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Effects of Inhabiting and Life Patterns on the UV Spectral Properties of Small Mammalian Herbivores' Urine

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Abstract

It is well known that avian predators can use prey excretions such as urine and feces to track their prey, and the urine and feces of small mammalian herbivores can reflect ultraviolet (UV) light and emit fluorescent light. There are still some debates as to whether UV visibility of small mammalian herbivores' urine is used as a hunting cue by avian raptors. Some studies in Europe have demonstrated that diurnal raptors are capable of utilizing these cues to target key prey species. However, researchers in Australia have argued that raptors do not use the UV visibility of urine while hunting. To our knowledge, there are no reports from Asia concerning the ultraviolet spectral characteristics of small mammal herbivores' urine. This study examined the UV spectral properties of urine from 6 small mammal herbivores species by comparing the UV reflectance and fluorescence spectra of urine from small mammalian herbivores living in plateau meadows, plateau shrubs, open marshland, farmland, and semi-desert grassland in China. In addition, we compared the UV spectral properties of urine from ground-dwelling species of rodents and subterranean species to determine whether ultraviolet visibility of small mammal herbivores' urine could be used as a visual signal by Asian vole-eating raptors. The results showed that: 1) the SC_{370} values of urine from four small mammal herbivores species were ordered as plateau pika (plateau meadow) > root voles (plateau bush) > reed voles (swampland) > Brandt's vole (desert grassland); and 2) UV fluorescence peak intensity and the wavelengths of urine from ground-dwelling species (such as the root vole, plateau pika, or Brandt's vole) were significantly higher than those of subterranean-dwelling species (mandarin vole and plateau zokor). These results indicate that UV visibility of small mammal herbivores' urine may act as a visual cue for raptors.

Keywords

Rodents Urine, Ultraviolet (UV) Light, Fluorescence

1. Introduction

It is well known that the UV visibility of rodent urine can also be a foraging cue for UV-sensitive predators (Koivula et al., 1997; Koivula & Korpimäki, 2001; Härmä et al., 2011; Hughes et al., 2010). Studies conducted in Finland have shown that certain diurnal raptors can locate prey using the UV reflectance and fluorescence of rodent urine (Koivula et al., 1999; Koivula & Viitala, 1999; Honkavaara et al., 2002). However, studies of small mammals in Australia have indicated that the UV visibility of urine does not provide foraging signals for raptors (Kellie, 2004; Hurst, 1990). Whether the UV visibility of small mammalian herbivores' urine can be used as a visual signal for raptor foraging thus remains controversial. In addition, as Finland is in Europe and Australia belongs to Oceania, the UV spectral characteristics of small mammalian herbivores' urine in Asia have not been reported.

Avian predators tend to search in open meadows or fields using their keen vision (Lind et al., 2013; Aschwanden et al., 2005). Therefore, the predation risk from avian raptors for small mammal herbivores living in open areas is much larger than those dwelling under bush and in subterranean burrows (Trejo & Guthmann, 2003; Kullberg, 1995). Subterranean species such as the plateau zokor (*Myospalax baileyi*) and mandarin vole *(Lasiopodomys mandarinus*) are rarely preyed upon by raptors. Furthermore, the species inhabiting high and dense plant communities are mainly preyed upon by snakes and carnivores (Korpimäki & Krebs, 1996; Lima, 1998; Mappes et al., 1993; Norrdahl & Korpimäki, 1993), though the reed voles (*Microtus fortis*) dwelling in dams and hills in the area of Dongting Lake are occasionally preyed on by kestrels (*Falco innocuous*) (Li et al., 2008). For small mammalian herbivores that are active on low, vegetated runways such as plateau pikas (*Ochotona curzoniae*) (Johnsingh, 1992; Honkavaara et al., 2002; Probst et al., 2002; Zampiga et al., 2006), their urine and feces may be a good foraging signal for raptors. It would be interesting to know whether there are differences among the UV spectral characteristics of urine of small mammal herbivores dwelling in different habitats. We predicted that there would be no significant differences among the UV spectra of urine of small mammal herbivores from different habitats, if urine does not function as a cue in raptor foraging.

