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Physical and behavioral indicators associated with hormonal changes during musth in zoo-housed and free-ranging Asian elephants (*Elephas maximus*)

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ABSTRACT

In-situ and *ex-situ* Asian elephant populations are threatened with extinction, and male elephants pose unique challenges to long-term sustainability. The heightened sexual state of "musth" is accompanied by a suite of physical, behavioral and physiological changes. Furthermore, musth is unique to male elephants and requires special consideration when developing short- and long-term management strategies for elephants in the wild and in human care. The purpose of this study was to identify associations between fecal hormone metabolites [fecal androgen metabolites, FAM; fecal glucocorticoid metabolites, FGM; and fecal triiodothyronine (T3) metabolites, FT3] and visible musth indicators [temporal gland secretions (TGS) and urine dribbling (UD)], and behavioral changes around musth. From fecal samples collected non-invasively from wild elephants in Wasgamuwa National Park, Sri Lanka, and zoo-housed elephants in the United States, we hypothesized that (1) TGS and/or UD would be associated with changes in FAM, FGM, and/or FT3 concentrations; (2) variation in fecal hormone metabolites would be associated with increased locomotion and chemosensory behavior, and decreased foraging; and (3) relationships we identified would be similar between wild and zoo-housed elephants. We found that FAM concentrations changed significantly with TGS and UD activity in both wild and zoo elephants. Further while FGM concentrations were higher with increased TGS and UD in zoo elephants, the opposite pattern occurred in wild elephants. We did not identify substantial change in FT3 concentrations with TGS/UD activity. Behavioral changes in zoo elephants were significantly associated with FAM concentration as predicted, but these relationships were more difficult to identify in wild elephants due to lower sample availability. Further, FGM concentration was directly related to time spent locomoting in zoo elephants, but no other apparent association existed between FGM concentration with other behaviors in zoo elephants, or in any behaviors in wild elephants. Likewise, we did not report associations between FT3 and any behaviors we measured. This study contributes to our understanding of the complex response patterns that male Asian elephants exhibit around musth, and it provides another example of complementary *in-situ–ex-situ* research that can be directly applied to improve the well-being of elephants and other wildlife.

1. Introduction

Asian elephants (*Elephas maximus*) are endangered with decreasing population trends across their range due to habitat loss and degradation [1]. Approximately 25 % of the 60,000 remaining Asian elephants in the world [2] are under human care (e.g., housed in zoos, wildlife parks, camps) [3]. These facilities are critically important to the continued existence of this species [3–5]. Historically, captive populations

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have been female-biased, as female elephants were preferentially imported from the wild due to their tractability [6]. However, there is now decreased reliance on wild importations as breeding success is steadily improving [7,8], and so the proportion of male elephants in *ex-situ* populations is increasing markedly [9]. This poses significant husbandry and management challenges. Male elephants are also of special interest to *in-situ* elephant population managers, as they are frequently implicated in human–elephant conflict [10–13]. Thus, investigation of male reproductive biology and behavior in wild and captive elephant populations is critical to conserve this endangered species [4,14], with the reproductive state of musth being of particular interest.

Musth is a reproductive event unique to male elephants, occurring regularly but asynchronously among post-pubertal males [15,16]. Thought to be triggered by a surge in plasma androgens (e.g., testosterone) [16–18], musth is accompanied by a suite of behavioral [19] and physiological changes [20] that can last from a few days to several months in Asian elephants [21,22]. Prolonged elevations in androgen concentrations (characteristic of musth) begin to occur around the time of sexual maturity [23,24], and while musth is not essential for successful reproduction to occur [i.e., non-musth males produce viable sperm [25]], it does facilitate inter- and intrasexual selection, signaling a male's reproductive intent to male and female conspecifics [26]. In wild Asian elephant populations, female elephants preferentially associate with mature males in musth [27–29], and musth males generally outcompete non-musth males for access to receptive females [30,31].

Musth also occurs in zoo-housed elephants [21,22], and adult males can be challenging to manage during this period, often requiring specialized care and housing, and posing problems for *ex-situ* breeding efforts. Males in musth are difficult to manage for reproductive procedures and may behave unpredictably around conspecifics when introduced for breeding [21,22,32]. Further, it can be challenging to collect blood from musth males, and thus determination of androgen status (i.e., to verify the male is in musth) can be difficult. Numerous additional physiological changes also can occur during musth that are relevant for health and management [e.g., nutrition, metabolism, and stress physiology [20,33]], not all of which are superficially apparent. Therefore, there is a need for additional methods to identify and more fully characterize the musth state, for both *in-situ and ex-situ* management.

Fortunately, development of robust methods for fecal endocrine analysis in wildlife has enabled acquisition of androgen data from musth males through entirely non-invasive means [34–37]. In additional to fecal androgens, two additional classes of hormones also may offer information about the musth state through fecal sampling: glucocorticoids and thyroid hormones [38,39]. Several studies on zoo-housed elephants have reported elevated glucocorticoids during musth [20,33], but other studies on wild elephants in musth have indicated lower glucocorticoids [36,40,41]. This indicates that musth may be associated with changes in the activity of the hypothalamic-pituitary-adrenal (HPA) axis, which orchestrates the stress response in vertebrates, releasing glucocorticoids (e.g., cortisol, corticosterone) after exposure to stressors to enact a range of short- and long-term responses [42]. Musth also may involve changes in the thyroid hormones triiodothyronine (T3) and thyroxine (T4), which drive metabolic rate in mammals and often reflect changes in metabolic or nutritional status [43–45]. *Ex-situ* studies indicate that musth may be metabolically limited, with only those male elephants with high enough body condition being able to maintain musth for sustained periods [4,46,47], suggesting a potential role for T4 or T3 in initiation or termination of musth. T4 and T3 also facilitate transitions between reproductive and non-reproductive states in some seasonally breeding species [48–50]. Relatively few studies have measured thyroid concentrations during musth [20,33], and relationships with androgens, behavior or the visual indicators are largely unknown.

During musth, male Asian elephants often display two visually conspicuous indicators associated with inter- and intrasexual chemical communication. First, temporal glands (modified apocrine sweat glands located on either side of the head behind the eye) exude chemical signals (i.e., temporal gland secretions, TGS) that have known signaling value [51]. Second, some males exhibit continuous urine dribbling (UD) on the medial side of the hind legs, producing dark stains with semiochemicals indicative of a male's musth status [52]. In Asian elephants, simultaneous TGS and UD are almost always limited to male elephants in musth, yet there appears to be significant individual variation in the occurrence and timing of these indicators [21,22]. Finally, behavioral changes during musth include increased locomotion, increased chemosensory behavior, and decreased foraging [19], but these musth-associated behaviors have never been directly linked to endocrine status in Asian elephants [but see 41, 53, 54].

In summary, by integrating datasets that include a wider array of hormonal, visual, and behavioral measures than has been attempted previously, we may more comprehensively characterize both short- and long-term features of musth in male Asian elephants. The purpose of this study, therefore, was to characterize the hormonal variation associated with visible musth indicators and behavioral changes in wild and zoo-housed male Asian elephants. Our approach involved non-invasive analysis of fecal hormone metabolites: fecal androgen metabolites (FAM; e.g., testosterone and related fecal metabolites), fecal glucocorticoid metabolites (FGM; e.g., corticosterone, cortisol, and related fecal metabolites), and fecal T3 metabolites (FT3), combined with behavioral observations and related visual indicators (TGS, UD). We hypothesized that (1) TGS and/or UD would correlate with FAM, FGM, and/or FT3 concentrations, as has been reported with plasma endocrine measures; (2) FAM, FGM and/or FT3 would also be associated with characteristic musth behaviors (increased locomotion and chemosensory behavior, and decreased foraging); and (3) wild and zoo-housed elephants would exhibit similar patterns in all these relationships, because of musth's adaptive significance.

2. Materials and methods

2.1. Study sites, subjects, and sample parameters

We sampled 26 male Asian elephants aged 9.2 to 57.0 years at ten zoo facilities throughout the US (Table 1) and 28 male Asian elephants in the wild at Wasgamuwa National Park, Sri Lanka. In wild elephants, we only included male elephants that were estimated to be older than ten years of age, gauged according to visual standards by Varma et al. [55], as this is the youngest age that males have been reported to leave their natal groups and/or exhibit signs of musth [24,29,31,56,57].

2.1.1. Sample collection from zoo-housed elephants

Zoo staff were asked to collect a weekly fecal sample from each of the study elephants for twelve months, beginning around July 2019. However, logistical challenges resulting from the COVID-19 pandemic affected the ability of some facilities to regularly collect samples beginning around March 2020, and as a result several of these facilities extended the collection period to obtain more samples on otherwise underrepresented elephants. The average ± SE number of samples collected per elephant was 50.3 ± 1.6 (min = 31, max = 61), for a span of 14.0 ± 0.4 mos (min = 9.3 mos, max = 16.7 mos), resulting in 1309 weekly fecal samples from zoo elephants in this study.

