Hen ranging behaviour in relation to light and ultraviolet intensity

Final Project Report | **October 2021**

A report for Australian Eggs Limited

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Australian Eggs Limited Publication No 31HS902CO

ISBN 978-1-920835-59-0

Project Title: Hen ranging behaviour in relation to light and ultraviolet intensity

Australian Eggs Limited Project Number 31HS902CO

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Published in February 2022

Foreword

This project was conducted to understand climatic conditions that may affect how many hens use the range area across the day with a specific focus on sunlight. Free range laying hen systems are prevalent within Australia and consumers find them favourable due to their naturalness. However, weather in Australia is extreme and birds may prefer to stay indoors if the sun intensity and ultraviolet radiation is high. The effect of sunlight on ranging may be particularly strong during the summer months but may vary across different regions of Australia.

This project conducted research in both controlled, indoor settings and directly on commercial farms to determine which wavelengths of sunlight hens preferred or avoided, how this varied across days and months on farm, and how use of range shelters will depend on what degree of sunlight filtering they provide. The project clarified sun-related variables that impact bird ranging and provides objective data for producers, consumers, and other stakeholders to understand factors that affect ranging for hens on commercial Australian farms.

This project was funded from industry revenue, which is matched by funds provided by the Australian Government.

This report is an addition to Australian Eggs Limited's range of peer reviewed research publications and an output of our R&D program, which aims to support improved efficiency, sustainability, product quality, education and technology transfer in the Australian egg industry.

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Acknowledgments

Thank you to the team of research technicians that ensured this project ran smoothly, even when faced with all the challenges of collecting data remotely and being restricted by border closures. The authors wish to express their gratitude to Jim Lea, Tim Dyall, Troy Kalinowski, and Andrew Cohen-Barnhouse who assisted with project set-up and data collection. Thank you also to the hard work and dedication of the PhD candidate, Md. Sohel Rana, who put in many hours to the project working through months of video footage to collect the necessary data. Thank you to L. Hewitt and S. Harris for all their constructive feedback throughout the project. Finally, the authors wish to thank all the commercial farms that participated in this study and hosted data collection on their properties. The farm staff were all incredibly willing and supportive, and it was a pleasure to be able to work with them to ensure we were able to answer our research questions directly on commercial properties. The authors are extremely grateful for everything the farm staff assisted with and acknowledge that the project would not have been a success without their unwavering support.

Australian Eggs Limited provided the funds that supported this project.

About the Authors

Dana Campbell is a Senior Research Scientist within the Animal Behaviour and Welfare Team of CSIRO, based in Armidale, NSW. She has a background in behavioural ecology and has worked with livestock species over the past 10 years. She has an interest in understanding how individual animals adapt to different types of housing systems and factors that can affect their behaviour and welfare. She has a particular interest in laying hens and has conducted many layer research projects to better understand environmental factors that have an impact on the behaviour and welfare of free range hens.

Caroline Lee is a Principal Research Scientist and is the Team Leader of the Animal Behaviour and Welfare Team. Caroline has worked on a number of livestock species for over 20 years, assessing their welfare through a range of behavioural, physiological, and affective state techniques. She has developed new tests for affective state assessment, and has been involved in developing new technologies for animal management. She has an interest in understanding how individual animals cope with their environments and what management strategies can be implemented to improve an individual’s welfare.

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Abbreviations

GLM	General linear model
GLMM	General linear mixed model
h	Hours
IR	Infrared
kPa	Kilopascal
LED	Light emitting diode
LSM	Least squares means
MEA	Measurement Engineering Australia
PAR	Photosynthetically active radiation
s	Seconds
TSR	Total solar radiation
UV	Ultraviolet
UVA	Ultraviolet A wavelengths
UVB	Ultraviolet B wavelengths
UVC	Ultraviolet C wavelengths
VIF	Variance inflation factor
VIS	Visual spectrum plus infrared wavelengths
W	Watt

Executive Summary

Sunlight intensity and ultraviolet (UV) radiation may affect free range hens' use of the outside range, particularly when sunlight is intense with a high UV index. However, it is uncertain what aspect of sunlight (brightness or UV) may be most aversive to hens to discourage them from leaving standard indoor lighting conditions to venture outdoors. A controlled indoor-based choice study was conducted to determine whether hens showed preferences for different light wavelengths and intensities that may affect outdoor range usage. Cage-reared ISA-Brown laying hens (n = 84) at 44 weeks of age in 3 groups (28 hens/group) were tested for preferences of indoor standard LED-White light (control) versus one of three different lights: (i) visible spectrum plus infrared wavelengths (VIS); (ii) visible spectrum plus UVA wavelengths (UVA); and (iii) visible spectrum plus UVA and UVB wavelengths (UVAB) presented successively at low, medium, or high levels of intensity.

Hens within each group were individually tested for 2 h in an apparatus with two compartments (control vs. treatment) connected by a tunnel on both sides. Videos of hens' time spent in each compartment and behaviours were decoded and analysed using GLMMs. Hens spent more time under the low intensity of the UVAB light treatment (62%), the low intensity of VIS light (61%), medium intensities of both UVAB light (60%), and UVA light (59%), and the high intensity of the VIS light (58%) when compared to control light (all $P < 0.05$). Hens spent less time feeding under all intensities of UVA light (all $P \leq 0.03$) and showed more foraging, ground pecking, and preening at lower levels of UVAB lights ($P < 0.05$). The study suggests that UVAB light (sunlight) may have positive effects for hen range use, but during peak sun intensities hens may need additional measures (e.g. shelter) to protect themselves.

To determine if range use was correlated with sunlight variables and other climatic factors on commercial free range farms, observations were carried out on three case study farms within Tasmania (2019/2020), Queensland (2019/2020), and Western Australia (2021) across the summer/autumn period. Video cameras were set up for daily video recording of hens ranging outside within a portion of one shed on each farm, starting around the early to peak lay period. The solar radiation spectrum [total solar radiation (TSR) (100 nm–1 mm, used to extra infrared (IR) wavelengths: 700–1000nm)/photosynthetically active radiation (PAR) (400–700 nm)/ultraviolet radiation (UVAB) (280–400 nm)] and weather data (temperature and relative humidity) were recorded through a weather station located on the farm.

Across a total of 66 to 102 days of video recordings per farm, image snapshots were taken at 30 min intervals from pop hole opening until sunset. The number of hens within direct sunlight or in the shaded areas was counted using Image-J software. General linear models showed the number of hens on the range increased in the autumn months relative to the summer months on each farm ($P < 0.05$). Across the day, there were typically fewer hens on the range in the direct sunlight in the middle to afternoon period of the day when the sunlight was at its most intense ($P < 0.05$). Ridge regression analyses were applied to determine the impacts of IR, PAR, UVAB, temperature and relative humidity on the number of hens in the sunlight or shade separately for each farm. In general, across all farms, temperature was a key factor affecting range usage, with hens avoiding the sunny areas when the temperatures were higher ($P < 0.05$). The different wavelengths of sunlight had varying impacts depending on the farm and the month of observation with PAR and UVAB showing greater impacts than the IR wavelengths. These results indicate hens are sensitive to the different wavelengths of sunlight, which are perceived as visibly bright and/or damaging to skin. Hens should have shelter on the range to access shade as needed, and lower range use during intense sunlight would be expected on commercial farms.

Artificial shelters within the range could provide protection from sunlight for free range hens in Australian climates with more extreme sunlight. A final study was conducted across two individual flocks (Flock A and Flock B) of a commercial free range laying farm (December 2020 to March 2021) in Queensland, Australia to assess hen preferences for artificial shelters that filtered 50%, 70%, and 90% UV radiation (3 replicates per shelter type). Both flocks comprised approximately 20,000 Hy-line Brown laying hens, and all video observations were taken between 34 and 40 weeks of age. A weather station was set up at the farm site with different sensors to record the irradiance of sunlight spectrums including ultraviolet radiation (UV_{AB}) (280–400 nm) (W/m^2), photosynthetically active radiation (PAR) (400–700 nm) ($\mu mol/m^2/s$), and total solar radiation (TSR) (100 nm–1 mm) [TSR was later used to extract infrared (IR) (W/m^2) radiation] along with an ambient air temperature and relative humidity sensor. A total of 14 days of video for Flock A and 17 days for Flock B were analysed, with image snapshots at 30-min intervals used to count the number of hens under the individual shelters. Data were analysed to test hens' shelter preferences across the day using GLMMs. The relationship of sunlight and weather variables with hens' shelter preferences was determined by fitting linear ridge regression models. Overall, the use of shelters by hens significantly varied across the time of day, with peaks in the morning and in the late afternoon compared to the mid-day (both Flocks, $P < 0.0001$). There was a significant interaction effect between UV-filtering shelter and time of day for hen preferences in both Flock A ($P < 0.0001$), and Flock B ($P < 0.0001$) where more hens preferred the 90%, followed by the 70%, then 50% UV-filtering shelters. Among the sunlight and weather variables, the majority of the variance in the models resulted from the ambient temperature in both study flocks, however, UV_{AB} was also significantly correlated with hens' shelter preferences in Flock B. The relationship between shelter use and weather/sunlight variables was negative, which may be due to fewer hens on the range during peak sunlight hours.

Overall Conclusions

The first controlled indoor study demonstrated that hens with minimal sunlight experience preferred lights that approximated daylight including high intensities of these lights. When a combination of UVA and B wavelengths were presented, preferences were reduced at the higher intensity, suggesting hens avoided the damaging radiation. Lower levels of UVAB resulted in more behavioural expression of foraging and comfort behaviours. This suggests that hens in a free range setting may positively respond to sunlight access but when the sunlight is intense, hens may need additional measures (e.g. shelter) to protect themselves from certain levels of UV radiation and intensity. It was impossible to completely mimic sunlight intensity and wavelengths in an indoor experimental setting for this study. Additionally, older cage-reared hens were used, which may have hindered behavioural expression. Therefore, further study is required to validate these findings in a free range setting.

The results of the range use observations across three commercial farms located in Tasmania, Queensland, and Western Australia showed that different wavelengths of sunlight will have differing impacts on range use. Ranges must have options for shade so hens may have a choice to be directly under the sun or seek shelter as required. Hens appear to be sensitive to the differing impacts of visual light (brightness), versus ultraviolet radiation (brightness and damaging), versus infrared (heat). Thus, it can be expected that as the intensity of these wavelengths change across the seasons, so will the range use by hens. Hens will avoid times of peak sun intensity and thus may not range as much during the summer months, particularly in regions of extreme sunlight. Heat consistently played a role in ranging behaviour with hens generally avoiding high temperatures, but sometimes seeking out the sun, presumably for warmth. These results are all consistent with how humans interact with sunlight and thus range design, and range use expectations should take this behaviour into account.

The results of the range shelter preference study showed that range shelters should be constructed of the highest filtering density when using shade cloth, but that trees on the range may be preferred over artificial structures. Across the summer months with intense sunlight and heat, hens are likely to prefer to remain inside across the middle of the day regardless of the shelter on the range. Shelter use decreased as the intensity of the sunlight and weather variables increased, which is likely a reflection of the low number of hens outside overall during the peak sunlight period within the day. Shelters were used in the evening with hens both underneath and on top of them indicating they are beneficial range enhancements even if hens still prefer to remain inside the shed during intense heat and sunlight.

1 Study 1: Laying hen preferences for different light spectrums and intensities

1.1 Introduction

Light in the laying hen industry can have significant impact on a hen's physiology, behaviour, production, and welfare (Manser 1996; Mohammed et al. 2010; Jacome et al. 2014). Typically, sources for artificial illumination in intensive commercial layer farms have been based on perception via human vision (Maddocks et al. 2001; Prescott et al. 2003). However, a chicken's visual perception is different (Bowmaker et al. 1997) as avian species can see part of the ultraviolet (UV) light spectrum (315–400 nm), namely the UVA wavelengths (Prescott et al. 1999). The UVB portion of the light spectrum (280–315 nm) is not visually perceived by hens but can penetrate the skin of a chicken's feet, comb and wattles, and play a key beneficial role in the production of vitamin D3, critical for bone mineralisation, bone growth, and eggshells (Rana & Campbell 2021). Too much UV radiation can cause cell damage.

The wavelengths of sunlight contain infrared radiation [700 nm–1 mm], human (and chicken) visible radiation [400–700 nm] and UV radiation [100–400 nm]. This UV radiation is comprised of three different types, with most UV radiation that reaches the earth's surface being UVA [315–400 nm] (95%), with a small amount of UVB [280–315 nm] (5%). All the UVC [100–280 nm] is screened out by the ozone layer (Holick 2016). This UVA and UVB radiation may affect how hens range in a free range system, as high UV intensities may be avoided. In addition to the effects of UV radiation, the intensity (brightness) of sunlight in the visual spectrum (human and chicken, 400–700 nm) may also impact hens' ranging behaviour. Indoor poultry lighting is typically kept at a lower lux level; in stark contrast to the light intensity that would be experienced outside under sunlight.

The objectives of this study were to use controlled indoor testing to determine hen preferences for different light wavelengths and intensities that may affect range usage. We predicted that hens would avoid light of high intensities, particularly the UVB wavelengths.

1.2 Methods

The study was conducted in the Rob Cumming Poultry Innovation Centre at the University of New England, Armidale, NSW, Australia. The research protocol was approved by the Animal Ethics Committee of the University of New England (AEC18-137).

1.2.1 Animals and husbandry

A total of 108 commercially supplied ISA Brown laying hens of 44 weeks of age were used. All hens were from the same caged flock with no outdoor exposure to natural daylight except during transport from the farm to the research facility (approximately 2 h duration). Hens were housed within nine home pens (3.2 m L x 1.75 m W) in a single room. Pens were supplied with nest boxes, perches, feeders, and drinkers with wood shavings as floor litter. The home pens were illuminated with poultry specific white LED bulbs (IP65 Dimmable LED Bulb, B-E27-10W-5K, Eco Industrial Supplies, China) with an average light intensity level of 20.3 ± 2.09 lux at bird's eye height level across the pens. The hens were maintained on a 16L:8D lighting schedule (lights on at 0500 h and off at 2100 h) during the study period. The home pens and testing room were environmentally controlled with mechanical ventilation set to maintain an average temperature of 21°C +/- 1 when possible, based on outdoor temperatures. Hens were allowed 12 days to adjust to the new facility before starting the testing process.

1.2.2 Light preference testing boxes

Six light preference testing boxes were set up in the adjacent room (testing room) at the facility. The 6 boxes were evenly distributed within the 9.6 m L x 6.2 m W room so that two boxes of the same light treatment were across from each other; test hens could hear but not see each other. Each of the black Formply, square-shaped boxes was divided in half, and comprised two identical adjacent compartments (180 cm L × 90 cm W × 60 cm H; Figure 1) joined by access tunnels (70 cm L × 20 cm W × 60 cm H) on each end. The tops of the compartments were covered with wire mesh to prevent the hens from escaping. Each compartment contained a round feeder and a water dish with wood shavings as floor litter. A temperature logger (Tinytag Plus 2, TGP-4500; Gemini Data Loggers Ltd, West Sussex, UK) was set up in each side of the box for 3 test boxes (Figure 1) all on the same side of the room to record temperature in 10 min intervals.

The testing room was illuminated by standard lighting similar to the home pens, with one side of each test box serving as the control condition where illumination was only the standard room lighting. The other side was the treatment condition where different lights under a round metal light shade were suspended just above the covering mesh (60 cm from the floor in the centre of the compartment; Figure 1). For balance, an empty metal light shade was placed on top of the centre of the mesh on the control side. Video cameras (Hikvision network turret cameras DS-2CD2355FWD-1) for each LPTA were installed at 150 cm above the floor and connected to a network video recorder in a separate room (Hikvision DS-7608NI-I2-8P CCTV NVR Recorder).

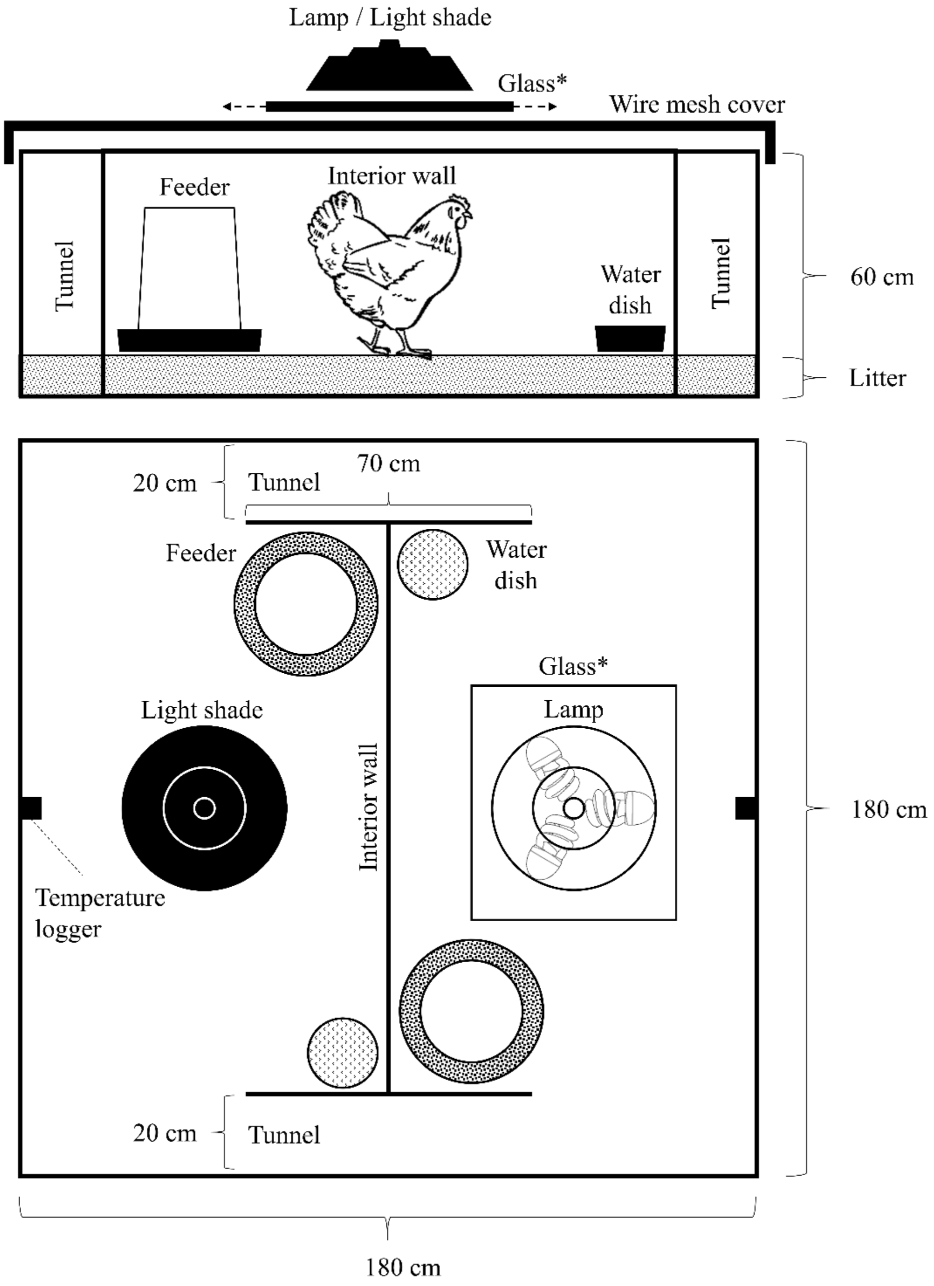


Figure 1 A schematic of the light preference testing box showing dimensions and placement of the food, water, lights and temperature loggers

* For the UVA treatments, 3 mm glass was placed between the lamp and the wire mesh cover to filter UVB wavelengths.

1.2.3 Test lights

The selected lights for preference testing were commercially available Exo Terra® (Rolf C. Hagen, Montreal, QC, Canada) pet reptile bulbs. The lights were selected to determine broadly what part of the light spectrum was aversive to the hens in high intensities (within the scope of what light spectrums were commercially available), and to understand what might be limiting factors for using the range on days with intense sunshine. Three different types were selected as being representative of: (1) the human/chicken visible spectrum (VIS) but including infrared wavelengths (Halogen Basking Spot

PT2181 50W, PT2182 75W, and PT2183 100W); (2) the human/chicken visible spectrum including UVA (Reptile UVB200, 25W, PT2341); and (3) the human/chicken visible spectrum including UVA and UVB (UVAB) (Reptile UVB200, 25W, PT2341; Figure 2). The peaks in the visible spectrum across the three bulb types were not equivalent. The same bulbs were used for the UVA and UVAB treatments with 3 mm glass placed under the bulbs in the UVA treatment to filter out the UVB wavelengths. Three light intensity levels (low, medium and high) of each three different light treatments were also tested by using different bulb wattages (treatment 1), or increasing the numbers of bulbs of the same wattage (in treatments 2 and 3, up to 3 bulbs were used). The treatment lights were suspended directly above the wire mesh covering (Figure 1). There were approximately 10 cm of wood shavings placed on the concrete floor within each testing box and thus standing chicken eye height was approximately 30 cm above the wood shavings or 20 cm from the light source. If the hens stretched their necks, they may have been only a few cm from the bulb. All spectral wavelengths and radiation intensities were measured using an Ocean Insight Flame-S-XR1 Spectroradiometer (200–1025 nm, Quark Photonics, Melbourne, VIC, Australia) at a distance of 20 cm (Figure 3a-d). Readings of the control light were taken at 15 cm due to the comparatively low intensity of this light. The lux of the lights was also measured using a digital luxmeter (Lutron Light Meter, LX-112850; Lutron Electronic Enterprise Co., Ltd, Taipei, Taiwan) and the ultraviolet index (UV index) was measured for the UVAB treatment using a reptile UV index meter (Solarmeter Model 6.5R UVI Reptile, Solarmeter Australia, Noosaville DC, QLD) (Table 1).

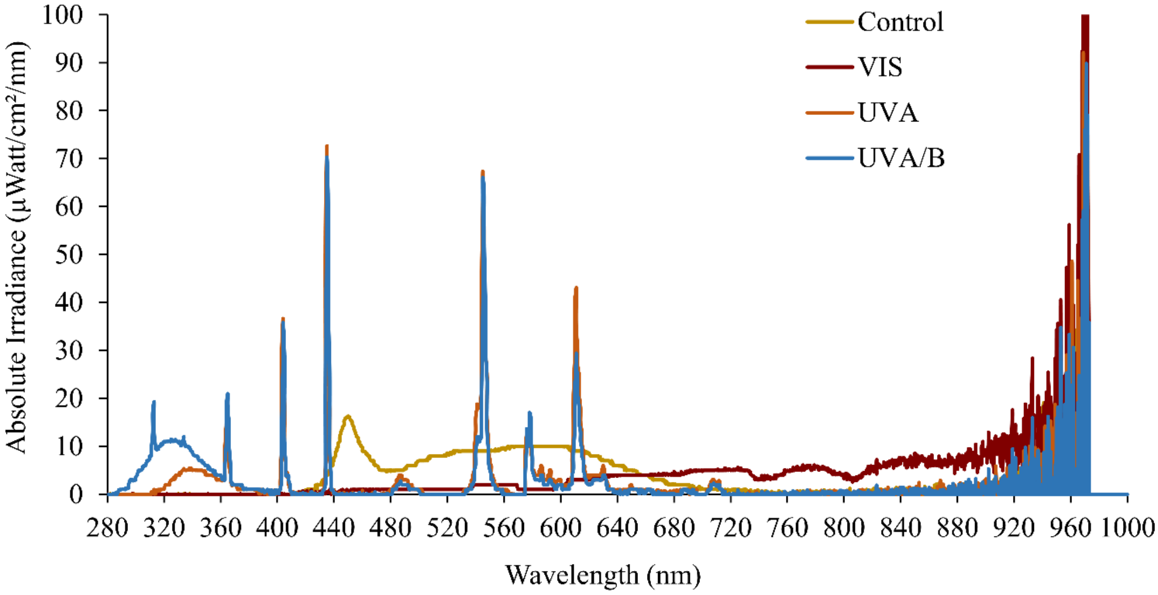


Figure 2 Spectral irradiance of different light treatments (high intensity) as measured by an Ocean Insight Flame-S-XR1 Spectroradiometer at 20 cm from the source (15 cm from source for control for visual appearance)

Control: Poultry specific LED-White bulb (IP65 Dimmable LED Bulb (420–724 nm)), B-E27-10W-5K.
VIS: Halogen Basking Spot Light (372–800 nm), PT2181 50W, Exo Terra®.
UVA: Reptile UVB200 light (320–712 nm), 25W, PT234, Exo Terra® with 3 mm glass placed under the bulbs.
UVAB: Reptile UVB200 light (288–714 nm), 25W, PT234, Exo Terra®.

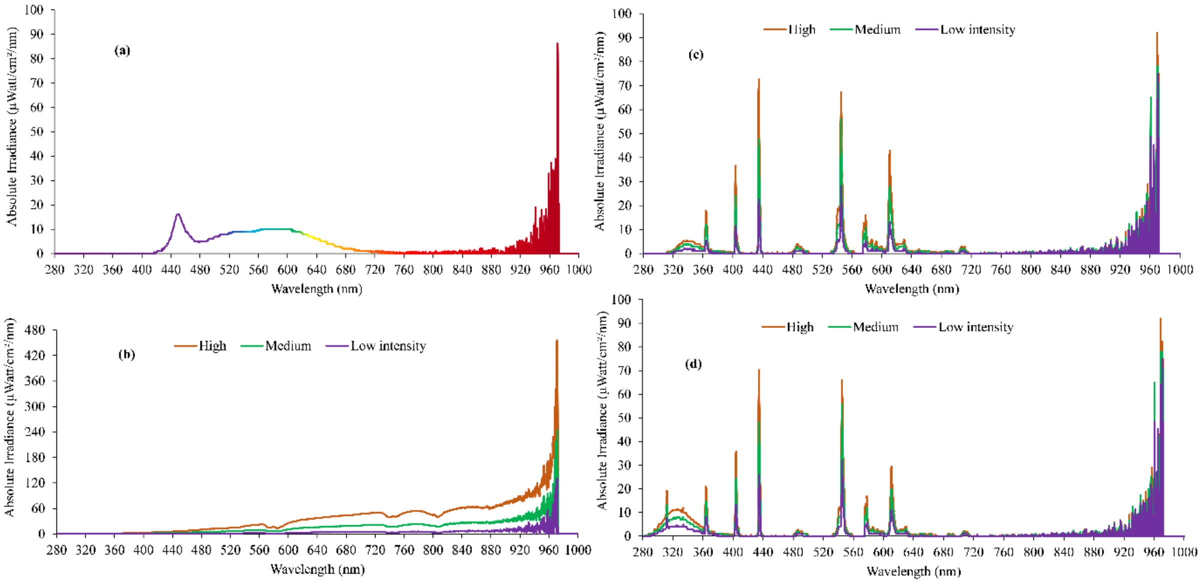


Figure 3 Spectral composition of the lights of different intensity as measured by an Ocean Insight Flame-S-XR1 Spectroradiometer at 20 cm from the source (15 cm from source for control for visual appearance)

(a) Control: Poultry specific white LED bulb (IP65 Dimmable LED Bulb, B-E27:10W–5K).

