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*Evaluating physiological stress in Asiatic black bears (*Ursus thibetanus*) rescued from bile farms in Vietnam*

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Abstract

*Asiatic black bears (*Ursus thibetanus*) face chronic stress in bile farms. In this study, we investigated whether bile-farmed bears show significantly high levels of stress at rescue and whether stress levels reduce over time in a bear sanctuary where the bears are supported with environmental enrichment and veterinary care to improve animal welfare. We measured stress hormone levels using faecal cortisol metabolites (FCM) in 16 Asiatic black bears freshly rescued from bile farms in Vietnam. Fresh faeces were collected from each bear on the rescue truck and on a weekly basis for a 22-week study period at a bear sanctuary in Vietnam. Results showed that for all 16 rescued bears (with one exception) individual FCM levels from truck samples were above mean baseline FCMs of bears previously rehabilitated to a bear sanctuary. This suggested the majority of the rescued bears were still capable of showing a stress endocrine response during the rescue operation despite being exposed to conditions causing chronic stress in bears on bile farms. Results showed that mean FCM levels of the rescued bears differed significantly between time-periods (higher at the rescue [on truck samples] compared to week 22 samples) and mean FCM levels showed an overall decline over the first 22 weeks after they arrived at the bear sanctuary. The bears also demonstrated acute FCM stress responses to management interventions at the sanctuary, such as veterinary health checks and transportation. In conclusion, rescued bears tend to modulate their stress endocrine response after rehoming at the bear sanctuary. This is an important result, indicating that the rescue effort and rehabilitation of bile-farm bears is effective. Whether this also coincides with behavioural adjustments in rehabilitating bears (eg lessening of stereotypic behaviour) warrants further investigation.*

Keywords: *animal welfare, bear bile farming, rehabilitation, rescue, stress,* Ursus thibetanus

Introduction

Asiatic black bears (*Ursus thibetanus*), also known as Himalayan black bears, moon bears and Tibetan black bears, are one of eight world bear species belonging to the Ursidae family. One of the major threats to the declining population of Asiatic black bears is bear bile farms, fuelled by the increasing demands for bear bile and bear parts for use in traditional Chinese medicine (Huygens *et al* 2003; Kikuchi 2012). Across Asia, multiple countries engage in bear farming and as many as 12,000 bears have been estimated to be housed in both illegal and legal bear farms (Bekoff 2009). Though some farms rely on captive breeding to provision bear products, many still depend on the capture of wild bears to support trade and risk natural populations. The welfare of bears on these 'farms' is generally considered very poor because bears are housed in small cages where they lack social or other forms of enrichment, receive poor nutrition, are exposed to surgical trauma and have a high risk of disease (Loeffler *et al* 2009).

Consequently, bears are often thought to be highly stressed, but the limited access to these facilities has made it difficult to quantify the extent of this stress (Malcolm *et al* 2013). Although bear-bile farming practice became illegal in Vietnam from 1992, the Ministry of Agriculture and Rural Development reported in 2015 that 1,245 bears were kept in small-scale bear-bile farms in Vietnam (Animals Asia Foundation 2015). As bear-bile farming is a sensitive issue, access to these 'farms' is extremely limited, resulting in a lack of systematic examinations of the bear-bile farming industry and its effects on the farmed bears' physiology. However, there is a handful of documented evidence of the impacts that bile farms have on the ethology of farmed bears (Maas 2000; Malcolm *et al* 2013).

A key initiative, alongside legislation to outlaw bear-bile farming, is for government or non-government organisations to remove incarcerated bears and relocate them into designated sanctuaries that offer considerably better environmental conditions and the provision of routine veterinary

Table 1 Information on name, identification, sex, age, weight, the farm from which they were rescued from and if the bears were anaesthetised during rescue on the Asiatic black bears from the Vietnam Bear Rescue Centre (VBRC) that were used in this study. The weight measurements were taken soon after arrival at the VBRC.

