

ELECTRONIC SUPPLEMENTARY MATERIAL

Do I stay or do I go? Shifts in perch used by lizards during morning twilight suggests anticipatory behavior

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Figure S1. Night-perching posture of the green-spotted grass lizard (*Takydromus viridipunctatus*) on the *Miscanthus* grass. This behaviour is thought to be an adaptation against nocturnal predators (e.g., rodents, shrews, or snakes) on the ground, and also forms camouflage under dim light condition.

Visual modelling of the avian predator

To model how conspicuous a lizard is in its environment to an egret, we quantified the habitat light environment or down-welling irradiance (vertical from the land surface), measured the spectral reflectance of the animal's body, and used published data on microspectrophotometry of the receiver's visual system.

Irradiance measurements were taken on a sunny day at the study site from one hour before to one hour after sunrise. During the 2-hr duration, we measured absolute irradiance ($\mu\text{Watt m}^{-2} \text{ s}^{-1} \text{ nm}^{-1}$) every minute using a spectrometer (Ocean Optics Flame UV-VIS model, Florida USA) using a fiber-optic (Ocean Optics QP600-2-SR) with a cosine-correcting probe (Ocean Optics CC-3-UV) simultaneously with the power meter. We recalibrated our spectrometer using a calibration source (Ocean Optics DH-3-P-BAL-CAL) prior to taking irradiance measurements.

We measured the spectral reflectance of lizards in both the breeding (10 males and 7 females) and non-breeding (10 males and 10 females) seasons in a dark room. Reflectance spectra were obtained using a reflectance probe (Ocean Optics QR600-7-SR-125F) connected to a Deuterium Tungsten Halogen light source (Ocean Optics DH-2000-BAL) and a portable spectrometer (Ocean Optics Flame UV-VIS model). The measurements were standardized using a diffuse white reflectance standard (Ocean Optics WS-1-SL, >98% reflective from 250-1500 nm). A black acrylic fixture was attached to the reflectance probe in order to

maintain the constant 5-mm distance and an angle of 90° between the end of the probe and the lizard's surface. The coincident normal (CN) ensured that both the angles of illumination and observation were orthogonal to the surface of the target [1]. We measured the reflectance spectra of seven body regions: head, dorsum, tail, cheek, lower jaw, anterior flank, and posterior flank, of both sexes (Fig. 1a). Three readings were taken from different locations within each region and averaged. After taking measurements, all individuals were released at their point of capture. We also measured the spectral reflectance of ten silver grass leaves and used the mean as background because most of the lizards chose to perch on this plant.

We used the receptor noise-limited (RNL) model [2–5] to estimate chromatic and luminance discrimination thresholds for known stimuli. This model assumes that the level of receptor noise sets the discrimination threshold, and the subsequent neural processes do not limit color discrimination. In a dim light environment, when the photon shot noise should be considered [5–7], calculation of the RNL model started with the absolute quantum catch (Q).

We calculate Q for each type of photoreceptor, using the following equation [5]:

$$Q_i(\lambda) = \Delta t \left(\frac{\pi}{4}\right)^2 \left(\frac{d}{f}\right)^2 D^2 \kappa \tau (1 - e^{-kA(\lambda)l}) L(\lambda) \quad (1)$$

where i denotes the four receptor types: VS, SWS, MWS and LWS. The definition of each symbol is given in Table S1. There is no MSP data for egrets, so we used data for the chicken [8], which is most similar in body and eye size among the limited list of available birds [8,9].

Because integration time is a function of light intensity, we used the maximum value for the

time interval of behavior when the ambient light was dim. Thus, the contrast (Δf) for the i^{th} type receptor is:

$$\Delta f_i = \ln \frac{Q_{i_{stim1}}}{Q_{i_{stim2}}} \quad (2)$$

where $Q_{i_{stim1}}$ and $Q_{i_{stim2}}$ denote the quantum catches of two stimuli [5].

