

THE SPACE USE BY PRZEWALSKI'S HORSES OF THE SEMI-FREE POPULATION DURING FIRST YEARS AFTER THEIR REINTRODUCTION TO ORENBURG STATE NATURE RESERVE, RUSSIA

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In 2015, the Joint Directorate of State Nature Reserves «Orenburg» and «Shaitan-Tau» (FSFI «Orenburg Reserves») launched the Programme on establishing a semi-free population of the Przewalski's horse *Equus ferus przewalskii* in the 165 km² Pre-Urals Steppe site of Orenburg State Nature Reserve (Russia). The aim of this study is to analyse the space use by two harem groups of the Przewalski's horse during the first years after release from acclimatisation enclosures. The study conducted from October 2016 to March 2019 and started when the first group of horses had been released from the acclimatisation enclosure to the main territory of Pre-Urals Steppe. Locations of harems were analysed separately for two periods: winter and snow-free. We compared location points in preferred areas (assessed by Kernel Density Estimation 50%) and other (random locations) with regard distance to acclimatisation enclosures, to a local zoo, and to ponds (only for snow-free period). The horses preferred to be closer to the acclimatisation enclosures and the local zoo during the winter period, but tended to avoid the zoo during the snow-free period. The distance between free-ranging harem groups was shorter during the winter than in the snow-free period. We also found a higher tendency of one group to be closer to the acclimatisation enclosures and the zoo. The presence of other horses within the area of release is an obvious attraction factor for free-ranging individuals and groups. We are of the opinion that it is due to needs to occasionally interact with horses of other groups regardless of whether it is aggression or attachment. Females seemed to contribute significantly in interband relationships of Przewalski's horses in Pre-Urals Steppe. Such social needs may facilitate a whole population's integrity, as acclimatisation enclosures with groups of horses inside will be an anchor that holds free-ranging horses within the site of reintroduction. On the other hand, free-ranging Przewalski's horses will leave an unfenced protected area with no guarantee to come back as soon as they detect other horses outside the reserve border. The difference in the distance between groups and the lower association needs in the snow-free period indicates the necessity for proper planning of a suitable area for free-ranging populations of Przewalski's horses.

Key words: distribution, *Equus ferus przewalskii*, Kernel Density Estimation, Pre-Urals Steppe, space use

Introduction

The habitat use patterns are a key parameter usually evaluated for animals; it allows to get the knowledge about the basic issues related to the microclimate, food base, habitat quality, etc. (e.g. Tweed et al., 2003; Moorhouse et al., 2009). It is also correlated with the social organisation of the population and the interaction of competing species and potential predators (e.g. Christensen & Burrows, 1995; Bahloul et al., 2001). Today, anthropogenic impacts play an important role in shaping spatial patterns of animals – many species adjust their spatial behaviour and daily activity to the distribution of human settlements, road or other infrastructure (e.g. Kuemmerle et al., 2010; Łopucki et al., 2017).

The issues described above become particularly important when carrying out conservation activities for rare and endangered animal species. This also applies to the Przewalski's horse *Equus ferus przewalskii* Poliakov, 1881, due to many efforts to reintroduce this species (Ryder, 1993; Pereladova et al., 1999; Zharkikh et al., 2002; Chen et al., 2008; King, 2012; Xia et al., 2014). These activities may include, for example, a multi-stage reintroduction of these animals in specially selected areas. The practice of these activities shows that such reintroductions of endangered species are not always successful, despite making large efforts and significant cost input (Reading et al., 2002; Landa et al., 2017). Such a situation appeared also in the case of Przewalski's horses in the Gobi desert (Kaczensky et al., 2011).

Therefore, there is a great need for detailed documentation of successful and unsuccessful re-introduction projects for a better understanding of all the mechanisms involved in the success of such conservation measures. Particularly the initial period of re-introduction during the first few years is considered the most critical, and research attention should focus on it.

In 2015, the Joint Directorate of State Nature Reserves «Orenburg» and «Shaitan-Tau» (FSFI «Orenburg Reserves») launched the Programme on establishing a semi-free population of the Przewalski’s horse in the Pre-Urals Steppe site of Orenburg State Nature Reserve, Russia. The Programme includes intensive monitoring after the release of the Przewalski’s horses into natural habitats. The monitoring allows also for more detailed analysis, including the spatial distribution of animals. These analyses are necessary to increase knowledge about this new population, but also to find general trends to minimise the risks of newly created populations. The aim of the study is to analyse the space use of two harem groups of the Przewalski’s horse in the Pre-Urals Steppe site of Orenburg State Nature Reserve in the first years after release.

