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Does the provision of environmental enrichment affect the behaviour and welfare of captive snakes?

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ABSTRACT

There is a wealth of evidence demonstrating the benefits of environmental enrichment across a range of different animal species. However, there is comparatively little such research into the effect of enrichment provision on captive reptiles. The aim of this study was therefore to ascertain if an increase in environmental complexity was beneficial to the behaviour and welfare of corn snakes (*Pantherophis guttatus*). The study used a combination of behavioural observations in the home enclosure, behavioural tests of anxiety, and a preference test. The snakes used the enrichment when it was available to them and enriched snakes showed changes in general behaviour reflective of improved welfare. However, the anxiety tests revealed few effects of enrichment provision on performance. In contrast, the snakes exhibited a strong preference for the enriched enclosure when given a choice. These findings suggest that the provision of environmental complexity to the enclosure was beneficial to the behaviour and welfare of captive corn snakes. We therefore recommend enrichment should be used when keeping captive snakes.

1. Introduction

Reptiles have very specific needs in captivity and failure to meet these can have serious consequences for their health (RSPCA, 2002; Burghardt, 2013). Many of the health problems that arise in reptiles appear to be linked to housing and husbandry issues (e.g. Oonincx and van Leeuwen, 2017; Benn et al., 2019). This seems to be due to owners' lack of understanding about the requirements of their reptile(s) rather than any lack of an emotional bond with their pets (Haddon et al., 2021). This may be due to a relative paucity of scientific research evaluating different aspects of reptile care, which makes providing evidence-based recommendations to reptile owners challenging - although there is some suggestion that uptake of available information can be poor (Howell et al., 2020). As reptiles are popular pets (PFMA, 2016), it is essential that this knowledge gap is filled in order to allow owners to make informed decisions on the provision of the necessary resources to keep their pet reptiles in good mental and physical health.

One aspect of resource provision that has an important impact on animal welfare is environmental enrichment - making changes to the animal's environment and/or husbandry to promote positive behaviours, reduce negative behaviours and so improve welfare (Young, 2003). This can be achieved in a variety of ways (e.g. social, occupational, physical, sensory and/or nutritional enrichment; Bloomsmith et al., 1991), and can take a variety of forms, including structural changes to the enclosure, cognitive tasks, food-based enrichment, social interaction and sensory enrichment (de Azevedo et al., 2007). Making appropriate changes to a captive animal's environment and husbandry regime can provide the animal with greater control over its environment and the opportunity to express species-specific behaviours (Mellen and MacPhee, 2001; de Azevedo et al., 2007; Alligood and Leighty, 2015). The ultimate goal of environmental enrichment is to improve an animal's welfare by providing for its physiological, psychological and behavioural needs (Mellen and MacPhee, 2001). One way to do this, as well as to potentially reduce stress and increase resilience to negative experiences (Burman et al., 2008), is to increase environmental complexity within the enclosure. Environmental enrichment has been successfully used to reduce stereotypic behaviours (Zoo mammals: Shyne, 2006); improve welfare and decrease stress, increase activity and exploration; improve body condition and mortality; reduce aggression (Amphibians: Hurme et al., 2003; Chum et al., 2013; Michaels et al., 2014; Fish: Näslund et al., 2013). Enrichment has also been shown to have neurological benefits, improve cognitive flexibility, and improve immune function (Fox et al., 2006; Hannan, 2014; Clemenson et al., 2015; Gelfo, 2019).

