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Welfare impacts associated with using aversive geofencing devices on captive Asian elephants

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ABSTRACT

Animal-borne aversive geofencing devices (AGDs, or satellite-linked shock collars) are commercially available and used on livestock to restrict their movement within a virtual boundary. This technology has potential application as a human-wildlife conflict mitigation tool, where problem animals might be conditioned to avoid human-dominated habitats by associating an audio warning with a subsequent electric shock, which is delivered if the audio warning is ignored. Ensuring that high standards of animal welfare are maintained when implementing such tools is important for acquiring manager and community acceptance of such approaches. We conducted two pilot experiments with eight captive Asian elephants using mild electric shocks from a modified dog-training collar fitted around the neck, as part of an ongoing effort to develop AGDs suitable for mitigating human-elephant conflict. As part of these experiments, we assessed elephants' behavioural and physiological stress before, during and after the experiments. During the experiments elephants wore collars for up to nine consecutive days and received a small number of electric shocks on 1–3 consecutive days. Bootstrapped principal component analysis showed that daily activity budgets of individual elephants on experiment days were not different from the pre-experiment days. Generalised linear mixed-effects model (GLMM) showed that anxiety/stress behaviours increased on the first day of acclimatising to the collar and on testing days (i.e. days they received shocks) of the first experiment, but not during the second experiment relative to pre-experiment days. Analysis of faecal cortisol metabolite (FCM) concentrations using GLMM showed that FCM concentrations were higher in samples collected ~24 hrs and ~48 hrs after testing days compared to baseline levels as expected given the lag time for excretion of cortisol metabolites. These elevated anxiety/stress behaviours and FCM concentrations returned to baseline levels shortly after the experiment. Therefore, we conclude that AGDs did not produce lasting behavioural or physiological stress effects in elephants during this short-term study but recommend further studies with a larger sample of elephants to confirm the transferability of these findings.

1. Introduction

Aversive conditioning involves influencing animals to modify an unwanted behaviour by associating it with an unpleasant stimulus, and has been applied in numerous ways to manage human-wildlife conflict (Appleby et al., 2017; Snijders et al., 2021, 2019). Virtual fencing using animal-borne electronic training collars (i.e. shock collars) is one such tool that has been tested on wild animals such as coyotes *Canis latrans* (Andelt et al., 1999), grey wolves *Canis lupus* (Rossler et al., 2012), dingoes *Canis familiaris* (Appleby, 2015), island foxes *Urocyon littoralis* (Cooper et al., 2005) and black-tailed deer *Odocoileus hemionus* (Nolte et al., 2003) to constrain their movement within a restricted space.

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Shock collars have been widely used on domestic dogs and livestock species for several decades (Anderson, 2007). Modern satellite-linked shock collars or aversive geofencing devices (AGDs) have the ability of real-time tracking of animals using Global Positioning Systems (GPS) and automatically deliver warning sounds followed by an electric shock when an animal crosses virtual boundaries (Boyd et al., 2022; Campbell et al., 2020; Lomax et al., 2019). The advantage of AGDs is that as the animal learns to associate the warning sound with the electric shock, they can predict and avoid receiving the electric shock (Lee et al., 2018). In other words, animals can control the receipt of electric shocks, thereby reducing the anxiety and stress caused to themselves (Kearton et al., 2020; Lee and Campbell, 2021) and thus minimise the welfare impact of AGDs on their wellbeing. Cattle *Bos taurus* and sheep *Ovis aries* learn to avoid the electric shock from AGDs after just a few attempts to cross virtual fences (Lee et al., 2009; Marini et al., 2018), and these devices are now commercially available for use on livestock (Goliński et al., 2023). However, aversive conditioning with electric shock has been frequently debated, with some studies showing unacceptable welfare impacts on animals (China et al., 2020; Schilder and van der Borg, 2004; Ziv, 2017). Therefore, despite its effectiveness, concerns remain about the welfare impact of using such tools on animals.

Use of AGDs have been suggested as a potential tool to mitigate conflict between humans and Asian elephants *Elephas maximus* (Cabral de Mel et al., 2022; Fernando, 2011). Human-elephant conflict (HEC) is a widespread problem across the 13 countries that Asian elephants inhabit (Fernando and Pastorini, 2011), and resolving HEC has become very challenging since most conventional mitigation efforts lose their efficacy in the long-term (Shaffer et al., 2019). Therefore, it is important to investigate novel tools (such as AGDs) to condition problem causing wild elephants to avoid human habitats. But, exactly how AGDs might affect elephant wellbeing is unclear given their complex cognitive and social systems (Bates et al., 2008; Hart et al., 2008). Initial studies with AGDs should be conducted under controlled conditions, for example, with captive animals so that their wellbeing can be properly evaluated (Lee and Campbell, 2021). It can be expected that during early stages, as animals learn to relate the audio warning from the AGD with the electric shock that follows, they would show acute stress responses, but after learning to avoid the electric shock, stress levels to be no different from baseline levels (Lee et al., 2018; Lee and Campbell, 2021). Welfare impact of shock collars on dogs and livestock has been assessed by studying their behavioural and physiological stress responses, demonstrating negligible effects of using them on the welfare of study animals (Campbell et al., 2019, 2017; Kearton et al., 2020, 2019; Schalke et al., 2007; Steiss et al., 2007). Conducting such assessments during preliminary investigations of AGDs with captive elephants will likewise help understand how AGDs could affect the wellbeing of wild elephants if used as an HEC mitigation tool.

Impact on the behavioural welfare of elephants to AGDs may be assessed by studying the changes in activity budgets (Veasey, 2006). Additionally, stereotypic behaviours, which are abnormal repetitive, invariant behaviours induced by stress or frustration (Mason, 2006, 1991) could be used as a measure of elephant welfare in response to AGDs (de Mel et al., 2013; Glaeser et al., 2021; Rees, 2009). Furthermore, self-directed behaviours (SDBs) are a type of displacement behaviour commonly associated with anxiety and stress in primates (Daniel et al., 2008; Thatcher et al., 2021; Wallace et al., 2019). SDBs such as trunk related behaviours (touch self and trunk swing) and foot swing are reported among African elephants *Loxodonta africana* (Kahl and Armstrong, 2000; Mason and Veasey, 2010; Poole, 1999) and have been used as a measure of their anxiety and stress (Manning et al., 2022). Elephants may also develop distinct collar-related behaviours such as touching, grasping and shaking the collar used to fit the AGD around the elephant's neck (pers. obs.). These SDBs and collar related behaviours may also be related to anxiety and stress when conducting experiments with AGDs. Adrenal glucocorticoid hormones such as cortisol are often measured as an indicator of physiological stress, as they increase in circulation in response to stressful events (Moberg, 2000; Mormède et al., 2007). Cortisol in elephants can be measured using serum (Brown et al., 1995), hair (Pokharel et al., 2021), saliva (Dathe et al., 1992; Menargues et al., 2008), urine (Brown et al., 2010, 1995) and faeces (Laws et al., 2007; Watson et al., 2013). However, quantifying faecal cortisol metabolites (FCMs) is preferred for elephants since it is a non-invasive method that would not cause additional stress on the animal and is not substantially affected by the temporal variation in secretion as it measures an accumulation of hormones over a longer period (Bansiddhi et al., 2020; Touma and Palme, 2005). The physiological and biological validity of using faeces to measure adrenal glucocorticoid hormones of both African and Asian elephants are well established (Fanson et al., 2013; Kumar et al., 2014; Laws et al., 2007; Millspaugh et al., 2007; Stead et al., 2000; Wasser et al., 2000). These behavioural and physiological stress indicators may be assessed to determine the impact of using AGDs on an elephant's wellbeing.

We previously conducted a preliminary study with captive Asian elephants using a modified dog-training collar to assess the potential of AGDs to manage elephant movement (Cabral de Mel et al., 2023) during which, two experiments were conducted; (1) to determine the optimum strength of the electric shock required, and the ideal location on the neck of the elephant which would generate the desired aversive responses and (2) to determine the ability to condition elephants to avoid receiving an electric shock and prevent reaching a food reward with an audio warning. Elephants showed desirable aversive behaviours in response to mild electric shocks received from the collar and the potential for elephants to learn to avoid an electric shock by associating it with an audio warning. The results of the experiments were promising, demonstrating the potential for AGDs to be used as an HEC mitigation tool, but the welfare effects of these experiments were not reported (Cabral de Mel et al., 2023). Therefore, in this paper we analysed the behavioural and physiological stress responses shown by the captive elephants that participated in the experiments using modified dog-training collars. The objectives of this study were to determine the changes in activity budgets, anxiety/stress related behaviours and FCM concentrations on experiment days compared to pre-experiment days (baseline levels). It was expected that conducting experiments would not influence the normal activity budgets of elephants. Further, it was expected that there could be increases in anxiety/stress behaviours and FCM concentrations corresponding to some experiment days (e.g., testing days on which elephants experience electric shocks) but these would return to baseline levels during the post-test monitoring days indicating minimum welfare impacts on animals.

