# Fitness Consequences of Group Living: Investigating Hormones and Behavior In the Colonial Tuco-Tuco *Ctenomys sociabilis*

By

Julie Ann Woodruff

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Committee in Charge:

Professor Eileen A. Lacey, Chair

Professor George E. Bentley

Professor Darlene D. Francis

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Abstract

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For many species, a critical component of an animal's environment is its social setting, specifically whether an animal lives alone or with conspecifics. Living in a group may confer benefits associated with cooperation but almost invariably comes with costs resulting from conflict and competition. In contrast, living alone may reduce costly interactions with conspecifics but likely requires greater effort when caring for young, finding food, or avoiding predators. My research investigates the adaptive (fitness) and physiological consequences of group living using the social system of the colonial tuco-tuco (*Ctenomys sociabilis*), a subterranean rodent endemic to southwestern Argentina. Unlike most species in the genus *Ctenomys*, colonial tuco-tucos are social with burrow systems shared by 2 – 6 females and, sometimes, one male. However, not all females live in groups. About one-third of the burrow systems in the population are comprised of females that have dispersed to live alone, providing a rare opportunity to explore the effects of naturally occurring social variation on the fitness and physiology of individuals living in groups and alone.

Previous studies of *C. sociabilis* have shown that living alone is associated with significant differences in survival and direct fitness compared to remaining in the natal group. Because social setting may also substantially impact proximate factors such as individual physiology, I examined the effects of intraspecific differences in social setting on measures

of baseline glucocorticoid (GC) levels. GCs are adrenal steroids critical to maintaining homeostasis. As such, GCs provide an appropriate gauge of the physiological response to social and physical stressors. By combining data on GC variation in free-living animals with experimental manipulation of housing conditions for captive individuals, I tested the hypothesis that living and breeding alone are associated with increased GC levels in this species. I also investigated how group size and composition correlate with GCs in group-living individuals. These resulting data indicate the social environment is an important determinant of baseline GC levels and that these effects vary with group composition. Collectively, these analyses yield important new insights into physiological consequences of sociality.

In colonial tuco-tucos, variation in the social environment also includes whether or not an adult male is present in the communal nest of this plural-breeding rodent. Due to high mortality, males are not found in all burrow systems. When a male is present in a colony, the time spent in the nest with pre-weaned offspring does not differ than that of females. Male assistance with young is rare for mammals, and for this part of the study, I tested two major hypotheses explaining male next attendance – the parental effort hypothesis and the mating effort hypothesis. Combining evidence from the field and lab, I found that male nest attendance did not increase the number or growth of young, nor future access to the females in the burrow system. However, the presence of an adult male increased the survival of male – but not female – pups. This result, along with observations in the lab indicating that adult males affected the pubescent onset of aggressive interactions between male pups, suggests that they may play a critical role in the development of male offspring, thereby affecting survival.

This work is dedicated in loving memory of my parents, Donald and Suzanne, who instilled in me a love of nature and a sense of self-worth, without which this great adventure could never have been undertaken, and to my kids, Jon, Chris, Andrew and Beth, who are a constant reminder of what's most important in this life. I love you.

… and to the cutest rodent on the face of this planet, the tuco-tuco!

# INTRODUCTION

Exploring the ultimate and proximate underpinnings of social behavior is critical to understanding the evolution of complex interactions between individuals. While exciting discoveries have come from both field and lab studies of the endocrine bases for social behavior, few have integrated information from animals in captive and "real" environments to gain insights into the interplay between physiology and behavior in a natural context. This work combines field and lab data to examine the endocrine bases for sociality in a plural-breeding rodent from southwestern Argentina, the colonial tuco-tuco (*Ctenomys sociabilis*). Using this non-model system (for which a detailed demography and comprehensive understanding of the natural history exists), I combined observations of a free-living population with manipulations of captive individuals to test hypotheses regarding the adaptive and physiological consequences of living in a group.

Hormones have been shown to coordinate appropriate behavioral responses to various environmental conditions – both social and physical (McEwen and Wingfield, 2003; Goymann and Wingfield, 2004). Among these are glucocorticoids, which are adrenal steroids produced upon activation of the hypothalamic pituitary adrenal axis. Glucocorticoids mediate physiological responses to environmental challenges by stimulating glucose release in order to maintain homeostasis, which can ultimately affect fitness and survival (Munck et al., 1984; Sapolsky et al., 2000; Romero 2004). Most mammalian studies investigating the effects of the social environment on glucocorticoids have examined singular breeding social systems in which a distinct hierarchy between dominant and subordinate individuals exists (Creel 2001). In contrast, my research examines a plural breeding social system, in which all female group mates breed (Lacey and Wieczorek 2004), in order to better understand the effect of group living *per se* (i.e., without a confounding intra-group hierarchy) on glucocorticoid levels.

In chapter one, I validate my method of measuring glucocorticoids in the colonial tuco-tuco using an adrenocorticotropic hormone challenge. Validation of an appropriate assay is critical to (1) determining the primary glucocorticoid hormone (i.e. corticosterone or cortisol) acting in a species and (2) insuring that the assay's antibodies bind with the desired hormone. With permission from John Wiley and Sons to re-publish my results (Woodruff et al. 2010; J. Exp. Zool. 313A, 2010. © 2010 Wiley-Liss, Inc.), I show that a commercial corticosterone assay (Cayman Chemical, Ann Arbor, Michigan) accurately measures fecal corticosterone metabolites (fCMs), which are thus used as a proxy for measuring corticosterone levels in my study species. In addition, I show that the environmental challenges faced by a free-living population of *C. sociabilis* result in much higher baseline fCMs relative to baseline fCMs in a captive population of this species. Finally, I explore the effect of reproductive status on fCMs and show that in both the field and lab, there is no difference in fCMs between lactating female *C. sociabilis* and non-reproductive females of this species (Woodruff et al. 2010). This chapter provides support for methods applied in the following chapter.

In chapter two, I examine the effect of the social environment on baseline fCMs by combining data obtained from a free-living population of *C. sociabilis* with experimental manipulation of a captive population of this species. Baseline glucocorticoids are naturally circulating hormones that are necessary for meeting the physiological demands of an individual (Sapolsky et al. 2000). Here I test the hypothesis that the social environment – specifically whether an individual lives in a group or alone – affects baseline fCMs in female colonial tuco-tucos. My data indicate that, for free-living animals, baseline fCMS are significantly higher for lone females. Experimental manipulation of the social environment of captive females reveals a similar pattern, suggesting a causal role of social environment on baseline fCMs. Closer inspection of intra-group differences in fCM levels indicate that group composition – specifically the number of older ($\geq$ 2 years) females in the group – affects fCMs in yearling females, suggesting that not all within-group relationships are the same.

In chapter three, I focus on the social environment of the communal nest in *C. sociabilis.* I consider the role of adult males in the nest and test several adaptive hypotheses to explain the presence of males in the nest during the period between the birth and weaning of young. Specifically, I examine the parental effort hypothesis, which predicts that males that remain in the nest increase their current direct fitness by increasing offspring growth and survival. I also examine the mating effort hypothesis, which predicts that male nest attendance leads to increased future direct fitness by increasing future mating opportunities with the females in the nest. Combining evidence from both free-living and captive populations of *C. sociabilis*, I find little support for the parental effort hypothesis and no support for the mating effort hypothesis. While evidence from both the field and the lab reveal that male *C. sociabilis* actively participate in juvenile care in the communal nest, neither the number of offspring nor the growth of pups are affected by the presence of a male. However, male nest attendance is associated with the survival to adulthood of male, but not female, pups. This apparent impact on juvenile survival may be related to delayed pubescent aggression in male pups that are raised with adult males, as observed in the captive population. Thus, understanding variation in social structure in this plural breeding species requires an understanding of the fitness consequences of communal nesting for males as well as females.

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Chapter 1

# Contrasting Fecal Corticosterone Metabolite Levels in Captive and Free-Living Colonial Tuco-Tucos (*Ctenomys sociabilis*)

ABSTRACT

The environment in which an animal lives can profoundly influence its biology, including physiological responses to external stressors. To examine the effects of environmental conditions on physiological stress reactions in colonial tuco-tucos (*Ctenomys sociabilis*), we measured glucocorticoid (GC) levels in captive and free-living members of this species of social, subterranean rodent. Analyses of plasma and fecal samples revealed immunoreactive corticosterone (metabolites) to be the most prevalent GC in this species. An adrenocorticotropic hormone (ACTH) challenge confirmed that fecal corticosterone metabolites are responsive to exogenous stressors and provided validation of the commercial enzyme immunoassay kit used to detect these metabolites. Comparisons of adult female *C. sociabilis* from natural and captive environments revealed significantly higher baseline concentrations of corticosterone metabolites and significantly greater individual variation in metabolite concentrations among free-living animals. These findings suggest that the natural environment in which these animals occur is more challenging and more variable than the captive housing conditions employed. In addition to providing the first evaluation of GC levels in captive and wild colonial tuco-tucos, our findings indicate that the influence of environmental conditions on stress physiology may have important implications for understanding the social behavior of this species in the laboratory and the field.

# INTRODUCTION

Field-based observations of free-living animals and laboratory studies of captive individuals offer valuable, often complementary insights into animal behavior (Calisi and Bentley, 2009). While the former have the advantage of examining behavior in the selective environments in which it typically occurs, the latter provide a critical opportunity to characterize behavior under controlled conditions. Each approach is also subject to limitations, the full effects of which may only become apparent when field and laboratory data are combined. As a result, integrating field and laboratory studies is essential to generating an accurate understanding of behavior (Calisi and Bentley, 2009).

One oft-cited criticism of laboratory studies is that captive conditions – including small cage sizes and contrived social groupings – may increase animal stress and therefore affect behavior (Korte et al., 2007; Swaisgood, 2007; Van de Weerd et al., 1997b; Morgan and Tromborg, 2007; Jordan, 2005). In this context, stress is generally viewed as a negative consequence of captivity (Balcombe et al., 2004; Morgan and Tromborg, 2007). In contrast, field studies tend to view stress as a natural part of life and to focus on the physiological *response* to stress, which includes activation of both the sympathetic nervous system and the endocrine system via the hypothalamic-pituitary-adrenal axis (HPA). While prolonged activation of the HPA axis can negatively impact reproduction, immune function, and growth (de Kloet, 2004; Goymann and Wingfield, 2004), short-term activation of this system can be beneficial, resulting in mobilization of energy reserves needed to respond to acute environmental challenges (Munck et al., 1984; Sapolsky et al., 2000; Romero 2004; Bonier et al., 2009). Given the complexity of these responses and the context-dependent manner in which they have been interpreted, data from both captive and free-living conspecifics can enhance our understanding of how environmental conditions affect physiological stress.

Glucocorticoids (GCs) are critical mediators of homeostatic balance and allostatic load (McEwen and Wingfield, 2003; Goymann and Wingfield, 2004). For example, GCs contribute to the regulation of feeding, locomotor activity and energy metabolism (Sapolsky et al 2000; Landys et al., 2006) as well as stimulate glucose production in the face of unexpected environmental challenges (Wingfield et al 1998; Goymann and Wingfield 2004). Consequently, these hormones can be measured to assess the physiological condition of an individual, which includes baseline and acute responses to external stressors (Sapolsky et al., 2000; Romero, 2002, 2004; Landys et al., 2006). Baseline GC concentrations reflect a rhythmic circadian pattern, which responds to predictable daily stressors in the environment (Ruis et al., 1997; Windle et al., 1998; Möstl and Palme, 2002; Touma et al., 2004); in contrast, acute changes in GCs represent an enhanced response to short-term, unpredictable challenges (Sapolsky et al., 2000; Landys et al., 2006). GCs have been used in a variety of species to assess HPA axis response to variation in social environment (Castro and Matt, 1997; Reeder et al., 2006; Raouf et al., 2006), habitat quality (von der Ohe et al., 2004), predation pressure (Mashburn and Atkinson, 2007), and anthropogenic disturbance (Tempel and Gutierrez, 2004; Garcia Pereira et al., 2006).

Studies of colonial tuco-tucos (*Ctenomys sociabilis*) provide an ideal opportunity to explore the impacts of environmental conditions on baseline GC levels in both captive and natural settings. This species of subterranean rodent is endemic to southwestern Argentina, where it inhabits mesic meadows in the eastern foothills of the Andes. Colonial tuco-tucos differ from most other members of the genus *Ctenomys* in that they are social, meaning that burrow systems are shared by multiple adults. Due to this unusual pattern of behavior, *C. sociabilis* has been the subject of long-term field research aimed at characterizing patterns of social structure, demography, and ecology (Lacey et al., 1997; Lacey, 2001; Lacey and Wieczorek, 2003, 2004). At the same time, a captive population of these animals established at the University of California, Berkeley, in 1996 has been the basis for studies of parental care (Soares, 2004), olfactory communication (Schwanz and Lacey, 2003), and circadian patterns of activity (Yan et al., in prep.).

As part of efforts to integrate behavioral information from free-living and captive members of this species, we examined endocrinological measures of stress in animals from both settings. Specifically, we used assays of fecal glucocorticoid metabolites to assess potential baseline differences in the physiological response to field and laboratory environments. The objectives of the study were to (1) validate a corticosterone enzyme immunoassay (EIA) for measuring fecal glucocorticoid metabolites (fGCM) as an appropriate measure of adrenocortical activity (and, hence, a proxy for physiological condition) in the colonial tuco-tuco and (2) compare baseline GC levels of free-living and captive individuals. In addition to providing one of the few comparisons of GC levels in a non-domesticated mammal housed under captive and natural conditions, it is also the first evaluation of GC levels in tuco-tucos.

## MATERIALS AND METHODS

### *Study animals.*

The free-living population of *C. sociabilis* sampled is located on Estancia Rincon Grande, Provincia Neuquén, Argentina (40°57'S, 71°03'W). The study site consists of ca. 20-ha area of open meadow dominated by seasonal grasses and sedges and containing several species of woody shrubs. Members of the study population have been live-trapped annually since 1992 as part of an intensive investigation of the behavioral ecology of this species. Animals were captured as they emerged to forage using hand-held nooses placed in the rim of active burrow entrances (Lacey et al., 1997; Lacey, 2004). Upon first capture, each animal was individually marked with a magnetically coded bead (IMI-1000 Implantable Transponders, BioMedic Data Systems, Seaford, DE) inserted beneath the skin at the nape of the neck; implanted transponders were read using a hand-held scanner (DAS 4001 Pocket Scanner, BioMedic Data Systems). Each individual captured was weighed; for females, reproductive condition (e.g., pregnant, lactating) was also assessed.

The laboratory population of *C. sociabilis* sampled consisted of ~ 45 captive-born individuals, all descended from an initial set of 12 individuals captured in Neuquén

Province, Argentina, and transported to the Berkeley campus in January of 1996. Captive animals were housed in artificial burrow systems constructed of clear acrylic. Each burrow system consisted of ~ 10 m of acrylic tunnels connecting three acrylic boxes. Two boxes (30 x 30 x 15 cm) served as nest chambers and latrines, and one box (30 x 50 x 40 cm) was used to introduce food to the burrow system. The floor of each box was covered with an ~ 2 cm layer of aspen bedding; in addition, ~ 100 $cm^3$ of shredded paper bedding was placed in one box for use in nest construction. Rooms housing the burrow systems were maintained at 20°C. The light:dark cycle in the rooms imitated seasonal changes in day length at 41°S, the latitude at which the original members of the population were captured. The animals were fed *ad-libitum* quantities of commercially available rat chow (Simonsen's Inc., Gilroy, CA) and were provided daily with fresh produce (corn, carrots and lettuce).

All procedures were approved by the University of California, Berkeley, Animal Care and Use Committee and followed guidelines established by the American Society of Mammalogists (Gannon and Sikes, 2007).

*Sample Collection*

In general, fecal samples were used to assess GC levels because such samples provide a temporally more comprehensive picture of baseline GC concentrations. While blood samples represent a single point in time and may be subject to extreme variation due to pulsatile GC secretion, fecal samples reflect a reliable average of integrated circulating GCs (Harper and Austad 2000; Touma and Palme 2005). Additionally, fecal samples can be obtained non-invasively without altering measured GC levels through the act of sample collection. In comparison, blood collection is invasive and must be completed rapidly to avoid altering measures of GC concentrations (LeMaho et al., 1992; Harper and Austad, 2000). This temporal constraint can be challenging when working with free-living animals that are difficult to capture or handle. Although fecal sampling provides a measure of GC metabolites rather than the whole hormone, it provides an excellent alternative that has been used to examine GC concentrations in multiple species, including several South American rodents (Wasser et al., 2000; Ponzio et al., 2004; Palme et al., 2005; Touma and Palme, 2005; Bauer et al., 2008; Soto-Gamboa et al., 2009).

Collection of feces: Individuals were temporarily (ca. 4-6 hours) removed from their artificial burrow systems and housed individually in standard polycarbonate rodent cages (1 m x 0.5 m x 0.25 m) with wire tops. Cages were checked every two hours and feces were collected directly from the cage bottom, with care taken to avoid fecal pellets lying in bedding that was soaked with urine. Individual samples were placed in cryogenic vials and stored in a -20° C freezer until assayed. Because some samples were collected nearer to the time of excretion than others, we compared fecal GC metabolite levels in samples frozen at different times. Fecal samples from six individuals were collected immediately following defecation and divided into two subsamples. One was frozen within 10 mins of collection; the other was frozen 3 hours later. All samples were assayed at the same time.

Collection of blood plasma: Because no previous studies of GC concentrations have been conducted for *C. sociabilis*, we used analyses of blood plasma to determine whether corticosterone or cortisol is the most prevalent circulating GC. Although corticosterone is typically considered the primary GC in rodents (Touma and Palme 2005), studies of an increasing number of rodent species, including many from South America, have revealed cortisol to be the primary GC in these animals (e.g. yellow-pine chipmunks: Kenagy and Place, 2000; Beldings' ground squirrels: Mateo and Cavigelli, 2005; degus: Kenagy et al., 1999; guinea pigs: Malinowska and Nathanielsz, 1974). Blood was collected from 10 females from the laboratory colony. Each individual was removed from her burrow system and anesthetized using Isoflurane. Blood (ca. 300 µl) was collected with a heparinized pipette from the retro-orbital sinus. All samples were secured within approximately 3 minutes of removing animals from their housing. Blood was centrifuged at 1500 *g* for 17 minutes at 4°C and plasma was saved and frozen at -20°C until assayed.

Field sampling. Immediately upon capture (see above), each animal was placed in a cloth bag and held until a minimum of 5 fecal pellets had been deposited (typically 10-15 min). Animals naturally deposit fecal pellets during routine marking, weighing, and handling. Fecal pellets were transferred to cryogenic vials and immediately flash frozen and stored in liquid nitrogen. All samples were transported to the laboratory on the Berkeley campus and stored at -80° C until assayed. The typical time elapsed between production of fecal pellets by an animal and freezing was 10 minutes.

*Steroid Extractions and Assays*

Following the methods of Mateo and Cavigelli (2005), we thawed fecal samples and dried them in an oven (95° C) for 4 hrs. Samples were then removed and crushed using a mortar and pestle, after which 0.2 g fecal powder were weighed into a microcentrifuge tube. Next, 1.5 ml of 100% ethanol was added to each tube and the sample was vortexed for ~8-10 sec. Samples were centrifuged at 2500 *g* for 45 minutes to eliminate all solid matter, after which the supernatant was collected and frozen at -20° C until assayed.

Plasma GC samples were purified and extracted by technicians at Cayman Chemical Company (Ann Arbor, MI). Specifically, each sample was mixed thoroughly with methlyene chloride using a vortexer. The methylene chloride layer was removed and then evaporated by heating to 30˚C with a gentle stream of dry nitrogen. Extracts were dissolved in buffer and diluted at 1:5 and 1:10 for assay.