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In the growing field of environmental enrichment, there is comparatively little work on reptiles (de Azevedo et al., 2007; Melfi, 2009; Burghardt, 2013; Alligood and Leighty, 2015). Those studies that have been carried out have generally found that enrichment is beneficial (e.g. Chelonia, Therrien et al., 2007; Case et al., 2005; Mehrkam and Dorey, 2014 and lizards Phillips et al., 2011; Hennig and Dunlap, 1978; Londoño et al., 2018; Bashaw et al., 2016). There are very few studies investigating snake welfare (e.g. Warwick et al., 2013; Van Waeyenberge et al., 2018) and even fewer papers considering the benefits of enrichment (Cardiff, 1996; Almli and Burghardt, 2006; Rose et al., 2014; Spain et al., 2020; Nagabaskaran et al., 2021). This makes it difficult to provide the necessary evidence-based guidance for how to house snakes in order to maximise their welfare. For instance, it is recommended by the RSPCA (2019) that snakes should be provided with resources that give them the opportunity to exhibit natural behaviours such as climbing (e.g. include branches) and hiding (e.g. include several size-appropriate hiding places). These are examples of the types of enrichment that might be expected to increase activity, helping to avoid health issues such as obesity, and reduce anxiety by allowing animals more control. However, in most cases, they have yet to be evaluated empirically. Cardiff (1996) found an increase in activity levels and the amount of time spent in an elongated position in Jamaican boas when provided with enrichment consisting of varied combinations of bark substrate, branches, and hides. More recent work has revealed that corn snakes (*Pantherophis guttata*) use enrichment if available (Rose et al., 2014), and that providing both additional environmental complexity and a larger enclosure size increased behavioural diversity and activity levels in Giant Hognose Snakes (*Leioheterodon madagascariensis*; Spain et al., 2020); although this study did not control for order effects. Crucially, none of the above studies looked beyond differences in within-enclosure behaviour to investigate enrichment effects on performance in preference tests, in which the animals could choose to spend time in the different environments, or behavioural/cognitive tests. Nagabaskaran et al. (2021) revealed that performance in a discrimination task differed with housing condition; when snakes were housed in *Enriched* enclosures they discriminated between familiar and unfamiliar human odours whereas when housed in *Standard* enclosures they did not. Almli and Burghardt (2006) also found that increasing cage complexity resulted in different behavioural profiles of ratsnakes (*Elaphe obsoleta*) as determined by a range of cognitive/behavioural tests. However, in their study Almli and Burghardt (2006) included the provision of live mammalian prey as part of their environmental enrichment (alongside climbing branches and hiding places). The use of live vertebrate prey comes with substantial ethical and welfare issues (Cooper and Williams, 2014). As such, it is essential to investigate whether increasing cage complexity *without* live food provision would have a similar impact.

Whilst a key aim of environmental enrichment is to increase natural species-specific behaviours, as determined by observing within-enclosure behaviour, it is also important to corroborate the apparently beneficial behavioural change observed in enriched animals with an understanding of their actual preference. By 'asking the animal' (Dawkins, 2008) which environment it prefers, we can obtain a more comprehensive interpretation of their welfare, identifying both what the animal wants, and if it is healthy (Dawkins, 2004). The aim of this study was therefore to determine whether providing enrichment improves the behavioural repertoire and welfare of snakes. Corn snakes, one of the most commonly kept species of snake in the UK, were used as a model species. We investigated the effect of enrichment provision on general behaviour within their home enclosures, their response in behavioural tests and also investigated how these measures related to the choices made by the snakes in a preference test.

2. Methods

2.1. Animals & husbandry

Fifteen captive bred, adult corn snakes (*Pantherophis guttatus*) of unknown sex, were used in this study. The snakes weighed between 110 g–375 g (average =277.62 g) at the start of the study. They were loaned from a reptile rescue centre, and thus had a varied range of backgrounds and experiences. Snakes were housed at the University of Lincoln for the duration of the study and then returned to the rescue centre from where they originated in order to be re-homed. The snakes were fed a dead mouse weekly on the same day, using tongs. Where possible snakes were handled on a daily basis for 2−5 min for acclimatisation in addition to handling for husbandry purposes. However, snakes were not handled (or involved in behavioural tests) for the two days after feeding nor during shedding (from when their eyes turned 'milky' until two days after completing their shed). This research study was approved by the University of Lincoln Research Ethics Committee (COSREC506).

2.2. Housing condition and overall design

All snakes were housed individually in glass-fronted enclosures measuring 83 × 35 × 39 cm (internal dimensions), solid walls prevented visual contact between neighbours. All enclosures contained a heat and light source and were kept on a 12 h light/dark cycle (on from 07:00 till 19:00) and with a thermal gradient of ~22 °C-31 °C. The enclosures were either *Standard* or *Enriched* (see Fig. 1), and they were distributed approximately evenly within the room to counterbalance for positional effects. The *Standard* enclosures each contained a small water bowl, a 'rock/cave' hide, and newspaper substrate. The *Enriched* enclosures each contained the same 'rock/cave' hide as the *Standard*, but, in addition, a real wood branch (to allow vertical climbing); a peg board (wood doweling pegs arranged in a vertical pattern designed to allow further climbing opportunity); a large water bowl (big enough for the snake to bathe in); a 'natural' substrate (Aspen shavings); a hanging hollow coconut hide; and a 'humid' hide (a plastic pot containing moistened moss and compost to allow digging/burrowing). Snakes were pseudo-randomly assigned to either *Enriched* or *Standard* enclosures upon their arrival at the University of Lincoln. Snakes were housed in their respective enclosures for a minimum of 32 days (some variation due to shedding). During this time their behaviour within the enclosure was monitored, they then experienced a series of behavioural tests to assess their affective state. After the tests had been completed, the snakes were switched to the type of enclosure that they had not previously experienced (either *Standard* to *Enriched* or *Enriched* to *Standard*). They then experienced this condition for a minimum of 32 days before again receiving the same set of tests. Following this they received a one-off preference test. At the end of the study all snakes were housed in *Enriched* enclosures until they were returned to the shelter for rehoming.