To estimate receptor noise, e_i , used the Weber fraction (ω), which is equivalent to the noise of discrimination thresholds [5]. The Weber fraction refers to the proportion of ΔI divided by a stimulus of intensity I ($\omega = \Delta I/I$), where ΔI is the smallest intensity difference that could be detected. In bright light conditions, the Weber fraction for chromatic contrast is constant and was set to 0.1 [5] — equal to noise e , which is referred to as Weber noise. In dim light conditions, Weber fractions increase so that the photon shot noise should be added to Weber noise [2,8,10,11]. Photon shot noise is calculated by the square root of the absolute number of absolute quantum catch Q , thus the noise parameter E for the i^{th} type receptor is:

$$E_i = \sqrt{\frac{e_i^2 Q_i^2 + Q_i^2}{Q_i}} \quad (3)$$

The chromatic contrast for a tetrachromatic animal ($i = 1$ to 4), ΔS_c , is then calculated:

$$\Delta S_c^2 = \frac{(E_1 E_2)^2 (\Delta f_4 - \Delta f_3)^2 + (E_1 E_3)^2 (\Delta f_4 - \Delta f_2)^2 + (E_1 E_4)^2 (\Delta f_3 - \Delta f_2)^2 + (E_2 E_3)^2 (\Delta f_4 - \Delta f_1)^2 + (E_2 E_4)^2 (\Delta f_3 - \Delta f_1)^2 + (E_3 E_4)^2 (\Delta f_2 - \Delta f_1)^2}{(E_1 E_2 e_3)^2 + (E_1 E_2 E_4)^2 + (E_1 E_3 E_4)^2 + (E_2 E_3 E_4)^2} \quad (4)$$

Finally, the chromatic contrast between lizards and their background (grasses) was estimated using the magnitude of "just noticeable difference" (JND) as the unit, where 1 JND indicates the detection thresholds.

The spectral sensitivity of MWS and LWS cones are summed together to simulate the

absorbance in the luminance channel. The Weber fraction was set to 0.2 [5], and the aforementioned equation (1–4) was applied. Because there is only one type of rod cells in bird eyes, i was changed to 1 and the equation 4 could be simplified:

$$\Delta S_a^2 = \left| \frac{\Delta f_i}{E_i} \right| \quad (5)$$

where ΔS_a refers the luminance contrast.

In order to test the difference in discriminability between the lizard and grass under the RNL model, the mean of all pair-wise chromatic distances between lizards and grass was calculated every minute during the twilight period. We used values from the dorsal and lateral regions of the lizards to estimate the chromatic contrast with the grass. For the lateral region, males and females were further separated based on sexual dichromatism [12]. A two-sample t-test was used to test the chromatic contrast between the timing of eyes opening and head movements, and that between head movements and body movements. All the statistical analyses and visual modeling were conducted using R (version 3.6.0).

Temperature effect was ignored in this system

Simultaneous air temperature of each experiment day, continuously recorded every minute, was acquired from two weather stations: 1) Hualin Weather Station belonging to Chinese Culture University, located just within the grassland where the experiment was conducted; and 2) Cyuchih Weather Station belonging to Central Weather Bureau of Taiwan, located

3970 m from the experiment site. Temperature records are exactly congruent between the two weather stations, showing a uniform weather status across this region.

The ambient temperature at sunrise depended on weather conditions of that day. It ranged between 12.9 °C (29 Oct 2018) and 24.8 °C (18 Sep 2018) in the non-breeding season. In the breeding season, it ranged between 16.7 °C (on May 21 and May 22, 2019) and 23.5 °C (on May 20 and Jun 4, 2019). Nevertheless, since the majority of behaviors occurred within a narrow time interval between -30 and +5 minutes from sunrise, the ambient temperature displayed low variation within this interval. In both breeding and non-breeding seasons, temperature fluctuation within this 35-min interval is usually 0, with a maximum of 0.6 °C in two occasions. As expected, temperature started to raise after sunrise. However, the lizards have already left the perch site long before the temperature raised. Therefore, the daily fluctuation of simultaneous air temperature could be ignored in this system.

