Analysis of Fecal Cortisol and Urinary Ketone Levels of Angolan Colobus (*Colobus angolensis palliatus*) as a Measure of Dry Season Stress in Diani, Kenya

A Thesis Submitted in Partial Satisfaction of the Requirements for the Honors
Program in Environmental Studies

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June 4, 1999

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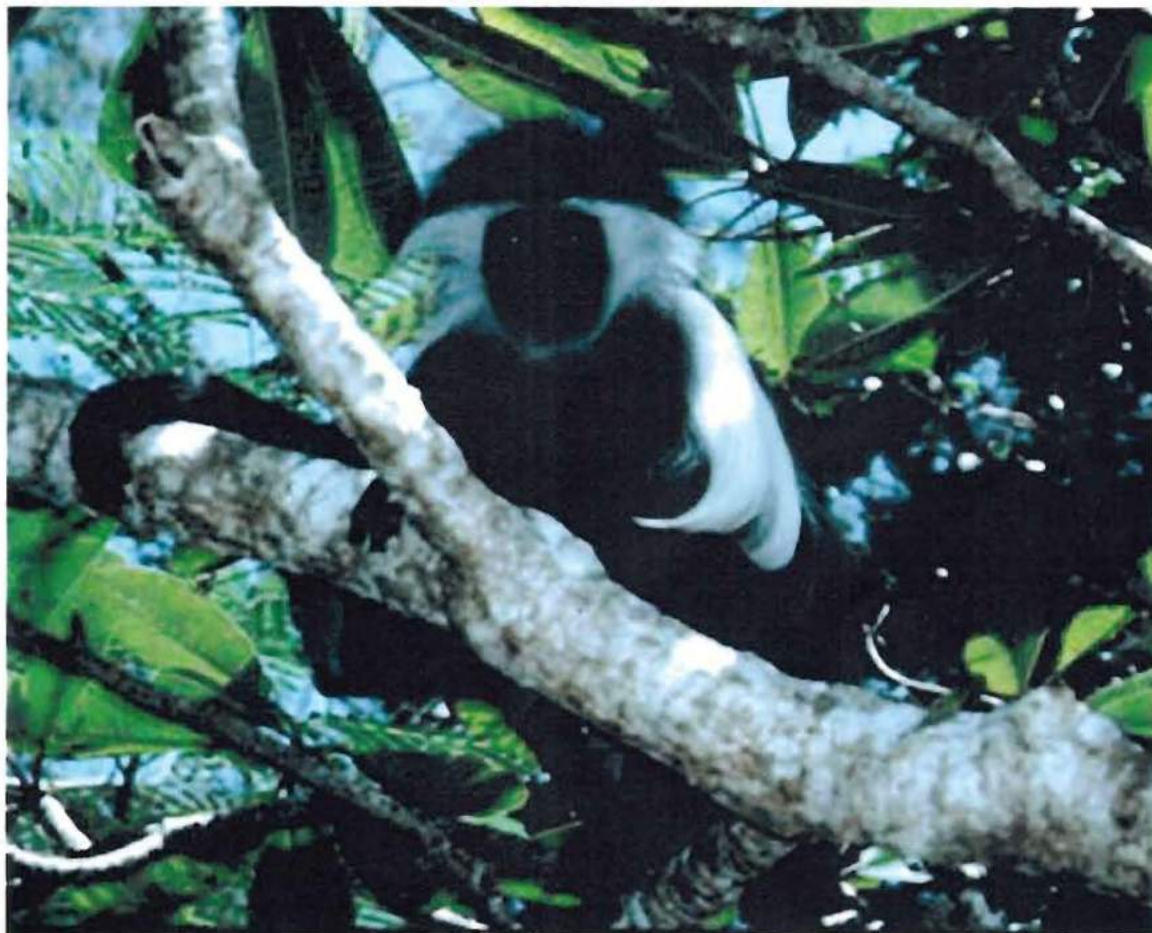Dartmouth College
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WAKULUZU
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Picture 1. Juvenile female Angolan Colobus at the Colobus Cottage.

Acknowledgements

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Abstract

Deforestation in Diani Beach, Kenya, has fragmented the remaining coastal forest such that home ranges of Angolan colobus' (*Colobus angolensis palliatus*) are now bisected by telephone lines, uninsulated powerlines, and roads. Patches of colobus habitat in Diani range in size and quality. This study attempts to quantify differences in five colobus habitat patches to measure habitat and food availability, and to compare these to physiological indicators of stress in individual monkeys. Fecal cortisol and urinary ketone levels were measured to assess stress levels and fat metabolism among colobus, respectively. Mean fecal cortisol levels for adult males in 5 habitat patches are significantly different ($p = 0.028$). Both mean total and mean basal cortisol levels correlate moderately with the stress index ($r \sim 0.65$, $p = 0.25$), showing that, in general, higher cortisol levels are found in habitats with higher stress index ratings. Gut retention time in colobus is approximately 48 hours, and samples taken two days after stressful and non-stressful encounters are significantly different ($p=0.037$). Encounters with other colobus groups, baboon groups, and forest workers could be the cause of these elevated cortisol levels. Some evidence of food stress in Diani during the dry season exists (10/83 urine tests were positive for ketones). Colobus in Diani experience both habitat and food stress in the dry season. Additional studies which determine wet season values and which focus on the factors affecting cortisol levels will help to place the results of this study in a broader context.

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Introduction

Deforestation and habitat alteration pose significant threats to ecosystem functioning and species survival. On the south coast of Kenya, clearcutting for subsistence agriculture and developments to support a thriving tourist industry have fragmented the remaining forest patches. At the turn of the century, the entire Kenyan coast was lined with a thin strip of forest. Today, forests on the north coast, from Mombasa to Somalia, have been completely cleared to make room for sisal plantations and residences. On the south coast, only a few, isolated patches have escaped development. Beginning 30 km south of Mombasa, Diani Forest and four *kayas*, sacred forests of the Diga people, are all that remain of this lowland forest. *Kayas* are protected by The National Museums of Kenya, but Diani Forest is unprotected and vulnerable to development. In the last twenty years alone, pressures of development have led to 75% deforestation in the Diani area (Eley, 1997).

Coastal forests south of Mombasa are categorized as coral rag forests because their unique group of flora thrive on a thin, nutrient poor layer of soil which forms over old coral. The close proximity of the coast, savannah, and forest creates an extremely diverse group of plant and animal species in these coral rag forests, many of which are not found in other places in Kenya or the world. Included in this diversity is the highest concentration of Angolan colobus monkeys (*Colobus angolensis palliatus*) found anywhere in the world. 258 of the estimated 1200 monkeys in the Kenyan population can be found in Diani (Wakuluzu Survey, 1998). *C. Angolensis palliatus* is only found in southeastern Kenya and northern

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Tanzania. The status of the Tanzanian population is unknown, and thus no determination about this sub-species' endangered status has been made by IUCN (Kahumbu, 1998).

Forest fragmentation poses a serious threat to the survival of the Angolan colobus in Kenya because the monkeys' diet is strictly foliverous (Oates *et al.*, 1994). In addition, colobus are not as resilient to disease and recover much more slowly from injuries than other coast primates (Kahumbu, 1998). Their sensitive health has not permitted them to adapt to habitat alterations as favorably as other coast primate species (Kahumbu, 1998). Forest losses continue to alter the forest ecosystem, largely due to support systems for residences and the area's thriving tourist industry. The only road, which serves the south coast, bisects the remaining forest patch in Diani. The location of this road has artificially separated natural home ranges of colobus groups. In a three-month period in 1996, 17 colobus were killed by vehicles while attempting to cross the road (Wakuluzu Trust). While aerial monkey bridges (called "colobridges") have been erected by the Wakuluzu Trust to help monkeys safely cross the road, the road still presents an unnatural break in the canopy and the speeding cars create a serious noise disturbance. Finally, telephone lines and uninsulated, 22,000-volt powerlines run through the east half of the forest, parallel to the road. Powerlines have been the second leading cause of colobus deaths, and non-fatal powerline encounters have left 3 females in the study groups with one arm (Wakuluzu Trust, 1998).

The purpose of this study was to compare cortisol and urinary ketone levels between Angolan colobus groups in 5 Diani forest patches. Colobus in Diani inhabit

small, fragmented forest patches with a much greater colobus density than in Zaire (Thomas, 1991), and unnatural forest disturbances (such as powerlines, humans, and residences) create a stressful environment that may ultimately lead to food constraints and long-term health ramifications for colobus. In order to quantify the effects of forest fragmentation, fecal and urine samples were collected from colobus in Diani to examine levels of cortisol and ketones, respectively. Cortisol levels are an indication of adrenal gland function in primates, and therefore provide a quantitative measure of stress (Bahr *et al.*, 1998). Ketone presence in urine is an indicator of internal fat metabolism caused by food stress or vigorous exercise (Robinson *et al.*, 1980). The combined data from these chemical analyses shed new light on the Diani forest environment and establish 1998 base levels of these biochemical indicators. From this new information, it will be possible to correlate cortisol and ketone levels with existing behavior studies of Angolan colobus in Diani and create more detailed recommendations for habitat conservation and species recovery.

The hypotheses tested in this study are:

• Colobus in forest patches with a high level of disturbance will have higher average fecal cortisol levels and more frequent elevated levels of cortisol than colobus living in less disturbed forest patches.

• Angolan colobus that inhabit smaller forest patches with less abundant and less diverse food resources will exhibit a higher rate of food stress during the dry season as seen in urinary ketone presence.