Additional fecal samples were collected daily coinciding with behavioral observations that began in July 2018 and ended in March 2021. Each zoo elephant was observed twice for five days: once when the elephant was in musth (i.e., exhibiting visual signs or behavior characteristic of an individual elephant's typical musth cycle, as determined by animal care staff), and another time when the elephant was not in musth. Not all elephants could be observed during musth, and not all elephants underwent musth over the study period. In total, 196 daily fecal samples were collected during matched behavioral observations (average ± SE samples per elephant = 7.5 ± 0.8, min = 4,

Table 1
Details of each zoo-housed elephant included in this study. Animals marked with † were born in the wild, and so birthdates were estimated to the nearest year; all other animals were born in captivity. Sampling schedule is indicated by the month of first sample, month of last sample, and the total number of weekly samples collected per elephant. The last column indicates whether the elephant experienced musth at least once over the study period, determined by elevated fecal androgen metabolite concentrations and concomitant visible musth indicators (temporal gland secretions and/or urine dribbling).

Facility	Animal	Birthdate (D-M-Y)	First sample (M-Y)	Last sample (M-Y)	No. weekly samples	Exhibited musth?
A	A1	19-Jan-09	Oct-19	Mar-21	50	Yes
A	A2†	1-Jan-71	Oct-19	Mar-21	42	Yes
A	A3†	1-Jan-73	Oct-19	Mar-21	52	Yes
A	A4	18-Nov-01	Oct-19	Mar-21	55	No
A	A5	1-Jun-05	Oct-19	Mar-21	53	Yes
A	A6	16-Aug-99	Oct-19	Mar-21	50	Yes
A	A7	21-May-02	Oct-19	Feb-21	48	No
A	A8†	1-Jan-63	Oct-19	Mar-21	52	Yes
B	B1	26-Jan-88	May-19	Jul-20	47	Yes
C	C1	27-Mar-09	Jul-19	Aug-20	46	No
C	C2	16-Jan-88	Jul-19	Aug-20	47	Yes
D	D1	17-Feb-08	Jul-19	Jul-20	56	Yes
D	D2	16-Apr-04	Jul-19	Jul-20	56	Yes
D	D3	15-Jul-08	Jul-19	Jul-20	57	No
D	D4†	1-Jan-71	Jul-19	Jul-20	57	Yes
D	D5	2-Nov-09	Jul-19	Jul-20	56	No
E	E1	4-Apr-91	Jun-19	Aug-20	60	Yes
E	E2	10-Jan-93	Jul-19	Aug-20	58	Yes
F	F1	4-May-10	Jun-19	Apr-20	31	No
F	F2†	1-Jan-65	Jul-19	Apr-20	32	No
F	F3	12-May-05	May-19	Jun-20	35	No
G	G1	2-Jul-81	Aug-19	Aug-20	49	No
H	H1	25-Nov-01	Jul-19	Aug-20	50	Yes
H	H2†	1-Jan-68	Jul-19	Aug-20	50	Yes
I	I1	8-May-97	Jun-19	Aug-20	61	Yes
J	J1	27-Dec-92	Jul-19	Sep-20	59	Yes

max = 18).

For each sample, zoo staff collected a single, small handful of fecal material (≲1 kg) from the middle of a bolus to minimize contamination from urine and the environment. Staff were asked to collect fecal material from multiple places within the bolus to produce a sample that adequately represented the much larger bolus. All samples were collected fresh within two hours of observed defecation. Samples were labeled and stored in plastic bags at –20 ºC until shipment with ice packs to George Mason University (GMU, Fairfax, VA) for analysis, where samples were again stored at –20 ºC until drying and extraction. Extraction occurred within 12 months of collection for all samples.

For each fecal sample, zoo staff noted the extent of visible musth indicators (TGS and UD) exhibited by the male when he defecated (Fig. 1), each with a score from zero for no visible secretions/dribbling to five, using scales from LaDue et al. [19] and Glaeser et al. [58]. Over the sample collection period, we asked each facility to note any major changes that occurred (e.g., births, deaths, and transfers of conspecifics; changes to exhibit spaces; major medical procedures). Husbandry conditions—including diet, feeding schedules, training, and enrichment—were consistent for each elephant over the study. Sample collection protocols were approved by GMU's IACUC (1168839-1) and by the research committees at each participating facility.

2.1.2. Sample collection from wild elephants

Fecal samples from wild elephants were obtained opportunistically over 57 days of fieldwork that occurred between December 2018 and April 2019, collected during Wasgamuwa's operating hours (06:00 to 18:00 daily), resulting in 37 samples from 31 elephants (average ± SE samples per elephant = 1.2 ± 0.1, min = 1, max = 3). Each day in the park, we drove one of three possible routes on roads in publicly accessible areas. Elephants were photographed and individually identified based on physical characteristics [59,60]. We collected samples only from elephants that we observed defecating, retrieving the sample only after the animal had moved away to a safe distance, with a maximum of one sample per elephant collected each day. All samples were collected less than six hours after defecation (average ± SE = 1.0 ± 0.2 h, minimum = 0.1 h, maximum = 5.7 h), which is well within the timespan before steroid metabolites degrade in Asian elephant feces in tropical environments [61]. Otherwise, sample collection protocols were identical to those used for zoo elephant sample collection. We noted the identity and extent of visible musth indicators (TGS and UD) of the male that defecated each sample and then sealed each sample in a plastic bag and placed it in a polystyrene cooler on ice until deposited in a –20 ºC freezer at the end of each day. Samples were transported to Rajarata University of Sri Lanka (RUSL, Mihintale, Sri Lanka) at the end of each month for drying and sifting (described below). Sample collection protocols were approved by GMU's IACUC (1168839-1) and by the Department of Wildlife Conservation of Sri Lanka (WL/3/2/57/18).

2.2. Fecal sample drying and hormone metabolite extraction

Fecal hormone metabolites were dried and extracted in the same way for zoo-housed and wild elephants, except that both drying and extraction took place for the zoo samples at GMU, while only extraction took place at GMU for the wild samples (wild elephant fecal samples were dried and sifted at RUSL, and the resulting fecal powder was shipped to GMU for subsequent analysis; for zoo elephants, samples were dried and sifted at GMU), The extraction protocol used in this study was based on those commonly used to analyze fecal steroid and thyroid hormone metabolites in elephants and other mammals [38,53,62,63], modified as follows. Fecal samples were transferred to individual paper bags and dried at 55ºC in a laboratory drying oven (Thermolyne model F30438CM; Thermo Scientific; Asheville, NC) for approximately 72 h; for a subset of samples (n = 20), we weighed each sample before and after drying to determine all moisture had evaporated after 72 h, and all other samples were visually inspected to confirm dryness after 72 h in the oven. Then, approximately 0.2500 g (mean ± SE = 0.2563 ± 0.0003 g, min = 0.2237 g, max = 0.2861 g) of sifted and mixed fecal powder was placed in a 16 × 100 mm borosilicate glass tube (Fisher Scientific; Pittsburgh, PA); each sample mass was recorded to the nearest 0.0001 g on a digital laboratory scale (Entris II BCE^{64}I–^{1}S; Sartorius Lab Instruments GmbH; Goettingen,

