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RESEARCH ARTICLE

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Effects of prey-based and non-prey-based scent enrichment on two zoo-housed monitor lizards

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ABSTRACT – Environmental enrichment is a powerful tool in maintaining positive welfare for captive animals but investigation of this in reptiles has been limited. Monitor lizards are active, intelligent animals that represent high priority targets for enrichment in a captive setting, but more data are required to develop evidence-based recommendations and to understand variation in the responses to enrichment of both individuals and species. We exposed two monitor lizards, *Varanus cumingi* and *Varanaus macraei*, both adult females, to olfactory enrichment comprising prey-based and non-prey-based scent trails and then measured changes in activity and tongue-flicking versus a control. Randomisation analysis, employed to deal with small sample size, showed that the *V. cumingi* significantly increased both behaviours in response to both enrichment types, versus control, while the *V. macraei* responded significantly only to the prey-based scent for both behaviours. These findings provide an evidence-based case study demonstrating the potential effectiveness of enrichment for captive monitors, but also the need for context-specific evaluation of enrichment strategies to ensure maximal benefit for a given individual animal.

INTRODUCTION

Enrichment is an animal husbandry principle aiming to enhance the psychological and behavioural well-being of captive animals (Burghardt, 2013). This can take many distinct forms though a core principle is the encouragement of species-specific natural behaviour. Enrichment strategies may include behavioural management (especially animal training), provision of novel objects and rearranging enclosures, modified presentation of food and olfactory stimulation (Swaisgood & Shepardson, 2005). The effectiveness of these interventions is usually assessed by the measurement of behavioural indicators (Zieliński, 2023).

Reptiles are a taxon devoid of domesticated species and elements of the natural behaviour exhibited by many species are often not appropriately catered for in captivity (Waterman et al., 2021). This, in combination with life in what are, relative to natural habitats, small and homogenous enclosures, means that reptiles are often susceptible to stereotypical behaviours (Warwick et al., 2013). In particular, many of the challenges of life in the wild are not presented (Van Waeyenberge et al., 2018). The apparently sedentary lifestyle shown by many reptile species may have, in part, led to the misconception that they do not require complex habitats or enrichment to ensure appropriate welfare (Warwick et al., 2019; Getsy et al., 2022). In reality, reptiles do have complex cognitive demands that require enrichment in order to avoid potential welfare issues that may arise from under-stimulation in a captive environment (Hoehfurtner et al., 2021). Consequently, environmental enrichment for reptiles has been found to improve longevity, physical condition, problem solving behaviour and enhance their behavioural repertoire; it also reduce the occurrence of abnormal behaviours (Waterman et al., 2021).

Environmental enrichment is one of the most effective and appropriate ways to increase behavioural development and plasticity (Almli & Burghardt, 2006) and encourage species-specific behaviours (Bashaw et al., 2016). The positive intended impact of enrichment may be maximised through careful selection of enrichment stimuli to suit the preferred sensory modalities of species and individuals (e.g. Clark & King, 2008; Januszczak et al., 2016; Londono et al., 2018; Waterman et al., 2021). However, it must be noted that not all attempts at providing enrichment are successful. Rosier & Langkilde (2011) showed no effect of environmental enrichment on several measured behavioural and physiological indicators of *Sceloporus* lizards, Januszczak et al. (2016) show only certain indicators of improved welfare are affected by the provision of an enrichment feeding device, and Michaels et al. (2020) describe abnormal repetitive behaviours in a false water cobra *Hydrodynastes gigas* resistant to strategies employed to reduce them.

Members of the genus *Varanus* are active foragers with excellent eyesight. They are capable of rapid learning and problem solving (e.g. Manrod et al., 2008), so unsurprisingly there have been several enrichment studies on the monitors (Cooper et al., 2020; Waterman et al., 2021; reviewed by Howard & Freeman, 2022). However, research on these lizards is limited when compared to, for example, similar studies on mammals or birds; indeed, research of this kind is limited for all reptiles, representing <10% of zoo and aquarium-based research between 2008 and 2017 (Binding et al., 2020). Therefore, more data are required to properly inform practice in order to provide meaningful information relevant to both more taxa and more enrichment types (Eagan, 2019). Given the small numbers of most monitor lizards maintained in zoological collections (Ziegler et al., 2016), an accumulation of small sample studies may be the most practical means of developing sufficient evidence to properly inform captive husbandry (Waterman et al., 2021). Monitor lizards are equipped with sensitive chemosensory organs and olfactory enrichment has been demonstrated to provide the most effective stimuli across several monitor species (Waterman et al., 2021). The present study adds to this literature by identifying the behavioural responses of two further species of monitor lizard in response to prey and non-prey scent-based olfactory enrichment.

