Seasonal habitat selection of the critically endangered Mangshan pitviper (*Protobothrops mangshanensis*), a species endemic to China

Bing Zhang [1] , **Bingxian Wu** [1] , **Daode Yang** [Corresp., 1] , **Xiaqiu Tao** [1] , **Mu Zhang** [1] , **Shousheng Hu** [2] , **Jun Chen** [2] , **Ming Zheng** [2]

[1] Institute of Wildlife Conservation, Central South University of Forestry and Technology, Changsha, Hunan, China
[2] Administration Bureau of Hunan Mangshan National Nature Reserve, Chenzhou, Hunan, China

Corresponding Author: Daode Yang
Email address: csfuyydd@126.com

Background. Habitat characteristics directly affect the population size and geographical distribution of wildlife species, including the Mangshan pitviper (*Protobothrops mangshanensis*), a critically endangered snake species endemic to China. Plots used by this species were paired with random plots to study habitat selection of Mangshan pitviper in spring, summer, and autumn, with the goals of gaining a better understanding of the mechanisms affecting its habitat requirements and testing the influence of seasonality on habitat selection. **Results.** We conducted the study in Hunan Mangshan National Nature Reserve in 2015 and 2016. We measured 14 habitat variables seasonally in used and paired plots: 20 (spring), 31 (summer), and 32 (autumn). Snakes tended to select open habitat with a relatively short distance to water, high fallen log density, and relatively gently sloping gradient in spring. In summer, habitats with relatively high fallen log density, shrub density, shrub height, and more gravel were most important, while in autumn these snakes tended to select habitats having relatively high fallen log density and shrub height, and those relatively close to water. **Conclusion.** The ecological variables making the important contribution to habitat selection of snakes changed with the season. Canopy cover and herb cover were important variables for seasonal discrimination. Due to the seasonal differences in habitat selection of Mangshan pitviper, some targeted measures should be carried out to improve conservation efforts in support of this critically endangered snake species.

Seasonal habitat selection of the critically endangered Mangshan pitviper (*Protobothrops mangshanensis*), a species endemic to China

Bing Zhang[1], Bingxian Wu[1], Daode Yang[1*], Xiaqiu Tao[1], Mu Zhang[1], Shousheng Hu[2], Jun Chen[2] and Ming Zheng[2]

[1] Institute of Wildlife Conservation, Central South University of Forestry and Technology, Changsha, Hunan, China
[2] Administration Bureau of Hunan Mangshan National Nature Reserve, Chenzhou, Hunan, China

Corresponding Author: Prof. Daode Yang[1]
498 South Shaoshan Road, Changsha, Hunan, 410004, China
Email address: csfuyydd@126.com

Abstract

Background. Habitat characteristics directly affect the population size and geographical distribution of wildlife species, including the Mangshan pitviper (*Protobothrops mangshanensis*), a critically endangered snake species endemic to China. Plots used by this species were paired with random plots to study habitat selection of Mangshan pitviper in spring, summer, and autumn, with the goals of gaining a better understanding of the mechanisms affecting its habitat requirements and testing the influence of seasonality on habitat selection.
Results. We conducted the study in Hunan Mangshan National Nature Reserve in 2015 and 2016. We measured 14 habitat variables seasonally in used and paired plots: 20 (spring), 31 (summer), and 32 (autumn). Snakes tended to select open habitat with a relatively short distance to water, high fallen log density, and relatively gently sloping gradient in spring. In summer, habitats with relatively high fallen log density, shrub density, shrub height, and more gravel were most important, while in autumn these snakes tended to select habitats having relatively high fallen log density and shrub height, and those relatively close to water.
Conclusion. The ecological variables making the important contribution to habitat selection of snakes changed with the season. Canopy cover and herb cover were important variables for seasonal discrimination. Due to the seasonal differences in habitat selection of Mangshan pitviper, some targeted measures should be carried out to improve conservation efforts in support of this critically endangered snake species.

Introduction

Most wild animals require multiple habitats types to obtain the various resources (*Raynor et al., 2017*; *Leite et al., 2018*), which would provide them opportunities for predation, reproduction, and shelter (*Doligez, Danchin*

& Clobert, 2002; *Hyslop, Cooper & Meyers, 2009*; *O'Hanlon, Herberstein & Holwell, 2015*). It is very important for wildlife conservation and management to master their habitat preference, habitat demand and the change of habitat selection mode in different seasons, especially endangered animals (*Willems & Hill, 2009*; *Ali et al., 2017*; *Mandlate, Cuamba & Rodrigues, 2019*). Although the distribution areas of many endangered animals are classified into nature reserves for special protection and management, the information of their habitat selection is often scarce because of the low population, narrow and remote distribution area, low public attention, and the danger of poisonous animals to investigators (*Rechetelo et al., 2016*; *Sutton et al., 2017*).

The relationship between the wild animals and their habitat is often variable (*Lunghi, Manenti & Ficetola, 2015*; *Ortega, Mencia & Perezmellado, 2016*). As ectothermic animals, snakes are very sensitive to thermal changes in their external environment, and therefore, habitat selection often varies in different seasons based on thermoregulatory requirements (*Weatherhead & Brawn, 2006*; *Sprague & Bateman, 2018*). In addition, breeding, prey availability, retreating and other factors are also important factors affecting the seasonal habitat selection of snakes (*Harvey & Weatherhead, 2006*; *Sperry & Weatherhead, 2009*; *Gardiner et al., 2015*). Snakes may also choose a preferred habitat factor that is not affected by the seasons, which brings them survival benefits and maximizing resource availability (*Sutton et al., 2017*; *Hecnar & Hecnar, 2011*). Such as, snakes may give priority to habitats that are easy to hunt for food and allowing a good place to escape (*Wasko & Sasa, 2011*; *Gardiner et al., 2015*).

The Mangshan pitviper (*Protobothrops mangshanensis*) is the largest species of Viperidae in China (up to 2 m long and 2–4 kg in weight) (*Gong et al., 2013*), but it has a very limited distribution (occupying c. 10,500 ha). Mangshan pitviper has been estimated to be less than 500 individuals (*Chen et al., 2013*; *Gong et al., 2013*), and as such, it is classified as an endangered species on the IUCN Red List of Threatened Species, listed in Appendix II of the CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) in 2013, and listed as critically endangered on the Red List of China's Vertebrates in 2016 (*Jiang et al., 2016*). The majority of studies of Mangshan pitviper have focused on venom (*Mebs et al., 2006*; *Murakami et al., 2008*; *Valenta, Stach & Otahal, 2012*), identification of individuals (*Yang et al., 2013*), and on population status and distribution (*Gong et al., 2013*). However, little is known about seasonal variation in habitat selection, which would provide basic information about how the snake meets its needs for survival; therefore, this information is especially crucial in efforts to preserve this at-risk species.

The primary objective of this study was: 1) to employ pairs of used and random selected plots to explore the mechanism responsible for habitat requirements of *P. mangshanensis*, and to better inform management decisions regarding the future conservation of this critically endangered species, 2) to test if habitat selection would change with season, and to study how environmental variables affect the habitat selection of snakes on each season, 3) to identify habitat elements that were consistently selected by snakes. We hypothesized that many environmental factors or structural resources that make up habitats are constantly changing, forcing snakes to choose habitats accordingly, and the snakes have their preferred habitat.

