

## No evidence for trade-off between clutch size and egg size in the spot-legged treefrog (*Polypedates megacephalus*)

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**Abstract.** The study of life-history traits in organisms mainly focuses on the female reproductive investment mode and its influencing factors. Offspring number and size as two plastic life-history traits, are commonly used to determine the reproductive investment. In this study, we evaluated the evolutionary trade-off between clutch size and egg size in the spot-legged treefrog (*Polypedates megacephalus*) along an altitudinal gradient. The results showed that females did not exhibit a significant correlation between body size, clutch size and egg size within each population, apart from the Leigong population. We did not find any evidence for the trade-off between clutch size and egg size within or among populations; however, clutch size and egg size from the highest-altitude population were larger than those from the lowest-altitude populations. Our findings suggest that the larger egg size may compensate for the increased mortality rates of their offspring at high altitudes.

**Key words:** breeding elevation, environmental gradient, fecundity, life history, parental investment, amphibians.

### Introduction

A major goal of life-history traits study is to gain insight into the patterns of variation in maternal reproductive investment and to identify the factors that influence these patterns. Offspring number and size, which simultaneously determine the reproductive investment, are two plastic life-history traits (Roff 2002). In most animals, large offspring have a higher chance of survival than small offspring (Stearns 1992, Badayev et al. 2001). Nevertheless, females only have a certain amount of body space available for storing reproductive output, which is the product of offspring number and offspring size (Smith & Fretwell 1974). For a given reproductive investment, a female can produce fewer but larger offspring or more but smaller offspring (Smith & Fretwell 1974). The evolutionary trade-off between offspring number and size is particularly important for taxa that determine not only the offspring number but also the offspring's fitness (Begon et al. 1990, Liao et al. 2014); however, numerous environmental factors (i.e. temperature, rainfall and predator pressure) and interactions determine the relationship between offspring number and size, and the trade-off between offspring number and size is non-significant in natural populations (Berven 1982).

Anurans vary clutch size and egg size in response to variations in environments when avail-

able resources are limited (e.g. Räsänen et al. 2008, Liao et al. 2015). Previous studies provide evidence for understanding life-history evolution in anurans across environmental gradients (Berven 1982, Howard & Wallace 1985, Lüddecke 2002, Morrison & Hero 2003, Liao & Lu 2010a,b, Liao & Lu 2011, Liao & Lu 2012, Li et al. 2013, Liao et al. 2015, Chen et al. 2016, Liao et al. 2016a,b, Jin et al. 2016, Mai et al. 2017). Indeed, environmental conditions at high altitudes (i.e. colder air temperatures, shorter breeding seasons and greater fluctuations in food resources) favor larger eggs as a strategy to improve offspring survival (Morrison & Hero 2003, Liao et al. 2014). For instance, individuals from high altitudes produce smaller clutches of larger eggs; whereas, in frogs, individuals from lower altitudes produce larger clutches of smaller eggs (Lüddecke 2002, Morrison & Hero 2003, Liao et al. 2014). However, more studies evaluating the evolutionary trade-off between clutch size and egg size among or within frog populations are required.

The spot-legged treefrog (*Polypedates megacephalus*) is widely distributed in China, at elevations ranging from 520 to 2200 m (Fei & Ye 2001). Breeding activity begins in mid-April and lasts until late July. It is a lekking species where males gather at ponds to wait for females. Males are quick to approach females as females are only present at night for mating in these ponds. The

amplectant pairs move to the edge of the ponds, where they release foam and eggs on the ponds' water surface. Immediately, other males come to join the pair, forming an amplecting group of two to five males. Female-biased sexual size dimorphism (SSD) does not result from phenotypic selection on females in *P. megacephalus* and age differences between males and females, but from the difference in growth rate between the sexes (Liao et al. 2017, Jin et al. 2016). So far, little information on the evolutionary trade-off between clutch size and egg size in *P. megacephalus* is available. In these cases, the aim of this study was to investigate altitudinal variation in evolutionary trade-off between clutch and egg sizes in *P. megacephalus* within and among populations. We investigated whether clutch size and egg size increases with increasing female body size within each population. We also tested whether or not clutch size and egg size increased with increasing altitude. Finally, we tested whether an evolutionary trade-off between clutch size and egg size occurs both within and among populations.

