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**Fecal Concentrations of Cortisol, Testosterone, and Progesterone in Cotton-Top  
Tamarins Housed in Different Zoological Parks: Relationships among Physiological  
Data, Environmental Conditions, and Behavioral Patterns**

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The aim of this investigation was to study the welfare of three captive groups of cotton-top tamarins housed in different zoological parks. Ethological observations were conducted over one year. In addition, fecal samples were collected and the concentrations of glucocorticoids,

androgens, and progestagens were measured. Within each group, no significant differences in fecal cortisol concentrations were found between subjects. The fecal concentrations of testosterone and progesterone significantly differed depending on the sexes and ages of the tamarins. A significant association was found among hormone concentrations, exhibit dimensions, and group composition. A highly significant correlation was found between all hormones considered and the space available for each subject. Significant differences in behavioral patterns were observed among groups, including social-individual, affiliative-aggressive, and anogenital-suprapubic scent marking. Correlations between hormone measurements and behaviors were detected. In conclusion, this study confirmed the associations between some behaviors exhibited by these nonhuman primates and both cortisol and testosterone; these data also highlight the role played by progesterone in these behaviors.

**Keywords:** captive management, hormones, stress, *Saguinus oedipus*

As the issue of nonhuman animal welfare has become increasingly important, many studies have assessed animal well-being in captivity (Candland & Bush, 1995; Kleiman, Allen, Thompson, & Lumpkin, 1992; Maple & Perkins, 1996; Moran & Sorensen, 1984), and zoological facilities now pay close attention to providing species with appropriate environments. Each species has different needs, so it is important to assess the best parameters for managing a given species in captivity to maintain animal health. This topic becomes even more essential when working with species at high risk of extinction and captive individuals who can be reintegrated into the wild.

Ethological observations are very important in the study of animal welfare (Hosey, 2005; Edwards, Sorkin, Rhodes, & Petersson, 2009). In nature, all species exhibit characteristic behavioral patterns, and the maintenance of these patterns in captivity is generally considered a sign of well-being. On the other hand, behaviors that differ from those in the wild, such as malaise, may be due to group composition (Box & Morris, 1980; Li, Jiang, Tang, & Zeng, 2007). Recent studies have considered numerous factors, including group composition, exhibit size (Burrell & Altman, 2006), environmental enrichment (Shepherdson, Mellen, & Hutchins, 1998; Savage, 1999; Mallapur, 2005), species-specific behaviors that usually occur in nature (Edwards et al., 2009), group social structure (Hershkowitz, 1977), and average size (Ferrari & Ferrari, 1989; Tardif, Richter, & Carson, 1984). However, most of these studies have examined behavioral aspects only and neglected the endocrine component, which is known to be of great importance in maintaining organisms' homeostasis and therefore adaptation to their environments and well-being.

Consequently, it is very important to analyze hormone levels, even if their fluctuations are not always easy to understand. The ability to collect physiological and behavioral data is of paramount importance for evaluating the stress responses and welfare of captive animals. To assess the impact of stress comprehensively, multiple components of the stress response (e.g., hormonal, behavioral, and immunological factors) should be monitored, and the links among these components should be considered as well (Peel, Vogelnest, Finnigan, Grossfeldt, & O'Brien, 2005).

One good indicator of stress is the measurement of cortisol levels (Möstl & Palme, 2002; Keay, Singh, Gaunt, & Kaur, 2006; Hoffman, Ayala, Mas-Rivera, & Mastripiéri, 2010). The primary factor responsible for differences among animals in cortisol levels may be social conflict (Peel et al., 2005). Moreover, constant high cortisol concentrations may be related to an unsuitable exhibit or group composition, or even to an inappropriate diet

(Cameron, 1996). In addition, environmental factors, such as temperature extremes and drought, are also considered stressors. These factors typically have seasonal patterns and may be responsible for changes in baseline cortisol concentrations (Sapolsky, 1986; Cavigelli 1999 cited in Weingrill, Gray, Barrett, & Henzi, 2004).

Stress may also be due to abiotic factors, including artificial lights, sounds, smells, exhibit substrates, and temperature, as well as environmental factors, such as small areas, forced proximity to humans, routine management, and unusual social groups (Mallapur, 2005; Hosey & Skyner, 2007; Morgan & Tromborg, 2007). In captivity, individuals are expected to exhibit species-specific baseline metabolic hormone concentrations, but fluctuations from baseline levels are expected to occur in association with social or induced stressors, as reported by several authors (e.g., Moberg & Mench, 2001; Palme, Rettenbacher, Touma, El-Bahr, & Möstl, 2005; Peel et al., 2005).

Little is known about how the hormones testosterone and progesterone vary in stressful situations, such as cases of prolonged isolation. Testosterone has been linked to aggressive behaviors and social rank (Czoty, Gould, & Nader, 2008), whereas progesterone is usually related to affiliative patterns (Carter, 1992; Alencar et al., 1995; Maestriperi, 1999; Razzoli, Cushing, Carter, & Valsecchi, 2003). To date, stressful situations have only been shown to affect cortisol levels (Reimers, Schwarzenberger, & Preuschoft, 2007).

