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Full Spectrum Lighting Induces Behavioral Changes and Increases Cortisol Immunoreactivity in Captive Arachnids

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ABSTRACT

The use of full spectrum illumination, including ultraviolet (UV), during captive husbandry of arachnids is common practice. The effect of this on captive arachnids has not been previously investigated. Comparison of key behavioral changes and hemolymph cortisol immunoreactivity was undertaken with and without full spectrum lighting. 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. 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. This study provides the first evidence of detectable cortisol immunoactivity in arachnid hemolymph. These levels changed in response to full spectrum illumination and were linked to behavioral changes. This suggests that a common husbandry practice may be detrimental to arachnids.

KEYWORDS

UV light; behavior; cortisol; tarantulas; 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 (Bennie, Loaring, & Trim, 2011; Pellett, Bushell, & Trim, 2015; Saul-Gershenz, 2009), 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., 2015); 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 & 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 (Smith & Vale, 2006). This response can be beneficial in the short term, as stress

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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 & 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 hemocytes of various molluskan 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, Barr, and Patterson, 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 hemolymph composition including an increase in the neurohormone octopamine which is structurally related to epinephrine (Adamo & Baker, 2011; Roeder, 1999). 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 hemocytes are responsive to corticotrophin releasing hormone and although the full pathway has not been fully characterized, it has been shown to lead to release of biogenic amines (Malagoli, Franchini, & Ottaviani, 2000). Chernysh (Chernysh, 2018, p. 88–89) has shown that an increase in cortisol-like molecules in the hemolymph 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 hormone signaling has never before been reported in arachnids although biochemical research undertaken on the hemolymph of the Chilean rose theraphosid (*Grammastola rosea*) did not detect cortisol using a biochemical analyzer (Kennedy, Warner, & Trim, 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 20°C 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 & Cummins, 1983). Solar UV radiation covers the wavelength range between 290 and 400 nm, subdivided into UV-B (290–320 nm) and ultraviolet-A UV-A (320–400 nm). A peak sensitivity at 370 nm 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 paralyze 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 & 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°C and 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 (three 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 did not include theraphosids or scorpions.

Fluorescent tubes, when operated on standard magnetic ballasts, flicker at 100 Hz owing to the 50 Hz alternating current supply. If the flicker fusion frequency (FFF) of the eyes of any species is greater than 100 Hz, a strobe-like effect would be visible to the animal. Some insects and birds have

Figure 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.

high FFF; flicker from fluorescent tubes has been shown as stressful to starlings, increasing their cortisol levels (Maddocks, Goldsmith, & Cuthill, 2001). However, arachnids have very low FFF, in the range of 10–37 Hz (Clark & 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.

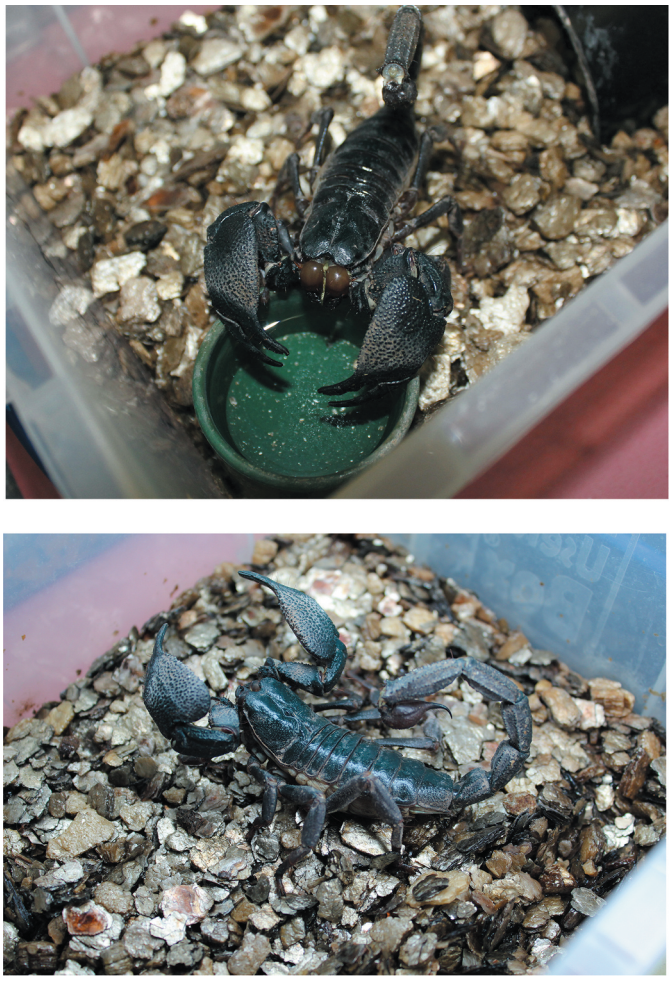


Figure 2. Different exoskeleton appearance in *H. swammerdammi*. (a) *H. swammerdammi* in diffuse ambient light. (b) *H. swammerdammi* fluorescing under full spectrum light.