#### Materials and methods

We collected 54 female spot-legged treefrogs from three populations in Guizhou province, Western China. The Fanjin population is located in the farmlands surrounded by several farmhouses with cultivated rice. The Shangzhong population is located in a farmland with abundant permanent ponds. The Leigong population is located close to a farmhouse, which has two natural temporary ponds. At the study sites, annual mean temperatures increase with increasing altitude across populations (Table 1).

Our fieldwork began in mid-April and last late-July during the breeding seasons between 2014 and 2015. Within each population, we caught paired frogs from natural ponds by hand at night, and took them to the lab. We then kept each pair in a separate tank (40 × 50 × 60 cm) filled with pond water until oviposition was completed. Body size (snout-vent lengths; SVL) of each female was measured to the nearest 0.1 mm using a vernier

caliper. We collected 53 clutches and counted the total number of eggs in each clutch. We placed approximately 100 randomly selected eggs on a glass plate to take a digital image. We then used a Motic Images 3.1 digital camera mounted on a Moticam 2006 light microscope to take digitalized photographs of egg diameter at 400× magnification. We avoided damaging any individuals or eggs during the experiment. We then released all eggs to the spawning habitats.

We estimated the correlations between body size and both egg size and clutch size within each population using Spearman's correlation analysis. We used partial correlation analysis to test the correlation between egg size and clutch size within each population with female size as controlled factor. We used a one-way ANOVA to test differences in SVL, egg size and clutch size across populations. We used the general linear models (GLMs) treating population as a fixed factor, and SVL as a covariate to test the egg size and clutch size variation across altitudes. All values are presented as mean ± SD and all analyses were performed using SPSS 22.0 (Statistical Product and Service Solutions Company, Chicago, USA).

#### Results

Female size was not correlated with the egg size in both the Fanjin and Shangzhong populations (Table 2), whereas there was a significant and negative correlation between female size and egg size in Leigong ( $r_s = -0.488$ ,  $P = 0.040$ ). We did not find any correlations between female size and clutch size for all populations (Table 2). Within each population, there was non-significant relationship between clutch size and egg size when removing female size effect (Table 3).

Average body size did not differ among populations (one-way ANOVA:  $F_{2,51} = 2.316$ ,  $P = 0.109$ ). However, LSD indicated that there were significant differences in female sizes between Fanjin and Shangzhong ( $P = 0.044$ ). We did not find any significant differences in female size between the Shangzhong and Leigong populations ( $P = 0.572$ ) as well as the Leigong and Fanjin populations ( $P = 0.352$ ). Mean clutch size differed significantly

**Table 1.** The altitude, temperature, locations, SVL, egg size and clutch size in *Polypedates megacephalus* populations.

Population	n	Altitude (m)	Mean annual temperature (°C)	Coordinates	SVL	Egg size (mm)	Clutch size
Fanjin	19	449	17.40	27°46.33'N, 108°44.67'E	59.70±0.60	2.19±0.04	192.37±5.00
Shangzhong	17	680	16.00	26°23.65'N, 108°43.64'E	56.80±1.10	2.19±0.03	192.24±4.40
Leigong	18	1300	9.00	26°22.73'N, 108°10.27'E	57.60±1.20	2.27±0.03	286.39±6.12
<i>F</i>					2.32	1.77	106.90
<i>P</i>					0.09	0.18	<0.001

Note: *F* = test value of one-way analysis of variance for differences in population. *n* = sample size.

