University of Alberta

The Relationship between Ovarian Hormones and Behavior in Female White-faced Capuchins, *Cebus capucinus*

by

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of the requirements for the degree of Master of Arts

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ABSTRACT

The relationship between ovarian hormones and behavior in white-faced capuchins (*Cebus capucinus*) was examined using fecal steroid hormones. Estradiol and progesterone were analyzed using radio and enzyme immunoassays to create hormone profiles for each female. Analysis revealed three pregnant, three non-cycling and four cycling females. Cycling females displayed between one to three ovarian cycles before they stopped cycling for unknown reasons. The average cycle length was 20 ± 6.1 days. Female white-faced capuchins show small increases in rates of proceptive behaviors directed towards adult males during the conceptive phases of their cycles. Male attractiveness and sexual behaviors directed towards females was the best indicator of the conceptive phase. Pregnant females mated at a higher rate with subordinate males, and cycling females mated at a higher rate with alpha males. This study demonstrates that female capuchins are inconspicuous in their proceptive behaviors and may use non-conceptive mating as a reproductive strategy.

Dedication

This thesis is dedicated to Czar

Acknowledgements

There are many people (and animals) to thank for helping me accomplish this huge task. First, I would like to extend a sincere thank you to Linda Fedigan for sharing with me all her wisdom and her enthusiasm for the monkeys and for being so patient with me, especially when my eyes were definitely bigger than my stomach. I would also like to thank the people who work at Santa Rosa National Park and the parks service of Costa Rica, specifically Roger Blanco-Segura, for allowing me and other researchers to continue to study the flora and fauna of that amazing forest. I would like to extend my gratitude to Toni Ziegler, Dan Wittwer and Steve Jacoris at the National Research Primate Center in Madison, Wisconsin for the opportunity to learn hormone analysis techniques and for not laughing every time I ruined a perfectly good assay with my not so perfect pipetting techniques. Thank you to Nancy Lovell, Pam Asquith and Norm Stacey for being part of my examining committee and providing helpful advice on the manuscript.

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Chapter One

General Introduction

The main objective of this project was to describe the ovarian cycle of the wild female white-faced capuchin monkey (*Cebus capucinus*) through fecal hormone analysis and document which behavioral patterns are more likely to occur in the periovulatory or conceptive phase of the ovarian cycle. Female *Cebus capucinus* do not display any physical cues advertising their conceptive periods, so field researchers do not know when females are ovulating, or even when they are cycling. It has been proposed for many primate species that "concealed ovulation" may be used by females as a mating strategy to confuse paternity and prevent infanticide (Manson *et al.*, 1997; cf. van Schaik *et al.*, 1999). Infanticide is known to occur in this population of white-faced capuchins but there is little information on the ovarian cycle and reproductive behavior of wild female capuchins (Fedigan, 2003). Moreover, there are only limited data on reproduction and behavior from captive studies on *Cebus apella* (brown or tufted capuchin) that can be used as a comparison (see Nagle & Denari, 1982). As with *Cebus apella*, it is thought that *C. capucinus* do not have an exclusive mating season because they may mate and give birth throughout the year (Janson, 1984; Fedigan & Rose, 1995; Lynch *et al.*, 2002). However, both species do exhibit a seasonal birth peak suggesting that conceptions, and therefore cycling and mating, are more common in some parts of the year than others (Freese & Oppenheimer, 1981; Manson *et al.*, 1997; Lynch *et al.*, 2002; Fedigan, 2003;).

A second part of this project developed after I completed my field work and was able to verify the reproductive status of my subject females. I found that my ten subject females were experiencing different reproductive states; they were non-cycling, cycling or pregnant. Therefore I compared behaviors across non-cycling, cycling and pregnant females and examined which behaviors might indicate the reproductive state of a female in the absence of hormonal data.

There are many unanswered questions concerning the reproductive patterns of female *Cebus capucinus* in the wild, some of which I address through my research. In addition to the documentation of ovarian cycle patterns and related behavior in this species, I also hope to contribute data that may support or negate the evolutionary theories of concealed ovulation, non-conceptive mating and mate choice strategies in this species and other non-human primates.

Fecal Steroid Hormones

Hormones are chemical compounds that are produced in specific tissues (i.e. endocrine glands), transported through blood and targeted to certain tissues to cause a specific effect (Dixson, 1998; Snowdon & Ziegler, 2000). Technically, any body fluid can contain hormones but not all hormones are found in all body fluids (Snowdon & Ziegler, 2000). Depending on the question being asked, researchers must consider the ease of collection for both the researcher and the subject when deciding which body fluid to analyze (*i.e.* frequency of sampling and degree of invasiveness; Snowdon & Ziegler, 2000). Blood, saliva, urine and feces have all been used to analyze for different types of hormones, each with its own advantages and disadvantages. The main advantage to using blood is that most hormones are readily released into the circulatory system; therefore, rapid changes in circulating hormones in response to stimuli can be immediately observed. The disadvantage is that an animal must be restrained each time blood is collected, and venipuncture and needles may cause undue stress to the individual (Snowdon & Ziegler, 2000).

In many primate studies, feces have been used to measure hormone levels. Recently developed collection and extraction techniques, designed to be used in the field, have now allowed researchers to study steroid excretion patterns in wild primates using non-invasive methods (*i.e.* no trapping or interfering with daily activities) (*Cebus apella*: Carosi *et al.*, 1999, Lynch *et al.*, 2002; *Saguinus oedipus, Callithrix jacchus*: Ziegler *et al.*, 1996; *Brachyteles arachnoids*: Strier & Ziegler, 1997; *Pithecia pithecia*: Shideler, 1994; *Macaca nemestrina*: Risler *et al.*, 1987; *Presbytis entellus*: Ziegler *et al.*, 2000; *Papio cynocephalus cynocephalus*: Wasser *et al.*, 1991; *Pan paniscus*: Heistermann *et al.*, 1996; *Propithecus verreauxi*: Brockman *et al.*, 1995). However, one of the disadvantages of using either feces or urine is that most steroids are metabolized into different hormone conjugates before being excreted, which can lead to a misrepresentation of actual circulating levels and therefore the study of fecal or urinary hormones must accommodate for this in the laboratory analysis (Ziegler *et al.*, 1997; Snowdon & Ziegler, 2000).

Metabolism of Steroid Hormones

Steroids that are released into the blood are found in an unmodified form and therefore blood samples result in more accurate measures of the hormone being studied (Snowdon & Ziegler, 2000). Hormones found in feces are filtered through the liver where most are metabolized and become conjugated and partly deactivated (Ziegler *et al.*, 1997; Snowdon & Ziegler, 2000). Conjugation of steroids is the process whereby either glucuronides or sulfates are attached to the steroid (Ziegler *et al.*, 1997; Snowdon & Ziegler, 2000). The new metabolite (either a monoconjugate or complex conjugate) becomes water-soluble and can be excreted into the bile and then into the intestine (Ziegler *et al.*, 1997; Snowdon & Ziegler, 2000). In humans and apes, microbial action within the intestine can break down some of the metabolites but in many other primate species (*i.e. Saguinus oedipus* and *Callithrix jacchus*), the conjugates must be broken down in the lab before accurate tests can be run (Ziegler *et al.*, 1997; Snowdon & Ziegler, 2000).

The process of conjugation of steroid hormones and their rate of excretion into the intestine is species-specific so knowledge of each species' patterns must be known before analysis is performed (Aldercreutz & Martin, 1976). Aldercreutz & Martin (1976) found that in humans 98% of fecal steroids are unconjugated whereas in some New World primates, 80% of estradiol is excreted as complex conjugates (*i.e. Saguinus oedipus* and *Callithrix jacchus,* Ziegler *et al.*, 1997).

Ovarian Hormones and the Female Reproductive Cycle

The ovarian cycle includes three distinct phases, the follicular phase, the periovulatory phase and luteal phase. During the follicular phase, an ovum (egg) develops to maturity within the ovary and during the periovulatory phase, it is released into the uterus to be fertilized. In the event that an egg is not fertilized the egg and uterine lining are shed (luteal phase and menstruation) and the cycle of egg development starts again.

The steroid hormones that are related to each phase of the ovarian cycle have been well studied for many species of animals but particularly for human and non-human primates (see Robinson & Goy, 1986; Dixson, 1998; Snowdon & Ziegler, 2000). The hypothalamus initiates the reproductive cycle in the female primate. A hormone called *gonadotropin-releasing factor* (GnRH) is released from the hypothalamus and targeted at the anterior pituitary gland. The anterior pituitary produces *follicle stimulating hormone* (FSH) which travels through the blood to the ovary and stimulates the development of a follicle. During the follicular phase of the cycle, the developing follicle, which houses a maturing ovum, produces estrogen that is released back into the blood. The periovulatory phase occurs when the levels of estrogen in the blood reach a threshold level and the anterior pituitary sends out a surge of *lutenizing hormone* (LH) targeted at the ovary. This hormone signals the follicle to burst and release the mature ovum into the fallopian tube (Dixson, 1998; Snowdon & Ziegler, 2000).

During the luteal phase, the follicle develops into the *corpus luteum* (yellow body), which takes over the production of estrogen and produces another steroid hormone, progesterone. In the case of fertilization of the ovum, estrogen and progesterone are both maintained at high levels for the duration of the gestational period. Conversely, menstruation occurs if the released egg is not fertilized. The corpus luteum degrades, progesterone production ceases and the uterine lining is shed. This triggers the pituitary to produce FSH and a new cycle begins (Robinson & Goy, 1986; Snowdon & Ziegler, 2000).

The reproductive physiology of the genus *Cebus* has been examined primarily in captive *Cebus apella* (brown capuchin). Nagle *et al.* (1979) and Nagle & Denari (1982) found the average cycle length for *C. apella* to be 21 ± 1.1 days and 20.8 days respectively (plasma samples) with a peak in estrogen (indicative of the start of the periovulatory period) occurring after 7 to 9 days from the start of the cycle. Carosi and Visalberghi (2002) found similar patterns with an average cycle length of 20.6 ± 1.6 days but with an overall range of 17-24 days (urine samples). The latter defined the periovulatory phase of *C. apella* to last about five days; three days before the significant rise in progesterone, the day of presumed ovulation and one day after to account for error and variability.

Cycle lengths among other New World primates appear to differ greatly (see Robinson and Goy, 1986, for details on cycle lengths listed below unless otherwise referenced). Small bodied squirrel monkeys (*Saimiri sciureus*) are reported to have cycle lengths between 7 to 12 days and common marmosets (*Callithrix jacchus*) have been found to have cycle lengths that range from 17 to 28 days. In other medium-bodied species (e.g. other than capuchins), female saki monkeys (*Pithecia pithecia*: Shideler *et al*., 1994) and owl monkeys (*Aotus trivirgatus*) both have average cycle lengths of 15 to 16 days. Among the larger bodied species, spider monkeys (*Ateles geoffroyi*) have cycle lengths of around 24 days (Hernandez *et al*., 1998) and mantled howling monkeys (*Alouatta palliata*: Dixson, 1998) of 15 to 16 days. In Old World monkeys, the most commonly studied rhesus macaque (*Macaca mulatta*) typically experiences cycles that range roughly from 28 to 30 days (Dixson, 1998).

The genus *Cebus* and most other neotropical primates (*i.e. Alouatta, Ateles, Saimiri*) differ from the Old World monkeys and apes in other aspects of their reproductive biology. Some primates' ovaries produce excess follicles that do not mature and are subsequently converted to accessory corpora lutea and then into interstitial glandular tissue that stays within the ovary. In New World primates, excess follicles are produced in a much larger quantity compared to Old World primates (Dixson, 1998). This difference is thought to explain why neotropical primates tend to have large ovaries compared to their body weights and produce very high levels of circulating progesterone (Dixson, 1998). Additionally, very few New World primates visibly menstruate, with the exception of *Cebus apella*; and blood loss is considerably lower compared to the Old World primates, apes and humans (Nagle *et al*., 1979).

Sexual Behavior and Ovarian Hormones

Traditionally the term "estrus" has been used to behaviorally describe the period of time when a female shows strong sexual desire and only at this time she will allow the male to mate with her (Dixson, 1998). For prosimians (e.g., lemurs, lorises and galagos), this is an applicable term as the females of these species display obvious sexually oriented behaviors and are sexually receptive to males for only short periods of time during their reproductive cycle. Additionally, prosimians are not known to mate outside of this distinct 'estrus' period. Presently, however, it is argued that the use of the term estrus to describe sexual behavior and receptivity in anthropoids is not suitable since most anthropoid females are often sexually receptive throughout their entire reproductive cycle. For instance, females will accept male solicitations and copulate when they are cycling, non-cycling and pregnant (Dixson, 1998). Therefore, alternate terms for describing sexual behavior in anthropoid species are used that do not refer to a restricted sexual phase but to behaviors that are directly or indirectly related to sexual activity and can occur at any time during the reproductive cycle.

Beach (1976) described three aspects of sexual behavior common to all female mammals that are now commonly used to describe sexual behaviors in anthropoid primates. These behaviors are considered peri-copulatory, or behaviors that occur around the time of copulation and often they are species specific (see Dixson, 1998). *Attractivity* is the degree to which the female acts as a sexual stimulus to males. This can be measured by the frequency of male approaches and male-maintained proximity to the female and may be regulated by behavioral and non-behavioral cues like odours or visual stimulation. *Proceptivity* occurs when the female actively solicits the male by means of affiliative behaviors such as vocalizations, body postures and maintenance of proximity. *Receptivity* is when the female accepts the male's advances and allows copulation to occur. Correlations have been found between fluctuating levels of estrogen and progesterone and these aspects of sexual behavior in primates (Beach, 1976; Baum, 1983; also see Dixson, 1998)

The combination of estrogens and progesterones are known to affect the sexual behavior of male and female primates but the exact influence of each hormone on attractivity, proceptivity and receptivity is still under investigation (Beach, 1976; Baum, 1983; Dixson, 1998). It has been suggested that high levels of estrogen increase the attractiveness and proceptive behaviors of the female and that increasing levels of progesterone significantly reduce both female proceptivity and attractiveness (measured by a decrease in male mounting; Beach, 1976; Baum, 1983). Changes in sexual behavior in relation to ovarian hormones are well documented in Old World monkey studies but data are largely lacking for the neotropical primates (Old World monkeys: *Macaca mulatta*: Johnson & Phoenix, 1976; *Macaca fuscata*: Enomoto *et al.*, 1979; *Erythrocebus patas*: Loy, 1981; *Cercocebus torquatus atys*: Gorden *et al.*, 1991; *Papio ursinus*: Saayman, 1970; New World monkeys: *Cebus apella*: Phillips *et al.*, 1994; Linn *et al.*, 1995; Carosi *et al.*, 1999; Carosi & Visalberghi, 2002; *Brachyteles arachnoids*: Strier & Ziegler, 1997).

If estrogens and progesterones influence sexual and social behaviors as has been suggested, and if these hormones fluctuate in a cyclical pattern in a normal adult female, it can be assumed that proceptivity and attractivity behavioral indicators will occur in a predictable cycle and should be easy to recognize. For many species of primates this is in fact the case. Many Old World monkeys and some great apes show conspicuous sexual swellings and characteristic behaviors during their conceptive or ovulatory phases (e.g. perineal swelling in the bonobo: Heistermann *et al.*, 1996; also see review in Hrdy & Whitten, 1987). However, in many New World monkeys, including the *Cebids*, periovulatory patterns are not conspicuous. Capuchins lack any external morphological change in relation to their ovarian cycle, appear to be very inconspicuous with their behavioral cues and seldom use olfactory signals (Manson *et al.*, 1997; Carosi *et al.*, 1999; Carosi & Visalberghi, 2002).

Study Species

***Cebus capucinus*, white-faced capuchin monkey**

The genus *Cebus* traditionally consists of four species: *C. apella* (brown or tufted capuchin), *C. albifrons* (white-fronted capuchin), *C. olivaceus* (wedge-capped capuchin) and *C. capucinus* (white-faced capuchin). Four other species are recognized by Groves (2001) but they are currently debated by others. *Cebus capucinus* is the only species that is found in both Central and South America, occupying a range that extends from south eastern Honduras into northern and western Columbia and Ecuador (Groves, 2001).

White-faced capuchins live in multimale/multifemale social groups that consist of related females, immigrant males and immature offspring. There is a moderate degree of sexual dimorphism in that adult males are 25-35% larger than adult females (the average male weighs ~ 3.25 kg) (Fedigan, 1990). There is also a discernable linear hierarchy within a group of capuchins and within the sexes (Perry, 1997). One male is usually dominant over the others but reportedly not to the point of exclusive access to females, as is commonly seen in *C. apella* or *C. olivaceus* (Janson, 1984; Robinson, 1988; Fragazy *et al.*, 2004). Females also exhibit a linear dominance hierarchy (Fedigan, 1993; Perry, 1996).

White-faced capuchins are omnivorous, opportunistic foragers (Rose, 1994). They eat fruit, buds and flowers as well as a vast variety of invertebrates and various small vertebrates (Rose, 1994). Their diet is comprised of 20% animal foods, 65% fruit and 15% green plant material (Fedigan, 1990; Rose, 1994). They utilize all layers of the canopy and often come to the ground to forage for insects or to play with one another.

Study Site

The study took place in Santa Rosa National Park (SRNP), the original sector of the Area de Conservación Guanacaste (ACG). This park is located in northwestern Costa Rica about 40 km south of the Nicaraguan border and 35 km north of Liberia, the capitol of Guanacaste Province. The boundaries of SRNP encompass 108 km^2 of dry deciduous forest fragments of semi-evergreen riparian and regenerating pasture land. The park reaches an elevation of 300 m along the central volcano range and extends west where it is bordered by the Pacific Ocean (Fedigan & Jack, 2001).

The forest type is classified as a 'tropical dry forest'. There is a distinct dry season (mid-December to mid-May) and a distinct wet season (mid-May to mid-December; Janzen, 1983). The area receives 800-2600 mm of rainfall annually (mean = 1473 mm) and temperatures range from 21.6° (September) to 34.4° (April) (Fedigan & Jack, 2001).

An extensive variety of flora and fauna occur within the park. In addition to the thousands of species of plants, the park is also home to about 115 species of mammals, 253 species of birds, 100 species of amphibians and reptiles and over 10,000 species of insects (Janzen, 1983; Boza, 1998). There are three species of non-human primates that live sympatrically within the park: the mantled howler monkey (*Alouatta palliata*); the black-handed spider monkey (*Ateles geoffroyi*); and the white-faced capuchin (*Cebus capucinus*).

Biennial censuses of the capuchins in SNRP document that the capuchin population has grown substantially since official protection of the forests and animals in the park began in 1971. From 1972 to 1999 the population grew at a rate of 3.7% per year, from 300 to 585 individuals (Fedigan & Jack, 2001). From the most recent census data taken in May, 2003 (Fedigan & Carnegie, unpub), we estimate the current number of capuchin groups in the park as 47, with an average group size of 14 and a total population of 668 capuchins in the Santa Rosa sector. In SRNP, capuchins have home ranges sizes of 1-2 km^2 (Freese, 1983).

Research Questions

The following research questions are the focus of the present study:

1) What is the ovarian cycle pattern in wild female white-faced capuchins?
2) Which behaviors do female white-faced capuchins display to and receive from adult males that signal the periovulatory phase of their ovarian cycle? Are there cycle-related changes in affiliative behaviors?
3) How do sexual and affiliative behaviors differ among cycling, non-cycling and pregnant females?

In chapter two I describe the sexual and affiliative behaviors observed during the ovarian cycle in female white-faced capuchins. By comparing behaviors between the periovulatory and non-ovulatory phases I hope to determine which proceptive behaviors and which attractivity indicators are more likely to occur around the time of ovulation. In chapter three I compare the differences in proceptive and attractivity indicators between females of three different reproductive states: non-cycling, cycling and pregnant. This was done to determine if it would be feasible in future studies to reliably infer the reproductive state of females using behavioral indicators alone, in the absence of hormonal data. In chapter four I summarize my findings and further discuss proximate and ultimate explanations for the reproductive behavior that I observed in this population of white-faced capuchins.

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Chapter Two

Sexual and peri-copulatory behavior during the ovarian cycle in female white-faced capuchins (*Cebus capucinus*)

INTRODUCTION

In many primate species, conspicuous behavioral and morphological changes are indicators of a change in the reproductive state of the female (*Macaca fuscata*: Enomoto *et al.*, 1979; *Cercocebus torquatus atys*: Gordon *et al.*, 1991; *Papio ursinus*: Saayman 1970; *Cebus apella*: Linn *et al.*, 1995; Carosi & Visalberghi 2002; also see review in Hrdy & Whitten 1987). However, several primate species lack physical cues to indicate their reproductive state and are also cryptic in displaying behavioral indicators of such (*Cercopithecus aethiops*: Andelman, 1987; *Presbytis entellus*: Hrdy, 1977; *Erythrocebus patas*: Hall, 1965; *Saguinus oedipus*: Ziegler *et al.*, 1993a). This lack of female cues to reproductive state has been referred to as "concealed ovulation", because the optimum conceptive stage of the ovarian cycle (*i.e.* ovulation) is not made obvious to the male (Sillén-Tulberg & Møller, 1993; Dixson, 1998; Nunn, 2000). Concealed ovulation is argued to be a reproductive strategy that has evolved in some multi-male species to promote paternal care (Alexander & Noonan, 1979) or to lower male-male competition and hence reduce the likelihood of infanticide (Hrdy, 1979).

It is difficult for field researchers to infer ovulation phases or estimate gestation lengths in primate species that exhibit concealed ovulation and non-seasonal mating patterns (*i.e. Brachyteles arachnoids*: Strier & Ziegler, 1997; *Cercopithecus aethiops*: Andelman, 1987), but laboratory studies have provided essential information on changes in behavior across the ovarian cycle in some species by providing controlled plasma, urinary and fecal hormonal data correlated with simultaneously collected behavioral data (*Cebus apella*: Linn *et al.*, 1995; Carosi *et al.*, 1999; *Macaca fuscata*: Enomoto, 1979; *Cercocebus torquatus atys*: Gordon *et al.*, 1991; *Saguinus oedipus*: Ziegler *et al.*, 1993b; *Pan paniscus*: Heistermann *et al.*, 1996). Fortunately, improved techniques now allow field researchers to accurately analyze ovarian hormones in feces collected from wild primates and to look for a relationship between ovarian hormone patterns and behavior in species that visually conceal their ovulation. This study examines ovarian hormone data from wild *Cebus capucinus,* collected in conjunction with behavioral data, to investigate the behavioral cues that may be reliable indicators of the periovulatory phase of the cycle.

Beach (1976) described three aspects of sexual behavior common to all female mammals. *Attractivity* is the degree to which the female acts as a sexual stimulus to males, *proceptivity* occurs when the female actively solicits the male for mating, and *receptivity* occurs when the female accepts the male's advances and facilitates the occurrence of copulation. Beach (1976) and Baum (1983) suggested that fluctuating levels of estrogens and progesterones correlate with these facets of sexual behaviour: high levels of estrogens increase the attractiveness and proceptive and receptive behaviors of the female, whereas increasing levels of progesterone significantly reduce female attractiveness. If these suggestions are correct, behaviors indicative of attractivity, proceptivity and receptivity should predict fluctuation in ovarian hormones.

Female white-faced capuchins do not exhibit any external morphological signs of ovulation and are not known to display any behavioral cues to indicate they are within their periovulatory phase (Manson *et al.*, 1997). *Cebus apella* is the most studied species in the genus *Cebus*, but the mating behavior of this species is quite different than *C. capucinus*. For instance, *C. apella* females actively solicit copulation with the alpha male via such behaviors as "eyebrow raising with vocalizations", "touching and running", "nuzzling" and "head cocking" (Philips *et al.*, 1994; Linn *et al.*, 1995; Carosi *et al.*,1999; Carosi & Visalberghi, 2002). These behaviors are observed during the peak of the periovulatory period (within the first few days of proceptivity) and directed only towards the alpha male. According to Janson (1984), females will also mate with subordinate males, but only near the end of their proceptive phase, when the presumed optimum conceptive period has passed. In white-faced monkeys (*C. capucinus*), males are almost exclusively responsible for initiating the courtship dance towards females who may or may not respond with a similar display of behavior (Manson *et al.*, 1997; per obs.). Furthermore, females of *C. capucinus* mate with all males in the group, although it has not been determined whether they restrict their copulations to the alpha male during their most fertile days (Oppenheimer, 1968; Fedigan, 1993; Manson *et al.*, 1997).

Perry (1996, 1997) described the *C. capucinus* courtship/dance display as follows. The male approaches the female making quiet whistling sounds and pursing his lips to produce a "duckface". If the female is interested, she starts producing a call and a facial expression similar to those of the male, while closely watching him. Then the pair will simultaneously approach and retreat from each other, the male sometimes turning his back and looking at the female upside down through his legs. Usually, the female stays in one place, turns her back and presents for mating. The male then approaches closely, grasps the female and copulation occurs. The mount may last for only a few seconds or up to a few minutes. Occasionally, the two may perform the "dance" again, followed by mounting, although not every dance display results in mounting (also see Freese & Oppenheimer, 1981).

