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Increased stress in Asiatic black bears relates to food limitation, crop raiding, and foraging beyond nature reserve boundaries in China

Karl D. Malcolm [a,b,g,*], William J. McShea [b], David L. Garshelis [c], Shu-Jin Luo [d], Timothy R. Van Deelen [a], Fang Liu [e], Sheng Li [a,b,f], Lin Miao [d], Dajun Wang [f], Janine L. Brown [b]

[a] *Department of Forest and Wildlife Ecology, University of Wisconsin-Madison, Madison, WI, USA*
[b] *Smithsonian Conservation Biology Institute, Front Royal, VA, USA*
[c] *Minnesota Department of Natural Resources, Grand Rapids, MN, USA*
[d] *College of Life Sciences, Peking-Tsinghua Center for Life Sciences, Peking University, Beijing, China*
[e] *Institute of Forest Ecology, Environment, and Protection, Chinese Academy of Forestry, Haidian, Beijing, China*
[f] *School of Life Sciences, Peking University, Beijing, China*
[g] *United States Department of Agriculture, Forest Service, Southwestern Regional Office, Albuquerque, NM, USA*

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ABSTRACT

Asiatic black bears (*Ursus thibetanus*) are declining throughout much of their range. In China they are partially protected by a nature reserve system and rely heavily on hard mast as a food source prior to winter denning. Bears may compensate for mast shortages by raiding agricultural crops and killing livestock, mainly outside reserves where they are exposed to increased threats of poaching. We hypothesized that stress would vary with availability of high-quality refugia and fluctuations in mast abundance. We collected fecal samples from free-ranging bears in and around nature reserves in southwestern China, recorded habitat characteristics at each fecal sample location, and quantified abundance of hard mast. We used feces for genetic and endocrine analysis and identified 106 individuals. Feces collected outside reserves, or in agricultural fields within reserves, contained elevated concentrations of glucocorticoid metabolites compared to samples collected in intact, mast-producing forests within reserves. Relationships with habitat variables indicated that the hypothalamic–pituitary–adrenal (HPA) axis of the Asiatic black bear is responsive to human activity, abundance of hard mast, extent of forest cover, and quality of diet. Our findings demonstrate biological reactions of a large mammal to variable forest quality, human threats, and foraging relative to boundaries of protected areas.

1. Introduction

Asiatic black bears (*Ursus thibetanus*) are threatened by loss of habitat and killing for commercial trade in bear parts (Garshelis and Steinmetz, 2008), a pervasive problem for many large mammals across much of Asia (Corlett, 2007). Poaching

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[*] Correspondence to: Southwestern Regional Wildlife Ecologist, United States Forest Service, 333 Broadway Boulevard SE, Albuquerque, NM 87102, USA. Tel.: +1 505 842 3262, +1 231 218 9464 (mobile).

E-mail addresses: karl.d.malcolm@gmail.com, kdmalcolm@fs.fed.us (K.D. Malcolm).

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of Asiatic black bears is facilitated by regular interaction with humans. Damage to crops and attacks on humans and livestock inspire retaliatory killing (Sathyakumar, 2001; Huygens et al., 2004) and foster negative attitudes toward bears (Liu et al., 2011).

In central Sichuan Province, the core of China's Asiatic black bear range, nature reserves established to protect giant pandas (*Ailuropoda melanoleuca*) also harbor robust populations of Asiatic black bears (Liu et al., 2009). Since the 1990s the number and total area of nature reserves in China increased rapidly (Li and Han, 2001), and anti-poaching patrols within reserves likely reduced illegal killing (Liu et al., 2011). However, a significant expanse of habitats occupied by Asiatic black bears remains outside protected areas. Moreover, many nature reserves may not be large enough to encompass the home ranges of resident bears, and poaching outside reserves can be intense (Hwang et al., 2010).

Asiatic black bears occupy a generalist ecological niche similar to that of American black bears (*Ursus americanus*, Reid et al., 1991; Steinmetz et al., 2011), but whereas American black bears are expanding in number and range (Garshelis et al., 2008) the opposite is true for the Asiatic species (Garshelis and Steinmetz, 2008). Both rely heavily on hard mast crops for food (Vaughan, 2002; Hashimoto et al., 2003); mast crop failures have been linked to increased use of anthropogenic foods (Noyce and Garshelis, 1997; Oka et al., 2004; Baruch-Mordo et al., 2014). Fluctuating abundance of natural food is an important source of variation in vulnerability of Asiatic black bears to human-induced mortality (Oi and Yamazaki, 2006).

Because Asiatic black bears hibernate in some parts of their range, including Sichuan Province (Schaller et al., 1989; Trent, 2010), acquiring sufficient energy stores during autumn is an important determinant of individual fitness and hence a strong driver of their behavior. In Japan, black bears traveled greater distances in autumns with poor mast (Kozakai et al., 2011) and experienced more conflicts with humans when searching for alternate foods.

