FACTORS AFFECTING THE REPRODUCTIVE SUCCESS OF ZOO-HOUSED GEOFFROY'S TAMARINS (*SAGUINUS GEOFFROYI*)

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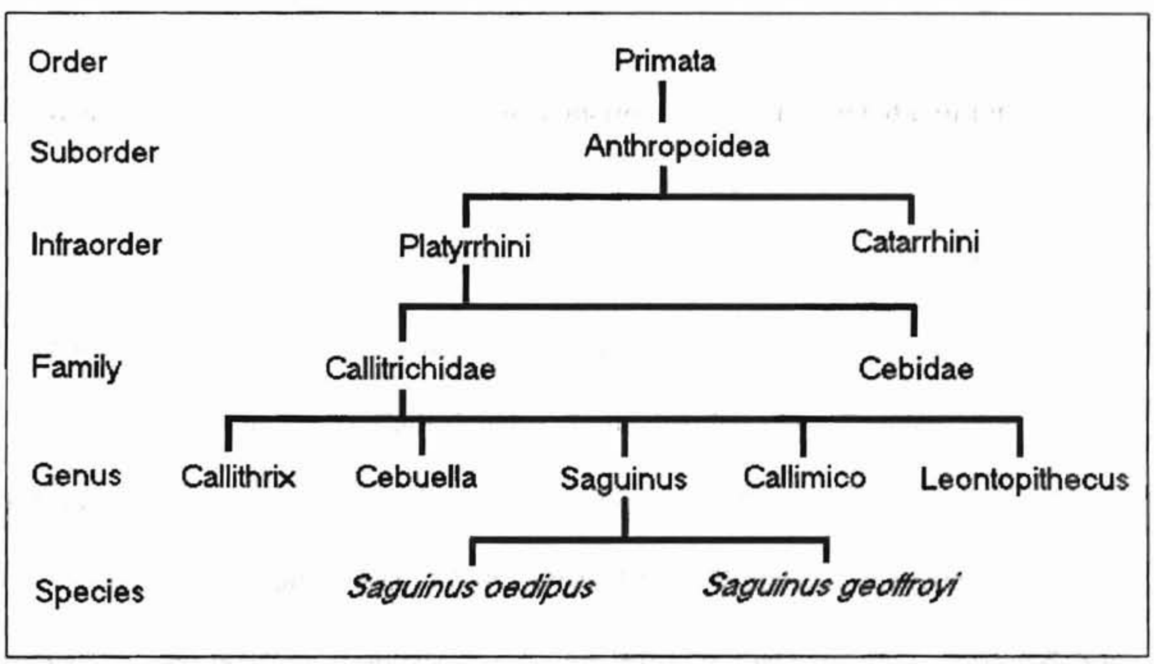
INTRODUCTION

Taxonomy

Geoffroy's tamarins (*Saguinus geoffroyi*), also called Panamanian tamarins (Moynihan, 1970; Skinner, 1986), are small New World primates that belong to the Suborder Anthropoidea, and the Infraorder Platyrrhini (Dolhinow & Fuentes, 1999). Geoffroy's belong to the family Callitrichidae (Figure 1), which consists of two genera of tamarins (*Saguinus* and *Leontopithecus*), two genera of marmosets (*Cebuella* and *Callithrix*), and the Goeldi's monkeys, also called *Callimico* (Jolly, 1985). There is considerable debate over the exact evolutionary relationship between the clades of New World monkeys (Schneider & Rosenberger, 1996). Although, there is little fossil evidence of the evolutionary history of these animals (Garber, 1992; Schneider & Rosenberger, 1996), the differentiation of the various species is thought to have occurred sometime during the Pleistocene (Hershkovitz, 1977; Kinzey, 1982).

Figure 1. Taxonomic classification of *Saguinus geoffroyi*.



Callitrichids are characterized by small body size, a high incidence of twinning, claws instead of nails, and communal parenting (Snowdon & Soini, 1988; Garber, 1992). Hershkovitz (1977) argued that tamarins and marmosets are among the most primitive of all the living primates. However, there is a growing consensus that these characteristics are derived, as opposed to primitive. Sussman and Kinzey (1984) argue that the traits that are characteristic of callitrichids are the result of evolution from a common ancestor that had adapted to a particular niche. Another theory is that these characteristics are the result of phyletic dwarfism (Ford, 1980). Small size may have resulted in the evolution of twinning to accommodate the high neonate to mother body weight ratio. Instead of giving birth to one relatively large infant, the female gives birth to multiple infants that are smaller and therefore easier to pass through the birth canal. Price (1992a) reported litters of cotton-top tamarins that account for up to 25% of the mother's body weight at the time of parturition. Litters of Geoffroy's tamarins have accounted for up to 35% of the mother's body weight at the time of parturition (pers. obs.). Insectivory, a characteristic of callitrichids, is common in small animals with high metabolisms. The claws that are common in marmosets and tamarins may be an adapted trait for insectivory.

There is little argument over the members of the family Callitrichidae (Schneider & Rosenberger, 1996). However, there is considerable debate regarding the relationships of the genera within the family Callitrichidae. Some theorists place *Saguinus* closely related to *Callimico* (Ford, 1986; Snowdon, 1993), while some place them more closely related to *Callithrix* and *Cebuella* (Kay, 1990; Garber, 1994). Whichever the relationship, the genus *Saguinus* is the most diverse of the New World primate genera (Snowdon & Soini, 1988). It consists of 10 or 11 species, depending on the actual classification of the Geoffroy's tamarin. Some taxonomists (Hershkovitz, 1977) place it as a subspecies of the cotton-top tamarin (*Saguinus oedipus oedipus* as the cotton-top and *Saguinus oedipus geoffroyi* as the Geoffroy's). More recently, Skinner (1986) has argued that Geoffroy's tamarin is a separate species on the basis of dental formulas. The current trend seems to consider the cotton-top and the Geoffroy's to be separate species, *Saguinus oedipus* and *Saguinus geoffroyi* (Mittermeier et al., 1988), although the World Conservation Union still considers them subspecies (Baillie & Groombridge, 1996). The separate species designation will be used for this paper, as this is the designation used by the Animal Record Keeping System (ARKS) used in most North American zoos (International Species Information System, 1996).

Natural History

Geoffroy's tamarins are native to Central and South America, weigh approximately 400 grams and measure approximately 24 centimeters in length. The face is mostly bare and black or dark gray, with a white triangular hair patch on the front of the crown (Moynihan, 1970). The rear of the head and nape are rufous. The dorsum is brindled black and grayish yellow, and the ventrum is white. They have a long (approximately 36cm.) tail that, although not prehensile, is used in balancing. The tail is dark rufous near the body and black at the tip. The underside of the hands and feet are hairless, and the fingers and toes have claws on all digits except the thumb and big toe, which have nails.

Geoffroy's tamarins, hereafter called GTs, have been found in the secondary growth forests (between 10 and 40 years old) throughout Panama, northern Colombia, and southeastern Costa Rica. The Rio Atrato in Colombia separates the ranges of GTs and the cotton-top tamarins. GTs occur on the western shore of the river, while cotton-tops occur on the eastern shore. There have been no censuses in the past 15 years and there are no estimations of current population size in the wild. It is thought that GTs are currently limited to the country of Panama.

GTs are the northernmost of the tamarin species. These animals appear to occupy the edges of middle and lower canopy regions of dry deciduous forests (Kinzey, 1997). They feed on insects, anoles, fruit, flowers, nectar, and plant exudates. Orthopterans are the most commonly consumed insect (Dawson, 1976).

Tamarins can be found occurring naturally in groups that average from 2 to 19 individuals (Dawson, 1976). These groups usually consist of a dominant reproductive pair and their offspring (Dawson, 1976; Epple and Niblick, 1997), however; multiple cycling females have been found in certain circumstances (Terborgh & Goldizen, 1985; McGrew & McLuckie, 1986). Tamarins have long been considered to be monogamous because of the tendency for only one active breeding pair to be present in a group. However, recent studies have shown instances of polygyny, polyandry, and polygynandry (Savage et al., 1996; Koenig, 1995).

Cooperative breeding, where non-breeding individuals remain in the group to assist in raising offspring, and twinning are characteristics of callitrichid societies. Groups of tamarins provide substantial infant care (Epple and Niblick, 1997; Price, 1992b; Dawson and Dukelow, 1976; Santos et al., 1997). Terborgh and Goldizen (1985) suggested that single pairs would not be able to raise young in the wild without the assistance of others. Infant carrying and food provisioning for the infants is energetically expensive (Santos et al., 1997; Tardif, 1994). Helping behavior that is common in Callitrichids is beneficial to both mother and helpers. The mother is provided relief from the high energetic demands of carrying twins, as well as, given an opportunity to forage and rest. The helpers, on the other hand, gain experience in infant rearing, which may be necessary in becoming a competent parent (Tardif, et al., 1984). Additionally, helpers may improve the odds of survival of their siblings (Emlen, 1984; Feistner & Price, 1990). The bonds developed with siblings may later assist the caretaker when it begins to breed (Feistner & Chamove, 1986). Price (1992a) also hypothesized that helpers are "paying" their parents to remain on their territory. Additionally, infant carrying has been hypothesized as a courtship strategy adopted by adult males (Tardif & Bales, 1997).

Groups of tamarins violently defend their territory from intruders. GTs vocally and physically defend areas against conspecifics, however; it appears that they are defending the area immediately surrounding the group rather than a geographical area, as was originally suspected (Dawson, 1978). The defensive behaviors are often focused male against male and female against female. Tamarins will attack a same sex conspecific when it is introduced in a captive environment. This behavior makes it difficult to house larger groups. Additionally, mother-daughter aggression often occurs when the daughter becomes of reproductive age and the mother is in estrous. As a result, tamarins are typically kept in breeding pairs with or without juvenile offspring. Occasionally, the adult male offspring of a pair is permitted to remain in the group, as this does not usually result in aggressive interactions between the males (Lamprey, 1997; Dietz & Baker, 1993; Kirkpatrick-Tanner et al., 1996). It has been hypothesized that polyandry may improve reproductive success in some species of tamarins (Lamprey, 1997). In this situation one male assumes a dominant role, and spends most of the time with the female, although it appears that the submissive male also gains breeding access to the female (Skinner, 1986). Studies have shown that males may actually inhibit the breeding success of each other indirectly, as they are forced to split the number of copulations with the female (Baker et al., 1999).

Most of the information regarding the ecology of GTs comes from Moynihan (1970), Garber (1980; 1984) and Dawson (1976; 1978). Other than a census conducted on the GT population in Panama in 1983 (Skinner, 1985), there has been little published on naturally occurring populations of GTs since these early works. Rasmussen is currently conducting research on several introduced groups of GTs on Isla Tigre in the canal zone of Panama (Rasmussen, 1998). Most of the captive data come either from extrapolating studies on closely related species, such as the cotton-top tamarin, or from Skinner's 1986 study. Skinner's study represents the only behavioral analysis of data on captive GTs to date.

Status of the Captive Population

The captive population of GTs is rapidly declining in North America. This rapid population decline is the result of the inability of most tamarins to successfully rear offspring, not the inability to conceive. In 1991, there were 101 *S. geoffroyi* in North American zoos (Sironen & Krentz, 1991). In 1998, only fifty GTs remained in captivity in the Western Hemisphere (Table I). Females regularly conceive and give birth to offspring, but the offspring often die soon after birth. As an illustration of this statement, twenty-four GTs were born in zoos in 1991, but 12 of those were dead within 24 hours (50%), another 4 within 7 days (Sironen & Krentz, 1991). Only 6 of those original 24 animals survived the year (25%).

