Integrated non-invasive investigations of captive Abyssinian colobus monkeys (*Colobus guereza*): behaviour, stress and parasitism

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

……………….. Johanna Rabineau

Abstract

The prime aim of the present study was to investigate the welfare of Abyssinian colobus monkeys (*Colobus guereza kikuyuensis)* held in captivity, by behavioural, endocrinological and parasitological approaches. Five groups of colobus monkeys were studied, in the UK at Port Lympne Zoo, Banham Zoo and Paignton Zoo (separate male and female groups) and in France at La Boissière du Doré Zoo.

Overall, the patterns of behavioural activity for captive colobus monkeys agree with observations of wild colobus monkeys, suggesting that there was generally good welfare at all zoos. However, local differences in group composition (i.e. age and gender) or management practices were shown to affect activity budgets. At Banham Zoo, where colobus monkeys had access to a large paddock and food was offered only twice a day, animals spent significantly more time foraging/feeding than other groups which received three meals a day. At Paignton Zoo (adult male only group) animals spent more time resting alone and less time resting socially or being social than other zoos. Generally, across zoos, older, higher ranking animals spent less time moving and more time feeding than younger, more subordinate ones.

Social dominance rank and linearity of the hierarchy in the five groups of colobus was determined. Large mixed groups of colobus monkeys including both males and females displayed strong linear dominance hierarchies, but in single sex groups, the strength of the hierarchy was found to depend on local events and group composition. Generally, male colobus monkeys displayed most of the dominance behaviour, however, females were an integral part of the dominance hierarchy with unidirectional dominant behaviour between both genders in large mixed groups.

Factors such as life history, which may influence social dominance ranking, were investigated. Index of success (another measurement of dominance rank) was mainly explained by age and gender. Social behaviours such as play behaviour and grooming were also associated with ranking. Older, more dominant animals were more often the recipient of grooming behaviour whereas younger more subordinate animals spent more time playing..

Faecal egg counts of the intestinal nematode parasite, *Trichuris trichiura*, were investigated in relation to individual index of success, age, gender, and husbandry practices at each zoo. No significant differences in egg count were observed between genders. Egg count was explained by the index of success and anti-helmintic practices at zoos. The highest count of eggs were observed at La Boissière du Doré Zoo, probably due to the fact animals were locked in for several months in

the winter, therefore favouring re-infection of infective larvae. The lowest count of *Trichuris* egg was at Paignton Zoo female group, who had the largest enclosure, with very low animal density. Counts of eggs were at their lowest in the winter compared to other season of the year. Differences between zoos seem to result from anti-helmintic practices coupled with the influence of enclosure size and husbandry regimes.

Faecal glucocorticoids and their metabolites, in colobus monkeys held in the five groups, were measured by radioimmunoassay and investigated in relation to index of success, levels of aggression, gender, age and reproductive status of females. The overall dominant animals in various groups had higher levels of faecal glucocorticoids. Males had significantly lower faecal glucocorticoid than females, and pregnant females had higher levels than others. The older, higher ranking colobus monkeys initiated significantly more acts of aggression than younger, lower ranking animals. Seasonal variation in faecal cortisol equivalents were observed in the autumn where higher levels were recorded compared to other seasons. Comparison of faecal content of cortisol equivalents of the different groups showed the lowest levels at Port Lympne Zoo and Banham Zoo while the highest levels were at Paignton Zoo (female groups). This suggests that the large multi-male, multi-female groups had the lowest levels of stress and associated high levels of welfare.

These studies lead to the recommendation that single sex groups of captive Abyssinian colobus monkeys should be avoided, particularly when this involves breaking down the social structure of established groups.

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Chapter 1
General Introduction

1.1 The concept of animal welfare

Animal welfare has been a topic of importance in the United Kingdom since as early as 1822 when the first bill in parliament offered protection from cruelty to cattle, sheep and horses. The definition of animal welfare is the subject of much debate, depending on the taxonomy of the species of interest. Essentially, it encompasses the concept that for an animal which is to be used by humans (either as food, research, material or entertainment), no unnecessary suffering, pain or distress should be encountered.

Much development of the animal welfare concept originated from the use of farm animals. In the 1960's, the UK government commissioned Professor Roger Brambell to undertake research into the welfare of intensively farmed animals. The resulting report led to the formation of the Farm Animal Welfare Council in 1979 which highlighted the need for animals to be able to "turn around, to groom themselves, to get up, to lie down and to stretch their limbs". These were the fundamental principles which led to the Five Freedoms (www.fawc.org.uk, FAWC, 1979), as the basis of modern animal welfare:

1) Freedom from thirst and hunger, by having ready access to fresh water and a diet to maintain full health and vigour
2) Freedom from discomfort, by providing an appropriate environment including shelter and a comfortable resting area
3) Freedom from pain, injury and disease, by prevention or rapid diagnosis and treatment
4) Freedom to express normal behaviour, by providing sufficient space, proper facilities and company of the animal's own kind
5) Freedom from fear and distress, by ensuring conditions and treatment which avoid mental suffering.

1.2 Assessing welfare in captive animals

Through evolution, animals become adapted to their natural environment or “niche” (Boere, 2001) and the degree of temporal or spatial variability associated with it (Wiepkema & Koolhaas, 1993). Although some level of stress may be associated with fluctuations in food availability or other seasonal variations in a natural environment and agonistic interactions, animals become equipped to deal with these types of variations, to a reasonable extent.

For good animal welfare to be provided for captive animals there must be appropriate housing and management conditions. Behaviours are performed by animals in the wild in order for them to survive and reproduce, sometimes termed thereafter as natural behaviours (Boere, 2001). In captivity, conditions are by definition different from those in the wild and some adaptation to the captive environment is essential (Wemelsfelder, 1994; Duncan & Fraser, 2000; Mallapur & Choudhury, 2003, Mallapur, 2005). A clear dichotomy between wild and captive behaviour may not necessarily exist if animals are housed adequately. For example, animals in free-ranging enclosure may show different behaviour to those restricted to very small “cages” (Carlstead, 1996). Therefore, welfare can be assessed by the ease with which an animal is able to ‘cope’ (survive) within the environment it lives in. Several methods have been used to assess the extent to which an animal is ‘coping’ within its environment. Physical measurements such as longevity, growth rate, reproduction, body and hair condition can all act as indicators of animal welfare by providing an estimation of health status; following the assumption that animals with poor health also have poor welfare (Clubb & Mason, 2002; Clubb *et al.,* 2008). Therefore, observations of recurrent skin disease or lacerations would be considered signs of poor welfare, and if observed in captive animals an inappropriate captive environment may be considered the cause (Boere, 2001). At the physiological level, poor welfare may be measured through the neuroendocrine response, and other measurements such as heart rate, blood pressure and body temperature (Clarke *et al*., 1994; Aureli *et al*., 1999).

Welfare can be determined through the observation of behaviours performed, where good welfare is associated with the presence of species-specific behaviours, which should be maintained by stimulation and adequate space (Carlstead, 1996). Measuring species-specific behaviours such as social aggressive interactions, maternal behaviour, grooming, foraging or play can therefore provide vital information about the welfare state of an animal (reviewed by Hosey *et al*., 2009). Behavioural studies of captive animals which are managed under different housing conditions can be compared with observations of wild conspecifics, which allow the impact of these captive conditions to be recorded (e.g. Melfi & Feistner, 2002). In some instances, behaviours are recorded in the captive individuals, which are not seen in their wild conspecifics and thus considered to be abnormal and deleterious (Petherick & Rushen, 2000; Mallapur *et al*., 2005). However, there are problems associated with determining animal welfare from wild captive comparisons. Animals in the wild may fight extensively, suffer infanticide or live in temporarily harsh environments with lack of water or food. Comparing levels of welfare in captivity to these potentially stressful conditions in the wild would not provide an accurate assessment. Therefore, long term studies in the wild to collect information over different seasons and local environments is necessary, to provide a true overall insight into the welfare of wild animals (Boere, 2001).

Maintaining a population with the full spectrum of natural behaviours is essential for zoological institutions as most are part of conservation programmes which aim to maintain the genetic and behavioural potential from the founder population (Kirkwood, 2003). If a population becomes adapted to its captive environment, it would be less suited to its original environment if re-introduction became possible in the future (Kirkwood, 2003) and potentially not be able to survive in the wild (Frankham *et al*., 1986). Comparing time spent performing behaviours, using activity budgets of captive and wild animals may be used as a tool to estimate similarities in behaviour between conditions. However, activity budgets may also be affected by management regimes (Melfi & Feistner, 2002).

The performance of abnormal behaviour patterns in captive animals can be described quantitatively or qualitatively. Quantitative abnormal behaviours are behaviours which are typical of animals in the wild, but performed at an abnormal intensity in captivity. For example, in the wild, food must be obtained and an animal may spend a considerable amount of time performing feeding and foraging behaviours to fulfil its energetic requirement. In captivity, however, food is provided by carers at determined times which alters the time allocated by the animals to feeding and foraging behaviours; time spent feeding and foraging by many captive animals is considered to be less than their wild conspecifics (Martin & Kitchen, 1996; Carlstead 1998; Mallapur *et al*., 2005). Additionally, captive conditions may include an inadequate social composition or impoverished environment, a group of animals may spend considerably less time performing social behaviours compared to groups in the wild, while spending more time resting (Broom, 1991; Carlstead, 1996; Kitchen & Martin, 1996).

In contrast, qualitative abnormal behaviours are not normally encountered in the wild. The most common example of this are stereotypies, in which an animal performs a particular behaviour which seems to have no purpose, and occurs in a repetitive and invariant manner, such as pacing, circling cages (Mason & Latham, 2004) or self-mutilation behaviours (Hosey & Skyner, 2007)

Although many types of abnormal behaviour can be used as indicators of bad welfare, surprisingly few specific behaviours are used as a sign of good welfare (Basset & Buchanan-Smith, 2007; Boissy *et al*,. 2007). Good welfare could be characterised by an increase in exploratory behaviours, and an increased range of behaviours (Young, 2003), coupled with a reduced frequency of behaviours indicative of bad welfare (such as stereotypies).

The most thorough approach to assess animal welfare would be to use all of the above (physical, physiological and behavioural) indicators (Mendoza, 1991, Owen *et al*., 2004), but in most studies, a combination of these is employed.

1.3 Stress and its relevance to animal welfare

Stress can be defined as a behavioural or physiological response of the body to a perceived stressor (Selye, 1935). Stressors can be defined as anything which challenge the state of homeostasis that would be maintained in a steady system (Selye, 1976), such as a physical challenge, like exposure to sudden change in temperature or combat, or the threat of a challenge such as a stare from a dominant animal (reviewed by Morgan & Tromborg, 2007). A large variation in stress responses can be observed between individuals and social status (reviewed by Honess & Marin, 2006).

Information regarding the environment (sensory, visual, auditory or olfactory), as well as emotions are received and processed by the hypothalamus. Vasopressin and corticotrophin-releasing hormones (CRH) are secreted by neuroendocrine neurons within the paraventricular nucleus of the hypothalamus. In turn these two hormones regulate part of the pituitary gland and stimulate the secretion of adrenocorticotropic hormones (ACTH). ACTH is then transported through the blood to stimulate the adrenal cortices in the production of glucocorticoid hormones from cholesterol. Glucocorticoids are a group of steroids, which are differentiated from mineralocorticoids and androgens by specifically binding to glucocorticoid receptors. This group contains the steroids of interest in the present study such as cortisol, cortisone, 21-deoxycortisol and corticosterone. Glucocorticoids act as a negative feedback on the hypothalamus suppressing CRH and on the pituitary to suppress ACTH (Padgett & Glaser 2003),

At a physiological level, stress is traditionally estimated through the adrenocortical response by measurement of cortisol, corticosterone or their metabolites: reviewed (Munck *et al*., 1984; Oliverio, 1986; Romero, 2004); in plasma (Shampous *et al* 1993; Johnson *et al*, 1995; Holekamp & Smale, 1998); faeces (Bahr *et al*., 2000; Schatz & Palme, 2001; Young *et al*., 2004); urine (Touma *et al*., 2003; Muller *et al*., 2004, McCallister *et al*., 2004; McCusker *et al*., 2004); saliva (Umeda *et al*., 1981; Kuhar *et al*., 2005); or hair (Koren *et al*., 2001). However, for accurate representation of stress levels, the identification of the animal producing the samples must be known and a strict protocol of sample collection must be followed as corticosteroids have been shown to follow circadian rhythms in some species (De Jong *et al*., 2000; Touma *et al*., 2003; Szeto *et al*., 2004) or to be affected by activity patterns (Touma *et al*., 2003).

Corticosteroids may improve fitness by energy mobilisation (Möstl & Palme, 2002) by increasing the availability of glucose in the bloodstream and promoting glucose production, while limiting their uptake and storage in tissues (Nelson, 2000) or by regulating the inflammatory response after injury (Morrow *et al*., 2002). Prolonged secretion of corticosteroids (often resulting during chronic stress) may on the other hand have deleterious effects, for example by causing immuno-suppression and thereby increasing the risk of disease (Balm, 1999), by causing tissue atrophy (Mason *et al*., 2008) or by changing gastrointestinal functions (Monnikes *et al*., 1994). Elevated glucocorticoid levels associated with chronic or excessive stress have been linked with abnormal stereotypic behaviour (Mason, 1991, Carlstead, 1998; Liu *et al*., 2006) and are therefore representative of poor welfare (Swaisgood, 2007).

The immune response is modulated by the central nervous system (CNS) in connection to the nervous, endocrine and immune systems (Padgett & Glaser 2003), as shown in Figure 1.1 (from von Borell, 2001). Stressful events can have an adverse effect on immunological mechanisms by affecting the natural balance of regulatory systems, leading to increased susceptibility to infection, inflammation, autoimmune disease, and therefore health (e.g. Kiecolt-Glasser *et al*., 1996; Glasser *et al*., 2000). For example, stress hormones have been shown to inhibit the trafficking of macrophages, antigen-presenting cells, natural killer cells, T and B lymphocyte, as well as suppress the production of proinflammatory cytokines and chemokines (reviewed by Padgett & Glaser, 2003).

Measuring the susceptibility to parasitic infections by counting parasite eggs in faecal samples has been used as a non-invasive measure of welfare (Melfi & Poyser, 2007) as it is expressed before pathological consequences are manifest (Swaisgood, 2007).

Fig 1.1: Mode of communication between the central nervous system (CNS), the endocrine system and the immune system, CRH is corticotrophin releasing hormones, from von Borell, 2001.

1.4 Factors affecting stress and welfare in captivity

In captivity, many factors such as new housing arrangements or physical restraint (Clarke *et al*., 1994), sounds (e.g. Tromborg & Coss, 1995, Birke, 2002), and light conditions (Ikeda *et al*., 2000; Grandin & Johnson, 2005) have been demonstrated to initiate behavioural and physiological stress responses.

Cage size may be an important factor in determining welfare. However, the way a space is constructed or filled with platforms, perches, and any age specific requirements is important (Honess and Marin, 2006). The complexity of the environment, maximising the space usable to animals, is a vital factor in determining the quality of enrichment (Kitchen & Martin, 1996). For example, colobus monkeys (*Colobus guereza*) are highly arboreal species found in the high tree canopy (Estes, 1991), therefore complex enclosures with trees or perches in a vertical dimension are required to simulate their natural environment and thus provide them with opportunities to perform natural behaviours (Kitchen & Martin, 1996; Boere, 2001; Mallapur *et al*., 2005, Mallapur, 2005). Similarly, covering the floor in different substrates such as leaves, bark chips or wood shavings can reduce the smell of excrement and urine and is visually pleasing, providing a more natural-looking, enriched enclosure, which can have long lasting effects on behaviour (Maple & Finlay, 1989, Hosey *et al*., 2009).

Housing conditions in relation to how they influence social interactions is a factor of importance in non-human primate welfare. Many studies have investigated the impact of long- term single housing on social primates (Marriner & Drickamer, 1994; Mootnick & Baker, 1994; Mallapur & Choudhury, 2003), and the effects of inappropriate group composition for a given primate species, both of which are associated with an increased performance of abnormal behaviours and decrease in normal behaviour patterns (Buchana-Smith, 1996). These abnormal behaviours (such as self injury or stereotypy) are very rarely observed in free-ranging primates or those housed in groups (Mallapur & Choudhury, 2003, Mallapur *et al*., 2005).

During infancy, the first and most important social contact for a primate infant is with its mother, and animals raised naturally by their mother tend to develop normal behaviour patterns (Anderson & Chamove, 1985; Mason, 1991). In colobus monkeys, all animals in the group may participate in infant rearing and handling (Estes, 1991).

Therefore, social contact with other conspecifics in play or grooming, is particularly important in developing the appropriate social skills of colobus monkeys, and will be determined by the formation of the captive group.

Enlarging an established group of primates or merging two groups together can have an adverse effect on the welfare of primates in captivity (Honess & Marin 2006). Alberts *et al*. (1992) found that cortisol levels of a group of yellow baboons (*Papio cynocephalus*), and particularly females, significantly increased after the immigration of an aggressive male. The immigrant animals also had higher levels of plasma cortisol after joining the group. However, the stress response of the enlarged group can attenuate with time, as was observed in a newly-formed large group of female long-tailed macaques, where levels of cortisol were found to continuously decrease at 4.5, 7.5 and finally 11 months following group formation (Stavinsky *et al*., 2001)

Group instability is another factor which may affect stress and welfare. In olive baboons, *Papio anubis*, the basal cortisol levels of dominant animals increased during instability, although they showed lower response to stress (Saplosky, 1983; 1992; 1995). Group instability may result after animal relocation between groups or any other alteration to group structure (such as removing animals), where affiliative bonds are severed (Boere, 2001).

In a zoo environment, animal welfare may be affected by the close proximity of numerous human visitors (Davis *et al*., 2005). For example, Wells *et al.* (2007) found that during low visitor density, zoo-housed gorillas (*Gorilla gorilla gorilla*) showed more relaxation behaviour, such as resting, while at high visitor density, more intra-group aggression, stereotypies and auto-grooming was observed. Similar negative impacts of visitors have been observed in many other studies of primates (Birke, 2002; Skyner *et al*., 2004; Davis *et al*., 2005). This highlights the need for adequate housing facilities with possible hiding places where animals are out of sight.

1.5 *Colobus guereza:* The Eastern black-and-white colobus

Eastern black and white colobus (*Colobus guereza;* Rüppel, 1835) are part of the Cercopithecidae family and the Colobinae subfamily. Colobines are a specialized type of folivorous monkey without cheek pouches but with sacculated stomach, and are found throughout Asia and Africa. Most of the early research on *Colobus guereza* was performed in the wild to collect data on their ecology, social structure and life history. Many of these studies date as far back as the 1960's and 1970's (Ullrich, 1961; Schenkel & Schenkel-Hullinger, 1967; Marler, 1969; Clutton-Brock, 1975; Oates, 1977 and reviewed by Baldwin *et al*., 1976). However, more recent studies have now extended the knowledge of *C. guereza* ecology (Krüger *et al*., 1998). Current studies have followed many of the same populations of *C. guereza* in certain parts of Africa, for example the populations in the Kakamenga Forest, Kenya (Fashing 2001a; 2001b; 2002; 2004) and Kibale Forest, Uganda (Harris, 2006; Harris & Monfort, 2006, Chapman *et al*., 2005; 2007). These monkeys are diurnal and arboreal, rarely descending to the ground, unless necessary to clear a gap in the tree.

1.5.1 General physical description

C. guereza are easily identified by the black and white colour of their coat. Most of the body is covered with black hair and there is a U-shaped ring of long white fur descending from their shoulders and around their back. Their face is grey with no fur and surrounded by a white beard. The top of their head is capped by two black domes of fur, which is more prominent in males than females (Photos 1.1-1.4). Another identifying feature is the presence of prominent ischial callosities that are separate in females and contiguous in males.

C. guereza are slightly sexually dimorphic primates, where males are larger and heavier than females. Adult males average 14.5 kg (but can be up to 23 kg, Kingston, 1971) whereas adult females weigh between 6.2-9.2 kg. The head and body length averages 59.3 cm in males and 55.4 cm in females. Colobus' tails are long, with an average length of 79 cm but vary in colour and shape between sub-species. For example, *C. guereza guereza* have mainly black, short-haired tails with a small amount of white at the tip, forming a pompom. However, the Abyssinian colobus (*Colobus guereza kikuyuensis*), have white hair spanning almost the entire length of the tail, with only a small amount of black hair visible at the base.

C. guereza live up to 20 years in the wild but have been known to leave up to 29 years in captivity (Hakeem *et al*., 1996)

Pictures 1.1: ***Colobus guereza***

1.5.2 Distribution

Eastern black and white colobus monkeys have a widespread distribution across central Africa which ranges from Nigeria in the West, to Ethiopia and Kenya in the East and from the Democratic Republic of the Congo in the South, to Sudan in the North. Other countries within this range include the Eastern Mountains of Uganda, Tanzania, the Republic of Congo, Gabon, Cameroon and the Central African Republic. *C. guereza kikuyuensis* has only been observed from the Ngong Escarpment, Mount Kenya and the Aberdare Mountain Range, Kenya (Kingdon *et al*., 2008).

1.5.3 Habitat

Eastern black and white colobus monkeys inhabit gallery, primary, secondary and swamp forests, bamboo thickets and isolated patches of trees. They are known to live in lowland, or up to 3,300 m high, from scrub forests to rainforests (Estes, 1991). But they are most abundant in secondary forest and in riparian environments. This species spends most of its time in the high canopy with small home ranges estimated to be between 15 and 16 ha (Estes, 1991). Smaller home ranges have been observed in groups with high animal density (Dunbar, 1987); home ranges as small as 1.7 ha and up to 6.2 ha have been measured for *C. guereza* (Krüger *et al*., 1998). Some overlap between adjacent groups is observed, with overlap as high as 80% in some cases (Krüger *et al*., 1998). However, the core part of the home range is defended in a territorial manner and the resident group chases other groups away, although not so as to permanently exclude them (Harris, 2006). Adult males use a loud 'roar', particularly in a morning chorus to advertise their home range to adjacent troops and maintain territorial spacing (Marler, 1969).

1.5.4 Diet & digestion

Colobus monkeys consume primarily large quantities of leaves (2-3 kg a day), sometimes rich in cellulose and often containing toxics secondary compounds such as alkaloids and phenolics. Young leaves are preferred over mature, tougher ones, (Oates, 1978), although *C. guereza* is capable of feeding for months on fibrous mature leaves without drinking (Clutton-Brock, 1975; Estes, 1991). Other parts of the diet include fruits, seeds, twigs and flower growth (Fashing, 2004).

C. guereza is typical of colobine species in having a highly adapted digestive system. Colobus monkeys have a tripartite stomach divided into the saccus gastrichus, the tubus gastricus and the pars pylorica. The vegetation consumed, high in cellulose, is retained long enough in this enlarged stomach to undergo fermentation by anaerobic bacteria (Estes, 1991). These bacteria also facilitate detoxification of leaves, fruits and seeds before digestion. The colobine fore-stomach has a high pH, measured at 6.5 and 7 in two animals in captivity (Kay *et al*., 1996), to sustain the bacterial population. Soil consumption has also been observed in some *C. guereza* (Oates, 1978). This behaviour was suggested to favour the intake of minerals in diets deficient in sodium, copper, manganese and zinc, while facilitating absorption of plant toxins and adjusting the pH of the fore-stomach (Oates, 1978).

Oates (1978) recorded that 50% of *C. guereza's* diet in Uganda's Kibale Forest originated from only one tree species, *Celtis durandii*. Clutton-Brock (1975) also observed groups of *C. guereza* feeding exclusively on mature leaves of two tree species for extended periods of time. Fashing (2001b) estimated that the African cherry tree (*Prunus africana*) accounted for 30-50 % of the diet of two groups of *C. guereza* in the Kakamega Forest, Kenya, over several months. Due to their diet of tree leaves, the home ranges required to support colobus population are relatively small. This allows *C. guereza* to occur at a high density in relatively small areas. For example, Krüger *et al*., 1998 reported a density of 347 animals per km^2.

1.5.5 Life history

Female Eastern black and white colobus show no external sign of oestrus and have an ovarian cycle of 24 days (Harris & Monfort, 2006). Copulation may be solicited by both sexes (Harris & Montfort, 2006). Females elicit copulation by presenting their ano-genital region to males (Estes, 1991), while males present an erect penis to a female and/or touch the female ano-genital region or rump area (Harris & Montfort, 2006). Copulation may occur at any time in the year, although in the wild, most births occur in the rainy season (Oates, 1977). A female may give birth every 19-24 months (Harris & Monfort, 2006). A single white offspring is produced after a median gestation of 158 days (Harris & Monfort, 2006), gradually taking on the adult colouration, at about three months. The newborn infants are handled by the mother and other members of the group, mainly females and more rarely by males (Horwich & Wurman, 1978; Estes, 1991). Handling of very young infants in this manner is an unusual feature among primates (Oates, 1977; Horwich & Wurman, 1978). When the infant acquires its adult colouring, attention from other members of the troop dramatically diminishes, or completely ceases. Infants as young as two to four months old start eating leaves in small amounts, and play and explore away from their mother. By the time they reach six months old, infant colobus monkeys become largely independent from their mother, although they will still suckle and associate with their mother during rest or while travelling, until they reach 10 months old. Young colobus over a year old are classified as juveniles and only associate with their mother during rest (Oates, 1977). Juvenile males mature at six years old, while females mature younger, at four years old (Oates, 1977).

1.5.6 Behaviour

The Eastern black and white colobus monkey is a highly territorial species, which defends its relatively small core territory in an aggressive manner towards neighbours. The common group structure of *C. guereza* includes one territorial male who lives with a group of related females and their offspring (Dunbar & Dunbar, 1974; Estes, 1991; Krüger *et al.*, 1998) forming troops of between 6-10 animals (Dunbar & Dunbar, 1974; Oates, 1977; 1978; Harris & Monfort, 2006). However, more recent studies have observed larger groups of *C. guereza* composed of between 13 (Krüger *et al.*, 1998) and 23 animals (Fashing, 2001). Several males have been observed to cohabit in mixed groups (Oates, 1977). However, as a rule, one of the males would be dominant over the other males, with exclusive access to females (Oates, 1977, Harris & Montfort, 2003). The number of males in a colobus group has been shown to be a function of the number of females in it, and multi-male groups were shown to have lower reproductive rates than one-male groups (Dunbar, 1987). *C. guereza* have at matriarchal society where males disperse from the group as sub-adults, while females normally remain with the natal troop (Estes, 1991). As a result, small all male groups have been observed (Marler, 1969) but only as a transitory phase when several males may associate, and often remaining in the periphery of existing groups, waiting to join or take over these groups (Oates, 1977).

Inter-group competition has been observed around food resources in several groups of *C. guereza*, notably in the Kakamega Forest, Kenya (Fashing 2001a) and Kibale Forest, Uganda (Harris, 2006). Within the troop, individuals are considered to be relatively peaceful and placid, with low levels of intra-group aggression (Estes, 1991). Most colobus spend their time resting, particularly in the morning when sunbathing behaviour and grooming is performed (Estes, 1991). The second activity taking up most of the time is feeding and foraging

Social grooming is often observed to account for as much as 6.2 % of all activity, with females and juveniles as the main performers of grooming behaviour, while the dominant male of the group rarely grooms others and receives little in return (Oates, 1977).

The oldest female in a group has been observed to determine the movement of the group and the dominant male generally initiates engagement in aggressive encounters (Estes, 1991).

C. guereza move through the forest quadrupedally (Fleagle, 1988) but can also be observed leaping long distances. This is done to cross particular large gaps in the canopy, to avoid predators or in territorial display by dominant males (Estes, 1991).

1.5.7 Conservation of colobus monkeys

The Eastern black-and-white colobus monkey was listed in the IUCN red data list in 2008 by Kingdon *et al*. as 'Least Concern'. However, some sub-species, such *as Colobus guereza percivali* are classified as endangered. Although restricted to a limited range, *C. guereza* remains abundant in many protected areas, with threats impacting at a local level. It is not thought to be declining fast enough to be categorized in a higher class of threat (Kingdon *et al*, 2008). *C. guereza* is listed on Appendix II of CITES and on Class B of the African Convention on the Conservation of Nature and Natural Resources. *C. guereza* is traditionally regarded as one of the few species relatively resistant to habitat degradation (Fashing, 2002), sometimes found in high densities in fragmented forest (Schenkel & Schenkel-Hulliger, 1967) or with increasing populations (Fashing, 2002). However, recent studies suggest that forest fragmentation may affect *C. guereza* population in an adverse manner (Fashing, 2004) and. Chapman *et al*. (2003) reported a 28% decline in a *C. guereza* population in fragmented forest in Kibale National Park, Uganda, over a five year period.

The main threat to *C. guereza* species is from hunting (especially in the Western parts of its range) and habitat loss through deforestation for timber, conversion to exotic forest plantations and conversion to agricultural land (von Hippel *et al.* 2000).

Human population is forever expanding, having a large impact on other animal species, often competing for space and limited resources (Kirkwood, 2003), so encroachment is inevitable, and requires the intervention of conservation strategies (Swaisgood, 2007). Captive-breeding in zoological institutions is a temporary solution, available until threats are controlled, and re-introductions possible. Zoological institutions have an essential role maintaining and managing the genetic diversity, through the use of studbooks, and by performing translocation between populations (Swaisgood, 2007).

Maintaining animals in zoological institutions has conservational value as well as educational and recreation value. Zoos provide an invaluable tool to perform research on many different species that would otherwise be difficult to observe, increasing the level of information available and allowing comparative studies between zoos (Melfi, 2005). Research in zoos has become an essential tool to better understand welfare and animals need in captivity, as well as to prepare captive-born animals for possible release to the wild (Vickery & Mason, 2004).

A third of all primate species are in danger of extinction, therefore understanding the ones kept in captivity is crucial, and any knowledge gained could improve conservation efforts (Melfi, 2005). However, in holding animals of conservation value in captivity, high standards of husbandry and welfare providing the physical and psychological needs of animals has become paramount (Melfi & Feistner, 2002; Hosey *et al*., 2009; Melfi *et al*., in press).

1.6 Aims

In the present study, one of the eight subspecies was investigated, *Colobus guereza kikuyuensis*, the Abyssinian colobus monkey *(*referred to as *Colobus guereza* or colobus monkey in the remainder of this thesis). *C. guereza* is a common species in zoological institutions, but there have been a surprisingly limited number of studies on their behaviour, physiology and welfare in captivity. Therefore, the prime aim of the present studies was to investigate the welfare of colobus monkeys in captivity by behavioural, endocrinological and parasitological approaches. Four zoos (five groups) of colobus monkeys were studied in the UK and France. The largest group was at Port Lympne Zoo, (UK) with 10 animals (five males and five females) spanning all ages from infant to adults. Port Lympne Zoo was visited seven times over a two year period (from March 2005 until October 2006). Banham Zoo (UK) was composed of seven animals during the first data collection period in March 2005 (two males and five females) and nine animals in October 2005 (three males and six females) also covering all ages from infant to adults. La Boissière du Doré Zoo (France) was composed of seven animals in March 2006 (five males, two females) and eight animals in November 2006 (six males, two females) with all age categories. Finally, two groups of colobus monkeys were observed at Paignton Zoo (UK). One group contained between three and four adult males and was visited three times in August 2005, March 2006 and October 2006. The other contained between four and six animals, all being adult females apart from a single juvenile male, and was also visited three times in August 2005, March 2006 and October 2006.

In Chapter 2, activity budgets were created to compare time allocation of the colobus monkeys to various behaviours in the different zoos. Seasonal patterns of the time spent performing each behaviour was investigated between groups, and the influence of gender, age and index of success was analysed. Finally, the mean activity budget of animals in captivity was discussed in comparison to those of colobus monkeys in the wild.

Chapter 3 examines the social dominance rank and linearity of the hierarchy in the five groups of colobus investigated. Data collected on social behaviour were analysed in connection to group structure (i.e. gender and age) and their impact on index of success (another measurement of ranking).

Chapter 4 reports on the results of faecal sample collection from the groups and was used to investigate how faecal egg counts of the parasite *Trichuris trichiura* related to the different aspects of social structure, group size and husbandry practices. *Trichuris trichuria* egg counts were investigated in relation to index of success, age, gender, season (and various variables linked to weather patterns) and anti-helmintic practices.

Chapter 5 examines the faecal glucocorticoids and their metabolites in colobus monkeys held in groups with variable social compositions (multi-male, multi-female; single male, multi-female; single female, multi-male, all male and all female). Faecal glucocorticoid content was compared between zoos and the factors affecting the variations (such as index of success, age, levels of aggression, and the effects of female reproductive status) were assessed

Finally, chapter 6 discusses overall results in relation to the welfare of colobus monkeys in captivity.

Chapter 2

Activity budget of *Colobus guereza*

2.1 Introduction

As time itself is limited, it becomes a factor affecting the amount of time available to carry out behaviours, and thus affects the choices animals make and their activity budget (Dunbar, 1988; 1992). Animals need to proportion their days, regardless of any limiting factors, to find enough food to fulfil their nutritional requirement to survive, maintain good body condition and develop, as well as reproduce. Time allocation to various activities, which cannot be performed simultaneously (activity budget), can give an overview of an animal's circumstances and be used as a tool to infer what factors affect behaviour. Studies of activity budgets can provide a basic understanding of an animal's interaction within a group and which behaviours are prioritised, based on species characteristics and life history. Information from activity budgets can then be used to compare behaviour between different groups with varying social composition and observe changing patterns of behaviour over time.

Activity budgets, as a measure of time spent performing various pre-defined behaviours, have often been used in ecological studies to investigate how a wide range of factors (both intrinsic and extrinsic) affect behaviour. These studies have included primates, such as the woolly monkey (*Lagothrix lagotricha poeppigii*), where time allocation patterns were used as a tool to explore foraging strategies, intraspecifically across their geographical range, and interspecifically compared to other species sharing similar habitat (Di Fiore & Rodman, 2000). Other activity budget studies, investigating extrinsic factors, have looked at the impact of food resource, distribution and seasonal variation and differences in food quality and abundance in a variety of primate species (reviewed by Chapman & Chapman, 2000) and specifically in the vervet monkey, *Cercopithecus aethiops* (Isbell & Young, 1993), the lion-tailed macaque, *Macaca silenus* (Menon & Poirier, 1996) or the Eastern black and white colobus, *Colobus guereza*, (Oates, 1977; Fashing, 2001 a,b,c).

Much of the data available on colobine activity budgets comes from studies of wild animals and includes a range of species, such as Western black-and-white colobus (*Colobus polykomos,* Dasilva, 1992), proboscis monkey (*Nasalis larvatus*, Boonratana, 2000), snub-nosed monkey, *Rhinopithecus roxellana*, (Kirkpatrick, 1998), François' langur, *Trachypithecus francoisi*, (Zhou *et al*., 2007) or black colobus (*Colobus satanas*, Fleury *et al*., 1999). The studies of most interest to the current research are the ones undertaken by Fashing (2001 a,b,c) and Oates (1977) in which activity budgets of wild *Colobus guereza* were recorded at different study sites in Africa. In Kibale forest, Uganda, *C. guereza* were recorded to spend up to 57 % of their time resting (Oates 1977), whereas, in Kakamega forest, Kenya, they spent more than 64 % of their time resting (Fashing, 2001). These results are similar to those found for other colobines, who also spend a large percentage of their time resting. For example, *Colobus polykomos* rest for up to 61 % of the time (Dasilva, 1992). The large proportion of time resting has been suggested to be due to the specialised diet of colobines (Oates, 1977; Dasilva, 1992; Fashing, 2001). Colobus monkeys differ from other Old World monkeys (sub-family cercopithecines) as they lack of cheek pouches and sharp, shearing crested molars (Strier, 2000) but have a large sacculated stomach divided into several compartments (Napier, 1985). This stomach is filled with bacteria, which facilitates fermentation and production of volatile fatty acids in the fore-gut (Ohwaki *et al*., 1974). The large proportion of time spent resting facilitates digestion (see Chapter 1, section 1.5.4). This is in marked contrast to other non-colobines, and other less nutritionally specialised species such as the mainly frugivorous woolly monkeys (*Lagothrix lagotricha*) which spend as little as 29.9 % of their time resting, 25.8 % of the time foraging, and up to 38.8 % of their time moving (Defler, 1995). Colobine monkeys follow the typical behavioural profile of folivorous animals adapted to a diet low in available energy, by displaying limited locomotion, social behaviour is generally low and long periods of rest are taken during the day to limit energy expenditure (Milton, 1983).

By definition, diurnal species perform 'active' behaviours largely during daylight hours during which, nutritional requirements must be met, therefore restricting their activity budgets. As the amount of time spent on an activity increases, the amount of time spent on others consequently reduces. Dunbar & Dunbar (1988) suggested a model based on gelada baboons (*Theropithecus gelada*) to meet the increased nutritional needs that animals may have during different life stages or events. As additional feeding time is required, animals reallocate the time they spent performing behaviours by preferentially reducing resting time, and then, if necessary, by reducing time spent in social interactions. Therefore, in periods of high food availability, when nutritional demands can be met quickly, one would expect resting time and social activity to increase. This is supported by the work of Defler (1995) who observed a significant increase in social activity of woolly monkeys (*Lagothrix lagotricha*) in the Colombian Amazon, when food supply increased.

Primates are highly social animals and as they live in a collective structure, time must be spent in establishing and maintaining social relationships that decrease the chance of conflict (Di Fiore & Rodman, 2000). In turn, group size will affect animals in terms of their access to resources and mates (Dunbar, 1992). As more animals are present in a group, competition for food may increase (Isbell, 1991). Subordinate animals have been observed to eat less than more dominant animals (van Schaik, 1983), but this may vary widely depending on the type of food available, food preferences and social structure (reviewed by Isbell, 1991). Frugivorous species (such as the woolly monkey, *Lagothrix lagotricha*) may suffer from higher intra-competition for food, as the availability of fruit is discrete and temporal. A clear relationship between the position in the social dominance hierarchy and access to these resources may exist, as fruits tend to be distributed in clumps and dominant animals would be expected to have priority access (Isbell, 1991).

Activity budgets allow investigation of the associations between group size and time spent moving or resting (Isbell & Young, 1993), in relation to their food preferences. Chapman & Chapman (2000) introduced the ‘ecological constraint model’, which suggests that as group size increases, so would the distance travelled to find adequate food supplies, which would be reflected by the increased time allocated to moving behaviour. O’Brien & Kinnaird (1997) constructed activity budgets for three groups of Sulawesi crested black macaques (*Macaca nigra*) in Sulawesi, Indonesia. The largest group moved further during the day, rested more, and socialized less than smaller groups. Daily movement was correlated with diet; the macaques moved shorter distances as the proportion of time spent feeding on fruit increased. However, food, in the form of leaves, may not be a limiting factor determining the activity budget of folivorous animals as leaves are dispersed and abundant. The availability of leaves should therefore, not affect day range (i.e. movement) as a function of group size (Isbell, 1991) or lead to the creation of a dominance hierarchy (McKenna, 1979). A study compared three groups of wild ursine colobus (*Colobus vellerosus*) who lived at the same study site and shared the same habitat and diet (Teichroeb *et al*., 2003). No effect of group size on time spent feeding, resting and moving was found in any of the groups studied.

However, even for folivorous animals, the abundance of leaves and their quality may vary over time and affect time allocation to behaviours to ensure sufficient energy is gained to fulfil vital requirements. In addition, it should be noted that some folivorous animals consume other food items which may be scarce and discreetly distributed, such as seeds, fruits or flowers (Dasilva, 1992; Guo *et al*., 2007). The availability of these items may lead to competition when they are rare and increase movement to reach them, regardless of group size. Colobines can display an active foraging strategy, increasing the time spent foraging during periods of food shortages. Alternatively, during low food availability, some colobine species can adopt a passive foraging strategy, spending less time moving, foraging and feeding (Oates, 1994). Guo *et al*. (2007) found that snub-nose monkeys spent the least time moving when food quality was at its lowest. Data are therefore contradictory about how different species react to decreased food availability, even within the colobine family.

In the wild, activity budgets often show some form of seasonality that mirrors food abundance and/or quality. For example, Francois' langurs spent significantly less time resting and grooming in the dry season (when food was scarce) compared to the rainy season (Zhou, 2007). Conversely, snub-nose monkeys spent more time moving in the autumn, when the food quality was at its highest, and less time moving in the winter when at its lowest (Guo *et al*., 2007). However, other studies have also shown no significant difference in activity budgets across seasons. For example, Li & Rogers (2004), found no significant seasonal change in activity budget of white-headed langurs (*Trachypithecus leucocephalus*) in Fusui, China in four types of habitat, ranging from high to low quality.

Disparity between sexes may be expected regarding the time allocation animals may spent on various activities. For example, in three groups of varying size and social composition of ursine colobus, the activity budgets varied between sexes, and according to group size (Teichroeb, 2003). Females in larger groups spent significantly more time feeding than males, but no more than females in smaller groups. This suggests a disparity between sexes in their nutritional requirement or their ability to acquire food. In the wild, female red ruffed lemurs (*Varecia rubra*) were observed to feed more than males in every season and during every reproductive state except gestation, when both sexes increased feeding and resting with little movement, most probably to allow energy accumulation prior to lactation (Vasey, 2006). Differences in activity budgets of males and females have therefore been observed in the wild (e.g. Sulawesi black crested macaques, O'Brien & Kinnaird, 1997) but have also been reported in captivity: Diana monkey, *Cercopithecus diana diana*, (Todd *et al.,* 2006); Japanese Macaques, Macaca fuscata (Jaman & Huffman, 2008). These disparities may reflect differences in the reproductive strategies of males and females (Matsumoto-Oda & Oda, 2000). When female mammals become pregnant or lactate, their nutritional requirements increase (Loudon, 1985), which can be reflected in their activity budget (Altman, 1980). For example, in lactating female brown lemurs (*Eulemur fulvu),* food intake significantly increased during the early lactating period (Tarnaud, 2006). In the yellow baboon (*Papio cynocephalus*), pregnant and lactating females spent more time feeding from foods that provide higher amounts of energy and protein than cycling females (Muruthi *et al*., 1991), reflecting the increased energetic demand at this stage of the reproductive cycle. Lactating woolly spider monkeys females *(Brachyteles arachnoids)* also spent significantly more time feeding than either pregnant or non-reproducing females (Strier, 1987). However, contrary results have been found in chimpanzees (*Pan troglodytes schweinfurthii*). Matsumoto-Oda & Oda (2001), found no significant difference in activity budget between lactating, oestrous and anoestrous wild females chimpanzees.

However, these patterns of time allocation to various behaviours by the different sexes vary according to species. Activity budgets are not significantly different in male and female wild woolly monkeys (Defler, 1995), vervet monkeys (Isbell & Young, 1993), woolly spider monkeys (Strier, 1987) or Milne-Edwards' sifaka (*Propithecus diadema edwardsi*), (Hemingway, 1999). In Milne-Edwards' sifaka, however, although, the time spent eating or performing other behaviour was not different between sexes, the quality of food consumed differed in one of the two study groups, in which females tended to select particularly nutritious plant parts, probably to boost nutrient and energetic intake to meet increased requirements during lactation.

Other important intrinsic factors influencing activity budget are age and body mass of the individual. Various studies have found significant differences in activity budgets of animals from different age categories. For example, immature wild Francois' langurs spend more time playing than other age classes, while adults allocate more time to resting, feeding and grooming (Zhou *et al*., 2007). Additionally, the activity budgets of white-headed langurs showed that juveniles spend significantly more time playing than adults (Li & Rogers, 2004). Similar results have been found in captivity with the capuchin monkey, *Cebus apella*, (Paukner & Suomi, 2008) and the Diana monkey (Todd *et al.,* 2006). Play behaviour is not always associated with young animals and has been linked to other functions, such as sexual interactions between adult males and females and as a tension reduction tactic (Palagi & Paoli, 2007). Differences in activity budget between age categories as been observed in captive Japanese macaques, in both vegetated and non-vegetated enclosures. Juveniles spent more time moving and feeding, and less time resting than adults (Jaman & Huffman, 2008). However, O'Brien & Kinnaird (1997) found that the behaviour of large juvenile Sulawesi crested black macaques were more similar to that of adults than to that of small juveniles.

Natural behaviours are performed by animals in the wild in order to survive and reproduce (Boere, 2001). In captivity, conditions are by definition different and some adaptation to the captive environment is essential (Wemelsfelder, 1994; Duncan & Fraser, 2000; Mallapur & Choudhury, 2003, Mallapur, 2005). For good animal welfare to be provided to captive animals, appropriate housing and management conditions must be provided. For example, food is provided to captive animals on a regular basis and at a relatively consistent quality, but nutritional makeup of this captive diet may deviate from the natural diet (Crissey & Pribyl, 1997; Calisi & Bentley, 2009). The difference in food quality and increased ease of access to food in captivity may affect the time spent feeding compared to that observed in the wild, which in turn, may affect welfare (Chapter 1, section 1.2). A comparative study of wild and captive black-and-white ruffed lemurs (*Varecia variegata*) found that captive animals spent more time on self-grooming and social behaviours, and less time feeding than wild animals, with a lack of manual manipulation of food items (Kerridge, 2005). However, other studies have found no significant difference between activity budget of wild and captive Sulawesi macaques, therefore suggesting good quality of management in the studied zoos (Melfi & Feistner, 2002).

The type of enclosure animals are confined in may have an impact on their activity budget. For example, Japanese macaques held in vegetated enclosures spent significantly less time resting and moving while feeding and grooming increased compared to the time spent feeding in non-vegetated enclosures (Jaman & Huffman, 2008). Macedonia (1986) studied the impact of housing conditions on sifakas (*Propithecus verreauxi*) and observed that animals kept in outdoor enclosures were significantly more active, spent less time resting, and more time feeding, moving and playing than those confined indoors. Similarly, Sulawesi macaques were found to display more wild-like behaviour as their enclosure became larger and more complex (Melfi & Feistner, 2002).

In this current study, five groups of colobus monkeys, maintained in four zoos in the United Kingdom and France, were observed and activity budgets were created to compare time allocation to various behaviours across zoos. Seasonal patterns in time spent performing behaviours were investigated across zoos, although due to the relatively stable environment created by captive conditions, no seasonality in behaviour was expected. The influence of gender and age on activity budgets was investigated across zoos and was expected to show significant differences. Finally, the mean activity budget of colobus monkeys in captivity was discussed in comparison to those in the wild. Different patterns were expected due to the large variation in environmental factors faced by wild animals.

2.2 Methodology

2.2.1 Age category definition

The criteria for age categories were defined as follows:

Infant: animals less than a year old and still dependant on their mother for feeding and general care

Juvenile: animals from one to two years old, still associated with their mother but independent for feeding and general care

Sub adult: animals from three to four years old for female and three to five years old for males

Adult: males older than five years old, females older than four years old
(based on Estes, 1991).

2.2.2 Study sites and subjects

Five groups of colobus monkeys were observed at four different zoos between March 2005 and October 2006 (in spring, summer, autumn and winter), with two separate groups housed at Paignton Zoo. Details of sex, group size, group composition, and times of visits are summarised in Table 2.1.

Zoos	Spring 05	Summer 05	Autumn 05	Winter 05	Spring 06	Summer 06	Autumn 06
Port Lympne	5.5 (9)	5.5 (9)	5.5 (9)	5.5 (9)	5.5.1 (9)	5.5.1 (9)	5.5.2 (9)
Paignton Male (A)		4.0 (5)			4.0 (5)		4.0 (5)
Paignton Female (B)		5.1 (6)			4.1 (5)		3.1 (5)
La Boissiere					5.2.1 (7)		6.2 (7)
Banham	2.5.2 (7)		3.6.2 (7)				

Table 2.1: Summary of colobus group composition at all zoos and times of visits. The first digit represents the number of males, the second, the number of females and the third (if present), the number of unsexed infants i.e. 5.5.1 represents five males, five females and one unsexed infant. The number in brackets is the number of days data were collected during the visit. Spring is March-April, summer is June-July, autumn is October-November and winter is January.

Port Lympne Zoo was visited a total of seven times, approximately every three months from March 2005 to October 2006; after this point, the group was dispersed, so data collection could not be continued. As data were collected over two years, the social composition of the groups varied throughout the study and is summarised in Table 2.2 a.

Two single sexed groups of colobus monkeys, housed separately, were studied at Paignton Zoo Environmental Park. Their compositions during each of the data collection periods are summarised in Table 2.2 b. The all male group was visited three times between August 2005 and October 2006. On the first two visits, three of the male colobus monkeys were housed together, but one was housed separately due to fighting (although kept within visual and auditory contact). On the last visit, all four animals were housed together.

a: Port Lympne	Animal	Sex	Date of birth	Mar 05	Jul 05	Oct 05	Jan 06	Mar 06	Jul 06	Oct 06

Zoo										
1	Katie	F	11/04/1991	A	A	A	A	A	A	A
2	Panda	M	30/03/1995	A	A	A	A	A	A	A
3	Ulna	F	17/11/1999	A	A	A	A	A	A	A
4	Radius	M	05/01/2000	A	A	A	A	A	A	A
5	Fibula	M	06/07/2001	S	S	S	S	S	A	A
6	Tibia	F	02/03/2002	S	S	S	S	S	S	S
7	Cranium	M	22/09/2002	J	J	S	S	S	S	S
8	Tarsal	M	11/11/2003	J	J	J	J	J	J	S
9	Dermis	F	18/02/2004	J	J	J	J	J	J	J
10	Femur	F	21/02/2005	I	I	I	I	J	J	J

b: Paignton Zoo	**Animal**	**Sex**	**Date of birth**	**Jun 05**	**Mar 06**	**Oct 06**
	Female group					
1	Lou	F	30/05/1985	A	A	A
2	Madonna	F	28/12/1991	A	n/a	n/a
3	Denny	F	21/03/1993	A	A	A
4	Jhazzie	F	27/11/1999	A	A	n/a
5	Salassie	F	28/11/1999	A	A	A
6	Joe	M	20/09/2004	J	J	J
	Male group			**Aug 05**	**Mar 06**	**Oct 06**
7	Millo	M	12/12/1997	A	A	A
8	Fergus	M	24/03/1999	A	A	A
9	Kabul *	M	18/06/2000	A	A	A
10	Hope	M	18/09/2001	S	S	A

Tables 2.2 a-b: Group composition, date of birth and age category classification of colobus monkeys at Port Lympne Zoo and Paignton Zoo during different visits. M= male, F= female. A= adult, S= sub-adult, J= juvenile and I= infant in the age categories. * housed separately in August 2005 and March 2006. n/a data not available as animal died.

The second group of colobus monkeys at Paignton Zoo was composed of five adult females and one juvenile male (Table 2.2 b, female group). This group was studied in June 2005, March 2006 and October 2006. Unfortunately, members of the group died over the time of the study and only three adult females and the juvenile male remained at the last visit.

The group studied at Banham Zoo was composed of seven colobus monkeys in March 2005 and nine in October 2005, as two infants became classified as juveniles. Details of each animal's sex and age category are presented in Table 2.3 a.

The group at La Boissière du Doré was composed of seven colobus monkeys in March 2006 and eight in November 2006 (the infant having become a juvenile). Group composition is presented in Table 2.3 b.

2.2.3 Housing and Husbandry

Enclosure size, access, feeding time, floor substrate and general husbandry at all the study sites are presented in Table 2.4.

2.2.3 a Port Lympne Zoo

At Port Lympne Zoo, the enclosure consisted of two areas: the inside area was heated and sheltered from the elements, divided into two parts and away from public view. The outside area was exposed to the elements, but with some shelters, and was exposed to public viewing, although not from all sides, but there were no hiding places.

The inside area contained climbing frames and shelves, deep enough for the animals to rest, feed and move about on. The outside area contained many climbing poles, ropes, ladders and platforms allowing feeding and resting at different levels, as well as shelters and enrichment devices.

2.2.3 b Paignton Zoo

Three areas were available to the male colobus monkeys. The main inside area, where the three males (Millo, Fergus and Hope) were kept together, was heated and viewed by the public via a large front glass window. The three males were also allowed unlimited access to two heated back cages, away from the public view.

a: Banham Zoo	Animal	Sex	Date of birth	Mar 05	Oct 05
1	Steffan	M	05/01/1990	A	A
2	Kivu	F	13/01/1995	A	A
3	Carmen	F	14/01/1996	A	A
4	Kiwi	F	28/01/2000	A	A
5	Sophie	F	28/09/2002	S	S
6	Monty	M	21/09/2003	J	J
7	Suzie	F	08/10/2003	J	J
8	Bea	F	25/09/2004	I	J
9	Bobby	M	16/10/2004	I	J

b: La Boissière du Doré	Animal	Sex	Date of birth	Mar 06	Nov 06
1	Ernesto	M	12/10/1992	A	A
2	Celize	F	08/11/1994	A	A
3	Fudgi	M	25/01/2000	A	A
4	Nimba	M	09/07/2000	A	A
5	Abyssian	M		S	S
6	Camara	M	18/12/2002	S	S
7	Maatai	F	27/07/2004	J	J
8	Swahili	M	01/12/2005	I	J

Tables 2.3 a-b: Group composition, date of birth and age category classification of colobus monkeys at Banham Zoo and La Boissière du Doré Zoo during different visits. M= male, F= female in sex category. A= adult, S= sub-adult, J= juvenile and I= infant in the age categories.

	Port Lympne	Paignton males	Paignton females	La Boissière du Dore	Banham
Inside Enclosure					
height	2.5m	n/a	n/a	4m	5m
area	7.5mx3m	$51m^2$	$63m^2$	6mx4m	9mx5m
substrate	Sawdust	Bark chippings	Some straw	Sawdust	Tiled
Outside enclosure					
height	9m	n/a	n/a	n/a	Small cage: 5m paddock: open top fence 3.75m
area	10.5mx9m	$315m^2$	$619m^2$	n/a	small cage: 13mx4m paddock: 38m diameter
substrate	Sand	Grass	Grass	n/a	small cage: grass and sand paddock: grass
Method of access	Three trap doors	One trap door	One trap door	n/a	One trap door and tunnel overhang
Locked in	No	Daily: am/pm* Overnight (16:30-08:00)	Overnight (16:30-09:00)	Always	Overnight (16:30-08:30)
out	10:30-11:30 or 13:00-14:00	08:00-08:30	09:00-09:45	n/a	08:30-09:30
Feeding time/regime	**09:00** - vegetables, fruits, seeds, primate pellets, nuts, cheese/chicken **12:30** - vegetables **16:30** - vegetables and fruits	**09:00** - primate pellets and seeds **14:30** - vegetable, eggs and bread, (and browse if available) * **16:30** - vegetable, eggs and bread **	**09:00** - primate pellets and seeds **14:30** - browse if available **16:30** - vegetables, eggs and bread	**09:00** - primate pellets **10:00** - vegetables **18:00** - vegetables	**09:00** - vegetables, fruits, nuts and seeds **16:30** - vegetables, fruits, bread, primate pellets and egg/meat

Table 2.4: Enclosure characteristics and husbandry practices at Paignton Zoo, La Boissière du Doré Zoo, Banham Zoo and Port Lympne Zoo (* when one colobus was separated from the other three and outside enclosure was alternated between them ** feeding time when all males where together in October 2006)

The outside enclosure was accessible to the three males in the morning. The singly housed animal (Kabul) remained in two not publicly viewed back cages, with no physical contact with the other three (to avoid any form of injury) and was allowed access to the main outside enclosure in the afternoon. The main enclosure, back cages and outside areas consisted up of numerous climbing frames, ropes and platforms for feeding and resting.

The females (and juvenile male) were all housed together in the main heated sheltered area with constant access to back cages away from the public, although these were very rarely used, and access to a large outside enclosure. The sheltered and outside areas were equipped with numerous climbing frames, ropes, large cargo nets, ladders and resting platforms. Hiding places away from the public view were available outside.

Both male and female groups of colobus monkeys at Paignton Zoo were provided with an environmental enrichment timetable which meant food was made available to the animals in a variety of ways every day, such as kebabs, stuffed in bags, in hanging baskets or in small cages with opening large enough to fit their hands); various sensory (olfactory such as perfume scented rolls or herbs and auditory, such as rattles) enrichment devices were also provided.

2.2.3 c Banham Zoo

Three areas were available at different times of day for colobus monkeys at Banham Zoo. The main inside enclosure consisted of a large sheltered area. A small outside cage and a large paddock were available throughout the day. The main enclosure was equipped with several large platforms for feeding and resting. The outside paddock contained a large tree trunk for climbing and resting, ropes and smaller tree stumps where food could be placed.

2.2.3 d La Boissière du Doré

This group was locked in during the winter season (from October until April) as it was suggested by zoo staff that the cold weather led to health problems. During the first visit (March 2006), the animals had been locked in for the past four months, while during the second visit, at the onset of winter (November 2006), animals had only been locked in for a few days before arrival. The sheltered enclosure was composed of one large heated room (Table 2.4) with many climbing posts, ropes, and platforms for feeding and resting. A small room was also available at the back but was only used to separate the animals from the main area while it was being cleaned. Information on dietary regimes and feeding times are given in Table 2.4.

2.2.4 Behavioural data collection

Data collection at all zoos followed the same procedure. Every 30 min between 08:00 and 17:00 instantaneous scan sampling was used to record the state behaviours listed in Table 2.5 and the location in the enclosure of each colobus monkey in the group was noted. Infants were not included in behavioural observations as they are too dependent on their mother (Chapter 1, section 1.5.5). Total number of scan per animal per visit are summarised in Table 2.6.

State behaviour	**Description**
Social	all interactions between two or more animals, normally within close proximity, including: *Playing:* physical contact between animals such as tumbling, wrestling or hugging *Mock fighting:* two animals are in physical non-aggressive contact, gentle biting, turning into play *Mating:* a male elicits mounting from a female or a male is presented by a female, both followed by copulation *Fighting:* two animals are in physical aggressive contact with hair erect and may include biting *Allogrooming:* between two animals where one cleans the fur and skin of another by removing foreign objects such as insects, ectoparasites, dead skin, leaves, dirt and twigs
Moving	any movement which covers any distance, walking, running, jumping including a*ggressive chase* (an animal pursues another, accompanied by threat vocalization, baring teeth and jumping with loud landings) and p*layful chase* (an animal pursues another, between play bouts) but excluding any movement which occurred during feeding
Resting alone	inactive and stationary, either sitting, lying, standing or sleeping (including vigilance behaviour where the environment was being scanned and autogrooming) with no contact with another animal (excluding tails)
Resting social	Same as above but in physical contact with another animal(s)
Feeding	handling or consuming food, as well as foraging (including movement during feeding)

Table 2.5: Ethogram of state behaviours recorded in colobus monkeys.

Zoos	Spring 05	Summer 05	Autumn 05	Winter 05	Spring 06	Summer 06	Autumn 06
Port Lympne	171	171	171	171	171	171	171
Paignton Male (A)		95			85		95
Paignton Female (B)		114			95		95
La Boissiere					133		133
Banham	133		133				

Table 2.6: Total number of scan per visit per zoo for each colobus monkey.

2.2.5 Data analyses

State behaviours were used to construct activity budgets, following the method of Altman (1974). The mean percentage time spent performing each behavioural category (Table 2.5) was calculated by adding all the daily data points, and averaged for the number of days of data collection.

Each state behaviour was transformed using arsin transformation (PAWS 18) in an attempt to reach normality. A Generalised Estimated Equation (GEE) model was performed for each state behaviour at a time (PAWS 18). The type of model was chosen according to the response (i.e. state behaviour) distribution. For normally distributed variables, an identity link function was selected. For skewed variables (i.e. social, moving and resting social), a Gamma or inverse Gaussian distribution with a log link function was chosen. In all five state behaviours, the same procedure was followed. The repeated subject variable was defined by the individual and the zoo identity. The within subject variable was identified by repeated data collection period (i.e. visit). The dependant variable was defined as the state behaviours of interest. Predictors were entered as factors i.e. zoo, season, index of success (see Chapter 3, section 3.2.3b) and sex, whereas age (in months) was entered as a covariate.

Initially, all predictors were included in the model as a main effect. Two-way interactions between terms were tested for significance, with the exception of the interaction between age and index of success as these were tightly correlated, Figure 2.1. Variables and covariates were then subsequently dropped from the model if lacking significance so the model only contained those terms which were significant yielding a ‘minimal model’. For non-normal variables, a hybrid method of parameter estimation starting with the Fisher scoring, then switching to the Newton-Raphson method was employed. In all models, scale parameter method was employed using the maximum-likelihood estimate. Statistics were performed with Type III analysis and 95% confidence interval level computing Wald statistics.

Contrast analysis was performed in the GEE model by use of estimated marginal means (EM means). The EM means were displayed in the PAWS 18 programme for factors only and pairwise comparison between each factor of relevance was performed and corrected with a Bonferonni adjustment for multiple contrasts.

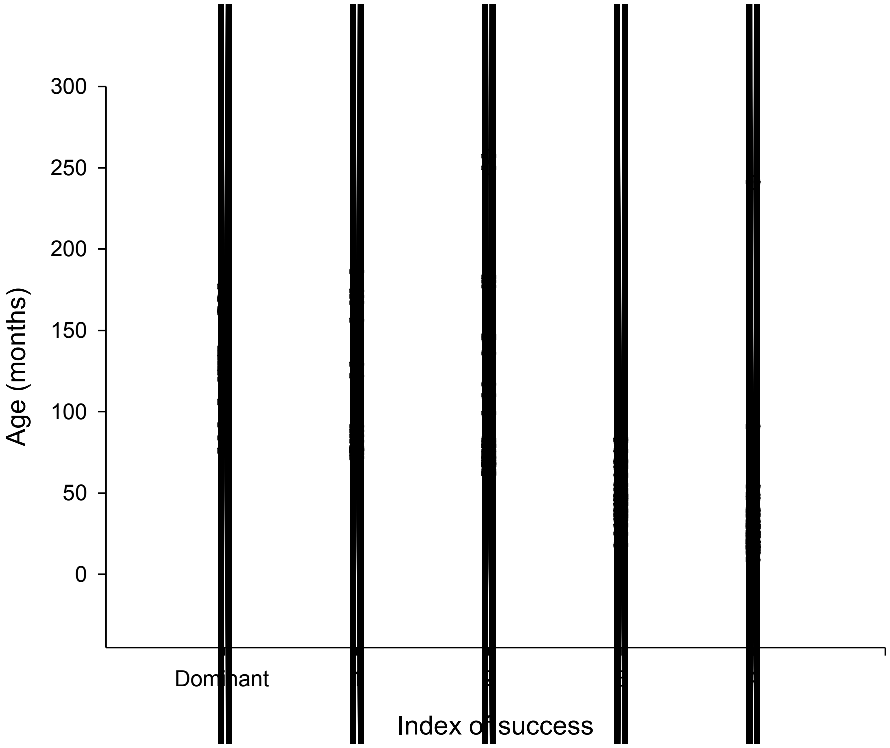

Fig 2.1: Age in months of colobus monkeys according to index of success.
Index of success (IS) was derived from the number of dominant encounter won divided by the total number of dominant encounters and scaled as dominant (the clear overall dominant animals in each group) and between 1-4 (from most dominant to most subordinate).

