The Old School, Brewhouse Hill, Wheathampstead,
Hertfordshire AL4 8AN, UK
www.ufaw.org.uk

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Effect of housing and husbandry practices on adrenocortical activity in captive Canada lynx (Lynx canadensis)

KV Fanson† and NC Wielebnowski‡*

† School of Life and Environmental Science, Deakin University, 75 Pigdons Road, Waurn Ponds, VIC 3216, Australia
‡ Oregon Zoo, 4001 Southwest Canyon Road, Portland, OR 97221, USA
* Contact for correspondence and requests for reprints: kerryfanson@gmail.com

Abstract

*In recent years, there has been an increasing focus on the study and assessment of animal welfare in captive settings, such as zoological gardens and aquaria. Canada lynx (*Lynx canadensis*) are a relatively common species in zoos, yet are known to exhibit frequent reproductive problems in captive environments. We provide an exploratory analysis of housing and husbandry factors that are associated with patterns of adrenocortical activity in lynx. Adrenocortical activity was assessed using the non-invasive technique of monitoring faecal glucocorticoid metabolites (FGM). First, we calculated baseline FGM values for each individual and controlled for sex, age class, and reproductive status. The residual values were used to determine how levels of adrenocortical activity correlated with institutional husbandry practices. Second, we compared the occurrence of FGM peaks to events and disturbances recorded by keepers. Our results highlighted that adrenocortical activity is strongly correlated with: (i) the size of the enclosure; (ii) the number of hiding locations available; and (iii) the social environment. Based on our findings, we recommend that lynx should generally be housed alone (unless with dependant offspring or temporarily paired up for mating purposes), in larger enclosures and with the provision of several species-appropriate hiding locations.*

Keywords: *animal welfare, Canada lynx, captive breeding, faecal glucocorticoid metabolites, felid, stress*

Introduction

The measurement of faecal glucocorticoid metabolites can provide a valuable window into the physiological and psychological condition of an animal as it responds to and copes with its environment. Glucocorticoids (GCs) are a product of the hypothalamic-pituitary-adrenal (HPA) axis which is one of the primary components of the vertebrate stress response and is activated by adverse or potentially threatening stimuli (Sapolsky 2002; Palme *et al* 2005). Understanding the stress physiology of a species and identifying normative patterns of adrenal activity is useful for management of captive and wild populations (Wielebnowski 2003; Schwarzenberger 2007). Some species and individuals seem particularly prone to stress-induced pathologies in captivity (Clubb & Mason 2007). However, even among species or individuals that do not exhibit pathological manifestations of stress, there may be sub-pathological variations in adrenal activity that contribute to the health, reproductive success, and overall well-being of the animal.

Canada lynx (*Lynx canadensis*) have a long history in captivity, both in public (eg zoos) and in private (eg fur-industry, pets) sectors. They are common as pets and ambassador/educational animals due to their 'easy-going' temperament. Since they appear relatively easy to maintain *ex situ,* little attention has been devoted to understanding factors that contribute to the well-being of lynx in captivity. However, with a recent trend toward lower reproductive success in the captive North American population (Goff 2008) and an increasing interest in animal welfare, it is important to consider factors that may contribute to lynx well-being and ultimately lynx conservation success. Captive Canada lynx in North America are maintained in a wide range of housing situations, from small enclosures with multiple individuals to large naturalistic enclosures with a single animal or a male-female pair.

We performed an exploratory analysis of housing and husbandry factors that may affect stress physiology in captive Canada lynx. We conducted a cross-institutional assessment of adrenocortical activity in lynx, as well as the husbandry practices adopted by each facility. Adrenocortical activity was quantified using the non-invasive technique of faecal glucocorticoid metabolite (FGM) analysis. Improving our understanding of the relationship between FGM concentrations and husbandry practices may allow us to elucidate future avenues of research that can ultimately help to improve the welfare of captive Canada lynx.