Materials & Methods

Study area

Hunan Mangshan National Nature Reserve (hereafter referred to as the Mangshan Reserve) is located in Yizhang County, Chenzhou City, Hunan Province, at the northern foot of the Nanling Mountains in China (24°53'00"–25°03'12"N, 112°43'19"–113°00'10"E). Elevations range from 436–1902.3 m, and the total area covers 198.33 km^2. Mangshan Reserve lies within the subtropical humid monsoon climatic zone of China, with an average annual temperature, relative humidity, and precipitation of 17.2°C, of 82.8%, and 1950 mm, respectively. This area features a frost-free period averaging 290 days. The seasons of the Mangshan Reserve are the following: spring = March–April; summer = May–August; autumn = September–October; winter = November–February (*Sun et al., 2011*). The vegetation type is mainly subtropical evergreen broad-leaved forest in areas < 1000 m a.s.l., with mainly coniferous and broad-leaved mixed forest at elevations > 1000 m a.s.l. (*Fu et al., 2012*).

Survey methods

We looked for *P. mangshanensis* through the transect survey. After a snake was discovered, we recorded the GPS location accurately using a global positioning system (GPS) unit (Beijing UniStrong Science and Technology Co., Ltd, Beijing, China). We used head patch pattern as a reliable biometric character to recognize Mangshan pitviper individuals (*Yang et al., 2013*). Based on field surveys conducted from 2012 to 2016, we identified 83 locations from different individuals for seasonal habitat studies (20 sites surveyed in spring, 31 in summer, and 32 in autumn; Fig. 1) using 10 m × 10 m plots. Plots used by snake individuals (used plots) were placed with the location used by *P. mangshanensis* as the center point. To compare used and available habitat, we conducted habitat studies at used plots and paired random plots (*Keating and Cherry, 2004*; *Johnson et al., 2006*). The direction and distance (between 50 and 150 m) of the paired plot from each used plot were determined using a random number generator (*Sprague & Bateman, 2018*). If the random plot occurred in an area that was not accessible to snakes, a new location was determined. Habitat variables were measured in used and paired random plots in April (spring), July (summer), and October (autumn) of 2015 and 2016.

Habitat variables

Based on a review of the current literature and data from our previous research (Fig. 2), we identified 14 important habitat variables for *P. mangshanensis* (*Baxley, Lipps & Qualls, 2011*; *Gardiner et al., 2015*; *Buchanan et al., 2017*; *Sutton et al., 2017*; *Sprague and Bateman, 2018*), including three categorical variables (gravel, slope aspect, and slope location) (Table S1)and 11 numerical variables. Habitat variables were measured as follows. (1) Gravel: rated based on the proportional area of gravel in sites where snakes take shelter. (2) Slope aspect: we walked vertically along the slope with a Samsung SM-T555C tablet computer (Seoul, Korea), looking at the direction of the pointer of Orux Map software (www.oruxmaps.com) using north as 0°, measuring clockwise from 0° to 360°. We defined 45°–134°, 135°–224°, 225°–314°, and 315°–44° as semi-sunny, sunny, semi-shady, and shady slopes, respectively. (3) Slope location was divided into three categories according to the elevation: upper, middle, and lower slope, including the upper, middle, and lower third of the hillside, respectively. (4) Elevation was obtained at the center of the plots by Orux Map software. (5) Slope gradient was measured from the lowest to the highest point in each plot using a Nikon Forestry 550 laser rangefinder (Nikon, Tokyo, Japan). (6) Distance to water: the linear distance between the plot and the nearest water was measured using a Nikon Forestry 550 laser rangefinder. (7) Tree density was measured as the number of trees in each plot/100 m^2 with diameter at breast height >4 cm. (8) Fallen log density: the number of fallen logs in each plot (diameter >4 cm). (9) Canopy cover: in the four corners and the center of the plot, we surveyed the vertical projection of trees and averaged the measured value. (10), (11), (12), (13), and (14) Shrub density, shrub height, herb cover, herb height, and deciduous coverage were measured in five small plots (1 m × 1 m) at the four corners and the center of the plot, with an average calculated for each variable. Shrub density was measured as the total number of shrubs in each small plot. Shrub height was the average height of shrubs in each small plot. Herb cover was the percent of herbaceous ground cover in each small plot/small plot area. Herb height was the average of maximum height of herbs in each small plot. Deciduous coverage was the area of deciduous leaf cover in each small plot/small plot area.

Statistical analyses

Kolmogorov-Smirnov Test of a Single Sample was used to test the normal distribution of the 11 numerical variables. Seasonal differences between habitat variables in used versus paired plots were compared using chi-square tests (for categorical variables), paired sample *t*-tests (for numerical variables consistent with normal distribution) and Mann-Whitey U Test in nonparametric test (for numerical variables that do not consistent with normal distribution). Statistical results were corrected by Bonferroni correction. Statistical significance was categorized as follows: extremely significant ($P \leq 0.01$), significant ($P \leq 0.05$), and insignificant ($P > 0.05$).

To determine the most important factors affecting the seasonal habitat selection of *P. mangshanensis*, the numerical variables need to be screened by principal component analysis (PCA) (Table S2) and Pearson correlation tests (Table S3-6). Principal Component Analysis was carried out for 11 numerical variables in spring, summer, autumn, and three seasons, respectively (Table S2). The principal component with the highest contribution rate was selected and the standardized eigenvectors of each variable were obtained. Then, paired Pearson correlation tests were performed for 11 numerical variables. If there was correlation between the two variables, the variable with larger eigenvectors was retained (Table S3-6). Finally, there were seven numerical variables retained in spring (elevation, distance to water, deciduous coverage, tree density, fallen log density, canopy cover, and shrub density). Eight numerical variables were retained in summer (elevation, distance to water, tree density, fallen log density, canopy cover, shrub density, shrub height and herb cover). Seven numerical variables were retained in autumn (elevation, slope gradient, distance to water, tree density, fallen log density, shrub height and herb cover). Nine numerical variables were retained in three seasons (elevation, slope gradient, distance to water, deciduous coverage, tree density, fallen log density, canopy cover, shrub height, and herb cover).

After screening numerical variables from spring, summer, and autumn, respectively, data were analyzed using binomial logistic regression to determine which factors have the strongest effect on habitat selection of Mangshan pitviper in different seasons. All possible models were considered for snakes that affect their habitat selection (R 3.5.1, The R Foundation for Statistical Computing, package “rJava, glmulti, and MuMIn”). The models were screened by Akaike's Information Criterion (AIC). The AIC, AICc (for small sample sizes, use the second-order AIC), ΔAICc ($\Delta AICc_i = AICc_i - \min AICc$, where $AICc_i$ is the AICc value for model i, and min AICc is the AICc value of the “best” model), and Akaike Weights (w_i, probability of becoming the “best” model in the candidate models) of each model were calculated, respectively. Two measures associated with the AIC can be used to compare models: the ΔAICc and w_i. The “best” model had a ΔAICc = 0, but we also considered all models with a ΔAICc < 2 (*Burnham & Anderson, 2002*; *Mazerolle, 2006*). Additionally, ecological variables in three seasons (data from used plots and random plots) were compared using canonical discriminant analysis to obtain canonical discriminant function coefficients of a linear combination of ecological variables. Any data that did not conform to a normal distribution was standardized. All data were processed by Excel 2016 and analyzed using SPSS 20.0, IBM.

