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Research article

Effects of enrichment and handling on the behavior of captive corn snakes (*Pantherophis guttatus*)

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Abstract

The vendors of pet snakes in exhibitions do not use a hide box in their enclosures, so there is a risk of inappropriate handling by customers. This study investigated the effects of enrichment (a hide box) and handling duration (5 and 10 minutes) on the behavior of captive corn snakes (n=8). During period 1 (weeks 1-4), snakes were kept in standard enclosures with sawdust and a water bowl. In period 2 (weeks 5-8), a hide box was added. An instantaneous time and scan sampling observation technique was used. Observations were conducted two hours per week during the 1st -3rd week of each period, recording the general behavior and body postures of the snakes every 5 minutes. In the 4th week of each period, the snakes were handled for 5 minutes on Saturday and 10 minutes on Sunday, with their behavior recorded for 5 minutes post-handling. Results showed that adding a hide box significantly decreased locomotion, digging and soaking (P<0.05). The snakes frequently used the hide box and were tightly coiled 25.61% of the observation time. However, the other body postures showed no significant differences between periods. There were no significant effects of periods and handling duration on behavioral duration, except that a 10-minute handling in period 2 made the snakes more inactive (P<0.05). After a 5-minute handling, the snakes were more active than after a 10-minute handling (P<0.05). In conclusion, adding a hide box to enclosures allowed snakes to exhibit their natural behavior. While enrichment and handling duration did not significantly affect overall behaviors, longer handling durations under enrichment conditions did influence activity levels. Further research is needed to find clearer explanations.

Keywords: Behavior, Captive, Corn snakes, Enrichment, Handling

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INTRODUCTION

Snakes are commonly kept in captivity in various situations and conditions, including as pets, zoological exhibits, and research animals (Warwick et al., 2019). From turtles to geckos or lizards to snakes, taking care of any reptile is a unique experience (Texas A&M Veterinary Medicine & Biomedical Sciences, 2015). In the past, snakes were raised only by a specific group of people. In 2019, the pet population of reptiles was 141.5 thousand in Thailand (Jueprasith, 2023). Nowadays, snakes have become more popular, especially beautiful snakes, such as ball pythons, corn snakes, milk snakes, king snakes, hognose snakes, and tree snakes (VPN, 2022). They are kept in relatively small boxes to large vivaria that are of greater length than the snake itself (Hoehfurtner et al., 2021a).

Racking systems are widely used and popular. These shelving systems feature trays which are made from clear acrylic or non-transparent plastic. A rack system consists of individual bins arranged as drawers. Some models have bins with separate lids, while others are open on top and flush with the upper shelf board. All bins include ventilation holes. Typically, racking systems lack built-in lighting, relying on ambient light for illumination. Heating elements, usually heating pads or cables, are installed at each drawer level and should be equipped with a thermostat to prevent overheating and undercooling (Hollandt et al., 2021). The advantages of a racking system have been described by Hollandt et al. (2021) as being quick and easy to clean. It takes only a little space and time to accommodate and raise many snakes and the risk of injury can be reduced by using minimal decoration.

If the stressor persists, the individual snakes may show abnormal behavior. These "displaced" behaviors may help the animal face the psychological aspect of the stressful event and reduce the full effect of its physiological response (Silvestre, 2014). In the wild, some snakes spend most of their time hiding in holes or under rocks and logs to protect themselves from other predators. Therefore, captive snakes have the same instinct to hide under things (Reptile Knowledge, 2023). The provision of additional resources and complexity to the enclosure was beneficial to the welfare of captive corn snakes (Hoehfurtner et al., 2021a). However, the accepted scientific and rational view is that snakes require enriched lives and that those kept without enrichment also experience good welfare, constitute mutually exclusive perspectives (Warwick et al., 2019).

However, it was found that the vendors of exotic pets at exhibitions, which may be an event of either one day or a few days where people can see, touch and buy exotic reptiles did not provide the hide boxes in the snake enclosures (D'Cruze et al., 2020; Yee, 2023), which would prevent the snakes from being clearly seen by the customers. One potential cause of stress in captive reptiles is interaction with humans; in particular, the effect of handling (Stockley et al., 2020). Inappropriate handling by customers can occur.

