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Variation in Stress Among Vervet Monkeys (Chlorocebus Pygerythrus) at Different Stages of Rehabilitation in South Africa

Auriana Iris Gilliland-Lloyd
University of Wisconsin-Milwaukee

VARIATION IN STRESS AMONG VERVET MONKEYS (*Chlorocebus pygerythrus)* AT DIFFERENT STAGES OF REHABILITATION IN SOUTH AFRICA

by

Auriana Gilliland-Lloyd

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ABSTRACT

VARIATION IN STRESS AMONG VERVET MONKEYS AT DIFFERENT
STAGES OF REHABILITATION IN SOUTH AFRICA

by

Auriana Gilliland-Lloyd

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This study observed behavior and hormonal responses among vervet monkeys (*Chlorocebus pygerythrus*) in two separate stages of the rehabilitation process at a rehabilitation and wildlife center in South Africa. The aim of this study is to determine whether groups undergoing the rehabilitation process exhibit significantly different mean fecal cortisol concentration or mean behavioral frequency rates, at the introduction and secondary semi-wild stages.

Females in the introduction group exhibited significantly higher rates of both affiliative, and agonistic behavior over their male introduction, and semi-wild counterparts. These findings suggest that rehabilitant vervets show stress patterns typically seen in wild vervets, and that female vervets experience heightened levels of behavioral variation. Despite introduction males having slightly higher cortisol rates, no significant difference was found in glucocorticoid expression among the groups. No other variables interacted significantly with cortisol rates.

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HORMONAL AND BEHAVIORAL VARIATION IN VERVET MONKEYS IN A REHABILITATIVE SETTING IN SOUTH AFRICA

INTRODUCTION

Animal rehabilitation programs receive thousands of animals each year in South Africa for a multitude of reasons such as injury, abandonment of offspring, and surrendered pets from the illegal pet trade (Wimberger 2010, Guy 2013). One goal of a rehabilitation center is to reintroduce rehabilitated groups back into the wild. Population reintroduction is recommended in situations where populations are endangered and at risk to decline (Baker 2002, Cowlishaw and Dunbar 2000). The rehabilitation process places stress on an individual animals (Abbott et al 2003; Clarke 1992). If an animal is to be released into a wild and uncontrolled environment, it is thought that they should exhibit behavior similar to what is observed in their wild counterparts. By learning typical or normally exhibited behaviors, rehabilitant animals learn how to survive in their natural environment (Guy and Curnoe 2013, Healy 2014, Wimberger 2010).

Cortisol is a glucocorticoid that acts as an indicator of stress, and is the predominant circulating glucocorticoid in primates (Abbott et al 2003). If specific sets of behaviors are associated with higher rates of cortisol, behaviors may act as predictors for when these animals are experiencing stress. Animals at different stages in the rehabilitation process may show varying levels due to their environment directly (e.g. electric wires, enclosed space), or due to the generally stressful process of social cohesion (e.g. unknown individuals). Chronic stress can lead to health issues and further social conflict (Abbott et al 2003, Clarke 1992, Dettmer 2014).

There are few studies that focus specifically on the environment and its relationship to social behaviors and stress in primates housed in a rehabilitative setting. Welfare and

environment are critical components to any discussion on primates in ex situ conservation. The ramifications of poor rearing can have severe consequences. This study analyzes cortisol and behavioral differences among vervet monkeys at two separate stages of rehabilitation: the introduction stage, and the semi-wild stage. Explanations for the differences between the two rehabilitant groups are discussed, and have the potential to add to the discussion on guidelines for vervet monkey rehabilitation.

VERVET MONKEYS (*Chlorocebus pygerythrus*)

Vervet monkeys (*Chlorocebus pygerythrus*) are ubiquitous to most of Africa, and the Caribbean. While vervets are not listed as endangered on the IUCN Red List, populations are still in decline due to human encroachment and conflict (Young and Isbell 1994, Cheney et al 1988). Vervets have adapted to many environments and have little genetic variability between populations (Warren et al 2015, Turner et al 2016).

Taxonomy

The classification for vervet monkeys has changed, shifting from genus *Cercopithecus* to *Chlorocebus* with currently six species and up to 25 subspecies recognized (Groves 2000, Grubbs 2003). Genus names are often used interchangeably, and considerable debate is ongoing. Within the literature, vervets are often referred to as *Chlorocebus pygerythrus*, but have been classified as *Cercopithecus aethiops* in the past (Cheney and Seyfarth 1980; Isbell et al 1990, Turner et al 2000). Vervets belong to the guenon primate group. Grubbs (2003) among other primate researchers argues for further evaluation of vervet taxonomy in the future, as future genetic analysis may provide further subspecies identification (Grubb 2003).

Morphology

Green monkey is the commonly used name to describe vervets, although their coloring ranges from sandy brown to olive green. The color of their coat is thought to be distinctive to their region, and fur length can also vary (Rodriguez et al 2016). A majority of the individuals observed in this study were of South African or Mozambiquen descent, which typically have sandy colored fur with light brown speckles. Male vervets have a bright pale blue scrotum that contrasts with a vibrant red penis and surrounding white fur (Rodriguez et al 2016, Anapol et al 2005, Cramer et al 2013). Vervet monkeys are medium-sized, and have cheek whiskers and a distinguishing band of fur on their brow.

Vervets are sexually dimorphic, with female growth leveling off at an earlier age than in males (Cramer et al 2013, Turner et al 2000). Males weigh between 8.6 and 17.6 lb, and measure in length between 1.4 and 2 ft. Females average between 7.5 and 11.7 lb, and measure in length between 11.8 inches and 1.6 ft. Vervets are born with black natal coats and bright pink faces that change to their adult coloration by 10 weeks of age (Cramer et al 2013, Struhsaker 1967). Vervet monkeys can have black to pink feet and hand pads, and the tip of their tails varies from golden colored to black. Vervet monkeys have distinctive white tufts of hair that surround the face.

Ecology

Vervets inhabit large sections of Sub-Saharan Africa, ranging from Egypt and Eritrea to South Africa (Anapol et al 2005, Groves 2000; Struhsaker 1967). Mountain ranges, forests, and savannahs separate species geographically, however hybridization still occurs (Turner et al

2000, Turner 1988, Louden et al 2014). Vervets are also found in the Caribbean on the islands of St. Kitts and Barbados. Brought over on slave ships in the early 1500's, they have flourished on the islands by adapting to the tropical island environment.

Limitations to the typical vervet environment are water availability and trees for sleeping. Vervets do not occupy arid open grasslands (Cheney and Seyfarth 1986, Anapol et al 2005, Groves 2001). South African vervets inhabit woodland and savannah habitats, with populations ranging from the lowveld to the highveld Cape Fold belt system mountains. This includes the Drakensberg Mountains, located in Limpopo, South Africa near the study site. Especially prevalent in wooded areas near rivers, vervets utilize the edges of forests rather than the interior.

Vervet monkeys are well known to adapt to different environments. They are extremely opportunistic and efficient foragers, and subsist on a wide variety of foods (Louden et al 2014; Brennan et al 1985). Their quick ability to adapt to varying ecological habitats brings them into direct conflict with humans (Grobler et al 2006, Loudon et al 2014, Wimberger 2010, Guy and Curnoe 2015). Vervets are known to farmers and gardeners as crop raiders and pests in nearly all environments where vervets exist with humans. Although vervets are the most widespread of the African monkeys, they still experience conservation challenges similar to other African primate species. Population surveys reveal that populations of vervets are on a steady decline (Guy et al 2015, Young and Isbell 1994). Stable isotope analysis revealed that vervets have varied diets depending on their close proximity to humans (Loudon et al 2014).

Vervet monkeys have the most omnivorous diet of all primates and eat all types of food that primates are known to eat: grass, fruit, shoots, seedpods, buds, invertebrates, birds, lizards, rodents, berries, and bird eggs (Struhsaker 1967, Isbell 1995, Isbell et al 2004). Fluctuations in food availability varies the vervet diet from month to month. Vervets can suffer severe nutritional stress as evident through hair loss and changes in skin color (Isbell 1995).

The average range size for a vervet troop changes depending on the season. Vervets forage less in the rainy season, and spend less time eating (Altmann 1988, Isbell et al 2004). Under conditions of food enrichment, protected vervets in Amboseli National Park spent 20% of their time foraging, while an unprotected free-roaming population of vervets spent 40% of their time feeding (Altmann 1988). The semi-provisioned vervets also spent more time resting and socializing, and moved far less than the wild un-provisioned animals (Altmann 1988).

The minimum recorded home range size for vervets is 24.7 acres, and the maximum-recorded home range is 237.2 acres (Struhsaker 1967, Isbell et al 1999, Isbell et al 2004). Resource abundance and distribution are key determinants in determining home range size and day range length for vervets. Vervets will alter their ranging patterns to take advantage of reduced predator presence (Isbell 1991, Cheney and Seyfarth 1983).

Smaller-sized monkeys are heavily susceptible to predation from a large number of mammalian, reptilian, and aerial predators. Crowned eagles appear to be the most pervasive predator for vervet monkeys living in forested areas. Other commonly observed predators include leopards, rock pythons, jackals, serval, caracals, hyenas, and lions (Isbell 2002).

Socioecology

Vervets live in multi-male/multi-female groups. The average troop consists of one to seven adult males, two to ten adult females, and their young offspring (Cheney and Seyfarth 1986). In all *Chlorocebus pygerythrus* populations where there is data, males disperse from their natal group at age 5, at the onset of sexual maturity (Struhsaker 1967,Cheney and Seyfarth 1983, Isbell 1990). Females are philopatric, living their entire lives in their natal groups. Males may transfer groups multiple times in their lifetime (Cheney and Seyfarth 1983). Benefits of dispersal include inbreeding avoidance, and increased opportunities for breeding mate suitability (Isbell 2004). Young male vervets transferring for the first time appear to be attracted by the presence of male peers in adjacent groups, including potential kin (Cheney & Seyfarth 1983).

One explanation for male dispersal and the existence of multiple males in a troop is the limited dispersal hypothesis. This hypothesis argues that males have limited dispersal abilities and opportunities because of the configuration of vervet habitat and the costs associated with dispersal (Isbell et al 2002). Because vervet group habitats line up along riverbanks one after another, rather than being scattered throughout savannah habitat, there are limited options for spatial dispersal. Males will disperse to adjacent groups in which other males (including relatives) already reside. One explanation for this dispersal pattern is that inclusive fitness benefits increase when males share residence with other males.

Vervets are known to engage in territorial range defense through vocalizations and active defense at boundaries. Both male and female vervets participate in this vigilance

behavior (Cheney 1980). Isbell and Van Vuren (1996) suggest that females are philopatric because transferring into unfamiliar territory, and heightened aggression from strangers makes female dispersal too costly. Exceptions occur when females fail to reproduce or are unlikely to reproduce in comparison to others in their group (Isbell 2004).