This study aimed to examine the welfare of cotton-top tamarins. Cotton-top tamarins (*Saguinus oedipus*) are listed under Appendix I of the *Convention on International Trade in Endangered Species of Wild Fauna and Flora* (CITES, 1973). These animals are characterized as “highly endangered” on the *International Union for Conservation of Nature Red List* (IUCN, 2010). Protection of this species in captivity is therefore essential.

*S. oedipus* is currently housed in many zoological gardens. The animals are considered easy to manage in captivity due to their small body size and high level of

sociability. However, aggressive episodes often occur, especially among animals who are housed in large groups (Snowdon & Pickhard, 1999). In captivity, individuals cannot migrate, set up their own social groups, or compete with other individuals to reproduce (Snowdon & Pickhard, 1999). Additionally, in comparison to conspecifics in the wild, captive *S. oedipus* often display inactivity and a decrease in social behaviors, such as contact and play (Glatston, Geilvoet-Soeteman, Hora-Pecek, & van Hooff, 1984).

The present work was designed to study the welfare of three colonies of cotton-top tamarins, considering a broad context of captive management. Behavioral approaches (ethological observations) and physiological measurements (monitoring cortisol, testosterone, and progesterone levels) were employed. The study groups were chosen to represent the different social groups, exhibit types, and management procedures, which are generally found in structures housing this species. We investigated the potential relationships among hormones, the social composition of groups, reproductive states, and environmental factors. We hypothesized that cortisol as well as testosterone and progesterone (which share the precursor cholesterol) are involved in stress-induced processes. According to our hypotheses, the levels of cortisol and testosterone increase during aggressive events, whereas progesterone would decrease in the same context.

## **MATERIALS AND METHODS**

### **Study Groups**

This research was carried out on three captive groups of cotton-top tamarins housed at the Pistoia Zoological Gardens (Pistoia—PT), Garda Zoological Park (Verona—VR), and Punta Verde Zoological Park (Udine—UD), all located in Italy. All outdoor areas were equipped according to the European Association of Zoos and Aquaria (EAZA) Husbandry Guidelines for the Callitrichidae (Carroll, 2002).

The Pistoia Zoological Gardens group consisted of two adult males (Table 1): Jens, who was permanently castrated; and Lou, who was intact. The group was housed in an exhibit with an internal area of 8 m<sup>2</sup> and a height of 2.9 m. This area was heated during the cold season so that the temperature never fell below 12 to 14°C. The group also had access to an outdoor area of 12 m<sup>2</sup> with a height of 2.9 m. A guillotine door managed access between the different environments.

The food supply was based on fruits and vegetables, combined with eggs and moths. Every morning the animals were provided with two spoons of Altromin MA610 Rieper. An agglomerate of Arabic gum was always available. Food was given twice per day in a bowl. The individuals were observed from October to December 2008 for a total of 283 hr. A total of 79 fecal samples were collected.

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Table 1 about here

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The Garda Zoological Park group was composed of nine individuals: six adults (four females and two males), two sub-adults (one female and one male), and one infant male born in April 2008 (Table 2). Zio and Zia were reproductively active, but were not breeding. All other subjects were the offspring of Mocio and Mamma. This group was housed in an internal area of 20.10 m<sup>2</sup> with a height of 3 m, located inside a tropical greenhouse where the temperature never fell below 18°C. The group also had access to an outdoor area of 12 m<sup>2</sup> with a height of 2.9 m. The internal area was equipped like the outdoor area, with branches and rich vegetation. The internal and outdoor areas were linked by a tunnel that was 10.28 m long, and access was managed by a guillotine door.

The diet of the Garda Zoological Park group was composed of vegetables, fruits, Arabic gum, eggs, boiled rice, insects, and “pappa Milupa” (homogenized food), with



integration of turkey and ricotta. Food was given twice per day in three bowls placed at a distance from each other to avoid feeding priority aggression. The individuals were observed from October to November 2008 for a total of 200 hr. A total of 49 fecal samples were collected.

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Table 2 about here

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The Punta Verde Zoological Park group consisted of two adult individuals, a mother and her son. Another female, Meta, was housed separately in a section that was not available to visitors. Only fecal samples, and no observation data, were collected for Meta (Table 3). The group was located in an internal area of 2.47 m<sup>2</sup> with a height of 1.90 m, linked to an outdoor area by a wooden bridge. The outdoor area was an island, approximately 14 m long and 4.5 m wide, surrounded by a ditch approximately 1 m wide filled with running water and without grates. These areas were separated by a guillotine door. During the cold season, the internal area was heated by two lamps and one stove. Meta was housed in an indoor area of 2.64 m<sup>2</sup> with a height of 2.40 m. Through a guillotine door, she had access to an outdoor area of 3.8 m<sup>2</sup> with a height of 2.30 m.