It is the persistent proceptive behaviors exhibited by *C. apella* females that are thought to stimulate males to mate; however, the case appears to be different for *C. capucinus* (Janson, 1984; Linn *et al.*, 1995; Manson *et al.*, 1997; Carosi *et al.*, 1999). In white-faced capuchins, the females rarely initiate the courtship dance display and the males are the primary initiators of sexual solicitations. Still, there may be subtle behaviors that females use to signal their readiness to males, behaviors that are not directly and noticeably part of copulatory patterns. Following Dixson (1998) I refer to actions that serve to bring the sexes into proximity and maintain them in close physical contact as "peri-copulatory behaviors". I have divided these peri-copulatory behaviors into proceptive and attractive indicators such that behaviors directed *to* the cycling female from an adult male are considered attractive, and behaviors directed *by* the cycling female, toward a male, are considered proceptive (Beach, 1976).

In *Cebus apella* (Linn *et al.*, 1995; Di Bitetti, 1997) and *Macaca fuscata* (Enomoto *et al.*, 1979), grooming tends to increase between males and females during the periovulatory period. White-faced capuchins are very social during rest periods and grooming is a common social activity; therefore, grooming may be one behavioral variable that is used to indicate either attractivity, proceptivity or both. Other behaviors that I investigate as possible peri-copulatory behaviors include follows, approaches and maintenance of spatial proximity (e.g. during feeding or resting). I predict that females will increase the rate of proceptive behaviors (*directed* grooming, approaches, follows) towards males during the periovulatory phase compared to the non-ovulatory phase in an effort to advertise their conceptive state. In parallel, I predict that during the periovulatory phase, adult males will increase their frequency of behaviors indicating attraction to the females – grooming, approaching and following females. I also predict that males and females will stay closer to each other during the periovulatory phase versus the non-ovulatory phase in order to facilitate copulation (see Hausfater & Skoblick, 1985).

Manson *et al.* (1997) suggested that mating in capuchins may serve functions other than procreation since white-faced capuchins perform courtship/dance displays and mate throughout the year during different reproductive states (e.g. pregnancy). In part, because of the non-seasonal mating behavior of white-faced capuchins it is difficult to determine if mating actually indicates that the female is conceptive (*i.e.* within her periovulatory phase). However, Carosi and Visalberghi (2002) found that in *Cebus apella* male sexual mounting of females occurs at a significantly higher rate during the periovulatory phase compared to the non-ovulatory phase (also see Linn *et al.*, 1995). Additionally, sexual behavior in many species of Old and New World primates increases during their conceptive phases. Therefore, I predict that during their periovulatory periods, the rate of copulations and courtship/dance displays will increase and females' will be more receptive to males at this time compared to their non-ovulatory periods.

Other social behaviors that do not necessarily qualify as proceptive or attractive may also vary with respect to cycle phase. For example, some neotropical primates including all species of capuchins display a behavior called "urine washing". This behavior consists of rubbing urine into the hands and feet (Robinson, 1979; Milton, 1985; Boinski, 1992; Perret, 1992). The most commonly proposed hypotheses for the function of urine washing include: thermoregulation (Robinson, 1979; Roeder & Anderson, 1991), communication of reproductive status (Boinski, 1992; Perret, 1992) and territoriality (but mainly in prosimians; Schilling, 1979). In white-faced capuchins, all age/sex classes exhibit this behavior, which suggests its function is common to all individuals (e.g. thermoregulation). However, male white-faced capuchins are occasionally seen smelling and licking the branch where a female had just performed a urine wash (per obs.). Since urine washing may function to communicate reproductive status, I predict the rate of urine washing will be higher during the periovulatory period.

This study compares the occurrence of sexual and peri-copulatory behaviors between the periovulatory and the non-ovulatory phases of female white-faced capuchin ovarian cycles. The purpose of the study is to determine if female white-faced capuchins exhibit behavioral cues that signal their conceptive period to adult males. This study is the first to compare behavioral data with hormonal cycles in *Cebus capucinus*.

Research Questions and Predictions:

1) **Does the occurrence of sexual behaviors vary between the periovulatory and non-ovulatory phases in white-faced capuchins?**

I predict that sexual behaviors (dance displays and copulations) will increase in the periovulatory period.

2) **Do attractivity and proceptivity indicators vary depending on ovarian cycle phase?**

I predict that behavioral indicators of proceptivity and attractivity such as approaches, grooms, and follows will increase in the periovulatory period. I also predict that individuals will maintain a closer proximity to each other (within three meters) during the conceptive versus the non-conceptive phases.

3) **Does the rate of urine washing change depending on ovarian cycle phase?**

I predict that the rate of urine washing by cycling females will increase during the periovulatory phases to advertise the conceptive phase to males.

METHODS

Study Site

My study took place in Santa Rosa National Park (SNRP); the original sector of the Area de Conservacion Guanacaste (ACG). This park is located in northwestern Costa Rica close to the Nicaraguan border and encompasses approximately 108 ha of dry deciduous forest fragments, semi-evergreen, riparian and regenerating pasture land. Santa Rosa reaches elevations of 300 m along the central volcano range and slopes downward to the west where it is bordered by the Pacific Ocean (Fedigan & Jack, 2001).

Study Subjects

The subjects for this study were 10 wild adult female white-faced capuchins who were members of two habituated groups; Cerco de Piedra (CP) and Los Valles (LV) (five per group). Females ranged in age from six to ~24 years and varied in rank within each group. Eight females were multiparous, and two were nulliparous at the start of the study. These female white-faced capuchins begin cycling by 5.5 years of age, since six years is the earliest recorded age at first birth in this population (unpublished data). Due to small group sizes, all of the adult females in each group were included in my sample. Additionally, there were six adult males: two alpha males and four subordinate males (CP: one alpha male, one subordinate male; LV: one alpha male, three subordinate males). Individual animals were identified by natural markings such as broken digits, scars, fur coloring, blotches on faces and the brow and peak shape. Both groups live in similar habitat types and are exposed to similar environmental pressures including water scarcity, predator pressures, food availability and level of human exposure. This population of capuchins has been part of LM Fedigan's research since 1983. Data included in the long term research that are applicable to this study are births, deaths, immigrations and emigrations. Capuchins are female bonded (Fragaszy *et al.*, 2004), so kinship relations among females are known for the last 20 years. Table 2.1 lists female ages, rank and reproductive states in 2002. Chapter three explains how reproductive states were interpreted and Appendix I lists CP and LV group composition data for 2002.

Table 2.1: Group, age, rank and reproductive state and history for each female in 2002

Female	Group	Age	Rank	Reproductive	Parity State	Age of last infant[1]
Limp	CP	24	alpha	cycling	multiparous	12
Kathy Lee	LV	13	alpha	cycling	multiparous	12
Blanquita	LV	20	subordinate	cycling	multiparous	9
Timone	CP	6	subordinate	cycling	nulliparous	0
Nyla	CP	8	high rank	non-cycle	multiparous	12
Seria	CP	13	subordinate	non-cycle	multiparous	7
Fiesty	LV	20	subordinate	non-cycle	multiparous	9
Pumba	CP	8	subordinate	pregnant	multiparous	24
Dos Leches	LV	11	high rank	pregnant	multiparous	12[3]
Salsa	LV	6	subordinate	pregnant	nulliparous[2]	0

[1] Age of infant in months from parturition to January 2002

[2] This female gave birth to her first infant during the study (May 2002)

[3] This female lost her last infant (disappeared) less than one month after it was born resulting in a shorter interbirth interval

Behavioral Data Collection

Between January and June, 2002, I collected a total of 443 hours of focal data (ranging from 39 to 47 hours per female) between 6 AM and 6 PM. I collected behavioral data using continuous focal animal sampling (Altmann, 1974). Focal sessions lasted for 15 minutes each, during which time all behaviors and interactions were recorded. I attempted to sample different individuals sequentially throughout the day so that each female was equally represented. However, if I could not find the target female within 15 minutes, I would move on to the next on the list. I also allowed for a 90 second "out-of-sight" period which refers to the time when the monkey is not in view but its approximate location is known and it is unlikely that confusion with another individual will occur (no more than 10% of total recording time). If this criterion was violated then that session was discarded.

I recorded behavioral data using a hand-held computer (PSION Workabout MX) and entered these data into a software program entitled "Behavior," that was designed by Syscan International Inc. (Montreal, Quebec) for the University of Alberta. This program

I recorded behavioral data using a hand-held computer (PSION Workabout MX) and entered these data into a software program entitled "Behavior," that was designed by Syscan International Inc. (Montreal, Quebec) for the University of Alberta. This program records the time each behavior code is entered so that durations of behavior can be accurately and easily calculated. I used an exhaustive ethogram developed over the years by previous *C. capucinus* researchers to identify and code behaviors (see Appendix II). I attempted to enter all behavior codes into the PSION as they happened but when fast moving events occurred, such as fights or sexual displays; I dictated the behavioral sequences into a micro-cassette recorder (Sony M-430). I later transcribed those sequences and integrated them into the behavioral data set for that day. To ensure inter-observer reliability of the data collected, my assistant and I spent several weeks together with one group identifying individuals and practicing data collection before I left her to work alone with one group (CP). Once per month we collected data together, on one set of females, and compared notes to remain consistent over the course of the study.

Fecal Collection and Field Extraction

I collected fecal samples from each female on each of the same days that I obtained behavioral data. A minimum of two to three samples per week per female is considered adequate to assess ovarian patterns in females (Hodges & Heistermann, 2003; Ziegler & Ward-Lynch, personal comm.) but I collected additional samples in case females could not be located in subsequent days. I collected feces within 10 minutes of defecation, placed them in plastic vials and stored them in a cold pack until the end of the day, taking note of the individual and date (Strier *et al.*, 2003). In total, my assistant and I collected over 600 samples from all 10 subject females. For initial field extraction of the steroids, I followed the techniques described in Strier and Ziegler (1997): 2.5 ml of distilled water and 2.5 ml of ethanol was added to 0.1 g of wet weight fecal matter in a 15 ml centrifuge tube. The tube was vortexed for 10 min and then centrifuged for 10 min at 2,000 x g. The resulting fecal pellet was discarded and the ethanol/water/steroid mixture was refrigerated until transported to the National Primate Research Center (NPRC) at the University of Wisconsin in Madison, Wisconsin for the laboratory analysis.

Laboratory Extraction and Analysis

Following the field season, I carried out hormone analyses at the NPRC (July and August 2002). Earlier, in February 2001, 10 fecal samples had been collected and field extracted, using the methods above, and sent to the NPRC in Wisconsin for validation of the steroid hormones. The 10 preliminary samples were pooled and used to validate the progesterone (P) and estradiol (E2) assays that were used. The accuracy with which the assay measures the steroid in each sample was evaluated by adding small volumes of the fecal pool to each point of the standard curve. Parallelism was determined through serial dilutions of the fecal pool along the standard curve. An intra- and interassay coefficient of variation was determined by running duplicate samples within and between each assay. The preliminary samples from 2001 were used for the validation tests and those samples indicated that this extraction technique is reliable for measuring these ovarian steroids in capuchin feces.

Analysis of the preliminary fecal samples also indicated that high concentrations of steroids were excreted in a conjugated form. Therefore before any assays could be performed, solvolysis was required to break the conjugated steroids into an un-conjugated form that would ensure an accurate estimation of the hormone levels. I combined 100 μL of saturated NaCl, 50 μl of 2.5 M H_2SO_4, and 4 ml of ethyl acetate with 1 ml of the water/ethanol/steroid sample in a glass extraction tube. The tubes were vortexed for 5 min and incubated in a water bath overnight (40° C). The following day, the tubes were vortexed and then centrifuged and the top ethyl acetate/steroid layer was aspirated off and transferred to glass culture tubes. The culture tubes were placed in a water bath which evaporated the ethyl acetate and left a discoloured residue. Finally, 1 ml of ethyl alcohol was added to each tube, vortexed and refrigerated until ready to assay. This solvolysis procedure is described in Ziegler *et al.* (1996). Once the samples had undergone solvolysis, I measured progesterone using enzyme immunoassays (see Ziegler *et al.*, 1996) and estradiol using radio-immunoassays (see Strier & Ziegler, 1994). In total, I analyzed about 400 samples out of the 600 that I had originally collected. Once the reproductive state of the pregnant and non-cycling females was established, not all of their samples required analyzing.

I also performed recovery tests for each steroid after solvolysis to determine the percentage of steroids that were recovered during the extraction process. Tritium (^{3}H) labelled progesterone and estradiol were added to capuchin fecal material in vials and placed in a liquid scintillation counter. Recoveries in the extracted samples were compared to initial levels by comparing the measurement of the radio-labelled known sample to the measurement of the radio labelled unknown samples (Wittwer, personal comm.).

Interpretation of Hormonal Data

Nagle & Denari (1979) found that, using *Cebus apella* urine samples, ovulation occurs at approximately the same time as excreted urinary progesterone. Carosi *et al.* (1999) further found in *C. apella* that there was a 0 to 1 day lag time in the excretion rate between urinary and fecal measurements of progesterone (P) and estradiol (E2). Therefore, because of the minimal lag time in excreted progesterone through feces compared to urine, ovulation is assumed to occur on the first day of the rise in fecal P in capuchins. Following Linn *et al.* (1995) and Carosi *et al.* (1999), I conservatively defined the periovulatory phase as being five days long; three days before the rise in P, the day of the rise, and one day after (to account for any error and variability). I calculated complete cycle lengths as the intervals between successive progesterone surges (after Strier *et al.*, 2003).

I used the results of the fecal assays of the 400 analyzed samples to create a hormone profile for each female, and from the profile I was able to infer the female's reproductive state. Out of the 10 subject females, only four were cycling. New World monkeys typically have very short follicular phases and luteal phases that show simultaneously elevated levels of estrogens and progestins over a 10-15 day period (Ziegler *et al.*, 1987; Ziegler *et al.*, 1993b; Dixson, 1998). However, it was very difficult to interpret the individual phases of the ovarian cycle (follicular, periovulatory and luteal) from the hormone profiles for the cycling females. The profiles for my subjects were often ambiguous and, therefore, on the advice of Toni Ziegler (NPRC), I chose to compare behavioral differences between the periovulatory and the non-ovulatory phases.

This procedure is also consistent with a study on *C. apella* by Carosi & Visalberghi (2002). The profiles of the 10 females were divided into periovulatory and non-ovulatory phases according to the criteria described above. All four cycling females ceased to cycle approximately mid-way through the study and three did not start cycling until one to two months into the study. Consequently, I used only behavioral data collected while they were cycling (periovulatory: 16.75 hrs; non-ovulatory: 38.5 hrs; total = 55.25 hrs).

Behavioral Analysis

The behavioral variables analyzed for phase variation were: copulations and courtship/dance displays and the hypothesized behavioral indicators of attractivity, proceptivity and receptivity. Behaviors that I considered as indicating proceptivity were approaches, follows and grooms *directed* by the subject female to adult males. I measured solicitations, frequency and duration of grooming. I considered the same behaviors as indicators of attractive behavior when they were *received* from adult males. I calculated the percentage of time males and females spent within three meters of each other as a measure of spatial proximity and compared these between phases. Additionally, I calculated a measure of which sex maintained proximity and compared these across phases. I also analyzed the rate of urine washing as a possible indicator of female reproductive status. Considering that *C. apella* females show preferential treatment to alpha males during their conceptive periods, I also compared the rates of pericopulatory behaviors between alphas versus subordinate males across ovarian phases to see if similar behavior exists in *C. capucinus*. Table 2.2 lists the behavioral variables analyzed and their definitions.

I compared each of the behavioral frequencies across cycle phases by calculating a mean rate (frequency per hour) for each of the subject females for each phase. I determined mean grooming duration (directed and received) by tallying the total amount of time females were involved in grooming bouts and calculating the proportion of that time spent grooming (or being groomed by) adult males. I measured receptivity as a percentage of male dance displays to which females responded positively by presenting for mounting and facilitating copulations.

Table 2.2: Description of Behavioral Variables

Behavior	Description
Sexual	
Copulation	Male monkey mounts a female ventro-dorsally with intense pelvic thrusts; both monkeys display a "duckface" and vocalize, penile insertion is assumed.
Dance Display	One monkey directs courtship behaviors toward another monkey – usually precedes copulations.
Peri-copulatory*	
Approach	One monkey advances toward another monkey to within three meters.
Follow	One monkey travels directly behind another monkey within five meters for an indefinite amount of time.
Groom	One monkey inspects and picks through the fur of another monkey.
Groom Solicit	One monkey approaches another and lies down in front of them, presenting a body part (back, head, limb) to be inspected by the other.
Spatial Proximity	Two monkeys maintain proximity to each other, within three meters.
Other Behaviors	
Urine Wash	One monkey urinates and rubs it around on hands, feet and tail.

* These behaviors may be *directed* to a male by a female (proceptive) or a female may *receive* the behavior from a male (attractivity)

I determined spatial proximity by tallying the total amount of time a subject female was within three meters of a male and dividing this by the total time I observed her (proportion of time in close proximity). I used Hinde's Index (Hinde & Atkinson, 1970) to decide which member of the male-female dyad was responsible for maintaining proximity. Hinde's Index is the proportion of all of the dyad's *approaches* directed by the subject female, minus the proportion of all of the dyad's *leaves* directed by the female. A negative index indicates that the male was responsible for maintaining proximity; a positive index suggests the female was responsible. *Approaches* to within three meters and *leaves* beyond three meters were used in this analysis.

Data were not normally distributed; therefore, I used non-parametric statistics for the analysis. I compared the mean scores for each subject female for each behavioral variable between the periovulatory and non-ovulatory phases using Wilcoxon signed ranks test for paired data (Siegal & Castellan, 1988; Zar, 1999). First, I ran the Wilcoxon tests with scores involving all adult males (by lumping all the alphas and subordinate males together). Second, I separated the scores involving either the alphas or the subordinates and ran the tests again to see if male dominance rank was affecting male and female behavior between the ovarian phases. The level of significance was set at $P<0.05$ and SPSS 11.5 was used for this analysis.

In each study group there was one alpha female and one subordinate female that cycled. Therefore, to evaluate behaviors between phases while controlling for female dominance rank, I further compared the mean scores of the two alpha females and the mean scores of the two subordinate females separately, between ovarian phases; and also in relation to male dominance rank. My sample sizes for this part of the analysis were too small to perform statistical analysis, so descriptive results are reported.

RESULTS

Hormone Validations and Profiles

Mean steroid recoveries were found to be within acceptable limits for progesterone (P=90.7%) and estradiol (E2=64.5%). Mean percent accuracy was high for both P and E2 assays (>100% accuracy). Percent bound values obtained from the pooled samples paralleled the percent bound values of the standard curves for both hormones (*i.e.* the observed hormone measurements mirrored the expected measurements). In addition, mean intra- and interassay coefficient of variation (CV) values were within acceptable limits (below 25% variation) for both progesterone and estradiol. Table 2.3 summarizes the results of the validation procedures.

Table 2.3: Summary of results from fecal assay validations

		Progesterone	Estradiol
Recoveries		90.70%	64.50%
Accuracy		107.25 ± 2.11%	101.28 ± 2.29 %
Parallelism*		t = 0.15, df = 24, p >.05	t = -1.74, df = 42, p > .05
CV†:	Intra	6.89% n=8	4.73% n=8
	Inter	8.56% n=8	8.20% n=8

* Slopes do not differ
† Coefficient of variation

Results from the hormone profiles revealed that three of the ten females failed to cycle for the entire duration of the study - January to June, 2002 (non-cycling), three females were pregnant at the start of the study (and subsequently gave birth during the study) and four females displayed regular cycling for a period of time and then stopped cycling even though they had not become pregnant. Appendix III contains the hormone profiles for each female (also see Chapter three).

Of the four cycling females, three did not show cycling until 14, 41 and 43 days after the first collected sample (Timone, Limp and Blanquita respectively). Observations for the fourth female, Kathy Lee, appeared to start right at the beginning of her luteal phase (indicated by elevated P). All four females stopped cycling midway through the study for unknown reasons. I determined the end of cycling for each female when the P levels dropped to baseline levels but did not elevate again to the previous (ovulatory) levels (Ziegler, personal comm.). I calculated one to three regular cycles for each of the cycling females for a total of nine cycles but only five of those were considered complete. A complete cycle was one that could be measured from one progesterone surge to the next (Strier *et al.*, 2003). The average length of a complete ovarian cycle was 20 ± 6.1 days (N=5; range 14-26 days). Complete cycle lengths did not differ significantly among females (χ^2=3.8, df=3, p=0.28). Figure 2.1 depicts one representative, complete ovarian cycle for one subject female illustrating the periovulatory and the non-ovulatory phases. Table 2.4 summaries the ovarian cycle patterns for each subject female including individual cycle lengths and the lengths of their cycling and non-cycling periods during the course of the study.

Behavioral Differences between Periovulatory and Non-Ovulatory Phases

None of the Wilcoxon tests on sexual behavior and proceptivity/attractivity indicators resulted in significant differences in rates of behavior between the two ovarian phases. This was true for both the lumped and the non-lumped male ranks, probably as a result of the small sample sizes (four cycling females; two alpha males; four subordinate males). However, descriptive statistics reveal some very interesting and noteworthy trends in the data and I have reported these below and in Table 2.5.

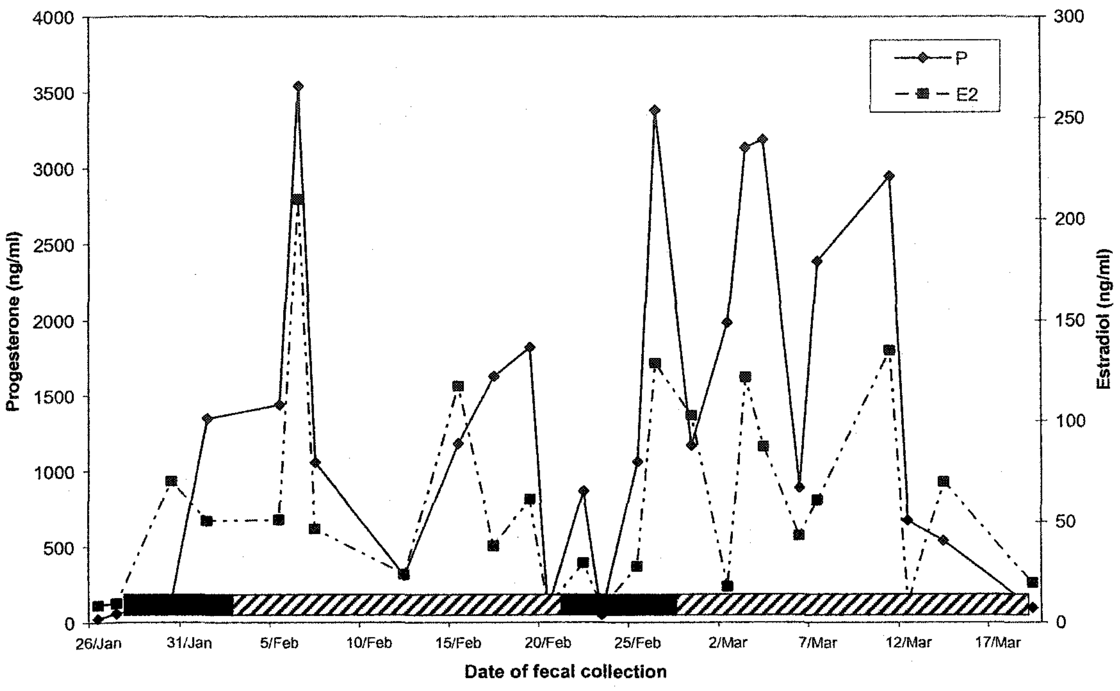

Figure 2.1: Representative hormone profile for a female white-faced capuchin (Timone). There was one complete ovarian cycle calculated from the first progesterone surge to the next (Jan 31 to Feb 25). A periovulatory phase covers a span of five days, day of P surge, one day before and two days after. The second non-ovulatory phase is illustrated to compare between the phases but this cannot be considered a "complete" cycle since P did not rise again. This female stopped cycling around March 17.

■ Periovulatory phase; ▨ Non-ovulatory phase

Table 2.4: Summary of Female Cycling Patterns: January to June, 2002

Female	Group	Date of first analyzed sample	Date of 1st P surge	Date of 2nd (3rd if applicable[1]) P surge	Length of Complete[2] Cycle 1 / Cycle 2 (days)	Length of Total Cycling Period (days)[3]	Date of Cycling Cessation	Length of non-cycling period (days)[4]	Length of time since last infant born (mths)[5]
Timone	CP	Jan 19	Feb 1	Feb 26 / -	25 / -	47	Mar 19	86	0
Limp	CP	Jan 21	Mar 2	Mar 22 / -	22 / -	41	Apr 12	62	12
Kathy Lee	LV	Jan 30	Jan 29[6]	Feb 26 / -	26 / -	48	Mar 16	73	12
Blanquita	LV	Jan 29	Mar 11	Mar 25 / Apr 6	14 / 13	38	Apr 18	40	9

[1] Only Blanquita had two complete cycles.

[2] A complete cycle was calculated from one progesterone (P) surge to the next.

[3] Length of cycling period was calculated from the first day of the P surge to the end of cycling when P levels decreased and did not rise again (see Appendix III for hormone profiles).

[4] Length of the non-cycling period was calculated from the date of cycling cessation to the date the last sample was collected

[5] This population of capuchins have a 2.5 yr interbirth interval (Fedigan, 2003); gestation lengths are approximately 5.5 mths (Nagle & Denari, 1982).

