

RESEARCH ARTICLE

Effect of Housing and Environmental Enrichment on Adrenocortical Activity, Behavior and Reproductive Cyclicity in the Female Tigrina (*Leopardus tigrinus*) and Margay (*Leopardus wiedii*)

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The objective of this study was to evaluate the effects of different captive housing conditions on reproductive cyclicity and adrenocortical activity in adult females of two small-sized felid species, the tigrina (*Leopardus tigrinus*; $n = 3$) and margay (*Leopardus wiedii*; $n = 2$). Females were housed as singletons and subjected to three enclosure conditions over successive time periods: Phase I—large, enriched enclosures for 3 months; Phase II—small, empty enclosures for 5.5 months; Phase III—the same small enclosures enriched with branches and nest boxes for 6.5 months. Fecal samples were collected five times weekly throughout the study for analysis of progesterone, estrogen, and corticoid metabolites. On the basis of observed behaviors, stereotypic pacing was more frequent before feeding for all cats, regardless of enclosure conditions. Both species displayed a bimodal activity pattern, with peaks occurring at nightfall and dawn. All animals exhibited

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agitated behavior, characterized by a high frequency and duration of stereotypic pacing, primarily during the first 3 days after moving to the small empty enclosures. On the basis of hormonal analyses, ovarian follicular activity decreased and corticoid concentrations increased in tigrinas after transfer to the small barren cages compared to the patterns observed in the initial large, enriched enclosures. Corticoid concentrations in tigrinas then declined after small cage enrichment. Margay females exhibited increased corticoid excretion during Phases II and III, but in contrast to tigrinas, concentrations remained high even after cage enrichment. It was further showed that enriching the small enclosures was insufficient to reestablish normal ovarian activity within the time frame of the study for both species. In summary, margay and tigrina females exhibited distinct elevations in corticoid concentrations after transfer from large enriched enclosures to smaller barren cages that corresponded with agitated behavior, especially immediately after transfer. Fecal corticoid concentrations were reduced after cage enrichment in tigrinas, but not in margays. Although only a few individuals were evaluated, data suggest there may be species differences in response to captive environmental conditions. Overall results emphasize the importance of enclosure dimensions and enrichment when designing species appropriate environments for improving the health and reproductive fitness of threatened species. *Zool Biol* 26:441–460, 2007. © 2007 Wiley-Liss, Inc.

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INTRODUCTION

The initial reaction to a stressor involves activation of the sympathetic-adrenomedullar and hypophyseal-adrenocortical systems [Selye, 1936, 1981]. If the stressor is intense (e.g., severe burns, extreme temperatures), death results in a few hours, whereas for stressors of lesser intensity or of shorter duration, the organism recovers to its original state. For some stressors (e.g., exposure to low temperatures or unavoidable physical effort), the body adapts by increasing the production of adrenal steroids, followed by suppression of functions such as growth, gonadal activity and immunologic resistance. If the stressor is severe and prolonged, adaptive mechanisms eventually fail and the organism dies. These responses, referred to as the “general adaptation syndrome” or “stress syndrome,” describe the negative consequences of an animal’s failure to cope with factors in its environment [Selye, 1936, 1981].

There is general agreement that stress is the biological response elicited when an individual perceives a threat to its homeostasis [Moberg, 2000]. The difficulty is determining what exactly constitutes stress [e.g., Broom and Johnson, 1993; Moberg and Mench, 2000]. Not all stressors have negative impacts on animal health [Antelman and Caggiula, 1980; Weiss et al., 1980; Moodie and Chamove, 1990]. Indeed, some stress is necessary to ensure survival and allow adaptation to changes in the environment [Ottaviani and Franceschi, 1996; Cook et al., 2000]. It is only a concern when the “stress response” threatens an individual’s well-being, causing “distress,” and exerts deleterious effects on the individual’s biological state [Moberg, 2000]. The ultimate impact of a challenging situation depends on an animal’s subjective perception of aversion or threat, modulated by the complexity of its environment and the response options it has [Hennessey and Levine, 1979]. Numerous studies have showed that captivity can adversely affect an animal’s behavior and physiology [Lindburg and Fitch-Snyder, 1994; Carlstead, 1996;

Estep and Dewsbury, 1996]. A variety of potential stressors exist in zoos; however, the deleterious effects occur only if an animal is unable to respond with appropriate behavioral and physiological coping responses (i.e., hiding, escaping, attacking, etc.). For many species, including felids, failure to reproduce in captivity has been attributed to stress ensuing from suboptimal housing and husbandry conditions [Mellen, 1991; Lindburg and Fitch-Snyder, 1994]. Thus, the goal of zoo managers is to mitigate the effects of potential stressors in zoos by improving behavioral response options (e.g., through environmental enrichment) [Carlstead and Shepherdson, 2000].

Analyses of corticosteroids in blood or urine can provide a physiological indicator of adrenal activity and stress responses in cats [Carlstead et al., 1992]. However, regular blood sampling is impractical in nondomestic felids and in itself can induce a stress response [Reinhardt et al., 1990; Cook et al., 2000]. Urine collection also is difficult in cats because many void by spraying. Development of noninvasive fecal steroid monitoring techniques in domestic and nondomestic felids has provided a new avenue for research on physiological stress responses. Furthermore, metabolism studies in the domestic cat showed that the majority of adrenal and gonadal metabolites are excreted in feces rather than in urine [Shille et al., 1990; Brown et al., 1994; Graham and Brown, 1996]. By representing an average pooled value for the previous 12–24 hr, fecal monitoring may be more appropriate for evaluating steroidogenic activity longitudinally in felids. This is especially relevant for assessing adrenal function because circulating cortisol fluctuates diurnally and in pulsatile fashion throughout the day in most mammals [Fulkerson and Tang, 1979; Thun et al., 1981; Monfort et al., 1993]. Measuring behavioral correlates of psychological stress is another valuable tool for assessing responses to environmental conditions [Mellen, 1991]. Studies using both behavioral and physiological measures of stress are more likely to provide informative and integrative information on how management strategies impact animal well-being.

Among felids, small-sized cats (i.e., with average body mass <20 kg) are known to be difficult to propagate consistently in captivity [Mellen, 1991; Swanson et al., 2003]. The poor reproduction of small cats may be related to heightened susceptibility to stress resulting from inappropriate husbandry and management [Carlstead et al., 1992; Swanson et al., 2003]. Many of these species, including several small cats native to Brazil, are considered threatened with extinction in the wild and represent priorities for development of effective captive breeding programs. This study was conducted because most of the captive small felids in South America are kept in small barren enclosures with poor reproductive results. The objective of this study was to evaluate the effects of different captive housing conditions on reproductive and stress physiology, as assessed by ovarian and adrenal fecal steroid concentrations, in two small-sized felids, the tigrina (*Leopardus tigrinus*) and margay (*Leopardus wiedii*). The hypothesis is that chronic stress owing to poor captive conditions increases corticoid production, which in turn suppresses ovarian follicular activity.

METHODS

Animals and Fecal Sample Collection

Adult female tigrinas ($n = 3$) and margays ($n = 2$) were housed off-exhibit as singletons at the Itaipu Binational Wildlife Conservation Center, located in Foz do

Iguassu City (altitude: 173 m; 25°32'45"S; 54°35'07"W), Paraná, Brazil. Individual identification, breeding category, and estimated age are presented in Table 1. Each female was categorized as a breeder if she had produced a kitten in captivity, or not determined if her reproductive history was not known. Two tigrina females were captive-born, whereas all others were wild-born and had been in captivity for at least 5 years before the start of the study. Females were maintained under natural photoperiod and provided a diet consisting of raw bovine meat, mineral, and vitamin supplement and water ad libitum. Occasionally, the diet was supplemented with whole prey (rat, fish, and chicken). All animals were fed once daily with no fasting days.

