

Effects of Sand Grain Size on Habitat Selection in Steppe Toad-headed Lizard (*Phrynocephalus frontalis*)

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Abstract To understand the effects of sand grain size on habitat selection, we conducted a field study on the steppe toad-headed lizard in Hunshandake Desert northern China. Methods of mark-recapture and transect survey were used to investigate the density of steppe toad-headed lizards and the environmental variables. The comparison on lizard densities among the habitats with different environmental factors revealed that: 1) population density of the steppe toad-headed lizard differed significantly among the habitats with different sand grain size indexes (SGSIs, representing roughness of sand substrate): the highest lizard density was found in the group with an SGSI of > 0.30, whereas the lowest density was found in the group with an SGSI of 0–0.15; and 2) vegetation cover, soil moisture, invertebrate diversity index, and abundance had no significant effects on the lizard density. These results implied that the sand grain size was the most important determinant of habitat selection for steppe toad-headed lizards in Hunshandake Desert. Steppe toad-headed lizards could avoid structural habitats that have negative effects on their maximal sprinting capabilities. Considering the changing sand grain size in the development phase of sand dunes, the sand lizard could be used as an indicator of the process of desertification.

Keywords habitat preference, agama lizard, sand substrate, desert grassland

1. Introduction

It is an important strategy for animals to select their habitats and survive in a changeable environment. Habitat selection has been widely studied in several taxa (Doligez *et al.*, 2002; Resetarits, 2005; Yahner, 2012; Beest *et al.*, 2015). A suitable habitat could temporarily improve the fitness and further support the development of evolutionary dynamics in a long time frame (Morris, 2003; Rodríguez-Robles *et al.*, 2005). Organisms occupy habitats that allow them to obtain sufficient food resources and reproductive opportunities and avoid predators and

competitors (Huey, 1991; Downes and Shine, 1998; Newbold and MacMahon, 2014).

Previous studies have demonstrated that habitat requirements are species-dependent (Huey, 1991; Yahner, 2012). As ectothermic animals, desert reptiles are lacking skin glands and live in arid ecosystems with low productivities due to little precipitation (Díaz *et al.*, 2006; Nemes *et al.*, 2006; Souter *et al.*, 2007; Lian *et al.*, 2012; Price-Rees *et al.*, 2013). Meanwhile, sand lizards, prevailing in the desert, are ideal species for studying habitat selection of desert reptiles (Nemes *et al.*, 2006; Gordon *et al.*, 2010; Korff and McHenry, 2011). Considering the importance of habitat selection for the survival of these lizards, several studies have been conducted to explore how the ambient factors such as vegetation cover, surface temperature, food resources, and human activities affect habitat used by sand lizards (Newbold and MacMahon, 2008; Price-Rees *et al.*, 2013; Newbold and MacMahon, 2014; Zeng *et al.*, 2014).

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The steppe toad-headed lizard, *Phrynocephalus frontalis*, is a common sand lizard in the central Asian desert with sparse vegetation (Wang and Fu, 2004; Munkhbaatar *et al.*, 2006; Liu *et al.*, 2008). As an insectivore, *P. frontalis* consumes a wide range of invertebrates and plays an important role in insect control in the arid ecosystem of Hunshandake Desert on the Mongolian Plateau (Lian, 2011; Li *et al.*, 2013; Zeng *et al.*, 2014). Consequently, loss of reptiles due to habitat alteration and climatic change reduces the effectiveness of insect control (D'Cruze and Kumar, 2011; Civantos *et al.*, 2012; Cosentino *et al.*, 2013). Sand grain size has a significant effect on the running speed of the sand lizard (Li *et al.*, 2011; Lian, 2011). However, there is a lack of knowledge about the habitat selection in this lizard. In particular, few studies have paid attention to the effect of sand substrate on the habitat selection of this sand lizard.

