Is behavioural enrichment always a success? Comparing food presentation strategies in an insectivorous lizard (*Plica plica*)

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Abstract

Staggering food availability through a delivery device is a common way of providing behavioural enrichment as it is usually thought to increase the amount of natural behaviour due to the unpredictability of the food source. Tree-runner lizards (*Plica plica*) are a Neotropical, scansorial, insectivorous species. We provided these lizards with an enrichment device that slowly released insect prey and tested its effect on the activity and frequency of a number of behaviours in comparison with a scatter control (where prey items were broadcast in the enclosure; standard food presentation for captive insectivorous lizards) and a non-feeding control. Both types of food increased activity and counts of several behaviours in comparison with the non-feeding control. However, we found the provision of the behavioural enrichment device led to a significantly lower frequency of almost all analysed behaviours in comparison with scatter control trials, mainly in behaviours associated with activity (unsuccessful strikes (= unsuccessful capture of prey) (p=0.004), locomotion (p=0.004), alertness (p=0.004) and the number of times a boundary in the enclosure was crossed ie. activity (p=<0.001)). The frequencies significantly increased in the enrichment trials (relative to the scatter control) were the number of successful strikes (= successful capture of prey; p=<0.001) and targeting prey (*p*=<0.001). There was no significant difference in latency to first strike (p=0.24), duration of hunting activity (p=0.83) or enclosure use (p>0.05) between scatter and enriched trials. The relative success of the scatter feed in promoting activity and increasing hunting difficulty was likely partly due to the enclosure design, where the complex physical environment contributed to the difficultly in catching the prey. However, when the feeding duration and enclosure use was analysed there was no significant difference between the scatter control and enrichment trails. The results from this study highlight the importance of evaluating enrichment strategies, and the role of complex enclosure design in creating effective enrichment for insectivores, which can contribute to their welfare in captivity.

Keywords: enrichment; behaviour; reptiles; lizards; activity; *Plica plica*

1.Introduction

Enrichment is an aspect of animal husbandry that is designed to promote natural behaviours and improved welfare and to reduce atypical behaviours or stereotypies in captive animals, often by mimicking an animal's natural environment and increasing its surrounding stimuli (Mason, 1991; Passos et al., 2014). Enrichment can be divided into environmental, behavioural and social categories, depending on whether an intervention targets an individual's physiological needs (environmental), or is intended to elicit natural behaviours either from individuals (behavioural) or between conspecifics (social) (Shepherdson, 1994; 1998). This can be achieved by increasing the 'behavioural repertoire' of an animal in captivity (Dawkins, 2006; Michaels et al., 2014).

It is now commonplace for behavioural enrichment to be provided to some taxa of captive animals, particularly mammals and birds (de Azevedo et al., 2007). However, there has been little research on the effects of enrichment on reptiles (Manrod et al., 2008; Burghardt, 2013; Michaels et al., 2014). It has only recently become possible to properly cater to basic reptile needs in captivity, due to progression in heat-and light-generating technology and a further understanding of the environmental requirements for reptile health (Divers and Mader, 2005). This historic absence of appropriate husbandry is perhaps one reason for the relative lack of interest in enriching reptiles (Rosier et al., 2011), as well as a relative lack of human empathy for this group alongside a common perception reptiles are too neurologically simple to suffer (Burghardt, 2013). This is despite the fact that more 'sophisticated' behaviours have been recognised in reptiles, such as long lasting parental care in crocodilians (Garrick et al., 1977), spatial learning in Eastern water skinks (*Eulamprus quoyii*; Noble et al., 2012), and parental care and sociality in Australian skinks (*Egernia whitii,* While et al., (2009) and *E. kingi,* Master and Shine (2002), respectively).

This deficiency in empirical data means that the husbandry of captive reptiles is either frequently based on anecdotal reports or human intuition, which can be particularly unreliable when applied to animals that are so phylogenetically different from ourselves (Langkilde and Shine, 2006). There is, however, a limited literature on the benefits of enrichment for a small number of reptile species: box turtles (*Terrapene*) were found to have a preference for an enriched environment over a ‘barren’ one (Case et al., 2005) and sea turtles displayed fewer stereotypic behaviours when they were provided with novel objects (Therrien et al., 2007). Among lizards, the Varanidae (Monitor lizard family) is known to show various behavioural characteristics that are usually attributed to ‘higher’ vertebrates, such as counting (Pianka et al., 2003) and problem solving (Manrod et al., 2008) and respond well to both environmental and behavioural enrichment as a part of their husbandry (Manrod 2008; Burghardt, 2013; Michaels et al., 2014). Conversely, one study suggests that for the eastern fence lizard *(Sceloporus undulatus)*, a non-varanid species, environmental enrichment does not have a measurable effect on behaviour and corticosteroid levels (Rosier et al., 2011); this study has been the centre of some controversy, however; see Burghardt (2013) for a discussion. There is not yet sufficient evidence to draw general conclusions or identify patterns about the effects of enrichment on reptiles,, and more research is required to broaden the variety of ecotypes and phylogenetic groups studied.