An animal that is regularly removed from its cage for routine procedures is likely to experience less stress than when one is only handled in an uncommon or one-off event (Kreger and Mench, 1993). The recommendation from the RSPCA (2019) for safe handling of corn snakes should be around 10-15 min, as any longer period will cause their core temperature to drop too low. Kreger and Mench (1993) claimed that the brief periods of handling in captivity thus do not appear to cause chronic stress in skinks, although restraint in a container appeared to result in short-term stress in pythons. Schuett et al. (2004) also found that there was no significant difference in the mean concentration of corticosterone levels between a control group (5 min) and a post handling group (15 min). However, there is little data available on whether the handling duration by customers affects the behavior of captive reptiles. It is necessary to improve the welfare of captive snakes in Thailand. Therefore, this study investigated the effects of enrichment (a hide box) and handling duration on the behavior of captive corn snakes.



MATERIALS AND METHODS

Animals and enclosure design

Eight healthy captive-bred blizzard corn snakes (*Pantherophis guttatus*) were born in 2022 (2 males, 6 females) from an exotic animal shop (Kratoey Exotic). They were fed a frozen mouse once a week (Monday) at 16:00 and provided water *ad libitum* (a water bowl, diameter 7 cm, Figure 1). They were housed individually in translucent plastic enclosures with ventilation holes, lids (L×W×H=50×36×16 cm) and sawdust (height 2-3 cm) as bedding materials during period 1 (weeks 1-4). The room temperature was not controlled while the ambient light provided the only illumination (Hollandt et al., 2021). A hide box (a paper bowl, diameter 9.5 cm, height 4.7 cm See Figure 2) was an enrichment item which was added during period 2 (weeks 5-8). A digital thermo-hygrometer was used. All procedures were approved by the Institutional Animal Care and Use Committee of Nakhon Ratchasima Rajabhat University (Approval number AE-004-2566). The experiments were conducted during July to October 2023.



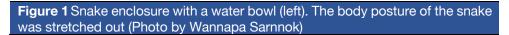




Figure 2 Snake was inside of the hide box (Photo by Wannapa Sarnnok)

Behavioral observations

The experiment was designed as a crossover study of two periods, each period lasting four weeks. During period 1 (weeks 1-4), a standard enclosure (only sawdust and water bowl) was used. In period 2 (weeks 5-8), a hide box was added



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to the standard enclosure. The observations were conducted twice at 10:00-11:00 and 14:00-15:00 on each Saturday. An instantaneous time and scan sampling observation technique was used (Martin and Bateson, 1986). The frequency of general snake behavior and body postures were recorded for two hours by scan sampling every 5 minutes for a total of 24 times from 1st -3rd week per period by using a smartphone (iPhone). Snake behavior such as locomotion, stationary, investigation, digging, bathing, drinking, resting, and hiding (modified from Spain, 2020, Table 1); the body postures were stretched out, loosely coiled, partially coiled or tightly coiled (Figure 3, Table 2) were recorded (modified from Hoehfurtner et al., 2021a; Hoehfurtner et al., 2021b).

Table 1	I Ethogram	of captive	snake	behavior
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Behavior	Description		
Active			
Locomotion	When $> 50\%$ of the body moves in any direction to a new position.		
Investigation	The head and neck area move around. To explore the surrounding area (with or without tongue flick)		
Digging	The head sinks under the bedding material.		
Drinking	drinking water		
Inactive			
Stationary	The head and body do not move, but the snake does not rest (e.g. appears to be aware of its surroundings and/or pauses while moving).		
Resting	The snake does not move around cage without tongue-flicking (Chemosensory investigation in which the tongue moves swiftly in and out of the mouth). Only short movements (such as adjusting position without moving from the current position) are still considered to be in the resting phase. The snake seems oblivious to its surroundings even when soaking in water.		
Hiding	>50% of the body is hidden from view by objects in the box (e.g. in the hide box).		
Not visible	Behavior being displayed by animal is not visible or is unclear (e.g. under sawdust)		
Other	Other behavior not described		
(Modified from S	pain, 2020)		