To quantify fecal GC metabolites and plasma GCs, we used commercially available cortisol and corticosterone enzyme immunoassay kits (Cayman Chemical Co., Ann Arbor, MI). According to the manufacturer, the corticosterone EIA kit cross-reacts with corticosterone at 100% versus < 1.0% for all other tested steroids. Similarly, the cortisol EIA cross reacts 100% with cortisol versus 22% with prednisolone, 6.1% with cortexolone, 2.0% with cortisone, 1.3% with corticosterone, and < 1.0% with all other tested steroids. All samples assayed yielded GC concentrations that were above the manufacturer's reported limit of detection for corticosterone and cortisol (38 pg/ml and 17 pg/ml at 80% binding, respectively). Sensitivity of the assay for corticosterone at 80% binding was 232

pg/ml. For cortisol, sensitivity at 50% binding was 80 pg/ml. Plasma samples were assayed in triplicate and fecal samples were assayed in duplicate. Samples were reanalyzed when the coefficient of variation exceeded 20%.

Intra- and inter- assay coefficients of variation for fecal corticosterone metabolites were 11.22% (N = 7) and 13.72% (N = 6), respectively. The intra-assay coefficients of variation for fecal cortisol (N = 8), plasma corticosterone (N = 6), and plasma cortisol (N = 6) were 11.01%, 10.58%, and 10.44%, respectively. No inter-assay coefficient of variation is reported for fecal cortisol or plasma GC immunoassays as only one plate was used for each assay.

*Validation of EIA*

Physiological validation of the corticosterone EIA was accomplished by using an adrenocorticotropic hormone (ACTH) challenge to confirm that the EIA kit employed detected elevated endogenous GC concentrations via measurement of their fecal metabolites (Palme et al., 2005; Touma and Palme, 2005). Ten females were isolated in individual polycarbonate cages, as described above, from October 3-8, 2006. The ages of these animals ranged from 1-4 years, with each age class represented in both experimental and control groups of females. All animals were fed their typical diet (see above) at 0800 hour each day. On 5 October, 6 females received an intramuscular injection of 0.0375 mg Cortrosyn® (Amphastar Pharmaceuticals, Inc., Rancho Cucamonga, CA), a synthetic form of ACTH, diluted in 0.3 ml of 0.9% NaCl; this dosage (12 IU/kg body mass) is comparable to those used in other studies (Wasser et al., 2000; Touma et al., 2004). The remaining 4 females received an intramuscular injection of 0.3 ml of 0.9% saline as a control. All females received their injections within a 20 min period between 1400 and 1420 hours, a time period just prior to the circadian trough of glucocorticoid secretion in many diurnal animals.

Fecal samples were collected from experimental and control females beginning 48 hours before injection and continuing until 72 hours after injection. Feces were collected every 24 hours throughout the experiment, with two additional collections at 8 and 16 hours post-injection. Peak concentrations of fGCM have been found up to 24 h after injection in several species of small mammals (Ponzio et al., 2004; Palme et al., 2005; Touma and Palme, 2005; Bauer et al., 2008; Soto-Gamboa et al., 2009). However, because the response time of colonial tuco-tucos to ACTH was unknown, we considered the additional post-injection collection times to be necessary.

Parallelism of fecal extracts with corticosterone standards was determined using pooled fecal samples (N = 5) serially diluted from 1:2 to 1:1024. Only those samples that fell within the linear range (20% - 80% binding) of the standard curve were included in the analysis. Regression curves for antibody binding versus GC concentration from fecal samples were compared to those from standards prepared using stock corticosterone supplied in the EIA kit.

*Field vs. Lab Comparisons*

To explore the potential effects of the lab environment on GC levels, we compared baseline concentrations of fGCM for 10 members of the captive population to concentrations for 10 members of the free-living population. Captive individuals were all non-reproductive females ranging in age from 1-3 years. Samples were collected during October 2006, which corresponds to the austral spring breeding period for the study species. The animals from the free-living population sampled represented a mix of non-reproductive and lactating individuals aged 1-3 years. All samples from free-living animals were collected during the austral spring (October and November).

*Data analyses*

Two-sample statistical comparisons were performed using t-tests unless the distribution of data points indicated that non-parametric tests were required. For the ACTH challenge study, we used Friedman's ANOVA (repeated measures) with post-hoc Wilcoxon sign tests to determine changes in GCM concentrations over successive sampling periods for control and experimental females. Follow-up comparisons were considered significant with a Bonferroni corrected $\alpha = 0.0033$. Additionally, Mann-Whitney U tests were used to identify differences for specific post-injection samples between experimental and control treatments. A Kruskal-Wallis ANOVA was used to compare non-reproductive laboratory animals with non-reproductive and lactating field animals followed by post-hoc Mann-Whitney U tests with a Bonferroni corrected $\alpha = 0.02$. An ANCOVA was used to test for parallelism between the slopes of the standards and the samples. Statistical analyses were performed using Statistica 6.0 (StatSoft, Inc. 1984-2008). All values are reported as 1± SEM.

RESULTS

*Corticosterone versus cortisol*

Analyses of fecal samples revealed that concentrations of fGCM measured with a corticosterone immunoassay were significantly higher than those measured with a cortisol assay (two-tailed t-test, $T = 5.71$, $N = 12, 12$, $P < 0.001$; Fig. 1). Similarly, analyses of plasma samples revealed that immunoreactive corticosterone concentrations were significantly higher than cortisol concentrations (two-tailed t-test, $T = 3.48$, $N = 9,8$, $P < 0.01$; Fig. 2). Accordingly, only the corticosterone EIA was used for all further analyses. No differences in corticosterone metabolite concentrations were detected between feces collected immediately after defecation and those frozen 3 hours afterwards (paired t-test: $T = 2.58$, $N = 6, 6$, $P = 0.79$).

*Biochemical Validation*

We found that the log-logit transformed slope derived from serially diluted pooled fecal samples paralleled the log-logit transformed slope of standards generated from stock solutions supplied with the assay kit ($F_{1, 11}$= 0.099, P > 0.70; Fig. 3).

*ACTH Challenge*

Concentrations of fGCM varied greatly between individuals in both control and experimental groups. Across sampling periods, we found significant differences among Cortrosyn-injected animals (ANOVA Chi Sqr. = 17.3; N = 6; df = 5; P < 0.004) and among saline-injected animals (ANOVA Chi Sqr. = 11.8; N = 4; df = 3; P < 0.04). However, no post-hoc comparisons of sampling periods were found to be significant. Prior to injection on day 0, there were no significant differences in metabolite concentrations between experimental and control females (MWU tests, Z = 0.68, N = 6,4, P = 0.49). However, at 24 hours post-injection, fGCM in Cortrosyn-injected animals peaked, showing significantly higher concentrations than in saline-injected animals (Fig. 4; MWU test, Z = 2.6; N = 6,4; P = 0.01). By 48 hours post-injection, experimental and control animals again exhibited no significant differences in concentrations of fGCM.

*Field vs. Lab Comparisons*

Females from the captive population exhibited significantly lower baseline concentrations of fGCM than either non-reproductive or lactating females from the free-living population (Kruskal-Wallis test: H (2, N = 30) = 19.2; P < 0.0001; Fig. 5). Post-hoc comparisons between these groups showed significant differences between the captive population and both categories of free-living females (MWU tests: non-reproductive: Z = -3.8, N = 10, 10, P < 0.001; lactating: Z = -3.7, N = 10,10, P < 0.001). Within the free-living population, there was no significant difference between concentrations of fGCM for non-reproductive and lactating females (MWU test, Z = -0.6, N = 10,10, P = 0.54). Thus, metabolite concentrations appeared to be influenced by environmental setting but not reproductive status. Individual variation in fGCM concentrations was also significantly greater for free-living versus captive animals (F-test Two-Sample for Variance: $F_{10, 20}$ = 0.001, P < 0.001). A comparison of the coefficients of variation reflected these differences as well (free-living animals: CV = 1; captive animals: CV = 0.35).

## DISCUSSION

Our analyses indicate that corticosterone metabolites in fecal samples provide a reliable means of evaluating baseline GC levels in colonial tuco-tucos. Comparisons of data obtained from fecal samples and blood plasma indicate that corticosterone is the primary circulating GC and the primary GC metabolite excreted by this species. The results of our ACTH challenge test revealed that concentrations of fecal GCM can be measured using a commercial corticosterone EIA kit and are responsive to exogenous ACTH, with a post-

injection lag time until the appearance of peak fGCM concentrations was 16-24 hrs, which corresponds to the lag time found in guinea pigs, a relatively closely related species of rodent (Bauer et al., 2008). The lag time from ACTH injection to peak concentrations of fGCM may be influenced by the diet of the captive animals (Möstl and Palme 2002; Millspaugh and Washburn 2004; Touma and Palme 2005) and thus should not be presumed to be identical to that for free-living individuals. Overall, however, our findings support the suitability of noninvasive, fecal sample analyses as a basis for future studies of physiological stress responses in colonial tuco-tucos.

GC concentrations in captive versus free-living animals can vary markedly between species. For example, while baseline GC levels for captive individuals are lower than those for free-living conspecifics in some species (Künzl and Sachser, 1999; Romero and Wingfield, 1999), others reveal the opposite tendency, with baseline levels being significantly higher for captive animals (Mashburn and Atkinson, 2007). Among guinea pigs, domesticated, wild, and recently cultivated varieties exhibit no differences in baseline GC concentrations, although wild animals show a greater GC response to acute stressors (Künzl et al., 2003). These findings demonstrate that the response to captivity is highly variable (Millspaugh and Washburn, 2004) and suggest that taxon-specific comparisons of natural and artificial environments are required to understand the effects of captivity on GC levels in a given species.

In female *C. sociabilis*, baseline concentrations of fGCM were significantly higher for free-living than for captive individuals, suggesting that HPA activity in response to challenges faced in the natural environment was greater than the response in captive conditions. Glucocorticoids are considered critical mediators of homeostatic balance and allostatic load in vertebrates (McEwen and Wingfield, 2003; Goymann and Wingfield, 2004). Sapolsky et al. (2000) have proposed that maintenance of elevated baseline GC concentrations may be an adaptive response to challenging environments that enhances an individual's ability to accommodate future stressors. The laboratory provides a highly predictable environment with regard to a number of variables, including lighting and temperature regimes, food availability, and the level of human activity in the vicinity of study animals. Although captive *C. sociabilis* are not domesticated, they are somewhat habituated to the presence of humans and common laboratory noises. In comparison, free-living tuco-tucos experience both predictable and unpredictable challenges in their environment. For example, while free-living animals may be able to prepare physiologically for seasonal changes in temperature or food availability, the same may not be true for unpredictable events such as a severe late-spring snowstorm. Thus, it seems reasonable to expect that laboratory animals that do not have to search for food, escape from predators, or compete for mates or territories should have lower baseline GC levels than free-living animals that routinely face these and other challenges associated with natural environments.

Baseline GC levels can also vary in response to other factors, including time of day (Ruis et al., 1997; Windle et al., 1998; Möstl and Palme, 2002; Lepschy et al., 2007), season (Kenagy and Place, 2000; Romero, 2002; Pride, 2005; Rosen and Kumagai, 2008), and reproductive condition (Kenagy et al., 1990, 1999; Boswell et al., 1994). Sample collection

for both captive and free-living animals was completed during similar portions of the day and year to minimize potential circadian and seasonal variation in our data set. There was no evidence that difference in reproductive status between captive and free-living females affected GC comparisons, as we found no significant differences in baseline corticosterone metabolite concentrations between lactating and non-reproductive females from the natural population of *C. sociabilis*. This result may not be particularly surprising given that most of the reproductive females sampled were nearing the end of lactation, when GC levels are expected to drop relative to early lactation (Meaney et al., 1989; Superina et al., 2009). All females – reproductive and non-reproductive – shared a communal nest with the offspring of reproductive group mates.

In addition to a significantly higher mean fGCM concentration, members of the free-living population of *C. sociabilis* exhibited significantly greater individual variation in baseline corticosterone metabolite levels. Variation between individuals can reflect differences in the perception of a stressor (Koolhaas et al., 1999) as well as variation in local habitats. In captive populations, variation in the conditions experienced by individuals is typically expected to be minimal. If GC response is heritable (Federenko et al., 2004; Evans et al., 2006; Bonier et al., 2009), then the tendency toward reduced inter-individual variability in GC response may be exacerbated if members of captive populations are inbred relative to free-living animals. In contrast, the natural environment can vary markedly among populations or even among different burrow systems within the same population and such variation may affect baseline GCs levels. The reduced variability in baseline GC metabolite concentrations among captive animals is consistent with the more controlled and presumably less challenging conditions under which these animals were housed relative to their free-living conspecifics.

Recently, Korte et al. (2007) redefined animal welfare based on the concept of allostasis. These authors argue that the *absence* of stressors as well as chronic exposure to environmental challenges can affect allostatic load and increase GC concentrations. The most common explanation for these problems in captive animals is lack of appropriate environmental enrichment. Multiple studies have demonstrated that individuals housed in enriched lab environments tend to be healthier and to exhibit decreased baseline GC levels relative to conspecifics in non-enriched environments (Van de Weerd et al., 1997a; Hutchinson et al., 2005; Baumans, 2005). Thus the optimal housing conditions for captive individuals may be those that provide the level of environmental challenge required to maintain homeostasis (Korte et al., 2007); deviations from this optimum in either direction (e.g., too much or too little challenge) may impact HPA response and may contribute to the variable results of studies that have compared baseline GC levels in captive and free-living conspecifics. The captive colonial tuco-tucos used in this study are housed in artificial burrow systems that allow the animals to run through tunnels, hide in multiple darkened refugia, and manipulate food and bedding much as they do in the wild. By imitating the burrows in which these animals naturally occur, we may have created an appropriately enriched lab environment that fosters homeostasis and contributes to the relatively lower baseline GC metabolite levels detected for captive members of this species.

Understanding how captive versus natural environments impact GC levels is also critical to interpreting patterns of animal behavior. The stress response is not limited to physiological changes, but often includes a behavioral component (Wingfield et al., 1998; Koolhaas et al., 1999; Touma and Palme, 2005; Spencer and Verhulst, 2007). Physiology and behavior function within a mutual feedback system, with each triggering responses in the other, thereby allowing an animal to adjust to current environmental demands (Boonstra, 2005; Reeder and Kramer, 2005). While this integrated response is generally thought to be adaptive in natural habitats, it may lead to unintended physiological and behavioral outcomes in laboratory settings. Behavioral attributes that may be affected are diverse and include activity patterns, communication, aggression, courtship, and parental care (Price, 1970; Berman, 1980; Künzl et al., 2003; Balcombe et al., 2004; Cortopassi and Bradbury, 2006). Thus, lab studies must consider the potential behavioral consequences of artificial environments that alter physiology, including GC levels. At the same time, studies of free-living animals must consider the role of physiology when attempting to elucidate the effects of naturally occurring environmental variation on behavior.

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FIGURES

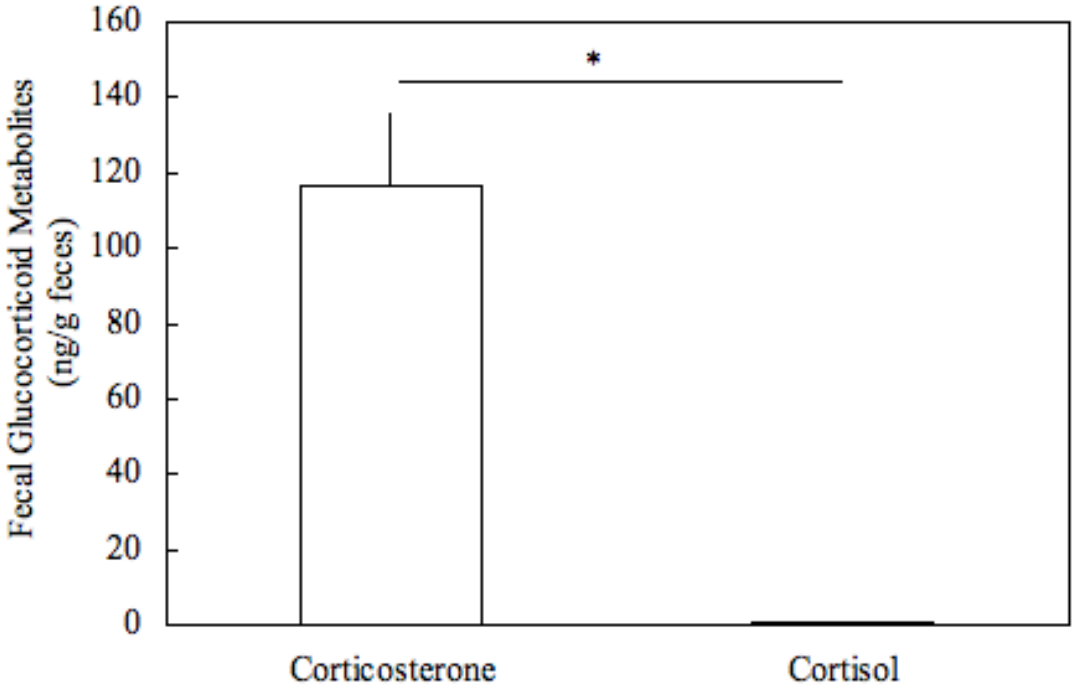


Figure 1. Concentrations of fecal corticosterone (N = 10) and fecal cortisol metabolites (N = 10) detected by respective EIA kits (Cayman Chemical, Ann Arbor, MI). Fecal samples were collected from captive nonreproductive adult female colonial tuco-tucos (*C. sociabilis*).

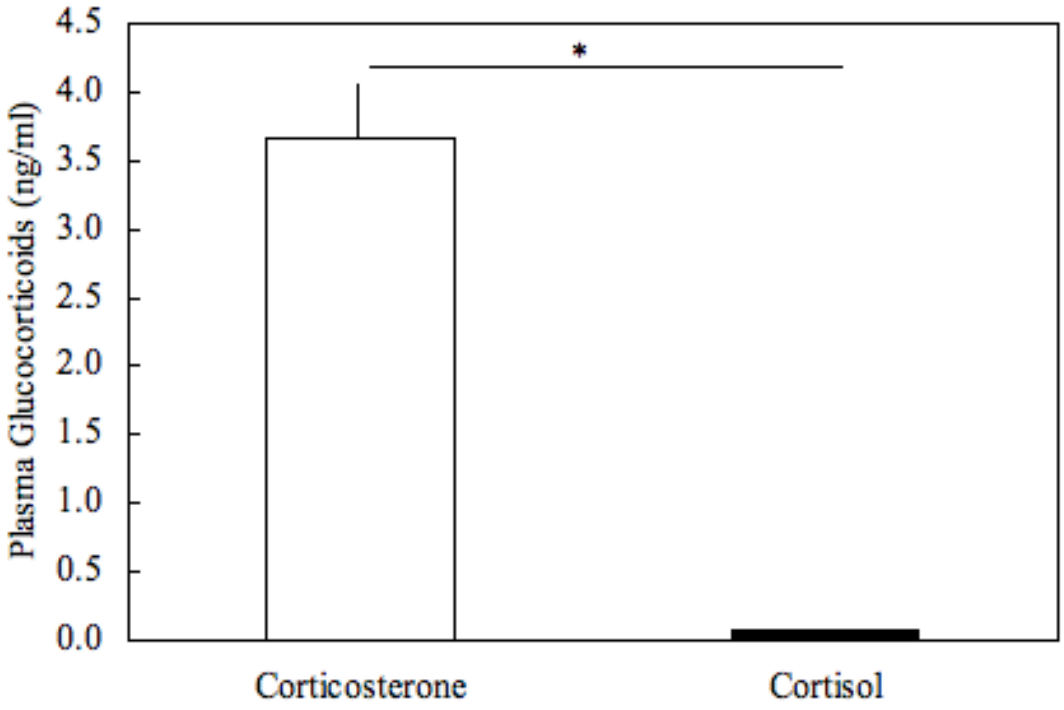


Figure 2. Concentrations of plasma corticosterone (N = 9) and cortisol (N = 8) detected by respective EIA kits (Cayman Chemical). Blood samples were collected captive nonreproductive adult female colonial tuco-tucos (*C. sociabilis*).