The aim of this study was to determine whether the urine of several ground-dwelling species and subterranean-dwelling species has UV properties that make it potentially visible to predators (Viitala et al., 1995), whether by absorbance, the reflectance of UV radiation, or fluorescence to UV. UV properties of the urine of small mammalian herbivores from different habitats (plateau meadows, bushes,

farmland, swamps, and semi-desert grassland) in the Chinese mainland were compared. If UV properties of urine are to be a cue for UV-sensing avian predators, there may be different UV properties indicating specific behavior patterns: e.g., ground-dwelling species vs. subterranean species. Furthermore, the UV properties of urine from small mammalian herbivores inhabiting open plateau meadows might be more visible to avian raptors compared to urine from species inhabiting bushes or prairies.

2. Materials and Methods

2.1. Study Species

Urine from 4 small mammalian herbivores found in China was examined; 4 species were considered as potential prey for raptors; plateau pikas, root voles, reed voles, Brandit voles. These species were defined as potential prey items based upon their behaviour (ground-dwelling) and distribution that broadly overlapped with those of raptors that prey upon mammals and live in plateau meadow, in dams and hilly regions in Dongting lake swamp areas, semi-arid grassland, where urine might be a potential cue for aerial predators. Two likely prey species for raptors were defined through their behavior (subterranean-dwelling) that made them less vulnerable to diurnal raptors, plateau zokor and Mandarin voles.

Reed voles were live trapped in the Dongting Lake area, Yuanjiang County, Hunan Province (28˚13'N-29˚55'N, 111˚11'E-113˚10'E) in 2014. The area's habitat is at the transition belt from subtropical to north subtropical zones. The Dongting Lake area is dotted with abundant broad-leaf forest. The plant community in lake swamp areas consists mainly of *Carex hirta* and *Erigeron annuus*. The farmland crop is rice paddy. Reed voles live in dams and hilly regions, and the main avian predator of reed voles is the kestrel (*Falco tinnulus*) (Li et al., 2008).

Mandarin voles were live trapped in farmland in the suburb of Xinzheng, Henan Province (34˚52'N, 113˚85'E) in 2014. Brandt's voles (*Lasiopodomys brandtii*) were live trapped in Xilingol desert grassland in Inner Mongolia (43˚02'N-44˚52'N, 115˚13'E-117˚06'E) in 2014. The desert grassland is mainly of the high plains type with an average elevation of 1000 - 1200 m. The average annual temperature is −2˚C, and the annual precipitation is around 250 - 400 mm. Vegetation types are mainly plateau drought-bearing plants such as *Stipa grandis*, *Aneurotepidimu chinense*, and *Cleistogenes squarrosa* (Wan et al., 2006).

Plateau pikas, root voles (*Microtus oeconomus*), and plateau zokor were live trapped at the Haibei alpine meadow ecosystem station of the Chinese Academy of Science (37˚29'N-37˚45'N, 101˚12'E-101˚23'E) in the Qing-Tibetan plateau in 2015. The alpine meadow is dominated by *Kobresia humilis, Kobresia tibetica,* and *Kobresia pygmaea*. The plateau pika is the keystone small mammal herbivore within the flat meadow (Qu et al., 2013). *Potentilla fruticosa* is the main alpine shrub, distributed in areas with high underground water levels and shady slopes. Root voles mainly inhabit the spaces under the *Potentilla fruticosa* shrubs

(Wang et al., 2013). Different from plateau pika and root voles, the plateau zokor is a subterranean-dwelling rodent of the Qing-Tibetan plateau (Shao et al., 2015).

The animals from whom urine samples were collected were live trapped in their wild habitats. Ten healthy individuals (five males and five females of similar weight) from each species were acquired. Each animal was confined to a single metabolic cage. All of the experimental animals were fed plant items collected from their habitats and were given water ad libitum. The urine was placed in portable liquid nitrogen and brought to Jishou University where it was kept at −86˚C. The samples were centrifuged at 4˚C, 5000 RPM for 20 min, and the supernatant was taken for the determination of the ultraviolet reflection spectrum and the fluorescence spectrum.

To determine whether the freezing process changed the UV spectrum of urine, we investigated the UV reflectance spectra and fluorescence spectra of the urine of reed voles; the sample treatments were fresh, frozen for 10 days, and frozen for 20 days.

The results showed that freezing time of reed vole urine did not affect the UV reflectance ($F_{2,27} = 0.554$, $P = 0.589$), λF_{max} ($F_{2,27} = 0.99$, $P = 0.380$), and F_{max} ($F_{2,27} = 1.071$, $P = 0.358$). Thus, the urine freezing process did not change the ultraviolet spectrum characteristics of the urine (**Figure 1** and **Figure 2**).

