Published by Associazione Teriologica Italiana
Hystrix, the Italian Journal of Mammalogy
Volume 29 (2): 195–201, 2018
Available online at:
http://www.italian-journal-of-mammalogy.it
doi:10.4404/hystrix–00063-2018

Research Article

Correlates of physiological stress and habitat factors in reintroduction-based recovery of tiger (*Panthera tigris*) populations

Manjari Malviya[1], Vinod Kumar[2], Dibyendu Mandal[1], Mriganka Shekhar Sarkar[1], Parag Nigam[1], Rajesh Gopal[3,4], Kalyanasundaram Sankar[5,1], Govindhaswamy Umapathy[2], Krishnamurthy Ramesh[1,*]

[1] *Wildlife Institute of India*
[2] *Laboratory for the Conservation of Endangered Species (LaCONES), Centre for Cellular and Molecular Biology*
[3] *National Tiger Conservation Authority*
[4] *Global Tiger Forum*
[5] *Sálim Ali Centre for Ornithology and Natural History*

Keywords:
terrain
glucocorticoids
large carnivores
breeding success
species-habitat relationship
anthropogenic disturbance

Article history:
Received: 8 February 2018
Accepted: 2 November 2018

Acknowledgements
This work was supported by the National Tiger Conservation Authority (NTCA), Government of India [NTCA Order No. 1-3/93-PT (Vol. II)]. We thank Wildlife Institute of India (P.R. Sinha and V.B. Mathur), the State Forest Departments of Madhya Pradesh (H.S. Pabla, N. Kumar, D. Shukla, S. Kumar and R.S. Murthy) and Rajasthan (V.K. Melhotra, R.S. Shekhawat and M. Parashar); colleagues (J.A. Johnson, S. Sen, Q.Qureshi, S. Bhattacharya, P. Chaurasia, D. Chatterjee, S.K. Bhardwaj, M. Naveen and S. Roamin) and field staff for providing support and facilitating field data collection. We are grateful to the reviewers and especially the editor of the manuscript for valuable inputs.

These tiger reintroductions were implemented as per the protocol issued by National Tiger Conservation Authority (NTCA), which is the supreme authority in India, as far as tiger management is concerned. In this protocol, NTCA has incorporated IUCN reintroduction specialist groups' guidelines. In term of field execution, the chief Wildlife Warden is empowered to carry out such management interventions, but because India is signatory to IUCN, the guidelines laid down by IUCN are generally followed and in this specific context has been incorporated through NTCA protocols.

Abstract

The ever-increasing human presence in tiger occupied landscapes mandates a better understanding of its effects on the species. The loss of tigers to conflict and poaching have been well established, while the indirect effects of human induced stress have not been widely discussed. Anthropogenic factors have driven tiger populations to extinction in Sariska and Panna Tiger Reserves in India. The reintroduction of tigers in these two reserves resulted in contrasting reproductive outcomes and population growth. In this paper, we demonstrate relationships between habitat factors and stress affecting reproduction of reintroduced animals in two contrasting wild tiger populations. The tiger population in Panna grew rapidly and reached carrying capacity within five years, while the Sariska population struggled with strikingly slow growth rate. Although past studies have linked anthropogenic disturbance to stress and low reproductive outcome in wild animals, we argue that it is the complexity and quality of the habitat that influence how animals perceive and cope with this disturbance, resulting in chronic stress and thereby poor reproduction. We quantified fecal glucocorticoid metabolite (FGM), prey density, terrain complexity, cover, water availability and anthropogenic disturbances at both study sites. As predicted, tigers in the population with low reproduction rate (Sariska) had higher FGM concentrations than in the population with high reproduction rate (Panna). We conclude that secure habitat conditions supported by terrain complexity, optimal prey, water availability and low anthropogenic disturbance determine levels of chronic stress, breeding success and population growth of tigers. Therefore, large carnivore reintroductions should consider physiological stress and suitable habitats at fine scale, for realistic population growth projections and adaptive management strategies.

Introduction

Global tiger (*Panthera tigris*) populations continue to face challenges from poaching, habitat loss, prey depletion and anthropogenic disturbances (Dinerstein et al., 2007). Additionally, tigers occupying fringe areas of reserves or unprotected forest patches in human dominated landscapes are exposed to anthropogenic pressures. This may lead to negative interactions that are stressful and sometimes fatal to both humans and tigers. Two tiger reserves in India, Sariska Tiger Reserve (STR) and Panna Tiger Reserve (PTR) experienced local extinction of tigers in 2008 and 2009, respectively, largely due to poaching, requiring reintroduction to mitigate the loss. Accordingly, eight adult tigers (three males and five females) were translocated from the nearby Ranthambore Tiger Reserve (RTR) to STR between 2008 and 2013 (Sankar et al., 2010). Similarly, seven tigers (two males and five females) were translocated from Bandhavgarh, Kanha and Pench Tiger Reserves to PTR between 2009 and 2015 (Sarkar et al., 2016).

Reintroduction of large mammals has emerged as one of the key management and conservation tools across the globe (Ripple and Beschta, 2003; Hayward, 2007, 2009). However, the reintroduction of a large carnivore such as tiger, in a populous country like India, with growing demands for land and increasingly degrading natural habitats, is a complex and challenging task (Johnsingh and Madhusudan, 2009). The success of reintroduction programs is generally measured in terms of settling behavior, fecundity and survivorship of the introduced animals and that of F1 (first generation) offspring (Weeks et al., 2011). The reintroduced population in PTR bred successfully and experienced rapid population growth (to >40 individuals in nine years) whereas no successful breeding was recorded in STR for the first four years after reintroduction and subsequent breeding contributed to only marginal population growth (to 15 individuals in ten years). Although the number of founder animals and broad contours of conservation problems and responses were similar in these two reserves, significant variation in breeding success and population growth patterns mandated detailed investigation because conservation investments have been significant in both sites. Furthermore, since these were the first successful reintro-

*Corresponding author
Email address: ramesh@wii.gov.in (Krishnamurthy Ramesh)

ductions of Indian tigers, it was important to learn from the challenges that these populations were facing, to be able to guide such endeavors in the future. We approached this investigation from the fundamental physiological response of tigers to a new environment, focusing on glucocorticoid (GC) responses to human disturbance and habitat correlates.

