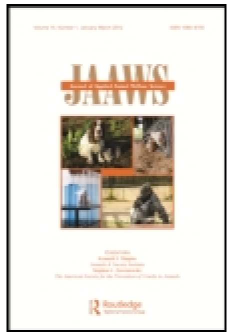
Full spectrum lighting induces behavioral changes and increases cortisol immunoreactivity in captive arachnids

Somerville, S., Baker, S., Baines, F., Trim, S. and Trim, C.M.



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ABSTRACT

The use of full spectrum illumination, including ultraviolet (UV), during captive husbandry of arachnids such as scorpions and theraphosids (tarantulas) is common practice in zoological institutions and amongst some hobbyists, as confirmed by a survey undertaken in this study. The effect of such lighting on captive arachnids has not been previously investigated. Comparison of key behavioral changes and haemolymph cortisol immunoreactivity was undertaken with and without full spectrum lighting. Two representative large arachnid species - king baboon spiders, *Pelinobius muticus* and Indian giant scorpions, *Heterometrus swammerdami* were selected for the study. Both organisms spent all their time hidden when exposed to full spectrum light compared to low-level ambient light except for one instance, in one spider, for one observation. There was no significant difference in burrowing and webbing in *P. muticus* when exposed to full spectrum lighting. There was a decrease in the number of behaviors or postures expressed in full spectrum lighting compared to ambient light for both species. Cortisol immunoactivity of both species were significantly elevated after exposure to full spectrum lighting compared to the same period of ambient light. This study provides the first evidence of detectable cortisol immunoactivity in arachnid haemolymph. These levels changed in response to full spectrum illumination and were linked to behaviorial changes. This suggests that a common husbandry practice may be detrimental to these animals.

Key words: UV light, behavior, cortisol, tarantulas, theraphosid, scorpions

INTRODUCTION

Arachnids have been maintained in zoological collections for over 100 years for entertainment, education and conservation. They are also increasingly popular with several research laboratories and specialist hobbyists. Although there is a growing knowledge about their husbandry requirements (Saul-Gershenz 2009; Bennie et al., 2011; Pellett et al., 2015), very little is known about stress in these animals or their response to full spectrum lighting that includes ultraviolet (UV) wavelengths. It is well known that reptiles and amphibians require ultraviolet-B (UV-B) light for optimal health, mainly to enable biosynthesis of Vitamin D_3 (Tapley et al., 2014); however, this seems to have resulted in the widespread use of daytime lighting containing UV-B in the vivaria of nocturnal arachnids. There is no evidence to suggest that these species benefit from, or indeed choose to expose themselves to daylight in the wild or to full spectrum lighting in captivity.

Unexpected occurrences, discomfort, or the perception of danger can result in metabolic changes in animals, such as the production of stress hormones, including cortisol (Mostl and Palme, 2002). In mammalian species studied so far, cortisol a major glucocorticoid, is stimulated by adrenocorticotropic hormone (ACTH), which, in turn, is stimulated by corticotropin-releasing hormone (CRH) in the hypothalamus (Addison, 2012). This response can be beneficial in the short term, as stress enables animals to respond more quickly, engage in courtship, hunt for food, or even simply move away from their current location; however, chronic stress is known to suppress the immune system and damage tissues (Mostl and Palme, 2002). Captive animals are unable to move to new surroundings; therefore, it is essential that potential stressors are understood and managed accordingly.

Signaling molecules involved in stress responses are present in many invertebrate taxa (Stefano et al., 2002). These include ACTH and cortisol-like molecules such as those that have been detected in the haemocytes of various molluscan species and research indicates that their stress-response is similar to that of vertebrates (Ottaviani, 2006). Crustacean hyperglycemic hormone (CHH) is thought to be the crustacean equivalent of cortisol and corticosterone Elwood et al 2009. Investigation of stress in insects has also demonstrated that behavioral changes to perceived predators, such as evasive actions are linked to biochemical changes in haemolymph composition including an increase in the neurohormone octopamine which is structurally related to epinephrine (Roeder, 1999; Adamo, 2011). This hormone has been found in other arthropods as well (Verlinden et al., 2010) and octopamine concentrations have been shown to be reduced in the brains of the theraphosid *Aphonopelma Hentzi* after agonistic encounters (Punzo & Punzo, 2001). Octopamine has also been found in the hemolymph of the scorpion *Leiurus quinquestriatus* (Ottaviani & Franceschi 1996). Invertebrate ~~heamocytes~~haemocytes are responsive to corticotrophin releasing hormone and although the full pathway hasn't been fully characterised, it has been shown to lead to release of biogenic amines (Malagoli, Franchini, & Ottaviani., 2000). Chernysh (2018, p88-89) has shown that an increase in cortisol like molecules in the haemolymph of *Calliphora vicina* was detected in response to stress. In spiny lobsters (*Panulirus homarus*) high levels of cortisol have been reported at a concentration of 1.59±0.28 nmol/l and low cortisol levels at 1.17±0.14 nmol/l (Lesmana, 2013). Cortisol immunoactivity ~~Stress~~ hormone signaling has never before been reported in arachnids although biochemical research undertaken on the haemolymph of the Chilean rose theraphosid (*Grammastola rosea*) did not detect cortisol using a biochemical analyser (Kennedy et al, 2019). The authors suggested using a more sensitive assay like an ELISA would be more appropriate. In the draft assembly of the only theraphosid genome sequenced so far, that of *Acanthoscurria geniculata* (Sanggaard et al, 2014) there is no mention of stress pathways in the manuscript. Regardless of this, the supplemental material does contain both DNA and protein sequences with similarity to other arthropod glucocorticoid genes. These are listed as L12438_T1/1_Tarantula_WB corticotropin-releasing factor receptor type, putative [*Ixodes scapularis*]; L21847_T1/1_Tarantula_WB PREDICTED: similar to corticotropin releasing hormone binding protein [*Tribolium castaneum*] and L15493_T1/1_Tarantula_S glucocorticoid induced transcript 1-like [*Nasonia vitripennis*] (Sanggaard et al, 2014, supplemental data 7 tarantula transcripts).

To investigate the effect of full spectrum lighting, at a natural level, in arachnids, two model species were chosen. These were king baboon spiders (*Pelinobius muticus*, Karsch 1885) and Indian giant scorpions (*Heterometrus swammerdami*, Simon 1872). Large mygalomorph spiders such as *P. muticus* have been wrongly referred to as tarantulas for decades, particularly in the pet trade. The authors support the zoological institutions in adopting theraphosid (derived from the family Theraphosidae) as the correct common name for this group of large mygalomorph spiders.

