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Patterns of testicular activity in captive and wild Canada lynx (*Lynx canadensis*)

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ABSTRACT

Canada lynx are listed as a threatened species in the contiguous US. Understanding the reproductive characteristics (i.e., mating system, behavior, physiology) of a species is useful for ensuring effective *in situ* and *ex situ* management plans. The goal of this study was to describe patterns of androgen expression in both captive and wild male Canada lynx using fecal hormone metabolite analysis. Among captive lynx, juvenile and castrated males had lower concentrations of fecal androgens (fA) than intact males, thereby demonstrating that the assay detects biologically meaningful differences in testicular activity. We found that captive males in general had much higher fA levels than wild males. All males showed strong seasonal variation in fA concentrations, with significantly higher levels being expressed during the breeding season (February and March) than during the non-breeding season. Among captive males, variation in seasonal fA levels did not correlate with latitude. Finally, males housed with intact cage-mates (either male or female) had significantly higher fA levels than males housed alone or with a neutered cage-mate.

1. Introduction

Canada lynx (*Lynx canadensis*) are the primary felid species inhabiting North America's boreal forest. They are meso-carnivores (9–11 kg) and feed primarily on snowshoe hares and squirrels (Nowak, 1999). In the northern part of their range, their dependence on snowshoe hares is so strong that their population size fluctuates with snowshoe hare density in 10-year cycles (Poole, 2003; Ruggiero et al., 2000). Lynx are primarily found in coniferous forests, particularly those with a mixture of forest age classes (Poole, 2003). Lynx are solitary, but there is some overlap of home ranges between males and females (Ruggiero et al., 2000). Southern populations of lynx have declined dramatically in the last century, and in 2000, they were listed as a threatened species by the US Fish and Wildlife Service (USFWS, 2000).

One poorly understood aspect of lynx biology is their reproductive physiology. Lynx reproduction fluctuates dramatically throughout the year, and also throughout their 10-year population cycle in northern populations (Poole, 2003). It is unclear whether these breeding restrictions are mediated solely by changes in female physiology, or whether males also exhibit physiological changes that may impact reproduction. Developing a better understanding of male reproductive physiology in Canada lynx may provide insights into how animals adapt to arctic environments, and may aid *in situ* and *ex situ* conservation efforts.

This study integrates information from both captive and wild lynx populations to establish basic knowledge about the reproductive physiology of male Canada lynx. We used the non-invasive technique of fecal hormone analysis to establish normative patterns of androgen expression in male lynx. Specifically, our objectives were to (1) validate an assay for monitoring testicular activity non-invasively, (2) characterize normative patterns of testicular activity, particularly around the breeding season, (3) compare patterns of hormone expression between captive and wild lynx, and (4) examine the effect of breeding status and housing situation on levels of fecal androgen metabolites (fA) in captive animals. This study provides the first longitudinal data on androgen levels in wild and captive Canada lynx. As such, it serves as an important foundation for future studies and the development of more effective management plans.

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2. Methods

2.1. Animals and fecal sample collection

2.1.1. Captive

This study included 28 captive Canada lynx males from 19 institutions (3 juveniles, 7 castrated males, and 18 intact, sexually mature males). All lynx were housed outdoors at least 50% of the time, and thus were exposed to natural photoperiod rhythms. Animal care staff collected fecal samples 2–4 times per week during routine cage cleanings. Most samples were collected during the breeding season (February–April), but sampling extended into other parts of the year for several individuals. When multiple lynx were housed together, icing dye (Wilton Industries, Wilton, IL) was delivered to each individual in a food treat, and then color was used to distinguish between feces.

2.1.2. Wild

Fecal samples were collected from wild adult males in Colorado (44 individual lynx, 339 samples), Maine (7 lynx, 51 samples), and Montana (9 lynx, 39 samples). The populations in Maine and Montana are naturally-occurring, while the population in Colorado has been reintroduced. All samples were collected by snow-tracking radio-collared lynx between December and April, a time period which includes their breeding season. Samples were collected from 1999 to 2008. Samples were generally collected within 24 h after defecation, and evidence from field experiments suggests that fA metabolites remain stable in winter field conditions for at least four days (Fanson, 2009). Therefore, we are confident that we were able to obtain meaningful results from samples collected in the field. To ensure that fA expression in reintroduced lynx was not affected by the translocation, we only included samples that had been collected at least six months post-release.

