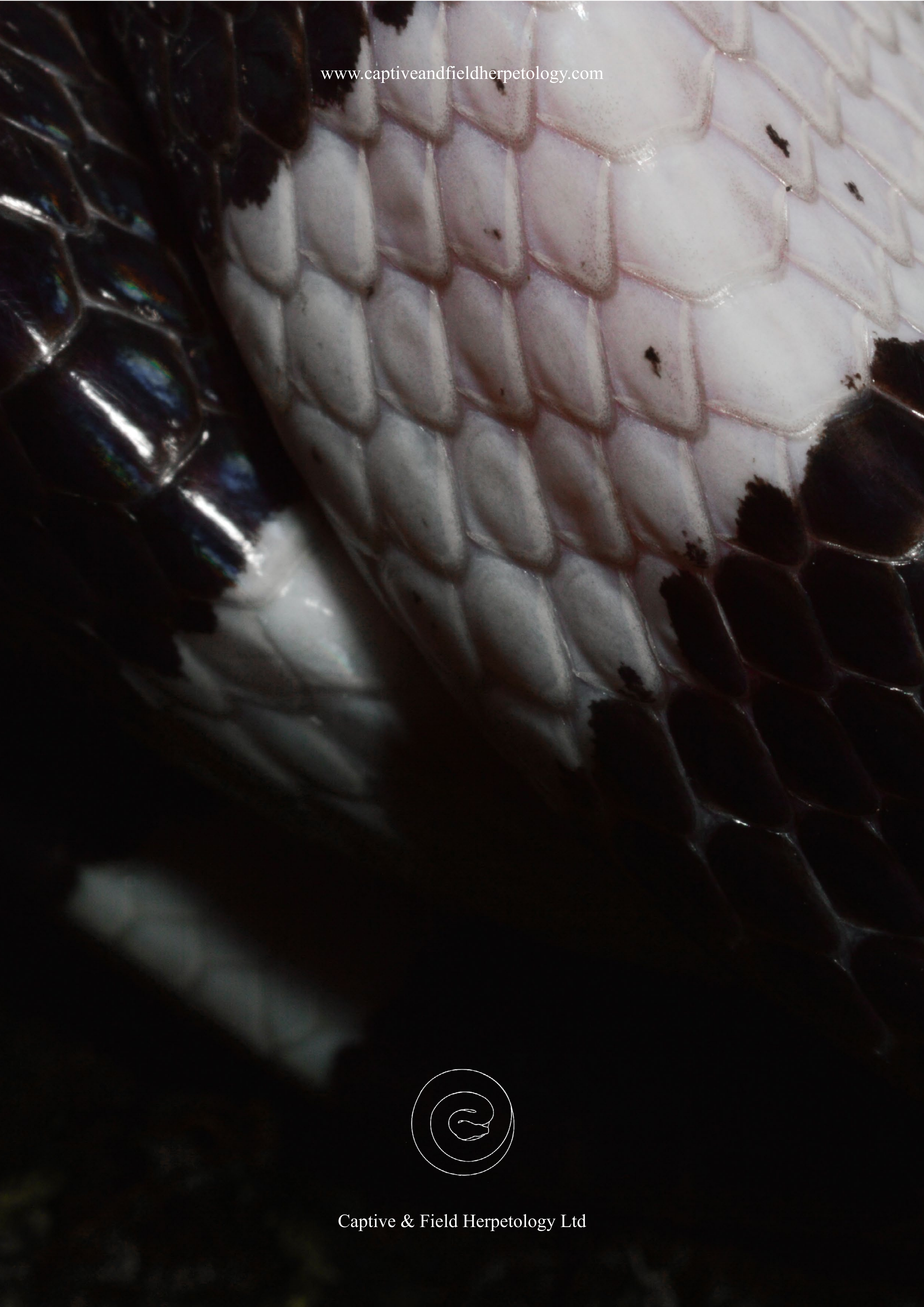

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Cover image and above:

Malayan or blue krait (Bungarus candidus), south Vietnam - John Benjamin Owens, Captive & Field Herpetology Ltd, 2017.

The Malayan or blue krait, *Bungarus candidus* is a highly venomous and medically significant elapid found throughout much of Southeast Asia. Responsible for a large number of bites every year. This species will often venture into homes, likely whilst foraging and leave victims with a painless but life-threatening bite. One of many venomous snake species in dire need of further research to better understand the ecology of snakebite.

Photographer:

John Benjamin Owens or Ben Owens is the founder of Captive & Field Herpetology Ltd, currently studying the population genomics of the UK's herpetofauna, he is also involved in the research of snakebite, primarily in India.

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Vogel, G. & David, P. & Pauwels, O. (2004) A review of morphological variation in Trimeresurus popeiorum (Serpentes: Viperidae: Crotalinae), with the description of two new species. *Zootaxa* **727**: 1-63.

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Responsible herping: A note on the ethics of reptile and amphibian interactions.

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Introduction

“Herping” is the act of searching for reptiles and amphibians, usually in a recreational manner, however, can also apply to scientific data collection in the field and any survey procedure directly seeking out herpetofauna. Unlike other wildlife-related activities such as birdwatching or safari, herping is often a hands-on activity, involving the capture and photography of the subject. Being hands-on with a subject is largely a more positive interaction, getting a closer look at the animal, taking more detailed and aesthetic pictures, and it is sometimes necessary if you intend to collect data on the individual. Despite this, being hands-on can introduce various negative impacts including stress for the animal, habitat destruction, and even disease. This raises several questions relating to the ethics, welfare, and responsibilities of herping, particularly on a recreational basis.

Herping is a somewhat “learn on the job” activity, the more time spent in the field, the more experience you get with handling different situations, interactions, and ultimately get better at it. Most people are introduced via peers and therefore have some guidance regarding best practices when herping. There is also a thriving online community consisting of social media, forums, blogs, and videos. These can be invaluable in gaining insight into how others operate around wildlife, both in a recreational and research capacity. Most would agree it is desirable to learn from as many peers as possible, expanding your theoretical repertoire regarding the unwritten guidelines of herping, however the rise of social media has facilitated views from various areas, angles and egos, often contrasting in the way they approach the activity.

This article aims to bring some objectivity to responsible herping (and general wildlife interactions), hopefully facilitating some balance between respect for animals and the environment, while continuing to enjoy observing and photographing these animals in the wild.

The first thing to consider when herping is the aim of your expedition. Whether you are collecting data on the herpetofauna of the area, answering a snake rescue call, or simply practicing your macro photography, know the reason why you are interacting with reptiles and amphibians, and adjust your responsibilities as appropriate. With a clear and concise goal in the back of your mind, you can assess what is necessary during your interaction; do you need to measure the animal, do you need to pose it for photos, do you even need to catch it?

Respect for the animal

It is not uncommon for herpers to preach about the ecological importance and conservation issues regarding reptiles and amphibians, whilst simultaneously irresponsibly wrangling snakes for the perfect picture for social media. What kind of message does this send to people interested in learning about herpetofauna, and indeed other herpers? If we want herpetofauna to be endeared as much as large mammals (e.g Tigers, Elephants), we need to start treating them less like objects and more like animals, demonstrating the beauty and nature of them in their natural environment (e.g. Figure 1 and Figure 2).

To consider the impact we have on the individual animal, we must explore what is important to the individual. Herpetofauna (mostly reptiles) are often

Figure 1. Multiple male European Adders (*Vipera berus*) basking outside a hibernaculum. From this in-situ photograph we can deduce proximity to a hibernaculum, sex, pre-slough condition, and kleptothermy.

Figure 2. Female Wagler's Pitviper (*Tropidolaemus wagleri*) photographed in-situ. A large individual, potentially gravid, and resting on a branch approximately 1.6m from the ground. No handling was needed for photography and observation.

encountered while basking. They may be easier to catch if they have not warmed up, and sometimes prioritise thermoregulation over predator avoidance (e.g . Herczeg *et al*., 2008; Webb *et al*., 2009) (however the converse can also be true; Webb & Whiting, 2005, also see Burger, 2001). While disturbing a basking reptile is not necessarily linked to mortality or survivorship, it is certainly an important process for them. Evidence shows that basking can be important for temperate species, where temperatures and sun exposure are limited throughout the whole year (Shine 2004). Olsson *et al*., (1997) demonstrated that when European Adders (*Vipera berus*) come out of hibernation they use up to 5% of their body mass on sperm production in preparation for the mating season, facilitated by sun exposure. Given that they often emerge from hibernation already in a poor body condition, this is obviously an important time of year for them (see Figure1 for in-situ observation).

Similar evidence demonstrates that even in tropical situations thermoregulation is important for embryonic development and survivorship, with Sup & Shine (1988) showing brooding female Diamond Pythons (*Morelia spilota spilota*) maintain a higher body temperature partly through basking. Whilst nocturnal herpetofauna do not directly benefit from solar radiation; and are constrained by the limited availability of suitable environmental temperatures, they are still able to thermoregulate behaviourally throughout the night, and appear to be quite efficient (Nordberg & Schwarzkopf, 2019). Basking duration is multi-factored and complex, depending on season, reproductive state, whether animal has fed, etc. Time spent thermoregulating can range from nothing, through minutes, to most of the day (Pearson & Bradford, 1976; Grigg *et al.*, 1979; Hammond *et al.*, 1988; Manning & Grigg, 1997; Herczeg *et al.*, 2003; Mukherjee *et al.*, 2018). Disturbing or catching a basking animal might be interrupting a crucial regulatory process but is not likely to affect mortality or a dramatic change in behaviour. This might be different when considering sites where animals risk being disturbed on multiple occasions, and the animal might not be able to effectively thermoregulate. This needs to be assessed on a case-by-case basis, given factors like time of year, reproductive status, etc., and should always be something carefully considered.

Occasionally, reptiles and amphibians are encountered in the middle of natural behaviours, which are, no doubt, important for the animal. Snakes, for example, are renowned for going long periods of time without eating (McCue, 2007) and, therefore when they do feed, it is an important event for them and they even change their digestive physiology in respect to this (Secor & Diamond, 1998; 2000). Disturbing a snake that is feeding or has recently fed can cause it to regurgitate, meaning the energy expenditure on prey capture, up-regulation of metabolism and the life of the prey animal are potentially wasted; a preventable and negative experience for the animals involved (See Figure 4 for in-situ observation). The same can be said for individuals mating, egg-laying, or involved in other courtship or combat behaviour. Although anecdotes and some field observations note that individuals appear focused on these behaviours, irrespective of disturbance (Sasa & Curtis, 2006), greatly disturbing these animals might inhibit them from successful reproduction and have a subsequent downstream effect on the whole population – particularly pertinent to species at risk. Observing and appreciating these natural behaviours from a distance is far more rewarding; and may even be note-worthy to contribute to the scientific literature in the form of a natural history note (see Figure 3 for in-situ observation).

General handling practices vary widely, from not at all, to “freehandling” venomous species. There are some generally accepted practices when it

Figure 3. An Ornate Gliding Snake (*Chrysopelea ornata*) wrestling with a large Tokay Gecko (*Gekko gecko*). The snake attempted to predate the gecko however as you can see the gecko is putting up a fight. Eventually the gecko succumbed, potentially from the venom, and/or constriction. A fascinating and exciting observation witnessed without intervention.

comes to handling, such as appropriate tools and restraint methods, which aim to reduce overall stress and safety for the handler and the handled. If our aim is to reduce stress to the animal, then the simplest solution is to simply not handle, which is somewhat antithetical to the definition of "herping". There is a huge swathe of literature assessing the physiological stress response of herpetofauna through corticosterone levels after capture and handling, and it is not surprising to hear that it almost always facilitates an increase. Associated increases in corticosterone levels can also influence other physiological processes, particularly reproduction (e.g. Moore *et al*., 1991; Lutterschmidt & Mason, 2010; Carr, 2011; Gomes *et al*., 2012), and while some of these studies are ex situ in nature, others explicitly used capture and handling to initially elicit the stress response. Despite this, there is nothing notable linking stress to individual survivorship, although there is perhaps some implication for reproductive success and downstream effects on populations dynamics (e.g Meylan *et al*., 2010; Tokarz & Summers, 2011). To put it in context, capture and handling of animals, particularly on multiple occasions, for long durations and in unnecessary situations, should be avoided. Given the lack of current evidence demonstrating mortality or direct causation of population instability through altered behaviour (as a result of capture and handling), completely abstaining from it is likely unnecessary, but some sensible level of the impact on the animals wellbeing should be considered, especially when considering that physical manipulation (and even sound) is likely more impactful than practices like flash photography (Huang *et al*., 2011; De Brauwer *et al*., 2019).