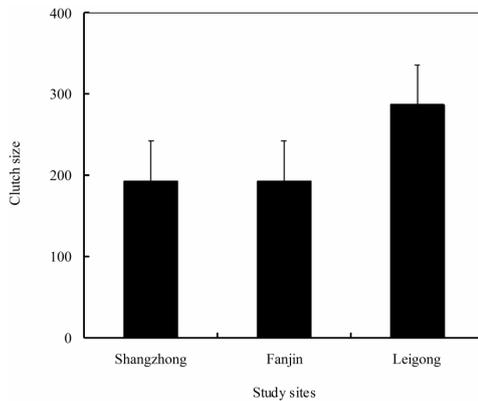
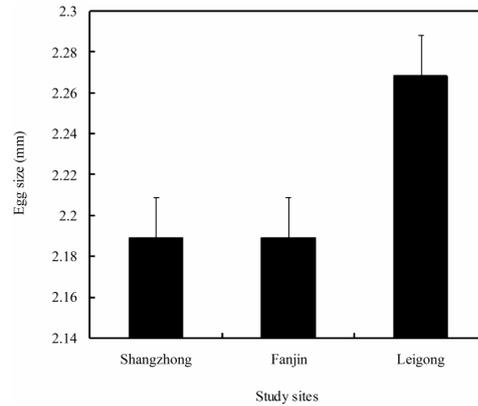
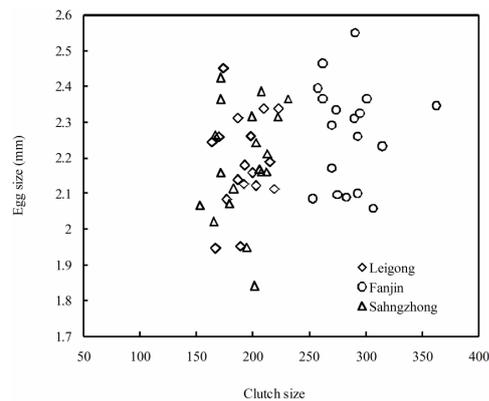
**Table 2.** The correlations between female size, clutch size and egg size in *Polypedates megacephalus* within each population.

Population	Fanjin			Shangzhong			Leigong		
	$r_s$	$P$	$n$	$r_s$	$P$	$n$	$r_s$	$P$	$n$
Female size									
Egg size	-0.39	0.10	19	0.19	0.46	17	-0.49	0.04	18
Clutch size	-0.26	0.28	19	-0.03	0.91	17	-0.33	0.19	18
Egg size									
Clutch size	0.31	0.20	19	0.12	0.65	17	0.12	0.67	18

**Table 3.** The correlation between the egg size and clutch size after controlling female size within each *Polypedates megacephalus* population.

Variables		Fanjin	Shangzhong	Leigong
		Egg size	Egg size	Egg size
Clutch size	$r$	0.15	0.17	-0.08
	$p$	0.27	0.27	0.38
	$df$	16	14	15

among the populations (one-way ANOVA:  $F_{2, 53} = 106.900$ ,  $P < 0.001$ ). When removing the effect of SVL, there was also significant difference in clutch size (GLMs:  $F_{2, 51} = 71.899$ ,  $P < 0.001$ ). LSD indicated a significant difference in clutch size between the Leigong and Fanjin populations ( $P < 0.001$ ) and between the Leigong and Shangzhong populations ( $P < 0.001$ ; Fig. 1). In addition, egg size did not differ significantly among the populations (one-way ANOVA:  $F_{2, 51} = 1.786$ ,  $P = 0.178$ ). When removing the SVL effect, egg size also did not differ among populations (GLMs:  $F_{2, 53} = 1.753$ ,  $P = 0.168$ ). However, LSD suggested that egg size from Leigong population was significantly larger than the Fanjin and Shangzhong populations (Fig. 2).

**Figure 1.** The variation in mean clutch size in *Polypedates megacephalus* across populations.**Figure 2.** The variation in mean egg size in *Polypedates megacephalus* across populations.**Figure 3.** The correlations between egg size and clutch size within each *Polypedates megacephalus* population.