2.3. Welfare assessment

To assess whether there were differences in the welfare of snakes kept in different housing conditions, a series of measures were used:

2.3.1. Behavioural observations

Observations of snake behaviour within the enclosure were conducted with the aid of video recordings. To ensure that snake behaviour was not affected by human presence, these recordings were made using a video camera without the experimenter being present. Snakes were filmed individually using one camera per enclosure. Each observation session lasted 30 min and a total of 2.5 h were recorded per snake per week (Monday to Friday). Observations were spread over the full 12 h light period to allow us to assess any changes in behaviour throughout the day, although they were not observed at night when they may also

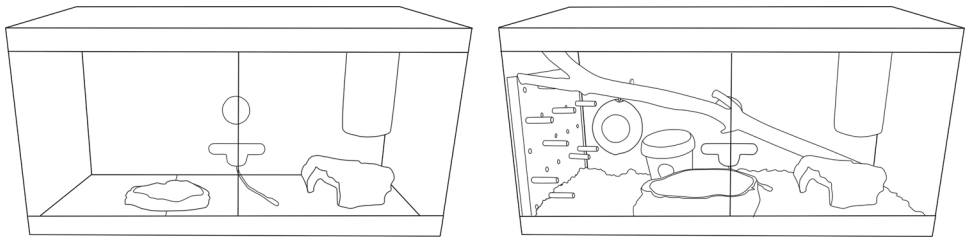

Fig. 1. Diagram of Standard (left) and Enriched (right) enclosures.

be active. Snakes were not handled for two hours prior to the observations taking place to avoid an effect of handling on behaviour. The order in which the snakes were recorded was pseudo-randomised to avoid effects of order and time.

2.3.2. Behavioural tests

The snakes were given behavioural tests to observe their response to novelty; a common test for anxiety-like behaviour that has been demonstrated to be appropriate for use with reptiles (Moszuti et al., 2017; Siviter et al., 2017; Stockley et al., 2020). These tests were presented twice to each animal and were carried out after the snakes had been in each enclosure type for a minimum of 32 days. The snakes were given two different behavioural tests, a novel environment test (introduction to a previously unfamiliar arena) and a novel object test (introduction to a previously unfamiliar object in a familiar arena). Temperature was recorded for each snake at the beginning of each trial. Each snake was tested individually and behaviour was recorded continuously for the 10 min trial duration. Recording started from the point at which the snake had been placed in the arena and the experimenter was no longer visible. Trials were video recorded for later analysis using a video camera placed above the arena. Both experimenters stayed in the room for the duration of the trial but stayed out of sight of the snakes. The arenas were covered with mesh to stop the snakes climbing out during testing.

2.3.2.1. Novel environment test. The snakes were placed at the same point in the arena at the start of the trial. The snakes were assigned to one of two novel arenas (83 × 83 cm) for their first test (after the first housing condition). These were balanced between conditions and differed in various aspects (e.g. floor/wall colour and texture) altered to enhance differentiation between them (e.g. Moszuti et al., 2017). In their second test (after the second housing condition) the snakes were tested in the arena they had not yet experienced (order counterbalanced across individuals) to ensure it was novel. The arena was cleaned with diluted disinfectant between each trial.

2.3.2.2. Novel object test. The same arena was used as in the novel environment tests. To ensure familiarity, the snakes received an additional four habituation sessions in the arena, before doing the novel object test. The arena was divided into four quadrants and a novel object was placed on a marker in the arena, either in the centre of the top left or top right quadrant, before the start of each trial. The snake was placed in the arena on a marker equidistant from the two novel object markers. The position of the novel object was counterbalanced across left and right quadrants between snakes. Each trial lasted five minutes and only one test per object was carried out for each snake. During each round of testing snakes received one object. Thus in total each snake received two objects from a selection of seven. The objects chosen were balanced between snakes and enclosures. The novel objects were household ornaments (3 ceramic ornaments, 2 aquarium ornaments and 2 plastic ornaments/toys, sizes varying from 8 × 12 × 8 cm to 16 × 13 × 10 cm). They were chosen to be unlikely to have been experienced by the snakes previously, and to be safe for the snakes to interact with if they chose. The arena and object were cleaned with diluted disinfectant between trials.