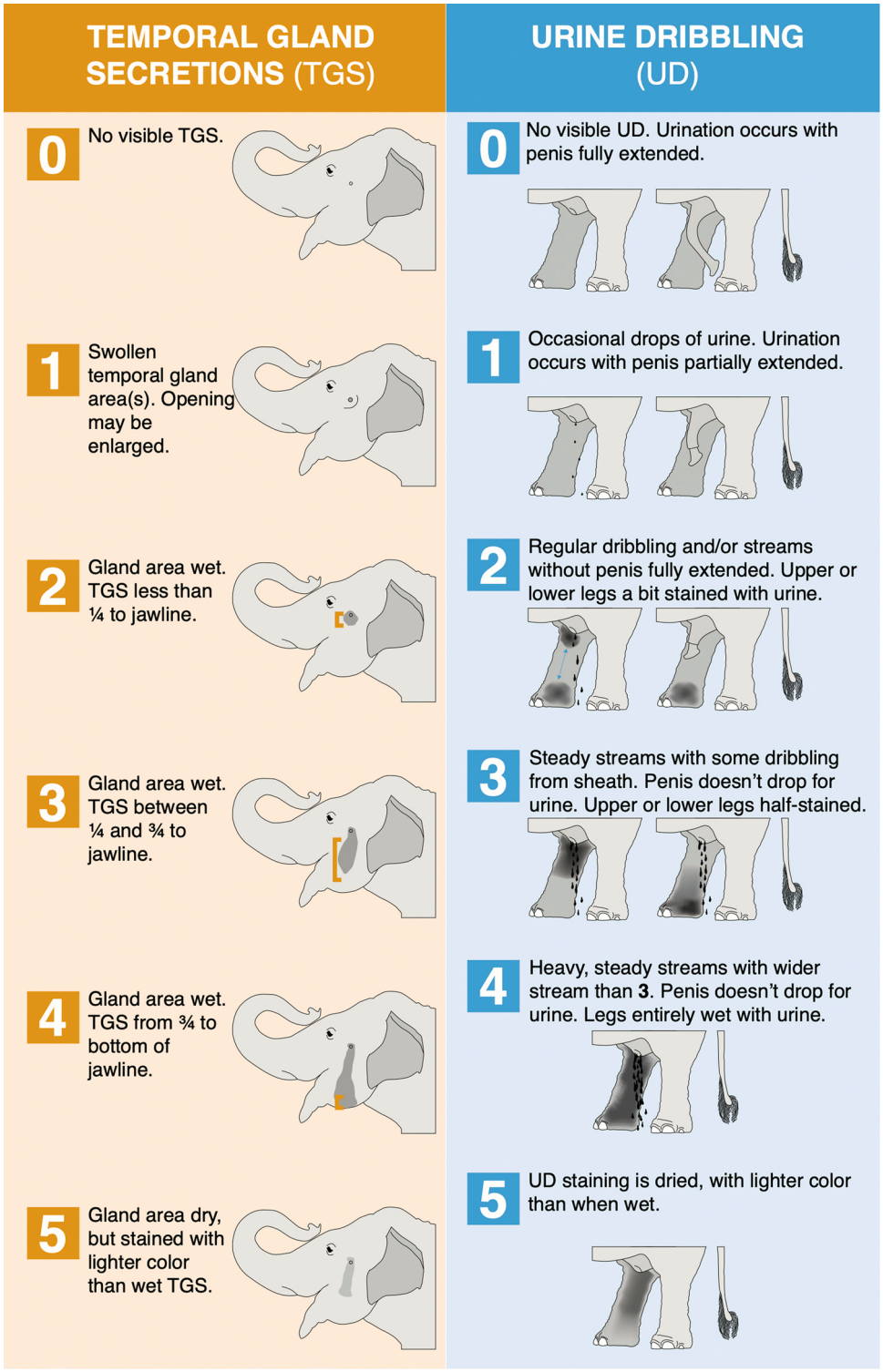

Fig. 1. Visible musth indicators recorded with each fecal sample collected in this study. Temporal gland secretions (TGS) and urine dribbling (UD) are typical indicators of musth in male Asian elephants and were each scored from 0 (non-musth) to 5 (post-musth). TGS and UD descriptions are adapted from Glaeser et al. [58]. Illustrations by C. LaDue, modified from LaDue et al. [19].

Germany). 5.00 mL of 100% methanol was added to each tube, followed by vortexing for 30 min on a large capacity mixer (Glas-Col; Terre Haute, IN; speed approximately 1000 rpm). Tubes were centrifuged for 5 min at 935 *g* (Thermo Scientific Sorvall ST Plus Series Centrifuge; Thermo Fisher Scientific; Waltham, MA), and 2.00 mL of supernatant was recovered and transferred to a 13 × 75 mm borosilicate glass tube (Fisher Scientific; Pittsburgh, PA) Not all supernatant was recovered due to the fact that high-fiber particles in the pellet (e.g., hay particles) retained much of the supernatant; results were corrected for percentage of supernatant lost. Recovered supernatants were dried using a vacuum concentrator (Savant SpeedVac SPD1030; Thermo Fisher Scientific; Waltham, MA) at 45 ºC for 75 min, reconstituted in 500 μL of assay buffer (buffer X065; Arbor Assays; Ann Arbor, MI), vortexed for 5 s each (Vortex Genie 2; Scientific Industries;

Bohemia, NY), and finally sonicated for 5 min (Branson M3800 Ultrasonic Cleaner; Emerson Electric; St. Louis, MO). Each resulting fecal extract (considered the "1:1″ extract, i.e., the full-strength extract) was transferred to a 2 mL polypropylene microcentrifuge tube fitted with an O-ring cap (Perfector Scientific; Atascadero, CA) and stored at –20 °C until analysis. All assays occurred within three months of extraction.

2.3. Enzyme immunoassay analysis

Fecal extracts were analyzed for FAM, FGM, and FT3 via double-antibody enzyme immunoassay (EIA) using commercially available kits (testosterone, catalog #K032; corticosterone, catalog #K014; T3, catalog #K056; Arbor Assays, Ann Arbor, MI). The manufacturer's reported cross-reactivities were as follows: testosterone antibody, 56.8 % with 5a-dihydrotestosterone, 2.34% with 11-ketotestosterone, and < 0.3 % for all other steroids; corticosterone antibody, 18.9 % with 11-dehydrocorticosterone, 12.3 % with desoxycorticosterone, 3.3 % with 1α-hydroxycorticosterone, 2.44 % with 11-dehydrocorticosterone, < 1 % for other steroids; T3 antibody, 0.88% with T4 and < 0.1 % with reverse T3. Manufacturer's protocols were followed, with extracts diluted to 1:49 for testosterone, 1:8 for corticosterone, and 1:8 for T3 based on parallelism validation results. These dilutions were selected as they fell near 50 % bound for each assay, the area of greatest assay precision. These antibodies were selected due to previous successful use for fecal hormone metabolites in a wide variety of mammalian species. Note that the corticosterone antibody is known to detect mammalian fecal metabolites of cortisol. Further discussion on assay validation is presented below.

Alongside fecal extracts, we also assayed a control in each assay plate consisting of a pooled sample at 1:128 for FAM and 1:16 for FGM and FT3. All assays included standards (with which to construct a standard curve for each plate), blanks (zeros), non-specific binding (NSB) wells, and a control (a pooled Asian elephant fecal extract diluted to 1:128 for FAM and 1:16 for FGM and FT3, used to measure inter-assay variation). Standards, blanks, NSBs, controls, and samples were run in duplicate. Samples were assayed in a pseudorandomized order for each hormone using a random number generator to minimize potential influences of intra- and inter-assay variation. Optical density of each well was read at 450 nm using a microplate reader (Epoch Microplate Spectrophotometer; Bio Tek Instruments; Winooski, VT), and metabolite concentration was calculated using a sigmoidal dose response curve in Prism version 9.3 (GraphPad; San Diego, CA). FAM, FGM, and FT3 concentrations are reported as ng/g of dried feces, corrected for volumetric differences during the extraction process. Any samples with < 10 % or > 90 % binding or with coefficients of variation (CVs) > 10 % were reanalyzed (using a more concentrated dilution or further reducing dilutions for high or low binding, as necessary). Intra-assay variation was < 10 % for all assays in this study. The inter-assay CV for the controls was 3.9% for testosterone (n = 49), 4.6 % for corticosterone (n = 51), and 3.4 % for T3 (n = 24).

Because FT3 concentrations remain relatively stable in male elephants over time [33], we analyzed monthly instead of weekly samples for each zoo elephant while he was not showing visible indicators of musth (i.e., TGS and/or UD). However, because we hypothesized that FT3 concentration would vary around musth, we analyzed weekly samples for FT3 for each male while he was exhibiting TGS and/or UD (TGS/UD = 1, 2, 3, 4, or 5). Therefore, analyses that investigated FT3 in weekly samples (discussed below) included only n = 595 samples. FT3 was analyzed in all daily zoo samples and in all samples from wild elephants.

2.3.1. Assay validation

For analytical validation of the EIAs, we first performed a parallelism test for each hormone consisting of 12 serial dilutions of pooled fecal extract assayed alongside known standards. Subsequently, we validated assays with accuracy tests (matrix effect tests), which assess the mathematical accuracy of the assays in the presence of any unusual sample matrix (i.e., fecal extract). To do this, we spiked standard curves of each hormone with equal volumes of low-concentration pool at 1:49 dilution for testosterone and 1:8 dilution for corticosterone and T3, and then assayed these along standards spiked with assay buffer, subsequently graphing observed dose versus. expected dose of the set of standards that had been spiked with pooled fecal extract. Finally, we performed biological validations for each of the hormones, which compare metabolite concentrations before and after events or conditions that are expected to elicit concomitant physiological responses. For the testosterone assay, we compared FAM concentrations in male elephants with no TGS (TGS = 0) to those that had extensive TGS (TGS = 3 or 4, based on expectations from previous studies [18,41]. For biological validation of the glucocorticoid assay, we assessed the effect of social changes reported by keepers over the study period (i.e., the introduction of new group members), which are known to result in increased glucocorticoid concentrations in zoo-housed elephants [64–66]. Following biological validation procedures for FT3 by Szott et al. [63], we compared the FT3 concentrations of zoo elephants with the lowest body condition in our study (BCS = 2) to those with the highest body condition (BCS = 5).