Reproduction

Vervet males reach sexual maturity on average at 60 months, and females at 48 months. Age at first birth for females on average is 60.8 months (Cheney et al 1988). There is a distinct mating season that typically occurs in April through October. The birthing season is October through December. Gestation lasts on average for 163 days; interbirth intervals for 13.3 months (Bramblett et al 1975).

PRIMATE BEHAVIOR

Evolutionary forces have shaped primate behavior over the course of millions of years, through the universal seeking of individuals to maximize their genetic contributions to the next generation (Isbell et al 2002, Isbell et al 2004, Cowlishaw and Dunbar 2000). Individual behavioral traits and life history (e.g. age, sex, rearing, ecology, and species) also affect social and environmental responses (Bernstein et al 1974). Behavioral strategies differ significantly between sexes because each invests energy in varying ways. Females invest far more energy in reproductive events, which includes the costs of egg production, gestation, and lactation. Males have the potential to produce offspring at a higher rate than females, which creates intense competition between reproductively fit males.

Female Behavior

Female behavior is a key determinant of male behavior in primates (Struhsaker 1967, Cowlishaw and Dunbar 2000). Female coalitions are observed in female-philopatric societies, and occur primarily among close kin (Teichroeb, 2015, Sterck et al. 1997). Evolutionary models suggest that alliances among kin are the most important factors shaping female social relationships in primates (van Hooff and van Schaik 1992, Sterck et al 1997). In highly competitive environments, related females should benefit from mutual support to protect resources.

Patterns of grooming competition among female vervets suggest that vervets are successful at rank recognition. When a lower ranking female left a grooming pair upon being supplanted by a higher-ranking female, the departure of the lower ranking female did not appear to be in response to the two higher-ranking females. They did not appear to threaten her, however it is argued that the lower female still recognizds that there was a status difference between them by leaving the grooming session (Cheney & Seyfarth 1980).

Dominance is asserted in vervet social relationships through intimidation, psychological harassment, or alliances, rather than through specific dyadic aggression (Teichroeb et al 2015, Abbott et al 2003, Cheney and Seyfarth 1986). Ranking for vervets centers on agonistic interactions, identifiable from submissive gestures, behaviors, vocalizations, and withdrawals.

Kinship

Kinship has potential buffering effects on stress caused in social interactions. The adaptive value of early kin relationships is evidenced by lower mortality rates found in captive and free-ranging vervet populations (Berman 2004). Because female vervets spend their lives in

their natal group, extensive networks of matrilineal kin have the potential to develop and associate with each other (Cheney and Seyfarth 2004, Isbell 2004, Kapsalis 2004). A plethora of field, captive, and laboratory studies have found maternal kinship to be one of the most influential factors that structure affiliative behavior. These studies include the following affiliative behaviors: spatial proximity, grooming, conflict intervention, alliances, reconciliation, and co-feeding (Kapsalis 2004). Patterns of grooming and alliance behavior begin in infancy, with daughters developing close and enduring affiliative relationships with their mothers, facilitators, and other maternal kin that continue into adulthood (Isbell 2004, Kapsalis 2004).

Males in female philopatric groups immediately sever their associations with their mothers and other female kin upon dispersal (Isbell 2004, Isbell and Enstam 2002). Dispersing with male kin would presumably reduce vulnerability for both males and females to predators, and access to familiar kin may be more important for males that need allies in order to successfully join extant troops (Cheney & Seyfarth 1983; Rendall 2004). Cooperative alliances between male kin have advantages for agonistic interactions with other males. However, cooperative genital displays towards females are less successful in attracting mates than when performed alone (Cramer et al 2013). Vervets can disperse from their natal groups with peers, however these associations would dissipate when older males transfer groups again (Cheney & Seyfarth 1983; Isbell 2004). Whether these associations are based on recognized kinship or some other criteria is unclear, but there is mutual benefit in transferring cooperatively rather than alone.

Captive Behavior

Vervet monkeys exhibit heightened intra-group aggression when living in close proximity to humans (Fourie et al 2015). Vervets in captivity live in human-managed environments, and in rescue centers are often hand-reared or peer-reared. Peer-only (PO) rearing occurs in non-related social groups comprised of similar ages, and occurs most often in *ex situ* conservation programs such as zoos, sanctuaries, and rehabilitation programs (Bramblett et al 1985; Clarke 1992). Behavioral differences between PO-reared monkeys are usually traced back to past experiences and biological make up. When there is no maternal bond, young primates living with non-related individuals can develop hyperattachments to their peers or human caretakers. Animal behaviorists consider hyperattachments to be dysfunctional, as an individual tends to appear fearful and will play significantly less than their mother-reared counterparts. Play deprivation during juvenility is known to lead to increased aggression as adults (Bramblett et al 1985).

Rehabilitation centers work towards mitigating abnormal behaviors that may occur due to PO-reared monkeys, offering increased opportunities for play through enrichment and by limiting competition with food availability (Personal Communication, Bob Venter; Guy and Curnoe 2015). There is limited published data on whether these strategies successfully treat abnormal behavior, and how such success is defined. An important aspect of introduction research is conditioning primates to form social bonds typically found in troop dynamics.

Stress Behavior

Chronic stress throughout early life is well known to produce adverse effects on mental and physical health in primates (Dettmer et al 2014). The neuroendocrine stress response system synchronizes the body's response to environmental stress. Rates of agonistic behavior remain relatively low among stable groups, although aggressive behaviors are a normal component of Old World monkey species behavioral repertoire (Clarke 1992). Free ranging troops experience periods of social instability with associated increases in the frequency of agonistic behavior, however in captivity the behaviors are magnified. Prolonged agonistic behaviors can result in injury or death. Chronic stress has considerable effects on cognitive and emotional development in nonhuman primates (Dettmer et al 2014).

Change of environment, introductions of new animals, and the formation of new social groups are stressful experiences for primate species (Clarke et al 1992, Guy and Curnoe 2015, Wimberger 2010). When space decreased, adult male aggression towards subordinate males increased (Clarke 1992). Female primates living in multi-male, multi-female groups typically undergo more stressors than their male counterparts. Associated stressors include birth, female bonding, infanticide, and constant vigilance (Isbell and Enstam 2002). Throughout the rehabilitation process, spatial factors and relocation affect the expression of agonistic behavior following troop formation and disruption of stable groups.

Prior studies on human-vervet conflict focus on cortisol rates and proximity to humans (Fourie et al 2015, Fairbanks et al 2011, Vigue 2008). Results show that males exhibit higher chronic cortisol rates than females when living closer to humans. Explanations for chronic

cortisol expression include abundance of foods, human tolerance of vervets, and higher-risk foraging behaviors. The interaction between high stress rates and environment were found only in male vervets, suggesting that females avoided higher risk behaviors, while males engage in more novel behaviors at higher frequencies (Fourie et al 2015).

HORMONAL PHYSIOLOGY

Glucocorticoids

The physiological stress response involves the release of catecholamine, norepinephrine and epinephrine, from the adrenal medulla and sympathetic nervous system, and the release of adrenocortical steroid hormones (glucocorticoids) (Abbot et al 2003). Other endocrine responses include the suppression of hormones related to anabolism, growth, and reproduction. The stress response aids in individual adaptation to acute stressors by stimulating hepatic glucose release and visceral lipolysis, which enhances the delivery of glucose, fatty acids, and triglycerides to skeletal muscle and the brain. This triage process is nonessential to immediate survival, however it triggers inflammatory and immune responses, such as growth, digestion, reproduction, and tissue repair (Abbott et al 2003).

While stress has adaptive functions, long-term ongoing stress can lead to negative physical repercussions such as hypertension, insulin-resistance (type II) diabetes, ulcerations, impotence, osteoporosis, and immunosuppression (Abbott et al 2003). There are also psychological implications for prolonged stress, and a number of psychiatric disorders are related to an overactive stress response. Behavioral and psychological variables such as physical

activity, immune function, food intake or nutritional status, and reproductive function all modulate cortisol levels.

Stress and Subordinance

Because of the social nature and structure of primates, subordinates are associated with chronically overactive stress as assessed by hypersecretion of glucocorticoids, high blood pressure, and greater rates of stress-related pathologies (Abbott et al 2003, Louden et al 2014). Stress differences among individuals are due to rank relations and control of resources, subject to aggression, and the establishment of predictable social relations. Subordinates typically have frequent stressors, few kin relatives, and little to no social support due to lower social ranking. Social outlets, such as social grooming, social contact, and engagement in displacement aggression can be particularly effective among primates (Abbott et al 2003, Guy and Curnoe 2015). It is logical to assume that subordinates without regular access to such outlets are more likely to exhibit hyper-cortisol expression.

Higher relative cortisol levels in subordinates are associated with societies in which behavioral interactions are unlikely to involve close kin (Abbott et al 2003). Kin selection is an important component of natural selection theory, and degrees of consistent aggression (and basal cortisol levels) can be a function of relatedness. Having kin is more likely to ameliorate stress via coalitional support, and coping outlets such as social grooming. Primate societies that have highly kin-based social networks tend to have lower basal cortisol levels, such as marmosets and tamarins (Ziegler 1990). Cortisol levels are also influenced by genetic contributions – under baseline conditions and under conditions of significant environmental

challenge (Fairbanks et al 2011). This suggests that chronic stress may be influenced by genetic factors, especially when undergoing long periods of environmental stress.

Results of ongoing stress and receptor down regulation include impaired negative feedback sensitivity at the brain, resulting in diminished neuroendocrine restraint on adrenocortical and basal cortisol levels (Abbott et al 2003). Diminished feedback resistance to glucocorticoids may occur in vervets when elevated levels for both displacement aggression and relative cortisol levels are experienced.

PRIMATE CONSERVATION

According to the IUCN Guidelines for *Ex Situ* Conservation, "*ex situ*" is defined as "conditions under which individuals are spatially restricted with respect to their natural spatial patterns or those of their progeny, are removed from many of their natural ecological processes, and are managed on some level by humans" (IUCN 2014). *Ex situ* conservation has the potential to address the primary cause of threats, offset the effects of threats, buy time, and restore wild populations (IUCN 2014).

Rehabilitation Strategies

Animal rehabilitation falls under two subcategories: ethical, or conservation release programs (Cowlishaw and Dunbar 2000). Ethical projects typically do not take implications of release, such as disease transmission, feeding competition, and outbreeding depression, into consideration when placing rehabilitated animals back into a natural environment. While ethical releases may create undesirable consequences, benefits can include eco-tourism and opportunities for education (Collins et al 2008, Cowlishaw and Dunbar 2000). Conservation

release programs rely on measures of biodiversity and holistic assessments of ecosystems. A single method of rehabilitation should not be relied upon to restock and restore wild populations of animals without taking into consideration other conservation priorities. For example, while eco-tourism may be a viable option for some populations, in many countries it is not an option for very remote areas or when there is little infrastructure in place for tourism. There are also negative ramifications of eco-tourism that can include ecosystem disruption, disease transmission, and animal habituation to humans.

