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The Elephant who Finally Crossed the Road – Significant Life Events Reflected in Faecal Hormone Metabolites of a Wild Asian Elephant

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Abstract. We used GPS-telemetry and faecal glucocorticoid metabolites (fGCM) to monitor a wild translocated female elephant in rainforests of Peninsular Malaysia. The elephant was GPS-tagged at translocation and her fGCM monitored within 11–22 months after translocation. The lowest fGCM concentrations were observed at the beginning of hormone monitoring, when she exhibited unusual movement patterns, moving repetitively alongside a major road without crossing it. Around the 16th month after translocation, the elephant delivered a calf and in the 18th month she crossed the road. In this period, she exhibited increased fGCM concentrations, presumably indicating response to challenging life events.

Introduction

It is challenging to study elusive Asian elephants (*Elephas maximus*) in the rainforest due to the dense foliage and limited sightings (Blake & Hedges 2004). Recent technological developments, such as GPS-telemetry and non-invasive molecular techniques, have considerably improved the wildlife ecologist toolbox and our capacity to study forest elephants. Similarly, ability to measure hormone metabolites from faeces, allows the physiological monitoring of free-ranging wildlife without the need to capture the animal.

Glucocorticoid hormones play a role in modulating daily energy needs and in helping to prepare the body to cope with challenges, managing the period of stress and in recovering after the challenge has passed (Sapolsky *et al*. 2000). Wildlife biologists can now use faecal glucocorticoid metabolites to gauge wildlife responses towards anthropogenic impacts such as tourism, logging, and translocation (Wasser *et al*. 1997; Thiel *et al*. 2008; Dickens *et al*. 2010; Wong 2017). In addition, it is considerably easier to obtain faecal samples in the field than saliva, urine, or blood (Palme *et al*. 2005), as wild Asian elephants are known to defecate up to 18 times per day (Hedges *et al*. 2005).

Furthermore, faecal glucocorticoid metabolites (fGCM) patterns are reflective of free glucocorticoid concentrations in the blood, after taking into account the gastrointestinal transit time (Touma & Palme 2005; Sheriff *et al*. 2010a), and are therefore a reasonable method to evaluate adrenal activity. However, the use of fGCM, requires various validation tests to ensure we are measuring actual adrenal responses instead of environmental or sampling artefacts (see reviews by Millspaugh & Washburn 2004; Goymann 2012). In previous studies, we have validated the use of fGCM in Asian elephants (Watson *et*

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al. 2013) and that fGCM samples are stable up to eight hours after defecation in a tropical rain forest environment (Wong *et al*. 2016).

In 1950, Selye introduced his theory of "General Adaptation Syndrome" outlining the adrenal glands' role in secreting glucocorticoids as response to stimulants (stressors). Since then, more researchers have identified links between glucocorticoid concentrations and health. In a "fight or flight" scenario when faced with a dangerous situation (e.g., zebra chased by a lion; Sapolsky 2004), within seconds to minutes, the body will release a cascade of hormones, including catecholamines and corticotropin-releasing hormone (CRH), into the blood stream; these effect a number of physiological changes in the body (Sapolsky *et al*. 2000; Sheriff *et al*. 2011). The CRH, secreted by the hypothalamus, stimulates the pituitary's secretion of adrenocorticotropic hormone (ACTH), which in turn, will stimulate the adrenal glands to release glucocorticoids minutes after the stressful encounter (Chrousos 1998; Sapolsky *et al*. 2000; Sheriff *et al*. 2011).

Glucocorticoids are steroid hormones that will exert a physiological effect on the body over a few hours, and will act through a negative feedback loop to receptors in the brain, to reduce the production of CRH and ACTH after the stressor ends (Sapolsky *et al*. 2000). In an acute stress scenario, glucocorticoids play a vital role in managing stress and assisting in recovery from stressors (Sapolsky *et al*. 2000), which includes mediating immune responses to prevent overshooting or autoimmunity, enhancing cardiovascular activation during stress, and maintaining the sensitivity of β-adrenergic receptors to catecholamines at vital locations in the body, including the heart (McEwen 1998; Sapolsky *et al*. 2000; Silverman *et al*. 2005).