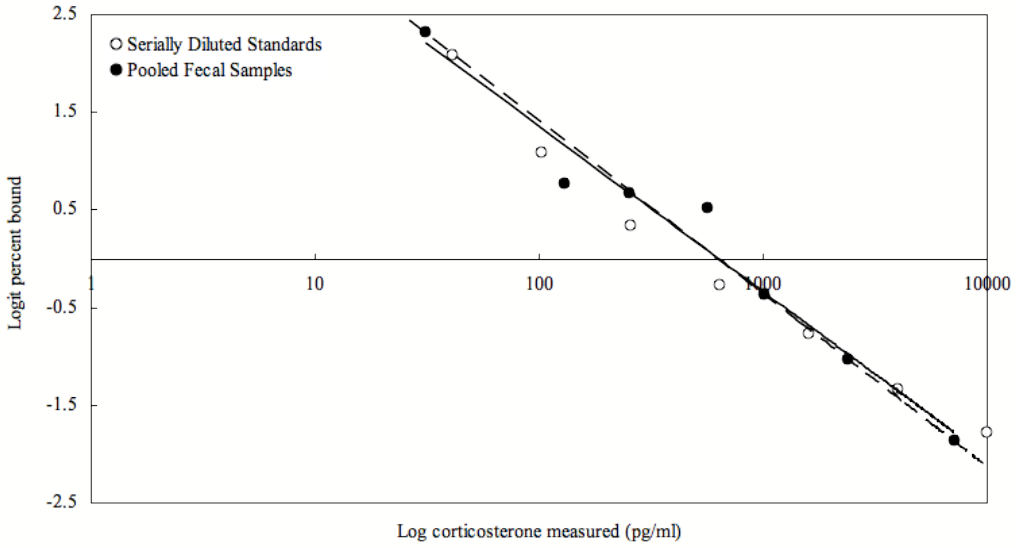


Figure 3. Parallelism between serially diluted standards and pooled fecal samples collected from captive adult female colonial tuco-tucos (N = 7). Log-logit transformed standard curve (filled circles): $y = -0.769\text{Ln}(x) + 4.96$, $r^2 = 0.967$; log-logit transformed curve of serially diluted fecal extract (open circles): $y = -0.734\text{Ln}(x) + 4.73$, $r^2 = 0.968$.

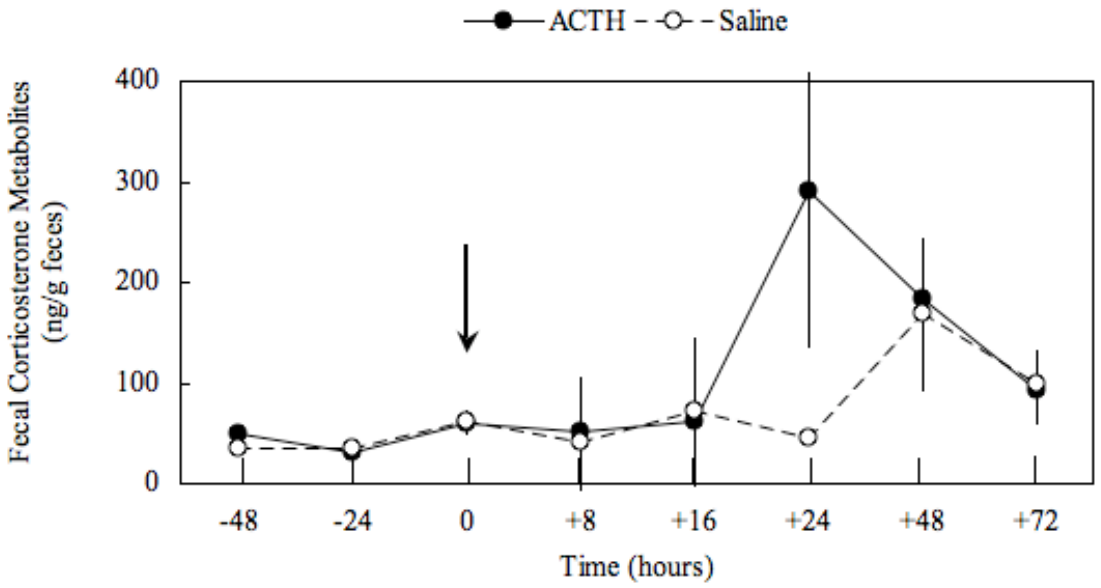


Figure 4. Mean fecal corticosterone metabolite concentrations measured before and after injection with synthetic ACTH (N = 6) or saline (N = 4). Data are from fecal samples collected from captive nonreproductive adult female colonial tuco-tucos. Time of injection is indicated by the arrow. Twenty-four hours after injection, GC levels in experimental animals were significantly higher than in control animals; no other significant differences were detected between experimental and control animals.

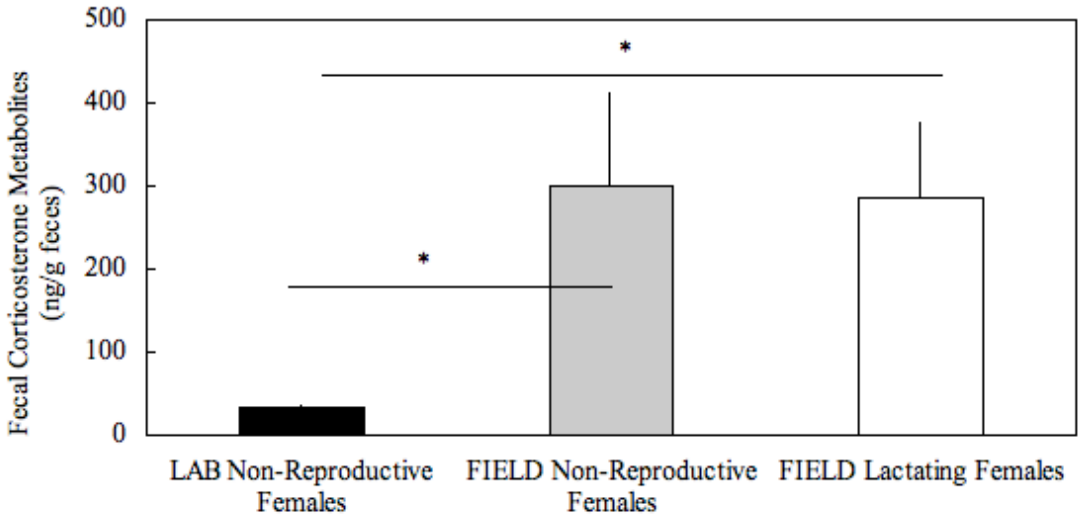


Figure 5. Mean (± SEM) baseline fecal corticosterone metabolite concentrations for captive and free-living female colonial tuco-tucos. Captive nonreproductive females (N = 10) had significantly lower baseline concentrations than either free-living nonreproductive females (N = 10) or lactating females (N = 10). There was no difference in baseline corticosterone metabolite concentrations between free-living nonreproductive and lactating females.

Chapter 2

# Social Mediation of Baseline Glucocorticoids In Colonial Tuco-Tucos, *Ctenomys sociabilis*

ABSTRACT

The social environment plays a powerful role in influencing the behavior and physiology of an individual. To complement studies that examine the adaptive (fitness) consequences of group living in colonial tuco-tucos (*Ctenomys sociabilis*), we investigated the physiological consequences of sociality using measures of baseline glucocorticoids (GCs) in this species. GCs (adrenal steroids produced upon activation of the hypothalamic pituitary adrenal [HPA] axis) are critical mediators of homeostasis, which provide a functionally relevant and biologically important measure of physiological response to environmental challenges. To determine if social environment is correlated with differences in the physiological stressors experienced by individuals, we compared baseline fecal glucocorticoid metabolite (fGCM) levels for captive and free-living *C. sociabilis* as a function of their social environment. These analyses revealed that baseline fGCMs in yearling females were significantly higher for lone animals. This difference appeared to arise because lone females did not exhibit the same afternoon reduction in fGCMs observed among group-living females. We found no relationship between fGCMs and either direct fitness or group size. However, group composition, specifically the number of older females in a group, was associated with difference is fGCMs in yearling females. To our knowledge, this is the first study to integrate studies of captive and free-living animals to examine the effects of the social environment on baseline GCs in a plural-breeding species of mammal.

# INTRODUCTION

The social environment – specifically, whether an animal lives alone or in a group – is a powerful force that affects numerous aspects of an individual's biology. Current conceptual understanding of sociality suggests that individuals choose between dispersing and living alone or being philopatric and remaining in their natal group (Emlen 1994, Solomon 2003). While the ultimate level or adaptive (fitness) consequences of this decision have been examined in detail for multiple species (Hayes 2000, Komdeur 1992, Randall et al. 2005, Silk 2007, Solomon and French 1997), proximate (e.g. physiological) consequences have been less thoroughly investigated. Hormones represent one such physiological mechanism that influences the development and expression of behavior. Specifically, hormones initiate meaningful behavioral responses to external environmental cues and challenges, which ultimately affect reproduction and survival (Bentley et al. 2006, Goldman 1999, Kriegsfeld and Silver 2006). Studies of the hormonal correlates of sociality complement ultimate-level adaptive explanations and enhance our understanding of social behavior (Adkins-Regan 2005 Reeder and Kramer 2005; Blumstein et al. 2010).

Measures of glucocorticoid hormones have been used to examine physiological correlates of social environment in multiple species (Wasser et al., 2000; Ponzio et al. 2004; Palme et al., 2005; Touma and Palme, 2005; Soto-Gamboa et al., 2009). Glucocorticoids (GCs) (adrenal steroids produced upon activation of the hypothalamic pituitary adrenal (HPA) axis) are critical mediators of homeostasis and allostatic load (Wingfield and Kitaysky 2002, McEwen and Wingfield 2003, Goymann and Wingfield 2004). Because GCs affect a diverse array of metabolic and other bodily processes (e.g. gluconeogenesis, reproduction), they provide a functionally relevant and biologically important measure of physiological response to both existing environments and changes in environment variables. Early studies of GCs focused on their role in response to unexpected environmental challenges, including life-threatening situations (Wingfield et al. 1998). Such unexpected challenges may be short in duration (e.g., a severe storm), generating relatively brief (i.e. acute) changes in GC levels or they may persist for longer periods of time (e.g., limited food availability throughout a breeding season), producing more enduring (i.e., chronic) GC changes, often with damaging results (McEwen and Wingfield 2003, Sapolsky et al. 2000, Wingfield 2003). Increasingly, investigators are realizing that baseline GCs play a critical role in response to predictable daily and seasonal conditions (Breuner et al. 1999, Romero 2002, Landys et al. 2006). Baseline levels reflect naturally occurring seasonal and circadian variation in GCs. As a result, they can provide an important indication of how individuals cope with existing environmental challenges (Bonier et al. 2009, Korte et al. 2005, McEwen and Wingfield 2003, Wasser et al. 1997).

Studies of free-living mammals that examine the relationship between the social environment and baseline GCs have been limited primarily to singular breeders in which a distinct reproductive hierarchy exists between dominant and subordinate individuals (Creel 2001; Hackländer et al. 2003; Raouf et al. 2005; Pride 2005). In contrast, few field studies have investigated the effects of the social environment on GCs in plural breeding systems (but see Ebensperger et al. 2011, Schradin 2008), in which most or all of the females in the

group reproduce. Because plural breeders lack a confounding intra-group hierarchy, studies of plural breeding systems provide an important opportunity to explore the effects of group living *per se* on baseline GCs.

The purpose of this study was to investigate how naturally occurring variation in the social system of colonial tuco-tucos (*Ctenomys sociabilis*) affects baseline GCs. Colonial tuco-tucos are subterranean rodents that are endemic to the Andean precordillera in southwestern Argentina (Lacey et al. 1997, Lacey and Wieczorek 2003). Unlike most members of the genus *Ctenomys*, this species is social. While ca one-third of yearling females have dispersed from their natal burrow system, the rest are still resident in their natal burrow (Lacey 2004), where they breed and share a communal nest. This intraspecific variation in social environment provides an important opportunity to assess the costs and benefits of living alone versus within a group. For example, comparative studies of lone and group living yearling females have revealed that lone females exhibit greater annual per capita direct fitness but are less likely to survive to a subsequent breeding season than are yearling females living in groups (Lacey 2004).

To complement these studies of ultimate-level correlates of social environment in *C. sociabilis*, this study explores the physiological consequences of this variation in social structure. Specifically, we sought to examine the effects of social setting on baseline GC levels in this species. If the decision to disperse and live alone increased physical challenges (e.g. caring for young, finding food, avoiding predators), then lone females should show higher baseline GCs relative to group living females. Alternatively, if social conflict between group members plays a greater role in GC production, then group living females would show higher GCs. We tested these predictions by comparing baseline fecal GC metabolite levels for lone and group-living females in a natural and a captive population of *C. sociabilis*. This study provides a distinctively comprehensive assessment of the effects of social environment on baseline GC levels, thereby yielding important new insights into consequences of sociality in plural breeding mammals.

## METHODS

### *Field Study*

The free-living population of *C. sociabilis* sampled was located on Estancia Rincon Grande, Provincia Neuquén, Argentina (40°57'S, 71°03'W). The study site consisted of ca 20-ha area of open meadow dominated by seasonal grasses and sedges and containing several species of woody shrubs. Members of the study population have been live-trapped annually since 1992 as part of an intensive investigation of the behavioral ecology of this species. Colonial tuco-tucos are subterranean, emerging only half a body length from their burrows to feed on surface vegetation. All individuals in the population were captured as they emerged to forage using hand-held nooses placed in the rim of active burrow entrances (Lacey et al. 1997; Lacey and Wieczorek 2004). Upon first capture, each animal was individually marked with a magnetically coded bead (IMI-1000 Implantable

Transponders, BioMedic Data Systems, Seaford, DE) inserted beneath the skin at the nape of the neck; implanted transponders were read using a hand-held scanner (DAS 4001 Pocket Scanner, BioMedic Data Systems).

*Determining Social Setting and Dispersal Histories*

We determined the social setting (i.e. group size and age composition) and dispersal histories of individual females by capturing effectively all animals – juveniles and adults – resident in the study population during form 2005 to 2009. Animals were captured from late October to December. Typically, members of a burrow system were captured as juveniles in that system first began to emerge above ground to forage for themselves; because captures occurred before young of the year could disperse from their natal burrow system, the natal group for each female was known. Lacey et al. (1997) describe the methods used to ensure that all residents of a burrow system were captured; in brief, animals were held for 24 hours to determine if there were additional individuals present within the colony. While animals were held, we monitored activity by placing twigs over all burrow entrances in the system. If twigs were not displaced over the course of 2 foraging periods (typically morning and late afternoon), we assumed that all animals from that burrow system had been captured. We also checked for evidence of new burrow entrances.

Radiotelemetry was used to confirm burrow sharing as well as to determine burrow system boundaries and nest locations. Past studies have used this technology to demonstrate the occurrence of communal nesting in *C. sociabilis* (Lacey et al. 1997; Lacey 2004). Adults captured were fitted with small (< 7 g) radio-collars consisting of an acrylic-encased transmitter (SM1-Mouse transmitters, AVM Instruments, Inc., Colfax, CA) attached to a plastic cable tie before being released. Radio-collared animals were followed using hand-held antennas and receivers (Yagi antennas, CE-12, AVM Instruments, Inc.) and fixed locations were marked every hour, 8-10 hours per day for 2 weeks. Animals that exhibited high spatial overlap (i.e. > 66%) were considered to be members of the same social group (Lacey et al. 1997).

*Quantifying Fitness and Survival*

We quantified per capita direct fitness for females by capturing all juveniles in each burrow system. For burrow systems that contained a single adult female, the number of pups weaned equaled the direct fitness of the female. However, due to low genetic variability (Lacey 2001) and the consequent inability to determine maternity of pups in burrow systems containing more than one lactating female, we determined per capita direct fitness in multi-female groups by dividing the number of pups weaned with the number of adult females captured (Lacey 2004).

Survival was determined by recapturing individuals in successive years. Only burrow systems for which all residents were captured were used to estimate survival. Dispersal by female *C. sociabilis* occurs only at the end of the juvenile season (Lacey and Wieczorek 2004) and thus the disappearance of an adult female from one year to the next was attributed to mortality (Lacey 2004).

*Fecal Sample Collection*

Fecal samples were used to assess GC levels by measuring GC metabolites (GCMs). Such samples provide a temporally more comprehensive picture of baseline GC concentrations. Specifically, while blood samples represent a single point in time and may be subject to extreme variation due to pulsatile GC secretion, fecal samples reflect a reliable average of integrated circulating GCs (Harper and Austad 2000; Millspaugh and Washburn 2004, Touma and Palme 2005). Additionally, fecal samples can be obtained non-invasively without altering measured GC levels through the act of sample collection. In comparison, blood collection is invasive and must be completed rapidly to avoid altering measures of GC concentrations (LeMaho et al. 1992, Harper and Austad 2000). This temporal constraint can be challenging when working with free-living animals that are difficult to capture or handle. Measures of fecal GCMs have been used to examine GC concentrations in multiple species, including several South American rodents (Wasser et al. 2000, Ponzio et al. 2004, Palme et al. 2005, Touma and Palme 2005, Bauer et al. 2008, Soto-Gamboa et al. 2009) and have been shown to be a reliable proxy for GC concentrations in colonial tuco-tucos (Woodruff et al. 2010).

Immediately upon capture (see above), each animal was placed in a cloth bag and held until a minimum of 5 fecal pellets had been deposited (typically 10-15 min). Animals naturally deposit fecal pellets during routine marking, weighing, and handling. Fecal pellets were transferred to cryogenic vials and immediately (≤ 10 minutes after collection) flash frozen in liquid nitrogen. All samples were shipped on dry ice to the Berkeley campus and stored at -80° C until assayed. Analyses comparing fGCMs for lone versus group-living female colonial tuco-tucos were restricted to lactating yearlings to control for breeding status and age; because most lone females in the field population are yearlings (Lacey and Wieczorek 2004), yearlings provide the most appropriate age group for comparisons of fGCMs. Samples were also collected and analyzed for older females to assess the effects of age and group composition of fGCMs.

*Laboratory Study*

The laboratory population of *C. sociabilis* sampled consisted of ~ 35 captive-born individuals, all descended from an initial set of 12 individuals captured in Neuquén Province, Argentina, in January 1996. The laboratory maintains an enriched housing environment, consisting of artificial burrow systems with three clear acrylic boxes connected by ~ 10 m of acrylic tunnels. Two boxes (30 x 30 x 15 cm) serve as nest chambers and latrines, and one box (30 x 50 x 40 cm) is used to introduce food to the burrow system. The floor of each box was covered with ~ 2 cm layer of aspen bedding. Rooms housing the burrow systems were maintained at 20°C. The light:dark cycle in the rooms imitated seasonal changes in day length at 41°S, the latitude at which the original members of the population were captured. The animals were fed *ad-libitum* quantities of commercially available rat chow (Simonsen's Inc., Gilroy, CA) and were provided daily with fresh produce (corn, carrots and lettuce).