2.2. Steroid extraction and analysis

All samples were stored in zip-lock bags at −20 °C and shipped on ice to the Brookfield Zoo for processing and hormone metabolite analysis. We extracted steroid metabolites by adding 5 ml of 80% ethanol to 0.5 g of well mixed, wet fecal material in polypropylene tubes. Capped tubes were placed on a rotator overnight and then centrifuged for 15 min at 1500 rpm. One ml of supernatant was transferred to a new polypropylene tube and diluted with 1 ml assay buffer (0.1 M phosphate-buffered saline (PBS) containing 1% BSA, pH 7.0). Extracts were stored at -20 °C.

Fecal androgen metabolites (fA) were quantified using a single-antibody enzyme-immunoassay (EIA). Testosterone polyclonal antibody R156/7 and the corresponding horseradish peroxidase (HRP) conjugate were obtained from C. Munro (University of California, Davis, CA). The testosterone antibody had the following cross-reactivities: 100% testosterone, 57% 5α-DHT, 0.3% androstenedione, and < 0.1% androsterone, DHEA, cholesterol, 17β-estradiol, progesterone and pregnenolone (deCatanzaro et al., 2003; Dloniak et al., 2004). Assay sensitivity was 0.039 ng/well.

Assay procedures were similar to those previously described (Atsalis et al., 2004; deCatanzaro et al., 2003). Briefly, 96-well microtitre plates (Nunc maxisorp) were coated with 50 μl of testosterone antibody solution (1:20,000) and incubated overnight at 4 °C. Plates were washed to remove unbound antibody. Immediately after being washed, 100 μl of standard, control, or diluted fecal sample extract and 50 μl of HRP–testosterone conjugate were added to each well. After incubating for 2 h at room temperature, plates were washed and 100 μl of substrate solution (1.6 mM hydrogen peroxide, 0.4 mM azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) in 0.05 M citrate buffer, pH 4.0) was added to each well. Plates were read when the optical density of the maximum binding wells was >0.6 using a single filter at 405 nm using an optical density plate reader (Dynex MRX Revelation, Dynex Technologies, Chantilly, VA). All samples were assayed in duplicate, and data are expressed as ng/g wet fecal weight.

The assay was biochemically validated for Canada lynx by demonstrating (1) parallelism between serially diluted extracts and the standard curve, and (2) significant (>80%) recovery of exogenous testosterone added to fecal extracts. To monitor precision and reproducibility, low (65% binding) and high (30% binding) quality control samples were run on each plate. Intra-assay coefficients of variation were 9.5% and 7.9% ($n = 14$) for low and high controls, respectively. The inter-assay coefficients of variation were 20.2% and 22.4% ($n = 97$), respectively. Although this degree of inter-assay variation is relatively high due to the extended duration of the study, the amount of inter-assay variation for a given individual was minimized by analyzing all of their samples at the same time, whenever feasible.

2.3. Statistical analysis

All data were analyzed using SAS 9.1 (Cary, NC) and hormone data were log transformed to meet assumptions of normality and homoscedasticity. Means provided in the text and figures are least-squares means that have been back-transformed, unless otherwise noted. One captive male (M13) showed unusually low fA values (similar to values observed in juveniles), even though he was an intact adult housed with an intact female. Therefore, he was excluded from all analyses except seasonality, in which he was included because his general pattern of fA expression was similar to other intact males.

2.3.1. Status

To assess the biological relevance of the assay, overall mean fA values were compared between juvenile ($n = 3$), castrated ($n = 7$), and intact, sexually mature ($n = 17$) males using a one-way ANOVA. Only captive males were included, since nearly all wild males were adults. Because two of the juveniles were housed together and their samples were indistinguishable, they were combined for statistical analysis. A Tukey–Kramer adjustment was used to correct for multiple pairwise comparisons.

2.3.2. Seasonality

We conducted a repeated measures ANOVA to test the effect of month on fA values for both captive and wild populations. The model also controlled for the effect of year (sample collection from wild lynx spanned 10 years; captive collection only spanned 4 years). Only intact, sexually mature males were included.

To test for differences between breeding and non-breeding seasons in captive males, we performed a post hoc linear contrast of monthly means. (This comparison was not possible for wild lynx due to the limited window of sample collection.) For this analysis, we compared the average fA concentrations during the breeding season (February and March) to the average of four non-breeding months for which we had the most data (May–August).

