

Seasonal and diurnal dynamics of glucocorticoids and behavior in giant pandas

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Abstract: Because of the suppressive effect of stress on reproduction and health, it is important to evaluate potential stressors that may compromise captive breeding programs for endangered species. However, behavioral and physiological measures of stress are sometimes difficult to interpret, and their relationship to stress can be obscured by factors unrelated to stress, such as seasonal and diurnal patterns. Here we present findings for daily and seasonal variation in glucocorticoid (GC) secretion and behavior from a 6-year study of 2 giant pandas (*Ailuropoda melanoleuca*). In the American black bear (*Ursus americanus*), seasonal patterns of corticoid secretion are putatively linked to metabolic demands of hibernation. Although pandas do not hibernate, we have found a similar pattern of GC dynamics. Using radioimmunoassay of urinary GC metabolites, we found seasonal variation in GC levels in an adult female and an adult male panda. As in black bears, winter and spring GC levels were significantly higher than summer levels. Additionally, in the female, GC levels during the period of parental care and lactation were higher, regardless of calendar season, than during other periods. Diurnal patterns were also detected in both the female and male panda, with elevated GCs in the morning sample. However, these diurnal patterns were not evident during the fall and summer months. Daily levels of several behaviors potentially indicative of stress also varied significantly with season in both male and female. Additionally seasonal dynamics of feeding behavior were documented. In the female, periods of elevated and diminished appetite were associated with embryonic diapause and post-implantation, respectively. The male had elevated feeding during the fall. Although these patterns are instructive for comparison with other species, we urge caution because our limited sample size does not allow us to extrapolate beyond the individuals studied.

Key words: *Ailuropoda melanoleuca*, behavior, circadian rhythm, giant panda, glucocorticoids, non-invasive methods, seasonality, stress, Ursidae

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Recent decades have seen a steady increase in research on organismic stress-response systems (Munck et al. 1984, Moberg 2000). Both physiological and behavioral stress responses have been elucidated, and as this line of research has developed it has prompted the use of these indices in the applied context of monitoring well being in captive and wild animals (Monfort et al. 1998, Carlstead and Shepherdson 2000, Creel et al. 2002). Ultimately, it is the intent of managers to determine what types of disturbance may negatively affect the health and re-

productive success of both captive and wild populations, thus enabling managers to make intelligent management and policy decisions. For endangered species this is particularly pertinent because time and money are typically limited, and therefore efficiency is essential to a successful conservation program.

By far the most widely used physiological index of stress is the non-invasive measure of glucocorticoid (GC) metabolites in either feces or urine. GCs perform a wide variety of physiological functions and influence the expression of various behaviors in a permissive, not deterministic, capacity (Sapolsky et al. 2000). In brief, GCs are secreted as the hypothalmo-pituitary-adrenal (HPA) axis is enlisted in response to a physiological need for increased metabolism. The increase in available

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energy may facilitate an appropriate behavioral response to a perceived threat or stressor. If an acute disturbance activates the HPA axis, GCs may increase temporarily. If an animal is exposed to a chronic disturbance, GCs may be chronically elevated. This chronic elevation is problematic and may be responsible for reduced immune function (Trindle et al. 1978, Stoskopf 1983, Napolitano et al. 2002) and reproductive success (Riviere et al. 1986, Johnson et al. 1990, Holmer et al. 2003).

Behavior is also a valid and frequently used measure of stress and well being, and the link between GCs and behavior has been described in numerous studies (Carlstead et al. 1992, Blanchard et al. 1998). GC secretion can have a triggering effect on behavior (Romero et al. 2000), or animals may be behaviorally expressive in the absence of a measurable hormonal response to disturbance (Owen et al. 2004). Behavioral data collection is non-invasive and therefore allows collection of data without negatively affecting the animal. As with GCs, a behavioral response to an acute stressor is likewise short-lived and would likely have minimal to no impact on health and well being. Chronic disturbance, however, may have a more lasting effect on behavior, potentially suppressing reproductively appropriate behaviors (Ziegler et al. 1995) and feeding (Cook et al. 2000) and may lead to development of abnormal behaviors such as stereotypies (Mason 1991).

But as valuable as these indices can be, the expression of behavior and secretion of GCs are also modulated by intrinsic biological phenomena as well as environmental influences with no relationship to "stress" at all. These behavioral and physiological adjustments are potentially a normal part of an animal's biology. Romero (2002) reviewed seasonal and reproductive variation in GC secretion in various vertebrate species, noting that not all taxa exhibit the same dynamic pattern. These differences underscore the need to understand how these physiological and behavioral parameters vary on a species-specific level and may mask or confound response to stressors.

Studies of American black bears (*Ursus americanus*) have shown that levels of GCs are higher in winter and spring (during hibernation) than summer (Palumbo et al. 1983, Harlow et al. 1990). Hibernation is a period of behavioral inactivity and little environmental disturbance (except for the substantial nutritional stress). These investigators posited that GCs serve an entirely metabolic function, not one that serves to initiate or maintain behavioral activity. Giant pandas (*Ailuropoda melanoleuca*) in the wild inhabit the temperate mountains of south central China, and although the environment is

seasonally variable with regard to temperature and weather conditions, they do not undergo the seasonal hibernation that other bears do. As such, we would not expect to see the same seasonal pattern of GC secretion in pandas that has been documented in black bears.

In recent years the plight of the giant panda has garnered much attention, and the export of these animals from China for research and captive breeding has increased. Running counter to these goals are many factors within the captive environment that could elicit a stress response (crowds, vehicular traffic, construction), negatively impacting health and reproductive success (Lindenmayer and Nix 1993, Zhang et al. 2004, Swaisgood et al. In press a). It is clear though that pandas are exposed to anthropogenic stressors in the wild as well, and therefore a thorough understanding of how pandas respond to disturbance is a timely component of *in situ* management, as human populations and activities continue to encroach on panda habitat (Lü and Liu 2004).

At the San Diego Zoo we began a program in 1996 of monitoring urinary GCs and behaviors to allow us to respond proactively to signs of stress or diminished well being in these pandas (Owen et al. In press). In an earlier paper (Owen et al. 2004), we showed that when exposed to loud ambient noise, the behavioral and hormonal stress response was influenced by reproductive condition. This prompted our interest in the effect of reproductive condition and season on basal and diurnal patterns of GC excretion. Thus the goal of this study was to elucidate any seasonal, reproductive, and diurnal patterns in these data to more appropriately interpret these measures of well being and integrate the results into our management strategy. We will also place our findings in phylogenetic context for other ursid species that diverge from pandas in their seasonal activity and GC patterns.

