ZENTRUM
FÜR BIODIVERSITÄT UND NACHHALTIGE LANDNUTZUNG
SEKTION
BIODIVERSITÄT, ÖKOLOGIE UND NATURSCHUTZ

– CENTRE OF BIODIVERSITY AND SUSTAINABLE LAND USE –
SECTION: BIODIVERSITY, ECOLOGY AND NATURE CONSERVATION

Effects of forest fragmentation on brown spider monkeys (*Ateles hybridus*) and red howler monkeys (*Alouatta seniculus*)

Dissertation
zur Erlangung des Doktorgrades der
Mathematisch-Naturwissenschaftlichen Fakultäten der
Georg-August-Universität Göttingen

vorgelegt von

Diplom-Biologin

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aus
Eschwege

Göttingen, August 2013

Referentin/Referent: Prof. Dr. Eckhard W. Heymann

Korreferentin/Korreferent: Prof. Dr. Peter M. Kappeler

Tag der mündlichen Prüfung: 04.09.2013

Brown spider monkey
(Ateles hybridus)

Red howler monkey
(Alouatta seniculus)

"*Biodiversity is the totality of all inherited variation in the life forms of Earth, of which we are one species. We study and save it to our great benefit. We ignore and degrade it to our great peril*."

Edward O. Wilson (on the homepage of his 'Biodiversity Foundation')

"*The one process now going on that will take millions of years to correct is loss of genetic and species diversity by the destruction of natural habitats. This is the folly our descendants are least likely to forgive us*."

Edward O. Wilson, *Biophilia* (1984), 121.

CONTENTS

GENERAL INTRODUCTION

The increasing intensity of anthropogenic land use has an immense impact on ecosystems worldwide, and especially in the tropics where at least 50%, or possibly even more than 90%, of all animal and plant species occur. Habitat loss and destruction pose a major threat to numerous animal species and are the main cause for species extinctions (Pimm et al. 1995; Dirzo & Raven 2003). In the process of habitat conversion and destruction habitat fragments are formed frequently. Fragments are patches of original vegetation that are imbedded within a matrix of modified habitat, such as pastures or agricultural fields (Andrén 1994). Species vary greatly in their resilience to anthropogenic disturbances and their ability to survive in fragments (Fritz et al. 2009; Thornton et al. 2011). Anthropogenic disturbances encompass on one hand habitat loss, degradation and fragmentation and on the other hand the utilization of forests (e.g. for logging activities) and their inhabitants (e.g. hunting) by human populations. These human-induced alterations can result in local extinctions, influence the behavior of individuals, affect population genetics and act as physiological stressors on animal populations. For many taxa we still lack a comprehensive understanding of which factors render them vulnerable to extinction and how they adapt to anthropogenic disturbances.

Here, I will first lay out why habitat loss and fragmentation pose serious threats to animals and discuss which species-specific traits are related to extinction risks. Then, I will address the effects of two specific types of anthropogenic disturbances - logging and hunting - that frequently accompany fragmentation. Following an overview of the general consequences of human disturbances on animal populations, I will introduce the current knowledge on the effects of human activities on the physiology and health of animals in disturbed environments. Subsequently, I will highlight why spider monkeys and howler monkeys are ideal study species to investigate the effects of fragmentation on animal populations and finally, I will describe the different approaches I used in this thesis. Overall, the aims of this thesis are to shed light on how the physiological stress levels of two Neotropical primate species are affected by habitat fragmentation, logging and hunting and to explain potential inter-specific differences in their physiological stress levels. Moreover, I aim to investigate how a species that exhibits fission-fusion dynamics adjusts its grouping patterns and its social behavior to the fruit availability in a small forest fragment, and how these factors influence its stress levels.

Habitat loss and fragmentation

To date approximately 40% of the global land surface has been converted for agricultural use and more than 50% of the terrestrial biosphere has undergone anthropogenic changes (Foley et al. 2005; Ellis et al. 2010). Due to this land conversion, vast areas of native vegetation have been destroyed and transformed into other vegetation types. Tropical forest is the biome that is lost most rapidly in this process (Myers 1994; Asner et al. 2005; Lepers et al. 2005; Wright 2005; Hansen et al. 2008). The disruption of once large areas of forest frequently leads to the formation of small fragments (Saunders et al. 1987). These fragments take on characteristics similar to those of habitat islands, depending on the degree of and the time since isolation from other forest and the type of matrix they are imbedded in. With elapsing time the number of fragments typically increases while the size of individual fragments decreases. Although tropical forests only cover a small proportion of the world´s land surface (6-7%), they contain at least 50% of all animal species worldwide (Wilson 1988; Groombridge & Jenkins 2002). Due to this concentration of biodiversity in tropical forests, processes like habitat loss, habitat destruction, and fragmentation pose major threats to the survival of many species (Wilcove et al. 1998; reviewed by: Turner 1996; Sih et al. 2000; Fahrig 2003, Ewers & Didham 2006). Currently, a large number of species from all major vertebrate taxa is already threatened with extinction (e.g. 25% of mammals, 13% of birds, 41% of amphibians; IUCN, 2012). Of all species listed by the IUCN as endangered 87% of birds, 88% of amphibians and 86% of mammals are threatened with extinction due to precisely these anthropogenic disturbances (Baillie et al. 2004). This is also reflected in current rates of species extinction, which are much higher than would be expected on the basis of geological records (Dirzo & Raven 2003; Butchart et al. 2010).

Due to the high rate of global forest cover loss and the resulting forest fragmentation, it is evident that fragments are valuable, and will become increasingly more crucial, for the long-term survival and conservation of numerous species. For some taxa fragments can serve as refuges, while others fail to survive in these. Knowledge on which factors render some species vulnerable to extinction and enable others to survive or even thrive in fragments is needed to minimize and prevent further extinctions of populations and species.

Which factors render species vulnerable to extinction in fragments?

The species-area relationship, used originally within the theory of island biogeography to explain patterns of species richness on oceanic islands (MacArthur & Wilson 1967), has also been applied to predict the loss of species numbers in habitat islands or fragments. As predicted by the species-area relationship, species richness generally declines with declining fragment size (Harcourt & Doherty 2005; Ferraz et al. 2007; Stouffer et al. 2009; Rybicki & Hanski 2013). Some species go locally extinct when confined to fragments (Miller & Harris 1977; Harris 1984; Bolger et al. 1997; Ferraz et al. 2003; Banks-Leite et al. 2010). The rate of extinction can accelerate in areas that experience very high levels of forest loss and result in a sudden, landscape-wide loss of forest-specialist taxa (Pardini et al. 2010; Swift & Hannon 2010). However, species generally differ greatly in their resilience to habitat fragmentation (Purvis et al. 2000; Fritz et al. 2009; Thornton et al. 2011). In general, species characterized by large area requirements, low population densities, slow population growth, small geographic distribution, and moderate dispersal abilities have been found to be at a high extinction risk in fragments (Cosson et al. 1999; Purvis et al. 2000; Owens & Bennett 2000; Henle et al. 2004; Cardillo et al. 2005; Neuschulz et al. 2013).

However, species diversity and animal populations are typically not immediately diminished after fragmentation. Instead, high population densities can often be found in recently formed fragments, which is termed 'crowding effect' (Lovejoy et al. 1986; Schmiegelow et al. 1997; Anderson et al. 2007; Irwin 2008; Boyle & Smith 2010; Link et al. 2010). Often animal populations go extinct only after some time has elapsed, this is the so-called 'time lag to extinction' or the 'extinction debt' (e.g. Tilman et al. 1994; Chalfoun et al. 2002; reviewed by Kuussaari et al. 2009). The duration of this 'extinction debt' can depend on inter-specific dynamics (e.g. predator-prey interactions, invasive species), species-specific life-history characteristics and a species´ ecological niche. Generally, the dietary specialization of a species can affect its susceptibility to extinction (Purvis et al. 2000; Hobbs 2001; Kamilar & Paciulli 2008). The degree of folivory of a species often influences its ability to survive after disturbances. In general, species with a more folivorous diet do better than more frugivorous species (Johns & Skorupa 1987; Jones et al. 2001; Kamilar & Paciulli 2008). One key factor causing these differences is very likely altered food resource availability in fragments. Although low-level disturbances (e.g. selective logging) can increase fruit production and leave quality (increased protein content (Ganzhorn 1995)), fruit availability is frequently reduced after medium- to high-level disturbances like habitat fragmentation (Johns 1988; Tutin 1999; Arroyo-Rodríguez & Mandujano 2006; Dunn et al. 2010). Conversely, young leaves are often highly abundant in fragments, especially at edge areas where light exposure is elevated and early successional vegetation can thrive (Johns 1988, 1991; Lynch & González 1993; Ganzhorn 1995, 1997; Irwin 2008). Therefore, species that can exploit this food resource will be at an advantage compared to species that cannot.

Altered resource availability within fragments is due to changes in plant species diversity and forest structure. These changes are caused, to some extent, by 'edge effects'. Edge effects describe the phenomenon of high wind exposure, elevated solar radiation, altered water and nutrient fluxes, and reduced humidity levels at fragment edges (Laurance 1991; Malcolm 1994; Laurance & Williamson 2001; for review see Murcia 1995). These effects can penetrate deep into fragments and can result in increased tree mortality (Lovejoy et al. 1986; Ferreira & Laurance

1997; Laurance et al. 1997, 1998, 2000; Mesquita et al. 1999). In addition, edge effects can reduce or prevent seed germination and regeneration of certain tree species (Janzen 1986; Chapman & Chapman 1997; Benitez-Malvido 1998; Cordeiro & Howe 2001). Consequently, tree species composition and diversity often differ between the forest interior and fragment edges (Laurance 1991; Jacinto Tabanez & Viana 2000; Hill & Curran 2001; Tabarelli et al. 2004, 2010).

Logging and hunting as a consequence of forest fragmentation

Two major consequences of fragmentation are that remaining forest patches 1) are easily accessible for logging activities and 2) facilitate the hunting of animals (Janzen 1986; Cullen Jr. et al. 2000; Peres 2001) that are ‘trapped’ in certain fragments because their movement or dispersal between fragments is restricted or entirely prevented by fragmentation (Proctor et al. 2005; Barlow et al. 2006; van Oort et al. 2011). Large-bodied mammals are the preferred prey of hunters (Souza-Mazurek et al. 2000; Cullen Jr. et al. 2001; Jerozolimski & Peres 2003; Isaac & Cowlishaw 2004). Hunting can aggravate the negative effects of fragmentation and habitat loss, it can prevent forest regeneration and negatively affect prey populations (Peres 1996; Cullen Jr. et al. 2000; Urquiza-Haas et al. 2011; Abernethy et al. 2013; Benchimol & Peres 2013; Effiom et al. 2013; Poulsen et al. 2013).

Moreover, intense logging activities can further facilitate access to fragments and reduce the fruit availability and quality for forest living species (Rode et al. 2006; Hardus et al. 2012). Because food resource availability is an important limiting factor for animal populations (White 1978; Boutin 1990), these changes can result in declining population densities (Felton et al. 2003). Population declines might be mediated through increased mortality rates and reduced reproductive output in logged areas (Thompson 1994). Some animal species seem not to be able to recover, even decades after logging activities have ceased (Chapman et al. 2000, 2005). Significant decreases in population densities of large-bodied - potentially seed dispersing - mammals can negatively affect the spatial structure of tree populations and lead to a consistent decline in local tree diversity (Gutiérrez-Granados & Dirzo 2009; Harrison et al. 2013; Poulsen et al. 2013). This, in turn, can further reduce food availability and negatively affect animal populations. However, smaller-bodied seed dispersers, that probably experience less severe hunting pressure, might be able to buffer some of these negative effects (Oliveira & Ferrari 2000; Culot et al. 2010).

Consequences of anthropogenic disturbances on animal populations

In response to fragmentation some species modify their population structure and group sizes (Wauters et al. 1994; Sumner et al. 1999; Chiarello & de Melo 2001; Clarke et al. 2002; Jepsen &

Topping 2004; Struhsaker et al. 2004), while others respond by adjusting their activity patterns, dietary strategies, and/or ranging and social behavior (Menon & Poirier 1996; Wong et al. 2006; Riley 2007; Irwin 2008; Boyle et al. 2009; Hardus et al. 2012; Linke et al. 2013; for a review see Banks et al. 2007). Altered resource availability in disturbed areas can change species abundances (Hargis et al. 1999; Mammides et al. 2009; Kankam & Sicotte 2013) and some species face local extinction (Cosson et al. 1999; Peres 2001; Cox et al. 2003). Due to space limitations and high population densities, average home-range size often decreases in small fragments (Koprowski 2005; Chapman et al. 2007a; Wong & Sicotte 2007; Irwin 2008; Arroyo-Rodriguez & Dias 2010). This can potentially intensify intra- and inter-specific competition for space and resources in a fragment, in particular, when resource availability is reduced. High levels of competition can increase the local extinction risk of animal populations and decrease individual survival and reproductive success (Gustafsson 1987; Bengtsson 1989; Eccard & Ylönen 2002; Gurnell et al. 2004).

Moreover, habitat fragmentation can limit the dispersal of individuals between fragments and thus, can restrict gene flow between groups or populations that are 'trapped' in different fragments. Subsequently, inbreeding within fragments can increase (Dietz et al. 2000; Keller & Largiadèr 2003; Stow & Sunnucks 2004; Fortes & Bicca-Marques 2008; Dixo et al. 2009). Genetic diversity can be lost in small and isolated populations through stochastic process such as genetic drift (Grativol et al. 2001; Dixon et al. 2007; Heller et al. 2010). Inbreeding and the loss of genetic diversity can negatively affect the long-term persistence of populations (Mills & Smouse 1994; Saccheri et al. 1998; Coltman et al. 1999; Crnokrak & Roff 1999; Reed & Frankham 2003; Vilas et al. 2006).

Physiology and health in disturbed environments

Not only the behavior and the genetic makeup of animals, but also their physiology and health can be influenced by anthropogenic disturbances (reviewed by: Busch & Hayward 2009; Cooke et al. 2013). Recent methodological advances have made it feasible to measure physiological parameters (e.g. heart rate, energy expenditure, immune system parameters, stress hormone levels) of wild animals and thus, to investigate the effects that human activities have on these parameters (e.g. Regel & Pütz 1997; Wasser et al. 1997; Ellenberg et al. 2006; Mbora & McPeek 2009). In this context, glucocorticoids (or 'stress hormones') have proven to be a useful biomarker to assess the physiological consequences of anthropogenic disturbances and habitat fragmentation.

The physiological stress response

The vertebrate stress response comprises a suit of behavioral and physiological changes that animals display after they experience a stressor. Physiological changes include an increase in glucocorticoids (GCs). This response is relatively nonspecific in a way that many different stressors (e.g. exposure to predators, aggression from a conspecific, food-deprivation, and severe storms) can elicit such a GC increase (Selye 1950). GCs are steroid hormones, and depending on the taxa either mainly cortisol or corticosterone. The magnitude of the GC increase depends on how a stressor is perceived by the organism (Hennessy et al. 1979). The perception of a stressor triggers the release of neurotransmitters (e.g. corticotropin-releasing hormone) and GCs by the hypothalamic-pituitary-adrenal (HPA) axis, which also regulates many other processes in the body, e.g. immune system functions, energy storage and expenditure (for a detailed review see Chrousos 1998). This rise in GCs helps to secure survival in emergency situations through the mobilization of readily available energy via gluconeogenesis (Selye 1956; Hennessy et al. 1979; Breazile 1987; Stratakis & Chrousos 1995), Therefore, short-term (or 'acute') elevations of GCs represent an adaptive response to energetically demanding conditions (Boonstra 2005; Monclús et al. 2005; Schulkin et al. 2005). During the stress response energetically demanding activities that do not increase the immediate survival, e.g. digestion, growth, reproduction and immune function, are suppressed (Landys et al. 2006). Consequently, long-term elevations of GC levels can chronically suppress these activities and thus, impair growth, reproduction and immune system activity, and ultimately survival and fitness (Pickering et al. 1991; Muehlenbein 2006; Charbonnel et al. 2008; Martin 2009; French et al. 2010; Satterthwaite et al. 2010; Setchell et al. 2010). This relationship between GC levels and fitness has been termed the 'Cort-Fitness Hypothesis', where elevated baseline GC (cortisol or corticosterone) levels are presumed to indicate that individuals are in worse condition and experience reduced fitness compared to individuals with lower GC levels (Bonier et al. 2009b). On account of this, GC measurements have been used as a biomarker to investigate the potential consequences of human-induced disturbances.

Effects of anthropogenic disturbances on glucocorticoids and health parameters

Anthropogenic disturbances have been associated with changes in both GC levels and health parameters. Animals´ susceptibility to parasites and diseases can increase in disturbed areas (Allan et al. 2003; Chapman et al. 2006; Schwitzer et al. 2010; Young et al. 2013; Sá et al. 2013). Many vertebrate taxa also show elevated GC levels: amphibians (Homan et al. 2003; Janin et al. 2011), birds (Wasser et al. 1997; Lucas et al. 2006) and mammals (Barja et al. 2007; Martínez-Mota et al. 2007; Gobush et al. 2008; Rangel-Negrín et al. 2009; Jaimez et al. 2012). '*Stress*', in response to anthropogenic disturbances, has also be documented using additional physiological (e.g. increased heart rate) and immune system (e.g. decreased leucocyte counts) parameters in vertebrates (Ellenberg et al. 2006; Behie et al. 2010; Irwin et al. 2010; Johnstone et al. 2012). Such changes in the physiology and health status can reduce the persistence and long-term viability of animals that are exposed to logging, hunting and/or that live in fragments. However, species differ in their sensitivity to disturbances and not all species respond with the predicated changes of physiological parameters (von der Ohe et al. 2004; Wasser et al. 2004; Munshi-South et al. 2008; Tecot 2008). The pervasive process of anthropogenic disturbances on natural ecosystems (reviewed in Foley et al. 2005) emphasizes the need to understand how these disturbances affect animals´ physiology, health and ultimately population survival, and how different taxa cope with anthropogenic activities.

Ateline primates as study species

Over 90% of all primate species occur in the tropics (Mittermeier et al. 2013), where habitat destruction and fragmentation are omnipresent (Achard et al. 2002). Habitat loss, fragmentation, and hunting pose significant threats to the survival of numerous primate species (Cowlishaw & Dunbar 2000; Chapman & Peres 2001). Neotropical primates are a particularly interesting taxa to examine the effects of fragmentation because 1) all species depend on forests, 2) they often comprise a high proportion of the vertebrate biomass in fragments (Cullen Jr. et al. 2001), 3) many species act as key seed dispersers and consequently influence forest structure and regeneration (Oliveira & Ferrari 2000; Link & Di Fiore 2006), 4) a large body of information is available for many species from undisturbed areas and 5) because many species frequently occur sympatrically (Mittermeier et al. 2013), cross-species comparisons in the same ecosystem (or even the same fragments with identical biotic and abiotic conditions) are feasible.

In this thesis, I focus on brown spider monkeys (*Ateles hybridus*) and red howler monkeys (*Alouatta seniculus*), two Neotropical primate species of the family Atelidae that co-occur in Colombia. The two genera *Ateles* and *Alouatta* appear to contrast strongly in their ability to cope with anthropogenic disturbances (Estrada & Coates-Estrada 1996; Michalski & Peres 2005), and the proximate causes for these differences are not yet well-studied. This makes them suitable study species to examine the proximate effects that anthropogenic disturbances have on animals.

Howler monkeys, and in particular red howler monkeys, can persist even in extremely small fragments (Lovejoy et al. 1986; Schwarzkopf & Rylands 1989; Gilbert 2003; Lopez et al. 2005; Michalski & Peres 2005; French et al. 2010; for review see Crockett 1998; Arroyo-Rodriguez & Dias 2010). Red howler monkeys have a broad distribution throughout large parts of South America and are able to occupy a wide array of different ecosystems, such as primary lowland rainforest, dry deciduous forest, Andean cloud forest, gallery forest and mangrove swamps (Boubli et al. 2008). They are currently not threatened with extinction (Boubli et al. 2008). Generally, they withstand hunting pressure better than other atelids, although their population density decreases when exposed to strong hunting pressure (Peres 1997). Typically, they require much smaller areas than spider monkeys (reviewed by Di Fiore et al. 2010), they form cohesive groups, and have a mainly folivorous, but flexible diet (Milton 1980; Julliot & Sabatier 1993; for review see Bicca-Marques 2003).

Conversely, spider monkeys are highly frugivorous and are considered ripe-fruit specialists (Symington 1988; van Roosmalen & Klein 1988; reviewed by Di Fiore et al. 2008). They adopt very flexible grouping patterns (fission-fusion dynamics) to cope with spatial and temporal changes in fruit availability and to reduce intra-group feeding competition (Klein and Klein, 1977; Symington, 1988). Their large body mass (6-10kg; reviewed by Di Fiore & Campbell 2007) makes them a preferred prey species of hunters (Peres 1990; Souza-Mazurek et al. 2000). High hunting pressure, together with slow reproductive cycles, large home-range requirements and a highly frugivorous diet (Milton 1981a; Fedigan & Rose 1995; for review see Di Fiore et al. 2010) make spider monkeys highly vulnerable to anthropogenic disturbances. This is reflected in the 'IUCN Red List of Threatened Species' where all species in the genus *Ateles* are listed as vulnerable, endangered or even critically endangered. The study species *A. hybridus* is critically endangered (Urbani et al. 2008) and considered to be one of the 25 most endangered primate species worldwide (Mittermeier et al. 2012). These differences make the two study species ideal candidates to examine the proximate effects of anthropogenic disturbances on animals, and utilizing a comparative approach is a promising method to gain further insights into the ability of different species to cope with human-induced changes.

Aims and approaches

This thesis aims to contribute to a better understanding of the proximate effects that anthropogenic disturbances have on animals and the coping mechanisms animals employ to adjust to fragmentation and to survive in fragments. For that purpose, I used a comparative and interdisciplinary approach, and combined data from behavioral observation and data on glucocorticoid levels - as an indicator for physiological stress - obtained from fecal samples. More precisely, the overall goal was approached at two different scales: a regional scale that includes data from several different forest fragments (**Chapter 2**) and a local scale, with data collected on identified individuals ranging in one small fragment (**Chapter 1, 3, 4**). The specific objectives are described in detail below:

In **Chapter 1**, I present the validation of an enzyme-immunoassay (EIA). This was a crucial pre-requisite to reliably determine GC levels in the endocrinologically unstudied species *A. hybridus* and *A. seniculus* because the metabolism and excretion route of GCs can differ substantially between species, even closely related ones. Thus, to assure that analyses will result in accurate and biologically meaningful data, a thorough validation of the endocrinological methods is essential and has to be conducted for every new study species. To validate the EIA, I used the well-documented stress response to anesthesia in both wild and captive animals and conducted reverse-phase high pressure liquid chromatography analysis (HPLC). Many different variables are known to potentially influence GC levels in vertebrates. Hence, to assure adequate interpretation of the results and to gain knowledge on which variables to include in subsequent analyses I investigated whether GC levels of both species were affected by several potentially confounding factors (e.g. age, sex, reproductive status, diurnal rhythm of GC output).

To examine the proximate mechanisms leading to the species-specific distinction in the ability to cope with anthropogenic disturbances, I investigated differences between both study species in their physiological responsiveness to human-induced disturbances (**Chapter 2**). In detail, I analyzed fecal samples of *A. hybridus* and *A. seniculus* collected in various forest fragments that differed in size as well as in the level of human impact (absence or presence of hunting and/or logging). I examined whether the GC output of both study species was influenced by these factors. Moreover, I assessed whether both species differ in their responsiveness to these factors according to the general notion that howler monkeys are more resilient and tolerant of human-induced disturbances than spider monkeys.

The potentially deleterious effects of chronically elevated GC levels and the typically reduced fruit availability in many forest fragments make it crucial to study the link between both factors in animal living in fragments. Currently, we still lack detailed information on how species that exhibit fission-fusion dynamics adjust to forest fragmentation. To shed light on how a species with such flexible grouping patterns adjusts to fragmentation, I studied two well-habituated groups of *A. hybridus* that range in one small forest fragment (65 ha). I examined how they adjust their grouping patterns and social behavior to changes in fruit availability, and assessed how their stress levels were influenced by fruit availability and aggression (**Chapter 3**).

In **Chapter 4**, I report cases of potential evidence of intense inter-specific competition for space and resources in a small fragment with a very high primate population density. I describe two incidents of inter-specific infanticide and several cases of infant-directed aggression from spider monkeys (*A. hybridus*) towards infant howler monkeys (*A. seniculus*) and one infant capuchin (*Cebus albifrons*).

CHAPTER 1

Validation of an enzyme immunoassay for assessing adrenocortical activity and evaluation of factors that affect levels of fecal glucocorticoid metabolites in two New World primates

with Eckhard W. Heymann, Andrés Link and Michael Heistermann

Abstract

Non-invasive methods to assess stress hormone output via fecal glucocorticoid metabolites (FGCMs) have become a powerful tool in behavioral studies and conservation biology because they allow exploring the link between behaviour, an animal´s socio-ecological environment and its adrenocortical activity. However, FGCM levels are influenced by numerous other factors which often confound their interpretation. Thus, before applying these methods, knowledge on the impact of these factors is important. In this study we investigated the effect of (1) time of day, (2) age, (3) sex and (4) female reproductive state on FGCM levels in brown spider monkeys (*Ateles hybridus*) and red howler monkeys (*Alouatta seniculus*). Initially, we validated a 11ß-hydroxyetiocholanolone enzyme immunoassay for monitoring the physiological stress response via fecal analysis in both species. We determined FGCM levels in fecal samples collected from two and six groups of wild spider monkeys (n=461 samples) and howler monkeys (n=166 samples), respectively. Our analyses revealed a strong effect of time of day on FGCM levels in spider monkeys, but no effect in howler monkeys. Adults of both species had significantly higher FGCM levels than subadults. In neither of the two species we found a sex-effect on FGCM output. Reproductive condition strongly affected FGCM levels in female spider monkeys which showed increasing concentrations with progressing gestation. This was not investigated in female howler monkeys due to an insufficient sample size. Our data indicate that the influence of the tested factors on fecal glucocorticoid metabolite output is species-specific, and that these variables need to be considered when interpreting FGCM levels in the species.

