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Investigating the Thermal Biology and Behaviour of Captive Radiated Tortoises

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Abstract

Thermoregulation is integral to the maintenance of reptile biological function and health, and therefore is a key area of investigation for herpetologists. To investigate the relationship between core body temperature and behaviour, a behavioural study was conducted in which iButton data loggers were placed on a group of captive radiated tortoises (Astrochelys radiata) located at Sparsholt College's Animal Management Centre, in Hampshire, UK. Correlations between core body temperature and specific behaviours were covered. Body mass had a significant effect on average core body temperature (P= <0.0001) with higher average temperatures recorded in larger individuals over longer periods. There was a significant positive relationship between mean body temperature and basking behaviour, (P= 0.001, r= 0.485), and a negative correlation between mean body temperature and feeding (P= 0.006, r= -0.155). Temperature did not significantly affect the prevalence of any other behaviours, though a trend toward greater expression of social behaviour, and fewer bouts of aggressive ramming, was observed when tortoises achieved higher body temperatures.

Introduction

The radiated tortoise (*Astrochelys radiata*) is categorised as Critically Endangered by the International Union for the Conservation of Nature (IUCN). Endemic to the most southern region of Madagascar, *A. radiata* is frequently collected for export for the pet trade, with seizures of hundreds of illegally imported animals reported. Given the imminent risk of extinction in the wild there is a need to house a sustainable, breeding population of *A. radiata* in captivity. Many zoos and aquaria have taken this reptile's plight into account: as of August 2020, the Zoological Information Management System (ZIMS) (Species360, 2020) recorded 1,237 *A. radiata* individuals held in zoos globally. Temperature regulation in chelonians has been covered in earlier studies, some dating back to 1939 [1]. As herbivorous ectotherms, tortoises must use behavioural thermoregulation to reach their preferred optimal temperature [2]. The ability to reach this optimal temperature affects tortoise digestion and immune function [3].

Heat provision presents some challenges for keepers of captive tortoises. The heat source should replicate, as much as possible, the type that the animal would receive in the wild [1]. Many chelonians are heliothermic, and therefore basking lamps are commonly used in captivity as heat sources [4]. Tortoise exhibits should also provide a heat gradient, allowing animals to control their internal temperature [1]. Most terrestrial tortoises operate within preferred ranges and hence exhibits must be carefully structured to ensure that the tortoises are able to achieve their preferred temperature [2].



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The radiated tortoise is found in a dry region consisting of brush and woodland habitats [5]. *A. radiata* is herbivorous and feeds by grazing on grasses, though it will on occasion feed on fruits and succulents, and it is well documented to indulge on invasive *Opuntia spp* cacti.

While body temperature has been previously researched in chelonians [1,2,6], comparisons between body temperature and behaviour would be valuable for animal managers. This could allow keepers to determine how behaviour changes as body temperature increases toward optimal.

Methods

The aims of our study were to determine whether the body temperatures of smaller and larger radiated tortoises differed across the normal daily routine in captivity, and to investigate the effect of tortoise body temperature on their activity budgets.

The study site

Five radiated tortoises (two males and 3 females) were located at Sparsholt College's Animal Management Centre, and housed in an open-top enclosure within a heated reptile room (W93" x D60" x H34"), with a surface area of 3.6 m². A temperature gradient design is implemented with the use of three heat lamps giving hot spots from between 29-37°C on one side and a heated area at the cool side around 25°C with UVA/B lamps along 3/4th of the enclosure. The substrate consisted of sand and small rocks along with a moss section, the animals had access to a large hide box in the cool area (Plate 1, Figure 1), a shallow water bowl and two food bowls. The tortoises wee custom seizures and therefore exact ages were unknown. Further details on sizes and weight can be found on (Table 1).

Activity and behavioural monitoring

Behavioural observations were conducted using instantaneous focal sampling at 60 second intervals for 1-hour time periods, resulting in 63 hours of observations per tortoise. The study took place between 0830-1730 on days with no classes such as weekends or holidays. Each tortoise was identifiable by physical characteristics and a GoPro video camera was used in tandem with first-person monitoring to ensure accuracy and identification of behaviour and of each individual. An ethogram consisting of nine state and six event behaviours was produced and refined from the extensive Ethograms provided by [7,8]. This was used to define exhibited behaviours (Table 2). A range of behaviours were observed and recorded in the study. The study intended to investigate thermal effects on all aspects of tortoise behaviour, rather than just those behaviours which are already known to be affected by temperature.