Methods

This study was conducted over a 6-year period (1997–2002) using a single female panda (7 years old in 1997) and a single male panda (about 20 years old in 1997) residing at the San Diego Zoo. The female panda, "Bai Yun" (studbook 371) was born in captivity, and the male "Shi Shi" was wild-born (studbook 381). Husbandry details are described elsewhere (Lindburg et al. 2001).

Behavioral observations

Observation sessions were 2 hours in duration, twice daily, 5 days a week. Morning sessions commenced

at 0730 hr and afternoon sessions commenced at 1300 hr. Focal animal observations were made using point-in-time, all occurrences and one-zero sampling procedures (Martin and Bateson 1993). Point-in-time and one-zero samples were collected at one-minute intervals. We recorded and analyzed the following behaviors for this study:

- (1) Active: percent time spent in non-rest behaviors.
- (2) Feed: percent time feeding.
- (3) Agitated: a composite of door-directed behavior and scratching. Door-directed is the percent time waiting restlessly at the keeper door (alert, investigating, or manipulating the door). This behavior typically indicates motivation to get off exhibit and into the bedroom area (Owen et al. 2004). Excessive scratching or other grooming behavior may also indicate an agitated psychological state (Maestripietri et al. 1992), and was clearly associated with other signs of distress in the male.
- (4) Honk vocalization (heard too infrequently in the female to merit analysis): percent of one-minute intervals in which the male vocalized ≥ 1 time. This vocalization indicated mild to moderate distress (Kleiman and Peters 1990, Swaisgood 1996).
- (5) Pace: percent time in stereotypic pacing, defined as locomotion in a repetitive pattern in which the same path is traversed more than three times consecutively. Pacing was documented only in the female subject.
- (6) Inter-observer reliability was assessed on all behavioral observers. Each observer was required to achieve a 90% agreement level.

Urine collection and corticoid assay

The morning (AM) urine was the first morning void collected when the animals were released from the bedrooms at 0700 hr. Urine was collected with a syringe from the bedroom tunnel floor. (Floors were cleaned daily, preventing sample contamination.) The time of collection varied between 0700 and 0730 and was never later than 0830. Samples were frozen in non-reactive, 1-mm plastic vials. To analyze seasonal dynamics of GC excretion, we used AM samples because they are more likely to reflect baseline values. Few disturbances preceded morning urine voiding because the zoo is closed overnight (the previous 12–14 hr). Afternoon (PM) urines were typically collected between 1300 and 1500 hr following procedures above. None were collected later than 1630. All urine samples collected during

periods of exposure to probable stressors were excluded from analysis.

Urinary GCs were measured by single antibody radioimmunoassay (RIA) previously validated on panda urine, and creatinine (Cr) content was determined via methods previously described in Owen et al. (2004). Results of hormone assays are presented as mass of steroid per milligram of Cr (ng/mgCr), and samples with a Cr value less than 0.1 mg Cr/ml were not used.

Data analysis

We conducted separate statistical tests on each individual panda. The intent of individual-animal analysis differs from traditional statistics in that it is only possible to generalize to the level of the *individual*, not the population (Bart et al. 1998:181). Thus, we cannot extrapolate our findings to the species. On the other hand, this method is not without merit because stress responsivity can be highly individualistic and managers of captive animals need to understand the likely effect of disturbances of each animal in their charge, not just general trends for the population (see Swaisgood et al. In press *a* for a discussion).

A total of 5,330 hours of behavioral data and 898 urine samples were collected from the female subject, and 3,626 hours of behavioral data and 295 urine samples were collected from the male. Weekly means were calculated for variables across years, thus substantially reducing degrees of freedom. All data were assigned to season: winter (21 Dec–20 Mar), spring (21 Mar–20 Jun), summer (21 Jun–20 Sep), fall (21 Sep–20 Dec). For the female's samples, categories for reproductive condition were: estrus (E), pregnant or pseudopregnant (P), lactation or parental care (L), or non-reproductive (NR). Estrus was 20 days before ovulation to 5 days after ovulation, with ovulation inferred from behavioral and hormonal changes associated with follicular development and ovulation (Lindburg et al. 2001, Steinman et al. In press). Pregnant or pseudopregnant was the end of the estrus period through the period of elevated progesterone, typically from early April to mid-August. Most females that ovulate display hormonal and behavioral signs of pregnancy regardless of whether they give birth. Our female experienced pseudopregnancy (or potential early embryonic death) in all years except 1997 and 2000 (in 2000, ovulation appeared to be suppressed by lactation). For a discussion of pregnancy and pseudopregnancy, see Swaisgood et al. (2003) and Steinman et al. (In press). Lactation or parental care was the period from birth to weaning and

Table 1. Mean (SE) urinary glucocorticoids (ng cortisol/mg creatinine) by season and time of day (morning = AM; afternoon = PM) in a captive female and male panda. Female panda averages are given according to reproductive condition.

Sex	Period	Time	Season			
			Winter	Spring	Summer	Fall
Female		AM	127.69 (13.0)	109.68 (4.4)	80.89 (6.6)	99.51 (7.2)
		PM	78.61 (6.8)	91.70 (10.3)	66.40 (7.0)	90.28 (10.6)
	Estrus	AM		102.15 (28.1)		
		PM		117.87 (24.4)		
	Pregnancy	AM		88.57 (11.3)	63.15 (13.7)	
		PM		101.53 (12.9)	58.57 (11.8)	
	Lactation	AM	151.54 (9.5)	113.52 (19.1)	160.95 (32.0)	136.68 (9.2)
		PM	127.58 (16.9)	97.65 (6.0)	139.16 (31.4)	85.57 (11.0)
	Non-reproductive	AM	139.66 (32.2)	94.52 (10.2)	62.59 (9.1)	69.61 (9.5)
		PM	58.24 (8.1)	70.14 (13.6)	49.06 (9.1)	99.90 (28.4)
Male	AM	163.59 (15.3)	142.53 (10.7)	75.00 (6.6)	120.15 (14.8)	
	PM	140.15 (13.5)	106.61 (7.6)	74.44 (7.8)	95.4 (16.9)	

separation from the cub (21 Aug 1999–11 Feb 2001). Non-reproductive applied to samples collected outside the periods defined above. To reduce the degrees of freedom in these analyses, weekly averages were taken across years and within a reproductive condition.

