RESEARCH ARTICLE

Lactating Coquerel's sifaka (*Propithecus coquereli*) exhibit reduced stress responses in comparison to males and nonlactating females

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Abstract

Cortisol is a glucocorticoid hormone that plays a principal role in metabolic function and stress responses in wild primates. Stressors are ubiquitous in environments and elicit a variety of physiological and behavioral responses. While stress responses are adaptive in the short-term, they can have negative effects when experienced over longer durations. As a physiological stressor, the process of lactation is an energetically expensive activity for mammals. Milk production increases water loss and increased hydration demands are amplified in mammalian species inhabiting xeric habitats, including lemur species living in northwestern Madagascar—the region for this research work. Here, sifakas give birth during the dry season (May–October) and wean infants during the subsequent wet season (November–April). The author collected fecal samples during the 24 weeks following infant births in 10 groups of Coquerel's sifaka in Ankarafantsika Park, Madagascar. The author analyzed the samples by comparing the first 12-week time block to the second 12-week time block, which corresponded to the dry and the beginning of the wet seasons, respectively. Analyses were based on 375 samples collected over two birth seasons (2010 and 2011). A linear mixed model determined the relationships between reproductive class and temporal cortisol variation. The three reproductive classes had significantly different cortisol concentrations. Lactating females had lower cortisol than adult males and nonlactating females in all weeks postnatal. Males had significantly higher cortisol in weeks 13–24 relative to weeks 1–12. Examining seasonal changes in cortisol concentrations demonstrates how lemurs respond physiologically to the energetic constraints of lactation during the critical life history stage of infant development.

KEYWORDS
cortisol, field endocrinology, lactation, life history, *Propithecus coquereli*

1 | INTRODUCTION

Lactation is the most energetically constraining behavior, in which female mammals engage; with daily energy expenditures escalating to 150% above the norm (reviewed in Gittleman & Thompson, 1988). Lactation strategies are characterized by prolonged length and frequency of nursing, large milk volume, and high energetic cost (Hinde & Milligan, 2011; Oftedal, 1984; Power, Oftedal, & Tardif, 2002). Milk is composed of dry matter (total solids), fat, protein, sugars, and minerals. The nutrient composition of milk is exceedingly variable, with primates typically producing relatively low quality, dilute milk relative to other mammals (Oftedal, 1984; Tardif, Power, Oftedal, Power, & Layne, 2001; Tilden & Oftedal, 1997). In general, lemur milk composition is more comparable to anthropoids

of similar body size and distinguished by a higher nursing frequency than other strepsirrhines (Hinde & Milligan, 2011). Lactation also increases demands to water balance (Gittleman & Thompson, 1988); a cost especially pronounced in species living in xeric habitats (Soholt, 1977), such as the dry deciduous forests in Madagascar. The length of lactation alters the concentration of milk nutrients. For example, energy density and milk yield increased as rhesus macaques (*Macaca mulatta*) infants aged and also influenced their growth rates (Hinde, Power, & Oftedal, 2009).

The "cortisol-adaptation (C-A) hypothesis" (Bonier, Martin, Moore, & Wingfield, 2009) is a theoretical reconsideration of the "cort-fitness (C-F) hypothesis" that suggests cortisol negatively covaries with reproductive fitness, where higher cortisol represents individuals in worse physical condition (Bonier, Martin et al., 2009). The "C-A hypothesis" incorporates all challenges related to reproduction as potential threats to homeostasis (Bonier, Martin et al., 2009). The inclusion of reproductive variables (e.g., postnatal effort) is essential to understanding lemur life histories, especially, since reproductive events and seasonal effects often occur in tandem (e.g., parturition and food scarcity; Tecot, 2013; Tecot, Irwin, & Raharison, 2019). A growing number of studies have explored links between environmental factors and stress responses in lemurs. Glucocorticoids (GCs) elevated with infant presence in Verreaux's sifaka (*Propithecus verreauxi*; Brockman, Cobden, & Whitten, 2009); the birth (dry) season in diademed sifaka (*Propithecus diadema*; Tecot et al., 2019); a greater number of males residing in groups (Gould, Ziegler, & Wittwer, 2005); droughts and cyclones in ring-tailed lemurs (*Lemur catta*; Fardi, Sauther, Cuozzo, Jacky, & Bernstein, 2018); and fruit scarcity in red-bellied lemurs (*Eulemur rubriventer*; Tecot, 2008; Tecot, 2013), collared brown lemurs (*Eulemur collaris*; Balestri et al., 2014), ring-tailed lemurs (Cavigetti, 1999), and gray mouse lemurs (*Microcebus murinus*; Hämäläinen, Heistermann, & Kraus, 2015).

Lemur reproductive events occur under stringent seasonal parameters resulting from food scarcity during the dry season (Wright, 1999). Forests in Madagascar are characterized by slow tree growth, low fruit quality (specifically low nitrogen content compared to other geographic areas containing nonhuman primates; Donati et al., 2017), and poor soil fertility (Wright, 1999). Lemurs have evolved behavioral and physiological strategies (e.g., female social dominance) to cope with these harsh conditions (Donati et al., 2017). Nonetheless, the hypervariable climate (i.e., droughts and cyclones) contributes to high infant mortality in Verreaux's sifaka (Richard, Rakotomanga, & Schwartz, 1991), ring-tailed lemurs (Gould, Sussman, & Sauther, 1999), and red-bellied lemurs (Tecot, 2010). *Propithecus* spp. are distinguished by a suite of slow life-history traits including delayed sexual maturity, biennial interbirth intervals with only one infant born per social group, and infant mortality rates exceeding 50% within the first postnatal year due to the severe environmental conditions (Kappeler, Mass, & Port, 2009; Richard, Dewar, Schwartz, & Ratsirarson, 2002). The "energy conservation hypothesis" suggests lemurs invest less in their offspring relative to other primates to save energy (Jolly, 1966; Wright, 1999). While lemurs may behaviorally invest less in the total length of time infants are dependent on mothers compared to haplorhines, Coquerel's sifaka (*Propithecus coquereli*) mothers spend considerable time as the primary caregivers during the first 6 months of life (Ross & Lehman, 2016). In western dry forests, sifaka infants are born during the period of decreased food quality and availability (June–August) and weaned as resources increase (January–February; Lewis & Kappeler, 2005; Young, Richard, & Aiello, 1990). This lean-season "challenge" (Tecot et al., 2019) ensures that higher-quality resources are available when infants start regularly consuming solid foods and females prepare for potential pregnancies.

