

Temporal Dynamics of Nutrition, Parasitism, and Stress in Colobus Monkeys: Implications for Population Regulation and Conservation

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KEY WORDS population regulation; primate conservation; parasitism; cortisol; colobus; Kibale national park, within-group competition

ABSTRACT The need to develop conservation plans calls for the ability to identify ecological factors that influence population density. Because stress is known to affect fecundity and mortality, increasing stress may provide a warning of potential population declines. We examined the effects of temporal variation in nutrition and parasitism on stress in endangered red colobus monkeys in Kibale National Park, Uganda. First, we tested the hypothesis that parasitism and nutrition would individually affect stress levels. We found that periods of poor-quality diet corresponded with an increase in cortisol. Similarly, increases in parasite infections were associated with increased cortisol. Next, we predicted that a poor-quality diet would facilitate increased parasite infections, and that together, they would lead to amplified

stress. However, we found no support for such amplification, likely because the quality of the diet had little effect on parasite infections. Third, we tested whether individuals in a larger group were subject to food stress due to greater within-group competition, which would intensify nutritional stress and parasitism, and lead to reduced reproduction. Although we found no evidence to support a group size effect on parasites, cortisol levels in the large group tended to be higher than those in the small group, and the large group had fewer infants per female. The results suggest that parasitism and poor nutrition lead to increased stress which, because they are known to be associated with reduced fecundity and increased mortality, may lead to population declines. *Am J Phys Anthropol* 000:000–000, 2007. © 2007 Wiley-Liss, Inc.

The interest in identifying the ecological factors that influence animal abundance has taken on a new importance with the need to develop informed conservation plans for endangered and threatened species. With respect to forest-dwelling primates, these issues are critical because tropical forests are undergoing rapid anthropogenic transformation. Countries with primate populations are losing ~125,000 km² of forest each year (Chapman and Peres, 2001), additional areas are degraded by logging and fire, and animal populations are further reduced by hunting (Oates, 1996). While there is great concern over these threats, few studies have gone beyond providing obvious solutions (e.g., decrease hunting, stop logging). Also, it has proven extremely difficult to develop techniques that forecast the effects of specific disturbances on population fitness, or even to develop a predictive understanding of the factors affecting the abundance of particular species (Wasser et al., 1997; but see Creel et al., 2002). This is partly due to the fact that reliable and broadly-applicable single-factor explanations for complex biological phenomena such as population density are unlikely, and long-term studies have highlighted the importance of multifactor explanations (Gulland, 1992; Milton, 1996).

We examine the effects of seasonal variation in nutrition and parasitism on stress levels (as indicated by fecal cortisol) in red colobus monkeys (*Procolobus rufomitratus*) in Kibale National Park, Uganda under natural conditions where the population has not experienced recent anthropogenic disturbance. We suggest that, since stress

can affect both fecundity and mortality, cortisol levels can provide an indication of potential population declines due to reduced fitness associated with these factors.

Nutritional status has been shown to affect reproduction, mortality, and stress in a variety of species (Sapolsky, 1992; Robbins, 1993; Murray et al., 1998). For these reasons, the quantity and quality of available food are among the primary variables of interest when examining variation in population density. Despite this obvious link, there are few general models available for predicting biomass. A notable exception is the protein to fiber model, developed for folivorous primates. Milton (1979) proposed that the protein to fiber ratio was a good

Grant sponsors: Canadian Research Chairs Program, Wildlife Conservation Society, Natural Science and Engineering Research Council of Canada, Leaky Foundation, National Science Foundation, Tomlinson Post-doctoral and Pre-doctoral Fellowships; Grant sponsor: NIH; Grant number: RR000167.

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Received 26 May 2006; accepted 1 May 2007

DOI 10.1002/ajpa.20664
Published online in Wiley InterScience
(www.interscience.wiley.com).

predictor of leaf choice. Subsequently, by measuring overall leaf acceptability as the ratio of protein to fiber, several studies have found positive correlations between folivore biomass and this index of leaf quality at local (Chapman and Chapman, 2002; Ganzhorn, 2002) and regional scales (Waterman et al., 1988; Oates et al., 1990).

As with poor nutrition, helminthic and protozoal parasites can affect host survival and reproduction. Severe parasitosis can lead to blood loss, tissue damage, spontaneous abortion, congenital malformations, and death (Collias and Southwick, 1952; Despommier et al., 1995). Less severe infections are more common, and may induce energetic costs that impair an individual's ability to travel, feed, escape predators, and compete for resources or mates (Hudson, 1986; Hudson et al., 1992; Coop and Holmes, 1996). Even upregulation of host immunity can reduce breeding success (Ilmonen et al., 2000). However, when hosts have adequate nutrient supplies, infections often appear to cause little or no effect on host energetics or fitness (Munger and Karasov, 1989; Gulland, 1992; Munger and Karasov, 1994; Milton, 1996).

Dietary stress may adversely affect resistance to parasitic infection by reducing the effectiveness of the immune system (Solomons and Scott, 1994; Holmes, 1995) and as a result, food shortages could result in greater parasite infections. This in turn could increase nutritional demands on the host and further compound the effects of food stress. In this way, nutrition and parasitism may operate together to affect host survival and reproduction. This relationship has been observed in wild howler monkeys (*Alouatta palliata*), where annual patterns of mortality resulted from a combination of effects, including age, physical condition, and the larval burden of a parasitic botfly (*Alouattamyia baeri*), which became especially critical during periods of nutritional stress (Milton, 1996). However, it is not clear under what conditions (e.g., level of dietary stress or anthropogenic habitat degradation) nutritional status and parasite infection will interact to affect the host population.

