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ANALYSIS OF HABITAT USE, ACTIVITY, AND BODY CONDITION SCORES OF PRZEWALSKI'S HORSES IN HORTOBAGY NATIONAL PARK, HUNGARY

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A founder population of Przewalski's horses (*Equus ferus przewalskii*) was set free in a semi-reserve called Pentezug in the Hortobagy National Park (Hungary) in 1997. Beside the Przewalski's horses reconstructed aurochs (*Bos taurus taurus*) were bred as well in the 24.5-km^2 large area. Both species reproduced successfully in this steppe reserve, which was once the homeland of their distant ancestors. The number of large grazers has grown so quickly that different strategies of population control had to be implemented for reconstructed aurochs and horses in 2007 and 2013, respectively. The activity, habitat use, and body condition of Przewalski's horses were monitored regularly throughout the 22 years. We detected seasonal changes in the activity of the horses but the proportions of the main activity types were similar in two distant time periods. In contrast, habitat use and body condition scores for the horses showed remarkable differences between early vs. late years of the project, implying that horses were forced to use non-preferable areas and their condition was worsened in parallel with the increased population density of the large grazers. During the cold spring in 2018, there was a massive loss of both horses and cattle. These observations suggest that limited food sources could lead to changes in habitat use and/or worse body condition and a greater death rate in extreme weather conditions. In summary, strict birth control measures must be implemented in dense Przewalski's horse populations, and the habitat use and body conditions must be monitored to balance the population size and carrying capacity of the areas.

Key words: *Bos taurus taurus*, carrying capacity, *Equus ferus przewalskii*, large grazers, population control, Pentezug Reserve, reconstructed aurochs, semi-reserve

Introduction

In the Holocene period, wild horses (*Equus ferus* Boddaert, 1785) inhabited both Europe and Asia (Vörös, 1987). Their descendant subspe-

Besides the reintroduction projects in Mongolia and China, many closed natural conservation areas are home to Przewalski's horses all over the world. The herd in the Pentezug Reserve (Horto-

the Hungarian Steppe. The aim of this project was to create an alternative grazing ecosystem with minimal human intervention compared to grazing livestock. The Pentezug Reserve is part of the steppe vegetation of HNP and provides adequate grass species and water supply (marshes and canals) for large grazers. However, apex predators of large herbivores are missing.

Equidae have a cecal digestion system (Janis, 1976), which means that the food goes through their digestion system quickly and they can live on vegetation with low nutritional content. As a consequence, horses choose habitats based on a green plant content (Duncan, 1983; King, 2002) and have to graze more in winter when the nutritional content of vegetation is lower (Boyd & Bandi, 2002; Souris et al., 2007). With a growing density of large grazers, horses might have to use areas that are not optimal (Beest et al., 2014), affecting their body condition.

In this study, we describe our observations on habitat use, activity, and body condition over several years. Seasonal changes and changes occurring over larger time intervals, parallel to the population growth, can be distinguished. The results, accumulated during more than 20 years, contribute to our understanding of the activity and habitat use of Przewalski's horses and may facilitate herd management or novel project designs.

Material and Methods

Study area

The HNP, established in 1972 as the first national park in Hungary, is located in the Eastern part of the country, 150 km from Budapest. The research area, called Pentezug Reserve (24.5 km^2), can be found in the middle of the HNP at 47.5175 N, 21.092778 E. The climate is dry continental with 550 mm annual precipitation. Four seasons can be differentiated, where the winter is cold and dry with a minimum temperature of -15°C, 20–40 day snow cover (2–15 cm) and the summer is hot with a maximum temperature of +35°C. There are moderate temperatures and more rainfall in autumn and spring. The reserve is part of the continental alkali grassland region that is characterised by salt and water content of the soil and the natural floods. The groundwater, which is rich in different salts and close to the surface, easily evaporates when the weather is hot. As a consequence, these salts accumulate in the upper soil level (Török et al., 2012). Due to these phenomena, these grasslands are not suitable for agricultural utilisation and have been used as pastures for centuries (Molnár, 2014). We can differentiate eight habitat types in the Pentezug Reserve with different plant associations showed in Table 1 (Deák et al., 2014a,b,c).

