

Age, growth and body size  
of *Bufo minshanicus* STEJNEGER, 1926,  
a toad native to the Tibetan Plateau  
(Anura: Bufonidae)

Alter, Wachstum und Körpergröße von *Bufo minshanicus* STEJNEGER, 1926,  
einer Kröte des Hochlandes von Tibet  
(Anura: Bufonidae)

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KURZFASSUNG

Nach der Bergmannschen Regel erreichen endotherme Tierarten in kalten Klimaten vielfach eine bedeutendere Körpergröße als in warmen. Einige Arten von Ektothermen darunter auch von Amphibien folgen dieser Regel, deren Allgemeingültigkeit jedoch bezweifelt werden kann.

Die vorliegende Untersuchung vergleicht Individuen dreier Populationen von *Bufo minshanicus* STEJNEGER, 1926 aus unterschiedlichen Höhenlagen entlang des Huangshui-Flusses im östlichen Hochland von Tibet (Volksrepublik China). Dabei war die mittlere Körpergröße der Tiere aus großer Höhe kleiner als bei solchen aus geringerer Höhe, was der Bergmannschen Regel widerspricht. Die Beobachtungen deuten darauf hin, daß es wachstumsverlangsamende Faktoren sind, welche die Körpergröße in hochgelegenen Populationen am stärksten beeinflussen. In allen drei Populationen waren die Weibchen signifikant größer als die Männchen. Es liegt nahe anzunehmen, daß sowohl das Alter als auch die Wachstumsrate zum geschlechtsabhängigen Größenunterschied beitragen, da sowohl die jährliche Wachstumsrate als auch das Alter bei den Weibchen höher war als bei den Männchen.

ABSTRACT

According to Bergmann's rule, endothermic species tend to grow larger in cold than warm climates. There are also ectotherms including some amphibian species, that conform to this rule, the validity of which is however disputed as to whether it is generally applicable to all anuran species.

Three populations of *Bufo minshanicus* STEJNEGER, 1926, from along the Huangshui River in the eastern Tibetan Plateau (People's Republic of China) showed that toads from high altitudes were on the average smaller in body size than toads from low altitudes, which is not in agreement with Bergmann's rule. The observations suggest growth-retarding factors to be the major contributor to the smaller body size in the high-altitude populations. The females were significantly larger than males across three sites at different altitudes suggesting that both age and growth rate contribute to the sexual difference in body size because annual growth rates as well as age were higher in females than males.

KEY WORDS

Amphibia: Anura: Bufonidae, *Bufo minshanicus*, Bergmann's rule, growth rate, high-altitude population, Tibetan Plateau, People's Republic of China

INTRODUCTION

Bergmann's rule has been found applicable to many endothermic vertebrates (BERGMANN 1847; ASHTON 2002a; BLACKBURN & HAWKINS 2004) and the mechanism underlying may be understood regarding to the heat conservation benefit enjoyed by larger individuals in colder climates (WALTERS & HASSALL 2006). Later, several researchers extended this rule to some ecto-

therms (reviewed by MORRISON & HERO 2003). However, whether and why they follow the rule remains ambiguous and contentious (BELK & HOUSTON 2002; ASHTON 2002b; ASHTON & FELDMAN 2003; ADAMS & CHURCH 2008; FENG et al. 2015).

So far, efforts have largely focused on exploring, in both ultimate and proximate contexts, why and how these organisms fol-

low Bergmann's rule while others reverse it (ANGILLETTA et al. 2004; WALTERS & HASSALL 2006; MA et al. 2009). In those species which follow Bergmann's rule, this is thought to be related to their short activity period available for growth, which delays the time at which sexual maturity is reached (MORRISON & HERO 2003; ANGILLETTA et al. 2004). Animals from colder environmental conditions therefore reach larger body size and older age at sexual maturity (MONNET & CHERRY 2002). By contrast, the recent explanations for arthropods following the converse Bergmann's rule assume that the short growth season at higher (colder) latitudes or elevations limits the time available for individuals to grow larger (MOUSSEAU 1997; BLANCKENHORN & DEMONT 2004; SCHUTZE & CLARKE 2008). Therefore, research on the body size in relation to age and growth rate for more amphibian species will help to better understand the proximate causes of the geographic variation in body size among the taxa.