(b) VIS: Halogen Basking Spot Light, Exo Terra®. Intensity of light considered as PT2181 50W (Low), PT2182 75W (Medium), and PT2183 100W (High).

(c) UVA: Reptile UVB 200, 25W, PT2341, Exo Terra® was used with 3 mm glass placed under the bulbs to block UVB wavelength. Intensity was considered as Low (1 x UVB 200 bulb), Medium (2 x UVB 200 bulb), and High (3 x UVB 200 bulb).

(d) UVAB: Reptile UVB 200, 25W, PT2341, Exo Terra®. Intensity was considered as Low (1 x UVB 200 bulb), Medium (2 x UVB 200 bulb), and High (3 x UVB 200 bulb).

Table 1 Parameters of treatment lights at different intensity levels

Treatment light	Wavelength (nm)	Level of intensity	Light intensity (lux)*	UV index**
LED-White	420–724	Control/ambient	20.3 ± 2.09	-
VIS	372–800	Low	1275.50	-
		Medium	6124.40	-
		High	14384.00	-
UVA	320–712	Low	1273.40	-
		Medium	2659.60	-
		High	3913.30	-
UVAB	288–714	Low	1147.60	5.1
		Medium	2166.40	9.4
		High	3057.30	16.3

* All the light measurements were taken at hen's eye height (30 cm above from the floor and/or 20 cm from the sources).

** The UV index was only available for the UVAB as this is calculated based on a range of factors including the relative contributions of both types of UV light.

1.2.4 Bird acclimation and preference testing

For acclimation to the testing box, hens were placed in the box on 4 separate occasions across different weeks first as a group and then as individuals, commencing at 46 weeks of age. In total, each hen spent 23 h within the box during the habituation phase. Hens were monitored via video cameras throughout the daytime habituation periods, to observe their movement. In the final habituation session, only hens that had exhibited movement between the two compartments via the tunnel (minimum 2 times between compartments was observed) were selected to proceed with testing (n = 84 hens).

Following habituation, the selected 84 hens (28 hens/treatment) were individually tested for different light preferences. An individual hen was only tested within one of the three treatment groups but was exposed to all three intensities within that treatment. The three intensities (low, medium, high) were tested sequentially to minimise the effect of previous exposure on the hens' preferences, and to simulate what a hen may experience when the pop holes first open within a free range facility. The hens would gradually be exposed to increasing intensities of light as they slowly venture outside for the first time following indoor rearing (typical practice within Australia and other countries). Individual hens were tested with each level of light intensity from 51 to 53 weeks of age. It took approximately 5 days to test all 28 hens of a specific treatment for one intensity. During testing, two test boxes were simultaneously used for each treatment, allowing 6 hens to be tested on each occasion. For consistency, birds were always placed in the treatment compartment first and allowed 2 h to exhibit a choice before being returned to their home pen. All the testing sessions were recorded by the video cameras for later analysis.

1.2.5 Video observations and data analyses

Six overhead video cameras recorded the position and behaviour of the hens throughout the testing period. Data were generated by watching the full length (2 h) video records of all tested hens (n = 252 test sessions) individually by a single observer using the "Behavioural Observation Research Interactive Software (BORIS)" (Friard & Gamba 2016).

The durations of time that hens spent in the treatment light compartment, in the standard light (control) compartment, or in the tunnels were expressed in total number of seconds for each of the 2 h test periods, and used to calculate the percentages of time that hens spent in each compartment. The frequencies of a hen's movement between the compartments were also used to measure the latency (s) until the hen first exited the treatment compartment, the number of transitions (visits to each compartment), and the duration of each compartment visit. Furthermore, during observation, each of the compartments was split into thirds (on screen) to document the time spent directly under the light sources (the middle) and in either side (right/left). The food and water were located in the right and left thirds. Behaviours of the hens during the testing periods were also recorded in each compartment by the same observer based on the ethogram displayed in Table 2. Behaviours of eating, foraging, drinking, and dust bathing were measured as state events in seconds, whereas other behaviours of body shaking, ground pecking, preening, leg stretching, wing flapping, and escape attempts were measured as point events (frequency).

All data were analysed in JMP® 14.0 (SAS Institute, Cary, NC, USA) with α level set at 0.05. Data were compiled per individual hen separately for each light treatment and level of intensity. These data included the proportion of the 2 h test period spent in each compartment (minus the time spent in the tunnels), the total time spent in the middle of each compartment (min), the latency to the first exit from the treatment compartment (hens were always placed on the treatment side), the number of visits to each compartment, and the mean duration (min) of each compartment visit. The temperature data were averaged per test session to provide a single value for the test period of an individual hen.

Data were transformed where necessary to enable parametric testing. General linear mixed models (GLMM) were applied to each parameter separately for different intensity levels for each light treatment, with compartment, and temperature nested within compartment included as fixed effects and hen ID as a random effect. GLMMs were also applied to compare data from the treatment side only between the different light treatments separately at each intensity level including the proportion of time spent, time spent in the middle, mean visit duration, mean number of visits, and latency to first exit. Light treatment and temperature nested within compartment were included as fixed effects and hen ID as a random effect.

For behavioural response data analysis, feeding time, drinking time, foraging time and dust bathing were recorded as state events and measured in seconds, which were converted to minutes in the final analysis and $\log_{10}$ transformed. However, dust bathing occurred infrequently and thus was not statistically analysed, but the data are presented in the tables. The behavioural responses including body shaking, ground pecking, and preening were recorded as point events and the count values were square-root-transformed with the raw values presented in the tables. There were insufficient observations of dust bathing, wing flapping, leg stretching and escape attempts for statistical analysis. GLMMs were fitted to analyse each behavioural response separately for different intensity levels of each light treatment, with compartment, and temperature nested within compartment included as fixed effects and hen ID as a random effect.

Table 2 Definition of time spent variables and behavioural ethogram used in the video observations

Parameters	Event type	Unit	Definition
		Time spent	
Time spent	State	min/hen	Average time spent in a compartment during the testing period.
Percentage of time spent	State	%	Time spent (min) ÷ 120 × 100%.
Inter-compartment transitions (visit)	Point	count	The number of visits a hen makes to a compartment within the testing period.
Mean visit duration	State	min/time	Time spent in a compartment during a single visit.
Time spent at middle	State	min/time	Average time spent in the middle of the compartment (under the light sources) during the testing period.
		Behavioural ethogram	
Feeding time	State	min/hen	Time spent at the feeder starting from when the hen commenced feeding until she turned away from the feeder. This time included brief pauses that the hen may have made during feeding.
Foraging time	State	min/hen	Scratching at substrate with feet followed by pecking on the ground.
Drinking time	State	min/hen	Time spent at the water dish starting from when the hen lowered her head and consumed water until the hen turned away from the dish. This time included brief pauses when the hen was not consuming water but was still facing the water dish.
Dust bathing	State	min/hen	Rolling or moving around in substrate, wings fluffed up, kicking substrate into the feathers.
Preening	Point	count	Grooming of feathers with beak.
Ground pecking	Point	count	Pecking at substrate or fallen feathers on the ground.
Body shaking	Point	count	Rapid whole-body movement associated with ruffling of the feathers that occurred randomly throughout the test period or at the end of the dust bathing sequence.
Wing flapping	Point	count	Opening of the wings while still standing.
Escaping (jump)	Point	count	Frequency of hens trying to escape from the apparatus by jumping upwards.
Leg stretching	Point	count	One leg stretched out on either side of the body.

1.3 Results

1.3.1 Time spent

Visible Spectral Light (VIS). The proportion of total time spent in the compartments was significantly affected by treatment for the low (*P* = 0.02) and high intensity (*P*= 0.03) of VIS light, with hens spending more time in the treatment side over the control side (Figure 4). In contrast, there was only a trend to spend more time on the treatment side at the medium intensity (*P* = 0.06; Figure 4). There were no significant differences in the time spent in the middle of the compartment (all *P* ≥ 0.15), the frequency of visits between the compartments (all *P* ≥ 0.08), and mean visit duration (all *P* ≥ 0.24) for any intensity levels of the VIS treatment but temperature did have a significant effect on the frequency of visits (*P* = 0.01), and mean visit duration (*P* = 0.04; Table 3) at the medium intensity level.

Ultraviolet A Light (UVA). The proportion of total time spent in the compartments was significantly affected by treatment for the medium intensity level (*P* = 0.04) with hens spending more time in the treatment side, but there was no effect of treatment for the low intensity level (P = 0.99) and a trend to spend more time on the treatment side at the high intensity level (*P* = 0.06; Figure 4). Hens also spent more time specifically in the middle of the treatment compartment at the medium intensity level (*P* = 0.05) but not at the low (*P* = 0.99) or high (*P* = 0.28) intensities (Table 4). Similarly, hens showed significantly more visits to the treatment side (*P* = 0.001) at the medium intensity level (*P* = 0.001) but not at the low (*P* = 0.24) or high (*P* = 0.18) intensities (Table 4). Temperature had a significant effect on the frequency of visits (*P* = 0.04) at the high intensity levels (Table 4). There was no significant effect of light treatment on the mean visit duration at any intensity (all *P* ≥ 0.24; Table 4).

Ultraviolet A/B light (UVAB). The proportion of total time spent in the compartments was significantly affected by treatment for the low (*P* = 0.03) and medium (*P* = 0.02) intensity levels, with hens preferring to spend more time on the treatment side (Figure 4). In contrast, there was no preference exhibited at the high intensity level (*P* = 0.17; Figure 4). Hens also spent significantly more time in the middle of the treatment compartment at the low (*P* = 0.01) intensity level but not at the medium (*P* = 0.21) or high intensities (*P* = 0.96; Table 5). There was no effect of treatment on the frequency of visits at any intensity level (all *P* ≥ 0.29; Table 5) but at medium intensity levels, temperature showed a significant effect on the frequency of hens' visits between the compartments (*P* = 0.04; Table 5). There were significantly longer visits to the treatment side at the low intensity level (*P* = 0.05) but not the medium or high intensities (both *P* ≥ 0.16; Table 5).

1.3.2 Relative preferences among light treatments

Hens' latency to first exit the treatment compartment did not differ among the treatment groups at any level of intensity (all *P* ≥ 0.15; Table 6), but there was an effect of temperature on the latency to exit at the medium intensity level (*P* = 0.03; Table 6). There were no significant effects of light treatment on hens' preferences for overall time spent and time spent at the middle of the compartment for any intensity level (all *P* ≥ 0.13, and all *P* ≥ 0.29 respectively; Table 6) except for a significant effect of temperature on hens' time spent at the medium intensity (*P* = 0.002; Table 6). Although there were no significant differences in the number of visits to the treatment compartment across all intensities (all *P* ≥ 0.24), hens did show a preference to spend a greater amount of time under the UVAB light and least amount of time under the UVA light during a single visit at the low intensity (*P* = 0.04; Table 6). There were no effects of treatment on the mean visit duration at the medium (*P* = 0.64) and high (*P* = 0.83) intensities (Table 6), but temperature did have a significant effect on the mean visit duration at the medium intensity (*P* = 0.01) and the frequency of visits at the high intensity (*P* = 0.01; Table 6).

1.3.3 Behavioural responses

There were no significant effects of the VIS light treatment on the time spent feeding, drinking, and foraging; or the frequency of ground pecking, and preening at any intensity level (all $P \geq 0.12$; Table 7), except for more body shaking observed under the low intensity of VIS treatment light ($P = 0.04$; Table 7). In the UVA light treatment, hens spent more time feeding under the standard light at all three intensity levels (all $P \leq 0.03$; Table 7) but there were no significant effects on any of the other measured behaviours (Table 7). In contrast, in the UVAB light treatment, hens showed more foraging under the medium intensity of the treatment light ($P = 0.002$; Table 7), more ground pecking under both the low ($P = 0.004$) and medium ($P = 0.01$) intensities of the treatment light and more preening under the low intensity of the UVAB light ($P = 0.01$; Table 7).

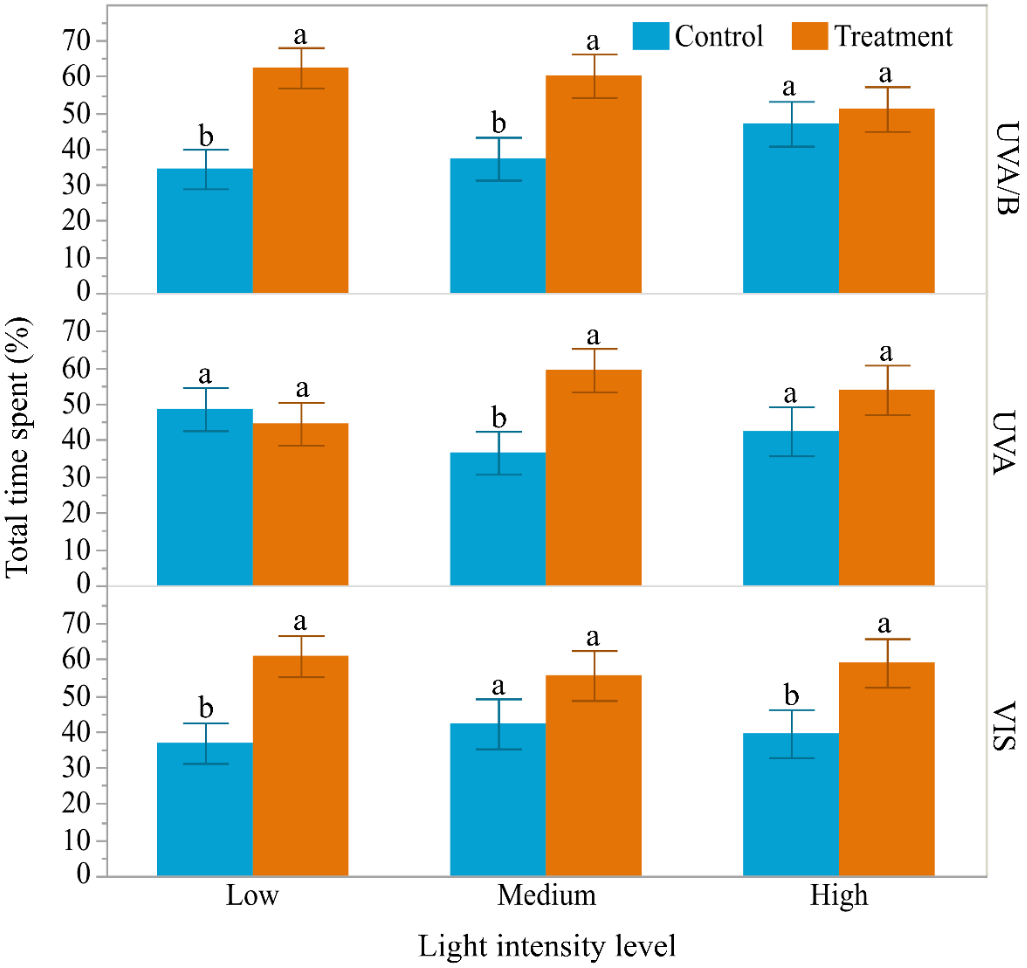


Figure 4 The least squares means ± standard error of the means for hens' percentage of time spent on the control or treatment side at different levels of VIS, UVA, and UVAB light intensity

Raw values are presented but analyses were conducted on transformed data.

[a,b] Dissimilar superscript letters indicate significant differences between control and treatment lights ($P < 0.05$) separately for each intensity level of each treatment.

Table 3 Hens' time spent at different intensity levels of the VIS light treatment

Variable	**Level of light intensity**	**Category**	**Time spent[1]** (min)	**Test statistics** (df, F-Ratio, *P*-value)	**Temperature (°C)** LSM ± SEM (Control/Treatment) (df, F-Ratio, *P*-value**)**
Time spent at middle	Low	Control	15.95 ± 5.49	$F_{(1, 31.34)}$ = 2.15, *P* = 0.15	24.44 ± 0.33 **/** 26.10 ± 0.33 $F_{(2, 30.73)}$ = 0.37, *P* = 0.69
		Treatment	36.01 ± 5.42		
	Medium	Control	21.55 ± 4.78	$F_{(1, 30.58)}$ = 0.60, *P* = 0.44	24.51 ± 0.27 **/** 26.05 ± 0.27 $F_{(2, 34.19)}$ = 3.10, *P* = 0.06
		Treatment	38.95 ± 4.74		
	High	Control	24.34 ± 5.63	$F_{(1, 26.25)}$ = 0.19, *P* = 0.67	23.60 ± 0.44 **/** 25.46 ± 0.44 $F_{(2, 28)}$ = 0.16, *P* = 0.85
		Treatment	30.07 ± 5.63		
Inter-compartment transitions (visits)	Low	Control	10.02 ± 3.12	$F_{(1, 48.45)}$ = 3.29, *P* = 0.08	$F_{(2, 38.54)}$ = 1.06, *P* = 0.36
		Treatment	11.55 ± 3.11		
	Medium	Control	12.62 ± 4.44	$F_{(1, 48.52)}$ = 0.33, *P* = 0.57	$F_{(2, 38.20)}$ = 5.35, ***P* = 0.01**
		Treatment	9.72 ± 4.43		
	High	Control	11.58 ± 2.94	$F_{(1, 37.12)}$ = 0.65, *P* = 0.43	$F_{(2, 36.41)}$ = 2.96, *P* = 0.06
		Treatment	9.68 ± 2.94		
Mean visit duration	Low	Control	16.52 ± 7.74	$F_{(1, 37.09)}$ = 1.13, *P* = 0.29	$F_{(2, 26.56)}$ = 0.18, *P* = 0.84
		Treatment	26.80 ± 7.65		
	Medium	Control	32.06 ± 8.52	$F_{(1, 39.63)}$ = 0.58, *P* = 0.45	$F_{(2, 32.69)}$ = 3.57, ***P* = 0.04**
		Treatment	42.71 ± 8.46		
	High	Control	16.40 ± 8.34	$F_{(1, 23.99)}$ = 1.47, *P* = 0.24	$F_{(2, 21.18)}$ = 1.93, *P* = 0.17
		Treatment	44.94 ± 8.35		

[1] The least squares means ± standard error of the means are presented for each variable.

[a,b] Dissimilar superscript letters indicate significant differences between control and treatment lights (*P* < 0.05). Significant *P*-values are indicated in **bold**.

Table 4 Hens' time spent at different intensity levels of the UVA light treatment

Variable	Level of light intensity	Category	Time spent[1] (min)	Test statistics (df, dfDen, F-Ratio, *P*-value)	Temperature (°C) LSM ± SE (Control/Treatment) (df, dfDen, F-Ratio, *P*-value)
Time spent at middle	Low	Control	24.12 ± 5.16	$F_{(1, 24.55)}$ = 0.0003, *P* = 0.99	25.33 ± 0.32 / 25.37 ± 0.32 $F_{(2, 31.35)}$ = 1.38, *P* = 0.27
		Treatment	26.73 ± 5.16		
	Medium	Control	19.63 ± 5.49^{b}	$F_{(1, 24.70)}$ = 4.40, ***P* = 0.05**	25.22 ± 0.19 / 25.36 ± 0.19 $F_{(2, 30.49)}$ = 0.54, *P* = 0.59
		Treatment	37.05 ± 5.49^{a}		
	High	Control	16.55 ± 5.50	$F_{(1, 21.17)}$ = 1.24, *P* = 0.28	24.29 ± 0.29 / 24.54 ± 0.29 $F_{(2, 25.72)}$ = 0.45, *P* = 0.64
		Treatment	28.14 ± 5.50		
Inter-compartment transitions (visits)	Low	Control	19.52 ± 5.49	$F_{(1, 26.46)}$ = 1.42, *P* = 0.24	$F_{(2, 35.08)}$ = 0.35, *P* = 0.71
		Treatment	19.65 ± 5.49		
	Medium	Control	10.49 ± 2.76^{b}	$F_{(1, 34.10)}$ = 12.75, ***P* = 0.001**	$F_{(2, 35.43)}$ = 0.03, *P* = 0.97
		Treatment	11.63 ± 2.76^{a}		
	High	Control	17.62 ± 7.72	$F_{(1, 46.61)}$ = 1.83, *P* = 0.18	$F_{(2, 36.11)}$ = 3.50, ***P* = 0.04**
		Treatment	17.43 ± 7.72		
Mean visit duration	Low	Control	17.50 ± 5.90	$F_{(1, 20)}$ = 0.98, *P* = 0.33	$F_{(2, 28.31)}$ = 0.89, *P* = 0.43
		Treatment	13.08 ± 5.90		
	Medium	Control	13.72 ± 6.96	$F_{(1, 17.95)}$ = 1.48, *P* = 0.24	$F_{(2, 25.31)}$ = 1.92, *P* = 0.17
		Treatment	28.86 ± 6.96		
	High	Control	16.34 ± 8.10	$F_{(1, 16.47)}$ = 0.01, *P* = 0.91	$F_{(2, 24.20)}$ = 1.95, *P* = 0.16
		Treatment	39.46 ± 8.10		

[1] The least squares means ± standard error of the means are presented for each variable.

[a,b] Dissimilar superscript letters indicate significant differences between control and treatment lights ($P < 0.05$). Significant *P*-values are indicated in **bold**.

Table 5 Hens' time spent at different intensity levels of the UVAB light treatment

Variable	Level of light intensity	Category	Time spent[1] (min)	Test statistics (df, dfDen, F-Ratio, *P*-value)	Temperature (°C) LSM ± SE (Control/Treatment) (df, dfDen, F-Ratio, *P*-value)
Time spent at middle	Low	Control	16.96 ± 3.93^{b}	$F_{(1, 26.32)} = 6.83$, **P = 0.01**	25.09 ± 0.33 / 25.59 ± 0.33 $F_{(2, 33.84)} = 0.30$, $P = 0.75$
		Treatment	33.50 ± 3.99^{a}		
	Medium	Control	16.54 ± 4.31	$F_{(1, 24.91)} = 1.66$, $P = 0.21$	24.88 ± 0.29 / 25.66 ± 0.29 $F_{(2, 32.91)} = 1.11$, $P = 0.34$
		Treatment	36.44 ± 4.31		
	High	Control	21.72 ± 3.97	$F_{(1, 27.41)} = 0.002$, $P = 0.96$	23.96 ± 0.38 / 25.01 ± 0.38 $F_{(2, 31.75)} = 0.89$, $P = 0.44$
		Treatment	26.74 ± 3.96		
Inter-compartment transitions (visits)	Low	Control	16.23 ± 4.50	$F_{(1, 47.82)} = 0.05$, $P = 0.82$	$F_{(2, 37.27)} = 1.61$, $P = 0.21$
		Treatment	15.30 ± 4.50		
	Medium	Control	14.17 ± 3.14	$F_{(1, 39.75)} = 0.26$, $P = 0.61$	$F_{(2, 35.83)} = 3.57$, **P = 0.04**
		Treatment	13.12 ± 3.14		
	High	Control	14.76 ± 5.19	$F_{(1, 36.65)} = 1.15$, $P = 0.29$	$F_{(2, 36.35)} = 2.00$, $P = 0.15$
		Treatment	15.24 ± 5.19		
Mean visit duration	Low	Control	12.22 ± 6.43^{b}	$F_{(1, 23.18)} = 4.35$, **P = 0.05**	$F_{(2, 31.31)} = 2.53$, $P = 0.10$
		Treatment	29.44 ± 6.43^{a}		
	Medium	Control	10.01 ± 6.31	$F_{(1, 26.40)} = 2.13$, $P = 0.16$	$F_{(2, 28.34)} = 1.58$, $P = 0.22$
		Treatment	33.28 ± 6.32		
	High	Control	21.86 ± 4.25	$F_{(1, 32.07)} = 0.12$, $P = 0.73$	$F_{(2, 29.29)} = 2.51$, $P = 0.10$
		Treatment	32.35 ± 7.23		

[1] The least squares means ± standard error of the means are presented for each variable.

[a,b] Dissimilar superscript letters indicate significant differences between control and treatment lights ($P < 0.05$). Significant *P*-values are indicated in **bold**.

Table 6 Comparisons of hens' time spent at different intensity levels of treatment lights

Light intensity level	**Light treatments**	**Time spent** (%)	**Time spent middle** (min)	**Mean visit duration** (min)	**Visits to treatment compartment** (n)	**Latency of first exit** (min)
Low	VIS	60.37 ± 5.97	36.56 ± 5.33	27.71 ± 7.34^{a}	11.74 ± 4.55	31.04 ± 7.54
	UVA	43.76 ± 5.91	27.21 ± 5.28	14.23 ± 7.27^{b}	19.35 ± 4.5	20.08 ± 7.47
	UVAB	62.72 ± 5.82	33.65 ± 5.20	28.53 ± 7.15^{a}	16.45 ± 4.44	26.31 ± 7.35
	Test statistics	$F_{(2, 78)} = 2.08$, $P = 0.13$	$F_{(2, 76)} = 1.27$, $P = 0.29$	$F_{(2, 78)} = 3.31$, **$P = 0.04$**	$F_{(2, 78)} = 1.45$, $P = 0.24$	$F_{(2, 78)} = 1.92$, $P = 0.15$
	Temperature x light treatments	$F_{(3, 78)} = 0.16$, $P = 0.92$	$F_{(3, 76)} = 0.17$, $P = 0.91$	$F_{(3, 78)} = 0.41$, $P = 0.74$	$F_{(2, 76)} = 1.27$, $P = 0.29$	$F_{(3, 78)} = 1.70$, $P = 0.17$
Medium	VIS	59.88 ± 6.22	34.64 ± 5.33	36.10 ± 8.00	10.31 ± 3.63	43.69 ± 8.34
	UVA	61.59 ± 6.19	39.03 ± 5.31	32.02 ± 7.97	11.42 ± 3.61	39.94 ± 8.30
	UVAB	60.21 ± 6.03	35.22 ± 5.17	29.01 ± 7.76	14.09 ± .052	38.97 ± 8.08
	Test statistics	$F_{(2, 78)} = 0.02$, $P = 0.98$	$F_{(2, 77)} = 0.66$, $P = 0.52$	$F_{(2, 78)} = 0.45$, $P = 0.64$	$F_{(2, 78)} = 1.41$, $P = 0.25$	$F_{(2, 78)} = 0.08$, $P = 0.92$
	Temperature x light treatments	$F_{(3, 78)} = 5.49$, **$P = 0.002$**	$F_{(3, 77)} = 2.0$, $P = 0.12$	$F_{(3, 78)} = 4.41$, **$P = 0.01$**	$F_{(2, 76)} = 1.27$, $P = 0.29$	$F_{(3, 78)} = 3.06$, **$P = 0.03$**
High	VIS	59.71 ± 6.72	29.25 ± 5.49	41.46 ± 9.31	10.40 ± 5.62	49.15 ± 9.52
	UVA	55.99 ± 6.74	27.72 ± 5.51	39.69 ± 9.33	21.37 ± 5.64	41.76 ± 9.55
	UVAB	51.10 ± 6.59	26.53 ± 5.38	31.10 ± 9.12	15.30 ± 5.51	36.19 ± 9.33
	Test statistics	$F_{(2, 78)} = 0.57$, $P = 0.57$	$F_{(2, 77)} = 0.50$, $P = 0.61$	$F_{(2, 78)} = 0.18$, $P = 0.83$	$F_{(2, 78)} = 5.53$, $P = 0.59$	$F_{(2, 78)} = 0.28$, $P = 0.76$
	Temperature x light treatments	$F_{(3, 78)} = 0.94$, $P = 0.43$	$F_{(3, 77)} = 0.18$, $P = 0.91$	$F_{(3, 78)} = 1.11$, $P = 0.35$	$F_{(2, 78)} = 3.86$, **$P = 0.01$**	$F_{(3, 78)} = 75$, $P = 0.52$

The least squares means ± standard error of the means are presented for each variable.