In order to further test whether the lizards' body temperature was representative of the ambient temperature, we used CENTER 309, a four-channeled datalogger thermometer (CENTER technology Corp., New Taipei City, Taiwan), to compare between ambient temperature and the temperatures obtained from three dead lizards (two males and one females, attached to the surface of a blade of grass) in the twilight moment. These specimens were originally collected for genetics, morphological measurements, and specimen preservation, which was approved by Institutional Animal Care and Use Committee

(IACUC), National Taiwan Normal University (license No. 106023). Measures between ambient temperature and body temperature of the lizards showed no difference during this period. Furthermore, since most of these behaviours occurred from -30 to +5 minutes, the lizards had not a chance to absorb the solar heat because they had already left the perch site.

Based on the above reasons, we concluded that the behavioural pattern of the lizards is triggered by environmental light condition instead of temperature condition. The temperature differences among different behavioural points could also be ignored in this system.

References

1. Hill GE, Hill GE, McGraw KJ, Kevin J. (Eds.). 2006 Bird coloration: mechanisms and measurements (Vol. 1). Harvard University Press.
2. Vorobyev M, Osorio D. 1998 Receptor noise as a determinant of colour thresholds. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **265**, 351–358.
3. Vorobyev M. 2003 Coloured oil droplets enhance colour discrimination. *Proceedings of the Royal Society London B* **270**, 1255–1261. (doi: 10.1098/rspb.2003.2381)
4. Osorio D, Smith AC, Vorobyev M, Buchanan-Smith HM. 2004 Detection of fruit and the selection of primate visual pigments for color vision. *American Naturalist* **164**, 696–708.
5. Olsson P, Lind O, Kelber A. 2018 Chromatic and achromatic vision: parameter choice and limitations for reliable model predictions. *Behavioral Ecology* **29**, 273–282.

6. Kelber A, Lind O. 2010 Limits of colour vision in dim light. *Ophthalmic and Physiological Optics* **30**, 454–459.
7. Kelber A, Yovanovich C, Olsson P. 2017 Thresholds and noise limitations of colour vision in dim light. *Philosophical Transactions of the Royal Society B: Biological Sciences* **372**, 20160065.
8. Olsson P, Lind O, Kelber A. 2015 Bird colour vision: behavioural thresholds reveal receptor noise. *Journal of Experimental Biology* **218**, 184–193.
9. Lind O, Kelber A. 2009 Avian colour vision: Effects of variation in receptor sensitivity and noise data on model predictions as compared to behavioural results. *Vision Research* **49**, 1939–1947.
10. Jarvis JR, Abeyesinghe SM, McMahon CE, Wathes CM. 2009 Measuring and modelling the spatial contrast sensitivity of the chicken (*Gallus g. domesticus*). *Vision research* **49**, 1448–1454.
11. Lind O, Chavez J, Kelber A. 2014 The contribution of single and double cones to spectral sensitivity in budgerigars during changing light conditions. *Journal of Comparative Physiology A* **200**, 197–207.
12. Tseng WH, Lin JW, Lou CH, Lee KH, Wu LS, Wang TY, Wang FY, Lin SM. 2018 Opsin gene expression regulated by testosterone level in a sexually dimorphic lizard. *Scientific reports* **8**, 1–10.

Table S1. Definition of symbols used for modelling absolute quantum catches

Symbol	Parameter (unit)	Value (range)	Ref.
Δt	Integration time (ms)	12–50 (min.–max.)	Lisney et al., 2011
d	Ellipsoid diameter (μm)	3.1	Olsson et al., 2015
f	Focal length (μm)	8300	Olsson et al., 2015
D	Pupil diameter (μm)	3500–4900 (min.–max.)	Olsson et al., 2015
κ	Quantum transduction efficiency (%)	50	Johnsen, 2012
τ	Transmission of ocular media (%)	80	Johnsen, 2012
k	Absorption coefficient	0.035	Bowmaker et al., 1977
A	Cone sensitivity	V-type eye	Endler & Mielke, 2005.
l	Cone outer segment length (μm)	30	Olsson et al., 2015
L	Radiance of subject (= reflectance \times ambient light)	Subject dependent	This study