[6] I have assumed that Kathy Lee's first P surge occurred one day before the first sample was collected as she appeared to have started cycling at that time.

Table 2.5: Mean Rates/Proportions of Sexual Behaviors, Proceptivity and Attractivity Indicators exhibited by Alpha and Subordinate Females in the Periovulatory and Non-Ovulatory Phases.

		Periovulatory			Non-Ovulatory		
Behaviors (D/R; N)		Alpha♀/	Sub♀*	Totals	Alpha♀ /	Sub♀	Totals
Sexual							
Copulations (3)	*r*			***0.23***			***0.0***
Alpha males*	r	0.18	0.0		0.0	0.0	
Subordinate males	r	0.0	0.07		0.0	0.0	
Dance displays (R; 11)	*r*			***0.36***			***0.13***
Alpha males	r	0.61	0.13		0.13	0.12	
Subordinate males	r	0.50	0.27		0.09	0.0	
Receptivity	%			***50***			***0***
Alpha males	%	67	0		0	0	
Subordinate males	%	0	50		0	0	
Proceptivity							
All Indicators	r			**0.42**			**0.28**
Approaches (D; 35)	*r*			***1.03***			***0.59***
Alpha males	r	1.22	0.34		0.66	0.25	
Subordinate males	r	0.22	0.21		0.11	0.21	
Grooming bouts (D; 21)	*r*			***0.63***			***0.50***
Alpha males	r	0.75	0.27		0.59	0.14	
Subordinate males	r	0.11	0.14		0.27	0.0	
Groom duration (D)	*%*			***18***			***13***
Alpha males	%	8.5	16		19	3	
Subordinate males	%	8.7	2.2		5.2	0	
Attractivity							
All Indicators	r			**0.96**			**0.44**
Approaches (R; 69)	*r*			***1.33***			***1.31***
Alpha males	r	2.25	0.0		0.62	0.38	
Subordinate males	r	0.34	0.75		1.30	0.32	
Follows (R; 26)	*r*			***1.65***			***0.14***
Alpha males	r	2.61	0.62		0.24	0.0	
Subordinate males	r	0.0	0.07		0.05	0.0	
Groom Solicits (R; 14)	*r*			***0.44***			***0.23***
Alpha males	r	0.61	0.27		0.24	0.10	
Subordinate males	r	0.0	0.0		0.06	0.05	
Grooming bout (R; 7)	*r*			***0.44***			***0.06***
Alpha males	r	0.61	0.27		0.07	0.06	
Subordinate males	r	0.0	0.0		0.0	0.0	
Proximity	*%*			***20***			***24***
Alpha males	%	36	1.7		24	3.4	
Subordinate males	%	0.7	0.06		19	1.4	
Other Behaviors							
Urine Washing (98)	*r*			***0.68***			***2.11***
	r	0.89	0.46		2.94	1.28	

- Behaviors are expressed as mean rates [rates (r), frequency/hour] or mean percentage of time (%).
- Totals refer to all adult females directing or receiving behaviors from all adult males.
- D = female direct behavior to a male; R = female receives behavior from a male; N = sample size

* alpha males: n=2; subordinate males: n=4; alpha females: n=2; subordinate females: n=2

Sexual Behavior

Table 2.5 lists the mean rates and percentages of sexual, attractivity, proceptivity and receptivity indictors compared across the periovulatory and non-ovulatory phases. The differences related to dominance rank are illustrated in the table but I also describe notable differences below.

I only observed three copulations during the periovulatory phases of the cycle and none during the non-ovulatory phases (periovulatory: 0.23/hr; Figure 2.2); two of these involved alpha males and alpha females and the third involved a subordinate male and a subordinate female. In total, all males directed 11 dance displays toward all adult females but directed them to females at over twice the rate during periovulatory phases compared to the non-ovulatory phases (periovulatory: 0.36/hr, n=6); non-ovulatory: 0.13/hr, n=5; Figure 2.2). Additionally, during the periovulatory phases, alpha males directed three of the four dance displays they performed, towards the alpha females (one display directed to a subordinate female).

Receptivity

During the periovulatory phases, adult females responded positively to 50% of all male dance displays through presenting for mounting and otherwise facilitating copulation (three out of six displays in periovulatory phase). Moreover, alpha females were only receptive to alpha males; but not every time they were solicited by them (two out of three; 67%). Subordinate females were solicited twice by subordinate males and were receptive to only one of the displays received (a subordinate female was solicited once by an alpha male but she did not respond positively). In the non-ovulatory phases, none of the subject females responded positively to any dance display received from any adult male (five dance displays observed).

Proceptivity

In total, proceptivity indicators (all *directed* approaches, follows, grooms) occurred at only slightly higher rates during the periovulatory phase compared to the non-ovulatory phase; and almost all of these occurred at higher rates between alpha females and alpha males (overall rates: periovulatory: 0.42/hr; non-ovulatory: 0.28/hr; Figure 2.3).

Breaking this total down into specific behaviors, the rate at which adult females directed approaches to all adult males was almost two times higher during the periovulatory as compared to the non-ovulatory phases (periovulatory: 1.03/hr; non-ovulatory: 0.59/hr; Figure 2.4a). During the periovulatory phases, all females directed low rates of approaches to subordinate males, but alpha females directed approaches to alpha males at about three times the rate of subordinate females. In the non-ovulatory phases, alpha females reduced the rate of approaches they directed to alpha males by about half compared to the periovulatory phases (see Table 2.5).

Interestingly, females were never observed to either follow or direct a groom solicit to either alphas or subordinate males regardless of the phase of the cycle. However, adult females directed grooming bouts to adult males at a slightly higher rate in the periovulatory phases compared to the non-ovulatory phases (periovulatory: 0.63/hr; non-ovulatory: 0.50/hr; Figure 2.4b). In both phases of the cycle, alpha females directed grooming bouts to alpha males at higher rates as did subordinate females (three and four times respectively).

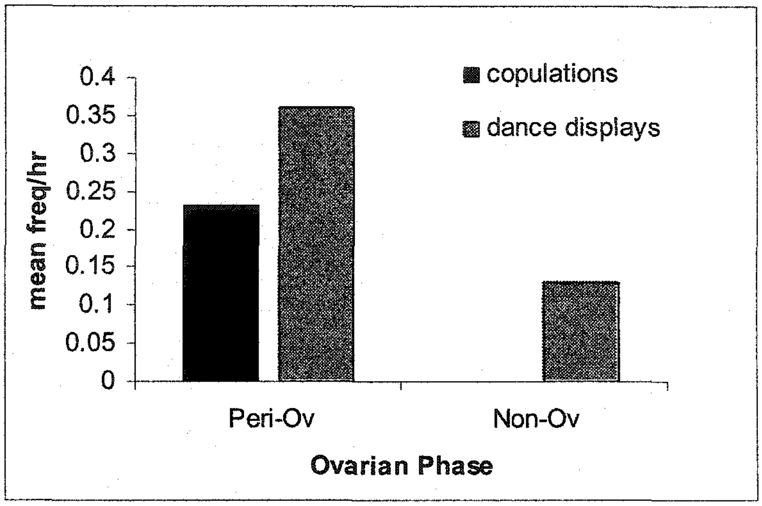

Figure 2.2: Mean rate of sexual behavior (copulations and dance displays) during the periovulatory and the non-ovulatory phases of the ovarian cycle.

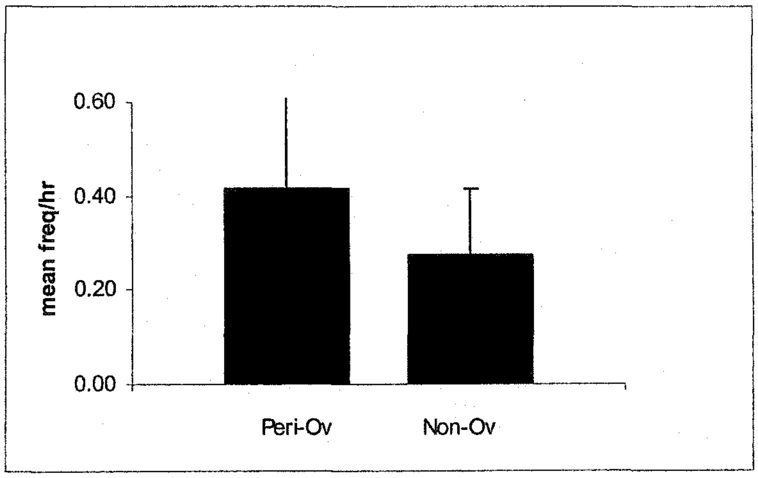

Figure 2.3: Mean rate of all proceptivity indicators (all *directed* approaches, follows and grooms) from all subject females toward all adult males.

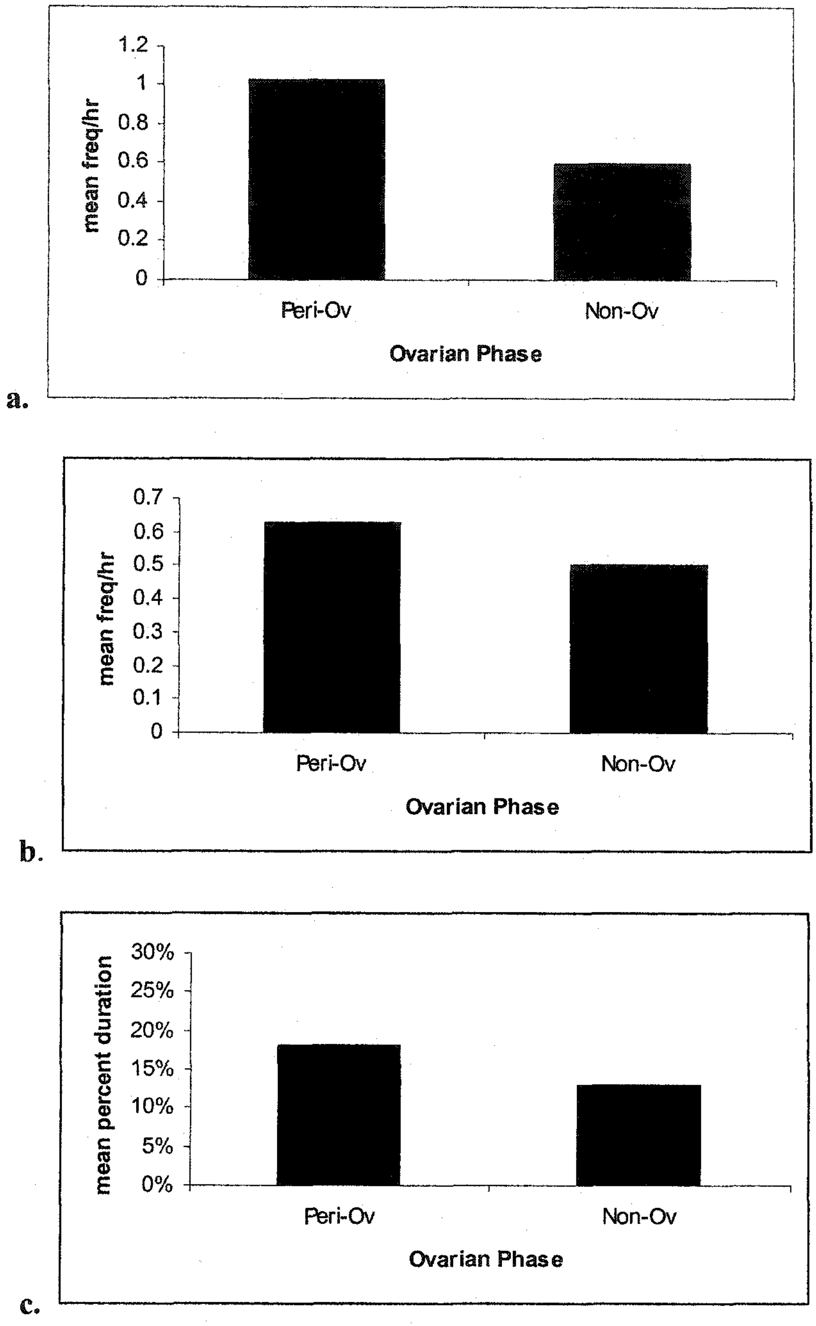

Figure 2.4: Changes in individual proceptivity indicators across ovarian phases (mean frequency/hour).

a. Mean rate of **approaches** directed by subject females to adult males.
b. Mean rate of **grooming bouts** directed by subject females to adult males.
c. Mean percentage of **time spent grooming** adult males by subject females (out of the total amount of time spent grooming).

The percentage of time adult females spent grooming adult males was only slightly greater during the periovulatory phases compared to the non-ovulatory phases (periovulatory: 18%; non-ovulatory: 13%; Figure 2.4c). During the periovulatory phases, subordinate females spent twice as much time grooming alpha males compared to the alpha females, whereas alpha females spent four times longer grooming subordinate males compared to the subordinate females. The situation partially reverses in the non-ovulatory phases, in that alpha females spent six times longer grooming alpha males (compared to the subordinate females), and were the only females observed to groom subordinate males.

In sum, proceptivity indicators (when lumped together) increase only slightly during the periovulatory phases of the cycle. In almost all cases during the conceptive phases, alpha females directed proceptive behaviors at higher rates than subordinate females and directed them more often to alpha males. The exception was the percentage of time spent grooming in the periovulatory phases, where subordinate females spent more time grooming alpha males.

Attractivity

In total, attractivity indicators (*received* approaches, follows, grooms) occurred at more than twice the rate during the periovulatory phases compared to the non-ovulatory phases (periovulatory: 0.96/hr; non-ovulatory: 0.44/hr; Figure 2.5); and all but one of the indicators (approaches) occurred at higher rates among alpha males and alpha females in both phases of the cycle.

The rate at which adult females received approaches (within three meters) from adult males was comparable in both phases of the cycle (periovulatory: 1.33/hr; non-ovulatory: 1.31/hr; Figure 2.6a). During the periovulatory phases, alpha males directed approaches <u>only</u> to alpha females, while subordinate males directed approaches to subordinate females at twice the rate compared to alpha females (see Table 2.5). In the non-ovulatory phases, alphas and subordinate males directed approaches to alpha females at two and eight times the rate respectively that they approached subordinate females.

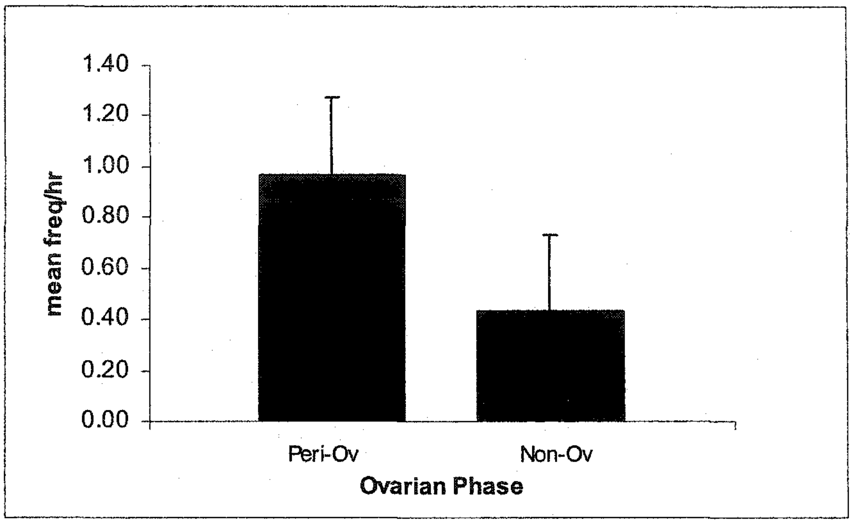

Figure 2.5: Mean rate of all attractivity indicators (all *received* approaches, follows and grooms) between the periovulatory and non-ovulatory phases between subject females and all adult males.

Adult females received follows from adult males at over 11 times the rate during the periovulatory phases compared to the non-ovulatory phases (periovulatory: 1.65/hr; non-ovulatory; 0.14/hr; Figure 2.6b). In the periovulatory phases, alpha males directed follows to alpha females at over four times the rate that they followed subordinate females. Subordinate males directed follows only to subordinate females but at low rates (see Table 2.5). In the non-ovulatory phase, only alpha females received follows from males (mostly the alpha males).

Adult females received groom solicits from adult males at two times the rate during the periovulatory phases compared to the non-ovulatory phases (periovulatory: 0.44/hr; non-ovulatory: 0.23/hr; Figure 2.6c). During both phases of the cycle, alpha males directed groom solicits at over twice the rate to alpha females compared to subordinate females (see Table 2.5). Subordinate males only directed groom solicits to females in the non-ovulatory phases, but directed them at low rates to all females.

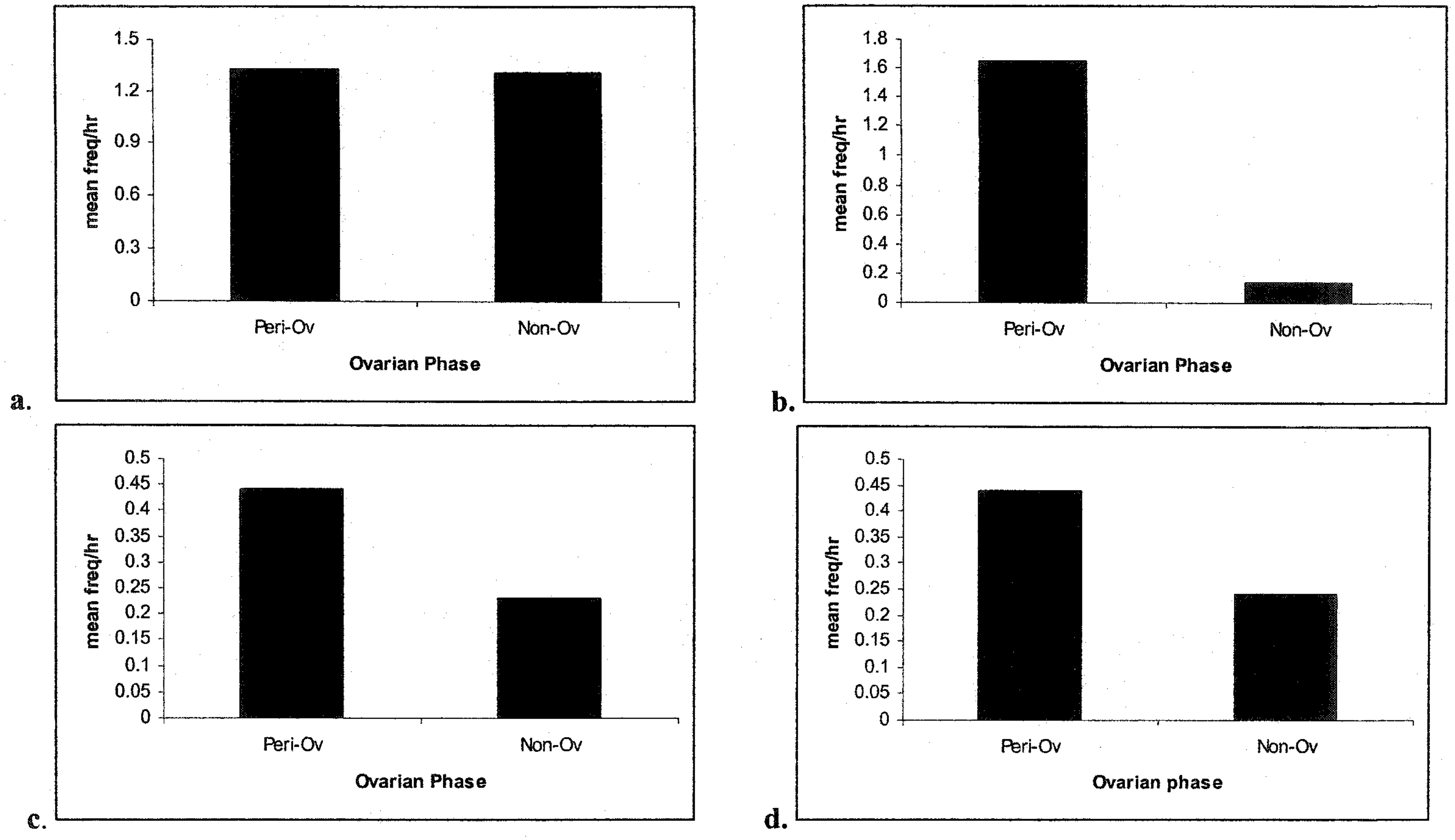

Figure 2.6: Changes in individual attractivity indicators across ovarian phases between all adult males and cycling females.

a. Mean rate of **approaches** received from adult males by subject females.

b. Mean rate of **follows** received from adult males by subject females.

c. Mean rate of **groom solicits** received from adult males by subject females.

d. Mean rate of **groom bouts** received from adult males by females.

Adult males directed grooming bouts to adult females at over seven times the rate in the periovulatory phases compared to the non-ovulatory phases (periovulatory: 0.44/hr; non-ovulatory: 0.06/hr; Figure 2.6d). During both phases of the cycle, the alpha male

was responsible for all grooming bouts directed to all adult females. However, alpha males groomed alpha females twice as much as subordinate females during the periovulatory phases but at comparable rates during the non-ovulatory phases (Table 2.5). The duration for which the alpha males groomed females was negligible; they would often start to groom but not maintain any duration.

In sum, females received all but one of the attractivity indicators (approaches) from males more often in the periovulatory phases compared to the non-ovulatory phases. The rate of approaches received from males was comparable in both phases of the cycle. However, it was more often the alpha males who directed attractivity indicators and more often towards alpha females, except during the non-ovulatory phases when subordinate males directed more approaches to alpha females than alpha males did.

Proximity

In total, all adult males and females spent slightly more time in close proximity (within three meters) during the non-ovulatory phase compared to the periovulatory phase (periovulatory: 20%; non-ovulatory: 24%; Figure 2.7). However, in the periovulatory phases, alpha males spent over 50 times more time in close proximity to alpha females, and 20 times more time close to subordinate females compared to the subordinate males (Table 2.5). During the non-ovulatory phases, both alpha and subordinate males spent more time close to alpha females than to subordinate females, and they did so at similar rates.

To examine which sex was responsible for maintaining proximity in each phase, Hinde's indices were calculated. A negative index implies the male was responsible for maintaining proximity and a positive index implies the female was responsible. During the periovulatory phases, indices could be calculated for six of the 12 possible male-female dyads. One male was never within proximity of a subject female; therefore, indices could not be calculated for those dyads. All four dyads that involved alpha males were negative and the two dyads involving subordinate males were positive (index range: -0.05 to 0.43). In the non-ovulatory phases, indices could be calculated for seven of the 12 possible male-female dyads. Of the four dyads that involved alpha males, two were negative and two were positive. All dyads involving the subordinate males (three dyads) were negative (index range: -0.08 to 0.25; Table 2.6).

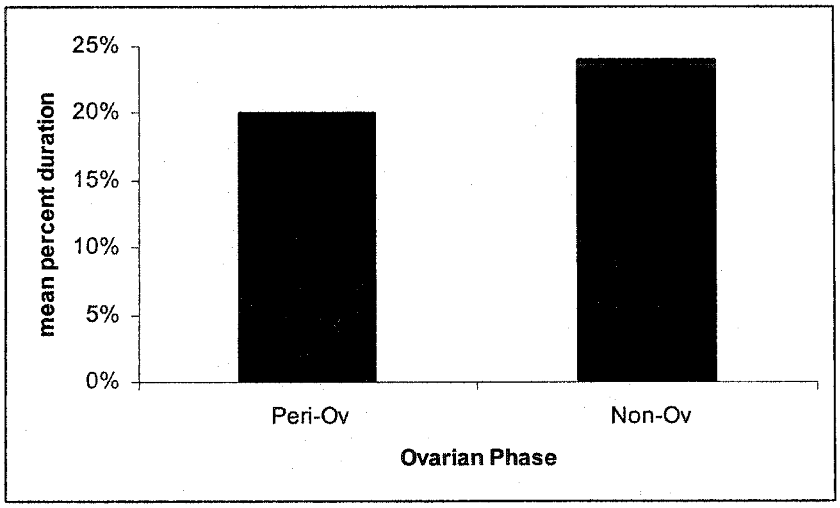

Figure 2.7: Mean percentage of time adult males and females spent within close proximity of each other (within three meters) during the periovulatory and non-ovulatory phases of the ovarian cycle.

Table 2.6: Hinde's Indices for Maintenance of Proximity

	Periovulatory	Non-Ovulatory
Timone		
Nose*	-0.67	-0.75
Trickle	+0.43	-----
Limp*		
Nose*	-0.63	+0.25
Trickle	+0.25	-0.66
Kathy Lee*		
Picante*	-0.30	-0.26
A-1	-----	-----
Chilli	-----	-0.40
Side	-----	-----
Blanquita		
Picante*	-0.05	+0.08
A-1	-----	-----
Chilli	-----	-0.08
Side	-----	-----

A negative index implies the male is responsible for maintaining proximity; a positive index implies the female is responsible

* denotes alpha individuals of CP and LV groups; other males listed are subordinate to the alpha male in each group.

----- Hinde's Index could not be properly calculated due to lack of data (i.e. "Side" was never involved in any approaches or leaves.)

Other Behaviors

Urine washing occurred at three times the rate in the non-ovulatory phase compared to the periovulatory phase of the cycle (periovulatory: 0.68/hr; non-ovulatory; 2.11/hr; Figure 2.8). In both phases of the cycle, alpha females urine washed at twice the rate of subordinate females (Table 2.5).

