RESEARCH ARTICLES
ОРИГИНАЛЬНЫЕ СТАТЬИ

ABIOTIC FACTORS CAN AFFECT FECAL GLUCOCORTICOID METABOLITE LEVEL OF *PANTHERA TIGRIS ALTAICA* (FELIDAE) IN THE WILD IN THE RUSSIAN FAR EAST

Sergey V. Naidenko[1,*], Anastasia Yu. Gorbunova[2], Evgeniy A. Ivanov[1], Jose A. Hernandez-Blanco[1], Elena I. Shevtsova[3], Dina S. Matiukhina[3], Ivan V. Seryodkin[4], Dale G. Miquelle[5], Svetlana V. Soutirina[6], Alexander Yu. Kalinin[7], Viatcheslav V. Rozhnov[1]

[1]*A.N. Severtsov Institute of Ecology and Evolution of the RAS*, *Russia*
[2]*University College Dublin, Ireland*
[3]*Joint Direction of Kedrovaya Pad' State Biosphere Nature Reserve and Land of Leopard National Park*, *Russia*
[4]*Pacific Geographical Institute FEB RAS*, *Russia*
[5]*Wildlife Conservation Society*, *USA*
[6]*Sikhote-Alin State Nature Reserve*, *Russia*
[7]*Bastak State Nature Reserve*, *Russia*
**e-mail: snaidenko@mail.ru*

Received: 04.10.2024. Revised: 16.05.2025. Accepted: 21.05.2025.

An estimation of welfare/stress level in wild animals helps to determine the factors affecting their conditions. *Panthera tigris* is a species, that has evolved in tropical Asia, but its subspecies *Panthera tigris altaica* (hereinafter – Amur tiger) lives under extreme climatic conditions in the Russian Far East. The aim of this study was to estimate the effect of low air temperature and high snow level as potential stressors for Amur tigers. Changes in glucocorticoids levels may be a good indicator to evaluate an animal's welfare/stress level. We collected a total of 209 fecal samples from Amur tigers. The samples were kept frozen until analysis. We used previously validated commercial kits to quantify fecal glucocorticoid metabolites (FGCM) in Amur tigers. We determined Amur tigers' FGCM levels in four Protected Areas, situating from the north to the south, suggesting that FGCM will be different in northern and southern locations (depending on weather conditions). We did not find significant differences in FGCM level related to the Amur tigers' location. However, we found correlation with the climatic conditions for these geographical points. Air temperature had a significant effect on FGCM levels in Amur tigers both in the study period as a whole ($F = 4.16$; $df = 1$; $p = 0.043$) and in the snowy period taken separately ($F = 4.92$; $df = 1$; $p = 0.028$). The FGCM level in Amur tigers increased significantly when the ambient temperature dropped. This correlation was significant over the whole studied period and during the snowy period. We assume that low ambient temperature should intensify the metabolic rate in such tropical species as the Amur tiger. The snow level did not affect considerably the FGCM level. However, we discovered a positive correlation between the level of hormones and snow depth. When the snow level exceeded 60 cm, FGCM concentrations declined by more than a half (from 1441 ± 561 ng/g to 668 ± 125 ng/g). Especially high snow depth led to a low FGCM level in Amur tigers, which may be related to the changes in their movement patterns and hunting efficiency. FGCM monitoring is an effective tool for the estimation of the Amur tiger's physiological status in the wild. However, the air temperature *per se* might affect significantly the FGCM level in the Amur tiger (tropical species) and it should be taken into account when scientists interpret the results.

Key words: air temperature, cortisol, geographic differences, hormonal monitoring, snow depth

Introduction

Measures of stress in individuals from animal populations in the wild can provide insight into population status and factors that may influence population persistence (Paquet & Darimont, 2010; Fanson et al., 2012; Creel et al., 2013; Bhattacharjee et al., 2015). One of the most reliable indices of stress is the level of glucocorticoids (GC) in blood plasma/serum. Glucocorticoids play a very important role in the regulation of metabolic processes in mammals and providing adaptive responses to any external impacts on the organism (Sapolsky et al., 2000; Romero, 2004). Such impacts may include unfavourable climatic factors like low air temperature, which was used in experimental studies on small mammals as «cold stress» (Novikov et al., 2015). The response to low temperatures requires an increase of metabolic rate and an increase of GC level in blood plasma. GC levels moderate the physiological responses to stress by mobilising internal resources of the organism, allowing it to overcome the negative effect of stressors (under acute stress). Under long-term stress, this physiological response can exhaust an organism's resources, sometimes leading to death associated with chronic stress. Therefore, changes in GC levels may be good indicators of animal welfare/stress levels (Möstl & Palme, 2002; Palme, 2012; Ugaz et al., 2013).

Usually, hormone measurements (including GCs) are conducted using blood plasma/serum (Naidenko & Erofeeva, 2005; Goritz et al., 2006). However, this method is most suitable for laboratory animals or mammals in captivity, where the sampling procedure takes little time and induces comparatively little stress. In the wild, this method is less reliable because the process of capturing (for sampling) can significantly increase GC levels in blood plasma/serum within 5–10 min. (Delehanty & Boonstra, 2012; Loschagina et al., 2017, 2018). An alternative approach is the use of non-invasive measurements of GC or their metabolites in various substrates (hairs, feces, urine) (Saltz & White, 1991; Davenport et al., 2006; Pribbenow et al., 2014). This method may be realised without any contact with or influence on the animal. Moreover, this approach gives the cumulative estimation of hormonal levels and individual hormonal status over an extended period, ranging from a few hours (from urine) to several months (from hairs) (Sheriff et al., 2011; but see Colding-Jørgensen et al., 2023). In felids (including *Panthera tigris* Linnaeus, 1758 (hereinafter – tiger)), glucocorticoid metabolites are excreted in feces about 24 h after perceiving a stressor (Pavlova & Naidenko, 2008; Naidenko et al., 2011). Such studies are now widely conducted in captive and wild mammals (Ayres et al., 2012; Bosson et al., 2009; Christofoletti et al., 2010; Dehnhard et al., 2001; Huber et al., 2003; Hunt et al., 2006; Dittami et al., 2008; Rangel-Negrín et al., 2009; Ganswindt et al., 2012; Mastromonaco et al., 2014), including some carnivores (Pavlova & Naidenko, 2008; Naidenko et al., 2011; Fanson & Wielebnowski, 2013; Pribbenow et al., 2014; Bhattacharjee et al., 2015).