Methods for teaching normal behaviors to rehabilitant vervets vary depending on the program and institution, and the standard behavioral learning period takes time. In animals from captive-bred environments, an extended period of time is necessary in order for an individual to learn necessary survival skills from fellow group members, as opposed to humans (Collins et al 2008, Wimberger 2010, Guy and Curnoe 2015, Cowlishaw and Dunbar 2000).

The Golden Lion Tamarin Conservation Program described in detail by Kleiman et al (1986), had humans systematically training individual tamarins how to feed, avoid danger, and locomote. Results from the program show that immature animals have higher successful adaptation rates than older individuals, by the quick pace in which younger tamarins started eating, sleeping, and using their new environment. The study also found that tamarins have difficulty adapting to three-dimensional spaces due to the enclosure conditions during the rehabilitation process. Modifications were subsequently made to make their environments naturalistic by removing plastics and toys not found in their scheduled release site (Kleiman 1986).

An issue with rehabilitation and release programs include the post-release, when animals will seek out human contact even after a period of stable living in a naturalistic environment (as seen in the Treves chimpanzee rehabilitation project) (Treves 1997). Chimpanzee releases that occurred from 1966 to 1985 saw mixed results upon reintroduction. Four out of five release groups had prerelease preparation and provisioning, and only two out of five resulted in successful reproducing populations (Hannah et al 1991). Unsuccessful releases can occur due to individuals being inadequately prepared for life in the wild. Hunting, theft by humans, or disease may also occur and limit a released groups potential for success (Guy & Curnoe 2013, Wimberger 2010, Collins et al 2008). Releases in which a group thrives in their new release site is attributed to prerelease training, extensive integration periods for troop formation, and costly post release support.

Rehabilitation and Stress

When discussing stress and welfare in *ex situ* conservation, the quality of the environment is as critical as the size. The complexity of the environment depends on the level of visual barriers, height of space, and whether the setting reflects natural animal environments. In a study conducted by Margaret Clarke and Darryl Mayeaux, rates of agonistic behavior decreased as the levels of complex housing increased in captive vervets not being rehabilitated for release. These results suggest that any change in a group’s enclosure can have long-term effects on rates of aggression (Clarke, Mayeaux 1992). An environmental shift to less complex housing can lead to persistent agonistic behavioral increases.

South African Primate Rehabilitation

The most common primate in rehabilitation centers in South Africa is the vervet monkey. Vervet monkeys arrive at rehabilitation centers as ex-pets, ex-laboratory animals, or because they were found injured or orphaned - mostly due to human conflict (Grobler et al 2006, Wimberger 2010, Guy and Curnoe 2015). The main goals of wildlife rehabilitation are to "(care) for injured and orphaned wildlife until release, or if necessary, euthanasia, as well as educating the public to prevent these problems" (Wimberger 2010 pg 481). All South African rehabilitation centers are required to have a permit in order to operate from the provincial government (Darren and Curnoe 2013, Guy and Curnoe 2015). As of 2010, only four out of the nine South African provinces have any guidelines specific to their province surrounding permit applications and the release of rehabilitates animals (Wimberger 2010).

There is ample discussion and interest, yet little regulatory practice in determining which centers are suitable for rehabilitation (Guy and Curnoe 2015). Two owners of vervet rehabilitation centers expressed the concern that several thousand injured, diseased, and orphaned animals pass through unregulated centers in South Africa, with little oversight from government agencies (Personal communication, Bob Venter). Regulation should rely on observation and minimum requirements for animal welfare and care. Standards are useful to provide instructions for how to care for injured and traumatized animal, especially when species-specific.

Limited scientific research on the efficacy of rehabilitation methods for care and release, along with post-release monitoring, has led to numerous differentiating techniques and

protocols. The dispute of whether animal rehabilitation and release is even a possibility is ongoing, as individual rehabilitant situations present difficulties for repopulation. Whether or not rehabilitation is a suitable conservation method is not addressed in this study. The criteria for successful rehabilitation should consider the behavioral and hormonal health at the individual level - which in turn has the potential to affect the longevity of the health of the troop.

Amanda Guy and Darren Curnoe published *Guidelines for the Rehabilitation and Release of Vervet Monkeys (2010)*, as there was no previously established framework in the literature for South African primate rehabilitation, and vervets specifically. Recommendations for their best-practice model center on traditional rehabilitation methods: natural enclosures, limited-human involvement and contact, and strict health assessments upon arrival, during the integration phase, and post-release for each individual. The authority and oversight to ensure that South African rehabilitation centers follow regulations is costly and limited in the current South African governing body. There is little accountability, and Riverside Wildlife Rehabilitation Center has received animals in poor health from neighboring centers that are legally realized as credible rehabilitation and education centers.

Methods for wildlife reintroduction vary across regions, and are changing rapidly due to the growing number of rehabilitation programs. The rising need for such programs due to human-wildlife conflict is all the more reason to research methods and best practice guidelines (Grobler et al 2006). This study does not, however, attempt to determine which methods are best. A trend in the collected observational data could point to influential variables that cause stress. Such applications of these data are out of the scope of this study.

RIVERSIDE WILDLIFE REHABILITATION CENTRE

The Riverside Wildlife and Rehabilitation Centre is located in the Limpopo province of South Africa, outside of Tzaneen in Letsitele. Orange farm fields surround the center, as does the Groot-Letaba River: an environment typical for free-ranging wild vervet troops. Bob and Lynne Venter established the center in 1992, with only a small group of orphaned vervet monkeys on their property. In the years since, they have released 19 troops of vervets, and 1 troop of baboons (Personal communication, Bob Venter). They also receive and care for thick tailed and lesser bush baby, samangos, and other indigenous South African animals, such as tortoises, duikers, and warthogs. Animals in the semi-wild condition are fed minimally prepared food once daily, younger animals and individuals in the earlier processes are fed twice daily. Vegetation (e.g. leaves, sticks, weeds) picked from the river is gathered every morning and hung from varying heights in the introduction enclosure. This encourages foraging behavior and teaches individuals knowledge of surrounding leafy vegetation.

Process of Rehabilitation

Intake and Quarantine

When an animal arrives at Riverside they are immediately taken to the quarantine house for assessment unless the individual is an infant, in which case the animal is cared for in close human contact. Animals that arrive together are kept together, and housed separately from other animals in quarantine. Each animal is placed into an individual cage, and further separated by concrete walls to aid in eliminating stress via noise cancellation and visual blocking. Larger animals are placed into small room enclosures with open fencing as one of the

walls. Animals are then sedated and physically assessed for any previous injuries or diseases. A tuberculosis eye test is administered, and hourly monitoring of the animals behavior and nutrition is ongoing throughout the duration of the quarantine period. All animals remain in quarantine for a minimum of 30 days before being placed in an introduction enclosure with other members of their species. The quarantine house is situated at least 25m away from other enclosures and residential areas.

The quarantine period is required in order to maintain the physical health of all animals at Riverside. The length of the quarantine time period also allows the individual sufficient time to acclimate to their new physical environment (e.g. temperature) and diet before being introduced to others. This is particularly important in the cases where they arrive as pets, never having lived in the outdoors or with another member of their species.

Introduction Condition

After quarantine, and depending on their age group, individuals will be placed in a

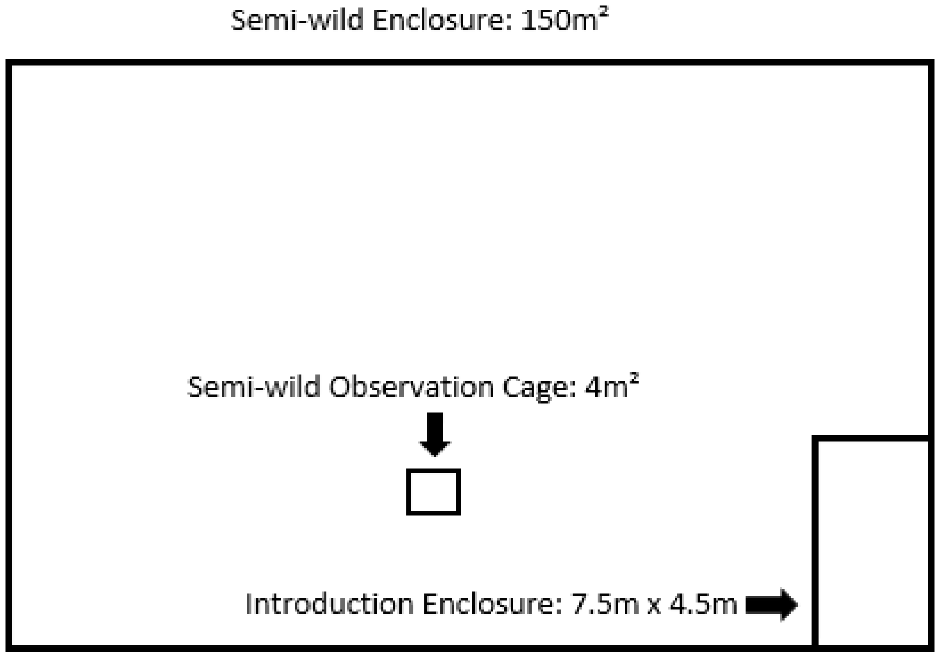

Figure 1: Layout of the Introduction and Semi-wild enclosures at the Riverside Wildlife and Rehabilitation Centre. The figure shows the shared fencing between the smaller Introduction enclosure and the larger Semi-wild enclosure. Samples and observations conducted in the Introduction enclosure were taken from the perimeter, while samples and observations for the Semi-wild were conducted in the Semi-wild observation cage, located in the lower middle of the enclosure.

nursery enclosure with other immature individuals, or if mature, will be placed in one of the four introduction enclosures. Each introduction enclosure is attached to a larger semi-wild enclosure. In each enclosure there are at minimum: two thick hanging branches for climbing, two thick branches bolted to the ground, water trough, and daily enrichment.

Once the quarantine period ends, monkeys are sedated and placed inside a pre-selected introduction enclosure, allowing the other group members to examine the newly introduced member prior to waking. This process can be traumatic for an individual who has not yet seen

other members of their species. This part of the integration process is thought to be the highest stress-inducing condition.

Introduction groups are fed and enclosures are cleaned once daily by center staff. When new individuals are placed, there is constant monitoring by volunteers and staff for at minimum a period of three days. Any abnormal or extreme physically violent behavior, and the severity of such behavior, is recorded. In situations where an individual is severely injured, they will be taken from the enclosure and to the veterinary house for further inspection. Because there are two different introduction and semi-wild enclosures for vervet monkeys, there is the option to try an alternative introduction group if the first does not accept the new individual. Animals will remain in the introduction enclosure until capacity has been reached.