Table 2 Body posture of the captive snakes

Body posture	Description
Stretched out	Snake is in a straight or nearly straight-line posture, with a mostly continuous portion of the snake being straight, (i.e., no lateral bending) with no self-contact.
Loosely coiled	Little contact with self, but with very elongated coils and/or lateral bending with some continuous straight portions. Includes no contact with self if snake was bent round multiple corners.
Partially coiled	Few gaps, but some gaps between coils. Mostly in contact with self.
Tightly coiled	No gaps between coils (this was assumed if the snake was in a hide). Full contact with self.
Invisible	Not visible (under the sawdust)

(Modified from Hoehfurtner et al., 2021a; Hoehfurtner et al., 2021b)





Figure 3 Body posture. Loosely coiled (a), Partially curled (b), Tightly coiled (c) (Photo by Nantakan Dongutit)

Human handling test

On Saturdays and Sundays during the 4th week of each period, the snakes were tested for human handling (modified from Stockley et al., 2020). One handler (experienced in handling snakes) randomly handled all snakes. Before each trial, the handler thoroughly washed his hands with unscented soap. The container was opened slowly with minimal noise. If the snake was in the hide box before the test, a light tap was made to alert it without startling. Each snake was gently removed from the cage by placing one hand underneath to support its body while the other hand lifted it out. The snake was allowed to remain on the arm or hand of the handler while the other hand gently held it. The handler held the snake for 5 minutes at 12:00 and 14:00 on Saturdays, and for 10 minutes at 12:00 and 14:00 on Sundays. After handling, each snake was returned to its enclosure, and once the snake was fully inside the enclosure, another experimenter recorded a video for 5 minutes (modified from Spain, 2020; Hoehfurtner et al., 2021a; Hoehfurtner et al., 2021b). The behavioral duration (seconds) such as active (movement, exploration, digging, drinking) and inactive (stillness, soaking, resting, hiding) were recorded by using a smartphone (iPhone).

Data analysis

The SPSS software (version 23.0; SPSS nc.; Chicago, IL, USA) was used for the statistical analysis. The general behavior and body posture data were calculated as a proportion of total observations. The durations of behavior during handling test were converted to proportions of observed time. The independent T-Test was used when comparing the percentages of general behavior, and body postures of the snakes between period 1 (standard enclosure) and period 2 (enriched enclosure). The effects of the periods and the handling duration (5 min, 10 min) on the behavior of the snakes were analyzed by two-way ANOVA. The periods and duration of handling were fixed effects. When there was an interaction between the periods and the duration of handling, all the means were compared. When there were no main effects or interaction effects, the impact of the main effects in different periods and the duration of handling were transformed prior to analysis. Means were compared using Duncan's multiple-range test and the significance was determined at P < 0.05.

RESULTS

Temperature and humidity

The average temperature during observation between 10:00 and 14:00 was 30.33 ± 0.48 °C and 31.60 ± 0.76 °C, respectively. The average humidity at 10:00 and 14:00 was $65.44\pm2.32\%$ and $59.11\pm4.18\%$, respectively.



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General behavior

The effects of enrichment (using the hide box) affected the general behavior of the captive corn snakes, such as locomotion, digging, and soaking (P<0.05). During observation the snakes were inactive most of the time. However, there were no significant differences in the total general behavior when classified as active or inactive, respectively, during periods 1 and 2 (Table 3). When a hide box was added in period 2, the percentage of locomotion (P=0.004, F=10.47, df=46), digging (P=0.001, F=34.94, df=46) and soaking (P<0.001, F=52.53, df=46) decreased significantly. However, the percentage of investigation, drinking, stationary, resting and being invisible were not significantly different in periods 1 and 2 (P=0.052, F=3.06, df=46). The hide box was used by snakes in period 2 during observation. Meanwhile, the snakes were also invisible (under the sawdust) in both periods.