2.4. Behavioral observation protocol

Matched pairs of behavioral observations and fecal samples were available opportunistically and coincided with sampling described in LaDue et al. [19]. Behavioral data were collected from 26 zoo-housed elephants between July 2018 and April 2021, and from 28 wild elephants between December 2018 and April 2019. Behavioral observations were matched to fecal samples collected the day after the observations occurred to account for the lag between hormone secretion and appearance of metabolites in feces [35,67]. However, accounting for this lag was not logistically feasible for wild elephants, so matched pairs consist of fecal samples collected and behavioral observations conducted on the same day.

Wild elephant observation sessions lasted 15 min each (minus any time the focal animal was out of view), with a maximum of three sessions per sighting of an elephant. Observation sessions of zoo-housed elephants lasted 60 min each (minus any time that the focal animal was out of view or interacting with care staff), and on most days when these observations took place at a facility, each male was observed once in the morning and once in the afternoon. Observation sessions of wild elephants were shorter due to the difficulty of following elephants across landscapes and in areas of dense vegetation; the controlled conditions of zoos permitted longer observations. If multiple male elephants were present in a group (for wild elephants, defined as all animals within 100 m of each other, estimated visually), we observed the males in a random order. For both wild and zoo-housed elephants, observation sessions in which the focal animal was out of view for greater than one-third of the observation time (5 min for wild elephants and 20 min for zoo-housed elephants) were excluded from subsequent analysis.

We used continuous and all-occurrence focal animal sampling [68] to record two state behaviors [*locomotion* (defined as movement from point A to B greater than one body length in three seconds), and *foraging* (defined as acquiring, processing, and/or consuming a food item)] and one event behavior [*chemosensory behavior* (including any exploratory behaviors involving the trunk, such as sniffing and the flehmen response [69])]. Other behaviors related to musth [e.g., alertness, object manipulation, self-maintenance [19]] were excluded from analysis due to very low occurrence of such behaviors. All observations before March 2020 were conducted live by a single observer (CAL) using ZooMonitor [70], but observations on zoo-housed elephants after March 2020 were recorded and later scored by the same observer (CAL) via video also using ZooMonitor, due to restrictions imposed by COVID-19. An index of concordance showed > 95 % agreement between

sampling efforts on the same observation session conducted at the beginning and end of the study [71].

2.5. Statistical analysis

Analysis was conducted in R version 4.1.0 [72] with the packages *afex*, *lme4*, *MuMIn*, and *tidyverse* [73–76]; for all analyses, statistical significance was set at $\alpha = 0.05$ when appropriate. After visual inspection with quantile-quantile (Q-Q) plots revealed non-normal distributions in FAM, FGM, and FT3 concentrations, we $\log_{10}$-transformed each of these measures to improve the distribution of these data in all subsequent analysis.

2.5.1. The influence of androgens on FGM and FT3 concentrations

Using all available samples for wild and zoo-housed elephants, we constructed LMMs using restricted maximum likelihood for FGM and FT3 concentrations, separately, to investigate a potential correlation of either hormone with FAM concentration. For each hormone, all models included animal identity as a random effect to account for uneven sampling. Additionally, models for wild and zoo elephants were analyzed separately, with results between the populations compared qualitatively due to lower sample size in wild elephants.

2.5.2. Association between fecal hormone metabolite concentration and visible musth indicators

For wild and zoo-housed elephants separately, we compared the $\log_{10}$-transformed concentration of each hormone metabolite in samples collected across all musth indicator scores (TGS and UD, separately) using LMMs via restricted maximum likelihood and Kenward-Roger approximations that minimize Type I error [77], resulting in six models for all hormone–musth indicator combinations (12 total models encompassing wild and zoo elephants). In each model, TGS/UD score was the fixed effect, hormone metabolite concentration was the response variable, and animal identity was included as a random factor to account for repeated sampling. Any samples for which TGS or UD scores were missing were excluded from their respective analyses. We identified significant differences between musth indicator scores using non-parametric pairwise Wilcoxon rank sum tests with Bonferroni corrections for multiple comparisons. For zoo elephants, we used only the weekly samples in this analysis to avoid over-representation of some elephants over others; all available wild elephant samples were used due to a lower sample size. Differences between wild and zoo-housed elephants were identified qualitatively.

The longitudinal collection of weekly fecal samples from zoo elephants allowed us to qualitatively investigate the timing of TGS and UD across musth episodes – we could not complete this with the wild elephant samples due to more sporadic, opportunistic collection. With the weekly zoo samples, we used an iterative process to identify musth episodes based on FAM concentration via the package *hormLong* in R version 4.1.0 [16,20,33,78,79]. In each iteration, all FAM concentrations exceeding the mean plus 1.5 times the standard deviation were removed [$\text{mean}_{\text{FAM}} + (1.5 \times \text{SD}_{\text{FAM}})$], and this process continued until no values exceeded this criterion. The remaining FAM values for each elephant described that individual's baseline, and the maximum baseline FAM concentration defined the upper baseline threshold (UBT) value. After that, we followed standards described by Chave et al. [20] to define the duration of a putative musth episode, with the following criteria: (1) a musth episode was sustained as long as the male's FAM concentration remained above the lower baseline threshold (LBT), defined as the mean FAM baseline plus two times the baseline standard deviation [$\text{mean}_{\text{baseline}} + (2 \times \text{SD}_{\text{baseline}})$]; and (2) a musth episode ended with two consecutive weeks below the LBT (the last day of the episode was the first day it dropped below the LBT). Single-point deviations above or below the UBT or LBT were assigned to be musth or non-musth based on the surrounding data points. We excluded episodes in which the male's FAM concentration remained sufficiently above the UBT but in which there were no accompanying external indicators of musth (i.e., TGS or UD) within one month of the beginning or end of the episode. This resulted in 32 confirmed musth episodes in 17 elephants. From these, we identified ten musth episodes (one each from $n = 10$ elephants) for which we had samples c. one month prior to the beginning of each episode, all throughout the episode, and c. one month after each episode. This allowed us to ascertain a rough estimate of when musth indicators began and ended around these episodes. We graphically analyzed TGS and UD activity with the progression of each episode; statistical analysis was not feasible due to low sample size.

2.5.3. Association between fecal hormone metabolites and behavior

We calculated the proportion of time the focal elephant was engaged in each state behavior (locomotion and foraging) by dividing the time engaged in that behavior by the total observation time (excluding time of out view or interacting with care staff). Likewise, rates of chemosensory behavior were calculated by dividing the count of each behavior by observable time during a session. For most matched pair samples of zoo-housed elephants—and occasionally for wild elephants—a single fecal sample was accompanied by multiple matching behavioral observations, accounted for by multiple matching behavioral observation sessions happening on a single day for these elephants. In these cases, we calculated the average proportions/rates of each behavior for that day, so that each fecal sample was only accompanied by one matching set of behavioral values. This resulted in 34 matched pair samples for wild elephants (range = 1 to 3 pairs per elephant, median = 1) and 196 matched pair samples for zoo-housed elephants (range = 4 to 18 pairs per elephant, median = 5).

We used a linear mixed model (LMM) approach to evaluate the influence of FAM, FGM, or FT3 concentration ($\log_{10}$-transformed) on each of the behaviors; each hormone was analyzed separately for wild and zoo-housed elephants. No other predictor variables were included due to potential confounding effects with musth (e.g., TGS, UD) and hormone concentration. Wild and zoo-housed elephants were analyzed separately because of the different environmental and social pressures they face that may also influence behavioral variation [19,29], and because of potential differences in the absolute values of hormone metabolite concentrations between these populations. Each LMM was evaluated using restricted maximum likelihood, including animal identity, and facility for zoo-housed elephants, as random factors. The statistical influence of each hormone metabolite on behavioral variation was evaluated via Kenward-Roger approximations. Finally, we also estimated the explanatory value of each model using the marginal coefficient of variation (R^2_c). These coefficients were used to elucidate qualitative differences in the hormone–-behavior relationships between wild and zoo elephants.