Oxytocin is a neuropeptide hormone responsible for the formation of mother–offspring bonds that downregulates hypothalamic–pituitary–adrenal (HPA) activity in response to stressors, thereby buffering against the adverse effects of chronic stress, in turn reducing cortisol levels in nonhuman and human primates (reviewed in Crockford, Deschner, & Wittig, 2017; Lee, Macbeth, Pagani, & Young, 2009; Saltzman & Maestripieri, 2011). Oxytocin decreases HPA activity during lactation and mitigates the stress response in a wide range of mammalian taxa including dairy cattle, rodents, nonhuman, and human primates (see Heinrichs, Neumann, & Ehlert, 2002; Saltzman & Maestripieri, 2011; Uvnäs-Moberg, Johansson, Lupoli, & Svennersten-Sjaunja, 2001). Environmental and social stressors have been specifically shown to instigate the release of oxytocin and cortisol in rodents (Smith & Wang, 2014; Torner, Plotsky, Neumann, & de Jong, 2017). Whether stressors can be specifically attributed to inducing oxytocin release is not yet explicitly clear in primates (reviewed in Crockford et al., 2017), though it is evident that hormones of the HPA axis regulate maternal behavior in all primate mothers (Saltzman & Maestripieri, 2011).

The author's goal was to better understand the adaptive value of reproductive seasonality by examining cortisol variation in Coquerel's sifaka to determine how lactating females, adult males, and nonlactating adult females physiologically respond to infants as they age in tandem with seasonal constraints. Based on the "C-A" hypothesis, the author hypothesized lactating females would experience higher fecal cortisol during postnatal Weeks 1–12, as food quality is poorer during those weeks compared to 13–24. The author further hypothesized that the same pattern of cortisol would occur in males and no-mothers, due to food scarcity in the first 12 weeks compared to the second 12 weeks. Lastly, the author hypothesized that lactating females would have lower cortisol compared to males and nonmothers, across all 24 weeks due to oxytocin decreasing HPA during lactation.

2 | METHODS

2.1 | Study site and species

The author conducted my study at the Ampijoroa Forestry Station in Ankarafantsika National Park (ANP), Madagascar. ANP is a dry

deciduous forest with a conspicuous dry season with little moisture extending from May to September (Alonso & Hannah, 2002; Du Puy & Moat, 1996). The majority of rainfall occurs in January and February (Rendigs, Radespiel, Wrogemann, & Zimmermann, 2003). *Propithecus coquereli* groups were initially located when infants had not been born. These same groups were checked weekly until an infant was present (*n* = 9). For example, Group Rambo was located with no infant present on 5/28/2011 and 6/6/2011, and then located with an infant on 6/11/11. Therefore, this infant was a maximum of 5 days old and placed within the category of Week 1 postnatal. If a group was first located with an infant present (*n* = 1), infant age was estimated based on relative infant body size when a precise birth date was known. Weeks 1–12 postnatal coincided with May–August; Weeks 13–24 postnatal coincided with September–December.

This research work complied with protocols approved by the University of Toronto Animal Care Committee (Protocol #: 2000), adhered to the legal requirements of Madagascar, and followed the American Society of Primatologists Principles for the Ethical Treatment of Nonhuman Primates. Research permits were issued in Madagascar by the Ministry of Environment, Water and Forests (Permit #: N°239/11/MEF/SG/DGF/DCB SAP/SCB).

2.2 | Fecal collection and preservation

Feces are a highly accurate biometric measure as they are representative of cumulative secretion and elimination over several hours, thereby reflecting steroid production rate and signifying a greater temporal aggregate of the stress response (Keay, Jatinder Singh, Gaunt, & Kaur, 2006; Sheriff, Dantzer, Delehanty, Palme, & Boonstra, 2011). Pooled cortisol contained in feces provides the opportunity for longitudinally examining stress responses in wild primates. Fecal samples were collected weekly from Coquerel's sifaka lactating adult females, adult males and nonlactating adult females in seven focal groups from July to December 2010 (season 1; *n* = 221) and three focal groups from June to December 2011 (season 2; *n* = 154) for a total sample size of 375. All lactating females were dominant, and all nonlactating females were subordinate. A total of 180 fecal samples were collected from lactating females, 133 from adult males, and 62 from nonlactating adult females. Samples were collected from lactating females and adult males from 1–24 weeks postnatal and nonlactating adult females from Weeks 4–23 postnatal. Weekly collections were uneven due to differences in the timing of infant births and/or no observed defecations with a positive ID. More than one adult male was present in 6 of the 10 groups and more than one nonlactating female was present in 2 of the 10 groups. The collection consistently ended after a sample from each reproductive class was obtained. However, it is possible on rare occasions, samples could have been collected from the same adult male and nonlactating female during a given week. The total amount of feces in each defecation was collected when groups began to move from their sleeping sites in the early morning. Feces were either caught before contacting the ground or collected immediately the

following defecation from the ground if the individual was clearly identified and no other group members defecated simultaneously. Feces were placed in aluminum foil and transported within 4 hrs to a field-made propane gas oven heated to 83℃ and dried for 2–3 hr or until thoroughly dried (following Brockman & Whitten, 1996). The temperature was measured using a water-resistant, max/min digital thermometer (HBE International Inc.) and monitored every 20 min to avoid overheating and potential hormone degradation. Dried feces were kept in manila paper coin envelopes in moisture barrier bags and stored in a plastic container with silica gel.