For the captive males, we also ran a simple regression to test the effect of latitude on the timing of peak fA values. Since reproductive seasonality in many temperate felids is influenced by photoperiod (Brown et al., 2002), we were interested if patterns of fA expression varied by latitude. This test included 13 males with the most complete fA profiles. Each individual's mean weekly fA values were used to determine when their maximum fA occurred (weeks were numbered 1–52, with week 1 starting January 1 of the year sampling occurred).

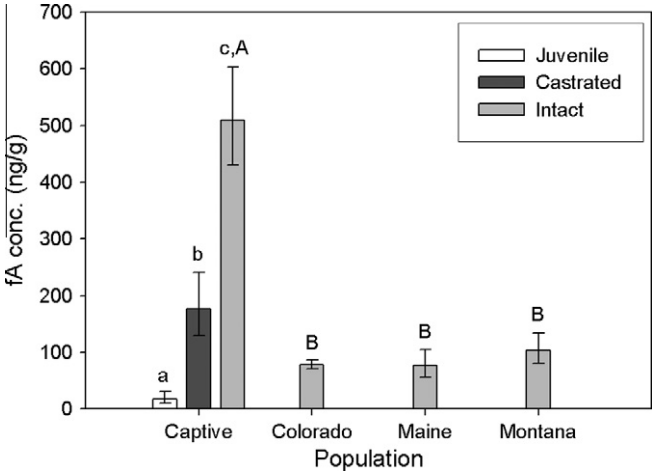

Fig. 1. Population and status differences in fA concentrations in male Canada lynx (back-transformed LS mean ± SE). Letters indicate statistically significant differences ($P < 0.05$): lower-case letters pertain to status comparisons within the captive population, and upper-case letters apply to comparisons of intact males across populations.

2.3.3. Population and age

We tested the effect of population and age using an ANCOVA. Mean age was calculated for each individual, and only intact, adult ($\geqslant$2 years old) males were included in this analysis. Captive lynx were treated as a single population, and the three wild populations (Colorado, Maine, and Montana) were considered separate populations. Captive males ranged in age from 3–18 years old, and wild males ranged from 2–12 years old. Two-way interactions were excluded if they were not significant. A Tukey–Kramer adjustment was used to correct for multiple pairwise comparisons between populations.

Unlike captive lynx, we had data spanning multiple years for several wild individuals ($n = 15$, difference in age = 2–5 years). Since all samples came from radio-collared lynx, age was estimated when the radio-collar was put on the male. In some cases, the male was trapped as a kitten so the year of birth was known, and in other cases, a more precise age estimate was obtained from tooth enamel (collected post-mortem). To see if there was any effect of age on fA within an individual, we ran a one-sample t-test using the difference in fA concentrations between the youngest and oldest age points for an individual as the response variable.

2.3.4. Past breeding success and housing situation

Keepers at each captive institution were asked to provide information about each male's past breeding success and housing situation. Past breeding success was scored as either proven breeder (has sired at least one litter), non-breeder (has been housed with a suitable mate for at least 2 years, but has not sired a litter), or unsure (not housed with suitable mate for at least 2 years). Information about housing included number, sex, and status (intact or neutered) of cage-mates.

Using mean fA levels as the response variable, we initially ran an ANOVA model with intact males housed with a cage-mate. Because of the limited number of housing situations, it was not possible to include interactions in the model. Neither the number of cage-mates, nor the sex of the cage-mate(s) had a significant effect on baseline fA levels ($P > 0.4$), so these variables were removed from the model. For the final model, we created a variable called 'housing situation' with three levels (housed alone, with an intact cage-mate, or with a spayed/castrated cage-mate). The final ANOVA included breeding success and housing situation. A Tukey–Kramer adjustment was used to correct for multiple comparisons between housing situations.

3. Results

3.1. Status

Male status had a significant effect on fA expression ($F_{2,29} = 17.17$, $P < 0.001$; Fig. 1). Both juveniles and castrated males had significantly lower mean fA values than intact adult males ($t_{29} = 5.50$, $P < 0.001$ and $t_{29} = 2.77$, $P = 0.03$, respectively). Interestingly, juveniles also had significantly lower fA concentrations than castrated males ($t_{29} = 3.56$, $P = 0.004$).