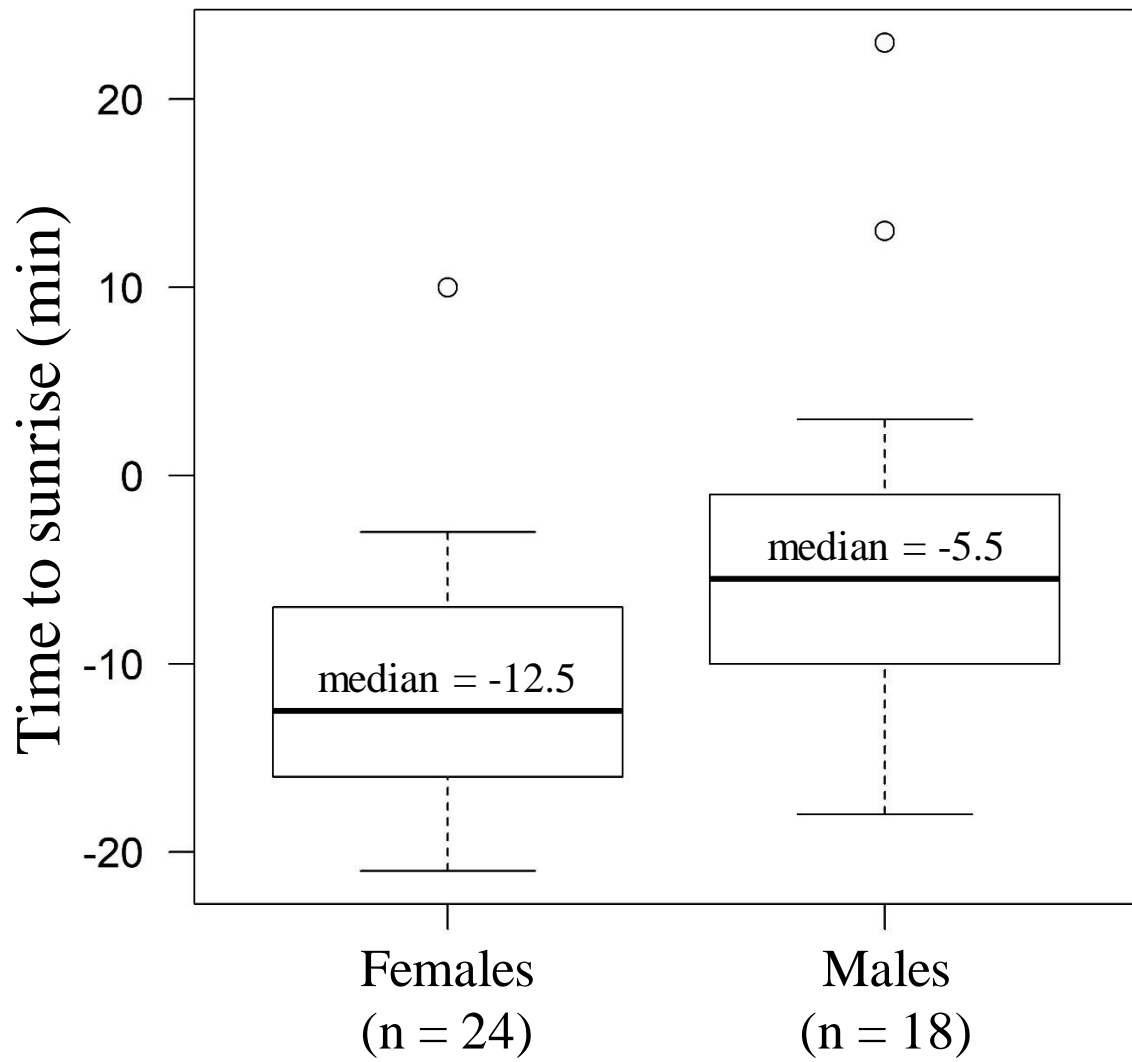


Figure S2. Effect of sex: females initiated body movements significantly earlier than males (ca. 7 mins prior to males) in the breeding season.