Table 1. Habitat types in Pentezug Reserve (Hungary)

Habitat Type	Association	Characteristics	Characteristic plant species	Habitat area in Pentezug (km^2)
Forest	Planted oak forest	along the river	*Quercus robur* L.	0.315887
Alkali marsh	*Bolboschoenetum maritimi*	shallow water cover, considerable salt content both in the soil and water	*Bolboschoenus maritimus* (L.) Palla	2.207703
Reed bed	*Phragmittetum communis*	deep water cover	*Phragmites australis* (Cav.) Trin. ex Steud.	0.712105
Weed	*Onoprodetum acanthi,*	on former livestock farms, high soil N	*Onopordium acanthium*	0.277978

In 2000, with the help of aerial photographs and field observations, these different habitat types and areas (i.e. polygons) were recorded and shape files were created using the ArcMap 10.1 program. Each polygon of the attributed table contains one identification code for each of the eight habitats. We calculated the area of each habitat with the help of the ArcMap 10.1 program. The Pentezug Reserve is surrounded by a three-lined electric fence. Inside the area, horses and cattle can move freely between habitat types. Other wild animals, such as red fox (*Vulpes vulpes* Linnaeus, 1758), roe deer (*Capreolus capreolus* Linnaeus, 1758) and wild boar (*Sus scrofa* Linnaeus, 1758) can cross the fence. Surplus food or water was not offered except occasionally in winter and any human intervention (such as herding and mowing) was banned.

Przewalski's horse and cattle populations in the Pentezug Reserve

The founders of the horse population were selected by the coordinator of the *European Endangered Species Programmes* (*EEP*) to create the widest genetic base. The coordinator uses the studbook data (which contains parentage, location, birth, and other important data on the horses) to calculate the mean kinship and genetic diversity of the horses and create a viable population with the help of PMX 0.1 software. According to the advice of the EEP coordinator, we imported 31 horses and exported 59 horses between 1997 and 2018. Przewalski horses are not marked by branding or by chips. However, most individuals are recognised by the HNP staff based on their gender, age, special marks/characters, and the harem they belong to. The marks/characters include coat colour, stripes on the legs, shoulder crosses, cowlick positions, and other special features, such as double shoulder crosses or missing eartips. The horses were identified and counted regularly (every this number to illustrate the yearly changes in the Pentezug Reserve.

Recognition and registration of horses in the Pentezug Reserve

Horses are registered in three databases, (1) a photo catalogue, (2) a group composition list, and (3) a DNA database. The photo catalogue consists of winter and summer portraits, summer and winter coats on both sides, and the back side of the animal. The group composition list consists of the positions of horses in different groups. The positions are recorded monthly from the beginning of the project with the exception of the years between 2008 and 2011, when there were only 1–4 observations annually. The genetic database of our horses is in the Davis Laboratory (Veterinary Genetics Laboratory, California, USA). We sampled each imported horse and all foals at the age of 1–2 year using a pneumatic rifle (operating with CO_2) and special biopsy needles (PneuDart and DanInject). In this way, small amounts of skin or biopsy samples can be taken without tranquilising animals. If we cannot identify a horse, we take a repeat sample and the Davis Laboratory can identify the horse in question.

Analysis of habitat use data

We recorded 258 and 291 GPS data in 2004–2006 and in 2013–2014, respectively. We collected data once a day at the most and transferred data to two Excel databases. We made shape files from the GPS Excel files in the ArcMap 10.1 program for both periods. We integrated these GPS shape files and a habitat map shape file using the ArcMap 10.1 program. In this manner, every GPS point had a habitat identification number. We calculated how many times horses stayed in different habitats in both periods and seasonally. Habitat preference was evaluated by comparing the proportion of

min. in 2013–2014 using a scan sampling method during daylight hours. The sampling lasted for 16 minutes and for 60 min. in 2008–2009 and in 2013–2014, respectively. We compared the activity percentage by calculating the ratio of the activity type to all recorded activities in the two periods. We examined the seasonal differences among activity types in 2013–2014 by calculating the proportion of time animals spent at a given activity compared to all activities in a season. We compared the three most remarkable activity types seasonally. Data visualisation and Kruskal-Wallis statistical tests were carried out using GraphPad Prism software.