The determination of the spectral properties of urine followed the methods of Lind et al. (2013) and Huitu et al. (2008). An uncovered box (30 × 12 × 6 cm) with 10 compartments was made from wood, and the surface was painted with black ink to serve as the UV reflectance spectrometric box (30 × 12 × 6 cm, **Figure 3**). Two compartments with the same background were treated with urine and distilled water. The control background was first measured with an AvaSpec-ULS3648 High-resolution Spectrometer in a dark room (temperature 20˚C ± 2˚C), humidity 22% - 26%, with a halogen lamp as the only light source. Light sources and optical fiber probe head were set at 45˚ angles to the sample, and the probe head bottom was 6 cm from the background. Then, 1 ml of distilled water

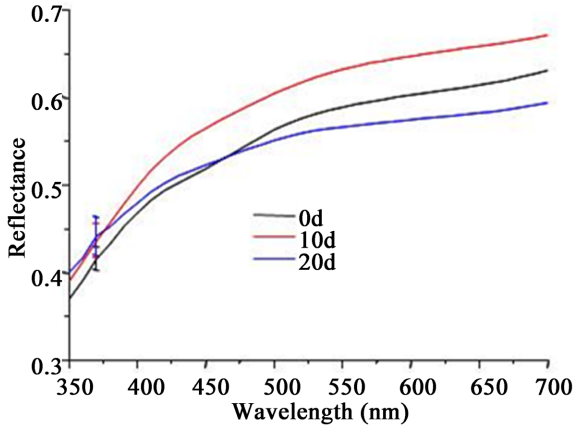

Figure 1. The effects of freezing time on the reflectance of *Microtus fortis* urine (Background: water-treated filter paper; SD of reflectance at 370 nm in the figure).

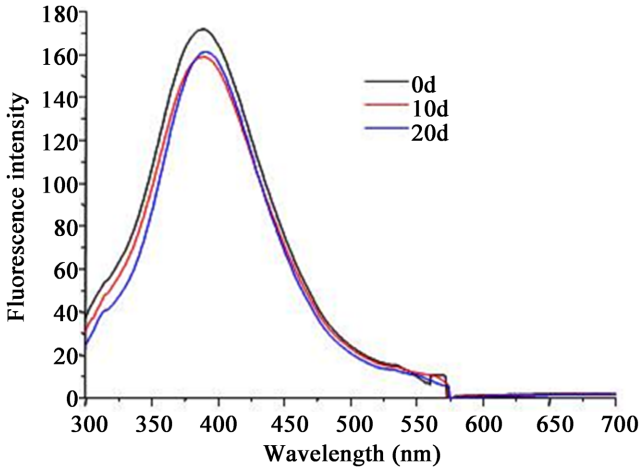

Figure 2. The effects of freezing time on the fluorescence spectrum of *Microtus fortis* urine (Background: water-treated filter paper; SD of reflectance at 370 nm in the figure).

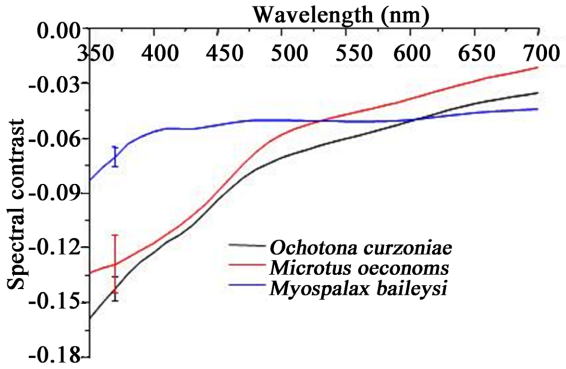

Figure 3. Spectral contrast of dried urine from three rodents from the plateau against the filter paper (SD of spectral contrast at 370 nm in the figure).

or urine was sprayed at the center of the background forming a circle with a radius of 2 cm, and the reflectance of the treatment area was determined immediately. Three successive measurements were made at different spots within the urine stain. The mean values of these three measurements were used in the determination of Spectral Contrast (SC) between the brightness of the sample and its background.

After the measurement, the background was replaced, and the sample was rearranged. In addition, 1 ml of urine and distilled water was sprayed on the filter paper, and the UV reflectance spectrum was determined after natural drying. The emission wavelength range of the spectrometer was set as 300 - 700 nm.