An individual's body typically releases GCs (cortisol and corticosterone), the hormones produced by activation of hypothalamic-pituitary-adrenal (HPA) axis, in response to challenges posed by its environment, which may be termed as stressors (Sapolsky et al., 2000; Reeder, 2005). The GCs are secreted in the body to help prepare an individual against external factors, channeling the body's energy to effectively cope with immediate stressful stimuli (Wingfield and Kitaysky, 2002). By doing so GCs help the body deal with the harmful effects of stress called "allostatic load" (McEwen, 2007). In case the stressful state continues for a prolonged period of time, excess GCs are released, leading to chronic stress (McEwen, 2007). Excess GCs often negatively affect reproduction. The various negative consequences of stress on reproductive capacity of animals include delay in timing of reproduction, low release of reproductive hormones, lower fertility, higher inter-birth intervals, abortions and impairment of erectile function in males (Sapolsky et al., 2000; Sapolsky, 2004; Schoech, 2009; Whirledge et al., 2013). Certain species that are semelparous or individuals that have very few breeding opportunities are able to reproduce even under stressful conditions, but the mechanism by which they cope with stress and successfully reproduce are genetically determined, as well as environment dependent (Weeks et al., 1995; Wingfield and Sapolsky, 2003). In contrast, an iteroparous species (such as tiger) which has a comparatively longer life span and would get multiple opportunities to reproduce, will make decisions related to parental investment cautiously and might choose its own survival over that of offspring, resulting in termination of pregnancy or litter abandonment (Frid and Dill, 2002).

Studies suggest that animals perceive humans as predators and as a result are stressed by their presence (Frid and Dill, 2002; Beale and Monaghan, 2004; Smith, 2017). The energy tradeoff strategy the animal applies to predation risk is also applied in case of non-lethal human activities (Frid and Dill, 2002; Stankowich, 2008). Previous studies have established that anthropogenic disturbances increase stress hormone levels in several species (Creel et al., 2002; Ellenberg et al., 2006; Van Meter et al., 2009; Janin et al., 2011; Creel et al., 2013; Knapp et al., 2013).

Regarding our study sites, there are 2216 families in the 28 villages inside STR, with a total population of 11964 humans and 32704 livestock (Rajasthan Forest Department Record 2008). Two state highways (Alwar-Thanagazhi-Jaipur and the Sariska-Kalighati-Tehla) which are over 44 km in length, traverse through the designated national park. According to STR management records, from mid-2011 to mid-2014, a total of 77794 vehicles entered through one of the gates of Sariska national park area. Within this period, more than 131000 visitors entered the designated national park area. Of these, 89827 were eco-tourists and 41178 were pilgrims. The high vehicle and visitor count can be attributed to STR being a famous tourist destination, both because of its diverse fauna as well as historical structures and temples. Tigers in STR have been reported to have high fecal glucocorticoid metabolites (FGM) concentrations, linked to the disturbance in their habitat (Bhattacharjee et al., 2015). PTR, on the other hand, has only few villages on its fringes and experiences low tourism pressure, but the FGM concentrations of its tigers has not been previously measured.

Given this background, we hypothesized that breeding success of a reintroduced tiger population could be a function of GC response and habitat constraints. To test this hypothesis, we examined the physiological response to anthropogenic disturbances in these two reintroduced tiger populations, with the aim of contributing to the scientific management of large carnivores and their reintroduction, globally.

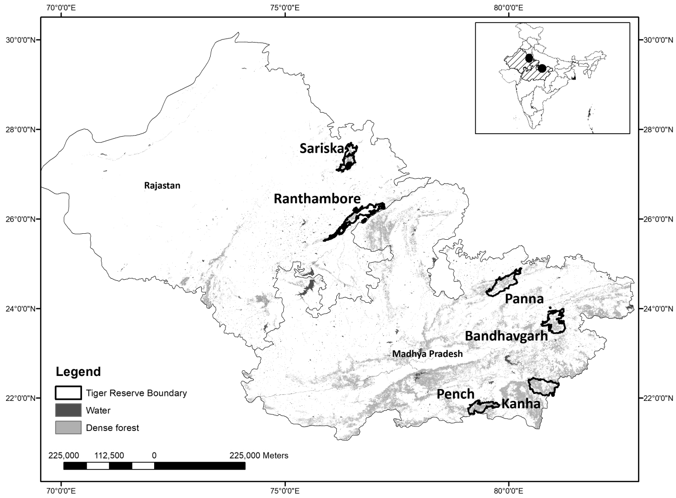

Figure 1 – Map showing location of Panna and Sariska tiger reserves along with their source population viz. Ranthambore, Kanha, Pench and Bandhavgarh tiger reserves.

Materials and methods

STR (27°5′ N to 27°33′ N and 76°17′ E to 76°34′ E) is situated in Alwar district of Rajasthan in India (Fig. 1, Tab. S1). This semi-arid tract lies in the Aravalli Hills, with altitude ranging between 540 and 777m a.s.l. and receives an annual rainfall of around 600 mm (Rodgers and Panwar, 1998). The major forest types here are tropical dry deciduous forest and tropical thorn forest (Champion and Seth, 1968). PTR (24°27′ N to 24°46′ N and 79°45′ E to 80°9′ E) is situated in Panna and Chhatarpur districts of Madhya Pradesh in India (Fig. 1, Tab. S1). The core zone comprises Panna National Park and Gangau Wildlife Sanctuary. Its altitude ranges between 330 and 540 m a.s.l. and it receives an annual rainfall of up to 1100 mm (Karanth et al., 2004). The major forest type here is dry deciduous forest (Meher-Homji, 1990).

Sample collection, hormone extraction and measurement

We collected fresh tiger scat samples from STR and PTR while monitoring reintroduced tigers based on VHF and GPS telemetry. Search efforts were focused on the home range of tigers and specifically around kill sites. Since all the tigers were radio-collared (minimum time between collaring and sample collection was approximately 1.5 months), they were monitored 24 hours a day, seven days a week. As such, it was possible to match fecal samples to individual tigers based on monitoring data, including inspection of kill sites. Freshness of samples was visually estimated, and only samples that were considered fresh were collected. We collected a total of 103 tiger scat samples from STR, and 144 from PTR between January 2013 and May 2014 for analyses of FGM concentrations and cataloged with its location (latitude and longitude), ID of the individual, date of collection and freshness. A total of 119 scat samples from PTR and all 103 from STR, were identified to individual tiger by correlating theirs and that of their kill's GPS location with that of scat, by tiger monitoring teams. Thereby, a total of nine individual tigers in PTR (of which five were founders and four were F1) and seven in STR (all founders), were sampled. The scat samples were oven dried at 80 °C for 48 hours. We extracted fecal steroid metabolites according to previously published protocols (Brown et al., 1994; Umapathy et al., 2013). We weighed 0.2-0.3 g of the dried, mixed and pulverized fecal sample and boiled it in 5 ml of 90% aqueous ethanol for 20 min. After centrifugation at 500 gf for 10 min, we recovered the supernatant and re-suspended the pellet in 5 ml of 90% aqueous ethanol, vortexed for one minute and re-centrifuged to recover the supernatant. We then combined both the ethanol supernatants, dried (in an oven at 40 °C), re-suspended in 1 ml of absolute methanol, vortexed for one minute and sonicated for 30 seconds (Branson Ultrasonics 250, CT, USA) to free particles adhering to the wall of the test tube. Samples were then stored at −20 °C until hormone assay. We examined extraction efficiency of protocols by adding known amount of radio labeled hormones in randomly selected samples before extraction. Extraction efficiencies were calculated as percentage of the amount of labeled hormones observed relative to the amount expected; these ranged between 85.2 and 93.4% (n=22).