Body condition scores

The body condition records were based on visual estimation (Rudman & Keiper, 1991). The body condition was scored by checking the hind quarter of the horse and giving a score between 0 (very bad condition) and 5 (very good condition). We collected body condition scores of 50 horses and 90 horses in 2005 and 2018, respectively. We used binoculars and went as close as possible (20–40 m) to the given animal and waited till we could see the bottom symmetrically. Each animal had one score between July and October in each year. Five groups of horses were classified based on age, sex, and position in the social structure. (1) Bachelors: members of the bachelor group, either males who left natal groups and do not have a harem yet or former harem stallions. (2) Harem stallions: owners of a harem at the time of scoring. (3) Adult females: females who left natal groups. (4) Young males: still in natal groups. (5) Young females: still in natal groups. We compared the data of each group for the two time periods. We only used data where a minimum of five records were collected in both years. In this way, we could compare the data of the harem stallion group (n = 5, n = 16), the adult female group (n = 21, n = 60), and the young male group (n = 9, n = 26). We used Mann-Whitney tests to examine whether the groups were different in the two time periods. Data visualisation and statistical tests were carried out using GraphPad Prism software.

Results

Population changes

Since 1997, cattle and horse populations have grown rapidly in the area (Fig. 1). The total number of animals was highest in 2017 (n = 950) and the population density reached 38.8 animals per km^2. Within one year the total number of animals dropped by 33% when the intensive decrease in the number involved both cattle and horses. The number of cattle was 400 at the end of 2018. The total number of calves was 1135 and 738 cattle were sold or died in the history of the Pentezug Reserve. The cattle population grew constantly until 2010 and stagnated for three periods (2010–2012, 2013–2014, 2016–2017). The robust decrease in the cattle population after 2017 was due to human intervention (transportation to other areas or to slaughter-houses). The cattle population consisted of mostly adult cows and their offspring, only two breeding bulls were temporarily transported to the area since 2007. At the end of 2018, the horse population consisted of 280 animals. Approximately 259 animals died between 1997 and 2018 and 567 foals were born. The horse population was increasing until 2015. Between 2015 and 2017, the population grew slower due to human interventions (transports and contraception treatment). In 2018 there was a massive loss because the cold spring devastated many weak individuals.

Table 2. Description of activity types

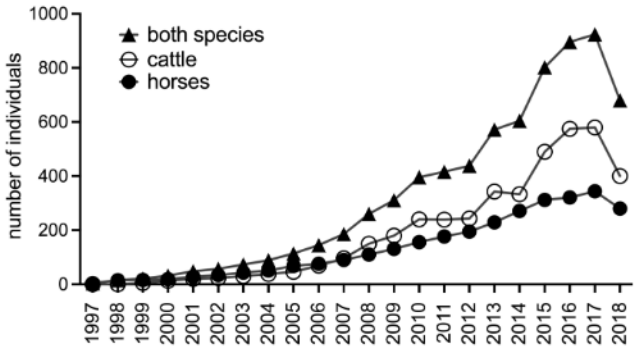
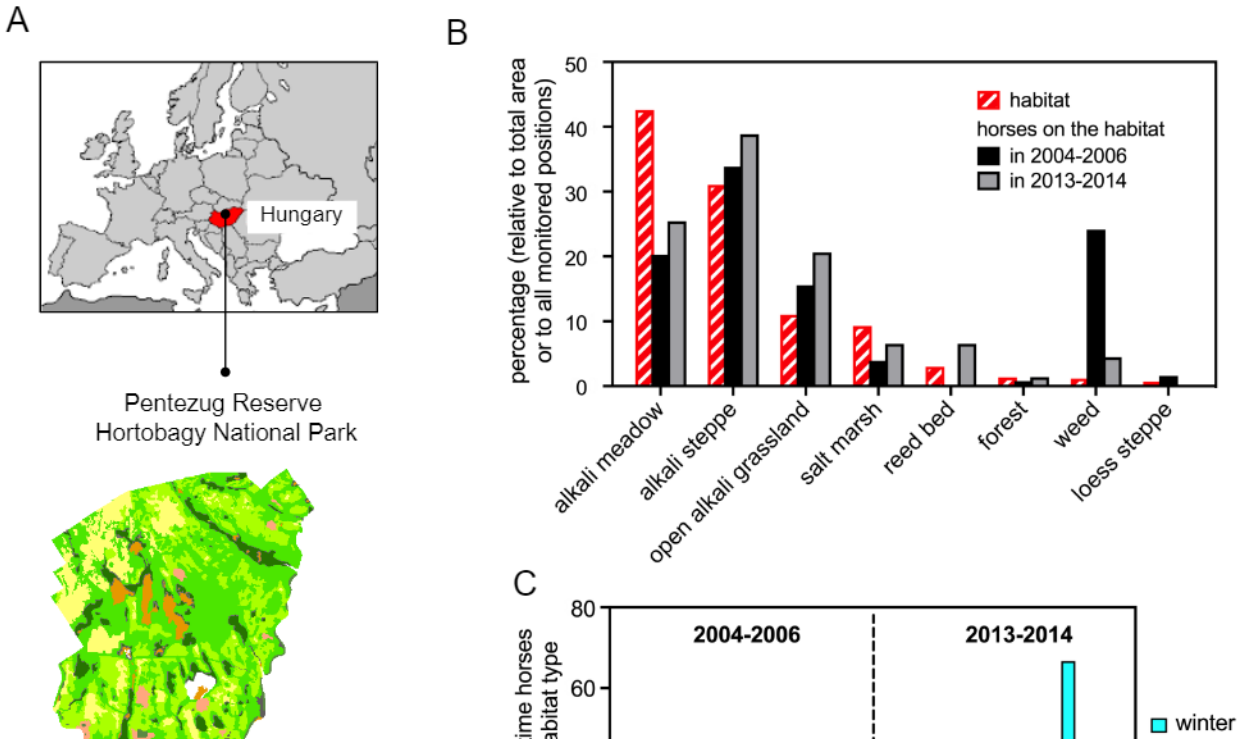