Behavior	Period 1	Period 2	P-value
Active	15.19±2.98	8.51±1.54	0.052
Locomotion	8.77±2.00	2.43±0.64	0.004
Investigation	4.51±1.13	5.90±1.25	ns
Digging	1.30±0.30	0.17±0.12	0.001
Drinking	0.26±0.14	0.00±0.00	N/A
Inactive	84.81±2.98	91.49±1.54	0.052
Stationary	9.32±1.31	8.55±1.63	ns
Soaking	17.45±4.19	0.35±0.20	<0.001
Resting	22.66±5.35	32.73±5.49	ns
Hiding (use hide box)	NA	22.40±4.30	N/A
Invisible	37.67±5.36	29.25±6.40	ns

Table 3 Effects of periods on percentages frequency of general behavior in captive corn snakes

Values are presented as Mean ± SE. Period 1=no enrichment, period 2=with enrichment.

ns means p-value >0.05 when compared with the data in the same row, NA means not available, N/A means not analyzed.

Body posture

It was found that there were four types of body postures (Figure 3) in the captive corn snakes, such as stretching, loosely coiled, partially coiled or tightly coiled (Table 4). When the hide box was available, 25.61% of the snakes were tightly coiled inside the hide box (P<0.001, F=55.35, df=46). However, the percentage of tightly coiled body posture (outside the hide box) in period 1 was significantly more than that in period 2 (P=0.037, F=0.64, df=46). However, the percentages of stretching, loosely coiled, partially coiled, or tightly coiled and being invisible were not statistically different in periods 1 and 2.

Human handling test

Table 5 shows that there were no significant effects of the periods (with a hide box, without a hide box) and handling duration (5 min, 10 min) on the behavior of the snakes, respectively. There was no interaction between periods and handling duration either (P>0.05). However, it was found that there was only the main effect of 10 min handling that had a significant impact on behavioral duration in period 2 (P=0.001, F<0.001, df=14). When a hide box was used in period 2, it was found that after 5 min handling the snakes were more active than those snakes which were handled for 10 min (P=0.031, F=0.60, df=14). In other words, after 10 min handling, the snakes were more inactive (P=0.001, F<0.001, df=14).



Behavior	Period 1	Period 2	P-value
Stretching	5.46 ± 1.56	3.04 ± 1.07	ns
Loosely coiled	10.85 ± 1.93	7.29 ± 2.65	ns
Partially coiled	7.73 ± 1.78	11.46 ± 2.47	ns
Tightly coiled (inside the hide box)	0.00 ± 0.00	25.61 ± 4.80	N/A
Tightly coiled (outside the hide box)	38.28 ± 5.24	23.35 ± 4.58	0.037
Total tightly coiled	38.28 ± 5.24	48.96±5.09	ns
Invisible	37.67 ± 5.36	29.25 ± 6.40	ns

Table 4 Effects of periods on percentages frequency of body posture in captive corn snakes

Values are Mean ± SE. Period 1=no enrichment, period 2=with enrichment,

ns means P-value >0.05 when compared with the data in the same row, N/A means not analyzed

Period (P)	Handling (H)	Active	Inactive	P-value
1	5 min	288.90	311.10	ns#
	10 min	274.03	325.98	ns#
	P-value	ns*	ns*	
2	5 min	328.81	271.19	ns
	10 min	172.52	427.48	0.001
	P-value	0.031	0.031	
P-value	Р	ns	ns	
	Н	ns	ns	
	P×H	ns	ns	
SE	Р	32.60	32.60	
	Н	32.60	32.60	
	P×H	46.10	46.10	

Table 5 Effects of periods and duration of handling on total behavior (seconds)

Values are present as Mean \pm SE. Period 1=no hide box, period 2=with a hide box, ns means P-value >0.05.

ns# means P-value >0.05 when compared with the data in the same row.

ns* means P-value >0.05 when compared with the data in the same column.

