Effects of environmental enrichment on behaviours and faecal glucocorticoid levels in captive sun bear (*Helarctus malayanus*)

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Abstract. Stereotypic behaviour is correlated to stress in wild animals in captivity. When wild animals are exposed to stress repeatedly, they may experience a chronic elevation of glucocorticoids (G.C.s), especially for captive sun bears. This study aimed to investigate the stress in captive sun bears by analysing the cortisol level using Faecal Glucocorticoid Metabolite (FGM) analysis before and after introducing environmental enrichments. 57 samples of faecal captive sun bears were collected from four selected study sites around Malaysia. The behavioural data were collected with an instantaneous sampling method. While for FGM analysis, glucocorticoids were analysed using enzyme immunoassays. As predicted, the stereotypic behaviour increased in pre and post enrichment and decreased during the enrichment period. For FGM analysis, the cortisol level was high in pre-enrichment and low in the enrichment period. In conclusion, the outcome of this study indicated that environmental enrichment might help reduce chronically elevated cortisol concentrations that are generally associated with distress in mammals.

Keywords: stereotypic behaviour, faecal glucocorticoid metabolite, stress, captive sun bear

1. Introduction
Keeping animals in captivity, such as zoos, is a widely accepted approach to conserving threatened species like sun bear. These animals will receive veterinary care and be free from starvation and predation [1]. This was proven by various studies on different animal species, which indicated that survival and fecundity rates are generally higher in captive than in wild populations [2]. However, captive animals live in an environment different from that in the wild [3]. For many captive wild species, many difficulties exist in captivity. One of the main concerns for animals kept in captivity is expressing abnormal behaviour such as stereotypical behaviour. It can be one of the indicators of poor animal welfare and often a sign of decreased welfare in an animal [4] because their choice to express
natural behaviour has become limited [1]. This issue has received much attention over several decades [1,5,6]. Restrictions in performing their normal species-specific behaviours may lead to stress and harm their welfare in captivity. This will lead to changes in their behavioural patterns, such as developing abnormal behaviour like stereotypic behaviours [7].

If an animal can show its normal, species-specific behaviour similar to those in the wild, it shows that the animal needs in the captive environment are being met, and it could have good health [3]. However, most bear species, including sun bear that were kept in captivity, exhibited abnormal behaviour. This is due to many factors, including the size of the enclosure [5,8], barren or unexciting environment [9,10], and no opportunity for exploration and foraging [3,10]. The abnormality in their behaviour is a potential indicator of pain, suffering, and injury in captive animals [11].

Even though stereotypies are categorised as abnormal behaviour, the behaviour is considered a typical behaviour for animals in captivity. Unfortunately, the mechanisms that lead to this situation are still poorly understood [12]. Mason [13] defined stereotypical behaviour as repetitive unvarying, and functionless behaviour. Stereotypic behaviour has been documented in many animal species kept in captivity, including American black bears (*Ursus americanus*) [14], giant pandas (*Ailuropoda melanoleuca*) [15], and polar bears [6]. This behaviour can be reduced by increasing the biological relevance of the captive environments through environmental enrichment, which can stimulate species-specific behaviour [11]. For sun bears, stereotypic behaviour includes pacing, circling, or walking in a figure of eight, head tossing, and swaying [5, 16].

Stereotypic behaviour is very much related to stress in captive animals, especially bears [17]. Besides the inability to perform some essential species-specific behaviour, stress can also contribute to the development of stereotypies in animals [18]. Stress, specifically chronic stress, is the leading cause of poor welfare in bears which has been widely accepted as one of the main factors that lead to stereotypic behaviours [19,20]. These stereotypic behaviours could be evaluated using direct observation methods and faecal corticoid analysis [4]. This non-invasive measurement of corticoid metabolites in faeces has been used repeatedly to determine individual differences in the physiological response to captive environments [6].

According to Sapolsky [21], the stress in animals like mammals is a complex condition that is controlled by the sympathetic nervous system and a steroid hormone (cortisol and corticosterone) called glucocorticoids (GC). GC measurement is a robust integrator of environmental stressors and animal's physical condition [22]. GC levels can be measured directly from blood (such as plasma and serum), saliva, or indirectly via assessment of their metabolite. Besides that, GC also can be excreted via urine and faecal [22]. The measurement of glucocorticoid metabolites excreted in the faeces is a non-invasive approach, allows for collection and analysis without any direct contact with the animals, thus reducing unnecessary stress to the animals. This is particularly important in large mammals and endangered species where repeated capture and handling is almost impossible or with high energetic costs. Thus, faecal glucocorticoid metabolite (FGM) analysis provides a non-invasive method for studying the physiological response of wildlife to a variety of stressors and is a ground-breaking monitoring technique in wildlife management and conservation [23], besides understanding the impacts of natural and anthropogenic disturbances on wild animals [24].