P. muticus are terrestrial members of the family Theraphosidae, native to East Africa. Temperatures in captivity range between 20ºC and 24°C, with a relative humidity (RH) of 60-70% (Bruins, 2001) As obligate burrowers, these theraphosids could potentially regulate the proportion of time exposed to sunlight, and hence UV, but no studies have been published on this so far. Their eight eyes all have dual spectral sensitivities, peaking at electromagnetic (EM) wavelengths of around 370 nm and 500 nm (Dhal & Granda, 1989). Human vision sensitivity peaks at 555 nm or 507 nm, depending on the quality of light available (Williamson and Cummins, 1983). Solar UV radiation covers the wavelength range between 290 - 400 nm, subdivided into UV-B (290 – 320nm) and ultraviolet-A UV-A (320 - 400nm). A peak sensitivity at 370nm would indicate that theraphosids are more sensitive to UV light than humans, and can perceive UV-A as well as the wavelengths visible to humans.

As with almost all spiders, theraphosids are predatory, using their strength and fast-acting venom to overcome and paralyse prey (Bennie et al., 2011). Unlike many spider families, theraphosid silk is not used for prey capture, but for building retreats, although any vibrations will alert the animal to danger or potential food. Previous research indicates that terrestrial theraphosids use silk to seal up their burrows from the external environment in times of increased vulnerability, such as cold weather or during a molt (Shillington and Verrell, 2010) as shown in Figure 1. Increased webbing could therefore be expected as a response to stress.

H. swammerdammi are members of the family Scorpionidae, indigenous to India and Sri Lanka. They are accustomed to temperatures between 24-27°C in captivity, with an RH of approximately 75% (Bruins, 2001). This particular genus is most comfortable with bark, peat and soil substrates and is known to burrow (Rubio, 2008). Scorpions have two central eyes and between two and five pairs of lateral eyes (3 pairs in *H.swammerdammi*). Previous research has shown that their central eye vision is not sensitive to UV; however their lateral eye vision shows a pronounced rise in sensitivity at 371 nm, indicating perception of UV-A, and a much lower peak in sensitivity at approximately 520 nm in the visible range (Machan, 1968). Interestingly, the same research demonstrated that if the lateral eyes are given time to adapt to any wavelength of visible light, there follows a marked reduction in sensitivity to UV light. Hu et al., 2014 have shown that non-salticid spider species with UV opaque corneas live in dim environments and species living in open areas had UV transmitting corneas but the study didn't include theraphosids or scorpions.

Fluorescent tubes, when operated on standard magnetic ballasts, flicker at 100Hz owing to the 50Hz alternating current supply. If the flicker fusion frequency (FFF) of the eyes of any species is greater than 100Hz, a strobe-like effect would be visible to the animal. Some insects and birds have high FFF; flicker from fluorescent tubes has been shown as stressful to starlings, increasing their cortisol levels (Maddocks et al. 2001). However, arachnids have very low FFF, in the range of 10-37 Hz (Clarke and Uetz 1990) so would not be expected to perceive flicker from the tubes used in this study. In some institutions and displays, UV "Black Lights" which emit UV-A are used such that the scorpions fluoresce a bright green; further research is also required to determine the level of stress caused by this, compared to full spectrum light. As scorpions are nocturnal animals, and can perceive UV-A, they may still experience stress under "Black Lights", and might avoid the UV-A if given the option.

Scorpions are nocturnal arthropods that fluoresce when exposed to UV light and this is evident, as a green glow, in full spectrum lighting containing UV (Figure 2 a-b). In the wild they fluoresce under moonlight – the fuller the moon, the brighter the glow. This fluorescence comes at a cost, however, as research has demonstrated that it deters flying insects to such an extent that significantly less prey is caught during a full moon than a new moon (Kloock, 2005). The ecological reason for this trait is unknown, which could be a coincidence of other beneficial factors or advantageous phenotype.

Here, in both *P. muticus* and in *H. swammerdami* we set about to identify if behavioral and hormonal changes occur in response to exposure to full spectrum light in these large arachnids. By investigating changes in cortisol immunoreactivity in arachnid haemolymph we aim to see if there is a hormonal effect in response to exposure to full spectrum light.

MATERIALS AND METHODS

Survey

A brief survey of professional arachnid suppliers and keepers was undertaken to gauge the current conditions supplied to their animals. The following three questions were asked: 1. Do you house tarantulas and/or scorpions with full spectrum lighting? 2. Whether yes or no, do you have a particular reason why? 3. If yes, then for how long each day is the full spectrum light switched on?

Animals

The microbiological status of the animals was not assessed prior to this study. All animals were acquired by Venomtech Ltd (Sandwich, Kent, UK), quarantined, and maintained in captivity for several years without direct lighting prior to this study. Although the species involved are not currently legally protected, they were maintained and treated under the ethos of the UK Animals Scientific Procedures Act 1986, (A(SP)A). Ethical approval for use of these animals in this study was obtained from Venomtech Ltd. prior to commencement.

Four female *H. swammerdammi*, mean weight 25.75 g (± 5.25) and four female *P. muticus*, mean weight 24.75 g (± 8.73), were housed individually in 5-litre polypropylene Really Useful Boxes® (RUBs) (Really Useful Products Ltd., Normanton, West Yorks, UK) with 2cm of vermiculite substrate as bedding. Hides were constructed from 15cm lengths of UPVC roundline guttering with a diameter of 11cm (Wickes, Broadstairs, Kent, UK), and placed length-wise in one corner of the box. Drinking water was provided *ad libitum* in 5 cm diameter plastic bowls (Penfolds Reptiles, Herne Bay, Kent), positioned in the corner diagonally opposite the hide. Each animal was offered a large, well fed locust (*Locusta migratoria*) once per month; any uneaten locusts were removed after three days. Temperature gradients were maintained at 22– 28 °C during light phase (09.00-21.00 hrs) and 26 °C during dark phase (21:00–09:00 hrs). Average room temperature was 24 ^{0}C (± 1.26). Average relative humidity was maintained at an average of 49.0 % (± 11.2).