Materials and methods

Study animals

This study included a total of 45 captive Canada lynx (21 males and 24 females) from 22 institutions. The mean age was 7.6 years (range: 2–18 years). Four males were castrated, and seven females were spayed. Samples collected during a pregnancy or pseudo-pregnancy were excluded from this study because FGM concentrations are significantly elevated at this time (Fanson *et al* 2010). All lynx were housed outdoors more than 50% of the time, and thus were exposed to natural photoperiod rhythms. Most lynx had been housed at the surveyed institution for at least one year prior to the study; the exception being one male on breeding-loan that had only been at the institution for one month.

Faecal sample collection and analysis

Animal care staff collected fresh faecal samples two to four times per week during routine cage cleaning. The duration of sample collection for individual lynx ranged from 2–12 months. Samples were frozen at –20°C and shipped to the Brookfield Zoo, Chicago, USA for analysis.

Faecal steroid metabolites were extracted and assayed as previously described (Fanson *et al* 2012). Faecal glucocorticoid metabolites (FGM) were quantified using a commercially available corticosterone enzyme-immunoassay kit (Assay Designs, Ann Arbor, MI, USA) that has been validated biologically for monitoring adrenocortical activity in Canada lynx (Fanson *et al* 2012). All samples were assayed in duplicate. Intra-assay coefficients of variation were 15.5 and 11.4% (n = 19) for low and high controls, respectively. The inter-assay coefficients of variation were 24.6 and 25.8% (n = 74), respectively. Data are expressed as ng g^{-1} wet faecal weight.

Keeper surveys

Questionnaires were distributed to each institution to obtain detailed information about the individual history, housing situation, and husbandry routine for each lynx (Table 1). A total of 21 variables were used in analyses. When possible, the *North American Canada Lynx Studbook* and personal visits to the institutions were used to corroborate information. Surveys were completed by the head keeper and/or curator.

Statistical analysis

All data were analysed using SAS 9.1 (Cary, NC, USA). For each individual, baseline FGM concentration was calculated through an iterative process excluding all points greater than the mean (± 3 SD) (Wielebnowski *et al* 2002). Peaks were defined as any points that were excluded from the baseline. Data were log-transformed as necessary to meet assumptions of normality and homoscedasticity.

Housing and husbandry effects

It was beyond the scope of this project to move or manipulate lynx in order to experimentally examine housing/husbandry effects. As a consequence, several variables were correlated with one another. Before testing the potential effects of housing and husbandry on FGM concentrations, we first controlled for the effect of biological factors that have been shown to influence adrenocortical activity (Fanson *et al* 2012). This was accomplished by running an ANCOVA to determine how baseline FGM values vary with sex, age class (young adult or mature adult), and reproductive status (intact or neutered). The residuals (*r*Baseline) of this biological model were used as the response variable in the husbandry model. The qualitative results obtained using the residuals were similar to those obtained using the raw data. For this analysis, if lynx were moved to a new enclosure at the same institution (n = 2), we only included the housing situation for which we had the most data.

To identify potentially influential husbandry factors, we screened the 21 husbandry variables by running all possible one- and two-factor ANOVAs. These results, in conjunction with graphical analyses, were used to identify 13 variables that were influential on *r*Baseline values (Table 1). Correlation coefficients were then calculated for these variables to examine issues of multicollinearity and identify clusters of variables. Although there was some degree of correlation among variables, there were no tight correlations or clusters, so we used all 13 variables for the next step.

To obtain an integrative understanding of how husbandry affects *r*Baseline, we constructed a general linear model using a backward step-wise procedure. This is an iterative procedure in which the least significant variable is removed from the model until the remaining variables meet a certain criterion (in this case, $P < 0.1$). There were five variables that remained in our final GLM model (Table 1). Tukey-Kramer adjustment was used to correct for multiple pair-wise comparisons. This multivariate approach allows us to get a better understanding of the interaction between variables, which cannot be obtained from simple correlations or one-way ANOVAs.

Correlates of FGM peaks

The previous analysis only considered differences in baseline FGM values. However, peaks in FGM concentrations can also provide useful information regarding how an animal perceives its environment. Keepers were asked to record any notable changes or potentially stressful events that occurred during the sampling period. We examined the occurrence of peaks (ie, points exceeding the baseline [± 3 SD]) relative to events recorded by the keepers. Excretion of FGMs lags behind changes in circulating GCs by approximately 24 h (Fanson *et al* 2012), and we took this excretion lag-time into account.