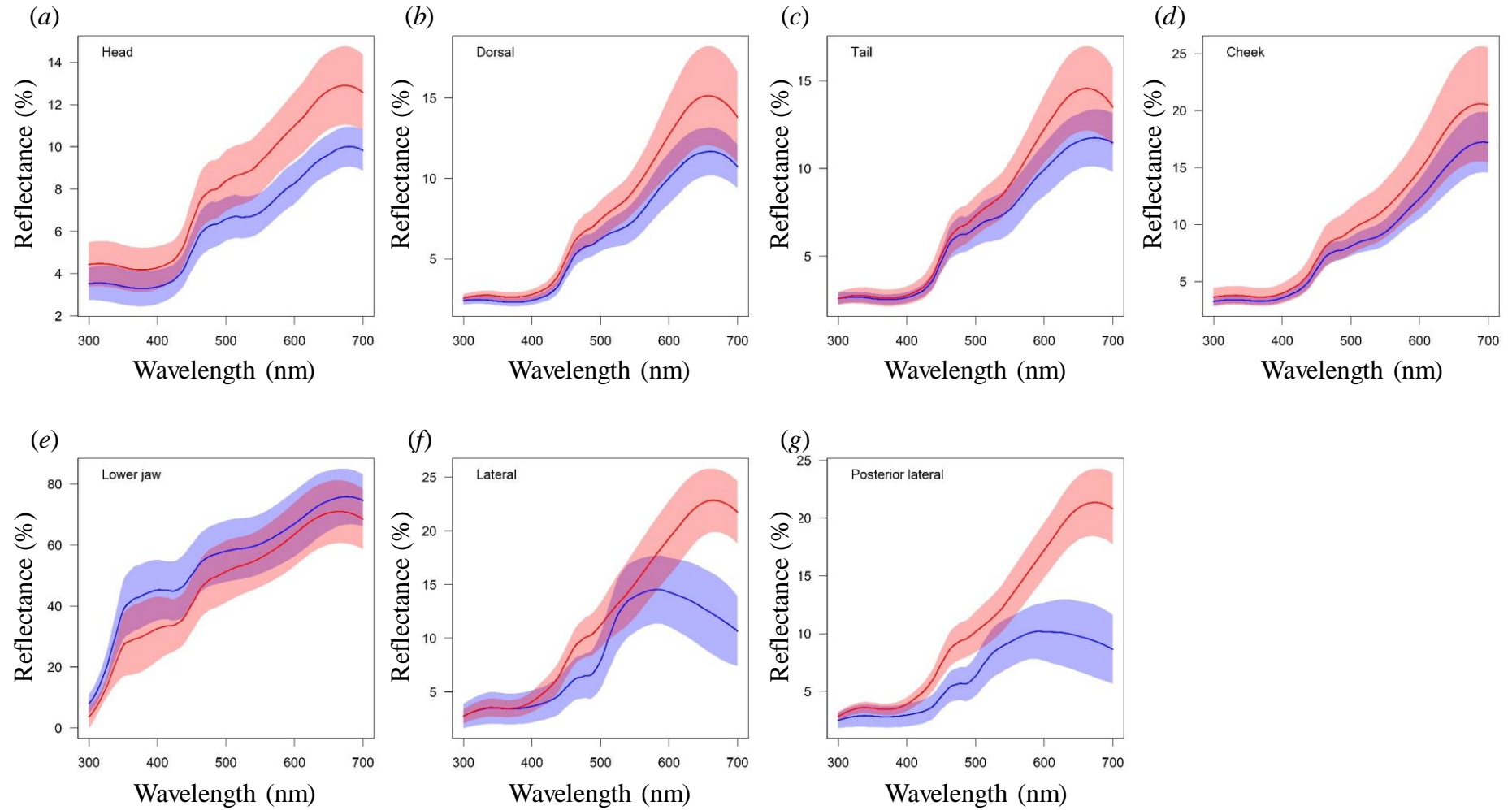


Figure S3. Reflectance spectra (means and standard deviation) of the males (blue) and females (red) in the non-breeding season. (a) to (g) correspond to the seven measurements in Fig. 1a, respectively.