Given that natural populations typically change size slowly in response to environmental stressors (Struhaker, 1976), we assume that these stressors will be reflected more immediately in cortisol levels. Glucocorticoids from the adrenal cortex are released to prepare the body to cope with a crisis. Specifically, cortisol promotes tissue catabolism so that circulating glucose is available for physical activity (Hadley, 1996). A great deal of research on captive mammals and humans has demonstrated that prolonged elevated glucocorticoid levels typically reduce survival, growth, reproduction, fecundity, and immunity (Munck et al., 1984; Moberg, 1985; Sapolsky, 1992; Wasser et al., 1997; Ferin, 1999; Bercovitch and Ziegler, 2002; Creel et al., 2002). These effects suggest that cortisol should provide an indication of individual and population health. However, because variation in cortisol can be due to many factors (e.g., positive or negative emotions (Pollard, 1995), group size (Pride, 2005c), dominance rank (Abbott et al., 2003), and inherent inter-specific and inter-individual differences (Muehlenbein, 2006)), the relationship between fecal cortisol and population health must be critically evaluated before its validity as an indicator can be determined.

Ideally, a test of these ideas would require data on the fitness effects of elevated glucocorticoid levels in the wild, but such data are limited and difficult to acquire. On the basis of captive studies, the expectation is that fitness will decrease as population-level stressors become

more severe or more prolonged (Boonstra and Singleton, 1993; Blanchard et al., 1995; Creel et al., 2002). There are a few recent field studies indicating relationships between glucocorticoid levels and fitness. For example, Pride (2005b) found that ring-tailed lemurs (*Lemur catta*) with high glucocorticoid levels had higher mortality rates than animals with lower levels (see Romero and Wikelski, 2001) for a similar finding with marine iguanas at a population level). Similarly, in an investigation of daily fluctuations in stress and women's reproductive hormones in Guatemala, researchers found that an increase in cortisol was associated with increases in gonadotropin and progesterone levels during the follicular phase. This is significant because such increases have been reported to negatively affect ovulation and reduce the chances of implantation (Ferin, 1999). The chronic activation of cortisol is also known to be related to food stress. Pride (2005a) demonstrated that among ring-tailed lemurs, females with low food intake had elevated cortisol levels. These studies demonstrate the link between food stress, cortisol, and fitness outcomes and suggest that cortisol levels can provide a measure of stress due to nutrition and parasite infections (among other factors). Cortisol may therefore also be a useful early indicator of potential population declines due to reduced fitness and increasing mortality associated with these factors.

HYPOTHESIS 1: PARASITISM AND NUTRITION INDIVIDUALLY AFFECT STRESS LEVELS IN RED COLOBUS

To test this hypothesis we examined three specific predictions using 23 months of data from a large group of red colobus monkeys. First, we predicted that poor nutrition would lead to elevated cortisol levels, and that there would be a time lag before the effect on cortisol became apparent. Second, we predicted that the important nutritional variable would be the protein to fiber ratio, while secondary compound levels would have little effect. This prediction was based on our previous research that has shown a consistent preference among colobus groups for a diet high in protein and low in fiber (Chapman and Chapman, 2002), the ability of the protein to fiber ratio of mature leaves to predict colobus biomass (Chapman et al., 2004), and the non-significant effect of secondary compounds on diet selection (Chapman and Chapman, 2002). Third, we predicted that parasite infections would lead to higher stress levels, and specifically that more-pathogenic parasites would have a larger impact on stress levels than less-pathogenic parasites. This prediction was based on the observation that there was a higher prevalence of more pathogenic parasites in edge habitats (Chapman et al., 2005) and forest fragments (Chapman et al., unpublished data) than in undisturbed habitats, that the forest fragment populations were declining, and that the rate of decline was related to the nature of the parasite infection (Chapman et al., 2006).

HYPOTHESIS 2: DIET QUALITY AND PARASITE INFECTIONS WILL INTERACT TO AFFECT STRESS LEVELS

Poor diet quality will lead to increased parasite infections (due to decreased immunity), which will together amplify the stress response.

HYPOTHESIS 3: THE EFFECT OF NUTRITION AND PARASITISM ON STRESS IS AFFECTED BY GROUP SIZE

On the basis of our previous research (Snaith and Chapman, 2005; Snaith and Chapman, in press), we suggest that individuals in large groups will experience more within-group scramble competition over food resources than individuals in small groups, resulting in decreased feeding efficiency (see also research by Dunbar, 1987; Koenig, 2000; Koenig and Borries, 2002; Saj and Sicotte, in press; Saj et al., in press). These factors may lead to greater stress (both nutritional and social) and ultimately to reduced reproductive success. Following this logic, we predicted that individuals in the large group would have increased parasite infections and higher stress levels relative to individuals in the small group. We also predicted that this would lead to individuals in the large group experiencing reproductive costs associated with larger group size, thus the infant/female ratio should be smaller in the large group than in the small group.

METHODS

The study was conducted in Kibale National Park, Uganda (795 km²), a mid-altitude, moist-evergreen forest. To address Hypotheses 1 and 2, dealing with relationships among nutrition, parasitism, and stress, we studied a large group of approximately 132 red colobus from June 2003 to April 2005 (23 months). To address Hypothesis 3, dealing with group size, a smaller group of 36 individuals was observed and its stress levels (March 2004–April 2005, 13 months), parasite infections (March 2004–May 2006, minus May and June 1995), and female to infant ratios (March 2004–May 2006, 27 months) were compared to those in the large group during the same period. These groups were selected because they differed markedly in size, but had overlapping home ranges (i.e., the small group's home range was totally encompassed within that of the larger group), and thus had similar available resources.

