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# Assessing adaptability and reactive scope: Introducing a new measure and illustrating its use through a case study of environmental stress in forest-living baboons



A.M. MacLarnon<sup>a,\*</sup>, V. Sommer<sup>b,c</sup>, A.S. Goffe<sup>a,1</sup>, J.P. Higham<sup>a,2</sup>, E. Lodge<sup>a</sup>, P. Tkaczynski<sup>a</sup>, C. Ross<sup>a,c</sup>

<sup>a</sup> Centre for Research in Evolutionary & Environmental Anthropology, University of Roehampton, Holybourne Avenue, London SW15 4JD, United Kingdom

<sup>b</sup> Department of Anthropology, University College London, Gower Street, London WC1E 6BT, United Kingdom

<sup>c</sup> Gashaka Primate Project, PMB 08, 663001 Serti, Taraba State, Nigeria

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## ABSTRACT

In order to maintain regulatory processes, animals are expected to be adapted to the range of environmental stressors usually encountered in their environmental niche. The available capacity of their stress responses is termed their reactive scope, which is utilised to a greater or lesser extent to deal with different stressors. Typically, non-invasive hormone assessment is used to measure the physiological stress responses of wild animals, but, for methodological reasons, such measurements are not directly comparable across studies, limiting interpretation.

To overcome this constraint, we propose a new measure of the relative strength of stress responses, 'demonstrated reactive scope', and illustrate its use in a study of ecological correlates (climate, food availability) of faecal glucocorticoid (fGC) levels in two forest-living troops of baboons. Results suggest the wild-feeding troop experiences both thermoregulatory and nutritional stress, while the crop-raiding troop experiences only thermoregulatory stress. This difference, together with the crop-raiding troop's lower overall physiological stress levels and lower demonstrated fGC reactive scope, may reflect nutritional stress-buffering in this troop. The relatively high demonstrated fGC reactive scope levels of both troops compared with other baboons and primate species, may reflect their extreme habitat, on the edge of the geographic range for baboons.

Demonstrated reactive scope provides a means of gauging the relative strengths of stress responses of individuals, populations, or species under different conditions, enhancing the interpretive capacity of non-invasive studies of stress hormone levels in wild populations, e.g. in terms of animals' adaptive flexibility, the magnitude of their response to anthropogenic change, or the severity of impact of environmental conditions.

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**Abbreviations:** DRS, demonstrated reactive scope; DRScv, coefficient of variation of demonstrated reactive scope; fGC, faecal glucocorticoids; uGC, urinary glucocorticoids.

\* Corresponding author.

**E-mail addresses:** [a.maclarnon@roehampton.ac.uk](mailto:a.maclarnon@roehampton.ac.uk) (A.M. MacLarnon), [v.sommer@ucl.ac.uk](mailto:v.sommer@ucl.ac.uk) (V. Sommer), [agoffe@dpz.eu](mailto:agoffe@dpz.eu) (A.S. Goffe), [jhigham@nyu.edu](mailto:jhigham@nyu.edu) (J.P. Higham), [emily.lodge@roehampton.ac.uk](mailto:emily.lodge@roehampton.ac.uk) (E. Lodge), [tkaczynp1@roehampton.ac.uk](mailto:tkaczynp1@roehampton.ac.uk) (P. Tkaczynski), [c.ross@roehampton.ac.uk](mailto:c.ross@roehampton.ac.uk) (C. Ross).

<sup>1</sup> Present address: Cognitive Ethology Laboratory, German Primate Centre, Kellnerweg 4, 37077 Göttingen, Germany.

<sup>2</sup> Present address: Department of Anthropology, New York University, 25 Waverly Place, New York, NY 10003, USA.

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## 1. Introduction

Understanding the physiological impact of different stressors on wild animals is pivotal to our comprehension of environmental adaptation (Wingfield 2005; Landys et al., 2006), and potentially also has an important role in the recognition and mitigation of impacts of anthropogenic change (Chapman et al., 2007; Wingfield, 2013). Animals experience stressors in two broad categories, ecological and social, and species are expected to be adapted to maintain regulatory processes in response to the particular types and severity of stressors usually encountered in their natural environment (Korte et al., 2005). Koolhaas et al. (2011) denoted the array of environmental conditions to which a species is adapted its 'regulatory range'; and Romero et al. (2009), building

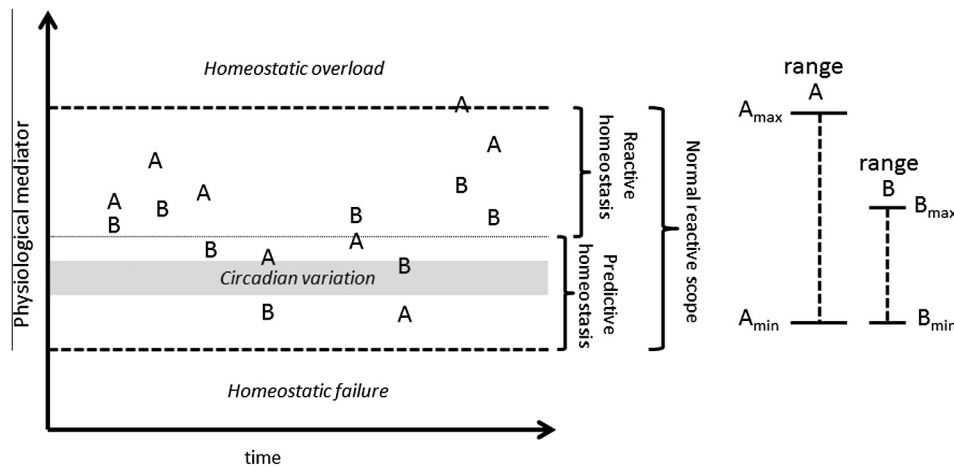
on the concept of allostasis, or maintaining stability through change (McEwen, 1998), termed a species' normal range of available stress response (physiological, behavioural or cognitive) its 'normal reactive scope'.

Physiological appraisal of the impact of stressors on wild mammals has been greatly enhanced by the development of non-invasive methods of hormone level assessment from excreta (Whitten et al., 1998). However, the inability to calibrate the relative strengths of detected stress responses hampers the interpretation of results in terms of animals' comparative responsiveness, robusticity and resilience (Wingfield, 2013). This is due to the lack of direct comparability of measures from different assays, of different hormone metabolites excreted by different species, in different matrices (urine or faeces), and differential faecal composition dependent on diet. Here, we propose quantifying a new measure, 'demonstrated reactive scope' (DRS), to overcome these constraints. This can be used to assess the relative stress experienced by different individuals, populations or species in response to the same or different stressors. We explore the analytical and interpretive value of this measure through a case study investigating variation in glucocorticoid levels in two troops of forest-living baboons (*Papio anubis*), in Gashaka Gumti National Park, Nigeria, in relation to climatic factors and food availability. Using the new measure, we assess the relative stress levels experienced by this population compared to other baboons and other primates, and consider the results both in relation to the location of the study troops, at the geographic and environmental edge of baboon distribution, and to the effects of anthropogenic impact.

Common vertebrate physiological stress responses function similarly across a broad range of stressors, both physical and psychological (Sapolsky, 1992). Activation of the hypothalamic–pituitary–adrenal (HPA) axis is a major component of the vertebrate

stress response battery that results in the release of glucocorticoids, which have a wide variety of effects. These include the direct mobilisation of energy, behavioural adjustments, and preparative processes which prime physiological systems for predictable events and changes such as circadian cycles of rest and activity, different life history stages including growth and reproduction, and seasonal climatic variation (Sapolsky et al., 2000; Romero, 2002; Wingfield, 2005); the corresponding glucocorticoid response levels are denoted the predictive homeostatic range in the Reactive Scope Model (Romero et al., 2009; Fig. 1). Greater glucocorticoid responses to more random, though still normal stressors for an animal's environment, such as injury, disease and predation avoidance (Wingfield, 2005), fall within the reactive homeostatic range of the Reactive Scope Model. Together, the predictive homeostatic range and the reactive homeostatic range comprise normal reactive scope (Romero et al., 2009; Fig. 1). The basic effects of the release of glucocorticoids are protective, and variation in glucocorticoid levels within normal reactive scope acts to achieve allostasis, or physiological stability, in the face of environmental challenges (McEwen and Wingfield, 2003; Landys et al., 2006). Such variation is adaptive. Conceptual models for the stress response, such as the Reactive Scope Model, envisage that there is a cumulative cost in terms of wear and tear, or allostatic load, to maintaining stability in response to such challenges, but that this is within an animal's adaptive capability (McEwen and Wingfield, 2003; Romero et al., 2009). Potentially, a species' habitat or geographic distribution may be restricted by its physiological limitations (Busch et al., 2011), such as the range of its normal reactive scope, which delimits its capacity to respond effectively and efficiently to varying environmental stressors, such as food availability or climate.

Extreme or unpredictable stressors may stimulate a stress response beyond the normal reactive scope of a species,



$$DRS_x = (X_{max} - X_{min}) / X_{min} \times 100$$

$$DRScv_x = (\text{standard deviation}_x / \text{mean}_x) \times 100$$

$$DRS_A > DRS_B$$

$$DRScv_A > DRScv_B$$

**Fig. 1.** Figure depicting the Reactive Scope Model (after Romero et al., 2009), for a non-seasonally reproducing organism, and two sets of data, A and B, which could be from two individuals, troops, species etc., on levels of a physiological stress mediator. The figure depicts the basic circadian range of physiological mediator levels; increases or decreases beyond this range, within the predictive homeostatic range, which occur in response to predictable changes and occurrences; and further increases within the range of reactive homeostasis, in response to more unpredictable events. All of this variation is incorporated within the normal reactive scope, within which the organism experiences wear and tear, but not pathological effects of stress. If physiological mediator responses rise above the range of normal reactive scope to levels of homeostatic overload, this may result in pathological effects. For the examples shown, Set A experiences a greater range of physiological mediator levels than Set B. Although the means of measurement for the two sets of data may be different, and hence the measures not directly comparable, values for demonstrated reactive scope (DRS) are higher for Set A than for Set B, reflecting Set A's full use of the normal reactive scope, whereas physiological mediator levels for Set B fall well within its bounds. The higher DRS and DRScv for Set A than Set B reflect the greater overall stress, and hence the greater wear and tear, experienced by the former Set.

corresponding to allostatic or homeostatic overload (McEwen and Wingfield, 2003; Romero et al., 2009; Fig. 1). Such overload may have damaging effects, particularly if sustained. In captive animals, chronically elevated glucocorticoid levels can result in deleterious effects such as diabetes, muscle wastage, fat accumulation, impaired memory, and the inhibition of the immune response and reproductive functions, which in turn affect fecundity, growth, reproduction and mortality (Sapolsky et al., 2000). Whilst the conditions promoting these effects may not normally be found in a species' natural environment (Wingfield, 2005; Boonstra, 2013), anthropogenic change such as habitat degradation or access to inappropriate or excessive human food sources, might pose similar challenges (Chapman et al., 2007; Rangel-Negrin et al., 2009). Measuring the physiological stress levels of a wild population might enable its wellbeing to be assessed more sensitively and sooner than through demographic measures such as population size or mortality data. Hence, for example, conservation needs may be recognised earlier and the efficacy of conservation interventions measured with greater precision (Chapman et al., 2007).