2.3 | Dried feces preparation

Laboratory assays were conducted at the Wisconsin National Primate Research Center in Madison, Wisconsin. Feces were pulverized using a ceramic mortar and pestle sterilized with ethyl alcohol to create a homogeneous distribution, sifted through a 1 mm mesh filter, and 0.1–0.2 g of feces was scooped into a polypropylene tube.

2.4 | Cortisol steroid extraction and recovery

The author added 2.5 ml distilled H_2O and 2.5 ml ethanol to the polypropylene tubes, vortexed for 10 min, and centrifuged at 2,000 rpm for 10 min. The supernatant was poured into minivials and 1 ml was aliquoted into glass extraction tubes. A total of 4 ml of ethyl acetate was added to extract cortisol from the H_2O/ethanol solution, vortexed for 8 min and centrifuged at 1,000 rpm for 3 min. The top serum layer was aspirated and evaporated in a hot water bath. The author added 1 ml of ethanol after evaporation and extracted samples were kept refrigerated until enzyme immunoassays. Cortisol extraction recovery rate was used to determine steroid extraction efficiency by measuring the procedural loss incurred during extraction. A fecal sample was randomly selected to determine the recovery rate. Titrated cortisol was extracted with an established recovery rate of 75%, which fell within the range of previous studies (see Brockman & Whitten, 1996). Cortisol concentrations were adjusted for this procedural loss before statistical analysis.

2.5 | Cortisol steroid validation

Validation was conducted to determine if circulating cortisol levels were reflected in the measured concentrations and to ensure the solution volume did not influence values (Toume & Palme, 2005). Cortisol was validated successfully on the standard curve after charcoal stripping. Charcoal stripping acts through adsorption, where the solid binds with the cortisol. Approximately 20 μl charcoal treated feces were used in validation and added to the standard curve during enzyme immunoassays.

2.6 | Enzyme immunoassays

Extracted cortisol, enzyme immunoassays (EIA) buffer, six standards, low/high pools were brought to room temperature and vortexed. Sample (20 µl) was aliquoted into culture tubes and 20 µl charcoal-stripped feces were added into standard tubes and evaporated in a hot water bath. The horseradish peroxidase (HRP) and EIA buffer solution were prepared by calculating the volume based on the number of EIA plates (e.g., 25 ml EIA buffer and 20 µl HRP for two EIA plates). EIA buffer (50 µl) was added to culture tubes, 300 µl EIA buffer was added to the nonspecific binding EIA tubes, 50 µl standard was added to standard tubes and 250 µl F: HRP was added to the culture and standard tubes, then vortexed and pipetted into a cortisol coated plate. The plate shook for 5 min and incubated for 2 hrs. The substrate (25 ml citrate, 80 µl H_2O_2, and 250 µl ABTS [color changing solution] for two EIA plates was applied to the plate, shook for 5 min and incubated for 45 min. The stop solution (12.5 ml hydrochloric acid, 30 µl ethylenediaminetetraacetic acid, and 15 ml distilled water) was added and the results read in a SpectraMax® Molecular Devices microplate reader. The low pool coefficient of variation was 15.46/5.17 and the high pool was 15.56/2.09, which fell within the expected range of variation. The same pools were used in 2011 and 2012. Cortisol concentrations were averaged if more than one sample per week was collected from the same individual.

2.7 | Data analysis

A linear mixed model (LMM) in SAS® Version 9.4 was applied to determine the relationships between cortisol and reproductive class. Reproductive class, weeks postnatal, and their interaction were treated as fixed effects. Subject and group ID were treated as random effects. The between-subject factor was reproductive class and the within-subject factor was weeks postnatal. The model contains two random effects, using both the repeated statement (weeks postnatal with Sub = Subject ID) and the random statement in the same LMM. Random effects were specified through repeated measures to account for the nesting structure between individual ID and reproductive class with the compound symmetry variance-covariance structure and to allow for a negative correlation among the observations from the same subject. The inclusion of two random effects gives two layers of covariance structure, with the correlation coefficients differing between observations from the same subject and observations from the same location.

Pseudoreplication was partially accounted for by the within-group and within-subject correlations, given that individuals from the same group are expected to be more correlated than individuals between groups. Since observations from the same individual were treated as observations from the same sifaka group, the within-subject correlation helps to account for individual dependence. LMM was used given that the assumption of normality using the residuals was satisfied ($\mu = 0.08$; $\Sigma = 53.98$).

3 | RESULTS

According to the Type III test from the mixed model, the three reproductive classes had significantly different cortisol concentrations ($F_{2,360} = 5.8$; $p = .003$). Overall, a comparison of Weeks 1–12 to 13–24 postnatal was not significant. A comparison of mean cortisol from Weeks 1–12 to 13–24 in lactating females ($n = 10$), adult males ($n = 19$), and nonlactating adult females ($n = 8$) is shown in Figure 1. Lactating females had significantly lower cortisol in all weeks postnatal relative to adult males ($p = .043$) and nonlactating adult females ($p = .002$; Table 1). Lactating females did not have significantly higher cortisol during Weeks 13–24 in comparison to Weeks 1–12 ($p = .564$; Table 1). Adult males had significantly higher cortisol during Weeks 13–24 relative to Weeks 1–12 ($p = .032$) (Table 1). Nonlactating females did not have significantly higher cortisol during Weeks 13–23 in comparison to Weeks 4–12 ($p = .660$; Table 1).