The tiger is one of the largest terrestrial carnivores in the world, and a flagship species for the ecosystem conservation across Asia. Over the XX century its total population has declined from an estimated 100 000 to 3000–3500 individuals (Dinerstein et al., 2007). An international tiger summit conducted in 2010 in Saint-Petersburg (Russia) aimed to double the number of wild tigers in the wild by 2022. The last data estimate the world tiger population as 4865–5697 individuals (WWF, 2022), approximating an increase of 1.4–1.9 fold. *Panthera tigris altaica* Temminck, 1844 (hereinafter – Amur tiger) lives in the extreme northern environments of the Russian Far East and northeast China. The range of Amur tigers extends in a north-south gradient for more than 1000 km, and in an east-west gradient from the Sea of Japan to several hundred kilometers inland. The tiger as a species has evolved in south-eastern Asia (tropical forests) (Luo et al., 2004; Driscoll et al., 2009) and later dispersed to the Russian Far East, where it encountered severe winter climatic conditions. In the northernmost areas, winter temperature may reach -45°C, and snow levels may exceed 1 m for several months.

Earlier, we validated a method for measuring fecal glucocorticoid metabolites (FGCM) levels in Amur tiger feces (using ACTH-test and helicopter transportation) (Naidenko et al., 2011) and showed that FGCM levels remain constant for approximately a week under conditions when air temperatures remain below +7°C (Naidenko & Rozhnov, 2009). The higher FGCM levels in wild Amur tigers in winter than in autumn and spring have been described earlier (Naidenko et al., 2011). The separate study in Russian zoos showed a significant negative correlation between FGCM levels and air temperatures in winter (Ivanov et al., 2017). We assumed that this negative correlation between air temperature and FGCM levels may exist in the wild. We hypothesised that low air temperatures and high snow levels are stressors for Amur tigers that will result in increased FGCM concentrations. Based on this idea, we also expected to see higher FGCM levels in northern areas with more severe climatic conditions. The aim of this study was to estimate the effect of air temperature and snow depth on FGCM level in Amur tigers at the four points situated in the north and south of the subspecies range.

Material and Methods

Samples were collected in 2011–2014 from four study sites in the Russian Far East. The sites (Fig. 1) represented both coastal and inland areas situated across the south-north gradient of Amur tiger range. The Land of the Leopard National Park (LLNP: 2620 km^2) represents the southernmost range of the Amur tiger in Russia, close to the Sea of Japan. The winter weather patterns in the LLNP are less continental than in other areas, resulting in higher temperatures and low snow levels. The Ussuriisky State Nature Reserve (UR: 405 km^2) is situated just east of the LLNP. It is similar in temperature conditions but shows higher depths of snow accumulation than the LLNP. Although the Sikhote-Alin State Nature Biosphere Reserve (SAR: 4016 km^2) is further north, winter temperatures are moderated by the proximity of the Sea of Japan, and snow depth is low during the winter. The Bastak State Nature Reserve (BR: 1271 km^2) is the northernmost and most inland of all sites surveyed, and consequently had the lowest winter temperature and generally the deepest snow.

Feces samples were collected mainly in winter (November – March) with some samples collected before or after the snow cover. Fecal samples were collected less than 24 h after deposition when air temperatures were higher than +5°C, and no more than 5 days after deposition when the air temperature was below +5°C. The age of feces samples was estimated based on the personal experience of researchers, changes in weather conditions (snowfall and thaw) and age of the Amur tiger tracks associated with the scat. Between 2 g and 20 g of fecal material were collected in plastic bags and labelled by location (GPS co-ordinates), date, sex (estimated from the size of the footprint) (Kerley et al., 2007; Yudin & Yudina, 2009), identity of the Amur tiger (when deposition coincided with camera trap data or a location of a GPS-collared individual) (Hernandez-Blanco et al., 2015). Amur tiger scat is easy to identify in winter by its large size because other large carnivores are either in hibernation during the survey period (both *Ursus arctos* Linnaeus, 1758 and *Ursus thibethanus* Guvier, 1823) or, in the case of *Canis lupus* Linnaeus, 1758, extremely rare in the study area (Miquelle et al., 2005). All samples were stored and transported frozen under -18°C. FGCM concentrations were measured in the laboratory of the Severtsov Institute of Ecology and Evolution of the RAS (Moscow, Russia) according to the method described earlier (Pavlova & Naidenko, 2008; Naidenko et al., 2019). Metabolite extraction was conducted with 90% methanol (Jewgenow et al., 2006). The FGCM concentration was estimated with the commercial kits of «Immunotekh» (Moscow, Russia), which were previously validated for the Amur tiger (Naidenko et al., 2011). This method for measuring FGCM levels in the Amur tiger's feces was validated using ACTH-test and helicopter transportation and showed that the level of steroid hormones remains constant for approximately a week under conditions when air temperatures remain below +7°C. The final concentration was calculated on 1 g of dry feces. For this purpose, we have calculated the relative humidity of each sample (the aliquot), drying it to constant mass (weighting them before and after it). This approach was earlier determined to be the most efficient for estimating the FGCM concentration in feces (Naidenko et al., 2019). Cross-reactivity of antibodies to cortisol was 6% for prednisolone and less than 1% for all other tested steroids. The sensitivity of the antibodies was 1.8 ng/ml of the extracts (about 45 ng/g of dry feces). The intra-assay coefficient was 2.89% (n = 209), the inter-assay coefficient was 11.23% (n = 7). The interassay coefficient was calculated for the sample with the FGCM concentration 200 ng/ml. Normally, two samples were used as quality controls with low (6.25 ng/ml) and high (200 ng/ml) concentrations.