2.3.3. Body weight

Snakes were weighed to the nearest 0.1 g every Tuesday and Friday (before feeding). Snakes were not weighed if they could not be handled due to shedding. To overcome differences as a result of snakes not being present in the dataset for that day an average weight was calculated using values either side of the missing values, if multiple values were missing a moving average was calculated. Weights were calculated as percentage weight change compared to week one of each condition.

2.4. Preference testing

After the second set of behavioural tests had been completed, snakes were given a preference test in which they could choose between the *Standard* and the *Enriched* enclosures (both of which they had by now experienced). For this test, an experimental enclosure was created in which a *Standard* and *Enriched* enclosure were joined on the long side so the animal could access both enriched and standard conditions. The top of the experimental enclosure was removed and replaced by a single 3 mm sheet of acrylic to allow filming from above. At the start of the preference test, the snake was placed in the centre of the experimental enclosure at 16:00 and removed the following day at 10:00 h. A video camera was placed directly above the set up and all snakes were recorded from 16:00−19:00.

2.5. Data analysis

2.5.1. Data

Videos were coded using BORIS (Friard and Gamba, 2016). Values are reported throughout as the mean followed by standard error.

In vivarium location was sampled every 2 min (Table S1) whereas behaviour was continuously recorded for 1 min and sampled at 5 min intervals (Table S2). All location data were calculated as proportion of total observations where the snake was visible (i.e. when it was possible to ascertain the relevant location information). 'Coiledness' was calculated as a proportion of time spent resting outside of the hide. Activity type (i.e. locomotion, partial movement, stationary), 'submerged', drinking, and 'nose rubbing' were calculated as a proportion of time spent active (i.e. not resting). Frequency data was used for gaping.

Behaviours coded for the behaviour tests are summarised in Table S3. For all behaviours both duration and frequency were extracted, so that each snake had an *Enriched* and a *Standard* value for each behaviour. Durations were converted to proportions of observed time and, where required, corrected for time when the snake's behaviour was not visible.

In the preference test, the proportion of time spent in each environment and the location at lights off were all recorded. The videos were sampled starting from after the snake had been placed in the arena and then sampled every minute until the last possible time point before the lights turned off at 19:00. The number of times each snake was observed in each side (i.e. *Enriched/Standard*) was divided by the total number of observations to calculate a proportion of time spent in each environment.

2.5.2. Statistical analysis

The study was a within-subjects design with each snake experiencing both conditions (balanced for order), thus differences of behaviours within-enclosure and in the behavioural tests, and body weight change between *Enriched* and *Standard* conditions were investigated using a mixed effects model using the function 'lmer' in the package lme4 (Bates et al., 2015). For each behaviour, a linear model was specified with Environment (*Enriched/Standard*) as the fixed effect, Snake (individual) specified as a random effect, and behaviour as the outcome variable. P-values were calculated using lmerTest (Kuznetsova et al., 2017). In the body weight analysis, Environment was included as a fixed effect, Week as a covariate, and body weight as the outcome variable. A Wilcoxon test was conducted to ascertain if there was a significant difference in body temperature between conditions. 26 % of the videos from the novelty tests were (blind) second coded and a Spearman's rank correlation revealed a strong positive correlation between observers (Novel Environment test: $\rho = 0.86$, $p < 0.001$, Novel Object Test: $\rho = 0.96$, $p < 0.001$). Analysis was conducted in R version 3.6.0 (R Development Core Team, 2019).

Proportion of time spent in each environment in the preference test was investigated using a one-sample T test to compare the differences to zero and using Binomial tests to assess frequencies. Significance was taken as $P < 0.05$. Analysis was carried out in SPSS version 23 (IBM Corp, 2013).

3. Results

3.1. Welfare measurements

3.1.1. Behavioural observations

When housed in the enriched enclosures, snakes were seen to use all the additional enrichment elements, including submersion in the larger water bath, climbing on the branches and 'peg boards', and resting in both the hanging coconut hide and moss pot. Due to the presence of the additional enrichment, snakes in the *Enriched* condition were able to utilise the full extent of the enclosure, spending significantly more time in the top section of the enclosure ($t = -13.52$, $df = 572.02$, $p < 0.001$).