Semi-Wild Enclosure

Riverside is one of many South African primate rehabilitation centers, yet unique in the large size of their open-air semi-wild enclosures. This phase of the reintroduction process can last from 1 to 4 years (Personal communication, Bob Venter). Riverside utilizes the natural environment by fencing in trees and vegetation, allowing individuals to locomote in the trees. There are four semi-wild enclosures measuring at least 100m by 100m, with the largest measuring up to 1,200 square meters. Each enclosure is secured with electrical wiring surrounding the top perimeter of the fencing, with no roofing or mesh above. Each enclosure is covered in dense brush and natural trees, shrubs, and grasses native to the woody environment typical of vervet monkeys. Over the lengthy rehabilitation process, the semi-wild enclosure

allows vervets to acclimate and adapt to a climate and vegetation they will presumably encounter upon release.

A strict no human contact rule once placed in the semi-wild enclosure applies to all who work at Riverside. Only for maintenance and animal removal purposes are workers allowed inside of the semi-wild enclosure. It is thought that physical distance from human contact conditions will allow the vervet group to form stronger social bonds, and discourage familial and friendly attitudes towards humans.

Vervets in the semi-wild enclosures are fed once daily in the mid-morning. The food varies depending on the season aside from oranges, which are available year round. Other fruits and vegetables include: cabbage, eggplant, mango, peppers, papaya, banana, and bread.

The process of integrating an introduction group to a semi-wild group can take up to a full year. The social interactions that took place prior to the introduction (through the fence perimeter) has a tension reducing effect, as the monkeys have already seen, smelled, and observed the hierarchy established in the semi-wild group.

Release

Troop releases are carefully planned so as to minimize pre-existing troop overlap, while also ensuring that there are troops in adjacent areas for future dispersal and survival of the group. Release site considerations include: access for post-release evaluation, territory overlap, land protection, inter-species competition, and effects on overall biodiversity (Personal communication, Bob Venter; Cowlishaw and Dunbar 2000).

Post-release, volunteers and staff from Riverside will travel to the release site and monitor troop adaptation to their new environment. These observational checkups are conducted as often as logistically possible, following the full week of monitoring immediately after the release occurs. On occasion, troops are released on protected private land. It can be logistically difficult to travel to release sites, and Riverside will communicate with the private landowner on the status of released groups. There is ongoing debate on whether rehabilitation is a viable option due to unexplored ramifications of releasing animals into the wild. Research on the rehabilitation process has the potential to support or discourage release processes. This study does not attempt to argue on behalf of or against animal release.

Aim of Study

There are many environmental and social variables that have the potential to affect stress and behavior. If social variables cause stress responses, then rehabilitant animals in different social and environmental conditions will reflect these responses.. In determining whether there is a patterned relationship, research can focus on specific variables selected for these associations. This study evaluates cortisol concentration rates, and both affiliative and agonistic behavior rates between four groups of monkeys, split up by categories of rehabilitation stage and sex.

Predictions

(1) Vervet monkeys in the semi-wild stage of rehabilitation should exhibit less agonistic behaviors, lower cortisol rates, and increased affiliative behaviors in comparison to the introduction stage due to enhanced social cohesion over time. (2) It is predicted that the

introduction group will exhibit higher mean rates of fecal cortisol concentration, and higher mean rates of agonistic behavior, due to being newly introduced to other individuals at the beginning of the rehabilitation process.(3) It is also predicted that males will exhibit higher mean rates of cortisol concentration, and higher rates of agonistic behavior, due to male vervet vigilance behavior. (4) It is also predicted that cortisol concentration rates are positively correlated to agonistic behavior, with agonistic behaviors acting as predictors for higher levels of fecal cortisol concentration.

METHODS

Subjects

Table 1: Subcategories of vervet monkeys under analysis: in the Cape Introduction and Cape Semi-wild Enclosures at the Riverside Wildlife and Rehabilitation Center

Introduction Males	Introduction Females
Semi-wild Males	Semi-wild Females

Table 1 shows the four separate subcategories under analysis. The aim is to determine whether there is a pattern of stress specific to each sex, or the introduction or semi-wild stage of rehabilitation. In doing so, the differences between conditions and behavioral dynamics by sex can be explored as potential reasons for behavioral and hormonal variation.

For the purposes of this paper, social cohesion is defined as an increase in typical physical behaviors and positive social interactions with individuals in both conditions (e.g. foraging, playing, grooming, and arboreal locomotion). The traumatic nature of being a lone animal introduced to a group of unknowns is expected to lead to heightened levels of stress.

A total of 12 individuals were selected for fecal and focal observation sampling. Table 3 provides physical and historical information about each of the six individuals under observation in the introduction group. There was limited data for the individuals under observation in the semi-wild group, and are not included. Individuals were distinguished by sex, size, and physical characteristics.

Behavioral Observations

Out of the three semi-wild enclosures at the Riverside Wildlife and Rehabilitation Centre, the Cape semi-wild enclosure had less dense brush and more visibility, and was chosen as the enclosure under observation. The Cape introduction enclosure was selected for observation because it was attached to the Cape semi-wild enclosure. From here on the Cape enclosures will be referred to as the introduction and semi-wild conditions or group. The individuals selected for observation in the introduction condition were randomly sampled in order to obtain an equally distributed sample population.

This study relied on both Jeanne Altmann and J.D. Patterson's methodology to create an ethogram suitable for the primates under observation (Altmann 1974; Paterson 2001). After eight hours of initial observations, an ethogram chart was created in relation to observed behavior within the specific eight-hour timeframe (See Table 2 for the full ethogram chart). When an individual was out of sight, the full duration was noted in the observational chart and deleted from the overall observational period.

Table 2: Ethogram with Descriptive Behaviors

Behavior Category	Behavior Descriptors
Affiliation	allogrooming, sitting in contact, clinging, touching, genital inspection, lipsmack, play, smell, embrace, muzzling
Agonistic	cageshaking, hitting, wrestling, biting, open mouth threats, brow raise, chase, lunge, displace, take object
Defensive	grimace, defensive touch, escape behavior, screaming, avoid, head bob
Solitary/Individual	self-grooming, object manipulation, solitary alert, locomotion
Abnormal	self-biting, self-huddle/clasp, floating limb, hair pulling, pace, repetitive movement, masturbate
Feeding/Resting	feeding/resting

A total of 86.2 observational hours were collected over the 6 week-observation period. The two behaviors under analysis for this study were affiliative and agonistic. Daily agonistic behavioral rates were obtained by the ratio of agonistic behavior duration over daily total observed time for each individual, and as a group; affiliative behavioral rates were obtained by the ratio of time spent engaging in affiliative acts, over daily total observed time for each individual, and as a group total. Individual counts of agonistic events, lasting less than 4 seconds and separated by at least 20 seconds, were considered in the daily time. A combined average behavioral, and combined average cortisol rate over the 6-week observation period for each sub-group is compared to detect whether a significant interaction exists for each condition and sex grouping.

Hormonal Data Collection

Because feces can be collected without capturing or interfering with animals, methods that use fecal steroid metabolites have become increasingly popular in both captive and free-ranging studies (Beehner et al 2004). Vervet feces have a typical length of 3-5 cm, and animals tend to excrete when they are terrestrial (Chame 2003). Feces were opportunistically collected in both conditions: introduction, and semi-wild. The collection process included: monitoring the individual monkeys until excretion, noting the exact time and date of the sample, and the individual.

A majority of samples in the semi-wild enclosure were obtained when the vervets were on top of the cage, while a long reach pole was used to collect samples through the fence of the introduction enclosure. Both condition samples were collected within the same one-hour period consecutively for a six day sampling period.

Field Extraction Methods

Fecal samples were labeled per individual on a daily basis for six consecutive days. The samples were stored using .1g of fecal material with 2.5ml of ethanol, and 2.5ml of distilled water. Steroids were then extracted by hand vortex, and centrifuged with a string by swinging the samples around above the head for a full ten minutes each. SPE columns were conditioned with .1ml of methanol followed by .1ml of water, and then .1ml of fecal extract was pushed through. The columns were then sent to the National Primate Center in Madison with full CDC approval for further assaying. Dr. Toni Ziegler at the University of Wisconsin’s National Primate Research Center advised fieldwork extraction methods.

Table 3: Individual background and physical information for each vervet monkey under observation in the introduction group.

ID	SEX	AGE	HT.	WT.	HISTORY	OTHER
VSARW09	M	3.7	100 cm	3.6 kg	Ex-Pet	top right canine broken, poor dental ecology; was thought to do well in introduction, but it took 6 days for the group to finally accept him
VSARW11	F	3.5	99 cm	3 kg	Born in KZN, kept in cage in Lanasia. Confiscated by Joburg SPCA	n/a
VSARW08	F	2.7	100 cm	2.4 kg	spent time in house after finding on land (person did not want any responsibility)	digit on right hand missing; 11/15/2014: surgical repair of laceration on thigh in Georgie (.5ml chloro), after this was moved to quarantine prior to going to intro; 11/18/2014: bandage clean (.2ml); 11/21/2014: bandage removal (.15ml); 11/25/2014: new leg incision (.15ml); 11/26/2014: bandage removal (.15ml); 12/12/2014: suture removal (.15ml); 2/5/2015: fight wound on lateral right hand; 4/27/2015: nasal discharge, hunched posture, oral ulcerated lesions, hypersalivation
VSARW05	M	+/- 6.5	122 cm	7 kg	Ex-Pet confiscated	1/26/2015: minor wounds on hands, most likely from self-harm on cages, minor wound on penis
VSARW04	F	+/- 3.5	107 cm	3.7 kg	Tame, possibly a pet	1/26/2015: left hand tip is missing, right hand is broken from an old injury; 2/4/2015: fight wounds on back and tail in CapeIntro, went back in to CINT on 2/5/2015
VSARW02	M	4.5	106 cm	3.6 kg	Born in KZN, kept in cage in Lanasia. Confiscated by Joburg SPCA	n/a

Lab Assaying Protocol

The UW-Madison National Primate Research Center lab assisted the cortisol assaying, and created the following protocol. First, the reagents were warmed up to room temperature in the water bath. Standards were vortexed and 50 ml were transerred into test tubes. A further 25µl sample was pooled to evaporate the EtOH. In a scintillation vial, F-HRP was made, and then 20µl was added to two plates, along with 30mL of an EIA-buffer. An additional 300µl of the F-HRP was slowly added to micro-tube samples, and pooled after evaporation and vortex. 250µl of the F-HRP and 300µl of NSB (non-specific binding) buffer were slowly added to the standard and vortexed. 100µl of this solution was pipetted into the plate, and then shaken for five minutes.

After putting the plates through the wash, a substrate solution of: 2467mL Citrate, 80µl of H_2O_2 (hydrogen peroxide) and 250µl of ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) were pipetted into the plates. Plates were shaken and incubated for 42 minutes total (recommended 35-40 minutes). A STOP solution of 15mL HF, 30µl EDTA, and 15mL of distilled H20 was added to the plates after the second wash cycle. A Spectra Max Plate Reader, along with SoftMax Pro software, analyzed the plates (Corbett, 2016)

Statistical Analysis

The aim of this study is to determine whether there are significant differences between behavior and cortisol expression between introduction and semi-wild groups, and males and females. Frequencies for mean behavior rates were calculated for each group under observation: semi-wild females, semi-wild males, introduction females, and introduction males.