Hartley's *F*max test was used on both behavioral and hormonal data to determine whether data met the assumptions of homoscedasticity. Residual plots were visually inspected to determine whether data were normally distributed. Hormone data were transformed using the log transformation (Sokal and Rohlf 1996). The effects of season and time of day on GC levels were analyzed with a 2-factor ANOVA (analysis of variance). We also evaluated the effect of reproductive condition in the female panda. Data classified by reproductive condition did not meet the assumptions of homoscedasticity of variance due to the high level of variability of both behavioral and physiological variables measured during the short estrus period. Therefore, these data were analyzed using Kruskal-Wallis ANOVA (Siegel and Castellan 1988). Time of day effects were tested with the Mann-Whitney *U*-test (Siegel and Castellan 1988). Because all possible reproductive conditions (E, P, L, and NR) did not occur in each season, a 2-factor Friedman's ANOVA (season, reproductive condition) could not be used. Thus, reproductive condition was analyzed within each season using a Kruskal-Wallis ANOVA or Mann-Whitney *U*-test (winter analyzed L and NR; spring: E, P, L, and NR; summer: P, L and NR; fall: L and NR).

Behavioral data were analyzed with a 2-factor (season, time-of-day) ANOVA. Scheffe's post-hoc tests were used to determine differences between seasons. To determine

which seasons had significant diurnal variation, planned comparisons were performed to compare morning and afternoon samples within each of the 4 seasons.

Results

Dynamics of GCs in the female panda

The female's GCs varied with respect to season ($F = 4.84$; 3,94 df; $P < 0.004$; Table 1) and reproductive condition (Kruskal-Wallis ANOVA: $H(3,4) = 38.3$, $P < 0.0001$; Table 1). Scheffe's post-hoc test showed that winter and spring GCs were significantly higher than summer ($P < 0.02$). Within each season Scheffe's post-hoc examination of reproductive condition showed that L was consistently higher than all other reproductive states (Table 1) except for L versus NR in Spring.

Urinary GC levels were significantly higher in the AM samples than in the PM samples ($F = 16.56$; 1,94 df; $P < 0.0001$). This pattern of diurnal variation was not influenced significantly by season (non-significant interaction of season \times time-of-day). However, examination of Table 1 shows that AM GCs were elevated in winter and spring, but not summer and fall. This observation was borne out by the planned intra-season comparisons for AM versus PM samples, where only winter ($t = 3.40$, 23 df, $P < 0.003$) and spring ($t = 1.98$, 24 df, $P = 0.05$) were associated with significant diurnal variation respectively.

Seasonal variation in behavior (female)

Univariate tests revealed that activity level varied with season ($F = 3.73$; 3,96 df; $P < 0.02$) as did time spent

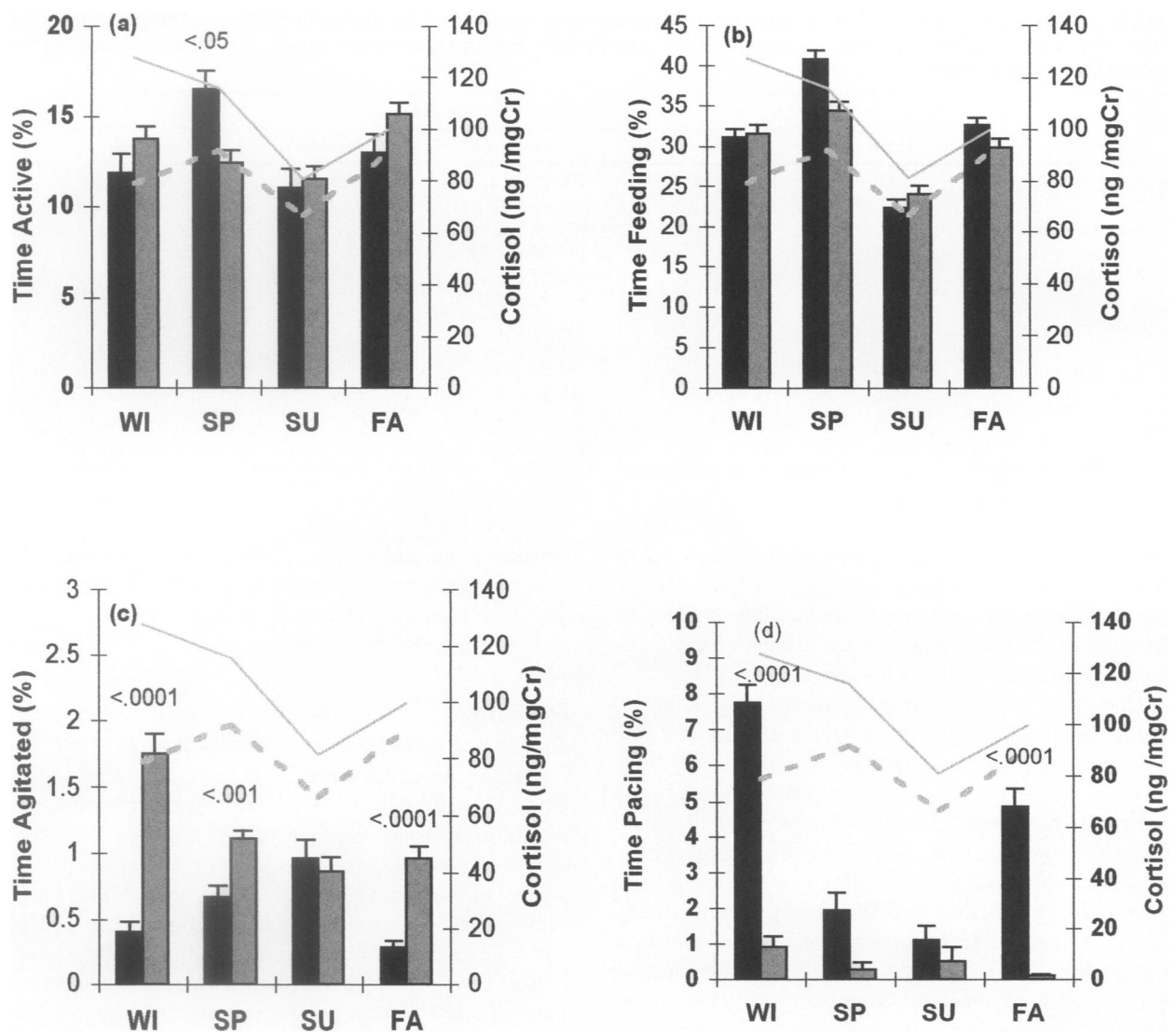


Fig. 1. Diurnal dynamics of cortisol level (solid = morning, dashed = afternoon) and activity for 4 behaviors (black = morning; grey = afternoon) for winter (WI), spring (SP), summer (SU), and fall (FA) in a captive female panda. Cortisol is measured in nanograms per milligram creatinine. Numbers above bars indicate P where differences are significant.