Respect for the habitat

Flipping rocks, logs, coverboards and other refugia, either natural or artificial can be quite effective in detecting herpetofauna, particularly small snake species (Halliday & Blouin-Demers, 2015). There is a large body of literature stressing the importance of microhabitats for herpetofauna, providing sheltering and thermoregulatory opportunities, with some studies demonstrating how destruction and removal of rocks can significantly reduce species abundance and richness (Schlesinger & Shine, 1994; Goode *et al*., 1998). Of course, if refugia is not removed or destroyed, it can continually be used by animals, and many anecdotes will demonstrate that the same individuals can be found under the same piece of cover even if disturbed. Contrary to this, Marsh and Goicochea (2003) reported that plethodontid salamanders start to avoid cover boards if they are extensively surveyed, potentially misleading population estimates and decreasing encounter rate. It is yet to be assessed whether this holds true for other species. Given the importance of refugia to herpetofauna, it is important to minimise disturbance as much as possible, by returning the refugia to its original position, taking care to not squash any animals (including invertebrates) residing there. The take home message is to leave things as you find them, impacting as little as possible.

Amphibians, on the other hand seem relatively resilient to habitat disturbance. With Canessa *et al*., (2013) showing that an endangered endemic amphibian, the Apennine yellow-bellied toad (Bombina variegata pachypus) breeds in sites that are frequently disturbed (both naturally and anthropogenically), leading to less aquatic and bank vegetation and fewer predators. Another study by Warren & Büttner (2010) examined the density of amphibians (including 2 endangered species) amongst breeding pools on military training areas in Bavaria, Germany. They found that most species, including the endangered ones, showed a preference for sites with more ground disturbance, characterised with bare ground and minimal vegetation. The authors do note that this may be conflated with habitat preference and on the macrohabitat level; the sites are a mosaic of disturbed and non-disturbed areas. These results may indicate support for the "Intermediate Disturbance Hypothesis", which itself has faced much criticism in overlooking the complexity within spatial-temporal distributions of animals (Wilkinson, 1999). Given that nearly all our wildlife and their respective environments face multiple threats and disturbances, it seems unnecessary to contribute more, so while habitat disturbance may not be as damning for

Figure 4. Common Asian Tree Frogs (*Polypedates leucomystax*) in amplexus, producing a foam nest. The smaller male (right) fertilises the spawn which the female deposits in a foam nest, formed by "fluffing" up with her hindlimbs. This breeding behaviour was witnessed in-situ without any need for disturbance, while still achieving aesthetic photographs.

amphibians, it is certainly something to consider. Perhaps of more concern to amphibians and water bodies, are diseases such as chytridiomycosis (chytrid). Caused by the fungus *Batrachochytrium dendrobatidis*, and its salamander-infecting counterpart *Batrachochytrium salamandrivorans*, it can manifest in lethal skin lesions, and is implicated in the decline or extinction of multiple amphibians (Skerratt *et al*., 2007). The fungus has proved remarkably resilient, surviving for up to 3 months in river sand, and hours of desiccation (Johnson & Speare, 2005). A major concern is that field herpers and researchers are unknowingly spreading the pathogen from one water body to another, through contaminated footwear and equipment. Biosecurity is now an integral part of most official survey procedures, and with other diseases such as Ranavirus and Snake fungal disease (Ophidiomycosis), it is time for us all to take this more seriously. This means thoroughly cleaning and disinfecting boots, buckets, bags and hooks between sites and between interactions, minimising any role we may play in spreading these pathogens.

Respect for others

Recreational herping occupies a unique social space in which individuals brag about "lifers" (the first time you observe a species in the wild) and spend hours getting the "money shot" photograph, while simultaneously gatekeeping the hobby and being reluctant to share data on the location and conditions of their observations. This is not entirely unjustified; if a site is publicly shared it may face more intensive disturbance and bad characters such as poachers and collectors. On the other hand, sharing observations and discoveries can endear reptiles and amphibians both in the public and amongst colleagues, perhaps leading to positive conservation practices and meaningful data collection. Ultimately, site-sharing comes down to a judgement call, and indeed this article is written to provide some guidelines on minimising disturbance so that herping can be shared amongst researchers, photographers, and the public alike.

Being a responsible herper is not just about respecting the animal and respecting the habitat, but also about being a responsible role model for anyone else interacting with wildlife, professional herpetologists and amateur hikers alike. By demonstrating in situ photography, safe and minimal handling, and biosecurity, we set an example of a respectful, ethical approach to human-wildlife interactions, instead of a competitive sport. Herping and photography can be a powerful tool in aid of conservation; but is all too often manipulated in the pursuit of personal glory. The default should not be "everyone else is doing it, so it's okay", it should be a contemplative assessment of what is appropriate in the context. This article has aimed to bring some of this "contemplative assessment" to the forefront of decision-making processes while interacting with reptiles and amphibians. It should also be noted that the responsibilities involved with removing specimens from the wild for personal collections, museum or zoological institutions, or consumption, where it is legal to do so, is not considered in this review, as whether or not it may beneficial to the parties involved must be taken on a case-by-case basis.

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Thanks to Jesse Goodyear for contributing some stunning in-situ photography of natural behaviours.

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Rethinking Captive Heating: Effect of Infrared Radiation on the Radiated Tortoise (*Astrochelys radiata*)

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Keywords: *Astrochelys radiata*, IR-A, IR-B, Heating, Husbandry

Abstract

In the wild, reptiles are exposed to the entire infrared spectrum (IR-A, IR-B and IR-C wavelengths), yet many zoological collections rely on IR-C to provide heat to their animals. This study investigated whether the addition of IR-A and B wavelengths affected the behaviour and enclosure use of a group of captive radiated tortoises (*Astrochelys radiata*). Modified Spread of Participation Index (SPI) was used to assess the evenness of exhibit use. This is the first published study using SPI on a reptile species, despite its practicality at addressing abiotic gradients and depth-based zones that are commonplace within herptile enclosures. The study identified a 31% reduction in tortoise basking behaviour when exposed to IR-A and B emitting bulbs ($p < 0.001$, $r = 0.402$) which simultaneously resulted in a wider variety of behaviours being expressed, including changes in inactivity ($p < 0.001$, $r = -0.309$) and drinking ($p < 0.001$, $r = -0.139$) behaviours. In addition to behavioural changes, the provision of IR-A light resulted in more even enclosure zone use, potentially making it an interesting application for improving herptile captive husbandry. IR-A and B lighting may be an important consideration for improving captive herptile welfare and facilitating an animal's expression of natural behaviours. Despite some interesting results, this study did not yield enough information to fully support the provision of IR-A lighting to captive reptiles, thus, future, more in-depth studies with larger sample size, observation counts and subjects amongst different taxa will be required. Potentially, with further studies and repetitions, this could have large and positive implications for the industry; ones that would derive from a relatively simple, and inexpensive/cost-effective change/modification of a light bulb. It is the author's aim that this work will provide a useful foundation for future studies.

Introduction

Reptiles within captivity are typically provided with a variety of light and heat sources for thermoregulation and vitamin D3 synthesis (Baines *et al.*, 2016). These lighting arrays should theoretically replicate natural heat sources as closely as possible so that wild type behaviours and correct homeostasis can occur (Beaupre & Zaidan, 2012; Michaels *et al.*, 2014).

Historically, keepers have concentrated on 1) ultraviolet B (UVB) provision and its effects on vitamin D3 production and 2) reptile heating systems to provide ecologically informed ambient temperatures. However, terrestrial organisms in the wild are exposed to the full solar spectrum, this includes infrared radiation (IR), which cannot be seen, but like visible light, can be perceived as heat (Wunderlich, personal communication, 2020; Barolet *et al.*, 2016; Schieke *et al.*, 2003). Much like UV, IR is not limited to just one type, but three different wavelength categories which are infra-red A, B and C (Barolet *et al.*, 2016; Schroeder *et al.*, 2008). IR-C is the furthest from visible light, this is often called 'far infrared' or FIR (Vatansever & Hamblin, 2012). Several reptile heating systems (ceramic heat emitters, heat mats, radiant panels, and cables) emit IR-C radiation (Figure 2), this begs the question: how important are the other wavelengths, IR-A/IR-B? Infrared-A, known as 'near-infrared' or NIR, has a short wavelength band that enables photons to penetrate deeper into the subcutaneous skin, promoting a 'deep warming' effect that is further distributed by the circulatory system (Divers & Stahl, 2019). This provides heat deep within the muscle tissues, warming the animal throughout just as it would in the wild from the sun (Muryn, personal communication, 2019), without significantly increasing skin temperature (Cho *et al.*, 2009). The penetrative depth of IR-A has been recorded to 5mm up to 100mm in human models, whereas IR-B has a penetration depth of approximately 1-3mm and IR-C is around 0.1mm (Currà *et al.*, 2019; Ruggiero *et al.*, 2016; Sowa *et al.*, 2013; Brown *et al.*, 2002).

Porter (1967) investigated the presence of black peritoneum and what its biological function is. It is believed that it protects the inner organs against harmful UV rays which counters the argument that is to aid in thermoregulation. However, one of the most interesting points in the study, was the measurements of transmission and absorption in each tissue layer for the full solar spectrum (290 nm - 2600 nm). Porter measured how much light was reflected and absorbed in the upper epidermal, dermis, the muscle, and how much radiation was transmitted into the inner body cavity for the side-blotched lizard (*Uta stansburiana*). The results indicate that the IR absorption is along a wide range of wavelengths, with each one being responsible for heating different cell types at varying depths. These results can be easily identified thanks to the recent work of Sarina Wunderlich (personal communication, 2020), who was able to use Porter's analysis and plotted the results in relation to the solar spectrum, helping display which wavelengths are absorbed in which tissue layer (see Figure 1).

The energy from IR-C sources, such as the commonly used Ceramic Heat Emitter (CHE) is mostly absorbed by animal surface tissues (stratum corneum), where it may cause localised overheating whilst leaving other regions of the body too cool (Highfield, 2015). IR-C releases its energy via water molecules, which can create a dry environment due to the reduction of ambient humidity, this may potentially result in dermatological/keratin issues (Courtney-Smith, 2018; Divers & Stahl, 2019).

