



## Altitudinal variation in organ size in *Polypedates megacephalus*

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Phenotypic flexibility of morphological characters is widespread in the animal kingdom. In the present paper, we investigated altitudinal variation in organ size (heart, lungs, livers, kidneys and digestive tract) in the spot-legged treefrog (*Polypedates megacephalus*) across three populations along an altitudinal gradient in southwestern China. Both heart and lung mass increased with altitude, and is possibly linked to a lower oxygen supply at high elevations; a higher liver mass suggested the increased need for energy stores. Despite significant differences between populations, we found no distinct cline in the length of the digestive tract according to altitude.

**Key words:** altitudinal variation, environmental gradients, organ size, *Polypedates megacephalus*

Phenotypic flexibility of morphological and physiological characters within species is a common phenomenon across animal taxa (Piersma et al., 1999; Hammond et al., 2001; Naya et al., 2009; Liao et al., 2015). In endotherms, the functional capacity and size of organs such as the lung and heart displays pronounced size variation along environmental gradients such as found across different altitudes (Hock, 1964; Hammond et al., 1999; Hammond et al., 2001; Naya et al., 2009). Storage of energy is an important factor influencing variation in life-history traits, and theory predicts that organisms allocate available energy between reproduction and survival (Bonnet et al., 1998; Liao et al., 2014; Liao et al., 2016a). The function of energy stores is often to allow organisms to reproduce, or to survive over periods when available food is constrained (Jonsson, 1997; Jin et al., 2016a; Liao et al., 2016a). For anurans, highly variable environmental constraints affect the needs and benefits of energy storage. For example, longer hibernation periods require more accumulated energy to survive during winter, and individuals at high altitudes and latitudes may be characterised by larger residual energy stores at post-hibernation emergence (Liao & Lu, 2010).

In amphibians, main energy sources are stored in tissues such as livers and fat bodies (Fitzpatrick, 1976).

Anurans often display phenotypic plasticity by changing organ size and function to meet energy requirement in changing environment (Naya et al., 2009; Lou et al., 2013). For example, common frogs (*Rana temporaria*) from northern latitudes have larger fat bodies and livers than their southern conspecifics (Jonsson et al., 2009). Likewise, the digestive tract length of Andean toads (*Bufo spinulosus*) in northern Chile declines with increasing altitude (Naya et al., 2009), whereas the Yunnan frog (*Pelophylax pleuraden*) exhibits a converse cline (Lou et al., 2013).

The spot-legged treefrog (*Polypedates megacephalus*) is widely distributed in the Qinling Mountains and Huaihe in southern China, at elevations ranging from 80 m to 1600 m (Fei et al., 2005; Chen et al., 2016). Matings frequently involve a single female and multiple males, and egg laying extends from early April to mid-August (Chang, 1989; Wu et al., 2010). However, while the natural history and population biology of *P. megacephalus* is relatively well known (Xu et al., 2005; Zhang et al., 2005; Xu et al., 2008; Wu et al., 2010; Jin et al., 2016b), no information on variation in organ size (i.e. heart, lungs, livers, kidneys and digestive tract) across geographical populations is available. Here, we explored differences in organ size in *P. megacephalus* across three populations along an altitudinal gradient. We also tested the predication that populations living in high altitude have larger relative organ sizes compared to their counterparts at low altitudes.

A total of 45 male treefrogs were collected from three populations after hibernation from April to June in 2014. The three populations were located at different altitudes in Shangzhong, Kaili (26°23'N, 108°43'E, 680m a.s.l.), Leigong Mountain (26°22'N, 108°10'E, 1300m a.s.l.) and Fanjing Mountain (27°46'N, 108°44'E, 449m a.s.l.) in the Guizhou province (see Fig. 1). Individuals were confirmed as adults by the presence of nuptial thumb pads in males, and an inflated abdomen in females. Before processing, the frogs were kept in rectangular tanks in the laboratory (1.0×0.5×0.4 m; L×W×H) with a water depth of 5 cm at room temperature. All individuals were killed using single-pithing (Liao et al., 2016b). Body size (snout-vent length: SVL) of each individual was measured to the

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**Table 1.** Descriptive information about the study sites, mean body length and organ mass of tree frog (*Polypedates megacephalus*). Values are given as means±SD.