Each group was observed for 6 days a month from just after sunrise and ending around the time that the group became inactive for the day. Instantaneous scan samples of five individuals were conducted every 15 min. When feeding was observed, species and plant part were recorded. The most frequently eaten foods (species/parts) were collected (typically 5–10 species/parts), dried using a dehydrator or kerosene lantern or light-bulb driven drying oven to maintain a low temperature during drying to prevent volatilization of secondary compounds or binding of protein. A max/min thermometer was used in the drying oven to assure that the temperature stayed below 50°C. Samples were analyzed in duplicate, and replicates for analyses were considered acceptable if the relative error was less than 2%. Protein (nitrogen) content was assessed using Kjeldahl procedures (Horowitz, 1970). Samples were digested using a modification of the aluminum block digestion procedure of Gallaher et al. (1975). The digestion mix contained 1.5 g of 9:1 K₂SO₄:CuSO₄, and digestion was conducted for at least 4 h at 375°C using 6 ml of H₂SO₄ and 2 ml H₂O₂. The nitrogen in the digestate was determined by semiautomated colorimetry. Measuring total nitrogen provides an estimate of crude protein (protein levels = $N \times 6.25$). Acid detergent fiber (ADF) was measured using the

methods of van Soest (1963). ADF is a measure of cell wall cellulose and lignin, which are refractory components of fiber. The presence of alkaloids was tested using a spot test with Dragendorff's reagent (Waterman, 1993). Saponin presence was indexed using the Froth Test using a 300-s criteria and a 12-mm cut-off criterion (Chapman and Chapman, 2002). The presence of hydrogen cyanide was determined by the Feigler-Anger test (Feigler and Anger, 1966).

We assumed that a high-quality diet includes foods that are high in protein and low in fiber (Milton, 1979; Oates et al., 1990; Ganzhorn, 1992; Chapman and Chapman, 2002; Chapman et al., 2004). The quality of the diet each month was determined by multiplying the feeding time of each of the 10 most frequently eaten food items by the protein to fiber ratio of each item and summing this product over the 10 items. This assumes that items have similar intake rates, but since 67% of the items were young leaves, this is a reasonable assumption. The proportion of the diet evaluated was standardized among months to 100%. Cyanogenic glycoside, alkaloid, and saponin analysis involved scoring their presence/absence rather than determining the total quantity per gm dry matter, thus levels of secondary compounds in a month's diet was evaluated multiplying the sum of these three secondary compounds presence/absence values (1 = present, 0 = absent) by the percent feeding value of each of the 10 most frequently eaten food items each month and summing the product.

To address hypotheses 1 and 2, 509 fecal samples were collected to assess fecal cortisol levels and parasite infections. September 2004 was excluded from analysis because of insufficient sample size ($n = 1$, other samples were discarded when their centrifuge tubes broke). Upon defecation, samples were immediately placed in vials stored on ice. At the end of the day the samples were transferred to a -20°C freezer. To compare parasite infections between the large and small group (Hypothesis 3), 1,043 samples were collected (small group $n = 415$; large group $n = 628$). On average 29.9 samples were collected each month for the large group (range 12–76) and 19.8 samples from the small group (range 14–43).

For parasite analysis, a portion of each fecal sample was stored individually in 10% formalin solution, and processed using concentration by sodium nitrate flotation (Sloss et al., 1994). Eggs and larvae of parasites were counted and identified on the basis of egg color, shape, contents, and size. Coprocultures and necropsies were used to match parasite eggs to larvae for positive identification (Gillespie et al., 2005a,b; Chapman, unpublished data). The parasite infections were described in terms of prevalence of infection, richness, multiple infections, and load (eggs/g). Prevalence is defined as the proportion of individuals sampled that are infected with a particular parasite. However, because we could not recognize individual animals, we use the proportion of fecal samples that are infected as our index of prevalence. High parasite prevalence can be a useful indicator of potential negative effects on host populations (i.e., population declines have been correlated to increased infection prevalence). See Huffman et al. (1997) for an evaluation of potential problems with this index. Richness is the number of unique parasite species documented in each sample and an increase in richness could be indicative of greater morbidity. The frequency of multiple-species infections (i.e., the proportion of samples with more than 1 species of parasite) can be another useful index as multiple-spe-

cies infections are associated with a greater potential for morbidity and mortality. For example, in humans, *Schistosoma mansoni* has an increased effect on the development of malnutrition in the presence of *Trichuris trichiura* (Parraga et al., 1996) and a range of parasites demonstrate greatly elevated pathogenic effects in the presence of HIV (Kaplan et al., 1996). Parasite egg production, or load (eggs/g), is frequently reported to describe infections (e.g., Gulland, 1992; Ezenwa, 2003), but because it is highly variable, it may not be indicative of actual infection intensity. We typically obtained <10 *Trichuris* sp. eggs/g from infected red colobus; however, in some groups at some times individuals consistently had much higher loads (i.e., over 300 eggs/g for all samples taken from a group for an extended time), suggesting that since very high loads are temporally and spatially specific, then load might be a useful index. We report parasite load, but recommend that these results be viewed critically and only be considered of interest if they are in concordance with other indices of parasite infection. To assess the frequency with which animals showed signs of sickness, we recorded all obvious symptoms (coughing, lethargy, etc.) between [March 2004 and May 2006 (27 months)]. Since we were unable to identify all animals, only one animal of a particular age/sex class exhibiting the same symptoms, was considered in the tally of sick individuals for each month. Thus, this index represents a minimum evaluation of the proportion of sick animals.