Fecal samples were collected from each female 5 days a week, at the same time of day, between 0730 and 0900 h, for 15 months to characterize longitudinal gonadal and adrenal steroidogenic activity. Animals usually defecated only once daily. Samples were placed into zip-lock bags, frozen immediately, and stored (−20°C) until transportation on dry ice to the Conservation and Research Center, USA, for analysis.

Enclosures and Experimental Design

Tigrinas and margays were exposed to three enclosure conditions over 15 consecutive months, designated as Phases I–III. In Phase I (3 months, from 1 April to 30 June), initial home enclosures were large (6.92 m length × 2.87 m width × 2.55 m height for tigrinas; 7.00 × 2.95 × 4.15 m for margays) and enriched with branches, tree trunks, plants, and a nest box. At the end of Phase I, the animals were restrained with catchpoles, placed in wood boxes, and moved without sedation to barren enclosures that were smaller in both horizontal and vertical space (3.43 × 1.61 × 2.0 m for both species), characterizing Phase II (5.5 months, from 1 July to 14 December). After this phase, the same small cages were enriched with tree trunks, plants, and a nest box for Phase III (6.5 months, from 15 December to 30 June).

Direct behavioral observations were conducted during routine husbandry procedures and at night, with emphasis on assessing signs of stress and estrus. At night, behavior was monitored using a monocular night vision scope with an illuminator that permitted observations in total darkness, a high magnification to capture images at a long distance (20 m), and a video camera designed for nocturnal observations (“zero lux”). Behavioral observations were conducted 3–4 times each day for periods of 15 min each and at night for a 1-hr period (evenly split between different time periods). This frequency of observation was used for each cat during the first phase and for all cats simultaneously during second and third phases, conducted 2 days in each week. Behaviors like resting (sitting or lying down and not exhibiting any other behavior), hiding (and in what location), moving (directional

TABLE 1. General information about tigrina and margay females used in this study

Animal ID	Species	Age (years)	Breeding category	Origin
Lti-1505	Tigrina 1	2.0	ND	Captivity
Lti-205	Tigrina 2	> 8.5	B	Wild
Lti-1058	Tigrina 3	6.0	B	Captivity
Lwi-1242	Margay 1	> 5.0	ND	Wild
Lwi-189	Margay 2	> 8.5	ND	Wild

B, breeder; ND, not determined.

movement or stereotypic pacing; repetitive movement, i.e., the same area is traversed repeatedly), self-grooming (licking; chewing; and cleaning fur; scratching with hind paws), and those commonly associated with estrus (rolling; object rubbing; vocalizing; pacing; grooming; urination; urine spraying; investigative activity; sniffing; lordosis) [Michael, 1961] were recorded at timed intervals (each 5 min) and calculated as the rate per 15 min observation set.

Hormonal Analysis

Fecal samples were analyzed for ovarian and adrenal metabolites as described previously [Moreira et al., 2001]. Samples were thawed and ~0.5 g of well-mixed wet feces were boiled in 5 ml aqueous ethanol (90%) for 20 min. After centrifuging at 500g for 10 min, the supernatant was recovered and the pellet resuspended in 5 ml aqueous ethanol (90%), vortexed for 1 min and recentrifuged. Both ethanol supernatants were combined dried completely, redissolved in 1 ml methanol and diluted in phosphate-buffered saline (0.01 M PO₄, 0.14 M NaCl, 0.5% BSA, 0.01% NaN₃, pH 7.4) before analysis.

Progestagen metabolites were quantified by a double-antibody radioimmunoassay (RIA) that used a monoclonal progesterone antibody (#331, provided by Dr. J. Roser, University of California, Davis, CA), a ¹²⁵I–progesterone label (ICN Biomedical, Inc., Costa Mesa, CA), and progesterone standards (Sigma Chemical Co., St. Louis, MO) [Brown et al., 1994]. The minimum detectable dose based on 90% of maximum binding was 50 pg/ml. The estradiol RIA used an antiestradiol-17β antiserum (provided by Dr. S. Wasser, University of Washington, Seattle, WA), a ³H-estradiol-17β label (New England Nuclear, PerkinElmer Life and Analytical Sciences, Inc., Wellesley, MA) and estradiol standards (Sigma Chemical Co., St. Louis, MO). The assay sensitivity was 5 pg/ml. A double-antibody ¹²⁵I RIA for corticosterone (ICN Biomedicals, Inc., Costa Mesa, CA), validated for felid feces, was used to quantify adrenal corticoid metabolites [Graham and Brown, 1996; Wasser et al., 2000; Wielebnowski et al., 2002; Young et al., 2004]. The assay sensitivity was 60 pg/ml.

Each RIA was validated for tigrina and margay feces by demonstrating: (1) parallelism between binding inhibition curves of extract dilutions and the appropriate steroid standard; and (2) significant recovery (>90%) of steroid standard added to fecal extracts. Steroid extraction efficiency ranged from 90 to 100% as determined by recovery of ¹⁴C-progesterone, ³H-estradiol-17β, or ³H-corticosterone added before extraction. Intraassay and interassay coefficients of variation for all assays were <10 and 15%, respectively. Fecal hormone concentrations are expressed on a per gram wet fecal weight basis.

Number of Samples

Approximately 1,500 samples were collected from each female. Weekly samples were processed and analyzed for corticoids, with more samples being analyzed immediately before and after each phase change. All samples were processed and analyzed for estradiol metabolites (five samples/week/female) because reproductive cycles are short in felids and characterization of follicular activity requires more frequent sample analysis. More than 300 samples were analyzed for progestagen metabolites (at least one sample/week/female) because characterization of luteal phases does not require as frequent a sampling regimen.

For Tigrina 1, five times weekly samples were analyzed for corticoids throughout Phase I and for 6 weeks after transfer to Phase II. For all other situations, samples for corticoid analysis were collected five times weekly for 1 week before through 2 weeks after each phase change.

Data Analysis

Estrual (hypothetically determined, because the females did not display overt estrous signs) and interestrual steroid values in each female were determined by an iterative process in which high values that exceeded the mean plus 1.5 standard deviations (SD) were excluded. Baseline values were determined after all high levels had been excluded and no values exceeded the mean plus 1.5 SD. The highest value within a cluster of high concentrations was considered a peak. The duration of the estrous cycle was calculated as the number of days between peaks in estrogen metabolite concentrations (presumed to be associated with estrus) for periods not exceeding 60 days (i.e., more than twice the estimated estrous cycle duration; Moreira et al., 2001]. Interestrogen peak intervals > 60 days were considered as anestrus periods, except in the case of apparent spontaneous ovulation when progestagen concentrations were elevated. Presumed postovulatory increases in progestagen metabolite excretion were considered significant if values exceeded the mean plus 2 SD of the preceding values and remained elevated for at least 1 week. Basal progestagen metabolite concentrations were calculated from values preceding preovulatory estradiol surges and after the return of fecal progestagens to the baseline values. Corticoid values that exceeded the mean plus 2 SD were considered significant elevations.