In captivity, insectivorous lizards are typically ‘broadcast’ or ‘scatter’ fed, whereby multiple prey insects are distributed around the enclosure at one time. Altering the way in which food is presented can be used to provide behavioural enrichment for captive insectivores (Hurme et al., 2003) by increasing physical activity and exploration of space and by eliciting a larger frequency and variety of behaviours; thus reducing the risk of psychological or physical diseases (mainly obesity, which can commonly occur in captive reptiles, (Dinse, 2004; Donoghue, 2006)).

We used tree-runner lizards (*Plica plica)* to provide information on enrichment in a group of lizards (Tropiduridae) that has not been studied previously. For our study, we assessed the impact of a feeding enrichment device on their behaviour and enclosure use in comparison with standard food presentation method (scatter or broadcast feeding) and a non-feeding control. Although the activity budget of tree-runners in the wild is currently unknown, the small size and relative simplicity of a typical captive environment means under stimulation is likely to cause problems with captive animal welfare.

Increased activity in captivity when engaged in natural behaviours is likely to suggest improved mental stimulation and will also contribute to the physical fitness of animals. Increased activity levels and movement in the enclosure while engaged in natural behaviours was therefore considered a desired outcome of enrichment, and that was what we were assessing in our experiment.

2.Materials and Methods

Ethics statement: all experiments were non-invasive, with all treatments falling within the scope of normal zoo husbandry, and did not compromise the welfare of the lizards. The study was approved by the Zoological Society of London (ZSL) zoo research coordinators before commencement.

2.1. Study animals

The study was conducted with five juvenile tree-runner lizards (*Plica plica*) at ZSL London Zoo, England. All animals were captive-bred and full siblings. Tree-runner lizards are found in rainforests in South America, in countries east of the Andes (i.e. Bolivia, Brazil and Colombia) and are scansorial, climbing on vertical rocks and smooth-barked tree trunks (Vitt, 1991; Murphjy and Jowers, 2013). The lizards were 99 (n=1), 56 (n=2) and 36 (n=2) days post hatching at the beginning of the study. The trials were completed between the 25th of June and the 6th August 2015.

2.2. Enclosures and husbandry

The trials were conducted in the enclosures where lizards were permanently housed. Each lizard was housed in a separate enclosure, side by side (see Figure 1, A). The enclosures consisted of a front-opening 45x45x45cm vivaria (Exo Terra; Rolf C. Hagen, Castleford, Yorkshire, UK), with a barkchip substrate, two similar cork-bark hides (one at the back and one at the front of the enclosure), and some thin branches to provide overhead cover (see Figure 1, B; the lizards did not climb on these, being adapted to locomotion across flat vertical surfaces). Two of each enclosure’s sides were completely covered with cork tiling and the back was covered with the proprietary polystyrene background supplied with the terrarium (Exo Terra; as above); this prevented lizards from being able to see into neighbouring enclosures. The top of the enclosure was composed of a fine wire mesh on which the lizards were able to climb. Hence the lizards could to climb on all sides except the glass front of the enclosure. Each enclosure contained a small water dish. Enclosures were held within a climate controlled room (at an ambient temperature of 24◦C) in which no other animals were maintained. Lighting was set on a 12 hour cycle, beginning at 07.00 h. Enclosures were lit using a warm-white fluorescent T5 lamp (OSRAM Lumilux T5 warm white HP 39W/830 DEL), a UVB-emitting T5 lamp (Arcadia D3 Reptile T5 Lamp 6% UVB) and a (GE R80 60W 240V Reflector) incandescant basking lamp. This lighting combination provided a UVB gradient between a UV index (see Michaels and Preziosi, 2013 for explanation) 0.0 to 3.0 in order to replicate the Ferguson zone into which these lizards are likely to fall (Ferguson et al., 2010) and a diurnal thermal gradient between 26 and 38°C. Photo- and thermo- gradients were correlated and identical in each of the enclosures. The enclosures had a night time temperature of 21◦C. UVB radiation is important in calcium metabolism for many reptiles and is an important aspect of their proper husbandry (Adkins et al., 2003).