Although glucocorticoids are often termed as "stress hormones", they have important functions outside the "fight and flight" stress response. Basal glucocorticoids have a circadian cycle in our body and play an important role in energy regulation. At low to moderate levels, glucocorticoids stimulate appetite; and appetite normally peaks when basal glucocorticoid concentrations are at their highest early in the morning (Sapolsky *et al*. 2000). When acute stress occurs, appetite is suppressed temporarily (less than an hour) and afterwards glucocorticoids may help build appetite to encourage metabolic intake and prepare the body for subsequent stressors (Sapolsky *et al*. 2000). If the timing and secretion pattern for glucocorticoids are disrupted (McEwen *et al*. 2015), the adrenal response is impaired (Dickens *et al*. 2009), or the body reaches exhaustion due to chronic stress (Selye 1950; Sapolsky 1999), then there could be negative impacts on health. Elevated glucocorticoids can have adverse effects on memory, learning, and cognitive function (Sapolsky 1999; McEwen *et al*. 2015). Although not so well known, low concentration of glucocorticoids is linked to acute adrenal crisis (a potentially life-threatening condition; Lee & Ho 2013), chronic fatigue syndrome (Edwards *et al*. 2011), and post-traumatic stress disorder (Raison & Miller 2003; Yehuda & Seckl 2011), amongst other health problems (Heim *et al*. 2000; Cicchetti & Walker 2001). Therefore, the ability to maintain an adequate concentration of basal glucocorticoids is vital for the body in managing daily activities (Sapolsky *et al*. 2000; Busch & Hayward 2009; Madliger & Love 2014), as well as in facing stressors or energy-intensive life-history stages such as migration (Sapolsky *et al*. 2000; Wingweld & Kitaysky 2002; McEwen & Wingweld 2003).

Peninsular Malaysia is home to an estimated population of 1223–1677 wild Asian elephants (Saaban *et al*. 2011) that, like elsewhere in the species range (Fernando & Pastorini 2011), are endangered due to the combined effect of habitat loss and human-elephant conflict (HEC). Translocation, moving elephants from conflict zones to protected areas, has been one of the main strategies to mitigate HEC in Peninsular Malaysia in recent decades, with approximately 10 to 25 wild elephants translocated every year since 1974 (Saaban *et al*. 2011; pers. comm. Nasharuddin Othman). Not much is known about the impact of translocation on elephants, nor how they fare after their release. The work presented here is part of the activity of the Management and Ecology of Malaysian Elephants (MEME)

project, a collaboration between university researchers and local wildlife authorities that aims to move towards an evidence-based conservation of elephants in Peninsular Malaysia (http://www.meme-elephants.org). Among other activities, MEME is using GPS-telemetry and fGCM monitoring to study elephant response to translocation, comparing the movement patterns (e.g. Wadey *et al*. 2018) and hormone profiles (Wong 2017) of translocated and local resident elephants at release sites. Here we present a case study that uses movement tracking and hormone profiles to gain insights into the physiological condition of a wild elephant.

Methods

Study site

The Belum-Temengor Landscape (BTL) is located in the northwest of Peninsular Malaysia (Fig. 1), and is mainly comprised of hill dipterocarp and upper dipterocarp forest. It covers the Royal Belum State Park (1175 km^2), Temengor Forest Reserve (1489 km^2), state land (131 km^2), indigenous villages, plantations, rivers and a large dam (Rayan & Linkie 2015). This landscape is bisected by the Gerik-Jeli East-West highway, of about 121 km in length (Wadey *et al*. 2018).