We hypothesized that environmental factors affect the habitat selection in the steppe toad-headed lizard. We also hypothesized that the lizard avoids sand substrates in which its maximal sprinting capabilities are impaired. To test these hypotheses, we investigated the density of the steppe toad-headed lizard in different selected transects. We also investigated the abiotic and biotic environmental factors, such as vegetation cover, soil characteristics, and distribution of invertebrates in those transects. After comparing the differences in the lizard density among the habitats with varying levels of environmental factors, we attempted to explain the relationship between the lizard densities and those environmental factors. We then discussed the potential and suitable habitat of the steppe toad-headed lizard in the desert.

2. Materials and Methods

2.1 Ethics statement All animals in this study were under animal research protocol IOZ-2006 approved by the Animal Care Committee of the Institute of Zoology, Chinese Academy of Sciences and cared for these animals in accordance with the principles and guidelines of the Ethics Committee of the Institute of Zoology, Chinese Academy of Sciences and the Chinese Wildlife Management Authority.

2.2 Study area We conducted our study in Sangendalai (115°54' E, 42°38' N), which is located on the southern margin of Hunshandake Desert in Inner Mongolia, China, with an altitude of 1813m above the sea level and a temperate semi-arid continental type of climate. The mean annual temperature is 1.7°C (maximum of 35°C in July and minimum of -33°C in January). The annual

precipitation ranges from 300 mm to 380 mm, with rains occurring primarily between July and September (Lian *et al.*, 2012; Zeng *et al.*, 2014). The study area was a sandy grassland with low to moderate levels of sparse vegetation dominated by *Artemisia ordosica* and scattered with *A. sphaerocephala*, *Agriophyllum pungens*, *Bassia dasyphylla*, *Corispermum declinatum*, *Inula salsoloides*, and *Poa sphondylodes* (Peng *et al.*, 2006; Zeng *et al.*, 2014).

2.3 Densities of steppe toad-headed lizards We randomly selected nine transects (each area is 20×100 m²) in an area of desert and semi-desert as sample quadrats. We used the mark-recapture method (Kacoliris *et al.*, 2009) to investigate the density of the steppe toad-headed lizard in each transect (Individuals/m²). We captured all steppe toad-headed lizard individuals by using insect nets in each transect from 08:00 to 18:00 for 6 days. Our team had two people and each person captured the lizards in one transect for 4-5h every experimental day. Captured animals were toe-clipped (Waddle *et al.*, 2008) and released at point of capture. The population number of sand lizard in each transect was estimated by the Schnabel method (Krebs, 1999). Then, the corresponding density of sand lizard could be figured out. All the investigations were conducted from June to August during the post-reproduction period for two years (2009-2010). We conducted the investigations in each transect for 10 times in total, and each survey was arranged at the beginning or end of the month.

2.4 Vegetation cover and soil characteristics To explore the environmental factors that may affect the density of the steppe toad-headed lizard, we used ten 1 m × 1 m wooden quadrats with two lines (each line had five quadrats with 20-m intervals and line space was 15 m) to systematically quantify the vegetation cover at each transect. Digital cameras were used for remote sensing even in a short distance, which is recently considered to be a reliable tool for measuring vegetation cover (White *et al.*, 2000; Chi *et al.*, 2007; Li *et al.*, 2013). We applied Photoshop 6.0 (©1990-2002, Adobe Systems Incorporated) to analyze the photos. The vegetation cover of the selected wooden quadrats measuring an area of 1 m² was calculated as follows:

$$C = G/A \times 100\%$$

where, *C* is the vegetation cover percentage of a quadrat; *G* is the number of green pixels in a quadrat, representing the area of plant coverage; and *A* is the number of total pixels in a quadrat. We categorized the variable of vegetation cover into five groups: the lowest (0–10%),

lower (10%–20%), moderate (20%–30%), higher (30%–40%), and the highest (> 40%), respectively.