Fig. 1. Number of horses and cattle between 1997 and 2018 in the Pentezug Reserve, Hortobágyi National Park, Hungary.

Habitat use of Przewalski horses

The three most typical habitats in Pentezug Reserve are the alkali grassland, open alkali steppe, and alkali meadow (Fig. 2A,B). These habitats together cover approximately 85% of the total area. Horses spent most of their time in these habitats irrespective of the year of observation (either 2004–2006 or 2013–2014). Forest and loess steppe patches were hardly used in both periods (Fig. 2B). A comparison of the proportion of a given habitat and the proportion of time horses spent in the habitat revealed their habitat preference (Fig. 2B). When the proportion of habitat was higher than the proportion of time spent there, then the habitat was not preferred. Horses did not prefer the alkali meadow and salt marsh, while they preferred the alkali steppe, alkali grassland, and weed. Interestingly, the weed habitat was highly preferred in 2004–2006, partly because this habitat could be found around small houses or former stables that could provide shelter for horses against the sun and wind, but they were broken down by 2013. Also, a high nitrogen and phosphorous content can be attractive for horses.



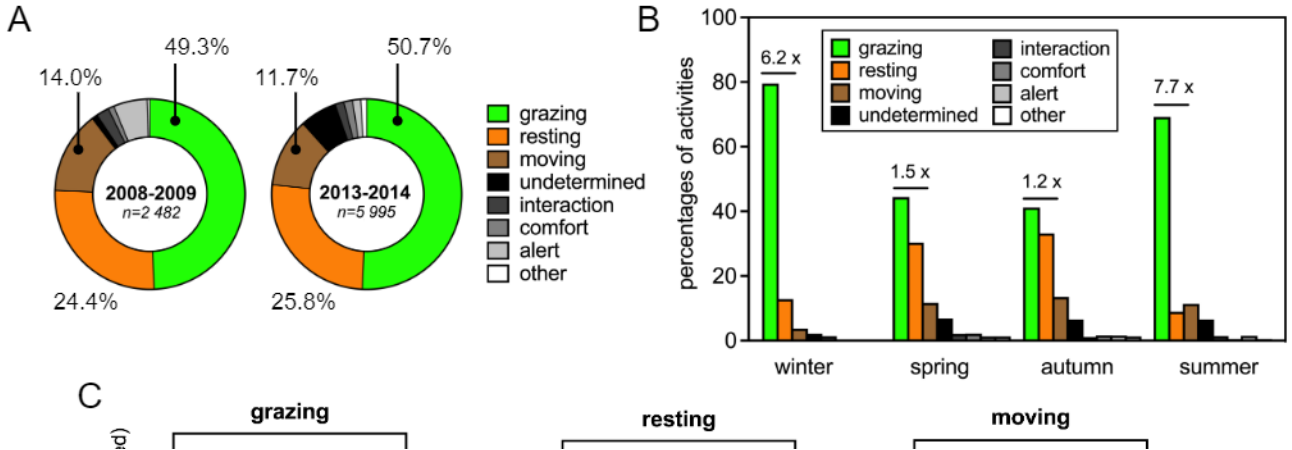
We also investigated whether habitat use exhibited seasonal changes in the two periods. Most of the habitats were used similarly in 2004–2006. Only open alkali grassland was used less frequently in winter. In 2013–2014, we detected seasonal differences in the case of two habitats. Animals spent more time on open alkali grassland in summer than in spring or autumn and they did not use the open alkali grassland in winter. Also, horses could be found on alkali meadows much more in the winter than in the other two habitat types (Fig. 2C). These data suggest that, in 2013–2014, the open alkali grassland was not used.