3. Results

3.1. Assay validations

Each assay showed good parallelism with slopes not significantly different from those of the standard curves: testosterone ($F_{1,11} = 0.315$, $P = 0.586$), corticosterone ($F_{1,8} = 2.763$, $P = 0.135$), and T3 ($F_{1,10} = 2.576$, $P = 0.140$), indicating that assay antibodies bound well to the fecal hormone metabolites (Fig. S1). We also found that all three assays demonstrated good accuracy upon inspection of slopes, intercepts, and linearity: testosterone, slope = 1.104, intercept = 118 pg/mL, pool = 57 pg/mL, R = 1.0000; corticosterone, slope = 1.008, intercept = 118 pg/mL, pool = 104 pg/mL, R = 0.9997; T3, slope = 1.242, intercept = 215 pg/mL, pool = 127 pg/mL, R = 0.9996 (Fig. S2). Finally, for biological validation, we found that males with no TGS had significantly lower FAM concentrations than males with TGS (Mann-Whitney U test: $W = 182{,}706$, $P < 0.001$; mean ± SE FAM no TGS = 94.7 ± 5.6 ng/g, mean ± SE FAM extensive TGS = 553.7 ± 42.0 ng/g, FGM concentrations were significantly higher in the one-month period after two zoo-housed males at different facilities were introduced to new social groups compared to one month before (Mann-

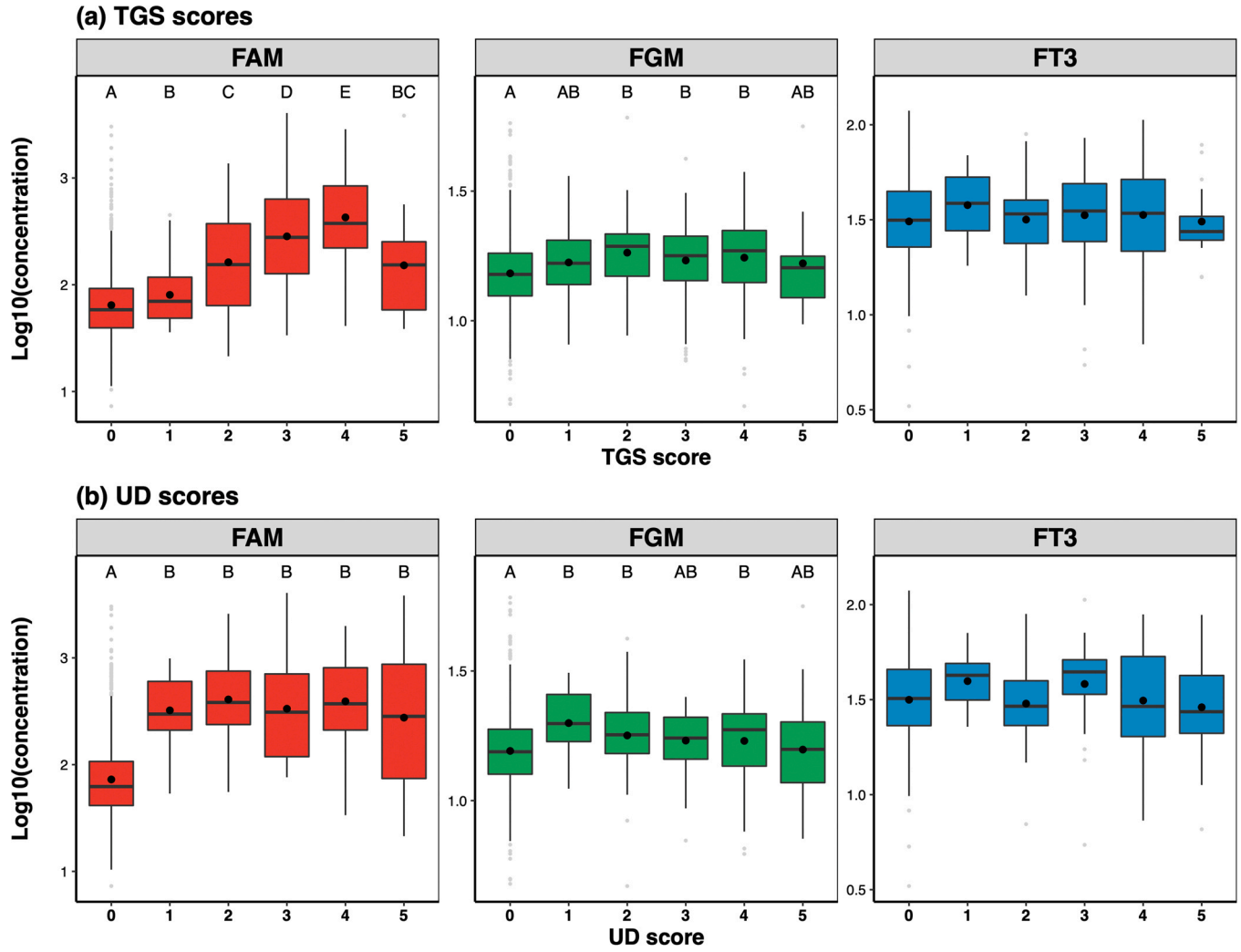

Fig. 2. Boxplots showing relationships between $\log_{10}$-transformed concentrations of fecal androgen metabolite (FAM), fecal glucocorticoid metabolite (FGM), and fecal T3 (FT3) and (a) temporal gland secretion (TGS) and (b) urine dribbling (UD) scores in male zoo-housed Asian elephants ($n = 26$). See Fig. 1 for TGS and UD score definitions. Letters above boxes indicate statistically significant ($P < 0.05$) differences between scores within the same hormone. Boxes extend from the first to the third quartile, with median indicated by a thick line; fences extend to 1.5 times the interquartile range, and gray circles indicate outliers outside this range. Black circles inside boxes show mean concentration for each TGS/UD score.

Whitney U test: $W = 56$, $P < 0.001$; mean ± SE FGM before = 17.1 ± 1.5 ng/g, mean ± SE FGM after = 33.9 ± 3.3 ng/g), and zoo elephants with the lowest body condition (BCS = 2) in our study had significantly lower FT3 concentrations than those with the highest body condition (BCS = 5) (Mann-Whitney U test: $W = 4478$, $P < 0.001$, mean ± SE FT3 for low BCS = 24.4 ± 1.2 ng/g, mean ± SE FT3 for high BCS = 41.6 ± 0.9 ng/g).

3.2. The influence of androgens on FGM and FT3 concentrations

In zoo-housed elephants, both $\log_{10}$[FGM] ($F_{1,\ 1785.00} = 291.06$, $P < 0.001$) and $\log_{10}$[FT3] ($F_{1,\ 1779.93} = 33.75$, $P < 0.001$) showed a positive relationship with $\log_{10}$[FAM]. However, this relationship was not evident in wild elephants for either $\log_{10}$[FGM] ($F_{1,\ 34.88} = 0.11$, $P = 0.743$) or $\log_{10}$[FT3] ($F_{1,\ 32.50} = 0.14$, $P = 0.711$).

3.3. Association between fecal hormone metabolite concentration and visible musth indicators

In zoo elephants, we found that FAM and FGM concentrations varied significantly across TGS (FAM: $F_{5,\ 1283.78} = 153.59$, $P < 0.001$; FGM: $F_{5,\ 1282.97} = 5.58$, $P < 0.001$) and UD scores (FAM: $F_{5,\ 1282.84} = 98.11$, $P < 0.001$; FGM: $F_{5,\ 1282.47} = 2.47$, $P = 0.031$). FAM concentrations increased steadily with increasing TGS score, falling back to early musth levels when TGS = 5 (Fig. 2a); similarly, there was a marked rise in FAM with UD = 1 that remained elevated with sustained UD (Fig. 2b). For FGM, concentrations increased with visible TGS and UD and returned to baseline levels once TGS or UD = 5 (Fig. 2a,b). However, FT3 concentrations remained stable across TGS and UD scores: TGS ($F_{5,\ 1274.97} = 0.88$, $P = 0.492$) and UD ($F_{5,\ 1276.04} = 1.00$, $P = 0.416$) (Fig. 2a,b).

Similarly, FAM and FGM concentrations in wild elephants were significantly different between both TGS (FAM: $F_{3,\ 29.77} = 3.51$, $P = 0.027$; FGM: $F_{3,\ 30.08} = 2.79$, $P = 0.044$) and UD scores (FAM: $F_{3,\ 29.22} = 2.88$, $P = 0.037$; FGM: $F_{3,\ 29.42} = 2.17$, $P = 0.040$). FAM concentrations were higher than baseline when TGS or UD = 3 or 4 (Fig. 3). However, in contrast to zoo elephants, FGM concentrations were lower when there was extensive TGS (TGS = 3 or 4) or UD (UD = 4) in wild elephants compared to when TGS/UD = 0 (Fig. 3). Additionally, while FT3 concentrations did not vary between TGS scores ($F_{3,\ 29.43} = 1.15$, $P = 0.345$), wild elephants with extensive UD (UD = 4) also exhibited significantly lower FT3 concentrations compared to baseline ($F_{3,\ 28.88} = 2.05$, $P = 0.034$) (Fig. 3).