Lighting

Full spectrum lighting was provided through two 1050mm 25-watt T8 fluorescent tubes (Arcadia Euro Range Desert 10%+ Lamp, Arcadia Products plc, Surrey, UK) with clip-on aluminium reflectors (Arcadia Reflector, Arcadia Products plc, Surrey, UK), operated on standard magnetic ballasts (Arcadia Fluorescent Lighting Controller 36-38 watts, Arcadia Products plc, Surrey, UK). The tubes were positioned directly over the middle of two rows of four enclosures, 9 cm above the lids (Fig. 3 a,b). Before this study was undertaken with the animals, the UV-B transmission properties of a new plastic polypropylene lid was assessed with hand-held broadband UV-B and UV Index (UVI) meters (Solarmeter Model 6.2 UVB and Model 6.5 UV Index meters, Solartech Inc., Harrison Township, Michigan, USA). The meter readings indicated transmission of 63% total UV-B (280 – 320nm) and 61% UVI (a measure of the shorter UV-B wavelengths used in vertebrate cutaneous vitamin D3 synthesis). To evaluate this further, spectral analysis of the UV and visible light from a sample of the same brand of fluorescent tube, as filtered by the polypropylene lid, was conducted using an Ocean Optics USB2000+ spectrometer (Ocean Optics Inc., Dunedin, Florida, USA). Figure 4 shows the lamp spectrum as recorded in the absence of the RUB and through the RUB when new. The plastic selectively filtered the shorter wavelengths, but allowed transmission of the entire UV and visible spectrum. Spectral analysis confirmed the meter readings, with a result of 61.5 % transmission of UV-B (296 - 320nm); 77.5% transmission of UV-A (320 - 400nm) and 87.5% transmission of visible light (400 - 750nm).

Since most plastics solarise under prolonged exposure to UV, the lid was then placed under intense UV-B (UVI 10.0) from an artificial source (Lucky Reptile Bright Sun Desert UV metal halide lamp, Lucky Reptile, Waldkirch, Germany) for 100 hours. Spectral analysis after this exposure revealed good stability under UV as very little reduction in transmission (11.2% of UV-B and 3.7% of UV-A) had occurred. Polypropylene (RUB) lids (Really Useful Products Ltd) were therefore considered suitable for use throughout the proposed study, during which time they would be subjected to much lower levels of UV radiation.

By adjusting the distance at which the fluorescent tube was hung over the lids, the lighting was set up to produce a UV index (UVI) of 1.6 - 2.0 within the RUB, outside of the animal's hide.

This is within the range which might be expected from natural sunlight before 08.30h in the tropics or in a moderate level of shade later on in the day. The fluorescent tubes also significantly increased the total visible light and UV-A in the enclosures and had a small heating effect.

The study was conducted for 58 days, sub-divided into two 29-day phases. In the first phase of the study, low level ambient light with no UV-B was provided through a small window adjacent to the study location. In the second phase, full spectrum lighting was provided every day for 12 hours, from 09.00h to 21.00h.

Behavioral observations

During each 29 day period instantaneous sampling was undertaken at random times between the hours of 09.00 - 16.00 recording each specimen's location within the enclosure (inside or outside of the hide), its body posture and the presence of web. For each condition 16 observations were undertaken in low level ambient light with no UV-B (the first 29 days) and 16 observations were undertaken in the full spectrum lighting phase of the experiment (the second 29 days). Any amount of web construction across the entrance to a hide was considered a positive observation for web presence. For the body posturing published guidelines were used for spiders in Figure 5 (Bennie *et al*., 2011) and scorpions in Figure 6 (Alexander & Ewer, 1958) but in the scorpions a couple of other postures were displayed in initial behavioral observations which were then added to Figure 6 (postures e and f). Paired two tailed T-tests were undertaken in Excel to detect if there are any significant differences ($P<0.05$) between the two lighting conditions and postures observed.

Haemolymph Extraction

Haemolymph was extracted 24-hours after transport between behavioral laboratory and collection laboratory. Previous research indicates that this timeframe is sufficient to allow raised cortisol levels stemming from acute stress to fully subside, whilst levels caused by chronic stress will remain elevated (Rotllant and Tort, 1997). For the same reason, behavioral observations were not recorded until at least 24 hours after arrival in the behavior laboratory. After acclimatisation, arachnids were anaesthetised with a rising concentration of carbon dioxide (CO_2) gas. The method used was a protocol optimised at Venomtech Ltd as per Baker *et al*., 2018 which has a slower rate of increase of CO_2 as this gives a smoother induction phase as reported by Dombrowski *et al* 2013. The *P. muticus* were placed in 3 litre RUBs, with a CO_2 flow-rate of 0.5 litres/minute, and the *H. swammerdammi* in 1 litre RUBs with a flow-rate of 0.1 litres/min. Average time to anaesthesia was 76 minutes (SD ±13.07) for *P. muticus*, and 80 minutes (SD ±20.00) for *H. swammerdammi*. Limb collection of haemolymph was piloted in this study based on the ability to dose spiders through these membranes as presented in Pellet *et al*., 2015. 50 µl of haemolymph was collected using a 25G sterile needle (Fisher Scientific, Loughborough, UK) and a p200 pipette (Gilson) for limb collection or 1 ml syringe for cardiac collection. Where possible, haemolymph was taken from the ventral membrane between the femur and patella in *H. swammerdammi* (Figure 7 a,b), and between the tibia and metatarsus in *P. muticus* (Figure 8a). If insufficient fluid was extracted, then cardiac puncture was used (only 2 occurrences with *P. muticus*) (Figure 8b). For safety, a 15 ml centrifuge tube (Fisher Scientific) was placed over the metasomas (commonly known as the tail) of the scorpions, to prevent any risk of envenomation due to early recovery. Haemolymph was collected in 1.5 ml centrifuge tubes (Fisher Scientific), containing 50 µl of molecular biology grade water (HyClone, GE Healthcare, Little Chalfont, UK) (to prevent coagulation), then frozen at -20 °C. To control for circadian fluctuations all samples were collected between 9am and 12pm.

Cortisol Assay

Cortisol levels were measured using a Cortisol Enzyme Immunoassay kit: K003-H1 (Arbor Assays Inc., MI, USA,). As the kit had not been validated for detecting cortisol in haemolymph, a number of dilutions of the haemolymph were tested with and without the dissociation reagent. The optimal results were obtained as follows: 5 µl sample (2.5 µl of haemolymph with 2.5 µl of water) was added to 5 µl dissociation reagent (DR) and 40 µl assay buffer and incubated at room temperature for 5 mins. This 50 µl of prepared sample was then used to continue the assay protocol according to the manufacturer's instructions, including a standard curve. This was undertaken in triplicate for each sample. Absorbance was measured at 450 nm using the FLUOstar Omega plate reader (BMG Labtech, Aylesbury, Bucks, UK). The cortisol concentrations were calculated from the standard curve. The data was analysed in Eexcel with a statistical significance assumed for $P<0.05$ using paired two tailed T-tests. Results are expressed as means ± SD ($N=4$).