4 | DISCUSSION

The author hypothesized that all reproductive classes would exhibit higher fecal cortisol during postnatal Weeks 1–12 compared to 13–24 due to decreased food quality and availability during the dry season. The author further hypothesized that lactating females would overall have lower cortisol concentrations relative to males and nonlactating females considering all 24 weeks as a result of oxytocin decreasing the HPA axis during lactation. The first part of my hypothesis was not supported: Lactating females and nonlactating females did not display differences in cortisol across the two time blocks, and males had significantly higher cortisol during Weeks 13–24. The second part of my hypothesis was supported: Lactating females exhibited a significantly reduced stress response compared to males and nonlactating females during all weeks postnatal.
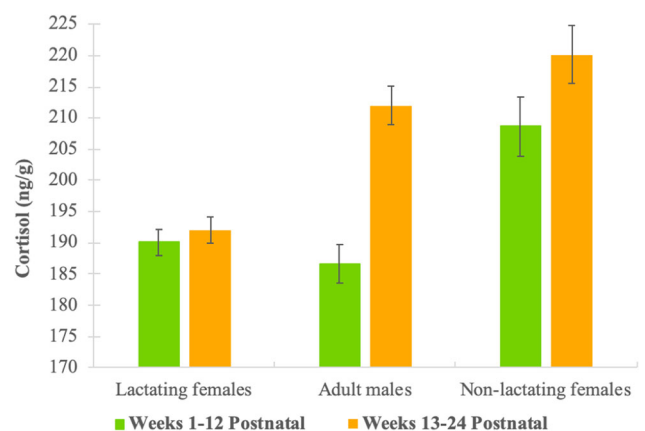

FIGURE 1 Comparison of weekly cortisol concentrations by reproductive class in Coquerel's sifaka

TABLE 1 Mixed model-based comparison of cortisol concentrations across reproductive class and weeks postnatal in Coquerel's sifaka

Reproductive class & weeks postnatal	Estimate	SE	DF	t Value	Pr > |t|
Lactating females; Weeks 1–12 vs. 13–24	−4.81	8.33	360	−0.58	0.564
Adult males; Weeks 1–12 vs. 13–24	−20.67	9.62	360	−2.15	0.032
LF vs. AM; All weeks postnatal	−10.05	4.95	360	−2.03	0.043
Non-lactating adult females; Weeks 1–12 vs. 13–24	−6.24	14.15	360	−0.44	0.660
LF vs. AF All weeks postnatal	−21.89	6.90	360	−3.17	0.002

Abbreviations: AF, adult female; AM, adult male; DF, degrees of freedom; LF, lactating females; SE, standard error.

4.1 | Lactation and HPA activity

Oxytocin is likely mitigating the stress response, thereby contributing to the decreased cortisol levels displayed by lactating sifakas. Reducing maternal stress may improve overall fecundity (Buck Louis et al., 2011) and reproductive fitness (Love & Williams, 2008). Given the low reproductive output characteristic of sifakas (e.g., delayed sexual maturity, 24-month interbirth intervals, singleton births, and high infant mortality; Kappeler et al., 2009; Richard et al., 2002), this may have meaningful implications for infant development and survivability. Maternal stress has been shown to generate adverse effects on child outcomes in humans, ranging from impaired development and disrupted cognitive function to increased psycho-pathological conditions (Class et al., 2014; Glover, 2014). Infant carriage is energetically costly (Altmann & Samuels, 1992) and sifaka mothers are the primary infant carriers (Ross & Lehman, 2016). Reduced stress response may help mothers modulate the responsibility of carrying infants while lactating during the most energetically challenging time of the year when food availability and quality are substantially reduced. Blue monkey (*Cercopithecus mitis*) females in late pregnancy and early lactation showed greater increases in fecal GCs (fGCs) than other females during intervals when preferred foods were less available (Foerster, Cords, & Monfort, 2012).

The impact of lactation on HPA reactivity to stressors in primates is variable (Saltzman & Maestripieri, 2011). Maternal cortisol levels are generally lower during lactation than gestation in white-faced capuchins (*Cebus capucinus*; Carnegie, Fedigan, & Ziegler, 2011), Geoffroy's spider monkey (*Ateles geoffroyi*; Rodrigues, Wittwer, & Kitchen, 2015), and mandrills (*Mandrillus sphinx*; Setchell, Smith, Wickings, & Knapp, 2008). Reference to more elevated cortisol levels appears with ring-tailed lemurs (Cavigetti, 1999) and rhesus macaques (Hoffman, Ayala, Mas-Rivera, & Maestripieri, 2010; Maestripieri, Hoffman, Fulks, & Gerald, 2008). While no hyporesponsiveness to stress when comparing gestating and cycling females has been reported with common marmosets (*Callithrix jacchus*; Abbott et al., 2003; Foerster et al., 2012). It is important to emphasize that although lactating females displayed significantly lower cortisol levels than their conspecifics, this does not signal a nonstress or even a low-stress state in lactating females. Rather, lactating females may be coping with stressors using undetermined behavioral or physiological mechanisms to increase infant survivability given high mortality (Kappeler et al., 2009; Richard et al., 2002). Understanding the interplay between oxytocin and cortisol will contribute to our understanding of how HPA activity differs between mothers and nonmothers. Evaluating oxytocin and cortisol simultaneously will help identify how seasonal stressors are modulated by oxytocin in sifakas.

4.2 | Female social dominance

Cortisol did not decrease despite infants becoming more independent and seasonal increases in food availability during Weeks 13–24 postnatal. Sifakas display female social dominance, with females outranking males in feeding priority, thereby gaining access to preferred and/or higher quality resources (Richard, 1974). Verreaux's sifakas display age and rank related asynchronous receptivity, where dominant females are the first group members to mate (Brockman, 1999). Dominant females were the only mothers in all groups, and subordinate Coquerel's sifakas were always nonmothers A. R. (personal communication, January 31, 2017), which could suggest hormonal suppression of ovulation in lower-ranking females and contribute to a reduced stress response. Feeding priority and increased social support of dominant, lactating females may contribute to elevated cortisol in males and nonlactating females. Future studies examining estradiol, pregnanediol-3-glucronide and luteinizing hormone will quantify the role of hormonal processes in female social dominance.

4.3 | Breeding season effects

Contrary to my hypothesis, males had significantly higher cortisol during Weeks 12–24. The 3-month breeding season occurs from January to March in Coquerel's sifaka (Richard, 1974; Richard, 1985), wherein females undergo a single half hour to 96-hr estrus period (Brockman, 1994). During this time, intergroup agonism and GCs increase (Fichtel, Kraus, Ganswindt, & Heistermann, 2007). Female and male Verreaux's sifakas regularly and equally participate in intergroup encounters (Koch, Signer, Kappeler, & Fichtel, 2016). Female participation is reduced when they have dependent infants, whereas increased male participation is associated with dominant social status (Koch et al., 2016). It is plausible that the approaching breeding season may contribute to an attenuated stress response in lactating females and the heightened response observed in males during the second time block, which ended in mid-December.