feeding ($F = 13.89$; 3,96 df; $P < 0.0001$; Fig. 1). Time engaged in agitated behaviors varied with season ($F = 7.72$; 3,96 df; $P = 0.0001$) and time of day ($F = 66.20$; 1,96 df; $P < 0.0001$), as did pacing ($F = 8.21$; 3,96 df; $P < 0.0001$; $F = 38.07$; 1,96 df; $P < 0.0001$). Additionally, the interaction was significant for all of these behaviors except feeding (active: $F = 3.86$; 3,96 df; $P < 0.02$; agitated: $F = 15.81$; 3,96 df; $P < 0.0001$; and pace: $F = 6.47$; 3,96; $P = 0.0005$), indicating that time-of-day effects on these behavior were influenced

by season. Scheffe's post-hoc pairwise comparisons showed that summer activity was lower than spring and fall and that spring activity was significantly higher than winter. Feeding peaked during spring, and feeding levels during summer were lower than all other seasons. Behavior indicating agitation was lowest in fall, and pacing was highest in winter.

Planned intra-season comparisons showed diurnal differences for most behaviors in all seasons except summer. In winter we observed differences between AM

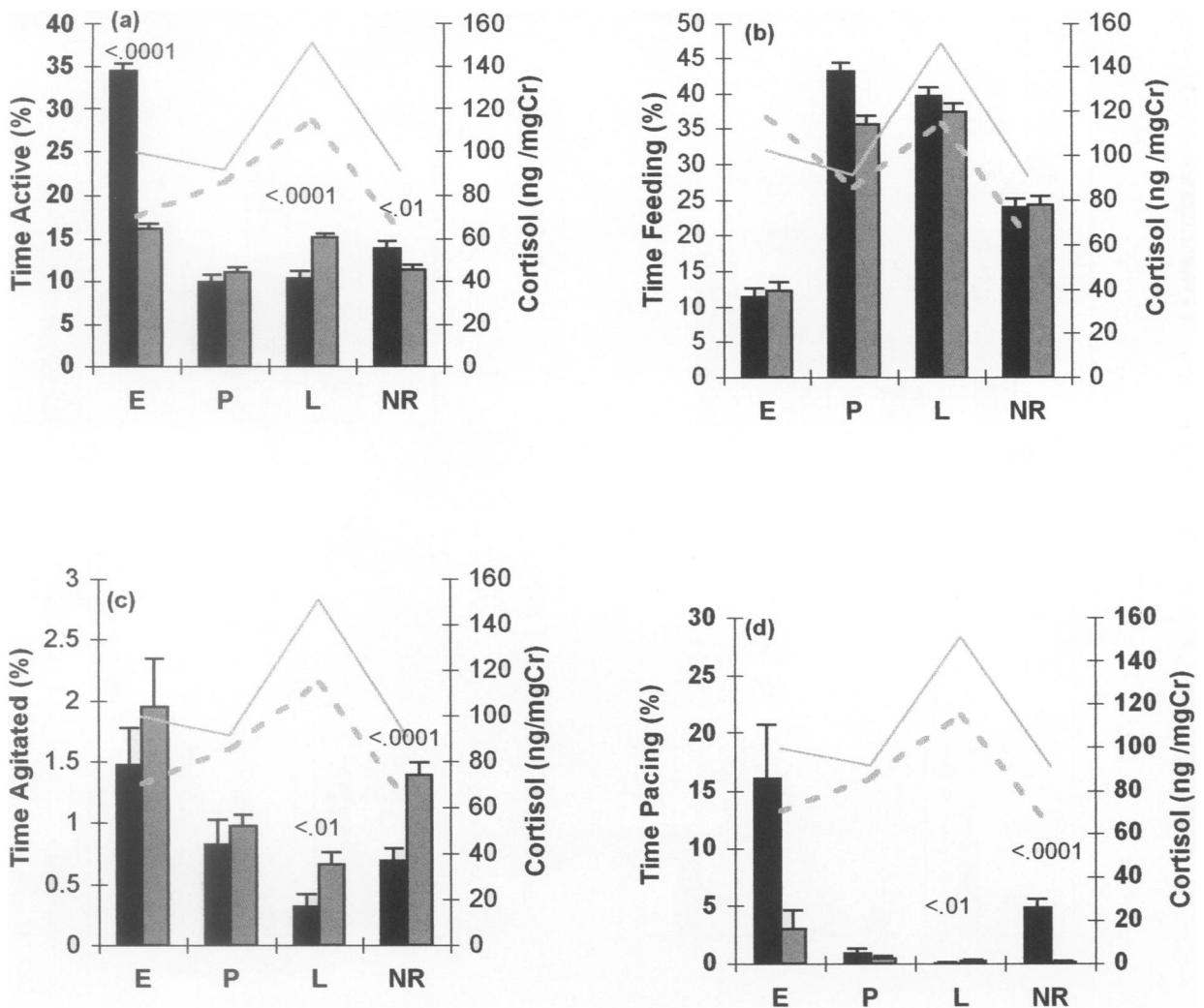


Fig. 2. Diurnal dynamics for cortisol levels (solid = morning, dashed = afternoon) and activity for 4 behaviors (black = morning; grey = afternoon) for reproductive condition (E = estrus; P = pregnant or pseudopregnant; L = lactation, NR = non-reproductive). Cortisol is measured in nanograms per milligram creatinine (ng/mgCr). Numbers above bars indicate P where differences are significant.

and PM levels of pacing ($t = 4.22$, 24 df, $P = 0.0003$) and agitated behaviors ($t = 7.71$, 24 df, $P < 0.0001$). In spring we observed differences between AM and PM levels of active ($t = 2.13$, 24 df, $P < 0.05$) and agitated ($t = 4.11$, 24 df, $P = 0.0004$). During fall we observed significant or near significant diurnal differences for all behaviors (active: $t = 1.75$, 24 df, $P < 0.09$; feed: $t = 1.89$, $P < 0.07$; pace: $t = 4.54$, 24 df, $P = 0.0001$; agitated: $t = 6.67$, 24 df, $P < 0.0001$). During summer there were no diurnal differences in behaviors measured.