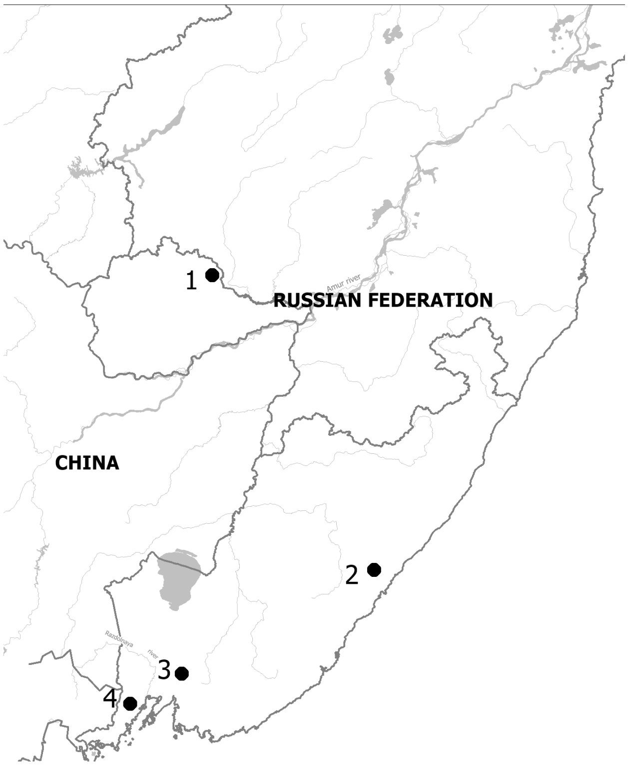

Fig. 1. Study sites on the Russian Far East. Designations: 1 – Bastak State Nature Reserve, 2 – Sikhote-Alin State Nature Biosphere Reserve, 3 – Ussuriisky State Nature Reserve, 4 – Land of the Leopard National Park.

The identification of animals using molecular-genetic methods was not conducted, raising the possibility that some samples could have been collected from the same animal. We attempted to minimise this possibility by identifying individuals whenever possible using camera-traps, satellite collars and size of paw print.

Daily weather indexes (average daily air temperature and snow depth) were estimated based on the data from the nearest weather broadcast stations of the Federal Russian meteorological stations (Birobidzhan, Terney, Artyom, Barabash), which are available at www.rp5.ru. We used the average value of weather parameters for three days prior to the estimated date of defecation. The time lag of glucocorticoid excretion in the Amur tiger constitutes about 24 h (Naidenko et al., 2011). We used the interval of three days because some samples were 2–4 days old (dating was imprecise), and excretion time of GC could have exceeded 24 h in some cases (Rozhnov et al., 2010; Naidenko et al., 2011). The samples that were not dated precisely were used only in the analysis of the «study site» effect.

The weather conditions at study sites are shown in Table 1. The sampling lasted longer in the Sikhote-Alin State Nature Reserve and covered the autumn and spring periods that affected the study site temperature. We paid attention to this point in results and discussion.

Statistics

To approach a normal distribution, we converted the FGCM concentrations by log10 and used a GLM (Generalised Linear Model) approach, using the factors air temperature, snow depth, and study site as parameters explaining the variation in FGCM concentrations. We also conducted an analysis excluding the study site parameter. The GLM was conducted for the whole data set and, separately, for the samples collected only in the snow period. The comparison of FGCM concentrations at various air temperatures or snow levels was conducted using the Mann-Whitney test for non-logged concentrations. The analysis was conducted in Statistica 12 (StatSoft, USA).

Results

In total, we collected 209 fecal samples from Amur tigers (Table 2). Differences in number of collected samples were related to the intensity of fieldwork and Amur tiger density on the various study sites. The samples where dating was not precise were excluded from the analysis of effect of air temperature and snow depth, but were used for the analysis of study site effect.

The air temperature had a significant effect on FGCM levels in Amur tigers both in the study period as a whole ($F = 4.16$; $df = 1$; $p = 0.043$) (Fig. 2) and in the snowy period taken separately ($F = 4.92$; $df = 1$; $p = 0.028$). The FGCM concentrations were at maximum at low air temperatures. When the air temperature was below -20°C, the FGCM level was three times higher than when the air temperature was between -10°C and 0°C (2561 ± 972 ng/g and 894 ± 202 ng/g, respectively). However, the FGCM concentrations by temperatures less than -20°C and -10–0°C did not differ considerably (Mann-Whitney test: $Z = 0.797$; $n_1 = 10$; $n_2 = 71$; $p = 0.43$). The FGCM concentrations at -20°C to -10°C were significantly higher than at warmer temperatures (-10°C to 0°C) (Mann-Whitney test: $Z = 3.697$; $n_1 = 71$; $n_2 = 71$; $p = 0.0002$).

Table 1. Average daily air temperature and snow depth during the sampling period at Bastak, Sikhote-Alin and Ussuriisky State Nature Reserves and Land of the Leopard National Park (Russia)

Study site	Mean air temperature, °C, M ± SE*	Snow depth, cm, M ± SE*
Bastak State Nature Reserve (BR)	-15.0 ± 2.4	27.1 ± 3.2
Sikhote-Alin State Nature Reserve (SAR)	-4.6 ± 0.7	23.0 ± 2.5
Ussuriisky State Nature Reserve (UR)	-13.0 ± 1.6	1.4 ± 0.6
Land of the Leopard National Park (LLNP)	-14.1 ± 0.6	2.6 ± 0.4

Note: M – average value, SE – standard error.

Table 2. Number of samples collected in the Bastak State Nature Reserve, Sikhote-Alin State Nature Reserve, Ussuriisky State Nature Reserve and Land of the Leopard National Park (Russia)

Study site	Number of samples in snow period	Number of samples in snowless period	Total number of samples
Bastak State Nature Reserve (BR)	14	0	14 (0)*
Sikhote-Alin State Nature Reserve (SAR)	56	32	95 (7)
Ussuriisky State Nature Reserve (UR)	4	3	27 (20)
Land of the Leopard National Park (LLNP)	50	11	73 (12)

Note: * – In brackets, the number of samples is added, where dating was not precise.

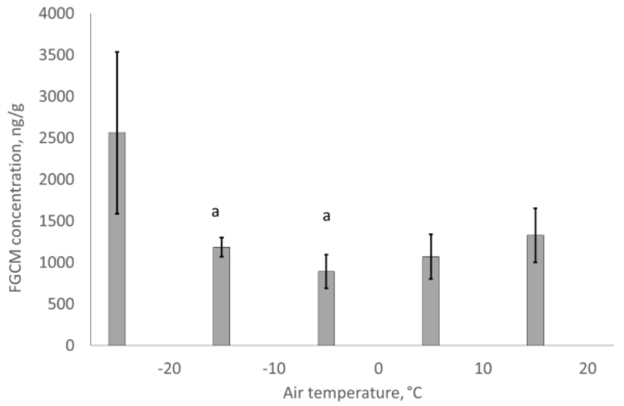

Fig. 2. Average (± standard error) FGCM level in dry Amur tiger feces at various air temperatures. Letter «a» shows statistically significant differences ($p < 0.05$) between two points (-20°C to -10°C and -10°C to 0°C).

