SALAMANDRA **56**(1): 16–26
15 February 2020 | ISSN 0036–3375

SALAMANDRA
German Journal of Herpetology

Habitat selection of the Asiatic toad (*Bufo gargarizans*) during hibernation in the Badagongshan National Nature Reserve, central China

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Manuscript received: 20 July 2019
Accepted: 26 October 2019 by Edgar Lehr

Abstract. Habitat selection refers to animals' choices and preferences relative to environmental conditions. Animals may select habitats based on external conditions such as climate, topographic traits, vegetation conditions, resource abundance, and human impacts. It also may be influenced by factors such as predator avoidance or an individual's physiological status. Identification of a species' essential habitat requirements is a fundamental component in understanding its ecology and conducting population management. Poikilothermic species, including amphibians, which experience harsh winter conditions, usually have strict requirements for hibernacula to ensure suitable conditions and promote their overwintering survival. To explore habitat utilization during hibernation by the Asiatic toad (*Bufo gargarizans*), we carried out field investigations in the Badagongshan National Nature Reserve, central China. We detected several ecological factors impacting on habitat selection and analysed the different degrees of preference exhibited by the toads. Based on an information-theoretic method and resource selection functions, our results revealed that distance to the nearest breeding pond, land-use type, soil texture, mean height of herbaceous plants, slope, and slope position were the main factors determining the hibernation habitat selection of *B. gargarizans*. The toads preferred croplands and vegetable patches with clayey soil and a mean height of herbaceous plants < 60 cm, and habitats at lower slope positions with < 20° of slope for hibernation. Our study provides valuable information about the overwintering ecology of *B. gargarizans*. It could be used as a scientific reference to population and habitat management of this toad species, and also expands our knowledge on the hibernating behaviour of amphibians.

Key words. Amphibia, Anura, Bufonidae, Badagongshan, *Bufo gargarizans*, ecological factors, habitat selection, hibernation.

Introduction

Habitat selection refers to choices and preferences of places to live in by animals (Marques et al. 2017). Preference, and hence selection, of habitat traits may be influenced by many factors, such as climate and topographic conditions, vegetation traits, food availability and abundance, interspecific competition, predator density, protection against predators, human activity impacts, and also, an animal's own characteristics and physiological status (Bélisle & Desrochers 2002, Semlitsch & Bodie 2003). Habitat selection is a dynamic process that varies with biological events (e.g., life-history stages). For example, animals may have different habitat requirements depending on seasons and thus choose different resources and regions or periodically migrate between seasonal habitats (Baldwin et al. 2006, Groff et al. 2016). It is important to explore and understand habitat requirements and utilization, because they could have profound effects on survival, breeding, behaviour, and population density, which are factors impacting on the fitness (Yan & Chen 1998, Yang et al. 2017). Therefore, habitat selection is considered a component fundamental to understanding animal ecology and conducting population management (Stuart et al. 2004, Ljubisavljević et al. 2017).

Hibernation is a physiological phenomenon prevalent in poikilothermic animals. During hibernation, individuals may increase survival rates by preventing intracellular ice formation, stabilizing membranes, and accumulating glucose in tissues (which serve as antioxidants, metabolic substrates, and metabolic regulators, respectively) in frozen environments (Xie et al. 2012, Don et al. 2014). The annual rhythm of hibernation affects reproductive behaviour and success in the following breeding season; thus compromised hibernation may cause local extinction and further the decline of a species (Turbill et al. 2011). Hi-

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bernation may be influenced by a multitude of processes, including hibernal habitat selection, which is considered to be one of the most important mechanisms (Tracy 1976, Pough et al. 1983). Hibernal habitat use has profound effects on the survival and population density of individual species and should maximize their fitness and ensure their long-term viability in an optimal scenario (Fretwell & Calver 1969, Stuart et al. 2004). Different habitat components of hibernacula can fulfil different environmental requirements, such as suitable micro-ambient temperature and humidity, which may have great impacts on the metabolism of the animals using them (Barica & Mathias 1979, Halliday & Blouin-Demers 2016). Good overwintering sites should also provide sufficient oxygen to their users, and should offer enough food, water, and cover (Turbill et al. 2011). For example, reports showed that some mammals remain inactive for fairly long periods in underground caves to avoid the damaging cold of winter, whereas some amphibian species burrow into soft soil or underwater silt to depths that are below the frost line to keep their bodies from being damaged (Breckenridge & Tester 1961, Kelleber &Tester 1969, Sinsch 1988, Kuyt 1991).

Anurans, being poikilotherms, commonly have low controlling abilities of body temperatures, and thus are sensitive to the traits and thermal characteristics of their habitats. They require both aquatic and terrestrial habitats throughout life, however, little is known about how exactly they utilize their habitats in many instances (Pilliod et al. 2002, Groff et al. 2016). Certain adult frogs and toads are able to move long distances overland not only from breeding ponds to terrestrial habitats, but also for foraging or searching for hibernacula, indicating that the payoff for correct choosing overwintering sites must be high (Campbell 1970, Sinsch 1988, Sinsch 1990). As summer progresses into autumn, with the associated environmental temperatures droping, displacements of anuran individuals obviously decrease until they will be confined to within quite small ranges (e.g., underground or in underwater caves or nests), which indicates that their hibernation period has started (Sinsch 1988). Thus, rather than being random, but to prevent themselves from freezing to death, anurans could be expected to be strict on the selection of hibernation sites due to their being sensitive to the conditions offered by the chosen environment (Stallard 2001). It has been reported that many frog species (e.g., *Rana kukunoris*, *R. chensinensis*) use aquatic habitats or remain in aquatic sediment while overwintering because of their limited excavating abilities, whereas, toads (e.g., *Bufo bufo*) were found to hibernate in the soil of forests and farms or around swamps and reservoirs (Laitinen & Pasanen 1998, Wu et al. 2007). Nevertheless, compared to those on breeding habitats, few studies have been entirely focused on anurans' hibernal habitat selection and hibernacula distribution (Groff et al. 2016), on which more systematic surveys are urgently needed.