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Effect of reproductive condition on behavior (female)

Each behavior analyzed showed significant effects of reproductive condition (Fig. 2) (Kruskal-Wallis ANOVA; active: $H(3,4) = 23.13$, $P < 0.0001$; feed: $H(3,4) = 86.61$, $P < 0.0001$; pace: $H(3,4) = 20.83$, $P < 0.0001$; agitated: $H(3,4) = 39.27$; $P < 0.0001$). Intra-season non-parametric tests showed significant, or near-significant ($P < 0.07$), effects of reproductive condition within each calendar season (Fig. 3) for all behaviors. The female was most active during spring estrus period,

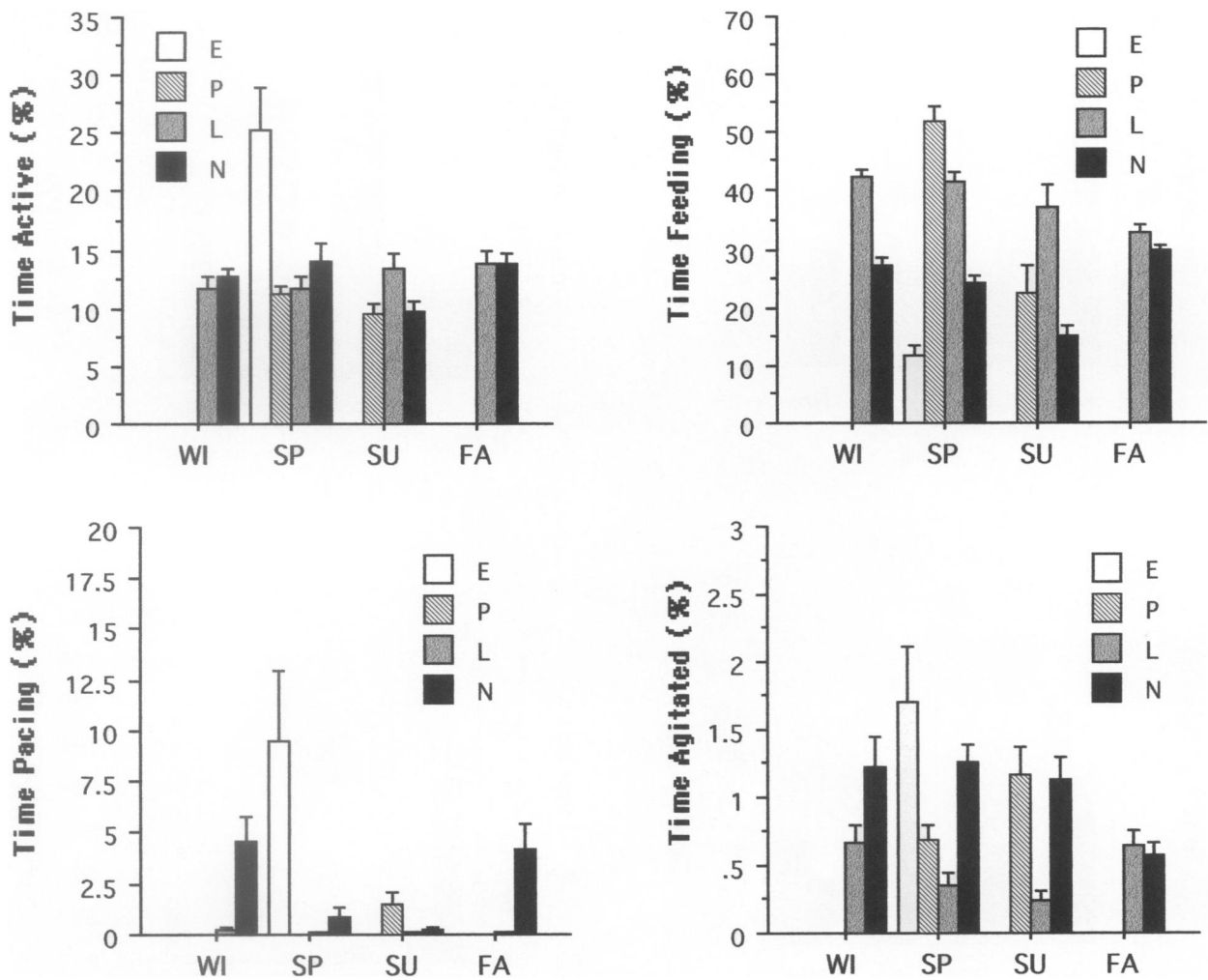


Fig. 3. The effect of reproductive condition (E = estrus; P = pregnant or pseudopregnant; L = lactation, and NR = non-reproductive) on seasonal dynamics (WI = winter, SP = spring, SU = summer, and FA = fall) of 4 behaviors in a captive female panda. Intra-season tests show a significant effect ($P < 0.005$) of reproductive condition except active, feeding, and agitated behaviors in fall and active behavior in winter.

spending approximately twice as much time active as in other periods. Pacing followed this same pattern, but levels were recorded at about 4 times that of any other period. Feeding behavior peaked during pregnancy or pseudopregnancy in the spring, which is generally the pre-implantation period of pregnancy. When lactating, the female showed consistently robust levels of feeding in all seasons. Signs of behavioral agitation were consistent throughout the winter, spring, and summer, when the female was not reproductively active, but we also found these same levels of agitation during the summer (post-implantation) period of pregnancy. Agitation levels were highest during spring estrus.

Dynamics of GCs in the male panda

The male's GCs also varied with respect to season ($F = 16.77$; 3,78 df; $P < 0.0001$, Table 1). As in the female, Scheffe's post-hoc examination showed that winter and spring GCs were significantly higher than summer GCs. Diurnal variation was seen in the male's GC levels ($F = 4.06$; 1,78 df; $P < 0.05$). Variation occurred only in spring ($t = 2.63$, 22 df, $P < 0.02$). GC levels did not differ among winter, summer, and fall.

