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Behavioural ecology of Przewalski's horses along their reintroduction process

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DECLARATION

I, Anna Bernátková, hereby declare that I have written this thesis entitled “Behavioural Ecology of Mongolian Ungulates” independently, except for chapters 4-7 where all the co-authors of the included articles were declared, and all of them agreed that the articles would be published as a part of this thesis. Moreover, I declare that the thesis includes the original text and all sources used have been quoted and acknowledged employing the complete reference list in the chapter General references according to the citation rules of FTA. I state that the work has not been submitted for any other degree to this or any other university within and outside Czechia.

In Prague, May 12th 2023

Ing. Anna Bernátková

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ABSTRACT

Reintroduction is a common technique for re-establishing threatened species. However, adapting to novel habitats with distinct conditions poses a risk of failure. Przewalski's horse (*Equus ferus przewalskii*) became extinct in the wild in the 1960s, with the last individual observed in the Great Gobi B Strictly Protected Area (GGBSPA), Mongolia. Thanks to the ongoing reintroduction efforts, the species was listed as Endangered in 2011. The current population of Przewalski's horses at the GGBSPA is composed of individuals with various origins (captive-breeding and wild-born), and the groups have different experiences with the area (recently reintroduced, long-term reintroduced and wild-born), therefore serving as an ideal natural model for behavioural studies. Despite substantial research on captive breeding and reintroduction over the last half-century, the long-term conservation of Przewalski's horses remains challenging, and information on the behavioural ecology of different groups of different origins is lacking. We studied selected wild-born and reintroduced Przewalski's horse groups in different seasons by direct observation and analysed the effect of the origin (wild-born vs reintroduced) on the response to environmental conditions, social network and habitat use at harem and individual levels. The origin of the horses was proven to be one of the most important factors influencing their behaviour. In general, wild-born horses in our study displayed better weather adaption, more complex social networks, and a selection of feeding locations with higher-quality vegetation compared to reintroduced horses. The need to acclimate to novel conditions was probably the cause of altering the behavioural pattern and habitat utilisation of recently reintroduced individuals. These horses also require sufficient time to form stable social relationships. The findings support the hypothesis of horses' gradual behavioural adaption to their new surroundings, which won't likely be complete until the second generation arrives. The results of this study may assist future reintroduction programs and the successful conservation of the species. Future research may focus on captive breeding conditions and their effects on subsequent adaptation to reintroduction at the individual level.

Keywords: Behaviour; Reintroduction; *Equus ferus przewalskii*; Gobi Desert; Mongolia; Soft-release

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CHAPTER 1

1. Introduction

1.1. General introduction

Przewalski's horse became extinct in the wild in the 1960s but was successfully recovered from extinction through captive breeding and reintroduction programs (Liu et al., 2014; van Dierendonck & de Vries, 1996; Xia et al., 2014). In China and Mongolia, breeding centres and reserves for the species have been developed. Currently, six main reintroduction areas with wild populations of Przewalski's horse are present in these two countries. With a significant increase in the number of wild Przewalski's horses, the International Union for the Conservation of Nature (IUCN) Red List of Threatened Species reclassified the species from Extinct in the Wild to Critically Endangered in 2005, and to Endangered in 2011 (King et al., 2015).

Despite substantial research on captive breeding and reintroduction over the last half-century, the long-term conservation of Przewalski's horses remains challenging (King et al., 2015). The reintroduction of animals into their former habitat is fraught with difficulties and requires immense effort. Individuals may have problems adapting to natural environments due to a lack of experience, adaptation, or physiological and morphological characteristics that are not appropriate. The key challenges in maintaining a wild population are selecting a suitable region, identifying the best stocks of animals for re-wilding, and implementing appropriate conservation management measures (IUCN, 2013). Interspecific interaction of Przewalski's horses with other animals and ungulate species in the release region, including breeding with domestic horses, must also be considered (King et al., 2015). Another challenge to reintroduction success is a lack of knowledge about the ecology of Przewalski's horses prior to their extinction in the native locations (Zhang et al., 2015).

The study of behaviour is a crucial aspect of long-term monitoring of reintroduced populations (Berger-Tal et al., 2011; IUCN, 2013) as the response of reintroduced animals to the environment is vital for their survival (Moorhouse et al., 2009; Rantanen et al., 2010). Moreover, it is possible to determine the state of reintroduced animals by observing their behaviour (Berger-Tal et al., 2011; Moorhouse et al., 2009; Rantanen et al., 2010). Therefore, behavioural research plays an essential role in monitoring and supporting reintroduction projects, and in the management of reintroduced species (Berger-Tal et al., 2020; Berger-Tal & Saltz, 2014; Seddon et al., 2007).

1.2. Aims of the thesis

Within the context mentioned above, this thesis aims to provide valuable insights into the ecology and behaviour of wild-born and reintroduced Przewalski's horses. To achieve these aims, the current dissertation seeks to: (1) Assess the influence of weather on the behaviour of Przewalski's horse in the Great Gobi B Strictly Protected Area (GGBSPA), Mongolia; (2) Analyse factors influencing the social behaviour of Przewalski's horse in the GGBSPA; and (3) Determine whether there is a difference in habitat use of wild-born and reintroduced Przewalski's horse in the GGBSPA.

1.3. Chapter overview

The thesis consists of three published papers and one manuscript under review. The research outputs are linked within the aims, research statements and outcomes as presented in Chapters 2-11 below.

The possible influence of weather on the behaviour of Przewalski´s horses is investigated in Chapter 4 (Paper 1, Bernátková et al. 2022). The research describes the effects of thirteen weather variables on selected behavioural categories (feeding, locomotion, resting, and social) of Przewalski´s horses in the GGBSPA. The influence of the group-history factors (recently reintroduced, long-term reintroduced and wild-born) on the selected behavioural categories is also analysed.

The second research (Chapter 5) evaluates the habitat use of selected Przewalski’s horse groups through ecological niche models (ENMs) and behavioural data. Three types of satellite-derived predictors (topography, ALOS; vegetation indexes, Landsat; land cover, Copernicus) are used to: 1) model habitat preferences for feeding and resting behaviour; 2) identify the influence of origin (wild-born vs reintroduced); and 3) describe the potential influence of human presence on the habitat selected by the horses for these behaviours (Manuscript 2, Bernátková et al. under review).

Based on the lacking knowledge comprising the social behaviour of different groups of Przewalski´s horses of different origins at different seasons, the third research (Chapter 6) aims to compare differences among individual group members and whole groups of wild-born, recently reintroduced, and long-term reintroduced Przewalski's horses in three different seasons in the GGBSPA, and to provide comprehensive information on this topic. Therefore, the study explores the influence of origin and season, but also sex, age, relative time belonging to the group, relatedness, and dominance rank on their social behaviour (Paper 2, Bernátková et al.2023).

Chapter 7 presents an observation of play behaviour between Przewalski's horse dominant harem stallion and members of a bachelor group. This chapter describes the first video recording of such an event and provides a summary of existing scientific knowledge on this behaviour, that is either unusual or has not been studied sufficiently (Paper 3, Bernátková et al. 2023).

Finally, the general discussion and conclusions summarise the research findings and provide recommendations for future research and conservation of Przewalski's horses.

CHAPTER 2

2. Literature review

2.1. Threats and conservation of wild animals

Humans are having an increasing impact on the populations of wild animals. Thus, effective measures for preserving endangered species are critically required in light of potentially disastrous biodiversity losses (Bainbridge, 2014). Even though standard methods such as law enforcement or indigenous community involvement have historically been successful for conservation management, further intrusive methods are becoming more and more necessary to guarantee the long-term existence of vulnerable populations (Challender & MacMillan, 2014; Keane et al., 2008).

Large, endangered mammals are frequently entirely removed from their natural habitats as a result of shrinking suitable areas, hunting or human-wildlife conflict. Therefore, placing and breeding endangered animals in ex-situ facilities is one type of potential conservation action (IUCN, 2013). Translocation of animals into their natural habitat and/or populations often represents a primary objective of such facilities (Banes et al., 2016). Reintroduction is a common translocation technique for in-situ conservation that attempts to return species to areas of their historical range. It is one of the most effective conservation tools for re-establishing and supporting threatened species populations and, in some cases, the only option. Nevertheless, the movement of animals to novel habitats poses a risk of failure (Armstrong & Seddon, 2008; Baguette et al., 2013; Seddon et al., 2007). Reintroduction strategies are complicated and involve multiple decisions, many of which could be more predictable (Fischer & Lindenmayer, 2000). The essential factors affecting a reintroduction's long-term success are access to vast areas of appropriate habitat and the genetic composition of the reintroduced population (Armstrong & Seddon, 2008; Baguette et al., 2013; Wolf et al., 1998). Insufficient knowledge of the reintroduced species' ecology before it vanished from its native habitat is another barrier to effective reintroduction. Some animal species have been reintroduced to the edge of their original habitat due to a lack of sufficient knowledge and the absence of large, suitable areas (Armstrong & Seddon, 2008; Baguette et al., 2013; Seddon et al., 2007).

2.1.1. Conservation of large ungulates

Among the charismatic species that have long drawn people's interest, admiration and became a subject of conservation attempts are numerous large terrestrial herbivores. Large herbivores can benefit rural communities by providing commodities and income, primarily by harvesting or indirectly by bringing enterprise and capital to the region, especially in locations with a thriving tourism sector. They are essential for maintaining ecosystems, dispersing seeds, cycling nutrients, and providing food for predators, including humans (Ripple et al., 2015). Despite the profit they bring to many humans, and their significance for ecosystem processes, the world's largest terrestrial herbivores are undergoing major population losses and range limitations worldwide. Forty-four of the 74 large terrestrial herbivores (≥100 kg) are recognised as threatened with extinction by the IUCN including 12 that are critically endangered or extinct in the wild, and 43 have declining populations (IUCN, 2022). The primary drivers of massive, large ungulate decline are an increasingly growing global population and rising per-person resource demand. Large ungulates are particularly vulnerable to human hunting and overexploitation, modifications in land use patterns, livestock competition for resources and space, and climate change (Luo et al., 2015; Ripple et al., 2015). Attempts are being made internationally to mitigate human impact on large ungulate habitats and to slow down excessive resource overuse. Still, imminent conservation measures should include efficient methods despite the rising human population density (Ripple et al., 2015).

2.1.2. History and conservation of Przewalski's horse

Przewalski's horse reintroduction is one of the most phenomenal and renowned examples of large ungulate conservation practice. Przewalski’s horse (*Equus ferus przewalskii*), or Takhi, once roamed through steppes of Central Asia until hunting and livestock competition depleted it to the brink of extinction (International Takhi Group, 2021). The last individual was seen in 1969 in the GGBSPA, Mongolia. Przewalski’s horse was listed as Extinct in the Wild in 1996, but thanks to the ongoing reintroduction became Critically Endangered in 2005 and is now listed as Endangered. It is currently classified as a subspecies of the extinct *Equus ferus*. Although Przewalski's horses have a distinct chromosomal karyotype (2n=66) to domestic horses (2n=64), the two species can interbreed and produce fertile offspring (King et al., 2015). DNA studies have proven that current domestic horses are not descended from Przewalski's horse, but have a common ancestor. The two subspecies split approximately 38,000 - 72,000 years ago (Der Sarkissian et al., 2015). However, current research suggests that Przewalski's horses are the feral descendants of horses domesticated by the Botai 5,500 years ago. In contrast, modern domestic horses evolved from horses domesticated later by different ethnicities (Gaunitz et al., 2018).

All living Przewalski's horses descend from 12 Przewalski's horse founders. Between 1901 and 1903, the first 11 Przewalski's horse founders were foals captured in southwest Mongolia. Orlitza III, the final wild founder, was apprehended in Mongolia in 1947 (Boyd and Houpt 1994, Bowling et al. 2003). One Mongolian domestic horse mare was also identified as a founder (the 13th founder; Bowling et al., 2003). Because of the limited size of the founding population, captive breeding programs have been concerned about handling high levels of inbreeding and the potential introgression of domestic horse genes into Przewalski's horse gene pool. Gene introgression remains one of the major conservation threats for the species today (Gaunitz et al., 2018; King et al., 2015; Turghan et al., 2022). Currently, there are more than 2500 Przewalski's horse individuals in the world. Among them, approximately 1360 horses live in the wild in Mongolia and China, 900 in European parks and zoos, and 120 in US parks (Jiang & Zong, 2019; Kerekes et al., 2021a).

2.1.3 Threats to the conservation of Przewalski's horse

It has been suggested that a number of factors contributed to Przewalski's horses' extinction in the area of current China and Mongolia. Major political and cultural shifts, hunting, military activity, climate changes, livestock competition, and expanding land use demand were probably the primary causes. However, by killing and scattering the adults, capture attempts by zoos, parks, and private collectors most likely additionally reduced Przewalski's horse numbers. It is highly possible that the severe winters in 1945, 1948, and 1956 also affected the limited population. Another important cause of the disappearance of Przewalski's horse from its natural habitat was the rising demand for water by herders and their livestock and the subsequent scarcity of waterholes in the species’ last strongholds (King et al., 2015; van Dierendonck & de Vries, 1996; Xia et al., 2014). Currently, small population size and restricted geographic distribution pose the biggest threat to the populations that have been restored, followed by the possibility of hybridisation with domestic horses and competition for resources with livestock. The possibility of hybridisation and disease transmission exists whenever Przewalski's horses come into contact with domestic horses. The survival of Przewalski's horse populations is also threatened by illegal land use, such as mining in protected areas (King et al., 2015).

As proposed by the IUCN Red List of Threatened Species (King et al., 2015), to improve the status and conservation of the species, the following management steps are fundamental:

- Disease surveillance for domestic and wild horses is necessary (Robert et al., 2005).
- All populations' health, fertility, mortality, habitat use, and social structure should be tracked using standardized methodologies (Wakefield et al., 2002).
- Contact between domestic horses and Przewalski's horses should be minimized.
- It is necessary to develop a unified population management strategy.
- All wild Przewalski's horses should have their ancestry documented, and steps should be taken to increase genetic variety (King et al., 2015)
- Governments should create and make available to locals and the public a protocol for hybrids´ management (King & Gurnell, 2005).
- It would be advantageous if all reintroduction centres communicate and work together more frequently.
- It would be favourable for the employees and scientists involved in this conservation initiative to have additional training and post-graduate study (King et al., 2015).

Threats to the survival of reintroduced Przewalski's horses can be reduced through strategic planning based on a knowledge of Przewalski's horse behaviour, habitat use and social structure. There has been intense research involving horses in zoos, parks, and reintroduction projects (King et al., 2015; Wakefield et al., 2002). However, a more profound understanding of the ecology of this endangered species is essential for optimal management, considering any differences between animals of wild-born and reintroduced origins. This need is urged by population increase, which necessitates bigger regions of appropriate habitat (Bernátková et al., 2022).

2.2. Reintroduction of Przewalski’s horse

Przewalski's horses were first released into vast fenced semi/reserves in Canada, the Netherlands, Germany, France, England, Hungary, Ukraine, Uzbekistan, and China in the 1980s as a pre-adaptation period. By 1990, four projects in China and Mongolia were prepared for the adaptation and reintroduction of Przewalski´s horses in the wild. In these two countries, Przewalski's horses are currently found in six areas (three separate locations throughout China and three separate locations throughout Mongolia; Bahloul et al., 2001; Jiang & Zong, 2019; Turghan et al., 2022).

2.2.1. Reintroduction of Przewalski’s horse in Mongolia

The Przewalski's horse reintroduction programme started in Mongolia in 1986. The GGBSPA, an International Biosphere Reserve in the southwest of Mongolia, where Przewalski's horses were last observed in the wild, and Hustain Nuruu National Park, a protected area north of the country's centre, about 130 km west of Ulaanbaatar, were chosen as the two first locations for the reintroduction of Przewalski's horses to the wild in their native habitat in Mongolia (van Dierendonck & Wallis de Vries, 1996; Wakefield et al., 2002).

The Great Gobi B Strictly Protected Area

The reintroduction efforts in the GGBSPA started in 1992 by transporting five adult Przewalski’s horses (two males and three females). In 1997, the first individuals were released from acclimatisation pens into the wild. Since then, a total of 93 horses were released till 2007 and 39 horses within the next period of transports (2011-2019) after the population crash in winter 2009/2010 (Jiang & Zong, 2019; ZIMS for Studbook for Przewalski’s horse, Šimek, 2022).

Hustai National Park

In Hustai National Park (Hustain Nuruu) the reintroduction efforts also started in 1992 by transporting 16 Przewalski's horses that were born in captivity. They were housed in enclosures in the park in preparation for a potential future reintroduction. In July 1994, the second set of 16 Przewalski's horses was delivered to the same location. In the following eight years, 89 Przewalski's horses were brought to the park. The first harem group was let out of the adaption enclosures in 1997 (Boyd & Bandi, 2002; Jiang & Zong, 2019; Wakefield et al., 2002).

Khomyn Tal

The third area of Przewalski’s horse reintroduction is located in the Khomyn Tal National Park, in the western part of Mongolia. Twelve horses that were born in captivity in France were transported to the Khomyn Tal in 2004. In 2005 a second transportation consisting of ten horses from France was made, and in 2011 four horses were transported from the Czech Republic (King, 2005).

2.2.2. Reintrioduction of Prewalski´s horse in China

While Przewalski's horse populations in Mongolia have received extensive coverage, data for China's program, for both captive and released populations, are severely deficient or imprecise (Jiang & Zong, 2019).

Xinjiang

A wild horse breeding facility was built in the semi-desert region of the Junggar Basin in Xinjaing in 1985 with the primary goal of creating a self-sustaining captive population from which animals can be reintroduced into the wild in the Kalamaili Nature Reserve. From 1985 until 2006, the centre received thirty captive Przewalski's horses in five groups from captive facilities in Germany, the United Kingdom, and the United States. In 1988, the first foal was born in the Xinjaing facility. In 2001 first horses from the Xinjaing facility were reintroduced into the wild in the Kalamaili Nature Reserve as the first step in the restoration program for the species throughout its previous ranges in

China, and since then, the reintroduction has taken place regularly (Jiang & Zong, 2019; Xia et al., 2014).

Wuwei-Dunhuang

Przewalski's horses in Wuwei Endangered Animal Breeding Centre and the Dunhuang West Lake Nature Reserve form the Wuwei-Dunhuang population. As part of the reintroduction initiative started by the Ministry of Forestry of the People's Republic of China, eighteen Przewalski's horses have been transferred from the US and Germany to the Wuwei Endangered Animal Breeding Centre since 1990. The horses were bred in the centre, and part of the population was released into the wild. The reintroduction took place twice: once in 2010 (seven horses) and once in 2012 (21 horses). However, the released Przewalski's horses were later taken back into the paddocks in order to provide extra feeding to improve winter survival and limit competition with the domestic horses kept by the local herders (Jiang & Zong, 2019; Liu et al., 2014).

Beijing-Anxi

David's Deer Park in Beijing City and the national natural reserve in Anxi County, Gansu Province represent the two parts forming the Beijing-Anxi area. Ten Przewalski's horses transported to the reserve in 1985 from the UK were housed in David's Deer Park for easier acclimatization. In 1997, two Przewalski's horses were kept in David's Deer Park, and ten horses were relocated to the reserve (Jiang & Zong, 2019; Wakefield et al., 2002). Five wild horses were present in David's Deer Park in 2018, but only one horse remained in 2021. Beijing-Anxi Przewalski's horse population now consists of 23 horses and still requires supplemental feeding (Jiang & Zong, 2019).

2.3. Behavioural monitoring in species conservation

Biodiversity conservation initially connected ecology (Soulé & Wilcox, 1980), research on population dynamics (Caughley & Gunn, 1996) and genetic studies (Schonewald-Cox et al., 1983) to understand how animals and their populations respond to environmental pressure and changes in their natural habitats. This knowledge was then applied to the preservation of the species. Historically, behavioural studies were not considered part of species conservation, and the proposed links between behavioural research and conservation have started with behavioural ideas and concepts rather than conservation issues (Caro, 2007). In the early 1990s, the first attempts to present the possibilities of the application of ethology to the conservation of fauna and its environments started. For example, MacDonald (1990) published a review on this subject in the yearbooks of the Fourth International Symposium on Wildlife Conservation. Another example of the initial steps towards behavioural conservation studies is the publication of Monaghan (1993) in the journal Etología. Both MacDonald (1990) and Monaghan (1993) underlined the contribution of ethology to conservation into a broader range of applications, which also included animal production. However, these examples only represented the efforts of rather few isolated researchers to spread the possibilities to apply the advances of ethological knowledge. In 1995, the first scientific meetings dedicated exclusively to the relationship between ethology and conservation were held (Cassini, 1999). These meetings were followed by two, in the view of behavioural conservation, historically most important symposia, one organized by the Animal Behavior Society in the United States and another organized by Oikos journal in Sweden (Cassini, 1999). After these key meetings, efforts to disseminate this field of knowledge through books, articles, sessions, congresses, and special gatherings were made more and more often (Caro, 2007; Cassini, 1999).