DISCUSSION

Each snake species has its own specific temperature needs. A corn snake prefers a slightly warmer environment from 29.4 to 31.1°C during the day (Sparks, 2024). According to the RSPCA (2019), it is recommended that the care of corn snakes for vivarium temperatures should range from 28 to 30 °C, with a cool temperature from 20 to 24 °C, and humidity at 40-50%. Although temperature and humidity were not controlled during our study when the average indoor temperature at 10:00 and 14:00 was around 30 °C, they were similar to the recommendations (RSPCA, 2019; Sparks, 2024). It is crucial to research and understand the specific temperature needs of the snake species to provide them with the best care possible in the future (Sparks, 2024). However, further controlled studies would be required to conclusively rule out any potential temperature effects.



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General behavior

Almli and Burghardt (2006) claimed that housing conditions can affect the behavior of captive snakes and produce improvements in behavior like those seen in mammalian enrichment studies. In the study of Hoehfurtner et al. (2021a, 2021b), all adult corn snakes were housed individually in glass-fronted enclosures $83 \times 35 \times 39$ cm (internal dimensions), or a 'large' enclosure $179 \times 58 \times 58$ cm (internal dimensions). The enclosure in the study of Spain et al. (2020) was a stainless steel enclosures (L×W×H=56×38×33 cm) with opaque sides and glass lids for Madagascar giant hognose snakes. Our enclosure was (L×W×H=50×36×16 cm) made of translucent plastic. Although we did not measure the length of the snakes, the snakes could perform a stretching posture in the enclosure (see Figure 1).

It was found that the western hognose snakes (*Heterodon nasicus*) on the enriched side were regularly found burrowing in the substrate, possibly because this behavior enhances crypsis, helps to modulate humidity levels, and provides a slight vertical thermal gradient (Nagabaskaran et al., 2022). Our results for corn snakes which were hiding or invisible (about 50% of the observation time) were higher than in the standard enclosure (about 37% of the observation time). Hoehfurtner et al. (2021a) who found that the corn snakes (about 60% of the snakes) hiding in the enriched enclosure were higher than those in the standard enclosure (about 12.5% of the snakes). Hoehfurtner et al. (2021a) also found that about 70% of the corn snakes stayed under substrate in the standard enclosure. Only less than 10% were under substrate in the enriched enclosure.

It was found that the snakes spent 17.45% of the observation time soaking in the standard enclosure, but this behavior was rarely observed in the enriched enclosure. One of the most common reasons a snake soaks in its water dish is to prepare for shedding its skin (Smith, 2024). The researchers observed shedding behavior during period 1, but it did not occur during the data collection period.

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. According to our results, the percentage of total activity of the snakes decreased (8.51%) when a hide box was added. Even when there was no enrichment used, the snakes were able to adapt themselves to the environment that best suited their behavioral needs, such as by staying under the substrate to hide. If a hide box was provided, then the snakes chose to use it. However, in a novel environment test, Hoehfurtner et al. (2021b) found that snakes in a standard environment spent significantly more time in full locomotion compared to those in the enriched environment. Whether each of our research periods was too short for the hiding box to be perceived as a novel stimulus for the snakes remains unclear, and further studies are needed.

Body posture

Snakes are universally identifiable by their elongated bodies that are often observed adopting coiled, straight lined or near straight-lined postures, both during locomotion and at rest (Warwick, 1995, cited in Warwick et al., 2019). However, the stretching of corn snakes in our study was rare (about 3 or 5 %) during observation time. Even though the racking system is popular in raising snakes, there is some evidence that keeping snakes in confined spaces (i.e., areas smaller than the length of the snake) may impede snake behavior such as stretching (Warwick et al., 2019; Hollandt et al., 2021; Warwick et al., 2021).

Snakes use coiled posture for a variety of purposes related to thermoregulation, defense, hunting and resting. When snakes coil up, it allows them to conserve body heat and maintain their preferred body temperature (Quora, 2024). The best-known species to use coiling as a defensive behavior is the ball python, but other snakes have also been observed to exhibit this behavior. The snake curls up and may move rapidly in this position or may remain still (Mayer and Bays, 2006).