Material and Methods

In 2015, the former military range of 165.38 km² was transferred into Orenburg State Nature Reserve ownership as the Pre-Urals Steppe site. It is situated in the Belyaevka district and Akbulak district, Orenburg Region, 120 km outside Orenburg. The centre of the site is located at 51.182600 N and 56.181700 E. There are several settlements around the site, local people work in agriculture, they are particularly engaged in livestock grazing including horse-herding (Fig. 1). A local Zoo (Breeding Centre for Steppe Animals as a part of the project «Orenburg Tarpania») lies adjacent to the border of Pre-Urals Steppe outside the area of the Orenburg State Nature Reserve. The Zoo keeps and breeds Przewalski’s horses, kiangs *Equus kiang* Moorcroft, 1841, yaks *Bos grunniens* Linnaeus, 1766, Bactrian camels *Camelus bactrianus* Linnaeus, 1758, and domestic goats *Capra hircus hircus* Linnaeus, 1758 (Chibilev et al., 2015).

Grasslands occupying more than 95% of the area represent rich bunchgrass, psammophytic, petrophytic, halophytic steppes, and their anthropogenic derivatives (Fedorov et al., 2018). There are three permanent ponds (filled with rain, melted water and partially with weak springs) and two semi-permanent ponds drying up during summer.



Fig. 1. The Pre-Urals Steppe site and surrounding territories (Orenburg Region, Russia).

In 2015, the Reintroduction Centre for the Przewalski’s horse was constructed in Pre-Urals Steppe. Two permanent acclimatisation enclosures of wire mesh of 0.45 km² in size each with natural steppe vegetation were constructed next to each other (Bakirova & Zharkikh, 2019). In 2016, a 2 m high and 52 km long wire mesh fence was constructed along the perimeter of the Pre-Urals Steppe site to prevent Przewalski’s horses from leaving the Protected Area.

There are five groups of founder horses, totaling 36 individuals, transported to Pre-Urals Steppe in 2015–2017. As there are two enclosures for acclimatisation of imported Przewalski’s horses, it was necessary to release earlier transported groups to have a place to acclimate the next groups (Table 1).

Two harem groups and three bachelor males have been released from the enclosures into the main territory of Pre-Urals Steppe since 2016 (Table 2). The study starting from release of the first group of horses, covered the period from 3 October 2016 to March 2019. During this period the released horses were observed during year-round routine monitoring. Other imported horses were still kept in the enclosures during the period of the study.

Table 1. Summary on the keeping of groups of Przewalski’s horses in acclimatisation enclosures in Pre-Urals Steppe, Orenburg State Nature Reserve, Russia

Acclimatisation enclosure 1		Acclimatisation enclosure 2	
Group	Period	Group	Period
1 st harem (Aven’s)	Oct 2015 – Oct 2016	2 nd harem (Makos’)	Nov 2016 – Sep 2017
Only-male group	Nov 2016 – present	Only-female group	Oct 2017 – May 2018
		3 rd harem	June 2018 – present

Table 2. Composition of studied horse groups in Pre-Urals Steppe, Orenburg State Nature Reserve, Russia

Group	Composition (adults) male/females	Keeping in acclimatisation enclosure	Roaming in Pre-Urals Steppe
Aven's harem	1/4 ¹	Oct 2015 – Oct 2016	since Oct 2016
Makos' harem	1/8	Nov 2016 – Sep 2017	since Sep 2017
	1/14 ^{2,3}		since Jun 2018
Bachelor males	1	Nov 2016 – Jan 2019	Jun and Jul 2018
	3		since Jan 2019

¹a foal was born on 18 September 2018;

²other six females joined Makos' harem in June 2018;

³four foals were born between May and October 2018.

The location of each studied group was marked with the GPS receiver Garmin eTrex 20 with indication of date, and harem stallion's name. The whole year observations were divided in: a) winter period – from November to March (with permanent snow cover), b) snow-free period – from April to October. The two harems were observed on average seven times per month during the winter period and 11 times per month during the snow-free period. Locations of bachelors were registered with 20 observations during the winter 2018/2019 and 32 observations during the snow-free period in 2018. In total we collected 559 observations, 228 during snow periods and 331 during snow-free periods.