Bear name	Bear ID	Sex	Age	Weight (kg)	Farm rescued from	Anaesthetised on rescue
Kay	V146	Female	Adult	91	Farm 1	No
Emy	V147	Female	Young	76.5	Farm 1	No
Rose	V148	Female	Adult	77	Farm 1	No
Long	V149	Male	Young	86	Farm 1	No
Hoa	V150	Female	Adult	92	Farm 1	No
Quang Yen	V151	Male	Adult	98	Farm 1	No
Tuffy	V152	Male	Adult	85	Farm 2	Yes
Autumn	V153	Female	Adult	156.5	Farm 3	No
Gloria	V154	Female	Adult	130	Farm 4	No
Popeye	V155	Male	Adult	184	Farm 5	Yes
Victoria	V156	Female	Adult	103	Farm 6	Yes
Sarah	V157	Female	Adult	61.5	Farm 7	Yes
Cliff	V158	Male	Adult	107.5	Farm 8	No
Sindy	V159	Female	Adult	89	Farm 8	No
Bong Bong	V160	Female	Adult	188	Farm 9	Yes
Hercules	V161	Male	Adult	159	Farm 10	No

care. Nevertheless, it is extremely important to evaluate the effectiveness of such rehabilitation practices to improve the physiological adaptation and ultimately the protection of threatened bear populations.

Non-invasive hormone monitoring using faecal cortisol metabolites (FCM) testing is routinely used to assess the stress physiology of animals (Millspaugh & Washburn 2004; Garcia *et al* 2005; Sgai *et al* 2010; Narayan *et al* 2012; Narayan 2013). Non-invasive hormone monitoring allows the endocrine status of target species to be evaluated without having to manually restrain animals to facilitate blood collection (Narayan *et al* 2013b). Non-invasive physiological markers of the neuroendocrine stress response (eg faecal glucocorticoid metabolites [FGMs]) have been widely used to assess the stress response of wildlife exposed to any environmental stressor or management intervention. To date, there has only been a single study to evaluate physiological stress responses of black bears to recovery in a sanctuary after removal from bile farms in China (Malcolm *et al* 2013). This study indicated that FGMs decreased post-rescue and hence suggested some benefits to the well-being of black bears. It is not known if such outcomes occur commonly with these rehabilitation practices in other Asian countries, such as Vietnam.

The primary aim of this study was to measure cortisol metabolites (either FGM or FCM; the latter will be used for the purpose of this paper) non-invasively in faecal extracts from Asiatic black bears that have recently been rescued from bear-bile farms in Vietnam. We determined whether individual bears' FCM levels were high at rescue and whether the levels reduced after removal from bile farms. We used mean baseline FCM levels of rehabilitated Asiatic black bears attained from previous work by Malcolm *et al* (2013) as our reference.

Materials and methods

Permissions

All research was undertaken with approval from the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) permit no PWS2016-AU-000156, Australian Quarantine and Inspection Services Biological Samples Import Permit No IP15002642 and Charles Sturt University (CSU) Animal Care and Ethics Committee (ACEC protocol number A16052).

Study population

By the 30th October 2015, the Animals Asia Foundation had rescued a total of 33 Asiatic black bears from bile farms in the Quang Ninh province, Vietnam. These bears were relocated to Animals Asia's Vietnam Bear Rescue Centre (VBRC) in Tam Dao National Park, where they joined another 112 bears consisting of the moon or Asiatic black bears that had been previously rescued. Since this study required samples to be collected from the very start of the bears' rescue, bears in the sanctuary more than one week after rescue could not be used in the study. Due to the timing of this study and the timing of

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the bears' rescue, only the last 16 of the 33 newly rescued bears were able to be included. A visual health check was performed on each bear on the bile farm before they were moved into the rescue truck. During the health check, sex, estimated age, weight and body condition were assessed and recorded. The bears' age was classified as young adult (2–4 years and not having reached sexual maturity) and adults (> 4 years or has reached sexual maturity). The body condition of each bear was scored on the basis of muscle cover, fat, pelvis and rib palpation and general appearance.

Information on the name, identification, sex, age, weight, the farm from which they were rescued and whether or not the bears were anaesthetised during rescue can be seen in Table 1.

All 16 newly rescued bears were quarantined in individual enclosures (1.5 × 1.5 × 1.5 m; length × width × height), with no access to outdoor enclosures or other bears for the first 45 days at the sanctuary (week 1–week 7 of the study period).

On arrival at the VBRC (this was the start of the 45-day quarantine period), bears were offered 300 g of dog food, apple, pumpkin, sweet potato, tomato and carrot twice a day until carers could determine the appetite and satiety of each individual. Thereafter, food amounts were decreased or increased depending, primarily, on the behaviour of the individual bear (eg their speed of eating and their behaviour immediately beforehand, such as anticipation and appetite and satiety post-feeding). Based on this, the food offered over the course of the following months ranged from 100 to 500 g with a range of different combinations of fruits and vegetables.