To evaluate enclosure influences and to compare between phases and species, data were normalized by application of a logarithm (base 10) and tested for normality. Shapiro–Wilk W test was used when the number of samples was lower than 2,000, and the Kolmogorov–Smirnov–Lilliefors test was used when the number of samples was higher than 2,000. When the reported P value was lower than 0.05, the distribution was not considered normal. Data means were calculated by phases as: Phase I (big and enriched enclosures), Phase II (small and empty enclosures), and Phase III (same small enclosures, but enriched). The homogeneity of group variances (after normalization) was tested using the Bartlett test ($\alpha = 0.05$). Although means were calculated, because of low animal numbers, some data were compared descriptively rather than statistically. Statistical comparisons of results were tested using analysis of variance (one-way) followed by comparisons for all pairs using Tukey–Kramer honestly significant difference test or for each pair using Student's t -test ($\alpha = 0.05$). Behavioral data were summarized as the total time animals spent being active, resting, or not visible. Means for each category were calculated for each female before averaging totals for the group and compared using Kruskal–Wallis and Mann–Whitney U -tests. All analyses were carried out using the software programs JMP (SAS Institute Inc., Version 4.0, 2000), Microsoft Excel 2003 (Microsoft Corporation) and STATISTICA for Windows (StatSoft Inc., Release 5.1 D, 1996). Values are presented as mean \pm SEM.

RESULTS

For all cats, active behavior (mostly stereotypic pacing) was more ($P < 0.05$) frequent for the 30 min before feeding ($92.0 \pm 2.1\%$ of the time in active behavior)

compared to 24-hr cycle ($18.7 \pm 1.9\%$), regardless of enclosure condition. Both species also displayed a circadian activity pattern, with peaks of stereotypic pacing occurring at nightfall and dawn.

Because the females were housed as singletons, only solitary behaviors were recorded. Females sometimes performed flehmen, albeit less frequently than males, for example when exploring a urine-marked room without another cat present [Hart and Leedy, 1987]. Of other behaviors noted, cheek rubbing, sharpening claws, and urine marking were more frequent especially at night, when they were more active. Flehmen (open mouth grimace after the sniffing of an object) and vocalizations were not observed. Under these conditions (i.e., without males), it was difficult to identify state of sexual receptivity. Behaviorally, none of the females showed regular signs of estrus in any of the enclosure situations. There also were no specific behaviors correlated with estradiol peaks.

There was no significant variation across individuals of the same species ($P > 0.05$) in activity peak time, nor in the percentage of time spent in active behavior, during the same enclosure treatment. The daily duration of active behavior (including stereotypic pacing) was significantly longer in the small enclosures, especially when they were empty (35% of observation time versus 26% of observation time for Phases II and III, respectively). At night, tigrinas initiated stereotypic pacing earlier (about 2200 h) than margays (about 2300 h). By 0100 h, stereotypic pacing ceased but resumed shortly before sunrise. During the day, animals spent most of the time resting on the trunks or inside the nest box, with a drop in active behavior during the hottest time of the day (from 1000 to 1600 h). Overall, there was a higher frequency of exploratory behavior (investigating, searching, pawing at, or trying to reach item; sniffing object) and territory definition (mark, spraying urine, scraping, or rubbing hind feet alternately on ground, sharpening front claws by scratching on objects; cheek rubbing against an object) in the enriched enclosures (Phases I and III) versus unenriched, especially at night. During Phase II, animals did not have trunks for sharpening front claws and no items were available to instigate exploratory behavior.

Longitudinal profiles of estradiol, progesterone, and corticoid metabolites for individual females are presented in Figures 1–5, whereas mean data are presented in Figures 6 and 7. Mean overall, baseline, and peak concentrations of corticoid metabolites for each individual are presented in Table 2. Agitated behavior, characterized by a high frequency and duration of stereotypic pacing, was exhibited by margays and tigrinas primarily for the first 3 days after moving to the small empty enclosures (70% of the observation time as compared to 35% before moving). Many corticoid peaks were consecutive during this time in tigrinas (Figs. 1–3). During Phase I of the study, Tigrina 1 exhibited three regular estrous cycles of 14–15 days in length (Fig. 1). With the exception of two random spikes at the beginning of the study, concentrations of fecal corticoids were comparatively low during Phase I. However, soon after transfer to the barren enclosure, corticoid concentrations increased and remained elevated for the next 2 months. A resumption in cyclicity coincided with the reduction in fecal corticoids and progesterone concentrations, 40 days after transfer to the barren enclosure in Phase II. After four regular estrous cycles, estrogen cyclicity ceased again, although baseline estrogens were elevated through the first half of Phase III, but not in a clear cyclic pattern. After that, the female exhibited another spontaneous ovulation followed by a luteal phase of

TABLE 2. Mean overall, baseline, and peak concentrations (\pm SEM) of fecal corticoid metabolites for individuals of tigrina and margay females examined by longitudinal steroid evaluation

Species	Samples (<i>n</i>)	Mean overall ^a	Mean baseline ^b	Mean peak ^c
Tigrina 1	291	752.0 \pm 52.2	582.1 \pm 28.4	2450.9 \pm 150.0
Tigrina 2	300	530.8 \pm 45.8	429.5 \pm 27.7	1703.1 \pm 120.7
Tigrina 3	260	544.0 \pm 45.3	441.9 \pm 25.6	1594.4 \pm 140.0
Tigrinas (overall means)	851	640.2 \pm 30.7	504.5 \pm 16.7	2117.3 \pm 107.7
Margay 1	250	185.6 \pm 35.5	144.4 \pm 11.0	1810.7 \pm 998.4
Margay 2	266	312.3 \pm 48.0	224.4 \pm 19.1	1683.8 \pm 416.3
Margays (overall means)	516	249.7 \pm 30.3	188.1 \pm 12.0	1871.3 \pm 388.2

^aAverage concentration of metabolites (ng/g feces) in all fecal samples (*n*) from the collection period.

^bAverage metabolite concentrations for all samples that fell below the overall mean+1.5 standard deviations (SD).

^cAverage metabolite concentration for all samples greater than the overall mean+1.5SD.

41 days. Fecal corticoids varied during Phase III with occasional spikes observed in relation to otherwise lower baseline concentrations.

Tigrina 2 cycled every 17–20 days during Phase I, but after transfer to the small, barren enclosure, cyclicity ceased and never resumed to the same degree (Fig. 2). This female had a spontaneous ovulation, followed by a luteal phase of 40 days at the beginning of Phase III. At the end of Phase III, two shorter-duration periods of elevated progestagens were observed after estrogen surges. Several corticoid peaks were observed for about 2 months after transfer to the barren cage and again at the end of this period. Corticoid increases were observed coincident with enclosure enrichment (December 14) and with handling and anesthesia for a clinical examination (March 3) to examine hair loss.

Fecal estrogen analysis indicated Tigrina 3 exhibited ovarian cycles throughout the study, although there were periods of reduced estrogen excretion during Phases II and III (Fig. 3). Midway through Phase III, the female experienced a nonpregnant luteal phase of 41 days, which was preceded by a large surge in fecal estrogens. Fecal corticoid concentrations were highest after transfer to the barren enclosure in Phase II, but thereafter were at baseline through the remainder of the study.

Margay 1 exhibited several cycles during Phase I, and continued cycling after transfer to the small enclosure in Phase II (Fig. 4). Ovarian cycle length was 23.9 \pm 3.4 days (range, 10–52). During Phase III she exhibited a spontaneous ovulation after enclosure enrichment followed by a luteal phase that lasted 28 days, and another one at the end of the period that lasted 40 days. Surges in fecal estrogens preceded each of the nonpregnant luteal phases. Fecal corticoid concentrations in this female were comparatively low, and remained at baseline throughout the study with the exception of a few low-amplitude spikes observed during Phase III.