Basing on the GPS-points we assessed the preferred areas (frequently visited areas) with Kernel Density Estimates 50% in Quantum GIS (version 3.4.5-Madeira) separately for both harem groups, bachelors and separately for the period type of the year (winter and snow-free). For bachelors, KDE 50% was made only for a visual comparison, due to the small number of observations. GPS-points inside core areas (KDE 50%) were regarded as preferred locations, whereas other points as random (not preferred). We also assessed distance of GPS-points to the acclimatisation enclosures and the local zoo for the winter period as well as distance of GPS-points the acclimatisation enclosures, the local zoo and ponds for the snow-free period.

For statistical assessment of the horses' distribution we used a logistic regression model with binary response variable, where locations of preferred areas (core areas KDE 50%) were marked as 1 and random locations as 0. In each separated model, for both harem groups and both period types of the year, we set covariates: (a) distance to acclimatisation enclosures and (b) distance to local zoo for the winter period, and: (a) distance to acclimatisation enclosures, (b) distance to local zoo, (c) distance to ponds for the snow-free period. We also compared distances between harem groups during the winter period and snow-free period, using GPS-points located during the same day for both groups.

The comparison was made with U-Mann Whitney test, because data were not normally distributed. All statistics were performed using SPSS software (version 24.0, IBM Corporation, Armonk, NY).

Results

Both groups of Aven and Makos were mainly located in the south-eastern part of Pre-Urals Steppe during winter period (Fig. 2a). Group locations were mixed within the explored area and preferred areas often overlapped. The preferred areas were mostly located close to the acclimatisation enclosures and covered 6.65 km² (Aven's group, ca. 4% of the total Pre-Urals Steppe area) and 5.71 km² (Makos' group, ca. 3.5% of the total Pre-Urals Steppe area). During snow-free periods a more separated distribution of groups was observed. Makos were mostly registered in the south-western part, while Aven roamed mostly in the eastern and northern part of Pre-Urals Steppe. Preferred areas overlapped only partially (Fig. 2b), and were larger than in the winter period: Aven's area covered 14.33 km² (ca. 8.7% of the total Pre-Urals Steppe area), Makos' area covered 8.08 km² (ca. 4.9% of the total Pre-Urals Steppe area).

All four logistic regression models were statistically significant ($p < 0.05$). Both groups showed a high preference to be closer to acclimatisation enclosures and the zoo during the winter period (Table 3). Nevertheless, Makos' group showed a higher chance/probability to be closer to acclimatisation enclosures as well as the zoo (Exp (B) = 0.457, and Exp (B) = 0.694 respectively) than Aven's group (Exp (B) = 0.746, and Exp (B) = 0.807 respectively). During the snow-free period both groups showed a weaker preference to be closer to acclimatisation enclosures, but again, Makos presented a higher chance to be closer than Aven (Exp (B) = 0.707 and Exp (B) = 0.840). Both groups avoided the zoo during snow-free period, with stronger avoidance of Aven than Makos (Exp (B) = 1.652 and Exp (B) = 1.233). Ponds did not statistically explain location of horses of both groups.

