Investigating temporary acyclicity in a captive group of Asian elephants (*Elephas maximus*): Relationship between management, adrenal activity and social factors

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**Abstract**

Routine faecal steroid monitoring has been used to aid the management of five captive Asian elephant (*Elephas maximus*) females at Chester Zoo, UK, since 2007. Progesterone analysis initially revealed synchronised oestrous cycles among all females. However, a 14- to 20-week period of temporary acyclicity subsequently occurred in three females, following several management changes (increased training, foot-care and intermittent matriarch removal for health reasons) and the initiation of pregnancy in another female. The aim of this study was to retrospectively investigate whether these management changes were related to increased adrenal activity and disruption of ovarian activity, or whether social factors may have been involved in the temporary cessation of cyclicity. Faecal samples collected every other day were analysed to investigate whether glucocorticoid metabolites were related to reproductive status (pregnant, cycling, acyclic) or management (training, foot-care, matriarch presence). Routine training and foot-care were not associated with adrenal activity; however, intensive foot-care to treat an abscess in one female was associated with increased glucocorticoid concentration. Matriarch presence influenced adrenal activity in three females, being lower when the matriarch was separated from the group at night compared to being always present. However, in the females that exhibited temporary acyclicity, there was no consistent relationship between glucocorticoids and cyclicity state. Although the results of this study do not fully explain this occurrence, the highly synchronised nature of oestrous cycles within this group, and the concurrent acyclicity in three females, raises the question of whether social factors could have been involved in the temporary disruption of ovarian activity.

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**1. Introduction**

Asian (*Elephas maximus*; (Choudhury et al., 2008)) and African (*Loxodonta africana*; (Blanc, 2008)) elephants are threatened with extinction in the wild, due primarily to habitat loss, human–wildlife conflict and poaching (Riddle et al., 2009). Captive populations of endangered species serve as important reservoirs to support declining *in situ* populations, allowing the generation of knowledge and act as conservation ambassadors to raise awareness for the plight of their wild counterparts. To fulfil these roles, populations must be self-sustaining and high levels of welfare maintained. There are currently around 700 Asian and 400 African elephants in zoological institutions around the world (AZA, 2011). However, historically these populations have not been self-sustaining (Clubb et al., 2009; Faust and Marti, 2011; Hutchins and Keele, 2006; Wiese and Willis, 2006), with irregular reproductive cyclicity, asymmetric reproductive ageing, reduced survivorship, high infant mortality and insufficient breeding males amongst the issues being reported (Brown et al., 2004b; Clubb and Mason, 2002; Clubb et al., 2008, 2009; Hermes et al., 2004; Hutchins and Keele, 2006). Additionally, the welfare of captive elephants has been questioned in recent years, with issues such as poor enclosure design, inappropriate social groupings, lack of
sufficient exercise, poor foot health, abnormal gait, and unsuitable nutrition being identified as significant problems (Clubb and Mason, 2002; Harris et al., 2008). To address some of these concerns, research is underway to better understand the welfare requirements of zoo-maintained elephants for optimal health and reproduction (Carlstedt et al., 2013; Posta et al., 2013).

In North America, comprehensive reproductive surveys and longitudinal hormone analyses have been conducted to assess cyclicity (Brown et al., 2004a,b; Dow et al., 2011; Proctor et al., 2010b) and found that irregular ovarian activity is a key factor in limiting fecundity; with as many as 25% of Asian and 46% of African elephants in North American zoos exhibiting either irregular cycles or complete acyclicity (Dow et al., 2011). Although a similar comprehensive survey has yet to be conducted in Europe; irregular ovarian activity has been observed (Hermes et al., 2004; S. Walker, personal communication). This acyclicity may be temporary, lasting a few weeks or months and often referred to as irregular cyclicity, or more long-term lasting several years (Brown et al., 2004a). Due to the prevalence of these issues, previous research has investigated a number of factors relating to acyclicity in African elephants, including social status, body condition and life histories (Freeman et al., 2009). However, exactly which aspects of the captive environment are involved in ovarian cycle problems have yet to be fully understood (Brown et al., 2004a; Proctor et al., 2010a).

In North America, 71% of non-cycling female African elephants have elevated serum prolactin concentrations (Dow and Brown, 2012), a condition that has been linked to stress in women (Sobrinho, 2005), and which can lead to the suppression of gonadotropins and oestrous cyclicity (Das and Khan, 2010). Although a previous study by Proctor et al. (2010a) did not find any relationship between social status, long-term acyclicity and chronic adrenal activity in female African elephants, potential relationships with temporary acyclicity, or indeed among Asian elephants have yet to be explored. There has been some suggestion that temporary cessation of ovarian cyclicity in captive elephants could be a distinct phenomenon from long-term acyclicity, and may be a stress response to social or environmental conditions (Hermes et al., 2004; Schulte et al., 2000). One of the biological responses to real or perceived stressors is the activation of the hypothalamic–pituitary–adrenal (HPA) axis (Moberg, 2000). This results in the production of glucocorticoids from the adrenal gland, which facilitates the mobilisation of energy stores and allows the individual to respond accordingly. Although this stress response is primarily adaptive, and can result from exposure to both positive and negative stimuli (Buwalda et al., 2012), prolonged activation can be detrimental and lead to suppression of immune (Khansari et al., 1990) and reproductive (Dobson and Smith, 2000) function. Captive elephants are faced with a myriad of challenges in their environment, which can include abnormal social groupings and inadequate enclosure design, as well as un-naturalistic substrates, lighting regimens and sounds, and husbandry routines (Clubb and Mason, 2002; Harris et al., 2008). These factors could potentially lead to increased adrenal activity (Morgan and Tromborg, 2007), and if individuals are unable to cope appropriately, could disrupt normal ovarian function leading to irregular oestrous cycles or acyclicity (Kalantaridou et al., 2004; Matteri et al., 2000).

