



Short Communication

Sexual Dimorphism of Internal Organ Mass in *Bufo gargarizans gargarizans* from Summer Population

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ABSTRACT

In this study, we investigated potential differences in internal organ mass between male and female Asiatic toads (*Bufo gargarizans gargarizans*), collected in central China during the summer 2015. Females differed significantly in the stomach, small intestine, large intestine, lung, and gonads compared to males; however, liver and fat bodies did not differ between the sexes. The sex difference of these organs may contribute to different energy requirements for reproduction between males and females. Our findings suggest that female toads invest considerable resources into gamete production compared to males, and greater internal organs in females are a consequences of high energy requirements for reproduction, which favors females to produce more high-energy eggs.

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Authors' Contributions

JG and JX designed the study.

Samples were collected and experiments were performed by JG, BD, ZL and MH. JG and BD analyzed the data. All others participated in writing manuscript.

Key words

Asiatic toad, *Bufo gargarizans gargarizans*, Internal organ, Sexual difference, Digestive system.

Sexual dimorphism is a widespread phenomenon throughout the animal kingdom (Andersson and Iwasa, 1996; Fairbairn *et al.*, 1997; Wells, 2007). An extensive examination of sexual dimorphism in amphibians has been carried out, and a variety of dimorphism characteristics have been detected, such as body size, body shape, vocal apparatus, head dimensions, coloration, reproductive behavior, and muscle mass (*e.g.*, Duellman and Trueb, 1994; Wojtaszek *et al.*, 1997; Kupfer, 2007; Wells, 2007; Mi, 2013). Selective forces favor the evolution of these sexual characteristics to increase reproductive success (Finkler *et al.*, 2014) and decrease resource competition (Serra-Cobo *et al.*, 2000). Internal organs are tightly related to the energy metabolism. Studies on the sex difference of internal organ mass can not only help to understand the dimorphism of physiological function, but also explain the evolution of internal organs. Until now, most studies have focused on seasonal variation of storage and expenditure organs (*e.g.*, Lu *et al.*, 2008; Naya *et al.*, 2010; Jönsson *et al.*, 2009; Chen *et al.*, 2013, 2015), while few studies focused on the sexual dimorphism of internal organ mass (Jönsson *et al.*, 2009; Finkler, 2013; Finkler *et al.*, 2014; Jin *et al.*, 2014).

The Asiatic toad *Bufo gargarizans gargarizans* is widely distributed throughout China, Russia, Japan, and Korea,

inhabiting a variety of habitats at elevations from sea level up to 1830 m (Fei *et al.*, 2006). The clutch in the form of two strings of eggs contains 1200-7400 eggs, thus this toad has been classified as an explosively breeding species (AmphibiaWeb, 2016). The breeding season is dependent on latitude and altitude, and reaches from January to May, predominantly during April (Fei *et al.*, 2006). Until now, the sexual difference of the internal organs mass in *B. gargarizans gargarizans* has not been reported. In this paper, we explored potential dimorphisms in internal organ mass of both male and female *B. gargarizans gargarizans*, collected from central China during the summer 2015. Since females invest more energy into gamete production than males during the whole process of reproduction (Bonnet *et al.*, 1998; Finkler, 2013), and since summer is the main season for supplying energy (Zhou and Sun, 1997), we hypothesized that females would have larger acquisition, distribution, storage, and expenditure organs than males.

Materials and methods

A total of 48 specimens (28 females and 20 males) were collected from the outskirts of Yiyang County (34°30'50.90" N, 112°10'15.77" E, 360 m above sea level), Henan Province, China in July, 2015. All individuals were killed via pithing and body weights were measured via an electronic balance to the nearest 0.01 g. The abdominal and thoracic cavities were opened, internal organs including heart, liver, lung, kidney, stomach, large intestine, small intestine, fat body, and gonad (tests and

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ovaries) of each individual were removed, then each internal organs were rolled on a blotting paper to remove excess fluid and subsequently weighed to the nearest 0.001 g using an electronic balance (wet weight).

Body mass was examined via t-tests between males and females. We regressed the organ mass on body mass per organ, and compared regression coefficients between sexes by regression analysis for homogeneity. To test for differences of internal organ mass between males and females, one-way analysis of covariance (ANCOVA) was utilized with internal organ mass as the dependent variable, sexes as the fixed factor, and body size as covariate. All variables were log-transformed to meet criteria of normality as well as homogeneity assumptions prior to analysis. All statistical tests were performed with SPSS software (Statistical Product and Service Solutions Company, Chicago, Version 22.0). Data are presented as means \pm SD, the statistical test was set to two-tailed, and the significance level was set at $p = 0.05$. All field and laboratory work was done under the license of the Wildlife Protection Law of China.