[a,b,c] Dissimilar superscript letters indicate significant differences between treatment lights (P < 0.05). Significant P-values are indicated in ***bold***.

Table 7 Hen's behavioural responses under lights of different levels of intensity

Variable	Level of light intensity	Category	VIS light		UVA light		UVAB light	
			LSM ± SEM	*P*-value*	LSM ± SEM	*P*-value*	LSM ± SEM	*P*-value*
Feeding (min)	Low	Control	5.30 ± 1.50	0.24	8.10 ± 1.58^{a}	**0.03**	4.83 ± 1.65	0.78
		Treatment	4.21 ± 1.48		3.92 ± 1.52^{b}		7.58 ± 1.65	
	Medium	Control	4.44 ± 1.76	0.56	4.61 ± 1.34^{a}	**0.03**	6.56 ± 2.10	0.60
		Treatment	3.47 ± 1.75		2.81 ± 1.34^{b}		5.02 ± 2.10	
	High	Control	2.31 ± 0.89	0.83	7.65 ± 2.06^{a}	**0.002**	5.63 ± 1.75	0.68
		Treatment	3.08 ± 0.89		2.93 ± 2.06^{b}		4.34 ± 1.75	
Drinking (min)	Low	Control	3.32 ± 1.24	0.22	0.72 ± 0.33	0.46	1.25 ± 0.51	0.58
		Treatment	2.01 ± 1.23		0.85 ± 0.33		1.49 ± 0.51	
	Medium	Control	2.07 ± 0.55	0.12	0.70 ± 0.36	0.28	0.59 ± 0.45	0.83
		Treatment	0.68 ± 0.56		0.98 ± 0.36		2.11 ± 0.45	
	High	Control	1.16 ± 0.84	0.48	1.41 ± 0.48	0.40	1.09 ± 0.57	0.23
		Treatment	2.09 ± 0.84		0.87 ± 0.48		2.50 ± 0.57	
Foraging (min)	Low	Control	0.87 ± 0.37	0.32	1.10 ± 0.87	0.61	0.56 ± 0.88	0.34
		Treatment	0.54 ± 0.36		1.77 ± 0.87		1.88 ± 0.88	
	Medium	Control	4.13 ± 1.43	0.58	0.42 ± 0.54	0.84	0.97 ± 0.74^{b}	**0.002**
		Treatment	0.45 ± 1.42		1.68 ± 0.54		1.85 ± 0.74^{a}	
	High	Control	3.83 ± 1.67	0.40	1.41 ± 0.51	0.18	1.86 ± 1.01	0.68
		Treatment	0.92 ± 1.67		1.40 ± 0.51		1.23 ± 1.01	
Dust Bathing (min)	Low	Control	1(4.37)	*	0	*	0	*
		Treatment	1(30.89)		0		1(10.60)	
	Medium	Control	3(9.83 ± 2.93)		0		0	
		Treatment	1(22.22)		0		2(9.74 ± 4.69)	
	High	Control	2(15.06 ± 12.92)		0		1(43.07)	
		Treatment	3(17.05 ± 9.79)		0		2(11.61 ± 4.79)	
Body Shaking	Low	Control	1.23 ± 0.42^{b}	**0.04**	2.15 ± 0.47	0.60	1.63 ± 0.42	0.08
		Treatment	2.41 ± 0.41^{a}		1.71 ± 0.47		2.44 ± 0.42	
	Medium	Control	1.81 ± 0.39	0.23	1.18 ± 0.31	0.25	1.28 ± 0.31	0.34
		Treatment	1.52 ± 0.39		1.66 ± 0.31		1.59 ± 0.31	
	High	Control	1.44 ± 0.42	0.36	1.52 ± 0.30	0.39	1.18 ± 0.24	0.97
		Treatment	1.70 ± 0.42		0.75 ± 0.30		1.11 ± 0.24	

Variable	**Level of light intensity**	**Category**	**VIS light**		**UVA light**		**UVAB light**	
			LSM ± SEM	***P*-value***	**LSM ± SEM**	***P*-value***	**LSM ± SEM**	***P*-value***
Ground pecking	Low	Control	2.53 ± 0.95	0.18	2.86 ± 0.72	0.62	1.93 ± 0.73^{b}	**0.004**
		Treatment	3.29 ± 0.94		2.94 ± 0.72		4.20 ± 0.73^{a}	
	Medium	Control	3.78 ± 0.94	0.49	2.46 ± 0.88	0.06	1.96 ± 0.58^{b}	**0.01**
		Treatment	2.43 ± 0.93		3.13 ± 0.88		3.81 ± 0.58^{a}	
	High	Control	3.22 ± 0.84	0.52	2.46 ± 0.59	0.79	2.30 ± 0.50	0.31
		Treatment	2.35 ± 0.84		1.61 ± 0.59		2.74 ± 0.50	
Preening	Low	Control	0.69 ± 0.35	0.16	1.45 ± 0.48	0.20	0.31 ± 0.23^{b}	**0.01**
		Treatment	1.42 ± 0.35		1.38 ± 0.48		1.23 ± 0.23^{a}	
	Medium	Control	0.37 ± 0.18	0.52	0.32 ± 0.20	0.56	0.31 ± 0.16	0.95
		Treatment	0.56 ± 0.18		0.55 ± 0.20		0.22 ± 0.16	
	High	Control	0.42 ± 0.27	0.65	0.71 ± 0.23	0.54	0.32 ± 0.15	0.88
		Treatment	0.56 ± 0.27		0.39 ± 0.23		0.32 ± 0.15	
Leg stretching	Low	Control	0	*	0	*	0	*
		Treatment	0		0		0	
	Medium	Control	0		0		0	
		Treatment	0		0		0	
	High	Control	0		0		0	
		Treatment	1(2)		1(1)		0	
Wing flapping	Low	Control	2(1 ± 0.0)	*	3(3.33 ± 1.86)	*	2(1 ± 0.0)	*
		Treatment	2(3 ± 0.0)		1(2 ± 0.0)		5(3.16 ± 2.23)	
	Medium	Control	3(2 ± 0.58)		2(1.5 ± 0.5)		2(4 ± 1.0)	
		Treatment	2(1.5 ± 0.5)		3(2.33 ± 1.33)		4(3.5 ± 1.03)	
	High	Control	4(2.75 ± 1.75)		4(2 ± 0.58)		2(6 ± 5.0)	
		Treatment	5(1.6 ± 0.4)		4(1.75 ± 0.48)		3(5 ± 3.51)	
Escaping	Low	Control	3(6.33 ± 2.73)	*	6(10 ± 4.91)	*	2(4 ± 2.0)	*
		Treatment	7(2.71 ± 1.29)		8(9.25 ± 4.09)		9(2.33 ± 0.83)	
	Medium	Control	2(11 ± 7.0)		5(12.8 ± 7.63)		1(23)	
		Treatment	9(9.89 ± 7.57)		6(12 ± 3.29)		3(11.67 ± 9.21)	
	High	Control	2(7 ± 6.0)		2(21.5 ± 10.5)		2(20 ± 16.0)	
		Treatment	5(2.4 ± 0.75)		5(7.2 ± 2.63)		3(7.67 ± 3.28)	

[a,b] Dissimilar superscript letters indicate significant differences between control and treatment lights ($P < 0.05$). Significant *P*-values are indicated in **bold**.

* Chi-square tests were not performed due to insufficient data, the raw values are presented as the number of hens that exhibited the behaviour and the mean (±SEM) durations or number of events in parentheses.

1.4 Discussion

The aims of this controlled indoor experiment were to test whether laying hen showed preferences towards light spectrums and intensities that free range hens may experience during outdoor ranging. The spectrums and intensities of visual and ultraviolet light tested approximated sunlight as close as logistically possible based on commercially available pet reptile light bulbs. The results showed that hens without substantial prior experience of daylight had significant preferences to spend more time under the different types of treatment lights over standard indoor lighting. The hens preferred the high intensity of the visual spectrum light and a trend towards the high intensity of the UVA, but did not prefer the high UVAB wavelengths. However, the hens did not actively avoid this high intensity and instead showed equal preference with the standard control lighting.

The typical habitat of the domestic chicken's jungle fowl ancestors may preclude preferences to spend the majority of time under the brighter light of natural spectrums. Hens in this study also showed preferences for higher intensities of the VIS light and UVA light (although the preference was only a trend at the highest UVA intensity), indicating the brightness of these lights was not aversive to the hens. Under the UVAB light treatment, hens showed clear preferences for the treatment light under the low and medium intensities but not at the high intensity. When focused specifically on the middle of the treatment compartment where the UV radiation would not yet have greatly dispersed, the hens only preferred to spend more time in this area at the low intensity level. This suggests that while some degree of UVB was preferred, the higher intensities supplied too much UVB radiation, which may have led to skin damage. This result is consistent with studies of free range chickens where birds range less on sunny days (Gilani et al. 2014; Bestman et al. 2019), during the midday period (Chielo et al. 2016; Fanatico et al. 2016), or with increasing solar radiation (Stadig et al. 2017a).

Hens showed more foraging, ground pecking, and preening at the low intensity of the UVAB light, suggesting hens were more comfortable and motivated to express active hen-typical behaviours (cf. sitting or standing) under this type of light. Many behaviours were not affected by treatment, and other studies have found no differences in hen behaviour or activity under different light types (Widowski et al. 1992; Mohammed et al. 2010; Huber-Eicher et al. 2013). Preferences for some lights were also affected by corresponding differences in temperature, but not consistently. There were some effects of the treatment lights on behaviours; however, behavioural expression may have been limited by the use of cage-reared hens.

1.5 Conclusions

This study demonstrated that hens with minimal sunlight experience preferred lights that approximated daylight including high intensities of these lights. When a combination of UVA and B wavelengths were presented, preferences were reduced at the higher intensity, suggesting hens avoided the damaging radiation. Lower levels of UVAB resulted in more behavioural expression of foraging and comfort behaviours. This suggests that hens in a free range setting may respond positively to sunlight access but when the sunlight is intense, hens may need additional measures (e.g. shelter) to protect themselves from certain levels of UV radiation and intensity. It was impossible to completely mimic sunlight intensity and wavelengths in an indoor experimental setting for this study. Additionally, older cage-reared hens were used, which may have hindered behavioural expression. Therefore, further study is required to validate these findings in a free range setting.

2 Relationship between sunlight and ranging of commercial free range hens

2.1 Introduction

The outdoor exposure in a free range system allows hens the opportunity to access natural vitamins from herbage, and vitamin D3 from ultraviolet (UV) radiation in sunlight (Van de Weerd et al. 2009; Singh & Cowieson 2013; Dunlop et al. 2017). Ranging outside may also reduce feather pecking behaviour, improving plumage coverage, and encourage greater expression of natural behaviours such as foraging, walking, and dust bathing (Campbell et al. 2020a). However, hens have a choice in accessing the outdoor area and, despite the apparent appeal of an outdoor range, usage can sometimes be low (Campbell et al. 2020b), which can result in negative perceptions of the industry by consumers.

Hen ranging can be affected by many variables, including the ambient weather. Temperature has previously shown a parabolic effect with hens showing maximum range use around 17°C in the UK (Hegelund et al. 2005). A clear linear relationship has been demonstrated by Richards et al. (2011) with increasing ranging for every degree rise in temperature (up to ~20° C). Wind speed, humidity, and rainfall will also impact ranging with hens preferring milder conditions, but the relative impacts of these variables depend on the season and surrounding temperatures (Hegelund et al. 2005; Richards et al. 2011; Richards et al. 2012). 'Mild' may also depend on what conditions hens are accustomed to. In both free range laying hens and broilers there are time of day effects, with more birds preferring to use the range in the morning and late afternoon/evening, and range use varies across seasons (Dawkins et al. 2003; Hegelund et al. 2005; Richards et al. 2011; Gilani et al. 2014; Chielo et al. 2016). Free range hens have been shown to increase range use with increasing hours of sunshine but this effect was only observed at lower temperatures, suggesting the sun had a warming effect (Richards et al. 2011). In contrast, free range broilers have been observed to range less when the sun is 'bright' versus covered by clouds (Dawkins et al. 2003) and will use shade more on sunny days in summer (Jones et al. 2007). Quantified solar radiation has also shown a negative relationship with range use, with fewer slow-growing broiler chickens ranging as the radiation increases, but this relationship is dependent on the type of shelter available on the range (Stadig et al. 2017a; Stadig et al. 2017b). However, while these aforementioned studies have demonstrated relationships between range use and climatic variables, they have all been conducted within European countries, which may not be directly applicable to the more extreme sunlight and temperatures experienced in Australia. Furthermore, these previous studies have only observed sunshine or recorded total solar radiation, which may limit understanding of the specific wavelengths of sunlight that may be affecting range usage.

Observations on ranging behavioural patterns in commercial free range chicken farms (laying hens and meat chickens) in Australia have documented lower range use across the mid-day/early afternoon period when the sun is most intense and during the summer months (Rault et al. 2013; Taylor et al. 2017). This may be a consequence of the damaging effect of high UV radiation (Lewis & Gous 2009; Weihs et al. 2012 as hens showed preferences for range structures that blocked the greatest amount of UV radiation (Rault et al. 2013), although UV radiation was not a significant predictor of ranging in meat chickens (Taylor et al. 2017). Alternatively, birds may be avoiding increased brightness (photosynthetically active radiation), which is visible to hens and humans (400–700 nm) and may be visually aversive (similar to human's preferring sunglasses on bright sunny days), or avoiding increased temperatures as a result of infrared sunlight radiation (> 700 nm).

The objective of this study was to determine if range use was correlated with sunlight variables across the summer/autumn period, across commercial Australian free range farms. It was predicted that the hens would show lower use of the range area when the sunlight was most extreme and that different wavelengths of sunlight would have varying impacts on ranging.

2.2 Methods

All the animal protocols and procedures of the study were approved by the Wildlife and Large Animal, Animal Ethics Committee of the Commonwealth Scientific and Industrial Research Organisation (Approval number: ARA2019-30).

2.2.1 Animals and housing conditions

Laying hens (*Gallus gallus domesticus*) housed in commercial free range systems were used in this study. Three different Australian commercial free range farms (Farms A, B, and C, as described below) with a diversity of climatic conditions, comprising a total number of approximately 60,000 (20,000/farm) hens that were housed and managed according to individual farm protocols, and current standards and guidelines. The study was conducted across the summer/autumn seasons in Tasmania (Farm A) and Queensland (Farm B) in 2019/20, and in Western Australia (Farm C) in 2021. On each farm, only a single shed and associated range area were selected to be used in the study. These indoor sheds were furnished with perches, feeders, drinkers and nest boxes to meet the national poultry guidelines, with littered floor and access to an outdoor range area. The lighting, temperature, and ventilation were automatically controlled, however, varied depending on the sites (see details in the Study Sites sections).

2.2.2 Study sites

Farm A – Tasmania

Tasmania (TAS) being the most southern island state of Australia has relatively cooler temperatures and lower UV indices. The study farm was located in the northern midlands of Tasmania. An estimated 20,000 Hy-Line Brown free range laying hens of 20 weeks of age in one flock were studied across the summer/autumn months (21 December 2019–31 March 2020). The day-old chicks were reared indoors until transfer at 14 weeks to the free range indoor shed, and housed with standard management practices up to 20 weeks of age. The indoor shed (93 m L x 15 m W x 3.5 m H) was in the northeast-southwest direction and contained an aviary system with a stocking density of 14 hens/m^2. The sidewalls of the shed were made of cool room panels from the ground to a height of 0.7 m, with the remainder covered with automatic curtains. Feed and water were provided *ad libitum* inside the shed only, not in the range area. Adjacent to the sidewalls was an outdoor range area on both the north and south sides, with a maximum outdoor stocking density of 6,666 hens/ha (equivalent to 0.67 hens/m^2). However, only the south side of shed was studied as the farm management indicated more birds ranged on that side. Hens could access the range area through pop holes from 20 weeks of age. Sixteen pop holes (0.9 m L x 0.6 m W) were set at 100 mm above the ground on the south side and regulated automatically, but the opening time varied based on the day lengths (opened at 1100 h and 1030 h during 21 December–21 February and 22 February–31 March, respectively) and closed at 2100 h. Most of the range area was covered with perennial ryegrass, clover, and native pasture starting at a distance of 15 m from the shed wall. Adjacent to the shed wall was 8.5 m of unevenly distributed stones of varying size, followed by a sloping area of approximately 7 m in width. There was no visible degradation of the grass in the range area during the study period. No trees were present on the range but seven rectangular shade cloth artificial covers (6.5 m L x 4.6 m W x 1 m H) were set within the gravel area at a distance of at least 1 m from the sidewall of the shed. However, one shade cover from each corner was shifted farther away from the shed on 3 March (a commercial decision, unrelated to the project aims), and remained there until the completion of the study. The boundaries of the entire outdoor range areas were wire fences.

Farm B – Queensland

Queensland (QLD) is the northeastern state, which experiences the highest average maximum temperature and the second-highest UV indices (after the city of Darwin) in Australia. The selected commercial free range laying hen farm for this study was situated in the southwest part of the Queensland state. The study was conducted within a flock of approximately 20,000 Hy-Line Brown laying hens across the summer/autumn months (23 December 2019–16 April 2020). In this commercial set-up, birds were reared indoors until transfer at 16 weeks of age to the free range indoor shed, where the pullets were managed as per the national guidelines. The indoor shed (120 m L x 20 m W x 8 m H) contained an aviary system and was long in the east-west position, with an indoor stocking density of 9 hens/m^2. *Ad libitum* feed and water were only provided inside the shed. The sidewalls were made of solid materials (poly panel) from the ground up to a height of 0.55 m, and curtains covered the remainder of the wall up to the ceiling. The shed had outdoor ranges on both the north and south sides, with an approximate stocking density of 1,500 hens/ha (equivalent to 0.15 hens/m^2). Hens within the shed could only access the range on either the north or south face due to an internal shed division, thus each shed actually contained 40,000 hens total. The south side of the shed was used for this study. Hens could access the outdoor range at 20 weeks of age via pop holes. In the south side, the sidewall of the shed had 14 pop holes (6 m L x 0.5 m W), but typically only half were opened across the shed length in an alternating pattern during the study period. The automatic pop holes were opened at 0900 h and closed at 2000 h daily; however, during adverse weather conditions such as extreme heat or storms they remained closed to ensure safety of the hens. The range area adjacent to the shed wall (2.5 m) was covered with evenly distributed compact gravel, followed by 12 m of range area covered with heavy weed fabric, then a distance of approximately 52 m was uncovered (dirt), and the remainder of the range area was covered with grass. A number of growing trees (*Eucalyptus sp.*) planted 8 m apart were present within the dirt area in four parallel rows, starting at 6.5 m from the weed fabric out into the range. There was visible degradation in the grassed area at the beginning of the study, which was gradually grown up during the study period. Distributed across the dirt range area were ten slatted wooden pallet shelters (1.2 m L x 2.2 m W) and five triangle shade cloth shelters (6.5 m L x 4.6 m W). The boundaries of the range area were wire fences.

Farm C – Western Australia

Western Australia (WA) is the largest state of Australia, with the farm selected for this study located in the Wheatbelt region that is characterised by its hot dry summers and mild winters. A flock of approximately 30,000 Lohmann Brown hens within one shed on the farm was studied across the summer/autumn months (28 January 2021–17 May 2021). Hens were transferred from a commercial rearing facility into the indoor shed (120.6 m x 16.5 m x 4.83 m) of the free range farm at 15 weeks of age. The housing practices followed the national guidelines. The indoor shed was in a north-south direction and contained an aviary system. Feed and water were provided *ad libitum* inside the shed only. The base of the shed sidewalls (1 m) was made from sandwich panel and the remainder of the walls were covered by curtains up to the ceiling. Hens could access the outdoor range from 19 weeks of age via pop holes on both sides of the shed, but only the east side of the shed was used for this study. The indoor and outdoor stocking density of hens was 10.75 hens/m^2 and 1,500 hens/ha (equivalent to 0.33 hens/m^2), respectively. Pop holes for range access were located 75 cm above ground in the sidewall. There were 15 pop holes (2.06 m L x 0.35 m W) in the south side through which hens had access to the range from 0900 to 1900. However, the pop holes remained closed preventing range access during extreme temperatures of 38° C or above. The range area adjacent to the shed wall (3.5 m) was covered with unevenly distributed gravel, then the immediate range area of approximately 10 m was uncovered (dirt), followed by approximately 12.5 m of range area covered with perennial rye pasture. A further approximately 40 m of range area had a mix of bottle brush trees (*Callistemon sp.*) and dirt as well. There were a few large trees (*Eucalyptus spp.)* grown at the southeast corner of the range area. The boundaries of the range area were wire fences.

2.2.3 On-farm weather stations

An MEA weather station (Green Brain, 41 Vine Street, Magill SA 5072, Australia) was set up on each farm site for recording sunlight variables and weather data every 15 min over the study periods. The weather station was mounted on a post (user supplied, 1 m height) with different sensors (SR-05 D1A3 pyranometer, QS5 PAR pyranometer, and UV3pAB UV sensor) for recording of sunlight variables including ultraviolet radiation (UVAB) (288–432 nm) (W/m²), photosynthetically active radiation (PAR) (400-700 nm) (µMol/m^2/s), and total solar radiation (TSR) (285 nm–3000 nm) (W/m^2). The TSR included UVAB wavelengths, PAR and infrared (IR) and was used to extract IR (700 nm–1 mm) (W/m²). Additionally, an air temperature and relative humidity sensor recorded weather variables including air temperature (˚C), relative humidity (%), barometric pressure (mBar), dew point (˚C), voltage (V), and vapour pressure deficit (kPa). As the study objective was to establish the relationship between sunlight variables and hen ranging behavioural patterns only the solar radiation spectrums, air temperature and relative humidity weather data were considered in the final analyses.

2.2.4 Video Recording

A high-resolution Hikvision (Hangzhou 310051, China) security camera system (Hikvision DS-7608NI-I2-8P CCTV NVR Recorder and Hikvision DS-2CD2355FWD-I2 CCTV 6MP Turret cameras) were installed at the southeast corner in both Farms A and B, and at the middle of the indoor shed just above the central pop hole in Farm C, to record hen range use across the study periods. Thus, we recorded part of the southeast corner portion of the range area on Farms A and B, and part of the middle portion of the range area on Farm C (Figure 5). The video recording was continuous daily across each study period. The shed's side and range area selection for the study were varied between the farms depending on the clear visibility of bird counts within the range through video cameras.

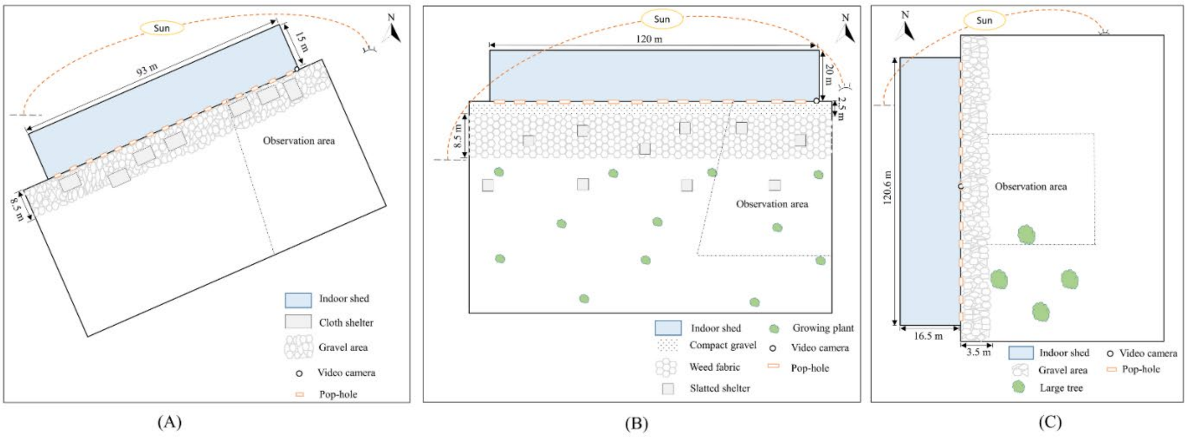


Figure 5 A schematic of Farms A, B and C showing the shed orientation, range layout and area of observation

2.2.5 Data collection

For observation of hen ranging behavioural patterns, the number of hens using the outdoor range in the sampled area for each farm was recorded daily across the study periods. A single observer took image snapshots at 30 min intervals of all available days during the period of ranging time, from pop hole opening until sunset, to count the number of hens ranging in the outside. The initial image snapshot was taken just 3 min after pop hole opening (i.e. if the pop hole opened at 0900 h, the first snapshot was taken at 0903, the next snapshot at 0930 h, 1000 h, and so forth until sunset).