The Asiatic toad (*Bufo gargarizans*) is an explosive breeder widely distributed in China and is listed as a Least Concern (LC) species in the IUCN Red List (Fei et al. 2012, IUCN SSC Amphibian Specialist Group 2019). With limited dispersal abilities and lek-patterned breeding behaviours, its populations are concentrated around permanent ponds and they breed during relatively short breeding seasons, commencing in early springs (Luo et al. 2014b, 2015). Previous research only reported that *B. gargarizans* prefers to hibernate in shallower soil close to ponds with low vegetation cover, or under the shrubs or in pre-harvest cereal fields near minor roads (Yu & Guo 2012), but detailed information on the habitat use during hibernation of this species is lacking. Due to its high value in Chinese traditional medicine, *B. gargarizans* populations experience intense hunting pressure during both breeding and hibernation periods and its habitats (including breeding and hibernation sites) are exposed to intensive human disturbance, e.g., from agriculture, roads, construction, and deforestation (Huusko et al. 2007). Therefore, studies on overwintering habitat use and hibernation site selection are urgently needed for the population monitoring and management of this toad species. Effects of hibernation site selection on other life historical processes of the toads cannot be adequately predicted until we gain an enhanced understanding of its hibernation habitat utilization and preferences.

Based on field surveys, we studied the hibernation site selection and overwintering habitat preference of *B. gargarizans*. Our aims were to: (1) observe the overwintering behaviour of *B. gargarizans* and describe its hibernation habitats and temporal rhythm; (2) investigate the critical factors that influence their hibernation site selection at microhabitat scale; and (3) analyse preferences of these ecological factors by this toad species during hibernation.

Materials and methods

Study area

Our study was carried out in the Badagongshan National Nature Reserve (29°38'–29°49' N, 109°41'–110°10' E), Hunan Province, which is located in the central part of China (Fig. 1). The nature reserve consists of three forest districts (i.e., Tianpingshan, Doupengshan, and Sanmujie; Fig. 1) and ranges in altitude from 350 to 2000 m (Wang et al. 2005, Lu et al. 2013). In this area, winter lasts for more than 160 days, with minimum temperatures of nearly -15°C in January, whereas spring stretches over >90 days, with heavy rainfalls in May (Wang et al. 2005).

Field surveys

We carried out field surveys in the Tianpingshan and Shanmujie forest districts of Badagongshan based on our previous studies (Luo et al. 2014a, Luo et al. 2016). Our field observations were made in winter from October 2015 through March 2016. As the Asiatic toads can move up to 3 km away from their breeding ponds after completing reproduction (Huusko et al. 2007), we opportunistically searched for individuals of *B. gargarizans* (both active and dormant) on

land and under water (in both permanent and temporary ponds, including breeding ponds) within 3 km around their breeding sites [four breeding sites were recorded in Tianpingshan, i.e., Xiaozhuangping, Futianya, Liaoyewan, Tuanping, and three in Shanmujie, i.e., Gaojiaping, Chajiaoxi, Lianjiawan; locations of these breeding sites were recorded using a GPS (Garmin-eTrex201x, USA)] each day during the investigation period (Fig. 1). Hibernating individuals were located by searching for traces of their activities, for example, overturned plant litter and entrances to underground cavities. No hibernating individual was recorded in underwater habitats during our surveys. When a hibernating individual was found, we measured the depth of its hibernaculum from the ground surface (mm), its body weight (g), body length (mm; SVL, snout–vent length), and the temperature (°C) in the hibernaculum, which we referred to as 'microhabitat temperature'. Then, using the location of the toad as the centre of a 5 × 5 m square, thirteen habitat parameters, which are indicative of human disturbance, land use, vegetation, and the terrain of the hibernation site, were recorded (Wang et al. 2011, Table 1). There are six land-use types within the study sites: primary arbour grove (dominated by *Agus lucida*, *Sassafras tzumu*, *Camellia pitardii*; with relatively dense vegetation cover; firewood collecting usually occurs during winter), artificial arbour grove (dominated by *Cortex phellodendri chinensis*, *Magnolia officinalis*; with medium vegetation cover; firewood collecting sometimes occurs during winter), cropland (dominated by farming cereals, potato, sweet potato; with relatively low vegetation cover; little human activity during winter), vegetable patch (dominated by farming radish, beans, cucumber, cabbages; with relatively low vegetation cover; little human activity during winter), meadow (dominated by Gramineae; with medium vegetation cover; firewood collecting sometimes occurs during winter), and bamboo shrub (dominated by *Indocalamus tessellatus*, *Phyllostachys heterocycla*; with relatively dense vegetation cover; firewood collecting usually occurs during winter). Based on Johnson (1980), Luo et al. (2014a, b, 2015), and Groff et al. (2017), we randomly established two 5 × 5 m squares around each hibernation location (distance to the hibernaculum was < 100 m) and collected their environmental data. Meanwhile, ambient temperature (°C) and relative humidity (%) around each breeding site were recorded with a hygrothermograph (AZ-8829, Taiwan, China) every day throughout the investigation period.