Due to IR-A penetrating further into the dermis than IR-C, it may result in IR-A emitting bulbs potentially improving and hastening the thermoregulation process. Thermoregulation is not the only application of infrared. Since the 1960s, infrared has been implemented in both medical and veterinary fields (Barolet *et al*., 2016; Min & Goo, 2013). and is shown to minimise scar

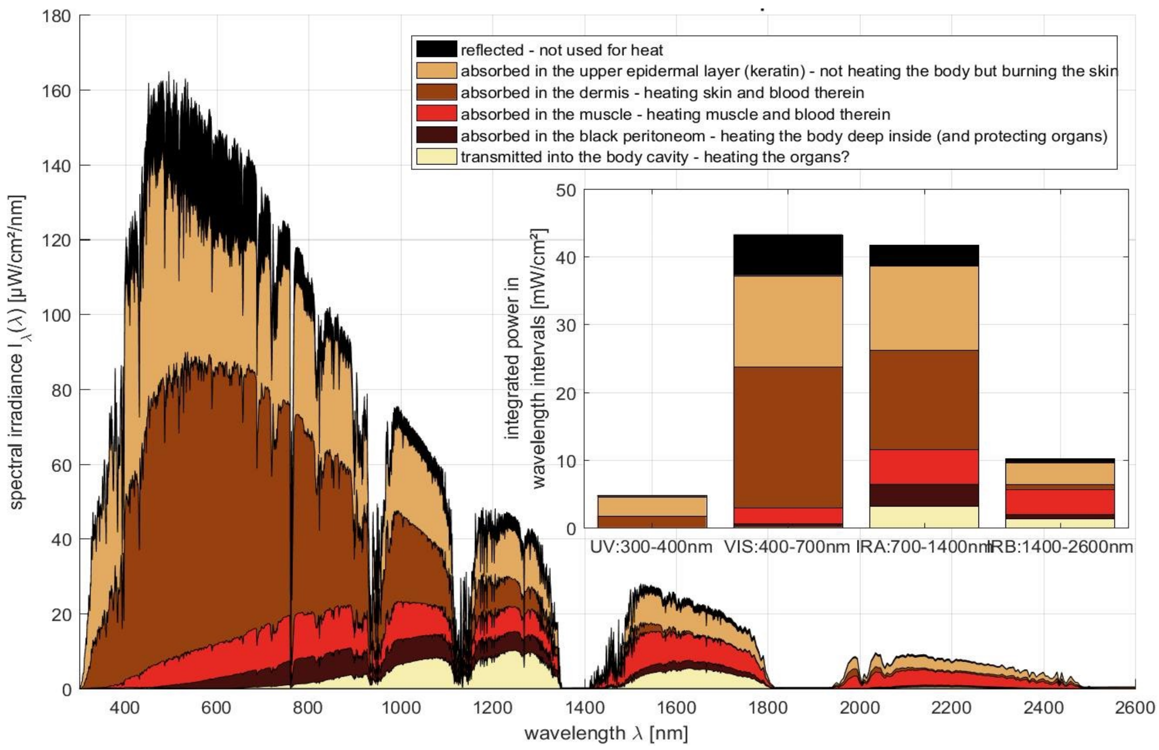


Figure 1. Penetration depth in relation to the solar spectrum for the side-blotched lizard (*Uta stansburiana*) and the result of integration along the wavelength-axis (ASTM reference spectrum AM1.5). Data was derived from Fig.5 from Porter(1967; Wunderlich, 2020). It is interesting to note that IRB (1400 nm - 2600 nm) did not heat the dermis yet managed to penetrate the muscle, and that IRA (700 nm – 1400 nm) and IRB had a larger heating effect on the muscles in comparison to VIS (400 nm – 700 nm).

tissue, downregulate inflammatory response and bruising, treat toxic insult and hypoxia, provide pain relief, combat autoimmune conditions via detoxification, and shorten recovery time (Baines pers. Comm, 2019; Cowles *et al*., 2013; Chung *et al*., 2011; Lopes *et al*., 2007). IR-A exposure has also been theorised to precondition the skin to protect against deleterious ultraviolet radiation through photoprevention (Barolet *et al*., 2016). Whilst the aforementioned examples may not be strictly related or necessary for husbandry practices, it is worth highlighting the medical significance of IR as to challenge the misperception that it is always deleterious to health (Barolet *et al*., 2016). However, as with most things, moderation is important. According to the International Commission on Non-Ionizing Radiation Protection (ICNIRP) statement, IR-A and IR-B poses a risk to corneas due to the penetration depth of the bands (Aly & Mohamed, 2011). unfortunately, no reliable data currently appears to exist on the incidence of IR-related optical conditions in captive reptiles.

Thomas *et al*., (2019) investigated IR effects by looking at the behaviour of two (1.1) captive blue tree monitors (*Varanus macraei*) when provided with two different lighting arrays, a mercury vapour lamp and a Deep Heat Projector® (DHP, Monkfield Nutrition Limited). The difference between these two bulbs was the amount of IR-A and B produced, as opposed to the absence or presence. The mercury vapour emits mostly IR-A with some IR-B and IR-C (Baines, personal communication, 2019), whilst the DHP emits a small amount of IR-A, with high levels of IR-B and IR- C (Figure 2). However, the Mercury vapour lamp also emits visible light and UV, resulting in the study having a 1.5-4 UVI difference between the two conditions. It has been demonstrated that UV can regulate behaviours such as; feeding, diurnal locomotion, courtship and influence agonistic, reproductive, and signaling behaviours (Reptile Lighting Guide, 2017; De Vosjoli, 2012; Lappin *et al*., 2006) which may have affected the results. Despite this, Thomas *et al*. (2019) reported a significant increase ($\chi2 = 12.200$, $P = 0.002$) in basking duration when the monitors were exposed to the DHP condition, which was associated with a statistical decrease ($\chi2 = 18.617$, $P < 0.001$) in resting. Thomas *et al*. state that these results may be due to the DHP having lower levels of IR-A, and not allowing the monitor lizards to reach optimal internal temperatures, resulting in the animals basking for longer durations; a theory that this paper intends to address.

This study aimed to further the investigations initiated by Thomas *et al*. (2019) by designing a methodology that focuses on the removal/addition

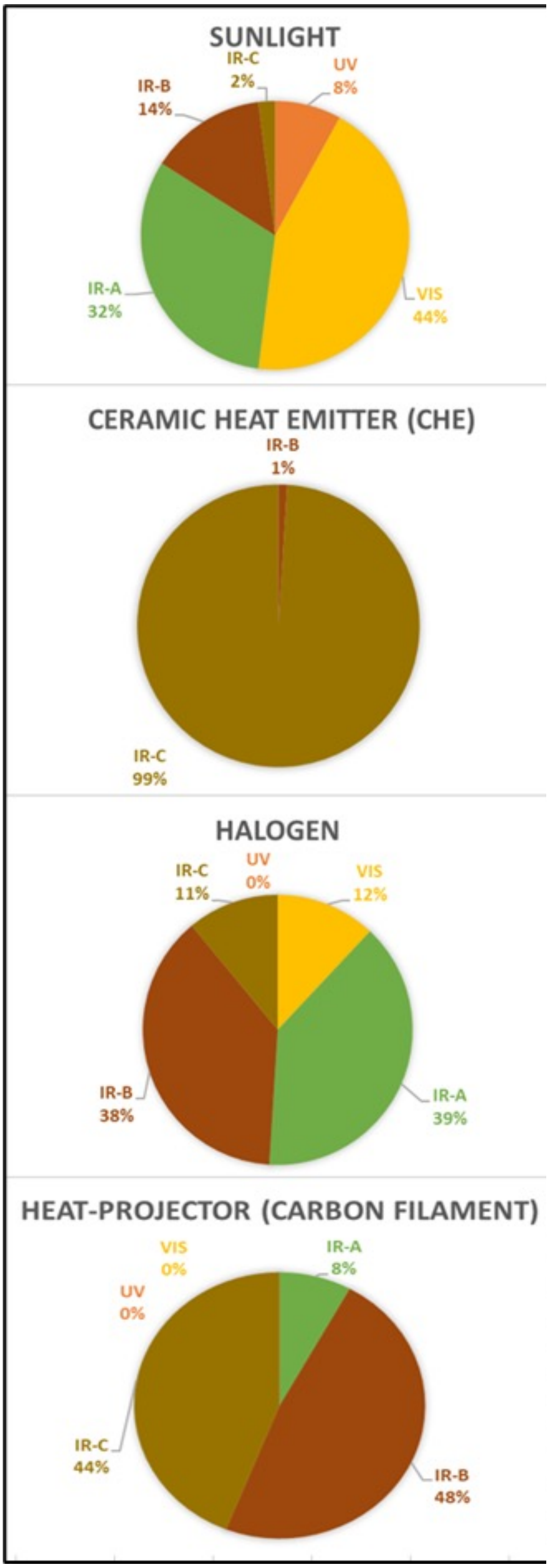


Figure 2. Charts display the percentage and type of wavelengths that are emitted from each bulb. A Spectrophotometer would have accurately measured the IR-spectrum, however, due to cost and logistics, it was out of scope for the purpose of this study (Authors own, 2020).

of IRA and B. Wavelength composition of sunlight, CHE, DHP and halogen bulbs were derived from theoretical blackbody radiation curves using kelvin (K) values from the manufacturer's specifications and the total solar irradiance (Figure 2). The charts identified that halogen lamps appear to replicate sunlight more accurately by emitting IR-A, B and C, whereas CHE produces primarily IR-C, making it appear to be the least effective at sunlight replication. The two bulbs fail to produce UV; however, UV bulbs can be provided additionally, which allow for the removal of an important extraneous variable. This is unfortunately not possible for visible light (VIS), as CHE is unable to emit this band, this was minimised by providing VIS in the form of LED lighting alongside the IR and UV lighting systems. However, the halogen condition will always produce more VIS, making it an extraneous variable; this might be an important factor if the subjects are heliothermic and use visible light as a cue for basking.

Methods

This study compares CHE with halogen bulbs (Richardson, 2001). The difference between CHE and halogen bulbs is the presence/absence of IR-A & B. This distinction allows a study to identify if IR-A and B wavelengths can influence tortoise behaviour and their choice in enclosure use. This study expands upon prior work by recording both state and event behaviours, and the observation of enclosure usage amongst the individual subjects, this was also supported with the implementation of a modified Spread of Participation Index (SPI).

Study subjects and location

The study was conducted at Sparsholt College, Winchester, UK, on a 2.3 group of radiated tortoises (*Astrochelys radiata*). Data was collected between 27/09/19 – 02/02/20, during the hours of 1100-1600. The research was approved by the Ethics Committee at the University Centre Sparsholt, Winchester and abided by the Association for the Study of Animal Behaviour Ethical Guidelines (ASAB, 2017).

The *A. radiata* group (Table 1) was housed in an indoor enclosure (W93" x D60" x H34"), with a surface area of 3.6m2 (Plate 1). The enclosure consisted of a hot and cool end, with a hide/shelter located at the cool end. A humid area containing sphagnum moss was also provided. Sand and soil were used as substrate with rocks situated around the enclosure.

Daily husbandry routines were kept the same to avoid any potential changes in subject behaviour. Main feeds were in the afternoon, this consisted of 300g of seasonal vegetation, such as cabbage, dandelions, plantain, hazel leaves and root vegetables. Portion sizes remained the same throughout the study.

Table 1. Subject information

Colour ID	Sex	Hatch Date	Weight (kg)	Remarks
Black	♂	2011	4.82	Captive bred (arrived at collection in 2014)
Blue	♂	Unknown	3.11	Wild caught (seizure from customs)
Pink	♀	Unknown	3.84	Wild caught (seizure from customs)
Red	♀	Unknown	3.29	Wild caught (seizure from customs)
Green	♀	Unknown	5.04	Wild caught (seizure from customs)

Data collection started an hour after the food was initially presented to avoid extended durations of observed feeding behaviour. As part of the tortoise's husbandry, the subjects had a "starve day", this was taking into consideration, the conditions, including repeats all had equal observed hours.

Experimental design

A quantitative case study approach was used to investigate if the addition of IR-A and B would affect behaviour and enclosure use amongst chelonia. The study consisted of two components: behaviour and enclosure-use observations, both of which were conducted simultaneously.

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).