In recent years, FGM analysis has become a valuable tool in wildlife field studies and conservation, e.g. [25,23,26-27] analysis has been used widely to determine stress in many species of animals, including small and large animals [28-31,24,6,23]. The study aims to measure the level of faecal glucocorticoid metabolites in captive sun bears via cortisol hormone regarding their stress level before and after enrichment programs in the different age-sex groups.

2. Material and Methodology

2.1 Study site

The faecal samples of sun bears were collected from captive and semi-captive environments in Malaysia from December 2017 to May 2019. Three study sites have been selected for captive sun bears to conduct the study, namely Zoo Negara, Kuala Lumpur; Zoo Taiping, Perak, and Lok Kawi
Wildlife Park, Sabah. While for semi-captive sun bears, the study has been done in Bornean Sun bear Conservation Centre, Sabah.

2.2 Subjects
Based on the record from both captive and semi-captive exhibits, the bear's ages were approximately between 4 to 25 years that comprised 17 adult males, 15 adult females, 13 juvenile males, and 12 juvenile females. Most of the animals were donated by the public, and some were rescued from the villages that kept the bears as a pet. The bears were housed in separate cages when the sites were closed and were released into the enclosure each morning. Similarly, at all study sites, the management routine included delivery of the primary daily feeding in the cages at the end of the day. So, the bears entered voluntarily when their cage doors were opened in the late afternoon.

2.3 Behavioural sampling methods
Observations were recorded for all bears at all study sites for 30 consecutive days at each study site. An ethogram composed from a literature review of sun bear behaviour [32-33] and modified with preliminary data collected during the pilot study using Ad Libitum sampling was used to record behavioural observations (Table 2.1). Observation refers to one animal's behavioural activity in a scan. The sampling protocol consisted of Instantaneous scan sampling conducted at a 15-min interval to record all individual activity data in two 3-hr segments each day, respectively, at 0900 – 1200 and 1300 – 1600 hr segment times [34-35]. No observations were made between 1200 – 1300 hr as the increased human-animal encounters occurring during this hour, which probably affected the bears' behaviour.

| Table 2.1 | Activities for the categories of active, passive, and abnormal behaviours in the sun bear captivity study |
| --- | --- |
| **Active Behaviour** | |
| Activities | Description |
| Exploring | Searching for things inside the enclosure such as stones, dead branches, walls, and artificial objects. |
| Locomotion | Moving from one location to another inside the enclosure bipedally or quadrupedally in the low-speed movement without sniffing the ground horizontally. |
| Climbing | Actively moving in a vertical motion, either ascending or descending movements typically associated with arboreal movements. |
| Digging | Breaking up soil or making a hole on the ground with its paws and claw. |
| Social | Interacting or engaging with another bear, including touching, chasing, playing, allo-grooming, and non-aggressive wrestling/fighting |
| Solitary | Playing all by itself with inanimate objects inside its enclosure. |
| Foraging | Actively searching for food items; feeding on edible material, drinking water. |
| Auto-grooming | Physical hygiene including cleaning body parts with its mouth and paws, scratches, licking, rubbing, examining its body parts. |
| **Passive Behaviour** | |
| Activities | Description |
| Resting | Non-locomotion behaviour (sits or lies, stationary with eyes open) |
| Sleeping | Sits or lies, stationary with eyes close. |
| Alert | Bear lies, sit, and stand with head up and eyes open and responds to any stimuli. |
Elimination | Urinating or defecating.
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| Activities | Description |
|---|---|
| Pacing | Bear moving in the same path repetitively (left to right, right to the left), stepping forward and backward without turning the body. |
| Head tossing | Moving the head up and down. |
| Swaying | Rocking of the head from side to side continuously. |
| Head throwing | Throwing head back and over a shoulder during locomotion. |
| Allo-sucking$^{a,b}$ | Repetitive sucking of a part of a conspecific's body area. |
| Self-sucking | A distinct humming vocalization often accompanies repetitive sucking of a part of their own body. |
| Begging$^a$ | Sitting or standing up while looking and staring at humans (bear keepers/visitors) to communicate with them. |
| Circling$^c$ | Locomotion tracing in circular path. |
| Vocalizations | Making loud sounds towards another individual |
| Aggressive | Showing aggressiveness towards another individual and other things |