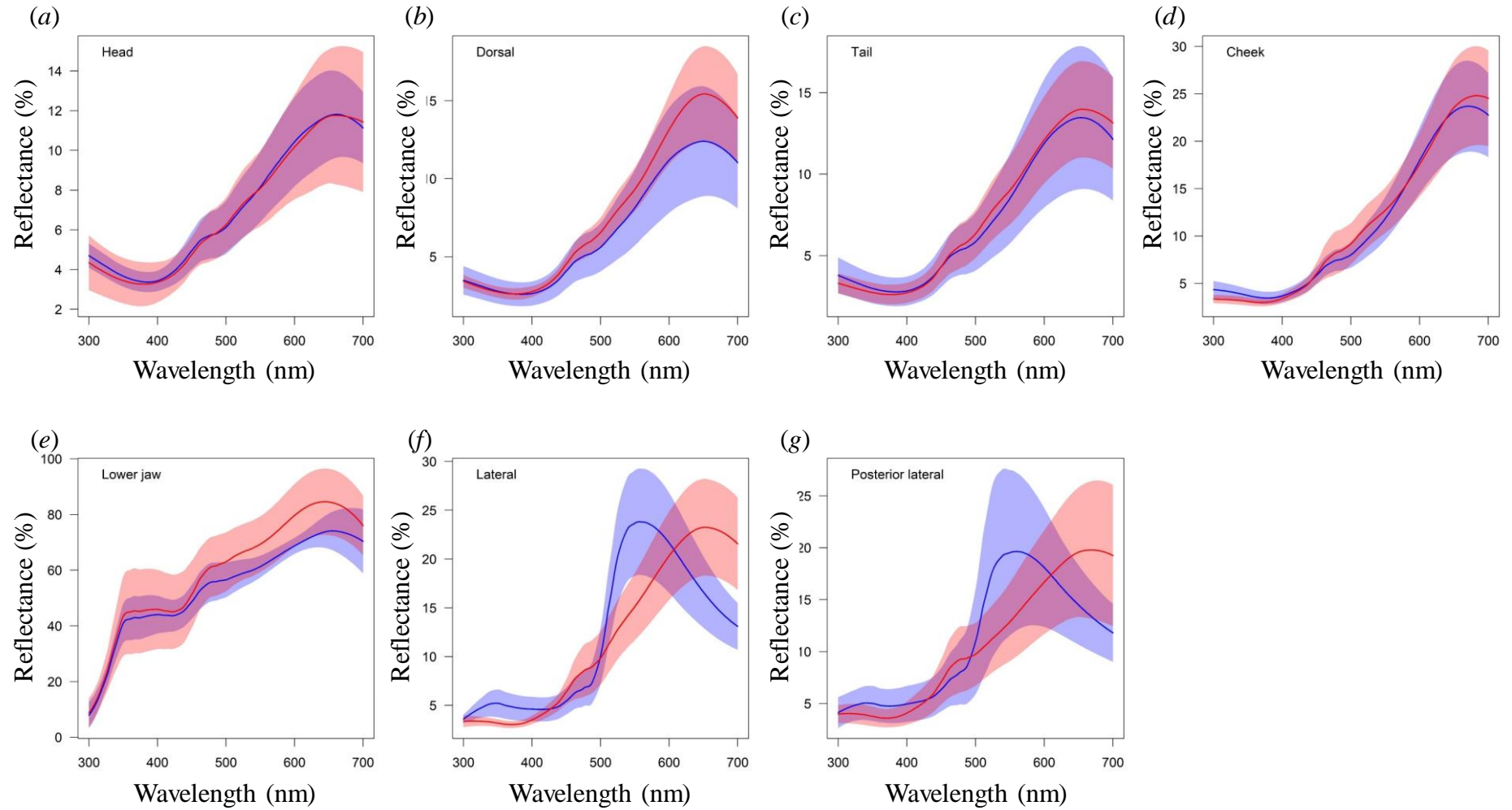


Figure S4. Reflectance spectra (means and standard deviation) of the males (blue) and females (red) in the breeding season. (a) to (g) correspond to the seven measurements in Fig. 1a, respectively.