General and Comparative Endocrinology, 191: 13-23 (2013)

Introduction

In recent years, there has been a substantial increase in the number of studies that investigate the interactions between animal behavior and steroid hormone levels in vertebrates of all major taxa (e.g., (Cavigelli 1999; Kenagy & Place 2000; Ganswindt et al. 2003; Goymann et al. 2003; Reeder et al. 2004; Engh et al. 2006; Bonier et al. 2009b)). These studies help to gain insight into the proximate factors underlying and modulating behavioral variation, life history traits, fitness, and survival of animals. Measurement of hormones is also employed in conservation research to assess and monitor the physiology, health and well-being of populations of endangered species in the wild (Franceschini et al. 1997; Wingfield et al. 1997; Hodges & Heistermann 2003; Chapman et al. 2006; Wikelski & Cooke 2006; Tarlow & Blumstein 2007; Cyr & Romero 2008; Van Meter et al. 2009) as well as to facilitate and ensure the propagation and welfare of animals in captivity (e.g., (Graham et al. 2002; Heistermann et al. 2004; Dehnhard et al. 2008; Pirovino et al. 2011).

In the latter contexts, glucocorticoids (cortisol and corticosterone) have received most attention. As front hormones of the vertebrate stress response that reflect physiological stress loads of individuals and populations, they have proven as an important biomarker when assessing the physiological consequences of anthropogenic disturbances and habitat fragmentation for individual and population health (Franceschini et al. 1997; Wingfield et al. 1997; Wasser et al. 1997; Chapman et al. 2006, 2007b; Wikelski & Cooke 2006; Martínez-Mota et al. 2007; Rangel-Negrín et al. 2009; Thiel et al. 2011). Generally, glucocorticoids and their metabolites can reliably be measured in blood, urine and feces using enzyme immunoassays (EIAs) (for detailed reviews see (Romano et al. 2010; Sheriff et al. 2011)). When studying stress physiology in wildlife, however, non-invasive methodologies based on the measurement of GC metabolites (GCM) in excreta (urine, feces) is the preferred approach because blood sampling is usually not feasible (and undesirable) in wild animals. Moreover, excreted GCM levels in urine and feces provide a more integrated measure of adrenocortical activity than point serum samples and thus diminish the influence of the pulsatile and episodic patterns of GC secretion (Whitten et al. 1998a). Since excreta can also be collected much more regularly than blood, analyses of urinary and fecal hormone metabolites provide the most suitable way to obtain longitudinal information on endocrine activity.

The metabolism and excretion route of glucocorticoids can differ substantially between species, even closely related ones (Bahr et al. 2000; Palme et al. 2005). Thus, prior to applying urinary or fecal analysis, it is crucial to biologically validate the respective method for each new species to assure that the data to be generated will be biologically meaningful (Whitten et al. 1998b; Palme et al. 2005; Touma & Palme 2005; Heistermann et al. 2006; Schwarzenberger

2007; Goymann 2012). In this respect, the validation should not only demonstrate that the GCM measurement reliably detects adrenocortical endocrine activity in response to a stressor, but should also evaluate the specificity of the measurement when immunological detection methods are used (Heistermann et al. 2006; Goymann 2012). The latter is particularly important given that metabolites of glucocorticoids and other steroids (e.g., testosterone) can be structurally very similar (Ganswindt et al. 2003). Since antibodies used for the quantification of glucocorticoids can potentially cross-react with those metabolites of different origin and function (Ganswindt et al. 2003; Palme et al. 2005; Heistermann et al. 2006), such cross-reactions can have major and distorting effects on the results obtained (see (Ganswindt et al. 2003; Goymann 2012)). Further, glucocorticoid metabolism can differ even between sexes within a given species (e.g., (Touma et al. 2003; Baltic et al. 2005)), making comparisons of GC levels between males and females potentially problematic and meaningless unless the immunological specificity of the assay used is demonstrated (for a detailed review see (Goymann 2012)).

In many species basal stress hormone levels are affected by a variety of intrinsic factors (for review see (Millspaugh & Washburn 2004; Keay et al. 2006; Goymann 2012)) such as age (Sapolsky 1992; Seraphin et al. 2008), sex (Sapolsky 1992; Ferreira Raminelli et al. 2001; Touma et al. 2003), reproductive state (Ziegler et al. 1955; Cavigelli 1999; Weingrill et al. 2004; Setchell et al. 2008; Carnegie et al. 2011) and body condition (Charbonnel et al. 2008), and they also often show diurnal variation (Ferreira Raminelli et al. 2001; Chapman et al. 2006; Bosson et al. 2009). All these factors may confound interpretation of GC levels generated in contexts such as behavioral studies or conservation research. Knowledge about whether and in which specific way these variables have an impact on stress hormone output in a given species is therefore of high importance when GC data is collected for such research questions.

The way and extent to which such factors influence adrenocortical activity appears to be species-specific, emphasizing the importance to assess their impact in every previously unstudied species. For example in Columbian ground squirrels (Bosson et al. 2009), common marmosets (Ferreira Raminelli et al. 2001) and red colobus monkeys (Chapman et al. 2006) a diurnal rhythm of glucocorticoid secretion is reflected in fecal glucocorticoid metabolite (FGCM) levels, while such variation is absent in other species (e.g. white rhinoceros (Turner et al. 2002), baboons (Beehner & Whitten 2004), western lowland gorillas (Shutt et al. 2012)). Correspondingly, adult male chimpanzees (Seraphin et al. 2008) and Assamese macaques (Ostner et al. 2008) (but only during the breeding season) have higher GC levels than subadult males and in rats glucocorticoid levels increase with increasing age (Sapolsky 1992), whereas there is no age-effect in other species (e.g. spiny mice (Nováková et al. 2008)).

In addition to these biological sources of variation, GC levels from feces can also be affected by methodological issues, in particular the way how samples are collected and stored (Khan et al. 2002; Lynch et al. 2003; Shutt et al. 2012). This presents a serious challenge especially for researchers that work in remote areas where there is no access to freezers. One solution to this problem is the immediate extraction of steroids from feces using on-site extraction methodologies (Beehner & Whitten 2004; Shutt et al. 2012; Murray et al. 2013) in combination with validated methods to store extracts under tropical conditions (Santymire & Armstrong 2010; Shutt et al. 2012). However, to date it remains unclear how versatile such methods are, i.e. to what extent they can be applied across multiple species.

As part of a larger project that investigates the impact of anthropogenic disturbances and habitat fragmentation on the stress physiology of wild brown spider monkeys (*Ateles hybridus*) and red howler monkeys (*Alouatta seniculus*) in Colombia, we examine here the effect of time of day, age, sex and female reproductive condition on fecal glucocorticoid excretion, information that does not exist for either of the two species. Brown spider monkeys are endemic to Colombia and Venezuela (Defler 2003). Due to their restricted distribution, their long inter-birth intervals (32–50 months) (Di Fiore & Campbell 2007), severe habitat loss and high hunting pressure the species is critically endangered (Urbani et al. 2008) and belongs to the 25 most endangered primate species in the world (Mittermeier et al. 2012). As other spider monkey species, they are mainly frugivorous, although young leaves can make up to 50% of their diet (Galvis et al. 2012). In contrast, red howler monkeys have a much wider distribution (Brazil, Ecuador, Peru, Venezuela and Colombia) and are not threatened with extinction (Boubli et al. 2008). They are highly folivorous and can persist even in extremely small forest fragments (Lovejoy et al. 1986; Estrada & Coates-Estrada 1996; Gilbert 2003).

For the present study we initially validated an EIA for assessing adrenocortical activity non-invasively from fecal samples of the two species and tested for potential storage effects on FGCM levels in fecal extracts stored for six month at high temperatures. For validation we used 1) the physiological stress response to anesthesia (e.g., (Sapolsky 1982; Whitten et al. 1998b; Martínez-Mota et al. 2008)) in zoo-housed and wild animals to test the suitability of four different EIAs in reflecting the stress-related FGCM increase in feces and 2) evaluated the specificity of the most suitable EIA in both sexes of both species by characterizing the pattern of immunoreactive metabolites measured using HPLC analysis. Thus, with this study, we procure the methodological basis for studying adrenocortical activity non-invasively in *A. hybridus* and *A. seniculus* and provide important comparative baseline information on the influence of several intrinsic variables on FGCM levels in these two endocrinologically unstudied species of New World primates.

Material and methods

Study sites and animals

Field

We collected fecal samples of two wild groups of brown spider monkeys (SJ1, SJ2) and six groups of red howler monkeys (C0, C1, C2, C3, C7, I) in which all individuals were individually recognized and fully habituated (Table 1). All groups ranged in a forest fragment located within the private cattle ranch ‘‘Hacienda San Juan del Carare’’ (06° 43´ N, 74° 09´ W; 150–200 m a.s.l) in Colombia. At the study site both study species have been habituated and studied since 2007 (Link et al. 2010). The fragment comprises 65 ha of seasonally flooded tropical rainforest and is located in the Magdalena River Valley between the eastern and central cordilleras of the Colombian Andes. The area shows two marked rainy seasons, typically one from March to May, and another from October to November. During the rainy seasons the fragment regularly floods for a period of several weeks up to three months. The area receives an annual medium rainfall of 3496.5 mm, shows a medium temperature of 27.9°C and a medium humidity of 80% (IDEAM 2008).

Table 1: Group composition and number of fecal samples collected per study group.

Species	Group	Group composition[a]	No. samples				
			AM	AF	SAM	SAF	total
Ateles hybridus	SJ1	3-4AM, 5AF, 1-3SAM, 0-2JM, 3-4JF, IF, IM	154	211	40	-	405
	SJ2	AM, 5AF, 2SAM, 3JF, 2IM, IF	11	44	1	-	56
Alouatta seniculus	C0	2AM, 3AF, SAM, JF, 2IF, IM	25	21	1	-	47
	C1	AM, 2AF, SAF, JM, JF	6	5	-	-	11
	C2	2-3AM, 4AF, SAF, SAM, 2JF, JM	17	20	2	1	40
	C3	3AM, 4AF, SAM, SAF, JM	6	15	5	2	28
	C7	AM, 2AF, SAM, JM	6	10	1	-	17
	I	1-2AM, 2AF, SAF, IM	11	10	-	2	23

[a] Adult male (AM), adult female (AF), subadult male (SAM), subadult female (SAF), juvenile male (JM), juvenile female (JF), infant male (IM) and infant female (IF).

Zoo

As part of the validation of a fecal GC assay, we collected fecal samples from a zoo-housed group of brown spider monkeys in the Zoological and Botanical Garden Stuttgart, Germany in 2011 (see below).The group consisted of one adult male, four adult females, two juvenile males, one juvenile female and one infant male. The animals had access to an indoor and an outdoor enclosure and they were fed twice a day with fruits and vegetables. Water was available ad libitum.

Fecal sample collection

Field

For the validation tests (see below), we collected fecal samples from two wild adult spider monkey males and one wild male adult red howler monkey before and after they were captured and anesthetized to place radio-collars on them as part of a behavioral study. In order to assess the impact of time of day, age, sex and female reproductive condition on FGCM levels, we collected 461 fecal samples from brown spider monkeys and 166 fecal samples from red howler monkeys between August 2010 and April 2012. To differentiate between adults and subadults we used age-related traits such as body size, coloration and size of primary sexual characteristics (e.g. testes, vulvae) and information on the date of birth (when available). Since in both study species pregnancy cannot reliably be detected by visual inspection, we used the date of parturition in combination with average gestation length (*Ateles* ~7.5 months, *Alouatta* ~6.3 months) (Di Fiore & Campbell 2007) to identify the samples that were collected during gestation. We categorized females as lactating for the period of time in which they were observed nursing their dependent offspring. Females that did not fall within these two categories (pregnant or lactating) were categorized as cycling (despite their actual cycle status was unknown). We collected fecal samples from all adult and subadult individuals usually on a weekly basis; however, in three less studied groups (C1, C3, C7) samples were collected more sporadically (Table 1).

For sample collection, we homogenized the fecal bolus and removed any obvious undigested matter (e.g. large seeds). We collected only samples uncontaminated with urine. We placed approximately 0.5 g of fresh feces into a 15 ml polypropylene tube pre-filled with 5 ml of 96% ethanol and shook the tube manually until the feces were suspended in the solvent (Shutt et al. 2012). Samples were kept at ambient temperatures until they were extracted after returning to the camp in the evening (see below).

Zoo

We collected 70 fecal samples (mainly in the morning) from zoo-housed brown spider monkeys during a capture-translocation event which took place in the Zoological and Botanical Garden Stuttgart, and during which all animals were anesthetized. Samples were collected before the capture commenced to assess pretreatment baseline FGCM levels and five days thereafter to monitor the FGCM response to the stressor. In addition, samples were collected 55 days following the stressful event, when the animals had completely settled into their new enclosure, to serve as a second control. On Day 3 of the sampling period, the zoo's veterinarian inspected the animals by observing them for a couple of minutes. This caused a high level of distress to all individuals, indicated by high arousal, screaming and defecation of diarrhea samples. During the period of sample collection, the animals could move freely between an indoor and an outdoor enclosure. Because of this and in combination with the large sizes of both enclosures, we could not systematically assign to which individual a certain defecated sample belonged. Thus, we could only assess the stress-related changes in FGCM concentrations on the group level, rather than in individual animals. Samples were collected usually within two hours after defecation and all samples were stored at -20°C until transportation on ice-packs to the endocrinology laboratory of the German Primate Center for analysis. The samples were processed and stored until analysis as described below.

Red howler monkeys are only kept in three European zoos. Unfortunately, during the study period none of these individuals was captured and anesthetized for a medical check-up or transport and stimulating adrenocortical activity using an ACTH challenge was also not possible. Thus, we were unable to collect fecal samples during a stressful event from captive animals as we did for *A. hybridus*.

Steroid extraction

Field

Prior to extraction we weighted the tube containing the sample to determine fecal wet weight. We then manually shook the fecal suspension firmly for 5 min (Shutt et al. 2012) and, thereafter, centrifuged the samples for 1 min using a manually-operated centrifuge (from Hettich GmbH & Co. KG Tuttlingen, Germany) to recover the supernatant containing dissolved steroids. We poured off ~2 ml of each fecal extract into 2 ml polypropylene tubes (PPT; SafeSeal Micro Tube; Ref. No. 72.695.200 from Sarstedt AG & Co. Nuernbrecht, Germany), sealed them with parafilm, labeled them with animal ID, date and time of day and stored them at ambient temperatures (~25 °C) in a dark place. Every two months we transported the extracts to the University of Los Andes, Bogotá, where we stored them at -20°C until shipment to the endocrinology laboratory at the German Primate Center for steroid analysis.

Zoo

We processed and extracted the fecal samples collected from zoo-housed spider monkeys following Heistermann et al. (1995). In summary, we lyophilized and pulverized the feces and extracted an aliquot representing 0.05-0.07 g of fecal powder in 3 ml of 80% methanol by vortexing the suspension for 15 min. Subsequently, we centrifuged the suspension, recovered the supernatant and stored it at -20°C until analysis.

Validation of an EIA to measure FGCM

We used the well-documented stress response to anesthesia (e.g., (Sapolsky 1982; Whitten et al. 1998b; Martínez-Mota et al. 2008) to test the ability of four glucocorticoid EIAs, described in detail by Heistermann et al. (2004, 2006), to detect the expected increase in FGCM levels following this stressor. Specifically, we analyzed fecal extracts with EIA systems designed to measure cortisol (CORT) (Palme & Möstl 1997), corticosterone (CCST) (Heistermann et al. 2006), 11oxo-etiocholanolone (Möstl & Palme 2002) and 11ß-hydroxyetiocholanolone (Ganswindt et al. 2003), the latter two assays representing group-specific assays for the measurement of 5ß-reduced GC metabolites with a 3α,11oxo- and 3α,11ß-dihydroxy structure. All four EIAs have been previously used successfully to monitor adrenocortical activity via FGCM analysis in other primate and non-primate species (Ganswindt et al. 2003; Weingrill et al. 2004; Heistermann et al. 2006; Fichtel et al. 2007; Ostner et al. 2008). In all EIAs antibodies used were raised in rabbit or sheep with steroids coupled to bovine serum albumin (BSA) via a carboxymethyloxime bridge. Detailed information on antibody characteristics, standards, and hormone labels as well as on other assay details, e.g., data on assay sensitivities, is given in Heistermann et al. (2006).

Steroid analysis

Based on the outcome of the validation tests, we analyzed all fecal samples collected from wild spider and howler monkeys in the 11ß-hydroxyetiocholanolone EIA. The EIA was performed as described in detail by Heistermann et al. (2004). Prior to steroid measurement, we diluted extracts 1:250-1:2000 (depending on concentration) in assay buffer and took duplicate aliquots to assay. Sensitivity of the assay was 1 pg/well. Serial dilutions of fecal extracts gave displacement curves parallel to those obtained with the 11ß-hydroxyetiocholanonole standard. Intra- and inter-assay coefficients of variation of high- and low-value quality controls were 6.1% (high, n = 16) and 7.8% (low, n = 16) and 7.4% (high, n = 37) and 13.0% (low, n = 37), respectively. All steroid concentrations are given as ng/g fecal dry (samples from zoo animals) or wet (samples from wild animals) weight.

HPLC analysis

We used reverse-phase high pressure liquid chromatography analysis (HPLC) to characterize the immunoreactive metabolites present in the feces of both species and measured by the 11ß-hydroxyetiocholanolone EIA. HPLC was carried out as described by Möhle et al. (2002) and Heistermann et al. (2006). To evaluate possible sex differences in 11ß-hydroxyetiocholanolone immunoreactivity profiles, we performed HPLC on both a male and a female sample from the wild-living animals of each species. HPLC also allowed us to evaluate whether certain fecal androgens, which could potentially be detected by antibodies raised against cortisol metabolites (Schatz & Palme 2001; Ganswindt et al. 2003), were commeasured by the 11ß-hydroxyetiocholanolone EIA.

Storage effect

To investigate whether FGCM levels changed during the two months of storage at ambient temperatures at the field site we conducted a storage experiment. We stored aliquots of eleven fecal extracts (from *A. hybridus*) at an elevated temperature of 30°C (to simulate storage conditions in the field) at the endocrinology laboratory at DPZ and measured each aliquot in the 11ß-hydroxyetiocholanolone assay immediately (time 0 control) and after one, three and six months of storage to test for a potential change in steroid concentrations as a function of storage duration. For these measurements, inter-assay coefficients of variation of high- and low-value quality controls were 6.1% (high, n = 4) and 7.4% (low, n = 4), respectively.

Statistical analyses

To assess the effects of time of day, sex, age and female reproductive state on FGCM levels we used a generalized linear mixed model (GLMM) (Baayen 2010) for each study species. We fit all models with the lmer function from the lme4 package (Bates & Maechler 2010) in R 2.15.1 (R Development Core Team 2012). Individual identity and group were used as random factors in the models. Sex, age (adult or subadult), time of sample collection (morning or afternoon) and female reproductive state (cycling, pregnant or lactating) were used as categorical variables. We tested for interactions between sex and age and between time and sex. These were not significant ($P \geq 0.05$) and thus, we did not include any interaction in the final models. We log transformed the response variable (FGCM levels) to achieve normal distribution and we checked that the assumptions of normally distributed and homogeneous residuals were fulfilled in every model by visually inspecting qqplots and the residuals plotted against the fitted values. We checked for model stability by excluding data points one by one from the data and comparing the estimates derived with those obtained for the full model. We derived variance inflation factors (Field 2005) using the function vif of the R-package car (Fox & Weisberg 2011) applied to a standard linear model excluding the random effect. To determine the significance of the full model (including all fixed and random effects) we compared it to the corresponding null model (including only the random effects) using a likelihood ratio test (R function 'anova'). To determine more reliable P-values we used the functions pvals.fnc of the package 'language R' (Baayen 2010). In this function P-values are based on Markov Chain Monte Carlo (MCMC) sampling (Baayen 2011).

Additionally, to examine changes in FGCM levels across the entire gestation period of female spider monkeys in more detail we divided the gestation period into seven monthly intervals and performed a Spearman rank correlation between month of gestation and respective FGCM levels (using Statistica 10). To back up the results of our GLMM we used a T-test (using Statistica 10) to test for potential differences between age categories in howler monkeys (as strongly indicated by Fig. 3f). All statistical tests were two-tailed and the statistical threshold was set at $P \leq 0.05$.

Results

Validation of an EIA to measure FGCM

Zoo-housed Ateles hybridus

In absolute terms, the highest levels of FGCMs were measured by the two group-specific assays (mean ± SE of all samples: 11ß-hydroxyetiocholanolone: 6094 ± 595 ng/g dry feces; 11oxo-etiocholanolone: 13842 ± 1123 ng/g dry feces), those measured by the CORT and CCST assay being generally much lower (CORT: 666 ± 119 ng/g dry feces; CCST: 501 ± 99 ng/g dry feces). Nevertheless, all four assays showed a clear response to the stress of anesthesia, reflected by a marked rise (200–320%) in FGCM levels within 24 h (Fig. 1). FGCM levels (except CCST) decreased on Days 2 and 3 post-anesthesia, but showed a second clear rise on Day 4 (exception CCST), which probably reflects the stress response to the marked distress caused by the visit of the veterinarian on Day 3 of the sampling period. On Day 5, levels of all four FGCM measures started to return to baseline and these low concentrations were also found two months later (Day 55) when the group had completely settled in their new environment. In terms of magnitude of response, the 11ß-hydroxyetiocholanolone, CORT and CCST assays were similar (all ca. 300% increase), while the response of the 11oxo-etiocholanoloneassay was slightly less pronounced (ca. 200%).

Wild Ateles hybridus

In the few samples collected from the two wild animals before and after the capture event, results were mixed (Table 2). With respect to 11ß-hydroxyetiocholanolone and 11oxo-etiocholanolone measurements, findings were similar to those obtained in the zoo-housed animals. Specifically, both individuals showed a 3- to 4-fold increase in 11ß-hydroxy-etiocholanolone and 11oxo-etiocholanolone levels in the sample collected 24.5 h after the capture and levels declined to pre-capture baseline concentrations in the samples collected following the stressful event (Table 2). The patterns for the CORT and CCST measures were inconsistent between individuals, i.e. a response to the stressor in terms of a clear rise in the respective FGCM levels was only seen in one of the two animals (Table 2). Based on the combined validation results (zoo and wild), we chose the 11ß-hydroxyetiocholanolone assay for all further analyses.

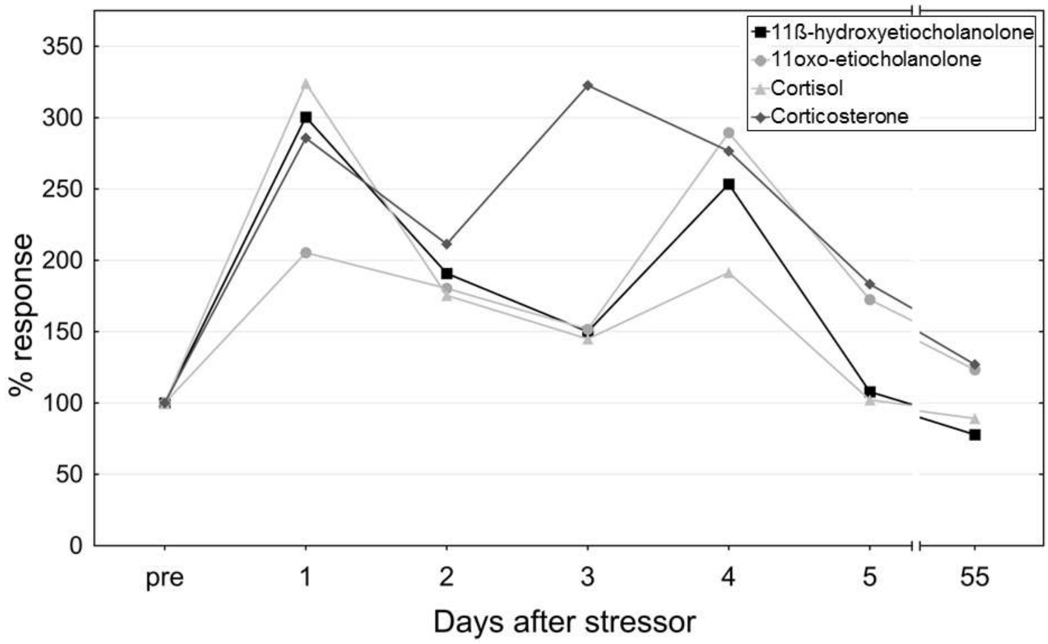

Figure 1. Percentage response (mean of all samples) in immunoreactive FGCM levels to a capture-translocation event and anesthesia in *A. hybridus*. Pre = samples collected before the anesthesia was applied. Note that on Day 3 the veterinarian checked on the animals which caused a high level of distress (see Methods).

Alouatta seniculus

FGCM levels in the individual male howler monkey showed a similar response to capture as in spider monkeys (Table 2). Specifically, 11ß-hydroxyetiocholanolone, 11oxo-etiocholanolone and CCST levels showed a clear rise (2.5–4.2-fold) within 24 h after capture, while CORT levels remained largely unchanged (Table 2). In contrast to spider monkeys, peak response in the two group-specific assays was, however, seen later, i.e. on Day 2 after capture. Levels of all FGCM measures showed a decline towards baseline in the sample collected on Day 3. Based on these findings we selected the 11ß-hydroxyetiocholanolone EIA for all further analyses (as for spider monkeys).

HPLC analysis

HPLC analysis indicated that the vast majority of immunoreactivity (>80% for *A. hybridus* and >90% for *A. seniculus*) was detected as distinct peaks between fractions 9 and 31 – positions where cortisol metabolites in our HPLC system elute (Fig. 2) (Heistermann et al. 2006). In both species the highest amounts of immunoreactivity were detected around fractions 16 and 25, the latter being the elution position of 11ß-hydroxyetiocholanolone, indicating a high abundance of this metabolite of cortisol in the feces of both species. The presence of only small amounts of immunoreactivity measured after fraction 40 (positions where certain potentially cross-reacting androgen metabolites elute (Ganswindt et al. 2003; Heistermann et al. 2006), suggests a low degree of co-measurement of these androgens in our assay (Fig. 2). In both species, HPLC profiles were very similar between males and females in terms of both number and elution position (i.e. characteristic) of metabolites measured.

Storage experiment

Our storage experiment revealed that 11ß-hydroxyetiocholanolone concentrations remained stable over the 6 months of storage at 30°C with levels deviating from time 0 control measurements by maximally 5.1% at any time point tested (1 month: -5.1%, 3 months: -2.4%, 6 months: +4.9%). The mean CV value calculated across the 11 individual CVs for each sample measurement over the 6 months of analysis was 6.6% (range 3.8%–10.7%) and was thus exactly in the range of our inter-assay variation (6.1% and 7.4%).

