.B. (1992): *Growth and reproduction*. In: Environmental physiology of the amphibians. Feder, M.E., Burggren, W.W., Eds, University Chicago Press, Chicago, Illionis.

Kaplan, R.H., King, E.G. (1997): Egg size is a developmentally plastic trait: evidence from long-term studies in the frog *Bombina orientalis*. Herpetologica **53**: 149-165.

Kaplan, R.H., Salthe, S.N. (1979): The allometry of reproduction: an empirical view in salamanders. Am. Nat. **113**: 671-689.

Kyriakopoulou-Sklavounou, P., Loumbourdis, N. (1990): Annual ovarian cycle in the frog, *Rana ridibunda*, in Northern Greece. J. Herpetol. **24**: 185-191.

Li, L.X., Li, D.H. (1991): A preliminary analysis on the age composition of *Rana chensinensis kukunoris* in alpine meadow, northern Qinghai. Alpine Meadow Ecosystems **3:** 209-212.

Lips, K.R. (2001): Reproductive trade-offs and bet-hedging in *Haly calypsa*, a neotropical treefrog. Oecologia **128**: 509-518.

Liu, H., Shi, H.Y. (2000): Hibernating habitats of *Rana kukunoris* in the Zoige wetlands. Sichuan J. Zool. **19**: 68-69.

Lu, X., Zeng, X.H., Du, B., Nie, C. (2008): Reproductive ecology of *Rana kukunoris* Nikolskii, 1918, a high-elevation frog native to the Tibetan Plateau. Herpetozoa **21**: 67-77.

Lüddecke, H. (2002). Variation and trade-off in reproductive output of the Andean frog *Hyla labialis*. Oecologia **130**: 403-410.

Marangoni, F., Tejedo, M., Gomez-Mestre, I. (2008). Extreme reduction in body size and reproductive output associated with sandy substrates in two anuran species. Amphibia-Reptilia **29**: 541-553.

Marangoni, F.,Tejedo, M. (2008). Variation in body size and metamorphic traits of Iberian spadefoot toads over a short geographic distance. J. Zool. **275**: 97-105

Morrison, C., Hero, J.M. (2003): Geographic variation in life-history characteristics of amphibians: A review. J. Anim. Ecol. **72**: 270-279.

Räsänen, K., Laurila, A., Merilä, J. (2005): Maternal investment in egg size: environment- and population-specific effects on offspring performance. Oecologia **142**: 546-553.

Roff, D.A. (1992): The evolution of life histories. Chapman and Hall, New York.

Roff, D.A. (2002): Life history evolution. Sinauer Associates, Sunderland, MA.

Ryser, J. (1988): Clutch parameters in a swiss population of *Rana temporaria*. Herpetol. J. **1**: 310-311.

Sinervo, B., Licht, P. (1991): Proximate constraints on the evolution of egg size, number, and total clutch mass in lizards. Science **252**: 1300-1301.

Tejado, M. (1992): Absence of the trade-off between the size and number of offspring in the natterjack toad (*Bufo calamita*). Oecologia **90**: 294-296.

Weddeling, K., Bosbach, G., Hatchel, M., Sander, U., Schmidt, P., Tarkhnishvili, D. (2005): *Egg size versus clutch size: variation and trade-offs in reproductive output of Rana dalmatina and Rana temporaria in a pond near Bonn (Germany)*. In: Herpetologia Petropolitana. Proc. of the 12th Ordinary General Meeting of the Societas Europaea Herpetologica, August 12–16, 2003, pp. 238-240. Ananjeva, N., Tsinenko, O., Eds, Societas Europaea Herpetologica, St. Petersbourg.

Wells, K.D. (2007): The ecology and behavior of amphibians. The University of Chicago Press, Chicago and London.