Plate 1: *A. radiata* enclosure. Basking area with UV on the left, UV in the middle and cool end to the right. Humid area situated above the hot end (Authors own, 2019).

 Table 1: Summary of information pertaining to identification

 number, gender, size and weight of radiated tortoises at the time

 of the study.

Tortoise Identification	Sex	Plastron length (Centimetres)	Body mass (Kilograms)
1	Male	24.7	4.26
2	Female	26	4.27
3	Male	23	3.28
4	Female	20.1	2.15
5	Female	21	2.51

Table 2: Ethogram used to record state and event behaviours of the radiated tortoises. Adapted from [7,8].

State Behaviours	Description	Event behaviours	Description
Resting	Eyes open or closed (could be sleeping), static position. limbs and head out or partially out.	Exploratory Sniff	Sniffing or putting its beak up to an object.
Alert	While sitting or standing, head elevated or level looking around.	Hiss	A noise made by the tortoise expelling air from its lungs.
Foraging	Head down, towards the substrate or subject to investigate area of interest.	Mandible Rub	Male places its head on a female's shell.
Feeding	Interaction involved with the process of eating such as biting and chewing food items. Includes drinking behaviour.	Ram	A tortoise ramming another.
Basking	Tortoise standing or sitting under heat source in any position in order to absorb warmth.	Social Sniff	Two animals are face to face exploring the head/face of the other.
Hiding	Animal in a position to attempt to be out of sight such inside its hides, under an object or the substrate.	Yawn	Opens mouth wide, usually momentary.
Soaking	Tortoise is situated in water. Water temperature varied across the project but was not recorded for this study.		
Walking	Moving at its regular gait. Tortoise is not eating during this time.		

Temperature

To investigate the body temperature of the tortoises, temperature/humidity data loggers (iButtons) were attached to both the carapace and plastron on each animal. They were secured via surgical tape and located on the 2^{nd} vertebral scute (Figure 2) and on the left abdominal scute in the concavity of the plastron to avoid being scraped off. The data loggers were attached at least 15 minutes before the commencement of the study to avoid interference of behavioural data. The error rate was +/- 0.1°C.



Plate 2: An illustration Showing iButton location placement on the carapace.

Statistical analysis

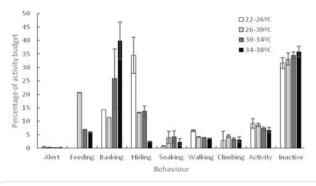
Collated data sheets were produced using means for plastron and carapace temperature and humidity along with behavioural observations per hour per individual. The body temperature of each tortoise was calculated by taking the mean of the carapace and plastron recorded temperatures. State behaviour percentages were calculated to present individual and group activity budgets for the tortoises.

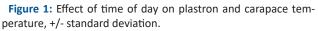
Statistical analyses were performed using Minitab version 23 and behavioural and temperature data were found to be not normally distributed. A Wilcoxon test was used to compare the iButton outputs for carapace and plastron temperatures. Spearman's Correlation Coefficient tests were used to find potential relationships between core body temperature and basking and feeding behaviour. We analysed the effect time of day and temperature on active or inactive behaviour.

Results

Difference between carapace and plastron temperature

The temperatures recorded from iButtons on the plastron and carapace of each tortoise were summarised for each hour of observations. The temperatures recorded from the carapace's iButtons were higher than those recorded from the plastrons, with average temperatures of 32.45°C and 28.55°C (+/- 2.07) respectively. A Wilcoxon test revealed that the difference between carapace and plastron temperatures was significant (Z= 70157, n=5, P<0.001). To produce an estimate for core body temperature, an average of the carapace and plastron was generated for use in statistical tests. Tortoises showed a predictable increase in body temperature throughout the day (Figure 1).





Effect of body size on temperature

On average, the temperature of the five tortoises used in the study differed. The larger tortoises, 1 and 2, showed considerably higher temperature means at 30.75°C and 32.36°C respectively. The smaller tortoises 3, 4 and 5 showed lower body temperature means at 29.27°C, 29.97°C and 30.5°C. The variance between large and small mean tortoise body temperatures was 1.64°C hotter for larger individuals. Mean body temperature showed a predictable change throughout the course of the day (Figure 2).

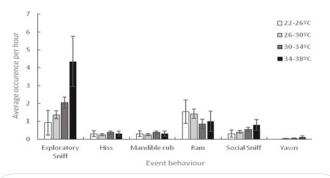


Figure 2: Relationship between time of day and average body temperature (as calculated using plastron and carapace temperatures), +/- standard deviation. Tortoises 1-5 are shown in different lines: See Table 1 for information on individuals.