Effect of time of day, age, sex and female reproductive state on FGCM levels

Fecal samples of *A. hybridus* collected in the morning (mean ± SE: 203 ± 10 ng/g) had significantly higher FGCM levels than samples collected in the afternoon (83 ± 13 ng/g; GLMM: P_{MCMC} = 0.0001, Fig. 3a). Adult individuals had significantly higher FGCM levels than subadults (adults: 196 ± 10 ng/g; subadults: 146 ± 21 ng/g; P_{MCMC} = 0.027, Fig. 3e) but there was no difference in FGCM concentrations between the sexes (males: 208 ± 15 ng/g; cycling females: 175 ± 21 ng/g; P_{MCMC} = 0.17, Fig. 3c). Within the females, however, reproductive state significantly influenced FGCM levels (X^2= 15.32, P= 0.001), with concentrations being significantly elevated during pregnancy compared to the cycling (P_{MCMC} = 0.0004) and lactating (P_{MCMC} = 0.003) condition (Fig. 4). Cycling and lactating females did not differ in FGCM levels (P_{MCMC} = 0.58). Furthermore, in pregnant females, FGCM levels were significantly and positively correlated with the duration of gestation (Spearman: R= 0.54, T= -3.59, P= 0.004; Fig. 5).

In *A. seniculus* neither time of day (morning: 284 ± 23 ng/g; afternoon: 287 ± 80 ng/g), nor sex (males: 296 ± 23 ng/g; cycling females: 311 ± 35 ng/g) significantly influenced FGCM levels (N = 166 samples, full versus null model X^2 = 3.46, df= 9, P= 0.62; Fig. 3b and d). However, in accordance with spider monkeys, adult howler monkeys (286 ± 16 ng/g) had significantly higher FGCM levels than subadult individuals (151 ± 12 ng/g; T= 2.74, df= 33, P= 0.009; Fig. 3f). Due to an insufficient sample size, the influence of female reproductive state on FGCM levels could not be tested in this species.

Table 2: Fecal glucocorticoid metabolite concentrations (as detected by four different assays) in response to anesthesia in individual spider monkeys and a howler monkey.

Animal[a]	11β-hydroxyetiocholanolone				11oxo-etiocholanolone				CORT				CCST			
	Pre[b]	Peak[c]	Post[d]	Lag[e]	Pre	Peak	Post	Lag	Pre	Peak	Post	Lag	Pre	Peak	Post	Lag
Wampi (A.h.)	140	466	83	24.5	378	1142	215	24.5	29	554	394	24.5	11	49	27	24.5
Roko (A.h.)	49	175	14	24.5	86	525	27	24.5	62	104	15	28	27	33	5	28
Cirilo (A.s.)	112	473	266	46	598	2083	1515	46	72	126	50	50.3	21	57	38	24

[a] A.h. = *Ateles hybridus*; A.s. = *Alouatta seniculus*

[b] Pre-treatment levels in ng/g wet feces (see Methods).

[c] Peak levels in response to stressor in ng/g wet feces.

[d] Wampi: levels 50 h after stressor; Roko: levels one week after stressor; Cirilo: 72 h after stressor.

[e] Lag time in hours between occurrence of the stressor and peak FGCM level.

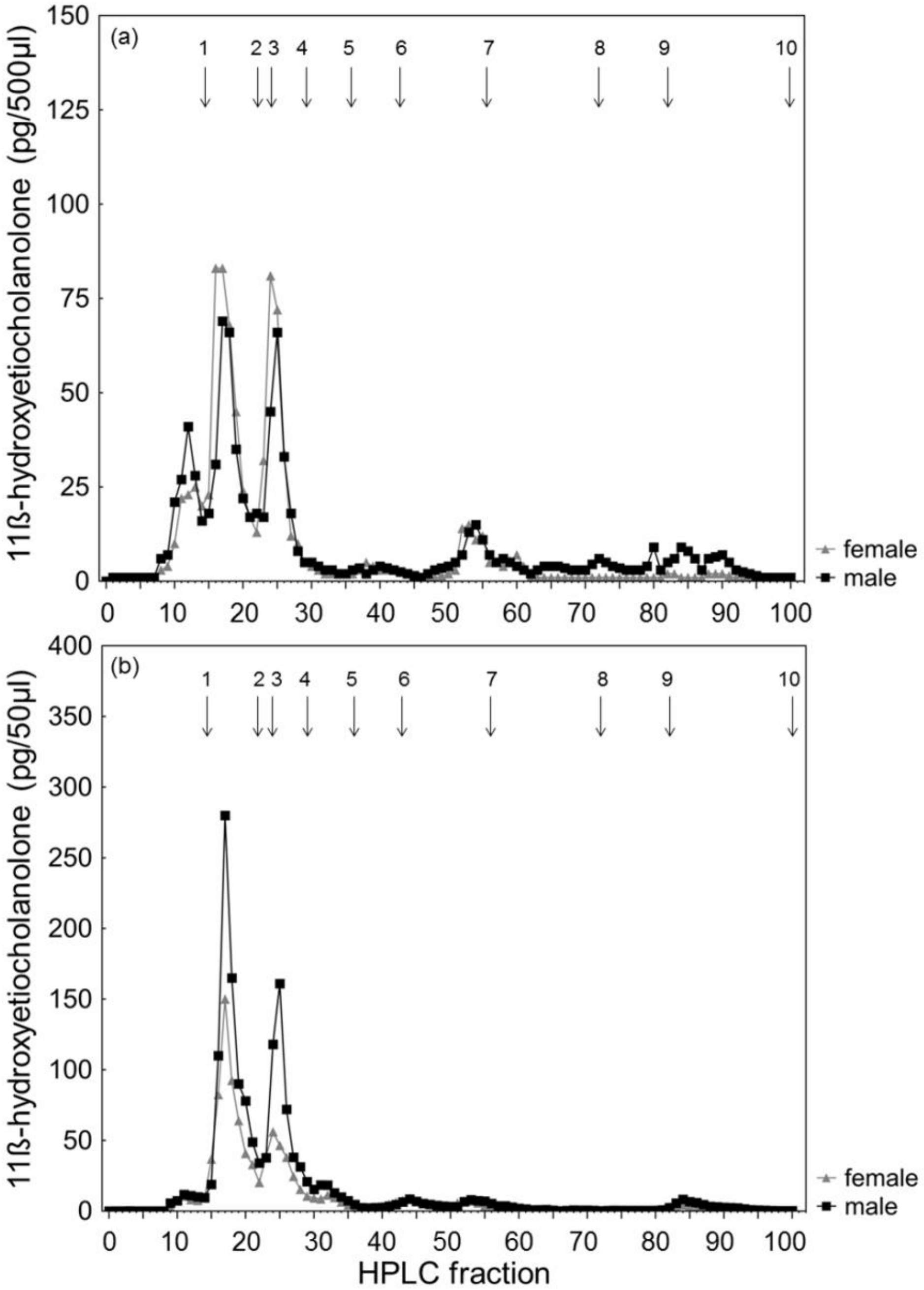

Figure 2. HPLC profiles of immunoreactivity detected with the 11ß-hydroxyetiocholanolone EIA in samples of a wild female and a male (a) spider monkey and (b) howler monkey. Arrows indicate elution positions of reference standards: [1] cortisol (fractions 14–15), [2] corticosterone (22), [3] 11ß-hydroxyetiocholanolone (24–25), [4] 11-oxoetiocholanolone (29–30), [5] 5ß-androstane-3,11,17-trione (36), [6] testosterone (43), [7] androstendione, dehydroepiandrosterone (55–56), [8] epiandrosterone, 5ß-DHT, 5ß-androstane-3ß-ol-17-one (72), [9] 5ß-androstane-3αol-17-one (82), and [10] androsterone (100).

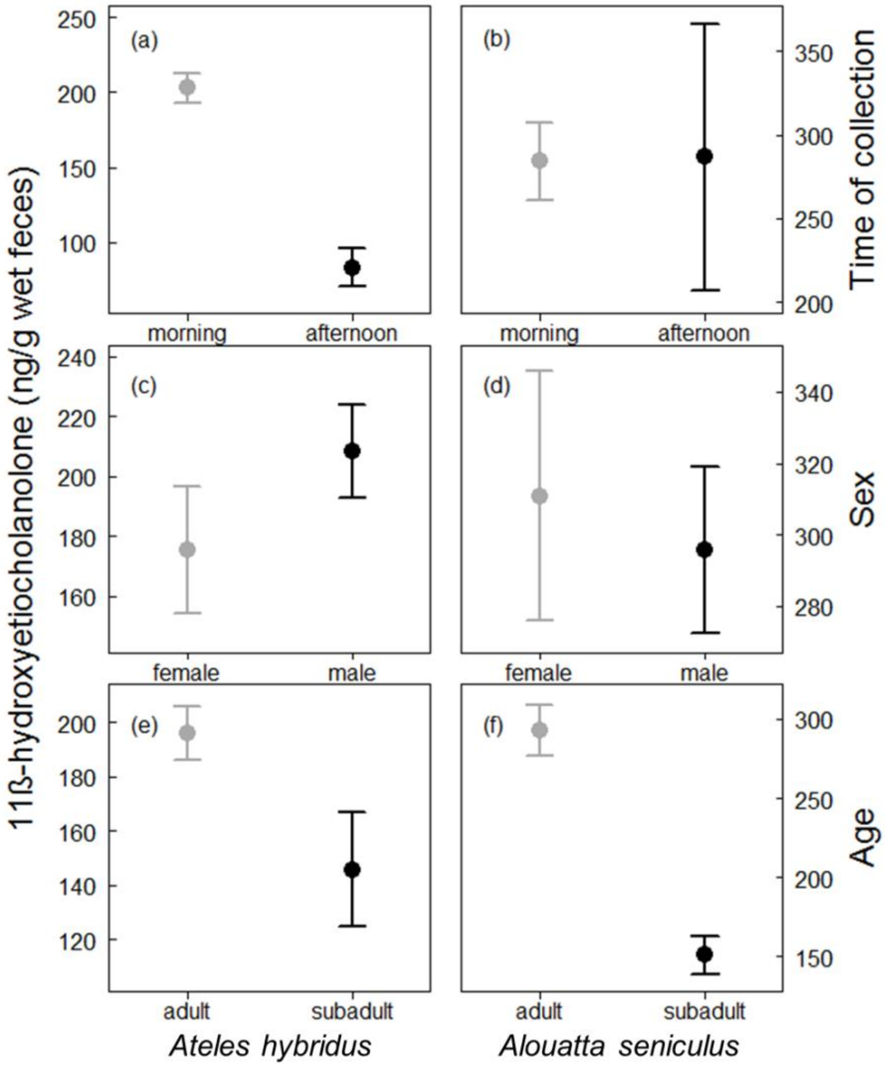

Figure 3. Mean ± SE FGCM levels of *A. hybridus* (left graphs) and *A. seniculus* (right graphs) in relation to time of day (a and b), sex (c and d) and age category (e and f).

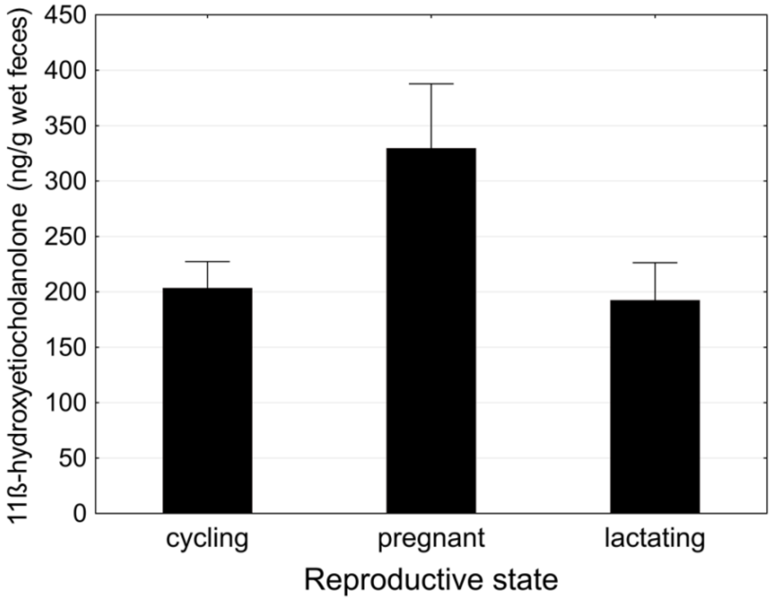

Figure 4. Mean ± SE FGCM levels in relation to female reproductive condition.

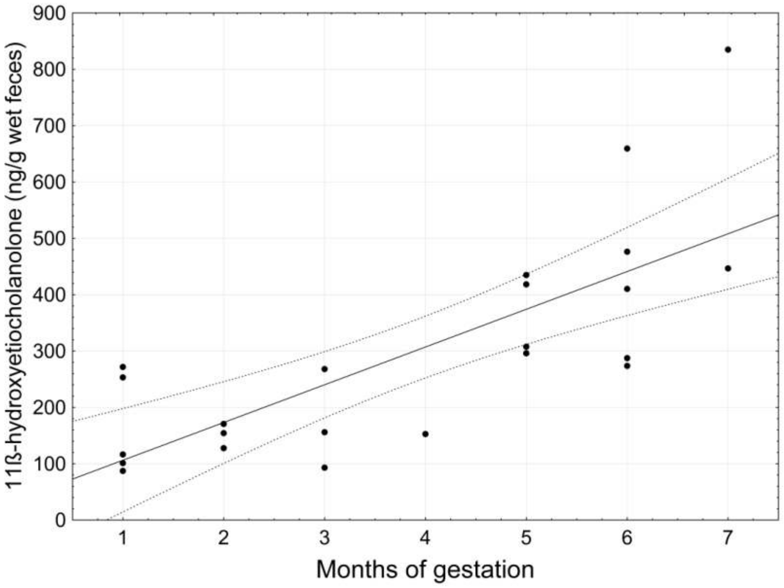

Figure 5. Mean ± SE FGCM levels in relation to months of gestation in female spider monkeys. Dashed lines indicate the 95% confidence intervals.

Discussion

This is the first study determining adrenocortical activity in brown spider and red howler monkeys and evaluating the potential impact of various intrinsic factors on FGCM output in these two species of New World monkeys. Our results demonstrate the validity of a group-specific EIA (11ß-hydroxyetiocholanolone) for monitoring the physiological stress response in both species by measuring 5-reduced GC metabolites with a 3α,11ß-dihydroxy structure in the feces. They also indicate that time of day, age and female reproductive state significantly influence FGCM output in *A. hybridus*, whereas age but not time of day affects FGCM levels in *A. seniculus*, and that sex did not affect FGCM concentrations in either species. Besides providing important information on the suitability of FGCM assays for tracking HPA axis activity in brown spider and red howler monkeys our study emphasizes species differences in terms of basic factors influencing adrenocortical activity.

Validation of an EIA to measure FGCM

Due to logistical constraints we were not able to determine the physiological stress responses individually in our zoo-housed spider monkeys and therefore can only present changes in FGCM concentrations on a group mean level. While this prevented us from obtaining information on individual variability in terms of the adrenocortical response to capture and anesthesia, it did not restrict our evaluation of the suitability of different GC assays for detecting the stressor associated changes in FGCM levels. Of the four assays tested, the two group-specific EIAs were the ones that responded consistently to the stressor(s) in both the captive and wild animals, indicating their higher biological sensitivity for tracking fluctuations in HPA axis activity compared to the cortisol and corticosterone EIAs tested. In terms of magnitude and time course of response, the two group-specific measures were within the range of those reported in other studies on primates (Whitten et al. 1998b; Heistermann et al. 2006; Martínez-Mota et al. 2008) and non-primate species (Wasser et al. 2000; Young et al. 2004). Although both assays thus appear to be of similar value, for practical reasons we selected the 11ß-hydroxyetiocholanolone assay for all subsequent analyses. Our HPLC data indicated that the 11ß-hydroxyetiocholanolone measure was largely specific for glucocorticoid metabolites and not affected by a substantial co-measurement of androgens which potentially can have distorting effects on FGCM results (Schatz & Palme 2001; Ganswindt et al. 2003; Goymann 2012). We found similar results for the red howler monkey used for biological validation, although the findings of this case study should be confirmed in a larger number of animals. The 11ß-hydroxyetiocholanolone assay has, however, been applied successfully to monitor stress physiology in many other primates, (lemurs (Fichtel et al. 2007); macaques (Ostner et al. 2008; Girard-Buttoz et al. 2009); gibbons (Pirovino et al. 2011); apes (Weingrill et al. 2011; Shutt et al. 2012)) indicating its versatility for non-invasively monitoring adrenocortical activity in primates of all major taxa. Based on these and our present findings, we are therefore confident that the 11ß-hydroxyetiocholanolone assay is valid for monitoring adrenocortical activity in the two study species.

Effect of time of day, age, sex and female reproductive state on FGCM levels

We extracted all fecal samples on-site in our field camp and stored fecal extracts at temperatures of about 25°C for up to two months before we were able to freeze them, the gold standard for storing hormone samples long-term (Hunt & Wasser 2003; Herring & Gawlik 2009; Shutt et al. 2012). Our storage experiment showed that FGCM levels were not largely affected by storing the alcoholic extracts at elevated temperatures for up to 6 months as indicated by changes that were within 5.1% of the controls. Our data thus confirm recent findings from a study on gorillas (Shutt et al. 2012) and baboons (Kalbitzer & Heistermann 2013), showing that FGCMs in alcoholic extracts remained stable for up to one year when stored unfrozen. As the variation in repeated sample measurements across the 6 months was almost identical to our measure of inter-assay variability, the small differences observed are very likely due to assay variation and thus do not reflect true changes in FGCM concentrations (see also (Shutt et al. 2012; Kalbitzer & Heistermann 2013)). Therefore, our method of storing fecal extracts for two months at elevated temperatures at the field site did not affect the FGCM levels obtained and thus is unlikely to have biased our data.

Using the newly established FGCM assay methodology we were able to examine the potential impact of time of day, age, sex and female reproductive state on adrenocortical activity in wild brown spider and red howler monkeys. Our results revealed species differences in this respect. For example, brown spider monkeys showed a marked diurnal variation in FGCM levels with morning samples showing, on average, 2.5-fold higher concentrations than afternoon samples. Similar results of the same magnitude have been found by Davis et al. (2005) for urinary cortisol excretion in closely related Colombian spider monkeys (*Ateles geoffroyi* [*sic*] *rufiventris*). By contrast, we did not find evidence for diurnal changes in FGCM levels in red howler monkeys, a finding that concurs with that reported for males of black howler monkey (*Alouatta pigra* (Van Belle et al. 2009); but see (Rangel-Negrín 2010)). These interspecific differences can presumably be ascribed to the distinct foraging strategies and differences in gut passage rates of the two species. Spider monkeys are mainly frugivorous and defecate very frequently (average of 13.7 times/ 12 h) (Link & Di Fiore 2006) as the gut passage time is very short, i.e. only 4.4 h on average (Milton 1981b). This should result in a high temporal resolution of endocrine information derived from feces which may explain why the diurnal variation in HPA axis activity is well reflected in FGCM levels in spider monkeys. In contrast, howler monkeys are mainly folivorous and have a bimodal defecation pattern (Andresen 2002) and an average gut passage rate of 20.4 h (Milton 1981b). Consequently, in howler monkeys information on endocrine state from feces is more dampened, and, therefore, it is likely that short-term temporal changes in FGCM output cannot be tracked from feces in howler monkeys. Overall, our data support the prediction made by Touma and Palme (2005), stating that it might be impossible to detect diurnal changes in FGCM levels in species with a long gut passage rate and/or animals that defecate rather infrequently. More importantly, our results emphasize that diurnal variation in HPA axis activity is an important factor to consider when interpreting FGCM data in brown spider monkeys and presumably other species of the genus.

In both species we found age-related differences where adult individuals showed significantly higher FGCM levels than subadult individuals. Our results are in accordance with findings from studies on wild chimpanzees (Seraphin et al. 2008) and wild Assamese macaques (Ostner et al. 2008) which both showed that fecal glucocorticoid levels were higher in aged individuals. Cortisol has also been reported to increase with age in humans (Touitou et al. 1983). Besides possible maturation-related changes in adrenocortical function, elevated FGCM levels in older individuals compared to younger ones may reflect the higher engagement of adult animals in physiologically demanding and potentially stressful activities such as territorial defense (Peres 1989; Pride 2005) and reproductive competition (Bercovitch & Ziegler 2002; Ostner et al. 2008). Consequently, for both species age needs to be recognized as a significant individual characteristic that interacts with social and ecological variables when shaping glucocorticoid production.

We did not find differences in FGCM levels between males and cycling females in either of the two primate species studied. We believe that this is a valid result because our HPLC data show that the 11ß-hydroxyetiocholanolone assay measures the same immunoreactive compounds in both males and females of both species. Thus, our quantitative comparison of FGCM concentrations between the sexes is not significantly biased by cross-reactivity of the antibody with particular sex-specific hormone metabolites, the latter being a major concern with respect to data interpretation when comparing absolute hormone levels between sexes (Preis et al. 2011; Goymann 2012). Absence of a sex-specific effect on FGCM levels as found in our two New World primate species has also been reported in other mammal species such as California mice (Harris et al. 2012) and black howler monkeys (*A. pigra*: (Martínez-Mota et al. 2007; Behie et al. 2010); but see (Rangel-Negrín 2010)). However, our results for the brown spider monkey contrast with those reported for a closely related species, the Yucatán spider monkey (*Ateles geoffroyi yucatanensis*) where captive-housed males show significantly higher fecal cortisol levels than females (Rangel-Negrín et al. 2009). Whether the latter finding may indicate a species-specific physiological difference in baseline adrenocortical activity within the genus *Ateles* or whether it merely reflects sex differences in the response to the potentially more stressful captive conditions remains unclear. However, a comparison of our results with those of other studies may be generally of limited value since the extent to which FGCM levels in males and females reflect measurements of the same or different metabolites (see (Goymann 2012)) is usually not reported.

Reproductive condition had a significant effect on FGCM concentrations in female spider monkeys, with levels increasing with progressive gestation. Unfortunately, due to small sample size we were not able to examine the effect of female reproductive state on FGCM output in howler monkeys. We envisage, however, that pregnancy-related increases in FGCM output are also present in howler monkeys given that this pattern is commonly found in female mammals, incl. primate species of all major radiations (e.g.: *Rattus norvegicus* (Atkinson & Waddell 1995); *Myotis lucifugus* (Reeder et al. 2004); *Lemur catta* (Cavigelli 1999); *Cebus capucinus* (Carnegie et al. 2011); *Saguinus oedipus* (Ziegler et al. 1955); *Papio ursinus* (Weingrill et al. 2004; Engh et al. 2006); *Mandrillus sphinx* (Setchell et al. 2008); *Macaca mulatta* (Hoffman et al. 2011)). Elevated glucocorticoid levels during gestation are likely caused by interactions of increased metabolic demands on the female and stimulation of the HPA axis by pregnancy-related increases in levels of estrogens (Jones et al. 1989; McLean & Smith 1999). In addition, elevated glucocorticoid levels during gestation may also arise from placental production of cortisol (Clapp et al. 1982). Our data indicate that female reproductive condition, specifically whether an individual is pregnant or not, strongly influences adrenocortical function and FGCM concentrations (at least in spider monkeys).

Collectively, the present study demonstrates that time of day, age and female reproductive state need to be considered in spider monkeys and to a lesser extent in howler monkeys when FGCM data generated as part of behavioral, ecological or conservation-related studies are interpreted. Specifically, by raising awareness to intrinsic factors confounding FGCM levels our findings should help to better interpret future data on the effects of anthropogenic disturbances and habitat fragmentation on stress hormone metabolite output in these two New World primate species. Using FGCM analysis as part of such conservation management studies might be especially important for the future survival of the critically endangered brown spider monkey. Subsequent studies may help to detect the most susceptible populations of *A. hybridus* and support the implementation of conservation plans for the species in Colombia and Venezuela.

Acknowledgments

We thank the Lalinde and De Greiff families who have allowed us to conduct our research at ‘‘Hacienda San Juan de Carare’’. We also thank the Ministerio de Medio Ambiente y Desarrollo Sostenible in Colombia for all the permits needed to conduct this study. We thank Associate Prof. Pablo R. Stevenson for logistic support with sample storage. We acknowledge the authorities of the Stuttgart Zoo for their support and the permission for the fecal sample collection. For their help with sample collection we thank Felipe Alfonso, Natalia Alvis, Nathalia Fuentes, Claudia Lodoño, Andrés Montes, Sara Ramirez and Luisa Salazar. For their support in the laboratory we acknowledge Andrea Heistermann and Petra Kiesel. We thank Cédric Girard-Buttoz for his advice concerning statistical analysis and helpful suggestions on earlier versions of the manuscript. Fundación Proyecto Primates provided all logistics and this study has been funded by the German Primate Center. This research was conducted with permission and in accordance with the Zoos’ research protocols and adhered to the legal requirements of Germany. The research at the field site in Colombia was authorized by the Ministerio de Medio Ambiente y Desarrollo Sostenible in Colombia under the research permit 4120-E1-110897 and CITES permits 32964 and 32965.

CHAPTER 2

Effects of human impact and forest fragment size on physiological stress levels of two sympatric ateline primates in Colombia

with Andrés Link, Michael Heistermann, Carolina Gómez-Posada, Nelson Galvis and Eckhard W. Heymann

Abstract

Habitat fragmentation and anthropogenic disturbances are of major concern to the conservation of endangered species because of their potentially negative impact on animal populations. Both processes can impose physiological stress (i.e. increased glucocorticoid output) on animals, and chronically elevated stress levels can have detrimental effects on long-term viability of animal populations. Here, we investigated the effect of fragment size and human impact (logging and hunting pressure) on glucocorticoid levels of two sympatric Neotropical primate species, the red howler monkey (*Alouatta seniculus*) and the critically endangered brown spider monkey (*Ateles hybridus*), two species that have been reported to contrast strongly in their ability to cope with anthropogenic disturbances. We collected fecal samples from eight spider monkey groups and 31 howler monkeys groups, living in seven and 10 different forest fragments in Colombia, respectively. We measured fecal glucocorticoid metabolite (FGCM) levels in both species using previously validated methods. Surprisingly, fragment size did not influence FGCM levels in either species. Spider monkeys showed elevated FGCMs in fragments with the highest level of human impact, whereas we did not find this effect in howler monkeys. This suggests that the two species differ in their physiological responsiveness to anthropogenic changes, further emphasizing why spider monkeys are at higher extinction risk than howler monkeys. If human impact persists on the long run elevated FGCM levels can lead to a state of chronic stress, which might limit the future viability of populations. We propose that FGCM levels should be used as a tool to monitor populations living in disturbed areas and to assess the success of conservation strategies such as corridors connecting forest fragments.

