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Response to novelty as an indicator of reptile welfare

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ABSTRACT

Whilst a great deal of research has been focused on identifying ways to assess the welfare of captive mammals and birds, there is comparatively little knowledge on how reptilian species are affected by captivity, and the ways in which their welfare can be accurately assessed. The present study investigated response to novelty – a commonly used approach to assess anxiety-like behaviour and hence welfare in non-human animals – in two species of reptile with the aim of determining whether this approach could be successfully translated from use in mammalian and avian species for use in reptiles, and whether we could also identify reptile-specific and/or species-specific behaviours. Eight red-footed tortoises (*Chelonoidis carbonaria*) and seventeen bearded dragons (*Pogona vitticeps*) were observed individually in both familiar and novel environments for 10 min time periods, and their behaviour recorded. Tortoises were found to begin locomotion sooner when placed in a familiar environment than when placed in a novel environment, they extended their necks further in a familiar environment and their neck length increased over time in both familiar and novel environments, suggesting an overall anxiety-like response to novelty as seen in non-reptilian species. In contrast, whilst bearded dragons exhibited significantly more tongue-touches in a novel, compared to a familiar, environment, they showed no difference between familiar and novel environments in their latency to move. This result suggests that, whilst the dragons appeared to discriminate between the two environments, this discrimination was not necessarily accompanied by an anxiety-like response. This study has confirmed the translatability of response to novelty as an approach to assess anxiety-like behaviour in one species of reptile, as well as identifying species-specific behaviours that have the potential to be used in future studies when assessing the welfare of reptiles in response to captive environments, but our results also highlight the need to be aware of species differences within a class as diverse as reptilia.

1. Introduction

Despite continued refinement of our ability to assess the welfare of captive animals using behavioural (*e.g.* optical flow, Dawkins et al., 2012), physiological (*e.g.* heart rate variability, Rehn and Keeling 2011) and cognitive indicators (*e.g.* cognitive bias, Harding et al., 2004) in conjunction with the assessment of motivations (*e.g.* Mason et al., 2001) and preferences (*e.g.* Nicol et al., 2009), the majority of welfare research has been focused on mammalian and avian species – whether they be laboratory, farm, zoo or companion animals – with comparatively little welfare research carried out on reptiles and/or amphibians. Yet, there has been a recent rapid increase, and continual growth, in the ownership of exotic pets (Whitehead and Forbes, 2013), and it is estimated that of the 11 million (40% of) households in the UK that have pets, around 3% of these keep reptiles, with estimates of UK captive reptile numbers at c.0.9 million (0.3 snakes, 0.3 turtles/tortoise, 0.3 lizards) (Pet Food Manufacturing Association, 2016).

These numbers indicate an urgent requirement for the creation of evidence-based guidelines for reptile husbandry and housing to ensure good standards of welfare (Altherr and Freyer, 2001), particularly given that the overall mortality rate for reptiles in the first year following acquisition in UK homes has been estimated from 3.6% (Robinson et al., 2015) to as high as 75% (Toland et al., 2012). However, little is known about how captive environments affect reptile welfare or even how welfare in reptilian species should be assessed (Burghardt, 2013). A few studies have shown initial research into reptile welfare (*e.g.* Kreger and Mench 1993 (ball python – *Python regius,* Blue-tongued skink – *Tiliqua scincoides)*, Schuett et al., 2004 (rattlesnakes – *Crotalus atrox*) Case et al., 2005 (box turtles – *Terrapene carolina carolina*), Kalliokoski et al., 2012 (green iguanas – *Iguana iguana*)) and there is literature that highlights the importance of this subject area (Burghardt, 2013; Hernandez-Divers, 2001; Stanford, 2013; Warwick et al., 2013). Experimental studies have revealed an effect of housing environment on behaviour and immune response, as well as environmental prefer-

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ences (Case et al., 2005) and impact of handling on behavioural and physiological measures (*e.g.* Schuett et al., 2004; Langkilde and Shine, 2006; Kalliokoski et al., 2012; although see Kreger and Mench 1993). But, before we can reliably assess the welfare of reptiles in response to the captive environments in which they are housed, further work is required to identify ways in which their welfare can be accurately assessed.

One approach to assess anxiety-like behaviour, and hence welfare, in non-human animals is the investigation of an animal's response when exposed to a novel environment (Langkilde and Shine, 2006).Validated in laboratory rodents using anxiolytic and anxiogenic drug treatments as well as environmental manipulations (*i.e.* the 'open field test', *e.g.* Prut and Belzung, 2003), this approach has been used in a range of species (birds *e.g.* Coppinger, 1970; mammals *e.g.* De Passillé et al., 1995; reptiles *e.g.* Langkilde and Shine, 2006) where observations are made of an animal's response to an environment that may be novel in a number of ways (*e.g.* size, shape, colour, illumination), with decreasing familiarity with the environment resulting in increased signs of anxiety (File and Seth 2003). Commonly recorded measures include: latency to move when introduced to the novel environment; total time spent moving around the environment; frequency of defecation/urination; immobility; 'escape' behaviour; self-grooming behaviour, feeding behaviour (if food provided) and heart rate (rats *e.g.* Jolles et al., 1979; cows *e.g.* De Passillé et al., 1995). The prediction being that individuals exposed to conditions (*e.g.* drug treatment or housing manipulation) of putatively negative impact will show a more anxiety-like response (*e.g.* longer latency to move) compared to controls, and vice versa for conditions of putatively positive impact. However, before this approach can be used in reptiles to determine the impact of different conditions (*e.g.* enclosure size) on their welfare, we first need to understand whether this approach (and those behavioural measures) is also appropriate for use in reptilian species.