Food was provided in the internal area twice per day in a bowl. The morning meal consisted of multigrain cream, a teaspoon of honey, and 3 g of PROTOVIT. In the evening, the subjects were supplied with fruits, vegetables, insects, eggs, and yogurt. These individuals were observed during October 2009 for a total of 245 hr, and 114 fecal samples were collected in total.

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Table 3 about here

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## **Behavioral and Physiological Data Collection**

Data collection was carried out between sunrise and sunset. In total, the cohort was observed for over 728 hr. Ethological observations of all group members ( $n = 13$ , excluding Meta) were carried out via two methods: sampling all occurrences of some behaviors and ad libitum sampling (Altmann, 1974). We based our observations on an ethogram, corresponding to a part of the ethogram published by Edwards et al. (2010). We recorded individual behaviors (i.e., feeding, scent-marking, self-grooming, and resting) and social activities (i.e., allo-grooming, affiliative, aggressive, sexual, and play behaviors).

Fecal samples of all subjects ( $n = 14$ , including Meta) were collected every morning during the behavioral observations, whenever defecation was observed and the identity of the animal was known. The samples were stored in a freezer at  $-20^{\circ}\text{C}$ . After every sampling period, the fecal samples were transferred in a refrigerated bag to the Dipartimento Morfofisiologia Veterinaria e Produzioni Animali of Bologna University for radioimmunoassay. In total, 240 fecal samples were collected.

## **Hormone Measurements**

Cortisol, testosterone, and progesterone concentrations were determined by radioimmunoassay (RIA). All concentrations were expressed in pg/mg of fecal matter. The extraction methodology was modified from the methods of Schatz and Palme (2001) and Accorsi et al. (2008). Five ml of a methanol:water (4:1 v/v) solution were added to 60 mg (wet weight) of feces in capped-glass tube vials. The vials were then vortexed for 30 min using a multitube pulsing vortexer. After centrifugation at  $1,500 \times g$  for 15 min, 5 ml ethyl ether (BDH Italia, MI, Italy) and 0.2 ml  $\text{NaHCO}_3$  (5%; Sigma Chemical Co., St. Louis, MO) were added to 1 ml of supernatant. This preparation was vortexed for 1 min and centrifuged

for 5 min at  $1500 \times g$ . The ether portion was aspirated with a pipet, and evaporated under an airstream suction hood at  $37^{\circ}\text{C}$ . The dry residue was redissolved into 0.5 ml of 0.05 M phosphate-buffered saline (PBS; pH 7.5).

Cortisol, testosterone, and progesterone were assayed in the fecal samples according to the methods of Tamanini, Giordano, Chiesa, & Seren, (1983), Gaiani, Chiesa, Mattioli, Nannetti, & Galeati (1984), and Seren, Leopold, & Bolelli (1974), respectively. The validation parameters of the analyses were as follows:

**Cortisol:** sensitivity 3.10 pg/100  $\mu\text{l}$ ; intra-assay variability 6.8%; interassay variability 9.3%; specificity (%): cortisol 100, corticosterone 9.5,  $11\alpha$ -hydroxyprogesterone 8.3, cortisone 5.3,  $11\alpha$ -desoxycortisol 5.0, progesterone 0.6, desoxycorticosterone 0.5,  $20\alpha$ -dihydrocortisone 0.4, testosterone 0.3, aldosterone 0.1, dehydroepiandrosterone  $<0.0001$ ,  $5\alpha$ -pregnenolone  $<0.0001$ ,  $17\beta$ -estradiol  $<0.0001$ , and cholesterol  $<0.0001$ .

**Testosterone:** sensitivity 2.80 pg/100  $\mu\text{l}$ ; intra-assay variability 5.7%; and interassay variability 10.1%; specificity (%): testosterone 100,  $5\alpha$ -dihydrotestosterone 30.6, androstenedione 0.6, epitestosterone  $<0.01$ , dehydroepiandrosterone  $<0.001$ , progesterone  $<0.001$ , pregnenolone  $<0.001$ , estrone  $<0.001$ , and cortisol  $<0.001$ .

**Progesterone:** sensitivity 2.72 pg/100  $\mu\text{l}$ ; intra-assay variability 9.28%; interassay variability 13.95%; specificity (%): progesterone 100,  $11\alpha$ -hydroxyprogesterone 83.3,  $11\beta$ -hydroxyprogesterone 15.7,  $17\alpha$ -hydroxyprogesterone 1.7,  $20\alpha$ -hydroxyprogesterone  $<0.1$ , cortisol 0.05, testosterone  $<0.002$ ,  $17\beta$ -estradiol  $<0.0001$ , and estrone  $<0.0002$ .

To determine the parallelism between hormone standards (Sigma Chemical Co.) and endogenous hormones in cotton-top tamarins, fecal samples from three different animals, containing high concentrations of endogenous hormones (100  $\mu\text{l}$ ), were serially diluted with 0.05 M PBS, pH 7.5 to obtain volumes of 50, 25, 10, and 5  $\mu\text{l}$ . Parallelism was assessed

between these serial dilutions and hormone standards prepared in buffer ranging in concentration from 7.81 to 1000 pg/100  $\mu$ l for cortisol, from 3.90 to 500 pg/100  $\mu$ l for testosterone, and from 3.90 to 1000 pg/100  $\mu$ l for progesterone.