In wild primates and other mammals, both social (e.g. Abbott et al., 2003; Goymann and Wingfield, 2004; Creel et al., 2013) and less commonly, ecological factors, such as climate and food availability, have been investigated for possible association with variation in physiological stress measures using non-invasive assessment of hormone levels. The results from studies of different species which excrete different glucocorticoid metabolites, using different assays with varying reactivity to different glucocorticoid metabolites, measured from different matrices (either faeces or urine), cannot be compared in quantitative terms. However, some general patterns can be detected. For example, in wild primate studies, variation in which ecological factors have been associated with variation in glucocorticoid levels can usually be ascribed to whether or not the range for a specific ecological factor includes extreme values, or extreme values for the species concerned. In a number of studies, in different species, reduced food availability and more challenging climatic factors are associated with higher glucocorticoid levels reflecting greater stress. Based on such intra-study comparisons, mostly across seasons, the evidence suggests that both nutritional and thermoregulatory factors may be involved. Studies to date are summarised in Table 1, and general patterns are described below, as well as examples highlighting the limitations of the means of analysis available to date.

Studies of wild primates have variously identified associations between very cold temperatures, very hot temperatures or dry conditions, which all potentially act as thermoregulatory stressors, and significant rises in faecal glucocorticoid (fGC) levels, although these are not always found and not consistently for both sexes (Table 1). For example in Amboseli, Kenya, which is characterised by high maximum temperatures, which can exceed 40 °C, low annual rainfall averaging 350 mm, and a 5-month long dry season, significantly higher fGC levels were associated with higher maximum temperatures for female yellow baboons (*Papio cynocephalus*), though not for males, and both sexes have significantly higher dry season fGC levels (Gesquiere et al., 2008, 2011). At other sites where minimum temperatures are more extreme, low temperatures are linked with high fGC levels, for example in male gelada in Ethiopia (*Theropithecus gelada*; Beehner and McCann, 2008); and in female chacma baboons (*Papio ursinus*) in South Africa, which have higher fGC levels in months with shorter daylight hours, suggesting a link with colder minimum temperatures in winter (Weingrill et al., 2004).

In contrast to these studies, others do not find associations between climate and fGC levels. Although it is not always easy to compare studies, sites where there are less extreme variations in temperature and/or rainfall seem less likely to show links between climate and fGC levels. For chacma baboons in Botswana, for

example, where minimum temperatures and rainfall are relatively high compared with other baboon sites, no associations between seasonal temperature variation or seasonal rainfall variation and fGC levels were found (Bergmann et al., 2005; Crockford et al., 2008). Similarly, male gelada in Ethiopia show no associations between seasonal rainfall variation and fGC levels (Beehner and McCann, 2008), and long-tailed macaques (*Macaca fascicularis*) in Sumatra, where mean monthly temperatures vary only by 0.9 °C, and, despite highly seasonal rainfall, there is rainfall every month, show no relationships between fGC levels and climatic factors (Girard-Buttoz et al., 2009).

Negative associations between seasonal variation in rainfall and variation in fGC levels, are usually considered to reflect variation in nutritional stress, as rainfall is interpreted as a proxy for food availability, and glucocorticoid levels may be raised to draw on stored energy reserves. Using intra-study seasonal comparisons, these studies most often report significantly higher fGC levels in the dry season, as for the Amboseli baboons described above, and for Mexican spider monkeys (Rangel-Negrin et al., 2009). However seasonal co-variation between food availability and climate may make relationships difficult to interpret. For example, in black howler monkeys (*Alouatta pigra*) variation in monthly maximum temperature was not correlated with fGC levels when fruit availability was included in the model, and analysis was further complicated by the correlation of both these ecological variables with rainfall (Behie et al., 2010). Reduced food availability may be the direct stressor causing fGC levels to vary seasonally in ring-tailed lemurs (Pride, 2005), although it is difficult to separate the effects of seasonality in food and reproductive factors in such highly seasonal breeders. In another study of female capuchins in Costa Rica, where the dry and wet seasons are very marked (Carnegie et al., 2011), fGC levels were significantly higher in the dry season when food was actually more available, suggesting that the dry heat itself may be the proximate factor causing thermoregulatory stress (Carnegie et al., 2011).

Behavioural plasticity may also have bearing on the stressfulness of environmental variation. Baboons and gelada commonly switch to consumption of underground plant storage structures in the dry season, which may mitigate any potential impact of the dry season via their effect on food availability (Beehner and McCann, 2008). However, in very hot and open environments, such as Amboseli, baboons are exposed to extreme ambient temperatures and direct sunlight whilst digging for subterranean food sources. Seasonal variation in fGC levels in such baboons may therefore be caused by either seasonal nutritional or thermoregulatory stress, or a combination of both.

Considering all these studies, evidence supports the interpretation that seasonality in climate may have both direct and indirect impacts on stress levels in wild primates, particularly where there is very marked fluctuation in rainfall or temperature. However, it is not possible from such comparisons to determine how relatively strong the stress responses measured in the different study populations are, and therefore how big a challenge is posed to their available adaptive capacity.

Rather than using rainfall as a proxy, a number of studies of wild primates have used direct food or feeding measures to test for the possible stress effects of variation in food availability (Table 1). In studies of red colobus monkeys (*Piliocolobus tephrosceles*), variation in food availability in forest fragments was marginally negatively related to variation in fGC levels within groups (Chapman et al., 2006), and dietary quality was negatively correlated with fGC levels in the subsequent month (Chapman et al., 2007). Among chimpanzees (*Pan troglodytes*), dietary quality was negatively correlated with urinary glucocorticoid (uGC) levels in lactating females only (Emery Thompson et al., 2010). Other studies have identified negative correlations between the availability of

**Table 1**

Summary of results from previous and present studies of faecal (fGC) or urinary (uGC) glucocorticoid levels in relation to climate and food availability in wild primates. Demonstrated reactive scope values for previous studies were most calculated from data presented in tables or figures, and are for monthly mean values, from multiple animals, unless otherwise indicated (only data based on similar periods are directly comparable). Coefficients of variation (cv) for DRS values are only presented where monthly mean values from multiple animals across the full calendar year were available. Positive (+ve), negative (-ve), non-significant (ns) correlations; T – temperature,  $T_{\min}$  – minimum temperature,  $T_{\max}$  – maximum temperature.

Species	Sex	Location	Matrix	Significant correlations with variation in glucocorticoid levels		Demonstrated reactive scope (cv of DRS)	References
				Climatic factors	Dietary factors		
<i>Pan troglodytes</i>	Lactating females	Kibale, Uganda	uGC		Dietary quality (-ve)	-	Emery Thompson et al. (2010)
<i>Pan troglodytes</i>	Non-lactating females	Kibale, Uganda	uGC		Dietary quality (ns)	-	Emery Thompson et al. (2010)
<i>Pan troglodytes</i>	Males	Kibale, Uganda	uGC		Availability of preferred foods (-ve)	175% (35%)	Muller and Wrangham (2004)
<i>Theropithecus gelada</i>	Males	Simien Mountains, Ethiopia	fGC	$T_{\min}$ (-ve) $T_{\max}$ (+ve) Altitude (+ve) (possibly indirect measure of T)		40% (12%) -	Beehner and McCann (2008)
<i>Papio ursinus</i>	Females	De Hoop, South Africa	fGC	Rain (ns) Daylight hours (-ve) (indirect measure of $T_{\min}$ )		- 170% (26%)	Weingrill et al. (2004)
<i>Papio cynocephalus</i>	Females	Amboseli, Kenya	fGC	$T_{\max}$ (+ve)	Indirect food availability: dry season vs wet season (higher fGC levels in dry season when food less available)	25% (8%)	Gesquiere et al. (2008)
<i>Papio cynocephalus</i>	Males	Amboseli, Kenya	fGC	$T_{\max}$ (ns)	Indirect food availability: dry season vs wet season (higher fGC levels in dry season when food less available)	-	Gesquiere et al. (2011)
<i>Papio ursinus</i>	Females	Moremi, Botswana	fGC	T (ns)			Bergmann et al. (2005)
<i>Papio ursinus</i>	Males	Moremi, Botswana	fGC	Rain (ns) T (ns)			Crockford et al. (2008)
<i>Macaca fascicularis</i>	Males	Sumatra, Indonesia	fGC	Rain (ns) T (ns)			Girard-Buttoz et al. (2009)
<i>Cercopithecus mitis</i>	Combined females + males	Gede Ruins, Kenya	fGC	Rain (ns)	Availability of preferred foods (-ve)	-	Foerster and Monfort (2010)
<i>Ptilocolobus tephrosceles</i>	Combined females + males	Kibale, Uganda	fGC		Food availability (marginally -ve)	-	Chapman et al. (2006)
<i>Ptilocolobus tephrosceles</i>	Combined females + males	Kibale, Uganda	fGC		Dietary quality (-ve)	50% (12%)	Chapman et al. (2007)
<i>Alouatta pigra</i>	Females & males	Monkey River, Belize	fGC	$T_{\max}$ (ns when fruit availability included in model)	Fruit availability (-ve)	65% (dry vs wet season)	Behie et al. (2010)
<i>Ateles geoffroyi</i>	Combined females + males	Yucatan, Mexico	fGC	Dry vs wet season (higher/ lower)		50% (dry vs wet season)	Rangel-Negrín et al. (2009)
<i>Cebus capucinus</i>	Females	Santa Rosa, Costa Rica	fGC	Hot dry season vs cooler wet season (higher/ lower)		25% (dry vs wet season)	Carnegie et al. (2011)
<i>Brachyteles arachnoides</i>	Males	Minas Gerais, Brazil	fGC	Less wet vs wetter rainy season (lower vs higher fGC)			Strier et al. (1999)
<i>Lemur catta</i>	Combined females + males	Berenty, Madagascar	fGC		Major fruit item availability (fGC levels highest in the one season of three with lowest availability)	36%	Pride (2005)
<i>Lemur catta</i>	Females	Beza Mahafaly, Madagascar	fGC		Seasonal feeding effort or foraging time (+ve)	90–160% (weekly means)	Cavigelli (1999)
<i>Papio anubis</i>	Females	Kwano, Gashaka, Nigeria	fGC	$T_{\max}$ monthly (+ve); $T_{\min}$ daily (+ve); Rain monthly (+ve); Humidity (ns)	Vine fruit availability daily & monthly (-ve); Tree fruit availability (ns)	236% (34%)	Present study
<i>Papio anubis</i>	Females	Gamgam, Gashaka, Nigeria	fGC	Rain monthly (+ve); Humidity daily (+ve); $T_{\max}$ , $T_{\min}$ (ns)	Vine and Tree fruit availability (ns)	187% (32%)	Present study

preferred foods and variation in fGC and uGC levels, for example in Sykes' monkey (*Cercopithecus mitis*) (Foerster and Monfort, 2010), black howler monkeys (Behie et al., 2010) and male chimpanzees (Muller and Wrangham 2004). Seasonal variation in relative feeding effort, or time spent actively foraging, was positively correlated with variation in fGC levels in female ring-tailed lemurs (Cavigelli, 1999), although, as before, reproductive seasonality makes interpretation difficult. Overall, it is again not possible to calibrate how marked the relative stress levels detected are for each of these studies.