MATERIALS & METHODS

Ethics

All methods were compliant with the British Herpetological Society Ethics Policy.

Animals and husbandry

Singly housed adult female *V. macraei* (9 years old, n = 1) and *V. cumingi* (9 years old, n = 1), both captive bred, were maintained in the herpetological collection at London Zoo. Husbandry followed best practice for the species, including heating, lighting and humidity management as deemed appropriate at the time. Both enclosures had themed, climbable walls, branching and both live and artificial plants. The enclosures measured 210 x 160 x 170 cm for *V. macraei* (snout-vent length [SVL] = 29 cm) and 540 x 220 x 230 cm for *V. cumingi* (SVL = 59 cm), length x width x height. Both animals had been historically exposed to a variety of enrichment without documentation.

Enrichment and data collection

An experimental period, comprising two five-weekday blocks either side of a weekend, was established for both the *V. cumingi* (January 2022) and *V. macraei* (November 2022). Both animals were fed twice weekly, resulting in four feeding days within the experimental period. Feed- and non-feed- days were randomly and equally allocated to either prey and non-prey scent trails. The scent trails were tracks of scented water within an enclosure, designed to stimulate olfactory senses and thereby activity and cognition. This type of enrichment is frequently employed in monitor and other groups of lizards in captivity (Loñdono et al., 2018; Waterman et al., 2021).

Both enrichment types were prepared by trained zookeepers. The scent object, a perforated frozen-thawed mouse for the prey scent and crushed basil leaves for the non-prey scent, was soaked in 1L of distilled water for one hour. The scent object was then removed, and the scented water decanted into a drinks bottle with a dripper cap. Bottles were sterilised between uses and a separate bottle was used for each scent type across observations to avoid cross contamination.

On each day, an observer (CS for *V. cumingi* and FN for *V. macraei*), who had been previously trained in observing the monitor lizards through practice sessions, watched the lizard in question from the public viewing window of the enclosure. Each observation session began with a 30-minute control phase, in which the lizard was observed prior to any enrichment. This was followed by an experimental phase in which the allocated enrichment scent was haphazardly dripped in a trail around the enclosure by a keeper. The *V. macraei* was present in the enclosure as the trail was laid. This was not the case with *V. cumingi* which, being a potentially hazardous animal, was recalled under trained behaviour to a locked compartment of the enclosure while the scent trail was laid. As soon as the trail was complete, typically in about 1 minute, the enclosure was closed and the locked den opened, giving the animal access to its enriched enclosure. The observer then monitored the lizard again for a further 30 minutes. The observer recorded two types of behaviour: activity was measured with a stopwatch as cumulative time, while tongue flicks were counted with a clicker-counter. A lizard was considered active when its centre of mass was in motion. A tongue flick was defined as a rapid protrusion and retraction of the forked tongue. Tongue flicking is the means by which monitor lizards engage their olfactory senses and is therefore a proxy for engagement with olfactory stimulation (Howard & Freeman, 2022; Loñdono et al., 2018; Waterman et al., 2021). Each observation session began at 11:00 h once lizards had had ample opportunity to thermoregulate and reach optimal body temperature for activity. On feed days, animals were fed at least two hours following the conclusion of the session.

The total time spent active and the total number of tongue flicks within each phase of each observation formed the dataset for analysis.