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 hemolymph 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-l polypropylene Really Useful Boxes® (RUBs) (Really Useful Products Ltd., Normanton, West Yorks, UK) with 2 cm of vermiculite substrate as bedding. Hides were constructed from 15 cm lengths of UPVC roundline guttering with a diameter of 11 cm (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°C (± 1.26). Average relative humidity was maintained at an average of 49.0% (± 11.2).

Lighting

Full spectrum lighting was provided through two 1050 mm 25-watt T8 fluorescent tubes (Arcadia Euro Range Desert 10%+ Lamp, Arcadia Products plc, Surrey, UK) with clip-on aluminum 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 (Figure 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–-320 nm) 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

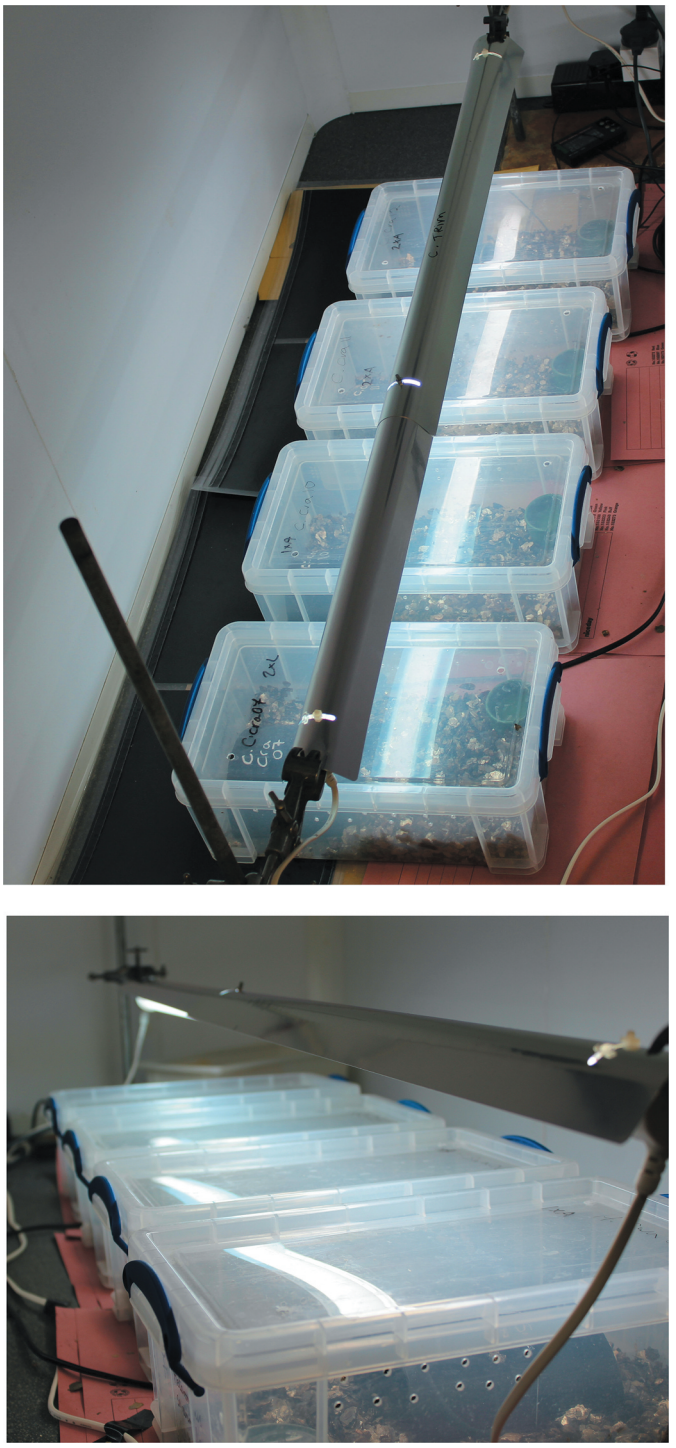


Figure 3. (a) Overhead view, showing lighting positioning over the middle of a row of Really Useful boxes, housing *P. muticus*. (b) Horizontal view, showing UV-B tube positioned 9 cm above the lids of Really Useful boxes, housing Indian giant scorpions.

and visible spectrum. Spectral analysis confirmed the meter readings, with a result of 61.5%