For assessing soil characteristics, we used a high-precision moisture-measuring instrument AZS-2 (Beijing Aozuo Instrumentation Ltd., China) to obtain the water content of the soil in each quadrat and then collected sand samples from each quadrat. By using a set of sieves, we sifted the sand into four different size groups according to their diameters: Group A: 1–2 mm, Group B: 0.5–1 mm, Group C: 0.25–0.5 mm, and Group D: 0.075–0.25 mm. The sand sample of each group was weighed and the proportion of each group in each quadrat was calculated. We used the sand grain size index (SGSI) to represent the overall roughness of a quadrat, which was calculated as follows:

$$SGSI = 1 \times A\% + 0.5 \times B\% + 0.25 \times C\% + 0.075 \times D\%$$

where, SGSI is the sand grain size index of a quadrat. Coefficients 1, 0.5, 0.25, and 0.075 as weighting of the sand grain size in different groups, are diameters of the sieve pores of different groups, and A%, B%, C%, and D% are sand weight proportions of each group in each quadrat, respectively. We categorized the variable of soil moisture into three groups: Dry (0–10), Moderate (10–20) and Wet (> 20). We also categorized the variable of sand grain size index into three groups: Low (range of SGSI was 0–0.15), Moderate (range of SGSI was 0.15–0.30), and High (range of SGSI was > 0.30).

2.5 Invertebrate diversity and abundance To investigate the diversity and abundance of invertebrates, we arranged 20 pitfall traps half-filled with a solution of sugar, vinegar, 75% ethanol, and water in the proportion of 1:2:1:20 in weight. The traps were placed at 10-m intervals along each transect. We also used an insect net to collect the insects above the traps. Invertebrate samples were collected and transferred to 70% ethanol for storage and later for identification on the basis of their morphology. We used the Shannon–Wiener index to quantify the diversity of the invertebrate species (Shannon and Weaver, 1949) and calculated the abundance simultaneously. We categorized the variable of diversity index of invertebrates into three groups: Low (0), Moderate (0–1), and High (> 1). We also categorized the variable of abundance of invertebrates into three groups: Low (individuals of invertebrates were 0–10), Moderate (individuals of invertebrates were 10–20), High (individuals of invertebrates were > 20).

2.6 Statistical analysis Statistical analyses were performed using SPSS-20 (SPSS, Inc., Chicago, IL,

U.S.A.). For non-normally distributed data even after transformation (one-sample Kolmogorov-Smirnov test, $P < 0.05$), the Kruskal-Wallis one-way ANOVA with multiple comparisons of all pairwise were used to compare the differences of lizard densities among/between the habitats with varying levels of environmental factors. In this process, when the Kruskal-Wallis test (the first step) does not show significant differences among those levels, multiple comparisons will be not performed. Vegetation cover was categorized into five grades, and other factors were sorted as three levels. All environmental factors were used as classified variables, while the densities of sand lizards were numerical variables. $P < 0.05$ was considered to be statistically significant for all tests.

3. Results

There was a significant difference in the lizard densities among the different groups of SGIs (Kruskal-Wallis test, $\chi^2 = 16.877$, $P < 0.001$). The highest lizard densities were found in the group with SGSI > 0.30, whereas the lowest density was found in the group with an SGSI of 0–0.15 (Table 1, Figure 1 A). Besides, the densities of sand lizards in SGSI 0.15–0.30 and SGSI > 0.30 were both significantly higher than that in SGSI 0–0.15 (multiple comparisons of pairwise, $\chi^2 = 17.686$, $P = 0.006$; $\chi^2 = 33.183$, $P = 0.001$), but no significant difference existed between the densities of sand lizards in SGSI 0.15–0.30 and SGSI > 0.30 (multiple comparisons of pairwise, $\chi^2 = -15.498$, $P = 0.253$).

Table 1 The statistical results of the effects of five environmental factors on steppe toad-headed lizard density (Kruskal-Wallis test).

Factors	Sample size	Chi-Square χ^2	Significance (2-tailed) P
Plant coverage	$n = 90$	6.076	0.194
Sand grain size index	$n = 90$	16.877	<0.001*
Soil moisture	$n = 90$	3.005	0.223
Invertebrate diversity index	$n = 90$	0.083	0.959
Abundance of invertebrates	$n = 90$	1.415	0.493

Note: *indicates the significant difference at 0.05.