As demonstrated in many of the studies described, seasonal variation in ecological factors provides the opportunity to investigate their stress impacts, comparing across seasons. However, the common synchrony of seasonality in different factors complicates identification of the direct and indirect causes of variation in stress levels. Primates mainly inhabit tropical and sub-tropical regions, where the amount and temporal distribution of rainfall in particular are related to the quantity and fluctuation of primary plant productivity, and therefore to variation in food availability. This in turn can influence the timing of reproductive activity (Brockmann and van Schaik, 2005), and reproductive factors are known to affect glucocorticoid and hence fGC levels (e.g. Strier et al., 1999; Ziegler et al., 2000). While studies of the ecological correlates of stress in wild primates to date have involved a variety of species, the majority are of baboons and gelada. When trying to tease apart the ecological factors affecting stress levels, baboons in particular have a number of useful features. Firstly, most populations demonstrate no, or limited reproductive seasonality, despite temporal fluctuations of climate and food sources throughout their range, so enabling statistical separation of reproductive and ecological factors on stress levels. Baboons are also highly unusual among primates in the broad range of habitats they inhabit, including some of the most extreme of all primate habitats in terms of physical and biological environment, being widely distributed across sub-Saharan Africa, and absent only from the highest altitudes and areas of very dense, wet forest. In addition, at several field sites, baboons have been studied over many years which enables analysis of inter-annual variation in environmental factors in addition to inter-seasonal comparisons. This approach has been exploited little to date in studies of glucocorticoid variation, probably because of the greater demands on data collection. Nevertheless, the finding of variation year to year in fGC levels relative to climatic factors for muriquis (*Brachyteles arachnoides*) (Strier et al., 1999) is indicative of its potential value. The case study presented here draws on all of these ecological characteristics of baboons and the analytical opportunities they provide.

However, comparisons within baboons are still limited by the difficulties of comparing field-derived glucocorticoid data from different studies even within the same species or genus (dependent on the baboon taxonomy used (Groves, 2001)). To overcome this, we suggest comparison of the ratios of maximum to minimum glucocorticoid levels, or the percentage increase from lowest to highest levels, as well as the scaled variance of glucocorticoid levels, calculated as the coefficient of variation, may provide useful, comparable measures of the relative stress levels experienced. Percentage increases and coefficients of variation could be considered to indicate relative stress loads, and therefore help to identify which stressors, in which populations or species, under which conditions, call upon adaptive flexibility or normal reactive scope to a greater or lesser extent. Following on from this, conditions might be identified that push populations or species into homeostatic overload, beyond their adaptive limits, resulting in their suffering detrimental effects, for example in terms of nutritional sufficiency or disease resistance. We propose that the range of an observed stress response is termed the 'demonstrated reactive scope' (DRS). This is defined as the percentage difference between the highest and lowest levels of stress

response seen, e.g. the difference between maximum and minimum observed glucocorticoid levels, as a percentage of the minimum level. The coefficient of variation of the demonstrated reactive scope (DRScv) is calculated using the standard formula, i.e. the standard deviation of glucocorticoid levels divided by the mean, expressed as a percentage. These two scale-free measures are not expected to be assay or matrix dependent, and hence they can be compared directly across different studies (Fig. 1).

In the case study presented here, using data from a long-term study of two troops of olive baboons at Gashaka Gumti National Park, Nigeria (Sommer and Ross, 2011a), we investigate potential ecological correlates of stress, both climatic factors and food availability, in relation to fGC levels in adult females across seasons and years. Few previous studies of the ecological correlates of stress levels have considered measures of both climatic and food resource variation together, which potentially enables separation of thermoregulatory and nutritional impacts on stress levels. The Gashaka baboons show no, or very limited, reproductive seasonality (Warren et al., 2011), so this potentially confounding factor is absent. Their diet predominantly comprises fruit and seeds (approx. 60%) which vary in availability seasonally (Ross et al., 2011). Of the two troops, Gamgam troop, whose home range incorporates cultivated fields, spends 9% of foraging time crop-raiding which may provide anthropogenic nutritional buffering against seasonal variation in fruit availability, while Kwano troop is totally wild-feeding (Warren et al., 2011). Unlike the majority of baboons studied to date, the baboons of Gashaka are forest-living, inhabiting a mosaic of open woodland and closed forest characterised by marked fluctuation between very dry and very wet seasons (Ross et al., 2011). They are located in an area in north-eastern Nigeria close to the edge of the baboon distribution. Nearby in even wetter regions with denser forest, they are replaced by drills (*Mandrillus leucophaeus*). Using measures of demonstrated reactive scope, we compare the relative stress levels and variance of these two troops with results from other baboon sites and other wild primate species, providing, for the first time, quantitative comparative assessment of stress levels in wild primate populations. We consider the results in relation to ecological factors, the geographical limits of the baboon distribution and anthropogenic change, exploring the analytical and interpretive power of demonstrated reactive scope.

## 2. Materials and methods

### 2.1. Study site & animals

Data were collected from adult and sub-adult females of the two study troops of olive baboons at Gashaka Gumti National Park (GGNP), Nigeria (Sommer and Ross, 2011b) from January 2004 to December 2009. Kwano and Gamgam troops have been monitored since 2000, and all individuals are identifiable. In the 72 months from 2004 to 2009, Kwano troop averaged 26.3 animals (range 15–38), including 9–10 adult females and 0–2 sub-adult females. Gamgam troop averaged 19.9 animals (range 14–24), including 4–5 adult females and 0–1 sub-adult females. Reproductive state was monitored daily for each female and categorised as cycling, pregnant (not cycling and showing reddening of the ano-genital area) or lactating (suckling an infant). In addition, the mean gestation period for the two troops (185 days,  $n = 4$ ; Higham et al., 2009a) was used to estimate the start of pregnancy from the date of birth, and this assessment was preferred over the ano-genital signs where any discrepancy occurred.

All research on these animals was assessed and approved by the Ethics Committees of the University of Roehampton, and adhered to all animal care, legal and ethical requirements of both Nigeria and the UK.

## 2.2. Climate data

Daily values were recorded at both field sites for minimum ( $T_{\min}$ ) and maximum ( $T_{\max}$ ) ambient temperature, rainfall (Rain) and humidity (recorded between 4 pm and 7 pm) for 2004–2009. Minimum and maximum temperatures are used, rather than daily averages, as these are expected to reflect better the significant stressors involved, as identified in previous studies. Data for some individual days are missing, and for some months (Kwano: all climate data March and April 2006, and humidity data October–December 2004; Gamgam: all climate data December 2008, rainfall data November 2006–March 2007, rainfall and maximum temperature data May 2008, and humidity data November 2005–January 2007 and March–December 2007). Both daily climate values and monthly averages are included in analyses. Although daily values are more direct measures of immediate climatic factors, in general daily values do not vary greatly over a month, and using monthly averages enables larger numbers of paired fGC values to be included as more daily climatic data points are missing. As monthly average rainfall and humidity values were highly collinear ( $r = 0.71$ ,  $n = 111$ ,  $p < 0.001$ ), with a correlation coefficient above the recommended cut-off of about 0.6 (Tabachnik and Fidell, 2013), only one of the two variables was entered into GLMM analyses using monthly climate data; rainfall was chosen as the data are more complete. For all other pairs of daily or monthly climatic variables, correlation coefficients were less than 0.6.

## 2.3. Food availability data

Data on habitat phenology are based on 985 trees and 815 associated woody climbers growing along 8 km of a line transect at the Kwano study site. This transect is a representative cross-section for the area's physical features and vegetation cover of closed forest and open woodland-savannah. Phenology was monitored every two weeks, and monthly fruit availability was assessed for the period 2004–2009 by summing the diameter-at-breast-height (DBH) of all fruit bearing trees (divided by 2 to produce monthly averages), to arrive at a fruit availability index for trees (Tree) (Chapman et al., 1992); fruit availability from vines was assessed similarly by summing the DBH of trees with fruit-bearing vines

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$$\text{Demonstrated reactive scope (DRS) for fGC (\%)} = \frac{(\text{max. monthly mean troop fGC} - \text{min. monthly mean troop fGC}) \times 100}{\text{min. monthly mean troop fGC}}$$

$$\text{Coefficient of variation of DRS (DRScv) for fGC (\%)} = \frac{\text{standard deviation of monthly mean troop fGC} \times 100}{\text{mean of monthly mean troop fGC}}$$


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(Vine). Although both Tree and Vine are measures of fruit availability for the baboons they are not combined as it is unclear how to convert the units of measurement meaningfully to a single scale. There are no corresponding fruit availability data for Gamgam. However, the Kwano and Gamgam habitats are very similar, except that Gamgam troop has access to cultivated fields (Warren et al., 2011), and so the Kwano phenology data are used in analyses for both troops. Neither of the food availability variables was correlated with any of the climatic variables with a correlation coefficient greater than 0.6, and so they were included in the same GLMM models.

## 2.4. Glucocorticoid data

A total of 1093 faecal samples was collected as part of four field projects from January 2004 to April 2005 (Kwano & Gamgam: JH,

$n = 753$ ), March–May 2007 (Kwano only: AG,  $n = 51$ ), and March–December 2009 (Kwano & Gamgam: PT,  $n = 80$ , March–April; EL,  $n = 209$ , March–December). Samples were collected every 2–7 days from each adult or sub-adult female. Most samples were collected early in the morning, but as we have previously shown that there is no circadian variation in glucocorticoid measurements for samples collected in the 2004–2005 season (Higham et al., 2009b), we included samples recovered at any time of the day. Samples were collected directly after defaecation, homogenised with a stick and approximately 2 g (wet weight) placed into 10 ml of 95% ethanol, following methods described in Hodges and Heistermann (2003). Samples were transported to the University of Roehampton at the end of each project for laboratory analysis. Faecal samples were extracted into 99.5% methanol using a double extraction method (based on Ziegler et al., 2000) immediately following their return from the field. The efficacy of the extraction procedure, determined by the recovery of tritiated estradiol added to 12 samples (not used elsewhere) prior to extraction, was  $97.5\% \pm 2.9$ . Faecal extracts were analysed for  $5\beta$ -androstane- $3\alpha$ ,  $11\beta$ -diol-17-one cortisol metabolites ( $3\alpha,11\beta$ -dihydroxy-CM, hereafter fGCs), using an enzyme immuno-assay previously applied to monitor glucocorticoid output in many other species of primates (e.g. Heistermann et al., 2006), and validated for use in olive baboons using an ACTH challenge test (A. Daspre, M. Heistermann, L. Rosetta and P.C. Lee, personal communication, 2007). The assay was carried out on microtitre plates according to the procedure described in detail by Heistermann et al. (2004). Sensitivity of the assays at 90% binding was 1.2 pg/well. Intra-assay variation, calculated from repeated measures of high and low concentration quality controls (7.5% high; 13.7% low), and inter-assay variation (11.9% high; 16.5% low), were satisfactory. All assay results were standardised for differences in extraction volume and faecal weight, and are presented as nanograms of hormone per gram dry faecal weight (DFW).