### **Statistical Analyses**

All statistical tests were conducted with SPSS (version 17.0) and were two-tailed. A  $p$ -value less than 0.05 was considered statistically significant. Comparisons of concentrations of the same hormone in two subjects were performed using the Student's  $t$ -test, and Levene's test was applied to verify whether the variances were equal. Multivariate analysis of variance (MANOVA) was conducted to determine if statistically significant differences existed in the average levels of hormonal concentrations due to exhibit dimension (in  $m^3$ , divided into categories of large, medium, and small), group composition (large, small, and isolation), and area ( $m^2$ ) available per individual.

A post-hoc Tukey's test was then applied to determine which combination was effectively significant. Ethological data were analyzed using contingency tables; the  $\chi^2$  test was applied to compare observed and expected frequencies (in the case of  $2 \times 2$  tables, the Yates' correction was applied). Spearman's correlation coefficients were computed between the hormone levels (i.e., testosterone, progesterone, and cortisol) and the behaviors (i.e., affiliative, aggressive, and scent-marking behaviors) observed in each subject. A nonlinear regression test was used to assess parallelism between standard and endogenous hormones.

## **RESULTS**

### *Hormonal Responses*

A high degree of parallelism ( $p < 0.01$ ) was observed between the hormone curves of the standards and the diluted endogenous samples, with a parallel drop in percent binding as the sample volumes and hormone standard concentrations increased.

Single-subject hormone concentrations (pg/mg) are shown for the subjects at Pistoia Zoological Gardens and Punta Verde Zoological Park, whereas the data for subjects from the Garda Zoological Park were grouped into three categories (males, females, and infant). For each subject (or category), the polynomial trendline is indicated (Figures 1, 2, and 3), and MANOVA was carried out for all groups. No significant differences in cortisol concentrations were detected between individuals in a given group (Figure 1).

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Figure 1 about here

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Differences in testosterone concentrations (Figure 2) were found in the Pistoia Zoological Gardens data between the intact and castrated males, with higher concentrations in the intact male ( $F = 26.576, p < 0.0001; t = 4.750, p < 0.0001$ ). There were differences in testosterone concentrations in the Garda Zoological Park data between males and females, with higher concentrations in the males ( $F = 3.234, p = 0.079; t = 2.130, p = 0.039$ ). Lastly, there were differences in testosterone concentrations in the Punta Verde Zoological Park data between all subjects (Cartagena-Bogotá:  $F = 1.046, p = 0.312; t = 2.007, p = 0.051$ ; Cartagena-Meta:  $F = 2.912, p = 0.94; t = -4.284, p < 0.0001$ ; and Bogotá-Meta:  $F = 6.686, p = 0.013; t = -6.476, p < 0.0001$ ).

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Figure 2 about here

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Differences in progesterone levels (Figure 3) were detected in the Pistoia Zoological Gardens data between the intact and castrated males, with higher levels in the castrated male ( $F = 6.448, p = 0.13; t = 2.392, p = 0.019$ ). Differences in progesterone levels were found in the Garda Zoological Park data between females and the infant, with higher levels in the infant ( $F = 13.396, p = 0.001; t = -1.120, p = 0.342$ ). There were differences in progesterone levels in the Punta Verde Zoological Park data between all subjects, with the highest levels in the isolated female Meta and the lowest levels in the male Bogotà (Cartagena-Bogotà:  $F = 0.824, p = 0.369; t = 3.914, p < 0.0001$ ; Cartagena-Meta:  $F = 7.604, p = 0.008; t = -5.984, p < 0.0001$ ; and Bogotà-Meta:  $F = 2.262, p = 0.139; t = -11.424, p < 0.0001$ ).

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Figure 3 about here

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There were highly significant correlations among hormone concentrations (Table 4), exhibit dimension (Table 5), group composition (Table 6), and the area (in m<sup>2</sup>) available for each subject (Table 7), according to MANOVA and the post-hoc Tukey's test.

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Table 4 about here

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Table 5 about here

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Table 6 about here

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Table 7 about here

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### **Behavioral Patterns**

We focused on three pairs of related behavioral patterns: social-individual, affiliative-aggressive, and anogenital-suprapubic scent marking. In Figures 4 and 5, the frequencies of behaviors exhibited by single subjects (converted to an hourly relative frequency) are shown for Pistoia Zoological Gardens and Punta Verde Zoological Park (data for the female Meta are not available). Data for the subjects at the Garda Zoological Park are grouped into three categories (males, females, and infant). The  $\chi^2$  values are reported for each pair of behaviors for comparisons made between individuals within each group and among all groups.