Chester Zoo, UK has a breeding herd of Asian elephants, and has been using faecal progestagen monitoring since 2007 to aid reproductive management. Five of the females were considered to be potential breeders, and were included in this programme. Apart from during pregnancy and lactation, all five females have exhibited regular oestrous cycles from the initiation of this monitoring programme to the present, and in fact have shown a high degree of synchrony among females during this time. However, one exception to this was in 2008, when three females experienced a temporary cessation in cyclicity lasting between 14- and 20-weeks. In more than 7 years since routine reproductive monitoring began on this group, this was the only period of acyclicity observed, so in an attempt to understand why this unique period of temporary acyclicity occurred, the following study was established to retrospectively explore some of the potential factors that could have been involved.

The elephants at Chester Zoo underwent the transition from a free-contact to a protected-contact management programme just prior to the observed period of acyclicity. This transition resulted in a number of husbandry changes taking place during this study period, including both the type and amount of training required to enable day to day husbandry practices and to perform a routine foot-care regime. In captive elephants, non-resolvable foot infections and arthritis are major causes of euthanasia (Fowler and Mikota, 2006); thus, regular preventative foot-care is a vital component of captive elephant management. During this same period, a decline in the health of the matriarch also required her separation from the rest of the herd to allow medical treatment and supplementary feeding. Due to their highly complex social structure and the importance of sociality for welfare and reproductive success in elephants (Schulte et al., 2000), even temporary physical separation could be perceived as a stressor by individual elephants. Therefore, removing the matriarch of this herd, even temporarily, could have consequences for the remaining individuals. One hypothesis for the observed temporary period of acyclicity was that the accumulation of multiple husbandry changes, namely matriarch presence and the type and frequency of training and foot-care, over a relatively short period could have been perceived as a stressor, and increased adrenal activity may have led to the disruption of ovarian activity.

Alternatively, there may have been a social basis for this disruption. In early July 2008, one of the females in the group was mated and successfully conceived. As these females’ oestrous cycles were highly synchronised, an alternative hypothesis for the temporary cessation in cyclicity is that it was in some way related to this pregnancy. The aim of the current study was therefore to determine the impact of changes in social management and routine husbandry on adrenal glucocorticoid activity of five potential breeding females within a captive herd of Asian elephants, using non-invasive faecal hormone analyses that were temporally-matched with husbandry data (Edwards et al., 2013). In addition, the degree of oestrous synchrony within this group was examined and analysed as a potential alternative explanation for the temporary cessation of ovarian cyclicity.

2. Materials and methods

2.1. Study subjects

This study was conducted on a captive herd of Asian elephants (E. maximus) at Chester Zoo, UK. At the time of the study, the herd was composed of three males (one breeding bull and two juveniles) and seven females (Table 1). The nucleus of this herd consisted of three generations of related females (CZF5, CZF6 and CZF7), along with four unrelated females. The oldest and most dominant female (CZF1) had been at the zoo since 1965, and although she was considered to be the herd matriarch, the dynamics within the group were not strictly linear at this time, with female CZF3 frequently challenging the matriarch, CZF1. Five females were included in the current study, all of which have been part of a non-invasive reproductive monitoring programme since February 2007. The other two older females (CZF1 and CZF2) were considered to be non-reproductive, and were not included in routine faecal monitoring or this study.

All seven female elephants were housed together as a single herd, including the two juvenile males (CZM2 and CZM3). The herd
Table 1 Composition and relationships of a herd of captive Asian elephants at Chester Zoo, UK at the start of the study period. Mean, standard deviation and range in faecal glucocorticoid metabolite concentrations are given for each of the five females included in the study, along with the number of samples (N) analysed per female.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Sex</th>
<th>Origin</th>
<th>Cycling</th>
<th>Offspring produced</th>
<th>Relationship to other group members</th>
<th>Study subject</th>
<th>Glucocorticoid metabolite concentration (ng/g faeces)</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Range</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>CZF1</td>
<td>~52y</td>
<td>F</td>
<td>w/c</td>
<td>Unknown</td>
<td>26/10/74 (U) 11/07/78 (M)</td>
<td>Unrelated</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CZF2</td>
<td>~42y</td>
<td>F</td>
<td>w/c</td>
<td>Unknown</td>
<td>25/04/98 (M) 18/07/00 (M) 07/10/04 (M)</td>
<td>Unrelated</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CZF3</td>
<td>~40y</td>
<td>F</td>
<td>w/c</td>
<td>Yes</td>
<td>–</td>
<td>Dam to CZM2</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CZF4</td>
<td>~24y</td>
<td>F</td>
<td>w/c</td>
<td>Yes</td>
<td>09/09/93 (U) 19/12/95 (F) 31/12/97 (F) 07/10/00 (M) 05/03/04 (U) 12/11/06 (M)</td>
<td>Dam to CZF6 and CZM3</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CZF5</td>
<td>~27y</td>
<td>F</td>
<td>w/c</td>
<td>Yes</td>
<td>–</td>
<td>Grand-dam to CZF7</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CZF6</td>
<td>9y 11m</td>
<td>F</td>
<td>c/b</td>
<td>Yes</td>
<td>–</td>
<td>CZF5’s daughter Dam to CZF7</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CZF7</td>
<td>3y 9m</td>
<td>F</td>
<td>c/b</td>
<td>Yes</td>
<td>–</td>
<td>CZF6’s daughter</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CZM1</td>
<td>13y 0m</td>
<td>M</td>
<td>c/b</td>
<td>N/A</td>
<td>07/03/04 (F) 12/11/06 (M)</td>
<td>CZF5’s granddaughter Sire to CZF7 and CZM3</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CZM2</td>
<td>3y 2m</td>
<td>M</td>
<td>c/b</td>
<td>N/A</td>
<td>–</td>
<td>CZF5’s son</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CZM3</td>
<td>1y 0m</td>
<td>M</td>
<td>c/b</td>
<td>N/A</td>
<td>–</td>
<td>CZF5 and CZM1’s son</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Sequential numbers within females reflect the relative dominance hierarchy.

b Age (years) at the start of the study period.

c Female cyclicity based on faecal progestagen metabolite analysis prior to the initiation of the study; CZF1 and CZF2 were non-breeding females, so were not monitored routinely.