Observations indicated hens rapidly accessed the range area within approximately two minutes of the pop holes opening. The exact area of the range that was sampled for counting hens varied among the farms based on hen visibility and their typical range occupancy (i.e. some areas within the image snapshot were rarely accessed by hens) but consistency in observational area was maintained within each farm over the study periods (Figure 5). Hen counts were categorised as the number of hens under the direct sunlight (sun), and the number of hens in the clearly visible shaded areas from range shelters or the shed (shadow). However, in cloudy conditions where shadow was not visually distinct, the same parts of the range area were marked from the previous sunny day. Moreover, in the late afternoon when the demarcation line between the shadow and sun was not visibly distinct, the number of birds was only considered as 'shadow'. Hens were counted using the Image-J 1.53a software (Wayne Rasband, National Institute of Health, USA) individually within the specified range area. However, when piling occurred (hens forming dense groups) and individual hens were not all clearly identifiable, the number of hens was estimated in the group by counting the hens within a certain area and then estimating the total count by multiplying the counted area. This same guideline was followed in future similar occurrences.

Hens under the shade and shelter that were clearly identifiable on the image snapshot were counted but any hens directly under the range shelter were unable to be seen. Farm A had a total of 102 days of available data of hen ranging in the outside; while Farm B and Farm C had a total of 94 days and 66 days, respectively. However, the days were not consecutive because of faults in the recording system as well as the restrictions on hens going outside during adverse weather conditions. As the hen ranging behavioural observations data were taken at 30 min intervals, the corresponding weather parameters across the 15-min period directly prior to the observation time point was matched accordingly. Due to the distinctness of each farm, databases were prepared and analysed separately.

2.2.6 Statistical analyses

The study generated data of the number of hens on the range across the day and this was correlated with recorded weather parameters on the farms. Only one shed was observed per farm and analysed separately due to the discrepancies in farms' structures and climatic conditions of the respective study sites. For Farm A, a total of 102 days of hen counts at 30 min intervals from pop hole opening (1030/1100) until sunset (2100) were analysed. For the analyses by month, data were grouped into December/January, February, and March. For Farm B, a total of 94 days of hen counts at 30 min intervals from pop hole opening (0900) until sunset (2000) were analysed. For the analyses by month, data were grouped into December/January, February, March, and April. For Farm C, a total of 66 days of hen counts at 30 min intervals from pop hole opening (0900) until sunset (1900) were analysed. For the analyses by month, data were grouped into January/February, March, April, and May.

The principal response variables were the number of hens under the direct sunlight (sun) and the number of hens in the shade of the range shelters/trees and the indoor shed's shadow (shadow) across the day from pop hole opening until sunset. Both 'sun' and 'shadow' data were analysed individually (6 separate datasets: 3 farms x 2 environments – sun/shadow). The independent variables were different levels of sunlight spectrums including UVAB, PAR, and IR, and the weather variables including ambient temperature and relative ambient humidity. TSR readings were used only for extracting IR by subtracting the UVAB and PAR. A conversion value (µmol/m^2/s to W/m^2), as described by Thimijan and Heins (1983), was applied to the PAR readings so all measures were in the same units for calculating the IR values. The hen count data of 'sun' were log (x+1) transformed to approach data normality as well as to include '0' values (when no hens were found in the sunny part of the range) and the hen counts for 'shadow' data were square-root transformed. The sunlight and weather data met the requirements of parametric statistics so no further transformations were required. General linear models (GLM) were applied using JMP® 14.0 (SAS Institute, Cary, NC, USA) with α level set at 0.05 on the number of hens in 'sun' to determine if the 'time of day' and 'month of year' as fixed factors had influence on the distribution of hens within the range. A separate model with the same parameters was also fitted to assess hens' presence in the 'shadow' area of the range. Finally, a GLM was performed with the same fixed factors for weather parameters to illustrate the variation of weather conditions across the daytime and between the months. The studentised model residuals were visually inspected for confirming homoscedasticity. Where significant differences were present, post hoc Student's t-tests were applied to the least squares means with Bonferroni corrections to the α level to account for multiple post-hoc comparisons.

To assess the effects of sunlight and climatic conditions on range use across the entire study period, multiple linear regression analyses with sunlight (UVAB, PAR, and IR) and weather (ambient temperature and relative humidity) variables as predictors were performed with the number of hens in 'sun' and 'shadow' separately for individual farms. However, before running the model, the collinearity among the independent variables were checked through determination of variance inflation factors (VIF). Because of multicollinearity (VIF ≥ 10) among the sunlight variables, we chose the ridge regression analyses instead of the linear regression (Schreiber-Gregory 2018) to best fit the predictors into the model. Therefore, we used the *'lmridge'* package in R statistical software (R Core Team, 2020) for the ridge regression. Moreover, the relative weight between the independent predictors in the regression model was estimated by the R package *'relaimpo'*. Initially, all independent variables were fitted in the model, then the non-significant variables ($p \geq 0.10$) were removed through backward stepwise elimination to reach the model of best fit based on the adjusted-R^2 values. To determine how sunlight and weather variables may affect the hens' use of range distribution across the months in different climatic conditions, separate ridge regression models were performed for each month(s) with the number of hens in the 'sun' included as the dependent variable, and the sunlight (UVAB, IR and PAR) and weather (ambient temperature and relative ambient humidity) variables included as independent variables. Through backward stepwise regression analyses any non-significant variables ($p \geq 0.10$) were removed to reach the model of best fit based on the adjusted-R^2 values. Similar ridge regression models were also applied for the number of hens in the 'shadow' to assess the relationship with the sunlight and weather parameters across the months at different study sites. Only raw values are presented in the figures.

2.3 Results

2.3.1 Farm A – Tasmania

Due to the farm layout, the shadow created by the indoor shed and range shelters increased steadily into the range across the day with maximum shade at the end of the day being approximately 9.5 m and 12 m (at the beginning and end of the study period, respectively) from the indoor shed sidewall in the sunlight (when the demarcation line between the sun and shadow portions were distinct). Thus, the shadowed area increased slightly across the day although there was still ample range area available in full sunlight.

2.3.1.1 Weather conditions

The average temperature and relative humidity during the study period was 20.71 ± 0.10˚C (ranged from 9.8˚C to 36.7˚C) and 51.94 ± 0.38% (ranged from 16.7% to 98.4%). Both temperature and relative humidity varied across the months ($F_{(2, 2082)} = 404.66$, $P < 0.0001$ and $F_{(2, 2082)} = 327.78$, $P < 0.0001$, respectively); specifically, the hottest month was December/January (23.03 ± 0.12˚C), followed by February (20.30 ± 0.14˚C) and March (17.85 ± 0.14˚C). The time of day also affected air temperature ($F_{(20, 2082)} = 30.78$, $P < 0.0001$) and relative humidity ($F_{(20, 2082)} = 18.702$, $P < 0.0001$) across the day with peak air temperature recorded between 1330 and 1800 ($P < 0.003$), and relative humidity between 1930 and 2100, and at 1100 and 1200 ($P < 0.003$). The lowest temperature was recorded during the evening (between 2000 and 2100), and lowest relative humidity between 1300 and 1830 ($P < 0.003$).

2.3.1.2 Effects of time of day on hens' distribution outside

The mean number of hens in the sun significantly varied across the day ($F_{(18, 1771)} = 22.51$, $P < 0.0001$; Figure 6) with peaks in the morning and evening ($P < 0.003$). The mean number of hens in the shadow was also affected by time of day ($F_{(18, 1771)} = 16.39$, $P < 0.0001$; Figure 6) but with an opposite pattern to that observed for hens in the sun, with the lowest values observed in the morning and evening ($P < 0.003$).

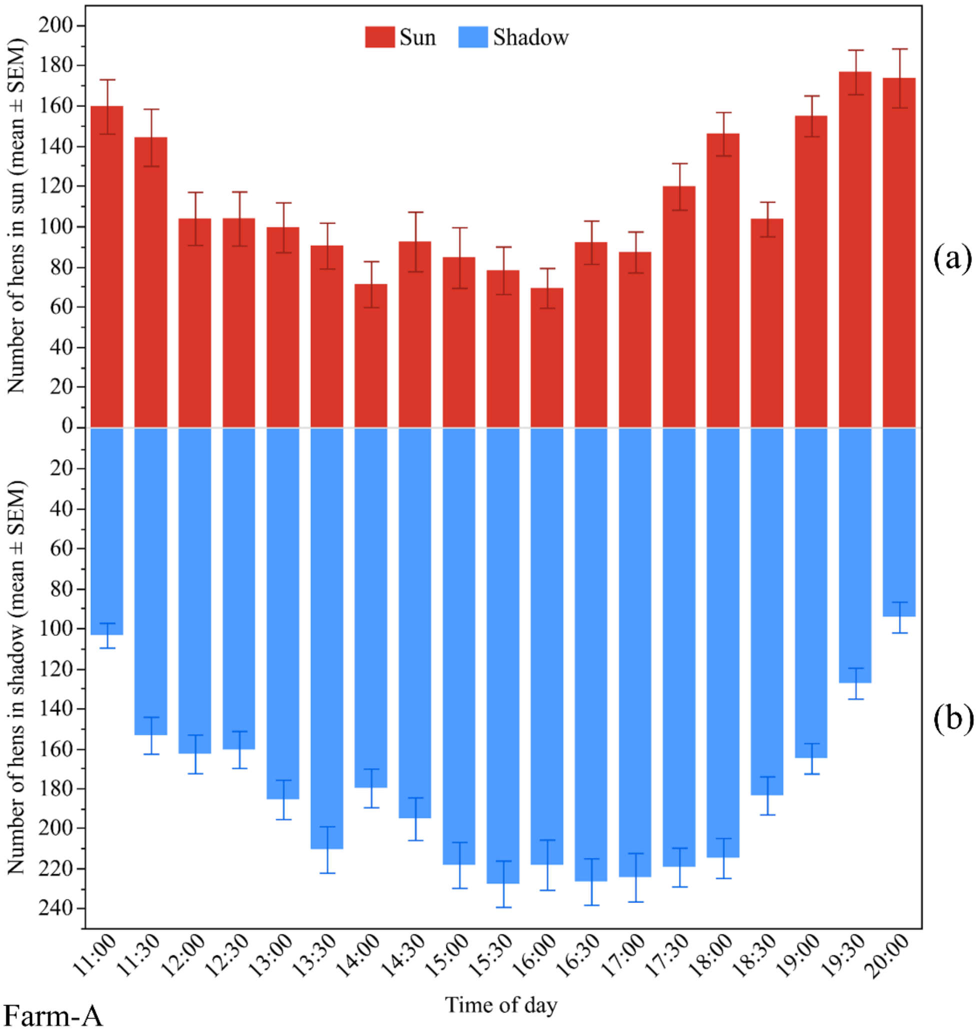


Figure 6 The mean (± SEM) number of hens on the range across the day in the sun (a) and shadow (b) areas on Farm A

2.3.1.3 Differences in hens' distribution across the months

There was a significant effect of month on the mean number of hens observed in sun ($F_{(2, 1771)} = 174.03$, $P < 0.0001$; Figure 7). The greatest number of hens ranged in the sun during the month of March, followed by February, then December/January. The mean number of hens in the shadow was also significantly influenced by the month of observation, with a peak in February and the fewest hens in March ($F_{(2, 1771)} = 38.66$, $P < 0.0001$; Figure 7).

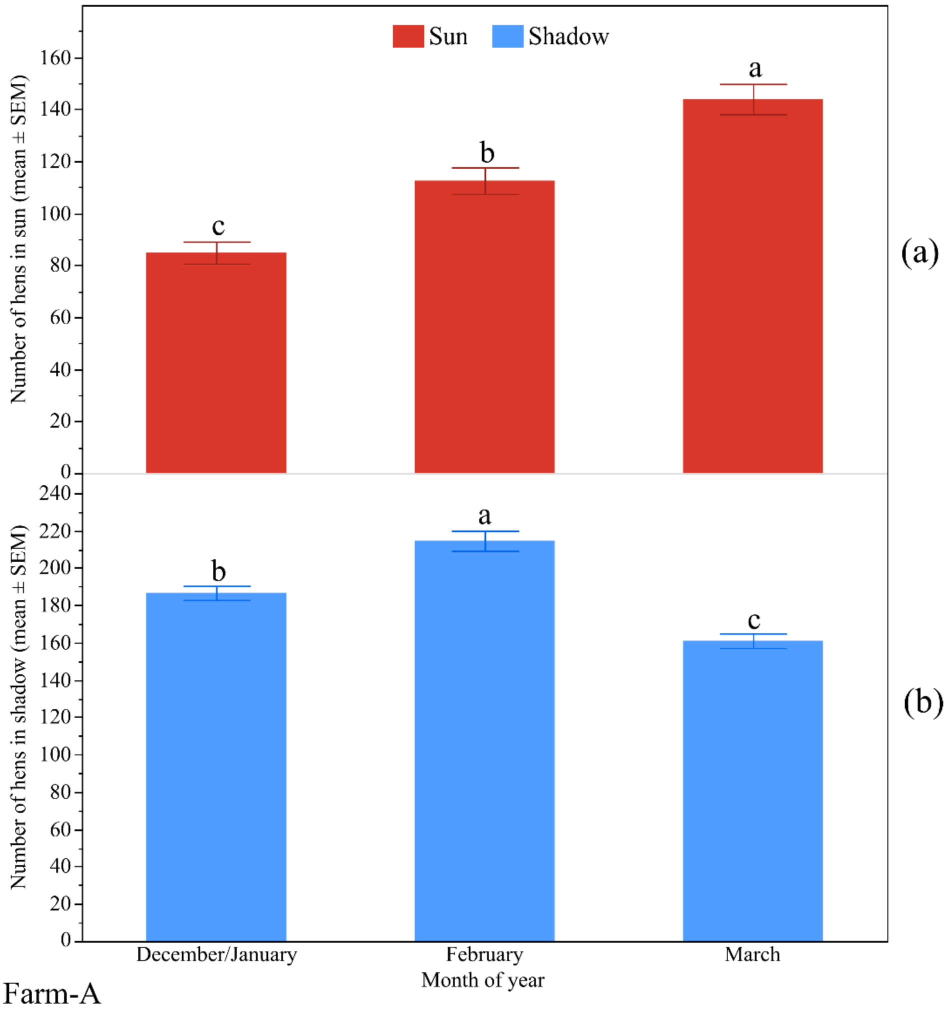


Figure 7 The mean (± SEM) number of hens on the range across the months in the sun (a) and shadow (b) areas on Farm A

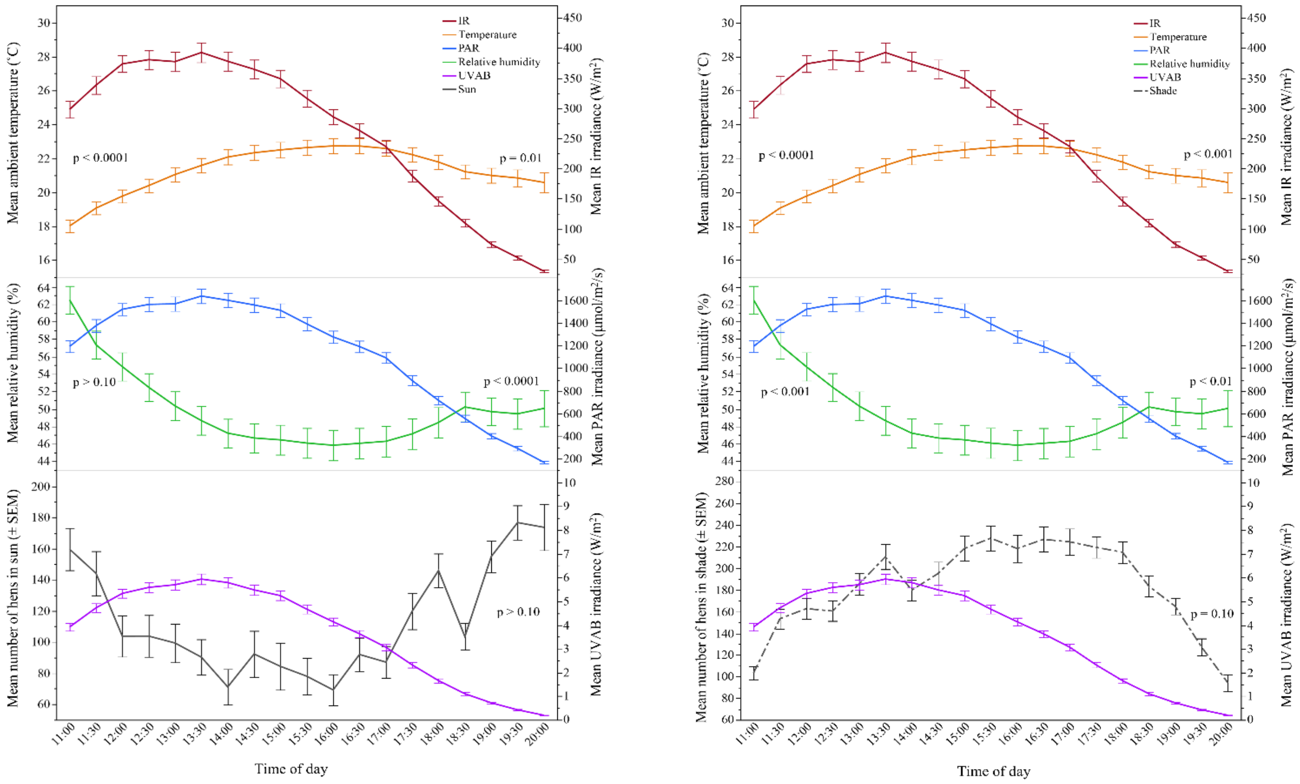
$^{a\text{-}c}$ Dissimilar superscript letters indicate significant differences among months separately for the sun and shadowed areas.

2.3.1.4 Relationship between sunlight and range use

The overall ridge regression model to determine the effects of sunlight and weather variables on the number of hens in the sun across the entire study period was significant ($F_{(2.17,\ 1789.64)} = 456.68$, $P < 0.0001$; Figure 8). The model showed that the number of hens in the sun could be predicted significantly by ambient temperature, PAR and IR, and all these factors explained 40.96% of the variance. Both ambient temperature and PAR were strongly negatively correlated and contributed to the model equally (37% and 35%, respectively) showing that increased air temperature and PAR intensity resulted in fewer hens ranging in the sun. IR had a positive relationship with the number of hens in the sun and caused 28.34% of the model variation. A similar model for the number of hens in the shadow showed that all the predictors included in the model had a significant positive relationship with the number of hens except for IR, which had a negative relationship. However, the model only explained 2.24% of the variation ($F_{(3.74,\ 1787.79)} = 13.92$, $P < 0.0001$; Figure 8).

The results of multiple ridge regression models to establish the relationship between the predictors and hens' distribution in the range are presented in Table 8. The maximum variance in the models accounted for by the sunlight and weather variables for the number of hens in the sun was found in

December/January (51.25%), with air temperature, PAR, and IR all showing negative associations. The next highest contributory model was found for February with air temperature, relative humidity and PAR causing 24.99% of the variance, where both air temperature and PAR had positive relationships, but there was a negative trend for relative humidity (Table 8). For hens in the shadow, only the model in February accounted for 23.85% of the variance with a strong positive correlation with air temperature suggesting that increases in temperature resulted in more hens in the shadowed areas. However, both the December/January and March models explained less than 6% of the variance, indicating poor fitting with the predictors (Table 8).



(A)

(B)

Figure 8 Relationship between hen range use, sunlight spectrums, and weather variables on Farm A

(a) Y-axis (left): the mean (±SEM) number of hens in sun, and the mean (±SEM) relative humidity and temperature.
Y-axis (right): the mean UVAB (ultraviolet radiation A and B wavelengths), PAR (photosynthetically active radiation), and IR (infrared radiation).

(b) Y-axis (left): the mean (±SEM) number of hens in the shadow, and the mean (±SEM) relative humidity and temperature.
Y-axis (right): the mean UVAB, PAR and IR.

p > 0.10 indicates the variable had no significant effect and was removed from the final model.

Table 8 Multiple ridge regression analyses (ridge parameter, k=0.02) on the number of hens in the sun and shadow across the day on Farm A

Month(s)	Distribution	Predictor[1]	β-coefficient (Standardised)[‡]	t-value	*P*-value	Adjusted R^2 and model's F-statistics	Relative weight of the predictors in the model
December/January	Sun	Ambient temperature	-0.38	-15.13	*<0.0001*	R^2-adjusted = 0.5125 $F_{(2.17,\ 779.63)} = 309.81$, $p < 0.0001$	32.71%
		PAR	-0.60	-11.78	*<0.0001*		36.48%
		IR	-0.12	-2.45	*0.01*		30.80%
	Shadow	Relative humidity	0.15	3.98	*<0.001*	R^2-adjusted = 0.0153 $F_{(2.19,\ 779.60)} = 8.19$, $p < 0.001$	65.07%
		PAR	0.26	3.33	*0.001*		18.11%
		IR	-0.21	-2.74	*0.01*		16.81%
February	Sun	Ambient temperature	-0.20	-5.16	*<0.0001*	R^2-adjusted = 0.2499 $F_{(2.93,\ 512)} = 60.15$, $p < 0.0001$	21.30%
		Relative humidity	0.07	1.76	*0.08*		15.77%
		PAR	-0.39	-9.27	*<0.0001*		62.93%
	Shadow	Ambient temperature	0.43	11.03	*<0.0001*	R^2-adjusted = 0.2385 $F_{(3.78,\ 510.78)} = 35.70$, $p < 0.0001$	62.04%
		Relative humidity	0.07	1.73	*0.08*		1.63%
		UVAB	0.23	2.20	*0.03*		9.62%
		PAR	0.16	1.65	*0.10*		14.36%
		IR	-0.16	-1.71	*0.09*		12.32%
March	Sun	Ambient temperature	-0.15	-3.29	*0.001*	R^2-adjusted = 0.0472 $F_{(3.48,\ 491.21)} = 3.48$, $p < 0.0001$	29.47%
		Relative humidity	-0.13	-2.25	*0.02*		9.73%
		UVAB	0.23	2.10	*0.04*		22.77%
		PAR	-0.44	-3.85	*<0.001*		38.03%
	Shadow	Ambient temperature	0.22	5.23	*<0.0001*	R^2-adjusted = 0.0582 $F_{(1.96,\ 493)} = 16.49$, $p < 0.0001$	80.28%
		UVAB	-0.12	-2.71	*0.01*		19.71%

Only variables that significantly contributed to the most parsimonious model are presented.

[‡] β-coefficient (standardised) of the predictor variables were estimated separately using the ridge regression coefficient in 'R' as the original ridge package did not include the 'β-coefficient' value in the regression outputs.

[1] UVAB (ultraviolet radiation A and B wavelengths), PAR (photosynthetically active radiation), and IR (infrared radiation).

2.3.2 Farm B – Queensland

This farm had more plants growing within the range area, but the shade created by these plants was minimal. The sun cast a shadow of the indoor shed into the range, which increased in size over the study period but reduced in size across the day. Thus, at the start of the observations, the maximum shadow measured approximately 2 m in width and increased up to 5.5 m at 0900 by the end of the study period. There was no shadow cast after 1530 on the first day of observations but it increased over time, and on the last day it measured 3 m at 1700. The majority of the range area experienced full sun.

2.3.2.1 Weather conditions

The mean temperature and relative humidity recorded during the study period on-site was 26.89 ± 0.09˚C (ranged from 17.7˚C to 37.2˚C) and 54.09 ± 0.42% (ranged from 16.5% to 99.5%), respectively. Air temperature and relative humidity varied across the months ($F_{(3,\ 1950)} = 263.33$, $P < 0.0001$ and $F_{(3,\ 1950)} = 216.19$, $P < 0.0001$, respectively). Broadly, December/January was the hottest month (30.06 ± 0.14˚C) and April was the coldest one (24.77 ± 0.21˚C). Average recorded temperature in February and March was 26.20 ± 0.14˚C and 25.44 ± 0.13˚C, respectively. The driest month(s) was December/January (43.99 ± 0.64% relative humidity), and the wettest month was February (66.21 ± 0.65% relative humidity). The average humidity in March and April was 55.46 ± 0.62% and 47.27 ± 0.96%, respectively. There were more rainy days observed during the hen counting in February compared to the other months. Time of day also impacted both air temperature ($F_{(20,\ 1950)} = 24.46$, $P < 0.0001$) and relative humidity ($F_{(20,\ 1950)} = 14.82$, $P < 0.0001$), where the highest temperature was recorded between 1200 and 1700 ($P < 0.003$), and the lowest temperature between 0900 and 0930, and 1830 and 1900 ($P < 0.003$). Relative humidity was maximum between 0900 and 1030, and 1830 and 1900, and the lowest humidity was measured between 1230 and 1700 ($P < 0.003$).

2.3.2.2 *Effects of time of day on hens' distribution outside*

There were significant effects of time of day on the mean number of hens in sun ($F_{(19,\ 1797)}$ = 30.97, *P* < 0.0001; Figure 9). Hens had similar preferences for ranging in the sun between 1000 and 1430, which then gradually increased after 1530 until the evening (*P* < 0.003). The mean number of hens in the shadow also significantly varied across the day ($F_{(16,\ 1437)}$ = 16.10, *P* < 0.0001) in an opposite pattern to the hens in the sun and with less variation across time (*P* > 0.003) (Figure 9).