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The Angolan Colobus

There are five species of black and white colobus monkeys: *Colobus guereza, Colobus angolensis, Colobus polykemos, Colobus vellerosus,* and *Colobus santanas* (Oates *et al.*, 1994). Black and white colobus are distributed across equatorial Africa (**Figure 1**). *Colobus angolensis* is endemic to the moist lowland rain forest of the Congo, eastern Uganda, northern Rwanda, southeastern Kenya, and northeastern Tanzania. The East African subspecies, *Colobus angolensis palliatus*, is only found on the south coast of Kenya and in Northern Tanzania. Very little is known about the Angolan colobus, and even less is known about *C. angolensis palliatus*. Since 1970, only 3 papers have been published on this subspecies.

Many Angolan colobus researchers look to the studies of *C. guereza* because of the wealth of information known about this closely related species. As more studies have been done however, differences in diet and behavior have been noted between the two species. For example, in Zaire, where the two species are sympatric, each utilizes different types of forest and food resources (Thomas, 1991). This introduction summarizes information from studies of *C. angolensis*, inferring information from *C. guereza* studies only when necessary. Although there are more than 30 species of colobines worldwide, references to non-African colobine genuses will also be limited. African colobines (red (*Procolobus*), and black and white colobus monkeys) are the most foliverous colobines (Cork, 1996) and are unique in their gut morphology (Milton, 1997). References to *Procolobus* species in

Figure 1. **Approximate distribution of colobus monkeys in Africa** (Hayes *et al.*, 1996). **(A)** *C. polykomos* (stripped), *C. santanas* (dotted), *C. angolensis* (black), and *C. angolensis palliatus* (open) *. **(B)** *P. verus* (dotted), and *C. guereza* (black). **(C)** *P. badius* (black).

relation to diet and stress will also be limited because they have slightly different gut morphology than black and white colobus (Eley, 1997).

The Wakuluzu Trust

The Wakuluzu Trust is a small coastal conservation group dedicated to the preservation of forest habitat, primate research and conservation, and community awareness. Since it's formation in 1996, the group has been influential in mobilizing community support for primate conservation and has undertaken a variety of long-term study projects. Research for this study was done under the auspices of the Trust, and many of the insights on *C. angolensis palliatus* in this paper come from research that has been conducted by the Trust.

Morphology

The name "colobus" comes from the Greek word "kolobus" which means "mutilated." The thumbs on colobus forelimbs are absent and on the rear limbs they are reduced to a stub, giving their hands a somewhat mutilated look in comparison to human hands. The lack of thumbs in colobus necessitates unique feeding behavior and movement. Colobus are specially adapted to an arboreal lifestyle, with hind limbs that are longer than their forelimbs and fingers that are comparatively longer than other primates. Angolan colobus are very adept at moving through the forest canopy and can jump gaps between trees up to 15 m in width (personal

observation). On the ground however, colobus are slow and awkward, moving quadrupedally in a hopping motion.

Colobus infants are all white at birth. At about 4 months, darker fur begins to replace most of the white hair. With the exception of *C. santanas*, all of the species of black and white colobus have a recognizable pattern of black and white fur. *C. santanas* have an all black coat (Oates *et al.*, 1994). *C. angolensis* are distinguishable by their white epaulets, cheeks, and lower half of the tail. However, individuals do not have unique coloration that allows them to be identified. While adult males are larger than adult females, Angolan colobus show sexual dimorphism at all ages in their genital region: males have a distinct white stripe, 4-6 cm in length (delineating the penis), while females have a shorter and wider white tuft in front of the vaginal opening.

Differentiation between individuals of the same sex can be accomplished from physical idiosyncrasies (tail coloration, missing limbs, and scars), but the age category of an individual can also be identified by size. Infants leave their mothers between 5 and 12 months and are then classified as juveniles. Juveniles are much shorter and thinner through the body than sub-adults (age = 1-2 years). Sub-adult males (2-5 years) and females (2-3 years) are nearly the same size as adult colobus, but are not sexually mature. Males sexually mature at 6 years and females at approximately 4 years.

Measurements of colobus in Diani have only been done *post mortem*. Adult males have been weighed between 8 and 10 kg (Wakuluzu Trust), very similar to Adult males in Zaire (9.9 kg) (Thomas, 1991). Adult males have a head and body

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length of 55-70 cm and an 80 cm tail. Slightly smaller, adult females measure 45-60 cm with a 70 cm tail and weigh between 6 and 8 kg. During survey work for the Wakuluzu Trust, colobus were observed in *kaya* Galu, a larger, more contiguous forest than Diani. Adult colobus in Diani appear to be smaller than colobus in *kaya* Galu (personal observation).

Colobus have a specially adapted, three-chambered stomach. The first section, the *saccus gastricus*, is more alkaline (pH 5.0-6.7) than other sections, and supports bacteria microflora, allowing microbial fermentation to take place (Chivers, 1995; Milton, 1998). The *saccus gastricus* may also be important in the detoxification of tannins and other chemicals in the colobus diet (Dasilva, 1992). This section is connected to the acidic (or true) stomach by a *tubus gastricus*. The acidic section of the stomach empties into the small intestine. Gut retention time has been estimated at 38 hours in the digestive tract, excluding the small intestine (Kay and Davies, 1995, as cited in Milton 1998). Colobus also have cusped molars which allow them to grind their food and enlarged saliva glands to buffer their forestomach, counteract the toxicity of plant defenses, and provide phosphorous to bacteria in their forestomach (Milton, 1998). However, colobines do not ruminate, and thus have inefficient microbial digestion (Dasilva, 1992).

Food and Habitat Preferences

Colobus are considered to be folivores, with the majority of their diet consisting of young leaves. They also consume quantities of mature leaves, flowers, seeds, and unripe fruits in varying amounts, causing some primatologists to

categorize them as frugivorous and foliverous (Thomas, 1991). Unlike the other diurnal coast primates that have adapted to forest loss by scavenging nearby crops and raiding garbage dumps, colobus are completely dependent on the diminishing forests for survival. The specialized colobus stomach is unable to digest sugary fruits. Instead, colobus prefer unripe fruits and seeds with lower sugar content and higher protein content. An ideal colobus diet combines high nitrogen and protein intake with a constant percentage of indigestible cell wall material to standardize gut retention time (akin to a diet of young leaves or seeds) (Milton, 1998).

Mature leaves are always present in equatorial forests, but they are not the preferred food source of colobus. Instead, studies have correlated colobus food choice throughout the year with the nutrient content of each plant part (Baranga 1982, 1984). As young leaves mature, the moisture content, crude protein, potassium, and phosphorus levels decrease, while the percentages of acid detergent fiber, lignin, calcium, and sodium increase, making the leaves more difficult to digest and a poorer nutrient source (Baranga, 1983). In a study of *C. polykemos*, a diet of 100% leaves was not able to meet the average daily metabolic needs of adults, based on daily energy expenditure calculations (Dasilva, 1992). Instead, colobus ate unripe tree seeds, leaf buds, and unripe fruits whenever possible as sources of protein and calories.

Studies by Moreno-Black and Bent in 1982 looked at secondary compounds as an indicator of food preference. As leaves mature or react to predation, they produce high concentrations of tannins and saponins that often deter herbivores. In addition to young leaves, seeds, flowers, and unripe fruits, evidence of a charcoal,

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soil, and coral eating has been found in East African colobus and in Indonesian red leaf monkeys (Davies and Baillie, 1988; Strusaker, 1998). It has been hypothesized that colobus eat soil, charcoal, and coral to help buffer the high concentrations of secondary toxins and tannins in the leaves of their diet. Two of the study troops were seen eating coral, and evidence of coral eating can be seen in fecal samples collected from a third group.

Habitat Preference and Activity Budget

Angolan colobus prefer secondary or mixed forests, and are generally found in the upper third of the canopy (Thomas, 1991; Kaspers, 1999). Colobus groups average six individuals and can survive on territories of 1-2 hectares of forest (Eley, 1997). Today, in Diani, many groups of eight or more colobus are living in habitat patches of half a hectare or less. Thus, during the dry season, when food pressures may be most intense, colobus in smaller habitat patches could be forced to expand their foraging ranges. Colobus have been described as low-energy strategists because it is very difficult for large folivores to meet their metabolic needs during the dry season (Dasilva, 1992). Milton (1997) also suggests that during seasonal extremes, colobus modify their behavior to account for the lack of food. Periods of inactivity have been shown to increase in the dry season (Lowe and Sturrock, 1998) and goal-directed travel has been observed, potentially as an energy saving technique.

Activity budget studies, which measure the amount of time spent on basic activities such as feeding, socializing, and resting, have found that colobus behavior varies throughout the year based on food availability (Lowe and Sturrock, 1998). In

a study of *C. angolensis* in Gendagenda South Forest Reserve, Tanzania, Lowe and Sturrock (1998) found that feeding behaviors varied significantly between the wet and dry seasons. The Gendagenda South Forest Reserve is approximately 150 km south of Diani and experiences a similar bimodal seasonal climate. The study troop of eight colobus ranged in an area of 30.6 ha during the dry season, but only covered a third of this area, 10.43 ha, in the wet season. Also, the group spent significantly more time feeding in the dry season as compared to the wet season (209 minutes per day versus 140.5 minutes per day) and had only one-third as much diversity in their diet during the wet season. Thus, habitat constraints may be particularly evident during the dry season, when a greater forest area and a greater diversity of feeding species may be needed to meet dietary requirements.

The increased feeding pressure during the dry season that Lowe and Sturrock observed in Tanzania has also been witnessed in Diani. Two activity budget studies conducted from June-September, 1997, found that colobus groups in smaller habitats spent more time feeding, on average, and fed from a higher number of species (Osore, 1997; Patel, 1997). These results suggest that a variety of habitat qualities exist in Diani, with group behavior modifying accordingly during the study period to account for food availability.

On average, colobus in Diani awake between 6:30 and 7:00 AM and feed intensively until approximately 8:30 AM. The entire troop then rests until around 11:00 AM when they move to a new location and feed until noon. Another period of rest occurs during the most intense heat of the day, followed by a period of light feeding and playing among juveniles and sub-adults until around 4:00 PM. The

third and final feeding bout lasts until 6:00 PM, and the entire troop convenes and sleeps in the same tree from 7:00 PM to 6:30 AM.