Mean frequencies were calculated by the amount of time each individual engaged in a specific behavior, over total time observed throughout the 6 week observation period. A two-way analysis of variance was performed to determine differences between affiliative and agonistic behavior means for each of the four groups over the six-week observation period. Because fecal samples were collected for 16 individuals over a one-week period, corresponding behavior means during this time were used to determine whether a correlation exists between cortisol concentration and agonistic behavior. A correlation regression analysis was performed, with significance for all statistical tests defined as a P-value < 0.05.

RESULTS

Prior to statistical analysis, mean cortisol values were transformed into a normally distributed variable using R statistical software and the associated Box-Cox transformation. A subsequent factorial ANOVA with type III sum of squares test was conducted to evaluate correlation significance for both mean agonistic and mean affiliative behavior rate and their association with cortisol. Along with the ANOVA test, t-tests also show that neither sex nor group differ in their significance to fecal cortisol concentration rates. There is no interaction between cortisol expression and agonistic behavior rates between groups, or sex.

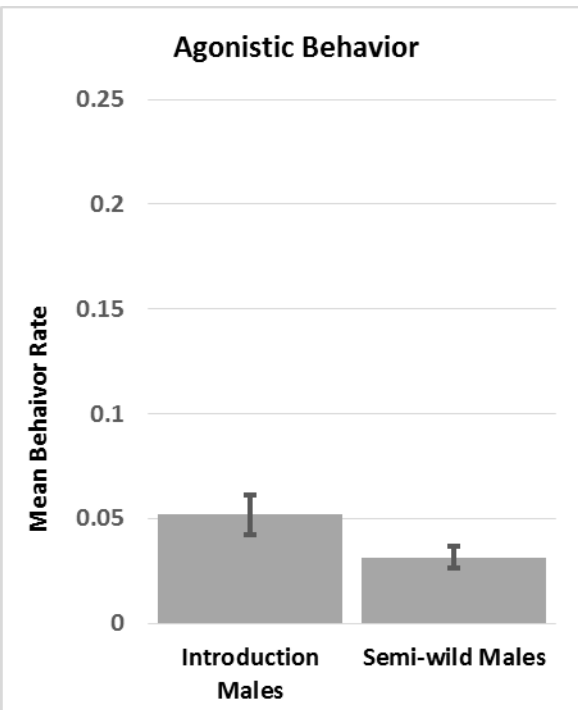

Figure 2: Mean Agonistic Behavior Rates for Males in the Introduction and Semi-wild Enclosure Groups. Male intro mean rate of 0.052, SE = 0.009. Male semi-wild mean rate of 0.031, SE = 0.005.

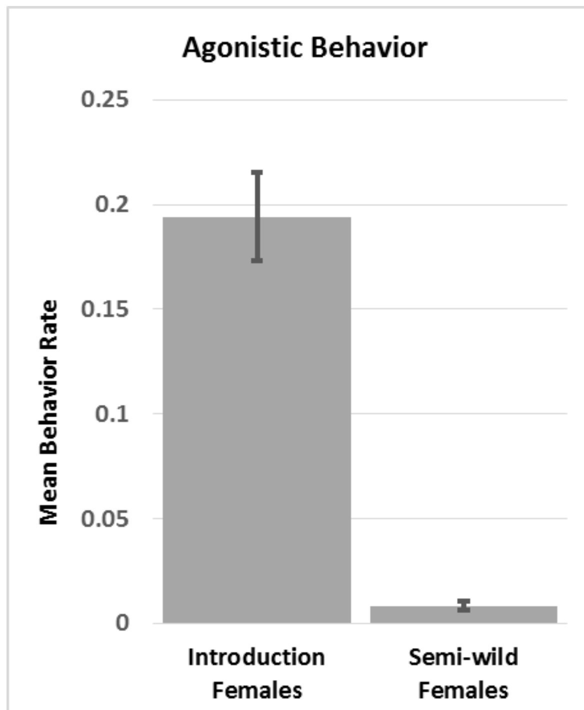

Figure 3: Mean Agonistic Behavior Rates for Females in the Introduction and Semi-wild Enclosure Groups. Female intro mean rate of 0.19, SE = 0.02. Female semi-wild mean of 0.008, SE = 0.002.

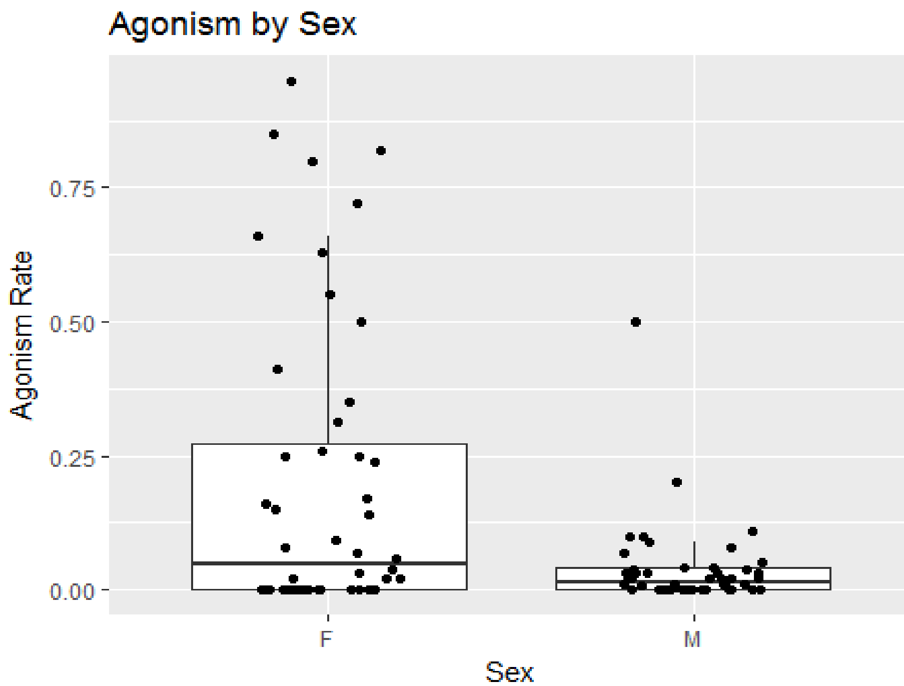

Figure 4: Mean frequency agonistic behavior rates for each observed individuals, broken down by sex. Females are on the left, and males are on the right. Mean for females is 0.2, SE = 0.04; mean for males is 0.04, SE = 0.01

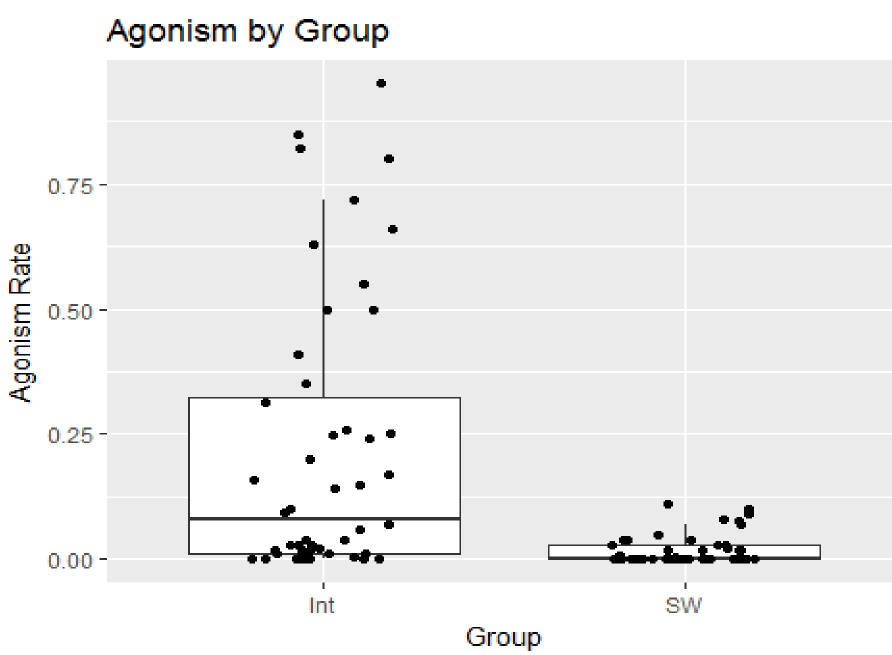

Figure 5: Mean frequency agonistic behavior rates for each observed individuals, broken down by group. Introduction rates are on the left, and semi-wild rates are on the right. Mean for the introduction group is 0.22, SE = 0.04; mean for the semi-wild group is 0.02, SE = 0

Table 4: Descriptive Statistics for Agonistic Behavior Frequency Rates among all stages and sex groupings.

Agonistic Behavior Frequency Rate			
GROUP	MEAN	MEDIAN	STANDARD DEVIATION
Introduction Males	0.052	0.00	0.107
Semi Wild Males	0.031	0.002	0.058
Introduction Females	0.194	0.097	0.237
Semi-wild Females	0.008	0.00	0.021

Analysis of agonistic behavior rates between groups, found a significant difference between both group type and sex. However, because the data for both agonistic and affiliative rates are non-normally distributed, results from the subsequent analyses are tentative. The

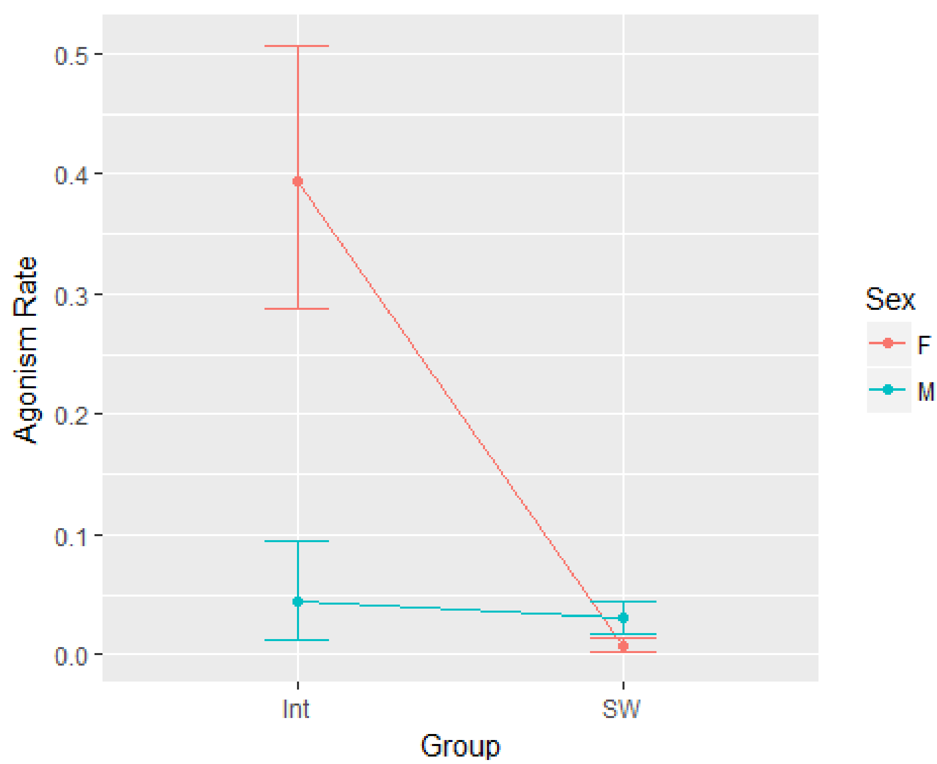

Figure 6: Interaction plot in mean frequency rates of agonistic behavior between groups, distinguished by sex. Introduction (Int) group is on the left, semi-wild (SW) group is on the right. Females are in red, males are in blue. Mean frequency rates are on the y-axis.

parametric t-test for agonistic behavior says the difference between the sexes is significant (p-value = 0.0003). There is a difference between males and females in agonistic behaviors. The test also declares that agonism is significantly higher in the introduction group than in the semi-wild group. Figures 3 and 6 shows how females in the introduction group appear to be responsible for all the elevated agonistic behavior.