2.3 Results

2.3.1 Overall activity budget

2.3.1 a Port Lympne Zoo

Figure 2.2 represents the mean activity budget for all animals at Port Lympne Zoo. Juveniles, spent between 5.09 % and 8.55 % moving and 12.60 % and 14.32 % on social behaviour, apart from the infant Femur who only spent 4.24% of her time being social. Adult animals ranged from 4.26 % to 6.83 % for social behaviour and from 0.94 % to 2.58 % for moving behaviour. Adult males spent only 2.77 %-8.36 % resting socially compared to 67.68 %-72.82 % resting alone. Adult females spent similar time resting alone or socially (between 36.49 %-41.89 % socially and 30.20 %-42.24 % alone). Sub-adult colobus monkeys like Fibula (male) and Tibia (female) showed intermediate values to those of the juveniles and adults (social behaviour: 7.32 % and 8.86 % respectively, moving: 3.32 % and 2.42 %, resting socially: 12.43 % and 12.79 % and resting alone: 5.13 % and 49.89%).

All animals showed relatively consistent amounts of time spent feeding, between 16.71 % and 25.60 % of time between 8:00 am and 17:00 pm.

2.3.1 b Paignton Zoo

Results of mean activity budget at Paignton Zoo are presented in Figure 2.3 and Figure 2.4 for the all male and all female groups respectively. The all male group showed very consistent results for each animal. Males spent little time on social (1.22 %-5.84 %), moving (1.57 %-6.13 %) and resting social behaviours (0 %-1.75 %). Most of their time was spent resting alone (67.92 %-74.55 %) and feeding (16.92 %-20.19 %).

The adult females spent little time moving (0 %-3.16 %) compared to the juvenile male, Joe (18.25 %). The four related females spent most time resting alone (51.75 %-63.16%), compared to the mother and son who spent similar time resting social together or alone (35.38 % and 34.91 % respectively). All animals spent similar time feeding, from 14.74 % to 21.58 %.

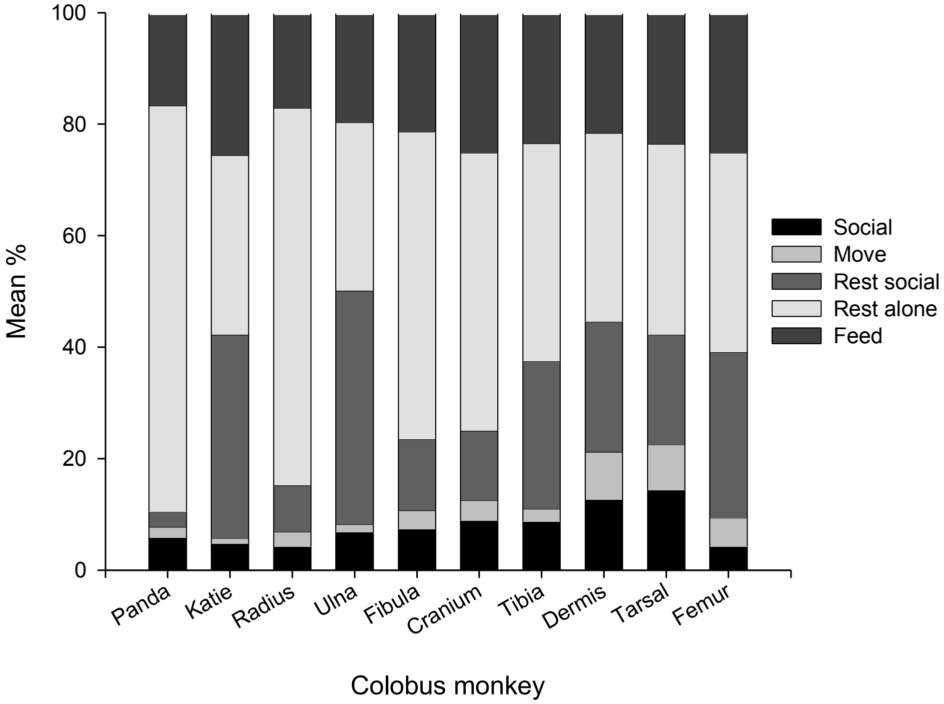

Fig 2.2: Mean % time spent performing state behaviours between March 2005 and October 2006 for each colobus monkey at Port Lympne Zoo.

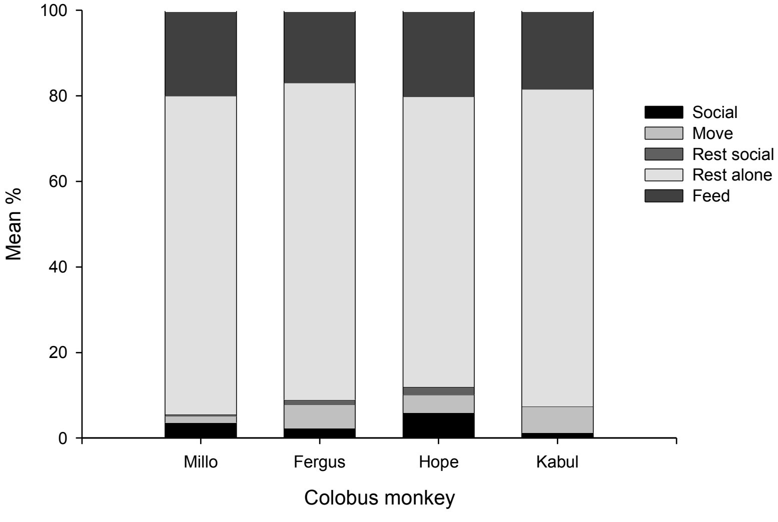

Fig 2.3: Mean % time spent performing state behaviours (from August 2005 to October 2006) for each colobus monkey at Paignton Zoo all male group.

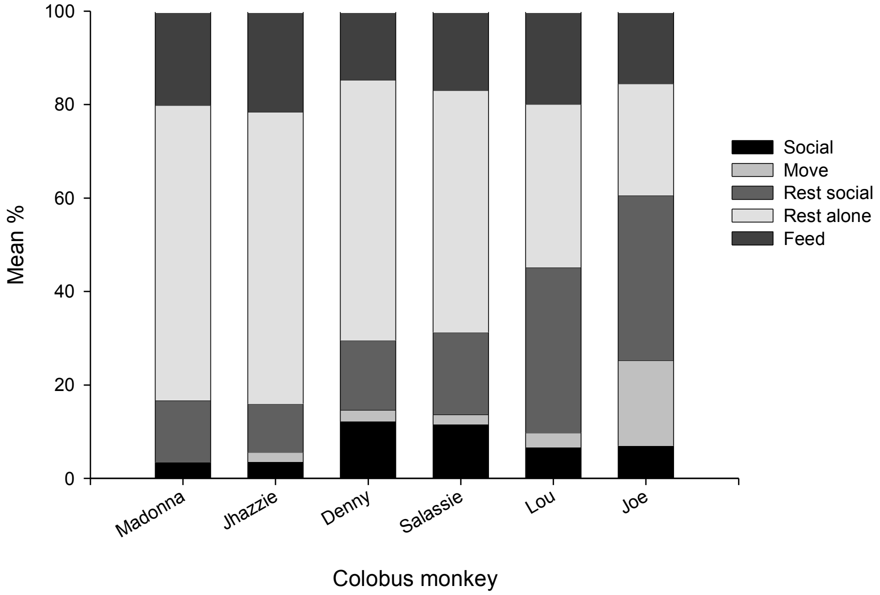

Fig 2.4: Mean % time spent performing state behaviours (from June 2005 to October 2006) for each colobus monkey at Paignton Zoo female group.

2.3.1 c Banham Zoo

Data for the mean time spent performing state behaviours for colobus monkeys at Banham Zoo are presented in Figure 2.5. Adults (Steffan, Kivu, Carmen and Kiwi: Table 2.3 a) spent 6.02 % to 9.02 % being social whereas sub-adults and juveniles allocated between 10.51 % and 16.10 % to this activity. Conversely, juveniles spent 9.70 % to 10.87 % moving compared to adults (0.00 % to 2.77 %). The adult male spent only 1.59 % of his time resting socially and 64.20 % resting alone. Adult females spent more time resting socially (between 46.33 %-49.77 %) than alone (15.08 %-18.67 %). All colobus monkeys spent a similar amount of time feeding (23.14 % to 31.67 %).

2.3.1 d La Boissière du Doré Zoo

As Figure 2.6 shows, results were very consistent across all colobus monkeys at La Boissière du Doré Zoo. Similar amount of time was spent being social (4.45 %- 7.99%) and feeding (13.35 %-22.26 %) across sexes and age categories. Adults and sub-adults spent between 1.34 % and 7.14 % moving while the juvenile (Maatai) spent 12.84 %. All animals spent similar time resting socially (11% -19.91 %) apart from the adult female (Célize) who was with an infant (47.89 %).

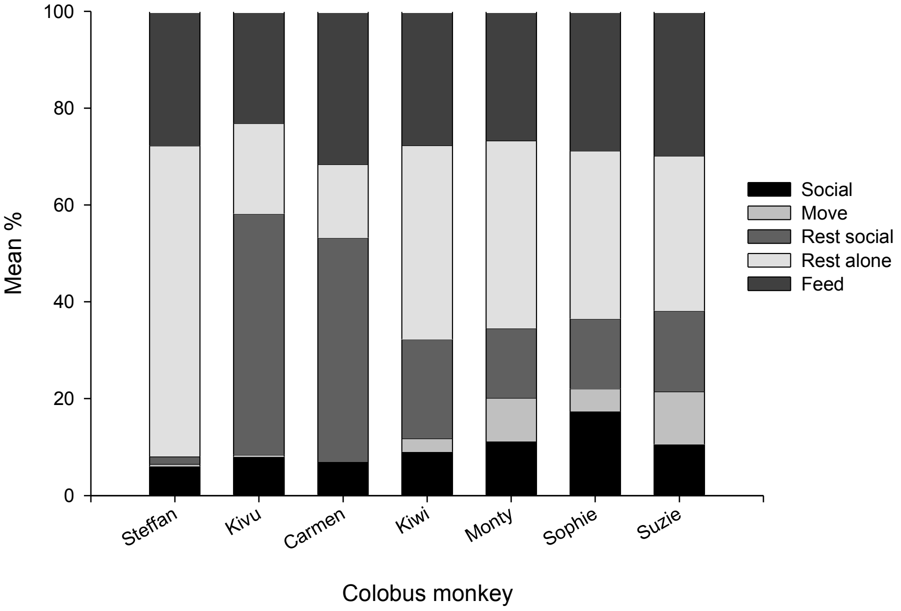

Fig 2.5: Mean % time spent performing state behaviours (in March 2005 and October 2005) for each colobus monkey at Banham Zoo.

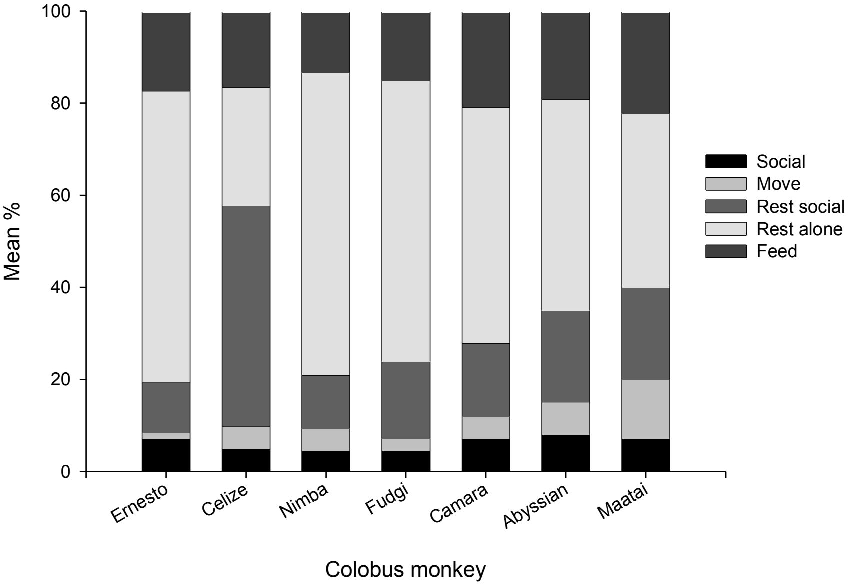

Fig 2.6: Mean % time spent performing state behaviours (in March 2006 and November 2006) for each colobus monkey at La Boissière du Doré Zoo.

2.3.2 Feeding behaviour

The analyses reported below (Table 2.7) investigated possible factors affecting the percentage of time spent feeding by colobus monkeys at various zoo over repeated data collection periods. Factors with significant power in the model were zoo, season, index of success (IS) and age.

Contrast analysis of factors was performed in the GEE model by use of estimated marginal means (EM means) on zoo, IS and season. Zoo was a significant factor in the analysis (x^2 = 103.549, df = 4, $p < 0.001$) and significant differences between EM were found between Banham Zoo and all other zoos (mean differences with Port Lympne Zoo: 0.0578, $p < 0.001$; La Boissière du Doré Zoo: 0.1022, $p < 0.001$; Paignton Zoo male group: 0.0862, $p < 0.001$ and Paignton Zoo female group: 0.1093, $p < 0.001$). Additionally, significant difference between the EM means was observed between Port Lympne Zoo and La Boissière du Doré Zoo (0.0444, $p < 0.01$), Figure 2.7.

Season was a significant factor in the analysis (x^2 = 18.385, df = 3, $p < 0.001$) and significant differences between EM were found between the autumn and all other seasons (mean differences with spring: 0.0339, $p < 0.001$; summer: 0.0293, $p < 0.05$ and winter: 0.0364, $p < 0.01$), Figure 2.8.

IS was a significant factor in the analysis (x^2 = 20.378, df = 4, $p < 0.001$) and significant differences between EM were found between the most subordinate individuals (IS = 4) and both the overall dominant animal (mean differences: 0.0702, $p < 0.01$) and animals with IS-1 (0.0572, $p < 0.01$). Additionally, significant difference between the EM means were observed between the overall dominant colobus and animals of IS-3 (-0.0573, $p < 0.05$), Figure 2.9.

Age was a significant covariate in the analysis (x^2 = 4.146, df = 1, $p < 0.05$). EM can not be used for covariate, however, Figure 2.10 suggest that younger animals spent more time feeding than older ones.

Full model

Terms	Type III		
	Wald Chi-Square	df	p
zoo	82.608	4	.000
season	18.522	3	.000
IS	15.891	4	.003
sex	.990	1	.320
age	3.699	1	.054

Minimal model

Terms	Type III		
	Wald Chi-Square	df	p
zoo	103.549	4	.000
season	18.385	3	.000
IS	20.378	4	.000
age	4.146	1	.042

Table 2.7: Factors affecting the percentage time spent feeding by colobus monkeys

Results are from a GEE model based on time spent feeding averaged per individual per data collection period from instantaneous state behaviour scan from 37 animals in 5 groups. Percentage time spent feeding was arsined transformed for the analysis.

Season was recorded as spring (March-April), summer (June-August), autumn (October-November) and winter (January). Index of success (IS) was derived from the number of dominant encounter won divided by the total number of dominant encounters and scaled between 0 (dominant) and 1-4 (from most dominant to most subordinate).

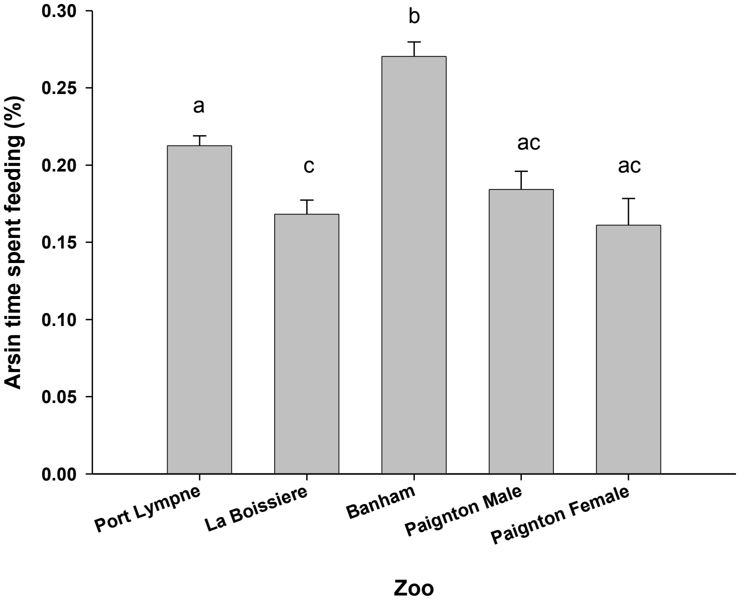

Fig 2.7: Mean (+SE) % time spent feeding by the groups of colobus monkeys at each zoo. Means and standard errors are predictions from the GEE model presented in Table 2.7, controlling for the variation in time spent performing state behaviour associated with the other significant predictors.

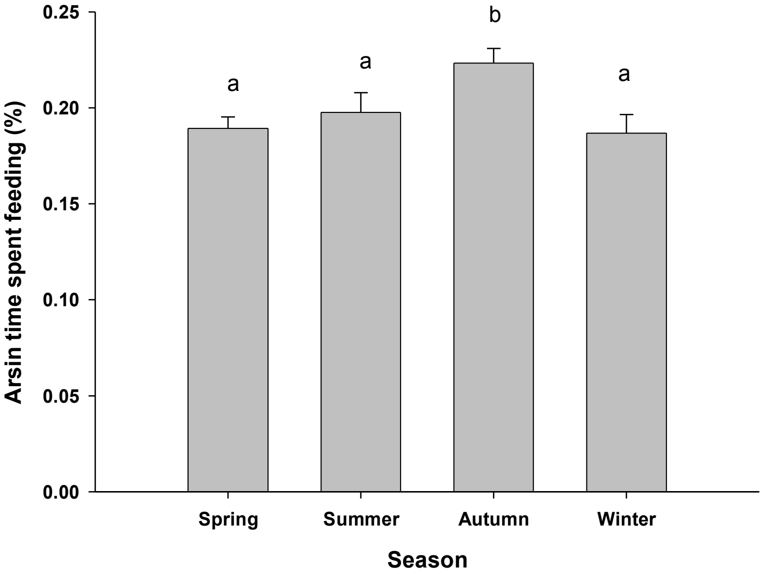

Fig 2.8: Mean (+SE) % time spent feeding by the groups of colobus monkeys during each season. Means and standard errors are predictions from the GEE model presented in Table 2.7, controlling for the variation in time spent performing state behaviour associated with the other significant predictors.

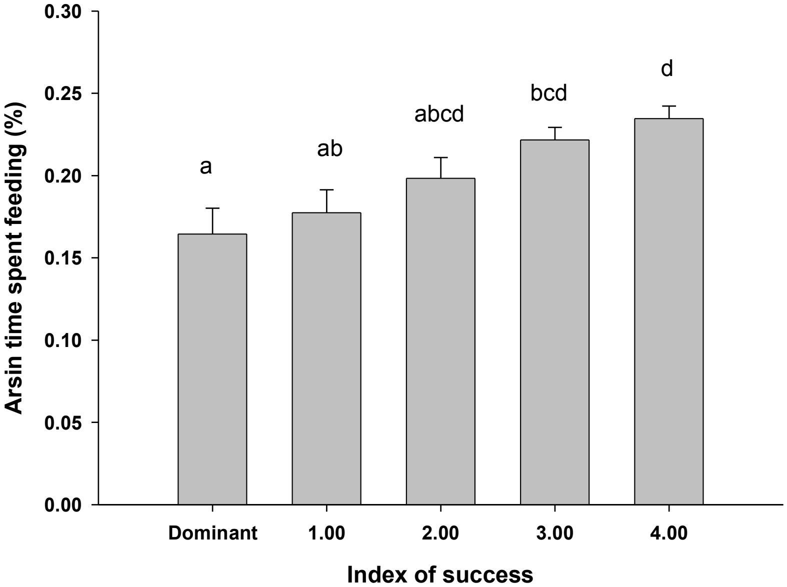

Fig 2.9: Mean (+SE) % time spent feeding by the groups of colobus monkeys according to index of success. Index of success (IS) was derived from the number of dominant encounter won divided by the total number of dominant encounters and scaled as dominant (the clear overall dominant animals in each group) and between 1-4 (from most dominant to most subordinate). Means and standard errors are predictions from the GEE model presented in Table 2.7, controlling for the variation in time spent performing state behaviour associated with the other significant predictors.

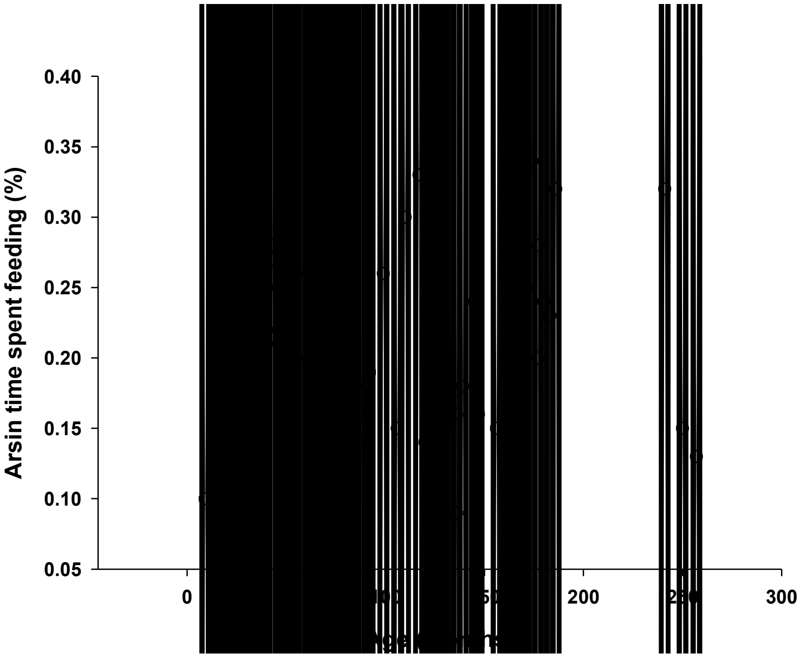

Fig 2.10: Percentage time spent feeding by the colobus monkeys according to their age.

2.3.3 Social behaviour

The following analyses (Table 2.8) investigated the possible factors affecting the percentage of time spent socially by colobus monkeys a various zoo over repeated data collection periods. Factors with significant power in the model were zoo, season and the index of success (IS).

Full model

Terms	Type III		
	Wald Chi-Square	df	Sig.
zoo	27.897	4	.000
season	17.234	3	.001
IS	14.028	4	.007
sex	.000	1	.997
age	.499	1	.480

Minimal model

Terms	Type III		
	Wald Chi-Square	df	Sig.
zoo	37.082	4	.000
season	17.770	3	.000
IS	30.738	4	.000

Table 2.8: Factors affecting the percentage time spent socially by colobus monkeys

Results are from a GEE model based on time spent socially averaged per individual per data collection period from instantaneous state behaviour scan from 37 animals in 5 groups. Percentage time spent feeding was arsined transformed for the analysis.

Season was recorded as spring (March-April), summer (June-August), autumn (October-November) and winter (January). Index of success (IS) was derived from the number of dominant encounter won divided by the total number of dominant encounters and scaled between 0 (dominant) and 1-4 (from most dominant to most subordinate).

Contrast analysis was performed in the GEE model by use of estimated marginal means (EM means) on zoo, IS and season. Zoo was a significant factor in the analysis (x^2 = 37.082, df = 4, $p < 0.001$) and significant differences between EM were found between the male group at Paignton Zoo and three other zoos: Port Lympne Zoo (mean difference: -0.0344, $p < 0.001$), La Boissière du Doré Zoo (mean difference: -0.0218, $p < 0.05$) and Banham Zoo (mean difference: -0.0462, $p < 0.001$). Additionally, significant differences were found between the EM at La Boissière du Doré Zoo and Banham Zoo (mean difference: -0.0244, $p < 0.05$), Figure 2.11.

Season was a significant factor in the analysis ($x^2 = 17.770$, df = 3, $p < 0.001$) and significant differences between EM were found between autumn and both spring (mean differences: -0.0225, $p < 0.01$) and winter (mean differences: -0.0224, $p < 0.01$), Figure 2.12.

IS was a significant factor in the analysis ($x^2 = 30.738$, df = 4, $p < 0.001$). More subordinate animals of IS-3 and IS-4 both had significant different EM to overall dominant animals (mean difference 0.0227, $p < 0.05$ and 0.0395, $p = 0.01$ respectively), animals of IS-1 (mean difference 0.0306, $p < 0.01$ and 0.0474, $p < 0.01$ respectively) and animals of IS-2 (mean difference 0.0196 $p = 0.01$ and 0.0365, p 0.05 respectively) Figure 2.13.

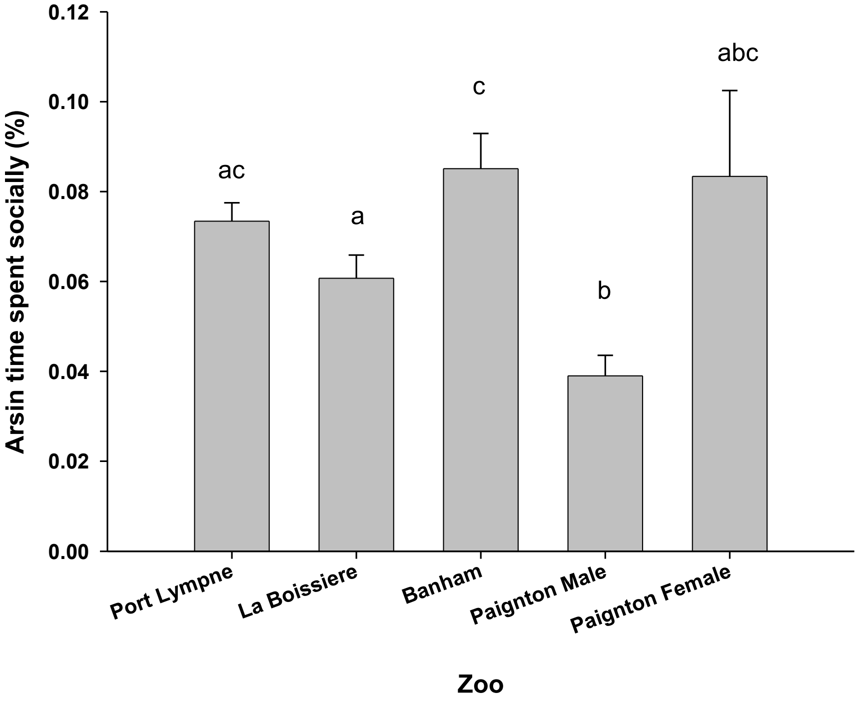

Fig 2.11: Mean (+SE) % time spent socially by the groups of colobus monkeys at each zoo. Means and standard errors are predictions from the GEE model presented in Table 2.8, controlling for the variation in time spent performing state behaviour associated with the other significant predictors.

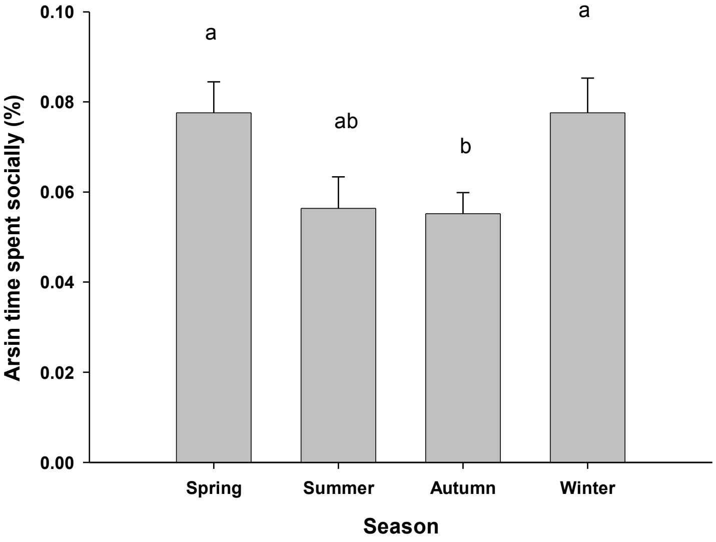

Fig 2.12: Mean (+SE) % time spent socially by the groups of colobus monkeys during each season. Means and standard errors are predictions from the GEE model presented in Table 2.8, controlling for the variation in time spent performing state behaviour associated with the other significant predictors.

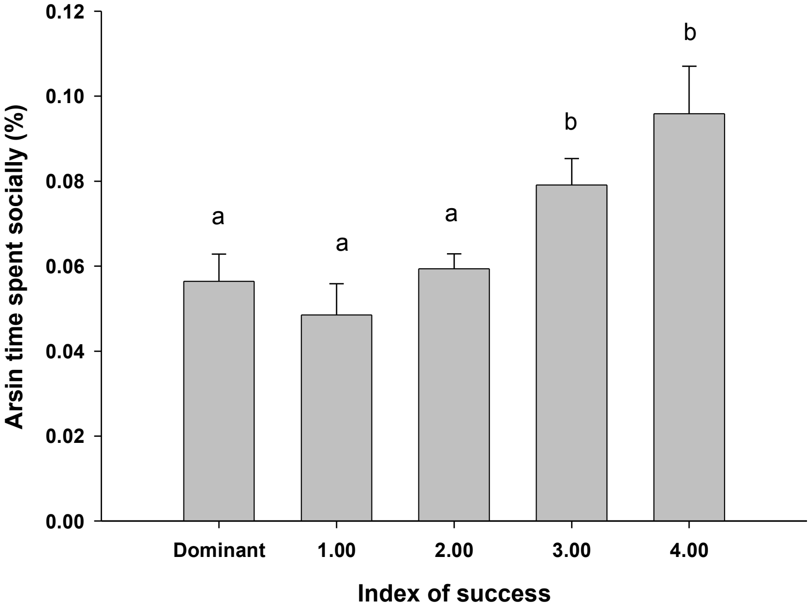

Fig 2.13: Mean (+SE) % time spent socially by the groups of colobus monkeys according to index of success. Index of success (IS) was derived from the number of dominant encounter won divided by the total number of dominant encounters and scaled as dominant (the clear overall dominant animals in each group) and between 1-4 (from most dominant to most subordinate). Means and standard errors are predictions from the GEE model presented in Table 2.8, controlling for the variation in time spent performing state behaviour associated with the other significant predictors.

2.3.4 Moving behaviour

The analyses reported below (Table 2.9) investigated possible factors affecting the percentage of time spent moving by colobus monkeys a various zoo over repeated data collection periods. Factors with significant power in the model were zoo, index of success (IS), sex, and age.

Contrast analysis was performed in the GEE model by use of estimated marginal means (EM means) on zoo, IS, and sex. Zoo was a significant factor in the analysis ($x^2 = 70.193$, df = 4, $p < 0.001$) and significant differences between EM were found between the female group at Paignton Zoo and three other zoos: Port Lympne Zoo (mean difference: 0.0873, p = 0.001), Banham Zoo (mean difference: 0.0853, $p < 0.01$) and Paignton Zoo male group (mean difference: 0.0723, $p < 0.05$), Figure 2.14.

Full model

Terms	Type III		
	Wald Chi-Square	df	Sig.
zoo	70.751	4	.000
season	6.640	3	.084
IS	39.691	4	.000
sex	15.092	1	.000
age	15.756	1	.000

Minimal model

Terms	Type III		
	Wald Chi-Square	df	Sig.
zoo	70.193	4	.000
IS	33.040	4	.000
sex	12.077	1	.001
age	16.086	1	.000

Table 2.9: Factors affecting the percentage time spent moving by colobus monkeys

Results are from a GEE model based on time spent moving averaged per individual per data collection period from instantaneous state behaviour scan from 37 animals in 5 groups. Percentage time spent feeding was arsined transformed for the analysis.

Season was recorded as spring (March-April), summer (June-August), autumn (October-November) and winter (January). Index of success (IS) was derived from the number of dominant encounter won divided by the total number of dominant encounters and scaled between 0 (dominant) and 1-4 (from most dominant to most subordinate).

IS was a significant factor in the analysis (x^2 = 33.040, df = 4, p < 0.001). Significant differences between EM were observed between the most subordinate animals of IS-4 and all other index of success (mean difference to overall dominant animal: 0.0664, p < 0.001, IS-1: 0.0627, p = 0.001, IS-2: 0.0549, p < 0.01 and IS-3: 0.0542, p = 0.001), Figure 2.15.

Sex was a significant factor in the analysis (x^2 = 12.077, df = 1, p = 0.001) with significant differences between EM of males and females (mean difference: 0.0203, p < 0.001), Figure 2.16.

Age was a significant factor in the analysis (x^2 = 16.086, df = 1, p < 0.001) where younger animals spent more time moving then older ones, Figure 2.17.

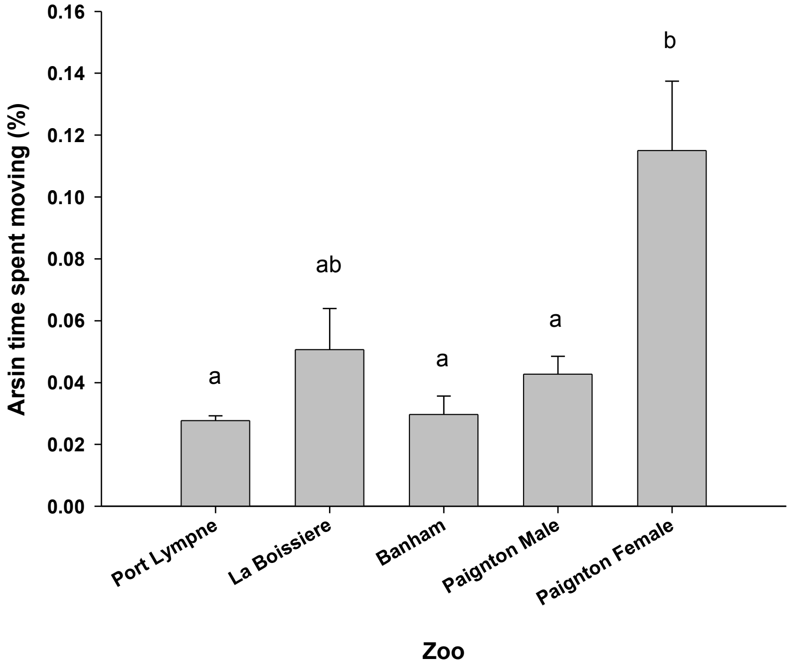

Fig 2.14: Mean (+SE) % time spent moving by the groups of colobus monkeys at each zoo. Means and standard errors are predictions from the GEE model presented in Table 2.9, controlling for the variation in time spent performing state behaviour associated with the other significant predictors.

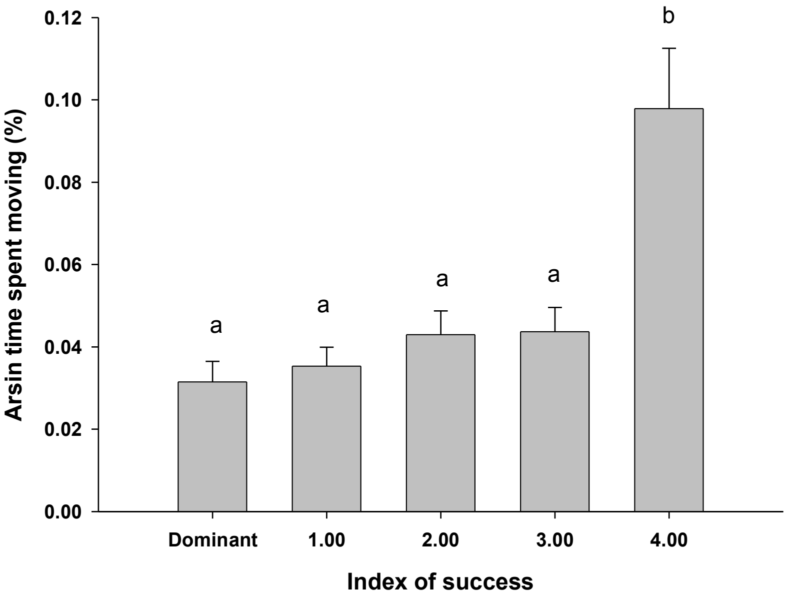

Fig 2.15: Mean (+SE) % time spent moving by the groups of colobus monkeys according to index of success. Index of success (IS) was derived from the number of dominant encounter won divided by the total number of dominant encounters and scaled as dominant (the clear overall dominant animals in each group) and between 1-4 (from most dominant to most subordinate). Means and standard errors are predictions from the GEE model presented in Table 2.9, controlling for the variation in time spent performing state behaviour associated with the other significant predictors.

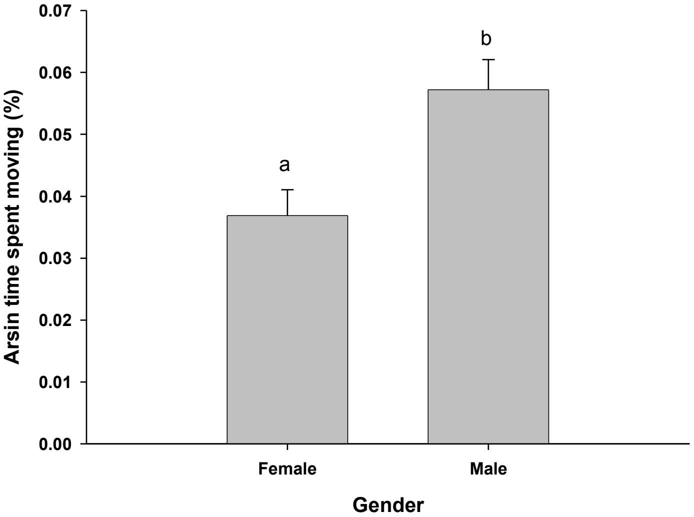

Fig 2.16: Mean (+SE) % time spent moving by the groups of colobus monkeys according to gender. Means and standard errors are predictions from the GEE model presented in Table 2.9, controlling for the variation in time spent performing state behaviour associated with the other significant predictors.

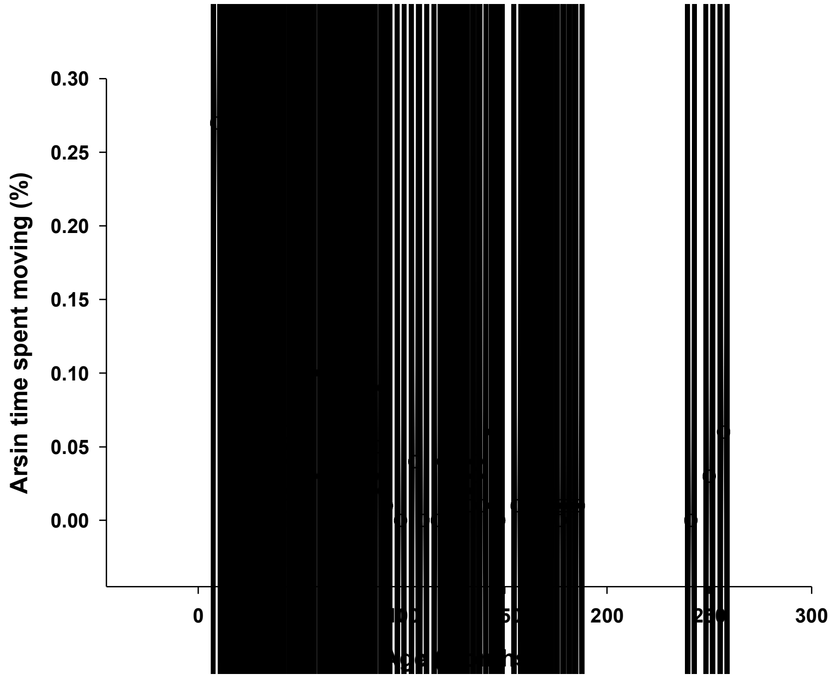

Fig 2.17: Percentage time spent moving by the colobus monkeys according to their age.

2.3.5 Resting socially behaviour

The following analyses (Table 2.10) investigated the possible factors affecting the percentage of time spent resting socially by colobus monkeys a various zoo over repeated data collection periods. Factors with significant power in the model were zoo, season, index of success (IS) and sex.

Contrast analysis was performed in the GEE model by use of estimated marginal means (EM means) on zoo, IS, sex and season. Zoo was a significant factor in the analysis (x^2 = 101.412, df = 4, $p < 0.001$) and significant differences between EM were found between the male group at Paignton Zoo and three other zoos: Port Lympne Zoo (mean difference: -0.0890, $p < 0.001$), La Boissière du Doré Zoo (mean difference: -0.2004, p = 0.001) and the female group at Paignton Zoo (mean difference: -0.2805, $p < 0.05$), Figure 2.18.

Season was a significant factor in the analysis (x^2 = 27.849, df = 3, $p < 0.001$) and significant differences between EM were found between the autumn and both spring (mean differences: -0.2649, $p < 0.05$) and summer (mean differences: -0.0884, $p < 0.001$). Additionally, a significant difference between the EM means of time spent resting socially was observed between summer and winter (0.1130, $p < 0.01$), Figure 2.19.

Full model

Terms	Type III		
	Wald Chi-Square	df	Sig.
zoo	89.582	4	.000
season	21.634	3	.000
IS	79.479	4	.000
sex	44.801	1	.000
age	.135	1	.713

Minimal model

Terms	Type III		
	Wald Chi-Square	df	Sig.
zoo	101.412	4	.000
season	27.849	3	.000
IS	19.698	4	.001
sex	32.975	1	.000

Table 2.10: Factors affecting the percentage time spent resting socially by colobus monkeys

Results are from a GEE model based on time spent resting socially averaged per individual per data collection period from instantaneous state behaviour scan from 37 animals in 5 groups. Percentage time spent feeding was arsined transformed for the analysis.

Season was recorded as spring (March-April), summer (June-August), autumn (October-November) and winter (January). Index of success (IS) was derived from the number of dominant encounter won divided by the total number of dominant encounters and scaled between 0 (dominant) and 1-4 (from most dominant to most subordinate).

IS was a significant factor in the analysis (x^2 = 19.698, df = 4, $p < 0.01$) and significant differences between EM were found between the dominant animals and all other indexes of success (mean differences IS-1: -0.0914, $p < 0.001$; IS-2: -0.1888, $p < 0.001$; IS-3: -0.2535, $p < 0.01$ and IS-3: -0.1847, $p < 0.001$), Figure 2.20.

Finally, sex was a significant factor in the analysis (x^2 = 32.975, df = 1, $p < 0.001$) with significant differences between EM between males and females (mean difference: -0.1963, $p < 0.001$), Figure 2.21.

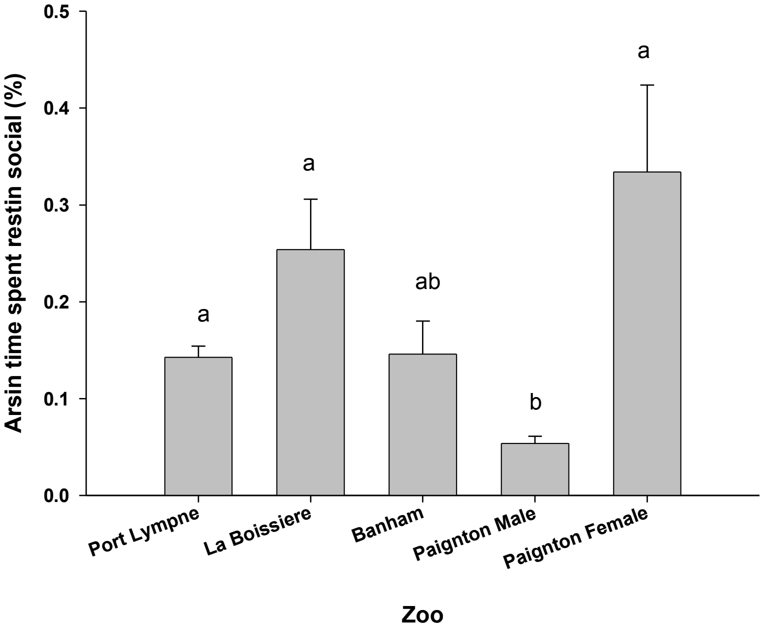

Fig 2.18: Mean (+SE) % time spent resting socially by the groups of colobus monkeys at each zoo. Means and standard errors are predictions from the GEE model presented in Table 2.10, controlling for the variation in time spent performing state behaviour associated with the other significant predictors.

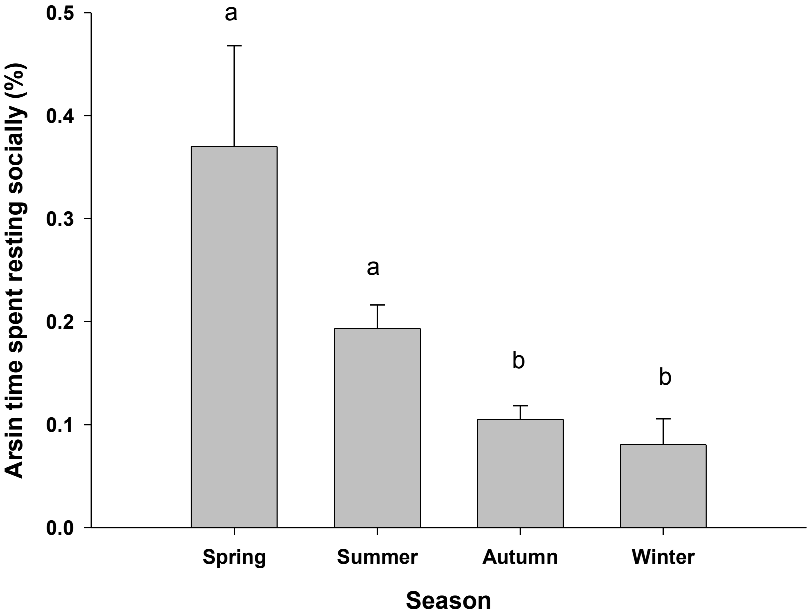

Fig 2.19: Mean (+SE) % time spent resting socially by the groups of colobus monkeys during each season. Means and standard errors are predictions from the GEE model presented in Table 2.10, controlling for the variation in time spent performing state behaviour associated with the other significant predictors.

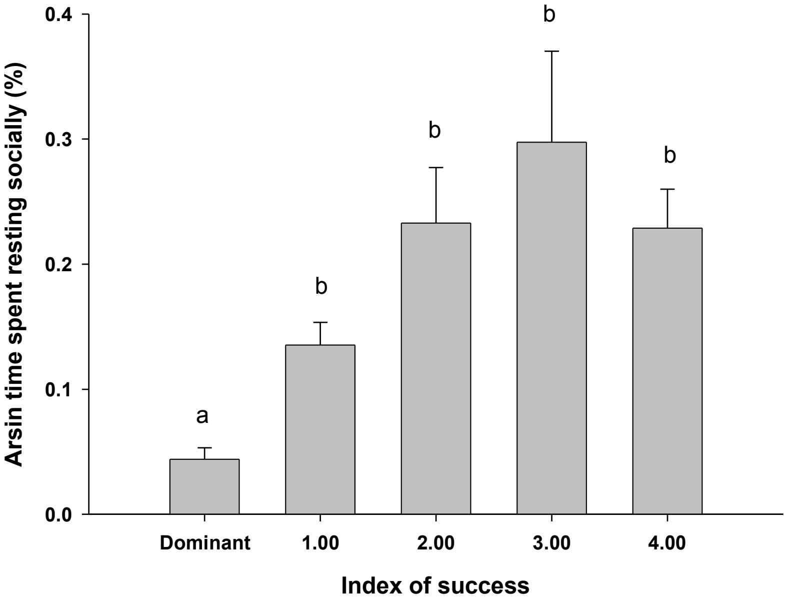

Fig 2.20: Mean (+SE) % time spent resting socially by the groups of colobus monkeys according to index of success. Index of success (IS) was derived from the number of dominant encounter won divided by the total number of dominant encounters and scaled as dominant (the clear overall dominant animals in each group) and between 1-4 (from most dominant to most subordinate). Means and standard errors are predictions from the GEE model presented in Table 2.10, controlling for the variation in time spent performing state behaviour associated with the other significant predictors.

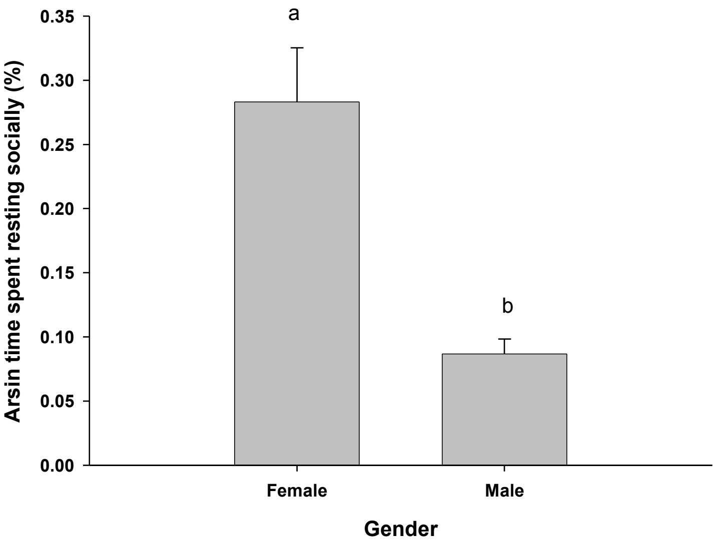

Fig 2.21: Mean (+SE) % time spent resting socially by the groups of colobus monkeys according to gender. Means and standard errors are predictions from the GEE model presented in Table 2.10, controlling for the variation in time spent performing state behaviour associated with the other significant predictors.

2.3.6 Resting alone behaviour

The following analyses (Table 2.11) investigated the possible factors affecting the percentage of time spent resting alone by colobus monkeys at various zoo over repeated data collection periods. Factors with significant power in the model were zoo, season, index of success (IS) and sex.

Full model

Terms	Type III		
	Wald Chi-Square	df	Sig.
zoo	38.967	4	.000
season	21.545	3	.000
IS	36.899	4	.000
sex	11.702	1	.001
age	2.242	1	.134

Minimal model

Terms	Type III		
	Wald Chi-Square	df	Sig.
zoo	42.707	4	.000
season	20.680	3	.000
IS	33.163	4	.000
sex	11.731	1	.001

Table 2.11: Factors affecting the percentage time spent resting alone by colobus monkeys.

Results are from a GEE model based on time spent resting alone averaged per individual per data collection period from instantaneous state behaviour scan from 37 animals in 5 groups. Percentage time spent feeding was arsined transformed for the analysis.

Season was recorded as spring (March-April), summer (June-August), autumn (October-November) and winter (January). Index of success (IS) was derived from the number of dominant encounter won divided by the total number of dominant encounters and scaled between 0 (dominant) and 1-4 (from most dominant to most subordinate).

Contrast analysis was performed in the GEE model by use of estimated marginal means (EM means) on zoo, season, IS and sex. Zoo was a significant factor in the analysis ($x^2 = 42.707$, df = 4, $p < 0.001$) and significant differences between EM were found between the male group at Paignton Zoo and three other zoos: Port Lympne Zoo (mean difference: 0.2203, $p<0.001$), La Boissière du Doré Zoo (mean difference: 0.1967, $p< 0.001$) and Banham Zoo (mean difference: 0.2867, $p < 0.001$), Figure 2.22.

Season was a significant factor in the analysis ($x^2 = 20.680$, df = 3, $p < 0.001$) and significant differences between EM were found between the spring and both summer (mean differences: -0.0832, $p<0.001$) and winter (mean differences: -0.0904, $p < 0.05$), Figure 2.23.

IS was a significant factor in the analysis ($x^2 = 33.163$, df = 4, $p < 0.001$) and significant differences between EM were found between the dominant animals and more subordinate animals with IS-2 (mean difference 0.1666, $p < 0.01$), IS-3 (mean differences: 0.2236, $p < 0.001$) and IS-4 (0.2935, $p < 0.001$), Figure 2.24.

Finally, sex was a significant factor in the analysis ($x^2 = 11.731$, df = 1, $p = 0.001$) with significant differences between EM between males and females (mean difference: 0.1681, $p = 0.001$), Figure 2.25.

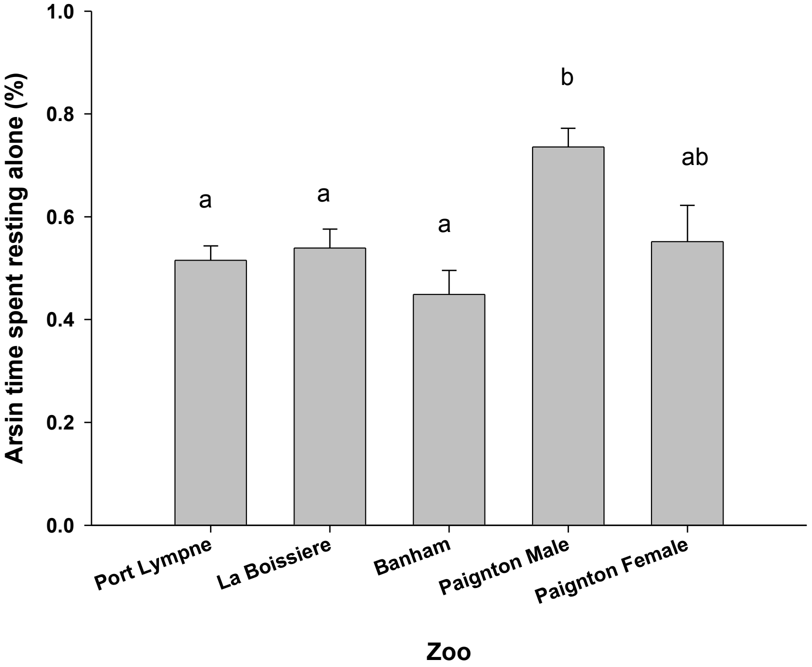

Fig 2.22: Mean (+SE) % time spent resting alone by the groups of colobus monkeys at each zoo. Means and standard errors are predictions from the GEE model presented in Table 2.11, controlling for the variation in time spent performing state behaviour associated with the other significant predictors.

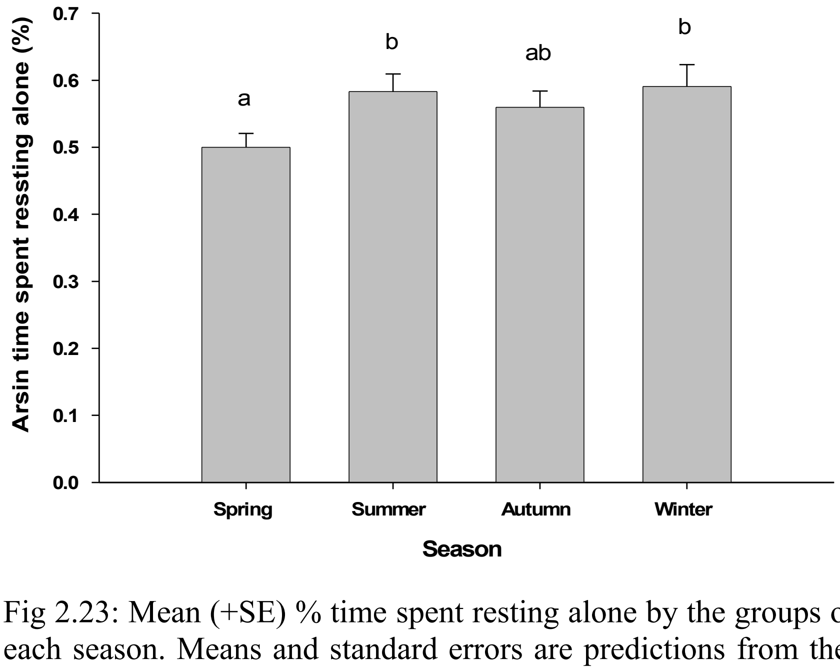

Fig 2.23: Mean (+SE) % time spent resting alone by the groups of colobus monkeys during each season. Means and standard errors are predictions from the GEE model presented in Table 2.11, controlling for the variation in time spent performing state behaviour associated with the other significant predictors.

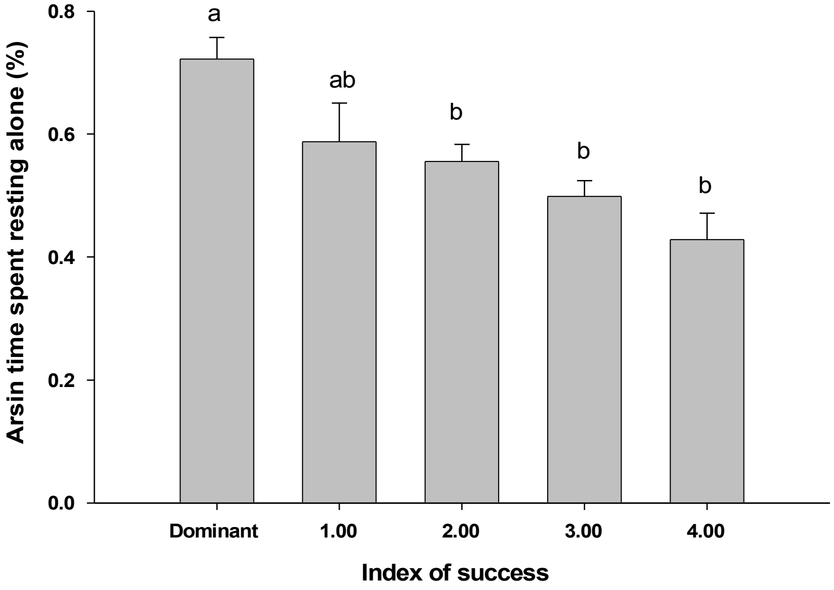

Fig 2.24: Mean (+SE) % time spent resting alone by the groups of colobus monkeys according to index of success. Index of success (IS) was derived from the number of dominant encounter won divided by the total number of dominant encounters and scaled as dominant (the clear overall dominant animals in each group) and between 1-4 (from most dominant to most subordinate). Means and standard errors are predictions from the GEE model presented in Table 2.11, controlling for the variation in time spent performing state behaviour associated with the other significant predictors.

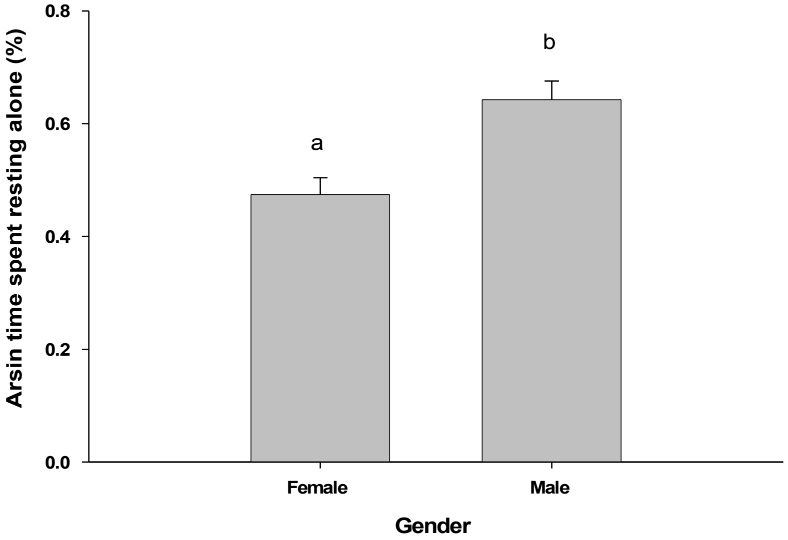

Fig 2.25: Mean (+SE) % time spent resting alone by the groups of colobus monkeys according to gender. Means and standard errors are predictions from the GEE model presented in Table 2.11, controlling for the variation in time spent performing state behaviour associated with the other significant predictors.

2.4 Discussion

2.4.1 Seasonality

Activity budgets of primates in the wild often show some form of seasonal pattern that is linked to food abundance and/or quality as shown in colobines such as the snub-nose monkey (Guo *et al.*, 2007) or the Francois' langur (Zhou, 2007). In captivity, food availability and quality varies greatly from conditions in the wild, as it is provided on a regular basis and has a relatively consistent quality (Crissey & Pribyl, 1997). Therefore, food was not anticipated to be a limiting factor on activity budgets, which were expected to be stable over seasons. However, for colobus monkeys in the present study, more time was spent feeding in the autumn season compared to all other seasons. This was probably due to the availability of extra natural palatable food materials at that time of the year. In autumn, leaves fell and settled on top of the cage (at Port Lympne Zoo, due to the unique design with a caged roof compared to other zoos) or in enclosures surrounded by trees. Animals were able to reach these extra food items, therefore spending significantly more time feeding.

Similarly, differences between time spent resting socially were observed. The highest proportion of time spent resting was in the spring and summer months. In all groups, during warmer periods, the colobus were observed to spend more time resting in the sun in the outside enclosures. This sunning behaviour has been observed in wild groups of colobus monkeys, particularly, first thing in the morning, when they may spend several hours at the top of the trees in the sun resting, and in between feeding bouts during the rest of the day (Estes, 1991; Dasilva, 1993; Fashing, 2001). In tropical habitats, sun seeking or avoidance, has been linked with thermoregulation needs in colobus species (Dasilva, 1993). However, in a temperate climate, temperatures seldom reach extremes. In 2005 and 2006, summers were relatively cool and colobus in the present study always seemed to seek the sun, with no perceived problems of overheating. Other factor at the individual zoo level may have affected the overall difference between resting behaviour across season. For example, at La Boissière du Doré Zoo, the resting behaviour was affected by the social event at the time. In November 2006 (autumn), the group as a whole attacked one of the large adult males (Nimba). This male became very determined to enforce his dominance over the male colobus, Fudgi, and solicited grooming repeatedly, to the point of disturbing the group as a whole, especially during rest. Eventually, after a few days, the group rebelled and attacked him; subsequently he remained on the floor at all times during daily data collection time. At this point, one or more animals (especially the sub-adult males, Abyssian and Camara, and the juvenile female, Maatai, Table 2.3b) would monitor him and if he attempted to move, they would attack as a group (although, the dominant male Ernesto and the adult female Célize never got involved in the fight). Therefore, in the autumn season, little time was spent resting compared to the spring, when the group was settled.

As the amount of time spent on an activity increases, the amount of time spent on others consequently reduces. Dunbar & Dunbar (1988) suggested that in gelada baboons, as feeding time increases, animals reallocate the time they spent performing behaviours by preferentially reducing resting time, and then, if necessary, by reducing time spent in social interactions. This model is in accordance with the present data where, as more time was spent feeding in the autumn season, less time was spent resting socially and being social compared to other seasons such as summer and spring.