Known threats to Asiatic black bears (i.e., habitat degradation and exposure to humans) and food abundance correlate with circulating levels of glucocorticoid hormones in other wildlife species (Busch and Hayward, 2009). While glucocorticoid hormones have increasingly been used as an indicator of physiological stress in wildlife species (Baker et al., 2013), they exhibit complex relationships with nutrition and stress. For example, they are variable at low to moderate stress levels, but increase at higher stress (Busch and Hayward, 2009). Glucocorticoids ensure that normal metabolic function is maintained by promoting appropriate levels of glucose and fatty acids in circulation (Harlow et al., 1990; Landys et al., 2006), but when an otherwise healthy individual cannot maintain energetic homeostasis or is exposed to noxious and unpredictable stimuli, circulating glucocorticoids rise above the stress threshold and trigger adaptive physiological and behavioral changes that may promote survival at a short-term cost (Wingfield and Kitaysky, 2002). For example, concentrations of glucocorticoids were elevated in Asiatic black bears farmed for bile (Malcolm et al., 2013).

We explored relationships between glucocorticoid production in free-ranging Asiatic black bears inside and outside Chinese nature reserves. We hypothesized that concentrations of glucocorticoids would increase with certain physical states (e.g., food limitation), behaviors (e.g., crop raiding), and habitats (e.g., less forested, unprotected, near roads), all of which relate to potentially higher risks of persecution by humans. We tested this hypothesis by comparing glucocorticoid concentrations in feces from bears that occupied areas with varying human activity, forest cover, availability of hard mast, and levels of protection.

2. Materials and methods

2.1. Study design

We quantified concentrations of glucocorticoid metabolites in fecal samples collected from free-ranging Asiatic black bears in Sichuan, Shaanxi, and Yunnan Provinces, China (Fig. 1). Given the link between energetic state and stress condition (in general: Wingfield and Kitaysky, 2002; and bears specifically: Bryan et al., 2013) and the predisposition of Asiatic black bears to experience conflict with humans during mast crop failures (Oka et al., 2004), we monitored spatial and temporal fluctuations in abundance of hard mast during each field season. Because primary dietary constituents were shown to influence fecal glucocorticoid concentrations in brown bears (*Ursus arctos*, von der Ohe et al., 2004), we examined and classified each fecal sample based on identifiable content to account for variation in glucocorticoids attributable to types of food consumed. We also genetically analyzed fecal samples that provided endocrine data to assess which samples were repeats of the same individual, to determine the minimum number of individuals sampled at each study site, and to test for sex-related differences in fecal glucocorticoid concentrations (Malcolm et al., 2013). We used the resulting data to model fecal glucocorticoid concentration as a function of landscape metrics (e.g., inside versus outside reserve boundaries, habitat type, etc.), hard mast abundance, diet, and season.

2.2. Study area

We collected fecal samples from Asiatic black bears in 8 nature reserves located in southwestern China (98°11′-107°42′ E, 24°47′-33°41′ N, Fig. 1). We also collected feces beyond reserve boundaries. We focused much of our sampling in and around 6 reserves (Meigu Dafengding [Meigu], Yele, Tangjiahe, Wanglang, Xiaohegou, and Xuebaoding) in the Liangshan–Xiangling–Minshan Mountain region of Sichuan Province. Additional sampling was conducted in Changqing Nature Reserve in Shaanxi Province and Gaoligongshan Nature Reserve in Yunnan Province. We collected fecal samples in six broad habitat categories: broadleaf deciduous, conifer, mixed broadleaf and conifer, agricultural (planted primarily

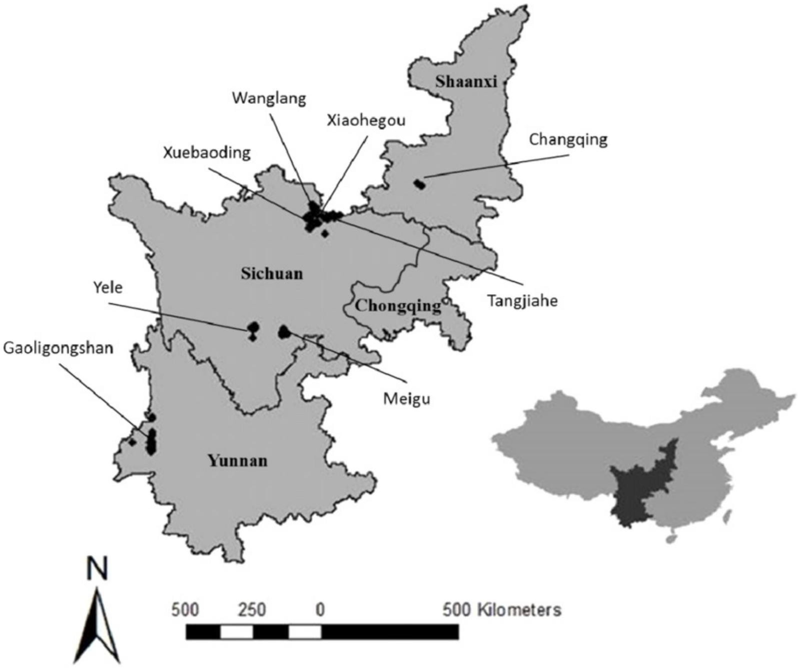

Fig. 1. Locations (black dots) where fecal samples ($n = 804$) were collected from free-ranging Asiatic black bears (and non-target species) during the autumns of 2008–2010. Samples were collected in and around the boundaries of 8 nature reserves located in southwestern China. Province names are given in bold while nature reserve locations are indicated with lines.

with corn, *Zea mays*), shrub, or bamboo (see Supporting Information for more details, Appendix A). Although forests within nature reserves throughout our study area provided excellent habitat for Asiatic black bears, human activity was typically high outside as well as within some reserves (Liu et al., 2009). Some reserves contained agricultural fields and free-ranging livestock. Reports of conflicts between people and bears in agricultural fields and at the interface of reserves and human settlements were common during the years leading up to this study (Liu et al., 2011).