Conservation Physiology, in revision

Introduction

Habitat loss, habitat fragmentation, and anthropogenic disturbances that accompany these processes (e.g. logging, increased hunting pressure) are of major concern to the conservation of endangered species due to their role in population declines (Peres 2001; Fahrig 2003). Currently, numerous species from all vertebrate groups are threatened with extinction (IUCN 2012). The pervasive process of anthropogenic disturbances of natural ecosystems (Hannah *et al*. 1995; Foley *et al*. 2005) emphasizes the need to understand the proximate effects these alterations have on the health and survival of animal populations. Generally, taxa with narrow dietary niches and that occupy few habitat types are at greater extinction risk than taxa that have broader niches and occupy several habitat types (Harcourt *et al.* 2002). Species respond to fragmentation and disturbances differently depending on factors such as life history, geographic range, ecological niche, and dispersal ability (Purvis *et al.* 2000; Henle *et al.* 2004; Cardillo *et al.* 2005). Some species manage to adjust aspects of their behavior and social system (Menon & Poirier 1996; Sumner et al. 1999; González-Solís et al. 2001; Blumstein et al. 2005; Umapathy et al. 2011) while species that cannot adjust, face local extinction (Cosson *et al.* 1999; Peres 2001).

Which role changes in physiological parameters play in population declines and ultimately species extinction is not yet well understood. Glucocorticoids (GC)- cortisol and corticosterone (depending on the species)- the frontline hormones of the vertebrate stress response (Sapolsky *et al*. 2000) potentially play an important role. While short-term elevations of GCs in emergency situations are considered adaptive (Monclús *et al.* 2005), long-term elevations can have deleterious effects on reproduction, growth and immune system activity (Pickering *et al.* 1991; Charbonnel *et al.* 2008; French *et al.* 2010; Setchell *et al.* 2010). Anthropogenic disturbances have been associated with GC elevations in many vertebrates (amphibians: Homan *et al.*, 2003; Janin *et al.*, 2011; birds: Wasser *et al*., 1997; Lucas *et al.*, 2006; mammals: Martínez-Mota *et al*. 2007; Gobush *et al*. 2008; Rangel-Negrín *et al*. 2009; Jaimez *et al.* 2012; Dunn *et al.* 2013). These GC elevations have been linked to negative effects on reproduction and immune system activity (e.g. Ellenberg *et al*. 2007; French *et al*. 2010), suggesting that elevated stress levels caused by human influence directly affect individual health and ultimately population viability.

We studied two primate species that occur sympatrically in Colombia, brown spider monkeys (*Ateles hybridus*) and red howler monkeys (*Alouatta seniculus*), to investigate whether and how anthropogenic disturbances influence GC output. The two genera *Ateles* and *Alouatta* contrast strongly in their ability to cope with anthropogenic disturbances (Bernstein et al. 1976; Bicca-Marques 2003; Michalski & Peres 2005). Brown spider monkeys are endemic to Colombia and Venezuela, and are considered to be one of the 25 most endangered primate species worldwide due to severe habitat loss and high hunting pressure (Mittermeier *et al.* 2012). Habitat fragmentation, slow reproductive cycles, large area requirements and their dietary niche (for review see: Di Fiore *et al.* 2010) make this species vulnerable to anthropogenic disturbances. Moreover, spider monkeys exhibit flexible grouping patterns (fission-fusion dynamics) to reduce intra-group feeding competition and to cope with changes in the availability of ripe fruit, their preferred food resources (e.g. Klein and Klein, 1977; Symington, 1988). Accordingly, the confinement to small fragments might reduce the flexibility of their grouping patterns and thereby lower their potential to minimize competition. This might in turn cause increased physiological stress.

In contrast, red howler monkeys seem less vulnerable and persist even in extremely small fragments (Crockett 1998; Lopez et al. 2005; Michalski & Peres 2005). They have a broad distribution range, occupy a wide array of ecosystems, withstand hunting pressure better than other atelids and are not threatened with extinction (Boubli *et al.* 2008). Typically, howler monkeys require much smaller areas than spider monkeys (reviewed by Di Fiore *et al.*, 2010). They from cohesive groups (Neville, 1972; for review see Di Fiore and Campbell, 2007) and have a mainly folivorous but flexible diet (Milton 1980; Julliot & Sabatier 1993; Bicca-Marques 2003).

The proximate mechanisms leading to such species-specific differences in the ability to cope with a changing environment have not yet been investigated. Therefore, the aim of this study was to determine how fragment size and level of human impact influence physiological stress levels - measured through fecal glucocorticoid metabolites (FGCMs) - in both species. We collected fecal samples from both species in forest fragments that differed in size and level of human impact (hunting pressure and logging). We predicted that FGCM levels increase with both, decreasing fragment size (as a proxy for fragmentation intensity) and increasing level of human impact. Due to differences in diet, area requirements and adaptation capabilities we predicted that howler monkeys generally react less strongly than spider monkeys to these disturbances and that they would show no or only a small elevation in GC levels compared to spider monkeys.

Methods

Study sites

Between April 2010 and April 2012 we collected fecal samples from eight spider monkey and 31 howler monkey groups, living in seven and 10 different fragments, respectively. Fragments differed in size and human impact (Table 1). Four fragments (San Juan, Quinchas, Jamaica and Juntas) are long-term study sites and study groups have been habituated to human observers previously (Aldana *et al.* 2008; Gómez-Posada *et al.* 2010; Link *et al.* 2010). We visited all other fragments only for fecal sample collection purposes (for 2-3 weeks). Nine of the fragments were occupied by both species and the other four (Jamaica, Juntas, LGPM, Cienaga) were only occupied by howler monkeys. Although most fragments contained both species, sometimes fecal sample collection was only feasible for one. We determined the level of current human impact through observations and surveys in which we interviewed farm owners and workers. We classified fragments without current human hunting pressure and absence of recent logging activity as level 0; fragments with either hunting pressure or logging activity as level 1, and fragments with both hunting pressure and ongoing logging activity as level 2.

Table 1. Number of fecal samples collected for each species in the different forest fragments (N= 13) which varied in size and level of human impact.

Fragment	Size (ha)	Human impact	No. of samples (no. of groups) *A. hybridus*	*A. seniculus*	Location
San Juan	65	0	411 (2)	289 (17)	6° 43'N, 74° 09'W
San Juan3	75	0	10 (1)	5 (1)	6° 43'N, 74° 07'W
LGPM	100	0	-	9 (1)	6° 41'N, 74° 09'W
Quinchas	250	0	46 (1)	21 (3)	6° 02'N, 74° 16'W
Terra Firme1	500	0	3 (1)	-	6° 41'N, 74° 08'W
LGPM2	500	0	-	4 (1)	6° 41'N, 74° 09'W
Jamaica	4.21	1	-	12 (1)	4° 23'N, 75° 48'W
Juntas	25.5	1	-	13 (1)	4° 25'N, 75° 47'W
India	500	1	3 (1)	-	6° 15'N, 74° 07'W
Cienaga	50	2	-	6 (2)	6° 42'N, 74° 08'W
Campo Capote	250	2	3 (1)	5 (1)	6° 34'N, 73° 51'W
Remedios	400	2	5 (1)	-	6° 53'N, 74° 34'W
Terra Firme2	500	2	-	9 (3)	6° 41'N, 74° 07'W
Total no. samples			**481 (8)**	**373 (31)**	

Fecal sample collection

We collected samples from adult and subadult individuals and noted sex and age-class, female reproductive state (when identifiable), collection time and date for every sample. The time-lag of GC metabolite excretion in feces is ~24 h in *A. hybridus* and ~46 h in *A. seniculus* (Rimbach *et al.* 2013). Thus, we avoided following unhabituated groups on 2-3 consecutive days (depending on the species) to reduce the influence of observer presence on FGCM levels. In unhabituated groups we did not recognize individuals and to avoid re-sampling of individuals we sampled each group or subgroup (in the case of *Ateles*) only once.

In both species pregnancy cannot reliably be detected by observation. Thus, to determine the approximate conception date and female reproductive state at sample collection we used parturition date (habituated groups) in combination with average gestation length (*Ateles* ~7.5 months, *Alouatta* ~6.3 months (Di Fiore & Campbell 2007)). We categorized females as lactating for the time in which they nursed dependent offspring. Females that were neither pregnant nor lactated were categorized as non-pregnant-non-lactating. However, in unhabituated groups we were able to categorize females merely as either lactating or non-lactating (pregnant and cycling females). We collected comparative numbers of samples from both sexes (*A. hybridus*: 51.8 % females, 48.2 % males; *A. seniculus*: 45.6% females, 54.4% males) per fragment and have no reason to assume that the collection was biased towards collecting samples from females in only certain reproductive conditions.

Before we collected samples, we homogenized the fecal bolus and removed any obvious undigested matter (e.g. large seeds). We placed approximately 0.5 g of feces into a 15 ml pre-weighted polypropylene tube pre-filled with 5 ml of 96% ethanol and shook the tube until the feces were suspended in the solvent (Shutt *et al*. 2012; Rimbach *et al.* 2013). We kept the samples at ambient temperatures until extracting them in the evening.

Hormone extraction and FGCM analysis

We determined fecal wet weight by calculating the difference between the weight of the tube before (tube plus ethanol) and after (tube, ethanol and feces) sample collection. Then we shook the tubes firmly for five min and thereafter, centrifuged the samples for one min using a manually-operated centrifuge (Shutt *et al.* 2012; Rimbach *et al.* 2013). We poured off ~2 ml of each fecal extract into 2 ml polypropylene tubes, covered them with parafilm, labeled and stored them at ambient temperatures (~25°C) in a dark place. Every two months we transported the extracts to the Universidad de Los Andes, Bogotá and stored them at -20°C until shipment to the Endocrinology Laboratory at the German Primate Center for analysis.

We analyzed all fecal samples using a previously validated (Rimbach *et al.* 2013) enzyme-immunoassay (EIA) for 11ß-hydroxyetiocholanolone, a group-specific measurement of 5beta-reduced GC metabolites (Ganswindt *et al.* 2003) with a 3α,11ß-dihydroxy structure. The EIA was performed as described in detail by Heistermann *et al*. (2004). Prior to hormone measurement, we diluted extracts 1:250–1:2000 (depending on original concentration) in assay buffer and thereafter, took duplicate aliquots to assay. Intra- and inter-assay coefficients of variation of were 6.1% and 7.8% for high-value and 7.4% and 13.0% for low-value quality controls. All hormone concentrations are expressed as ng/g fecal wet weight.

Statistical analyses

To assess the effects of fragment size and level of human impact on FGCM levels we used linear mixed models (LMM, (Baayen 2010)). We fit all models with the lmer function from the lme4 R-package (Bates & Maechler 2010) in R2.15.1 (R Development Core Team 2012). We used restricted maximum likelihood methods to estimate the models since they are robust against unequal sample sizes (Keselman *et al.* 2001). We utilized one model per species, and group and fragment ID were used as random factors. GC excretion often shows a diurnal rhythm and GC levels can be affected by a variety of potentially confounding factors (for review see (Millspaugh & Washburn 2004; Keay et al. 2006; Goymann 2012)). Thus, we used sex, age, female reproductive state, group size and sample collection time as control variables in the models (as some of these variables have been shown to affect FGCM levels in the study species (Rimbach *et al.* 2013)). We tested for interactions between fragment size and human impact, and between group size and fragment size. These were not significant ($P \geq 0.05$) and not included in the final models.

We log transformed the response variable (FGCM levels) to achieve normal distribution and checked that the assumptions of normally distributed and homogeneous residuals were fulfilled by visually inspecting qqplots and the residuals plotted against the fitted values for each model. To assess model stability we run diagnostics (dfbetas) that did not suggest the existence of influential cases. We used the function vif of the R-package car (Fox & Weisberg 2011), applied to a standard linear model excluding the random effects, to derive variance inflation factors (Field 2005). We used a likelihood ratio test (R function ‘anova’) to determine the significance of the full model (all fixed and random effects) compared to the corresponding null model (only random effects). We used the functions pvals.fnc of the R-package ‘language R’ (Baayen 2010) to determine P-values based on Markov Chain Monte Carlo (MCMC) sampling (Baayen 2011). All statistical tests were two-tailed and the statistical threshold was set at $P \leq 0.05$.

Results

In both species, FGCM levels varied substantially between different fragments (Fig. 1). Fragment size did not affect FGCM levels in spider monkeys (N= 481 samples; LMM: X^2= 2.06, df= 1, P= 0.15, Fig. 1a), whereas human impact had an influence (LMM: X^2= 11.22, df= 2, P= 0.003; Fig. 2a). Specifically, spider monkeys living in fragments with both kinds of human influence had FGCM levels (mean ± SD= 496.7 ng/g ± 90.0 ng/g) which were more than double as high as those found in animals living in fragments with no disturbance (mean ± SD= 206.74 ng/g ± 7.5 ng/g; P_{MCMC}= 0.01, Table 2) or only one type (mean ± SD= 137.16 ng/g ± 45.5 ng/g; P_{MCMC}= 0.04) of disturbance. There was no difference between the FGCM levels of spider monkeys living in fragments with no or only one type of human impact (P_{MCMC}= 0.56, Table 2). In howler monkeys neither fragment size (Fig. 1b) nor human impact (none: mean ± SD= 245.9 ng/g ± 10.6 ng/g; one: 159.4 ng/g ± 31.0 ng/g; both: 251.2 ng/g ± 32.9 ng/g; Fig 2b) influenced FGCM levels (N= 373 samples, full vs. null model X^2= 13.76, df= 9, P= 0.13).

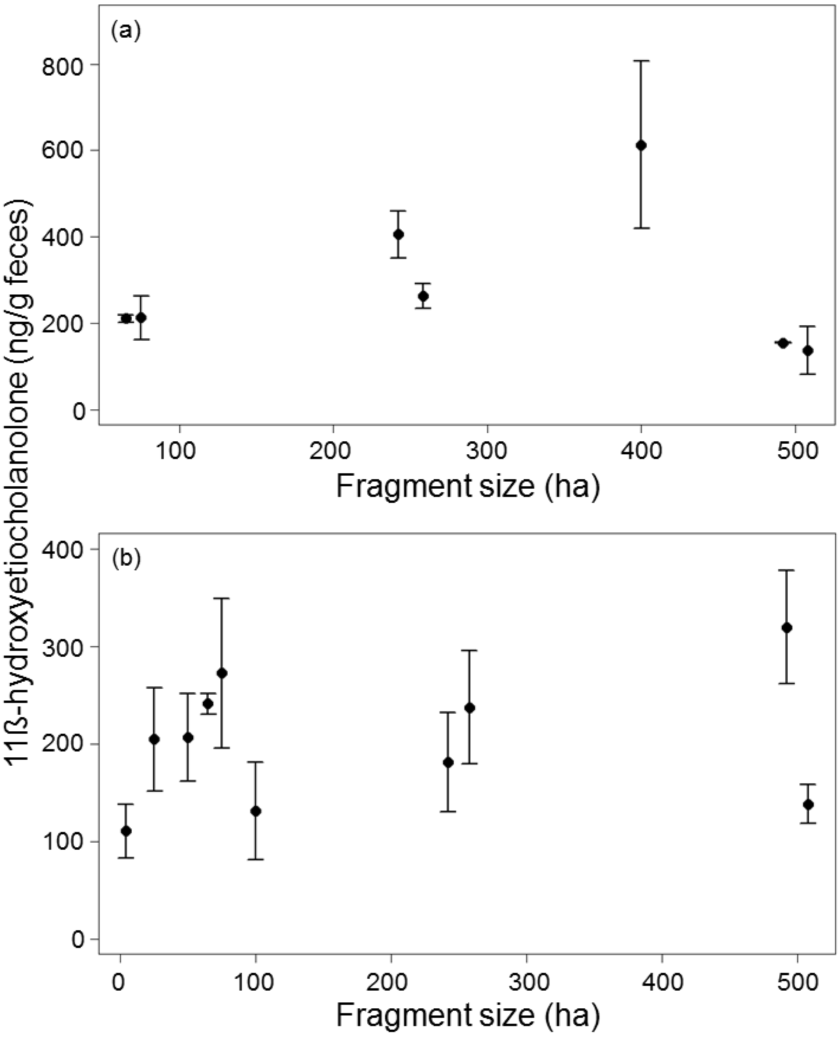

Figure 1. Mean ± SE of FGCM levels of a) *Ateles hybridus* and b) *Alouatta seniculus* in relation to fragment size.

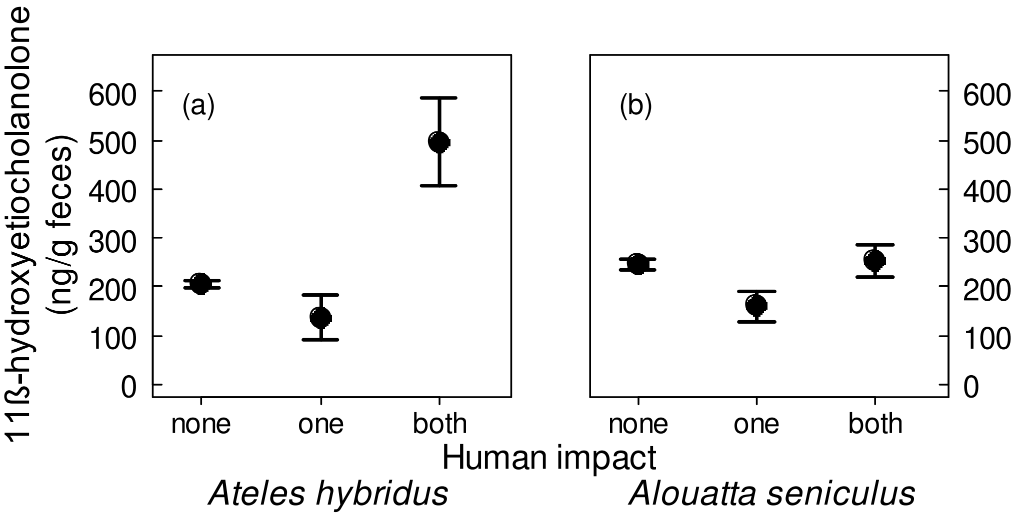

Figure 2. Mean ± SE of FGCM levels of a) *Ateles hybridus* and b) *Alouatta seniculus* in relation to level of human impact.

Table 2. Results of the LMM examining the effect of fragment size and level of human impact on log transformed FGCM levels in *Ateles hybridus*. Variables that significantly influenced FGCM levels appear in bold.

Null vs. full model	*df*	X^2	*P*
	9	85.23	**<0.001**
Variable	*Estimate ± SE*	*t*	P_{MCMC}
Intercept	5.96 ± 0.33	17.85	**0.0001**
Fragment size	0.08 ± 0.05	1.44	0.2252
Impact: both-none	-0.95 ± 0.32	-2.92	**0.0152**
Impact: both-one	-1.27 ± 0.57	-2.20	**0.0420**
Impact: one-none	0.31 ± 0.60	0.52	0.5666
Group size	0.05 ± 0.04	1.05	0.2925
Time	-0.25 ± 0.03	-7.10	**0.0001**
Sex	0.05 ± 0.10	0.54	0.6564
Age	-0.39 ± 0.12	-3.24	**0.0014**
Female reproductive state	*df*	X^2	*P*
	4	12.43	**0.014**

Discussion

Consistent with our predictions, spider monkeys had higher FGCM levels in fragments with the highest level of human impact compared to less impacted fragments. We did not find such an effect in howler monkeys. In contrast to our predictions, fragment size did not influence FGCM levels of either species. Time of sample collection, age and female reproductive state influenced FGCM levels in *A. hybridus* (possible explanations are discussed elsewhere (Rimbach *et al.* 2013)), but were controlled for in data analyses. This study reinforces previous results concerning species-specific differences in the ability to cope with anthropogenic disturbances and strengthens the assumption that spider monkeys are more susceptible to human alterations than howler monkeys.

Proximity to humans, hunting pressure and logging are likely perceived as threatening by many animals. Red deer (*Cervus elaphus*) that were chased by humans (Bateson & Bradshaw 1997) and African elephants (*Loxodonta africana*) that experienced high hunting risk (Gobush *et al.* 2008) have elevated GC levels compared to conspecifics that did not experience the disturbance. The presence of humans leads to elevated GC levels in several animal taxa (birds: (Fowler 1999; Müllner *et al*. 2004; Thiel *et al*. 2011); reptiles: (French *et al.* 2010); mammals: (Creel *et al*. 2002; Barja *et al.* 2007; Behie *et al.* 2010; Piñeiro *et al.* 2012; Muehlenbein *et al.* 2012; Zwijacz-Kozica *et al*. 2012)). Proximity to humans can impair the breeding success of animals (Ellenberg et al. 2006; Hinam & St. Clair 2008; Strasser & Heath 2013), which might be caused by increased GC levels (Ellenberg *et al.* 2007; Charbonnel *et al.* 2008). Furthermore, logging activities can result in elevated GC levels (Wasser *et al.* 1997). Concordant with previous studies, we found elevated FGCM levels of spider monkeys in fragments where logging and hunting occurred, whereas no such effect was observed in howler monkeys. These results suggest clear species-specific differences in the sensitivity to react to anthropogenic disturbances with an activation of the HPA axis. Whether this elevation of FGCM levels indicates a state of chronic stress with potential negative consequences on health and fitness is difficult to assess and beyond the scope of this paper (for a discussion of chronic stress see e.g. (Boonstra 2013)).

Fruit availability often declines in small and established (> 10 years) fragments (Putz et al. 1990; Cordeiro & Howe 2001; Arroyo-Rodríguez & Mandujano 2006; Dunn et al. 2010) and low food availability can cause elevated GC levels in primates (Cavigelli 1999; Muller & Wrangham 2004; Chapman et al. 2007b; Behie et al. 2010). Surprisingly, spider monkeys living in small fragments did not show elevated FGCM levels compared to those living in larger ones, although they potentially experience low levels of food availability and high resource competition. Although some fragments included in this study are very small, drastic changes in food availability might not have occurred yet because most of these fragments have been created rather recently (< 10 years). This might explain why fragment size did not influence FGCM levels of either species. Alternatively, it could be that elevated GC levels were associated with low food availability in some fragments. But we are lacking the data to test this assumption. However, two very small fragments (Jamaica and Juntas) have been isolated about 100 years ago and food availability is extremely low (Gómez-Posada *et al.* 2010). Nevertheless, howler monkeys are able to persist in these fragments and seem to maintain relatively low GC levels.

In contrast to frugivores, folivores are often able to persist in moderately disturbed areas (Johns & Skorupa 1987), likely because leaf quantity and quality are often higher in disturbed areas; especially at edges, where light exposure is high (Johns 1988; Ganzhorn 1995; Irwin 2008). In the case of howler monkeys, altered leaf availability and quality might compensate for negative effects associated with small fragments and human impact because of their mainly folivorous diet. This supports the notion that they are capable to habituate to human activities, which likely explains why they do not show increased FGCM levels when living in small and disturbed fragments.

The observed inter-specific differences in responsiveness to human impact could also be the result of a different 'perception' of stressful factors. High population densities in fragments (*Alouatta pigra*: Ostro *et al*. 2001; *Alouatta caraya*: Rumiz 1990) might be a more crucial factor determining GC levels of howler monkeys than the variables we tested in this study. Due to the lack of data on population densities for most fragments, we were not able to include this variable. Future research should aid to unravel whether both species respond differently to various levels of population density.

Generally, our results support previous findings emphasizing a species-specific effect of human disturbance and habitat fragmentation on adrenocortical activity. This specificity might be the reason for inconsistent results revealed by previous studies that used GC levels as markers of physiological stress in a conservation-context (review in: Busch & Hayward 2009). While several studies report elevated GC levels in response to anthropogenic disturbances (Wasser *et al.* 1997; Lucas *et al.* 2006; Martínez-Mota *et al.* 2007; Rangel-Negrín *et al.* 2009; Janin *et al.* 2011; Jaimez *et al.* 2012), others found the reversed or no effect at all. For instance, red-bellied lemurs (*Eulemur rubriventer*: Tecot 2008) and African forest elephants (*Loxodonta africana cyclotis*: Munshi-South *et al.* 2008) show higher GC levels in undisturbed areas than conspecifics in disturbed habitats. Canadian grizzly bears (*Ursus arctos*) exhibit lower GC levels in areas with high poaching activity compared to less disturbed areas (Wasser *et al.* 2004), whereas Alaskan brown bears (*Ursus arctos horribilis*), a closely-related subspecies, show no effect of human presence on GCs (von der Ohe *et al.* 2004). This demonstrates that species likely differ in their sensitivity to disturbances and that not all species respond with a predicable change in GC levels, or, not mutually exclusive, that such a physiological response depends on the degree of the threat perceived.

One important limitation of our study is the lack of data from continuous forests and uneven samples sizes between fragments. Small sample size in fragments where primates are being hunted reflects the challenge of encountering and following arboreal animals that are wary and fearful of humans. Although we only have few samples of *A. hybridus* from two fragments with both types of human impact, FGCM levels of all samples are much higher than those collected in other fragments. Thus, it is conceivable that these differences in FGCM levels reflect true differences, although additional studies should be conducted to back up these results. Another impeding factor for sample collection is the high degree of fragmentation of the remaining habitat of *A. hybridus* (Urbani *et al.* 2008) that exacerbated the access to large forests. Our sample size of fragments with only one type of human impact is also small and future research may clarify which of the two factors (logging or hunting pressure) drives the observed FGCM level elevation in spider monkeys. Nevertheless, by controlling for many variables that can potentially confound GC levels (of which some have been neglected previously) and by comparing GC levels of two species that occur (at least partly) in the same fragments, we provide important evidence for species-specific differences in physiological responsiveness and susceptibility to anthropogenic disturbances.

This study reveals that some species (e.g. howler monkeys) may not be negatively influenced by a moderate level of human activity and suggests that agricultural ecosystems could be of use to conserve them. However, our results also demonstrate that GC levels of some species are elevated in response to anthropogenic disturbances. To what extent these increased FGCM levels reflect a situation of chronic stress with potentially negative fitness consequences or are merely a reflection of an acute adrenocortical reaction to ongoing human activities and as such, might be adaptive to cope with a short-term challenge (without consequences on fitness) is impossible to assess in the absence of longer-term investigations on fitness parameters. It is conceivable, however, that if human impact persists on the long run, this can potentially lead to a state of chronic stress, which might limit the future viability of populations. This study emphasizes the need for the active protection of continuous forests for the conservation of species with low coping abilities (e.g. spider monkeys). Measurements of physiological stress levels should be used to monitor populations living in disturbed areas and to assess the success (concerning amelioration or minimization of stress) of conservation strategies such as corridors connecting fragments and the promotion of alternative sources of animal protein for the human population (e.g. to decrease hunting pressure).

Funding

This work was supported by the German Primate Center; Margot Marsh Biodiversity Foundation´s Primate Action Fund; Rufford Small Grants Foundation and Idea Wild.