For social and individual behaviors (Figure 4), differences were found between observed and expected frequencies within the group (Pistoia Zoological Gardens,  $\chi^2 = 177.608$ ,  $p < 0.0001$ ; Garda Zoological Park,  $\chi^2 = 149.909$ ,  $p < 0.0001$ ; Punta Verde Zoological Park,  $\chi^2 = 668.281$ ,  $p < 0.0001$ ) as well as among all groups in the cohort ( $\chi^2 = 11194.974$ ,  $p < 0.0001$ ). For affiliative and aggressive behaviors (Figure 5), no differences were detected between individuals in the Pistoia Zoological Gardens data ( $\chi^2 = 2.369$ ,  $p = 0.124$ ). In contrast, differences in affiliative and aggressive behaviors were observed in the data from Garda Zoological Park between males, females, and the infant ( $\chi^2 = 285.410$ ,  $p < 0.0001$ ) and in the data from Punta Verde Zoological Park between the individuals ( $\chi^2 = 105.466$ ,  $p < 0.0001$ ).

There was a difference in affiliative and aggressive behaviors among all groups ( $\chi^2 = 737.820$ ,  $p < 0.0001$ ). For anogenital and suprapubic scent marking (Figure 6), the only difference was observed at Punta Verde Zoological Park between the two individuals ( $\chi^2 = 1182.399$ ,  $p < 0.0001$ ), and there were no significant differences in the data from Pistoia

Zoological Gardens ( $\chi^2 = 0.544, p = 0.461$ ) and Garda Zoological Park ( $\chi^2 = 3.352, p = 0.187$ ). However, for anogenital and suprapubic scent marking, there were differences in these behaviors among all groups ( $\chi^2 = 1146.107, p < 0.0001$ ).

### **Correlations between Hormones and Behaviors**

Application of Spearman's test showed there were three correlations in the data from Pistoia Zoological Gardens (Lou's testosterone levels and received affiliative behaviors,  $r_s = -0.700, p = 0.016$ ; Jean's progesterone levels and performed aggressive behaviors,  $r_s = 0.613, p = 0.045$ ; and Lou's progesterone levels and scent-marking behaviors,  $r_s = 0.861, p = 0.001$ ). There were also three correlations for the data from Punta Verde Zoological Park (Cartagena's cortisol levels and performed aggressive behaviors,  $r_s = -0.717, p = 0.030$ ; Bogotà's cortisol levels and performed affiliative behaviors,  $r_s = -0.655, p = 0.021$ ; and Bogotà's cortisol levels and received affiliative behaviors,  $r_s = -0.757, p = 0.004$ ). No significant correlations were detected for the data from Garda Zoological Park.

## **DISCUSSION**

The goal of the present study was to examine the welfare of the individuals belonging to three colonies of cotton-top tamarins, considering a broad context of captive management and by applying both behavioral observations and physiological measurements. Cortisol, testosterone, and progesterone were retrieved from fecal samples to minimize disturbances to the animals. Indeed, although various methods are available for collecting physiological data, extracting hormones from feces is noninvasive and minimizes interaction between the observer and subjects (Keay et al., 2006). In addition to its advantages for animal welfare, this form of noninvasive sampling allows for repeat sampling and comprehensive long-term



monitoring to gain a more complete understanding of the dynamics of the stress response (Peel et al., 2005).

## **Cortisol**

In our study, cortisol concentrations did not significantly differ among members of the same colony. Fecal cortisol concentrations are considered to be a good indicator of stress and adaptation to the environment (Möstl & Palme, 2002; Yamaguchi, Kikusui, Takeuchi, Yoshimura, & Mori, 2005). Therefore, this result may reflect good adaptation of all animals to the environments in which they are housed.

In contrast, in the among-colony comparison, the multi-male/multi-female group from Garda Zoological Park exhibited the highest cortisol concentrations, especially in the early weeks of the observation period when the most aggressive events were recorded. This association between high cortisol concentrations and a high frequency of antagonistic events is in agreement with a previous report (Coe et al., 1978). The high fecal cortisol concentrations in this group may be due to the greater number of group subjects and social instability (Creel, 2001). We observed much more aggressive behavior in this colony than in the other colonies. Internal group struggles may have been taking place to establish a hierarchy, especially because young individuals were exploring their role within the colony.

Social instability can lead to stress, as demonstrated in baboons by Gesquiere et al. (2011), and it may be related to the social role (alpha or beta) of the individuals themselves. For alpha subjects, chronic activation of the stress axis depends on the continuous challenges that the animals must undertake to maintain their ranks, as well as on the higher energetic and psychological costs required to maintain their social role within the group. For beta individuals, activation depends on the strain caused by the persistent expectation of possible aggression by dominant individuals (Gesquiere et al., 2011).

Environmental conditions, seasonal variations, and diet can affect hormone levels, but we believe that these factors did not affect our results. The zoological gardens housing the three study groups complied with the EAZA Husbandry Guidelines for the Callitrichidae (Carroll, 2002) and have a shared geographical area. Therefore, several factors potentially affecting hormone levels may be excluded, including artificial lights, exhibit substrate, smells, sounds, forced proximity to visitors, temperature extremes, and drought.