Standard veterinary care was provided for all bears at the VBRC. Veterinarians performed health checks on all bears when they first arrived and performed operations on bears that required medical attention throughout the rehabilitation period.

The study bears were rescued from ten different small bile farms in Quang Ninh province, Vietnam within the same season. The conditions of the bile farms varied but, in general, bears were kept, permanently, in small, outdoor cages for a number of years without any form of enrichment. Five of the study bears had to undergo general anaesthesia (for details, see Malcolm *et al* 2013) during the rescue (see Table 1). These five individuals were kept in areas either too small or too large, making direct transfer into transport cages difficult. Once anaesthetised, the bears had to be carried from the farm cage to the transport cage.

The bears' daily routine consisted of the following; all bears were fed dog food in the morning (around 0830h), fruit and vegetables at around 1000h, given enrichment at around 1130h, fruit and vegetables and enrichment at around 1430h, given dog food at around 1645h and given browse (herbaceous materials for feeding and nesting) around 1700h.

After the 45 days of quarantine period, 12 of the rescued bears were kept within individual indoor-only dens (3.5 × 4 × 2.8 m; length × width × height), with no outdoor access, in the River House/Mountain House. These bears included Kay (V146), Rose (V148), Autumn (V153), Gloria (V154), Victoria (V156), Sarah (V157), Cliff (V158), Sindy (V159), Bong Bong (V160), Tuffy (V152), Popeye (V155) and Hercules (V161).

Of the remaining four bears, Long (V149) was moved into 'House 1' and Emy (V147) was moved into 'House 2'. Both of these houses had the same indoor den sizes (4 × 9 × 3.6 m; length × width × height) and also included access to a semi-natural outdoor enclosure that had a total area of 2,534 m^2 for House 1 and 2,596 m^2 for House 2. Hoa (V150) and Quang Yen (V151) were moved to 'House 5' which had individual dens sized 3.4 × 5 × 3.2 m (length × width × height).

All of the buildings and enclosures were built to standard environmental guidelines and met the international standards for holding bears in captivity. The enclosures and dens had extensive enrichment made from local materials. Enrichment structures included various artefacts, such as wooden climbing structures, ledges, swings, hammocks and artificial pools. They were also provided with balls, ice-blocks, Kongs® filled with food, rawhide and given the opportunity to nest or hibernate.

Collectively, the process from rescue and integration with other bears included the following key steps: (i) quarantine in River House/Mountain House; (ii) move to a bear house with an enclosure; (iii) possibly, by this time, integration with cohorts from the same rescue; (iv) access to a small, fenced off e-fence enclosure; (v) access to the main enclosure; and (vi) integration with the main group at the house (not discussed in the current study). The dimensions of the outdoor enclosures (e-fenced areas) were flexible in shape and size and hence exact dimensions are not available.

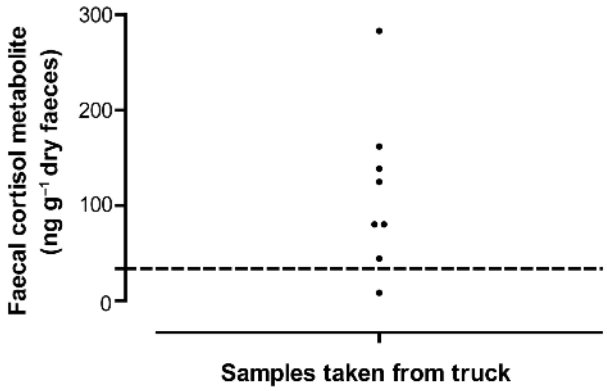
Faecal sampling

Fresh faecal samples were collected on the rescue truck during the bears' rescue, 24 h and/or three days after the bears arrived at the sanctuary, then once every week for 22 weeks starting from one week after arrival at the sanctuary. The truck samples were limited because not all bears defaecated on the truck. Faeces that were collected on the truck were fresh and, as each bear was in an individual transport cage, it was readily apparent which sample had originated from which individual. Moreover, each cage was cleaned after previously being occupied.

The individual bear dens were cleaned every morning so that old and fresh faeces were not mixed. At the sanctuary, faeces were collected in the morning, during routine cleaning, when bears were moved from their dens to the holding enclosure. The freshest pile of faeces was collected by picking up a small handful with the collector's hand inside a plastic zip-lock bag (10 × 15.6 cm; length × width) turned inside out to enable uncontaminated collection. Each sample weighed 2–12 g and was then labelled with the date of collection, the bear's name and ID number, and which week of the study it was collected using a permanent marker both directly on the plastic bag and on a paper label sticker which was also stapled to the bag. A faecal sample may not have been collected in those instances where the animal did not defaecate within the sampling period or been removed from the individual den for veterinary assessment.