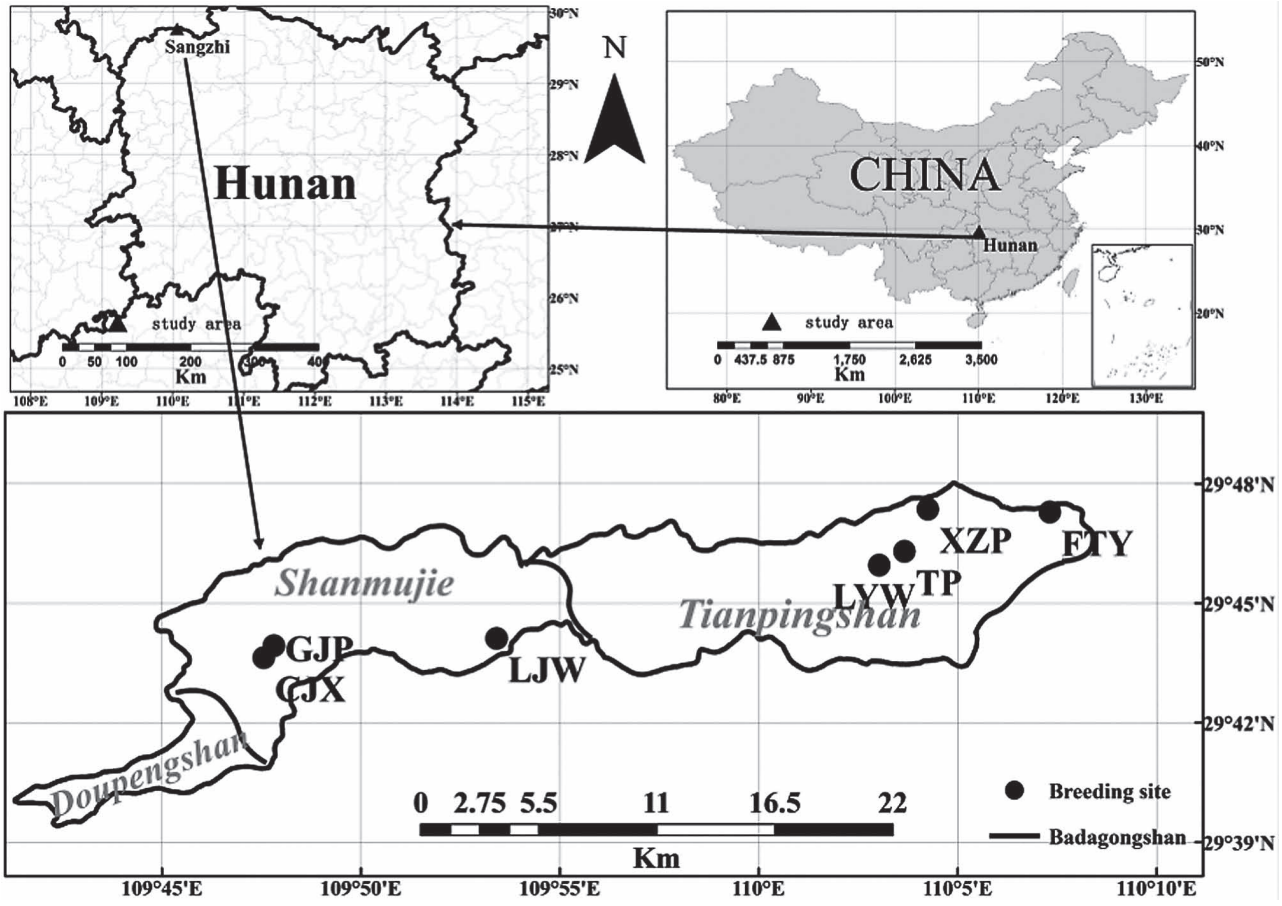


Figure 1. Location of the study area. Breeding sites: GJP, Gaojiaping; CJX, Chajiaoxi; LJW, Lianjiawan; XZP, Xiaozhuangping; FTY, Futianya; LYW, Liaoyewan; TP, Tuanping.

Table 1. Ecological factors recorded in the surveys of hibernation site selection of *Bufo gargarizans* in Badagongshan.

Factors	Descriptions
Distance to the nearest breeding pond (m; DB)	We calculated the distance from the centre of each square to the nearest breeding pond with a GPS device.
Distance to the nearest dwelling (m; DH)	We calculated the distance from the centre of each square to the nearest building with a GPS device.
Distance to the nearest water source (m; DW)	We calculated the distance from the centre of each square to the nearest pond or stream with a GPS device.
Land-use type (LT)	Six categories were recorded within the study area: primary arbour grove, artificial arbour grove, cropland, vegetable patch, meadow, bamboo shrub.
Gravel degree (%; GD)	Percentage of gravel covering the ground in each square, e.g., 50% means that the area of gravel-covered surface accounts for 50% of the total area of the square.
Soil texture (ST)	Three categories were recorded according to the international system of soil texture-grading standards (www.cqates.com/cqsoil/gjztrzdfjbz.htm) in the study area: clayey, sandy clay loam, sandy soil.
Moss cover (%; MC)	Percentage of moss covering the ground in each square, e.g., 50% means that the area of moss-covered surface accounts for 50% of the total area of the square.
Mean height of herbaceous plants (cm; HP)	We randomly measured the heights of 25 herbaceous plants in each square and calculated their mean heights.
Leaf litter depth (cm; LL)	We randomly selected 10 spots within each square, measured the depths of leaf litter using a measuring tape, and calculated a mean value.
Vegetation cover (%; VC)	Percentage of likely area of the cover for aboveground herbage and shrubs in each square.
Aspect (A)	Five categories were recorded: flat, south-facing slope (S67.5°E–S22.5°W), west-facing slope (N22.5°W–N67.5°W), north-facing slope (S67.5°W–N22.5°E), east-facing slope (N22.5°E–S67.5°E).
Slope (°; S)	We measured the sloping of each square with a digital compass.
Slope position (SP)	Three categories of slope position were recorded: lower, middle, upper.