RESULTS

Survey

The survey of UK arachnid suppliers and zoo keepers indicated that, whilst full spectrum lighting is not regarded as essential for these species, it is not generally believed to cause stress. Of the twelve establishments contacted during this study, six were housing their theraphosids or scorpions under full spectrum light - either for aesthetic display purposes, or simply because lighting was already in place over the vivaria when obtained. Of the six that were not using full spectrum light, only one keeper stated that he believed it would cause stress - the remainder advised that it just wasn't necessary, or they did not have full spectrum light available. This survey confirmed that there is a lack of knowledge regarding possible costs or benefits of providing full spectrum lighting.

Behavior

H. swammerdammi were observed outside of their hides on 32 out of 64 observations (50%) under ambient light, but never observed outside (100% of observations) whilst under full spectrum light displaying a difference in behavior between the two conditions see Table 1. *P. muticus* were rarely viewed outside of their hide; on only 6 out of 64 observations (9.4%) under ambient lighting and 1 out of 64 (1.6%) under full spectrum light, thus there was no difference between the conditions see Table 2. The silk webs produced by *P. muticus* were observed on more occasions in full spectrum light. In total 43 silk webs were observed across the entrance to the theraphosid hides compared to ~~only~~ 38 in ambient light conditions so the differences were not significant see Table 3. Even though the difference in the amount of webbing was show to be not significant one individual did produced webs on 7 occasions (in ambient light) compared to 15 occasions (in full spectrum light). One individual didn't produce any webs at all during the study.

Both species exhibited a variety of postures, although there is some research in spiders (Bennie et al, 2011) and in scorpions (Alexander and Ewer, 1958) there is still very little research on understanding these. The scorpions in particular ranged from lying completely relaxed with their metasomas flat behind them, to crawling up their hides with claws and metasomas raised high. Posture was monitored as part of the behavioral study. It was observed that a wide range of behaviors were observed in ambient light compared to full spectrum lighting. In the theraphosids four different postures were observed in ambient light (Figure 5 postures a,b,c and d,) but only two different postures were observed in full spectrum lighting (Figure 5 postures a and b). Five different postures were observed for the scorpions in ambient light (Figure 6 postures a,b,c,e and f,) compared to only three in full spectrum light (Figure 6 postures a,b and e). Plotting the average number of observations for each behavior in a bar graph shows that for the theraphosids there are a wider range of ~~more~~ behaviors expressed under ambient light (Figure 9a). Looking at the behaviors in scorpions there are differences between behaviors expressed and there are also behaviors expressed under ambient light that are not expressed under full spectrum lighting

(Figure 9b). When a T-test was undertaken to see if the differences in behaviors exhibited in ambient temperature compared to full spectrum lighting were significant, they were found not to be in the spiders. In the scorpions posture f where the scorpion is vertical against the side of the box was found to be significant (P value 0.0498) but the other postures were shown not to be significant.

Cortisol Immunoreactivity Levels

Mean cortisol immunoreactivity levels after full spectrum light was 886 pg/ml (SD ±233) or 2.44nmol/l for theraphosids and 668.935 pg/ml (SD ±115) or 1.85nmol/l for scorpions and before full spectrum light was 314.184 pg/ml (SD ±58) or 0.866nmol/l for theraphosids and 387.785 pg/ml (SD ±25) or 1.070nmol/l for scorpions (Figure 10). A significant increase in cortisol immunoreactivity was detected in the haemolymph of both species after full spectrum light exposure (Scorpion 1.7 fold increase (P value 0.039), theraphosid 2.8 fold increase (P value 0.0215) comparing full spectrum lighting to ambient light (Figure 10). Fold changes for individual scorpions ranged from 1.57-2.42 and for the theraphosids (1.72-4.09). Smaller differences in cortisol immunoreactivity levels were observed in both groups between baseline test and first test in controlled conditions and may be a result of movement between labs.

DISCUSSION

This is the first study of the effects of full spectrum light in arachnids, and the first description of cortisol immunoreactivity in arachnid haemolymph.

This study shows that the cortisol immunoreactivity levels detected were raised under full spectrum lighting compared to ambient light conditions. Our findingsstudy haves also shown that the high and low levels of cortisol detected in archnids in our study are comparable (particularly in the case of the scorpions) detectable cortisol levels similar to levels thatdetected in spiny lobsters (Lesmana, 2013). (see introduction).

Although it is difficult to find cases in invertebrates, there have been several examples of ultraviolet radiation causing stress in fish. Manek et al., 2014 detected higher levels of cortisol in the blood of fathead minnows *Pimephales promelas* subjected to UV radiation than non-exposed controls. A significant increase in cortisol levels were detected in the serum of the

African catfish *Clarias gariepinus* exposed to UVA compared to a control group (Ibrahim, 2015). This has not been shown before in invertebrates to the authors knowledge.

Is this an indicator that the animals are under stress? Are there shelters they are provided with in the laboratory not as good as burrows they would have produced in the wild? Do spiders and scorpions experience this level of stress in the wild under full spectrum lighting? The theraphosids utilized in this study are obligate burrowers and the scorpions were nocturnal so in the wild so if they didn't venture out during the day they would not usually be exposed to full spectrum lighting. This is why full spectrum lighting in captive invertebrates needs to be considered carefully.

There were no significant differences in burrowing and webbing in *P. muticus* when exposed to full spectrum lighting which is surprising as they are burrowing species. If the shelters were not suitable why didn't the animals burrow more under the full spectrum light conditions? For both species studied there was a decrease in the number of behaviors or postures expressed in full spectrum lighting compared to ambient light. Comparing the difference between each behavior under each condition showed that the differences displayed were not significant except with posture f in the scorpions. Further work is needed to understand these postures and what they mean as the work undertaken by Bennie et al 2011 was with arboreal theraphosids compared to our work with terrestrial theraphosids.

This study could have been undertaken with spiders that sit out more often during the day but these spiders tend to be the ones that contain urticating's hairs which can lead to a safety issue for a study like this if these animal kick their hairs. This would be a useful study in the future as these animals are kept in zoo's and owned by private keepers. Future studies would include obtaining more data to get normal and stressed ranges in theraphosids and scorpions of cortisol like molecules

How does cortisol immunoreactivity relate to amines? Malagoli, Franchini, & Ottaviani., 2000 have stated that Invertebrate haemocytes are responsive to corticotrophin releasing hormone and although the full pathway hasn't been fully characterised, it has been shown to lead to release of biogenic amines.