Results

The body mass ranged from 38.37 g to 99.25 g for females ($n = 28$, mean = 66.99 ± 16.23 mm), and from 35.01 g to 71.05 g for males ($n = 20$, mean = 56.04 ± 8.95 mm). On average, females were significantly larger than males in body mass ($t = 2.993$, $df = 43.640$, $p = 0.005$).

As shown in Table I, the mean mass of each internal organ in females was larger compared to that of males. The results of linear regression analysis revealed that the mass of each internal organ correlated significantly with body mass (even when males and females were analyzed separately, $p < 0.05$ for all case). Larger individuals of both sexes had significantly higher internal organ mass than small ones (Supplementary Fig. 1, Supplementary Table S1). Slopes were homogeneous ($p > 0.05$) for all comparisons between males and females.

The results of ANCOVA indicated that the lung, gonad, stomach, small intestine, and large intestine differed significantly between the sexes when the influence of body mass was controlled, and other internal organs showed no sexual differences (Table I). Females had larger values for lung, gonad, stomach, small intestine, and large intestine.

Discussion

As predicted, the sex difference is present in acquisition (stomach, small intestine, and large intestine), distribution (lung), and expenditure (gonad) organs; however, storage organs (liver and fat bodies) did not show sex differences. The different reproductive roles of males and females, which impose selection on different organ

systems, can cause this sex difference of organs (Bonnet *et al.*, 1998). For example, females invested more energy into gamete formation and reproductive tract proliferation than males (Finkler, 2013) resulting in enlarged alimentary tracts, fat bodies, and livers (Bonnet *et al.*, 1998). Thus, the sex difference of internal organs may be ascribed to the energy requirements for reproduction.

Table I.- The statistics of internal organ size (g) and the results of one-way ANCOVA between sexes in *Bufo gargarizans gargarizans*.

Organs	Females (n=28)	Males (n=20)	ANCOVA
	Mean (Range)	Mean (Range)	
Heart	0.36 \pm 0.01 (0.22-0.56)	0.32 \pm 0.01 (0.19-0.45)	$F_{1,45}=0.024$, $p=0.878$
Liver	3.73 \pm 0.26 (1.57-6.65)	2.74 \pm 0.18 (1.21-4.07)	$F_{1,45}=1.132$, $p=0.293$
Lung	0.99 \pm 0.05 (0.66-1.79)	0.79 \pm 0.03 (0.52-1.25)	$F_{1,45}=4.649$, $p=0.036$
Kidney	0.56 \pm 0.03 (0.34-0.81)	0.46 \pm 0.02 (0.28-0.66)	$F_{1,45}=1.798$, $p=0.187$
Fat bodies	0.93 \pm 0.12 (0.18-2.85)	0.67 \pm 0.09 (0.13-1.87)	$F_{1,45}=0.249$, $p=0.620$
Gonad	3.27 \pm 0.43 (0.47-9.65)	0.25 \pm 0.02 (0.13-0.51)	$F_{1,45}=246.687$, $p<0.001$
Stomach	2.01 \pm 0.07 (1.26-2.86)	1.52 \pm 0.05 (1.18-1.94)	$F_{1,45}=28.611$, $p<0.001$
Small intestine	1.45 \pm 0.07 (0.80-2.14)	1.10 \pm 0.05 (0.70-1.53)	$F_{1,45}=5.727$, $p=0.021$
Large intestine	0.64 \pm 0.03 (0.42-0.96)	0.52 \pm 0.02 (0.37-0.70)	$F_{1,45}=6.059$, $p=0.018$

Females were found to have larger values for stomach, small intestine, and large intestine than males in this study. Stomach, small intestine, and large intestine are important components of the digestive system, which represents a functional link between the intake of food and the energy and nutrients required to meet all vital functions, including survival, growth, and reproduction (Secor, 2001; Naya *et al.*, 2014). The digestive system of amphibians features obvious flexibility, which can be varied due to the changes of artificial or natural environmental conditions (Scheiner, 1993, 2002); thus it was regarded as one of the most responsive and sensitive systems react to environmental cues (Naya and Bozinovic, 2004). The sexual dimorphism of the digestive system has also been found in other species (Lou *et al.*, 2013). Sex differences in the digestive tract may result from different energy requirements of females and males (Pullianinen, 1976). Growth, maintenance, and reproduction are the main functions requiring energy in organisms (Duffitt and Finkler, 2011). Investments for reproduction are obviously different between females