Statistical analysis

For both lizards, the alternative hypothesis was that enrichment would increase activity and tongue flicks compared with the baseline established during the control phase. Randomisation analysis was used to test the hypothesis. Randomisation is a valid strategy for analysing small- and single-n samples, where assumptions of traditional statistical analyses are violated (Dugard et al., 2012; Michaels et al., 2020). Paired analyses were used, with each control-experimental pair forming a datapoint, to control for daily variation in baseline behaviour. For each lizard, each scent-behaviour combination was analysed separately. All analyses were conducted in R 4.2.2 in RStudio 2023.03.1, using base R (R Core Team, 2022). For each analysis, a test statistic was calculated as the observed mean difference between control-experimental pairs. Treatment labels were then randomly shuffled 9,999 times and, after each shuffle, the simulated mean pairwise difference was again calculated. A seed was set prior to this stage to permit repeatability between code runs. The randomised simulations formed a frequency distribution of mean pairwise difference under the null hypothesis that there is no difference between enriched and control phases. The observed test statistic, forming the 10,000th observation, was then compared to this frequency distribution to derive a two-tailed p value as the proportion of absolute values greater than or equal to the test statistic. A one-tailed p value was then calculated as half the two-tailed value. A one-tailed p value was used due to the directionality of the alternative hypothesis.

Table 1. Mean and standard deviation (sd) duration of activity and counts of tongue flicks under control and enriched conditions for *Varanus macraei* and *Varanus cumingi* monitor lizards, with associated one-tailed p values derived from paired randomisation analyses. Significant p values are in bold.

Species	Behaviour (units)	Scent vs. control	Mean (sd) before enrichment	Mean (sd) after enrichment	One-tailed p
Varanus macraei	Activity (seconds)	Prey	424.7 (371.1)	1069.6 (577)	**0.031**
		Non-Prey	471.3 (427.6)	693.3 (579.2)	0.125
	Tongue flicks (count)	Prey	215 (188.4)	973.2 (552.2)	**0.032**
		Non-Prey	248 (230.8)	539 (538.9)	0.157
Varanus cumingi	Activity (seconds)	Prey	32.6 (59.4)	732.4 (231.8)	**0.031**
		Non-Prey	40 (54.8)	470.8 (182.9)	**0.032**
	Tongue flicks (count)	Prey	29 (47.8)	1766.4 (774.9)	**0.031**
		Non-Prey	27.6 (41.2)	1124.2 (1155.3)	**0.031**

We did not compare prey and non-prey scents in head-to-head comparisons due to substantial heterogeneity in baseline activity and tongue flicking. The paired analyses controlled for this but, given that the processes driving daily baseline variation could not be modelled, it was inappropriate to compare enrichment types in unpaired analyses. Relative efficacy of scent types can only be inferred qualitatively relative to the control condition.

RESULTS

Randomisation analysis showed that for both *V. macraei* and *V. cumingi*, prey-based scent trails were associated with a significant increase in both activity and tongue flicks (p = 0.031–0.032; Table 1; Fig. 1). Non-prey scent trails were associated with a significant increase in tongue flicks for *V. cumingi* (p = 0.031, Table 1; Fig. 1) and in activity (p = 0.032; Table 1; Fig. 1), but in neither behaviour for *V. macraei* (Table 1).

DISCUSSION

In this study, we evaluated the behavioural response of two zoo-housed species of monitor lizard to exposure of prey- and non-prey based scent enrichment. These forms of enrichment were chosen due to their suitability for use with varanid lizards, being the only group of lizards that use their tongues exclusively for sensory function (Smith, 1986; Murphy et al., 2019) and the ease of preparation and delivery to target recipients, which make scent-based enrichment an attractive strategy (Clark & King, 2008). Our results showed a difference between animals in responses, such that while the *V. cumingi* significantly increased target behaviours