Statistical analyses

First, we mapped the fluctuations in humidity, ambient temperature, and the number of active individuals observed aboveground across time to visualize their hibernation rhythm and predict the hibernation stages of *B. gargarizans*. Then, linear and curve estimations were used to pinpoint the relationships of depth of hibernaculum to body weight, body length, and microhabitat temperature. We used the Kolmogorov-Smirnov (K-S) test to assess the normality of each continuous variable and then conducted ln-transformations for the non-normally distributed continuous parameters. To detect the difference of each ecological factor between used and random habitat squares, independent-sample T tests and Mann-Whitney U tests were developed for continuous (ln-transformed if not normally distributed) and categorical parameters, respectively (Bull 2006). In order to reduce the impacts of multicolinearity between variables on our further analyses, we calculated the pairwise Pearson's correlation coefficients between ecological factors. If the coefficient of a variable pair was > |0.7|, we removed the variable with the higher standard deviation.

An information-theoretic approach was used to establish best-fit models and explore the importance of habitat factors for hibernation habitat selection of the Asiatic toads in R 3.5.1 software (Venables et al. 2018). Using presence-absence as the response variable and all the combinations of habitat parameters as the independent variable sets, we fitted generalized linear models (GLMs) by the dredge function in the package MuMIn (Barton 2018). Then, corrected Akaike's information criteria (AICc) were calculated to rank and select the candidate models (Boyce et al. 2002, Burnham & Anderson 2002, Wang & Wang 2005). As models with $\Delta AICc < 2$ are considered to be competing best-fit habitat selection models, we computed the Akaike weight ($AICc\omega$) of each model to assess its confidence and relative likelihood within the model set ($\Delta AICc < 2$) (Burnham & Anderson 2002). If no single model is clearly superior to the others ($AICc\omega > 0.9$), a weighted model averaging approach was used based on the model set and $AICc\omega$ (Burnham & Anderson 2002, Gibson et al. 2004). As this approach reduces model selection bias, estimates and relative variable importance (RVI) indexes were calculated for the environment variables included in the model set (Guisan & Zimmermann 2000, Burnham & Anderson 2002, Barton 2018).

To analyse preference characteristics of *B. gargarizans* for each habitat factor, we classified the factors based on their means and maximum–minimum variations and calculated the resource selection index (ε) of each factor class as follows (Boyce & McDonald 1999, Li et al. 2001, Boyce et al. 2002, Manly et al. 2002, McDonald et al. 2006, Irwin et al. 2012, Luo et al. 2014a):

$$\omega_{ij} = (\gamma_{ij}/\rho_{ij}) / \Sigma (\gamma_{ij}/\rho_{ij})$$

$$\varepsilon_{ij} = \left(\omega_{ij} - \frac{1}{n}\right) / \left(\omega_{ij} + \frac{1}{n}\right)$$

where γ_{ij} is the resource amount of class j in the factor i that has been utilized; ρ_{ij} is the resource amount of class j in the factor i that can been utilized; ω_{ij} is the resource coefficient of class j in factor i; and n is the number of classes of a given habitat factor. The value of ε ranges from -1 to 1 and its value of > 0.1, < -0.1, and between -0.1 and 0.1 indicate that the individuals prefer, avoid, or randomly select a resource class, respectively (Boyce & McDonald 1999, Boyce et al. 2002, McDonald et al. 2006).

Results

Overwintering behaviour

Based on our field observations of overwintering behaviour and climate data, the hibernation period of *B. gargarizans* in Badagongshan can be generally classified in three stages: pre-hibernation, deep hibernation, and post-hibernation (Fig. 2). With the decrease of mean ambient temperatures after these had reached 10°C in late October, hibernation was likely to have commenced when the numbers of toads observed above ground were conspicuously reduced (pre-hibernation stage). When mean temperature dropped below 5°C from early November through mid-/late February of the next year, no surface-active toads could be recorded, which suggested that they were deeply hibernating. Then, toads were occasionally observed moving about above ground when temperatures began to rise, indicating that their post-hibernation stage had started, especially as a significant increase of individual aboveground activity was noted at under 5°C during the period late February to early March (Fig. 2).

Our observations revealed that *B. gargarizans* generally hibernated underground and not underwater. These toads used hibernacula cavities under stones on the ground, in layers of forest litter or humus and, moreover, burrowed into soft soil up to 20 cm (mean ± SE: 9.250 ± 5.067 cm) deep to build caves with their hind legs. We found no significant relationship between cave depth and the physical condition of individual toads ($p > 0.05$ for all linear and curve regression analyses between cave depth, body weight, and body length). However, a positive relationship was detected between the depth of the hibernaculum and microhabitat temperature ($p = 0.021$, $R^2 = 0.299$; Fig. 3), suggesting that increased cavity depth enabled the toads to enjoy a relatively warmer environment during hibernation (Fig. 3).