2. Methods

This study follows on from our previous study on two experiments conducted with captive Asian elephants using a modified dog-training collar (Cabral de Mel et al., 2023) conducted between June 2019 and May 2022. This study received approval from the University of Southern Queensland Animal Ethics Committee (19REA007) in Australia, and the Institute of Biology in Sri Lanka (ERC IOBSL 193 04 2019 and 252 08 2021). Permission was also granted by the Department of National Zoological Gardens, Sri Lanka (DZG/DEV/02/Research work/2019) to conduct this study. We quantified the welfare impacts of the experiments on the elephants in this study, which were conducted under the constant supervision of veterinarians, mahouts and researchers at all times. Mild pain or discomfort (i.e. aversion) was an expected part of this study. However, no animal had to be removed from the study at any point in time because undesirable reactions, and excessive pain or discomfort were not observed. We could not replace the use of live animals in this research given that we were exploring the effects of a novel tool on live animals, but we did reduce or limit the number of animals used in our pilot studies to those few needed to achieve our objectives. We further refined our methods by using a small number of captive elephants under constant supervision rather than using larger numbers of wild elephants where supervision would have been difficult or impossible.

2.1. Animals

This study was conducted with eight adult female Asian elephants (K1, M1, M2, M3, M4, S1, S2, S3 with ages 50, 40, 51, 31, 24, 24, 32 and 37 years respectively) held captive at Pinnawala Elephant Orphanage (PEO), Sri Lanka. All except S3 were born in the wild. M3 had been a resident of PEO for 16 years but had been in captivity for a much longer period before arriving at PEO. All other wild born elephants in the study were brought to PEO as orphans at an age between < 1–5 years and have been held at PEO for 24–46 years. All elephants, except M3, were part of a herd comprising of about 25 elephants, released daily into a ~3 ha open area to forage at 08:30 h. They were shepherded to and from a water body about 500 m away from the open area twice each day between 10:00 h and 12:00 h and between 14:00 h and 16:00 h. While in the open area they were supplemented with food (e.g. branches of jak fruit, coconut etc.) and water and were free to range within the entire area. These elephants were herded to their overnight sheds after returning from the water body in the evening, before being let free again the following morning. M3 was an individually managed working elephant that worked for about 1 hr each day delivering food to other elephants within PEO. It was taken to the water body twice a day between 09:45 h and 11:00 h and again between 14:00 h and 15:00 h, while it remained in the shed during the rest of the day. When taken to the water body M3 often laid recumbent and submerged in water, and while in the shed it was provided with food and water. These typical daily routines sometimes varied for all elephants. For example, if there were high water levels in the water body resulting from heavy rains and flooding elephants were not taken to the water body for their bath.

2.2. AGD experiments

A detailed description of our two experiments can be found in Cabral de Mel et al. (2023), along with results discussing the efficacy of AGDs at managing elephant movement. Here, we briefly summarise the experimental design (Table 1) and instead focus our discussion on the animal welfare effects of these experiments.

The two experiments were conducted using a modified dog-training collar, delivering an electric shock of 4 kV with no resistance at variable strengths (varying pulse frequencies). Experiment 1 involved testing different strengths of electric shocks, on two different locations on the neck of eight elephants to determine the ideal position and the optimum strength that would generate desired responses from elephants such as touching the neck/collar and stopping or changing the direction of movement. Experiment 2 was conducted several months later with five of the same elephants from Experiment 1. This involved conditioning elephants walking along a path towards a food reward, to avoid receiving an electric shock by responding appropriately to the prior audio warning by modifying its movement and not continuing towards the food reward. These two experiments were conducted between 08:30 h and 11:00 h, and each experimental session ranged between 30 and 45 min. The two experiments involved wearing a collar around the neck for 3–9 consecutive days and receiving one or more electric shocks on 1 day during Experiment 1 and 3 consecutive days during Experiment 2. Collars were removed each evening, on days elephants were fitted with them when the elephants returned to the sheds for the night and re-fitted in the following morning.

2.3. Monitoring welfare impacts of the AGD experiments

2.3.1. Assessment of behavioural welfare of elephants

An ethogram (Table 2) was constructed based on preliminary observations conducted at PEO and elephant behaviour categories described in published sources (Asher et al., 2015; de Mel et al., 2013; Glaeser et al., 2021; Manning et al., 2022; Olson, 2004; Wilson et al., 2006). Instantaneous focal sampling of behaviour (Martin and Bateson, 2007) was conducted every 15 s for 15 min during four sessions of the day; 08:00 h – 10:00 h, 10:00 h – 12:00 h, 12:00 h – 14:00 h and 14:00 h – 16:00 h for a total of one hour per day per animal to obtain a general sample of diurnal behaviour shown by each animal. Visual observations were conducted while elephants were not directed by mahouts. If at any time an elephant was interacting with a mahout during observation or if

Table 1
Summary of steps involved in Experiment 1[a] and Experiment 2[b] with days receiving electric shocks (see Cabral de Mel et al., 2023 for further details).

Day	Activity	Details		Receipt of electric shock by each elephant on experiment days; received (√) not received (x): K1	M1[d]	M2	M3	M4	S1	S2[d]	S3[d]
Experiment 1[a]											
Day 1	Acclimatisation to wearing a collar	Elephants wore a dummy collar during the day and continued with their normal routine.		←			NA				→
Day 2	Acclimatisation to wearing a collar										
Day 3	Acclimatisation to wearing a collar										
Day 4	Testing day	Elephants K1, M1 and S1, (n = 3)[c] wore the shock collar during testing, which was removed soon after. Elephants did not wear a collar during the rest of the day.	Elephants M2, M3, M4, S2 and S3, (n = 5)[c] wore the shock collar during testing which was replaced with the dummy collar soon after and continued to wear it for the rest of the day.	√	√	√	√	√	√	√	√
Day 5	Post-test monitoring day	Elephants did not wear a collar during Days 5 and 6 and continued with their normal routine.	Elephants wore a dummy collar during Days 5 and 6 and continued with their normal routine.	←			NA				→
Day 6	Post-test monitoring day										
Day 7	Post-test monitoring day	Post-test monitoring not conducted on Days 7, 8 and 9[c].	Elephants did not wear a collar during Days 7, 8 and 9 and carried on with their normal routine.								
Day 8	Post-test monitoring day										
Day 9	Post-test monitoring day										
Experiment 2[b]											
Day 1	Training day	Elephants wore a dummy collar and were trained to walk along a path (~100 m) to a food reward, five times. Elephants then continued to wear a dummy collar and carried on with their normal routine during the rest of the day.		←			NA				→
Day 2	Training day										
Day 3	Training day										
Day 4	Testing day 1	Elephants wore a shock collar during testing. Shock collar was then replaced by the dummy collar, and elephants continued their normal routine for the rest of the day.		√		√	√	√	√		
Day 5	Testing day 2			x	NA	√	x	√	√	NA	NA
Day 6	Testing day 3			√		√	x	√	√		
Day 7	Post-test monitoring day	Elephants wore a dummy collar during the day and carried on with their normal routine.		←			NA				→
Day 8	Post-test monitoring day										
Day 9	Post-test monitoring day										

[a] Experiment 1- Assessing responses of elephants to mild electric shocks from a modified dog-training collar fitted on the neck.
[b] Experiment 2- A food attractant trial experiment conducted to condition elephants to associate an audio warning with a mild electric shock from a modified dog-training collar.
[c] Post-test monitoring procedure modified after conducting the experiment with first three elephants.
[d] M1, S2 and S3 were not involved in Experiment 2.

Table 2
Ethogram of elephant behaviours with subcategories considered in this study.