Results

Habitat selection in spring

At the 100 m^2 plot scale, fallen log density ($t = 3.90$, df = 19, $P = 0.002$) affected the probability of habitat selection by Mangshan pitviper positively, while slope gradient ($t = -4.41$, df = 19, $P < 0.001$), distance to water ($t = -3.26$, df = 19, $P = 0.008$), shrub height ($t = -4.49$, df = 19, $P = 0.000$), herb cover ($t = -2.63$, df = 19, $P = 0.034$), and herb height ($t = 3.68$, df = 19, $P = 0.004$) had a negative effect (Table 1). The “best” model with min ΔAICc shows that distance to water and deciduous coverage were most important variables affecting habitat selection of *P. mangshanensis* (Table 2). The statistics related to the relative important values for each variable used in the models show that the contribution rate of distance to water and fallen log density were relatively high, followed by deciduous coverage, and the contribution rate of the tree density was relatively small (Table 3). In spring, snakes tended to select habitats near water and with a relatively high fallen log density (Table 1-3).

Habitat selection in summer

Significant or extremely significant differences were observed in gravel ($X^2 = 5.994$, df = 2, $P = 0.05$), fallen log density ($t = 3.69$, df = 30, $P = 0.002$), shrub density ($t = 3.02$, df = 30, $P = 0.010$), and shrub height ($t = 2.36$, df = 30, $P = 0.050$) between used and paired random plots (Table 1). According to the "best" model, fallen log density, shrub density, and canopy cover were most important for habitat selection of snakes (Table 2). The statistics of relative important values for each variable used in the models show that the contribution rates of fallen log density and shrub density were relatively high, followed by shrub height, indicating the Mangshan pitviper's preference for these variables; meanwhile, the contribution rate of the canopy cover was relatively low (Table 3). Snakes most frequently selected the habitats with relatively high fallen log density, shrub density, and shrub height in summer, indicating the prediction probability of habitat selection by the snakes (Table 1-3).

Habitat selection in autumn

Habitat variables in used versus paired plots were significant differences were observed in fallen log density ($t = 2.91$, df = 31, $P = 0.014$), shrub height ($t = 2.70$, df = 31, $P = 0.022$), and distance to water ($t = -2.55$, df = 31, $P = 0.032$) (Table 1). Two optimal models were constructed and the "best" model shows that fallen log density and shrub height were most important for habitat selection of *P. mangshanensis*, proving that snakes have strong selectivity for these variables (Table 2). The statistics of relative important values were consistent (Table 3). In autumn, snakes significantly preferred the habitats with relatively high fallen log density and shrub height that were relatively close to water (Table 1-3).

Comparison of habitat selection variables in different seasons

The main ecological variables affecting habitat selection of Mangshan pitviper were shown to change with seasons (Table 1–3). Deciduous coverage and tree density are important ecological variables affecting snake habitat selection only in spring. Shrub density and canopy cover are important ecological variables affecting snake habitat selection only in summer. Fallen log density is the only important ecological variable affecting snake habitat selection in all three seasons, which proves that the habitat element is preferred by snakes and not affected by seasons. Results from the stepwise discriminant function analyses showed that spring, summer, and autumn can be distinguished by two typical discriminant functions (Table 4). The first discriminant function could distinguish the three seasons significantly with a contribution rate of 98.2%. Based on the contribution rates, the important variables for the seasonal discrimination were canopy cover and herb cover (Table 4). The discriminant plot established by two discriminant functions (Fig. 2) showed that habitat selection variables were effectively separated in spring, summer, and autumn. Among them, there was less overlap among the variables between spring and summer or between autumn and summer.

Discussion

Due to the heterogeneity of the environment, habitat selection of *P. mangshanensis* varied in different seasons. Overall, snakes selected habitat that was close to water, had a relatively high fallen log density, low shrub as well as herb height, less herb cover, and had a relatively gentle slope gradient in spring. In summer, they selected habitats with relatively more gravel, higher fallen log density, higher shrub density, and higher shrub height. In autumn, they tended to select habitats with a relatively high fallen log density, shrub height, and that were relatively close to water.

Shelter and water considerations

Snakes usually choose rocks, vegetation, and burrows as shelter (*Webb, Shine & Pringle, 2005*; *Hyslop et al., 2009*). The need for thermoregulation and the location of potential prey influenced the site selection of snakes seeking shelter (*Whitaker & Shine, 2003*; *Webb, Shine & Pringle, 2005*). A lack of adequate shelter can perturb behaviors, increase stress levels, and thus alter physiological performance (e.g. digestive, immune, or reproductive functions) for snakes (*Bonnet, Fizesan & Michel, 2013*). Our data indicated that *P. mangshanensis* tended to select habitat with relatively high fallen log density in all three seasons analyzed here. The snakes were also often discovered beside fallen logs in the field and gave priority to fallen logs when crawling according to our observation (Fig. 2). The body color of the snakes is similar to that of a fallen log, which makes it difficult to find them. Therefore, one of the most appropriate types of shelter for this snake species is fallen logs. This habitat element was preferred by snakes, and they consistently selected it in all three seasons. The habitat element preferred by animals is related to animal's response to habitat heterogeneity (*Price-Rees, Brown & Shine, 2013*). Resources may be unevenly distributed in space in habitats within the home range of animals, so that the animals must move about to seek the best locations, which can influence their acquisition of nutrients (*Sperry & Weatherhead, 2009*), perfect mimicry (*O'Hanlon, Herberstein & Holwell, 2013*; *Skelhorn and Ruxton, 2013*), and help with thermoregulation (*Ortega & Perez-Mellado, 2016*). As a sit-and-wait predator, Mangshan pitviper may have evolved phenotypic characteristics that lure prey to the snakes because the snake resembles a food item. The Mangshan pitviper has a white tail, but the body color and markings are similar to the moss on fallen logs. Mangshan pitviper may also elicit predatory responses from prey by waving the distal portion of their tails, just like other snakes (*Nelson, Garnett & Evans, 2010*). Therefore, we predict that the snakes prefer fallen logs that maximize the efficacy of their deceptive signal and the likelihood that signal receivers are successfully deceived, which is an optimal foraging strategy and under optimal foraging theory (*O'Hanlon, Herberstein & Holwell, 2013*). In brief, fallen logs may offer *P. mangshanensis* protection from predators and convenience for predation.

Water availability and distribution are important determinants of behavior and habitat selection in snakes (*Halstead, Wylie & Casazza, 2010*; *Sprague & Bateman, 2018*). Our data indicated that *P. mangshanensis* tended to select habitat that was relatively close to water in spring and autumn, which indicated that their habitat selection behavior was affected by the distribution of water (Table 1). In addition, based on four months of continuous tracking of three individuals, Mangshan pitviper did not visit streams to drink water or regulate body temperature. We observed that small mammals were more abundant in habitats that were relatively close to water. Such habitats might provide improved foraging opportunities to Mangshan pitviper.

Thermoregulatory aspects of habitat selection

Because snakes are ectotherms, thermoregulation of snakes directly affects their movement, digestion, growth rate, physiological behavior, and habitat selection (*Webb & Shine, 1998*; *Blouin-Demers & Weatherhead, 2008*; *Row & Blouin-Demers, 2006*; *Lelièvre et al., 2011*). To achieve their preferred temperatures during different physiological periods, snakes selected habitats with certain thermodynamic characteristics (*Bruton et al., 2014*). For example, the broad-headed snakes (*Hoplocephalus bungaroides*) actively selected thin (< 15-cm thick) unshaded rocks in spring and avoided thin exposed rocks when temperatures exceeded 40°C in the summer (*Webb & Shine, 1998*).

