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Effects of Ultra-violet B on the behavior of red-eared sliders (Trachemys scripta elegans)

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Effects of Ultra-violet B on the behavior of red-eared sliders (*Trachemys scripta elegans*)

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Abstract

Ultra-violet B (UVB) is a light ray given off by the sun that is a vital component for vitamin D synthesis in reptiles. Studies have found turtle retinas spectral sensitivity to be in the 370nm-380nm ranges, to the author's knowledge studies have not been conducted on how turtles regulate their activity in order to acquire adequate levels of UVB. Red-eared sliders (*Trachemys scripta elegans*) were used as a model in this experiment, due to their ability to thrive in a variety of environments. For the current study, the role of different types of artificial ultra-violet B exposure on the behavior of red-eared sliders was studied. Twenty-one subjects were randomly assigned to one of three different conditions (only UVA, UVA/UVB from same source, or UVA/UVB from two different sources) and their position was plotted, in order to determine the effect of UVB exposure on locomotive behavior. The subjects were also observed in a Y-maze to test for their ability to differentiate between two different sources of light. These studies tested the predictions that (1) turtles raised under mercury vapor artificial lamps will display the greatest amount of time spent in ultra-violet zones, and (2) turtles are able to differentiate between UVB and non-UVB light sources. Prediction 1 was partially supported, while prediction 2 was met. Differences in housing conditions were significant in relation to their choices in the y-maze. Moreover, only zone 2 of experiment 1 showed significant differences in the amount of time spent basking between each of the conditions.

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As human populations increase, biodiversity is put at greater risk worldwide. Declines in biodiversity have prompted a need to manage wild populations in captivity in order to prevent endangerment and/or extinction (Walker, 1992). Ex-situ conservation is a key component in saving species where in-situ conservation is no longer an option, due to habitat being destroyed, lost, etc. While there are multiple obstacles and complications with captive breeding (such as bottle neck effect, maintaining healthy age structures, and disease prevention) (Willoughby, 2014), efforts are being made to investigate and implement optimal conditions to maintain stable populations within zoos (Snyder, 1996).

Most reptiles are ectothermic vertebrates that are also unable to endogenously produce sufficient level of Vitamin D and thus rely heavily on their environment for temperature modulation and obtaining Vitamin D. Basking by reptiles is thought to have evolved for thermoregulation (Ferguson et al., 2003). Some other possibilities for this behavior may be to reduce parasite load by drying off their carapace upon leaving the water, fever response to infection, and stimulation of Vitamin D_3 synthesis (Ferguson et al., 2003). The importance of Vitamin D in reptiles is indicated by studies in which captive and wild animals exposed themselves to ultra-violet B (UVB radiation). Ultra-violet B is one of the three types of invisible light rays (together with ultra-violet A and ultra-violet C) given off by the sun. UVB is a medium wavelength that is biologically active but is mostly filtered out by the atmosphere. The primary function of vitamin D_3 in vertebrates is to maintain and regulate calcium homeostasis. This is possible due to the increased absorption of calcium through the intestinal tract, which aids in bone mineralization (Ferguson, 2009). In many vertebrates, UVB radiation causes provitamin D (7-dehydrocholesterol) in the skin to form previtamin D_3 (Ferguson et al., 2002). After hydroxylation in the liver, the inert vitamin becomes 25-hydroxyvitamin D_3 (Ferguson et al., 2002). Then, this vitamin is carried to the kidney where it becomes the active protein form 1,25 dihydroxyvitamin D or calcitriol, which is a hormone whose main function is to facilitate the uptake of calcium in the intestinal tracks of adults or juveniles, or from yolks and shells of developing embryos (Ferguson et al. 2002).