Study subject

The elephant in this case study is a female (named Mek Jalong) from the south of Perak that was translocated 94 km to BTL on the 20th May 2012 (day 0). Jalong was fitted with a GPS-satellite collar (~17 kg, Africa Wildlife Tracking, South Africa), which tracked her movements every two hours from day 0 to day 669. We started monitoring Jalong's fGCM in the 11th month (day 341) after her translocation and stopped in the 22nd month (day 669), when her GPS-collar failed. During this latter period (days 341–669), Jalong underwent two presumably challenging (in terms of stimulation of the HPA axis) events. The first event was the birth of her calf (Fig. 2), which took place around the 16th month after translocation. This indicates that Jalong was four to six months pregnant when she was translocated. The second event was when she crossed the East-West highway for the first time during the study period, 18 months (day 537) after translocation.

Field sample collection

We tracked Jalong on the ground by first using the GPS collar's location coordinates to know where she had been in the previous few hours and, then, using the strength of the collar's VHF signal and forest signs (e.g. footprints, disturbed

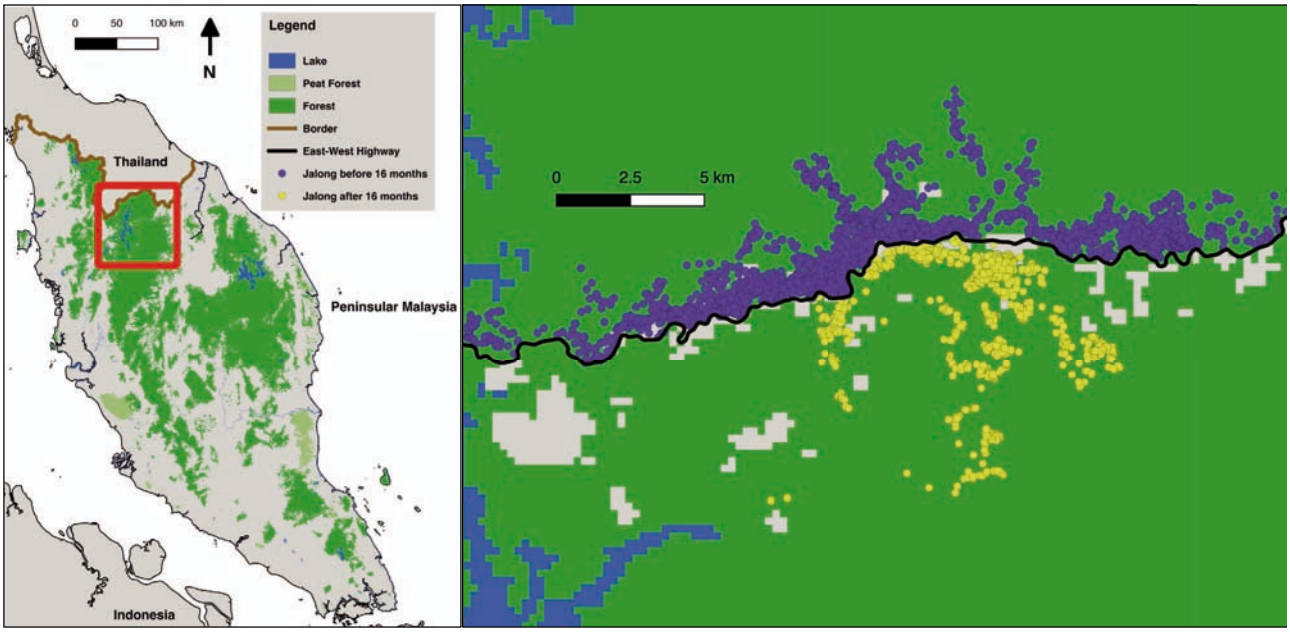


Figure 1. Combined tracklog (days 0–660) of Mek Jalong's movement before (blue dots) and after (yellow dots) crossing a major highway (black line) in the Belum-Temengor landscape for the first time on day 537 after translocation.

vegetation, and the sound of flapping ears and feeding) to narrow down Jalong's position. Once the tracking team was at close distance from the elephant (within visual or auditory range), we closely tracked her movements to look for fresh dung assumed to have been produced by her. Since the elephant never joined any other elephant (except her calf) and we were tracking her at very close range, it is highly unlikely that the dung samples we collected could have been produced by any other elephant. Once we found a dung pile, we recorded the GPS location and environmental variables associated.