Activity of horses

We recorded 2482 and 5995 activity events in 2008–2009 and in 2013–2014, respectively. In both monitored years, we found that three activity types, namely grazing, resting, and moving, covered 90% of the yearly activity (Fig. 3A). The ratio of these activities did not change from 2008 to 2013. The only remarkable changes were in the undetected activities, which increased in 2013 compared to 2008 (Fig. 3A) because monitoring groups with more members resulted in more undetected horses. Seasonal activity ratios in 2013–2014 demonstrate that grazing is the most common activity in all seasons. However, horses spent almost the same amount of time resting as grazing in spring and summer (Fig. 3B). Similar seasonal changes can be found in 2008–2009 (data not shown). Grazing, resting, and moving were significantly different seasonally (Fig. 3C). Horses spent more time grazing in autumn and winter than in spring and summer. On the other hand, they rested much more in warmer seasons than in colder ones. Horses spent almost the same amount of time moving in the spring, summer, and autumn, but much less in the winter.

Changes in body condition

The condition scores of adult females and young males decreased from 2005 to 2018 (Mann-Whitney test $p < 0.0001$). Female scores varied between 3–4.5 and 2.5–4.5 in 2005 and in 2018, respectively. Scores of young males varied between 3.5–4.0 and 2.5–4.0 in 2005 and 2018, respectively. The scores for harem stallions did not differ significantly between the two time periods (Mann-Whitney test $p = 0.87$) and the more animals we could score, the more variable results we got. The scores of bachelors and young females could not be compared, because of inadequate data (n = 3 for bachelors in 2018 and n = 3 for young females in 2005).



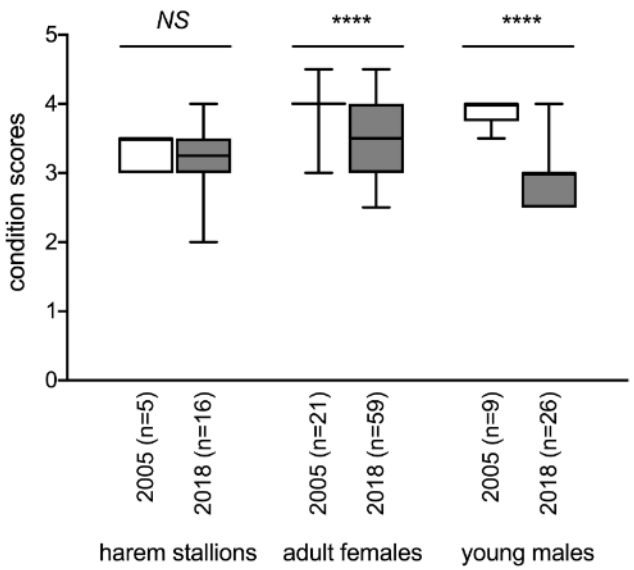


Fig. 4. Condition scores of Przewalski's horses in Pentezug Reserve. The number of monitored individuals is shown in brackets. The condition scores obtained from two years were compared separately for each group using Mann-Whitney statistical tests (NS – not significant; **** – $p < 0.0001$).

Discussion

The number of Przewalski's horses and reconstructed aurochs has remarkably increased in the Pentezug Reserve since launching the project. We can see similar phenomena in other areas where grazers were introduced and the former apex predator was missing (Caughley, 1981; Coulson et al., 2001). Since these two species compete for grasslands to a certain degree (Duncan et al., 1990; Menard et al., 2002), it is important to consider the cattle population when examining the horses' behaviour or activities. In 2007, the number of cattle started to grow dramatically. Two different strategies were selected to control the population growth of the two species. Horses were treated as wild animals but cattle were caught once a year (in winter) for the purpose of population regulathorities, where all our animals are registered. Immunocontraception is an option for management of large horse populations (Kirkpatrick et al., 1990). In 2013, following the recommendation of the EEP coordinator of Przewalski's horses, we decided to use Pig Zona Pellucida (PZP) immunocontraception (Turner et al., 2002) to slow down the population growth. In 2015, the population growth did slow down as an effect of this treatment. Nevertheless, we had an enormous loss of horses (40–50 horses) and cattle (20–30 cows) in 2018, most likely due to a combination of a very cold spring (snow and 15 degrees below zero) in March, a massive drought in 2016–2017, and a large number of both cattle and horses. This phenomenon can also be observed in areas occupied by feral horses (Scorolli et al., 2006). We decided to reduce the number of both species as soon as possible. We sold more cattle compared to the number born, resulting in a fast reduction in the animal population. We are planning to increase the number of PZP-treated female horses.

