Does exposure to UVB light influence the growth rates and behaviour of hatchling Corn Snakes, *Pantherophis guttatus*?

(photo taken by the author 6/11/2010)

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Reaseheath College in collaboration with the University Of Chester

DECLARATION

I certify that this work is original in its entirety and has never before been submitted for any form of assessment.

The practical work, data analysis and presentation and written work presented are all my own work unless otherwise stated.

Signed ……………………………………………………………….

Abstract

The benefit of UV light for reptiles is a long, much debated area and results and opinions are often mixed. To investigate the growth and behaviour effects of UVB exposure on *Pantherophis guttatus,* twelve snakes were used. These were split into three groups of four snakes, and exposed to different levels of UVB; 2% UVB (1), 6% UVB (2) and a control group (3). Due to health reasons, two snakes had to be removed from group 2, therefore number of participants was: group 1 – four snakes, group 2 – two snakes, group 3 – four snakes. Results showed no significant difference in growth for snake length. However, a significant difference was noted in snake weight between groups 1 and 3, the potential reasons for which are discussed. No significant difference was found in basking high, however a significant difference was observed in basking medium (the best position for UVB exposure) between groups 1 and 2. The control group was observed more times than group 1 in this behaviour, perhaps in attempts to access UVB, although this was not statistically significant. Again, the potential reasons for this behaviour are discussed. Group 1 were observed significantly more actively moving than group 2, and also group 3, but this was not statistically significant. Group 1 were observed in the UVB basking area on level 3 considerably more times than group 2, but only slightly more than group 3; 39%, 8% and 35% respectively. *P. guttatus* are known to be able to synthesis vitamin D_3 through exposure to UVB and this may also influence other metabolic processes in this species. This study shows that *P. guttatus* will voluntarily expose themselves to UVB, the effects of which can increase activity levels. This increase in activity levels may be due to the possible influence of UVB on metabolic processes. Therefore, a low level of UVB, such as a 2% bulb, can be provided to *P. guttatus* without detriment.

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Acknowledgements

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1.0 Introduction

The physiology and biology of reptiles is extremely different to mammals and birds and how they see the world is one of the main differences. Reptiles are tetrachromats; they have an extra cone type which allows them to see UV-A below 400nm (Brames, 2007). UV enables reptiles to distinguish conspecifics from interspecifics, aids in intersexual recognition and the contrast and brightness are important for foraging and motion perception (Brames, 2007). As well as the importance to their vision, UV, specifically UVB, regulates the endosynthesis of vitamin D_3 (Holick, 1996).

Whilst both lizards and snakes have been observed basking directly in the sun (Ferguson, Brinker, Gehrmann, Bucklin, Baines, & Mackin, 2010) it has generally always been thought that snakes perform this behaviour purely for the benefits associated with maintaining optimal body temperature (Kauffeld, 1969). Reptiles do, however voluntarily expose themselves to UVB radiation (Ferguson et al., 2010) and in 2003, Adkins et al stated that further research on snakes is required before a definitive answer on UV requirements can be given. Under captive conditions the benefits of UV lighting is greatly documented for a variety of lizard species; reducing risk of metabolic bone disease, increased hatchling success and wellbeing (Ferguson, et al., 2003; Ferguson, Gehrmann, Chen, Dierenfeld, & Holick, 2002; Ferguson, et al., 1996; Laszlo, 1969; Logan, 1969). Research for snakes however, is limited with just two scientific experiments currently completed. It is thought that snakes gain adequate vitamin D_3 through their carnivorious diet (Baines, Beveridge, Hitch, & Lane, 2005c). However, Acierno, Mitchell, Zachariah, Roundtree, Kirchgessner and Sanchez-Migallon Guzman (2008) found that *Pantherophis guttatus* have the ability to biosynthesise vitamin D and Bellamy and Stephen (2007) found increased behavioural activity in the Jamaican Boa, *Epicrates subflavus*, with the provision of UVB lighting. The results of these studies show that snakes, or at least *P. guttatus* and *E. subflavus*, are able to utilise UVB effectively both physiologically and psychologically.

The author is not aware of any other research that investigates the effects of providing UVB to snakes; other than the two aforementioned studies. Therefore research carried out on amphibian and lizard species provides the basis for the research plan along with communications with experts in relevant fields.

Many species of snake are currently being raised in captivity as pets and, due to the potential life span, it is important to fully understand their husbandry requirements (Acierno et al., 2008) including providing the correct spectrum of lighting.

The purpose of this study is to ascertain whether the provision of UVB lighting enhances the growth and behaviour of *P. guttatus* and what constitutes the best percentage of UVB. This was done by providing different levels of UVB output for comparison.

The experiment was conducted over a period of seventeen weeks. Snake growth, weight and length, were monitored weekly and behaviour was observed in two hours sessions throughout the study. The hypotheses tested were that *P. guttatus* which are exposed to UVB light have a faster growth rate compared with control snakes and that UVB light positively affects their behaviour.

2.0 Literature Review

2.1 *Pantherophis guttatus*

2.1.1 History, biology and distribution of *P. guttatus*

Snakes are listed under the order Squamata. This order contains approximately 7900 species of lizard and snake. Figure 2.0 shows the percentage of species in each order under the class Reptilia (O'Shea & Halliday, 2002, p. 12).

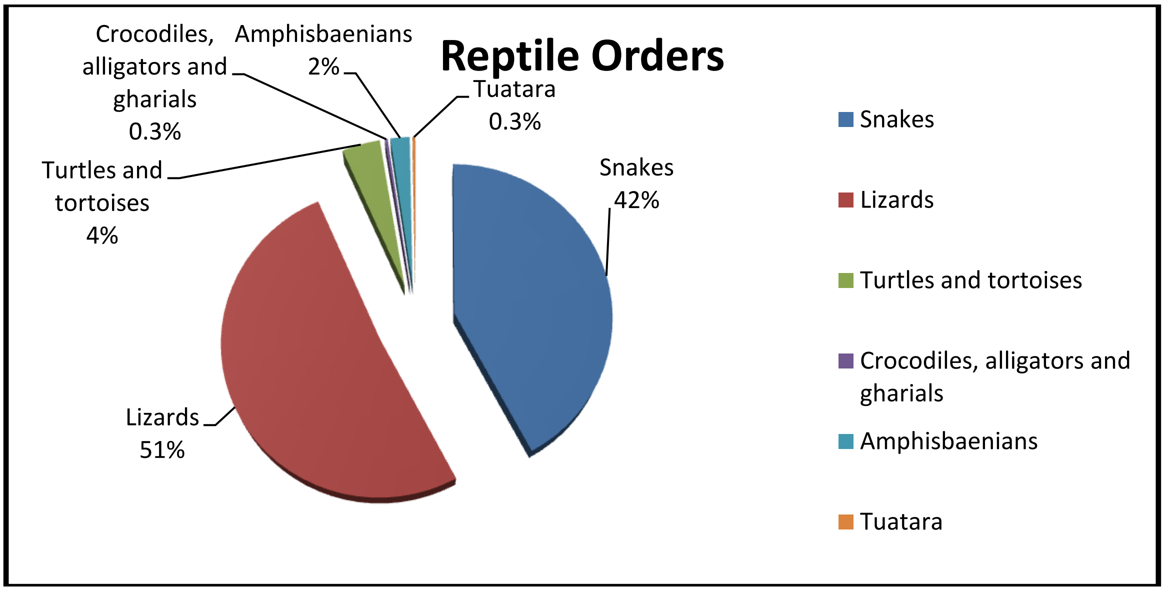

Fig 2.0 Percentage of species in the class Reptilia (O'Shea & Halliday, 2002)

Snakes form the suborder Serpentes, which is split into two infra-orders; Alethinophidia and Scolecophidia. The colubridae family are found under the infra-order Alethinophidia. There are some 1726 species documented under this family. Colubridae, aka ‘typical snakes’, are further split into seven subfamilies; Colubrinae, Grayiinae, Calamariinae, Dipsadinae, Pseudoxenodontinar, Natricinae and Scaphiodontophiinae (Uetz & Hallermann, n.d). The Corn Snake is one of the 668 species that form the family Colubrinae (Uetz & Hallermann, n.d). There are four subspecies of corn snake; *Pantherophis guttatus, P. emoryi, P. vulpinus, P. gloydi and P. bairdi* (Hammerson, 2007). *P. guttatus* is the best known and inhabits south eastern USA (O'Shea & Halliday, 2002). There is a suggestion that this genus belongs in Pituophis, however the IUCN currently retain them under *Pantherophis* until further information of the relationships in this taxa are available (Hammerson, 2007).

P. guttatus inhabit the Nearctic (Mattison, 1994). They are endemic to eastern and southern eastern America where its range extends from southern Florida up to southern New Jersey and eastwards towards Kentucky, Southern Mississippi and Alabama and south-eastern Louisiana. See Figure 2.1 for an illustration of their geographical range (Hammerson, 2007).

They are a constrictor which primarily feeds on small mammals/birds and whilst primarily terrestrial, their bodies are adapted for climbing (Whitfield Gibbons & Dorcas, 2005; Werler & Dixon, 2004; Jose, 2006). Juveniles generally eat small tree frogs and lizards, progressing onto small mammals as they grow (Whitfield Gibbons & Dorcas, 2005). Found in a variety of habitats, from dry woodland and farmlands to swamps (O'Shea & Halliday, 2002) these snakes gained their common name from their nature of hunting rodents in corn stores (Nickerson, Krysko, & King, n.d). Log piles and leaf litter are suitable natural hides for these snakes in the day; however they are also found around human settlements and may inhabit abandoned barns or houses (Werler & Dixon, 2004).

The IUCN Red List of Threatened Species state the species as ‘least concern’ due to their current abundance (Hammerson, 2007). Wild types are generally orange with red saddles and a black and white chequered underbelly (Werler & Dixon, 2004). They are a relatively placid snake that reach lengths of between 35.5in-47.6in (Jensen, Camp, & Gibbons, 2008) and have a reluctance to bite making them excellent pets for novice herpetologists. Sexual maturity occurs in females when body length reaches around 26.5in, males mature at a slightly smaller size (Jensen et al., 2008).

P. guttatus are known to be active during the day in the cooler seasons and at night during the summer (Whitfield Gibbons & Dorcas, 2005; Jose, 2006). They are active from April to October or early November, however this varies according to the climate in specific areas (Whitfield Gibbons & Dorcas, 2005; Werler & Dixon, 2004). During the winter they may brumate for several weeks, again this depends on the local climate. During brumation they locate areas for warmth such as rotting trees and vegetation and tunnels of burrowing animals. In the warmer southern regions they are active almost all year round (Whitfield Gibbons & Dorcas, 2005).