*$^a$ = only been performed by bears in Lok Kawi Wildlife Park  
$^b$ = only been performed by bears in National Zoo  
$^c$ = only been performed by bears in BSBCC

2.4 Environmental Enrichment
Following the baseline data collection, enrichment tools were installed in all enclosures at each study site. In this study, three types of enrichment (food, sensory, and occupational enrichments) were introduced to the bears.

Before the bears were sent out, the enrichment tools were placed in the exhibit area each morning. Each tool, i.e., buoy filled with dog food (occupational enrichment), gunny sack filled with cinnamon powder (sensory enrichment), honey mixed with fruits such as banana and honeydew (food enrichment) were scattered placed and hidden around the enclosure. Each of the enrichment tools was introduced on a rotational basis for ten consecutive days during the enrichment period. Every morning the enrichment tools were placed in the exhibit area before the bears were sent out. All enrichment materials were refilled daily. The data was collected by using the same method applied for baseline data collection.

Post-enrichment data was collected after removing all the enrichment tools from enclosures at all study sites. The data was collected for 180 hours at all study sites using the same methods for collecting baseline data.

2.5 Faecal sample collections
Faecal Glucocorticoid Metabolite (FGM) analysis was used as an indirect measure of serum cortisol since it is non-invasive [6]. Fresh faecal from each bear at each participating zoo and center was collected before and after introducing the environmental enrichment program. Fresh faecal samples from 57 individuals were collected early morning by bear keepers of each study site. The samples were mixed thoroughly to equalize the hormone distribution throughout the sample and immediately stored at -20°C upon returning from the field until extraction procedure using ELISA technique was carried out.
2.6 Extraction of Steroid from faeces (Vortexing extraction method)
To extract glucocorticoid in faecal samples, the vortexing extraction method was applied following [23]. About 0.6g of faeces sample was placed in a centrifuge tube containing 2 ml of 90% methanol. The mixture was vortexed for 30 minutes, then continued with centrifugation at 2500 rpm for 20 minutes. The supernatant was then diluted with Phosphate Buffered Saline (PBS) and stored at -20°C for further analysis [36]. Four samples were serially diluted to test for parallelism with the standard curve.

2.7 Enzyme-Linked Immunosorbent Assay (ELISA)
Before starting the test, all reagents and specimens were kept at room temperature 20 minutes in advance and were mixed well. The ELISA test was conducted in 96-well microplates using the Elabscience Cortisol ELISA Kit and following the manufacturer's instruction provided in the kit (catalog.no. E-EL-0030; Elabscience, Houston, Texas, United States). 50 µl of serum samples and standards were added to wells in duplicate, followed by 50 µl Biotinylated Detection Antibody solution into each well. One well was served as a control where it remained blank with no standards or enzyme conjugate was added into it. The microplate was sealed and incubated for 45 minutes at 37 °C. After incubation, 350 µl of wash buffer was added to each well, soaked for 1 minute and aspirated. The washing step was repeated three times. In each well, 100 ul of HRP Conjugate solution was added and incubated for 30 minutes at 37 °C. The mixture was then removed and washed five times with wash buffer. After wash, 90 µl of Substrate reagent was added to each well and incubated for 15 minutes at 37 °C. The reaction was stopped by adding 50 µl of Stop solution into each well. The optical density (OD value) of each well was read at 450 nm, using a microplate reader (Thermo Scientific™ Multiskan).

2.8 Calculation of Cortisol Concentration
The cortisol concentration index was calculated following Vimalraj and Jayathangaraj [36]. The calculated results were expressed as A/Ao X 100%, where Ao was the average absorbance of the 0 ng/g cortisol standard.

2.9 Validation
Validation of the FGM assay was determined through biological validation by monitoring the bears during a stressful event. This validation is essential to determine the cortisol response during the event. The captive bears at all study sites were left unfed for 16 hours, starting at 4 pm to 8 am the following day which caused the bear to become more stressed to stimulate the production of cortisol hormone. The faecal samples before and after this event were collected during pre and post-event to ensure that the bear released the cortisol hormone. The collection process was done by a bear keeper who could identify known individuals.