When comparing those behaviours that could be exhibited in both conditions (Fig. 2–3), there was no significant difference between amount of time spent in the rock hide ($t = 0.50$, $df = 523.29$, $p = 0.616$), however, *Enriched* snakes spent significantly more time in hides overall ($t = -12.33$, $df = 523.54$, $p < 0.001$). Snakes in the *Enriched* enclosure also spent significantly more time on the (aspen) substrate ($t = -3.559$, $df = 523.23$, $p < 0.001$), whereas those in a *Standard* enclosure spent significantly more time under the (newspaper) substrate ($t = 18.25$, $df = 523.25$, $p < 0.001$). Despite spending more time in hides, snakes spent significantly more time visible when in the *Enriched* enclosure ($t = 11.830$, $df = 572.00$, $p < 0.001$). There was no significant difference between the amount of time spent resting in the two conditions (Table S4), however, while active, *Enriched* snakes spent more time in full locomotion ($t = -3.289$, $df = 103.75$, $p = 0.001$), whereas *Standard* snakes spent more time stationary ($t = 2.528$, $df = 104.14$, $p = 0.013$). In addition, *Enriched* snakes spent significantly more time loosely coiled while resting outside of a hide ($t = -3.549$, $df = 101.15$, $p < 0.001$). Nose rubbing and gaping were insufficiently frequent for statistical analysis.

3.1.2. Behavioural tests

For both tests only 14 snakes completed the tests each time. For the first set of tests one individual was removed due to laying eggs during the testing period, for the second set of tests a different individual was removed due to ill health. There was no significant difference between the two environments in the temperature of the snakes at testing (novel environment: $V = 26.5$, $p = 0.1958$, novel object: $V = 31$, $p = 0.1937$).

3.1.2.1. Novel environment test. Snakes in *Standard* environment spent significantly more time in full locomotion compared to those in the *Enriched* environment ($t = 2.306$, $df = 13.78$, $p = 0.037$). There were no significant differences between snakes housed in the *Enriched* and *Standard* conditions for any of the other measures in the novel environment test (Table S5).

3.1.2.2. Novel object test. Two snakes did not complete the second novel object test and were thus were removed from the frequency analysis, as durations of behaviours were calculated as a proportion of total duration of trial it was possible to include these individuals in analysis of duration. Due to the quality of the recording one snake was only included in analysis of object related behaviours. Snakes in the *Enriched* condition spent a significantly greater proportion of time in partial movement in the novel object test compared to those kept in the *Standard* condition ($t = -3.119$, $df = 12.74$, $p = 0.008$), however, full locomotion and inactivity did not differ significantly between conditions, nor did the proportion of time other behaviours were performed (Table S6).

Snakes in the *Enriched* condition entered the quadrant with the object in ($t = -3.296$, $df = 13.08$, $p = 0.006$) significantly more frequently than those in the *Standard* condition. No other behaviours differed

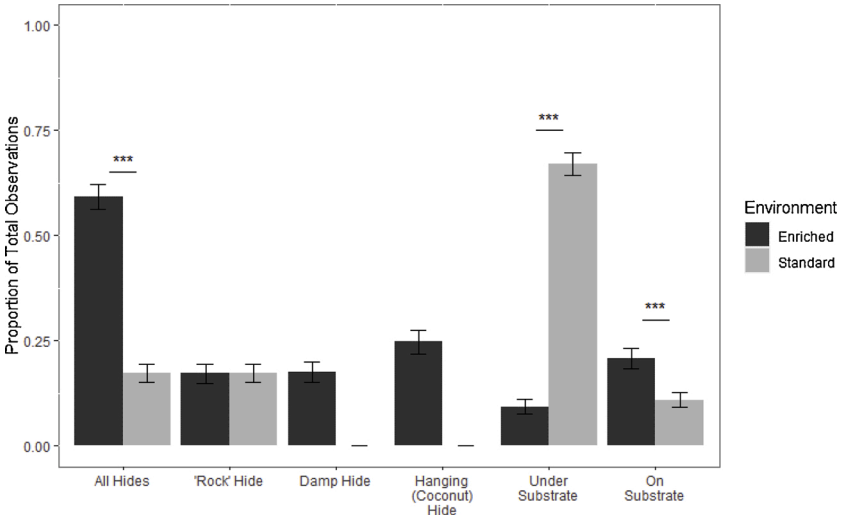

Fig. 2. Proportion of observations that snakes spent in closest proximity to resources in their enclosures. Items included only in the Enriched enclosures (Damp hide; Hanging hide) are included to show use, not to allow for comparison. Data are means ± standard errors.