We measured FGM concentration by cortisol enzyme immunoassay (EIA) (C. Munro, University of California, Davis), which has been successfully validated in various mammals, including tigers (Young et al., 2004; Narayan et al., 2013; Bhattacharjee et al., 2015). The cortisol polyclonal antibody (R4866) was diluted to 1:9000, HRP-conjugated cortisol 1:250000 and cortisol standards (1000-1.95 pg/well). The cross-reactivity of cortisol antibody with cortisol was 100%, prednisolone 9.90%, prednisone 6.30%, cortisone 5%, and <1% with corticosterone, desoxycorticosterone, 21 desoxycortisone, testosterone, androstenedione, androsterone, and 11-desoxycortisol (Young et al., 2004). Assay sensitivity was calculated at 90% binding and found to be 1.95 pg/well. To calculate inter and intra assay coefficients we pooled the fecal extracts and divided these into high (spiked with standards) and low (not spiked) quality controls, and used these on each EIA plate (n=10). The intra and inter coefficient of variation (CV) were 5.30% (n=10) and 9.60% (n=10) respectively. The cortisol EIA demonstrated parallel displacement curves between pooled serial dilutions of fecal samples and standards. We performed the EIA procedure as described previously (Young et al., 2004; Kumar et al., 2014; Umapathy et al., 2015).

Prey availability estimation

We used distance-sampling method for tiger prey species population estimation (Buckland et al., 2001). We surveyed a total of 41 and 48 line transects each up to 2 km in length in PTR and STR respectively, during the winter 2012-13 and 2013-14. All the line transects in both the reserves were walked in replicates of three. We combined data for the entire study period and calculated prey encounter rate for each transect. Further data analysis was done using conventional distance sampling engine in the software Distance 6.2 (Thomas et al., 2010). We selected suitable truncation distances to achieve better model fit of the data. Models were selected on the basis of quantile-quantile plot, chi-square goodness of fit test and lowest value of Akaike information criteria (AIC) (Buckland et al., 2001; Thomas et al., 2010).

Assessment of habitat conditions

We quantified vegetation indices viz. canopy cover and shrub abundance in 15 m circular plots laid at every 400 m on the line transects (Jhala et al., 2009). A total of 235 and 234 circular plots were laid in STR and PTR, respectively. Within each plot, we made visual estimation for canopy cover and scored shrub cover according to its abundance.

For further understanding the vegetation cover, we calculated normalized difference vegetation index (NDVI) (Rouse et al., 1974) for both the reserves based on LANDSAT 8 (OLI/TIRS) scenes; downloaded for STR (scene id `LC81470412013140LGN01`, downloaded on 24 December 2014) and PTR (scene id `LC81440432013119LGN01`, downloaded on 20 April 2015) from USGS website for the month of May and April 2013, respectively. We used raster calculator in ArcGIS 10.1 (ESRI, 2012) for calculating NDVI by the formula: $\mathrm{NDVI} = \frac{(\text{near infrared}-\text{red})}{(\text{near infrared}+\text{red})}$.

We downloaded Advanced Spaceborne Thermal Emission and Reflection Radiometer (ASTER) Global Digital Elevation Model (GDEM) data from the USGS global visualization viewer website, for both the sites. We used slope tool in spatial analyst in ArcGIS 10.1 to calculate slope from DEM layers (ESRI, 2012). Additionally, we calculated topographic ruggedness index or terrain ruggedness index (TRI) that measures elevation difference between a cell and mean of its eight neighboring cells (Riley, 1999) for both reserves. We used raster calculator in ArcGIS 10.1 (ESRI, 2012) to calculate TRI by the formula $\mathrm{TRI} = \sqrt{\left|(\max(3\times3)^2 - \min(3\times3)^2)\right|}$ (Cooley, 2016).

The scenes we used for calculating NDVI, were also used to calculate normalized difference water index (NDWI) (McFeeters, 1996) with raster calculator in ArcGIS 10.1 (ESRI, 2012) by the formula $\mathrm{NDWI} = \frac{(\text{green}-\text{near infrared})}{(\text{green}+\text{near infrared})}$.

Status of anthropogenic disturbances

We quantified anthropogenic disturbance indices in the circular plots (as discussed under section "Assessment of habitat conditions"), at both the sites. In each plot, we counted all the lopped (only branches were cut) and cut (cut to stump) trees and ranked weed abundance. We divided tiger occupied area in both STR and PTR into 2×2 km grids and deployed camera trap (Cuddeback Attack) pairs, within these grids. In PTR, we deployed camera traps in 109 locations accounting for 7459 trap nights, in STR, camera traps were deployed in 104 locations accounting for 1827 trap nights. We then manually counted the number of livestock, humans, and vehicles captured in each camera trap and calculated encounter rates (total no. of captures/total trap nights). We considered livestock as an anthropogenic disturbance factor since it was often accompanied by humans and overgrazing by domestic stock can lead to habitat degradation.

Statistical analyses

We subjected FGM concentrations obtained for both the reintroduced tiger populations to descriptive statistics. After checking for normality, we log transformed FGM values and used these as dependent variable in linear mixed effect model (LMM) with tiger id as random effect and tiger reserve id and sex as fixed effects, to explore the effects of tiger reserve, sex and individual tiger on FGM concentrations.