Results

Housing and husbandry effects

*r*Baseline values were most strongly influenced by sex of cage-mates, area of enclosure, and number of hiding spots (Table 1). Lynx housed with both male and female cage-mates had significantly higher *r*Baseline values than lynx housed with only one sex of cage-mate (Figure 1). Lynx housed alone had the lowest *r*Baseline values. *r*Baseline values were negatively correlated with area of enclosure

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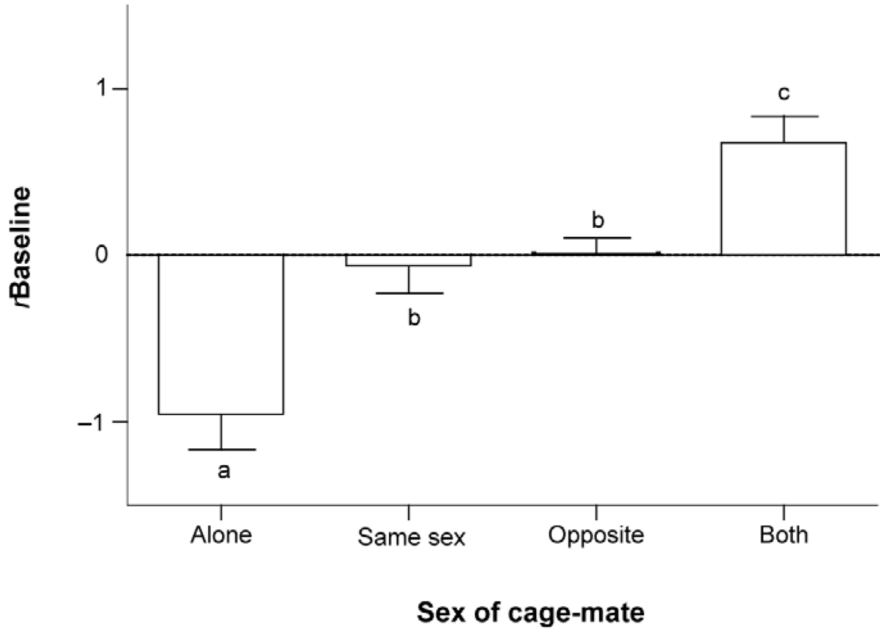
Table 1 List of husbandry variables.

Variable	Distribution	Initial trend	Final GLM results
Sex of cage-mates	Same sex 9 Opposite sex 22 Both 8 (Alone 6)	Alone < Same < Opposite < Both	$F_{3,36}$ = 10.51 P < 0.001
Total area	2,500 feet^2 (150–15,000)	Negative	β = –0.0001 $F_{1,36}$ = 19.58, P < 0.001
Number of hiding spots in exhibit	4.5 hiding spots (1–7)	Negative	β = –0.11 $F_{1,36}$ = 6.72, P = 0.014
Closest possible visitor proximity to lynx	4.9 feet (0–15)	Negative	β = 0.05 $F_{1,36}$ = 3.67 P = 0.063
Total number of keepers	3.2 keepers (2–6)	Negative	β = –0.10 $F_{1,36}$ = 3.04, P = 0.089
Number of cage-mates	1.3 cage-mates (0–3)	Positive	
Area per lynx	1,000 feet^2 per lynx (150–7,500)	Negative	
Highest perch in exhibit	10 feet (3–17)	Negative	
Number of dens in exhibit	2 dens (0–4)	Positive	
Number of sides of the enclosure that visitors can view lynx	2 sides (1–4)	Positive	
Relationship to cage-mates	Unrelated 27 Sibling 12	Sibling < Unrelated	
Birth location	Captivity 36 Wild 9	Wild < Captivity	
Primary component of diet	Chicken 22 Red meat 19 Dry catfood 2 Mix (fish) 2	Mix < Red meat < Dry < Chicken	
Number of dens per lynx	0.9 dens per lynx (0–2)	None	
Keeper time (average number of hours per week that keepers spend with lynx)	4.3 h per week (0.8–15)	None	
Enrichment schedule	3.3 days per week (0–7)	None	
Rearing	Hand 26 Mother 10 Unknown 9	None	
Keeper interaction	Hands-off 0 Moderate 31 Hands-on 14	None	
Most ecologically threatening nearest neighbour	Large carnivore 29 Meso-carnivore 9 Herbivore 7	None	
Shifted between exhibit and holding	Never 33 Daily 12	None	
Status of cage-mates	Intact 30 Neutered 6 Both 3	None	