* Although this represents the average dominance hierarchy, the direction of these dominance relationships was sometimes reversed, with CZF3 being dominant over CZF1.

was occasionally separated into two groups either due to aggression within the herd or so that the breeding bull (CZM1) could be given access to a sub-set of females for breeding purposes. In addition, individuals were temporarily separated from the herd for management purposes, as described in Section 2.5. The elephant enclosure consists of an indoor house (1000 m² plus separate 280 m² bull pen), and an outdoor paddock (5490 m²) with a large (300 m²) pool. Elephants had access to both indoor and outdoor enclosures between May and September, and were kept inside at night between October and April.

2.2. Faecal sample collection

Routine monitoring of faecal progestagen metabolite concentration to facilitate reproductive management of the five potential breeding females in this herd had begun in February 2007. Faecal samples were collected approximately every other day between 0600 h and 0900 h, and frozen immediately after collection for routine analysis of progestagen metabolites. The herd is managed using protected contact, so if defecation was not observed directly, close-circuit television recordings and indigestible faecal markers were used for proper sample identification. During the current study, faecal markers were used in two females. One female was fed 100 g dry corn mixed with molasses in a loaf of bread twice a day; the other was fed 200 g dry wheat mixed with pellets that had been soaked in water to form a mash, once a day. To investigate the occurrence of temporary acyclicity within this group of elephants, this study utilised a 16-month subset of the longitudinal dataset (day 0–486), designated to incorporate the acyclic period along with approximately two oestrous cycles prior and one oestrous cycle after. During this defined study period, faecal samples (n = 956; 173–217 samples/individual) were analysed for glucocorticoid metabolites in addition to routine progestagen monitoring. Female CZF4 was translocated to another institution at day 411 of the study period, thus data collection for this female ceased at that time.

2.3. Faecal extraction and enzyme immunoassays

Faecal samples were processed using a wet-weight shaking extraction technique adapted from Walker et al. (2002). In brief, 0.50 g of well-mixed faecal material was combined with 5.0 ml 90% methanol, shaken overnight at room temperature on an orbital shaker and centrifuged for 20 min at 598 g. The methanol fraction was decanted and evaporated to dryness, before being resuspended in 1.0 ml methanol and stored at −20 °C until analysis.

Faecal progestagen (fPGM) and glucocorticoid (fGCM) metabolites were analysed using previously described optimal enzyme immunoassay protocols Edwards et al. (2014) and Watson et al. (2013) adapted from Munro and Stabenfeldt (1984). Each EIAs utilised an antiserum (monoclonal anti-progesterone CL425 or polyclonal anti-corticosterone CJM006; C.J. Munro, University of California, Davis), corresponding horseradish peroxidase (HRP) conjugated label (C.J. Munro, University of California, Davis), and standards (P0130 and C2505; Sigma–Aldrich, UK) conducted on a Nunc-Immuno Maxisorp (Thermo-Fisher Scientific, UK) microtitre plate. Firstly, 50 µl/well of antigen (1:10,000 for progesterone or 1:15,000 for corticosterone diluted in coating buffer (0.05 M NaHCO₃, pH 9.6)) was loaded and incubated overnight at 4 °C. The following day, plates were washed five times (0.15 M NaCl, 0.05% Tween 20), before 50 µl/well standards (progesterone, 0.78–200 pg/well or corticosterone 3.9–1000 pg/well) or samples diluted (1:40 or 1:10, respectively) in EA buffer (0.1 M NaPO₄, 0.149 M NaCl, 0.1% bovine serum albumin, pH 7.0) were loaded; followed by 50 µl/well HRP-conjugated label (1:35,000 for progesterone or 1:15,000 for corticosterone diluted in EA buffer). According to optimal protocols for each assay, plates were incubated for 2 h at room temperature either in constant light (progesterone)
or in the dark (corticosterone). They were then washed five times (0.15 M NaCl, 0.05% Tween 20) and 100 µl/well of substrate (0.4 mM 2,2’-azino-di-(3-ethylbenzthiazoline sulphonic acid) diammonium salt (ABTS), 1.6 mM H₂O₂, 0.05 M citrate, pH 4.0) was added, incubated at room temperature either in constant light (progesterone) or in the dark (corticosterone), then absorbance measured at 405 nm.