Habitats with the preferred thermal conditions can provide greater fitness rewards, in terms of both reproductive output and growth rate (*Paterson & Gabriel, 2018*; *Sprague & Bateman, 2018*). Nocturnal reptiles use sun-exposed shelters for diurnal thermoregulation, especially in spring (*Webb, Shine & Pringle, 2005*). Compared with summer and autumn, in spring *P. mangshanensis* tended to choose a more open habitat with a lower slope gradient, shrub height, herb cover, and herb height (Table 1), and so they had more opportunities to select basking spots in the sunshine and enhance their body temperature (Fig. 2C). In summer, when the air temperatures were higher and there was less demand for sunlight, snakes tended to select habitats with more gravel, greater shrub density, and higher shrub density. Thermoregulation is an important factor affecting the habitat selection in nocturnal Mangshan pitviper.

Seasonal habitat differences

The role of seasonality in habitat selection of ectotherms is important, and their habitat needs may change seasonally (*Brito, 2009*; *Hyslop et al., 2009*; *Sprague & Bateman, 2018*). The ecological variables making an important contribution to habitat selection of *P. mangshanensis* changed with seasons (Table 1-3; Fig. 3). The seasonal variation in their habitat selection was unique compared with other snakes (*Row & Blouin-Demers, 2006*; *Hyslop, Cooper & Meyers, 2009*; *Sprague & Bateman, 2018*). In spring, *P. mangshanensis* chose more open habitat with relatively close to water with high fallen log density, and more gently sloping habitats, possibly for foraging, economizing on physical energy, and thermoregulation, which was different from their selected habitats in summer and autumn (Table 1-3). Unfortunately, such habitats also allow poachers to more easily find individual snakes. Their habitat selection in autumn was similar to that in spring. In spring and autumn, the snakes tended to select habitats with a relatively high fallen log density that were relatively close to water, which may be beneficial in that the cryptic coloration of the snakes may aide in their search for food. These results emphasize that habitat selection of snakes change with season, and the important environmental variables affecting the habitat selection of snakes during each season are different, which also reflect the behavioral flexibility of snakes to adapt to seasonal variations. Among the three seasons analyzed here, little overlap was observed between the variables that were important in summer and the other two seasons (Fig. 3). The variables that caused this difference were mainly canopy cover and herb cover, which showed that the snake's requirements for vegetation cover in summer were different from those in the other two seasons. This difference may be due to seasonal variations in solar radiation, which also leads to changes in environmental temperature and plant growth (*Ortega & Perez-Mellado, 2016*). Environmental factors or structural resources that make up habitats are constantly changing. In order to meet their needs for survival needs, snakes choose habitats accordingly. In any case, our results studying the mechanism responsible for habitat requirements of *P. mangshanensis* during spring, summer, and autumn support the conclusion that seasonality is the most important factor affecting habitat selection of this snake.

Conclusions

Mangshan pitviper select an open habitat with relatively gently sloping gradients and closer distance to water in spring, shaded habitat (relatively high gravel ground cover as well as high vegetation density and cover) to avoid high temperatures in summer, and relatively high shrub height in autumn. The distribution of water affects habitat selection of *P. mangshanensis* in spring and autumn. *P. mangshanensis* prefer fallen logs as a shelter in spring, summer, and autumn. Seasonal variations significantly affected the habitat selection of *P. mangshanensis*. Canopy cover and herb cover were the main factors leading to seasonal differences in the habitat selection of *P. mangshanensis*. Based on the habitat selection requirements of *P. mangshanensis* and the current management status of the Mangshan Reserve, we offer the following suggestions for the continued conservation of this critically endangered snake species. (1) Snakes often were disturbed or caught by bamboo shoot collectors and poachers in spring. The Administration Bureau of Mangshan Reserve must actively work to reduce the illegal collection of bamboo shoots and prevent poaching in the Mangshan Reserve. (2) A scientifically sound plan should be designed to promote the value of ecological tourism but at the same time prevent damage to vegetation and changes in the distribution of streams. (3) Some of the natural shelters used by this species have been destroyed by the construction of hydropower stations, man-made water channels, and tourist trails, so methods should be explored to rehabilitate the lost or degraded habitat of *P. mangshanensis* by building artificial shelters that mimic the appropriate physical characteristics of their preferred fallen log shelters.

Acknowledgements

We thank Prof. Richard Shine and Prof. Melanie Elphick for providing kind help with references and manuscript preparation, and Dr. Yayong Wu, for providing valuable advice on this manuscript. We express special appreciation to Guoxing Deng, Jianguo Tan, Tianbin Liu, Desheng Chen, and Yuanhui Chen from Administration Bureau of Hunan Mangshan National Nature Reserve for their assistance in field work.

References

Ali AH, Ford AT, Evans JS, Mallon DP, Hayes MM, King J, Amin R, Goheen JR. 2017. Resource selection and landscape change reveal mechanisms suppressing population recovery for the world's most endangered antelope. *Journal of Applied Ecology* **54:**1720-1729 DOI 10.1111/1365-2664.12856.

Baxley DL, Lipps GJ, Qualls CP. 2011. Multiscale Habitat Selection by Black Pine Snakes (*Pituophis melanoleucus lodingi*) in Southern Mississippi. *Herpetologica* **67:**154-166 DOI 10.1655/herpetologica-d-10-00029.1
Blouin-Demers G, Weatherhead PJ. 2008. Habitat use is linked to components of fitness through the temperature dependence of performance in ratsnakes (*Elaphe obsoleta*). *Israel Journal of Ecology & Evolution* **54:**361-372 DOI 10.1560/ijee.54.3-4.361.
Bonnet X, Fizesan A, Michel CL. 2013. Shelter availability, stress level and digestive performance in the aspic viper. *Journal of Experimental Biology* **216:**815-822 DOI 10.1242/jeb.078501.
Brito JC. 2009. Seasonal variation in movements, home range, and habitat use by male *Vipera latastei* in Northern Portugal. *Journal of Herpetology* **37:**155-160 DOI 10.1670/0022-1511(2003)037[0155:svimhr]2.0.co;2.
Bruton MJ, McAlpine CA, Smith AG, Franklin CE. 2014. The importance of underground shelter resources for reptiles in dryland landscapes: A woma python case study. *Austral Ecology* **39:**819-829 DOI 10.1111/aec.12150.
Burnham KP, Anderson DR. 2002. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. 2nd ed. Berlin Heidelberg: Springer-Verlag.
Chen SK, Yang DD, Yang WC, Chen YH. 2013. Artificial incubation and snakelet captive breeding of Mangshan pitviper (*Protobothrops mangshanensis*). *Chinese Journal of Ecology* **32:**3048-3053.
Doligez B, Danchin E, Clobert J. 2002. Public information and breeding habitat selection in a wild bird population. *Science* **297:**1168-1170 DOI 10.1126/science.1072838.
Fu Q, Yan DD, Fei DB, Mo JW, Song YC. 2012. Amphibian resources in Mangshan National Nature Reserve of Hunan Province. *Chinese Journal of Zoology* **47:**62-67.
Gardiner LE, Somers CM, Parker DL, Poulin RG. 2015. Microhabitat Selection by Prairie Rattlesnakes (*Crotalus viridis*) at the Northern Extreme of their Geographic Range. *Journal of Herpetology* **49:**131-137 DOI 10.1670/12-266.
Gong SP, Yang DD, Chen YH, Lau M, Wang FM. 2013. Population status, distribution and conservation needs of the endangered Mangshan pit viper *Protobothrops mangshanensis* of China. *Oryx* **47:**122-127 DOI 10.1017/s0030605311001037.
Harvey DS, Weatherhead PJ. 2006. A test of the hierarchical model of habitat selection using eastern massasauga rattlesnakes (*Sistrurus c. catenatus*). *Biological Conservation* **130:**206-216 DOI 10.1016/j.biocon.2005.12.015.
Halstead BJ, Wylie GD, Casazza ML. 2010. Habitat suitability and conservation of the giant gartersnake (*Thamnophis gigas*) in the sacramento valley of California. *Copeia* **2010:**591-599.
Hecnar SJ, Hecnar DR. 2011. Microhabitat Selection of Woody Debris by Dekay's Brownsnake (*Storeria dekayi*) in a Dune Habitat in Ontario, Canada. *Journal of Herpetology* **45:**478-483 DOI 10.1670/10-219.1.