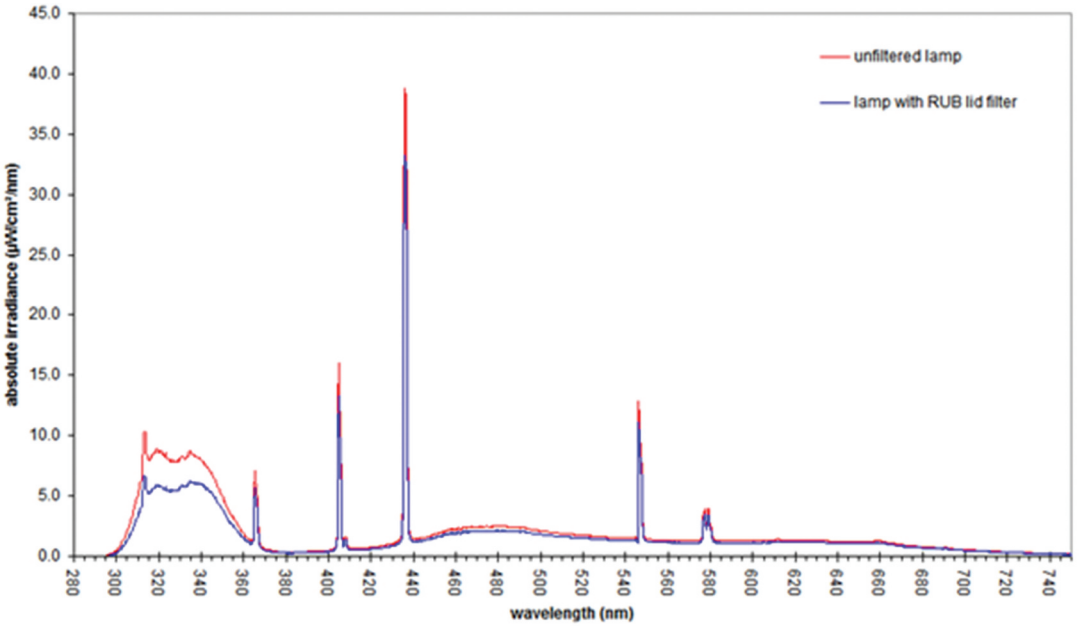


Figure 4. Spectral irradiance (UV and visible light) from Arcadia Euro Range 10%+ UVB fluorescent tube with and without filtering through lid of Really Useful Box as used in this study. Light source: distance = 10 cm.

transmission of UV-B (296–320 nm); 77.5% transmission of UV-A (320–400 nm) and 87.5% transmission of visible light (400–750 nm).

Since most plastics solarize 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 08.30 h 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 09.00 h to 21.00 h.