## 2.5. Demonstrated reactive scope

Using the mean troop fGC values for each month, which were calculated by averaging the means for each month for individual troop females, demonstrated reactive scope (DRS) values and coefficients of variation for the DRS were calculated for each troop:

Similar values for DRS were calculated from published data, including data estimated from figures, for as many previous studies in wild primates of relationships between fGC or uGC levels and climatic or food availability variables as possible (Table 1). These calculations mostly utilised monthly mean values for fGC or uGC from multiple animals; exceptions are noted on Table 1. Similar values for DRScv were calculated using only the published studies of wild primates which presented monthly mean values for fGC or uGC from multiple animals across the full calendar year (Table 1).

## 2.6. Statistical analysis

General linear mixed models (GLMM), which take into account the non-independence of multiple samples from the same individuals, were used to analyse variation in fGC levels. All analyses were

performed using MLwiN version 2.20 (Rasbash et al., 2009). fGC level,  $\log_{10}$  transformed to normalise the data, was the dependent variable, and sample and animal ID were entered as nested random factors. Fixed factors were two monthly fruit availability variables (Tree, Vine), three monthly ( $T_{\min}$ ,  $T_{\max}$ , Rain) or four daily ( $T_{\min}$ ,  $T_{\max}$ , Rain, Humidity) climatic variables, and reproductive status (3 categories: cycling (reference), pregnant, lactating). Daily climatic values were from two days prior to the date of faecal sample collection, to take into account the delay in steroid hormone excretion (Wasser et al., 1994).

Stepwise model building produced some model instability, with changes in the relative significance of factors as others were added, and so all possible models were tested, using all combinations of one to five monthly climatic factors, or one to six daily climatic factors, with one or two fruit availability variables, plus reproductive status, and the models with the lowest AIC values selected as best fit. In addition, all models with AIC values differing from the best fit models by two or less were selected as substantially supported (Burnham and Anderson, 2002), and results compared to establish the overall best supported sets of correlated factors.

### 3. Results

#### 3.1. Climate

The climate at Gashaka was generally hot (average temperature 2004–2009: Kwano 26.3 °C, Gashaka 26.6 °C) with high annual rainfall (average annual rainfall 2004–2009: Kwano 2012 mm, Gamgam 1919 mm), but with marked seasonality between the dry and wet seasons (Fig. 2). The overall climate and patterns of seasonal variation are very similar at Gamgam and Kwano (Fig. 2). Mean monthly minimum temperature ( $T_{\min}$ , Fig. 2a) was lower from December to February and plateaued at higher values from March to November. Mean monthly maximum temperature ( $T_{\max}$ , Fig. 2b) rose from January to a peak in March, fell again to June, and was more or less constant from June to January; Kwano had slightly higher  $T_{\max}$  values from August to December in the cooler season, values were very similar at Gamgam and Kwano as they rose from January to March, and slightly higher at Gamgam during the latter part of the hot season from March to June (Fig. 2b). Mean monthly values for daily rainfall (Fig. 2c) were markedly higher from April to October, the wet season, than from November to March, the dry season. The seasonal pattern for humidity (Fig. 2d) was very similar to that for rainfall. For both rainfall and humidity, Kwano had somewhat higher values than Gamgam. There was only limited variation in climatic variables across years from 2004 to 2009 at both sites (Fig. 2).

#### 3.2. Fruit availability

Mean monthly sum DBH of fruiting trees (Tree) at Kwano (Fig. 3a) varied from 495 to 1983 mm. The annual distribution is bimodal, peaking in January to April and again in July to September. In general, tree fruit availability fell from 2004 to the first half of 2008 and then rose. Mean monthly sum DBH of trees bearing fruiting vines (Vine) (Fig. 3b) varied from 34 to 4523 mm. Vine fruit availability is very low from July to December, rising from January to a peak around March to May before falling sharply. The general annual pattern of rising and falling fruit availability was similar in all years, but peak vine fruit availability rose steadily from 2004 to 2009. Data from 2010 to 2011 show that vine fruit availability fell after this period, as part of a pattern of multi-year cyclicality in fruit availability at Gashaka (G. Jesus and V. Sommer, unpublished data for 2002–2012). Missing data for November–December 2004 and November–December 2009 were estimated as the average of val-

ues from the preceding and following years. Given the regularity of both within and between year variation for both the Tree and Vine data, these estimates should be reasonably reliable.

#### 3.3. Variation in glucocorticoid levels by troop, season and reproductive state

Pooling all females, in all reproductive states, annual variation in mean monthly fGC levels (Fig. 4) is bimodal in both troops, peaking March to April and again in August to October. Both peaks, particularly the earlier one, are less marked in Gamgam troop than in Kwano troop. Mean monthly fGC levels did not vary significantly by reproductive state within troop, but were consistently higher in all reproductive states in Kwano troop than in Gamgam troop (one-way analysis of variance: cycling,  $F = 15.4$ ,  $n = 401$ ,  $p < 0.001$ ; pregnant,  $F = 23.8$ ,  $n = 21$ ,  $p < 0.001$ ; lactating,  $F = 17.3$ ,  $n = 190$ ,  $p < 0.001$ ). As reproductive state may still have an impact on individual fGC levels, it is included in the GLMM models.

#### 3.4. Demonstrated reactive scope

For each troop, demonstrated reactive scope and the coefficients of variation of demonstrated reactive scope, are presented in Table 1, together with values calculated from fGC or uGC levels for previous studies of wild primates.

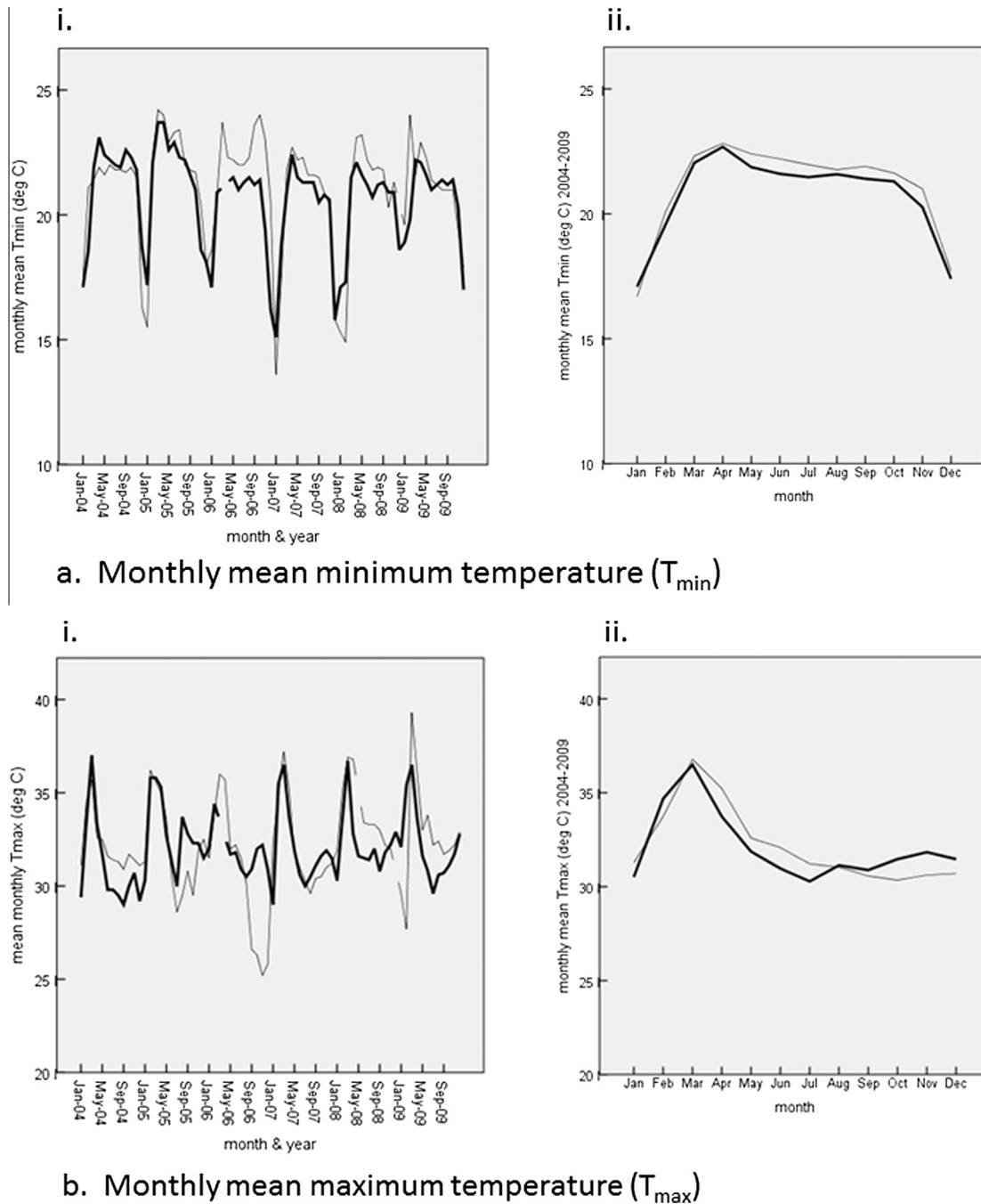
#### 3.5. GLMM results

Details of the best fit GLMM models for explaining variation in fGC levels, for each troop separately, using both monthly and daily climatic variables are presented in Table 2. Table 3 lists outline details for these models together with all other substantially supported models (as defined in Section 2.6). For Kwano, both climatic and food availability factors are significantly correlated with variation in fGC levels and the results of the best-fit and the substantially supported models are mostly the same: for the monthly climatic analyses,  $T_{\max}$ , Rain and Vine are significantly correlated in both the best-fit and the one substantially supported model; for daily climatic analyses,  $T_{\min}$  and Vine are significantly correlated with variation in fGC levels in all three models, with Humidity also a significant factor only in one of the substantially supported models. For Gamgam, only climatic factors are significantly correlated with variation in fGC levels, and the results of the best-fit and substantially supported models are completely consistent: for monthly climatic analyses, Rain, and for daily climatic analyses, Humidity are significantly correlated.