Future work could investigate the levels of heat shock proteins and levels of octapamine in this type of experiment. HPLC and mass spectrometry could be utilised to further investigate this cortisol like molecule

Under full spectrum lighting one animal (a *P. muticus*) was recorded outside of its hide. Since members of this species rarely left the hides even during the ambient light condition, this was unexpected. This specimen moved its hide horizontally across the middle of the enclosure, directly beneath the UV tube, and blocked up both ends of the hide with heavy web. When the hide was lifted to check the animal, it became very defensive, raising its front legs, with fangs exposed. After intrusions into their hides, the theraphosids usually settled down fairly quickly, but the following day this individual was observed out of its hide, hunched up in a corner exposed to the full light. When the hide was returned to its original position in the corner of the enclosure the animal retreated into it. This animal also appeared dehydrated when presented for haemolymph collection and had the highest increase in cortisol level. More research is needed on the range of cortisol concentrations experienced by these animals. This animal also completed ecdysis three weeks after the study. Ecdysis is a particularly stressful process for theraphosids (Shillington and Verrell, 2010), which draws their energy resources in the preceding weeks as the new exoskeleton is formed beneath the existing one (Herzig, 2010). ~~Our study has shown detectable cortisol levels similar to that in spiny lobsters (see introduction).~~

Overall, the results of this study indicate that provision of full spectrum light does cause a physiological change which could be interpreted as stress in *H. swammerdami* and *P. muticus* compared to low level ambient lighting. This has been demonstrated by an increase in haemolymph cortisol immunoreactivity levels, and that *H. swammerdami* actively avoids exposure to this light. However, the full spectrum lighting used in this study provided a significantly increased intensity, within the enclosure, of visible light, UV-A and UV-B, and minor additional heat. Therefore, more studies are needed to determine whether any specific component of this lighting regime was particularly responsible for creating the response observed.

This pioneering study raises many questions about the physiology of arachnids and therefore this study is expected to have a significant impact on arachnid husbandry in zoological institutions and the growing field of invertebrate veterinary medicine.

CONCLUSION

Full spectrum lighting equivalent to dappled sunlight induces behavioral changes in *P. muticus* and *H. swammerdammi*. Full spectrum lighting equivalent to dapped sunlight elevates detectable cortisol immunoreactivity in *P. muticus* and *H. swammerdami* haemolymph. Further work is required to determine the effect of these changes on the health of these animals in long term captivity.

LIST OF SYMBOLS AND ABBREVIATIONS

ACTH	adrenocorticotropic hormone
CO_2	Carbon Dioxide
CRH	corticotrophin releasing hormone
DR	Dissociation reagent
ELISA	Enzyme-Linked Immunosorbent Assay
EM	electromagnetic
FFF	flicker fusion frequency
UV	ultraviolet
UV-A	ultraviolet-A
UV-B	ultraviolet-B
UVI	ultraviolet index

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COMPETING INTERESTS

The authors have no disclosures or conflicts of interest to report.

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FIGURE LEGENDS

Fig. 1. Terrestrial theraphosid hide with silk web covering the entrance. The substrate seen here may not necessarily be used as a building material, as these mounds tend to be a natural consequence of the animal burrowing inside the hide.

Fig. 2. Differening exoskeleton appearence in *H. swammerdammi*. (A) *H. swammerdammi* in diffuse ambient light. (B) *H. swammerdammi* fluorescing under full spectrum light.

Fig. 3. (A) Overhead view, showing lighting positioning over the middle of a row of Really Useful boxes, housing *P. muticus* . **(B) Horizontal view**, showing UV-B tube positioned 9 cm above the lids of Really Useful boxes, housing Indian giant scorpions.

Fig 4. Spectral irradiance (UV and visible light) from Arcadia Euro Range 10%+ UVB fluorescent tube with and without filtering through lid of Really Useful Box as used in this study. Light source: distance = 10cm.

Fig 5. Theraphosid postures as developed by Bennie et al., 2011. (A & B) Resting (C) Alert (D) Walking: prosoma and opisthosoma slightly elevated (E) Feeding: prosoma and opisthosoma raised high off the ground, prey tightly held between chelicerae and fangs (F) Threat display frontal view: note exposed fangs, raised pedipalps and raised legs I and II (G) Threat display side view: note the steep angle at which both the prosoma and the opisthosoma are raised, chelicerae pushed forward to expose fangs.

Fig 6. Scorpion postures redrawn from Alexander and Ewer, 1958 with the addition of further postures observed in initial observations in the study. (A) Resting stance (B) Stilted pose, whole body is lifted (C) Stilted pose mesosoma is sharply elevated (D) Rather rare stilted pose (E) completely relaxed, claws flat on substrate, tail limp (F) Vertical against side of box.

Fig. 7. (A) Anaesthetised *H. swammerdammi*, ventral view, indicating joint between femur and patella. (B) Extracting haemolymph, with safety tube over the metasoma.

Fig. 8. (A) Anaesthetised *P. muticus* ventral view, indicating joint between tibia and metatarsus. (B) Extracting haemolymph via cardiac bleed.

Fig. 9 Postures exhibited by the theraphosid *P. muticus* (A~~-B~~) and scorpion *H. swammerdammi* (B~~C-D~~) during the study in response to change in lighting conditions. Comparison of ambient lighting with full spectrum lighting ~~. A and C~~ showing ~~the total number of observations and B and D show the~~ average number of observations with standard error (N=4 for each species).

Fig. 10. Cortisol concentrations in the haemolymph of *H. swammerdammi* and *P. muticus* at baseline and then before and after exposure to full spectrum light. Results presented as individuals **(A)** Scorpions (N=4) and **(B)** Theraphosids (N=4) and (C) together as means +/- sd. (*N=4* for each species).

TABLES

H. swammerdammi	**Ambient light**		**Full spectrum lighting**	
	In hide	**Out of hide**	**In hide**	**Out of hide**
1	14	2	16	0
2	5	11	16	0
3	6	10	16	0
4	7	9	16	0
Total	**32**	**32**	**64**	**0**

Table 1 – Observations investigating if individual scorpions (*H. swammerdammi*) are located in the hide compared to out of the hide, comparing ambient light with full spectrum lighting (n=4). 16 observations were undertaken for each individual under each condition.