The aim of this study was therefore to determine whether the response to novelty approach commonly used to assess the welfare of mammalian and avian species could be successfully translated for use in different reptile species, and whether we could also identify reptile-specific and/or species-specific behaviours. To do this, we compared the behaviour of red-footed tortoises (*Chelonoidis carbonaria*) and bearded dragons (*Pogona vitticeps*) when exposed to both a novel and a familiar environment, as these species are popular pets and represent two of the four extant reptile orders: Testudines and Squamata.

2. Methods

2.1. Subjects, housing & husbandry

2.1.1. Tortoises

Eight red-footed tortoises were used in this study. They were housed in a group of six and a group of two and their plastron sizes ranged from 7.2 cm to 15.5 cm. The tortoises were housed in a room maintained at approximately 28 °C in open topped raised enclosures (L = 153 cm, W = 92 cm, D = 20 cm) with a UV and heat lamp at one end and shelters throughout. The housing contained an orchid bark substrate and slate tile underneath the lights for basking. They had *ad libitum* access to a water dish and were fed once a day (fruit and vegetables) with one day a week as a starve day.

2.1.2. Bearded dragons

Seventeen adult bearded dragons (*Pogona vitticeps*), seven males and ten females, were used in this study. They were housed in groups of two or three and ranged in body length (Snout – vent length) from 14.5 cm to 17.2 cm. They were housed in vivariums with a UV strip bulb along the back wall and a heat lamp at one end. With the room temperature maintained at 27 °C, the vivariums maintained a temperature gradient of approx. 45 °C, directly under the heat lamp, to 27 °C at the opposite end of the vivarium. Heat lamps were set to turn off for an hour, twice during the day and from 7pm-7am. They had sheltered areas and climbing branches within the vivarium, ad libitum access to water and were fed leafy greens once a day and received live food, including locusts and crickets, three time a week.

2.2. Apparatus

We used two testing environments that, at the onset of the experiment, were both novel to the animals. They differed from one another in terms of environment shape, floor substrate and wall covering, all selected to provide contrasting contextual cues (*e.g.* Burman and Mendl, 1999): (1) Environment 1 – The arena was rectangular in shape (38.25 cm × 120 cm × 31 cm) with a large 'O' (3.5 cm diameter) on the base that served as a marker on which animals were initially positioned. There was a bubble wrap flooring substrate and an animal print wall covering. This testing environment was set up on the floor; (2) Environment 2 – The arena was almost square in shape (77 cm × 80.5 cm × 31 cm) with a large 'X' (3.5 cm square) on the base that served as a marker on which animals were initially positioned. There was a sawdust flooring substrate and a decorative wrapping paper wall covering. This testing environment was set up on a table 72.5 cm off the ground. Tripods and cameras were set up approximately 90 cm above the environments and at the centre of the arena sides, closest to the start marker, at a height of 35 cm to observe the animals from different positions. All aspects were the same for both species except that a fine black fibreglass mesh was secured over the top of both arenas when testing the bearded dragons to prevent the possibility of escape.

2.3. Experimental procedure

2.3.1. Habituation trials

Animals were assigned to groups pseudo-randomly to match for age, as response to novelty changes over age in some species (Casadesus et al., 2001), as well as matching for experience to housing, previous experimental experience and conspecifics.

Half of the subjects (group 1) were habituated to environment 1 and the other half (group 2) to environment 2, making these their 'familiar' environments. A habituation trial involved an animal being picked up out of a travel container and individually placed into the allocated environment on the marker and allowed to move freely around for a period of 10 min per day for four consecutive days. Trials took place at the same time each day for individual subjects. Following their 10 min exposure to the environment, animals were returned to their home enclosures. The environments were cleaned before each trial in order to prevent any olfactory cues influencing behaviour. This required the sawdust substrate to be mixed around and re-laid and the bubble wrap substrate to be wiped over using a diluted disinfectant cleaner (safe4). This method has shown to be effective in other studies (Wilkinson et al., 2010). This habituation process was carried out for both tortoises and bearded dragons. After experiencing the four habituation trials that allowed familiarisation to one of the two environments, the animals received two test trials.

2.3.2. Test trials

On Test day 1, half of the animals from each group, selected at random, were tested in the *same* familiar environment in which they already had been habituated, whereas the remainder were tested in the other, *novel*, environment with which they had no previous experience. For Test day 2, those individuals that had initially been tested in the familiar environment were tested in the novel environment, whilst those that had first been tested in the novel environment were returned to their familiar environment for the second test (see Fig. 1). Thus, all individuals were tested once in both a familiar and a novel environment, balanced for order of exposure (*i.e.* half the animals experienced the novel (Test day 1) and then the familiar environment (Test day 2)