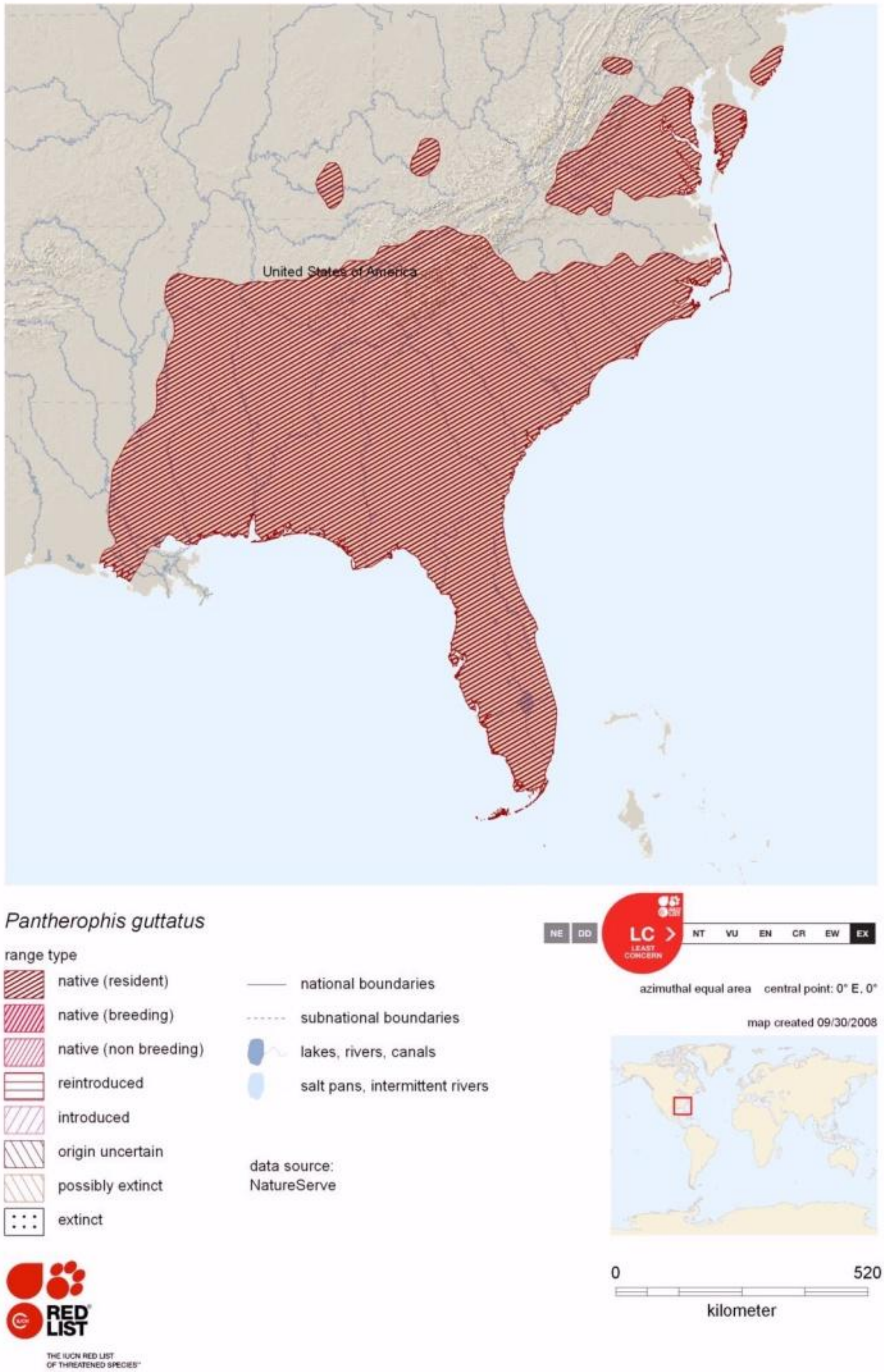

Fig 2.1 Geographical range of P. guttatus (Hammerson, 2007)

Like all snakes *P. guttatus* is ectothermic; behaviourally selecting their environment to obtain their preferred thermoregulation temperature (Warwick, Frye, & Murphy, 1995). Heat can be obtained from a variety of sources including warm rock surfaces (thigmothermic) and basking directly in the sun (heliothermic).

P. guttatus are a placid snake, however some will show defensive postures when threatened (Werler & Dixon, 2004). This involves forming a series of 's' shaped loops with its head pulled near to the top of these loops; they may also rattle their tail. A fast strike may occur if further threats are made, although contact is rarely made. This technique is thought to discourage the threat. When handled they may bite and excrete faeces as a defense, although other *P. guttatus* may not react at all (Werler & Dixon, 2004). Generally, within a few days in captivity these behaviours fade and they become tractable (Werler & Dixon, 2004)

2.1.2 *P. guttatus* in captivity

P. guttatus have been kept for many years in captivity and are therefore a well known snake. They are an abundant species that is readily available in the pet trade. Due to their size, temperament, ease of breeding and availability in a variety of colour morphs they make excellent pets for the novice herpetologist (Werler & Dixon, 2004). In captivity colours vary due to breeding in recessive genes. The three main variants are albino or 'snow corns', amelanistic (lack of black pigment) and anerythristic (lack of red or orange pigment) (Bartlett, Griswold, & Bartlett, 2001).

All snakes are ectothermic and therefore, when being kept in captivity, provisions must be in place to accomodate this requirement. Being ectothermic means that they rely on their external environment for heat (Warwick et al., 1995). This affects almost all of their physiology and influences ecological characteristics, such as activity patterns, and behavioural charateristics (Warwick et al., 1995).

Appropriate day time temperatures for *P. guttatus* are between 24-30°c with night time temperatures ranging from 21-24°c (Mitchell & Tully, 2009). Heat can be provided by either a heat bulb (guarded to prevent thermal burns) or a heat mat; both should be controlled automatically via an appropriate thermostat (Love & Love, 2005). A heat gradient should be provided so that the snake can perform thermoregulatory behaviour. To ensure an appropriate heat gradient is provided a small to medium sized vivarium should be provided. The minimum recommend size is around 12in (w) x 30in (l) x 12in (h); however a larger vivarium would allow a greater area for exercise (Love & Love, 2005). It is important to provide adequate ventilation, which can be simple circular air vents located at the top and bottom of the back board or in both sides of the vivarium. Substrate should be provided and can vary from newspaper to an aspen type bedding depending on the preference of the owner. Hides need to be provided in both the cool and warm end of the vivarium so that the snake can feel secure regardless of where they are located. A small sturdy water dish should be provided to prevent dehydration and to ensure adequate humidity levels; required for successful shedding (Love & Love, 2005). Decor can also be incorporated to create a naturalistic enclosure. Branches, that will allow climbing can add a three dimensional look and create extra places for the snake to investigate. *P. guttatus* do have the physical ability to climb (Werler & Dixon, 2004) however, the diameter of the climbing equipment should be chosen according to the size of the snake (Astley & Jayne, 2007).

2.2 UV Light

Ultraviolet (UV) light forms part of the Electromagnetic Spectrum (Jagger, 1967) which is not visible to the human eye. Reptile vision, however, does extend into the UV spectrum (Adkins, et al., 2003; Baines, Beveridge, Hitch, & Lane, 2005b). There are three types of UV light, all measured in nanometres (nm); UVA (320-400nm), UVB (280-320nm) and UVC (180-280nm) (Adkins, et al., 2003).

2.2.1 Spectrum

UVA light is important to some reptiles as it enables identification on conspecifics through reflective markings (Baines et al., 2005b). It has also proved to be important in the behavioural repitoire and activity levels of some reptiles; see section 4 for examples (Moehn, 1974; Baines et al., 2005b; Whiting, Stuart-Fox, O'Connor, Firth, Bennett, & Blomberg, 2006).

UVB cannot penetrate glass and most plastics therefore it needs to be provided from within an enclosure (Baines et al., 2005b). It is this spectrum that many species of reptile require for the photo-biosynthesis of pre-vitamin D_3 (Adkins, et al., 2003). Vitamin D is important for the skeletal system; deficiency in vitamin D can result in metabolic bone disease; a softening of the bone tissue resulting in malformed bones (Adkins, et al., 2003).

UVC does not reach the Earth as it is filtered out by the ozone layer (Ultraviolet Radiation, 1994). It is harmful to living organisms and is never present in artificial lighting (Baines et al., 2005b).

2.2.2 Costs and benefits associated with UV

Calcediol, hydroxylated vitamin D3 (25-OH-D3, see figure 2.2), plays a vital role in the functioning of other organs as well as having advantageous effects on the cardiovascular system, immune system and controlling cell division resulting in the prevention of cells becoming cancerous (Baines, Beveridge, Hitch, & Lane, 2005a). Although readily available and therefore considered relatively harmless (Jagger, 1967) the main problem with UV lies with over exposure; this can cause permanent blindness, skin damage and death in reptiles (Logan, 1969).

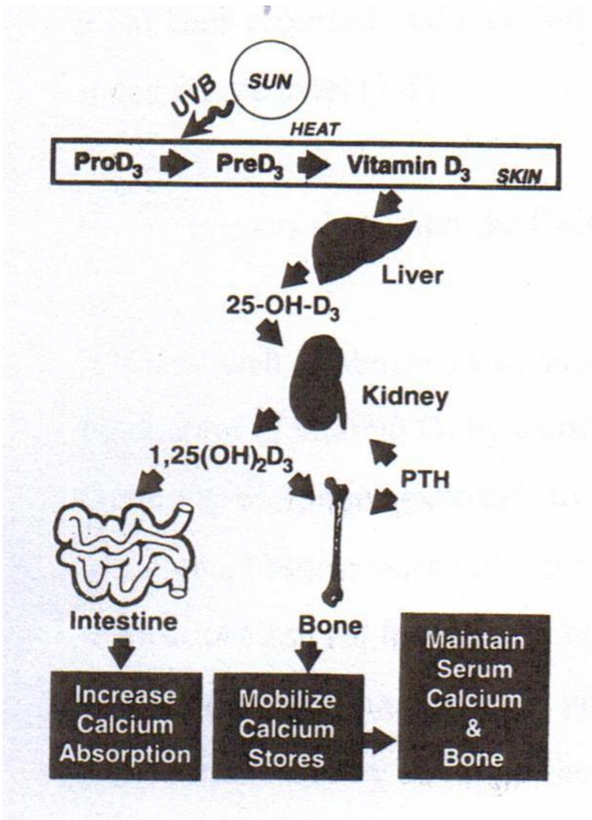

Fig 2.2 Illustration of the pathway of previtamin D_3 (Holick, 1996)

2.2.3 Absorption

Ultraviolet light is required for the biosynthesis of the previtamin D_3 (Adkins, et al., 2003). This vitamin is hydroxylated in the liver to calcediol. Calcediol circulates around the bloodstream where, in the kidneys, it is converted to the active hormone calcetriol (Baines et al., 2005a). This active hormone controls the absorption of calcium from the bones and gut. See figure 2.2 for an illustration of the pathway (Holick, 1996).

2.2.4 Sources

Ultraviolet light is available naturally, through the sun, and artificially. Artificial sources include incandescent sources, gas discharges, electric discharges, fluorescent lamps and lasers (Ultraviolet Radiation, 1994). The two types used in reptile husbandry are gas discharges and fluorescent lamps. Mercury vapour lamps are classed as gas discharges. Typically they work via the electrical excitation of gas or vapour (Ultraviolet Radiation, 1994). Mercury vapour lamps provide an excellent form of ultraviolet light and can be particularly beneficial to sick reptiles (Baines et al., 2005c). There are many variants available on the market today. Fluorescent lamps are most commonly used for application of low-pressure discharge (Ultraviolet Radiation, 1994). They consist of two electrodes and a mixture of mercury vapour and a rare gas, typically argon. The glass envelope of the tube is coated with phosphor and variances of these are used to create different levels of visible, UVA and UVB light (Ultraviolet Radiation, 1994). The spectrum is determined by the nature of the phosphor (Ultraviolet Radiation, 1994).

2.3 Ultraviolet light and amphibians, lizards, turtles and crocodilians

As amphibians are listed under the class Reptilia, it must be considered important to understand the effect that ultraviolet light has on them. UV light is known to be harmful to a variety of amphibians (Bancroft, Baker, & Blaustein, 2007) and there have been many studies on the effects of UVB on amphibians. Certain species don't seek to avoid UVB; preferring higher temperatures regardless of UVB exposure present (Bancroft, Baker, Searle, Garcia, & Blaustein, 2008). That said, a previous study by Belden, Moore, Mason, Wingfield, and Blaustein (2003) found that the Cascades Tree frog tadpoles (a species tested by Bancroft et al, 2008) may not be able to perceive UVB radiation and found that after 42 days survival was significantly lower than those that were protected from UVB exposure. Marco, Lizana, Alvarez, and Blaustein (2001) found increased survivorship when egg-wrapping behaviour was performed and 90-95% of exposed embryos died within 14 days of exposure; mortality of protected eggs was 20%. A study carried out on two sympatric species of Australian frogs also found that exclusion of UVB significantly enhanced survival rates (Broomhall, Osborne, & Cunningham, 2000). That said, Pahkala, Laurila, & Merila (2003) found positive affects on the growth two species of amphibian larvae and no affect on survival rates, concluding that moderate amounts of UVB can enhance tadpole growth rates of certain species.

It appears that whilst the majority of amphibians may be adversely affected by exposure to UVB there may be some species that would benefit. Further research into the effects on different species is required.