Nowadays, research on animals´ ecology and behaviour is considered as a crucial component in species conservation. It represents a useful tool that might be utilized in long-term monitoring of reintroduced populations and in reintroduction success evaluation (Berger-Tal et al., 2011; IUCN, 2013). For instance, the health of the reintroduced population can be determined by observing the movement, spatial utilization, feeding habits, vigilance behaviour, and reproductive behaviour of the reintroduced individuals (Berger-Tal et al., 2011). Observation of social structure is also crucial in reintroduction monitoring as it may influence a response of animals to the environmental factors. Knowledge on the social structure may help to avoid social transmission of problem behaviour and assist to design the most sufficient reintroductions. Therefore, understanding the relationships between animals can be profitably applied by wildlife managers and conservationists (Snijders et al., 2017). The behavioural ecology has shown that animal behaviour is not a fixed character but varies plastically between individuals and environmental conditions. Further, the fundamental role that behaviour plays in the patterns of distribution and abundance of animal populations has been discovered (Cassini 1999). Advances in behavioural research have begun to influence the theory and practice of conservation biology, but this influence has been greater in the field of the preservation and management of individual species (Berger-Tal & Saltz, 2014; Blumstein et al., 2019; le Flohic et al., 2015). However, there is the perspective of a future impact on the conservation of whole protected areas, landscapes and ecosystems with behavioural studies representing valuable tools applicable to wildlife conservation (Tobias & Pigot, 2019).

2.3.1. Behavioural monitoring and Przewalski's horse conservation

The research on the behaviour of Przewalski´s horses was considered vital for the species' conservation even before the start of its reintroduction, and it was stressed that the lack of information on the species' behaviour before its extinction in the wild was threatening its successful preservation (Boyd, 1991). Therefore, various studies have been carried out on the captive and free-ranging horses, both prior to and after the reintroduction to deepen knowledge on Przewalski's horse (e.g., Berger et al., 1999; Boyd & Bandi, 2002; Boyd, 1988; Boyd et al., 1988; van Dierendonck et al. 1996). Studies on the populations in China and Mongolia have shown that their behaviour resembles the one of the feral horses (Kerekes et al., 2021b; King et al., 2016; Wakefield et al., 2002). Przewalski's horses typically live in stable groups with strong social bonds. In Przewalski's horses, there are two main types of social grouping: harem groups, which consist of one stallion, mares, and foals and bachelor groups, which are made up of young males (King et al., 2015). The harem stallion protects the family group from intruding stallions and possibly also from predators. Harems typically keep in a distance from each other but might occasionally overlap (Kerekes et al., 2021a; Xia et al., 2014). Like wild and free-ranging horses, Przewalski's horses thrive best on steppe-like grasslands, and the availability of essential supplies may have an impact on how they utilise their habitat (Kajiwara et al., 2016). Przewalski's horses favour flat, lowland regions near water sources, forests, and simple plant communities that contain their preferred species (Kaczensky et al., 2008; Kajiwara et al., 2016). In the summer, they regulate their body temperature by resting in the shade of trees (Kajiwara et al., 2016), and to escape flies, they climb to windswept hillsides (King & Gurnell, 2010).

It was anticipated that the reintroduced Przewalski´s horses might experience difficulties in adaptation to the novel conditions. Some places (e.g., the GGBSPA the harsh semi-desert area) were expected to be more challenging for the species than others (e.g., Hustai National Park, an area with more abundant feed, water and tree cover; Mongolian Takhi Strategy and Plan Work Group, 1993; Souris et al., 2007). Moreover, it has been stressed that GGBSPA represents the edge of the native habitat of Przewalski´s horses (Gao et al., 1989; Wakefield et al., 2002). The expectations of demographic and adaptational problems of Przewalski´s horses in certain selected reintroduction areas became a reality during the dzud (extreme winter conditions) in Mongolia in 2000/2001 and 2009/2010. While Przewalski’s horse population in the GGBSPA experienced mortality rates of 67% during the extreme winter of 2009/2010, at that time, the population in Hustai National Park accounted loss of 10% (Kaczensky et al., 2011). After these events, the necessity of knowledge on the response of the reintroduced horses to their environment and weather factors, especially in the harsh release sites, became apparent. Nonetheless, thanks to deliberate management and ongoing reintroduction efforts, the wild Przewalski’s horse populations are continuously increasing (Jiang & Zong, 2019; King et al., 2015; Turghan et al., 2022). However, these populations are still confronted with present and future threats such as habitat loss, domestic horse hybridization or human-wildlife conflict. In order to support free-ranging Przewalski's horse populations in the wild, careful monitoring of the ecology and behaviour of these animals should be conducted in all ongoing and future programmes (Turghan et al., 2022).

CHAPTER 3

3. General materials and methods

3.1. Study area

The GGBSPA is a part of the Great Gobi Biosphere Reserve and was established in 1975. In 2019, the GGBSPA was extended and currently encompasses ≈18 000 km^2 of desert steppe and semi-desert habitat (≈9 000 km^2 before the enlargement; ITG International Takhi Group, 2021). This protected area in SW Mongolia is a reintroduction site for Przewalski's horse and an important refuge for several other endangered species (Kaczensky et al., 2004; Kaczensky et al., 2008). Despite its protected area status, the GGBSPA is used by herders. In 2018 it was used by about 130 families with close to 70 000 heads of livestock (3 070 domestic horses). Since the area was enlarged (2019), herders and livestock numbers have also increased. In 2021 the GGBSPA signed a cooperation contract with 228 herder families that own 122 897 livestock heads: 11 829 big livestock (horses, cows and camels) and 111 068 small livestock (goats and sheep; GGBSPA administration, personal communication). Herders and their livestock are mainly present in the GGBSPA in winter and during the spring and fall migration as they move to their summer camps in different areas for the summer season (National Statistical Office of Mongolia, 2021)

The temperatures in the GGBSPA differ significantly both during the day and night and between the seasons. The altitude ranges from 1,100 to 2,900 mamsl, and the annual mean temperatures are below zero (ITG International Takhi Group 2021). The GGBSPA is situated between the Altai Mountains and borders with China. The amount of precipitation is within the typical range of a semi-desert climate (around 150 mm per year). Most of the precipitation falls in the summer rains. However, the drought, the large temperature gradient, and the enormous annual differences are characteristic of the habitat. Plants must cope with water stress and a high-temperature gradient. The high degree of mobility of animals is essential in order to be able to find suitable habitats depending on the situation (ITG International Takhi Group, 2021).

3.2. Studied animals

In July 2018, the initial data in the GGBSPA, Mongolia, was collected. Three easy-to-follow groups were recorded (wild-born Azaa, wild-born Tsetsen and reintroduced Mares18). Azaa harem consisted of 17 individuals – 1 dominant stallion, 6 breeding mares, 2 two-year-old males, 1 one-year-old male, 3 one-year-old females, 2 male and 2 female foals. At the end of the observation period, the average age of foals was 46 days. All the members of Azaa harem, except for one breeding mare, were born in the wild – GGBSPA. Tsetsen harem consisted of 8 individuals – 1 dominant stallion, 3 breeding mares (one of them was only two years old), 2 two-year-old males, 1 one-year-old male and 1 one-year-old female. All the members of Tsetsen harem were born in the wild – GGBSPA. Group of Mares18 consisted of 4 mares – 3 three years old and 1 four years old. All mares were bred ex-situ, transported to GGBSPA by Prague Zoo and released to the acclimatisation fence on 20.6.2018. All four mares came from three European breeding facilities. Two of them were born in the Helsinki Zoo, one in the Swiss Wildnispark Zürich Langeberg, and one in the German Wisentgehege Springe. The four mares were observed in the acclimatisation enclosure during this research period.

The data collection continued in late May - June summer 2019. Same groups as in 2018 and one other group were recorded (wild-born Azaa, wild-born Tsetsen, reintroduced Hustai1 – previously Mares18 – and reintroduced Mares19). Azaa harem consisted of 14 individuals – 1 dominant stallion, 5 breeding mares, 2 two-year-old females, 2 one-year-old males, 1 one-year-old female, 2 male and 1 female foal. One two-year-old female left the group just at the beginning of our observation period, on 21.5.2019. At the end of observation, the average age of foals was 26 days. All the members of Azaa harem, except for one breeding mare, were born in the wild – GGBSPA.

Tsetsen harem consisted of 12 individuals – 1 dominant stallion, 4 breeding mares, 1 three years old male (which was observed breeding with mares in the harem several times), 1 two-year-old male, 1 two years old female, 1 one-year-old male and 3 female foals. At the end of observation, the average age of foals was 28 days. All the members of Tsetsen harem were born in the wild – GGBSPA.

Hustai1 consisted of the Mares18 and a stallion which joined them by getting into the acclimatisation fence. This stallion was seven years old and was born in Hustai Nuruu National Park and transported to GGBSPA on 21.07.2016. Hustai and "his" mares were released from the acclimatisation fence on 20.5.2019, immediately before our observation period. The Hustai1 harem, Azaa and Tsetsen harem were observed in the wild.

Mares19 consisted of 3 mares – 2 five years old and 1 six years old. All mares were born ex-situ, transported to GGBSPA by Prague Zoo and released to the acclimatisation fence 19.6.2019, so just three days of recordings were collected. All three mares came from three European breeding facilities. Two of the three mares come from Prague Zoo, Czech Republic. One mare was born directly in Prague, and one in the Prague Zoo breeding facility in Dolní Dobřejov. One mare was born in Springe, Germany. The three mares were observed in the acclimatisation enclosure during this research period.

The last data collection was conducted in the autumn of 2019. The same groups as in summer 2019 were recorded (wild-born Azaa, wild-born Tsetsen, reintroduced Tanan (previously Mares18/Hustai1) and reintroduced Hustai2 (previously Mares19). Azaa harem consisted of 14 individuals – 1 dominant stallion, 5 breeding mares, 2 one-year-old males, 1 one-year-old female, 3 male foals and 2 female foals. At the end of observation, the average age of foals was 100 days. All the members of Azaa harem, except for one breeding mare, were born in the wild – GGBSPA.

Tsetsen harem consisted of 12 individuals, the same as in summer 2019. The average age of foals (at the end of observation) was 128 days.

Tanan consisted of the mares from Hustai1 harem from summer 2019 and the stallion Tanan, which joined them after he defeated Hustai stallion on 3.8.2019. This stallion was eight years old and was born in GGBSPA 21.07.2016. The Tanan harem, Azaa and Tsetsen harem were observed in the wild.

Hustai2 consisted of the Mares19 and Hustai stallion, which joined them by getting into the acclimatisation fence after losing his original harem to Tanan stallion. The three mares and Hustai stallion were observed in the acclimatisation enclosure during this research period.

3.3. Data collection

The data collection was conducted in three seasons (late spring 2019, summer 2018, and autumn 2019). The harems were localised by binoculars during daily monitoring routines. Once one of the target harems was localised, the harem was approached and filmed from a close distance (from 150m to 800m) by the 4K Panasonic VX1 video camera with a tripod. Kestrel 4500 Pocket Weather Tracker on a stabilised tripod was used to collect weather conditions. Weather variables were summarised by the device in 10-minute intervals together with GPS position of the observation point. Distance between the observation point and between each of the two most distant members of the group was measured with a rangefinder Nikon MONARCH 2000 and digital compass, also every 10 minutes so location and dispersion of the group could be calculated later on. In the summer 2018, 45 hours of recording were collected. In late spring 2019, 108 hours of videos were recorded. In autumn 2019, 88.5 hours of videos were recorded. The time of recording was similarly distributed among all studied groups. For categorisation of the behaviours, an ethogram established during the initial data collection was used and complemented with other published material for horses (McDonnell & Haviland, 1995; McDonnell & Poulin, 2002). Full descriptions of specific behavioural categories and behaviours are found in each chapter (Chapter 4, Chapter 5, Chapter 6, respectively).

Behavioural Observation Research Interactive Software (BORIS; Friard and Gamba, 2016) was used for event logging, coding and observation of the captured videos. Data from BORIS was transferred to excel. For the social behaviour measures, this data was further analysed in SOCPROG 2.9 for MATLAB2018B (analysis of data on the social structure, Whitehead, 2015) and DomiCalc (Tools for dominance analysis, de Silva et al., 2017). In DomiCalc, only agonistic interactions were analysed. All the other social network measures were calculated in SOCPROG.

Satellite-derived remote sensing predictors of high spatial resolution (~ 100 m) were used to represent the main dimensions of the ecosystem geomorphology and dynamics in the study area. Products from several sensors onboard different satellites were categorised into three groups: i) topography (ALOS topographic attributes); ii) vegetation indexes (Landsat vegetation index); and iii) land cover (Copernicus). Considering both topography and land cover datasets as static predictors in time, the mean of the maximum Normalized Difference Vegetation Index (NDVI) for the different selected periods was calculated. All native and derived satellite predictors at their original spatial resolutions were computed and aggregated to target 100 m grid cells using the cloud-based Google Earth Engine (GEE) computational platform (https://earthengine.google.com; Gorelick et al., 2017).

3.4. Data analysis

All statistical analyses were conducted in IBM® SPSS® Statistics 27, IBM® SPSS® Statistics 28 (IBM, Armonk, New York) and R software version 4.2.0. Mapping was performed in the R software version 4.2.0 and QGIS 3.28.0 'Firenze'. Full descriptions of the analysis techniques are found in each chapter (Chapter 4, Chapter 5, Chapter 6, respectively).

CHAPTER 4

Influence of weather on the behaviour of reintroduced Przewalski's horses in the Great Gobi B Strictly Protected Area (Mongolia): implications for conservation

Wild-born Przewalski's horses in the Great Gobi B Strictly Protected Area, Mongolia.
(Photo by Anna Bernátková)

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4.1. Abstract

Background: Reintroduction is a common technique for re-establishing threatened species. However, the adaptation to novel habitats with distinct conditions poses a risk of failure. Weather conditions affect the behaviour of animals, and thus, their adaptation to new conditions and survival. Reintroduced Przewalski's horses living in Mongolia's continental arid climate with extreme temperature and precipitation variability, serve as an ideal model species for studying the behavioural response of selected groups to these harsh conditions.

Methods: The research was conducted in The Great Gobi B Strictly Protected Area, Mongolia. In summer 2018, three groups were recorded (Azaa, Tsetsen and Mares18) involving 29 individuals. In Spring 2019, 4 groups were recorded (Azaa, Tsetsen, Hustai1 and Mares19) involving 34 individuals. In Autumn 2019, 4 groups were recorded (Azaa, Tsetsen, Hustai2 and Tanan) involving 35 individuals. Thirteen weather variables were recorded in 10-min intervals, together with the percentage representation of selected behavioural categories (feeding, locomotion, resting, and social). The effect of weather on behaviour was analysed through GLMM. Influence of the group-history factors (recently reintroduced, long-term reintroduced and wild-born) was also analysed.

Results: Feeding significantly increased with cloudy and windy conditions and was more frequent in autumn than spring and summer. Locomotion was positively explained by temperature and cloudiness and was higher in summer than spring and autumn. Resting behaviour decreased with altitude and cloudiness, and the dispersion of the group was lower when resting. Increased social interactions were observed with higher temperatures and were more frequent in summer compared to spring and autumn. Differences were found in the display of the behaviours among the selected harems, showing interesting patterns when grouping them according to their origin and experience.

Conclusions: Weather patterns seem to influence the behaviour of Przewalski's horse. These results might assist in further management plans for the species, especially in the view of intensifying climate change and alteration of weather patterns. As previously suggested, after approximately 1 year, horses adapt to novel conditions and display the typical behavioural pattern of wild-born Przewalski's horses.

Keywords: Activity budget; Arid environments; *Equus ferus przewalskii*; Gobi Desert; Soft-release

4.2. Background

Reintroduction is an attempt to bring back species to areas of their historical range, and it is a common technique for in situ conservation. It is one of the most effective conservation techniques for re-establishing and supporting threatened species populations, sometimes the only way, however they have a relatively low success rate (Seddon et al. 2007 Armstrong and Seddon 2008; Baguette et al. 2013). Access to large enough areas of suitable habitat (especially important for larger species) and the genetic makeup of the reintroduced population are the most critical determinants of a reintroductions long-term success (Wolf et al. 1998; Armstrong and Seddon 2008; Baguette et al. 2013). As a result of scarcity of such large suitable areas, some animal species, such as northern bobwhite (*Colinus virginianus*), roe deer (*Capreolus capreolus*), or Pzewalski's horse (*Equus ferus przewalskii*) have been reintroduced to the edge of their original habitat (Gao et al. 1989; Macaluso et al. 2017; Paklina & Pozdnyakova 1989; Wakefield et al. 2002; Wallach et al. 2007; Zhang et al. 2015).

Another obstacle in reintroduction is the lack of knowledge on the ecology of the reintroduced species prior to its disappearance from the native area (Wallach et al. 2007; King et al. 2005; Zhang et al. 2015). In general, most reintroductions pose a risk of failure as they require the movement of animals from a relatively secure environment (such as zoos and breeding facilities) to a harsh environment which might often be located on the edge of their original habitat (IUCN 2013). After the translocation animals require time and space in order to be able to recover from the stress of transportation and to maintain and develop key cognitive processes such as habitat utilisation, anti-predatory behaviours, social behaviours and territory or home range establishment (Teixeira et al. 2007; Dickens et al. 2010; Swaisgood 2010). Therefore, the preferred method of release for many reintroduced species worldwide, including mammals, is soft-release. It allows animals to become accustomed to its new environment, while they are still being fed and/or protected from predators. (Jefferies et al. 1986; Fritts et al. 1997; Truett et al. 2001; Mateju et al. 2012; Wanless et al. 2002; Moehrenschlager and Macdonald 2003). Time spent in the acclimatisation facility allows animals to develop affinity to novel habitat as well as with opportunities to adjust to local environmental conditions (Miller et al. 1999; Letty et al. 2000; Moehrenschlager and Macdonald 2003).

Przewalski's horse was listed as Extinct in the Wild from 1996, with the last individual seen in 1969 in the Guntamga spring of the Great Gobi B Strictly Protected Area (GGBSPA). Thanks to the ongoing reintroduction efforts the species is listed as Endangered from 2011 (King et al. 2015). The Przewalski's horse is a flagship species which could be used for conservation of the whole habitat (Berger et al. 1996). But reintroduction into its former habitat and further conservation is fraught with challenges and requires immense effort (Van Dierendonck et al. 1996).

At the GGBSPA, which is the part of Great Gobi Biosphere Reserve, the first reintroduced horses were released from acclimatisation enclosures in spring of 1997 and currently, there is a mixture of recently reintroduced, long-term reintroduced and wild-born animals (wild-born individuals are in majority; ITG International Takhi Group 2021). At present, there are 349 Przewalski's horses, involving 24 harems, and 3-5 bachelor groups. 231 of the horses are females and 148 are males. From 1992 to 2019, a total of 131 horses were transported from the zoos and reserves into the GGBSPA. Of these, 36 were males and were 95 females. Of the currently living horses, 315 were born in the Gobi, the remaining 34 horses were translocated to the area (personal communication with Dalaitseren Sukhbaatar, Takhi researcher, GGBSPA). Inland continental and arid climate of the GGBSPA is defined by extreme temperature and precipitation variability through the year with temperatures varying from -40 to 40 degrees Celsius. Extreme drought of the warm-season and snow cover limiting animals from grazing in the cold season can occur in the same year. Dzud (extremely severe winter) induced wild-horse mortality rates of 21% in 2000/2001 and of 60% in 2009/2010 (livestock mortality was even higher, 67% in 2009/2010). This is a huge challenge for small and isolated animal populations such as the reintroduced population of Przewalski's horse (Kaczensky et al. 2011). Therefore, horses living in these extreme conditions serve as an ideal model species for monitoring the relationship between reintroduced animals and their environment.

In reintroduced and endangered species habitat use is one of the key studied factors (Massaro et al. 2018; Viola et al. 2019). In equids, forage abundance is the most important determinant for habitat use because of their digestive anatomy (King & Gurnell 2005; Schoenecker et al. 2016) and they may spend more than half of their daily time budgets grazing to get enough nutrients (King et al. 2016). However, inconsistent and contradictory information has been described by previous observations of the feeding behaviour and daily budget of horses (feral horses: Duncan 1985; Pratt et al. 1986; Berger et al. 1999; wild horses: King 2002; King et al. 2016; Van Dierendonck et al. 1996). In Hustai National Park, Mongolia, harems of Przewalski's horses usually feed in the morning and evening, before and after the walk to the water source (Van Dierendonck et al. 1996; King 2002). In the spring, summer and autumn, groups typically migrate up to higher altitudes to rest in shade during the day. In the cold season, they tend to relax on sunny south-facing areas and spend more time at lower altitudes (King 2002). Under semi-natural conditions, average feeding rate of Przewalski's horses in spring is much higher than at any other period of year, and the level of activity is generally high. In summer, the feeding levels are lowest compared to the rest of the year and activity shifts to the night. In autumn, the level of activity and feeding is usually high. In winter, the average daily activity is lower than at any other period of the year and the level of feeding is usually high (12 Przewalski mares, Berger et al. 1999).

It is apparent, that feeding behaviour of Przewalski's horses is influenced by the seasonal changes in their habitat. The GGBSPA is considered to be the edge of the original habitat of, Przewalski's horses (Gao et al. 1989; Paklina & Pozdnyakova 1989; Wakefield et al. 2002). In such dry habitat, they need to stay close to water points to be able to drink at least two times per day (Zhang et al. 2015) and their behaviour is affected by this need (Rubenstein et al. 2016). Ranging behaviour of the Przewalski's horses in this area is also widely fluctuating, forcing them to sustain excessive heat load. Only after the sunset and during cooler climatic conditions, they are able to move to more favourable feeding areas (Gersick & Rubenstein 2017). At the GGBSPA, there is a mixture of recently reintroduced, long-term reintroduced and wild-born animals (ITG International Takhi Group, 2021), and behaviour and habitat use of the recently reintroduced animals is possibly influenced by the need to adapt to novel conditions as identified by Scheibe et al. (1997). The study of Scheibe et al. (1997) analysed activity and feeding behaviour of the herd of Przewalski horses over a 2.5-year period in a semi-reserve in Europe. The observation cycle involved an adaptation to nature-like conditions in the first year. But only after the first winter in the semi-reserve, horses showed a similar annual trend to that observed by other studies aimed at the annual budget of Przewalski's horses (Scheibe et al. 1997).