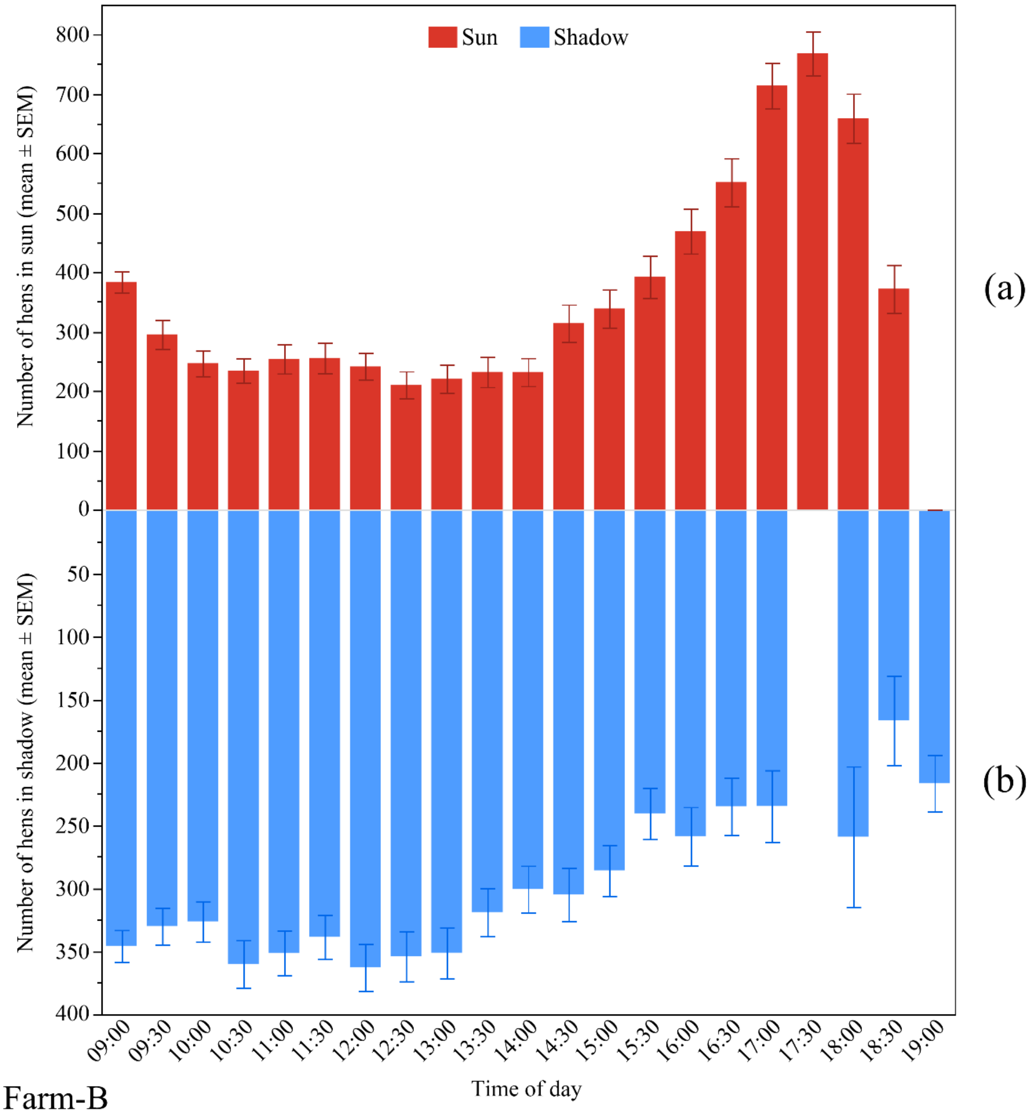


Figure 9 The mean (± SEM) number of hens on the range across the day in the sun and shadow areas at Farm B

2.3.2.3 Differences in hens' distribution across months

The distribution of hens on the range in the sun significantly varied across the months ($F_{(3,\ 1797)} = 37.92$, $P < 0.0001$; Figure 10). The most hens were observed in the sun during the month of April, with no differences between December/January and February, where fewer hens ranged in the sun (Figure 10). Significant effects on shadow usages were also seen for month of year ($F_{(3,\ 1437)} = 253.93$, $P < 0.0001$; Figure 10). The fewest hens were found to use the shadow in December/January, which linearly increased across the months ($P < 0.05$; Figure 10).

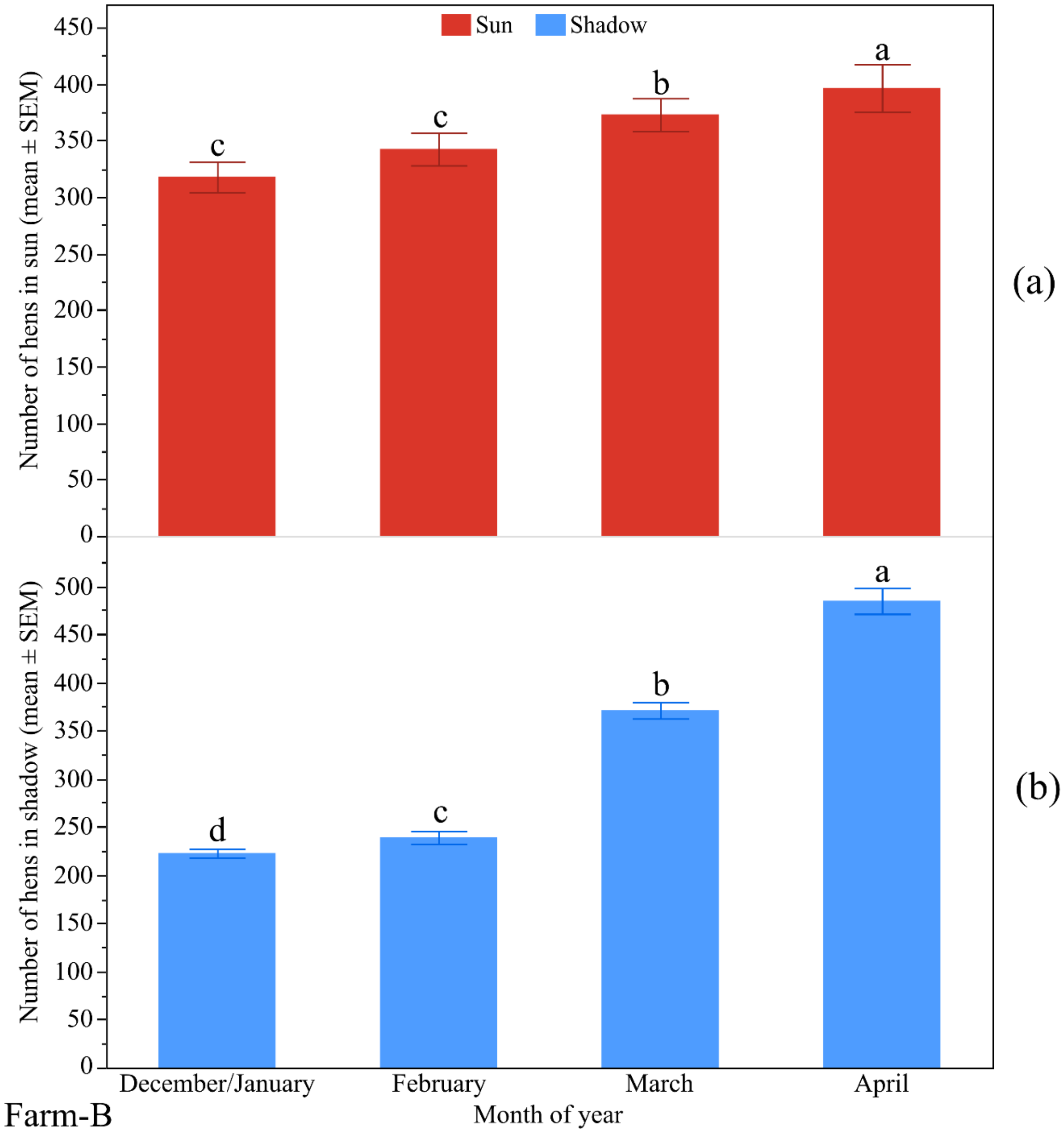


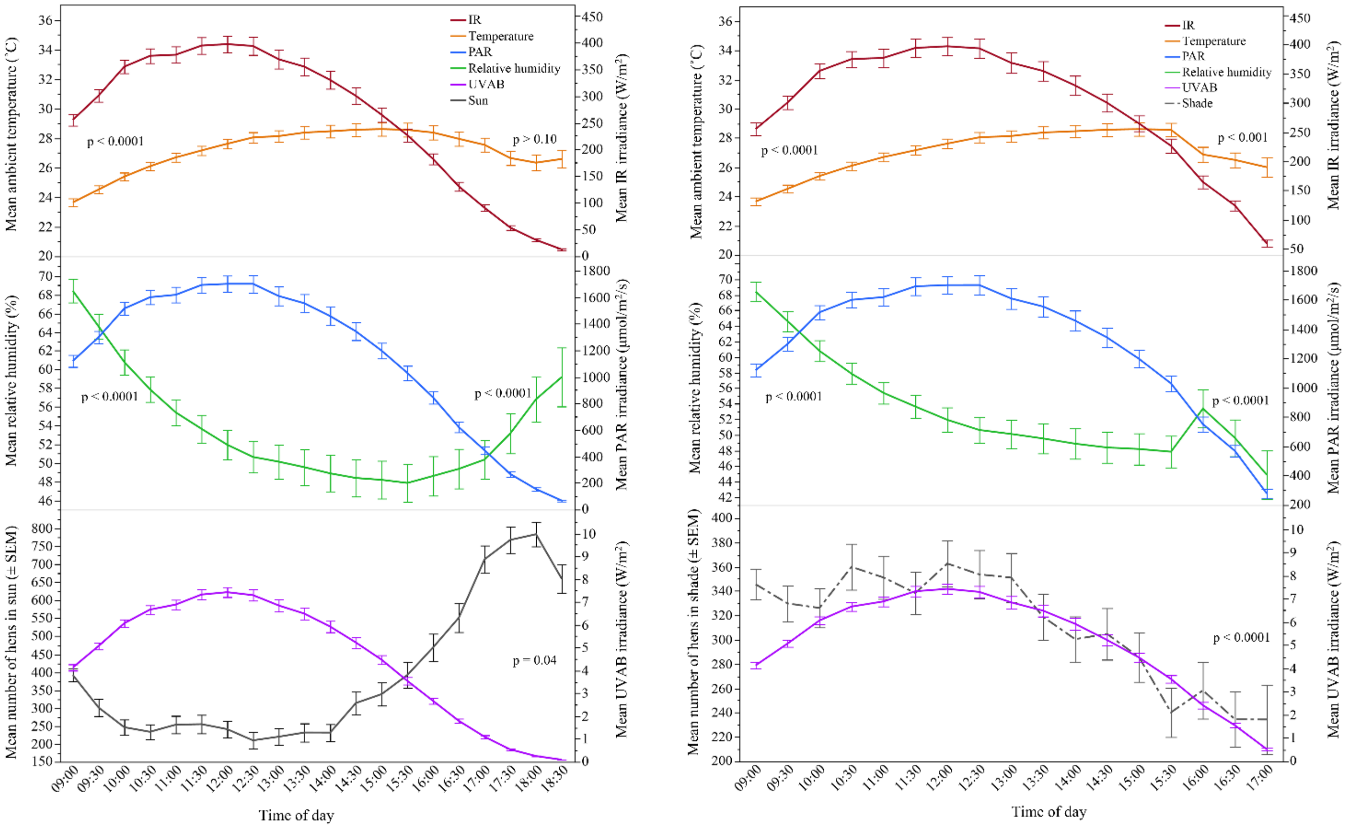
Figure 10 The mean (± SEM) number of hens on the range across the months in the sun (a) and shadow (b) areas on Farm B

$^{a\text{-}d}$ Dissimilar superscript letters indicate significant differences among months separately for the sun and shadow areas.

2.3.2.4 Relationship between sunlight and range use

The relationship of the predictors with the ranging behaviour of hens in the sun has been illustrated in Figure 11. The overall model was significant with temperature, relative humidity, UVAB , and PAR as contributing factors ($F_{(3.40,\ 1816.26)} = 226.21$, $P < 0.0001$) explaining 31.82% of the variance. The model indicates that hens were more active in the sun when all the associated predictors decreased. PAR accounted for 40.77% of the variation in the model, followed by UVAB (36.62% variance). For hens in the shadow, all the predictors influenced their distribution and described 24.09% of the variance ($F_{(4.02,\ 1452.46)} = 114.02$, $P < 0.0001$; Figure 11). Temperature had strong negative correlation along with relative humidity and PAR, and both the UVAB and IR were positively associated with the use of the shadowed area.

The effects of the predictors on hen ranging patterns in both the sun and shadow across the months are presented in Table 9. For the number of hens in the sun, the greatest variance (58.38%) explained by the sunlight and weather variables was in December/January, where all the predictors showed a negative correlation. However, among the predictors, the sunlight variables explained more than 90% of the variation with almost equal contribution to the model (Table 9). The second largest explained variance (40.12%) was found in April with temperature, relative humidity and PAR all negatively associated with hens in the sun, where PAR alone accounted for 73.46% of the variation. For the number of hens in the shadow, the best fitted model explained 44.27% of the variance with temperature, relative humidity and UVAB as significant contributors across December/January (Table 9). Increased temperature reduced the number of hens in the shadow but both relative humidity and UVAB showed positive relationships with hen use of the shadowed areas. In all other models, the sunlight and weather variables explained less than 30% of the variation for hens in the shadow, while only relative humidity and IR significantly contributed to the models in March and April (Table 9).



(A)

(B)

Figure 11 Relationship between hen range use, sunlight spectrums, and weather variables on Farm B

(a) Y-axis (left): the mean (±SEM) number of hens in the sun, and the mean (±SEM) relative humidity and temperature. Y-axis (right): the mean UVAB (ultraviolet radiation wavelengths A and B), PAR (photosynthetically active radiation), and IR (infrared radiation).

(b) Y-axis (left): the mean (±SEM) number of hens in the shadow, and the mean (±SEM) relative humidity and temperature. Y-axis (right): the mean UVAB, PAR and IR.

p > 0.10 indicates the variable had no significant effect and was removed from the final model.

Table 9 Multiple ridge regression analyses (ridge parameter, k=0.02) on the number of hens in the sun and shadow across the day on Farm B

Month(s)	Distribution	Predictor[1]	B-coefficient (Standardised)[‡]	t-value	*P*-value	Model's F-statistics	Relative weight of the predictors in the model
December/January	Sun	Ambient temperature	-0.23	-5.24	*<0.0001*	R^2-adjusted = 0.5838 $F_{(3.56,\ 533.99)} = 159.05$, $p < 0.0001$	5.69%
		Relative humidity	-0.10	-2.22	*0.03*		3.41%
		UVAB	-0.31	-3.95	*<0.001*		29.85%
		PAR	-0.30	-5.10	*<0.0001*		31.13%
		IR	-0.13	-2.08	*0.04*		29.93%
	Shadow	Ambient temperature	-0.51	-9.07	*<0.001*	R^2-adjusted = 0.4427 $F_{(2.87,\ 375)} = 110.72$, $p < 0.0001$	42.54%
		Relative humidity	0.20	3.35	*<0.001*		19.98%
		UVAB	0.54	12.76	*<0.0001*		37.49%
February	Sun	Relative humidity	0.04	1.47	*0.14*	R^2-adjusted = 0.1780 $F_{(1.95,\ 518)} = 59.11$, $p < 0.0001$	19.80%
		PAR	-0.43	-8.63	*<0.0001*		80.20%
	Shadow	Ambient temperature	-0.38	-4.57	*<0.0001*	R^2-adjusted = 0.2790 $F_{(3.61,\ 396.83)} = 37.01$, $p < 0.0001$	8.32%
		Relative humidity	-0.24	-3.04	*<0.01*		6.66%
		UVAB	0.57	4.96	*<0.0001*		3.32%
		PAR	-0.20	-1.9	*0.06*		25.03%
		IR	0.22	2.15	*0.03*		26.83%
March	Sun	Ambient temperature	-0.40	-8.35	*<0.0001*	R^2-adjusted = 0.2758 $F_{(3.14,\ 544.60)} = 56.06$, $p < 0.0001$	32.18%
		Relative humidity	-0.23	-4.61	*<0.0001*		7.85%
		PAR	-0.20	-2.56	*0.01*		30.88%
		IR	-0.21	-2.65	*0.01*		29.10%
	Shadow	Relative humidity	-0.10	-2.56	*0.01*	R^2-adjusted = 0.3064 $F_{(1.95,\ 474)} = 110.77$, $p < 0.0001$	16.98%
		IR	0.50	12.41	*<0.0001*		83.02%

April	Sun	Ambient temperature	-0.37	-5.68	*<0.0001*	R^2-adjusted = 0.4012	22.78%
		Relative humidity	-0.16	-2.49	*0.01*	$F_{(2.91, 211)} = 52.06$, $p < 0.0001$	3.76%
		PAR	-0.54	-10.52	*<0.0001*		73.46%
	Shadow	Relative humidity	-0.26	-4.03	*<0.0001*	R^2-adjusted = 0.1642	25.62%
		IR	0.29	4.54	*<0.0001*	$F_{(1.96, 493)} = 21.12$, $p < 0.0001$	28.84%

Only variables that significantly contributed to the most parsimonious model are presented.

[ǂ] β-coefficient (standardised) of the predictor variables were estimated separately using the ridge regression coefficient in 'R' as the original ridge package did not include the 'β-coefficient' value in the regression outputs.

[1] UVAB (ultraviolet radiation A and B wavelengths), PAR (photosynthetically active radiation), and IR (infrared radiation).

2.3.3 Farm C – Western Australia

The indoor shed on this farm was in a north-south direction where hens could range on both the east and west side. The central part of eastern side was selected for observation. There were large trees located at the southeast corner, with only one tree captured in the video recordings used for observations. Therefore, the shadow within the observation area was created by the shade of the indoor shed as well as the large tree and this varied across the day. The indoor shed shadow increased in the range area over the day resulting in a reduction of the counting area for hens in the sun and a corresponding increase in the shadow area.

2.4.2.1 Weather conditions

The weather conditions of this study site included a mean air temperature of 24.20 ± 0.12˚C (ranged from 8.6˚C to 38.1˚C) and mean relative humidity of 49.09 ± 0.44% (ranged from 14.4% to 92%). Across the months, both air temperature ($F_{(3, 1448)} = 140.69$, $P < 0.0001$) and relative humidity ($F_{(3, 1448)} = 69.52$, $P < 0.0001$) varied significantly. The warmest month was January/February (26.26 ± 0.21˚C), then March (25.13 ± 0.17˚C), April (24.26 ± 0.12), and May (21.58 ± 0.15), respectively. Average relative humidity was highest in March and May (53.15 ± 0.77% and 54.07 ± 0.68%), followed by April (48.51 ± 0.56%), and January/February (38.40 ± 0.95%). Time of day affected both air temperature ($F_{(22, 1448)} = 78.97$, $P < 0.0001$) and relative humidity ($F_{(22, 1448)} = 31.99$, $P < 0.0001$). The lowest temperature was recorded at 0900, which gradually increased with the highest temperature recorded between 1500 and 1700 ($P < 0.003$). The air contained the highest percentages of humidity between 0900 and 1030, then gradually reduced until 1230, with no differences in the observation time points between 1300 and 1830 ($P < 0.003$).

2.4.2.2 Effects of time of day on hens' distribution outside

Time of day had effects on the hens' distribution both in the sun ($F_{(19, 914)} = 15.32$, $P < 0.0001$) and in the shadow ($F_{(21, 1059)} = 97.45$, $P < 0.0001$; Figure 12). Generally, a higher number of hens was observed in the sun between 0900 and 1130 and comparatively lower numbers between 1400 and 1730, with the fewest observed between 1800 and 1830 ($P < 0.003$) (Figure 12). Few hens were observed in the shadow between 0900 and 1130, followed by gradual increases up to 1730 ($P < 0.003$) (Figure 12).

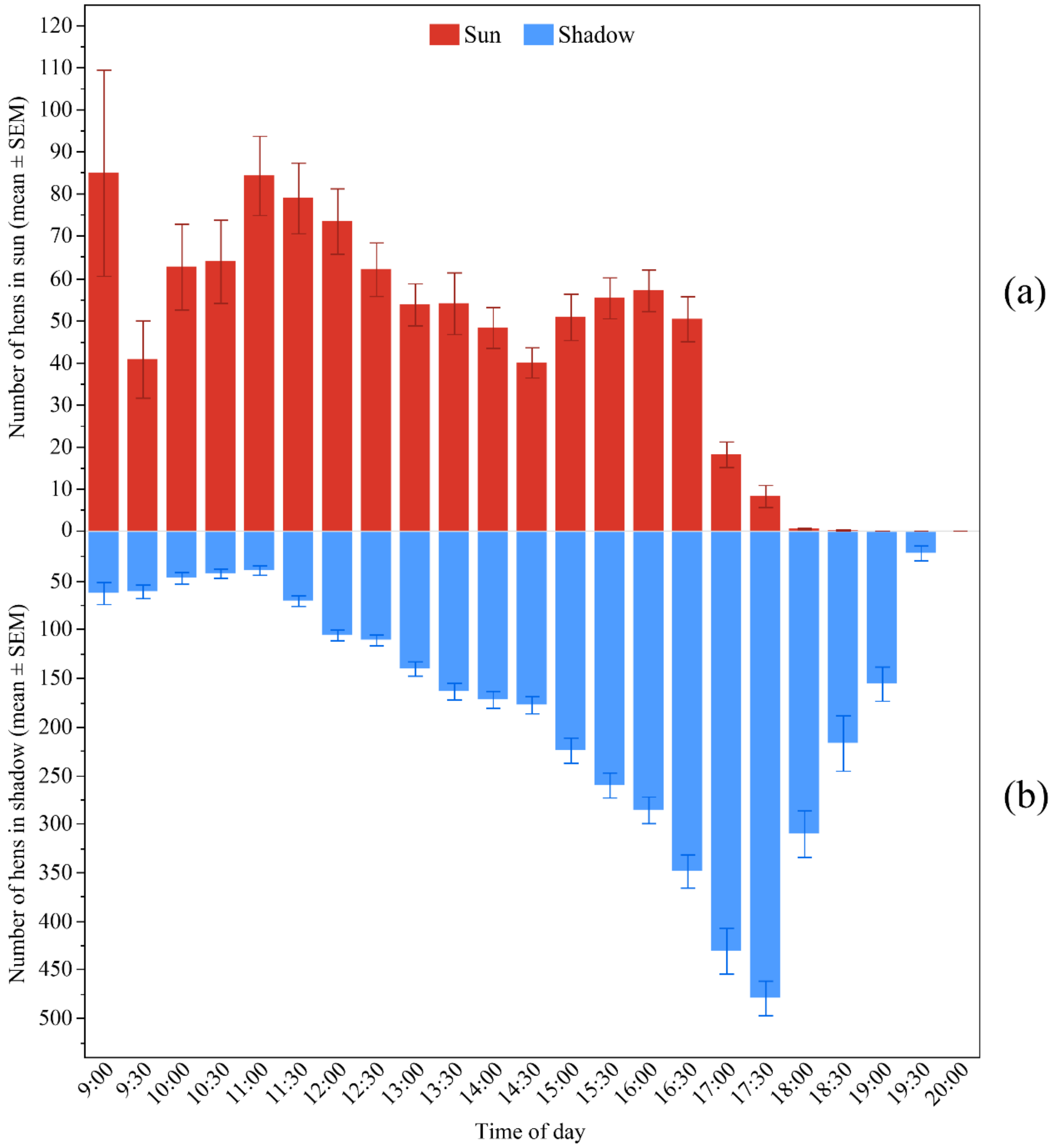


Figure 12 The mean (± SEM) number of hens across the day in the sun (a) and shadow (b) areas on Farm C

2.4.2.3 Differences in hens' distribution across months

There was significant variation between the months for hens ranging in the sun ($F_{(3,\ 914)} = 82.08$, $P < 0.0001$) and as well as in the shadow ($F_{(3,\ 1059)} = 114.32$, $P < 0.0001$). Fewer hens ventured into the sunny area in January/February and March, with significantly linear increases across the following months of study ($P < 0.05$) (Figure 13). There were more hens in the shadow in April and May, and the fewest in January/February ($P < 0.05$) (Figure 13).

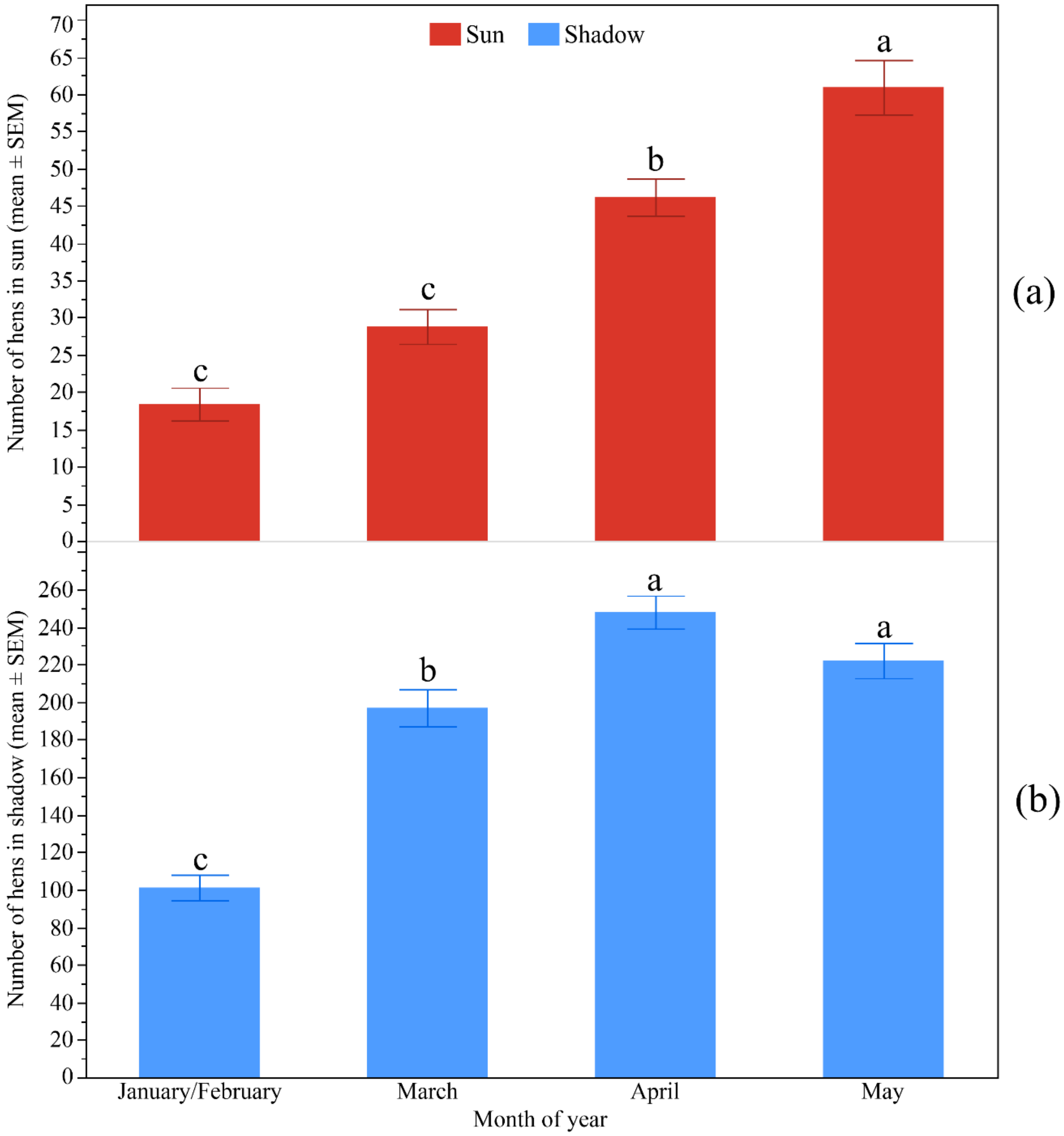


Figure 13 The mean (± SEM) number of hens across the months in the sun (a) and shadow (b) areas on Farm C

$^{a\text{-}c}$ Dissimilar superscript letters indicate significant differences among months separately for the sun and shadow areas.