Cleveland Metroparks Zoo (CMZ) houses nearly half of the GTs in North American zoos (47%). In 1986 Skinner reported that the Cleveland group of GTs exhibited cannibalism of infants. Cannibalism of infants continued to be reported in the Cleveland group over the past several years (pers. obs.). Over the same period CMZ has also exhibited a rate of stillborn infants near 45%. This is higher than the averages published for cotton-top tamarins (Kirkwood et al., 1983) and golden lion tamarins, *Leontopithecus rosalia,* (Kleiman et al., 1982) in the early 1980's. The captive population of golden lion tamarins was thought to be in danger of extinction in captivity until changes in management practices, such as improved diet and complete visual isolation of groups, resulted in an increase in survival rates and thriving captive populations of both species.

Table I. Captive North American population of Geoffroy's tamarin as of 4/15/98.

Institution	AZA Affiliated	Males	Females	Unsexed	Total
Bronx Zoo	Yes	3	3	0	6
Buffalo Zoo	Yes	2	3	2	7
Cleveland Metroparks Zoo	Yes	8	8	1	17
Henry Doorly Zoo	Yes	2	1	0	3
Little Rock Zoo	Yes	2	2	0	3
Jardin Gaia	No	0	1	0	1
Private Owners	No	7	6	0	13

Thirty-six of the captive GTs in North America (72%) were housed in institutions that are affiliated with the American Zoo and Aquarium Association (AZA). These animals are managed collectively to maintain genetic diversity and demographic stratification within the population. The New World Primate Taxon Advisory Group (NWP TAG) is a voluntary committee of members of the AZA. The TAG prepares a Regional Collection Plan that identifies captive breeding priorities for each region and provides direction to institutions as they develop their own collection plans (Baker, 1998).

Geoffroy's tamarin is listed as threatened by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), and has been listed as endangered by the United States Fish and Wildlife Service since 1976. Due to the extensive use of small New World primates in biomedical research, other callitrichid species are abundant in captivity, including the closely related cotton-top tamarin (*Saguinus oedipus*). However, GTs have not been used as a research animals, and as a result, less time and energy has been invested in establishing a successful captive population. Of the 101 captiveanimals that were alive in 1991, only 12 were known to be wild-born. The endangered status of GTs limits the importation of new wild-born, mother-raised animals, while the few founder animals that remain in the captive population continue to age. The offspring of these founder animals are not raising infants as successfully as did the founders.

Reproduction And Parental Behavior

One theory on the poor infant rearing behaviors of GTs relates to the past practice of removing infants from their parents soon after birth to be hand-raised. This practice shortened the interbirth interval, which temporarily increased the population size. Studies on related primate species have shown that parental contact at an early age may be related to future breeding success (*Saguinus fuscicollis*: Epple, 1975; *Leontopithecus rosalia*: Hoage, 1978; Tardif et al., 1984; *Saguinus oedipus*: Price, 1992b). If maternal contact is necessary in acquiring infant rearing skills, the protocol of pulling infants from their mothers may have resulted in a population of tamarins that does not possess the necessary skills to adequately raise infants.

A closely related phenomenon may involve the process of young adults helping to rear infant siblings. The experience of carrying infants prior to their own reproduction may be necessary for tamarins to develop into competent parents (Cleveland & Snowdon, 1984; Snowdon et al., 1985, Tardif et al., 1990; 1992). Cleveland & Snowdon (1984) have shown that animals 7-14 months of age are the primary play partners for new infants, and those greater than 14 months are more involved in carrying their siblings. The process of pulling infants from their parents deprives infants of the social experiences of group life. As infants mature in a social group they have the opportunity to carry the younger offspring produced by their parents. If the individuals are pulled to be hand-reared and are not incorporated into a family group prior to adulthood, parenting skills may not be learned. To gain proper parenting skills, the *Cotton-top Tamarin Species Survival Plan Husbandry Manual* (Savage, 1995) recommends that "an individual should remain with its family group for at least two litters". This idea is recurrent in other publications (Buchanan-Smith, 1997; Kleiman et al., 1982).

Another theory regarding low infant rearing success rates for GTs is based on the high energetic demands involved in raising infant tamarins. Studies in captivity on other tamarin species, including the closely related cotton-top tamarin (*Saguinus oedipus*), have shown infant mortality rates to range from 40-55% (International Zoo Yearbook, 1994;

EEP Yearbook, 1994/95; de Avila-Pires, 1972; Snowdon et al., 1985; Garber et al., 1984). This has often been linked to the high energetic demands involved in raising infant tamarins. Tamarin infants are often up to 25% of the mother's body weight at birth (Eisenberg, 1978; Price, 1992a). Lactation and the demands of carrying one, two, or even three relatively large infants creates a very large energetic demand (Goldizen, 1987; Kirkwood & Underwood, 1984). It has been hypothesized that the callitrichid social system has evolved to meet these high energetic demands (Garber & Leigh, 1997; Eisenberg, 1978; Goldizen, 1990; Leuteneger, 1973; Ford & Davis, 1992). Field studies have shown that larger groups have greater success rearing infants than do smaller groups (Savage et al., 1997). Traditionally, Geoffroy's have been housed in pairs, but the energetic demands of raising offspring may be higher than those that the parents are able to meet without assistance.

<u>Reproduction and the Physical Environment</u>

The physical environment in which an animal is housed may create sufficient stress to increase mortality. Abiotic factors, such as low temperatures or humidity levels may result in stress. The *Cotton-top Tamarin Husbandry Manual* (Savage, 1995) suggests a minimum temperature of 25°C, preferably 29°C. This temperature level is recurrent in other studies (Kilborn et al., 1983; Dronzek et al., 1986). However, one study has shown that when tamarins are subjected to temperatures lower than 32°C, they are being subjected to temperature regulation stress (Stonerook et al., 1994). Temperature regulation stress is thought to be the cause of colon cancer in captive cotton-top tamarins.

The *Cotton-top Tamarin Husbandry Manual* (Savage, 1995) suggests a relative humidity range of 50-75%. All studies on effective captive management of callitrichids listed 50% relative humidity as a minimum level for captive breeding. However, a thorough literature search revealed no studies that showed the effects of low humidity levels on primates.

Additionally, light has been mentioned as a source of reproductive failure in captive primates (Reinhardt, 1997; Buchanan-Smith, 1997). Animals housed under poor lighting conditions have been shown to exhibit extended ovulatory cycles, low levels of steroid hormones, and low fecundity (Reinhardt, 1997).

Reproduction and Social Stress

Social factors in housing may also create unnecessary stress on the tamarins. Skinner (1986), as noted previously, found GTs in Cleveland to exhibit high rates of cannibalistic behavior toward infants. She attributed this to the social stress resulting from living within view of an unfamiliar group of conspecifics. The *Cotton-top Tamarin Husbandry Manual* (Savage, 1995) states that visual isolation is critical when housing multiple groups of cotton-top tamarins. Territoriality displayed by these tamarins will result in displaced aggression. This can result in a disruption of the normal behavior or reproduction of the tamarins. Bardi et al. (1998) displayed a relationship between the rearing environment and the amount of infant abuse that occurs in cotton-top tamarins.

The breeding of captive animals involves the management of a limited gene pool (Soule et al., 1986; Bennett, 1990; Poole, 1992). The difficulty in maintaining genetic diversity with a small founder population is compounded when a large number of animals that reproduce will not successfully raise their offspring. A literature search revealed only one study examining the captive behaviors of GTs. Since Skinner's 1986 report, no studies have examined GTs in captivity and no long-term studies have examined behavior in the wild. As was the case with golden lion tamarins twenty years ago, the captive population of GTs seems to be headed toward extinction. In 1966, 58 golden lion tamarins existed in captivity (Kleiman et al., 1982). Following a period of extensive research and alteration of management styles, the population has grown to over 500 captive golden lion tamarins (Stoinski et al., 1997). The alteration of management style included: preventing visual interactions between groups, ensuring juveniles obtain experience in caring for infants, developing successful hand rearing techniques, and improving nutrition and housing. If there are management issues that may assist in the reproductive success of GTs in a similar fashion, it is vital that these issues are discovered, and changes are implemented.

Hormones and Behavior in Callitrichids

Callitrichids have become important animals in biomedical research because of their small body size, high fecundity, and ease of handling (Kuederling & Heistermann, 1997). The common marmoset has been used in reproductive and periodontal disease studies, and the tamarins of the genus *Saguinus* have been used in immunology, virology, and oncology studies (Epple & Katz, 1983). This has lead to extensive analysis of the breeding patterns of these animals, because application of knowledge of the breeding behavior can increase the reproductive success of the colonies that supply these animals for research.

Most of the reproductive analysis of callitrichids has come from the radioimmunoassay of urine or fecal samples, as opposed to blood samples. This is because of the excitable nature of these small animals. Repeated capture of these animals for blood draw has been shown to cause anemia or deviation from normal reproductive behaviors (Ziegler et al., 1989). Studies on captive reproductive biology have shown a few characteristics that are unique to callitrichids. Reproductively active callitrichid females are capable of hormonally suppressing the ovulation of other females in the group (French et al., 1984; Ziegler et al., 1987a). Reproductive suppression keeps the estrogen levels of post-pubescent females at pre-pubescent levels so that the dominant female is the only one in the group to ovulate. The primary mechanism for reproductive suppression in tamarins seems to be olfactory, with information being conveyed through scent marking (Snowdon et al., 1993). It has been shown that there is a positive correlation between scent marking in females and estrogen levels (Ziegler et al., 1987a).

According to Snowdon et al. (1993), reproductive suppression is critical in maintaining groups in which only one female reproduces. There have been reports of groups in which more than one female ovulates (McGrew & McLuckie, 1986; Price & McGrew. 1991), but they have also shown that generally only one female actually raises her young, and that these groups tend to be unstable (Price & McGrew, 1991). Reproductive suppression may be a method of preventing competition between mothers and daughters. Despite reproductive suppression, eldest daughters are often violently evicted from groups (McGrew & McLuckie, 1986; McGrew, 1997). In contrast, males emigrate on their own (McGrew, 1997), and groups with multiple males tend to be more stable (Price & McGrew, 1991).

Callitrichids are unusual in that the analysis of urinary estrone and estradiol metabolites do not show a preovulatory estrogen peak, as they do in other primates

(Ziegler et al., 1987a). Thus, urinary steroid levels may not accurately reflect the phases of the ovarian cycle (Ziegler et al., 1989). Also, up to 50% of the circulating estradiol is metabolized into estrone before it is excreted, and up to 95% of circulating progesterone is excreted in feces as opposed to urine (Ziegler et al., 1989). Ziegler et al. (1996) showed that there is a preovulatory peak in circulating estradiol, but there have been no studies that compared the relationship between circulating estrogen levels and metabolized levels of estrogen in urine. Despite the uncertainty surrounding the relationship between circulating and metabolized estrogen levels, an analysis of urine samples for excreted estrogen conjugates will provide valuable information regarding the cycles of these animals.