We masked rasters viz. NDVI, NDWI, DEM, Slope, and TRI, with STR and PTR boundary polygons using ArcMap's extract by mask tool (ESRI, 2009). We then obtained means and standard deviations (SD) of all the listed variables from these rasters for both the reserves. Difference between NDVI, NDWI, ungulate density, livestock density, DEM, slope and TRI, of the two reserves, were tested using independent samples t-test and their standard deviations using f (variance ratio) test. We also compared means of canopy cover, prey, human, livestock, and vehicle encounter rates, and, cutting and lopping intensity rates between the reserves, using Mann Whitney U test (data were not normally distributed). All statistical tests and modeling were done in statistical package for the social sciences (SPSS) versions 15 and 23 (SPSS 2006; IBM, 2015). Statistical significance levels were set at $p<0.05$.

We interpolated canopy cover, prey, human, livestock, and vehicle encounter rates, cutting and lopping intensity rates, as well as their SD to create rasters, using the Geostatistical wizard in ArcMap 10.1 (De Smith et al., 2007; Cressie, 2015; ESRI, 2012). After trimming the outliers, testing for assumptions and transforming the data where necessary, we used Inverse distance weighting (IDW), Ordinary kriging (OK) and Empirical Bayesian Kriging (EBK) interpolation tools to create rasters. Only in case of vehicle encounter rate for STR we used IDW tool, since its probability distribution was highly non-Gaussian, even after transformation. The rasters were selected after comparing variograms and root mean square errors, for all listed variables, for both the reserves (Tab. 1).

Table 1 – Comparison of root mean square errors (RMSE) of rasters created using different interpolation tools for all habitat variables of Panna and Sariska tiger reserves.

	Panna Tiger Reserve				Sariska Tiger Reserve			
Variable (stressor)	**IDW**[1]	**OK**[2]	**EBK**[3]	**MS**[4]	**IDW**[1]	**OK**[2]	**EBK**[3]	**MS**[4]
Human encounter rate	1.1	1.07	1.06	EBK	1.43	1.38	1.39	OK
Vehicle encounter rate	5.12	5.19	4.92	EBK	0.95			IDW
Livestock encounter rate	2.61	3.1	3.13	OK	4.27	4.1	4.2	OK
Prey encounter rate	2.21	2.3	2.01	EBK	0.9	0.88	0.93	OK
Cutting frequency	2.16	2.01	1.99	EBK	1.6	1.5	1.5	EBK
Lopping frequency	0.97	0.9	0.89	EBK	6.85	6.4	6.5	OK
Canopy cover	0.16	0.15	0.15	EBK	0.16	0.15	0.16	OK

[1] IDW: Inverse distance weighting
[2] OK: Ordinary Kriging
[3] EBK: Empirical Bayesian Kriging
[4] MS: Method selected

On an average, daily movement of the tiger was 6.90 km/day for STR (no significant difference between sexes) (Bhattacharjee et al., 2015). Considering the daily movement as the radius, we drew buffers around each scat location. We extracted habitat and disturbance variables for each buffer from the rasters (created using interpolation tool, as discussed previously) using the zonal statistics as table 2 tool in ArcMap 10.5 (ESRI, 2016). Bivariate Pearson correlation was run and highly correlated variables (>0.7) were not used together in models.

We constructed LMM (Henderson et al., 1959; Duchateau et al., 1998) to explore the variables linked to log transformed FGM levels in STR in SPSS (IBM, 2015) with tiger id as random effect to account for repeated measures of FGM for individual animals. We also performed LMM for PTR, but found none of the variables to show significant effect on FGM levels, possibly due to inherently low stress in the population and low variance among the individuals. Therefore, we focused our analyses and interpretation primarily on the more disturbed STR population, which required management inputs to address the stress issues and improve reproductive outcome. This decision allowed us to streamline the content of the paper while avoiding the redundancy from PTR analyses. The transformed FGM values (dependent variable) had a normal distribution, and the residuals of all variables showed homoscedasticity and linear relationships. We used maximum likelihood or full model (FML) for model reduction and restricted maximum likelihood (REML) for the final model to get more accurate estimates of random effect. The significance of explanatory variables (fixed effect) was tested using Wald test. We explored canopy cover and its SD, prey, human and livestock encounter rates, vehicle encounter rate SD, cutting and lopping intensity rates, lopping SD, NDVI, NDWI, DEM, slope as well as, their SD (s), and, TRI, as fixed effects. We checked the variables for multicollinearity, only variables with variance inflation factor less than 10, were used together in a model. Stepwise backward model selection was based on partial p-values and model selection was based on AIC values.

Results

FGM concentrations of reintroduced tiger populations

The mean FGM concentration in the STR tiger population (mean±SD=50.14 ± 42.84 ng/g; n=103) was significantly higher than in the PTR population (20.29 ± 16.34 ng/g; n=144) (Tab. 2, 3). Tiger ID did not have a significant random effect on the covariance structure of our model (Wald's χ^2= 0.45, p=0.65).

There were no significant differences in FGM concentrations between the sexes, in either of the reserves (sex effect and sex by reserve interaction; all p>0.05; Tab. 3). In STR, FGM concentration for female tigers was 53.25 ± 42.62 ng/g (n=74) while for males it was 42.19 ± 43.12 ng/g (n=29). In PTR, it was 19.65 ± 16.11 ng/g (n=71) and 20.00 ± 14.09 ng/g (n=57) for females and males, respectively.

Prey and habitat conditions

Prey density estimates (including livestock) were 52.80 ± 7.10 n/km^2 in PTR and 199.50 ± 12.30 n/km^2 in STR. STR had higher wild ungulate prey density (Tab. S1, Fig. 2) and livestock density (85.20 ± 11.90 n/km^2) than PTR (39.80 ± 10.90 n/km^2). Average canopy cover and shrub abundance were higher for PTR (0.33 and 1.81, respectively) than STR (0.29 and 1.72, respectively). The difference was significant for shrub abundance (p=0.01) but not for canopy cover (p=0.53). NDVI during summer was higher for STR than PTR (Tab. S1). Mean elevation, slope and associated SD were higher in STR than PTR (Tab. S1). However, the TRI value was higher for PTR than STR. (Tab. S1). During summer in STR mean NDWI was lower than PTR (Tab. S1).

Regarding human disturbance, both lopping and weed presences were higher in STR (67% and 91% plots, respectively), as compared to PTR (22% and 71% plots, respectively), although woodcutting evidence (both old and new) was slightly higher in PTR (40% plots) than STR (31% plots). Mean lopping in STR (6.37 ± 8.19) was higher than in PTR (0.60 ± 1.50; p<0.001). There was no difference between sites in mean woodcutting (STR 1.06 ± 2.11; PTR 1.39 ± 3.28).