P. muticus	**Ambient light**		**Full spectrum lighting**	
	In hide	**Out of hide**	**In hide**	**Out of hide**
1	16	0	16	0
2	13	3	16	0
3	14	2	15	1
4	15	1	16	0
Total	**58**	**6**	**63**	**1**

Table 2 – Observations investigating if individual theraphosids (*P. muticus*) are located in the hide compared to out of the hide, comparing ambient light with full spectrum lighting (n=4). 16 observations were undertaken for each individual under each condition.

Theraphosid number	**Ambient light**	**Full spectrum lighting**
1	7	15
2	0	0
3	15	15
4	16	13
Total	**38**	**43**

Table 3 – Webbing observed during the 16 observations for each condition for each individual theraphosid *P. muticus* (n=4) under each lighting condition.

Word count: ~~5639~~6677

Fig. 1. Terrestrial theraphosid hide with silk web covering the entrance. The substrate seen here may not necessarily be used as a building material, as these mounds tend to be a natural consequence of the animal burrowing inside the hide.

1828x1219mm (72 x 72 DPI)

Fig. 2. Differening exoskeleton appearence in H. swammerdammi. (A) H. swammerdammi in diffuse ambient light. (B) H. swammerdammi fluorescing under full spectrum light.

878x655mm (72 x 72 DPI)

Fig. 2. Differening exoskeleton appearence in H. swammerdammi. (A) H. swammerdammi in diffuse ambient light. (B) H. swammerdammi fluorescing under full spectrum light.

1828x1219mm (72 x 72 DPI)

Fig. 3. (A) Overhead view, showing lighting positioning over the middle of a row of Really Useful boxes, housing P. muticus . (B) Horizontal view, showing UV-B tube positioned 9 cm above the lids of Really Useful boxes, housing Indian giant scorpions.

604x804mm (72 x 72 DPI)

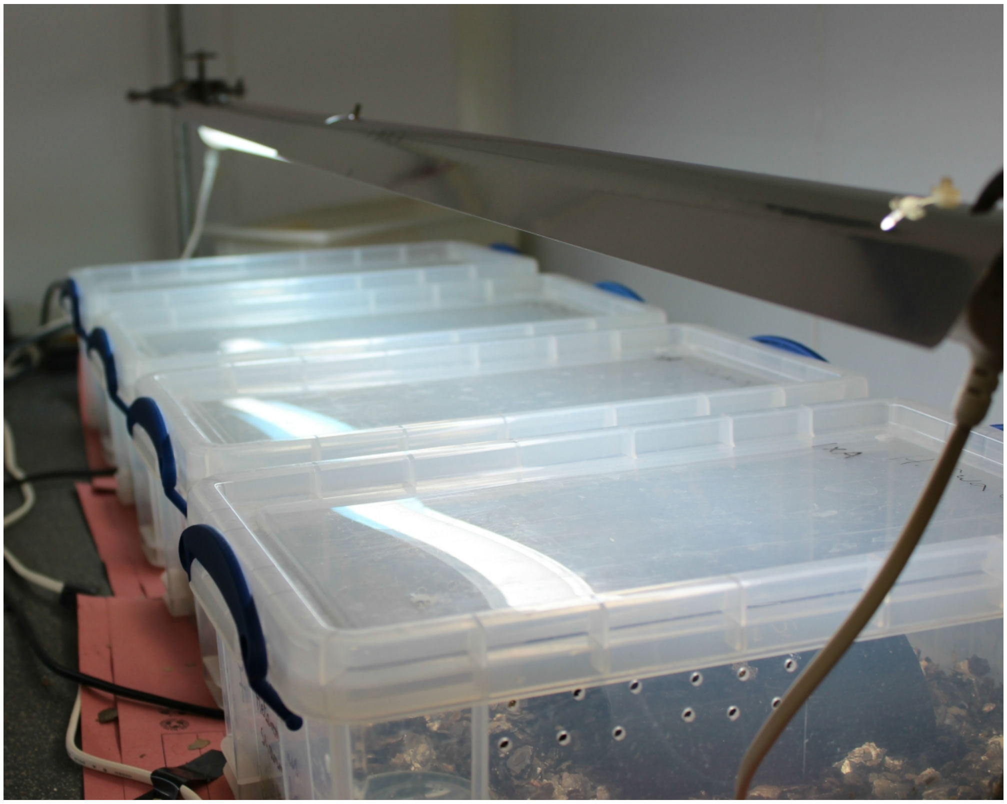


Fig. 3. (A) Overhead view, showing lighting positioning over the middle of a row of Really Useful boxes, housing P. muticus . (B) Horizontal view, showing UV-B tube positioned 9 cm above the lids of Really Useful boxes, housing Indian giant scorpions.

971x773mm (72 x 72 DPI)