Behaviour and temperature

Average body temperatures derived from the carapace and plastron iButton were compared against the behaviour of tortoises. An activity budget was developed (Figure 3), comparing the behaviour of tortoises under a range of temperature categories. Some behaviours, notably basking and inactivity, appeared to become more prevalent when tortoises were warm, whereas hiding and feeding decreased as environmental temperature decreased. Similarly, body temperature categories were developed to compare the occurrence of behaviours (Figure 4). Ramming occurred less frequently when tortoises were warmer, whereas both social and exploratory sniffs became more common.

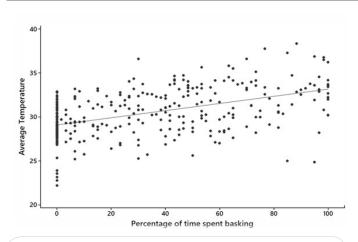


Figure 3: Activity budget for tortoises at different temperatures, as recorded using an average of plastron and carapace temperatures.

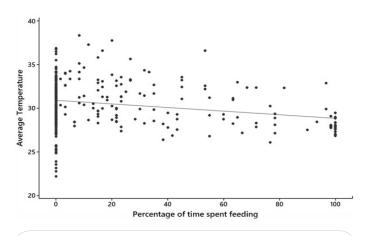


Figure 4: Number of occurrences of each event behaviour per hour, categorised by average carapace and plastron body temperature recordings.

Carapace and plastron body temperature recordings

Spearman's rank correlations were selected to investigate the relationship between behaviour and temperature. Overall, there was a significant positive correlation between the average body temperature of a tortoise and the percentage of time it spent basking (r= 0.485, P<0.001) (Figure 5). There was also a weak negative association identified between average body temperature and feeding (r= -0.155, P= 0.006) (Figure 6).

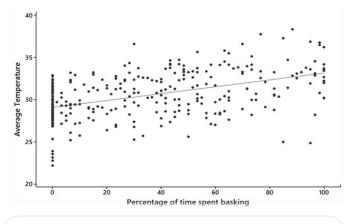


Figure 5: Correlation between basking behaviour and mean temperature recorded (as calculated using plastron and carapace temperatures) from iButtons.

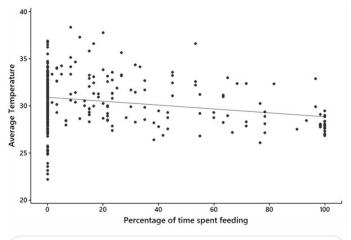


Figure 6: Correlation between feeding behaviour and mean temperature (as based on recorded from iButtons).

Discussion

The purpose of this project was to investigate the behaviour changes of radiated tortoises in association with daily changes in environmental temperature. Several significant changes in behaviour were identified in relation to carapace and plastron temperature. Specifically, there was a weak, negative correlation between feeding and body temperature, and a positive correlation between basking and body temperature.

Effect of time on temperature

Overall, the current study identified a significant difference between the iButton temperatures gathered from the carapaces and plastrons of radiated tortoises. However, both plastron and carapace temperatures may be affected by environmental variables due to their contact with substrates or light sources respectively [9]. When both measures were considered in the form of a mean temperature, the values showed some similarity to skin and carapace measures collected by [9] for the leopard tortoise (Stygmochelys pardalis) in captivity. Alternative techniques for measuring core body temperature include surgically inserted or cloacal probes, or ingested sensors (Huot-Daubremont et al., 1996) [10]. However, many animal collections prohibit invasive procedures, and as such many techniques for assessing body temperature are unavailable. This method of using a mean of both carapace and plastron temperatures may therefore have value in captive collections where ethics might prevent alternative strategies. It may allow reptile keepers to better identify whether their heating strategies are adequate for captive chelonians.

The largest female showed the highest body temperature, and also the greatest capacity to retain heat throughout the day. This correlates with the findings of previous literature [1,11]. The ability of large chelonians to retain body heat may have considerable value in terms of their management. For example, large, grazing tortoises might be provided with additional outdoor space, even in temperate climates, if they are able to sufficiently warm themselves before and after going outside. However, it is not known whether large tortoises can heat themselves sufficiently to use outdoor zones in cooler weather. By contrast, smaller individuals will struggle to maintain their temperature for long periods of time without returning to bask [12]. Enclosures could therefore be developed with this in mind: larger enclosures for small tortoise species or youngsters may benefit from many heat spots interspersed throughout the

zones.