Colobus monkeys are unusual amongst primates as they often share the handling of newborn infants (Dunbar & Dunbar, 1976; Oates, 1977; Horwich & Wurman, 1978). The presence of babies in a group affects many aspects of social behaviour due to their considerable interest for many female primate species (Nicholson, 1987). The mother and other members of the groups, mainly females are the main handlers (Horwich & Wurman, 1978), and more rarely males (Horwich & Wurman, 1978; Estes, 1991). Females in a group will show the highest interest in new born infant, with many repeated transfer amongst themselves (Horwich & Wurman, 1978). However, the interest paid to the mother or the baby may not always be in a positive manner, such as increased grooming, and can potentially turn to harassment with a harmful outcome (Zucker & Clarke, 1998). For example, at Port Lympne Zoo, in April 2006, when the female, Ulna gave birth to her baby, she was attacked by the dominant female who then took the baby away, leaving Ulna with a leg injury. In the present study, although handling of newborn infants from females other than the mother was observed, it was not generally a peaceful transfer until after the newborn lost its white colour (after three months).

In spring, few "white" offspring were present (in March 2005: Femur and in April 2006, Ulna's offspring, both at Port Lympne Zoo), most young animals classified into the infant category. These infants were fairly independent from their mothers and interacted in a social manner with all members of the group. In autumn there were two or three “white offsprings” (two from Banham Zoo in 2005/2006, and one from Tibia at Port Lympne Zoo in October 2006). Although much attention was provided to the offspring itself and to the mother, to gain access to the offspring (Zucker & Clarke, 1998), females noticeably preferred to care and rest socially with their newborn alone, avoiding other members of the group. This could be a significant factor explaining the increased time spent resting socially in spring/summer compared to autumn/winter. Additionally, all colobus monkeys showed a significant reduction in social activity from spring to autumn probably due to the arrival of new offsprings in the group. Due to their presence, the adult females were less available for social activity with other observed individuals such as grooming (as most social grooming originates from females in colobus monkeys, Oates, 1977), and were more focused on their young offsprings. The younger females were particularly interested in the presence of “white” offsprings and were often observed attempting trying to hold them, which consequently, reduced the time they had available to spend in social (mainly play) behaviour with the infants.

2.4.2 Influence of age/sex and index of success on activity budgets

As expected, the index of success was highly correlated and older animals tended to be more dominant. Younger colobus monkeys of more subordinate status spent significantly more time moving compared to other animals, while resting less. These patterns are comparable to those previously observed in captivity with Japanese macaques (Jaman & Huffman, 2008) and with colobines in the wild such as the white-headed langur (Li & Rogers, 2004), the Francois’ langur (Zhou *et al*., 2007) and *C. guereza* (Oates, 1977).

Similarly, younger more subordinate animals spent more time feeding than older more dominant ones, which is in accordance with previous studies on Japanese macaques (Jaman & Huffman, 2008) but in contradiction with van Schaik (1983) where subordinate animals were observed to eat less than more dominant animals. Dominant animals are often considered to have priority access to resources (Fashing, 2001) however they often have to assert their status. Competition over resources, being females or food, is essential in determining social dominance hierarchy (Fahsing, 2001; Harris, 2006). During feeding time, the overall dominant animals and those of high dominance status were observed to spend considerable amount of time displacing others (see Chapter 3 section 3.3.1) away from valuable resources (food and/or females), resulting in the less time spent feeding than others.

The time spent resting social was significantly lower for the overall dominant animal compared to others in the group, with more time spent resting alone. Additionally, females generally spent more time resting socially than males. Resting socially is not specifically measured in many species and is more often amalgamated together by researchers who consider rest to encompass animals alone and those in contact with other animals. It was felt in the present study that patterns of social resting could provide an invaluable insight into animal social relationship. Social rest was measured in a study on bearded sakis (*Chiropotes satanas*) in the Eastern Brazilian Amazonia (Silva & Ferrari, 2009). In this study, social rest was recorded as one of the most important behaviours (36 %) contributing to social behaviour. However, no further information was provided regarding links to gender or age. Another study on Sichuan snub-nosed monkeys (*Rhinopithecus roxellana*) where males in one-male sub-units (one male, several females and infants) in larger groups, rested in a solitary manner, while other unit members (females and juveniles) tended to rest socially together (Zhang *et al*., 2006). However no clear measurements of social rest were reported. In a study on wild *C. guereza*, Dunbar & Dunbar (1976) also reported that in small one-male groups, the dominant male would often sit and rest alone, away from females. Similar results for *C. guereza* in the wild were reported by Oates (1977). To my knowledge, this is the first time that clear patterns in social resting have been reported for *C. guereza*, where females spent significantly more time resting socially than males.

2.4.3 Activity budget comparison between zoos

The type of enclosure animals are confined to has an impact on activity budgets (Boere, 2001; Mallapur *et al*, 2004; Mallapur *et al*., 2005, Mallapur, 2005; Jaman & Huffman, 2008). At Banham Zoo, access to a large paddock covered with lush grass, resulted in the colobus monkeys spending considerably more time feeding than at most of the other zoos. Animals at Banham zoo were observed to spend a noticeably larger amount of time on the ground grazing, especially the two adult females (Kivu and Carmen). These behaviour patterns observed at Banham Zoo are comparable to those reported by Jaman & Huffman (2008) on captive Japanese macaques, which spent significantly more time feeding in vegetated enclosures than in non-vegetated enclosures.

Although both groups of colobus monkeys at Paignton Zoo also had access to grassed areas outside, and husbandry regimes were similar to those at Banham (i.e. locked up at night, therefore anticipation of reaching the outside enclosure in the morning), grazing was observed much less frequently at Paignton Zoo. Colobus monkeys at Banham Zoo only received two feeds a day, compared to three at La Boissière du Doré, Paignton Zoo and Port Lympne Zoo. The restricted feeding regime at Banham Zoo may have encouraged animals to spend more time feeding on other available resources such as grass or bamboo often provided in the outside paddock.

Colobus monkeys at Banham Zoo and Port Lympne Zoo spent significantly more time being social than animals at Paignton Zoo all male group. These groups were composed of one or several males with many females, which is the typical social structure of wild *C. guereza* (Estes, 1991). Similarly, the female group at Paignton Zoo also exhibited higher levels of social behaviour than the all male group. The social behaviours in this group were mainly compiled from grooming activities. In many primate species, females are often the initiators of grooming behaviour. This is true of vervet monkeys (Isbell & Young, 1993), in which females form the stable core of the group and remain in their natal group, while males migrate between groups (Seyfarth, 1980). This behaviour pattern is similar in *C. guereza,* in which males migrate between groups while females remain within their natal group (Estes, 1991), and can therefore be expected to be at the core of social interactions in the group. It is not surprising therefore, that, when a group does not include females (such as the Paignton male group), the resulting social behaviour is very different to that seen in a group with many females. The absence of females probably explains the very low level of social interaction among the Paignton Zoo all male group, which spent the least time on social behaviour compared to any other group. Additionally, as the group at Paignton Zoo male group was composed exclusively of males, it is not surprising that animals in this group would spent significantly more time spent resting alone than resting socially compared to other groups, as resting alone was associated with male, while resting socially was found to be more important in females.

Chapman & Chapman's (2000) 'ecological constraint model' suggests that as group size increases, so would the distance travelled to find adequate food supplies, which would be reflected by the allocation of more time to moving. Two of the largest groups (Banham Zoo and Port Lympne Zoo) had the lowest amount of time spent on moving behaviour (while being the two groups who spent the most time actually feeding). In a captive environment, food is not a limiting factor and should have only a partial impact on the activity budgets of colobus monkeys regarding moving behaviour. The current results agree with Isbell (1991), who suggested that when food is abundant and not limiting (i.e. it is provided in sufficient quantity to sustain the needs of all animals), day range (i.e. movement) and time spent feeding do not act as function of group size.

2.4.4 Activity budget in captivity compared to in the wild

C. guereza in the wild have been reported to spend large amounts of time resting, from 57 % in the Kibale forest, Uganda (Oates, 1977) to 64 % in the Kakamega forest, Kenya (Fashing, 2001). In the present study, the mean time spent resting for all zoos was 67 ± 2.6 % (mean ± se, considering all zoos), which is consistent with that observed in wild *C. guereza* of both the Kakamega forest (Fashing, 2001) and the Kibale forest (Oates, 1977). Dasilva (1992) suggested that increased time resting in *Colobus polykomos* on Tiwai Island, Sierra Leone, could be an adaptation to thermoregulation needs, where by reducing the amount of time spent moving or actually feeding, more time could be allocated to resting behaviour and digestion during low food availability. In the present study, the issue of low food availability is not relevant as the animals being studied are in captivity and food is made available to them all year round in adequate quantities. The quality of food provided is relatively constant compared to that in the wild, as diets provided mainly contained vegetables, seeds, a protein source (egg or meat) and ripe fruits rich in sugars and carbohydrates (Milton, 1993). The colobus monkeys held in captivity in zoos may spend a large proportion of time resting, simply because food is provided for them.

In captivity, food is provided by carers at determined times which may alter the time allocated by an animal to feeding and foraging behaviours (Martin & Kitchen, 1996;

Carlstead 1998; Mallapur *et al*., 2005). The mean time spent feeding for all groups in the present study was 20.91 ± 1.9 % (mean ± se) of the day. This is comparable to that of reported values for several groups of *C. guereza,* which spent between 22.9 % and 28.3 % of the day feeding (Fashing, 2001). In the wild, colobus monkeys eat considerable amounts of leaves (Dasilva, 1992), which can make up most of their diets; for example, 40 % of the diet of *C. guereza* in the Kibale Forest was made up of only one species (*Celtis durandii*) of tree leaves (Oates, 1977). Mature leaves are often eaten, although younger leaves are preferred if available, as seen in other folivorous species, such as woolly spider monkeys (*Brachyteles arachnoids)* (Milton, 1983). Animals in the present studies were fed on a diet made up of fruit or vegetable (see Table 2.4). These are high in fibre content, but much easier to digest and chew than the leaves that they would come across and eat in the wild (Crissey & Pribyl, 1997). Therefore, a shorter period of time would be needed to break down the food down before ingestion and digestion. However, in most zoos, extra feeding items in the form of browse (bamboo, lime tree, bark and grass), high in cellulose, were offered to the animals. Milton (1983) found that the time spent feeding by woolly spider monkeys increased when the animals were feeding mainly on mature leaves since these require longer chewing time before swallowing and processing those takes longer. The similarity in time spent feeding between captive and wild animals may be due to the balanced diet, rich in cellulose offered to the colobus monkeys throughout the day.

The values calculated for time spent in social behaviour, were consistent with previous studies of wild animals. When data were pooled for colobus in all groups at all zoos, an average of 6.75 ± 1.2 % (mean ± se) time was spent in social activity. Oates (1977) observed *C. guereza* spending 6 % of their day in social behaviour and Fashing (2001) observed *C. guereza* spending between 5.9 % and 8.3 % in social behaviour (with the latter figures recalculated to match the classification criteria of the present study i.e. by removing categories that were not taken into account in the present study and re-calculating percentage time). In captivity, with an inadequate social composition or impoverished environment, a group of animals may spend considerably less time performing social behaviours compared to groups in the wild, while spending more time resting (Broom, 1991; Carlstead, 1996; Kitchen & Martin, 1996). The similarity of the two datasets for social activity suggests that the general welfare of colobus monkeys in the zoos included in the present study was good.

Similarly, moving behaviour, which occupied 4.78 ± 0.4 % of time (mean ± se value for all groups) in the present study, was comparable to 5 % observed by Oates (1977) and values of 1.8 % and 4.2 % for two groups of colobus monkeys observed by Fashing (2001). It should be noted that the low figure, of 1.8 %, belonged to a group that was noticeably more frugivorous (where fruits composed up to 81 % of the diet) than other *C. guereza* in the study by Fashing (2001). Colobus monkeys in captivity did not follow the ‘ecological constraint model’ (Chapman & Chapman, 2000) which suggests that as group size increases, so would the distance travelled to find adequate food supplies, which would be reflected by the increased time allocated to moving behaviour. In all zoos, sufficient food to cater for all animals in the group was provided, and only limited movement was necessary to reach it, due to the restricting environment of the enclosure.

2.5 Summary & Conclusions

The impact of group composition on activity budgets was evident across state behaviours. Index of success was confounded by age. Younger, more subordinate animals were found to spend more time moving and feeding than others. Conversely, adult more dominant animals spent more time resting, and less time feeding, moving and being social than younger ones. The gender of the animal was important in the time spending resting socially or alone, and consequently the time allocated to moving behaviour. Males spent significantly more time resting alone than females and vice versa. Males also spent more time moving than females.

Some seasonality in state behaviours was observed, often in conjunction with local history event (such as increased feeding in the autumn) or temporary group composition (presence of newborn).

Overall, the comparison of activity budgets between the present study of captive colobus monkeys and previous studies of wild colobus monkeys (Oates, 1977; Fashing, 2001), where age was not taken into consideration and all results were pooled, show a surprising level of agreement and only relatively little variation of time allocation to various state behaviours. The colobus monkeys in the present study follow the typical behavioural profile of other Colobine monkeys and folivorous animals adapted to a diet low in available energy, by displaying limited locomotion, having reduced social behaviour and by spending long periods of rest (Milton, 1983).

Chapter 3
Social dominance

3.1 Introduction

3.1.1 Social dominance

Primates are particularly social animals and a large proportion of them live in groups (Estes, 1991). Inter-group social interactions are often aggressive, as animals defend territories to protect resources, particularly food or mates (Fashing, 2001; Harris, 2006). Intra-group interactions are often more complex, and can be interpreted as affiliative, neutral or agonistic (Singh *et al*., 2003). Affiliative and agonistic encounters are of particular interest as they are an indication to the social relationship between two animals; for dyadic interactions, those involving two individuals.

Dominant animals are often considered to have priority access to resources (Fashing, 2001) and this determines the type and frequency of interactions they have with other group members. They often have to defend their status within a group by displaying to potential challengers that they are fit and healthy, and essentially could win any physical contest; these behaviours are often termed dominant behaviours (Table 3.1). For example, the dominant male colobus monkey has the monopoly on reproduction with exclusive access to females (Oates, 1977, Harris & Montfort, 2003). He would also have to defend and assert his access over mates (Saplosky, 1992). Subordinate animals in turn may adapt their behaviour to gain favour or support from dominant animals during conflicts (Seyfarth, 1977). When two animals encounter each other, it is the subordinate animal which will behave in a way to terminate the encounter such as fleeing or signalling submission; sometimes termed as being displaced (Bernstein, 1981).

Several methods have been established to measure social dominance in a group of animals, these are mostly based on interactions between two animals only (dyadic interactions). In most cases, the social status of an animal, relative to others in a group, is determined by the outcome of social encounters and can be measured by the number of encounters in which it displaces (is dominant) or is displaced (is subordinate) another animal; simply the number of displacements it receives and gives (Izawa & Watanabe, 2008). This method has been used in a wide variety of non-human primate studies, for example, the rhesus monkey, *Macaca mulatta* (Bernstein & Ehardt, 1985), the Sulawesi crested macaque, *Macaca nigra* (Reed *et al*., 1997), the Japanese macaque, *Macaca fuscata* (Nakamichi *et al*., 1995), the gentle lemur, *Hapalemur alaotrensis* (Waeber & Hemelrijk, 2003), the brown capuchin, *Cebus apella (*Pannozzo *et al*., 2007), and the Assamese macaques, *Macaca assamensis* (Cooper & Bernstein, 2000).

3.1.2 Social dominance hierarchy

The dominance status of animals in a group can be classified into hierarchical systems, of which there are three main categories. In the *despotic system*, one animal dominates every other member of the group and there is no difference in rank amongst other animals, while in the e*galitarian system* every animal is as likely to win or loose an encounter with another member of the group. In contrast, in the l*inear system,* there is a linear order of ranked animals, from the most dominant to the most subordinate.

The strength of hierarchy in social dominance can be determined by several methods. The first one was developed by Landau (1951 a & b), as described in Equation 3.1. This method calculates the degree of linearity of the dominance hierarchy using the number of individuals in a group, compared to the number of individuals each animal dominates.

$$h = [12 / (n^3 - n)] \sum_{a=1}^{n} [V_a - (n - 1) / 2]^2 \qquad \text{Equation 3.1}$$

h is the strength of hierarchy, *n* is the number of individuals in the group and *Va* is the number of individuals in the group which the a^{th} animal dominates. The addition of the constant $[12/ (n^3 - n)]$ to Equation 3.1 permits a value of between 0 and 1 to be obtained, where 0 represents no linearity in dominance rank hierarchy and 1 represents a perfect linear dominance rank hierarchy. However, this method does not take into account missing data cells in dominance matrices, which occur when two animals are not observed to interact in a dominant/submissive manner. Neither does it encompass dominance reversals, which occur when a normally subordinate animal manages to win an encounter with a usually more dominant animal. By assuming that in dyadic interactions, when animal A dominates animal B, it does so *all* the time (i.e. with no reversal), data analysed using the Landau method can overinflate linearity, resulting in a high Landau index (h) (Singh *et al.*, 2003).

Kendall's coefficient (Kendall, 1962), measures the degree of linearity in a social dominance hierarchy within a group of animals, based on circular triads (where animal A dominates B, B dominates C, and C dominates A), rather than dyadic interactions. The number or circular triads in a group is calculated using Equation 3.2.

$$[n\ (n-1)\ (2n-1)/12] - (0.5) \sum_{i=1}^{n} (S_i)^2 \qquad \text{Equation 3.2}$$

where S_i is the total number of individuals that the ith individual dominates over, and n is the number of individuals in the group. The index of linearity, K, is then given by Equation 3.3 when n is even and Equation 3.4 when n is odd (Kendall, 1962).

$$K = 1 - [24\ (\text{number of circular triads})] / \ n^3 - 4n \qquad \text{Equation 3.3}$$

$$K = 1 - [24\ (\text{number of circular triads})] / \ n^3 - n \qquad \text{Equation 3.4}$$

In Kendall's method, the information on the number of dominant interactions between a pair of individuals is not used, therefore limiting its use in large groups (Kasuya, 1995). De Vries (1995) suggested that Landau's method is preferable to Kendall's if tied dyads occur, i.e. if two animals show the same amount of dominant behaviour to one another.

Appleby (1983) developed the approach used by Kendall but incorporated investigation of statistical significance of a hierarchy by constructing a matrix of interactions (by looking at all possible interaction between all dyads). This method also dealt with the issue of missing data cells, where no interaction between individuals was observed. The null hypothesis for the test of linearity of a dominance hierarchy is that the relationship between any two individuals predicts nothing about the relationships that each has with others (Appleby, 1983). In Appleby's test of linearity, dominant animals (based on pre-defined dominant behaviours that vary between species) are allocated a value of 1, subordinates have a value of 0, and where no interactions are observed, a value of 0.5 is assigned, which represents an equal chance of winning an interaction for both animals. The obvious problem with this method is that the probability of the two individuals assigned a score of 0.5 winning an encounter may not necessarily be equal. An animal dominant over high-ranking animals may be more likely to be dominant over more subordinate, even if an encounter has not been observed.

Rather than assigning a value of 0.5 to missing data cells, Singh *et al*. (2003), calculated a corrected Landau's index (*h'*) by calculating the outcome probability for empty cells in the matrix. In cases where dyadic interactions had not been observed, the percentage chance of winning or losing the encounter was calculated by using data from encounters with other members of the group. This method takes account of reversal of dominance, and therefore gives a more accurate estimation of linearity than Landau's index, Kendall's coefficient or Appleby's test of linearity. In colobine species (Borries *et al*., 1991; Koenig *et al*., 2004) and howler monkeys, *Alouatta palliate* (Jones,1980), reversal of dominance seems to be regularly observed, whereas in macaques (Nakamichi *et al*., 1995, Reed *et al*., 1997) or vervet monkeys *(Cercopithecus aethiops)*, these are rare or absent (Isbell & Young, 2002). The corrected Landau's index (Singh *et al*., 2003) has been used successfully in several analyses, including studies of the bonnet macaque, *Macaca radiate* (Cooper *et al*., 2004), the lion-tailed macaque, *Macaca silenus* (Singh *et al*., 2006) and the Assamese and rhesus macaques (Cooper & Bernstein, 2000).

The use of matrices is a powerful way of tabulating social dominance as it takes into account interactions between each opponent and gives an accurate description of the dominance hierarchy (Poisbleau *et al*., 2006). In the present study, all animals were in captivity and easily identified, so all animals were included in the matrix and dominance linearity was calculated using Singh's methods (Singh *et al*., 1992; Singh *et al*., 2003).

3.1.3 Linear dominance hierarchy

Linear systems are the most common type of hierarchy observed in non-human primate species. For example, Cooper & Bernstein (2000) found that in both Assamese and rhesus macaques, mixed-sex and single-sex dominance hierarchies showed high degree of linearity. Female blue monkeys (*Cercopithecus mitis stuhlmanni*) were found to compete aggressively and displayed linear dominance hierarchy (Pazol & Cords, 2005). Studies of colobine monkeys have also shown linearity in dominance hierarchy of the Hanuman langurs, *Presbytis entellus* (Borries *et al*., 1991) and the Phayre's leaf monkey, *Trachypithecus phayrei* (Koenig *et al*., 2004). However, no hierarchy was observed in a captive group of female *Colobus guereza* (Grunau & Kuester, 2001). This is in marked contradiction with the study on wild *C. guereza* where animals were ranked in a linear dominance hierarchy (Harris, 2006). Differences in dominance hierarchy patterns of captive and wild populations have been observed in other species of monkeys; for example, Liu (1989) reported linear dominance hierarchies in an all male group of captive Sichuan snub-nose monkeys (*Rhinopithecus roxellana).* Males of this species, in the presence of females, also showed high levels of aggression towards other males with a high frequency of fighting (Shi *et al*., 1982). These observations are in marked contradiction with behavioural patterns observed in groups of wild Sichuan snub-nose monkeys, where there was no clear dominance hierarchy and agonistic interactions were rarely observed (Ren *et al*., 1998). However, another study (Zhang *et al*., 2008) examined several all male groups of Sichuan snub-nose monkeys in the wild and found strong linear dominance hierarchies in all groups. Therefore dominance hierarchies, whether in the wild or in captive groups seem to vary even within a particular species.

3.1.4 Social dominance hierarchy, life history and social behaviours

An individual's dominance rank relative to other individuals in its social group and the strength of linearity of the group's hierarchy are related to many aspects of social behaviour (Hemelrijk & Thierry, 2003; Hemelrijk *et al*., 2005). The study of dominance rank can therefore provide a useful tool to explore social interactions in primates (Bernstein & Cooper, 1999; Koening *et al*., 2004; Klinkova *et al*., 2005; Garcia *et al*., 2006; Paoli *et al*., 2006; Gould & Ziegler, 2007; Hanya *et al*., 2007).

3.1.4 a Social dominance hierarchy in relation to gender

Mammalian females have often been reported to be less aggressive than males (Smuts & Smuts, 1993; Pazol & Cords, 2005). However, this pattern seems to vary from species to species, and depends on the social composition of the group, the type of social dominance hierarchy and the dominance relationship between males and females. For example, Teas *et al*. (1980) found that male rhesus macaques were more aggressive than females, whereas Anderson *et al*. (1972) reported more contact aggression between female pigtail macaques than males.

In primates, in mixed sex groups, dominance over the group is often attributed to the alpha male. However, this is not always the case, as shown by many species of lemurs, where females have consistently been found to be dominant over males (Raps & White, 1995; Pereira & Kappeler, 1997; Waeber & Hemelrijk, 2003).

Social dominance in captive and wild primates is often investigated by considering one sex at a time (Klinkova *et al*., 2005; Garcia *et al*., 2006; Gould & Ziegler, 2007; Hanya *et al*., 2007). However, females' dominance over some males (known as female co-dominance) in typically male-dominated species has been observed (Hemelrijk *et al*., 2003). In species where dominant behaviours are performed and received by both males and females, a mixed sex social dominance hierarchy can be obtained. For example, this has been found in the bonobo, *Pan paniscus* (Paoli *et al*., 2006) and the stumptail macaque, *Macaca arctoides (*Butovskaya & Kozintsev, 1996).

The typical group composition of *Colobus guereza* in the wild is one male with several females and their offsprings (Dunbar & Dunbar, 1976; Estes, 1991; Krüger *et al*., 1998). However, multi-male, multi-female groups have also been observed (Dunbar & Dunbar, 1976; Oates, 1977; Estes, 1991; Harris & Montfort, 2006). In captivity, group composition is dependant upon management decisions and practicalities; therefore, multi-males, multi-females or single sex groups are often encountered. Husbandry practices would therefore be expected to affect the social dominance hierarchy due to the differences between sexes regarding competition. For males, reproduction is a prime consideration and male fitness determines mating and fertilization success. Access to mates can lead to intense and frequent aggressive encounters between males (Smuts and Smuts, 1993), which would lead to a strongly linear social dominance hierarchy. Competition amongst females is more often linked to food resources (Silk 1993). For example, Koening *et al*. (2004) found that agonistic interactions in female Phayre leaf monkeys occurred more frequently in the presence of food.

In the wild, as leaves are dispersed and abundant, it may not be a limiting factor affecting competition and leading to the creation of a dominance hierarchy (McKenna, 1979). In a zoo environment, little effort is required to obtain food that is plentiful and regularly provided by keepers (Abbot *et al*., 2003) therefore limiting the "clumping" effect of food availability, which favours contest competition and therefore, strong linear hierarchies. However, food offered to the captive animals is not always in the form of leaves but also includes fruit, vegetables and highly desirable items such as eggs or cheese (Chapter 2, Table 2.4). These food items are scarce and discreetly distributed, and may lead to competition and the creation of dominance hierarchies. For example, in captivity, Scott & Lockard (2006) found that high ranking female gorillas (*Gorilla gorilla*) monopolized food patches and restricted the access of low ranking females to the food with threat vocalizations or by chasing them away.

3.1.4 b Social dominance hierarchy and life history

Species life history is an important factor in determining social dominance rank in many primates and more generally mammals (Bernstein & Cooper, 1999; Koening *et al*., 2004). Social dominance rank has been linked with intrinsic factors such as age in a variety of mammals (for both males and females). In many species, for example brown capuchin, the older the animal gets, the more dominant it becomes (Bernstein & Cooper, 1999). Different patterns have also been observed in other species, notably female Phayre's leaf monkeys, where young adult females held top rank, old adult females were of middle rank and the youngest females were at the bottom of the social dominance hierarchy (Koening *et al*., 2004). However, kinship, based on family status instead of individual age can influence the outcome of interactions between females (Datta & Beauchamp, 1991; Nakamichi *et al*., 1995; Kutsukake, 2000). Closely related animals may have inherited dominance status, which would affect their likelihood of winning an encounter with, for example, another animal from a lower ranking family (e.g. the Japanese macaque, *Macaca fuscata,* Kutsukake, 2000).

3.1.4 c Social dominance hierarchy and grooming behaviour

In primates, social behaviour between individuals in a group, such as grooming, is often associated with social dominance rank. Waeber & Hemelrijk (2003) found that dominant female gentle lemurs were groomed more often by males than lower ranking females. In another study, dominant brown capuchin monkeys were found to spend significantly more time grooming subordinates (Parr *et al*., 1997). In Assamese macaques, females directed more grooming towards subordinate females, and thus appeared to 'groom down the hierarchy' (Cooper & Bernstein, 2000). In an earlier study, Bernstein & Cooper (1999), found no correlation between social dominance rank of Assamese macaques and the directionality of grooming by females, while males tended to groom dominant animals ('groom up the hierarchy'). However, these results were confounded by age, as the more dominant males were older while the males who performed the majority of grooming interactions were younger.

Some studies have found no relationship between social dominance and grooming. For example, Sambrook *et al*. (1995) found no relationship between grooming and dominance rank of female olive baboons in a large group, although in small troops, the higher ranking individuals received more grooming. Similarly, blue monkey females (Pazol & Cords, 2005) or mixed sex groups of captive Sulawesi crested black macaques (Bernstein & Baker, 1988) showed no correlation between social dominance hierarchy and grooming behaviour. However in another study high-ranking Sulawesi crested macaque males received more grooming from adult females (Reed *et al*., 1997).

Therefore, results seem to be difficult to interpret and often contradictory depending on species or study sites. No clear pattern exists to predict directionality of grooming behaviour across all primate species. Very often, correlations between dominance and various aspects of behaviours are reported, but these are often confounded by factors like age and sex (Bercovitch, 1997). For example, in captive female *Colobus guereza*, the youngest and oldest females were the least active groomers (Grunau & Kuester, 2001). However, females distributed their grooming equally between all other females, showing no kin or demographic preferences and all grooming was bidirectional (Grunau & Kuester, 2001).

The aim of the studies reported in this chapter was to investigate social dominance rank and the linearity of the hierarchy in the five groups of colobus observed. Social dominance hierarchy in mixed sex groups in captivity was expected to be linear, matching conditions met in the wild (Harris, 2006). In the all female group at Paignton zoo, no linearity was expected as females have been reported to follow an egalitarian social dominance (Grunau & Kuester, 2001). Behavioural and life history data were expected to influence dominance ranking. Age was expected to influence ranking order with older individuals being more dominant than younger individuals. Social behaviours were expected to vary according to social ranking. High ranking individuals were expected to be more attractive as recipients of grooming, due to the prospect of support they may offer during agonistic interaction (Seyfarth, 1977).

3.2 Methodology

3.2.1 Dominance related activities

Social interactions between all possible dyads, where a clear outcome of dominant and subordinate behaviour could be established were collected opportunistically throughout the day (from 08:00 until 17:00), using the descriptors in Table 3.1 (ethogram). Information recorded included, the initiator, the target individual, the behaviour and the result of the encounter. Dominant animals were described as those which initiated displacement, threats and aggressive encounters resulting in the target moving away from or showing submissive behaviour to the dominant animal (as described by Bernstein, 1981). Subordinates were described as those that received displacement, threats and aggression and either moved away from the initiator or showed submissive behaviour (Table 3.1).

Behaviour such as social mounting, forward mount ("a standing individual forces the other's head down with its hand, then extend its face and chest over the other's back with its pelvic area over the recipient's head", Hprwich & Wurman, 1978), instantaneous presenting (not as a result of a threat behaviour), threat with no clear reaction, or fight with no clear winners were not used in the creation of a dominant/submissive matrix as their implications were ambiguous.

3.2.2 Focal sampling of behaviours

Each day, two or three 10 min focal follows were performed per animal. An average of 43 (sd ± 4) follows per animal per visit was conducted. Focal follows were randomly assigned to individuals and balanced evenly across the hours of the day. During each 10 mins focal follow, instantaneous sampling every 15 s was used to record behaviour, as outlined in the ethogram shown in Table 3.2.

Behaviour displayed by dominant animals, all resulting in the target moving away:	
Displacement	The initiator moves towards or pushes the target, and takes over its emplacement
Open mouth, stare threat	The initiator opens its mouth briefly while staring at the target
Gape, stare threat	The initiator opens its mouth for at least a second while staring at the target
Yawn threat	The initiator yawns, exposing its teeth towards the target
Forward threat	The initiator crouches on hands and feet with the head extended, and may stare towards the target
Tongue clicking threat	The initiator makes a clicking noise with its tongue towards the target, and may stare
Penile display threat	The initiator sits with its legs extended, penis erect in plain view of target, and may stare towards the target
Stiff legs display threat	The initiator sits with its ankles hanging down, legs half bent towards the target, and may show a penile display, stare and yawn
Fight	Two animals are in physical aggressive contact with hair erect and may include biting
Behaviour displayed by subordinate animals:	
Moving away	The target distances itself from the initiator
Grimace	The target retracts its lips, showing teeth, often accompanied with squealing and crouching
Presenting	The target lowers its body, presenting its ano-genital area to the initiator

Table 3.1: Ethogram of dominant and submissive behaviours recorded and used in the establishment of the social dominance hierarchy.

Autogrooming	An animal cleans its fur and skin by removing foreign objects such as insects, ectoparasites, dead skin, leaves, dirt and twigs.
Allogrooming	Same as above but between two animals where one performs the cleaning and the other receives it
Sex	A male elicits mounting from a female or a male is presented by a female, both followed by copulation
Play	Physical contact between animals such as tumbling, wrestling or hugging
Aggressive Chase	An animal pursues another, accompanied by threat vocalization, baring teeth and jumping with loud landings
Playful chase	An animal pursues another, between play bouts
Rest	An animal is stationary, sitting or sleeping in no physical contact with other members if the group
Rest social	An animal is stationary, sitting or sleeping in physical contact with other members of the group, excluding tails
Move	An animal is in motion to cover any distance, but not in a social manner such as aggressive or playful chasing
Feed	An animal is eating food or searching for it
Other	Any behaviour not mentioned above and described individually
Out of sight	When a physical barrier prevented observation of the animal

Table 3.2: Ethogram of behaviours recorded during focal sampling.

3.2.3 Data Analysis

3.2.3 a Social dominance hierarchy

Matrix tables were created, including all members in a group except infants. All submissive/dominant behaviours were tallied and compiled in the matrix. Only encounters between two animals were used; encounters involving three or more animals would affect the dyadic context of the matrix system (Singh *et al*., 2003), but were very rare.

Dominance linearity was calculated using the method described by Singh *et al.* (1992) in which the strength of linearity is calculated by working out the proportion of encounters won by an individual, in matrices without empty cells. In most matrices, some pairs of animals never expressed or received dominant/subordinate interactions, or the outcome of these interactions was unclear. In these cases, the Equation 3.5 from the method described by Singh *et al*. (2003) was followed to calculate the strength of hierarchy (h'):

$$h' = [12 / (n^3 - n)] \sum_{a=1}^{n} [d_a - (n - 1) / 2]^2 \qquad \text{Equation 3.5}$$

Where $d_a = \sum_{a=1}^{n} P_a$ and P_a is the proportion of encounters won by an individual in a dyadic interaction. d_a is the total proportion of encounter won by an individual. In cases where dyadic interaction had not been observed, the percentage chance of winning or loosing the encounter was calculated using Equation 3.6.

$$P_a = d_a \text{ individual a} / (d_a \text{ individual a} + d_a \text{ individual b}) \qquad \text{Equation 3.6}$$

where P_a refers to the probability of winning an encounter for individual *a* over individual *b*, using the original d_a in the matrix before adjusting for added proportions:

Once these values were calculated, they were added to the actual observed d_a values to give a new total $d_a(2)$ value, where all cells of the matrix had then been taken into consideration. Once the adjusted d_a value was evaluated, strength of linearity (h) could be calculated using Equation 3.5. Table 3.3 shows an example of data used in calculating the strength of the dominance linearity (h').

Animal displacing	Animal being displaced												
	Panda	Katie	Radius	Ulna	Fibula	Tibia	Cranium	Tarsal	Dermis	d_a	$d_a(2)$	$[da-(n-1)/2]^2$	
Panda		2/2=1	19/19=1	1/1=1	15/15=1	4/4=1	8/8=1	11/11=1	4/4=1	8.00	8.00	16.00	
Katie	0/2=0		5/(5+6) =0.45	1/1=1	5/(5+3) =0.63	1/1=1	6/6=1	3/3=1	7/7=1	5.00	6.08	4.32	
Radius	0/19=0	6/(6+5) =0.55		4/4=1	11/11=1	3/3=1	3/3=1	10/10=1	3/3=1	6.00	6.55	6.48	
Ulna	0/1=0	0/1=0	0/4=0		0/2=0	4/4=1	4/4=1	9/10=0.9	1/1=1	3.90	3.90	0.01	
Fibula	0/15=0	3/(3+5)=0.37	0/11=0	2/2=1		1/1=1	3/(3+1) =0.75	7/7=1	3/(3+0.33)=0.9	3.00	5.03	1.05	
Tibia	0/4=0	0/1=0	0/3=0	0/4=0	0/1=0		4/4=1	8/8=1	4/4=1	3.00	3.00	1.00	
Cranium	0/8=0	0/6=0	0/3=0	0/4=0	1/(1+3) =0.25	0/4=0		0/2=0	1/1=1	1.00	1.25	7.56	
Tarsal	0/11=0	0/3=0	0/10=0	1/10=0.1	0/7=0	0/8=0	2/2=1		2/3=0.66	1.77	1.77	4.99	
Dermis	0/4=0	0/7=0	0/3=0	0/1=0	0.33/(0.33+3)=0.1	0/4=0	0/1=0	1/3=0.33		0.33	0.43	12.72	
											Total	**54.14**	**h'=0.902**

Table 3.3. Example of dominance matrix at Port Lympne, October 2006. Panda displaced Radius 19 times out of 19, therefore giving a P_a of 1. D_a in the black font is the original sum of each row of P_a excluding empty cells. D_a in the blue font is the adjusted d_a after filling the empty cells by calculating its probability based on the original d_a (cells filled with blue calculations). h' is the measure of the strength of linearity calculated using Equation 3.5 (Singh *et a.l,*2003).

3.2.3 b Index of success in relation to age, sex, linearity of the hierarchy and social behaviours

Because each zoo was composed of different number of animals, ranking itself was not comparable across zoo. Instead, an index of success was derived from dominant aggressive interactions between animals. The number of encounter won by an animal was divided by the total amount of encounter involving that animal, giving a value between 0 and 1, where 1 was most successful. These results were then attributed to an index of success (IS) category from 1 to 4 (IS-1 = 0.75 to 1, IS-2 = 0.5 to 0.74, IS-3 = 0.25 to 0.49 and IS-4 = 0 to 0.24). Overall dominant animal, showing a clear distinction from the rest of the group, were attributed a score of 0 to differentiate them from the rest.

To investigate factors affecting the index of success across zoo, a Generalised Estimated Equation (GEE) model was performed (PAWS 18). A multinomial distribution was attributed to IS with a cumulative logit link function (i.e. multinomial ordinal logistic regression). The repeated subject variable was defined by the individual and the zoo identity. The within subject variable was identified by repeated data collection period (i.e. visit). The dependant variable was defined as IS results. Predictors were entered as factors and covariate. The only factor was gender. Covariates were age (in months), the linearity of the hierarchy (see Table 3.4) and social behaviours, including grooming provided or received, play behaviour and social rest. These behaviours were recorded every 15 s for each 10 min focal follow, added up and divided by the number of scans over data collection period to provide a value for relative time spent performing the behaviours per visit.

Initially, all predictors were included in the model as a main effect. Two-way interactions between terms were tested for significance, with the exception of the interaction between age and index of success as these were tightly correlated (Figure 2.1). The interaction between age and play behaviour was included, as younger animals spend typically more time playing than older ones. The interaction between sex and grooming provided or received was also included in the model as female primates tend to be more involved with grooming behaviour than males. Factors and covariates were then subsequently dropped from the model if lacking significance so the model only contained those terms which were significant yielding a 'minimal model'. Due to the multinomial nature of the data, a hybrid method of parameter estimation starting with the Fisher scoring, then switching to the

Newton-Raphson method was employed. The scale parameter method was employed using a fixed-value. Statistics were performed with Type III analysis and 95% confidence interval level computing Wald statistics. Due to the multinomial nature of the data, contrast analysis was not possible in the present logistic GEE model.

3.3 Results

3.3.1 Dominance matrices and hierarchy indices

Matrices at Port Lympne Zoo are presented in Figures 3.1 a-d and Figures 3.2 a-c. The corrected Landau's indices (*h*') were high during all seven data collection periods (summarized in Table 3.4), with low overall levels of reversals (2.39 %). Throughout the entire two year study, the male (Panda) remained dominant. Similarly, the four most subordinate animals in this group remained stable within the hierarchy. Most of the variation in rank position within the group occurred between the adult males and females (Chapter 2, Table 2.2a), excluding the dominant male.

Matrices at Banham Zoo are presented in Figures 3.3 a-b. Both data collection periods showed high values of linearity (*h'* = 0.969 in March 2005 and *h'* = 0.888 in October 2005, Table 3.4) with low overall levels of reversals (1.80 %). In both cases, the adult male (Steffan) was dominant. In March 2005, a dominance reversal was observed between the adult females Kiwi and Carmen (see Figure 3.3 a), and in October, these two animals had swapped dominance ranks. The three younger animals remained consistent in their ranking order.

Matrices at La Boissière du Doré Zoo are presented in Figures 3.4 a-b. This group displayed the highest linearity found in the five groups (*h'* = 0.966 in November 2006 and *h'* = 1 in March 2006) and had a stable hierarchy with only one swap of rank order between the two data collection periods (Table 3.4). The male (Ernesto) remained dominant on both occasions. Most dominant interactions (40 % in March 2006 and 66 % in November 2006) were between the three adult males (Figures 3.4 a-b).

a: March 2005	Animal being displaced								
Animal displacing	Panda	Radius	Katie	Ulna	Fibula	Tibia	Cranium	Tarsal	Dermis
Panda(M)		31	4	1	9	9	8	1	1
Radius(M)	2			2	9	8	6	1	2
Katie(F)				9	13	17	4	2	5
Ulna(F)					1		1	2	
Fibula(M)				2			2	1	1
Tibia(F)							1		
Cranium(M)						1			
Tarsal(M)									
Dermis(F)									

b: July 2005	Animal being displaced								
Animal displacing	Panda	Katie	Radius	Ulna	Fibula	Tibia	Cranium	Tarsal	Dermis
Panda(M)		1	9	4	3	13	2	1	2
Katie(F)				12	3	20	2		2
Radius(M)				2	9	5	3	1	4
Ulna(F)					8	1			
Fibula(M)			1			7	1	1	
Tibia(F)					2				
Cranium(M)				1					
Tarsal(M)									
Dermis(F)									

c: October 2005	Animal being displaced								
Animal displacing	Panda	Katie	Radius	Ulna	Fibula	Tibia	Cranium	Tarsal	Dermis
Panda(M)		1	24	6	13	11	7	6	11
Katie(F)			2	8	4	12	4	1	3
Radius(M)				1	21	7	8	6	
Ulna(F)					4	2	3	2	1
Fibula(M)	1			1		5	4	2	2
Tibia(F)									1
Cranium(M)									1
Tarsal(M)									2
Dermis(F)								1	

d: January 2006	Animal being displaced								
Animal displacing	Panda	Radius	Katie	Fibula	Ulna	Tibia	Cranium	Tarsal	Dermis
Panda(M)		28		20	5	22	7	6	3
Radius(M)	1			6	13	4	5	6	
Katie(F)						1	9	12	4
Fibula(F)					3	8	3	8	1
Ulna(F)				3		2	1	2	
Tibia(F)	1						4	3	2
Cranium(M)								1	
Tarsal(M)		1							
Dermis(F)									

Fig 3.1 a-d: Dominance matrices based on all observations of displacement behaviours in March 2005, July 2005 October 2005 and January 2006 at Port Lympne Zoo. Panda is most dominant with decreasing social rank order left to right to Dermis, the most subordinate. (M = male, F = female).

a: April 2006

Animal being displaced

Animal displacing	Panda	Radius	Katie	Fibula	Ulna	Tibia	Cranium	Tarsal	Dermis
Panda(M)		11		11	1	14	9	6	11
Radius(M)				18	1	4	5	4	3
Katie(F)					2		3	1	4
Fibula(M)		2				3	4	6	6
Ulna(F)						3	1	1	3
Tibia(F)	1				1		8	1	2
Cranium(M)								3	1
Tarsal(M)									2
Dermis(F)				1					

b: July 2006

Animal being displaced

Animal displacing	Panda	Radius	Fibula	Katie	Ulna	Tibia	Cranium	Tarsal	Dermis
Panda(M)		40	4	1	1		3	2	3
Radius(M)			9		4	4	4	5	1
Fibula(M)	1			1	3	4	6	4	7
Katie(F)					6			3	
Ulna(F)						1	1	1	1
Tibia(F)							3	2	2
Cranium(M)								3	4
Tarsal(M)									
Dermis(F)									

c: October 2006

Animal being displaced

Animal displacing	Panda	Radius	Katie	Fibula	Ulna	Tibia	Tarsal	Cranium	Dermis
Panda(M)		19	2	15	1	4	11	8	4
Radius(M)				11	4	3	10	3	3
Katie(F)					1	1	3	6	7
Fibula(M)					2	1	7		
Ulna(F)						4	9	4	1
Tibia(F)							8	4	4
Tarsal(M)					1			2	2
Cranium(M)									1
Dermis(F)							1		

Fig 3.2 a-c: Dominance matrices based on all observations of displacement behaviours in April 2006, July 2006 and October 2006 at Port Lympne Zoo. Panda is most dominant with decreasing social rank order left to right to Dermis, the most subordinate. (M = male, F = female).

Visit	Port Lympne Zoo	***h***'
03-05	*Panda>Radius*>Katie>Ulna>*Fibula*>Tibia>*Cranium>Tarsal*>Dermis	**0.953**
07-05	*Panda*>Katie>*Radius*>Ulna>*Fibula*>Tibia>*Cranium>Tarsal*>Dermis	**0.918**
10-05	*Panda*>Katie>*Radius*>Ulna>*Fibula*>Tibia>*Cranium>Tarsal*>Dermis	**0.931**
01-06	*Panda>Radius*>Katie>*Fibula*>Ulna>Tibia>*Cranium>Tarsal*>Dermis	**0.890**
04-06	*Panda>Radius*>Katie>*Fibula*>Ulna>Tibia>*Cranium>Tarsal*>Dermis	**0.879**
07-06	*Panda>Radius>Fibula*>Katie>Ulna>Tibia>*Cranium>Tarsal*>Dermis	**0.857**
10-06	*Panda>Radius*>Katie>*Fibula*>Ulna>Tibia>*Tarsal>Cranium*>Dermis	**0.902**

Visit	Paignton Zoo all male group	
08-05	*Millo>Fergus>Hope*	**0.750**
03-06	*Fergus>Millo>Hope*	**0.444**
10-06	*Millo>Kabul>Hope>Fergus*	**0.900**

Visit	Paignton Zoo all female group	
06-05	Madonna>Denny>Jhazzie>Salassie>Lou>*Joe*	**0.780**
03-06	Jhazzie>Denny>Lou>Salassie>*Joe*	**1.000**
10-06	Denny>Lou>Salassie>*Joe*	**0.778**

Visit	Banham Zoo	
03-05	*Steffan*>Kivu>Carmen>Kiwi>*Monty*>Sophie>Suzie	**0.969**
10-05	*Steffan*>Kivu>Kiwi>Carmen>*Monty*>Sophie>Suzie	**0.888**

Visit	La Boissiere du Dore Zoo	
03-06	*Ernesto>Fudgi>Nimba*>Celize>*Camara>Abyssian*>Maatai	**1.000**
11-06	*Ernesto>Nimba>Fudgi*>Celize>*Camara>Abyssian*>Maatai>*Swahili*	**0.966**

Table 3.4: Summary of dominance order (most dominant on the left to most subordinate on the right) and hierarchy indices (*h*') (Singh *et al.,* 2003) at all zoos during each data collection period. Names in italic are males.

Animal being displaced

Animal displacing

a: March 2005	Steffan	Kivu	Carmen	Kiwi	Monty	Sophie	Suzie
Steffan (M)	■	2	6	7	2	2	3
Kivu (F)		■	5	7	3	3	4
Carmen (F)			■	1	2	1	2
Kiwi (F)			1	■	8	6	2
Monty (M)					■	4	3
Sophie (F)						■	3
Suzie (F)						1	■

Animal being displaced

Animal displacing

b: October 2005	Steffan	Kivu	Kiwi	Carmen	Monty	Sophie	Suzie
Steffan (M)	■	1	1	1	4	2	1
Kivu (F)		■		7		2	3
Kiwi (F)			■	1	2	1	6
Carmen (F)				■		1	
Monty (M)					■		2
Sophie (F)						■	
Suzie (F)							■

Fig 3.3 a-b: Dominance matrices based on all observations of displacement behaviours in March 2005 and October 2005 at Banham Zoo. Steffan was most dominant decreasing in social dominance rank order to Suzie, the most subordinate (M = male, F = female).

Animal being displaced

Animal displacing

a: March 2006	Ernesto	Fudgi	Nimba	Celize	Camara	Abyssian	Maatai
Ernesto (M)	■	13	22	2	3	8	3
Fudgi (M)		■	3	2	3	5	5
Nimba (M)			■	1	3	6	2
Celize (F)				■	6	3	2
Camara (M)					■		1
Abyssian (M)						■	1
Maatai (F)							■

Animal being displaced

Animal displacing

b: October 2006	Ernesto	Nimba	Fudgi	Celize	Camara	Abyssian	Maatai	Swahili
Ernesto (M)	■	27	17	1	3	3		5
Nimba (M)	1	■	40	1	4	3	1	
Fudgi (M)		1	■		4	1		2
Celize (F)				■	3	6		
Camara (M)					■	4		1
Abyssian (M)						■	2	1
Maatai (F)							■	
Swahili (M)								■

Fig 3.4 a-b: Dominance matrices based on all observations of displacement behaviours in March 2006 and November 2006 at La Boissière du Doré Zoo. Ernesto was the most dominant decreasing in social dominance rank order to Swahili, the most subordinate (M = male, F = female).

Matrices at Paignton Zoo for the female group are presented in Figures 3.5 a-c. In June 2005, the strength of linearity was relatively low (h' = 0.780; Table 3.4). Most of the dominant behaviours (85 %) were directed towards Lou and Joe, the two most subordinate in the group. In March 2006, the females displayed a perfect linearity in the matrix with no missing cell and no dominance reversals observed (Figure 3.5 b). Most of the dominant behaviours (56 %) were directed towards the youngest member of the group (Joe). The female Lou increased her status in the group and became dominant to the younger animal, Salassie. In October 2006 (Figure 3.5 c), the strength of linearity decreased to h' = 0.778 with only four animals left in the group. The target of most dominant behaviours (82 %) was again aimed at the youngest animal in the group (Joe).

Matrices at Paignton Zoo for the all male group are presented in Figures 3.6 a-c. In August 2005, the strength of linearity was low (h' = 0.750) (see Table 3.4). Both adult animals (Millo and Fergus) displayed dominant behaviours, whereas the sub-adult (Hope) only received them (Chapter 2, Table 2.2). In March 2006, the index of linearity was reduced to h' = 0.444. In both August 2005 and March 2006, dominance reversals were observed. In October 2006, high dominance linearity was restored (h' = 0.900), and no dominance reversals were observed.

Animal displacing	Animal being displaced					
a: June 2005	Madonna	Denny	Jhazzie	Salassie	Lou	Joe
Madonna (F)	■		1		1	1
Denny (F)		■			5	1
Jhazzie (F)			■	1		
Salassie (F)				■		3
Lou (F)					■	
Joe (M)						■

Animal displacing	Animal being displaced				
b: March 2006	Jhazzie	Denny	Lou	Salassie	Joe
Jhazzie (F)	■	1	7	7	9
Denny (F)		■	4	1	15
Lou (F)			■	3	1
Salassie (F)				■	5
Joe (M)					■

displacingAnimal	Animal being displaced			
c: October 2006	Denny	Lou	Salassie	Joe
Denny (F)	■	2		8
Lou (F)		■	1	1
Salassie (F)			■	5
Joe (M)				■

Fig 3.5 a-c: Dominance matrices based on all observations of displacement behaviours in June 2005, March 2006 and October 2006 at Paignton Zoo for the female group (including the juvenile male, Joe). Madonna was the most dominant decreasing in social dominance rank order to Joe, the most subordinate (M = male, F = female).

displacingAnimal	Animal being displaced		
a: August 2005	Millo	Fergus	Hope
Millo	■	1	9
Fergus	1	■	
Hope			■

displacingAnimal	Animal being displaced			
b: March 2006	Millo	Kabul	Hope	Fergus
Millo	■	5	3	1
Kabul		■		2
Hope			■	1
Fergus				■

displacingAnimal	Animal being displaced		
c: October 2006	Fergus	Millo	Hope
Fergus	■	5	2
Millo		■	4
Hope	1		■

Fig 3.6 a-c: Dominance matrices based on all observations of displacement behaviours in August 2005, March 2006 and October 2006 at Paignton Zoo for the all male group. a) Millo was the most dominant, decreasing in social dominance rank order to Hope, the most subordinate.

3.3.2 Life history factors and social behaviours in relation to index of success

The following analysis investigated the possible predictors affecting the index of success of colobus monkeys at various zoos over repeated data collection periods. Factors with significant power in the model were sex and age (Figure 3.7), play (Figure 3.8), grooming given (Figure 3.9) and received (Figure 3.10) and the interaction of grooming given and sex (Figure 3.11), as summarised in Table 3.5.

Full model

Terms	Type III		
	Wald Chi-Square	df	Sig.
Sex	9.594	1	.002
Linearity	2.306	3	.511
Age	17.779	1	.000
PL	4.077	1	.043
GR	13.141	1	.000
GG	13.138	1	.000
sex * GR	.171	1	.679
sex * GG	6.629	1	.010
age * PL	.834	1	.361
RS	.005	1	.946

Minimal model

Terms	Type III		
	Wald Chi-Square	df	Sig.
sex	16.192	1	.000
age	12.719	1	.000
PL	7.936	1	.005
GR	11.817	1	.001
GG	13.999	1	.000
sex * GG	4.456	1	.035

Table 3.5: Factors affecting the index of success (IS) of colobus monkeys.

Results are from a GEE model based on IS from 37 animals in five groups. IS was derived from the number of dominant encounter won divided by the total number of dominant encounters and scaled between 0 (dominant) and 1-4 (from most dominant to most subordinate).

Social behaviours were recorded every 15 s for each 10 min focal follow, added up and divided by the number of scans over data collection period to provide a value for relative time spent performing the behaviours per visit. PL: play, GR: grooming received, GG: grooming given, RS: social rest.

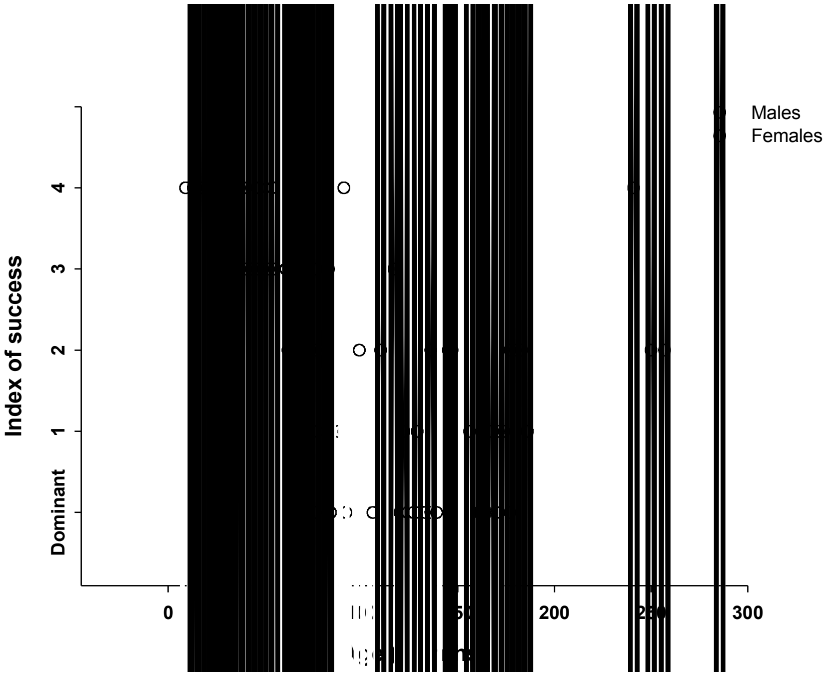

Fig 3.7: Age in months and gender of colobus monkeys according to index of success.
Index of success (IS) was derived from the number of dominant encounter won divided by the total number of dominant encounters and scaled as dominant (the clear overall dominant animals in each group) and between 1-4 (from most dominant to most subordinate).

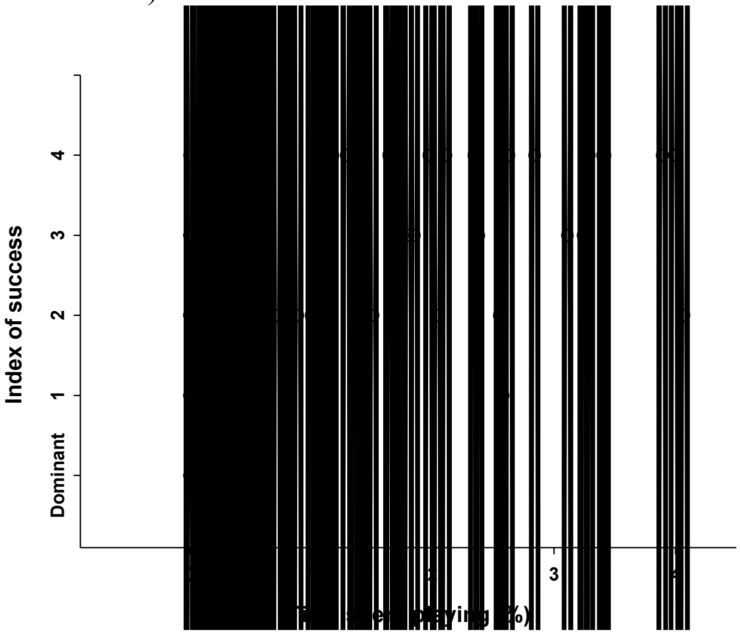

Fig 3.8: Percentage time spent playing by the colobus monkeys according to their index of success. Index of success (IS) was derived from the number of dominant encounter won divided by the total number of dominant encounters and scaled as dominant (the clear

overall dominant animals in each group) and between 1-4 (from most dominant to most subordinate).

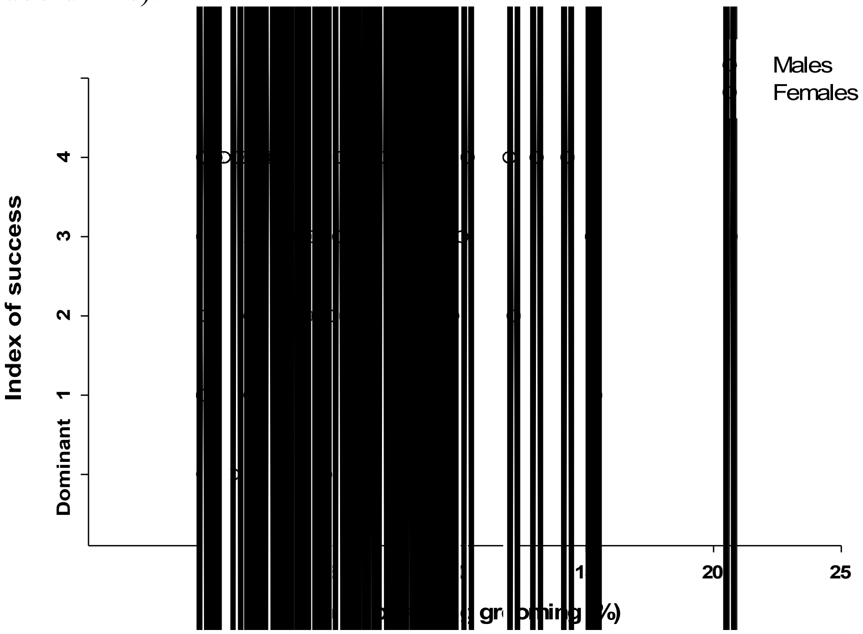

Fig 3.9: Percentage time spent providing grooming by the colobus monkeys according to their index of success and gender. Index of success (IS) was derived from the number of dominant encounter won divided by the total number of dominant encounters and scaled as dominant (the clear overall dominant animals in each group) and between 1-4 (from most dominant to most subordinate).

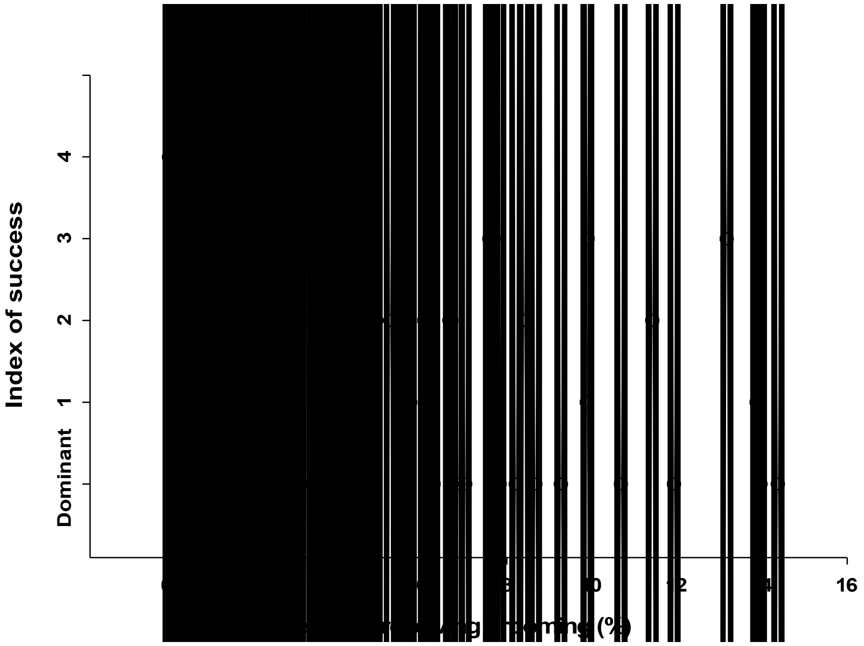

Fig 3.10: Percentage time spent receiving grooming by the colobus monkeys according to their index of success. Index of success (IS) was derived from the number of dominant encounter won divided by the total number of dominant encounters and scaled as dominant

(the clear overall dominant animals in each group) and between 1-4 (from most dominant to most subordinate).

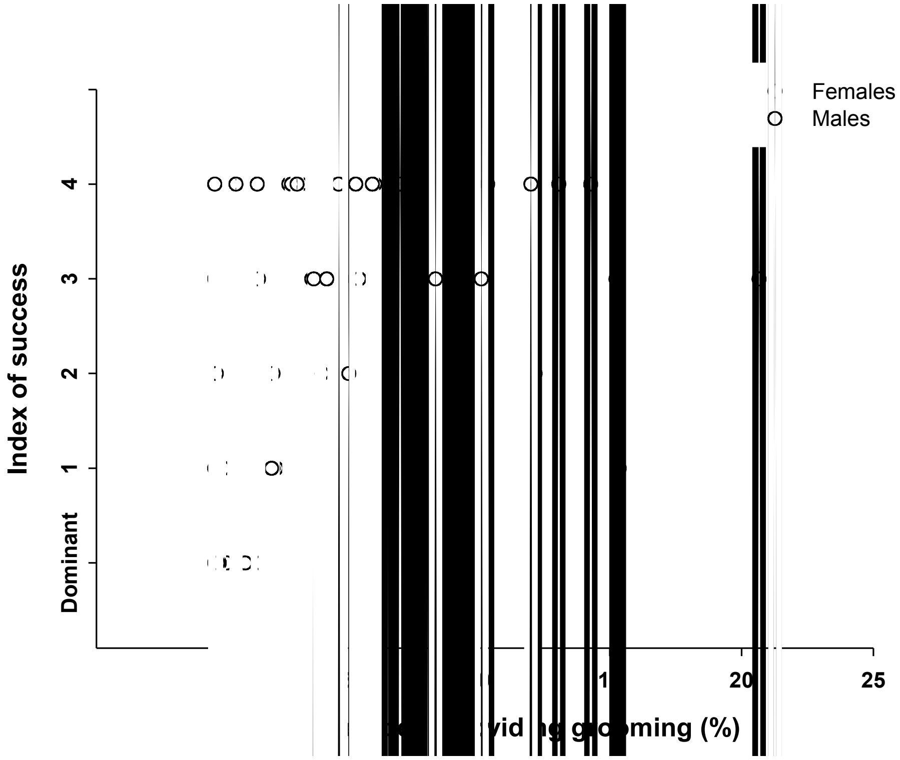

Fig 3.11: Percentage time spent providing grooming by the colobus monkeys according to gender and index of success (IS).