2.10 Statistical analysis
All statistical data were analysed using Statistical Package for Social Sciences (SPSS). A non-parametric test was selected since the data was not normally distributed, as showed through the Kolmogorov-Smirnov test after the data was further transformed using log transformation. Data on cortisol levels between pre-enrichment and during enrichment period over sex and age were analysed using the Mann-Whitney U test. Spearman Rank order correlation was performed to determine the correlation between stereotypic behaviour and FGM concentration. For all tests, a value of P<0.05 was considered significant.

3. Result
Differences in the proportion of abnormal behaviours were studied for captive and semi-captive bears before the enrichments were introduced to the bears. Stereotypic pacing occurred at a significantly higher level in the captive bears compared to semi-captive bears (U = 3.69, DF = 19, P < 0.05). Allo-
sucking and begging behaviour has only been performed by captive sun bears. While for circling behaviour, it only is performed by a bear in semi-captive (Figure 3.1).

![Abnormal Behaviour Graph]

**Figure 3.1:** Mean percentage of observations of abnormal behaviour for sun bear across captive and semi-captive exhibits (*P< 0.05, Mann-Whitney U)

The different concentrations of cortisol in ng/g in the faecal samples gained for pre, during, and post-enrichment from sun bears of all the four study sites were tabulated in Table 3.2 together with the absorbance values 450 nm.

For pre-enrichment, among the faecal samples collected from the captive exhibit, the mean values of cortisol concentration in the faecal samples of Zoo Negara were 175.7ng/g, the mean value was 184ng/g of cortisol concentration in samples obtained from Zoo Taiping, and the mean values were 178.6ng of cortisol concentration in samples obtained from Lok Kawi Wildlife Park. While in the semi-captive exhibit, BSBCC, the mean value of cortisol concentration in the faecal samples collected was 56.4.7ng/g. There is no significant difference (H = 2.250, P > 0.05) between captive centers (Zoo Negara, Zoo Taiping, and Lok Kawi Wildlife Park) for glucocorticoid concentration. Therefore, data for captive centers were pooled to compare the glucocorticoid concentration between captive and semi-captive exhibits. However, there is a significant difference between captive centers and semi-captive centers (U = 3.60, P < 0.05), with a higher concentration of cortisol seen in captive sun bears compared to semi-captive sun bears (Table 3.1).

| Centres                | Captive Exhibit | Semi-captive exhibit |
|-----------------------|-----------------|----------------------|
|                       | Zoo Negara (n=7) | Zoo Taiping (n=6) | Lok Kawi Wildlife Park (n=7) | BSBCC (n=37) |
| Mean values of cortisol Concentration (ng/g) | 175.7ng/g | 184ng/g | 178.6ng/g | 56.4ng/g |

During enrichment, the mean values of cortisol concentration in the faecal samples of Zoo Negara were 93.9ng/g, the mean value was 104.4ng/g of cortisol concentration in samples obtained from Zoo Taiping, and the mean value was 98.6ng/g of faeces in samples obtained from Lok Kawi Wildlife Park (Table 3.2). In BSBCC, there was no data gained for the enrichment period as no enrichment activities had been conducted in this study area.
Table 3.2 Cortisol Concentration at all study sites during enrichment in the captive exhibit

| Captive Exhibit | Sites (n)       | Mean values of Cortisol Concentration (ng/g) |
|-----------------|----------------|---------------------------------------------|
| Zoo Negara (n=7)| 93.9ng /g      |                                             |
| Zoo Taiping (n=5)| 104.4ng/g    |                                             |
| Lok Kawi Wildlife Park (n=7) | 98.6ng /g |                                             |

For post-enrichment, the mean values for the cortisol concentration of the faecal sample at Zoo Negara were 142.7ng/g, 148ng/g mean values for cortisol concentration samples from Zoo Taiping 144.6ng/g for faecal samples from Lok Kawi Wildlife Park (Table 3.3).