The effect of the «snow level» factor over the whole period of study and in the snowy period was not statistically significant ($F = 0.464$; df = 1; $p = 0.500$ and $F = 0.000$; df = 1; $p = 0.99$, respectively). However, we observed a clear tendency of a FGCM concentration increase with the increase of snow levels from 0 cm to 60 cm (from 761 ± 105 ng/g to 1441 ± 561 ng/g (n = 24–68)). The FGCM level correlated positively with a snow depth from 0 cm to 50 cm ($k = 0.16$; $p < 0.05$) (Fig. 3). During a very high snow level (exceeding 60 cm), the FGCM concentrations in Amur tigers were low (668 ± 125 ng/g (n = 3)). These differences in FGCM concentrations were not considerable at various snow levels.

The location (i.e. study site) significantly affected the FGCM concentrations in Amur tigers over the whole study period ($F = 2.660$; df = 1; $p = 0.050$), but not in the snowy period ($F = 2.307$; df = 1; $p = 0.080$). The FGCM concentration was the lowest in SAR and the highest in BR (Fig. 4). However, differences were significant only between SAR in comparison to the largely similar data set size UR and LLNP ($Z = 2.350$; $n_1 = 95$; $n_2 = 27$; $p = 0.018$ and $Z = 2.704$; $n_1 = 95$; $n_2 = 73$; $p = 0.007$, respectively). It is necessary to note that weather conditions (especially air temperature) were less severe in SAR over the sampling period than on the other three study sites (Table 1). To exclude the effect of air temperature, only the samples (n = 20), collected under the average temperature -12.1 ± 0.5°C (similar to the other study sites (Table 1)), were used for this analysis. The average level of FGCM in SAR in this case was 883.4 ± 135.3 ng/g, and the GLM model did not show any effect of «study site» factor on the FGCM level in Amur tigers ($F = 0.065$; df = 1; $p = 0.80$).

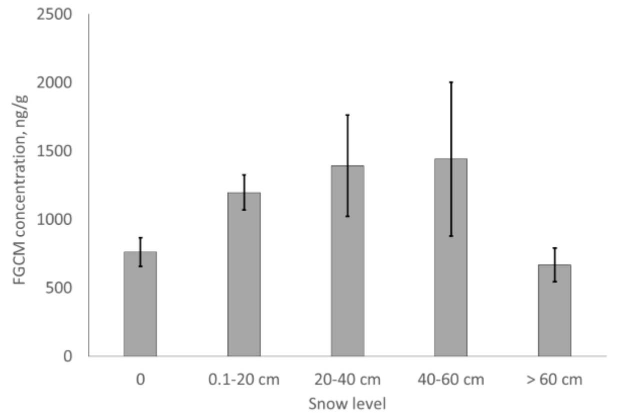

Fig. 3. Average (± standard error) FGCM level in dry Amur tiger feces at various snow levels.

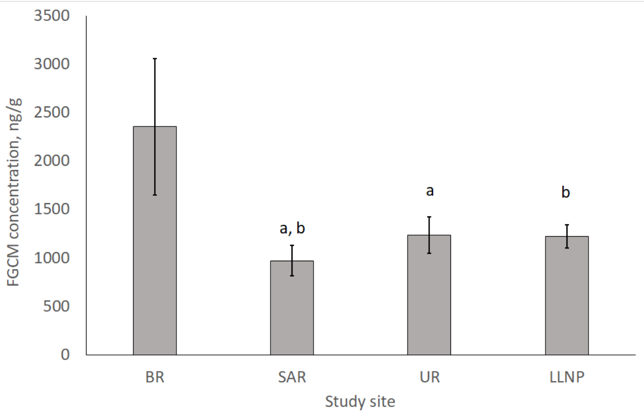

Fig. 4. Average (± standard error) FGCM level in dry Amur tiger feces on the study sites. Designations: BR – Bastak State Nature Reserve, SAR – Sikhote-Alin State Nature Reserve, UR – Ussuriisky State Nature Reserve, LLNP – Land of the Leopard National Park. Letters «a» and «b» show statistically significant differences ($p < 0.05$) between SAR and UR, letter «b» between SAR and LLNP.

Discussion

We hypothesised that Amur tigers in northern study areas will have higher FGCM levels compared to south areas, because we assumed lower winter temperatures and higher snow level there. However, this hypothesis was not confirmed. Amur tigers on the northernmost study site (Bastak State Nature Reserve) had higher levels of FGCM by two to three times than on the other three study sites; however, because of the small sample size these differences need to be interpreted with caution. However, at the second northern study site (Sikhote-Alin State Nature Reserve) the FGCM level was significantly lower than in both southern areas. This may be explained in part by less severe weather conditions in the Sikhote-Alin State Nature Reserve during the sample collection period, since the average daily temperature was 10°C higher than on the other three study sites. In captivity, the decrease of air temperature in winter resulted in an increase of glucocorticoid level in Amur tigers (Ivanov et al., 2017). It may be also explained by a more prolonged sampling period in SAR than on the other three study sites, which covered autumn and spring. Earlier we described higher concentrations of FGCM in Amur tigers in winter than in autumn in the wild (Naidenko et al., 2011).

Moreover, on the southern sites, the concentrations of FGCM in Amur tigers may be affected by other factors, such as anthropogenic disturbance or prey availability/density. Anthropogenic disturbance may lead to an increase of FGCM levels in various mammals (Rangel-Negrín et al., 2009), including carnivores (Creel et al., 2002) and Amur tigers in particular (Bhattacharjee et al., 2015; Tyagi et al., 2019). In the Amur tiger range, the human density is higher in the southern part of its range. This may lead to a noticeable increase of FGCM level of «southern» Amur tigers because of anthropogenic stress (higher number of people, including hunters, in the forest; more intensive traffic) (Ivanov, 2013). Food availability and diet may also affect significantly the level of FGCM (Goymann, 2012; Bryan et al., 2013). The diet of Amur tigers may depend on the preferable prey distribution in various study sites as well as on several other factors (including climate ones) that affect the killing rate of various prey. Therefore, clearly estimating the effect of «study site» was not possible because it is overlapped with climatic conditions, among other factors.