On the basis of fecal estrogen excretion, Margay 2 cycled at 22.2 \pm 2.8-day intervals (range, 14–29) except during periods of elevated fecal progestagens, which occurred in each phase of the study (Fig. 5). These nonpregnant luteal phases were preceded by surges in fecal estrogens, and lasted 29–37 days. Fecal corticoid concentrations were low during Phase I, with several spikes in corticoid excretion occurring during Phases II and III.

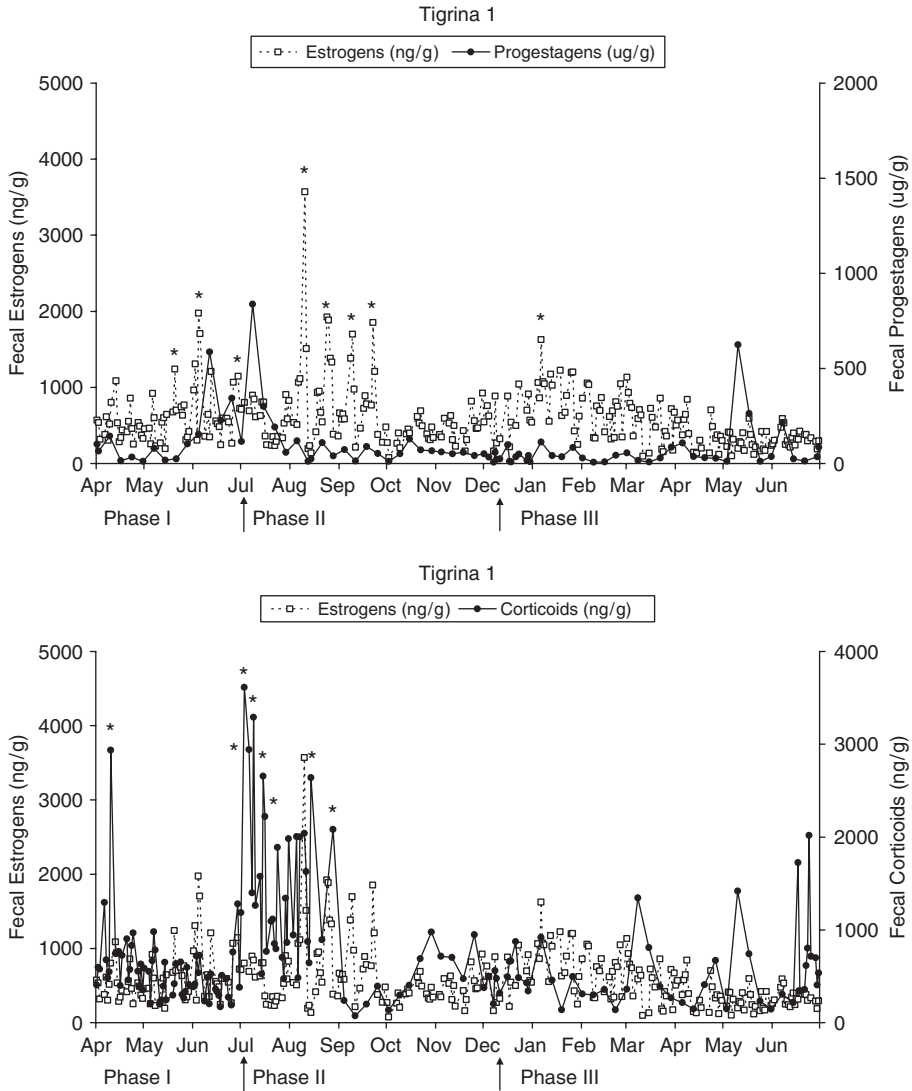


Fig. 1. Longitudinal profiles of fecal estrogen (dashed lines and empty squares, top and bottom panels), progesteragen (filled circles, top panel), and corticoid (filled diamonds, bottom panel) excretion in an adult, female tigrina (*Leopardus tigrinus*) housed singly at the Itaipu Wildlife Conservation Center. Phases refer to different housing enclosures: Phase I, large enriched enclosure; Phase II, transfer to small barren enclosure; Phase III, enrichment of the small enclosure. *Estrogen (top panel) and corticoid (bottom panel) peaks.

Overall for tigrinas, the concentration of fecal estrogens was highest during Phase I and lowest during Phase III ($P < 0.05$) (Fig. 6). Conversely, overall mean corticoids were elevated during Phase II and returned to baseline after cage enrichment (Fig. 6) ($P < 0.05$). For margays, overall concentrations of fecal estrogens were less affected by transfer to smaller enclosures (Fig. 7). In these females, corticoids increased, even after enrichment (Phase III) ($P < 0.05$) (Fig. 7).

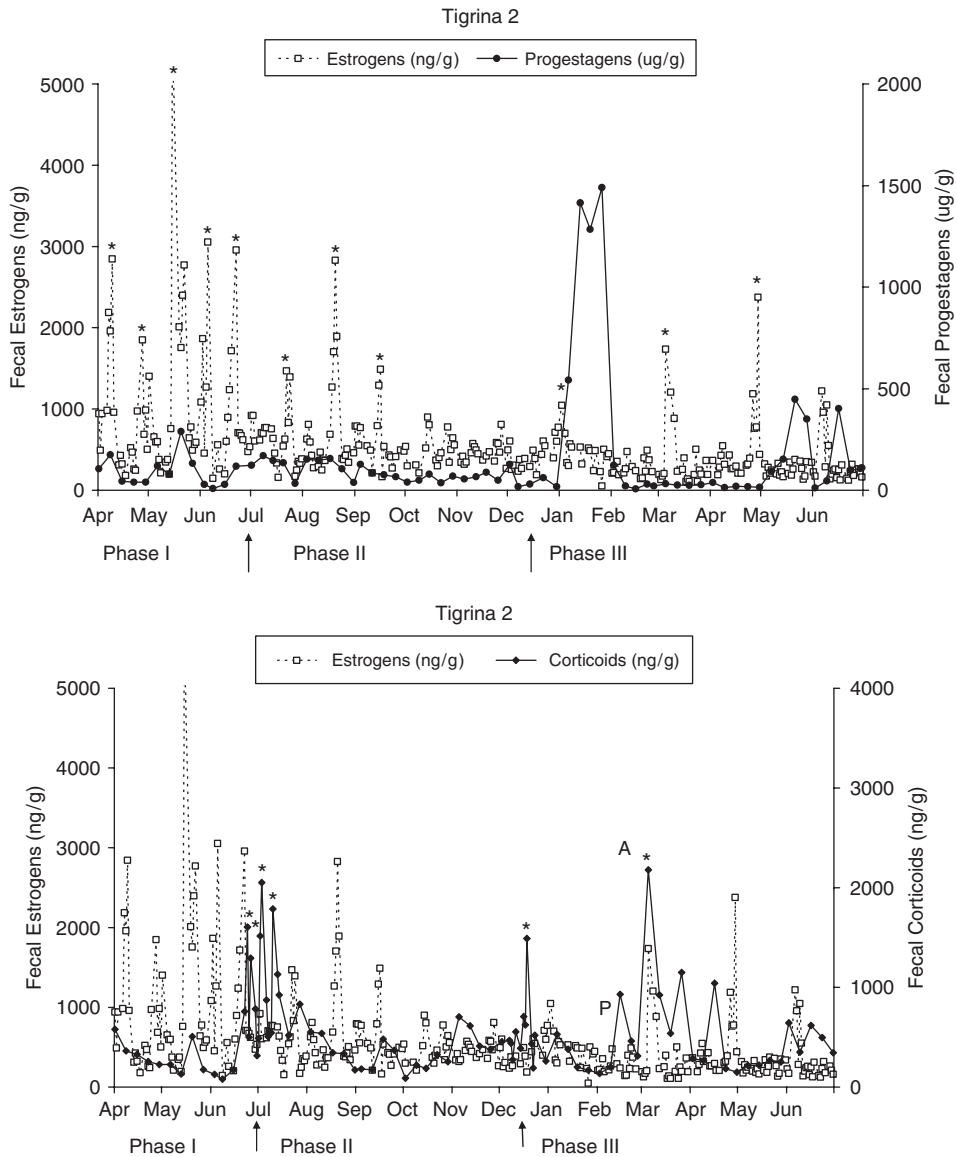


Fig. 2. Longitudinal profiles of fecal estrogen (dashed lines and empty squares, top and bottom panels), progesterone (filled circles, top panel) and corticoid (filled diamonds) excretion in an adult, female tigrina (*Leopardus tigrinus*) housed at the same conditions described in Figure 1. The letter “P” designates the observation of hair loss as a result of plucking behavior. The letter “A” designates the time this cat was anesthetized for clinical examination. *Estrogen (top panel) and corticoid (bottom panel) peaks.