Table 3.3 Cortisol Concentration at all study sites during the post-enrichment study of the captive exhibit

| Captive Exhibit | Sites (n)       | Mean values of Cortisol Concentration (ng/g) |
|-----------------|----------------|---------------------------------------------|
| Zoo Negara (n=7)| 142.7 /g       |                                             |
| Zoo Taiping (n=5)| 148.4ng/g    |                                             |
| Lok Kawi Wildlife Park (n=7) | 144.6ng /g |                                             |

There was a significant variation between the cortisol levels in faecal samples from pre-enrichment and during enrichment at all captive exhibits for both sexes and age-structure; adult and juvenile (U= 0.25, P < 0.05) (Figure 3.2 and 3.3). However, there was no significant difference between pre and post-enrichment periods for the cortisol concentration level (U= 3.14, P > 0.05).

The cortisol concentration level significantly decreased after the exposure to environmental enrichment and increased again in the post-enrichment period in both male and female sun bears in captive centres (U =2.24; P<0.05) (Figure 3.2). Male showed a higher cortisol concentration level than females at all three periods of the enrichment program (U =3.634; P<0.05).

Figure 3.2 Mean cortisol concentration level in pre-enrichment, enrichment, and post enrichment of male and female sun bears in the captive exhibit (*P < 0.05, Mann-Whitney U).

While for the age group, the cortisol concentration level was also decreasing significantly after the exposure to an environmental enrichment programme in both adult and juvenile sun bears in the captive exhibit (U = 3.14; P < 0.05). However, the cortisol concentration level increased again in the
post-enrichment period (Figure 3.3). In addition, adults showed higher cortisol concentration levels than juveniles at all three periods of the environmental enrichment program ($U = 1.497; P < 0.05$).

![Figure 3.3](Image)

**Figure 3.3** Mean cortisol concentration level in pre-enrichment, enrichment, and post enrichment of adult and juvenile sun bear in captive exhibit (*$P < 0.05$, Mann-Whitney U).

The occurrence of stereotypic pacing and the level of faecal glucocorticoid concentration for captive sun bear in three different phases (pre-enrichment, enrichment, and post-enrichment) were correlated (Figure 3.4; $r = 1.41$, $P < 0.05$).

![Figure 3.4](Image)

**Figure 3.4** Relationship between cortisol concentration and stereotypic pacing frequency in captive sun bears (N=20). ($P < 0.05$, Spearman Rank Order Correlation).

4. **Discussion**

4.1 Sun bear and stereotypic behaviour

Sun bears in captivity spent most of their time on abnormal behaviour compared to semi-captive bears. They spent about 67% of their time pacing all day long. This may be due to a lack of enrichment tools related to the size of the enclosure. A previous study on captive sun bears in Peninsula Malaysia
concurred with this finding where the bears in captivity showed the most frequent resting and stereotypic pacing activities [5]. This finding was also supported by a study conducted on captive giant pandas in National Zoo, Malaysia, whereby the pandas spent most of their time on inactive behaviour [37]. Other species of captive bears from other regions, such as American black bear [14], spectacled bear (*Tremarctos ornatus*) [38], Asiatic black bear (*Ursus thibetanus*) [12], and sloth bears [32] also showed similar results with this finding. This showed that abnormal behaviours were common among captive bears. It could be because of many reasons, including the smaller size of the enclosures that will limit the opportunity to exhibit their natural behaviour [14,5]. Cless *et al.* [39] stated that among the reasons for stereotypic behaviours is the enclosure limitations, inappropriate design, and size of the enclosure. The small size of the enclosure will restrict bears to perform their natural foraging and hunting habits. When the enclosure is larger and more natural, little abnormal behaviour is observed in bears of the zoological institutions [40].

Less abnormal behaviour was recorded in BSBCC than other enclosures due to smaller enclosures sizes in captive study sites than the open enclosure in BSBCC. A study on the size and design of enclosure of captive sun bears in two zoos in Peninsular Malaysia showed that indoor bears engaged in stereotypic behaviours were more frequent than bears housed outdoors [5]. Vickery and Mason [12] reported that Asiatic black bears and sun bears performed a high frequency of stereotypic pacing when the size of the indoor enclosure was smaller than the outdoor enclosure. Other mammals such as giant pandas (*Ailuropoda melanoleuca*) also performed less stereotypic behaviours when kept in the semi-natural enclosure than concrete enclosure [41]. The pacing was suggested to adapt to spatial limitations [42], especially for a carnivore species with a wide home range. For sun bears in the wild, the average home range size was 14.8 km² and ranged from 6.2 to 20.6 km² [43].