Behaviour category	Subcategory	Description
1. Feeding	Feeding	Depositing food items (or sometimes mud) in mouth, chewing and swallowing
	Foraging	Searching, acquiring, processing and picking up food item (or sometimes mud) using trunk
2. Movement		Taking two or more steps in any direction from one point to another using feet. Also included wading in shallow water (< 2 feet)
3. Environmental investigation		Investigating things in the environment. Included sniffing air, ground, urine, faeces and other inanimate objects other than food items using the tip of the trunk. Also included placing non-food material in mouth and moving its jaw in a chewing motion (e.g. plastic bottles) without ingestion of material.
4. Standing		Showing no movement, simply standing still on all four legs for short durations- momentary (< 5 s), with little or no leg movement and not showing any other behaviour
5. Comfort	Relaxing	Standing still upright, relaxed, eyes open or half closed for longer durations (> 5 s). The trunk may be still, and the tip may be lying on the ground
	Dozing	Standing still with no movements and eyes closed
	Lying down	Lying flat on either side of body (in lateral recumbence)
	Leaning	Leaning entire or part of body on another elephant or object, eyes open or half closed.
	Leg rest	Crosses one hind-leg in front of the other while standing so that one leg does not touch the ground
	Rubbing or scratching	Rubbing head, body or trunk on an object, wall or another elephant or scratching self with either trunk or legs or a stick
	Trunk resting	Placing trunk on an object or another elephant's body or holding trunk in mouth or laying the distal end of the trunk on the ground
	Dust bathing	Pick up dust using trunk and spraying over body
	Mud bathing	Pick up mud using trunk and spraying over body
	Wallowing	Lying down and wriggling body back and forth to cover the body in mud, dirt or sand
	Water spraying	Collecting water in trunk and spray or throw on body or into nearby space
	Fly swatting	Swatting flies by slapping branches against the skin
	Bathing	Lying in water or standing submerged in water (> 2 feet)
	Urinating	Discharging urine
	Defecating	Discharging dung
	Drinking	Collecting water in trunk and putting in mouth
6. Social	Antagonistic	Tail biting (biting the tail of another elephant), chasing (chasing another elephant- aggressively)
	Play	Head-to-head sparring (head-to-head contact between two elephants), trunk wrestling (trunk entwined with another elephant and pull or push another), mounting, chasing, rolling on one another
	Affiliative	Sniff/touch other elephants with tip of trunk, trunk extended towards another elephant for several seconds, placing trunk in another elephant's mouth or gentle head or body contact with another elephant (which does not lead to play or aggression)
7. Anxiety/stress	Stereotypy	Weaving (repeated moving of body from side to side), head bobbing (moving head up and down), head oscillation (weaving and head bobbing shown together resulting in a figure 8 movement of head)
	Self-directed behaviours	Touching self anywhere on the body with the tip of its own trunk or flick trunk in a swinging motion in and out and slaps own skin or directional trunk swing (back and forth) sometimes repetitively
	Collar	Touch, pull or hit collar using the trunk, shake collar using trunk or by rapidly shaking head.
8. Other		Any other behaviour not listed here

the elephant was not within the visual range, observations were paused and continued after the elephant had stopped interacting with the mahout or was located again, thus completing 15 min of observations during each session of the day. Behavioural observations were conducted for a total of 154 hrs. Elephants were first observed during the pre-experiment period (six months prior to conducting Experiment 1) to obtain a sample of baseline behaviours of each elephant. This included a total of 46 hrs, i.e., six different days or 6 hrs for each elephant, except for M4 which was observed for only four days. Then during Experiment 1 elephants were observed for 63 hrs, i.e., 6–9 days or 6–9 hrs for each elephant (n = 8) and during Experiment 2 elephants were observed for 45 hrs, i.e., 9 days or 9 hrs for each elephant (n = 5) (Table 1). Observations were conducted by a single observer (SJC). Intra-observer reliability was measured with the index of concordance (Martin and Bateson, 2007) using a video taken at the beginning of the study which was observed during the initial stage and then at the end of the study with 90% agreement between observations.

2.3.2. Assessment of physiological stress level of elephants

The physiological stress level of elephants was assessed using FCM concentration. The method for faecal sample collection and extraction of cortisol was adopted and modified for this study based on published sources (Palme et al., 2013; Wasser et al., 2000, 1996). A sample of ~50 g of fresh faeces (< 6 hrs since defecation) was collected between 08:00 h and 08:30 h from each elephant on pre-experiment and experiment days from the sheds where the elephants had spent the night. A total of 163 faecal samples were collected and analysed during this study. Fifty faecal samples were collected from the elephants to measure pre-experiment or baseline FCM concentrations for Experiment 1. This included 5–7 faecal samples from each elephant (n = 8) collected on different days within a three-month period prior to Experiment 1, which also included the sample collected on the morning of Day 1 before fitting the collar to begin Experiment 1. Only the faecal sample collected on the morning of Day 1 before fitting the collar to begin Experiment 2 was used to measure the pre-experiment or baseline FCM concentration for each elephant (n = 5) in Experiment 2. Faecal samples corresponding to each experiment day was then collected ~24 hrs later, i.e., on the following morning of each experiment day. This included 63 faecal samples, i.e., 6–9 samples from each elephant (n = 8) during Experiment 1 and 45 faecal samples, i.e., nine samples from each elephant (n = 5) during Experiment 2. During faecal sample collection, two to three portions from the centre of the bolus were taken, given cortisol distribution in faeces is not homogenous (Wasser et al., 1996) and cross-contamination with urine or other faecal matter is possible if portions had been taken from the outside of the bolus (Ganswindt et al., 2003). Each sample was placed in a well-sealed, labelled container, immediately stored in ice, and then stored in a −20 °C freezer at PEO within 1–2 hrs of collection. All collected samples were later transferred to a −20 °C freezer in the laboratory via a cool box with ice within 1.5 hrs at the end of each field visit.

To extract FCM, faecal samples were first thawed at room temperature. Wet faecal samples were mixed well for ~5 min with latex-gloved hands ensuring equal distribution of FCM in the sample. Approximately 0.6 g (avoiding large undigested material) of each well-mixed faecal sample was placed in a 15 ml centrifuge tube and mixed with 2 ml of 80% ethanol by vortexing for 30 min using a multitube vortex mixer.

Samples were then centrifuged at 2500 rpm for 25 min. The liquid supernatant was collected in 2 ml micro-centrifuge tubes, evaporated to dryness under a stream of nitrogen at 35 °C and stored at −20 °C until further analysis.

The concentration of FCM in faecal extracts was estimated using a commercially available multispecies enzyme-linked immunoabsorbent assay (ELISA) kit specific for cortisol (DetectX® Cortisol Immunoassay (EIA) kit, Arbor Assays, Ann Arbor, MI, USA). Following the steroid extraction protocol provided for DetectX Steroid Immunoassay Kits, dried faecal extracts were dissolved in 100 μl of ethanol and 25 μl of the supernatant was added to 475 μl of assay buffer and mixed (to reduce the ethanol concentration of the sample to ≤ 5%, as recommended) immediately prior to analysis. Protocol in the assay kit was followed and the absorbance was measured at 450 nm using a microplate reader. Serial dilution (n = 5) of pooled samples displaced parallel to the standard cortisol curve (ANCOVA, $F_{1,8} = 0.52$, $P = 0.50$), mean percentage recovery of pooled sample spiked with a set of standards with known cortisol concentrations was 98.9 ± 15.1%, intra-assay and inter-assay coefficient of variation was 7.1% and 9.2% respectively. FCM concentrations are expressed as ng/g of wet faeces. FCM extracts of samples collected from a particular elephant during one experiment was run on a single assay to minimise errors due to inter-assay variation.

2.4. Data analysis

All statistical analysis were conducted in RStudio (v 2022.07.2 Build 576) and R (v 4.2.2) (R Core Team, 2022).

2.4.1. Comparison of activity budgets

Activity budgets (number of observations of each behaviour category per day converted into percentages) during experimental days were compared with their pre-experiment (baseline) activity budgets using principal component analysis (PCA), followed by a bootstrapped PCA. This method was first described in Catlin-Groves et al. (2009) and adapted to analyse behaviour by Stafford et al. (2012), allowing comparison of entire activity budgets, and avoiding problems with dependence on repeated measures of behavioural data. The results of the bootstrapped PCA are displayed as a three-dimensional sphere plot using the RGL library and 'rgl.spheres' function for R (Murdoch and Adler, 2023). Each sphere represents the overall activity budget of each day, with the centre and the radius representing the mean of the first three principal components and the 95% confidence interval respectively. Overlapping of spheres indicate that the activity budgets are not significantly different (α = 0.05). The cumulative proportion of variance explained by the first three principal components for each analysis were ≥ 0.95, thus the plots can be considered reliable. This analysis was conducted following the instructions and the code provided by Stafford et al. (2012), modifying it only to suit the number of cases (days in this study) for each analysis.