It is well known that certain species of reptiles require UVB to survive (Allen, Oftedal, & Horst, 1996; Baines et al., 2005c; Ferguson et al,. 2003; Gehrmann, Ferguson, Odom, Roberts, & Barcellona, 2005). The importance of lighting for reptiles was documented by Lazlo (1969). He stated that “the fluorescent tubes of Optima and, especially, Vita-lite type can be important factors in the successful maintenance of captive reptiles, particularly those that are traditionally short-lived in zoo collections” (Laszlo, 1969, p. 13). There are many lizard species that have been observed basking in the sun for prolonged periods of time, exposing themselves to elevated temperatures and UVB levels. These species are aptly termed as ‘sun worshippers’ and include the Bearded dragon, Uromastyx and Chuckwalla (Baines et al., 2005c). These behaviours led to researchers questioning why they would expose themselves for such prolonged periods and risk predation. One observation by Pawley, (1969) on a Mata turtle saw old feeding habits return with renewed vigour when exposed to UVB. It was reported as being close to death when an incandescent ‘Sun lamp’ bulb was installed for the cohabiting Caiman lizard. The turtle aroused within ten minutes of the lamp being switched on and postioned itself directly under the lamp, exposing itself to the most intensive spot of UV light. A year on and the turtle was reported to have a renewed vigor. Karsten, Ferguson, Chen, and Holick (2009) and Ferguson et al. (2003) reported Panther Chameleons basking under UVB lamps when deficient in vitamin D_3. Allen et al. (1996) varied levels of dietry vitamin D3 in the Giant Day gecko, a diurnal species, without the provision of UVB lighting. Mortality began within fifty days; all gecko’s died apart from the three in the control group (UV provided) and one from an experimental group. The gecko’s were reported as “growing poorly”, although this is not elaborated on, and soft bones were noted in all specimens. It was concluded that the deaths were possibly due to vitamin D deficiency. A study on juvernile Green Iguanas was also carried out by Allen et al. (1996). The Iguana's were exposed to different fluorescent lamps and their health monitored when the UVB was removed. Exposed groups had significantly higer calcidiol (the susbtance used to test vitamin D levels in reptiles) levels but not calcitriol (formed in the kidneys) levels. Two years after the study finished six Iguana's had died. Bone demineralization was shown to be the cause with blood calcidiol levels of less than 5mg/mL (12.5nmol/L). UVB lights were immediately implemented as a new study to assess whether the effects could be reversed. Radiographs that were carried out showed healing of prior fractures and animal vigor improved. The study concluded stating that UVB is extremely important to Iguanas.

The level and type of lighting is also important as Ferguson, et al. (1996) found during their study on the effects of dietry vitamin A and D and ultraviolet irridation. No clear benefits were found with the provision of high UVA exposure and, in fact, the provision of UVA slowed female growth. Hatchling success was substantially improved with the provision of high UVB exposure and a medium vitamin A diet. The best possible results were found by providing intermediate levels of dietry vitamin A and D along with the provision of high UVB irridation.

Ultraviolet light has also proven to be an important factor in the behaviour of lizards. Both Moehn (1974) and Whiting et al. (2006) found that ultraviolet light facilitated agonistic behaviour in Iguanid and Agamid species and the Augrabies flat lizard.

From the studies discussed it has become obvious that the welfare of lizards and turtles is at risk if the provision of UVB lighting is withheld. From the biosynthesis of pre-vitamin D_3 and successful reproduction to encouraging natural behaviours it can be seen that UVB has benefits that cannot be disputed.

2.4 Ultraviolet light and snakes

Currently there is insufficient evidence to imply that the provision of UV light would benefit snakes whilst under captive conditions. Despite this, several authors have endeavoured to evaluate the effect of UV light on the health and welfare of snakes, yielding mixed results. For example, Laszlo (1969) reported the significant positive effects he had witnessed when providing a variety of species of snakes with an Optima tube bulb. These included long starving snakes feeding and more natural behaviours expressed; although it was not stated what these behaviours were. Nocturnal species were also included in his experiments and, interestingly, 'active sunning' was observed in these species.

Similarly, Kauffeld (1969) investigated the effect of altitude, UV light and humidity on captive reptiles. However, he stated that there are many cases of snakes being kept and living long lives without access to natural or artificial light, stating that "I am quite certain that no snake needs direct sunlight or ultra-violet light in any form, and I am equally certain that growth of the young can progress quite normally in ordinary daylight, or even in darkness" (Kauffeld, 1969, p. 8). Whilst snakes have been observed basking, Kauffeld (1969) states that this is for thermoregulation and not for access to ultraviolet light. Whilst this is still the case with many species he does make note of two species of rattlesnake (Eastern diamond-back rattlesnake, *Crotalus adamanteus*, and Western pigmy rattler, *Sistrurus miliarius streckeri*) that do not do well in captivity, putting it down to potential effects of latitude. Interestingly, Laszlo (1969) actually reported on ‘active sunning’ behaviour in a Black-tail rattlesnake, *Crotalus molossus,* during his observations; maybe the provision of the Optima tube bulb may have influenced their life span in captivity. The comments and conclusions in Kauffeld’s article were based on personal observations and not scientific experiments as were Laszlo’s. However, the bold statements made may well have influenced many people’s perspectives on how snakes should be kept in captivity.

Not all reports on the provision of UV lighting though have been positive. Logan (1969) reported fatalities caused by infrared radiation in a Sidewinder, *crotalus cerastes*, and stated that overexposure to mid-range ultraviolet may be dangerous to certain reptiles causing health issues such as permanent blindness and skin tissue destruction and may result, in some instances, in death.

Adkins, et al. (2003) answered many questions in a roundtable discussion on the role of ultraviolet light for reptiles and amphibians. In particular question seven asked for the UVB requirements of different reptiles, including snakes, and amphibians. A variety of answers were provided. Owen had no recommendations, whilst Ogle firmly believes that they may well be long term health and propagation benefits in providing UVB lighting with many taxa of snakes (Adkins, et al., 2003). Gehrmann, Ferguson and Gyimesi all state that further research is required, however if provided appropriately it may be beneficial to many species (Adkins, et al., 2003). Following on from this, Ferguson et al. (2010) researched the levels of ultraviolet light that a variety of reptiles exposed themselves to in the field. They monitored levels from four different species of snake along with a variety of lizards. The Texas rat snake (*Elaphe obseleta*) was observed exposing itself to an ultraviolet index of between 0 – 0.8 and this could be a good basis to look at for *Pantherohpis* species as they are a very similar species.

Historically it was thought that snakes gained all their vitamin D_3 requirements through their diet (Adkins, et al., 2003), however research carried out by Acierno et al. (2008) found that *P. guttatus* are able to biosynthesise vitamin D_3. Their research found a significant difference in plasma concentrations of 25-hydroxyvitamin D_3 in the group of *P. guttatus* exposed to UVB light. This research provides important information regarding *P. guttatus's* ability to biosynthesis vitamin D. The research was concluded by stating that further research is required to determine the health and development consequences of UVB exposure (WL 290 to 320nm).

The one study that has been carried out on the effects of UVB on behaviour was carried out by Bellamy and Stephen (2007). They conducted a study on the effects of UVB and vitamin D3 supplementation on the Jamaican Boa. They had four groups: Group 1 – 2% UVB, Group 2 – Vitamin D3 supplementation, Group 3 – control group, Group 4 – monthly UVB exposure (20 minute exposure once a month). Their findings saw a significantly higher rate of basking on the higher perch for Group 3 and 4, suggesting that the reason for this was that they increased basking behaviour in an attempt to obtain UVB. They also found that Group 1 and 2 were significantly more active during the day and night compared to groups 3 and 4.

2.5 Study Justification

From the literature presented it is obvious that research on UVB lighting is biased towards lizards and amphibians and even that is scarce. Literature on snakes is starting to come through however, more research is required before a definitive answer on what UV sources should be provided can be determined. It is obvious from the current research that UVB plays an important role in the biosynthesis of pre-vitamin D_3 although all of the research, apart from Acierno et al (2008), is based on lizard species. It is empirically thought that a snakes requirement of vitamin D_3 is gained through their carnivorous diet and, as they have been kept successfully in captivity for a number of years without the provision of any form of UV lighting, that they do not require it (Adkins, et al., 2003). However, there are snakes that do not feed exclusively on mammals and therefore their need for UV lighting should be questioned. An example of this is the Egg Eating snake which feeds exclusively on eggs. One of the earliest accounts depicting the benefits of UV lighting for snakes was in 1969 by Laszlo, however it is not understood why further research has not been conducted since then. As with amphibians, the UVB requirements of reptiles can vary considerably, therefore it is necessary for further research on all species to be carried out before it can be concluded whether or not to provide UVB to snakes in captivity. The study on the Jamaican Boa by Bellamy and Stephen (2007) is a big step forward on ascertaining the potential benefits of providing UVB light and provides a useful experimental design for future projects.

3.0 Methods

3.1 Subject species

P. guttatus were chosen as the subject species for this experiment as they are very well represented within the reptile industry. Available in a variety of colours, *P. guttatus* are a very popular pet and ideal for novice keepers. The outcome of this study therefore, has the potential to have huge implications on captive husbandry routines and could also provide a basis for further studies of a similar nature on other commonly kept snake species.

3.2 Preparation of subjects

Ten snakes were obtained from Reaseheath College and two were loaned from a local pet shop (Discount Koi and The World of Reptiles, Stoke on Trent). The snakes obtained from Reaseheath College were all weighed and measured and the average taken across all snakes. This was carried out so that the two additional snakes required could be matched accordingly so to reduce confounding variables. The snakes were then split randomly into three treatment groups; each group containing four snakes. Prior to the commencement of the experiment it was found that the snakes from Reaseheath College had mites. Therefore the start date was delayed for four weeks whilst they received treatment and confirmed clear of mites. Treatment consisted of submerging the snakes in water to remove any surface mites and then olive oil was liberally applied and left on for twenty four hours to suffocate any remaining mites. This was followed by a weekly treatment of Frontline Mite

Spray. All treatment was recommended and carried out by the reptile keeper at Reaseheath College.

Other than for weighing and measuring purposes the snakes were not handled, again this was enforced to eliminate any effect handling may have had on their behaviour.

3.3 Treatment

Group one were provided with an Arcadia “Natural Sunlight” 2% UVB, 18watt T8 (1” diameter, length 60cm) fluorescent tube (0.519 UV Index). Group two were provided with an Arcadia “D3 Reptile Lamp” 6% UVB, 18watt T8 (1” diameter, length 60cm) fluorescent tube (1.223 UV Index). Group three (control group) were provided with an Arcadia “D3+ Reptile Lamp” 12% UVB, 18watt T8 (1” diameter, length 60cm) fluorescent tube (0.000 UV Index); the UVB of the 12% bulb was blocked using glass. All UVB lights were supplied by Arcadia Products plc, Redhill, Surrey, UK. The bulbs were placed on top of the faunariums above the mesh lids. Each bulb provided lighting for two faunariums; therefore each faunarium had a middle and end section of a bulb (see fig 3.0 for picture of the layout). The ends of a UVB bulb show a significant reduction in UVB output; therefore it was necessary to ensure that each tank had the same exposure (F Baines, personal communication, July 10, 2011). Reflectors were used on each bulb to increase light reflection back into the faunariums, therefore increasing UV output (J Courtney-Smith, personal communication, November 4, 2010).

Frances Baines was consulted when choosing which bulbs to use for this study (personal communication, October 5, 2010). Zoomed and Arcadia were highly recommended due to the results of recent tests (not yet published) that Frances Baines has conducted and Arcadia bulbs were chosen due to their spectrums and availability. The bulbs were 'burnt in' for a period of 100 hours; this eliminates the rapid fall in UVB that initially occurs (F Baines, personal communication, October 5, 2010). Readings were taken from a set distance of 3 inches below the bulb once a week. This was the closest basking spot to the lamp to which the snakes had access. The Solarmeter 6.5 UV Index meter was used to take weekly readings. This piece of equipment was recommended by Frances Baines (personal communication, October 4, 2010) and has previously been used by Ferguson et al. (2010) during their field study of voluntary reptilian exposure to UV light. The UV Index (UVI) is a World wide recognised measurement which was developed by the National Weather Service and the Environmental Protection Agency. It indicates solar UV radiation strength and is measured on a scale from 1 (low) to 11 (extremely high) (UV Index, 2010).