Several authors have linked changes in cortisol levels with the season (Lynch, Ziegler, & Strier, 2002; Behie, Pavelka, & Chapman, 2010; Gesquiere et al., 2008). In our study, the variation in cortisol levels between the colonies cannot be attributable to climatic conditions because the collection of fecal samples was carried out in the same period (i.e., October to November/December 2008 and October 2009). Furthermore, some authors (Barrett, Shimizu, Bardi, Asaba, & Mori, 2002; Fichtel, Kraus, Ganswindt, & Heistermann, 2007; Lynch et al., 2002; Strier, Ziegler, & Wittwer, 1999) reported that competition for access to mates creates social stress for male primates. In seasonally breeding species, cortisol levels increase immediately before or during the mating season. We did not consider this possible factor because the males were outside of their mating season.

Another possible cause of variation in cortisol concentrations is the animals' diet. Indeed, some primates show increased cortisol levels when food availability is low, including chimpanzees (*Pan troglodytes*; Muller & Wrangham, 2004) baboons (*Papio anubis*; Sapolsky, 1986), and ring-tailed lemurs (*Lemur catta*; Cavigelli, 1999). The diets fed to the animals during this study were qualitatively and quantitatively very similar and filled the daily needs of the animals. Therefore, we believe that this variable cannot account for the differences in cortisol levels among the colonies.

## **Testosterone**

The testosterone measurements were similar to the cortisol measurements. In the Pistoia Zoological Gardens group, the intact (dominant) male displayed significantly higher testosterone levels than the castrated one, whereas in the Garda Zoological Park (multi-male/multi-female) group, the males showed higher concentrations of testosterone than the females. As with cortisol, the higher testosterone concentrations principally occurred in the first weeks of observation. Once again, these observations underline the relationship between higher testosterone concentrations and more aggressive behaviors.

Testosterone concentrations are closely associated with aggressive behaviors and are related to the species and to the animal's social standing. High testosterone concentrations have been reported in species with polygamous males who are characterized by a strong commitment to territory defense and mate acquisition (Jonsen, 1998; Wingfield, Hegner, Dufty, & Ball, 1990; Gleason, Fuxjager, Oyegbile, & Marler, 2009). However, in species with monogamous males who are also involved in parental care, testosterone concentrations only increase when the males engage in aggressive behaviors, returning to baseline concentrations after the aggressive interactions (Cavigelli & Pereira, 2000; Creel, Wildt, & Monfort, 1993; Ross, French, & Patera, 2004; Wingfield et al., 1990). In contrast to the scenario for cortisol, Gesquiere et al. (2011) observed that alpha and beta males had equally high testosterone concentrations.

Our data from the Punta Verde Zoological Park (mother-son) group are more difficult to interpret. The highest testosterone concentrations were exhibited by the female Meta, which may be due to her isolation. The relationships among testosterone concentrations, social isolation, group density, and territoriality are well known, although some data appear to be contradictory (Shimozuru, Kikusui, Takeuchi, & Mori, 2008). Moreover, the effects of social deprivation vary according to age at the time of isolation (neonatal, post-weaning, adulthood), the type of isolation (from the mother, from peers), and the length of time (Hall,

1998). For example, in male rats, isolation increases motor activity (Einon & Morgan, 1978), aggression (Wongwitdecha & Marsden, 1996), anxiety (Wright, Upton, & Marsden, 1991), and endocrine responses to stressful events (Weiss, Pryce, Jongen-Relo, Nanz-Bahr, & Feldon, 2004).

Behavioral changes induced by rearing in isolation vary according to species (Weiss, Di Iorio, Feldon, & Domeney, 2000); for example, isolation induces more major changes in mice than in rats (Einon, Humphreys, Chivers, Field, & Naylor, 1981; Syme, 1973). The effects of social deprivation are influenced by the species' social structure. Subjects belonging to gregarious species show larger changes in behavioral and neuroendocrine/chemical patterns as a result of social deprivation than animals belonging to nonsocial species (Shimozuru et al., 2008).

In addition, testosterone is implicated in the regulation of aggressiveness in females, although with species-dependent differences (Ross et al., 2011). Increased testosterone levels and aggressive interactions have been associated with dominance rankings in wild female baboons (Beehner, Phillips-Conroy, & Whitten, 2005). French, Schaffner, Shepherd, & Miller (1995) reported that aggressive behavior in females differs depending on the species and on their reproductive mechanisms. Females also display higher testosterone concentrations during encounters with other females than during encounters with males, even if the aggression does not involve contact between subjects.

### **Progesterone**

As expected, progesterone concentrations significantly differed between the intact and the castrated males, with higher levels in the castrated male (Pistoia Zoological Gardens group). In the Punta Verde Zoological Park (mother-son) group, the progesterone concentrations significantly differed among the subjects. Moreover, we detected a difference between the

colonies from Pistoia Zoological Gardens (male-male) and Garda Zoological Park (multi-male/multi-female), which have low values, and the Punta Verde Zoological Park (mother-son) colony, where the progesterone concentrations were very high for all subjects.