There were no significant differences in the lizard densities among the groups of different vegetation cover, water content in the soil, invertebrate diversity indexes, and abundance (Table 1, Figure 1 B, Figure 2, and Figure 3).

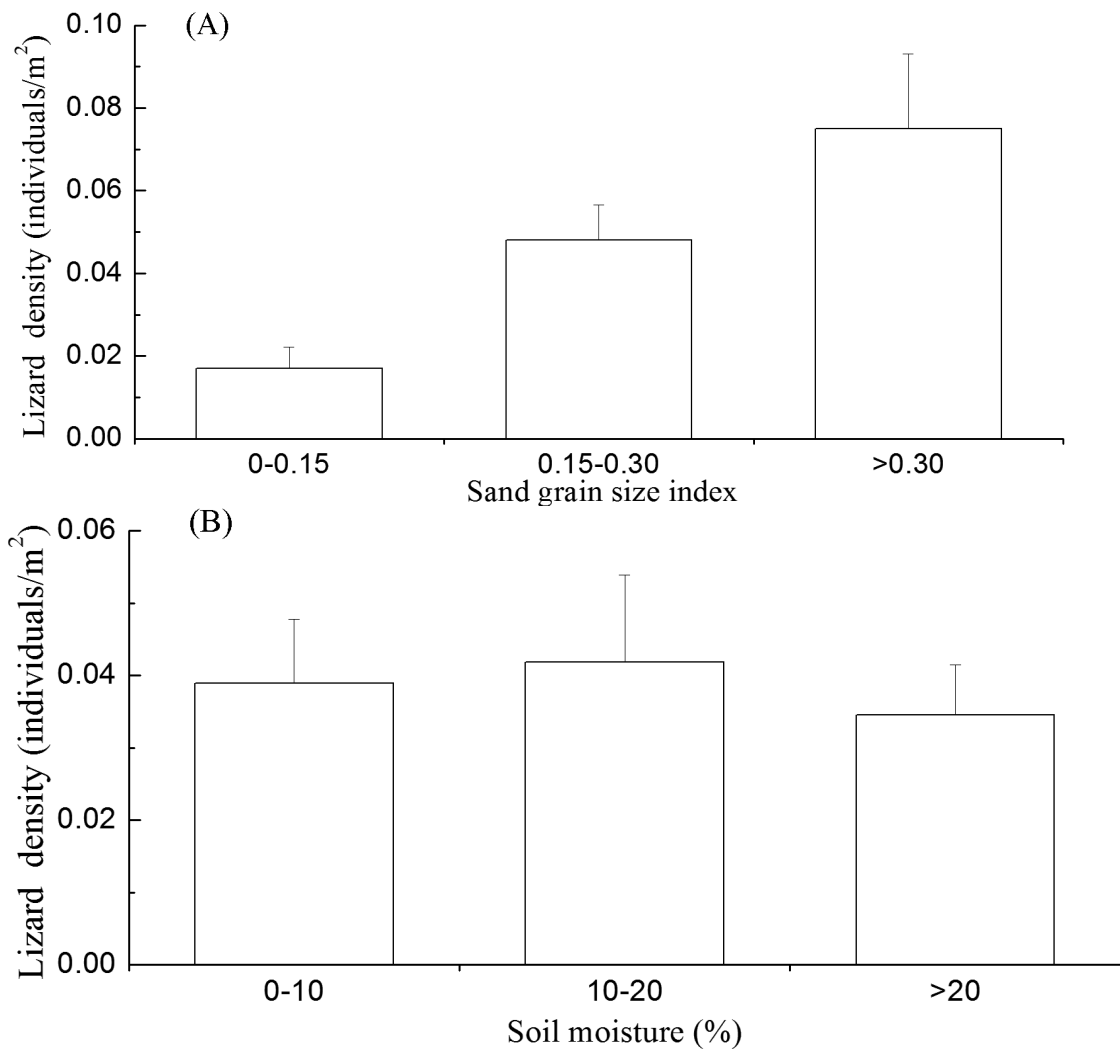


Figure 1 Steppe toad-headed lizard density in habitats with different soil characteristics. (A) was sand grain size index (SGSI) and (B) was soil moisture. Error bars represented the standard error (SE). The number of SGSI measurements in each category: 0–0.15 ($n = 36$), 0.15–0.30 ($n = 44$), > 0.30 ($n = 10$); the number of soil moisture measurements in each category: 0–10 ($n = 42$), 10–20 ($n = 22$), > 20 ($n = 26$).