Social Structure

Angolan colobus live in different units based on the type of forest they inhabit. In the Nyungwe Forest, Rwanda, multi-male groups of up to 50 members have joined together into aggregate groups of 300 (Wakuluzu Trust). The smaller, more defendable habitat areas present in East African and in riverine forests in Zaire support much smaller colobus groups with one or two males (Moreno-Black, 1982). Family units in Diani Forest average six individuals (one male, several adult females, and juveniles or infants), similar to *C. angolensis* in Ituri Forest, Zaire (average group size of 6.6 individuals) (Thomas, 1991). Colobus group size seems to be dependent on the size and quality of the habitat. The total colobus density in a forest has been found to correlate positively with the protein/fibre ratio of mature tree foliage (Oates, 1996) and with the number of trees in a group's habitat area (Dunbar, 1987).

A study in Diani of intra-group behavior found matriarchal dominance, with the alpha female receiving the most attention from other troop members (based on grooming behaviors), followed by the alpha male, beta male, and then the sub-adult females (Kaspers, 1999). Adult males generally initiate group movement and engage in aggressive encounters with other groups of colobus or other species. From birth until the infant begins to develop its black and white coloring around 4

months, every female in the group, not just the mother, carries the all-white infant. This behavior is unique to black and white colobus (Eley, 1997).

Predation Risks

In central and western Africa, human poached poses the largest predation threat to colobus. In Zaire and Uganda, chimpanzees kill colobus for food. Busse (1997) reported that chimpanzees in Gombe, Tanzania, took as much as 8-13% of the red colobus population in two years (as cited in Oates, 1996). In eastern Africa, the largest risk for colobus survival is human encroachment on forests, while human poaching is not a significant factor (Oates, 1996). However, natural predators are also important. The largest threat to colobus in Kenya is the crowned hawk-eagle (*Stephanoaetus coronatus*), the largest predatory bird in Africa (Wasser, 1993). Since colobus spend the majority of their time in the upper canopy of the forest, they are very susceptible to aerial attack, especially when all-white infants are present. Leopards, pythons (and other forest snakes), and monitor lizards also pose predation risks for colobus (Mturi, 1993, Wasser, 1993). The presence of ground predators has been cited as a deterrent for colobus to come to the ground to feed (Mturi, 1993).

Study Site

Diani Beach, Kenya

Diani Beach is located in southeastern Kenya, south of Mombasa, at approximately 4°20'S and 39°20'E (**Figure 2**). The 12 km beach and remaining forest is separated from Tiwi Beach in the north by a fresh-water estuary. More forest fragments, beginning with *kaya* Galu, exist south of Diani.

Climate

The coastal climate is cooler and more humid than central areas of Kenya. Constant daytime highs from 25°-35°C and relative humidity over 70% year-round provide an ideal microclimate for coastal forests (Ojany and Ogendo, 1992, 65; Burgess *et al.*, 1996). There are two main rainy seasons on the coast, from March to May (wettest in May) and from October to Mid-December. However, the coast gets rain in every month of the year, averaging 145 rainy days per year (Ojany and Ogendo, 1992). The mean annual rainfall for the south coast is 1204 mm/year (Hawthorne, 1993). July and August are the driest and coolest months of the year, with average daytime highs around 28°C and evening lows around 20°C (Ojany and Ogendo, 1992, 66).

Forests

The forests on the south coast of Kenya have been categorized as lowland dry forest on coral rag (Hall *et al.*, 1984 as cited in Hawthorne, 1993). The total area of dry

KENYA-MAIN ROAD NETWORK

SUDAN

LAKE TURKANA

LODWAR

LOYENGALANI

MARSABIT

UGANDA

SOMALIA

KITALE

MARALAL

ELDORET

KABARNET

NYAHURURU

NANYUKI

MERU

KISUMU

NAKURU

KERICHO

NYERI

EMBU

GARISSA

KISII

HOMA BAY

NAIVASHA

NAROK

THIKA

NAIROBI

TANZANIA

SULTAN HAMUD

NAMANGA

EMALI

GARSEN

LAMU

TSAVO RIVER

LOITOKITOK

VOI

MALINDI

TAVETA

MOMBASA

INDIAN OCEAN

From <u>Kenya</u>, Kenya Board of Tourism, 1991.

coastal forest is estimated around 3100 km² in Eastern Africa, making coastal forests the most threatened forest type in Africa (Burgess *et al.*, 1996). In Kenya, surveys have listed 97 forests as dry coastal forests totaling 661 km² (ave.=6.81 km²/forest) (Burgess *et al.*, 1996). Coral rag forests, in particular, exist in flat, coastal areas where coral platforms and breccia were uplifted and sea levels changed in the quaternary era of the Cainozoic period (Hawthorne, 1993).

The canopy of the forest in Diani is dominated by *Combretum schumannii*, but other species such as *Adansonia digitata, Tamarindus indica, Ficus spp.*, and *Diaspyros squarrosa* are also quite prevalent (Moreno-Black *et al.*, 1977; Hawthorne, 1993). Below the forest canopy, coastal scrub brush and species of *Grewia, Fagara chalybea*, and *Meyna tetraphylla* form a dense understory. Coconut palms (Cocos nucifera) and Casuarina equisetifolia dominate the eastern edge of the forest. As noted above, there are two dry seasons in southern Kenya. During the long dry season (June-October), many trees lose their leaves and very few of them produce fruit. In a study of 12 tree species that form the mainstay of the colobus diet (Anderson *et al.*, 1999), seasonal variations in food availability were found to follow the seasonal pattern of rain (see **Appendix A** for data). **Figure 3** summarizes the data for six of the most-preferred food species: *Azadracta indica, Delonix regia, Ficus sycamorous, Grewia plagiophyla, Lecaniodiscus fraxinifolious*, and *Sideroxylon inerme* (Anderson *et al.*, 1999). Note the lower availability of young leaves, flowers, and fruits during August-October. Studies have matched this low food availability with increased feeding time, on average, during the dry seasons (Lowe and Sturrock, 1998).

Figure 3. Colobus food availability in Diani Beach, Kenya, from January, 1998 to December, 1998. Values shown as percentage of sample trees for Young Leaves, Flowers, and Fruits. Data were collected by Bakari Garise and Hamisi Mwahari of the Wakuluzu Trust.

Trees are rarely able to put down a solid root system on top of the old coral, and blowdowns from the strong tradewinds, erosion, thunderstorms, and old age create gaps in the forest canopy. The natural regeneration of forest in these patches creates a mosaic of forest patches at different stages of growth. Moderately disturbed forests can be more rich in lean-season folivore foods than undisturbed forests, allowing for a higher primate density (Oates, 1996). However, trees that are heavily predated or disturbed may fruit irregularly (Wasser, 1975, as cited in Baranga, 1986).

Unnatural forest fragmentation creates what are known as "edge effects". Edges of forests are the most prone to invasion by non-natural species of plants and

animals. Dryer and more exposed than the original coastal forest climate, edges favor species that can adapt to these changes. Edge effects can extend up to 300 m into a forest. The small Kenyan coastal forests may therefore be regarded as entirely edge (Burgess *et al.*, 1996).

Primates

Three additional species of diurnal primates inhabit the forest patches in Diani (**Pictures 2, 3, 4**). Yellow baboons (*Papio cynocephalus*) live in large, multi-male troops in Diani and have become quite a menace in the local area. In a more wild setting, baboons feed on roots, grasses, insects, and are opportunistic scavengers of eggs, rodents, and other ground prey (Eley, 1997). In Diani, many of the baboon troops feed exclusively from garbage dumps located near the major hotels and residential areas. Baboons are also adept crop raiders, and local farmers have been forced to keep dogs and use barbed wire fencing to keep baboons away from their crops. Reproduction rates have increased dramatically in recent years, and now the Diani area is overrun with yellow baboons, presenting a problem for locals, tourists, and the Kenya Wildlife Service. Baboons spend the majority of their time on the ground, but are equally adept in the forest canopy. Adult males present a frightening facade, weighing as much as 30 kg and stand more than 50 cm at the shoulders (Eley, 1997). Whether on the ground or in the canopy, interactions with colobus are most often agonistic in nature, and usually end with the baboon groups displacing the colobus.

Two species of Cercopithecus monkeys also inhabit the forests along Diani. The coastal sub-species of sykes monkey, *Cercopithecus albogularis albogularis*, are

slightly smaller than colobus. Sykes monkeys prefer to stay in the lowest parts of the canopy, feeding from leaves, seeds, and ripe fruits. Cercopithecus monkeys have simple stomachs that do not permit them to digest cellulose. Sykes are omnivorous, eating insects and eggs when available. In Diani, sykes monkeys coexist peaceably with colobus (Moreno-Black, 1982), although at times colobus displace them from their feeding positions. Sykes monkeys in Tanzania did not have as much spatial and dietary overlap as in Diani, however (Wasser, 1993). It is possible that the confined forest space in Diani forces species to be sympatric when they would otherwise not coexist.

The vervet, or green monkey (*Cercopithecus aethiops*), has adapted to the coast conditions in a manner similar to the baboon. Vervets are opportunistic scavengers and thrive on hotel dump sites and tourist lunch plates. Slightly smaller than sykes monkeys (adult males are 4-6 kg versus 6-9 kg for sykes), they spend roughly equal amounts of time in the canopy and on the ground (Wakuluzu Trust). In recent years, vervets have also sustained high reproduction rates and are now prevalent in large troops on the coast (as many as 30 individuals). Colobus and vervets do not coexist in Diani, with colobus displacing vervets in nearly all encounters (Patel, 1997).