3.2. Seasonality

For both populations, month strongly affected fA expression in intact male lynx (captive: $F_{11,69} = 3.87$, $P < 0.001$; wild: $F_{4,128} = 3.52$, $P = 0.01$; Fig. 2). There were also significant differences in fA concentrations among years for wild lynx ($F_{9,121} = 2.84$, $P =$

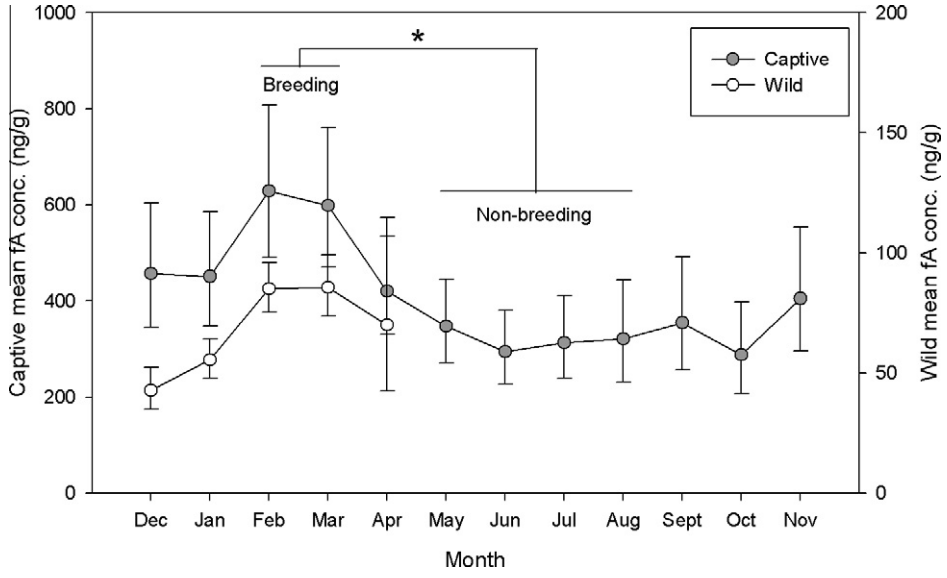

Fig. 2. Seasonal patterns of androgen expression in captive and wild male lynx (back-transformed LS mean ± SE). Asterisk indicates a statistically significant difference between breeding and non-breeding seasons for captive males. Note that different *y*-axis scales are used for wild and captive populations.

0.004), but not for captive lynx ($F_{3,48.6} = 0.07$, $P = 0.97$). There was no obvious trend to the changes in fA between years.

In the captive population, fA concentrations were significantly higher during the breeding season than the non-breeding season (breeding – February/March: mean = 520.25 ng/g, 95%CI = 331.96–815.42; non-breeding – May–August: mean = 329.18 ng/g, 95%CI = 212.85–509.08; $t_{78.2} = 4.72$, $P < 0.001$). While this comparison was not possible for wild lynx, the monthly trends are similar, with the highest fA concentrations in February and March. Latitude did not have a significant effect on the timing of an individual's maximum weekly fA in captive lynx ($F_{1,11} = 0.03$, $P = 0.88$).

3.3. Population

Fecal androgen levels differed significantly between populations ($F_{3,65} = 28.92$, $P < 0.001$). The captive population had significantly higher fA values than all three wild populations (CO: $t_{65} = 9.27$, $P < 0.001$; ME: $t_{65} = 5.14$, $P < 0.001$; MT: $t_{65} = 5.05$, $P < 0.001$), but the wild populations were not significantly different from each other ($P > 0.7$; Fig. 1).

3.4. Age

Age did not have a significant effect on fA concentration in adult, intact males ($\beta = 0.01 \pm 0.03$ (ln(ng/g))/year of age, $F_{1,65} = 0.12$, $P = 0.73$; Fig. 3). Although not statistically significant, the two populations exhibited opposite trends for the effect of age: in captive males, there was a positive relationship between age and fA, while in wild males, it was negative. For wild males, the one-sample t-test, which examined individual-level changes in fA with age, also indicated there was not a significant relationship between fA concentration and age ($t_{14} = 0.12$, $P = 0.91$, $n = 15$).