### 4. Discussion

We have presented a case study analysing the impact of environmental stressors on forest-living baboons at Gashaka, Nigeria. Here we firstly consider in more detail the probable stressors involved, which include independent climatic and food availability factors. Then, using values of demonstrated reactive scope, interpretation is broadened through comparisons of the strengths of the physiological stress responses of the Gashaka baboons with those found in other baboon populations and primate species, illustrating the usefulness of this new measure. Finally, we make suggestions for further development of the concept of demonstrated reactive scope, and the types of questions that could be tackled using it.

Our evidence suggests that climatic factors and variation in food availability independently both affect the physiological stress levels of the female baboons of Gashaka. For Kwano troop, in the monthly analysis, higher  $T_{\max}$ , higher rainfall and lower vine fruit



**Fig. 2.** Seasonal variation in climatic variables at Gamgam (thin line) and Kwano (thick line) sites: (i) from January 2004–September 2009; (ii) means for months 2004–2009.

availability, and in the daily analysis, higher  $T_{\min}$  and lower vine fruit availability, correlate consistently and significantly with higher fGC levels. Seasonal trends in each of these variables suggest that the peak in Kwano troop's physiological stress levels at the very end of the dry season/start of the rainy season (March–April) is associated with high  $T_{\max}$  and  $T_{\min}$  temperatures, and the wet season peak (August–October) with high rainfall and  $T_{\min}$ , and low vine fruit availability. For the monthly analysis, rainfall is highly correlated with humidity, which was therefore not included in the monthly model, and so a more direct effect on glucocorticoid levels could be caused by humidity, which can be as high as 98.5% at Kwano. Both high  $T_{\max}$  and high  $T_{\min}$  may act directly as thermoregulatory stressors. At Kwano, on a daily basis,  $T_{\max}$  reaches 44 °C

and  $T_{\min}$  can be as high as 30 °C. High humidity, which reaches 99% on individual days at this site, would increase the difficulties of keeping body temperature within the thermoregulatory zone through sweating. Low levels of fruit availability could necessitate the release of stored energy, and hence raised glucocorticoid levels. The evidence suggests that Kwano troop is under marked thermoregulatory and nutritional stress at some points of the year. For Gamgam troop, only climatic variables are significantly correlated with variation in fGC levels: rainfall, which is correlated with humidity for the monthly analyses, and humidity itself for the daily analyses. Gamgam has only one major peak in physiological stress levels, in the rainy season, when both rainfall and humidity are at their highest. Humidity reaches a maximum of 96% on indi-



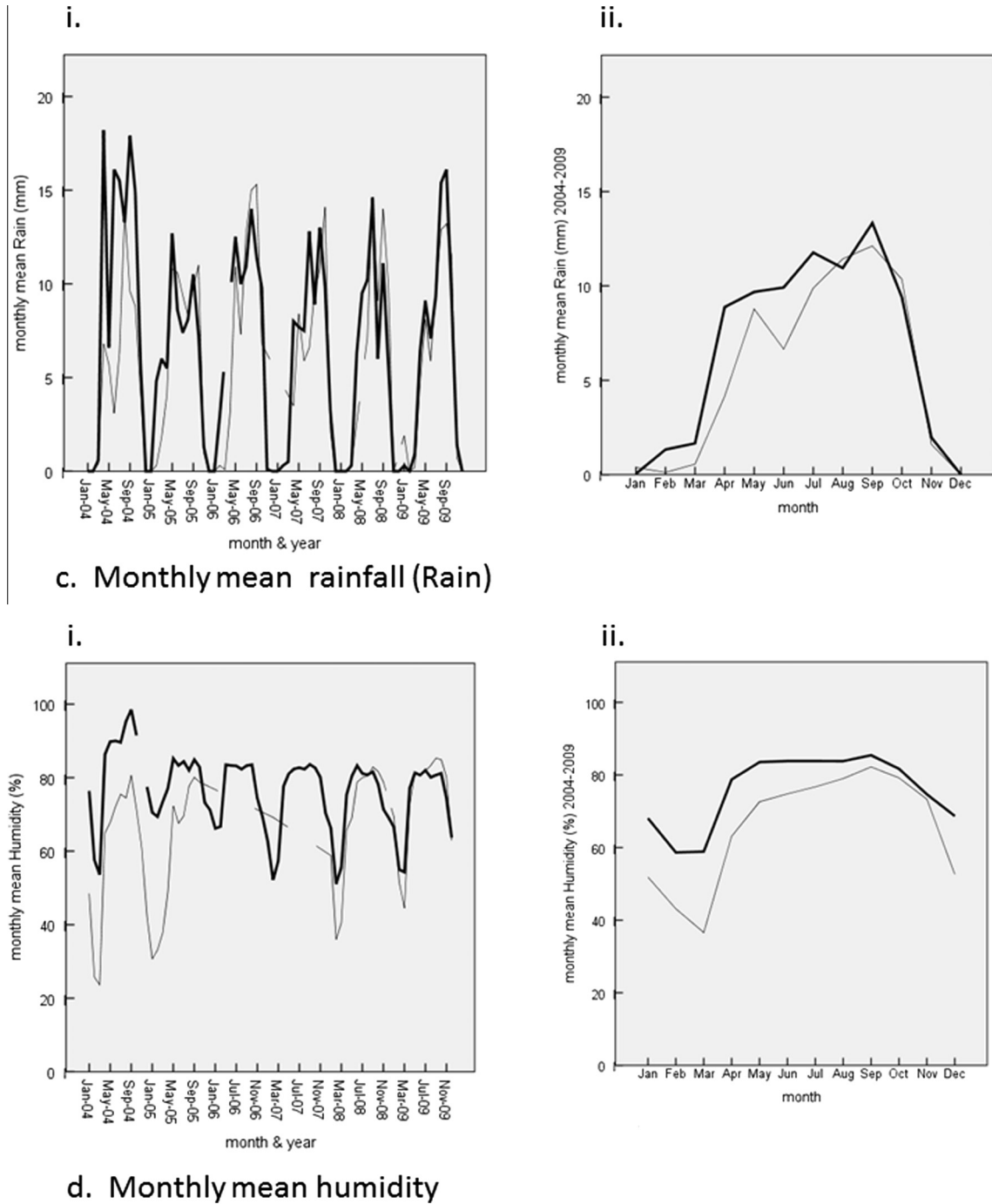
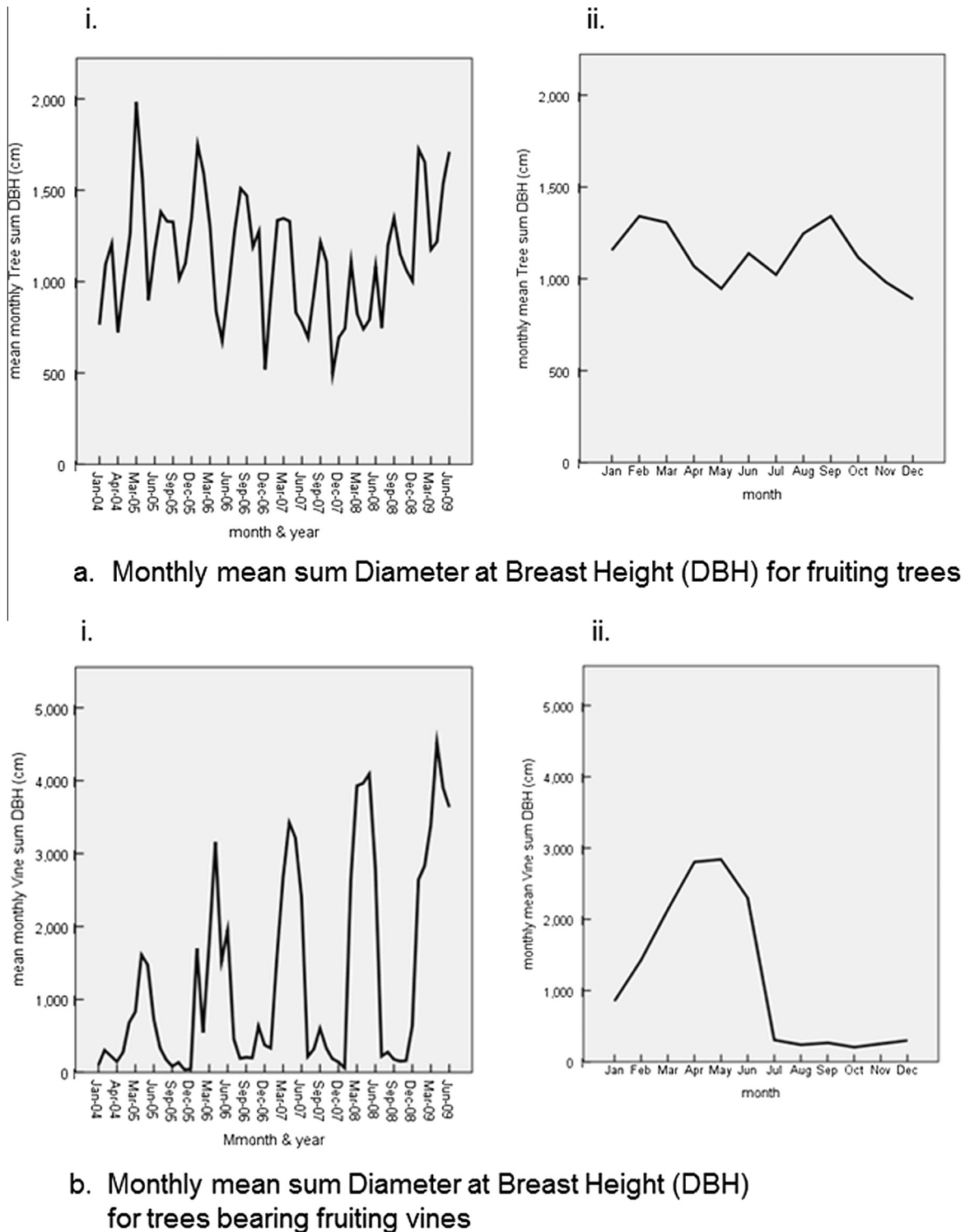


Fig. 2 (continued)

vidual days at Gamgam, comparable to levels at Kwano, which may cause similar thermoregulatory stress, through high costs for maintaining body temperature in the thermoregulatory zone when cooling by sweating is greatly impeded. Unlike at Kwano, though, there is no evidence for a nutritional stressor at Gamgam, which may be because the latter troop supplements its wild food consumption through crop-raiding.