Although contrast analysis was not possible in the present logistic GEE model, due to the multinomial nature of the data, clear patterns can be observed in the variables significantly explaining the index of success of colobus monkeys in the present study. Figure 3.7 shows that the overall dominant animals were always older males (except in the all female group at Paignton Zoo). Further down the hierarchy, from IS 1-4, more dominant animals tended to be older, and within the IS categories 1-2, females were older than males. In Figure 3.8, clear patterns emerged where more subordinate animals spent more time playing than dominant ones. Figure 3.9 and 3.10 showed the grooming variables, significant in explaining variations in the index of success of colobus monkeys. More dominant animals received more grooming from others than subordinates, while providing less to others. The relationship between grooming provided differed significantly for the two sexes of colobus monkeys (Figure 3.11). On average, females provided three times the amount of grooming compared to males

3.4 Discussion

3.4.1 Dominance hierarchy linearity

Many matrices constructed in the present study contained missing cells and rank reversals occurred during observations, so the method used by Singh *et al*. (2003) was particularly appropriate, in preference to other methods, such as those of Appleby (1983) or Landau (1951 a & b), which do not take into account the amount of time a dyadic interaction is reversed. The method of filling empty cells with the probability of an animal winning an encounter, rather than assigning a fifty percent value (as suggested by Appleby, 1983), succeeded in measuring the strength of linearity of the colobus hierarchies in the five groups at the four zoos studied. Singh *et al*. (2003) recommended recording over a short time to avoid changes in rank order. In the present studies, recording over a maximum of 10 days reduced the possibility of changes of rank order during the observation period.

Generally, colobine species such as Hanuman langurs (Borries *et al*., 1991; Koenig *et al*., 2004), and other non-human primates such as howler monkeys (Jones, 1980) show low levels of reversals in social dominance hierarchies. These low levels of reversals suggest stability in a group where the position of the dominant animal is rarely contested by more subordinate animals. As far as I am aware, levels of reversals in the wild *C. guereza* have not been recorded. A study by Dunbar & Dunbar (1976) studying groups of colobus monkeys suggested that, in the presence of three large males in a group, more agonistic interaction were present in the mixed-sexes group. “All eight encounters between males involved attacks on the peripheral males by the central male, in each of which the latter was dominant” (Dunbar & Dunbar, 1976). This comment therefore hints towards non-existent reversals in the wild, however, concrete measurements were not performed and therefore, this statement should be interpreted cautiously. In the present study, reversals were rare or absent. None were observed at Paignton Zoo female group. Banham Zoo only had 1.80% of hierarchy reversal, probably due to the fact that only one large male was present, and none of the females ever contested his position. La Boissière du Doré Zoo had the highest number of adult males (n = 3) but only had 1.63% of reversals in the matrices, however, these were exclusively between the three large males. Port Lympne Zoo had 2.39% reversals observed, between, both male and female animals, which were dominant or subordinate. Paignton all male group had the highest level of reversal (6.1%) between the three males of similar age (Chapter 2, Table 2.2 b). Reversal rate <5% are typical of reported species with strong dominance hierarchy whereas values about 15% are attributed to species with weak and indiscernible dominance hierarchies (Isbell & Young, 2002).

3.4.1 a Social dominance in large mixed sexes groups

Indices of linearity for the groups of colobus monkeys at Port Lympne Zoo (n= 9 and 10), Banham Zoo (n = 7) and La Boissière du Doré Zoo (n = 7) were high i.e. above or close to h'=0.9 value, which suggests a strong linear hierarchy (Martin & Bateson, 1993). This is in agreement with several studies in the wild of colobines such as Hanuman langurs, (Borries *et al*., 1991). Phayre's leaf monkeys (Koening *et al*., 2004) and *C. guereza*, (Harris, 2006) and other non-human primates such as multi-male, multi-female groups of Assamese macaques (Bernstein & Cooper, 1999). The group sizes at Port Lympne Zoo, Banham Zoo and La Boissière du Doré Zoo, matched those observed in the wild with an average of between 6-10 animals (Maler, 1969; Dunbar & Dunbar, 1976; Oates, 1977; 1978; Krüger *et al*., 1998; Harris & Monfort, 2006). The common group structure of *C. guereza* is one territorial male with a group of related females and their offspring (Dunbar & Dunbar, 1976; Estes, 1991; Krüger *et al*., 1998). However, several males have been observed to cohabit in mixed groups (Dunbar & Dunbar, 1976; Oates, 1977; Harris & Montfort, 2006).

The group with the highest number of males, Port Lympne (n = 5) and La Boissière du Doré Zoo (n = 5), exhibited the highest amount of dominant behaviours with an average of 16.7 interactions per animals (total of 1115 for all seven visits) at Port Lympne and 16.1 (total of 225 in two visits) at La Boissière du Doré Zoo. Banham Zoo (males: n = 2) displayed half the amount of dominant behaviours compared to Port Lympne Zoo and Banham Zoo with an average of 8.1 interactions per animals (total of 113 in two visits). The highest percentage of dominance interactions were initiated by adult males, which explains the difference between these zoos. Males were at the centre of dominant agonistic interactions which is in agreement with Smuts & Smuts (1993) who suggested that male non human primates performed more intense and frequent aggressive interactions than females. This is also supported by other studies of primates such as the male rhesus macaque (Teas *et al*., 1980). At La Boissière du Doré Zoo, only one adult breeding female and one juvenile female were present, and all other members of the group were males (Chapter 2, Table 2.3 b). Males in groups with unequal sex ratios are thought to have more competition for mates (Hill, 1994), which would explain the higher levels of aggression at Port Lympne Zoo and La Boissière du Doré Zoo compared to Banham Zoo. Adult male colobus monkeys at La Boissière du Doré Zoo contributed to 40 % of the dominant interactions in March 2006 and 66 % in November 2006. This group displayed the highest linearity (*h'* = 0.966 in November 2006, *h'* = 1 in March 2006. Similarly, at Port Lympne Zoo, male colobus monkeys were the originators of 73 % of all dominant agonistic interactions. In all zoos, male colobus monkeys showed the highest level of dominant interactions at feeding time (60 % at Paignton Zoo all male group and La Boissière du Doré Zoo, 63 % at Banham Zoo and 75 % at Port Lympne Zoo, suggesting that competition over resources other than females (i.e. food) is also essential in determining social dominance hierarchy (Fahsing, 2001; Harris, 2006).

A study of wild *Colobus vellerosus (*Saj *et al*., 2007) suggested that, contrary to male colobines, dominant agonistic interactions between females are low (in mixed multi male, multi females groups), and that they are an egalitarian species. A study of captive female *Colobus guereza* observed aggression between females, exchanged bi-directionally, but no rank relations could be established from the direction of aggression and retreat (Grunau & Kuester, 2001). These workers found that the female dominance hierarchy in multi-male, multi-female groups followed an egalitarian dominance style without formalized rank relations. In the present studies, however, females in the mixed sex groups at Port Lympne Zoo, La Boissière du Doré Zoo and Banham Zoo, displayed clearly dominant unidirectional behaviour towards other animals (males and females) and were entirely part of the social dominance hierarchy. Actual displacements were common amongst females (particularly at Port Lympne Zoo), as well as physical threats (most often launching) leading to the displacement of the target animal. Although females did not use facial expressions, vocalizations or displays (such as yawning and teeth bearing), physical threats were often used such as forward threats (with the body or the hand). Also, fights were observed between females in all groups, at several times these escalated to the point of injury. For example at Port Lympne Zoo fighting occurred between the matriarch Katie and second female Ulna, and at La Boissière du Doré Zoo fighting occurred between Nimba and the juvenile female Maatai, who initiated them. Interactions were unidirectional and rank reversals between females were rare, only occurring three times in any of the zoos studied, therefore suggesting stability in rank hierarchy amongst females.

3.4.1 b Dominance hierarchies at Paignton Zoo male and female groups

The strength of linearity of the hierarchy for the female group at Paignton Zoo was variable, depending on the social structure and local events in the group. In June 2005, the females were settled in their enclosure and showed very little dominant behaviour, which was reflected in a relatively low strength of linearity (h' = 0.780). Before the second visit in March 2006, the dominant female Madonna passed away. During the following data collection period, a complete dominance hierarchy was displayed (h' = 1) with the highest levels of dominance behaviours (64 % of the three visits). The second oldest female (Denny) did not take over the group, but the daughter of the deceased matriarch (Jhazzie) did. In March 2006, Jhazzie was still establishing her new position in the group by undertaking much dominant behaviour and re-establishing the social dominance hierarchy with the juvenile male as the most common target (82 % of all interactions). Finally, in October 2006, the strength of dominance hierarchy linearity decreased again to h' = 0.778. Only four individuals remained in the group as the dominant female (Jhazzie) also died. These results suggest that female groups, under settled conditions, do not show a strong linearity in the dominance hierarchy. This conclusion agrees with the study on captive female colobus monkeys by Grunau & Kuester (2001) but differs from the behaviour of female colobus monkeys in mixed sexes groups (see previous section).

In the all male group of colobus monkeys at Paignton Zoo, only three individuals were housed together during the first two visits (see Chapter 2, section 2.2.2). During both visits, the dominance linearity indices were low (h' = 0.750 in August 2005 and h' = 0.444 in March 2006). As only three individuals were present, dominance behaviours were scarce and dominance reversals were observed, therefore, the dominance hierarchy itself must be interpreted cautiously. However, in all cases, the dominant animal was one of the older males (Millo or Fergus), and the younger male (Hope) remained subordinate, which is a similar pattern to that observed in the large mixed sex groups. In October 2006, the four males were brought back together, which allowed more convincing social dominance hierarchy data to be obtained. The introduction of the fourth individual had profound consequences on the dynamic of the group and more dominant behaviours were observed.

Both groups at Paignton Zoo showed the lowest number of dominant agonistic interactions, with a mean of 5.7 per animal for the female group and 3.6 for the all male group. Low levels of dominant behaviours could be an effect of group size, or could result from the particular captive conditions, with males housed without females. Male-male aggression in primates has been shown to increase when more females are present and competition for access to females augments in multi-male groups with female-skewed sex ration (Bercovitch, 1983; Janson, 1984). For example, higher levels of aggression were observed in captive male Sichuan snub-nose monkeys towards other males with higher frequency of fighting when in the presence of females (Shi *et al*., 1982).

3.4.1 c Differences in social dominance hierarchy of females in single sex and mixed sexes groups

Food is often seen as a critical resource for females reproductive success as gestation and lactation are nutritionally demanding (Pazol & Cords, 2005). As food resource becomes a limiting factor, competition to access this resource should increase and in turn, shape the patterns of social relations among females (Isbell, 1991; Isbell & Young, 2002). If the limiting factor (food resource) is also found in a quality patch (i.e. feeding table at a zoo), it can be monopolized (Isbell *et al*., 1998) leading to agonism and the creation of linear dominance hierarchies which determine the priority access to these resources (Pazol & Cords, 2005). This suggests that most dominant interactions between females are likely to occur at feeding time as was shown in a study on a colobine species, the Phayre's leaf monkey (Koening *et al*., 2004). This was also the case in the large groups in this study where many females were present. At Port Lympne Zoo, where there were five females, 81 % of dominant interactions were observed at feeding time. At Banham Zoo, also with five females, 70 % of dominant interactions were observed while food was present. In these zoos, food was generally placed on feeding tables (normally the same ones every day). In captivity, Scott & Lockard (2006) found that high-ranking female gorillas

(*Gorilla gorilla*) monopolized food patches and kept the low ranking females at bay with threat vocalizations or by chasing them away. Increased competition over preferred items has been observed in wild studies of forest guenon, where more aggressive competition took place over access to fruits (Cords, 2000; Payne *et al*., 2003). The high levels of dominant behaviour to access preferred food items in the large mixed sexes groups at Port Lympne Zoo and Banham Zoo suggest competition for resources. Higher ranking females in these groups accessed the table with the preferred food items (such as bananas or nuts) while the others would eat less favoured but more abundant foods (Nakagawa 1989; Saito, 1996) such as lettuce or tomatoes, after snatching it and running away to feed on a different table than that used by the dominant animals. This observation agrees with the suggestion of Isbell (1991) that where food can be monopolized, contest competition would be expected and strict rank relations established.

However, the opposite results were observed with the Paignton female group where the majority of dominant agonistic interactions (63 %) happened during non-feeding activities. If a limiting resource is found in a disperse manner, scramble competition would be expected where low levels of agonism are observed and even if linear dominance is observed, it may be weak and unstable (Pazol & Cords, 2005). The female group at Paignton Zoo was always fed on the floor area and individuals were observed picking up food, then quickly moving up to perches to feed. Only short amounts of time were therefore spent in the vicinity of one another, which avoided aggressive encounters.

Similarly, at La Boissière du Doré Zoo, few dominant interactions were observed at feeding time, as only two females were present in the group of which one was a juvenile. The low aggression at feeding time probably derived from the fact these females were in a group with several large, more dominant males. Dominant behaviour at feeding time could be dangerous and detrimental to them as the large males were dominant to them. All the female dominance behaviours at La Boissière du Doré Zoo were initiated by the matriarch and only towards the two young sub-adult males and the juvenile female (Figure 3.4a-b).

3.4.2 Index of success in relation to age, sex and social behaviours

The variables that were found to predict the index of success of the colobus monkeys in the five groups studied were sex, age, and social behaviours such as play, grooming given, grooming received and the interaction between sex and grooming given. Although the present social behaviours were entered in the model as potential predictors of rank, it is not clear whether these behaviours cause rank, or are caused by rank. It is possible that, although these behaviours were investigated as a cause to rank in the present analysis, the correlations observed between behaviours and rank may actually reflect effects of rank on behaviour.

Age has previously been observed as a confounding factor in determining dominance in other primate species such as the brown capuchins (Bernstein & Cooper, 1999). In the groups of colobus monkeys at Port Lympne Zoo, Banham Zoo and La Boissière du Doré Zoo, the older animals were at the top of the social hierarchy. Throughout data collection periods at these zoos, the oldest male in the group remained dominant with the breeding monopoly. Younger animals of both sexes remained low and stable within the hierarchy. Similar patterns have been observed in other primate studies such as the one on rhesus monkeys (*Macaca mulatta*) where older animals were more often involved in social dominance interaction and younger animals were significantly less involved as the performer or receiver of agonistic interactions (Irwin *et al*., 1985). Social dominance based on age was observed in the colobine species, Phayre's leaf monkeys (Koening *et al*., 2004) and other primates such as Hanuran langurs (Borries *et al*, 1991) and howler monkeys (Jones, 1980), however in these three studies, rank was inversely related to age. Most of the variations within rank position in Port Lympne Zoo, Banham Zoo and La Boissière du Doré Zoo were between the larger, older animals but included both males and females. Most intense aggression and dominant behaviour would be expected to occur between close ranking animals and the nearest ranking subordinate to an animal would have the highest chance to win agonistic interaction and gain access to contested resource (Reed *et al*., 1997).

Another important variable to explain the index of success was the gender of the animal, which has been previously described as a factor influencing dominance in primates (Klinkova *et al*., 2005; Garcia *et al*., 2006; Gould & Ziegler, 2007; Hanya *et al*., 2007). Within older animals belonging to the adult category (Chapter 2, section 2.2.1), males tended to be dominant over females both in La Boissière du Doré Zoo, Banham Zoo and Port Lympne Zoo. In turn, adult females tended to be dominant over both sub-adult males and sub-adults females. In the juvenile category, male tended to be dominant over females at Port Lympne Zoo and Banham Zoo. When adult males and females were in a mixed sex group, the oldest male was dominant and controlled the entire group. Females did not form kinship relationships, where related females are ranked closely in the hierarchy, but appear to be individualistic with age-dependant social dominance. This contradicts work with other non-human primate studies (Datta & Beauchamp, 1991; Nakamichi *et al*., 1995; Kutsukake, 2000). In kin-based society, closely related animals may have in-born dominance status, which would affect their likelihood of winning an encounter with, for example, another animal from a lower ranking family (e.g. the Japanese macaque, *Macaca fuscata,* Kutsukake, 2000).

Play behaviour was also found to be a main predictor of variation in index of success. In most primate species play behaviour is initiated and performed by the younger animals in a group and directed to other young animals. This has been observed in Francois' langurs where immature animals spent more time playing than other age classes (Zhou *et al*., 2007). Li & Rogers (2004) also found that juvenile white-headed langurs spent significantly more time playing than adults. Similar results have also been observed in wild *C. guereza* where infants (Fashing, 2001), juveniles and sub-adults were most involved in play behaviours. Results in the present study were consistent with these previous findings. Play behaviour showed consistent patterns across the groups of colobus monkeys where the youngest animals, both male and female, displayed most play activity. Adult, more dominant animals were often solicited to play but were rarely observed to actively take part and if they did it was only for a short amount of time with the youngest individual in the group and almost never amongst themselves.

Grooming is a form of affiliative behaviour, and providing grooming to non-kin may increase the probability of future benefit, in the form of alliance, tolerance or reduced aggression (Fairbanks, 1980). In vervet monkeys, Seyfarth (1984) observed that non-kin animals would support those who had recently groomed them if there was a dispute. The impact of grooming on bond formation between animals was such that, non-kin often responded to support aggressed recent grooming partner at higher levels than kin-related animals. Grooming a dominant animal may be more attractive in return of possible support during agonistic interactions (Seyfarth, 1984; Barrett *et al*., 1999). This theory was supported by the present results. Grooming was performed more often by subordinate animals. On the occasion it was provided by dominant males, it was in very small amounts and to very few animals. For example, at Port Lympne Zoo, the dominant male (Panda) was rarely seen grooming other individuals and this was mostly restricted to his reproductive female and the large second male in the dominance hierarchy. Similar patterns were observed at Banham Zoo and La Boissière du Doré Zoo. These patterns of grooming by the dominant males are in accordance with previous observation of grooming behaviours in wild groups of *Colobus guereza* (Estes, 1991).

Grooming received was a significant variable explaining the variation in index of success. More dominant animal received more grooming, i.e. the direction of grooming was up the hierarchy. These results are similar to that seen in studies of olive baboons, where in small troops, the higher ranking individuals received more grooming (Sambrook *et al*., 1995). Another study by Reed *et al*. (1997) found that high-ranking Sulawesi crested macaque males received more grooming from adult females. Similarly, dominant Alaotran gentle lemurs (*Hapalemur griseus*) received more grooming than other animals in a group (Waeber & Hemelrijk, 2003).

3.5 Summary & Conclusions

Social dominance hierarchy in mixed sex groups in captivity was strongly linear (above or close to $h' = 0.9$), matching observations of *C. guereza* groups in the wild (Harris, 2006) and other colobines species (Borries *et al., 1991;* Koening *et al., 2004)*

In the all female group at Paignton zoo, the strength of the social dominance hierarchy was variable: from a weak linearity, matching other study of captive female colobus monkeys (Grunau & Kuester, 2001), to strongly linear, depending on the social structure and local events in the group. These results highlight the impact of husbandry procedures on social dominance hierarchy. Similarly, in the all male group at Paignton Zoo, when only three males were present, dominance behaviours were scarce and dominance reversals were observed. As husbandry procedures changed and all four males were brought back together, more convincing social dominance hierarchy data were obtained.

As expected, social behaviours (including play, grooming given and received) were associated with dominance ranking. Although the relationship of cause and effect between ranking and social behaviours remains unclear, distinct trends could be observed. Social grooming behaviours varied according to social ranking. More dominant animals received more grooming, i.e. the direction of grooming was up the hierarchy, as found in other primate species (Sambrook *et al*., 1995; Waeber & Hemelrijk, 2003). High ranking individuals can be expected to be more attractive as recipients of grooming, due to the prospect of support they may offer during agonistic interaction (Seyfarth, 1977, 1984; Barrett *et al*., 1999), or the formation of alliance, tolerance or even reduced aggression (Fairbanks, 1980). Similarly, grooming was performed more often by subordinate animals as found in many other primate species (Bernstein, 1970; Isbell & Young, 1993).

Life history (age and sex) data also influenced ranking where older animals were more dominant than younger ones, as observed in other primate species (Bernstein & Cooper, 1999). Males tended to be dominant over females in mixed sexed groups and older females reached similar dominance status as much younger males. In mixed sex group, the oldest male was always the overall dominant and controlled the entire group.

Chapter four

Trichuris trichiura burden in captive *Colobus guereza*

4.1 Introduction

4.1.1 The importance of Trichuriasis in relation to health

Trichuris worms, also known as the whipworm, are part of the Trichuroidea superfamily, a common nematode. Different species are usually associated with different hosts, such as *T. ovis* in sheep and goat, *T. globulosa* in catlle, *T. suis* in pigs, *T. vulpis* in dogs and *T. trichiura* in humans and non-human primates (referred to as primates from forth). However, humans have been known to host species such as *T. vulpis* (Stephenson *et al*., 2000). *Trichuris spp* seldom achieves the public health significance it deserves, since most infections are light and asymptomatic (Cooper & Bundy, 1988), and although present in livestock, they can be controlled well by anti-helmintics (Urquhart *et al*., 1988). However, *T. trichiura* infection does pose a major health risk worldwide, particularly in developing countries where heavy infections hinder growth and mental development in children (Cooper & Bundy, 1988). The level of infection in humans is often classified by counting the eggs per gram of faeces (epg), where infections under 1000 epg are described as light, between 1000 - 9999 epg thought to be moderate and above 10,000 epg considered heavy (Stephenson *et al*., 2000). Each level of infection is associated with different responses varying from abdominal pain, diarrhoea, vomiting, and nausea, leading to decreased food intake and weight loss. As the food intake reduces, so does nutrient uptake, which may lead to stunting (growth retardation). Prevalence of *T. trichiura* in tropical and sub-tropical countries can reach up to 90% in children (Crompton, 1999) and affects India, Africa, Indonesia, Malaysia, China and various other Asian countries, Latin America, the Caribbean and the middle East (Stephenson *et al*., 2000).

Trichuris is a known zoonosis (Carithers, 1998; Stephenson *et al*., 2000; Gillespie *et al*., 2005), i.e. it can be transmitted between humans and other animals, which can be achieved through the faeco-oral route. As such it poses a threat to both humans and animals. As populations in developing countries increase, causing communities and primates to live in closer proximity and in indirect contact through water and soil, the risk of infection increases (Muriuki *et al*., 1998; Legesse *et al*., 2003).

Measuring the *Trichuris*-primate interaction in captivity is a vital part of monitoring health, and therefore welfare in captive groups as well as broadening the understanding of the parasite-host relationship and factors which may affect it. There is also an obligation to monitor and control potential zoonosis so that the health of keepers and zoo staff is ensured (Munene *et al*., 1998) as direct contact is favoured (Muriuki *et al.,* 1998) and clinical outbreaks of zoonotic parasite in animal caretakers has been reported (Hamlen & Lawrence, 1994).

4.1.2 *Trichuris spp* life cycle

Trichuris eggs are voided in faecal samples and deposited in the soil, where, after 15-30 days of incubation at temperatures between -8˚C and 50˚C, the infective larvae (L1) develop within the egg (after going through a two cell stage and advanced cleavage phases). Below and above these temperatures, the eggs may die but in optimal conditions, eggs can survive for over two years (Larsen & Roepstorff, 1999). *Trichuris* is a hardy species, which can endure harsh environmental conditions (Mabosa *et al*., 2004).

Ideally, the incubation conditions would be in moist, warm soil, without exposure to direct sunlight (Smyth, 1994; Stephenson *et al*., 2000) or in tree bark (Chapman *et al*., 2005), as *Trichuris* is sensitive to desiccation (Duval, 2002). The infection takes place by ingestion of embryonated (L1) eggs found in soil and water, or deposited on food handled with contaminated hands. Therefore, *Trichuris* is particularly abundant in humans or primates who practise geophagia (soil eating) behaviour (Cooper *et al*., 1995). Once ingested, the larvae hatch from the eggs and are thought to temporarily penetrate the duodenum for a period of growth (about a week) after which, they re-enter the intestinal lumen and migrate to the caecum and burrow into the mucosal surface (Stephenson *et al*., 2000; Bundy & Jackson, 2004). Adult worms (about 4 cm long) develop in 30-90 days after ingestion and are shaped like whips (Rothman & Bowman, 2003). They can live up to a year within the caecum. After copulation, females lay new eggs (between 1,000 and 46,000 per female per day, Cheng, 1973) and these are defecated with the faeces, and the cycle starts again.

4.1.3 Trichuriasis in primates and factors affecting it

Investigation of faecal parasites in *Colobus guereza* housed at Paignton Zoo (Melfi & Poyser, 2007) identified *Trichuris trichiura* (the whipworm) as the helminth of major importance. The main host of this parasite is man (Stephenson *et al*., 2000) but pigs, lemurs and monkeys are also able to carry *T. trichiura* . The whipworm parasite has been recorded in high levels in many primates such as yellow baboons, *Papio cynicephalus (*Hausfater & Watson, 1976; Hahn *et al*., 2003), vervet monkeys, *Cercopithecus aethiops*, de brazza monkeys, *Cercopithecus negletus*, black and grey mangabeys, *Cercocebus torquatus & C. albigena* (Muriuki *et al*., 1998), Eastern black and white colobus, *C. guereza* (Gillespie *et al*., 2005; Chapman *et al*., 2005), red colobus, *Procolobus rufomitratus* (Chapman *et al.,* 2005; 2006; 2007), olive baboons, *Papio cynocephalus* and Sykes monkeys, *Cercopithecus mitis (*Munene *et al*., 1998), and rhesus monkeys, *Macaca mulatta,* (Phillippi & Clarke, 1992).

A study of wild colobines focused on parasitic load in the red colobus, *Piliocolobus tephrosceles*, Eastern black and white colobus and the Angolan colobus, *Colobus angolensis* (Gillespie *et al*., 2005). In all three species, *Trichuris sp* were found at the highest prevalence (78.99 % in *C. guereza* and 100 % in *C. angolensis*) compared to other nematodes (< 11 %), cestode (< 1 %), trematode (<1 %) and protozoans (< 16 %). Prevalence was significantly higher in the Eastern black and white colobus and Angolan black and white colobus than in the red colobus (37.75 %). However, prevalence in either species of colobus did not differ between age and sex classes (Gillespie *et al*., 2005). Other studies confirmed that *Trichuris* is the most common parasite infecting Eastern black-and-white and red colobus (Chapman *et al*., 2005; 2006; 2007).

Different infection levels associated with age have been observed in other parasites which infect primate species. For example, juvenile and sub-adult rhesus monkeys have been reported to have more pathogenic species of parasites than adults (Philippi & Clarke, 1992). Age also has a significant impact on the prevalence of strongyloides sp in olive baboons (MullerGraf *et al*., 1996). Heavy infections with *T. trichiura* in humans often occurs in children (Bundy *et al*., 1987; 1991), probably because of relatively low immunity which increases as the individuals grow older (Kessler *et al*., 1984), and also due to differences in behaviour. A study by Bundy *et al*. (1987) found that the prevalence of *T.*

trichiura in St Lucia reached a plateau of 80-100 % for hosts aged 5-10 yrs old but that the mean worm burden seemed to decrease after 15 years of age.

However, adult males *Colobus guereza*, held in captivity at Paignton Zoo, were found to have higher faecal counts of *T. trichiura* eggs than other age categories (Melfi & Poyser, 2007). This is in contradiction with results from wild *C. guereza* (Gillespie *et al*., 2005) and opposite to patterns observed in the human population (Cooper & Bundy, 1988; Bundy *et al*., 1991). Whether this is a general phenomenon is not known and requires further investigation of captive colobus at different zoos. The higher egg count in adult males at Paignton zoo in this case could be due to immune senescence hindering physiological processes (Coe, 1992). An animal's ability to expel gastrointestinal helminths is dependant upon the CD4+ T-cells, which produce cytokines and induce gut inflammatory responses (Onah & Nawa, 2000). As animals grow older, control of CD4+ T cells is diminished, which can lead to an increased parasite burden (Onah & Nawa, 2000).

Previous research into the parasite burden of captive *Colobus guereza* housed at Paignton Zoo suggested a significant correlation between social dominance ranking and the *Trichuris trichiura* burden (Melfi & Poyser, 2007). The older, more dominant male colobus carried the highest worm burden. In humans, males have been suggested to be more susceptible to parasite infections than females (Klien, 2000) due to the immunosupression associated with male sex steroids (Klien, 2000; Remoue *et al*., 2000; Beery, 2003), and if similar effects occur in colobus monkeys they could influence parasite loading. However, further studies of captive colobus are required to determine whether the relationship between social dominance rank and Trichuriaris that is apparent at Paignton Zoo (Melfi & Poyser, 2007) is a general phenomenon.

Helminth infections tend to be closely correlated with levels of rainfall, with faecal egg counts peaking during the wettest months (Hammond *et al*., 1997; Ocaiso *et al*., 1999; Yospeh *et al*., 2005). This can be explained by the high vulnerability of *Trichuris* eggs to desiccation, reducing infective viability during dry weather. Seasonal fluctuations in faecal egg count have been observed in many parasitic infections (Sheridan *et al*., 2000; Hammond *et al*., 1997). For example, in dairy and beef cattle, faecal egg counts of *Trichuris* spp peak in spring and are significantly affected by temperature and rainfall (Jiménez *et al*., 2007). Similarly, a study on wild elk found that faecal egg counts of *Trichuris* spp increased from January until April (Hines *et al*., 2007). Further studies have investigated the efficacy of anti-helmintics on *T. trichuria* and re-infections at one zoo (Fox & Melfi, 2006). While both anithelmintics (Panacur® and Oramec®) succeeded in eliminating *T. trichiura* re-infection showed a seasonal pattern such that Trichuriasis returned faster in autumn than spring. It was anticipated that the results for *Trichuris* egg counts from each of the 4 zoos in the current study could provide guidance for the effectiveness of the different methods employed for treating Trichuriasis in captive colobus monkeys'.

However, in studies of wild *C. guereza*, no correlation between, season, rainfall and *Trichuris spp* parasite burden was apparent (Gillespie *et al*., 2005). Similarly, in a study of captive *C. guereza* in a single zoo there was no seasonal pattern in *T. trichiura* burden (Melfi & Poyser, 2007). Therefore, results seem contradictory and further studies are needed to investigate the *Trichuris trichiura* burden of captive housed colobus and to establish the scope of variation within this species. The interacting influences of season, social structures and husbandry issues on *Trichuris* infection of captive colobus can only be examined by investigations at several zoos, with different practices.

The studies reported in this chapter aimed to collect faecal samples from a variety of individually identified animals in several groups at selected zoos (see Chapter 2, section 2.1) that have different social structures, group size and husbandry practices. By increasing the sample size in the current study, compared to the previous studies of captive colobus monkeys at Paignton Zoo, the aim was to investigate whether the correlation between *Trichuris* burden and social rank of male colobus monkeys at Paignton Zoo (Melfi & Poyser, 2007) is a species-wide phenomenon or simply a feature of a particular group.

4.2 Methods

4.2.1. Sample collection

A standardised protocol was used to collect faecal samples for analysis of *Trichuris* egg counts. All faeces voided between 12:30 and 16.30 were marked on a map of the enclosure with the donor identified. At Port Lympne Zoo, the researcher was permitted access to the enclosure at all times, and samples were therefore collected immediately after they were voided; as appropriate so as to avoid disturbing the animals. At all other zoos, sample collection had to be delayed until feeding time, in the late afternoon (see Chapter 2, Table 2.4) when the animals were locked away and the researcher had access to the enclosure enabling collection of faecal samples. During faecal sample collection for analysis of *Trichuris* egg burden, it was important that the samples were collected in the same time frame (afternoon), to ensure consistency within the dataset. Density of eggs/gram of faeces recorded in faecal samples appears to exhibit a circadian rhythm; where afternoon faecal egg/gram of faeces is significantly higher than in the morning (Melfi & Poyser, 2007). Additionally, faeces voided indoors are preferable for collection as some studies suggest that weather patterns (mainly rain) may affect egg density in faeces (Washburn & Millspaugh, 2002; Jiménez *et al.*, 2007). Samples were always collected within five hours of defecation. Samples voided outdoors were collected only during dry weather, as too much rain may have degraded them. Any faecal samples contaminated with another faecal sample from a different animal were discarded. Each sample was collected into a plastic tube, labelled with the date, time and name of the donor.

Whole stools were collected as the concentration of parasite eggs may vary along the length of the faecal sample (often accumulating at the anterior end of the stool). After collection, faecal samples were placed in a freezer at -20 ºC at the end of each day. At the end of each visit to a zoo all samples from the site were placed in a polystyrene box covered by frozen gel packs and transported to the University of Exeter. Samples were then stored at -20 ºC until processed.

4.2.2 Identification, extraction and measurement of *Trichuris* eggs in faeces

Trichuris sp. eggs are easily recognisable by their barrel shape and opercular plug at each end (photo 4.1-4.3). Eggs in faecal samples were separated from faecal matter and counted after flotation technique in McMaster chambers at x10 magnification, using a compound microscope, eyepiece 17 mm (Wild M20E) to provide an estimate of eggs per gram (epg) of faeces (Urquart *et al.*, 1988). Estimation of parasite burden is most commonly achieved thought the determination of presence and/or the number of helminth eggs in faecal samples (Ward *et al.*, 1997; Cringoli *et al.*, 2004). Correlation between faecal egg count and adult worm burden has been demonstrated for several parasites (Bryan and Kerr, 1989; Murrell *et al*, 1989),

A few faecal samples were taken out of the freezer at a time and allowed to reach room temperature, which took about an hour. Once defrosted, each sample was thoroughly mixed in the tube using a glass rod to ensure an even distribution of eggs. Once mixed, three grams of faeces was weighed in a small weighing boat and placed in a plastic jar with about 45 glass balls. Distilled water (42 ml) was added to the jar, which was sealed with a lid and shaken for at least one minute or until all faecal matter was broken down. The amount of distilled water was reduced for small samples (< 3g) to avoid the sample being over-diluted (Dunn & Keymer, 1986; Cringoli *et al*., 2004). Therefore, 25 ml distilled water was added to one to two grams of faecal samples and 15 ml distilled water was added to faecal samples of <1 g, although samples this small were rare. The mixture was then filtered through a metal sieve with a 0.15 mm mesh and the strained fluid collected.

The strained fluid was thoroughly mixed using a glass rod for 30 s and an aliquot of 15 ml was poured into a clean 15 ml plastic centrifuge tube (Greiner). The tube was centrifuged (MSE, Mistral 3000) for two minutes at 120 g, and was then carefully removed from the centrifuge to avoid disturbing the pellet (containing the eggs). The supernatant was discarded and 15 ml saturated NaCl added to the tube (Cringoli *et al*., 2004). The tube was covered and inverted several times to break up the pellet and then mixed thoroughly using a plastic pipette (1 ml Pastette, Alpha Laboratories Limited) After mixing an aliquot was rapidly removed with the Pasteur pipette and placed in the chamber. The aliquot was sampled about two centimetres from the surface and transferred to four McMaster chambers. These chambers were individual improved egg counting chambers (depth 1.5 mm, ruled area 1 cm x 1 cm, with a total volume of 0.3 ml, Weber Scientific International Ltd). The chambers were covered by the ruled cover slip and left to stand to allow the eggs to float (see section 4.2.3c for tests of floatation time). Each chamber was then carefully moved to the microscope (to avoid disturbing the floating eggs) and examined under the microscope. Eggs within the grid were counted and were included if touching the right hand or bottom of the grid. The final egg count was calculated from the count for each slide using values for the exact amount of faeces extracted and the total amount of water added, using the following equation:

Eggs/ gram faeces = (egg count*volume water added/chamber volume)/faecal weight (g)

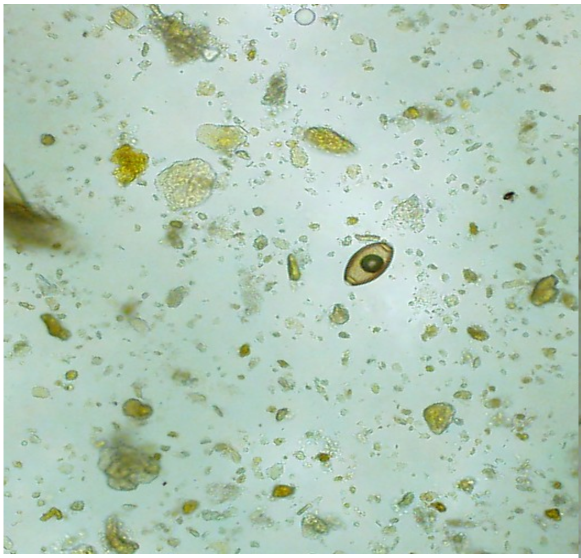

Photo 4.1: *Trichuris* egg showing bipolar ends.

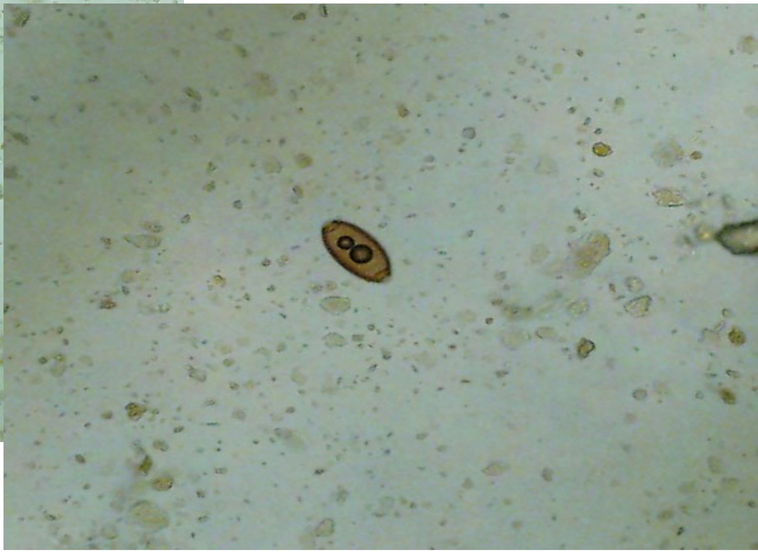

Photo 4.2: Two-cell stage of egg life cycle (before cleavage and embryonation).

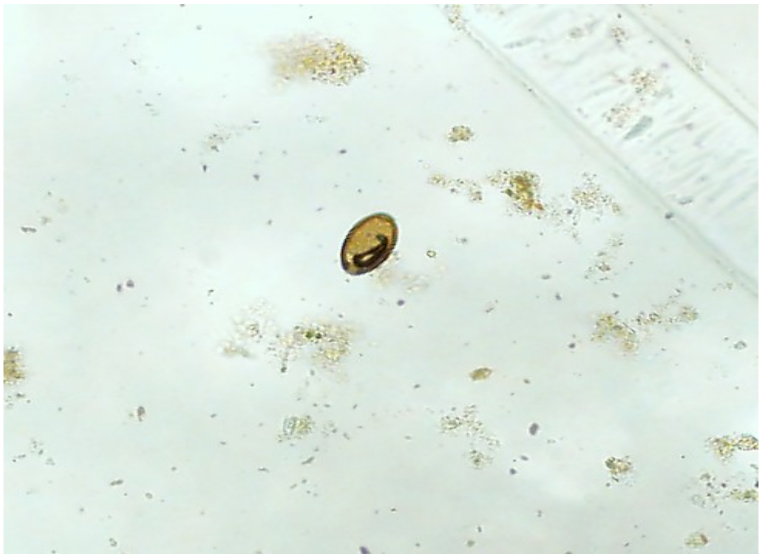

Photo 4.3: Embryonated egg ready for infective stage.

4.2.3 Validation of separation methods

4.2.3 a Extraction of Trichuris eggs from faecal samples

To extract *Trichuris* eggs from faecal samples, 3 g of faeces were mixed with 42 ml of distilled water in a lidded jar, and the mixture was strained (section 4.2.2). In this single stage procedure, debris left on the mesh was discarded. To investigate if all eggs are extracted by a single-stage procedure, the debris was washed off in a clean pot with another

42 ml of distilled water, shaken for another minute, and strained. The sieved fluid was used to obtain an egg count (using the remainder of the method in 4.2.2) and provided an estimate of eggs missed by the single stage extraction. This procedure showed that a second stage extraction recovers 4.4 % ± 1.9 (mean ± SE, n=23) of the total number of eggs per gram of faeces (epg).

4.2.3 b Assessment of eggs lost in supernatant

Trichuris eggs were extracted from faecal samples by mixing 3 g of faeces with 42 ml of distilled water in a lidded jar. The mixture was strained and a thoroughly mixed 15 ml aliquot was poured into a clean 15 ml plastic centrifuge tube. Tubes were centrifuged for two minutes at 120 g and the supernatant discarded on the assumption that all the eggs in the mixture were in the pellet (see section 4.2.2). To determine the loss of eggs in the supernatant, after centrifugation, the fluid was carefully poured into a clean tube and re-centrifuged, as before. The centrifuge tube was refilled with 15 ml saturated NaCl, mixed and aliquots were placed in McMaster counting chambers. The counts made allowed estimation of the number of eggs lost in the supernatant. This showed that 3.7 % ± 1.7 (mean ± SE, n = 25) of the total number of eggs per gram of faeces were lost in the supernatant.

The validation tests suggest that less than 9 % of the total number of eggs is lost from counts based on a single stage extraction procedure. This method was therefore considered to give a reliable estimate of egg counts and was used to analyse all the samples in the present study.

4.2.3 c Floatation time

The last step in the procedure employed to extract *Trichuris* eggs was to float the faecal samples in the McMaster's chambers. The amount of time a sample should be left to float in the McMaster's chambers, allowing all the eggs in the sample to reach the surface of the grid and become visible under the microscope was determined. A previous study showed that floatation time influenced the estimated number of parasite eggs (Dunn &

Keymer, 1986). Several samples were prepared (see section 4.2.2) and placed in separate McMaster chambers. The total number of eggs floating (clear in one plane in the microscope) were then counted every five minutes for 30 minutes, and then at 60, 120 and 180 minutes. This determined the minimum and maximum amount of time a sample should be left to float to obtain the highest reading of egg count. Counts obtained showed a plateau between 25 and 60 minutes of flotation (Figure 4.1) after which the number of eggs slightly decreased. This was probably due to the fact that at room temperature, water started to evaporate and the salt started to crystallised, decreasing the surface area of liquid in contact with the cover slip. For counts of *Trichuris* eggs, all samples were left to float for about 30 min.

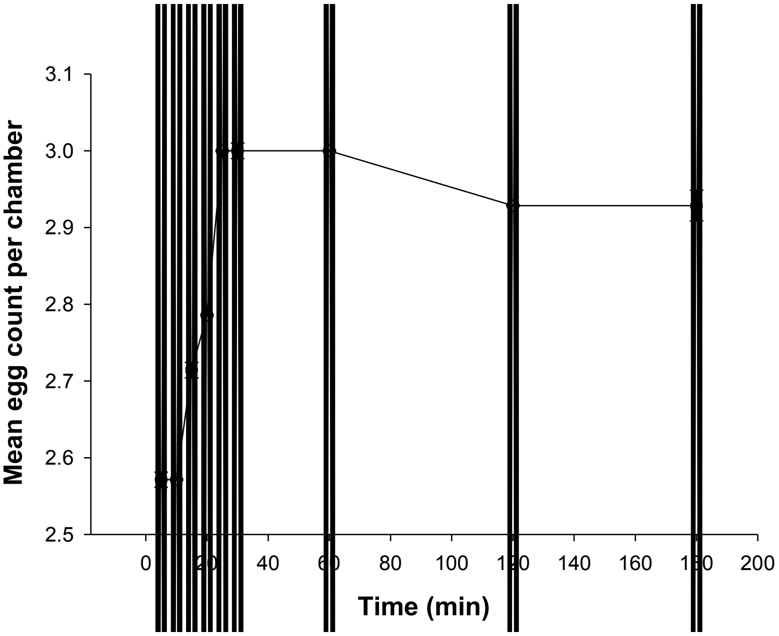

Fig 4.1: Mean (± SE) *Trichuris* egg counts in samples left to float for up to 180 min in McMaster chambers (n=14).

4.2.4 Anti-helmintic practices

All zoos visited had preventative health routines, which included the provision of anti-helmintics, with varying drugs formulation, dosage and administration methods.

Veterinary check-ups for the presence of *Trichuris* eggs at Port Lympne Zoo was ad-hoc, either using random mixed group faecal samples, or using known individual faecal sample. Figure 4.2 is a summary of all parasitological work and actions undertaken at Port

Lympne Zoo as a group or for particular individuals. Dates at which anti-parasitic treatments were carried out and data collection periods when samples were collected are highlighted. Anti-helmintic was injected both at the end of June 2005 and end of July 2005.

Summaries of anti-helmintic practices and data collection periods at Paignton Zoo are in Figure 4.3 (female group) and (Figure 4.4) male group. Zerofen, as a thick liquid, was administered to the whole group or particular individuals in 2005 and 2006. The animals in the male group were part of a training programme at Paignton Zoo, and trained to accept oral medication from a syringe, while the female group received Zerofen mixed with their food.

Banham Zoo routinely wormed the group of colobus monkeys, twice a year. In 2005, during both visits (19th - 25th March and 6th - 12th October), Zerofen 4 % had been recently administered, on the 12th February for three days (28.5 g between the whole group) and 20th of August for two days (29 g between the whole group). The anti-helmintic was in the form of a fine powder, spread over the food.

Zerofen, as a fine powder spread over food was also employed at La Boissière du Doré Zoo who routinely wormed their group of colobus monkeys twice a year. In 2005, when both data collections were performed (03rd - 09th March and 4th -11th November), Zerofen was administered to the whole group in April and October for three days at 50 mg/kg.

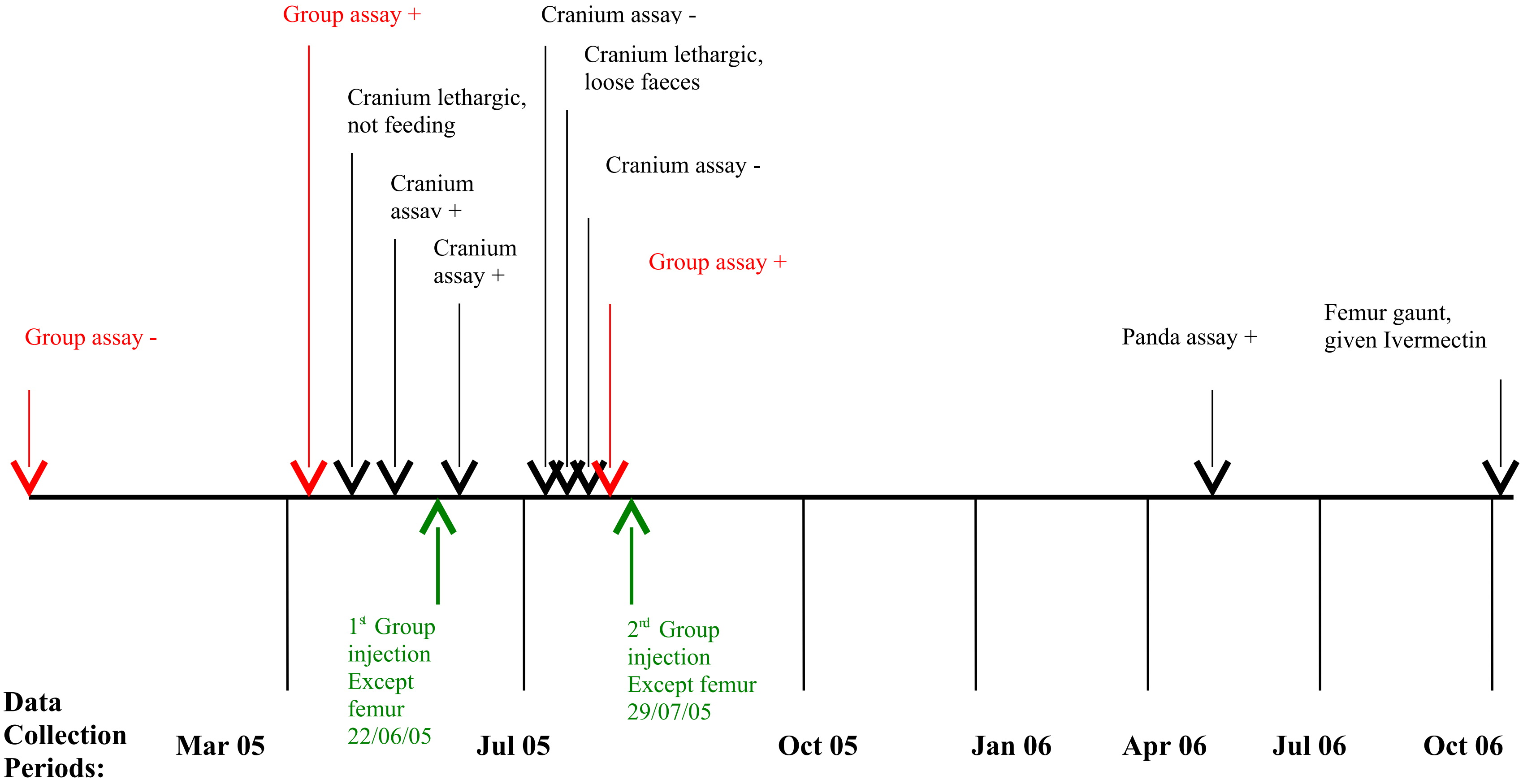

Fig 4.2: Faecal-parasitological work, anti-helmintic treatments and data collection periods undertaken between 20-01-2005 and 30-10-2006 at Port Lympne Zoo. Entries indicated by arrows refer to 'group' when identical treatment was administered to all individuals or to named animal. Assays done at Port Lympne were by zinc-sulphate floatation and referred to as + (positive) or – (negative) for presence of *Trichuris trichiura* ova. The 1st injection was levamisole and pyrantel and the 2nd injection was levamisole, moxidectin and pyrantel.

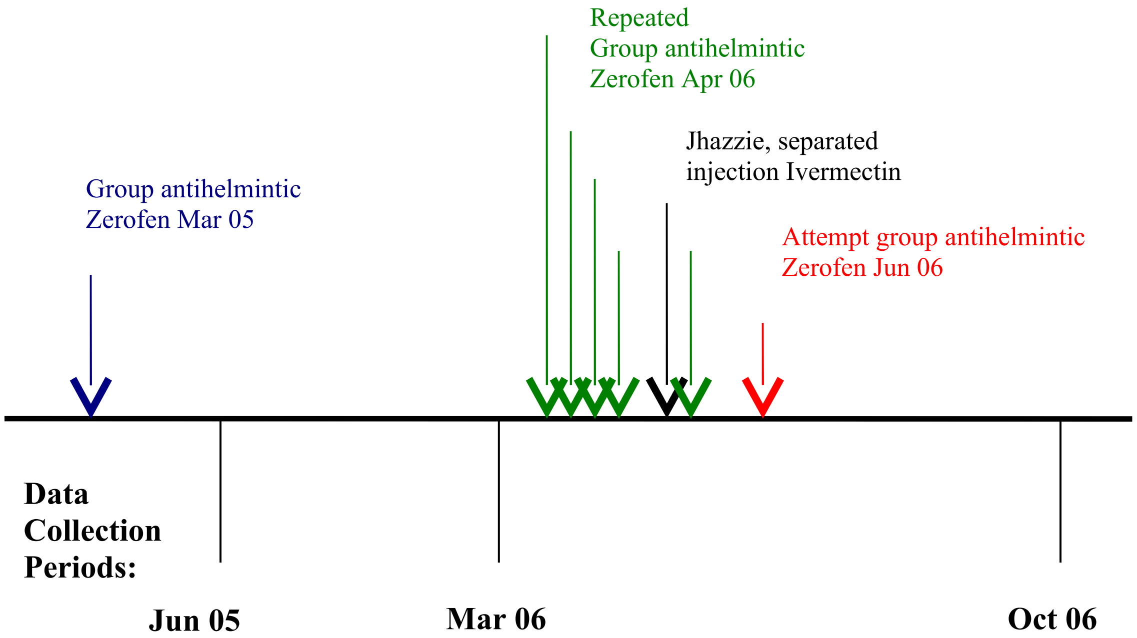

Fig 4.3: Anti-helmintic treatments and data collection periods undertaken between March 2005 and October 2006 at Paignton Zoo female group. Entries indicated by arrows refer to 'group' when identical treatment was administered to all individuals or to named animal. Zerofen used at 22%, 50 mg/kg administered for 3 days.

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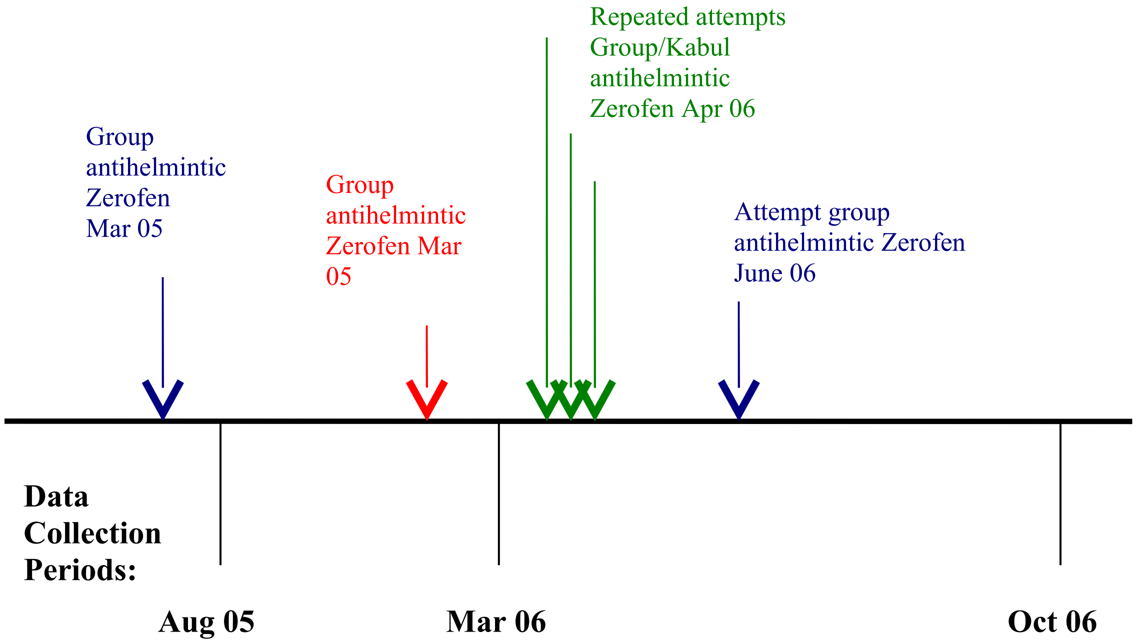


ig 4.4: Anti-helmintic treatments and data collection periods undertaken between March 2005 and October 2006 at Paignton Zoo all male group. Entries indicated by arrows refer to

‘group’ when identical treatment was administered to all individuals or to named animal. Zerofen used at 22%, 50 mg/kg administered for 3 days.

4.2.5 Data analyses

Four McMaster chambers were prepared for each faecal sample, therefore, mean *Trichuris* egg counts per gram faeces were derived for each sample. Several samples were available for each animal during each data collection period and are summarized in Table 4.1. Normality of data was checked using the Kolmogorov-Smirnov test. All data were initially log transformed as normality was not met.

To investigate factors affecting the counts of *Trichuris* eggs across zoo, a Generalised Estimated Equation (GEE) model was performed (PAWS 18). After being log transformed, *Trichuris* egg count was normally distributed therefore an identity link function was selected. The repeated subject variable was defined by the individual and the zoo identity. The within subject variable was identified by repeated data collection period (i.e. visit) and the day the samples were being collected (as several samples were available per visit). The dependant variable was defined as *Trichuris* egg count. Predictors were entered as factors and covariate. Factors in the model were season, index of success, gender, group (zoo) and worming routine. Animals were considered under worming regime if having received anti-helmintic within two months prior to data collection. Covariates were age (in months), rainfall, maximum temperature and minimum temperature, as gathered from the weather history website www.weatheronline.co.uk.

Initially, all predictors were included in the model as a main effect. Two-way interactions between terms were tested for significance, with the exception of the interaction between age and index of success as these were tightly correlated (Figure 2.1). Factors and covariates were then subsequently dropped from the model if lacking significance so the model only contained those terms which were significant yielding a ‘minimal model’, see Table 4.2.

Scale parameter method was employed using the maximum-likelihood estimate. Statistics were performed with Type III analysis and 95% confidence interval level computing Wald statistics. Contrast analysis was performed in the GEE model by use of estimated marginal means (EM means). The EM means were displayed in the PAWS 18 programme for factors only and pairwise comparison between each factor of relevance was performed and corrected with a Bonferonni adjustment for multiple contrasts.

Port Lympne Zoo	Mar-05	Jul-05	Oct-05	Jan-06	Apr-06	Jul-06	Oct-06
Panda	3	4	5	3	5	6	6
Katie	6	3	9	5	8	5	3
Radius	5	4	3	5	8	4	7
Fibula	8	5	5	5	6	5	3
Ulna	5	7	4	7	5	6	8
Tibia	7	8	3	9	7	6	8
Cranium	4	2	3	7	3	4	5
Tarsal	5	3	5	5	7	3	7
Dermis	5	3	6	8	5	3	6
Femur	3	-	1	3	4	4	3

Paignton Zoo- female	Jun-05	Mar-06	Oct-06
Madonna	6	-	-
Denny	4	5	4
Jhazzie	5	4	-
Salassie	2	4	5
Lou	5	5	5
Joe	1	1	1

Paignton Zoo- male	Aug-05	Mar-06	Oct-06
Millo	5	3	5
Fergus	6	4	4
Kabul	4	4	4
Hope	5	4	4

Banham Zoo	Mar-05	Oct-05
Steffan	4	5
Kivu	4	3
Carmen	6	4
Kiwi	3	4
Sophie	6	5
Monty	1	3
Suzie	5	4
Bobby	-	2
Bea	-	2

La Boissiere du Dore Zoo	Mar-06	Nov-06
Ernesto	4	4
Fudgi	5	5
Nimba	5	4
Celize	6	4
Abyssian	3	4
Camara	4	3
Maatai	2	3
Swahili	-	4

Table 4.1: Number of samples for each colobus monkey at Port Lympne Zoo, Paignton Zoo (both groups), Banham Zoo and La Boissière du Doré Zoo, during each data collection period.

4.3 Results

4.3.1 Mean *Trichuris* egg count per data collection period in relation to anti-helmintic practice.

Actual count of *Trichuris* egg count averaged for all animals in a group per data collection period are presented in Figure 4.5 for Port Lympne Zoo, Figure 4.6 for La Boissière du Doré Zoo, Figure 4.7 for Banham Zoo, Figure 4.8 for Paignton Zoo all male group and Figure 4.9 for Paignton Zoo all female group.

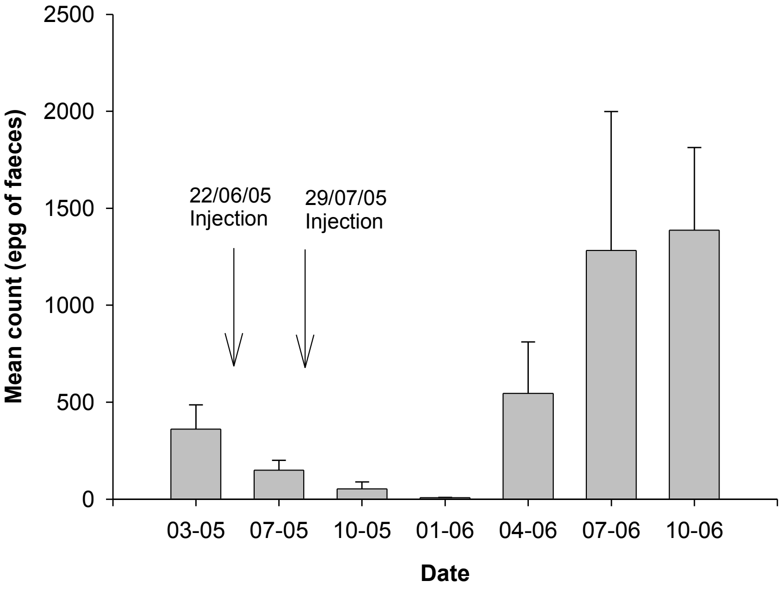


Fig 4.5: Mean (+ SE) *Trichuris* count of eggs per gram (epg) of faeces for the group of colobus monkeys at Port Lympne Zoo for each data collection period. Anti-helmintic injections were given at times indicated by arrows.

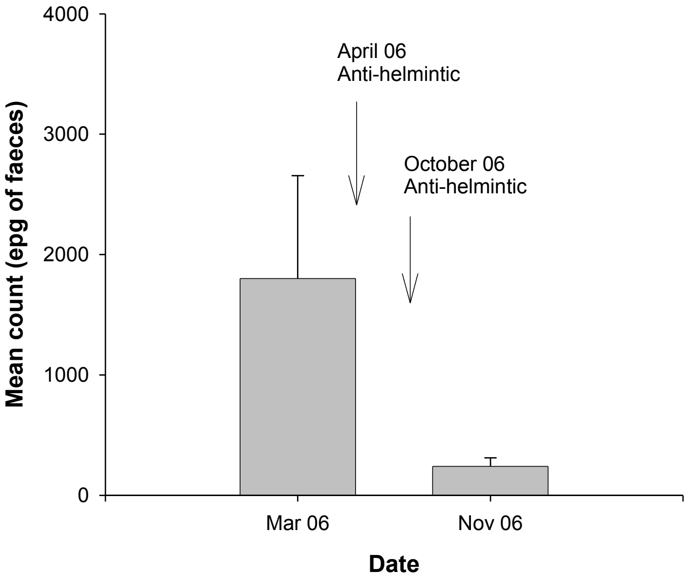


Fig 4.6: Mean (+ SE) *Trichuris* count of eggs per gram (epg) of faeces for colobus monkeys at La Boissière du Doré Zoo for each data collection period. Anti-helmintic treatments to group are shown by arrows.