Picture 2. Adult female (presenting) and adult male yellow baboons near the Colobus Cottage.

Picture 3. Adult female sykes monkey and infant in the Colobus Cottage patch.

Picture 4. Adult male vervet monkey in the Warandale forest patch.

Study Groups

Habitat Area	Total	Adult Males	Adult Females	Sub-Adult Males	Sub-Adult Females	Juveniles	Infants
Colobus Cottage	9	2	1	1	3	1	1
Mosin	8	1	2	0	1	2	2 * *
Warandale	4	1	1	0	0	1	1
Tradewinds 1	5	1	2	0	2	0	0
Tradewinds 2	9	2	2	0	1	2	2

Table 1. Breakdown of Study Troops by age category. Only adult males were used for fecal cortisol analysis, but all individuals were sampled for urinary ketone presence. ** Infants born 7/19/99 and 7/28/99.

Study Sites

Map of Diani Coast, see Figure 4.

Study Sites

The five study habitat areas represent a variety of the factors of habitat fragmentation faced by colobus in Diani. The Colobus Cottage group (**CC**) occupies the largest area in the study. Their forest patch is protected by the Wakuluzu Trust and has a contiguous, high canopy, with the greatest diversity of colobus food species. The area is relatively undisturbed by humans, although it does border the main tarmac road and has uninsulated, 22,000 volt powerlines which bisect the patch. The Warandale group (**W**) occupies the northernmost habitat; a piece of forest that has been significantly altered to build 10 residences and 4 support buildings for a "cottage community". **W** colobus use the roofs of residences to travel between trees, for display behaviors, and to sun themselves on a regular basis. The

MOMBA[SA]

Frere Town
Nyali
Likoni
Kwale
Matuga
Waa
Tiwi
Ukunda

Diani Beach

Ras Mwachema
Gombato Estate
Kongo
Diani P.S.
Mwakola
Mwabungu
Galu
Kenya Sugar Estate
Kinondo
Gazi
Gongoni Forest Sawmills
Chale Point
Chale Island
Chale Reef
Mafisaha (Gazi) Bay
Kisima Chande

SHIMBA FOREST
Makadara Forest
Pengo Hill 1471
Cha Shimba
Lango Ya Mwagandi Forest
Kisumoni Forest
Radio Sta. 1068

Port Reitz
Mombasa Port Reitz
Mkunguni
Mtongwe
Mihongani
Kaya Pungu
Ngombeni
Tangila 423
Slji
Makondeni
Kigato
Vinunyi
Kidzumo
Mivumoni 342
Bowa

Godoni Forest 477
Golini 1318
Raoro Sta.
Forestry Dept.
Vuga
Marere Forest
Water Works
Kiamtoi Forest
Kiamtoi Hill

Vikolani 761
Chirimachagona
Kibandaongo
Yava
Mbungo
Mwakunde
Mwambalo
Ng'onzini Kibaoni
Mitokoni

Mwachi Forest
Mamduchi
Mipirani
Dzivani
Majumbani
Mteza
Mumbogni
Thicket
Mwaluganze
Itsantse
Lunguma
Banga
Mbuguni Mkt.
Manjera
Pump Ho.
Lime

Giriama
Mwabria 306
Shamu
Magutu
Muhaka Mbavu
Muhaka Forest
Mwaweche
Kilole
Njele
Tongonyika
Mkomba
Nzosuni
Mtwani
Nguluku 543
Magodi
Kidiani
Eshueshu
Ngwaru
Buda Forest
Nguruweni
Bomani
Msambweni
Tumbe
Sawa Sawa
Kirafuni P.P.
Copra
Chingwede
Kiviogo Store
Mwaembe
Nzovuni
Ramisi

Mweie Mdogo Hill
Ziwani
SHIMBA FOREST

Map from A. O. Thompson, _Geological Survey of Keny[a]_
Nairobi: Government Printers, 1956

24

Mosin group (M) occupies a neighboring patch of forest to the W group. Aggressive interactions between the groups were not witnessed during the study period, and very few trees are in the overlapping section of habitat. M lives in a narrow band of forest that is bisected by 3 dirt roads. The final two study troops, Tradewinds 1 and 2 (TW1 and TW2) share a forest patch that borders a large tourist hotel. The area is not equally split between the two groups and agonistic interactions between alpha males and cross-troop copulations were regularly observed during the study. Although the hotel was not open for business during the study period, grounds-keepers and staff were still working and moving through the colobus habitat. Additionally, TW1 has the smallest range and the fewest number of food species in its habitat area. A summary and comparison of all of the habitat characteristics can be found in Table 2, and maps of each of the study sites can be seen in Appendix C.

Table 2. A Summary of Habitat Area Characteristics

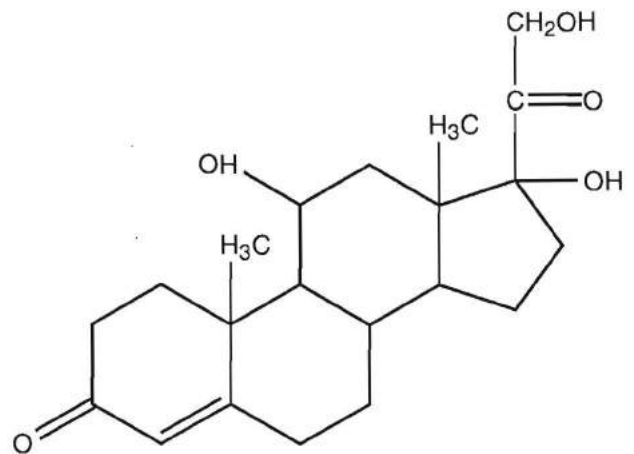| Habitat Factor	Colobus Cottage	Mosin's	Warandale	TW1	TW2
Colobus in Group	10	8	4	5	9
Habitat Area (m^2)	23629	5125	6900	2836	4490
Area per Ind. (m^2)	2362.9	640	1725	567	499
Total Trees	233	93	138	83	101
Feeding trees	207	85	97	69	101
Feed trees/Ind.	20.7	10.63	24.25	13.8	11.2
Feed tree spp.	26	11	10	10	15
Rest trees	52	15	11	11	34
Other colobus	0	4	8	9	5
Sykes	12	10	10	15	15
Vervets	8	5	18	10	10
Roads (km)	0.146	0.18	0.05	0.05	0
Buildings	4	2	14	6	4

Cortisol and Stress

Introduction

Levels of cortisol in the body are a measure of adrenal gland function. Cortisol levels in non-stressed animals remain low. In a "fight or flight" response to a stressful circumstance, increases in adrenal gland function cause levels of cortisol to rise. High levels of physiological stress, in response to environmental pressure, reduce an animal's resistance to disease, and long-term elevated levels can lower survival and reproduction rates (Wasser *et al.*, 1996).

Cortisol

Cortisol is a steroid metabolite of adrenocorticotrophin (ACTH), a hormone which is produced by the adrenal medulla in response to external stimuli and controls the conversion of glycogen into glucose in the liver. Cortisol is widely accepted as a quantitative index of the activity state of the

Cortisol. Taken from Schimmer and Parker (1996), pg. 1462.

hypothalamic-pituitary-adrenal cortex axis (HPA), and therefore, a quantitative index of physiological stress (Bahr *et al.*, 1998). The HPA axis maintains appropriate levels of corticosteroids in the body through three levels of regulation: diurnal rhythm in basal steroidogenesis, negative feedback regulation by adrenal

corticosteroids, and increased steroidogenesis in response to stress (Chrousos, 1995 as quoted in Schimmer and Parker, 1996). Corticosteroids provide the capacity for an organism to resist stressful circumstances such as environmental changes by overriding normal negative feedback mechanisms in the HPA axis, leading to an increase in plasma concentrations of adrenocortical steroids (Schimmer and Parker, 1996). While this short-term ability of an animal to deal with a stressor is very important to the animal's survival, long-term exposures to elevated adrenocortical steroids inhibit a variety of building processes:

> They slow digestion; they block the release of brain, pituitary, and gonadal hormones needed for reproduction; and, by depriving cells of nutrients and interfering with the secretion of growth hormone, they inhibit growth. Finally, by disabling and destroying lymphocytes, glucocorticoids suppress the immune and inflammatory responses, which guard against infections and repair bodily injury (Sapolsky, 1996).

In humans, the presence of high levels of cortisol is rare, and elevated levels are generally an indication of a very stressful event, adrenal hyperplasia, or adrenal adenoma (Cushings syndrome) (Jacobs *et al.*, 1990). In a situation of severe stress (fight or flight response), daily production rates of cortisol in humans can rise at least 10-fold (Schimmer and Parker, 1996). Studies of primates in captivity have linked induced stress to a rise in blood, urine, and fecal cortisol levels (Brown *et al.*, 1997).

Fecal analysis is an ideal method for cortisol monitoring because it is non-invasive and can still provide an indication of physiological condition and habitat suitability (Wasser, 1994). Fecal cortisol levels are also useful for studies of free-ranging animals because the levels are stable and continue to reflect in vivo changes

corticosteroids, and increased steroidogenesis in response to stress (Chrousos, 1995 as quoted in Schimmer and Parker, 1996). Corticosteroids provide the capacity for an organism to resist stressful circumstances such as environmental changes by overriding normal negative feedback mechanisms in the HPA axis, leading to an increase in plasma concentrations of adrenocortical steroids (Schimmer and Parker, 1996). While this short-term ability of an animal to deal with a stressor is very important to the animal's survival, long-term exposures to elevated adrenocortical steroids inhibit a variety of building processes:

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over time. Isolation and analysis of plasma cortisol levels in humans is a common clinical laboratory procedure (Jacobs *et al.*, 1990). From clinical technology, a method for fecal cortisol analysis has been developed that is equally as accurate as blood plasma analysis or urinalysis (Wasser, 1995; Whitten *et al.*, 1998). Laboratory studies on primates and other large mammals have been conducted with anesthesia (Whitten *et al.*, 1998), a known stressor, and with ACTH injections (Bubenik and Bantis, 1993). Levels of cortisol appear in the blood within minutes of the induced stressor, in the urine the same day, and in fecal samples after about 48 hours (Whitten *et al.*, 1998). With the extraction and radioimmunoassay procedures perfected by Wasser *et al.* in the study of free-ranging yellow baboons, researchers have gained an important new ability to effectively analyze the impacts of habitat destruction and disturbances on free-living animals.