Acknowledgments

We thank the Lalinde and De Greiff families for permission to work at ‘Hacienda San Juan de Carare’ and all the persons who allowed us to visit their farms and forests. We thank Prof. Pablo R. Stevenson and the Laboratorio de Ecología de Bosques Tropicales y Primatología for logistic support with sample storage. For their help with sample collection we thank Felipe Alfonso, Natalia Alvis, Nathalia Fuentes, Miguel García, David Gongora, Claudia Lodoño, Natalia Mejía, Jennifer Rey-Goyeneche, Germán Ríos and especially Andres Montes. For their support in the laboratory we acknowledge Andrea Heistermann and Petra Kiesel. We thank Cedric Girard-Buttoz for his statistical advice. Fundación ProAves provided all logistic support for work at Reserva El Paujil. We also thank the Ministerio de Medio Ambiente y Desarrollo Sostenible in Colombia for the permits needed for this study. Fundación Proyecto Primates provided all logistics. The study was conducted completely non-invasively.

CHAPTER 3

Behavioral and physiological responses to fruit availability of spider monkeys ranging in a small forest fragment

with Andrés Link, Andrés Montes-Rojas, Anthony Di Fiore, Michael Heistermann and Eckhard W. Heymann

Abstract

Numerous animal species currently face habitat loss and fragmentation. This might force them to adjust their behavior and dietary strategies in order to survive, especially because fragmentation frequently reduces fruit availability for forest-dwelling species. Food scarcity can result in elevated physiological stress levels, and chronic stress can have detrimental effects on long-term population viability. Few animal species exhibit flexible fission-fusion dynamics and theory predicts that these species reduce intragroup feeding competition by modifying their subgroup size according to resource availability. So far we are lacking detailed knowledge on how species that exhibit fission-fission dynamics adjust their social behavior and grouping patterns in fragments and on how altered food availability influences their stress levels. We collected data on fruit availability, stress hormone levels (measured though fecal glucocorticoid metabolites (FGCM)), subgroup size and aggression of two brown spider monkey (*Ateles hybridus*) groups in a small fragment in Colombia and examined whether fruit availability influences these variables. Against our predictions, spider monkeys ranged in smaller subgroups and had higher FGCM levels when fruit availability was high compared to when it was low. Also contradictory to our predictions, aggression rates of both same-sex and opposite-sex dyads were higher when fruit availability was high. Moreover, the level of female-female aggression was surprisingly high compared to other studies on *Ateles*. Both groups have a more folivorous diet than other spider monkey populations, which might reduce intragroup competition for fruits and permit their atypical grouping patterns. Overall, our findings illustrate that the relationship between fragment size, resource availability, grouping patterns, aggression rates and stress levels can be more complex than assumed so far and that fragmentation can reduce spider monkeys´ ability to decrease intragroup feeding competition via subgroup size. Additional studies are needed to investigate the long-term consequences on the health and long-term persistence of spider monkeys in fragmented habitats.

To be submitted to American Journal of Primatology

INTRODUCTION

The spatio-temporal dynamics of animal groups varies from almost constant cohesion of the same individuals to groups with highly flexible grouping patterns. Most group-living animals form cohesive groups, whereas few others exhibit flexible fission-fusion dynamics, like giant noctule bats (Popa-Lisseanu et al. 2008), giraffes (Shorrocks & Croft 2009), spotted hyenas (Holekamp et al. 1997), dolphins (Würsig 1978), elephants (Couzin 2006), spider monkeys (Klein 1972; Fedigan & Baxter 1984), chimpanzees and bonobos (Itani & Suzuki 1967; Kano 1982; Nishida & Hiraiwa-Hasegawa 1987). In these species members of a stable group form smaller subgroups that vary in size and composition (Kummer 1971; Klein 1972; Symington 1987; Aureli et al. 2008). It has been proposed that these flexible grouping patterns are exhibited to balance the costs and benefits of group-living and to cope with changes in the spatial and temporal availability of food resources (Kummer 1971; Klein & Klein 1977; Dunbar 1987; Symington 1988; Chapman 1990a, 1990b; Strier 1992; Shimooka 2003). Consistent with that notion, several studies have shown a positive relationship between measurements of habitat-wide food availability and subgroup size in both spider monkeys and chimpanzees (Symington 1988; Chapman 1990b; Shimooka 2003; Basabose 2004; Itoh & Nishida 2007; Asensio et al. 2009; Potts et al. 2011).

Most studies that have examined changes in food availability and subgroup size in primates have been conducted in large and continuous forests. However, currently many animal species are confronted with and threatened by habitat loss and habitat fragmentation (Turner 1996; Janson 2000; Pimm & Raven 2000). To ensure their survival animals often have to adjust aspects of their behavior and/or demographics (e.g. activity patterns, population densities, social behavior, group size) in response to these anthropogenic disturbances (Wauters et al. 1994; Menon & Poirier 1996; Hargis et al. 1999; Cristóbal-Azkarate et al. 2004; Boyle & Smith 2010; Umapathy et al. 2011). In addition, many species (particularly frugivorous ones) modify their diet in fragments because fragmentation often results in reduced fruit availability for forest-dwelling animals (Putz et al. 1990; Laurance et al. 1997; Cordeiro & Howe 2001; Arroyo-Rodríguez & Mandujano 2006; Dunn et al. 2010). Availability of leaves, in contrast to fruit, can increase in fragments due to changed microclimatic parameters (Johns 1988; Ganzhorn 1995). Consequently, primates living in fragments are generally forced to reduce the amount of fruit and to increase the amount of leaves in their diet (Tutin 1999; Juan et al. 2000; Lopez et al. 2005; Irwin 2007; González-Zamora et al. 2009; Dunn et al. 2010; Chaves et al. 2012; Abondano & Link 2012; Tesfaye et al. 2013). Primates with flexible grouping patterns might additionally modify subgroup sizes in order to adjust to reduced levels of fruit availability.

Periods of food scarcity, during which animals cannot feed on their preferred food resources and might experience a decreased caloric intake, result in an elevation of glucocorticoid levels in many vertebrate taxa (Cavigelli 1999; Kitaysky et al. 1999; Foley et al. 2001; Chapman et al. 2007b; Gesquiere et al. 2008; Behie et al. 2010). Glucocorticoids (GC) are a class of steroid hormones (in primates mainly cortisol) that play an important role in the vertebrate stress response (Selye 1956; Sapolsky et al. 2000). Short-term elevations of GCs are considered adaptive responses because they provide readily available energy (through gluconeogenesis) which enables vertebrates to respond to acute stressors (Selye 1956; Breazile 1987; Stratakis & Chrousos 1995). Long-term GC elevations, however, can have deleterious effects on reproduction, growth and immune system activity (Pickering et al. 1991; Ellenberg et al. 2007; Charbonnel et al. 2008; Martin 2009; French et al. 2010; Setchell et al. 2010). The current rate of habitat loss and fragmentation worldwide make it crucial to study the link between fruit availability and animal GC levels in forest fragments, especially with regard to the potentially deleterious effects of long-term GC elevations and frequently reduced levels of fruit availability in fragments. Low availability of fruit might increase intragroup feeding competition and thus, species with fission-fusion dynamics are an interesting system in which to study this link because they might be able to reduce high levels of feeding competition by adjusting their grouping patterns.

In this study we investigated the relationship between fruit availability and subgroup sizes in brown spider monkeys (*Ateles hybridus*) ranging in a small forest fragment (65 ha) in Colombia. In addition to this “traditional approach” we incorporated GC measurements as a proxy for physiological stress levels and investigated how fruit availability influenced stress levels. Moreover, we examined the relationship between aggression rates and GC levels, and between fruit availability and aggression rate. We collected data on habitat-wide fruit availability, fecal glucocorticoid metabolite (FGCM) levels, subgroup size, and agonistic behavior of two study groups. Due to the physiological function of GCs in the vertebrate stress response (energy release), we predicted that FGCM levels of brown spider monkeys would be lower when fruit availability was high compared to when fruit availability was low. We also predicted that spider monkeys would reduce their subgroup size in times of reduced fruit availability to avoid intragroup feeding competition, and increase subgroup size in times of high fruit availability (Chapman 1990b; Shimooka 2003; Asensio et al. 2009). Additionally when resource availability is low, intragroup feeding competition might increase and result in more aggressive interactions (Boccia et al. 1988; Barton et al. 1996). Thus, we predicted that aggression rates between spider monkeys would be higher during times of lower fruit availability. The relationship between fruit availability and aggression might be more pronounced in females due to the tight link between access to resources and their reproductive success (Trivers 1972). Moreover, high population densities (often found in small fragments) can result in high levels of aggression (Cristóbal-Azkarate et al. 2004, 2006; Macdonald et al. 2004). Thus, we predicted that levels of aggression could be elevated compared to studies in continuous forests. Many studies have found a positive relationship between aggression and increased GC levels in many vertebrate taxa (Creel et al. 1996; Wallner et al. 1999; Goymann et al. 2001; Hackländer et al. 2003; Creel 2005; Pride 2005; Crockford et al. 2008; Emery Thompson et al. 2010). Thus, we predicted that FGCM levels would be higher when aggression rates were higher in the study groups.

METHODS

Study Site and Study Species

This study was conducted in a small forest fragment located within the private cattle ranch "Hacienda San Juan del Carare" (06° 43' N, 74° 09' W; 150-200 m a.s.l) in Colombia. At the site spider monkeys have been habituated, identified and studied since 2007 (Link et al. 2010). The fragment comprises 65 ha of seasonally flooded tropical rainforest, located between the Central and eastern cordilleras of the Andes in the Magdalena River Valley. The area has bimodal rainy seasons, with peak rains occurring from March to May and from October to November. During intense rainy seasons, the fragment regularly floods entirely for a period of several weeks up to three months. The area receives an annual median rainfall of 3496 mm, has a mean temperature of 27.9 °C and a mean humidity of 80% (IDEAM 2008).

Brown spider monkeys are endemic to Colombia and Venezuela. They are considered to be one of the 25 most endangered primate species worldwide (Mittermeier et al. 2012) and are listed as critically endangered by the IUCN because they have experienced a dramatic population decline over the past 45 years. The most severe threats for the species´ survival are habitat loss, fragmentation and high hunting pressure (Urbani et al. 2008; Link et al. 2013).

Data Collection

Fecal Sample Collection

Between July 2010 and April 2012 we collected a total of 470 fecal samples from two groups of brown spider monkeys (Table 1). Due to severe floods, fecal sample collection, as well as behavioral and phenological data collection, were interrupted from November to December (2010), March to June 2011, and November to December (2011). These floods were due to heavy rains produced by the 'La Niña' phenomenon. We collected fecal samples from identified adult and subadult individuals and recorded sex and age-class, reproductive state of females (when identifiable), collection time and date for every sample. For the time in which females were nursing dependent offspring we categorized them as lactating. Pregnancy cannot reliably be detected by observation in this species. Therefore, we inferred the pregnancy status of females post hoc, based on their known parturition dates in combination with average gestation length of spider monkeys (~7.5

months (Di Fiore and Campbell, 2007)) to determine the approximate conception date and to assess which fecal samples had been collected during gestation. We categorized all females that did not fall within either of these two classes (pregnant or lactating) as cycling (even though their actual cycle status was unknown).

We homogenized the fecal bolus and removed undigested matter such as large seeds before collecting a sample. We placed approximately 0.5 g of feces into a 15 ml polypropylene tube pre-filled with 5 ml of 96% ethanol and subsequently shook the tube until the feces were suspended in the solvent (Shutt et al. 2012; Rimbach et al. 2013). We kept the samples at ambient temperatures until we extracted them in the evening (see below).

Table 1. Group composition and number of fecal samples collected per individual in both study groups.

Study group	Adult females	No. of fecal samples	Adult males	No. of fecal samples	Subadult males	No. of fecal samples
SJ-1	Ba	48	Nw	50	Vt	9
	Pe	43	Wa	39	Db	11
	Vi	34	Pk	31		
	Dl	49	Rk	56		
	Ku	39				
SJ-2	Cle	13	Ky	14	Het	1
	Gat	12				
	Man	11				
	Mel	7				
	Iwa	3				

Subgroup Size and Aggressions

We conducted all-day focal animal sampling on all adult and subadult individuals of both study groups and collected a total of 1503 h of focal data on SJ-1 (2010: 392 h; 2011: 783 h; 2012: 328 h) and a total of 186 h of focal data on SJ-2 (2010: 36 h; 2011: 122 h; 2012: 28 h) between July 2010 and April 2012. All individuals could reliably be identified and we attempted to balance sampling effort amongst all individuals. We recorded subgroup composition upon encountering a subgroup and thereafter every 15 min. for the entire length of the focal animal sample. Changes in subgroup composition were recorded whenever one or more individuals left (“fissioned”) or joined (“fused”) the subgroup containing the focal animal. We considered individuals as belonging to the same subgroup when they were at a distance of ≤50 m from at least one other subgroup member following a chain rule (Asensio et al. 2009, 2012).

Finally, given the conspicuous nature of aggressive interactions (chasing, grabbing, and biting) in spider monkeys, we recorded all aggressions during both continuous focal observations and ad libitum sampling and identified the actor and recipient of each antagonistic encounter whenever possible.

Fruit Availability

Biweekly, we monitored eight phenological transects (6.45 km) following the methods described in detail by Stevenson (2002). Briefly, we registered all trees that bore fruit and/or flowers and, recorded the plant species or morphospecies. In order to estimate the sampling area we measured the perpendicular distance of each tree to the center of the transect. To calculate the sampling area we determined the average perpendicular distance to the transect (multiplied it by two) and multiplied it by the total length of transects monitored. Finally, we measured each tree´s diameter at beast height (DBH) at approximately 1.3 m high in order to derive estimates of fruit availability based on "basal area" estimation (see below).

Data Analysis

Steroid Extraction and Analysis

After fecal sample collection, we determined fecal wet weight by calculating the difference between the weight of the tube before (tube plus ethanol) and after (tube, ethanol and feces) addition of the sample to the tube. For hormone extraction, we shook the tubes rigorously for 5 min and thereafter, centrifuged the fecal suspension using a manually-operated centrifuge for 1 min (Shutt et al. 2012; Rimbach et al. 2013). We decanted ~2 ml of each resultant fecal extract into 2 ml polypropylene tubes, covered them with parafilm, labeled each tube and stored them at ambient temperatures (~25°C) in a dark place. In a previous study we showed that storing fecal extracts this way did not affect FGCM levels (Rimbach et al. 2013). Every two months we transported the extracts to the Universidad de Los Andes, Bogotá where we stored them at -20°C until shipment to the endocrinology laboratory at the German Primate Center for analysis.

We analyzed all fecal samples using a previously validated (Rimbach et al. 2013) group-specific 11ß-hydroxyetiocholanolone enzyme-immunoassay (EIA), designed to measure 5beta-reduced metabolites of glucocorticoids (Ganswindt et al. 2003) with a 3α,11ß-dihydroxy structure. We performed the EIA as described in detail by Heistermann et al. (2004). Depending on the original concentration of the sample, we diluted extracts 1:250–1:2000 in assay buffer prior to steroid measurement and took duplicate aliquots to assay. Intra- and inter-assay coefficients of variation were 7.4% and 13.0% for low-value and 6.1% and 7.8% for high-value quality controls.

Subgroup Size and Aggressive Interactions

We only included adult and subadult individuals in calculating subgroup size because infants and juveniles always ranged in the same subgroup as their mothers. To avoid pseudoreplication of subgroup sizes during the day, we used the size of the first subgroup encountered each day for subsequent analyses (Asensio et al. 2009; Schaffner et al. 2012). However, the size of the first subgroup could potentially be smaller than subgroups during the rest of the day (individuals might sleep dispersed). Therefore, we also calculated the modal subgroup size per day and ran all analyses with both measurements of subgroup size. The analyses did not reveal differences and therefore we only report the results of the analyses including first subgroup size.

To calculate aggression rates we used only agonistic interactions for which all participants, actor(s) and recipient(s), were identified (N= 294 aggressive interactions). We calculated aggression rates per dyad and counted coalitionary aggressions (e.g. when two individuals jointly aggressed one individual) as two aggressive interactions. For example when A + B aggressed C, we counted this as one aggression from A towards C and one from B towards C. To account for the fact that aggressions can only occur between individuals that are present in the same subgroup, we corrected for the time actor and recipient were observed together in a subgroup by dividing the number of aggressions between a dyad by the time both individuals were observed together in the same subgroup.

Fruit Availability Index (FAI)

We calculated two different fruit availability indices (FAI) (1) number of fruiting trees/area (density) and (2) basal fruiting area of trees (m^2/ha). To avoid overestimation of fruit production by trees that have long fruiting periods we assumed a triangular distribution of fruit production for the calculation of the basal fruiting area. Thus, we assumed that the fruit production for each individual tree increased up to the median fruit period and subsequently decreased (Stevenson et al. 1994). Accordingly, for trees that bore fruit for more than one consecutive phenological sampling period (i.e. for longer than two weeks) we distributed their basal area over the sampling periods in a way that the proportion of the basal area in each period followed the coefficients of the Pascal´s triangle. Both indices of fruit availability were correlated (Spearman: R= 0.61, T= 3.68, P= 0.001 N= 25), and because basal fruiting area of a tree is a reasonably accurate index of its fruit crop size (Leighton & Leighton 1982; Peters et al. 1988), we used only basal fruiting area in further analysis.

Statistical Analyses

To assess the effect of fruit availability on FGCM levels (Model 1), we matched each fecal sample with the corresponding FAI on the date the fecal sample had been collected (FAI was kept constant in a way that the subsequent two weeks were presumed to be the same as the FAI at the start of the period). We used overall aggression rate in the groups (the total of all aggression events seen per day) to investigate the effect of aggression on FGCM levels and we investigated the effect of fruit availability on dyadic aggression rates. To investigate the effect of aggression rate on FGCM levels (Model 2), we matched each fecal sample with the overall aggression rate observed on the day before sample collection (thereby accounting for a ~24 h time-lag before glucocorticoid metabolite excretion in feces of *A. hybridus* (Rimbach et al. 2013)). We used linear mixed models (LMM, (Baayen 2010)) with a Gaussian error distribution for both models. As control variables we used sex, age, female reproductive state, fecal sample collection time (Model 1 +2), and size of first subgroup (Model 1) as some of these variables have been shown to affect FGCM levels in *A. hybridus* (Rimbach et al. 2013). We used group and individual ID as random factors in both models. In exploratory runs of these models, we tested for an interaction between fruit availability and subgroup size. The interaction was not significant (P≥0.05) and thus, not included into the final models.

Before running the models, we checked the distributions of the response and all predictor variables. To achieve a more symmetrical distribution, we log transformed the response variable (FGCM levels) and to obtain comparable estimates we z-transformed all predictor variables. By visually inspecting qqplots and scatterplots of the residuals plotted against fitted values, we checked for the assumptions of homogeneous and normally distributed residuals. The plots did not reveal any obvious violations of these assumptions. To assess model stability, we ran diagnostics (dfbetas) that did not suggest the existence of influential cases, and variance inflation factors indicated that there was no collinearity between variables (Field 2005; Zuur et al. 2009, 2010). To derive variance inflation factors we used the function vif of the R-package car (Fox & Weisberg 2011). We fit the models with the lmer function from the lme4 package (Bates & Maechler 2010) in R 2.15.1 (R Development Core Team 2012). To determine the significance of the full models (all fixed and random effects) compared to the corresponding null models (only random effects) we used likelihood ratio tests (R function ‘anova’). Finally, we used the function pvals.fnc of the package ‘language R’ (Baayen 2010) to determine P-values based on Markov Chain Monte Carlo (MCMC) sampling (Baayen 2011).

We performed a Spearman rank correlation to test whether the two fruit availability indices (density and basal fruiting area of trees) were correlated (using Statistica 10). We used Mann-Whitney U tests to assess whether the size of the first subgroup changed between years and to test whether the size of subgroups differed between times of high and low fruit availability (using Statistica 10). To evaluate whether aggression rates of different dyads (same-sex and opposite-sex dyads) differed between times of high and low fruit availability we also used Mann-Whitney U tests. To avoid type I errors we adjusted the level of significance through a Bonferroni correction to a critical value of P≤0.01. We defined an index of fruit availability as ‘high’ when it was higher than the median and as ‘low’ when it was lower than that. Results from nonparametric tests are presented as the median and quartiles (range when appropriate). All statistical tests were two-tailed, and the significance threshold was set at P≤ 0.05.

All research was non-invasive and complied with protocols approved by the German Primate Center and the Animal Welfare and Use and IACUC committees at New York University and the University of Texas at Austin. Further, the research adhered to the legal requirements of Colombia and to the American Society of Primatologists Principles for the Ethical Treatment of Non-Human Primates.

RESULTS

Aggression rates ranged between 0.01/h – 0.5/h (Fig. 1) and differed between different types of dyads. The aggression rate of female-female and male-female dyads was significantly higher when fruit availability was high compared to when it was low (Table2, Fig. 1). Aggression rates of male-male and female-male dyads (the latter only includes subadult males) did not differ between times of high or low fruit availability.

Table 2. Median hourly aggression rates of different dyad types when fruit availability (FAI) was high and low (only includes adult and subadult individuals).

Dyad (actor-recipient)	Median (range) aggression rate/h low FAI	high FAI	Mann-Whitney U	No. of aggressions (% of all observed)
female-female	0.018 (0.012-0.3)	0.028 (0.012-0.5)	Z= 4.09 P< 0.0001	70 (23.8)
female-male[1]	0.019 (0.012-0.16)	0.020 (0.012-0.028)	Z= -0.50 P= 0.61	15 (5.1)
male-female	0.020 (0.015-0.11)	0.033 (0.013-0.25)	Z= 4.04 P< 0.0001	88 (29.3)
male-male[2]	0.023 (0.015-0.13)	0.038 (0.02-0.11)	Z= 1.53 P= 0.12	54 (18.3)

[1] Only includes subadult males. [2] In 62.9 % of these aggressions a subadult male was the recipient.

Fruit availability in the forest fragment ranged from 1.73 m^2/ha to 60.52 m^2/ha during the study period (median= 15.77 m^2/ha; Fig.2). Median subgroup size was five adult and subadult individuals in SJ-1 and three in SJ-2. Subgroup size of SJ-1 significantly increased from 2010 to 2011 (Mann-Whitney U test: Z= -2.99, P= 0.002) and from 2011 to 2012 (Mann-Whitney U test: Z= -3.57, P< 0.0001; Fig. 3.a). Subgroup size of SJ-2 did not change significantly between 2010 and 2011 (Mann-Whitney U test: Z= 1.32, P= 0.18), but increased significantly from 2011 to 2012 (Mann-Whitney U test: Z= -2.96, P= 0.003; Fig. 3.b). Subgroup size was significantly larger in times of low fruit availability than when fruit availability was high (Z= 3.28, P= 0.001; Fig. 4).

Overall, the full model (Model 1) estimating the influence of fruit availability on FGCM levels differed significantly from the null model (X^2= 123.43, df= 7, P< 0.0001, N= 371 fecal samples). More specifically, fruit availability had a positive effect on FGCM levels (LMM: Estimate ± SE: 0.22 ± 0.03, T= 5.82, P_{MCMC}= 0.001; Table 3). The full model (Model 2) investigating the effect of aggression rate on FGCM levels did not differ from the null model (X^2= 3.34, df= 3, P= 0.34, N= 97 fecal samples).

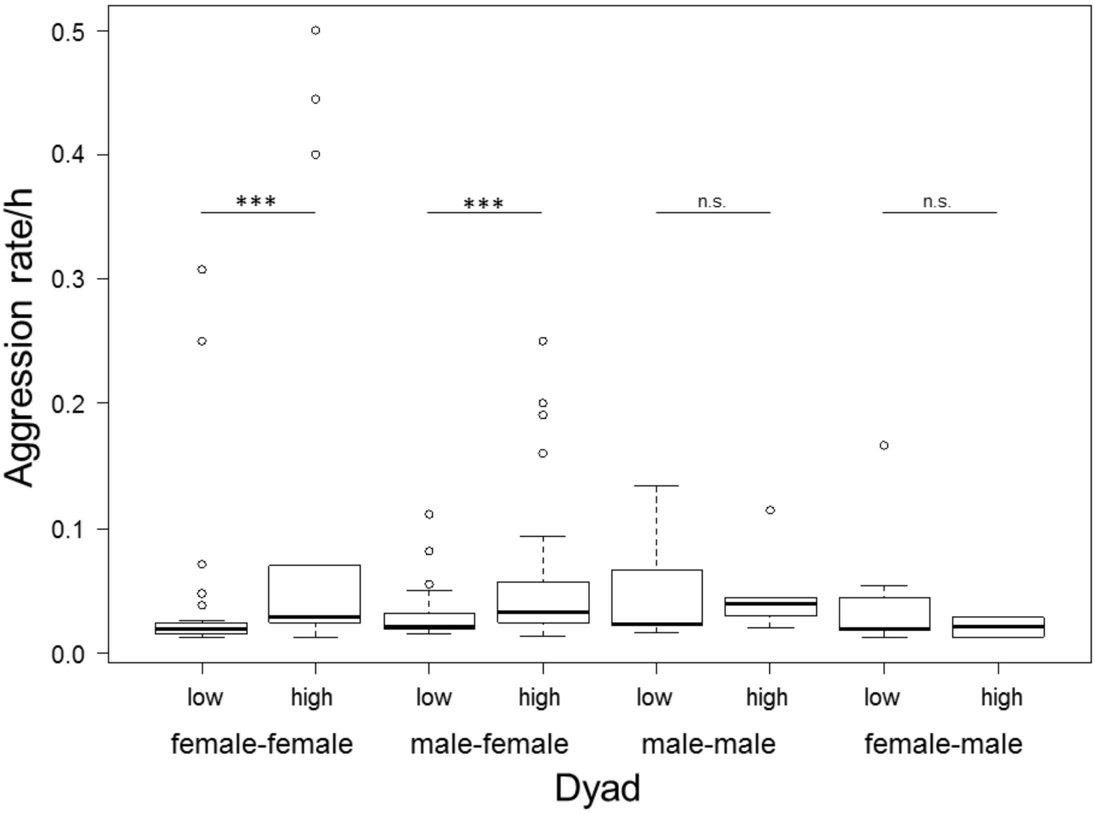

Figure 1. Box plots showing the range, upper and lower quartiles, and medians for aggression rates of different dyad types in times of high and low fruit availability.