Animals can obtain vitamin D_3 through food sources or synthesize it through the skin (Karsten et al. 2009). Evidence exists that provitamin D_3 is located in the membrane fraction of skin cells (Holick at al. 1995). UV radiation exposure is a more efficient manner of vertebrates increasing the levels of circulating 25-hydroxyvitamin D_3 than supplemental concentrations of vitamin D through food sources. Also, reptiles seem to regulate the intake of UVB through exposure to UV light better than the regulation of dietary supplementation, so regulation of exposure to UV light may be preferential to the supplementation of vitamin D to the food of reptiles (Ferguson et al. 2002). While food supplementation may be important, research shows that at least in some species (i.e. Hermann's Tortoise [*Testudo hermanni*]) biosynthesis of vitamin D is dependent on UVB radiation (Selleri et al., 2002).

Many reptile curators and herpetoculturists believe that natural lighting is the best type of light for reptiles (Selleri et al. 2012). However, in captivity, there exist various sources of UVB radiation (i.e. self-ballasted mercury vapor lamps) that are available for captive reptiles. In order to provide adequate levels of UV through these artificial sources, attention must be paid to distance of reptiles from light source, as well as any barrier used to enclose the said animal. Research has shown that consideration of UVB attenuation by light-transmitting materials, such as enclosure tops, is equally important as the assessment of the quality and quantity of UVB emitted from light sources (Burger et al. 2007).

In the past four decades, it has been found that a number of vertebrate species possess vision in the UV wavelength. Behavioral studies have shown that birds, fish, and turtle are sensitive to UV light (Ventura, 1998). The ability to discriminate in the UV was demonstrated in turtles (Arnold & Neumeyer, 1987), pigeons (Emmerton, 1983), and hummingbirds (Ventura & Takase, 1994). Research finds that sea turtles are well adapted to seeing UV light. Studies by Wang (2013) show that the mean sea turtle capture rate was reduced by 39.7% in UV-illuminated nets compared to nets without illumination. This finding in conjunction with the possibility that up to 10% of the cones in the retina are designed specifically to pick up sources of UVB raises the question if turtles visually seek out sources of UVB.

Some species of animals are tetrachromatic, meaning they have four color-sensitive cone types. However, UV cones are not exclusively present in tetra chromatic retinas, they can also be found in some dichromatic mammals (Ventura et al., 2001). The functional architecture of the turtle visual system has been extensively studied and has supplied a large amount of information on chromatic processing (Ventura et al., 2001). Turtle retinas were measured to have maximal spectral sensitivity in the ultraviolet range 370-380nm (Arnold et al., 1987). This wavelength, however, is in the Ultraviolet-A range (315-400nm). Ultraviolet-B, which is responsible for the synthesis of vitamin D, falls within the range of 280-315nm.

Research so far has suggested considerable variation among species in the importance of UVB and the production of vitamin D. Species with naturally high levels of vitamin D in their diet, such as dogs, cats, and polar bears, seem unable to endogenously produce vitamin D from UVB exposure. By contrast, reptiles such as iguanas and bearded dragons with naturally low levels of vitamin D in their diet mainly use the UVB- mediated endogenous source (Allen et al., 1999). UVB radiation can be obtained through exposure to natural sunlight or some source of artificial light. Research on green iguanas suggests that the dietary route is not only less efficient, but insufficient to maintain proper vitamin D levels. To gather enough UVB radiation through dietary measures alone, the huge amount of supplementation needed would lead to vitamin D toxicity (Allen et al., 1999).

The species in focus for this experiment is the red-eared slider (*Trachemys scripta elegans*). This is a subspecies of the common pond turtle (*T. scripta)*. The red-eared slider is widely distributed on all continents except Antarctica, because of its preference to shallow water with aquatic vegetation in lakes, channels, and rivers of slow stream (Ernst and Barbour 1972; Gibbons et al. 1979). Thermal preferences are between 25 and 31 degrees Celsius, and heat tolerance reaches 43 degrees Celsius (Hutchinson et al. 1966). Its natural distribution ranges from the Mississippi area in Illinois to the southern Gulf of Mexico in North America (Hutchinson 1992). Because of its high adaptability, this species has become a pest in many countries, notably Australia. Slider populations have forced the Department of Agriculture and Food in western Australia to declare it a class 1 pest (Department of Agriculture and Food, Western Australia 2015). The study aims to help answer broader questions, including why this species has the ability to acclimate to such varied environments, and what mechanisms may play a role in helping chelonians thrive and metabolize the energy from the sun.