2.4.2. Analysis of anxiety/stress behaviours and FCM concentrations

The number of anxiety/stress behaviours observed per day (i.e., the frequency of anxiety/stress behaviours) during different experiment days were compared to that observed on pre-experiment days (baseline levels) using a generalised linear mixed-effects model (GLMM). Initial analyses with a Poisson distribution and log link function showed overdispersion (i.e. the variance of the counts was significantly greater than the mean), therefore, data were fitted with a negative binomial distribution and a log link function. FCM concentrations on days of Experiment 1 and 2 were compared with the respective baseline FCM concentrations using a GLMM with a gamma distribution and an inverse link function. Days of experiment were included as the fixed effect and elephant identity was included as a random effect to control for repeated measures taken of the same elephant for the GLMM of anxiety/stress behaviours and FCM concentrations. Analysis were conducted using the functions 'glmer' and 'glmer.nb' in the R package lme4 (Bates et al., 2015).

3. Results

3.1. Assessment of behavioural welfare of elephants

Comparison of daily activity budgets of individual elephants during pre-experiment days and the experiment days using sphere plots showed that all spheres overlap with each other (Figs. 1 and 2). This indicated that there is no detectable difference between the overall daily activity budgets. Percentage contribution of each behaviour category for individual elephants on different days of the study, are given in supporting information (Table S1). The behaviour of interest, the anxiety/stress behaviours shown by elephants during pre-experiment days, Experiment 1 and Experiment 2 ranged from 0–7% (mean = 2.57 ± 1.92%), 0–15% (mean = 3.65 ± 3.19%) and 0–6% (mean = 2.60 ± 1.80%) of their activity budgets respectively. There was an increase in the observed anxiety/stress behaviours on Day 1 (mean = 6.50 ± 5.01%, range = 1–15%), and Day 4 (mean = 5.62 ± 4.93%, range = 0–15%) during Experiment 1 compared to pre-experiment days (see Table 3). This trend in overall anxiety/stress behaviour can be seen in Fig. 3a, with collar related behaviours and SDBs particularly contributing to the total anxiety/stress behaviour on Days 1 and 4 respectively. Peaks on Days 1 and 4 can be clearly observed when the frequency of anxiety/stress behaviour shown by individual elephants are considered (Fig. 3b). Even though such differences of anxiety/stress behaviours were not detected in Experiment 2 (see Table 3 and Fig. 4a), an increase in this behaviour on Day 4 (first testing day) compared to other days could be observed in Fig. 4b with some elephants (e.g. K1 and M4). Stereotypic behaviour on pre-experiment days was only observed in M3 who followed a different daily routine (tethered in her shed during some periods during the day) with few other elephants (K1, M2 and S1) showing stereotypy on some experiment days (see Table S1).

3.2. Assessment of physiological stress level of elephants

FCM concentrations in samples collected ~48 hrs after the testing day of Experiment 1 (i.e. Day 5, mean = 2.93 ± 1.19 ng/g of wet faeces, range = 1.76–4.63, n = 8) was high compared to pre-experiment days of Experiment 1 (mean = 2.12 ± 0.69 ng/g of wet faeces, range = 0.84–3.65, n = 8) (Table 4, Fig. 5a). FCM concentrations in samples collected ~24 hrs after the first testing day of Experiment 2 (i.e. Day 4, mean = 3.66 ± 0.77 ng/g of wet faeces, range = 1.41–5.73, n = 5) were also higher than the FCM concentration in the pre-experiment samples collected prior to beginning Experiment 2 (mean = 2.38 ± 0.61 ng/g of wet faeces, range = 1.73–3.23, n = 5) (Table 4, Fig. 6a). Days on which peaks were observed varied between individual elephants during Experiment 1 (Fig. 5b) and Experiment 2 (Fig. 6b) with some elephants showing peaks for samples on Day 4 or 5 (i.e. in samples collected ~24 or 48 hrs after testing day of Experiment 1 and in samples collected ~24 hrs after the first and second testing day of Experiment 2). An increase in FCM concentrations in response to wearing a collar can be observed for M1 in the sample corresponding to Day 2 in Experiment 1 (Fig. 5b). Unexpected increases in FCM concentrations were also observed in some animals 3 days (~72 hrs) after receiving a shock. For example, M1, M2 and M4 (Fig. 5b) show an increase in FCM concentration on Day 6 or later during Experiment 1.

4. Discussion

Aversive conditioning of captive Asian elephants using AGDs appears to be effective at restricting their movements, but their possible welfare effects are largely unknown (Cabral de Mel et al., 2023). Our assessment of the welfare impacts of AGDs on elephants revealed that daily elephant activity was not affected by the receipt of the electric shocks (Figs. 1 and 2). Anxiety/stress behaviours and FCM concentrations temporarily

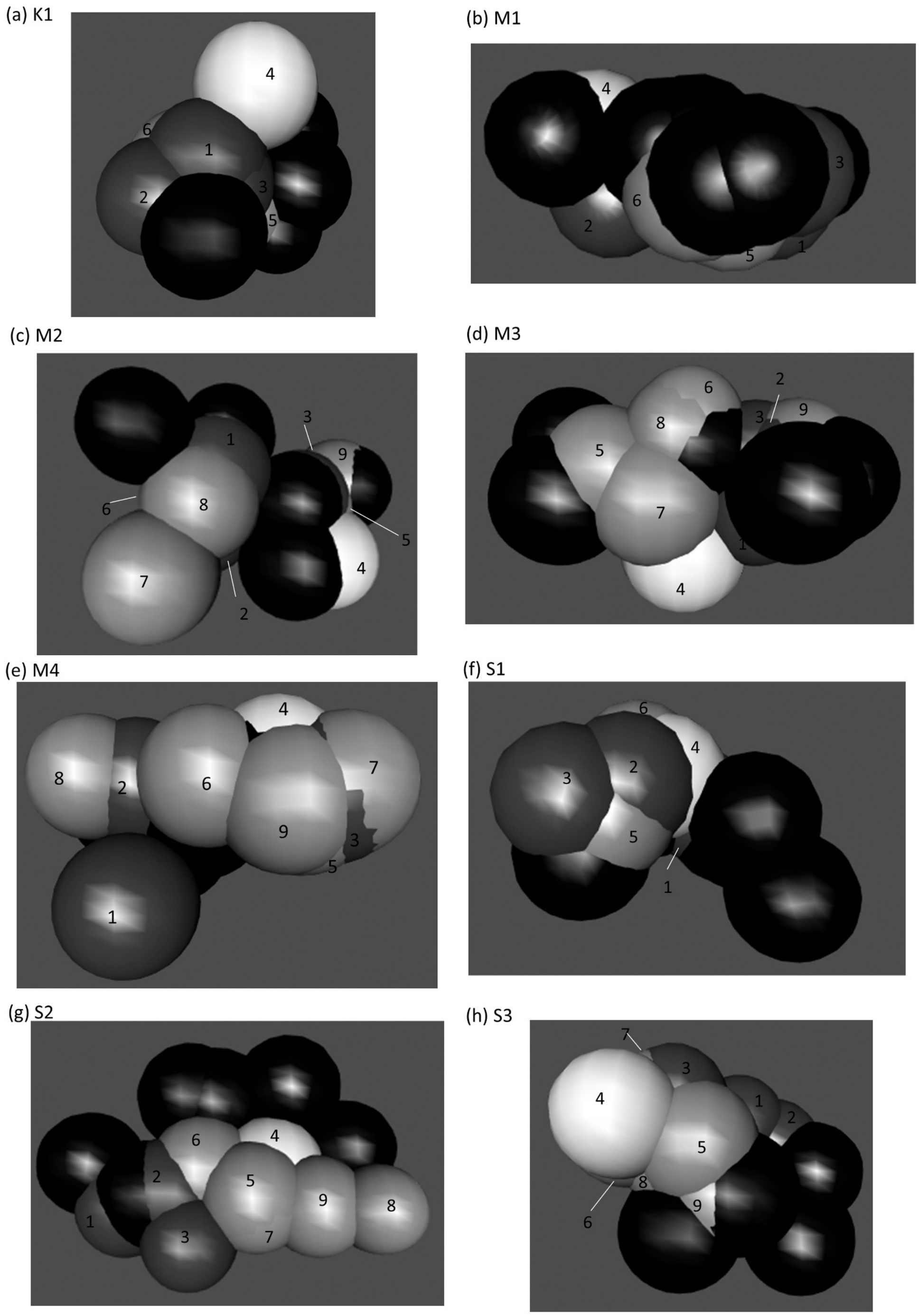

Fig. 1. Three-dimensional principal component sphere plot of activity budgets of the eight elephants; K1, M1, M2, M3, M4, S1, S2 and S3 (a–h respectively) that participated in Experiment 1 (assessing elephants' responses to mild electric shocks from a modified dog-training collar fitted on the neck). Black- pre-experiment days, dark grey- Days 1–3 (days of acclimatising to the dummy collar), white- Day 4 (testing day), light grey- Days 5–9 (post-test monitoring days). The first three principal components explain $\geq$ 95.0% of the total variance of the dataset.

increased as expected on initial days of wearing a collar and/or on days electric shocks were given but returned to prior baseline levels in the following days (Tables 3 and 4, Figs. 3–6). These results confirm that elephants show acute stress responses on specific days as expected, but do not show any lasting welfare effects after experiencing electric shocks. This suggests that AGDs might be used more broadly on Asian elephants with confidence that this management tool does not unduly influence normal elephant behaviour or cause unnecessary anxiety or stress.