The cross-reactivities of the antibodies CL425 and CJM006 have been reported elsewhere (Walker et al., 2008; Watson et al., 2013). The immunoassays were biochemically validated for measuring progestagen and glucocorticoid metabolites in female Asian elephant faecal extracts through parallelism and matrix interference assessment. Firstly, serial dilutions of faecal extract yielded a displacement curve parallel to the standard curve (progesterone: \( y = 0.664x + 21.441, R^2 = 0.967, F_{1,7} = 208.057, P < 0.001 \); corticosterone: \( y = 0.743x + 32.711, R^2 = 0.902, F_{1,7} = 64.702, P < 0.001 \)). This indicates that for each EIA, the antibody was recognising the faecal metabolites proportionally to the synthetic standard curve. Secondly, there was no evidence of matrix interference on either EIA, as addition of diluted faecal extract to synthetic standards did not alter the amount observed (progesterone: \( y = 0.584x – 0.568, R^2 = 0.992, F_{1,7} = 824.269, P < 0.001 \); corticosterone: \( y = 0.693x + 6.262, R^2 = 0.994, F_{1,7} = 1145.114, P < 0.001 \)). The corticosterone EIA was biologically validated for assessing adrenal status via fGCM in female Asian elephants in a previous study conducted in the same laboratory (Watson et al., 2013). The progesterone EIA has previously been shown to be biologically valid for African elephant faeces (Freeman et al., 2011), and was similarly validated for Asian elephants in this study by showing clear increases in fPGM during gestation, increasing gradually for the first 3–4 months and remaining elevated above baseline for a total of 21–22 months. Intra- and inter-assay coefficients of variations (CVs) were <10% and <15% respectively for both assays.

### 2.4. Characterising reproductive cyclicity

Oestrous cycles were determined from fPGM profiles and characterised according to a previously established method (Brown et al., 1999b; Glaeser et al., 2012), whereby samples with baseline hormone concentrations were distinguished from those with elevated hormone concentrations using an iterative process. To calculate a baseline fPGM concentration for each elephant, sample concentrations that exceeded the mean plus 1.5 standard deviations (SD) were removed and the process repeated until no samples exceeding 1.5 SD from the mean remained. This iterative process was performed in R version 3.1.1 (R Core Team, 2014) using the package ‘hormLM’ (Fanson and Fanson, 2014). Oestrous cycles were defined according to the criteria for Asian elephants, as described by Glaeser et al. (2012): (1) the onset of the luteal phase was considered to be the first sample where fPGM concentration exceeded 1.5 SD above the mean, and remained elevated for at least two consecutive weeks; (2) the end of the luteal phase was considered to be when fPGM concentration went below the threshold of 1.5 SD above the mean, and remained low for at least two consecutive weeks; and (3) single point increases or decreases in fPGM were assigned to the same phase as the surrounding points. Cycle length was determined from the first follicular phase sample to the last luteal phase sample.

To determine the degree of synchrony between females’ oestrous cycles, smooth splines were fitted to fPGM data for each female, using R version 3.1.1 (R Core Team, 2014). Peak fPGM concentration was estimated from the maxima of these spline curves, and the variance in day of peak concentration between females calculated for each cycle. Synchrony was then calculated as 1/between individual variance, and compared to previously published values (Weissenböck et al., 2009).

### 2.5. Management factors

Information on management factors are routinely recorded into the Animal Records Keeping System (ARKS) and the elephant keepers’ daily diaries, and were compiled retrospectively for each elephant every day during the 16-month study period (days 0–486). Management factor categories and their relative frequencies for each of the five subject females are given in Table 2.

Four months prior to the start of the current study, management of the herd was changed from free-contact (FC) to protected-contact (PC). As part of this transition, all individuals received PC training to enable routine husbandry procedures to be carried out. This involved separating the elephant and keeper by a fence line, and obtaining desired behaviours through voluntary cooperation by the animal, with food rewards as positive reinforcement. Individual elephants were separated from other group members for between 10 and 40 min during these training sessions.

### Table 2

<table>
<thead>
<tr>
<th>Reproductive state</th>
<th>CZF3</th>
<th>CZF4</th>
<th>CZF5</th>
<th>CZF6</th>
<th>CZF7</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Management factors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foot-care</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No foot-care</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Routine foot-care</td>
<td>14.8%</td>
<td>13.8%</td>
<td>5.5%</td>
<td>12.2%</td>
<td>0.0%</td>
</tr>
<tr>
<td>(preventative care – includes filing and trimming)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intensive foot-care</td>
<td>1.2%</td>
<td>4.4%</td>
<td>4.5%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>(treatment of specific problems, such as abscesses)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Training</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No training</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Routine training (carrying out pre-established behaviours as required for husbandry)</td>
<td>27.5%</td>
<td>11.4%</td>
<td>15.6%</td>
<td>4.1%</td>
<td>4.5%</td>
</tr>
<tr>
<td>Intensive training (introducing new behaviours as required for husbandry)</td>
<td>7.6%</td>
<td>14.3%</td>
<td>5.1%</td>
<td>2.7%</td>
<td>2.9%</td>
</tr>
<tr>
<td><strong>Matriarch presence</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day: matriarch (CZF1) is separated from the group at night, but out on the paddock with the group during the day</td>
<td>53.2%</td>
<td>68.9%</td>
<td>47.2%</td>
<td>53.0%</td>
<td>52.0%</td>
</tr>
<tr>
<td>Night: matriarch (CZF1) is mixed in with the group in the indoor enclosure overnight, but separated during the day</td>
<td>9.2%</td>
<td>1.5%</td>
<td>9.9%</td>
<td>9.9%</td>
<td>9.9%</td>
</tr>
<tr>
<td>Both: matriarch (CZF1) is mixed with the group in the indoor enclosure overnight, and out with the group on the paddock during the day</td>
<td>14.2%</td>
<td>19.4%</td>
<td>18.9%</td>
<td>18.5%</td>
<td>18.9%</td>
</tr>
<tr>
<td>Neither: matriarch (CZF1) is separated from the group at night, and separated during the following day</td>
<td>23.4%</td>
<td>10.2%</td>
<td>24.0%</td>
<td>18.3%</td>
<td>19.3%</td>
</tr>
</tbody>
</table>

1 Reference category to which each term is compared in the GLMM.
sessions. A training session on a given day was categorised as ‘routine training’ if it included only previously established behaviours as required for husbandry and health checks, or ‘intensive training’ when a new behaviour was being established to improve access to the individual and their level of husbandry.