Seasonal variation in behavior (male)

Univariate tests revealed seasonal and diurnal variation (respectively) (Fig. 4) in activity levels ($F = 8.94$;

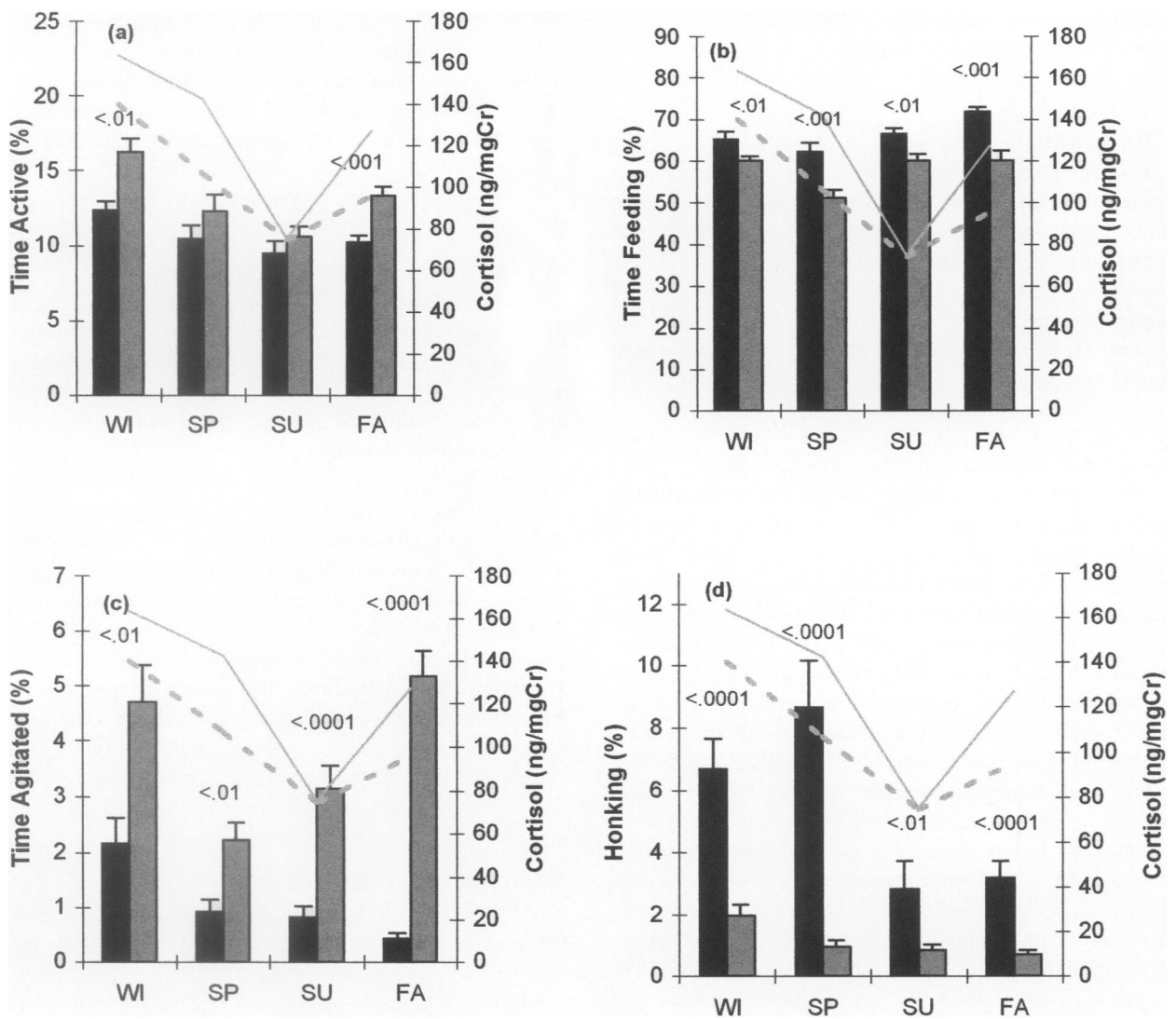


Fig. 4. Diurnal dynamics of cortisol level (solid = morning, dashed = afternoon) and activity for 4 behaviors (black = morning; grey = afternoon) for winter (WI), spring (SP), summer (SU), and fall (FA) in a captive male panda. Cortisol is measured in nanograms per milligram creatinine (ng/mgCr). Numbers above bars indicate *P* where differences are significant.

3,96 df; $P < 0.0001$; $F = 19.07$; 1,96 df; $P < 0.0001$), time feeding ($F = 9.92$; 3,96 df; $P < 0.0001$; $F = 48.75$; 1,96 df; $P < 0.0001$), agitated behaviors ($F = 8.86$; 3,96 df; $P < 0.0001$; $F = 94.22$; 1,96 df; $P < 0.0001$) and the honking vocalization ($F = 8.82$; 3,96 df; $P < 0.0001$; $F = 63.46$; 1,96 df; $P < 0.0001$). Additionally, the interaction was significant for agitated behavior ($F = 6.71$; 3,96 df; $P = 0.0004$) and honking ($F = 44.49$; 3,96 df; $P = 0.0007$). Scheffe's post-hoc tests showed that activity level was highest in the winter and that feeding

levels peaked in the fall. Agitated behaviors were highest during winter and honking vocalization was heard most frequently during winter and spring.

Diurnal activity levels were significantly higher in the afternoon during winter and fall. Feeding levels were highest in the mornings year-round. Again this likely reflects consistent presentation of fresh bamboo in the morning. Conversely, and possibly related to this pattern of food availability, agitated behavior was significantly higher in the afternoons during all seasons. The honking

vocalization was significantly higher during morning during winter, spring, and summer.

Discussion

We documented both seasonal and diurnal variation in behavior and urinary GCs in these giant pandas. We also found significant variation in these variables in the female during differing reproductive conditions. These results suggest that although both GCs and behavior are important tools for monitoring stress, variation by season, time of day, and reproductive condition can affect these variables to such an extent that care must be taken when interpreting results.

Seasonal variation in glucocorticoids

GCs for baseline morning samples were most elevated during winter and spring and lowest during summer for both subjects. Interestingly, American black bears follow this same pattern (Palumbo et al. 1983, Harlow et al. 1990) despite markedly different ecological and physiological constraints. (However, these black bear studies did not report time of day the samples were collected, and GC concentrations were assessed via serum and not urine.) In black bears this pattern has been attributed to metabolic demands of stored fat and protein catabolism during hibernation. Because GCs can alter metabolic pathways and affect energy mobilization in various species (Goymann et al. 1999), it is tempting to consider a metabolically-based hypothesis for the dynamics in these pandas. However, given the relatively consistent food supply exploited by pandas (both in the wild [Schaller et al. 1985, Johnson et al. 1988] and captivity), it is unlikely that metabolic needs are primarily responsible for the seasonally dynamic elevation of basal GCs. However, we documented consistently elevated GCs during lactation. As in other species, energy expenditure can be elevated during this period to support increased nutritional and behavioral demands on the mother while rearing and nursing young (see below).