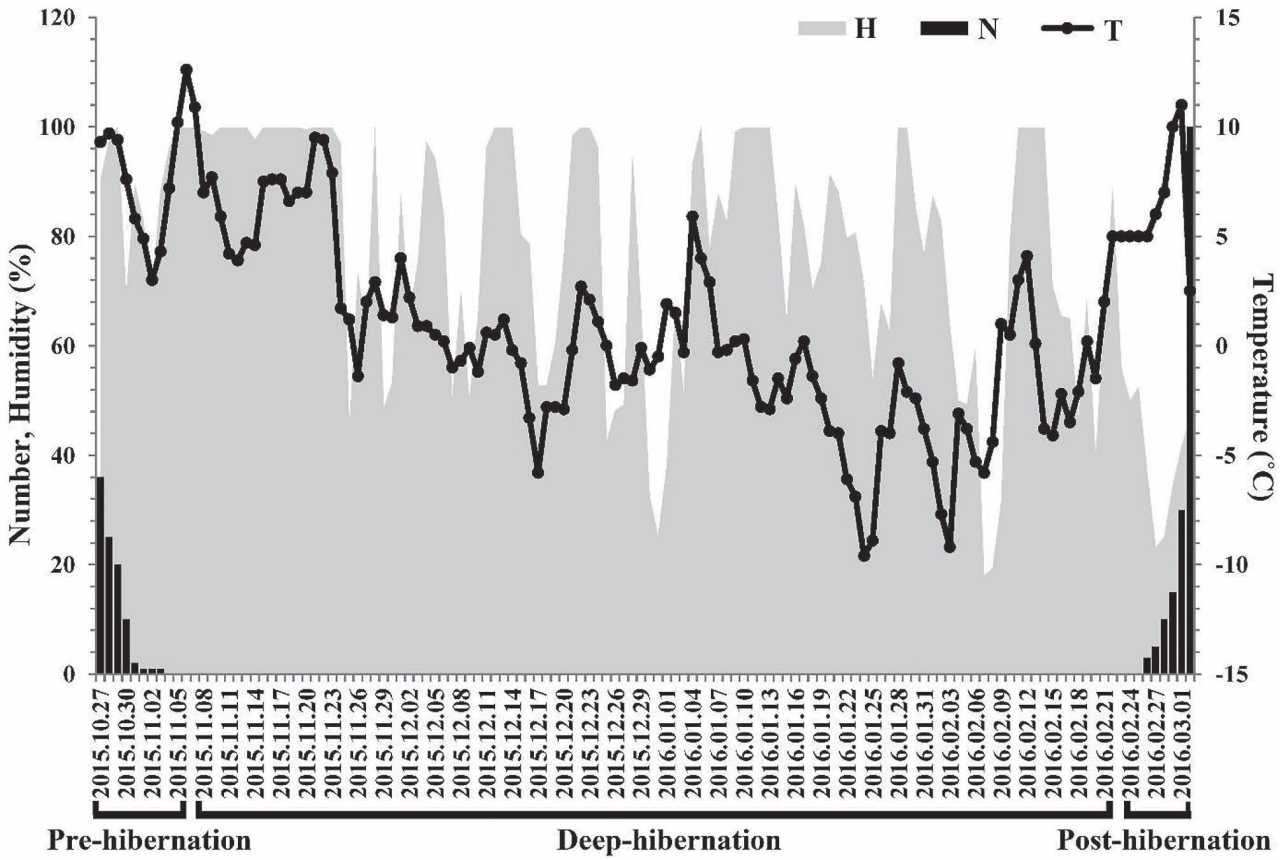


Figure 2. Hibernation stages of *Bufo gargarizans*, dynamics of the number of observed aboveground individuals, environment temperature, and humidity during the study period. T, temperature; H, humidity; N, number of individuals recorded above ground.

Table 2. Results of one-way ANOVA and Mann-Whitey U tests of the ecological factors affecting used and random habitats of *Bufo gargarizans* in Badagongshan. * non-normally distributed and ln-transformed variable; ** *F* value of independent-sample T test for continuous parameter; *** *Z* value of Mann-Whitney U test for categorical parameter.

	Used habitat (n = 84)		Random habitat (n = 168)				
Factor	Mean	SE	Mean	SE	F**	Z***	p
DB*	0.079	0.097	-0.039	0.079	3.738		0.371
DH*	0.136	0.107	-0.057	0.075	0.645		0.137
DW*	0.115	0.099	-0.054	0.078	1.603		0.197
LT	2.726	0.147	2.286	0.116		-3.365	0.001
GD*	-0.302	0.079	0.164	0.079	12.181		<0.001
MC*	-0.092	0.091	0.062	0.075	5.063		0.195
HP*	-0.114	0.111	0.059	0.074	1.083		0.189
ST	1.488	0.064	2.125	0.060		-6.047	<0.001
LL*	-0.067	0.097	0.084	0.069	0.241		0.209
VC*	-0.207	0.094	0.146	0.070	1.299		0.003
A	1.559	0.166	2.292	0.104		-3.923	<0.001
S*	-0.337	0.085	0.216	0.070	3.789		<0.001
SP	1.179	0.048	1.381	0.052		-2.259	0.024

Differences between used and random habitats

A total of 252 habitat squares, i.e., 84 used and 168 random ones, were analysed in this study. Following the Kolmogorov-Smirnov (K-S) tests, all continuous variables had significant departures from normality (K-S tests $p < 0.05$) and thus were subjected to ln-transformations (Table 2). Based on independent-sample T tests (for ln-transformed continuous variables) and Mann-Whitney U tests (for categorical variables), LT ($p = 0.001$), GD ($p < 0.001$), ST ($p < 0.001$), VC ($p = 0.003$), A ($p < 0.001$), S ($p < 0.001$), and SP ($p = 0.024$) showed significant differences between habitats used by *B. gargarizans* and random selections, whereas limited differences were detected in other ecological factors (Table 2).