The air or environmental temperature is an important factor affecting glucocorticoid levels in animals. A decrease in air temperature requires changes in metabolic rates (an increase or decrease depending on species) that are achieved through changes of glucocorticoid level (Hanna et al., 2008; Haase et al., 2016). A cold air temperature clearly represents «stress» on a variety of animals (Hanna et al., 2008; Novikov et al., 2015). In some species, the glucocorticoid level increases in cold weather to intensify heat production to retain a constant body temperature. However, even closely related mammal species vary in their response to low temperatures. In contrast to Amur tigers (Naidenko et al., 2011), *Panthera uncia* Schreber, 1775 and *Otocolobus manul* Pallas, 1776 did not show glucocorticoid changes corresponding to air temperature (Ivanov, 2013). These differences support the hypothesis that the Amur tiger, as a species that evolved in the tropics (Luo et al., 2004) is sensitive to low temperatures (less than -10°C according to our data). The increase of the FGCM level in winter, which was earlier noted in the wild (Naidenko et al., 2011) seems to be largely a response to air temperature.

The effect of the air temperature on FGCM levels in Amur tigers was detected during the analysis of the whole data set, as well as for the snowy period. The FGCM level was at a maximum when the air temperature was lower than -20°C. Analysis of data overall shows that the FGCM level increased when the air temperature fell below -10°C. We did not observe considerable changes in the FGCM level in an air temperature higher than -10°C. The effect of air temperatures on stress levels of other felids has not been measured in the wild. We hypothesise that other felids of tropical origin (e.g. *Panthera pardus* Linnaeus, 1758) will respond to such conditions similarly to Amur tigers.

The snow level is a very crucial factor for carnivores (as well as for many other mammals) because it influences mobility and ability to hunt and to kill prey. Matjushkin (1978) has shown that *Lynx canadensis* Kerr, 1792 and *L. lynx* Linnaeus, 1758 have adapted to deeper snow levels with broader paw size (reducing the load pressure) and long legs. The Amur tiger males and females average body mass is 176 kg and 118 kg, respectively (Slaght et al., 2005), with no evidence of a broadened paw size to reduce the strain. In fact, the index of body mass pressure on 1 cm^2 in the Amur tiger (158 g) is the highest for all felids inhabiting the temperate climatic zone (in *L. lynx* is 37 g) (Formozov, 1946). For this reason, it is not surprising that the FGCM level in Amur tigers slightly increased with an increase of snow depth (requiring an increase of metabolic rate to overcome the difficulties of movements). But, in fact, the FGCM level decreased with the deepest snow (i.e. more than 60 cm). We suspected that there may be reasons to explain this effect. Firstly, as the snow level has increased over a certain depth level (for example, more than 60 cm) Amur tigers begin to use forest roads and animal trails (where snow is already trampled) more actively to decrease the energy costs of locomotion (and consequently decrease FGCM levels). Secondly, the snow level also restricts the movement of their prey (mainly, *Sus scrofa* Linnaeus, 1758 and *Cervus nippon* Temminck, 1838). Their indexes of body mass pressure on 1 cm^2 are about 800 g and 900 g respectively (Heptner & Sludskii, 1972) (about 5 times higher than in Amur tigers). It means that prey species have even more problems with moving through the deep snow. In deep snow winters *Cervus nippon* stays under trees eating branches and moves little (personal observations of the authors), making hunting for Amur tigers much easier. Hence, Amur tigers can spend more time near kills and reduce the locomotion and its costs and corresponding FGCM levels. These two possible explanations are not mutually exclusive, and both may act simultaneously to decrease the cortisol level.

We found that climatic factors (air temperature and snow depth) have some effects on wild Amur tigers' «stress level», or, more correctly, on the level of glucocorticoids (their metabolites). Low temperatures affected the physiological status negatively, by increasing the FGCM, and it corresponds with the data from zoos where an extremely low temperature led to the highest cortisol level (Ivanov et al., 2017). The effect of snow depth was different: it has an approximation of a threshold at approximately 60 cm depth: when the snow layer was less than 60 cm deep, the deeper snow led to a higher glucocorticoid level. However, when it became deeper, the cortisol level decreased sharply. We assume that it is related to the changes in hunting behaviour of Amur tigers. Further studies may help with the estimation of the effect of human activity, prey availability and presence of conspecifics.

Conclusions

The hypothesis that the ambient low temperatures will have significant effect on glucocorticoid (FGCM) levels in the Amur tiger (possibly, due to the species having evolved in tropical areas) was proven largely correct. While the temperature and snow levels affected the FGCM concentrations as expected, demonstrating an increase in stress in response to the increase in difficulty of hunting and movement, we also discovered a decrease of this index at extremely low (-10°C) temperatures and/or in conditions of a snow cover of more than 60 cm. We hypothesise that the decrease of the stress factor correlates with the periods of vulnerability and inactivity in potential prey due to the weather conditions. The adaptability of the Amur tiger to such conditions requires further study for the purposes of conservation of this threatened species.

Acknowledgements

This study was supported by the Russian Geographical Society and non-governmental foundation «Far-Eastern leopards».

References

Ayres K.L., Booth R.K., Hempelmann J.A., Koski K.L., Emmons C.K., Baird R.W., Balcomb-Bartok K., Hanson M.B., Ford M.J., Wasser S.K. 2012. Distinguishing the Impacts of Inadequate Prey and Vessel Traffic on an Endangered Killer Whale (*Orcinus orca*) Population. *PLoS ONE* 7(6): e36842. DOI: 10.1371/journal.pone.0036842

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. DOI: 10.1371/journal.pone.0127626

Bosson C.O., Palme R., Boonstra R. 2009. Assessment of the stress response in Columbian ground squirrels: laboratory and field validation of an enzyme immunoassay for fecal cortisol metabolites. *Physiological and Biochemical Zoology* 82(3): 291–301. DOI: 10.1086/597530

Bryan H.M., Darimont C.M., Paquet P.C., Wynne-Edwards K.E., Smits J.E.G. 2013. Stress and reproductive hormones in grizzly bears reflect nutritional benefits and social consequences of a salmon foraging niche. *PLoS ONE* 8(11): e80537. DOI: 10.1371/journal.pone.0080537

Christofoletti M.D., Garcia Pereira R.J., Duarte J.M.B. 2010. Influence of husbandry systems on physiological stress reactions of captive brown brocket (*Mazama gouazoubira*) and marsh deer (*Blastocerus dichotomus*) – noninvasive analysis of fecal cortisol metabolites. *European Journal of Wildlife Research* 56(4): 561–568. DOI: 10.1007/s10344-009-0350-8