2.3. Fecal sample collection

Field teams consisting of researchers and nature reserve staff collected fecal samples during September through November, 2008–2010. We limited sampling to this autumnal period to minimize variation in adrenal activity related to season (Romero, 2002; Malcolm et al., 2013). Also, we were specifically interested in the interplay between abundance of fall hard mast and fecal glucocorticoids. We divided each fecal sample into two parts, for DNA and hormone extraction.

We located feces along wildlife and hiking trails and by searching in areas where nature reserve staff and local people reported recent bear activity. In 2010 we employed a trained dog and handler team (Working Dogs for Conservation, Three Forks, Montana, USA) to aid in finding fecal samples outside nature reserves, where habitat tended to be more degraded and the density of bear sign, including feces, was lower. The dog was released to search by scent and indicated the detection of feces by sitting nearby. For each fecal sample we recorded location and elevation using GPS, and habitat category based on vegetation characteristics in the immediate area. Fecal samples were placed in plastic bags and frozen (−20 °C) within 12 h until processing.

2.4. Evaluation of habitat and quantification of natural food abundance

At each nature reserve we used strip transects to quantify the density of hard mast-producing trees (chestnut [*Castanea*], oak [*Quercus*], and walnut [*Juglans*]) and their annual production of nuts following the protocols outlined in Liu et al. (2009) (see Supporting Information for more details, Appendix A). We repeated most transects and quadrats at the same locations during each of the 3 years.

2.5. Fecal DNA extraction and genetic identification of species, sex and individual

We extracted DNA from thawed feces at least twice for each sample using QIAamp DNA Stool Mini Kit (QIAGEN). We amplified and sequenced segments of mitochondrial DNA control region and *cytochrome b* to verify the quality of DNA and the species identity of each sample, following Yasukochi et al. (2009). We conducted sex identification of Asiatic black bears using a PCR assay of *amelogenin* gene (Yamamoto et al., 2002). Genetic identification to individual was based on a panel of 11 microsatellite markers developed for *Ursus* species (see Supporting Information for more details, Appendix A).

2.6. Diet and hormonal analysis

We thawed frozen fecal samples at room temperature and documented their primary undigested content in broad categories: soft mast, hard mast, green vegetation, corn, insect remains, flesh and hair, vegetation plus flesh or indecipherable. We dried feces using a conventional oven at 100 ° C for 12 h and pulverized them into a fine powder, which was sifted through a wire mesh to remove large particles (>2 mm, e.g., seeds). We extracted hormones from fecal powder following Graham and Brown (1996) modified for Asiatic black bears (Malcolm et al., 2013).

We used a cortisol enzyme immunoassay (Munro and Lasley, 1988) validated for Asiatic black bear fecal extracts (Malcolm et al., 2013) to quantify glucocorticoid metabolites (see Supporting Information, Appendix A). We expressed concentrations of fecal glucocorticoid metabolites as nanograms per gram of dry, powdered sample material (ng/g).

2.7. Statistical analysis

Fecal samples that failed to yield quantifiable DNA were considered degraded and corresponding glucocorticoid data were excluded from analyses (Vynne et al., 2012). For the subset of bears identified to sex, we compared mean fecal glucocorticoid concentrations between males and females using a t-test. Based on findings that female brown/grizzly bears tended to produce elevated hair cortisol compared to males in parks and protected areas (Bourbonnais et al., 2013), we used a two-way ANOVA with sex and location with respect to reserve boundaries as independent variables. We compared capacity for hard mast production among reserves using the density of mast-producing tree species as an index. We compared mean concentrations of glucocorticoids in samples collected from bears in different nature reserves using ANOVA, and identified sources of variation using Tukey's Honestly Significant Difference (HSD) test. We had adequate sample sizes for this analysis from seven reserves ($n = 24$–149), but excluded Changqing Nature Reserve due to an inadequate sample size ($n = 3$). We used simple linear regression, with each reserve as a data point, to test: (1) whether mean fecal glucocorticoids were related to the abundance of hard mast-producing tree species, (2) whether agricultural land use was correlated within and adjacent to nature reserves and (3) whether the abundance of mast-producing trees was related to amounts of agricultural land use.