3.4 Housing and heating

All snakes were housed individually in Exo Terra twelve inch square faunarium tanks. Plain white paper was taped to the sides of each tank for two reasons; to eliminate any potential behavioural effect from neighbouring snakes and to create the feeling of a more secure environment. All faunariums were set up to allow expression of natural behaviours. This included the provision of 2cm of substrate (Easibed) to allow for burrowing, a small hide (Exo Terra) to provide a secure retreat, a small water bowl (Exo Terra) for bathing and drinking, and a flexible branch, for climbing (Werler & Dixon, 2004; Love & Love, 2005). All housing equipment was supplied by Hagen (U.K.) Ltd., Castleford, W. Yorkshire WF10 5QH and was purchased by Reaseheath College. The faunariums were set up identically and positioned on a three tier shelf system; four faunariums per shelf (see fig 3.0 for a picture of the tier system). The employed layout differed from the original proposal due to the type of flexible branching obtained and as no heat mat was required (see section 3.5 Heating) (see fig 3.1 for the proposed and employed layouts).

Fig 3.0 Picture illustrating the layout of the experiment. Note the position of the UVB bulbs (taken by the author 11/01/2011)

The digestive efficiency of Corn Snakes is reported to be 85% at 20°C and 89% at 31°C, indicating that their digestive efficiency is somewhat insensate (Greenwald & Kanter, 1979), therefore no temperature gradient was provided to reduce any temperature bias between groups. The room temperature was regulated at 25°c ± 1°c by animal centres heating system.

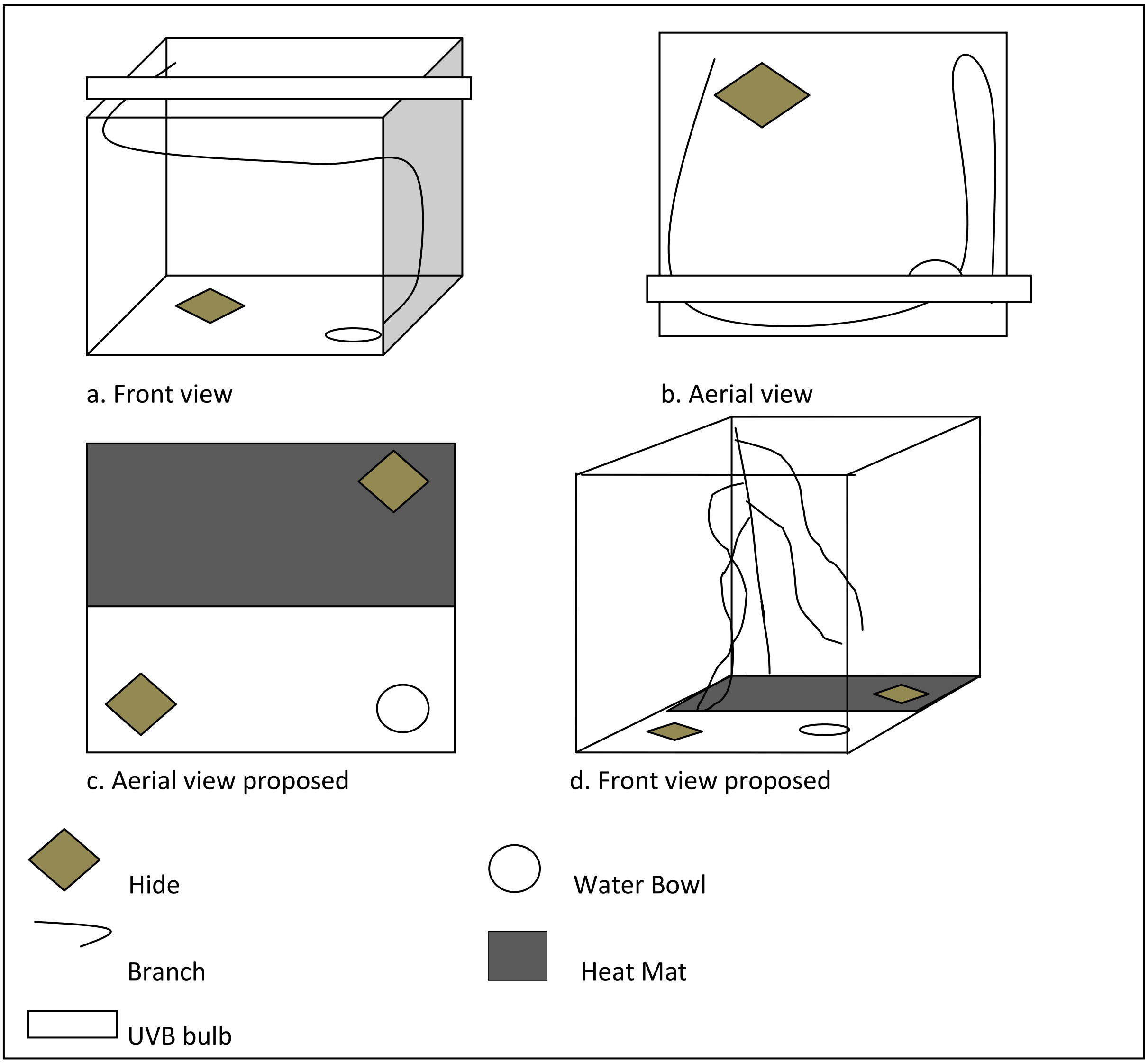

Fig 3.1 Layout of enclosures. a. Front view of the employed enclosure design. b. Aerial view of the employed enclosure design. c. Aerial view of original enclosure design. d. Front view of original enclosure design

3.5 Light cycle

Lighting was provided on a timer of 12hr day/night cycle for all three study periods. This lighting cycle was imperative as the snakes may have entered a period of brumation if the day lengths were around nine hours (Love & Love, 2005) and therefore a reduction of behavioural activity may have been observed. Pre and post experiment lighting was provided by the standard lighting feature in the room. During the experiment the lighting was provided by the UVB bulbs controlled by an electronic timer to ensure consistency.

3.6 Feeding

All snakes were fed twice a week (Tuesday and Friday) on pinkie mice; pinkie mice were aged between one and five days old. Twelve similar sized prey items were chosen, weighed and allocated randomly to each snake. The prey items were weighed to a thousandth of a gram due to their small size.

3.7 Measuring

Snakes were measured and weighed weekly, with the same scales and box used to ensure uniform weighing; the snakes were weighed to the nearest gram. Snakes were photocopied, to scale, for measuring purposes; they were placed onto the photocopier glass and held in place with a piece of disposable hand towel (see fig 3.2 for example). Each photocopy of each snake was then measured three times and the average taken. Total length was measured as the vent was not always clear on the photocopies. This way of measuring has been used previously in the field by Kevin Palmer (personal communication, October 8, 2010). Prior to this the snakes were measured by using a piece of string; starting at the snout and following the length of their spine to the end of their tail. This method however, can prove difficult especially in juvenile snakes due to their small size and rapid movements.

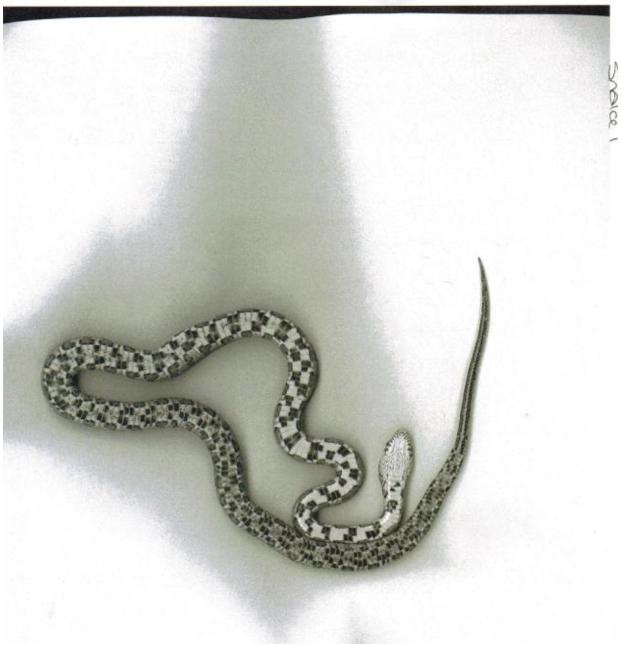

Fig 3.2 Example of a photocopy of a snake for measuring purposes

3.8 Observations

In order to assess the relative activity of snakes between treatments, the position of each snake was observed. The faunariums were divided into 64 grids; each grid measured 3 inches square, width was labelled A to D, depth X to Z and height 1 to 4 (see fig 3.3 for a picture depicting the grid system), and was constructed out of three pieces of cardboard. The location of the snakes head was taken as their grid position. The grid system has been successfully used by Dickinson and Fa (1997) and Bellamy & Stephen (2007) whilst studying enclosure usage. However, inspection of the raw data found many unused grid squares therefore it was decided to alter the grid plan for the purpose of carrying out statistical tests. Originally there were 64 grid locations, each measuring 3 inch squared. This was changed to 16 grid locations, each measuring 6 inch squared. See fig 3.4 for altered grid reference layout.

The snakes were observed for six hours prior to being provided with UVB light, twenty two hours whilst under the UVB light, and sixteen hours after the UVB light was removed (see appendix A for the behaviour check sheet). It would have been ideal to ensure the snakes were observed for an equal amount of time for each study period; however the pressure to start the project lent only for one week preliminary studies and due to deadlines only four weeks post treatment studies. All snakes were monitored during each of the observations, which were carried out between 9am - 12noon and 1pm - 5pm, for a period of two hours. Night time observations could not be carried out, due to health and safety, as no staff members would have been present in the animal centre. Interval sampling was used for each observation. This method was chosen due to the relative inactivity of snakes compared to mammals and also because of the inability to undertake continuous sampling for all snakes at the same time due to lack of cameras. However, to account for periods of activity the interval was set at five minutes. This sampling method was chosen as it has previously been successfully used on ectotherms by Dickinson and Fa (1997) and Bellamy & Stephen (2007).

Grid reference and behaviour were noted during all observations. Behaviours monitored were: out of site (OOS), basking on substrate (BS), basking high (BH), basking medium (BM), basking low (BL), visible in hide (VH), hiding in substrate (HS), actively moving (AM) and in water bowl (W). See appendix B for ethogram. Bellamy and Stephen (2007) also monitored these behaviours during their research with the Jamaican Boa. To validate the ethogram a Cohen's Kappa inter-observer reliability test was conducted. A result of at least 0.6 is classed as a good level of agreement.

The snakes were left to acclimatise to their new enclosures for a period of seventy two hours before preliminary observations began. During the first four weeks of UVB light treatment no observations were carried out. This was due to the promise of camera's being installed. After this time period however it had to be assumed that the cameras would not be available and so physical observations commenced.

Fig 3.3 Picture illustrating the grid system used for observations (taken by the author 20/01/2011)

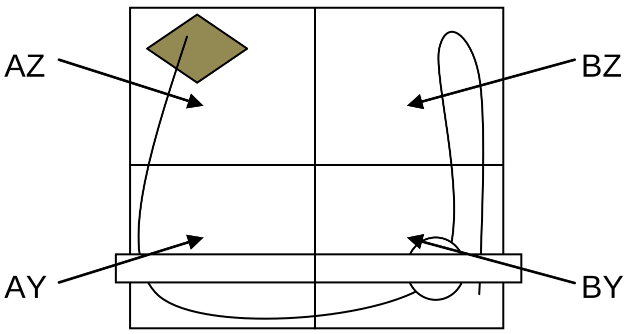

Fig 3.4 Illustration of the altered grid reference layout

3.9 Data analysis

All data analysis was undertaken using SPSS (Statistical programme, IBM SPSS Version 19). See appendix C for raw data. All tests were conducted working to a 95% significance level. Therefore, the p value must be less than 0.05 to be considered significant (Hawkins, 2009).

3.9.1 Normality tests

Prey weights, snake weight and snake length, percentage of grid reference occurrence and percentage of behaviour occurrence were all tested for normality using the Kolmogorov-Smirnov test. Normal distribution can be assumed if the p value is more than 0.05 (Field, 2000). However, as this test "is notoriously affected by large samples in which small deviations from normality yield significant results" (Field & Miles, Discovering Statistics using SAS, 2009) histograms were also conducted.