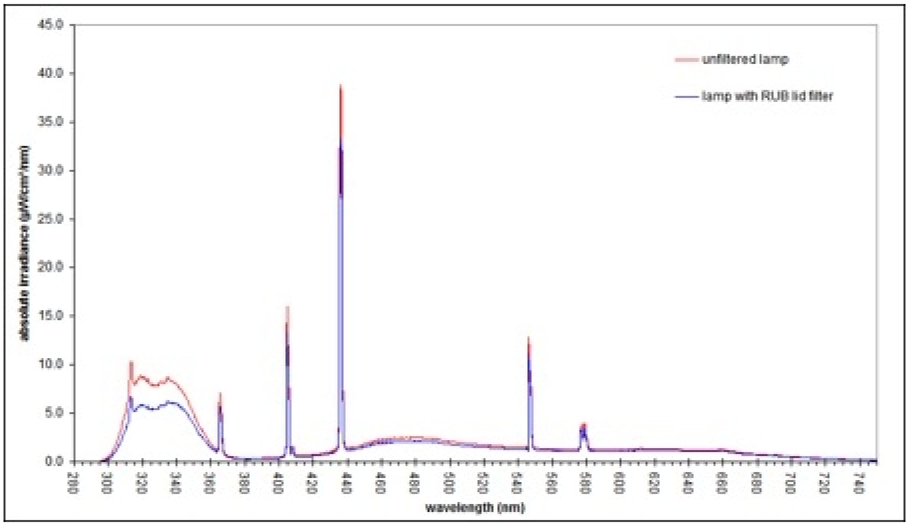
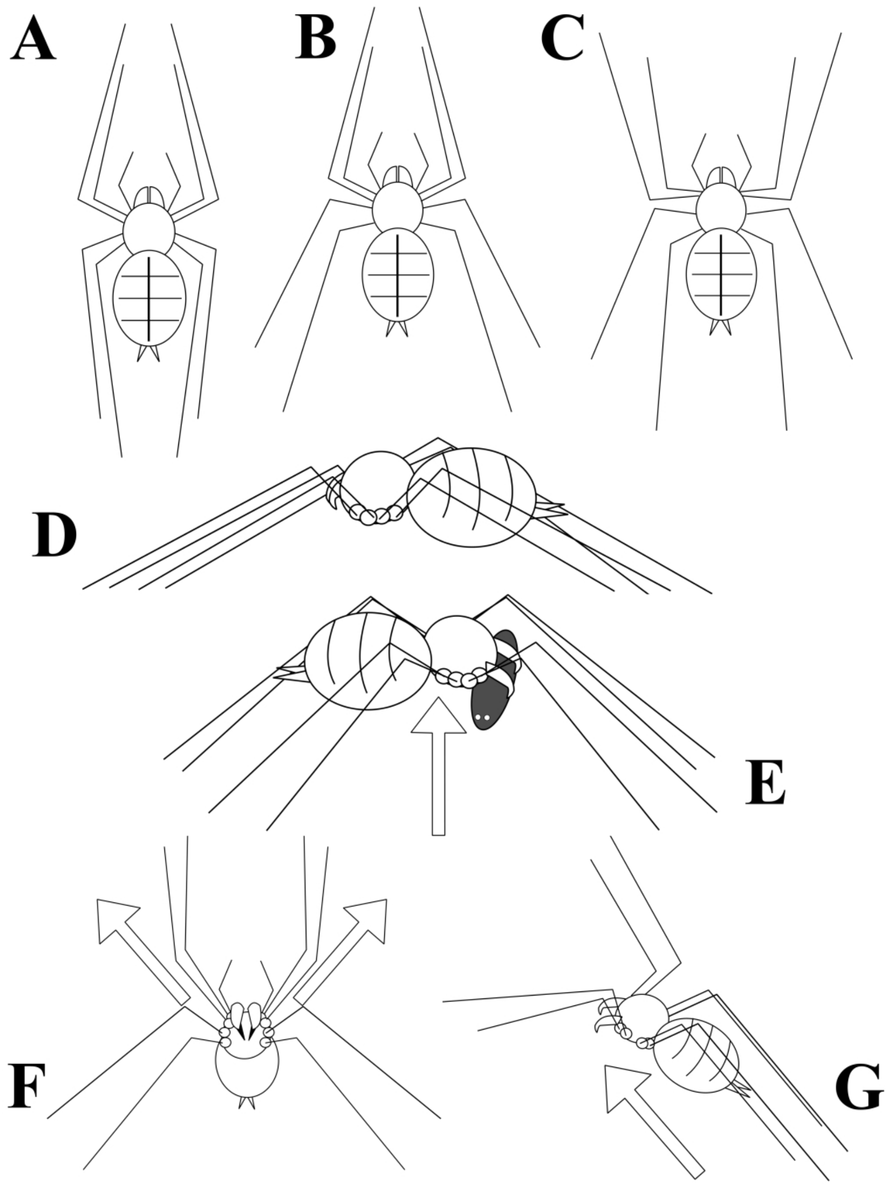


Fig 4. Spectral irradiance (UV and visible light) from Arcadia Euro Range 10%+ UVB fluorescent tube with and without filtering through lid of Really Useful Box as used in this study. Light source: distance = 10cm.

159x91mm (72 x 72 DPI)



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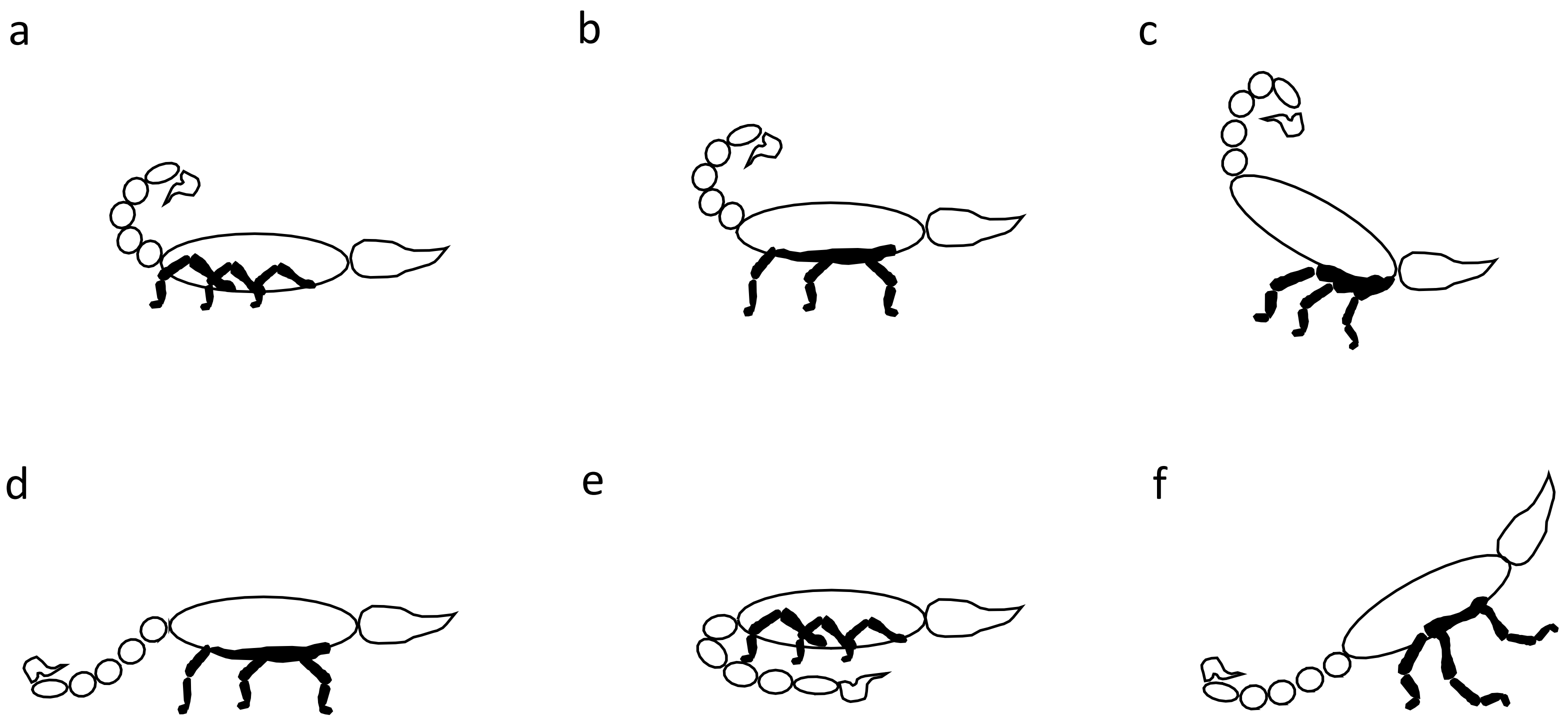
305x394mm (300 x 300 DPI)


a
b
c
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e
f

Fig. 7. (A) Anaesthetised H. swammerdammi, ventral view, indicating joint between femur and patella. (B) Extracting haemolymph, with safety tube over the metasoma.