We noted differences in the occurrence of TGS and UD around musth in zoo-housed elephants. Of the 10 musth episodes for which we

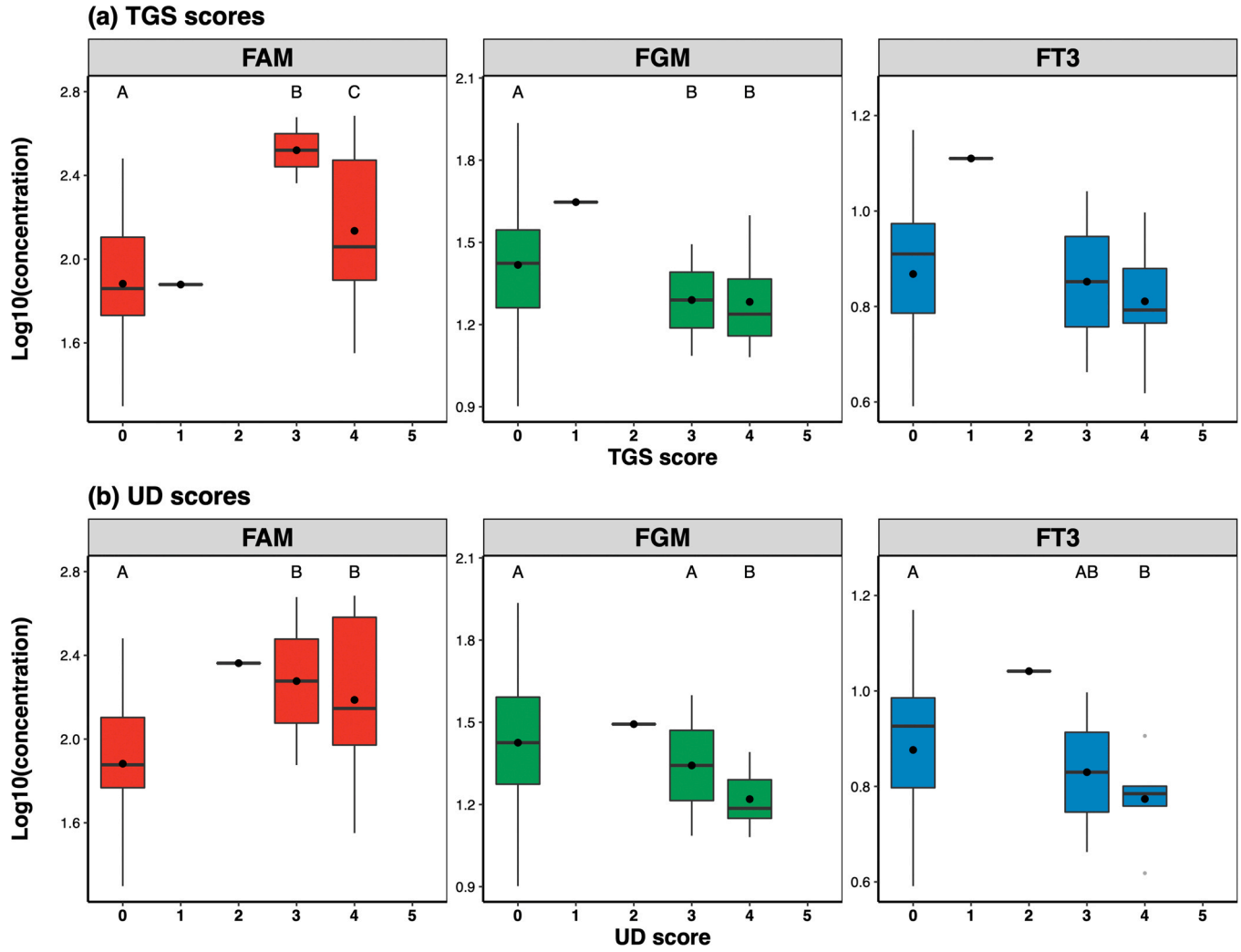

Fig. 3. Boxplots showing relationships between $\log_{10}$-transformed concentrations of fecal androgen metabolite (FAM), fecal glucocorticoid metabolite (FGM), and fecal T3 (FT3) and (a) temporal gland secretion (TGS) and (b) urine dribbling (UD) scores in male wild Asian elephants (n = 28). See Fig. 1 for TGS and UD score definitions. Letters above boxes indicate statistically significant ($P < 0.05$) differences between scores within the same hormone. Boxes extend from the first to the third quartile, with median indicated by a thick line; fences extend to 1.5 times the interquartile range, and gray circles indicate outliers outside this range. Black circles inside boxes show mean concentration for each TGS/UD score. Empty TGS/UD scores indicate no data for those respective scores.

also had samples before and after the musth episode, all were associated with TGS activity during at least part of the episode (Fig. 4a), with most TGS observed in the latter half of musth. However, only 50 % of these ten musth episodes (n = 5 elephants) involved UD (Fig. 4b). Either indicator of musth typically began within two weeks of the onset of musth (defined by increased FAM), but one elephant (E1) did not display TGS or UD until about four months after musth began. After including all musth episodes of zoo elephants (i.e., even those without one month of sampling prior to and following the musth episode, n = 32), TGS activity was associated with all musth episodes (i.e., during all musth episodes in all zoo elephants) and UD activity with 21 of 32 episodes (65.6 %) in 14 of 17 elephants (82.4 %). Still, TGS and UD activity was not limited to periods we identified as musth episodes: we observed TGS associated with 46 (5.98 %) and UD with 6 (1.04 %) of 769 non-musth samples from zoo elephants.

3.4. Association between fecal hormone metabolites and behavior

In zoo-housed elephants, FAM concentrations showed significant positive relationships with locomotion and chemosensory behavior, and a significant negative relationship with foraging (Fig. 5a). FGM concentrations also were positively related to locomotion. Models that tested the relationship between either FGM or FT3 concentrations and foraging or locomotion demonstrated strong predictive value ($R_c^2 > 0.4$) despite not necessarily yielding statistically significant relationships, indicating that much of the variation in these cases was predicted by animal identity and/or facility.

In wild elephants, for which we had a lower sample size, FAM concentrations also demonstrated a significantly positive relationship with locomotion (Table 2), but had no significant relationship with chemosensory behavior or foraging—though the latter two variables exhibited similar trends as in zoo-housed elephants (Fig. 5b). Random intercepts (i.e., individual identity) for various behavior–hormone pairs (forage–FAM, forage–FT3, and locomotion–FGM) significantly impacted the predictions of these models.

4. Discussion

In this study, we found that fecal hormone metabolites were associated with physiological changes that occur during musth in Asian elephants, and that the physical (TGS and UD), behavioral, and hormonal correlates of musth were similar between *in-situ and ex-situ* populations. Specifically, significant differences were apparent in FAM and FGM concentrations between various TGS and UD scores