To test experimentally the effects of social environment on GC levels, we housed captive yearling females either alone or in female-female pairs for a period of 30 days. More specifically, we randomly assigned each of 10 yearling females to be housed alone or with one of her recently weaned female offspring; the housing experiment was conducted when offspring were ca 4 months old, which corresponds to the period shortly after which those free-living females that disperse leave their natal burrow system. While all juvenile male *C. sociabilis* disperse at puberty, most juvenile females are philopatric (Lacey and Wieczorek), such that it is typical to find mothers and daughters sharing the same burrow. Females from both treatment groups were subject to the same husbandry procedures. The same protocol was applied to a sample of fifteen 2-year old females to examine the effects of age on GC response to these experimentally generated differences in social environment.

At the end of 30 days, fecal samples were collected for GC analysis. Fecal samples were collected during the afternoon to parallel sample collection in the field. To collect samples, females were temporarily (ca 4-6 hours) removed from their artificial burrow systems and housed individually in standard polycarbonate rodent cages (1 m x 0.5 m x 0.25 m) with approximately 2 cm of aspen bedding. Cages were checked every two hours and feces were collected directly from the cage bottom, with care taken to avoid fecal pellets lying in bedding that was soaked with urine. Individual samples were placed in cryogenic vials and stored in a -20° C freezer until assayed.

All procedures involving live animals were approved by the University of California, Berkeley, Animal Care and Use Committee and followed guidelines established by the American Society of Mammalogists (Gannon and Sikes, 2007).

*Steroid Extractions and Assays*

Following the methods of Mateo and Cavigelli (2005), we thawed fecal samples and dried them in an oven (95° C) for 4 hrs. Samples were then removed and crushed using a mortar and pestle, after which 0.2 g fecal powder was placed into a microcentrifuge tube. A 1.5 ml aliquot of 100% ethanol was then added to each tube and the sample was vortexed for ~8-10 sec. Samples were centrifuged at 2500 *g* for 45 minutes to eliminate all solid matter, after which the supernatant was collected and frozen at -20° C until assayed.

To quantify fecal GC metabolites, we used a commercially available corticosterone enzyme immunoassay kit (Cayman Chemical Co., Ann Arbor, MI), which had previously been validated for fecal corticosterone metabolites (fCMs) in colonial tuco-tucos (Woodruff et al. 2010). All samples assayed yielded GC concentrations that were above the manufacturer's reported limit of detection for corticosterone (38 pg/ml at 80% binding). Sensitivity of the assay for corticosterone at 80% binding was 232 pg/ml. Fecal samples were assayed in duplicate and reanalyzed when the coefficient of variation exceeded 20%. Intra- and inter- assay coefficients of variation for fecal corticosterone metabolites were 11.2% (N = 7) and 13.7% (N = 6), respectively.

*Data analyses*

Two-sample statistical comparisons were performed using t-tests unless the distribution of data points indicated that non-parametric tests were required. An ANCOVA was used to determine the relationship between baseline fCMs and the per capita number of pups in single or multiple female social systems. A non-parametric Kruskal-Wallis ANOVA was used for between-group analyses, with subsequent Mann-Whitney U tests used for post-hoc comparisons. We used general regression models (GRM) for simple regression analyses. Statistical analyses were performed using Statistica 6.0 (StatSoft, Inc. 1984-2008). All values are reported as 1± SEM.

RESULTS

*Field Samples*

Between 2005 and 2009, 175 free-living *C. sociabilis* from 29 colonies were captured as part of this study. Of these, 37 were yearling females from which fecal samples were collected for fGCM analysis. Twelve of these yearlings were lone females; the remaining 15 lived in groups containing $\geq$ 2 adult females. We found no effect on fCMs with respect to the year in which fecal samples were collected (ANOVA: $F_{year}$=2.17, N=12,15, P=0.116) and thus data from all years were pooled for subsequent analyses. For group-living females, the presence of a male in the burrow system during the period when samples were collected had no effect on fCMs (Unpaired T test: $t$ = 1.94, N = 6, 17, df = 6, p = 0.93) and thus data from all multi-female groups were used in our analyses. Due to the small number of male-female pairs detected during this study (N = 1), we could not determine if the presence of a male had an effect on fCMs for females living in this social setting and thus data for this pair were excluded from our analyses. As a result, social units consisting of a lone female were those in which the adult female was the only adult present in the burrow system.

*Effects of Time of Day*

To determine if GC production in our study population fluctuated with time of day, we examined GC concentrations with respect to sampling time. Specifically, we compared fCMs from samples collected before 1300 hours to those collected after 1300 hours. This timeline reflects the generally bimodal pattern of diurnal activity observed in the study population. For different individuals captured on different days (i.e., one sample per individual), there was a significant effect of time of day but not of social system on fCM levels (Two-way ANOVA: $F_{socialsystem}$ = 0.147, df = 1, p = 0.70; $F_{timeofday}$ = 10.3, df =1, p = 0.005; Figure 1). Post-hoc comparisons showed significant differences in fCMs between the morning and afternoon samples from group-living females (MWU: U = 16.0, Z = 2.84, N = 15, 8, p = 0.005) but not between the morning and afternoon samples from females living alone (MWU: U = 21.0, Z = 0.45, N = 7, 7, p = 0.65). While no differences in fCMs were found between the morning samples of group living females and females living alone

(MWU: U = 44.0, Z = 0.59, N = 15, 7, p = 0.55), nearly significant differences were found between the afternoon samples of group living females and females living alone (MWU: U = 12.0, Z = -1.85, N = 8, 7, p = 0.06). Consequently, to control for circadian variation in GCs, we restricted subsequent analyses to fecal samples that had been collected from free-living and captive animals after 1300 hrs.

*Effects of Age*

We investigated the influence of age on GCs in free-living female *C. sociabilis* by comparing fCMs in the same individual in successive years (i.e., at one and two years of age). As yearlings, four of the sixteen individuals lived alone, while the remaining 12 lived in groups. However, all 16 individuals were group living as two year olds. We found no effect of yearling social environment on the average difference in fCMs between years (ANOVA: F = 0.124, df =1, N = 4, 11, p = 0.72). When data from all yearling females were pooled, we found that fCMs were significantly lower in yearlings than they were in two-year old females (One-tailed paired T test: $t$ = -1.92, N = 16, df = 15, p = 0.037; Fig. 2). The same pattern was observed in the captive colony, although samples were not collected from the same animals across multiple years (One-tailed unpaired T test: $t$ = -2.62, N = 9, 11, df = 10, p = 0.013). Additionally, we found that a female's fCM concentration as a yearling was a significant positive predictor of her fCM concentration as a 2 year old (General Regression Model: $r^2 = 0.85$, F = 83.3, df = 14, $p < 0.0001$; Fig. 3).

*Effects of Social Environment*

Among free-living *C. sociabilis*, fCMs were significantly higher for yearling females living alone versus those living in groups (One-tailed T Test: t = 1.75, df = 15, N = 12,15, p = 0.039; Fig. 4a). Results from experimental manipulations of housing conditions for captive females were similar: captive females housed alone had significantly higher fCMs than those housed in pairs (Mann Whitney U: Z = 2.02, N = 5,5, p = 0.03; Fig. 4b). The same pattern was observed in older (> 1 year) free-living and captive *C. sociabilis*, although these differences were not significant (Field: One-tailed T test: $t$ = 1.89, N = 6, 10, df = 7, p = 0.18; Lab: One-tailed T test: t = 1.77, N = 7, 8, df = 13, p = 0.12; Fig. 5a, b).

We also considered other factors in the social environment that might influence fCMs by examining the effect of the number of pups in a social unit. Neither the per capita number of pups in a burrow system (ANCOVA: $F_{\text{pup:mom ratio}} = 1.32$, df = 1, p = 0.26; $F_{\text{social setting}} = 0.067$, df = 1, p = 0.79; Fig. 6) nor the absolute number of pups in a burrow system (ANOVA: F = 0.916, df = 1, p = 0.34; Fig. 7) were associated with significant differences in fCMs in either lone or group-living free-living female *C. sociabilis*.

*Effects of Group Structure*

To explore potentially more subtle impacts of social setting on GC levels, we investigated how group size and age structure correlated with GCs in group-living individuals. Analysis of free-living yearling female *C. sociabilis* in groups showed no significant relationship between fCMs and group size (GRM: r = 0.15, F = 0.29, df = 14, N = 15, p = 0.59; Fig. 8).

Because female *C. sociabilis* live in groups composed of mothers, sisters, daughters, and similar categories of kin, the age structure in groups varies from burrow system to burrow system. To examine how this variation affects GCs, we examined fCMs for yearling females living in three different group types: groups composed only of yearling females, groups with one older (> 1 year) female, and groups with more than one older female. We found that fCMs varied significantly with age composition (Kruskal-Wallis ANOVA: $H(2, N = 45) = 6.9$, $p = 0.032$; Fig. 9). Post-hoc comparisons revealed that yearling females living in groups with one older female exhibited significantly higher fCMs than yearling females living in groups with more than one older female (Mann Whitney U: $U = 48.0$, $Z = 2.5$, $N = 18,12$, $P=0.011$). The number of older females in a group was significantly correlated with group size (GRM: $r = 0.77$, $F = 40.1$, $df = 1$, $N = 30$, $p < 0.001$). Eighty-three percent of the 12 yearling females in groups with more than one older female were from groups with five or more females, while about half of the yearlings in groups with one older female were from groups of three females. Thus, despite the absence of a direct relationship between yearling fCMs and group size, group size may contribute indirectly to fCMs through size-related differences in group composition.

## DISCUSSION

The social environment can be a powerful influence on multiple aspects of an individual's biology and physiology. In this study, we found a significant effect of social setting on glucocorticoid concentrations. Yearling female *C. sociabilis* living alone exhibited higher fCMs than females living in groups; this was true of both the free-living and the captive animals studied. Because females in the latter were randomly assigned to be housed in pairs or alone, this outcome suggests a causal relationship between social environment and fCMs. This difference appeared to result from socially-mediated differences in diurnal patterns of fCM production. While fCM levels for group-living yearlings declined significantly during the afternoon, a comparable decrease was not detected for lone females. Our analyses focused on samples collected during the afternoon, suggesting that the higher GC levels detected for lone females were due to the maintenance of high fCM levels throughout the day.

### *Potential Influences on GCs*

Social environment is not the only factor that may influence fCM concentrations. For example, glucocorticoid concentrations have been shown to increase with age in some studies (Thompson et al. 2010, Novakova et al. 2008, Wang et al. 1997, Sapolsky 1992), but not in others (Harper and Austad 2000, Van Kampen and Fuchs 1998). We found that fCM concentrations in *C. sociabilis* increased with age in both free-living and captive females, suggesting that age is a potentially significant confound in studies of glucocorticoid levels in the species. However, because we restricted our analyses of social setting to comparisons of yearling females, data for these animals were not confounded by age differences. In *C. sociabilis,* lone females are typically yearling animals that dispersed from their natal burrow system at the end of their juvenile season (Lacey and Wieczorek

2004). Because lone yearlings typically accumulate philopatric daughters (i.e., become group living) in subsequent years, restricting our analyses to yearling animals was biologically relevant and captured the age class during which differences in social setting are most pronounced.

We found no evidence that reproductive success influenced fCMs in yearling females. While elevated GC levels have long been thought to lead to decreased reproductive success, Bonier et al. (2009) have argued that GCs may increase with reproductive effort due to the additional physical challenges associated with producing and rearing offspring. Although Ebensperger et al. (2011) found that cortisol increased with the per capita number of pups in a group of free-living degus (*Octodon degus*), our data suggest that this is not the case for *C. sociabilis*. In general, GCs levels can vary with life history stage (e.g. breeding or lactating, Boswell et al. 1994, Kenagy et al. 1990, 1999), suggesting that reproductive condition should be considered when comparing GC levels of conspecifics. In this study, however, data were collected after pups were effectively weaned (Lacey and Wieczorek 2004) and all females sampled were in effectively the same reproductive condition. Thus, although Woodruff et al. (2010) did not find significant differences in fCMs between non-reproductive and lactating female *C. sociabilis*, our data were not confounded by differences in GC levels resulting from differences in breeding status.

*GCs and Plural Breeding*

Most studies of GC responses in free-living social mammals have been limited to singular breeders in which a distinct reproductive hierarchy exists between dominant and subordinate individuals (Creel 2001; Hackländer et al. 2003; Raouf et al. 2005, Young et al. 2006). In contrast, few studies have examined GCs in plural breeding mammalian groups, in which most or all females breed. Plural breeders provide an opportunity to look at group living *per se*, without the confounding hierarchy observed in singular breeding social groups. To our knowledge, only Ebensperger et al. (2011) specifically address the influence of the social environment on GCs in *O. degus*, a free-living plural-breeding mammal. These authors reported that baseline GCs increased with the number of pups in a social group but did not vary predictable with group size (number of adult females). Thus, in degus, reproductive effort appears to be a more important determinant of GC levels than does social setting (group size). This is in contrast to our data, which suggest that social environment – specifically the distinction between living alone versus with conspecifics – is a key determinant of baseline glucocorticoid levels. Because Ebensperger et al. (2010) did not explicitly compare GC levels for lone versus group living females, direct comparisons of data from the two species are difficult. However, the variation in the outcomes reported for degus and colonial tuco-tucos suggest that relationships between social setting, reproductive success, and baseline GC levels may vary among plural breeding mammals.

*Social versus Physical Challenges*

Because GCs mediate a general response to a variety of challenges, it is difficult to distinguish the effects of the social environment from those of the physical environment,

especially in free-living animals. For example, wild female chimpanzees show increased cortisol response to both aggressive social interactions with conspecifics as well as the energetic demands of breeding (Thompson et al. 2010). In our study population, females that live alone must do all of the work involved in maintaining and digging tunnels in the burrow, finding food, and caring for offspring; in contrast, group-living females are able to share these tasks, perhaps resulting in a per capita decrease in the effort that must be expended on these activities. On top of this, lone females have higher per capita direct fitness than do group-living females (Lacey 2004), so that in addition to performing all foraging and burrow maintenance activities on their own, the former are also likely to be investing more in offspring care. Because one function of GCs is to maintain homeostasis by increasing the availability of glucose to meet energetic challenges imposed by the environment (Sapolsky et al. 2000, Goymann and Wingfield 2004), it seems likely that lone females must sustain higher levels of GCs as an adaptive means of meeting the energetic demands associated with living alone.

Balanced against these physical challenges are GC responses to increased social contacts among conspecifics. Our experimental manipulation of the social conditions under which captive female *C. sociabilis* were housed provide convincing evidence that the social environment influences baseline GCs in this species. In other words, when variation in physical demands and individual phenotypes are controlled for, interactions with conspecifics do not result in higher baseline GC levels. Woodruff et al. (2010) demonstrated that baseline GC levels were significantly higher for free-living tuco-tucos than for captive animals, suggesting that the former face greater physical challenges associated with life in "real" environments. How individuals respond to those challenges may be mediated by the social environment. In other words, in *C. sociabilis*, the effects of social environment on baseline GC levels may be amplified in free-living animals by the physical challenges associated with living alone.

*Group Structure and GCs*

While group living was associated with lower baseline GCs among yearling females, not all social groups were the same. To investigate potentially more subtle impacts of social groups on GCs, we also looked at the effects of group size and group composition. Previous studies have demonstrated considerable interspecific variation regarding the influence of group size on individual fitness, behavior, and physiology (Armitage and Schwartz 2000, Hayes et al. 2009, Izquierdo and Lacey 2008, Lacey 2004, Silk 2007). With regard to GCs, Pride (2005) found that ring-tailed lemurs (*Lemur catta*) exhibited high fecal cortisol levels in groups that were atypically large or small. However, Ebensperger et al. (2011) did not find that group size predicted mean cortisol levels in adult female degus (*O. degus*). Similarly, we did not find that group size influenced fCMs in yearling female tuco-tucos. Thus, group size alone does not seem to be a consistent predictor of variation in baseline GC levels among plural breeding mammals.

Group composition also varies within our study species. While most studies investigating the effects of group composition on GCs have focused on kinship among group members (Silk 2007), others have examined the effects of differences in sex ratio (McGuire et al.

2002). Given the social structure of *C. sociabilis*, differences in sex ratio were not a salient feature of our sample. Similarly, because groups are typically composed of close female kin, differences in kin structure were not immediately apparent. In contrast, however, the age structure of social groups did vary markedly from groups composed only of yearling females breeding for the first time to groups composed of several generations of adult females. While there is no direct evidence that females maintain an age-related dominance hierarchy, age has been found to influence dominance interactions and GC responses in other specie of social mammals (e.g., *Marmota marmota*: Hackländer et al. 2003). In our study population, baseline GC levels differed as a function of the number of older adult females in a group, with yearling females in groups containing only a single older female having higher fCMs than yearlings in groups with none or more than one older female. These data suggest that not all within-group relationships are the same, implying that group composition may be an important component of the social environment of *C. sociabilis*. This finding has important implications for a number of aspects of the social structure of this species, including the possibility of modest reproductive skew among group mates. Future studies of *C. sociabilis* will examine relationships between group composition, GC levels, and direct fitness in greater detail.

*Conclusion*

This is the first study to examine -- using both captive and free-living mammals -- the effects of the social environment on baseline GCs in a plural-breeding species in which individuals live alone and in groups. Numerous laboratory studies have examined relationships between social setting and GC concentrations, with emphasis on how social isolation and overcrowded conditions elicited a GC response (Marchlewska-Koj 1997, Serra et al. 2000, Viveros et al. 1988). While subsequent studies have emphasized the impacts of naturally occurring variation among group-living individuals (e.g. maternal affects, Liu et al. 1997, aggression, Castro and Matt 1997), few studies have considered the effects of group living *per se* on baseline GC levels. Our studies, in particular our integration of data from free-living and captive colonial tuco-tucos, indicate that whether individuals live alone or in groups is a significant determinant of baseline GC response to external challenges. More specifically, our data suggest that the social environment is an important mediator of response to physical as well as social challenges that impact glucocorticoid physiology.

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FIGURES

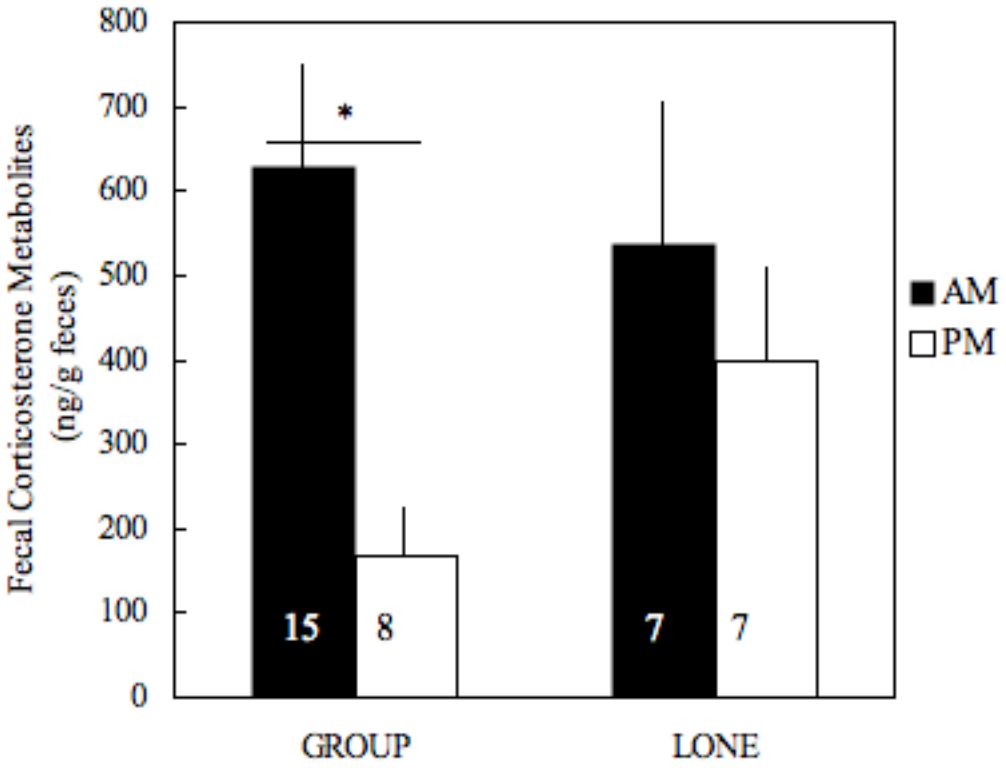


Figure 1. Fecal corticosterone metabolites from samples collected in the morning and the afternoon for free-living yearling colonial tuco-tuco females living in groups or alone. fCMs were significantly different between the morning and afternoon samples in group-living females (One-tailed T test: * denotes $P < 0.05$) and nearly significantly different between the afternoon samples of females living in groups and females living alone.