Males could be ramping up hormonally during the several months directly preceding the breeding season in anticipation of increased stressors. GCs were significantly higher during mating and birth seasons than other times of the year in male red-fronted lemur (*Eulemur rufus*), which the authors attributed to heightened intra-group competition and the increased risk of infanticide (Ostner, Kappeler, & Heistermann, 2008). Dominant males had higher GCs than subordinates and aggressive behaviors increased during the mating season in Verreaux's sifaka due to the energetic demands associated with male reproduction (Fichtel et al., 2007).

4.4 | Food quality and availability

Cortisol was not elevated during Weeks 1–12 (May–August) relative to 13–24 (September–December), in contrast to my hypothesis. The transition from the dry to the wet season may have been too gradual for the two-time blocks to capture a distinct seasonal change. Examining cortisol levels in conjunction with climatic data during the height of the wet season (January–February; Rendigs et al., 2003) would help detect more distinct seasonal differences in stress responses. Food quality and availability during the peak to the end of the dry season is likely the lowest of the year (Tecot et al., 2019) and coincides with later infant development in Coquerel's sifaka (Ross & Lehman, 2016). Cortisol increases in females during the dry opposed to the wet season in white-faced capuchins (Carnegie et al., 2011) and ring-tailed lemurs (Cavigetti, 1999). All diademed sifaka study groups exhibited elevated fecal GCs during the lean season, wherein a substantial peak occurred during the birth season (Tecot et al., 2019). The same study found that GCs were negatively correlated with both fruit and leaf production (Tecot et al., 2019). FGCs were highest during the convergence of parturition and food scarcity, with high fGCs prevailing until fruit became more abundant and infants were independent (Tecot, 2013). Sifakas have evolved an expanded hindgut and large small intestine to assist with extracting nutrients from foods, including assisting in fiber digestion (Lambert, 1998). This specialized digestive anatomy could allow sifakas to maximize nutrient extraction from lower-quality resources more prevalent during the dry season, in turn contributing to the reduced stress response exhibited during Weeks 1–12.

4.5 | Maternal and allomaternal care

The lower cortisol concentrations displayed by lactating females may be a strategy to allocate more resources towards immediate infant caregiving and future pregnancies. Sifaka infants are more altricial than other lemurs during the first 6 months of life, especially considering their rapid postnatal growth (Young et al., 1990). For example, though *Varecia* and *Propithecus* spp. have comparable adult body weights (3.1–3.6 and 3.7–4.3 kg, respectively; Mittermeier et al., 2010), black-and-white ruffed lemur (*Varecia variegata*) infants develop considerably more quickly than sifakas, with infants reaching

independence 3–4 months postnatal (Baden, 2011; Morland, 1990) in contrast to longer than 6 months in Coquerel's sifaka (Ross & Lehman, 2016).

Allomaternal care in Coquerel's sifakas relatively low (Ross & Lehman, 2016); however, infrequent occurrences should not be disregarded since they may still have an effect on elevated male stress responses. Adult males have been documented carrying infants most frequently in Week 1 postnatal (6.17% of the time) in comparison to less than 5.50% from Weeks 2–25 postnatal (Ross, 2017; Ross & Lehman, 2016). Males did not carry infants during Week 26 in contrast to lactating females that still carried infants 26.42% of the time (Ross, 2017; Ross & Lehman, 2016). The infant mortality rate during my study was 9% in the first 6.5 months postnatal ($n = 11$ infants). This is a striking contrast to 50% mortality in Verreaux's sifaka infants during the first 12 months postnatal (Kappeler et al., 2009; Richard et al., 2002). The potential rise in mortality during late infant development could incite elevated cortisol during later lactation in adult males, who are potential fathers, since this is when infants spend greater durations off their carriers (Ross & Lehman, 2016). Younger, more altricial infants that spend the majority of time physically attached to their mothers could be less of a stressor than older infants.

5 | CONCLUSIONS AND FUTURE RESEARCH

Reproductive class may profoundly influence cortisol levels, which supports the "C-A hypothesis" (Bonier, Moore, Martin, & Robertson, 2009). Future studies using fecal isotope analysis to quantitatively measure the weaning process will help determine exact interbirth intervals and the length of each lactation phase while contextualizing stress responses. Isolating seasonal effects will assist in recognizing causal relationships between reproductive events and the stress response. High infant mortality coupled with slow life histories makes identifying the proximate and ultimate causes of stressors an imperative conservation tool for sifakas.

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REFERENCES

Abbott, D., Keverne, E., Bercovitch, F., Shively, C., Mendoza, S., Saltzman, W., & Sapolsky, R. (2003). Are subordinates always stressed? A comparative analysis of rank differences in cortisol levels among primates. *Hormones and Behavior*, *43*, 67–82. https://doi.org/10.1016/s0018-506x(02)00037-5

Alonso, L., & Hannah, L. Rapid Assessment Program (Conservation International) (2002). *Introduction to the reserve naturelle integrale and reserve forestiere d'Ankarafantsika and to the rapid assessment program. A biological assessment of the Reserve Naturelle Integrale d'Ankarafantsika, Madagascar*. Washington, D. C.: Conservation International, Center for Applied Biodiversity Science, Dept. of Conservation Biology.

Altmann, J., & Samuels, A. (1992). Costs of maternal care: Infant-carrying in baboons. *Behavioural Ecology and Sociobiology*, *29*, 391–398. https://doi.org/10.1007/BF00170168

Baden, A. (2011). *Communal infant care in black-and-white ruffed lemurs (Varecia variegata)* (PhD thesis). Stony Brook University, New York, NY.