To determine cortisol levels, fecal cortisol and metabolites were solubilized from a 0.5 g sample of fecal material using a 5.0 pH citrate buffer and 95% ethanol solution that was mixed for 21–27 h. After extraction, samples were placed in a centrifuge for 30 min. The supernatant (with solubilized hormones) was processed by passing 2 ml of supernatant through a primed Alltech maxi-clean filter cartridge. These cartridges were analyzed at the National Primate Research Center at the University of Wisconsin-Madison, following the methods reported in Ziegler et al. (1995), and resulted in a measure of cortisol and metabolites in each sample (ng/g dry feces). The dry weight of each sample was calculated in the field by drying to constant weight and calculating the percent water. The cartridges were washed with 1 ml of 20% methanol and the columns were eluted with 2-ml methanol. This methanol was dried, resuspended in 1-ml ethanol, and 50 μ l was taken for enzymeimmunoassay. As fecal steroid levels vary with age, reproductive status, and time of day (Bercovitch and Clarke, 1995; Sousa and Ziegler, 1998; Chapman et al., 2006; Wasserman and Chapman, unpublished data;), the conditions under which samples were collected were carefully defined. Samples were collected only from adult males and adult females with young infants (presumed not pregnant; pregnancy cannot be accurately visually assessed until near the time of birth). All samples were collected between 8:00 and 13:00 and the average time of collection differed between the two groups by only 24 min.

To compare cortisol levels between the small and large group, two approaches were used. First, a simple *t*-test is used. Second, a paired *t*-test was run using the values from the two groups during the same month as the pair. This second analysis controls for seasonal variation when comparing the groups. However, the degree to which months represent true pairs is debatable since in some months the large group ranged to areas not avail-

able to the small group; however, since the approaches produced similar findings (i.e., the significant levels led to the same conclusion, see below) we just present the paired *t*-test. Cortisol levels were exceptionally high in September 2004 for the small group; almost twice the average (1.96, while the next highest month was only 1.38 times the average). In this month all individuals had similarly high levels. We view this month to be an anomaly that could have been caused by a stressful event like a predator attack or severe aggression within the group. This month was removed from the analysis comparing the small and large group (including this month converts a significant difference between groups to an insignificant difference).

We used path analysis to examine whether there was an amplification of the effect of diet quality on stress because of low diet quality facilitating higher parasite infections. Path analysis is a useful statistical approach since the path (beta) coefficients among the variables reveal the magnitude of both direct (i.e., diet quality on stress) and indirect effects (i.e., diet quality on parasite infections on stress). Although independent variables are often measured with different units, path (beta) coefficients are standardized regression coefficients that allow the relative effects of each independent variable to be compared. As with most statistical techniques, path analysis does not confirm causation (Shipley, 1999). Path analysis begins with the construction of a path diagram showing the presumed relationships among the variables in the system based on *a priori* knowledge (Kingsolver and Schemske, 1991). Our path diagram is based or rooted on changes in food quality (protein to fiber intake), which was presumed to have a direct effect on stress, and/or an indirect effect on stress via parasite infections. Only those indices of parasite infections that were found to be correlated to stress were included in the path analysis. Path analysis was run in the module available through SPSS (version 15.0).

RESULTS

Parasite community of the red colobus study groups

The parasite community described from the 1,043 red colobus fecal samples collected from both groups included the following nematodes: *Trichuris* sp. (Superfamily Trichuroidea), *Oesophagostomum* sp. (Superfamily Strongyloidea), *Strongyloides fuelleborni*, *S. stercoralis* (Superfamily Rhabditoidea), *Colobenterobius* sp. (Superfamily Oxyuroidea), and an unidentified strongyle. We also identified two protozoans, likely *Entamoeba coli*, and *E. histolytica/dispar*. Because of the small sample size for *Strongyloides fuelleborni*, *S. stercoralis*, *Colobenterobius* sp., and the unidentified strongyle, these parasites are not considered individually. Further, *E. histolytica* and *E. dispar* have cysts that are morphologically indistinguishable and it was only recently that *E. dispar* was considered a distinct species (Gatti et al., 2002). However, *E. histolytica* is pathogenic, while *E. dispar* is not. Thus, we do not consider the *E. histolytica/dispar* complex further with the exception of analyses of multiple infections and the richness of the parasite community, where they are considered as one species. *Strongyloides fuelleborni*, *S. stercoralis*, and *Colobenterobius* sp. are only considered in analyses of multiple infections and species richness. In June 2006 we darted 30 red colobus as part of a complete health assessment. This

TABLE 1. A description of the monthly cortisol level, quality of diet (protein/fiber index; secondary compound index), and parasite infections of a large group of red colobus (*Piliocolobus tephrosceles*) in Kibale National Park, Uganda

Month	Cortisol (ng/g)	Protein/fiber	Secondary compounds	<i>Trichuris</i> sp. prevalence	<i>Oesophagostomum</i> prevalence	Richness	Multiple infections
Jun-03	76.35	143.52	180.77	19.0	4.8	0.38	9.5
Jul-03	123.4	94.19	128.49	45.5	0.0	0.52	1.8
Aug-03	132.91	69.35	109.08	62.5	5.0	0.95	17.50
Sep-03	118.82	86.56	111.92	37.8	2.2	0.63	13.30
Oct-03	114.64	65.72	94.83	26.1	0.0	0.46	6.50
Nov-03	116.57	76.51	94.23	37.5	2.1	0.52	2.10
Dec-03	88.13	81.4	115.99	55.3	2.1	0.78	10.60
Jan-04	117.32	87.6	148.96	20.4	2.0	0.39	6.10
Feb-04	108.93	98.59	108.49	45.6	2.2	0.50	4.30
Mar-04	108.93	121.38	145.34	27.5	0.0	0.27	0.00
Apr-04	122.07	109.04	132.23	36.4	0.0	0.36	0.00
May-04	90.57	34.39	107.11	43.8	0.0	0.53	6.30
Jun-04	82.97	76.19	112.42	52.6	0.0	0.53	0.00
Jul-04	74.17	48.16	68.6	46.0	0.0	0.46	0.00
Aug-04	84.56	122.49	157.89	47.9	0.0	0.60	2.10
Sep-04	106.36	84.72	124.26	31.0	0.0	0.34	0.00
Oct-04	102.26	100.45	133.12	40.0	0.0	0.41	0.00
Nov-04	72.02	76.45	73.93	28.6	2.9	0.31	0.00
Dec-04	131.74	104.24	134.59	54.5	0.0	0.55	0.00
Jan-05	115.70	73.91	55.82	59.4	0.0	0.59	0.00
Feb-05	81.67	69.86	106.63	45.5	0.0	0.45	0.00
Mar-05	110.10	112.54	148.48	45.6	0.0	0.44	0.00
Apr-05	120.10	60.72	99.44	37.5	0.0	0.38	0.00
Average	104.36	86.87	117.07	41.1	1.0	0.49	3.48