Individual faecal samples were then immediately placed into a –20°C freezer for a week, stored at –80°C and transported

Figure 1



Range of individual faecal cortisol metabolite level in Asiatic black bears measured in samples taken from the truck (n = 8). Mean baseline FCM level recorded in the study of Malcolm *et al* (2013) is provided using horizontal dashed line.

from Vietnam to Australia within four months under the Australian biosecurity and CITES permits. The samples were securely packed in styrofoam and plastic boxes and sent by air freight (DHL, Vietnam). This process took six days. Each box weighed 10 kg (9 kg of frozen ice gel packs and 1 kg of samples) so the samples remained intact and frozen during transportation. Samples were then stored in a –80°C freezer immediately on receipt and assayed within two months.

Faecal cortisol metabolites extraction

Faecal cortisol metabolites were extracted from the Asiatic black bear faecal samples using previously published methods (Narayan *et al* 2013a). The samples were freeze-dried in the lyophiliser (Alpha 2-4 LD plus, Christ™, Martin Christ, Germany) for 48 h to eliminate all water content. The dried samples were then ground up in a mortar and pestle, sieved and mixed thoroughly to ensure the faecal powder was homogenous. Then, 0.2 (± 0.01) g of the powdered faeces was weighed into polystyrene tubes with the sample ID to which 2 ml of 90% aqueous ethanol was added. Each tube was then vortexed for 30 s then placed into a hot water bath pre-set at 80°C for 10 min. The supernatant of each sample was then poured into labelled 1 ml eppendorf tubes and centrifuged at 10,000 rpm for 5 min. A micropipette was used to transfer 600 µl of the supernatant into another set of labelled 1 ml eppendorf tubes and these were left open under a laminar flow chamber for up to five days to allow for the remaining ethanol to completely evaporate, leaving only the extracted sample as residue on the sides of the eppendorf tubes. The residue was reconstituted by adding 1 ml of assay buffer (39 mM $NaH_2PO_4H_2O$, 61 mM $NaHPO_4$, 15 mM NaCl and 0.1% bovine serum albumin; pH 7.0) then centrifuged at 10,000 rpm for 10 min before pipetting 850 µl of clean supernatant into newly labelled eppendorf tubes. These faecal extracts were stored at –80°C until analysed.

Faecal cortisol metabolite (FCM) enzyme-immunoassay

An FCM enzyme-immunoassay (EIA) was used to analyse the faecal extracts by following the detailed protocol reported by Malcolm *et al* (2013). Concentrations of FCMs were determined by indirect enzyme-immunoassay using a polyclonal anti-cortisol antiserum (R4866; procured from the University of California Davis, USA) diluted 1:15,000 which was then reacted with horseradish peroxidase (HRP) conjugated cortisol label diluted 1:80,000 and cortisol standards (1.56–400 pg $well^{-1}$). Specificity of the assay was determined by cross-reactivity of the R4866 anti-cortisol antiserum, which was reported as 100% with cortisol and less than 10% with other steroids tested (Narayan *et al* 2010). The same FCM EIA was used recently to assess FCM concentrations in other mammals, including a sub-population of Asiatic black bears from China (Malcolm *et al* 2013).

Faecal extracts were assayed on Nunc Maxisorp™ (Thermo Scientific Nunc®, USA) plates (96 wells) that were coated with 50 µl of diluted cortisol antibody (1:100) and coating buffer (50 mmol L^{-1} bicarbonate buffer; pH 9.6) then incubated for at least 12 h at 4°C. Plates were not incubated for more than 24 h to minimise the risk of the antibody coating buffer drying over time, possibly affecting the results. On the second day, after the Nunc Maxisorp™ plates had been incubated for at least 12 h, the plates were washed using an automated plate washer supplied with phosphate-buffered saline containing 0.5 ml L^{-1} Tween 20 (Sigma, Sigma Aldrich, USA), used to rinse away any unbound antibody. Stocks of standards, high- and low-binding internal controls, faecal extracts and HRP labels were diluted to the appropriate concentration in assay buffer. Stocks of plate-coating buffer, EIA buffer, substrate, wash buffer and stop solution were prepared within two weeks of the analysis.