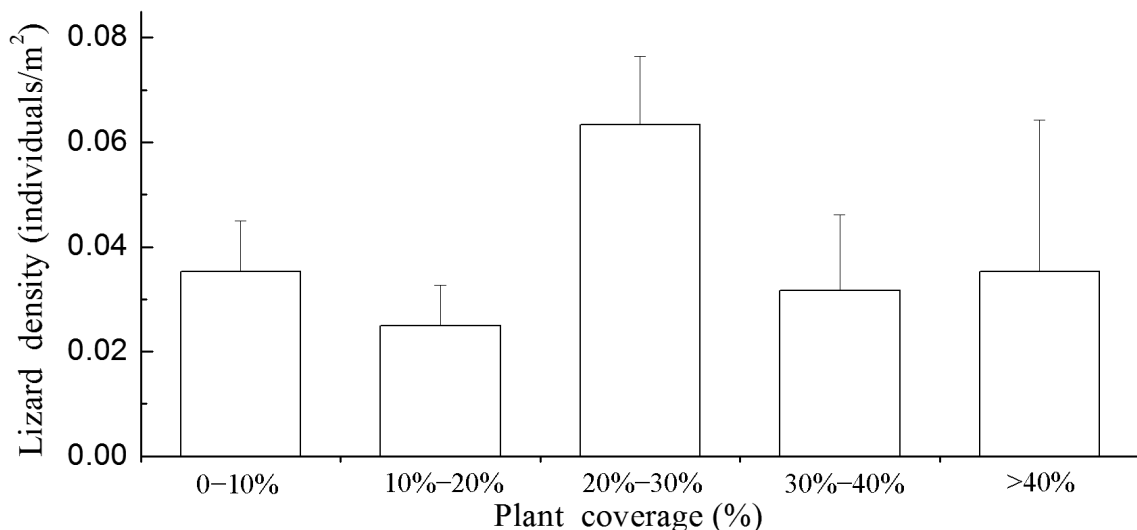


Figure 2 Steppe toad-headed lizard density in habitats with different plant coverage. Error bars represented the standard error (SE). The number of measurements in each category: 0–10% ($n = 29$), 10%–20% ($n = 24$), 20%–30% ($n = 20$), 30%–40% ($n = 12$), > 40% ($n = 5$).