The difference in fecal progesterone concentrations among colonies is likely related not only to the sex of the members, but also to their ages and to the reproductive activities of the females. At Punta Verde Zoological Park (mother-son group), the female Meta exhibited typical ovarian activity, with high progesterone concentrations in the luteal phase and lower concentrations in the follicular phase of the estrous cycle. In this species, the estrous cycle occurs approximately every 15 days (Hershkowitz, 1977).

Similarly, as observed for cortisol and testosterone, progesterone concentrations may also be related to female aggressiveness. The aggressive behaviors at Garda Zoological Park (multi-male/multi-female group) were greater than those observed in the other colonies, and the females' behaviors were similar to those expressed by males. In females, progesterone plays an important role in controlling these behaviors, and the concentration changes according to the period of the reproductive cycle.

Moreover, in some species, the aggressive behaviors of females are similar to those seen in males during intraspecific encounters (Mayer & Rosenblatt, 1987; Stockley & Bro-Jorgensen, 2011; Vom Saal, Franks, Boechler, Palanza, & Parmigiani, 1995). The few studies that have examined female aggressive behaviors and the function of hormones not associated with maternal roles have reported contradictory results. Research carried out on Siberian hamsters (Scotti, Place, & Demas, 2007) and California mice (Davis & Marler, 2003) failed to uncover any association between gonadal steroid hormones and female aggressiveness.

## **Group Composition and Exhibit Dimensions**

Previous studies showed that parameters such as group composition and exhibit type affect animal welfare and consequently the stress response (Li et al., 2007). We verified there is a significant relationship between these parameters and hormone concentrations. As expected, the level of significance varied depending on the parameter pairs examined. In particular, both exhibit type and group size were significantly related to testosterone and progesterone levels. Small dimension ( $m^2$ /subject) was associated with high testosterone and low progesterone levels. Isolation was associated with an increase in testosterone levels.

It is difficult to discriminate between the effects of group composition and exhibit type because they influence each other, and together they deeply affect animal welfare. Therefore, it seems more appropriate to discuss them together. Previous research has shown that the amount of space available to animals can affect their cortisol concentrations, particularly in relation to the animals' ranks. In pens that are too small, subordinate subjects display higher cortisol concentrations due to their inability to escape from dominant aggressive behaviors (McLeod, Moger, Ryon, Gadbois, & Fentress, 1996). The low involvement of cortisol in our data is not surprising because the concentrations of cortisol within each colony were similar, indicating that the tamarins had adapted to their housing and to their group dynamics.

Hierarchical reorganization within a group and the formation of new groups are highly stressful events for animals (Bartoš, Schams, Bubenik, Kotrba, & Tománek, 2010), as observed in the Garda Zoological Park (multi-male/multi-female) group. During the observation periods in this study, no new subjects were introduced to any colony. New introductions can be stressful, even without hierarchical changes within the group (Bartoš et al., 2010).

In our study, fecal testosterone concentrations seemed to be strongly influenced by group composition and exhibit type, and this was most likely related to the role this hormone

plays in territorial behavior and in social rank. Previous research demonstrated that social isolation modifies testosterone basal concentrations in rodents (Sachser, 1986; Sayegh, Kobor, Lajtha, & Vadasz, 1990). However, the results are contradictory; in rats, for example, Fulgheri, Di Prisco, & Verdarelli (1975) and Gamallo, Villanua, Trancho, & Fraile (1986) observed an enhancement of endocrine responses, whereas Esquifino, Chacon, Jimenez, Reyes Toso, & Cardinali (2004) and Sanchez, Aguado, Sanchez-Toscano, & Saphier, (1995) reported its suppression. Group composition and exhibit type also affect fecal progesterone concentrations, but in this case we suggest that the cyclic activity of the animal may influence this effect.

We also considered the relationship between the number of square meters available per subject and hormone concentrations. Our observations appear to contradict previous reports. We detected no significant differences in testosterone and progesterone levels, and a borderline significant difference for cortisol, for areas between 5 m<sup>2</sup> and 33 m<sup>2</sup> per subject. We suggest that these results may be partially influenced by dynamics among group members. The contradiction between our observations and previously reported results may be attributable to the difficulty in separating the effects of different variables (group composition, exhibit type, and square meters available per subject) because they are closely connected. Again, group dynamics are likely far larger determinants than the single variables taken separately.

In the groups from Pistoia Zoological Gardens (male-male) and Punta Verde Zoological Park (mother-son), where only two subjects were present, there were few interactions between group members, although *S. oedipus* is a social species and social relations play a key role in group life. In Garda Zoological Park, where the multi-male/multi-female composition of the group was much closer to natural conditions, interactions were more frequent; nonetheless, this colony was unstable during the observation period.

Instability often occurs in captive conditions, where individuals are not free to leave the group and tend to compete among themselves (Snowdon & Pickhard, 1999). In the Garda Zoological Park (multi-male/multi-female) group, reconciliation behaviors were also noted. These behaviors are often observed after aggressive events (Strier, 2003; Konecki et al., 2007). Affiliative behaviors, such as grooming, are often manifested to strengthen social bonds (Strier, 2003) and to create alliances between group members (Kappeler & Pereira, 1996; Strier, 2003; Konecki, Luecke, & Fuentes, 2007).