For each assay, 50 µl of cortisol standard, internal control for Asiatic black bears and diluted faecal extracts were immediately pipetted into each well corresponding to a set plate map, followed by 50 µl of the corresponding HRP label using a micropipette. The loaded plates were then incubated at room temperature for exactly 2 h. The plates were then washed and 50 µl of substrate buffer (0.01% tetramethylbenzidine and 0.004% H_2O_2 in 0.1 M acetate citrate acid buffer; pH 6.0) was added to each well and incubated at room temperature for 15 min to allow for a colour change before stop solution (0.5 mol L^{-1} H_2SO_4) was added. This incubation time was based on visual inspection of the plates so that the optical density of the zero wells read between 0.7 and 1.0, which usually occurs after 10–15 min. Plates were then read at 450 nm (reference 630 nm) on a microplate reader to quantify the concentration of FCM in each sample. All faecal data were expressed (ng g^{-1}) as net dry faeces.

The FCM dilution factor for Asiatic black bears was determined by the concentration of pooled samples that resulted in 50% binding on the parallelism curve. Assay sensitivity for FCM EIA was 3.44 (± 0.67) pg $well^{-1}$ (n = 10). The intra- and inter-assay coefficients of variation determined from internal control samples (~30 and 70% bound) included in all assays. Intra-assay coefficients of variation were 1.8 and 6.94% for low- and high-percentage-bound controls, respectively, while inter-assay coefficients of variation were 10.5 and 4.9%.

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Statistical analysis

Statistical analyses were performed using Systat (Systat Software Inc, San Jose, CA, USA) and Prism (Graphpad Software Inc, La Jolla, CA, USA). All FCM concentrations were log-transformed to meet the assumptions of homogeneity variances (Levene's test) and normality (Shapiro Wilk). A General Linear Model ANOVA was used to determine whether there were significant differences between mean FCM concentrations with bear ID and time (weeks) as fixed factors and FCM as dependant variable. Sex and anaesthesia were eliminated as factors because initial unpaired *t*-tests showed that there were no significant differences between male and female bears (anaesthetised/unanaesthetised) during rescue at the bile farm ($P > 0.05$). Significant differences were found between mean FCM concentrations by time so *post hoc* testing followed. Unpaired *t*-tests with unequal variances were used to compare significant differences in mean FCMs between time-periods during the study. Due to the unavailability of FCM data from previously rehabilitated bears at the VBRC, baseline FCM data from the study of Malcolm *et al* (2013) were utilised for reference. Malcolm *et al* (2013) obtained a baseline value of 33.75 (± 0.72) ng g^{-1} (range, 0.17–108.5 ng g^{-1}) averaging all values under the overall mean FCM (± 1.5 SD). Malcolm *et al* (2013) used eleven bears that had been in residence at the Chinese Bear Rescue Centre (CBRC) for a year or more as controls (females, n = 7; males, n = 4). Level of significance was set at $P < 0.05$.

Results

Sample sizes obtained from individual bears over the 22-week study period ranged from 8–21 individual faecal samples. Total sample size (n = 16 bears) used for the analysis was 245 faecal samples.