The distribution is given for each variable. For continuous variables, we report the mean first and the total range in parentheses; for categorical variables, we report the frequency for each treatment level. We ran all possible one- and two-way ANOVAs to screen for potentially influential variables. For factors that emerged as influential, we describe the general effect the variable had on FGM values ('Initial trend'). These 13 variables were then used to construct a general linear model using a backward step-wise procedure to remove non-significant factors (P > 0.1). For the 5 factors remaining in the final model, we report the slope (β), test statistic, and P-value.

Figure 1



Effect of cage-mate sex on *r*Baseline values (residuals for baseline FGM concentration after controlling for sex, reproductive status, and age class). Bars represent least square means (± 1 SE). Superscripts indicate statistically significant differences at $P < 0.05$ between groups.

Table 2 Occurrence of FGM peaks in relation to events recorded by keepers.

Category	Specific events	Number of times event was noted	Number of FGM peaks	
			Females	Males
Visitor activity	Special events, programmes, tours, zoo/exhibit opening	54	1	2
Loud noises	Construction, logging, fireworks, thunderstorms	19	1	2
Mating	As implied	10	0	3
Conspecific interaction	Fighting, being mounted by same-sex cage-mate	7	2	1
Training	Working with squeeze cage	6	0	0
Transfer/move	Moved to new enclosure or institution	3	0	2
Medical	Darting, exam	2	0	1
Unknown		n/a	27	11

and number of hiding locations. Two variables had marginally significant effects: (i) the closest possible proximity of visitors to the enclosure; and (ii) the total number of keepers caring for a given lynx at a given institution.

Correlates of FGM peaks

In general, there was very little agreement between events that keepers perceived as stressful, based on notes, and events that lynx perceived as stressful (based on the occurrence of FGM peaks; Table 2). The majority (~70%) of the FGM peaks were not associated with any recorded event. The stressor with the highest occurrence of FGM peaks was movement to a new enclosure or institution (peaks observed in two out of three cases; Figure 2). There were no events that were consistently associated with peaks. Even amongst cage-mates, a given stimulus often elicited an adrenocortical response in only one individual. There was a higher proportion of unexplained peaks in females than in males.

Discussion

Understanding the factors that contribute to the well-being of animals in captivity is a complex task. There is no simple measure of well-being, and there are numerous facets of captive animal management to consider. Furthermore, species and even individuals may vary in their response to particular husbandry practices (Wielebnowski *et al* 2002; Christofoletti *et al* 2010; Vick *et al* 2011). Rigorous experimental studies with a completely balanced design are difficult with exotic species. Therefore, we must adopt other approaches to learn about animal well-being. In this study,