Variables	Fanjingshan	Shangchong	Leigongshan
Altitude (m)	449	680	1300
SVL (mm)	44.2±2.9	43.8±2.8	43.6±3.1
Sample size	16	15	14
Digestive tract length (mm)	4.6±0.8	5.5±0.9	5.1±0.6
Heart wet weight (mg)	17.9±6.7	20.4 ±7.4	18.5±5.6
Heart dry weight (mg)	2.5±0.8	3.1±0.7	3.5±1.0
Lung wet weight (mg)	22.1±6.8	32.1±8.6	29.1±28.9
Lung dry weight (mg)	3.4±1.1	4.4±1.2	4.5±1.2
Liver wet weight (mg)	63.7±17.9	69.9±18.4	70.7±19.7
Liver dry weight (mg)	15.7±3.1	16.1±3.2	22.5±7.4
Kidney wet weight (mg)	29.4±10.9	38.4±9.0	36.1±12.5
Kidney dry weight (mg)	5.3±1.3	6.2±1.2	7.2±1.8
Spleen wet weight (mg)	1.4±0.6	1.9±0.9	2.0±0.8

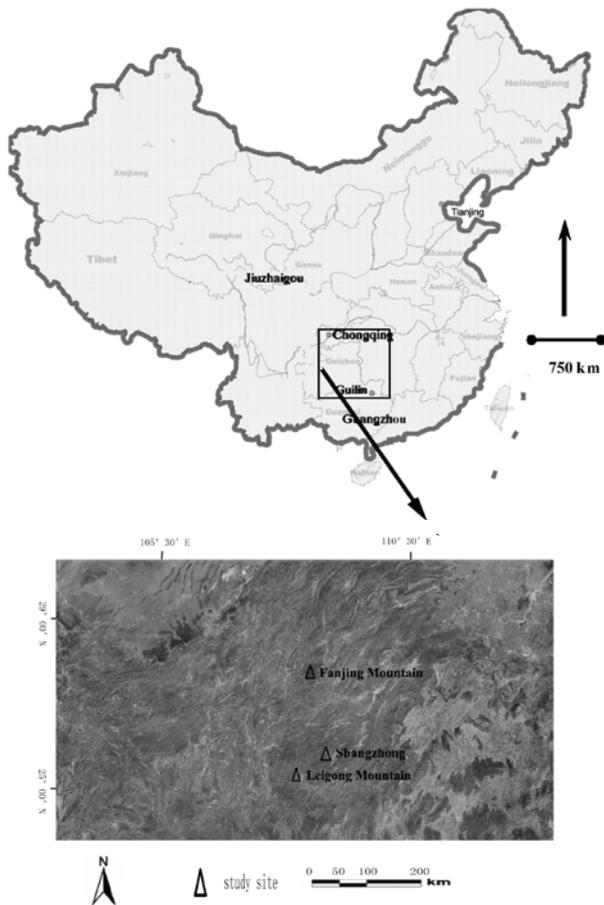
nearest 0.1 mm using a vernier caliper. Frogs had their testes removed for a related study, and were weighed to the nearest 0.1 mg using an electronic balance.

After five months of preservation in 4% buffered formalin in a phosphate buffer for tissue fixation, the heart, lungs, livers, kidneys and the digestive tract were

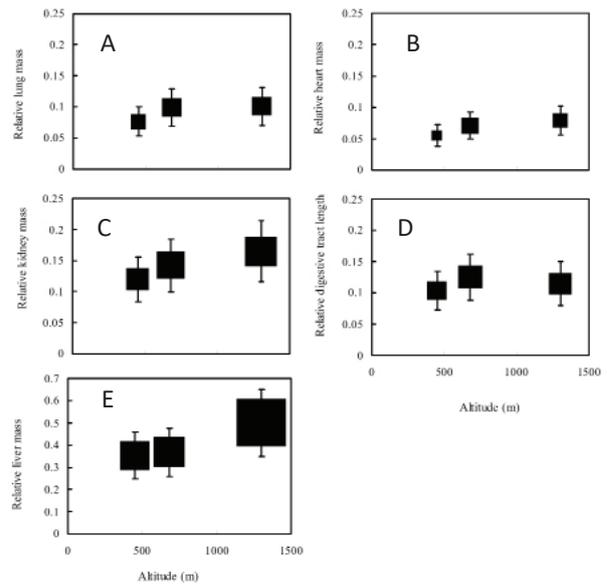
dissected out. All organs except the digestive tract were placed in a thermostat drier (60°C) for 48 hours, and weighed (dry mass) with an electronic balance to the nearest 0.1mg. We used a vernier caliper to measure the length of digestive tract to the nearest 0.1 mm.

Data analysis was conducted using SPSS v. 20.0 (Statistical Product and Service Solutions Company, Chicago). Differences in body size among populations were investigated using one-way ANOVA. We used a general linear models (GLMs) with organs as dependent variables, altitude as a fixed factor, and SVL as covariate to test for differences in organs among populations. Post-hoc SLD comparisons were used to test for difference in organs between pairs of populations.

The raw data are presented in Table 1. We found



**Fig. 1.** Topographic map showing the locations of the three *Polypedates megacephalus* populations in southwestern China



**Fig. 2.** Altitudinal variations in relative mass of organ (A: lung; B: heart; C: kidney; D: digestive tract; E: liver) in *Polypedates megacephalus*. Relative mass of organ is calculated from organ mass (mg) / SVL (mm).