3.9.2 Snake growth (weight)

A Spearman's Rank Correlation was used to test for an association between prey weight and snake growth. This test was chosen as it tests for an association between independent variables (Hawkins, 2009). A Kruskal Wallis test was used to test for a significant difference between snake weight and treatment group. The Kruskal Wallis test was chosen as it is a non parametric test that analyses if there is a significant difference between the medians of three or more variables.

If there was a significant difference found a Mann-Whitney U test would be conducted. This test was chosen as it is a non parametric test used to analyse whether there is a difference between the medians of two experimental conditions.

3.9.3 Snake growth (length)

Snake length did meet the assumptions of the normality tests (see section 3.9.1) and so the parametric one-way repeated measure ANOVA was conducted to analyse if there was an association between prey weight and snake length. An ANOVA test was chosen as it is a parametric test that analyses if there is a significant difference between the means of two or more experimental conditions; data must be at the scale level (Hawkins, 2009).

3.9.4 Snake behaviour

Tests were carried out on grid location where basking under the UVB light would occur and on grid level (4 levels). Tests were conducted to determine if there was a difference in space usage and behaviour per treatment group.

Grid reference

Grid reference occurrence did not meet the assumptions for normality as mentioned in section 3.9.1. A Kruskal-Wallis test was then used to determine if differences in grid reference occurred on each level between treatment groups. A Kruskal-Wallis test was used as it is a non parametric test that analysing whether there is a difference between 3 or more variables. To ascertain whether variances occurred on each grid level between treatments a Chi-Squared cross tabulation goodness of fit test was conducted. Speaman's Rank Correlation was then conducted to ascertain whether there was a correlation between grid reference and treatment group.

Behaviour

Behaviour occurrence did not meet the assumptions for parametric testing as discussed in section 3.9.1. Therefore non parametric tests were used to further analyse the data. A Kruskal Wallis test was carried out to determine if there was a difference between percentage of behaviour occurrence and treatment. This test was chosen as it looks for a difference between two or more variables that are measured on an ordinal or scale level (Hawkins, 2009). Chi-Squared cross tabulation goodness of fit tests was used to determine whether the frequency of all behaviours varied between treatment groups. This test was chosen as it looks for a difference between the frequencies of more than two variables (Hawkins, 2009).

Kruskal Wallis tests were carried out on basking behaviour to determine if there was a significant difference between percentage of occurrences and treatment group. Mann-Whitney U tests were carried out to determine where the significance occurred if Kruskal Wallis tests were significant. This test was chosen as it is a non parametric test that looks for a difference between two samples measured on an ordinal or scale level.

Finally, Friedman's tests were carried out to determine if there was a difference in the occurrence of behaviours pre, during and post treatment. This test was chosen as it tests for differences between three or more related groups (Field, 2000).

3.9.5 UV Index (UVI)

UVI was measured using the Solarmeter 6.5 UV Index meter. The mean, median, mode and range figures were used for gauging average UVI measurements.

4.0 Results

4.1 Snake growth

4.1.1 Normality Tests

Snake weights and lengths were checked for normality using a Kolmogorov-Smirnov test. The results showed that the p value was less than 0.05 for snake weight ($p = 0.047$) showing that the data was of non-normal distribution and more than 0.05 for snake length ($p = 0.384$) indicating a normal distribution. Histograms were also conducted (see fig 4.0 and fig 4.1) as the Kolmogorov-Smirnov test 'is notoriously affected by large samples in which small deviations from normality yield significant results' (Field & Miles, Discovering Statistics using SAS, 2009).

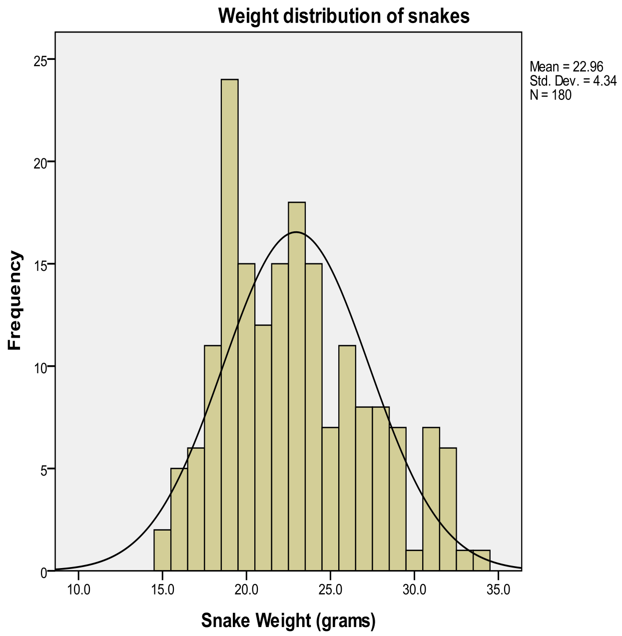

Fig 4.0 Histogram showing snake weights

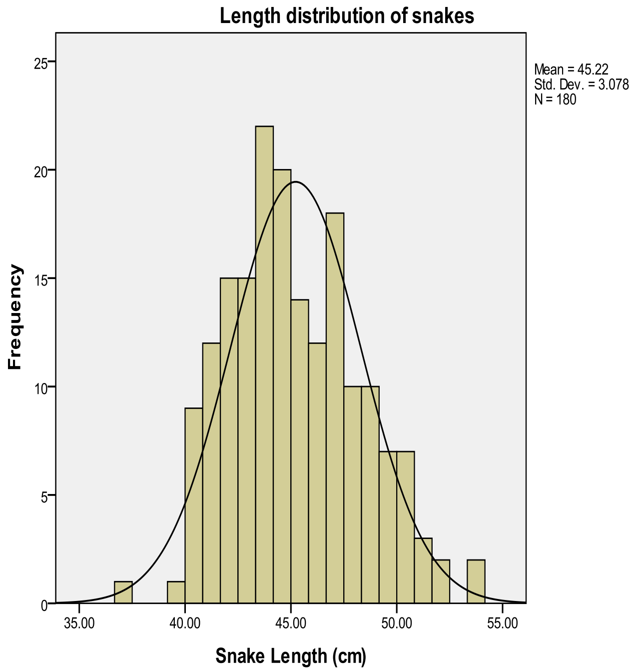

Fig 4.1 Histogram showing snake lengths

4.1.2 Length

H_0 - UVB does not influence the growth length of juvenile *P. guttatus*.

A one-way repeated measures ANOVA was conducted to compare the length of *P. guttatus* between treatment groups, there was a not significant difference in the length of *P. guttatus* between treatment group, $F_{2, 177} = 0.306$, $p = 0.737$. Therefore the null hypothesis can be accepted, UVB does not influence the growth rates of juvenile *P. guttatus*.

4.1.3 Weight

H_0 – UVB light does not influence the growth weight of juvenile *P. guttatus*.

A Kruskal Wallis test was carried out to compare snake weights to the different treatments groups (see fig 4.2 for the supporting box plot graph). There was a significant difference in the weight of the *P. guttatus* when compared to treatment ($X_2 = 7.759$, $n1 = 72$, $n2 = 36$, $n3 = 72$, $P = 0.021$). Therefore the null hypothesis can be rejected, UVB does influence the growth weight of juvenile Corn snakes, *P. guttatus*.

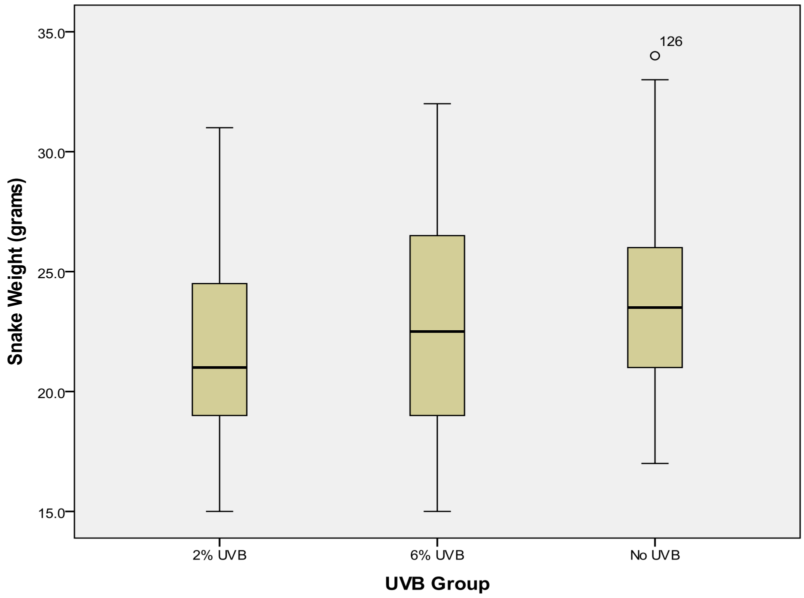

Fig 4.2 Box plot illustrating snake weight differences in relation to treatment group.

A Spearman's rank correlation test was conducted to compare the weight of *P. guttatus* between treatment groups, again this showed a strong positive correlation between the weight of *P. guttatus* and treatment group, r_s=0.207, n=180, p=0.005.

Furthermore, Mann-Whitney *U* tests were conducted to compare the weights of *P. guttatus* between the different treatment groups to determine where the significant difference occurred (see table 4.0 for results).

Table 4.0 Results of Mann-Whitney U tests comparing weights of *P. guttatus* between treatment groups

Group 1 vs. Group 2	**Group 1 vs. Group 3**	**Group 2 vs. Group 3**
U = 1177.000, n1 = 72, n2 = 36, p = 0.436	U = 1892.500, n1 = 72, n2 = 72, p=0.005	U = 1089.000, n1 = 36, n2 = 72, p=0.176

The results showed that the significant difference occurred between groups 1 and 3 ($p = 0.005$). As prey weight was not normally distributed (see section 4.3) it was important to determine if it was this that produced the significant difference between groups 1 and 3. As fig 4.3 illustrates, there was a positive association between prey weight and snake weight between these two groups, however fig 4.9, illustrating the median value of prey weight, shows that prey weight was not a determining factor in the increased weight of group 3. Mean weights for groups 1, 2 and 3 were 22.056, 22.778 and 23.944 respectively.

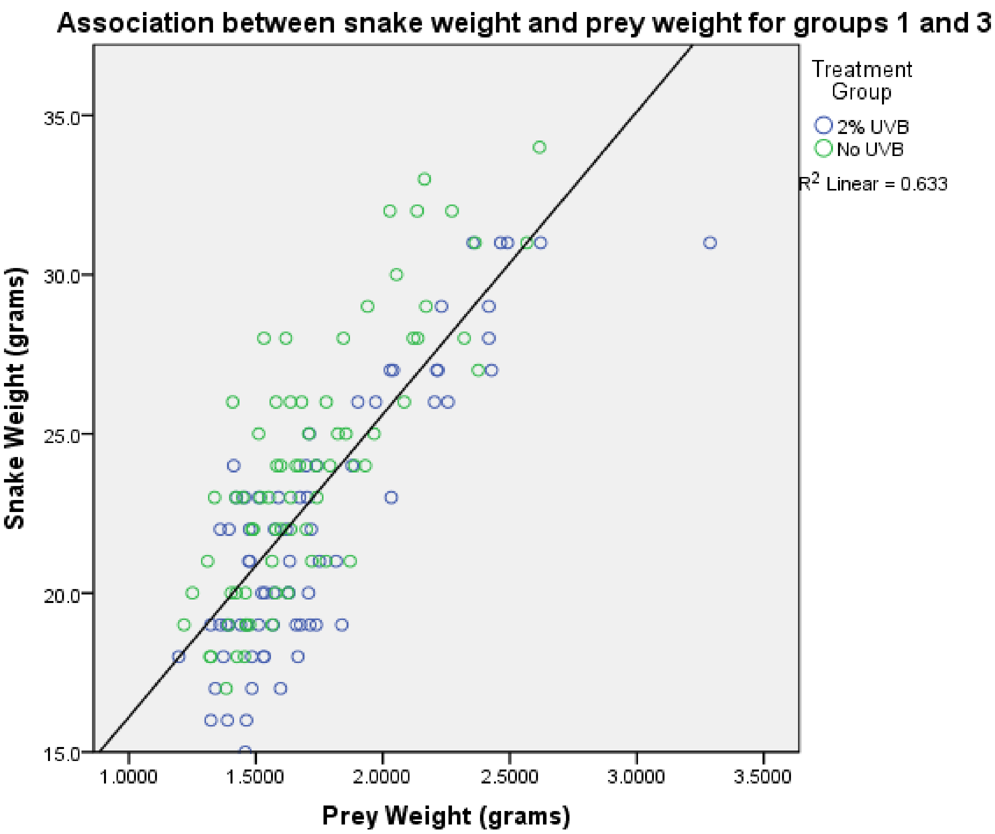

Fig 4.3 Scatterplot illustrating the relationship between snake weight and prey weight.