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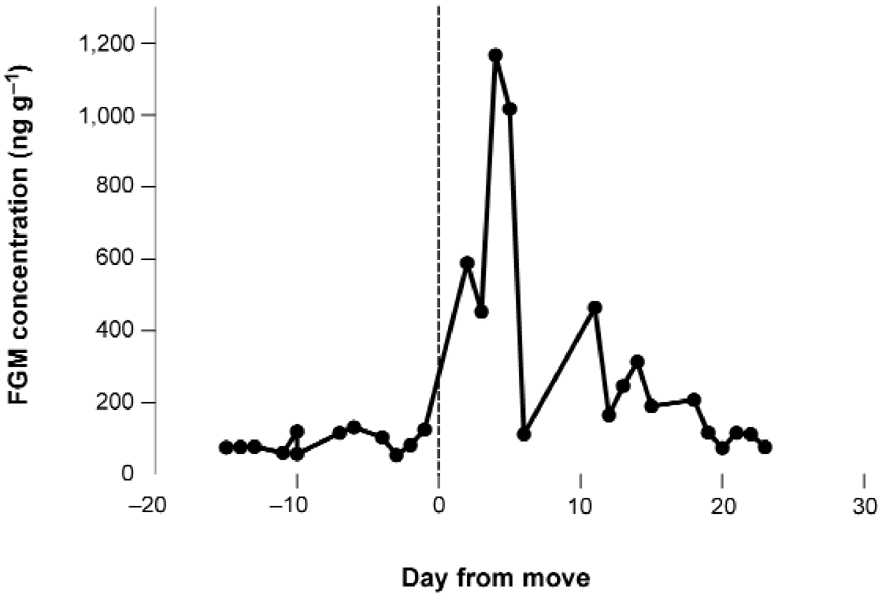
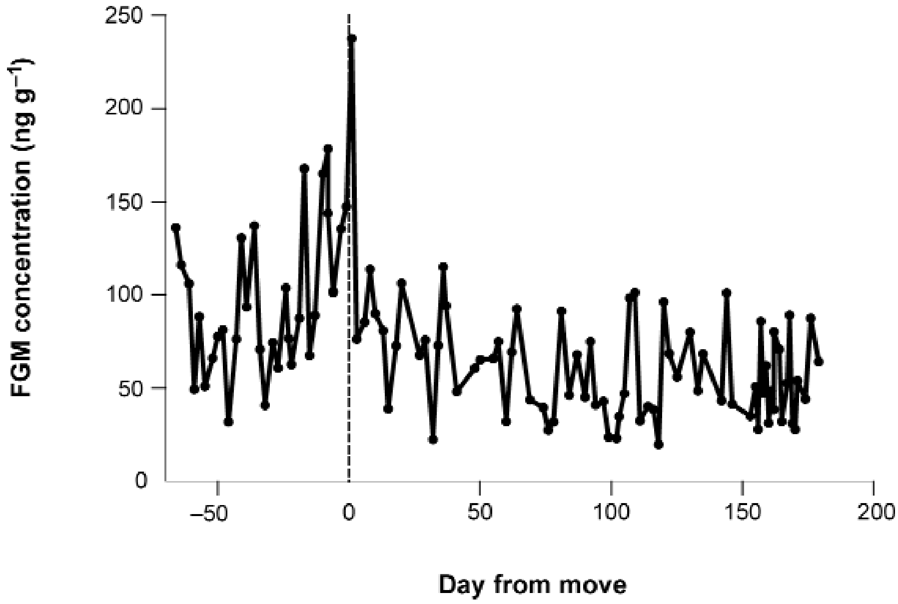
Figure 2



Longitudinal FGM profile for a male that was transferred between two institutions that were approximately 450 miles apart.

Figure 3



Longitudinal FGM profile for a male that was moved to a new, larger enclosure at the same institution. Mean FGM concentrations fell by nearly half following the move (before: 97.5 [± 38.6] ng g^{-1}; after: 57.9 [± 25.8] ng g^{-1}).

we examined the effect of management practices on Canada lynx well-being using the quantification of faecal glucocorticoid metabolites (FGM) as an index of animal condition.

We identified three factors that were strongly correlated with adrenocortical activity: (i) total area of enclosure; (ii) sex of cage-mates; and (iii) number of hiding locations. During preliminary data investigations, we considered different permutations of our analysis. Although the specific variables remaining in the final model varied slightly, the general conclusions remained consistent. For example, some models identified 'total area of enclosure' as being significant, whereas other models identified 'area per lynx'. Either way, it highlights the fact that space is important. Both graphical and statistical analyses repeatedly indicated that three factors are important predictors of adrenocortical activity in Canada lynx: space, hiding locations and social environment.