For the successful management of this endangered species, knowledge on its ecology considering possible differences of animals of different origins is vital. This need is exacerbated by the constant population growth, demanding larger areas of suitable habitat. There is a lack of research on the influence of weather conditions on Przewalski's horse behaviour and, in addition, most of the published data is from captive populations. It is critical to have more information about the effect of environmental conditions on daily budget and habitat use of the horses translocated to the novel habitat and to apply such knowledge to the management of the species.

In this paper, we aimed to present the most important patterns of behavioural response of the Przewalski's horse to selected weather factors in the highly demanding environment of the GGBSPA. We hypothesized that the response would differ according to the group origin and experience, as by previous research it was described, that after release, Przewalski´s horses do not present the typical behavioural pattern of wild individuals. Our finding could servefor the selection of future reintroduction sites, as climate change-altered habitats may become a new norm and the ability of appropriate response of the species to the changing environmental conditions may present the biggest conservation challenge in the future.

4.2. Materials and Methods

4.2.1. Study area

The GGBSPA (established in 1975) is a part of the Great Gobi Biosphere Reserve. Currently, it encompasses over 18 350 km2 of desert steppe and desert habitat (ITG International Takhi Group, 2021). This protected area located in SW Mongolia is a reintroduction site for the Przewalski's horse and an important refuge for several other endangered species (Kaczensky et al. 2004; Kaczensky et al. 2008). Despite its protected area status, the GGBSPA is used by about 130 families with close to 70 000 heads of livestock mainly in winter and during spring and fall migration (National Statistic Office of Mongolia 2021).

The climate of the GGBSPA is continental and very dry. The temperatures differ significantly both during the day and night and between the seasons. The altitude ranges from 1,100 to 2,900 metres above mean sea level (mamsl) and the annual mean temperatures are below zero (°C) (ITG International Takhi Group 2021). The GGBSPA is situated between the Altai Mountains and borders with China. The amount of precipitation is within the typical range of semi-desert climate (around 150 mm per year). Most of the precipitation falls in the summer rains. However, not only the drought and the large temperature gradient, but also the enormous annual differences are characteristic for the habitat. Plants have to cope with water stress and a high temperature gradient. The high degree of mobility of animals is essential in order to be able to find suitable habitats depending on the situation (ITG International Takhi Group 2021).

4.2.2. Data collection

Authorisation for working with this endangered species in the protected area was granted by the director of the Strictly Protected Area.

The data collection was conducted in three different seasons (late spring, summer, autumn) in the groups described in Table 4.1. The season was defined according to the consultation with rangers of GGBSPA and local inhabitants (the herders move from winter to summer camps in spring and back in autumn). The studied harems were classified as recently reintroduced (from 2 days up to 3 months after the transport from Europe, fenced area), long-term reintroduced (released after a one-year acclimatization period), and wild-born (born in the wild). The harems were localised by binoculars during daily monitoring routines. Once one of the target harems was localised, the harem was approached and filmed from a close distance (from 150m to 800m) by the 4K Panasonic VX1 video camera with tripod. The video recordings were made every day across the whole study period (in Summer, Spring, Autumn respectively) and each observation day was dedicated to one group. We typically spent time from morning till afternoon or from midday to evening with each group. When possible, we aimed to change the group every day and spend equal percentage of morning-afternoon/midday-evening period with each group to collect comparable data in terms of environmental conditions and hours of observation. For the collection of weather conditions Kestrel 4500 Pocket Weather Tracker on a stabilised tripod was used. Weather variables (magnetic heading, true heading, wind speed, crosswind calculation, headwind/tailwind, temperature, wind chill, relative humidity, heat stress index, dewpoint temperature, wet bulb temperature, barometric pressure, altitude, density altitude, cloudiness) were summarised by the device in 10-minute interval together with GPS position of the observation point. The ranges for the data collected in each of the three study periods is shown in Table 4.2. Distance between the observation point and between each of the two most distant members of the group was measured with a rangefinder Nikon MONARCH 2000 and digital compass, also every 10 minutes so location and dispersion of the group could be calculated later on (Figure 4.1). In summer 2018, 45 hours of recording were collected. In late spring 2019, 108 hours of videos were recorded. In autumn 2019, 88.5 hours of videos were recorded. Time of recording was similarly distributed among all studied groups.

For categorisation of the behaviours, an ethogram established during the initial data collection was used and complemented with other published material for horses (McDonnell & Haviland 1995; McDonnell & Poulin 2002). The ethogram (Table 4.3) consisted of 5 Categories (feeding, locomotion, resting, social and other) which included specific behaviours. The category Other was computed to calculate accurately the percentage of the previous categories but was not used in further analyses. The average time dedicated to each behavioural category in each studied period is shown in Table 4.2. No interactions between selected herds or competition for resources was observed along the study.

Behavioural Observation Research Interactive Software (BORIS) was used for event logging, video coding and observation of the captured videos. Data from BORIS was transferred to Excel. This data was divided in 10 minutes intervals in order to make them comparable to group position, group dispersion and weather data. Time dedicated to each behavioural category in every 10-minute interval (same as those intervals used for weather and dispersion) was calculated for each individual, then summarised for each group and finally, a percentage representation of each behavioural category was calculated.

Table 4.1. Description of the studied groups.

Group	Season/Year*	Observation hours	Group experience**	Individuals	Number of breeding mares	Stability index***	Age of foals (d)****
Azaa	Summer/18	18.5	wild-born	1 dominant ♂, 9 ♀, 3 ♂, 4 foals	6	0.86	46
Mares18	Summer/18	17.5	recently reintroduced	4 ♀	4	0.00	-
Tsetsen	Summer/18	11.2	wild-born	1 dominant ♂, 4 ♀, 3 ♂	3	0.00	-
Azaa	Spring/19	30.0	wild-born	1 dominant ♂, 8 ♀, 2 ♂, 3 foals	5	0.83	26
Hustai1	Spring/19	25.5	long-term reintroduced	1 dominant ♂, 4 ♀	4	0.00	-
Mares19	Spring/19	21.0	recently reintroduced	3 ♀	3	0.00	-
Tsetsen	Spring/19	31.5	wild-born	1 dominant ♂, 5 ♀, 3 ♂, 3 foals	4	0.75	28
Azaa	Autumn/19	22.5	wild-born	1 dominant ♂, 6 ♀, 2 ♂, 5 foals	5	1.00	100
Tanan	Autumn/19	21.0	long-term reintroduced	1 dominant ♂, 4 ♀	4	0.00	-
Hustai2	Autumn/19	20.0	recently reintroduced	1 dominant ♂, 3 ♀	3	0.00	-
Tsetsen	Autumn/19	25.0	wild-born	1 dominant ♂, 5 ♀, 3 ♂, 3 foals	4	1.00	128

Only animals older than one year (inclusive of) were used in our research. Foals are shown in the table to illustrate the general information on the studied groups.

*Summer/18 period accounts for July 7th – July 22nd 2018, Spring/19 accounts for May 19th – June 23rd 2019 and Autumn/19 accounts for September 10th – October 10th 2019.

**Group experience: recently reintroduced (from 2 days up to 3 months after the transport from Europe, fenced area); long-term reintroduced (after one year acclimatization period, released); wild-born (born in the wild). One mare in the wild-born Azaa harem was born in captivity in Europe and reintroduced to the GGBSPA in 2004.

*** Stability index was counted as the inter-yearly changes in the number of breeding mares (in %; the number of mares present in the herd during the previous and the observation year, divided by the number of mares present in one or another year. Zeros represent groups which existed for less than one year.

****Average age of foals (in days) at the end of each selected observation.

Table 4.2. Range of values recorded at each of the study periods for the different weather variables studied (mean is shown in parentheses); and mean time percentage dedicated to each behavioural category studied.

	Summer 2018	**Spring 2019**	**Autumn 2019**
WS (m/s)	0 - 8.4, (3.6)	0 - 12.9, (3.4)	0 - 8.8, (2.6)
CW (m/s)	0 - 7.6, (2.8)	0 - 12.8, (2.6)	0 - 8.8, (1.8)
HW (m/s)	-5.1 - 6.9, (0.7)	-8.4 - 7.6, (0.0)	-5.9 - 6.7, (-0.7)
TP (°C)	16.2 - 32.5, (23.8)	9.0 - 29.1, (20.2)	3.1 - 34.6, (18.4)
WC (°C)	15.3 - 32.5, (23.8)	5.5 - 29.1, (19.9)	3.1 - 27.4, (18.0)
RH (%)	1.9 - 67.3, (25.5)	0.2 - 69.7, (20.5)	3.0 - 67.4, (20.8)
HI (°C)	11.0 - 36.1, (21.6)	7.7 - 25.7, (17.7)	4.8 - 31.0, (16.0)
DP (°C)	-24,6 - 15.1, (-0.2)	-50.0 - 10.6, (-6.7)	-23.2 - 8.5, (-8.3)
WB (°C)	8.1 - 19.4, (11.4)	2.1 - 14.3, (8.2)	0.3 - 13.3, (6.8)
BP (mb)	820.1 - 837.7, (829.6)	818.0 - 842.7, (830.9)	816.3 - 852.7, (836.5)
AL (m)	1583 - 1746, (1653)	1524 - 1767, (1640)	1429 - 1784, (1586)
DA (m)	2051 - 2774, (2363)	1738 - 2566, (2217)	1568 - 2522, (2085)
clouds (%)	0 - 75, (15)	0 - 100, (18)	0 - 100, (17)
Feeding (%)	15.3	22.0	36.0
Locomotion (%)	14.0	11.7	8.6
Resting (%)	33.6	31.9	29.1
Social (%)	14.1	7.2	7.2
Other (%)	22.9	27.3	19.1

WS (m/s) = windspeed, CW (m/s) = crosswind calculation, HW (m/s) = headwind/tailwind, TP (°C) = temperature, WC (°C) = windchill, RH (%) = relative humidity, HI (°C) = heat stress index, DP (°C) = dewpoint temperature, WB (°C) = wet bulb temperature, BP (mb) = barometric pressure, AL (m) = altitude, DA (m) = density altitude, clouds (location) = % of cloud cover in the place of observation

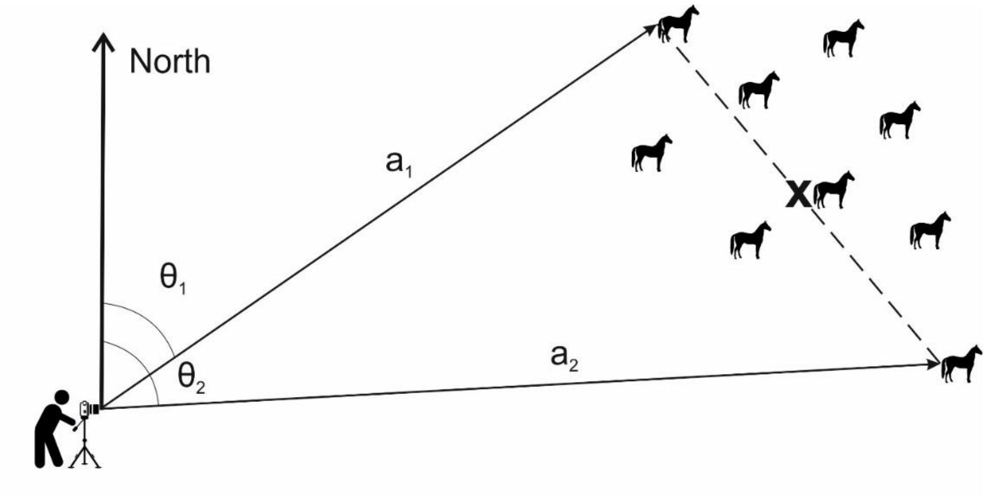

Figure 4.1. Method followed for the calculation of dispersion and herd location. The two animals which were visually most distant in the group were located by the observer and their distance and angle (between animal and that of true north) from the location of the observer were measured. Dispersion of the group was counted afterwards by basic trigonometric function formulas.

Table 4.3. Ethogram showing the categories used for describing the behaviour of the studied Prezewalski´s horses.

Category	Behaviour	Definition
Feeding	Feeding	Food acquisition and investigation, very slow movement connected to feeding is categorized as feeding and not as walking.
Locomotion	Lead	Movement to a particular direction while leading other group members, may be accompanied by head movements (up and down) and/or herding.
	Follow	Move along the path of another horse (typically leading mare), usually at the same gait as the horse being followed. There is no attempt to direct the movement, attack, or overtake the leading horse.
	Running	Fast movement to a particular place, fast movement towards and object or other horse is categorized as approach. Fast movement while following or leading some horse(s) is categorized as lead/follow.
	Walking	Slow movement to a particular place, very slow movement connected to feeding is categorized as feeding, slow movement towards and object or other horse is categorized as approach, slow movement while following or leading some horse(s) is categorized as lead/follow.
	Leaving the group	Movement performed in order to increase the distance of an individual from a group resulting in the group abandonment. The abandonment might last from several hours to days or become permanent

	Coming back	Movement performed in order to decrease the distance of an individual from a group resulting in connection to the group. This behaviour typically follows leaving the group.
Resting	Standing alert	Rigid stance with the neck elevated and the head oriented toward the object or animal of focus. The ears are held stiffly upright and forward, and the nostrils may be slightly dilated. The whinney vocalization may accompany this stance.
	Stand resting	Standing relaxed with head down.
	Resting	Laying down.
Social	Approach	Movement of a horse in attempt to initiate a behaviour towards other horse.
	Play	Behaviour appearing to have no immediate use or function for the animal, involving a sense of pleasure. Various behaviours presented while playing with other individual/s.
	Grooming	Two members standing beside one another, usually head-to shoulder or head-to-tail, grooming (each) other’s neck, mane, rump, or tail by gentle nipping, nuzzling, or rubbing.
	Head resting	Horse is placing it´s head on the other horse´s body.
	Head rubbing	Horse is rubbing it´s head on the other horse´s body. Not connected to sexual activity.
	Olfactory investigation	Olfactory investigation involves sniffing various parts of another horses’ head and/or body. Considered friendly if followed by another friendly behaviour.
	Arched neck threat	Neck tightly flexed with the muzzle drawn toward the chest. Arched neck threats are observed during close aggressive encounters and ritualized interactions.
	Bite threat	No contact is made. The neck is stretched, and ears pinned back as the head swings toward the target horse giving the warning to maintain distance.
	Bite	Opening and rapid closing of the jaws with the teeth grasping the flesh of another horse. The ears are pinned, and lips retracted.
	Chase	One horse pursuing another, usually at a gallop. The chaser typically pins the ears, exposes the teeth, and bites at the pursued horse’s rump and tail. The horse being chased may kick out defensively with both rear legs.
	Ears back	Ears pressed caudally against the head and neck. Typically associated with intense aggressive interaction.
	Fight	Various behaviours associated with fighting, not a single aggressive movement. More than one of the aggressive attempts must be present. Striking, rearing, mounting, lunge, levade, repeated biting/kicking.
	Head bump	In two horses: a rapid lateral toss of the head that forcefully contacts the head and neck of another horse. Usually, the eyes remain closed and the ears forward.

Herding	Combination of a threat (usually bite) and ears laid back with forward locomotion, apparently directing the movement of another horse.
Interference	Disruption of combat of horses by moving between the fighting individuals, pushing, attacking, or simple approaching the combatants. One or more horses may simultaneously interfere with an encounter.
Kick threat	Similar to a kick, but without sufficient extension or force to make contact with the target. The hind leg(s) lifts slightly off the ground and under the body in tense “readiness”.
Kick	One or both hind legs lift off the ground and extend towards another horse, with apparent intent to make contact.
Push	Pressing of the head, neck, shoulder, chest, body or rump against another in an apparent attempt to displace target horse.
Retreat	Movement that maintains or increases an individual’s distance from an approaching horse or a horse initiating some behaviour. The head is usually held low, and ears turned back. The retreat can be at any gait, even very slow and little movement from the initiator.
Retreat chewing	Moving the lower jaw up and down in a chewing motion. A sucking sound may be made. Typically, the head and neck ate extended, with the ears relaxed and oriented back or laterally.
Threat	Giving the general appearance of a warning to maintain distance. Threats are typically not directed toward the particular part of the body of other horse.
Olfactory investigation social	Olfactory investigation involves sniffing various parts of other horses’ head and/or body. Considered agonistic if followed by another agonistic behaviour.
Copulation	Stallion mounting the female from behind, neck arched over her back and forelegs resting on her sides.
Copulation attempt	Attempt for copulation without successful completion.
Defecating over	Defecation over faeces of other group members (typically females) presented by the stallion in a characteristic sequence: sniff faeces, step forward, defecate, pivot or back up, and sniff faeces again.
Female presentation	Mare presents herself facing away from the stallion, lifting her tail, posturing her body with hind legs slightly apart and often turning her head toward her posterior.
Head rubbing (sexual)	Stallion proceeds by rubbing his head on the female’s flanks and (or) resting his chin on her back, usually extends his penis out of the prepuce.
Reproductive tending	Close following of an oestrous female by the male, without the directional driving observed in herding behaviour.

	Sniffing faeces/urine	Sniffing, typically followed by flehmen, defecations and urinations performed by stallion towards faeces of females in oestrus.
	Nursing	Recorded in mares. The foal's muzzle is in contact with the mare's udder, movement of milk down the oesophagus is visualized, or the foal is seen to grasp and hold the teat with its lips.
	Suckling	Recorded in foals. The foal's muzzle is in contact with the mare's udder, movement of milk down the oesophagus is visualized, or the foal is seen to grasp and hold the teat with its lips.
	Suckling attempt	The foal is trying to reach mare's udder with its muzzle.
	Vocalization	Emitting a sound produced through the action of respiratory system, used in communication.
Other	Defecating	The discharge of faeces from the body.
	Drinking	The action or habit of consuming water.
	Erection	Fully extended and tumescent penis.
	Head bow	Repeated, exaggerated, rhythmic flexing of the neck such that the muzzle is brought toward the point of the breast.
	Insect elimination	Waving of tail, kicking around and/or biting itself or hiding itself under the tail of another horse in attempt of insect elimination.
	Masturbation	Erection with rhythmic drawing of the penis against the abdomen, with or without pelvic thrusting. Solitary or group activity (bachelors).
	Solitary olfactory investigation	Olfactory investigation involves sniffing various parts of a ground or objects.
	Rolling	Dropping from standing to sternal recumbency, then rotating one or more times from sternal to dorsal recumbency. Typically occurs on dusty or sandy areas.
	Urinating	The discharge of urine from the body.

4.3.2. Data analysis

All analyses were conducted in IBM® SPSS® Statistics 27. Pearson's correlations among all the weather variables were studied (Table S4.1, Appendix 1), and with the percentage representation of each studied behavioural categories (Table S4.2, Appendix 1), were conducted in order to measure the statistical relationship between these variables. Due to the high degree of correlation observed among the weather variables, the raw variables were grouped by Principal Component Analysis using a varimax rotation procedure. Six variables with an eigenvalue higher than 1 were selected: F1-T related to temperature, F2-H related to humidity, F3-Mag related to the magnetic heading, F4-Alt related to altitude, F5-Wind related to wind speed, and F6-Cloud related to cloudiness. The contribution of the original variables to the extracted factors, eigenvalue and percentage of variance explained by each new factor is shown in Table 4.4.

Generalized Mixed Models were built in order to study the influence of the 6 selected weather factors, the group dispersion and the season on the percentage representation of each of the four selected behavioural categories. Data structure was set based on group and period, since most of the studied groups were studied in different periods. Group also entered the model as random factor. Data was also weighed based on the number of adult horses in the herd, which differed between the herds observed. Gamma distribution with log function was set for the models. From all the possible solutions of the model, we selected those with all significant or marginally significant variables and lower AIC value.

One-way ANOVA with post-hoc Tukey test was used to detect differences between the studied groups in the display of the studied behaviours, separately for each studied season.

Table 4.4. Scores of the six weather variables used in the factor analysis.

	F1-T	**F2-H**	**F3-Mag**	**F4-Alt**	**F5-Wind**	**F6-Cloud**
% Variance explained	28.234	13.653	13.511	13.414	11.839	11.300
Eigenvalue	4.517	2.185	2.162	2.146	1.894	1.808
MG (mag)	0.099	0.100	0.940	0.099	0.172	0.047
TR (True)	0.099	0.100	0.940	0.099	0.172	0.047
WS (m/s)	0.042	-0.045	0.135	0.069	0.955	0.054
CW (m/s)	0.039	0.027	0.182	0.094	0.942	0.082
HW (m/s)	-0.023	0.016	0.493	0.042	0.017	0.081
TP (°C)	0.984	-0.149	0.028	0.073	0.041	-0.008
WC (°C)	0.987	-0.132	0.013	0.064	-0.014	-0.010
RH (%)	-0.393	0.866	0.076	-0.011	-0.050	0.219
HI (°C)	0.991	-0.026	0.046	0.079	0.044	0.015
DP (°C)	0	0.962	0.115	0.090	0.013	0.099
WB (°C)	0.740	0.631	0.114	0.086	0.020	0.146
BP (mb)	-0.182	-0.056	-0.124	-0.970	-0.086	0.015
AL (m)	0.182	0.057	0.124	0.970	0.085	-0.013
DA (m)	0.889	-0.038	0.079	0.444	0.067	0.001
Clouds (location)	-0.053	0.143	0.055	-0.034	0.075	0.931
Clouds (whole)	0.094	0.134	0.160	0.009	0.060	0.917

The significant structuring variables (>0.8, following Budaev, 2010) are indicated in bold characters.