The enclosures were sprayed with water daily, and the animals fed every other day using small (c. 8mm) black crickets (*Gryllus bimaculatus*) dusted with a vitamin and mineral supplement (Nutrobal; Vetark UK). In nature, Plica feed primarily on ants, but other invertebrates compose of 30% of their diet (Vitt, 1991). In captivity, ants are not available as viable food source and instead crickets form the staple diet of these and other captive insectivorous species.

All crickets were fed on mixed fruit and vegetables for at least 24 hours prior to being consumed to improve nutritional value. All routine maintenance and husbandry was performed by the observing individual (I. Januszczak).

2.3. Enrichment device

The enrichment device was designed to deliver ten small black crickets randomly over 40 minutes. Although ants form a large proportion of the diet of wild *Plica* lizards, they are not obligatory ant feeders (Vitt, 1991) and, moreover, crickets are a staple insect diet typically used to feed a wide range of insectivorous lizards, including this species, in captivity. Film canisters were used due to their size; they are also easy to manipulate and sterilise and are commonly used as way of dispensing insects to captive insectivores (pers. obs. Michaels, C). The dispenser consisted of a white film canister (48x30mm), upright without a lid, with a 7x9x70mm piece of cork inside that emerged from the top, which the crickets used to climb out of the canister (Figure 2). The canister was deep enough that the lizards could not access prey while the insects were still in the canister.

Prior to our experiment we tested the canisters to ensure they were dispensing crickets over a period of time. Ten crickets were placed into the canister in environmental conditions identical to the lizard enclosures and their emergence was timed. 30 replicates were performed. We calculated the cricket emergence time (mean: 13.96, standard error: 1.28, range: 1-30 minutes), the inter-insect escape time (mean: 13.96, standard error: 1.28, range: 0-28 minutes) and the total time for the enrichment device to empty (mean: 15.5 minutes, standard error: 1.46, range: 5-36 minutes) and concluded this device was appropriate to stagger cricket emergence, increase unpredictability of prey emergence and would not dispense them into the enclosure at the same time.

The enrichment and scatter control feeds were always placed in the back right hand side of the enclosure floor (see Figure 1, B).

2.4. Behaviour assessment

Based on observation of lizards feeding without the presence of the enrichment device before formal trials began, an ethogram was devised for the lizards, with event behaviours recorded (see Table 2. for recorded behaviours and their definitions). We used focal sampling every minute to tally any event behaviours observed within that minute on an ethogram timeline. The lizards moved only in very short, extremely rapid bursts, hence any form of whole body movement (‘locomotion’; Table 1.) was categorised as an event, rather than a state, behaviour. The time of the first successful strike and the last successful strike was used to calculate the feeding duration of the lizards in each trial.

Type of behaviour	Recorded behaviour	Definition
Event	Successful strike	Lizard successfully captures and eats the prey. Includes any actions observed straight after the strike for example chewing.
	Unsuccessful strike	Lizard unsuccessfully attempts to capture a prey.
	Locomotion	Any form of whole body movement.
	Targeting prey	A head tilt aimed in the direction of any potential prey.
	Alertness	A head tilt aimed away from any potential prey, instead acting as a way of observing surroundings - usually visible if the lizard is startled or sometime after a successful strike.

Table 1: Table to show the definitions behind the recorded behaviours of the tree-runner lizards (*Plica plica*)

The enclosures were also divided into grid cells (Figure 1, C) and the location was recorded every time a lizard moved into a new section in the enclosure. The number of times a lizard 'crossed over' into a cell was later totalled to be analysed (as 'Times boundary crossed') as a measure of their activity. These data were also used to quantify the enclosure use of the lizards in response to the different trials.

For feeding trials, we also recorded the latency to first successful strike and the duration of feeding behaviour within the trial (time between first and last successful strike or the end of the observation period).

2.5. Feeding trials

The lizards experienced three types of feed trial; a 'scatter control' (ten crickets broadcast into the back right corner of the enclosure; the standard feeding method for most captive insectivores), enriched (ten crickets presented using the enrichment device) and 'non-feeding control' (no food offered). The purpose of the non-feeding control was to determine the baseline activity levels of the lizards when no food was present. The purpose of the scatter control was to have a baseline activity level of the lizards when presented with the standard broadcast feeding they would normally experience in captivity. Both controls were used to assess the success of the enrichment device in promoting activity outside these baseline levels. In total, each lizard was observed 11 times for each type of trial (33 trials per lizard). Each trial was 40 minutes long, beginning as soon as food was placed into the vivarium. An acclimatisation period was not necessary as the lizards would start feeding as soon as they detected the food. For the trials, the 40-minute observation period started as soon as the observer was ready. As the lizards were fed every other day the enrichment trails and scatter control feed trials were done on alternate feed days for the 42 days of the experiment. The non- feeding control trials were carried out on the non-feeding days in between the enrichment and scatter control feed trials.