Mean livestock encounter rate for STR (μ=4.09 ± 5.94) was higher than for PTR (μ=3.19 ± 9.60; p<0.001). In contrast, vehicle presence was significantly higher in PTR (μ=4.57 ± 7.72) than STR (μ=0.50 ± 1.20; p<0.001). There was no difference in human encounter rate (STR μ=1.40 ± 1.97; PTR μ=1.02 ± 1.20; p=0.37).

Stress and stressor relationship

The selected LMM model retained tiger id as the random effect and included human encounter rate, canopy cover, NDWI SD (positive effects on FGM), TRI and DEM SD (negative effects on FGM) as fixed effects (Wald's χ^2=6.66; p< 0.001; Tab. 4). The random effect of tiger id was statistically not significant (Wald's χ^2= 0.69, p=0.49) implying that the repeated measures of same individuals in STR does not affect the model's covariance structure.

Table 2 – Fecal glucocorticoid metabolites (FGM) concentration of tigers sampled in Sariska tiger reserve (STR) and Panna tiger reserve (PTR).

Sl. no.	Tiger Reserve	Tiger ID	Generation	Sex	No. of samples	FGM (ng/g)	SD
1	STR	ST2	Founder	Female	12	39.5	19.37
2	STR	ST3	Founder	Female	25	55.01	54.75
3	STR	ST4	Founder	Male	8	42.87	29.82
4	STR	ST5	Founder	Female	14	52.95	37.5
5	STR	ST6	Founder	Male	21	41.94	47.87
6	STR	ST9	Founder	Female	15	63.42	45.24
7	STR	ST10	Founder	Female	8	49.86	28.25
1	PTR	T1	Founder	Female	16	26.13	13.98
2	PTR	T2	Founder	Female	28	25.81	21.89
3	PTR	T3	Founder	Male	16	18.73	17.63
4	PTR	T4	Founder	Female	5	27.14	23.05
5	PTR	T5	Founder	Female	7	13.26	13.57
6	PTR	P111	F1	Male	14	15.73	12.14
7	PTR	P112	F1	Male	4	19.72	12.97
8	PTR	P212	F1	Male	14	21.94	12.86
9	PTR	P213	F1	Female	15	14.49	9.6

Table 3 – Results of linear mixed-effect model to explain FGM concentrations' association with tiger reserve and sex of the sampled individuals (Dependent variable log FGM).

Parameter	Estimate	SE	df	t	p	95% CI	
Intercept	1.22	0.1	10.24	12.67	0.001	1.00	1.43
TRID	0.28	0.09	10.03	3.25	0.01	0.09	0.47
SEX=F	−0.05	0.11	9.81	−0.5	0.63	−0.29	0.18
SEX=M	−0.02	0.11	10.77	−0.18	0.86	−0.26	0.22
TRID * SEX	0.16	0.11	9.54	1.55	0.15	−0.07	0.40

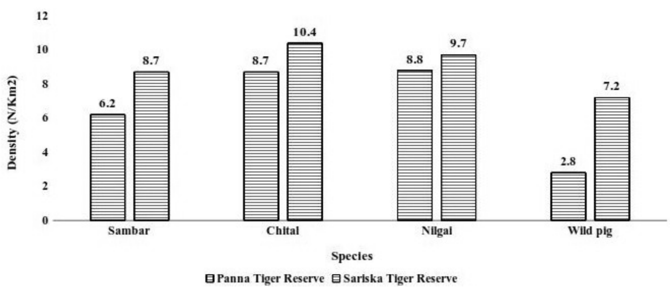

Figure 2 – Comparison of major tiger prey species densities between Panna and Sariska tiger reserves.

Discussion

Monitoring stress in reintroduced animals is important to ascertain animal wellbeing and reintroduction success (Teixeira, 2007; Gelling, 2012). The results of this study agree with our earlier findings (Bhattacharjee et al., 2015) that reintroduced tigers in STR have high indices of chronic stress. The mean FGM concentration of the Sariska tiger population was more than twice to that of Panna tiger population. We did not have pre-translocation FGM values for the studied tigers, so we cannot rule out completely a possible founder effect from the source site. However, we are convinced that this is unlikely. If the founders suffered chronic stress in their original habitat (RTR), the reproductive outcome would have been impaired in the source as well. RTR is one of the high-density tiger areas in India with a high reproductive rate (Jhala et al., 2015; Sadhu et al., 2017). Therefore, we presume that the individuals released were not affected by chronic stress. In terms of acute stress due to translocation, any stress hormone remains in body for a maximum period of 72 hours and stress levels are known to peak between 22 to 26 hours post stressful activity in big cats (Young et al., 2004). The scat samples used in this study were collected after a few months of translocation, excluding an effect of post translocation stress on our FGM measures.

High physiological stress in Sariska tigers necessitated the identification of habitat constraints that could act as stressors. For large carnivores such as tigers, preferred habitat parameters have been identified mainly as high prey density, forest contiguity, cover, thick understory, high altitude, steep slopes, proximity to water and low human impact, among others (Miquelle et al., 1999; Karanth and Sunquist, 2000; Sunarto et al., 2012; Takahata et al., 2014). Prey, the first essential requisite for tiger presence, was available in high densities to tigers in Sariska, and ungulate densities were higher in Sariska than in Panna. Given the low population of tigers and the overall high wild prey density in Sariska, the problem of demand-supply does not appear to exist and nutritional stress can be ruled out as the reason behind the poor breeding. Water, the second essential requisite, was a constraint in Sariska, with low water presence during summer months. Vegetation cover was high in Sariska and comparable to Panna. However, areas with high canopy cover in Sariska are frequented by both people and tiger; humans use it for fuelwood and fodder collection, tigers to get adequate cover from tropical heat and for hunting. Consequently, the role of terrain/topographic features which also provide cover, becomes significantly important. Topographic features not only provide cover to both prey and predator to avoid detection by each other (Canon et al., 1997; Lingle, 2002; Gorini, 2012), but also, impart protection and cover to animals from human and human-induced disturbances (Peyton, 1980; Sawyer, 2007). Low values of TRI in Sariska as compared to Panna, indicate inadequate terrain complexity. In Panna, the steep and rugged gorges act as escape cover and provide a secure environment for breeding tigers, which is absent in case of Sariska. Even so, the high FGM values in Sariska cannot be explained by insufficient water availability and terrain complexity alone. Low water availability can, however, elevate the basal cortisol levels and low terrain complexity reduce the chances of animals successfully avoiding encounters with humans, so that additional stress in the form of anthropogenic disturbance can lead to ‘allostatic overload’ in the animals (McEwen, 2007).