## Discussion

Our results uncovered non-significant negative correlations between female body size and egg size in the spot-legged treefrog (*P. megacephalus*) apart from the Leigong population. Furthermore, clutch size and egg size was highest in the Leigong Mountain and was lowest in the Fanjin Mountain

(Figs 1, 2). Inconsistent with predication of life-history theory, we did not observe an evolutionary trade-off between clutch size and egg size both within and among populations.

There is some evidence of a positive relationship in anurans between maternal size and investment in offspring (Roff 1992). For *P. megacephalus*, there was no positive correlation between egg size and body size. Liao et al. (2014) found that egg size decreases with increasing female body size. Hence, further studies need to solve the exact mechanisms causing these results. Larger females produce larger clutch size due to higher total capacities in reproduction, as confirmed for other anurans (reviewed in Duellman & Trueb 1986, Roff 2002, Liao et al. 2014, Liao et al. 2015); however, clutch size did not increase with increasing female body size in *P. megacephalus*. The reasons for this non-significant relationship between female size and clutch size need to be explained by studying more samplings and populations.

The life-history hypothesis predicts that the decreasing breeding site temperature and shorter breeding seasons select larger eggs in high altitudes, thus exhibiting a positive relationship between egg size and altitude (Johnston & Leggett 2002). In this study, we found that egg size increased with increasing altitude in *P. megacephalus*. This finding is in accordance with previous studies among populations with wide geographical ranges, supporting the idea that larger eggs in colder localities improve egg survival (Berven & Gill 1983, Morrison & Hero 2003, Liao et al. 2014). Moreover, a larger amount of yolk and nutrients in larger eggs can overcome the energy requirements posed by physiological stress or limited resources in cold environments (Komoroski et al. 1998).

Small clutch size and large eggs at high altitudes suggests that investment in larger eggs come at a cost of the egg numbers (Stearns 1992). In contrast to basic life-history theory, in our study clutch size was highest at highest altitudes. It has been suggested that male investment in sperm production might be related to the variation in clutch size; namely, greater clutch size has been predicted to select for larger testes mass in males due to the higher demand for sperm (Emerson 1997). It is possible that the observed variation in clutch size among populations is a result of colder environments in high-altitude habitats, which had higher sperm demand and larger testes mass in

males (Chen et al. 2016).

Before accounting for female body size, egg number-size relationships in anurans may be negative (Lüddecke 2002, Liao & Lu 2011, Liao et al. 2014) or unrelated to each other within populations (Tejedo 1992). When controlling the effect of body size, there is a negative relationship in frogs (reviewed in Duellman & Trueb 1986, Liao et al. 2014). For three *P. megacephalus* populations, we did not observe the egg number-size trade-off for a given female size. Nonetheless, inconsistent with the prediction that stronger trade-offs between egg number and egg size should occur in stressful environments (Olsen & Vøllestad 2003), our data indicated that clutch size tended to be positively correlated with egg size in highest altitude.

In most anurans, higher altitude individuals experiencing lower temperatures and shorter growth periods favor smaller clutches and larger eggs (Berven 1982, Lüddecke 2002, Morrison & Hero 2003, Liao & Lu 2012, Liao et al. 2014). The interpopulation trade-off at the high altitude could be a result of adaptation of larger offspring and low survivorship to harsh highland environments, as has been shown by theoretical (Smith & Fretwell 1974, Parker & Begon 1986) and empirical studies (Badayev et al. 2001, Räsänen et al. 2005, Liao et al. 2014). In this study, we did not find any inter-population trade-off between clutch and egg size and moreover, the advantages of larger eggs and small clutch size at high altitudes in anurans have been demonstrated in many other characteristics (development rate, Merilä et al. 2000, size at metamorphose, Kaplan & King 1997, juveniles survival, Altwegg & Reyer 2003). For *P. megacephalus*, the survival advantage associated with larger eggs produced by females at high altitude is, consistent with life-history theory (Roff 2002).