**Table 2.** The influence of population and altitude on variation in heart, lung, liver, kidney and digestive tract length across three populations of tree frog (*Polypedates megacephalus*) when correcting for SVL using GLMs.

Variables	Factor	F	p
Heart dry weight	Altitude	13.431	<0.001
	SVL	39.117	<0.001
Lung dry weight	Altitude	8.700	0.001
	SVL	28.496	<0.001
Liver dry weight	Altitude	12.455	<0.001
	SVL	31.722	<0.001
Kidney dry weight	Altitude	7.455	0.002
	SVL	12.651	0.001
Digestive tract length	Altitude	5.814	0.006
	SVL	7.723	0.008

no significant differences in body size across the three populations (one-way ANOVA:  $F_{2,42}=0.152$ ,  $p=0.851$ ). When controlling for the effect of SVL, the relative mass of heart, lungs, livers, kidneys and the relative length of the digestive tract differed significantly among populations (Table 2; Figs. 2-6). *Post-hoc* comparisons revealed that frogs at high altitudes had larger hearts and livers than those at mid and low altitudes (heart: both  $p<0.021$ ; livers:  $p<0.006$ ), while the difference was not significant between mid and low altitudes (heart:  $p=0.390$ ; liver:  $p=0.774$ ). *Post-hoc* comparisons revealed that frogs at high and mid altitudes had significantly larger lungs than those at low altitudes (both  $p<0.018$ ), while there was no difference between high and mid altitude ( $p=0.915$ ). *Post-hoc* comparisons revealed significantly larger kidneys at high altitude than at low altitude ( $p=0.003$ ), but no differences between high and mid altitudes ( $p=0.244$ ), and between mid and low altitude ( $p=0.058$ ). Frogs at mid altitude had longer digestive tracts than those at high and low altitudes ( $p=0.005$ ). There were no differences in digestive tract length between high and low altitude ( $p=0.113$ ), and between high and mid altitude ( $p=0.201$ ).

The results demonstrate that *P. megacephalus* exhibits striking variation in organ size (mass of heart, lungs, livers, kidneys and length of digestive tract) across populations along an altitudinal gradient, and that the relative mass of heart, lungs, livers and kidneys display an increase with altitude. The higher mass of hearts and lungs in high altitude may be a result of lower oxygen partial pressures, and the larger livers at high altitude may result from the higher energy storage demands during hibernation and at post-hibernation emergence. However, in contrast to our prediction of larger digestive organs at high altitude, our results show that individuals at mid altitude had the longest digestive tracts.

To adapt for the decreased oxygen supply at high altitude, animals may experience limitations to aerobic activities such as exercise and heat production (Lenfant, 1973; Chappell et al., 1988). Previous studies demonstrated that for example deer mice at high altitudes had larger hearts and lungs than at low altitude (Hock, 1964; Hammond et al., 1999). In our study, the mass of hearts and lungs increased with altitude, suggesting a possible

link to lower partial pressure of oxygen at high elevations. However it should be kept in mind that the on average lower temperatures at higher altitudes also result in an overall lower metabolism in ectotherms such as anurans.

Individuals living at high altitudes generally have increased energy demands and intakes (Chappell et al., 1988). We found for *P. megacephalus* that livers mass increased towards high-altitude populations. For members of the genus *Rana*, liver weight and liver glycogen contents increases towards higher altitudes and latitudes (Pasanen & Koskela, 1974; Jonsson et al., 2009; Chen et al., 2011). Testes mass in *P. megacephalus* from the high-altitude population is larger than at low altitude (Chen et al., 2016), and larger energy stores may be linked to more uncertain environmental conditions (McNamara & Houston, 1990; Jonsson, 1997). However, in contrast to the finding that kidney size decreases with altitude due to the lower oxygen supply (Tucker & Horvath, 1973), we found for *P. megacephalus* that kidney size increases with altitude.

Both endotherms and ectotherms are able to adjust their digestive traits in response to environmental changes (Hock, 1964; Hammond et al., 1999; Hammond et al., 2001; Sassi et al., 2007; Naya et al., 2009). Lou et al., (2013) found that the relative length of the digestive tract increases with altitude in *Pelophylax pleuraden*, whereas it decreases with altitude in *Bufo spinulosus* (Naya et al., 2009). Contrary to our observations, we predicted an increase in digestive organ mass for *P. megacephalus* at high altitude attributable to lower ambient temperatures, and possibly a shift in diet towards less easily digestible food (as observed in Sassi et al., 2007). For *B. spinulosus*, in turn, an increased proportion of animal-based food was attributed to reduced gut lengths at high altitude (Naya et al., 2009), which could also be the case for *P. megacephalus*.

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