The results of various anthropogenic disturbance indices as assessed by us revealed that overall anthropogenic disturbance was significantly higher in Sariska as compared to Panna. Sariska had higher lopping, weed and livestock presence than PTR. In STR, the intensity of lopping was higher than cutting, reflecting that people are using the reserve forest for their subsistence, exploiting it for fuelwood and fodder. Human encounter rate was also higher in STR, but the difference was not statistically significant. Deeper examination of the data revealed that people frequently captured in PTR were forest department staff, carrying out various management related activities in and around the roads, in contrast, in STR, the people captured were mostly villagers moving inside the reserve to collect fuelwood and fodder or grazing livestock, infiltrating the forest irrespective of presence of roads and trails. Thus, even though similar number of people may be moving inside these reserves, the manner in which they are moving and the purpose of their movement are different. In Sariska, movement of people contributes to forest degradation, while in Panna it contributes to forest management. Further, in STR villages are located within small distance of tiger home ranges unlike in PTR where villages are located outside or in the fringe area of tiger habitats (Tab. S1).

Table 4 – Results of linear mixed-effect model to explain FGM concentrations in tiger population of Sariska tiger reserve (Dependent variable log FGM).

Parameter	Estimate	SE	df	t	*p*	95% CI	
Intercept	18.42	6.23	88.58	2.96	0.001	6.04	30.81
Human encounter rate	1.48	0.62	68.11	2.40	0.02	0.25	2.72
Canopy cover	4.96	1.71	42.39	2.89	0.01	1.50	8.42
NDWI SD	1.45	0.67	94.62	2.17	0.03	0.12	2.78
DEM SD	−4.21	1.71	84.87	−2.47	0.02	−7.60	−0.82
TRI	−2.02	1.37	94.93	−1.47	0.15	−4.74	0.71

Only some carnivores such as wolves have behavioral plasticity and reproduction capability that makes it possible for them to survive in close proximity to human beings. Species such as bears and tigers that have specialized requirements related to habitat quantity and quality are susceptible to threats posed by anthropogenic pressures (Weaver et al., 1996). High FGM concentrations in STR tiger population were positively linked to the presence of humans; high canopy cover, low water availability and to homogeneous elevation and low TRI values, within their habitat. Therefore, at a fine scale, it seems that the simple act of a villager going inside the forest areas frequented by tigers, is leading to increased physiological stress in tigers of STR. Stress is reduced where tigers are occupying areas with homogenous distribution of water and the opportunity provided by topographic features to avoid detection. In PTR, stressful encounters for tigers are few and far apart, with the opportunity to navigate through escarpments, resulting in low average FGM concentrations of the population and high reproductive output.

Thus, we can infer that tigers experience physiological stress in case of frequent encounters with human beings, which is exacerbated in a habitat that does not provide sustainable space to negotiate or avoid these disturbance elements, which, eventually, seems to affect their reproductive output. In comparison, if there is low human presence within their habitat, coupled with complex terrain and optimum water availability, tigers experience low stress and thereby attain healthy population growth.

Implications for tiger conservation

Tiger conservation efforts are increasingly focused on creating large undisturbed habitats, which involve voluntary relocation of people and legislative safeguards. However, the conservation objective can only be accomplished by spatial prioritization and rationalization of the resources, and accordingly management inputs have to be modified. It is also important to address anthropogenic issues before tiger translocation is implemented. In India, the key policy document for conservation of tiger that was drafted after extinction of tiger in Sariska (Narain et al., 2005) recommended reintroduction as one of the conservation agendas. With knowledge and experience gained through these tiger reintroductions in Sariska and Panna, this reintroduction agenda can now be improved through specific policy inputs in terms of land management and field operation strategies.

To the best of our knowledge, this is the first time that habitat-stress-reproduction relationships, incorporating multiple habitat elements across two contrasting populations were studied for wild tigers in general, and reintroduced tiger populations in specific. The results of this study affirm that costly conservation efforts for tigers, such as reintroduction programs, need to consider the levels of anthropogenic disturbance and availability of secure environments as major drivers of success/failure of breeding and population growth. This is especially true for habitats that lost their original population to anthropogenic factors. For such reintroduced populations, it is prudent to remove/reduce the disturbance elements while continuing to closely monitor the habitat and physiological responses of the tigers. Finally, our results suggest that successful reintroduction and population growth is a function of habitable space availability, including terrain complexity and low anthropogenic interactions. Therefore, conservation success of future reintroductions should be modeled and visualized keeping in view these parameters.

References

Beale C.M., Monaghan P., 2004. Human disturbance: people as predation-free predators? J. Appl.Ecol. 41(2): 335–343.

Bhattacharjee S., Kumar V., Chandrasekhar M., Malviya M., Ganswindt A., Ramesh K., Sankar K., Umapathy G., 2015. Glucocorticoid stress responses of reintroduced tigers in relation to anthropogenic disturbance in Sariska Tiger Reserve in India. PLoS ONE 10(6): e0127626.

Brown J.L., Wasser S.K., Wildt D.E., Graham L.H., 1994. Comparative aspects of steroid hormone metabolism and ovarian activity in felids, measured noninvasively in feces. Biol.Reprod. 51(4): 776–786.

Buckland S.T., Anderson D.R., Burnham K.P., Laake J.L., Borchers D.L., Thomas L., 2001. Introduction to distance sampling: estimating abundance of biological populations, Oxford University Press, Oxford.

Canon S.K., Bryant F.C., 1997. Bed-site characteristics of pronghorn fawns. J. Wildl. Manage. 61(4): 1134–1141.

Champion H.G., Seth S.K., 1968. Revised forest types of India. Government of India, New Delhi.

Cooley S.W., 2016. Terrain Roughness - 13 ways. GIS4Geomorphology. Available at http://gis4geomorphology.com/watershed [Accessed January 2017]

Creel S., Christianson D., Schuette P., 2013. Glucocorticoid stress responses of lions in relationship to group composition, human land use, and proximity to people. Conserv. Physiol.1(1) cot021.

Creel S., Fox J.E., Hardy A., Sands J., Garrott B., Peterson R.O., 2002. Snowmobile activity and glucocorticoid stress responses in wolves and elk. Conserv. Biol. 16: 809–814.

Cressie N., 2015. Statistics for spatial data. John Wiley & Sons, New Jersey.