MG (mag) = magnetic heading, TR (True) = true heading, WS (m/s) = windspeed, CW (m/s) = crosswind calculation, HW (m/s) = headwind/tailwind, TP (°C) = temperature, WC (°C) = windchill, RH (%) = relative humidity, HI (°C) = heat stress index, DP (°C) = dewpoint temperature, WB (°C) = wet bulb temperature, BP (mb) = barometric pressure, AL (m) = altitude, DA (m) = density altitude, clouds (location) = % of cloud cover in the place of observation, clouds (whole) = % of cloud cover in the observable surrounding.

4.4. Results

The models selected for each of the behavioural categories studied is shown in Table 5. The occurrence of feeding behaviour significantly increased with cloudiness (t=2.013) and under windy conditions (t=1.987) and was also more frequent in autumn compared to spring and summer (Figure 4.2a). Locomotion was positively explained by temperature (t=3.251) and cloudiness (t=1.923), and also varied with the period being higher in summer compared to spring and autumn (Figure 4.2b). The occurrence of resting behaviour decreased with altitude (t=-2.892, i.e., the animals preferred to rest in lowlands) and with cloudiness (t=-3.291), and the dispersion of the group was lower (t=-2.920, i.e., the animals were closer to each other while resting). Horses showed increased social interactions under higher temperature (t=2.633). Social interactions were also affected by period, being more frequent in summer compared to spring and autumn (Figure 4.2c).

Table 4.5. General linear mixed models (GLMM) assessing the effects of weather variables on the occurrence of different behavioural categories displayed by horses.

	Feeding	**Locomotion**	**Resting**	**Social**
df	4, 1119	4, 436	3, 1205	4, 405
F1-T	ns	F=10.569 p=0.001	ns	F=6.932 p=0.009
F2-H	ns	ns	ns	ns
F3-Mag	ns	ns	ns	ns
F4-Alt	ns	ns	F=8.361 p=0.004	ns
F5-Wind	F=3.950 p=0.047	ns	ns	ns
F6-Cloud	F=4.051 p=0.044	F=3.697 p=0.055	F=10.829 p=0.001	ns
Dispersion	ns	ns	F=8.527 p=0.004	ns
Period	F=65.293 p<0.001	F=17.835 p<0.001	ns	F=3.367 p=0.026

ns = not significant

FX-X = Factors after PCA analysis (see Table 4.4) related to temperature, humidity, magnetic heading, altitude, wind speed, and cloudiness respectively.

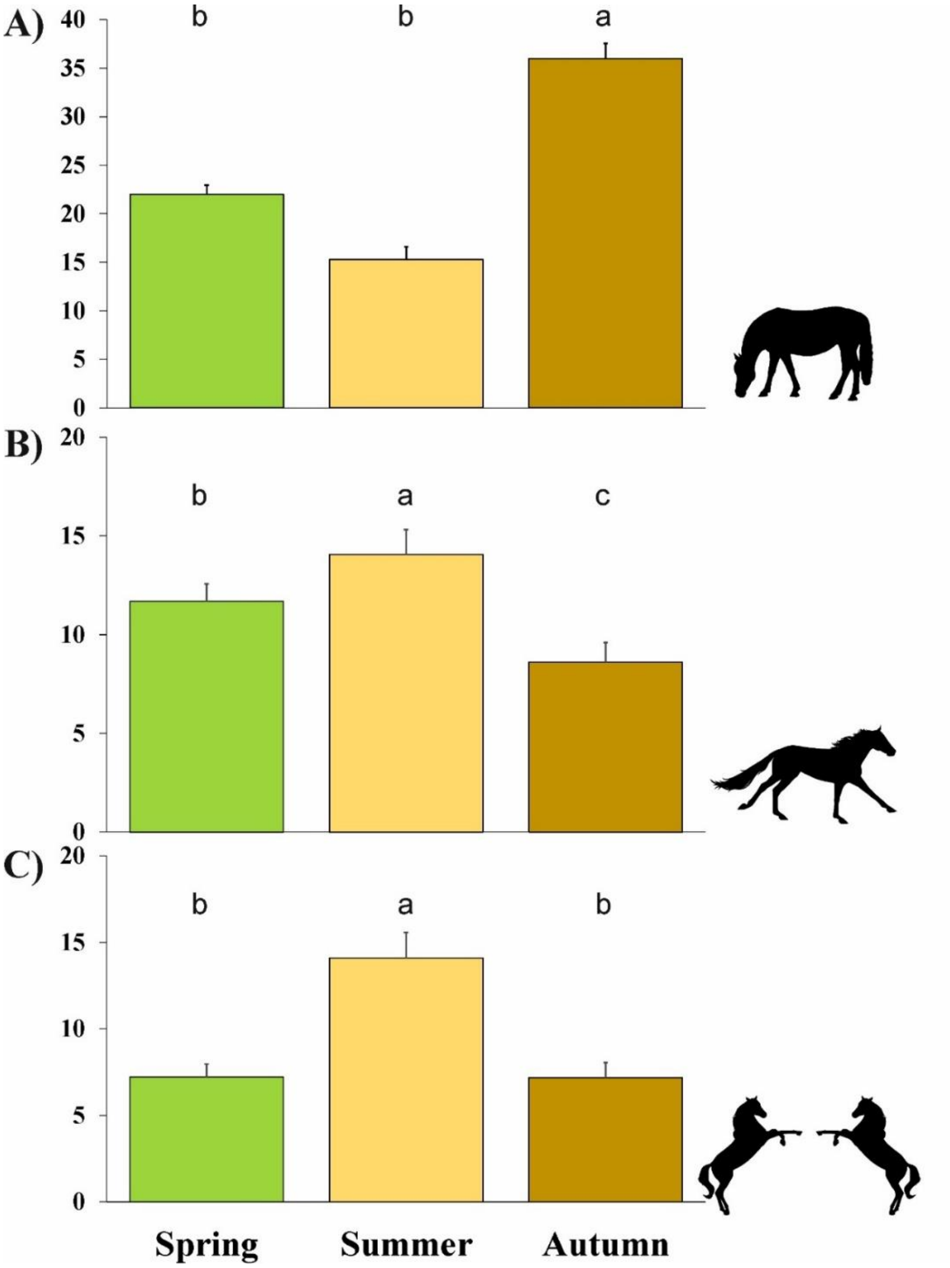

Figure 4.2. Influence of the season (green=spring, beige=summer, brown=autumn) on the percentage representation of each studied behavioural categories (A = feeding, B = locomotion, C = social). a, b, c superscripts indicate significant differences after Tukey test.

ANOVA analyses showed differences in the display of the studied behaviours among the studied harems. Overall inter-harem differences in spring were found for feeding (F=19.613, p<0.001), locomotion (F=33.060, p<0.001), resting (F=8.690, p<0.001) and social (F=4.928, p=0.002). In Summer, inter-harem differences were found for feeding (F=5.295, p=0.006), locomotion (F=23.394, p<0.001), resting (F=5.370, p=0.005), and social (F=5.957, p=0.003). In Autumn, inter-harem differences were found for feeding (F=17.405, p<0.001), locomotion (F=12.269, p<0.001) and resting (F=2,904, p=0.034), but not for social (F=2.484, p=0.060). Differences between specific harems are shown through superscripts in the figures, respectively for feeding (Figure 3), locomotion (Figure 4.4), resting (Figure 4.5) and social (Figure 4.6). These differences also show interesting patterns when grouping the harems according to their origin and experience in the area, thus the harems are grouped in the figures according to that (wild-born harems in dark green; long-term released harems in light green; recently reintroduced harems in white).

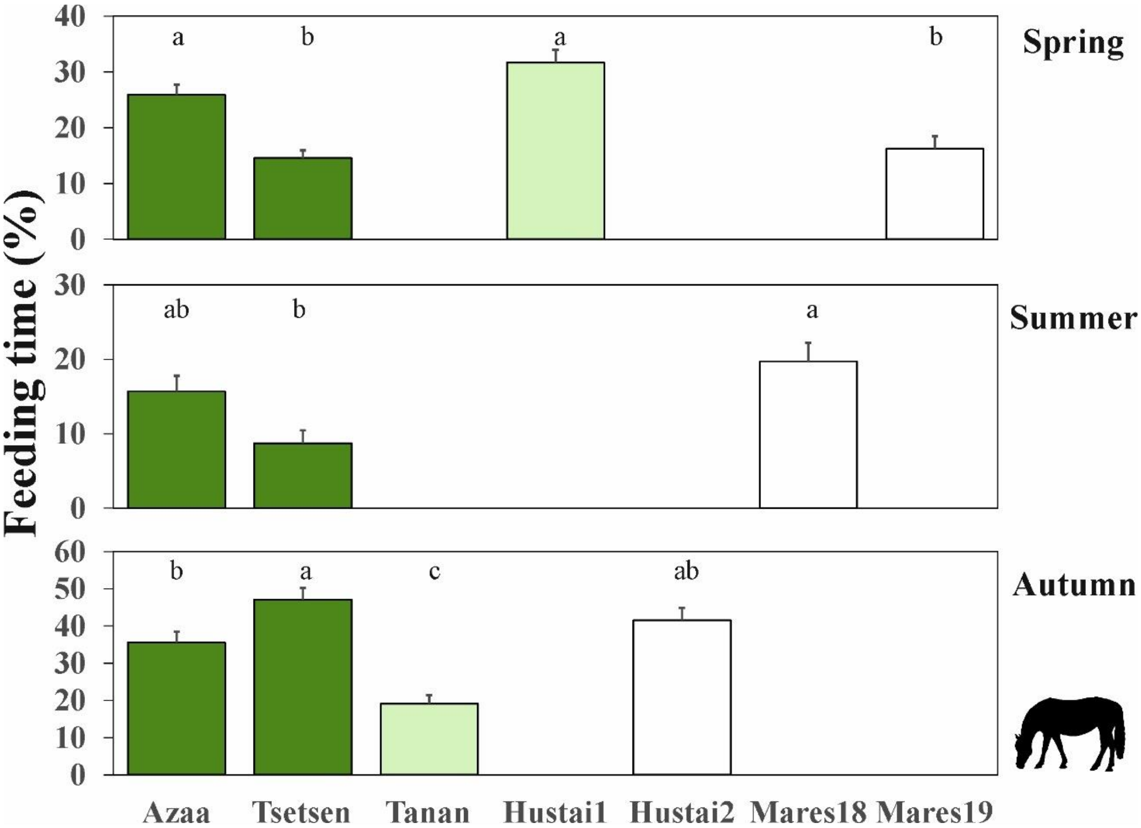

Figure 4.3. Differences between specific harems for the time spent feeding. The harems are grouped in the figures according to their origin and experience in the area (wild-born harems in dark green; long-term released harems in light green; recently reintroduced harems in white). a, b, c superscripts indicate significant differences after Tukey test.

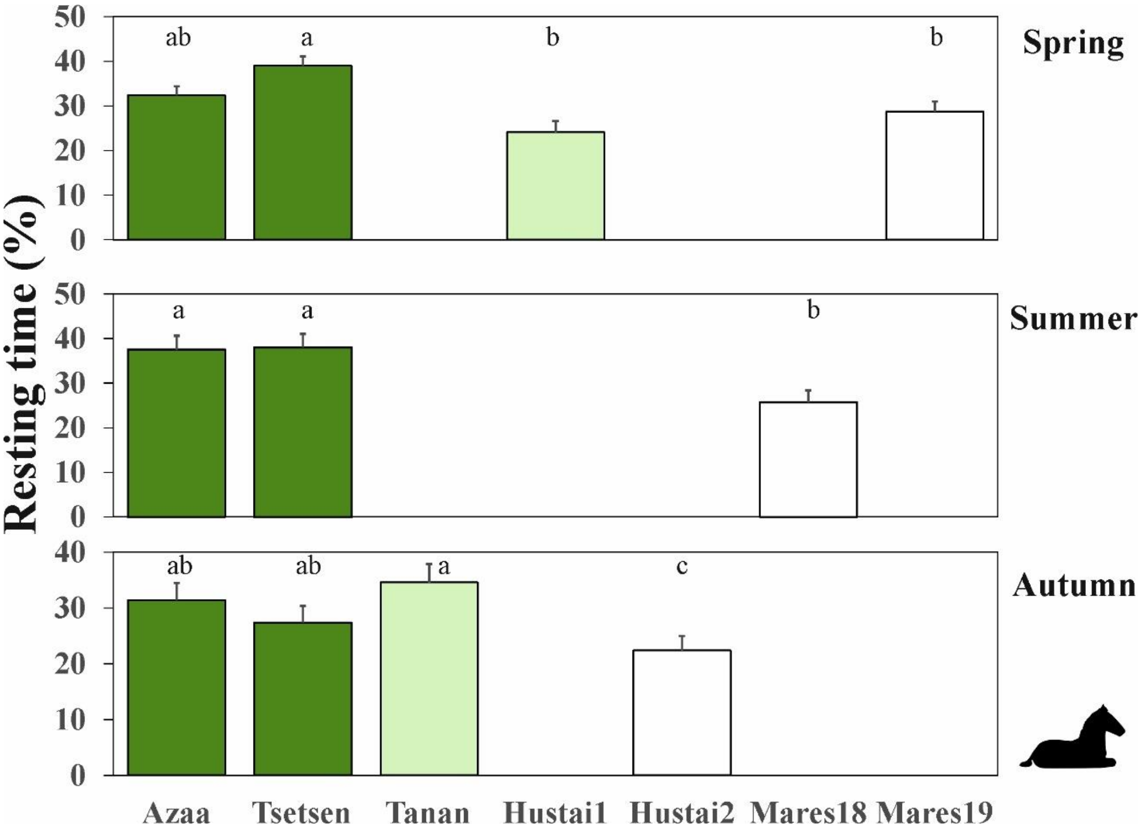

Figure 4.4. Differences between specific harems for the time spent resting. The harems are grouped in the figures according to their origin and experience in the area (wild-born harems in dark green; long-term released harems in light green; recently reintroduced harems in white). a, b, c superscripts indicate significant differences after Tukey test.

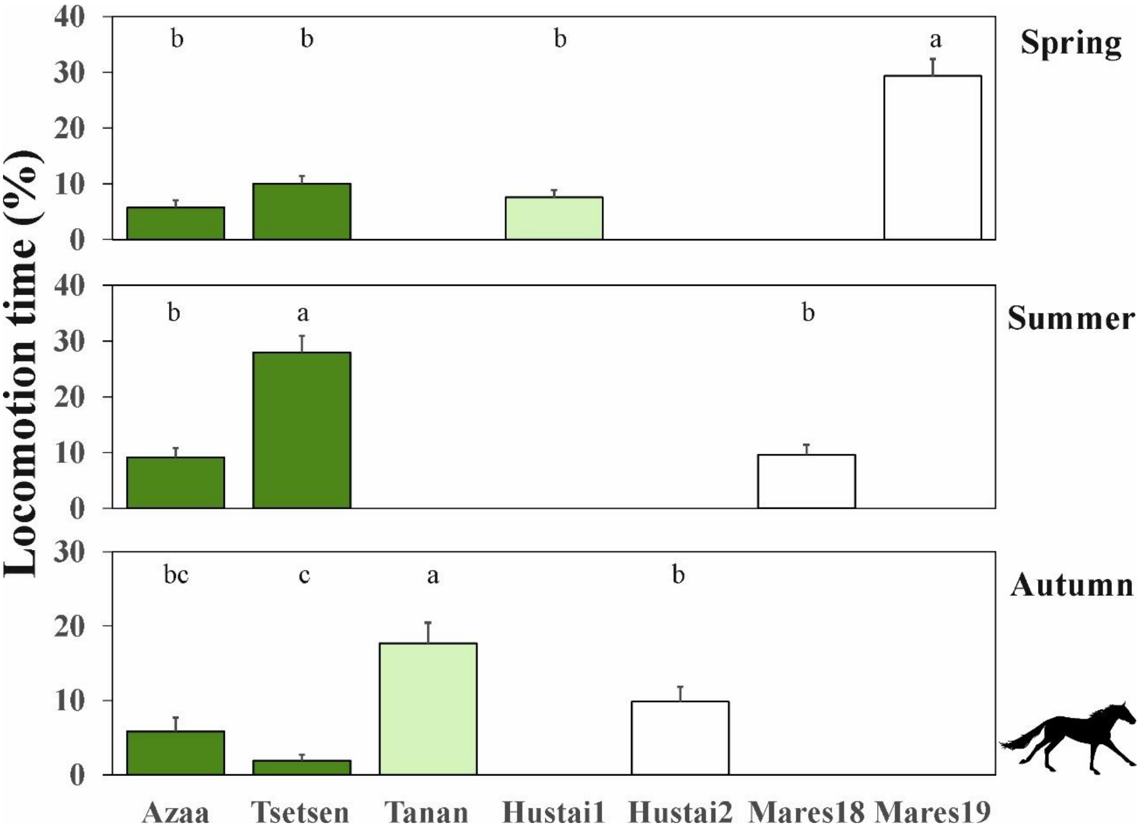

Figure 4.5. Differences between specific harems for the time spent on locomotion. The harems are grouped in the figures according to their origin and experience in the area (wild-born harems in dark green; long-term released harems in light green; recently reintroduced harems in white). a, b, c superscripts indicate significant differences after Tukey test.

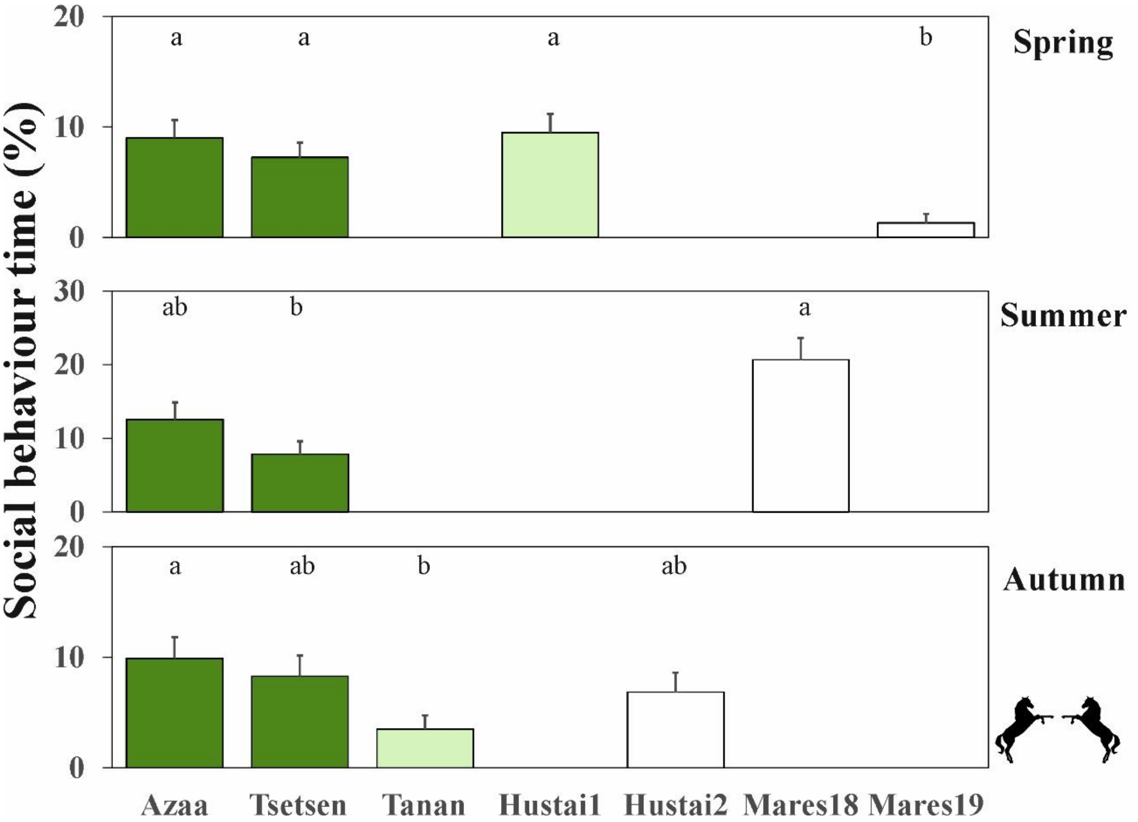

Figure 4.6. Differences between specific harems for the time spent on social interactions. The harems are grouped in the figures according to their origin and experience in the area (wild-born harems in dark green; long-term released harems in light green; recently reintroduced harems in white). a, b, c superscripts indicate significant differences after Tukey test.

4.5. Discussion

4.5.1. Weather effect

Weather conditions are known to affect the behaviour of animals and the response of animals to the environment is vital for their survival. That is especially true for Mongolia's continental and arid climate, defined by extreme temperature and precipitation variability. Our results indicate that there are seasonal changes in behaviour of Przewalski's horses, and that weather conditions influence the behaviour of the species. These findings may help for future conservation of endangered Przewalski's horses, especially considering global climate change.