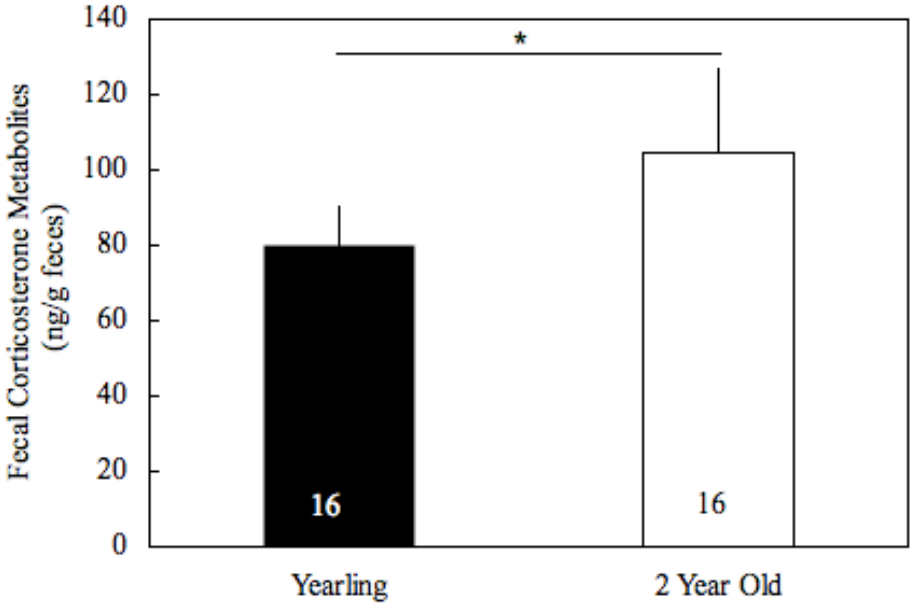


Figure 2. Free-living female *C. sociabilis* showed a significant increase in fecal corticosterone metabolites from one year to two years of age (Paired T Test: * denotes $P < 0.05$), regardless if they were living alone or in a group as yearlings. All females were group living as 2-year olds.

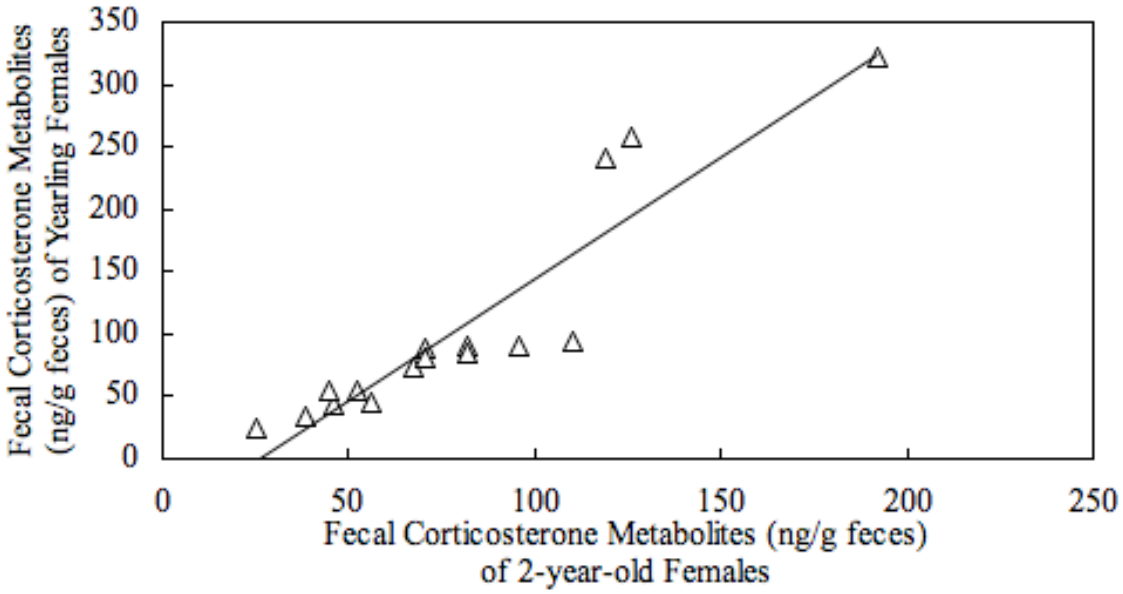


Figure 3. A significantly positive correlation was found between fCMs in female colonial tuco-tucos as yearlings and in the same individual at 2 years old (GRM: $r^2 = 0.86$, N = 16, p = < 0.0001).

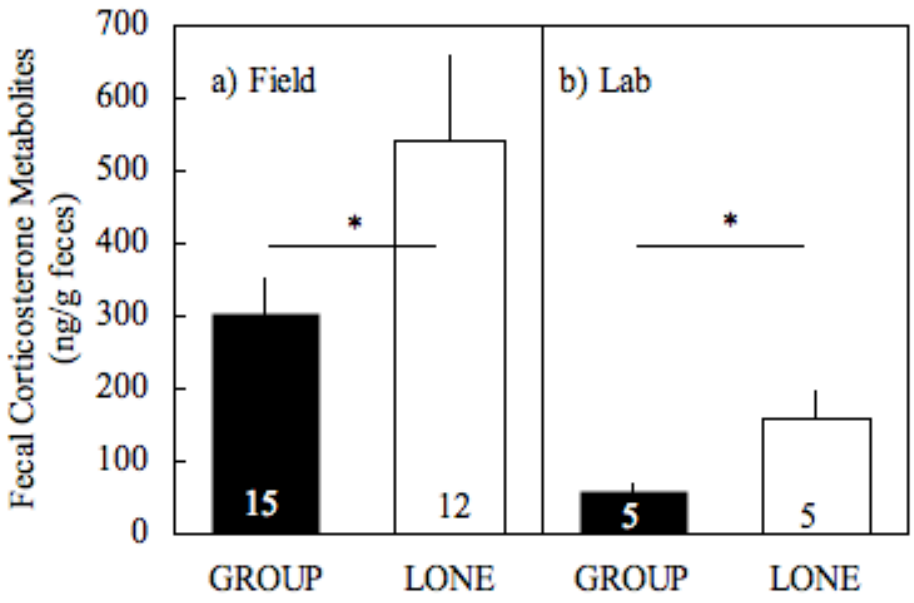


Figure 4. Fecal corticosterone metabolite concentrations for lone and group-living female colonial tuco-tucos: a) field data from yearling females collected 2005-2009; b) lab data collected from yearling females experimentally housed alone or in pairs in 2011. (field: One-tailed T test; lab: Mann-Whitney U Test; * denotes $P < 0.05$).

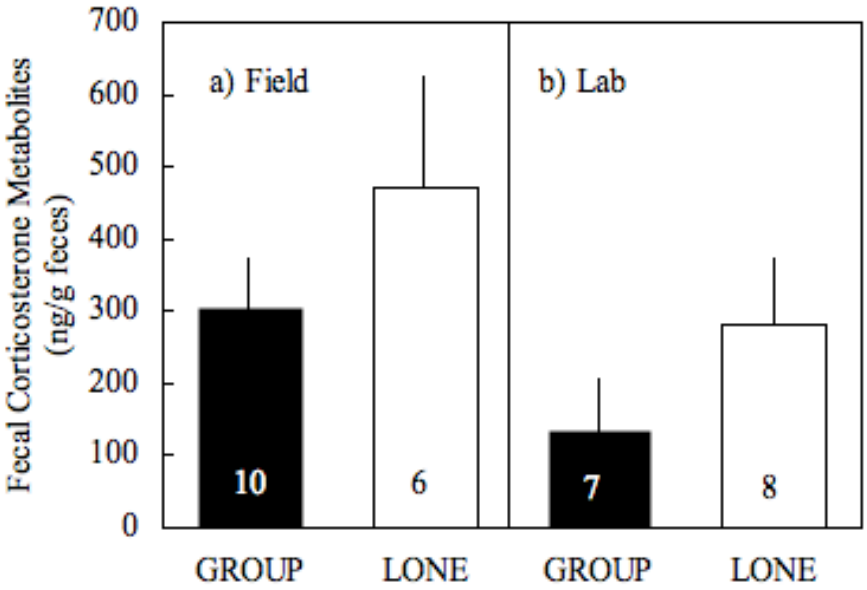


Figure 5. Fecal corticosterone metabolite concentrations for older (> 1 year) lone and group-living female colonial tuco-tucos: a) field data collected 2005-2009; b) lab data collected from females experimentally housed alone or in pairs in 2008-2009. Similar patterns to yearling females (see Fig. 4) were observed, though differences were not significant (T test: $p > 0.05$).

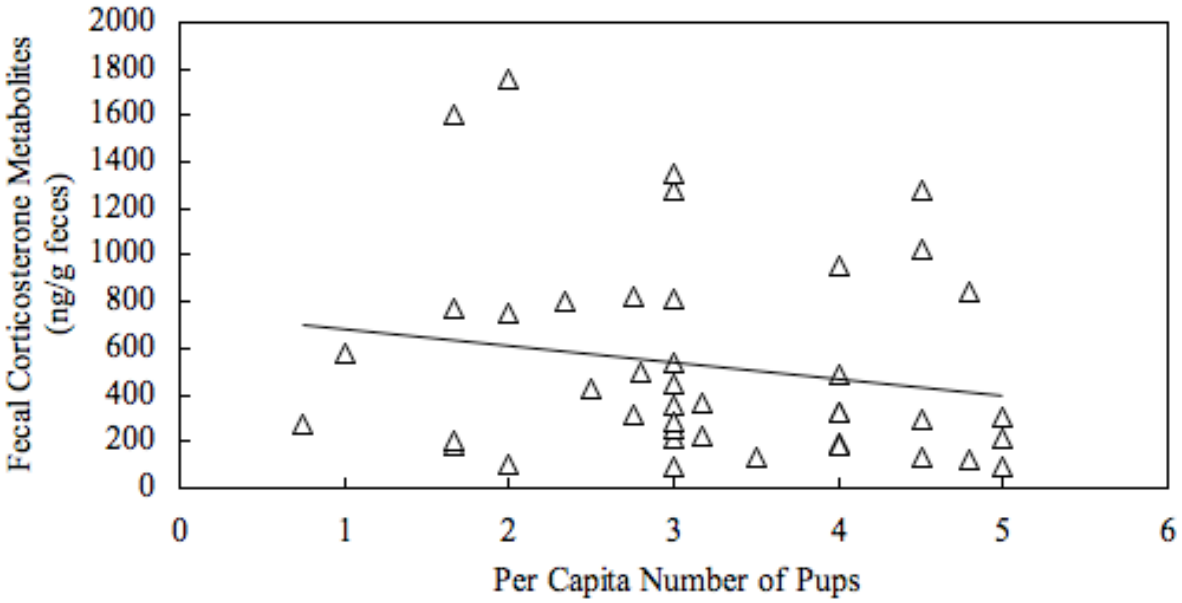


Figure 6. Scatterplot showing the relationship of fecal corticosterone metabolite concentrations to the per capita number of *C. sociabilis* pups per colony of free-living colonial tuco-tucos. There was a non-significant trend ($r^2 = 0.035$) for fCMs to go down with increasing number of pups (ANCOVA: $N = 42$, $p > 0.05$).

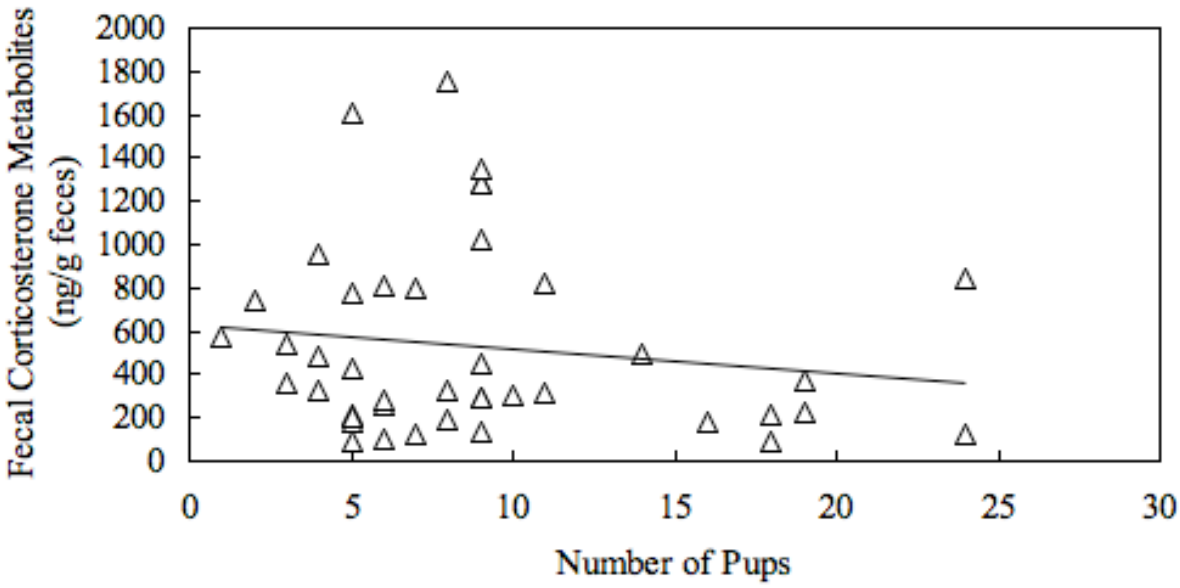


Figure 7. Fecal corticosterone metabolites did not differ with respect to the absolute number of *C. sociabilis* pups in colony (ANOVA, $r^2 = 0.02$; N = 42, p = 0.34).

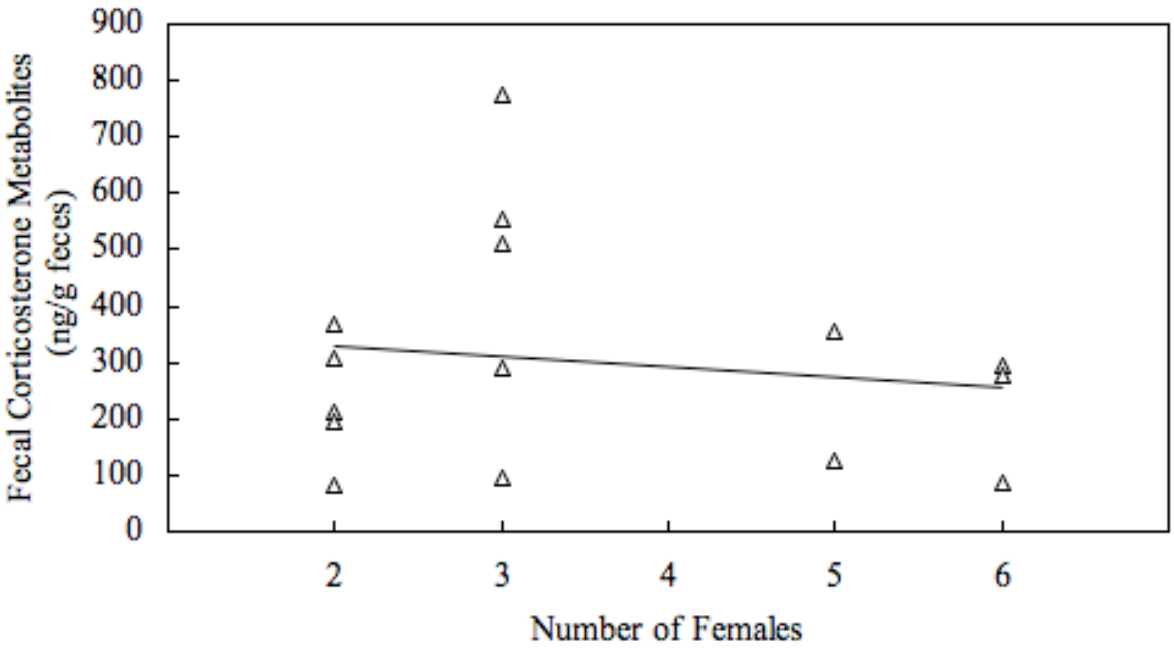


Figure 8. Fecal corticosterone metabolite concentration as a function of the number of adult female *C. sociabilis* in a group. Each triangle represents a yearling female in a group of specific size (for each group size, 2, 3, 5, and 6, N = 5, 5, 2, and 3, respectively). No significant correlation was found between fCMs in yearling females and the number of females resident in a colony (GRM, N = 15, $r = 0.15$, $p > 0.05$).

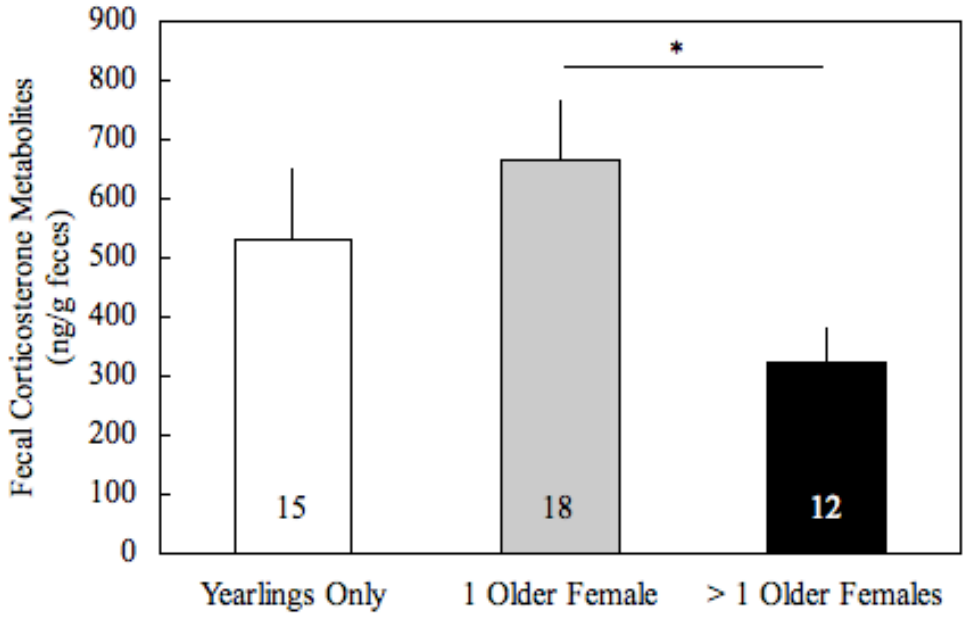


Figure 9. Fecal corticosterone metabolite concentrations (fCMs) for free-living yearling female *C. sociabilis* living in groups consisting of: 1) yearlings only, 2) yearlings and 1 older female, or 3) yearlings and $\geq$ 2 older females. Yearling females in groups with 1 older female exhibited significantly higher fCMs than those living in groups with $\geq$ 2 older females (Kruskal-Wallis ANOVA, * denotes $p < 0.05$).