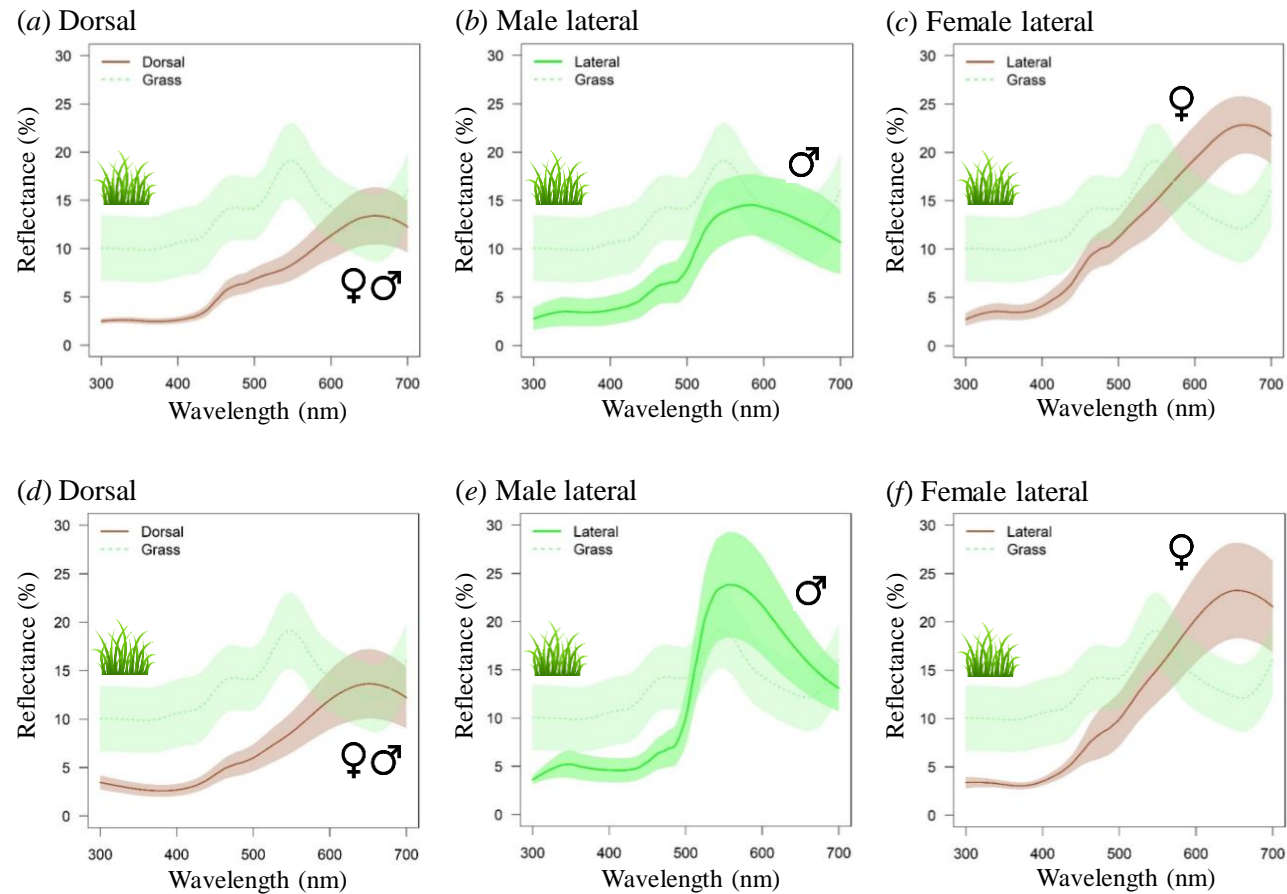


Figure S5. Reflectance spectra (means and standard deviation) of the lizards' dorsal and lateral regions compared with the grasses as background in the non-breeding season (*a, b, c*) and the breeding season (*d, e, g*). The dorsal coloration had no difference of sex or season (*a* and *b*). Males and females were significantly different for lateral sides (*b* vs. *c* and *e* vs. *f*), and males showed marked differences between breeding and non-breeding seasons (*b* and *e*).