Habitat selection modelling

Our pairwise Pearson's correlation coefficients showed no obvious multi-colinearity between parameters ($|r| < 0.7$ for all parameter pairs), and thus we included all of our selected ecological factors in the following modelling. According to the AIC-based model selection process, the first 25 models had $\Delta AICc < 2$ (Table 3). Because these models had relatively low Akaike weights (AICcω ranged from 0.030 to 0.070) and low variations of Akaike weights (the maximum AICcω difference was 0.040), and no single model was apparently superior to any other one, we included all of them in the best-fit model set and used an integrative (weighted) habitat selection model (Tables 3, 4).

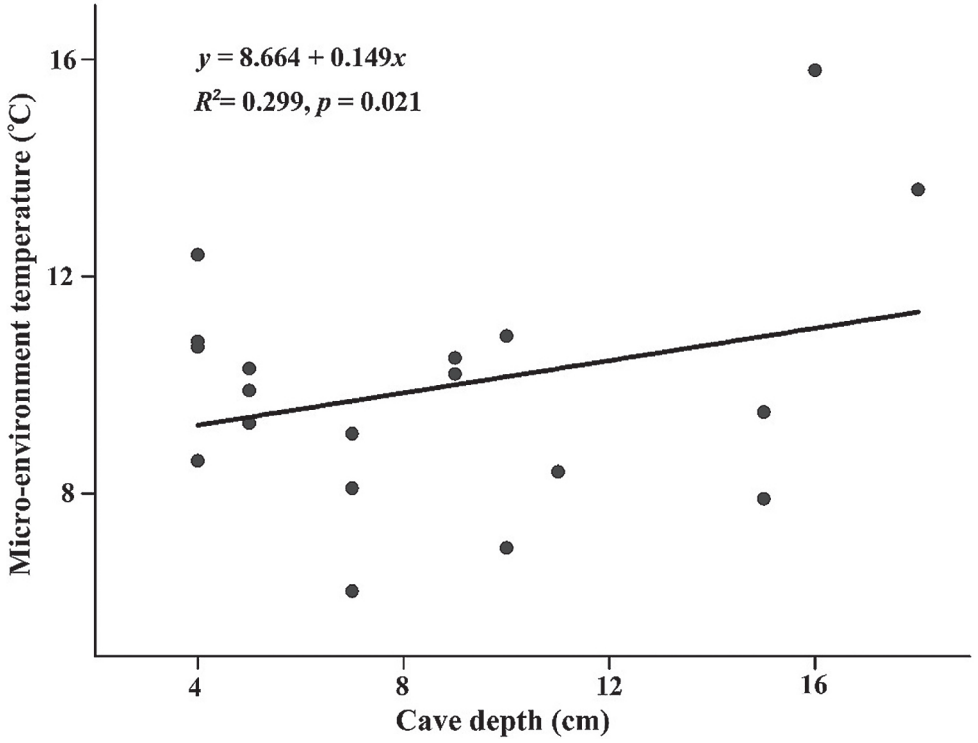


Figure 3. The relationship between hibernaculum depth and microhabitat temperature.

Table 3. Top 25 generalized linear models (ΔAICc < 2) of habitat selection by *Bufo gargarizans* in Badagongshan. The log(L) is the maximized log-likelihood function; k is the number of parameters used in the model

Model		log (L)	k	AICc	ΔAICc	AICcω
1	DB + HP + LT + S + SP + ST + VC	-157.620	7	331.840	0.000	0.070
2	DB + HP + LL + LT + S + SP + ST	-157.720	7	332.030	0.190	0.060
3	DB + DH + HP + LL + LT + S + SP + ST	-156.770	8	332.290	0.440	0.060
4	DB + HP + LT + MC + S + SP + ST + VC	-156.800	8	332.350	0.500	0.060
5	DB + DH + HP + LT + S + SP + ST + VC	-156.820	8	332.380	0.530	0.050
6	DB + HP + LT + MC + S + SP + ST	-157.960	7	332.510	0.670	0.050
7	DB + HP + LT + S + SP + ST	-159.180	6	332.830	0.990	0.040
8	DB + DH + HP + LT + MC + S + SP + ST + VC	-155.960	9	332.840	0.990	0.040
9	DB + HP + LL + LT + S + SP + ST + VC	-157.050	8	332.840	0.990	0.040
10	DB + DH + HP + LT + MC + S + SP + ST	-157.090	8	332.930	1.090	0.040
11	DB + HP + LL + LT + MC + S + SP + ST	-157.100	8	332.950	1.100	0.040
12	DB + LT + MC + S + SP + ST + VC	-158.260	7	333.100	1.260	0.040
13	DB + DH + HP + LL + LT + MC + S + SP + ST	-156.150	9	333.220	1.380	0.040
14	DB + DH + HP + LL + LT + S + SP + ST + VC	-156.150	9	333.220	1.380	0.040
15	DB + DH + HP + LT + S + SP + ST	-158.350	7	333.300	1.450	0.030
16	DB + LL + LT + S + SP + ST	-159.490	6	333.430	1.590	0.030
17	DB + LT + S + SP + ST + VC	-159.490	6	333.430	1.590	0.030
18	DW + DB + HP + LT + S + SP + ST + VC	-157.370	8	333.480	1.640	0.030
19	DW + DB + HP + LL + LT + S + SP + ST	-157.390	8	333.520	1.680	0.030
20	DB + GD + HP + LT + S + SP + ST + VC	-157.400	8	333.540	1.700	0.030
21	DB + LL + LT + S + SP + ST + VC	-158.480	7	333.560	1.710	0.030
22	HP + LT + MC + S + SP + ST + VC	-158.550	7	333.700	1.860	0.030
23	DB + LL + LT + MC + S + SP + ST	-158.600	7	333.800	1.960	0.030
24	A + DB + HP + LT + S + SP + ST + VC	-157.540	7	333.820	1.980	0.030
25	A + DB + HP + LL + LT + S + SP + ST	-157.540	8	333.830	1.980	0.030