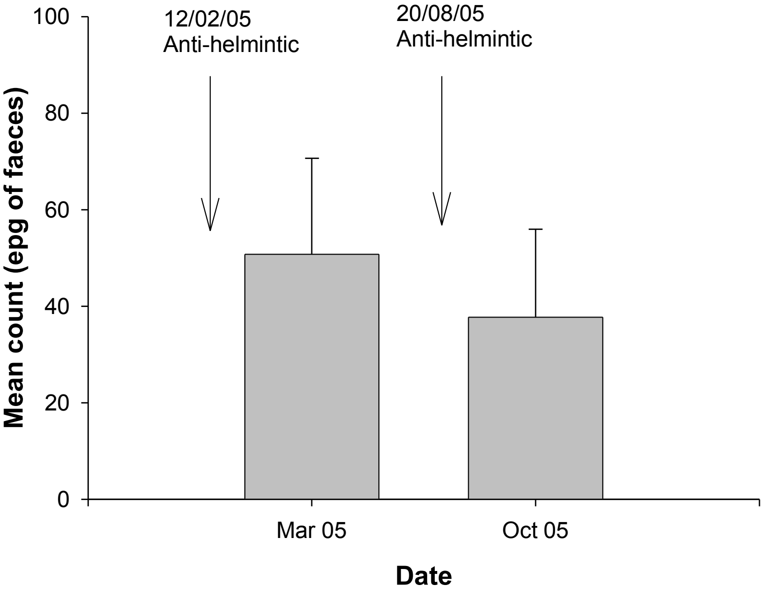


Fig 4.7: Mean (+ SE) *Trichuris* count of eggs per gram (epg) of faeces for colobus monkeys at Banham Zoo for each data collection period. Anti-helmintic treatments to group are shown by arrows.

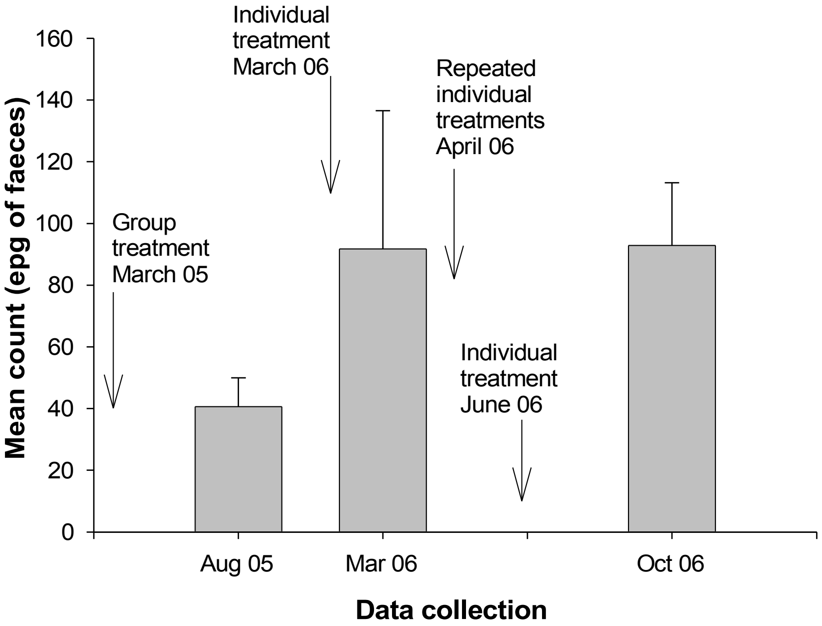


Fig 4.8: Mean (+ SE) *Trichuris* count of eggs per gram (epg) of faeces for male group of colobus monkeys at Paignton Zoo for each data collection period. Anti-helmintic treatments to group or individuals are shown by arrows.

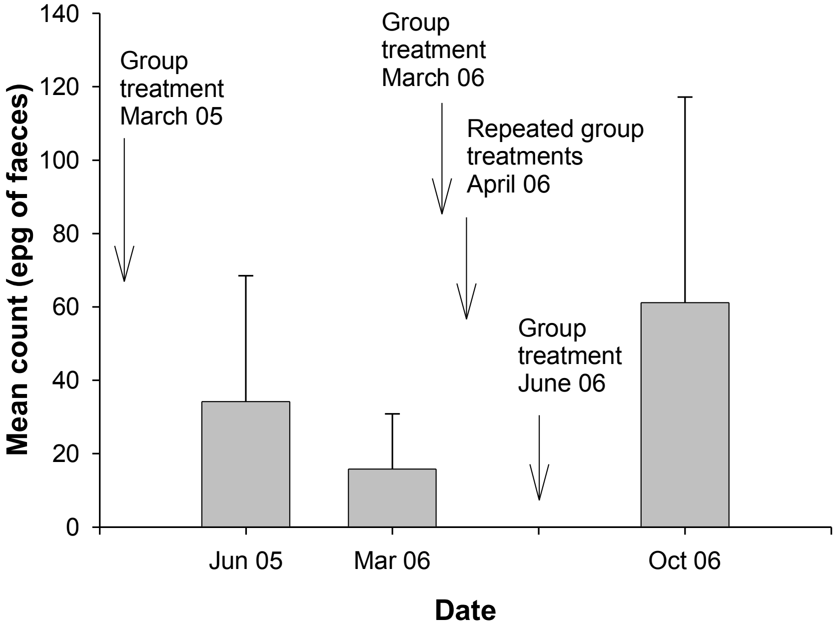


Fig 4.9: Mean (+ SE) *Trichuris* count of eggs per gram (epg) of faeces for female group of colobus monkeys at Paignton Zoo for each data collection period. Anti-helmintic treatments to group are shown by arrows.

4.3.2. Generalised estimated equation of factors affecting the counts of *Trichuris* eggs

The analyses reported below (Table 4.2) investigated possible factors affecting *Trichuris* egg counts of colobus monkeys at various zoos over repeated data collection periods. Predictors with significant power in the model were zoo, season, worming routine and age ($x^2 = 8.362$, df = 1, $p < 0.01$) where older animals tended to have higher faecal egg count of *Trichuris* sp, Figure 4.10.

Contrast analysis was performed in the GEE model by use of estimated marginal means (EM means) on zoo, season and worming routine. Zoo was a significant factor in the analysis ($x^2 = 94.233$, df = 4, $p < 0.001$) and significant differences between EM were found between the female group at Paignton Zoo and all other groups (mean difference Port Lympne Zoo: -2.0527, $p < 0.001$; La Boissière du Doré Zoo: -2.5600, $p < 0.001$; Banham Zoo: -1.6365, $p < 0.001$; and Paignton Zoo male group: -1.5976, $p < 0.001$). Additionally, significant difference were observed between La Boissière du Doré Zoo and both Banham Zoo (mean difference: 0.9234, $p < 0.01$) and Paignton Zoo male group (1.0524, $p < 0.01$), Figure 4.11.

Season was a significant factor in the analysis ($x^2 = 130.285$, df = 3, $p < 0.001$) and significant differences between EM were found between the winter and all other seasons (mean difference spring: -1.6895, $p < 0.001$; summer: -1.8031, $p < 0.001$ and autumn: -1.7294, $p < 0.001$), Figure 4.12.

Worming routine was a significant factor in the analysis ($x^2 = 18.800$, df = 1, $p < 0.001$) with higher *Trichuris* egg count when no anti-helmintic was administrated within two months prior to data collection (mean difference: 0.7619, $p < 0.001$), Figure 4.13.

Full model

Terms	Type III		
	Wald Chi-Square	df	Sig.
zoo	89.052	4	.000
season	78.660	3	.000
is	7.104	4	.130
sex	4.696	1	.030
worm	13.657	1	.000
age	7.182	1	.007
rain	.167	1	.683
tmax	2.337	1	.126
tmin	2.358	1	.125

Minimal model

Terms	Type III		
	Wald Chi-Square	df	Sig.
zoo	94.233	4	.000
season	130.285	3	.000
worm	18.800	1	.000
age	8.362	1	.004

Table 4.2: Factors affecting colobus monkeys faecal egg count of *Trichuris sp*.

Results are from a GEE model based on *Trichuris* egg count per day, individual and data collection period from 37 animals in 5 groups. *Trichuris* egg counts were log transformed for the analysis.

Season was recorded as spring (March-April), summer (June-August), autumn (October-November) and winter (January). Index of success (IS) was derived from the number of dominant encounter won divided by the total number of dominant encounters and scaled between 0 (dominant) and 1-4 (from most dominant to most subordinate).

Age was in months and “worm” refers to animals under worming regime if having received anti-helmintic within two months prior to data collection. “Rain” was rainfall (mm), “tmax” was maximum temperature (T˚C) and “tmin”was minimum temperature (T˚C).

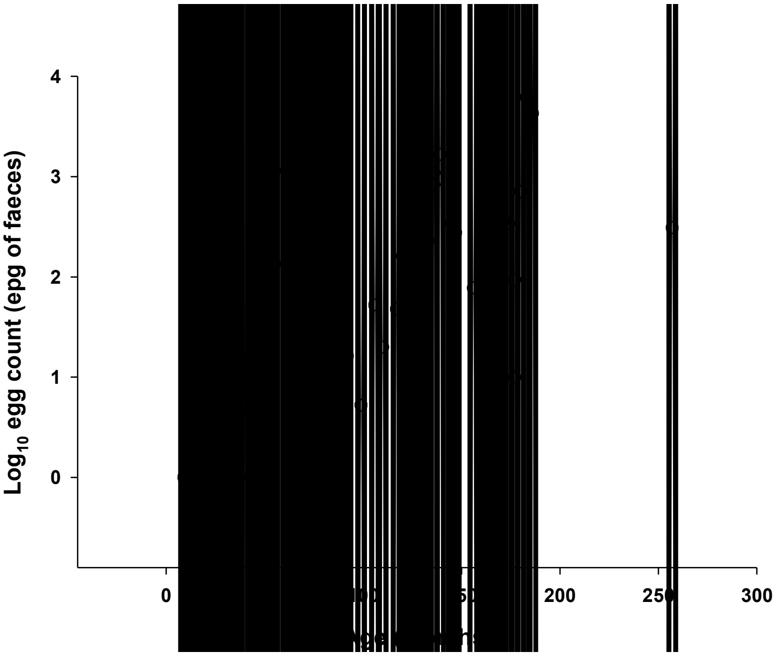


Fig 4.10: *Trichuris* egg count according to age in colobus monkeys.

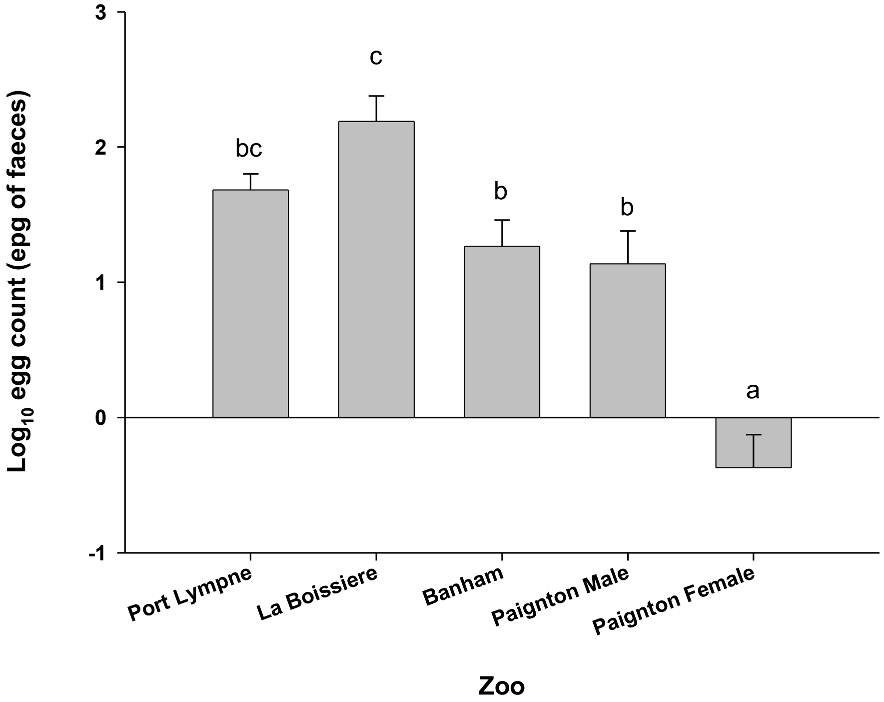


Fig 4.11: Mean (+SE) *Trichuris* egg count per gram of faeces (epg) for colobus monkeys at various zoos. Means and standard errors are predictions from the GEE model presented in Table 4.2, controlling for the variation in *Trichuris* egg count associated with the other significant predictors.

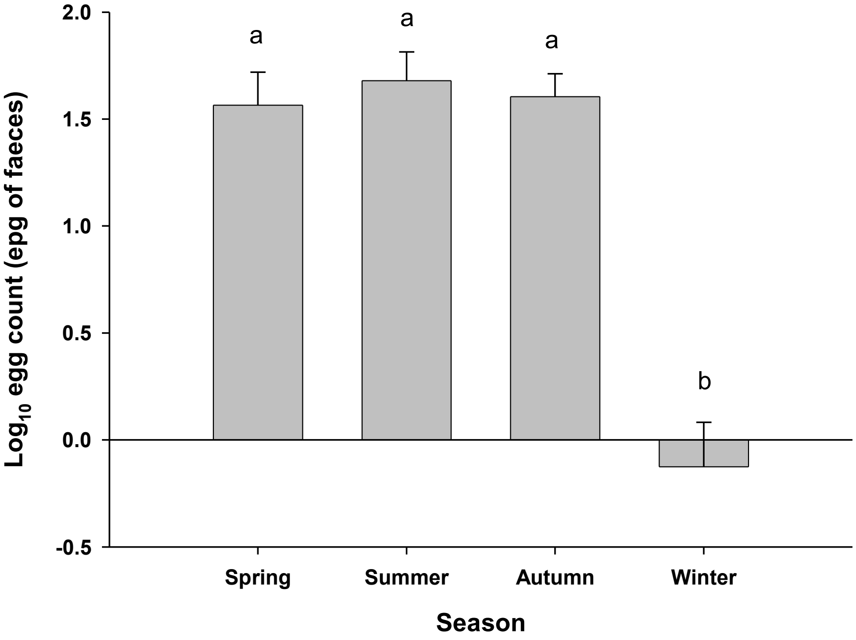


Fig 4.12: Mean (+SE) *Trichuris* egg count per gram of faeces (epg) for colobus monkeys during each season. Means and standard errors are predictions from the GEE model presented in Table 4.2, controlling for the variation in *Trichuris* egg count associated with the other significant predictors.

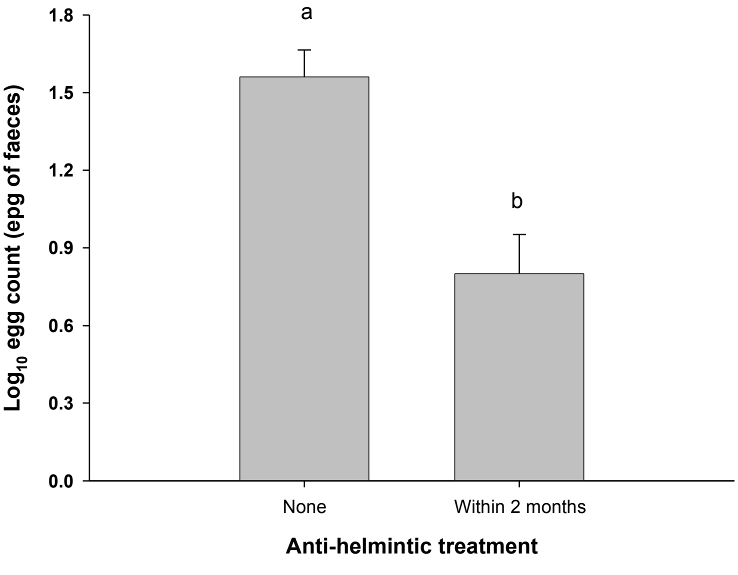


Fig 4.13: Mean (+SE) *Trichuris* egg count per gram of faeces (epg) for colobus monkeys according to anti-helmintict treatment. Means and standard errors are predictions from the GEE model presented in Table 4.2, controlling for the variation in *Trichuris* egg count associated with the other significant predictors.

4.4 Discussion

4.4.1 *Trichuris* egg count, age and index of success

Association between rank and parasite burden, at least within the male group structure of several primates, has been observed in previous studies. For example, among adult and sub-adult yellow baboon males, high ranking individuals were reported to have higher *Trichuris* faecal egg loads than subordinates, although the egg burden of females was not correlated with dominance rank (Hausfater & Watson, 1976). Similarly, in a captive group of mixed male and female colobus monkeys (six males, two females), a significant positive correlation between male rank and faecal *Trichuris* egg counts was observed (Melfi & Poyser, 2007). However, in the present study, index of success was not a significant predictor of *Trichuris* egg count. These data agrees with a lack of correlation between parasite infection (including *Trichuris spp*) and social rank, in olive baboons (MullerGraf *et al.,* 1996). Ranking of primates is often related to age or body mass which can be confounding factors when investigating parameters affected by rank, as reported for captive colobus monkeys at Paignton Zoo, in a mixed sex group (Melfi & Poyser, 2007). Similar results were observed in the current study where the interaction of age and index of success was significant with older animals being more dominant.

Heavy infections of *Trichuris trichiura* in humans are often found in children (Bundy *et al*., 1987; 1991), and similar age-related patterns of infection have been observed in a variety of parasites in primate species, such as the rhesus monkey (Philippi & Clarke, 1992) and the olive baboon (MullerGraf *et al*., 1996). In the present study, faecal *Trichuris* egg count was significantly predicted by age, however older animals tended to have higher count of *Trichuris* eggs. The association between high parasite burden and older animals seems to contradict the expected patterns where younger, less immunologically competent individuals were expected to be more vulnerable to disease and infection (Kessler *et al*., 1984). In humans, heavy infections with *T. trichiura* most often occur in children with the highest prevalence occurring at between five and ten years of age, decreasing after 15 (Bundy *et al*., 1987; 1991). It is not known at what age the colobus immune system develops but would be of interest in the future to determine how comparable their datasets are with human data, regarding immunological competence. Similarly, high levels of infection are often associated with children due to behavioural habits and lack of hygiene

(Bundy *et al*., 1987; Stephenson *et al*., 2000). These would not be expected to be relevant factors affecting primates, and may therefore not lead to significant differences from younger animals.

The present pattern of older animals having higher load of *Trichuris* eggs is in agreement with the preliminary study of colobus monkeys at Paignton Zoo where older males had higher burden of eggs (Melfi & Poyser, 2007). The patterns observed in both the present study and Melfi & Poyser (2007) study could be based on the concept of immune senescence (Coe, 1992; Onah & Nawa, 2000). These authors reports that immune responses in old animals changes and can be associated with disease and promote many age-related immune alterations towards the end of their life span. In wild *Colobus guereza*, no significant differences in *Trichuris* faecal epg were observed between age categories (Gillespie *et al*., 2005). The apparent association between age and parasite burden may be a by-product of captivity where animals are cared for, allowing them to reach much older age than they would otherwise in the wild.

4.4.2 *Trichuris* egg count and gender

In primates, females are often the instigator and performer of most grooming behaviour (Bernstein, 1970; Isbell & Young, 1993). This grooming may facilitate re-infection via the faecal-oral route (Munene *et al*., 1998), so higher parasite burdens would be expected in females compared to males. However, in wild *C. guereza*, there was no significant difference in *Trichuris* burden of males and females (Gillespie *et al*., 1992). This agrees with results for olive baboons (MullerGraf *et al*., 1996) and data from the present study in which no significant gender-related differences in *Trichuris* egg counts were identified. In a study of captive *C. guereza* at Paignton Zoo, opposite results were reported where adult males had significantly higher faecal epg than adult females and juvenile females (Melfi & Poyser, 2007). This study however, included data from two different groups. One made up of four females, and one made up of six males (four of which were fully grown adult males) and two females. The apparent difference between sexes could be due to other factors such as enclosure size, stocking density (see below) or behavioural differences. For example, in the large group with many males, competition and aggression between males may have been higher than the all female group (Chapter 3, section 3.4.1).

All animals in the mixed sex group were fed on one big platform indoors. The dominant male may have asserted his dominance at feeding time, displacing other males away from valuable resources such as food and/or females, resulting in the males feeding on the floor, therefore increasing their contact with contaminated faecal material and increasing possible re-infection. Unfortunately, further data are not available and a clear conclusion cannot be drawn. However, these results suggest that patterns within the captive environment seem inconsistent.

4.4.3 Seasonality of *Trichuris* epg

Results of seasonality for *Trichuris* egg count, while controlling for the variation associated with the other significant predictors, suggested that levels of infection were at their lowest in winter. All other season showed consistent levels of *Trichuris* egg count.

Seasonal patterns in *Trichuris* faecal egg counts has been observed in a variety of mammalian species based on temperature variation and rainfall (Jiménez *et al*., 2007; Hines *et al*., 2007). The increase in faecal egg output of other helminths has also often been linked to high levels of rainfall (Hammond *et al*., 1997; Ocaido *et al*., 1999; Yospeh *et al*., 2005). However, neither rainfall nor temperature, were significant predictor of *Trichuris* egg count in the present study. The present results suggest that seasonal variation in *Trichuris* egg count was present, with lowest parasite burden in January however, a tight link to rainfall or temperature was not apparent.

It is possible that the low *Trichuris* egg count in January emanated from behavioural differences of colobus monkeys in winter and other seasons. Colobus monkeys at various zoos spent little time in the outside enclosure in the winter, and therefore would not come into such regular contact with infective egg reserves contained in soil (Smyth, 1994; Stephenson *et al*., 2000). Animals were always fed indoors in the winter on feeding platforms that were disinfected each day. In contrast, throughout the rest of the year feeding was often on outdoor platforms often contaminated with faeces, some food fell to the floor also contaminated and cleaning did not occur daily. These behavioural and husbandry differences which occurred in winter, are likely to have altered re-infection rates in the group. In spring, the colobus monkeys fed and spent more time outdoors, increasing the possibility of re-infection from embryonated eggs in the soil. In summer, the monkeys also spent most of their time outdoors and were often observed eating soil from the outside enclosure which would maximise the possibility of re-infection (Cooper *et al*., 1995; Stephenson *et al*., 2000) increasing parasite loads during the summer visit, which would consequently remain apparent in October, due to the delay between infection by embryonated eggs and maturation of adults to start producing more eggs (Stephenson *et al*., 2000; Rothman & Bowman, 2003; Bundy & Jackson, 2004). The apparent seasonal variation observed in the faecal *Trichuris* epg of colobus monkeys may be an artefact of captivity and husbandry regimes rather than any natural seasonality of parasite infection *per se*. This is supported by data on the parasite burdens of wild *Colobus guereza* in the Kibale National Park, Uganda*,* where there was no correlation between *Trichuris* levels (or any of the other parasites monitored), and monthly rainfall, an indication of seasonal difference (Gillespie *et al*., 2005). A previous study of *Colobus guereza* held in captivity at Paignton Zoo under a consistent husbandry regime found no significant difference in *Trichuris* epg in faecal samples collected in autumn and spring (Melfi & Poyser, 2007).

It is therefore not possible to completely rule out a possible natural annual cycle of *Trichuris* intensity in the present study, but in a captive environment, seasonality of parasites is difficult to interpret due to varying husbandry regimes. Seasonal patterns in captive animals may only become apparent when the individuals are not exposed to an anti-helmintic regime for a substantial amount of time, letting natural patterns develop and become discernible such as the wild study type on yellow baboons (Hausfater & Watson, 1976).

4.4.4 The impact of anti-helmintic on *Trichuris* epg

A study of rhesus monkeys in captivity found significant differences in the number of pathogenic species when Ivermectin ® (a broad spectrum anti-helmintic) was administered (Phillippi & Clarke, 1992). The drug was injected intramuscularly in autumn and spring, and resulted in reduced numbers of *Trichuris* epgs in these seasons. Similar patterns were observed in the present study of colobus monkeys. Worming routine was a significant predictor of *Trichuris* egg count and animals not exposed to anti-helmintic had higher *Trichuris* egg count than those treated.

All zoos, apart from Port Lympne Zoo used the same brand of anti-helmintic (Zerofen, also known as Panacur), which has the active ingredient fenbendazole. This compound belongs to the benzimidazole family of anti-helmintics, successfully used as a broad spectrum agent against gastrointestinal parasites, lungworms and some tapeworms (Beier *et al*., 2000). However, it is not effective against the whole life cycle of *Trichuris* (Carithers *et al*., 2000); and no anti-helmintic is effective against the larval stage (Quihui-Cota, 2004). Consequently, re-infection is inevitable and zoos must maintain a continuous anti-helmintic programme to avoid heavy infections.

4.4.5 Enclosure size and husbandry

The two groups of colobus monkeys at Paignton Zoo were the subjects of a previous study (Melfi & Poyser, 2007), when held as a mixed sex larger group. Faecal *Trichuris* egg counts were much lower in the present study compared to the previously reported findings. Averages per visit for the two groups of animals rarely reached over 100 epg faeces, whereas Melfi & Poyser (2007) reported a lowest average of approximately 1000 epg faeces and up to 6000 epg. However, the group composition and husbandry regime were markedly different between the two studies. In Melfi & Poyser's study (2007), all animals were housed together in a large multi male, muti female group. In the present study, this group was split up into two one sex groups, males and females, and located in different enclosures, thus preventing direct comparison of the datasets. However, another study (Fox & Melfi, 2006) compared *Trichuris* egg load in faeces of the same colobus before and after moving to the new larger enclosure at Paignton Zoo, and found significantly reduced egg counts in the new enclosure. The enclosure at Paignton Zoo during the data collection by Melfi & Poyser (2007) was very small (only 76 m^2) and would have favoured re-infection, which could explain the high level of *Trichuris* at the time. Parasite prevalence and diversity have been shown to be closely correlated with stocking density (Freeland, 1979; Gillespie *et al*., 2005a). As more animals are housed together, in a restricted space, levels of social contact increase (Altizer *et al*., 2003), which favours parasite transmission due to the increased contact with contaminated material (Gillespie *et al*., 2005). Comparable results were found in groups of rhesus monkeys;

when 12 or more animals were housed together the number of samples containing helminths doubled (Phillippi & Clarke, 1992). Fox & Melfi (2006) suggested that when the stocking densities of colobus monkeys at Paignton Zoo decreased (the males moved from an enclosure with the overall area 76 m^2 to one of 476 m^2 and the females from an enclosure of 76 m^2 to one of 764 m^2), rates of re-infection would be reduced, leading to lower egg count. Female colobus monkeys at Paignton Zoo had the lowest faecal *Trichuris* egg counts than any other group. The enclosure the females were moved to was twice the size that of the males, and was brand new, with no other primate species having lived in it. The turf was freshly laid, with no known pre-existing egg bank in the soil, to facilitate re-infection (Fox & Melfi, 2006). The males were moved to an enclosure which was much older, and where other primate species had previously leaved, which may have facilitated some re-infection, however in minimal amount compared to other zoos such as Port Lympne or La Boissière du Doré.

Enclosure size and animal density may have been a factor affecting faecal *Trichuris* epg at La Boissière du Doré Zoo. This zoo had some of the highest levels of *Trichuris* egg count compared to Banham Zoo and Paignton Zoo male and female groups. In spring, this group had the highest egg count of all zoos studied. This group was fairly large (n=8), but more importantly, animals were locked in for most of the winter (normally between October and March). Consequently, in March 2006, these animals had been locked in for several months, and despite every effort to clean the enclosure each day, the probability of re-infection via ingestion of fertilised eggs would be greater as restricted to a closed environment (Gillespie *et al*., 2005). Additionally, all animals in the group were regularly observed eating the bark of perches and wooden beams from the walls of the enclosure which probably also favoured re-infection by embryonated eggs (Chapman *et al*., 2005). Spatial proximity increases the probability of parasite exchange and parasites with direct life cycle (such as *Trichuris* sp) are more likely to increase in prevalence with increasing host density (Chapman *et al*., 2005). Additionally, the enclosure these animals were confined in was relatively small compared to other zoos. In March 2006, before anti-helmintic treatment and after confinement, the egg count per gram of faeces averaged around 1800. These results were comparable to those reported by Melfi & Poyser (2007). Although conclusions could not definitely be drawn, results by Fox & Melfi (2006) and trends in the present study seem to suggest that animals confined in small enclosures with no anti-helmintic treatment display higher levels of infection than those in larger enclosures.

Finally, the two zoos with the highest level of infections were always Port Lympne Zoo and La Boissière du Doré Zoo. It may also be significant that these were the zoos where pressure washing was not possible within the cage. Similar results were obtained by Phillippi & Clarke (1992) while studying captive rhesus monkeys. The enclosures housing these animals were pressure-washed on a daily basis and displayed reduced parasitism compared to others, probably due to the restricted chance of repeated infections. In the present studies, Banham Zoo was unusual compared to others in being one of the largest groups but having some of the lowest egg counts. However, the colobus monkeys at Banham Zoo had daily access to a particularly large paddock area (see Chapter 2, Table 2.4) where on sunny days, they were fed. At night, this group was locked in, but the inside housing was pressure-washed every day reducing the probability of re-infection. The Paignton Zoo female group showed the lowest levels of infection (with some individuals never showing any eggs present in the faeces). This group was relatively small (five individuals at most) in a particular large enclosure with inside and outside housing, and pressure washed every day.

4.5 Conclusions

Patterns of Trichuriasi*s* in all zoos followed the expected association between faecal egg counts and social rank/age as observed in the preliminary study at Paignton Zoo (Melfi & Poyser, 2007). Older animals had higher count of *Trichuris* egg in faecal samples.

No significant differences in faecal epg were observed between gender, which contradicts the results of the preliminary studies at Paignton Zoo (Melfi & Poyser, 2007), but matches data on wild colobus studies.

Seasonal patterns of infection were observed but additional factors such as animal behaviour and husbandry practices appear to be an important influence. However, these are difficult to differentiate because of the non-invasive nature of the studies. The observed differences between zoos seem to result from a combination of parameters such as group composition and behaviour, enclosure size and husbandry regimes.

Chapter 5

Non invasive monitoring of faecal cortisol equivalents in relation to rank, age, gender and time

5.1 Introduction

5.1.1 Measurement of stress from plasma and faecal samples

Stress can be evaluated by measuring the levels of cortisol, corticosterone or their metabolites. Corticosteroids may improve fitness by energy mobilisation (Möstl & Palme, 2002) where the availability of glucose in the bloodstream increases and glucose production is promoted while tissue uptake and storage is limited (Nelson, 2000) or by regulating inflammatory response to injury (Morrow *et al*., 2002), (Chapter 1). Prolonged secretion (often resulting during chronic stress) may on the other hand have deleterious effects on individuals, for example by causing immuno-suppression and thereby increasing the risk of disease (Balm, 1999), by causing tissue atrophy (Mason *et al*., 2008) or by changing gastrointestinal functions through brain mediation (Monnikes *et al*., 1994).

The corticosteroid hormones are released into circulation during stress, after activation of the hypothalamo-pituitary-adrenal axis resulting in elevated concentrations in blood plasma (Munck *et al*., 1984). Plasma corticosteroids, extracted from blood samples, have been widely used to evaluate stress levels in animals (e.g. Bercovitch & Clarke, 1994; Johnson *et al*., 1996). This technique presents several significant problems. Initially, the animal must be caught and handled to provide a blood sample. This may affect the results particularly in free/semi-free and even captive populations, as the process itself may induce stress (it is invasive) and may therefore lead to an increase in corticosteroid concentration (Möstl & Palme, 2002; Good *et al*., 2003). Additionally, even assuming blood samples are rapidly obtained, the measurement of plasma corticosteroids at best provides a snapshot of current status, which may not reflect long-term patterns (Möstl & Palme, 2002). Non-invasive approaches to investigate the stress status in animals are now favoured as these avoid ethical issues and can provide more reliable long-term information on stress status.

Elevated circulatory concentrations of steroid hormones have been reported to result in elevated salivary (Umeda *et al*., 1981; Kuhar *et al*., 2005), urinary (Touma *et al*., 2003; Muller *et al*., 2004) and faecal corticosteroids or their metabolites in a variety of species (Whitten *et al*., 1998; Good *et al*., 2003; Young *et al*., 2004). Saliva, urine and faeces therefore provide samples that can be used to investigate the stress status of animals non-invasively (for example, in the chimpanzee using urine: Muller *et al*., 2004). Salivary cortisol provides information on the immediate levels of corticosteroids, but urinary or faecal measurements of corticosteroids and their metabolites are preferred as a longer term, integrated measure of welfare and stress (Möstl & Palme, 2002). Data may be collected from urine or faeces, although the collection of faeces is more widespread due to obvious practical difficulties of collecting urine samples from most wild and captive animals. Another non-invasive method has been developed in the last few years, using hair (Koren *et al*., 2001), which also provides long-term information of hormonal profiles. The advantage of non-invasive monitoring of steroids comes from the longitudinal aspect of the data as they reflect the total amount of cortisol/corticosterone or their metabolites excreted (Palme *et al*., 1999).

The time necessary to observe the effect of a stressful event on levels of corticosteroid metabolites in faeces is often dependant on the intestinal passage rate of the species under study (Touma *et al*., 2003) whereas in blood plasma, it is almost instantaneous. This was shown by Palme *et al*. (1996) by infusing various species with radioactive cortisol and measuring the amount excreted via faeces and urine and the time it took to be excreted. The lag time between a peak of cortisol in blood plasma and its appearance in the faecal sample seems to vary widely between species from a few hours (for example in the common marmoset, *Callithrix jacchus,* Bahr *et al*., 2000), between 22 and 26 hours in the long-tailed macaque (*Macaca fascicularis),* and the chimpanzee, *Pan troglodytes* (Bahr *et al*., 2000), and up to 48 hours in the pig (*Sus domestica)* and other hindgut fermenters such as the white rhinoceros (*Ceratotherium simum*) and a variety of primates such as the cotton top tamarin (*Saguinus oedipus*), macaques and the ring-tailed lemur, *Lemur catta* (reviewed by Schwarzenberger *et al*., 1996). A lag time between plasma and faecal samples results from conjugation of circulating hormones in the liver, before they are excreted via the bile, and sometimes further hydrolysis by bacteria during passage through the gut (Möstl *et al*., 2002; Beehner & Whitten, 2004).

Non-invasive faecal monitoring of glucocorticoids and their metabolites has proved successful in a variety of species investigating various factors affecting stress in wild, captive or domestic animals. This method had been applied to measure faecal cortisol metabolites in the roe deer, *Capreolus capreolus* (Dehnhard *et al*., 2001) and to look at the impact of capture and season on faecal corticosteroid levels in deer mice (*Peromyscus maniculatus*) and red-backed voles, *Clethrionomys gapperi* (Harper & Austad, 2001). Faecal corticoids have also been examined to investigate the effect of female dominance in ring-tailed lemurs (Cavigelli *et al*., 2003), to examine the effect of dominance and aggression in wild populations of wolves, *Canis lupus* (Sands & Creel, 2004) and the impact of stressors such as translocation or social tension on captive spotted hyenas, *Crocuta crocuta* (Goymann *et al*., 1999).

5.1.2 Steroid pathway

With the exception of retinoic acid, all steroid hormones are derived from cholesterol. The C_{27} of cholesterol is converted directly into the 21-carbon steroid hormone, pregnenolone, in the adrenal cortex. The cleavage of the C_{27} to C_{21} is catalysed by desmolase (a P450-linked side chain cleaving enzyme, P450ssc), found in the mitochondria of steroid-producing cells. Pregnenolone then moves into the cytosol to be converted to androgens, mineralocorticoids or glucocorticoids by enzymes of the endoplasmic reticulum. Glucocorticoids are a group of steroids, which are differentiated from mineralocorticoids and androgens by specifically binding to glucocorticoid receptors. This group contains the steroids of interest in the present study such as cortisol, cortisone, 21-deoxycortisol and corticosterone.

Steroid synthesis in the zona fasciculata is regulated by the adrenocorticotropic hormone (ACTH) of the anterior pituitary gland. ACTH receptors in the plasma membrane activate the enzyme, adenylate cyclase with the production of a second messenger, cAMP. The impact of ACTH on corticosteroids is regulated by a negative feedback loop regulating the levels of corticotrophin releasing hormone (CRH) produced by the hypothalamus, ACTH and corticosteroids produced by the adrenal gland. The pathway of cholesterol metabolism to produce cortisol is summarised in Figure 5.1:

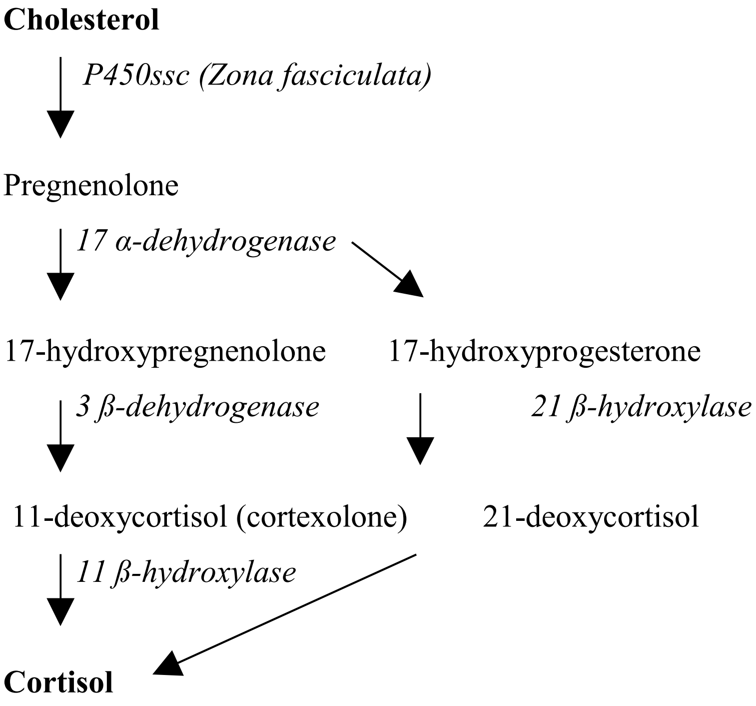


Figure 5.1: Summary of metabolic pathway from cholesterol to cortisol.

Cortisol is metabolised in the liver to cortisone (by catalysation with 11-ß-steroid dehydrogenase). Cortisone itself is reduced to dihydrocortisone, which in turn is hydroxylated to tetrahydrocortisone, mainly in the liver. Tetrahydrocortisones can either be converted into glucuronides by conjugating to a hydroxyl group (mainly in the liver and to a lesser extent in the kidney) or be reduced into cortolone (a 17-ketosteroid precursor). Cortisol itself is reduced to dihydrocortisol, which in turn is hydroxylated to tetrahydrocortisol. Tetrahydrocortisoles can either be converted into inactive water soluble glucuronides by conjugating to a hydroxyl group or be reduced into cortol (another 17-ketosteroid precursor). Oxidation of cortolone and cortol produces 17-ketosteroids, which are excreted in urine as sulphate conjugates.

Hormone secretions in many species have been shown to exhibit a circadian rhythm (De Jong *et al*., 2000; Touma *et al*., 2003; Szeto *et al*., 2004) or be affected by activity patterns (Touma *et al*., 2003). It is therefore important in endocrinology studies to collect samples within the same time frame (to ensure consistency within the dataset). Activity patterns for colobus monkeys, being a diurnal species (see Chapter 1, section 1.1) would vary greatly between night time (therefore affecting morning faeces collection) and day time. Collection of faeces at different times of day could therefore invalidate the data (Chapman *et al.,* 2006).

5.1.3 Presence of glucocorticoid and their metabolites in faeces and urine

The proportion of glucocorticoid metabolites found in faeces and urine varies between species. Mammals such as mice, *Mus musculus f. domesticus* (Touma *et al*., 2003) and cats, *Felis catus* (Schatz & Palme, 2001) were found to excrete most of their glucocorticoids metabolites in faeces, rather than urine. However, by measuring the excretion of an injection of radiolabelled cortisol, Schatz & Palme (2001) found opposite results in dogs (*Canis lupus familiaris*), where the majority of glucocorticoids metabolites were found in the urine. Other species including the hare, *Lepus europaeus* (Teskey-Gerstl *et al*., 2000), horses (*Equus ferrus caballus*) and pigs, *Sus domestica* (Möstl *et al*., 1996) and various ungulates, clouded leopard *(Neofelis nebulosa*), Malayan sun bear (*Helarctos malayanus)*, long-tailed macaque and northern spotted owl, *Strix occidentalis caurina* (Wasser *et al*., 2000) were found to excrete most of their cortisol metabolites in urine. A study by Bahr *et al*. (2000) looking at the common marmoset, long-tailed macaque and chimpanzee, also indicated that more than 81 % of the radiolabelled cortisol was excreted in the urine. This in itself does not affect results from faecal samples, but does restrict the comparison of the total amount of corticosteroid and their metabolites in different species that may vary in the percentage excretion in urine and faeces.

Levels of authentic cortisol or corticosterone recorded in faeces vary significantly between species. In the chimpanzee and long-tailed macaque (Bahr *et al*., 2000) recovery of cortisol was virtually non-existent. This agrees with data for other species such as the African elephant, *Loxodonta africana* (Ganswindt *et al*., 2003), domestic cat and dog (Schatz & Palme, 2001), the hare (Teskey-Gerstl *et al*., 2000), and ruminants (Möstl *et al*., 2002), the Barbary macaque, *Macaca sylvanus* (Wallner *et al*., 1999), various ungulates, the clouded leopard, Malayan sun bear, long-tailed macaques and the northern spotted owl

(Wasser *et al*., 2000), carnivores such as the sloth bear (*Melursus ursinus*), cheetah (*Acinonyx jubatus*), black-footed ferret (*Mustela nigripes*), slender-tailed meerkat (*Suricata suricatta*), and red wolve, *Canis rufus* (Young *et al*., 2004) and sheep (Palme & Möstl, 1997). However, other studies suggest that measureable amounts of cortisol are excreted in faeces (Whitten *et al*., 1998; Bahr *et al*., 2000; Young *et al*., 2004; Peel *et al*., 2005; Heistermann *et al*., 2006; Li *et al*., 2007).

A study by Heistermann *et al*. (2006) was devised to specifically compare results using various EIAs for several species of primates including the common marmoset, long-tailed macaque, Barbary macaque, chimpanzee and gorilla (*Gorilla gorilla*). Two assays (11b-hydroxy etiocholanolone and 11-oxo etiocholanolone) used group specific antibodies raised against cortisol metabolites (5b-reduced cortisol metabolites with a 3a,11-oxo and 3a,11b-dihydroxy structure) and two raised against cortisol and corticosterone respectively. Group specific antibodies measure glucocorticoid metabolites which share part of their structure as opposed to a specific antibody which measures the authentic compound. In the long-tailed macaque and chimpanzee, authentic cortisol was virtually undetectable, and group-specific assays provided much stronger signals of glucocorticoid output. In the other three species, (marmoset, Barbary macaque and gorilla), after an adrenocorticotropic hormone challenge, authentic cortisol was clearly detectable in extracted faecal samples separated by high performance liquid chromatography from. These species showed valuable results in the cortisol specific assay. Other species have been found to excrete substantial amounts of cortisol in their faeces. This has been shown in the Himalayan black bear (*Ursus thibetanus*) and clouded leopard (Young *et al*., 2004), Père David's stag deer, *Elaphurus davidianus* (Li *et al*., 2007), lowland gorilla (Peel *et al*., 2005), several primates such as the chimpanzee (Whitten *et al*., 1998) and the common marmoset (Bahr *et al*., 2000). Therefore, results regarding the presence of authentic cortisol in faeces appear contradictive at times. For example, it was absent in the chimpanzee in studies by Bahr *et al*. (2000) and Heistermann *et al*. (2006), but present in studies by Whitten *et al*. (1998). No clear pattern exists, even for closely-related species. This is probably due to the species-specific differences in steroid metabolism and the variation in gut micro-flora, which may create a large range of metabolites varying between species

(Wasser *et al.*, 2000). So far, there have been no studies of faecal corticosteroids metabolism in the Abyssinian colobus monkey.

Two methodologies are now preferred for analysis of glucocorticoid metabolites extracted from faecal samples. The first method is by enzyme immunoassay (EIA) and has been used widely in a variety of mammalian species including domestic cat and dog (Schatz & Palme, 2001), the African elephant (Ganswindt *et al.*, 2003), hare (Teskey-Gerstl *et al.*, 2000), hyena (Goymann *et al.*, 1999), roe deer (Dehnhard *et al.*, 2001), horse and pig (Möstl *et al.*, 1999), sheep (Möstl *et al.*, 2002) and a variety of primate species (Bahr *et al.*, 2000; Heistermann *et al.*, 2006). Radioimmunoassay (RIA) using ^{125}I cortisol and antibodies raised against cortisol have also been used to measure faecal glucocorticoids and their metabolites in a variety of mammals including the ring-tailed lemur (Cavigelli, 1999; Cavigelli *et al.*, 2003), the Père David's deer stag (Li *et al.*, 2007), the wolf (Sands & Creel, 2004), and the chimpanzee (Whitten *et al.*, 1998). Other ^{125} I RIAs have also been used, such as a corticosterone RIA in dairy cattle (Morrow *et al.*, 2002), Stella sea lion, *Eumetopias jubatus* (Hunt *et al.*, 2004) and bison, *Bison bison* (Mooring *et al.*, 2006).

5.1.4 Faecal glucocorticoid content in relation to social dominance hierarchy

A large proportion of primates live in social groups with a defined hierarchy (Estes, 1991). Different relationships between stress levels and dominance hierarchy have been found, depending on the primate species (and gender) investigated and their type of social structure. For example, in some species, higher ranking animals seem to exhibit higher levels of faecal glucocorticoids than subordinates, as reported for the male captive mouse lemur, *Microcebus murinus* (Perret, 1992). For females, a correlation between high rank and high levels of glucocorticoids has often been explained as a consequence of the monopoly of breeding by the dominant females, as in the common marmoset (Saltzman *et al.*, 1994). In a group with singular breeders, dominant animals would be expected to be more socially stressed than subordinates because of the need to maintain their status and access to a mate (Creel, 2001). However, similar patterns have also been observed in cooperative breeders such as ring-tailed lemurs (Cavigelli *et al.*, 2003; Gould *et al.*, 2005), suggesting that the type of reproduction itself may not be the primary factor determining glucocorticoid levels.

This positive relationship between faecal glucocorticoids and rank is often observed in species with low levels of aggression, such as the common marmoset, (Lazaro-Perea, 2000). However, this sort of pattern would also be expected in highly unstable groups where dominance would be contested (Sapolsky, 1992).

Profiles of glucocorticoid secretion compared to dominance may be reversed, with a negative relationship where lower ranking animals have higher levels of faecal glucocorticoids than dominant ones. This was observed in male olive baboons, *Papio anubis* (Sapolsky, 1992; Sapolsky *et al*., 1997) and female cynomolgus monkeys, *Macaca fascicularis* (Shively *et al*., 1997). This relationship between stress levels and dominance hierarchy may be expected where resources are unevenly distributed and animals compete for them in a highly aggressive manner, putting the lower ranking animals under greater social and physical stress (Abbott *et al*., 2003). Finally, some animals such as male rhesus macaques, *Macaca mulatta* (Bercovitch & Clarke, 1995), male ring-tailed lemur (Gould *et al*., 2005), and tufted capuchins, *Cebus apella* (Lynch *et al*., 2002) have shown a lack of correlation between rank and faecal glucocorticoids.

Other studies have looked at the impact of aggression rather than ranking on the level of faecal glucocorticoids in primates. Cavigelli (1999) and Cavigelli *et al*. (2003) found no relationship between aggression levels and faecal glucocorticoids in male ring-tailed lemurs, whereas in females (who are dominant over males in this species), faecal glucocorticoids were related to aggression given (for dominant female) and received (for low-ranking females). Other studies have also found a correlation between aggression and faecal glucocorticoids in species such as the Chacma baboon, *Papio hamadryas ursinus* (Bergman *et al*., 2005) or the chimpanzee, (Muller & Wrangham 2004).

Abbott *et al*. (2003) proposed a model to explain the different patterns between faecal glucocorticoids and rank, based on kinship, frequency of stressors and social support. The model suggests that lower ranking animals would have higher levels of faecal glucocorticoids when exposed to frequent aggression from the more highly ranked animals, while receiving low levels of support. When levels of aggression are reduced, similar faecal glucocorticoid levels between low and high ranking animals would be expected.

Colobus in their natural habitat live in groups, each with a dominant male and several related females and their offspring, although groups with multi males have also been observed (Estes, 1991). Only the dominant male normally mates with the females, but all colobus in the group (mainly females though) take care of the young. In the present study, one group (Banham Zoo) complied with this social formation, and it was predicated that animals in this group would have relatively low levels of stress, especially the dominant male, which had no competition from other males to mate with the females. Others in the group would also be expected to have low stress levels due to the generally reduced aggressive climate and the abundance of kin support (Abbot, 2003). In contrast, two groups (at Port Lympne and La Boissière du Doré Zoos) consisted of several adult and sub-adult males, mixed with females (only one adult female at La Boissière du Doré Zoo). Therefore, in these groups, higher levels of faecal glucocorticoid might be anticipated based on the increased levels of aggression, particularly between adult males. The dominant male of each group would be expected to have the highest levels of faecal glucocorticoids because of the need to defend his access to the females (i.e. contested dominance: Saplosky, 1992). It was predicted that at Paignton Zoo, the all female group would show very low levels of faecal glucocorticoids since other studies have indicated a weak dominance hierarchy in all female groups (Grunau & Kuester, 2001) and low levels of aggression, although this may depends on the particular species and whether data have been collected in captivity or in the wild (Shi *et al*., 1982; Ren *et al*., 1998; Zhang *et al*., 2008). The Paignton Zoo all male group, however, was expected to show higher levels of faecal glucocorticoids, decreasing down the dominance hierarchy, because of the high levels of aggression between males.

The analysis of faecal corticosteroids and their metabolites provides a valuable integrated index of physiological status and offers the potential to be employed without harm to an animal to evaluate environmental conditions in captivity and changes in management strategies (Möstl & Palme, 2002). The aim of this study was to measure faecal glucocorticoids in several groups of Abyssinian colobus with a variety of group structures (multi-male multi-female, single male multi female, single female multi male, all male and all female) and compare faecal glucocorticoid contents between zoos and to index of success, age, levels of aggression, sex, age and reproductive status of females.

5.2 Materials and methods

5.2.1 Faecal sample collection

Animals were observed between 08:00 and 17:00 to record behaviour (Chapter 2, section 2.2.4) and to collect faecal samples from known individuals for parasitology and endocrinology. Faeces voided between 12:30 and 16:30 were marked on a cage map and the donor was identified. At Port Lympne Zoo, samples were collected immediately after defecation (but stored and only frozen after data collection finished every day as the other zoos). At all other zoos, sample collection had to be delayed until feeding time in the late afternoon, when the animals were locked away to permit entrance to the cage.

Faeces voided indoors are preferable as some studies suggest that weather patterns (mainly rain) can affect hormone content of the faeces (Washburn & Millspaugh, 2002). However, as samples were always collected within four hours of being deposited and therefore only had limited exposure to the elements, all faecal samples voided outside during dry weather were collected and analysed.

Any faecal sample voided on top of another sample from a different animal was discarded. Similarly, faecal samples contaminated by urine, either because the faeces were voided on urine itself, or because an animal urinated while defecating, were rejected from hormonal analyses. This is because urine also contains excreted steroids that could interfere with measurement of faecal hormonal levels.

Each sample was collected into a plastic tube labelled with the date, time and the name of the donor. As far as possible, entire faeces were collected (and not split for parasitological data, see Chapter 4, section 4.2.1) as some studies have suggested that steroids are unevenly distributed in faecal matter (Brown *et al*., 1994; Palme *et al*., 1996; Wasser *et al*., 1996; Millspaugh & Washburn, 2003). Samples were then placed in a freezer at -20 ºC at the end of each day of collection, as suggested by Möstl & Palme (2002), to limit conversion by bacteria and bacterial enzymes. All samples were frozen within 5.5 h, which was within the time limit of 6 h recommended by Huber *et al*. (2003 b). At the end of each visit, all samples were placed in a polystyrene box on and covered by frozen gel packs and taken to Exeter University (maximum of 12 h from France), where they were stored at -20 ˚C until processing.

5.2.2 Lyophilisation

Within 2 weeks of collection, all faecal samples were lyophilized (Edwards freeze-drier, -40 ˚C) to remove all water, which varied between samples and could influence extraction of faecal glucocorticoids. Lyophilisation also permits the later grinding that allows the removal of foreign elements such as hair and seeds, and which would also affect the measurement of faecal cortisol equivalents in the RIA.

Initially, the time required to achieve complete lyophilisation was determined using 32 samples from different colobus monkeys (four from each of eight animals) at Paignton Zoo (male and females). These samples were weighed and placed in a freeze-drier, vacuuming at -40 ˚C. After an initial period of 72 h and then at 96 h, 120 h and 144 h, samples were taken out and weighed again. The declining weight during drying is shown in Figure 5.2. A repeated measures ANOVA on rank was performed on all data and was significant ($\chi^2 = 110.775$, $p<0.01$, df = 4). After 72 h, samples did not decrease significantly in weight ($q = 3.466$, $p>0.05$). As a measure of precaution, faecal samples were routinely lyophilised for 96 h and then stored at -20 ˚C until extraction.

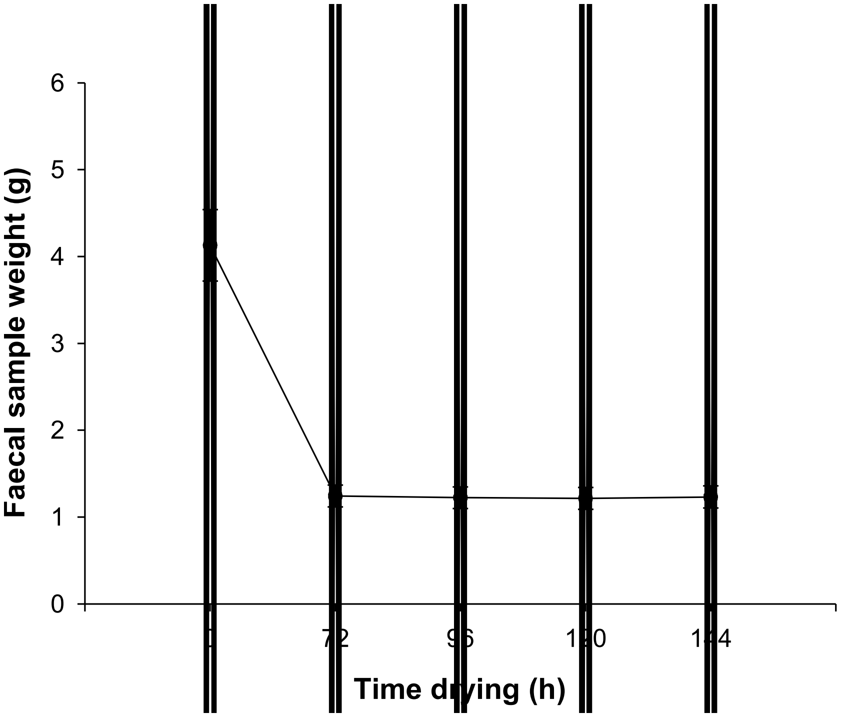


Fig 5.2: Mean (± SE) drying time for samples (n = 32) lyophilised in vacuum freeze drier.

5.2.3 Grinding

Each dried sample was taken out of the freezer and allowed to reach room temperature for one hour. The samples were then individually ground in a clean pestle and mortar. At this stage, hair, seeds and fibres were removed using clean tweezers. The samples were then sieved sequentially with grinding through 250 µm and 125 µm stainless steel sieves (Endecotts) and then transferred to acid-washed glass tubes with screw-top lids through a glass funnel. The tubes were capped and stored at -20 °C. All equipment was disinfected with Virkon (Antec International, Sudbury, Suffolk, UK) and rinsed twice with distilled water between each sample.

After preparing several samples by grinding and extracting them, a large disparity in the amount of fibre in faecal samples between zoos, and sometimes between colobus monkeys within a zoo was noticed. With all complete groups left available, each sample was weighed before grinding. Seeds and large pieces of fibre and any material that was removed from the sieve were collected and weighed to give an approximate value for the fibre content of each sample. These values were expressed as a percentage of initial mass of the sample.

5.2.4 Extraction of faecal samples

Faecal glucocorticoids were extracted in 95 % HPLC grade methanol at 4 ml per 0.5 g of dry faecal powder. There was a large disparity between the mass of dry faecal powder per sample and if the entire sample had been extracted, the amount of methanol added would have varied between 0.5 ml and 6.5 ml, giving rise to difficulties in deriving assay blanks. Therefore, 0.2 g of faecal powder was routinely extracted in 1.6 ml 95 % methanol, maintaining the 1:8 ratio (g faeces: ml methanol) until 1 ml methanol was used. For the few samples where there was less than 0.125 g of faecal powder available, a ratio of 1:16 (g faeces: ml methanol) was used to allow adequate pellet formation during the centrifugation step.

The faecal/methanol mixture was capped and sealed with parafilm, vortexed for 10 s and shaken for 30 min with another 5 s vortex after 15 min (Scientific Industries, Vortex

Genie 2). After shaking, the tubes were re-vortexed for 10 s to remove all particles from the side of the tube and centrifuged at 3000 g for 20 min at 4°C (MSE Mistral 3000).

After centrifugation, each supernatant was pipetted with a new glass Pasteur pipette into a small acid-washed glass tube, capped, labelled and stored at -20ºC until radioimmunoassay (which was performed within 90 days of extraction as suggested by Khan *et al*., 2002 to minimise variations in glucocorticoid content).

Each extraction included a procedural blank in which the same steps as described above were followed, using 1.6 ml methanol, but with no faecal matter.

5.2.5 Extraction efficiency

Extraction efficiency was estimated by adding a known amount of ^{125}I cortisol (approximately 1000 cpm in 50 µl phospho-buffered saline (PBS: Sigma) to several samples of faecal powder (0.2 g) and extracting in 1.6 ml of 95 % methanol (as described in section 5.2.4). After centrifugation, approximately 1 ml of spiked methanol was recovered. Of this, 500 µl were removed and diluted with 500 µl PBS in LP4 tubes. This procedure was repeated for 12 faecal samples from different zoos, taking samples from males and females, adults, sub-adults and juveniles. Separately, 50 µl of ^{125}I cortisol was placed in triplicate LP4 tubes (Thermo Fisher Scientific) and diluted to 1 ml with 950 µl 50 % methanol, so to be comparable to the sample volumes and concentration. The ^{125}I cortisol was counted in a gamma counter (Cobra auto-gamma counter, Packard) and counts were compared to those extracted from spiked faecal samples. The mean recovery rate was 96.88 ± 3.49 % (n = 12). Although recovery was very high, all data for samples were corrected using this value.

5.2.6 Radioimmunoassay

5.2.6 a Assay methanol concentration

During the assay development, several methanol dilutions (5 %, 10 % and 20 %) for the samples and the standard curves were investigated. Three sets of standard curves were prepared (as described in section 5.2.6c) and assayed with all other variables held constant. Three samples were also analysed to investigate the impact of the standard curve dilution on the sample reading. As shown by Figure 5.3a, a methanol concentration of 20 % seemed to have an impact on the standard curve by rendering it more variable and making the readable part of the curve less reliable, and only reaching a maximum percentage binding of about 70 %. Figure 5.3b shows the standard curve at 5 % methanol. Although the curve displayed the normal sigmoidal shape, when samples were diluted to match 5 % methanol, a large proportion of the samples were too dilute to be readable. Finally, Figure 5.3c shows the assay curve with samples in 10 % methanol. This shows a good sigmoidal standard curve shape and allows all samples to be read. The middle part of the curve had the steepest slope, and all samples fell on this part of the curve.

5.2.6 b Antiserum and ^{125}I cortisol concentration

To investigate the most appropriate anti-serum concentration to employ in the RIA, a dilution curve was established to find the optimum percentage binding, at around 20 %. The range of dilutions used was from 1:1000 to 1:100,000. Aliquots of 100 µl were placed in duplicate LP4 tubes and 100 µl radio labelled ^{125}I cortisol, Cortisol-3-(O-carboxymethyl) oximino-(2-[^{125}I]iodohistamine (GE Healthcare, Amersham 2000 Ci/mmol, 3.7 MBq/ml, 100 µCi/ml), at 2500 cpm was added to each tube. As a total volume of 250 µl would be used in the final assay, another 50 µl of 10 % methanol was also added to the tubes to make volumes comparable. Two 'totals tubes' (containing only ^{125}I cortisol) and two blank tubes (without antiserum), were also prepared, following the RIA method described in section 5.2.6c. The concentrations of antiserum which gave the optimum percentage binding of around 20 % was between 1:20,000 and 1:30,000 as shown in Figure 5.4.

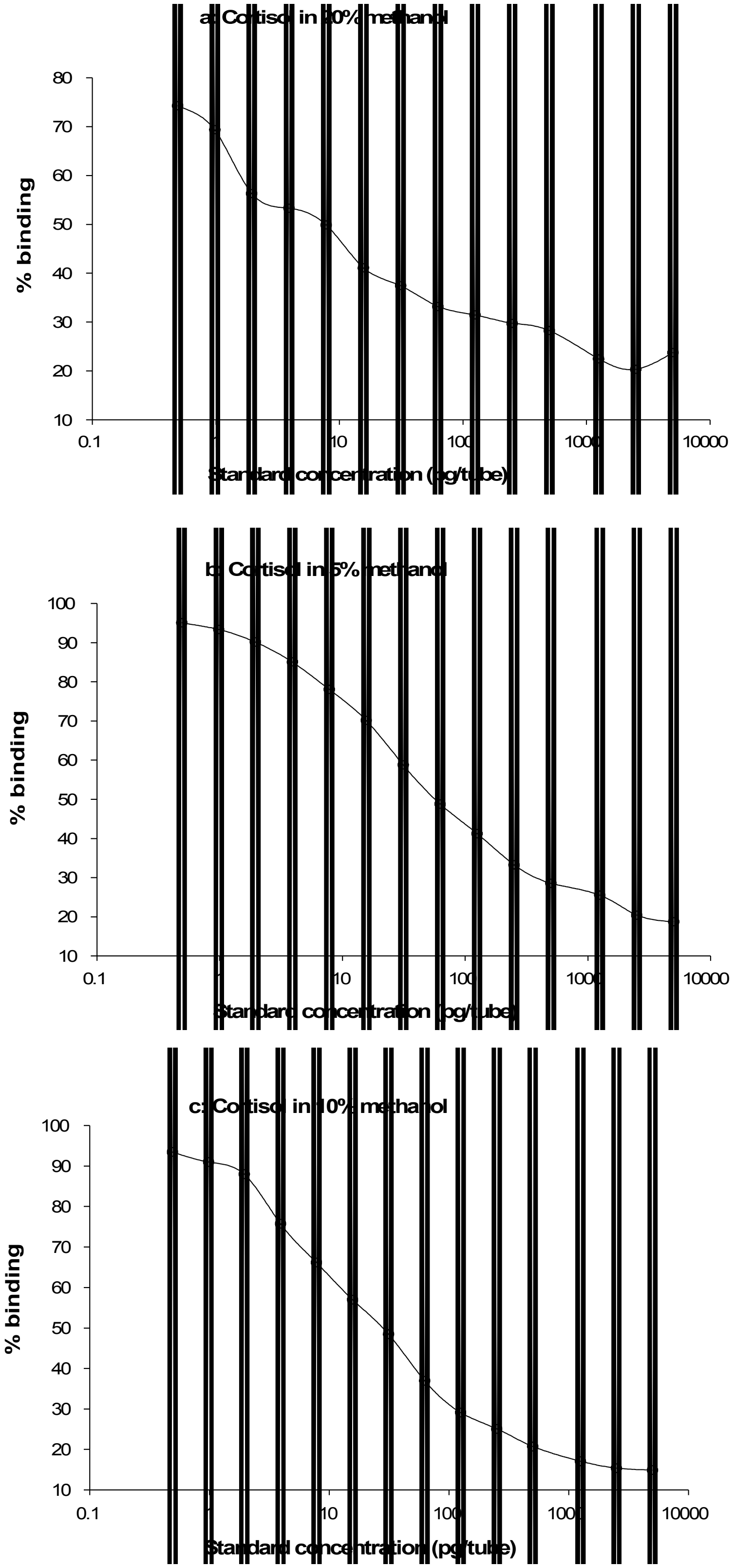


Fig 5.3 a-c: Standard curves showing percentage binding of standards made up in different methanol concentrations.