DISCUSSION

This study examined how captive tigrinas and margays responded to the potential stress of being housed in small barren enclosures as compared to enriched

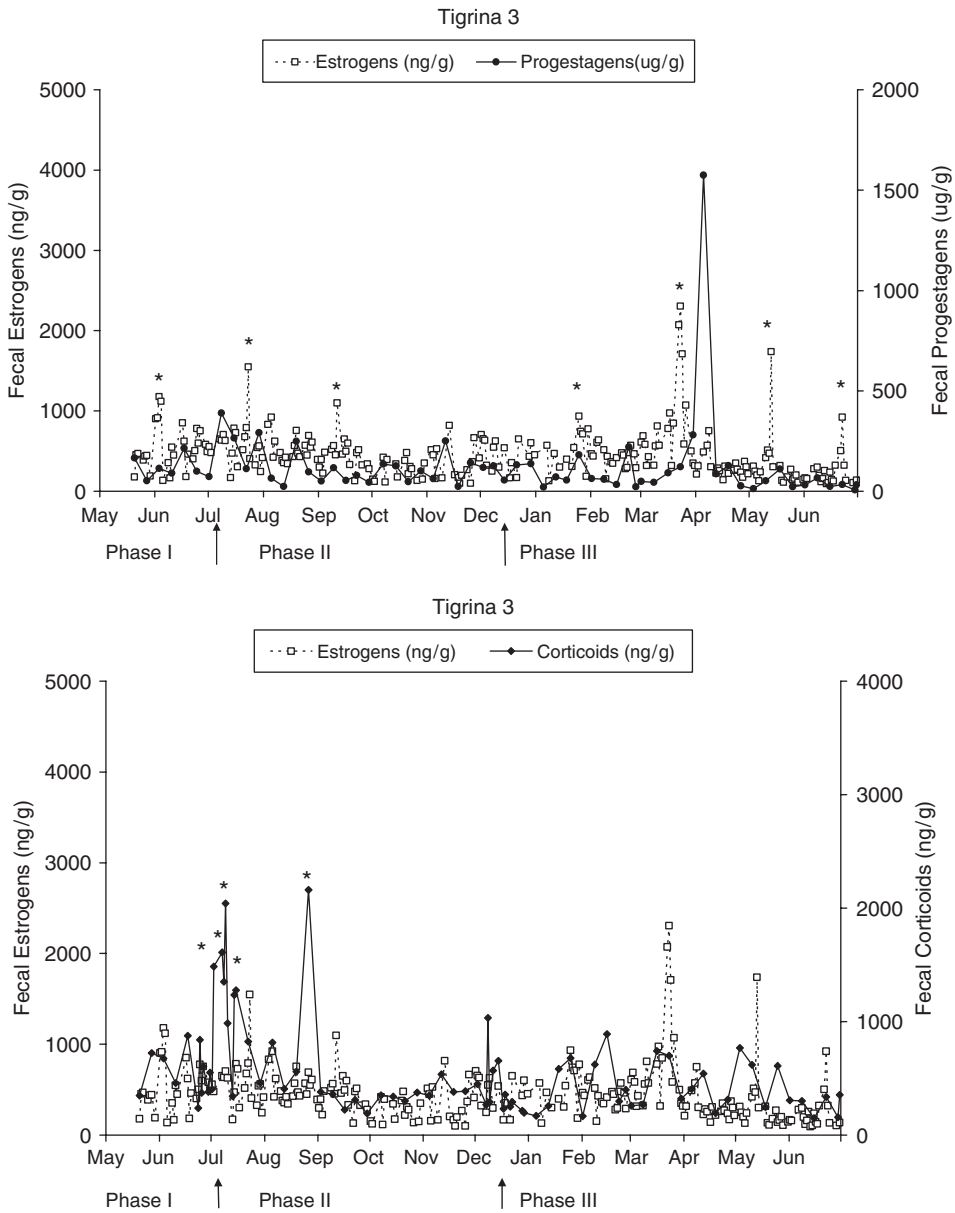


Fig. 3. Longitudinal profiles of fecal estrogen (dashed lines and empty squares, top and bottom panels), progesteragen (filled circles, top panel), and corticoid (filled diamonds, bottom panel) excretion in an adult, female tigrina (*Leopardus tigrinus*) housed at the same conditions described in Figure 1. *Estrogen (top panel) and corticoid (bottom panel) peaks.

enclosures of varying sizes. Exposure to new situations of an unexpected or uncertain nature is an especially potent inducer of adrenocortical activity [Mason, 1968a,b]. In this case, it was the effect of environmental change on behavior, stress hormones, and ovarian steroid activity that was of particular interest. First, normal baseline