Trials were always conducted after 12 noon, to allow for sufficient basking time for the lizards so that they could raise their metabolic rate before hunting. Lizards were observed from a distance of 170 cm.

Five trials (one for each lizard) were conducted consecutively in the same afternoon. The order in which lizards were observed was systematically changed each day to account for the different times the lizards were fed. The enrichment devices used for the enrichment trials were rotated in a similar fashion, to account for any variation within the enrichment devices themselves. Devices were thoroughly washed and disinfected between trials and fresh latex gloves were used to touch any part of the enclosures or enrichment devices.

2.6. Statistical analysis

All of our analyses were conducted using SPSS 22 (IBM) for Windows. Prior to analysis, we tested our data for normality using Kolmogorov-Smirnov tests and decided that parametric analysis was appropriate. General Linear Models (GLMs) were conducted to test for effects of treatment (non- feeding control , enriched, scatter control) on the total frequencies of each behaviour in trials. We analysed all trials and included individuals (Lizard) as a factor in the model, positioned first and using sequential sums of squares, to partition the variation explained by individuals and the treatments to statistically deal with the problem of pseudoreplication. We tested for effects on successful strike, unsuccessful strike, locomotion, targeting prey, alertness and the number of times a boundary in the enclosure was crossed (see Methods for behaviour definitions). Fisher's Least Significant Difference test was used *post-hoc* to compare means after the GLM in each case. A Bonferroni correction was used to correct for the number of tests, so all significance thresholds were moved from 0.05 to 0.0083.

We used 1-tailed paired Wilcoxon sign tests to compare latency to first strike and duration of feeding under enriched and scatter control conditions.

Using our records of the locations of animals within the grid-square layout during trials, we calculated Simpson's Measure of Evenness ($E_{1/D}$; see Payne et al., 2005) for the mean observations for each lizard in each treatment to quantify evenness of enclosure use, where $E_{1/D} = (1/D)/s$, where $D = \Sigma p_i^2$, and p_i is the proportion of observations in grid square i and s is the total number of grid squares. This is a modified version of the reciprocal Simpson's index (Simpson, 1949) sometimes used to quantify evenness of spatial distribution in ecology (e.g. Payne et al., 2005). Values close to 0 mean patchy or skewed distributions, values close to one mean evenly spread distributions. This index is useful in that it is relatively robust against small numbers of observations at some sites (Payne et al., 2005). We then used 1-tailed paired Wilcoxon sign tests to compare treatments.

3. Results

The effect of 'lizard' was significant for locomotion (n=55, $F_{4, 51}$= 7.71, p=<0.001), targeting prey (n=55, $F_{4, 51}$=5.18, p=<0.001) and alertness (n=55, $F_{4, 51}$= 4.28, p=0.003), but not significant (p>0.0083) for all other recorded behaviours.

We found there was a significant effect of the three treatments on all recorded behaviours (see Table 2. for a summary of the GLM results, and Figure 3 for a graphical representation). It was found that the interaction between the covariates, the lizard and the treatment, was not significant for all recorded behaviours.

Both feeding types ('enriched' and 'scatter control') increased behaviour frequencies against no food being present at all (non-feeding control trial); see Table 3. *Post hoc* tests show there was a significant difference between the 'non-feeding control' and 'enriched' trials in the frequency of all recorded behaviours, except for the number of unsuccessful strikes

(p=0.046, see Table 3), as very few strikes were unsuccessful in the enriched trials and the lizards did not exhibit any striking behaviour in the non-feeding control trials. The number of successful strikes indicate the number of crickets eaten per trial. The significantly higher number of successful strikes in the enriched trials (see Figure 3, A) show that on average more crickets were eaten in enriched trials compared to the scatter control trials within the 40 minutes experimental period. There was a significant difference in the frequency of all the recorded behaviours between the non-feeding control and scatter control trials (Table 3).

All behavioural frequencies were significantly higher in the 'scatter control' trials than the 'enriched' trials (unsuccessful strikes (p=0.004), locomotion (p<0.001), alertness (p=0.004), times boundary in the enclosure was crossed (p<0.001), See Figure 3; B, C, E, F), except for successful strikes (p=0.001) and targeting prey (p<0.001), which was higher in the 'enriched' trial (See Figure 3; A and D; Table 3).