4.2 Snake behaviour

A Cohen's Kappa test was conducted to test the ethogram for inter-observer reliability. The reliability was found to be Kappa = 1.0 ($p < 0.001$). This figure is interpreted as an almost perfect agreement; however, it must be noted that only two behaviours were performed during the observation.

4.2.1 Normality tests

Kolmogorov-Smirnov tests were carried out on the percentage of grid reference occurrences over all observations (see fig 4.4 for histogram) and percentage of behaviour occurrences over all observations (see fig 4.5 for histogram). The data was of non normal distribution ($p=0.000$) for both sets of data.

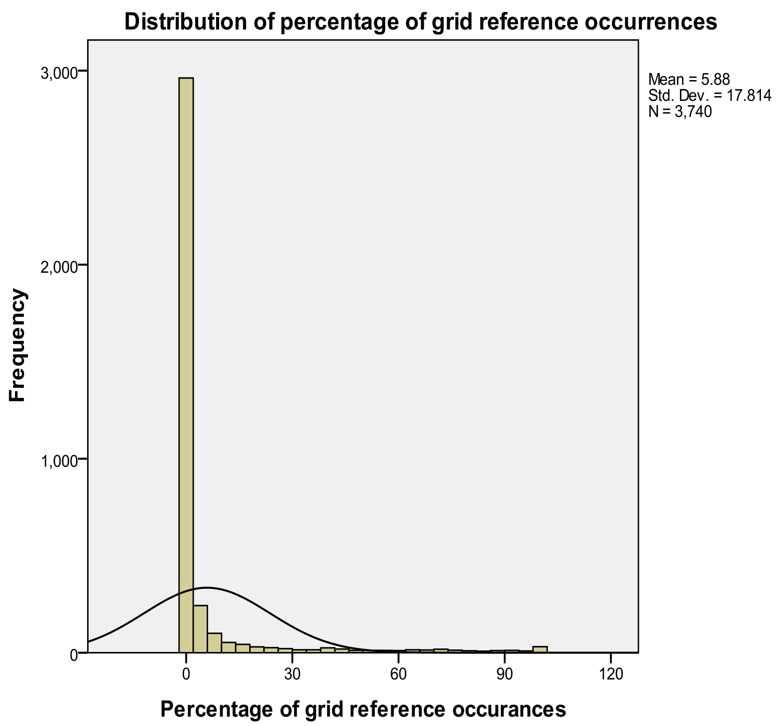

Fig 4.4 Histogram showing the distribution of percentage of grid reference occurrence during all observations.

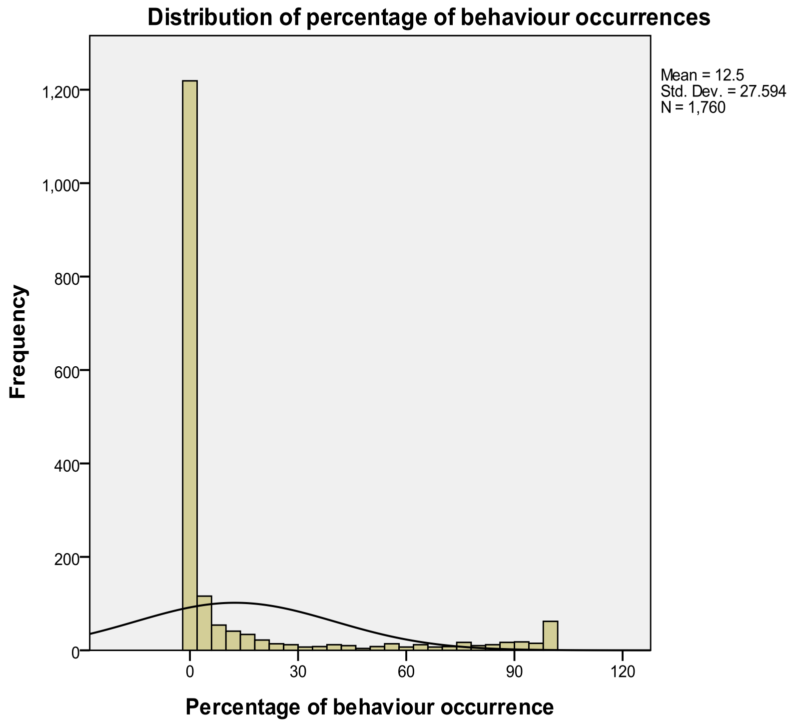

Fig 4.5 Histogram showing the distribution of percentage of behaviour occurrences during all observations.

4.2.2 Occurrences by grid reference

A Kruskal-Wallis test was carried out to determine if there was a difference between the percent of grid reference occurrence on each level and treatment group (see table 4.1 for the results of these tests). Box plot graphs were produced to illustrate the results of these tests (see fig 4.6).

Table 4.1 Results of Kruskal-Wallis test. Is there a difference between the percent of grid reference occurrence on each level between treatment groups?

Level 1	**Level 2**	**Level 3**	**Level 4**
X_2 = 43.361, n1 = 3928, n2 = 2572, n3 = 3864, p = 0.000	X_2 = 36.134, n1 = 116, n2 = 4, n3 = 72, p = 0.000	X_2 = 44.845, n1 = 1456, n2 = 264, n3 = 1208, p = 0.000	X_2 = 154.762, n1 = 3300, n2 = 1560, n3 = 3656, p = 0.000

The results indicate that there was a significant difference between the percent of grid reference occurrence on all levels between treatment groups. All four tests were by weighted by percentage of grid reference occurrence and filtered by each level accordingly when conducted.

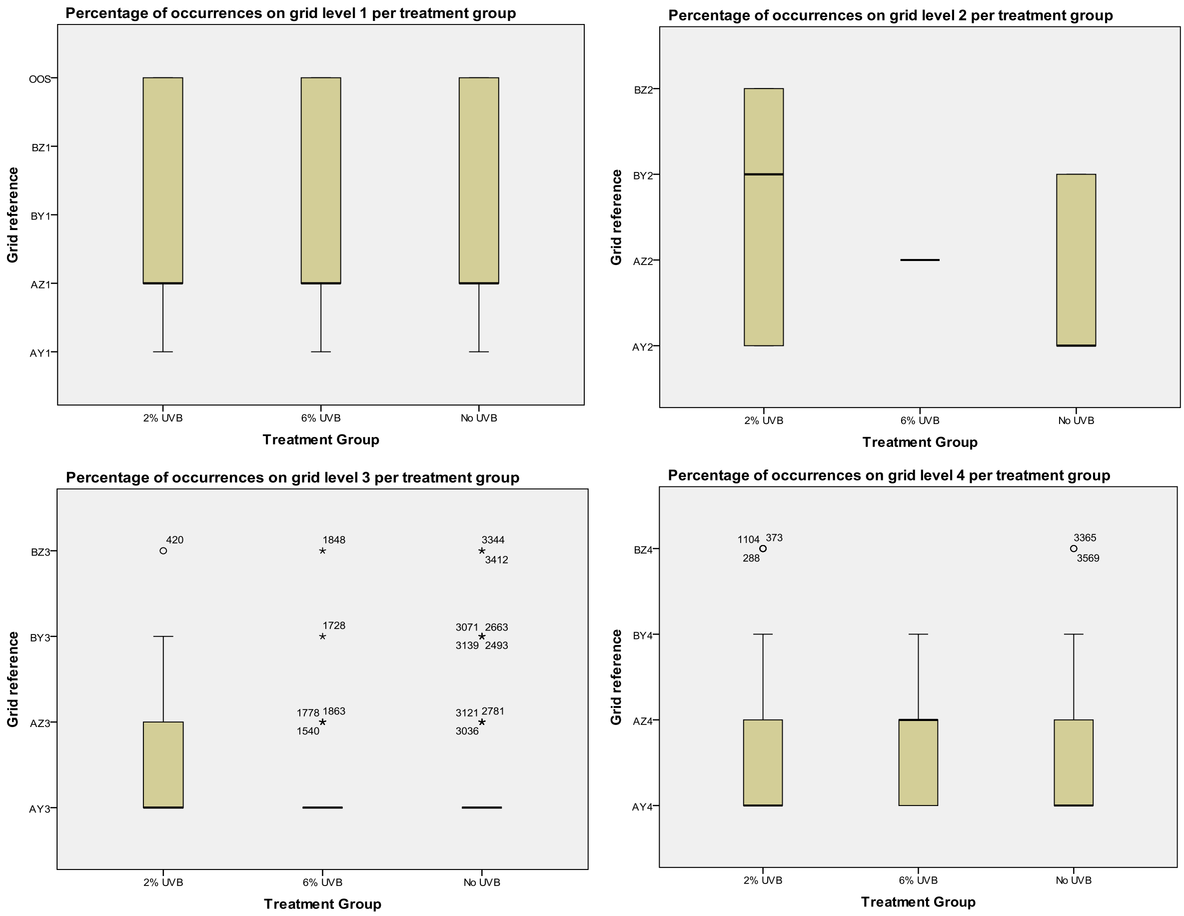

Fig 4.6 Box plot illustrating the percentage of grid reference occurrences per grid level between treatments

A Chi-Squared cross tabulation goodness of fit test was carried out to determine whether the frequencies of occurrences on each grid level varied between treatment group (see table 4.2 for results). All four tests were weighted by frequency of occurrence and filtered by each level accordingly when conducted.

Table 4.2 Results of Chi-Squared cross tabulation goodness of fit test. Do the frequencies of occurrences on each level vary between treatment group?

Level 1	Level 2	Level 3	Level 4
$X^2 = 122.221$, n = 1675, p = 0.000	$X^2 = 20.744$, n = 48, p = 0.002	$X^2 = 13.829$, n = 732, p = 0.032	$X^2 = 71.715$, n = 2145, p = 0.000

The results showed that the frequencies of occurrences on each grid level did vary between treatment groups, therefore Spearman's Rank Correlation tests were carried out on grid references AY and BY and AZ and BZ on each level to determine if there was a correlation between grid reference and treatment group (see table 4.3 for results). All tests were weighted by percentage of gird reference occurrence and filtered by grid reference and level accordingly. The results show that there was a correlation between occurrences in grid references AY and BY on levels 1, 2 and 3, and in grid references AZ and BZ on levels 1, 2 and 4 between treatment groups.

Table 4.3 Results from Spearman's Rank Correlation. Is there a correlation between occurrences in grid reference and treatment group?

	AY and BY	AZ and BZ
Level 1	$r_s = 0.080$, N = 976, P = 0.012	$r_s = -0.095$, N = 5724, P = 0.00
Level 2	$r_s = -0.286$, N = 144, P = 0.001	$r_s = -0.630$, N = 48, P = 0.000
Level 3	$r_s = -0.090$, N = 2428, P = 0.000	$r_s = 0.071$, N = 500, P = 0.113
Level 4	$r_s = 0.012$, N = 4620, P = 0.400	$r_s = -0.061$, N = 3896, P = 0.000

4.2.3 Occurrences by behaviour

A Kruskal-Wallis test was carried out to analyse if there was a difference between percent of behaviour occurrence and treatment. There was a significant difference between percent of behaviour occurrence and treatment ($X^2 = 9.363$, n1 = 8800, n2 = 4400, n3 = 8800, p = 0.009). Further testing on the frequency of behaviours was undertaken to determine which behaviours varied in what appeared to be a significant manner.

A Chi-squared cross tabulation goodness of fit test was carried out to ascertain whether the frequencies of all behaviours observed vary between treatment groups, the results showed that the frequencies of all behaviours did vary between treatment groups (X^2 = 196.473, n = 5500, p = 0.000). Pie charts are also used to show the percentage of behaviour occurrences within groups (see fig 4.7).