Much of the information regarding the reproductive cycle of female Geoffroy's tamarins has been extrapolated from hormonal data on captive cotton-top tamarins or from field observations on Geoffroy's tamarins. A thorough literature search revealed no studies on the hormone cycle of GTs, but they have been estimated to have a gestation period of 140-145 days (Dawson and Dukelow, 1976) and an estrous cycle of approximately 14-21 days (Skinner, 1986). GTs do not exhibit visual indications of ovulation. Data from captive groups have not previously been obtained.

Field studies on GTs have shown that both sexes are equally likely to emigrate (Dawson, 1978), but captive studies on cotton-top tamarins have shown that females are much more likely to leave their natal group (McGrew & McLuckie, 1986; McGrew, 1997). Male tamarins appear not to undergo reproductive suppression similar to that of females. In contrast, they appear to avoid inbreeding via behavioral controls. Baker et al. (1999) conducted experiments that determined that fathers did not reproductively suppress their sons when they were housed in their natal group. The sons interacted sexually with their mothers significantly less often than with an unfamiliar female regardless of whether the father was present. This study suggests that tamarin males tend to exhibit incest avoidance. Mothers and sons did not completely avoid sexual contact, however. This may indicate that incest avoidance is not expressed consistently, and groups that have no opportunity to outbreed may be likely to exhibit incestuous behaviors.

Analysis of estrogen and progesterone conjugates will indicate if Cleveland Metroparks Zoo's Geoffroy's tamarin colony is undergoing reproductive suppression. Additionally, the analysis of hormone excretion patterns will allow for verification of information, such as gestation period and cycle length, which has been previously extrapolated from data on similar species.

Cortisol and Behavior

Cortisol is a corticosteroid that is produced by the adrenal gland during stress. Stress can occur when an internal need or environmental demand is out of the individual's control (Clark et al., 1997). Corticosteroids are released by the adrenal gland in response to a physical or psychological stressor and are critical for adaptation to an undesirable situation (Sapolsky, 1994). The secretion of cortisol is an activation of the body's stress response and provides energy for the fight or flight response in an effort to return to its original homeostatic condition (Sapolsky, 1994). As a result, the presence of elevated cortisol levels may simply be an indicator of an animal's response to a stressor and not an indicator of an unacceptable situation. Alternatively, the failure to respond to a stressor, i.e. cortisol suppression, has been shown to be related to some illnesses, such as post-traumatic stress disorder (Yehuda et al., 1991; 1993).

In primates, cortisol has been shown to be released in response to acute stressors, such as capture or immobilization, or long-term stress, such as anxiety from social interactions (Coe et al., 1978; Eberhart et al., 1980). In humans, elevated cortisol levels have been shown to be associated with chronic anovulation due to long distance running (Glass et al., 1987) and anorexia nervosa (Casper et al., 1979). In research with rodents, an increase in population density results in decreased fertility (Christian, 1980). The behavioral and physiological mechanisms that regulate this type of response are thought to be an increase in aggression and an increase in corticosteroid secretion.

Typically, elevated cortisol levels are associated with stress. Studies have shown, however, that although stress is considered to be associated with reproductive suppression, anovulatory subordinate callitrichid females do not show higher levels of cortisol (Abbott et al., 1981; Saltzman et al., 1994; Ziegler et al., 1995). As a result, if cortisol is an indicator of stress, then reproductive suppression in callitrichid females is not stress induced.

Studies on cotton-tops have shown a rise in cortisol levels near ovulation and in response to altered social conditions (Ziegler et al., 1995). The rise near ovulation can be attributed to the increase in ovarian steroids such as progesterone, which is the precursor to cortisol. However, this study also showed a decrease in cortisol in the beginning of a pregnancy and an increase in cortisol later in the pregnancy. Throughout this time ovarian steroids remained elevated. They hypothesized that this may be attributed to fluctuating levels of cortisol binding globulins throughout the pregnancy. Other primates have shown a cortisol response to altered social conditions. Gorillas (Bettinger et al., 1998a), rhesus macaques (Scallet et al., 1981; Bercovitch et al., 1995; Boyce et al., 1995) and vervet monkeys (McGuire et al., 1986) have been shown to exhibit an increase in cortisol secretion in response to an unfamiliar social situation.

There have been conflicting results in studies which examined the relationship between rearing history and cortisol secretion. Some studies have shown that nursery-reared rhesus macaques have a higher basal cortisol level than mother-reared infants (Champoux et al., 1989). Other studies have shown that nursery-reared rhesus macaque infants have lower basal cortisol levels than do mother-reared infants (Clarke, 1993). No studies were found which analyzed the effects of rearing history on basal cortisol levels in callitrichids.

Lack of elevated cortisol levels does not necessarily indicate the lack of stressful conditions. Bettinger et al. (1998a) emphasized the evaluation of an animal's ability to show an acute stress response, as well as its ability to show a diurnal shift in cortisol secretion. Failure to show an acute stress response may indicate a chronically stressed animal. Additionally, animals that do not show a decrease in cortisol production throughout the day may also be chronically stressed. Care should also be taken to consider individual variation in mean cortisol levels among animals (Bettinger et al., 1997, 1998b).

There have been no published studies that examine the cortisol levels of GTs. This study will determine mean concentration of excreted cortisol in *S. geoffroyi* and determine if there is a correlation with other variables, i.e., housing situation, rearing history, and abiotic factors.

Evaluating Factors Affecting Reproductive Success

Geoffroy's tamarins at CMZ abuse and cannibalize infants. This behavior was reported for a different population of tamarins housed in the Primate, Cat, & Aquatics Building (PC&A) in the 1980's (Skinner, 1986). Over the past few years, two females have each produced two litters annually. Cricket has abused and cannibalized infants each time she has reproduced. She is housed such that she can see, hear, and smell other groups of GTs (hereafter termed the control condition). Alternatively, Josephine has successfully reared at least one offspring in each litter she has produced during the past several years. She has never cannibalized infants and has raised twins successfully with her mate. Josephine is housed where she cannot see, hear, or smell other groups of GTs (hereafter termed the experimental condition). Additionally, Elly reproduced several times and cannibalized the infants while she was housed where she had contact with other groups of GTs. She was removed from PC&A and housed in the rainforest. Thus, she was no longer housed where she could see, hear, and smell other GTs. Less than one year later she successfully raised twins.

Circumstantial evidence implies that housing GTs within contact of other groups of GTs may contribute to the high rate of infant abuse and cannibalism at CMZ. The two females, which are housed where they cannot see, hear or smell other GTs, are the only GTs to successfully rear offspring at CMZ in the past 10 years. Skinner (1986) hypothesized that the presence of extra-cage conspecifics may have created a stressful situation that resulted in abnormal parenting behaviors, including infant abuse.

As a result of the small sample size, direct assessment of factors involving higher rates of infant mortality in certain situations for GTs is impractical. This study will evaluate several factors to determine if they affect the behavioral and hormonal patterns of groups of captive GTs at Cleveland Metroparks Zoo. These factors include:

1) The abiotic conditions of the enclosures:

Analysis of the temperature, relative humidity, and light levels in each exhibit will determine if GTs are being housed in environments that are in compliance with recommendations of housing and breeding conditions for other closely related species. Differences in abiotic factors may result in differences in behaviors and/or account for differences in rearing success between the two housing conditions.

2) Behavior patterns of mother-reared and hand-reared tamarins:

Differences in specific behavior patterns between mother-reared and hand-reared animals imply differences in temperament and responses to stimuli. If hand-rearing infants alters the behavioral pattern of these animals, it may alter their ability to successfully rear offspring. If the frequencies of some behaviors between mother-reared and hand-reared animals differ significantly, this may lend support to the theory that contact and learning associated with mother-rearing helps to shape behavior patterns of these animals.

3) Behavior patterns of *S. geoffroyi* housed in experimental and control conditions:

Higher rates of aggressive behaviors under the control condition may indicate that the proximity of GTs in nearby social groups causes the higher levels of these behaviors. Heightened levels of aggressive behaviors indicate a stressed or agitated state. This behavioral state may result in more frequent incidences of infant abuse and cannibalism in the control housing condition.

4) Urinary estrogen and progesterone levels:

Reproductive suppression in cotton-top tamarins resulted in sex hormones being excreted at barely detectable levels [< 0.1 micg/mg Creatinine for E_1C] (Snowdon et al., 1993). The presence of adequate concentrations of estrogen and progesterone metabolites in urine at higher levels would indicate that these animals are not reproductively suppressed. Additionally, information regarding the excretion profile of these compounds in urine would provide baseline information for future studies of reproduction in Geoffroy's tamarin.

5) Cortisol levels in urine:

As cortisol level is an indicator of stress in primates, a comparison of cortisol levels for both rearing histories and housing condition provides additional information regarding the animal's stress levels. Animals that are stressed may not show behavioral differences, but elevated cortisol levels would indicate that the animal is under stress. High stress levels may contribute to the high rates of infant abuse and cannibalism.

MATERIALS AND METHODS

This study was conducted at Cleveland Metroparks Zoo between May 1998 and April 1999. A total of 15 Geoffroy's tamarins housed in 4 locations throughout Cleveland Metroparks Zoo were used. The information regarding these animals is summarized in Table II. All animals that would be at least 18 months old by the conclusion of the study were included. The study consisted of two housing situations. No alterations of the social groups were made prior to the start of the study, as existing groups were balanced for rearing history.

All groups housed under the control condition were subjected to visual, auditory, and acoustic access to other groups. Three groups and a solitary female were housed in the control condition. These groups were housed in the basement of the Primate, Cat, and Aquatics Building, and were exposed to visual, auditory and olfactory contact with various other species, including cheetahs (*Acinonyx jubatus*), sloths (*Choloepus didactylus*), and howling monkeys (*Alouatta caraya*). Each group was housed in a 3 x 3 x 3 meter walk-in enclosure (Figure 2). The sides of the enclosure were painted steel. The walls were solid up to approximately 1.2 m. The remainder of the sides was mesh, and the entire ceiling was mesh. The floor of the enclosures was cement.

Each enclosure was equipped with a .6 x .3 x .3 meter nest box, heat lamp, and various combinations of nylon rope, grapevine, and 2.5 x 2.5-cm pieces of wood which were used as climbing structures. Fluorescent lights were on a timer set for 12 hours on and 12 hours off. Enclosures were separated by a hallway that the keepers used to access the enclosures. Sheets of opaque black plastic were placed on the mesh sections to provide visual barriers between groups (Figure 2). Seams between sections of plastic and around the doorways provided minimal visual access to other groups.

Table II. Basic information on Geoffroy's tamarin used in this study. All information is based on records of Cleveland Metroparks Zoo.