Our study showed that the repeated use of AGDs did not influence the activity budgets of elephants. This result complements previous studies conducted with African elephants wearing GPS collars (Horback et al., 2012) and livestock species with electric shock collars (Aaser et al., 2022; Campbell et al., 2019, 2017; Marini et al., 2022; Verdon et al., 2021), which likewise showed no or negligible changes to their normal daily activity patterns as a result of wearing collars or receipt of shock. Collar-related behaviours contributed substantially to the increase in anxiety/stress behaviours on Day 1 of Experiment 1 (Fig. 3a), suggesting displeasure, hostility, or frustration in elephants towards having to wear a novel object on their body. But the low frequency of collar related behaviour during the rest of Experiment 1 and all days of Experiment 2 except on Day 4; the first testing day (Figs. 3a and 4a), indicates that elephants were no longer treating the collar as a novel or hostile object and had gradually habituated to wearing it. A study conducted on cattle showed similar results (Ranches et al., 2021). SDBs contributed substantially to the increase in anxiety/stress behaviours on Day 4 (testing day) of Experiment 1, suggesting a discomfort and distress caused by the electric shocks on that day. Although an increase in anxiety/stress

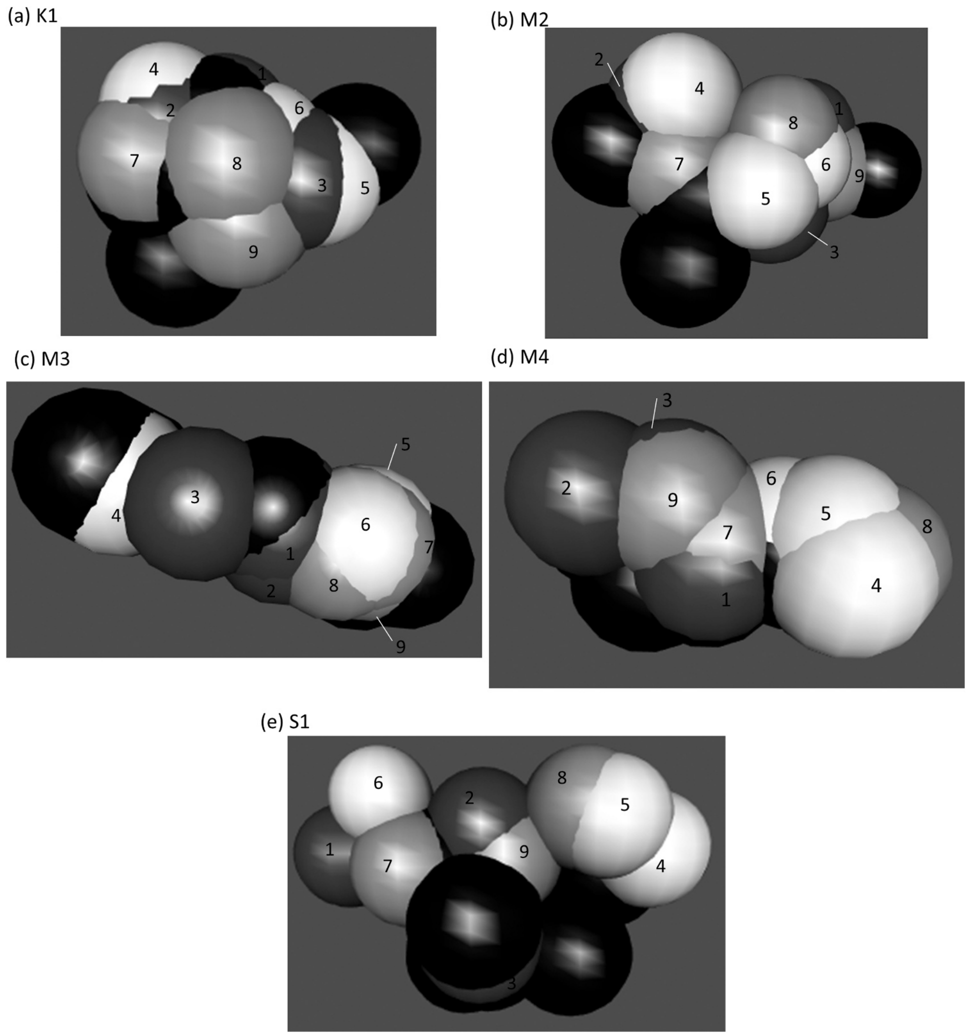

Fig. 2. Three-dimensional principal component sphere plot of activity budgets of the five elephants; K1, M2, M3, M4 and S1 (a–e respectively) that participated in Experiment 2 (a food attractant trial experiment conducted to condition elephants to associate a warning sound with a mild electric shock from a modified dog-training collar). Black- pre-experiment days, dark grey- Days 1–3 (training days), white- Days 4–6 (testing days), light grey- Days 7–9 (post-test monitoring days). The first three principal components explain ≥ 95.0% of the total variance of the dataset.

Table 3
Generalised linear mixed-effects model with negative binomial distribution and log link function for the frequency of anxiety/stress behaviours shown on different days of Experiment 1[a] and Experiment 2[b] with elephant identity as a random effect.

Experiment 1[a]				Experiment 2[b]			
Day	Estimate	Standard error	*P* value	Day	Estimate	Standard error	*P* value
Intercept (PE)[c]	1.816	0.150	< 0.001	Intercept (PE)[c]	1.897	0.187	< 0.001
Day 1	0.917	0.249	< 0.001	Day 1	0.123	0.269	0.646
Day 2	0.059	0.268	0.825	Day 2	-0.207	0.283	0.465
Day 3	0.436	0.257	0.089	Day 3	0.165	0.264	0.533
Day 4	0.711	0.250	< 0.001	Day 4	0.422	0.256	0.099
Day 5	0.237	0.261	0.362	Day 5	-0.572	0.306	0.062
Day 6	-0.142	0.277	0.608	Day 6	-0.306	0.289	0.289
Day 7	-0.128	0.336	0.703	Day 7	0.039	0.269	0.884
Day 8	-0.479	0.363	0.186	Day 8	-0.585	0.311	0.060
Day 9	0.196	0.326	0.547	Day 9	-0.431	0.299	0.150

[a] Experiment 1- Assessing elephants' responses to mild electric shocks from a modified dog-training collar fitted on the neck (n = 8).
[b] Experiment 2- A food attractant trial experiment conducted to condition elephants to associate a warning sound with a mild electric shock from a modified dog-training collar (n = 5).
[c] PE- Pre-experiment.

behaviours during Experiment 2 was not revealed (Table 3), some indication of distress caused by electric shock is evident from the increase in collar related behaviours (Fig. 4a) and peaks in anxiety/stress behaviours of individual elephants, except M2 (Fig. 4b) on Day 4 of Experiment 2.

In captive elephants, stereotyping is generally associated with being tethered, in limited space and in isolation (Greco et al., 2017, 2016; Horback et al., 2014; Varadharajan et al., 2016). This was also evident in our study. Only M3, who was tethered during some periods of the day, showed stereotypic behaviour throughout the study (on both pre-experiment and experiment days). But of those free-ranging elephants, only few showed stereotypic behaviour on some of the