In conclusion, our analyses of life-history strategies for three *P. megacephalus* populations showed that female size did not contribute to reproductive traits, suggesting that selection did not favor larger clutches and egg size in larger females. Individuals from high altitudes had larger egg sizes to compensate for the increased mortality rates of their offspring. We did not find evidence for any inter- or intra- population trade-off between clutch size and egg size due to limited resources. More data need collect to study the relationship between clutch size and egg size in future.

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## References

- Altwegg, R., Reyer, H.U. (2003): Patterns of natural selection on size at metamorphosis in water frogs. *Evolution* 57: 872-882.
- Badayev A.V., Ghalambor, C.K. (2001) Evolution of life histories along elevational gradients: Trade-off between parental care and fecundity. *Ecology* 82: 2948-2960
- Berven, K.A., Gill, D.E. (1983): Interpreting geographic variation in life-history traits. *American Zoologist* 23: 85-97.
- Berven, K.A. (1990): Factors affecting population fluctuations in larval and adult stages of the wood frog (*R. sylvatica*). *Ecology* 71: 1599-1608.
- 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.
- Chen, C., Huang Y.Y., Liao, W.B. (2016): A comparison of testes size and sperm length in *Polypedates megacephalus* between two populations at different altitudes. *Herpetological Journal* 26:249-252.
- Duellman, W.E., Trueb, D.L. (1986): *Biology of amphibians*. McGraw-Hill Inc, New York.
- Emerson, L.A. (1997): Sexually transmitted disease control in the armed forces, past and present. *Military Medicine* 162: 87-91.
- Fei, L., Ye, C.Y. (2001): *The colour handbook of amphibians of Sichuan*. China Forestry Publishing House, Beijing.
- Howard, J.H., Wallace, R.L. (1985): Life history characteristics of populations of the long-toed salamander (*Ambystoma macrodactylum*) from different altitudes. *American Midland Naturalist* 113: 361-373.
- Jin, L., Yang, S.N., Liao, W.B., Lüpold, S. (2016): Geographical variation in male reproductive investments: Altitude is positively with testis mass and sperm length in *Fejervarya limnocharis*. *Behavioral Ecology and Sociobiology* 70: 1197-1208.
- Johnston, T.A., Leggett, W.C. (2002): Maternal and environmental gradients in the egg size of an iteroparous fish. *Ecology* 83: 1777-1791.
- 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.
- Komorowski, M.J., Nagle, R.D., Congdon, J.D. (1998): Relationships of lipids to ovum size in amphibians. *Physiological & Biochemical Zoology* 71: 633-641.
- Li, S.T., Wu, X., Li, D.Y., Lou, S.L., Mi, Z.P., Liao, W.B. (2013): Body size variation of Odorous Frog (*Odorrana grahami*) across altitudinal gradients. *Herpetological Journal* 23: 187-192
- Liao, W.B., Chen, W. (2012): Inverse Rensch-rule in a frog with female-biased sexual size dimorphism. *Naturwissenschaften* 99: 427-431.
- Liao, W.B., Lu, X. (2012): Adult body size =  $f(\text{initial size} + \text{growth rate} \times \text{age})$ : explaining the proximate cause of Bergman's cline in a toad along altitudinal gradients. *Evolutionary Ecology* 26: 579-590.
- Liao, W.B., Lu, X. (2010a): Age structure and body size of the Chuanxi tree frog *Hyla annectans chuanxiensis* from two different elevations in Sichuan (China). *Zoologischer Anzeiger* 248: 255-263.