In addition to PC training, routine foot-care was administered as part of a preventative health care protocol. Routine foot-care consisted of filing nails and trimming the pad or cuticle (Roocroft and Oosterhuis, 2001); during treatment individuals were separated from the group for between 10 and 40 min. More intensive foot-care was given whenever warranted, for example during the treatment of a foot injury or abscess. During the study period, intensive foot-care was conducted on three females (CZF3, CZF4, and CZF5) for the treatment of abscesses or foot injuries.

During the study period, the herd matriarch (CZF1) was periodically separated from the rest of the group for health reasons. This separation was not always continuous, but occurred during the day, during the night, or both, depending on her husbandry requirements. When separated during the day, the matriarch received intensive foot-care to alleviate foot problems, and was completely separated from the group with no visual or tactile contact for periods of greater than 4 h. When separated at night, she was removed at 1700 h and returned at 0800 h the following day. This was due to a decline in sleep while present with the herd, and to allow supplementary feeding to increase her body condition. In contrast to the daytime separations, at night the matriarch was physically removed from the group, but remained in visual and tactile contact across a fence line. Matriarch presence was defined as being in the same enclosure as other group members without being separated by a fence line, and categorised as: (1) being present during the previous night only; (2) during the day only; (3) both during the day and the previous night; or 4) neither.

2.6. Statistical analyses

Data were analysed using generalised linear mixed models (GLMM) in MLwiN version 2.02 (Rasbash et al., 2005). This allowed random effects to be incorporated into the model to control for repeated faecal samples and management observations from individual elephants over the study period. The dependant variable for all models, fGCM, was log10 transformed to normalise the distribution of data (log10 fGCM), and is presented in this format throughout. As we were interested in investigating individual-level effects, separate models were created for each elephant. All models incorporated fixed factors from day n related to log10 fGCM from day n + 1, to allow for a 24 h time-lag from hormone production to excretion in faeces (Fuller et al. (2011). Observation day was included as a random effect in all models; the fixed effects and their reference categories are defined in Table 2.

For each individual, models were run to investigate any differences in log10 fGCM concentration according to their reproductive state (pregnant (where applicable), cycling, acyclic (where applicable)), and the impact of management practices (matriarch presence, training, foot-care) on log10 fGCM. All fixed effects were entered into the GLMM together before non-significant terms were dropped sequentially until only those that explained significant variation in log10 fGCM concentration remained. All statistics reported are taken from this, the minimal model, and any non-significant terms of interest were re-entered individually into the minimal model to determine their level of non-significance. A normal error structure was used for all models of log10 fGCM, and the significance of each fixed effect was determined using the Wald statistic and chi-squared ($\chi^2$) distribution, with alpha set to 0.05. Glucocorticoid data are presented as the mean prediction ± standard error (SE) taken from the minimal model of log10 fGCM, to control for non-independence of data.

3. Results

3.1. Reproductive cyclicity

Progestagen data is presented spanning 2 years (day –94 to 636; Fig. 1), extending before and after the study period (day 0–486) during which both fPGM and fGCM were measured. Based on fPGM profiles, all five females in the group were exhibiting clear oestrous cycles for three months prior to, and at the beginning of the study period (Fig. 1; day –94 to day 101). This included the juvenile female (CZF7), who was already exhibiting clear oestrous cycles at the start of this monitoring period, age 3.7 years. Average cycle length among all five females during this two-year period (mean ± standard deviation) was 102.3 ± 16.8 days, comprised of the follicular phase (40.4 ± 24.8 days) and luteal phase (64.8 ± 25.8 days).

Peak concentrations of fPGM during the first two cycles were temporally synchronised between all five females, with peak fPGM concentrations occurring between days –39 and –35, and between days 58 and 62, respectively. The four females that exhibited a non-pregnant luteal phase during cycle three also had synchronised peak fPGM concentrations between days 174 and 180. The degree of synchrony among the group was 0.31, 0.36 and 0.15 during these three periods. Although peak fPGM concentrations were temporally synchronised between these five females, the concentrations observed were highly variable among females. Baseline values ranged from 4.8 to 37.6 ng/g faeces, and peak luteal concentrations ranged from 33.1 to 154.4 ng/g faeces (Fig. 2). Furthermore, peak concentration during pregnancy in this group ranges from 99.1 to 244.3 ng/g faeces (S. Walker, unpublished data).

Following the first two synchronised cycles, female CZF3 was mated (day 128) and successfully conceived as illustrated by a sustained elevation in fPGM. The remaining four females completed their luteal phase before returning to baseline. A second female, CZF6, then conceived at the beginning of the third cycle (day 236), again illustrated by a sustained elevation in fPGM. However, the remaining three females (CZF4, CZF5 and CZF7) exhibited prolonged periods of baseline fPGM with no evidence of oestrous cyclicity, lasting 138, 183 and 103 days, respectively (Figs. 1 and 2), which were categorised as acyclic periods. Following these periods, CZF4 resumed cycling before being translocated to a new institution on day 411; CZF5 conceived immediately following her acyclic period (day 379); and CZF7 recommenced cycling and re-synchronised with CZF3, who had subsequently lost her pregnancy around day 335 and resumed cycling shortly thereafter. These two females (CZF3 and CZF7) again exhibited synchronised peak fPGM concentrations occurring between days 485 and 487, and between days 584 and 588, respectively (Fig. 1). The degree of synchrony between these two females was 0.50, 0.13 for these two cycles, and in fact remained synchronised for the following two years, until CZF7 was mated and successfully conceived (data not shown).