Stressors in Diani

Colobus in Diani are susceptible to a series of environmental stressors. Interactions with other colobus groups, encounters with predators, injury, and low food availability are all potential sources of elevated cortisol in Diani. The proximity of neighboring colobus groups and the frequency of encounters with baboon troops are both important measures of this stress. Injuries from powerlines and fights could also trigger a rise in cortisol, as would the presence of predatory birds, snakes, and monitor lizards within a habitat patch (Sapolsky, 1996). Finally, food stress also induces adrenocortical hormone releases (Jacobs *et al.*, 1990).

Dry season food stress has been witnessed for colobus in Tanzania. Lowe and Sturrock (1998) found that during the dry season, the study troop of C. *angolensis* spent significantly more time feeding at the expense of resting, had a higher dietary diversity, and ranged over a much greater area than during the beginning of the wet season. Activity budgets of colobus groups in Diani have shown a range of behaviors in the dry season. The variations in behavior among Diani groups could suggest that certain habitat patches are more stressful than others.

Deforestation in Diani threatens to decrease the dietary diversity available to colobus. Without this dietary diversity, colobus may be forced to range further from the center of their habitat. More frequent use of the periphery of their habitat increases the rate of encounters with other stressors: neighboring colobus groups, humans, roads, powerlines, and other potentially stressful situations. Total forest size has also been found to influence colobus group size. Dunbar (1987) found that groups would fission and members would migrate to new forest areas when there were fewer than 10 trees per individual. In this study, the TW2 and M groups inhabit small areas where the number of trees per individual approaches this threshold (11.2 and 11.6 trees per individual, respectively).

The current situation in Diani is potentially dangerous for colobus: smaller habitats are less resistant to natural disturbances and will likely promote more frequent interaction with the surrounding human population. For example, in a larger forest, colobus groups would migrate to avoid a fight with another group. In small, isolated forest patches (like Diani), colobus groups might prefer aggressive interactions to the risks of migration (Dunbar, 1987). In previous cortisol studies,

aggressive interactions and territory stress were confined to males, and physiological stress associated with offspring was confined to adult females (Wasser, 1997). In a study of western lowland gorillas, high postpartum stress in mothers decreased the chances of offspring survival (Bahr *et al.*, 1998). Noting the differences in male and female colobus cortisol levels will be important to understand the dynamics of stress in Diani. During the dry seasons, the size and quality of the colobus habitat patch may be an important factor in determining colobus cortisol levels and, therefore, colobus fitness. Monitoring the degree and frequency of stressful events by fecal cortisol analysis will help to form more comprehensive habitat and species management plans for the future.

Methods

Field Collection

Initially, six troops were chosen for this study, representing a variety of the habitat sizes and qualities on the coast. The most wild troop, which inhabits the Chale Island *kaya*, was dropped from the study because of the difficulty following and collecting from the group. **Table 1** shows the age breakdown of the five study troops. The initial goal was to collect at least one sample from every study individual once per week. This goal was met, and a total of 230 samples were collected from 7/2/98 to 8/20/98 according to the following method.

Upon location of the colobus group within the forest patch, troop activity was monitored from a distance with 8 x 40 field glasses. Notes on troop behavior and location were made in conjunction with field collections. Collections of fecal samples were made as non-invasively as possible. First, collection was done alone to prevent alterations in colobus behavior from an intimidating human presence in the forest (from a group of researchers). Second, care was taken to move slowly and quietly when entering and moving within the forest, with the head lowered in a submissive posture. Third, fecal samples were not collected until the troop moved to a new feeding location to avoid disturbing the colobus group, unless the troop was in the high canopy (over 20 m). Samples were collected in sterile, 4 oz. Whirlpak bags and carried back to the Colobus Cottage. Contamination of samples from forest litter was not a problem, since dried leaves, seeds, and coral chips were removed before drying and again before laboratory analysis. Only samples where the donor individual could be accurately traced were kept for analysis. A large

charcoal drying oven was made from plywood, metal sheeting, and chicken wire on a 2 x 4 inch wood frame (Picture 5). Fecal samples were dried in the oven (approximately 175-250° F) for 2-3 hours, or until completely dry. Once the samples were dried, they were placed in new Whirlpaks and stored frozen (-15° C) until transported to the U.S.

Laboratory Analysis

Once back in the U.S., samples were again frozen until analysis. Cortisol levels in each fecal sample were analyzed as follows. For a complete account of laboratory procedures, please see **Appendix C**. Due to equipment budget and time constraints, only 76 samples were analyzed by this method, modeled after Diamandis and D'Costa, 1987; and Whitten *et al.*, 1998. All adult male samples were analyzed (64), as were 12 samples from the two adult females in the Mosin group. The average inter-sample interval for all samples was 5.02 days, while the inter-sample interval for males was only 4.85 days.

0.25 g of each fecal sample was ground with a mortar and pestle into a fine powder. Large seeds, coral chips and other inert materials were removed before weighing. The powdered samples were placed in 15 ml, 17 x 120 mm screw cap tubes (Sarstedt Inc., Newton, NC) and extracted with 2 ml of methanol/acetone mixture (4:1). Tubes were vortexed for 1 minute to ensure thorough mixing. The elute was poured into a 3 mL syringe and solid impurities were removed by forcing the elute through a 0.2 μm syringe filter (Whatman Inc., Clifton, NJ).

Picture 5. Author and Bakari Garise drying fecal samples outside the Colobus Cottage.

Picture 6. Men clearing understory at the Tradewinds forest patch (8/17/98).

Reverse chromatography was used to separate cortisol from other extracted materials. C18 Sep Pak VAC columns were used for this purpose (Waters Corp, Milford, MA) with a vacuum manifold which allowed 12 columns to be run at once. Columns were prepared according to the manufacturer's directions. The column was washed with 5 ml water, activated with 1 ml of methanol, and then the sample was loaded on the column. The sample was subsequently washed with 5 ml of water, and then the cortisol fraction was eluted with 2 ml of methanol. Methanol fractions were transferred into glass test tubes and dried under a stream of nitrogen. Samples were reconstituted with 300 µl of dichloromethane, and transferred by pipet into the antibody-coated tubes provided in the radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, CA, Coat-a-Count Cortisol). This assay kit was chosen for its high specificity for cortisol (percent crossreactivity for corticosterone= 0.94%, Cortisone= 0.98%, 11Deoxy-corticosterone= 0.26%, 11-Deoxycortisol= 11.4%, Tetrahydrocortisol= 0.34%) and low inter-assay variation (DPC pamphlet, 7). Samples were analyzed according to the manufacturer's directions. Dichloromethane extracts were dried under a stream of nitrogen in the antibody-coated tubes and reconstituted with 25 µl of human serum and 1 ml of I^{125}-labeled cortisol. Tubes were then covered with parafilm, vortexed for 15 seconds to ensure mixing, and incubated for 45 minutes at 37°C. After the incubation, tubes were emptied into a radioactive waste depository and allowed to drain upside down to dryness. Radioactive counts were done for one minute with a gamma counter (Beckman Coulter, Inc., Fullerton, CA) and compared to a standard curve made

from known cortisol values. Replicate tests and samples that were spiked with a known cortisol concentration had less than 15% variation in the final method.

Stress Index

A habitat stress index was created in order to rank the quality of the habitats for comparison with cortisol values. Six categories were chosen to comprise the index based on a combination of personal observations and literature references. The following categories, listed in order from most stressful to least stressful, were included: colobus/colobus interaction between neighboring groups, number of trees per colobus, human disturbance of habitat, proximity to roads, habitat area per colobus, and residences neighboring the forest patch. Within each category, the study habitats were ranked from 1 to 5, and that rank was multiplied by the weighting factor listed in parentheses to form the overall stress index.

The most stressful factor of colobus' habitat areas in Diani is the proximity of neighboring colobus groups and the frequency of interaction between them. Colobus are territorial, and confined forest areas in Diani bring neighboring groups of colobus in contact with one another on a regular basis. Encounters of this kind are most often agonistic (Patel, 1997; von Hippel, 1998), and display behaviors and chasing between males is likely to be very stressful (**Factor = 0.3**). The number of trees per colobus has been correlated with colobus group size (Dunbar, 1987) and with total food availability (Oates, 1996). The number of trees above 5 m tall is also an indication of the density of the forest canopy, which forms a protective barrier

from sun and aerial predators (**Factor = 0.2**). Human interactions with colobus are limited in several of the study patches. However, human activities in other patches are much more disturbing because of noise or activity. In the Warandale forest patch, colobus flee from Kenyans because the workmen who constructed the cottages within the forest threw rocks at them in the past (**Factor = 0.15**). Diani habitats were also ranked based on their proximity to the main beach road or to a heavily trafficked dirt track (**Factor = 0.15**). The amount of habitat area per colobus is an important indicator of the physical separation from bordering habitat stressors (**Factor = 0.1**). The final category accounts for the number and style of residences, huts, or larger structures which interrupt the forest canopy within the colobus' habitat area (**Factor = 0.1**).

Components of the Stress Index were evaluated from habitat data collected in the field. Ranking each habitat was accomplished by a simple comparison of values, but determining the weighted factors for each of the components reflects personal observations of colobus behaviors and noted impacts of habitat factors (roads, powerlines, etc.) by the Wakuluzu Trust and previous colobus studies. The Stress Index was calculated prior to obtaining cortisol values from laboratory work.