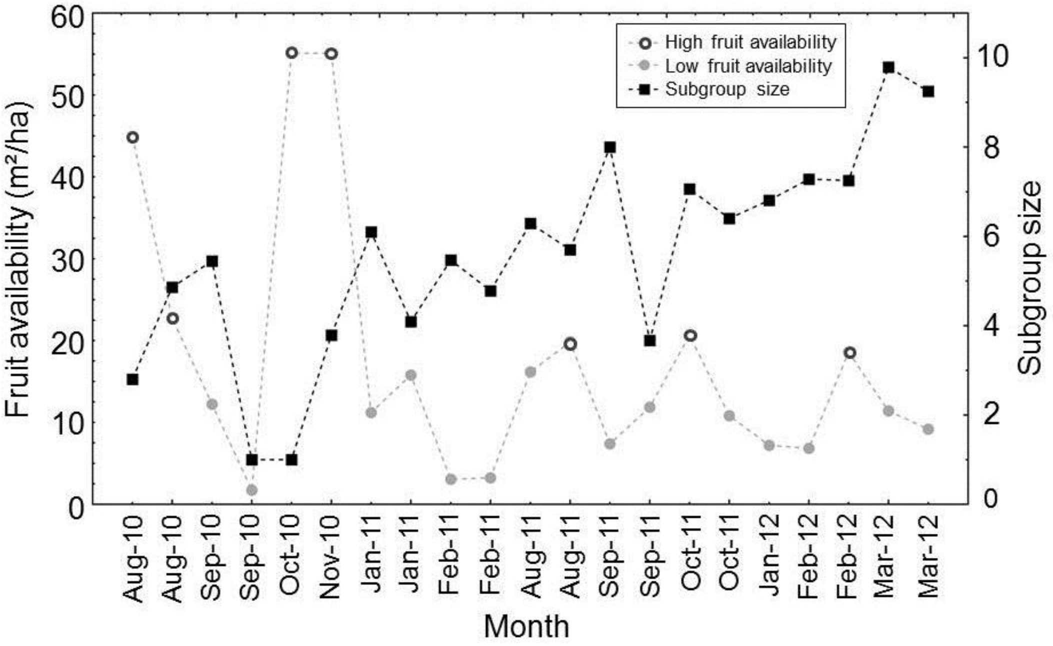

Figure 2. Temporal variation in fruit availability (basal fruiting area) and subgroup size.

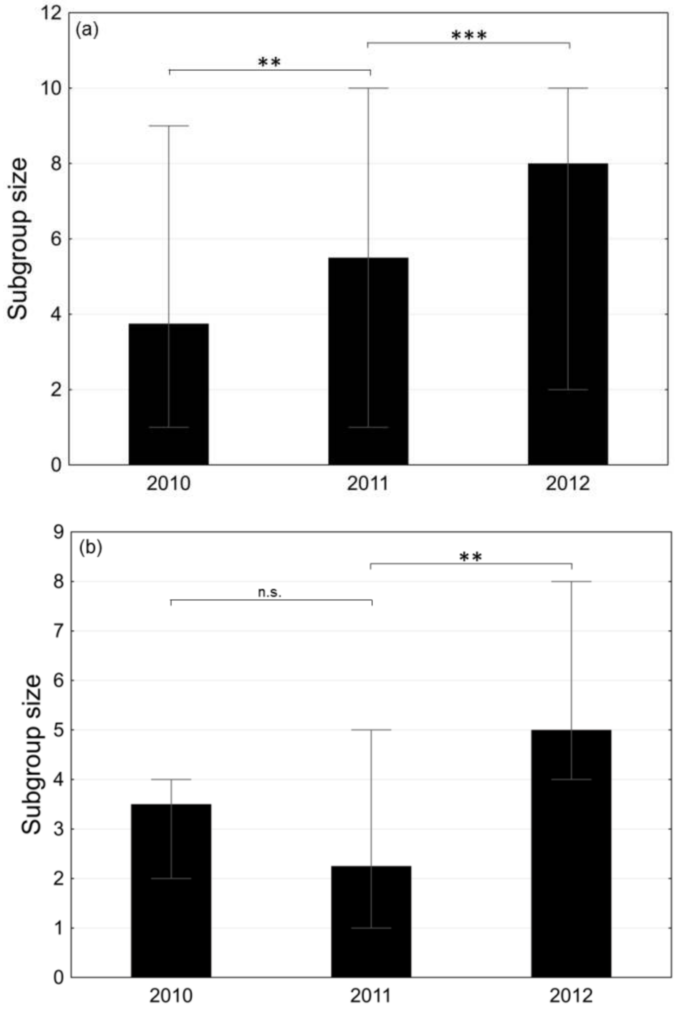

Figure 3. Annual variation in median subgroup size in (a) SJ-1 and (b) SJ-2 (bars indicate the range of observed subgroup sizes).

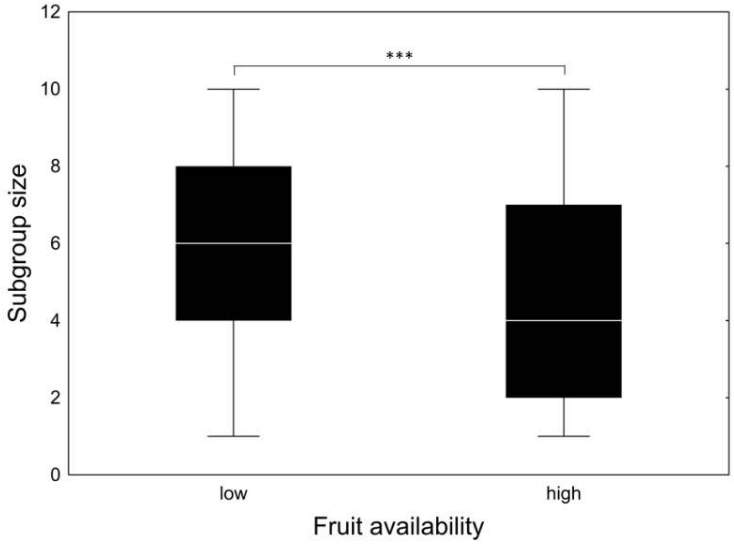

Figure 4. Range, upper and lower quartiles and medians for subgroup sizes in times of high and low fruit availability (median indicated by grey line).

Table 3. Results of Model 1 examining the effect of fruit availability on log transformed FGCM levels. Variables that significantly influenced FGCM levels appear in bold.

Variable	*Estimate ± SE*	*t*	P_{MCMC}
Intercept	5.56 ± 0.12	43.89	**0.0190**
Fruit availability	0.22 ± 0.03	5.82	**0.0001**
Subgroup size	0.05 ± 0.03	1.45	0.1458
Time	-0.00 ± 0.00	-9.16	**0.0001**
Sex	0.37 ± 0.14	2.65	**0.0176**
Age	-0.43 ± 0.16	-2.67	**0.0118**
Lactating-cycling	0.06 ± 0.14	0.48	0.6180
Pregnant-cycling	0.70 ± 0.12	5.80	**0.0001**
Pregnant-lactating	-0.63 ± 0.14	-4.39	**0.0002**

DISCUSSION

Overall, our analyses of the relationship between fruit availability and FGCM levels, subgroup size, and agonistic interactions in brown spider monkeys produced unexpected results. Against our prediction that FGCM levels would be higher when fruit availability was low, our results show the opposite pattern where spider monkeys showed elevated FGCM levels in times of high fruit availability. Also contrary to our predictions, spider monkeys ranged in smaller subgroups when fruit availability was high compared to when it was low, and the aggression rate did not influence FGCM levels. Aggression rates at San Juan were generally comparable to other studies conducted on *Ateles* (Fedigan & Baxter 1984; Symington 1987; Asensio et al. 2008; Slater et al. 2009). Interestingly, though, we observed high rates of aggression between adult females. Female-female aggression is usually very rare in spider monkeys and the high level observed in this population might be a response to a high level of feeding competition.

Generally, primates that exhibit high fission-fusion dynamics increase subgroup size when feeding competition is low and decrease subgroup size when it is high (e.g., Symington, 1988; Chapman et al., 1995; Itoh and Nishida, 2007). However, the relationship between these two variables is not in all cases predictable, and several studies report results deviating from this general pattern (Newton-Fisher et al. 2000; Hohmann & Fruth 2002; Hashimoto et al. 2003; Moscovice et al. 2007; Wakefield 2008). In accordance with the latter studies, our results show a negative relationship between fruit availability and subgroup size. This demonstrates that additional determinants, e.g. demographic and social factors (Chapman 1990b; Lehmann & Boesch 2004; Shimooka 2005), might also affect grouping patterns of species with fission-fusion dynamics, especially when considering populations living in intervened habitat.

In small-sized forest fragments, space is restricted and consequently, primates might be forced to reduce the size of their home range (Cristóbal-Azkarate & Arroyo-Rodríguez 2007; Wong & Sicotte 2007; Irwin 2008; Arroyo-Rodriguez & Dias 2010). In addition, fruit availability is often reduced in fragments (e.g. Putz et al., 1990; Arroyo-Rodríguez and Mandujano, 2006). Both of these processes can intensify intra- as well as intergroup competition for space and food resources. During most of the study period habitat-wide fruit availability in the study fragment was relatively low and both study groups have a more folivorous diet than is generally observed for spider monkeys (> 40% of feeding time (Montes-Rojas 2012; Abondano & Link 2012)). This pattern of dietary shift towards leaves has previously been observed in primates inhabiting small fragments, including other populations of spider monkeys (González-Zamora et al. 2009; Chaves et al. 2012). While this diet likely reflects the low availability of fruit, it may also be due to, in part, the fact that young leaves are often highly abundant in fragments, especially at edges with elevated light exposure (Johns 1988, 1991; Lynch & González 1993; Ganzhorn 1995, 1997; Irwin 2008). Further, it is possible that spider monkeys are able to rely on leaves in this fragment because there are many fig trees (*Ficus* spp.) that permanently provide new leaves (Montes-Rojas 2012). In addition, leaves growing in this particular fragment might be highly nutritional because the soil is likely to be very rich in nutrients, given that during floods the entire forest fragment receives nutrients carried by the Magdalena River (Restrepo et al. 2006). Moreover, young leaves are typically more digestible than mature leaves, have fewer chemical defenses (e.g. phenols and alkaloids) and a higher nutritional value compared to mature leaves (Waterman & Kool 1994; Bilgener 1995; Coley & Barone 1996), and therefore can constitute an important food resource. It seems likely that spider monkeys at San Juan avoid or reduce direct feeding competition by increasing the amount of young leaves in their diet when fruit availability is low. In turn, it seems that this diet permits their atypical pattern of ranging in larger subgroups when fruit availability is low.

The pattern of high levels of female-directed aggression by males is commonly observed in spider monkeys and is interpreted as a form of social control over females (Campbell 2003; Slater et al. 2008; Link et al. 2009), which fits the general notion that access to females is the main limiting factor of male reproductive success, whereas food resources are the limiting factor for females (Trivers 1972; Lee 1987; Koskela et al. 1998; Higginbottom 2000). Interestingly, we observed higher rates of aggression in times of high fruit availability in all dyads (albeit this difference was only significant for male-female and female-female dyads). Further, we did not find an effect of aggression rate on FGCM levels. This is unusual and does not necessarily mean that GC levels of spider monkeys do not increase in response to aggression. This result might rather be due to the fact that we were not able to collect fecal samples of all individuals involved in every aggressive interaction on the day after those incidents.

Moreover, we observed an unusually high level of aggressive interactions between adult females. The rareness of female-female aggression in most spider monkey populations has been interpreted as evidence that they generally manage to reduce or avoid intragroup feeding competition through fission-fusion dynamics. This seems to be impaired in the study population, and higher levels of female-female aggression when fruit is more abundant might reflect a higher level of feeding competition than when fruit is less abundant. This could potentially also explain the unexpected results of higher FGCM levels in times of high fruit availability, which might be the proximate cause that triggers the decrease in subgroup size. However, FGCM levels might also have been influenced by other social factors (e.g., grooming) that we did not include in this study.

Further, both study groups might experience a high level of intergroup competition for space. The forest fragment is very small (65 ha), and the home range of both study groups only comprises roughly 30 ha. Thus, size of the entire fragment available to these two groups is much smaller than the typical home-range size for a single group (average 278 ha, range: 80 ha – 963 ha) in other spider monkey populations in continuous forests (reviewed in Di Fiore et al., 2011). Moreover, spider monkey population density is very high in this small fragment (42.8 ind./km^2 (Link et al. 2010)), a phenomenon that frequently occurs in fragments immediately after forest fragmentation (Lovejoy et al. 1986; Estrada et al. 2002; Boyle & Smith 2010). High population density and small home-range size can intensify intergroup competition for space and potentially also resources. In this study, one group increased its median subgroup size over each year of the entire study period and the other one between 2011 and 2012. Ranging in larger subgroups can be advantageous during intergroup encounters and can increase the probability of success in competition for space and resources (Packer et al. 1990; McComb et al. 1994; Scarry 2013). In line with that, we have observed an increase in home-range overlap between the groups and an increase in both frequency and intensity of intergroup encounters, especially in 2012 (Rimbach, Link, Montes-Rojas; pers. observations), supporting the idea that intergroup competition is one important factor shaping the grouping patterns of these groups.

Although we derived our predictions from current socio-ecological and behavioral theory, not all results of this study meet our predictions. This fact illustrates that forest fragmentation can influence the grouping patterns of spider monkeys in an unexpected manner and that the relationship between fragment size, resource availability, aggression rates and stress levels is more complex than assumed so far. To fully understand how food availability influences their grouping patterns in fragments, it will be crucial to investigate also changes in the availability of other food items (e.g., young leaves, flowers and decaying wood). Future studies are necessary to clarify the exact relationship between ‘complete’ resource availability, subgroup size and GC levels in spider monkeys, and the potential effect on population health and viability in fragments. Such studies could incorporate measurements of body condition and energetic balance (using urinary C-peptide measurements; e.g., Deschner et al. 2008; Emery Thompson & Knott 2008; Girard-Buttoz et al. 2011). Even though our results suggest that adopting a more folivorous diet permits spider monkeys to adjust to reduced fruit availability and to persist in small fragments, it is less than certain that these groups can be sustained in the long-term in such small fragments. Therefore, the conservation of large forests is needed to ensure their long-term survival.

ACKNOWLEDGMENTS

We thank the Lalinde and De Greiff families for allowing us to conduct our research at "Hacienda San Juan de Carare". For the permits needed to conduct this study we thank the Ministerio de Medio Ambiente y Desarrollo Sostenible in Colombia. We thank Associate Prof. Pablo R. Stevenson for logistic support with sample storage and we thank Felipe Alfonso, Natalia Alvis, Nathalia Fuentes, Miguel Garcia, Claudia Lodoño, Sara Ramirez and Luisa Salazar for their help with sample collection. We acknowledge Andrea Heistermann and Petra Kiesel for their support in the laboratory. Fundación Proyecto Primates provided all logistics. This study has been funded by the German Primate Center, Ecopetrol, National Science Foundation and National Geographic Society and the research adhered to the legal requirements and animal care regulations of Germany and Colombia. The Ministerio de Medio Ambiente y Desarrollo Sostenible in Colombia authorized the research at the field site in Colombia.

CHAPTER 4

Interspecific infanticide and infant-directed aggression by spider monkeys (*Ateles hybridus*) in a fragmented forest in Colombia

with Alejandra Pardo-Martinez, Andrés Montes-Rojas, Anthony Di Fiore and Andrés Link

Abstract

Interspecific aggression amongst nonhuman primates is rarely observed and has been mostly related to scenarios of resource competition. Interspecific infanticide is even rarer, and both the ultimate and proximate socio-ecological factors explaining this behavior are still unclear. We report two cases of interspecific infanticide and five cases of interspecific infant-directed aggression occurring in a well-habituated primate community living in a fragmented landscape in Colombia. All cases were initiated by male brown spider monkeys (*Ateles hybridus*) and were directed toward infants of either red howler monkeys (*Alouatta seniculus*: *n* = 6 cases) or white-fronted capuchins (*Cebus albifrons*: *n* = 1 case). One individual, a subadult spider monkey male, was involved in all but one case of interspecific infanticide or aggression. Other adult spider monkeys participated in interspecific aggression that did not escalate into potentially lethal encounters. We suggest that competition for food resources and space in a primate community living in high population densities and restricted to a forest fragment of ca. 65 ha might partly be driving the observed patterns of interspecific aggression. On the other hand, the fact that all but one case of interspecific infanticide and aggression involved the only subadult male spider monkey suggests this behavior might either be pathological or constitute a particular case of redirected aggression. Even if the underlying principles behind interspecific aggression and infanticide are poorly understood, they represent an important factor influencing the demographic trends of the primate community at this study site.

American Journal of Primatology, 74:990-997 (2012)

Introduction

Interspecific high-intensity aggression - or aggression seemingly aimed at wounding or killing an individual of another species - is a rarely observed behavior in mammals (Cassini 1998; Peiman & Robinson 2010; Cotter et al. 2012) except in the context of predator–prey interactions. Among primates, the most common form of interspecific lethal aggression is also observed in those few species that prey upon other sympatric primates (e.g., chimpanzees: (Stanford et al. 1994; Stanford 2002; Watts & Mitani 2002)). Although rarely observed, some cases of interspecific aggression have been also reported among primate species outside of predator–prey interactions, mainly in the context of interspecific resource competition at actual feeding sites (Heymann 1990; Stevenson et al. 2000). For example, in Costa Rica, aggression between white-faced capuchins (*Cebus capucinus*) and black-handed spider monkeys (*Ateles geoffroyi*) can arise at heavily contested food resource sites (Rose et al. 2003). At the same study site, Rose et al. (2003) also observed aggression between white-faced capuchins (*C. capucinus*) and mantled howler monkeys (*Alouatta palliata*) that was not obviously associated with resource competition.

Infanticide - the act of killing a dependent offspring usually committed by a conspecific - is a fairly common behavior in a wide variety of taxonomic groups, including beetles (Trumbo 1990), fish (Kondoh & Okuda 2002), birds (Weisheit & Creighton 1989), rats (Boice 1972), lions (Packer & Pusey 1983), nonhuman primates (Butynski 1982; Newton 1988; Agoramoorthy & Rudran 1995) and humans (Riches 1974; Hausfater 1984). Although many of the hypotheses forwarded to explain the occurrence of infanticide are related to male reproductive strategies or intersexual conflict, some individuals commit infanticide without any obvious prospect of a personal fitness gain. Several adaptive hypotheses have been proposed to explain the potential benefits of infanticide, particularly to males, who are responsible for most cases of infanticide reported in the primate literature: [1] the use of an infant as a food resource, [2] the elimination of handicapped or ill-timed offspring by a parent which could lead to improved lifetime reproductive success, [3] the elimination of a potential future competitor for resources, and, specifically for males, [4] the elimination of the offspring of male rivals and the hastening of a female's return to normal cycling following loss of her infant (the "sexual selection" hypothesis: (Hrdy 1979; van Schaik & Janson 2000).

Additionally, the "social pathology" hypothesis for infanticide has been proposed to explain certain cases of infanticide as an apparently maladaptive social behavior or an epiphenomenon associated with aggression generally, which conveys no apparent benefits to committing infanticide (Curtin 1977; Curtin & Dolhinow 1978; Hrdy 1979; Sussman et al. 1995). Under some circumstances, wild animals might be exposed to high stress levels (e.g., in captivity or in recently disturbed areas) and might display unnatural and destructive behaviors (Dolhinow 1977). Under this scenario, infanticide should be unpredictable and often associated with the vulnerability of young animals (Curtin & Dolhinow 1978).

Interspecific infanticide is an extremely rare behavior amongst mammals (see (Cassini 1998)). One of the few examples of interspecific infanticide among mammals has been documented for South American sea lions (*Otaria flavescens*), which occasionally kill infants of South American fur seals (*Arctocephalus australis*) at islands where both species reproduce in sympatry (Cassini 1998). Subadults are the main perpetrators of such infanticide, and it has been hypothesized that these cases of infant killing are in fact a byproduct of sexual conflict among these individuals (Cassini 1998). Interspecific infanticide has also been reported in tropical house wrens (*Troglodytes aedon*) by rufous-and-white wrens (*Thryothorus rufalbus*) (Freed 1987). Cases of infanticide between these two wren species were almost certainly based on food resource competition, since nestlings were killed only during a pronounced food shortage. Barn owls (*Tyto alba*) have been reported to increase their reproductive success by killing the broods of tawny owls (*Strix aluco*) in times of breeding place shortage (Mátics et al. 2008). The underlying causes of interspecific infanticide are still poorly understood, as the key adaptive hypothesis proposed to explain conspecific infanticide (e.g., the “sexual selection” hypothesis: (Hrdy 1979; van Schaik & Janson 2000) cannot be applied to cases of interspecific infant killing.

Here, we report several cases of interspecific infanticide and infant-directed aggression that we observed in a well-habituated primate community living in a fragmented landscape in Colombia. We describe all events where at least one member of a group of brown spider monkeys (*Ateles hybridus*) initiated high intensity or lethal aggression toward infants of other sympatric primates.

Methods

Study Site

The study took place in a forest fragment located within the private cattle ranch “Hacienda San Juan del Carare” (06°43´N, 74°09´W), where studies on the behavioral ecology of brown spider monkeys (*A. hybridus*), red howler monkeys (*Alouatta seniculus*) and white-fronted capuchin monkeys (*Cebus albifrons*) have been conducted since 2007. The study site is located near the Magdalena River between the eastern and central cordilleras of the Andes of Colombia. The study site is a thin forest patch of roughly 65 ha of seasonally flooded tropical rainforest. The forest fragment runs along the western bank of the San Juan River and is surrounded on its western border by natural savannas and wetlands and on its eastern border by the San Juan River. In 2007, prior to the onset of our long-term work at the site, the forest was isolated from a larger expanse of forest by the clearing of trees and conversion to pastures of land to the north and south of the fragment. Although dispersal to other nearby fragments is feasible for *C. albifrons*, which can use low vegetation to cross between fragments, it is likely to be severely constrained for both *A. seniculus* and *A. hybridus*. All three diurnal species in the primate community at the study site live at high population densities: 42.8 ind./km2 of *A. hybridus*, 98.8 ind./km2 of *Alouatta seniculus,* and 130.3 ind./km2 of *C. albifrons* (Link et al. 2010).

Data Collection

Data were collected on one of the two groups of brown spider monkeys that live within the forest fragment at San Juan. The study group consisted of three adult males (one of whom disappeared partway through the study, in June 2011), five adult females with offspring, and one subadult male; all group members were well habituated and individually recognized. We conducted behavioral follows from dusk to dawn on focal individuals from this group and used all day focal animal sampling (Altmann 1974) to collect behavioral, ranging, and foraging data. These data were complemented with ad libitum data on conspicuous behaviors such as aggression and intergroup encounters. We also conducted behavioral follows on six groups of howler monkeys and two groups of white-faced capuchins at the site. During the study period (January 2010 to December 2011), all three sympatric taxa were followed by at least one researcher, obtaining roughly 3000, 1500, and 800 hr of behavioral follows on groups of *A. hybridu*s, *Alouatta seniculus,* and *C. albifrons*, respectively. All cases of interspecific infanticide were recorded during behavioral follows, and, given the conspicuous nature of intergroup aggression, we were able to record in detail all such events. This research adhered to the American Society of Primatologists principles for the ethical treatment of primates, complied with protocols approved by IACUC at New York University and adhered to the legal requirements of the Colombian legislation.

Results

Over the course of the study, we observed two cases of interspecific infanticide and five cases of interspecific infant-directed aggression which not led to the death of the infant. These are summarized in Table I and described in more detail below.

Observations

Case 1 (Infanticide)

On August 16, 2010, we followed a subgroup of spider monkeys, which contained the only subadult spider monkey male of the group as the focal animal. The subadult male was traveling apart from the rest of the subgroup, and at 08:50, we heard loud “growling” vocalizations (which are typically emitted during aggressive interactions). We approached the location of those vocalizations, and at 08:54, we found a newborn male red howler monkey (with a piece of umbilical cord still attached) on the forest floor. We kept at a distance from the infant, and at 09:04, an adult female howler monkey (presumably the newborn’s mother) descended to the forest floor and retrieved the infant. As she climbed back onto the tree, we observed the subadult male spider monkey directing aggression toward the howler monkey female. He grabbed at the infant, which then fell again to the forest floor (Fig. 1). At 09:39, shortly after the subadult male spider monkey left the area, the female picked up the injured infant again. After the second fall, the infant was not able to hold onto the female, and the female was not able to support him while climbing in the tree. He fell again to the ground at 09:40. During the course of the day, he fell four more times to the forest floor and died during the late afternoon that same day. Although the other members of the howler monkey group (an adult male, another adult female, and a juvenile male) were present during the entire event, neither did they emit alarm calls nor interfere with the subadult spider monkey’s aggression. The dead infant’s body had several small round wounds (arranged in a half circle) on his back, which we infer were caused by the teeth of subadult spider monkey.

Table I. Observed cases of interspecific infanticide and infant-directed aggression by spider monkeys.

Case	Type	Date	Target species	Age-sex class of aggressor(s)	Age-sex class of victim
1	Infanticide	August 16, 2010	*Alouatta seniculus*	**One subadult male**	**One infant male**
2	Infant-directed aggression	August 18, 2010	*Alouatta seniculus*	**One subadult male**	**One infant (unknown sex)**
3	Infant-directed aggression	January 17, 2011	*Alouatta seniculus*	**One subadult male** One adult male	**One infant (unknown sex)** One adult male
4	Infant-directed aggression	June 21, 2011	*Alouatta seniculus*	**One subadult male** Two adult males Five adult females	**One infant male**
5	Infanticide	June 30, 2011	*Alouatta seniculus*	**One subadult male**	**One infant (unknown sex)**
6	Infant-directed aggression	July 05, 2011	*Alouatta seniculus*	**One subadult male**	**One infant (unknown sex)**
7	Infant-directed aggression	August 20, 2011	*Cebus albifrons*	**Two adult males**	**One infant (unknown sex)**

Figure 1. Infant male howler monkey (*A. seniculus*), after the second fall described in Case 1, while still alive. (Photograph by R. Rimbach).

Case 2 (Infant-directed aggression)

On August 18, 2010 (two days after case 1), we were again following a subgroup of spider monkeys containing the same subadult male. He was moving at the rear of the subgroup. At 17:06, we again heard “growling” vocalizations and immediately noticed another newborn red howler monkey on the forest floor. One of the two females of the howler monkey group (presumably the mother) retrieved the infant (of unknown sex) from the ground. The subadult spider monkey male immediately approached the female, grabbed the infant by its tail, and chased the female howler monkey for ca. 4 min. before leaving the area. The female did not alarm call and again none of the other group members interfered with the aggression nor vocalized. This infant howler monkey survived the incident with no apparent injuries and subsequently was carried by a female of the group.

Case 3 (Infant-directed aggression)

On January 17, 2011, we followed a subgroup of spider monkeys that consisted of two adult females, their offspring, an adult male, and the same subadult male. At 09:44, the subadult male initiated aggression toward a howler monkey group, and the other adult spider monkey male present in the subgroup joined in. The subadult male chased a female howler monkey carrying an infant of about one month old. He grabbed the infant from its mother, threw it to the ground, and then continued chasing the female. The infant stayed on the ground for a few seconds and then climbed up into a shrub, where the female then retrieved it. She rapidly moved away from the subadult spider monkey, while he stayed several meters behind staring at her. Meanwhile, the adult male spider monkey attacked an adult howler monkey male and chased him for 20 meters until the howler monkey turned and confronted him. The adult male spider monkey stopped the attack and the adult male howler monkey left the tree. During the aggression, the other howler monkey group members neither interfered nor vocalized.