We used R Version 2.12.1 (R Development Core Team, 2010) to fit linear mixed-effects regression models of the influence of habitat type, landscape metrics (inside or outside reserve boundaries, extent of agricultural land use, distance to nearest village, distance to nearest road, forest cover), diet, hard mast abundance, and month on concentrations of glucocorticoid metabolites in fecal samples from Asiatic black bears (additional details provided in Supporting Information, Appendix A). We compiled data on landscape characteristics for the study area, including primary and secondary roads, nature reserve boundaries, and village locations using the Chinese National Fundamental Geographic Information System (Chinese National Administration of Surveying, 1999) to determine whether each fecal sample was collected inside or outside official reserve boundaries, distance from point of collection to nearest village, and distance to nearest road. We constructed buffers with radii of 3 km around each fecal sample location to estimate percent forest cover in the vicinity of the point of collection. We selected this buffer size because it corresponds to a conservative home range estimate (27 km^2) of Asiatic black bears (Hwang et al., 2010). Forest coverage was calculated using the European Space Agency's Global Land Cover Map, with 300 m resolution (GlobCover, 2009). We classified percent forest cover in each buffer as low ($<70\%$), moderate (70%–90%), or high ($>90\%$). We categorized reserves as having low ($<5\%$) or high ($>5\%$) agricultural land cover within their boundaries and compared mean concentrations of fecal glucocorticoids in those categories to each other and to samples collected outside reserves using ANOVA, and identified sources of variation using Tukey's Honestly Significant Difference (HSD) test. We also constructed 3-km buffers around each nature reserve to compare the amount of agriculture within and immediately adjacent to each reserve. We included the month of collection as an explanatory variable in a subset of candidate models (Malcolm et al., 2013). We applied various power functions to transform variables to meet the assumption of normality. We log-transformed fecal glucocorticoid concentration ($\log[FGC]$), and applied Box–Cox transformations to distance to nearest road ($\text{bc}[DistRoad]$, $\lambda = 0.5$) and distance to nearest village ($\text{bc}[DistVillage]$, $\lambda = 1.2$). We selected λ values for Box–Cox transformations using maximum likelihood (Venables and Ripley, 2002).

We posited *a priori* candidate fixed effects structures that represented different, plausible explanations of variation in fecal glucocorticoids by free-ranging Asiatic black bears. Candidate models included a null model, a habitat model (variables: *HabitatType, InOutReserve, PercentAgInReserve, DistVillage, DistRoad, ForestCover*), a diet model (*FoodType*), a mast abundance model (*MastAbundance*), a season model (*Month*), and combinations of those sets of explanatory variables. The null model included only the random effects terms accounting for variation among individual bears and variation among nature reserves with no fixed effects. The hypothesis underlying models with landscape metrics was that higher fecal glucocorticoids would be found in Asiatic black bears responding to anthropogenic landscape alteration (e.g., deforestation, proximity to settlements, agricultural activity, establishment and enforcement of nature reserve boundaries). We evaluated our hypothesis that fecal glucocorticoids were related to the abundance of hard mast by including a subset of models that had the *MastAbundance* term. We accounted for variation in primary dietary constituents using the *FoodType* covariate (Table S1, Appendix A).

3. Results

3.1. Glucocorticoid comparisons by sex

Of 804 samples collected, 511 contained adequate DNA for species assignment and, of those, Asiatic black bears produced 468 (92%). Of the identified Asiatic black bear feces, 212 succeeded in individual identification with microsatellite genotypes from at least eight loci. These accounted for 106 unique individuals including 52 females, 40 males and 14 of unknown sex. Mean ± SEM concentrations of fecal glucocorticoid metabolites were similar for male (19.49 ± 3.25 ng/g) and female (13.46 ± 2.22 ng/g) bears ($t(90) = -1.58$, $p = 0.12$), as was the mean number of samples per genotyped individual of each sex ($\bar{x}_{male} = 1.93 \pm 0.19$, range 1–6; $\bar{x}_{female} = 2.25 \pm 0.30$, range 1–12; $t(90) = 0.85$, $p = 0.40$). We eliminated one anomalous sample from a female bear in Meigu Reserve that contained >63 times (1472 ng/g) the overall mean concentration of fecal glucocorticoids (23 ng/g), a level indicative of severe distress, injury, or disease (Malcolm et al., 2013). A two-way analysis of variance of the subset of samples to which sex could be attributed indicated that the effect of location with respect to reserve boundaries was significant ($F(1, 199) = 9.41$, $p < 0.01$), as was the interaction between that variable and sex ($F(1, 199) = 8.00$, $p < 0.01$), while the sex term alone was not ($F(1, 199) = 1.22$, $p = 0.27$). Females had lower concentrations of fecal glucocorticoids inside versus outside reserve boundaries. Within reserves, glucocorticoids were lower in feces from females than males (Tukey HSD $p < 0.05$). Because most feces with identifiable Asiatic black bear DNA could not be differentiated by sex, data were pooled for multiple linear regression. Thirteen feces with Asiatic black bear DNA were excluded from glucocorticoid analysis due to incomplete collection records, leaving a total of 454 samples available for linear mixed-effects regression.

3.2. Spatial and temporal distribution of feces from identifiable bears

We identified 2 (Gaoligongshan and Yele) to 47 (Tangjiahe) individual bears among samples collected in and around each nature reserve (Table S2, Appendix A). Fifty of the 92 known bears (29 females, 21 males, 54%) were detected exclusively within protected areas, 37 (20 females, 17 males, 40%) were detected exclusively outside of protected areas and the remaining 5 (3 females, 2 males, 5%) were detected on both sides of a protected area boundary. Although searches for scats were conducted inside and outside of reserve boundaries at all study sites, most bears located at Meigu, and Tangjiahe were sampled exclusively within reserves, whereas most at Wanglang, Xiaohegou, and Xuebaoding were detected outside (sample sizes were too small at other sites; Table S2, Appendix A).