Hyslop NL, Cooper RJ, Meyers JM. 2009. Seasonal shifts in shelter and microhabitat use of *Drymarchon couperi* (eastern indigo snake) in Georgia. *Copeia* **2009:**458-464 DOI 10.1643/ch-07-171.

Jiang ZG, Jiang JP, Wang YZ, Zhang E, Zhang YY, Li LL, Xie F, Cai B, Cao L, Deng GM, Dong L, Zhang ZW, Ding P, Luo ZH, Ding CQ, Ma ZJ, Tang SH, Cao WX, Li CW, Hu HJ, Ma Y,Wu Y, Wang YX, Zhou KY, Liu SY, Chen YY, Li JT, Feng ZJ, Wang Y, Wang B, Li C, Song XL, Cai L, Zang CX, Zeng Y, Meng ZB, Fang HX, Ping XG. 2016. Red List of China's Vertebrates. *Biodiversity Science* **24:** 500–551 DOI 10.17520/biods.2016076.

Johnson CJ, Nielsen SE, Merrill EH, Mcdonald TL, Boyce MS. 2006. Resource selection functions based on use-availability data: theoretical motivation and evaluation methods. *Journal of Wildlife Management* **70:**347-357 DOI 10.2193/0022-541x(2006)70[347:rsfbou]2.0.co;2.

Keating KA, Cherry S. 2004. Use and interpretation of logistic regression in habitat-selection studies. *Journal of Wildlife Management* **68:**774-789 DOI 10.2193/0022-541x(2004)068[0774:uaiolr]2.0.co;2.

Leal M, Fleishman LJ. 2003. Differences in visual signal design and detectability between allopatric populations of *Anolis* lizards. *American Naturalist* **163:**26-39 DOI 10.1086/379794.

Leite GA, Farias IP, Goncalves AL, Hawes JE, Peres CA. 2018. Coarse- and fine-scale patterns of distribution and habitat selection places an Amazonian floodplain curassow in double jeopardy. *PeerJ* **6:**e4617 DOI 10.7717/peerj.4617

Lelièvre H, Blouin-Demers G, Pinaud D, Lisse H, Bonnet X, Lourdais O. 2011. Contrasted thermal preferences translate into divergences in habitat use and realized performance in two sympatric snakes. *Journal of Zoology* **284:**265-275 DOI 10.1111/j.1469-7998.2011.00802.x.

Lunghi E, Manenti R, Ficetola GF. 2015. Seasonal variation in microhabitat of salamanders: environmental variation or shift of habitat selection? *PeerJ* **3:**e1122 DOI 10.7717/peerj.1122

Mandlate LC Jr, Cuamba EDL, Rodrigues FHG. 2019. Postrelease monitoring habitat selection by reintroduced burchell's zebra and blue wildebeest in southern mozambique. *Ecology and Evolution* **00:**1–10 DOI 10.1002/ece3.5221

Martin TE. 1998. Are microhabitat preferences of coexisting species under selection and adaptive? *Ecology* **79:**656–670 DOI 10.2307/176961.

Mazerolle MJ. 2006. Improving data analysis in herpetology: using Akaike's Information Criterion (AIC) to assess the strength of biological hypotheses. *Amphibia-reptilia* **27:**169-180 DOI 10.1163/156853806777239922.

Mebs D, Kuch U, Coronas FI, Batista CV, Gumprecht A, Possani LD. 2006. Biochemical and biological activities of the venom of the Chinese pitviper Z*haoermia mangshanensis*, with the complete amino acid sequence and phylogenetic analysis of a novel Arg49 phospholipase A_2 myotoxin. *Toxicon* **47:**797-811 DOI 10.1016/j.toxicon.2006.01.031.

Miller GJ, Smith LL, Johnson SA, Franz R. 2012. Home range size and habitat selection in the Florida Pine Snake. ***Copeia*** **2012:**706-713 DOI 10.1643/ce-12-054.

Murakami M, Kuch U, Betzel C, Mebs D, Arni R. 2008. Crystal structure of a novel myotoxic Arg49 phospholipase A_2 homolog (zhaoermiatoxin) from *Zhaoermia mangshanensis* snake venom: insights into Arg49 coordination and the role of Lys122 in the polarization of the C-terminus. *Toxicon* **51:**723-735 DOI 10.1016/j.toxicon.2007.11.018.

Nelson XJ, Garnett DT, Evans CS. 2010. Receiver psychology and the design of the deceptive caudal luring signal of the death adder. *Animal Behaviour* **79:**555-561 DOI 10.1016/j.anbehav.2009.12.011.

O'Hanlon JC, Herberstein ME, Holwell GI. 2015. Habitat selection in a deceptive predator: maximizing resource availability and signal efficacy. *Behavioral Ecology* **26:**194-199 DOI 10.1093/beheco/aru179.

Ortega Z, Pérez-Mellado V. 2016. Seasonal patterns of body temperature and microhabitat selection in a lacertid lizard. *Acta Oecologica* **77:**201-206 DOI 10.1016/j.actao.2016.08.006.

Ortega Z, Mencia A, Perezmellado V. 2016. Adaptive seasonal shifts in the thermal preferences of the lizard *Iberolacerta galani* (Squamata, Lacertidae). *Journal of Thermal Biology* **62:**1-6 DOI 10.1016/j.jtherbio.2016.10.005.

Paterson JE, Gabriel BD. 2018. Density-dependent habitat selection predicts fitness and abundance in a small lizard. *Oikos* **127:**448-459 DOI 10.1111/oik.04758.

Raynor EJ, Beyer HL, Briggs JM, Joern A. 2017. Complex variaton in habitat selecton strategies among individuals driven by extrinsic factors. *Ecology and Evolution* **7:**1802-1822 DOI 10.1002/ece3.2764.

Rechetelo J, Grice A, Reside AE, Hardesty BD, Moloney J. 2016. Movement patterns, home range size and habitat selection of an endangered resource tracking species, the black-throated finch (*Poephila cincta cincta*). *PloS One* **11:**e0167254 DOI 10.1371/journal.pone.0167254.

Price-Rees SJ, Brown GP, Shine R. 2013. Habitat selection by bluetongue lizards (*Tiliqua*, scincidae) in tropical Australia: a study using GPS telemetry. *Animal Biotelemetry* **1:**7 DOI 10.1186/2050-3385-1-7.

Shine R, Sun LX, Zhao E, Bonnet X. 2002. A review of 30 years of ecological research on the Shedao pitviper, *Gloydius shedaoensis*. *Herpetological Natural History* **9:**1-14.

Skelhorn J, Ruxton GD. 2013. Size-dependent microhabitat selection by masquerading prey. *Behavioral Ecology* **24:**89-97 DOI 10.1093/beheco/ars139.

Sperry JH, Weatherhead PJ. 2009. Does prey availability determine seasonal patterns of habitat selection in Texas Ratsnakes. *Journal of Herpetology* **43:**55-64 DOI 10.1670/08-058r1.1.

Sprague TA, Bateman HL. 2018. Influence of seasonality and gestation on habitat selection by northern Mexican gartersnakes (*Thamnophis eques megalops*). *PloS One* **13:**e0191829 DOI 10.1371/journal.pone.0191829.