Case 4 (Infant-directed aggression)

On June 21, 2011, we followed the entire group of spider monkeys, which consisted at the time of five adult females, their offspring, two adult males, and the subadult male. At 11:00, the subadult male initiated an aggression toward a group of howler monkeys, shaking branches and chasing them, and he was joined by the other adult spider monkeys. The subadult male attempted to take an infant howler monkey off the back of its mother. The other howler monkey group members retreated without defending either the female or the infant. One minute later, the subadult male spider monkey pulled the infant by its tail while at the same time its mother tried to hold him by its arms. At 11:04, the infant fell to the ground from ca. 20 meters up in the canopy, and the female was not able to descend to the ground to retrieve the infant because the subadult male continued his aggression toward her. She was forced to leave the tree, and soon after the subadult male spider monkey descended to the ground three times to observe the infant. The male infant was about two months old. He had a deep wound on one leg and about a third of its tail was ripped off. The infant vocalized while lying on the ground, and 2 hr later the female howler monkey appeared again. She tried to recover the infant but the subadult spider monkey again directed aggression toward her. At 14:47, the female finally descended to the ground and successfully retrieved the infant. She went up into the trees and left the area rapidly. We could not confirm whether or not the infant survived.

Case 5 (Infanticide)

On June 30, 2011, the same subadult spider monkey male was resting with three adult females, their offspring, and the two adult males. At 12:30, the subadult male and another adult male left the other group members and approached a group of howler monkeys, which immediately started alarm calling and began to leave the tree. The subadult male then attacked a female howler monkey with an infant on her back. He chased the female, grabbed the infant, and pulled it by its tail. At 12:45, the subadult male bit the infant’s body inflicting a large open wound on its abdomen. The attack lasted a few seconds, and the infant fell to the ground while the female escaped. The subadult male remained at approximately 1.5 m watching the infant for several minutes. The infant was still alive, although it had serious wounds in the abdomen that left its internal organs exposed (Fig. 2). The subadult male descended to the ground, observed the infant, and then returned to the tree and kept watching it for 5 min more. At 12:52, he left the area. The other howler monkeys did not interfere with the aggressor, and the female did not return to check on her infant. We did not retrieve the infant's body when we left because it was still alive and kept vocalizing. But due to the severity of its wounds, it is highly probable that the infant died.

Figure 2. Infant howler monkey after being attacked by a subadult spider monkey described in Case 5. (Photographs by A. Pardo Martinez).

Case 6 (Infant-directed aggression)

On July 5, 2011, we followed a female spider monkey, her offspring, and the subadult male. At 13:02, the subadult male moved toward group of howler monkeys that was resting about 20 m away. He emitted several growling vocalizations and then began directing aggression toward an adult female howler monkey that had an infant on her back. The female rapidly jumped into a small tree close to the forest ground, and the subadult male followed her. When the female was approximately 2 m above the ground, the subadult male stopped chasing her but vocalized and displayed by shaking branches at her. He remained staring at her for about 2 min before leaving them at 13:05. The other howler monkey group members emitted alarm calls during the aggression.

Case 7 (Infant-directed aggression)

On August 20, 2011, we followed a subgroup of spider monkeys that consisted of five adult females, their offspring, and two adult males. At 09:05, the adult males were foraging far from the rest of the group close to a group of white-fronted capuchin monkeys that was also foraging. At 09:11, a female capuchin monkey with an infant on her back passed in front of the spider monkey males, and they began directing aggression toward her. The female capuchin tried to escape from the males, but they reached her and took off her infant. The female struggled with them and the infant fell from around 10 m to the ground. It stayed there for a few seconds and then went up a small tree. The female escaped from the males by descending to the ground and hiding in the shrubs where they could not follow her. The aggression ended at 09:14, and the infant survived the incident without injuries.

Discussion

Interspecific aggression and infanticide is a rare and poorly understood behavior amongst primates. Nonetheless, although other behaviors such as conspecific infanticide and predation are also rarely observed in most primatological field studies, they still feature prominently in contemporary socioecological models concerning the evolution of primate sociality (Isbell 1994; van Schaik 1996). During our two-year study, we observed seven cases of interspecific conflict between spider monkeys and two other sympatric primate species (the only other diurnal species found in the community), at least two of which escalated into lethal aggression. Interestingly, most populations of spider monkeys live sympatrically with howler monkeys and capuchin monkeys, but even during long-term studies conducted on *Ateles* at other sites, this type of behavior has not been reported previously, although spider monkey males have been observed to cooperatively chase and aggressively interact with other mammal species like coatis (*Nasua nasua*) and sloths (*Bradypus variegatus*) (Link et al., personal observation). Furthermore, at most study sites, juvenile spider monkeys have been observed playing for extended periods with other juvenile primates, including howler monkeys and woolly monkeys (Link, Di Fiore, personal observations). In all cases of interspecific infanticide or infant-directed aggression reported in this study, male spider monkeys exclusively directed the aggression to females that carried dependent offspring and attempted to inflict injuries on their infants.

Interestingly, in all but one of the cases we describe, a single individual (the only subadult male spider monkey) was involved in and acted as the initiator and principal perpetrator of aggressive interspecific interactions. It is thus possible that this subadult male spider monkey is a particularly aggressive individual (or at least was particularly aggressive during this specific observation period), or that his behavior is somehow “pathological,” or both. Alternatively, his behavior could be a result, in part, of the fact that he was reaching adulthood throughout this study, a period when male spider monkeys are exposed to high levels of stress because of intraspecific aggression targeted at them from older males, which can sometimes even escalate to lethal aggression (Campbell 2006; Valero et al. 2006). In fact, most of the aggression we observed within the spider monkey group was directed toward this particular subadult male (Link et al., unpublished data), and thus, his pervasive participation in infanticide-related behaviors could conceivably reflect “redirected aggression”. For many primates, it has been found that levels of aggression of males rise during sexual maturation (Kraemer et al. 1982;

Pereira & Altmann 1985; Anestis 2006). Cassini (1998) reports that only subadult sea lion males (*O. flavescens*) -and never adults- attack and sometimes even kill seal pups (*A. australis*). Still, the fact that the subadult male spider monkey was repeatedly joined in his aggressive interspecific interactions by other adult individuals (mostly, but not exclusively, adult males) suggests that such aggression at San Juan might constitute more than merely individual pathological behavior.

Out of the four possible adaptive hypotheses positing fitness gains for the perpetrators of infanticide (Hrdy 1979), those related to improving reproductive opportunities for infanticidal males after killing infants of females of another taxon can be ruled out. The elimination of potential competitors for food resources remains as a potential explanation for the reported cases. Under conditions of high population density, species that overlap in their ecological niches may experience increased resource competition; thus, during these times infanticide could conceivably be used as a strategy to eliminate potential future competitors for resources or space. Accordingly, it has been suggested that resource competition is the most plausible explanation for male infanticide in male bank voles (*Myodes glareolus*). At high population densities, aggressiveness of male bank voles toward pups of their own species increases (Korpela et al. 2010). Moreover, resource competition also consistently affects interspecific aggressive interactions and nest predation between bank voles and common shrews (*Sorex araneus*) (Liesenjohann et al. 2011). Among primates, Rose et al., (2003) observed multiple cases of aggression directed by white-faced capuchins (*C. capucinus*) toward mantled howler monkeys (*A. palliata*) as well as cases of reciprocal aggression (mostly chases and threats) between capuchins and black-handed spider monkeys (*A. geoffroyi*) at the same site. Most of the confrontations between the latter two primate species arose over heavily contested resources, suggesting resource competition might be driving this interspecific aggression.

The members of the primate community we studied at San Juan live at very high density (Link et al. 2010) in a small forest fragment of only 65 ha. Under these conditions, resource competition for food and space may indeed be a possible explanation for the observed cases of interspecific aggression and infanticide we report here. Although none of the cases of infant-directed aggressions we observed took place during contest competition for feeding resources, the dietary overlap between spider monkeys and howler monkeys is considerably high in this forest fragment. Depending on the season, the diet of spider monkeys can contain a very high percentage of new leaves 26–57% (Aldana Saavedra 2009) -considerably higher than that reported at other study sites where leaves comprise a much smaller fraction of the diet (e.g., 8.3% (Nunes 1998); 9% (Dew 2001); 12% (Russo et al. 2005); 17.2% (Campbell 2000); reviewed in (Di Fiore et al. 2011)). At the same time, howler monkeys seasonally consume a high percentage of ripe fruits at the study site (45–75%: San Juan (Aldana Saavedra 2009)). In contrast, there is much less dietary overlap between capuchins and spider monkeys because capuchins have a very flexible diet and feed on fruits, insects, small vertebrates, and mammals.

Even though interspecific infanticide has been seldom documented in primate studies, it seems that it could be an important factor influencing the population dynamics of howler monkeys at San Juan. If it turns out that interspecific infanticide in this situation is largely explained by the “pathological” behavior of a single individual, then the phenomenon may only be relevant for this *particular* community and only during the next few years. On the other hand, if interspecific infanticide represents a more general phenomenon influenced by high population densities and resource competition in recently fragmented forests, then we might predict that similar behaviors will increasingly be observed in populations of primates that are being isolated in forest fragments at high population density as habitat fragmentation and isolation due to human activity increases worldwide.

Acknowledgments

We thank the Lalinde and De Greiff families who have allowed us to conduct our research at Hacienda San Juan de Carare. We also thank the Ministerio de Medio Ambiente y Desarrollo Sostenible in Colombia for all the permits needed to conduct this study. We are very grateful to Dr. Eckhard W. Heymann, Dr. Paul Garber, and two anonymous reviewers for the constructive comments they provided on an earlier version of this article. Fundacion Proyecto Primates provided all logistic support. Several field assistants collaborated with this research in the field, and we especially thank Nathalia Fuentes. Our broader study on the conservation and social behavior of wild spider monkeys at San Juan has been generously funded by the National Geographic Society, the Ecopetrol Diversity Grants program, Fundación Proyecto Primates, and the Deutsches Primatenzentrum (DPZ).

GENERAL DISCUSSION

In this general discussion I will first briefly summarize the main findings of chapters 1, 2, 3 and 4. Then I discuss which role different aspects of phenotypic flexibility might play in animals´ ability to cope with human-induced disturbances. Following the depiction of the need for science-based conservation efforts, I examine glucocorticoid measurements as a potential conservation tool. Finally, I provide an outlook in which I suggest possible approaches for future studies inspired by this thesis.

Effects of anthropogenic disturbances on glucocorticoid output and behavior

The overall aim of this thesis was to broaden our understanding of the effects anthropogenic habitat disturbances have on animals, and how different species cope with these disturbances in order to survive. The current rate of habitat conversion and deforestation make a better understanding of these aspects essential to ensure the survival of numerous animal species and their ecosystems worldwide.

One of the main goals was to use a physiological approach to assess the effect of anthropogenic disturbances on stress hormone levels - measured through fecal glucocorticoid metabolites (FGCM) - in two primate species, brown spider monkeys (*Ateles hybridus*) and red howler monkeys (*Alouatta seniculus*). As an essential pre-requisite to obtain biologically meaningful results I first validated an enzyme-immunoassay (EIA) for both study species using the well-documented stress response to anesthesia (**Chapter 1**). I evaluated the suitability of four different EIAs and demonstrated the validity of the 11ß-hydroxyetiocholanolone EIA to detect adrenocortical endocrine activity in both species. Further, using HPLC I could show the specificity of the validated EIA by demonstrating that there were no cross-reactions between metabolites of GCs and other steroids (e.g. testosterone), and that the EIA measures the same GC metabolites in males and females of both species. This is crucial knowledge for an adequate interpretation of the results, especially because sex-specific differences in the hormone metabolism have been reported for some mammal species (e.g. Touma et al. 2003; for a detailed review see: Goymann 2012). Such differences could render a meaningful interpretation of data collected on both sexes impossible. In addition, the results emphasize inter-specific differences in terms of basic factors influencing adrenocortical activity which have to be considered in subsequent studies. For instance, spider monkeys show a diurnal rhythm of GC excretion while howler monkeys do not.

Using the validated EIA I examined the physiological responsiveness of both study species to forest fragment size and level of human impact (determined through the presence or absence of hunting and/or logging activities). Previous observational studies demonstrated that spider monkeys are usually negatively affected by anthropogenic alterations, while howler monkeys seem more resilient (Bernstein et al. 1976; Bicca-Marques 2003). The results of my study support the inter-specific differences reported in previous studies (**Chapter 2**). While fragment size did not influence FGCM levels of either species, the level of human impact did. Spider monkeys showed elevated FGCM levels in fragments where hunting and logging occurred, whereas howler monkey did not show such a response. This demonstrates that the two species differ in their physiological responsiveness to anthropogenic disturbances and further emphasizes why spider monkeys are at a higher extinction risk than howler monkeys. These results suggest that hunting and logging activities (facilitated through forest fragmentation) may create long-term stress for spider monkeys which can negatively affect their population viability in the future. Further, this study demonstrates the potential use of GC measurements as a tool for population monitoring in disturbed areas.

Species-specific differences in the ability to cope with anthropogenic disturbances may also be influenced by the social organization of a species. Albeit both study species are group-living, they differ in their spatio-temporal cohesion. Howler monkeys typically live in cohesive groups whereas spider monkeys belong to the few primate species that exhibit fission-fusion dynamics. The underlying mechanisms of fission-fusion dynamics have been studied, so far, primarily in continuous forests. Accordingly, we currently still lack detailed information on how these species adjust to living in forest fragments. Therefore, another goal of this thesis was to use spider monkeys as a model species to examine their grouping patterns in a small fragment (**Chapter 3**). Typically, spider monkeys and chimpanzees, who also exhibit fission-fusion dynamics, range in smaller subgroups when fruit availability is low and in larger ones when fruit availability is high (e.g. Symington 1988; Itoh & Nishida 2007). In contrast to this pattern, the two study groups ranged in smaller subgroups in times of higher fruit availability compared to times with lower availability. Furthermore, FGCM levels of the study groups were higher when fruit availability was high. This was unexpected because of the function GCs play in the release of energy during the vertebrate stress response and because consequently, GC levels generally increase in times of low resource availability (e.g. Chapman et al. 2007b; Behie et al. 2010). I propose that both high FGCM levels and smaller subgroup sizes in times of high fruit availability reflect that in this fragment the level of intra-group feeding competition is higher when fruit availability is higher compared to times of low fruit availability. Although this seems counterintuitive, it is likely that the study groups adapt this grouping pattern to avoid even higher levels of intra-group feeding competition in times of low fruit availability. A more folivorous diet (> 40% of total feeding time spent on young leaves) might permit this atypical grouping pattern. This is further reinforced by the observation of an, for spider monkeys, unusually high level of female-female aggression. In accordance with this interpretation, aggressions between females were more often observed when fruit availability was high. The findings of this study demonstrate that forest fragmentation can reduce spider monkeys´ ability to decrease intra-group feeding competition via flexible subgroup sizes, which might drive the adjustment of their dietary strategy.

Habitat fragmentation can result in reduced home-range sizes compared to intact forest (Irwin 2008; Arroyo-Rodriguez & Dias 2010) and thus, can lead to high levels of intra-specific competition for resources and space. In fragments, where population densities are often high and resource availability is frequently altered, confinement to a small area can furthermore result in inter-specific competition for space and resources, especially if species overlap in their ecological niches to some degree. This might result in aggressive interactions between species or even inter-specific infanticide (**Chapter 4**). This behavior might be "pathological", or it might represent a strategy to eliminate potential future competitors for space or resources.

In conclusion, this study confirms differences in the ability to cope with anthropogenic disturbances between both study species. The results suggest that these differences can be, to some extent, attributed to differences in their physiological responsiveness to human-induced alterations. This thesis also illustrates that the relationship between ecological variables of a certain fragment, social and grouping behavior and stress levels can be complex in disturbed habitats, and it describes how forest fragmentation can change the ability of spider monkeys to decrease intra-group feeding competition via flexible grouping patterns.

The potential role of flexibility in animals´ ability to cope with anthropogenic disturbances

Species-specific traits such as geographic range, dispersal ability, life-history variables and ecological niche are known to influence whether a species will be able to cope with human-induced changes and disturbances (e.g. Purvis et al. 2000). However, phenotypic flexibility might also play a crucial role in the adaptive response of individuals or populations to anthropogenic disturbances (Parmesan 2006; Riley 2007; Hendry et al. 2008; Tuomainen & Candolin 2011; Tesfaye et al. 2013). Here, I define phenotypic flexibility as *reversible* modifications of the phenotype of an organism caused by processes in its environment (following Angelier & Wingfield 2013). Many authors use 'flexibility' and 'plasticity' interchangeably, while others contrast between both terms and define plasticity as processes that lead to *irreversible* modifications of the phenotype of an organism (e.g. Angelier & Wingfield 2013). Hereafter, I will use the term flexibility following the aforementioned definition (which will also incorporate studies that have used the term 'plasticity' in a similar manner).

Generalists, i.e. species that express a high level of flexibility, seem to be able to cope better with human-induced changes than specialist (for reviews see: Colles et al. 2009; Clavel et al. 2011; Tuomainen & Candolin 2011). Phenotypic flexibility can be expressed in several different ways, for example in the behavior or the ecology of a species. Behavioral flexibility can enable individuals or populations to modify their social organization in response to anthropogenic disturbances (for detailed reviews on behavioral flexibility see: Schradin 2013; Kappeler et al. 2013). For instance both colobus and howler monkeys, usually live in cohesive groups and can adopt flexible grouping patterns in disturbed areas (Struhsaker et al. 2004; Dias & Rodríguez Luna 2006; Aguilar-Melo et al. 2013). Also, several ungulate species adjust their group composition in response to human disturbance (Averbeck et al. 2012). Moreover, the results presented in **Chapter 3** also demonstrate that spider monkeys modify their grouping behavior to the conditions in a small forest fragment. Individuals or populations that express ecological flexibility might be able to shift or extend their ecological niche in response to human-induced changes, which could provide them with the ability to use anthropogenically altered habitats such as crop fields and plantations (Estrada & Coates-Estrada 1996; Haupt et al. 2006; Bonilla-Sánchez et al. 2012; Pozo-Montuy et al. 2013).

Recently it has been suggested that flexibility in the vertebrate stress response (physiological flexibility) might be one mechanism that could enable animals to respond to anthropogenic disturbances (Wingfield 2013). Physiological flexibility can be defined as processes that cause reversible modifications of an organisms´ physiological phenotype (Piersma & Drent 2003; Angelier & Wingfield 2013). It is conceivable that individuals or populations that manage to survive anthropogenic disturbances might have a more flexible stress response than those that cannot and decline in numbers. Flexibility in the stress response could enable individuals to reduce or avoid potentially negative effects of long-term GC elevations on growth, immunofunction and reproduction. However, this is a very new field in physiological research and therefore, individual flexibility in the GC stress response is largely unstudied and the underlying mechanisms of such flexibility are not well-understood (Angelier & Wingfield 2013).

The expression of phenotypic flexibility (in behavior, ecology and/or physiology) can permit populations or species to persist in anthropogenically disturbed habitats. Nonetheless, if the intensity of anthropogenic disturbances further increases, phenotypic flexibility may reach its limits. Beyond these limits buffering the negative effects is no longer possible and species might go extinct, which - depending on the position of a species within an ecological network - could also have cascading effects on ecosystem functioning. For example, declines in populations of important seed dispersing primate species (e.g. spider monkeys) can hamper forest regeneration (Chapman & Onderdonk 1998; Nunez-Iturri et al. 2008; Gutiérrez-Granados & Dirzo 2009; Effiom et al. 2013).

The need for evidence-based conservation

Meta-analyses and biodiversity scenarios have predicted that in the near future numerous species will face extinction (Alkemade et al. 2009; Pereira, Leadley et al. 2010). Even though some species currently do not seem threatened, they might go extinct through cascading effects and functional extinctions within the ecosystem (Anderson et al. 2011; Säterberg et al. 2013). Functional extinctions are extinctions of one species caused by a decrease in the population abundance and an increase in the mortality rate of another species. Such extinctions can have far-reaching effects for the functionality of entire ecosystems. For instance, many plant species rely on animals as seed disperses and pollinators (Howe & Westley 1988; Stiles 2000; Culot et al. 2010) and anthropogenic disturbances and habitat fragmentation can disrupt such animal-plant interactions (Rathcke & Jules 1933; Goverde et al. 2002; Aguilar et al. 2006; Anderson et al. 2011). Local extinctions of pollinators and seed dispersers can threaten the survival of plant and animal species and disrupt ecosystem functioning (Chapman 1995; Babweteera & Brown 2009; Effiom et al. 2013; Levi & Peres 2013; Galetti & Dirzo 2013).

To reduce the pervasive threats to global biodiversity and ecosystem functioning, science-based knowledge on the cause-and-effect relationships of anthropogenic disturbances is needed. To gain such information interdisciplinary frameworks, that integrate and apply methods like behavioral and ecological observations, genetics, endocrinology and conservation management, pose a promising approach. For example, knowledge on how animals migrate, disperse and select a new home range is essential to identify suitable areas for conservation (e.g. for the planning of national parks) and to plan corridors between these areas that will be used by the target species (Mansergh & Scotts 1989; Kokko & López-Sepulcre 2006; Kertson et al. 2011). Detailed behavioral knowledge is also important to predict responses of animals to novel environments to ensure the success of relocations or translocations (Swaisgood 2007). Further, knowledge gained in such interdisciplinary research could be used to predict which species are most vulnerable to extinction and why, where species are most likely to go extinct, and where conservation effort should be concentrated. The latter is especially important because resources for conservation are limited. Moreover, such knowledge should be used to inform politicians and decision-makers, and to build the foundation for management interventions and conservation practices (Cooke & O'Connor 2010). In this context physiological tools, including glucocorticoid measurements, can be a powerful tool to gain deeper insight into conservation-relevant cause-and-effect relationships (Carey 2005; Cooke et al. 2012, 2013).

Glucocorticoid measurements as a conservation tool

The recent advances in the procedures and application of physiological methods have made “field endocrinology” a valued method to investigate the effects of human-induced changes on animals. Applying non-invasive methods to wild and eventually threatened species is advantageous because they do not inflict additional physiological stress on the animals during capture and blood sampling. Besides, they permit and facilitate the repeated collection of samples of the same individuals or populations (e.g. to compare pre- and post-disturbance levels or levels before and after restoration or translocation). However, an adequate validation of every new method for every previously unstudied species is crucial and should be conducted before its application (see **Chapter1**).

Applying these methods to conservation-related research has recently been termed “conservation endocrinology” or “conservation physiology” (Wikelski & Cooke 2006). This discipline could improve our understanding of the underlying mechanisms of animal responses to human-induced alterations (Wingfield 2008). Furthermore, the discipline can potentially have a high impact on management decisions and practices because its methods can be applied to all major vertebrate taxa and a wide array of different objectives (for reviews see: Wikelski & Cooke 2006; Cooke et al. 2013). They can be used to assess how animals respond to environmental change, seemingly benign activities such as ecotourism and severe anthropogenic disturbances such as fragmentation, hunting and logging (e.g. Müllner et al. 2004; Walker et al. 2005; Maréchal et al. 2011, **Chapter 2**). They can also be used to identify sources and consequences of different stressors on species viability. Besides, they may be used to identify species-specific thresholds of stressors (to avoid maladaptive levels of stress) and to improve and evaluate the success of restoration plans (e.g. reconnection of fragments) and translocations of animals. Together with studies on reproductive hormones (e.g. progesterone and testosterone), GC measurements can provide information on the reproductive biology of animals, and assist in the understanding of reproductive failure and causes of high mortality e.g. after translocations or relocation (Teixeira et al. 2007; Dickens et al. 2010). When the link between baseline GC levels and fitness is known, they could also be used as “early warning signals” for population declines and to inform management executives where to intervene (for examples see: Wikelski & Cooke 2006).

However, the relationship between baseline GC levels and fitness still remains unclear and needs further investigation. The link between both variables might depend on the species or taxa under investigation, as in some species a negative link between GC levels and fitness has been found, whereas in others a positive or no link has been reported (for detailed reviews see: Breuner et al. 2008; Bonier et al. 2009a). Furthermore, it has been suggested that the underlying relationship between GC levels and fitness might be non-linear as generally assumed, but possibly log quadratic (Busch & Hayward 2009). Therefore, further studies are needed to understand species-specific relationships between GC levels and fitness. However, to obtain direct measurements of fitness may be challenging in long-lived animals with slow life histories.

In conclusion, this thesis further demonstrates that species differ in their ability to cope with anthropogenic disturbances and that this variation can be, at least to some extent, attributed to different levels of physiological responsiveness to human-induced pressures. Moreover, it suggests that hunting and logging activities may create long-term stress for spider monkeys that can impair their population viability in the future. This thesis also describes how spider monkeys have to adjust their grouping patterns to the conditions in a small fragment. In order to conserve global biodiversity further interdisciplinary research is needed, which will help to understand how species adapt to anthropogenic disturbances.

Outlook

This thesis demonstrates that species differ in their physiological responses to anthropogenic disturbances, which might impair the long-term viability of populations. But this thesis also emphasized the need for a deeper understanding of the underlying mechanisms of animal responses to disturbances and raised questions concerning the ultimate effects of anthropogenic alterations on animal fitness and long-term population viability. Based on this thesis, I encourage research to provide further clarification of the underlying mechanisms of animals´ ability to adjust to anthropogenic disturbances:

(1) To fully understand which effects forest fragmentation and other human activities have on physiological stress levels of animals (**Chapter 2**) and ultimately on fitness, we need to improve our understanding of 'chronic stress' and its consequences. Concerning the study species, we would need fecal samples from larger fragments or preferably undisturbed continuous forests (though this might be very difficult for *A. hybridus*) as a reference or baseline for comparisons. To investigate whether elevated GC levels of *A. hybridus* reflect 'chronic stress' that will impair its population viability and fitness, long-term data on the physiology of the species, on birth rates, individual survival and demographic changes is necessary (collected in fragments as well as continuous forest).

(2) Long-term elevations of GC levels have been associated with a decreased activity of the immune system and increased susceptibility to diseases and parasites. Moreover, proximity to human settlements can affect parasite occurrence, richness and the intensity of infections in primates (Wenz et al. 2010; Wenz-Mücke et al. 2013; Hussain et al. 2013). Both study species show a great variation of FGCM levels between fragments (**Chapter 2**). Accordingly, differences

in parasite prevalence and (multiple) infections can be predicted between fragments exposed to different levels of human impact. High parasite load can negatively affect individual survival (Gillespie & Chapman 2008; Hussain et al. 2013) and thus, measuring parasite infections (possibly in combination with GC measurements) could be one additional method to evaluate the effects of anthropogenic disturbances on health and indirectly on fitness.