3.5. Past breeding success and housing situation

Past breeding success did not have a significant effect on mean fA levels in intact males ($F_{2,13} = 0.29$, $P = 0.75$). However, housing condition did significantly affect mean fA levels ($F_{2,13} = 7.98$, $P = 0.006$; Fig. 4). Males housed with intact cage-mates, either male or female, had significantly higher fA levels than males housed alone ($t_{13} = 2.77$, $P = 0.04$) or with neutered cage-mates ($t_{13} = 3.16$, $P = 0.02$). Mean fA levels were not significantly different between intact males housed alone and males housed with a neutered cage-mate ($t_{13} = 0.49$, $P = 0.88$). Since the initial model revealed that sex of the cage-mate did not have a significant effect on fA levels, it was not included in the final model.

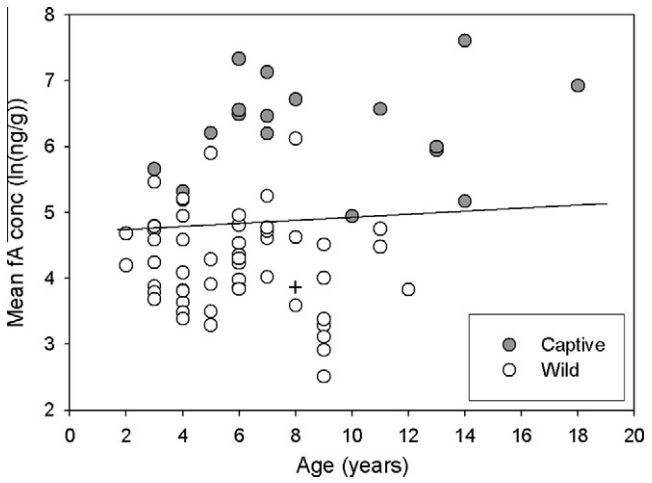

Fig. 3. Effect of age on fA concentration in intact Canada lynx. The "+" represents the excluded male (M13, see text for discussion). Note that the *y*-axis is in log-scale.

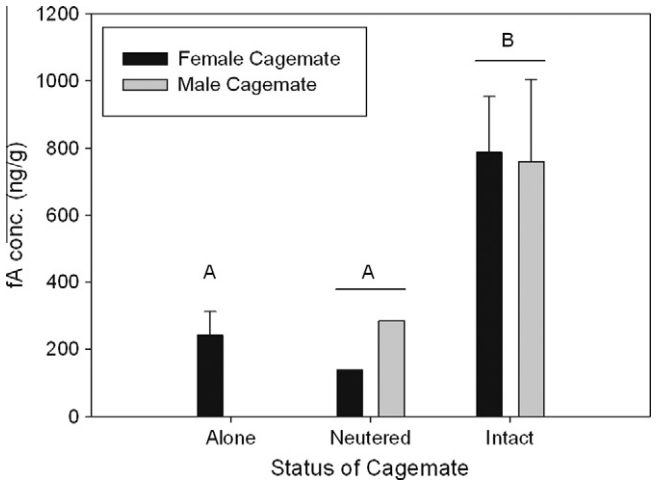

Fig. 4. fA concentrations in intact male lynx housed alone, with a neutered cage-mate, or with an intact cage-mate (mean ± SE). Letters indicate statistically significant differences ($P < 0.05$). The 'neutered cage-mate' bars do not have error bars because there was only one of each sex.

4. Discussion

Although fecal hormone analysis is becoming increasingly common and has been applied to several felid species, studies of *Lynx* species are just starting to emerge. In this study, we successfully validated an EIA for monitoring testicular activity in Canada lynx. Using this technique, we were able to provide valuable information about normative patterns of androgen expression in wild and captive Canada lynx.

The testosterone assay successfully detected biologically relevant differences in testicular activity, as indicated by the fact that intact, reproductively mature males had significantly higher fA levels than castrated or juvenile males. However, there were still detectable levels of fA in juvenile and castrated males. This suggests that some of the androgen metabolites detected by the assay may have been of adrenal origin. While androgens are primarily secreted by the testes, they are also secreted by other glands, such as the adrenals (Meikle et al., 1991). Prior to excretion, steroid hormones undergo extensive metabolism by the liver and intestinal bacteria (Palme, 2005). Therefore, it is the steroid hormone *metabolites* rather than parent hormone molecules that are being measured in the feces. In fact, studies on other felids have revealed that very little testosterone is excreted in its native form (Brown et al., 1996; Jewgenow et al., 2006b). It was beyond the scope of this study to determine whether some immunoreactive androgen metabolites may indeed have been of adrenal origin. Nonetheless, we are confident, based on the overall results, that our assay primarily measured testicular activity.