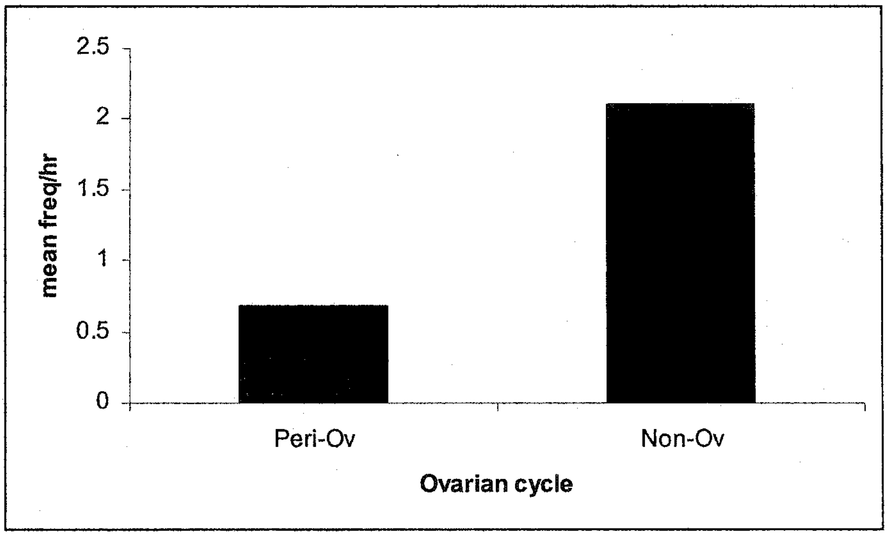

Figure 2.8: Mean rates of urine washing by all subject females during the periovulatory and non-ovulatory phases of the ovarian cycle

DISCUSSION

Fecal Hormone Analysis and Interpretation of Hormone Profiles

The results from the hormone assays revealed that the collection and extraction techniques used in the field and the laboratory were successful methods for studying ovarian steroids obtained from white-faced capuchin feces. The reproductive states of the subject females were easily recognized from the hormone profiles (cycling, non-cycling or pregnant), however among the cycling females, the follicular and luteal phases of the ovarian cycle were not clearly distinguished. Therefore, I compared the periovulatory phases to the non-ovulatory phases, a procedure also used by Carosi and Visalberghi (2002) in their study of capuchin sexual behavior throughout the ovarian cycle.

The reason why the follicular and luteal phases were not more distinctive is presently unknown. The water content or the amount of undigested food in the feces could have affected the quality of some fecal samples analyzed. To remedy this, Hodges and Heistermann (2003) suggested that samples should be freeze dried which compensates for water content, allows easier removal of non-fecal material and produces a homogeneous powder that can be easily weighed and stored. However, I did not have the proper field setting (*i.e.* consistent electricity for freezer) to freeze dry my samples and many of the hormonal studies on wild primates that have used the same methods as I have, have produced accurate results profiling ovarian cycles (Strier & Ziegler, 1997; Ziegler *et al.*, 1997; Strier *et al.*, 1999, 2003; Lynch *et al.*, 2002).

The four cycling females displayed between one to three ovarian cycles before they stopped cycling. After their cycles stopped, the hormone levels fluctuated slightly but stayed low and never reached and sustained baseline levels compared to the profiles of the non-cycling females. It was a surprise to find that these females stopped cycling mid way through the study, and that they did not start cycling again and did not get pregnant. According to currently published literature this ovarian pattern has not been documented before. However, Recabarren *et al.* (2000) may provide an explanation for why the females did not get pregnant and for why the cycles were not more distinctive. They describe a period of time called "lactational residual infertility", which occurs after the interval of lactational amenorrhea (occurs after parturition when the ovary ceases to produce ovarian hormones). Lactational residual infertility is a period of time where females may still be nursing and begin to cycle but the hormone patterns are abnormal and most likely anovulatory. The suggested function of this extra infertile period is to extend the interval between pregnancies (interbirth interval) and it has been previously documented in apes (chimpanzees: Tutin & McGrew, 1973), Old World monkeys (baboons: Altmann *et al.*, 1978) and some New World monkeys (howler and squirrel monkeys: McNeilly, 1994). Nursing is thought to prolong this residual infertility period, but exactly how it does so is unknown as suckling, in *C. apella*, tends to decrease after 6 to 7 months (Recabarren *et al.*, 2000).

Recabarren *et al.* (2000) stopped measuring hormone levels after 150 days so it is unclear if their female capuchins also stopped cycling before conceptive cycles started. However, the exception in the present study to the "lactational infertility" explanation is that one female, Timone, had never been pregnant (nulliparous) so it would have been impossible for her to have been experiencing residual infertility. Her cycles may have represented the beginning of her ovarian cycling as she did have the most distinct cycles of all females, but again, why she stopped cycling is still unknown (see Figure 2.1).

The same four cycling females from this study are likely to have experienced regular ovulatory cycles later in 2002 (around November to December) because they gave birth the following year (between April and May, 2003; gestation length is assumed to be similar to *C. apella*; ~155 days; Nagle & Denari, 1982). These cycle patterns were unexpected and therefore not the main focus of this study; however, this phenomenon is definitely worthy of future studies and I believe it would be most beneficial to monitor *C. capucinus* ovarian activity throughout entire interbirth intervals.

Sexual Behavior and Mate Choice

The behavioral analyses revealed interesting aspects of the sexual and pericopulatory behaviors of white-faced capuchins. Dominance appears to play a major role in terms of mate choice for both males and females in this species. Alpha males more often choose the alpha females to solicit and alpha females more often choose the alpha male as a mating partner. Brown capuchins (*C. apella*) and talapoin monkeys (*Miopithecus talapoin*) are also known to preferentially choose the alpha male as a mate (Dixson & Herbert, 1977; Janson, 1984). However, *Cebus apella* females are also known to mate with subordinate males after their most fertile days have passed, near the end of their periovulatory period (Janson, 1984; Linn *et al.*, 1995; Carosi *et al.*, 1999). Unfortunately, I am not able to suggest that the same behavior occurs in *C. capucinus*. Given that I observed copulations rarely among the cycling females, and only one female (a subordinate) was observed mating with a subordinate male, it would be premature to suggest that mating with the alpha male was restricted to the females' most fertile days. In fact, subordinate males directed very few sexual solicitations to any adult females during their conceptive phases, and even so, neither of the alpha females mated with them. This suggests that the alpha males may have exclusive mating rights to conceptive females, and that alpha females preferentially choose the alpha males as mates. To further support this, recent paternity studies on this population have shown that the alpha males are siring most of the infants within their groups (Jack & Fedigan, 2003). Subordinate males do mate with adult females but this is observed more often with pregnant or "post-conceptive" females; a topic that will be further discussed in Chapter Three.

What remains unclear from these results is why the alpha males did not mate more often with the conceptive, subordinate females. An ideal reproductive strategy for a sexually mature male is to mate with as many fertile females as possible in order to maximize his reproductive output or "success" (Fedigan, 1993; Dixson, 1998; Strier, 2000). Theoretically, if the conceptive females in each group had over-lapping periovulatory phases, and the alpha males had exclusive mating rights to females, it would be expected that the alpha males would try and guard all fertile females from other males. If they could not successfully do so, a good strategy for alpha males would be to mate mainly with high ranking females. High rank confers better access to resources (*i.e.* water, food, grooming) compared to a low ranking individual and, therefore, it is argued that the infants belonging to the higher ranking female would have a greater chance of survival (Fedigan, 1983; Dixson, 1998; Strier, 2000). However, in this study the conceptive phases of each female (in each group) did not overlap in time and the alpha male could have easily guarded each female from other males. Nonetheless, the alpha males persistently followed and courted the alpha females at a greater rate than they did the subordinate females during their respective conceptive phases. It has been found in other primate species that males do show preferences for certain sexual partners over others (*i.e.* baboons: Saayman, 1970; macaques: Enomoto, 1978; Matsumura, 1993 and patas monkeys: Herbert, 1968), so it is not entirely unusual that these capuchin males appear to prefer the high ranking females as mates. However, it is important to realize that the alpha females are not the only reproducing females in each group as all adult females in this population (both high and low ranking) do conceive and produce young and, as mentioned above, the alpha males are most often the sires.

In light of my findings on possible male mate choice in these monkeys there is one important point that can not be ignored. In CP I observed the alpha male, Nose, solicit a subordinate female, Timone, but she was not receptive to him (by presenting for mating). According to recent paternity studies of this group of capuchins (Fedigan & Jack personal comm.), Nose is the sire of Timone. Of course, it is unknown if either Nose or Timone is aware of this relationship but perhaps by not being receptive to Nose, Timone was preventing inbreeding. The one copulation I observed involving subordinate individuals occurred between Timone and the beta male in the group, Trickle. Escobar-Parama (1999) found in her study on *C. apella* that the dominant male does sire the majority of the infants born during his reign, except for those that are born of his daughters, which are sired by other males in the group. Her study provides evidence that inbreeding avoidance behavior is occurring in *C. apella* and, comparing Timone's lack of receptivity, perhaps it also occurs in *C. capucinus*. However, this does not explain why the alpha male in LV group, Picante, did not solicit and display high rates of attractive behaviors to the subordinate female (Blanquita) as he did towards the alpha female (Kathy Lee) during their conceptive phases. To investigate the presence of inbreeding avoidance behavior in white-faced capuchins, more paternity studies are needed.

Proceptivity and Attractivity: Indicators of Ovarian Phase

The increase in sexual behavior during the periovulatory phases suggests that adult males are at least somewhat aware of the onset of the females' conceptive phases. Given that white-faced capuchins lack any known morphological cues to their conceptive phase, I predicted that adult males might be responding to the increased rate of the females' proceptive behavior during their periovulatory periods. However, I found that females' proceptive behaviors occurred at only slightly higher rates during the periovulatory compared to the non-ovulatory phases. Compared to many primate species that display overt proceptive behaviors around the time of ovulation (*Cebus apella*: Linn *et al.*, 1995; Carosi *et al.*, 1999; *Macaca fuscata*: Enomoto *et al.*, 1979; *Cercocebus torquatus atys*: Gordon *et al.*, 1991; *Papio ursinus*: Saayman, 1970; review in Hrdy & Whitten, 1987) female white-faced capuchins are very inconspicuous in this respect.

Dominance rank appears to play a major role in proceptivity. The alpha females displayed the highest rates of proceptive behaviors and they did so most often towards the alpha males. Affiliative behaviors such as grooming and close spatial proximity (*i.e.* during feeding) are commonly seen at higher rates between high ranking individuals than between high ranking and low ranking individuals (Dixson, 1998; Strier, 2000). Moreover, females may choose to be more proceptive toward and mate more often with males of higher rank because it is frequently argued that an individual who can maintain a high rank position is reproductively superior (*i.e.* good genes) to those that can not (Agrell *et al.*, 1998; Dixson, 1998; Strier, 2000), although high dominance rank does not always confer greater reproductive success (see Fedigan, 1983). The exception in this study is that subordinate females spent a greater amount of time grooming the alpha males, when they had the opportunity, compared to the length of time alpha females spent grooming. This could be interpreted as a social strategy that the subordinate females are using not only to help increase their rank within the group (by forming a close bond with the highest ranking individual) but also to gain an ally to help protect against predators and infanticidal males (Dixson, 1998; Strier, 2000).

Attractivity indicators appeared to provide the best estimate of the female's ovarian state and, during the periovulatory periods, these behaviors were almost exclusively displayed by the alpha males. Specifically, the rate at which the alpha males followed the females was probably the best behavioral indicator of the conceptive phase. Additionally, alpha males directed more grooming bouts to females when they were conceptive, which is noteworthy because adult males very rarely groom females in this species. This initiation of sexual behaviors by males in *C. capucinus* contrasts greatly with the pattern in *C. apella* where males become sexually attracted to females only after the females have intensely solicited them for mating. In white-faced capuchins, the high rate of following and the increased rate of grooming towards conceptive females does not appear to be preceded by any conspicuous female behavioral signals.

Urine washing has been suggested as a method to communicate reproductive state and therefore could also be considered a non-affiliative, proceptivity indicator (Boinski, 1992; Perret, 1992). However, contrary to what I had originally predicted, this behavior occurred more often in the non-ovulatory phase, which does not support this hypothesis. Nonetheless, males were often seen smelling and licking the branches where a female had just performed a urine wash. There may be multiple functions to this behavior, since urine washing is seen in all age/sex classes. Considering the hot and arid climate of the tropical dry forest, the behavior may also function to regulate body temperature (Robinson, 1979; Roeder & Anderson, 1991). Presently there is a lack of data on the circumstances surrounding urine washing behavior in capuchin populations.

Overall, my study suggests that female white-faced capuchins increase their frequency of affiliative proceptive behaviors towards males during their conceptive phases, but at very low rates. Furthermore, from my view as the observer, it was the males' persistent and overt behaviors towards the females that indicated which phase of the ovarian cycle she was in. The very subtle increases in proceptive behaviors may still act as the cue for the males, but to support this notion more data must be collected, from a larger sample size of cycling females and over a longer amount of time (including phases where conceptions occur) so that statistical analyses can be conducted. Nevertheless, proceptive behaviors in white-faced capuchins are very inconspicuous compared to many species and especially compared to *Cebus apella* (which is one of the most closely related species), so the question still remains; why do white-faced capuchins inconspicuously conceal their ovulation from males?

Of the available explanations for concealed ovulation, the reduction of male-male competition appears to be the most appropriate in this case (Hrdy, 1979). Hrdy (1979) suggested that concealed ovulation evolved in multi-male species to help reduce competition between males and confuse paternity to reduce the likelihood of infanticide. To do this, females who visibly conceal their ovulation may employ different reproductive behavioral counter-strategies (Agrell *et al.*, 1998). In *C. capucinus* infanticide is known to occur more often than previously thought (Fedigan, 2003). In fact, Fedigan (2003) found in this study population that 14 infants died/disappeared after nine aggressive male takeovers, and three of these infants were observed to be fatally wounded by a new adult male.

One possible counter-strategy for a female is to mate only with the dominant male during her most fertile period, thereby ensuring for him that the newly born infant is his own (Agrell *et al.*, 1998). By choosing this strategy, the female may ensure the safety of her infant by benefiting from the dominant male's willingness and ability to better defend the group from invading males compared to lower ranked males (Agrell *et al*, 1998). As two of the three observed copulations in this study involved the alpha males, and recent paternity studies on this population show that the alpha males are siring the majority of the infants (Jack & Fedigan, 2003) this may be one of the counter strategies used by female *C. capucinus.* It could also explain why most of the proceptive indicators involved the alpha males at higher rates than subordinate males. If the female stays closer to the alpha males by either grooming or by just resting in close proximity this may facilitate more mating opportunities.

Burt (1992) and Nunn (2000) suggested that truly concealed ovulation does not exist in primate species. Females have evolved in a way to make their ovulations unpredictable. This means there is a large degree of variance in the duration of the follicular phase and a lack of correlation between proceptive behaviors (or maximum sex skin swelling) and the actually timing of ovulation. In this way, unpredictable ovulation further functions to confuse paternity in multi-male groups (Nunn, 2000). I agree that white-faced capuchins are not truly concealing their ovulation; primarily because of my finding that females are more attractive to males during the periovulatory phases. Perhaps they do experience unpredictable ovulation in that peaks in female proceptive behaviors do not correspond to actual ovulation, which helps to confuse paternity and reduce the threat of infanticide. However, to further complicate things, if female white-faced capuchins do experience lactational residual infertility (Recabarren *et al.*, 2000) the ovarian cycles and behavior examined in this study are representative of that phenomenon. Further study of white-faced capuchin reproductive biology is worthwhile in order to investigate if residual infertility exists in this species, if unpredictable ovulation is used as another reproductive strategy, and to gain a clearer understanding of the reproductive behavior between males and females of different dominance ranks.

Conclusions

Many of my original predictions about the changes in behavior between the periovulatory and non-ovulatory phases of the ovarian cycle were supported, although not reaching statistical significance due to small sample sizes. There were some exceptions as described below. In addition, dominance appeared to play a role in mate choice for both males and females in these subjects. In almost all cases the alpha males and females exhibited higher rates of the relevant behaviors than the subordinate individuals.

- The rates of sexual behaviors (both copulations and courtship dance displays) are higher during the periovulatory phases of the cycle.
- The rates of proceptivity indicators increased very little during the periovulatory compared to the non-ovulatory phases.
- The rates of attractivity indicators increased two-fold during the periovulatory compared to the non-ovulatory phases of the cycle.
- The rate of urine washing occurred at three times the rate in the non-ovulatory compared to the periovulatory phases.

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Chapter Three

Sexual and affiliative behaviors associated with reproductive state in female white-faced capuchins (*Cebus capucinus*)

INTRODUCTION

In many species of non-human primates, sexual and social behaviors are known to vary across a female's reproductive stages and thus numerous studies have focused on the relationship between behavior and hormonal fluctuations during the ovarian cycle (*Macaca fuscata*: Enomoto, 1979; *Macaca mulatta*: Mallow, 1981; *Papio ursinus*: Saayman, 1970; *Erythrocebus patas*: Loy, 1981; *Cebus apella*: Linn *et al.*, 1995; Carosi *et al.*, 1999; *Brachyteles arachnoids*: Strier & Ziegler, 1997). However, fewer primate studies have compared sexual and social behaviors across different reproductive states; namely cycling, non-cycling and pregnant (*Cercocebus torquatus atys*: Gorden *et al.*, 1991; Gust, 1994; *Pan troglodytes*: Wallis, 1982)

The recent development of non-invasive fecal collection and hormone extraction techniques that can be successfully used in the field has led to a growing number of studies that relate hormones to behavior in wild primates (*Propithecus verreauxi*: Brockman *et al.*, 1995; *Brachyteles arachnoids*: Strier & Ziegler, 1997; *Macaca nemestrina*: Risler *et al.*, 1987; *Papio cynocephalus cynocephalus*: Wasser *et al.*, 1991). This study uses hormone profiles, created from the analysis of fecal ovarian steroids, to determine the reproductive status of wild female white-faced capuchins, and then examines the sexual and affiliative behaviors that are exhibited across three reproductive states (cycling, non-cycling and pregnant). The ultimate objective of this study is to determine if there are behavioral indicators that could be used to reliably infer the reproductive status of wild female capuchins in the absence of hormonal data.

Hrdy (1974, 1979) suggested that mating during non-conceptive periods (*i.e.* when females are either pregnant or anovulatory due to lactation) is a behavioral counter-strategy by females to reduce the threat of infanticide by invading males (also see Agrell *et al.*, 1998). Mating after conception (here on referred to as "post-conceptive mating") has been reported in a number of primate species who also experience infanticide (*Cercocebus torquatus atys*: Gordon *et al.*, 1991; *Papio hamadryas*: Zinner & Deschner, 2000; *Cebus capucinus*: Manson *et al.*, 1997; *Pan troglodytes*: Wallis, 1982). For instance, female sooty mangabeys (*Cercocebus torquatus atys*) and hamadryas baboons (*Papio hamadryas*) are known to experience post-conceptive swellings that mimic the swellings experienced during their conceptive periods, thereby potentially confusing males about female reproductive state. Furthermore, the subordinate male sooty mangabeys only mated with pregnant females during their post-conceptive swelling phases, and alpha males mated only with females during their fertile swelling phases. Thus, the authors inferred that the alpha males were able to discriminate between the fertile and the non-fertile swellings. However, they were unable to explain how the alpha males could tell the difference (Gust, 1994).

Manson *et al.* (1997) examined non-conceptive mating in white-faced capuchins and found that pregnant females mated more frequently than the potentially conceptive females. They concluded that post-conceptive mating functions to confuse paternity and reduce the risk of infanticide from immigrating males. In white-faced capuchins, infant deaths and disappearances are often associated with male takeovers of groups (Fedigan, 2003). However, for this counter strategy to infanticide to be successful, males must not be able to detect the ovulatory period, and they also must not associate the timing of mating with the timing of births (Zinner & Deschner, 2000).

Ovarian hormones have been suggested to correlate with aspects of sexual and social behavior (Baum, 1983; Beach, 1976). For instance, high levels of estrogen are associated with an increase in proceptive behaviors and female attractiveness. In contrast, high levels of progesterone significantly reduce female attractiveness and frequency of proceptive behaviors.

As reported in chapter two of this thesis, I found that non-cycling females experienced very low, non-fluctuating levels of estradiol and progesterone. In contrast, cycling females experienced cyclical, fluctuating hormone levels typical of regular ovarian cycles. In pregnant females, both ovarian hormones remained at elevated concentrations throughout the gestational period, and dropped to baseline levels at parturition. Given the likely influence of ovarian hormones on behavior, and the variable hormone patterns of non-cycling, cycling and pregnant females, I predict that there will be differences in sexual and social behaviors across three different reproductive states in female white-faced capuchins.

Specifically, I predict that sexual behavior (copulations and courtship displays) will vary between the three categories of females; and that non-cycling females will receive the lowest rate of sexual solicitations and copulations from adult males because they produce low levels of stimulating estrogen. Also, in accordance with previous findings (*i.e.* sooty mangabeys) and theoretical models (*i.e.* paternity confusion), I predict that pregnant females will interact sexually with subordinate males more often than with alpha males.

I found that proceptivity and attractivity indicators both increase during the periovulatory phase, when estrogens are climbing and progesterone is low (see Chapter two). Therefore, I predict that proceptivity and attractivity behavioral indicators will also vary according to reproductive state, and that pregnant and cycling females (with their high, fluctuating levels of hormones) will direct and receive these indicators at higher rates compared to the non-cycling females.

The function of urine washing (rubbing urine into the hands and feet) has been suggested to communicate reproductive status (Boinski, 1992; Perret, 1992) where it may act as a non-affiliative proceptive behavior. Urine washing occurs in many primate species including squirrel monkeys, woolly spider monkeys, and all species of capuchins (Robinson, 1979; Milton, 1985; Boinski, 1992). However, as reported in chapter two of this thesis I found that urine washing is more frequent during the non-ovulatory phases of the ovarian cycle suggesting that it may not function entirely as a reproductive signal. Nevertheless, male white-faced capuchins are often seen smelling and licking branches where a female had performed a urine wash (personal obs.). Thus, I believe urine washing may play a communicative role in *C. capucinus* females but perhaps on a larger scale (*i.e.* advertising reproductive state as opposed to ovulatory phase).

Research Questions and Predictions:

1) **Does the frequency of sexual behavior vary between cycling, non-cycling and pregnant females?**

I predict that the rate of sexual behaviors will be higher among cycling and pregnant females compared to non-cycling females.

2) **Do proceptivity and attractivity indicators vary depending on reproductive state?**

I predict that both proceptivity and attractivity indicators will vary according to reproductive status. Cycling and pregnant females will direct more proceptive behaviors to males and receive more attractivity indicators from adult males compared to non-cycling females.

3) **Do adult males and females show preference for certain individuals for mates or social activities depending on reproductive state?**

I predict that cycling females will show a preference for alpha males and pregnant females will show a preference for subordinate males for both sexual (copulations and dance displays) and affiliative behaviors. I predict that non-cycling females will not show any particular preference for any adult male.

METHODS

Study Site

My study took place in Santa Rosa National Park (SNRP); the original sector of the Area de Conservacion Guanacaste (ACG). This park is located in northwestern Costa Rica about 40 km south of the Nicaraguan border. The forest type is classified as a dry, deciduous forest which experiences a distinct dry season (January to April) and wet season (May to December; average annual rainfall is 1473 mm; Fedigan & Jack, 2001). There is an extensive variety of flora and fauna that occur within the park including three species of primates: *Cebus capucinus* (white-faced monkey), *Alouatta palliata* (mantled howling monkey) and *Ateles geoffroyi* (black handed spider monkey). SRNP extends along the central volcano range (reaching elevations of 300m) and slopes down ward to the west where it is bordered by the Pacific Ocean (Fedigan & Jack, 2001).

Study Subjects

The subjects for this study consisted of 10 wild adult female white-faced capuchins who were part of two habituated groups; Cerco de Piedra and Los Valles (five per group). Females ranged in age from 6 to ~24 years (females start to cycle at 5 to 6 of age). Additionally, there were six adult males: two alpha males and four subordinate males. Individuals were identified by natural markings such as broken digits, scars, fur coloring and the brow and peak shape. This population of capuchins has been part of LM Fedigan's research since 1983. Table 2.1 (see Chapter two) lists female ages, rank and reproductive history at the time of the study. Appendix I lists further group composition data for 2002.

Behavioral Data Collection

I collected behavioral data using continuous focal sampling methods (Altmann, 1974). Focal sessions lasted for 15 minutes each, during which time I recorded all behaviors and interactions. Behavior codes were recorded using a hand-held computer (PSION Workabout MX) and a micro-cassette recorder (Sony M-430; see Appendix II for ethogram codes). I collected a total of 443 focal hours between January and June 2002 and between the hours of 6AM and 6PM (ranged from 39 to 47 hours per female).