4.2 The correlation between FGM (Cortisol) level and stereotypic behaviour

As an assessment of animal welfare should integrate more than one indicator, a non-invasive adrenal activity approach to measure the faecal glucocorticoid metabolite (FGM) level via cortisol concentration in faeces has been conducted. This study showed the biological reliability of FGM assessment obtained through the ELISA kit and the applicability of the faecal sampling method in captive and semi-captive sun bears. The measurement of glucocorticoid metabolites in faeces has become an important new information for other researchers interested in using FGM analysis to monitor stress physiology in captive animals, especially sun bears, using various parameters.

As predicted, there was a positive correlation between FGM level and stereotypic behaviour, particularly on pacing behaviour for captive sun bears. This concurred with previous studies on mammals kept in captivity, such as housed polar bears by Shepherdson *et al.* [6] on clouded leopard by Wielebnowski *et al.* [48] and on captive panda in Beijing Zoo by Liu *et al.* [41]. All these studies showed that FGM was associated with stereotypic pacing in the animals. This underlines the significance of stereotypic pacing as an indicator of animal well-being, and therefore it is vital to eliminate it from the repertoire of captive sun bear behaviour. Furthermore, the level of circulating cortisol or corticosterone hormones is associated with stress, especially in mammals such as primates, bears and canines [49-51,41,6]. This shows that the sun bears in captivity are in stress conditions, which might be associated with reduced well-being.

FGM levels were found to be higher for bears in exhibits with smaller areas [6]. This statement supports the finding of this study which also found both FGM level and stereotypic pacing behaviour were higher in captive sun bears, which have smaller exhibits areas than semi-captive sun bears. This is not surprising as a large mammal such as sun bears required an extensive home range in the wild.
Therefore, a proper exhibit design is also essential to reduce stress level, which is associated with FGM level in captive sun bears.

4.3 FGM level in pre-enrichment, enrichment, and post-enrichment period.

Besides the size and design of the exhibit, the other factors most strongly associated with reduced proportions of stereotypic behaviours in zoo animals are the provision of diverse enrichment [6, 52]. In the pre-enrichment period, there was significant variation between the standard curve and the cortisol level of faecal samples obtained from BSBCC. However, no significant difference was found between the standard curve and the faecal samples collected from Zoo Negara, Zoo Taiping, and Lok Kawi Wildlife Parks, which showed that level of cortisol during validation and pre-enrichment in the sun bears at these study sites was parallel. This result might be attributed to the fewer enrichment activities offered to the captive sun bears. During the pre-enrichment period, the bears in captive exhibits were only provided with a climbing structure such as an artificial tree, a small tree trunk and a culvert for the bears to rest and sleep. Some captive centres did provide a structural enrichment for bears; however, based on personal observation, this enrichment was not fully utilized by the bears, probably because of the size and shape of the structure that the bears did not prefer. Therefore, this situation may lead to increased FGM levels in all captive bears, which indicates stress. These present results seem to support the theory that analysis of FGM concentrations can increase baseline differences caused by chronic stress [53].

During the enrichment period, the FGM level decreased in all captive bears. In captive brown capuchins (Cebus apella), enrichment positively affects behavioural and physiological stress responses, enhancing psychological well-being in a single individual [50]. A study on snub-nosed monkeys also showed that the faecal cortisol level decreased significantly after introducing enrichment to the monkeys [54]. FGM level also showed a reduction in rabbits after the introduction of enrichment tools [55]. This showed that the welfare of the bears was improved after enrichment that led to the reduction of FGM in the bears. Shepherdson et al. [6] suggested that some environmental tools can effectively reduce the stereotypic pacing behaviour of captive polar bears. However, this finding contradicts the study on pandas in Beijing Zoo, which found that enrichment stimulus did not show significant effects on stereotypic behaviour or faecal cortisol level [41]. This is perhaps because the small sample size did not support their prediction that predicted enrichment could reduce stereotypic behaviour and faecal cortisol level.

However, in this present study, the FGM level increased again in the post-enrichment period, which showed a positive correlation between FGM and stress. All enrichment tools were removed from the enclosure at this period, making the bears perform more stereotypic behaviour, especially on stereotypic pacing, as can be seen in the pre-enrichment period.

In summary, FGM concentration was elevated in the pre-enrichment period but was reduced during the enrichment period. However, FGM returned to the pre-enrichment level again during the post-enrichment period. Overall, the outcome of this study indicated that environmental enrichment might help reduce chronically elevated cortisol concentrations that are generally associated with distress in mammals.

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