De Smith M.J., Goodchild M.F., Longley P., 2007. Geospatial analysis: a comprehensive guide to principles, techniques and software tools. Troubador Publishing Ltd., Leicester.

Dinerstein E., Loucks C., Wikramanayake E., Ginsberg J., Sanderson E., Seidensticker J., Forrest J., Bryja G., Heydlauff A., Klenzendorf S., Mills J., O'Brien T., Shrestha M., Ross S., Songer M., 2007. The fate of wild tigers. BioScience 57: 508–514.

Duchateau L., Janssen P., Rowlands J., 1998. Linear mixed models. An introduction with applications in veterinary research, ILRI (International Livestock Research Institute), Nairobi, Kenya.

Ellenberg U., Mattern T., Seddon P.J., Jorquera G.L., 2006. Physiological and reproductive consequences of human disturbance in Humboldt penguins: The need for species-specific visitor management. Biol. Conserv. 133(1): 95–106.

ESRI (Environmental Systems Research Institute), 2009. ArcMap 9.3. ESRI, Redlands, California.

ESRI (Environmental Systems Research Institute), 2012. ArcMap 10.1. ESRI, Redlands, California.

ESRI (Environmental Systems Research Institute), 2016. ArcMap 10.5. ESRI, Redlands, California.

Frid A., Dill L., 2002. Human-caused disturbance stimuli as a form of predation risk. Conserv. Ecol. 6(1) 11. http://www.consecol.org/vol6/issl/artll/

Gelling M., Johnson P.J., Moorhouse T.P., Macdonald D.W., 2012. Measuring animal welfare within a reintroduction: an assessment of different indices of stress in water voles Arvicola amphibius. PLoS ONE7 7: e41081.

Gorini L., Linnell J.D., May R., Panzacchi M., Boitani L., Odden M., Nilsen E., 2012. Habitat heterogeneity and mammalian predator-prey interactions. Mamm. Rev. 42(1): 55–77.

Henderson C.R., Kempthorne O., Searle S.R., Von Krosigk C.M., 1959. The estimation of environmental and genetic trends from records subject to culling. Biometrics 15(2): 192–218.

Hayward M.W., Somers M., 2009. Reintroduction of top-order predators, John Wiley & Sons, New Jersey.

Hayward M.W., Kerley G.I., Adendorff J., Moolman L.C., O'brien J., Sholto-Douglas A., Bissett C., Bean P., Fogarty A., Howarth D., Slater R., 2007. The reintroduction of large carnivores to the Eastern Cape, South Africa: an assessment. Oryx 41(2): 205–214.

IBM Corp. Released 2015. IBM SPSS Statistics for Windows. Version 23.0. Armonk, NY.

Janin A., Léna J.P., Joly P., 2011. Beyond occurrence: body condition and stress hormone as integrative indicators of habitat availability and fragmentation in the common toad. Biol. Conserv. 144: 1008–1016.

Jhala Y.V., Qureshi Q., Gopal R., 2015. The status of tigers, copredators & prey in India 2014. National Tiger Conservation Authority, New Delhi and Wildlife Institute of India, Dehradun. TR2015/021

Jhala Y.V., Qureshi Q., Gopal R., Amin R., 2009. Field Guide: Monitoring tigers, co-predators, prey and their habitats, 3rd ed. Wildlife Institute of India and National Tiger Conservation Authority, Dehradun.

Johnsingh A.J.T., Madhusudan M.D., 2009. Tiger reintroduction in India: conservation tool or costly dream? In: Hayward M.W., Somers M.J. (Eds.) Reintroduction of top-order predators. Wiley-Blackwell, West Sussex. 146–163.

Karanth K.U., Sunquist M.E., 2000. Behavioural correlates of predation by tiger (*Panthera tigris*), leopard (*Panthera pardus*) and dhole (*Cuon alpinus*) in Nagarahole, India. J. Zool. 250(2): 255–265.

Karanth K.U., Chundawat R.S., Nichols J.D., Kumar N., 2004. Estimation of tiger densities in the tropical dry forests of Panna, Central India, using photographic capture-recapture sampling. Anim. Conserv. 7(3): 285–290.

Knapp C.R., Hines K.N., Zachariah T.T., Perez-heydrich C., Iverson J.B., Sandra D., Romero L.M., 2013. Physiological effects of tourism and associated food provisioning in an endangered iguana. Conserv. Physiol.1 cot032.

Kumar V., Reddy V.P., Kokkiligadda A., Shivaji S., Umapathy G., 2014. Non-invasive assessment of reproductive status and stress in captive Asian elephants in three south Indian zoos. Gen. Comp. Endocrinol. 201: 37–44.

Lingle S., 2002. Coyote predation and habitat segregation of white-tailed deer and mule deer. Ecology 83(7): 2037–2048.

McEwen B.S., 2007. Physiology and neurobiology of stress and adaptation: central role of the brain. Physiol. Rev. 8: 873–904.

McFeeters S.K., 1996. The use of the Normalized Difference Water Index (NDWI) in the delineation of open water features. Int. J. Remote Sens. 17(7): 1425–1432.

Meher-Homji V.M., 1990. Vegetation types of India in relation to environmental conditions. In: Daniel J.C., Serrao J.S. (Eds.) Conservation in developing countries: problems and prospects. Oxford University Press, Bombay. 95–100

Miquelle D.G., Smirnov E.N., Merrill T.W., Myslenkov A.E., Quigley H.B., Hornocker M.G., Schleyer B., 1999. Hierarchical spatial analysis of Amur tiger relationships to habitat and prey. In: Seidensticker J., Christie S., Jackson P. (Eds.) Riding the Tiger: tiger conservation in human-dominated landscapes. Cambridge University Press, Cambridge. 71–99.

Narain S., Singh S., Panwar H.S., Gadgil M., 2005. Joining the Dots: the report of the Tiger Task Force. Project Tiger, Union Ministry of Environment and Forests, New Delhi.

Narayan E.J., Parnell T., Clark G., Martin-Vegue P., Mucci A., Hero J.M., 2013. Faecal cortisol metabolites in Bengal (*Panthera tigris tigris*) and Sumatran tigers (*Panthera tigris sumatrae*). Gen. Comp. Endocrinol. 194: 318–325.

Peyton B., 1980. Ecology, distribution, and food habits of spectacled bears, *Tremarctos ornatus*, in Peru. J. Mammal. 61(4): 639–652.

Reeder D. M., Kramer K. M., 2005. Stress in free-ranging mammals: integrating physiology, ecology, and natural history. J. Mammal. 86(2): 225–235.