(3) Spider monkeys ranging in forest fragments might have to adopt a much more leafy diet than conspecifics in continuous forest (Chaves et al. 2012; Abondano & Link 2012, **Chapter 3**). This can impair the nutritional status of these groups, which can affect reproduction, mortality, and physiological stress levels. Therefore, it would be important to examine whether changes in dietary strategy affect the nutritional status of populations and their persistence in fragments. For such studies detailed nutritional analyses of food items (Chapman et al. 2006; Felton et al. 2010), stable isotope biogeochemistry (Hobson et al. 1993; Dammhahn & Kappeler 2010; Crowley 2012; Sandberg et al. 2012) and urinary C-peptide measurements are promising methods (Deschner et al. 2008; Girard-Buttoz et al. 2011)).

(4) Species-specific differences in phenotypic flexibility (behavioral, ecological and physiological) could potentially be used to predict the ability of species to be able to cope with anthropogenic disturbances and climate change. The underlying mechanisms are still not well-studied and consequently represent a promising field for future studies.

(5) Howler monkeys frequently cross the matrix habitat between fragments and they have been observed to cross rivers too, whereas many other (primate) species avoid crossing open areas such as the matrix (e.g. spider monkeys). Thus, it would be interesting to study the genetic variation or the loss thereof in populations living in fragments that differ in their isolation distances from other fragments or continuous forest.

SUMMARY

The increasing intensity of anthropogenic land use and destruction has an immense impact on ecosystems worldwide. The process of forest conversion into agricultural fields or plantations often results in forest fragments. Habitat fragmentation and other anthropogenic disturbances such as logging and hunting, pose major threats to numerous animal species worldwide. Species vary greatly in their resilience to these disturbances and in their ability to survive in forest fragments. Animals living in fragments might have to adjust their behavior, their grouping patters and/or dietary strategies. Moreover, human-induced alterations can result in elevated glucocorticoid (GC) levels and long-term elevations of these 'stress' hormones can have deleterious effects on growth, reproduction and immune system activity of animals.

I applied an interdisciplinary approach and combined behavioral observations with data on fecal glucocorticoid metabolite (FGCM) levels. I studied two Neotropical primate species, brown spider monkeys (*Ateles hybridus*) and red howler monkeys (*Alouatta seniculus*), to broaden our understanding of the effects that anthropogenic disturbances have on the physiological responsiveness of animals and to investigate how animals cope behaviorally with these disturbances. The specific aims were to 1) validate an enzymeimmunoassay (EIA) for the analyses of FGCM levels of both study species, 2) to investigate the species-specific differences in the physiological responsiveness to anthropogenic disturbances and 3) to examine how spider monkeys adjust their grouping patterns and social behavior in a small forest fragment.

The validation of the EIA, for which I used the stress response to anesthesia and reverse-phase high pressure liquid chromatography analysis (HPLC), was a crucial pre-requisite for the analyses of FGCM levels. The results demonstrated that both species differ in terms of basic factors influencing their adrenocortical activity (e.g. diurnal rhythm of GC excretion). To investigate the physiological responsiveness to anthropogenic disturbances of both species I collected fecal samples in several forest fragments in Colombia that differed in size (4.21 ha - 500 ha) as well as the level of human impact (determined through the occurrence and/or absence of hunting and logging activities). Using the validated EIA I examined species-specific differences in the physiological responsiveness to both factors. Fragment size did not influence FGCM levels of either species. But spider monkeys showed elevated FGCM levels in fragments where both hunting and logging occurred, whereas howler monkey did not show such a response. This suggests that hunting and logging activities might create long-term elevations of GC levels in spider monkeys which could impair their future population viability. These results emphasize why spider monkeys are at a higher extinction risk than howler monkeys when living in anthropogenically altered habitats.

To better understand how spider monkeys cope with habitat fragmentation, I studied two well-habituated groups living in a small fragment (65 ha). To investigate if and how they modify their grouping patterns and social behavior, I collected data on subgroup sizes, aggression and habitat-wide fruit availability. In addition, I collected fecal samples to investigate how FGCM levels are influenced by these factors. Both study groups ranged in smaller subgroups and showed higher FGCM levels when fruit availability was high compared to when fruit availability was low. These results were unexpected because (1) species like spider monkeys or chimpanzees, which exhibit fission-fusion dynamics, typically show the opposite grouping pattern, and (2) a major function of GCs is the release of energy during the response to a stressor. Consequently, GC levels typically increase in times of low resource availability. Spider monkeys are generally considered to be ripe fruit specialists. However, both study groups have a more folivorous diet than other spider monkey populations, which seems likely to be a way to reduce the level of competition for fruit in times of low availability. Thus, it seems reasonable to assume that when fruit availability is relatively high in this fragment, the level of intra-group feeding competition for fruit might also be higher. This would also explain why FGCM levels are higher and subgroup size is smaller in times of high fruit availability. This interpretation is further reinforced by the observation of unusually high levels, for spider monkeys, of female-female aggression, which was also more often observed when fruit availability was high. This illustrates how fragmentation can change the ability of spider monkeys to decrease intra-group feeding competition via flexible adjustments of subgroup size and that the relationship between fragment size, resource availability, grouping patterns, aggression rates and stress levels can be more complex than assumed so far.

Population densities are often high in forest fragments and resource availability is frequently altered. Moreover, animals that live in fragments often have to reduce the size of their home range. This can potentially lead to high levels of intra-specific competition for resources and space. The confinement to a small amount of space can furthermore result in inter-specific competition, especially between species that overlap in their ecological niches. I report several cases of severe aggression from spider monkeys directed at infant howler monkeys and capuchins in a small fragment with high primate population densities. This behavior might be either “pathological” or a strategy to eliminate potential future competitors for resources or space.

In conclusion, this study demonstrates species-specific differences in the ability to cope with anthropogenic disturbances. These differences can be, at least partly, attributed to different levels of physiological responsiveness to anthropogenic pressures. In addition, the results suggest that hunting and logging activities may create long-term stress for spider

monkeys that could impair their long-term population viability. This thesis also illustrates that the relationship between ecological variables of a certain fragment, social and grouping behavior and stress levels can be more complex in disturbed habitats than assumed so far. Importantly, the results of this study illustrate the potential use of GC measurements as a tool to monitor populations in disturbed areas and for the assessment of the success of conservation strategies (e.g. corridors connecting fragments). Moreover, it demonstrates that interdisciplinary research could improve conservation efforts and will be necessary to ensure the conservation of the global biodiversity.

ZUSAMMENFASSUNG

Weltweit bedrohen anthropogene Störungen - wie Habitatfragmentierung, Holzeinschlag und Jagd - das Überleben zahlreicher Spezies. Tierarten variieren stark in ihrer Widerstandsfähigkeit gegenüber diesen Störungen, sowie in ihrer Fähigkeit in Waldfragmenten zu überleben. Diese Störungen können sich auf das Verhalten der Tiere, ihre Gruppierungsmuster und ihre Ernährung auswirken. Zusätzlich können diese anthropogenen Einflüsse als Stressoren auf die Tiere einwirken.

Um zu verstehen welche Auswirkungen anthropogene Störungen auf die Physiologie und das Verhalten von Tieren haben, habe ich Daten von zwei südamerikanischen Affenarten, Braunen Klammeraffen (*Ateles hybridus*) und Roten Brüllaffen (*Alouatta seniculus*), erhoben. Während Brüllaffen selbst in sehr kleinen Fragmenten überleben können, werden Klammeraffen in der Regel davon negativ beeinflusst und sterben oft in zu kleinen Fragmenten aus. Ich nutzte einen interdisziplinären Ansatz, um zu untersuchen welche proximaten Ursachen es für diese artspezifischen Unterschiede gibt: ich kombinierte Verhaltensbeobachtungen mit Messungen von Glucocorticoiden in Kotproben. Um für beide Arten einen Enzym-Immunoassay (EIA) zu validieren, habe ich die Stressreaktion auf Anästhesie genutzt und außerdem Hochleistungsflüssigkeitschromatographie (HPLC) angewandt. In diesem Rahmen habe ich des Weiteren untersucht, welche potentiellen Störfaktoren die Konzentration von Glucocorticoidmetaboliten im Kot (FGCM) beider Arten beeinflussen. Anschließend sammelte ich in verschiedenen Waldfragmenten Kolumbiens Kotproben beider Arten. Die Fragmente unterschieden sich sowohl in der Größe (4,21 ha - 500 ha), als auch in der Ausprägung der anthropogenen Störung (bestimmt durch das Vorkommen beziehungsweise der Abwesenheit von Jagd und Holzeinschlag). Mit Hilfe des validierten EIA untersuchte ich artspezifische Unterschiede in der physiologischen Reaktion (FGCM Level) auf Fragmentgröße und den Level des menschlichen Einflusses. Bei keiner der beiden Arten wurde der FCGM Level von der Fragmentgröße beeinflusst. Klammeraffen zeigten jedoch erhöhte FGCM Level in Fragmenten, in denen sie sowohl gejagt wurden, als auch Holzeinschlag betrieben wurde. Im Gegensatz dazu, zeigte sich dieser Effekt nicht bei Brüllaffen. Dies deutet darauf hin, dass Jagd und Abholzung langfristige Erhöhungen der FGCM Level bei Klammeraffen hervorrufen können, welche möglicherweise die Langzeit-Überlebensfähigkeit der Population beeinträchtigen kann. Diese Ergebnisse unterstreichen, warum Klammeraffen generell stärker vom Aussterben bedroht sind als Brüllaffen.

Um ein detaillierteres Verständnis darüber zu erhalten, wie Klammeraffen in Fragmenten überleben, beobachtete ich das Verhalten von zwei habituierten Gruppen in einem

kleinen Waldfragment (65 ha). Dabei nahm ich Daten über die Größe der Untergruppen, Aggressionen zwischen den Tieren sowie deren Nahrungsverfügbarkeit auf. Gleichzeitig sammelte ich Kotproben zur Analyse von FGCM Level. Mit diesen Daten untersuchte ich, wie Klammeraffen ihre flexiblen Gruppierungsmuster (auch ‚fission-fusion' Dynamiken genannt) und ihr Sozialverhalten an die Bedingungen des Fragmentes anpasst haben. Wenn die Verfügbarkeit von Früchten in dem Fragment hoch war, formten beide Gruppen kleinere Untergruppen und zeigten höhere FGCM Level – wenn die Verfügbarkeit von Früchten niedrig war, formten sie größere Untergruppen und zeigten niedrigere FGCM Level. Diese Ergebnisse waren unerwartet, weil (1) Arten, die flexible ‚fission-fusion' Dynamiken aufweisen (wie Klammeraffen und Schimpansen), typischerweise ein genau umgekehrtes Gruppierungsmuster zeigen. Und weil (2) eine wichtige Funktion von Glucocorticoiden (GC) die Freisetzung von Energie als Reaktion auf einen Stressor ist. Folglich sind GC Level normalerweise in Zeiten geringer Ressourcenverfügbarkeit erhöht. Obwohl Klammeraffen allgemein als Frucht-Spezialisten gelten, haben beide Studiengruppen ihre Ernährungsweise in Richtung Folivorie geändert. Dies scheint es ihnen ermöglicht zu haben, die Konkurrenz um Früchte, in Zeiten niedriger Verfügbarkeit, zu reduzieren. Es scheint dass gerade dann, wenn die Verfügbarkeit von Früchten in diesem Fragment hoch ist, dann auch die Konkurrenz um eben diese steigt. Dies würde auch erklären, warum in dieser Zeit die FGCM Level höher sind und die Tiere kleinere Untergruppe bilden. Diese Interpretation wird weiterhin durch ein - für Klammeraffen - ungewöhnlich hohes Level an Weibchen-Weibchen Aggression verstärkt, zumal dieses höher war wenn die Verfügbarkeit von Früchten ebenfalls hoch war.

Im Vergleich mit intakten Wäldern ist die Populationsdichte von Tieren in Fragmenten oft hoch; die Ressoucenverfügbarkeit nicht selten durch mikroklimatische Veränderungen verändert. Zudem müssen in Fragmenten lebende Tiere oft die Größe ihrer Streifgebiete reduzieren. Dies kann zu hoher intraspezifischen Konkurrenz um Ressourcen und Habitat führen. Aufgrund des beschränkten Raumes in Fragmenten, kann dies weiterhin zu interspezifischer Konkurrenz führen. In einem kleinen Fragment mit hoher Primatendichte habe ich mehrere starke Aggressionen von Klammeraffen gegenüber Jungtieren von Brüllaffen und Kapuzineraffen beobachtet. Dieses Verhalten könnte "pathologisch" sein oder aber eine Strategie darstellen, mögliche zukünftige Konkurrenten um Ressourcen und Habitat zu beseitigen.

Zusammenfassend zeigt diese Studie artspezifische Unterschiede in der Fähigkeit, sich an anthropogene Störungen anzupassen. Diese Unterschiede lassen sich, zumindest teilweise, auf Unterschiede in der physiologischen Stressantwort auf anthropogene Störungen zurückführen. Des Weitern zeigt diese Studie die mögliche Verwendung von Glucocorticoidmessungen zum Monitoring von Populationen in gestörten Habitaten sowie für die Beurteilung des Erfolgs von Naturschutzmaßnahmen (z.B. Habitatkorridore zwischen Fragmenten). Darüber hinaus zeigt diese Studie, dass der Erfolg von Naturschutzmaßnahmen dadurch verbesserten werden könnte, wenn Naturschutz mit wissenschaftlicher Forschung kombiniert wird. Dies würde zum Erhalt der globalen biologischen Vielfalt beitragen.

RESUMEN

El incremento en la destrucción e intensidad del uso de tierras por factores antropogénicos han impactado considerablemente los ecosistemas mundiales. Los procesos de transformación de los bosques a plantaciones o a campos de agricultura, con frecuencia generan fragmentación de hábitat. Fragmentación y otros disturbios antropogénicos tales como cacería y tala, son las mayores amenazas para numerosas especies de animales en el mundo. Especies de animales varían ampliamente en su resistencia a estos disturbios y en su habilidad para sobrevivir en hábitats fragmentados. Alteraciones inducidas por humanos pueden afectar el comportamiento de animales, sus patrones de agrupación, estrategias de alimentación, y pueden actuar como estresores para ellos. Elevaciones en términos cortos de tiempo de los niveles de glucocorticoides "hormona de stress", son considerados como respuestas adaptativas porque aseguran la supervivencia de individuos en situaciones de emergencia. Elevaciones de glucocorticoides en términos largos de tiempo, pueden tener efectos deletéreos sobre el crecimiento, reproducción y actividad del sistema inmune.

Por lo tanto, el principal objetivo de esta tesis fue ampliar nuestro entendimiento de los efectos de los disturbios antropogénicos, sobre la sensibilidad fisiológica de animales silvestres y la diferencia inter-específicas entre ellos. Además, el objetivo fue investigar como comportamentalmente una especie puede hacer frente a estos disturbios. Yo aplique un acercamiento interdisciplinario en el cual combine observaciones comportamentales, con datos de niveles de metabolitos de glucocorticoides fecales (FGCM). Los objetivos específicos fueron: (1) validar un inmunoensayo de enzimas (EIA) para análisis de niveles de FGCM para ambas especies de estudio; (2) investigar las diferencias específicas de las dos especies en la sensibilidad fisiológica a las perturbaciones antropogénicas; (3) examinar como los monos araña café ajustan patrones de agrupación y comportamiento social, a la disponibilidad de frutos en un fragmento de bosque. Yo escogí dos especies de primates Neotropicales, los monos araña café (*Ateles hybridus*) y los monos aulladores rojos (*Alouatta seniculus*) como especies de estudio, porque ellos han sido reportados por contraerse fuertemente para contrarrestar los efectos de las perturbaciones antropogénicas. Los monos araña son generalmente menos resistentes a la fragmentación, mientras que los monos aulladores pueden prevalecer aun en pequeños fragmentos.

Para la validación del EIA, utilice la respuesta al estrés a anestesia y análisis de cromatografía liquida de alta presión en fase reversa (HPLC), siendo un crucial pre-requisito para el análisis de los niveles de FGCM. Para asegurar la significancia de los resultados, adicionalmente investigue cuales cofactores (ej. sexo, edad, etc.) influencian los niveles de

FGCM en ambas especies. En Colombia colecte muestras fecales de ambas especies de estudio, en diferentes fragmentos de bosques que diferían en tamaño (4.21 ha – 500 ha), también el nivel del impacto humano (determinado a través de ocurrencia y/o ausencia de cacería y actividades de tala). Usando el EIA validado, examine las diferencias específicas de los dos especies en la sensibilidad fisiológica al tamaño del fragmento y a los niveles de impacto humano. El tamaño del fragmento no influenció los niveles de FGCM de ninguna de las especies. Pero los monos araña mostraron niveles elevados de FGCM en fragmentos con presencia de cacería y actividades de tala, mientras que los monos aulladores no mostraron tal respuesta. Esto sugiere que cacería y actividades de tala, podrían crear a largo plazo incremento de los niveles de GC en monos araña, los cuales pueden perjudicar la viabilidad de sus poblaciones. Estos resultados además enfatizan por qué monos araña se encuentran en alto riesgo a la extinción cuando viven en hábitats antropogénicamente alterados.

Así, para un mejor entendimiento de como los monos araña hacen frente a la fragmentación de hábitat, estudie dos grupos bien habituados que viven en un fragmento pequeño (65 ha). Para investigar si y como ellos modifican sus patrones de agrupaciones y comportamiento social, colecte datos del tamaño del subgrupo, agresiones, disponibilidad de frutos en el fragmento y niveles de FGCM. Ambos grupos de estudio se movieron en subgrupos pequeños, mostrando niveles altos de FGCM cuando la disponibilidad de frutos fue alta, comparado cuando la disponibilidad de frutos fue baja. Estos resultados fueron inesperados porque (1) especies como monos araña o chimpancés, quienes exhiben dinámicas fusión-fisión, típicamente muestran el patrón de agrupación opuesto, y (2) una mayor función de glucocorticoides es la movilización de energía durante la respuesta a un estresor. Consecuentemente niveles de GC generalmente incrementan con el tiempo de baja disponibilidad de recursos. Los monos arañas son considerados como especialistas en frutos maduros. Sin embargo, ambos grupos de estudio tienen una tendencia hacia una dieta más folivora, parece que probablemente sea una manera de reducir el nivel de competencia por frutos en tiempos de baja disponibilidad. Por lo tanto, parece razonable suponer que cuando la disponibilidad de frutas es relativamente alta en este fragmento, el nivel de competencia alimenticia intra-grupal por frutas también podría ser alta. Esto también podría explicar porque los niveles de FGCM son altos y el tamaño de los subgrupos es pequeño en épocas de alta disponibilidad de frutos. Esta interpretación es además reforzada por las observaciones de monos araña, con inusuales niveles altos de agresiones hembra-hembra, las cuales son mas frecuentes cuando la disponibilidad de frutos fue alta.

Animales que viven en fragmentos de bosque a menudo tienen que reducir el tamaño de sus rangos de hogar. Esto puede potencialmente conducir hacia altos niveles de competencia intra-especifica por recursos y espacio. En fragmentos, en donde las densidades son a menudo altas y la disponibilidad de recursos es frecuentemente alterada, el confinamiento a un espacio pequeño puede provocar competencia inter-especifica, especialmente entre especies que comparten nichos ecológicos. Reporte, varios casos de agresiones severas de monos araña dirigidas hace infantes de monos aulladores y capuchinos en un fragmento pequeño con alta densidad poblacional de primates. Este comportamiento podría ser “patológico” o una estrategia para eliminar competidores potenciales futuros por espacio o recursos.

En conclusión, este estudio demuestra diferencias entre especies en la habilidad para hacer frente al disturbio antropogénico. Estas diferencias pueden ser al menos parcialmente atribuidas a diferentes niveles de sensibilidad fisiológica a las presiones antropogénicas. Además, esta tesis describe como la fragmentación de bosque cambia la habilidad de los monos araña a disminuir la competencia intra-grupal por alimento ajustando el tamaño del subgrupo. Es muy importante resaltar, que los resultados de este estudio ilustran el potencial uso de medidas de GC como una herramienta para monitorear poblaciones en áreas perturbadas y para la evaluación de las estrategias de conservación (ej. corredores conectando fragmentos). Además, demuestra el potencial beneficio para la conservación de la biodiversidad global, combinando investigación científica con esfuerzos de conservación.

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ACKNOWLEDGEMENTS

Foremost I want to thank my supervisor Eckhard Heymann for the opportunity to conduct this project. I am very grateful for your ongoing support, trust and scientific guidance since my diploma thesis in 2008. I very much appreciate that you were always there when I needed advice but that you also let me find my own way in so many ways.

I am grateful to Peter Kappeler for co-supervising this dissertation. I further thank Peter Kappeler, Julia Ostner, Oliver Schülke and Kerstin Wiegand for agreeing to be members of my evaluation committee.

This work would not exist and would not have been possible without Andrés Link. Thank you for introducing me to paradise, the spider monkey world and for not giving up on me during my first weeks in TBS. Also thanks for teaching me about the, sometimes hard to embrace, ease of Colombian life. I am very grateful that you and Tony Di Fiore have welcomed me in the Proyecto Primates family and later in San Juan. Moreover, thank you (and Gaby, Manu and Maya) so much for opening up the doors to your house (even at 5 a.m.).

In Colombia I want to thank Pablo Stevenson and the LEBTYP for support with sample storage, the acquisition of export permits and the exportation of the fecal extracts to Germany. I also want to thank Fransisco Henao Diaz for his help with the endless process of obtaining the export permits. I am grateful to the Lalinde and De Greiff families for their permission to work at ‘Hacienda San Juan de Carare’ and all the persons who allowed me to visit their farms and forests. I also want to thank Fundación ProAves for the permission to work at Reserva El Paujil and the Ministerio de Medio Ambiente y Desarrollo Sostenible for all permits needed for this work.

I would like to thank Michael Heistermann for teaching me all I know about hormones and EIAs, for giving me the opportunity to work in his lab and for numerous constructive discussions. Andrea Heistermann and Petra Kiesel always had an open ear for questions, were always very helpful in the lab and I really enjoyed working with them. Thanks so much for that.

I want to thank the authorities of the Wilhelma (Zoo Stuttgart) for welcoming me in their Zoo twice and for the great cooperation of the animal caretakers (especially Thali Bauer) during fecal sample collection.

For financial support I want to thank the German Primate Center, Margot Marsh and Ruffords Small Grants Foundation. Idea Wild generously provided some of the equipment used for the project.

I want to thank all the field assistants of Proyecto Primates that have helped with the sample collection and during long nights of sample extraction. Especialmente quiero agradecer a Laura Abondano (especialmente por enseñarme a remar), Felipe Alfonso (por tantas caminatas en el sol y horas esperando a los micos enfrente del “bajo”), Natalia Alvis (por tu fantasía y energía con los niños), Andrea de la Cadena (por muy buena comida y mucho tinto), Liz Diaz (por tu involucración con los animales), Nathalia Fuentes (por tu amor por todos los bichos que hay y tu amistad), Miguel Garcia (por hacerme reír tanto), Andrés Montes (realmente por todo!),

Alejandra Pardo (por tu buen humor y gran fe en ‘que todo va a salir bien’), Sandro Pianda (por todo tu ayuda en el ‘otro bosque’), Luisa Salazar (por la buena energía) y Boris Villanueva Tamayo (por todo tu ayuda, especialmente al principio de mi trabajo). Estoy muy agradecida por vivir tanto tiempo con ustedes en nuestra casita, por disfrutar el bosque, los miquitos, por tantos buenos, graciosos y tristes experiencias que hemos tenidos juntos. Muchas gracias por enseñarme tantas cosas sobre Colombia, el español (sea de Colombia o España), su cultura y la música. Muchas gracias por su amistad y por ser mi segunda familia! Y muy especialmente quiero agradecer a Andrés y Aleja por ayudarme tanto con las muestras, los tubos y por su entusiasmo y amistad.

También quiero agradecer a TODA la familia de Nelson por recibirme como alguien de ellos, por darme una cama para dormir, mucha mucha comida y clases de salsa!!

I want to thank Ana C. Palma for teaching me many things about “how to be a field biologist” (better be safe than sorry!), for taking out spines from the weirdest places (together with Mia Marek and Monica Ramirez) and for (rice-free) culinary and cultural expeditions in Bogotá! And thank you for opening my eyes to so many little but important things in this world.

I want to thank all my current and former colleagues of the Behavioral Ecology and Sociobiology Unit for very much appreciated scientific (and unscientific) discussions. Especially I want to thank Melanie for much appreciated exchange of ideas and Anna for her help during my first steps in R.

For his excellent support with technical questions and problems I thank Henning Lahmann.

I want to thank Gisela Kopp, Laëtitia Maréchal, Julie Duboscq, Deike Terruhn and Pascal Marty for their helpful comments on this thesis.

I am grateful to all my friends in and around Göttingen for making these years full of joy and stories. Especially I want to thank Gisela, Matthis, Flavia, Anna G., Anna S., Anni, Pascal, Sally, Marlies, Christof, Cay, Inga, Deike, Dirk, Hilka, Jenny and Verena for their support, very good times, plenty of great memories, and humorous and serious discussions about work and all facets of life. I want to thank Cedric for invaluable and sound statistical support, for always being willing to help, for great discussions in our kitchen and for being a good friend. I also want to thank all my housemates and Anne for feeding me during the final phase and for keeping up the good mood (in spite of my grumpiness). Special thanks go to Julie, Chris and Tabby for your encouragement, support, humor and friendship especially during the last months of writing up! Tabby and Chris, our fruitful discussions on the roof terrace made the final spurt much more bearable and enjoyable! Thanks to all of you!

I am deeply grateful to my parents and my ‘little’ brother hobs. I want to thank you for your endless support and faith, especially when I was on the other side of the planet. Thank you for never questioning this unusual job and thanks for believing in me!!

Y al fin: Nelson, gracias por todo, por ser mi amigo, mi compañero de campo, por ser un soñador y por compartir tus sueños e ideas conmigo, por quedarte conmigo, aun si fuimos separados por tanto tiempo y por tanta distancia. Gracias por tu apoyo y amor, tu fe en mí, este trabajo y un futuro para nosotros!

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2013	Nacey Maggioncalda Foundation. Major Grant (US $ 10,000)
2013	Conservation International (Margot Marsh Biodiversity Foundation) (US $ 2,830)
2012	The Rufford Small Grants Foundation (UK £ 5,577)
2011	Conservation International (Margot Marsh Biodiversity Foundation) (US $ 2,000)

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