2.4.3.4 Relationship between sunlight and range use

The relationship of the predictors with the ranging behaviour of hens in the sun is illustrated in Figure 14. The overall model was significant with temperature, relative humidity, UVAB , and IR as contributing factors ($F_{(3.68,\ 933.05)}$ = 143.35, *P* < 0.0001) explaining 36.50% of the variance (Figure 14). Both temperature and UVAB contributed equally (32.36% and 31.17%) to the model, and had a negative correlation with hens in the sun. For the number of hens in the shadow, all predictor variables were significant ($F_{(4.20,\ 1079.31)}$ = 121.42, *P* < 0.0001; Figure 14), accounting for 21.40% of the variance**.** UVAB and PAR were negatively correlated with hens in the shadow and temperature and IR were positively correlated. IR (48.75%) and PAR (24%) showed the greatest contributions to the model.

The model effects across different months are presented in Table 10. There were only 9 days of available data together from January and February, which may limit the interpretations of the model. However, in March the model showed temperature, UVAB and PAR accounts for 35.63% of the variation with temperature and UVAB showing a negative relationship, and PAR a positive relationship with hens in the sun (Table 10). Irrespective of variables, the models in other months explained less than 20% of the variation, with temperature showing the greatest contribution (more than 50%) and a negative relationship. In terms of hens in the shadow, UVAB did not account for any variation in either model while temperature and IR were positively correlated, and PAR had negative association in the models of March, April and May (Table 10).

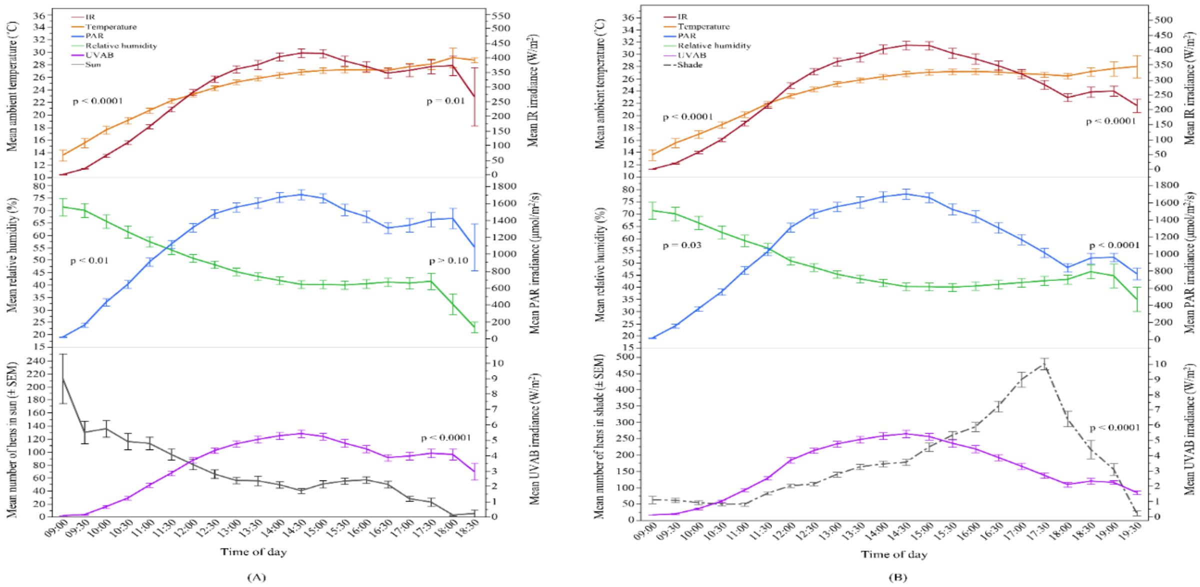


Figure 14 Relationship between hen range use, sunlight spectrums, and weather variables on Farm C

(a) Y-axis (left): the mean (±SEM) number of hens in the sun, and the mean (±SEM) relative humidity and temperature. Y-axis (right): the mean UVAB (ultraviolet radiation wavelengths A and B), PAR (photosynthetically active radiation), and IR (infrared radiation).

(b) Y-axis (left): The mean (±SEM) number of hens in the shadow, and the mean (±SEM) relative humidity and temperature.
Y-axis (right): the mean UVAB, PAR and IR.

p > 0.10 indicates the variable had no significant effect and was removed from the final model.

Table 10 Multiple ridge regression analyses (ridge parameter, k=0.02) on the number of hens in the sun and shadow across the day on Farm C

Month(s)	Distribution	Predictor[1]	B-coefficient (Standardised)[‡]	t-value	*P*-value	Model's F-statistics	Relative weight of the predictors in the model
January/February	Sun	Relative humidity	0.39	5.62	*<0.0001*	R^2-adjusted = 0.3674 $F_{(2.76, 136.04)}$ =33.73, $p < 0.0001$	50.83%
		UVAB	0.65	4.93	*<0.0001*		17.42%
		IR	-0.78	-5.67	*<0.001*		31.76%
	Shadow	Relative humidity	0.16	2.18	*0.03*	R^2-adjusted = 0.1498 $F_{(3.23, 140.33)}$ = 13.51, $p < 0.0001$	7.01%
		UVAB	-0.32	-1.67	*0.10*		10.59%
		PAR	-0.88	-4.25	*<0.0001*		25.09%
		IR	1.21	7.01	*<0.0001*		57.31%
March	Sun	Ambient temperature	-0.44	-6.94	*<0.001*	R^2-adjusted = 0.3563 $F_{(2.65, 193.10)}$ = 42.94, $p < 0.0001$	53.32%
		UVAB	-0.65	-4.80	*<0.0001*		29.48%
		PAR	0.48	3.62	*<0.0001*		17.20%
	Shadow	Ambient temperature	0.22	3.93	*<0.001*	R^2-adjusted = 0.2920 $F_{(3.64, 219.08)}$ = 59.41, $p < 0.0001$	7.13%
		Relative humidity	0.10	1.74	*0.08*		2.97%
		PAR	-1.57	-14.56	*<0.0001*		46.70%
		IR	1.57	13.72	*<0.0001*		43.20%
April	Sun	Ambient temperature	-0.29	-5.39	*<0.0001*	R^2-adjusted = 0.1986 $F_{(1.95, 347)}$ = 45.09, $p < 0.0001$	57.35%
		UVAB	-0.22	-3.95	*<0.0001*		42.65%
	Shadow	Ambient temperature	0.26	6.57	*<0.0001*	R^2-adjusted = 0.3004 $F_{(2.68, 410.08)}$ = 113.72, $p < 0.0001$	20.12%
		PAR	-1.25	-14.71	*<0.0001*		34.63%
		IR	1.33	15.41	*<0.0001*		45.25%

May	Sun	Ambient temperature	-0.19	-2.66	*<0.0001*	R^2-adjusted = 0.1842 $F_{(1.93,\ 251)} = 29.86$, $p < 0.0001$	42.34%
		Relative humidity	0.29	4.06	*<0.001*		57.66%
	Shadow	Ambient temperature	0.43	7.06	*<0.0001*	R^2-adjusted = 0.3463 $F_{(3.55,\ 300.14)} = 48.17$, $p < 0.0001$	40.92%
		Relative humidity	0.13	2.34	*0.02*		7.62%
		PAR	-0.41	-3.77	*<0.001*		19.37%
		IR	0.69	6.03	*<0.0001*		32.09%`

Only variables that significantly contributed to the most parsimonious model are presented.

[‡] β-coefficient (standardised) of the predictor variables were estimated separately using the ridge regression coefficient in ‘R’ as the original ridge package did not include the ‘β-coefficient’ value in the regression outputs.

[1] UVAB (ultraviolet radiation A and B wavelengths), PAR (photosynthetically active radiation), and IR (infrared radiation).

2.4 Discussion

The aim of this study was to determine what sunlight and climatic factors may affect whether a hen uses the range or not across the summer months on three farms in different regions of Australia. While hens of similar ages were observed, each farm was distinct in its layout, range design and exact recording period. Thus, while some general patterns were similar among the farms, the results from the farms are recommended to be interpreted individually as case studies as the causes for variation were likely multifactorial and not solely based on region of observation.

Each farm had varying degrees of shaded area available on the range, whether this resulted from shelters placed on the range, or the shadow of the shed across the day. Thus, hens may be outside on the range, but within the shaded areas that are more protected from the sunlight. To account for this, hens on each farm were assessed as being present directly in the sunlight as well as in the shadows on the range. The results on each farm were similar in that the relationship between hens outside and sunlight/climatic variables was greater for hens directly in the sun rather than in the shade. Across all farms, temperature played a strong role in the number of hens in the sunny range areas, with fewer hens in the sun when the temperatures were higher. But specific wavelengths of sunlight also affected range usage and to varying degrees. Hens were shown to avoid PAR, which is visible light and accounts for the brightness outside on sunny days, as well as UVAB radiation, particularly on the Queensland and Western Australian farms where there would be greater ultraviolet radiation than that experienced in Tasmania. The ultraviolet radiation assessed combined both the A and B wavelengths. Hens are able to see UVA light and UVB has more damaging effects. Thus, it is not certain whether hens were avoiding the UV as a result of an increase in brightness they may see, or whether they were avoiding potential skin damage, or a combination of both factors. The results do indicate hens are sensitive to different wavelengths of sunlight, and these contribute to lower numbers of hens on the range across the summer months.

As may be expected, across all farms, hens varied in their range use across the day, with lower ranging in the peak sun period in the middle of the day, and they increased their ranging into the autumn months. This is further support for the impacts of sunlight on ranging, although it is possible that hen age also played a role, with hens growing more accustomed to the range across time. The wavelengths of sunlight that correlated with ranging also changed across time. For example, on the Queensland farm, UVAB played a role across the summer months but was no longer a significant factor into the autumn. Across all farms, temperature still was a significant contributing factor across the months, with hens primarily avoiding higher temperatures. In some cases, however, more hens were observed out in the sun with increasing temperatures, which could indicate they were using the sun as warmth, similar to what has been observed in studies conducted in the UK (Richards et al. 2011).

2.5 Conclusions

Overall, different wavelengths of sunlight will have differing impacts on range use across commercial farms. Ranges must have options for shade so that hens may have a choice to be directly under the sun or seek shelter as required. Hens appear to be sensitive to the differing impacts of visual light (brightness), versus ultraviolet radiation (brightness and damaging), versus infrared (heat). Thus, it can be expected that as the intensity of these wavelengths change across the seasons, so will the range use by hens. Hens will avoid times of peak sun intensity and thus may not range as much during the summer months, particularly in regions of extreme sunlight. Heat consistently played a role in ranging behaviour with hens generally avoiding high temperatures, but sometimes seeking out the sun, presumably for warmth. These results are all consistent with how humans interact with sunlight and thus range design, and range use expectations, should take this behaviour into account.

3 Preference of commercial free range laying hens for shelters of different sunlight filtering percentages

3.1 Introduction

Important attributes of free range (including organic) laying hen farming are the birds' access to an outdoor range, exposure to natural daylight (sunlight), increased space to better regulate social interactions, and opportunities to express natural behaviours (Miao et al. 2005; Knierim 2006). The number of hens using the range in the first few weeks following the opening of the pop holes is typically low, which gradually increases upon adaptation to the outdoor environment (de Koning et al. 2019; Campbell et al. 2020b). However, a range of external factors impact hens' daily outdoor range use even after acclimation including weather conditions (Nicol et al. 2003; Richards et al. 2011; Pettersson et al. 2016), season of the year (Dawkins et al. 2003; Gilani et al. 2014), time of the day (Rault et al. 2013; Chielo et al. 2016), and range enrichments (Hegelund et al. 2005; Nagle & Glatz 2012). Hens' distribution on the range can depend on range features such as shed walls, fences, vegetation, and other enrichments (Rault et al. 2013; Dal Bosco et al. 2014; Larsen et al. 2017b).

The outdoor range needs to be attractive to increase hens' use by offering different kinds of natural or artificial shelters and/or shade within the range (Zeltner & Hirt 2008; Nagle & Glatz 2012; de Koning et al. 2019). These shelters may increase hen ranging through facilitating protection from predators (Zeltner & Hirt 2003; Hegelund et al. 2005) or direct intense sunlight (Rault et al. 2013; Stadig et al. 2017a; Larsen & Rault 2021). Artificial shelters that provide protection from sunlight may be particularly important for free range hens in climates with more extreme sunlight conditions such as those experienced in Australia across the summer months.

Previous studies assessing shelter preferences on commercial farms within Australia have shown artificial vertical structures attracted more hens to the range, but the shelter preferences varied with time of the day and the strength of the UV filtering shade cloth (Rault et al. 2013). During sunshine hours, hens preferred the higher density shade cloth structures that filtered the most UV radiation (Rault et al. 2013). Larsen and Rault (2021) also investigated artificial shelter preferences of commercial laying hens, focusing on shelter height, orientation, and cover density (% of UV filtering). Hens preferred the highest UV filtering but there were interactions among all factors in their preferences indicating the complexity around designing optimal artificial structures (Larsen & Rault 2021). Further confirmation of what features of artificial shelters hens prefer, particularly when there is high sunlight intensity and UV radiation, is important for optimising free range systems in hot climates.

This study was conducted to assess hens' use of different sunlight filtering shade cloth shelters on the range) of a commercial free range laying hen farm in Australia. The study hypothesised that hens would prefer the shelters that blocked a greater amount of UV radiation, particularly on days of high sunlight intensity.

3.2 Methods

The Commonwealth Scientific and Industrial Research Organisation (CSIRO) Wildlife and Large Animal, Animal Ethics Committee approved all research procedures for this study (AEC approval number:

2020-27) although husbandry and management of the birds fell under the responsibility of the commercial farm.

3.2.1 Hens and study site

The study was conducted across two individual flocks (Flock A and Flock B) of a single commercial free range laying hen farm during the summer months (December 2020–March 2021) in Queensland, Australia. Both flocks, comprising approximately 20,000 Hy-line Brown laying hens each were studied from 34 to 40 weeks of age. The birds were from the same hatchery and reared indoors for 16 weeks (pullet stage) with the same resources, feed, and housing management before shifting into the free range facility. From 16 to 20 weeks, the hens were housed inside the indoor aviary with standard farm management protocols and resource access. At 20 weeks of age, hens were provided range access via pop holes (0900–2000). Hens were given 14 weeks of range acclimation before the study commenced.

The study sites each had distinct land layout and vegetation within the range but identical resources inside the sheds and the same management practices. Each site had an indoor shed, which was longer in the east-west position with an outdoor range at both the north and south face. Hens within the shed could only access the range on either the north or south face due to an internal shed division, thus each shed actually contained 40,000 hens total. The south side of each shed was used for this study. The indoor sheds included an aviary system, furnished with feeders, drinkers, nest boxes and perches. Feed and water were provided *ad libitum* inside the shed only. The base of the shed sidewalls (0.62 m) was made from solid materials (poly panel) and the upper parts were covered by curtains up to the ceiling. The indoor shed temperature and relative humidity were maintained both mechanically by lowering and raising the curtains and automatically with fans throughout the study periods. Each of the indoor sheds measured 120 L x 20 W x 8 m H with an indoor stocking density of 9 hens/m^2. The outdoor stocking density was 1,500 hens/ha (equivalent to 0.15 hens/m^2). Pop holes for range access were located at 0.55 m above the ground in the sidewalls. There was a total of 14 pop holes (6 m L x 0.62 m W) on each side but typically only half were open across the shed length. The range area adjacent to the shed wall (2.5 m) was covered with compact gravel, then the immediate range area (12 m) was covered with heavy weed fabric, followed by approximately 25 m of uncovered (dirt) area, and the rest of the range was covered with grass. A number of trees (*Eucalyptus spp*.) were establishing within the range area, planted at varying distances from the shed past the gravel and plastic-covered areas. The boundaries of the range area were wire fences. During the daytime, the average minimum and maximum outdoor ambient temperatures in Flock A were recorded as 24.09 ± 0.10˚C and 26.57 ± 0.10˚C respectively, and average relative humidity was 51.41 ± 0.27%; in Flock B, the average minimum and maximum outdoor ambient temperatures were recorded as 24.01 ± 0.11˚C and 27.31 ± 0.11˚C respectively, and average relative humidity was 49.29 ± 0.17%.

3.2.2 Experimental set-up

To test hens' preferences for shade cloth shelters of different densities, three types of shade cloth shelters with three replicates each were used including: (i) 50% UV block (Coolaroo, 484866, Shade cloth, Rainforest); (ii) 70% UV block (Garden shield, SC303610CG, HDPE, Cottage Green where supplier labelling indicated 30% UV filtering but controlled testing showed it was actually 70% UV block; Figure 15); and (iii) 90% UV block (Coolaroo, 486921, Shade cloth, Rainforest) across 2 flocks consecutively. The UV filtering percentage of the treatment shelter cloths were confirmed using an Ocean Insight Flame-S-XR1 Spectroradiometer (200–1025 nm, Quark Photonics, Melbourne, VIC, Australia). Measurements were taken at a distance of 20 cm with each type of shade cloth placed over a set of three Exo Terra® (Rolf C. Hagen, Montreal, QC, Canada) pet reptile bulbs: Reptile UVB200, 25W, PT2341 used as a standard, controlled source of UV radiation. Although the shade cloths are marketed as blocking UV radiation, they also filtered out solar radiation in the visible and infrared spectrums (Figure 15).

Each shelter (4 m L x 3 m W x 1 m H) was positioned in a straight line parallel with the shed 10.5 m away from the pop holes. Shelters were placed 3 m apart following the repeating pattern of 90%, 70%, and 50% UV block shade cloth in Flock A, and 70%, 90%, and 50% UV block shade cloth in Flock B. The structure of the shelter was made of stainless steel, and shade cloth was stretched tight over the frame to minimise its movement in the wind. Temperature and humidity loggers (Tinytag Plus 2, TGP-4500; Gemini Data Loggers Ltd, West Sussex, UK) were placed under each shelter on the rear left post at a 300 mm height with automated logging at 15 min intervals. The position of these loggers resulted in them sometimes being shaded and sometimes being under direct sunlight, depending on the position of the sun across the day.

A high-resolution security camera system (Hikvision DS-7608NI-I2-8P CCTV NVR Recorder) was installed with a camera (Hikvision DS-2CD2355FWD-I2 CCTV 6MP Turret cameras) on a stand 1.6 m in front of each shelter to capture the entire shelter and the shadows that were cast across the day. Each Internet Protocol camera was individually cabled back to a small enclosure mounted within the range that contained a Hikvision Ethernet POE Switch (Model DS-3e0109P-E(C)) that powered the cameras, as well as a set of NanoBeam® – ACs (model NBE_5AC-Gen2, Ubiquiti Inc.) that wirelessly routed the cameras back to the NVR system set up in the site office.

An MEA weather station (Green Brain, 41 Vine Street, Magill SA 5072, Australia) was set-up on the respective farm site for recording sunlight variables, and recorded weather data every 15 min over the study periods. The weather station was mounted on a post (user supplied) at a height of 1 m and included different sensors (SR-05 D1A3 pyranometer, QS5 PAR pyranometer, and UV3pAB UV sensor) for recording sunlight variables including the total solar radiation (TSR) (285 nm–3000 nm) (W/m²), photosynthetically active radiation (PAR) (400–700 nm) (µmol/m2/s), and ultraviolet radiation (UVAB) (288–432 nm) (W/m²), respectively. Additionally, an air temperature and relative humidity sensor recorded the ambient air temperature (˚C), relative humidity (%), barometric pressure (mBar), dew point (˚C), voltage (V), and vapour pressure deficit (kPa). As the study was focused on the hen preferences for different shelters relative to sunlight variables, only the solar radiation spectrums, air temperature and relative humidity data were considered in the final analyses.

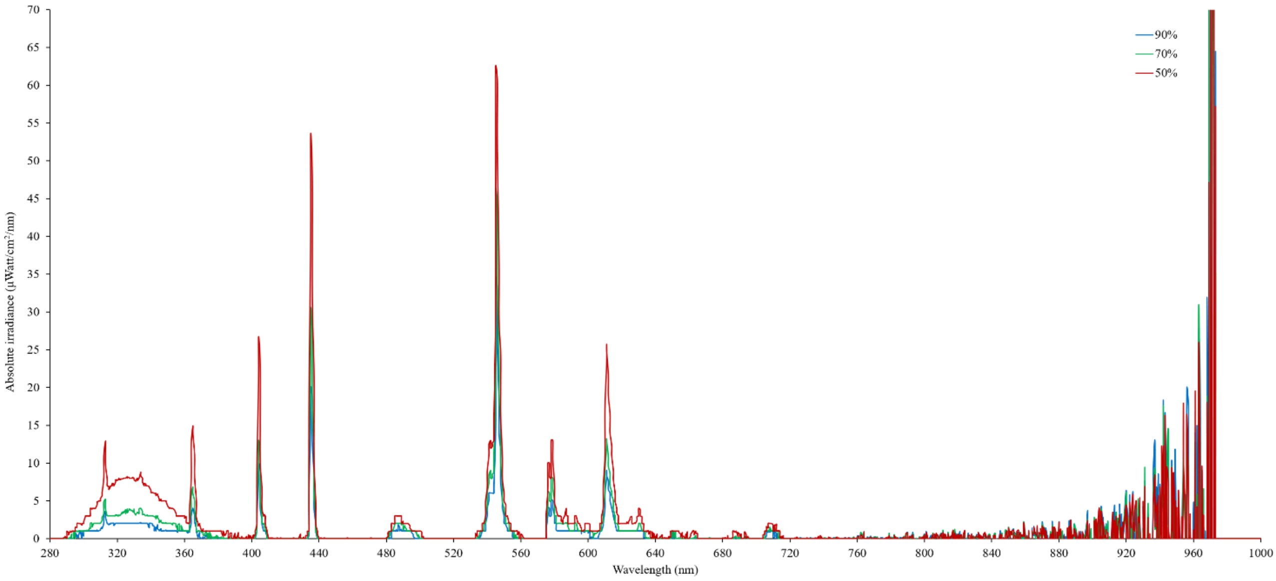


Figure 15 The wavelengths (nm) and irradiance of a standard UVAB bulb as filtered by shade cloth with 90%, 70%, and 50% UV filtering capacity

3.2.3 Observations and data collection

The shelters were installed when the hens were 34 weeks of age, with 2 weeks allowed for habituation to the range shelters before the study observations began. Recording was continuous across daylight hours for approximately 5 weeks for Flock A and 4 weeks for Flock B. Due to temporary failures in video recording, a total of 14 days videos for Flock A and 17 days for Flock B were analysed and these days were not consecutive within the recording period. For assessing shelter preferences, image snapshots from video records were taken at 30 min intervals, from 30 min after pop hole opening (i.e. 0930) until just before sunset (i.e. 1830). The images were imported into Image-J 1.53a software (Wayne Rasband, National Institute of Health, USA) and an observer counted the number of hens both under the individual shelters and on top of the shelters (Figure 16). On sunny days, the area for counting the hens under the shelter was defined by the shadow that the shelter cast (the exact position of the shadow varied across the day). On cloudy days without a prominent shadow, the counting area was considered as the area directly underneath the shelter frame. If the individual hens could not be clearly identified due to crowding under the shelter, the number of hens was estimated in the group by counting the birds within a certain area and then multiplying that number across the counted area (this occurred on 41 occasions out of 5,580 observations across both flocks).

Figure 16 One of the shelters showing hens underneath and on top

This image is from later in the afternoon (1730), where there is no clear shadow from the shelter.

3.2.4 Data and Statistical Analyses

All observations for each flock were analysed separately. A total of 5,301 observations were made over the 14-day period in Flock A (2,394 observations), and 17-day period in Flock B (2,907 observations) to count both the hens underneath and on top of the shelters. The number of hens counted in each observation was matched with the corresponding weather parameters across the 15-min period directly prior to the observation time point. Weather parameters included the UVAB, PAR, TSR, ambient temperature and relative ambient humidity, and temperature and relative humidity readings from the loggers underneath the shelters. The hen count data contained a considerable number of '0' values (when no hens were under or on top of the shelters) and were not normally distributed, thus these data were log (x+1) transformed to include the '0' values in the analyses as well as to approach data normality. To test hens' preferences to be underneath the shelters across the study period data were analysed using JMP® 14.0 (SAS Institute, Cary, NC, USA) with α level set at 0.05. General linear mixed models (GLMM) were applied with the different UV-filtering percentages, time of day, and their interaction included as fixed effects and shelter replicate nested within UV-filtering percentage as a random effect. A separate model with the same parameters was fitted to assess hens' preferences to be on top of the shelters. The studentised model residuals were visually inspected for confirming homoscedasticity. Where significant differences were present, post hoc Student's t-tests were applied to the least squares means with Bonferroni corrections to the α level to account for multiple post-hoc comparisons. The means of the underneath shelter temperatures and relative humidity were plotted along with the mean ambient temperature and humidity across the day, but these data were not statistically analysed as their positioning on the rear leg resulted in the loggers sometimes being under direct sunlight.

To investigate the effects of sunlight variables on hens' shelter use across the day (presence under the shelter regardless of shelter type), an overall linear regression model was constructed for each flock using a summarised dataset where values within each UV-filtering percentage were averaged across all three replicates for each time point for each day ($n = 798$ per UV-filtering percentage in Flock A, and $n = 969$ per UV-filtering percentage in Flock B). Before setting the model, IR spectrum values were extracted from the TSR readings by subtracting UVAB and PAR. A conversion value ($\mu mol/m^2/s$ to W/m^2) as described by Thimijan and Heins (1983), was applied to the PAR readings so all measures were in the same units for calculating the IR values. The number of hens underneath the UV-filtering shelters was included as the dependent variable, whereas sunlight variables (UVAB, PAR and IR), ambient temperature, and relative ambient humidity were included as independent variables in the model. Prior to running the model in R statistical software (R Core Team, 2020), the collinearity among the independent variables was checked through determination of variance inflation factors (VIF). Due to collinearity ($VIF \geq 10$) among the sunlight variables, we chose ridge regression (Schreiber-Gregory 2018) to best fit the predictors into the model using the 'lmridge' package in R. The relative contributions of the predictors in the regression model were estimated by the R package 'relaimpo'. All independent variables were initially included in the model with non-significant variables ($p \geq 0.10$) removed through backward elimination until the model of best fit was produced based on the adjusted R2 values. To specifically determine how sunlight and weather variables may affect the use of the different shelter types, individual linear ridge regression models were performed separately for each UV-filtering percentage with the number of hens underneath included as the dependent variable, and the sunlight variables (UVAB, IR and PAR), ambient temperature, and relative ambient humidity included as independent variables. Non-significant variables ($p \geq 0.10$) were removed through backward elimination. The raw values are plotted in the figures.