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

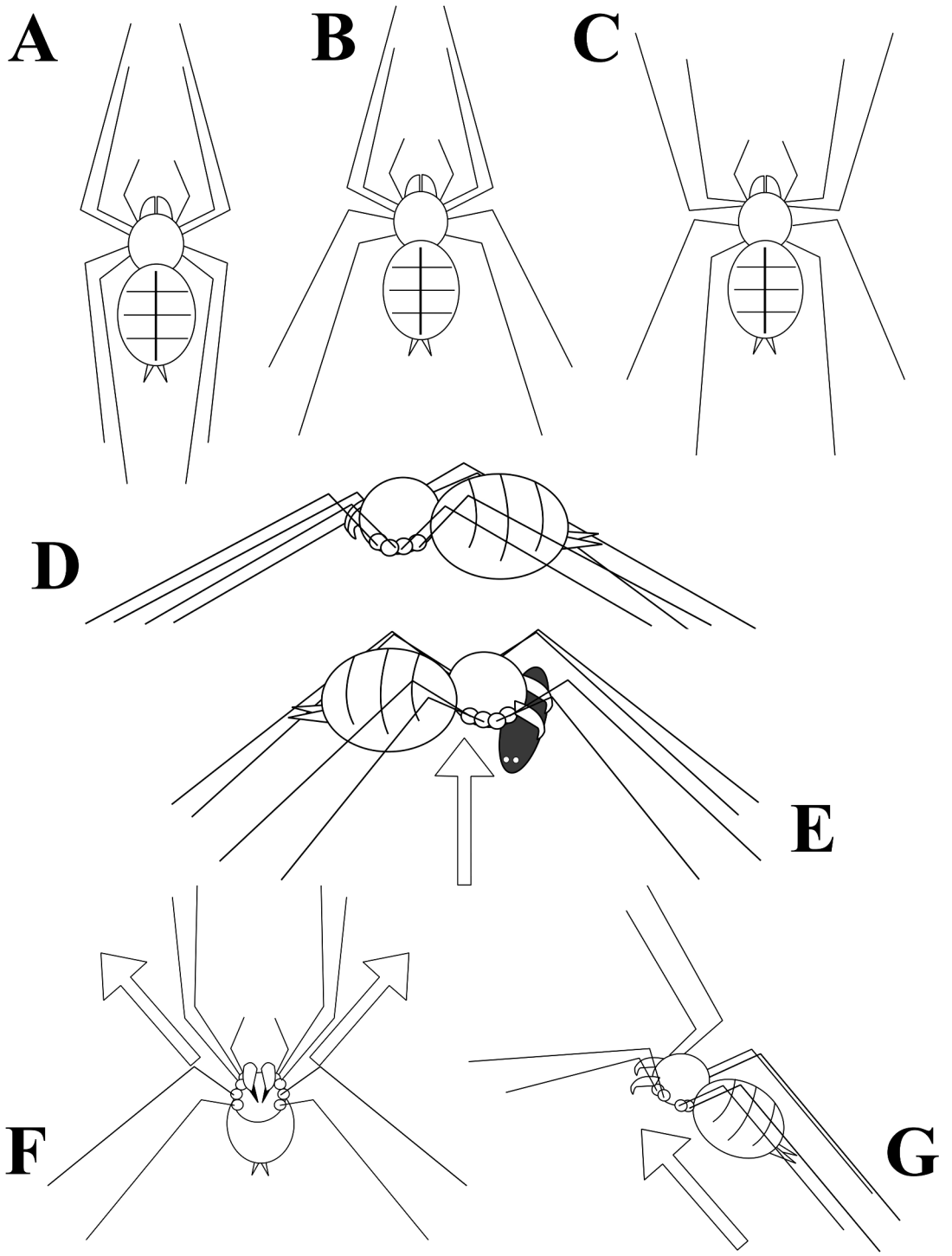


Figure 5. Theraphosid postures as developed by Bennie et al. (2011). (a & b) Resting (c) Alert (d) Walking: prosoma and opisthosoma slightly elevated (e) Feeding: prosoma and opisthosoma raised high off the ground, prey tightly held between chelicerae and fangs (f) Threat display frontal view: note exposed 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.

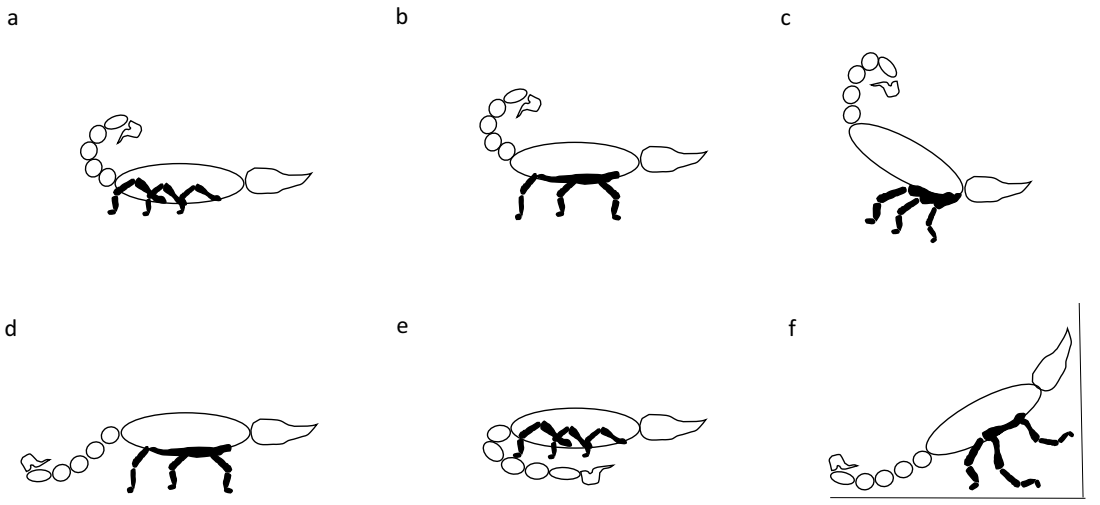


Figure 6. Scorpion postures redrawn from Alexander and Ewer (1958) with the addition of further postures observed in initial observations in the study. (a) Resting stance (b) Stilted pose, whole body is lifted (c) Stilted pose mesosoma is sharply elevated (d) Rather rare stilted pose (e) completely relaxed, claws flat on substrate, tail limp (f) Vertical against side of box.

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 ($P < .05$) between the two lighting conditions and postures observed.