Subjects (N= 5) were classified into two conditions, baseline (CHE) and experimental (halogen), these were then repeated for validation in an ABAB experimental design. The first condition, the baseline consisted of overhead IR-C wavelengths, whilst the second condition, the experimental stage consisted of IR-A, B and C wavelengths, this was achieved by replacing the CHE bulbs with halogen bulbs. Apart from the heating arrays, all other lighting, equipment, furnishings, and husbandry remained the same throughout the entirety of the study. The IR-C emitting bulb in the baseline experiment was positioned at 35cm from the surface, whilst the IR-A/B emitting bulb during the experimental stage, was positioned at 45cm from the surface, this allowed for a similar enclosure temperature amongst both conditions (Plate 2-5), however, a change in bulb height resulted in a basking area difference between the two conditions, this was difficult to quantify, but was attempted with a laser thermometer, it was estimated that the conditions had an area difference of +/- 8-15cm diameter; whilst this may be minimal, it may be enough of a difference to restrict the individuals from attempting to bask at the same time, especially when accounting for social hierarchies. Temperature was measured by FLIR meters (One Pro & E5) to produce a thermal image (Plate 1-5). UVI readings were also monitored by a UVI meter (ZooMed Digital UV Index Radiometer 6.5). Visible light was measured with a digital light meter (URCERI MT-912), due to the halogen bulb emitting visible light, the basking area was brighter by an average of 300-325 LUX.

Data collection was carried out by one of the authors (NW) to avoid out of sight observations and to accurately record subtle behaviours and zone occupancy. Researcher position was semi-concealed and 3m away. Each condition/repeat was monitored for equal durations of 15 hours each, resulting in a total of 60 hours of observation.

The bulbs had a burn-in period of >100 hours prior to data collection, at the beginning of each condition, a break-in period of seven days was observed, this allowed the subjects to get accustomed to the new bulb. As a precaution, the subjects were routinely examined (remotely) to check for signs of dehydration (McArthur, Wilkinson & Meyer, 2008).

Behaviour

Instantaneous focal sampling (Martin and Bateson, 2007) was carried out at 60-second intervals for 60-minutes per observation. Ethograms were used to define behaviours (Table 4-5). Both state and event behaviours were recorded (Martin and Bateson, 2007). 12 state behaviours were identified, these were recorded using instantaneous

Table 2. Baseline Condition: UVI measurements taken 30cm from the surface, T5 12% UV bulb had measurements taken from both ends and the centre to account for UV gradients.

Lighting/heating bulbs	UVI	Avg. Surface Temperature (°C)
• 2x T5 UV 12% (Arcadia 54W) • 160w active UV basking lamp • 2x T5 Plant growth lights • LED (Arcadia jungle dawn 22W) • 2x CHE (Arcadia 150w)	3-4 6.5 N/A N/A N/A N/A	27 30 27 27 36

Table 3. Experimental condition: UVI measurements taken 30cm from the surface, T5 12% UV bulb had measurements taken from both ends and the centre to account for UV gradients.

Lighting/heating bulbs	UVI	Avg. Surface Temperature (°C)
• 2x T5 UV 12% (Arcadia 54W) • 160w active UV basking lamp • 2x T5 plant growth lights • LED (Arcadia jungle dawn 22W) • 2x PAR38 Halogen (Philips 175w)	3-4 6.5 N/A N/A N/A N/A	27 30 27 27 37

focal sampling method described above. Six event behaviours were chosen and recorded using continuous focal sampling (Martin & Melfi, 2016).

Enclosure usage

Enclosure use was measured by recording the location of each subject, this was achieved by dividing the enclosure into seven zones (Table 6 and Figure 3). Using the same instantaneous focal sampling method as described above, the zone occupancy of each subject was observed and recorded after each minute. Thus, presenting whether individual enclosure use is influenced by the type of infrared wavelength received. Seven zones were selected and measured (Figure 3), these were based on a combination of resources and landmark features such as heat gradients, UV basking areas, hides and type of substrate. Zones defining locations of basking importance were calculated using laser temperature guns, FLIR meters and solarmeters, this helped to identify and outline heat gradients.

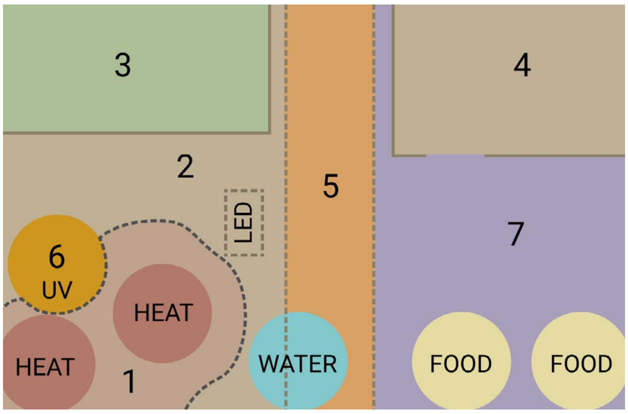
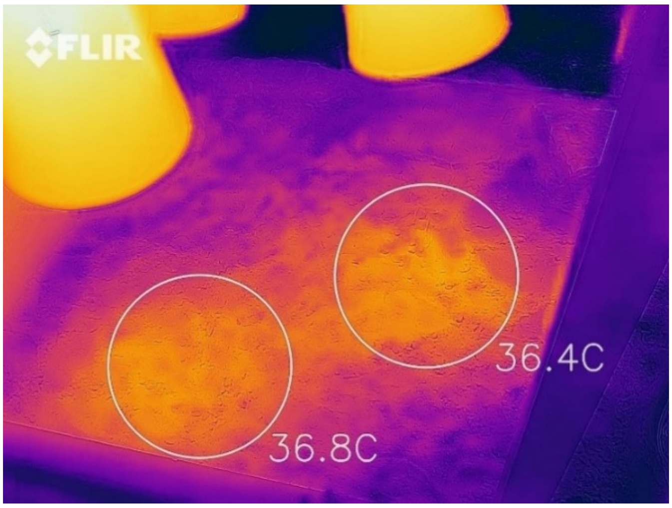


Figure 3. Diagram showing how the seven zones within the enclosure (Authors own & Neilson, 2020).



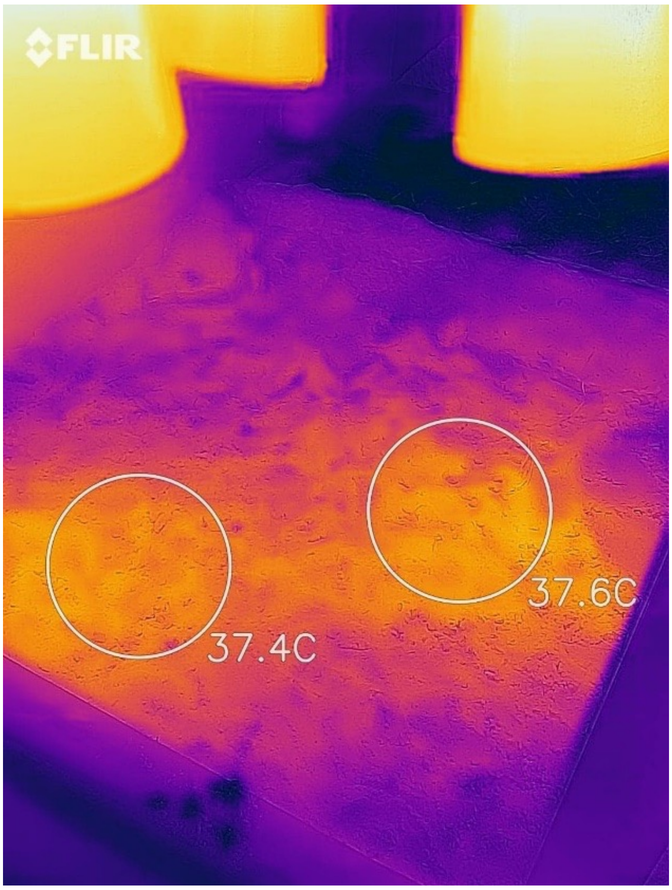


Plate 2-3. Infrared images of the basking spots (zone one) for both conditions. (above) is the baseline (right) is the experimental condition (Authors own, 2019). The CHE bulb was 150W whilst the halogen was 175W; the difference in wattage resulted in a difference in surface temperature; this was minimised by adjusting the height of the bulbs between the conditions.

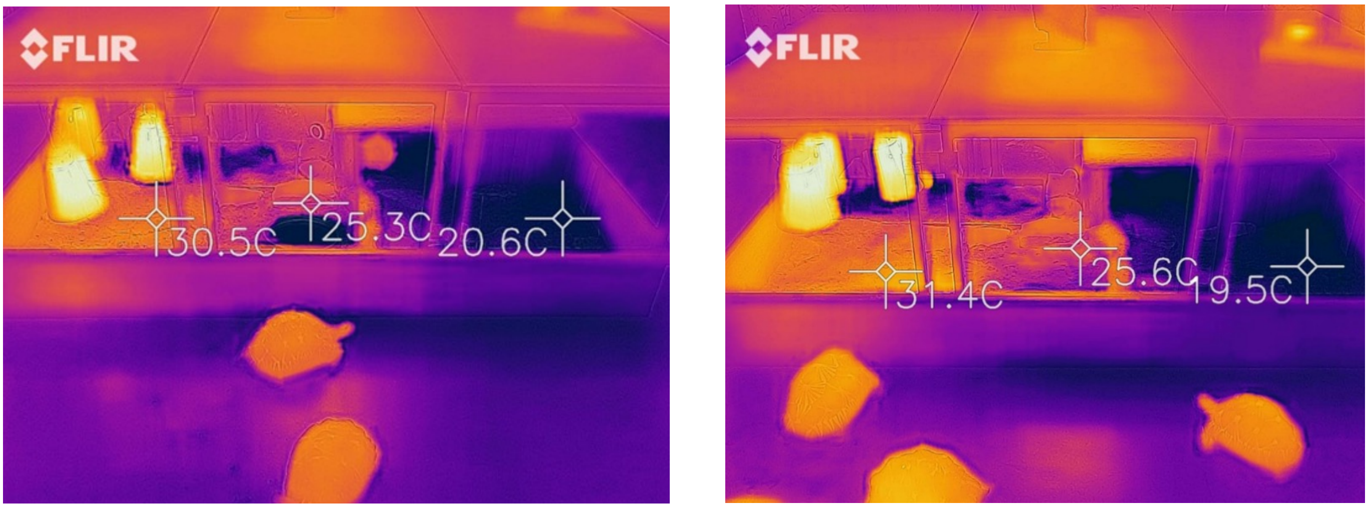


Plate 4-5. Heat gradients within the enclosure, left is the baseline condition whilst right is the experimental. At the time, the ambient temperature in the room was 27°C for both conditions. (Authors own, 2019).

Table 4. *A. radiata* state behaviours.

Basking (Infrared)	Absorbing heat from either a CHE or halogen bulb via laying semi-motionless to increase metabolism rate and aid in thermoregulation. Animal is under the heat source, within the optimal basking range of >30°C (Zone 1).
Basking (UV)	Absorbing UV from a UV emitting light source via laying semi-motionless. Animal is under the light source, within the optimal UV index range of >UVI 3 (Zone 5 and 6).
Locomotion	Tortoise movement from one objective to another, using the front and hind limbs to move across the enclosure. Minimum distance was set at 5cm.
Burrowing	The tortoise uses its front claws to remove sediment to place its carapace in the cooled ditch.
Inactive	A lack of mobility for a prolonged period, >1 minute. No visible locomotion.
Feeding	The interest in a food item, followed by the consumption, mastication, and ingestion of said food item for a prolonged period, >5 seconds. May occur alongside other behaviours.
Drinking	An interest in the water bowl, followed by the using of its mandible and gullet to quench thirst from the water source. Water is imbibed through the external nares.
Climbing	Use of its front limbs to hook onto a vertical surface or another tortoise, using its hind limbs to lift the front of the carapace over a more vertical surface.
Investigating	Use of head or limbs to manipulate an object or food item.
Excretion	The release of waste products due to the digestive processes. Uric acid and faeces are expelled out through the cloaca.
Out of Sight	The tortoise has moved away from observer view, making the ability to individually identify or see the species preforming a behaviour an impossibility.
Other	A state behaviour that has not featured within the list but is seen performing.