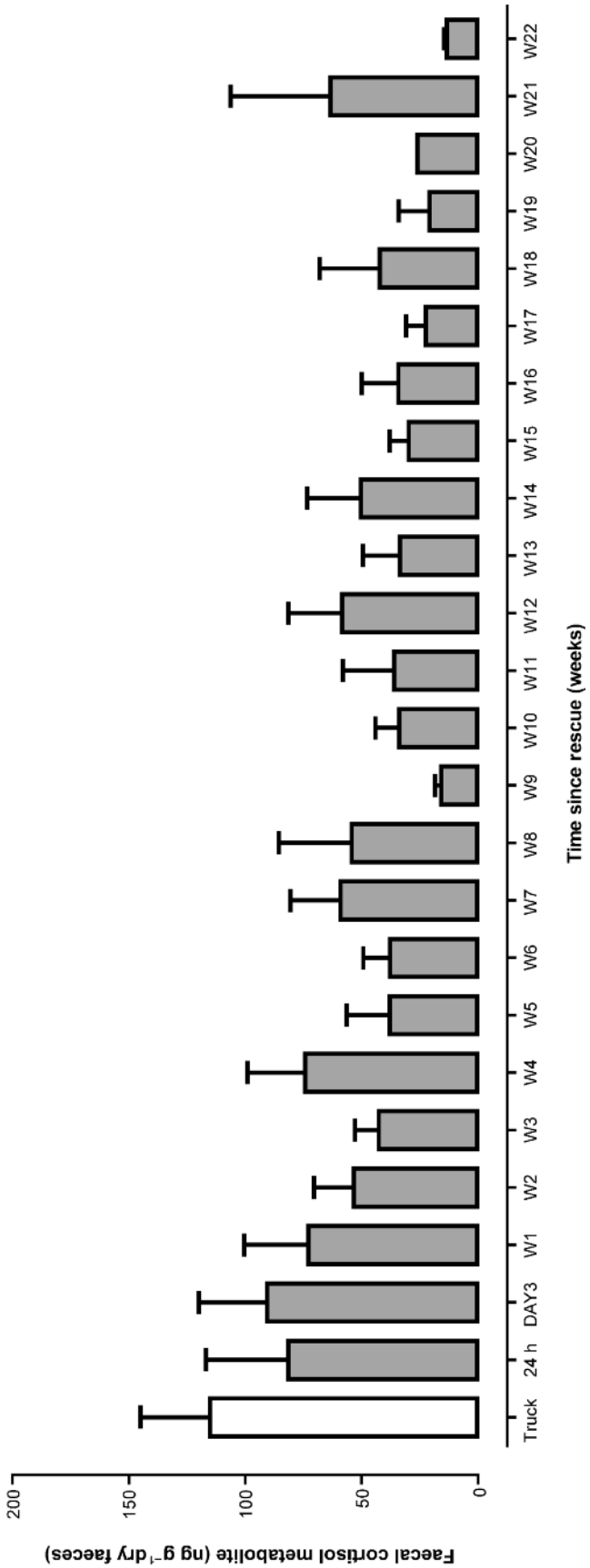
Stress hormone levels in rescued Asiatic black bears

The average FCM level of the rescued bears (n = 16) over 22 weeks (including truck samples) was 47.82 ng g^{-1}. Concentrations of FCMs in extracts of faecal samples taken from the rescue truck ranged from 8.45–283.05 ng g^{-1} (n = 8 samples; Figure 1). Mean (± SEM) FCM levels from the truck samples (115.1 [± 29.85] ng g^{-1}) were significantly greater than control baseline FCMs recorded in the study by Malcolm *et al* (2013) ($t = 2.27$; $P = 0.029$).

Furthermore, as shown in Figure 1, all FCM levels of individual bears from truck samples were above the mean control baseline FCM level recorded in Malcolm *et al*'s 2013 study, with the exception of one data point.

Overall, the GLM ANOVA results showed that mean FCM concentrations were significantly different between time (weeks) periods ($F = 1.733$, df = 24, 204; $P = 0.022$; Figure 2). Mean FCM concentration from truck samples was significantly greater than mean FCM concentration from samples taken at week 7 (time point for release of bears from quarantine) ($P = 0.035$), week 9 (two weeks after release of bears from quarantine; $P = 0.0023$) and week 22 (end-point of study; $P = 0.0017$). Mean FCM concentration of samples collected on the truck was not significantly

Figure 2



Mean (± SEM) faecal glucocorticoid concentrations in Asiatic black bears for each sampling period taken for 22 weeks after arrival at the Animals Asia Foundation Vietnam Bear Rescue Centre.

Table 2 Identified stressors and the range of FCM concentrations measured and the range of percentage above baseline levels (33.75 [± 0.72] ng g^{-1}).

Stressors	FCM range (ng g^{-1})	Percentage increase from baseline FCM (%)
Rescue and transport from bile farms	113.153–338.22	235–903
Moving out of quarantine	39.831–283.06	18–739
Moving to new enclosures	71.72–128.83	113–282
Health checks and/or dentals	125.44–269.36	272–699
Operations and/or castrations	188.27	458
Infections	102.459	203
Injuries	141.33	319

different to mean FCM concentration of samples collected 24 h and three days after arrival at the sanctuary ($t = 2.13$; $P = 0.26$ and $t = 2.22$; $P = 0.59$, respectively). Interestingly, there was no significant differences in mean FCM concentration between samples taken at week 9 and week 22 ($t = 2.22$; $P = 0.85$) (Figure 2).

Acute physiological stress responses were noted in the bears during exposure to routine management interventions at the sanctuary. Some of the stressors identified included rescue and transport from bile farms, moving out of quarantine, moving to new enclosures, health checks, castrations, dental procedures, infections and injury.