Sun SP, Zhang L, Hou W, Feng GL. 2011. A non-linear similarity method for season division in China. *Acta Physica Sinica* **60:**803-809.

Sutton WB, Wang Y, Schweitzer CJ, Mcclure CJ. 2017. Spatial ecology and multi-scale habitat selection of the Copperhead (*Agkistrodon contortrix*) in a managed forest landscape. *Forest Ecology and Management* **391:**469-481 DOI 10.1016/j.foreco.2017.02.041.

Valenta J, Stach Z, Otahal M. 2012. *Protobothrops mangshanensis* bite: first clinical report of envenoming and its treatment. *Biomedical Papers-Olomouc* **156:**183-185 DOI 10.5507/bp.2012.021.

Wasko DK, Sasa M. 2012. Food resources influence spatial ecology, habitat selection, and foraging behavior in an ambush-hunting snake (Viperidae: *Bothrops asper*): an experimental study. *Zoology* **115:**179-187 DOI 10.1016/j.zool.2011.10.001.

Weatherhead PJ, Brawn JD. 2006. Habitat use and activity of prairie kingsnakes (*Lampropeltis calligaster calligaster*) in illinois. *Journal of Herpetology* **40:**423-428 DOI 10.1670/0022-1511(2006)40[423:huaaop]2.0.co;2.

Webb JK, Shine R. 1998. Using thermal ecology to predict retreat-site selection by an endangered snake species. *Biological Conservation* **86:**233-242 DOI 10.1016/s0006-3207(97)00180-8.

Webb JK, Shine R, Pringle RM. 2005. Canopy removal restores habitat quality for an endangered snake in a fire suppressed landscape. *Copeia* **2005:**894-900 DOI 10.1643/0045-8511(2005)005[0894:crrhqf]2.0.co;2.

Whitaker PB, Shine R. 2003. A radiotelemetric study of movements and shelter-site selection by free-ranging brownsnakes (*pseudonaja textilis*, elapidae). *Herpetological Monographs* **17:**130-144 DOI 10.1655/0733-1347(2003)017[0130:arsoma]2.0.co;2.

Willems EP, Hill RA. 2009. Predator-specific landscapes of fear and resource distribution: effects on spatial range use. *Ecology* **90:**546–555 DOI 10.1890/08-0765.1.

Yang DD, Chen SK, Chen YH, Yan YY. 2013. Using head patch pattern as a reliable biometric character for noninvasive individual recognition of an endangered pitviper *Protobothrops mangshanensis*. *Asian Herpetological Research* **4:**134-139 DOI 10.3724/sp.j.1245.2013.00134.

Figure 1

Figure 1 Distribution map of habitat study sites of *Protobothrops mangshanensis* showing used versus control plots in different seasons.

The coincidence plot indicated where snake individuals were located in the same sites for two or three seasons. All the snake individuals were found only in the eastern part of the Hunan Mangshan National Nature Reserve. The core zone is the most strictly protected part of the reserve. The buffer zone surrounds or is contiguous to the core area. Only scientific research is allowed in the buffer zone. The experimental zone is the outer area of the reserve and is less strictly regulated. An inset map shows the general location of the study area in China.

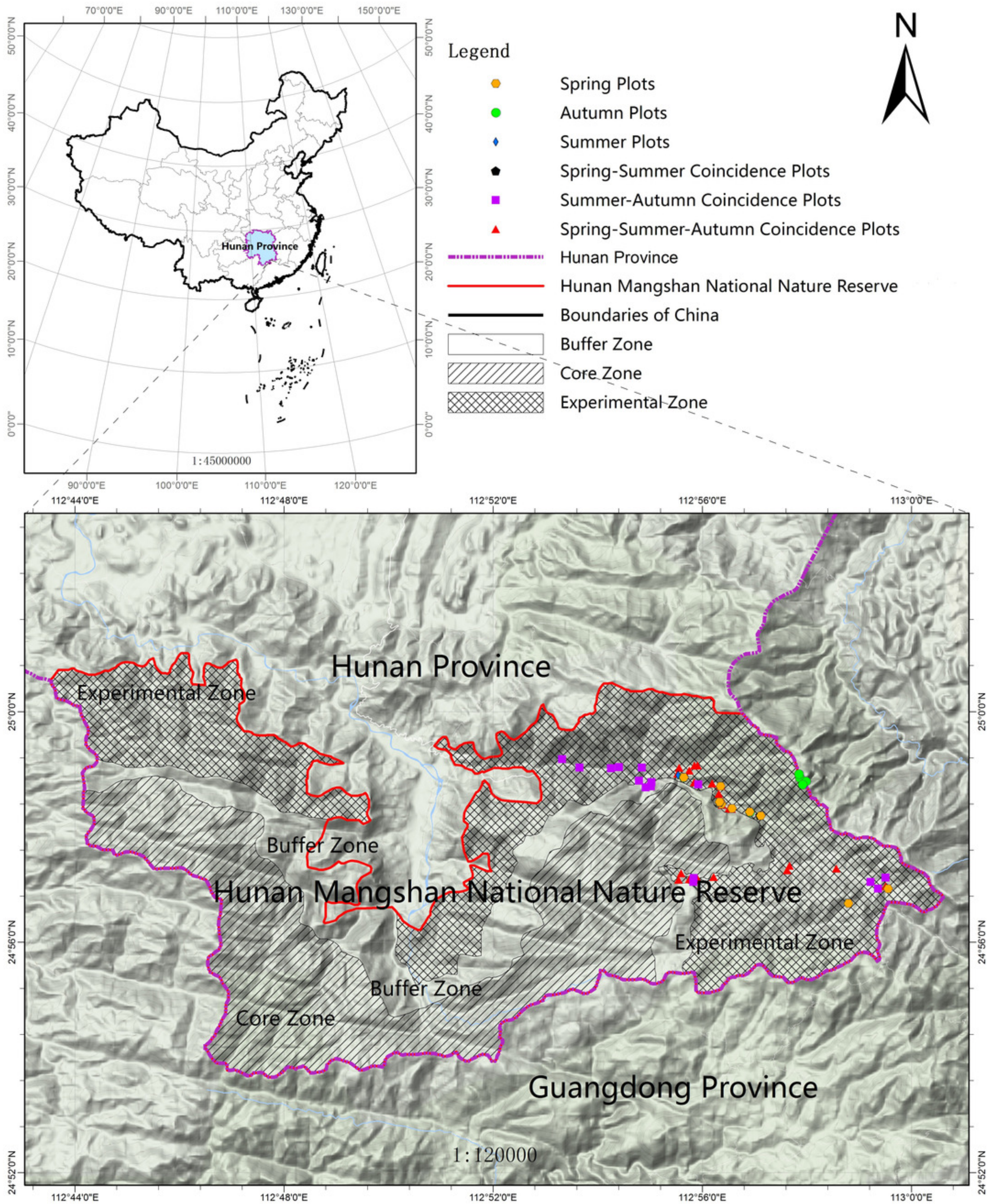
Legend
Spring Plots
Autumn Plots
Summer Plots
Spring-Summer Coincidence Plots
Summer-Autumn Coincidence Plots
Spring-Summer-Autumn Coincidence Plots
Hunan Province
Hunan Mangshan National Nature Reserve
Boundaries of China
Buffer Zone
Core Zone
Experimental Zone
N
Hunan Province
1:45000000
Hunan Province
Experimental Zone
Buffer Zone
Hunan Mangshan National Nature Reserve
Experimental Zone
Buffer Zone
Core Zone
Guangdong Province
1:120000
112°44'0"E
112°48'0"E
112°52'0"E
112°56'0"E
113°0'0"E
25°0'0"N
24°56'0"N
24°52'0"N

Figure 2

Figure 2 The Mangshan pit viper (*Protobothrops mangshanensis*) and its habitats.