The focus of this study is to better understand the effects of Ultraviolet-B (UVB) on chelonians. Research has shown that at least one reptile species (i.e. panther chameleons (*Furcifer pardalis*) is able to differentiate between levels of exposure to UVB, and regulate their exposure dietary vitamin D intake (Ferguson et al., 2002). The order of Chelonia is a highly vulnerable group within our animal kingdom. Adaptations in their retina have equipped them with the ability to detect and discriminate UV stimuli (Hart et. al 2006). Diverse behavioral data have been gathered on turtles' and tortoises' abilities to discriminate between different colored stimuli, but not on their ability to discriminate between ultraviolet ranges of the light spectrum. To the author's knowledge, there also appears to be a lack in data on the effect UVB has on activity budgeting, specifically locomotive behavior. Measuring the effects of different sources of UVB radiation on their activity budget and testing the turtles' ability to discriminate between light sources of different UVB wavelengths are two ways in which we can better understand the effects that UVB rays have on the behavior of the red-eared slider. It is predicted that the mercury vapor bulb condition will produce the greatest amount of time spent in the UVB zone of the enclosure. Also, it is predicted that the turtles will in fact be able to differentiate between two identical light sources with different wavelengths of UVB.

Research Design, Materials, & Methods

Twenty-one unsexed Red-Eared Sliders were obtained from the Staten Island Zoo's reptile department. They were approximately 30 days old and measured roughly 2.54 cm in carapace length. The subjects were set up in order to observe their behavior under ultraviolet-B radiation. Two experiments were conducted, both during 2015. In the first experiment, the turtles were housed in AGA aquariums measuring 76.2 cm x 30.48 cm x 30.4 cm. The water level was 10 cm deep, totaling about 16 liters of occupied water volume. Each enclosure was provided a basking platform on each end of the enclosure measuring roughly 30.48 cm x 15.24 cm. Room temperatures were held constant at 24.0 degrees C and all enclosures were given a 37.0 degrees C basking spot and a water temperature of 24.0 degrees C. Three light bulb arrangements were provided (Table 1), with each of the lighting cycles set on a 12:12 day/night cycle.

TABLE 1: Description of light treatments

Treatment	Heat Light source	UVB Light source	Hours per day
1	Zoo Med Repti halogen bulb (100-watt)	None	10
2	Zoo Med Repti halogen	Zoo Med ReptiSun	10

	bulb (100-watt)	5.0 T5 HO (24-watt)	
3	Zoo Med Mercury Vapor Lamp (100-watt)	Zoo Med Mercury Vapor Lamp (100-watt)	10