TABLE 2. A comparison of the parasite infections of red colobus inhabiting eight forest fragments with the large group of *Colobus* found in the main forest of Kibale National Park, Uganda

Variable	Fragment	Fragment seasonal maximum	Forest	Forest seasonal maximum	Difference of averages	Difference of maximum
Cortisol (ng/g)	264.17	445.12	104.36	132.90	159.81	340.76
Richness	0.67	1.29	0.49	0.95	0.18	0.80
Prevalence of Nematode	57.59	83.33	41.63	65.00	15.96	41.70
Prevalence of <i>Trichuris</i>	47.92	63.41	41.13	62.50	6.79	22.28
Prevalence of <i>Oesophagostomum</i>	5.63	16.67	1.01	5.00	4.62	15.66
<i>Trichuris</i> eggs/g	3.94	9.20	2.41	6.90	1.53	6.79
All Nematodes eggs/g	4.71	9.68	2.43	4.00	2.28	7.25
Proportion with multiple infections	8.73	33.33	3.48	17.50	5.25	29.85

allowed us to determine that ectoparasite infections were relatively low: 93% of the animals had lice, but all at low levels, 43% had what appeared to be botfly-like parasitic larvae, but only 13% had more than one larva and the greatest number of larvae was 3. In addition, one animal (3%) had a tick (species undetermined).

Characterizing the infection of the red colobus based on the large group monitored between June 2003 and April 2005, illustrates that with the exception of *Trichuris* sp., whose mean monthly prevalence was 41.1% (range among months 19.0%–62.5%), the prevalence of parasitic infections in the large group was low ($n = 509$ samples; June 2003 to April 2005, $n = 23$ months). For example, the mean monthly prevalence of *Oesophagostomum* sp. was 1.01% (range among months 0–4.8%). The mean monthly percentage of samples that had multiple infections was 3.5%, but varied among months from 0% to 14.5%. Considering all samples, the richness of most parasite infections was low (mean 0.49, range 0.27–0.78). Considering only infected samples, the mean richness increased to 1.08 (range 1–1.33; Table 1).

It is difficult to know whether the observed levels of parasitic infection will have a biologically significant impact on the population. We offer two means of evaluat-

ing this. First we compared the parasite infections and cortisol levels of the large group of red colobus for which we had the longest record of parasite data, with the infections of red colobus from eight fragments outside of Kibale National Park (Chapman et al., 2006) (Table 2). In general, the levels of infections and the maximum levels of infections were lower in the group in the national park than in the forest fragments (Table 2). From our previous work in these fragments we know that a number of these indices of parasite infections are correlated with the changes in colobus population size. Second, we looked for evidence of illness in the study population. On 8.6% of observation days we noted evidence of illness. The most commonly observed symptoms were sneezing and signs of nasal discharge (7.1% of the days; sneezing alone not considered—See Struhsaker, 1975). Animals that appeared lethargic were seen on 0.8% of the days and in one case a recognizable adult male was so lethargic that he trailed the group throughout the day by up to 100 m and was even seen moving on the ground. The following day we returned to the site where he was last seen, but could not find him and it is possible he died in the evening. The remaining symptoms (0.7%) included swollen and blistered lips and jaws and inflamed genitals.

Hypothesis 1: Parasitism and nutrition individually affect stress levels in red colobus

Our first hypothesis was that poor nutrition and parasite infections would each individually lead to elevated cortisol levels, and that there might be a time lag before a rise in cortisol was detected. Mean monthly cortisol level in the large group was 104 ng/g, but there was considerable variation among months (72–133 ng/g; Table 1). There was also a large variation in diet quality; the greatest protein to fiber value was four times the lowest, and the highest secondary compound index was thrice the lowest. As expected given the methodological variation of characterizing an infection from a single sample, there was no correlation between the cortisol level of individual samples and any of the continuous measures of the parasite infections ($P > 0.20$ in all cases).

While we found no relationship between indices of diet quality, parasite infection, and cortisol level in a given month, there were a number of correlations between nutrition/parasite indices and cortisol levels in the subsequent month. Specifically, if a low quality diet was consumed in one month (i.e., low protein to fiber index), cortisol levels were higher in the next month ($r = -0.415$, $P = 0.027$, $n = 23$ months; June 2003 to April 2005). Surprisingly, we found that months with high cortisol levels were preceded by months where the group consumed less secondary compounds ($r = -0.484$, $P = 0.011$). This relationship may be a consequence of the strong positive correlation between protein to fiber intake and secondary compound intake ($r = 0.774$, $P < 0.001$).

With respect to parasite infections, as predicted, we found that multiple infections and more highly-pathogenic parasites led to a greater stress response. An increase in the potentially pathogenic *Oesophagostomum* sp. ($r = 0.376$, $P = 0.043$) and the proportion of multiple parasite infections ($r = 0.484$, $P = 0.011$) corresponded to an increase in the next month's cortisol level. However, cortisol did not increase with an increase in the previous month's prevalence of the less-pathogenic *Trichuris* sp. ($r = 0.053$, $P = 0.408$).