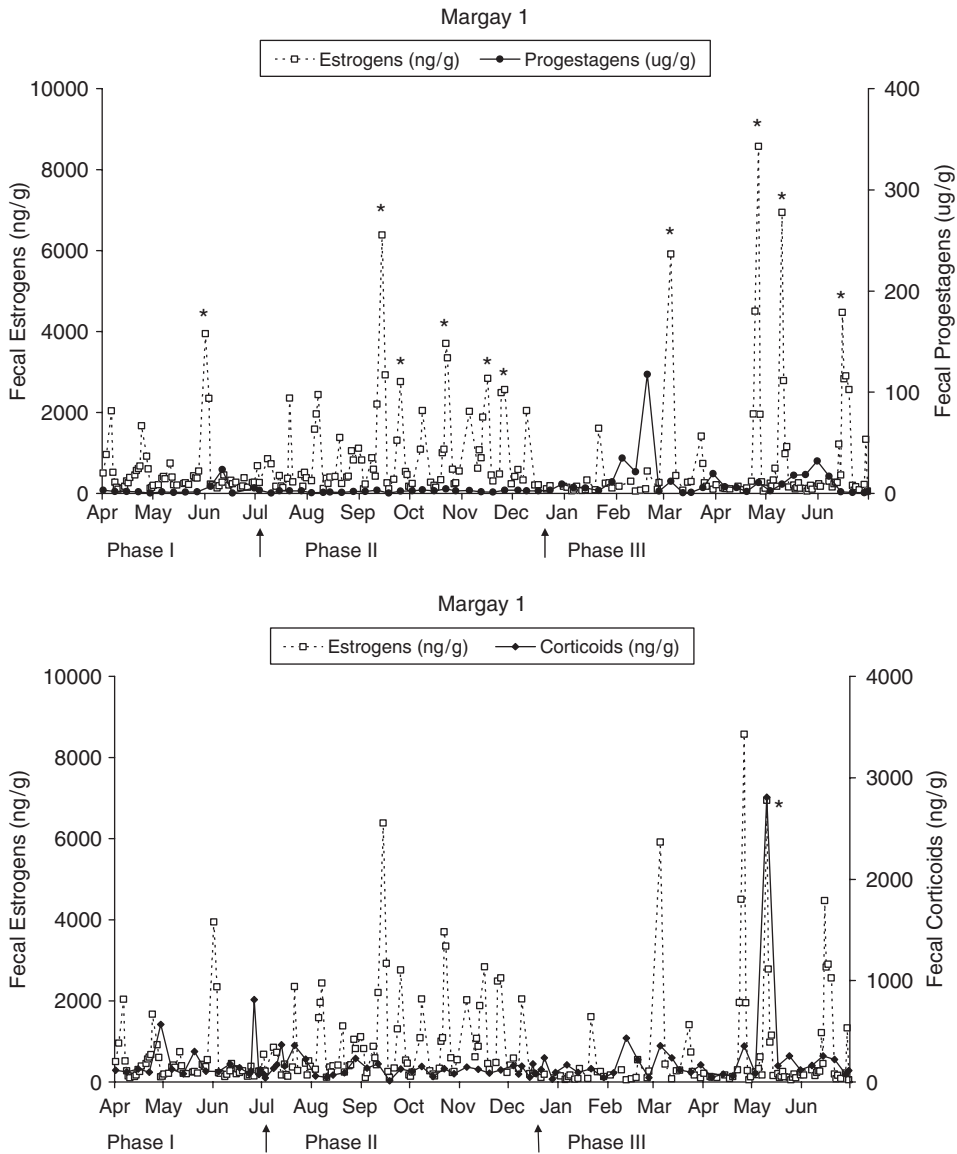


Fig. 4. Longitudinal profiles of fecal estrogen (dashed lines and empty squares, top and bottom panels), progesteragen (filled circles, top panel), and corticoid (filled diamonds, bottom panel) excretion in an adult, female margay (*Leopardus wiedii*) housed at the same conditions described in Figure 1. *Estrogen (top panel) and corticoid (bottom panel) peaks.

gonadal and adrenal excretory patterns were established for each animal when housed in large and enriched home enclosures (Phase I). During this period, each animal exhibited a circadian pattern of behavior, active at night and resting during the day, which resembled that reported for captive and wild ocelots and margays [Petersen, 1977; Emmons, 1988; Konecny, 1989; Weller and Bennett, 2001]. Activity peaks were crepuscular, occurring after sunset and again before sunrise. For both

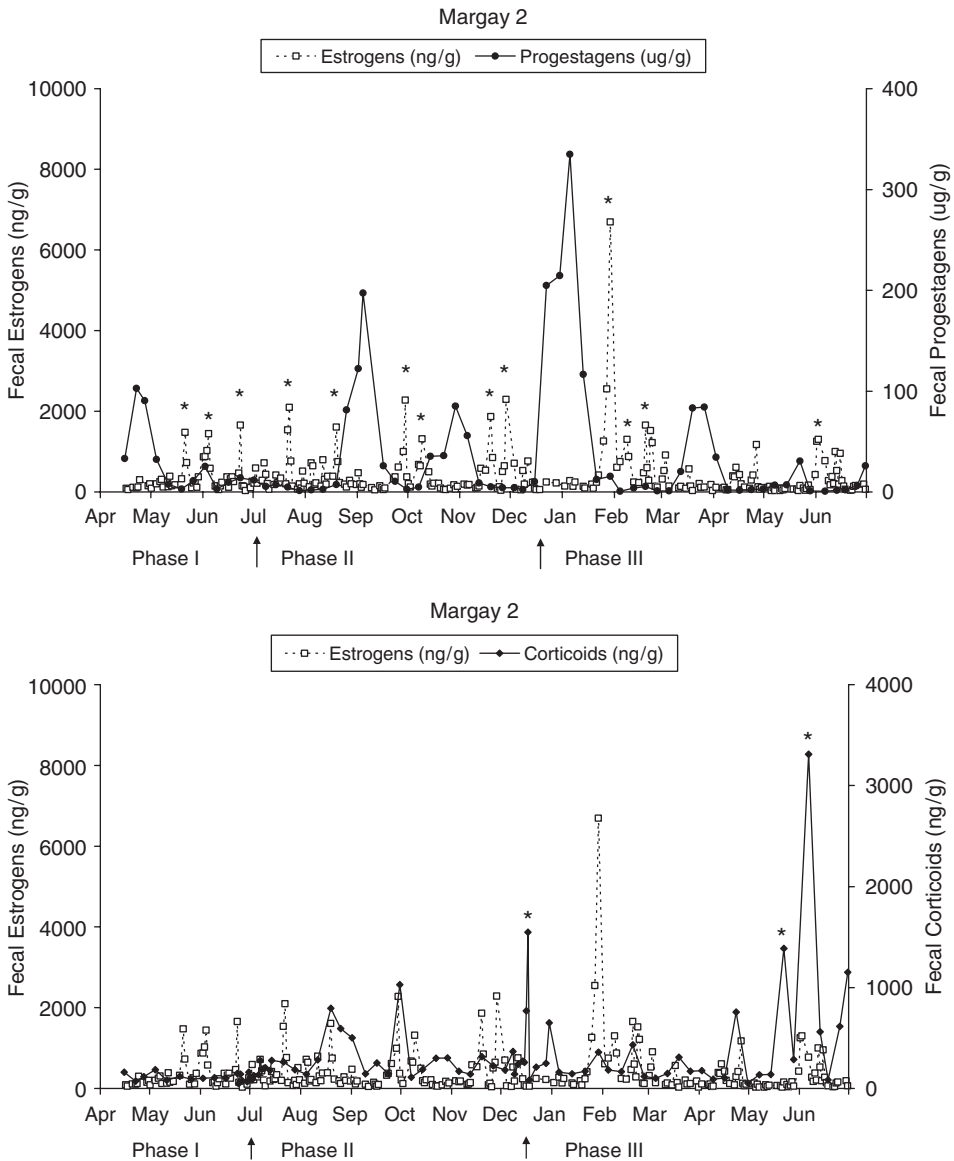


Fig. 5. Longitudinal profiles of fecal estrogen (dashed lines and empty squares, top and bottom panels), progesteragen (filled circles, top panel), and corticoids (filled diamonds, bottom panel) excretion in an adult, female margay (*Leopardus wiedii*) housed at the same conditions described in Figure 1. *Estrogen (top panel) and corticoid (bottom panel) peaks.

species, stereotypic pacing increased before feeding, coincidentally with characteristic sounds, such as opening of enclosure doors and keeper sounds, reflecting an anticipatory response. All females exhibited at least some ovarian steroidogenic activity during the study suggestive of estrous cyclicity, but because they were solitary they did not exhibit obvious estrual behaviors as described by Mellen [1989] and Michael [1961]. It is unlikely the variation in ovarian or corticoid responses were