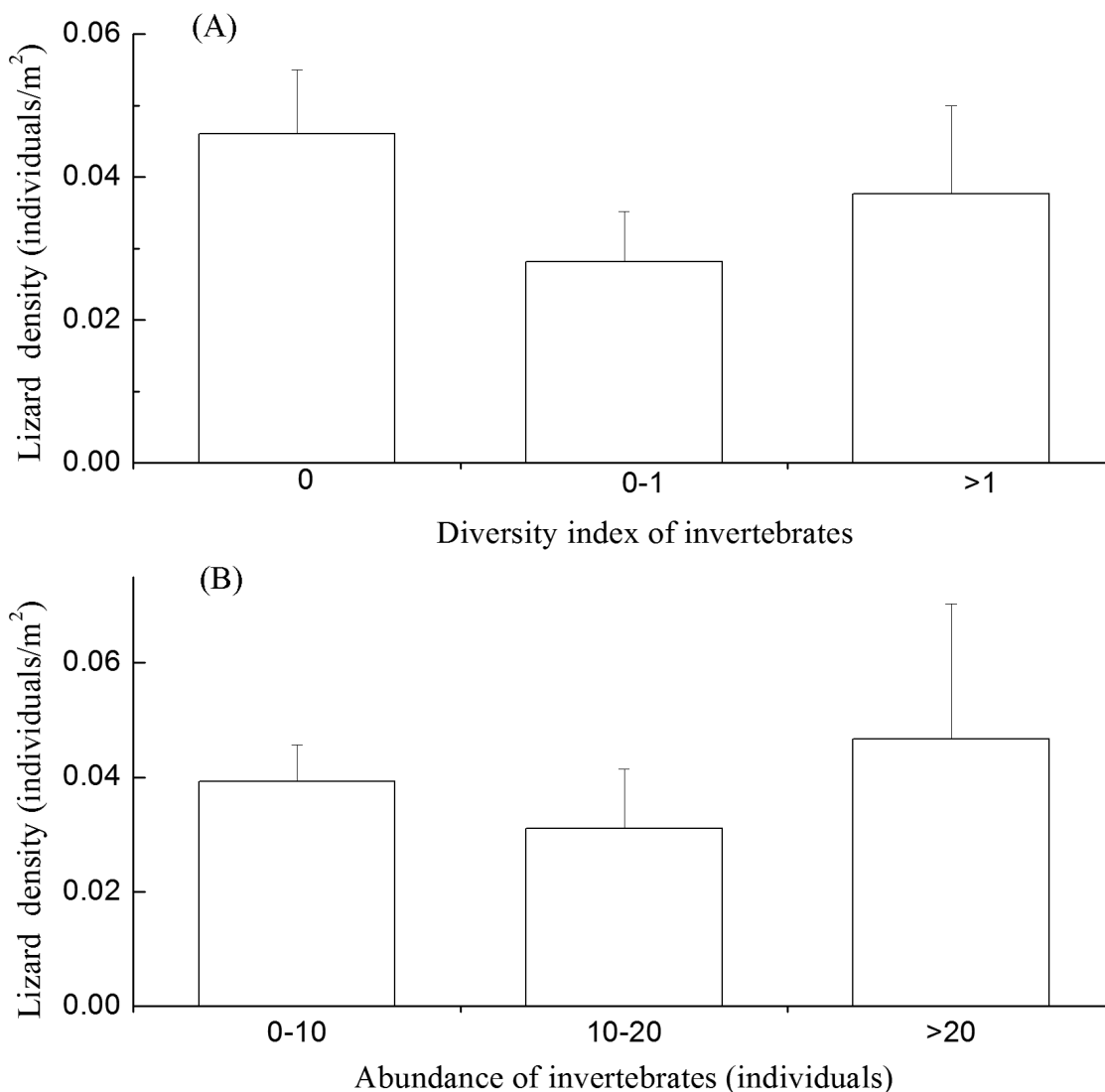


Figure 3 Steppe toad-headed lizard density in habitats with different invertebrate species diversity (A) and abundance (B). Error bars represented the standard error (SE). The number of diversity measurements in each category: 0 ($n = 42$), 0-1 ($n = 30$), > 1 ($n = 18$); the number of abundance measurements in each category: 0-10 ($n = 70$), 10-20 ($n = 15$), > 20 ($n = 5$).

4. Discussion

Our results indicated that the steppe toad-headed lizard preferred the habitat with rougher sand (higher SGSI), which are consistent with previous finding that this lizard performed maximal and sub-maximal running speed when they ran on sand of grain sizes 0.5–1 mm and 1–2 mm, respectively (Li *et al.*, 2011). Therefore, we assumed that the steppe toad-headed lizard avoided structural habitats in which their maximal sprinting capabilities were impaired. Similar results that lizards maintain a high running speed as an essential factor in habitat selection was also found in *Anolis* species (Luke, 1986; Irschick and Losos, 1999) and other sand lizards (Irschick and Jayne, 1998; Korff and McHenry, 2011). The occurrence