Hypothesis 2: Diet quality and parasite infections will interact to affect stress levels

In a simple correlation, the quality of the diet was not related to any of the indices of parasite infections ($P < 0.10$ in all cases) in the current or subsequent months. We applied path analysis to examine the direct and indirect effects of these factors on stress. We considered only those indices of parasite infections that were related to cortisol levels in the subsequent month (i.e., *Oesophagostomum* sp. prevalence, multiple infections) along with the protein to fiber ratio of the diet in the path analyses. Diet quality corresponded with significant or marginally significant increases in the cortisol levels (*Oesophagostomum* model, nutritional quality to cortisol path coefficient = -0.369 , $P = 0.037$, $n = 23$ months; multiple infection model, nutritional quality to cortisol -0.0348 , $P = 0.061$; Fig. 1). In contrast, the quality of the diet had no significant effect on parasite infections (*Oesophagostomum* sp. prevalence, 0.162 , $P = 0.230$, multiple infections -0.078 , $P = 0.358$). However, elevated parasite levels had a significant or marginally significant effect on cortisol level (*Oesophagostomum* 0.355 , $P = 0.057$; multiple infections 0.432 , $P = 0.026$; Fig. 1). In both cases, the indirect path (nutrition to parasite infec-

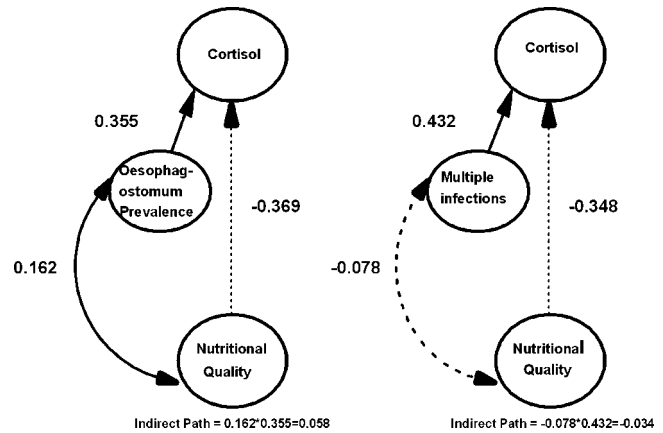


Fig. 1. Path analyses of factors predicted the cortisol levels of a large group of red colobus monkeys (*Procolobus rufomitratus*) in Kibale National Park, Uganda over 23 months. Positive effects are indicated by solid lines and negative effects by dashed lines.

tion to stress) was very weak (*Oesophagostomum* $0.162 \times 0.355 = 0.058$; multiple infections $-0.078 \times -0.432 = -0.034$; Fig. 1). These analyses provide further support that both nutritional stress and parasite infections influence cortisol levels, but did not support our hypothesis that these factors interact to create an amplified stress response.

Hypothesis 3: The effect of nutrition and parasitism is affected by group size

Overall, the large group had significantly higher cortisol levels (mean = 99.8 ng/g) than the small group (mean = 85.4 ng/g; $t = -1.930$, $P = 0.039$; $n = 13$ months, September 2004 excluded), with the large group's cortisol levels being higher in 9 of the 13 months. However, we found no evidence to support a group-size effect on parasite infections. There were no differences between the small and large group with respect to percentage of multiple infections, richness of infections, prevalence of *Trichuris* sp. or *Oesophagostomum* sp., or the eggs/g of *Trichuris* sp. or *Oesophagostomum* sp. ($P > 0.10$ in all cases; Table 3). Nonetheless, as predicted, the large group had fewer infants per adult female than the small group (large group 0.31 infants/female; small group 0.61 infants/female). Similar differences were apparent if older juveniles were considered (large group 0.52 infants+juveniles/female; small group 0.95 infants + juveniles/female).

DISCUSSION

We used natural seasonal variation to examine the relationships between nutritional stress, parasite infections, and cortisol levels across 23 months in a large group of red colobus monkeys. As predicted, we found that periods of presumed nutritional stress (i.e., months of low protein to fiber content in the diet) were followed by months with elevated parasitic infections (i.e., high prevalence of *Oesophagostomum* sp. and multiple infections) and periods of high cortisol levels. Secondary compound ingestion and other less pathogenic parasite infections did not lead to similar increases in stress hormones, and we found no evidence of an amplified stress response due to the interaction of these stressors.

TABLE 3. The parasite infections and cortisol levels of a large and a small group of red colobus (*Ptilocolobus tephrosceles*) in Kibale National Park, Uganda