Winter and spring peaks occurred when day length was increasing, whereas the GC nadir occurred when days were long, but getting shorter. This pattern suggests that GC secretion may be regulated by photoperiod. An alternative explanation is that cooler winter months encouraged activity, and the relationship between physical activity and GC production (Weiss 1968) accounted for this seasonal pattern. However, the absence of seasonal variation in PM samples, which should have reflected activities earlier in the day, argues

against this hypothesis. By contrast, AM samples were collected following the least active period (i.e., night). Moreover, seasonal variation in nighttime temperatures was much less pronounced in the dry San Diego climate than were daytime temperatures. Thus, photoperiod regulation of GCs is more plausible than any effects of ambient temperature or physical activity.

Diurnal variation in glucocorticoids

The significantly higher AM levels of GCs were detectable only during winter and spring for both animals (PM samples were remarkably consistent from season to season). Lack of a difference during summer and fall makes it clear that the AM samples generated the observed seasonal GC pattern.

Additionally, GCs collected during reproductively active periods (estrus, pregnancy or pseudopregnancy, and lactation) did not vary with time of day. The absence of diurnal variation reflects elevated PM GCs during lactation (in winter, summer, and fall) and a decrease in AM GCs for estrus and pre-implantation pregnancy. Our management objective during periods of estrus and pregnancy was to optimize the female's environment and eliminate or reduce environmental stressors. As such, thoroughly mapping these patterns of GC excretion can lead to more effective interpretation of data and implementation of appropriate management.

Seasonal dynamics of behavior

We found ample evidence that the behavior of both male and female were significantly influenced by season, time of day, and—for the female—reproductive condition. Many co-varying factors may be posited as the cause of such variations in behavior, including photoperiod, temperature, climatic factors, management, and changes in underlying physiology, including glucocorticoids. We also recognize that changes in behavior may be partially responsible for causing changes in GC levels (the effects of physical activity on GCs). Because our study was correlational, excessive speculation on causal relationships and functional significance of each finding is not warranted. Here, we offer a few suggestions regarding the possible meaning of some patterns in the data.

Pacing is perhaps the most important behavior measured in this study because of its possible relationship with poor well being (Mason 1991). The female's pacing peaked during the brief spring estrus, and it was significantly elevated during the winter and fall, periods of lowest public attendance at the zoo. Pacing was

consistently highest during the morning, before the zoo was opened to the public, and thus we can conclude that this female's pacing was not a response to human disturbance. On the contrary, we suggest that high levels of pacing during the winter and spring estrus were related to the approaching mating season. The high level of pacing noted during estrus reflected a general anxiousness noted during the mating season for this female (Lindburg et al. 2001) and was probably related to a general increase in locomotor behavior and range expansion among females just prior to annual estrus (see Swaisgood et al. 2002). During this period, females search for and advertise their presence to males through scent marking. A similar increase in pacing has been found during the breeding season for other bear species (Carlstead and Seidensticker 1991), suggesting that sexual interest is one of the motivational states underlying pacing in ursids (but not the only one: see Carlstead et al. 1991, Swaisgood et al. 2001). During the summer months pacing was at its lowest levels, but a partitioning of the summer data by reproductive condition showed that during post-implantation, pregnancy pacing (and behavioral agitation) was significantly higher than during other conditions in summer. We speculate that this activity may be related to motivation to find a suitable den. In the wild, pandas descend several hundred meters in elevation and select a den during this stage of pregnancy (Yong et al. 2004). Thus, we tentatively conclude that for pacing, our most direct behavioral measure of well being, seasonal patterns are best explained by changes in internal motivation, rather than external factors (such as disturbance) that can be mitigated by altered husbandry and management practices. However, this does not mean that pacing is an invalid measure of well being. On the contrary, such seasonal changes in motivation may indicate only that animals find their captive surroundings more or less aversive depending on what behaviors they are motivated to perform. Indeed "frustrated" motivation, in which an animal is thwarted from obtaining a goal, is one of the most powerful predictors of stereotypic behavior (Hughes and Duncan 1988, Mason 1991).

Signs of behavioral agitation also signal the possibility of frustrated motivation. Our measures of agitation were door-directed behavior, indicating thwarted attempts to leave the exhibit, and scratching, which also can be associated with re-direction of a primary motivation that cannot be fulfilled (Maestriperi et al. 1992). Thus, the expression of these behaviors may indicate a motivational precursor to the development of stereotypic behavior, and indeed door-directed behav-

iors and scratching can become rigid and stereotypic (Swaisgood et al. 2001). These behaviors followed a similar pattern to that observed for pacing, with the highest levels observed during spring estrus and winter, although levels were low during the fall. Thus, the seasonal patterns of behavioral agitation may be explained by motivational factors similar to those advanced for pacing. Interestingly, pacing and behavioral agitation conformed to differing diurnal patterns, with pacing peaking in the morning in the female and behavioral agitation more predominant in the afternoon in both the male and female. While we cannot rule out some internal biorhythm, the most plausible explanation lies in timing: afternoon data were collected shortly before the animals went off exhibit. They were highly motivated to get off exhibit because they expected to be fed upon return to the bedroom areas or because they had been on display for several hours. The female's pacing in the morning may have been because she had just been released from the bedroom into the larger exhibit area.

Although sexual motivation during estrus may explain elevated levels of pacing at that time, the demands of maternal care and lactation apparently suppressed behaviors that potentially indicated poor well being (agitation and pacing). Interestingly, the female also had by far the highest levels of glucocorticoids during this period. We propose that this inverse relationship between behavior and hormones suggests a causal explanation. The demands on a panda mother are unusually high, even compared with other ursids (Zhu et al. 2001), and much of the mother's activity budget is devoted to maternal care (Snyder et al. 2004). Meeting these demands, coupled with self-imposed confinement during the denning phase, may evoke a stress response. More importantly, the mother's behavioral options at this time are constrained by the need to provide care for the cub, and she may not be able to resort to the usual coping mechanisms to deal with the stress of a difficult environment. Indeed the performance of stereotypic behavior is believed to be one means of coping with aversive situations (Mason 1991), suggesting that the mother's inability to resort to these behaviors during the maternal care period may actually exacerbate the physiological stress response. Alternatively, elevated GCs may be the simple consequence of increased metabolic needs for lactation. Future research needs to evaluate these hypotheses.