Bears of both sexes congregated at concentrated food sources during September–October. The most striking examples were two sites of about 0.5 km^2, separated by ~3 km, in southeastern Tangjiahe during 2008, when hard mast was especially abundant in that reserve (Table 1). One site yielded feces from 10 unique genotypes (7 females, 3 males) collected during a 39-day period. Eleven (8 female, 3 male) unique individuals, none from the first site, were detected at the second Tangjiahe site based on samples collected over 25 days. Other concentrations included a 5-km^2 site in the center of Meigu where 7 bears (5 females, 2 males) were detected in 2009, and a 3-km^2 site north of Xuebaoding where 6 bears (2 females, 4 males) were detected in 2010. Mast densities in the vicinity of the Meigu and Xuebaoding congregation sites averaged 5.7 and 8.0 nuts/m^2 respectively, and were considerably higher than reserve averages in those years (Table 1).

3.3. Fecal sample characteristics

Of 454 fecal samples included in our analysis of glucocorticoids, 190 (42%) were collected within nature reserve boundaries at 1165–3310 m elevation, in broadleaved forests (52%), mixed broadleaf conifer forests (25%), conifer (11%) and shrub (4%) vegetation types. Ten fecal samples (5%) found within reserves were in agricultural fields. The remaining 58% of feces were collected outside reserves at 1250–3300 m elevation where they were similarly distributed among habitat types (broadleaved forests 53%, mixed broadleaf conifer 32%, shrub 7%, conifer 6%, and agricultural fields 2%). Both males and females engaged in crop raiding based on samples collected in agricultural fields (10 female feces [8% of female samples], 3 male feces [4% of male samples]) and those with corn as a primary dietary constituent (3 female, 2 male).

Ranked in descending frequency of occurrence, primary constituents in fecal samples collected inside reserves were soft mast (36%; e.g., *Elaeagnus umbellata*, *Actinidia* spp.), hard mast (23%), non-fruit vegetation (13%), flesh (12%), mixed flesh and vegetation (8%), and corn (7%). Primary constituents in feces collected outside reserves were soft mast (50%), hard mast (13%), non-fruit vegetation (10%), flesh (10%), corn (9%), mixed flesh and vegetation (4%), and insect (4%). A larger proportion of samples collected within reserves contained hard mast. Fecal glucocorticoid concentration, distance to the nearest village, distance to road, and percent forest cover each varied considerably among samples (Table S3, Appendix A).

3.4. Habitat characteristics and influence on fecal glucocorticoids

Mast production varied among nature reserves and across years within each reserve, with 2009 being highest in 5 of 6 reserves sampled during all 3 years. Some variation in hard mast abundance was attributable to differing densities of chestnut, oak, and walnut trees, which varied 18-fold among reserves (Table 1). A remote sensing-based analysis of agricultural land cover components inside and adjacent to nature reserves revealed that 2%–14% of the land within reserves

Table 1

Extent of agricultural cover types within and adjacent to (3 km buffer) reserve boundaries, densities (trees/km^2) of hard mast producing tree species (diameter at breast height $\geq$ 9 cm) and densities of hard mast (nuts/m^2) in nature reserves where Asiatic black bear fecal samples were collected. Extent of agriculture was based on remote sensing data. Tree densities were a pooled average of all transects completed in each reserve; mast densities were from a pooled average of all quadrats within the reserve for the given year (range of 48–80 quadrats/reserve/year). Dashes indicate that reserves were not sampled (for feces or mast) in a given year.

Reserve name	Mean fecal glucocorticoids	% Agriculture in reserve/buffer	Years surveyed	Transects/year	Total transects	Chestnut tree density	Oak tree density	Walnut tree density	Total mast tree density
Yele	32.24	14%/13%	3	9	27	620	1 269	0	1 889
Meigu	20.59	10%/11%	3	8	24	146	3 354	2156	5 656
Wanglang	20.70	8%/4%	3	10	30	0	5 725	0	5 725
Xiaohegou	28.34	5%/4%	3	10	30	2650	5 350	2650	10 650
Xuebaoding	24.58	4%/7%	3	8	24	323	5 104	813	6 240
Gaoligongshan	14.70	3%/6%	2	10	20	8050	5 025	850	13 925
Tangjiahe	11.35	2%/3%	3	10	30	250	17 250	1117	18 617
Changqing	32.54	2%/3%	1	9	9	9194	23 806	889	33 889
Average	20.06	6%/7%				2654	8 360	1059	12 074
Standard error	1.00	2%/1%				1340	2 773	330	3 644

Reserve Name	2008 mast density	2009 mast density	2010 mast density	Average mast density	Standard error
Yele	0.01	2.61	1.78	1.46	0.77
Meigu	0.56	4.08	0.02	1.56	1.27
Wanglang	0.08	15.45	0.02	5.18	5.13
Xiaohegou	4.65	10.37	2.28	5.77	2.4
Xuebaoding	0.01	9.78	4.11	4.63	2.83
Gaoligongshan	–	7.23	10.68	8.96	1.73
Tangjiahe	15.62	4.58	0.45	6.88	4.53
Changqing	–	–	1.41	1.41	–
Average	3.49	7.73	2.59	4.6	
Standard error	2.54	1.69	1.25	1.83	

and 3%–13% of land outside reserves (within 3 km) were agricultural (Table 1). Amounts of agricultural land use within and adjacent to each reserve were correlated ($r^2 = 0.67, p < 0.05$), and reserves with higher densities of mast-producing trees tended to contain lower amounts of agriculture ($r^2 = 0.70, p < 0.05$).