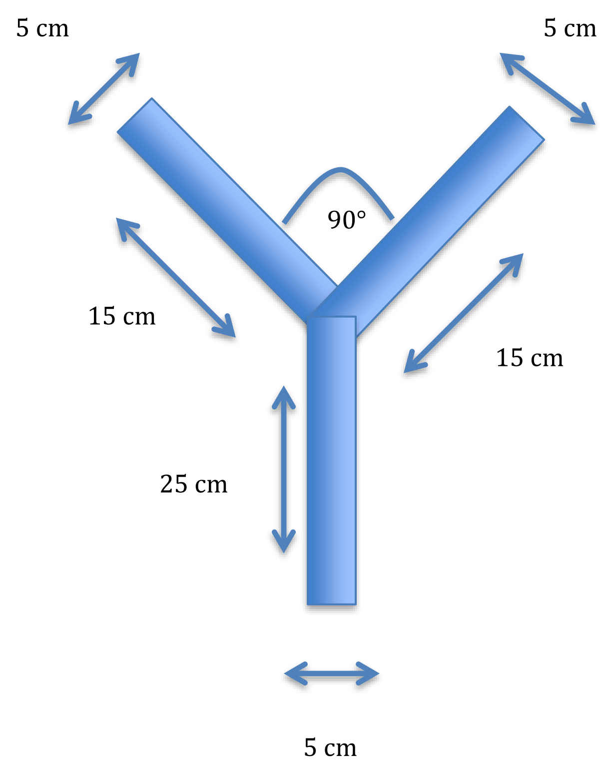
Research by Gopar-Canales et. al. has shown the importance of circadian regulation of locomotor activity. The turtles were then randomly assigned into one of the three treatment groups, with each treatment group containing seven subjects. They were marked with individual accession numbers from 1-21 using a white marker. Diet was then restricted to Zoomed natural aquatic turtle diet without supplemental Vitamin D3, four times per week. For this experiment, the independent variable was the type of exposure to ultraviolet light (UVB). The control group did not receive a UVB source, only a UVA source provided by an incandescent bulb. Group 1 received a concentrated UVB source from a Zoomed Power sun mercury vapor bulb, which provided a focal source of UVA and UVB. The third Group received UVB through a Zoomed t5 HO (high output) bulb measuring 76 cm. in length with a separate UVA source (Figure 2). The dependent variable was the time spent in each zone. In order to measure the time spent in each area, a camera was placed overhead and the location of each turtle was plotted every 5 minutes. A marker outside of the aquariums was used as reference to divide the aquarium into 3 equal 25 cm x 30 cm sections (Figure 1).

FIGURE 1: Overhead view of each condition used in Experiment 1 (Left to Right; Condition 3 (UVB/UVA same source), Condition 2 (UVB/UVA separate sources), Condition 1 (UVA, no UVB)

After data was gathered, a mixed model analysis was run using JMP statistical software to compare results between conditions.

In experiment number 2, the same 21 subjects were housed in identical conditions as experiment one. Furthermore, they were subjected to a maze (Figure 2) built by Dr. Henning Voss. The maze was rested on top of a 4 cm platform under the starting position in order to facilitate movement of the turtles in the direction of the exit. The maze consisted of an enclosed Y-shaped PVC piped, crafted out of plastic with a 3D printed.

FIGURE 2: Schematic representation of the Y-maze apparatus used in the UVB maze experiments



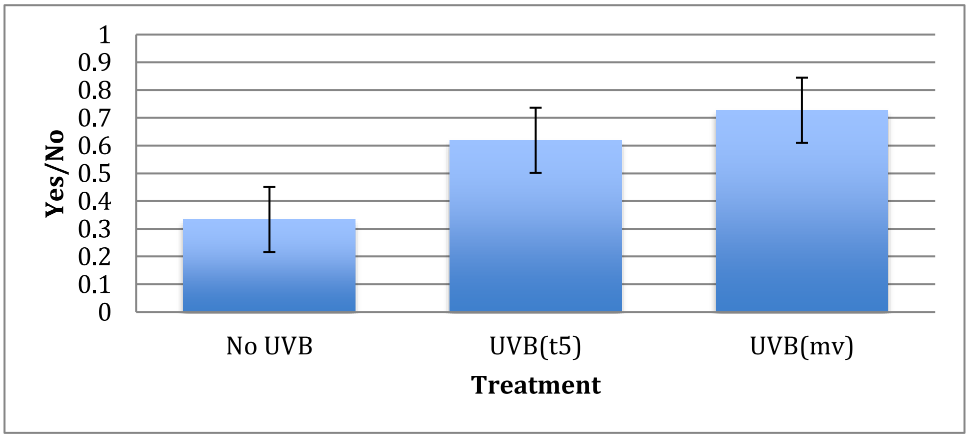
During experiment number 2, an enclosed Y-shaped maze was placed in an AGA aquarium measuring 76.2 cm x 30.48 cm x 30.4 cm. At the end of the aquarium, the split end of the maze was separated by a glass sheet (30.48 cm x 30.48cm x 0.1cm) perpendicular to the floor and met with the mesh aquarium topper. This sheet of glass prevents UVB rays from penetrating from one side to the other. Each side of the maze had its own source of light. It was switched after every trial in order to prevent bias. A Zoomed Repti-Halogen 100-watt bulb provided the non-UVB light, whereas a 100-watt Zoomed Mercury Vapor bulb provided the UVB light. These bulbs were chosen because of the similar light spectrum but absence of UVB from one bulb. Using a Zoomed digital ultraviolet radiometer, safe levels of UVB were maintained through the duration of the experiment. Each turtle was inserted into the maze in no particular order and allowed infinite time to exit through either one of the ends past the Y-junction (Figure 3). Choices were recorded after each trial and analyzed using a nominal logistic regression.