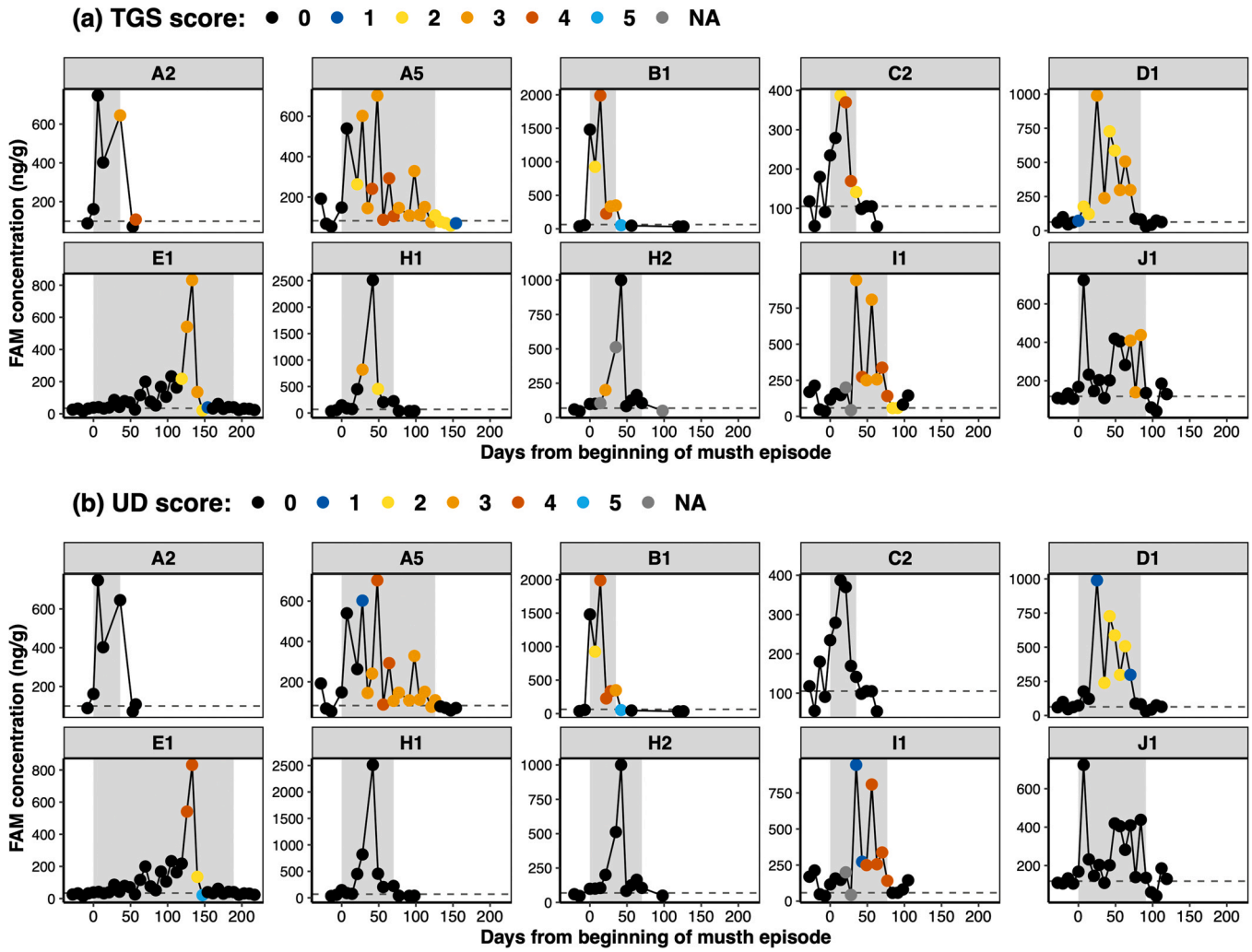

Fig. 4. Relationship between progression of musth episodes and (a) temporal gland secretion (TGS) scores and (b) urine dribbling (UD) scores in male Asian elephants housed in zoos (n = 26). Each panel represents a confirmed musth episode of elephant designated by alphanumerical code (see Table 1). Shaded region represents defined musth episode in each panel, with dashed lines indicating upper baseline threshold (UBT) that helps define start of musth (see text for details). Circles represent individual samples, colored based on TGS/UD score (see Fig. 1 for definitions). NA = missing TGS/UD score.

(especially when comparing TGS/UD = 0 to all other scores), but we did not observe associations between FT3 concentrations and these visible musth indicators. Moreover, all musth episodes that were identified in zoo elephants via FAM concentrations were associated with TGS activity but not necessarily with UD. As expected, we also documented significant relationships between characteristic behavioral changes during musth (locomotion, foraging, and chemosensory behavior) and FAM concentrations; we also identified a significant correlation between FGM concentration and locomotion in zoo-housed elephants. While the relationships between hormonal variation and behavior are more tenuous for wild elephants and did not always reach significance (possibly due to lower sample size), they do motivate further research. Together, these results underscore the wide-ranging impacts of musth physiology on male Asian elephants.

The TGS and UD scores we utilized in this study correspond to major behavioral changes that occur around musth in Asian elephants (e.g., decreased foraging, increased locomotion and exploratory behaviors) [19]; we show in the present study that TGS and UD are also associated with distinct hormonal changes. FAM concentrations increased with each progressive TGS score until TGS = 5 (post-musth), when the average FAM concentration declined to a level just above baseline. Conversely, the presence of any UD indicated significantly elevated FAM concentrations. For both TGS and UD scores, FGM concentrations were significantly higher as musth progressed (TGS = 2, 3, and 4; UD = 1, 2, 3, and 4). Therefore, we suggest that TGS and UD are useful as a visual proxy for the general physiological state of a male elephant in musth. Still, there appears to be a wide range of variation in the timing and combination of musth indicators, both between and within individual male elephants [data shown in [80]]. For example, in zoo elephants, TGS and UD activity lagged behind the first elevation in androgen levels by a few weeks, but males also tended to exhibit these indicators with oscillating intensities (i.e., not proceeding in a linear fashion from a low TGS/UD score to a high score). Additionally, TGS activity was observed during all musth episodes, but we observed UD activity in only 50–65 % of episodes. Further, though both indicators tended to occur within two weeks of elevations in FAM, some individual elephants did not demonstrate TGS or UD until months later, and both indicators occasionally occurred in the absence of musth. Therefore, we suggest that TGS may be a defining physical characteristic of "true" musth in Asian elephants and not necessarily UD (although UD has been suggested to be a true indicator of musth in *L. africana* [23,53]). Even so, long-term studies are needed to better reveal how these musth indicators change with physiological, health, social, and environmental factors and as male elephants experience successive musth episodes. Further research will also elucidate if there are practical means to accurately quantify changes in TGS and/or UD intensity.

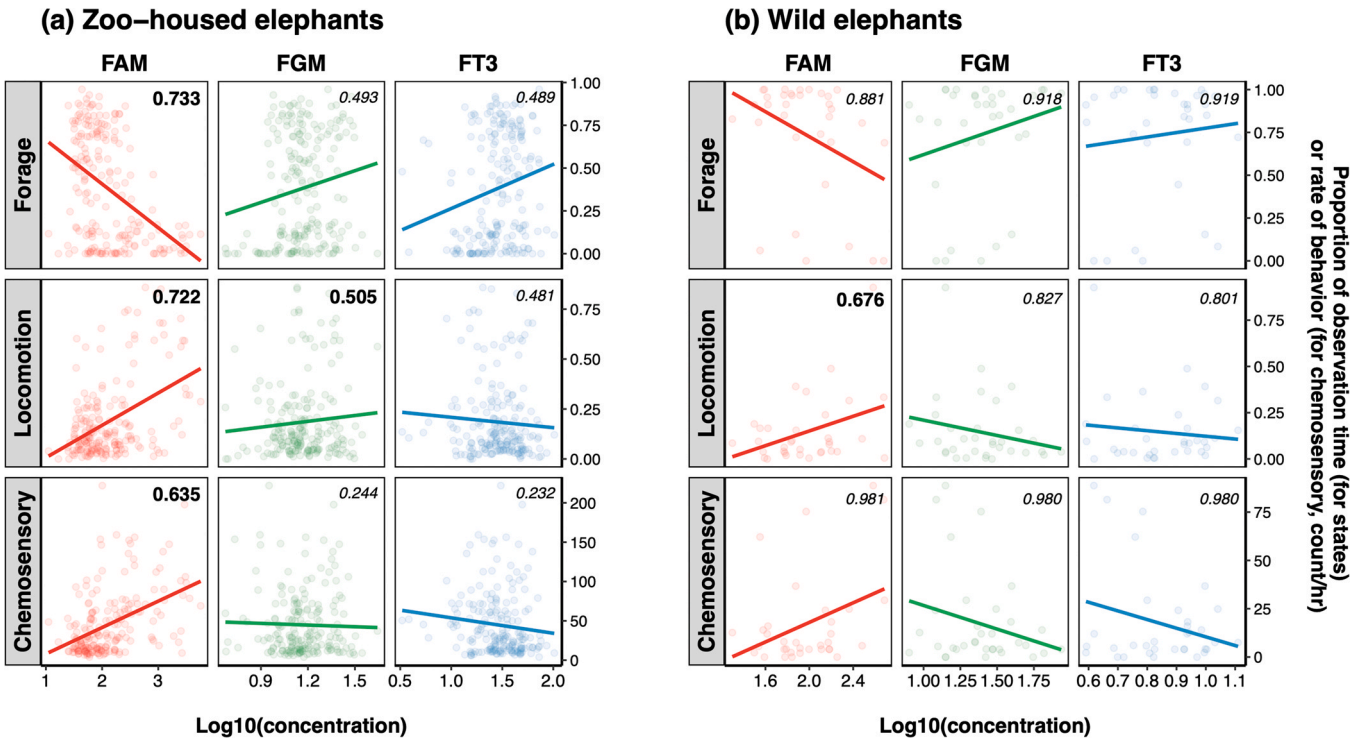

Fig. 5. Linear relationships between fecal hormone metabolite concentrations [fecal androgen metabolites (FAM), fecal glucocorticoid metabolites (FGM), and fecal T3 metabolites (FT3)] and behavioral variation in (a) zoo-housed, n = 26, and (b) wild male Asian elephants, n = 28. Points represent individual observation sessions, and solid lines show linear model predictions, assuming no random effects. Values in the upper righthand corner of each panel display marginal coefficients of variation (R^2_c) for the full model (including random effects of animal identity, and for zoo elephants, facility) of each relationship; bolded values were statistically significant ($P < 0.05$).