3.2. Adrenal activity

The range of fGCM concentrations for each subject, along with mean and standard deviation are given in Table 1. Mean fGCM concentrations were very similar across females, and there was no significant effect of age (GLMM $\chi^2 = 0.114$, df = 1, $P = 0.74$) or dominance rank (GLMM $\chi^2 = 0.003$, df = 1, $P = 0.96$) on log10 fGCM concentrations across females. However, there was considerable variation within each female during the study period (Fig. 2).

3.3. Glucocorticoids, reproductive state and management

In two of the three females that conceived during the study period, reproductive state was a significant predictor of log10 fGCM

concentration, with lower concentrations during the early stage of pregnancy compared to when they were cycling (CZF3 log10 fGCM pregnant 1.17 ng/g ± 0.02 ng/g, cycling 1.24 ng/g ± 0.02 ng/g: GLMM $\chi^2 = 10.003$, df = 1, $P = 0.002$; CZF6 log10 fGCM pregnant 1.25 ng/g ± 0.02 ng/g, cycling 1.36 ng/g ± 0.02 ng/g: GLMM $\chi^2 = 26.840$, df = 1, $P < 0.001$). This was not the case for the third female, with no difference between cycling and pregnant periods (CZF5 log10 fGCM pregnant 1.27 ng/g ± 0.02 ng/g, cycling 1.25 ng/g ± 0.02 ng/g: GLMM $\chi^2 = 0.218$, df = 1, $P = 0.64$). In the three females that temporarily ceased cycling, there were no differences in log10 fGCM concentration between cycling and acyclic periods (CZF7 log10 fGCM cycling 1.19 ng/g ± 0.01 ng/g, acyclic 1.18 ng/g ± 0.02 ng/g: GLMM $\chi^2 = 0.259$, df = 1, $P = 0.61$; CZF5 log10 fGCM cycling 1.25 ng/g ± 0.02 ng/g, acyclic 1.23 ng/g ± 0.03 ng/g: GLMM $\chi^2 = 0.585$, df = 1, $P = 0.44$; CZF4 log10 fGCM cycling 1.25 ng/g ± 0.02 ng/g, acyclic 1.22 ng/g ± 0.04 ng/g: GLMM $\chi^2 = 1.456$, df = 1, $P = 0.23$).

Controlling for any significant differences according to each female's reproductive state, three females within the group had lower log10 fGCM concentration when the matriarch, CZF1, was separated from the group at night but reintroduced the following day, compared to when she was always present (CZF5 log10 fGCM day 1.18 ng/g ± 0.01 ng/g, both 1.18 ng/g ± 0.02 ng/g: GLMM $\chi^2 = 0.000$, df = 1, $P = 1.000$), or on female CZF3 (log10 fGCM day 1.25 ng/g ± 0.03 ng/g, both 1.26 ng/g ± 0.03 ng/g: GLMM $\chi^2 = 0.057$, df = 1, $P = 0.81$) (Fig. 3).

Routine training and foot-care were not associated with any change in adrenal activity in any of the subject females (all $P > 0.05$). However, in one female who experienced a foot abscess during the study period, intensive foot-care was associated with an increase in log10 fGCM (CZF5 log10 fGCM intensive footcare 1.41 ng/g ± 0.05 ng/g, none 1.26 ng/g ± 0.02 ng/g: GLMM $\chi^2 = 9.250$, $P = 0.002$). Furthermore, log10 fGCM also tended to be higher during periods of intensive training in the same female (CZF5 log10 fGCM intensive training 1.33 ng/g ± 0.04 ng/g, none 1.26 ng/g ± 0.02 ng/g: GLMM $\chi^2 = 3.419$, $P = 0.064$).

### 4. Discussion

The potential for increased adrenal activity to disrupt reproduction has previously been demonstrated in a range of domestic (Dobson et al., 2003; Rivier and Rivest, 1991; Tilbrook et al., 2002) and wildlife (Boonstra et al., 1998; Moreira et al., 2007; Williams et al., 2007) species. Disruption can occur at many stages, including secretion of GnRH from the hypothalamus, follicular development and ovulation (Dobson and Smith, 2000); all of which could potentially lead to the observation of acyclicity. Previous research has indicated that in the white rhinoceros (Ceratotherium simum), mean glucocorticoid concentration did not differ between...
Fig. 2. Individual profiles of faecal progestagen (fPGM, black) and glucocorticoid (fGCM, red) metabolite concentration in the five study females between days 0 and 486 ((a) CZF3 to (e) CZF7). The prolonged period where fPGM did not increase above baseline in three of the females is illustrated by the dotted black lines (b, c and e). Panels below indicate the occurrence of routine foot-care (orange triangles), intensive foot-care (green triangles), routine training (yellow triangles), intensive training (blue triangles) and abscesses (purple line), where relevant. Arrows represent mating and solid black lines represent confirmed pregnancies. As in the GLMM, fGCM data is log₁₀ transformed, and all hormone data is adjusted by 24 h to match up with management factors occurring the previous day, allowing for a 24 h excretion time in this species. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
females that were cycling normally and those that were acyclic, but the variability in glucocorticoids was significantly greater amongst the latter group. However, in elephants, two previous studies have found no relationship between acyclicity and either the concentration or variability in serum glucocorticoid concentration (Brown et al., 2004b; Proctor et al., 2010a). This is consistent with the findings of the current study, as observed acyclicity was not explained by increased adrenal activity, with no differences
in glucocorticoids observed between the acyclic period and normal ovarian activity. Thus, the temporary cessation of oestrous cyclicity observed in this group of Asian elephants may not necessarily have been in response to altered adrenal activity, but could be related to other stimuli.