The SC was calculated with the reflectance spectra of the treated (fresh urine, dry urine trace, and water) and control backgrounds (Chávez et al., 2003; Huitu et al., 2008). The value was calculated as SC = (A − B)/(A + B), where A is the reflectance of urine and B is the reflectance of the background. SC < 0 indicates that the sample reflects less light than the background at a given wavelength (i.e., it absorbs more light than the background), and SC > 0 indicates that the sample reflects more light than the background. SC (SC_{370}) of urine at the emission wave-

length of 370 nm was compared (Chávez et al., 2003; Huitu et al., 2008).

The fluorescence measurements of urine samples followed the methods of Kellie et al. (2004) and Huitu et al. (2008) and were made using a fluorescence spectrometer (F-7000, Hitachi High-Tech Company, Japan). Samples were drawn in liquid form into a 0.5-mm path length quartz tube (1 mm in diameter, 0.5 mm optical path, and height 60 mm) that was placed in a custom-fabricated cuvette holder (made of organic glass, a 12.5 mm × 12.5 mm × 25 mm solid cuboid with a 1 mm diameter hole drilled in the center). The wavelength range of excitation light was set from 230 nm to 350 nm; the emission spectrum of the urine sample was pretested at 5 nm intervals between 360 nm and 380 nm, and the optimal excitation wavelength was determined to be 285 nm. Therefore, the excitation wavelength of the fluorometer was set at 285 nm. The wavelength range of the emission spectrum was 300 - 700 nm; the excitation slit was set at 10 nm, with the emission slit of 10 nm. The scanning speed was set at 1200 nm/min. Each sample was measured in three tubes, and each tube was scanned twice. Distilled water was used as the reference material for fluorescence determination. When the sample was analyzed, the fluorescence emission spectrum of the urine was subtracted from the emission spectrum of distilled water. The peak value of urine fluorescence spectrum (F_{max}) and its wavelength (λF_{max}) were compared.

2.2. Statistics

SPSS 17.0 statistical software was used to analyze the data. Data were tested for normality and homogeneity of variance to meet the parametric test conditions. Single-factor analysis of variance (one-way ANOVA) was used to compare the effects of different habitats and way of life on rodent urine ultraviolet spectral reflectance. Multiple comparisons were performed using the LSD test. Before repeated measurement analysis of variance, Mauchly's sphericity test was performed. If $P > 0.05$, the spherical hypothesis was satisfied, and the unitary analysis results were adopted. If the spherical hypothesis was not satisfied, the correction result of Greenhouse-Geisser was adopted. The data were expressed as the mean ± standard error, with $P < 0.05$ as the significant difference level.

3. Results

SC_{370} of the dry urine trace from the plateau pika was significantly higher than that of the plateau zokor ($P = 0.001$), and that of root vole was significantly higher than that of the plateau zokor ($P = 0.001$) (**Figure 3**). The SC_{370} values of urine from three kinds of plateau small mammal herbivores on filter paper were significantly different ($F_{2,27} = 47.398$, $P = 0.001$). SC_{370} of urine of the plateau pika was significantly higher than those of root voles and the plateau zokor ($P = 0.001$). SC_{370} of the urine from the root vole was significantly higher than that of the plateau zokor ($P = 0.005$). For the background after water treatment, there was also a significant difference in SC_{370} of urine among the three small mammal herbivores ($F_{2,27} = 56.715$, $P = 0.001$). SC_{370} of the urine from the plateau pika was significantly higher than those of root voles and the plateau zokor ($P =$

0.001). SC_{370} of urine from root voles was significantly higher than that from the plateau zokor (P = 0.002) (Figure 4).

There was a significant difference in SC_{370} of urine dry stains on filter paper among the three plain voles ($F_{2,27}$ = 7.371, P = 0.007). SC_{370} of urine of reed voles (marsh grass) was significantly higher than mandarin voles (dwelling underground) in farmland (P = 0.002). SC_{370} of urine of Brandt's voles (desert ground-dwelling) was significantly higher than mandarin voles (P = 0.038) (Figure 5).

The SC_{370} values of urine from three plain voles on filter paper were significantly different ($F_{2,27}$ = 14.107, P = 0.001). SC_{370} of the reed vole's urine was significantly higher those that of Brandt's vole and the mandarin vole (P = 0.004). After the background was treated with distilled water, there was a significant difference in SC_{370} among the three species' urine ($F_{2,27}$ = 14.843, P = 0.001). SC_{370} of the reed vole's urine was significantly higher than those of the Brandt's vole and mandarin vole (P = 0.002). Reed vole and mandarin vole results are shown in Figure 6.