Male lynx show strong seasonal changes in fA concentrations. Fecal androgens increased dramatically in February and remained high in March, which corresponds to the approximate breeding period for lynx. Androgens can influence both physiological and behavioral aspects of reproduction; however, androgen expression does not always correlate with other reproductive parameters (Brown et al., 1996). In some felids, testosterone expression varies by season, but sperm production and quality remain constant (Byers et al., 1990; Wildt et al., 1986). Conversely, in other species, increases in androgen expression are associated with increases in testicular volume and increases in seminal quality 1–2 months later [Pallas' cats (Brown et al., 1996; Newell-Fugate et al., 2007);

Eurasian lynx (Göritz et al., 2006; Jewgenow et al., 2006a); snow leopards (Johnston et al., 1994); ocelots, tigrinas, margays (Morais et al., 2002)]. Based on North American Canada lynx studbook records (Goff, 2008), females conceive around mid-March. If fA expression is related to other reproductive parameters in lynx, then the increase in fA concentrations in February may result in higher sperm quality by the time the female becomes receptive.

Many felid species exhibit some degree of reproductive seasonality (Brown, 2006). Male felids often exhibit some variation in at least one reproductive parameter (testosterone expression, testicular volume, sperm quantity or quality) throughout the year [domestic cats (Blottner and Jewgenow, 2007); jaguars (Morato et al., 2004); ocelots, tigrinas, margays (Morais et al., 2002); leopards (van Dorsser and Strick, 2005); clouded leopards (Wildt et al., 1986)]. However, in many felid species, these fluctuations do not preclude successful breeding throughout the year, and are much less tightly regulated than changes in females (Spindler and Wildt, 1999). From an evolutionary perspective, if even a few females become receptive outside the breeding season, the benefit to males of maintaining reproductive functionality throughout the year may outweigh any costs. However, in species inhabiting more northerly regions with harsh winters, female receptivity may be so tightly restricted that it is not beneficial for males to maintain reproductive function outside the breeding season. This is supported by the fact that seasonal changes in male reproductive physiology are much more pronounced in more temperate species [Pallas' cats (Brown et al., 1996; Newell-Fugate et al., 2007); Eurasian lynx (Göritz et al., 2006; Jewgenow et al., 2006a; Jewgenow et al., 2006b); snow leopards (Johnston et al., 1994)].

Lynx species have the shortest breeding seasons among felids (Jewgenow et al., 2006b; Tumlison, 1987). Pronounced male seasonality has been documented in all four lynx species [bobcats, *L. rufus* (Crowe 1975); Eurasian lynx, *L. lynx* (Göritz et al. 2006; Jewgenow et al. 2006a; Jewgenow et al. 2006b); Iberian lynx, *L. pardinus* (Jewgenow et al. 2006b)]. Harsh winter conditions, which are associated with food shortages and other environmental constraints, may prevent females from becoming receptive outside of the breeding season, and contribute to the seasonality in males. However, there is one interesting caveat. In Eurasian lynx, if a female loses a litter, she can begin cycling again in June or July (Göritz et al., 2006; Jewgenow et al., 2006a). Correspondingly, male Eurasian lynx have a second increase in testosterone expression and testicular size in May and June (Göritz et al., 2006; Jewgenow et al., 2006a). There is some anecdotal evidence that female Canada lynx can also give birth in August, which means there would be a second period of estrous in May or June (J. Vashon and J. Tremblay, pers. comm.). However, we did not see a second increase in fecal androgens in May or June for any of the males in the study. The frequency of this "second estrous" in females is not known for either species. However, given the differences in androgen expression between male Eurasian lynx and male Canada lynx, we can speculate that a second estrous is more common in Eurasian lynx.