Colding-Jørgensen P., Hestehave S., Abelson K.S.P., Kalliokoski O. 2023. Hair glucocorticoids are not a historical marker of stress – Exploring the time-scale of corticosterone incorporation into hairs in a rat model. *General and Comparative Endocrinology* 341(11): 114335. DOI: 10.1016/j.ygcen.2023.114335

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. *Conservation Biology* 16(3): 809–814. DOI: 10.1046/j.1523-1739.2002.00554.x

Creel S., Christianson D., Schuette P. 2013. Glucocorticoid stress responses of lions in relationship to group composition, human land use, and proximity to people. *Conservation Physyology* 1(1): cot021. DOI: 10.1093/conphys/cot021

Davenport M.D., Tiefenbacher S., Lutz C.K., Novak M.A., Meyer J.S. 2006. Analysis of endogenous cortisol concentrations in the hair of rhesus macaques. *General and Comparative Endocrinology* 147(3): 255–261. DOI: 10.1016/j.ygcen.2006.01.005

Dehnhard M., Clauss M., Lechner-Doll M., Meyer H.H.D., Palme R. 2001. Noninvasive monitoring of adrenocortical activity in roe deer (*Capreolus capreolus*) by measurement of fecal cortisol metabolites. *General and Comparative Endocrinology* 123(1): 111–120. DOI: 10.1006/gcen.2001.7656

Delehanty B., Boonstra R. 2012. The benefits of baseline glucocorticoid measurements: maximal cortisol production under baseline conditions revealed in male Richardon's ground squirrels (*Urocitellus richardsonii*). *General and Comparative Endocrinology* 178(3): 470–476. DOI: 10.1016/j.ygcen.2012.07.009

Dinerstein E., Loucks C., Wikramanayake E., Ginsberg J., Sanderson E., Forrest J., Bryja G., Heydlauff A., Klenzendorf S., Leimgruber P., Mills J., O'Brien T.G., Shrestha M., Simons R., Songer M. 2007. The fate of wild tigers. *BioScience* 57(6): 508–514. DOI: 10.1641/B570608

Dittami J., Katina S., Möstl E., Eriksson J., Machatschke I.H., Hohmann G. 2008. Urinary androgens and cortisol metabolites in field-sampled bonobos (*Pan paniscus*). *General and Comparative Endocrinology* 155 (3): 552–557. DOI: 10.1016/j.ygcen.2007.08.009

Driscoll C.A., Yamaguchi N., Bar-Gal G.K., Roca A.L., Luo S., Macdonald D.W., O'Brien S.J. 2009. Mitochondrial phylogeography illuminates the origin of the extinct Caspian tiger and its relationship to the Amur tiger. *PLoS ONE* 4(1): e4125. DOI: 10.1371/journal.pone.0004125

Fanson K.V., Wielebnowski N.C. 2013. Effect of housing and husbandry practices on adrenocortical activity in captive Canada lynx (*Lynx canadensis*). *Animal Welfare* 22(2): 159–165. DOI: 10.7120/09627286.22.2.159

Fanson K.V., Wielebnowski N.C., Shenk T.M., Lucas J.R. 2012. Comparative patterns of adrenal activity in captive and wild Canada lynx (*Lynx canadensis*). *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology* 182(1): 157–165. DOI: 10.1007/s00360-011-0597-8

Formozov A.N. 1946. *Snow cover as an environmental factor, its importance in the life of mammals and birds*. Moscow: MOIP. 152 p. [In Russian]

Ganswindt A., Tordiffe A.S.W., Stam E., Howitt M.J., Jori F. 2012. Determining adrenocortical activity as a measure of stress in African buffalo (*Syncerus caffer*) based on faecal analysis. *African Zoology* 47(2): 261–269. DOI: 10.1080/15627020.2012.11407558

Goritz F., Dehnhard M., Hildebrandt T.B., Naidenko S.V., Vargas A., Martinez F., Lopez-Bao J.V., Palomares F., Jewgenow K. 2006. Non Cat-Like Ovarian Cycle in the Eurasian and the Iberian Lynx – Ultrasonographical and Endocrinological Analysis. *Reproduction in Domestic Animals* 44(S2): 87–91. DOI: 10.1111/j.1439-0531.2009.01380.x

Goymann W. 2012. On the use of non-invasive hormone research in uncontrolled, natural environments: the problem with sex, diet, metabolic rate and the individual. *Methods in Ecology and Evolution* 3(4): 757–765. DOI: 10.1111/j.2041-210X.2012.00203.x

Haase C.G., Long A.K., Gillooly J.F. 2016. Energetics of stress: linking plasma cortisol levels to metabolic rate in mammals. *Biology Letters* 12(1): 20150867. DOI: 10.1098/rsbl.2015.0867

Hanna S.K., Haukenes A.H., Foy R.J., Buck C.L. 2008. Temperature effects on metabolic rate, swimming performance and condition of Pacific cod *Gadus macrocephalus* Tilesius. *Journal of Fish Biology* 72(4): 1068–1078. DOI: 10.1111/j.1095-8649.2007.01791.x

Heptner V.H., Sludskii A.A. 1972. *Mammals of the Soviet Union. Vol. 3: Carnivores (Feloidea)*. Moscow: Vysshaya Shkola. 551 p. [In Russian]

Hernandez-Blanco J.A., Naidenko S.V., Chistopolova M.D., Lukarevskiy V.S., Kostyrya A., Rybin A., Sorokin P.A., Litvinov M.N., Kotlyar A.K., Miquelle D.G., Rozhnov V.V. 2015. Social structure and space use of Amur tigers (*Panthera tigris altaica*) in Southern Russian Far East based on GPS telemetry data. *Integrative Zoology* 10(4): 365–375. DOI: 10.1111/1749-4877.12140

Huber S., Palme R., Arnold W. 2003. Effects of season, sex, and sample collection on concentrations of fecal cortisol metabolites in red deer (*Cervus elaphus*). *General and Comparative Endocrinology* 130(1): 48–54. DOI: 10.1016/s0016-6480(02)00535-x

Hunt K.E., Rolland R.M., Kraus S.D., Wasser S.K. 2006. Analysis of fecal glucocorticoids in the North Atlantic right whale (*Eubalaena glacialis*). *General and Comparative Endocrinology* 148(2): 260–272. DOI: 10.1016/j.ygcen.2006.03.012