3.3 Results

3.3.1 Shelter preferences

There was a significant interaction effect between UV-filtering shelter and time of day for hen preferences in both Flock A ($F_{(36,\ 2331)} = 3.49$, $P < 0.0001$), and Flock B ($F_{(36,\ 2844)} = 2.63$, $P < 0.0001$) (Figure 17). In general, at most observation points across the day, more hens were seen under the 90% UV-filtering shelters in both flocks, but at some time points their preferences were similar across all filtering percentages ($P > 0.001$) (Figure 17).

Overall, more hens were found underneath the 90% UV-filtering shelters (LSM mean ± SEM, Flock A: 16.88 ± 2.67 hens; Flock B: 29.10 ± 1.52 hens), followed by the 70% (LSM mean ± SEM, Flock A: 9.67 ± 2.67 hens; Flock B: 15.70 ± 1.52 hens) then 50% UV-filtering shelters (LSM mean ± SEM, Flock A: 5.16 ± 2.67 hens; Flock B: 8.43 ± 1.52 hens) in both study flocks (Flock A: $F_{(2,\ 6)} = 16.25$, $P = 0.004$, and Flock B: $F_{(2,\ 6)} = 134.09$, $P < 0.0001$). The use of the shelter shade by hens were varied across the day in both Flock A ($F_{(18,\ 2331)} = 44.64$, $P < 0.0001$) and Flock B ($F_{(18,\ 2844)} = 75.11$, $P < 0.0001$) with peaks in the morning and in the late afternoon compared to the mid-day ($P < 0.003$) (Figure 18).

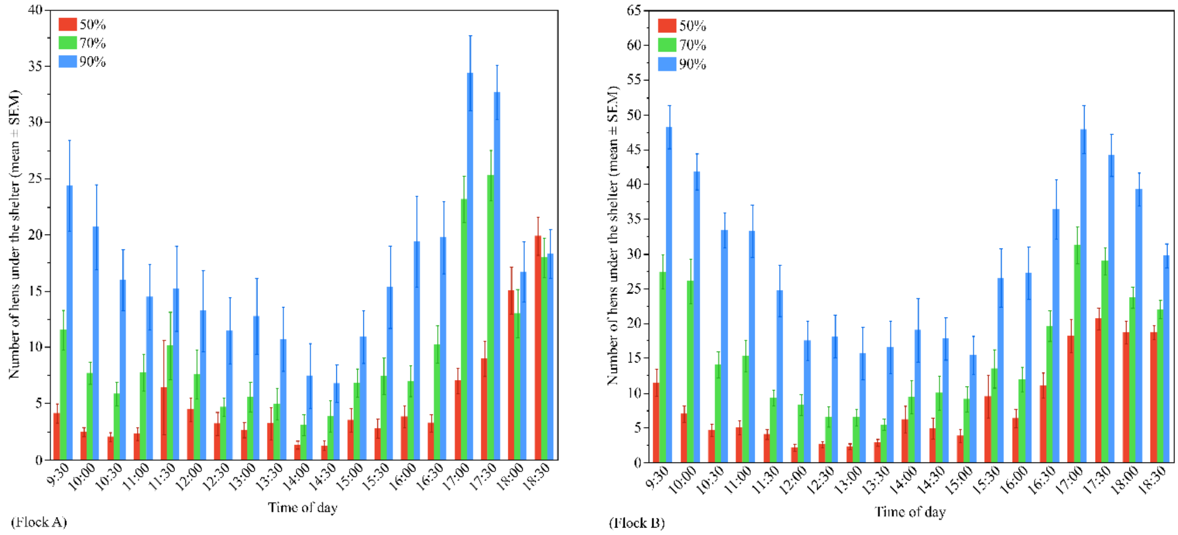


Figure 17 The mean (±SEM) number of hens underneath the shelters of different UV-filtering percentages across the day for two separate flocks (A and B)

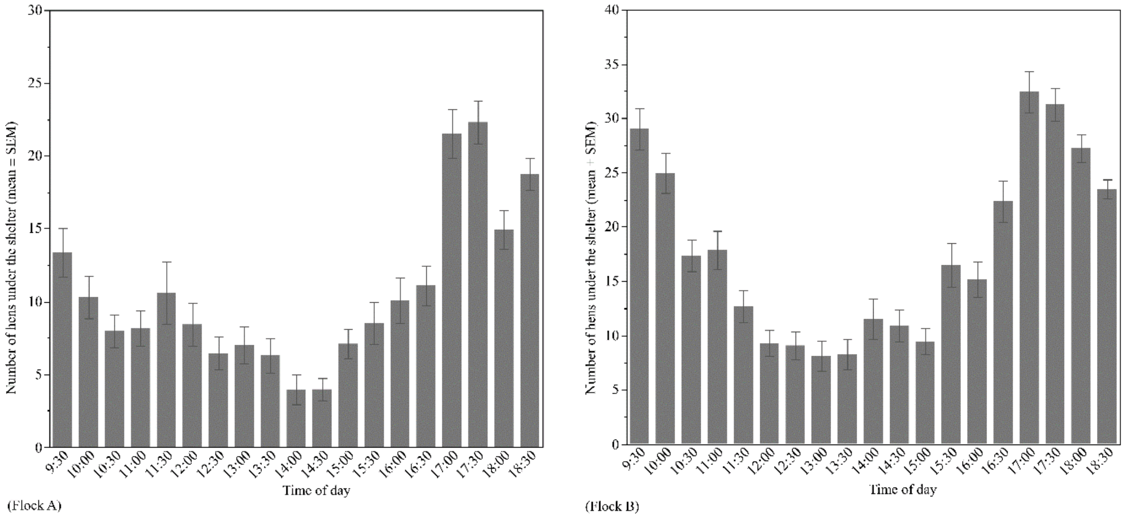


Figure 18 The mean (± SEM) number of hens across the day under all UV-filtering shelters in both study flocks (A and B)

In contrast, there was no significant interaction effect between UV-filtering shelter and time of day for the number of hens on top of the shelters in Flock A ($F_{(36,\ 2331)}$ = 0.89, *P = 0.65*); whereas an interaction effect was found in Flock B ($F_{(36,\ 2844)}$ = 0.2.68, $P < 0.0001$) (Figure 19). Across the day in Flock B, there was a general pattern of more hens on top of the 90% UV-filtering shelter in the morning and late afternoon relative to both the 50% and 70% shelters ($P > 0.001$) (Figure 19).

Overall, there was no differences for the number of hens on top of the shelters between different degrees of UV-filtering percentages in Flock A (LSM mean ± SEM, 50%: 0.52 ± 0.18, 70%: 0.75 ± 0.18, 90%: 0.96 ± 0.18, $F_{(2,\ 6)}$ = 1.37, $P = 0.32$), but the time of day had an effect on the number of hens across the day ($F_{(18,\ 2331)}$ = 41.78, $P < 0.0001$) with a gradually increasing trend after 1700 h compared to the rest of the day ($P < 0.003$) (Figure 20). In Flock B, more hens were found on top of the 90% UV-filtering shelter with no differences between the 50% and 70% shelters (LSM mean ± SEM, 50%: 1.14 ± 0.24, 70%: 1.38 ± 0.24, 90%: 2.54 ± 0.24, $F_{(2,\ 6)}$ = 9.14, $P = 0.02$). Time of day had an effect on the number of hens on top of the shelters ($F_{(18,\ 2844)}$ = 36.56, $P < 0.0001$) with more hens observed in the late afternoon ($P < 0.003$) (Figure 20).

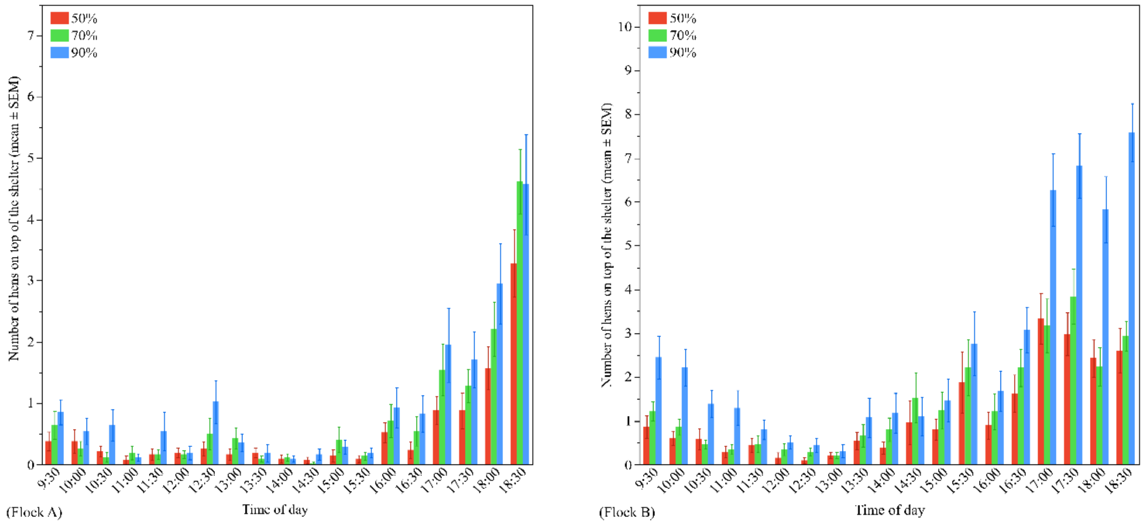


Figure 19 The mean (±SEM) number of hens on top of the shelters of different UV-filtering percentages across the day for two separate flocks (A and B)

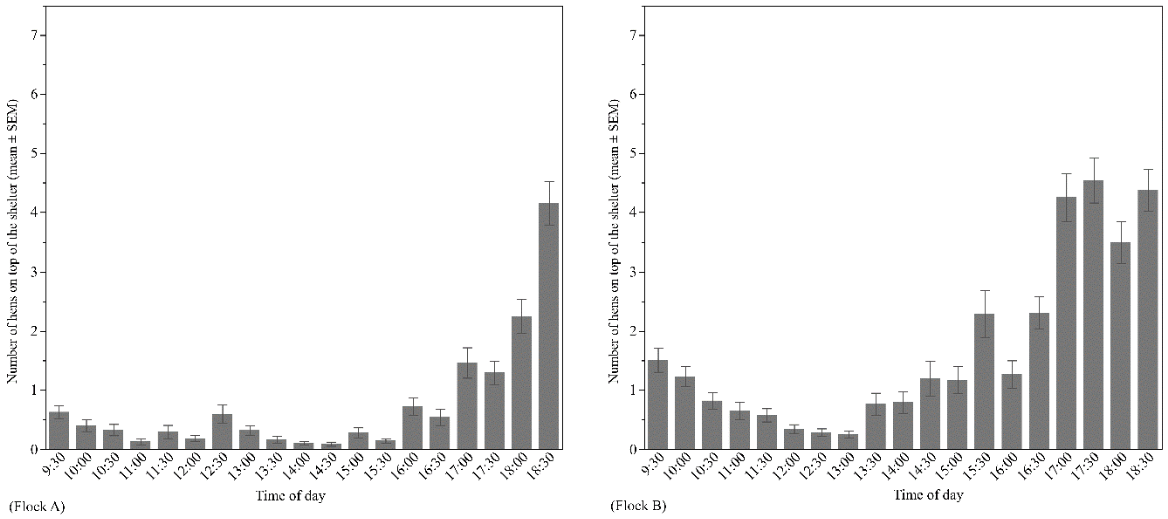


Figure 20 The mean (± SEM) number of hens across the day on top of the shelters of different UV-filtering percentages in both flocks of study (A and B)

The temperature and humidity loggers underneath the shelters were intended to provide measurements on ambient conditions the chickens may have been experiencing. However, the placement of loggers at chicken eye height on one of the rear posts of the shelters resulted in the loggers sometimes being under direct sunlight and sometimes being under the shelter shade. Figure 21 displays the temperature and relative humidity readings under each shelter type relative to the ambient temperature and relative ambient humidity readings obtained from the weather station, which was placed 1 m above ground. The temperature under the shelters was higher than the ambient temperature, whereas relative humidity was lower than the relative ambient humidity across the daytime (Figure 21). The temperatures and relative humidity across the different shelter types were visually similar but these data were not statistically analysed, as the loggers did not capture data as originally intended.

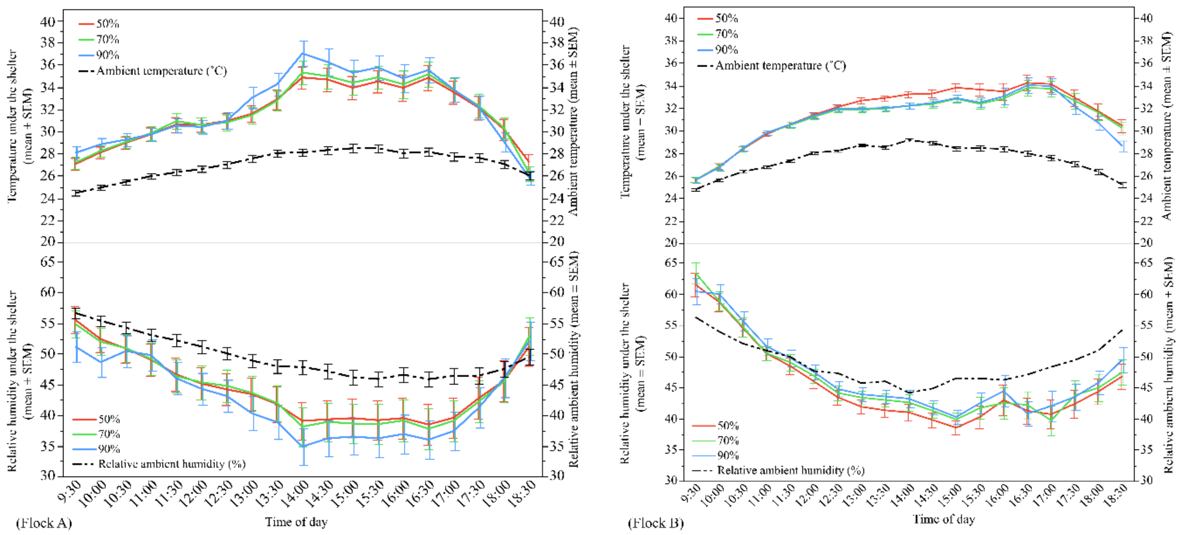


Figure 21 The mean (±SEM) ambient temperature and relative humidity and mean (±SEM) temperature and relative humidity underneath shelters of different UV-filtering percentages (50%, 70% and 90%) across the day for two flocks of hens (A and B)

3.3.2 Sunlight effects

A ridge regression model for each flock was performed to investigate the relationship between the number of hens underneath the shelters across all of the UV-filtering percentages and the sunlight variables, the ambient temperature and relative ambient humidity. The best-fit model results are presented in Table 11. In Flock A, the model accounted for 34.21% of variance in the use of all the UV-filtering shelters across the day. The ambient temperature, UVAB, IR, and relative ambient humidity contributed significantly to the model, respectively ($F_{(3.35,\ 794.24)} = 120.50$, $P < 0.0001$). However, all these predictors had a negative correlation with the number of hens under the shelters across the day (Table 11).

In Flock B, the model accounted for 35.77% variance in the number of hens under the shelters with respect to sunlight and weather variables considered within the model. The majority of the variance was explained by the ambient temperature (49.01%), however, IR, UVAB and PAR also significantly contributed to the model ($F_{(2.68,\ 965.98)} = 146.64$, $P < 0.0001$; Table 11). The ambient temperature, UVAB, and IR were negatively correlated, and PAR was positively correlated with the number of hens underneath the shelters (Table 11).

Table 11 Two ridge regression analyses (ridge parameter, k=0.02) on the number of hens under the shelter across the day (adjusted R^2 = 0.34 and 0.36 in Flock A and Flock B, respectively)

Flock	Predictor[1]	β- coefficient (standardised)‡	t-value	P-value	Model's F-statistics	Relative weight of the predictors in the model
Flock A	Ambient temperature	-0.70	-12.61	<0.0001	$F_{(3.35,\ 794.24)} = 120.50, P < 0.0001$	33.54%
	Relative ambient humidity	-0.46	-8.29	<0.0001		13.86%
	UVAB	-0.15	-2.17	0.03		25.36%
	IR	-0.24	-3.37	0.001		27.24%
Flock B	Ambient temperature	-0.41	-15.90	<0.0001	$F_{(2.68,\ 965.98)} = 146.64, P < 0.0001$	49.01%
	UVAB	-0.27	-3.78	<0.001		16.88%
	PAR	0.10	2.07	0.04		16.84%
	IR	-0.19	-3.70	<0.001		17.28%

Only variables that significantly contributed to the most parsimonious model are presented.‡ β-coefficient (standardised) of the predictor variables were estimated separately using the ridge regression coefficient in 'R', as the original ridge package did not include the 'β-coefficient' value in the regression outputs.

[1] UVAB (ultraviolet radiation A and B wavelengths), PAR (photosynthetically active radiation), and IR (infrared radiation).

Table 12 Multiple ridge regression analyses (ridge parameter, k=0.02) on the number of hens under different UV-filtering shelters across the day

UV-filtering shelter	Flock	Predictor[1]	B-coefficient (Standardised)	t-value	P-value	Model's F-statistics	Relative weight of the predictors in the model
50%	A	Ambient temperature	-0.59	-7.16	*<0.0001*	R^2-adjusted = 0.52 $F_{(2.79,\ 263.03)} = 108.58$, $P < 0.0001$	24.62%
		Relative ambient humidity	-0.36	-4.42	*<0.0001*		10.52%
		PAR	-0.56	-13.25	*<0.0001*		64.86%
	B	Ambient temperature	-0.40	-11.24	*<0.0001*	R^2-adjusted = 0.58 $F_{(2.53,\ 320.19)} = 156.77$, $P < 0.0001$	34.26%
		UVAB	-0.29	-3.18	*<0.01*		32.43%
		IR	-0.28	-3.08	*<0.01*		33.31%
70%	A	Ambient temperature	-0.77	-8.48	*<0.0001*	R^2-adjusted = 0.40 $F_{(2.79,\ 263.03)} = 71.33$, $P < 0.0001$	38.52%
		Relative ambient humidity	-0.46	-5.11	*<0.0001*		15.64%
		UVAB	-0.41	-8.87	*<0.0001*		45.85%
	B	Ambient temperature	-0.44	-10.63	*<0.0001*	R^2-adjusted = 0.44 $F_{(2.68,\ 319.98)} = 71.26$, $P < 0.0001$	45.57%
		UVAB	-0.33	-2.85	*<0.01*		18.10%
		PAR	0.13	1.66	*0.10*		17.91%
		IR	-0.22	-2.70	*0.01*		18.42%
90%	A	Ambient temperature	-0.93	-9.99	*<0.0001*	R^2-adjusted = 0.35 $F_{(3.08,\ 262.54)} = 51.13$, $P < 0.0001$	40.75%
		Relative ambient humidity	-0.68	-7.44	*<0.0001*		18.85%
		PAR	0.20	1.84	*0.07*		19.14%
		IR	-0.53	-4.92	*<0.0001*		21.26%
	B	Ambient temperature	-0.54	-12.28	*<0.0001*	R^2-adjusted = 0.38 $F_{(2.68,\ 319.98)} = 56.45$, $P < 0.0001$	71.58%
		UVAB	-0.31	-2.51	*0.01*		9.20%
		PAR	0.24	2.85	*<0.01*		9.58%
		IR	-0.15	-1.78	*0.08*		9.63%

Only variables that significantly contributed to the most parsimonious model are presented.

[‡] β-coefficient (standardised) of the predictor variables were estimated separately using ridge regression coefficient in 'R', as the original ridge package did not include the 'β-coefficient' value in the regression outputs.

[1] UVAB (ultraviolet radiation A and B wavelengths), PAR (photosynthetically active radiation), and IR (infrared radiation).

The separate ridge regression models for each UV-filtering percentage showed differences in the relative impacts of the sunlight and weather variables on the number of hens underneath the shelters. For the 50%, 70% and 90% UV-filtering shelter preferences, both sunlight and weather variables accounted for: 51.71% (Flock A: $F_{(2.79,\ 263.03)} = 108.58$, $P < 0.0001$) and 57.94% (Flock B: $F_{(2.53,\ 320.19)} = 156.77$, $P < 0.0001$) of the variance for the 50% shelters; 40.35% (Flock A: $F_{(2.79,\ 263.03)} = 71.33$, $P < 0.0001$) and 44.29% (Flock B: $F_{(2.68,\ 319.98)} = 71.26$, $P < 0.0001$) of the variance for the 70% shelters; and 31.16% (Flock A: $F_{(3.08,\ 262.54)} = 51.13$, $P < 0.0001$) and 37.77% (Flock B: $F_{(2.68,\ 319.98)} = 56.45$, $P < 0.0001$) for the 90% shelters (Table 12).

The ambient temperature significantly affected hens' shelter preferences for each shelter type in both flocks (Flock A: all $P < 0.0001$; Flock B: all $P < 0.0001$) (Figure 22), with it being the greatest contributing factor for use of the 70% and 90% UV-filtering shelters. The temperature accounted for 38.52% and 45.57% of the variation in Flock A for the 70% and 90% shelters respectively, and 40.75% and 71.58% of the variation in Flock B for the 70% and 90% shelters respectively. The results indicated that increased ambient temperature resulted in fewer hens under the shelters (Table 12).

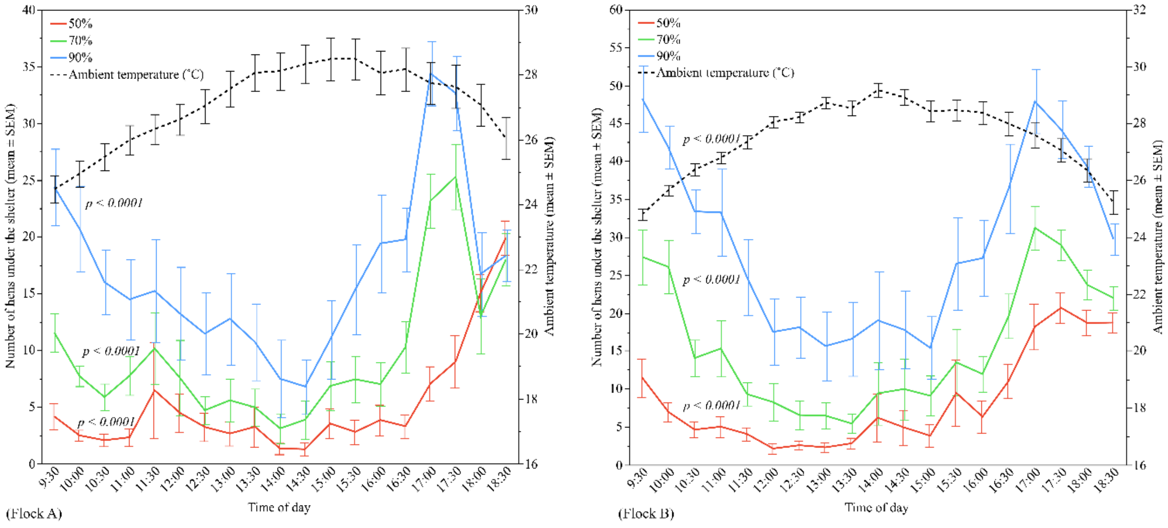


Figure 22 The mean (±SEM) number of hens under the different UV-filtering shelters (50%, 70% and 90%) and the mean (±SEM) ambient temperature across the day for two hen flocks (A and B)

The relative ambient humidity also significantly contributed to hens' shelter preferences for each shelter type in Flock A (all $P < 0.0001$) but did not show an effect in Flock B (Figure 23). However, in Flock A, the relative contribution of the relative ambient humidity was less than 20% in the respective models of 50%, 70% and 90% UV filtering shelter (accounting for 10.52%, 15.64% and 18.85% of the variation, respectively), and had a negative correlation with the number of hens under the shelters (Table 12).

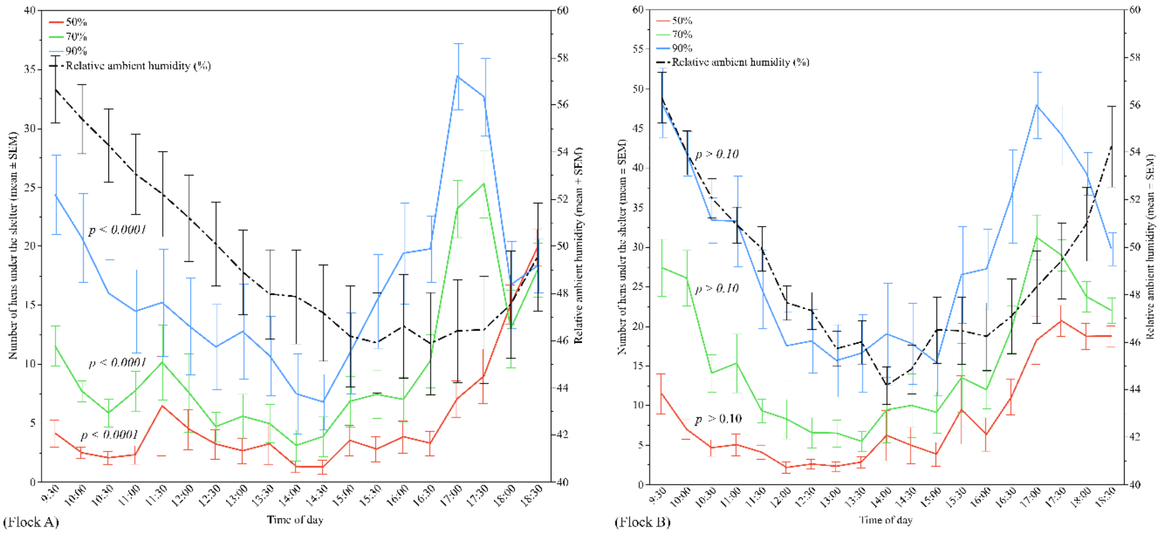


Figure 23 The mean (±SEM) number of hens under the different UV-filtering shelters (50%, 70% and 90%) and the mean (±SEM) relative humidity across the day for two hen flocks (A and B)

p > 0.10 indicates the variable had no significant effect and was removed from the final model.