The faecal samples were collected using clean surgical gloves and stored in a zip-lock bag; approximately 100 g of faecal material was removed from the middle of the bolus from an average of three intact boli. The fGCM samples were mixed thoroughly in the zip-lock bag and placed immediately in a cooler bag with ice packs, before transferring it to a portable car compressor freezer (–15°C to –18°C; Mobicool CF18C and CDF-11, Germany, powered by car AC socket) or chest freezer (–20°C) at our field station. Following Wong *et al*. (2016), only samples stored in the freezer within eight hours after defecation were used in the analysis.

In the 11 months in which we monitored Jalong's fGCM, we obtained a total 13 dung samples from nine different sampling occasions.

Laboratory analysis

We used a wet-weight extraction technique (Watson *et al*. 2013), whereby 5 ml of 90% methanol were used to extract fGCM overnight from 0.5 g (± 0.003) of a well-mixed dung sample. Extracts were dried and reconstituted in 1 ml of 100% methanol, and stored at –20°C until being analysed with a corticosterone enzyme immunoassay (CJM006, Coralie Munro, UC Davis). The biological and biochemical validation for the assay was previously carried out by Watson *et al*. (2013). Only data with an intra-assay coefficient of variation (CoV) of less than 10% and inter-assay CoV less than 15% were used for subsequent analyses.



Figure 2. Mek Jalong and her newborn calf.

Results

In the approximately 16 months after her translocation, Jalong remained solitary although there were other elephant groups in the area. She moved up and down repetitively along the northern side of the East-West highway, always close to the road but never crossing it (Fig. 1). When Jalong's fGCM monitoring started on day 341 post-translocation, for 4.5 months, Jalong's mean fGCM was 7.3 ± 1.2 ng/g (SD; Fig. 3).

Jalong was first noticed to be with her calf on day 481 post-translocation during a field track. Soon after that (day 536), Jalong crossed the East-West highway for the first time (Fig. 1). In the period between these two events, Jalong's fGCM fluctuated (mean±SD fGCM = 10.9 ± 3.9 ng/g; Fig. 3). We recorded Jalong's highest fGCM value shortly before she crossed the highway (15.9 ng/g, highest concentration throughout the monitoring period of 11 months). After crossing the highway, Jalong's movement changed and she began exploring new areas away from the road (see Fig. 1), while her fGCM concentration persisted around 11.2 ± 1.4 ng/g for the remaining four months (Fig. 3) until monitoring terminated at day 669 post-release, when the GPS satellite housing detached from the collar.

Discussion

At the beginning of the study, Jalong roamed alone and showed repetitive movements alongside the highway (i.e. she seemed attracted to the highway

but avoided crossing it). This is a very unusual movement pattern compared to other elephants monitored in this landscape (Wadey *et al*. 2018). Jalong's initial fGCM concentration was similar to fGCM concentrations found in other translocated elephants in the year immediately after translocation (8.5 ± 1.9 ng/g, N = 5; all males; Wong 2017) but lower in comparison to local resident elephants in the same landscape (11.4 ± 2.8 ng/g, N = 4, 3 males and 1 female; Wong 2017). Although the East-West highway has a negative impact on elephant movements in the area, the elephants in the landscape are still able to cross it. Wadey *et al*. (2018) found that translocated elephants in this landscape were 14 times less likely to cross the road than local ones, suggesting that road crossing is particularly challenging to elephants not familiar with the road. In separate studies, we have found that wild elephants are attracted to this highway due to the availability of grasses and other early succession plants, mainly monocots, in the area (Yamamoto-Ebina *et al*. 2016; Terborgh *et al*. 2017).