In many temperate felids, reproductive seasonality is influenced by photoperiod (Brown et al., 2002). However, we did not find any evidence that seasonal patterns of fA expression (specifically, the timing of peak fA concentrations) were influenced by latitude. The latitudes at which the captive males in this study were held represent a change in photoperiod (i.e., change in day length between summer and winter solstice) from ~4.5 h for the southernmost male, to ~8 h for the northernmost male. While this is still much less than the 16+ h change in photoperiod experienced by lynx in the northern part of their range, it nonetheless represents a fairly wide range. If peak fA was regulated by photoperiod in lynx, we would have expected to see some effect of latitude. There are two, non-mutually exclusive explanations for why we did not observe an effect of latitude. First, it is possible that photoperiod may affect a component of androgen expression that we were not able to characterize well (e.g., onset or duration of elevated androgen expression). Alternatively, androgen levels may peak in response to different cues, such as female receptivity. Other mammalian males are known to exhibit peak androgen levels when they are exposed to a female or when they copulate (Dloniak et al., 2006; Hesterman et al., 2005; Kretzschmar et al., 2004). In such cases, changes in androgen expression are a response to (rather than preparation for) a breeding opportunity/situation. Dloniak et al. (2006) speculated that when breeding opportunities are unpredictable, this endocrine response may help synchronize male and female behavior/physiology. Given that lynx are solitary and can be widely dispersed, this could be a possible mechanism for ensuring successful breeding encounters. Males in this study were permanently housed with their cage-mates, so the peak in fA would not have been triggered by the presence of the female alone. We only received notes about breeding activity for three males. Peak fA values for one of the males did coincide with mating activity. However, for the other two males, peak fA levels occurred 1–2 weeks after mating was recorded.

While there was no correlation between latitude and peak fA timing, there was much more variation in the timing of peak fA values in the south than in the north (low latitudes 34–36°: $n = 6$, range = 11 weeks; middle latitudes 39–42°: $n = 5$, range = 5 weeks; high latitudes 47–50°: $n = 2$, range = 2 weeks). Due to small sample sizes at northern latitudes, we were not able to analyze this statistically. However, the observed trend may suggest that the breeding season does not shift across latitudes; rather, it may be more constricted at northern latitudes.

A unique aspect of this study is our ability to compare fecal hormone concentrations between wild and captive populations. We found that captive males had dramatically higher (~7× greater) fA levels than wild males. For the few other studies that have considered this comparison, results are quite variable depending on the species and the hormone (Fujita et al., 2001; Terio et al., 2004; Ziegler et al., 1997). In contrast to our results, Terio et al. (2004) found that fA concentrations were lower in captive cheetahs than wild cheetahs. However, they also found that fecal corticoids were higher in captive cheetahs, and this, in conjunction with other physiological data obtained during medical exams, suggested that captive cheetahs experienced chronic stress that resulted in suppression of testicular activity. In a study of spotted hyenas, there was no notable difference in fA concentrations between captive and wild populations (S. Dloniak, pers. comm.). We suspect that this difference is not related to differences in gonadal activity, but may be explained by other factors.

In female Canada lynx, we also found that both fecal estrogens and progestagens were significantly higher in captive lynx (Fanson et al., this issue). Similarly, for both sexes, fecal glucocorticoids were also higher in captive lynx than in wild lynx (Fanson, 2009). This suggests that the mechanism underlying the observed population difference is neither hormone nor gender specific. Furthermore, as mentioned previously, one of the captive males (M13) was excluded from most analyses because his fA levels were very low compared to the other captive males (see Fig. 3). It is unlikely that his low fA values were caused by testicular dysfunction, because (1) he exhibited normative seasonal changes in fA expression, (2) his fA values fell within the range of healthy wild male fA values, and (3) in other felids, testicular dysfunction is associated with unusually high serum testosterone values, rather than low values (Wildt et al., 1986). M13 was one of our northernmost males, so he would be exposed to similar climatic conditions as wild lynx. He also had the largest enclosure of all males (4000 m^2 larger than the second largest enclosure), so his activity levels/energetic demands may be greater than other captive males.

One possible explanation for this population difference is that it is due to differences in diet, metabolic rate, and/or body condition – all factors which can affect steroid production and metabolism (von der Ohe and Servheen, 2002; Wielebnowski and Watters, 2007). Individuals in captivity generally have more regular access to food, lower energy expenditure, and greater mass than wild lynx (11–20 kg for captive lynx vs. 7–11 kg for wild lynx). In humans, obesity is associated with changes in steroid production and binding globulin concentrations (Hajamor et al., 2003). This includes not only glucocorticoids, which have obvious connections to energy regulation, but also sex hormones, which can impact metabolism, adipose tissue, and energy regulation (Tchernof et al., 1996). Essentially, there is a complex network of interactions between glucocorticoids, sex steroids, and metabolic hormones (e.g., leptin and insulin), and perturbations to this network may have cascading impacts on other physiological systems.