Fecal Collection and Field Extraction

I collected fecal samples from each focal female on every day that behavioral collection occurred. A minimum of two to three samples per week per female was enough to assess ovarian patterns in females but additional samples were collected in case females could not be located in subsequent days (Hodges & Heistermann, 2003; Ziegler & Lynch, personal comm.). After the pregnant females gave birth, I collected only one to two samples per week per female which was sufficient to monitor post-partum ovarian function. In total, my assistant and I collected about 600 samples from all

10 females. Fecal collection and initial extraction of the steroids in the field followed the techniques described in Strier and Ziegler (1997) and Strier *et al.* (2003) and Chapter two. I refrigerated the samples until I transported them to the National Primate Research Center (NPRC, University of Wisconsin) in Madison for the laboratory analysis.

Laboratory Extraction and Analysis

I carried out the hormone analyses at the NPRC (University of Wisconsin) between July and August 2002. Before my arrival, 10 previously collected fecal samples (January 2001) were validated for the EIAs and RIAs that were used in order to assess the progesterone (P) and estradiol (E2) values respectively. These 10 samples were also used for the validation tests for accuracy and parallism. I analyzed about 400 of the 600 samples that I collected because once the reproductive state of the non-cycling and pregnant females was determined, not all samples were needed. I analyzed all of the samples collected from the cycling females to get a clear picture of their ovarian cycle (*i.e.* try to pinpoint day of ovulation). Solvolysis was first required to break conjugated steroids into un-conjugated forms before assays could be preformed. This procedure has been previously described in Ziegler *et al.* (1996), Strier *et al.* (1999) and in chapter two of this thesis.

Interpretation of Hormonal Data

I used the results of the fecal assays (performed on the 400 samples) to create hormone profiles for each female and from the profiles I was able to infer their reproductive state. I identified non-cycling females by their non-fluctuating and sustained baseline levels of estradiol and progesterone. The gestational period of the pregnant females was identified by their elevated levels of both steroid hormones. After parturition, the hormone levels dropped drastically to baseline and remained there until the end of the study. Cycling females were identified by their fluctuating levels of estradiol and progesterone which are representative of the periovulatory and non-ovulatory phases of their ovarian cycles (Dixson, 1998; Ziegler personal comm.; also see Chapter two).

Behavioral Analysis

All four cycling females ceased to cycle approximately mid-way through the study and three did not start cycling until one to three months into the study. Therefore, for this analysis, I used only behavioral data collected while they were cycling (cycling: 55.25 hrs). From the three pregnant females, I analyzed behavioral data collected only while they were pregnant (68.75 hours). For consistency, I analyzed behavioral data collected from the non-cycling females until April 30th (68.5 hours). I choose this date because most of the cycling females had stopped cycling at this time and there was only one pregnant female still to give birth (her parturition date was May 13).

The behavioral variables analyzed for reproductive state variation were: copulations and courtship/dance displays (sexual behavior) and the hypothesized behavioral indicators of attractivity, proceptivity and receptivity. Proceptivity indicators were approaches, follows and grooms (frequency of groom solicits and frequency and duration of grooming bouts) *directed* by the subject female to adult males. I considered the same behaviors as indicators of attractive behavior when they were *received* from adult males. I calculated a measure of spatial proximity (percentage of time males and females were within three meters of each other) and recorded which sex was responsible for maintaining that proximity. I also analyzed the rate of urine washing as a non-affiliative indicator of reproductive state. Because *C. apella* females show preferential treatment to the alpha males during their conceptive phases (also see Chapter two), I compared pericopulatory behaviors exhibited by alpha versus subordinate males, with the females in the three different reproductive states. Table 2.2 (Chapter two) lists the behavioral variables analyzed and their definitions.

I compared each of the behavioral frequencies across each group of females by calculating a mean rate (frequency per hour) for each of the subject females for each reproductive state. I determined mean grooming duration by tallying the total amount of time females spent in grooming bouts and calculating the proportion of that time they spent grooming (or spent being groomed by) adult males. I measured receptivity as a percentage of male dance displays to which females responded positively by presenting for mounting and facilitating copulations.

I determined spatial proximity by tallying the total amount of time a subject female was within three meters of a male and dividing this by the total time I observed her (proportion of time in close proximity). I used Hinde's Index (Hinde & Atkinson, 1970) to decide which member of the male-female dyad was responsible for maintaining proximity. Hinde's Index is the proportion of all of the dyad's *approaches* directed by the subject female, minus the proportion of all of the dyad's *leaves* directed by the female. A negative index indicates that the male was responsible for maintaining proximity; a positive index suggests the female was responsible. *Approaches* to within three meters and *leaves* beyond three meters were used in this analysis.

I used non-parametric statistics as my data were not normally distributed and my sample sizes were small (Siegal & Castellan, 1988; Zar, 1999). I used Kruskall Wallis one-way analysis of variance to compare behavioral frequencies among reproductive states (cycling, non-cycling and pregnant). All frequencies showing a significant difference were further analyzed using a multiple comparison test to determine where the difference existed among the three groups of females (Siegel & Castellan, 1988). Level of significance for all tests was set at $P < 0.05$.

RESULTS

Hormone Validations and Profiles

Mean steroid recoveries were found to be within acceptable limits for progesterone (P=90.7%) and estradiol (E2=64.5%). Pooled samples compared for accuracy and parallelism to the standard curve were high for both P and E2 assays (> 100% accuracy). Additionally, mean intra- and interassay coefficient of variation (CV) values were within acceptable limits. Table 2.3 (see Chapter two) summarizes the results of the validation procedures.

The hormone profiles created from the fecal analysis clearly revealed that three females were non-cycling throughout the study, three females were pregnant at the beginning of the study and four females displayed regular cycling for a period of time and then stopped mid-way through the study period without getting pregnant. Figures 3.1

to 3.3 illustrate representative hormone profiles for non-cycling, cycling and pregnant females respectively. Appendix III contains the hormone profiles for all 10 subject females.

Non-cycling females (Nyla, Fiesty and Seria) showed baseline levels of progesterone and estradiol throughout the duration of the study. Mean levels of P were 211.84 ng/ml (range: 166.92 to 237.71 ng/ml). Mean levels of E2 were 10.87 ng/ml (range: 9.93 to 12.22 ng/ml).

Three pregnant females (Dos Leches, Pumba and Salsa) displayed typical patterns of elevated hormones during pregnancy that dropped drastically to baseline levels the day of parturition (Dixson, 1998). Since the females were pregnant when the study commenced I was unable to estimate a gestation length but I was able to collect data for the last six weeks for one female (Dos Leches) and the last 15 weeks for the other two (Pumba and Salsa). The mean level of progesterone during pregnancy was 3099.37 ng/ml (range: 1773.10 to 7719.06 ng/ml). The mean level of estradiol was 105. 99 ng/ml (range: 64.16 to 222.95 ng/ml). Table 3.1 summarizes the average estradiol and progesterone levels for the non-cycling and pregnant females.

Of the four cycling females, three did not show cycling until 14, 41 and 43 days after their first collected sample (Timone, Limp and Blanquita respectively) and observations for the fourth female, Kathy Lee, appeared to start at the beginning of her luteal phase. The onset of cycling was indicated by the abrupt rise in the concentrations of progesterone and estradiol from near baseline levels. Regular cycling for all females ceased when both steroid hormones dropped to near baseline and did not elevate again to the previous (ovulatory) levels (Ziegler, personal comm.). I calculated between one to three regular cycles for each female that each extended over an average of 43.5 days (range: 40 to 86 days; Table 2.4 in Chapter two lists the number and lengths of cycles and the length of the cycling period for each female).

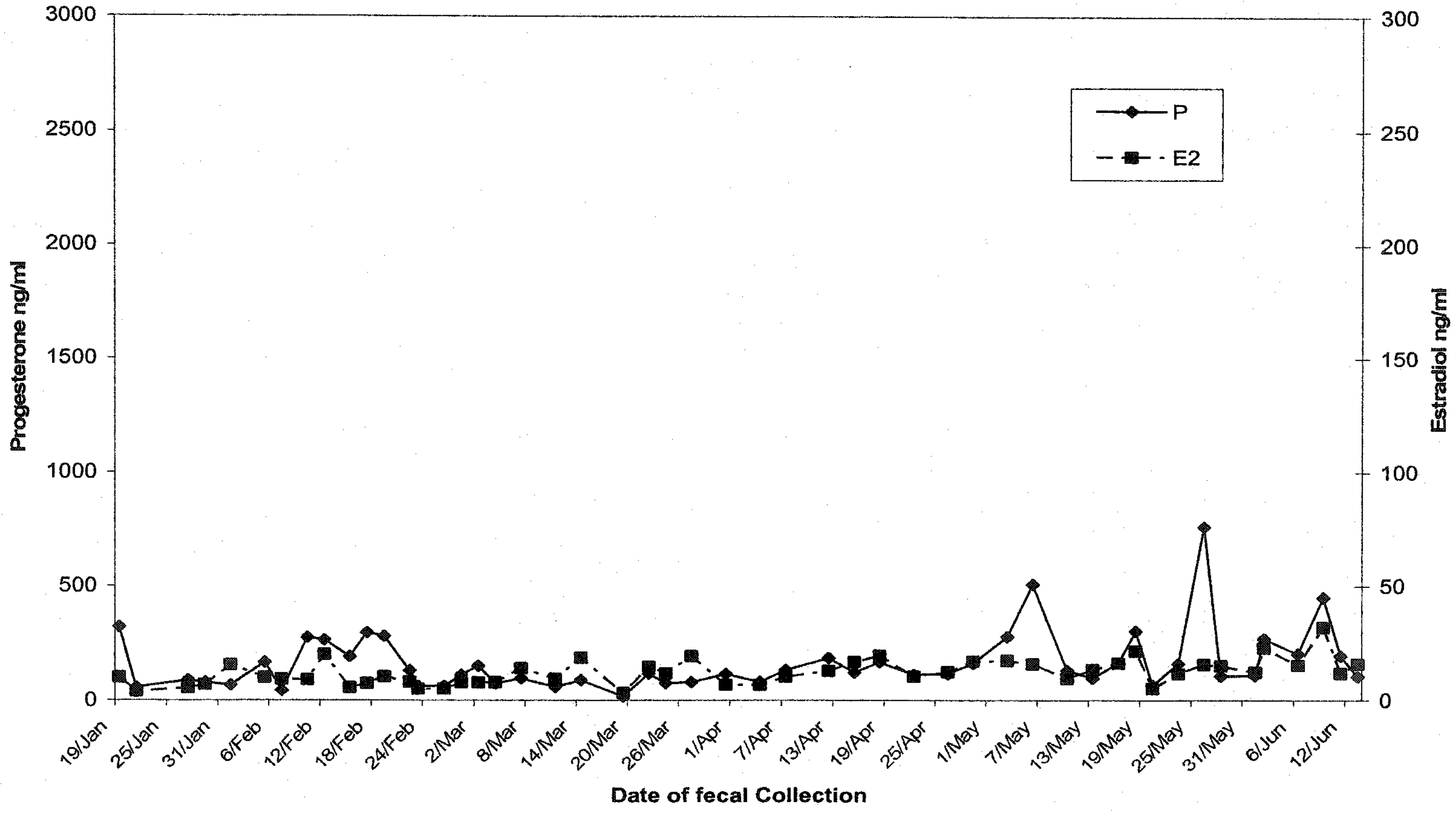

Figure 3.1: Representative hormone profile for one non-cycling female white-faced capuchin. Estradiol (E2) and progesterone (P) remained near baseline levels for the entire duration of the study. Baseline levels: P< 250ng/ml; E2< 25ng/ml.

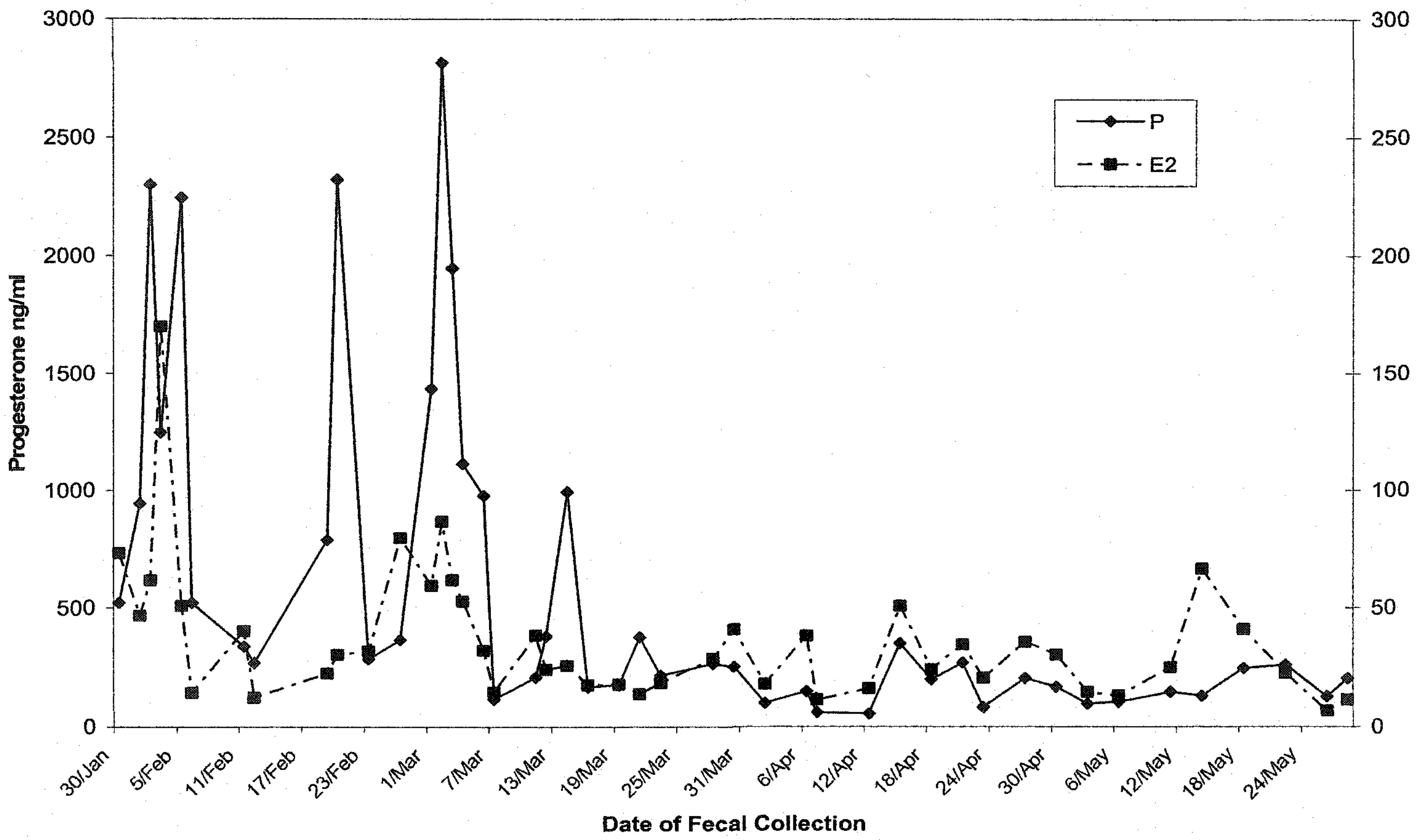

Figure 3.2: Representative hormone profile for one cycling female white-faced capuchin. This female appeared to have started cycling when observations commenced and displayed two cycles before she stopped in early March.

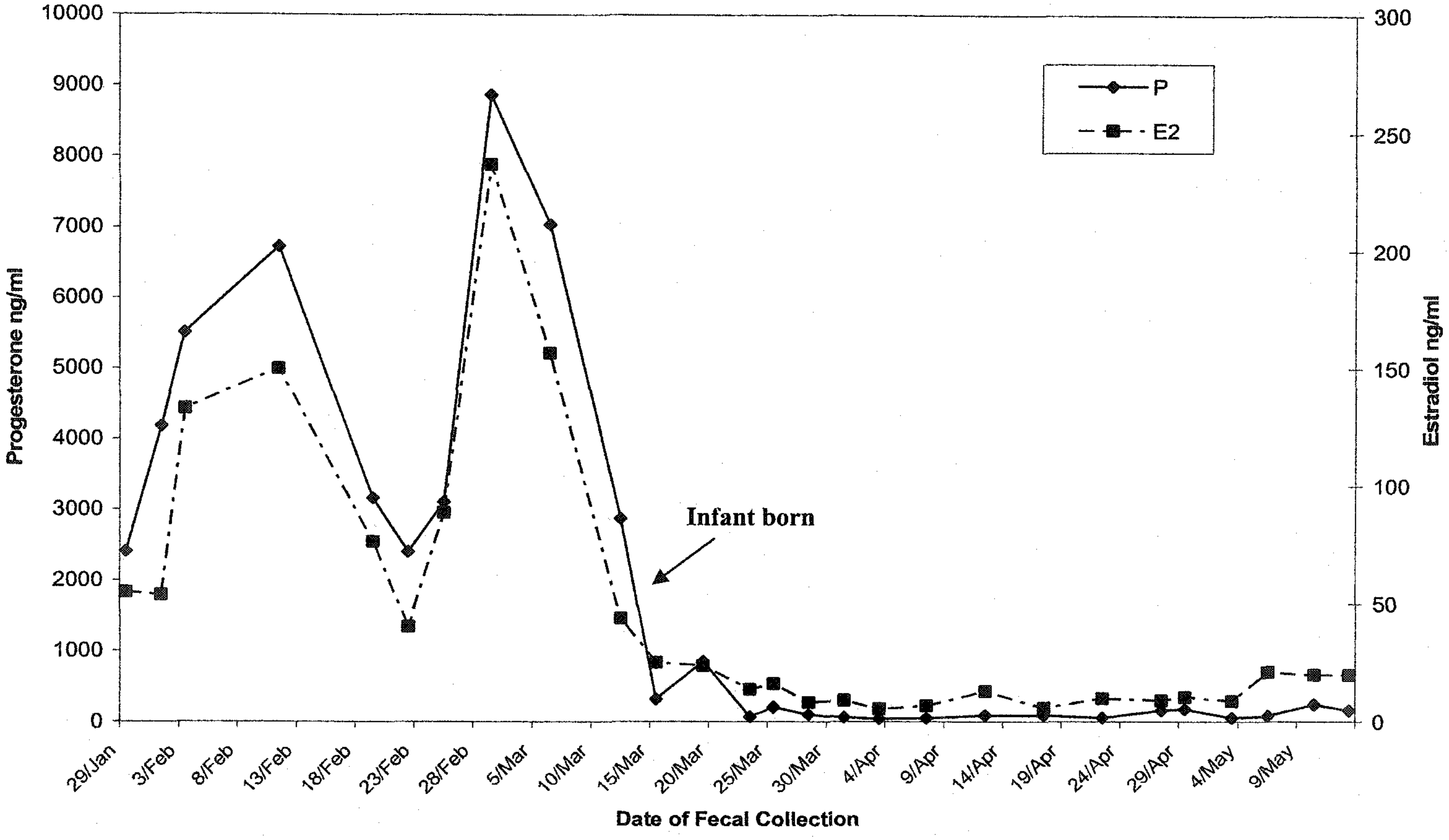

Figure 3.3: Representative hormone profile for one pregnant female white-faced capuchin. Pregnancy is represented by elevated progesterone (P) and estradiol (E2) which drop to baseline levels 1 to 2 days after parturition.

Table 3.1: Mean levels of estradiol and progesterone in non-cycling and pregnant females.

	Estradiol (ng/ml)[1]	Progesterone (ng/ml)
Non-Cycling	10.87	211.84
Range	*9.93 – 12.22*	*166.92 – 237.71*
Pregnant[2]	105.99	3099.37
Range	*64.16 – 222.95*	*1773.10 – 7719.06*

[1] nanograms per millimetre of water/ethanol/steroid mixture.
[2] mean levels during gestation only

Behavioral Variation between Cycling, Non-Cycling and Pregnant Females

Sexual Behavior

I observed copulations and courtship dance displays only in pregnant and cycling females. I found significant differences between the three reproductive states in the rate of three behavioral variables: 1) rate of copulations with subordinate males; 2) rate of dance displays received from alpha males; 3) rate of dance displays received from subordinate males (copulations with subordinate males: χ^2=7.45, df=2, P=0.024; dance displays received from alpha male: χ^2=8.22, df=2, P=0.016; dance displays received from subordinate males: χ^2=7.17, df=2, P=0.028).

Multiple comparison tests revealed that the pregnant females copulated at a significantly higher rate with *subordinate males* (0.29/hr) compared to both cycling (0.02/hr) and non-cycling females (no copulations; Figure 3.4). In total, there were 22 copulations observed among all subject females and 19 of these (86%) involved pregnant females and subordinate males. Furthermore, two pregnant females copulated once each with a subordinate male, one and two days respectively *after* they gave birth. Only the cycling females were observed to mate with the alpha males.

Pregnant females also received significantly more dance displays from *subordinate males* (0.33/hr) compared to both cycling (0.05/hr) and non-cycling females (none). There was no difference in the rate of received dance displays from the alpha males between the pregnant (0.05/hr) and cycling females (0.15/hr) but non-cycling females received significantly less than both those groups (none; Figure 3.5).

Pregnant females responded positively to 90% of the dance displays they received from males by presenting for and thereby facilitating copulation, whereas cycling females responded positively to only 25% of received dance displays. Non-cycling females never received dance displays.

Proceptivity

Only one of the proceptivity indicators that I compared for reproductive state variation showed a significant difference between states. The rate of *grooming bouts directed to subordinate males* by subject females varied significantly between states (χ^2 = 6.85, df = 2, p = 0.033). Multiple comparison tests revealed that pregnant females directed grooming bouts at a significantly higher rate to subordinate males (0.41/hr) than did cycling females (0.08/hr) and non-cycling females (0.10/hr; Figure 3.6).

Attractivity

Two of the attractivity indicators that were compared showed a significant difference between reproductive states. The rate of *groom solicits received from alpha males* by subject females varied significantly between reproductive states (χ^2=6.82, df=2, p=0.032). Further comparison tests showed that cycling females received groom solicits from the alpha males at a significantly higher rate (0.22/hr) compared to the non-cycling females (0.01/hr; Figure 3.7).

The second attractivity indicator that varied significantly was the rate of *grooming bouts received from alpha males* by subject females (χ^2=7.63, df=2, p=0.02). Comparison tests revealed that cycling females received grooming bouts from the alpha males at a significantly higher rate (0.13/hr) compared to both the pregnant (0.02/hr) and non-cycling females (none; Figure 3.8).

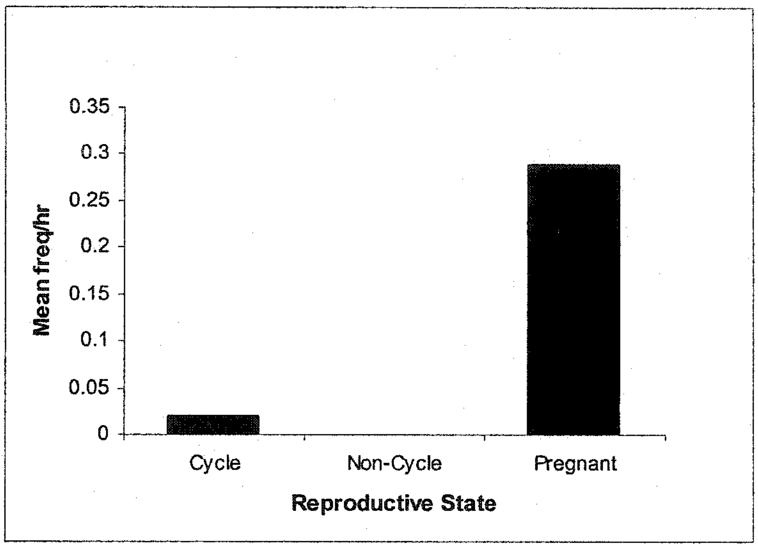

Figure 3.4: Mean rate of copulations *with subordinate males* for cycling, non-cycling and pregnant females.

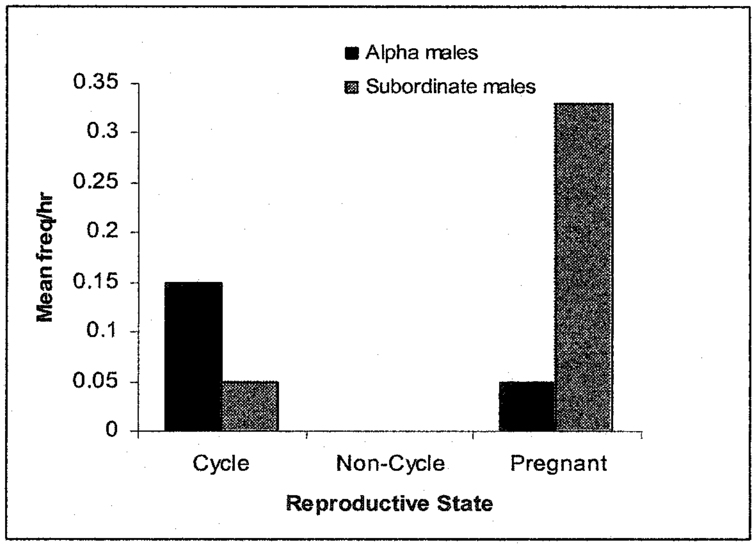

Figure 3.5: Mean rate of dance displays (sexual solicitations) that cycling, non-cycling and pregnant females received from alpha and subordinate males.