Chapter 3

Being There:

Male Nest Attendance in a Communally Nesting Rodent,

The Colonial Tuco-Tuco (*Ctenomys sociabilis*)

ABSTRACT

While many studies of communally nesting, plural breeding mammals have examined the costs and benefits to females, few have investigated the adaptive consequences of participation by males. Male nest attendance is rare in mammals but has been shown to occur in both monogamous and polygynous systems. In this study, we explored the potential adaptive benefits of nest attendance to males in the communally breeding colonial tuco-tuco (*Ctenomys sociabilis*). Using data from free-living and captive populations of this species, we tested two hypotheses, the parental effort hypothesis, which states that males remain in the nest to enhance their current direct fitness (e.g., by increasing offspring growth and survival) and the mating effort hypothesis, which asserts that males remain in the nest to enhance their future direct fitness (e.g., enhanced access to additional mating opportunities). On average, males spent the same percentage of time in the nest with pre-weaned young as did females. We found no evidence that the presence of the male contributed to the number of offspring reared to weaning or the growth of pups. However, male nest attendance was associated with increased survival to breeding age by male, but not female, pups. Overall, we found little support for the parental effort hypothesis and no support for the mating effort hypothesis. Instead, it is possible that males remain in the communal nest because the costs of doing so are less than those of leaving the group and establishing residence elsewhere.

# INTRODUCTION

Studies of mammalian plural breeders that nest communally have generally emphasized the costs and benefits to breeding females (Becker et al. 2007; Boyce and Boyce 1988b; Ebensperger et al. 2007; Gilchrist et al. 2004; Hayes 2000; Hayes et al. 2009). Far less attention has been given to males that participate in these social groups. In part, this is due to the fact that, in mammals, females play the role of primary caregiver to the offspring, having the obligate responsibilities of gestation and lactation, leaving males free to pursue additional reproductive opportunities elsewhere. Therefore, male nest attendance in mammals is rare (< 10%; Kleiman and Malcolm, 1981; Woodroffe and Vincent, 1994) and, in all probability, even rarer in communally nesting species in which there are multiple females available as caregivers. However, in a number of species, males are present and have been observed to provide care to unweaned offspring in the nest (e.g. marmosets, Ziegler et al. 2009; fox, Wright 2006; rodents, Gubernick et al. 1993, Dewsbury 1985). Such examples include several communally nesting species (e.g. degus, Ebensperger et al. 2010, banded mongoose, Gilchrist et al. 2004; striped mice, Schubert et al. 2009). While data are available to examine the adaptive consequences of male nest attendance in several socially monogamous species of rodents (Gubernick et al. 1993, Oliveras and Novak 1986, Silva 2008), few, if any, studies have examined the adaptive importance for polygynous males in communally nesting species.

Several adaptive hypotheses have been proposed to explain why a male should remain in the nest rather than seek mating opportunities elsewhere. Chief among these is the parental effort hypothesis (Westneat and Sherman 1993; Magrath and Komdeur 2003), studies of which have shown that the presence of a male in the nest increases his *current* direct fitness by positively impacting offspring growth and survival (Dudley 1974; Gubernick et al. 1993; Luis et al. 2004, Schradin and Pillay 2004; Wright 2006). In contrast, the mating effort hypothesis asserts that males might improve their *future* direct fitness by remaining with a female, thereby increasing the male's chances of attaining additional mating opportunities (Fernandez-Duque et al., 2008; Prates and Guerra, 2005; Féron and Gouat, 2007). Few studies, however, have examined both hypotheses in the same species.

Studies of colonial tuco-tucos (*Ctenomys sociabilis*) present an ideal opportunity to explore potential adaptive benefits of male nest attendance in both natural and captive settings. This species of subterranean rodent is endemic to southwestern Argentina, where it inhabits mesic meadows in the eastern foothills of the Andes. Colonial tuco-tucos differ from most other members of the genus *Ctenomys* in that they are social, living in burrow systems occupied by 1 to 6 adult females and, ca 40% of the time, a single adult male (Lacey et al. 1997; Lacey 2004; Lacey and Wieczorek 2003, 2004). Females give birth once a year in early to mid-spring (October-November), with no evidence of a postpartum estrous. When unweaned young are present, all animals in the burrow system, including the male, share a common nest site (Izquierdo and Lacey 2008). Although low genetic variability (Lacey 2001) has precluded genetic determination of paternity, the effects of male nest attendance on production of young and on future mating opportunities can still

be assessed. In 1996, a captive population of these animals was established at the University of California, Berkeley, providing an unusual opportunity to compare data on nest attendance from free-living and captive conspecifics.

The purpose of this study was twofold: (1) to characterize nest attendance by males and (2) to examine the fitness consequences of this attendance. More specifically, we examined the current and potential future direct fitness consequences of male nest attendance using complementary data from free-living and captive animals. If the parental effort hypothesis applies, we would expect the presence of a male to increase production, growth and/or the survival of young. If the mating effort hypothesis applies, we would expect the presence of a male to increase the chance that he will mate with the same females in the following season. In addition to providing one of the few studies examining potentially adaptive benefits of male nest attendance in a communally nesting rodent, it also presents the first evidence of male nest attendance in a ctenomyid.

## MATERIALS AND METHODS

### *Field Study Site*

The free-living population of *C. sociabilis* sampled was located on Estancia Rincon Grande, Provincia Neuquén, Argentina (40°57'S, 71°03'W). The study site consisted of ca 20-ha area of open meadow dominated by seasonal grasses and sedges and containing several species of woody shrubs. Members of the study population have been live-trapped annually since 1992 as part of an intensive investigation of the behavioral ecology of this species. Colonial tuco-tucos are subterranean and only emerge half a body length from their burrows to feed. Animals were captured as they emerged to forage using hand-held nooses placed in the rim of active burrow entrances (Lacey et al., 1997; Lacey, 2004). Upon first capture, each animal was individually marked with a magnetically coded bead (IMI-1000 Implantable Transponders, BioMedic Data Systems, Seaford, DE) inserted beneath the skin at the nape of the neck; implanted transponders were read using a hand-held scanner (DAS 4001 Pocket Scanner, BioMedic Data Systems).

### *Captive Population*

The laboratory population of *C. sociabilis* sampled consisted of ~ 45 captive-born individuals, descended from an initial group of 12 individuals captured in Neuquén Province, Argentina, and transported to the Berkeley campus in January of 1996. Captive animals were housed in artificial burrow systems constructed of clear acrylic. Each burrow system consisted of ~ 10 m of acrylic tunnels connecting three acrylic boxes. Two boxes (30 x 30 x 15 cm) served as nest chambers and latrines, and one box (30 x 50 x 40 cm) was used to introduce food to the burrow system. The floor of each box was covered with ~ 2 cm layer of aspen bedding; in addition, ~ 100 $cm^3$ of shredded paper bedding was placed in one box for use in nest construction. Rooms housing the burrow systems were maintained at 20°C. The light: dark cycle in the rooms imitated seasonal changes in day length at

41°S, the latitude at which the original members of the population were captured. The animals were fed *ad-libitum* quantities of commercially available rat chow (Simonsen's Inc., Gilroy, CA) and were provided daily with fresh produce (corn, carrots and lettuce).

To simulate naturally occurring patterns of reproductive behavior, members of our captive study population were paired in late May of each year from 1998 to 2009. Captive female *C. sociabilis* produced only a single litter of 1–6 young per year, with parturition occurring between October and December, which is similar to the annual breeding cycle in nature. Because spatial constraints prevented us from creating multi-female colonies in the laboratory, we used only male-female pairs to investigate male nest attendance. Within 24 hrs of the birth of a litter, we randomly assigned individual females to male or no male treatment groups, both of which reflect naturally occurring patterns of social behavior in this species (Lacey 2004; unpublished data). For each pair, adult males were allowed to remain with their mates and offspring until juveniles were weaned. For lone females, adult males were removed immediately at parturition. Each male-female pair and lone female used in this study was represented by different individuals.

Groups vs. Pairs. Although laboratory analyses were restricted to data from male-female pairs, for free-living animals we explored whether patterns of nest attendance differed between male-female pairs and multi-females groups. In keeping with the primary theme of this study (i.e., importance of male nest attendance in communally breeding groups), we used data from free-living groups of all sizes but emphasized contrasts between lone female and male-female pairs in order to better match data obtained from members of the captive study population.

*Quantifying Nest Attendance*

Field. We used radiotelemetry to determine which adults shared a burrow system and nest site. Detailed descriptions of these procedures are provided in Izquierdo and Lacey (2008) (see also Lacey et al., 1997). Briefly, adults captured were fitted with small (< 7 g) radio-collars consisting of an acrylic-encased transmitter (SM1-Mouse transmitters, AVM Instruments, Inc., Colfax, CA) attached to a plastic cable tie before being released. We determined colony boundaries and nest locations by following radio-collared animals using hand-held antennas and receivers (Yagi antennas, CE-12, AVM Instruments, Inc.). Nest attendance for *C. sociabilis* was monitored for 75 individuals in 39 social units resident on the study site from 1996 – 2000; these are the same individuals and social groups monitored by Izquierdo and Lacey (2008). Eighteen of the social units were inhabited by a lone female; the other 14 were communally nesting groups comprised of 2, 3 or 4 females with or without a male. We also monitored seven colonies that were inhabited by male-female pairs.

Each colony (N = 25) was monitored for 5 to 40 days (mean = 23 ± 10 days) between the birth and weaning of young. Telemetry data were collected by sitting at the nest and checking relevant collar frequencies every 5 minutes for 30 minutes. Typically, 3 30-min scan periods were completed per colony per day between the birth and weaning of young.

We calculated the percentage of scans that each individual, male and female, was in the nest.

Laboratory. Patterns of nest attendance by adults and young were documented from videotapes made during the first two weeks following the birth of a litter. To minimize disturbance to neonates and their parents, we did not begin filming nest attendance until 24 hrs after birth. For each 2-hr filming session, a video camera was focused on the acrylic box containing the focal litter; after the camera was set to record, the person operating the camera left the animal room to avoid disturbing the animals during filming. Typically, we collected 2–6 hours of videotape per day for each litter studied. Data collection continued until pups were 14 days old; by this age, young were very active, making it difficult to quantify nest attendance by individuals.

Videotapes were decoded by recording the times at which individuals (adults and juveniles) entered and exited the nest. To characterize nest attendance by adults, we calculated the percentage of total time videotaped per day that each parent was in the nest and averaged across days. Behaviors related to nest attendance were also recorded from the videotapes whenever possible; these behaviors included greeting, retrieving, grooming, huddling, and feeding (i.e. bringing food back to the nest) (Soares 2004).

Analyses. To quantify nest attendance in the field, Izquierdo and Lacey (2008) used time series analyses to assess whether the date or the time of day at which scans were completed influenced adult behavior. For the captive population, we used repeated measures ANOVA to examine potential temporal variation in adult nest attendance across days and examined time in nest as a function of pup age. For both the field and lab, we then calculated an average across days of the percent time in the nest, with separate estimates for each adult monitored. Comparisons of mean percent time in the nest for males versus females were completed using paired *t* tests. To confirm that data from free-living male-female pairs were generally representative of adult behavior, we compared nest attendance by these animals to patterns of nest attendance by adults in multi-female groups that contained an adult male.

Because females provide primary care to the young and therefore have the greatest impact on offspring growth and survival, we also evaluated the effect of the male on female nest attendance. We used pair-wise tests to examine the time that nests were left unattended in colonies with and without males. Additionally, regular filming in the lab provided an in-depth examination of daily nest attendance patterns between males and their female partners, male-female pairs and lone females, and paired females and lone females. Paired *t* tests were used to identify significant differences between group types on specific days during the first two weeks after parturition.

*Quantifying Number of Pups Reared*

All individuals in the free-living population, including pups, were captured and marked annually (see above). Lacey et al. (1997) describe in detail the methods used to ensure that all residents of a burrow system were captured. In brief, animals were held for 24 hours to determine if there were additional individuals present within the colony. We monitored activity by placing twigs over all entrances to a burrow system. If no twigs were displaced over the course of 2 foraging periods (typically morning and late afternoon), we assumed that all animals from that burrow system had been captured. We also checked for evidence of new burrow entrances. The number of pups reared to weaning by lone females versus male-female pairs were compared using paired two-sample tests. We also examined the mean number of pups weaned as a function of group size in colonies with a male present and colonies without a male, using ANCOVA and regression analyses.

*Estimating Juvenile Growth*

We examined juvenile growth rates in 97 free-living pups from 30 litters trapped over a 12-year period (1994–2006). All pups included in the data set were captured $\geq 2$ times within the first month after weaning. Pups were weighed in a cloth bag with a 300-gram Pesola scale. Because females within a group give birth somewhat asynchronously, at capture the pups within a colony represented multiple ages. As a result, we calculated the rate of growth for individual pups. This was done by dividing the difference between the two weight measures for an individual by the number of days between measures (i.e. $\Delta$ weight/time). The average number of days between weight measures was $12 \pm 6$ days.

Unlike free-living juveniles, pups in the captive population were weighed at known ages, creating uniformity in the growth data. As a result, a precise growth curve was established for these animals and compared to weights of field animals. Data were collected for 16 captive litters of pups born between October 2003 and January 2010. Litters ranged in size from three to five pups and contained a total of 32 males and 41 females. Pups were individually marked with distinctive patterns using hair bleach (Jerome Russell, Canoga Park, CA; cream peroxide, 30 Volume, 9%). From the first day after birth and every other day thereafter, we weighed each pup to the nearest tenth of a gram using an Acculab VI-600 scale. In order to capture the entire lactation period, we collected data for six weeks following the birth of a litter.

Because juvenile *C. sociabilis* are sexually dimorphic for body weight (Woodruff and Young, unpubl. data), separate analyses of growth rates were conducted for male and female juveniles for both the captive and free-living animals examined. Growth curves for captive male and female pups were generated from a quadratic regression model. Residual vs. fitted graphs confirmed that this was the correct functional form for examining growth rates from birth to 6 weeks old. Because the weights of free-living pups corresponded to the weights of 2-4 week-old captive animals, we used that age range to examine growth rates in the captive animals. Growth rates were regressed against *weight at first capture* for free-living animals and against *weight at 2 weeks* for captive animals. We used a general

linear model (GLM) for multiple regression analyses, testing for the effects of weight at first capture, the presence of the male, and the interaction between the two. We also tested for effects of two other potentially operative variables, the ratio of pups to adult females in each social group and the ratio of male to female pups in a social group.

*Estimating Survival*

In addition to looking at the number of pups reared to weaning, we examined survival of juveniles to their yearling season as an additional measure of current direct fitness. We used Chi-Square tests to determine if male and female pups survived better than expected in colonies with an adult male present. Expected values were derived by calculating the percentage of pups that came from male versus no-male colonies. Assuming that juvenile survival was equal between these two types of social groups (null hypothesis), we used those percentages to determine the expected number pups from male versus no-male colonies that survived to their yearling season.

To explore the effects of male nest attendance on future direct fitness, we determined the frequency with which males remained in the same burrow system for two successive breeding seasons (thereby potentially allowing males to mate with the females with which they had shared a nest during the previous breeding season). First, we calculated percent male survival to a second breeding season. This was done by recapturing all individuals on the study site during the period when young were first beginning to forage above ground for themselves (October-December). We only calculated survival if all individuals in the burrow system were captured (see methods for quantifying number of pups reared). We then determined if surviving males were resident in the same burrow system in successive breeding seasons. Fidelity to a burrow system was considered a proxy for fidelity to females, since females are philopatric and do not disperse from their natal colony after their juvenile season.

All procedures were approved by the University of California, Berkeley, Animal Care and Use Committee and followed guidelines established by the American Society of Mammalogists (Gannon and Sikes, 2007).

*Statistical Analyses*

Two-sample statistical comparisons were performed using *t*-tests unless the distribution of data points indicated that non-parametric tests were required. All statistical analyses were completed using Statistica 6.0 (StatSoft, Inc. 1984-2008, Tulsa, OK). Means are reported ± 1 SD.

## RESULTS

### *Male nest attendance*

Using the same data set on nest attendance employed here, Izquierdo and Lacey (2008) showed that the percentage of time that the nest was left unattended (no adult present) did not vary predictably with the date of data collection, suggesting that patterns of adult activity did not change as a function of juvenile age. Similarly, we did not find a significant effect of pup age on the percentage of time that no adult was present in the nest in the captive population (repeated measures ANOVA: $F = 2.02$, $df = 12$, $P > 0.05$). In the field, males spent an average of 40.7 % (±14.5) scans per day (N = 10 males over 23 days) in the nest and females spent an average of 48% (±11.4) scans per day in the nest (N = 24 females over 23 days) (paired T test: $t = 1.45$, N =10, $P = 0.18$; Figure 1). In the captive population, we found that while males spent on average 57.5% ± 9.5 of their time in the nest (N = 13 males) and females spent on average 71.7% ± 9.0 of their time in the nest (N = 13 females), there was no significant tendency for a male to spend more time in the nest than his female partner (Paired *t* test: $T = 1.56$, $df = 10$, N = 11, $P = 0.15$; Fig. 2).

### *Effects of males on female nest attendance*

To determine how males contributed to the overall percentage of time that the nest was attended, we examined the effect of the presence of males on female nest attendance in the captive population. In the field, group size affected the time that the nest was left unattended (Izquierdo and Lacey 2008). Similarly, we found that captive lone females left the nest unattended significantly more than male-female pairs (Two-tailed *t* test: $T = 2.10$, $df = 18$, N = 13, 13, $P < 0.001$).

In the lab, analyses of data obtained from daily filming revealed more subtle differences in nest attendance that were not evident when comparing overall means of time spent in the nest between male and female (Fig. 3). There was no significant difference between the time lone females and paired females spent in the nest (repeated measures ANOVA: $F = 0.162$, $df = 1$, $P = 0.69$, Fig. 3c). Although paired females spent significantly more time in the nest than their male partners on day 2 after the birth of a litter (Paired *t* test: $T = 3.64$, $df = 6$, N = 7, 7, $P = 0.01$), no significant differences between members of a pair were detected for days 3-14 following the birth of a litter (Fig. 3b). There were significant differences between male-female pairs and lone females in time spent in the nest on multiple days occurring at the start and end of the two-week period (repeated measures ANOVA: $F = 8.02$, $df = 1$, $P = 0.016$; Fig. 3a). Specifically, male-female pairs spent more time in the nest than lone females on days 3–5 and days 12 -14 (See Table 1 for significant *t* test results).

### *Male behavior toward juveniles*

Male behavior toward pups was quantified for 11 litters in the captive population. Soares (2004) found that males performed all of the same pup-care behaviors as females except

nursing (Fig. 4). In most cases, there was no difference in the frequency of observed behaviors between the male and the female. However, females spent more time than males retrieving offspring (i.e. bringing them back to the nest) (Soares 2004).