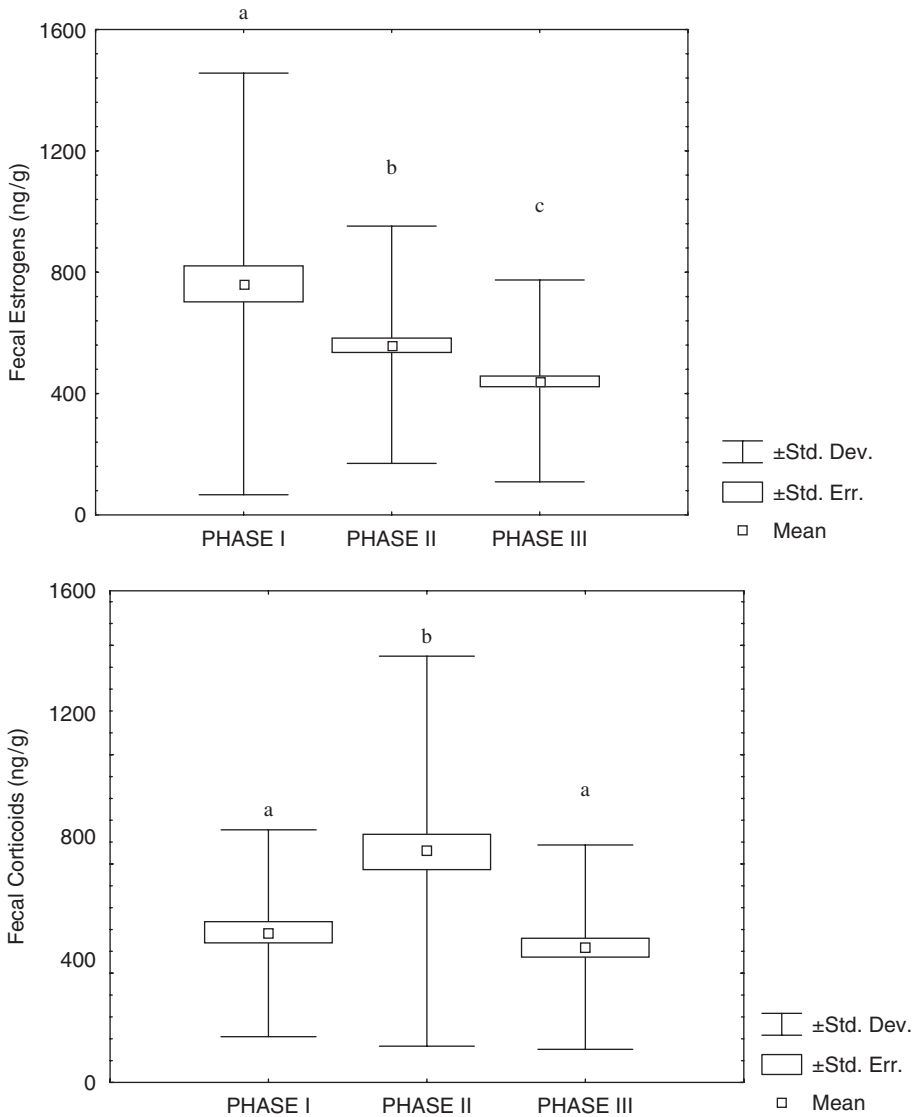


Fig. 6. Estrogens and corticoids concentrations during the three housing phases for tigrina females. Phases refer to different housing enclosures: Phase I, large enriched enclosure; Phase II, transfer to small barren enclosure; Phase III, enrichment of the small enclosure. Different superscript letters depict significant differences ($P < 0.05$).

due to seasonal effects, given our previous research showing neither margays nor tigrinas are seasonal breeders [Moreira et al., 2001].

All animals in this study exhibited some signs of stress immediately after moving and while housed in the small, barren enclosures (Phase II). These responses were reflected to varying degrees by changes in behavior, adrenal activity, and ovarian cyclicity. Transfer from the large, enriched enclosures (Phase I) to the small, barren ones (Phase II) required restraint and transport, and was associated with

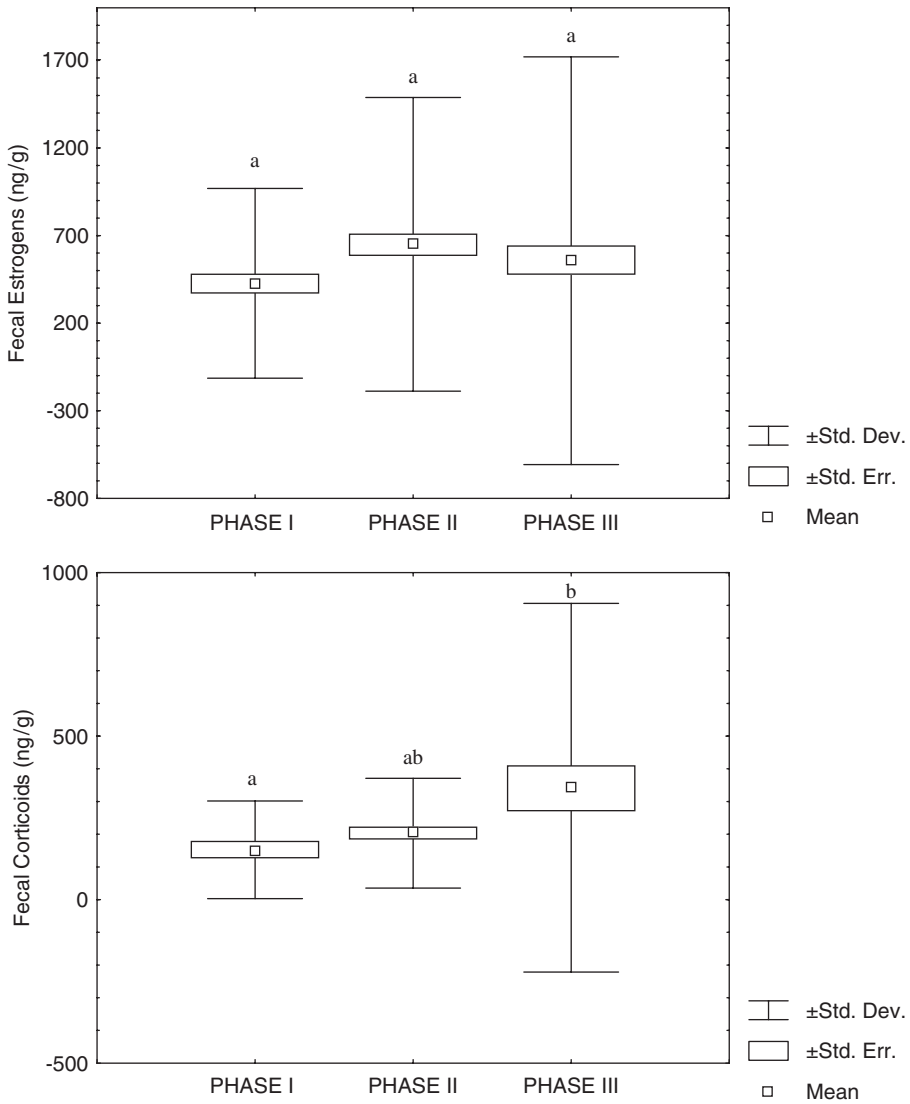


Fig. 7. Estrogens and corticoids concentrations during the three phases for margay females. Phases refer to different housing enclosures: Phase I, large enriched enclosure; Phase II, transfer to small barren enclosure; Phase III, enrichment of the small enclosure. Different superscript letters depict significant differences ($P < 0.05$).

acute increases in fecal corticoid excretion. Elevated adrenocorticoid activity in response to acute stressors, like anesthesia and translocation, has been described for these same species [Morais et al., 1997] and for cheetahs [Terio et al., 1999]. Overall, tigrina females exhibited a more dramatic increase in fecal corticoid excretion than did margays after transfer from large, enriched to smaller, barren enclosures. The high corticoid concentrations coincided with increased agitated behavior, especially in the days immediately after transfer. The most pronounced behavior suggestive of agitation was an increased frequency of stereotypic movement, characterized by

repeated pacing from one side of the enclosure to the other. After enrichment of the small enclosures (Phase III), the concentration of corticoids returned to initial levels, at least in tigrinas, supporting the importance of supplying hiding places and branches to minimize captivity stress for felids [Carlstead et al., 1993]. A review and meta-analysis of the literature evaluating the effectiveness of enrichment in reducing stereotypic behavior indicates that enrichment is a successful technique for reducing stereotypic behavior in zoo animals [Swaisgood and Shepherdson, 2005]. The differences noted in stress responses among animals and between species were not unexpected given that these are mainly psychological processes resulting from an individual's perception of the situation [Carlstead et al., 1992; Holst, 1998]. Behavioral and hormonal reactions will vary depending on the individual coping strategies used.