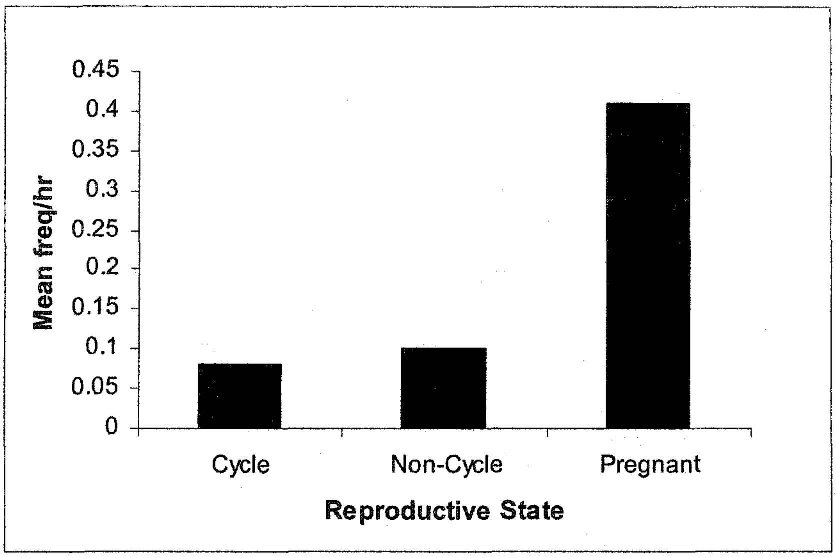

Figure 3.6: Mean rate of *grooming bouts directed to subordinate males* by cycling, non-cycling and pregnant females.

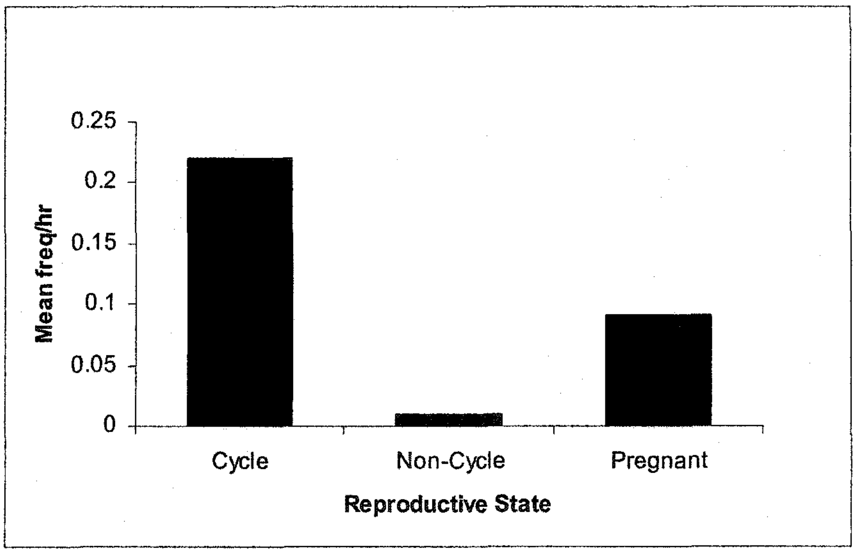

Figure 3.7: Mean rate of *groom solicits* that cycling, non-cycling and pregnant females *received from alpha males.*

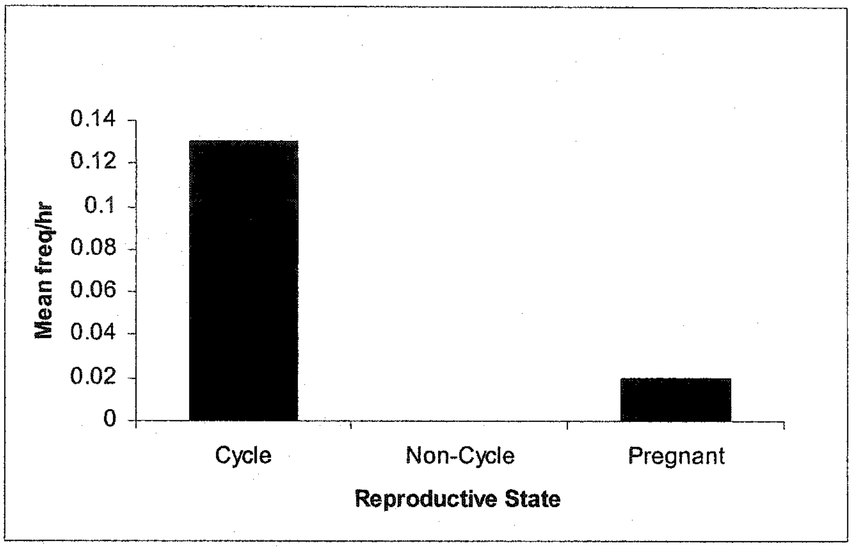

Figure 3.8: Mean rate of *grooming bouts* that cycling, non-cycling and pregnant females *received from alpha males.*

Proximity

The amount of time adult males and females spent in close proximity to each other (within three meters) did not vary significantly between cycling, non-cycling or pregnant females. However, after calculating Hinde's indices to examine which sex was responsible for maintaining the proximity there were some noteworthy results. Table 3.2 lists the percentages of dyads for which either the male (alpha or subordinate) or the female maintained the proximity in each reproductive state. Figure 3.9 illustrates the percentage of dyads maintained by both the alphas and subordinate males for each group of females.

In the cycling female group, only 10 out of a possible 12 dyads could be calculated as one male was never within proximity to subject females. All four dyads involving the alpha males were negative which implies the alpha males were responsible for maintaining proximity 100% of the time. Four of the six dyads involving the subordinate males were negative (67%) and the two remaining dyads were neutral (33%)

which implies that there was no difference between the sexes in who maintained the proximity.

In the pregnant female group, eight out of a possible 10 dyads could be calculated. Two of the three dyads involving the alpha males were negative (67%) and one was positive (33%), which implies the female was responsible for maintaining proximity in that dyad. Of the five dyads involving the subordinate males, four were negative (80%), and the remaining dyad was neutral (20%).

Among non-cycling females, eight out of the possible 10 dyads could be calculated. In contrast to the other females, all three of the dyads involving the alpha males were positive (100%) which implies that the non-cycling females were entirely responsible for maintaining proximity with the alpha males. In contrast, all five of the dyads involving the subordinate males were negative (100%).

Table 3.2: Percentage of dyads for which either the male or the female maintained proximity in each reproductive state.

	Cycling	Non-cycling	Pregnant
Male Maintained			
Alpha	100*	0	67
Subordinate	67	100	80
Female Maintained			
Alpha	0	100	33
Subordinate	0	0	0
Neutral	33	0	20

* all values reported as percentages

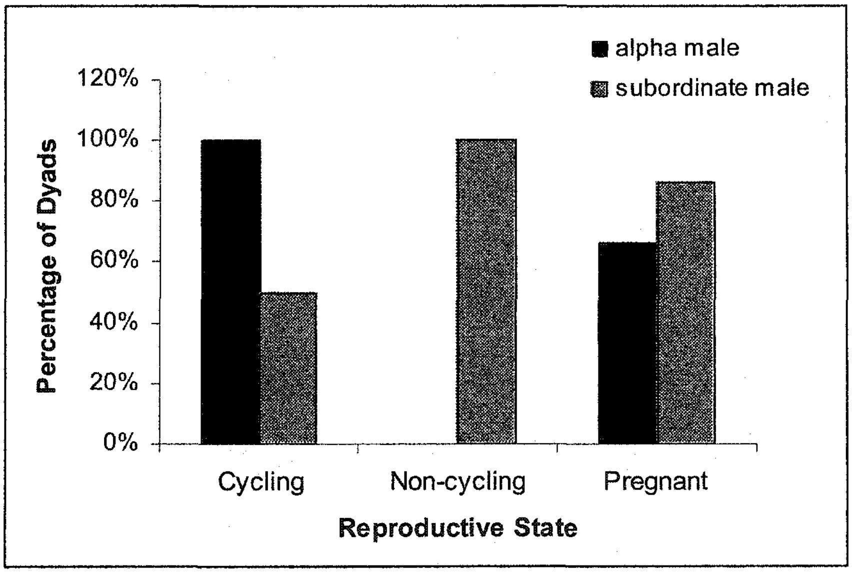

Figure 3.9: Percentage of heterosexual dyads where it was the male that maintained proximity (< 3 meters) to cycling, non-cycling or pregnant females.

Non-Affiliative Behaviors

There was a significant difference in the rate of urine washing between cycling, non-cycling and pregnant females (χ^2=6.56, df=2, p=0.03). Cycling females performed urine washes at a significantly higher rate (3.12/hr) compared to both pregnant (1.54/hr) and non-cycling females (1.67/hr; see Figure 3.10).

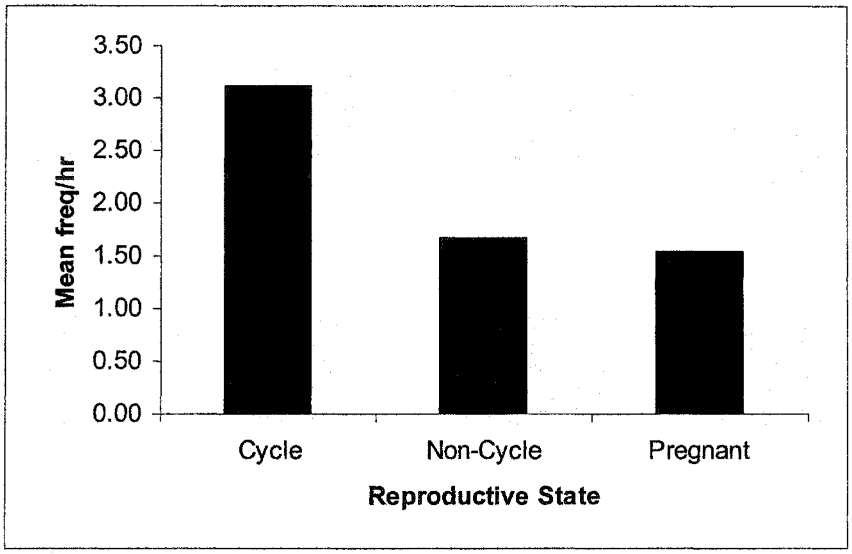

Figure 3.10: Mean rate of urine washing displayed by cycling, non-cycling and pregnant females.

DISCUSSION

Fecal Hormone Analysis and Interpretation of Hormone Profiles

The results from the hormone assays revealed that the collection and extraction techniques used in the field and the laboratory were successful methods for studying ovarian steroids from white-faced capuchin feces. Non-cycling, cycling and pregnant females were easily recognized from the hormone profiles created from the fecal analysis.

The three non-cycling females were identified by their sustained baseline levels of estradiol and progesterone that lasted throughout the study. Lactational amenorrhea (when ovulation ceases due to lactation) is one possible explanation for their lack of cycling since each female still had a nursing infant (Dixson, 1998). However, two of the non-cycling females had infants of the same age as two of the cycling females (within two months) and all four of those infants were still suckling. Therefore, these four females presumably conceived and gave birth to their infants at approximately the same time so why did they not all resume cycling at approximately the same time as well? Appendix I lists group compositions and Table 2.2 lists reproductive status of females at the time of the study.

Recabarren *et al.* (2000) describes lactational residual infertility as a period of time after lactational amenorrhea where the female begins to cycle but hormone patterns are abnormal and often anovulatory. If this occurs in *C. capucinus* it may be one explanation for the cycling discrepancy between the cycling and the non-cycling females, as there is likely a degree of individual variation in either the timing of the onset of residual infertility and/or the end of lactational amenorrhea. Lactational residual infertility has also been recorded in chimpanzees (Tutin & McGrew, 1973), Old World monkeys (baboons: Altmann *et al.*, 1978) and some New World monkeys (howler and squirrel monkeys: McNeilly, 1994).

The four cycling females stopped cycling after each experienced between one to three cycles. After the cycles stopped, hormone levels stayed low and fluctuated slightly but never reached or sustained baseline levels compared to the non-cycling females. It is unclear why these four females stopped cycling and did not get pregnant. They may have been experiencing residual infertility and, if so, perhaps these abnormal cycles cease before the "normal" cycles begin, but at present not enough is known about residual lactational infertility in wild primates to further explain this (see Chapter two). The following year (2003), all four of these females gave birth between the months of February to May; therefore, they must have experienced regular conceptive cycles starting in September of 2002 (assuming a 5.5 month gestation period; Nagle & Denari, 1982).

All of the three pregnant females were already pregnant at the beginning of the study, so gestation lengths could not be calculated. Since it is assumed that white-faced capuchins have gestation lengths similar to *C. apella*, of 22 to 23 weeks (Nagle & Denari, 1982), my study females probably conceived between October and December of 2001.

Sexual Behavior

One of the most interesting results of this study was that pregnant females mated more often than any of the other females and they did so almost always with subordinate males. In contrast, the cycling females mated only with the alpha males and the non-cycling females did not mate at all. Furthermore, pregnant females received significantly more sexual solicitations from subordinate males and were over three times more likely to respond positively to solicitations from all males compared to the cycling females. Pregnant and cycling females both received sexual solicitations from the alpha males but cycling females received them at three times the rate. These results raise two questions about mate choice in this species: 1) Why were the pregnant females the most common mating partner for subordinate males and 2) why did the pregnant females choose to mate more often with the subordinate males?

As reported in Chapter two I found that the alpha males intensely followed and maintained a close proximity to cycling females during their periovulatory phases and subordinate males were rarely observed to either sexually solicit or mate with the same periovulatory females. It is possible that the alpha males of *C. capucinus* may have exclusive mating rights to conceptive females and/or group males may not directly compete (*i.e.* with outright aggression) for females during their ovulatory phase. Consequently, mating may be more common between pregnant females and subordinate males simply because lower ranked males are inhibited from mating with conceptive females.

Another explanation for this mating behavior between pregnant females and subordinate males may be related to the elevated levels of stimulating estrogen that pregnant females produce during gestation; however, these levels of estrogen are accompanied by high levels of progesterone – a hormone which has been suggested to act as an 'anti-stimulant'. However, if pregnant females are sexually attractive, then why did the alpha males not solicit the pregnant females as often as they did the ovulating females?

Gordon *et al.* (1991) and Gust (1994) found that alpha male sooty mangabeys (*Cercocebus torquatus atys*) discriminate between a female's fertile and non-fertile or post-conceptive swellings. The researchers found that alpha males only mated with females during their fertile swellings and never mated with pregnant females, whereas subordinate males mated with the pregnant females during their non-fertile swelling phases. They found no difference in the concentrations of estradiol or progesterone between the two types of swellings, but the concentration of lutenizing hormone (LH) was significantly higher in the fertile females. It is possible that the alpha males are sensitive (*i.e.* through smell) to the differences in LH concentrations, and this difference helps them to discriminate between a conceptive and non-conceptive female. There is some evidence that supports this notion that LH stimulates sexual behavior in male rats and male primates; however, it is inconclusive and more research is still needed (see Dixson, 1998).

From the present study it is still unclear why subordinate males would sexually solicit pregnant females when there is obviously no reproductive advantage to this behavior. Gust (1994) could not conclude that the hormonal differences in female sooty mangabeys accounted for the behavioral differences observed between the alphas and subordinate males; but if alpha males can discriminate between female's reproductive status, it is more than likely that subordinate males can do the same. Therefore, it is possible that post-conceptive mating may have little or nothing to do with increasing reproductive fitness but instead functions to build social bonds and increase sexual familiarity and/or reduce tension between males and females (Gust, 1994; Manson *et al.*, 1997).

As to my second question, pregnant females may be selected to mate with subordinate males in order to confuse the male as to the paternity of the soon to be born infant (Hrdy 1974, 1979). Infanticide does occur in this species and it is often carried out by immigrating and/or lower ranked males (Fedigan, 2003). It is argued that if males are uncertain of the paternity of the infant, and if they have a positive past history with the mother, they are less likely to harm an infant and instead will stay in the group to help protect the infant from predators and invading males (Hrdy, 1974; Agrell *et al.*, 1998; Fedigan, 2003).

Previously, I have found that these capuchin females mate more often with the alpha males during their conceptive phase (two out of three copulations), and paternity studies have shown that the alpha males sire almost all of the infants within their groups (Jack & Fedigan, 2003). Therefore, it can be assumed that the pregnant females have already mated and conceived infants with the alpha male during their regular ovarian cycling period. Since lower ranked (and immigrating) males may pose a high risk to a new infant in terms of infanticide, by being receptive to the male's solicitations throughout gestation and even within days of parturition, the pregnant female may form a positive bond with the male and confuse him as to paternity.

Proceptivity and Attractivity

I found that pregnant females groomed the subordinate males significantly more often than did the cycling females, and, even though there was no significant difference, they directed grooming bouts to subordinate males at over twice the rate as the non-cycling females. If the risk of infanticide is high from subordinate males then this finding may support the idea that grooming is being used by females as a strategy to form and maintain good social relationships between individuals (Fedigan, 1992). For instance, pregnant females may use this behavior to form a close bond with lower ranked males in the hope that the male would hesitate to kill her infant if that situation arises (Palombit, 2003). Additionally, the close proximity that is required for grooming means that the female is also accessible to that male for mating.

Cycling females received groom solicits and grooming bouts (attractivity indicators) from alpha males significantly more often than did the non-cycling and pregnant females. Pregnant females also received groom solicits from alpha males, but did so at less than twice the rate compared to the cycling females. Since alpha males conspicuously approach, follow and maintain proximity to females during their ovulating phase, these affiliative behaviors may help the males maintain a close proximity and facilitate potential mating opportunities.

There was no difference in the amount of time cycling, non-cycling and pregnant females spent in close proximity to males but there were interesting differences in which sex and rank maintained the proximity within each of the three groups. For cycling females, the alpha males maintained close proximity in all of the cases that they were involved in whereas subordinate males maintained proximity in just over half. Almost the opposite result was found for pregnant females. In this case, subordinate males maintained proximity in 80% of the cases they were involved in, and in alpha males just over half. These proximity findings lend further support to the sexual behavior I observed because by maintaining a close proximity to a potential mate (pregnant or cycling female) either through affiliative social behaviors like grooming or by just resting within a close distance, males may increase their chances of copulations since females are spatially accessible. However, among the non-cycling females all dyads involving the alpha males were maintained by the females themselves which is completely opposite to the case for the cycling females. Among the three non-cycling females, only one was very high ranking and the other two were low ranking and often remained on the outskirts of the group; therefore, it is possible that the two females were trying to maintain a close proximity to the alpha males in an effort to associate with high ranking individuals. By staying close to the alpha male (and usually other high ranking individuals) and therefore more central within the group, they might have had better access to resources or benefit from greater group protection in the incident of an inter-group encounter or predator attack (Fedigan, 1992).

In sum, the affiliative pericopulatory behaviors displayed and received by female capuchins and the proximity patterns found, appear to facilitate the sexual behavior patterns and mate choices I observed in these white-faced capuchins.

Non-affiliative Behavior

Urine washing did vary significantly between the three groups and it was the cycling females who performed urine washes more often than the pregnant or non-cycling females. In Chapter two I had incorrectly predicted that the rate of this behavior would increase among periovulatory females, which is interesting because it is suggested that a function of urine washing is to advertise ovarian phase (Boinski, 1992). However, in this study, the potentially conceptive cycling females performed more urine washes than the two groups of non-conceptive females (pregnant and non-cycling). Is it possible that one of the functions of urine washing in this species is to advertise reproductive state as opposed to actual ovarian cycle phase (*i.e.* ovulation)? The use of olfaction within anthropoid primates is not as well studied as in prosimians, which are known to use olfactory cues for reproduction and territoriality (Schilling, 1979). The function of this behavior in *C. capucinus* and other anthropoid primates that are thought to conceal their ovulation is definitely worthy of future research in order to further understand how olfaction is used in *Cebids* and other anthropoids.

Conclusions

All of my original predictions about the variations in sexual and pericopulatory behaviors between non-cycling, cycling and pregnant females were supported by the results of this study.

- The rate of sexual behavior (copulations and dance displays) was higher among cycling and pregnant females compared to non-cycling females.

- The majority of the observed copulations occurred between pregnant and subordinate males. Cycling females only copulated with alpha males. Non-cycling females were never involved in any sexual behavior with males.
- Of the proceptivity indicators, pregnant females groomed subordinate males at a significantly higher rate than cycling females and at over twice the rate of non-cycling females.
- Of attractivity indicators, cycling females received groom solicits and grooming bouts at a significantly higher rate from the alpha males compared to non-cycling females and at two times the rate of pregnant females.
- Alpha males maintained proximity to cycling females in every case, subordinate males maintained proximity to pregnant females in over 80% of the cases and non-cycling females maintained proximity to the alpha males in all cases.
- Urine washing occurred at significantly higher rates among the cycling females.

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Chapter Four

General Discussion and Conclusions

In this project I have addressed several aspects of wild white-faced capuchin reproductive behavior as it relates to ovarian hormonal status. This study was the first to use feces to measure ovarian hormones in this species, and to compare hormonal state with corresponding behavioral data. In Chapter two I discussed how the sexual and pericopulatory behaviors vary between the periovulatory and non-ovulatory phases of the ovarian cycle. In Chapter three I compared sexual and affiliative behaviors between non-cycling, cycling and pregnant females. My findings from both chapters have shown that ovarian hormones (estrogen and progesterone) are related to the sexual and pericopulatory behaviors observed in both male and female capuchins. Additionally, I found that *C. capucinus* reproductive behavior may be influenced by the risk of infanticide that is known to occur in this species. I believe that the behaviors I observed in this population of white-faced capuchins provides some evidence to support theories of the evolution of concealed ovulation and the practice of non-conceptive mating for paternity-confusion.

Since this study was the first to measure ovarian hormones in this species, there was no previous research to which I could compare my hormone results. I used information collected on *Cebus apella* to compare cycle and gestation lengths and reproductive behavior but even so, there is limited hormone data that exists on wild populations of *C. apella*. Fortunately, the use of feces to study ovarian hormone patterns in primates has become more "field friendly", which has allowed researchers to conduct non-invasive studies of endocrine function in conjunction with behavior in wild primates. Since fecal hormone analysis was effective for this study, it now opens the door for future endocrine studies to be done successfully with *C. capucinus*.

In Chapter two my purpose was to determine which behavioral cues a female capuchin displays when she is within the periovulatory or conceptive phase of her ovarian cycle. I found that female capuchins display very low rates of proceptive behaviors directed towards adult males during their periovulatory phases, and that the best indicator of a female's conceptive phase is the intensity of the male's attractiveness towards her. I also found some evidence of mate choice and exclusivity in this species. Particularly, alpha males seemed to more often choose the high ranking alpha females for mating compared to the lower ranked females, even though all females cycled at different times. Additionally, alpha males persistently pursued conceptive females whereas the subordinate males rarely solicited or mated with these females.

Female white-faced capuchins are thought to "conceal" ovulation in that the optimum conceptive period of the ovarian cycle is undetectable to males. It has been argued that this helps to confuse paternity in multi-male societies to either promote paternal care or to lower male-male competition to reduce the risk of infanticide (Hrdy 1974, 1979). Female capuchins morphologically conceal ovulation (*i.e.* do not show any sex skin swellings) but do they also behaviorally conceal ovulation from males? The fact that they displayed low rates of proceptive behaviors toward adult males shows that they may not entirely conceal their conceptive state after all. However, the rates were very low and due to my sample size it is premature to conclude that females advertise ovulation with behavioral changes. On the other hand, the males were more attracted to females during the periovulatory phases, which suggest that ovulation is not completely concealed in this species. I propose that either proceptive behaviors (regardless of intensity) or an olfactory cue is being used to advertise this state but more research needs to be done before solid conclusions can be made.

I found that alpha males to mate more often with conceptive females than subordinate males, which has not been proposed for this species before but is not surprising as other species of capuchins also show this behavior (*C. apella*: Janson, 1984; *C. olivaceus*: Robinson, 1988). As well, the recent paternity studies on this population show that alpha males are siring most of the infants (Jack & Fedigan, 2003). What was surprising was that alpha males pursued alpha females more persistently compared to subordinate females during their periovulatory periods, even though none of the females (within each group) had overlapping conceptive phases. I can not fully explain why I observed this behavior as it does not make a lot of sense in regards to the male's reproductive fitness, but I suspect with a larger sample of males and females it would become clearer if this male choice of mates actually exists.

Another surprising finding of my study was that cycling females stopped cycling even though they had not become pregnant. One explanation for this is the possibility of "post-partum lactational residual infertility". If it exists in this species, then the females in my study may have been experiencing anovulatory cycles that ceased before regular cycles began later in the year. However, the exception to this is that one female had never before been pregnant but she also ceased to cycle mid-way through the study. If this is to be understood, then the ovarian cycle of females will have to be monitored throughout entire interbirth intervals.

In Chapter three my purpose was to investigate the differences in sexual and affiliative behaviors among non-cycling, cycling and pregnant females. I found that pregnant females mated more often than any of the other females (including the conceptive females) and they did so more often with subordinate males. As well, subordinate males sexually solicited pregnant females more often compared to other females. Alpha males solicited and mated with cycling females more than any of the other females, which is consistent with my findings from Chapter two. Among the pregnant and cycling females, the affiliative behaviors that I measured appeared to have facilitated the sexual behavior observed by bringing the sexes into closer proximity. Additionally, I found that non-cycling females were never involved in sexual encounters, and participated at low rates in affiliative behaviors with all adult males.