Further analysis of interactions between agonistic behavior and groupings was conducted using a factorial ANOVA with type III sum of squares. The interaction effect is significant (p-value = 7.270e-12) with the plot in Figure 6 showing a strong sex effect on the introduction group, but no sex effect in the semi-wild group.

The parametric t-test for affiliative behavior says the difference between the sexes is significant (p = 1.629e-08), and the non-parametric Wilcox rank sum test agrees (p = 2.441e-09).

Table 5: Descriptive Statistics for Affiliative Behavior Frequency Rates among all stages and sex groupings.

Affiliative Behavior Frequency Rate			
GROUP	**MEAN**	**MEDIAN**	**STANDARD DEVIATION**
Introduction Males	0.179	0.100	0.196
Semi Wild Males	0.044	0.035	0.0491
Introduction Females	0.377	0.333	0.2808
Semi-wild Females	0.215	0.141	0.202

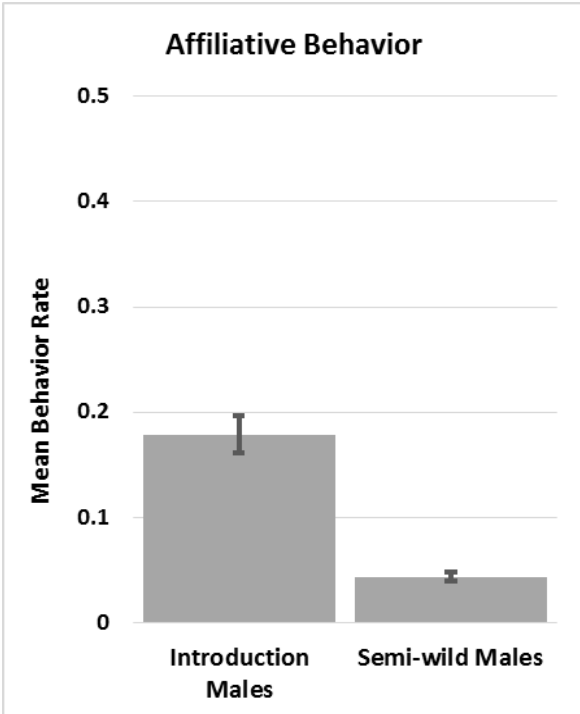

Figure 7: Mean Affiliative Behavior Rates for Males in the Introduction and Semi-wild Enclosure Groups. Male intro mean rate of 0.179, SE = 0.017. Male semi-wild mean rate of 0.043, SE = 0.004.

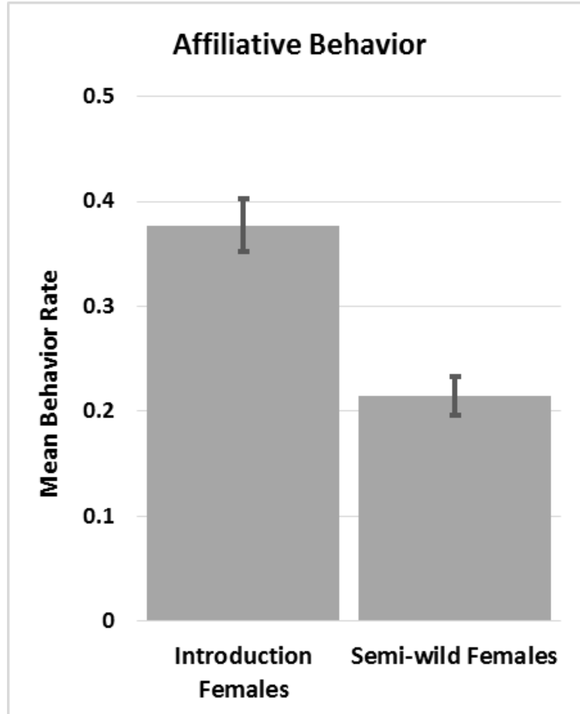

Figure 8: Mean Affiliative Behavior Rates for Females in the Introduction and Semi-wild Enclosure Groups. Female intro mean rate of 0.376, SE = 0.025. Female semi-wild mean rate of 0.215, SE = 0.018.

There is a difference between males and females in affiliative behaviors. The test also declares that mean affiliative behavior rates are significantly higher in the introduction group than in the semi-wild group. Both tests indicate the groups differ on affiliation, with the introduction group having a higher rate. Figure 9 plots each individuals mean frequency affiliative behavior rate over the six week observation period.

Further analysis of an interaction between affiliative behaviors and groupings was not significant. The main effects of sex and group however, are significant (p-value = 2.106e-06 for sex, p-value = 0.0005288 for group).

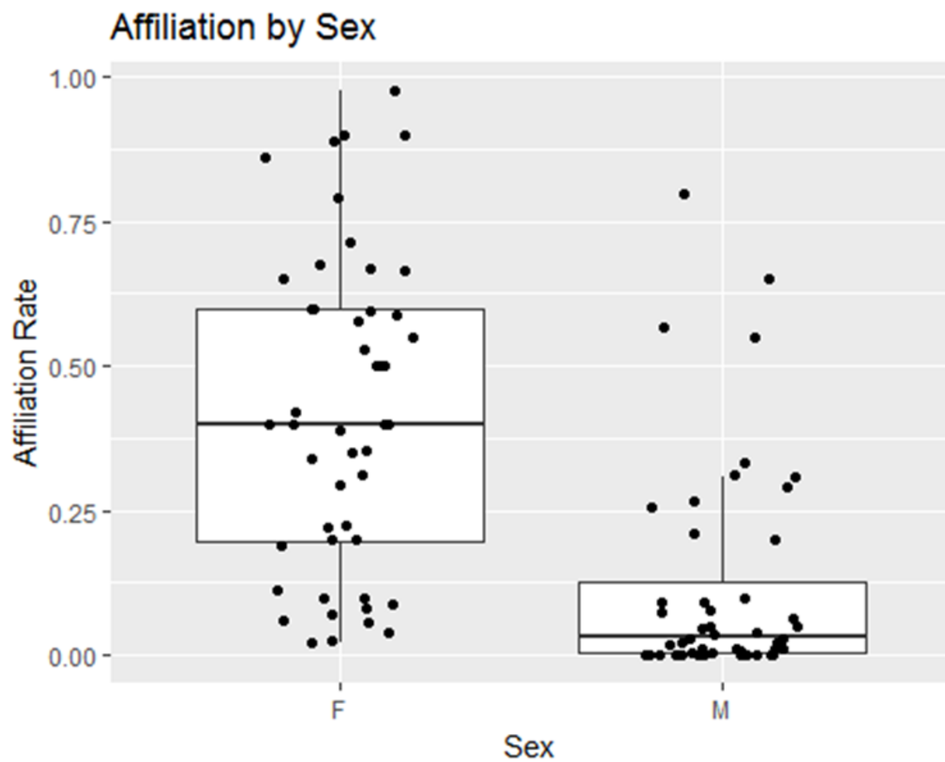

Figure 9: Mean frequency affiliative behavior rates for each observed individual, broken down by sex. Females are on the left, and males are on the right. Mean for females is 0.53, SE = 0.06; mean for males is 0.31, SE = 0.05

Cortisol Analysis

Despite males (mean rate of 0.31) having slightly higher mean cortisol concentration rates than females (mean rate of 0.28), there is no significant difference between sexes, or group in fecal cortisol expression. Both the parametric t-test and the non-parametric Wilcox rank sum test indicate the sex difference in cortisol is not statistically significant. Figures 10 and 11 show the mean (ng/n) cortisol rate for each group broken down further by sex. Each group and sex exhibited similar mean cortisol concentration rates over the 7-day sampling period.

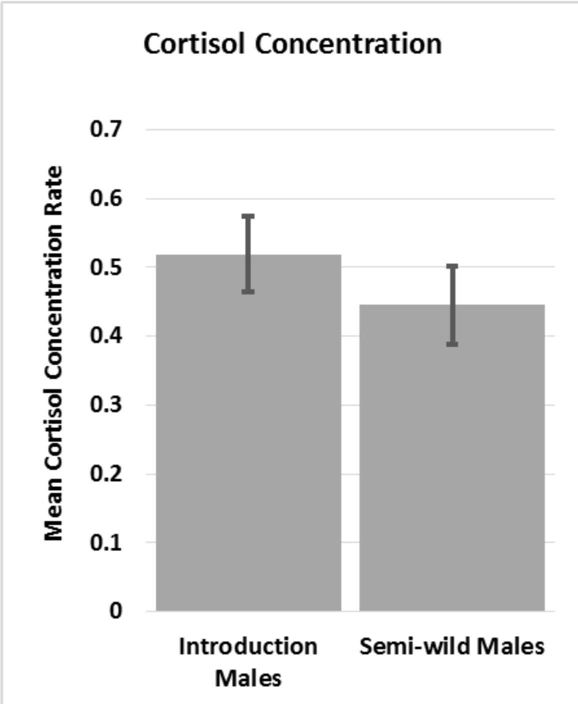

Figure 10: Mean Cortisol Concentration Rate over the three-week Sampling Period for Males in the Introduction and Semi-wild Enclosure Groups. Male intro mean (ng/n) rate of 0.518, SE = 0.055. Male semi-wild mean (ng/n) rate of 0.445, SE = 0.056.