Hemolymph extraction

Hemolymph was extracted 24 h 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 & Tort, 1997). For the same reason, behavioral observations were not recorded until at least 24 hours after arrival in the behavior laboratory. After acclimatization, arachnids were anesthetized with a rising concentration of carbon dioxide (CO_2) gas. The method used was a protocol optimized 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, De Voe, and Lewbart (2013). The *P. muticus* were placed in 3 liter RUBs, with a CO_2 flow-rate of 0.5 l/minute, and the *H. swammerdammi* in 1 l RUBs with a flow-rate of 0.1 l/min. Average time to anesthesia was 76 minutes (SD ±13.07) for *P. muticus*, and 80 minutes (SD ±20.00) for *H. swammerdammi*. Limb collection of hemolymph was piloted in this study based on the ability to dose spiders through these membranes as presented in Pellett et al. (2015). 50 µl of hemolymph was collected using a 25 G sterile needle (Fisher Scientific, Loughborough, UK) and a p200 pipette (Gilson) for limb collection or 1 ml syringe for cardiac collection. Where possible, hemolymph 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 8(a)). If insufficient fluid was extracted, then cardiac puncture was used (only two occurrences with *P. muticus*) (Figure 8(b)). 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. Hemolymph was collected in 1.5 ml centrifuge tubes (Fisher Scientific), containing 50 µl

Figure 7. (a) Anaesthetised *H. swammerdammi*, ventral view, indicating joint between femur and patella. (b) Extracting hemolymph, with safety tube over the metasoma.

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 9 am and 12 pm.

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 hemolymph, a number of dilutions of the hemolymph were tested with and without the dissociation reagent. The optimal results were obtained as follows: 5 µl sample (2.5 µl of hemolymph 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 were analyzed in Excel with a statistical significance assumed for $P < .05$ using paired two-tailed T-tests. Results are expressed as means ± SD ($N = 4$).

Figure 8. (a) Anaesthetised *P. muticus* ventral view, indicating joint between tibia and metatarsus. (b) Extracting hemolymph via cardiac bleed.

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 12 establishments contacted during this study, six were housing their theraphosids or scorpions under full spectrum light – either for esthetic 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 was not 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 38 in ambient light conditions so the differences were not significant see Table 3. Even though the difference in the amount of webbing was shown to be not significant one individual did produce webs on seven occasions (in ambient light) compared to 15 occasions (in full spectrum light). One individual did not 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 & 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))

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.

	Ambient light		Full spectrum lighting	
H. swammerdammi	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**

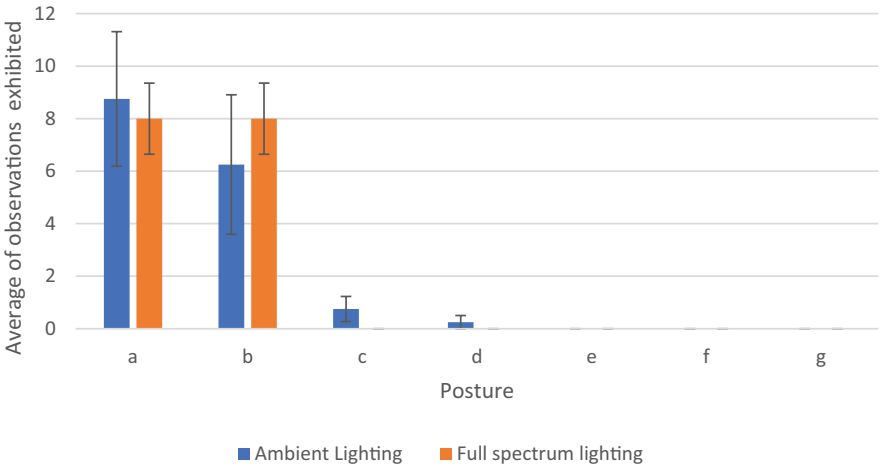
Table 2. Observations investigating if individual theraphosids (*P. muticus*) are located in the hide compared to out of the hide, comparing ambient light with full spectrum lighting (n = 4). 16 observations were undertaken for each individual under each condition.

	Ambient light		Full spectrum lighting	
P. muticus	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 3. Webbing observed during the 16 observations for each condition for each individual theraphosid *P. muticus* (n = 4) under each lighting condition.