Riley S.J., DeGloria S.D., Elliot R., 1999. A terrain ruggedness index that quantifies topographic heterogeneity. Intermt. J. Sci. 5(1–4): 23–27.

Ripple W.J., Beschta R.L., 2003. Wolf reintroduction, predation risk, and cottonwood recovery in Yellowstone National Park. Forest Ecol. Manag. 184(1): 299–313.

Rodgers W.A., Panwar H.S., 1988. Planning a wildlife protected area network in India. Government of India, New Delhi.

Rouse J. Jr., Haas R.H., Schell J.A., Deering D.W., 1974. Monitoring vegetation systems in the Great Plains with ERTS. NASA special publication 351, 309.

Sadhu A., Jayam P.P.C., Qureshi Q., Shekhawat R.S., Sharma S., Jhala Y.V., 2017. Demography of a small, isolated tiger (*Panthera tigris tigris*) population in a semi-arid region of western India. BMC Zool. 2(1): 16.

Sankar K., Qureshi Q., Nigam P., Malik P. K., Sinha P. R., Mehrotra R. N., Gopal R., Bhattacharjee S., Mondal K., Gupta S., 2010. Monitoring of reintroduced tigers in Sariska Tiger Reserve, Western India: preliminary findings on home range, prey selection and food habits. Trop. Conserv. Sci. 3(3): 301–318.

Sapolsky R., 2004. Social status and health in humans and other animals. Annu. Rev. Anthropol. 33 393–418.

Sapolsky R.M., Romero L.M., Munck A.U., 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr. Rev. 21(1): 55–89.

Sarkar M.S., Ramesh K., Johnson J.A., Sen S., Nigam P., Gupta S.K., Murthy R.S., Saha G.K., 2016. Movement and home range characteristics of reintroduced tiger (*Panthera tigris*) population in Panna Tiger Reserve, central India. Eur. J. Wildl. Res. 62(5): 537–547.

Sawyer H., Nielson R.M., Lindzey F.G., Keith L., Powell J.H., Abraham A.A., 2007. Habitat selection of Rocky Mountain elk in a nonforested environment. J. Wildl. Manage. 71(3): 868–874.

Schoech S.J., Rensel M.A., Bridge E.S., Boughton R.K., Wilcoxen T.E., 2009. Environment, glucocorticoids, and the timing of reproduction. Gen. Comp. Endocrinol. 163(1): 201–207.

Smith J.A., Suraci J.P., Clinchy M., Crawford A., Roberts D., Zanette L.Y., Wilmers C.C., 2017. Fear of the human "super predator" reduces feeding time in large carnivores. Proc. R. Soc. B 284(1857): 20170433.

SPSS Inc. Released 2006. SPSS for Windows, Version 16.0. SPSS Inc., Chicago.

Stankowich T., 2008. Ungulate flight responses to human disturbance: a review and meta-analysis. Biol. Conserv. 141(9): 2159–2173.

Sunarto S., Kelly M.J., Parakkasi K., Klenzendorf S., Septayuda E., Kurniawan H., 2012. Tigers need cover: Multi-scale occupancy study of the big cat in Sumatran forest and plantation landscapes. PLoS ONE 7(1): e30859.

Takahata C., Nielsen S.E., Takii A., Izumiyama S., 2014. Habitat selection of a large carnivore along human-wildlife boundaries in a highly modified landscape. PLoS ONE 9(1): e86181.

Teixeira C., Deazevedo C., Mendl M., Cipreste C., Young R., 2007. Revisiting translocation and reintroduction programmes: the importance of considering stress. Anim. Behav. 73(1): 1–13.

Thomas L., Buckland S.T., Rexstad E.A., Laake J.L., Strindberg S., Hedley S.L., Bishop J.R.B., Marques T.A., Burnham K.P., 2010. Distance software: design and analysis of distance sampling surveys for estimating population size. J. Appl. Ecol. 47(1): 5–14.

Umapathy G., Deepak V., Kumar V., Chandrasekhar M., Vasudevan K., 2015. Endocrine profiling of endangered tropical chelonians using noninvasive fecal steroid analyses. Chelonian Conserv. Biol. 14(1): 108–115.

Umapathy G., Kumar V., Kabra M., Shivaji S., 2013. Detection of pregnancy and fertility status in big cats using an enzyme immunoassay based on 5α-pregnan-3α-ol-20-one. Gen. Comp. Endocrinol. 180: 33–38.

Van Meter P.E., French J.A., Dloniak S.M., Watts H.E., Kolowski J.M., Holekamp K.E., 2009. Fecal glucocorticoids reflect socio-ecological and anthropo-genic stressors in the lives of wild spotted hyenas. Horm. Behav. 55: 329–337.

Weaver J.L., Paquet P.C., Ruggiero L.F., Ruggierot L.F., 1996. Resilience and conservation of large carnivores in the Rocky Mountains. Conserv. Biol. 10(4): 964–976.

Weeks A.R., Sgro C.M., Young A.G., Frankham R., Mitchell N.J., Miller K.A., Byrne M., Coates D.J., Eldridge M.D., Sunnucks P., Breed M.F., 2011. Assessing the benefits and risks of translocations in changing environments: a genetic perspective. Evol. Appl. 4(6): 709–725.

Whirledge S., Cidlowski J.A., 2013. A role for glucocorticoids in stress-impaired reproduction: beyond the hypothalamus and pituitary. Endocrinology 154(12): 4450–4468.

Wingfield, J.C., Kitaysky, A.S., 2002. Endocrine responses to unpredictable environmental events: stress or anti-stress hormones? Integr. Comp. Biol. 42(3): 600–609.

Wingfield J.C., Sapolsky R.M., 2003. Reproduction and resistance to stress: when and how? J. Neuroendocrinol. 15(8): 711–24.

Wingfield J.C., O'Rielly K.M., Astheimer L.B., 1995. Modulation of the adrenocortical responses to acute stress in arctic birds: a possible ecological basis. Am. Zool. 35(3): 285–294.

Young K.M., Walker S.L., Lanthier C., Waddell W.T., Monfort S.L., Brown J.L., 2004. Noninvasive monitoring of adrenocortical activity in carnivores by fecal glucocorticoid analyses. Gen. Comp. Endocrinol. 137(2): 148–165.

Associate Editor: L.A. Wauters

Supplemental information

Additional Supplemental Information may be found in the online version of this article:

Table S1 Comparison of Sariska tiger reserve against Panna tiger reserve, across different habitat parameters.