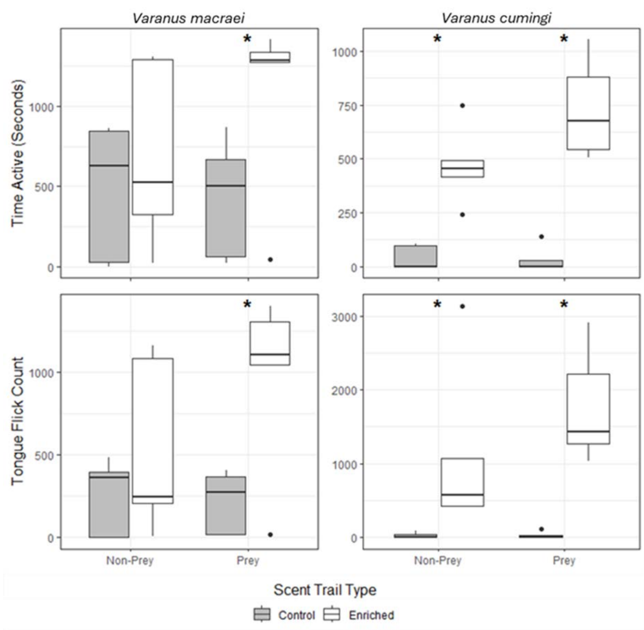

Figure 1. Boxplots of time spent active and tongue flick count before and after enrichment with prey nor non-prey scent trails in *Varanus macraei* and *Varanus cumingi* monitor lizards. Asterisks indicate a significant increase between control and enriched phases.

associated with physical and cognitive stimulation (activity and tongue-flicks) in response to both scent types, the *V. macraei* responded significantly only to the prey scent in terms of both behaviours, despite the basil scent being novel to both of these lizards.

These results, combined with those of other studies (Waterman et al., 2021; see review by Howard & Freeman, 2022) reinforce the utility and broad applicability of prey scents in enrichment strategies for monitor lizards. Under the prey-based scent enrichment, the *V. macraei* increased its average proportion of time spent active within the 30-minute observation window from approximately 25% to approximately 70%, while the *V. cumingi* increased from engaging in little to no activity on average to spending approximately 35% of the observed time active. Both species approximately tripled their counts of tongue flicking. The increase in both tongue flicking and activity strongly associates the behavioural impact of enrichment specifically with the presence of olfactory stimuli and improves health, welfare and physical fitness through increased movement, and likely cognitive stimulation through the processing of sensory information (Howard & Freeman, 2022). Given that obesity and other pathologies resulting partly from reduced activity levels are a concern in captive reptiles and, specifically in monitor lizards (Mitchell, 2007; Latney, 2016), the value of this increase in activity may be substantial.

While it is useful to extend data concerning response to prey scents to two as yet unstudied *Varanus* species, the behavioural response of predators to prey-based scents is unsurprising. The comparison of prey- and non-prey-based scents in this study was designed to investigate the use of scents not associated with food as a means to stimulate cognition and exploration beyond the elicitation of hunting behaviour, and of accessing a much broader array of available scents to incorporate in enrichment strategies. The difference between animals in response, or lack thereof, to the non-prey scent illustrates that enrichment strategies may not be transferable between monitor lizard species or individuals. The reason for differences between individuals in this study is not clear and cannot be investigated with the current data given the conflation of individual and species; trends may be driven by aspects of personality, species adaptation, individual history and learning or other factors (Clark & King, 2008; Akhund-Zade, 2019; Waterman et al., 2021). However, from a pragmatic perspective, the data demonstrate that scents may be drawn from a wider palette for the benefit of the *V. cumingi* in this context but might be better limited to prey-based scents for the *V. macraei*. This reinforces the need to evaluate the impact of enrichment at an individual level (Clark & King, 2008). Further investigation of more scents, individuals and species would be beneficial, and such data collection may be easily accommodated in long-term husbandry. Additionally, a better understanding of the duration of response to enrichment would be useful to inform enrichment management, but due to limited resources this was not feasible in the present study.

The presented data are subject to limitations associated with small sample size and incomplete individual histories, which are typically unavoidable in the context of opportunistic data collection from small numbers of long-lived and uncommon animals forming part of a long-standing zoological assemblage (Waterman et al., 2021; Getsey et al., 2022). Consequently, while the within-study conclusions may be considered to be valid due to the use of appropriate statistical methods, it may not be robust to extrapolate the results of this study and assume transferability to other individuals of the included species. Rather, findings should be treated as a case study of potential responses to enrichment and combined with other data to provide accumulated insight into relevant trends and patterns that may collectively inform enrichment strategies for monitor lizards in zoos. To facilitate this, further investigation of enrichment for captive reptiles, even in the context of small sample size, is therefore encouraged.

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