Table 2 depicts the FCM range and the percentage increase from baseline FCM (33.75 [± 0.72] ng g^{-1}) that each stressor produced.

All of the management-related stressors have produced peaks which subsided within 24–48 h to baseline FCM levels. Baseline FCM value recorded in the study of Malcolm *et al* (2013) has been shown as dashed lines in individual graphs (Figure 3).

Discussion

This study applied non-invasive faecal glucocorticoid metabolite monitoring to evaluate physiological stress in Asiatic black bears rescued from bile farms in Vietnam. The results showed overall significantly higher FCM levels at rescue and overall reduction in mean FCM concentrations over 22 weeks post-relocation of the rescued bears into the sanctuary. FCM levels in samples taken immediately on arrival at the sanctuary (truck samples) were significantly higher than samples taken at week 22 (end of study) and, also, the FCM level of individual rescued bears (with the exception of one individual) were higher than the mean baseline level of rehabilitated bears quantified in the study by Malcolm *et al* (2013). Since cortisol metabolites measured in each faecal sample represent the amount of cortisol in the animal's blood around 24–48 h before sampling, high FCM levels in the truck samples (see Figure 1) indicated that the bears were capable of showing physiological stress responses during the rescues. This was a very interesting result because, as was similarly highlighted in the study of Malcolm *et al* (2013), the acute response during rescue indicates that bears on bile farms were able to maintain responsiveness of the HPA axis during farming and the rescue process and this result further suggests that the bears on bile farms may not have suffered adrenal exhaustion or shutdown, however this could be associated with behavioural and health-related problems. The results also showed that mean FCM concentrations during rescue were significantly higher than samples taken immediately after quarantine (week 9) and rehabilitation (week 22), which reinforces that FCM levels reduced over time in the Vietnam bear sanctuary as seen in the mean FCM concentrations for each sampling period.

Implications of the general decline in stress levels for rehabilitating bears

Prolonged environmental trauma and/or human-induced stressors can create physiological stress in animals which also end up with chronic stress (Narayan & Williams 2016). For instance, dogs that were abandoned during the prolonged refugee situation during the nuclear incident resulting from the Fukushima earthquake in Japan showed higher levels of urinary cortisol and lowered aggression towards unfamiliar people compared to those sampled from unaffected areas (Nagasawa *et al* 2012). Likewise, in humans, it has been shown that chronic stress induces psychological imbalance of the neuroendocrine stress system (McEwen 2004; Juster *et al* 2010). In the current study, mean stress levels of bears decreased during the 22-week sampling period. This suggests that the bears were adapting physiologically to their new home in the sanctuary and were able to modulate their stress levels after rehoming. However, it is also possible that the bears that have experienced chronic stress on bile farms might never fully recover in the sanctuary, showing extreme signs of ill health and abnormal behaviour. It has been shown in human studies that patients that have experienced extreme stress tend to have malfunctioning learning ability which is linked to high levels of glucocorticoids (Layton *et al* 2002).

It can be postulated that the bears were responding physiologically to the management interventions as depicted in the acute stress responses of certain individuals during rehabilitation. These acute stress responses were identified to known stressors, however, there were some peaks that were not able to be explained by known stressors. This could be because there was incomplete information from logbooks. Acute stress responses were not consistent among the 16 study bears at rescue and during rehabilitation. Some bears had FCM levels that increased and/or decreased throughout the study, either above or below the mean baseline levels, while others showed relatively stable basal FCM concentrations with only one or two peaks above baseline. This demonstrates how the stress endocrine responses are unique to each individual animal and may reflect differences in each bear's ability to cope within its new environment. We provide a caveat that the FCM levels could have also fluctuated in response to the