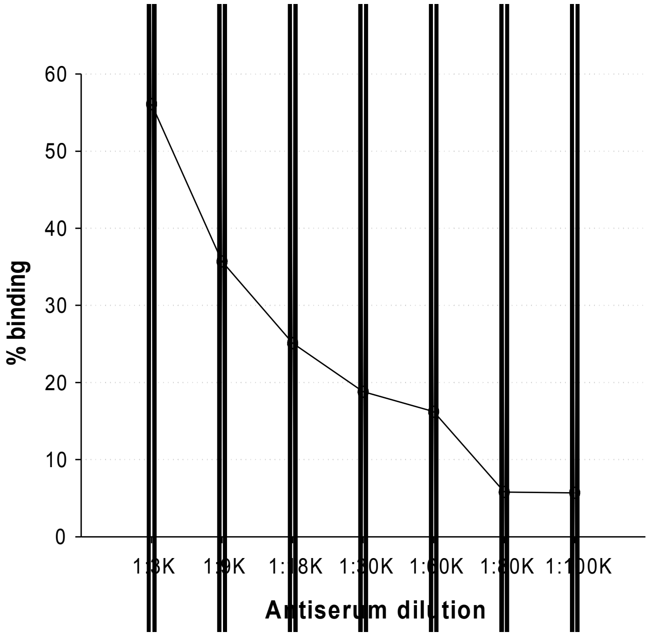


Fig 5.4: Antiserum dilution curve showing the percentage binding of ^{125}I cortisol at a range of antibody dilutions and where K represents x1000.

To find the most sensitive concentration of antiserum to be used in the radioimmunoassay, several standard curves were run using a range of antiserum concentrations varying from 10,000 to 30,000 fold dilution. All volumes were kept constant and approximately 2500 cpm ^{125}I cortisol was used, as described above. As shown in Figure 5.5 the curve resulting in the greatest displacement (therefore being the most sensitive) was at the 1:30,000 anti-serum concentration. This dilution was adopted throughout the whole study.

Similarly, the optimum radioactive concentration for ^{125}I cortisol was investigated by running several standard curves at different concentration of ^{125}I cortisol (1000 cpm, 2500 cpm and 4000 cpm) with all other variables held constant and with the antiserum concentration set at the optimal dilution of 1:30,000. Assay curves for different amounts of ^{125}I cortisol are presented in Figure 5.6. The curve which showed the most displacement, and therefore the greatest sensitivity, was at 2500 cpm. This concentration was employed throughout the entire study.

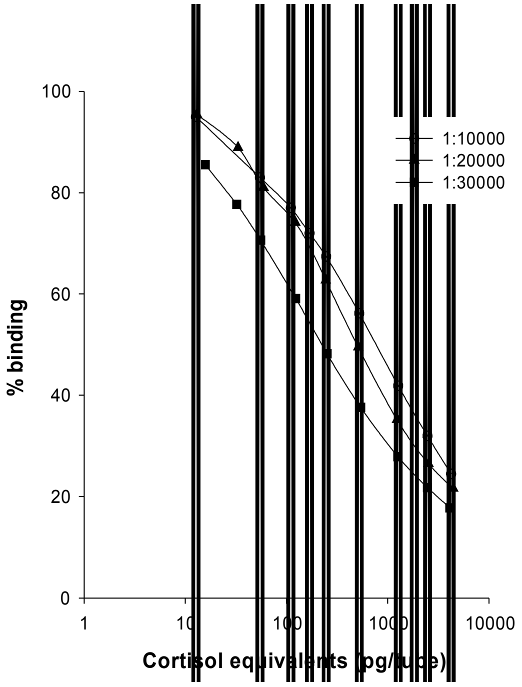


Fig 5.5: Effect of the dilution of the antiserum on the standard curve using the ^{125}I cortisol RIA. The percentage binding was calculated using a zero standard which was assumed to have 100 % binding.

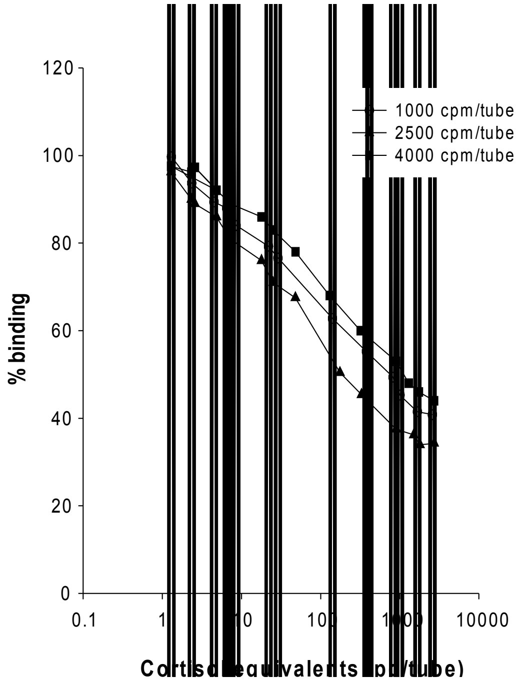


Fig 5.6: Effect of the amount of ^{125}I cortisol on the standard curve using the ^{125}I cortisol RIA. The percentage binding was calculated using a zero standard which was assumed to have 100 % binding.

5.2.6 c ^{125}I cortisol radioimmunoassay

A ^{125}I cortisol radioimmunoassay (RIA) was used to evaluate cortisol, and/or cortisol metabolite concentrations by using the antigen-antibody principle where cortisol equivalents in samples or standards compete with added radiolabelled ^{125}I cortisol for the limited number of binding sites of the antiserum.

Standards were prepared from 10 mg hydrocortisone (Sigma H0888) in 100 % (HPLC grade) ethanol. The whole weighing boat, containing 10 mg hydrocortisone, was placed in an acid-washed and autoclaved glass bijou bottle. Ethanol (10 ml) was added and the bottle (now containing solution A) was wrapped in foil (to protect from day light) and placed on a shaker for 24 h (R100 Rotatest shaker, Luckham). Dilutions were then made up in 10 % methanol (HPLC grade). Solution A (10 μl) was diluted to 10,000 ng/ml by adding 990 μl of 10 % methanol. This solution was further diluted by taking (10 μl) and adding 990 μl of 10 % methanol to provide standard assay 1, at 100 ng/ml. For the assay, standards were serially-diluted using a positive displacement pipette (Microman M1000) to provide solutions giving 5000 pg/tube to 0.488 pg/tube in 14 standards, as shown in Table 5.1.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8	Std 9	Std 10	Std 11	Std 12	Std 13	Std 14
Cortisol in pg/tube	5000	2500	1250	500	250	125	62.5	31.25	15.63	7.81	3.91	1.95	0.976	0.488
Cortisol in ng/ml	100	50	25	10	5	2.5	1.25	0.625	0.3125	0.156	0.078	0.039	0.0195	0.0097

Table 5.1: Standard (Std) number and cortisol in pg/tube and ng/ml.

Standard 4 was prepared from standard 1, in 10 % HPLC methanol, and several aliquots of both standards were stored in eppendorfs and kept at -20 ˚C, ready for serial dilution to obtain all other standards in the curve. This step reduced the risk of variation between standard curves. Tubes containing no cortisol (zeros), blanks, and 'totals tubes' were included in each assay.

Prior to radioimmunoassay, samples were taken out of the freezer and left for one hour to reach room temperature. They were then diluted to 10 % methanol in acid-washed tubes (using PBS) to match the standards. Higher concentrations of methanol were found to interfer with the assay (see section 5.2.6a).

Standards(50 μl), zeros (50 μl 10 % methanol only) and samples were placed in triplicate in LP4 assay tubes and 100 μl cortisol antiserum (Biogenesis, 2330-5004, anti-cortisol raised against rabbit) was added to all the tubes using a repetitive pipettor (Eppendorf Multipipette Plus), apart from triplicate blanks and 'total tubes'. Tubes (containing ^{125}I cortisol) were vortexed and left at 4 ˚C for one hour.

The antiserum was made up in PBS containing 0.1 % (g/ml) gelatine (BDH) at a dilution of 1:30,000 (antibody: PBS). Aliquots (100 μl) of ^{125}I cortisol made up in PBS with 0.1 % gelatin, at a count of approximately 2500 cpm per aliquot, were repetitively pipetted into each tube. Tubes were then vortexed for 5 s and left to incubate at 4 ˚C overnight.

The following day, 100 μl of solid phase second antibody coated cellulose suspension (sac cell: anti rabbit, AA-SAC1 IDS) was repetitively pipetted to all tubes (apart from 'totals tubes'), which were vortexed and left to incubate at room temperature for 30 min, to allow time for binding to the first antigen-antibody complex. The reaction was stopped by adding 1 ml of deionised water to each tube using a repetitive pipette. Tubes were then centrifuged (MSE Mistral 3000), in batches, for 5 min at 4 ˚C and 3000 g. The supernatant (containing unbound ^{125}I cortisol) was aspirated using a water tap vacuuming system attached to a glass Pasteur pipette and bound radioactivity remaining in the pellet was counted (Cobra auto-gamma, Packard) for two minutes per tube.

Assay blank tubes were set up containing 150 μl 10 % methanol. As these tubes contained no known cortisol or antiserum, no antibody-antigen complex should form and be bound by the second antibody, giving a very low final count. Zeros contained no known cortisol but had antiserum and ^{125}I cortisol. No competition for binding sites by ^{123}I cortisol should occur, giving the highest count per minute. 'Totals tubes' were made up exclusively of 100 μl ^{125}I cortisol (approximately 2500 cpm) and were used to calculate percentage binding. Procedural blanks were treated in the same manner as samples.

The cortisol equivalents in extracted faecal samples were calculated against the standard curve obtained from the radioimmunoassay software (AssayZap, Biosoft). Values were then corrected for procedural blanks (between 2.19 and 8.69 pg/ tube) and calculated to take into account the amount of faecal powder extracted and the volume of methanol added. The final value for cortisol equivalents was expressed in ng g^{-1} dry faecal powder. This value was also corrected for recovery (see section 5.2.5).

5.2.7 Assay checks

5.2.7 a RIA coefficients of variation and minimum detectable limit (MDL)

The intraassay coefficient of variation was measured in two way: using a single batch of standard solution 8 (Table 5.1), at the steepest point of the curve (31.25 pg/tube, 0.625 ng/ml) diluted in 10 % methanol from the hydrocortisone stock solution (1 mg/ml in 100 % ethanol) and using a pooled sample extracted as the method in section 5.2.4 (from three males: Nimba, adult at La Boissière du Doré, the adult Radius and the juvenile Cranium at Port Lympne Zoo and three females: the adult Katie at Port Lympne Zoo, the adult Celize and the juvenile Maatai at La Boissière du Doré Zoo). Aliquots (50 µl) of standards or samples were pipetted into eighteen LP4 tubes each and assayed as described in section 5.2.6c. The intraassay coefficient of variation (CV) was then calculated using the following relationship:

CV= standard deviation/mean*100

In this study, the intraassay coefficient of variation was 11.93 % (n = 18) for the standard solution and 10.24 % (n = 12) for the pooled sample.

The minimum detectable limit (MDL) was assessed to measure the sensitivity of the assay. Methanol (50 µl at 10 %) was used as a zero and was pipetted into twelve LP4 tubes and assayed as described previously. MDL was then calculated as two standards deviation from the reading for the zero. MDL was 2.06 pg/tube (n = 12).

Finally, the inter-assay coefficient of variation was measured to give an indication of the variability between assays. Standard 8 was used, as it is mid-range in the assay curve. A large quantity was derived from the hydrocortisone stock solution and aliquots (200 µl) were stored at -20 ˚C. Each time an assay was run, an aliquot was included. Interassay coefficient of variation, calculated as above was 14.3 % (n = 12).

These results are comparable to, or lower than those of other studies (e.g. Cavigelli *et al*., 2003; Ganswindt *et al*., 2003).

5.2.7 b Parallelism of faecal extracts and standard curve

The parallelism of faecal extracts and the standard curve was investigated using a pool faecal extract known to contain a high quantity of cortisol equivalents (see 5.2.7 a).

This procedure is widely employed to examine whether samples contain other compounds that could interfere with the assay (Cavigelli, 1999; Strier *et al*., 1999; Wasser *et al*., 2000; Morrow *et al*., 2002; Good *et al*., 2003; Li *et al*., 2007). The pooled sample was doubly diluted 5 times, and triplicate aliquots of each dilution were run in the ^{125}I cortisol RIA, as described in section 5.2.6c, with antiserum dilution at 1:30000 and ^{125}I cortisol counts at 2500 cpm.

Results for parallelism between the standard curve and a serially-diluted pool of faecal samples are shown in Figure 5.7. The slope from each set of dilution was compared to the slope from the standard curve using a Student's t test (significant threshold 0.05) and shows good parallelism between the two slopes with no significant difference (t_{19}= 1.57, p = 0.726).

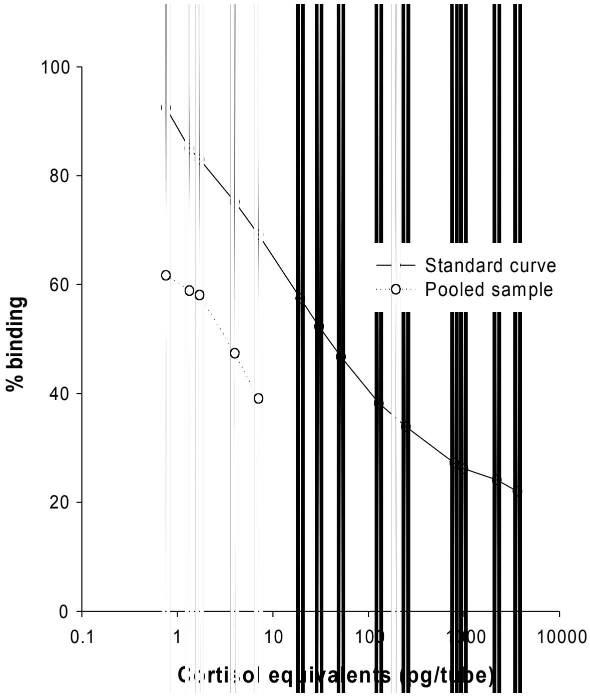


Fig 5.7: Serially diluted pooled sample compared to cortisol standard curve to show parallelism between the curves.

5.2.7 c Biological validation

The validation of the cortisol equivalent RIA was performed biologically by evaluating the potential changing concentration of cortisol equivalents between gender (17 males and 17 females across zoos), by comparing possible differences across female reproductive stages (from immature, cycling, lactating, mating to pregnant) and by investigating the impact on cortisol equivalent content during known stressor situations. As an example of stressful circumstance, detail results of cortisol equivalents at La Boissière du Doré in November 2006, when the group targeted one particular animal and violently attacked him were presented.

5.2.7 d Assay cross-reactivity

Most antisera are specific to the antigen put into the assay, but they may still have affinity for closely-related compounds with similar structure. To investigate cross reactivities, several closely-related steroids were assayed and percentage binding was calculated. A summary of all the compounds assayed is shown in Table 5.2 and skeleton structures of these compounds, compared to cortisol and corticosterone are presented in Figure 5.8. All test compounds were initially made up in 100 % ethanol (HPLC grade) as described for hydrocortisone (section 5.2.6c). A series of dilutions of each compounds were made up in 10 % (HPLC grade) methanol using a Microman pipette (piston and capillaries) and kept at 4 ˚C until assayed. To have a broader understanding of interaction in the cortisol radioimmunoassay, a larger range of concentrations were used in each assay, including much higher concentrations as summarised in Table 5.3.

Aliquots of each standard (50 µl) were pipetted into LP4 tubes, in triplicate. Each assay also contained one set of ^{125}I cortisol in triplicate (‘totals tubes’), one set of blank tubes in triplicate (with no antiserum) and one set of zero tubes, in triplicate. The method described in section 5.2.6c was then applied to each standard curve.

Readings of cortisol immunoreactivity (pg/tube) obtained from the radioimmunoassay software (AssayZap, Biosoft) were compared to cortisol standards by calculating percentage binding of each concentration of standard for each compound. The radioimmunoassay software was set up to provide Equivalent Dose (ED) values at 20 %, 50 % and 80 % binding for all curves. Values at 50 % binding were used to calculate cross reactivities using the following equation:

Cross-reactivity = ED (50) cortisol / ED (50) test compounds * 100

Compounds showing some degree of cross reactivity were further investigated using High Pressure Liquid Cromatography (HPLC).

5.2.8 Cross-reacting compounds investigated using HPLC

The two compounds of interest showing significant cross-reactivities in the radioimmunoassay were cortisone and cortexolone. HPLC was used as a tool to investigate the presence of these compounds within faecal samples.

Initially, each compound's position was identified with the gradient and flow conditions specified below, by spiking an extract of faecal sample, to avoid matrices effects that could affect the speed of travel through the column.

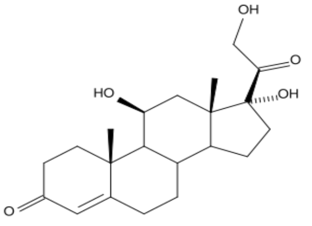
Once the position of each compound was confirmed, several samples, covering various aspects such as age and sex and collected from different zoos were run though the HPLC system to investigate the presence or absence of each substance in faeces.

Compound		Source	Pathway
21 deoxycortisol	4-pregnene-11β, 17α-diol-3, 20-dione	Sigma	Precursor
Aldosterone		Sigma	Mineralocorticoid
Cholesterol	S-cholesten-3β-ol	Sigma	Precursor
Corticosterone		Sigma	Glucocorticoid
Estrone		Aldrich	Estrogen
Estriol	1,3,5[10]-estratriene-3, 16α, 17β-triol	Sigma	Estrogen
Cortexolone	4, pregnene-17α, 21-diol-3, 20-dione	Sigma	Precursor
Progesterone	4-pregnene-3, 20-dione	Sigma	Precursor
B estradiol	1,3,5 [10]-estratriene-3, 17β-diol	Sigma	Estrogen
Testosterone	4, androsten-17β-ol-3-one	Sigma	Androgen
Pregnenolone		Aldrich	Precursor
1-dehydrotestosterone	1,4-androstadien-17β-ol-3-one	Sigma	Androgen
Cortisone	4, pregnene-17α-21-diol3, 11, 20-trione	Sigma	Metabolite

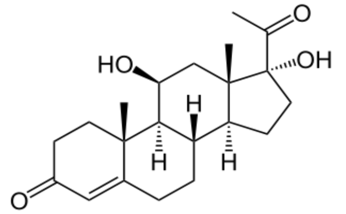
Table 5.2: List of compounds used in radioimmunoassay cross-reactivity checks.

Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8	Std 9	Std 10
5,000,000	2,500,000	500,000	50,000	25,000	5,000	2,500	500	5	0.5
Standard in pg/tube									
100,000	50,000	10,000	1,000	500	100	50	10	0.1	0.01
Standard in ng/ml									

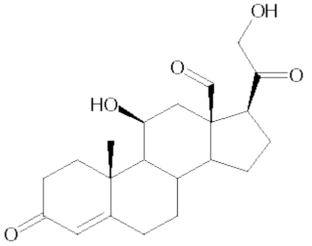
Table 5.3: Compounds standard (Std) dilution in pg/tube and ng/ml.



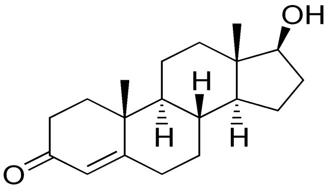
Cortisol



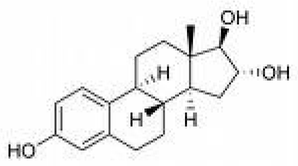
21-deoxycortisol

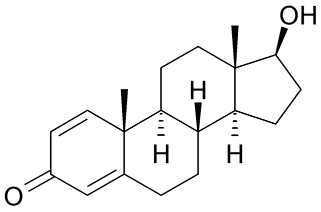


Aldosterone

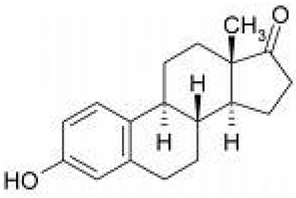


Testosterone

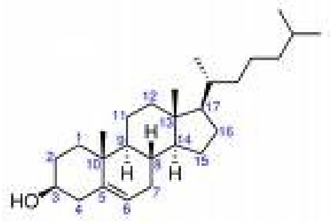




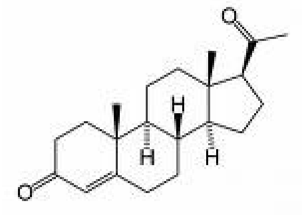
1-dehydrotestosterone



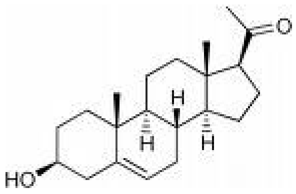
Estrone



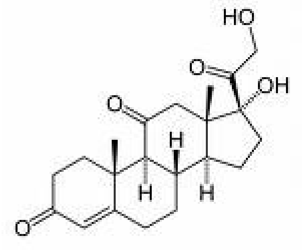
Cholesterol



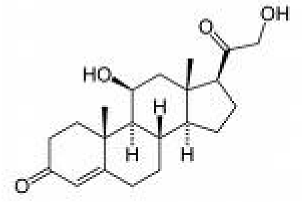
Progesterone



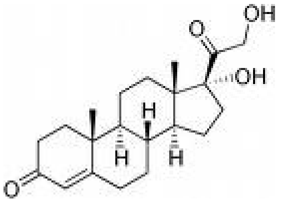
Pregnenolone



Cortisone



Corticosterone



Cortexolone (Reichstein's substance S)

Estriol Fig 5.8: Skeleton structure of hormones used in the cross-reactivity assays (diagrams taken from www.wikipedia.org).

5.2.8 a Sep-Pak (C18) purification of faecal samples

In all HPLC work, samples were initially extracted as described in section 5.2.4. A cleaning step was then applied as pigments and hydrocarbons remained in the methanol extract.

Extracted samples (in 95 % methanol) were diluted 1:10 with HPLC grade water by taking 0.5 ml of extracted sample and vortexing for 30 seconds with 4.5 ml HPLC grade water. Solid-phase extraction cartridges (Sep-Pak C-18 Waters Vac) were primed using 5 ml of 100 % HPLC grade methanol, slowly loaded with a 5 ml syringe. Each cartridge was rinsed with 20 ml HPLC grade water (loaded by hand with a 20 ml syringe). Diluted extracts were then loaded onto the Sep-Pak using a 5 ml syringe mounted onto an infusion pump (Meltec limited at 66, 65 DN). Cartridges were then washed slowly with 5 ml of HPLC water, twice, injected by hand using a 5 ml syringe. The sample was eluted into an acid-washed glass bijou bottle, using 5 ml of 100 % HPLC grade methanol delivered from the infusion pump. Samples were then dried down under a stream of nitrogen and reconstituted in 1 ml of 50 % methanol prior to HPLC.

5.2.8 b HPLC system

All the work was done using a Schimadzu HPLC system with a UV detector (SPD-10A-VP), set at 240 nm, column heater set at 35˚C (CT0-10AS), dual pump (LC-10ADVP) and a low pressure gradient flow control valve (LC-10AVP), set at a minimum pressure of 10 psi, and a maximum of 4000 psi. A gradient was run from 50 % HPLC methanol: 50 % HPLC water to 100 % methanol (in two solvent bottles) over 40 min. The flow rate of the mobile phase was 1 ml/min. Samples were loaded onto an auto-sampler (HPLC 360) and injected as 200 µl. The HPLC system was connected to a computer and the Kromasystem 3000 (Biotek Instruments) program was used to display data.

5.2.8 c HPLC analysis

Each day, an initial blank (50 % HPLC methanol) was injected to check for cleanliness of the column. Two faecal samples, from an adult colobus female from Banham Zoo and a juvenile female from La Boissière du Doré, were selected to locate the position of pure compounds spiked into these samples. For each run, an original unspiked aliquot of faecal sample (200 µl) for each animal was also injected.

Each compound was diluted from the stock solutions used in cross-reactivity checks (see section 5.2.7c). A cortisol spike was added to a 200 µl aliquot of each sample and run in the HPLC system. Cortisone was also added to another aliquot of the spiked sample (now containing both cortisol and cortisone) and the sample was re-run. Cortexolone was then added and a third run, compared an un-spiked aliquot to the spiked sample, now containing all three substances

To investigate the repeatability of these results, four more unspiked samples from another zoo (2 males and 2 females at Port Lympne) were run through the HPLC, without spiking.

To investigate the cross reactivity in the RIA of the peaks identified by HPLC, one of the unspiked sample (the adult male at Port Lympne Zoo) was run through the HPLC system. After injections, fractions were collected every 30 seconds. These fractions were dried down under nitrogen and reconstituted in 10 % methanol for RIA, as described in section 5.2.6 c. A procedural blank was incorporated in the RIA and its value was deducted from all data. The fractions showing readings within the RIA could then be matched to the fractions from spiked compounds to check for cross-reactivity, as described by Strier *et al.*, 1999.

5.2.9 Data analyses

Several samples were available for each animal during each data collection period and are summarized in Table 5.4. Normality of data was checked using the Kolmogorov-Smirnov test. All data were initially log transformed as normality was not met.

To investigate factors affecting the faecal glucocorticoid content across zoo, a Generalised Estimated Equation (GEE) model was performed (PAWS 18). After being log transformed, data of faecal glucocorticoid content were normally distributed therefore an identity link function was selected. The repeated subject variable was defined by the individual and the zoo identity. The within subject variable was identified by repeated data collection period (i.e. visit) and the day the samples were being collected (as several samples were available per visit).

Port Lympne Zoo	**Mar-05**	**Jul-05**	**Oct-05**	**Jan-06**	**Apr-06**	**Jul-06**	**Oct-06**
Panda	7	6	7	3	6	6	7
Katie	4	4	9	5	4	5	6
Radius	5	7	4	7	5	5	8
Fibula	7	6	6	8	7	7	4
Ulna	6	7	6	5	6	6	8
Tibia	7	9	4	9	6	6	9
Cranium	8	4	5	8	8	5	6
Tarsal	5	5	6	8	5	5	8
Dermis	4	4	6	6	3	4	8
Femur	1		2	4	3	3	5

Paignton Zoo- female	**Jun-05**	**Mar-06**	**Oct-06**
Madonna	6	-	-
Denny	5	2	3
Jhazzie	5	5	-
Salassie	2	3	4
Lou	6	5	2
Joe	1	2	2

Paignton Zoo- male	**Aug-05**	**Mar-06**	**Oct-06**
Millo	5	5	5
Fergus	5	3	5
Kabul	3	3	5
Hope	3	4	5

Banham Zoo	**Mar-05**	**Oct-05**
Steffan	6	6
Kivu	5	2
Carmen	5	5
Kiwi	4	5
Sophie	5	4
Monty	4	1
Suzie	4	4
Bobby	-	2
Bea	-	3

La Boissiere du Dore Zoo	**Mar-06**	**Nov-06**
Ernesto	4	4
Fudgi	5	4
Nimba	5	5
Celize	5	5
Abyssian	3	5
Camara	5	4
Maatai	2	3
Swahili	-	4

Table 5.4: Number of faecal samples for each colobus monkey at Port Lympne Zoo, Paignton Zoo (both groups), Banham Zoo and La Boissière du Doré Zoo, during each data collection period.

The dependant variable was defined as faecal cortisol equivalents. Predictors were entered as factors and covariate. Factors in the model were season, index of success, gender, group (zoo) and reproductive status. Females were classified as pregnant, lactating, cycling, immature or mating. For the purpose of analysis, males were included and scored as a zero category. Covariates were age (in months), aggression given and aggression received. Animals could either be receiving aggression leading to a movement away from the initiator, or be the one displaying dominance over others. Index of success is a categorical concept derived from the proportion of successful displacements compared to all displacement behaviour between animals but does not take into account the rates of displacements and aggressive behaviours.

Initially, all predictors were included in the model as a main effect. Two-way interactions between terms were tested for significance, with the exception of the interaction between age and index of success as these were tightly correlated (Figure 2.1). Factors and covariates were then subsequently dropped from the model if lacking significance so the model only contained those terms which were significant yielding a ‘minimal model’.

Scale parameter method was employed using the maximum-likelihood estimate. Statistics were performed with Type III analysis and 95% confidence interval level computing Wald statistics.

Contrast analysis was performed in the GEE model by use of estimated marginal means (EM means). The EM means were displayed in the PAWS 18 programme for factors only and pairwise comparison between each factor of relevance was performed and corrected with a Bonferonni adjustment for multiple contrasts.

5.3 Results

5.3.1 Antiserum cross-reactivity tests

Several assays were necessary to measure the cross-reactivity of all compounds. The assay curves are presented in Figures 5.9-5.11. Those compounds shown in Figures 5.9 and 5.10, generally demonstrate very low cross-reactivity with the antiserum, with only 21-deoxycortisol showing measureable cross-reactivity, at 5.52 %. Figure 5.11 shows the data for compounds with the most significant cross-reactivity: cortisone (17.32 %), corticosterone (77.86 %) and cortexolone. Cortexolone gave a very high value for cross-reactivity (116 %). This is mainly due to the shape of the curve. As can be seen on Figure 5.11, the cortexolone binding curve is at 50 % binding level with a left-handed displacement compared to the cortisol standard curve.

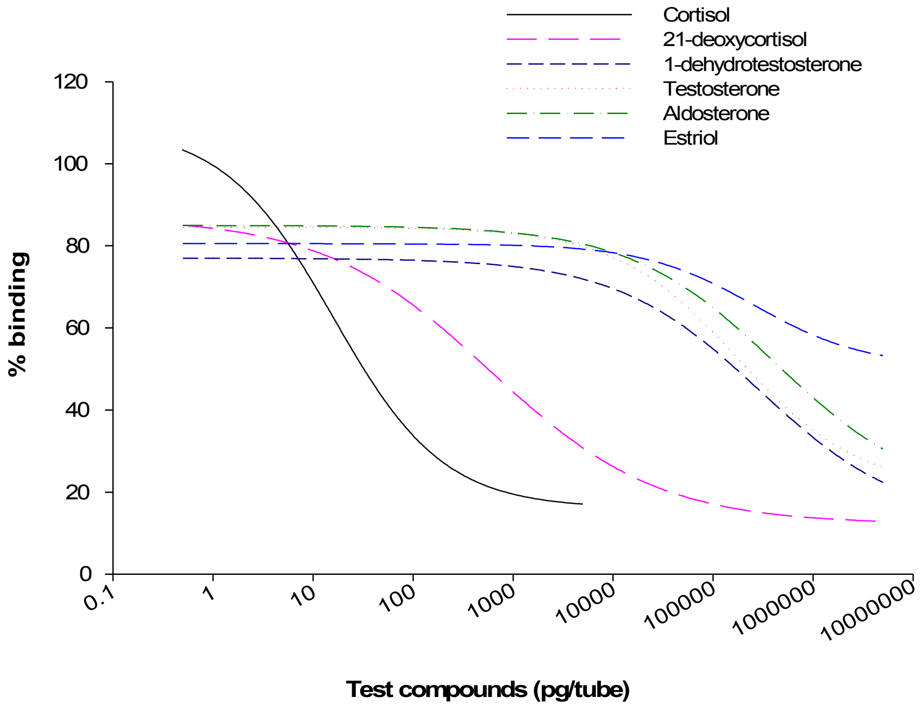


Fig 5.9: Percentage binding of cortisol and test compounds at various concentrations.

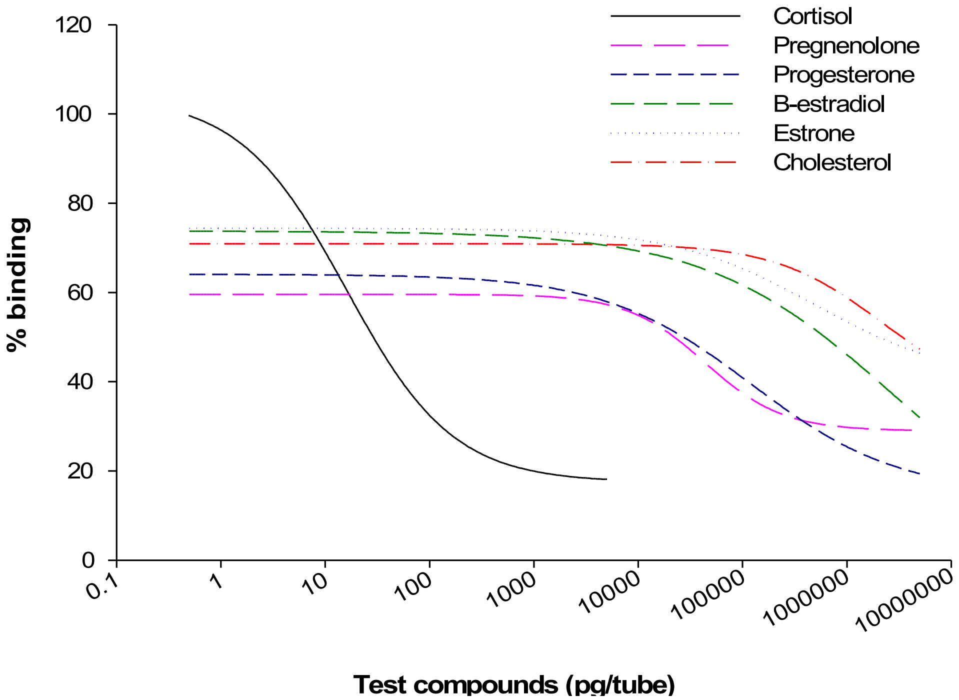


Fig 5.10: Percentage binding of cortisol and test compounds at various concentrations.

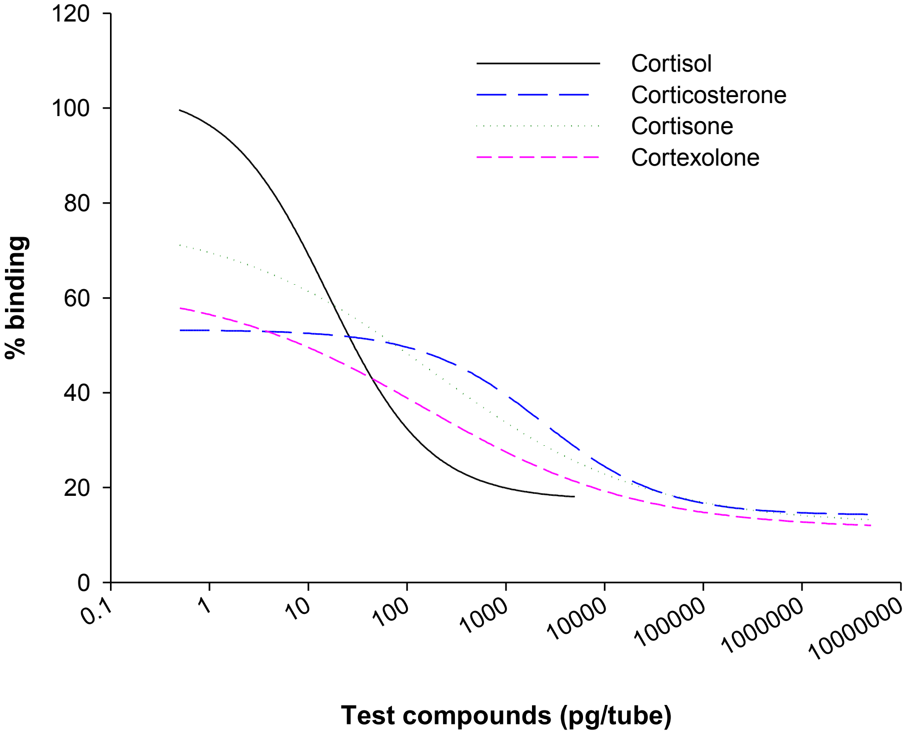


Fig 5.11: Percentage binding of cortisol and test compounds at various concentrations.

5.3.2 HPLC results for faecal samples and cross-reacting compounds

Graphs for HPLC results have been shortened to 16 or 18 min as all the relevant information is included within this elution time. Results for hormone spiking of two samples are presented in Figures 5.12a-b, 5.13a-b and 5.14a-b. In all samples, spiked peaks are very clear and highly repeatable between runs. The injection front is clearly observed in all figures between 2-3 min. Cortisol shows a consistent elution time of between 11-11.5 min in all cases. Cortisone elutes at between 9.5-10 min whereas cortexolone is between 15-15.5 min in all injections. Repeatability of each un-spiked sample is also very high between runs and shows consistent peaks. In both samples, peaks are observed at 11-11.5 min and 15-15.5 min. Another peak is also consistently observed, between 8.5-9 min.

Results from the four unspiked faecal samples from two males and two females at Port Lympne Zoo are presented in Figures 5.15a-d.These figures suggest that cortisone is not detected at the set wavelength (λ=240 nm) after HPLC separation of most faecal samples. Only the juvenile male (Figure 5.15b) shows a slight peak at the corresponding elution time. All samples show a peak around 11-11.5 minutes, which matched the cortisol elution time. A peak also appears between 8.5-9 min, which is more substantial in the two adult animals than in the two juveniles. The nature of this peak remains unknown. The corresponding peak to cortexolone is also more significantly observed in adult animals and not juveniles. All four colobus monkeys at Port Lympne show a new unidentified peak between 13-13.5 min, which is again more substantial in adults than juvenile.

Results (in pg/tube) for each fraction from the adult male (Figure 5.15c) are presented in Figure 5.16. Although a large unidentified peak with an elution time of 8-9 min is present in the HPLC separation (see Figure 5.15c), this peak shows very low amount of immunoreactivity with the cortisol RIA used in this study. Similarly, peaks observed at 15-15.5 min which correspond to cortexolone, do not show any immunoreactivity with the cortisol RIA. An unidentified peak of immunoreactivity is present at 14 min elution time. Although cortisone does not appear in the absorption data after HPLC separation, a small amount appears to be detected in the RIA, in the fraction collected at 9.5 min, although this peak is difficult to interpret due to noisy baseline.

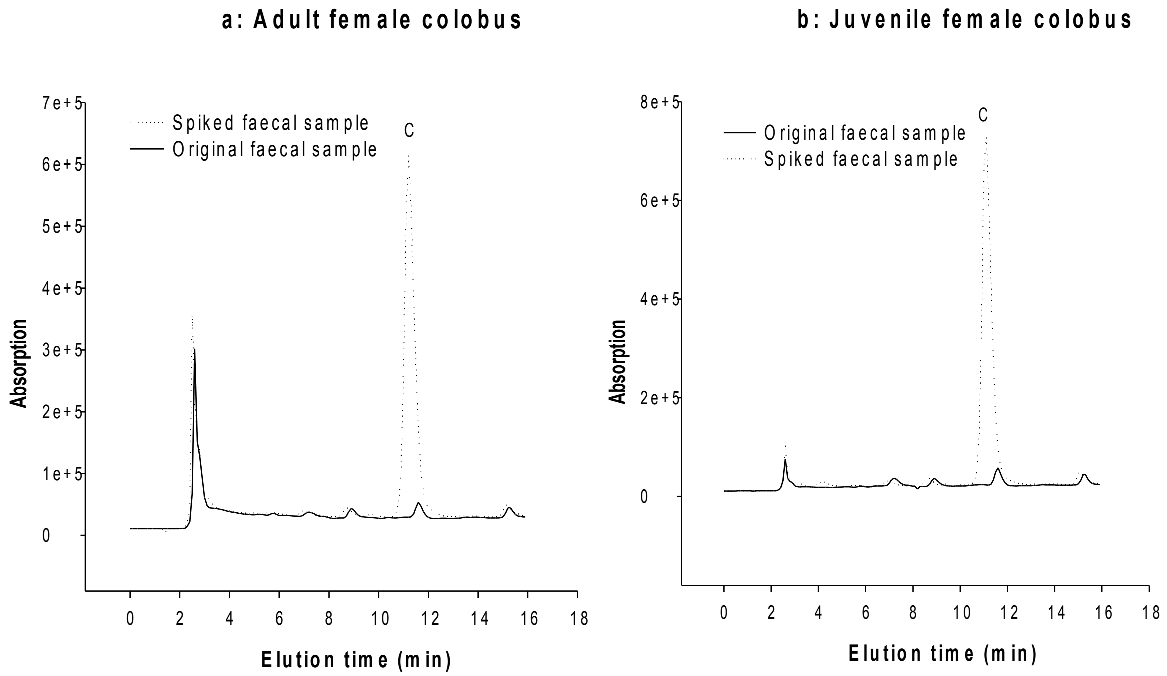


Fig 5.12 a-b: Comparison between an original faecal sample aliquot and an aliquot of the same faecal sample, spiked with cortisol (C) for the adult female at Banham Zoo and the juvenile female at La Boissière du Doré. HPLC, λ=240 nm, 1 ml/min.

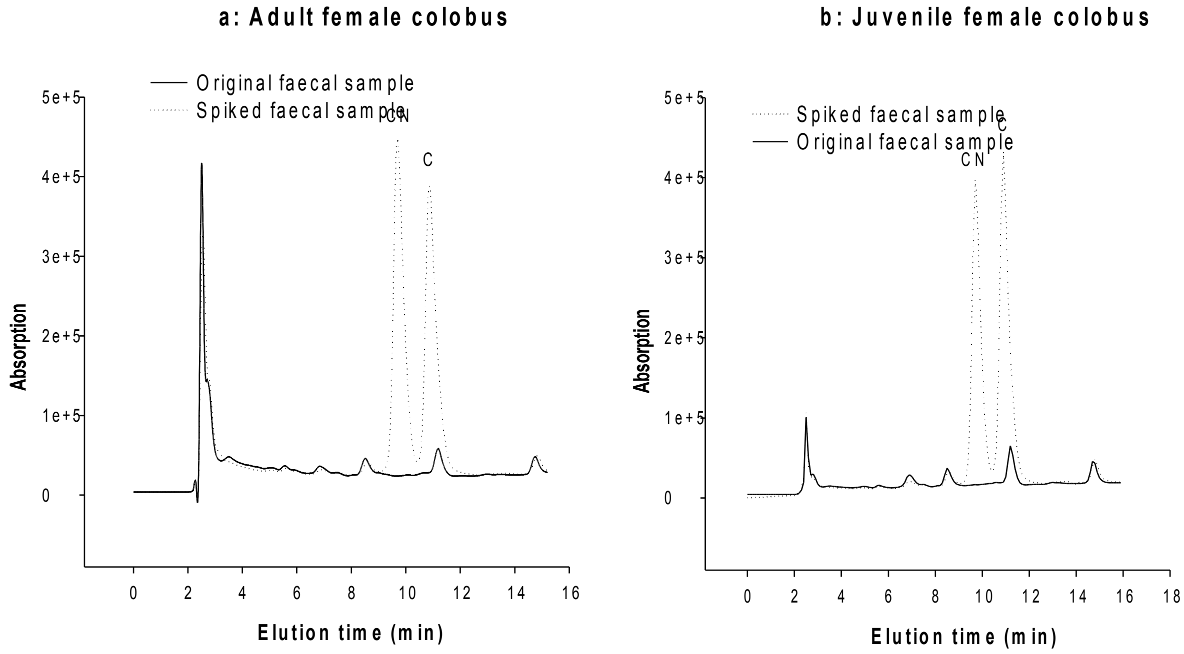
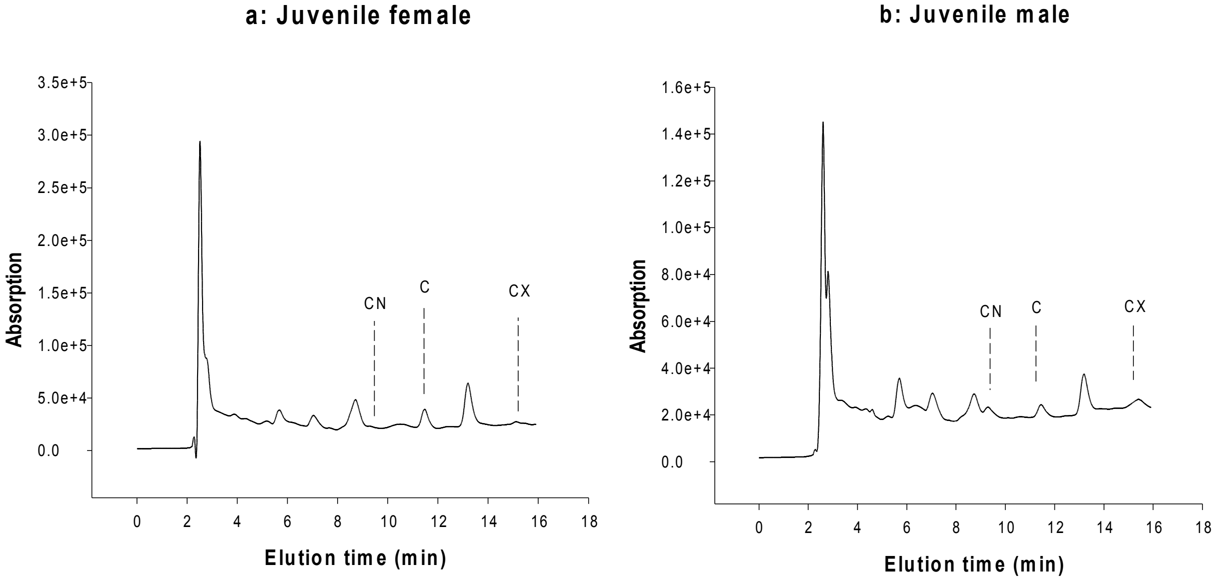


Fig 5.13 a-b: Comparison between the original faecal sample aliquot and an aliquot of the same faecal sample, spiked with cortisol (C) and cortisone (CN) for the adults female at Banham Zoo and the juvenile female at La Boissière du Doré. HPLC, λ=240 nm, 1 ml/min.

Fig 5.14 a-b: Comparison between the original faecal sample aliquot and an aliquot of the same faecal sample, spiked with cortisol (C), cortisone (CN) and cortexolone (CX) for the adult female at Banham Zoo and the juvenile female at La Boissière du Doré. HPLC, λ=240 nm, 1 ml/min.



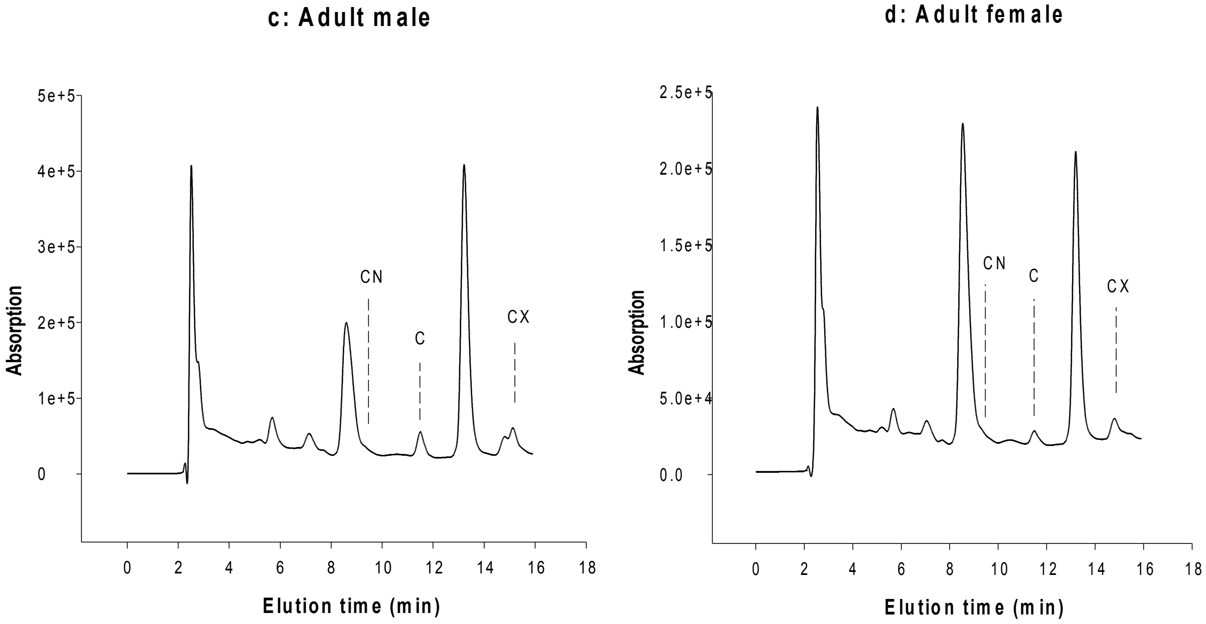


Fig 5.15 a-d: HPLC results (λ=240 nm, 1 ml/min) for four colobus monkeys at Port Lympne Zoo (all samples collected in July 2006). Faecal samples were unspiked and positions of known compounds (C= cortisol, CN= cortisone and CX= cortexolone) identified.

The largest reading of immunoreactive cortisol is for the fraction collected at 11 min, which corresponds to the elution time for cortisol (see Figures 5.12a-b). It is not possible to state that the assay solely measures cortisol, as shown by the inclusion of an unknown peak of immunoreactivity in the fraction collected at 13.5 to 14 min. However, HPLC separation followed by RIA confirms that the RIA measures primarily cortisol. As a result, for the remainder of this study, results will be referred to as measuring “cortisol equivalents”.

Fig 5.16: Radioimmunoassay results of faecal sample (Fibula, Port Lympne Zoo, July 2006) after HPLC separation (λ= 240 nm, 1 ml/min). Fractions were collected every 30 s, dried down and reconstituted in 500 µl of 10 % methanol prior to RIA. C=cortisol, CN=cortisone, CX=cortexolone, and ? are unknown peaks observed in samples.

5.3.3 GEE of predictors affecting faecal content of cortisol equivalents.

The analyses reported below (Table 5.5) investigated possible factors affecting faecal glucocorticoids of colobus monkeys at various zoos over repeated data collection periods. Predictors with significant power in the model were zoo, season, index of success (IS), gender, reproductive status, aggression given and aggression received. As the level of aggression given increased, faecal cortisol equivalent contents tended to increase, (Figure 5.17), whereas as levels of aggression received increased, faecal cortisol equivalent contents tended to decrease (Figure 5.18).

Contrast analysis was performed in the GEE model by use of estimated marginal means (EM means) on zoo, season, IS, gender and reproductive status. Zoo was a significant factor in the analysis ($x^2 = 94.909$, df = 4, $p < 0.001$) and significant differences between EM were found between Port Lympne Zoo and all other groups (mean difference La Boissière du Doré Zoo: -0.2581, $p < 0.001$; Banham Zoo: -0.1356, $p < 0.05$; Paignton Zoo male group: -0.3291, $p < 0.001$ and Paignton Zoo female group: -0.4285, $p < 0.001$). Banham Zoo was also had significantly different EM from a further three zoos: La Boissière du Doré Zoo (-0.1224, $p < 0.05$), Paignton male group (-0.1935, $p < 0.01$) and Paignton female group (-0.2929, $p < 0.001$). Additionally, significant differences were observed between Paignton Zoo female group and La Boissière du Doré Zoo (mean difference 0.1705, $p < 0.05$), Figure 5.19.

Season was a significant factor in the analysis ($x^2 = 36.776$, df = 3, $p < 0.001$) and significant differences between EM were found between the autumn and all other seasons (mean difference spring: 0.1437, $p < 0.001$; summer: 0.1609, $p < 0.001$ and winter: 0.1357, $p < 0.01$), Figure 5.20.

IS was a significant factor in the analysis ($x^2 = 8.898$, df = 4, $p < 0.01$) and significant differences between EM were found between the overall dominant animal and other animals in the group (mean difference to IS 1: 0.2521, $p < 0.001$; to IS 2: 0.2637, $p < 0.001$; to IS 3: 0.2058, $p < 0.05$; to IS 4: 0.2617, $p < 0.001$), Figure 5.21.

Gender was a significant factor in the analysis ($x^2 = 11.796$, df = 1, $p = 0.001$) with higher content of faecal glucocorticoids in females than males (mean difference: 0.2939, $p = 0.001$), Figure 5.22.

Finally, reproductive status was a significant factor in the analysis ($x^2 = 31.293$, df = 4, $p < 0.05$) with higher content of faecal glucocorticoids in pregnant females than others (mean difference to lactating females: 0.2571, $p < 0.001$; to cycling females: 0.1899 $p < 0.05$; to immature females: 0.2835, $p < 0.001$ and to mating females: 0.2035, $p < 0.05$), Figure 5.23.

Full model

Terms	Type III		
	Wald Chi-Square	df	Sig.
zoo	65.489	4	.000
season	40.043	3	.000
IS	6.531	4	.003
sex	7.957	1	.002
Reproductive status	31.141	4	.000
age	.713	1	.399
Aggression given	4.403	1	.036
Aggression received	5.802	1	.016

Minimal model

Terms	Type III		
	Wald Chi-Square	df	Sig.
zoo	94.909	4	.000
season	36.776	3	.000
IS	8.898	4	.004
sex	11.706	1	.001
Reproductive status	31.293	4	.000
Aggression given	8.413	1	.004
Aggression received	5.842	1	.016

Table 5.5: Factors affecting colobus monkeys faecal glucocorticoid equivalent levels.

Results are from a GEE model based on faecal glucocorticoids per day, individual and data collection period from 37 animals in 5 groups. Faecal glucocorticoid contents were log transformed for the analysis.

Season was recorded as spring (March-April), summer (June-August), autumn (October-November) and winter (January). Index of success (IS) was derived from the number of dominant encounter won divided by the total number of dominant encounters and scaled between 0 (dominant) and 1-4 (from most dominant to most subordinate).

Reproductive status encompassed pregnant, lactating, cycling, immature or mating females. Covariates were age (in months), aggression given and aggression received. Animals could either be receiving aggression leading to a movement away from the initiator, or be the one displaying aggression over others.

Fig 5.17: Faecal cortisol equivalents according to the proportion of aggression given.

Fig 5.18: Faecal cortisol equivalents according to the proportion of aggression received.

Fig 5.19: Mean (+SE) faecal glucocorticoids (ng/g) for colobus monkeys at various zoos. Means and standard errors are predictions from the GEE model presented in Table 5.5, controlling for the variation in faecal glucocorticoid content associated with the other significant predictors.

Fig 5.20: Mean (+SE) faecal glucocorticoids (ng/g) for colobus monkeys during each season. Means and standard errors are predictions from the GEE model presented in Table

5.5, controlling for the variation in faecal glucocorticoid content associated with the other significant predictors.

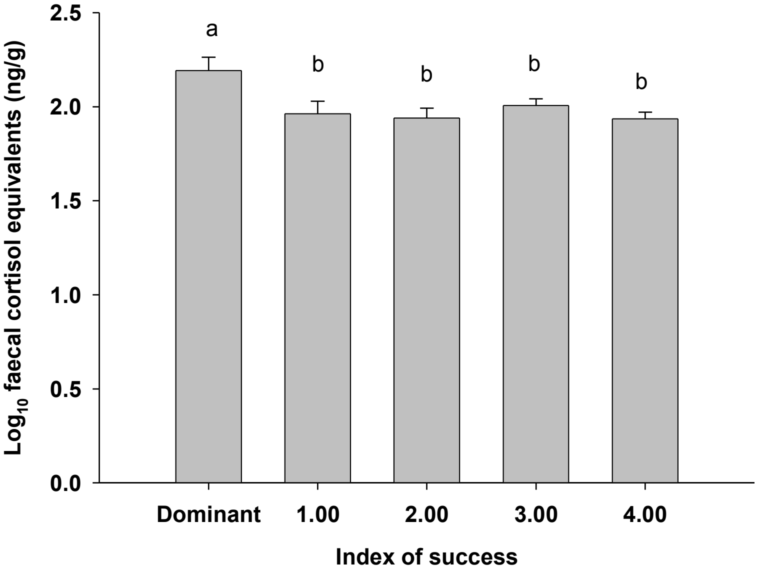


Fig 5.21: Mean (+SE) faecal glucocorticoids (ng/g) for colobus monkeys according to index of success. Index of success (IS) was derived from the number of dominant encounter won divided by the total number of dominant encounters and scaled as dominant (the clear overall dominant animals in each group) and between 1-4 (from most dominant to most subordinate). Means and standard errors are predictions from the GEE model presented in Table 5.5, controlling for the variation in faecal glucocorticoid content associated with the other significant predictors.

Fig 5.22: Mean (+SE) faecal glucocorticoids (ng/g) for colobus monkeys according to gender. Means and standard errors are predictions from the GEE model presented in Table 5.5, controlling for the variation in faecal glucocorticoid content associated with the other significant predictors.

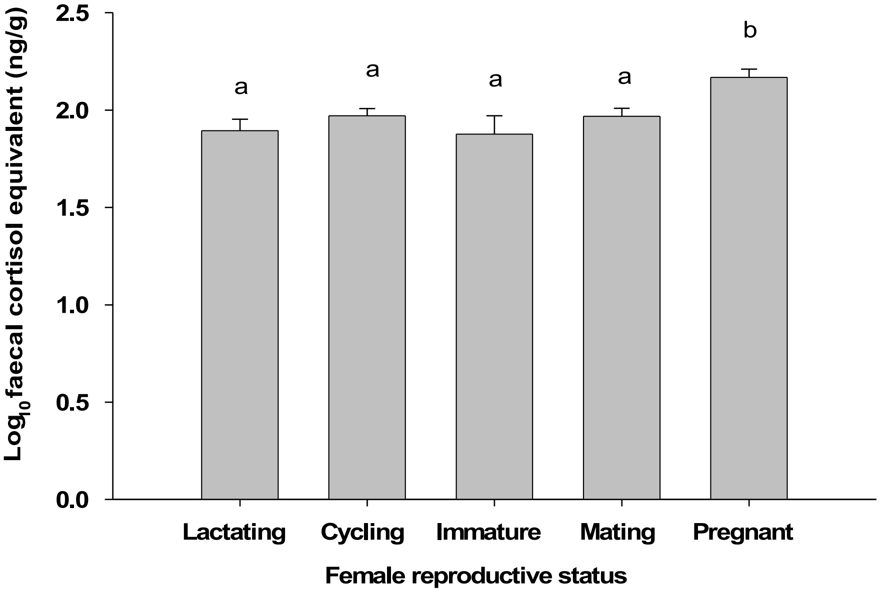


Fig 5.23: Mean (+SE) faecal glucocorticoids (ng/g) for female colobus monkeys according to reproductive status. Means and standard errors are predictions from the GEE model presented in Table 5.5, controlling for the variation in faecal glucocorticoid content associated with the other significant predictors.

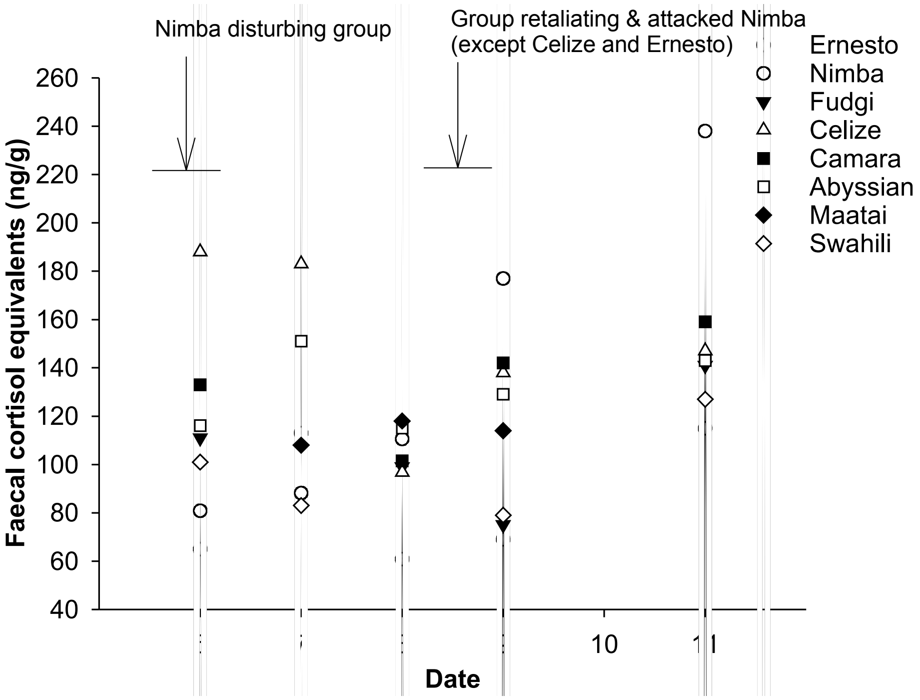


Fig 5.24: Faecal cortisol equivalents (ng g^{-1}) for each colobus monkey at La Boissière du Doré during the November 2006 visit. No data are available for 10-11-2006 as the animals were allowed access to the outside to ease the tension.

5.3.4 Detailed results of cortisol equivalents at La Boissière du Doré Zoo

Because of the attack event at La Boissière du Doré Zoo during the November visit (see Chapter 2, section 2.4.1) Figure 5.24 was included to look more closely at data obtained for that visit. This figure suggests an increase in cortisol equivalents towards the end of the period of faecal collection (especially by Nimba, who was at the receiving end of the attacks). His levels of faecal cortisol equivalents started at 80.9 ng g^{-1} of faeces before the attack, and increased up to 248.1 ng g^{-1} of faeces two days after the attack.