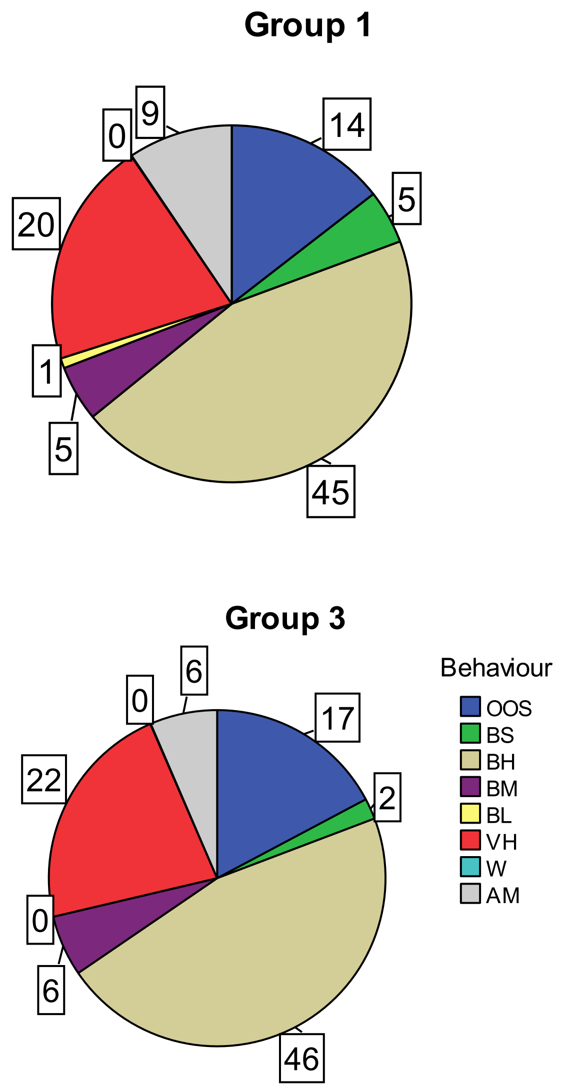

Fig 4.7 Pie charts displaying percentage of behaviour occurrences within treatment group

A Chi-squared cross tabulation goodness of fit test was used to ascertain if there was a difference between out of site (OOS) and visible in hide (VH) behaviour between treatment groups (see fig 4.7 for supporting pie charts). These two behaviours were grouped together as when a snake was OOS they were in their hide. There was not a significant difference between OOS and VH behaviour and treatment group ($X_2 = 5.812$, $n = 2222$, $p = 0.055$). Mean scores of OOS/VH for groups 1, 2 and 3 were 21.09, 23.11, and 21.88 respectively.

Mann-Whitney U tests were carried out to determine if there was a difference between actively moving (AM) behaviour and treatment groups. There was a significant difference between groups 1 and 2 ($U = 1401.500$, $n1 = 88$, $n2 = 44$, $p = 0.005$) but not between groups 1 and 3 ($U = 3377.500$, $n1 = 88$, $n2 = 88$, $p = 0.114$) and groups 2 and 3 ($U = 1665.500$, $n1 = 44$, $n2 = 88$, $p = 0.139$). These results show that group 1 showed significantly more occurrences of AM than group 2 and although group 1 showed more occurrences of AM (see fig 4.7 for pie charts illustrating occurrence within treatment group) than group 3 it was not statistically significant. Mean scores of AM for groups 1, 2 and 3 were 9.41, 2.18, and 6.45 respectively.

Kruskal Wallis tests were carried out to determine if there was a significant difference between basking high (BH) and basking medium (BM) behaviours between treatment groups. BH behaviour was not significantly different between treatment groups ($X_2 = 2.739$, $n1 = 88$, $n2 = 44$, $n3 = 88$, $p = 0.254$). Mean scores of BH for groups 1, 2 and 3 were 44.77, 35.91, and 46.23 respectively. However, BM behaviour was significantly different between treatment groups ($X_2 = 76.231$, n1 =

111, n2 = 50, n3 = 129, p = 0.000). Mann-Whitney U tests were then carried out to determine where the significance occurred. Significant differences occurred between groups 1 and 2 (U = 1634.000, n1 = 88, n2 = 44, p=0.044), and groups 2 and 3 (U = 1612.500, n1 = 44, n2 = 88, p = 0.033) but not between groups 1 and 3 (U = 3832.000, n1 = 88, n2 = 88, p = 0.881). Mean scores of BM for groups 1, 2 and 3 were 5.05, 4.55, and 5.86 respectively.

To determine if a difference in behaviour occurred pre, during and post treatment a Friedman's test was carried out (see table 4.4 for results).

Table 4.4 Results of Friedman's Tests. Is there a difference between behaviour occurrence pre, during and post treatment

Group 1	**Group 2**	**Group 3**
X^2 (1) = 2766.992 P = 0.000	X^2 (1) = 1397.027 P = 0.000	X^2 (1) = 3332.041 P = 0.000

The results confirm that there was a difference in behaviour pre, during and post treatment which suggests that the treatment did have an influence on behaviour.

4.3 Prey weight and Snake weight/length

Prey weights were checked for normality using a Kolmogorov-Smirnov test. The result showed that the p value (0.000) was less than the significant level of 0.05 therefore the data was not normally distributed. This shows that prey weight means differed across treatment groups. A histogram was also conducted to verify this result (see fig 4.8).

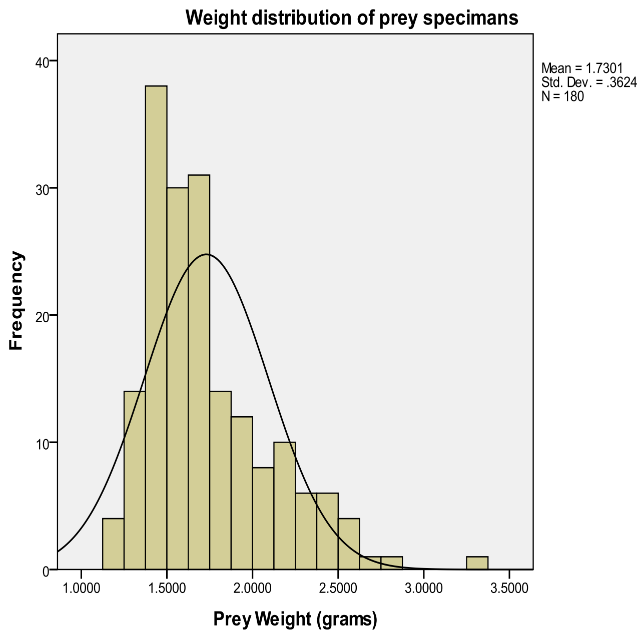

Fig 4.8 Histogram showing prey weights

A Spearman's Rank Correlation test was chosen to determine if there was an association between prey weight and snake weight and prey weight and snake length. There was a strong positive association between prey weight and snake weight (r=0.728, n=180, p=0.000) and prey weight and snake length (r=0.696, n=180, p=0.000). Therefore it can be concluded that snake growth is positively correlated with prey weight. Prey consumption was not analysed as, with the exception of two incidents, all prey was consumed when presented.

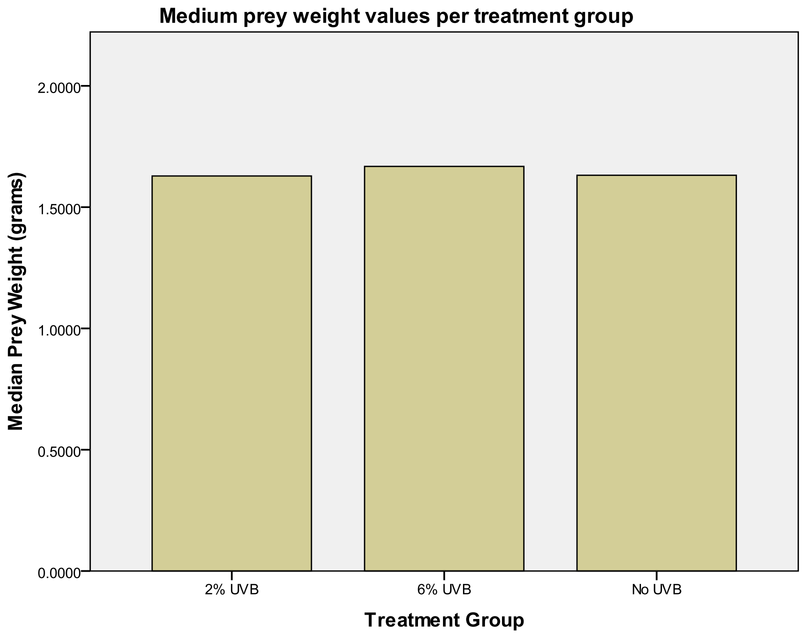

Fig 4.9 Bar chart illustrating the median values of prey weight per treatment group

4.4 UV Index (UVI)

UVI was measured for all groups using the Solarmeter 6.5 UV Index meter (see table 4.5). The mean UVI was 0.519 for group 1, 1.223 for group 2 and 0.000 for group 3.

Table 4.5 Mean, median, mode and range figures for the UVI measurements taken during the study

	Group 1 (2% UVB)	**Group 2 (6% UVB)**
Mean	0.519	1.223
Median	0.500	1.200
Mode	0.5	1.2
Range	0.2	0.4

5.0 Discussion

5.1 Snake behaviour

5.1.1 Occurrences by behaviour

The behaviours that were of most interest were basking high (BH) and basking medium (BM), due to their relative position near and under the UVB light, actively moving (AM) and out of site (OOS) and visible in hide (VH); when the snake was OOS they were positioned in the hide, therefore these two behaviours were grouped together.

There was no significant difference between treatment groups for BH behaviour ($p = 0.254$). This suggests that this position, situated on the branch on the left side of the enclosure, is either favourable regardless of the treatment or due to the limited places off the ground with which to occupy. The frequency counts of BH also showed that this behaviour was observed more frequently than any other behaviour (see appendix D for SPSS output). This coincides with personal observations of juvenile *P. guttatus* tending to be more arboreal than adults which may possibly be an indication of a survival technique. The BH area of the enclosure may have also been seen as a more secure area for the snakes due to the position of the branch; close to the top of the enclosure. Bellamy and Stephen (2007) however did find significant differences in their study on basking on the higher perch behaviour. The UVB exposed group and the Vitamin D_3 supplement group where observed significantly less in this behaviour than the control group.

The BM area of the enclosure, situated on the middle part of the branch at the front of the enclosure, was directly under the UVB light. Therefore, basking position in the enclosure was relative to UVB output. This area was more exposed than other areas in the enclosure; however for snakes to access UVB in the wild they would have to move from a covered, protected area into an open, exposed area so this was not seen as an issue. There was a significant difference in BM behaviour between groups 1 and 2, and 2 and 3 but not between groups 1 and 3. This result shows that group 1 were observed significantly more times BM, and therefore exposing themselves to UVB, than group 2. Group 2 may have been observed BM less due to the higher UVB output of their bulb, the reason for this could be because they did not need to expose themselves to the UVB as much as group 1 because of the higher UVB output. Therefore, less time basking was needed to gain their required vitamin D_3. As group 2 were observed BM it can be concluded that they did not actively avoid this area and therefore the UVB. Group 3 however, were observed basking more times than group 1, although it was not statistically significant (mean scores for groups 1, 2 and 3 were 5.05, 4.55, and 5.86 respectively). Although not specifically tested for, the explanation for this could be because of vitamin D_3 levels. Potentially they sought out the light to access the associated UVB however, as vitamin D_3 levels would not increase, due to the UVB being filtered out, they persisted with their exposure in a further attempt to increase levels. This may indicate that they were actively seeking out UVB associated with light. This behaviour has been noted in the Panther Chameleon. When low in dietary Vitamin D_3, they increased their basking behaviour, under the UVB, in order to synthesis this vitamin through their skin (Ferguson, et al., 2003; Ferguson, et al., 2002). However, if group 2 limited their exposure to the 6% UVB bulb because of the reason stated above, surely group 3 would have been able to detect that there was no UVB from the bulb they were basking under and therefore not persisted in BM behaviour. Belden et al. (2003) has suggested that the Cascades Frog, *Rana cascade*, may not be able to percieve UVB radiation due to a lack of difference in corticosterone levels between UVB exposed and non-exposed tadpoles (see section 2.3) and so whether snakes can actually perceive UVB light is something that would need to be investigated in the future.