In our final GLM, five ecological factors stood out as significant in the regression analyses, that is, LT, S, and ST had $p < 0.01$ and SP and DB had $p < 0.05$ (Table 4). Because these factors had high RVIs (> 0.95; Table 4), we considered them as critical factors, affecting hibernation habitat selection by the toads. Although the p value of HP was slightly over 0.05, it also had a high RVI (0.84; Table 4). Whereas the influences of the other seven factors on the model were limited in the results (Table 4), we also found that DB, LT, HP, and SP were all positively correlated with the presence of these toads, but ST and S were negatively correlated (Table 4).

Table 4. The integrative (weighted) habitat selection model for *Bufo gargarizans* in Badagongshan. RVI, relative variable importance index.

Variable	Estimate	SE	Z	p	RVI
DB	0.001	0.001	2.259	0.024	0.970
DH	-0.001	0.001	1.296	0.195	0.300
DW	0.001	0.001	0.746	0.456	0.060
LT	0.099	0.018	5.482	< 0.001	1.000
GD	-0.099	0.150	0.657	0.511	0.030
MC	0.183	0.135	1.354	0.176	0.360
HP	0.003	0.001	1.900	0.057	0.840
ST	-0.112	0.040	2.766	0.006	1.000
LL	0.011	0.007	1.485	0.137	0.420
VC	0.218	0.139	1.561	0.119	0.520
A	-0.010	0.021	0.492	0.623	0.050
S	-0.005	0.002	2.889	0.004	1.000
SP	0.127	0.053	2.368	0.018	1.000

Preference of habitat factors

Resource selection indexes demonstrated that, in winter, *B. gargarizans* preferred cropland (ε = 0.121) and vegetable patch (ε = 0.163) and avoided primary arbour grove (ε = -0.454) and bamboo shrub (ε = -0.180) habitats (Table 5). They preferred a mean height of herbaceous plants of < 60 cm (ε > 0.100) and avoided that of more than 90 cm (ε = -1.000); preferred clayey soil (ε = 0.259) and avoided sandy soil (ε = -0.677); preferred slope of < 20° (ε = 0.312) and lower slope position (ε = 0.183) and avoided 20–40° (ε = -0.257) and > 60° (ε = -0.416) of slope and upper slope position (ε = -0.422) (Table 5). In addition, selection was apparently random in the other factor classes (Table 5).

Table 5. Resource selection indexes of important ecological factors of habitats selected by *Bufo gargarizans* in Badagongshan. *A: avoided (-1 < ε < -0.1); R: randomly selected (-0.1 < ε < 0.1); P: preferred (0.1 < ε < 1).

Factor	Class	Resource selection index (ε)	Preference*
DB (m)	< 100	0.051	R
	100–200	0.033	R
	200–300	0.041	R
	300–400	-0.211	A
	> 400	0.041	R
LT	primary arbour grove	-0.454	A
	artificial arbour grove	0.070	R
	cropland	0.121	P
	vegetable patch	0.163	P
	meadow	0.053	R
	bamboo shrub	-0.180	A
HP (cm)	< 30	0.166	P
	30–60	0.202	P
	60–90	0.046	R
	> 90	-1.000	A
ST	clayey	0.259	P
	sandy clay loam	0.051	R
	sandy soil	-0.677	A
S (°)	< 20	0.312	P
	20–40	-0.257	A
	40–60	0.043	R
	> 60	-0.416	A
SP	lower	0.183	P
	middle	0.068	R
	upper	-0.422	A

Discussion

Overwintering behaviour

Hibernation is one of the adaptive strategies of cold-blooded animals to survive under freezing temperatures and food shortages in winter. Our study indicates that *B. gargarizans* has a hibernation duration of about three and a half months (from early November through mid-/late February) in Badagongshan, which is consistent with previous results (Fei et al. 2012). As other studies have reported (e.g., Yu & Guo 2010), ambient temperature is the main factor that will determine whether and when amphibians begin brumation. The temporal hibernation rhythm of the Asiatic toad was closely related to temperature decrease, and three stages were detected (pre-hibernation: ambient temperature approaching 5°C; deep hibernation: ambient temperature below 5°C; post-hibernation: ambient temperature above 5°C; Fig. 2). However, the timing of hibernation (onset and termination) of *B. gargarizans* may vary, depending on local climate and/or yearly variation. More related observations and comparative analyses are required to fully characterize the hibernation traits of this toad species.