During our study, occurrence of feeding behaviour significantly increased with cloudiness and during windy conditions and was also more frequent in autumn compared to spring and summer. This result complements the research conducted by Van Dierendonck et al. (1996) who described that the maximum number of hours spent grazing in free-living Przewalski horses in Mongolia was documented during fall and winter seasons. Souries et al. (2007) described, that feeding is negatively influenced by temperature, this corresponds with our result as in autumn the temperature is typically lower than in summer and late spring. As described by Berger et al. (1999) food intake accounts for 40% of total activity of Przewalski's horses in the summer season (the lowest amount of food intake through the year) which also fits with our results. However, during their research horses spent 62% of time feeding throughout the spring season, representing the highest level of the year. This outcome is contradictory to our result and may be explained by the fact that Berger et al. (1999) observed horses in semi-reserve, possibly with more favourable climatic conditions and feed composition. In grasslands of central Asia, the leaf/stem ratio of plant decreases with rising temperatures, promoting the formation of structural carbohydrates, resulting in reduced digestibility of pasture fodder (Shi et al. 2013). For this reason, Przewalski's horses in Gobi might be forced to dedicate more time to grazing in autumn, as the quality of forage after warm summer is considerably lower in these areas.

In the GGBSPA, Przewalski's horses live on the edge of their original habitat (Gao et al. 1989; Paklina & Pozdnyakova 1989; Wakefield et al. 2002) and are highly water limited (Rubenstein et al. 2016). The need to stay in relative proximity to water points to be able to drink at least two times per day is forcing them to feed on non-preferred or even normally avoided plants during warmer days. Only after the sunset and during cooler climatic conditions (windy and cloudy weather) they are able to move to more favourable feeding areas (Rubenstein et al. 2016). Even during late spring, the temperatures could be considerably high (reaching up to 26 °C, National Statistical Office of Mongolia 2019), limiting the movement of horses. The proximity of mostly undesirable feed sources and inability to move to different areas might cause horses to switch from feeding to alternative behaviours.

Wind plays an important role in habitat use and feeding behaviour of ungulates. During the warm season, ungulates often move to windy and sparsely vegetated sites to avoid biting insects (Blank 2020). Activity of biting insects is positively correlated with air temperature and negatively influenced by wind speed (Baldacchino et al. 2013). Therefore, windy weather provides horses of Gobi with the opportunity to feed undisturbed and to choose areas preferred for feeding without the necessity of searching shelter from insects. This phenomenon has been already described in previous research stating that serious harassment from biting insects might decrease feed intake in ungulates and resting period, impacting their well-being and body condition (Helle et al. 1992; Weladji et al. 2003)

As described in previous research, Przewalski's horses significantly decrease their metabolic rate during the winter season to cope with food scarcity and harsh weather (Scheibe & Streich 2003; Kuntz 2006; Arnold et al. 2004). Therefore, the increase in feeding activity during autumn might be explained by the attempt to make the most of the still available pasture to prepare for upcoming rough climate conditions.

Horses observed in our research showed higher locomotion activity in summer compared to spring and autumn, and their movement was positively explained by temperature and cloudiness. This outcome is consistent with the research by Arnold et al. (2006) who stated that Przewalski's horses in semi-natural conditions present much lower locomotion activity in the cold season in comparison to spring and summer season. However, previous research conducted in the GGBSPA was contradictory to our results and stated that locomotion was more or less constant over the whole observation period and did not change with temperature (May to September, Souries et al. 2007). Nonetheless that study was done considering only recently released horses. Blood-sucking insects are very possibly a significant factor influencing locomotive behaviour of Przewalski's horses in summer forcing them to frequently move and seek shelter from insect bites. As demonstrated by Blank (2020) the largest proportion of insect-repelling behaviour in ungulates occurs during the warm and windless summer season, which is the time of year when most activity of biting insects occurs. Ungulates frequently move to specific landscape features to find high and unvegetated sites with reduced temperatures and higher wind speeds to avoid insect.

The horses preferred to rest in the lowlands during our investigation, and the frequency of resting decreased with cloudiness. While resting, horses maintained lower distance among each other. Souries et al. (2007, research conducted on Przewalski´s horses from May to September) and Ihle (1984, research conducted on domestic horses year-round) found that resting is positively influenced by temperature. Resting behaviour usually implies lower metabolic rate, and a reduction in metabolic rate is a typical reaction to extreme temperatures (Arnold et al. 2004). In Gobi, where almost no shadow exists, cloudy weather may provide relieve from constant radiant heat and allow horses to stop resting and engage in more active behaviours.

Resting behaviour is heavily influenced by feed availability. It lasts longer when the feed sources are abundant (Boyd & Houpt 1994; Van Dierendonck et al. 1996; Berger et al. 1999; Woodfine 2003) and is almost non-existent when there is not enough feed (Boyd & Houpt 1994; Van Dierendonck et al. 1996; Berger et al. 1999; Woodfine 2003; Hechler 1971; Zeitler-Feicht 2008). The positive relationship between resting and lowlands, as, in the studied area, food is usually more plentiful and of higher quality in lowlands than at higher altitudes, could be explained by this fact (1550 – 1805 mamsl, own data). This fits with the research of Heintzelmann-Gröngröft (1984) who stated that it is typical for wild horses to rest in open grasslands.

Studies measuring spatial proximity in resting horses showed significant variations in the distance measured between individuals (Cameron et al. 2009; Christensen et al. 2002; Crowell-Davis et al. 1986; Heitor et al. 2006; Kimura 1998; Sigurjónsdóttir et al. 2003). However, in ungulates vulnerable to predation (such as the Przewalski's horses in Gobi, Robert et al. 2005) greater group cohesion supports the dilution effect (Hamilton 1971; Dehn 1990) and increases confusion in predators (Neill & Cullen 1974; Milinski 1977) by creating “safety in numbers”. When group members are closer to each other, the predator gets more confused, and it might be harder to aim the attack on a particular animal (Lehtonen & Jaatinen 2016). Lower group dispersion observed during resting might be explained as anti-predatory behaviour (higher vigilance) in wild Przewalski's horses.

4.5.2. Social interactions

During our research, horses showed increased social interactions under warmer weather conditions. Social interactions were also affected by period, being more frequent in summer compared to spring and autumn. Berger et al. (1999) stated that over the period of the year, the general pattern of activity and feeding of semi-wild group of Przewalski's horse females was closely linked to the time of sunset and sunrise and during daytime most of the social activity occurred.

In some mammalian species, no correlation was found between weather and social interactions (Ross & Giller 1988). However, in some other (Bernstein & Baker 1988; Young et al. 2008; Taraborelli & Moreno 2009) including ungulates (Fattorini 2018; Ismail et al. 2011) the social interactions might decrease with increasing environmental pressure to reduce the risk of thermal stress. Following this logic, horses in Gobi should engage less in interactions during warm weather conditions and during summer season, when temperatures are generally high, and environment is more demanding than in autumn and spring. However, we observed opposite trend. It could be explained by the fact that during the warm season, the insect harassment is the highest as described by Blank (2020) and therefore horses might tend to engage more in social insect repelling behaviour, such as grooming (although we did not distinguish between positive and agonistic interactions in our analyses). The higher frequency of social interactions could not be explained by sexual activity of the selected groups, as it was the highest in spring period during our research (spring: 31 sexual interactions observed; summer: 7; autumn: 22; own data). This result is in contradiction to the study by Stevens (1990) who described that the number of social interactions in feral horses was significantly higher in the breeding period.

Przewalski's horses have been translocated to a variety of environments, most typically to mesic habitats and semi-desert areas (Wakefield et al. 2002). True arid-living species have phenotypic, physiological, and behavioural characteristics to deal with high heat and water stress (Bartholomew & Dawson 1968; Cain et al. 2006). Przewalski's horses, as a mesic evolved species, are unlikely to have structural or physiological characteristics that would help them to survive in the desert. However, behavioural mechanisms like social structure (Rubenstein 1989), daily budget (Jarman 1977), and locomotion patterns (Fryxell & Sinclair 2008) may be modified to allow them to cope with these relatively unfamiliar and severe environments and they can thrive in arid environments as long as they have regular access to water sources (Van Dierendonck 1996).

4.5.3. Group behavioural patterns

We observed an interesting pattern when comparing occurrence of the selected behaviours in different studied groups: The free-ranging, wild-born groups Azaa and Tsetsen shared common behavioural pattern for feeding in summer. However, in spring and autumn, they differed. These differences could be caused by the fact that Tsetsen is an inexperienced harem-holder, and his harem had not existed for a significant period when our observations were conducted. His first harem (Tsetsen) was formed in 2018 only two months before the start of our fieldwork. As described by Klimov (1988) a harem led by an inexperienced stallion might shift to anomalous behaviours and might not present typical daily activity budget of stable groups.

Moreover, the feeding behaviour displayed by Hustai1 harem, which consisted of mares brought in summer 2018 and a wild stallion Hustai, was very similar to that of the wild-born and stable Azaa harem. Hustai1 harem showed such similar behaviour to that of the Azaa harem after approximately one year, supporting the research by Scheibe et al. (1997) who found that Przewalski's horses adapt to novel conditions and start to present the typical yearly behavioural pattern of wild-born horses living in the native habitat one year after being translocated.

Nevertheless, when the same females were led by Tanan stallion (who defeated Hustai stallion) the time dedicated to feeding greatly differed from both wild-born groups. Even if these mares were in Gobi for more than one year and clearly started to present the typical behavioural pattern of wild-born horses in spring, their behaviour was indeed influenced by the change of the stallion. Stallions may play an important role when exploring a novel environment (Wolter et al. 2014) and have the ability to play a unique role in the decision-making process. Indisputably, holding females together is part of their reproductive strategy, and specialised acts such as herding allows them to affect the behaviour of the entire group (Briard et al. 2017). The fact that Hustai2 harem, consisting of the stallion Hustai and mares brought in spring 2019, shared similar feeding pattern with both wild-born groups in autumn, support the stallion influence interpretation.

The wild-born, free-ranging groups Azaa and Tsetsen shared common behavioural pattern for resting during all studied periods. Both, Azaa and Tsetsen, shared similar resting pattern with free-ranging Tanan in autumn, and in spring, Azaa shared the pattern with free-ranging Hustai1 and Mares19 (enclosure). Mares18 (enclosure) differed from both, Azaa and Tsetsen and Hustai2 (enclosure) different from all three free-ranging groups in autumn. This variation in resting time might be caused by the fact, that Hustai2 and Mares18 were observed only in the enclosure. As described by Keiper and Receveur (1992), size of the area used by horses has a significant influence on time dedicated to resting and in general, horses in smaller enclosures tend to rest less. Nonetheless, one of the enclosed groups, Mares19, did not differ from free-ranging Azaa and Hustai1 in the resting pattern in spring.

Keiper and Receveur (1992) also found that locomotion in Przewalski´s horses is influenced by the size of enclosure. However, we only observed this trend in spring, when Mares19 (enclosure) differed in the locomotion pattern from free-ranging Azaa, Tsetsen and Hustai, who shared similar pattern. During our research in summer and autumn this trend was not observed.

In spring, we found that free-ranging groups of Azaa, Tsetsen and Hustai1 shared similar pattern in social behaviour, and enclosed Mares19 differed from these three groups. In previous research it was described that area size influences the rate of social interactions among horses, as individuals in smaller enclosures were reported to engage more in social interactions than those on large pastures (Hogan et al., 1988; Keiper & Receveur 1992). Nonetheless, we did not find such differences between enclosed and free-ranging groups in autumn and summer.

During our research, we detected influence of the harem characteristic on the activity budget. One of the factors was harem origin and experience. It was described in previous research that after the release, wild horses might show differences from the typical pattern in specific behaviours, such as locomotion and resting up to one year (Scheibe et al. 1997), or even two years after the release (Boyd & Bandi 2002). In general, differences from the typical annual behavioural pattern of Przewalski's horses presented in our results can be explained by the period of adaptation of recently reintroduced horses to the seasonal change of climatic and nutritional conditions, as also described by Scheibe et al. (1997), and Boyd and Bandi (2002).

Nonetheless, we also detected influence caused by the characteristics of harem holder (dominant stallion) and area size on the behavioural pattern of Przewalski's horses.

During our research, only two groups (Azaa and Tsetsen) were observed in all three seasons as the structure of other groups was changing through the observation period or included the observation of horses transported from Europe in 2019 (i.e., new groups).

Winter monitoring was not possible due to low accessibility to the area (frozen paths) and impossibility to record the herds due to the extremely low temperatures. For this reason, we emphasize a need for further research concerning more groups (if possible, same ones) and all seasons (including winter).

4.5.4. Implications for conservation

It was stressed by several studies that appropriate response of animals to the environment is vital for their survival (Knight 2001; Snijders et al. 2017; Macdonald 2015).

The disastrous winter of 2009 and 2010, resulting in the loss of the 60% of Przewalski's horse population, demonstrated how sensitive are the small and spatially restricted populations to severe climatic and environmental changes (Kaczensky et al. 2011). As stated by Slotta-Bachmayr (2004) the severity level of natural climatic conditions has the highest influence on extinction risk and population size of Przewalski's horses in the Great Gobi B, according to population simulation model VORTEX. The extinction risk for severe weather disasters measured by Slotta-Bachmayr (2004) was 37%, even for initial population size larger than 500 individuals. The Przewalski's horse population in the GGBSPA is still significantly smaller and thus highly susceptible to severe weather conditions.

According to the 2014 Mongolia Second Assessment Report on Climate Change, the impact of climate change is already visible in Mongolia and this country is highly vulnerable being ranked 8th among over 100 countries according to the Global Climate Risk Index. Winters are becoming warmer and snowier; the temperature is expected to increase continuously in all seasons and there is a high probability of climate anomalies happening more often in future (Dagvadorj et al 2014). In this study we found out that there is an important link between the behaviour of Przewalski's horse and different weather factors. These findings might assist in the successful selection of future reintroduction sites and in further conservation of the species, especially in the view of intensifying climate change and alteration of weather patterns.

4.6. References

Armstrong DP, Seddon PJ. Directions in reintroduction biology. Trends Ecol Evol. 2008;23:20-5. https://doi.org/10.1016/j.tree.2007.10.003.

Arnold W, Ruf T, Kuntz R. Seasonal adjustment of energy budget in a large wild mammal, the Przewalski horse (*Equus ferus przewalskii*) II. Energy expenditure. J. Exp. Biol. 2006;22:4566-73. https://doi.org/10.1242/jeb.02536.

Arnold W, Ruf T, Reimoser S, Tataruch F, Onderscheka K, Schober F Am. 2004. Nocturnal hypometabolism as an overwintering strategy of red deer (*Cervus elaphus*). J Physiol Regul Integr Comp Physiol. 2004;286:174-81. https://doi.org/10.1152/ajpregu.00593.2002.

Baguette M, Blanchet S, Legrand D, Stevens VM, Turlure C. Individual dispersal, landscape connectivity and ecological networks. Biol. Rev. 2013;88:310-26. https://doi.org/10.1111/brv.12000.

Bartholomew GA, Dawson W. Desert Biology. Cambridge, Massachusetts: Academic Press, Inc.; 1968.

Baldacchino F, Puech L, Manon S, Hertzog LR, Jay-Robert. Biting behaviour of Tabanidae on cattle in mountainous summer pastures, Pyrenees, France, and effects of weather variables. Bull Entomolog Res. 2014;471–479. https://doi.org/10.1017/S0007485314000170.

Berger A, Scheibe KM, Eichhorn K, Scheibe A, Streich J. Diurnal and ultradian rhythms of behaviour in a mare group of Przewalski horse (*Equus ferus przewalskii*), measured through one year under semireserve conditions. Appl. Anim. Behav. Sci. 1999;64:1-17.

Bernstein IS, Baker SC. Activity patterns in a captive group of Celebes black ape (*Macaca nigra*). Folia Primatol. 1988;61-75.

Blank DA. Insect-repelling behaviour in goitered gazelles: Responses to biting fly attack. Eur. J. Wildl. Res. 2020;66:1-2. https://doi.org/10.1007/s10344-020-01382-1.

Bousquet, CAH. Group decision-making in meerkats (*Suricata suricatta*) [Doctoral dissertation]. [Zurich, Switzerland]: University of Zurich Rämistrasse; 2011.

Boyd L, Houpt KA. Activity Patterns. In: Boyd L, Houpt KA (Eds) Przewalski's Horse: The history and biology of an endangered species. State University of New York Press, New York; 1994. p. 195-227

Boyd L, Bandi N. Reintroduction of takhi, Equus ferus przewalskii, to Hustai National Park, Mongolia: time budget and synchrony of activity pre-and post-release. Appl. Anim. Behav. Sci. 2002;87-102. https://doi.org/10.1016/S0168-1591(02)00088-6.

Briard L, Deneubourg JL, Petit O. How stallions influence the dynamic of collective movements in two groups of domestic horses, from departure to arrival. Behav. Processes. 2017;142:56-63. https://doi.org/10.1016/j.beproc.2017.05.014.

Brown C, Day RL. The future of stock enhancements: lessons for hatchery practice from conservation biology. Fish Fish. 2002;79–94. https://doi.org/10.1046/j.1467-2979.2002.00077.x.

Budaev SV. Using principal components and factor analysis in animal behaviour research: caveats and guidelines. Ethology. 2010;116:472-80. https://doi.org/10.1111/j.1439-0310.2010.01758.x.

Cain JW, Krausman PR, Rosenstock SS, Turner JC. Mechanisms of Thermoregulation and Water Balance in Desert Ungulates. Wildl. Soc. Bull. 2006;34:570–81. https://doi.org/10.2193/0091-7648(2006)34[570:MOTAWB]2.0.CO;2.

Cameron EZ, Setsaas TH, Linklater WL. Social bonds between unrelated females increase reproductive success in feral horses. P Natl Acad Sci.2009; 50-53. https://doi.org/10.1073/pnas.0900639106.

Christensen JW, Zharkikh T, Ladewig J, Yasinetskaya N. Social behaviour in stallion groups (*Equus przewalskii* and *Equus caballus*) kept under natural and domestic conditions. Appl Anim Behav Sci. 2002;76, 11–20.

Conradt L, Krause J, Couzin ID, Roper TJ. “Leading according to need” in self-organizing groups. Am. Nat. 2009:304-12. https://doi.org/10.1086/596532.

Crowell-Davis SL, Houpt KA, Carini CM. Mutual grooming and nearest-neighbor relationships among foals of Equus caballus. Appl Anim Behav Sci. 1986;113–123.

Dagvadorj D, Batjargal Z, Natsagdorj L. 2014. Mongolia Second Assessment Report on Climate Change 2014. Ministry of Environment and Green Development of Mongolia. Ulaanbator, Mongolia.

Dehn MM. 1990. Vigilance for predators: detection and dilution effects. Behav. Ecol. Sociobiol. 1990;26:337-342.

DeVries AC, Glasper ER, Detillion CE. Social modulation of stress responses. Physiol Behav 2003;79:399–407. https://doi.org/10.1016/S0031-9384(03)00152-5.

Dickens MJ, Delehanty DJ, Romero LM. Stress: an inevitable component of animal translocation. Biol. Conserv. 2010;143:29–41. https://doi.org/10.1016/j.biocon.2010.02.032.

Van Dierendonck MC, Wallis de Vries MF. Ungulate reintroductions: Experiences with the takhi or Przewalski horse (*Equus ferus przewalskii*) in Mongolia. Biol. Conserv. 1996;10:728–740.

Duncan, P. Time budgets of Camargue horses. III. Environmental influences. Behaviour 1985;92:188-208.

Fattorini N, Lovari S, Brunetti C, Baruzzi C, Cotza A, Macchi E, Pagliarella MC, Ferretti F. Age, seasonality, and correlates of aggression in female Apennine chamois. Behav. Ecol. Sociobiol. 2018;72:1-7. https://doi.org/10.1007/s00265-018-2584-5.

Fritts SH, Bangs EE, Fontaine JA, Johnson MR, Phillips MK, Koch ED, Gunson JR. Planning and implementing a reintroduction of wolves to Yellowstone National Park and central Idaho. Restor. Ecol. 1997;5:7–27. https://doi.org/10.1046/j.1526-100X.1997.09702.x.

Fryxell J, Sinclair A. Seasonal migration by white-eared kob in relation to resources. Afr. J. Ecol. 1988;26:17–31. https://doi.org/10.1111/j.1365-2028.1988.tb01125.x.

Gao X, Gu, Zhou J. 1989 The change on the distribution area of the wild horse in the modern times. Arid Zone Res. 1989;2:10.

Gersick AS, Rubenstein DI. 2017 Physiology modulates social flexibility and collective behaviour in equids and other large ungulates. Phil. Trans. R. Soc. B 372: 20160241. http://dx.doi.org/10.1098/rstb.2016.0241.

Hamilton WD. Geometry for the selfish herd. J. Theor. Biol. 1971;31:295-311.

Hechler B. Beitrag zur Ethologie des Islandpferdes [Doctoral dissertation]. [University of Gießen, Germany]: University of Gießen, Ludwigstraße; 1971.

Heitor F, Oom MM, Vicente L. Social relationships in a herd of Sorraia horses Part II. Factors affecting affiliative relationships and sexual behaviours. Behav Process 2006;73, 231–239. https://doi.org/10.1016/j.beproc.2006.05.005.

Heintzelmann-Gröngröft B. Spezielle Ethologie – Pferd. In: Bogner H, Grauvogl A, editors. Verhalten landwirtschaftlicher Nutztiere. Eugen Ulmer, Stuttgart; 1984. p. 87 – 147.