However, the proximate factors producing the highest physiological stress levels in Gashaka baboons may be correlates of the climatic measures, rather than the climatic factors themselves, particularly in the rainy season. Disease associated with higher parasite loads or other disease vectors is likely to be more

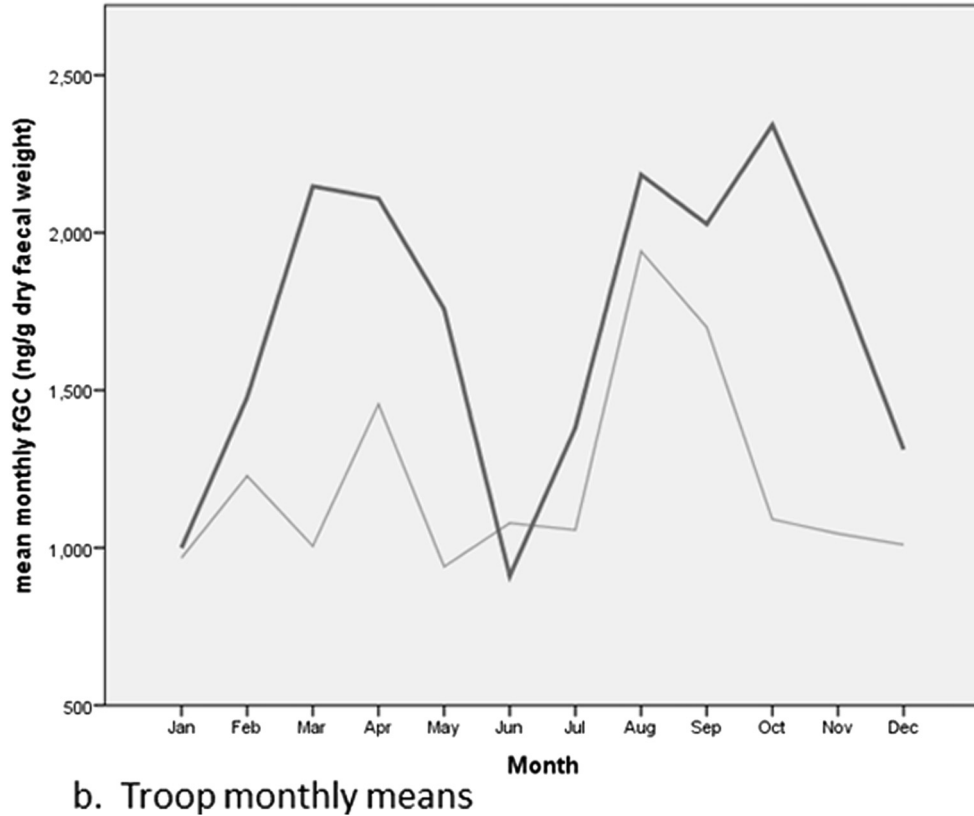
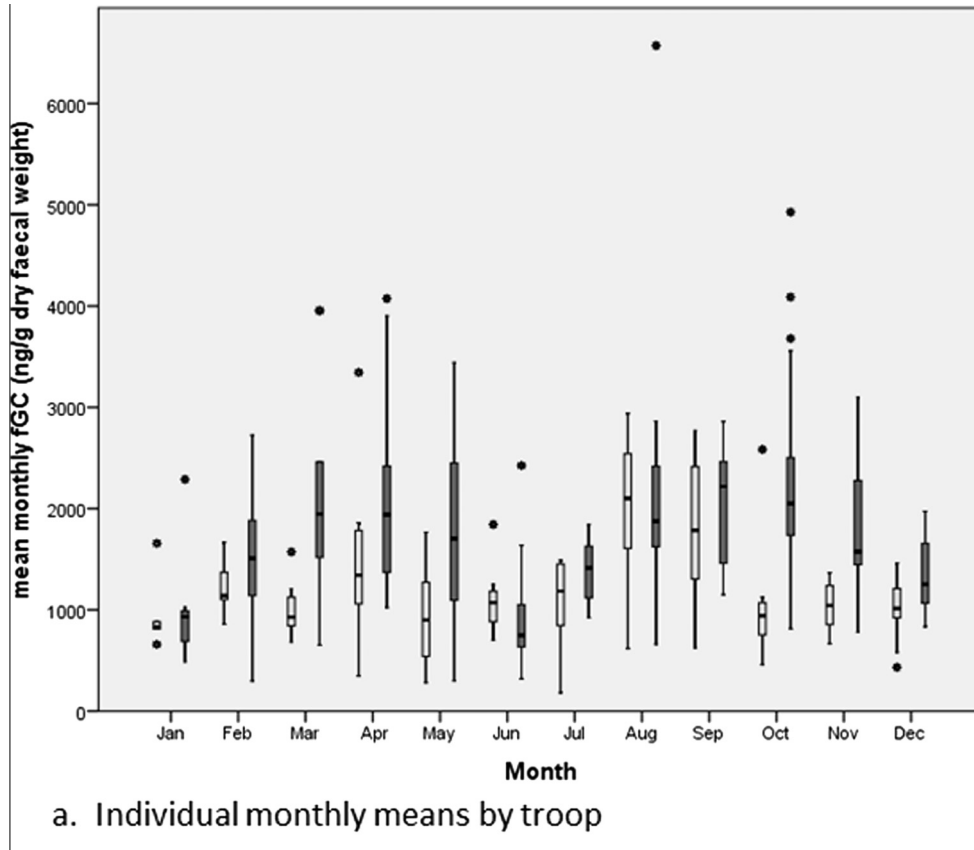
prevalent in the extremely wet, humid conditions of the rainy season, and may be the primary cause of the high physiological stress levels of female baboons at this time of year (Higham et al., 2009a). Glucocorticoids suppress immune function in multiple ways (McEwen et al., 1997), and fGC levels were found to be positively correlated with intestinal parasite loads in wild chimpanzees (Muehlenbein, 2006), indicating that glucocorticoid levels may reflect levels of parasite infestation, and potentially disease state more generally. Another possibility is that a combination of factors results in the high rainy season stress levels, particularly the higher levels at Kwano. At a time of relatively lower wild fruit availability, the Kwano baboons, unlike the



**Fig. 3.** Seasonal variation in fruit availability at Kwano site: (i) from January 2004–September 2009; (ii) means for months 2004–2009.

crop-raiding Gamgam troop, may experience a degree of nutritional stress, exacerbated by long periods of heavy rainfall, during which they travel less and spend less time foraging (Lodge, 2012). Unlike many other populations, Gashaka baboons do not switch to substantial amounts of wild underground food items at any time of the year, which may reflect the high primary productivity of their environment (Ross et al., 2011), but may also be affected by limited storage organ production by plants in

wetter environments (Richards, 1952), as well as the metabolic costs to the baboons of activity in conditions of very high humidity. Although the average daily energy intake of Gashaka baboons is greater in the wet season, it decreases with increasing rainfall across the year (Lodge, 2012), and on very wet days they travel very little or not at all (Higham et al., 2009a; Lodge, 2012), which supports the suggestion that the thermoregulatory costs of combating climatic stressors are restricting at this time



**Fig. 4.** (a) Variation in individual monthly means of faecal glucocorticoid levels (fGC) by troop. Boxes reflect the upper and lower quartiles of the sample range, divided by a line at the median value; lower whiskers encompass points falling up to one and half times as far below the median, and upper whiskers up to one and half times as far above the median; asterisks indicate samples falling outside these levels. Lighter boxes: Gamgam troop; darker boxes: Kwano troop. (b) Seasonal variation in troop mean glucocorticoid levels (fGC) for all females. Thin line: Gamgam troop; thick line: Kwano troop).

**Table 2**

Best fit GLMMs for faecal glucocorticoid levels ( $\log_{10}$ ), for both troops, from all combinations of one to five monthly climatic plus fruit availability variables, and one to six daily climatic plus fruit availability variables and reproductive status (reference category 'cycling'; non-significant categories not shown).

	Effect	SE	Wald	p-value	
<b>(a) Kwano</b>					
(i) Monthly climatic variables, $n = 390$ , AIC = 5.0					
Fixed terms					
	$T_{max}$	0.039	0.008	24.728	<0.001
	Rain	0.012	0.002	34.780	<0.001
	Vine	-0.00003	0.00001	25.995	<0.001
Random terms					
	Sample	0.008	0.004		
	Individual	0.055	0.004		
(ii) Daily climatic variables, $n = 298$ , AIC = 43.2					
Fixed terms					
	$T_{min}$	0.043	0.009	20.638	<0.001
	Vine	-0.00004	0.00001	9.948	0.002
	Humidity	-0.0028	0.0015	3.455	0.063
Random terms					
	Sample	0.007	0.004		
	Individual	0.062	0.005		
<b>(b) Gamgam</b>					
(i) Monthly climatic variables, $n = 392$ , AIC = 75.6					
Fixed terms					
	Rain	0.014	0.003	21.821	<0.001
	Reproductive status: lactating	-0.092	0.038	5.816	0.016
Random terms					
	Sample	0.003	0.002		
	Individual	0.069	0.005		
(ii) Daily climatic variables, $n = 386$ , AIC = 87.7					
Fixed terms					
	Humidity	0.002	0.001	11.209	<0.001
	Reproductive status: lactating	-0.114	0.040	8.114	0.004
Random terms					
	Sample	0.003	0.002		
	Individual	0.071	0.005		

**Table 3**

Ecological variables for best fit and substantially supported GLMMs for faecal glucocorticoid levels ( $\log_{10}$ ) for both troops, from all combinations of one to five monthly climatic plus fruit availability variables, and one to six daily climatic plus fruit availability variables and reproductive status. Substantially supported models are defined as those with AIC values differing from best fit models by  $\leq 2$ .

Model No.	AIC	Ecological variables	
		Significant	Non-significant
<b>(a) Kwano</b>			
(i) Monthly climatic variables, $n = 390$			
1	5	$T_{max}$ , Rain, Vine	
2	5.1	$T_{max}$ , Rain, Vine	Tree
(ii) Daily climatic variables, $n = 298$			
1	43.2	$T_{min}$ , Vine	Humidity
2	44.6	$T_{min}$ , Humidity, Vine	Rain
3	44.7	$T_{min}$ , Vine	
<b>(b) Gamgam</b>			
(i) Monthly climatic variables, $n = 392$			
1	75.6	Rain	
2	75.7	Rain	Tree
(ii) Daily climatic variables, $n = 386$			
1	87.71	Humidity	
2	87.74	Humidity	Tree
3	88.85	Humidity	$T_{max}$ , Vine
4	88.92	Humidity	$T_{max}$ , Tree, Vine
5	89.6	Humidity	$T_{max}$ , Rain, Tree, Vine

of year. Energy needs may also increase with increasing disease load in the wet season at Gashaka (Lodge, 2012), and hence despite higher average daily energy intakes, animals may be in poorer physical state. There is evidence that mortality and mor-

bidity are seasonally variable at Gashaka, peaking in the rainy season, particularly in Kwano troop (Higham et al., 2009a; Lodge, 2012).