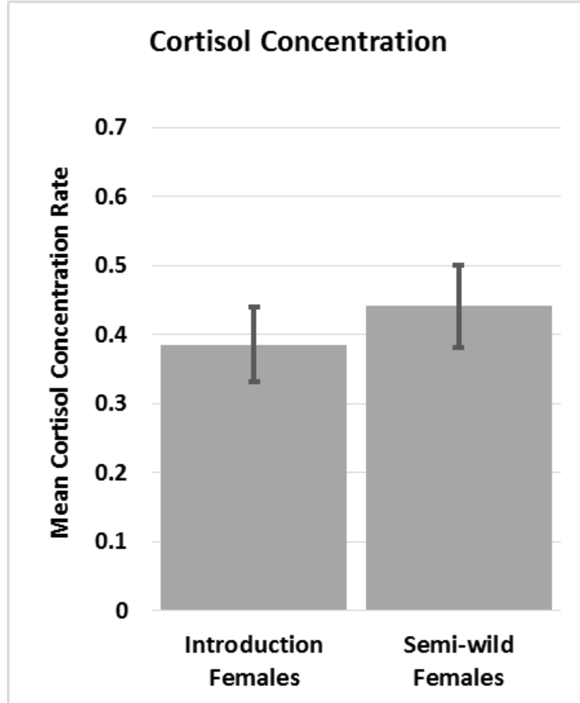

Figure 11: Mean Cortisol Concentration Rate over the three-week Sampling Period for Males in the Introduction and Semi-wild Enclosure Groups. Female intro mean (ng/n) rate of 0.385, SE = 0.053. Female semi-wild mean (ng/n) rate of 0.441, SE = 0.059.

Table 6: Descriptive Statistics for Cortisol Concentration among all stages and sex groupings.

Cortisol Concentration (ng/n)			
GROUP	MEAN	MEDIAN	STANDARD DEVIATION
Introduction Males	0.519	0.580	0.277
Semi Wild Males	0.445	0.370	0.294
Introduction Females	0.386	0.305	0.273
Semi-wild Females	0.441	0.385	0.301

Wild vervet fecal cortisol values provided by Jennifer Danzy-Cramer were used to compare both the introduction and semi-wild groups to cortisol means found in a free-roaming vervet population (n= 46) to determine significant differences between groups (Danzy-Cramer 2010). The introduction and semi-wild values were first multiplied by 100. A normality test determined samples to be non-normally distributed. A non-parametric Wilcox rank sum test conducted in R-statistical software revealed no significant differences between groups (Introduction p-value = 0.0473; semi-wild p-value = 0.03222). Figure 12 shows mean cortisol rates on the y-axis, and the three different groups under analysis on the x-axis. While cortisol levels are lower for the wild populations than the two rehabilitant groups, there is no significant difference in cortisol rates between groups.

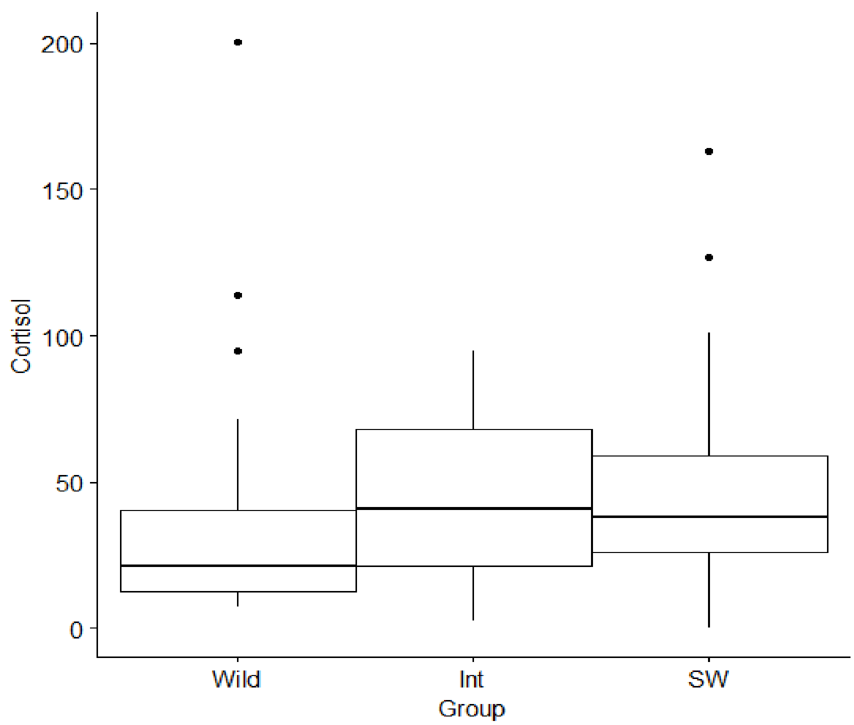

Figure 12: Mean Cortisol Concentration Rates between Wild samples, and the Introduction (Int) and Semi-wild (SW) Enclosure Groups. Mean wild (ng/n) rate of 32.58, SE = 0.055. Introduction mean (ng/n) rate of 45.09, SE = 0.056. Semi-wild mean (ng/n) rate of 44.32, SE = 0.054.

A plotted regression between agonism and affiliation rates with sex is not accurate for males, however the female regression line suggests that for females, higher rates of agonism are positively correlated with higher rates of affiliation (Figure 12). A Pearson's correlation test found that for females, the positive correlations are statistically significant (p-value = 0.006659). However, there is no relationship for males (p-value = 0.8075).

A similar relationship is found when plotting the same regression between agonism and affiliation rates by group. The introduction correlations are significantly positive (p-value = 0.002066), whereas the semi-wild correlations are significantly negative (p-value = 0.00678).

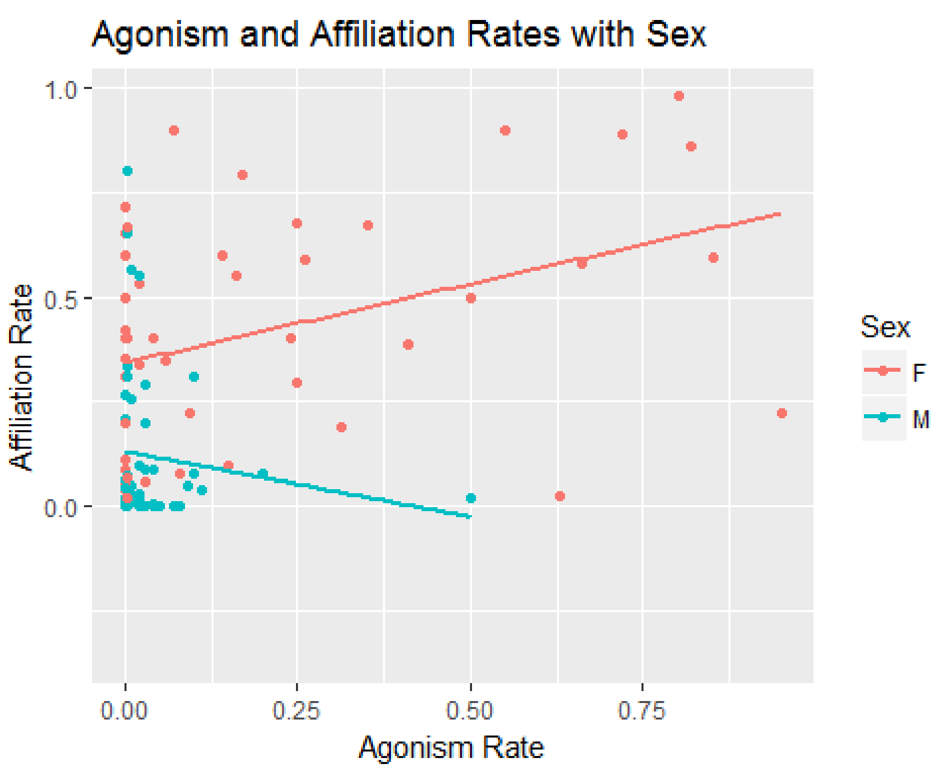

Figure 13: Plotted regression between agonistic and affiliative behavior frequencies by sex. Females are in red, showing a positive linear relationship. As agonistic behavior increases, affiliative behavior increases. Male rates are in blue, showing a negative linear relationship. As agonistic behavior increases, affiliative behavior decreases among males.

An analysis of behavioral rates and cortisol expression over time revealed no significant differences between days (agonistic behavior p-value = 0.4071; affiliative behavior p-value = 0.1299). An interpretation of behavior over time is not under evaluation in this study, due to the lack of difference between observations.

DISCUSSION

Results were anticipated to show an increase in cortisol concentration and agonistic behavior rates among the introduction group. Individuals in the semi-wild group were expected to show decreased levels of cortisol concentrations, decreased agonistic behaviors, and increased affiliative behaviors due to the prolonged integration period. This prediction is supported as evidenced by the reduction in agonistic behaviors among individuals in the semi-wild stage, however cortisol rates were stagnant for both stages of rehabilitation, sex, and group. Affiliative behaviors also show differences among sex and group that do not support the prediction, but rather suggest that vervets undergoing the initial introduction stage experience enhanced social affiliative behaviors. The overall trend of reduced cortisol responses among all four groups at Riverside, suggests that vervets undergoing the rehabilitation process are not experiencing high stress rates. Because the literature associates agonistic, as well as abnormal behaviors with high stress and traumatic events, it is expected for rehabilitant groups to exhibit higher stress response rates. Results did not find any variable to be associated with cortisol – meaning there is no behavioral, sex, or group (introduction v semi-wild) predictor for cortisol expression in vervets undergoing rehabilitation.

It was predicted that vervet monkeys in the beginning introduction group, both male and female, would exhibit higher cortisol concentration rates and agonistic behavior rates because of the highly stressful nature of animal introduction. Stress is a result of heightened competition, and unfamiliarity between primates (Teichroub 2015, Struhsaker 1967). Vervets that came to the center as orphans or pets have little to no experience in normal social behaviors. The process of integration can be traumatic for vervet monkeys, and it takes time in order for social hierarchies to develop. Furthermore, some individuals may never reach heightened social status and be subjected to chronic stress. While agonistic behaviors are expected to occur in any socially healthy primate group, higher rates of agonistic behavior among all individuals may suggest an imbalance in the social cohesion of a group. Below the predictions made in the Methods section (pg 24) are repeated to frame the discussion of the statistical results.

(1) Vervet monkeys in the semi-wild stage of rehabilitation should exhibit less agonistic behaviors, lower cortisol rates, and increased affiliative behaviors in comparison to the introduction stage due to enhanced social cohesion over time.

There was no statistically significant difference in cortisol expression among groups, however females in the introduction enclosure exhibited significantly higher affiliative, and agonistic behaviors over the observation period.

(2) It is predicted that the introduction group will exhibit higher mean rates of fecal cortisol concentration, and higher mean rates of agonistic behavior, due to being newly introduced to other individuals at the beginning of the rehabilitation process.

Female vervets in the introduction enclosure exhibited higher rates of agonistic behavior, while males did not.