The body color of Mangshan Pit viper blends well into the surrounding environment. a) Typical habitats of the Mangshan Pit viper; b) an individual Mangshan Pit viper on fallen log; c) an individual Mangshan Pit viper selects a basking spot in sunshine and enhances its body temperature; d) an individual Mangshan pit viper crawling on a fallen log.

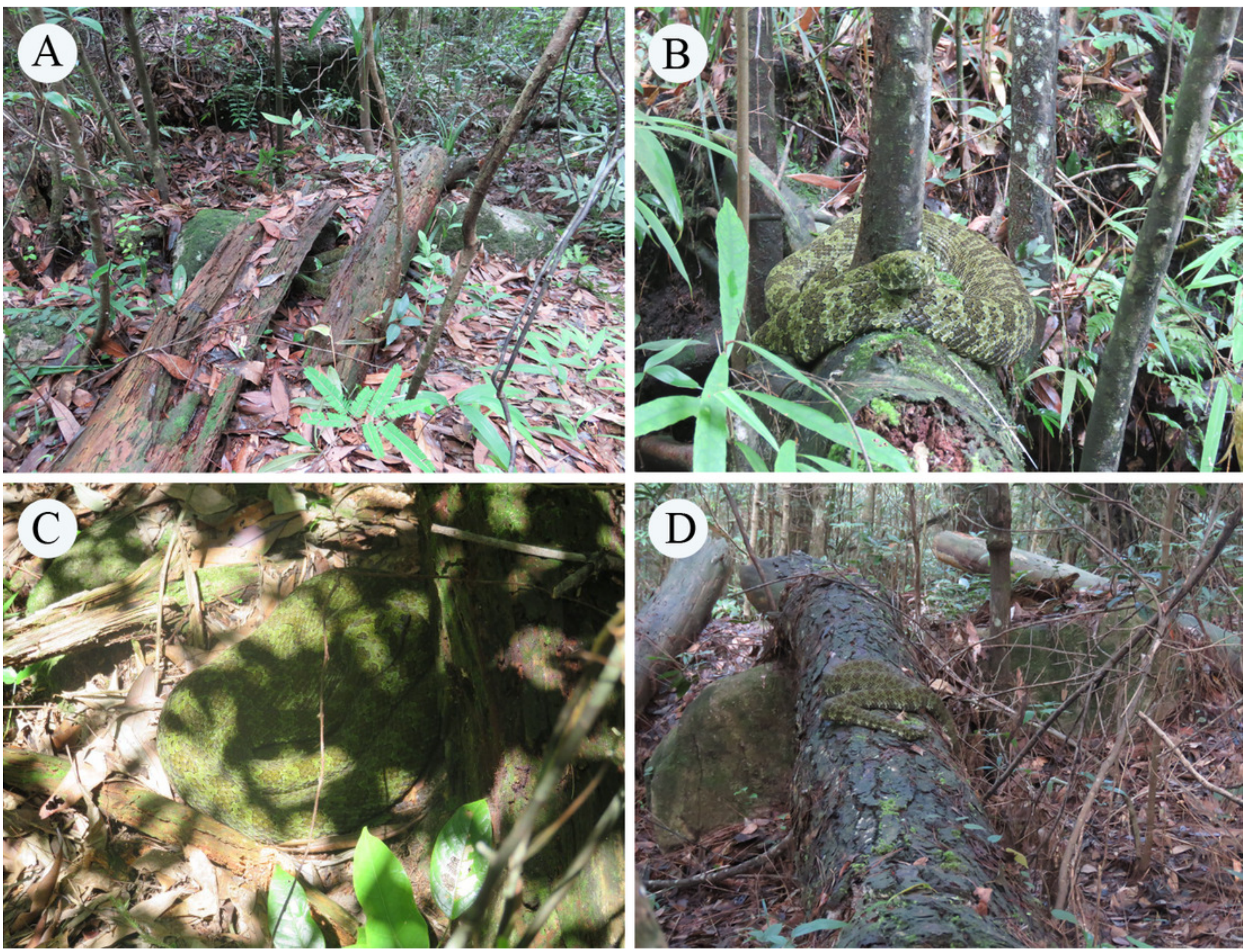

Figure 3

Figure 3 Seasonal discrimination in the habitats of the Mangshan pit viper (*Protobothrops mangshanensis*).

The results of seasonal discrimination analysis were calculated by the numerical ecological factor in plots used during spring, summer, and autumn.

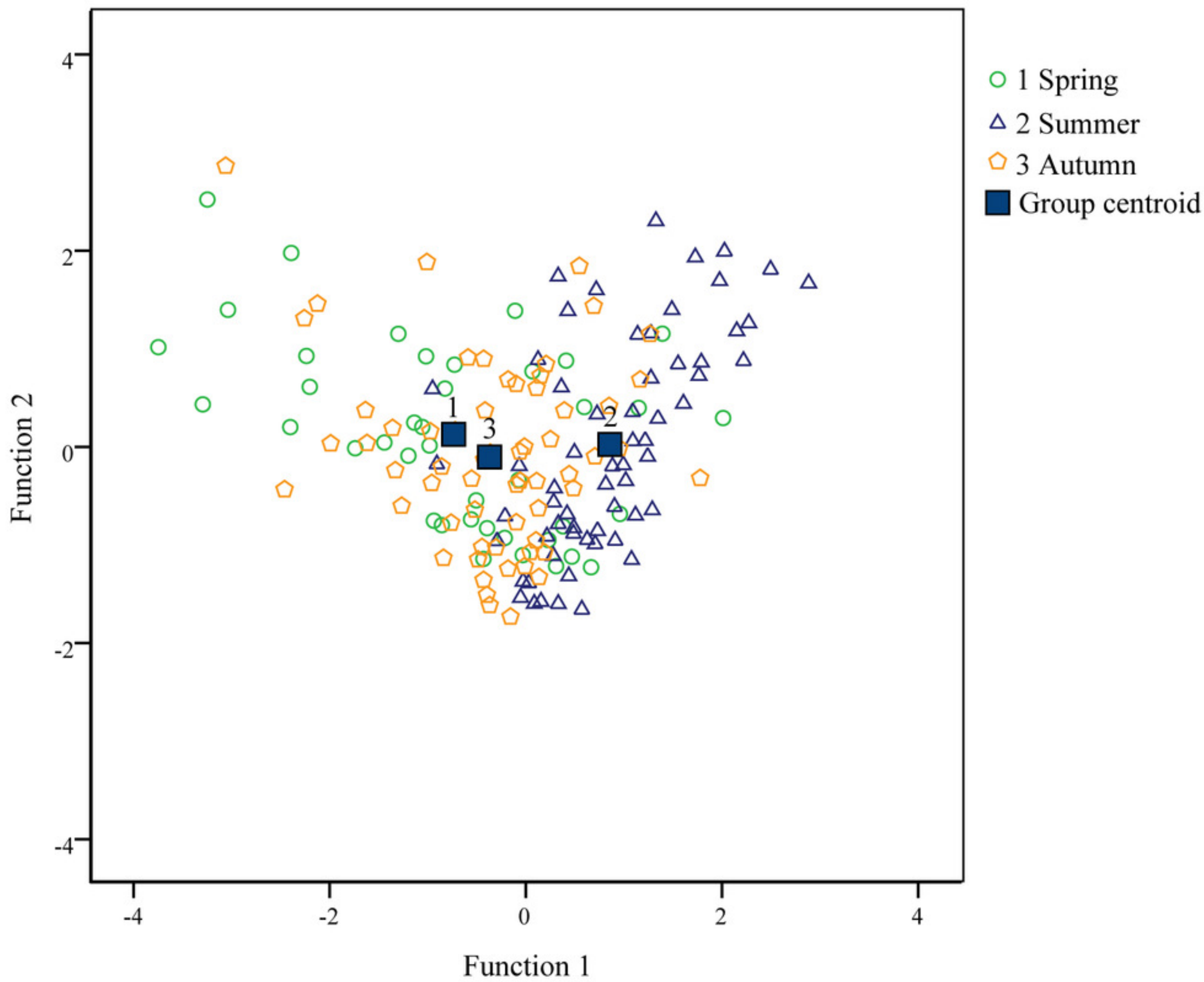

Table 1(on next page)