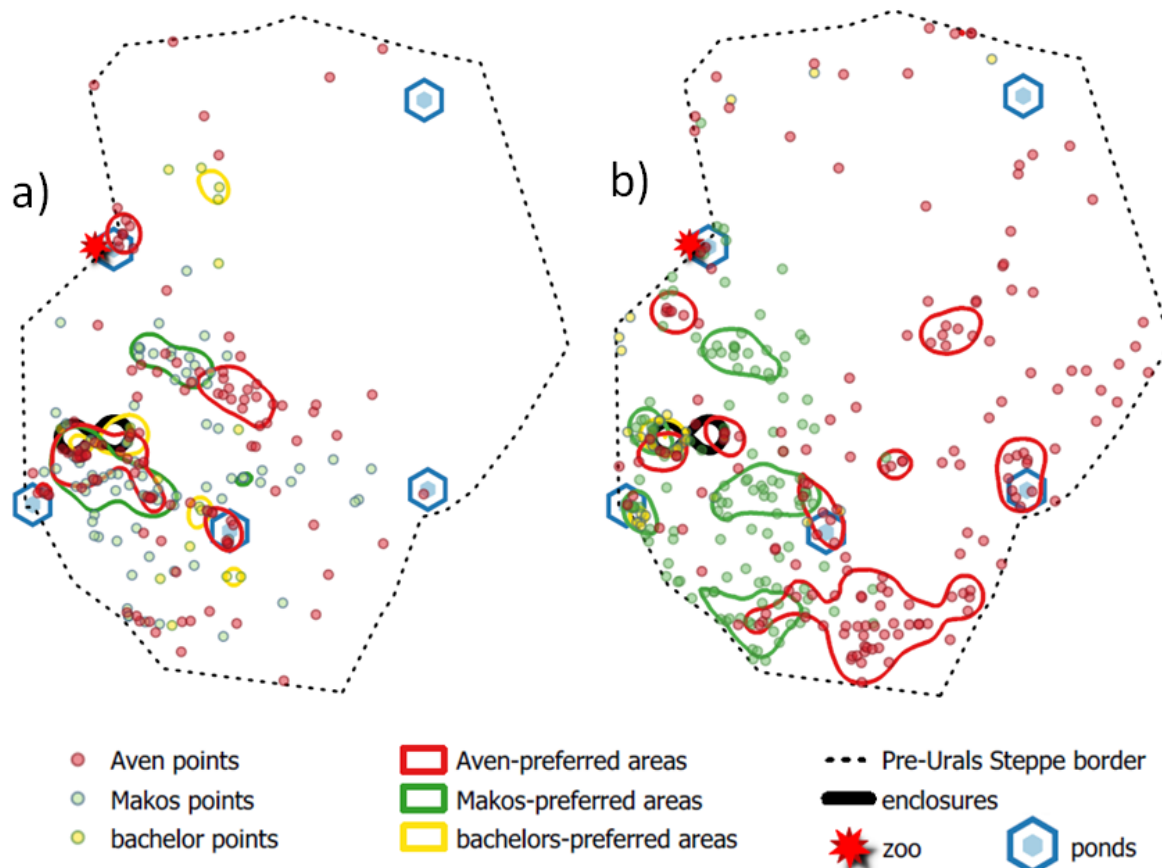


Fig. 2. Distribution of Przewalski’s horses during a) winter and b) snow-free period in Pre-Urals Steppe (2016–2019), Orenburg State Nature Reserve, Russia.

Table 3. Main summary statistics or logistic regression models of preferred/random locations of Przewalski’s horses and explanatory variables (distance to acclimatisation enclosures, zoo and ponds)

		Enclosure P / Exp (B)	Local zoo P / Exp (B)	Ponds P / Exp (B)	Whole model P / χ^2
Winter period	Aven	0.000 / 0.746	0.025 / 0.807	–	0.000 / 31.2
	Makos	0.000 / 0.457	0.000 / 0.694	–	0.000 / 45.3
Snow-free period	Aven	0.000 / 0.840	0.000 / 1.652	0.586 / NS	0.000 / 50.1
	Makos	0.001 / 0.707	0.024 / 1.233	0.819 / NS	0.002 / 15.1

An average distance between groups marked during the same day of survey was 2.53 km during the winter period and 6.91 km during the snow-free period. Groups were statistically closer to each other during winter than in the snow-free period ($p = 0.000$, Fig. 3).

Discussion

Numerous studies reported that despite the frequently observed overlapping of home ranges of different bands of free-ranging horses, it is not unusual for bands to utilise different core areas; bands mutually avoid each other to a possible extent (see Waring, 2003). When the recourses were abandoned and evenly distributed in the habitat, home ranges need not overlap.

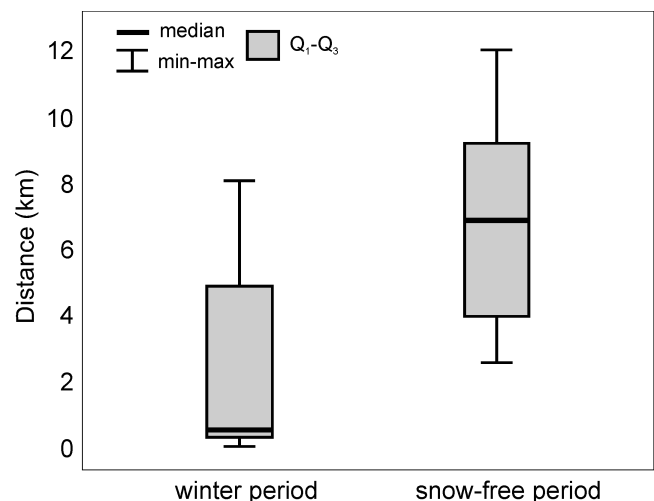


Fig. 3. Distance between harem groups of Aven and Makos during winter and snow-free season (statistically significant in U-Mann Whitney test, $p = 0.000$), Orenburg State Nature Reserve, Russia.