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

a



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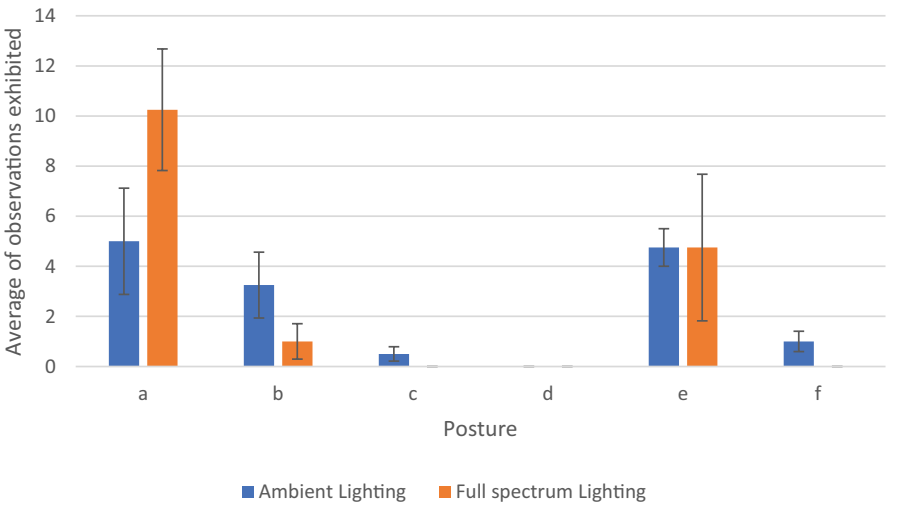

Figure 9. Postures exhibited by the theraphosid *P. muticus* (a) and scorpion *H. swammerdammi* (b) during the study in response to change in lighting conditions. Comparison of ambient lighting with full spectrum lighting showing average number of observations with standard error (N = 4 for each species).

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 behaviors expressed under ambient light (Figure 9(a)). Looking at the behaviors in scorpions there are differences between behaviors expressed and there are also behaviors expressed

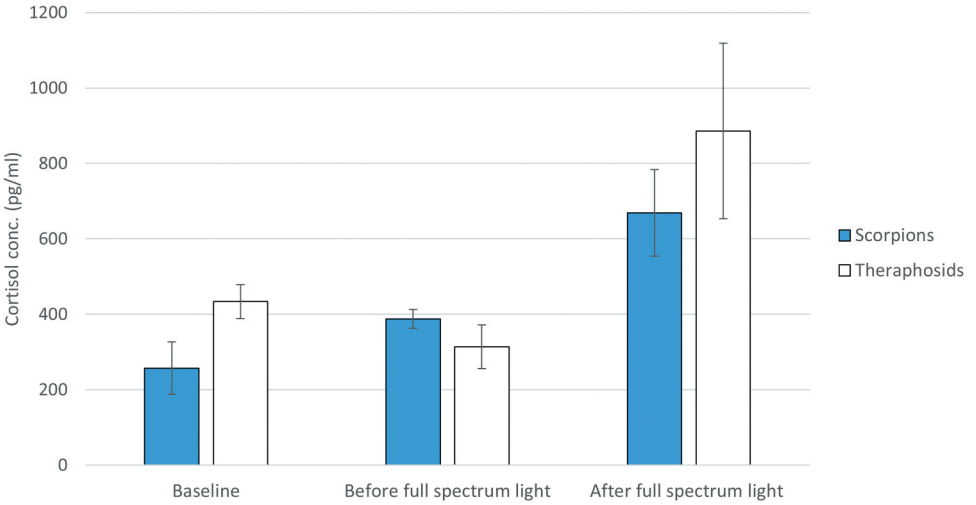

Figure 10. Cortisol concentrations in the hemolymph of *H. swammerdammi* and *P. muticus* at baseline and then before and after exposure to full spectrum light. Results presented as individuals (a) Scorpions (N = 4) and (b) Theraphosids (N = 4) and (c) together as means ± SD. (*N = 4* for each species).

under ambient light that are not expressed under full spectrum lighting (Figure 9(b)). 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 2.44 nmol/l for theraphosids and 668.935 pg/ml (SD ±115) or 1.85 nmol/l for scorpions and before full spectrum light was 314.184 pg/ml (SD ±58) or 0.866 nmol/l for theraphosids and 387.785 pg/ml (SD ±25) or 1.070 nmol/l for scorpions (Figure 10). A significant increase in cortisol immunoreactivity was detected in the hemolymph 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 hemolymph.

This study shows that the cortisol immunoreactivity levels detected were raised under full spectrum lighting compared to ambient light conditions. Our findings have 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) to levels detected in spiny lobsters (Lesmana, 2013).

Although it is difficult to find cases in invertebrates, there have been several examples of ultraviolet radiation causing stress in fish. Manek, Ferrari, Niyogi, and Chivers (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 did not 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 did not 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 et al. (2000) have stated that Invertebrate hemocytes are responsive to corticotrophin releasing hormone and although the full pathway has not been fully characterized, 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 utilized 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 hemolymph 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 & Verrell, 2010), which draws their energy resources in the preceding weeks as the new exoskeleton is formed beneath the existing one (Herzig, 2010).