### **Marking Behavior**

In *S. oedipus*, chemical communication occurs primarily through two specialized glands located in the anogenital and suprapubic areas. These glands are much more developed in females than in males, and females mark more often than males (Omedes & Carrol, 1980). In particular, anogenital scent marking is expressed with greater frequency than suprapubic marking (French & Snowdon, 1981). In this study, members of all colonies displayed much more anogenital scent marking than suprapubic marking, and this behavior was performed especially by females. It is interesting to note that the castrated male marked very often, particularly in comparison with the intact male. Castration may favor feminine-type behaviors, although this hypothesis contrasts with the findings of Epple (1979).

### **Correlations between Hormones and Behaviors**

Finally, we analyzed the correlations between hormones and behaviors. Cortisol has been reported to increase in relation to attacks both performed (Abbott et al., 2003) and received (Ziegler, Scheffler, & Snowdon, 1995; Abbott et al., 2003). The increase in testosterone level is linked to aggressive behavior and subjects who suffer aggressive episodes have lower testosterone levels (Czoty et al., 2008). On the other hand, progesterone



is generally linked to affiliative events (Archer, 1991; Carter, 1992). Therefore, we expected a positive correlation between these two variables. Because scent marking is predominantly a female behavior (Omedes & Carroll, 1980), we investigated its correlation with sex hormones, particularly progesterone.

We observed positive correlations between cortisol and testosterone levels and aggressive behaviors in agreement with the correlations reported in the literature. In contrast, the positive correlation between Jens's progesterone level and performed aggressive behaviors disagrees with previous studies. Other factors may affect progesterone levels, but we cannot exclude the possibility of a link between progesterone and stress. The positive correlation between Lou's low progesterone levels and scent marking may be explained by assuming that marking behavior, which is predominantly performed by females, may be related to progesterone, a female sex hormone.

## CONCLUSION

In conclusion, the results of this study allow us to confirm the roles of cortisol and testosterone in determining some behaviors exhibited by *S. oedipus* and highlight the role played by progesterone in these behaviors. Cortisol is a good indicator of animal welfare within each colony, and it is correlated with aggressive, affiliative, received, and performed behaviors. Testosterone was linked to hierarchical and performed affiliative behaviors. In addition, testosterone is considered an indicator of malaise in animals subjected to social deprivation, although this hypothesis requires further investigation. Progesterone also seems to be involved in aggressive behaviors, which are not related to the reproductive phases of females, as well as marking behavior. Progesterone's possible role as a stress indicator deserves further study.

Despite differing proportions, the fecal concentrations of the three hormones measured in this study were influenced by group composition, exhibit type, and the number of square meters available for each subject, although it is difficult to extrapolate the importance of each variable on these hormonal changes. We think that the importance of these variables to animal welfare depends on other factors, such as the dynamics of the social group. Large differences often exist between the compositions of groups in captivity and in the wild; in many cases, captive groups consist of few individuals, who are easier to manage. For this reason, it is important to balance the needs of the zoological gardens and the animals' well-being. It is therefore critical to analyze the behavioral and physiological aspects of small groups carefully.

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**FIGURE 1** Cortisol concentrations. Polynomial trendlines are indicated for the observation periods at (A) Pistoia Zoological Gardens (Lou, grey dots, continuous trendline; Jens, black triangles, broken trendline), (B) Garda Zoological Park (males, black squares, continuous trendline; females, grey dots, broken trendline; infant, grey triangles, dotted trendline), and (C) Punta Verde Zoological Park (Cartagena, grey dots, continuous trendline; Bogotà, black squares, broken trendline; Meta, grey triangles, dotted trendline).

**FIGURE 2** Testosterone concentrations during the observation periods at (A) Pistoia Zoological Gardens, (B) Garda Zoological Park, and (C) Punta Verde Zoological Park. Symbols and trendlines are as defined in Figure 1.

**FIGURE 3** Progesterone concentrations during the observation periods at (A) Pistoia Zoological Gardens, (B) Garda Zoological Park, and (C) Punta Verde Zoological Park (C). Symbols and trendlines are as defined in Figure 1.

**FIGURE 4** Social and individual behaviors at (A) Pistoia Zoological Gardens, (B) Garda Zoological Park, and (C) Punta Verde Zoological Park. Values are expressed in hourly relative frequencies.

**FIGURE 5** Affiliative and aggressive behaviors at (A) Pistoia Zoological Gardens, (B) Garda Zoological Park, and (C) Punta Verde Zoological Park. Values are expressed in hourly relative frequencies.

**FIGURE 6** Anogenital and suprapubic scent marking at (A) Pistoia Zoological Gardens, (B) Garda Zoological Park, and (C) Punta Verde Zoological Park. Values are expressed in hourly relative frequencies.