of this phenomenon could attribute to the following reasons. Firstly, the steppe toad-headed lizard generally inhabits desert, semi-desert, or grassland habitat with low and sparse vegetation, where animals move farther for food and spend more time foraging for the compensation of low food availability (Zhao *et al.*, 1999). In this situation, the risk of being preyed by predators increases in the open field (Hinsley, 2000; Attum and Eason, 2006; Zeng *et al.*, 2014). For the steppe toad-headed lizard, smaller sand grain size could reduce the running speed to escape predation by potential avian predators such as the gray shrike (*Lanius excubitor*), magpie (*Pica pica*), red-footed falcon (*Falco amurensis*), kestrel (*Falco tinnunculus*), and cuckoo (*Cuculus canorus*) (Zhao, 2001; Zeng *et al.*, 2014). Secondly, bigger sand grains

could effectively bolster burrows excavated by the steppe toad-headed lizard for inhabitation (Zaady and Bouskila, 2002). Considering that the SGSI could well represent the sand grain size structure in our study, we suggest that steppe toad-headed lizards prefer the sand substrate when they make choices about the kind of habitat.

In the desert ecosystem, vegetation cover is considered as a vital factor for the survival and distribution of the sand lizard because it provides food resources and shelter for thermoregulation and evading predators (Martín and López, 2000; Attum and Eason, 2006; Newbold and MacMahon, 2014; Zeng *et al.*, 2014). In general, steppe toad-headed lizards choose to stay in the shade of shrub canopies or flee into the burrow to evade predators or excessive temperatures (Zhao, 2001). However, our results demonstrated that the steppe toad-headed lizard showed no significant preference for the habitat with higher vegetation cover. This paradox could be explained by the fact that the even distribution of grasses and shrubs diluted the effects of the vegetation cover on the distribution of the steppe toad-headed lizard in Hunshandake Desert (Lian, 2011). Another possible explanation is that the dominant habitat of the steppe toad-headed lizard is of higher ground temperatures and less vegetation cover, whereas the lizard prefers warmer thermal environments of these habitats with sparse vegetation (Zhao, 2001; Liu *et al.*, 2008; Zeng *et al.*, 2014). Similarly, varanid lizards (*Varanus gouldii*) prefer to stay in habitats with sparse vegetation created after artificial burning (Bird *et al.*, 2013). In addition, in our study, we demonstrated that soil moisture was not a limiting factor on the habitat selection of the steppe toad-headed lizard in Hunshandake Desert. Most reptiles in the desert adapted to the environment lacking water (Warner and Shine, 2008). For example, the eggs of *Tropidonophis mairii* and *Sceloporus undulatus* can absorb moisture from relatively dry substrates (Warner and Andrews, 2002; Brown and Shine, 2005).

Steppe toad-headed lizards primarily prey on insects of the Coleoptera, Hymenoptera, and Hemiptera orders (Li *et al.*, 2013). Our results showed that there was no significant difference in the lizard densities among those habitats that are varied in diversity and abundance of invertebrates. Thus, the invertebrates, as a primary food resource, did not appear to be a significant determinant of the distribution of the steppe toad-headed lizard. Souter *et al.* (2007) and Newbold *et al.* (2014) suggested that sand lizards did not rely on a particular invertebrate community as their prey and this is consistent with our results. Moreover, the sufficient food resource in this

region is another factor that eliminates the weak effect of food resource on the habitat selection of the steppe toad-headed lizard (Li *et al.*, 2013).

In conclusion, in this study, we implied that the sand substrate of the environment was the primary determinant, whereas other environmental factors and food abundance were not the limiting resources of habitat selection of the steppe toad-headed lizard in the arid ecosystem. In addition, stabilized sand dunes may primarily consist of sand grains measuring 0.001–0.05 mm, and those with a size of 0.25–1 mm are mostly moving sand dunes in the arid environment (Zhang *et al.*, 2004; Wang *et al.*, 2006; Li *et al.*, 2011). Therefore, our study highlighted the potential use of the steppe toad-headed lizard as an environmental indicator of sand dune stability. However, further research is needed to investigate the level of sensitivity of the steppe toad-headed lizard to the change of sand substrate.

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