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 hemolymph 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* hemolymph. Further work is required to determine the effect of these changes on the health of these animals in long-term captivity.

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References

Adamo, S. A., & Baker, J. L. (2011). Conserved features of chronic stress across phyla: The effects of long-term stress on behavior and the concentration of the neurohormone octopamine in the cricket. *Gryllus Texensis. Hormones and Behavior*, *60*(5), 478–483. doi:10.1016/j.yhbeh.2011.07.015

Alexander, A. J., & Ewer, D. W. (1958). Temperature adaptive behavior in the scorpion, *Opisthophthalmus Latimanus* Koch. *Journal of Experimental Biology*, *35*, 349–359.

Baker, S., Knight, E., Pellett, S., Roberts, K., Smulders, E., & Trim, S. A. (2018). Spider and chips - the use of Internal Radio Frequency Identification (RFID) chips as a minimally invasive method to measure internal body temperatures in invertebrates. *Animal Technology and Welfare*, *17*(1), 1–7.

Bennie, M., Loaring, C., & Trim, S. (2011). Laboratory husbandry of arboreal tarantulas (Theraphosidae) and evaluation of environmental enrichment. *Animal Technology and Welfare, 10*(1), 163–169.

Bruins, E. (2001). *The complete encyclopedia of the terrarium*. Grange Books Ltd, London.

Chernysh, S. I. (2018). *Hormones and metabolism in insect stress* (J. Ivanović & M. Janković-Hladni Eds.), (1st ed.) CRC Press Taylor & Francis group, UK.

Clark, D. L., & Uetz, G. W. (1990). Video image recognition by the jumping spider, Maevia inclement (Araneae: Salticidae). *Animal Behaviour*, *40*(5), 884–890. doi:10.1016/S0003-3472(05)80990-X

Dhal, R. D., & Granda, A. M. (1989). Spectral sensitivities of photoreceptors in the Ocelli of the Tarantula Aphonopelma chalcodes (Araneae, Theraphosidae). *The Journal of Arachnology*, *17*(2), 195–205.

Dombrowski, D. S., De Voe, R. S., & Lewbart, G. A. (2013). Comparison of isoflurane and carbon dioxide anesthesia in Chilean Rose Tarantulas (*Grammostola rosea*). *Zoo Biology*, *32*(1), 101–103. doi:10.1002/zoo.21026

Elwood, R. W., Barr, S., & Patterson, L. (2009). Pain and stress in crustaceans? *Applied Animal Behaviour Science*, *118* (3–4), 128–136. doi:10.1016/j.applanim.2009.02.018

Herzig, V. (2010). Ontogenesis, gender, and molting influence the venom yield in the spider Coremiocnemis tropix (Araneae, Theraphosidae). *Journal of Venom research*, *1*, 76–83.

Hu, Z., Xu, X., Chen, Z., Li, H., Wang, X., Wu, L., … Li, D. (2014). The spectral transmission of non-salticid spider corneas. *Journal of Experimental Biology*, *217*(15), 2698–2703. doi:10.1242/jeb.099069

Ibrahim, A. T. (2015). Negative impacts of ultraviolet-A radiation on antioxidant and oxidative stress biomarkers of African catfish Clarias gariepinus. *Photochemical & Photobiological Sciences*, *14*(7), 1337-1345. doi:10.1039/c5pp00112a

Kennedy, B., Warner, A. S., & Trim, S. A. (2019). Reference intervals for plasma biochemistry of haemolymph in subadult chilean rose tarantula (*Grammastola rosea*) under chemical restraint. *Journal of Zoo and Wildlife Medicine*, *50*(1), 127–136. doi:10.1638/2018-0037

Kloock, C. (2005). Aerial insects avoid fluorescing scorpions. *Euscorpius*, (21), 1–7. doi:10.18590/euscorpius.2005.vol2005.iss21.1

Lesmana, D. (2013) Evaluation of compartments using floating cage culture on the level of stress and growth of Spiny Lobsters *Panulirus homarus*, PhD thesis, Bogor Agricultural University.

Machan, L. (1968). Spectral sensitivity of scorpion eyes and the possible role of shielding pigment. *Effect Journal of Experimental Biology*, *49*(1), 95–105.