An individual's perception of control in an environment or situation is important to their well-being (Sapolsky 2002), and captivity inherently reduces an animal's control over its environment (Morgan & Tromborg 2007). However, by increasing the size of the enclosure and/or the number of hiding locations, lynx may have more options regarding how to spend their time and thus may perceive a greater degree of control. During the study, one male was moved to a larger enclosure at the same institution and FGM concentrations decreased notably following the move (Figure 3). Usable space and environmental complexity have been shown to be critical determinants of well-being in a number of studies (eg Wielebnowski *et al* 2002; Li *et al* 2007; Ross *et al* 2011). Our findings reinforce the idea that enclosures should be designed to allow animals to engage in their full range of natural behaviours and provide choices whenever possible.

Group size is also an important determinant of the well-being of captive animals (Price & Stoinski 2007). Species-inappropriate group sizes can have severe repercussions for the health and reproductive success of the animals. For example, it has been documented in felids that larger group sizes are associated with poor reproductive success (Mellen 1991). Our findings reveal that for Canada lynx, adrenocortical activity is not simply affected by group size, but more specifically by the sex-composition of the group. Lynx housed alone had the lowest levels of adrenocortical activity, and we suggest that this may be the optimal housing arrangement for lynx. This agrees with the solitary nature of Canada lynx in the wild (Ruggiero *et al* 2000), and with findings for other felids (Mellen 1991). Conversely, mixed-sex groups of three or more were associated with the highest levels of adrenocortical activity, and are not recommended.

There were two factors that were included in the final GLM model, but were only marginally significant ($P > 0.05$) and exhibited a somewhat counter-intuitive relationship with FGM expression. The first factor was 'total number of keepers,' which was negatively associated with adrenocortical activity, in contrast to other studies. High quality keeper-animal relationships (specifically, fewer keepers but more time spent with the animal) have been associated with lower levels of adrenal activity and higher reproductive success in several felids (Mellen 1991; Wielebnowski *et al* 2002). The second factor was 'closest possible visitor proximity to lynx'. Although this variable showed the predicted negative relationship in initial screening, once we controlled for other housing factors the relationship became positive (Table 1). In other words, there was a trend toward lower FGM values when visitors were allowed to get closer to the enclosure. It is likely that both variables were confounded with other institutional factors. For example, the number of keepers may be inversely related to keeper workload, and thus the amount of time keepers spend with the lynx. To conclusively determine the effect of these factors on lynx well-being, further investigation is required.

We found that several of the potentially stressful events that keepers reported were not associated with FGM peaks, and *vice versa*. The stressors that were most commonly reported by keepers were changes in visitor activity — special events, evening programmes, opening for the season (for zoos that close in winter), etc. However, we found very few FGM peaks associated with such events. A keeper's generally anthropocentric perception of the environment is likely to be very different than that of an animal. Furthermore, keepers are very busy and only spend a small portion of the day with the animal. Therefore, it is quite likely that they may be unaware of stressors that occur when they are not present. Another reason for the discrepancy between reported stressors and FGM peaks is that the peak may have occurred in a sample that was not collected.

The strongest association between reported stressors and observed subsequent peaks in adrenocortical activity (FGM) was when animals were moved to a new enclosure or institution. For example, in one male lynx that was moved between institutions, FGM values increased ~ten-fold following the transfer and reached the highest peak five days post-transport (Figure 2). It has been documented in other felids that transport and introduction to novel environments results in a significant, though relatively short-lived, increase in GC expression (Carlstead *et al* 1992, 1993; Dembiec *et al* 2004). Furthermore, this GC increase is associated with changes in behaviour, such as reduced activity and increased hiding (Carlstead *et al* 1993; Dembiec *et al* 2004).

Animal welfare implications and conclusion

Here, we provide an exploratory analysis of the relationship between husbandry factors and adrenocortical activity in captive Canada lynx. Our analyses highlighted three factors that are significantly associated with adrenocortical activity in Canada lynx: space, places to hide, and social environment. Based on our findings, we recommend that lynx should generally be housed alone (unless with dependant offspring, or temporarily paired up for mating purposes) and should be provided with several hiding locations. Although space is an issue for all zoos, bigger enclosures are likely better for lynx well-being.

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