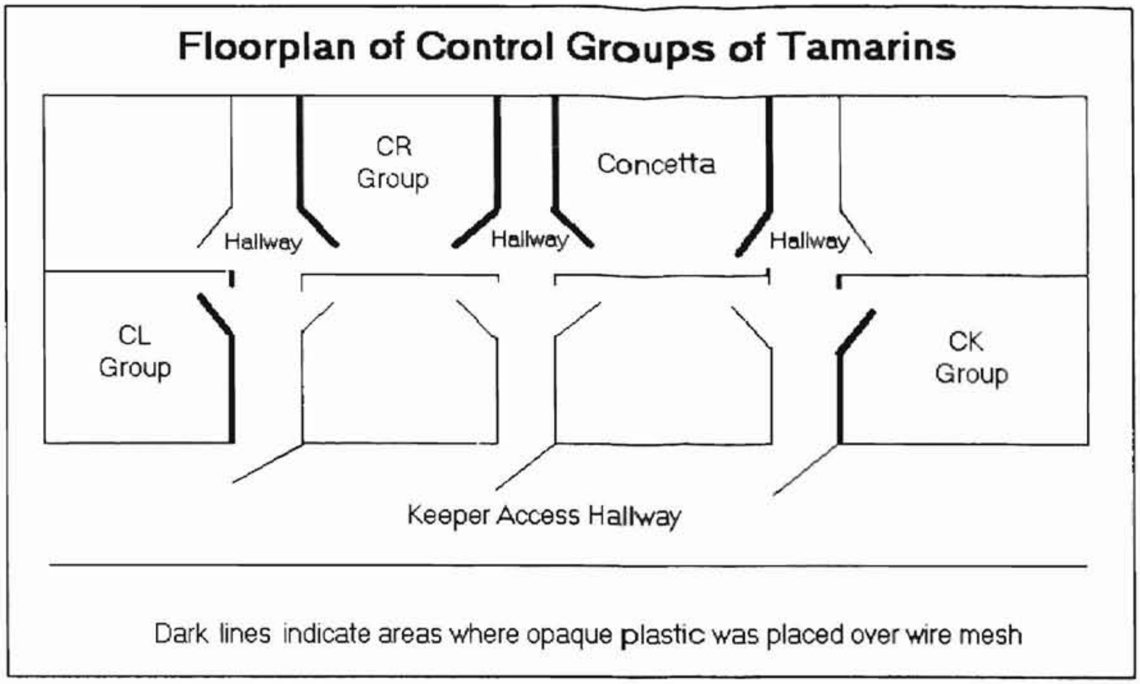
Tamarin	ID#	Studbook #	Sex	Age (years)	Rearing	Group	Housing
Goober	950440		M	~3	Unknown	CL	Control
Clairese	950321		F	2	Mother	CL	Control
Concetta	840515	67	F	18	Unknown	CO	Control
Junior	940436		M	3	Mother	CR	Control
Crabtree	930455		F	4	Hand	CR	Control
Bob	891008	340	M	9	Unknown	CK	Control
Cricket	861209	293	F	11	Hand	CK	Control
Joey	900722	392	M	7	Hand	JP	Experiment
Josephine	880725	337	F	9	Unknown	JP	Experiment
Spaz	961215		M	1	Mother	JP	Experiment
Clinger	850503	156	M	13	Unknown	EL	Experiment
Jamie	890309	357	M	8	Mother	EL	Experiment
Elly	850935	264	F	12	Mother	EL	Experiment
Peanut	890911	365	M	8	Mother	BE	Experiment
Bear	890303	353	F	8	Mother	BE	Experiment

Three groups were housed in the experimental condition. These groups were housed in three different areas in the zoo, thus preventing visual, auditory, and olfactory access to other GT groups. JP group was housed on exhibit in the Primate, Cat, and Aquatics Building. This enclosure was approximately 3 x 3 x 4 meters. It consisted of a concrete floor and three concrete walls. The front of the exhibit was 2.5 cm Plexiglas through which the public could view the animals. Grapevines and dead trees were provided as climbing structures, and a hollow log was provided as a nest box. A heat lamp was present in this exhibit only during the introduction of a hand-reared infant. Skylights in the ceiling provided natural light during the day.

BE group was housed in an off-exhibit area in the Primate, Cat, and Aquatics Building. The exhibit was shared with an agouti (*Dasyprocta cristata*), and measured 4 x 5 x 4 meters. It consisted of concrete floor and walls. The enclosure also contained a nest box, feeding platform, and ropes, grapevines and tree branches. Heat lamps and fluorescent lighting illuminated the room.

EL group was housed on exhibit in the Rainforest Building. This group had two areas, an exhibit and night holding area. The exhibit measured 3 x 3 x 3 meters with a concrete floor and three walls. The front of the enclosure consisted of piano wire. The public, therefore, could be seen and heard easily by the tamarins in the exhibit. The exhibit area had grapevines for climbing and a concrete ledge for feeding. No nestbox or heat lamps were provided on exhibit. The holding area consisted of three interconnected stainless steel cages. Each cage measured approximately 0.5 x 0.75 x 2 meters. A nestbox, feeding platform, and heat lamps were provided. The tamarins were on exhibit from 10:00 until 16:00 each day. None of the three groups housed in the experimental condition were able to see, hear, or smell other GTs.

Figure 2. Floorplan of the control groups of Geoffroy's tamarin.



In JP and EL groups, adult animals were ear tagged to insure proper identification. In all of the other groups, identification was possible by determining sex of the animal or other physical differences.

In each enclosure, a HOBO data logger (Onset Computer Corporation, Pocasset, MA) was installed. These loggers measured temperature, relative humidity, and light intensity once per hour. Each device was installed on the nest box in the exhibit to standardize location as much as possible. In JP group the HOBO data logger was mounted on the wall of the enclosure as close to the nesting log as possible because it was not possible to mount the data logger to the nesting log. For EL group, a data logger was mounted on the next box in the night quarters and on the wall just below the feeding ledge. This location was used because it was underneath the feeding area, which the animals visited frequently throughout the day.

Data were collected from the loggers using a data shuttle (Onset Computer Corporation, Pocasset, MA). The information was translated using BoxCar Pro for Windows 3.5 (Onset Computer Corporation, Pocasset, MA) and summarized using Microsoft Excel 97 and a Gateway GP6-333 desktop computer. Average values per month were calculated for each exhibit. A two sample T-test was conducted to determine if there were differences in temperature, humidity, and light intensity between the two housing conditions.

Behavior Data Collection

An ethogram [Appendix A] was prepared using a combination of behaviors from Moynihan's survey of GTs in the wild (Moynihan, 1970), Skinner's study of GTs in captivity (Skinner, 1986), and Knox and Sade's study of agonistic behavior in *Saguinus imperator* (Knox & Sade, 1991). Behaviors were sampled every 30 seconds on a focal animal using instantaneous sampling (Altmann, 1974). Additionally, aggressive behaviors were recorded using one-zero sampling (Altmann, 1974) and a 30 second interval. All instances of scent marking were recorded throughout the observation period. Data were recorded on prepared data sheets [Appendix B]. Observation order of groups and individuals within groups was conducted in a predetermined randomized order. Recording of observations began 5 minutes after the observer arrived at the enclosure to minimize bias and the animal's initial interest in the observer upon arrival.

Data collection was balanced for time of day as much as was possible considering the general maintenance schedule of the keepers. A total of 46 observations on each animal were collected. A grand total of 115 hours of data were used in the analysis. A repeated measures ANOVA was conducted using Systat Version 8.0. Analysis of variance compared levels of scent marking behaviors, aggressive behaviors, sexual behaviors, cuddling, aberrant behaviors, and inactivity level against rearing history, housing condition, and gender.

Urine Sample Collection

First morning void urine samples were collected from each study animal once per week throughout the study, more often when possible. I turned on the lights and entered the enclosure between 5:30-6:00 AM. I had conditioned the tamarins to sit on a branch near me until they urinated in exchange for a reward. The reward was usually a mealworm or raisin. The urine was collected in a clean paper cup. The urine was then drawn up with a plastic syringe, and pipetted into a 1ml cryotube. The urine samples were labeled with the name of the animal, date, and time, then frozen at -70°C until time of assay.

The sampling schedule was altered by the birth of infants. Samples were not collected from groups immediately following the birth of infants. Although valuable data regarding the hormone levels of the mother and other group members was missed, it was considered to be too great a risk to stress the new mother considering the instability of the population. If an infant died at birth or had to be removed for hand-rearing, sample collection resumed the following day. If the infant survived, sample collection resumed approximately two weeks after birth, or as soon as the mother voluntarily gave samples while carrying the infant.

Sex Hormone Assays

Urine samples were shipped to the California Regional Primate Research Center where I conducted the estrogen and progesterone enzymeimmunoassays under direction of staff at the primate center. Creatinine was assayed using the Taussky method (Taussky, 1954) as described in Monfort et al. (1987). Estrone conjugates and immunoreactive pregnanediol-3-glucuronide were assayed using the method described in Shideler et al. (1990).

For analysis of estrone conjugates 0.050 ml of R522 antibody was added to each well of a 96-well microtiter plate and incubated overnight at room temperature. Wells were emptied and rinsed twice with a wash solution consisting of NaCl, Tween, and distilled H_20. After wells were patted dry, 0.100 ml phosphate buffer was added to each well in the blank rows, and 0.050 ml were added to the other wells. The plates were allowed to incubate for 30 minutes. Next, 0.040 ml of sample was added to the active wells, and 0.050 ml of E_1C horseradish peroxidase was added to each well. Plates were allowed to incubate overnight. The following morning, plates were rinsed twice with wash solution, patted dry, and substrated with 0.100 ml of 2,2'-azino-di-(3-ethylbenzthiazoline sulphonic acid) diammonium salt (ABTS) in a citrate buffer solution. Plates were incubated on a shaker for approximately 60 minutes and read on a Dynatec MR580 automatic microelisa plate reader at 405 nm. Standard curves and sample concentrations were generated using an in-house computer program (Munro & Stabenfelt, 1984).

For analysis of pregnanediol-3-glucuronide concentrations, 0.050 ml of Tilson progesterone antibody was added to each well of a 96-well microtiter plate and incubated overnight at 4°C. The following morning, plates were emptied and rinsed twice with a wash solution consisting of NaCl, Tween and distilled H_2O. After plates were patted dry, 0.100 ml phosphate buffer was added to each well in the blank rows, and 0.050 ml of buffer was added to the other wells. The plates were allowed to incubate for 30 minutes. Next, 0.020mls of sample was added followed by .050mls of horseradish peroxidase. The plates were sealed and allowed to incubate at room temperature overnight. The plates were then washed twice and 0.100mls of 2,2'-azino-di-(3-ethylbenzthiazoline sulphonic acid) diammonium salt (ABTS) in a citrate buffer solution was added. Plates were incubated on a shaker for approximately 60 minutes and read on a Dynatec MR580 automatic microelisa plate reader at 405 nm. Standard curves and sample concentrations were generated using an in-house computer program (Munro & Stabenfelt, 1984). All urinary hormone levels were indexed by creatinine to correct for variations in urine sample concentration.

Cortisol Assays

Following assessment for sex hormones, the urine samples were then sent to St. Louis Zoological Park. Staff at the St. Louis zoo conducted the cortisol raidoimmunoassays. The creatinine assays were done using the Taussky method (Taussky, 1954) as described in Monfort et al. (1987). All samples were indexed by creatinine to control for differences in concentration of urine using the DSL-2100 ACTIVE Cortisol Coated-Tube Radioimmunoassay Kit (Diagnostic Systems Laboratories Inc., Webster, TX).

The urine samples were thawed at 37°C, and the reagents were allowed to come to room temperature. Uncoated and Anti-Cortisol-Coated tubes were labeled in duplicate. Cortisol results were indexed by creatinine to correct for urine sample concentrations. 0.025 ml of standards, controls and urine samples were added to the tubes. Immediately, 0.500 ml of I-125 labeled cortisol in a buffer containing sodium azide was added to all tubes. The tubes were mixed by shaking the test tube rack gently by hand, then incubated at 37°C for 120 minutes. Tubes were decanted into a radioactive disposal container and inverted for 2 minutes to allow them to drain. The tubes were then counted using a gamma counter for one minute.