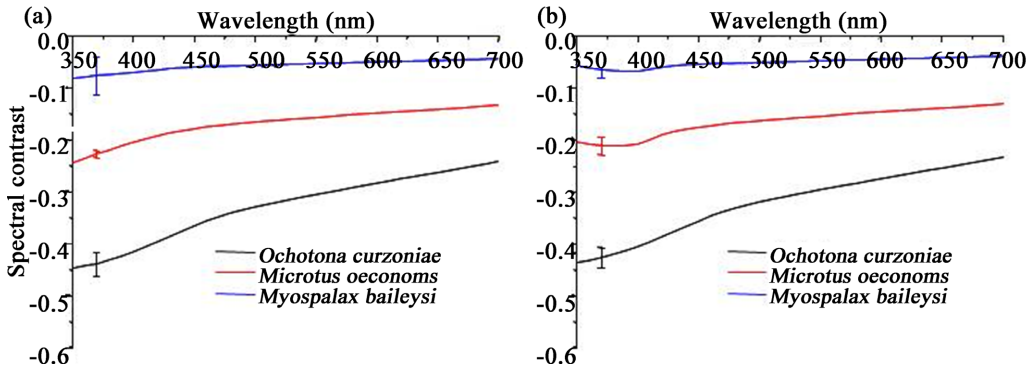

Figure 4. Spectral contrast of moist urine from three rodents living in the plateau against the filter paper (SD of spectral contrast at 370 nm in the figure). (a) Untreated background; (b) water-treated background, the same below.

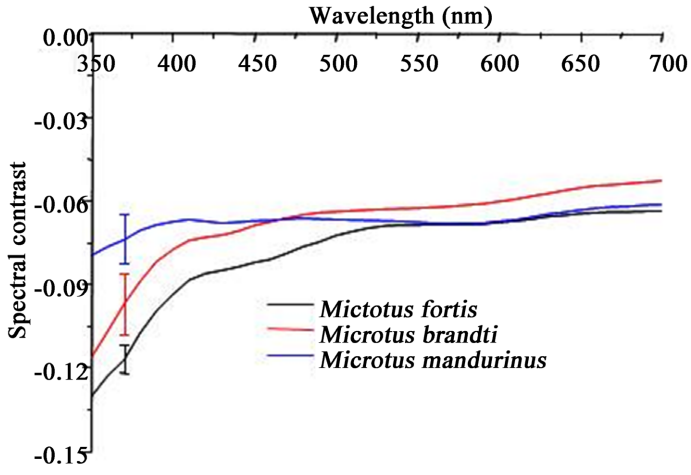

Figure 5. Spectral contrast of dried urine from three rodents against a filter paper background (SD of spectral contrast at 370 nm in the figure).

There was a significant difference in SC_{370} of dry urine stains on filter paper among rodent species from different habitats ($F_{4,45}$ = 4.702, P = 0.008). SC_{370} of the dry urine trace of the plateau pika was significantly higher than those of the reed vole, Brandt's vole, and the mandarin vole (Figure 7).

Repeated measures ANOVA results showed that there were significant differences between the two groups ($F_{2,27}$ = 7.378, P = 0.005) and F_{max} ($F_{2,27}$ = 4.109, P = 0.024). F_{max} of the plateau pika's urine was significantly higher than those of the root vole (P = 0.002) and plateau zokor (P = 0.025).

There was a significant difference in F_{max} ($F_{2,27}$ = 17.139, P = 0.001) among the three urine samples. F_{max} of urine of reed voles was significantly higher than those of Brandt's voles and mandarin voles (reed voles and Brandt's vole: P = 0.001; reed vole and mandarin vole: P = 0.001) (Figure 8).