One potential cause of temporary acyclicity is environmental and/or seasonal effects. Elephants are considered to be non-seasonal breeders, and cycle continuously throughout the year (Hildebrandt et al., 2011). However, in a group of three female African elephants at a zoo in North America, five out of six periods of temporary acyclicity occurred during the winter when females spent significantly less time outside (Schulte et al., 2000). Ayclicity was not considered to be due to increased agonistic interactions among the females whilst housed in closer proximity, but it was suggested that a build-up of chemicals or olfactory stimuli may have caused suppression of oestrous cycles (Schulte et al., 2000). Although the period of acyclicity observed in the current study (September–March) also occurred over the winter when females were kept inside overnight (September–May), in more than 7 years since routine reproductive monitoring began on this group, this was the only period of acyclicity observed. Furthermore, between November and May of the previous year, when housing conditions were the same as those during the acyclic period, the females were all cycling synchronously.

In African elephants, ovarian inactivity has been found to correlate with dominance rank, with the dominant female within a group generally more likely to exhibit acyclicity (Freeman et al., 2004, 2009). However, in one group of African elephants where all females exhibited periods of temporary acyclicity, the dominant female exhibited the shortest overall duration (Schulte et al., 2000). In the current study, dominance status was not directly related to the short-term period of acyclicity, as the three females that underwent a temporary cessation were relative ranks 4, 5 and 7 within the group, whereas the higher ranked female (CFZ3) was cycling regularly, and conceived immediately prior to the occurrence of acyclicity. Although there were two older, more dominant females within this captive herd, both were considered to be post-reproductive, and were not included in the reproductive monitoring programme. Although this meant that we could not determine the extent of ovarian activity in these two older females, the resident bull was not observed exhibiting sexual interest in either of these older females in the months prior to or during the study, perhaps indicating that they may not have been exhibiting oestrus during this time-frame.

To investigate the issue of acyclicity in elephants, we perhaps need to make a distinction between temporary and long-term ovarian inactivity, which may not represent the same phenomenon. The latter has so far been found to correlate with dominance rank, body condition and the length of time housed at a particular facility (Freeman et al., 2009), associated with hyperprolactinaemia (Brown et al., 1997), and can be pathological in nature (Brown et al., 1999a). In contrast, there has been less research conducted on temporary acyclicity, but it has been suggested that temporary acyclicity could be an adaptive mechanism in response to changes or challenges in the environment that may make reproduction temporarily unfavourable, and so may be a facultative mechanism to prevent asymmetric reproductive ageing (Hermes et al., 2004). This is supported by the data of Schulte et al. (2000), where environmental factors were considered to be the cause of temporary cessation. However, as the females in this current study group were highly synchronised prior to the acyclic period, and three out of five females became acyclic at the same time, an alternative hypothesis is that instead of being adaptive in this instance, social stimuli related to their synchronicity could have contributed to this period of temporary ovarian quiescence.

Female oestrous synchronicity has been reported in a number of social species, including humans (McClintock, 1971), non-human primates (French and Stribley, 1987; Wallis, 1985), rodents (Handelmann et al., 1980; McClintock, 1978), and other small mammals (Jordan et al., 2011; Stockley, 1996), where social cues...
have been demonstrated to affect the timing of female oestrous cycles. Females living in close proximity to one another may simultaneously accelerate or decelerate the phase of their own oestrous cycle to synchronise with conspecifics. Alternatively, the subordinate female within a dyad may adjust her cycle length to that of the dominant female (Handelmann et al., 1980). There are a number of benefits to this synchronisation of oestrus among females. Firstly, if all females within a social group or within an area of habitat come into oestrus at around the same time, they may be able to attract more males, thereby gaining better choice of potential mates. Secondly, there may be benefits of birth synchrony to offspring care and survival, as females within a group giving birth at the same time may allow opportunities for allomothering (Schulte, 2000), and reduce individual risk of infanticide (Hodge et al., 2001; Poikonen et al., 2008) or predation (Gregg et al., 2001; Sinclair et al., 2000).

This phenomenon of oestrous synchrony has long been suspected in elephants (Bechert et al., 1999; Dublin, 1983; Poole, 1989; Rasmussen and Schulte, 1998; Slade et al., 2003), and has recently been demonstrated in captive African elephants (Weissenböck et al., 2009). However, perhaps due to the relative difficulty in conducting detailed longitudinal studies of reproductive physiology in wild elephants, the presence of oestrous synchrony has not yet been confirmed outside of captivity. Conspecific elephants use pheromones to gain information from each other, including social status, metabolic state and inter- and intra-sexual exchange of reproductive state (Rasmussen and Krishnamurthy, 2000). Additionally, two volatile compounds found in female urine during the luteal phase may represent a route of communicating, and possibly even influencing, ovarian function in members of a group (Dehnhard et al., 2001). However, the potential role that these substances play, the mechanism by which females synchronise oestrous cycles, and the identity of the female or females that control the synchrony remains unclear. Although we cannot determine which female may have been controlling the synchrony in this case, the temporary period of acyclicity...
observed in this group occurred at the same time in three females, and coincided with the initiation of pregnancy in CZF3. It therefore seems plausible that this relatively high-ranking female may have been mediating the oestrous synchronicity in this group. The oestrous synchrony observed in captive African elephants by Weissenböck et al. (2009) also appears to have been controlled by the dominant female. In that study, the females exhibited asynchronous oestrous cycles for most of the study period, but became synchronised with the dominant female when she recommenced cyclicity following lactational anoestrus. The Asian elephants in this current study were even more tightly synchronised than previously reported in African elephants by Weissenböck et al., with variance in peak FPGM concentration across all five females just 3.2 and 2.8 days during the first two oestrous cycles. Indeed, when CZF3 lost her pregnancy and resumed cycling, she did so synchronously with the only other non-pregnant female present at that time (CZF7), and they remained synchronised for the following two years. The observed period of temporary acyclicity could perhaps have been an artefact of the removal of some signal, preventing the three females from ovolating during the following interluteal period, and instead entered into a period during which progesterogens remained at baseline. However, the fact that one female in this group (CZF6) did not undergo the period of acyclicity along with her herd-mates, and instead conceived during the following oestrus suggests that not all individuals within this group responded equally. This highlights the need for further data to be compiled before we can understand the mechanisms involved in oestrous synchrony in elephants.