5.4 Discussion

5.4.1 Radioimmunoassay (RIA) of faecal cortisol equivalents

The HPLC separation of faecal extracts, collection of fractions and measurement of cross-reactivity in the cortisol RIA suggests that the main hormone measured in this study was authentic cortisol. Substantial amounts of cortisol have been found in the faeces of a variety of mammals, including Himalayan black bear and clouded leopard (Young *et al*., 2004), Père David’s stag deer (Li *et al*., 2007) and also in a variety of primates, such as the common marmoset, (Bahr *et al*., 2000; Heistermann *et al*., 2006), the Barbary macaque and the gorilla (Heistermann *et al*., 2006), the lowland gorilla (Peel *et al*., 2005), and the chimpanzee (Whitten *et al*., 1998).

Excretions of faecal glucocorticoids are best investigated by injection of radiolabelled cortisol or corticosterone and measurement of the excretion of radiolabelled steroids and their metabolites (Palme *et al*., 1996; Palme & Möstl, 1997; Wasser *et al*., 2000; Möstl & Palme, 2002; Touma *et al*., 2004). Unfortunately, due to legislation, and possible health hazard to the animals (Schwarzenberger, 2007), this was not feasible in this study. Although the polyclonal cortisol antibody used in the present studies was developed to measure cortisol specifically, it may also behave as a group-specific antibody (see 5.1.3), as observed in the study by Young *et al*. (2004), and it is probable that other metabolites also reacted in the present RIA. The cross-reactivity in the RIA of cortisone, the major metabolite in plasma was 17.32 % and cortexolone, a precursor to cortisol cross reacted at 116 %. However, RIA results of the faecal sample after HPLC separation (Figure 5.16) showed that the largest peak of reading was from cortisol itself and to a lesser extend cortisone and an unknown peak. Faeces may contain a large array of corticosteroid metabolites (Schatz & Palme, 2001; Millspaugh & Washburn, 2004; Schwarzenberger, 2007) and it is not possible to affirm that the present ^{125}I RIA solely measured cortisol, more accurately it measures cortisol equivalents (due to the small percentage of cross reactivity to cortisone and its reduced presence in fraction measurement). Ideally, the excretion of authentic cortisol and radiolabelled metabolites should be investigated in the future.

Studies by Schatz & Palme (2001) and Ganswindt *et al*. (2003) highlighted a potential problem of importance in some immunoassays, due to their cross-reactivity with androgen metabolites (due to their similar structures, as highlighted in Figure 5.8). For example, Ganswindt *et al*. (2003) studied the efficiency of various enzyme immunoassays (cortisol, 11-oxo-etiocholanolone I, 11-oxo-etiocholanolone II and 11ß-Hydroxy-etiocholanolone) in measuring the variation in urinary and faecal glucocorticoids in African elephants in musth (i.e. when androgen levels are high). Two of these assays (11-oxo-etiocholanolone II and cortisol) showed substantial cross-reactivity with testosterone and failed to differentiate between increased faecal testosterone during musth and increased faecal glucocorticoids. The other two EIAs, which did not cross react with androgens (11-oxo-etiocholanolone I and 11ß-Hydroxy-etiocholanolone), showed no elevation in cortisol metabolites profile during or out of the musth season. Heistermann *et al*. (2006) checked the validity of various EIAs on several species of primates and found that group-specific assays (to measure 5b-reduced cortisol metabolites with a 3a, 11-oxo and 3a,11b-dihydroxy structure) tended to cross-react with androgens. In the present study, a cortisol specific antiserum was employed in the RIA, and the androgens (testosterone and 1-dehydrotestosterone) and progesterone steroids showed little cross-reactivity with the antibody. The assay can therefore be reliably used to measure glucocorticoids in faecal samples from both male and female colobus monkeys.

Accuracy is normally assessed by investigating the recovery of known amounts of cortisol which has been added to samples with a cortisol concentration below the detection limit of the assay. Accuracy can also be assessed by comparing the theoretical and measured cortisol concentrations for a reference standard. Accuracy tests were not performed in the present study and therefore, the method can not be confirmed to be quantitatively accurate. However, the actual quantity of faecal cortisol equivalents found in the present study closely match those obtained by Chapman *et al.* (2006) while working with wild red colobus monkeys. In their study, the authors found averaged cortisol levels of 264 ng/g (with a range of 140 to 446 ng/g) in samples originating from red colobus in forest fragments. For red colobus monkeys leaving in intact forest of the Kibale National Park, levels were 3.5 times lower than the average value of the fragment forest samples: averages of 76.4 ng/g, 82.9 ng/g and 66.3 ng/g depending on group or year (ranging from 44.6-154.4 ng/g, 42.9-153.2 ng/g and 31-145.4 ng/g respectively). Results for faecal cortisol equivalents (ng/g) for colobus monkeys at Port Lympne Zoo ranged within 12.6 and 211.15 ng/g (average: 67.35 ± 4.33 ng/g) The mean faecal cortisol equivalent content for colobus monkeys at La Boissière du Doré Zoo (including data from the attack event) was 119.10 ± 9.42 ng/g (ranging from 65.00-248.1 ng/g). At Banham Zoo, faecal cortisol equivalents were between 32.86 and 376.63 ng/g (mean value 101.62 ± 20.35). Finally, the mean faecal cortisol equivalent values for colobus monkey at Paignton Zoo were 132.65 ±18.20 ng/g for the all male group and 181.83 ± 20.92 for the all female group. The latter showed some of the highest levels of faecal cortisol equivalents seen in the present study: up to 1000 ng g^{-1} in one sample in March 2006. Altogether, these results are extremely close to those of red colobus monkeys observed in the wild.

Behavioural evidence supports the idea that the cortisol RIA provided data that are indicative of adrenocortical activity and stress status. During the study, at two separate zoos, colobus were exposed to a particularly stressful event. At La Boissière du Doré, one animal (Nimba) was attacked by the rest of the group, half way through the study. Throughout the visit, as the tension mounted up to the ultimate attack event, levels of faecal cortisol equivalents in the attacked colobus monkey steadily increased from 80.9 ng/g before the attack, to about 177 ng/g on the day of the attack and up to 248.1 ng/g two days after the attack (Figure 5.25). The lag time for intestinal passage of glucocorticoids metabolites is reported to be 38 h in Abyssinian colobus (Kay *et al*., 1994). Therefore the measurements of faecal cortisol equivalents two days after the attack are indicative of a rise in circulating cortisol just after the attack. Similarly the steep increase in faecal cortisol equivalents on the day of the attack is indicative of a sharp increase in the two days preceding, when Nimba was disturbing the rest of the group (see Chapter 2, section 2.4.1)

At Port Lympne Zoo, in July 2005, one colobus (Cranium) had to be separated from the group due to illness. When this animal was re-introduced into the group, its faecal immunoreactive cortisol content doubled from 94.7 ng/g (when it was held in a separate cage but with visual, auditory and tactile contact with the rest of the group) to 180 ng/g two days after being re-introduced (no data were available on the day of reintroduction). Both of these colobus monkeys showed an increase in faecal cortisol equivalents of more than the group mean plus three SD, which was the limit suggested by Goymann *et al*. (1999) to highlight significant reaction to stressors. There were other cases during this study where the faecal immunoreactive cortisol contents exceeded the group mean plus three SD, but the stressors were not known. Behavioural data were only collected between 8 am and 5 pm and significant stressors could have occurred at any time outside this bracket. Samples with high faecal cortisol equivalents per gram of faecal powder compared to other samples within each group were re-analysed to check the accuracy of the data. All values were consistent throughout repeated assays. This suggests that high values either resulted from procedural errors (such as contamination with urine) or that the higher values reflect real changes in glucocorticoid levels. Previous studies have used cut-off points to determine outliers. Most commonly a mean value for all faecal samples within a group are taken to represent a baseline value, and values higher than the mean plus 1.5 standard deviations (SD) are considered outliers (Young *et al*., 2004). Additionally, Goymann *et al*. (1999) introduced the idea of values above the mean of a group plus 3 SD. In this study, a limited amount of data were available for each colobus monkey and removing data points was considered unwise and would significantly affect the results. Outliers are always by definition a man made cut off point. Data between 1.5 SD and 3 SD could equally be a reaction to various stimuli but with milder measurable consequences, therefore omitting them could have serious consequences to the data set. Therefore, it was decided to include all data, from its full range, and include all data points in graphs (even those exceeding the mean plus 3 SD range). In one case, at Port Lympne Zoo in October 2006, every value (5 samples) from one colobus monkey (Katie) exceeded 1.5 SD of the mean. Clearly, every single point could not be due to errors. It seems likely that this animal had much higher faecal content of cortisol equivalents than other animals.

5.4.2 Faecal cortisol equivalents in relation to index of success, gender and reproductive status

One important question in this study was to investigate the relationship between faecal content of cortisol equivalents (as a measure of stress) and the relative ranking of individual colobus monkeys. Previous studies on a large variety of primates are contradictory with some species, such as female cynomolgus monkeys, (Shively *et al*., 1997) and male olive baboons (Sapolsky *et al*., 1997) showing a negative correlation with lower ranking animals which have higher levels of faecal glucocorticoids than dominant ones, while other species such as male captive mouse lemurs, (Perret, 1992) and female ring-tailed lemurs (Gould *et al*., 2005) suggest a positive correlation with high ranking animals exhibiting higher levels of faecal glucocorticoids than subordinates. Finally, some studies have shown no correlation between rank and faecal glucocorticoids, for example in rhesus macaques (Bercovitch & Clarke, 1995), tufted capuchins, (Lynch *et al*., 2002) and male ring-tailed lemur (Gould *et al*., 2005). In the present study, index of success was a significant predictor of faecal glucocorticoid and showed significant difference between the overall dominant animal and other members of the group.

Dominant colobus monkeys, in multi-male groups, were anticipated to show higher levels of faecal glucocorticoid, especially in the male hierarchy as dominant animals have the monopoly on reproduction (see Chapter 1, section 1.1.6) and would have to defend and assert their access over mates (Saplosky, 1992). High levels of competition were observed between male colobus monkeys, and a large proportion of displacement was observed between adult males in multi-male groups. Chapter 3 showed that females exhibited a linear social hierarchy in large groups (but not kin-based). Therefore, some degree of competition was also expected, although not based on the monopoly of reproduction, as all females in a group may breed when they reach sexual maturity (Harris & Monfort, 2006). Competition between female colobus monkeys was expected mainly at feeding time, as the most common context for conflict in female primates is concentrated around access to food supplies (Silk 1993), or in relation to social contact (Barrett *et al*., 1999). Although some competition was observed, especially at feeding time (see Chapter 3, section 3.4.1 a-c), this competition was mainly directed towards access to a preferred item, not food in general, and would not be likely to result in a correlation between rank and faecal glucocorticoids. In a zoo environment, little effort is required to obtain food that is plentiful and regularly provided by keepers (Abbot *et al*., 2003) therefore limiting the "clumping" effect of food availability, which favours contest competition. Similar results were found by Cavigelli *et al*. (2003) in their study of ring-tailed lemurs, provided with a daily food supply. The lemurs showed a diminished effect of dominance on glucocorticoid contents compared to free-ranging animals. Most of the dominance and aggression in colobus monkeys was in the form of display and rarely involved contact. Instead, animals moved away to avoid conflict therefore making the energetic cost of obtaining and maintaining dominance relatively low when compared to other species (Goymann & Wingfield 2004).

Some seasonality of faecal glucocorticoids was observed with significantly higher levels in autumn compared to other seasons. Seasonal changes in faecal glucocorticoid content could be related to photoperiod, as shown by mammalian species such as mice (Harper & Austad, 2001), or related to fluctuations in temperature, as shown by red deer (Huber *et al*., 2003), where the highest faecal glucocorticoid content was observed in December and January. However, in a zoo environment, temperature influences are doubtful as animals always have access to heated areas. Interestingly, the increased levels of faecal glucocorticoids in the autumn match the increased time spent on feeding due to extra palatable food item, and reduced time spent being social and resting socially (see Chapter 2). Therefore it is possible that with unpredictable extra food availability, some more natural form of competition may have arisen, leading to higher levels of faecal glucocorticoids.

Statistical differences between the faecal cortisol equivalents of male and female colobus monkeys were observed where female monkeys had higher faecal glucocorticoid levels than males. Similar, patterns have been found in other mammalian species (Reeder & Kramer, 2005), such as the red deer, (Huber *et al*., 2003) and primates such as baboons (Beehner *et al*., 2005). This significant difference across zoos could derive from a disparity in faecal metabolite formation cross reactivities, in parallel to other mammalian species such as mice where females formed significantly more polar metabolites in the faeces with higher immunoreactivity than those in male (Touma *et al*., 2003).

Previous studies have suggested a correlation between glucocorticoid levels and copulation/reproduction in other primate species, such as the woolly spider monkeys, *Brachyteles arachnoides* (Strier *et al*., 1999), in which glucocorticoid levels increased during the copulatory season. In the present study of colobus monkeys, the mating period was encountered three times with three different females at Port Lympne Zoo in October 2005, January 2006 and April 2006, and with two females at Banham Zoo (both in March 2005), however, levels of faecal glucocorticoids in mating females was not significantly higher than those cycling, lactating or immature ones. Pregnant females on the other hand had significantly higher levels of faecal glucocorticoids than cycling, immature, lactating or mating females. Similar results were found in female ring-tailed lemurs where faecal cortical levels were higher during late gestation period (Cavigelli, 1999) or baboons (Altmann *et al*., 2004; Weingrill *et al*., 2004; Beehner *et al*., 2005; Crockford *et al*., 2008). These elevated levels are thought to be an adaptation to cope with the increased energetic demand associated with gestation (Brockman *et al*., 2009). Additionally, some studies suggest that elevated glucocorticoid levels in female prepartum are associated with increased affiliative bond formation with infants (Nguyen *et al*., 2008).

5.4.3 Faecal cortisol equivalents in relation to aggression

Although the index of success was based on aggression, (see Chapter 3, section 3.2.3 for the definition of dominance), the total amount of aggressive behaviour was not taken into account, only the consistency of the direction of the aggressive behaviour and the outcome (i.e. winning or losing). Looking at the amount of aggression either received or given provides a potentially better understanding of possible links to faecal glucocorticoids.

In the present study, colobus monkeys receiving the least amount of aggression, while giving the most, also had the highest levels of faecal glucocorticoids. This is in agreement with previous study of female ring-tailed lemurs (who are dominant over males in this species), where faecal glucocorticoids were related to aggression given for dominant females, and aggression received for low-ranking females (Cavigelli, 1999; Cavigelli *et al*., 2003). In the ring-tailed lemur, the best predictor of faecal corticoid contents in high ranking females was the proportion of aggression initiated. In lower ranking female ring-tailed lemurs, the best predictor of elevated faecal corticoid levels was aggression received (Cavigelli *et al*., 2003). Other studies have also found a correlation between aggression and faecal glucocorticoids in species such as the Chacma baboon, *Papio hamadryas ursinus* (Bergman *et al*., 2005) or the chimpanzee, (Muller & Wrangham 2004).

5.4.4 Comparison of faecal content of cortisol equivalents of colobus monkeys in different zoos

Contrary to the expected outcome outlined in the aim of this chapter, the group of colobus monkeys at Port Lympne Zoo had the lowest levels of faecal glucocorticoids compared to other zoos. Additionally, the group at Banham Zoo had significantly lower faecal glucocorticoids than animas at La Boissière du Doré and Paignton Zoo (male and female groups). The comparisons of faecal glucocorticoids across zoo suggest that, rather than being based on the amount of male present in a group, differences between faecal glucocorticoid content exist due to group composition, where in large, stable mixed group levels of faecal glucocorticoids are lower than in abnormal formation group (i.e. small and single sex).

A recent review by Morgan & Tromborg (2007) investigated possible sources of stress in captivity and highlighted abnormal group formation as a possible factor increasing abnormal behaviour and stress. Honess & Marin (2006) also reviewed group formation and its impact on stress and aggression levels in captive primates. In a newly mixed sex group of pig-tailed macaques, cortisol levels were significantly elevated compared to previous housing arrangements, which indicated increased levels of stress (Gust *et al*., 1996).

The Paignton female group was created for husbandry purposes by merging four related females who had always lived together, and two females (one with a juvenile) from a previous large mixed sex group. During the merging process and prior to data collection, the younger female from the mixed-sex group was attacked by the four females and eventually died from her injuries. This group had some of the highest levels of cortisol equivalents (significantly higher than all mixed sexed groups), which is in accordance with

Honess & Marin (2006) who suggested that merging groups can lead to increased cortisol levels.

Port Lympne Zoo, Banham Zoo and La Boissière du Doré Zoo were large mixed sex groups of colobus monkeys and had some of the lowest faecal glucocorticoid contents but the highest numbers of recorded agonistic interactions. Port Lympne Zoo had an average of 16.7 interactions per animals, 16.1 at La Boissière du Doré Zoo and 8.1 at Banham Zoo (see Chapter 3, section 3.4.1a). In contrast, at Paignton Zoo, the higher levels of faecal glucocorticoid contents coincided with the lowest levels of aggression; 3.6 interactions per animal for the male group and 5.5 for the female group. This suggests that an important predictor of stress levels, as measured by faecal cortisol equivalents, is the stability and natural structure of the group, rather than the actual aggression rates observed between animals.

In Port Lympne, Banham and La Boissière du Doré Zoos, the dominant male remained the same throughout the study. High-ranking males won most of the aggressive encounters that they initiated and reversals in the hierarchy were only observed with lower ranking colobus monkeys. Therefore, the outcome of aggressive behaviour was highly predictable and the hierarchy was stable (Muller & Wrangham 2004). This is in marked contradiction with the two single sex groups at Paignton Zoo. These had highly unpredictable dominance hierarchies and frequent reversals possibly leading to stress, and increased faecal content of glucocorticoids and their metabolites.

Aggression itself may not be the sole indicator of variation in faecal glucocorticoids when comparing colobus monkeys within a group. Other factors such as husbandry conditions and diet, or exposure to the public may be additional factors. There were clear differences in husbandry conditions at the zoos investigated in the studies that may influence aggression and faecal glucocorticoids. Aggression of Père David’s stag deer has been linked to the size of the enclosure (Li *et al.*, 2007). Stags had significantly higher levels of faecal cortisol and showed more conflict behaviour when held in a display enclosure compared to a free-ranging deer. In the present study, the two zoos where the groups of colobus monkeys showed the highest levels of aggression were Port Lympne and La Boissière du Doré, and these were also the two groups with the smallest enclosures (see

Chapter 2, Table 2.4). However, the faecal content of cortisol equivalents in these two groups was less than that of the Paignton Zoo male and female groups (who both had access to large outside areas). No simple association between enclosure size and faecal glucocorticoid was therefore apparent.

Diet is known to influence gut passage and levels of faecal glucocorticoids in mammalian species (Morrow *et al*., 2002; von der Ohe *et al*., 2004). All zoos provided foliage to their animals (either in the form of bamboo or other browse such as lime) and dry food supplements (primate and/or leaf eater pellets). Paignton Zoo and La Boissière du Doré offered a strictly vegetarian diet to their animals with added proteins in the form of eggs (Paignton Zoo) or meat (La Boissière du Doré). Both Port Lympne and Banham Zoos provided a mixture of vegetables and fruits, with added proteins in the form of meat (chicken at Port Lympne and horse meat at Banham) to their colobus monkeys. Therefore, no obvious dietary factors separated Paignton Zoo from others, although further analysis of the nutritional values of recorded food (at each visit for each zoo) should be analysed and incorporated for verification. Most studies which found a significant impact of diet on the levels of faecal glucocorticoids were based on wild animals with extreme variation in nutrition. In zoos, although some degree of variation between diets is inevitable, differences are likely to be of less importance than in the wild, due to the control of nutritional input. Other studies specifically investigating feed intake on steroid secretion in faecal samples found no relationship between food availability or quality, and elevated faecal steroid concentration (Cook *et al*., 2002, Rabiee *et al*., 2001). Seasonal differences in faecal cortisol metabolites in a variety of species have been found to be influenced by endogenous rhythms rather than diet (Huber *et al*., 2003, Weingrill *et al*., 2004).

The impact of exposure to the public at the different zoos in the present study should be considered, since other studies have shown that public exposure can affect faecal glucocorticoids. For example, black rhinoceros, held in a larger perimeter enclosure, which increased public viewing, had higher levels of faecal glucocorticoids (Carlstead & Brown, 2005). In the present studies, the zoos had different enclosure designs. Colobus monkeys at Port Lympne Zoo had a completely private house with no public access or viewing. Paignton Zoo male and female groups also had private back cages with no public access and outside platforms sheltered from public viewing. However, back cage entrances for the female group were situated at ground level and were never used. Colobus monkeys at Banham Zoo had no private area for use during the day, although the extremely large paddock area meant that there was a substantial amount of distance between animals and humans (although visual contact was always maintained). Finally, La Boissière du Doré had no private area in use during the day and animals were exposed to the public viewing, with no possibility of escape to the outside paddock, as they were locked in. However, during both visits the zoo was only opened at weekends therefore exposure to public viewing was greatly limited.

Examination of faecal content of cortisol equivalents in relation to the various housing conditions showed no clear patterns. Colobus monkeys at Paignton Zoo had many possibilities to be sheltered from viewing, but faecal cortisol equivalents were still some of the highest.

5.5 Conclusions

Patterns between index of success and faecal glucocorticoid contents emerged where the overall dominant colobus monkeys had higher levels of faecal glucocorticoids than other members of the group. This pattern was expected due to the need of the dominant animal (always a male when both genders were present) to defend his access to the females (i.e. contested dominance: Saplosky, 1992) or other resources such as females or food (Isbell, 1991).

In the present study the groups at Port Lympne and La Boissière du Doré Zoos were expected to have higher levels of faecal glucocorticoids due to the presence of many males. These groups were anticipated to have increased levels of aggression, particularly between adult males, which would lead to higher levels of faecal glucocorticoids. Reciprocally, it was predicted that at Paignton Zoo, the all female group would show very low levels of faecal glucocorticoids since other studies have indicated a weak dominance hierarchy in all female groups (Grunau & Kuester, 2001) and low levels of aggression. Opposite results were found where the groups at Port Lympne and La Boissière du Doré Zoos had some of the lowest glucocorticoids values, whereas the group of females at Paignton Zoo had some of the highest, therefore suggesting that the stability and natural structure of the group, rather than the actual aggression levels observed between animals is an important predictor of stress levels, as measured by faecal cortisol equivalents.

Results between genders followed previously observed patterns where females had higher levels relation to faecal cortisol equivalent content than males (Beehner *et al*., 2005; Reeder & Kramer, 2005). Pregnant females in particular had significantly higher levels of faecal glucocorticoids than cycling, immature, lactating or mating females, which has been observed in other species of primates (Cavigelli, 1999; Altmann *et al*., 2004; Weingrill *et al*., 2004; Beehner *et al*., 2005; Crockford *et al*., 2008).

Chapter 6
General discussion and future directions

The aim of the present studies was to collect behavioural, endocrinological and parasitological data on captive *Colobus guereza* in an attempt to evaluate their welfare. Although this species is commonly found in zoological institutions, there are only a limited number of studies about its behaviour, physiology and welfare in captivity. Data were therefore collected at four zoos, comprising five groups of colobus monkeys, and activity budgets were created to compare the time allocation to various behaviours by colobus in the different zoos. These data were investigated to see whether there was any seasonal pattern in the time spent performing each type of behaviour, to examine the influence of sex, age and index of success, and whether there were differences between each zoo. Social dominance rank and linearity of the hierarchy in the five groups of colobus was investigated. Data collected on social behaviours were analysed in connection to life history factors (i.e. sex and age) and their influence on social dominance ranking were investigated. Egg count of *Trichuris trichiura* were measured from faecal samples and linked to different aspects of social structure such as age, gender, husbandry practices and index of success. Faecal glucocorticoids (and their metabolites) of colobus monkeys held in the five groups were measured, taking into account the variable social compositions (multi-male, multi-female; single male, multi-female; single female, multi-male, all male and all female). These results were investigated using index of success, age, levels of aggression, sex, reproductive status of female and age.

Keeping primates in captivity has become essential as a third of all primate species are in danger of extinction (Melfi, 2005). However, in holding animals of conservation value in captivity, high standards of husbandry and welfare are essential (Wemelsfelder, 1994; Duncan & Fraser, 2000; Mallapur & Choudhury, 2003, Mallapur, 2005, Melfi & Feistner, 2005, Hoisey, 2009). Welfare in captivity can be assessed by the state of an animal coping with the environment it lives in through the performance of behaviour where, for good welfare to exist, species-specific behaviours should be maintained (Mallapur & Choudhury, 2003; Mallapur, 2005, reviewed by Calisi & Bentley, 2009).

Chapter 2 discussed the state behaviours of colobus monkeys in captivity, in five groups held in four zoos, in comparison to those in the wild. These behaviours were highly comparable and therefore suggest that the frequency of behaviours observed in captivity did not diverge from those expressed in the wild (Melfi & Feistner, 2005). The whole range of behaviour was maintained which suggested there was generally good welfare at all zoos. In captivity, food is provided by carers, at determined times, and this could have a major effect on the time allocated by an animal to feeding and foraging behaviours (Martin & Kitchen, 1996; Carlstead 1998; Mallapur *et al*., 2005). The captive environment may be seen as less rich in experiences, and as reducing the possibility of finding food naturally and in novel ways which would normally stimulate the animals (Boere, 2001). However, similar time was spent feeding in captivity (between 18 % and 28 % of time) compared to in the wild with 22.9 % to 28.3 % (Fashing, 2001).

In most zoos, the use of enrichment devices to provide food may have contributed to favour the occurrence of natural behaviour, by increasing the time spent foraging and feeding and improve animal welfare (Kitchen & Martin, 1996) by making food acquisition more difficult and time consuming. For example, both the male and female groups at Paignton Zoo were provided with an environmental enrichment timetable (Dobbs & Fry, 2006). The types of enrichment provided were food based, manipulative or sensory (olfactory, visual and auditory). Food was an important part of the enrichment timetable and was made available to the animals in a variety of ways and on a regular basis, such as food kebabs, stuffed in bags, in hanging baskets or in small cages with opening large enough to fit their hands (Dobbs & Fry, 2006). However, the actual effectiveness of such devices should be assessed, particularly since natural preferences for certain enrichment devices has been reported to be species specific (Visalberghi *et al*, 2002). The groups of colobus monkeys at Paignton Zoo spent 18.15 ± 1.14 % for the female group and 18.71 ± 0.71 % for the male group foraging and feeding (i.e. slightly less time than other group although not significantly different), which suggests that the feeding enrichment devices may not have been particularly well suited to colobus monkeys. Colobine monkeys have been reported to be a particularly difficult species to enrich (Melfi, pers. comm.). For example, in the female group, no attempts were made to reach the food when hidden in bags. Other zoo based study have investigated auditory enrichment on *Colobus guereza caudatus*, by exposing the animals to recorded vocalization of *Colobus guereza* and Western red colobus, *Piliocolobus badius* (Cooke, 2006). In that particular case, exposure of the black and white colobus to the vocalization from red colobus lead to undesirable behaviours (decrease social play and feeding/foraging) and was abandoned. This is a prime example of the necessity of monitoring studies where enrichment actually had a negative effect and lead to decreased welfare. The efficacy of food based enrichment devices offered to colobus monkeys in captivity needs to be further investigated in a systematic way in future studies, to find the most appropriate devices for this highly specialized folivorous species.

At Banham Zoo, no enrichment programmes were in place, but a large paddock with grass was available to the animals. Colobus monkeys at Paignton Zoo also had access to large grassed areas but were more rarely observed feeding from them. One possible explanation for the disparity between these two groups could be based on the nutritional intake from the different diets at the two zoos. At Banham Zoo, the diet was a mixture of dry food, fruit and vegetables and protein in the form of meat (see Chapter 2, Table 2.4) offered only in the morning and late afternoon. Colobus monkeys at Paignton Zoo were fed a high fibre diet, composed exclusively of vegetable and protein in the form of eggs three times a day. Further research into the diet composition offered to different groups may highlight nutritional differences, which could affect the time spent feeding by animals in captivity. Such differences could question the relevance of using feeding time as a behavioural indicator of welfare. Animals at Banham Zoo may have fed extensively on grass during the day because food was only offered to them twice a day and may have been of lower nutritional value, therefore boosting the relative length of time spent feeding, compared to Paignton Zoo. Diet composition and quantity were recorded in the present study but not examined in detail. The animals at Banham Zoo were given fruits, which may have altered the bacterial flora of the highly folivorous species that is the colobus monkeys.

Commercial fruits offered to captive monkeys have been shown to contain higher levels of sugar while having lower fibre content (Plowman, 2000). The high consumption of grass at Banham Zoo may have been a way for animals to cope with the diet offered to them and increase their levels of fibre. The diet of colobus monkeys at Paignton Zoo have been the subject of much improvement after studies on nutritional comparison between frugivorous and folivorous primates within the zoo. At the time, the groups of colobus monkeys at Paignton Zoo were fed a variety of vegetable and fruits (banana, kiwi, orange, tomato, pineapple, apple). The studies revealed that, although the diet was sufficient for daily nutritional requirement, most vitamins and minerals were above the daily allowance which may have affected the specialized digestive flora. The increased citric acid from fruits may have affected the alkaline condition of the forestomach, lowering the pH and reducing its efficiency at digesting (Chivers & Langer, 1990). Since these studies, the diet offered to colobus monkeys at Paignton Zoo has improved dramatically, tailor made to provide high levels of fibre in the form of vegetable and protein as eggs. Therefore, at Paignton Zoo, nutritional needs may have been met by the highly specialized diet on offer, without the need for the animals to consume vast quantities of grass. Further comparison of diet quality between zoos could highlight differences in activity budget of time spent feeding between groups in captivity under varying husbandry regimes.

In all zoos, the cages were furnished with platforms, perches and ropes, which provided a simulation of the natural environment and potentially stimulated the performance of natural behaviours (Kitchen & Martin, 1996; Boere, 2001; Mallapur *et al*., 2005, Mallapur, 2005). The environment made available to the animals was complex, maximising the usable space which all contributed to increased welfare. However, if groups are held in captivity with an inadequate social composition or impoverished social environment, then a group of animals may spend considerably less time performing social behaviours compared to groups in the wild, while spending more time resting (Broom, 1991; Carlstead, 1996; Kitchen & Martin, 1996). In the wild, *C. guereza* were observed to spend between 6 % (Oates, 1977) and 8.3 % (Fashing, 2001) of their time on social behaviours and between 57 % (Oates, 1977) and 64 % (Fashing, 2001) of their time spent resting. These values match the results obtained in the present studies in which the mean time spent being social was 6.75 ± 1.2 % (mean ± SE, considering all zoos) and 67 ± 2.6 %

(mean ± SE) was spent resting. However, the Paignton Zoo all male group was clearly different from other groups, with only an overall mean of 2.58 ± 0.66 % of time spent being social and the highest percentage time spent resting alone. Therefore, although as a whole, the zoos showed comparable values of activity budgets to those in the wild, some disparities between groups were identified. These differences suggest distinct differences in the welfare of colobus monkeys held at the different zoos in the studies. However, it is important to stress that these differences may be relatively slight as qualitative abnormal behaviours, not normally encountered in the wild, such as stereotypies (self injury or pacing) were never observed at any of the zoos studied. Only cage circling was observed but always in anticipation of feeding time, which may not reflect stereotypy *per se*. In all zoos, the whole range of behaviours observed in the wild was present and results of moving, exploratory behaviours in captivity (mean of 4.78 ± 0.4 %) was comparable to values of 4.2 % (Fashing, 2001) and 5 % (Oates, 1977) observed in wild. In summary, the welfare of the individuals in the five groups at four zoos studied can be considered to be good, as the whole spectrum of behaviours observed in the wild were also observed in captivity, with a matching level of exploratory behaviour and no behaviours were observed that can be considered to be indicative of poor welfare, such as stereotypies welfare (Young, 2003; Basset & Buchanan-Smith, 2007).

Behavioural measurements other than state behaviours were used in the present study in an attempt to understand the social organisation of colobus monkeys in captivity and highlight possible links to welfare. Chapter 3 examined the social dominance rank and linearity of the hierarchy in the five groups of colobus at Port Lympne Zoo, Banham Zoo, La Boissière du Doré Zoo and male and female groups at Paignton Zoo in relation to social behaviour and life history. The studies described in Chapter 3 showed that variables such as age, gender, play behaviour, grooming given and received were all predictors of the index of success of colobus monkeys. Index of success itself was found to be a predictor with probable indices of welfare such as parasite egg count (Chapter 4), aggression and stress levels, the latter as measured by faecal cortisol equivalents (Chapter 5).

The present studies developed methods to allow the first measurements of faecal cortisol equivalents in the Abyssinian colobus monkeys, which were used to assess basal status in relation to rank. Levels of stress hormones are commonly used as an indicator of health and relative condition in individuals and populations (Wikelsi & Cooke, 2006). In most studies, higher levels of baseline cortisol are assumed to be an indication of an animal or population with reduced fitness, following the Cort-Fitness Hypothesis (Bonier *et al*., 2009a). Bonier *et al*. (2009b) reviewed studies investigating Cort-Fitness Hypothesis and found little evidence to support it. Additionally, in their review, species with social dominant systems were excluded from analysis due to the reduced fitness inherent to suppressed reproductive system of subordinate. Therefore, rank itself could affect glucocorticoid levels. Large variations in glucocorticoid levels of individuals linked to their social status have been observed in a variety of primates (reviewed by Honess & Marin, 2006), but not all species (*Macaca mulatta*, Bercovitch & Clarke, 1995; *Cebus apella nigritus*, Lynch *et al*., 2002; *Lemur catta*, Gould *et al*., 2005).

Based on the method developed in the present study, investigation of faecal glucocorticoid content of wild colobus monkeys is now feasible. Although animals in the wild are adapted to their natural environment or "niche" (Boere, 2001), a degree of temporal and spatial variability exists (Wiepkema & Koolhaas, 1993), and could affect an animal's levels of stress through the fluctuation in food availability or other seasonal variations. These factors could lead to a different relationship between faecal glucocorticoid content and social rank than that observed in captivity. A method to measure faecal cortisol equivalents has already been developed for another colobines species, the red colobus (Chapman *et al*., 2006; 2007) using the enzyme immuno-assay method developed by Ziegler *et al*. (1995). However, in these studies, individual animals were not recognized, and faecal samples could not be attributed to single individuals. Additionally, samples were only collected from adult males and adult females with young infants. Therefore, differences between gender or age category could not be investigated and reported. Furthermore, the Eastern black-and-white colobus and the red colobus are socially, ecologically and physiologically very removed from one another (Estes, 1991; Chapman *et al*., 2005). Therefore although the enzyme immuno-assay may be adequate for the red colobus, it may not be suited to *C. guereza* as steroid metabolism and the variation in gut micro-flora is highly species-specific and may create a large range of metabolites varying between species (Wasser *et al*., 2000). A novel assay to measure faecal glucocorticoid in *C. guereza* was therefore required. Additionally, individual recognition of *C. guereza* is possible and has been applied in several wild type studies (Von Hippel, 1996; 1998; Harris, 2006; Harris & Montfort, 2006; Harris *et al*., 2006). Therefore, the application of the radio-immunoassay developed in the present study would be applicable to wild studies, where samples from known animal could be collected, processed and analysed in relation to social dominance hierarchy.

Elevated glucocorticoid levels associated with chronic or excessive stress are generally considered to be representative of poor welfare (Swaisgood, 2007). One animal in the present study, Femur at Port Lympne Zoo, showed abnormally high levels of faecal immunoreactive cortisol equivalents compared to other members of its group. Femur was separated from her mother only a few months after birth. During the two successive data collection periods (October 2005 and January 2006), this animal had some of the highest faecal cortisol equivalents. This animal also showed an abnormal behaviour activity budget, spending little time playing, or showing any interest in social behaviour, moving slowly and generally acting very sedately. During infancy, the first and most important social contact for a primate infant is with its mother, and animals raised naturally by their mother tend to develop normal behaviour patterns (Anderson & Chamove, 1985; Mason, 1991). The data obtained for Femur throughout the present studies, suggest that the mother-infant bond may have been damaged when the mother was moved from the group, due to veterinary needs. This subsequently affected the normal development of this animal. Romero (2004) highlighted the fact that long maternal separation resulted in life-long hypersecretion of glucocorticoids when exposed to moderate stressor. It is known that elevated levels of glucocorticoids resulting from chronic stress can result in calcium loss, bone mass reduction and inhibition of growth (Stewart, 2003; Romero, 2004). Glucocorticoids also suppress the immune system and this may, to some extent, explain the elevated *Trichuris* egg counts seen in the present study for this animal. Although formal conclusions cannot be drawn, an association between elevated faecal glucocorticoids, the poor coat condition and reduced growth rate compared to healthy individuals, coupled with withdrawn behaviour and large parasitic load does seem to be implied. In future, separation of young animals from their mother should be avoided in captivity as long term effects seem to remain and affect behavioural development and physiological and endocrine status of colobus monkeys.

In colobus monkeys, all animals in the group may participate in infant rearing and handling, which is an unusual feature for primate species (Horwich & Wurman, 1978; Estes, 1991). Therefore, social contact with other conspecifics in play or grooming, is particularly important in developing the appropriate social skills of colobus monkeys. This will be determined by the formation of the captive group, which differed markedly in the zoos studied (see Chapter 2). The group of female colobus monkeys at Paignton Zoo was formed by bringing together a group of four adult females and a mother from a separate group, with her infant, Joe. This infant became a juvenile while separated from all former members of his group, and in the company of an unknown group of adult females.

Enlarging an established group of primates or merging two groups together has been reported to potentially have adverse effects on the welfare of captive primates (Honess & Marin 2006), and the results of data collected from the group of female colobus at Paignton Zoo suggests that this can apply to colobus monkeys. Some of the highest values for faecal content of immunoreactive cortisol observed in the present study were seen in the female group at Paignton Zoo. This suggests that faecal analysis of immunoreactive cortisol, in conjunction with other possible indices such as behaviour which also elude to the same conclusions, can provide a valuable index of welfare in colobus monkeys, even when measuring a mix of compounds, as has been shown in Chapter 5. Originally, when the two groups were merged, another female (Deanna) was present but was attacked by the four related female and was euthanized due to the extent of the injury. Although the overall activity budgets of the group were comparable to colobus monkeys in the wild, the social interactions were particularly restrictive, and occurred mainly between individuals of former group formation. All grooming behaviours and contact proximity was confined to the four related females or in separate associations between the mother and offspring. As far as I am aware, such restrictive social patterns amongst Colobus monkeys have not been reported previously. These results emphasise that, although group activity budgets are a good indication of welfare, grossly abnormal behaviours may exist and greater detail on the direction of social behaviours are essential to truly evaluate welfare in captivity.

Group instability is another factor that can affect stress and welfare of primates, particularly when animals are relocated between groups or when there is an alteration in group structure (such as removing animals), and affiliative bonds are severed (Boere, 2001). Colobus monkeys in the all male group at Paignton Zoo initially belonged to a large multi male-multi female group which was separated (removing the mother and offspring, Joe, mentioned above). The dominant male in the group and another high ranking male died, leaving a group of four males. Due to persistent fighting, one of the males (Kabul) remained physically separated from the others until re-introduction was finally successful. As in the female group at Paignton Zoo, some of the highest faecal content cortisol equivalents were measured for these colobus monkeys. Social activity patterns were significantly lower than other zoos, probably due to the lack of females in the group. The Paignton Zoo all male group separated itself from others due to the pronounced lack of aggression between the males. The 'challenge hypothesis' developed as a result of research on birds suggests that testosterone levels in adults is closely linked to mating and male-male aggression (Wingfield *et al*., 1990). In the absence of mating behaviour, levels of testosterone would be expected to be low, only sufficient to maintain the feedback regulation of gonadotrophin release. These ideas are supported by work on ursine colobus monkeys (*Colobus vellerosus*) in which the levels of faecal testosterone were positively correlated with male-male aggression, with no relationship to rank. Such studies have yet to be carried out for the colobus monkey. Measurement of faecal testosterone, alongside faecal glucocorticoids in the single sex group of male colobus monkeys could help to understand the probable links between the low levels of aggression but high faecal cortisol equivalents that were observed in this group in the absence of reproductive females.

Both groups at Paignton Zoo suffered from group composition alteration (by merging, separating and animal death), which were clearly highlighted by measurement of faecal content of cortisol equivalents and the social behaviours compared to other groups. These studies lead to a recommendation for husbandry practices avoiding single sex groups, particularly when it involves breaking down the social structure of established groups. However, if the groups were separated, to reform multi male, mutli female or single male, multi females breeding group and increase genetic variability, more positive benefits would emerge. This practice was observed at Port Lympne Zoo, to prevent inbreeding and further investigation of the impact of group re-structuring, when a normal social structure is re-instated would be valuable in guiding zoo management practices.

Reference List

Abbott, D. H., Keverne, E. B., Bercovitch, F. B., Shively, C. A., Medoza, S. P., Saltzman, W., Snowdon, C. T., Ziegler, T. E., Banjevic, M., Garland, T. & Sapolsky, R. M. 2003. Are subordinates always stressed? A comparative analysis of rank differences in cortisol levels among primates. *Hormones and behavior* 43: 67-82.

Abraham, G. 1974. Radioimmunoassay of steroids in biological material. *Acta endocrinologica* 74 (suppl.): 7-42.

Adedokun, O. A., Adedokun, R. A. M., Emikpe, B. O., Ohore, O. G., Oluwayelu, D. O. & Ajayi, O. L. 2005. Concurrent fatal helminthosis and balantidosis in red monkey (*Erythrocebus patas*) in Ibadan, Nigeria. *Nigerian veterinary journal* 23(2): 56-59.

Alberts, S. C., Sapolsky, R. M. & Altmann, J. 1992. Behavioural, endocrine, and immunological correlated of immigration by an aggressive male into a natural primate group. *Hormone and Behaviour* 26: 167-178.

Alexander, S. L. & Irvine, C. H. G. 1998. The effect of social stress on adrenal axis activity in horses: the importance of monitoring corticosteroid-binding globulin capacity. *Journal of Endocrinology* 157: 425-432.

Altmann, J. 1974. Observational study of behavior: sampling methods. *Behavior* 47: 227-262.

Altmann, J. 1980. Baboon mothers and infants. Harvard University Press, Cambridge, Massachusetts. 242pp.

Alvarez, L., Martin, G. B., Galindon, F. & Zarco, L.A. 2003. Social dominance of female goats affects their response to the male effect. *Applied Animal Behaviour Science* 84: 119-126.

Anderson, B., Erwin, N., Flynn, D., Lewis, L. & Erwin, J. 1977. Effects of short-term crowding on aggression in captive groups of pigtail monkeys (*Macaca nemestrina*). *Aggressive Behaviour* 3: 33-46.

Anderson, J. R. & Chamove, A. S. 1985. Early social experience and the development of self-aggression on monkeys. *Biology of Behaviour* 10: 147-157.

Aureli, F., Preston, S. D. & de Waal, F. M. B. 1999. Heart rate response to social interactions in free-moving rhesus macaques (*Macaca mulatta*): A pilot study. *Journal of Comparative Psychology* 113(1): 59-65.

Bahr, N. I., Pryce, C. R., Dobeli, M. & Martin, R. D. 1998. Evidence from urinary cortisol that maternal behavior is related to stress in gorillas. *Physiology & Behavior* 64: 429-437.

Bahr, N. I., Palme, R., Möhle, U. & Hodges, J. K. 2000. Comparative aspects of the metabolism and excretion of cortisol in three individual nonhuman primates. *General and Comparative Endocrinology* 117: 427-438.

Baldwin, L.A., Teleki, G. & Kavanagh, M. 1976. Filed research on Colobus, Guenon, Mangabey, and Patas monkeys: an historical, geographical, and bibliographical listing. *Primates* 17(2): 233-252.

Bardi, M., Bode, A. E., Ramirez, S. M. & Brent L.Y. 2005. Maternal care and development of stress responses in baboons. *American Journal of Primatology* 66(3): 263-278.

Barrett, L., Henzie, S. P., Weingrill, T., Lycett, J. E. & Hill, R. A. 1999. Market forces predict grooming reciprocity in female baboons. *Proceedings of The Royal Society for Biological sciences Series B* 266: 665-670.

Barroso, F. G., Alados, C. L. & Boza, J. 2000. Social hierarchy in the domestic goat: effect on food habits and production. *Applied Animal Science* 69: 25-53.

Bassett, L. & Buchanan-Smith, H. M. 2007. Effects of predictability on the welfare of captive animals. *Applied Animal Behaviour Science* 102: 223-245.

Beery. T. A. 2003. Sex differences in infection and sepsis. *Critical Care Nursing Clinic North America*. 15(1): 55-62.

Beehner, J. C. & Whitten, P. L. 2004. Modifications of a field method for faecal steroid analysis in baboons. *Physiology & Behavior* 82: 269-277.

Beehner, J. C., Bergman, T. J., Cheney, D. L., Seyfarth, R. M. & Whitten, P. L. 2005. The effect of new alpha males on female stress in free-ranging baboons. *Animal behaviour* 69: 1211-1221.

Beier, E., Lehenbauer, T. W. & Sangiah. S. 2000. Clinical efficacy of fenbendazole against gastrointestinal parasites in llamas. *Small Ruminant Research* 36: 17-23.

Beloeil, P. A., Chauvin, C., Fablet, C., Jolly, J. P., Eveno, E., Madec, F. & Reperant, J. M. 2003. Helminth control practices and infections in growing pigs in France. *Livestock Production Science* 81: 99-104.

Berard, J., Schmitdke, J. & McGeehan, L. 1990. Male reproductive success in a free-ranging colony of Rhesus macaques. *American Journal of Primatology* 20: 173-181.

Berard, J., Nrunberg, P., Epplen, J. & Schmitdke, J. 1993. Male rank, reproductive behavior, and reproductive success in free-ranging rhesus macaques. *Primates* 34: 481-489.

Bercovitch, F. 1983. Time budgets and consortships in olive baboon (*Papio Anubis*). *Folia primatologica* 41: 180-190.

Bercovitch, F. B. & Clarke, A. S. 1995. Dominance rank, cortisol concentrations and reproductive maturation in male Rhesus macaques. *Physiology & Behavior* 58: 215-221.

Bercovitch, F. B. 1997. Reproductive strategies of rhesus macaques. *Primates* 38: 247-263.

Berdanier, C. D. 1989. Role of glucocorticoids in the regulation of lipogenesis. *Faseb Journal* 3: 2179-2183.

Bergman, T. J., Beehner, J. C., Cheney, D. L., Seyfarth, R. M. & Whitten, P. L. 2005. Correlates of stress in free-ranging male chacma baboons, *Papio hamadryas ursinus*. *Animal Behaviour* 70: 703-713.

Bernstein, I. S. 1970. Primate status hierarchies. In Rosenblum, L. A. (Ed) Developments in field and laboratory research (pp. 71-109). New York: Academic Press.

Bernstein, I. S. 1981. Dominance: the baby and the bathwater. *Behavioural Brain Science* 4: 419-457.

Bernstein, I. S. & Ehardt, C. L. 1985. Age-sex differences in the expression of agonistic behavior in rhesus monkey (*Macaca mulatta*) groups. *Journal of Comparative Psychology* 99: 115-132.

Bernstein, I. S. & Baker, S. C. 1988. Activity pattern in a captive group of Celebes black apes (*Macaca nigra*). *Folia Primatologica* 54: 61-75.

Birke, J. F. 2002. Effects of browse, human visitors and noise on the behaviour of captive orang-utans. *Animal Welfare* 11: 189-202.

Boere, V. 2001. Environmental enrichment for neotropical primates in captivity. *Ciência Rural, Santa Maria*, 31(3): 543-551.

Boissy, A., Manteuffel, G., Jensen, M. B., Moe, R. O., Spruijt, B., Keeling, L. J., Winckler, C., Forkman, B., Dimitrov, I., Langbein, J., Bakken, M., Veisser, I. and Aubert, A. 2007.

Assessment of positive emotions in animals to improve their welfare. *Physiology and Behavior* 92: 375-397

Bonier, F., Quigley, H. & Austad, S. N. 2004. A technique for non-invasively detecting stress response in cougars. *Wildlife Society Bulletin* 32: 711-717.

Bonier, F., Moore, I. T., Martin, P. R. & Robertson, R. J. 2009b. The relationship between fitness and baseline glucocorticoids in a passerine bird. *General and Comparative Endocrinology* 163: 208-213.

Bonier, F., Martin, P. R., Moore, I. T. & Wingfield, J. C. 2009b. Do baseline glucocorticoids predict fitness? *Tree* 1141: 1-9.

Borell von, E. H. 2001. The biology of stress and its application to livestock housing and transportation assessment. *The journal of animal sciences* 79 (E suppl.): E260-E267.

Borries, C., Sommer, V. & Srivastava, A. 1991. Dominance, age, and reproductive success in free-ranging Hanuman langurs (*Presbytis entellus*). *Animal behaviour* 31: 600-608.

Boswell, T., Woods, S. C. & Kenagy, G. J. 1994. Seasonal changes in body-mass and glucocorticoids of free-living golden-mantled ground squirrels. *General and Comparative Endocrinology* 96: 339-346.

Bradley, A. J., McDonald, I. R. & Lee, A. K. 1980. Stress and mortality in a small marsupial (*Antechinus stuartii*, Macleay). *General and Comparative Endocrinology* 40: 188-200.

Bradley, J. E. & Jackson, J. A. 2004. Immunity, immunoregulation and the ecology of trichuriasis and ascariasis. *Parasite Immunology* 26: 429-441.

Brann, D. W. & Mahesh, V. B. 1991. Role of corticosteroids in female reproduction. *Faseb Journal* 5: 2691-2698.

Brockman, D. K., Whitten, P. L., Richard, A. F., and Schneider, A. 1998. Reproduction in free-ranging male *Propithecus verreauxi*: The hormonal correlates of mating and aggression. *American Journal of Physiological Anthropology* 105: 137-151.

Broom, D. M. 1991. Assessing welfare and suffering. *Behavioural Processes* 25: 117-123.

Brown, J. L., Wasser, S. K., Wildt, D. E. & Graham, L. H. 1994. Comparative aspects of steroid hormone metabolism and ovarian activity in felids, measured non-invasively in faeces. *Biology of Reproduction* 51: 776-786.

Buchana-Smith, H. M. 1996. Enriching the lives of marmosets and tamarins in captivity. *Shape Enrichment* 5(4): 3-5.

Bulgar, J. B. 1993. Dominance rank and access to oestrous females in male savannah baboons. *Behaviour* 127: 67-103.

Bundy, D. A. P. & Cooper, E. S. 1989. *Trichuris* and trichuriasis in humans. *Advances in Parasitology* 28: 107-173.

Bundy, D. A. P., Lillywhite, J. E., Didier, J. M., Simmons, I. & Bianco, A. E. 1991. Age-dependency of infection status and serum antibody levels in human whipworm (*Trichuris trichiura*) infection. *Parasite Immunology* 13: 629-638.

Butovskaya, M. L. & Kozintsev, A. G. 1996. Gender-related factors affecting primate social behaviour: grooming, rank, age and kinship in heterosexual and all-male groups of stump-tail macaques. *American Journal of Physical Anthropology* 101: 39-54.

Calisi, R.M. & Bentley, G.E., 2009. Lab and field experiments: Are they the same animal? *Hormone and Behavior* 56: 1-10.

Carlson, A. A., Young, A. J., Russell, A. F., Bennett, N. C., McNeilly, A. S. & Clutton-Brock, T. 2004. Hormonal correlates of dominance in meerkats (*Suricata suricatta*). *Hormones and behavior* 46: 141-150.

Carithers D. 1998. Fact and Faecal. *Medical Veterinary Bulletin*.

Carlstead, K. 1996. Effect of captivity on the behaviour of wild mammals. In Kleiman, D.G., Allen, M.E., Thompson, K.V. (Ed) Wild mammals in captivity (pp. 317-333). Chicago: the University Chicago.

Carlstead, K. 1998. Determining the cause of stereotypic behaviour in zoo carnivores: towards developing appropriate enrichment. In: Shepherdson, D.J., Mellen, J. & Hutchins (Eds.). Environmental Enrichment for Captive Animals. Smithsonian Institute Press (pp. 285-295). Washington DC, USA.

Carlstead, K. & Brown, J. L. 2005. Relationships between patterns of faecal corticoid excretion and behavior, reproduction, and environmental factors in captive black (*Diceros bicornis*) and white (*Ceratotherium simum*) rhinoceros. *Zoo Biology* 24: 215-232.

Cavigelli, S. A. 1999. Behavioural patterns associated with faecal cortisol levels in free-ranging female ring-tailed femurs, *Lemur catta*. *Animal behaviour* 57: 935-944.

Cavigelli, S. A., Dubovick, T., Levash, W., Jolly, A. & Pitts, A. 2003. Female dominance status and faecal corticoids in a cooperative breeder with low reproductive skew: ring-tailed lemurs (*Lemur catta*). *Hormones and behavior* 43: 166-179.

Champoux, M., Zanker, D. & Levine, S. 1993. Food search demand effort effects on behaviour and cortisol in adult female squirrel-monkeys. *Physiology & Behavior* 54: 1091-1097.

Chan, M. S., Medley, G. F., Jamison, D. & Bundy, D. A. P. 1994. The evaluation of potential global morbidity attributable to intestinal nematode infections. *Parasitology* 109: 373-387.

Chapman, D. I. 1979. Radioimmunoassay. *Chemistry in Britain* 15: 439-447.

Chapman, C. A., Balcomb, S. R., Gillespie, T. R., Skorupa, J. P. & Struhsaker, T. T. 2000. Long-term effects of logging on African primate communities: a 28-year comparison from Kibale National Park, Uganda. *Conservation Biology* 14: 207-217.

Chapman, C. A., Lawes, M. J., Naughton-Treves, L. & Gillespie, T. R. 2003. Primate survival in community-owned forest fragment: are metapopulation models useful amidst intensive use. In: Marsh, L.K. (Ed.), Primates in Fragments: Ecology and Conservation (pp 63-78). Kluwer Academic/Plenum Publishers, New York.

Chapman, C. A. & Pavelka, M. S. M. 2004. Group size in folivorous primates: ecological constraints and the possible influence of social factors. *Primates* 46: 1-9.

Chapman, C. A., Gillespie, T. R. & Speirs, M. L. 2005. Parasite prevalence and richness in sympatric colobines: effect of host density. *American Journal of Primatology* 67: 259-266.

Chapman, C. A., Wasserman, M. D., Gillespie, T. R., Speirs, M. L., Lawes, M. J., Saj, T. L. & Ziegler, T. E. 2006. Do food availability, parasitism, and stress have synergistic effects on red colobus populations living in forest fragments? *American Journal of Physical Anthropology* 131: 525-534.

Chapman, C. A., Naughton-Treves, L., Lawes, M. J., Wasserman, M. D. & Gillespie, T. R. 2007. Population declines of Colobus in Western Uganda and conservation value of forest fragments. *International Journal of Primatology* 28: 513-528.

Charmandari, E., Kino, T. & Chrousos, G. P. 2004. Glucocorticoids and their actions - An introduction. *Annals of the New York Academy of Sciences* 1024: 1-8

Chatani, K. 2003. Positional behavior of free-ranging Japanese macaques (*Macaca fuscata*). *Primates* 44: 13-23.

Cheng, T. C. 1973. General Parasitology. New York: Academic Press. 965p.

Chivers, D.J.& Langer, P., 1990. The digestive system of mammals. Cambridge University Press.

Clarke, A. S., Mason, W. A. & Mendoza, S. P. 1994. Heart rate patterns under stress in three species of macaques. *American Journal of Primatology* 33(2): 133-148.

Clarke, K. R. & Warwick, R. M. 2001. Change in marine communities: an approach to statistical analysis and interpretation, 2nd edition. PRIMER-E: Plymouth.

Clubb, R. & Mason, G. 2002. Animal welfare: captivity effects on wide-ranging carnivores. *Nature* 425: 473-474.

Clubb, R., Rowcliffe, M., Lee, P., Mar, K. U., Moss, C., and Mason, G. 2008. Compromised survivorship in zoo elephants. *Science* 322(5908): 1649.

Clutton-Brock, T. M. 1975. Feeding behaviour of red colobus and black and white colobus in East Africa. *Folia Primatologica* 23: 165-207.

Coe, C. L., Mendoza, S. P., Davidson, J. M., Smith, E. R., Dallman, M. F. & Levine, S. 1978. Hormonal response to stress in squirrel -monkey (*Saimiri sciureus*). *Neuroendocrinology* 26: 367-377.

Coe, C. L., Mendoza, S. P. & Levine, S. 1979. Social status constrains the stress response in the squirrel-monkey. *Physiology & Behavior* 23: 633-638.

Coe, C. L., Chen, J., Lowe, E. L., Davidson, J. M. & Levines, S. 1981. Hormonal and behavioural changes at puberty in the squirrel-monkey. Hormones and behavior 15: 36-53.

Coe, C. L., Savage, A. & Bromley, L. J. 1992a. Phylogenetic influences on hormone levels across the primate order. *American Journal of Primatology* 28: 81-100.

Coe C. L., Ershler W. B., Champoux M., Olson J. 1992b. Psychosocial factors and immune senescence in the aged primate. *Annuals of the New York Academy of Sciences* 15(650): 276-282.

Coe, C. L. & Levine, S. 1995. Diurnal and annual variation of adrenocortical activity in the squirrel-monkey. *American Journal of Primatology* 35: 283-292.

Cooke, L. 2006.The effect of auditory enrichment in the behaviour of black and white colobus monkeys (*Colobus guereza caudatus*). Abstract.

Cool, R. C., Cook, J. G., Garrott, R. A., Irwin, L. L. & Monfort, S. L. 2002. Effects of diet and body condition on fecal progestagen excretion in Elk. *Journal of Wildlife Diseases* 38: 558-565.

Cooper, E. S. & Bundy, D. A. P. 1988. *Trichuris* is not trivial. *Parasitology Today* 4: 301-306.

Cooper, E. S., Duff, E. M. W., Howell, S. & Bundy, D. A. P. 1995. 'Catch-up' growth velocities after treatment for *Trichuris* dysentery syndrome. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 89: 653-653.

Cooper, M. A., Aureli, F. & Singh, M. 2004. Between-group encounters among bonnet macaques (*Macaca radiata*). *Behavioral Ecology and Sociobiology* 56: 217-227.

Cooper, M. A. & Bernstein, I. S. 2008. Evaluating dominance styles in Assamese and Rhesus macaques. *International Journal of Primatology* 29: 225-243.

Cords, M. 2000. Agonistic and affiliative relationships in a blue monkey group. In: Whitehead, P. & Jolly, C. (Eds) Old world monkeys (pp 453-479). Cambridge Press, Cambridge.

Corradino, C. 1990. Proximity structure in a captive colony of Japanese monkeys (*Macaca fuscata fuscata*): an application of multidimensional scaling. *Primates* 31(3): 351-362.

Crabtree, G. R., Gillis, S., Smith, K. A. & Munck, A. 1979. Glucocorticoids and immune response. *Arthritis and Rheumatism* 22: 1246-1256.

Creel, S., Creel, N. M. & Monfort, S. L. 1996. Social stress and dominance. *Nature* 379: 212.

Creel, S., Creel, N. M. & Monfort, S. L. 1997. Radiocollaring and stress hormones in African wild dogs. *Conservation Biology* 11: 544-548.

Creel, S. 2001. Social dominance and stress hormones. *Trends in Ecology & Evolution* 16: 491-497.

Creel, S., Fox, J. E., Hardy, A., Sands, J., Garrott, B. & Peterson, R. O. 2002. Snowmobile activity and glucocorticoid stress responses in wolves and elk. *Conservation Biology* 16: 809-814.

Cringoli, G., Rinaldi, L., Veneziano, V., Capelli, G. & Scala, A. 2004. The influence of flotation solution, sample dilution and the choice of McMaster slide area (volume) on the reliability of the McMaster technique in estimating the faecal egg counts of gastrointestinal strongyles and *Dicrocoelium dendriticum* in sheep. *Veterinary Parasitology* 123: 121-131.

Crissey, S. D. & Pribyl, L. S. 1997. Utilizing wild foraging ecology information to provide captive primates with an appropriate diet. *Proceeding of the nutrition Society* 56: 1083-1094.

Cromtpon, D. W. T. 1999. How much human helminthiasis is there in the world? *The journal of Parasitology* 85: 397-403.

Cubicciotti, D. D., Mendoza, S. P., Mason, W. A. & Sassenrath, E. N. 1986. Differences between *Saimiri sciureus* and *Callicebus moloch* in physiological responsiveness-implications for behaviour. *Journal of Comparative Psychology* 100: 385-391.

Dasilva, G. L. 1992. The western black-and white colobus as a low energy strategist: activity budgets, energy expenditure and energy intake. *British Ecological Society* 61: 79-91.

Davila Ross, M. & Geissmann, T. 2007. Call diversity of wild male orang-utans: a phylogenetic approach. *American Journal of Primatology* 69: 305-324.

Davis, N., Schaffner, C. M. & Smith, T. E. 2005. Evidence that zoo visitors influence HPA activity in spider monkeys (*Ateles geoffroyii rufiventris*). *Applied Animal Behaviour Science* 90: 131-141.

Datta, S. B. & Beauchamp, G. 1991. Effects of group demography on dominance relationships among female primates.1) mother-daughter and sister-sister relations. *The American Naturalist* 138: 201-226.

Davis, N., Schaffner, C.M. & Smith, T.E. 2005. Evidence that zoo visitors influence HPA activity in spider monkeys (*Ateles geoffroyii rufiventris*). *Applied Animal Behaviour Science* 90: 131-141.

Defler, T. R. 1993. The time budget of a group of wild woolly monkeys (*Lagothrix lagotricha*). *International Journal of Primatology* 16: 107-120.

Dehnhard, M., Clauss, M., Lechner-Doll, M., Meyer, H. H. D. & Palme, R. 2001. Non-invasive monitoring of adrenocortical activity in roe deer (*Capreolus capreolus*) by measurement of fecal cortisol metabolites. *General and Comparative Endocrinology* 123: 111-120.

Diamandis, E. P. & D'Costa, M. 1988. Selective determination of urinary free cortisol by liquid chromatography after solid state extraction. *Journal of Chromatography-Biomedical Applications* 426: 25-32.

Dobbs, T. & Fry A. 2006. The development and progression of the primate enrichment timetables at Paignton Zoo. *Proceedings of the 1st Regional Environmental Conference*. Paignton Zoo Environmental Park, UK.

Drews, C. 1997. The concept and definition of dominance in animal behaviour. *Behaviour* 125: 283-313.

Dunbar, R. I. M. & Dunbar, E. P., 1974. Ecology and population dynamics of *Colobus guereza* in Ethiopia. *Folia Primatologica* 21(3-4): 188-208.