The combination of climatic and food availability stressors identified for the Gashaka baboons adds to the evidence from studies of a range of primate species that both categories of factors may impact significantly on physiological stress levels in wild primates. The particular factors involved vary between studies, and for climate, generally reflect the most extreme factor in a particular species' location, which may be temperature (maximum or minimum), or rainfall (high or low). Although it has commonly been suggested that climatic factors primarily act through their impact on food availability, and other research has demonstrated more directly an impact of food availability on physiological stress levels, only one previous study of wild primates combined climatic and food availability measures in analyses (Behie et al., 2010). In this study of howler monkeys, however, it was not possible to separate the potential effects of rainfall and fruit availability on fGC levels as they were highly correlated temporally. Our own findings indicate that climatic and food availability factors can be independently correlated with physiological stress levels in wild primates. This suggests that both direct thermoregulatory and nutritional effects may be involved, although disease factors, associated with one or both categories may be, or may also be, the proximate causes of high stress.

Average monthly fGC levels varied across the year by 187% at Gamgam and 236% at Kwano, with coefficients of variation of 32% and 34%, respectively. These are extremely high levels of demonstrated reactive scope compared to baboons at other sites and other primate species (Table 1). Much more typical values are between 20% and 70% for demonstrated reactive scope, particularly

if only monthly mean values are included, and coefficients of variation for these studies of 10–25%. Whilst using DRS enables direct comparison of stress responses measured in different species, from different media, using different assays, some other study differences may affect the values calculated. One previous study based on weekly means (Cavigelli, 1999), has a high value for demonstrated reactive scope, but this may have been lower if monthly averages had been used, which may dampen variation. Two studies which used seasonal averages (Behie et al., 2010; Carnegie et al., 2011) may have had lower values than monthly data would produce for the same reason.

The high DRS and DRScv values for the Gashaka troops are most closely matched by those for a community of chimpanzees at Kibale (Muller and Wrangham, 2004). The animals in this study, the Kanyawara community, spend more time foraging and less time resting than another Kibale community, Ngogo; they live at one third the density and apparently have a lower quality diet (Potts et al., 2011). Nutritional stress may underlie the high demonstrated reactive scope of the Kanyawara community. Comparing data from other baboon sites, the high levels of demonstrated reactive scope at Gashaka are only approached at the De Hoop Nature Reserve, in South Africa (Weingrill et al., 2004), where the DRS was 155%, with a DRScv of 26%. This site is at the southern tip of Africa, and its climate has been characterised as Mediterranean (Hill et al., 2003), though with less rainfall seasonality (Weingrill et al., 2004). Temperatures at De Hoop are low for baboons, particularly minimum temperatures, which dip to monthly averages below 5 °C for 2–3 months during the winter (from Fig. 2 in Weingrill et al., 2004), and may be the major environmental stressor. Like the De Hoop baboons, the Gashaka troops inhabit an extreme climatic zone in terms of the baboon range. High rainfall and humidity in the rainy season, high temperatures in the dry season, which can reach 46 °C on a daily basis and a monthly average of 39 °C, and high minimum temperatures for much of the year, contrast with the hot but drier savannah and open woodland norms of much of the baboon range. From about 60 km south of Gashaka, there are no baboons; as the forest becomes denser, and the climate even wetter and more humid, they are replaced by drills. The evidence presented here suggests that the very high demonstrated reactive scope of fGC levels exhibited by Gashaka baboons may reflect their location in an extremely testing physical environment for *Papio* species. The DRS and DRScv of Gamgam baboons, whilst still very high, are lower than those of Kwano troop, perhaps as a result of energetic buffering through crop-raiding.

Anthropogenic impacts can be negative on wild primate populations, in terms of habitat destruction and disease introduction (Paterson and Wallis, 2005). However, the availability of human foods may provide food enhancement, reducing the likelihood of nutritional stress. Evidence presented here adds to this interpretation of crop-raiding for Gamgam troop. The two troops live only about 10 km apart, in habitats which are broadly similar except that Gamgam's home range incorporates farmland from which the baboons crop-raid (Warren et al., 2011). Unlike wild-feeding Kwano baboons, Gamgam troop's physiological stress levels are not significantly affected by wild-fruit availability, and their access to crop foods may account for why both their fGC levels across the year, as well as their DRS and DRScv values, are lower. Gamgam troop has both higher energy intake and energy balance than Kwano troop (Lodge et al., 2013). Evidence also indicates that fertility is higher and mortality lower at Gamgam compared with Kwano, which may well reflect higher nutritional status in the former troop, as a result of crop-raiding (Higham et al., 2009a; Lodge et al., 2013).

Baboons are highly flexible in a number of ways, including the range of habitats and climatic zones they successfully inhabit, from dry, hot tropical and sub-tropical savannahs, to cooler Mediterranean-like climes, and open and more closed woodland. However,

it seems from the evidence presented here that one reason they do not extend into an even greater range of environments, including thicker, wetter forest, may be that they are physiologically unable to maintain stability within their normal reactive scope, under what are more challenging conditions for baboons. Climatic factors such as temperature and humidity, food availability and disease factors may all be involved, directly or more indirectly. The measurements of demonstrated reactive scope proposed here, the range and variance of monthly average fGC values, may provide means of assessing relative stress load, and range values as high as 200% and more, with coefficients of variation around 35%, appear to be very extreme, potentially reflecting the maxima of normal reactive scope.

The particular measures of demonstrated reactive scope employed here, the percentage difference between maximum and minimum measures, and the coefficient of variation, were calculated from published summary data for a number of studies of baboons and other primate taxa, enabling assessment of the relative breadth of normal reactive scope utilised under different conditions. Where raw data are available, more detailed comparisons of demonstrated reactive scope may also prove informative. Group mean monthly measures were used in most of the studies compared here, but demonstrated reactive scope values could also be compared for individuals, populations or species, for annual, daily or point sample data. For example, the relative strengths of stress responses elicited by male takeover of a primate group, group fission, an extreme storm, or major predation event, which would be expected to make greater use of the available normal reactive scope, could be compared. The questions of interest that demonstrated reactive scope could be used to tackle range across all aspects of stress biology. For ecological and evolutionary studies, these might include assessing ecological adaptation and flexibility, and the challenge of understanding biogeographic constraints (Gaston, 2003, 2009). Demonstrated reactive scope might usefully be employed to investigate various other topics related to conservation, such as the impact of habitat degradation, climate change, specific conservation measures and wildlife management interventions. Analysis here might involve identifying stress responses that fall outside a species' normal reactive scope, in the range of homeostatic overload, and hence the potential for detrimental consequences. In studies of animal welfare, investigating the effects of different husbandry techniques or dietary regimes might similarly utilise the comparative power of demonstrated reactive scope, whilst recognising that in conditions of chronic stress, continually raised levels of stress response would potentially dampen the values calculated. However, in many circumstances, measures of demonstrated reactive scope may provide useful means of overcoming the limitations of comparing stress responses only within studies, or between studies in the same species with the same measurement methodology, enabling interpretation in more general terms of the Reactive Scope Model. Values of demonstrated reactive scope could also potentially be compared across different stress response modes: behavioural and cognitive, as well as physiological. Further work is needed to investigate the interpretive merit of comparing values of demonstrated reactive scope, but the case study presented here suggests this could be a useful means of quantifying and comparing relative stress loads measured in many different situations, and hence enabling assessment of animals' robustness, responsiveness and resilience (Wingfield, 2013) in the face of different challenges, both those normal to their natural habitat, and those outside their usual adaptive range.