Honking, a vocalization associated with an aversive situation or thwarted access to resources (Kleiman and Peters 1990), was a primary behavioral index of the

male's well being (Owen et al. 2004). The seasonal pattern for these 2 behavioral indices may be influenced by seasonal management changes. For example, beginning the weeks preceding the breeding season and continuing until the end of the female's estrus (late winter, early spring), the male and female were given access to one another through cage bars and placed in the same pen as part of a breeding management strategy (Swaigood et al. In press *b*). The elderly male, who never mated or showed sexual interest in the female, invariably tried to avoid the female and, when pushed, responded aggressively. It is thus not surprising that honking increased during this period. Our data show that the male often engaged in long bouts of honking following interaction with the female.

Seasonal variation in behaviors less associated with stress and well being were also illuminating. The female had higher levels of activity and feeding in spring versus other seasons and had significantly lower activity in the summer than other seasons. The summer depression of activity could have been a response to warmer weather, or it may also have reflected energy conservation during pregnancy. A closer look at the female's behavioral data by reproductive period (Fig. 3) supports both explanations, as the female's activity level during non-reproductive and pregnancy did not differ during summer, and both showed a trend toward lower activity levels than during lactation. However, she was significantly more active during the spring (pre-implantation) period of pregnancy than the summer (associated with post-implantation) period of pregnancy. Her feeding behavior was also elevated during pre- versus post-implantation period. The dynamics of these behaviors likely reflect different physiological needs of the female during pre- and post-implantation pregnancy.

Elevated levels of feeding during the post-estrus, pre-implantation spring period may parallel the pre-denning hyperphagia documented in *Ursus* species (Nelson et al. 1983, Craighead et al. 1995, Harlow et al. 2002). This is the period of embryonic diapause in pandas, and it may be advantageous to stock up on nutrient stores as do black and brown bears (*U. arctos*), especially after the striking appetite suppression documented during estrus; see Fig. 3. The same argument holds for pseudo-pregnancy because behavioral and hormonal patterns are virtually indistinguishable and the panda's biology seems wired to prepare for the possibility of pregnancy. Of course, giant panda females do not fast *per se* during the prepartum denning period, and as such panda females might not be expected to stock up on nutrients during the period of blastocyst arrest. However,

a distinct, and often drastic, prepartum decrease in appetite has been documented in this and other captive and wild female pandas during the post-implantation period (Zhu et al. 2001, Swaigood et al. 2003). Appetite decrease has been documented as early as about 1 month prepartum and fasting is often observed for 2 weeks peripartum. This appetite suppression continues several weeks after birth while the female remains in the den tending to the constant needs of her offspring. Later, during lactation, the female spent more time feeding (Fig. 3).

Contrary to the pattern seen in the female, feeding levels peaked in the fall for the male, and activity levels were highest during the fall and winter. Perhaps cool ambient temperature played a role in this elderly male's activity and appetite levels, as suggested by the high afternoon activity levels in winter when afternoon temperatures were coolest. We might expect, however, that a male interested in mating would have higher activity levels during the spring breeding season.

Conclusions

In general, seasonal and diurnal dynamics of GC secretion in these 2 pandas was more readily explained by factors unrelated to stress and more likely governed by factors such as photoperiod that prepared the animals for seasonally varying physiological needs. Another line of evidence arguing against the stress hypothesis is that behaviors potentially indicative of stress did not follow the same seasonal pattern as GCs. By contrast, GC and behavior patterns coincided in a previous study specifically designed to test the stress-provoking effects of noise (Owen et al. 2004).

Pandas, like *Ursus* species, are seasonal breeders and as such the behavioral and physiological demands of reproduction may be responsible for patterns observed in this study. Photoperiod may trigger the circa-annual patterns noted (Nelson and Demas 1996), and behavioral or reproductive needs may facilitate the maintenance of GC levels, as well as levels of activity and feeding. In other species inhabiting temperate latitudes, photoperiod has been identified as the primary signal by which key physiological and behavioral phenomena are either initiated or terminated (Nelson and Demas 1996, Hofman 2004). These factors could also play a synergistic role, working in concert to trigger or maintain these dynamic biological phenomena. In the female American black bear, seasonal GC elevation is coupled to, and perhaps enables, the fulfillment of the metabolic needs of fasting during post-implantation hibernation

(Harlow et al. 1990). We observed a similar seasonal GC pattern in our female panda, but it did not coincide with the same stage of reproduction, raising the interesting hypothesis that seasonal GC dynamics in pandas may result in part from phylogenetic inertia from ancestral ursid adaptation in temperate zones to seasonally limited food resources. Consistent with other bears, our female panda fed more during the period of blastocyst arrest even though this reproductive stage occurs during a different season in the panda than it does in the black bear. Perhaps the seasonal changes in GC excretion are triggered by changes in photoperiod, and the seasonal dynamics in feeding behavior are associated with the fulfillment of the metabolic needs of reproduction (hyperphagia during blastocyst arrest and a period of appetite suppression and fasting during the post-implantation period of pregnancy). It is important to note also that male American black bears also have a pre-hibernation hyperphagic period during the fall. Our male panda spent more time feeding during the fall than all other seasons. Clearly, the parallels between the behavior and physiology of pandas and other bears should be more thoroughly investigated with larger sample sizes, and we suggest that similar studies be done in other bear species.

Anthropogenic disturbance is of great concern to animal managers, especially those charged with the stewardship or care of endangered species in the wild. All animals can cope with disturbance to some degree, but the point at which the effects of disturbance overload an animal's physiological and behavioral coping systems is variable. If managers are to use these bioindicators to assess individual or population level well being, a thorough understanding of the range of variation, and non-stress related causes of this variation must underlie interpretation of results. Clearly, behavioral and hormonal systems are regulated by a complex set of factors, all subject to synergistic interplay. Our study of these individuals raises many interesting hypotheses, but alone cannot elucidate these complex patterns, nor place the panda in phylogenetic context with its ursid kin. We hope this work will stimulate further research with larger sample sizes in pandas and other ursid species. Information gained in these pursuits will enable these data to be used to build better management systems to maintain the well being of ursids in captivity. Perhaps more importantly, as human populations and activities continue to encroach on the habitat of wild bears, wildlife managers will rely ever more heavily on non-invasive techniques to detect and monitor the impact of these encroachments. A thorough

understanding of naturally occurring patterns in GCs and behavior may increase the efficacy of these often-used bioindicators by enabling field researchers to more accurately interpret data.

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