We assume that the changes in number will not only alter the vegetation, but also the behaviour of the Przewalski's horse population. Horses prefer a habitat with grass species dominated by *Festuca pseudovina* Hack. ex Wiesb. This species can grow even if the temperature is below zero providing small but continuous amounts of green plant parts that are important for horses when choosing habitat (Duncan, 1983; King, 2002). Open alkali grasslands are attractive in summer in higher animal density, when horses destroy its vegetation by trampling and create dust baths to avoid insects. As a consequence, these habitats cannot provide food in winter. On the other hand, grass species on alkali meadow grow and dry quickly but provide quite a large

Souris et al., 2007). Berger at al. (1999) found a similar pattern, although they recorded horse movement even during the night. Horses have to increase food intake during winter, when the food is less nutritious (Berger et al., 1999). We expected that the growing population and decreasing amount of food in the area would alter the grazing-rest ratio. This ratio, however, did not change in the two periods, which suggests the population number did not alter the activity percentage in Pentezug.

If we compare the body condition in 2005 and in 2018, we can conclude that the condition of most horses greatly decreased probably because of the larger density and less available food (Rudman & Keiper, 1991). Environmental effects have more impact on young horses while they are still growing and cannot collect fat deposits. The harem stallions' physical condition did not change over the years, but the data show a high variability for this group. Harem stallions go through different stages when owning a harem. In the beginning, they have to put a lot of effort into protecting the harem and keeping the new members together. Also, when they are old or injured their physical condition quickly decreases and they often lose the harem.

Conclusions

We demonstrate that the Pentezug Reserve has been an adequate area for both Przewalski's horses and reconstructed aurochs for the last 22 years. To avoid overpopulation of the area, human interventions (birth control, transport, etc.) are essential. Monitoring the habitat use and body conditions of Przewalski's horses are also important because changes in these characteristics could indicate the necessity for human interventions.

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АНАЛИЗ ПАРАМЕТРОВ ИСПОЛЬЗОВАНИЯ ТЕРРИТОРИИ, АКТИВНОСТИ И ФИЗИЧЕСКОГО СОСТОЯНИЯ ЛОШАДЕЙ ПРЖЕВАЛЬСКОГО В НАЦИОНАЛЬНОМ ПАРКЕ ХОРТОБАДЬ, ВЕНГРИЯ

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Популяция лошади Пржевальского (*Equus ferus przewalskii*) был основана в полурезервате Пентезуг в Национальном парке Хортобадь в 1997 г. Кроме лошадей Пржевальского, там также разводят восстановленных туров (*Bos taurus taurus*) на огороженной территории площадью 24.5 км^2. Оба вида успешно размножились в степном заповеднике, некогда бывшим родиной их далеких предков. Количество крупных травоядных росло так быстро, что для восстановленных туров и лошадей пришлось применять разные стратегии контроля популяции в 2007 и 2013 гг., соответственно. На протяжении 22 лет проводился постоянный мониторинг активности, использования территории и физического состояния лошадей Пржевальского. Мы обнаружили сезонные изменения в активности лошадей, но соотношение основных типов активности были схожи в два разных периода времени. Напротив, были выявлены значительные различия в использовании территории и физическом состоянием лошадей в ранние и поздние годы проекта. Имеется в виду, что лошади были вынуждены использовать непривлекательные районы, и их состояние ухудшалось по мере увеличения плотности популяций крупных травоядных. Во время холодной весны 2018 г. произошла массовая гибель как лошадей, так и туров. Исследования показали, что ограниченные запасы кормов могут привести к изменениям в использовании среды обитания и/или ухудшению физического состояния, увеличению смертности при экстремальных погодных условиях. Таким образом, строгие меры контроля над рождаемостью лошадей Пржевальского должны применяться в популяциях с высокой численностью, и необходимо контролировать использование территории и физическое состояние животных, чтобы находить баланс между численностью популяции и емкостью угодий.

Ключевые слова: *Bos taurus taurus*, *Equus ferus przewalskii*, восстановленный тур, емкость угодий, заповедник Пентезуг, контроль численности, крупные травоядные, полурезерват