Previous literature states that some adult toads choose underwater sites for hibernation (e.g., *B. viridis*, Hoffman & Katz 1989). Other studies reported that the Asiatic toad chose rock crevices, hollow tree trunks, and dense vegetation as overwintering shelters, or even hibernated underwater (Denton & Beebee 1993, Schwarzkopf & Alford 1996, Seebacher & Alford 1999, Yu & Guo 2010), which is contrary to our observations. In addition, frogs and some species of *Bufo* are stated to burrow cavities as hibernacula, with depths, diameters, and shapes of these cavities being positively correlated to the body sizes of the individuals (Schwarzkopf & Alford 1996, Jofré et al. 2007). Our analyses did not find such relationships, but a positive and linear trend was revealed between microhabitat temperature in the cavity and its depth (Fig. 3). We suspect that, besides the impacts of body condition, *B. gargarizans* burrows in soil to take advantage of the stable and moderated environment found at depth. That means, although burrowing is an energy-intensive process, the toads might invest into it so as to benefit from relatively warmer and therefore safer underground shelters and thus maximise winter survival. This might constitute a trade-off between fitness benefits from better hibernal environments and energetic costs of burrowing by *B. gargarizans*. In addition, because of their permeable skins and weak controlling abilities on evaporation, moist environments and wet skins play crucial roles in the respiration processes of anurans that are especially critical during hibernation (Pan et al. 2001). Deep underground shelters are helpful in reducing the loss rates of body fluids in toads (Schwarzkopf & Alford 1996).

Hibernation habitat selection and preferences

We found that overwintering *B. gargarizans* generally used natural terrestrial nests, such as cavities beneath stones on the ground, forest litter or humus, and cavities they had burrowed themselves in Badagongshan. Our results indicated that human interference, land use, vegetation, and terrain traits had significant impacts on overwintering habitat utilization of the toad individuals.

Migration is an energy-consuming task especially for amphibians whose dispersal abilities are quite limited (Yu et al. 2010, Luo et al. 2014b, 2015). When temperatures decrease, toads and frogs usually burrow cavities after moving short distances from, or choose natural shelters near, their breeding ponds as their hibernacula for the coming winter (Hacheol et al. 2007), which was consistent with our monitoring results. Interestingly, our study found *B. gargarizans* used habitats that were relatively distant from reproductive sites for overwintering in Badagongshan. We considered that this could be a trade-off between energy expenditure and human disturbance during the toads' selection process of hibernation habitats. As the permanent ponds or temporary pools within which they

breed often serve as water sources for local residents, livestock, and poultry in winter, the surrounding areas generally experience high intensities of anthropogenic disturbance (Huusko et al. 2007). Thus, the toads had to migrate further, especially to croplands or vegetable patches where human activities are less intensive during winter, to avoid human-related influences and improve their chances of surviving winter.

Previous studies reported that microhabitat temperature and humidity are major determinants of activity frequency and habitat use of anurans in winter in temperate zones (Sinsch 1984, Smits 1984, Preest & Pough 1989). Our analyses detected that the Asiatic toad has a preference for overwintering shelters in croplands and vegetable patches. Possibly, the open skies of these types of habitat promote the accumulation of snow and snow cover preservation, which could lead to relatively warmer underground environments and more stable and predictable temperatures in hibernacula under a thick snow cover (Packer 1971, Winkler 2001, Pauli et al. 2013). Moreover, these habitats often host relatively low plants and a thick ground-level vegetation cover. These factors may provide cover from predators, have a lower degree of human interference, and provide good foraging opportunities (live insects etc.) for the post-overwintering period. Microhabitat humidity characteristics driven by morning dew, early snowmelt, and plant transpiration, may also be factors that promote the use of these habitats (Yu et al. 2010, Yu & Guo 2010). In addition, habitats such as these usually have loose soils with low stone contents, presumably due to tillage (Gerson 1982). This was consistent with our results on soil texture selection in that the toads preferred soils with a high proportion of clayey particles (< 2 mm), which could help to keep water in the soil and on the skin, facilitating efficient respiration by the toads (Rossa-Feres et al. 2004).

According to this study, a relatively low plant height (< 60 cm) was preferred by the Asiatic toads of Badagongshan. We considered it to be their strategy for pursuing warmer temperatures and greater humidity in winters, as they are not able to control the evaporation rate of body fluids via the skin (Shoemaker 1964, Wygoda 1988). Although the toads can withstand relatively high levels of water loss (up to 10% of body mass), they cannot regularly allow themselves to become extremely dehydrated and need to replenish their body fluids whenever moisture is available (Jørrgensen 1991). Low herbaceous vegetation in the habitat is conducive to the accumulation of snow, which could serve as a blanketing cover for the plants and animals beneath. A thick snow cover could be valuable for reducing the toads' loss of body fluids through evaporation, supplement them from melting snow (Packer 1971, Winkler 2001), and moreover, reduce exposure to dangerously low temperatures and temperature fluctuations, as has been reported for many amphibian species (e.g., the Chinese giant salamander, *Andrias davidianus*; Zhang et al. 2014).

As for the terrain factors, *B. gargarizans* used positions on lower slopes and small slope habitats in Badagongshan. One of the most likely explanations for this is that they can reduce energy consumption when moving to their hibernacula, although upper slope positions and steep slopes may help with spotting and escaping predators (Irwin et al. 2018). Furthermore, rainwater is not accumulated in higher positions and steep areas as much as in lower, more gently sloped ones, which could cause the ground to dry out and render it unsuitable for establishing hibernacula with an adequate water supplementation. Humid and moist micro-environments at lower and small slopes are more conducive to their water requirements, which would increase the toads' chances of survival under the life-threatening conditions of winter.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (No. 31770427), China Scholarship Council (No. 201706775008), and the Youth Science and Technology Plan of Hubei Province. We thank Z. Li for helping with data collection and the staff of Badagongshan National Nature Reserve for their support in our field investigations. We thank H. Wu, M. Zhao, W. Zhang, and S. McCann for providing valuable advice on early drafts of this research report. Our field studies were conducted under a permit issued by the Management Bureau of the Badagongshan National Nature Reserve. Our observations, capturing and measuring of specimens did not induce significant stress or suffering to the study animals and their reproductive success, behavioural rhythm, or the ultimate survival of the toads.

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