and males. Females have a higher investment in gamete production, both in energetic contribution to ovarian follicle development as well as in the proliferation of the reproductive tract (Wells, 2007; Finkler, 2013). However, males have a higher investment in the reproductive action (such as calling, active seeking of females, and amplexus interference) (Gatz, 1981; Howard, 1988; Sullivan, 1992). Generally, females have greater overall energetic cost of reproduction compared to males (Finkler *et al.*, 2014). Thus, the sexual dimorphism of the digestive tract discovered in this study may contribute to the energy requirement of reproduction, which requires females to intake and absorb more energy. Furthermore, Jin *et al.* (2010) reported that female *B. gargarizans gargarizans* have higher values for the index of the food diversity compared to males. This may demonstrate that different food quality, causing the response of the sexual dimorphism of digestive tract (Moss, 1983; Lou *et al.*, 2013), which is present between sexes, also attributed to the sexual dimorphism of internal organs.

Among the distribution organs, only the mass of lungs showed sex difference, where females have large values. However, Jin *et al.* (2014) found that male *Pelophylax nigromaculata* (collected during summer, autumn, and spring) have large values of dry mass of lung than females; however, both sexes featured similar wet mass of lung. Lungs provide oxygen from inhaled air to the bloodstream and enable the exhalation of carbon dioxide. The sex difference of lungs found in this study may also be attributed as a consequence of energy requirements. As mentioned above, the reproductive investment of females is large than that of males; consequently, females need to acquire more food to provide more energy and nutrients for more eggs. As a result, hunting action, consumption, and transition of more energy and nutrients all require more oxygen. Furthermore, more carbon dioxide was produced due to the increase of hunting action, consumption, and transition; thus, had to be exhaled in time. Therefore, these reasons result in an enhancement of the female lung function.

Liver and fat bodies, the storage organs (Naya *et al.*, 2010), did not feature sex differences in this study. Both liver and fat bodies provide energy for reproduction or for survival during periods when feeding is constrained for one reason or another (Jönsson *et al.*, 2009). In the process of reproduction, fat bodies are major energy stores for the gonads (Fitzpatrick, 1976), and the liver processes fats and proteins required for egg production in females (Dahle *et al.*, 2003). However, the pattern of energy storage differs among population in different environments or individuals faced with different energetic expenditures (Lu *et al.*, 2008; Jönsson *et al.*, 2009). Males typically store more energy in the liver, fat bodies, and carcass tissues, while females store in the ovaries and oviducts. Significant

differences of storage organs between sexes are common in pre-breeding amphibians (e.g., Lu *et al.*, 2008; Jönsson *et al.*, 2009; Duffitt and Finkler, 2011; Finkler *et al.*, 2014; Chen *et al.*, 2015). The lack of sex difference found in liver and fat body mass is likely a result of the annual cycle of storage organs in this study. The season, at the time of our sampling, was post-breeding of *B. gargarizans gargarizans*. During this season, the stored energy has been consumed, and new energy has not been stored yet.

Not surprisingly, gonad mass was substantially larger in females compared to males. Significant difference in gonad mass is very common in amphibians (e.g., Finkler, 2013; Finkler *et al.*, 2014; Chen *et al.*, 2015). Sex difference in gonad contributes to the energy investment in gametogenesis and females invest more during gametogenesis than males (Finkler, 2013). Furthermore, females have already invested their energy into reproduction in the given year at the time of our sampling, while the main reproductive activities of the male occurred later in the season (Jönsson *et al.*, 2009). This was demonstrated by unmaturing eggs, which were found in the ovaries during dissection of females.

Conclusion

Significant differences in acquisition, distribution, and expenditure organs have been discovered between male and female *B. gargarizans gargarizans*; however, storage organs did not reveal sex differences. This sexual difference in internal organs indicated that females invest more energy during gametogenesis compared to males, which increase the energy requirements for reproduction. The findings of this study highlight physiological dimorphisms between male and female *B. gargarizans gargarizans*, related to fundamental differences in their energy acquisition, distribution, and expenditure.

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Supplementary material

There is supplementary material associated with this article. Access the material online at: <http://dx.doi.org/10.17582/journal.pjz/2017.49.4.sc12>

Statement of conflict of interest

Authors have declared no conflict of interest.

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