- Liao, W.B., Lu, X. (2010b): Age and growth of a subtropical high-altitude torrent frog, *Amolops mantzorum*, in Western China. *Journal of Herpetology* 44: 172-176.
- Liao, W.B., Liu, W.C., Merilä, J. (2015): Andrew meets Rensch: Sexual size dimorphism and the inverse of Rensch's rule in Andrew's toad (*Bufo andrewsi*). *Oecologia* 177: 389-399.
- Liao, W.B., Lu, X., Jehle, R. (2014): Altitudinal variation in reproductive investment and trade-off between egg size and clutch size in the Andrew's Toad (*Bufo andrewsi*). *Journal of Zoology* 293: 84-91.
- Liao, W.B., Lu, X., Shen, Y.W., Hu, J.C. (2011): Age structure and body size of two populations of the rice frog *Rana limnocharis* from different altitudes. *Italian Journal of Zoology* 78: 215-221.
- Liao, W.B., Wu, Q.G., Barrett, K. (2012): Evolution of sexual dimorphism in the forelimb muscles of Andrew's toad (*Bufo andrewsi*) in response to putative sexual selection. *Animal Biology* 62: 83-93.
- Liao, W.B., Luo, Y., Lou, S.L., Jehle R. (2016a): Geographic variation in life-history traits: growth season affects age structure, egg size and clutch size in Andrew's toad (*Bufo andrewsi*). *Frontiers in Zoology* 13: art.6.
- Liao, W.B., Lou, S.L., Zeng, Y., Kotschal, A. (2016b): Large brains, small guts: The expensive tissue hypothesis supported in anurans. *American Naturalist* 188: 693-700.
- Liao, W.B., Zhong, J.M., Cheng, C., Jin, L., Wu, Z.J. (2017): No evidence of phenotypic selection on large females leading to female-biased sexual size dimorphism in the frog *Polypedates megacephalus*. *Salamandra* 53: 231-236.
- Lüddecke, H. (2002): Variation and trade-off in reproductive output of the Andean frog *H. labialis*. *Oecologia* 130: 403-410.
- Mai, C.L., Liao, J., Zhao, L., Liu, S.M., Liao, W.B. (2017): Brain size evolution in the frog *Fejervarya limnocharis* does neither support the cognitive buffer nor the expensive brain framework hypothesis. *Journal of Zoology* 302: 63-72.
- Merilä, J., Laurila, A., Pakkala, M., Räsänen, K., Laugen, A.T. (2000): Adaptive phenotypic plasticity in timing of metamorphosis in the common frog *R. temporaria*. *Ecoscience* 7: 18-24.
- Morrison, C., Hero, J.M. (2003): Geographic variation in life-history characteristics of amphibians: a review. *Journal of Animal Ecology* 72: 270-279.
- Olsen, E.M., Vøllestad, L.A. (2003): Microgeographical variation in brown trout reproductive traits: possible effects of biotic interactions. *Oikos* 100: 483-492.
- Parker, G.A., Begon, M. (1986): Optimal egg size and clutch size: effects of environment and maternal phenotype. *American Naturalist* 128: 573-592.
- Räsänen, K., Laurila, A., Merilä, J. (2005): Maternal investment in egg size: environment- and population-specific effects on offspring performance. *Oecologia* 142: 546-553.
- Räsänen, K., Söderman, F., Laurila, A., Merilä, J. (2008): Geographic variation in maternal investment: acidity affects egg size and fecundity in *Rana arvalis*. *Ecology* 89: 2553-2562.
- Roff, D.A. (2002): *Life-history evolution*. Sinauer Associates, Sunderland.
- Roff, D.A. (1992): *The evolution of life histories: theory and analysis*. Chapman and Hall, New York.
- Smith, C.C., Fretwell, S.D. (1974): The optimal balance between size and number of offspring. *American Naturalist* 108: 499-506.
- Stearns, S.C. (1992): *The evolution of life histories*, Oxford University Press, Oxford.
- Tejedo, M. (1992): Absence of the trade-off between the size and number of offspring in the natterjack toad (*B. calamita*). *Oecologia* 90: 294-296.