Mating either non- or post-conceptively has been documented in many primate species and it is argued that females may use this behavior as a counter-strategy to reduce the risk of infanticide (Hrdy 1974, 1979). If the risk of infanticide is greatest from immigrating and/or low ranked males within a multi-male group, it would be adaptive for females to confuse paternity by mating promiscuously – including mating while pregnant - to confuse a male of the paternity of the newly born infant. However, for this to be successful, females must trust that males can not associate the timing of mating with the birth of the infant (*i.e.* gestation length). My findings in this study are also consistent with

Manson *et al.* (1997) who found that pregnant white-faced capuchins mated more often than any of the other females and did so with subordinate males.

The reason why subordinate males solicited and mated with pregnant females as often as they did is still unclear. I proposed that subordinate males mated with pregnant females because they are inhibited from mating with conceptive females, since alpha males seem to have exclusive mating rights. I also suggested that males are attracted to the high levels of stimulating estrogens that are produced during pregnancy. However, if this is the case, I would expect that alpha males would be equally interested in pregnant females; but they were not. Similarly to sooty mangabeys (Gorden *et al.*, 1991), alpha male white-faced capuchins may also be able to discriminate between cycling and pregnant females. As mentioned in Chapter three, subordinate males are probably also able to discriminate between females so they may instead be interested in mating with pregnant females to build social bonds and/or increase their sexual familiarity with females.

Both chapters of my thesis have demonstrated that fluctuating ovarian hormones influence the reproductive behavior in both male and female capuchins. Both chapters also reveal that females may have adapted specific reproductive strategies that have helped to ensure male protection of infants, and have helped to reduce the risk of infanticide in multi-male groups. I concluded that cycling females do not conceal their ovulation from males but they do choose to mate with only alpha males. These males are considered to be the most reproductively superior males in the group, and are argued to be the most effective defenders of females and their offspring. On the other hand, pregnant females are choosing to mate promiscuously with subordinate males, perhaps in an attempt to confuse paternity for the males who pose the greatest infanticide risk for their infant.

This project has revealed that there are some very interesting aspects of white-faced capuchin reproductive behavior and ovarian cycling patterns that have not been previously reported to exist in this species. My findings have created many more questions than I have been able to properly address in this thesis. For instance: are there other cues that signal the conceptive phase to males? What is the function of urine washing in the species? Does olfaction in any way play a part in advertising the periovulatory phase of the ovarian cycle? Why did the cycling females stop cycling and not get pregnant? Do females experience a period of residual lactational infertility that functions to increase the interbirth interval? If it does, how does this affect the commencement of regular ovarian cycles? Does male mate choice exist in this species? Do alpha males have exclusive mating rights to conceptive females? What influences subordinate males to actively sexually solicit pregnant females? Further studies on capuchin reproductive behavior is definitely worthwhile. I believe that to fully examine the above questions it would be worth repeating and expanding this study to incorporate more cycling females and observe them over entire interbirth intervals.

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Appendix I

Group Composition Lists for the 2002 Field Season

Cerco de Piedra (CP)

Monkey	ID	Sex	Age Class	*Rank (1=highest)	Maternity/Birth Year
Nose	NN	M	Adult	1	unknown
Trickle	TK	M	Adult	4	unknown
Limp	LL	F	Adult	2	unknown
Nyla	NY	F	Adult	3	Limp/94
Pumba	PP	F	Adult	7	Patches/93
Seria	SS	F	Adult	10	Tuft/89
Timone	TT	F	Adult	9	Seria/96
Rafiki	RR	M	Subadult	6	Limp/96
Simba	SI	F	Lg Imm.	5	Limp/98
Zazu	ZZ	F	Lg Imm.	11	Seria/99
Ed	ED	F	Sm Imm.	8	Pumba/00
Sarabi	SA	F	Sm Imm.	-	Limp/01
Shenzi	SH	F	Sm Imm.	-	Nyla/01
Mowgli	MO	M	Infant	-	Seria/01
Kiara	KI	F	Infant	-	Pumba (born Apr 2002)

Los Valles (LV)

Monkey	ID	Sex	Age Class	Rank (1=highest)	Maternity/Birth Year
Picante	PI	M	Adult	1	unknown
A-1	A1	M	Adult	2	unknown
Chili	CC	M	Adult	5	unknown
Side	SD	M	Adult	10	unknown
Kathy Lee	KL	F	Adult	3	Gringa/89
Dos Leches	DL	F	Adult	4	Gringa/91
Fiesty	FF	F	Adult	10	unknown
Blanquita	BB	F	Adult	9	unknown
Salsa	SL	F	Adult	8	Blanquita/96
Tobasco	TO	M	Subadult	7	Blanquita/94
Alien	AA	M	Subadult	6	Kathy Lee/96
Mayo	MM	F	Lg Imm.	-	Kathy Lee/98
Chutney	CH	F	Lg Imm.	-	Blanquita/99
Caramelo	CA	F	Sm Imm.	-	Blanquita/01
Tahini	TA	M	Sm Imm.	-	Fiesty/01
Mostaza	MO	M	Sm Imm.	-	Kathy Lee/00
Pesto	PE	M	Infant	-	Dos Leches (Mar 2002)
Nutello	NN	M	Infant	-	Salsas (born May 2002)

* rank determined by the number of supplants one individual directed towards another and recorded within a matrix to determine the linear hierarchy

APPENDIX II

C. capucinus Ethogram / Psion behavior codes

Column 1: Type of Data

P - self directed behavior in proximity
C - self directed behavior in contact
S - self directed or socially directed behavior
D - focal directs the behavior
R - focal receives the behavior
T - triadic interaction
O - out of sight
I - focal back in view (insight)
M - mutual,more then focal engaging in activity
D - insert dictophone data
E - edit previous line(s), enter the number of the lines above to edit

Columns 2 & 3: Behavior Codes

Affilative Behaviors:

RE rest (s,p,c)
PF - playface (d.r)
FR - fur rub (s)
PL - play (m)
FL - follow (d,r)
GR - groom (d,r)
GS - groom solicit (d,r)
VI - visual inspect/sniff (d,r)
AP - approach (d,r)
LV - leave (d,r)
FI - food interest (d,r)
SF - steal food (d,r)
RF - resist food share/steal (d,r)
SD - share food (d,r)
CO - climb on (d,r)
CA - carry (d,r)
MO - mount (d,r) (display or play)

Self Directed or Generally Directed Behaviors:

DP - display (s,p,c)
BB - branch break/bounce (s,p,c)
SN - scan (s,p,c)
VG - vigilant (s,p,c)
WA - watch (s,p,c)
SG - self groom (s,p,c)
UW - urine wash (s,p,c)
FO - forage (s,p,c)

VF - visual forage (s,p,c)
DR - drink (s,p,c)
TR - travel (s,p)
SB - sniff branch (s,p,c)
FA - face rub (on a branch or monkey) (s,p,c)
SH - sniffhand (d,r)
FS - fingersuck (d,r)

Aggressive Behaviors:

TF - open mouth threat face (d,r)
BI - bite (d,r)
NI - nip (d,r)
HI - hit (d,r)
PU - push/pull (d,r)
CH - chase (d,r)
SW - swing at (d,r)
LU - lunge (d,r)
HO - hold (d,r)

Triadic / Coalitionary Aggression (enter: T<behaviorcode>actor,victim,partner)

DT - double threat (T)
OL - overlord, with an open mouth threat face (T)
EB - embrace (T)
HF - headflag (T)

Fear and Submission Behaviors:

SU - supplant (d,r)
AV - avoid (d,r)
FG - fear grimace (d,r)
JS - jump startle (s,p,c)
TC - tail chew/tail lick (s,p,c)
NW - nervous watch (s,p,c)

Vocalizations:

BV - bird alarm (s,p,c)
PV - terrestrial predator alarm (s,p,c)
SV - snake alarm (s,p,c)
UA - unknown alarm (s,p,c)
CV - cough vocalization (s,p,c)
FC - food call (s,p,c)
LC - lost call (s,p,c)
TW - twitter (s,p,c,d,r)
CC - contact call (s,p,c)
GL - gargle (s,p,c,d,r)
WH - wheeze (s,p,c,d,r)
XG - sex grunt (d,r)
SQ - sex squeaks (d,r)
SA - scream (s,p,c,d,r)
YE - yelp (s,p,c,d,r)
FQ - fear squeaks (d,r)
TS - threat squeaks (d,r)

CU - coo (s,p,c)
UV - unknown vocalization
BK - bark vocalization
PU - purr vocal.

Sexual Behaviors:

DF - duck face (d,r)
SM - sex mount (d,r)
PA - pace (s,p,c)
PI - pirouette (s,p,c)
TH - thrust (d,r)
DM - dismount (d,r) (either for mating, display or play)

Mother/Infant Behaviors:

NU - nurse (d,r)
RN - refuse nurse (d,r)
JO - jump off back (d,r)
TO - throw off back (d,r)
TA - tantrum (d,r)

Columns 4 & 5: Identification Codes

Dyadic = ID of the monkey interacting with the focal
Triadic = ID of the actor of the behavior
enter: T<behavior code>actor,victim,partner

ID Codes

Unidentified Monkeys and Others:

XX - no idea
XM - unidentified male
XF - unidentified female
XJ - unidentified juvenile
XI - unidentified infant
X1, X2, X3 etc. = unknown multiple monkeys

UU - unidentified allospecific
ZH - howler monkey
ZS - spider monkey
ZK - snake
OO - observer
OH - other humans

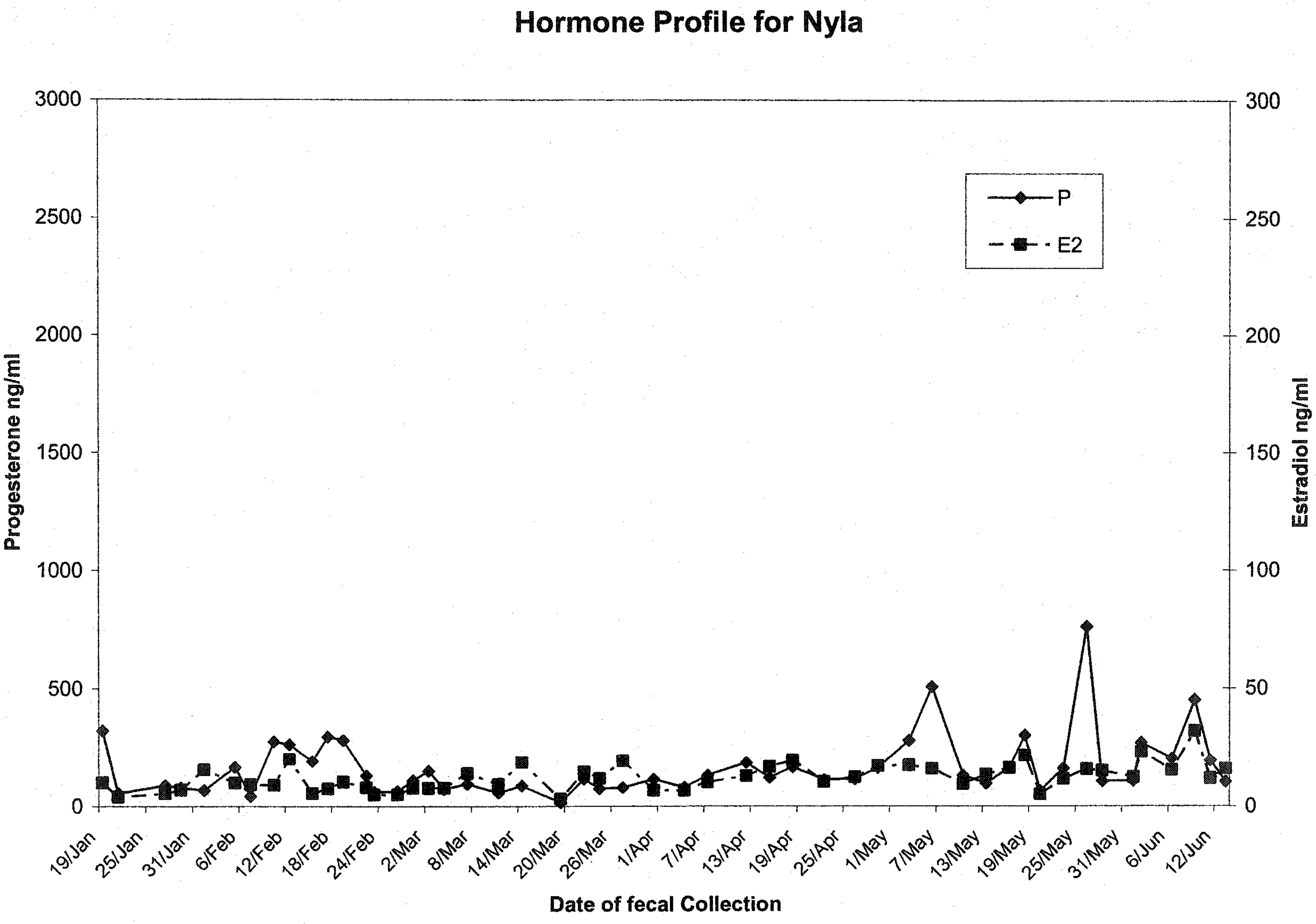
APPENDIX III

Individual hormone profiles for each subject female in 2002.

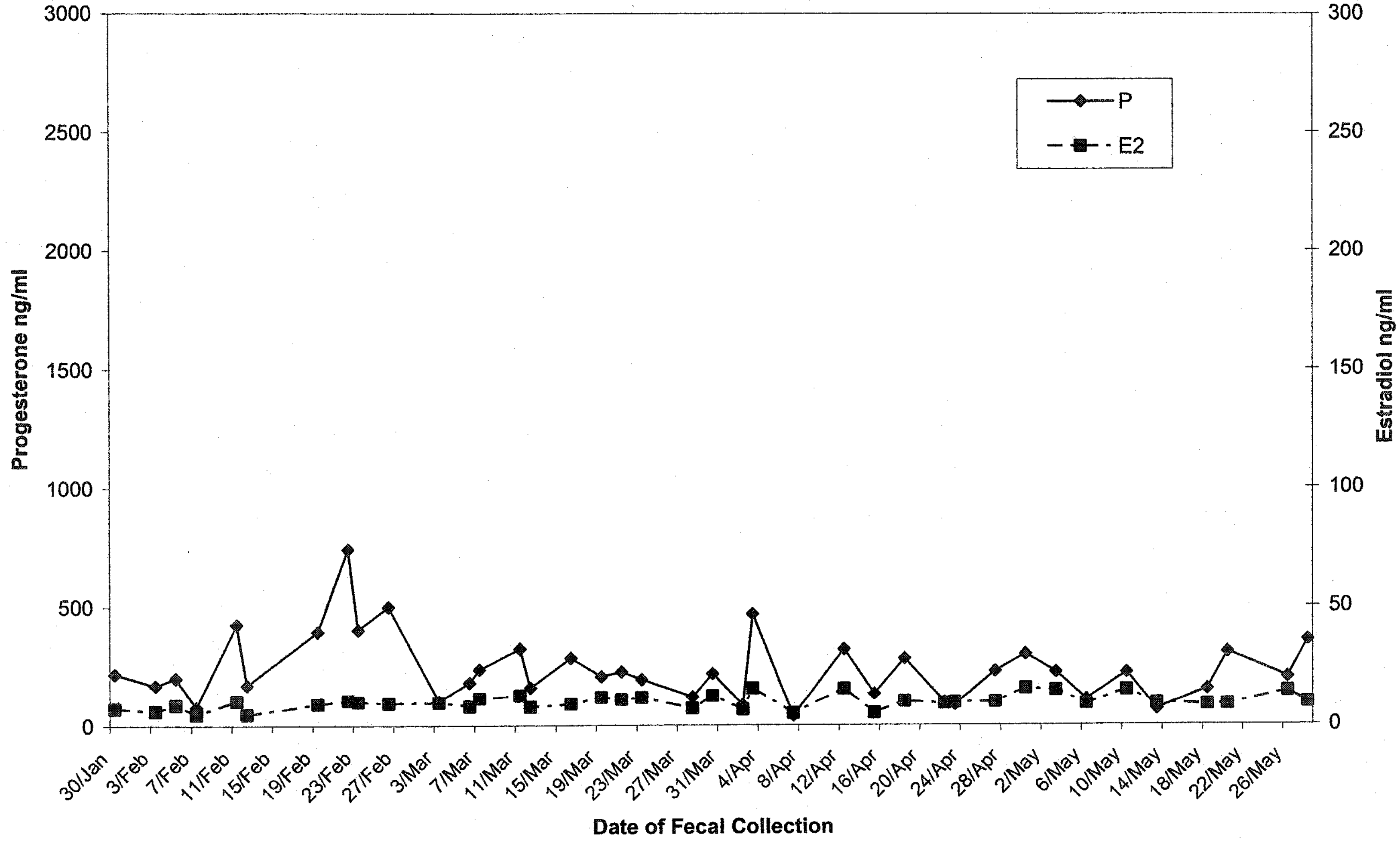
The explanation of how I interpreted the following hormone profiles is found in Chapter two of this thesis. I analyzed about 400 of the 600 samples collected as once the reproductive states of the non-cycling and pregnant females was determined, not all of their samples were assayed. I analyzed all of the samples collected from the cycling females to try and accurately estimate the day of ovulation.

All of the cycling females stopped cycling about mid-way through the study for unknown reasons and did not get pregnant. Some of the profiles are ambiguous and therefore it was difficult to distinguish the individual phases of the ovarian cycle (*i.e.* follicular, periovulatory and luteal). Upon the advice of Toni Ziegler at the National Primate Research Center in Madison, Wisconsin, I compared behavioral differences between the periovulatory and the non-ovulatory phases.

Non-cycling females were easily identified by their non-fluctuating levels of progesterone and estradiol. Pregnant females were identified by their elevated levels of both ovarian hormones, which dropped drastically to baseline levels at parturition (as indicated).


Hormone Profile for Nyla
Progesterone ng/ml
Estradiol ng/ml
3000
2500
2000
1500
1000
500
0
300
250
200
150
100
50
0
P
E2
19/Jan
25/Jan
31/Jan
6/Feb
12/Feb
18/Feb
24/Feb
2/Mar
8/Mar
14/Mar
20/Mar
26/Mar
1/Apr
7/Apr
13/Apr
19/Apr
25/Apr
1/May
7/May
13/May
19/May
25/May
31/May
6/Jun
12/Jun
Date of fecal Collection

Hormone Profile for Fiesty



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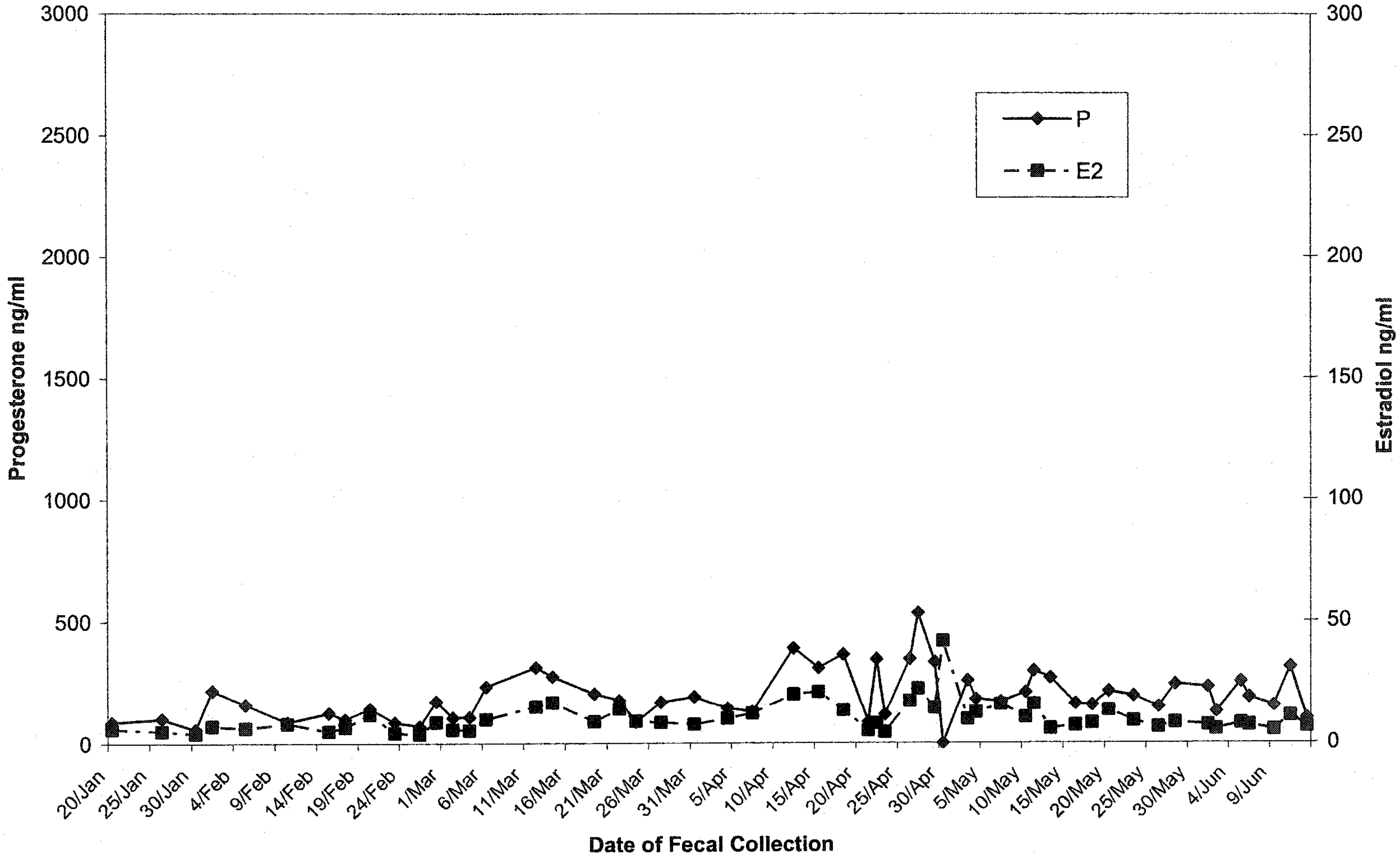
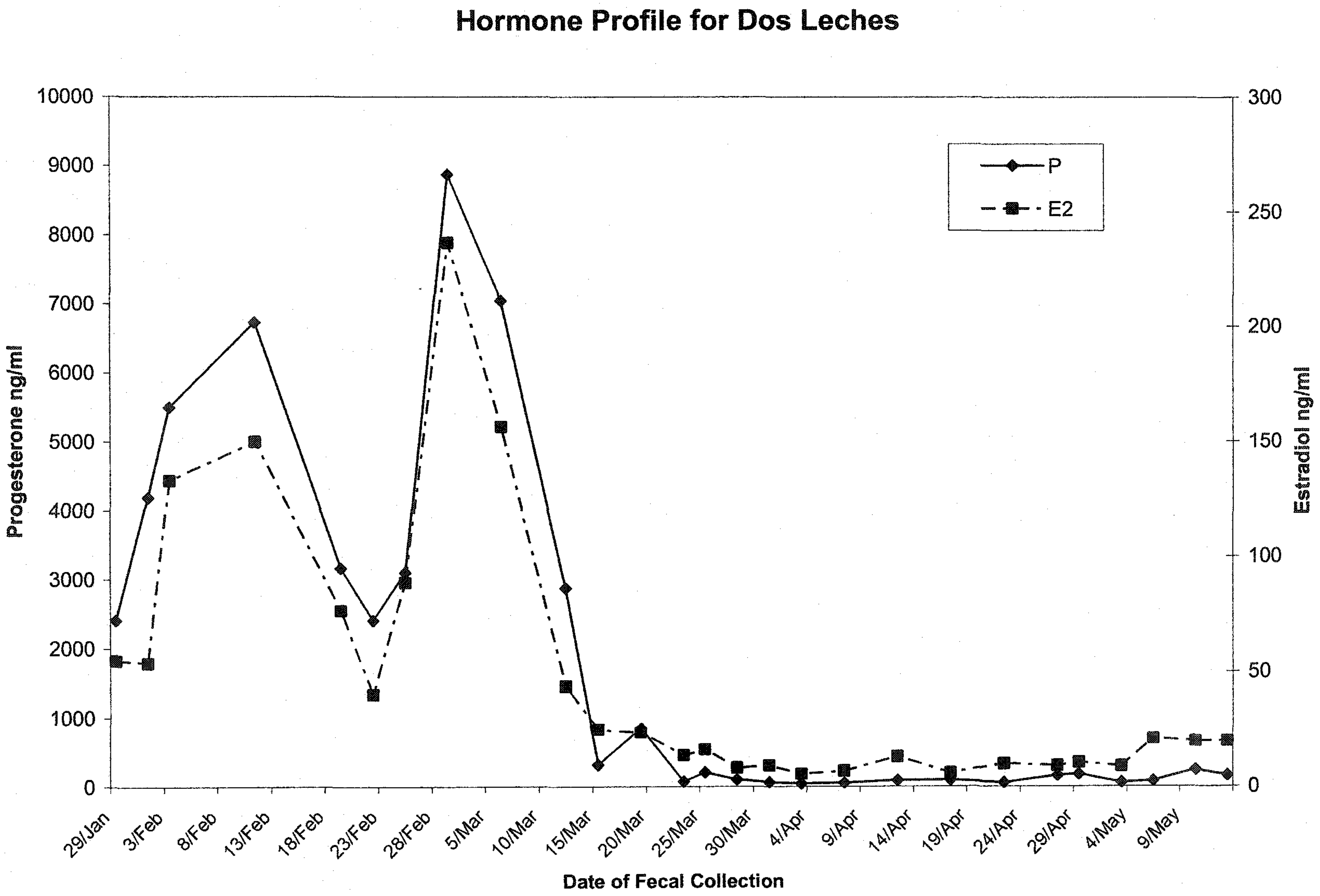
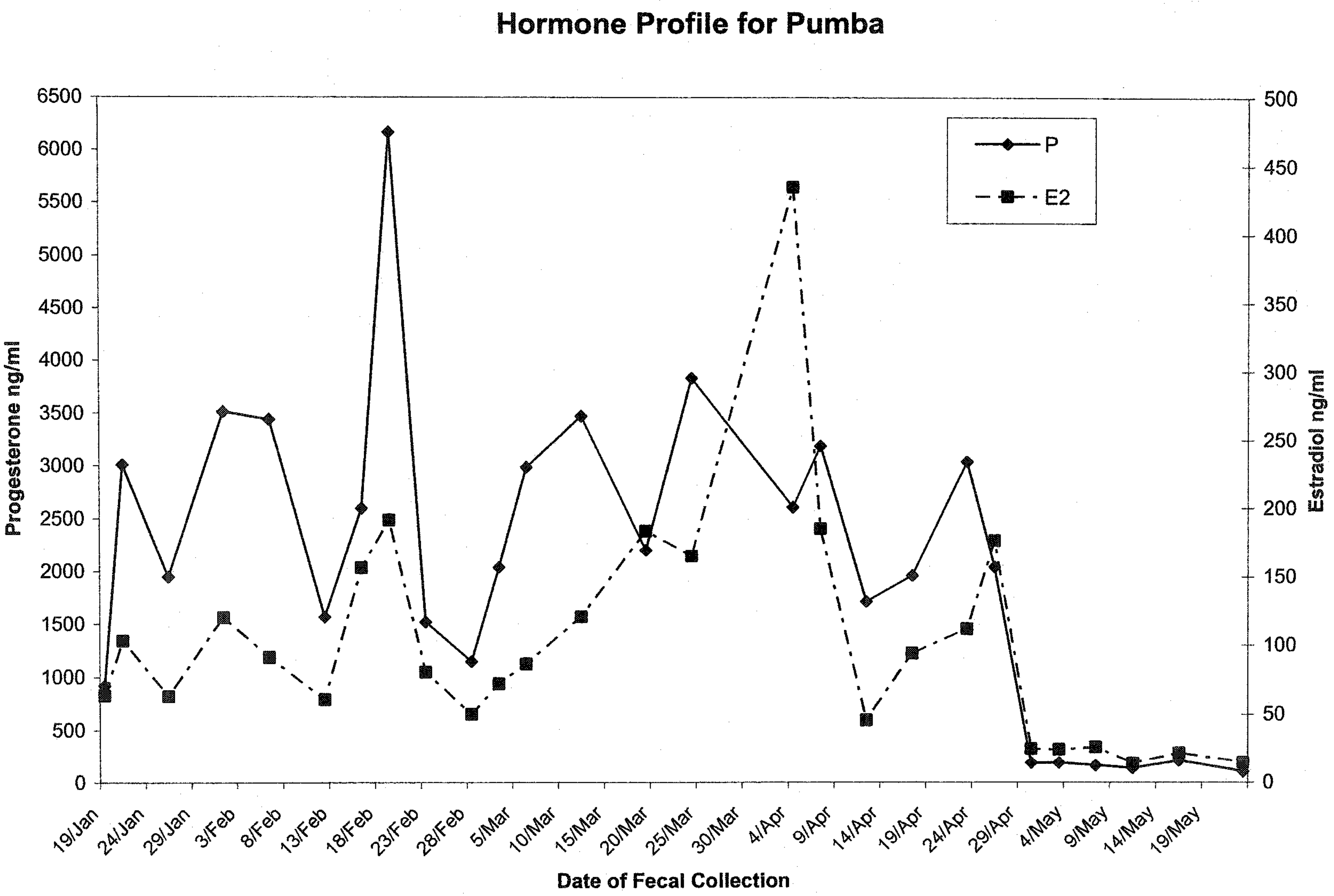