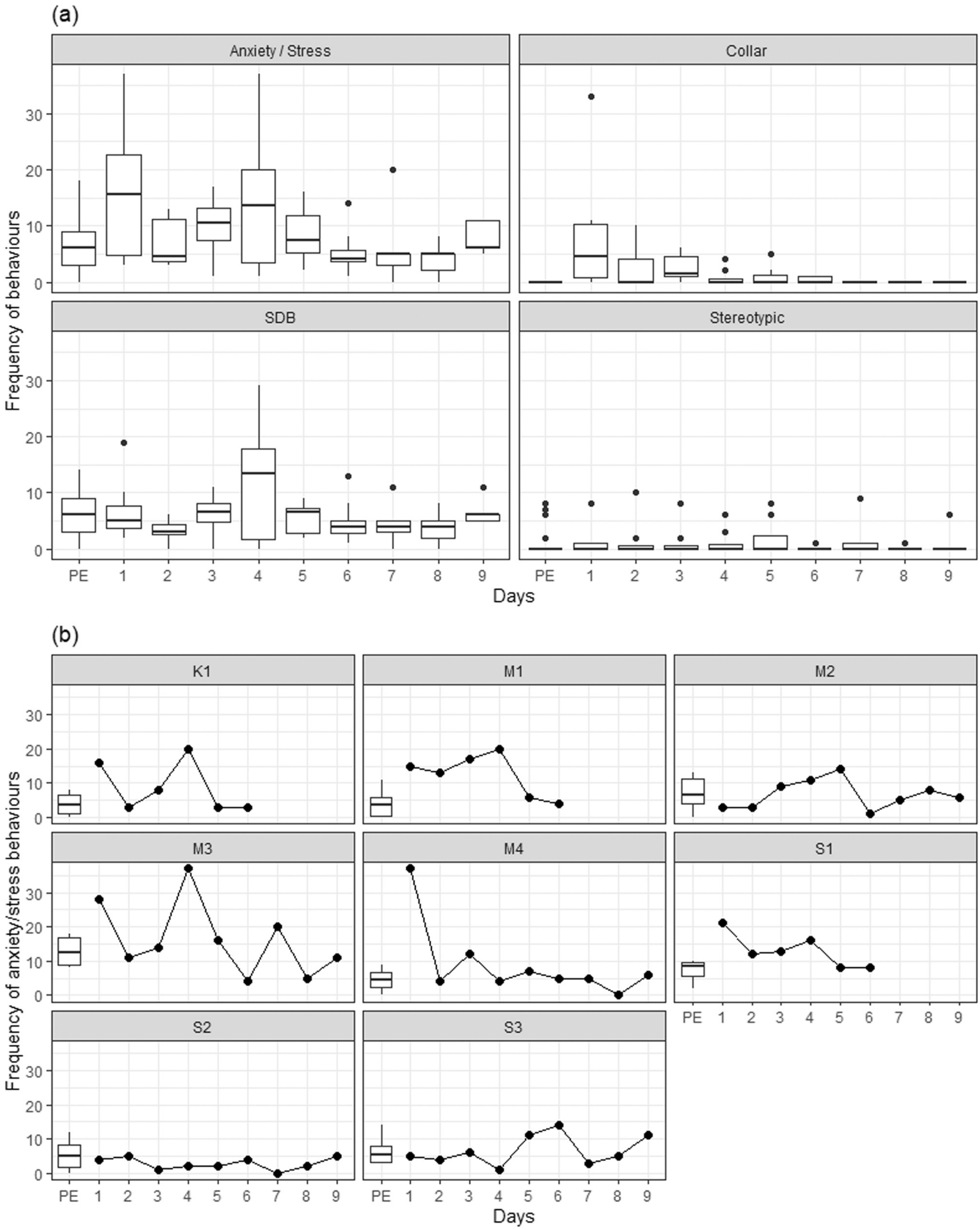

Fig. 3. Frequency of anxiety/stress behaviours of eight elephants on different days of Experiment 1 (assessing elephants' responses to mild electric shocks from a modified dog-training collar fitted on the neck). PE- pre-experiment days, Days 1–3- days of acclimatising to the dummy collar, Day 4- testing day, Days 5–9- post-test monitoring days. a. Box plots of anxiety/stress behaviours (Anxiety/Stress) and its subcategories; collar related behaviours (Collar), self-directed behaviours (SDB), stereotypic behaviours (Stereotypic) of the eight elephants. b. Box plot of anxiety/stress behaviours on pre-experiment days and connected scatter plot of anxiety/stress behaviours shown on experiment days by individual elephants (K1, M1, M2, M3, M4, S1, S2 and S3).

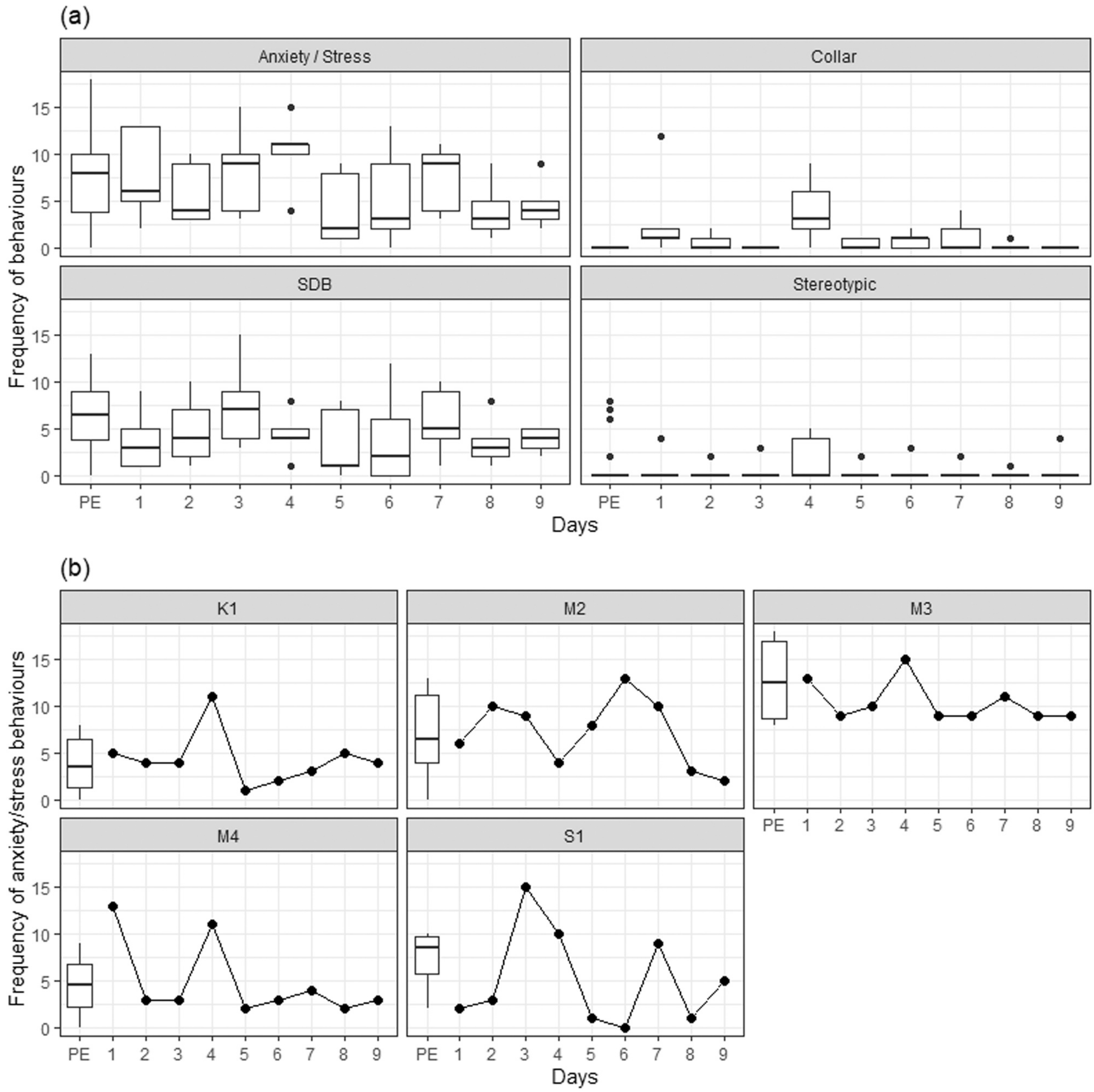

Fig. 4. Frequency of anxiety/stress behaviours of five elephants on different days of Experiment 2 (a food attractant trial experiment conducted to condition elephants to associate a warning sound with a mild electric shock from a modified dog-training collar). PE- pre-experiment days, Days 1–3- training days, Days 4–6- testing days, Days 7–9- post-test monitoring days. a. Box plots of anxiety/stress behaviours (Anxiety/Stress) and its subcategories; collar related behaviours (Collar), self-directed behaviours (SDB), stereotypic behaviours (Stereotypic). b. Box plot of anxiety/stress behaviours on pre-experiment days and connected scatter plot of anxiety/stress behaviours shown on experiment days by individual elephants (K1, M2, M3, M4 and S1).

experiment days (Table S1). Further, stereotypic behaviour may not always reflect the current welfare state, but instead may have developed and persisted due to an old stressor that may no longer exist (Mason and Latham, 2004), meaning that such behaviours may not always be a good welfare indicator on its own. SDBs are not yet well established as a welfare indicator for elephants (Mason and Veasey, 2010). But our study concurs with Manning et al. (2022) and suggests that they may be a useful indicator to assess behavioural welfare in captive Asian elephants, especially when they are free ranging in an open space.