Dunbar, R. I. M. & Dunbar, E. P. 1976. Contrasts in social structure among black-and-white colobus monkey groups. *Animal Behaviour* 24: 84-92.

Dunbar, R. I. M. & Dunbar, E. P. 1977. Dominance and reproductive success among female gelada baboon. *Nature* 266: 351-352.

Dunbar, R. I. M. 1987. Habitat quality, population dynamics, and group composition in Colobus monkeys (*Colobus guereza*). *International Journal of Primatology* 8(4): 299-330.

Dunbar, R. I. M. & Dunbar, E. P. 1988. Maternal time budgets of gelada baboons. *Animal behaviour* 36: 970-980.

Dunbar, R. I. M. 1992. Time: a hidden constraint on the behavioural ecology of baboons. *Behavioral Ecology and Sociobiology* 31: 35-49.

Duncan, I. J. H & Fraser, D. 2000. Understanding animal welfare. In: Appleby, M.C., Hughes, B.O. (Eds). Animal Welfare (pp 19-32). CAB International, Oxon, UK.

Dunn, A. & Keymer, A. 1986. Factors affecting the reliability of the McMaster technique. *Journal of Helminthology* 60: 260-262.

Emikpe, B. O., Ayoade, G. O., Ohore, O. G. & Olaniyan, O. O. 2002. Fatal Trichuriasis in a captive baboon (*Papio anubis*) in Ibadan Nigeria: a case report. *Tropical veterinarian* 20: 36-39.

Engel, J. 2004. Time sampling for dummies-An overview of data collection methods with particular reference to time sampling. *Addendum to Proceedings of the sixth annual symposium on zoo research*. Conference Proceeding.

Fashing, P. J. 2001a. Male and female strategies during inter-group encounters in guerezas (*Colobus guereza*): evidence for resource defence mediated through males and a comparison with other primates. *Behavioral Ecology and Sociobiology* 50: 219-230.

Fashing, P. J. 2001b. Feeding ecology of guerezas in the Kakamega Forest, Kenya.: the importance of Moraceae fruit in their diet. *International Journal of Primatology* 22: 579-609.

Fashing, P. J. 2001c. Activity and ranging patterns of guerezas in the Kakamega Forest: inter-group variation and implication for intra-group feeding competition. *International Journal of Primatology* 22: 549-574.

Fashing, P. J. 2002. Population status of black and white colobus monkeys (*Colobus guereza)* in Kakamega Forest, Kenya: are they really on the decline? *African Zoology* 37(2): 119-126.

Fashing, P. J. 2004. Mortality trends in the African cheer (*Prunus africana*) and the implications for colobus monkeys (*Colobus guereza*) in Kakamega Forest, Kenya. *Biological Conservation* 120: 449-459.

Finlay, T. & Maple, T. 1986. A survey of research in American zoos and aquariums. *Zoo Biology* 5: 261-268.

Fiore Di, A. & Rodman, P. S. 2003. Time allocation patterns of lowland woolly monkeys (*Lagothrix lagotricha poeppigii*) in a neotropical terra firma forest. *International Journal of Primatology* 24: 743-758.

Ganswindt, A., Palme, R., Heistermann, M., Borragan, S. & Hodges, J. K. 2003. Non-invasive assessment of adrenocortical function in the male African elephant (*Loxodonta africana*) and its relation to musth. *General and Comparative Endocrinology* 134: 156-166.

Garcia, C., Lee, P. C. & Rosetta, L. 2006. Dominance and Reproductive Rates in Captive Female Olive Baboons, *Papio anubis*. *American Journal of Physical Anthropology* 131(1): 64-72.

Gebo, D. L. & Chapman, C. A. 1995. Positional behaviour in 5 sympatric old-world monkeys. *American Journal of Physical Anthropology* 97: 49-76.

Gebo, D. L. & Chapman, C. A. 1995. Habitat, annual and seasonal effects on positional behaviour in red colobus monkeys. *American Journal of Physical Anthropology* 96: 73-82.

Gillespie, T. R., Greiner, E. C. & Chapman, C. A. 2005. Gastrointestinal parasites of the colobus monkeys of Uganda. *The Journal of Parasitology* 91(3): 569-573.

Glaser, R., Sheridan, J., Malarkey, W. B., MacCallum, R.C. & Kiecolt-Glaser, J. K. 2000. Chronic stress alters the immune response to a pneumococcal pneumonia vaccine. *Psychosomatic Medicine* 62: 804-807.

Gomez, M. S., Gracenea, M., Montoliu, I., Feliu, C., Monleon, A., Fernandez, J. & Ensenat, C. 1996. Intestinal parasitism - protozoa and helminths - in primates at the Barcelona Zoo. *Journal of Medical Primatology* 25: 419-423.

Gonzalez, C. A., Hennessy, M. B. & Levine, S. 1981. Subspecies differences in hormonal and behavioral responses after group formation in squirrel monkeys. *American Journal of Primatology* 1: 439-452.

Good, T., Khan, M. Z. & Lynch, J. W. 2003. Biochemical and physiological validation of a corticosteroid radioimmunoassay for plasma and fecal samples in old-field mice (*Peromyscus polionotus*). *Physiology & Behavior* 80: 405-411.

Gordon, T. P., Bernstein, I. S., and Rose, R. M. 1978. Social and seasonal influence on testosterone secretion in the male rhesus monkey. *Physiology and Behaviour* 21: 623-627.

Gould, L., Ziegler, T.E. & Whittwe, D.J. 2005. Effects of reproductive and social variables on fecal glucocorticoid levels in a sample of adult male ring-tailed lemurs (*Lemur catta*) at the Beza Mahafaly Reserve, Madagascar. *American Journal of Primatology* 67(1): 5-23.

Gould, L. & Ziegler, T. E. 2007. Variation in fecal testosterone levels, inter-male aggression, dominance rank and age during mating and post-mating periods in wild adult male ring-tailed lemurs (*Lemur catta*). *American Journal of Primatology* 69(12): 1325-1339.

Goymann, W., Möstl, E., Van't Hof, T., East, M. L. & Hofer, H. 1999. Non-invasive fecal monitoring of glucocorticoids in spotted hyenas, *Crocuta crocuta*. *General and Comparative Endocrinology* 114: 340-348.

Goymann, W., East, M. L., Wachter, B., Honer, O. P., Möstl, E., Van't Hof, T. J. & Hofer, H. 2001. Social, state-dependent and environmental modulation of faecal corticosteroid levels in free-ranging female spotted hyenas. *Proceedings of the Royal Society of London Series B-Biological Sciences* 268: 2453-2459.

Goymann, W. & Wingfield, J. C. 2004. Allostatic load, social status, and stress hormones - The costs of social status matter. *Hormones and behavior* 46: 130.

Graham, L. H. & Brown, J. L. 1996. Cortisol metabolism in the domestic cat and implications for non-invasive monitoring of adrenocortical function in endangered felids. *Zoo Biology* 15: 71-82.

Grandin, T. & Jonhson, C. 2005. Animals in translation. Scribner, New York, NY.

Grencis, R. K. 2001. Cytokine regulation of resistance and susceptibility to intestinal nematode infection - from host to parasite. *Veterinary Parasitology* 100: 45-50.
Grencis, R. K., Else, K. J. Bancroft, A. J. & Bundy, D. A. P. 1993. Trichuris update '93. *Parasitology Today* 9: 309-310.

Guo, S., Li, B. & Watanabe, K. 2007. Diet and activity budget of *Rhinopithecus roxellana* in the Qinling Mountains, China. *Primates* 48: 268-276.

Guo, T., Chan, M. & Soldin, S. J. 2004. Steroid profiles using liquid chromatography-tandem mass spectrometry with atmospheric pressure photoionization source. *Archives of Pathology and Laboratory Medicine* 128: 469-475.

Gust, D. A., Gordon, T. P., Wilson, M. E., Brodie, A. R., Ahmed-Ansari, A. & McClure, H. M. 1996. Group formation of female pigtail macaques (*Macaca nemestrina*). *American Journal of Primatology* 39: 236-273.

Hahn, N. E., Proulx, D., Muruthi, P. M., Alberts, S. & Altmann, J. 2003. Gastrointestinal parasites in free-ranging Kenyan baboons (*Papio cynocephalus* and *P-anubis*). *International Journal of Primatology* 24: 271-279.

Hakeem, A., Sandoval, R., Jones, M. & Allman, J. 1996. Brain and life span in primates. J. Birren (Eds). In Handbook of the Psychology of Aging (pp. 78-104). Academic Press.

Hamel, S. & Côté, S. D. 2008. Trade-offs in activity budget in an alpine ungulate: contrasting lactating and non-lactating females. *Animal behaviour* 75: 217-227.

Hamlem, H. J. & Lawrence, J. M. 1994. Giardiasis in laboratory-housed squirrel monkeys: a retrospective study. *Laboratory Animal Science* 44: 235-239.

Hammond, A. C., Williams, M. J., Olson, T. A., Gasbarre, L. C., Leighton, E. A. & Menchaca, M. A. 1997. Effect of Rotational vs. continuous intensive stocking of bahiagrass on performance of Angus cows and calves and interaction with sire type on gastrointestinal nematode burden. *Journal of Animal Science* **75**: 2291-2299.

Hanya, G., Matsubara, M., Hayaishi, S., Zamma, K., Yoshihiro, S., Kanaoka, M. M., Sugaya, S., Kiyono, M., Nagai, M., Tsuriya, Y., Hayakawa, S., Suzuki, M., Yokota, T., Kondo, D. & Takahata, Y. 2007. Food conditions, competitive regime, and female social relationships in Japanese macaques: within-population variation on Yakushima, 49(2): 116-125.

Harper, J. M. & Austad, S. N. 2001. Effect of capture and season on fecal glucocorticoid levels in deer mice (*Peromyscus maniculatus*) and red-backed voles (*Clethrionomys gapperi*). *General and Comparative Endocrinology* 123: 337-344.

Harris, T. R. & Monfort, S. L. 2006. Mating behavior and endocrine profiles of wild black and white Colobus monkeys (*Colobus guereza*): Toward an understanding of their life history and mating system. *American Journal of Primatology* 68: 383-396.

Harris, T. R. & Monfort, S. L. 2003. Behavioral and endocrine dynamics associated with infanticide in a black and white Colobus monkey (*Colobus guereza*). *American Journal of Primatology* 61: 135-142.

Harris, T. R. 2006a. Within- and among-male variation in roaring by black and white colobus monkeys (*Colobus guereza*): What does it reveal about function? *Behaviour* 143: 197-218.

Harris, T. R. 2006b. Between-group contest competition for food in a highly folivorous population of black and white colobus monkeys (*Colobus guereza*). *Behavioral Ecology and Sociobiology* 61: 317-329.

Harris, T. R., Fitch, W. T., Goldstein, L. M. & Fashing, P. J. 2006. Black and white Colobus monkeys (*Colobus guereza*) roars as a source of both honest and exaggerated information about body mass. *Ethology* 112: 911-920.

Hausfater, G. & Watson, D. F. 1976. Social and reproductive correlates of parasite ova emission by baboons. *Nature* 262: 688-689.

Hayashi, K. T. & Moberg, G. P. 1987. Influence of acute stress and the adrenal axis on regulation of LH and testosterone in the male rhesus monkey (*Macaca mulatta*). *American Journal of Primatology* 12: 263-273.

Hayes, R. A., Morelli, T. L. & Wright, P. C. 2006. Volatile components of lemur scent secretions vary throughout the year. *American Journal of Primatology* 68: 1202-1207.

Hayssen, V., Harper, J. M. & DeFina, R. 2002. Fecal corticosteroids in agouti and non-agouti deer mice (*Peromyscus maniculatus*). *Comparative Biochemistry and Physiology A-Molecular and Integrative Physiology* 132: 439-446.

Heistermann, M., Palme, R. & Ganswindt, A. 2006. Comparison of different enzymeimmunoassays for assessment of adrenocortical activity in primates based on fecal analysis. *American Journal of Primatology* 68: 257-273.

Heleski, C. R., Shelle, A. C., Nielsen, B. D. & Zanella, A. J. 2002. Influence of housing on weanling horse behavior and subsequent welfare. *Applied Animal Behaviour Science* 78: 291-302.

Hemelrijk, C. K. & Thierry, B. 2003. Special issue - Models and empirical studies of causes and consequences of dominance hierarchies - Introduction. *Behaviour* 140: 1189-1192.

Hemelrijk, C. K., Wantia, J. & Dätwyler, M., 2003. Female co-dominance in a virtual world: ecological, cognitive, social and sexual causes. *Behaviour* 140: 1247-1273.

Hemingway, C. A. 1999. Time budgets and foraging in a Malagasy primate: do sex differences reflect reproductive condition and female dominance? *Behavioural Ecology and Sociobiology* 45 (3-4): 311-322.

Hepwoth, G. & Hamilton A.J. 2001. Scan sampling and waterfowl activity budget studies: design and analysis considerations. *Behaviour* 138: 1391-1405.

Hill, D. 1994. Affiliative behaviour between adult male and female macaques: Sexual consortships. *Primates* 28: 439-456.

Hines, A. M., Ezenwam V. O., Cross, P. & Rogerson, J. D. 2007. Effects of supplement feeding on gastrointestinal parasite infection in elk (*Cervus elaphus*): Preliminary observations. *Veterinary Parasitology* 148: 350-355.

Hippel von, F. A. 1996. Interaction between overlapping multimale groups of black and white colobus monkeys (*Colobus guereza)* in the Kakamega Forest, Kenya. *American Journal of Primatology* 38: 193-209.

Hippel von, F. A. 1998. Use of sleeping tree by black and white colobus monkeys (*Colobus guereza*) in the Kakamega Forest, Kenya. *American Journal of Primatology* 45: 281-290.

Hippel von, F. A., Havard, F. & Cleland, E. 2000. Population decline of black-and-white colobus monkey (*Colobus guereza*) in Kakamega Forest, Kenya. *African zoologist* 35: 69-75.

Holdo, R. M. 2000. Altitudinal distribution of two primates in the Mount Kenya forest. *African Journal of Ecology* 38: 369-371.

Holekamp, K. E. & Smale, L. 1998. Dispersal status influences hormones and behavior in the male spotted hyena. *Hormones and behavior* 33: 205-216.

Honess, P. E. & Marin, C. M. 2006. Behavioural and physiological aspects of stress and aggression in non-human primates. *Neuroscience and Biobehavioural review* 30: 390-412.

Hope, K., Goldsmith, M. L. & Graczyk, T. 2004. Parasitic health of olive baboons in Bwindi Impenetrable National Park, Uganda. *Veterinary Parasitology* 122: 165-170.

Horne, T. J. & Ylonen, H. 1996. Female bank voles (*Clethrionomys glareolus*) prefer dominant males; but what if there is no choice? *Behavioral Ecology and Sociobiology* 38: 401-405.

Horwich, R. H. & Wurman, C. 1978. Socio-maternal behaviours in response to an infant birth in *Colobus guereza*. *Primates* 19(4): 693-713.

Hosey, G. R. & Skyner, L. J. 2007. Self-injurious behaviour in zoo primates, *International Journal of Primatology* 28: 1431–7.

Hosey, G. R., Melfi, V. A. & Pankhurst, S. J. P. 2009. Zoo Animals: behaviour, management and welfare. University of Oxford Press.

Hrdy, S. B. & Hrdy, D. B. 1976. Hierarchical relations among female hanuman langurs (Primates: Colobinae, *Presbytis entellus*). *Science* 193: 913-915.

Huber, S., Palme, R. & Arnold, W. 2003a. Effects of season, sex, and sample collection on concentrations of fecal cortisol metabolites in red deer (*Cervus elaphus*). *General and Comparative Endocrinology* 130: 48-54.

Huber, S., Palme, R., Zenker, W. & Möstl, E. 2003b. Non-invasive monitoring of the adrenocortical response in Red deer. *The Journal of Wildlife Management* 67(2): 258-266.

Huber, S., Palme, R., Zenker, W. & Möstl, E. 2003. Non-invasive monitoring of the adrenocortical response in red deer. *Journal of Wildlife Management* 67: 258-266.

Hunt, K. E., Trites, A. W. & Wasser, S. K. 2004. Validation of a fecal glucocorticoid assay for Steller sea lions (*Eumetopias jubatus*). *Physiology & Behavior* 80: 595-601.

Ikeda, M., Dagara, M. & Inoue, S. 2000. Continuous exposure to dim illumination uncouples temporal patterns of sleep, body temperature on the lying behaviour and productivity of growing finishing pigs. *Transactions of the ASAE* 47: 1773-1782.

Isbell, L. A. 1991. Contest and scramble competition: pattern of female aggression and ranging behavior among primates. *Behavioral Ecology* 2: 143-155.

Isbell, L. A. & Young, T. P. 1993. Social and ecological influences on activity budgets of vervet monkeys and their implications for group living. *Behavioral Ecology and Sociobiology* 32: 377-385.

Isbell, L. A., Pruetz, J. D. & Young, T. P. 1998. Movements of vervet *(Cercopithecus aethiops*) and patas monkeys (*Erythrocebus patas*) as estimators of food resource size, density and distribution. *Behavioral Ecology and Sociobiology* 42: 123-133.

Isbell, L. A. & Young, T. P. 2002. Ecological models of female social relationships in primates: similarities, disparities and some directions for future clarity. *Behaviour* 139: 177-202.

Janson, C. J. 1984. Female choice and mating system of the brown capuchin monkey *Cebus apella*, *Zeitschrift fur Tierpsychologie* 65: 177-300.

Jiménez, A. E., Montenegro, V. M., Hernández, J., Dolz, G., Maranda, L., Galindo, J., Epe, C. & Schnieder, T. 2007. Dynamics of infections with gastrointestinal parasites and *Dictyocaulus viviparous* in dairy and beef cattle from Costa Rica. *Veterinary Parasitology* 148: 262-271.

Johnson, E. O., Kamilaris, T. C., Carter, C. S., Calogero, A. E., Gold, P. W. & Chrousos, G. P. 1996. The biobehavioral consequences of psychogenic stress in a small, social primate (*Callithrix jacchus jacchus*). *Biological Psychiatry* 40: 317-337.

Jones-Engel, L., Engel, G. A., Schillaci, M. A., Froehlich, J., Paputungan, U. & Kyes, R. C. 2004. Prevalence of enteric parasites in pet macaques in Sulawesi, Indonesia. *American Journal of Primatology* 62(2): 71-82.

Kalema-Zikusoka, G., Rothman, J. M. & Fox, M. T. 2005. Intestinal parasites and bacteria of mountain gorillas (*Gorilla beringei beringei*) in Bwindi Impenetrable National Park, Uganda. *Primates* 46: 59-63.

Kay, R. N. B., Hoppe, P. & Maloiy, G. M. O. 1976. Fermentative digestion of food in the colobus monkeys, *Colobus polykomos*. *Experientia* 32: 485-486.

Kay, R. N. B., Davies, A. G., 1994. In: Davies, A.G., Oates, J.F. (Eds.). Colobine monkeys their ecology, behaviour and evolution. Cambridge University Press. Cambridge, UK.

Kenagy, G. J., Place, N. J. & Veloso, C. 1999. Relation of glucocorticosteroids and testosterone to the annual cycle of free-living degus in semiarid Central Chile. *General and Comparative Endocrinology* 115: 236-243.

Kenagy, G. J. & Place, N. J. 2000. Seasonal changes in plasma glucocorticosteroids of free-living female yellow-pine chipmunks: Effects of reproduction and capture and handling. *General and Comparative Endocrinology* 117: 189-199.

Kerridge, F. J. 2005. Environmental enrichment to address behavioral differences between wild and captive black-and-white ruffed lemurs (*Varecia variegata). American Journal of Primatology* 66(1): 71-84.

Khan, M. Z., Altmann, J., Isani, S. S. & Yu, J. 2002. A matter of time: evaluating the storage of fecal samples for steroid analysis. *General and Comparative Endocrinology* 128: 57-64.

Khansari, D. N., Murgo, A. J. & Faith, R. E. 1990. Effects of stress on the immune system. *Immunology Today* 11: 170-175.

Koenig, A., Larney, E., Lu, A. & Borries, C. 2004. Agonistic behavior and dominance relationships in female Phayre's leaf monkeys-preliminary results. *American Journal of Primatology* 64: 351-357.

Kiecolt-Glaser, J. K., Glaser, R., Gravenstein, S., Malarkey, W. B. & Sheridan, J. 1996. Chronic stress alters the immune response to influenza virus vaccine in older adults. *Proceedings of the National Academy of Sciences* 93(7): 3043-3047.

Kingdon, J., Struhsaker, T., Oates, J. F., Hart, J. & Groves, C. P. 2008. *Colobus guereza*. In: IUCN 2008. 2008 IUCN Red List of Threatened Species. <www.iucnredlist.org>.

Kingston, T. J. 1971. Notes on the black and white colobus monkey in Kenya. *East African Wildlife Journal* 9: 172-175.

Kirkwood, J. K. 2003. Welfare, husbandry and veterinary care of wild animals in captivity: changes in attitudes, progress in knowledge and techniques. *International Zoo Yearbook* 38: 124-130.

Kitchen, A. M. & Martin, A. A. 1996. The effects of cage size and complexity on the behaviour of captive common marmosets, *Callithrix jacchus jacchus*. *Laboratory Animals* 30: 317-326.

Klien, S. L. 2000. The effects of hormones on sex differences in infection: from genes to behaviour. *Neuroscience Biobehaviour Review* 24(6): 627-38.

Klinkova, E., Hodges, J. K., Fuhrmann, K., de Jong, T. & Heistermann, M. 2005. Male dominance rank, female mate choice and male mating and reproductive success in captive chimpanzees. *International Journal of Primatology* 26(2): 357-484.

Korstjens, A., Nijssen, E. & Noe, R. 2005. Intergroup relationships in western black-and-white colobus, *Colobus polykomos polykomos*. *International Journal of Primatology* 26: 1267-1289.

Korte, S. M., Bouws, G. A. H. & Bohus, B. 1993. Central actions of corticotropin-releasing hormone (CRH) on behavioural, neuroendocrine and cardiovascular regulation-brain corticoid receptor involvement. *Hormones and behavior* 27: 167-183.

Kruczek, M. & Zatorska, M. 2008. Male rank affects reproductive success and offspring performance in bank voles. *Physiology & Behavior* 94: 611-615.

Krüger, O., Affeldt, E., Brackmann, M. & Milhahn, K. 1998. Group size and composition of *Colobus guereza* in Kyambura Gorge, Southwest Uganda, in relation to chimpanzee activity. *International Journal of Primatology* 19 (2): 287-296.

Kuhar, C. W., Bettinger, T. L. & Laudenslager, M. L. 2005. Salivary cortisol and behaviour in an all-male group of western lowland gorillas (*Gorilla g. gorilla*). *Animal Welfare* 14: 187-193.

Kumar, R. S., Mishra, C. & Sinha, A. 2008. Foraging ecology and time-activity budget of the Arunachal macaque *Macaca munzala*- a preliminary study. *Current Science* 93: 532-539.

Kummer, H. 1968. Social organization of Hamadryas Baboons. A field study. Karger, Basel & New York.

Kutsukake, N. 2000. On dominance rank and kinship of a wild Japanese monkey troop in Arashiyama. *Primates* 41(3): 321-335.

Ladewig, J. 1986. Endocrine aspects of stress: evaluation of stress in farm animals. In Wiepkema, P. R. & Van Adrichem, P. W. M. (Eds) Biology of stress in farm animals: an integrative approach (pp. 13-25). Martinus Nijhoff Publishers.

Larsen, M. N, Roepstorff, A. 1999. Seasonal variation in development and survival of *Ascaris suum* and *Trichuris suis* eggs on pastures. *Parasitology* 119 (2): 209-20.

Lazaro-Perea. C., Castro, C. S. S., Harrison, R., Araujo, A., Arruda, M. F. & Snowdon, C. T. 2000. Behavioural and demographic changes following the loss of the breeding female in cooperatively breeding marmosets. *Behavioral Ecology and Sociobiology* 48: 137-146.

Legesse, M. & Erko, B. 2004. Zoonotic intestinal parasites in *Papio anubis* (baboon) and *Cercopithecus aethiops* (vervet) from four localities in Ethiopia. *Acta Tropica* 90: 231-236.

Li, Z. & Rogers, E. 2004. Habitat Quality and Activity Budgets of White-Headed Langurs in Fusui, China. *International Journal of Primatology* 25: 41-54.

Li, C., Jiang, Z., Tang, S. & Zeng, Y. 2007. Influence of enclosure size and animal density on faecal cortisol concentration and aggression in Père David's deer stags. *General and Comparative Endocrionology* 151: 202-209.

Liu, S. F. 1989. A preliminary investigation of the golden monkey in Qinling Mountains. In Chen, F. G. (Ed) Progress in the study of golden monkey. (pp. 201-262). Xi'an: Northwest University Press.

Liu, J., Chen, Y., Guo, L., Gu, B., Liu, H., Hou, A., Liu, X., Sun, L. & Liu, D. 2006. Stereotypic behaviour and faecal cortisol level in captive giant pandas in relation to environmental enrichment. *Zoo Biology* 25: 445-459.

Locati, M. & Lovari, S. 1991. Clues for the dominance in female chamois: age, weight, or horn size. *Aggressive Behavior* 17: 11-15.

Lopuch, S. & Matula, B. 2008. Is there a relationship between dominance rank and condition in captive male bank voles, *Clethrionomys glareolus*? *Acta ethologica* 11: 1-5.

Loudon, A. S. 1985. Lactation and neonatal survival of mammals. *Symposia of the Zoological Society of London* 54: 183-207.

Lynch, J. W., Ziegler, T.E. & Strier, K. B. 2002. Individual and seasonal variation in fecal testosterone and cortisol levels of wild male tufted capuchin monkeys, *Cebus apella nigritus*. *Hormones and Behavior* 41: 275-287.

Lynch, J. W., Khan, M. Z., Altmann, J., Njahira, M. N. & Rubenstein, N. 2003. Concentrations of four fecal steroids in wild baboons: short- term storage conditions and consequences for data interpretation. *General and Comparative Endocrinology* 132: 264-271.

Mabosa, M. L. H., Appleton, C., Higher, J. C. & Gouwa, E. 2004. Hookworm (*Necator americanus*) transmission in inland areas of sandy soil in Kwazulu-Natal, South Africa. *Tropical Medicine and International Health* 9(4): 471-476.

Macedonia, J. M. 1986. Effects of housing differences upon activity budgets in captive sifakas (*Propithecus verreauxi*). *Zoo Biology* 6(1): 55-67.

MacWhirter, R. B. 1991. Effects of reproduction on activity and foraging behaviour of adult female Columbian ground squirrels. *Canadian Journal of Zoology-Revue Canadienne de Zoologie* 69: 2209-2216.

Mallapur, A. & Choudhury, B. C. 2003. Behavioural abnormalities in captive non-human primates. *Applied Animal Welfare Science* 6: 275-284.

Mallapur, A., Waran, N. & Sinha, A. 2005. Factors influencing the behaviour and welfare of captive lion-tailed macaques in Indian zoos. *Applied Animal Behaviour Science* 91: 337-353.

Maloney, M. A., Meiers, S. T., White, J., & Romano, M. A. 2006. Effects of three food enrichment items on the behaviour of black lemur (*Eulemur macaco)* and ringtail lemurs (*Lemur catta*) at the Henson Robinson Zoo, Springfield, Illinois. *Journal of Applied Animal Welfare Science* 9(2): 111-127.

Maple, T.L. & Finlay, T.W. 1989. Applied primatology in the modern zoo. *Zoo Biology* 1: 101-116.

Marler, P. 1969. *Colobus guereza*: Territoriality and group composition. *Science* 163: 93-95.

Marriner, L. M. & Drickamer, L. C. 1994. Factors influencing stereotyped behaviour of primates in a zoo. *Zoo Biology* 13: 267-275.

Mashburn, K. L. & Atkinson, S. 2004. Evaluation of adrenal function in serum and feces of Steller sea lions (*Eumetopias jubatus*): influences of molt, gender, sample storage, and age on glucocorticoid metabolism. *General and Comparative Endocrinology* 136: 371-381.

Mason, W. A. 1991. Effects of social interaction on well-being development aspects. *Laboratory Animal Science* 41(4): 323-328.

Mason, G. J. & Latham, N. R. 2004. Can't stop, won't stop: is stereotypy a reliable animal welfare indicator? *Animal Welfare* 13: 557–69.

Mason, L. W., Oakley, J. E., & Wilson, E. B. 2008. Soft tissue atrophy following repeated corticosteroid injection for De Quervain's Tenosynovitis. *The Internet Journal of Orthopedic Surgery* 10(1): 1-3.

Matsumoto-Oda, A. & Oda, R. 2001. Activity budgets of wild female chimpanzees in different reproductive states. *Journal of ethology* 17-21.

McCallister, J.M., Smith, T.E. & Elwood, R.W. 2004. Validation of urinary cortisol as an indicator of hypothalamic-pituitary adrenal function in the bearded emperor tamarind (*Saguinus imperator subgrisescens*). *American Journal of Primatology*, 63: 17-23.

McCusker, C., Elwood, R.W. & Smith T.E. 2004. Dominance status and faecal and urinary cortisol in captive ring-tailed lemurs, *Lemur catta*. *Folia Primatologica* 75: S1: 301.

McKenna, J. J. 2008. The evolution of allomothering behavior among colobine monkeys: function and opportunism in evolution. *American Anthropology* 81: 818-840.

Melfi, V. A. & Feistner, A. T. C. 2002 A comparison of the activity budgets of wild and captive Sulawesi crested black macaque (*Macaca nigra*). *Animal Welfare* 11(2): 213-222.

Melfi, V. A. 2005. The applicance of science to zoo-housed primates. *Applied Animal Behaviour Science* 90: 97-106.

Melfi, V. A. & Poyser, F. 2007. *Trichuris* burdens in zoo-housed *Colobus guereza*. *International Journal of Primatology* 28: 1449-1456.

Mendoza, S. P. 1991. Sociophysiologie of well-being in non-human primates. *Laboratory Animal Science* 41(4): 344-349.

Menon, S. & Poirier, F. E. 1996. Long-tailed macaques (M*acaca silenus*) in a disturbed forest fragment: Activity patterns and time budget. *International Journal of Primatology* 17: 969-985.

Mes, T. H. M. 2003. Technical variability and required sample size of helminth egg isolation procedures. *Veterinary Parasitology* 115: 311-320.

Michaud, C., Tantalean, M., Ique, C., Montoya, E. & Gozalo, A. 2003. A survey for helminth parasites in feral New World non-human primate populations and its comparison with parasitological data from man in the region. *Journal of Medical Primatology* 32: 341-345.

Miller. W., Hobbs, N. T. & Sousa, M. C. 1991. Detecting stress responses in rocky mountain bighorn sheep (*Ovis canadensis canadensis*) - reliability of cortisol concentration in urine and faeces. *Canadian Journal of Zoology-Revue Canadienne de Zoologie* 69: 15-24.

Millspaugh, J. J., Woods, R. J., Hunt, K. E., Raedeke, K. J., Brundige, G. C., Washburn, B. E. & Wasser, S. K. 2001. Fecal glucocorticoid assays and the physiological stress response in elk. *Wildlife Society Bulletin* 29: 899-907.

Millspaugh, J. J. & Washburn, B. E. 2003. Within-sample variation of fecal glucocorticoid measurements. *General and Comparative Endocrinology* 132: 21-26.

Millspaugh, J. J., Washburn, B. E., Milanick, M. A., Slotow, R. & van Dyk, G. 2003. Effects of heat and chemical treatments on fecal glucocorticoid measurements: implications for sample transport. *Wildlife Society Bulletin* 31: 399-406.

Millspaugh, J. J. & Washburn, B. E. 2004. Use of fecal glucocorticoid metabolite measures in conservation biology research: considerations for application and interpretation. *General and Comparative Endocrinology* 138: 189-199.

Milton, K. 1981. Food choice and digestive strategies of two sympatric primate species. *American Nature* 117: 476-495.

Milton, K. 1993. Diet and primate evolution. *Scientific American* 269(2): 86-93.

Milton, K. 2008. Habitat, diet, and activity patterns of free-ranging woolly spider monkeys (*Brachyteles arachnoides* E. Geoffroy 1806). *International Journal of Primatology* 5: 491-514.

Monnikes, H., Schmidt, B. G., Tebbe, J., Bauer, C. & Tache, Y. 1994. Microinfusion of corticotrophin releasing factor into the locus coeroleus/subcoeroleus nuclei stimulates colonic motor function in rats. *Brain Research* 644: 101-107.

Mooring, M. S., Patton, M. L., Lance, V. A., Hall, B. M., Schaad, E. W., Fetter, G. A., Fortin, S. S. & McPeak, K. M. 2006. Glucocorticoids of bison bulls in relation to social status. *Hormones and Behavior* 49: 369-375.

Mootnick, A. R. & Baker, E. 1994. Masturbation in captive Hylobates (gibbons). *Zoo Biology* 13: 345-353.

Morgan, K. & Tromborg, C. T. 2007. Sources of stress in captivity. *Applied Animal Behaviour Science* 102: 262-302.

Morrow, C. J., Kolver, E. S., Verkerk, G. A. & Matthews, L. R. 2002. Fecal glucocorticoid metabolites as a measure of adrenal activity in dairy cattle. *General and Comparative Endocrinology* 126: 229-241.

Möstl, E., Messmann, S., Bagu, E., Robia, C. & Palme, R. 1999. Measurement of glucocorticoid metabolite concentrations in faeces of domestic livestock. *Journal of Veterinary Medicine Series A-Physiology Pathology Clinical Medicine* 46: 621-631.

Möstl, E. & Palme, R. 2002. Hormones as indicators of stress. *Domestic animal endocrinology* 23: 67-74.

Möstl, E., Maggs, J. L., Schrotter, G., Besenfelder, U. & Palme, R. 2002. Measurement of cortisol metabolites in faeces of ruminants. *Veterinary Research Communications* 26: 127-139.

Muller, M. N. & Wrangham, R. W. 2004. Dominance, cortisol and stress in wild chimpanzees (*Pan troglodytes schweinfurthii). Behavioral Ecology and Sociobiology* 55: 332-340.

MullerGraf, C. D. M, Collins, D. A. & Woolhouse, M. E. J. 1996. Intestinal parasite burden in five troops of olive baboons (*Papio cynocephalus Anubis*) in Gombe Stream National Park, Tanzania. *Parasitology* 112(5): 489-497.

Munck, A., Guyre, P. M. & Holbrook, N. J. 1984. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. *Endocrine Reviews* 5: 25-44.

Munene, E., Otsyula, M., Mbaabu, D. A. N., Mutahi, W. T., Muriuki, S. M. K. & Muchemi, G. M. 1998. Helminth and protozoan gastrointestinal tract parasites in captive and wild-trapped African non-human primates. *Veterinary Parasitology* 78: 195-201.

Muriuki, S. M. K., Murugu, R. K., Munene, E., Karere, G. M. & Chai, D. C. 1998. Some gastro-intestinal parasites of zoonotic (public health) importance commonly observed in old world non-human primates in Kenya. *Acta Tropica* 71: 73-82.

Najjar, S. M. & Weintraub, B. D. 1993. Radioimmunoassay and radioreceptor assay: past, present, and future. Handbook of endocrine research techniques. (pp. 3-23). Academic press.

Nakamichi, M., Itoigawa, N., Imakawa, S. & Machida, S. 1995. Dominance relations among adult females in a free-ranging group of Japanese monkeys at Katsuyama. *American Journal of Primatology* 37: 241-251.

Nakagawa, N. 1989. Feeding strategies of Japanese monkeys against deterioration of habitat quality. *Primates* 30:1-6.

Nelson, R. J. 2000. An introduction to behavioural endocrinology. Sunderland, Massachusetts: Sinauer.

Newton-Fisher, N. E. 2004. Hierarchy and social status in Budongo chimpanzees. *Primates* 45: 81-87.

Nicholson, N. A. 1987. Infants, mothers and other females. In Smuts, B., Cheney, D., Seyfarth, R., Wrangham, R. & Struhsaker, T. (Eds.), Primate Societies (pp. 330-342). University of Chicago Press, Chicago.

Nokes, C., Grantham McGregor, S. M., Sawyer, A. W., Cooper, E. S., Robinson, B. A. & Bundy, D. A. P. 1992. Moderate to heavy infections of Trichuris trichiura affect cognitive function in Jamaican school children. *Parasitology* 104: 539-547.

Oates, J. F. 1977. The social life of a black and white colobus monkey, *Colobus guereza*. *Zeitschrift fur Tierpsychologie* 45: 1-60.

Oates, J. F. 1978. Water-plant and soil consumption by Guereza monkeys (*Colobus guereza*): a relationship with mineral and toxins in the diet? *Biotropica* 10(4): 241-253.

Oates, J. F. 1994. The natural history of African colobines. In Davies, A. G. & Oates, J. F. (Eds) Colobine monkeys: their ecology, behavior and evolution. (pp. 75-128). London: Cambridge University Press.

O'Brien, T. G. & Kinnaird, M. F. 1997. Behavior, diet, and movements of the Sulawesi crested black macaque (*Macaca nigra*). *International Journal of Primatology* 18(3): 321-351.

Ocaido, M., Sielfert, L. & Baranga, J. 1999. Seasonal changes of impala (*Aepyceros melampus*, Lichtenstein, 1812) faecal helminth egg counts through a one year period. *African Journal of Ecology* 37: 327-333.

Ohe der, C. G. & Servheen, C. 2002. Measuring stress in mammals using fecal glucocorticoids: opportunities and challenges. *Wildlife Society Bulletin* 30: 1215-1225.

Ohe der, C. G., Wasser, S. K., Hunt, K. E. & Servheen, C. 2004. Factors associated with fecal glucocorticoids in Alaskan Brown bears (*Ursus arctos horribilis*). *Physiological and Biochemical Zoology* 77: 313-320.

Oki, C. & Atkinson, S. 2004. Diurnal patterns of cortisol and thyroid hormones in the Harbor seal (*Phoca vitulina*) during summer and winter seasons. *General and Comparative Endocrinology* 136: 289-297.

Oliverio, A. 1987. Endocrine aspects of stress: central and peripheral mechanisms. In Wiepkema, P. R. & Van Adrichem, P. W. M. (Eds) Biology of stress in farm animals: an integrative approach (pp. 3-12). Martinus Nijhoff Publishers.

Onah, D. N., Nawa, Y. 2000. Mucosal immunity against parasitic gastrointestinal nematodes. *The Korean Journal of Parasitology* 38(4): 209-236.

Padgett, D. A. & Glaser, R. 2003. How stress influences the immune response. *Trends in Immunology* 24: 444-448.

Palagi, E. & Paoli, T. 2007. Social play in bonobos: not only an immature matter. In developments in primatology and prospects. Furuichi, T. & Thompson, J. (Eds). The bonobo, behaviour, ecology and conservation (pp. 55-74). Springer, New York.

Palme, R., Fischer, P., Schildorfer, H. & Ismail, M. N. 1996. Excretion of infused ^{14}C-steroid hormones via faeces and urines in domestic livestock. *Animal Reproduction Science* 43: 43-63.

Palme, R., Robia, C., Messmann, S., Hofer, J. & Möstl, E. 1999. Measurement of faecal cortisol metabolites in ruminants: a non-invasive parameter of adrenocortical function. *Wiener Tierarztliche Monatsschrift* 86: 237-241.

Pannozzo, P. L., Phillips, K. A., Haast, M. E. & Mintz, E. M. 2007. Social monitoring reflects dominance relationships in a small captive group of brown capuchin monkeys (*Cebus apella*). *Ethology* 113: 881-888.

Paoli, T., Palagi, E. & Borgognini Tarli, S. M. 2006. Re-evaluation of the dominance hierarchy in bonobos (*Pan paniscus*). *American Journal of Physical Anthropology* 130(1): 116-122.

Parr, L. A., Matheson, M. D., Bernstein, I. S. & deWaal, F. M. B. 1997. Grooming down the hierarchy: allogrooming in captive brown capuchin monkeys (*Cebus apella*). *Animal behaviour* 54: 361-367.

Paukner, A. & Suomi, S. J. 2008. Sex differences in play behavior in juvenile tufted capuchin monkeys (*Cebus apella*). *Primates* 49(4): 288-291.

Pavelka, M. S. M. & Knopff, K. H. 2004. Diet and activity in black howler monkeys (*Allouatta pigra*) in southern Belize: does degree of frugivory influence activity level? *Primates* 45: 105-111.

Payne, H. F. P., Lawes, M. J. & Henzi, S. P. 2003. Competition and the exchange of grooming among female samango monkeys (*Cercopethicus mitis erythrarchus*). *Behaviour* 140: 453-472.

Pazol, K. & Cords, M. 2005. Seasonal variation in feeding behavior, competition and female social relationships in a forest dwelling guenon, the blue monkey (*Cercopithecus mitis stuhlmanni*), in the Kakamega forest, Kenya. *Behavioral Ecology and Sociobiology* 58: 566-577.

Peel, A. V., Vogelnest, L., Finnigan, M., Grossfeldt, L. & O'Brien, J. K. 2005. Non-invasive faecal hormone analysis and behavioural observations for monitoring stress responses in captive lowland gorillas (*Gorilla gorilla gorilla*). *Zoo Biology* 24(5): 431-445.

Pelletier, F. & Festa-Bianchet, M. 2004. Effects of body mass, age, dominance and parasite load on foraging time of bighorn rams, *Ovis canadensis*. *Behavioral Ecology and Sociobiology* **56**: 546-551.

Perret, M. 1992. Environmental and social determinants of sexual and steroid hormones in the male lesser mouse lemur (*Microcebus murinus*). *Folia Primatologica* 59: 1-25.

Petherick, J. C. & Rushen, J. 2000. Behavioural restriction. In: Appleby, M.C., Hughes, B.O. (Eds.), Animal Welfare (pp. 89-106). CAB International, Oxon, UK.

Poisbleau, M., Fritz, H., Guillon, N. & Chastel, O. 2005. Linear social dominance hierarchy and corticosterone responses in male mallards and pintails. *Hormones and behavior* 47: 485-492.

Poyser, F., Melfi, V. & Sayers, G. 2003. A *Trichuris spp* in zoo-housed colobines. In: *Proceedings of the British Veterinary Zoological Society Meeting*. Edinburgh Zoo, Scotland (Abstract).

Pusey, A., Williams, J. & Goodall, J. 1997. The influence of dominance and reproductive success of female chimpanzees. *Science* 277: 828-831.

Quihui-Cota, L. 2004. Prevalence and intensity of intestinal parasitic infections in relation to nutritional status in Mexican school children. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 98(11): 653-659.

Rabiee, A. R., Macmillan, K. L. & Schwarzenberger, F. 2001. The effect of level of feed intake on progesterone clearance rate by measuring faecal progesterone metabolites in grazing dairy cows. *Animal Reproduction Science* 67: 205-214.

Randon, T. W. & Ranson, B. S. 1971. Adult male-infant relations among baboons (*Papio Anubis*). *Folia Primatologica* 16: 179-195.

Reed, C., O'Brien, T. G. & Kinnaird, M. F. 1997. Male social behavior and dominance hierarchy in the Sulawesi crested black macaque (*Macaca nigra*). *International Journal of Primatology* 18: 247-260.

Reeder, D. M., Kosteczko, N. S., Kunz, T. H. & Widmaier, E. P. 2004. Changes in baseline and stress-induced glucocorticoid levels during the active period in free-ranging male and female little brown myotis, *Myotis lucifugus* (Chiroptera: Vespertilionidae). *General and Comparative Endocrinology* 136: 260-269.

Reeder, D. M., Kunz, T. H. & Widmaier, E. P. 2004. Baseline and stress-induced glucocorticoids during reproduction in the variable flying fox, *Pteropus hypomelanus* (Chiroptera : Pteropodidae). *Journal of Experimental Zoology Part A-Comparative Experimental Biology* 301A: 682-690.

Reinhardt, V., Reinhardt, A., Bercovitch, F. B. & Goy, R. W. 1986. Does inter-male mounting function as a dominance demonstration in Rhesus monkeys. *Folia Primatologica* 47: 55-60.

Remoue, F., To Van, D., Schacht, A. M., Picquet, M., Garraud, O., Vercruysse, A., Capron, A. LY. A. & Riveau, G. 2000. Gender-dependant specific immune response during chronic human Schistosomiasis haematobia. *Clinical Experimental Immunological* 124; 62-68.

Robles, T. F., Glaser, R. & Kiecolt-Glaser, J. K. 2005. Out of Balance: A new look at chronic stress, depression and immunity. *Current directions in psychological science* 14: 111.

Rose, R. M., Holaday, J. W. & Bernstein, I. S. 1971. Plasma testosterone, dominance rank and aggressive behaviour in male rhesus monkeys. *Nature* 231: 366-368.

Roth J.A. 1985. Cortisol as mediator of stress-associated immunosuppression in cattle. In Moberg & G.P. (Eds) Animal Stress (pp. 225-243). Baltimore, Maryland: Waverly Press Inc.

Rothman, J. & Bowman, D. D. 2003. A Review of the endoparasites of mountain gorillas. In: Companion and Exotic Animal Parasitology, Bowman, D.D. (Ed.). International Veterinary Information Service. New York.

Ruckstuhl, K. E. & Festa-Bianchet, M. 1998. Do reproductive status and lamb gender affect the foraging behaviour of bighorn ewes? *Ethology* 104: 941-954.

Rutberg, A. T. 1986. Dominance and its fitness consequences in American bison cows (*Bison bison*). *Behaviour* 96: 62-91.

Saito, C. 1996. Dominance and feeding success in female Japanese macaques, *Macaca fuscata*: Effects of food patch size and inter-patch distance. *Animal Behaviour* 51: 967-980.

Saj, T. L., Marteinson, S., Chapman, C. A. & Sicotte, P. 2007. Controversy over the application of current socioecological models to folivorous primates: *Colobus vellerosus* fits the predictions. *American Journal of Primatology* 133: 994-1003.

Saltzman, W., Schultz-Darken, N. J., Scheffler, G., Wegner, F. H., Wittwer, D. J. & Abbott, D. H. 1994. Social and reproductive influences on plasma cortisol in female marmoset monkeys. *Physiology & Behavior* 56: 801-810.

Sambrook, T. D., Whiten, A. & Strum, S. C. 1995. Priority of access and grooming patterns of females in a large and a small group of olive baboons. *Animal behaviour* 50: 1667-1682.

Sands, J. & Creel, S. 2004. Social dominance, aggression and faecal glucocorticoid levels in a wild population of wolves, *Canis lupus*. *Animal behaviour* 67: 387-396.

Sannen, A., Van Elsacker, L., Heistermann, M. & Eens, M. 2004. Urinary testosterone-metabolite levels and dominance rank in male and female bonobos (*Pan paniscus*). *Primates* 45: 89-96.

Sapolsky, R. M. 1983. Endocrine aspects of social instability in the olive baboon (*Papio Anubis*). *American Journal of Primatology* 5: 365-379.

Sapolsky, R. M. 1992. Neuroendocrinology of the stress response. In: Becker, J.B., *et al* (Eds.), Behavioural Endocrinology (pp. 287-324). Massachusetts Institute of Technology Press, Massachusetts.

Sapolsky, R. M. 1995. Social subordinates as a marker of hypercortisolism: some unexpected subtleties. G. P. Choursos, R. McCarty, K. Pacak, G. Cizza, E. Sternberg, P. W. Gold, & R. Kvetnansky, (Eds.). In "Stress Basic Mechanisms and Clinical Implications" (Vol 771, 626-639). Ann. N.Y. Academy Science, New York.

Sapolsky, R. M., Alberts, S. C. & Altmann, J. 1997. Hypercortisolism associated with social subordinance or social isolation among wild baboons. *Archives of General Psychiatry* 54: 1137-1143.

Schaffner, C. M. & French, J. A. 1997. Group size and aggression: recruitment incentives in a cooperatively breeding primate. *Animal Behaviour* 54(1): 171-180.

Schatz, S. & Palme, R. 2001. Measurement of faecal cortisol metabolites in cats and dogs: A non-invasive method for evaluating adrenocortical function. *Veterinary Research Communications* 25: 271-287.

Schenkel, R. & Schenkel-Hullinger, L. 1967. On the sociology of free-ranging colobus (*Colobus guereza caudatus*, Thomas, 1885). In Starck, D., Schneider, R. & Kuhn, H. J. (Eds.), Neue Ergebnisse der Primatologie, Gustav Fischer-Verlag, Stuttgart.

Schiml, P. A., Mendoza, S. P., Saltzman, W., Lyons, D. M., & Mason, W. A. 1996. Seasonality in squirrel monkeys (*Saimiri sciureus*). *Physiology and Behavior* 60: 1105-1113.

Schwarzenberger, F., Möstl, E., Palme, R. & Bamberg, E. 1996. Faecal steroid analysis for non-invasive monitoring of reproductive status in farm, wild and zoo animals. *Animal reproduction science* 42: 515-526.

Schwarzenberger, S. 2007. The many uses of non-invasive faecal steroid monitoring in zoo and wildlife species. *International Zoo Yearbook* 41: 52-74.

Selye, H. 1935. A syndrome produced by diverse nocuous agent. *Nature* 138: 32-33.

Selye, H. 1973. Evolution of stress concept. *American Scientist* 61: 692-699.

Seyfarth, R. M. 1977. A model of social grooming among adult female monkeys. *Journal of Theoretical Biology* 65: 671-698.

Sheridan, L. A. D., Poulin, R., Ward, D. F. & Zuk, M. 2000. Sex differences in parasitic infections among arthropod hosts: is there a male bias? *OIKOS* 88: 327-334.

Shi, D. C., Li, G. H. & Hu, T. Q. 1982. Preliminary study on the ecology of golden haired monkey. *Zoological Research* 3: 105-110.

Shively, C. A, Laber-Laird, K. & Anton, R. F. 1997. Behaviour and physiology of social stress and depression in female cynomolgus monkeys. *Biological Psychiatry* 41: 871-882.

Silk, J. B. 1982. Altruism among female *Lacaca radiata*: explanation and analysis of patterns of grooming and coalition formation. *Behaviour* 79: 162-188.

Singh, M., Singh, M., Sharma, A. K. & Krishna, B. A. 2003. Methodological considerations in measurement of dominance in primates. *Current Science* 84: 709-713.

Singh, M., Krishna, B. A. & Singh, M. 2006. Dominance hierarchy and social grooming in female lion- tailed macaques (*Macaca silenus*) in the Western Ghats, India. *Journal of Biosciences* 31(3): 369-377.

Skyner, L. J., Amory, J. R. & Hosey, G. 2004. The effects of visitor on the self-injurious behaviour of a male pileated gibbon (*Hylobates pileatus*). Zool Garten N.F.

Smuts, B. B. & Smuts, R. W. 1993. Male aggression and sexual coercion of females in nonhuman primates and other mammals: evidence and theoretical implications. *Advances in the Study of Behavior* 22: 1-63.

Smuts, B. B. & Smuts, R. W. 2007. Male aggression and sexual coercion of females in nonhuman primates and other mammals: evidence and theoretical implications. *Advances in the Study of Behavior* 22: 1-63.

Smyth, J. D. 1994. Sphasmid Nematoda. In Smyth J.D. (Ed) Introduction to Animal Parasitology (pp. 388-389). Cambridge: Cambridge University Press.

Stanford, C. B. 1991. The capped langur in Bangladesh: behavior, ecology and reproductive tactics. In Slazay, F. S. (Ed) Contribution to primatology (pp. 1-179). Basel: Karger.

Stead, S. K., Meltzer, D. G. A. & Palme, R. 2000. The measurement of glucocorticoid concentrations in the serum and faeces of captive African elephants (*Loxodonta africana*) after ACTH stimulation. *Journal of the South African Veterinary Association-Tydskrif Van Die Suid-Afrikaanse Veterinere Vereniging* 71: 192-196.

Stephenson, L. S., Holland, C. V. & Cooper, E. S. 2000. The public health significance of *Trichuris trichiura*. *Parasitology* 121: S73-S95.

Sterck, E. H. M., Watts, D. P. & van Schaik, C. P. 1997. The evolution of female social relationships in nonhuman primates. *Behavioral Ecology and Sociobiology* 41: 291-309.

Strier, K. B. 1992. Causes and consequences on nonaggression in woolly spider monkeys. In J. Silverberg and J.P. Gray (Eds). Aggression and Peacefulness in Humans and Other Primates (pp 100-116). Oxford University Press, New York.

Strier, K. B., Ziegler, T. E. & Wittwer, D. J. 1999. Seasonal and social correlates of faecal testosterone and cortisol levels in wild male muriquis (*Brachyteles arachnoides*). *Hormones and behavior* 35: 125-134.

Stuart, M., Pendergast, V., Rumfelt, S., Pierberg, S., Greenspan, L., Glander, K. & Clarke, M. 1998. Parasites of wild howlers (*Alouatta* spp.). *International Journal of Primatology* 19: 493-512.

Sussman, R. W., Garber, P. A. & Cheverud, J. M. 2005. Importance of cooperation and affiliation in the evolution of primate sociality. *American Journal of Physical Anthropology* 128: 84-97.

Swaisgood, R. R. 2007. Current status and future directions of applied behavioural research for animal welfare and conservation. *Applied Animal Behaviour Science* 102: 139-162.

Szeto, A., Gonzales, J. A., Spitzer, S. B., Levine, J. E., Zaias, J., Saab, P. G., Schneiderman, N. & McCabe, P. M. 2004. Circulating levels of glucocorticoid hormones in WHHL and NZW rabbits: circadian cycle and response to repeated social encounter. *Psychoneuroendocrinology* 29: 861-866.

Tarnaud, L. 2006. Feeding behaviour of lactating brown lemur females (*Eulemur fulvus*) in mayotte: influence of infant age and plant phenology. *American Journal of Primatology* 68: 966-977.

Teas, J., Richie, T., Taylor, H. & Southwick, C. 1980. Population patterns and behavioral ecology of rhesus monkeys (Macaca mulatta) in Nepal. In Lindburg, D. G. (Ed) The macaques: Studies in ecology, behavior and evolution. (pp. 247-262). New York: Van Nostrand Reinhold.

Teichroeb, J. A., Saj, T. L., Paterson, J. D. & Sicotte, P. 2003. Effect of group size on activity budgets of *Colobus vellerosus* in Ghana. *International Journal of Primatology* 24: 743-758.

Teskey-Gerstl, A., Bamberg, E., Steineck, T. & Palme, R. 2000. Excretion of corticosteroids in urine and faeces of hares (*Lepus europaeus*). *Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology* 170: 163-168.

Thierry, B., Gauthier, C. & Peignot, P. 1990. Social grooming in Tonkean macaques. *International Journal of Primatology* 11: 357-374.

Todd, P. A., Macdonald, C. & Coleman, D 2006. Within-group differences in captive Diana monkey (*Cercopithecus diana diana*) behaviour. *Journal of Ethology* 26(2): 272-278.

Todd, P. A., Macdonald, C. & Coleman, D. 2007. Visitor-associated variation in captive Diana monkey (*Cercopithecus diana diana*) behaviour. *Applied Animal Behaviour Science* 107: 162-165.

Touma, C., Sachser, N., Möstl, E. & Palme, R. 2003. Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. *General and Comparative Endocrinology* 130: 267-278.

Touma, C., Palme, R. & Sachser, N. 2004. Analyzing corticosterone metabolites in fecal samples of mice: a non-invasive technique to monitor stress hormones. *Hormones and behavior* 45: 10-22.

Tromborg, C. T., & Coss, R. C. 1995. Denizens, decibels and dens. In: Annual Proceeding of the American Association and Aquariums (pp. 521-528). Seattle, WA.

Turner, J. W., Tolson, P. & Hamad, N. 2002. Remote assessment of stress in white rhinoceros (*Ceratotherium simum*) and black rhinoceros (*Diceros bicornis*) by measurement of adrenal steroids in feces. *Journal of Zoo and Wildlife Medicine* 33: 214-221.

van Schaik, C. P., van Noordwijk, M. A., de Boer, R. J. & den Tonkelaar, I. 1983. The effect of group size on time budgets and social behaviour in wild long-tailed macaques (*Macaca fascicularis*). *Behavioral Ecology and Sociobiology* 13: 173-181.

Ullrich, W. 1961. Zur Biologie und Soziologie der Colobusaffen (*Colobus guereza caudatus*, Thomas, 1885). Zool. Garte., 25: 305-368.

Umeda, T., Hiramatsu, R., Iwaoka, T., Shimada, T., Miura, F. & Sato, T. 1981. Use of saliva for monitoring unbound free cortisol levels in serum. *Clinica Chemica Acta* 110: 245-253.

Urquhart, J. W., Armour, J., Duncan, A. M. & Jennings, F. W. 1988. McMaster's technique. In: Veterinary Parasitology (pp. 270–271). London, UK: Longman.

Vasey, N. 2005. Activity budgets and activity rhythms in red ruffed lemurs (*Varecia rubra*) on the Masoala Peninsula, Madagascar: seasonality and reproductive energetics. *American Journal of Primatology* 66: 23-44.

Vervaecke, H., Stevens, J. M. G., Vandemoortele, H., Sigurjonsdottir, H. & De Vries, H. 2007. Aggression and dominance in matched groups of subadult Icelandic horses (*Equus caballus*). *Journal of Ethology* 25: 239-248.

Visalberghi, E., Myowa Yamakoshi, M., Hirata, S. & Matsuzawa, T. 2002. Response to novel food in captive chimpanzees. *Zoo Biology* 21: 539-548.

Vitale, A. F., Visalberghi, E. & Delillo, C. 1991. Responses to a snake model in captive crab-eating macaques (*Macaca fascicularis*) and captive tufted capuchins (*Cebus paella*). *International Journal of Primatology* 12(3): 277-286.

de Vries, H. 1995. An improved test of linearity in dominance hierarchies containing unknown or tied relationships. *Animal behaviour* 50: 1375-1389.

de Vries, H. 1998. Finding a dominance order most consistent with a linear hierarchy: a new procedure and review. *Animal behaviour* 55: 827-843.

Vylitova, M., Miksik, I. & Pacha, J. 1998. Metabolism of corticosterone in mammalian and avian intestine. *General and Comparative Endocrinology* 109: 315-324.

Waeber, P. O. & Hemelrijk, C. K. 2003. Female dominance and social structure in Alaotran gentle lemurs. *Behaviour* 140: 1235-1246.

Wallner, B., Möstl, E., Dittami, J. & Prossinger, H. 1999. Fecal glucocorticoids document stress in female Barbary macaques (*Macaca sylvanus*). *General and Comparative Endocrinology* 113: 80-86.

Ward, M. P., Lyndal-Murphy, M. & Baldock, F. C. 1997. Evaluation of a composite method for counting helminth eggs in cattle faeces. *Veterinary Parasitology* 73: 181-187.

Washburn, B. E. & Millspaugh, J. J. 2002. Effects of simulated environmental conditions on glucocorticoid metabolite measurements in white-tailed deer feces. *General and Comparative Endocrinology* 127: 217-222.

Wasser, S. K. 1996. Reproductive control in wild baboons measured by fecal steroids. *Biology of reproduction* 55: 393-399.

Wasser, S. K., Hunt, K. E., Brown, J. L., Cooper, K., Crockett, C. M., Bechert, U., Millspaugh, J. J., Larson, S. & Monfort, S. L. 2000. A generalized fecal glucocorticoid assay for use in a diverse array of non-domestic mammalian and avian species. *General and Comparative Endocrinology* 120: 260-275.

Weingrill, T., Gray, D. A., Barrett, L. & Henzi, S. P. 2004. Faecal cortisol levels in free-ranging female chacma baboons: relationship to dominance, reproductive state and environmental factors. *Hormone and Behaviour* 45: 259-269.

Wells, D. E., Hepper, P. G., Coleman, D. & Challis, M. G. 2007. A note on the effect of olfactory stimulation on the behaviour and welfare of zoo-housed gorillas. *Applied Animal Behaviour Science* 106 (1-3): 155-160.

Wemelsfelder, F. 1994. Animal boredom: consequences for housing and feeding conditions. *Science and Animal Care* 5: 1-4.

Wiepkema, P. R. & Koolhaas, J. M. 1993. Stress and animal welfare. *Animal welfare* 2: 195-218.

Wikelski, M. & Cooke, S. J. 2006. Conservation physiology. *Trends in Ecology & Evolution* 21: 38-46.

Wingfield, J. C., Hegner, R. E., Dufty, A. M. & Ball, A. M. 1990. The ‘challenge hypothesis’: theoretical implications for patterns of testosterone secretion, mating systems and breeding strategies. *The American Naturalist* 136: 829-846.

Wingfield, J. C., Maney, D. L., Breuner, C. W., Jacobs, J. D., Lynn, S., Ramenofsky, M. & Richardson, R. D. 1998. Ecological bases of hormone-behavior interactions: The "emergency life history stage". *American Zoologist* 38: 191-206.

Wittemyer, G. & Getz, W. M. 2007. Hierarchical dominance structure and social organization in African elephants, (*Loxodonta africana*). *Animal behaviour* 73: 671-681.

Wong, M. S. & Bundy, D. A. P. 1990. Quantitative assessment of contamination of soil by the eggs of *Ascaris lumbricoides* and *Trichuris trichiura*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 84: 567-570.

Yoseph, S. I., Smith, D. G., Mengistu, A., Teklu, F., Fire, T. & Betere, Y. 2005. Seasonal variation in the parasite burden and body condition of working donkeys in east Shewa and west Shewa regions of Ethiopia. *Tropical Animal Health and production* 37(1): 35-54.

Young, K. M., Walker, S. L., Lanthier, C., Waddell, W. T., Monfort, S. L. & Brown, J. L. 2004. Non-invasive monitoring of adrenocortical activity in carnivores by fecal glucocorticoid analyses. *General and Comparative Endocrinology* 137: 148-165.

Zhang, P., Watanabe, K., Li, B. & Qi, X. 2008. Dominance relationships among one-male units in a provisioned free-ranging band of the Sichuan snub-nose monkeys (*Rhinopithecus roxellana*) in the Qinling Mountains, China. *American Journal of Primatology* 70: 634-641.

Zhou, Q., Wei, F., Huang, C., Li, M., Ren, B. & Luo, B. 2007. Seasonal variation in the activity patterns and time budgets of *Trachypithecus francoisi* in the Nonggang Nature Reserve, China. *International Journal of Primatology* 28: 657-671.

Zucker, E. L. & Clarke, M. R., 1987. Patterns of male-female spatial relationships in a mutli-male group of howling monkeys (*Alouatta palliate*) in Costa Rica. *American Journal of Primatology* 12: 379-380 (Abstract).

Zucker, E.L. & Clarke, M.R., 1998. Agonistic and affiliative relationships of adult female howlers (*Alouatta palliate*) in Costa Rica over a 4 year period. *International Journal of Primatology* 19(3): 433-449.