Table 1 Descriptive statistics and comparison of values of twelve ecological variables in used versus paired plots for habitat of the Mangshan pitviper (*Protobothrops mangshanensis*) in three different seasons (mean ± SE).

* Significant difference between habitat variables in used versus paired plots ($p \leq 0.05$). ** Extremely significant difference between habitat variables in used versus paired plots ($p \leq 0.01$).

Table 1 Descriptive statistics and comparison of values of twelve ecological variables in used versus paired plots for habitat of the Mangshan pitviper (*Protobothrops mangshanensis*) in three different seasons (mean ± SE). * Significant difference between habitat variables in used versus paired plots ($p \leqslant 0.05$). ** Extremely significant difference between habitat variables in used versus paired plots ($p \leqslant 0.01$).

Ecological variable	Spring			Summer			Autumn		
	Used plots (*n*=20)	paired plots (*n*=20)	*P*-value	Used plots (*n*=31)	paired plots (*n*=31)	*P*-value	Used plots (*n*=32)	paired plots (*n*=32)	*P*-value
Elevation (m)	1039±38	1025±41	0.696	992±48	988±49	0.144	1064±57	1061±57	0.477
Slope gradient (°)	17.66±1.67	27.78±2.11	<0.01**	29.59±2.68	28.09±3.07	0.665	28.23±2.39	27.60±2.76	0.849
Distance to water (m)	16.70±1.83	26.70±2.27	0.008**	26.58±5.19	31.58±5.19	0.096	24.38±1.02	30.25±2.37	0.032*
Deciduous coverage (%)	74.40±4.81	82.71±3.1	0.066	84.24±2.17	83.47±2.53	0.772	83.94±1.81	85.90±1.85	0.282
Tree density (trees/100 m^2)	18.45±1.37	17.45±1.3	0.515	18.50±1.44	18.16±1.47	0.845	19.48±1.45	18.47±1.36	0.586
Fallen log density (number/100 m^2)	7.85±0.79	4.80±0.51	0.002**	7.35±0.63	4.81±0.44	0.002**	7.18±0.42	5.43±0.46	0.014*
Canopy cover (%)	68.95±3.86	71.37±3.1	0.574	84.77±1.11	83.11±1.27	0.286	73.92±2.12	75.79±1.78	0.452
Shrub density (trees/m^2)	6.79±0.62	7.30±0.64	0.527	7.92±1.11	4.60±0.55	0.01**	7.78±0.63	7.18±0.62	0.418
Shrub height (cm)	136.48±5.59	163.39±7.26	<0.01**	187.90±6.11	173.54±6.58	0.05*	177.18±9.42	150.17±7.08	0.022*
Herb cover (%)	17.30±2.05	27.42±2.8	0.034*	30.13±3.18	35.46±3.58	0.27	21.68±2.49	22.26±2.26	0.848
Herb height (cm)	19.61±1.24	37.80±5.21	0.004**	30.32±3.7	28.28±2.45	0.665	30.04±3.24	31.55±3.19	0.747

Table 2(on next page)

Table 2 Models for predicting habitat selection by Mangshan pitviper (*Protobothrops mangshanensis*), Akaike's Information Criterion (AIC), and the difference of Akaike Weights (w_i) in three different seasons.

DW: distance to water; TD: tree density; FD: fallen log density; CC: canopy cover; SD: shrub density; SH: shrub height; DC: deciduous coverage; (+) and (-) indicate: the variables values of used plots were significantly or extremely higher or lower than those paired random plots, respectively. Models were screened according to Akaike's Information Criterion (AIC).

Table 2 Models for predicting habitat selection by Mangshan pitviper (*Protobothrops mangshanensis*), Akaike's Information Criterion (AIC), and the difference of Akaike Weights (w_i) in three different seasons. DW: distance to water; TD: tree density; FD: fallen log density; CC: canopy cover; SD: shrub density; SH: shrub height; DC: deciduous coverage; (+) and (-) indicate: the variables values of used plots were significantly or extremely higher or lower than those paired random plots, respectively. Models were screened according to Akaike's Information Criterion (AIC).

Seasonal	Model ID	Model	AIC	*K*	AICc	ΔAICc	Exp(ΔAICc)	w_i	Correctly classified (%)
Spring	1	DW(-) + DC	50.32	4	51.47	0.00	1.00	0.53	75.00
	2	DW(-) + FD(+)	51.67	6	52.81	1.34	3.82	0.27	70.00
	3	DW(-) + FD(+) + DC + TD	50.86	6	53.41	1.94	6.96	0.20	77.50
Summer	1	FD(+) + SD(+) + CC	84.06	5	83.37	0.00	1.00	0.44	69.40
	2	FD(+) + SD(+)	82.30	4	83.70	0.32	1.38	0.38	66.10
	3	FD (+)+ SD(+) + SH(+)	82.99	5	85.13	1.76	5.81	0.18	74.20
Autumn	1	FD(+) + SH(+)	86.65	4	87.35	0.00	1.00	0.50	75.00
	2	FD(+) + DW(-)	86.68	4	87.39	0.04	1.04	0.50	73.20

Table 3 (on next page)

Table 3 Relative important values for each variable used. Each sum stands for the sum of Akaike weights in all the models including the given variable.

Table 3 Relative important values for each variable used. Each sum stands for the sum of Akaike weights in all the models including the given variable.

Seasonal	Ecological variable	Sum of w_i
Spring	distance to water	1.00
	fallen log density	0.73
	deciduous coverage	0.47
	tree density	0.2
Summer	fallen log density	1.00
	shrub density	1.00
	shrub height	0.44
	canopy cover	0.18
Autumn	fallen log density	1.00
	shrub height	0.50
	distance to water	0.50

Table 4 (on next page)

Table 4 Results of the stepwise discriminant function analyses of numerical variables in the habitats of the Mangshan pitviper (*Protobothrops mangshanensis*) used for seasonal discrimination.

Table 4 Results of the stepwise discriminant function analyses of numerical variables in the habitats of the Mangshan pitviper (*Protobothrops mangshanensis*) used for seasonal discrimination.

Ecological variable	Discriminant function coefficient		Wilks' Lambda	*F*	*P*
	Function 1	Function 2			
Canopy cover	0.900	-0.495	0.793	21.262	=0.000
Herb cover	0.688	0.763	0.676	17.486	=0.000
Wilks' Lambda	0.676	0.992	–	–	–
F, df, *p*	63.528, 4, =0.000	1.362, 1, =0.242	–	–	–
Eigenvalue	0.466	0.008	–	–	–
Contribution rate	98.2%	1.80%	–	–	–
Cumulative contribution rate	98.2%	100.0%	–	–	–