Helle T, Aspi J, Lempa K, Taskinen E. Strategies to avoid biting flies by reindeer: field experiments with silhouette traps. Ann Zool Fennici. 1992;29:69–74.

Henry JP, Stephens PM. Stress, health and the social environment: a sociobiological approach to medicine. Springer, Berlin, Germany: Springer, Inc.; 1977.

Hennessy MB, Kaiser S, Sachser N (2009) Social buffering of the stress response: diversity, mechanisms, and functions. Front Neuroendocrinol. 2009;30:470–482. https://doi.org/10.1016/j.yfrne.2009.06.001.

Hinde RA. Interactions, relationships, and social-structure. Man. 1976;11:1–17.

Jefferies DJ, Wayre P, Jessop RM, Mitchell-Jones AJ. Reinforcing the native otter *Lutra lutra* population in East Anglia: an analysis of the behaviour and range development of the first release group. Mamm Rev. 1986;16:65–79.

Ihle P. Ethologische Studie über den Tagesrhythmus von Pferden in Abhängigkeit von der Haltungsform. [Doctoral dissertation]. [University of Gießen, Germany]: University of Gießen, Ludwigstraße; 1984.

Ismail K, Kamal K, Plath M, Wronski T. Effects of an exceptional drought on daily activity patterns, reproductive behaviour, and reproductive success of reintroduced Arabian oryx (*Oryx leucoryx*). J. Arid Environ. 2011;75:125-31. https://doi.org/10.1016/j.jaridenv.2010.09.017.

ITG International Takhi Group. Great Gobi B Biosphere [Internet]. ITG; 2016 [cited 2021 Oct 20]. Available from: https://savethewildhorse.org/en/great-gobi-b-biosphere//

The IUCN Red List of Threatened Species. Goitered Gazelle [Internet]. IUCN; 2007 [cited 2021 Oct 15]. Available from: https://www.iucnredlist.org/species/8976/50187422#population//

IUCN, SSC. "Guidelines for reintroductions and other conservation translocations." Gland Switz Camb UK IUCNSSC Re-Introd Spec Group (2013).

Jarman P. Behaviour of topi in a shadeless environment. Zool. Afr. 1977;12:101–11.

Kaczensky P, Enkhsaihan N, Ganbaatar O, Samjaa R, von Wehrden H, Walzer C. Takhis, Khulans, Wolves and Vegetation – An Ecosystem Approach for Takhi Conservation. Mong. J. Biol. Sci. 2004;2: 60.

Kaczensky P, Ganbaatar O, von Wehrden H, Walzer C. Resource selection by sympatric wild equids in the Mongolian Gobi. J Appl Ecol. 2008;45:1762–1769. https://doi.org/10.1111/j.1365-2664.2008.01565.x.

Kaczensky P, Ganbataar O, Altansukh N, Enkhsaikhan N, Stauffer C, Walzer C. The danger of having all your eggs in one basket—winter crash of the re-introduced Przewalski's horses in the Mongolian Gobi. PLoS One. 2011;612:28-57. https://doi.org/10.1371/journal.pone.0028057.

Kimura R. Mutual grooming and preferred associate relationships in a band of free-ranging horses. Appl Anim Behav Sci. 1998;59, 265–276.

King SRB. Delinquency Behavioural ecology of Przewalski horses (*Equus przewalskii*) reintroduced to Hustai National Park, Mongolia [Doctoral dissertation]. [Queen Mary, UK]: University of London, London; 2002.

King SRB, Gurnell J. Habitat use and spatial dynamics of takhi introduced to Hustai National Park, Mongolia. Biol. Conserv. 2005; 124:277–290. https://doi.org/10.1016/j.biocon.2005.01.034.

King SR, Boyd L, Zimmermann W, Kendall BE. *Equus ferus* [Internet]. IUCN; 2015 [cited 2021 Sep 30]. Available from: https://dx.doi.org/10.2305/IUCN.UK.2015-2.RLTS.T41763A45172856.en.

King SRB, Asa C, Pluhacek J, Houpt KA, Ransom JI. Behaviour of horses, zebras, and asses. In: Ransom JI, and Kaczensky P, editors. Wild Equids: ecology, management, and conservation. John Hopkins University Press. Baltimore, Maryland, USA; 2016. p. 23–40.

Knight J. If they could talk to the animals. Nature. 200;414:246–247. https://doi.org/10.1038/35104708.

Klimov VV. Spatial-ethological organization of the herd of Przewalski horses (*Equus przewalskii*) in Askania-Nova. Appl. Anim. Behav. Sci.. 1988;21:99-115.

Kuntz R, Kubalek C, Ruf T, Tataruch F, Arnold W. Seasonal adjustment of energy budget in a large wild mammal, the Przewalski horse (*Equus ferus przewalskii*) I. Energy intake. J. Exp. Biol. 2006;209:4557-65. https://doi.org/10.1242/jeb.02536.

Lehner PN. Handbook of ethological methods. 2nd ed. Cambridge, UK: Cambridge University Press, Inc.; 1996.

Lehtonen J, Jaatinen K. Safety in numbers: the dilution effect and other drivers of group life in the face of danger. Behavioral Ecology and Sociobiology. 2016 Apr;70(4):449-58. http://www.jstor.org/stable/44857058.

Letty J, Marchandeau S, Clobert J, Aubineau J. Improving translocation success: an experimental study of anti-stress treatment and release method for wild rabbits. Anim. Conserv. 2000;3:211–219. https://doi.org/10.1111/j.1469-1795.2000.tb00105.x.

Leonardi M, Boschin F, Giampoudakis K, Beyer RM, Krapp M, Bendrey R, Sommer R, Boscato P, Manica A, Nogues-Bravo D, Orlando L. Late Quaternary horses in Eurasia in the face of climate and vegetation change. Science advances. 2018;4:55-89. https://www.science.org/doi/10.1126/sciadv.aar5589.

Massaro M, Chick A, Kennedy ES, Whitsed R. Post-reintroduction distribution and habitat preferences of a spatially limited island bird species. Anim. Conserv. 2008;2:54-64. https://doi.org/10.1111/acv.12364.

Macaluso W, Williams CK, Terhune II TM. Testing northern bobwhite reintroduction techniques in the northern edge of their range. National Quail Symposium Proceedings. 2017;8:49.

Macdonald, David W. Animal behaviour and its role in carnivore conservation: examples of seven deadly threats. Anim. Behav. 2016;20:197-209. https://doi.org/10.1016/j.anbehav.2016.06.013.

Mateju J, Říčanová Š, Poláková S, Ambros M, Kala B, Matějů K, Kratochvíl L. Method of releasing and number of animals are determinants for the success of European ground squirrel (*Spermophilus citellus*) reintroductions. Eur. J. Wildl. Res. 2012;58:473-82. https://doi.org/10.1007/s10344-011-0597-8.

McDonnell SM, Haviland JC. Agonistic ethogram of the equid bachelor band. Applied Animal Behaviour Science. 1995;43:147-88.

McDonnell SM, Poulin A. Equid play ethogram. Applied Animal Behaviour Science. 2002;78:263-90. https://doi.org/10.1016/S0168-1591(02)00112-0.

Milinski, M. Experiments on the selection by predators against spatial oddity of their prey. Zeitschrift für Tierpsychologie. 1977;43:311-325.

Miller B, Ralls K, Reading RP, Scott JM, Estes J. Biological and technical considerations of carnivore translocation: a review. Anim. Conserv. 1999;2:59–68.

Moehrenschlager A, Macdonald DW. Movement and survival parameters of translocated and resident swift foxes *Vulpes velox*. Anim. Conserv.2003;6:199–206. https://doi.org/10.1017/S1367943003251.

National Statistic Office of Mongolia. 1212. [Internet]. 1212; 2021 [cited 2021 Oct 20]. Available from: https://www.1212.mn/default.aspx?fbclid=IwAR0BEXeCvXQhyRjP_kUAmDcNmEHTl40knH-nMilphtaOsOvy2UZLDZAr7x8//

Neill S, Cullen JM. Experiments on whether schooling by their prey affects the hunting behaviour of cephalopods and fish predators. J. Zool. 1974;172:549-69.

Nowzari H, Hemami M, Karami M, Kheirkhah Zarkesh MM, Riazi B, Rubenstein DI. Habitat use by the Persian onager, *Equus hemionus onager* (Perissodactyla: Equidae) in Qatrouyeh National Park, Fars, Iran. J. Nat. Hist. 2013;47:795-814. https://doi.org/10.1080/00222933.2013.802040.

Paklina N, Pozdnyakova M. Why the Przewalski horses of Mongolia died out. Przewalski Horse. 1989;24:30-4.

Pratt RM, Putman RJ, Ekins JR, Edwards PJ. Use of habitat by free-ranging cattle and ponies in the New Forest, Southern England. J. Appl. Ecol. 1986;23, 539-557.

Pyritz LW, Fichtel C, Huchard E, Kappeler PM. Determinants and outcomes of decision-making, group coordination and social interactions during a foraging experiment in a wild primate. PloS one. 2013;8:53-54. https://doi.org/10.1371/journal.pone.0053144.

Robert N, Walzer C, Rüegg SR, Kaczensky P, Ganbaatar O, Stauffer C. Pathologic findings in reintroduced Przewalski's horses (*Equus caballus przewalskii*) in Southwestern Mongolia. J. Zoo Wildl. Med. 2005;36:273-85. https://doi.org/10.1638/03-035.1.

Rodriguez M, Cohen S. Social support. In: Friedman H, editor. Encyclopedia of mental health. Academic Press, New York; 1998. p. 537–644.

Ross RA, Giller PS. Observations on the activity patterns and social interactions of a captive group of blackcapped or brown capuchin monkeys (*Cebus apella*). Primates. 1988;3:307-17.

Rubenstein DI. Life history and social organisation in arid adapted ungulates. J. Arid Environ. 1989;17:145-56.

Rubenstein DI. The ecology of female social behaviour in horses, zebras and asses. In: Jarman P, Rossiter A, editors. Animal Societies: individuals, interactions, and organisation. Kyoto, Japan: Kyoto University Press.1994.p. 13–28.

Rubenstein DI, Cao QS, Chiu J. 2016 Equids and ecological niches: behavioural and life history variations on a common theme. In: Ransom JI, and Kaczensky P, editors. Wild Equids: ecology, management, and conservation. John Hopkins University Press. Baltimore, Maryland, USA; 2016. p. 56-68.

Schoenecker, K. A., King, S. R. B., Nordquist, M. K., Nandintsetseg, D., and Cao, Q., 2016. Habitat and diet of equids. Behaviour of horses, zebras, and asses. In: Ransom JI, and Kaczensky P, editors. Wild Equids: ecology, management, and conservation. John Hopkins University Press. Baltimore, Maryland, USA; 2016. p. 41-57.

Scheibe KM, Lange B, Lange V, Eichhorn E, Scheibe A, Streich J. Przewalskipferde in einem Semireservat–Verhaltensuntersuchungen zur Vorbereitung einer Auswilderung. Curr. Res. Appl. Ethol. 1997;376:120–131.

Scheibe KM, Streich WJ. Annual rhythm of body weight in Przewalski's horses (*Equus ferus przewalskii*) Biol. Rhythm Res. 2003;34:383–395. https://doi.org/10.1076/brhm.34.4.383.26227.

Seddon PJ, Armstrong DP, Maloney RF. Developing the science of reintroduction biology. Conserv. Biol. 2007;212:303-12. https://doi.org/10.1111/j.1523-1739.2006.00627.x.

Sigurjónsdóttir H, Van Dierendonck MC, Snorrason S, Thórhallsdóttir AG. Social relationships in a group of horses without a mature stallion. Behav. 2003;140:783–804. https://doi.org/10.1163/156853903322370670.

Slotta-Bachmayr LE, Boegel R, Kaczensky P, Stauffer C, Walzer C. Use of population viability analysis to identify management priorities and success in reintroducing Przewalski's horses to southwestern Mongolia. J. Wildl. Manag. 2004;684:790-8. https://doi.org/10.2193/0022-541X(2004)068[0790:UOPVAT]2.0.CO;2.

Snijders L, Blumstein DT, Stanley CR, Franks DW. Animal social network theory can help wildlife conservation. Trends Ecol. Evol. 2017;38:567-77. https://doi.org/10.1016/j.tree.2017.05.005.

Souris AC, Kaczensky P, Julliard R, Walzer C. Time budget-, behavioural synchrony- and body score development of a newly released Przewalski's horse group *Equus ferus przewalskii*, in the Great Gobi B Strictly Protected Area in SW Mongolia. Appl Anim Behav Sci. 2007;107:307-321. https://doi.org/10.1016/j.applanim.2006.09.023.

Stamps JA, Swaisgood RR. Someplace like home: experience, habitat selection and conservation biology. Appl. Anim. Behav. Sci. 2007;102:392-409. https://doi.org/10.1016/j.applanim.2006.05.038.

Swaisgood RR. The conservation-welfare nexus in reintroduction programmes: a role for sensory ecology. Anim. Welf. 2010;19:125-37.

Tao D. The Fossils of the Przewalski’s horse and the climatic variations of the Late Pleistocene in China. Papers in honour of Vera Eisenmann (Proceedings of the 9th ICAZ Conference, Durham) 2006 (Vol. 240). Oxford, UK: Oxbow Books.

Taraborelli P, Moreno P. Comparing composition of social groups, mating system and social behaviour in two populations of *Micvocavia australis*. Mamm. Biol. 2009;74:15-24. https://doi.org/10.1016/j.mambio.2008.08.002.

Teixeira CP, De Azevedo CS, Mendl M, Cipreste CF, Young RJ. Revisiting translocation and reintroduction programmes: the importance of considering stress. Anim Beh. 2007;73(1):1-3. https://doi.org/10.1016/j.anbehav.2006.06.002.

Truett JC, Dullum JA, Matchett MR, Owens E, Seery D. Translocating prairie dogs: a review. Wildl. Soc. Bull. 2001;863-72.

Viola K, Katalin O, István S, Zsolt V, Csilla C, Bettina N, Timea S, Lajos S, Zoltán B. Analysis of habitat use, activity, and body condition scores of Przewalski's horses in Hortobagy National Park, Hungary. Nat. Conserv. Res. 2019;4:1-2. https://doi.org/10.1016/j.gecco.2020.e01407.

Wakefield S, Knowles J, Zimmermann W, Van Dierendonck M. 2002 Status and action plan for the Przewalski's horse (*Equus ferus przewalskii*). In Moehlman PD, editors. Equids, zebras, asses and horses status survey and conservation action plan. Cambridge, UK; 2002. p. 82-92.

Wallach AD, Inbar M, Scantlebury M, Speakman JR, Shanas U. Water requirements as a bottleneck in the reintroduction of European roe deer to the southern edge of its range. Can. J. Zool. 2007;85:1182-92. https://doi.org/10.1139/Z07-098.

Wanless RM, Cunningham J, Hockey PA, Wanless J, White RW, Wiseman R. The success of a soft-release reintroduction of the flightless Aldabra rail (*Dryolimnas cuvieri aldabranus*) on Aldabra Atoll, Seychelles. Biol. Conserv. 2002;107:203–10. https://doi.org/10.1016/S0006-3207(02)00067-8.

Weladji RB, Holand Ø, Almøy T. Use of climatic data to assess the effect of insect harassment on the autumn weight of reindeer (*Rangifer tarandus*) calves. J Zool. 2003; 260:79–85. https://doi.org/10.1017/S0952836903003510.

Whitehead H. Analysing animal societies: quantitative methods for vertebrate social analysis. Chicago, USA: The Unviersity of Chicago Press., Inc; 2008.

Whitehead, H., 2009. SOCPROG programs: analysing animal social structures. Behav. Ecol. Sociobiol. 2009;63:765–78. https://doi.org/10.1007/s00265-008-0697-y.

Whitehead H. SOCPROG: Programs for analysing social structure. Nova Scotia, Canada: Dalhousie University, Inc.;2015.

Wolf CM, Garland Jr T, Griffith B. Predictors of avian and mammalian translocation success: reanalysis with phylogenetically independent contrasts. Biol. Conserv. 1998;86:243-55.

Wolter R, Pantel N, Stefanski V, Moestl E, Krueger K. The role of an alpha animal in changing environmental conditions. Physiol. Behav. 2014;133:236-43. https://doi.org/10.1016/j.physbeh.2014.05.025.

Woodfine T. The Eelmoor Marsh Przewalski's horse Project: The Complementary Management of an Endangered Equid and a British Nature Reserve [Doctoral dissertation]. [Lexington, Kentucky]: University of Kentucky, Kentucky; 2003.

Young JK, González-Suárez M, Gerber LR. Determinants of agonistic interactions in California sea lions. Behaviour. 2008;797-810.

Zhang Y, Cao QS, Rubenstein DI, Zang S, Songer M, Leimgruber P, Chu H, Cao J, Li K, Hu D. Water use patterns of sympatric Przewalski's horse and khulan: interspecific comparison reveals niche differences. PloS one. 2015;10:7. https://doi.org/10.1371/journal.pone.0132094.

Zeitler-Feicht M. Handbuch Pferdeverhalten. Stuttgart, Germany: Eugen Ulmer, Inc.; 2008.

CHAPTER 5

Habitat use differs in released and wild-born Przewalski's Horses of the Great Gobi B Strictly Protected Area (Mongolia)

Wild-born Przewalski's horses in the Great Gobi B Strictly Protected Area, Mongolia.
(Photo by Anna Bernátková)

Bernátková A, Arenas-Castro S, Oyunsaikhan G, Komárková M, Sillero N, Šimek J, Ceacero F. Habitat use differs in released and wild-born Przewalski's Horses of the Great Gobi B Strictly Protected Area (Mongolia), under review.

5.1. Abstract

The Przewalski’s horse (Extinct in the Wild in 1996) is currently listed as Endangered. First individuals were reintroduced to the Great Gobi B Strictly Protected Area (GGBSPA), Mongolia, in 1997. We observed selected horse groups in the GGBSPA between intra-annual (2019) selected periods and used ecological niche models (ENMs) to: 1) model habitat preferences for ‘feeding’ and ‘resting’ with a binomial logistic regression; 2) identify the influence of origin (Wild-born vs Reintroduced); and 3) describe the potential influence of human presence on the habitat selected by the horses for these behaviours. We used three types of satellite-derived predictors: i) topography (ALOS); ii) vegetation indexes (Landsat); and iii) land cover (Copernicus). We assessed the spatial similarity between Reintroduced vs. Wild-born models with pairwise comparisons of the two response variables (‘feeding’ and ‘resting’). We found significant differences between the horses’ origin in habitat preferences. Predictors showed opposite signals for Wild-born and Reintroduced horses’ ‘feeding’ behaviour (positive and negative, respectively). For the successful reintroduction of Przewalski's horses, habitat suitability, anthropogenic pressure, and reintroduced group size should be considered key factors. High spatial resolution remote sensing data provide robust habitat predictors for ‘feeding’ and ‘resting’ areas selected by Przewalski's horses.

Keywords: Gobi; habitat use; Mongolia; Przewalski’s horse; reintroduction; satellite remote sensing

5.2. Introduction

Habitat use is a significant feature of every animal species. It helps researchers to identify how essential factors such as feed and water sources, habitat conditions, and environmental factors are linked to behaviour, social structure and interspecific and intraspecific interactions (Bahloul et al., 2001; Moorhouse et al., 2009; Tweed et al., 2003). Habitat use is connected to feeding as to survive, thrive, and reproduce, or in other words, to increase their fitness, free-living ungulates have to make foraging decisions on a routine basis. The major reason for the necessity of these foraging choices are differences between qualitative and quantitative dietary demands and the properties of the available food. The foraging strategy, i.e. how the conflict between the need for food and the internal and external constraints is resolved, results in habitat use (Illius & Gordon, 1993). Habitat use of animals is affected by trade-offs between specific behaviours which require different types of areas and therefore result in different habitat selections. One of the conflicts might be observed in habitats selected for feeding and resting, i.e. foraging typically occurs in regions with abundant food but a poor protective cover and resting typically occurs in areas with a good protective cover but low forage availability (Mysterud & Ims, 1998). Moreover, animal behaviour is commonly affected not only by environmental and social factors but also by human activities. The close presence of humans and livestock might result in changes in the daily budget, habitat use and selection of feeding patches in many wild animal species, especially herbivores (Kuemmerle et al., 2010; Łopucki et al., 2017).

The Przewalski's horse (*Equus ferus przewalskii*) was listed as Extinct in the Wild (EW) in 1996, with the last individual seen in 1969 (King et al., 2015). The reintroduction efforts for the species started in Mongolia in 1992 and in 1997, the first individuals were released from acclimatization pens into the wild in the GGBSPA, Mongolia. Since then, a total of 93 horses were released till 2007 and 39 horses within the next period of transports (2011-2019) after the population crash in winter 2009/2010 (ZIMS for Studbook for Przewalski's horse & Šimek J, 2022). Currently, there is a mixture of reintroduced and wild-born individuals (International Takhi Group, 2021). Habitat use of the recently reintroduced horses is possibly influenced by the need to adapt to novel conditions, as identified by Scheibe et al. (Scheibe et al., 1996), who detected an adaptation period of one year after reintroduction: just after the first year, the horses started to show a similar activity budget as the wild individuals and groups. It has already been described that reintroduced animals may use habitats inappropriately or even prefer low-quality habitats, resulting in lower survival and breeding success rates (Battin, 2004; Rantanen et al., 2010). At GGBSPA, Przewalski’s horses are confronted with two main challenges: very harsh environmental conditions and the need to coexist with nomadic and resident herders and their livestock (Kaczensky et al., 2017), including feral horses, which are (as all equids) commonly found in more mesic areas with abundant food and water sources (Rubenstein, 2011). Because of that, the last remaining wild horses were likely forced to reside in more desertic areas, on the outskirts of their historic range distribution. Although the last population managed to survive in such conditions, there is little evidence of how well they performed in such demanding habitats (Wakefield et al., 2002). Horses usually prefer wide grasslands, but the use of specific places for feeding and resting varies throughout the year and is dependent on vegetation resources (Duncan et al., 1990; Kerekes et al., 2019; Zhang et al., 2015). During the vegetative period, horses prefer locations with high green phytomass density (Duncan, 1983).