RESULTS

Abiotic Data

The average temperature, humidity, and light intensity for each exhibit is summarized in Table III. A two sample T-test showed no difference in average temperature, humidity, or light level between the experimental and control housing conditions ($\propto$=.05).

Table III. Abiotic values for the control and experimental conditions.

	Control Condition		Experimental Condition	
Abiotic Factors	**Mean**	**Std. Dev.**	**Mean**	**Std. Dev.**
Temperature	78.2395	2.7888	77.8990	4.5638
Humidity	37.0434	16.8075	38.3472	16.3441
Light Intensity	4.6124	17.8605	7.5166	7.1581

Behavior Data

Summarized frequencies of behaviors for individuals are shown in Table IV. Concetta was excluded from calculations involving the behavior category "huddle" because she was solitarily housed and therefore could not "huddle" with another tamarin. She was included in calculations involving "aggressive behaviors" and "sexual behaviors" because she exhibited these behaviors despite being housed alone by interacting with GTs in adjacent enclosures. She was seen presenting, tongue flicking, head shaking, and displaying (Appendix A) at various times during the study.

Table IV. Frequency of behaviors per observation for Geoffroy's tamarins at Cleveland Metroparks Zoo.

Individual	Scent Marking	Aggressive Behaviors	Sexual Behaviors	Inactive	Huddle	Aberrant Behaviors
Crabtree (C)	.609	1.413	1.804	7.913	4.609	.022
Junior (C)	1.283	.435	2.652	7.196	2.957	.022
Clairese (C)	11.391	1.435	.130	5.109	.283	0
Goober (C)	2.435	.065	.065	6.217	.022	.065
Cricket (C)	1.196	.326	.413	7.717	.283	2.065
Bob (C)	2.022	.630	.630	7.326	.870	.043
Concetta (C)	11.261	.522	.370	6.522	NA	.130
Elly (E)	.609	.283	.109	9.630	.696	0
Clinger (E)	.174	0	.319	10.109	.391	0
Jamie (E)	.652	.348	.217	9.00	.957	0
Bear (E)	3.761	.456	.065	5.130	.022	1.826
Peanut (E)	1.283	.065	.543	6.217	.196	.152
Joey (E)	.370	.130	.1095	11.065	0	0
Josephine (E)	1.435	.043	.130	9.326	.043	.043
Spaz (E)	.652	.022	.022	10.326	0	0

(E) indicates the animal was housed in the experimental condition
(C) indicates the animal was housed in the control condition.

Although these animals are known to be extremely active, several of the animals were inactive for over 50% of the observation time. Aberrant behaviors, which consisted of hair plucking, coprophagia, and self-mutilation, occurred infrequently, with some animals never exhibiting these behaviors. However, some of the animals engaged in these types of behaviors up to 10% of the observations. The one-zero sampling method is more effective at recording behaviors that are events, such as aggressive and sexual behaviors, than behaviors that are states, such as inactivity. Therefore, aggressive behaviors and sexual behaviors were analyzed using the data collected using the one-zero sampling method, as they occurred only briefly and they were less detectable using the instantaneous sampling method. Aggressive behaviors were analyzed together and consisted of: confrontation, wide open-mouth grimace, display stance, lunge, chase, bite, grab face, bat, head shake, hiss, and bark. Sexual behaviors consisted of: mount, thrust, ano-genital sniff/lick, and present.

Scent marking, aggressive behaviors, sexual behaviors, inactivity, huddling, and aberrant behaviors were chosen for analysis considering they should be indicators of stress or reproductive suppression. Average frequencies of these behaviors were compared against rearing history using a repeated measures ANOVA. No differences were found for average frequencies of behavior per observation by rearing history ($\propto$=0.05).

Frequencies of behaviors showed no significant differences when compared against gender ($\propto$=0.05). There was, however, a trend for females to mark more often than males, although this was not statistically significant (P=0.087).

Significant differences in patterns of aggressive and inactive behavior between housing conditions were shown. Aggression was higher and inactivity lower in the control group. The results of a repeated measures ANOVA are summarized in Table V. Although not statistically significant, trends were found for animals in the control condition to scent mark, huddle and exhibit sexual behaviors more often than those animals in the experimental condition.

Table V. Differences in mean frequencies of behavior between control and experimentally housed Geoffroy's tamarins using a repeated measures ANOVA.

Housing Condition	**Scent Marking**	**Aggressive Behavior**	**Sexual Behavior**	**Inactive**	**Huddle**	**Aberrant Behavior**
Control	4.314	0.696	0.711	6.857	1.289	0.339
Experiment	1.117	0.168	0.198	8.851	0.288	0.258
Significance Level	P=0.089	*P=0.017	P=0.081	*P=0.034	P=0.093	P=0.809

*Indicates a significant difference.

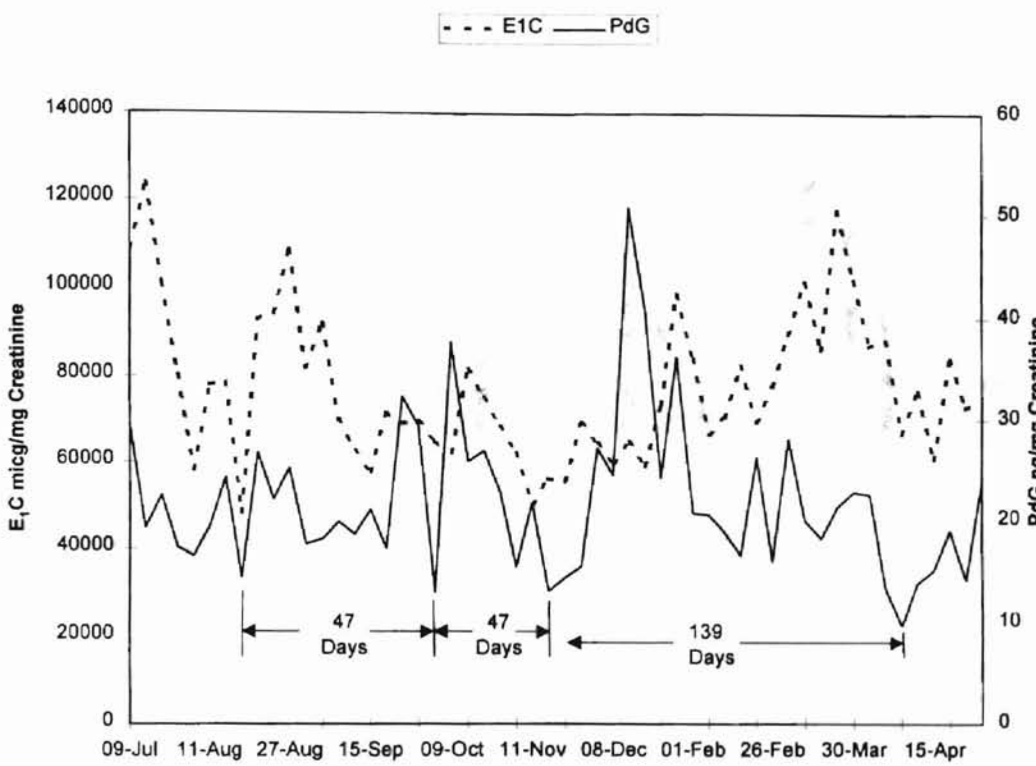
Sex Hormones

The assays for estrone conjugates and progesterone glucuronide showed that all individuals in the study were excreting levels of sex hormones, suggesting regular estrous cycles. As Concetta was not housed with a male during the study, her values were used to estimate the excretion profile of E_1C and PdG in GTs(Figure 3). A true assessment of cycle length from this data is difficult, as samples were not collected on a daily basis. Other females were not included in the estimation of cycle length, as it was impossible to determine accurately if a female was pregnant or cycling. Analysis of the PdG and E_1C values for Concetta showed extremely low levels at regular intervals. Of the four lowest levels of PdG recorded, three were 47 days apart from each other. The fourth was 139 days from the others. Each of these intervals is divisible by 23, which is the estimated cycle length for cotton-top tamarins (Ziegler et al., 1987a). The missing low points in the excretion profile are probably attributed to lack of samples on the low days of the cycle. It should be noted, however, that she is the oldest female in captivity (18 years) and although tamarins are not reported to exhibit reproductive senescence, the data should be confirmed with data on younger females.

Mean values of sex hormones leading up to each birth (n=7) provide an illustration of the hormonal pattern during pregnancy for GTs (Figures 4 and 5). Excretion profiles for cotton-top tamarins have shown high levels of hormones during the middle of pregnancy, with a decline in the weeks preceding parturition and a peak on the day before and day of parturition. Although the peak in hormone levels is not detectable during this study, the mean excretion profiles for GTs follows this pattern. The lack of a peak prior to parturition may be the result of a protocol of collections only twice per week.

Alternatively, the peak in hormone levels may not be detectable in the urine of *S. geoffroyi*, but this seems unlikely, as the excretion profile matches closely in all other cases.

Figure 3. Urinary excretion profile of E_1C and PdG for Concetta. Arrows show the time passed between the four lowest levels of PdG.



The mean interbirth interval during this study was 205 days. A 23-day cycle followed by a 180-day gestation period (the estimated gestation in *S. oedipus*) would seem to indicate that many of the infant tamarins are conceived during the first post-partum estrous, which quickly follows parturition.

Figure 4. Mean excretion of PdG in urine for pregnant female Geoffroy's tamarins (n=7) during the 28 weeks prior to parturition.