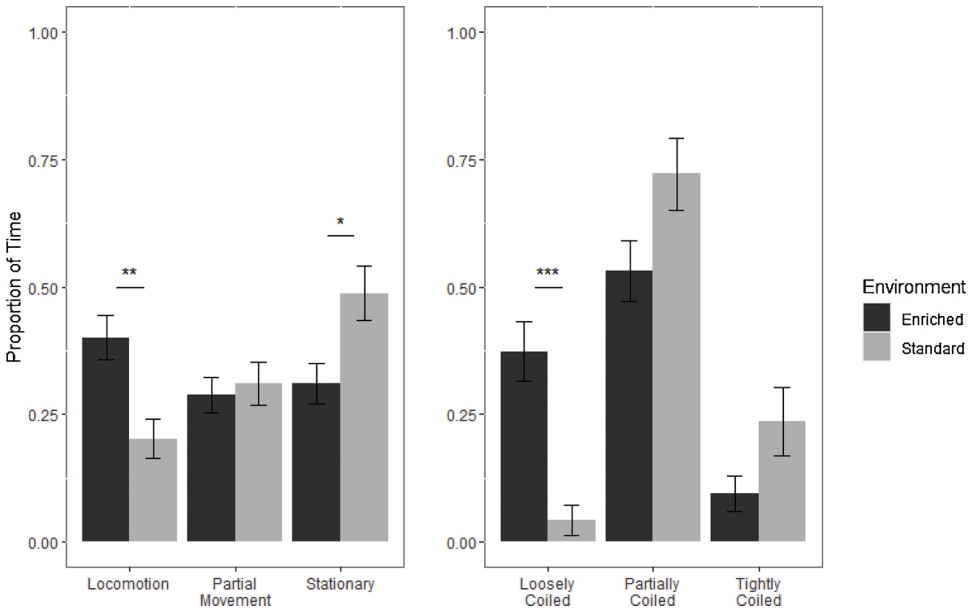

Fig. 3. Average proportion of time spent, engaged in different movements while active (left) and the amount of time they spent tightly coiled, partially coiled and loosely coiled while resting outside of a hide (right) in the two different housing conditions. Data are means ± standard errors.

significantly in their frequency between the two housing conditions (Table S6).

3.1.3. Weights

Snakes experienced a significant increase in weight over time ($F_{5,132} = 7.9833$, $p < 0.001$). However, there was no overall effect of condition (*Enriched/Standard*, $F_{1,132} = 0.859$, $p = 0.3557$) or interaction between time and condition ($F_{5,132} = 0.2068$, $p = 0.9592$).

3.2. Preference tests

Fourteen snakes took part in the preference tests. Both the location of the head (either in the *Enriched* or the *Standard* side) and the majority of the body were investigated as a proxy of 'preference', but only the location of the head is reported here as the results were very similar. Snakes spent significantly more time in the *Enriched* side, an average of 82.5 ± 5.9 % ($t = 5.502$, $df = 13$, $p < 0.001$, Fig. 4). Significantly more (12/14) snakes were located in the *Enriched* side when lights went off at 19:00 (Binomial (0.5) $p = 0.0056$).

4. Discussion

Our findings revealed that the provision of additional resources and complexity to the enclosure was beneficial to the welfare of captive corn snakes, as reflected in a variety of behavioural measures, and so can be confirmed as being enriching. We found that, when available, the snakes