4. Discussion

Small mammals, especially males, often mark their territories and runways with

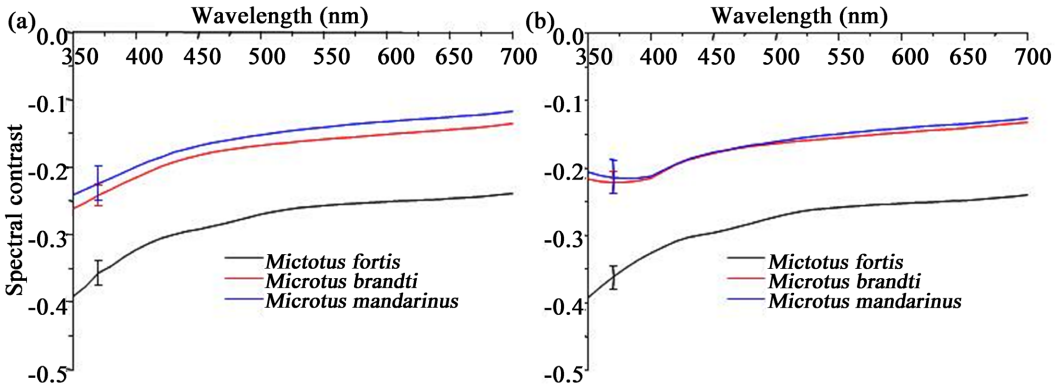

Figure 6. Spectral contrast of moist urine from three rodents against a filter paper background (SD of spectral contrast at 370 nm in the figure).

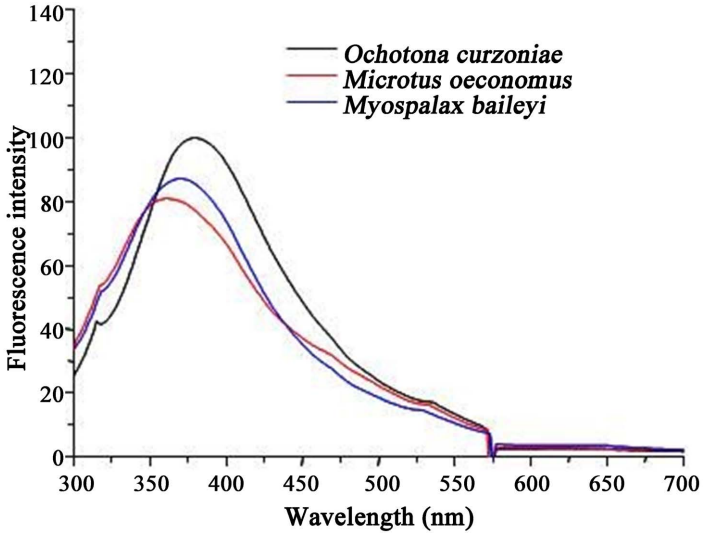

Figure 7. Fluorescence spectra of urine from three rodents (SD of spectral contrast at 370 nm in the figure).

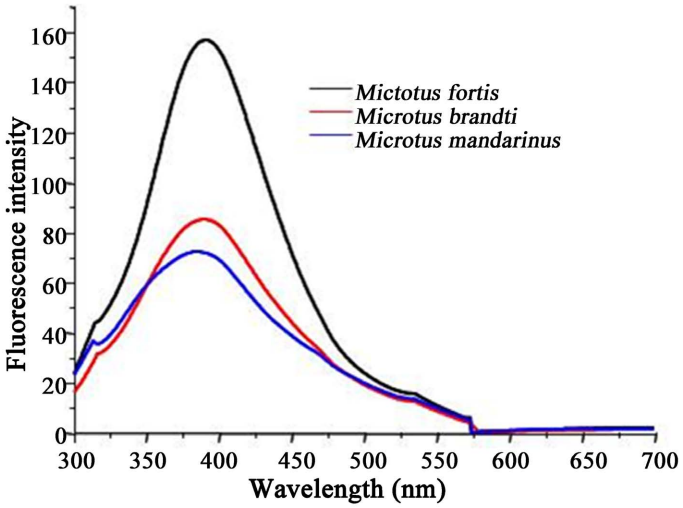

Figure 8. Fluorescence spectra of urine from three rodent species (SD of spectral contrast at 370 nm in the figure).

urine, feces, or sebum from anogenital scent glands to increase their chances of finding food or competing for mates (Desjardins et al., 1973; Brown & Macdonald, 1985; Chávez et al., 2003). Excreta reflect individual characteristics, gender characteristics, kinship relations, social status, and other crucial information (Brown & Macdonald, 1985; Fen & Macdonald, 1995; Palme et al., 2005; Peichl et al., 2005). The spectral absorption (or reflection) values of small herbivores' urine may indicate the visibility of the animals to avian predators with UV vision (Viitala et al., 1995). Avian predators having UV vision are more sensitive to urine with a higher absorbance (or reflectance) value (Janzen, 1980). This study has examined the spectral characters of small mammal herbivores' urine. SC_{370} of fresh urine was significantly different among small mammalian herbivores inhabiting different habitats, whether the background was white filter paper. The ordering of the measured spectral property (SC_{370}) was plateau pika (plateau flat meadow) > root voles (plateau bu-sh) > reed vole (swamp) > Brandt's vole (desert grassland). Furthermore, the absolute value of SC_{370} of the urine of ground-dwelling rodents (plant pika, root vole, reed vole, and Brandt's vole) was significantly higher than those of subterranean rodents (the plant zokor and mandarin vole).