FIGURE 3: Overhead view of Experiment 2 y-maze

Results

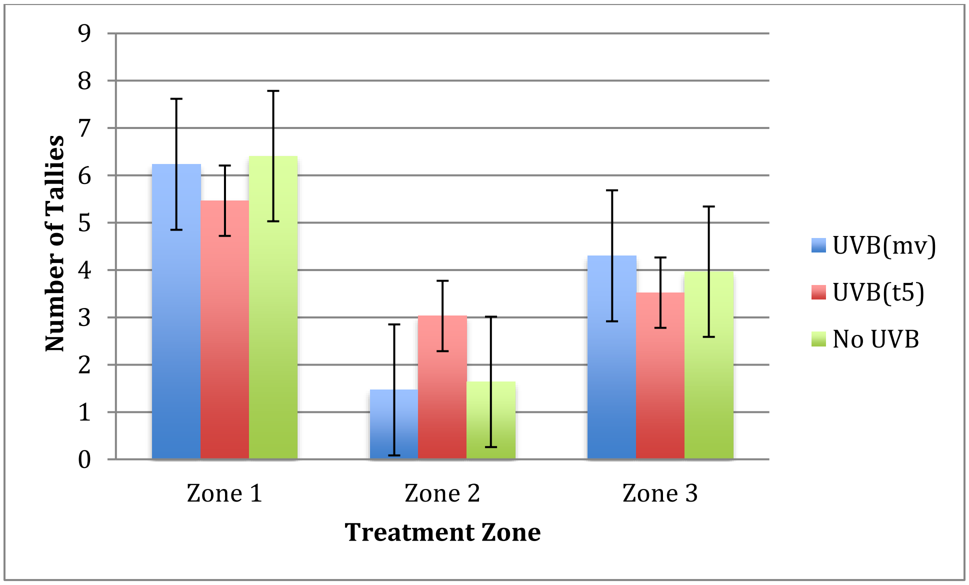
I conducted a nominal logistic regression in JMP with treatment as the predictor variable, to test for red-eared sliders ability to distinguish between a UVB and non-UVB light source. Differences in the choices between UVB and non UVB light sources was significant, X^2=25.33,p<0.0001. As shown in figure 4, the average number of "yes" choices in the UVB choice maze increased from condition 1 to condition 3. Subjects from conditions 2 (UVB from t5) and 3 (UVB from mercury vapor bulb) on average chose the side of the maze with the UVB component 62 and 72 percent, respectively. This can be compared to the first condition (no UVB), which chose the UVB side of the maze only 32 percent of the time.

FIGURE 4: Average number of yes choices in UVB maze



To test for differences in amount of time spent in each zone of the assigned conditions, I conducted a mixed model analysis in JMP with treatment as the predictor variable and ID and Date as the random effects. There was no significant difference reported in zone 1, $F(2, 17)=0.97$, $p>0.40$. Neither was there a significant difference in zone 3, $F(2,17)=0.80$, $p>0.47$. However, there was a significant difference in zone 2, $F(2,17)=7.95$, $p>0.0037$. As shown in figure 5, red-eared sliders raised in condition 2 spent a significantly larger amount of time in zone 2 than the turtles raised in conditions 1 and 3.