This study examined whether there is an altitudinal variation in body size of Minshan's Toad, *Bufo minshanicus* STEJNEGER, 1926, a species endemic to the eastern Tibetan Plateau (FEI & YE 2001, 2004). It inhabits forests, fields and open alpine marshes from 1,700 to 3,700 m elevation, and is among the highest-living amphibians globally (FEI & YE 2001, 2004). The timing of breeding is later in higher altitudes, which provided the opportunity to sequentially investigate the altitudinal pattern of breeding adults' body size. Although some researchers reported habitat selection and daily activity rhythm of *B. minshanicus* (DAI et al. 2005; ZHANG et al. 2007), little is known about its body size variation with altitude and associated life-history traits. In this paper, the authors test the prevalence of the altitudinal pattern of demographic variables in *B. minshanicus* using skeletochronological methods to better understand the evolution of body size in correlation with environmental conditions among certain amphibians.

## MATERIALS AND METHODS

Data was collected from three localities at different altitudes along the Huangshui River in the eastern Tibetan Plateau (Table 1). Annual mean air temperature and precipitation decreases with increasing altitude (Table 1). The beginning of the spawning period of *B. minshanicus* tends to be the later the higher the altitude is (2,999 m: mid-April; 2,588 m: late March; 2,434 m: late March). Toads were caught by hand in spawning ponds when they emerged from hibernation in spring 2008 and 2015.

For each specimen age was estimated from its size, sex was determined from external features and body length was measured (from snout to vent, SVL, to the nearest 0.1 mm) with a caliper. Male toads were identified as sexually mature if they displayed nuptial pads on the fore digits whereas females were classified sexually mature if they had well-developed oocytes (which were readily visible through the inflated abdomen). Within each population, the longest (fourth) phalange of the left hindlimb was removed from randomly selected adults and preserved in 10 % aqueous solu-

tion of formaldehyde for histological section. However, the hind limbs originating from the highest site dissolved due to improper preservation and thus dropped out of the skeletochronological analysis. All toads examined were released to the site where they originally had been collected.

Paraffin sections and Ehrlich's haematoxylin stain were used to produce histological sections of the phalanges. Age was determined by counting the number of lines of arrested growth (LAG) in the sections. In total, 1,260 adults were examined in this study of which 198 from the two lower altitudes were skeletochronologically aged.

To describe the growth of the toads, growth equations were computed according to von Bertalanffy's model (VON BERTALANFFY 1957), using a nonlinear regression procedure:  $SVL_t = [SVL_{max} - (SVL_{max} - SVL_{met})] - k_t$ , where  $SVL_t$  is SVL at age  $t$ ,  $SVL_{max}$  is the estimated asymptotic SVL,  $SVL_{met}$  the mean length at metamorphosis, and  $k$  is a growth coefficient relating to the rate of decline in growth as toads attain maximum SVL. Because data for body size

Table 1: Geographic and climatic study site details, including mean snout-vent-length and age for the three high-altitude populations of *Bufo minshanicus* STEINEGER, 1926, studied in the east Tibetan Plateau, Peoples Republic of China. The descriptive statistics in columns 6-9 include arithmetic mean  $\pm$  standard error of the mean and sample size (in parentheses). F/t - F or t is the test value of one-way analysis of variance or Student's t tests of variance for differences in population means.

Tab. 1: Geographische und klimatische Kenngrößen der hochgelegenen Untersuchungsstellen im östlichen Hochland von Tibet, Volksrepublik China, sowie mittlere Kopf-Rumpf-Länge und Alter der untersuchten Individuen in den dortigen Populationen von *Bufo minshanicus* STEINEGER, 1926. Die deskriptiven Statistiken der Spalten 6-9 geben den arithmetischen Mittelwert  $\pm$  Standardfehler des Mittelwertes und die Stichprobengröße in Klammern wieder. F/t - F bzw. t sind Testwerte der einfaktoriellen Varianzanalyse bzw. des Student t-Tests für Unterschiede in den Populationsmitteln.