As described by Kaczensky et al. (Kaczensky et al., 2017), the food composition of Przewalski's horses before the species went extinct was different to the composition of the current reintroduced individuals. Diet transited from being a mixed feeder in the winter and a grazer in the summer in the past to being a year-round grazer presently. Kaczensky et al. (Kaczensky et al., 2017) suggest that the transition might be caused by the positive attitude of the current human inhabitants of the Gobi that allows Przewalski's horses to share the limited, grass-dominated pastures with herds of domestic animals. In contrast, the ancestors of today´s Przewalski's horses were often forced into less productive areas dominated by browse. This highlights the importance of the positive relationship of locals towards Przewalski's horses and the danger of being threatened by competition with livestock and human activities. However, the reintroduction process may be difficult for the new animals not only because of potential conflict with herders (Kaczensky et al., 2007) but also because of the possible failure in adaptation and competition with the well-adapted wild-born animals (Bernátková et al., 2022; Rantanen et al., 2010).

Ecological niche models (ENMs) (Sillero, 2011), also called Species distribution models (SDMs), are effective tools in conservation management for the identification of the most suitable environmental conditions and habitat use of a given species over large geographic areas (Arenas-Castro et al., 2019; Guisan & Zimmermann, 2000; Hu & Jiang, 2010; Kuemmerle et al., 2011; Morueta-Holme et al., 2010; Sillero et al., 2021). Therefore, in this study, we used ENMs to 1) model habitat preferences of Przewalski's horses for ‘feeding’ and ‘resting’ activities; 2) identify the potential influence of the horses’ origin (wild-born vs reintroduced harems); and 3) describe the potential influence of human and livestock presence on the habitat selected by the horses. We hypothesised that the wild-born horses would select more suitable habitats for these major activities than recently reintroduced horses, which should require a sufficient adaptation period to make an effective habitat selection. We also expected a lower tendency to avoid conflicts with humans and livestock in the reintroduced individuals.

5.3. Materials and Methods

5.3.1. Study area

The Great Gobi B Strictly Protected Area (GGBSPA; Figure 5.1) was established in 1975, and it is a part of the Great Gobi Biosphere Reserve, Mongolia. It is situated between the Altai Mountains and borders with China. In 2019, the GGBSPA was extended and currently encompasses ≈18 000 km^2 of desert steppe and semi-desert habitat (≈9 000 km^2 before the enlargement, (International Takhi Group, 2021; Kaczensky, Enkhsaikhan, et al., 2008). This protected area in SW Mongolia is a reintroduction site for Przewalski's horse and an important refuge for several other endangered species (Kaczensky, Enkhsaikhan, et al., 2008; Kaczensky, Oyunsaikhan, et al., 2008). Despite its protected area status, the GGBSPA is used by herders. In 2018 it was used by about 130 families with close to 70 000 heads of livestock (3 070 domestic horses). Since the area was enlarged (2019), herders and livestock numbers have also increased. In 2021 the GGBSPA signed a cooperation contract with 228 herder families, that own 122 897 livestock heads: 11 829 big livestock (horses, cows and camels) and 111 068 small livestock (goats and sheep; GGBSPA administration, personal communication). Herders and their livestock are mainly present in the GGBSPA in winter and during the spring and fall migration as they move to their summer camps in different areas for the summer season (National Statistic Office of Mongolia, 2021). The climate of the GGBSPA is continental and very dry. The altitude ranges from 1100 to 2900 metres above the mean sea level (m a. s. l.). The temperature differs significantly during the day and night and between the seasons (varying from − 40 to 40 degrees Celsius), and the annual mean temperatures are below zero (International Takhi Group, 2021; Kaczensky et al., 2011). The amount of precipitation is within the typical range of a semi-desert climate (around 150 mm per year). Most of the precipitation falls in the summer rains (International Takhi Group, 2021). Main water bodies are unevenly distributed, with almost no water in the central or western section of the area (Kaczensky, Enkhsaikhan, et al., 2008). Plants have to cope with water stress and a high-temperature gradient. Thus, the species living in this area need a high degree of mobility to be able to find suitable habitats depending on the environmental conditions (International Takhi Group, 2021). The area is not enclosed by fences, except for the border with China, which is fenced in certain areas (Kaczensky, Enkhsaikhan, et al., 2008).

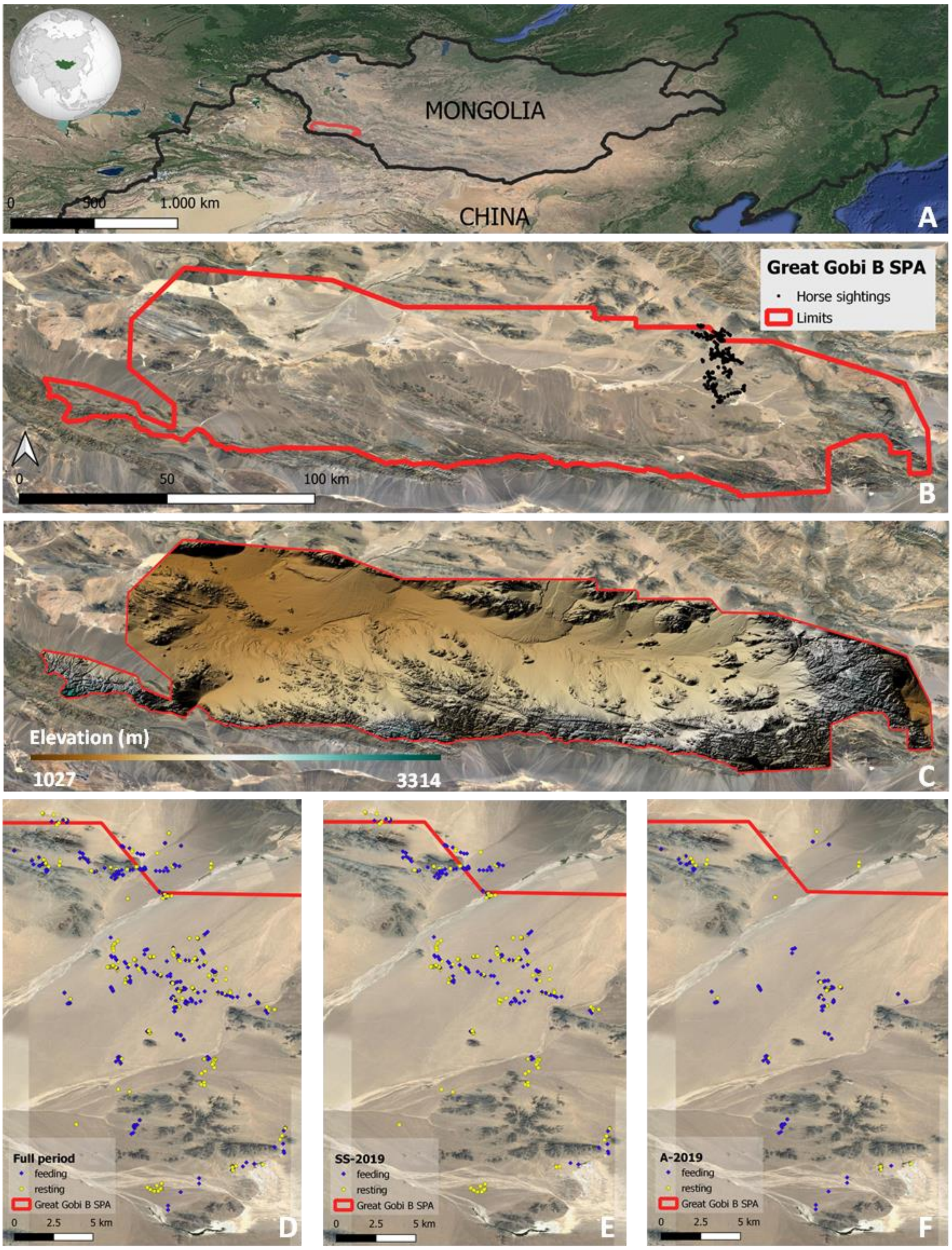

Figure 5.1. Study area in the context of Mongolia country (A); full horses' sightings and the limits of the protected area (B); the elevation gradient in the study area (C); horses' sightings separated by behavioural types (feeding vs. resting) for the Full period (SS-2019 and A-2019; D), Spring/Summer 2019 (SS-2019; E) and Autumn 2019 (A-2019; F). Geographical coordinate system of maps is WGS 84.

5.3.2. Data collection

Behavioural patterns of horse harems

We conducted the data collection (behavioural data, GPS data) in two different seasons, Spring/Summer 2019 (SS-2019) and Autumn 2019 (A-2019) in the groups described in Table 5.1. The period “Full” consisted of both SS-2019, and A-2019 datasets merged. We classified the studied harems as Reintroduced (transported from Europe and released after a one-year acclimatization period in an enclosure in GGBSPA) and Wild-born (born in the wild). We localised the harems with binoculars during daily monitoring routines. All Przewalski´s horses in the GGBSPA are recognised by rangers' observation of physical characters so we could distinguish each of the selected harems and each individual of our study. Once one of the target harems was localised, we filmed the harem from a close distance (from 150m to 800m) with a 4K Panasonic VX1 video camera with a tripod. During the video recordings, we recorded the position of the observation point with a GPS receiver in 10-minute intervals. At the same time, we measured the distance and the angle between the observation point and the harem with a rangefinder (Nikon MONARCH 2000). This way, the exact position of the horses could be calculated later.

Table 5.1. Description of the studied groups.

Group	Season/Year	Observation hours	Group experience*	Individuals	Number of breeding mares	Stability index**	Age of foals (d)***
Azaa	SS-2019	30	Wild-born	1 dominant ♂, 8 ♀, 2 ♂, 3 foals	5	0.83	26
Hustai1	SS-2019	25.5	Reintroduced	1 dominant ♂, 4 ♀	4	0.00	-
Tsetsen	SS-2019	31.5	Wild-born	1 dominant ♂, 5 ♀, 3 ♂, 3 foals	4	0.75	28
Azaa	A-2019	22.5	Wild-born	1 dominant ♂, 6 ♀, 2 ♂, 5 foals	5	1.00	10
Tanan	A-2019	21	Reintroduced	1 dominant ♂, 4 ♀	4	0.00	-
Tsetsen	A-2019	25	Wild-born	1 dominant ♂, 5 ♀, 3 ♂, 3 foals	4	1.00	128

We only used animals older than one year (inclusive of) in our research. Foals are shown in the table to illustrate the general information on the studied groups.

*Group experience: Reintroduced (after one year acclimatization period, released); Wild-born (born in the wild). One mare in the Wild-born Azaa harem was born in captivity in Europe and reintroduced to the GGBSPA in 2004. The stallions in Hustai1 and Tanan Reintroduced harems were wild-born; all mares from these harems were born in captivity.

** Stability index was counted as the inter-yearly changes in the number of breeding mares (in %; the number of mares present in the herd during the previous and the observation year, divided by the number of mares present in one or another year.

***Average age of foals (in days) at the end of each selected observation.

Despite the selection of various groups of different origins, the position of the horses was relatively concentrated, considering the extensive size of the GGBSPA (Figure 5.1). We were able to observe only the four selected groups as other groups in the area are very suspicious of human presence. Nevertheless, most of the groups of Przewalski's horses in GGBSPA move exclusively around this area, which is the main release site in the NE part of the protected area (a smaller part of the GGBSPA horses' population ranges around the secondary release site in the North-western part; (Michler LM et al., 2021). It is typical for the reintroduced Przewalski's horses to keep close to water sources and have very conservative movement habits (31). In any manner, the presented points distribution represents the whole study area features.

We used the Behavioural Observation Research Interactive Software (BORIS, v. 8.5) for event logging, video coding and observation of the recorded videos. For the categorisation of the behaviours, we established an ethogram during the initial data collection (Bernátková et al., 2022) and complemented it with other published material for horses (McDonnell & Haviland, 1995; McDonnell & Poulin, 2002). We reduced the ethogram to two main categories: 'feeding' and 'resting' (including standing and laying down). The category 'other' included any other behaviour in the complete ethogram to accurately calculate the percentage of the previous categories, but we did not use it in further analyses. We divided BORIS data into 10-minute intervals finishing at the same time when the position of the harems was recorded. This way, we could match the behaviour at each location. We calculated the time dedicated to each behavioural category in every 10-minute interval for each individual, then summarised for each group, and finally calculated a percentage representation of each behavioural category for each interval. We classified each observation as 'resting' or 'feeding' when the behavioural category had the highest percentage of occurrence, and the difference between the 'resting' and 'feeding' percentages was greater than 10%. When this difference was lower, or the category 'other' was the most common, we excluded the observations from the analyses since these could not be clearly assigned as 'resting' or 'feeding' observations (238 observations excluded out of 1253 records).

Environmental predictor variables

To know which variables could better represent the main ecosystem dimensions of the study area, we selected the predictors with several criteria such as expert knowledge, previous literature on the ecology of the target species, and the construction of preliminary statistical models or exploratory analysis (e.g., testing the associations between predictors; see below). Considering the absence of other types of environmental data at a higher spatial resolution, we exclusively used satellite-derived remote sensing predictors of the high spatial resolution (~ 100 m) to represent the main dimensions of the ecosystem geomorphology and dynamics in the study area. We selected products from several sensors onboard different satellites categorized into three groups: i) topography (ALOS topographic attributes); ii) vegetation indexes (Landsat vegetation index); and iii) land cover (Copernicus) (Table 5.2). Considering both topography and land cover datasets as static predictors in time, we calculated the mean of the maximum Normalized Difference Vegetation Index (NDVI) for the three periods, SS-2019, A-2019 and Full (the combination of the previous two).

Table 5.2. Summary of the satellite-derived predictor variables.

Type	Mission	Product name	Code	Pixel size	Description	Rationale
Topography	Advanced Land Observing Satellite (ALOS)	ALOS Digital Surface Model	ALOSdem	30m	ALOS World 3D is a global digital surface model.	Height above sea level (in meters).
		ALOS Continuous Heat-Insolation Load Index	ALOSchi	90m	As a surrogate for effects of insolation and topographic shading on evapotranspiration.	Ranging from 0 (very cool) to 255 (very warm).
		ALOS Multi-Scale Topographic Position Index	ALOSmtpi		The ALOS-derived mTPI is calculated using elevation data for each location subtracted by the mean elevation within a neighbourhood.	Distinguishes ridge (positive) from valley (negative) forms.
		ALOS Landforms	ALOSldfs		The ALOS Landform dataset provides landform classes created by combining the ALOS CHILI and the ALOS mTPI datasets.	Classes from peak/ridge (warm) to valley (narrow).
Vegetation Indices	Landsat 8	Maximum of the Normalized Difference Vegetation Index	NDVImx	30m	The Normalized Difference Vegetation Index is generated from the Near-IR and Red bands of each scene as (NIR - Red) / (NIR + Red).	As a proxy for vegetation cover quality, productivity and status. It ranges in value from -1.0 to 1.0, where values around 0.1 or less represent areas of barren rock, sand, or snow, approximately 0.2 to 0.5 are for sparse vegetation (e.g., shrubs, grasslands or senescing crops), and approximately 0.6 to 0.9 correspond to dense vegetation or crops at the peak growth stage.

Land cover	Copernicus	Land cover discrete classification	LCdcl	100m	The Copernicus Global Land Service - Dynamic Land Cover (CGLS-LC100) product provides a primary land cover scheme. Next to these discrete classes, the product also includes continuous field layers for all basic land cover classes that provide proportional estimates for vegetation/ground cover for the land cover types.	It ranges from 0 (Unknown. No or not enough satellite data available) to 200 (oceans, seas; can be either fresh or salt-water bodies).
		Land cover discrete classification - proba	LCdclpro		Quality indicator (classification probability) of the discrete classification.	Pixel value ranges from 0 to 100 (%).

We computed all native and derived satellite predictors at their original spatial resolutions and aggregated them to target 100 m grid cells using the cloud-based Google Earth Engine (GEE) computational platform (https://earthengine.google.com; Gorelick et al., 2017). We measured multicollinearity effects with the pairwise Pearson's correlation coefficient $< |0.75|$ (Figures SM5.1-SM5.3) and a variance inflation factor (VIF) < 4 (Figures SM5.4). We finally included seven independent predictors in our models (Figures SM5.5-SM5.9: ALOS-derived Digital Elevation Model (ALOSdem), Continuous Heat-Insolation Load Index (ALOSchi), Topographic Position Index (ALOSmtpi) and Landforms (ALOSldfs), Landsat-derived Normalized Difference Vegetation Index (NDVI), and Copernicus-derived Land Cover Discrete Classification (LCdcl) and its probability (LCdclpro) (Table 5.2).

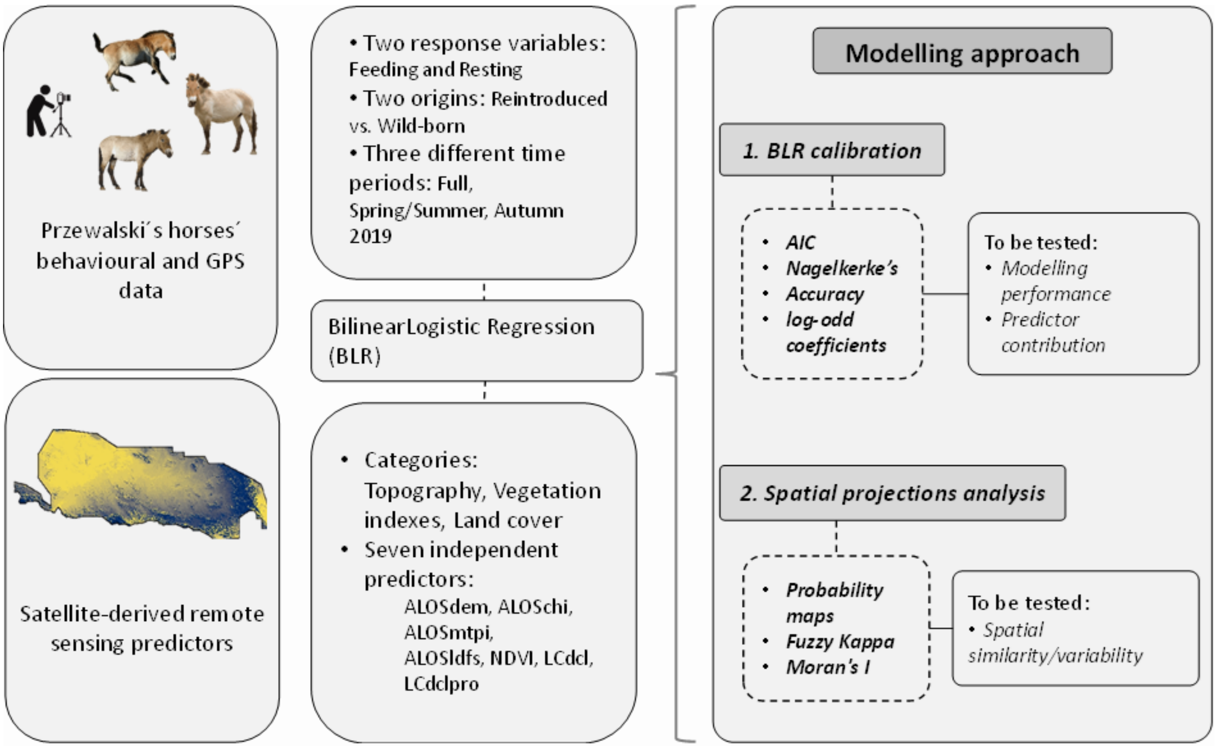

Figure 5.2. Workflow of the modelling approach.

Modelling approach

To identify the environmental factors that are associated with the behaviour ('feeding'/'resting') within the groups of Reintroduced and Wild-born horses in the study area by each period, we used the binomial logistic regression technique (BLR) with the function ‘glm’ of the R package ‘stats’ version 3.6.2 (Figure 5.2). A BLR predicts the probability that an observation falls into one of two categories of a dichotomous dependent variable (‘feeding’/‘resting’) based on independent variables (predictors) that can be either continuous or categorical. Thus, we modelled the bivariate response variable as a vector of two names (‘feeding’/‘resting’) and the (log-)odds of the outcomes as a linear combination of the predictor variables. Then, the BLR predicted the level of horses’ behaviour as a function of the three groups of predictors, topography, land cover and vegetation features.

We randomly split our data set into a training set and testing set with the command ‘sample.split’ of the ‘caTools’ R package and a split ratio of 0.75. Thus, we selected 75% of the data as the training set to build the models and the remaining 25% as the testing set to test the models. We used different evaluation metrics to examine the model performance: i) the Akaike Information Criterion (AIC) to rank the models based on a trade-off between their goodness-of-fit and complexity (Burnham KP & Anderson DR, 2002); ii) the Nagelkerke's pseudo-R^2 to evaluate the goodness-of-fit of the logistic regression models, and iii) the Accuracy percentage from the confusion matrix that measures how accurate a model is in predicting outcomes. The Accuracy is defined as TP+TN/Total, where TP = true positives, TN = true negatives, and Total = the sum of all cases (true and false). For comparison, we used the AIC from a baseline ‘null model’ containing a single intercept term. We computed all AIC, Nagelkerke's pseudo-R2 and Accuracy metrics from the results of the ‘glm’ function from the ‘stats‘ R package.