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## References

- Abbott, D.H., Keverne, E.B., Bercovitch, F.B., Shively, C.A., Medoza, S.P., Saltzman, W., Snowdon, C.T., Ziegler, T.E., Banjevic, M., Garland, T., Sapolsky, R.M., 2003. Are subordinates always stressed? A comparative analysis of rank differences in cortisol levels among primates. *Horm. Behav.* 43, 67–82.
- Beehner, J.C., McCann, C., 2008. Seasonal and altitudinal effects on glucocorticoid metabolites in a wild primate (*Theropithecus gelada*). *Physiol. Behav.* 95, 508–514.
- Bergmann, T.J., Beehner, J.C., Cheney, D.L., Seyfarth, R.M., Whitten, P.L., 2005. Correlates of stress in free-ranging male chacma baboons, *Papio hamadryas ursinus*. *Anim. Behav.* 70, 703–713.
- Behie, A.M., Pavelka, M.S.M., Chapman, C.A., 2010. Sources of variation in fecal cortisol levels in howler monkeys in Belize. *Am. J. Primatol.* 72, 600–606.
- Boonstra, R., 2013. Reality as the leading cause of stress: rethinking the impact of chronic stress in nature. *Funct. Ecol.* 27, 11–23.
- Brockmann, D.K., van Schaik, C.P., 2005. Seasonality and reproductive function. In: Brockmann, D.K., van Schaik, C.P. (Eds.), *Studies of Living and Extinct Human and Non-Human Primates*. Cambridge University Press, Cambridge, pp. 269–305.
- Burnham, K.P., Anderson, D.R., 2002. *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*, second ed. Springer, New York.
- Busch, D.S., Robinson, W.D., Robinson, T.R., Wingfield, J.C., 2011. Influence of proximity to a geographic range limit on the physiology of a tropical bird. *J. Anim. Ecol.* 80, 640–649.
- 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*). *Am. J. Primatol.* 73, 861–869.
- Cavigelli, S.A., 1999. Behavioural patterns associated with faecal cortisol levels in freeranging female ring-tailed lemurs, *Lemur catta*. *Anim. Behav.* 57, 935–944.
- Chapman, C.A., Chapman, L.J., Wrangham, R., Hunt, K., Gebo, D., Gardner, L., 1992. Estimators of fruit abundance of tropical trees. *Biotropica* 24, 527–531.
- Chapman, C.A., Wasserman, M.D., Gillespie, T.R., Gillespie, T.R., Speirs, M.L., Lawes, M.J., Saj, T.L., Ziegler, T.E., 2006. Do food availability, parasitism, and stress have synergistic effects on red colobus populations living in forest fragments? *Am. J. Phys. Anthropol.* 131, 525–534.
- Chapman, C.A., Saj, T.L., Snaith, T.V., 2007. Temporal dynamics of nutrition, parasitism, and stress in colobus monkeys: implications for population regulation and conservation. *Am. J. Phys. Anthropol.* 134, 240–250.
- Creel, S., Dantze, B., Goymann, W., Rubenstein, D.R., 2013. The ecology of stress: effects of the social environment. *Funct. Ecol.* 27, 6–80.
- Crockford, C., Wittig, R.M., Whitten, P.L., Seyfarth, R.A., Cheney, D.L., 2008. Social stressors and coping mechanisms in wild female baboons (*Papio hamadryas ursinus*). *Horm. Behav.* 53, 254–265.
- Emery Thompson, M., Muller, M.N., Kahlenberg, S.M., Wrangham, R.W., 2010. Dynamics of social and energetic stress in wild female chimpanzees. *Horm. Behav.* 58, 440–449.
- Foerster, S., Monfort, S.L., 2010. Fecal glucocorticoids as indicators of metabolic stress in female Sykes' monkeys (*Cercopithecus mitis albobularis*). *Horm. Behav.* 58, 685–697.
- Gaston, K.J., 2003. *The Structure and Dynamics of Geographic Range*. Oxford University Press, Oxford.
- Gaston, K.J., 2009. Geographic range limits: achieving synthesis. *Proc. Roy. Soc. B* 276, 1395–1406.
- Gesquiere, L.R., Khan, M., Shek, L., Wango, T.L., Wango, E.O., Alberts, S.C., Altmann, J., 2008. Coping with challenging environment: effects of seasonal variability and reproductive status on glucocorticoid concentrations of female baboons (*Papio cynocephalus*). *Horm. Behav.* 54, 410–416.
- Gesquiere, L.R., Onyango, P.O., Alberts, S.C., Altmann, J., 2011. Endocrinology of year-round reproduction in a highly seasonal habitat: environmental variability in testosterone and glucocorticoids in baboon males. *Am. J. Phys. Anthropol.* 144, 169–176.
- Girard-Buttoz, C., Heistermann, M., Krummel, S., Engelhardt, A., 2009. Seasonal and social influences on fecal androgen and glucocorticoid excretion in wild male long-tailed macaques (*Macaca fascicularis*). *Physiol. Behav.* 98, 168–175.
- Goymann, W., Wingfield, J.C., 2004. Allostatic load, social status and stress hormones: the costs of social status matter. *Anim. Behav.* 67, 591–602.
- Groves, C., 2001. *Primate Taxonomy*. Smithsonian Institution Press, Washington DC.
- Heistermann, M., Ademmer, C., Kaumanns, W., 2004. Ovarian cycle and effect of social changes on adrenal and ovarian function in *Pygathrix nemaeus*. *Int. J. Primatol.* 25, 689–708.
- Heistermann, M., Palme, R., Ganswindt, A., 2006. Comparison of different enzymeimmunoassays for assessment of adrenocortical activity in primates based on fecal analysis. *Am. J. Primatol.* 68, 257–273.
- Higham, J.P., Warren, Y., Adanu, J., Umaru, B.N., MacLarnon, A.M., Sommer, V., Ross, C., 2009a. Living on the edge: life-history of olive baboons at Gashaka-Gumti National Park, Nigeria. *Am. J. Primatol.* 71, 293–304.
- Higham, J.P., MacLarnon, A.M., Heistermann, M., Ross, C., Semple, S., 2009b. Rates of self-directed behaviour and faecal glucocorticoid levels are not correlated in female wild olive baboons (*Papio hamadryas anubis*). *Stress* 12, 526–532.
- Hill, R.A., Barrett, L., Gaynor, D., Weingrill, T., Dixon, P., Payne, H., Henzi, S.P., 2003. Day length, latitude and behavioural (in)flexibility in baboons (*Papio cynocephalus ursinus*). *Behav. Ecol. Sociobiol.* 53, 278–286.
- Hodges, J.K., Heistermann, M., 2003. Field endocrinology: monitoring hormonal changes in free-ranging primates. In: Setchell, J., Curtis, D. (Eds.), *Field and Laboratory Methods in Primatology*. Cambridge University Press, Cambridge, pp. 282–294.
- Koolhaas, J.M., Bartolomucci, A., Buwalda, B., de Boer, S.F., Flügge, G., Korte, S.M., Meerlo, P., Murison, R., Olivier, B., Palanza, P., Richter-Levin, G., Sgoifo, A., Steimer, T., Stiedl, O., van Dijk, G., Wöhr, M., Fuchs, E., 2011. Stress revisited: a critical evaluation of the stress concept. *Neurosci. Biobehav. Rev.* 35, 1291–1301.
- Korte, S.M., Koolhaas, J.M., Wingfield, J.C., McEwen, B.S., 2005. The Darwinian concept of stress: benefits of allostasis and costs of allostatic load and the trade-offs in health and disease. *Neurosci. Biobehav. Rev.* 29, 3–38.
- Landys, M.M., Ramenofsky, M., Wingfield, J.C., 2006. Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. *Gen. Comp. Endocrinol.* 148, 132–149.
- Lodge, E., 2012. *Energetics and Life-History of Olive Baboons (Papio hamadryas anubis) in the Gashaka Gumti National Park*. (Ph.D. dissertation). Centre for Research in Evolutionary and Environmental Anthropology, University of Southampton, UK.
- Lodge, E., Ross, C., Ortmann, S., MacLarnon, A.M., 2013. Influence of diet and stress on reproductive hormones in Nigerian olive baboons. *Gen. Comp. Endocrinol.* 191, 146–154.
- McEwen, B.S., 1998. Protective and damaging effects of stress mediators. *N. Engl. J. Med.* 338, 171–179.
- McEwen, B.S., Biron, C.A., Brunson, K.W., Bulloch, K., Chambers, W.H., Dhabhar, F.S., Goldfarb, R.H., Kitson, R.P., Miller, A.H., Spencer, R.L., Weiss, J.M., 1997. The role of adrenocorticoids as modulators of immune function in health and disease: neural, endocrine and immune interactions. *Brain Res. Rev.* 23, 79–133.
- McEwen, B.S., Wingfield, J.C., 2003. The concept of allostasis in biology and biomedicine. *Horm. Behav.* 43, 2–15.
- Muehlenbein, M.P., 2006. Intestinal parasite infections and fecal steroid levels in wild chimpanzees. *Am. J. Phys. Anthropol.* 130, 546–550.
- Muller, M.N., Wrangham, R.W., 2004. Dominance, cortisol and stress in wild chimpanzees (*Pan troglodytes schweinfurthii*). *Behav. Ecol. Sociobiol.* 55, 332–340.
- Paterson, J.D., Wallis, J. (Eds.), 2005. *Commensalism and Conflict: the Human-Primate Interface*. American Society of Primatologists, Norman, Oklahoma.
- Potts, K.B., Watts, D.P., Wrangham, R.W., 2011. Comparative feeding ecology of two communities of chimpanzees (*Pan troglodytes*) in Kibale National Park, Uganda. *Int. J. Primatol.* 32, 669–690.
- Pride, R.E., 2005. Optimal group size and seasonal stress in ring-tailed lemurs (*Lemur catta*). *Behav. Ecol.* 16, 550–560.
- Rangel-Negrín, A., Alfaro, J.L., Valdez, R.A., Romano, M.C., Serio-Silva, J.C., 2009. Stress in Yucatan spider monkeys: effects of environmental conditions on fecal cortisol levels in wild and captive populations. *Anim. Conserv.* 12, 496–502.
- Rasbash, J., Steele, F., Browne, W.J., Goldstein, H., 2009. *A User's Guide to MLwiN, version 2.10*. Centre for Multilevel Modelling, University of Bristol.
- Richards, P.W., 1952. *The Tropical Rain Forest: An Ecological Study*. Cambridge University Press, Cambridge.
- Romero, L.M., 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. *Gen. Comp. Endocrinol.* 128, 1–24.
- Romero, L.M., Dickens, M.J., Cyr, N.E., 2009. The Reactive Scope Model – A new model integrating homeostasis, allostasis, and stress. *Horm. Behav.* 55, 375–389.
- Ross, C., MacLarnon, A.M., Warren, Y., Adanu, J., Higham, J.P., 2011. How different are Gashaka's baboons? Forest and open country populations compared. In: Sommer, V., Ross, C. (Eds.), *Primates of Gashaka: Socioecology and Conservation in Nigeria's Biodiversity Hotspot*. Developments in Primatology: Progress and Prospects. Springer, New York, pp. 333–359.
- Sapolsky, R.M., 1992. Neuroendocrinology of the stress-response. In: Becker, J.B., Breedlove, S.M., Crews, D. (Eds.), *Behavioral Endocrinology*. The MIT Press, Cambridge, Mass, pp. 287–324.
- Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr. Rev.* 21, 55–89.
- Sommer, V., Ross, C., 2011a. Exploring and protecting West Africa's primates. In: Sommer, V., Ross, C. (Eds.), *Primates of Gashaka: Socioecology and Conservation in Nigeria's Biodiversity Hotspot*. Developments in Primatology: Progress and Prospects. Springer, New York, pp. 1–37.

- Sommer, V., Ross, C. (Eds.), 2011b. Primates of Gashaka: Socioecology and Conservation in Nigeria's Biodiversity Hotspot. Developments in Primatology: Progress and Prospects. Springer, New York.
- Strier, K.B., Ziegler, T.E., Wittwer, D.J., 1999. Seasonal and social correlates of fecal testosterone and cortisol levels in wild male muriquis (*Brachyteles arachnoides*). *Horm. Behav.* 35, 125–134.
- Tabachnik, B.G., Fidell, L.S., 2013. Using Multivariate Statistics, sixth ed. Pearson, London.
- Warren, Y., Higham, J.P., MacLarnon, A.M., Ross, C., 2011. Crop-raiding and commensalism in olive baboons: the costs and benefits of living with humans. In: Sommer, V., Ross, C. (Eds.), Primates of Gashaka: Socioecology and Conservation in Nigeria's Biodiversity Hotspot. Developments in Primatology: Progress and Prospects. Springer, New York, pp. 307–332.
- Wasser, S.K., Monfort, S.L., Southers, J., Wildt, D.E., 1994. Excretion rates and metabolites of oestradiol and progesterone in baboon (*Papio cynocephalus cynocephalus*) faeces. *J. Reprod. Fertil.*, 213–220.
- Weingrill, T., Gray, D.A., Barrett, L., Henzi, S.P., 2004. Fecal cortisol levels in free-ranging female chacma baboons: relationship to dominance, reproductive state and environmental factors. *Horm. Behav.* 45, 259–269.
- Whitten, P.L., Brockman, D.K., Stavisky, R.C., 1998. Recent advances in noninvasive techniques to monitor hormone-behavior interactions. *Yearb. Phys. Anthropol.* 41, 1–23.
- Wingfield, J.C., 2005. The concept of allostasis: coping with a capricious environment. *J. Mammal.* 86, 248–254.
- Wingfield, J.C., 2013. The comparative biology of environmental stress: behavioural endocrinology and variation in ability to cope with novel, changing circumstances. *Anim. Biol.* 85, 1127–1133.
- Ziegler, T., Hodges, J.K., Winkler, P., Heistermann, M., 2000. Hormonal correlates of reproductive seasonality in wild female Hanuman langurs (*Presbytis entellus*). *Am. J. Primatol.* 51, 119–134.