Differences in habitat quality in and around reserves accounted for some variation in mean concentrations of fecal glucocorticoids among reserves (ANOVA, $F_{6,444} = 11.18, P < 0.001$). Feces collected from Yele contained, on average, higher concentrations of glucocorticoids than those from Gaoligongshan, Tangjiahe, and Wanglang. Mean fecal glucocorticoid concentration in samples from Tangjiahe was lower than that of Xiaohegou, and Xuebaoding, while Gaoligongshan was lower, on average, than Xiaohegou (Tukey HSD $p < 0.05$). Bear feces from in and around reserves with lower densities of mast-producing trees tended to have higher mean glucocorticoids ($r^2 = 0.79, p < 0.05$, Fig. 2). Comparing samples from reserves with low amounts (<5%) of agriculture, reserves with high (>5%) agricultural cover, and areas outside reserves indicated that glucocorticoids were lowest in reserves with little agriculture, whereas concentrations were similar for reserves with substantial agriculture and areas outside reserves (ANOVA, $F_{2,451} = 11.15, P < 0.001$, Tukey HSD < 0.01, Fig. 3).

3.5. Relationship of fecal glucocorticoids to habitat, natural food abundance, diet, and season

The model of fecal glucocorticoid concentration that received the greatest support included covariates reflecting landscape characteristics, dietary constituents, hard mast abundance, and month of sample collection ($w_{Saturated} = 0.61$). The two other models that received support both also contained landscape and month covariates (Table S1, Appendix A). The second-ranked model ($w_{Habitat\&Mast\&Month} = 0.33$) included the mast abundance covariate, while the third-ranked model ($w_{Habitat\&Diet\&Month} = 0.05$) accounted for diet. Unconditional parameter estimates for samples collected in agricultural fields (*HabitatAgriculture*) and conifer forests (*HabitatConifer*) did not include 0 in their 95% confidence intervals, indicating significant effects on fecal glucocorticoid levels. Fecal glucocorticoid concentrations detected in samples collected from those habitat types were elevated relative to those found in broadleaved deciduous forest. Feces collected within reserve boundaries (*InsideReserve*) contained significantly lower concentrations of glucocorticoids than those collected outside reserves. Other parameter estimates that emerged as significant were: total forest coverage (*ForestLow*, inversely related to glucocorticoids), mast abundance (*MastLow*, inversely related to glucocorticoids), distance to the nearest road (*DistRoad*, higher glucocorticoids farther from roads), and season (*MonthNovember*, later samples had lower glucocorticoids; Table S4, Appendix A).

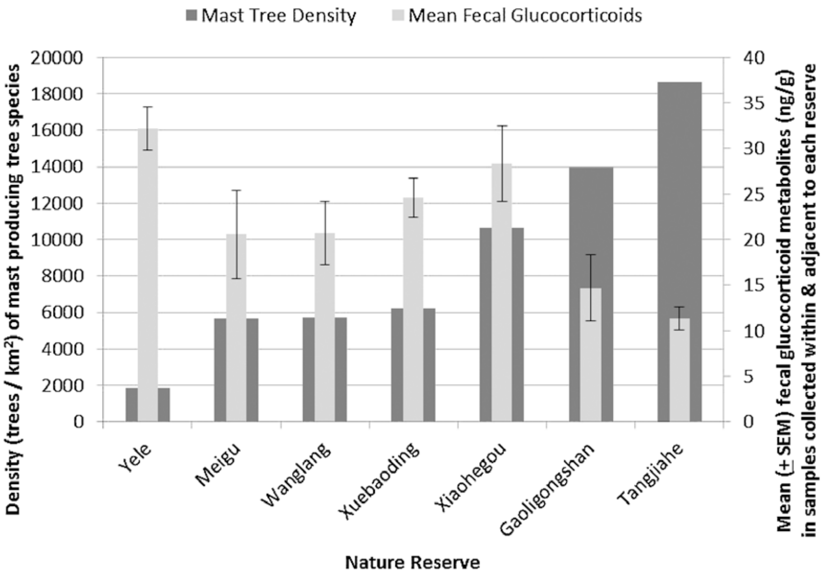

Fig. 2. Mean concentrations of fecal glucocorticoids (light gray) were negatively related to the density of mast-producing tree species within nature reserves based on summed transects (dark gray). Changqing Nature Reserve, which had the highest density of mast-producing trees, was excluded from this analysis due to an insufficient sample of scats.