Altitude a.s.l. (m)	Höhe ü. M. (m)	Geogr. Breite (°)	Geogr. Länge (°)	Longitude (°)	Annual mean precipitation (mm)	Annual mean temperature (°C)	Snout-vent-length (mm)			Age (yrs)	
							Male Männchen	Female Weibchen	Kopf-Rumpf-Länge (mm)	Male Männchen	Female Weibchen
2,434		36.61	101.49		576	5.1	74.35 $\pm$ 0.34 (181)	85.19 $\pm$ 0.54 (100)	3.82 $\pm$ 0.12 (59)	4.90 $\pm$ 0.19 (39)	
2,588		36.67	101.34		540	4.7	74.44 $\pm$ 0.18 (573)	84.00 $\pm$ 0.28 (313)	4.47 $\pm$ 0.16 (60)	5.20 $\pm$ 0.15 (40)	
2,999		36.90	101.01		450	1.7	67.94 $\pm$ 0.54 (76)	78.83 $\pm$ 1.24 (17)			
F/t							73.66	11.36	-3.26	-1.26	
P							< 0.001	< 0.001	0.001	0.212	

at metamorphosis was available for three different temperatures of lab-reared toads, the average value of SVL<sub>met</sub> (9.53 mm) was used as an approximation for each population. Growth rate was calculated applying the equation used by e.g., LIAO et al (2016):  $\Delta\text{SVL} / \Delta t = k (\text{SVL}_{\text{max}} - \text{SVL}_{\text{mean}})$ , where SVL<sub>mean</sub> is the mean SVL of adult toads within the population.

Student t-tests were used to test for differences in mean age and mean body size between sexes, and chi-square tests were used to compare differences in age distributions between sexes. ANCOVA with age as covariate was used to check the statistical significance of differences in body size between sexes. One-way ANOVA was used to discriminate populations by means of body size. If the overall ANOVA results were significant, Bonferroni post hoc multiple comparisons were applied to evaluate differences between populations. SPSS ver. 20.0 (SPSS Inc., Chicago, Illinois, USA) was used for all analyses. All probabilities were two-tailed, and the significance level was set at  $P = 0.05$ .

## RESULTS

Sexual differences in adult age and body size.— The average age was younger in males than females (both  $P < 0.002$ , Table 1) and the minimum age at sexual maturity was two years for males and three or four years for females in the populations of known age distribution at 2,434 or 2,588 m a.s.l., respectively (Table 2). The age distribution differed significantly between sexes in these populations (2,434 m: Pearson Chi-Square test:  $\chi^2 = 26.10$ ,  $df = 6$ ,  $P < 0.001$ ; 2,588 m:  $\chi^2 = 15.19$ ,  $df = 6$ ,  $P < 0.019$ ).

Females were significantly larger in SVL than males in all three populations studied (all  $P < 0.001$ ). This result persisted when age was controlled using the known age data of the two lower altitude populations (ANCOVA, both  $P < 0.001$ ).

Altitudinal differences in adult age and body size.— The mean age of adult males was significantly older in the middle than low altitude population (Table 1), whereas the age of adult

Table 2: Age frequencies (%) by sex in adult *Bufo minshanicus* STEJNEGER, 1926, from two populations at different altitudes in the east Tibetan Plateau, Peoples Republic of China.  $N$  – Sample size

Tab. 2: Häufigkeitsverteilung (%) des Alters bei den Geschlechtern von *Bufo minshanicus* STEJNEGER, 1926 in zwei Populationen aus unterschiedlicher Höhenlage im östlichen Hochland von Tibet, Volksrepublik China.  $N$  – Stichprobengröße.

Altitude a.s.l. (m) Höhe ü. M. (m)	$N$	Age (yrs) / Alter (a)								
		2	3	4	5	6	7	8	9	
Males										
Männchen										
2,343	59	3.4	39.0	32.2	23.7	1.7				
2,588	60	5.0	16.7	31.7	26.7	13.3	6.7			
Females										
Weibchen										
2,343	39		2.6	43.6	30.8	12.8	7.7			2.6
2,588	40			22.5	45.0	25.0	5.0	2.5		

females was similar among these populations (Table 1).

In both sexes mean body size varied significantly between the three populations studied (Table 1). Males and females from the middle and low altitude were significantly larger than the corresponding sexes from the high altitude site (Bonferroni Post Hoc test,  $P < 0.001$ ), while each sex was similar in size when low and middle altitude populations were compared ( $P = 0.108$ ).