965x553mm (72 x 72 DPI)

Fig. 7. (A) Anaesthetised H. swammerdammi, ventral view, indicating joint between femur and patella. (B) Extracting haemolymph, with safety tube over the metasoma.

861x540mm (72 x 72 DPI)

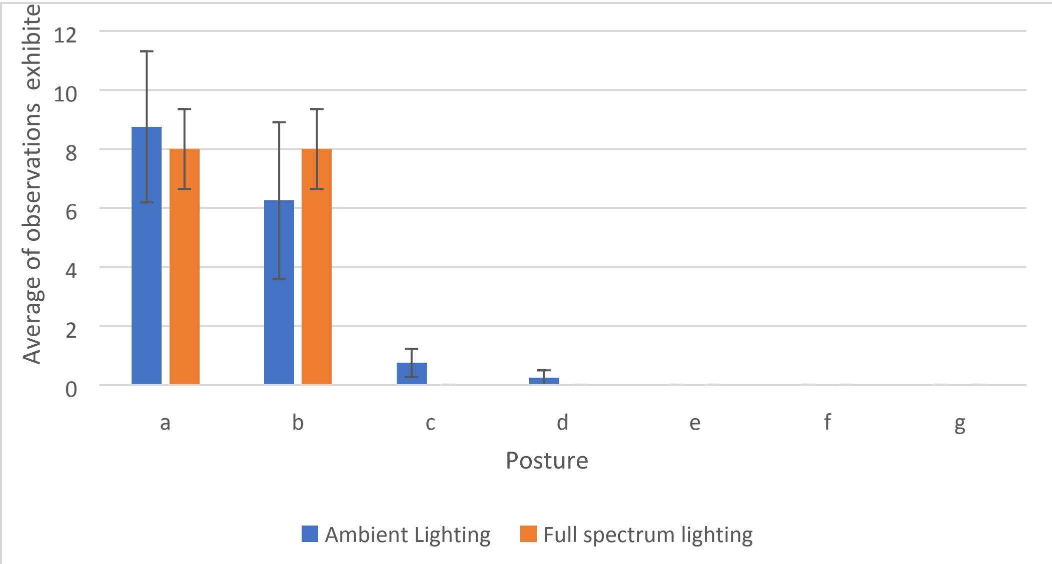
Fig. 8. (A) Anaesthetised P. muticus ventral view, indicating joint between tibia and metatarsus. (B) Extracting haemolymph via cardiac bleed.

808x682mm (72 x 72 DPI)

Fig. 8. (A) Anaesthetised P. muticus ventral view, indicating joint between tibia and metatarsus. (B) Extracting haemolymph via cardiac bleed.

875x572mm (72 x 72 DPI)

a


Average of observations exhibite
12
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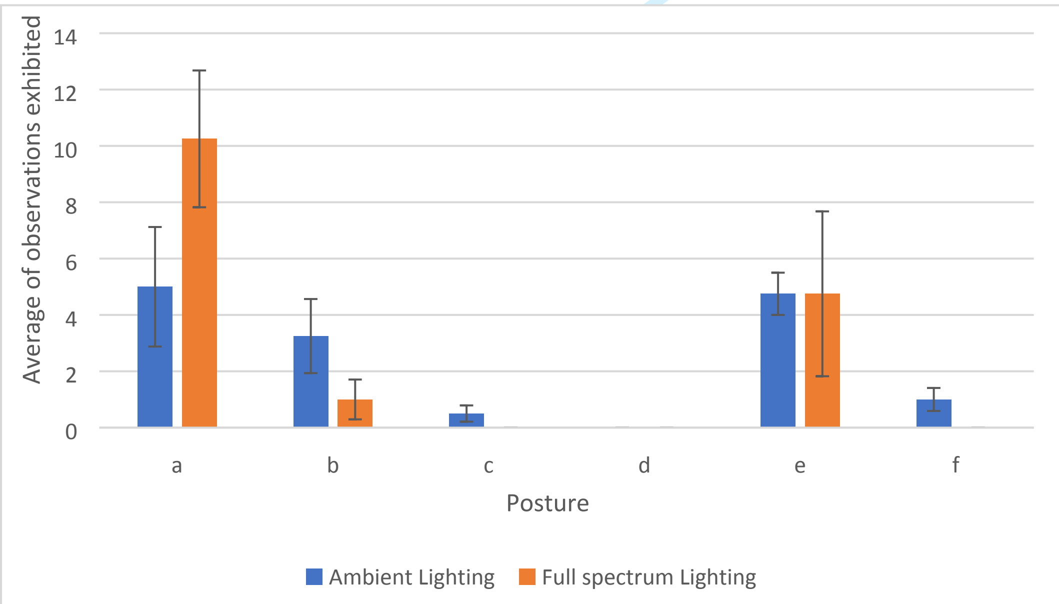
Posture
Ambient Lighting
Full spectrum lighting

b


Average of observations exhibited
14
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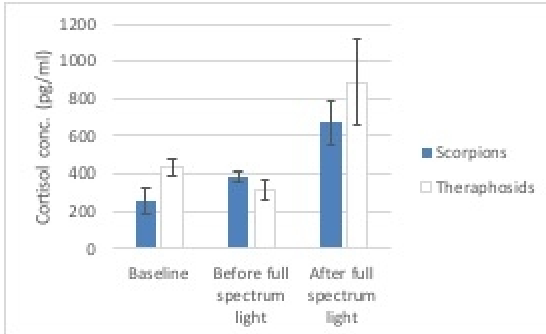
Posture
Ambient Lighting
Full spectrum Lighting



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97x59mm (72 x 72 DPI)