A second, though not mutually exclusive explanation for elevated fA levels in captive males, is that physiological or psychological stressors associated with captivity may cause generalized adrenal stimulation. As mentioned, we observed higher fecal glucocorticoid levels in captive lynx, and adrenal glands are capable of producing androgens (Meikle et al., 1991). While elevated fA concentrations may reflect greater levels of adrenal activity in captive males, it is unclear whether this degree of adrenal stimulation is pathological. It is interesting to note that, with the one exception of M13, elevation of fA levels in captive males was very consistent and did not seem to be affected by institution type (e.g., public, private, encounter programs) or management practices (e.g., enrichment, human interaction, etc.). If specific aspects of captive management function as stressors, one would expect a larger spread in the variation of fA levels of captive males, and more of a continuum or gradation in fA levels between wild and captive males. Instead, we observed a fairly bi-phasic split between the two populations. Because steroid over-production is associated with metabolic dysfunction and other pathologies, it is important to develop a better understanding of the significance and potential implications of elevated steroid hormone levels in captive lynx.

Housing situation was another factor that had a significant effect on baseline fA levels in captive males. Males housed with intact cage-mates had higher fA levels than males housed alone or with castrated cage-mates, regardless of whether the cage-mate was male or female (Fig. 4). Androgens, which mediate both reproductive and aggressive behaviors, may be notably modulated by social environment (Hirschenhauser and Oliveira, 2006). Therefore, both the presence of intact males (potential reproductive competitors) and intact females (potential mates) may cause androgen levels to be elevated. Interestingly, the data indicate that lynx can discern between intact and neutered individuals. This suggests that lynx use more than just the physical presence or absence of conspecifics to assess their sociosexual situation. Potential cues for such an assessment could be behavioral (e.g., aggressive behaviors from males or receptive behaviors from females) or chemical (e.g., pheromones).

Black rhinoceros males exhibit similar patterns of androgen expression in captivity: serum testosterone is higher in males housed with females *or* males than in those housed alone (Christensen et al., 2009). In a survey of six small felid species, Swanson et al. (2003) found that housing situation did not affect serum testosterone levels, but did affect other reproductive parameters. Males housed alone or with a single female had increased testis size, semen quality, and sperm count compared to males housed with another male or in larger groups. Since we did not measure other reproductive parameters, we do not know if sex of the cage-mate may have affected other aspects of male reproductive physiology.

Age did not have a significant effect on fA levels in adult, intact males, either captive or wild. Even at the individual level, there was no evidence for an effect of age on androgen expression. Male lynx reach sexual maturity at 2 years of age (Poole, 2003), so all males in this study should have been reproductively mature. Although the effect of puberty on androgen expression has been extensively documented in many species, there is very little literature that describes the trajectory of androgen expression beyond the point of sexual maturity in non-human species. In sun bears, older males have higher fA than younger (but still reproductively mature) males (Hesterman et al., 2005). Younger leopards (3–7 years old) exhibit higher sperm quantity and quality compared to older males, but hormone expression was not monitored (van Dorsser and Strick, 2005).

Among captive males, past breeding success was not significantly related to fA concentrations. Even if we sub-divided proven breeders into 'historic breeders' (had successfully sired litters in the past, but not during the study) and 'current breeders' (sired a litter during the study period), there was still no relationship between fA and breeding status. Similarly, in cheetahs, neither serum testosterone levels nor semen quality were correlated with breeding status (Wildt et al., 1993). Conversely, in a survey of eight different felid species, Swanson et al. (2003) found that reproductive status was significantly correlated with serum testosterone; successful breeders had higher testosterone levels.

In conclusion, we were able to validate an EIA for non-invasively monitoring testicular activity in Canada lynx. Using this technique, we provide the first description of testicular activity for this species, and also provide a unique comparison between captive and wild males. Our key findings include: (1) both captive and wild male Canada lynx exhibit seasonal increases in fA during the breeding season, (2) captive males have much higher fA concentrations than wild males, which may be related to differences in metabolic rate, diet, body condition, and/or adrenal activity and (3) captive males housed with an intact cage-mate exhibit higher fA concentrations than those housed with neutered cage-mates. Understanding not only the basic reproductive physiology of male Canada lynx, but also the degree of plasticity in these reproductive traits, is critical for developing successful captive breeding programs and designing sound conservation strategies. Furthermore, this information can be used to understand the reproductive limitations of this genus in the face of environmental and climatic changes.

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