UVAB radiation only had a significant effect for the 70% UV-filtering shelter preferences ($P < 0.0001$) in Flock A, where it was the most contributory effect (45.57% variation) in that specific model (Figure 24). In contrast, UVAB radiation showed a significant relationship with the use of all shelter types in Flock B (all $P \leq 0.01$) (Figure 24). The relative contribution of UVAB among the predictors in Flock B for 50%, 70% and 90% UV-filtering shelter was 32.43%, 18.10% and 9.20%, respectively, with the number of hens under the shelter decreasing with increasing UVAB radiation (Table 12).

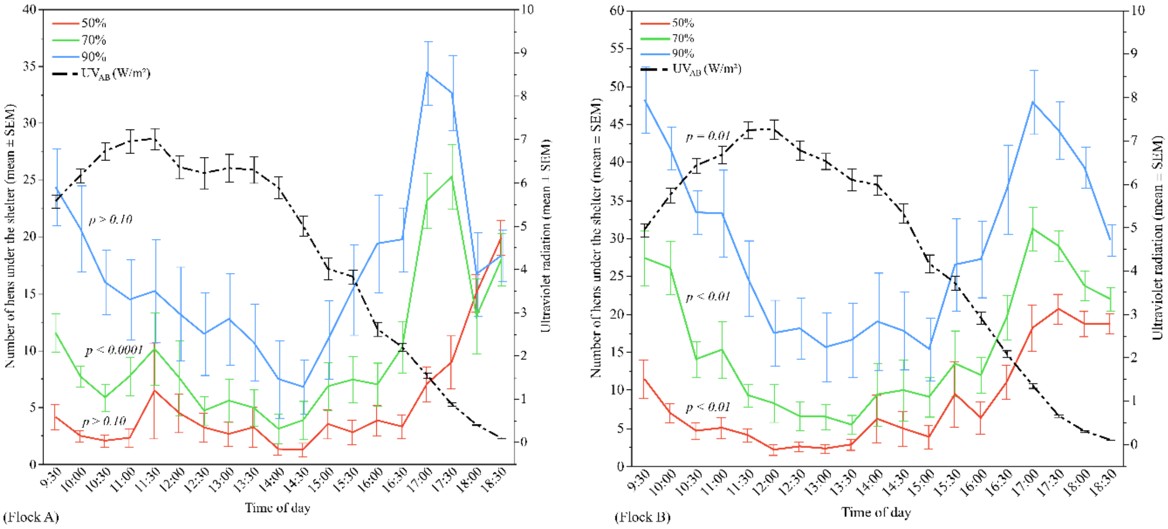


Figure 24 The mean (±SEM) number of hens under the different UV-filtering shelters (50%, 70% and 90%) and the mean (±SEM) ultraviolet (UV_{AB}) radiation across the day for two hen flocks (A and B)

p > 0.10 indicates the variable had no significant effect and was removed from the final model.

In Flock A, PAR had a significant negative correlation with the hens' use of the 50% UV-filtering shelter ($P < 0.0001$) showing the greatest contributory effect (64.86% variation; Table 12) in the model, and a positive trend for the 90% shelters ($P = 0.07$), but no association with hens' use of the 70% UV-filtering shelters (Figure 25). Whereas, in Flock B, PAR was a significant contributing variable for hens' use of the 90% UV-filtering shelters ($P < 0.01$), and it had a trend effect for the 70% shelters ($P = 0.10$) but no significant contribution for the 50% UV-filtering shelters (Figure 25). While the relative weight of PAR in the models of 90% and 70% UV-filtering shelter preferences was 9.58% and 17.91%, respectively, it had a positive relationship with the number of hens under the respective shelters, indicating that increases in PAR also increased hens' use of shelters (Table 12).

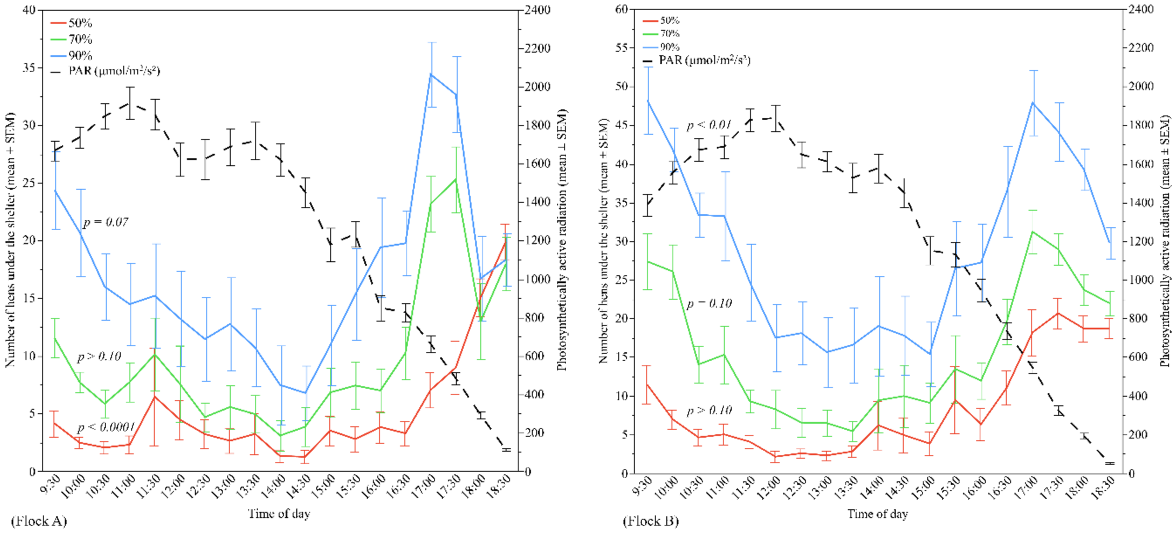


Figure 25 The mean (±SEM) number of hens under the different UV-filtering shelters (50%, 70% and 90%) and the mean (±SEM) photosynthetically active radiation (PAR) across the day for two hen flocks (A and B)

p > 0.10 indicates the variable had no significant effect and was *removed* from the final model.

IR significantly affected hens' shelter use of only the 90% UV-filtering shelters ($P < 0.0001$) in Flock A. However, in Flock B, IR significantly influenced shelter use of both the 70% and 50% UV-filtering shelters, and had a trend of an effect for the 90% UV-filtering shelters ($p = 0.08$) (Figure 26). However, a negative correlation between IR and use of shelters indicated that the number of hens under the shelters decreased when IR increased (Table 12).

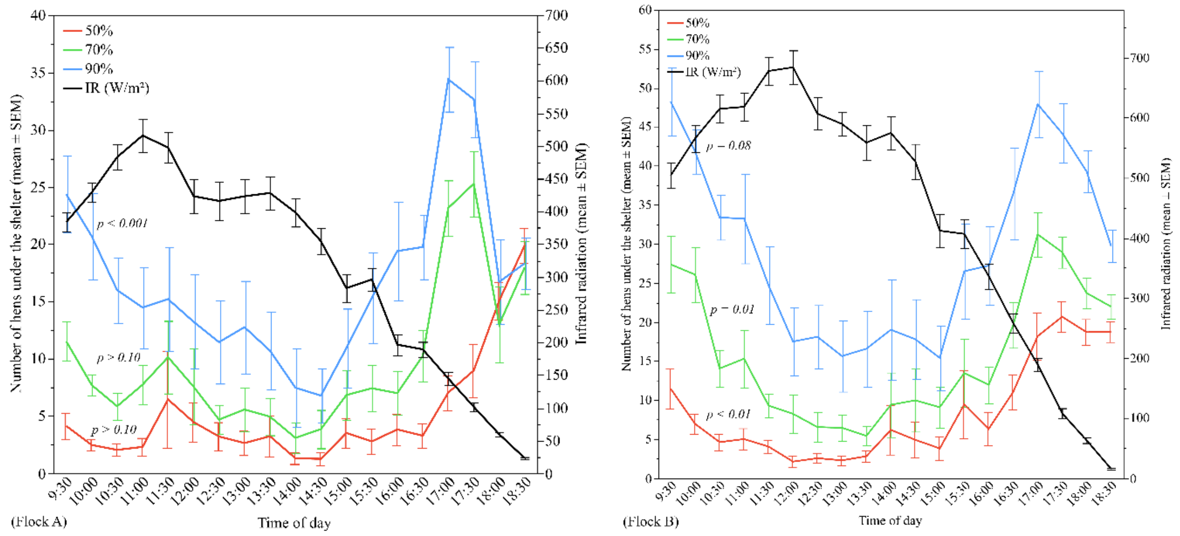


Figure 26 The mean (±SEM) number of hens under the different UV-filtering shelters (50%, 70% and 90%) and the mean (±SEM) infrared radiation (IR) across the day for two hen flocks (A and B)

p > 0.10 indicates the variable had no significant effect and was removed from the final model.

3.4 Discussion

The objective of this trial was to determine if hens had preferences for artificial shelters on the range that filtered different degrees of sunlight through the use of shade cloth of varying densities. The trial was conducted across the summer months when the sunlight is most intense and when hens may have the greatest motivation to seek shelter. The study also measured different wavelengths of sunlight rather than just total solar radiation, as it was hypothesised that specific components of the sunlight may have different impacts on shelter use. Both temperature and humidity were also included as these are factors that have previously been correlated with ranging behaviour in hens (Richards et al. 2011).

Similar to other studies that have been conducted on commercial farms within Australia (Rault et al. 2013; Larsen & Rault 2021), hens showed clear preferences for the higher densities of the shade cloth, with a linear relationship between use of the shelter and percentage of sunlight it filtered. These results confirm that hens are able to differentiate between shaded environments and will preferentially select the environment that provides the greatest amount of shaded protection. However, anecdotal observations of the video recordings from each shelter indicated that hens used shade created by trees on the range preferentially over shade created by the shelters (Figure 27). Thus, artificial shelters may still not provide the same protection as trees. Future observations should determine if shelters of 100% filtering capabilities are preferable to shade cloth, or if there are other features of trees that lead hens to prefer the shade they create (e.g. do hens prefer natural versus artificial shelters). The shade created by the trees may also be significantly cooler for the hens than what artificial shelters can provide. However, presence of avian predators in established trees may be a concern if this results in significantly increased predation and hen mortality.

When considering the surrounding weather and sunlight variables that affected the use of the shelters, temperature was a clear influencing factor with all wavelengths of sunlight (infrared – IR; photosynthetically active radiation – PAR; ultraviolet radiation – UVAB) also contributing to shelter use, although the exact impacts of the different wavelengths varied across the two flocks. This variation could have been related to the time of year, as the flocks were observed sequentially rather than simultaneously. When considering the weather and sunlight factors that affected use of a specific density of shelter, again temperature was a key factor, with the exact impact of sunlight wavelengths variable across the densities and flocks. Overall, the measured variables only accounted for approximately half of the variation in the data, indicating there were other factors in addition to the factors measured in the study that were influencing the use of the shelters. This could be other weather parameters not measured (e.g. wind speed), or social factors between the hens.

Contrary to predictions, the weather and sunlight parameters showed a negative relationship with the number of hens under the shelters. That is, when there were increases in temperature, humidity and sunlight intensity, there were actually fewer hens underneath the shelters. This relationship is likely due to the overall patterns of range use that the hens exhibited. While only hens underneath the shelters were counted, and not hens on the range area surrounding the shelters, anecdotal observations indicated when there were fewer hens underneath the shelters, there were also fewer hens outside on the range overall (Figure 28). This pattern of ranging corresponds with what has been observed in many previous studies (Dawkins et al. 2003; Rault et al. 2013; Richards et al. 2011), as well as in the second study within this report. Thus, shelter on the range may still be insufficient to draw hens outside in the peak sun, where inside the shed likely offers cooler temperatures as well as sunlight protection.

Use of the shelters increased in the evenings, even when the temperatures were cooler and sunlight intensity had dropped substantially. This suggests the shelters were also used for the protection they may offer from overhead predators and contribute to the hens potentially feeling sheltered and protected, as they would in their natural habitat of forest understory. However, many hens did go on top of the shelters in the evenings which would negate the overhead protection benefits and may be indicative of hens' motivation to roost in the evenings. Even if the shelters are unable to increase ranging during the middle of the day, they are still used by the hens and are therefore beneficial to include on the range. Further research could determine how shelter use changes across the seasons of the year, and whether daytime shelter use increases across colder temperatures if more hens are outside at these times.

Figure 27 Hens in the shade of one of the artificial shelters (foreground) and crowded underneath the shade created by trees on the range (background)

Note: Video recordings were aimed to capture the shelter, and did not adequately record the area around the trees to be able to collect data on use of the trees' shade.

Figure 28 Snapshots of one of the 90% UV-filtering shelters in Flock B, showing use of the shelter and surrounding range area across one day

3.5 Conclusions

Overall, these results show that range shelters should be constructed of the highest filtering density when using shade cloth, but that trees on the range may be preferred over artificial structures. Across the summer months with intense sunlight and heat, hens are likely to prefer to remain inside across the middle of the day regardless of the shelter on the range. Shelter use decreased as the sunlight and weather variables increased, which is likely a reflection of the low number of hens outside overall during the peak sunlight period within the day. Shelters were used in the evening, with hens both underneath and on top of them, indicating that they are beneficial range enhancements even if hens still prefer to remain inside the shed during intense heat and sunlight.

4 References

Bestman, M., Verwer, C., van Niekerk, T., Leenstra, F., Reuvekamp, B., Amsler-Kepalaite, Z. and Maurer, V. (2019) Factors related to free-range use in commercial laying hens. *Applied Animal Behaviour Science*, 214, 57-63.

Bowmaker, J.K., Heath, L.A., Wilkie, S.E. and Hunt, D.M. (1997) Visual pigments and oil droplets from six classes of photoreceptor in the retinas of birds. *Vision Research*, 37, 2183-2194.

Campbell, D.L.M., Bari, M.S. and Rault, J.-L. (2020a). Free-range egg production: its implications for hen welfare. *Animal Production Science*, 61, 848-855.

Campbell, D.L.M, Dyall, T.R., Downing, J.A., Cohen-Barnhouse, A.M. and Lee, C. (2020b) Rearing enrichments affected ranging behavior in free-range laying hens. *Frontiers in Veterinary Science*, 7, 446.

Chielo, L., Pike, T. and Cooper, J. (2016) Ranging behaviour of commercial free-range laying hens. *Animals*, 6, 28.

Dawkins, M.S., Cook, P.A., Whittingham, M.J., Mansell, K.A. and Harper, A.E. (2003) What makes free-range broiler chickens range? In situ measurement of habitat preference. *Animal Behaviour*, 66, 151-160.

Dal Bosco, A., Mugnai, C., Rosati, A., Paoletti, A., Caporali, S. and Castellini, C. (2014) Effect of range enrichment on performance, behavior, and forage intake of free-range chickens. *The Journal of Applied Poultry Research*, 23, 137-145.

de Koning, C., Kitessa, S.M., Barekatain, R., Drake, K. (2019) Determination of range enrichment for improved hen welfare on commercial fixed-range free-range layer farms. *Animal Production Science*, 59, 1336-1348.

Dunlop, E., Cunningham, J., Sherriff, J.L., Lucas, R.M., Greenfield, H., Arcot, J., Strobel, N. and Black, L.J. (2017) Vitamin D3 and 25-hydroxyvitamin D3 content of retail white fish and eggs in Australia. *Nutrients*, 9, 647

Fanatico, A.C., Mench, J.A., Archer, G.S., Liang, T., Gunsaulis, V.B., Owens, C.M. and A.M. Donoghue. (2016) Effect of outdoor structural enrichments on the performance, use of range area, and behavior of organic meat chickens. *Poultry Science*, 95, 1980-1988.

Friard, O. and Gamba, M. (2016) BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods in Ecology and Evolution*, 7, 1325-1330.

Gilani, A.M., Knowles, T.G. and Nicol, C.J. (2014) Factors affecting ranging behaviour in young and adult laying hens. *British Poultry Science*, 55, 127-135.

Hegelund, L., Sørensen, J.T., Kjaer, J. and Kristensen, I.S. (2005) Use of the range area in organic egg production systems: effect of climatic factors, flock size, age and artificial cover. *British Poultry Science*, 46, 1-8.

Huber-Eicher, B., Suter, A. and Spring-Stahli, P. (2013) Effects of colored light-emitting diode illumination on behavior and performance of laying hens. *Poultry Science*, 92, 869-873.

Holick, M.F. (2016) Biological effects of sunlight, ultraviolet radiation, visible light, infrared radiation and vitamin D for health. *Anticancer Research*, 36, 1345-1356.

Jacome, I., Rossi, L.A. and Borille, R. (2014) Influence of artificial lighting on the performance and egg quality of commercial layers: a review. *Revista Brasileira de Ciencia Avicola*, 16, 337-344.

Jones, T., Feber, R., Hemery, G., Cook, P., James, K., Lamberth, C. and Dawkins, M. (2007) Welfare and environmental benefits of integrating commercially viable free-range broiler chickens into newly planted woodland: A UK case study. *Agricultural Systems*, 94, 177-188.

Knierim, U. (2006) Animal welfare aspects of outdoor runs for laying hens: a review. *NJAS-Wageningen Journal of Life Sciences*, 54, 133-145.

Larsen, H., Cronin, G.M., Gebhardt-Henrich, S.G., Smith, C.L., Hemsworth, P.H. and Rault, J.L. (2017b) Individual ranging behaviour patterns in commercial free-range layers as observed through RFID tracking. *Animals*, 7, 21.

Larsen, H. and Rault, J.-L. (2021) Preference for artificial range enrichment design features in free-range commercial laying hens. *British Poultry Science*, 62, 311-319.

Lewis, P.D. and Gous, R.M. (2009) Responses of poultry to ultraviolet radiation. *World's Poultry Science Journal*, 65, 499-510.

Maddocks, S.A., Cuthill, I.C., Goldsmith, A.R. and Sherwin, C.M. (2001) Behavioural and physiological effects of absence of ultraviolet wavelengths for domestic chicks. *Animal Behaviour*, 62, 1013-1019.

Manser, C. (1996) Effects of lighting on the welfare of domestic poultry: a review. *Animal Welfare*, 5, 341-360.

Miao, Z., Glatz, P. and Ru, Y. (2005) Free-range poultry production-A review. *Asian-Australasian Journal of Animal Sciences*, 18, 113-132.

Mohammed, H., Grashorn, M. and Bessei, W. (2010) The effects of lighting conditions on the behaviour of laying hens. *Archiv für Geflügelkunde*, 74, 197-202.

Nagle, T.A. and Glatz, P.C. (2012). Free range hens use the range more when the outdoor environment is enriched. *Asian-Australasian Journal of Animal Science*, 25, 584-591.

Nicol, C., Pötzsch, C., Lewis, K. and Green, L. (2003) Matched concurrent case-control study of risk factors for feather pecking in hens on free-range commercial farms in the UK. *British Poultry Science*, 44, 515-523.

Pettersson, I.C., Freire, R. and Nicol, C.J. (2016) Factors affecting ranging behaviour in commercial free-range hens. *World's Poultry Science Journal*, 72, 137-150.

Prescott, N., Wathes, C.M. and Jarvis, J. (2003) Light, vision and the welfare of poultry. *Animal Welfare*, 12, 269-288.

Prescott, N.B. and Wathes, C.M. (1999) Spectral sensitivity of the domestic fowl (*Gallus g. domesticus*). *British Poultry Science*, 40, 332-339.

R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rana, M.S. and Campbell, D.L.M. (2021) Application of ultraviolet light for poultry production: A review of impacts on behavior, physiology, and production. *Frontiers in Animal Science*, 2, 699262.

Rault, J.L., Van De Wouw, A. and Hemsworth, P. (2013) Fly the coop! Vertical structures influence the distribution and behaviour of laying hens in an outdoor range. *Australian Veterinary Journal*, 91, 423-426.

Richards, G., Wilkins, L., Knowles, T., Booth, F., Toscano, M., Nicol, C. and Brown, S. (2011) Continuous monitoring of pop hole usage by commercially housed free-range hens throughout the production cycle. *Veterinary Record*, 169.

Richards, G., Wilkins, L., Knowles, T., Booth, F., Toscano, M., Nicol, C., Brown, S., 2012. Pop hole use by hens with different keel fracture status monitored throughout the laying period. *Veterinary Record*, 170, 494.

Schreiber-Gregory, D.N. (2018) Ridge regression and multicollinearity: An in-depth review. *Model Assisted Statistics and Applications*, 13, 359-365.

Singh, M. and Cowieson, A. (2013) Range use and pasture consumption in free-range poultry production. *Animal Production Science*, 53, 1202-1208.

Stadig, L.M., Rodenburg, T.B., Ampe, B., Reubens, B. and Tuyttens, F.A.M. (2017a) Effect of free-range access, shelter type and weather conditions on free-range use and welfare of slow-growing broiler chickens. *Applied Animal Behaviour Science*, 192, 15-23.

Stadig, L., Rodenburg, T., Ampe, B., Reubens, B. and Tuyttens, F. (2017b) Effects of shelter type, early environmental enrichment and weather conditions on free-range behaviour of slow-growing broiler chickens. *Animal*, 11, 1046-1053.

Taylor, P.S., Hemsworth, P.H., Groves, P.J., Gebhardt-Henrich, S.G. and Rault, J.-L. (2017) Ranging behaviour of commercial free-range broiler chickens 1: Factors related to flock variability. *Animals*, 7, 54.

Thimijan, R.W. and Heins, R.D. (1983) Photometric, radiometric, and quantum light units of measure: A review of procedures for interconversion. *Hortscience*, 18, 818-822.

Van de Weerd, H., Keatinge, R. and Roderick, S. (2009) A review of key health-related welfare issues in organic poultry production. *World's Poultry Science Journal*, 65, 649-684.

Weihs P., Schmalwieser A.W. and Schauberger G. (2012) UV Effects on Living Organisms. In: Meyers R.A. (eds) Encyclopedia of Sustainability Science and Technology. Springer, New York, NY.

Widowski, T.M., Keeling, L.J. and Duncan, I.J. (1992) The preferences of hens for compact fluorescent over incandescent lighting. *Canadian Journal of Animal Science*, 72, 203-211.

Zeltner, E. and Hirt, H. (2003) Effect of artificial structuring on the use of laying hen runs in a free-range system. *British Poultry Science*, 44, 533-537.

Zeltner, E. and Hirt, H. (2008) Factors involved in the improvement of the use of hen runs. *Applied Animal Behaviour Science*, 114, 395-408.

5 Plain English Summary

<table>
<tr><th>Project Title:</th><th>Hen ranging behaviour in relation to light and ultraviolet intensity</th></tr>
<tr><td>Australian Eggs Limited Project No</td><td>31HS902CO</td></tr>
<tr><td>Researchers Involved</td><td>D.L.M. Campbell, and C. Lee</td></tr>
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<tr><td>Objectives</td><td><p>This project was conducted to understand climatic conditions that may affect how many hens use the range area across the day, with a specific focus on sunlight for free range hens within Australia. The project comprised three components to address three research objectives.</p>
Test hen behavioural preferences for different UV and light intensity levels in a controlled indoor environment.
Validate hen ranging on commercial farms in relation to UV and light.
Test hen range use in an experimental free range facility using different shade cloth filters.</td></tr>
<tr><td>Background</td><td>Free range laying hen systems are prevalent within Australia and consumers find them favourable due to the naturalness. However, weather in Australia is extreme and birds may prefer to stay indoors if the sun intensity and ultraviolet radiation is high. The effect of sunlight on ranging may be particularly strong during the summer months but may vary across different regions of Australia. Understanding of what climatic factors affect range use will provide objective data for producers, consumers and other stakeholders as well as inform on range design.</td></tr>
<tr><td>Research</td><td><p>Firstly, a controlled indoor experiment was conducted to determine the preferences of individual hens to lights of different wavelengths and intensities when given a choice between the treatment lights versus standard control lighting.</p><p>Secondly, observations were conducted on three case study free range farms located in Tasmania, Queensland, and Western Australia across the summer and autumn months. Hens on the range were counted over several months as located in either the direct sun or the shade, and these numbers were correlated with different sunlight wavelengths, temperature, and relative humidity as measured by weather stations directly on the farms.</p><p>Finally, artificial shelters that filtered different degrees of sunlight were placed on a commercial farm in Queensland, and hen use of the shelters was observed across a period of approximately 2 weeks across 2 different flocks. Use of the shelters was also correlated with sunlight wavelengths, temperature, and relative humidity measured directly on-farm.</p></td></tr>
</table>

Outcomes	The first controlled indoor study demonstrated that hens with minimal sunlight experience preferred lights that approximated daylight including high intensities of these lights. When a combination of UVA and B wavelengths was presented, preferences were reduced at the higher intensity suggesting that hens avoided the damaging radiation. Lower levels of UVAB resulted in more behavioural expression of foraging and comfort behaviours. The second study demonstrated that hens appear to be sensitive to the differing impacts of visual light (brightness), versus ultraviolet radiation (brightness and damaging), versus infrared (heat). Thus, it can be expected that as the intensity of these wavelengths changes across the seasons, so will the range use by hens. Hens will avoid times of peak sun intensity and thus may not range as much during the summer months, particularly in regions of extreme sunlight. Heat consistently played a role in ranging behaviour with hens generally avoiding high temperatures, but sometimes seeking out the sun, presumably for warmth. The final study showed range shelters should be constructed of the highest filtering density when using shade cloth, but that trees on the range may be preferred over artificial structures. Across the summer months with intense sunlight and heat, hens are likely to prefer to remain inside across the middle of the day regardless of the shelter on the range. Shelters were used in the evening with hens both underneath and on top of them indicating that they are beneficial range enhancements even if hens still prefer to remain inside the shed during intense heat and sunlight.
Implications	These results demonstrate that hens are sensitive to different wavelengths of sunlight and this will affect their use of the range, particularly across the summer period. In times of high sun intensity, the indoor shed is preferable but the range should have shelters available so hens can access shade as needed.
Key Words	ultraviolet; free range; commercial farm; sunlight; infrared; temperature; behaviour; ranging; PAR
Publications	Rana, M.S., Cohen-Barnhouse, A.M., Lee, C. and Campbell, D.L.M. (2021). Preference testing for UV light spectrum and intensity in laying hens. *Poultry Science*, 100, 6, 101063. https://doi.org/10.1016/j.psj.2021.101063 Rana, M.S., Cohen-Barnhouse, A.M., Lee, C. and Campbell, D.L.M (2020). Preference testing for ultraviolet light spectrum and intensity in laying hens. *Proceedings of the International Society for Applied Ethology*, Virtual Conference, August 6-7. Rana, M.S.; Lee, C.; Lea, J.M.; Campbell, D.L.M. Commercial Free-Range Laying Hens' Preferences for Shelters with Different Sunlight Filtering Percentages. *Animals* 2022, 12, 344. https://doi.org/10.3390/ani12030344