Table 5. *A. radiata* event behaviours

Biting	The use of the mandible and beak to snap at an individual, either in front of the targeted individual or catching skin or shell of the other individual in quick succession.
Jaw Snapping	The rapid closing of the mandible to the cranium, producing a cracking sound, displaying the aggression of one tortoise to another.
Bill Scraping	When a viscous substance is being removed from the jaw using the beak in an abrasive action.
Head Bobbing	When two male tortoises are competing for a mate, the neck and the skull vigorously move up and down.
Shell Bashing	The clashing of one carapace of one tortoise against another tortoise as a sign of dominance, fighting and aggression.
Other	An event behaviour not described on the ethogram.

Spread of Participation Index

To determine how *A. radiata* utilised their enclosure, a modified spread of participation index (SPI, Plowman, 2003) was conducted using the observation counts. The formula (Table 7) was chosen over the original formula by Dickens (1995), due to it accounting for enclosures with unequal zones, such as thermal and UV gradients, and features like bodies of water (Dickens, 1955; Rose *et al*., 2018). Modified SPI has not

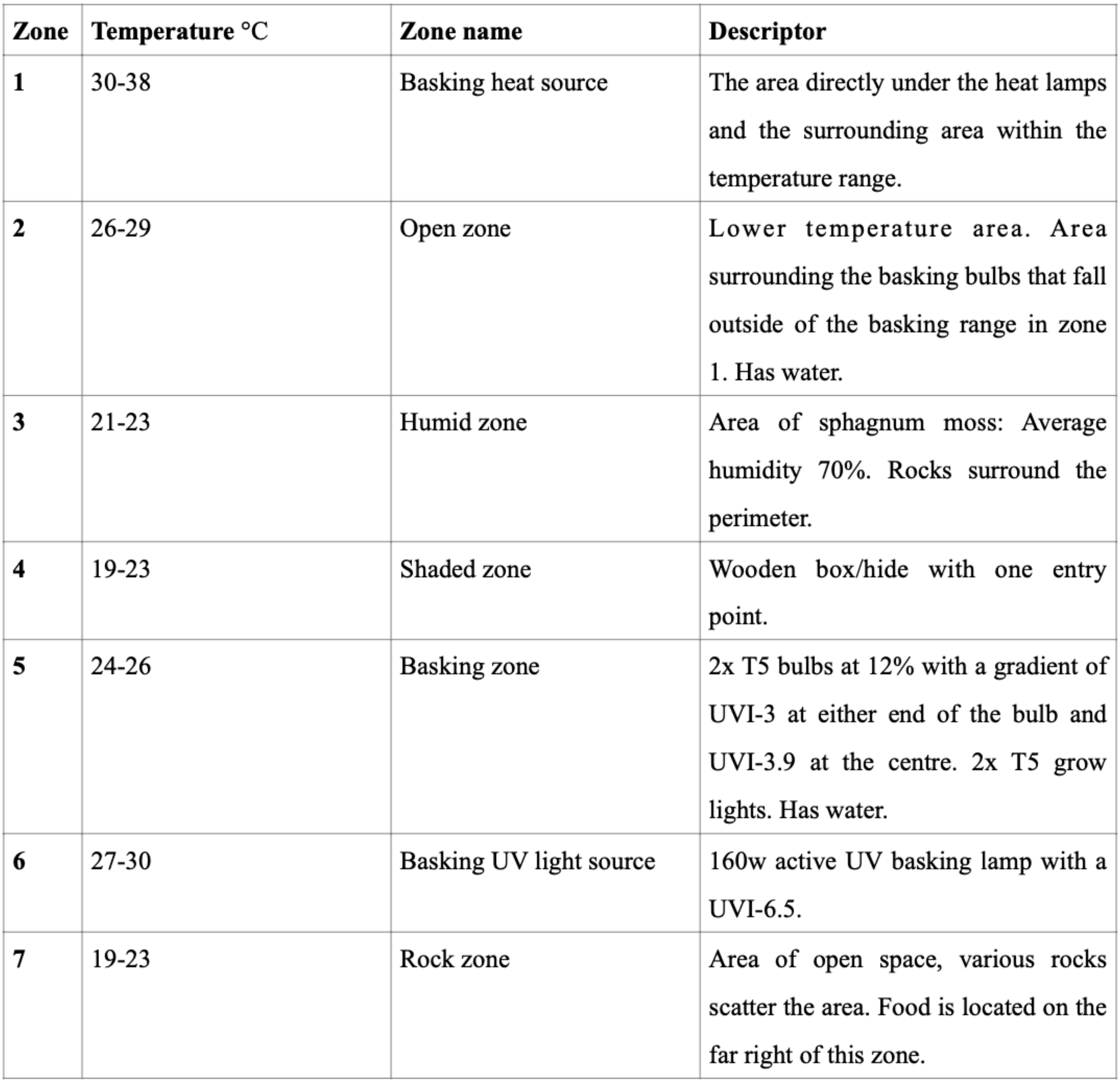
Table 7. The modified SPI uses the below formula which reports a value ranging from 0 to 1. With 0 representing maximum enclosure use (i.e., all zones occupied equally) and a value of 1 indicating minimum utilisation of the enclosure (i.e., only one zone occupied).

$\mathrm{SPI} = \dfrac{\sum \lvert f_o - f_e \rvert}{2\,(N - f_{e\,\mathrm{min}})}$	f_o is the frequency of observations in a zone, f_e is the expected frequency of observations in a zone, based on zone size assuming even use of the whole enclosure, the numerator $\lvert f_o - f_e \rvert$ is the absolute value of the difference between f_o and f_e, which is summed for all zones Σ. N is the total number of observations in all zones and f_{emin} is the expected frequency of observations in the smallest zone (Rees, 2015). 0 = all zones used equally 0.2 = zones used fairly equally 0.4 = zones used reasonably equally 0.6 = unequal use of zones 0.9 = very unequal use of zones 1.0 = all animals in one zone SPI values closer to 0 indicate that the enclosure is used equally, whereas 1 indicates use of only one zone.

yet been applied to reptilian or amphibian taxa, despite use in other areas (Brereton, 2020).

SPI allows keepers to analyse an enclosure based on its biological resources, helping to identify how animals best utilise their enclosure. SPI measurements range from 0 to 1 (Table 7). SPI measurements are a useful tool to incorporate into behavioural studies and can help support behaviour data (Brereton, 2020). The tool can also help identify individual characteristics and enclosure preferences which can be useful in welfare

Table 6. Descriptions of the seven zones that were used to divide the enclosure.



Zone	Temperature °C	Zone name	Descriptor
1	30-38	Basking heat source	The area directly under the heat lamps and the surrounding area within the temperature range.
2	26-29	Open zone	Lower temperature area. Area surrounding the basking bulbs that fall outside of the basking range in zone 1. Has water.
3	21-23	Humid zone	Area of sphagnum moss: Average humidity 70%. Rocks surround the perimeter.
4	19-23	Shaded zone	Wooden box/hide with one entry point.
5	24-26	Basking zone	2x T5 bulbs at 12% with a gradient of UVI-3 at either end of the bulb and UVI-3.9 at the centre. 2x T5 grow lights. Has water.
6	27-30	Basking UV light source	160w active UV basking lamp with a UVI-6.5.
7	19-23	Rock zone	Area of open space, various rocks scatter the area. Food is located on the far right of this zone.

assessments (Quintavalle Pastorino *et al*., 2017).

Data analysis

The data was inputted into Microsoft Excel®, descriptive statistics such as bar graphs and interval plots helped display activity budgets and enclosure use. Microsoft Excel® was also used to calculate individual SPI values per hour from the enclosure use count data; these values were averaged to represent the total individual average for each of the two conditions. Inferential statistical analysis was conducted using Minitab® 2019. The effect of the condition (baseline versus experimental) was investigated for each tortoise state behaviour, using a series of General Linear Models (GLM). The additional covariates of tortoise identity, humidity (%), and temperature (oC) were included in the model.

Poisson regressions were performed to determine the predictors of tortoise zone use for each of the seven zones. The outcome was the amount of time that tortoises spent in each zone, and the predictors included were the individual tortoise, the condition (baseline versus experimental), the temperature and humidity.

Results

Overall, there were sizeable differences in terms of tortoise behaviour between baseline and

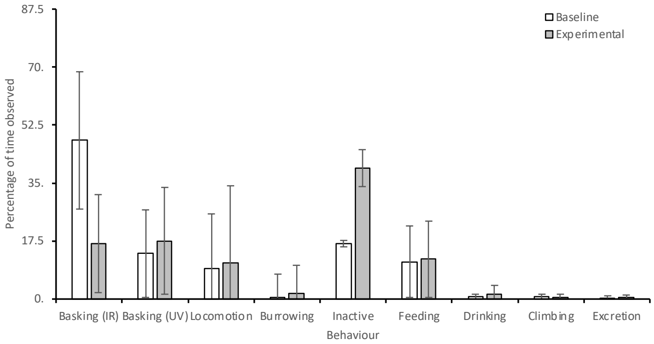


Figure 4. Activity budget for tortoise behaviour during baseline and experimental observation +/- standard error.

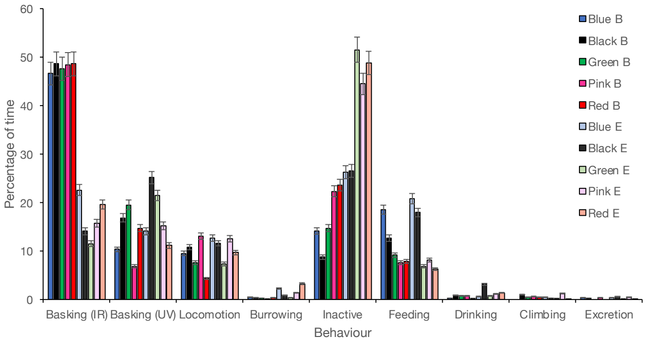


Figure 5. Activity budget of individual tortoises during baseline (B) and experimental (E) observation +/- standard error.

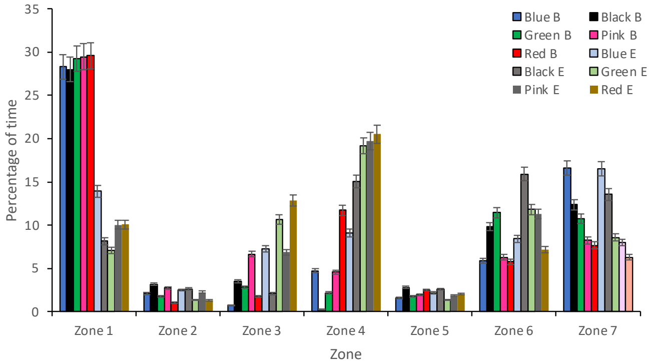


Figure 6. Zone occupancy of tortoises under baseline (B) and experimental (E) conditions +/- standard error.

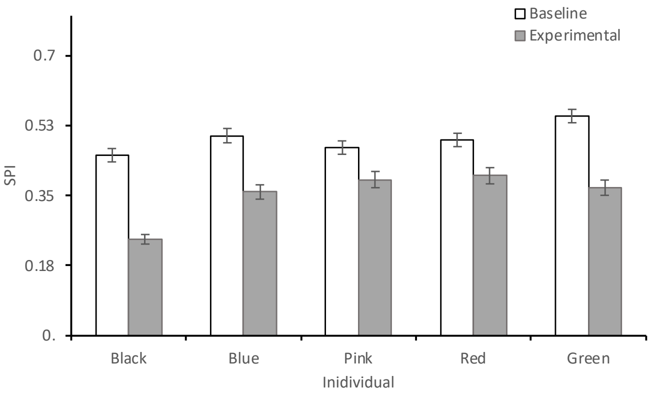


Figure 7. Spread of Participation scores for individual tortoises under baseline and experimental conditions +/- standard error.

experimental observations (Figure 4 and 5). The models for basking (IR), locomotion, inactivity, feeding, drinking, and climbing were significant. However, only Basking (IR), inactivity and drinking can possibly be attributable to the condition, as reported in the general linear model (GLM).