Growth patterns.— There was a positive correlation between age and body size in both sexes at the two lower altitude

sites (2,434 m: males,  $r_s = 0.61$ ,  $N = 59$ ; females,  $r_s = 0.66$ ,  $N = 39$ ; 2,588 m: males,  $r_s = 0.66$ ,  $N = 60$ ; females,  $r_s = 0.51$ ,  $N = 40$ ;  $P < 0.001$  in all cases). The results demonstrated that females were generally larger than males throughout their life cycle. Additionally, growth rate was lower in males than females (Table 3). The mean individual annual growth rates resulting from von Bertalanffy's models showed that individuals from higher altitude had smaller growth rates than those from lower altitude in either sex (Table 3).

## DISCUSSION

Data from three *B. minshanicus* populations living at different altitudes showed that individuals from higher altitudes were smaller in body size than those from lower altitudes. This observation is contrary to Bergmann's rule and has previously been observed in other amphibians (ASHTON & FELDMAN 2003; ANGILLETTA et al. 2004; MATTHEWS & MIAUD 2007; MA et al. 2009; CHEN et al. 2011). Additionally, *B. minshanicus* showed lower growth rates with increasing altitude.

Based on von Bertalanffy's model, age and growth rate contribute to the asymptotic body size. They may do this to different degrees depending on the environmental conditions prevailing. In anuran species which follow Bergmann's rule the effect of age on body size appears to exceed that of growth

rate (reviewed by MA et al. 2009), whereas, species in which growth rate contributes to body size more than age will follow a converse Bergmann's rule (reviewed by LIAO & LU 2012). In the present study of populations from different altitudes, mean age of sexually mature individuals was similar among each sex. Hence, not age but growth-retarding factors appear to be the major contributor to the small body size in high-altitude populations.

As a prevalent pattern among amphibians, sexual maturation of female *B. minshanicus* took longer and body size was larger than in males. This is in accordance with most other studies on anurans (e.g., MONNET & CHERRY 2002; CHEN et al. 2011; JIN et al. 2017). Also, the resource allocation theory may explain the female-biased size

Table 3: Mean annual growth rates of adult *Bufo minshanicus* STEJNEGER, 1926, from two populations at different altitudes in the east Tibetan Plateau, Peoples Republic of China. Snout-vent-length (SVL) given in mm. Annual growth rates were calculated using the formula: annual growth rate =  $k(SVL_{\max} - SVL_{\text{mean}})$ , where  $k$  is the growth coefficient of the von Bertalanffy model,  $SVL_{\max}$  the asymptotic SVL according to the model, and  $SVL_{\text{mean}}$  the mean body size of a given population.

Tab. 3: Mittlere jährliche Wachstumsraten adulter *Bufo minshanicus* STEJNEGER, 1926 bei zwei Populationen aus unterschiedlicher Höhenlage im östlichen Hochland von Tibet, Volksrepublik China. Kopf-Rumpf-Länge (SVL) in mm. Die jährlichen Wachstumsraten wurden berechnet nach der Formel: Jährliche Wachstumsrate =  $k(SVL_{\max} - SVL_{\text{mean}})$ . Dabei ist  $k$  der Wachstumskoeffizient des von Bertalanffy Modells,  $SVL_{\max}$  die asymptotische Kopf-Rumpf-Länge nach diesem Modell und  $SVL_{\text{mean}}$  die mittlere Kopf-Rumpf-Länge der Population.

Altitude a.s.l. (m) Höhe ü. M. (m)	Males / Männchen				Females / Weibchen			
	SVL <sub>max</sub>	SVL <sub>mean</sub>	k	Growth rate Wachstumsrate	SVL <sub>max</sub>	SVL <sub>mean</sub>	k	Growth rate Wachstumsrate
2,434	78.38	74.34	0.780	3.146	92.94	85.19	0.495	3.834
2,588	78.24	74.44	0.809	3.073	91.49	83.95	0.494	3.723

dimorphism occurring in these and other amphibian populations (SHINE 1979; KOZŁOWSKI 1992), since larger females feature higher fecundity and enjoy a greater lifetime reproductive success. By contrast, energy costs of male reproduction are relatively lower as spermatogenesis is far less costly

than vitellogenetic growth of oocytes (JØRGENSEN 1992). The authors of the present study suggest that both age and growth rate contribute to the sexual difference in body size of *B. minshanicus* because annual growth rate as well as age were larger in females than males.

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