Elevated FCM concentrations were recorded in faecal samples collected ~48 hrs after the testing day of Experiment 1 and ~24 hrs after first testing day of Experiment 2, compared to pre-experiment levels (Table 4). This was as expected given the excretion lag time for glucocorticoids which depends on the time taken for digesta to pass through the gut, which peaks after 12–58 hrs for Asian and African elephants (Ganswindt et al., 2003; Laws et al., 2007; Stead et al., 2000; Turczynski, 1993; Wasser et al., 1996). Similar increases in FCM concentrations in faecal samples have been observed in an Asian elephant two days after a stressful event (transportation and relocation) (Laws et al., 2007). Differences in the times of observing peaks between individual elephants and within the same elephant during the two experiments could be due to the differences in diets (e.g. differences in the amount of food

Table 4
Generalised linear mixed-effects model with a gamma distribution and inverse link function for the faecal cortisol metabolite concentrations (ng/g wet faeces) on days of Experiment 1[a] and Experiment 2[b] with elephant identity as a random effect.

Experiment 1[a]				Experiment 2[b]			
Day	Estimate	Standard error	*P* value	Day	Estimate	Standard error	*P* value
Intercept (PE)[c]	0.501	0.041	< 0.001	Intercept (PE0)[d]	0.544	0.087	< 0.001
Day 1	-0.022	0.048	0.653	Day 1	-0.018	0.065	0.776
Day 2	-0.078	0.043	0.069	Day 2	-0.044	0.062	0.482
Day 3	-0.016	0.049	0.748	Day 3	-0.038	0.063	0.542
Day 4	-0.080	0.043	0.062	Day 4	-0.130	0.055	0.019
Day 5	-0.129	0.038	< 0.001	Day 5	-0.083	0.059	0.162
Day 6	-0.064	0.044	0.152	Day 6	-0.007	0.066	0.916
Day 7	-0.101	0.054	0.063	Day 7	-0.074	0.060	0.217
Day 8	0.026	0.070	0.714	Day 8	-0.083	0.059	0.162
Day 9	-0.035	0.063	0.579	Day 9	-0.028	0.064	0.661

[a] Experiment 1- Assessing elephants' responses to mild electric shocks from a modified dog-training collar fitted on the neck, (n = 8).
[b] Experiment 2- A food attractant trial experiment conducted to condition elephants to associate a warning sound with a mild electric shock from a modified dog-training collar, (n = 5).
[c] PE- Pre-experiment samples collected prior to Experiment 1.
[d] PE0- Pre-experiment samples collected on the first day before beginning Experiment 2.

consumed on different days and preference for different types of food by individual elephants) or hepatic and gastrointestinal function of each animal on the specific days (Wasser et al., 1993). During Experiment 2, K1 who experienced electric shocks on Day 4 and 6, showed two FCM peaks in the samples collected ~48 hrs after each experience; M3, who received shocks only on Day 4, showed a peak in FCM concentration in the sample collected ~24 hrs later. The other three elephants received shocks on all three testing days (Days 4, 5 and 6) during Experiment 2, but showed only one peak in the sample collected ~24 hrs after the first testing day (Fig. 6b). Our failure to detect multiple peaks associated with multiple shocks may indicate that the elephants were no longer stressed by the shocks, and could instead predict the receipt of the electric shock from their previous experience (Lee et al., 2018). In other words, elephants appeared to be less stressed or anxious after the initial experiences with electric shocks.

Our results accord with previous studies on livestock species where elevated cortisol levels in response to electric shock returned to baseline levels immediately afterwards (Kearton et al., 2019; Lee et al., 2008). Similar observations have also been made with elephants whose elevated cortisol levels due to stressful relocation/transportation events returned to baseline levels with time (Dathe et al., 1992; Laws et al., 2007; Millspaugh et al., 2007). The frequency of anxiety/stress behaviours also followed the same pattern, returning to pre-experimental levels on post-test monitoring days. The additional peaks in FCM concentrations observed in samples collected after ~72 hrs (3 days) of receiving shocks in some elephants may have occurred due to hepatic circulation of the metabolites (Palme et al., 1996; Stead et al., 2000) or due to other stressful events that may have occurred after the testing day.

There was variation in the increase in the frequency of anxiety/stress behaviours and FCM concentrations in response to electric shocks by individual elephants. Some elephants showed only minor or negligible deflections (e.g. S2 and S3 in Fig. 3b, M2 and M3 in Fig. 4b, M3, S2 and S3 in Fig. 5b) while some others showed very sharp increases from baseline levels (e.g. M3 and M4 in Fig. 3b and K1, M1, S1 in Fig. 5b). This could be because of the differences in how shock is perceived by individuals, their sensitivities (Lines et al., 2013; Norell et al., 1983; Reinemann et al., 1999), temperament (Finkemeier et al., 2018; Réale et al., 2007), or their personalities (Found and Clair, 2018). Cortisol increases in response to different stressful situations in elephants have been shown to vary with individual personalities (Fanson et al., 2013), age, sex (Hambrecht et al., 2021), ovarian cycle phase, reproductive state (Boyle et al., 2015; Glaeser et al., 2020; Oliveira et al., 2008) and even seasonality (Menargues Marcilla et al., 2012). Similarly, behaviour of elephants are also known to depend on ovarian cycle phase (Slade-Cain et al., 2008) and may also vary depending on management routines (Elzanowski and Sergiel, 2006), availability of resources (food and space) (Lasky et al., 2021; Powell and Vitale, 2016) and environmental factors (Rees, 2002). For these reasons, a sound understanding of individual elephants' baseline or pre-treatment behaviour and cortisol levels is important for interpreting changes caused by using AGDs.

Conditions that affect stress levels of elephants could have potential consequences for their conservation (Pokharel et al., 2017; Tang et al., 2020) as stress levels may have an influence on their fitness, reproduction and survival (Busch and Hayward, 2009; Hing et al., 2016). Although our study showed that stress responses of captive elephants to AGDs are short-lived, it would be important to conduct long-term studies to determine how intermittent exposure to stimuli from AGDs could affect elephants' wellbeing. A recent study on cattle showed that animal-borne devices may influence social behaviours of animals (Buijs et al., 2023). GPS collars have been in use on wild elephants to monitor their movements for several decades and adverse impacts on their social behaviour have not been reported (de la Torre et al., 2021; Fernando et al., 2015; Pastorini et al., 2015; Sampson et al., 2018; Wadey et al., 2018). But how other elephants in a herd would respond if only the matriarch is wearing an AGD and is responding to the stimuli from AGDs would need to be investigated. Ensuring that there is negligible impact on elephants' wellbeing when using AGDs is vital and such assurance will also help gain acceptability and support of stakeholders to implement AGDs as an HEC mitigation tool in the future.

5. Conclusion

Use of electric shocks to manage animal behaviour have long been a controversial subject, but AGDs have been proven successful at managing the movement of domesticated livestock species with minimum welfare impact. AGDs have been identified as a potentially useful tool for resolving HEC, but a lack of knowledge on the potential welfare effects of AGDs has limited the adoption of this new technology. Our results give confidence that AGDs can be safely used to control elephant movement without lasting adverse effects on elephant welfare. We therefore encourage the continued development and use of AGDs on Asian elephants as an effective non-lethal tool to mitigate HEC. More broadly, we also encourage continued assessment of the animal welfare effects of these and other novel wildlife management tools so that stakeholders can have confidence that such tools can be used in an acceptable way.