Study Individuals

Samples from 9 individuals were used for this study. All adult males' samples were analyzed. There are two males (an alpha and beta male) at the Colobus Cottage site which are denoted by CCA and CCB, respectively. The male in

the Mosin habitat is called M, and the Warandale male W. In the Tradewinds Hotel forest patch, there are two groups, and three males. The Tradewinds 1 adult male is called TW1, and the alpha and beta males in the Tradewinds 2 group are called TW2a and TW2b, respectively. Samples from two adult females in the Mosin group were also analyzed. One of the adult females gave birth to an infant during the study period. This individual is called Mamom, while the other female is called MAF.

Elevated Cortisol Values

Elevated cortisol values (spike values) were determined by a threshold cutoff after statistical definitions for outlier points were ruled out. The standard statistical means of defining outlier points is a data point which is more than 1.5 times the inter-quartile range away from the mean (Tukey, 44). By this strict statistical definition, only two data points would be considered outliers because of the large variation in the data set. However, spike values of cortisol are not necessarily outlying points. Since stress levels vary throughout a wide range of values, spike levels of cortisol are not necessarily anomalies. Thus, a threshold was determined to reflect the most accurate cutoff of spike levels possible. Ideally, threshold levels would be determined on a per individual basis, since individual variation in cortisol levels are possible. For the purposes of this study however, a comparison of mean values relies on a uniform threshold level. Male cortisol values show a clustering at a low level, with spike levels that are 3 to 6 times the mean basal level.

Thus, for CCA, CCB, M, TW1, and W, the cutoff between basal and spike levels is at 25 µg/dl (1 µg/g dry weight), and this value is used for the study. Literature values are often reported in grams cortisol/gram dry weight of feces since no internal standard exists for fecal analysis. For ease of comparison in this study, concentrations are left in µg/dl instead (25 µg/dl = 1 µg/g dry weight).

Statistical Analysis

Statistical analysis of data was done with Microsoft Excel 98, JMP 3.2, and Prism. A logarithmic transformation was performed on my complete male data set. After the transformation, data were found to be normally distributed with equal variances ($p > 0.05$), and parametric tests were used (One-way Anova, Student's t-test). For the male basal levels, male spike levels, and female levels, non-parametric tests (Wilcoxon and Tukey-Kramer HSD) were used for comparison because the data were not normally distributed.

Results

Adult Males

Cortisol values were obtained by comparing sample radioimmunoassay values with a standard curve generated from known cortisol concentrations. The standard curve which was generated for this purpose shows a high level of accuracy ($r = 0.99$). The cortisol values for male samples ($n = 64$) which were extrapolated from this curve can be seen in **Figure 5**.

Mean cortisol values per individual are significantly different among males (One-way Anova, d.f. = 6, $p = 0.028$). A comparison of means between individuals using a Students-T test found significant differences between six pairs: TW1 and TW2b with each of M, TW2a and W ($t = 2.00$, d.f. = 6, $p<0.05$). Correlation of mean male cortisol values per habitat patch with the Stress Index was moderate ($r = 0.59$, d.f. = 4, $p = 0.29$), and a general association between higher mean cortisol levels in each patch and higher habitat stress can be seen (**Figure 6**). However, it may be more accurate to look at the data set after first categorizing elevated or spike levels of cortisol. Results from each individual show a concentration of values at a basal level with higher, spike values of stress. This threshold value between basal and spike levels is at 25 µg/dl (1 µg/g dry weight) for CCA, CCB, TW1, M, and W adult males, and was therefore used as the spike-level cutoff for all males.

After re-categorizing the data to account for spike values (**Figure 7**), a significant difference exists between mean basal cortisol levels (Wilcoxon/Kruskal-Wallis Test, d.f. = 6, $p= 0.022$). A comparison of mean basal levels between pairs of

Figure 5. Cortisol Levels from 7 Adult males in Study Groups. Mean values among males are significantly different (p = 0.028), as are the following six pairs of individuals: TW1 and TW2 beta with each of M, TW2a, and W (p<0.05)).
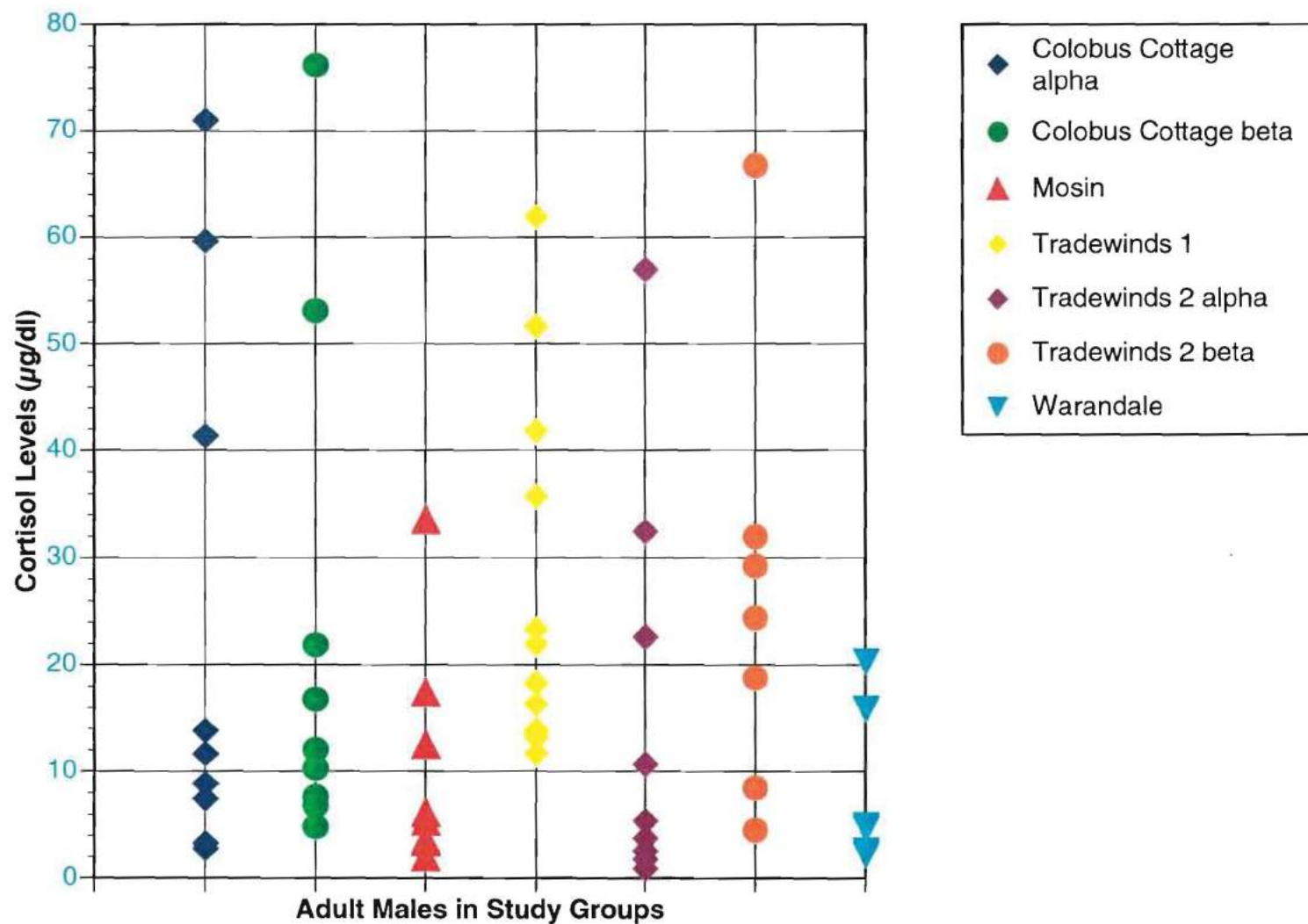
Figure 6. Mean Adult Male Cortisol Values With Respect to Stress Index (r = 0.59). Note that both alpha (**a**) and beta(**b**) males are shown for CC and TW2 habitat patches.
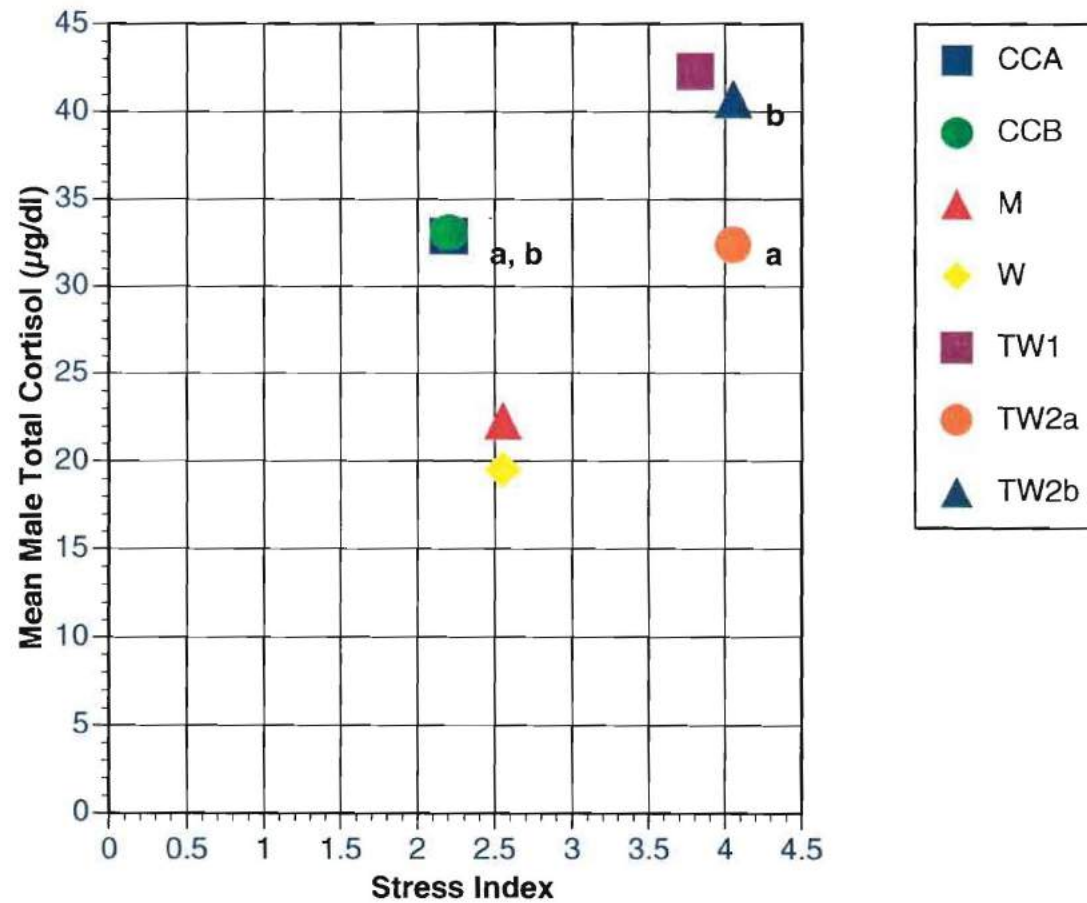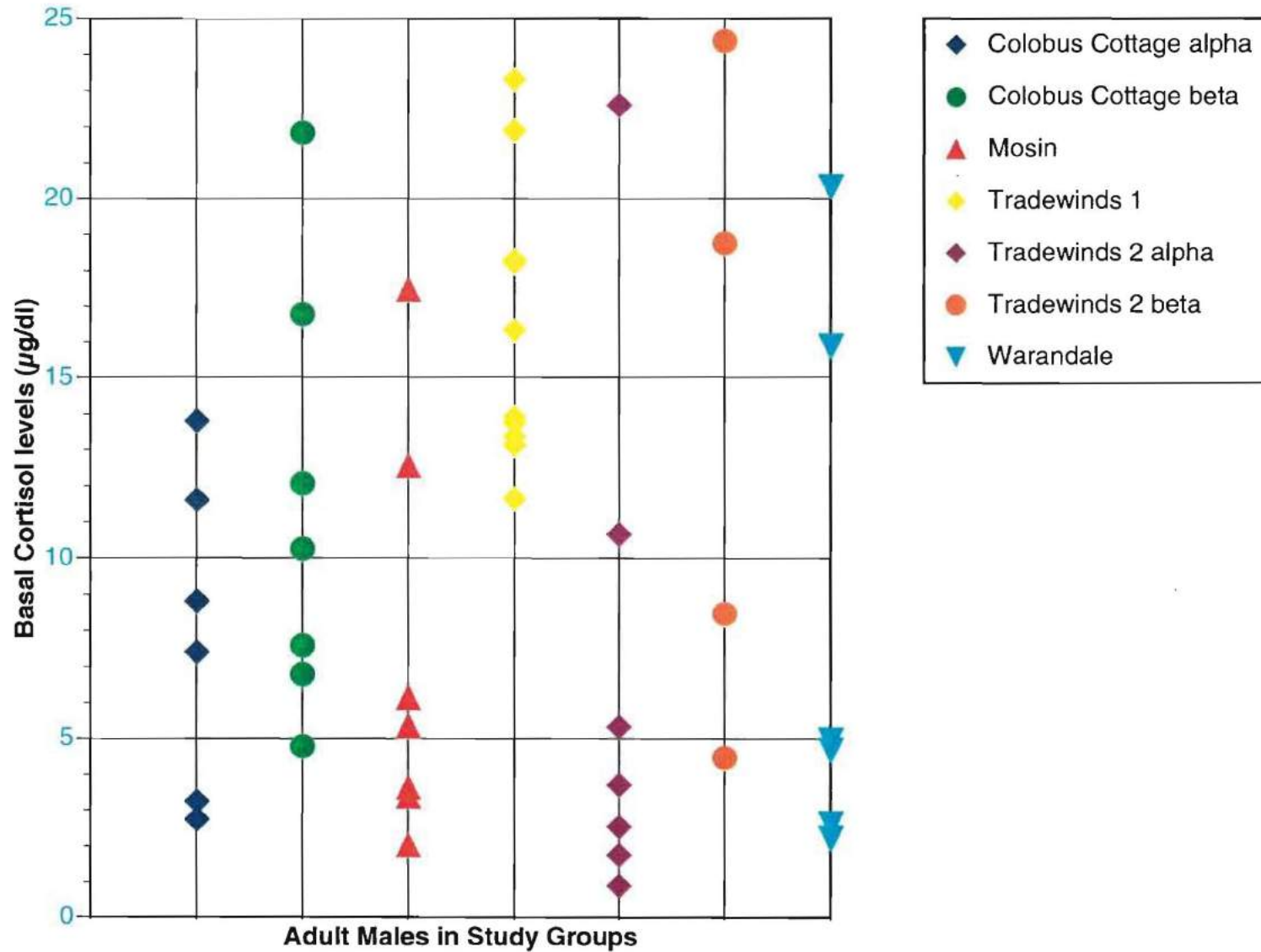
Figure 7. Basal Cortisol Levels in 7 Adult males in Study Groups. Mean basal levels among males are significantly different (p = 0.022), and TW1 and TW2a are significantly different (p<0.05).