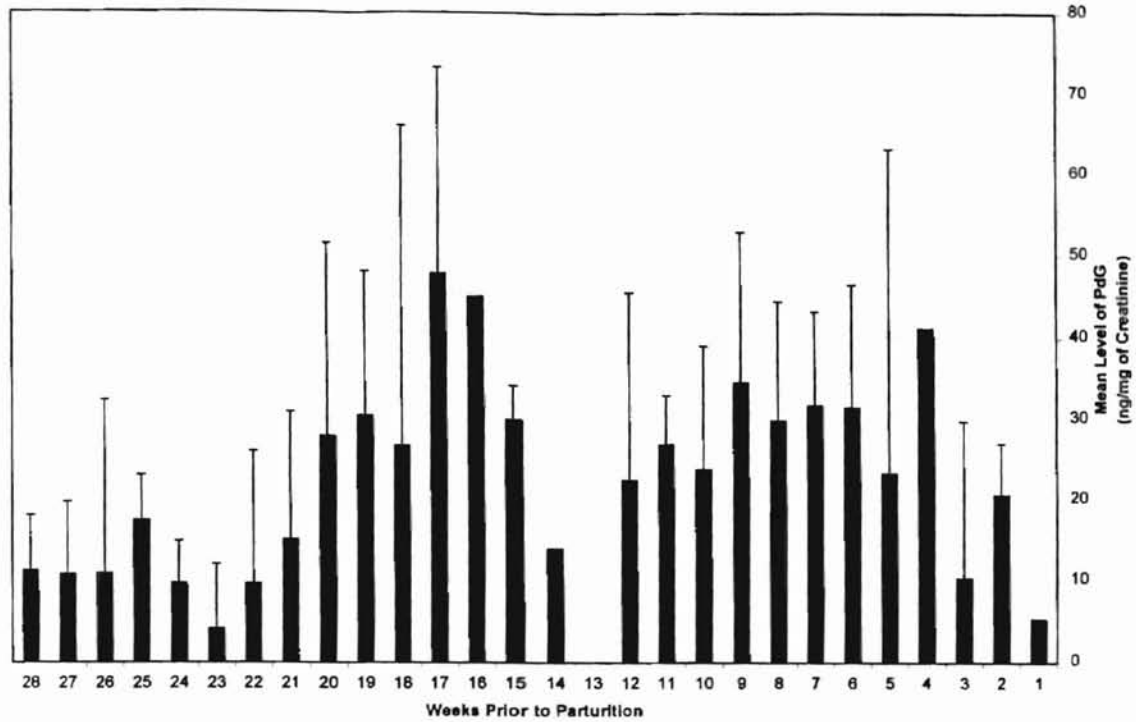
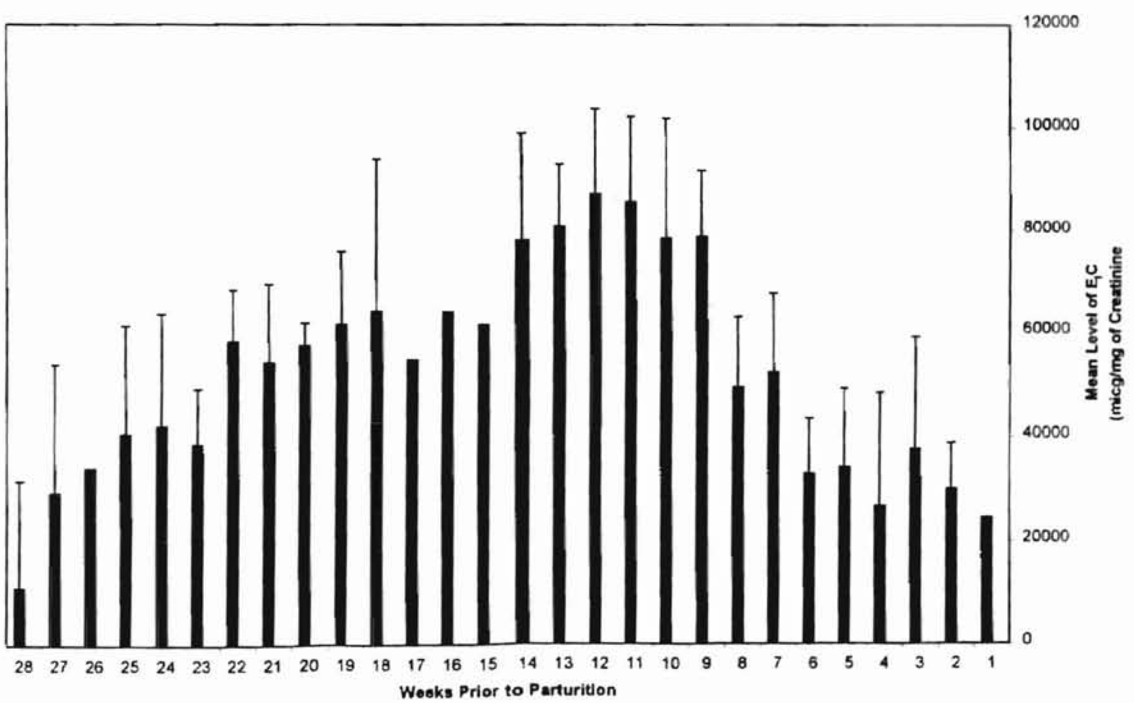


Figure 5. Mean excretion of E_1C in urine for pregnant female Geoffroy's tamarins (n=7) during the 28 weeks prior to parturition.



Concetta showed the highest mean levels of PdG in the colony (Table VI). This female is housed singly, and therefore cannot show elevated levels of progesterone due to pregnancy. She is also the oldest tamarin in the colony, and according to studbook records she is the oldest GT in captivity.

Table VI. Summary of mean E_1C and PdG levels in urine of female Geoffroy's tamarins.

Individual	**Estrone Conjugates (micg/mg Creatinine)**	**Progesterone Glucuronide (ng/mg Creatinine)**
Bear (E)	15.7611	35484.8
Elly (E)	32.6001	29453.7
Josephine (E)	29.0388	59671.0
Concetta (C)	21.8528	77202.4
Clairese (C)	13.0420	20610.9
Cricket (C)	38.6969	36985.3
Crabtree (C)	30.8242	59327.8

(C) indicates the animal was housed in the control condition.
(E) indicates the animal was housed in the experimental condition.

Cortisol

Table VII summarizes mean and standard deviation of cortisol excretion for each animal. The assays for cortisol showed no independent variable measured had an effect on the cortisol excretion in urine (Table VII). Excretion profiles for the females showed a linear increase in cortisol excretion as they neared parturition (Figure 6). The rises in cortisol coincided with rises in E_1C excretion and PdG excretion.

Table VII. Means and standard deviations of cortisol excretion in urine for Geoffroy's tamarins.

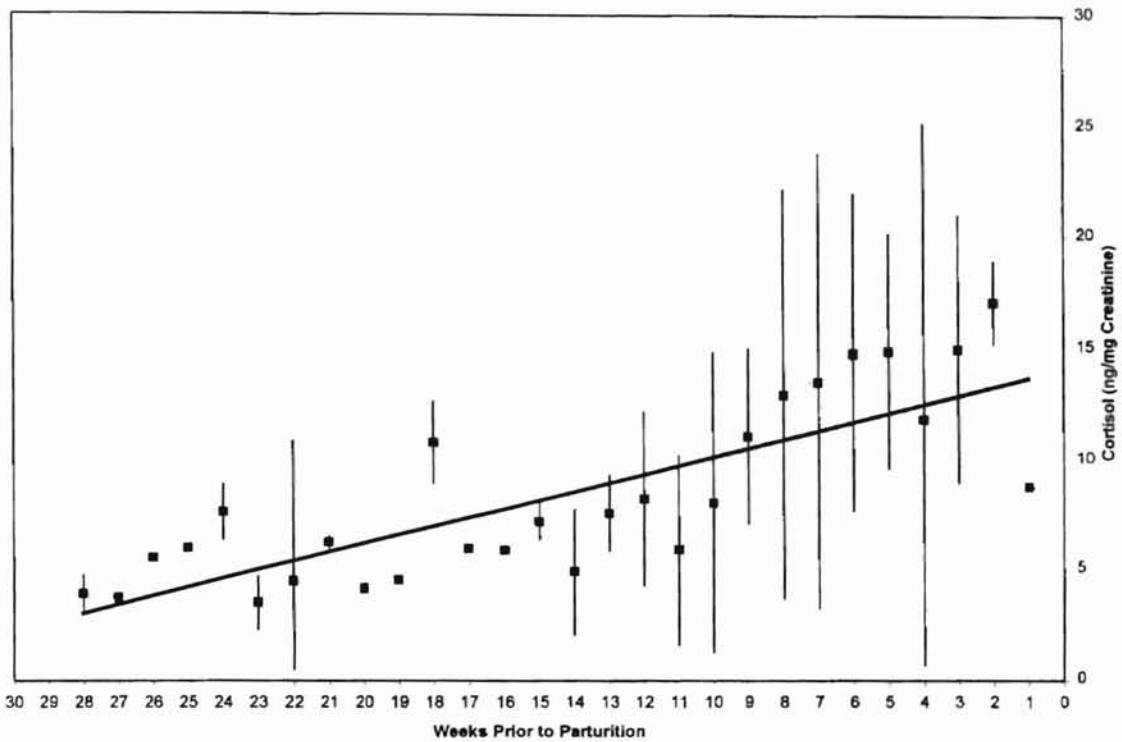
NAME	MEAN	STANDARD DEVIATION
Bob(C)	3.657	2.604
Clairese (C)	7.994	6.242
Concetta (C)	6.094	1.584
Crabtree (C)	4.929	4.325
Cricket (C)	7.234	4.886
Goober (C)	5.556	4.058
Junior(C)	5.798	8.113
Bear (E)	9.052	7.377
Clinger (E)	3.432	0.629
Elly (E)	5.904	1.476
Jamie (E)	3.440	1.877
Joey (E)	4.600	4.573
Josephine (E)	12.218	7.367
Peanut (E)	15.464	6.939
Spaz (E)	3.500	1.234

(C) indicates animal was housed in the control condition.
(E) indicates the animal was housed in the experimental condition.

Table VIII. ANOVA results for mean cortisol excretion by housing condition, rearing history, and gender of the animal.

	Independent Variable	**Cortisol Secretion (micg/mg Creatinine)**	**ANOVA Results**
Housing Condition	Control	5.9	P=0.448
	Experimental	7.3	
Rearing History	Hand	5.57	P=0.767
	Mother	7.31	
	Unknown	6.36	
Sex	Female	7.63	P=0.314
	Male	5.79	

Figure 6. Mean levels of cortisol excreted in urine for pregnant female Geoffroy's tamarins (n=7) during the 28 weeks prior to parturition.



It should be noted that female Elly was ill for much of the second half of the study. She suffered spontaneous leg fractures that were attributed to degenerative bone disease. In April of 1999 she was placed in a smaller cage within the holding area. This was to decrease her movement, but still involve her with the group socially. During this time her cortisol excretion increased from an average of 5.9 micg/mg of Creatinine to a high of 96.3 micg/mg of Creatinine on May 4. This nearly 20 fold increase may be attributed to the small cage she was in, rather than her illness, but it is an indication that cortisol levels increase in response to an acute stressor. She was actually in better physical condition at the time of this high sample than she had been previously. Considering this possibility, the sample values obtained while Elly was in the small cage were excluded from the analysis. This lowered her average cortisol excretion value greatly, but made no difference in the statistical analysis.

It should also be noted that although the control groups had a lower mean cortisol excretion, they had a larger variance (Control=5.226, Expermental=3.287) in excretion levels. Although the difference was not statistically significant ($t=1.515$, $P=0.154$), it may be biologically significant. Because of the high level of individual variation within the population and the small sample size, it may be impossible to determine whether an animal is being stressed by examining mean levels of cortisol secretion. However, cortisol levels for an animal that showed a high degree of variability, i.e. a large standard deviation, may better provide information on the fluctuation of cortisol levels, and also stress levels, within an animal. Further research is needed to determine whether this variation in cortisol secretion is biologically significant.

Births

All females except Concetta became pregnant at least once during the study. A summary of births and their results is given in Table IX.

Table IX. Summary of births for Geoffroy's tamarins at Cleveland Metroparks Zoo between June 1998 and May 1999.

Mother	Date	Offspring Produced	Offspring Result
Josephine (E)	10/4/98	triplets	1 fell from mother (died) 1 fell from mother (hand reared) 1 raised
Josephine (E)	4/30/99	twins	1 fell from mother (died) 1 raised
Bear (E)	4/6/99	singleton	1 died following day
Elly (E)	7/15/98	twins	2 raised
Elly (E)	2/28/99	singleton	1 died at birth
Cricket (C)	10/12/98	twins	1 cannibalized 1 pulled from mother (hand raised)
Cricket (C)	4/29/99	triplets	1 cannibalized 2 pulled from mother (hand raised)
Crabtree (C)	9/11/98	twins	2 died at birth
Clairese (C)	1/15/99	singleton	1 stillborn

(C) indicates the animal was housed in the control condition.
(E) indicates the animal was housed in the experimental condition.