Balestri, M., Barresi, M., Campera, M., Serra, V., Ramanamanjato, J., Heistermann, M., & Donati, G. (2014). Habitat degradation and seasonality affect physiological stress levels of *Eulemur collaris* in littoral forest fragments. *PLOS One*, *9*, e107698. https://doi.org/10.1371/journal.pone.0107698

Bonier, F., Martin, P., Moore, I., & Wingfield, J. (2009). Do baseline glucocorticoids predict fitness? *Trends in Ecology and Evolution*, *24*, 634–642. https://doi.org/10.1016/j.tree.2009.04.013

Bonier, F., Moore, I., Martin, P., & Robertson, R. (2009). The relationship between fitness and baseline glucocorticoids in a passerine bird. *General and Comparative Endocrinology*, *163*, 208–213. https://doi.org/10.1016/j.ygcen.2008.12.013

Brockman, D. (1994). *Reproduction and mating system of Verreaux's sifaka, Propithecus verreauxi, at Beza Mahafaly, Madagascar* (PhD thesis). Yale University, New Haven, CT.

Brockman, D. (1999). Reproductive behavior of female *Propithecus verreauxi* at Beza Mahafaly, Madagascar. *International Journal of Primatology*, *20*, 375–398. https://doi.org/10.1023/A:1020500804442

Brockman, D., & Whitten, P. (1996). Reproduction and free-ranging *Propithecus verreauxi*: Estrus and the relationship between multiple partner matings and fertilization. *American Journal of Physical Anthropology*, *100*, 57–69. https://doi.org/10.1002/(SICI)1096-8644(199605)100:1<57::AID-AJPA6>3.0.CO;2-A

Brockman, D., Cobden, A., & Whitten, P. (2009). Birth season glucocorticoids are related to the presence of infants in sifaka (*Propithecus verreauxi*). *Proceedings of the Royal Biological Society B*, *276*, 1855–1863. https://doi.org/10.1098/rspb.2008.1912

Buck Louis, G. M., Lum, K. J., Sundaram, R., Chen, Z., Kim, S., Lynch, C. D., & Pyper, C. (2011). Stress reduces conception probabilities across the fertile window: Evidence in support of relaxation. *Fertility and Sterility*, *95*(7), 2184–2189. https://doi.org/10.1016/j.fertnstert.2010.06.078

Carnegie, S. D., Fedigan, L. M., & Ziegler, T. E. (2011). Social and environmental factors affecting fecal glucocorticoids in wild, female white-faced capuchins (*Cebus capucinus*). *American Journal of Primatology*, *73*(9), 861–869. https://doi.org/10.1002/ajp.20954

Cavigetti, S. (1999). Behavioural patterns associated with faecal cortisol levels in free-ranging female ring-tailed lemurs, *Lemur catta*. *Animal Behaviour*, *57*, 935–944. https://doi.org/10.1006/anbe.1998.1054

Class, Q. A., Abel, K. M., Khashan, A. S., Rickert, M. E., Dalman, C., Larsson, H., & D'Onofrio, B. M. (2014). Offspring psychopathology following preconception, prenatal and postnatal maternal bereavement stress. *Psychological Medicine*, *44*(1), 71–84. https://doi.org/10.1017/S0033291713000780

Crockford, C., Deschner, T., & Wittig, R. (2017). The role of oxytocin in social buffering: What do primate studies add? In H. R. & G. V. (Eds.), *Behavioral pharmacology of neuropeptides: Oxytocin. Current topics in behavioral neurosciences* (35 Germany: Springer Verlag. https://doi.org/10.1007/7854_2017_12

Donati, G., Santini, L., Eppley, T. M., Arrigo-Nelson, S. J., Balestri, M., Boinski, S., ... Ganzhorn, J. U. (2017). Low levels of fruit nitrogen as drivers for the evolution of Madagascar's primate communities. *Scientific Reports*, *7*, 14406. https://doi.org/10.1038/s41598-017-13906-y

Du Puy, D., & Moat, J. (1996). A refined classification of the primary vegetation of Madagascar based on the underlying geology: Using GIS to map its distribution and assess its conservation status. In W. Lourenço (Ed.), *Proceedings of the international symposium on the biogeography of madagascar* (pp. 205–218). Paris, France: Editions de l'Orstrom.

Fardi, S., Sauther, M., Cuozzo, F., Jacky, I., & Bernstein, R. (2018). The effect of extreme weather events on hair cortisol and body weight in a wild ring-tailed lemur population (*Lemur catta*) in southwestern Madagascar. *American Journal of Primatology*, *80*, 80. https://doi.org/10.1002/ajp.22731

Fichtel, C., Kraus, C., Ganswindt, A., & Heistermann, M. (2007). Influence of reproductive season and rank on fecal glucocorticoid levels in free-ranging male Verreaux's sifaka (*Propithecus verreauxi*). *Hormones and Behavior*, *51*, 640–648. https://doi.org/10.1016/j.yhbeh.2007.03.005

Foerster, S., Cords, M., & Monfort, S. (2012). Seasonal energetic stress in a tropical forest primate: Proximate causes and evolutionary implications. *PLOS One*, *7*, e50108. https://doi.org/10.1371/journal.pone.0050108

Gittleman, J., & Thompson, S. (1988). Energy allocation in mammalian reproduction. *American Zoologist*, *28*, 863–875. https://doi.org/10.1093/icb/28.3.863

Glover, V. (2014). Maternal depression, anxiety and stress during pregnancy and child outcome; what needs to be done. *Best Practice & Research Clinical Obstetrics & Gynaecology*, *28*(1), 25–35. https://doi.org/10.1016/j.bpobgyn.2013.08.017

Gould, L., Sussman, R. W., & Sauther, M. L. (1999). Natural disasters and primate populations: The effects of a 2-year drought on a naturally occurring population of ring-tailed lemurs (*Lemur catta*) in southwestern Madagascar. *International Journal of Primatology*, *20*, 69–84. https://doi.org/10.1023/A:1020584200807