This study also investigated the potential impacts of a number of management factors on adrenal activity, including the presence of the matriarch. Similar to other captive groups of elephants (Freeman et al., 2009; Schulte, 2000), not all of the females within this group were related. However the occurrence of the oestrous synchrony suggests close bonds may exist among even unrelated females within a social group. Indeed, when the matriarch was periodically removed from the group to allow additional husbandry to be carried out, FGC concentration in three of the females was altered. Interestingly however, IGCM was not affected in the most dominant of the subject females (CZF3), nor in the youngest member of the group (CZF7). One explanation for the latter is that CZF7 resides in the presence of close relatives (dam, CZF6 and grand-dam, CZF5); thus, removal of the unrelated matriarch had minimal impact in this instance. In the case of the adult female CZF3, she was behaviourally dominant over the other four females in the study, and was often involved in agonistic interactions with the two older females in the group (CZF1 and CZF2), at times being observed to be dominant over the matriarch (CZF1). Perhaps this fairly dominant position within the group may have influenced her response to matriarch removal. Although a decrease in FGC was observed when the matriarch had been present the previous day, but was separated from the group the preceding night, as opposed to an increase as reported in wild African elephants following removal of the matriarch (Gobush et al., 2008), the alteration in adrenal activity suggests that these females were indeed affected by this removal and subsequent re-introduction of the unrelated matriarch. Further investigation would be required to understand why adrenal activity may have been decreased in this instance, but any change from normal activity could be an indicator that individuals are affected by a potential stressor. Indeed, the suppression of glucocorticoid concentration was considered to be a sign of distress in translocated rhinoceros (Linklater et al., 2010), where it was associated with suppressed reproductive hormone concentrations.

The impact of training and husbandry on adrenal activity were also investigated. As a result of the transition from free-contact to protected-contact management in the months prior to the start of this study, the type and frequency of training received, and the way in which foot-care required to maintain foot health was performed had all been altered. Despite these changes, routine training and foot-care had no significant physiological effect upon adrenal activity in any of the five subject females based on FGC concentration present in faeces the following day. However, for one individual (CZF5), intensive foot-care was required to treat an abscess, and was related to increased fGCM concentration. It was not possible to determine whether the elevated FGC concentration was due to the etiology of the abscess itself, or to its treatment, but because foot infections can lead to euthanasia in elephants (Fowler and Mikota, 2006), it is necessary to aggressively treat abscesses whenever they occur. This same female also showed a tendency for FGC concentration to be higher following days when she had received intensive training. This may have been related to the need to train in new behaviours to treat her developing abscess, as a period of intensive training was required to establish the behaviours needed for foot baths and abscess treatment to be performed. Additionally in this case, this may also be related to this female’s temperament, as keepers regard her to be more easily disturbed by changes in her surroundings or to her daily routine (J. Trotter, personal observation).

Although a number of relationships were identified, the management factors investigated here likely do not explain all of the variation observed in FGC across the study period. Activation of the HPA axis can occur in response to a wide variety of stimuli (Buwalda et al., 2012), and fluctuations over time are normal. One such example is the normal changes in glucocorticoid secretion across the oestrous cycle which may help promote normal ovarian function, including ovulation and formation of the corpus luteum (Tetsuka, 2007). Indeed, in a recent study by Fanson et al. (2014), female Asian elephants exhibited cyclic patterns in cortisol concentration with cortisol peaks among parous females occurring at the end of the follicular phase. In this current study, four out of five females exhibited peak log10 fGCM concentrations between day 14 and 31, and two females again between days 121 and 134, both at the beginning of the luteal phase. Although the timing of these peaks were slightly later relative to that observed by Fanson et al., and the pattern was not observed consistently across all females or all oestrous cycles, this is one possible explanation for some of the variability in FGC observed. In addition, there may have been stimuli other than the management factors investigated here, which could have led to temporal variation in FGC across the study period.

5. Conclusion

To improve the performance of captive breeding programmes, we need to understand what contributes to sub-optimal reproduction, and how social and environmental factors may affect adrenal activity and acyclicity. This study has illustrated how longitudinal faecal hormone analysis can be a useful tool to determine cyclicity status through FPGM concentration, and along with detailed keeper records, IGCM concentration can be used to assess an individual’s response to potentially challenging events. Although we did not identify the exact cause of this cessation in normal ovarian activity, results illustrate that this was not associated with increased adrenal activity in response to the management changes that occurred around this time. The techniques used in the current study have wide implications for captive management, as frequent hormone analysis along with a record of daily management activities provides a practical, non-invasive method for further investigation of reproductive health and welfare in relation to social and environmental factors, and to the routine husbandry practices required when these animals are maintained in captivity.
This is the first study to confirm oestrus synchrony in a captive group of Asian elephants, and the occurrence of this short-term acyclic period at the time when a more dominant female within the group conceived suggests that oestrus synchrony could have an influence on the reproductive function of other individuals within a social group. Whether the temporary acyclicity was merely an artefact of this synchrony or otherwise, this has important implications for captive elephants, as even unrelated females could influence the reproductive success of their herd-mates; an important consideration when establishing groups or translocating individuals for breeding or management purposes.

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