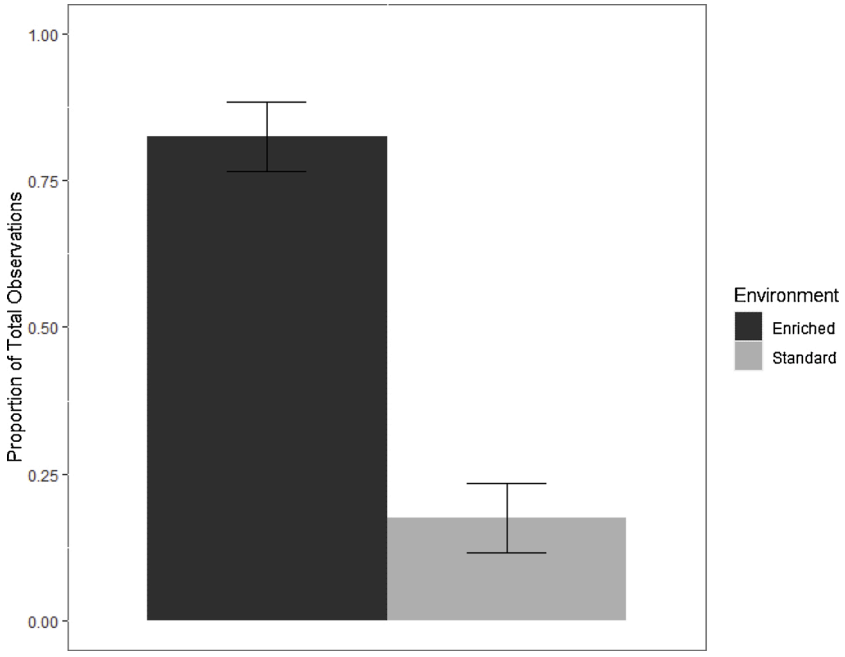

Fig. 4. The proportion of total time the snakes spent with their head in the Enriched and Standard sides of the preference test set up. Data are means ± standard errors.

interacted with the enrichment. Whilst we observed little difference in the behavioural tests, the snakes exhibited a strong preference for the enriched enclosure when given a choice. Taken together, this suggests that enrichment was beneficial to the snakes and we recommend that it should be provided in order to improve welfare, as not providing enrichment is likely to be a welfare concern.

The first step to identifying successful enrichment is to determine whether the animal interacts with those additional resources provided, though this measure alone is not sufficient to confirm a welfare improvement. Bearing this caveat in mind, in addition to the snakes in this study being observed engaging with all elements of the enrichment and so demonstrating an enhanced, and more natural, behavioural repertoire (e.g. using different shelters, climbing, submerging themselves in the water bowl), the way that the snakes utilised the space in their enclosures also differed; presumably as a consequence of the different resources provided throughout their enclosure. *Enriched* snakes spent significantly more time at the top of their enclosure (i.e. above the line of the top of the rock hide). Whilst this is to be expected, as the additional hides, branch and peg board gave the opportunity to utilise that space in a way that was not possible when housed in the *Standard* enclosure, that they did so suggests this is something for which they were motivated. This in in line with other studies that found that snakes utilised climbing opportunities and spent significant amount of times off the ground (Jamaican boas: Cardiff, 1996; corn snakes: Rose et al., 2014). However, Rose et al. (2014) found that corn snakes only utilised climbing opportunities when further enrichment was also provided, highlighting the importance of the combination of individual resources/items provided as enrichment. Spain et al. (2020) found that increased enrichment and enclosure size together resulted in snakes being more active and visible a greater proportion of time. Rose et al. (2014) also found that the provision of enrichment and the opportunity to climb resulted in corn snakes being visible a significantly greater proportion of time, a finding that was mirrored in our study, despite the snakes having more places to hide in the *Enriched* enclosure. Further, our recent work (Nagabaskaran et al., 2021) found that when snakes were housed in *Enriched* enclosures they were able to discriminate between the odours of familiar and unfamiliar humans, whereas when housed in *Standard* enclosures they did not. Taken together, these findings suggest a benefit to owners as well as their snakes, with interactions likely to be improved as a result of pet snakes being able to discriminate between people, and owners likely to appreciate the increased opportunities to observe their pet; inclusion of enrichment will therefore likely contribute towards an improved pet-owner bond (e.g. Alba et al., 2017).

We found that, when *Enriched,* snakes spent more time in a hide and on top of the aspen substrate, whereas when in the *Standard* enclosures they spent more time under the paper substrate. Similarly, in a study looking at the provision of enrichment for Jamaican boas, a hide box was used preferentially over hiding under the substrate (regardless of whether the substrate was a paper towel or bark, Cardiff, 1996). The finding that the snakes in our study used the rock hide (available in both conditions) for equal amounts of time, but overall used hides more often when *Enriched* (i.e. when multiple hide options were available) is indicative of the importance of giving the snakes choice, as each hide was intended to provide different benefits (e.g. contrasting thermal properties/humidity/height). For example, the provision of a damp substrate may be particularly important around shedding, and a lack of this could result in shedding difficulties (dysecdysis). As this is one of the most common reasons for snakes to visit the vets (Hoehfurtner et al., 2021) this is likely to be a key provision. Furthermore, the lack of a choice of hiding places in the *Standard* condition resulted in the snakes using suboptimal hiding places (i.e. under the newspaper), as seen in Eastern box turtles who took shelter under the paper substrate or water dish when no hiding places were provided (Case et al., 2005).