1-tailed paired Wilcoxon sign tests showed there was no significant difference in the feeding duration (W_5 = 0; p= >0.05; SE = 24.724) or the latency to first strike (W_5 = 0; p>0.05; SE = 3.617) between the enrichment and scatter feed trials.

Simson's measure of evenness was significantly higher in Scatter Control (Mean $E_{1/D}$ = 0.150; W_5 = 1, p<0.05) and Enriched (Mean $E_{1/D}$ = 0.127; W_5 = 1, p<0.05) trials than in Control trials (Mean $E_{1/D}$ = 0.097). There was no significant difference between Scatter Control and Enriched trials, however (W_5 = 0, p>0.05). Enclosure use in the three trials is summarised in Figure 4.

	Lizard			Treatment			Interaction Lizard * Treatment		
	F	d.f	*P*	*F*	d.f	*P*	*F*	d.f	*P*
Successful strike	1.30	4, 51	0.271	419.86	4, 51	**<0.001**	2.12	4, 51	0.031
Unsuccessful strike	0.86	4, 51	0.492	12.52	4, 51	**0.004**	1.34	4, 51	0.226
Locomotion	7.71	4, 51	**<0.001**	149.64	4, 51	**<0.001**	2.67	4, 51	0.009
Targeting prey	5.18	4, 51	**0.001**	131.28	4, 51	**<0.001**	2.04	4, 51	0.045
Alertness	4.28	4, 51	**0.003**	41.06	4, 51	**0.004**	0.85	4, 51	0.561
Times boundaries crossed	3.22	4, 51	0.014	89.10	4, 51	**<0.001**	1.70	4, 51	0.104

Table 2: Results of the General Linear Models summarised by the effect of the lizard, treatment and the interaction between those two covariates. The significance was compared to p=0.0084. The significant p values are displayed in bold.

Treatment 1	Treatment 2	P values for the Fishers Least Significant Difference test between treatment means					
		Successful strike	Unsuccessful strike	Locomotion	Targeting prey	Alertness	Times boundary crossed
Non-fed Control	Enriched	**0.000**	0.046	**0.000**	**0.000**	**0.000**	**0.000**
	Scatter	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
Enriched	Control	**0.000**	0.046	**0.000**	**0.000**	**0.000**	**0.000**
	Scatter	**0.001**	**0.004**	**0.000**	**0.000**	**0.004**	**0.000**
Scatter control	Control	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
	Enriched	**0.001**	**0.004**	**0.000**	**0.000**	**0.004**	**0.000**

Table 3: Results of Fisher's Least Significant Difference test to compare means after the GLM. The significance was compared to p=0.0084. The significant p values are displayed in bold.

4. Discussion

We compared the activity levels and the frequency of certain behaviours of five tree-runner lizards (*Plica plica*) during non-feeding control, scatter control and enrichment trials. An increase in activity and enclosure use in association with an increased frequency of normal behaviours was the desired outcome of this experiment, as it was thought to result in the improved mental stimulation and physical fitness of animals. Staggering food availability though an enrichment device (in comparison to the scatter feed, where all their prey was delivered simultaneously) was hypothesised to result in this increase their activity within the observation periods. Instead, our results suggest that, although both forms of food delivery promote increased activity levels and enclosure use in comparison with the non-feeding control, in this instance a scatter feed out-performed the enrichment device in most of the measures recorded. However, when the feeding duration and enclosure use was further analysed the differences between the enriched and scatter control treatments decreased, highlighting the imperfections in the enrichment device itself which are discussed here.

In all but two recorded behaviours (number of successful strikes and targeting prey), there was a significantly higher frequency of behaviours in the scatter control feed trials than in the enriched feed trials. The higher frequency of 'targeting prey' behaviour in the enriched trials suggests the enrichment device allowed the lizards to prepare to 'strike' more effectively for prey, as it emerged from a singular spot. This not only resulted in the higher successful strike count (more crickets were consumed in the trial period) but also a decrease in unsuccessful strikes, i.e. hunting became easier.

When analysing the latency to catch the first cricket and the duration of feeding in both scatter control and enrichment trials; although the 'first strike' time was similar in both treatments, there was no significant difference in the feeding duration between the enrichment and scatter feed trials. The maximum feeding duration recorded (across all 5 lizards) was 38 minutes in the enrichment trials and 39 minutes in the scatter control feed trials. This highlights how the enrichment device (although effective in staggering the cricket emergence) reduced the number of noted behaviours despite having a similar feeding duration to the scatter feed.