Table 2
Summary of fixed effect coefficients from linear mixed models (LMM) that evaluated the association between log_{10}-transformed concentrations of fecal hormone metabolites [fecal androgen metabolites (FAM), fecal glucocorticoid metabolites (FGM), and fecal T3 metabolites (FT3)] and behaviors of interest in zoo-housed ($n = 26$) and wild Asian elephants ($n = 28$). Estimates (Est.) of the associations between hormone concentrations and state behaviors (forage, locomotion) are indicated in proportion of observation time, and chemosensory behavior is reported as a rate (count of behaviors per hour). Statistically significant associations ($P < 0.05$) are bolded. SE = standard error. df = approximated degrees of freedom.

		Zoo-housed elephants					Wild elephants				
		Est.	SE	df	*t*	*P*	Est.	SE	df	*t*	*P*
Forage	FAM	**–0.357**	**0.033**	**191.31**	**–10.77**	**< 0.001**	–0.252	0.140	17.866	–1.797	0.089
	FGM	0.043	0.143	192.01	0.301	0.763	0.215	0.202	21.597	1.067	0.298
	FT3	0.105	0.099	192.62	1.062	0.290	–0.036	0.299	12.475	–0.121	0.906
Locomotion	FAM	**0.201**	**0.019**	**188.13**	**10.380**	**< 0.001**	**0.172**	**0.084**	**31.319**	**2.051**	**0.049**
	FGM	**0.177**	**0.080**	**192.72**	**2.207**	**0.029**	–0.174	0.118	30.352	–1.477	0.150
	FT3	–0.045	0.057	192.89	–0.803	0.423	–0.007	0.194	20.466	–0.036	0.971
Chemosensory behavior	FAM	**43.877**	**4.232**	**192.02**	**10.369**	**< 0.001**	–0.059	5.010	5.026	–0.012	0.991
	FGM	15.400	17.080	145.77	0.901	0.369	–6.189	9.532	8.310	–0.725	0.488
	FT3	–16.370	11.75	102.20	–1.393	0.167	–4.125	11.173	6.380	–0.369	0.724

From the behavioral results, it is clear that musth—defined here by increased FAM concentration—is associated with distinct behavioral changes. In male mammals, androgens influence sexual behavior through a variety of mechanisms that may be species-specific [81]. Traditional experiments on the role of testosterone in behavior involve ablation–replacement study designs [82–84], which is not logistically feasible in elephants. However, several zoo-housed male elephants have been castrated (surgically or chemically) for management purposes, most often resulting in eliminated, weakened, and/or postponed musth behavior [85–90]. In combination with the results from this study, these cases provide anecdotal evidence that similar relationships between androgens and behavior exist in male Asian elephants as in other male mammals.

Our results also suggest that musth in Asian elephants is associated with altered HPA activity. FGM concentrations were positively correlated with FAM concentrations, supporting prior findings from zoo-housed Asian elephants that indicate musth is a physiologically stressful state [20,33]. Further, FGM concentrations were higher with increased TGS/UD scores in zoo-housed elephants. Androgens and glucocorticoids are commonly associated in vertebrates, as the often complex factors involved in locating, attracting, and/or breeding with a receptive mate can be stressful for males [91]. Still, an elevated adrenal response (reflected in increased glucocorticoids) is only one potential indicator of the complex physiological stress response [92], and additional measures of short- and long-term stress are necessary to fully understand how male elephants may cope with the possibly stressful state of musth.

Further, diurnal variation in the relationship between serum concentrations of androgens and glucocorticoids are evident in zoo-housed elephants [93]. While this variation is often muted in fecal steroid metabolites, the physiological significance of this relationship between androgens and glucocorticoids warrants additional investigation.

However, wild elephants showed an opposite pattern, with FGM generally declining in musth, corroborating results from free-ranging male Asian [36] and African [35,41,53] elephants during musth. This wild–zoo difference should be interpreted with caution, as our sampling was limited in Sri Lanka, but it may be accounted for by a few non-exclusive explanations. First, our longitudinal sampling of zoo elephants afforded us the opportunity to track within-individual patterns across time, while studies of wild Asian elephants (including our sampling in Sri Lanka) often rely on relatively few samples on a limited number of individuals. Additional studies emphasizing repeated sampling of wild individuals (e.g., with noninvasive fecal techniques) could prove fruitful in filling this data gap. There may also be interspecies differences in musth physiology between *L. africana* and *E. maximus* (although see [33] and [20]), hormonal differences between wild and captive populations, and/or differences that arise in measuring circulating hormones versus excreted hormone metabolites (described further below). Finally, it is possible that the zoo environment imparts inherent challenges during musth that stimulates an elevated stress response in male elephants. For example, both wild and zoo-housed elephants exhibit increased locomotion during periods of musth [19]; the smaller spaces afforded by zoos may contribute to perceived stress among managed elephants but not wild elephants.

FGM also was associated with behavior, with zoo-housed male elephants showing increased activity and locomotion in conjunction with elevated glucocorticoid secretions. These relationships may be attributed to musth, as elevated FGM concentrations are associated with musth in this population. Wild elephants did not show this pattern, however. A shortcoming of our data for wild elephants is that fecal samples had to be collected on the same day as observations, but FGM concentrations in elephants are thought to represent the physiological state of approximately one day prior to defecation. Additionally, we had relatively few repeated samples from wild elephants; longitudinal sampling is desirable for FGM-behavioral studies due to typically high inter-individual variation in behavior as well as in FGM metabolism [39,94]. As noted above, future longitudinal studies employing repeated sampling in wild elephants, ideally with multiple-day observations to resolve the issue of fecal excretion lag time, would help to clarify these hormone-behavior relationships in wild elephants.

Although FAM and FT3 concentrations were positively correlated in this study, we did not find that FT3 concentrations were associated with visible musth indicators (although there were lower FT3 concentrations with higher UD scores in wild elephants, but this may be an artifact of lower sampling). Thyroid hormone concentrations are expected to decline with increased metabolic stress [95,96], and previous studies have documented lower concentrations in circulating T3 and T4 in serum around musth [20,33], so we expected that FT3 would decrease during musth to reflect the transition to a metabolically taxing state, but this did not occur. There appeared to be substantial FT3 variation between individuals and elephant facilities (for zoo elephants). Other studies have described changes in circulating thyroid hormones [including thyroid-stimulating hormone (TSH), and free and total T3 and T4] associated with musth in zoo-housed elephants [20,33], but these patterns seem to be individually variable, with only some male elephants demonstrating clear associations between elevated androgens indicative of musth and thyroid hormones. There also were no significant relationships between FT3 and behavior. However, in wild elephants, it is interesting that FAM and FT3 exhibited opposite relationships with certain behaviors (i.e., foraging, locomotion, chemosensory behavior); most of these relationships were statistically non-significant but suggest possible avenues for future research. Future studies on FT3 in male elephants also should investigate differences in nutrition and health that may influence thyroid hormone activity in this species. Many other factors may be important as well; generally in vertebrates, thyroid hormones can reflect a wide variety of factors including not just metabolic state but also reproductive status, age, and thermal environment [97].

5. Conclusions

The long-term sustainability of Asian elephants and other threatened species depends in part on integrative, animal-centered approaches to conservation. For elephants, musth poses a challenge to short- and long-term management strategies. We show that non-invasive fecal sampling can be used to effectively measure FAM and FGM concentrations alongside musth, and that these metabolites vary with successive stages of musth determined by visible musth indicators (TGS and UD) and with certain characteristic musth behaviors. Such information is useful for wildlife managers who cannot readily measure hormone concentrations in their animals to gauge their physiological status; these visible indicators thus reflect the physiological status and stress experienced by a male elephant. Further research should explore other biomarkers (e.g., insulin, triglycerides, prolactin) that reflect the internal state of male elephants and also may be indicated by TGS and/or UD activity, as there may be other physiological correlates that we were unable to measure via fecal hormone metabolites [20,33]. In this regard, this study also provides another example of the value of conducting complementary *in-situ–ex-situ* studies to inform the management of wild and captive wildlife populations. Populations in managed care (e.g., zoos) permit ample access to individual animals for sample collection, and often the complete life histories of these animals are well-documented. Conversely, while wild populations can be complicated to access and individual animals difficult to follow, they offer insight into how and why biological processes evolved in natural settings. Still, field studies should make an effort to conduct longitudinal hormonal and behavioral sampling when possible, as zoo studies demonstrate the clear value of being able to sample from known individuals over consecutive days. Nonetheless, synergistic efforts to study *in-situ and ex-situ* Asian elephant populations will help us further elucidate the complexities of musth to contribute to the conservation of this endangered species.

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Declaration of Competing Interest

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.therwi.2022.100011.

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