Ivanov E.A. 2013. *An estimation of welfare of endangered feline species (tiger, leopard and Pallas' cat) in the wild by noninvasive methods*. PhD Thesis. Moscow. 128 p. [In Russian]

Ivanov E.A., Rozhnov V.V., Naidenko S.V. 2017. The effect of ambient temperature on glucocorticoid level in the Amur tiger (*Panthera tigris altaica*). *Russian Journal of Ecology* 48(3): 294–297. DOI: 10.1134/S1067413617030079

Jewgenow K., Naidenko S.V., Goeritz F., Vargas A., Dehnhard M. 2006. Monitoring testicular activity of male Eurasian (*Lynx lynx*) and Iberian (*Lynx pardinus*) lynx by fecal testosterone metabolite measurement. *General and Comparative Endocrinology* 149(2): 151–158. DOI: 10.1016/j.ygcen.2006.05.015

Kerley L.L., Goodrich J.M., Smirnov E.N., Miquelle D.G., Nikolaev I.G., Arjanova T., Slaght J.S., Quigley H.B., Hornocker M.G. 2007. Chapter 7. Morphological parameters of Amur Tigers. In: D.G. Miquelle, E.N. Smirnov, J.M. Goodrich (Eds.): *Tigers in Sikhote-Alin State Nature Reserve: Ecology and Conservation*. Vladivostok: PSP. P. 36–42. [In Russian]

Loschagina J.A., Tsvey A.L., Naidenko S.V. 2017. Plasma corticosterone concentrations in European robins during spring and autumn migration. *Doklady Biological Sciences* 473(1): 61–63. DOI: 10.1134/S0012496617020089

Loschagina J.A., Tsvey A.L., Naidenko S.V. 2018. Baseline and stress-induced corticosterone levels are higher during spring than autumn migration in European robins. *Hormones and Behavior* 98: 96–102. DOI: 10.1016/j.yhbeh.2017.12.013

Luo S.J., Kim J.H., Johnson W.E., van der Walt J., Martenson J., Yuhki N., Miquelle D.G., Uphyrkina O., Goodrich J.M., Quiqley H.B., Tilson R., Brady G., Martelli P., Subramaniam V., McDougal C., Hean S., Huang S.Q., Pan W., Karanth U.K., Sunquist M., Smith J.L.D., O'Brien S.J. 2004. Phylogeography and genetic ancestry of tigers (*Panthera tigris*). *PLoS Biol* 2(12): e442. DOI: 10.1371/journal.pbio.0020442

Mastromonaco G.F., Gunn K., McCurdy-Adams H., Edwards D.B., Schulte-Hostedde A.I. 2014. Validation and use of hair cortisol as a measure of chronic stress in eastern chipmunks (*Tamias striatus*). *Conservation Physiology* 2(1): cou055. DOI: 10.1093/conphys/cou055

Matjushkin E.N. 1978. *Der Luchs. Die Neue Brahm Bucherei 517, A*. Wittenberg Lutherstadt: Ziemsen Verlag. 160 p.

Miquelle D.G., Stephens P.A., Smirnov E.N., Goodrich J.M., Zaumyslava O.J., Myslenkov A.E. 2005. Competitive exclusion, functional redundancy, and conservation implications: tigers and wolves in the Russian Far East. In: J.C. Ray, K.H. Berger, R.S. Steneck, J. Redford (Eds.): *Large Carnivores and the Conservation of Biodiversity*. Washington, Covelo, London: Island Press. P. 179–207.

Möstl E., Palme R. 2002. Hormones as indicators of stress. *Domestic Animal Endocrinology* 23(1–2): 67–74. DOI: 10.1016/s0739-7240(02)00146-7

Naidenko S.V., Erofeeva M.N. 2005. Changes in steroid hormone level in lynx (*Lynx lynx* L.) blood during the early postnatal ontogenesis. *Doklady Biological Sciences* 400(1): 51–53. DOI: 10.1007/s10630-005-0041-x

Naidenko S.V., Rozhnov V.V. 2009. An application of non-invasive methods to estimation of reproductive status of resource species of mammals. *Uspekhi Sovremennoi Biologii* 129: 610–619. [In Russian]

Naidenko S.V., Ivanov E.A., Lukarevskii V.S., Hernandez-Blanco J.A., Sorokin P.A., Litvinov M.N., Kotlyar A.K., Rozhnov V.V. 2011. Activity of the hypothalamo-pituitary-adrenals axis in the Siberian tiger (*Panthera tigris altaica*) in captivity and in the wild, and its dynamics throughout the year. *Biology Bulletin* 38(3): 301–305. DOI: 10.1134/S1062359011030095

Novikov E., Kondratyuk E., Petrovski D., Krivopalov A., Moshkin M. 2015. Effects of parasites and antigenic challenge on metabolic rates and thermoregulation in northern red-backed voles (*Myodes rutilus*). *Parasitology Research* 114(12): 4479–4486. DOI: 10.1007/s00436-015-4691-9

Palme R. 2012. Monitoring stress hormone metabolites as a useful, non-invasive tool for welfare assessment in farm animals. *Animal Welfare* 21(3): 331–337. DOI: 10.7120/09627286.21.3.331

Paquet P.C., Darimont C.T. 2010. Wildlife conservation and animal welfare: two sides of the same coin?. *Animal Welfare* 19(2): 177–190. DOI: 10.1017/S0962728600001433

Pavlova E.V., Naidenko S.V. 2008. Noninvasive monitoring of glucocorticoids in feces of the bengal cat (*Prionailurus bengalensis euptilurus*). *Zoologicheskii Zhurnal* 87(11): 1375–1381. [In Russian]

Pribbenow S., Jewgenow K., Vargas A., Serra R., Naidenko S., Dehnhard M. 2014. Validation of an enzyme immunoassay for the measurement of faecal glucocorticoid metabolites in Eurasian (*Lynx lynx*) and Iberian lynx (*Lynx pardinus*). *General and Comparative Endocrinology* 206: 166–177. DOI: 10.1016/j.ygcen.2014.07.015

Rangel-Negrín A., Alfaro J.L., Valdez R.A., Romano M.C., Serio-Silva J.C. 2009. Stress in Yucatan spider monkeys: effects of environmental conditions on fecal cortisol levels in wild and captive populations. *Animal Conservation* 12(5): 496–502. DOI: 10.1111/j.1469-1795.2009.00280.x