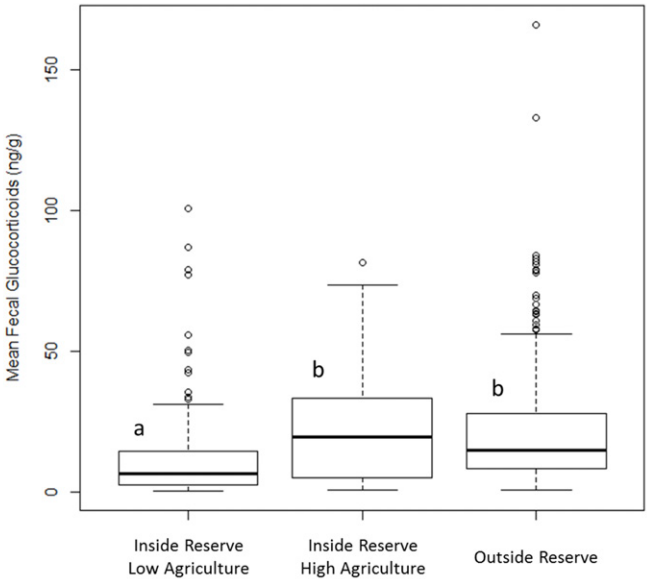

Fig. 3. Mean concentrations of glucocorticoids in feces collected from free-ranging Asiatic black bears (*U. thibetanus*) in low agriculture reserves ($<5\%$ land cover, $n = 136$), high agriculture reserves ($>5\%$ land cover, $n = 54$), and outside reserve boundaries ($n = 264$) in Sichuan, Shaanxi, and Yunnan Provinces, China, during the late summers and early autumns of 2008–2010. Different letters denote significance ($p < 0.01$).

High ($>90\%$) forest cover and dense hard mast (>5 nuts/m^2) corresponded with significantly lower fecal glucocorticoids than low ($<70\%$) forest cover or sparse (0–2.5 nuts/m^2) hard mast. Forest cover, mast abundance, and collection in agricultural fields were the significant explanatory variables with the largest effect sizes, all comparable in magnitude (Table S4, Appendix A).

4. Discussion

Results presented here support our hypotheses that concentrations of glucocorticoids in Asiatic black bears increased with food limitation, crop raiding, and use of habitats with less forest and less protection from human persecution. We predicted that all of these would be stressors for bears, although some may be more directly perceived than others.

4.1. *Limited fall food abundance as a stressor*

Availability of hard mast was a key predictor of fecal glucocorticoid concentrations during September–November. Limited abundance of hard mast prior to denning may relate to behavioral modifications mediated by the HPA axis, which is consistent with reports of increased conflicts between bears and humans during failures of hard mast crops (e.g., Oka et al., 2004). Likewise, Bryan et al. (2013) observed long-term cortisol responses by grizzly bears to varying availability of another seasonally and spatially constrained food resource that has profound effects on their nutrition—Pacific salmon (*Oncorhynchus* spp.).

Facing food limitation when they can afford it least, bears may be more likely to engage in innovative and exploratory foraging including targeting anthropogenic food resources and occupying riskier habitats (e.g., agricultural fields, less forested habitats beyond reserve boundaries, closer to human settlements) to compensate for shortfalls in naturally available foods (Baruch-Mordo et al., 2014). Acquiring sufficient energy stores prior to predictable periods of food scarcity is a behavioral adaptation that Asiatic black bears in the temperate part of their range share with other bear species occupying habitats where food availability is highly seasonal. Hard mast abundance can affect body condition (Oi and Furusawa, 2008) and reproductive performance (e.g., Costello et al., 2003; Seger et al., 2013). Therefore, poor fall mast crops represent an unpredictable, costly, and potentially severe condition for Asiatic black bears.

Unfavorable environmental triggers with these characteristics can induce behavioral and physiological responses collectively dubbed the ''emergency'' life history stage, and there is evidence that associated changes are mediated by levels of circulating glucocorticoids (Wingfield et al., 1998). Responses to elevated HPA activity include increased foraging, elevated glucogenesis, migration in search of alternate food sources and habitats, improved spatial memory, and willingness to return to feeding sites after being disturbed (Wingfield et al., 1998; Pravosudov, 2003; Busch and Hayward, 2009). These behaviors may predispose bears to greater risk of poaching. Increased exposure to hunter-induced mortality was documented in American black bears that were more easily attracted to hunters' bait sites during years when natural food abundance was low (Noyce and Garshelis, 1997). We postulate that, when natural foods (particularly hard mast) are abundant, bears prefer to forage in intact forests isolated from human disturbance. Bears failing to meet their energetic needs may have been driven by hunger and emboldened by endocrine secretions to forage in relatively dangerous and disturbed areas within and outside of nature reserves where alternate foods (e.g., corn, honey, livestock, and abundant soft mast) were available. Glucocorticoids diminished later in autumn, possibly associated with metabolic changes associated with denning (Blumenthal et al., 2011).