AM behaviour was observed significantly more frequently in group 1 than in group 2 but was not significant between groups 1 and 3, and 2 and 3. However, the non significant result between groups 1 and 3 was not expected due to the difference in frequency of occurrences observed (group 1 - 207, group 2 - 24, group 3 - 142). Analysing the frequency of occurrences it appears that UVB has a positive effect on the occurrences of AM behaviour. Chang and Zheng (2003) found that UVB may increase metabolic and enzyme activity and an increase in appetite and activity have also been shown to be stimulated by UVB (Adkins, et al., 2003; Dickinson & Fa, 1997). This may account for the increase in activity levels seen in this study. Similarly, Jamaican Boa’s, another crepuscular species, were found to be more active during the day when provided with UVB light, although this was not statistically significant. They did however, show statistically higher activity levels during night time observations (Bellamy & Stephen, 2007). Unfortunately, night time observations were not possible in the present study for a direct comparison.

No significant difference was found between OOS/VH behaviour and treatment groups. Looking at the frequencies of occurrences of these behaviours group 3 was observed performing these behaviours more times. However, when the smaller sample size of group 2 is taken into account the results may have indicated that group 2 were OOS/VH on significantly more occasions. This could indicate that the higher UVB levels induced more frequent hiding behaviour. Chang and Zheng (2003) note that high UVB may cause damage to reptile skin and so OOS behaviour may have been performed as a protective measure. This result contrasts with the findings by Bellamy and Stephen (2007) on Jamaican Boas who found the highest proportion of significant difference occurred in OOS behaviour.

Only on three occasions, one per treatment group, were noted of *P. guttatus* being present in the water bowl (W), therefore no further analysis was undertaken. One interesting finding of Bellamy and Stephen’s (2007) study found that the Jamaican Boas spent a significant amount of time in the water bowl. The control group spent significantly more time in the water than the other three groups. Research by Packard and Packard (1989) found increased efficiency in calcium and phosphorus metabolism when embryos where incubated on wet substrate as opposed to dry. However, this study was conducted on embryos of the Snapping turtle, *Chelydra serpentine*, a species that lives predominately in the water and so may be the best comparison for terrestrial snake species.

5.1.2 Occurrences by grid reference

Tests conducted to analyse if there was a difference between grid reference occurrence on all four levels and treatment group proved significant (p = 0.000 for all levels). Grid references AY and BY on levels 3 and 4 were classed as the available UVB basking areas and so tests for differences between treatment groups were carried out. Percentage of occurrence on grid reference AY and BY differed on levels 1, 2 and 3 between treatment groups but not on level 4 (see fig 3.4 for grid reference layout). This result was not expected due to the results of frequency distribution between these grid references and treatment group (see Appendix D – SPSS Outputs, for grid reference frequency counts). However, juvenile *P. guttatus* tend to be more arboreal (personal observations) which may explain the non significant result on level 4. Group 1 were observed on considerably more occasions in these grid references on level 3 compared to group 2 but not to group 3. This indicated that level 3 was the most preferable level for basking behaviour under the UVB light.

A study by Huey (1991) investigated ectotherms making habitat selection choices based on their operative environmental temperature, stating that they behaviourally select their environment in accordance to the temperature required to function normally. This may also be the case with UVB. By basking on level 3, rather than level 4, it could be interpreted that they were behaviourally selecting a position that may optimise their UVB exposure. This is an area that would require further investigation.

The frequencies showed, what seemed to be, a significant difference between the occurrences in these grid references of group 1 and 3 to that of group 2; even taking into consideration that group 2 consisted of two snakes as opposed to the four snakes in groups 1 and 3. On level 4, Group 3 were observed basking more times directly under the (filtered) UVB light, followed by group 1 and then group 2. Although the difference was not significant, it could be that group 3 were trying to expose themselves to the light in order to gain access to UVB (see section 5.1.1) and as this was filtered out their counts were higher. The counts in the basking area then appear to filter down as the UVB index got higher so that group 3 did not need to spend as much time under the UVB light to gain their required UVB exposure.

5.2 Snake growth

5.2.1 Weight

It was originally thought that group 1 may have been heavier due to an increase in calcium absorption in the bones. Upon investigation, however, it was found that group 3 were significantly heavier than group 1. As prey weight was of non normal distribution (see section 5.3) this may have been a factor in the significant differences in snake weight, however as fig 4.9 (methods section) shows, this was not the case. Mean weights for each group show that group 2 were heavier, on average, than group 1 so it can be assumed that UVB does not have a negative effect on weight. It is not clear why there was a difference between the weights of these two groups however there are a few theories:

- The UVB levels group 1 experienced may have encouraged hiding behaviour during the day time and higher activity levels at night, this extra activity may have depleted fat stores.
- All UVB bulbs also produce a small amount of UVA, this along with the light levels may have influenced spring/summer hormone levels in group 1 and a winter hormone balance, favouring fat storage, in group 3 due to the UVB and UVA being filtered out (F Baines, personal communication, July 10, 2011).
- Vitamin D_3 levels may also increase metabolism and enzyme activity which may have affected snake weights in group 3 (Chang & Zheng, 2003; F Baines, personal communication, July 10, 2011).

These theories however, do not correspond with the heavier weight found in group 2. Therefore, above all, the small sample size must be taken into consideration as there were only four snakes in each of the two groups. A greater sample size may allow for greater individual weight variation. This appeared to be the case in the Jamaican Boa project where 6 snakes were used per group and no significant difference in snake weight was found (Bellamy & Stephen, 2007).

5.2.2 Length

Tests on snake length confirmed that the data was normally distributed ($p = 0.84$). Upon further analysis no significant difference was found in snake length between the groups, therefore it can be concluded that exposure to UVB does not influence growth in length. No significant difference in snake length was also found by Bellamy and Stephen (2007).

5.3 Prey weight

Tests on prey weight showed non normal distribution (p = 0.000) and the histogram (fig 4.1) illustrated a positive skew. This indicates that prey weight was not equal between groups which may have had an influence on snake growth. Tests proved that snake growth, weight (p=0.000) and length (p=0.000), were directly correlated with prey weight. This has been noted previously in different species (Smith, 1976; ASIH, 1979; Forsman, 1991) and so can be elimiated as a potential bias for snake growth when compared to UVB exposure.

5.4 Implications for captive husbandry

It is well known among herpetologists that snakes are not the most active of creatures, mainly due to them being ectotherms, and many zoo visitors will complain that 'they don't do anything!' (personal observations). The reasons for this inactivity may be due to our lack of understanding of their true requirements. This study indicates that juvenile *P. guttatus* will voluntarily expose themselves to UVB light if provided and the provision of UVB can also enhance behaviour. Therefore, this study shows that *P. guttatus* may benefit behaviourally from the provision of UVB lighting. As group 1 were observed basking under the UVB bulb more than the other groups, this output should be considered a good starting point for captive provisions. The area of which the snakes spent most time basking measured between 0.5 and 0.6 UVI (see section 4.4), positioned approximately 4inches from the bulb. This can be provided by supplying a 2% UVB bulb. The bulb should be guarded, with a similar mesh to that of an exo terra enclosure, to ensure the snake cannot come into direct contact with the bulb. However, further testing to ascertain whether the 2% bulb is preferable would be required before a definitive answer on captive provisions can be made.

5.5 Study limitations

The original proposal incorporated twenty snakes split into four groups; control group and three treatment groups. It was envisaged that twenty snakes would be obtained from four different clutches of similar hatch dates (five snakes from each clutch) from Monkfields (a UK reptile supplier). One snake from each clutch would then be randomly allocated a group. However, due to limited funding the amount of snakes was reduced to twelve and the groups reduced to three; control group and two treatment groups. Due to a reduction in the number of subjects the 12% UVB group was omitted as this spectrum is used for desert species and so would be considered an unnatural UVB exposure to *P. guttatus* and is likely to be of limited use as a result. In addition to the initial reduction in study specimen numbers two snakes from group 2 had to be removed due to a mite problem. This may have altered feeding and influenced behaviour to an extent that it may have resulted in false reflection of the results. The reduction in the number of study specimens may have affected the validity of the study. However, small study numbers in captivity is common (Mundry & Fischer, 1998). de Azevedo, Cipreste and Young (2007) state that a minimum of four subjects should be used to enable a statistically significant result, however, six subjects are generally considered to constitute the minimum sample size. Future studies should therefore endeavour to instigate rigorous quarantine periods to eliminate any potential problems related to health for future studies.

It was envisaged that video cameras would be used to enable twenty four hour recording periods to be carried out. Constraints regarding the availability of video surveillance equipment meant that this could not be implemented. To compensate, physical observations were carried out. Observations carried out during the night may have provided further data to show an increase/reduction in activity as seen in Bellamy and Stephen (2007).

Due to time restrictions it was not possible to carry out observations on the snakes for an equal period of time before, during and after the treatment. Because of this, the observations carried out prior to treatment being commenced may not have given a good indication of behaviour occurrence. Although this was only a small part of the research it must be noted. It is recommended that the amount of time observed during treatment should be matched prior to and after treatment has been implemented to ensure equal observations periods.

5.6 Considerations for future studies

Future studies should incorporate a larger sample size, ideally at least 8 snakes per group; to ensure adequate data is collected. This means that results can be interpreted without the small sample size bias that generally occurs with captive research (Mundry & Fischer, 1998). A larger sample size would also allow for weight variation, a potential limitation of one area of this study, and for any potential loss of specimens.

If possible, dual-energy X-ray absorptiometry (DXA) should be carried out to determine if exposure to UVB increases bone density in comparison to a control group (Secor & Nagy, 2003). Therefore, providing evidence that exposure to UVB light increases calcium absorption in the bones of snakes.

Improvements to the experimental design of this study are as follows:

- As mentioned in section 3.3 (methods section), the output of UVB tapers off towards the ends of the bulbs. Therefore if future studies use one bulb for two enclosures they should ensure that the set ups are a mirror image to ensure basking sites in each tank are exposed to the same amount of UVB (see fig 5.0 for an illustration).
- Flexible branches should be replaced by fixed areas for basking to reduce any bias on basking location between enclosures. Although this was generally not a problem the flexible branches had to be readjusted on numerous occasions. It may also be an idea to provide basking sites at different levels so the snake can chose the best exposure as discussed in section 5.1.2.

An alternative experimental design for ascertaining whether snakes require UVB light is to create a choice or preference test. This would eliminate the bias for preferred basking heights and allow the snake to choose to bask between non UVB emitting light and a range of UVB emitting lights. Although the snakes in group 2 were not observed basking under the UVB light as much as group 1 one cannot eliminate this group purely based on this. Therefore a choice of UVB bulbs should be provided and the snakes allowed to choose their preference.

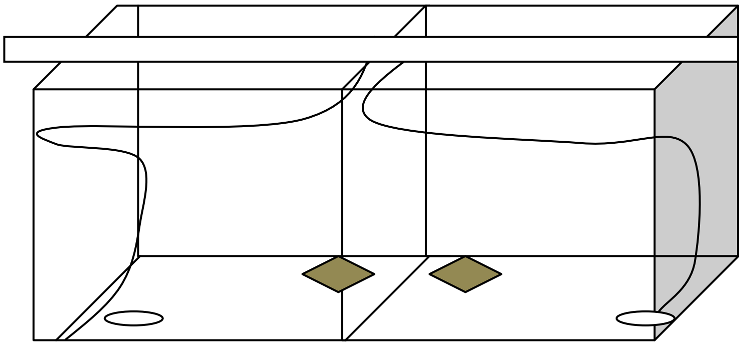

Fig 5.0 Illustration of recommended mirror image enclosure set up for future studies

5.7 Conclusion

In conclusion, this study shows that snakes will voluntarily expose themselves to UVB light and that it has a positive effect on their activity levels. UVB exposure of 2% saw a notable difference in behavioural activities when compared to the other two groups in this study. It is known that *P.* guttatus can synthesis vitamin D_3 through exposure to UVB (see section 2.4) and so it is important to recognise that it may therefore have an influence on other metabolic processes. Future studies should incorporate a greater number of individuals to eliminate the small sample size bias seen in captive studies. This study provides further, much needed, evidence of the potential health benefits of providing UVB to snakes in captivity.

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