Hormone Profile for Dos Leches


Hormone Profile for Pumba
P
E2
Progesterone ng/ml
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Estradiol ng/ml
500
450
400
350
300
250
200
150
100
50
0
19/Jan
24/Jan
29/Jan
3/Feb
8/Feb
13/Feb
18/Feb
23/Feb
28/Feb
5/Mar
10/Mar
15/Mar
20/Mar
25/Mar
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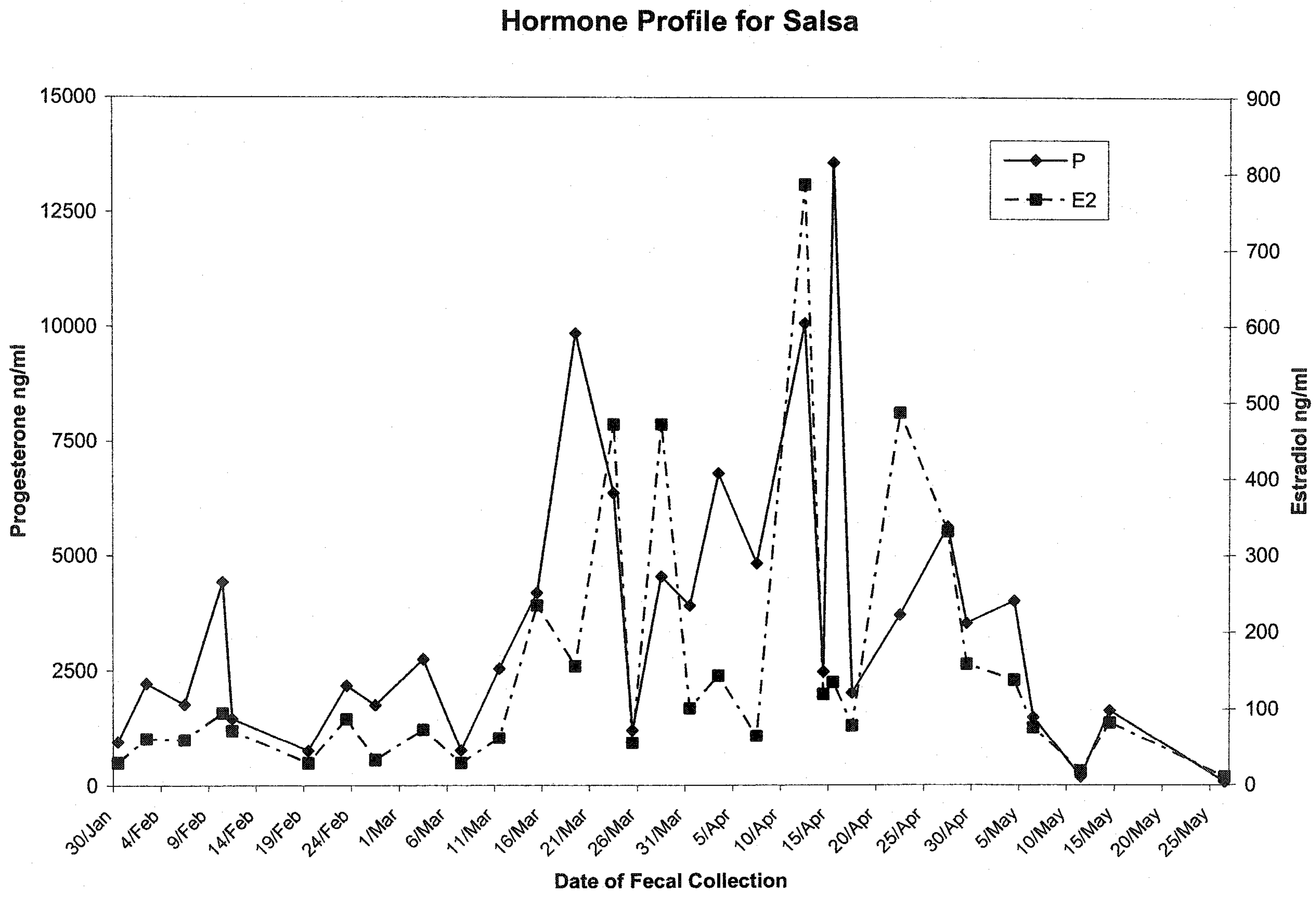
14/May
19/May
Date of Fecal Collection


Hormone Profile for Salsa
Progesterone ng/ml
15000
12500
10000
7500
5000
2500
0
Estradiol ng/ml
900
800
700
600
500
400
300
200
100
0
P
E2
30/Jan
4/Feb
9/Feb
14/Feb
19/Feb
24/Feb
1/Mar
6/Mar
11/Mar
16/Mar
21/Mar
26/Mar
31/Mar
5/Apr
10/Apr
15/Apr
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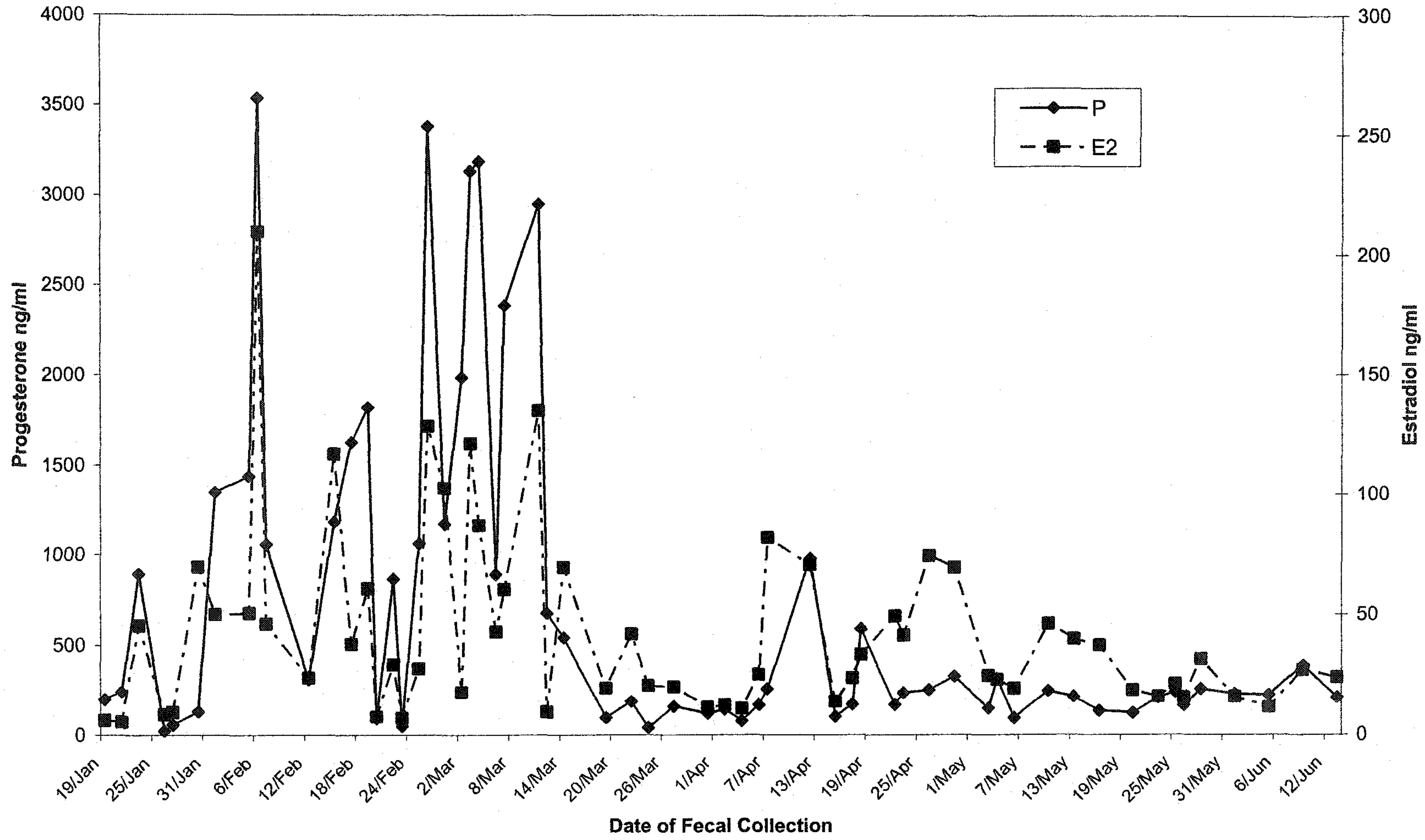
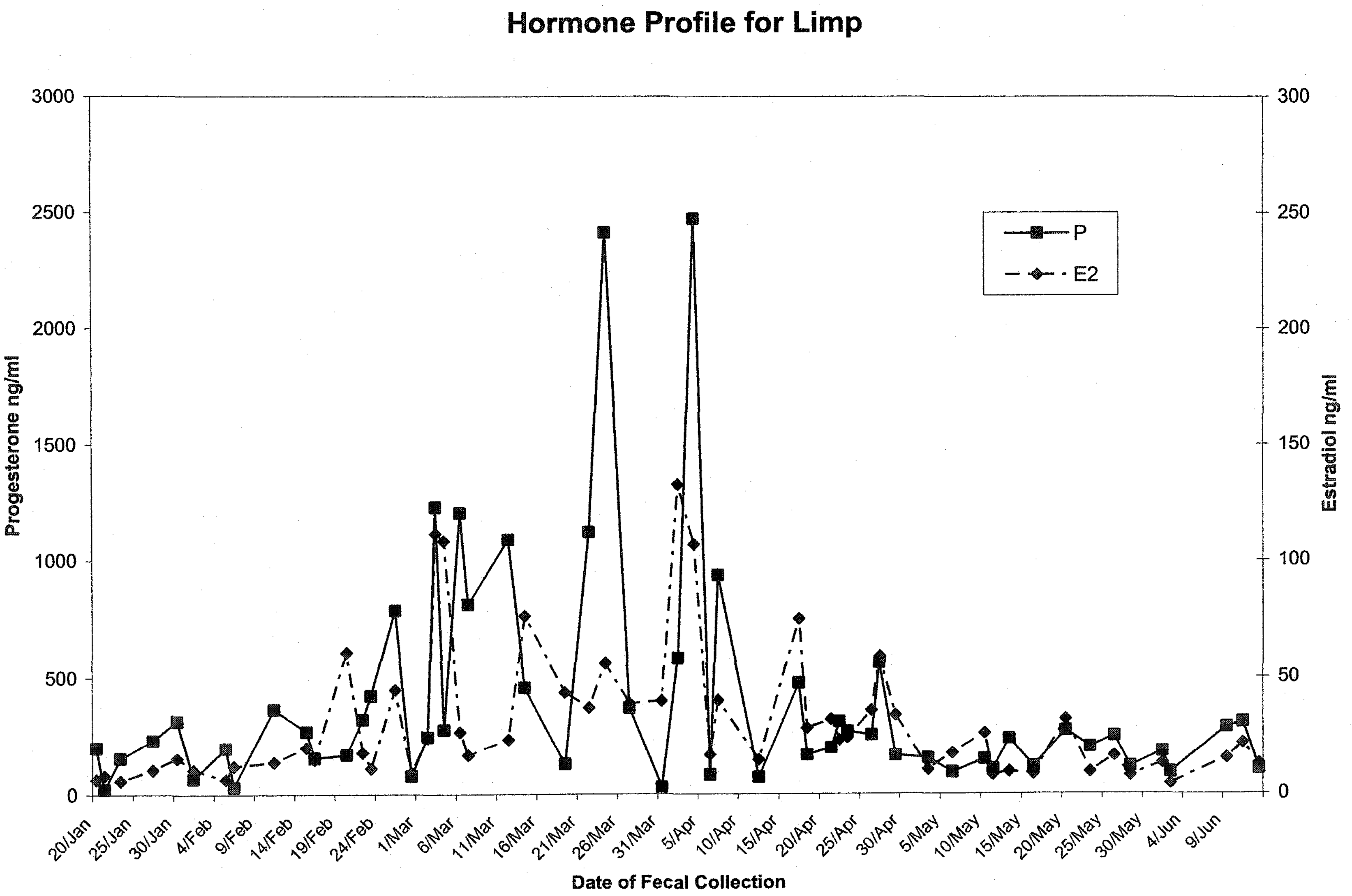
5/May
10/May
15/May
20/May
25/May
Date of Fecal Collection

Hormone Profile for Timone

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Estradiol ng/ml
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2500
2000
1500
1000
500
0
300
250
200
150
100
50
0
P
E2
20/Jan
25/Jan
30/Jan
4/Feb
9/Feb
14/Feb
19/Feb
24/Feb
1/Mar
6/Mar
11/Mar
16/Mar
21/Mar
26/Mar
31/Mar
5/Apr
10/Apr
15/Apr
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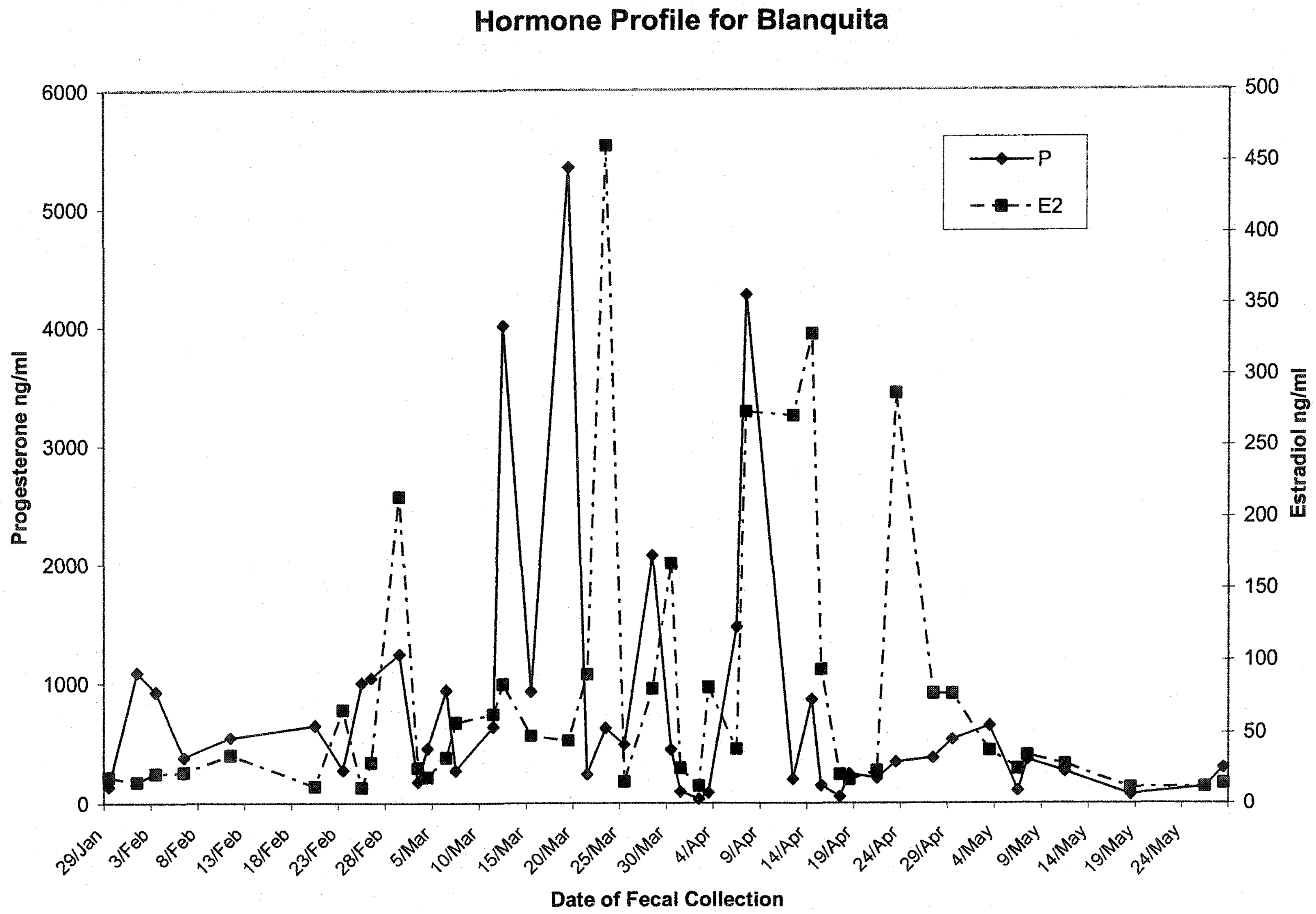
4/Jun
9/Jun
Date of Fecal Collection


Hormone Profile for Blanquita
Progesterone ng/ml
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1000
2000
3000
4000
5000
6000
Estradiol ng/ml
0
50
100
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200
250
300
350
400
450
500
P
E2
29/Jan
3/Feb
8/Feb
13/Feb
18/Feb
23/Feb
28/Feb
5/Mar
10/Mar
15/Mar
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25/Mar
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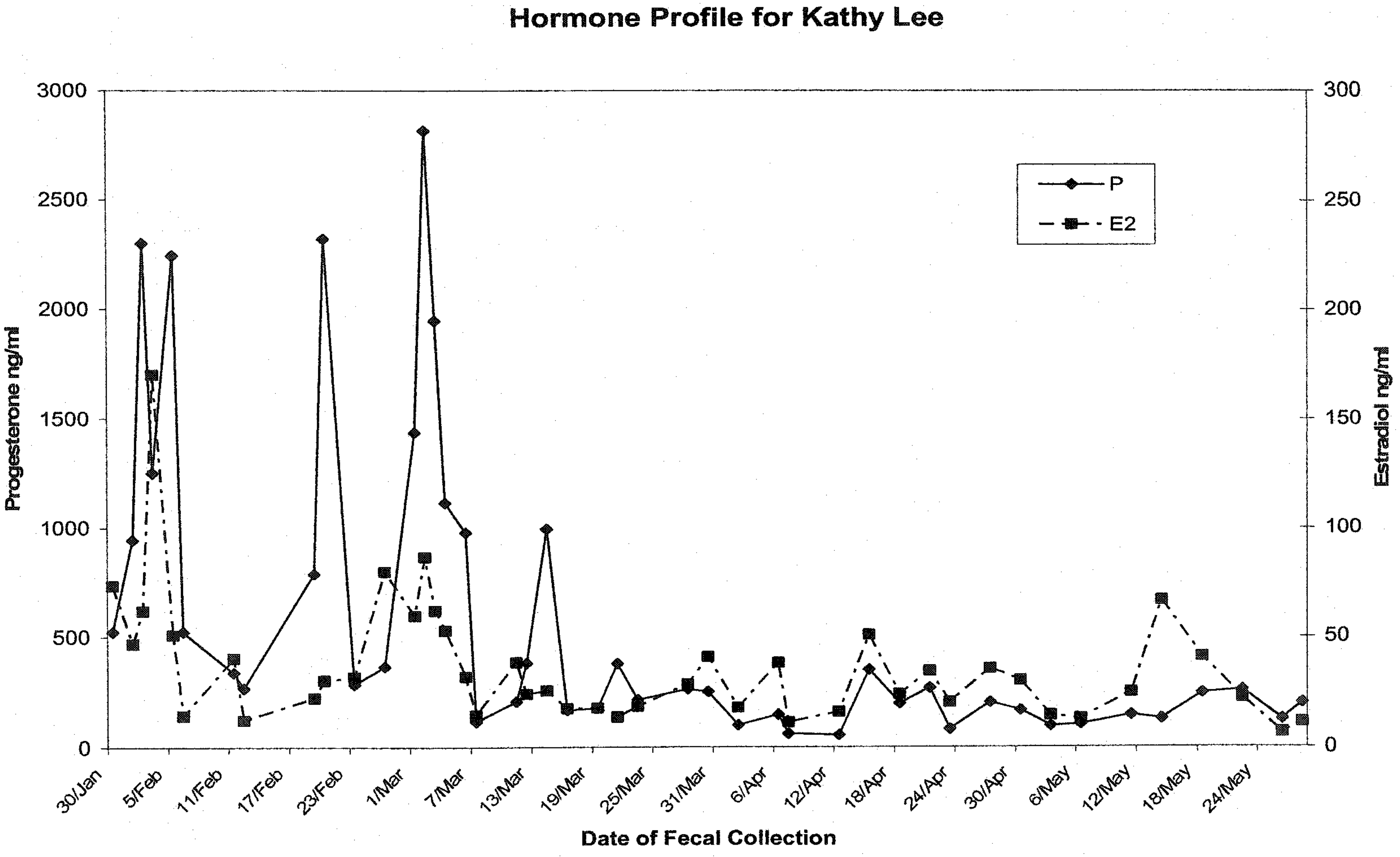
19/May
24/May
Date of Fecal Collection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3000
2500
2000
1500
1000
500
0
Estradiol ng/ml
300
250
200
150
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0
P
E2
30/Jan
5/Feb
11/Feb
17/Feb
23/Feb
1/Mar
7/Mar
13/Mar
19/Mar
25/Mar
31/Mar
6/Apr
12/Apr
18/Apr
24/Apr
30/Apr
6/May
12/May
18/May
24/May
Date of Fecal Collection