4.2. *Perceived risk of mortality as a stressor*

Bears within nature reserves in central China showed less stress than bears that lived or wandered outside reserves. Also notably, female bears inside reserves had lower concentrations of glucocorticoids than male bears inside reserves and females outside reserves. Higher concentrations of stress hormones in male bears within reserves compared to female bears within reserves may relate to greater intrasexual competition among males at higher densities (Bryan et al., 2013). Findings that females inside reserves had relatively low stress hormone concentrations contrast with grizzly bears in Alberta, Canada (Bourbonnais et al., 2013), where female bears inhabiting parks and protected areas had higher cortisol than bears in surrounding landscapes with more human disturbance. However, a key distinction is that abundance of quality food (primarily herbaceous vegetation) in the Alberta study area was associated with anthropogenic disturbance rather than with intact, mast-producing forests as was the case in our study. Reserves that conserve hard mast-producing forests offer both refuge and calorically rich habitat, making it difficult to parse the benefits of sanctuary versus food. Likewise, higher concentrations of glucocorticoids in feces collected in agricultural fields and areas with less forested cover could have either driven forays into those habitats (in response to limited natural food availability elsewhere), been a product of perceived risk, or a combination of the two.

Clear differences existed among reserves in terms of their ability to provide high quality natural food sources and refuge from human disturbance. Reserves with the lowest densities of mast-producing forests tended to contain more agriculture both within and near their boundaries. Bears were responsive to these differences, based on relationships between fecal glucocorticoids and density of mast-producing trees and extent of agriculture. We unexpectedly collected a higher proportion of usable feces in agricultural fields inside nature reserves than we did outside, and our remote imaging assessment of land cover within reserve boundaries revealed that croplands accounted for up to 14% of the landscape of reserves we studied. Furthermore, nearly the same proportion of feces within (7%) and outside (9%) nature reserves contained corn as a primary constituent. Bears were also likely to encounter agriculturalists, herdsmen, and other human activity within several of the reserves we studied (e.g., Yele, Meigu, Wanglang). Thus, in some reserves, there was no clear distinction in habitat and human threat inside versus outside, and accordingly, in these cases bears showed no difference in glucocorticoid concentrations inside or outside the reserve (Fig. 3). The same bear–human conflicts, stemming from crop and livestock raiding, that pose threats to bears outside of reserves might also therefore exist within the boundaries of some protected areas.

We have sparse anecdotal information about the extent of bear poaching in our study area during the years of our study, but based on work completed immediately prior we believe that it occurred more regularly outside reserves than within (Liu et al., 2011). As such, reserves with good supplies of hard mast likely retain more bears within their borders, thus offering not only greater protection but also a better source of nutrition, thereby enhancing reproduction. Possibly because of the rich mast supplies, we detected the highest number of bears in Tangjiahe Nature Reserve (Tables 1, S2). Notably, the two dense concentrations totaling 21 bears that we discovered in this reserve included both males and females, whereas previously described congregations of this species in fall feeding areas contained almost exclusively males (Huygens and Hayashi, 2001, Hwang and Garshelis, 2007).

Potential for bear–human conflicts and poaching in landscapes surrounding reserves with poorer habitat is exacerbated by the apparently inadequate size of protected areas and the fact that much of the region's black bear habitat is beyond the scope of formal protection. This point is underscored by the finding that 42 of 92 genotyped bears in our study were detected beyond patrolled reserve boundaries on at least one occasion. Hwang et al. (2010) documented that half of the Asiatic black bears captured in the core of a 1055 km^2 protected area in Taiwan ventured beyond the bounds of protection. Seven of eight reserves included in our study were smaller (160–636 km^2) than the Taiwan site, therefore it is conceivable that a substantial number of bears that we detected exclusively within protected areas also spent some portion of their lives in unprotected habitats.

Fecal glucocorticoids also increased with distance from the nearest road, contradictory to our initial hypothesis. One possible explanation for the unexpected positive relationship between distance from roads and fecal glucocorticoids is that roadways, which tend to follow lower elevation routes, frequently coincide with relatively productive riparian forest types, including those that produce soft and hard mast. Bears could theoretically access and exploit these resources without being unduly exposed to humans. Cattet et al. (2002) similarly linked superior body condition in grizzly bears to higher food availability outside the bounds of mountainous protected parks. Furthermore, disturbance to forestlands adjacent to roadways could boost productivity and food availability for bears relative to undisturbed landscapes (Nielson et al., 2004; Roever et al., 2008), thereby creating a trade-off between distance from human disturbance and caloric richness (i.e., high risk, high-reward habitats, Bourbonnais et al. 2013).

Our findings reinforce the paradigm that managers should maintain ecological integrity of landscapes that provide diverse natural foods for bears, including alternate foods that will be available when crops of primary forage species are limited (Garshelis and Noyce, 2008). Reforestation programs aimed at maintaining the integrity of sloping lands and conversion of agricultural areas to forest (Yan-qiong et al., 2003) would be more beneficial to Asiatic black bears and people if they incorporated vegetation types that produced some combination of hard and soft mast rather than monocultures of coniferous tree species. Considering the behavioral and ecological similarities between Asiatic and American black bears (Reid et al., 1991), the latter of which are thriving in diverse and in some cases highly degraded, human-dominated habitats, Asiatic black bears may too have the capacity to flourish in landscapes with considerable human activity. The key caveat is that they be effectively protected from unsustainable exploitation.

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Appendix A. Supplementary data

Supplementary material related to this article can be found online at http://dx.doi.org/10.1016/j.gecco.2014.09.010.

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