Maddocks, S. A., Goldsmith, A. R., & Cuthill, I. C. (2001). The influence of flicker rate on plasma corticosterone levels of European starlings, Sturnus vulgaris. *General and Comparative Endocrinology*, *124*(3), 315–320. doi:10.1006/gcen.2001.7718

Malagoli, D., Franchini, A., & Ottaviani, E. (2000). Synergistic role of cAMP and IP3 in corticotropin-releasing hormone-induced cell shape changes in invertebrate immunocytes. *Peptides*, *21*(2), 175–182. doi:10.1016/S0196-9781(99)00203-X

Manek, A. K., Ferrari, M. C., Niyogi, S., & Chivers, D. P. (2014). The interactive effects of multiple stressors on physiological stress responses and club cell investment in fathead minnows. *The Science of the Total Environment*, *476–477*, 90–97. doi:10.1016/j.scitotenv.2013.12.042

Mostl, E., & Palme, R. (2002). Hormones as indicators of stress. *Domestic Animal Endocrinology*, *23*(1–2), 67–74. doi:10.1016/S0739-7240(02)00146-7

Ottaviani, E. (2006). Molluscan immunorecognition. *Invertebrate Survival Journal*, *1*(3), 50–63.

Ottaviani, E., & Franceschi, C. (1996). The neuroimmunology of stress from invertebrates to man. *Progress in Neurobiology*, *48*(4–5), 421–440. doi:10.1016/0301-0082(95)00049-6

Pellett, S., Bushell, M., & Trim, S. (2015). Tarantula husbandry and critical care. *Companion Animal*, *20*(2), 54–60. doi:10.12968/coan.2015.20.2.119

Punzo, F., & Punzo, T. (2001). Monoamines in the brain of tarantulas (*Aphonopelma hentz*) (Araneae, Theraphosidae): Differences associated with male agonistic interactions. *The Journal of Arachnology*, *29*, 388–395. doi:10.1636/0161-8202(2001)029[0388:MITBOT]2.0.CO;2

Roeder, T. (1999). Octopamine in invertebrates. *Progress in Neurobiology*, *59*(5), 533–561. doi:10.1016/s0301-0082(99)00016-7

Rotllant, J., & Tort, L. (1997). Cortisol and glucose responses after acute stress by net handling in the sparid red porgy previously subjected to crowding stress. *Journal of Fish Biology*, *51*(1), 21–28. doi:10.1111/j.1095-8649.1997.tb02510.x

Rubio, M. (2008). *Scorpions: Everything about purchase, care, feeding, and housing*. New York: Barron's Educational Series, USA.

Sanggaard, K. W., Bechsgaard, J. S., Fang, X., Duan, J., Dyrlund, T. F., Gupta, V., Jiang, X., Cheng, L., Fan, D., Feng, Y., Han, L., Huang, Z., Wu, Z., Liao, L., Settepani, V., Thøgersen, I. B., Vanthournout, B., Wang, T., Zhu, Y., Funch, P., Enghild, J. J., Schauser, L., Andersen, S.U., Villesen, P., Schierup, M. H., Bilde, T., Wang, J. (2014). Spider genomes provide insight into composition and evolution of venom and silk. *Nature Communications*, *6*(5), 3765. doi:10.1038/ncomms4765

Saul-Gershenz, L. (2009) Laboratory culture techniques for the Goliath tarantula Theraphosa blondi (Latreille, 1804) and the Mexican red knee tarantula, Brachypelma smithi (Araneae: Theraphosidae). AAZPA Annual Conference Proceedings, USA.

Shillington, C., & Verrell, P. (2010). Sexual strategies of a North American 'Tarantula' (Araneae: Theraphosidae). *Ethology*, *103*(7), 588–598. doi:10.1111/j.1439-0310.1997.tb00170.x

Smith, S. M., & Vale, W. W. (2006). The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues Clin Neurosci*, *8*(4), 383-395. doi:10.31887/DCNS.2006.8.4/ssmith

Stefano, G. B., Cadet, P., Zhu, W., Rialas, C. M., Mantione, K., Benz, D., … Slingsby, B. (2002). The blueprint for stress can be found in invertebrates. *Neuro Endocrinol Letters*, *23*(2), 85–93.

Tapley, B., Rendle, M., Baines, F. M., Goetz, M., Bradfield, K. S., Rood, D., … Routh, A. (2015). Meeting ultraviolet B radiation requirements of amphibians in captivity: A case study with mountain chicken frogs (Leptodactylus fallax) and general recommendations for pre-release health screening. *Zoo Biology*, *34*(1), 46–52. doi:10.1002/zoo.21170

Verlinden, H., Vleugels, R., Marchal, E., Badisco, L., Pflüger, H.-J., Blenau, W., & Broeck, J. V. (2010). The role of octopamine in locusts and other arthropods. *Journal of Insect Physiology*, *56*(8), 854–867. doi:10.1016/j.jinsphys.2010.05.018

Williamson, S., & Cummins, H. Z. (1983). Light and color in nature and art. In *Light and color in nature and art* (pp. 173). New York: John Wiley & Sons, USA.