Zone occupancy graphs were developed to show the zone use of tortoises under both baseline and experimental conditions (Figure 6). Spread of participation scores were calculated for individual tortoises under both baseline and experimental conditions (Figure 7). Poisson regressions were run on the count data for zone use. In most cases, both the condition (baseline versus experimental) and the other predictors were significant, all except Zone 5 and zone 7.

Discussion

The study investigated whether the type of infrared wavelength provided to basking tortoises influenced their behaviour and enclosure use. Significant differences in both individual behaviour and enclosure usage were identified. Three state behaviours were significantly influenced by the condition: basking, inactivity, and drinking. A significant increase in "shell bashing" behaviour was also observed. The condition affected enclosure usage, with five out of the seven zones reporting a significant change.

Behaviour

When exposed to a halogen bulb that emitted IR-A, B and C, the subjects decreased basking behaviour by 31% ($P< 0.001$). The results support that of Thomas *et al* (2019), who reported a significant decrease in basking when varanids were exposed to a heating array with increased IR-A output. However, this study observed two conditions that shared the same UVI levels and instead focused on the addition/removal of IR-A; although visible light was different between the two conditions, this was only reported as 300-325 LUX (30 FC). Due to the enclosure being situated in a room with no windows, this reading did not fluctuate.

Whilst a decrease in basking time was observed, it is not possible to ascertain whether this was due to more efficient thermoregulation, the subjects may have simply had a preference to spending larger proportion of their time under an IR-C emitting bulb, perhaps they found the halogen bulb aversive. Future studies could possibly investigate this by implementing a preference component by installing both a CHE and a halogen bulb at either end of an enclosure, the addition of internal dataloggers could also help quantify intradermal temperatures.

A. radiata are heliothermic and diurnal, with basking occurring primarily in the early morning

(Castellano *et al*., 2013), and other behaviours such as foraging and locomotory activity initiated once optimal activity temperatures is achieved (Rose & Judd, 2014; Ernst & Lovich, 2009). In contrast, it appeared that when exposed to the IR-C emitting bulbs, tortoises may have not achieved optimal intradermal temperatures, supported by the fact that subjects spent a larger percentage of time basking. Meek (1984) reports that Hermann's tortoises (Testudo hermanni) display increased activity when internal body temperatures of 34-35°C are reached, basking duration is increased in cloudy conditions and reduced in summer; a strategy which has also been observed in the Greek tortoise (*Testudo graeca graeca*, Meek and Jayes, 1982).

A reduction in basking time may be beneficial to ectotherms; central bearded dragons (*Pogona vitticeps*) have been observed to actively reduce basking duration by utilising adaptive skin colouration to increase IR absorption (Smith *et al*., 2016; Vitt & Caldwell, 2014). The benefits of a reduction in basking behaviour can include extra time to forage, mate and to potentially reduce the time spent out in the open (Meek, 1995; 1988). By hastening thermoregulation, computer modelling predicts that *P. vitticeps* save approximately 85 hours during the energy-intensive breeding season (Smith *et al*., 2016). Currylow *et al* (2017) shed some light on wild, free-ranging *A. radiata* activity by conducting behavioural observations over a duration of 22 months; interestingly, the findings report that basking contributes to just 3.5% of their daily budgets, suggesting that reducing basking times is important for replicating wild behaviour. Literature, both peer reviewed and anecdotal could not be sourced regarding captive energy budgets for *A. radiata*, however, a wild versus captive budget was found for the angonoka (*A. yniphora*) (Currylow *et al*., 2017). The study identified that basking duration between wild and captive A. yniphora were 1% and 7% respectively, indicating that captive animals may spend larger percentage of time basking.

Due to a significant reduction in basking (IR) behaviour, subjects exposed to the halogen bulb had extra time within their activity budget to execute other behaviours, with this extra time allocation, the five individuals increase inactivity levels by 137%. Inactivity could be interpreted as sleeping, relaxing, digesting, or perhaps lack of stimulation. Despite this, wild *A. radiata* and A. yniphora are known to spend a large proportion of their time being inactive, often including periods of up to four days (Currylow, Louis & Crocker, 2017; Paquette *et al*., 2006). Wild *A. radiata* activity budgets conducted by Currylow *et al* (2017) report that "resting" behaviour contributes towards 61-76% of tortoise's daily budget; and that wild A. yniphora resting behaviour accounted for 85% and around 70% in captivity. Prior studies amongst other Genus help further support the theory that a high percentage of inactivity may be a natural chelonian behaviour; Greek tortoises (*T. graeca graeca*) 93%, Russian tortoise (*Agrionemys horsfieldi*) >90% and desert tortoise (*Gopherus agassizii*) >90% (Lagarde *et al.*, 2008; Lagarde *et al.*, 2003).

The only behaviours which were found to be purely influenced by IR wavelengths were Basking (IR) and drinking. The predictor "humidity" appeared to be solely responsible for significance found within tortoise locomotion; this was also identified within captive A. yniphora in Madagascar, the animals walked more often in higher humidity ground conditions (75.0 ± 1.9%).

A significant increase ($p < 0.001$) in shell bashing was reported for tortoises exposed to the halogen bulb, however, a low effect size ($r = 0.033$) implies low variation between the conditions. Interestingly, 86% of shell bashing behaviour was observed between the two males, this could be considered as increased aggression towards conspecifics, notably towards the same sex, it is not known why this was observed. The males may have had more energy to display agonistic behaviour, either for female competition or for priority basking resource rights; due to the increased height of the halogen bulb condition, the basking size could have been smaller, resulting in resource competition.

All five subjects were observed spending similar basking durations during the CHE condition (+/- 2%), however, basking duration began to vary between individuals when the subjects were exposed to the halogen bulb. Subject "black" spent a large proportion of his time basking in the halogen condition (14%), this was originally assumed to be due to his size and thus needing more time to effectively reach a comfortable optimal temperature, however, the largest tortoise, subject "green", was observed spending the least amount of time basking (7%), suggesting that in this study, basking duration cannot be explained by weight or sex. Subject "Black" was the darkest tortoise amongst the subjects, with a carapace containing very little yellow/orange pigmentation, primarily being black. It was theorised that due to this, energy absorbency would be hastened, resulting in a shorter basking duration being required, a theory derived from simple physical principles (Rice and Bradshaw, 1980), however, the opposite was observed. Subject "black" had the highest IR basking budget compared to other tortoises: it is unclear this individual spent such a large proportion of time basking.

Enclosure usage

While individual differences in enclosure use were observed (Figure 7), these were often non-significant. The tortoises tended to change their enclosure use in similar ways in response to the experimental condition. In the IR-A and B condition, tortoises spent 19% more time in zone four (sheltered refugia). The primary behaviour that tortoises were exhibiting in zone four was inactivity. The behavioural study conducted by Currylow *et al* (2017) also included location data, the findings report that wild *A. radiata* spend on average, 25.5% of their activity budget resting whilst under shelter, this value is close to the experimental condition value for zone four (27%). This similarity may possibly indicate that tortoises exposed to IR-A and B wavelengths are expressing more natural enclosure use usage. Sheltered refugia is an important resource for effective thermoregulation (Rasoma *et al.*, 2013), they can act as a microclimate allowing herptiles to optimise energy expenditure through behavioural variation (Huey & Tewksbury, 2009), additionally, they can provide animals with security, potentially helping with predator avoidance, which may potentially reduce animal stress. A stress measurement component would be an interesting future study regarding IR heating provisions.

The subjects spent more time within the moist/humid area (zone three) when exposed to the halogen condition. The choice in zone use may have been a thermoregulatory strategy in response to increased intradermal temperature, this theory may also explain the extended use of the sheltered refugia. Though non-significant, increased burrowing behaviour was also observed, this may have been attributable to thermoregulatory behaviour, a response that is reported amongst Testudines species, including *A. radiata* (Wilson, Nagy, Tracy, Morafka & Yates, 2001).

A modified SPI reported that all five tortoises utilised their enclosure more evenly when exposed to the halogen bulb (Figure 7), this could be attributed to the fact that the tortoises were more active from an increased internal temperature and were utilising the enclosure more, specifically the humid, sheltered and drinking zones for thermoregulation. It was evident that zones two and five were underutilised and were unaffected by the type of heating array. Zone five was a secondary UV basking resource situated within the middle of the enclosure; this zone had a reduced UVI output in comparison to the more favoured UV basking resource in zone six. It is a possibility that the tortoises had a preference over a stronger UVI or may have preferred basking in a zone with a higher ambient basking temperature. The results may suggest the necessity to enhance the underutilised areas with more fitting biologically relevant or enriching structures that can potentially induce the subjects to use them (Brereton, 2020).

Use within captivity

The provision of IR-A and B wavelengths to herptiles could be double-edged, whilst it may have beneficial welfare applications, it also may have some unfavourable consequences. Visitors attend zoological institutions to view animals and this study suggests that the lights may increase inactivity (Roe, McConney & Mansfield, 2014). Additionally, increased aggressive behaviours that may potentially be caused by IR-A and B, may increase the chance and risk of injuries for species housed within communal enclosures. These attributes are unattractive qualities for captive animals to exhibit, ones that keepers actively try to discourage. However, this needs to be balanced against the fact, that as professionals and welfare advocates, it is important to encourage and replicate natural behaviours where possible (Hosey, Melfi & Pankhurst, 2013).

This study is not suggesting that IR-C emitting arrays such as CHE should be abandoned, they remain an essential lighting choice for night-time heating. When the Sun sets, IR-A and B are no longer being emitted, however, surfaces around 30-100°C that have received IR-A lighting during the day do emit IR-C, a significant amount of this heat is transferred to the surrounding via conduction (Wunderlich, personal communication, 2020); IR-C energy can be released for long periods of time, hence the reason why there is not a sudden temperature drop after dark (Jones, 2018). This makes IR-C heating arrays a good night-time solution for replicating natural heating after dark (Courtney-Smith, 2018).

Future directions

The findings of this study demonstrate that the provision of IR-A and B lighting may have been responsible for a significant effect on reptile behaviour and enclosure usage. The findings may however be due to the time window in which data was collected, it is a possibility that basking and other behaviours occurred earlier in the morning, prior to observation. Data collection occurred between the hours of 1100-1600, thus activity budgets were not full 24-hour representation. Future studies could address this by monitoring morning, afternoon, and evening observation points such as implemented by Thomas *et al* (2019).

The power output between the bulbs was not ideal (150W vs 175W), this resulted in the conditions having different basking temperatures and height, which affected basking area size. Best efforts were made to minimize both variables, however, these were still evident. At the time of the study, a PAR 38 150W Halogen flood bulb could not be sourced, if repeated, this should be addressed, and uniformity of wattage should be a priority.

Conclusion

IR-A and B emitting bulbs may allow captive reptiles to express behaviours that are not commonly observed by either A) having more energy and B) having more free time within the activity budget to express certain behaviours. Based on wild activity budgets and enclosure use studies, to better replicate *A. radiata* natural behaviour, reduced basking durations, increased inactivity and prolong shelter use should be further encouraged within captivity, the findings of this study appear to show that by providing natural bioavailable heat in the form of IR-A and B, this could potentially be accomplished. The concept of promoting natural behaviour is a key element in current policymaking on animal welfare, as such, it is an area that demands further research. The answer to the question of whether IR-A and B emitting arrays are beneficial for captive herptiles is unfortunately out of scope for this paper, however, IR-A and B provision is a progressive area of study within the field of reptile husbandry, as such, it warrants extensive consideration and research. This will help aid in the advancement of captive welfare and the development of husbandry guidelines.