Before Jalong crossed the highway for the first time, we detected an increase in fGCM concentrations. The time of the road crossing event, however, also coincided with Jalong's delivery of her calf, which also may result in an increase in fGCM concentrations (Brown 2000). Jalong's fGCM concentration remained elevated after crossing the road; this could be related to the challenges of exploring a new environment

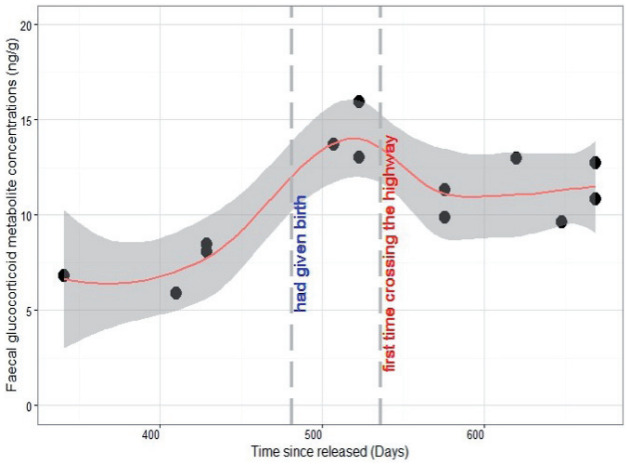


Figure 3. Faecal glucocorticoid metabolites profile for Mek Jalong. The red line was drawn using loess smoothing (span 0.75) and the shaded grey area represents standard error 95%.

(there was a change in movement patterns and Jalong was exploring areas further away from the road) and the need to be vigilant when caring for offspring (Rees *et al*. 2004). Jalong's fGCM concentration values, which persisted after crossing the highway, however, were within the usual fGCM range for local elephants in the area who crossed the highway regularly (Wong 2017). In retrospect, the increase in fGCM in Jalong's case could be due to many other reasons, but we speculate it is a positive indication that she was actively coping with challenges in her surroundings and in caring for her young calf. Although there could be innate fGCM differences between male and female elephants, both will respond to challenging situations in the field and show an increase in fGCM (Vijayakrishnan *et al*. 2018).

The unusual nature of Jalong's movements before crossing the road and her relatively low fGCM concentration compared with local elephants in the landscape (Wong 2017) could be of concern, since prolonged periods of low glucocorticoid concentrations can be associated with health problems (e.g. Dickens *et al*. 2009; Linklater *et al*. 2010; Pawluski *et al*. 2017). In future studies, researchers should investigate the importance of having an adequate amount of basal glucocorticoids in helping humans and animals to manage challenges in their surrounding (Sapolsky *et al*. 2000; McEwen & Wingweld 2003).

Alteration of the mother's glucocorticoid concentrations during pregnancy can exert influence on her offspring's (F1) and grandchildren's (F2) stress response, physiology, and health (Franklin *et al*. 2010; Sheriff *et al*. 2010b; Matthews & Phillips 2012; Khan *et al*. 2016). This also means that Jalong's translocation could potentially affect her calf's health and behaviour.

This case study demonstrates that (1) GPS-tracking can be successfully combined with fGCM to monitor the physiological condition of wild Asian elephants in tropical rainforest and (2) significant life events can be reflected in wild elephants' fGCM concentrations. Although our sample size is too small to draw conclusions, our

results and those in Wong (2017) suggest that translocation could affect elephants' health and behaviour. More research is needed to understand the relationship between elephant exposure to prolonged stress and changes in glucocorticoid concentrations, and when these hormonal changes can be harmful for the elephants. In this context, we call for the precautionary principle in managing wild elephant populations.

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