Whilst resting outside of their hides, snakes in *Enriched* enclosures spent more time loosely coiled than when housed in *Standard* conditions. Similarly, the use of a bark substrate increased the amount of time spent in an elongated position in Jamaican boas (Cardiff, 1996), suggesting that resting outside of the hide while loosely coiled (i.e. a more elongated position) may be a behavioural indicator of good welfare, possibly as an expression of reduced anxiety and increased relaxation/security, similar to the relaxed 'exposed' resting positions observed in other species (Meagher et al., 2013; Owczarczak-Garstecka and Burman, 2016).

In the behavioural tests of anxiety, the few statistically significant effects that were observed (e.g. snakes, when *Enriched*, more frequently moving into close proximity with the novel object) appeared in line with a tentative suggestion of reduced anxiety and increased confidence (Stockley et al., 2020). However, it should be noted that with so few significant results in the behavioural tests used here these results should be taken with caution. Although these behavioural tests have been found to be effective in tortoises (Moszuti et al., 2017) and bearded dragons (Siviter et al., 2017; Stockley et al., 2020), it may be that they are less appropriate for snakes - possibly due to the influence of individual differences (e.g. Spain et al., 2020).

No difference in body weight change was found between the housing conditions, although the snakes did gain weight over the course of the study. Change in bodyweight can be an ambiguous measure of welfare as increases might be positive in some contexts (e.g. no inhibition of growth) or negative in other contexts (e.g. obesity; Mcmillan, 2013). Our findings differ to a previous study in rat snakes where individuals gained significantly more weight and grew significantly more when enriched (Almli and Burghardt, 2006). However, the snakes used in that study were all yearling snakes and so would be expected to experience greater changes in size.

Preference and choice tests are considered as a key measure of animal welfare and can be used as a benchmark against which other measures can be validated (Dawkins, 2008). In our study, the snakes showed a clear preference for the *Enriched* enclosure in their preference test. This preference emerged whether the snakes' most recent experience had been in the *Enriched* enclosure (i.e. the last four weeks) or the *Standard* one. The outcome of preference tests is usually limited to those resources that were offered to the subject, so a preferred resource can only be interpreted as being the most desired of the available options (i.e. *Enriched* > *Standard*) rather than being the 'best' possible resource; and so, in this instance, the *Enriched* enclosure that we provided was preferred to the *Standard* enclosure, rather than being the optimal environment for housing corn snakes. There have been few preference tests applied in reptiles, but Case et al. (2005) identified a distinct preference for an enriched, rather than a barren, enclosure in eastern box turtles (*Terrapene carolina carolina*), regardless of their previous housing experience. Further applications of this approach are therefore strongly recommended given its importance and apparent effectiveness in reptile welfare assessment.

Reptiles have complex cognitive demands (reviewed by Matsubara et al., 2017), thus it logically follows that they would 'suffer' in an under-stimulating environment that lacks sufficient complexity. We found that there was a clear usage of the various items provided in the enriched enclosures. Snakes were observed using all elements of the enriched enclosure. Being able to express a behavioural need (Dawkins, 1988) and having control over one's environment (e.g. Bassett and Buchanan-Smith, 2007) are key aspects of good animal welfare, as is showing an increased range of natural behaviours (Young, 2003). So, taken together, this suggests that providing enrichment to the snakes was beneficial - in that they chose to use the resources when they were available and, feasibly, would therefore have had their welfare compromised by the thwarting of this behavioural need (Mason and Burn, 2011).

5. Conclusions

In conclusion, based on the preference of the snakes, within-enclosure behavioural observations and, to a lesser extent, their

response in behavioural tests, it appears that the provision of complexity to the enclosure was enriching, and beneficial to the behaviour and welfare of captive corn snakes. Our recommendation would therefore be that enrichment is the default provision for captive snakes. However, due to the comparison made in this study (*Enriched* vs. *Standard*), we are unable to determine what specific resource, or combination of resources, of our enriched enclosure was most beneficial, and so would suggest that all the options studied here are provided, including: resources that allow use of vertical space (climbing); a choice of shelters (raised and at ground level, humid and dry); and a water bowl sufficiently large to allow submersion. Further investigation is needed to determine the relative importance of these different resources and to investigate other resources not included in this study that may meet additional behavioural needs.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.applanim.2021.105324.

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