*Effects on juveniles*

We found no difference between the mean number of pups reared to weaning in lone female versus male-female burrow systems for either free-living (MWU: U = 249.0, Z = -0.77, N = 36, 16, P = 0.44; Fig. 5a) or captive animals (MWU: U = 71.5, Z = -1.03, N = 17, 11, P = 0.30; Fig. 5b). We found no effect of group size or the presence of a male on the per capita number of pups (ANCOVA: $F_{size}$ = 0.44, df = 1 N = 28, P = 0.52; $F_{male}$ = 2.14, df = 1, N = 18, 10, P = 0.16). However, when we separated data by male-absent and male-present groups, we found a difference in regression slopes. The per capita number of pups reared to weaning in groups without an adult male decreased significantly as group size increased ($r^2$ = 0.35, F = 8.61, df = 1, N = 18, P = 0.01). In contrast, the per capita number of pups reared to weaning did not differ with group size in burrow systems in which an adult male was present ($r^2$ = 0.008, F = 0.062, df = 1, N = 10, P = 0.81).

Growth curves generated from data for juveniles reared in captivity (Fig. 7) indicated that males grew more rapidly than females although no differences were found in growth rates for either male or female pups as a function of the presence of an adult male (GLM; *males*: $F_{1,45}$ = 2.08, P = 0.157; *females*: $F_{1,56}$ = 0.517, P = 0.478). Similarly, the presence of an adult male did not affect growth rates for free-living male and female pups (GLM; *males*: $F_{1,51}$ = 0.007, P = 0.934, Fig. 8a; *females*: $F_{1,38}$ = 2.4, P = 0.131, Fig. 8b). For males, neither the ratio of pups to adult females nor the ratio of male to female pups affected growth rate (GLM; *pups to adult females*: $F_{1,45}$ = 0.008, P = 0.93; *male to female pups*: $F_{1,45}$ = 0.095, P = 0.76). In contrast, growth rates for female pups were significantly influenced by the ratio of pups to adult females ($F_{1,38}$ = 7.81, P = 0.01).

We looked at the survival of juveniles to their first year as a function of being raised in burrow systems with and without males (Fig. 9). Survival of juvenile females to their yearling season did not differ from expected for either lone females or male-female pairs ($\chi 2$ = 0.09, N = 29, 16, P > 0.05). In contrast, survival of juvenile males to their yearling season was significantly greater than expected for burrow systems containing an adult male ($\chi 2$ = 6.5, N = 99, 52, P < 0.05).

*Patterns of male breeding dispersal*

From 1998 to 2009, an average of 11.6% (± 6.3) of the male pups reared in the population each year survived to their yearling season. In half of these years, 1.7 % (± 2.2) of yearling males survived to a second breeding season, while no yearling males survived to a subsequent season in the other six years (Fig. 10). Overall, male survival to a second breeding season was significantly lower than survival to a first year (Paired *t* Test: T = 5.29, df = 11, p < 0.001). Males surviving to a second breeding season did not typically remain in the same burrow system that they had inhabited during their first breeding

season; 8 of 9 males that survived to a second breeding season changed burrow systems between years.

## DISCUSSION

While many studies of plural-breeding mammals have examined the costs and benefits to females of communal nesting, few have investigated the adaptive consequences to males of this behavior (but see Ebensperger et al. 2010, Gilchrist et al. 2004, Schradin and Pillay 2004). In this study, we considered the role of male colonial tuco-tucos in communally nesting groups and explored potential adaptive benefits of nest attendance to males. We found that, on average, there was no difference in the percentage of time spent in the nest between males and females. The presence of a male did not affect the number of young reared to weaning or to the growth rates of pups. However, male nest attendance did have an apparent effect on the survival of male, but not female, pups.

### *Nest attendance*

We determined that males and females did not differ with respect to mean percentage of time spent in the nest. Further, we found that males did not contribute to the overall time that an adult was present in the nest in multi-female groups, although the presence of a male did significantly decrease the percentage of time that the nest was attended when comparing lone females to male-female pairs. In communally nesting species in which multiple individuals (i.e. > 2) share the same nest, the effect of a single individual (e.g. the male) may be reduced in larger groups. The absence of a male effect on maternal nest attendance has been observed in other species, such as collared lemmings, the southern grasshopper and white-footed mouse, and mandarin voles (Shilton and Brooks 1989, McCarty and Southwick 1977, Smorkatcheva 2003).

Studies of socially monogamous mammals have shown that males play a critical role in caring for young (Gubernick et al. 1993, Oliveras and Novak 1986, Silva 2008). Even in some polygynous species, males have been shown to assist with offspring (Barash 1975, Ebensperger et al. 2006, Schubert et al. 2009), supporting the idea that, while males cannot participate in female-specific behaviors (e.g. nursing offspring), their presence can contribute to other important functions related to caring for young (Woodroffe and Vincent 1994). Males in the captive population of colonial tuco-tucos studied engaged in all the same care behaviors as females except nursing. Because tuco-tucos are fully subterranean, we could not compare directly the frequencies of these behaviors for males versus females, but based on data indicating that free-living males and females spend comparable amounts of time in the nest, it seems reasonable to conclude that males contribute substantially to juvenile care. Minimally, by huddling in the nest with young, adult males may contribute to improved thermoregulation of young, a contribution that may be particularly important during the first two weeks following birth (Cutrera et al. 2003, Vickery and Millar 1984, Gilbert et al. 2010).

*Mating effort hypothesis*

The mating effort hypothesis predicts that remaining in the communal nest enhances a male's future direct fitness by increasing his access to future mating opportunities with the females with which he cohabits. Féron and Gouat (2007) found that in the mound-building mouse, *Mus spicilegus* inter-litter intervals were shortened, when males attended the young in the nest, thereby increasing opportunities to mate with the same females. *C. sociabilis* breeds only once a year and, while there is no evidence of a postpartum estrous, males may benefit by remaining in the communal nest if this increases their chances of surviving to a second breeding season and thus achieving future matings with females resident in that burrow system. The predictions of this hypothesis, however, were not met. Males that survived to a second breeding season dispersed between years, such that they occupied different burrow systems in successive breeding seasons. Although we do not know if the males that disappear between years (and were presumed dead) dispersed before dying, the observation that most 2 year old males changed burrow systems between years suggests that it is unlikely that males mated with the same female(s) in two successive years. Thus, the mating effort hypothesis does not appear to explain male nest attendance in *C. sociabilis*.

*Parental effort hypothesis*

The parental effort hypothesis predicts that males gain current direct fitness benefits by remaining in the communal nest and providing care to their offspring. Support for this hypothesis comes from studies of multiple mammalian species (Woodroffe and Vincent, 1994; Dewsbury, 1985; Wright and Brown, 2000). In *C. sociabilis*, low genetic variability (Lacey 2001) has precluded genetic determination of paternity; although it is likely that the male resident in a burrow system is the father of some to all of the juveniles in that system, we cannot confirm that male care is directed to offspring. We found no evidence that the mean number of pups reared to weaning differed between colonies with and without adult males in either captive or free-living colonial tuco-tucos. Similar to Lacey (2004), we found that as group size increased, the mean per capita number of pups weaned decreased. However, while the presence of an adult male did not appear to affect this relationship, regression analyses indicated that, in groups in which an adult male was present, the per capita number of young weaned did not decrease with group size, suggesting a subtle influence of males on the number of pups reared to weaning.

The growth rates of pups did not differ between burrow systems with and without adult males. We did not expect differences in offspring growth in the controlled environment of the lab, since access to food was unrestricted and temperatures were constant. Studies of free-living mammals, however, have shown differences in offspring growth when males were present in the nest. For example, Schradin and Pillay (2005) showed that striped mice (*Rhabdomys pumilio*) grew more quickly when an adult male was present. In contrast, paternal care did not affect the growth of offspring in *Peromyscus californicus* (Gubernick et al. 2000). Similarly, we did not find any differences in juvenile growth rates between free-living colonies with and without adult males. Thus, nest attendance did not lead to increased male direct fitness via increases in growth.

Offspring survival is a critical measure of direct fitness because only offspring that survive to breeding age will produce offspring of their own (Dewsbury, 1985; Woodroffe and Vincent, 1994; Wright and Brown, 2000). A number of studies of socially monogamous species such as *Peromyscus californicus* have shown that survival was greater for young reared by both a male and a female compared to those reared by only a female (Gubernick and Teferi 2000). To our knowledge, there are currently only two species on which offspring survival has been examined as a function of the presence of a male in a communal group. While studies of degus (*Octodon degus*, Ebensperger et al. 2010) revealed no effect of a male, studies of striped mice (*Rhabdomys pumilio*, Schubert et al. 2009) indicated that the effect of a male was dependent on environmental temperature. Although the mean per capita number of juveniles tuco-tucos reared to weaning did not differ as a function of the presence of a male, the number of juveniles males surviving to their yearling season was significantly greater than expected in burrow systems containing an adult male. Thus, nest attendance by adult males did appear to enhance one component of the male's direct fitness.

Observations of juveniles reared in captivity suggest a possible mechanism by which the presence of an adult male may enhance the survival of male pups. While many female *C. sociabilis* are philopatric and remain in their natal burrow system for life (Lacey and Wieczorek 2004), all juvenile males disperse. As juvenile males approach weaning, aggressive interactions between male littermates increase (Woodruff, pers. obs.). We have observed that juvenile males raised in burrow systems without males begin to fight much earlier than do juvenile males raised in burrow systems containing a male. It is possible that by suppressing aggression among male littermates, adult males delay natal dispersal by juveniles, thereby allowing young to grow larger, more competitive, and more likely to survive to their yearling season.

*Other explanations*

In sum, we found no evidence to support the mating effort hypothesis and only limited support for the parental effort hypothesis. Although our analyses focused on these two potential explanations for male behavior, it is possible that adult males remain in the communal nest for other reasons. For example, males may remain in the nest because this is safer than attempting to disperse and establish residence elsewhere. In other words, nest attendance by males may reflect somatic effort – actions that increase an individual's survival (Magrath and Komdeur 2003). Male *C. sociabilis* are vulnerable to aerial predators, especially when dispersing above ground to new burrow systems. Males may reduce their risk of predation by remaining in the communal nest for as long as possible before engaging in breeding dispersal; this option may be particularly beneficial if opportunities to move to other burrow systems are limited.

As with any social system, communal nesting varies among species and this variation includes differences in the number of males per social group. This variation may reflect differences in male behavior and the associated fitness consequences. Most research has focused on females; these studies have revealed that for females, direct fitness may increase, decrease, or be unaffected by group size (Ebensperger et al. 2011, Hayes 2000,

Hayes and Solomon 2004, Lacey 2004, Randall et al. 2005). The fitness consequences to males, however, may be quite different. For example, while per capita direct fitness for female *C. sociabilis* decreases with increasing group size, the direct fitness of the single male per group should increase as the number of females per group increases. Thus, understanding variation in social structure in communally breeding species requires understanding of fitness consequences of communal nesting for males as well as females.

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FIGURES

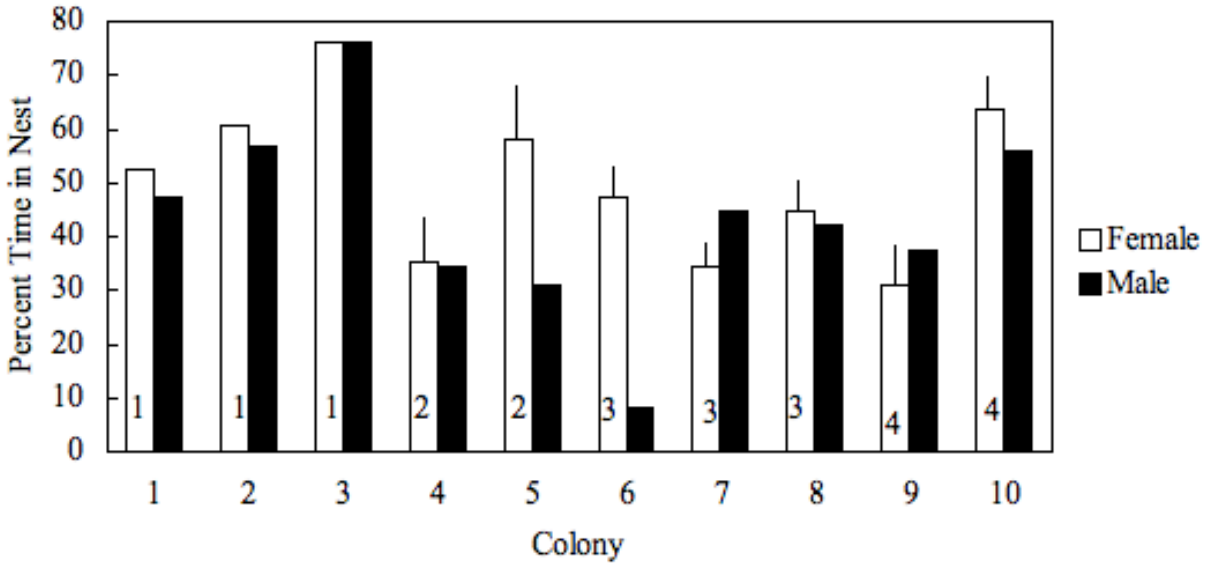


Figure 1. Focal nest telemetry scans of free-living colonial tuco-tuco colonies (N = 10), each with one male and 1-4 females (see number in bar). There was no significant difference between females and males in time in nest (Paired *t* test: p = 0.18).

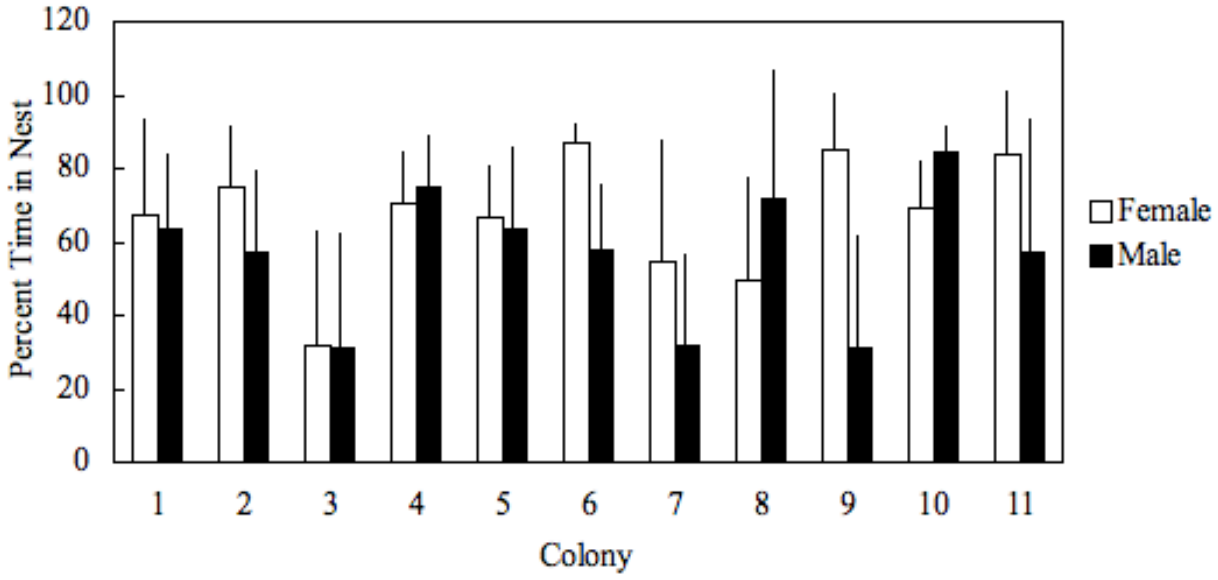


Figure 2. Mean percent time that captive male and female colonial tuco-tucos (N = 11) spent in the nest with their unweaned offspring. There was no significant difference between females and their male partner (Paired *t* test: p = 0.15).

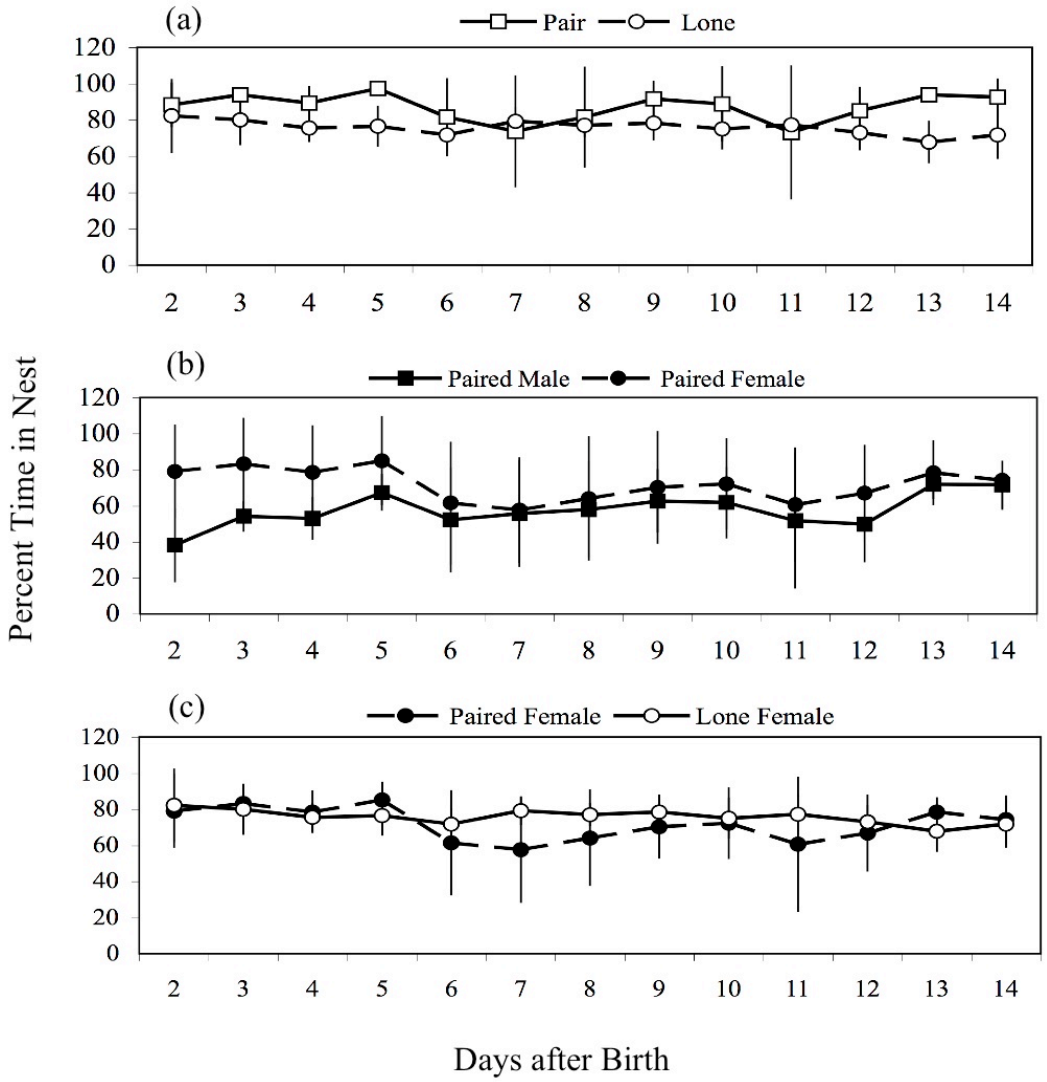


Figure 3. Daily nest attendance patterns of captive colonial tuco-tucos over the first two weeks after parturition: (a) male-female pairs and lone females, (b) paired males and paired females, and (c) paired females and lone females. Significant differences in nest attendance on days 3 – 5 and days 12 – 14 were found between male-female pairs and lone females ($P < 0.05$) and on day 2 between paired males and their female partners ($P < 0.05$). Paired females and lone females showed no significant difference in patterns of nest attendance on any day two weeks after parturition.