	Cortisol (ng/g)	Trichuris prevalence		Oesophagostomum prevalence		Multiple infections		Richness		Trichuris (eggs/g)	
		Large	Small	Large	Small	Large	Small	Large	Small	Large	Small
Mar-04	90.68	27.5	28.6	0.0	0.0	0.0	0.0	0.27	0.29	1.4	3.5
Apr-04	113.03	36.4	20.0	0.0	0.0	0.0	0.0	0.36	0.2	0.8	0.2
May-04	126.83	43.8	15.0	0.0	5.0	0.1	0.8	0.53	0.25	1.1	0.3
Jun-04	66.26	52.6	34.8	0.0	0.0	0.0	0.0	0.53	0.35	2.1	2.3
Jul-04	80.20	46.0	46.7	0.0	0.0	0.0	0.0	0.46	0.47	5.3	3.5
Aug-04	75.94	47.9	14.3	0.0	0.0	2.1	0.0	0.6	0.14	4.6	0.6
Sep-04	180.90	31.0	33.3	0.0	0.0	0.0	0.0	0.34	0.33	0.6	0.3
Oct-04	105.09	40.0	69.2	0.0	0.0	0.0	0.0	0.41	0.69	2.1	4.0
Nov-04	51.81	28.6	75.0	2.9	0.0	0.0	0.0	0.31	0.75	1.8	6.0
Dec-04	69.36	54.5	61.5	0.0	0.0	0.0	0.0	0.55	0.62	3.7	1.2
Jan-05	79.71	59.4	58.8	0.0	0.0	0.0	0.0	0.59	0.59	6.9	2.6
Feb-05	98.87	45.5	64.2	0.0	0.0	0.0	0.0	0.45	0.64	1.9	4.1
Mar-05	82.90	45.5	65.0	0.0	0.0	0.0	0.0	0.44	0.65	1.9	2.8
Apr-05	69.30	37.5	55.0	0.0	0.0	0.0	0.0	0.38	0.55	1.4	1.4
Jul-05	NA	37.5	76.9	0.0	0.1	0.0	0.1	0.42	0.85	2.5	3.4
Aug-05	NA	58.8	50.0	0.0	0.0	0.0	0.0	0.58	0.5	2.5	2.6
Sep-05	NA	40.0	23.7	0.0	0.0	0.0	0.0	0.38	0.33	2.5	1.5
Oct-05	NA	50.0	66.7	0.0	0.0	0.1	0.1	0.54	0.72	2.1	2.0
Nov-05	NA	43.8	63.2	0.0	0.0	0.0	0.0	0.37	0.63	2.1	3.7
Dec-05	NA	61.1	85.0	0.0	0.0	0.0	0.1	0.61	0.85	2.6	6.2
Jan-06	NA	35.0	50.0	0.0	0.0	0.0	0.0	0.35	0.5	2.3	1.4
Feb-06	NA	70.5	68.8	0.0	0.0	0.0	0.0	0.76	0.7	2.7	2.1
Mar-06	NA	61.1	57.1	0.0	0.0	0.0	0.0	0.64	0.6	4.0	3.4
Apr-06	NA	62.0	60.0	0.0	0.0	0.0	0.0	0.33	0.56	0.5	3.2
May-06	NA	42.0	52.0	0.0	0.0	0.0	0.0	0.38	0.52	2.5	2.9
Average		46.32	51.79	0.12	0.20	0.09	0.04	0.46	0.53	2.48	2.61

TABLE 4. A comparison of the results of a study from forest fragments (Chapman et al., 2006) and this seasonal study in terms of the effect of predictor variables on either stress or population change

	Seasonal study	Fragment study
Diet effect on stress	Yes	Yes
Parasite effect on stress	Yes	Yes
Diet to parasites to stress ^a	No	No
Diet effect on population change	n/a	Yes
Parasite effect on population change	n/a	Yes
Diet to parasite to population	n/a	Yes
Stress effect on population	Yes, but 2 points	No

^a Diet increases parasite infections which have a combined effect on stress.

Because elevated cortisol levels may lead to increased mortality rates (Pride, 2005b), such stressors may have fitness consequences. The finding with respect to secondary compounds requires further studies because the role they play in primate nutrition is not clear. Secondary compounds are typically considered to make foods less palatable; however, it is also possible that some secondary compounds might act as antihelminthic compounds (Huffman et al., 1997). For example, tannins, which have received the most attention for their affect on nematodes (Hoste et al., 2006), have been shown to decrease nematode larva viability and interfere with egg hatching in sheep and goats (Min and Hart, 2003).

We recently completed a study in a system of forest fragments adjacent to Kibale National Park to examine whether food availability, parasite infections, and stress levels influenced red colobus abundance in a fragmented landscape (Chapman et al., 2006). We monitored gastrointestinal parasites, fecal cortisol levels, and changes in food availability by conducting complete tree inventories in eight fragments in 2000 and 2003. Red colobus populations declined by an average of 21.0% and the cumulative basal area of food trees declined by an average of 29.5%. Some of the results of this fragment study were similar to those of the current study (Table 4). Elevated cortisol levels were marginally related to decreasing food availability and were related to only some indices of parasite infections. Path analysis revealed no indirect effects of food availability on stress levels via increased parasite infections. The fragment study then went a step further to examine the relationships of these factors to changes in population size. Food availability and some indices of parasite infections were related to changes in population size, but, interestingly, elevated cortisol levels were not. Path analysis suggested that changes in food availability had a strong direct effect on population size, as well as an indirect effect via parasite infections.

The lack of association between stress and population size in the fragment study is troubling as it casts doubt on our suggested application of cortisol level as an index of population health, because this application rests on a causal chain linking poor nutrition and parasite infections to stress to population declines. The current studies validate some, but not all, of the links in this chain. Cortisol may not be an appropriate indicator of the cost of nutritional and parasite stressors, or may not have a significant effect on mortality and reproduction. Further study is required to more rigorously test these relation-

ships. However, the lack of association between cortisol levels and population declines in the fragments may be due to our sampling regime: we only collected fecal samples for cortisol at one time from each fragment and thus some measurements may have been from populations that were only experiencing short-term stress (e.g., temporary food shortage), while cortisol would have to be chronically elevated to correspond to a decline in population size. Thus, evaluation of cortisol levels in the populations in the forest fragments over an annual cycle might have illustrated an association between stress and population change. Furthermore, there is a need to critically assess methods of evaluating and interpreting cortisol data to address such conservation questions. Some animals naturally have higher cortisol levels than others (Gesquiere et al., 2005; Muehlenbein, 2006), and cortisol varies due to a number of factors (Pollard, 1995). Thus, it is important to evaluate sources of variation in cortisol levels so that these can be controlled in any future applications of this tool.