The results of this study showed the occurrence of some distinct phenomena characterising the space use by Przewalski's horses in the Pre-Urals Steppe site. The first is the difference between periods of the year. The horses preferred to be closer to the acclimatisation enclosures and the local zoo during the winter period, and to avoid the local zoo during the snow-free period. Moreover, the distance between free-ranging harem groups was shorter in the winter than in the snow-free period. The second phenomenon was that the preferred areas for different groups partially overlapped but it could not be explained by the lack of pastures and/or water resources in Pre-Urals Steppe. The third phenomenon was a higher tendency of the Makos' group to be closer to the acclimatisation enclosures and the local zoo.

Ponds did not statistically explain location of horses in Pre-Urals Steppe, regardless that they were often observed near ponds during routine monitoring. This was probably due to the regular distribution of the water sources within the area of the Pre-Urals Steppe site, where horses had a good access to the water.

Other studies reported that Przewalski's harems showed different patterns in the change of home range over years after their release: some preferred staying close to their previous enclosures, others moved farther. This was attributed to that the horses tended to remain in an area with which they were familiar and that they could find all necessary resources in this area so they have no need to travel farther (Bouman, 1998; Pereladova et al., 1999; King, 2002, 2012). For Pre-Urals Steppe, we are of the view that the main reason of this behaviour was the presence of other horses in the enclosures. Free-ranging groups came regularly to interact distantly – to see, sniff and hear other horses. They were often observed near the enclosures trying to make contact with captive individuals. Przewalski's horses introduced into the Chernobyl Exclusive Zone, Ukraine, showed similar spatial behavioural patterns. The first group of young bachelor males released from the Acclimatisation Centre in Chernobyl Exclusive Zone had been roaming in the vicinity of the enclosures until a harem group was released. After that, the bachelors moved farther. For the next years the harem's home range adjoined to the Acclimatisation Centre where other Przewalski's horses and domestic horses were kept (Zharkikh et al., 2002). Similar interactions were probably the reason of preference to be closer to the local zoo in this study, where other Przewalski's horses were kept. We also

speculate that the presence of free-ranging groups near the south-western part of the Pre-Urals Steppe site was a result of a similar interaction with bands of domestic horses outside the Orenburg State Nature Reserve border.

It seems that females have a noticeable tendency to seek social contacts with other females even if they belong to different groups. When studied harems came to the acclimatisation enclosures, the females were staying close to the fence with captured individuals of either the only-female group or the 3rd harem, displayed a wide range of facial and body gestures, occasionally produced nickers, whinny and other acoustical expressions. Harem stallions demonstrated common rituals including faecal pile displays, and then tried to herd their females away from the fence, but they (females) were coming back to the fence of the enclosure for a while. This supposition is also confirmed by observations in Askania Nova Biosphere Reserve, Ukraine. Surplus females (40 to 60 specimens) were free-roaming in one group (T.L. Zharkikh, unpublished data). In contrast, 15 to 20 bachelor males kept in an isolated enclosure, were often separated into several distinct groups (Zharkikh & Andersen, 2009).

Makos' group presented a higher need to be closer to other horses and the acclimatisation enclosures throughout the year than Aven's group. We speculate that Aven's spatial behaviour (more distant preferred areas to other horses) was the result of his weaker position toward Makos. There were observed numerous fights between both stallions, and Makos always won. A lower position in the hierarchy may result in the avoidance of other horses (Bailey, 2016), which is more justified in the case of Aven, who can lose the mares in contact with another stallion.

Differences between periods of a year were probably an effect of changes in horse behaviour. Przewalski's horses in natural and semi-natural conditions usually start the reproductive season in April or May and end usually in September (e.g. Zharkikh, 2000; Chen et al., 2008; Dorj & Namkhai, 2013). During this period, stallions are more focused on defence against other stallions, potential competitors for mares. During the reproductive season, the frequency of agonistic interactions between stallions increases, and they tend to be more aggressive than bachelors (e.g. Boyd, 1988; Zimmermann et al., 2009). For this reason, groups may have a tendency to keep farther away from each other. This phenomenon is confirmed by differences

in the distance between free-ranging horses, as the distance was larger during the snow-free period. The results coincide with reports from Mongolian Gobi, where close associations of the Przewalski's horse harems were observed only outside the mating season (Kaczensky et al., 2008). In Uzbekistan, although home ranges overlapped, during summer the groups were rarely recorded at a distance of visual contact (Bahloul et al., 2001). The lower tendency in contacts with other horses during the mating season explains the avoidance of the local zoo and the weaker preference of being closer to acclimatisation enclosure during the snow-free period.