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Coiling up is also a natural resting position for snakes, allowing them to conserve energy when not actively hunting or moving around (Quora, 2024). In the wild, snakes spend time resting hidden in a curled position to protect themselves from predators and to retain heat (Fitch, 1949, cited in Hoehfurtner et al., 2021a). Coiling up could be a relaxed basking pose in ball pythons (I-M-Sneks, 2019). Whilst resting, snakes in the large enclosure spent significantly more time loosely coiled, however, while snakes in the small enclosure spent significantly more time partially or tightly coiled (Hoehfurtner et al., 2021a). Hoehfurtner et al. (2021b) found that snakes in longer enclosures would coil more, though the significance was unclear.

Kish (2018) claimed that a loose body posture is an example of relaxed body language. However, the tightly wrapping of an object that is not prey or tightly squeezing into a hide would be one of the moderate or acute stress signals. Although Mariah (2024) found that corn snakes coil up when they feel unsure, our study found that the tightly coiled posture was not affected by providing a hiding box. There are several online resources for information about snake body language which is shared by experienced reptile keepers and breeders (Kish, 2018; I-M-Sneks, 2019; Melanie, 2023; Mariah, 2024; Quora, 2024), there is still rare scientific evidence on captive snake behavior. Our study did not evaluate the body posture in relation to the mental status, but it would be a very useful for future study.

The snakes in our study which were in the standard enclosure adopted significantly more tightly coiled positions even while resting outside a hide. RSPCA (2019) recommend that hides must be large enough for the snake to fit inside, but not so large that their body does not touch the sides when coiled up. It would be interesting to find a suitable hiding size or have a preference test of hiding items for captive snakes in a racking system in a future study.

Handling test

Stockley et al. (2020) found no significant differences in movement following the handling of bearded dragons during both 5-minute and 15-minute periods. Moreover, Pike et al. (2005) indicated that for gopher tortoises the short-term effects of handling and marking were limited. However, our study showed that after handling, the snakes were returned to their normal enclosure. In practice, if there is no hide box in the enclosure, the handling time does not affect snake activity. However, if a hide box was used and the snakes were handled for 10 min before being returned to the enclosure, they were more inactive. This might be because increased inactivity is likely to be associated with negative affective states in response to a perceived threat stimulus such as freezing, tonic immobility or hiding responses (Fureix and Meagher, 2015). However, it is unclear in the research on snakes whether increased activity or decreased activity is likely to be associated with positive affective states. Further study of human-snake relationships is needed.

Our study was a short-term investigation aimed to examine the effects of adding a hideas the observed trend of decreasing active behavior has not been studied for short-term impacts. Therefore, further research is necessary to investigate whether short-term or long-term enrichment impacts the behavior of snakes. This study was only able to use short-term behavioral data with a small sample size compared to other studies. For instance, Hoehfurtner et al. (2021a) used 15 corn snakes as the total number of experimental animals. However, our study required snakes of the same age and morph, and the exotic pet store where the samples were obtained could only provide a total of 8 snakes. For future studies, it is recommended that different snake species which primarily inhabit environments outside of terrestrial habitats (e.g. in trees, water) should be explored. Healthy but stressed reptiles, such as those that react poorly to handling may be important to assess their emotional state (Warwick et al., 2013). It is not clear about

the relationship between general behavior, body posture and body temperature of captive corn snakes in an uncontrolled indoor environment (Gaywood, 1990).

In conclusion, providing enrichment (a hide box) for captive corn snakes allowed the snakes to respond and behave according to their natural needs. The duration of handling can affect the activity of corn snakes. In the enriched enclosure, handling up to 10 minutes impacted their activity, but handling for only 5 minutes did not. The researchers recommend that exotic pet shops specializing in snake husbandry who use the rack system or traditional storage system, and hides should be added to provide a positive experience for the snakes thereby improving their welfare. However, as behavioral studies of captive snakes are rare, further research is needed to understand whether the duration of handling will affect snake behavior and welfare.

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AUTHOR CONTRIBUTIONS

Xin Huo: Conceptualization, Methodology, Project administration, Investigation, Data analysis, Writing-original draft, Writing-Review & Editing, Supervision

Nantakan Dongutit: Project administration, Investigation, data collection Wannapa Sarnnok: Project administration, Investigation, data collection Panich Kumroptanasan: Data analysis advise Phakphume Saowaphak: Review & Editing

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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