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Tarantula binge: Predation of a large tarantula by *Leptodactylus* frog in the Peruvian Amazon

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Diet information is important for understanding species natural histories and how they interact in their ecosystems (Ceron *et al*., 2018). *Leptodactylus* is a genus of large frog widely distributed across the South American continent (Maneyro *et al*., 2004; Heyer, 2005). They occupy a range of habitats including tropical rainforests, grasslands and rocky substrate. They are regarded as opportunistic sit-and-wait ambush predators, consuming prey types from upwards of 18 taxonomic groups (Thomas *et al*., 2017; do Couto *et al*., 2018; Ceron *et al*., 2018). Prey can include toads, lizards, spiders, scorpions, centipedes, millipedes, ants, termites, beetles, and crabs (França *et al*., 2004; do Couto *et al*., 2018; Ceron *et al*., 2018; Augusto Pena Correa *et al*., 2020). The feeding on large tarantulas by *Leptodactylus* are reported in Brazil and Ecuador (Duellman, 1978; do Couto *et al*., 2018) but to date tarantulas are not documented in the diet of *Leptodactylus* in the Peruvian Amazon. We report here, a predation even by a young *Leptodactylus* frog on a tarantula of almost equal size, suggesting that large tarantulas are part of the diet of *Leptodactylus* species in Peruvian populations.

At 2222 h on the night of 17 July 2018 in the Pacaya-Samiria National Reserve, Peru (-4.853850, -74.366700), during field work, one of the authors (EK) observed a juvenile *Leptodactylus* species (SVL ca. 4.5 cm) subduing a comparably sizable tarantula (body size ca. 6 cm) (Figure. 1) on the forest floor. The juvenile frog engaged the tarantula from behind, and successfully consumed the opisthosoma (Figure. 1). The tarantula struggled for approx. 30 seconds before remaining still, as the frog continued to consume the tarantula whole.

Leptodactylus typically consume manageable sized prey, but some reports suggest that they occasionally consume exceptionally large and potentially dangerous prey in comparison to their size (do Couto *et al*., 2018). Due to time constraints, and so as not to disturb the interaction, the frog was left to continue engulfing its meal

Figure 1. *Leptodactylus* species consuming a large tarantula in the Peruvian Amazon (photo taken by Ellen King).

undisturbed, and therefore a formal identification to species level was not obtained. Consequently, some limitations to this observation are that the frog could only be identified to the genus level. Although it is confirmed that large tarantulas are successfully consumed by *Leptodactylus* (do Couto *et al*., 2018), in a previous observation, an attempt to consume a large tarantula resulted in the frog being envenomed after one fang penetrated the frog's upper lip (Augusto Pena Correa *et al*., 2020). However, in this observation, the positioning of the frog and the lack of struggle from the prey after 30 secs suggest it is likely the frog successfully consumed the tarantula without retaliation.

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The great escape: earthworm survives the digestive tract of Indian bullfrog (*Hoplobatrachus tigerinus*)

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Introduction

The Indian bullfrog (*Hoplobatrachus tigerinus*) (Daudin, 1802) is a large dicroglossid frog distributed amongst the Indian subcontinent, and has established invasive populations on the Andaman Islands and Madagascar (Mohanty *et al*., 2020). It is one of the largest species of anuran in South and Southeast Asia, reaching a snout to vent length of 170 mm, and classified as "Least Concern" on the IUCN Red List (Padhye *et al*., 2008).

The tadpoles of the Indian bullfrog are obligate carnivores, feeding on sympatric tadpoles and scavenging animal matter (Khan, 1996). As adults they are generalists, feeding mostly on invertebrates, but only limited by what can fit in their mouth, including vertebrates (Khan, 1973; Corlett, 2011; Rahman *et al*., 2012), with some notable predation notes on other large frog species (Datta & Khaledin, 2017; Tripathi, 2018).

There are few records of prey items escaping through the digestive tract of a predator, most unpalatable or indigestable prey items are regurgitated instead of swallowed. Snails have been demonstrated to consistently survive the digestive system of birds (Wada *et al*., 2012), and other molluscs have been identified to survive the digestive tracts of fish both in situ and ex situ (Brown, 2007). These are hypothesised to be advantageous dispersal mechanisms, by surviving the digestion process and excreted with faeces.

Some more examples exist from anuran predators. Sugiura (2020) reports the escape of the aquatic beetle *Regimbartia attenuata* (Coleoptera: Hydrophilidae) from the vents of five frog species via the digestive tract, and experimentally demonstrates they actively use their legs to hasten their escape. O'Shea *et al*., (2013) observed the defecation of a live blind snake (*Ramphotyphlops braminus*) from a Common Asian Toad (*Duttaphrynus melanostictus*), which despite being small and slender, is still a remarkable feat for a vertebrate to survive. The blindsnake did, however, die shortly after expulsion, presumably from exposure to digestive chemicals or anoxia (Pizatto *et al*., 2012).

Observation

Here we report the excretion of a live earthworm (*Opisthopora*) by an Indian bullfrog, by Dehra, Himachal Pradesh, India. On the 31st of August, 2019, at approximately 2230, during a night-time herpetofaunal survey, we encountered a sub-adult Indian bullfrog (*H. tigerinus*) on a concrete path. The surrounding surfaces and vegetation were wet from recent light rain, and invertebrate and amphibian activity appeared to be elevated. Upon closer inspection, we identified a live, moving annelid worm emerging from the cloaca of the frog. The frog was approximately 40 mm snout to vent length, and approximately 55 mm of the worm was visible (Figure1). The presence of the clitellum and the direction of locomotion demonstrated the worm was emerging anterior first, with the posterior part still within the frog. By appearance of its rectilinear locomotion, the worm appeared to be actively "escaping" from the frog's cloaca, however it is impossible to discount any potential effect of muscular contractions from the frog. The frog remained motionless during the encounter, with

Figure 1. Sub-adult Indian Bullfrog (*Hoplobatrachus tigerinus*) nearing the end of its excretion of a live earthworm (*Opisthopora*).

no behaviour inferring discomfort. The remaining excretion continued for approximately three minutes, until the worm had fully escaped. Both the worm and the frog appeared unharmed. The entire length of the worm was estimated at 65 mm. Although both predator and prey were alive once we had finished observing, it is possible the worm died after the event. A full video of the encounter is available at https://figshare.com/articles/media/VID_20190831_210833_mp4/14673756.

Conclusion

Given the voracious nature of the Indian bullfrog, the likely scenario included the predation and ingestion of the worm without much jaw pressure, allowing it to survive entry to the digestive tract. The worm than either actively, or passively, or likely a combination of both, navigated through the digestive tract to the cloaca, of which we observed. Despite annelid worms respiring through their skin, they are notable for their regenerative properties, and given previous literature regarding the excretion of live animals from anurans, perhaps the digestive system of frogs are not particularly strong, or some live prey items may be more tolerant of the conditions. These factors combined may have worked synergistically to explain our observation.

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The use of an artificial niche dimension by the introduced *Anolis cristatellus* (C. Duméril and Bibron, 1837) in the Caribbean lowlands of Costa Rica.

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Few animal species engage both in diurnal and nocturnal activities, or shift between the two (Abom *et al*., 2012; Fraser *et al*., 1993) because diel activity patterns normally are well established (Abom *et al*., 2012). Most animals have set activity times and as such species can be classified as diurnal, crepuscular, nocturnal, or cathemeral (Abom *et al*., 2012; Kronfeld-Schor & Dayan, 2003; Toms *et al*., 2022). Most lizards of Gekkota (geckos and pygopodoids) are nocturnal, however, the majority of other lizards predominantly are diurnal (Stark *et al*., 2020; Vidan *et al*., 2017; Vieira *et al*., 2020). Moreover, species of lizards excluding *Gekkota* that are active both diurnally and nocturnally are extremely rare, as are those that may shift from diurnally to at least partially nocturnality if conditions allow (Amadi *et al*., 2021), as has been found in some snakes (Abom *et al*., 2012).

Although the environmental factors associated with lizard nocturnal activity largely remain unknown (Vidan *et al*., 2017), it is known that activity levels of ectotherms, including lizards, primarily is correlated with availability of external heat sources (Afsar *et al*., 2018; Pianka & Vitt, 2006; Vidan *et al*., 2017; Underwood, 1992). As heliothermic organisms, diurnal lizards depend on sunlight and are active during the day; however, they sometimes can be active in the absence of this abiotic factor (Afsar *et al*., 2018; Nordberg & Schwarzkopf, 2019; Rose, 1981; Vieira *et al*., 2020). As a result, over 40 diurnal reptile species, particularly anole lizards (*Anolis* spp., Squamata: Dactyloidae), have expanded their niche from diurnal to nocturnal habits (e.g., Baxter-Gilbert *et al*., 2021), although these statements have in general been informed by anecdotal reports (Amadi *et al*., 2021; Baxter-Gilbert *et al*., 2021;

Maurer *et al.*, 2019). The transition from diurnality to nocturnality generally occurs under particular conditions. Large lizards such as iguanas have thermal inertia that allows them to transition in their tropical ranges (Mora, 1986). Other species take advantage of the availability of additional food: for example, insects that are attracted to artificial lights (Owens & Lewis, 2018). Because lizards are ectotherms, nocturnal activity in the vicinity of artificial light comes with the requirement to cope with lower night-time temperatures due to the lack of external heat source (Gaston, 2019; Nordberg & Schwarzkopf, 2019; Vidan *et al.*, 2017). As a result, this activity niche, often referred to as the night-light niche, is not exploited commonly (Amadi *et al.*, 2020; Amadi *et al.*, 2021; Gaynor *et al.*, 2018). Besides, patterns of diel activity normally are fixed firmly to function most successively at the time of day when individuals are most likely to be active, as determined by eye morphology, intraspecific communication methods, and body coloration (Abom *et al.*, 2012). These diel activity cycles are one of the key niche partitioning elements among lizard species (Pianka & Vitt, 2006); it therefore is rare to find species of lizards that are active both diurnally and nocturnally (Amadi *et al.*, 2021; Gaynor *et al.*, 2018). However, increased urbanization and the concomitant presence of artificial light at night (ALAN; Gaston *et al.*, 2014) have facilitated the transition of several normally day-active lizard species to extend or even change their typical diurnal behavior patterns to extend into crepuscular and even nocturnal activity (Maurer *et al.*, 2019; Perry *et al.*, 2008). This is notably true for anoles, whose dominant sense for prey acquisition is sight (Maurer *et al.*, 2019), and particularly for introduced species, where 14 out of 20 known introduced species have been observed using ALAN (Thawley & Kolbe, 2020).

Anoles are one of the most species rich of all the lizard groups (Pianka & Vitt, 2006; Pyron *et al.*, 2013; but see Nicholson *et al.*, 2012). Interspecific competition among species of anoles is avoided by using distinct microhabitats within their geographic ranges; the use of distinct microhabitats within ecosystems results over evolutionary time in distinct and predictable evolutionary trajectories (Crandella *et al.*, 2014; Losos, 2009). This group of lizards therefore have been hypothesized as recurrently evolving into occupancy of a distinct set of niches (Losos *et al.*, 2003) wherein each species is associated with a specific suite of morphological and ecological characteristics (Mora & Escobar-Anleu, 2017; Walguarnery *et al.*, 2012).