(3) *It is also predicted that males will exhibit higher mean rates of cortisol concentration, and higher rates of agonistic behavior, due to male vervet vigilance behavior.*

Males did not exhibit significantly higher rates of cortisol concentration or agonistic behavior in either the introduction or semi-wild phase of rehabilitation.

(4) *It is also predicted that cortisol concentration rates are positively correlated to agonistic behavior, with agonistic behaviors acting as predictors for higher levels of fecal cortisol concentration*.

There was no correlation found between cortisol expression and behavior. Agonistic behaviors do not act as predictors for cortisol rates, and thus do not act as indicators of stress.

Variation in Cortisol Response

It was predicted that higher cortisol concentration rates are positively correlated to high rates of antagonistic behavior among all vervet monkeys under observation. It was also predicted that the semi-wild enclosure provides increased opportunities and time for learned typical vervet behavior, and should result in decreased cortisol concentration rates among semi-wild individuals compared to the introduction group. However, all subcategories of vervets under observation exhibited similar low levels of cortisol concentration. The lack of variation can be explained by limited resource competition, reduced predator pressure, and the prolonged duration of the rehabilitation period.

Cortisol Concentration Values

Results show no significant differences in cortisol values between wild vervets and rehabilitant vervets under observation. No behavioral data were acquired for wild vervets, thus only cortisol values were compared in this study. Prior studies comparing wild cortisol values to

isolated and peer-raised vervets show varying results. These studies attribute differences to diet, daily schedule, and proximity to other vervets (Fairbanks 2011, Fourie 2015). This study does not attempt to explain discrepancies in cortisol values between groups. However, the similar levels of cortisol expressed by rehabilitant vervets suggests that vervets undergoing rehabilitation do not experience heightened levels of stress compared to their wild counterparts. Rather, it is possible that the strategy of a prolonged duration of rehabilitation used at the Riverside center may lower overall stress experienced in the semi-wild and introduction groups.

Resource Competition

A lack of resources creates heightened competition among individuals and groups, which in turn directly affects the stress response. Both the semi-wild and introduction groups were fed fresh food once daily, and groups had near unlimited access to fresh food. Food is abundant in both enclosures, leading to lessened competition in comparison to what is observed among free-roaming vervets. Food abundance may contribute to the similar mean cortisol concentration levels between groups found during analysis.

Predation Pressure

Vervets undergo predation pressure from eagles, leopards, and humans, which can lead to both physical and mental stressors. Females especially will suffer reduced reproductive success when lacking nutrition and safety from predators. These stressors are shown in poor body condition and low birth rates (Whitten 2008). The introduction enclosure is completely closed and predators have no access to individuals in this setting. While the semi-wild enclosure

is open-air, there is very little predation pressure due to the electrified fences that surround the perimeter, and the dense tree brush to keep predator animals out. The lack of predation pressure among both groups and sexes may have limited vigilance behavior and cortisol response.

Duration of Rehabilitation Period

The rehabilitation process can take up to 4 years from when an individual arrives at the Riverside Wildlife and Rehabilitation Centre, to when they are released with a group. Because many animals brought to Riverside have traumatic backgrounds, it is critical that each individual learns behaviors necessary to survive in the wild without human interference. The lengthy process Riverside utilizes, allowing individuals to form tightly knit social bonds, may explain the lower cortisol concentration levels observed across all groups in this study. When the Riverside staff transfers vervets into a semi-wild enclosure, individuals are moved together as a group from the introduction enclosure that shares a fence with the semi-wild location. Because introduction groups are introduced together as a group of at least 12-16 individuals, it may lessen the overall stress during this transitional period. The shared fencing also allows individuals to familiarize with members of the semi-wild group that they will soon reside with. This extensive familiarization with conspecifics may also lessen cortisol expression.

Prior to the transfer of introduction groups to semi-wild enclosures, individual vervets are placed in introduction enclosures on a trial basis. If placement to an introduction enclosure after the quarantine period does not prove to be a good fit for an animal, staff will attempt placement in the three other vervet introduction enclosures at the centre. This holistic, trial and

error process provides multiple opportunities for an individual to fit within a group. Establishing dominance and troop hierarchies occurs in both phases of the rehabilitation process, and if groups are given sufficient time to acclimate to one another, cortisol expression may be less than it would if groups were not given ample time to form social bonds. At the time of fecal sampling, the introduction group had been in the introduction enclosure together for at minimum six weeks. The semi-wild group had been in the semi-wild enclosure together for at minimum three months. It is possible that the extended duration of each stage of rehabilitation contributed to the overall low cortisol concentration rates among all groups and sexes.

Variation in Behavior

Statistical analysis revealed that there are significant differences in behavioral expression among groups and between sexes at Riverside. Males were expected to exhibit higher rates of agonistic behavior due to heightened stress living in close proximity to humans. However, females in the introduction enclosure exhibited significantly higher mean rates of agonistic behavior. Affiliative behavior rates differed significantly between sexes, with females in both groups exhibiting higher rates of affiliative behavior. The Introduction group also exhibited significantly more affiliative behavior than the semi-wild, although all groups exhibited normal behaviors associated with social bonding. Grooming, play bouts, and huddling in close proximity were the most frequent affiliative behaviors observed. The regulatory benefits of affiliative behavior include social bond reinforcement, and stress reduction. While cortisol rates were not correlated with affiliative behavior in this study, positive social behaviors have been associated with stress reduction in other analyses (Abbott et al 2003; Clarke 1992).

Differences in agonistic behavior expression may be explained by dominance behavior, female and kin behavior, and the enclosure environment.

Dominance Behavior

The dominant vervet in the Introduction enclosure was a female aged 3.5 years (VSARW11), which may have influenced the mean frequency of agonistic behavior observed among this group. In order to maintain dominance, specific agonistic behaviors were exhibited by this alpha female, including: hitting, wrestling, open mouth threats, chase, lunge, displace, and taking objects. In the semi-wild stage, nearly all agonistic behavior observed were seen in males. Males were more likely to venture closer to the observation cage, were more likely to attempt to interact with me during fecal sample collection with the sampling pole, and were more visible throughout observations than females. Perhaps these group dynamics and troop hierarchies influenced agonistic behavior expression for both groups, with the introduction alpha being female, and the semi-wild males acting more dominant.

Female and Kin Behavior

Females in the wild remain with their natal groups throughout their lifetime and form tightknit bonds as a result of living with their kin. Because kin relationships in the rehabilitation process are rare, agonistic behavior expression may be heightened among females in all groups. However, only females in the introduction enclosure exhibited such high rates of agonistic behavior. While the data was limited, staff at the centre has regularly noticed rehabilitant vervets having offspring while living in the semi-wild enclosures. Due to the limited visibility and limited access given to humans to the semi-wild groups, data on offspring are limited. However

it is highly likely that vervets in the semi-wild group had offspring due to the estimated age of individuals within the semi-wild group that were not listed in the intake records at the centre.

Kin relationships occur within the semi-wild group and do not occur in the introduction group. Not only would having kin reduce levels of agonistic behavior, they would also encourage affiliative behavior. Studies have found maternal kinship to be one of the most influential factors that structure affiliative behavior. Having kin is more likely to ameliorate stress via coalitional support with coping outlets such as social grooming. It would make sense that Introduction females exhibit higher rates of agonistic behavior, and lower rates of affiliative behavior – however, introduction females *also* exhibited significantly higher rates of affiliative behavior as well.

Enclosure Environment

Moving individuals out of their environment can have long-lasting effects on captive nonhuman primates (Clarke 1992). Environmental shifts are important mediators of behavior. Vervet monkeys are placed into introduction enclosures in groups of at least two successfully quarantined monkeys to reduce the likelihood of injury and aggression aimed at one individual. Socially integrated introduction groups can range from eight to twenty individuals and are introduced into the connected semi-wild enclosure together. The process of forming introduction groups can last from one month, to eight months. It is critical that social bonds are solidified among the introduction group before being combined with an already integrated semi-wild group. The environmental disruption in moving the introduction group is less severe, as the monkeys have already acclimated to the specific environment to which they will be moved by sharing a fence and meeting semi-wild individuals.

While enclosure environment directly effects cortisol expression, it also effects behavioral expression. Spatial and demographic variation between the two enclosures may further explain the behavioral discrepancies between groups. The introduction enclosure is much smaller than the semi-wild enclosure, and offers no room to hide or flee from other individuals. The small enclosure offers more opportunity for social interaction, and may have led to more observed behaviors in general. Looking at figure 12, mean frequency rates of both affiliative and agonistic behaviors are higher in introduction individuals. There were more females in the introduction enclosure than males, despite observing an equal number of males and females. Female alliances in the introduction enclosure may explain the higher rates of both behaviors in the introduction group.

Because the semi-wild enclosure was so large, and there were many non-visible areas of the enclosure from the observation cage, when focal animals went out of the viewable range the observation was stopped. Females typically stayed further away from the observation cage, and were less visible throughout the observation period, despite selecting an equal number of female and male focal observation individuals. Less observable interactions during the observation period for the semi-wild group may largely have to do with the size of the enclosure. The increased space may have provided more typical vervet behaviors, while the smaller introduction enclosure forced more observable social interactions to occur.

CONCLUSION

This study compared behavioral and hormonal differences between vervets in the beginning introduction stage of the rehabilitation process, and the secondary semi-wild stage.

There is no significant difference between sexes, or stages of rehabilitation in mean cortisol concentration rates. Explanations for similarity in cortisol concentrations between groups and sex include limited resource competition, reduced predator pressure, and the prolonged duration of the rehabilitation period. Further statistical analysis also shows that there is no relationship between cortisol expression and behavior, sex, or stage of rehabilitation. It is important to note that cortisol values in this study are tentative at best, due to the low mean cortisol concentrations in comparison to wild female chacma baboon cortisol expression.

Females in the introduction stage of rehabilitation exhibited significantly higher rates of affiliative and agonistic behaviors over their male introduction and semi-wild counterparts. Reasons for this behavioral variation in females can be attributed to dominance behavior, female and kin behavior, and the enclosure environment.

Before considering populations for release, it is argued that individuals should be provided with specific husbandry and welfare (IUCN 2014). It is critical to prepare them for a life in the wild. This includes behaviors such as how to move in natural vegetation, forage for food, where to sleep, and how to interact with other members of their species. There is also a need to mitigate animal-human conflict and ensure that they understand that they are not bonded to humans, or will seek out human attention once released. Welfare standards are helpful to determine what space and enrichment is necessary to keep the animals healthy and mentally stimulated. These can benefit *ex situ* captive populations, and be used as minimum guidelines for housing animals with the purpose of reintroduction.

Because there are few institutional standards for primate rehabilitation centers in South Africa, data should continue to be collected and compared between rehabilitation centers that utilize different enclosure methods. In order to combat declining primate populations, and the unethical treatment of wild primate populations, a combined approach of many strategies should be used. Rehabilitation programs, such as the one at Riverside, are one tactic in the ongoing fight for populations to live free of human-primate conflict.

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