Behaviour and temperature

The current study found increased behaviour shifts with increased body temperatures. Environmental temperature directly determines the activity of tortoises and warmer temperatures promote increased activity, whereas low temperatures and extreme highs limit tortoise movement [13]. The radiated tortoises in this study were most active and exhibited a greater range and frequency of behaviours when body temperatures were between 26°C and 34°C. This is similar to research undertaken in the wild on Hermann's tortoises [14] (*Testudo hermanni*), leopard tortoises (*Geochelone pardalis*) [15] and Aldabra tortoises (*Aldabrachelys gigantea*) [11] which showed similar temperatures and were most active between 26°C and 32°C.

In the current study, basking and hiding behaviour correlated with high and low temperatures respectively. Ramming, an aggressive behaviour, was seen most commonly when tortoises were cooler. This has been observed in gopher tortoises (*Gopherus polyphemus*) where competition for thermal hotspots further constrain effective thermoregulation [16]. However, it should be noted that [16] study was conducted on wild individuals, therefore the motivation for ramming may be different. For the radiated tortoises in the study, ramming may have been associated with competition over the most effective basking spots.

In contrast, social and environmental behavioural interactions were more prevalent at warmer body temperatures. It has long been known that reptiles become more alert once they have reached their optimal body temperature [17]. However, newer studies have also demonstrated heightened social and problem solving abilities in red footed tortoises (*Geochelone carbonaria*) when at warmer temperatures [18]. While radiated tortoises are not highly gregarious in the wild [5], it is interesting to note that aggressive behaviours decreased and more affiliative social behaviours increased once body temperatures were higher.

One behaviour associated with the loss of heat, and also potentially rehydration, is soaking. Tortoises appeared to make use of their water and engage in soaking behaviour most when at body temperatures between 26°C and 34°C. Movement into water is known to be an effective strategy for cooling for both endotherms and ectotherms, and tortoises have been seen engaging in this behaviour both in the wild and in captivity [8]. The limited use of water when body temperatures were cooler suggests that the primary purpose of soaking was to cool down. As large, ectothermic herbivores, there are also challenges associated with the digestion of food. At cooler temperatures, ectotherm digestion may be slowed [1,12], potentially resulting in reduced appetite or feeding behaviour. In the current study, a negative correlation was identified, in which feeding behaviour declined with body temperature. This may be an artefact of the body temperatures observed: the lowest temperatures recorded were over 20°C, whereas the mean highest body temperatures (using plastron and carapace temperatures combined) were closer to 40°C. It is possible that temperature was not a limiting factor for feeding behaviour, even at the cooler temperatures. The higher temperatures, by contrast, may have resulted in the tortoises seeking shade rather than feeding.

Future applications

As a study that shows radiated tortoise activity budgets under different temperature gradients, this study has some application to captive collections. The behavioural and temperature results could be used by curators of animal collections, to make comparisons with their own stock. For example, early career researchers such as undergraduates may not always be aware of the impact of temperature on reptile behaviour, particularly if they have only studied endotherm behaviour previously [19-22]. Further investigations of the behaviour and temperature of captive tortoise species may help keepers to better evaluate the suitability of their captive environments in relation to the species they keep. The study methodology could also be applied to other commonly housed species, such as the Horsefield (*Agrionemys horsefieldii*) or Hermann's tortoise.

The iButton placement technique employed during this study, including the fitting process, was performed rapidly with minimal handling. Brief handling has little effect on stress as opposed to subjection of longer-term handling and added stressors, which in turn influences the behaviour of radiated tortoises [5]. Alternative techniques involving ingestion or implantation, are invasive methods that could cause high stress and hence distort behaviour. Furthermore, risk of choking or impaction in smaller individuals is a cause for ethical concern and could result in research being prohibited. The method implemented in the current study aims to reduce stress and maintain animal well-being as well as being universally repeatable on various sizes and species of animals.

Conclusion

Overall, the current study found that iButtons could detect daily changes in tortoise temperature. Larger tortoises had higher mean body temperatures, which is in-keeping with prior research. The behaviour of the captive radiated tortoises also changed as body temperatures changed, with more basking and social interaction shown as temperature increased. iButtons may be used in future for other captive animal collections where there are restrictions on the types of invasive measurements that can be used, in order to better assess the thermal suitability of exhibit design.

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