DISCUSSION

Abiotic Factors

The analysis of the abiotic data revealed that temperature, relative humidity, and light intensity did not differ between the housing conditions. A few trends were observed in the data, however. None of the exhibits were maintained at the recommended temperature for breeding related *Saguinus* species in captivity. In fact, the average temperature for four of the exhibits was lower than the recommended minimum temperature for captive breeding of cotton-top tamarins. All of the exhibits had average temperatures lower than the preferred minimum temperature (29°C) for breeding of cotton-tops. However, because there were no significant differences between the housing conditions it was not possible to determine if these temperatures are affecting reproductive success. Differences in behaviors or rearing success between the housing conditions in this colony of GTs, therefore, cannot be attributed to differences in temperature.

There have been no studies conducted on the effects of temperature-induced stress on cortisol production in Callitrichids. However, it has been shown that temperature induced stress may be the source of disease in captive callitrichid primates (Stonerook et al., 1994). Further investigation of the effects of sub-optimal temperatures on New World monkeys is necessary.

All of the exhibits in the control setting were provided with heat lamps. P119 and RFH were also equipped with alternative heat sources. P105 and RFE were not equipped with heat sources during the study because the design of the exhibit did not allow for safe placement of heat lamps in the exhibits. A heat lamp was provided for the infant introduced into the exhibit in P105 during the study, but the adults in the exhibit were not observed to be using this heat source. The tamarins in the other exhibits, however, were observed to be sitting near the heat sources during the day. The temperature at these heat sources was not measured. In studies that have examined the effect of temperature induced stress, no provisions were made in the enclosures for the animal to be able to regulate its temperature by moving toward and away from a heat source. It is possible that although the ambient temperature in the exhibit is lower than recommended, the animals are not suffering temperature-induced stress because they are able to thermoregulate by their behaviors.

The exhibits in the control category are housed in the basement of the Primate, Cat & Aquatics building of the zoo. This basement area is adjacent to the night quarters of the cheetahs. The cheetah holding areas have access to the outside exhibit. As a result, the basement area is much more susceptible to the temperature fluctuations of the seasons in northeast Ohio. The temperature of the basement exhibits fell drastically during the winter months. Additionally, the average temperature for each of the four exhibits was related to its position in relation to shift door in the cheetah exhibit. P015 is closest to the door and it had the lowest average temperature. P021 was also close to the hallway leading to the cheetah exhibits. It, however, was further away and had a slightly higher temperature. The exhibits that were farther from this hallway (P017 & P018) had higher temperatures than the exhibits adjacent to the hallway. The connection to the elements that the shift doors in the cheetah exhibits provide creates a management problem for maintaining temperature and humidity levels. Moving the tamarin colony to an area of the building that was less drastically influenced by the outside temperature would enable the temperature and humidity levels of the exhibits to be more easily managed.

Additionally, Buchanan-Smith et al. (1993) showed that cotton-top tamarins exhibited high anxiety when presented fecal scents of margay and tayra. They suggested that captive animals should not be held in areas where prey species can detect predators via olfaction. Although cheetah are not natural predators to GTs, they are felids and natural predators. Humans can detect the scent of cheetahs in this area, and it can be assumed that the tamarins can detect their presence as well. It is possible that the presence of these feline predators may also be stressful to the groups in the control condition.

Although high levels of humidity are recommended for these animals, humidity will increase the amount of evapo-transpiration that occurs on the skin of these animals. Evapo-transpiration has a cooling affect on the skin. High humidity levels may be increasing the amount of heat that is lost by these animals in sub-optimal temperature conditions and magnify any negative effects that low ambient temperature is having on these animals.

The average relative humidity levels did not reach the recommended levels of humidity in the Cotton-top Tamarin Husbandry Manual. The recommended level of 50-75% relative humidity was not reached by any of the average humidity levels in the exhibits in the study. Although the humidity level recommendation was found in the literature, no studies stating the effects of inadequate humidity levels were found. The logic behind these humidity levels seems to be that these are the relative humidity levels of the countries of origin of these primates, but GTs have been found on the dry Pacific side of Panama (Dawson, 1978). It is possible that humidity may have no affect in altering the behavior or physiology of these or other New World monkeys. Further research is needed to determine the role humidity plays in the biology of these animals.

Another issue seems to be the reliability of the loggers themselves. The data for the month of April for several exhibits was lost when the data shuttle did not download the files properly. This system has no means for backing up files until they have been downloaded into the BoxCar program. Several months of data in the Rainforest (exhibits RFE & RFH) were lost or incomplete. This was due in part to corrosion, which occurred within the loggers. Often the internal components of the loggers were extremely wet when the data was downloaded, but the logger still recorded the humidity within the rainforest at between 35 and 37%. It is possible that these loggers were not accurately reading the humidity levels of this building. The building is intended to have a high level of humidity and is equipped with a high pressure misting system to keep the humidity in the building at high levels. Although the loggers were calibrated at the beginning of the study, further investigation needs to be conducted to determine the reliability of this set of data loggers.

No significant differences were found in light intensity. The variation between exhibits would seem to be more related to the placement of heat lamps within the exhibits. The light intensity in these exhibits meets standards for maintaining captive animals.

Rearing History and Conditions

Analysis of the behaviors indicated that no differences in the frequencies of behaviors could be attributed to the rearing history of the animals. Therefore, in this study failure to successfully rear infants cannot be attributed to a particular behavioral repertoire or tendencies related to the rearing history of the individual.

It should be pointed out, however, that there are five individuals in this study that have unknown rearing histories. Bob, Clinger, and Concetta are wild caught individuals. No record exists of the age of these animals when they were brought into captivity. Probably their parents raised them. The probability of humans successfully rearing an infant tamarin is extremely low. Records for other primate species indicate an extremely poor success rate of raising infant offspring during the capture of exotic animals for the pet trade and zoo market (Wallis, 1997). Goober was a pet and was placed in a zoo setting in 1994. Records do not indicate the source of the animal or his age at capture. Most likely he is a wild caught animal that was raised by his parents.

Josephine has an unknown rearing history as well. She was transferred to CMZ in 1988, but no information regarding her birth type or rearing history was transferred from the original zoo. Josephine is the most prolific female Geoffroy's tamarin at CMZ, and possibly in captivity. She has reproduced twice a year for the past 4 years. She regularly raises at least one infant out of the litter, and has successfully reared twins. It is because of her and her partner's parenting ability that a single hand reared infant, and hand reared twins have successfully been introduced into her group. Information on her early experience would be extremely valuable.

If the rearing history was known for those five animals, differences in behaviors might become apparent. Future studies on a larger population may reveal differences in behavior between parent-raised and hand-raised animals. However, the data shows no behavioral differences between different rearing histories based on this limited sample.

Gender

Gender did not affect frequencies of behaviors. There was a trend for females to scent mark more than males, but this was not statistically significant. The slightly higher frequencies of scent marking in females could be attributed to an increase in estrogen during pregnancy. Ziegler et al. (1987b) found that scent marking in female cotton-top tamarins correlated positively with estrogen level. Most females were pregnant during this study and would have elevated estrogen levels. Because urine samples were not collected on a daily basis an in-depth analysis of the correlation between scent marking and estrogen level in *S. geoffroyi* was not possible.

The fact that there are no differences in behavior frequencies between the sexes is characteristic of a cooperatively breeding species. Both males and females are approximately the same size and engage in many of the same activities. Males are not able to dominate females because they are similar in size. Additionally, because of the energetic demands of rearing offspring (Sanchez et al., 1999; Price, 1992a, 1992b; Tardif, 1994), both sexes must participate in raising the infants and other general duties. Therefore, there is no segregation of duties by gender. Both sexes participate in all aspects of tamarin life and show the same behavior patterns.

Housing Condition

Housing condition resulted in a difference in behaviors by the GTs. Increased levels of aggression and sexual behaviors in the control condition may be a result of incomplete visual separation. Previous studies with cotton-top tamarins have shown an increase in aggressive behaviors when they were moved into a position where they could see other tamarins. These animals exhibited much lower levels of aggression when they were housed in areas where they could hear and smell, but not see other tamarins. In this study aggression in the control condition was directed at individuals in other exhibits. The tamarins in this housing situation spent long periods of time sitting in areas of the exhibit where they could see the other tamarins. Lunges, confrontations, head shakes, and tongue flicks occurred at the borders where other tamarins were visible. Additionally, Crabtree had become adept at picking the lock to her enclosure. Once they had escaped their enclosure, Crabtree and Junior initiated violent confrontations with Clairese and Goober. These confrontations have resulted in bite wounds on faces, hands, and feet. Usually, the attacks are male-male and female-female.

The increased frequencies of aggressive behaviors to extra-pair individuals, sexual behaviors, scent marking, and huddling are common characteristics of monogamous animals under natural conditions (Fernandez-Duque et al., 1997). The higher levels of aggression and scent marking may be an attempt to stake out their territory and drive away the perceived "competing" individual of the same sex. Once the intruder has been driven away frequent copulations and huddling maintain the pair bond and increase the likelihood that any offspring that result are the offspring of the partner and not the intruder. If this is the perception at CMZ, the tamarins presumably would be stressed because the stranger is never driven away and the conflict is never resolved. The inability to resolve the conflict may create stress.

Cortisol

Analysis of the cortisol data indicates that there is no difference in the cortisol excretion in the two housing conditions. In fact, there is no difference in cortisol excretion for any of the independent variables. Therefore, either the housing condition is not stressful, or urinary cortisol assays are not an accurate measure of an animal's stress level. The ineffectiveness of the assays as a measure of stress level seems unlikely because of two factors. First, Ziegler et al. (1995) showed that urinary cortisol could be used as an indicator of stress in captive cotton-top tamarins. It seems logical that urine samples from GTs would show similar results for urinary cortisol assays. Second, the cortisol response of Elly to a known stressor was observable from the data.

As stated previously, Elly was placed in a small cage within her home exhibit to minimize her movements but provide social interactions. During this time, Elly proceeded to pluck the hair from her legs, abdomen, and tail. Her cortisol level increased nearly 20 fold. This acute response to a stressor demonstrates the ability of the assay to detect high fluctuations in cortisol.

Another possibility for the lower than expected cortisol levels in the control groups would be an increased sensitivity to cortisol resulting in a downregulation of glucocorticoid receptors that would decrease cortisol levels. This explanation was offered by Ziegler et al. (1995) to explain lower than expected levels of cortisol in long-term cycling female cotton-top tamarins. The test that would seem to determine the accuracy of this hypothesis would be to switch the current grouping of tamarins at CMZ. Placing the experimental groups into the colony condition and moving the control groups to conditions where they could not see, hear, or smell other GTs may stimulate increased cortisol excretion in the animals that are moved into the control setting. Ziegler et al. (1995) showed cotton-tops to have elevated cortisol levels when placed in a situation where they were housed adjacent to unrelated groups of cotton-tops.