Analysis of enclosure use (Figure 4) shows that the lizards' movements across the enclosure, although both showing a greater spread of usage than the control trials, did not significantly differ between the two fed treatments. Increased use of the entire enclosure is usually thought to improve welfare (e.g. Ross et al., 2009) and so an effective enrichment device should lead to higher $E_{1/D}$ values. Although in this respect the scatter control did not outperform the enrichment device, these data further indicate that the enrichment device failed to promote activity and exploration of the environment beyond that achieved by the standard food presentation method. In both feeding treatments, lizards tended to stay on the back wall grid squares (C1-4) from which they could most easily access insects escaping from the device or emerging from refugia post scatter feeding (see Figure 4), It is important to note that even though feeding did increase enclosure use, spatial distributions were still highly skewed and further attempts at enrichment in this species should aim to substantially increase $E_{1/D}$ values.

In this instance an enrichment device that staggered food in both time and space (rather than just in time) would have had the ideal effect. However, it transpired that scatter feeding combined with a complex environment achieved these qualities without the use of a dedicated enrichment device.

It is likely that the physical complexity of the environment in the enclosures in this study contributed to the relative success of scatter feeding in promoting the noted natural behaviours. Although the crickets were left in the same area of the enclosure in the feeding trials, when presented as a scatter feed, crickets rapidly hid within the substrate and refugia in the lizard enclosures, thereby increasing the difficulty with which lizards could locate and catch prey items. The enclosure may have acted as a form of enrichment beyond the environmental sense (providing stimuli through physical heterogeneity), by providing behavioural enrichment and eliciting more hunting behaviour, acting, as it were, as a giant food dispenser. The influence of context on the 'success' of an enrichment device has been previously demonstrated in a narrower sense in rat snakes (*Elaphe obsolete*; Almli and Burghardt, 2006), and our results highlight the importance of taking this into account when designing enrichment interventions.

Due to time constraints we were unable to measure the effects of the enrichment device in the long term. Our GLM results showed that there was no effect of trial number in our results and therefore that there was no evidence of habituation in our data over the 42-day trial period. However, it is worth noting that in order to fully understand the future potential of this device, and any effects on physical fitness, more longitudinal data would be required.

Sample size was limited to five lizards, which was the maximum number available at the time of study. Limited sample size is frequently a limiting factor when working with non-model organisms, particularly in a zoo setting, but by doing so we were able to address enrichment in an as yet unstudied group. Although a large number of trials were done to counteract the small sample size, it may be difficult to extrapolate these particular results to all tree-runners; however, the underlying principle that enclosure complexity may provide more behavioural enrichment than a dedicated device is an important finding.

The oldest and youngest lizards varied in age by 60 days (36 versus 96 days). Our experiment lasted for 42 days and during that time there was no variation in the results (i.e. no significant effect of 'trial number'). This suggests that the age differential was not important in determining the responses to the enrichment device. In terms of the potential effect of the sexes of the lizards; the sexes of the lizards remained unknown throughout the experiment. As juveniles this lizard species shows no sexual dimorphism and sexing the lizards before our experiment was something that was outside the scope of the study. No lizards reached sexual maturity during the study (this occurs at approximately one year of age in captivity, Michaels, C. pers. obs.) and so effects of sex are less likely to have been important.

Despite the results of this study this does not imply that feeding enrichment devices are unnecessary with captive insectivorous lizards. There is no doubt that the provision of live food played a large role in the success of the scatter feed and these results highlight its effectiveness when combined with the right enclosure complexity. The effectiveness of live food as a part of an enrichment device has been reported anecdotally (Rosier et al., 2011), and there has been some attempt to quantify its importance in the literature; Phillips et al. (2011) found blue-tongued skinks (*Tiliqua scincoides*) displayed more foraging behaviour when fed live mealworms as a scatter feed rather than from a food bowl. Similarly, green anoles (*Anolis carolinensis*) and five-lined skinks (*Plestiodon fasciatus*) respond more to movement (in live mealworms) regardless of mealworm size (Burghardt, 1964). However, most research involving enrichment still suggests that scatter feeding of any kind of food is not as beneficial compared to a manipulated feeding device, although concrete data especially for reptiles is still rare. Puzzle feeders were found to increase feeding time in fly river turtles (*Carettochelys insculpta)* (Bryant and Kother, 2015), but the turtles in this study were housed in a relatively simple enclosure and offered unmoving food, both of which prevented environmental complexity from impeding food discovery. These contrasting results show the importance of choosing the correct enrichment method for a particular species and in the context of a particular enclosure design, and that more research is needed to inform these decisions.