Table 1. Significant results of pair-wise *t* tests for nest attendance from days 2 to 14 after parturition between: (a) male-female pairs and lone females and (b) paired males and paired females.

| Pair-wise Groups | Days after Birth | N | df | *t* | p |
|---|---|---|---|---|---|
| (a) male-female pairs vs. lone females | 3 | 7,6 | 6 | 2.45 | 0.02 |
| | 4 | 6,6 | 10 | 2.23 | 0.01 |
| | 5 | 7,6 | 5 | 2.57 | 0.003 |
| | 12 | 7,6 | 11 | 2.20 | 0.05 |
| | 13 | 6,6 | 6 | 2.45 | 0.005 |
| | 14 | 7,6 | 10 | 2.23 | 0.01 |
| (b) paired males vs. paired females | 2 | 6,6 | 5 | 2.57 | 0.01 |

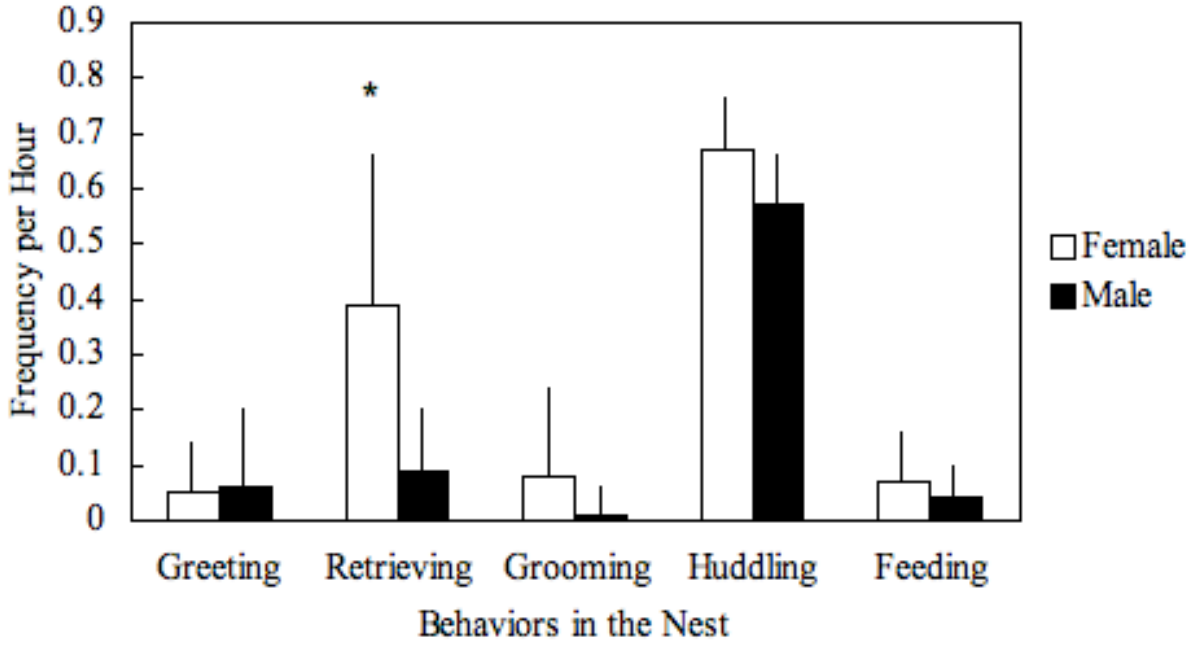


Figure 4. Parental care behaviors performed by captive male and female colonial tuco-tucos (N = 14 pairs). For each behavior, the mean frequency across litters (± SD) is reported as number of occurrences per hour. Asterisks indicate significant difference. Total number of hours of observation = 41.4 ± 23.3. Redrawn from Soares (2004)

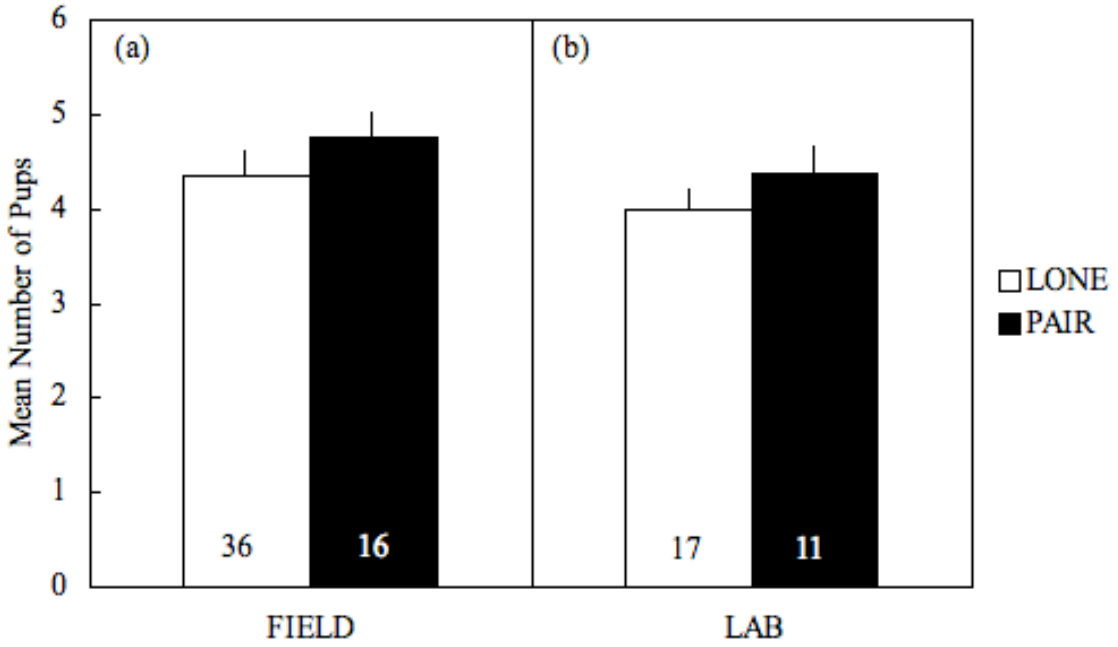


Figure 5. The mean number of pups at weaning did not differ (P > 0.05) between colonies with a lone female and those with a male-female pair in either (a) free-living (N = 36, 16) or (b) captive (N = 17, 11) colonial tuco-tucos.

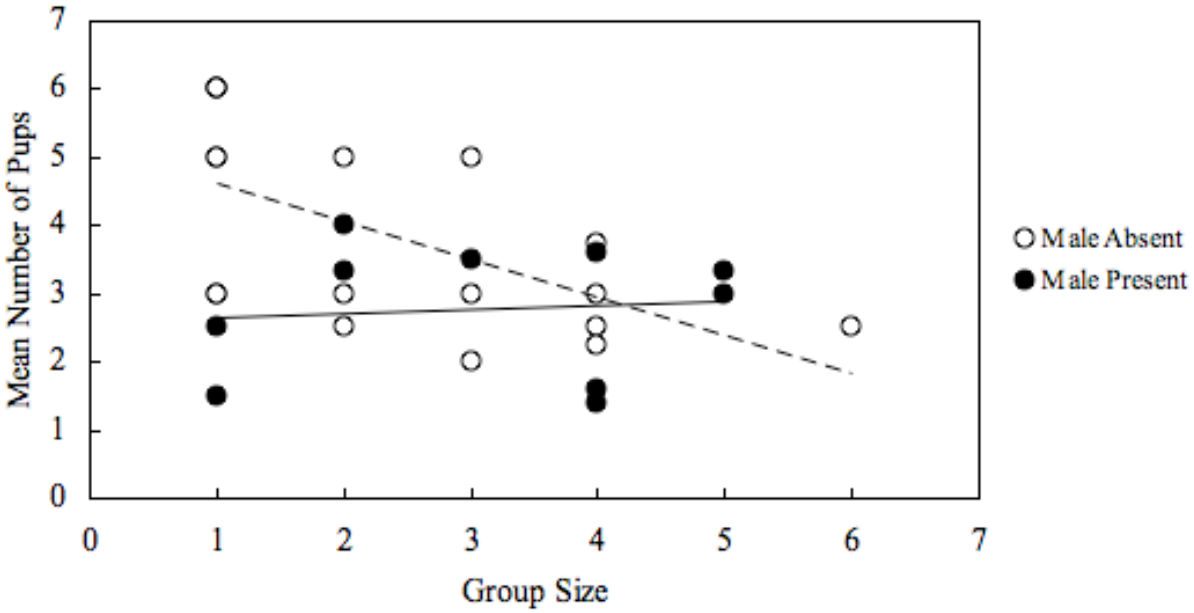


Figure 6. The mean number of pups at weaning did not differ as a function of group size in free-living colonies in which there was a male present (solid trendline; $P > 0.05$). However, the number of pups in colonies in which adult males were absent significantly decreased as group size increased (dashed trendline; $P < 0.01$).

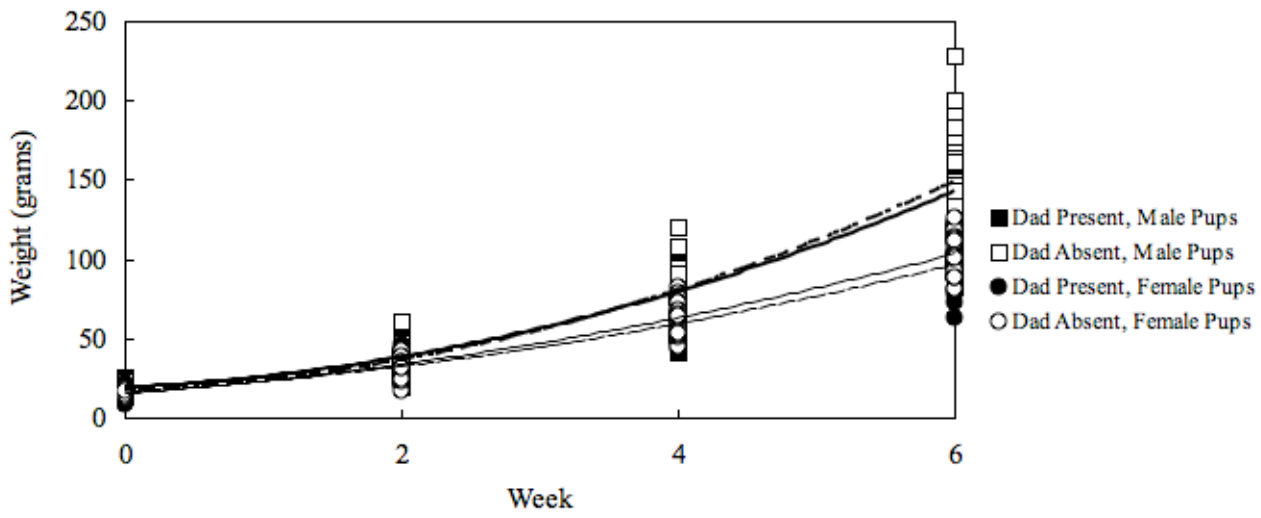


Figure 7. Growth curves generated for captive male and female colonial tuco-tuco pups in colonies with (solid trendlines) and without (broken trendlines) an adult male present. There was no significant difference in growth rates between male pups in colonies with and without a male present ($P = 0.16$) or between female pups with and without a male present ($P = 0.48$).

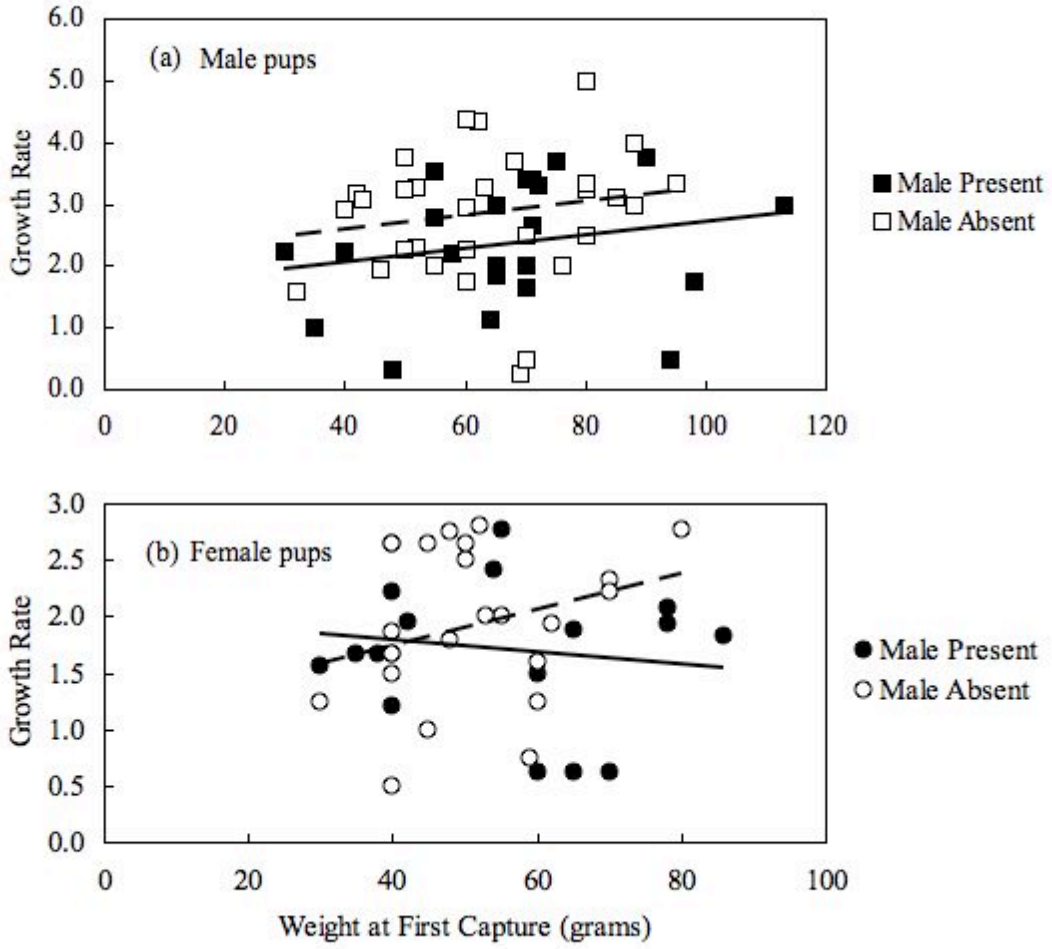


Figure 8. Growth rates (i.e. Δ weight/time) of (a) free-living male and (b) female colonial tuco-tuco pups in colonies with (solid line) and without (broken line) an adult male present. No significant differences were found in male pups (P = 0.93) or female pups (P = 0.13).

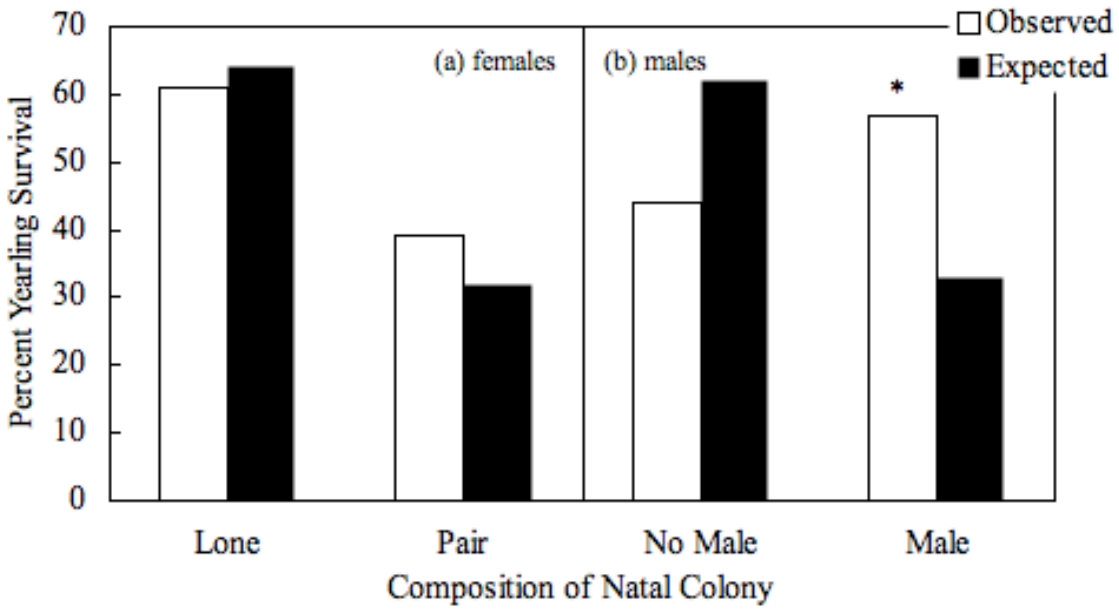


Figure 9. Observed and expected percent survival of free-living colonial tuco-tuco female (a) and male (b) yearlings raised in colonies with and without an adult male present. $\chi 2$ tests show no significant difference between observed and expected survival for females raised in colonies with a lone female (N = 29) or in colonies with a male-female pair (N = 16) ($P > 0.05$). However, male survival was significantly greater than expected when raised in colonies with an adult male present (N = 99) than when raised in colonies without an adult male present (N = 52) ($P < 0.05$).

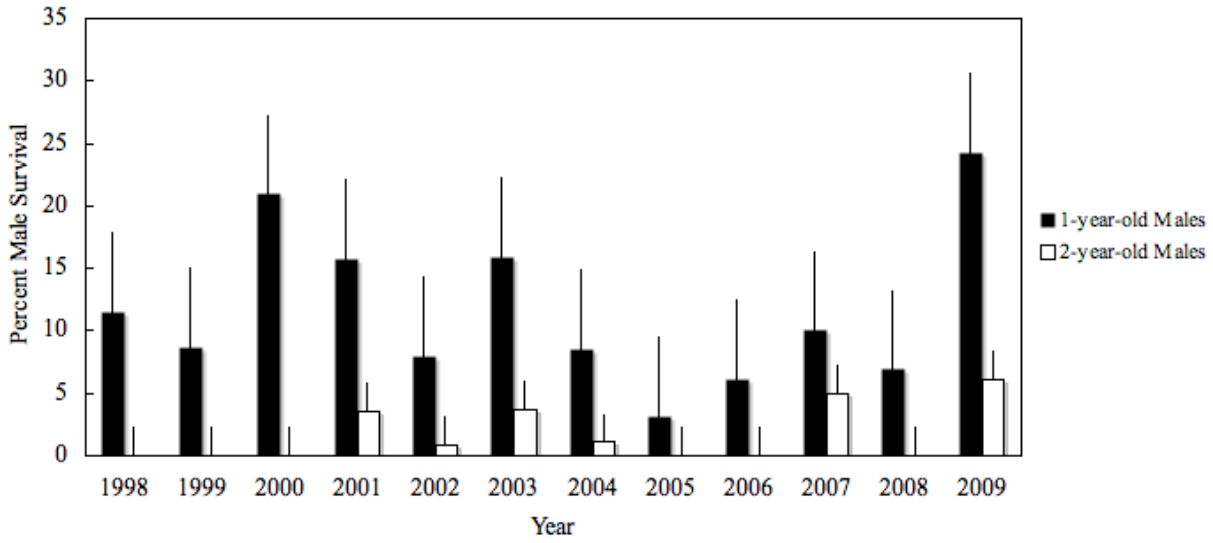


Figure 10. Percent survival of free-living male colonial tuco-tucos to their first and second breeding seasons. Male survival to their first year was significantly higher than survival to their second year ($P < 0.001$).

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