Cortisol did show a relationship to the level of sex hormones excreted. Cortisol excretion profiles, similar to the excretion profiles of E_1C and PdG, showed a marked increase near the perinatal period and near the periovulatory period. Ziegler et al. (1995) showed a similar relationship and attributed the increase to the activity of the ovarian steroids. It may be that the hypothalamic-pituitary-adrenal axis, which is involved in stimulation of secretion of cortisol, may be involved in the ovulatory process in some primates. Additionally, progesterone is the base steroid in the formation of both estrogen and cortisol. It is possible that the higher levels of progesterone and estrogen that are present near birth cause an increase in the production of cortisol. It was noted that cortisol levels do not remain high throughout a pregnancy and this may be the result of fluctuating levels of cortisol binding globulin (Ziegler et al., 1995). A similar effect is shown here, as cortisol levels rise near what we would assume to be the periovulatory period, fall, and then rise again near parturition. The relationship between the gonadal steroids and cortisol needs further definition, but the correlation between these hormones, found in other studies, is present in this study.

Complete reproductive suppression is not occurring in these groups. All females that were paired with a male became pregnant during the course of the study. Ziegler et al. (1987b) defined detection of a pregnancy by use of enzyme immunoassays as an elevated and sustained level of E_1C greater than 4,000 ng/g Creatinine, and PdG levels greater than 8 μg/mg Creatinine. All pregnancies during the study fit this description.

Clairese (control group) and Bear (experimental group) became pregnant for the first time during the study. Clairese had a stillbirth on January 15, 1999. Bear gave birth on

April 6, 1999. This infant died the next day. Necropsy revealed that it had not suckled. Staff were unsure whether the mother produced milk, or simply failed to nurse. Crabtree (control group) gave birth to twins on September 11, 1998. The infants were found dead. A necropsy was not performed, therefore, we were unsure whether or not the infants were stillborn.

Cricket, Josephine, and Elly each gave birth twice during the study. The average interbirth interval for Geoffroy's tamarins during the course of this study was 210 days. Crabtree and Clairese, however, only reproduced once during the study. Crabtree and her mate Junior were involved in the greatest number of sexual behaviors. It is possible that Crabtree and Clairese were pregnant, but had stillbirths and consumed the fetuses. It is also possible that the fetuses were resorbed. Resorption has been attributed to social stress and reproductive inhibition in wild groups of cotton-tops (Savage et al., 1997). Metabolic demands on a female may force resorption. The golden lion tamarin breeding program made huge gains with an improved diet for their tamarins (Kleiman et al., 1982). Additionally, resorption of fetuses in the wild has been attributed to the lack of food during a dry season. It is possible that although the control environment may not actually be stressful according to the cortisol levels, the amount of energy being expended by the tamarins, in both aggressive interactions and regulating body temperature, may be having a detrimental effect on reproductive success. If the tamarins in the control setting are expending energy at a greater rate than those tamarins in the experimental setting, they would have less energy to expend in fetus development, parturition, and rearing offspring.

Other than Josephine, the only tamarin in the colony to successfully rear offspring over the past 10 years is Elly. Prior to the fall of 1997 she had been housed in the control setting. She had reproduced in the control condition, but had always cannibalized her offspring. She successfully reared the first litter she produced following her move to the rainforest building. This movement to the rainforest building was the first time she had been housed outside of sensory contact of GT groups. Her poor health during the second half of the study is the most likely cause of her stillbirth in February 1999.

There are two factors that Elly and Josephine have in common. They are both housed in the experimental condition. They are not able to see, hear, or smell other Geoffroy's tamarins. Additionally, they are both housed with two adult males. Elly is housed with Clinger and his adult son Jamie. Josephine is housed with Joey and their adult son Spaz. The presence of two adult males has been suggested as a successful breeding strategy (Lamprey, 1997). Which factor is contributing most to the success of these groups is impossible to determine at this time. Further research is needed to separate these two factors.

The basement colony presents three major problems. First, the absence of complete visual barriers is resulting in high levels of aggression, and may be resulting in chronic stress. Second, the possibility of animals escaping and injuring themselves in escalating fights with other GT groups. Third, the potential for disease transmission among the groups is extremely high. In addition to the proximity of the groups, there is only one drain in the area in which the GTs are housed. When the enclosures are cleaned all of the waste must pass through the other enclosures to reach the drain. Thus, all groups have potential contact with the urine and feces of other groups. This small basement colony represents 14% of all of the Geoffroy's tamarins in the captive population. Loss of these animals would severely decrease the already low captive population numbers.

Conclusions

1. There were no differences in the average temperature, humidity, or light intensity between the control and experimental housing conditions.

2. The average temperature and humidity for each exhibit was lower than the recommended temperature and humidity for breeding of cotton-top tamarins, a closely related species.

3. No differences existed in the average frequency of sexual behaviors, aggressive behaviors, huddling, inactivity, scent marking and aberrant behaviors for different rearing histories and condition.

4. No differences existed in the average frequency of sexual behaviors, aggressive behaviors, huddling, inactivity, scent marking and aberrant behaviors by gender.

5. Animals housed in the control condition engaged in significantly more aggressive behaviors, and less time was spent inactive than those housed in the experimental condition. Additionally, animals housed in the control condition showed a tendency to engage in more sexual behaviors, huddling, and scent marking.

6. No differences were found in excreted cortisol levels by housing condition, rearing history, or gender.

7. All females are cycling and all females housed with a male became pregnant during the study. Thus, reproductive suppression was not occurring.

8. Further research is necessary to determine if lower than expected levels of cortisol are found in the control housing condition because of a downregulation of cortisol receptors in response to chronic high levels of cortisol.

9. Separation of the GTs in the basement colony of Cleveland Metroparks Zoo is recommended because of the higher levels of aggressive behaviors, which have the potential of resulting in injury and may be contributing to the insufficient parenting behavior exhibited by these pairs. Additionally, the proximity of the animals to each other put them at risk of rapid disease transmission.

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APPENDIX A

Geoffroy's Tamarin (*Saguinus geoffroyi*) Ethogram

SEXUAL BEHAVIORS

Ano-genital Sniff/Lick-(SNF)-tamarin puts face/nose to the ano-genital region of another tamarin.

Mount-(MNT)-tamarin climbs on another tamarin's back from the rear, the arms encircling the mountee's waist (Knox & Sade, 1991).

Present Rear-(PRES)-presenting tamarin brings his or her ano-genital region into the view of another tamarin (Knox & Sade, 1991).

Thrust-(THR)-while mounting is taking place, forward pelvic movements directed by the mounting individual.

AGONISTIC BEHAVIORS

Bark-(BK)-corners of the mouth are retracted upward as the animal emits a harsh vocalization. Often accompanied by the Wide-Mouth Grimace, Fleeing, or both (Knox & Sade, 1991).

Bat-(BT)-tamarin swiftly cuffs another tamarin, usually on the face (Knox & Sade, 1991).

Bite-(BIT)-attacking tamarin strikes at another tamarin with its teeth (Knox & Sade, 1991).

Chase-(CH)-one tamarin rapidly pursues another tamarin. May end with the charging tamarin biting the fleeing tamarin (Knox & Sade, 1991).

Confrontation-(CONF)-rapid and erratic movement toward and away from another individual, may be accompanied by piloerection, arched back, open mouth, and staring.

Display Stance-(DIS)-Standing quadrupedally, the tamarin, usually a male, directly faces another, everting the shoulders, and arching the back. Makes tamarin appear broader than usual. Often accompanied by piloerection (Knox & Sade, 1991).

Flee-(FL)-the tamarin that is pursued evades the attack by suddenly running away. There may be less flexion and extension of the vertebral column than in normal locomotion (Knox & Sade, 1991).

Grab Face-(GRBF)-hand is extended toward the face of another tamarin and the claws are used to grab the pelage, which is then immediately released (Knox & Sade, 1991).

Head Shake-(HDSHK)-head is rapidly turned from side to side while staring at the target tamarin (Knox & Sade, 1991).

Hiss-(HS)-tamarin gives a quick hissing vocalization while looking at another tamarin. Sometimes accompanied by head shaking (Knox & Sade, 1991).

Lunge-(LUN)-tamarin's head and shoulders are rapidly thrust toward another tamarin. The mouth may be partially opened (Knox & Sade, 1991).

Neck Bite-(NBIT)-tamarin bites another tamarin on the neck. The bite is not delivered with full force (Knox & Sade, 1991).

Piloerection-(PILO)-individual raises all or almost all the hair on its head, body, limbs, and tail.

Submission-(SUB)-individual bends head and lowers neck slightly, while directly facing another, so that the red/brown nape patch is easily viewed. Forelimbs may be raised to the face.

Tongue Flick-(**TFLK**)-mouth is partially opened; the tongue protrudes from the mouth and is moved rhythmically up and down (Knox & Sade, 1991).

Wide Open-Mouth Grimace-(**GRIN**)-tamarin's mouth is completely opened and the lips are drawn back so that both the upper and lower teeth are visible (Knox & Sade, 1991).

SOCIAL BEHAVIORS

Approach-(**APP**)-an individual locomotes directly toward another individual in order to maintain proximity.

Grooming-(**GRS-Groom Self; GRG-Groom Given; GRR-Groom Received; GRM-Groom Mutual**)-one animal picks through the hair of another animal with its forelimbs or mouth. This behavior can either be directed from one individual to another (GRG; GRR), it can be mutual (GRM) or it can be self-directed (GRS).

Huddling-(**HUD**)-one animal is in contact with another animal. This behavior is not scored if any of the other behaviors on this ethogram are occurring (Skinner, 1986).

Move Away-(**MA**)-an individual locomotes away from another individual in order to maintain or establish physical separation.

Wrestle-(**WR**)-an individual is involved in grappling or biting with another animal. This behavior is not accompanied with vocalizations.

ABERRANT BEHAVIORS

Coprophagia-(CO)-an individual manipulates and/or consumes feces.

Hair Pluck-(**HP**)-individual removes hair from itself or another individual with its mouth or its hands.

Self Mutilate-(SM)-an individual bites at or chews on any part of its body (usually the tail).

OTHER BEHAVIORS

Eat-(EAT)-an individual is consuming food or water.

Examine/Explore-(EXXP)-an individual is actively manipulating an object or structure in the enclosure.

Idle-(IDL)-focal animal is not engaged in any of the behaviors on this ethogram.

Nest Box-(NB)-focal animal is inside of the nest box.

Out of View-(OOV)-the focal is not in view or the behavior is not able to be determined on the interval.

Public Orient-(PO)-an individual is attentive or attempting to interact with the observer/visitor.

Scent Marking-(MK)-this consists of ano-genital scent marking and sternal scent marking. The animal either presses the ano-genital area against the substrate and either rubs from side to side or drags itself along the substrate with its forelimbs, or the animal lies down on its stomach and pulls itself forward with its forelimbs.

Travel-(TR)-an individual is moving from one area of the exhibit to another, not one of the other locomotion behaviors, APP, MA, CH, etc.

APPENDIX B

Geoffroy's Tamarin Data Sheet

Focal Animal: ____________________ Location: ____________ Observer: _______
Date: __________ Time: _____________ Enclosure Mates: __________________

	NN	BEHAVIOR	AGON	SEX
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SCENT MARKING:

VITA

Christopher William Kuhar

Candidate for the Degree of

Master of Science

Thesis: FACTORS AFFECTING THE REPRODUCTIVE SUCCESS OF ZOO-HOUSED GEOFFROY'S TAMARINS (*SAGUINUS GEOFFROYI*)

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