Conclusion

Behavioural enrichment devices are commonly used for captive animals to encourage activity and the exhibition of natural behaviours. Despite their growing importance as a vital part of animal husbandry, their effectiveness is rarely empirically evaluated with captive reptile species, especially in comparison with the number of studies found on enrichment devices in mammals and birds. Our data show that even enrichment devices designed for animals with particular prey items in mind may be less effective than simpler methods of enrichment provision and that the success of a device may be dependent on its environmental context. In future studies, there should be a larger emphasis on the natural feeding methods of the animal, the effects of enclosure design and the provision of live food on the success of an enrichment device, especially with reptiles, in order that their husbandry is not compromised due to a lack of understanding.

Acknowledgements

Thank you to Dr. Beatrice Gini for assistance with the statistical analysis. We would also like to thank the two anonymous reviewers who provided guidance on this paper during submission. This study was carried out in fulfilment of the Wild Animal Biology MSc degree at the Royal Veterinary College and the Zoological Society of London. Funding was provided by the Royal Veterinary College.

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Figure captions for the figures submitted to Applied Animal Behaviour Science, for the paper 'Is behavioural enrichment always a success? Comparing food presentation strategies in an insectivorous lizard (*Plica plica*)', Januszczak et al.

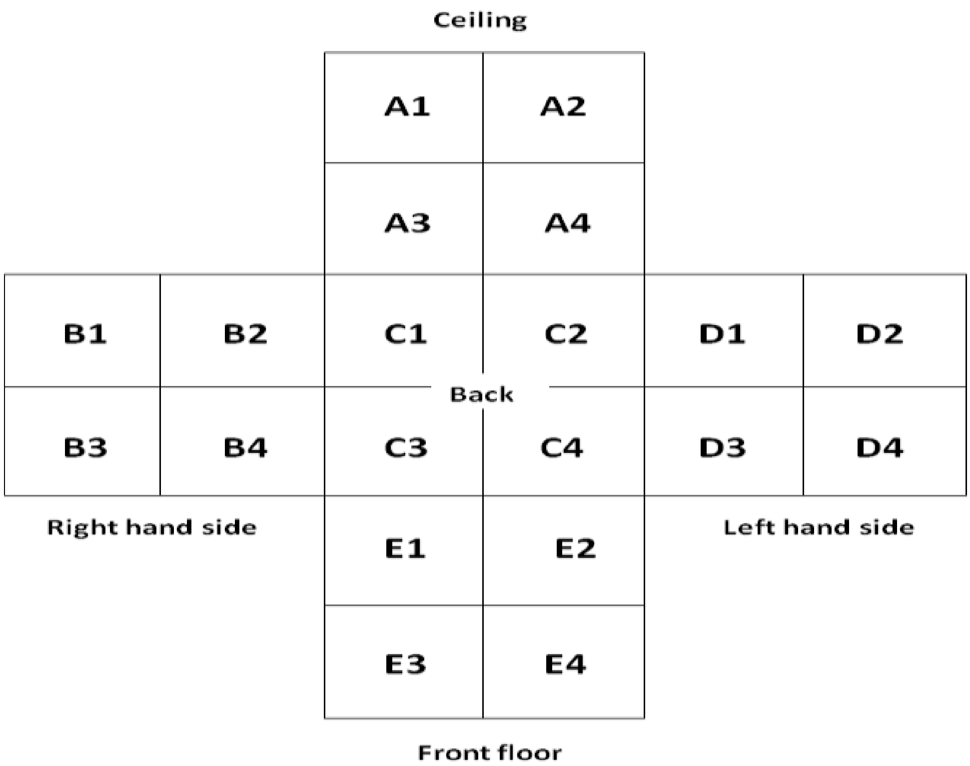
Figure 1: Photographs and 2D representations of different components of the experiment

A) Setup of the experimental tanks containing the lizards. The tanks were numbered one to five; left to right; **B)** Close up of the general layout of each tank; **C)** 2D representation of the enclosure labelling **D)** A tree-runner lizard (*Plica plica*) inside one of the tanks (on the polystyrene backing). Photo credit: I. Januszczak.

A.

B.

C.


Ceiling
A1
A2
A3
A4
B1
B2
C1
C2
D1
D2
Back
B3
B4
C3
C4
D3
D4
Right hand side
E1
E2
Left hand side
E3
E4
Front floor

D.

Figure 2: Photo of the enrichment device

Photo of the enrichment device used. Photo credit: I. Januszczak.

Figure 3: Graphs displaying the means of different recorded behaviours in the control, enriched and scatter trials

95% confidence intervals as error bars. The asterisks indicate significant differences (if present) between different treatments (p<0.0083). The graphs show the mean frequencies of successful strikes (A), unsuccessful strikes (B), locomotion (C), targeting prey (D), alertness (E), times boundary crossed (F) and proportion of successful strikes (G).