Gould, L., Ziegler, T., & Wittwer, D. (2005). Effects of reproductive and social variables on fecal glucocorticoid levels in a sample of adult male ring-tailed lemurs (*Lemur catta*) at the Beza Mahafaly Reserve, Madagascar. *American Journal of Primatology*, *67*, 5–23. https://doi.org/10.1002/ajp.20166

Hämäläinen, A., Heistermann, M., & Kraus, C. (2015). The stress of growing old: Sex- and season-specific effects of age on allostatic load in wild grey mouse lemurs. *Oecologia*, *178*, 1063–1075. https://doi.org/10.1186/s12983-015-0118-9

Heinrichs, M., Neumann, I., & Ehlert, U. (2002). Lactation and stress: Protective effects of breast-feeding in humans. *Stress (Amsterdam, Netherlands)*, 5(3), 195–203. https://doi.org/10.1080/1025389021000010530

Hinde, K., & Milligan, L. (2011). Primate milk: Proximate mechanisms and ultimate perspectives. *Evolutionary Anthropology*, 20, 9–23. https://doi.org/10.1002/evan.20289

Hinde, K., Power, M., & Oftedal, O. (2009). Rhesus macaque milk: Magnitude, sources, and consequences of individual variation over lactation. *American Journal of Physical Anthropology*, 138, 148–157. https://doi.org/10.1002/ajpa.20911

Hoffman, C. L., Ayala, J. E., Mas-Rivera, A., & Maestripieri, D. (2010). Effects of reproductive condition and dominance rank on cortisol responsiveness to stress in free-ranging female rhesus macaques. *American Journal of Primatology*, 72(7), 559–565. https://doi.org/10.1002/ajp.20793

Jolly, A. (1966). *Propithecus verreauxi* verreauxi, *Lemur behavior: A madgascar field study* (pp. 20–68). Chicago, IL: University of Chicago Press.

Kappeler, P., Mass, V., & Port, M. (2009). Even adult sex ratios in lemurs: Potential costs and benefits of subordinate males in verreaux's sifaka (*Propithecus verreauxi*) in the Kirindy Forest CFPF, Madagascar. *American Journal of Physical Anthropology*, 140, 487–497. https://doi.org/10.1002/ajpa.21091

Keay, J., Jatinder Singh, B., Gaunt, M., & Kaur, T. (2006). Fecal glucocorticoids and their metabolites as indicators of stress in various mammalian species: A literature review. *Journal of Zoo and Wildlife Medicine*, 37, 234–244. https://doi.org/10.1638/05-050.1

Koch, F., Signer, J., Kappeler, P. M., & Fichtel, C. (2016). Intergroup encounters in Verreaux's sifakas (*Propithecus verreauxi*): Who fights and why? *Behavioral Ecology and Sociobiology*, 70, 797–808. https://doi.org/10.1007/s00265-016-2105-3

Lambert, J. (1998). Primate digestion: Interactions among anatomy, physiology, and feeding ecology. *Evolutionary Anthropology*, 7, 8–20. https://doi.org/10.1002/(SICI)1520-6505(1998)7:1<8::AID-EVAN3>3.0.CO;2-C

Lee, H. J., Macbeth, A. H., Pagani, J. H., & Young, S. W. (2009). Oxytocin: The great facilitator of life. *Progress in Neurobiology*, 88, 127–151. https://doi.org/10.1016/j.pneurobio.2009.04.001

Lewis, R. J., & Kappeler, P. M. (2005). Seasonality, body condition, and timing of reproduction in *Propithecus verreauxi* verreauxi in the Kirindy Forest. *American Journal of Primatology*, 67, 347–364. https://doi.org/10.1002/ajp.20187

Love, Oliver P., & Williams, Tony D. (2008). The adaptive value of stress-induced phenotypes: Effects of maternally derived corticosterone on sex-biased investment, cost of reproduction, and maternal fitness. *The American Naturalist*, 172(4), E135–E149. https://doi.org/10.1086/590959

Maestripieri, D., Hoffman, C. L., Fulks, R., & Gerald, M. S. (2008). Plasma cortisol responses to stress in lactating and nonlactating female rhesus macaques. *Hormones and Behavior*, 53(1), 170–176. https://doi.org/10.1016/j.yhbeh.2007.09.013

Mittermeier, R., Louis, E., Jr., Richardson, M., Schwitzer, C., Langrand, O., Rylands, A., ... Mackinnon, J. (2010). Indriidae, *Lemurs of madagascar* (3rd Ed., pp. 481–597). Bogotá, Colombia: Conservation International.

Morland, H. (1990). Parental behavior and infant development in ruffed lemurs (*Varecia variegata*) in a northeast Madagascar rain forest. *American Journal of Primatology*, 20, 253–265. https://doi.org/10.1002/ajp.1350200402

Oftedal, O. (1984). Milk composition, milk yield and energy output at peak lactation: A comparative review. *Symposium Zoological Society of London*, 51, 33–85.

Ostner, J., Kappeler, P., & Heistermann, M. (2008). Androgen and glucocorticoid levels reflect seasonally occurring social challenges in male redfronted lemur (*Eulemur fulvus* rufus). *Behavioral Ecology and Sociobiology*, 62, 627–638. https://doi.org/10.1007/s00265-007-0487-y

Power, M., Oftedal, O., & Tardif, S. (2002). Does the milk of callitrichid monkeys differ from that of larger anthropoids? *American Journal of Primatology*, 56, 117–127. https://doi.org/10.1002/ajp.1068

Rendigs, A., Radespiel, U., Wrogemann, D., & Zimmermann, E. (2003). Relationship between microhabitat structure and distribution of mouse lemurs (Microcebus spp.) in northwestern Madagascar. *International Journal of Primatology*, 24, 47–64. https://doi.org/10.1023/A:1021494428294

Richard, A. (1974). Intra-specific variation in the social organization and ecology of *Propithecus verreauxi*. *Folia Primatologica*, 22, 178–207. https://doi.org/10.1159/000155624