Conclusions

The analysis of the space use by Przewalski's horses revealed some behaviour peculiarities of the newly formed population after release. The presence of other horses within the area of release is an obvious attraction factor for free-ranging individuals and groups. We are of the opinion that it is due to needs to occasionally interact with horses of other groups regardless of whether it is aggression or attachment. Females seemed to contribute significantly in interband relationships of Przewalski's horses in Pre-Urals Steppe.

Such social needs may facilitate a whole population's integrity as acclimatisation enclosures with groups of horses inside will be an anchor that holds free-ranging horses within the site of reintroduction. On the other hand, free-ranging Przewalski's horses will leave unfenced protected area with no guarantee to come back as soon as they detect other horses outside the reserve border.

The difference in the distance between groups and the lower association needs in the snow-free period indicates the necessity for proper planning of a suitable area for free-ranging populations of Przewalski's horses.

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ИСПОЛЬЗОВАНИЕ ТЕРРИТОРИИ ЛОШАДЬМИ ПРЖЕВАЛЬСКОГО ПОЛУВОЛЬНОЙ ПОПУЛЯЦИИ В ПЕРВЫЕ ГОДЫ ПОСЛЕ ИХ РЕИНТРОДУКЦИИ В ГОСУДАРСТВЕННЫЙ ПРИРОДНЫЙ ЗАПОВЕДНИК «ОРЕНБУРГСКИЙ», РОССИЯ

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В 2015 г. ФГБУ «Заповедники Оренбуржья» (Объединенная дирекция государственных природных заповедников «Оренбургский» и «Шайтан-Тау») начали Программу по созданию полувольной популяции лошади Пржевальского *Equus ferus przewalskii* на участке Предуральская степь площадью 165 км² в государственном природном заповеднике «Оренбургский» (Россия). Цель настоящего исследования – проанализировать использование территории двумя гаремными группами лошади Пржевальского в первые годы после их выпуска из акклиматизационных загонов. Исследования проводились с октября 2016 г. по март 2019 г., и были начаты, когда первая группа лошадей была выпущена из акклиматизационного загона на основную территорию Предуральской степи. Местонахождения гаремов анализировались отдельно для двух периодов: зимний и бесснежный. Мы сравнили места встреч животных в предпочтительных местообитаниях (рассчитанных с помощью ядерной оценки плотности распределения KDE 50%) и других (случайные местоположения) с точки зрения расстояния до акклиматизационных загонов, до местного зоопарка и прудов (только для бесснежного периода). Лошади предпочитали быть ближе к акклиматизационным загонам и местному зоопарку в зимний период, но, как правило, избегали зоопарка в бесснежный период. Расстояние между вольными гаремными группами зимой было меньше, чем в бесснежный период. Мы также обнаружили более высокую склонность одной группы держаться ближе к акклиматизационным загонам и зоопарку. Присутствие других лошадей в зоне выпуска является очевидным фактором привлекательности для отдельных особей и групп. Мы считаем, что это связано с необходимостью время от времени взаимодействовать с лошадьми других групп независимо от того, является ли это агрессией или привязанностью. По-видимому, кобылы играли значительную роль в установлении межгрупповых отношений у лошадей Пржевальского в Предуральской степи. Подобные социальные потребности могут способствовать целостности популяции, так как акклиматизационные загоны с группами лошадей внутри будут якорем, который удерживает вольно пасущихся лошадей в месте их реинтродукции. С другой стороны, вольные лошади Пржевальского покинут неогороженную охраняемую территорию без гарантии возвращения, как только обнаружат других лошадей за пределами границы заповедника. Разница в расстоянии между группами и более низкими потребностями в группировании в бесснежный период указывает на необходимость правильного выбора подходящей территории для вольных популяций лошадей Пржевальского.

Ключевые слова: *Equus ferus przewalskii*, использование территории, Предуральская степь, распределение, ядерная оценка плотности распределения