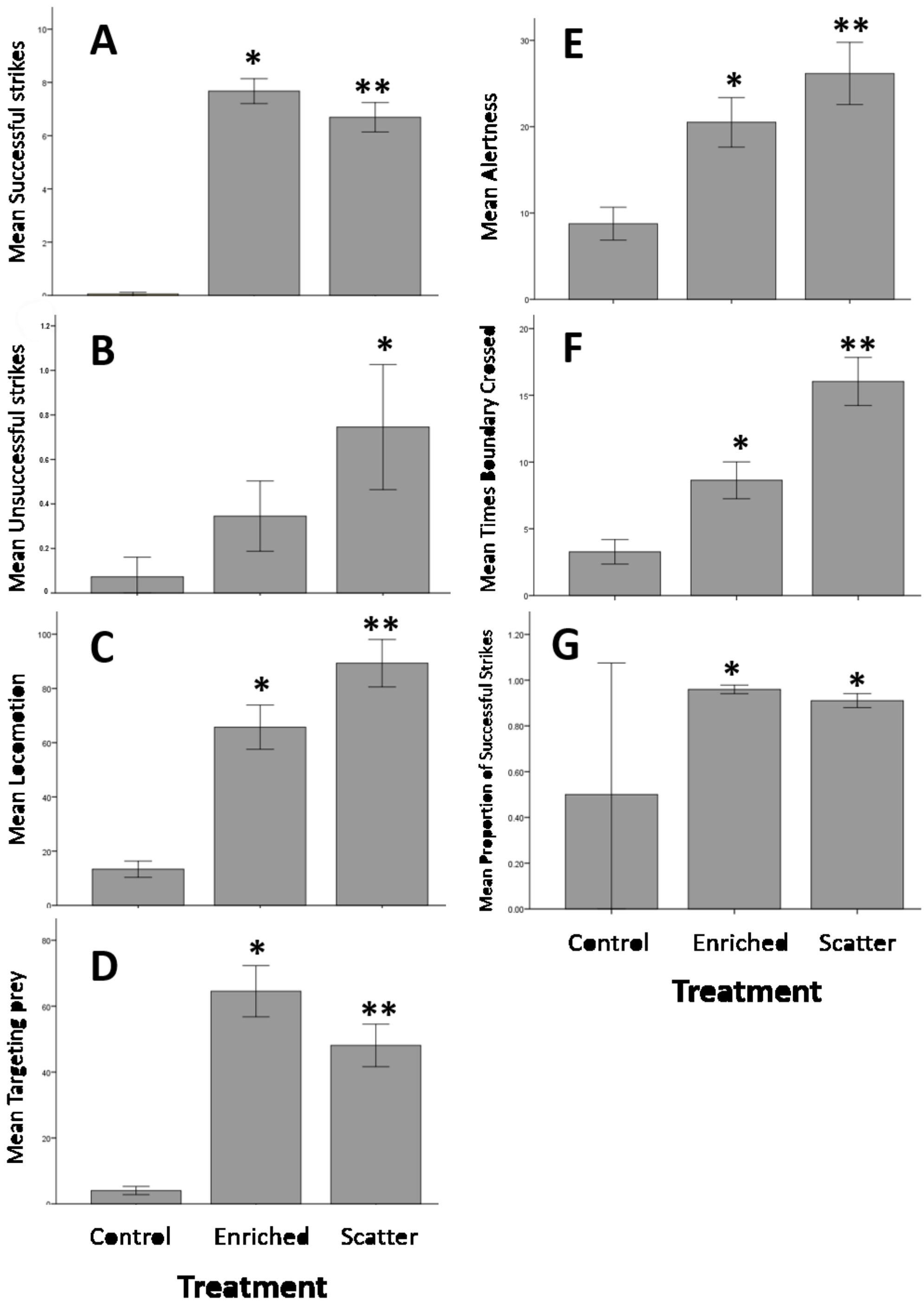


Figure 4: ‘Heat map’ style charts, with a colour key representation of the percentage enclosure use of the lizards in the three treatments

2D charts showing the percentage enclosure use of the lizard in each grid square in the enclosure in the control, enriched and scatter control trials. The greyscale is quantified by the accompanying colour key which shows the corresponding percentage enclosure use. The number of colours presented in the colour key has been minimised as necessary to differentiate more clearly between the grids. The black dot in grid ‘C2’ represents where the enrichment device was placed in the trials (the back right hand side of the enclosure floor).