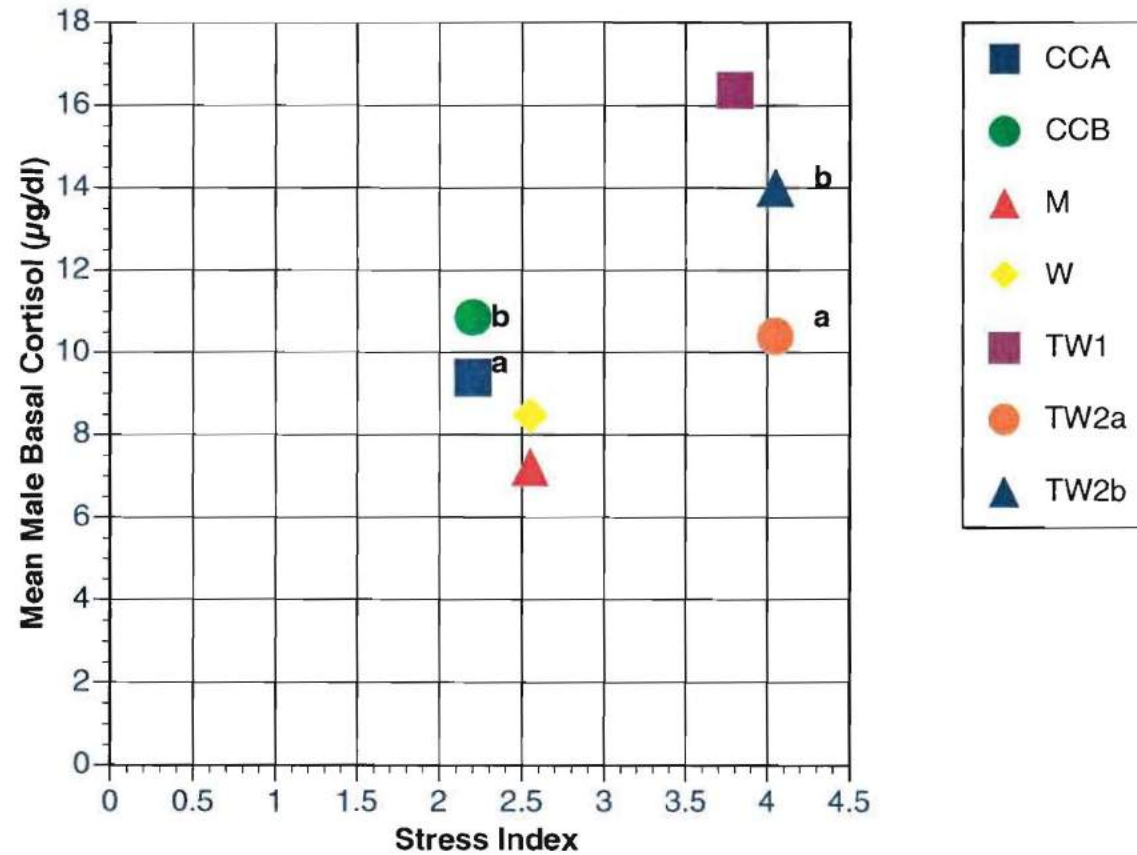males shows significant differences between TW1 and TW2a (Tukey-Kramer HSD, $q = 3.10$, $p<0.05$). In addition, mean basal cortisol levels show a stronger correlation with the Stress Index ($r=0.66$, d.f. = 4, $p = 0.22$) than total mean levels (**Figure 8**). Again, a general trend can be seen within the male data at this point: higher basal cortisol levels are found in habitats with a higher Stress Index rating. When the number of spike values (>25 μg/dl) are compared with the Stress Index, the correlation was also moderate ($r=0.59$, d.f. = 4, $p = 0.28$). The percentage of spike values per total tests (after the data were arcsine-square root transformed) did not correlate well with the stress index ($r = 0.42$, d.f. = 6, $p = 0.34$).