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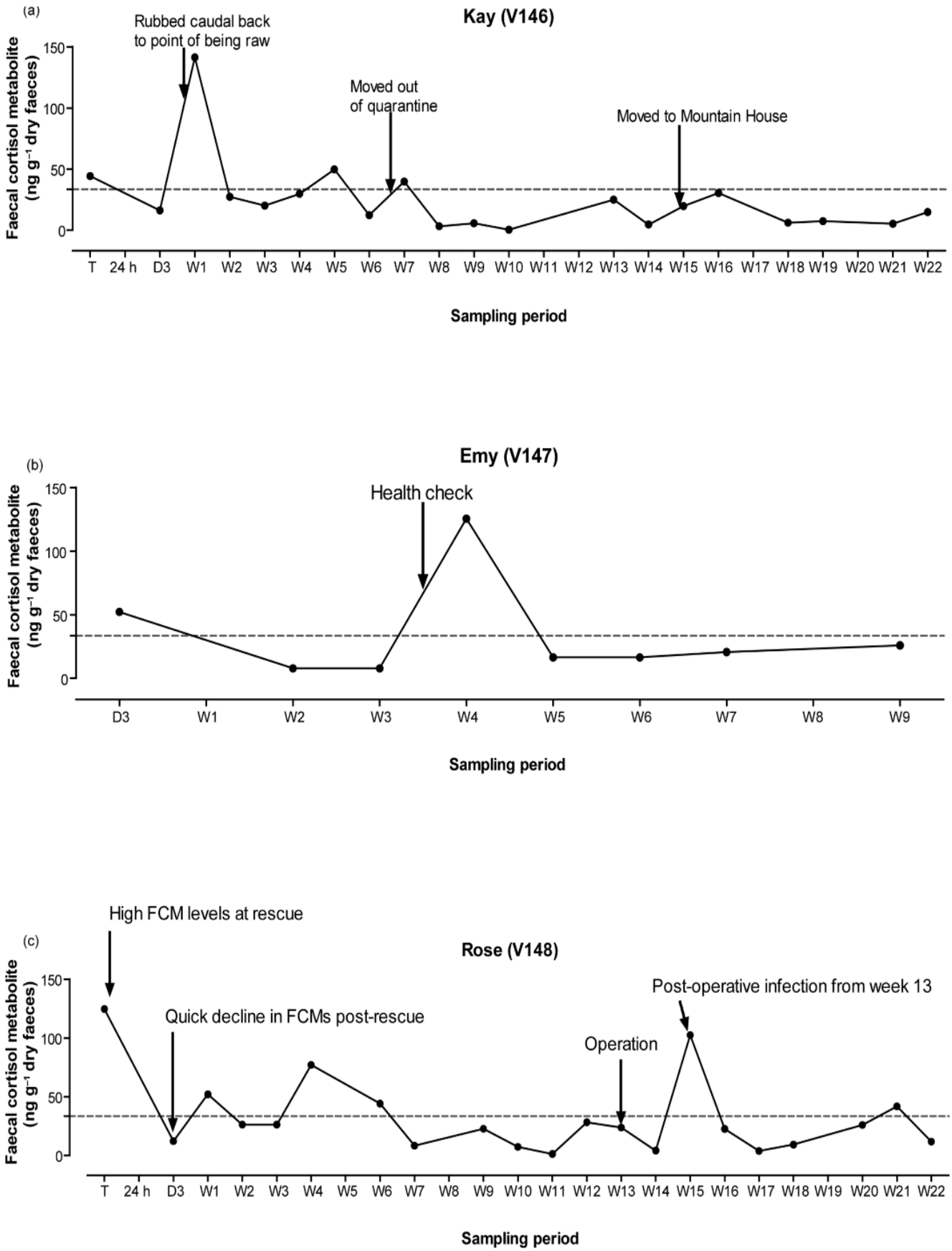
Figure 3



Individual faecal cortisol metabolite levels of a sample of rescued Asiatic black bears over 22 weeks after their arrival at the Animals Asia Foundation Vietnam Bear Rescue Centre. The horizontal dashed line on each graph represents baseline FCM levels (Malcolm *et al* 2013).

veterinary treatment, however we were unable to attain more frequent samples from individual bears so it was difficult to determine if veterinary intervention alone was enabling bears to re-settle quickly (within 22 weeks) into the sanctuary post-rescue. It is most likely that the combination of veterinary intervention and environmental enrichment provided the best opportunity for the rescued bears to rehabilitate in the sanctuary.

Conclusion

In conclusion, non-invasive faecal cortisol metabolite measurements provide a powerful tool for detecting and monitoring physiological stress in Asiatic black bears. Implementing this method in management protocols for captive animals will be essential for increasing our understanding of the Asiatic black bear's stress response in captive environments. Adopting these protocols in captive management should provide the best possible environments to maximise the effectiveness of rescue and rehabilitation efforts. There is variation between individual bears which demonstrates that each bear's response is unique and been shaped by previous experiences as well as their ability to adapt or cope with environmental stressors — highlighting the importance of individually monitoring each bear in captivity. The reduction in faecal cortisol metabolite concentrations over the 22-week study period strongly suggests that husbandry and management protocols at the Vietnam Bear Rescue Centre are effective in rehabilitating rescued Asiatic black bears.

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