Anolis cristatellus (Dactyloidae: Squamata: Reptilia) is a species native to Puerto Rico and the

British Virgin Islands; in Costa Rica is a highly adaptable invasive species that is tolerant of habitat alteration (Hall & Warner, 2018; Kolbe *et al*., 2021; Thawley *et al*., 2019). It initially was found in Costa Rica on the giant fig trees of Parque Vargas, Port of Limón (Savage, 2002), but now is commonly found in city parks, roadside vegetation, and within homes and other structures, throughout much of the southern Atlantic coastal plain region of Costa Rica (Leenders, 2019) as well as in a few other localities in Limón and Cartago provinces (Savage, 2002). It is a diurnal species (Garber, 1978) that perches on the lower trunks of trees, on the ground, and on walls and rafters of wooden structures, where it sits and waits for prey, principally arthropods, moving along the ground (Leenders, 2019; Savage, 2002). *Anolis cristatellus* appears to be restricted to open habitats (Garber, 1978): in Cahuita, also in Limón, for example, only a small creek separates the town from a national park (Parque Nacional Cahuita; creek at ca. 9.7363889°N, 82.83917°W), but the natural forest milieu appears to constitute an impenetrable—or certainly unsuitable—ecological matrix for this species.

Anolis cristatellus is a moderate-sized anole (205 mm total length; tail ca. 60 to 65% of total length; Savage, 2002). They are dull brown with several transverse dark bars in males and overlapping diamond-shaped blotches in many females (Savage, 2002). This species is easily recognized because they have a distinct caudal crest, more developed in males, and the dewlap is greenish yellow with the free margin of burnt orange to reddish, smaller in females (Savage, 2002).

Artificial light at night is one of the many consequences of contemporary human development, and although its impacts on biodiversity as a component of anthropogenic global change is increasingly being recognized, they remain poorly understood (Maurer *et al*., 2019). Animals such as anoles that use sight as a primary sense in prey acquisition have as a result obtained new opportunities to exploit the night-light niche (Kolbe *et al*., 2021; Maurer *et al*., 2019). At least seventeen species of anoles have been documented using ALAN (Maurer *et al*. 2019; Perry *et al*., 2008). However, the ecological consequences of this nocturnal activity by anoles, and other organisms, also largely remain unknown (Maurer *et al*., 2019; Rutschmann *et al*., 2021). In laboratory conditions, brown anoles (*Anolis sagrei*) exposed to ALAN increased growth and did not suffer apparent negative consequences (Thawley & Kolbe, 2020). Individuals exposed to ALAN developed earlier egg-laying, probably by mimicking a longer photoperiod, and increased reproductive output without reducing offspring quality and likely increasing fitness (Thawley & Kolbe, 2020). *Anolis cristatellus* normally exhibits diurnal behaviour; here we report its nocturnal activity in Costa Rica under ALAN.

On 06 March 2018 at 2134 h we observed a large male *Anolis cristatellus* perched close to a white, fluorescent light source (Figure 1). This male was hunting in the inside upper part of the outside dining room of Las Veraneras hotel, in Manzanillo, Limón (9.630276° N, 82.660276°W; 8 m asl). The individual remained at its perch hunting insects until at least 2300 h, when we left the site. We visited this site again on 27 June 2019 and observed another individual in the same location. On both occasions we saw individuals of this species in the vicinity of the dining room during the day, but not in the upper part where the individuals observed at night on the two recorded occasions were located.

An increase in the prevalence of ALAN is an important component of global environmental

Figure 1. An adult male *Anolis cristatellus* perched close to a white, fluorescent light source, nocturnally foraging for insects at an open dining room, Manzanillo, Limón, Costa Rica. Photo: José M. Mora.

Figura 1. Un macho adulto de *Anolis cristatellus* posado cerca de una fuente de luz fluorescente blanca, en busca de insectos durante la noche en un comedor abierto, Manzanillo, Limón, Costa Rica. Foto: José M. Mora.

change, however, its biological impacts only now are beginning to be recognized. Artificial lighting attracts and repels animals differentially according to each particular taxonomic group's ecological preferences (Mora *et al*., 2018). ALAN exposition may negatively affect a variety of organisms by means of disrupting key functions such as physiology, growth, stress, and reproduction, thereby resulting in adverse conditions for many species in urban areas (e.g. Gaston *et al*., 2015; Ouyang *et al*., 2017; Ouyang *et al*., 2018). However, it also may be favourable to other species, such as brown anoles, although those results are derived from laboratory conditions (Thawley & Kolbe, 2019). ALAN as part of the urbanization process drastically transforms the environment, and can create new habitats with different elements and dynamics that opportunistically can be leveraged by certain species (Badillo-Saldaña *et al*., 2016; McKinney, 2006; Perry *et al*., 2008). Lizards will adapt to such changes depending on whether the species under consideration are negatively impacted or whether they have the behavioural flexibility to exploit novel environmental conditions (Amadi *et al*., 2020).

Several reptile species, among many other organisms, have expanded nocturnal foraging in the presence of ALAN (Garber, 1978; Rydell, 1992; Thawley & Kolbe, 2020). ALAN has caused diurnal lizards adapted to living in urban areas to alter their diel cycles (Perry *et al*., 2008; Powell, 2015). Nocturnal activity facilitated by ALAN has been reported for several *Anolis* species (e.g. Badillo-Saldaña *et al*., 2016; Brown & Arrivillaga, 2017; Thawley & Kolbe, 2020). It is possible that anoles as well as other organisms could be resistant to at least some of the negative effects of ALAN, and even could take advantage of the novel niche space ALAN creates (Thawley & Kolbe, 2020). Most reports on anole nocturnal activity are from Tropical environments, most likely because the ecological and physiological characteristics such as the optimal body temperatures typical of these lizards allow them to exploit available resources depending on the ambient temperature, which is less variable from day to night in the tropics than in temperate regions (Badillo-Saldaña *et al*., 2016; Janzen, 1967; Medina *et al*., 2016). Tropical temperatures enable anoles to maintain high body temperatures both day and night allowing diurnal species become cathemeral resulting in changes in movements patterns (Abom *et al*., 2012).

Many species of lizards are insectivorous, and insects are influenced strongly by lighting (Owens & Lewis, 2018). Nocturnal activity by diurnal lizards may allow for maintenance and importantly, expansion of territories, as well as opportunities for courtship and reproduction and avoidance of competition and predation (Gaston, 2019; Kolbe *et al.*, 2021; Maurer *et al.*, 2019; Rich & Longcore, 2006). It has been hypothesized that nocturnal exposure and movement by spotted turtles (*Clemmys guttata*) provide them with increased time for foraging or mate-seeking, investing daytime hours for basking (Toms *et al.*, 2022). Night-time also can be used for basking, as shown by Krefft's river turtles (*Emydura maquarii krefftii*) in Australia (Nordberg & McKnight, 2020). For most ectotherm terrestrial animals in tropical and desert areas, the principal thermal challenge is not to attain high body temperatures but rather to stay cool (Kearney *et al.*, 2009). This means that in the tropics, diurnal, sight-dependent species, such as anoles, potentially could "compensate" for the hours of activity precluded by excessively high temperatures with periods of nocturnal activity within more suitable temperature ranges when ALAN is provided. This could particularly be the case in lizard species adapted to live in urban areas such as *Anolis cristatellus*. This species perches on broader, smoother, artificial substrates such as concrete walls and metal fences rather than the trunks of trees found in natural habitats (for Puerto Rico, see Tyler *et al.*, 2016); preference for broad substrates accordingly resulted in niche expansion for this species in Miami, Florida (Battles *et al.*, 2018).

Some diurnal lizards that are active under ALAN conditions potentially and eventually could be active in such areas under conditions absent artificial light if temperature allows activity. It was reported that *Anolis cristatellus* may be active under moonlight (on Dominica: Brisbane & van den Burg, 2020). We hypothesize that presence of artificial light is the first stimulus for nocturnal activity on the part of some lizard species, given the opportunity for extended foraging and consequent increased energy acquisition (Dwyer *et al.*, 2013). They then would transition to nocturnal activity in the presence of moonlight if temperature and other environmental conditions allow it. Several reports, albeit isolated, have reported nocturnal or crepuscular activity in lizard species and other reptiles otherwise characterized as diurnal (Arenas-Moreno *et al.*, 2018; Arenas-Moreno *et al.*, 2021; Lara-Resendiz, 2020; Nordberg & McKnight, 2020; Rutschmann *et al.*, 2021; Toms *et al.*, 2022). Warm environments as are the Caribbean lowlands of Costa Rica may allow *Anolis cristatellus* to be active at night because absence of an external heat source is not a limiting factor. It has been shown that anoles are behaviourally capable of exploiting novel resources, and many species have been observed foraging nocturnally under ALAN in urbanized areas where they are invasive (e.g. Badillo-Saldaña *et al.*, 2016; Brown & Arrivillaga, 2017; Kolbe *et al.*, 2016;

Lapiedra *et al*., 2017; Maurer *et al*., 2019; Perry *et al*., 2008; Thawley & Kolbe, 2020; Winchell *et al*., 2018). Invasiveness in some reptiles may depend on their ability to exploit the night-light niche (Perry *et al*., 2008; Thawley & Kolbe, 2020). Negative and positive impacts of ALAN may play a determining role selecting which species invade and exploit urban environments (Thawley & Kolbe, 2020).

Buffering for the hours of activity precluded by excessively high temperatures with periods of nocturnal activity within more suitable temperature ranges when ALAN is provided could be an alternative for tropical lizards affected by warmer conditions due to climate change. In addition, warmer nocturnal temperatures can advance reproduction timing and increase offspring quality, as was shown in the Otago gecko (*Woodworthia* "Otago/Southland"; Moore *et al*., 2020). However, increases in nocturnal temperatures may provide body operating temperatures leading to increased individual performance but reducing optimal resting time and raising energetic costs of rest (Rutschmann *et al*., 2021). ALAN may alter activity towards night time in environments where daily temperatures exceed critical maximum temperatures (Lara Resendiz, 2019; Nordberg & Schwarzkopf, 2019). However, these changes may have consequences over community and ecosystem structure by their effects on dispersal strategies, population dynamics, and intra- and interspecific interactions (Toms *et al*., 2022). The plasticity evidenced by *Anolis cristatellus* in expanding its foraging niche to a nocturnal milieu points to a potentially important suite of behavioural characters that may enable this and other species equipped with such behavioural flexibility to weather impending increases in environmental temperature regimes.

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Introducing a new society

Later this year, Captive & Field Herpetology will be forming a society with the intent of creating a bridge between field herpetologists and herpetoculturists. The Captive & Field Herpetology Journal was originally formed to initiate the bridging of both fields and we believe the society is the next step in bringing herpetologists, herpetoculturists and field herpers together.

Details are being kept under wraps as we prepare the final parts of this venture but you can expect events, field excursions, grants, merchandise and much more! The society will bring with it a number of positions to sit on its committees, please get in touch with us at captiveandfieldherpetology@gmail.com if interested.

We plan to release the society with volume 6 of The Captive & Field Herpetology Journal later this year.

Final call for Mizoram Monsoons 2022

July 2022 will see Captive & Field Herpetology return to the field with an expedition to Mizoram in northeastern India. This expedition is open to 10 participants with a number of places already filled during the time of writing this. The expedition will run from the 11th of July until the 22nd of July and will see us explore the habitats of king cobra, nunerous vipers and the Indian cobra in West Bengal where we will begin and end the journey.

For costs, details on joining and any other details visit our website at www.captiveandfieldherpetology.com or contact us as captiveandfieldherpetology@gmail.com.

Cover image submissions

The Captive and Field Herpetology Journal welcomes image submissions for use on the covers of upcoming volumes. Images should be of high quality and free of signatures/watermarks. Preference will be given to images in landscape orientation as images are spread across the front and back covers. Preference will also be given to darker images or images which feature primarily dark backgrounds. Please see previous journal releases for examples and email any image submissions to captiveandfieldherpetology@gmail.com.

Image: A male European adder (*Vipera berus*) shortly after emerging from its hibernacula, north Wales, 2022.