Since the role of alpha and beta males within a colobus troop may be different (Kaspers, 1999), it may be important to compare the five alpha males in the habitat patches. Running the same order of tests, total cortisol levels are significantly different among alpha males (Wilcoxon/Kruskal-Wallis Test, d.f. = 4, $p = 0.035$), but no significant differences exist between pairs of males ($p > 0.05$). Basal cortisol levels are also significantly different among alpha males (Wilcoxon/Kruskal-Wallis Test, d.f. = 4, $p = 0.016$), and two pairs of alpha males are also significantly different: TW1/M, and TW1/TW2a (Tukey-Kramer HSD, $q = 2.89$, $p < 0.05$). When compared to the Stress Index, correlations between both total ($r = 0.22$, d.f. = 4, $p = 0.72$) and basal ($r = 0.4$, d.f. = 4, $p= 0.49$) cortisol levels are not significant. The percentage of alpha male spike values did not correlate well with the Stress Index ($r = 0.33$, d.f. = 4, $p = 0.57$).

Spike cortisol levels in Diani males appear to be linked to stressful situations two days before the sample was collected. The behavior records allow 21/62 samples

Figure 8. Mean Male Basal Cortisol Levels with Respect to Stress Index Values (r = 0.66). Note that both alpha (**a**) and beta (**b**) males are shown for CC and TW2 habitats.

to be judged to be from stressed or non-stressed days. For the remaining 41 tests, insufficient data exists two days prior to sampling to be included in this test. The mean non-stressed value is 7.66 μg/dl (n = 9), and the mean stressed cortisol value is 29.68 μg/dl (n = 12) for this test. When compared per individual (n = 6, CCA could not be used because no non-stressed points were found), stress and non-stress samples are significantly different (Paired t-test, t = -2.83, d.f. = 5, p = 0.037). On this basis, association of stressful events with cortisol values seems valid.

While many variables exist, spike cortisol values were found two days following forest disturbances, interactions with baboons, and mating behavior. For example, on 8/16/98, groundskeepers at Tradewinds Hotel began clearing the underbrush in the forest behind the hotel block (**Picture 6**). For two days, four men with machetes slashed and cleared ground vegetation, forcing both colobus groups and sykes monkeys to relocate to the periphery of their habitat. At one point, both colobus groups were in the same tree. During this incident, colobus appeared skittish (personal observation). All three males showed peak cortisol levels on 8/17 or 8/18, likely in response to this disruption and forced coexistence. At the Colobus Cottage, the local baboon troop displaced the colobus group on 7/19 and 7/21. The highest cortisol level for CCB was on 7/21 (76.1 μg/dl) but CCA was unaffected (11 μg/dl), supporting Kaspers' (1999) theory of task differentiation between males. Mating behavior may also produce high levels of cortisol. TW1 was observed copulating with one of the adult females in his group 3 times on the morning of 7-22. A sample collected on the morning of 7-24 revealed a peak cortisol level of 41.75 μg/dl. W was heard grunting on the morning of 7/29, a sign of territoriality or

stress. For four days prior to 7-29, the Warandale group had been confined to small patch (4 trees) with poor food resources because of disturbances by workmen in the area. A sample collected that day had the highest value for that individual (20 µg/dl), although this level is below the study peak-level cutoff. These associations suggest that spike levels in feces are present following stressful events.

Group Cortisol Trends

Unlike urinary cortisol concentrations, which are highest during the morning, male fecal cortisol values in this study are more randomly distributed (**Figure 9**). Study samples show spike values throughout the day; however, clusters
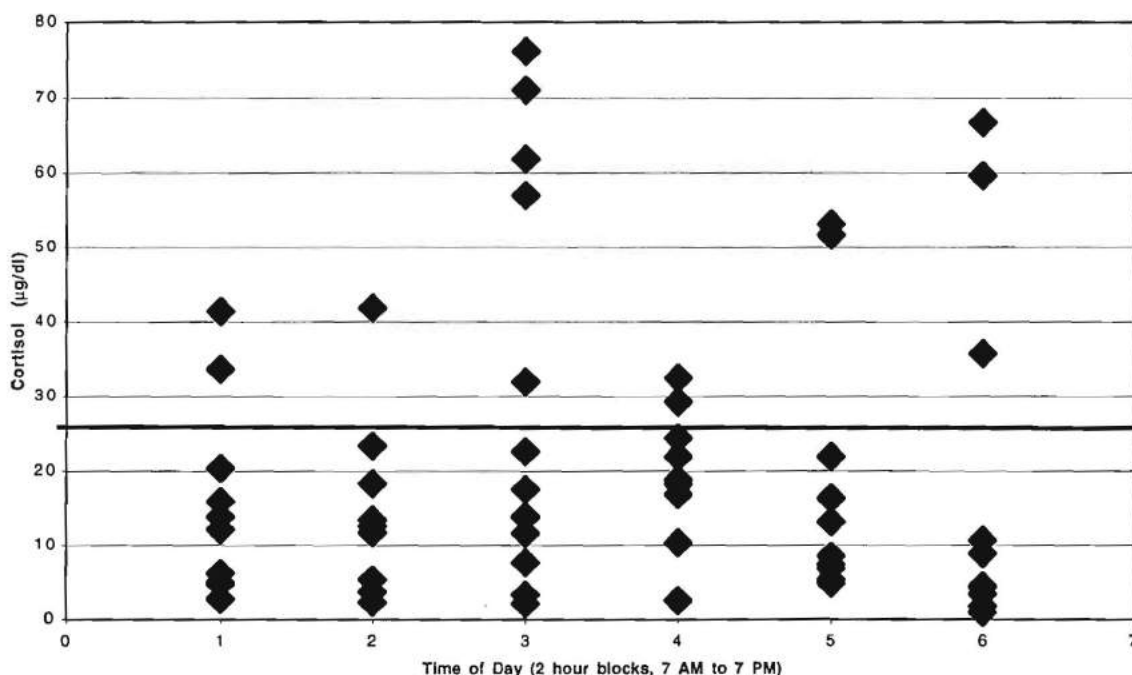

Figure 9. Male Cortisol Values as a function of Time of Day. Horizontal Line at 25 µg/dl is the Spike Value Cutoff. (1= 7:00-8:59 AM, 2 = 9:00-10:59 AM, 3 = 11:00-12:59, 4 = 1:00 to 2:59 PM, 5 = 3:00-4:59 PM, 6 = 5:00-6:59 PM).

of spike values can be seen in the 11:00-12:59 block and again from 3:00-7:00 PM. There are 3 spike values before 12:00 PM (mean = 39.6) and 12 spike values after 12:00 PM (mean = 52.6). These values are not statistically different, but they show an interesting trend towards elevated afternoon cortisol (Wilcoxon Rank test, p = 0.28, Chi-Square = 0.09). In addition, male cortisol does not appear to increase progressively with the length of the dry season, but instead seem variable throughout the study period (**Figure 10**).
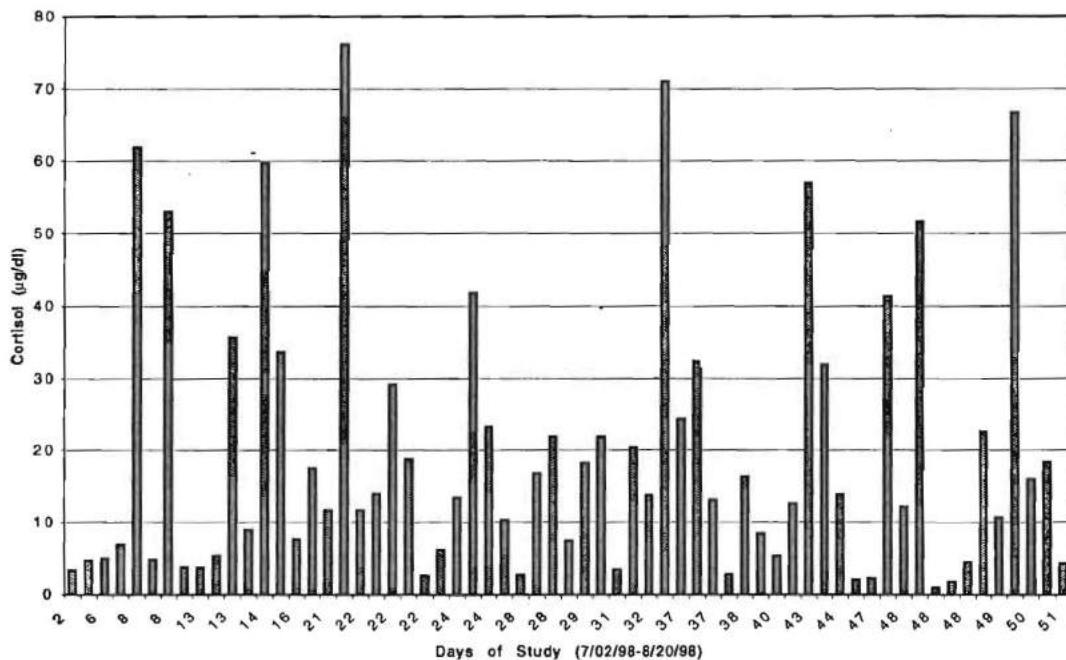

Figure 10. Cortisol values over the duration of the study period. Note that spike levels are randomly distributed, with no evidence of increases as the season progresses.

Adult Females, Mosin Group

Data for Mosin group adult females were analyzed to compare stress levels of pregnant and non-pregnant adults. Female data are displayed in **Figure 11**. Mean cortisol levels between Mamom and MAF are significantly different (Wilcoxon Rank Sums Test, d.f. = 1, p=0.022; Tukey-Kramer HSD, q = 2.20, p < 0.05). Adult female cortisol values were not regressed against the Stress Index because both females are from the same forest patch. MAF's highest stress level was found three days after Mamom gave birth to her infant. Since all female colobus carry infants while they are all white, evidence for non-mother stress associated with an infant in the group is an important insight on this behavior.

Figure 11. Cortisol Values from Adult Females in Mosin Group. Mean cortisol levels between females are significantly different (p = 0.022). **Mamom** is new mother, **MAF** is a non-pregnant adult female.