SC_{370} of dry urine traces of the plateau pika was significantly higher than those of the root vole and plateau zokor, and the value of the root vole was significantly higher than that of the plateau zokor. The main activities of the plateau pika, including foraging, chasing, mating, and vigilance, take place within flat plateau meadows. This species is the main prey of avian predators. Compared to the plateau pika, root vole foraging and other behaviors take place under plateau bushes, meaning that avian predation risk is much lower (Roger et al., 2007). Plateau zokors spend 85% - 90% of their lifetime in underground nests in Alpine meadows performing activities such as burrowing, consuming vegetation, and producing excrement at a depth of 3 - 20 cm (Zhang et al., 2003; Peichl, 2005; Su, 2001). It

has been assumed that most large predatory birds on the Tibetan plateau, such as golden eagles (*Aquila chrysaotos*), upland buzzards (*Buteo hemilasius*), and saker falcons (*Falco cherrug*) depend primarily on the plateau pika as a food resource (Li et al., 2008; Anthony et al., 2008).

The climate of the Tibetan plateau is harsh, and the plant growth period is short; illumination intensity is strong, and plants contain high levels of secondary compounds (Dai et al., 2014). These biotic and abiotic factors may contribute to SC_{370} of root voles (inhabiting the plateau) being highest among the three species of voles.

The reflective spectra of urine at 370 nm from small mammalian herbivores living in different habitats displayed significant differences in the ratio of the fluorescence peak in the ultraviolet band and the associated wavelength. Compared to subterranean rodents, ground-dwelling small mammalian herbivores' urine had an ultraviolet fluorescence peak located between 370 nm and 380 nm, while for raptor eyes, the range 370 - 380 nm is corresponding to the most sensitive visual pigments. Therefore, the UV visibility of small mammalian herbviores' urine can be used as a visual signal by raptors.

As urine is volatile, the moisture level of urine may affect its UV spectral characteristics. Over time, the water and volatile substances in the urine are lost, and urine forms a dry trail in the small mammalian herbivores' running paths. SC_{370} of small mammalian herbivores' dry micturition tracks differed among species. SC_{370} of dried urine traces of ground-dwelling small mammalian herbivores was significantly higher than that of subterranean rodents and thus more likely to be discovered by birds of prey. This is consistent with Chávez et al. (2003) who found that the fresh urine of Chilean Degu rats had a reflection peak at 360 nm, while dried urine trace reflectance was low in the UV band, with no reflection peak. However, some studies have shown that the reflectance spectrum produced by dry and wet urine in voles is similar (Koivula et al., 1999; Koivula & Viitala, 1999). However, there was no significant difference in the ultraviolet absorption properties between small mammal prey and non-prey species of avian raptors in Australia (Kellie et al., 2004).

This study concludes that the fluorescence of small mammalian herbivores' urine excited by UV irradiation had a fluorescence peak in the near-UV band (320 - 400 nm). This is consistent with reports that the fluorescence of urine may be masked by sunlight under natural conditions during the day, reducing the potential effect of urine fluorescence as a visual signal (Viitala et al., 1995). However, this view is based on the fact that human visual perception is different from that of birds (Goldsmith, 1990). When the environment gets dim, the relative proportion of UV in natural light sources tends to increase (Koivula et al., 1997; Endler et al., 1993). At the same time, many rodents and their predators become more active at dawn and dusk (Halle, 1993). Therefore, urine fluorescence may play an important role in the foraging of predators.

5. Conclusion

Small mammalian herbivores' urine from animals living in varied habitats in

China displayed different fluorescence peak wavelengths. Therefore, UV-visible small mammalian herbivores' urine could be used as a visual signal by raptors while foraging. The role of small mammalian herbivore urine in the risk of raptor predation may be varied in different regions.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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