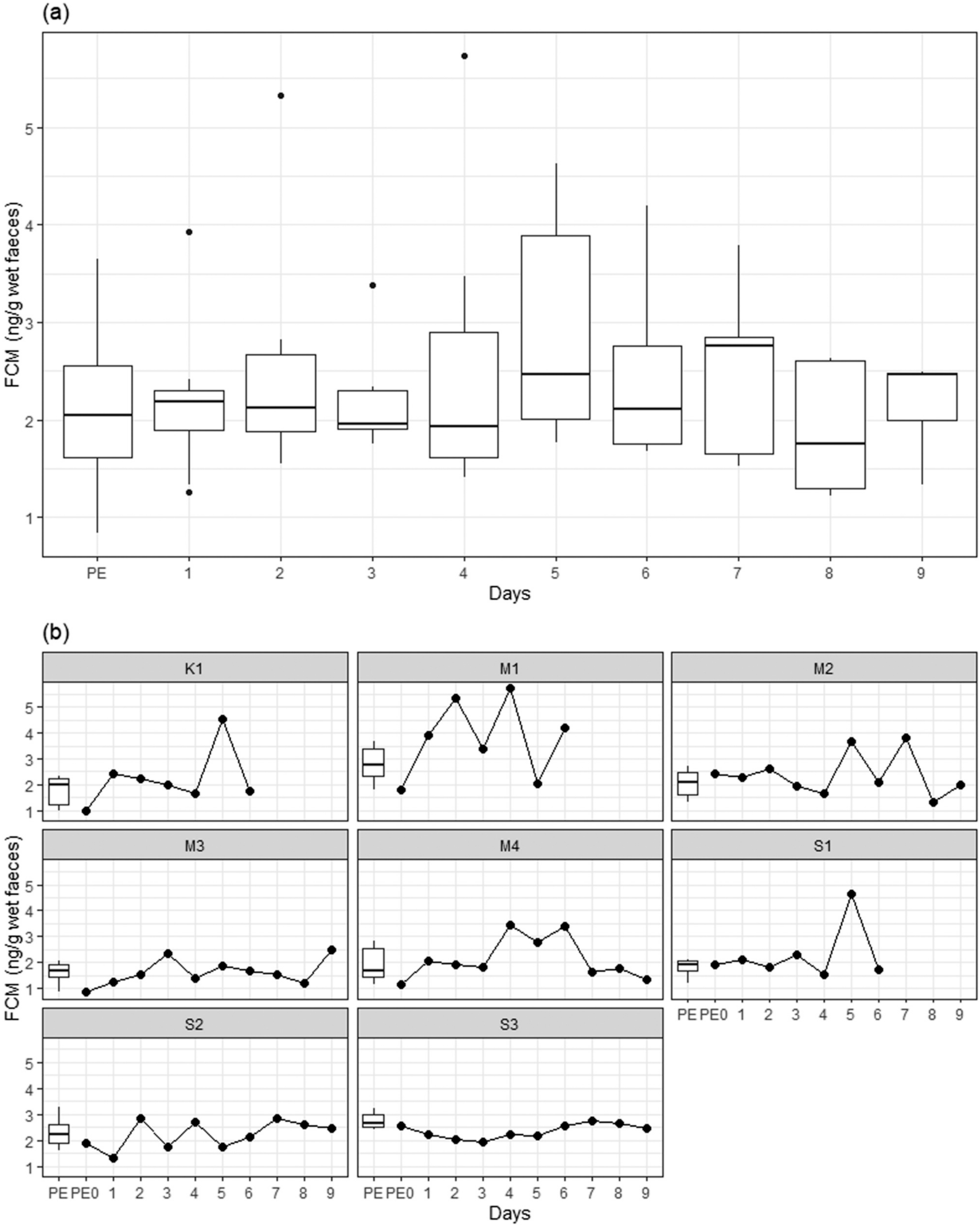

Fig. 5. Faecal cortisol metabolite (FCM) concentrations (ng/g wet faeces) in samples collected ~24 hrs after each day of Experiment 1 (assessing elephants' responses to mild electric shocks from a modified dog-training collar fitted on the neck) of eight elephants. PE- pre-experiment days, PE0- pre-experiment faecal sample collected on Day 1 before beginning Experiment 1, Days 1–3- days of acclimatising to the dummy collar, Day 4- testing day, Days 5–9- post-test monitoring days. a. Box plot of FCM concentrations of all elephants. b. Box plot of FCM concentration on pre-experiment days and connected scatter plot of FCM concentration on experiment days of individual elephants (K1, M1, M2, M3, M4, S1, S2 and S3).

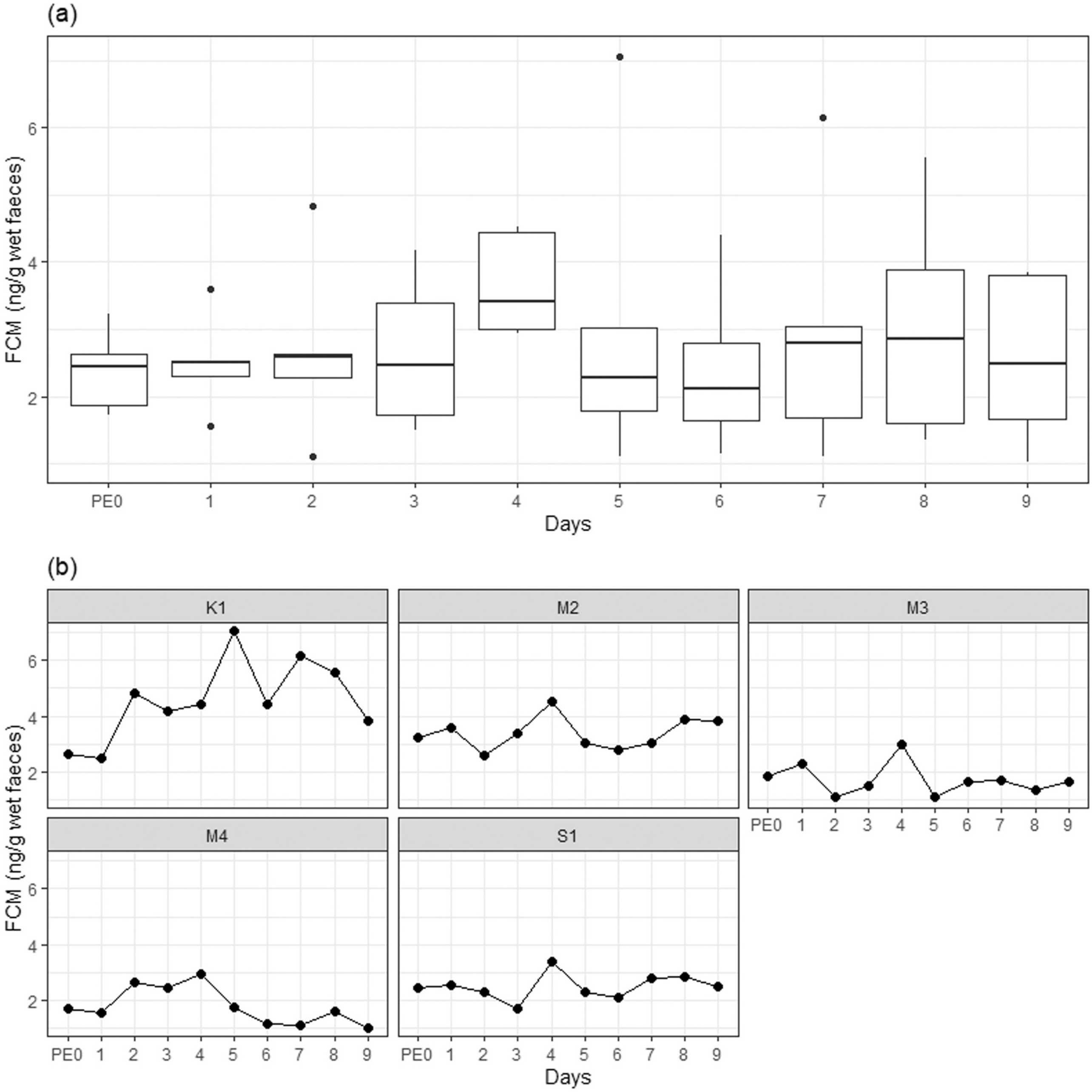

Fig. 6. Faecal cortisol metabolite (FCM) concentrations (ng/g wet faeces) in samples collected ~24 hrs after each day of Experiment 2 (a food attractant trial experiment conducted to condition elephants to associate a warning sound with a mild electric shock from a modified dog-training collar) of five elephants. PE0- pre-experiment faecal sample collected on Day 1 before beginning Experiment 2, Days 1–3- training days, Days 4–6- testing days, Days 7–9- post-test monitoring days. a. Box plot of FCM concentrations of all elephants. b. Connected scatter plot of FCM concentration on experiment days by individual elephants (K1, M2, M3, M4 and S1).

CRediT authorship contribution statement

Surendranie Judith Cabral de Mel: Conceptualisation, Design of study, Acquisition of data, Analysis and interpretation of data, Project administration, Writing – original draft, Writing – review and editing. **Saman Seneweera**: Funding acquisition, Project administration, Writing – review and editing, Supervision. **Ruvinda Kasun de Mel**: Design of study, Acquisition of data, Writing – review and editing. **Ashoka Dangolla**: Project administration, Writing – review and editing, Supervision. **Devaka Keerthi Weerakoon**: Design of study, Project administration, Writing – review and editing, Supervision. **Tek Maraseni**: Project administration, Writing – review and editing, Supervision. **Benjamin Lee Allen**: Conceptualisation, Design of study, Project administration, Writing – review and editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.applanim.2023.105991.

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