Romero L.M. 2004. Physiological stress in ecology: lessons from biomedical research. *Trends in Ecology and Evolution* 19(5): 249–255. DOI: 10.1016/j.tree.2004.03.008

Rozhnov V.V., Lukarevskiy V.S., Hernandez-Blanko J.A., Sorokin P.A., Litvinov M.N., Kotlyar A.K., Udin V.G., Naydenko S.V. 2010. Non-invasive approach to estimation of hipothalamus-pituitary-adrenals in Amur tigers. *Doklady Biological Sciences* 430: 57–59. DOI: 10.1134/S0012496610010199

Saltz D., White G.C. 1991. Urinary cortisol and urea nitrogen responses to winter stress in mule deer. *Journal of Wildlife Management* 55(1): 1–16. DOI: 10.2307/3809235

Sapolsky R.M., Romero L.M., Munck A.U. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews* 21(1): 55–89. DOI: 10.1210/edrv.21.1.0389

Sheriff M.J., Dantzer B., Delehanty B., Palme R., Boonstra R. 2011. Measuring stress in wildlife: techniques for quantifying glucocorticoids. *Oecologia* 166(4): 869–887. DOI: 10.1007/s00442-011-1943-y

Slaght J.C., Miquelle D.G., Goodrich J.M., Nikolaev I.G., Smirnov E.N., Schleyer B.O., Traylor-Holzer K., Christie S., Arjanova T., Smith J.L.D., Karanth K.U. 2005. Chapter 6. Who's king of the beasts? Historical and recent body weights of wild and captive amur tigers, with comparisons to other subspecies. In: D.G. Miquelle, E.N. Smirnov, J.M. Goodrich (Eds.): *Tigers in Sikhote-Alin State Nature Reserve: Ecology and Conservation*. Vladivostok: PSP. P. 25–35. [In Russian]

Tyagi A., Kumar V., Kittur S., Reddy M., Naidenko S., Ganswindt A., Umapathy G. 2019. Physiological stress responses of tigers due to anthropogenic disturbance especially tourism in two central Indian tiger reserves. *Conservation Physiology* 7(1): coz045. DOI: 10.1093/conphys/coz045

Ugaz C., Valdez R.A., Romano M.C., Galindo F. 2013. Behavior and salivary cortisol of captive dolphins (*Tursiops truncatus*) kept in open and closed facilities. *Journal of Veterinary Behaviour* 8(4): 285–290. DOI: 10.1016/j.jveb.2012.10.006

WWF. 2022. *WWF Tigers Alive Annual Report*. 62 p.

Yudin V.G., Yudina E.V. 2009. *The Tiger of the Far East of Russia*. Vladivostok: Dalnauka. 483 p. [In Russian]

АБИОТИЧЕСКИЕ ФАКТОРЫ МОГУТ ВЛИЯТЬ НА УРОВЕНЬ МЕТАБОЛИТОВ ГЛЮКОКОРТИКОИДОВ В ЭКСКРЕМЕНТАХ *PANTHERA TIGRIS ALTAICA* (FELIDAE) В ПРИРОДЕ НА ДАЛЬНЕМ ВОСТОКЕ РОССИИ

С. В. Найденко[1,*], А. Ю. Горбунова[2], Е. А. Иванов[1], Х. А. Эрнандес-Бланко[1], Е. И. Шевцова[3], Д. С. Матюхина[3], И. В. Середкин[4], Д. Г. Микуэлл[5], С. В. Сутырина[6], А. Ю. Калинин[7], В. В. Рожнов[1]

[1]*Институт проблем экологии и эволюции имени А.Н. Северцова РАН, Россия*
[2]*Университетский колледж Дублина, Ирландия*
[3]*Объединенная дирекция государственного природного биосферного заповедника «Кедровая падь» и национального парка «Земля леопарда» имени Н.Н. Воронцова, Россия*
[4]*Тихоокеанский институт географии ДВО РАН, Россия*
[5]*Общество сохранения диких животных, США*
[6]*Сихотэ-Алинский государственный природный заповедник, Россия*
[7]*Государственный природный заповедник «Бастак», Россия*
**e-mail: snaidenko@mail.ru*

Оценка уровня благополучия/стресса диких животных помогает выявить факторы, влияющие на их состояние. *Panthera tigris* – вид, который эволюционировал в тропической Азии. Однако его подвид *Panthera tigris altaica* (далее – амурский тигр) живет в экстремальных климатических условиях на Дальнем Востоке России. Целью настоящего исследования было оценить эффект низкой температуры воздуха и глубокого снежного покрова как потенциальных стресс-факторов на состояние амурских тигров. Изменения уровня глюкокортикоидов могут быть хорошим индикатором для оценки уровня стресса/благополучия. Мы собрали 209 проб экскрементов амурских тигров. Пробы держали замороженными до проведения анализа. Мы использовали предварительно валидированные коммерческие наборы для оценки уровня метаболитов глюкокортикоидов (FGCM) в экскрементах амурских тигров. Мы оценили уровень FGCM у амурских тигров на четырех особо охраняемых природных территориях (ООПТ) с севера на юг, предполагая, что он будет наиболее высоким у животных на севере (из-за погодных условий зимой). Мы не выявили достоверных различий в концентрации FGCM в зависимости от месторасположения ООПТ. Однако он коррелировал с климатическими условиями в этих географических точках. Температура воздуха существенно влияла на уровень FGCM у амурских тигров как на протяжении всего периода исследований ($p = 0.043$), так и в снежный период ($p = 0.028$). Мы полагаем, что низкие температуры окружающего воздуха могли приводить к увеличению скорости метаболизма у такого тропического вида, как амурский тигр. Высота снежного покрова не влияла достоверно на уровень FGCM. Однако была выявлена позитивная связь между уровнем гормонов и глубиной снега. При экстремально высоком снежном покрове уровень FGCM у амурских тигров был низким, что могло быть связано с изменениями в характере перемещений и эффективности охоты. Определение уровня FGCM – это эффективный подход для оценки физиологического статуса амурских тигров. Однако температура воздуха может достоверно влиять на этот показатель у амурского тигра (тропического вида), что необходимо принимать во внимание при интерпретации результатов.

Ключевые слова: географические различия, глубина снега, кортизол, мониторинг гормонов, температура воздуха