Furthermore, it is interesting that the fragment study found an interactive effect of nutrition and parasite infection on population size, while this study did not. This suggests that further investigation is required to determine the conditions under which diet quality will facilitate greater infections and thus amplify the cortisol effect. Possibly, this interaction will only occur under extreme conditions, such as those created by acute anthropogenic disturbance, fragmentation, and habitat degradation. This inference is supported by the very high mean cortisol levels observed in the fragments compared to those of the contiguous-forest groups in this study (fragments 267.2 ng/g; large group 104.4 ng/g; small group 85.4 ng/g), suggesting generally more extreme stress levels in the fragments. Similarly stressful times may occasionally occur in undisturbed populations due to natural stochastic environmental variation (e.g., drought).

In our third hypothesis we proposed that the effects of food stress and parasitism would be intensified in large groups due to the costs of increased within-group food competition and parasite transmission risk and that these costs would lead to elevated cortisol levels and decreased reproductive success. While we did not find that the large group had greater parasite infections, we did find that they had higher cortisol levels and fewer infants per female. However, this cortisol difference should be viewed cautiously as the number of months being compared is small ($n = 14$) and one analogous month was excluded. In a classic study, Freeland (1979) found that the number of intestinal protozoan species in mangabeys (*Lophocebus albigena*) increased with group size. In a meta-analysis that synthesized the results of a number of field studies, Cote and Poulin (1995) demonstrated a positive association between group size and the intensity and prevalence of contagious parasites. In contrast, we found no such relationship for red colobus. This is in accordance with a number of studies in other vertebrate systems (Poulin, 1991; Ezenwa, 2004; Vitone et al., 2004). It is difficult to evaluate the generality of the association between group size and parasitism as there is probably a bias in the literature towards reporting significant findings. Furthermore, it is somewhat ironic that if elaborate defenses have evolved to reduce the risk of parasite transmission or their impact on hosts in groups, then correlations between parasitism and group size may not be found (i.e., the "ghost of parasitism

past"; (Nunn and Altizer, 2006; Chapman et al., in press). Thus, some populations or species might live in large groups, but have low parasite levels because they have evolved counterstrategies, such as changing group spread or decreasing the frequency of re-visitation to potentially infectious areas to reduce transmission risk.

Two previous studies demonstrated a group-size effect on cortisol levels, and one of them linked this effect to food availability. Among African elephants (*Loxodonta africana*), cortisol levels were elevated in a large group compared to a small and medium sized group (Foley et al., 2001) and among ring-tailed lemurs, individuals in intermediate sized groups had lower cortisol levels than individuals in larger and smaller groups, although individuals in the largest groups had the highest levels when food was scarce (Pride, 2005c). Our results are consistent with these findings.

Our application of assumptions regarding the intensification of food competition in large groups to folivores runs counter to the current consensus that folivorous primates experience little within-group food competition (Wrangham, 1980; Isbell, 1991; Sterck et al., 1997), and that group size is not ecologically constrained and individuals in larger groups will not experience feeding stress. However, some of our research in Kibale suggests that the data and assumptions that underlie these ideas may require reevaluation (Gillespie and Chapman, 2001; Chapman et al., 2002; Snaith and Chapman, 2005). There are now a number of lines of evidence to suggest that folivores experience within-group scramble competition (Snaith and Chapman, in press). For example, some folivores deplete food patches (Snaith and Chapman, 2005) and some defend food resources (Harris, 2005), and in some cases group size is related to day range, habitat quality, and/or reproductive success (Gillespie and Chapman, 2001; Snaith and Chapman, in press).

In combination with the emerging data regarding increasing stress in groups, this leads to a paradoxical situation. Because females are the dispersing sex (Struhsaker, 1975), they should emigrate from a large group if it imposes costs that reduce their fitness. This suggests that either there is some other benefit of large group size, or that the cost of dispersal is very high. Dispersal costs can be high if resident females actively prevented immigrating females from joining the group; however, Struhsaker and Pope (1991) found that female red colobus were readily accepted into new groups and their intergroup transfer was relatively rapid with little time, if any, spent as a solitary. To understand the fitness costs and benefits associated with red colobus groups of different sizes we need data from individually recognizable animals over significant time periods. This would allow a quantification of the connections between attributes of individuals, such as nutritional stress, and parasite infections, and indices of fitness, such as stress, survival, and reproduction.

CONCLUSION

We examined the direct and combined effects of variation in nutrition and parasitism on stress levels in red colobus monkeys in Kibale National Park, Uganda. Periods of dietary stress (i.e., a diet with low protein to fiber ratio foods) and certain parasite infections were significantly associated with subsequent periods of increased cortisol. In contrast, the quality of the diet had little effect on parasite infections, and we found no statistical

support for an amplification of the effect of nutritional stress due to parasite infections. We found no evidence to support a group-size effect on parasite infections; however, cortisol levels in a large red colobus group were higher than those in a small group, and the large group had fewer infants per female. These results suggest that parasitism and nutritional stress may cause increased stress hormone levels. Because elevated cortisol is known to be associated with reduced fecundity and increased mortality, these stressors may be among the ecological factors leading to population declines in threatened and endangered species. We thus suggested that fecal cortisol levels may provide an indication of potential population declines due to reduced fitness associated with these stressors, and that measure of dietary quality, parasite infections, and/or cortisol levels in wild populations may prove to be a useful conservation tool by providing an early warning system of possible population declines.

ACKNOWLEDGMENTS

Permission to conduct this research was given by the National Council for Science and Technology, and the Uganda Wildlife Authority. Authors thank Toni Zeigler, Dan Witter, and the Wisconsin National Primate Research Center Assay Services for conducting the stress hormone analysis. L. Chapman, L. Fedigan, E. Greiner, M. Huffman, J. Lambert, P. Sicotte, M. Wasserman, and T. Zeigler provided helpful comments on this research and E. Greiner aided in parasite identification.

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