Richard, A. (1985). Social boundaries in a Malagasy prosimian, the sifaka (*Propithecus verreauxi*). *International Journal of Primatology*, 6, 553–568. https://doi.org/10.1007/BF02692288

Richard, A., Rakotomanga, P., & Schwartz, M. (1991). Demography of *Propithecus verreauxi* at Beza Mahafaly, Madagascar: Sex ratio, survival, and fertility, 1984–1988. *American Journal of Physical Anthropology*, 84, 307–322. https://doi.org/10.1002/ajpa.1330840307

Richard, A., Dewar, R., Schwartz, M., & Ratsirarson, J. (2002). Life in the slow lane? Demography and life histories of male and female sifaka (*Propithecus verreauxi* verreauxi). *The Zoological Society of London*, 256, 421–436. https://doi.org/10.1017/S0952836902000468

Rodrigues, M. A., Wittwer, D., & Kitchen, D. M. (2015). Measuring stress responses in female Geoffroy's spider monkeys: Validation and the influence of reproductive state. *American Journal of Primatology*, 77(9), 925–935. https://doi.org/10.1002/ajp.22421

Ross, A. (2017). *Maternal effort, food quality, and cortisol variation during lactation in Propithecus coquereli in northwestern Madagascar* (PhD dissertation). University of Toronto, Toronto, Canada.

Ross, A., & Lehman, S. (2016). Infant transport and mother–infant contact from 1 to 26 weeks postnatal in Coquerel's sifaka (*Propithecus coquereli*) in northwestern Madagascar. *American Journal of Primatology*, 78, 646–658. https://doi.org/10.1002/ajp.22529

Saltzman, W., & Maestripieri, D. (2011). The neuroendocrinology of primate maternal behavior. *Progress in Neuro-psychopharmacology & Biological Psychiatry*, 35(5), 1192–1204. https://doi.org/10.1016/j.pnpbp.2010.09.017

Setchell, J. M., Smith, T., Wickings, E. J., & Knapp, L. A. (2008). Factors affecting fecal glucocorticoid levels in semi-free-ranging female mandrills (*Mandrillus sphinx*). *American Journal of Primatology*, 70(11), 1023–1032. https://doi.org/10.1002/ajp.20594

Sheriff, M., Dantzer, B., Delehanty, B., Palme, R., & Boonstra, R. (2011). Measuring stress is wildlife: Techniques for quantifying glucocorticoid. *Oecologia*, 166, 869–887. https://doi.org/10.1007/s00442-011-1943-y

Smith, A., & Wang, Z. (2014). Hypothalamic oxytocin mediates social buffering of the stress response. *Biological Psychiatry*, 76, 281–288. https://doi.org/10.1016/j.biopsych.2013.09.017

Soholt, L. (1977). Consumption of herbaceous vegetation and water during reproduction and development of Merriam's kangaroo rat, *Dipodomys merriami*. *The American Midland Naturalist Journal*, 98, 445–457. https://doi.org/10.2307/2424992

Tardif, S., Power, M., Oftedal, O., Power, R., & Layne, D. (2001). Lactation, maternal behavior and infant growth in common marmoset monkeys (*Callithrix jacchus*): Effects of maternal size and litter size. *Behavioral Ecology and Sociobiology*, 51, 17–25. https://doi.org/10.1007/s002650100400

Tecot, S. (2008). *Seasonality and predictability: The hormonal and behavioral responses of the red-bellied lemur, Eulemur rubriventer, in Southeastern Madagascar* (PhD Dissertation). University of Texas, Austin, TX.

Tecot, S. (2010). It's all in the timing: Birth seasonality and infant survival in *Eulemur rubriventer*. *International Journal of Primatology*, 31, 715–735. https://doi.org/10.1007/s10764-010-9423-5

Tecot, S. (2013). Variable energetic strategies in disturbed and undisturbed rain forests: *Eulemur rubriventer* fecal cortisol levels in south-eastern Madagascar. In J. Masters, M. Gamba & F. Génin (Eds.), *Leaping ahead: Advances in prosimian biology* (pp. 185–195). New York, NY: Springer-Verlag. https://doi.org/10.1007/978-1-4614-4511-1_21

Tecot, S., Irwin, M., & Raharison, J. (2019). Faecal glucocorticoid metabolite profiles in diademed sifakas increase during seasonal fruit scarcity with interactive effects of age/sex class and habitat degradation. *Conservation Physiology*, 7, coz001. https://doi.org/10.1093/conphys/coz001

Tilden, C., & Oftedal, O. (1997). Milk composition reflects patterns of maternal care in prosimian primates. *American Journal of Primatology*, 41, 195–211. https://doi.org/10.1002/(SICI)1098-2345(1997)41:3<195::AID-AJP3>3.0.CO;2-S

Torner, L., Plotsky, P., Neumann, I., & de Jong, T. (2017). Forced swimming-induced oxytocin release into blood and brain: Effects of adrenalectomy and corticosterone treatment. *Psychoneuroendocrinology*, 77, 165–174. https://doi.org/10.1016/j.psyneuen.2016.12.006

Toume, C., & Palme, R. (2005). Measuring fecal glucocorticoid metabolites in mammals and birds: The importance of validation. *Annals of the New York Academy of Sciences*, 1046, 54–74. https://doi.org/10.1196/annals.1343.006

Uvnäs-Moberg, K., Johansson, B., Lupoli, B., & Svennersten-Sjaunja, K. (2001). Oxytocin facilitates behavioural, metabolic and physiological adaptations during lactation. *Applied Animal Behaviour Science*, 72(3), 225–234. https://doi.org/10.1016/S0168-1591(01)00112-5

Wright, P. (1999). Lemur traits and Madagascar ecology: Coping with an island environment. *Yearbook of Physical Anthropology*, 42, 31–72. https://doi.org/10.1002/(sici)1096-8644(1999)110:29+<31::aid-ajpa3>3.0.co;2-0

Young, A., Richard, A., & Aiello, L. (1990). Female dominance and maternal investment in strepsirhine primates. *American Naturalist*, 135, 473–488. https://doi.org/10.1086/285057