FIGURE 5: Comparison between average times tallied in each zone per treatment



Discussion

Results from this study partially support the hypothesis that turtles would spend more time in the UV zone of the mercury vapor lamp condition. Even though the turtles did spend more time in the UV zone, it was condition two (t5 fluorescent lamp) that produced the most significant results. Moreover, the y-maze experiment supported the hypothesis that the subjects would be able to differentiate between two equal light sources with different UVB outputs. According to Arnold (1987), the maximal spectral sensitivity of turtles is 370-380 nm. To the best of the author's knowledge, the mechanism used to detect these wavelengths is still unknown. Further behavioral studies need to be conducted in order to determine if the presence of this fourth UV cone works in conjunction with UV receptors in the skin to attract these turtles towards UVB sources, or if there may be another component (i.e. neurological, physiological) that allows them to voluntarily expose themselves to ultraviolet rays.

Placement patterns showed the greatest disparity in time spent in zone 2. Turtles in condition 2 spent roughly twice the amount as condition 1 and 3 in the UV zone 2. Because exposure to UV radiation is a more effective way of raising 25-hydroxyvitamin D_3 than dietary supplementation of vitamin D (Karsten, 2009), and because reptiles such as panther chameleons show the ability to regulate UV exposure based on dietary levels of vitamin D, exposure to vitamin D synthesizing wavelengths may be more beneficial. Acierno (2006), showed that pre vitamin D_3 concentrations differed significantly between turtles provided supplemental UV radiation than those not provided supplemental UV radiation, whether from artificial UVB sources or natural sunlight. Hermann's tortoises were also documented to have higher circulating levels of previtamin D_3 when exposed to UVB. Also, it was noted that Hermann's tortoises kept in captivity outside of their natural range were more effectively synthesizing vitamin D when exposed to artificial UVB sources than tortoises that were kept in sunlight at higher latitudes (Ferguson et al., 2002). Experiment 1 shows us that red-eared sliders may also be amongst the species able to willingly regulate their exposure to UV rays. While blood samples were not collected in order to test circulating levels of the previtamin D_3, it is plausible to hypothesize that red-eared sliders in condition 2—which had access to a UVB component separated from a UVA Heat bulb—were regulating their exposure to UVB.

Results from other studies (Ferguson, 2009; Karsten, 2009) have indicated that some reptilian species (i.e. red-eared sliders, veiled chameleons) produce optimal levels of vitamin D through a balance of diet and UV exposure. Bearded dragons (*Pogona* vitticeps) have also been noted in other experiments to eat more vitamin D rich foods when circulating levels of previtamin D_3 are low (Selleri et al., 2012). Although the extent to which reptiles can regulate their vitamin D on diet alone is unknown, vitamin D toxicity is possible if over-consumed. The red-eared sliders in this study were kept on the same diet of nutritionally balanced commercial pellets for aquatic terrapin species. Again, without blood samples it is not possible to determine if these subjects had adequate levels of vitamin D before the start of the experiment or during the trials. It is possible, however, to note that whether adequate levels of vitamin D were met in each subject or not, there was a significant behavioral difference between subjects kept in an enclosure with separate UVB and UVA heating components.

Results of this study provide important behavioral data regarding the ability of red-eared sliders to differentiate between light sources emitting UVB and those not emitting UVB. If combined with blood samples, this data may better help us understand the optimal setting for raising turtles in captivity. Additional long-term studies are needed to see the impact artificial UVB bulbs have on animals that may be released back into the wild or on long-term biological success (i.e. hatchling success rate, growth rates, mating success) of captive individuals. However, the findings of this study suggest that turtles with the option of a UVB source away from a heat source will spend more time "basking" in these zones.

The findings of this study create the possibility of red-eared sliders being used as an indicator species in ecological monitoring, especially in the context of assessing dangerous level of UVB penetration through our atmosphere. If connections can be made between optimal levels of UVB exposure in red-eared sliders and safe levels of human absorbance of UVB, this species may become a valuable indicator as a UVB index. With significant changes in physical and biological systems occurring all over the world, anthropogenic climate change is a real concern to global physical and environmental systems (Rosenzweig, 2008). If a better understand is gathered on the way UVB affects red-eared sliders biologically and behaviorally as a system, this species may prove to be a valuable indicator species.

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