In association with elevated corticoids, tigrina females exhibited a decrease in overall estradiol concentrations as a consequence of reduced ovarian activity when transferred from large enriched enclosures to smaller barren ones. However, enrichment of the smaller enclosures was not sufficient to elicit a return to normal ovarian activity. Often when animals experience a loss of control over stresses in their environment, gonadotropin secretion can be diminished, and the self-preservation, fight or flight catecholamine response takes priority [Henry and Wang, 1998]. A decrease in estradiol levels during stressful situations could be the result of an inhibition in gonadotropin-releasing hormone, or its ability to stimulate ovarian activity and hormone-dependent sexual behavior [Kime et al., 1980; Moberg, 1987; Rabin et al., 1988]. The decrease in fecal estrogen excretion that occurred in conjunction with elevated corticoids during Phase II in tigrina females is suggestive of the resistance phase of the stress syndrome described by Selye [1936, 1981], which states that gonadal activity is suppressed during stress because it is a nonessential function. Several studies in other species have linked reduced cage space and barren enclosures with impaired animal well-being and increased pathologic conditions [Clarke et al., 1982; Barnett et al., 1984, 1985, 1992; Jeppesen and Pedersen, 1991]. Enclosure size and complexity are known to substantially affect behavior in some species [Carlstead et al., 1993; Carlstead, 1996], and a study in carnivores and primates found overall enclosure size to be correlated with reproductive success [McCusker, 1978].

The responses of Tigrina 1 highlight the changes observed in the adreno-ovarian axis as a result of environmental manipulations. Immediately after transfer to the small barren enclosure, this female responded with an increased excretion of fecal corticoids accompanied by a decrease in ovarian activity. Only after 40 days in the enriched enclosure did she return to normal cyclicity, at least temporarily. This response is similar to that reported for laboratory mice where the stress response was maintained for an extended period of time before coping mechanisms took over [Schoorman, 1981]. These results can be compared to an experimental study that suggested a causal relationship between the provision of additional hiding spaces and a decline in fecal corticoid concentrations in clouded leopards [Shepherdson et al., 2004].

After a period of initial agitation, both tigrinas and margays exhibited more subdued behavior when housed in the small barren enclosures. Animals ceased most activities, a behavioral characteristic of chronic stress and adapted states of helplessness [Henry, 1986, 1992]. This passive reaction is often associated with an increase in hypophyseal-adrenocortical activity, whereas the activity of the

sympathetic-adrenomedullary system remains more or less unaffected [Henry, 1992]. With increasing anxiety, active coping shifts to a more passive mode [Henry, 1992], and this type of behavior was exhibited by all females in the study during Phase II. In a study of clouded leopards, Wielebnowski et al. [2002] reported that sleeping, hiding, appearing “fearful-tense,” and exhibiting self-mutilating behaviors were associated with increased fecal corticoid excretion. In domestic cats, behavioral studies showed a decrease in general activity (e.g., walk, run), exploratory behavior (e.g., sniff, rub), and grooming in association with elevated serum cortisol levels [Rochlitz et al., 1995]. Other studies on domestic and nondomestic felids cite behavioral apathy, hiding, and low activity levels as indicators of distress [Carlstead et al., 1993; Kessler and Turner, 1997]. Furthermore, studies in other mammalian species indicate that increased sleep may be a sign of physiological and biological distress [Rampin et al., 1991; Rushen, 2000].

It is generally accepted that environmental enrichment for captive felids should use a variety of methods that meet the physiological and psychological needs of the individual. For example, provision of tree trunks allows cats to use the vertical components of the enclosure in a naturalistic way, in addition to providing a place to sharpen nails. Each enclosure should include at least one visual barrier and elevated platforms that provide hiding places throughout the enclosure. In our experience, providing natural substrates like grass has been effective in stimulating natural behaviors. For example, in this study cats were observed eating grass after it was made available during the enrichment phase of the study. On the basis of enclosure recommendations [Mellen, 2001] by the American Zoo and Aquarium Association, the width and height of the small enclosures used in Phases II and III were below minimum standards, whereas cage length exceeded the recommendation. Our findings that behavioral inactivity and elevated fecal corticoid excretion were observed in the smaller enclosures indicate that adequate cage space is important to animal well-being, and that provision of hiding places alone may be insufficient to prevent animal distress.

In a previous study, we noted that tigrina females did not ovulate spontaneously, whereas margays did [Moreira et al., 2001]. In this study, tigrinas housed alone and without manipulation did exhibit spontaneous ovulations (four among the three females over a 15-month period); however, not as frequently as those observed in margays (five among the two females). It is known that the rate of spontaneous ovulations differs among felid species, so that within this taxon ovulatory mechanisms vary, regulated to a greater or lesser degree by species-specific and even individual-specific responses to as yet unidentified stimuli of a physical and psychosocial nature [Brown, 2006]. There were no obvious reasons why the tigrinas in this study exhibited spontaneous ovulations, and they did not seem to be related to any experimental manipulations. Rather, it just may be another example of how much individual variation there is, even within the same species.

In sum, many aspects of the captive environment can directly impact the psychological and physiological welfare of felids. Today, emotional “loads” are considered the most common reason for stress and increased adrenal activity in humans [Holst, 1998] and, as described by Ursin and Olf [1993], emotional stressors are commonly used to study physiological responses in animal research. Activation of the adrenocortical system can be induced without emotional stimuli (e.g., surgery, anesthesia, illness, injury). However, it is the impact of psychological stress on welfare

that is of most concern to captive animal managers. Ensuring that animals respond appropriately to various stressors in their environment requires well-coordinated interactions among the higher nervous centers, hypothalamus, hypophysis, and adrenal glands. It is when coping mechanisms fail that individuals experience the negative consequences of stress, which can then affect general health and reproduction. For many wildlife species held in captivity, a successful *ex situ* breeding program is considered an important conservation tool. The degree to which captive conditions facilitate reproductive success is directly related to the provision of adequate social and physical environments for normal breeding behavior [Carlstead and Shepherdson, 1994; Kreger et al., 1998]. Providing conditions that meet psychological needs can ultimately improve health and welfare, including optimizing reproductive potential. Overall, these results emphasize the importance of enclosure dimensions and enrichment when designing species-appropriate environments.

CONCLUSIONS

1. Transfer of female tigrinas and margays from large enriched enclosures to small barren ones induced stress responses in both species as indicated by altered behavior and increased fecal corticoid concentrations.
2. Increases in adrenocortical activity were associated with decreased ovarian steroidogenic activity in both tigrina and margay females.
3. Enrichment of small barren enclosures with tree trunks, plants, and hiding places decreased the stress response for tigrina females, but was ineffective in reinitiating normal ovarian steroidogenic activity in margays.
4. Although margay females seemed to be less affected, based on fecal estrogens, by transfer to smaller enclosures, corticoid levels increased (not remained elevated) even after cage enrichment.

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