To measure the association between independent variables and dependent variables, we estimated the odds ratios from the BLR models by obtaining the antilog with the ‘exp’ function applied to the model coefficients. Thus, if X increases by one unit, the odds of Y increase by k unit, given the other variables in the model are held constant. In other words, the coefficient of a variable that is attained by the BLR function refers to the increase in relative odds of an event happening against the base event with a unit increase in the chosen variable. Although the odds ratio is expressed as a single number, it has an implicit reference to 1. An odds ratio of 1 means that both events have the same probability. Thus, an odds ratio greater than 1 means that the successful event is more likely than the failure event and the reverse is less than 1. To avoid the effect of predictors expressed in different measurement units, we standardized the variables for each period (Full, SS-2019 and A-

2019) before running the models. Once we calculated the BLR models, we used the ‘predict’ function with the option “response” in the ‘stats’ R package to spatially predict the model results in terms of probability based on the input data.

To examine the contribution of each environmental predictor, we calculated the relative variable importance by using the ‘h2o.varimp’ function from the ‘h2o’ R package. We compared the relative importance of predictors across the two sets of models (Reintroduced vs. Wild-born) for each period. To facilitate ecological interpretability of the effect of the most relevant predictors, we provided the response curve (maximum sensitivity range and sense of the slope) of each predictor for each model combination.

Finally, we assessed the similarity and the spatial agreement between Reintroduced vs. Wild-born models based on the two response variables (‘feeding’ and ‘resting’) by comparing their spatial projections for each period and between them. Firstly, we analyzed the similarity between spatial projections by using the improved fuzzy Kappa algorithm implemented in the Map Comparison Kit software version 3.2.3 (Visser & de Nijs, 2006); http://mck.riks.nl/) for categorical maps. The fuzzy Kappa algorithm expresses the mean agreement between two maps, compared to the expected agreement from randomly relocating all grid squares in both maps, ranging between -1 (totally different maps) and 1 (identical maps). It considers the fuzziness of the location for near ‘grid square-by-grid square’ agreement while also accounting for autocorrelation in the changes amongst maps (Hagen-Zanker, 2009; Visser & de Nijs, 2006). Secondly, we computed the agreement between continuous prediction maps by pairwise comparisons between spatial projections based on Spearman's correlation coefficient.

We performed all analyses and mapping in the R software version 4.2.0 and QGIS 3.28.0 'Firenze'.

5.4. Results

5.4.1. Data analysis

The frequencies of occurrences of the two response variables (‘feeding’ and ‘resting’) within the origin groups (Reintroduced vs. Wild-born) and the three different time periods (Full, Spring/Summer 2019 – SS-2019, and Autumn 2019 – A-2019) were well balanced (46,87% ‘feeding’, 53,13% ‘resting’; Table SM5.1).

Range values (minimum and maximum) of the predictors per time period (Full, SS-2019 and A-2019) are shown in Table SM5.2. Only the NDVI values changed between periods; topography and landcover remained the same over time. (Table SM5.2). Spatial projections of the satellite-derived remote sensing predictor variables for the GGBSPA study area at 100 m squares for the Full period are shown in Figures SM5.8 and SM5.9.

5.4.2. Model outputs

Overall, models showed significant differences in AIC values between null models and BLR models and moderate-high performance (Table 5.3). For the A-2019 period, Reintroduced horses presented the most parsimonious model (AIC=122.82) with the greatest predictive ability (Nagelkerke's pseudo-R^2 =0.41). For this same period, models could also accurately identify Reintroduced and Wild-born horses ‘feeding’ against ‘resting’ in the study area, with test set accuracy equal to 72% and 63%, respectively. Models for the other two periods, the SS-2019 and the Full ones, showed good performances for Reintroduced and Wild-born horses from accuracies equal to 65% and 66%, and 66% and 54%, respectively (Table 5.3).

Table 5.3. Regression coefficients of standardized predictors and their confidence intervals (within brackets). Model performance based on the Akaike Information Criterion value (AIC), the Nagelkerke pseudo-R^2 and the Accuracy are also showed. For comparison, a baseline ‘null model’ containing a single intercept term was used (AIC in brackets). Significance values in AIC cells correspond to an ANOVA test between null models and logistic models. Feeding was considered as the base class for each model. RE = Reintroduced; WB = Wild-born; Spring/Summer 2019 (SS-2019); Autumn 2019 (A-2019).

Predictors	***Full***		***SS-2019***			***A-2019***
	RE	**WB**	**RE**	**WB**	**RE**	**WB**
ALOSchi	0.06	0.49*	0.19	-0.03	-0.03	0.41
	(-0.10 – 0.23)	(-0.03 – 1.02)	(-0.06 – 0.46)	(-0.84 – 0.77)	(-0.67 – 0.51)	(-0.51 – 1.37)
ALOSdem	-0.46**	-0.05	-1.15**	1.05***	0.89	-0.28
	(-0.88 – -0.05)	(-0.37 – 0.28)	(-2.20 – -0.14)	(0.45 – 1.68)	(-0.07 – 2.18)	(-0.92 – 0.35)
ALOSldfs	-0.23	0.07	-0.72***	0.19	0.24	0.15
	(-0.59 – 0.13)	(-0.14 – 0.28)	(-1.28 – -0.20)	(-0.12 – 0.51)	(-0.57 – 1.01)	(-0.2 – 0.51)
ALOSmtpi	0.28**	0.29	-0.11	0.75	0.95**	-0.03
	(0.04 – 0.53)	(-0.22 – 0.81)	(-0.48 – 0.24)	(-0.2 – 2.1)	(0.24 – 1.81)	(-1.36 – 1.33)
LCdcl	-0.71**	0.2	-0.83	-0.52*	0.3	0.23
	(-1.38 – -0.06)	(-0.05 – 0.46)	(-2.03 – 0.32)	(-1.08 – 0.01)	(-1.23 – 1.95)	(-0.17 – 0.63)
LCdclpro	0.44	-0.13	0.07	0.66**	0.27	-0.06
	(-0.13 – 1.02)	(-0.41 – 0.14)	(-0.89 – 1.04)	(0.12 – 1.23)	(-0.97 – 1.53)	(-0.67 – 0.57)
NDVImx	-0.15	0.08	-0.29	1.45***	0.44	-0.92**
	(-0.51 – 0.20)	(-0.09 – 0.27)	(-0.82 – 0.23)	(0.77 – 2.19)	(-0.36 – 1.29)	(-1.83 – -0.22)
Constant	0.62*	-0.21*	1.69***	-0.51	-1.39*	0.29*

	(-0.01 – 1.28)	(-0.47 – 0.04)	(0.58 – 2.89)	(-0.97 – 0.1)	(-3.4 – 0.23)	(-0.22 – 0.8)
AIC	347.62 (379.86)**	831.38 (866.28)***	203.35 (215.61)*	408.32 (461.53)**	122.82 (156.33)***	342.01 (369.22)***
pseudo-R^2	0.12	0.02	0.12	0.17	0.41	0.11
Accuracy (%)	66	54	65	66	72	63

Note: *p<0.1; **p<0.05; ***p<0.01

Considering the odds-ratio coefficients of the models (Figure 5.3), there was no predictor with a significant effect shared between the three periods. Still, there were differences between pairwise periods and horse types. For the Full and A-2019 periods, the probability of ‘feeding’ increased by 1.32 and 2.58 when increasing the ALOSmtpi variable, respectively, in Reintroduced horses (Figures 3A and 3E). The variable ALOSdem was another significant predictor shared by periods and horse types. While the probability of ‘feeding’ decreased by 0.63 and 0.32 when decreasing the ALOSdem variable for the Full and SS-2019 periods, respectively, in Reintroduced horses (Figure 5.3A and 3C), it increased by 2.87 for the Wild-born horses in the SS-2019 period (Figure 5.3D). The ALOSldfs variable also showed a significant effect on ‘feeding’ for the Reintroduced horses in SS-2019 period (Figure 5.3C), as the probability significantly decreased by 0.48 when decreasing the predictor values.

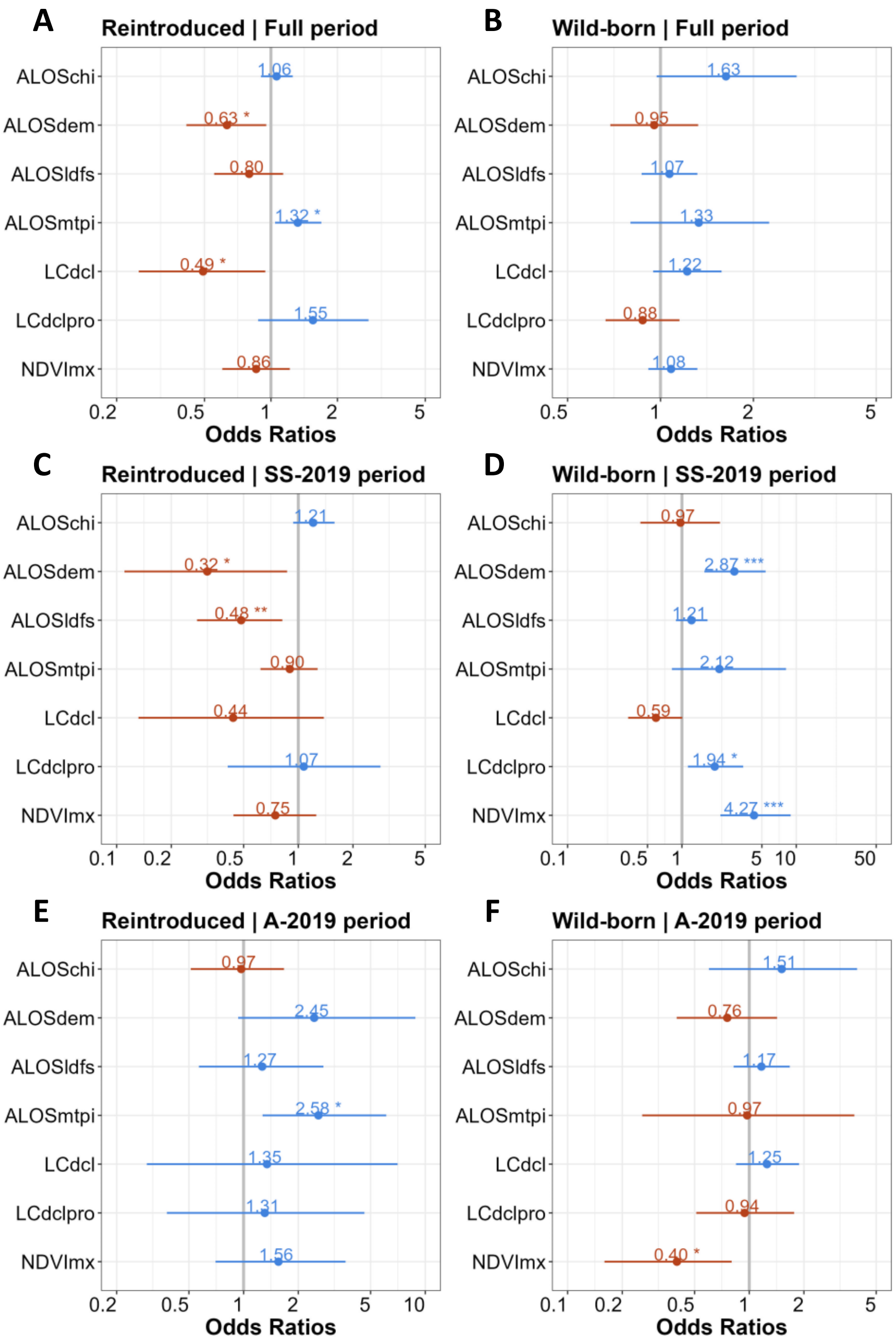

Figure 5.3. Comparison of Odds Ratios (after exponentiation of the log of the probability of horses presenting 'feeding' behaviour divided by the probability of not presenting it) considering the different environmental predictors for: A-B) the Full period (Spring/Summer and Autumn 2019); C-D) the Spring/Summer 2019 period (SS-2019); and E-F) the Autumn 2019 period (A-2019). Ratios tend to increase (blue colour) and decrease (red colour) with a unit increase/decrease in the response variable (feeding) values. The (*) means a significant value for p<0.05.

Regarding land cover, the two LCdcl and LCdclpro variables showed different effects on 'feeding' behaviour depending on the horse type and period. While the probability of 'feeding' decreased by 0.49 for the Reintroduced horses for the Full period (Figure 5.3A) when the LCdcl decreased, the probability increased by 1.94 for the Wild-born horses for the SS-2019 period (Figure 5.3D) as the LCdclpro values increased.

Regarding the NDVImx, this variable showed a significant and different effect on 'feeding' only for the Wild-born horses, increasing by 4.27 for the SS-2019 period (Figure 5.3D) as the predictor values increased from 0.1, and decreasing by 0.4 for the A-2019 period for NDVImx values above 0.05 (Figure 5.3F). Although not significant, we noted a relationship between the probability of 'feeding' and NDVImx by the Reintroduced horses, opposite to the results showed by the Wild-born horses for both SS-2019 (Figure 5.3C) and the A-2019 (Figure 5.3E) periods.

Predictor contribution and response curves

The importance of each predictor in the models, ranging between 0 (no importance) and 1 (high importance), is shown in Figures SM5.10-SM5.12. Overall, topographic (ALOSdem) and landform classes (LCdcl) were the most important predictors for most models regardless of the horses' origin. More specifically, the vegetation quality (NDVImx) was the most important predictor for the Wild-born horses for the SS-2019 and A-2019 periods. In contrast, for the Reintroduced horses the most important predictor was the Land Cover discrete classification (LCdcl) for both the Full and SS-2019 periods, and the ALOSmtpi for the A-2019 period.

According to the response curves, we found a relationship between the 'feeding' behaviour of horses and some environmental factors (Figure 5.4). Overall, the topographic predictors (represented mainly by ALOSdem and ALOSmtpi) determined the horses' behaviour. For the Reintroduced horses, the probability of 'feeding' decreased as the elevation increased (approx. 1600-1650 m) for the Full and SS-2019 periods (Figure 5.4A-C) but increased for the A-2019 (Figure 5.4E); while for the Wild-born horses, the higher the elevation, the higher the probability of 'feeding' for the SS-2019 period (Figure 5.4D). Regarding the ALOSmtpi, although both types of horses apparently move between similar values of this predictor, the probability of 'feeding' for the Reintroduced horses increased as a function of a larger range of values (from -10 to 20, that is, valleys and ridges) than the Wild-born horses, which are more conservative. The same is shown by the effect of the ALOSldfs variable on ‘feeding’ for the Reintroduced horses for the SS-2019 period, as the probability decreased while the landform became a lower slope or a valley (Figures 5.4C and SM5.9). It should be noted that, although not significant, there is a certain relationship between the probability of ‘feeding’ and temperate or warm areas shown by the Wild-born horses for both the Full and the A-2019 periods (Figures 5.4B and 5.4F and SM5.8 and SM5.9).

The other most significant variable was the NDVImx, but only for the Wild-born horses and with opposite effects. While the probability of ‘feeding’ increased as the NDVImx values ranged between 0.1-0.3 (sparse vegetation and grasslands) (Figure 5.4D and SM5.9) for the SS-2019 period, the probability decreased for values above 0.05 (areas of barren rock, sand, or snow) for the A-2019 period (Figure 5.4F and SM5.9).

The two LCdcl and LCdclpro variables also showed significant effects on ‘feeding’ behaviour depending on the horse type and period. Although not shown in Figure 5.4, while the probability of ‘feeding’ decreased for the Reintroduced horses for the Full period (Figure 5.3A) when the land cover classes moved to less productive areas, the probability increased for the Wild-born horses for the SS-2019 period (Figure 5.3D) to more productive areas with less than 10 % of shrub cover but higher herbaceous vegetation (Figures SM8 and SM9).

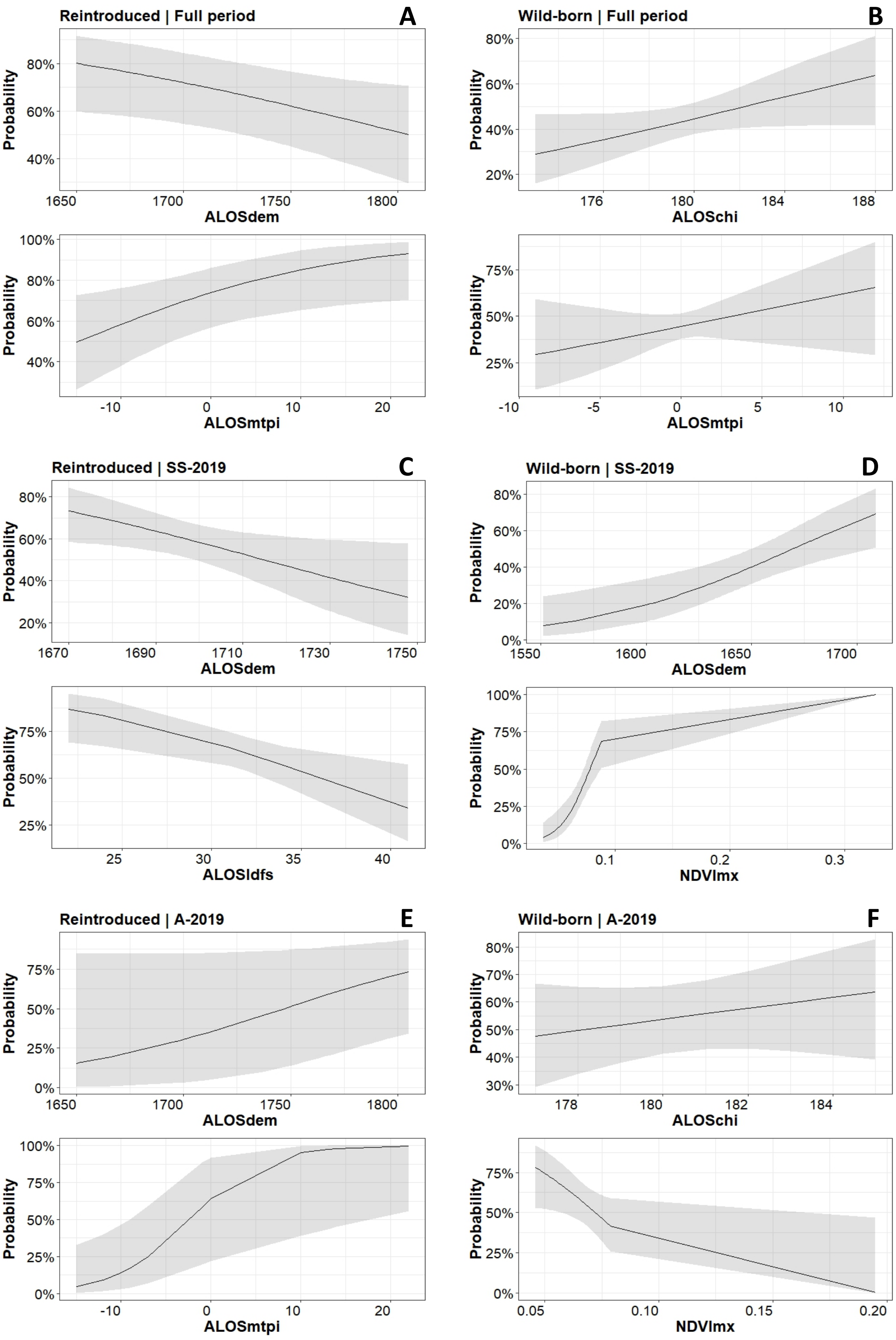

Figure 5.4. Response curves of predicted ‘feeding’ against the most important satellite-derived predictor variables for: A-B) the Full period (Spring/Summer and Autumn 2019); C-D) the Spring/Summer 2019 period (SS-2019); and E-F) the Autumn 2019 period (A-2019).

Spatial projections analysis

Both probability (Figures 5.5) and binary (Figure SM5.13) predictive maps between areas for ‘feeding’ and ‘resting’ behaviours of Reintroduced and Wild-born horses were not similar in all periods (Table 5.4). The greatest dissimilarities corresponded to the spatial projections of probability for the seasonal periods: SS-2019 (Fuzzy Kappa = -0.63 and Spearman coefficient = -0.94) (Figure 5.5B) and A-2019 periods (Fuzzy Kappa = -0.52 and Spearman coefficient = -0.82) (Figure 5.5C). In addition, maps showed low similarity when both seasonal time periods were compared for the Reintroduced (Fuzzy Kappa = -0.61 and Spearman coefficient = -0.93) and Wild-born type horses (Fuzzy Kappa = -0.68 and Spearman coefficient = -0.9) (Table 5.4). In terms of predicted area, the SS-2019 model predicted a more partially suitable area than A-2019 for Reintroduced horses (51.73%) (Figure SM5.14), while the A-2019 model prediction was higher for the Wild-born (40.05%) (Figure SM5.14). The lowest overlaid area (1.72%) between Reintroduced and Wild-born horses was shown for the A-2019 model. Wild-born horses used a larger area than the Reintroduced horses when comparing seasonal periods (Figures SM5.14).

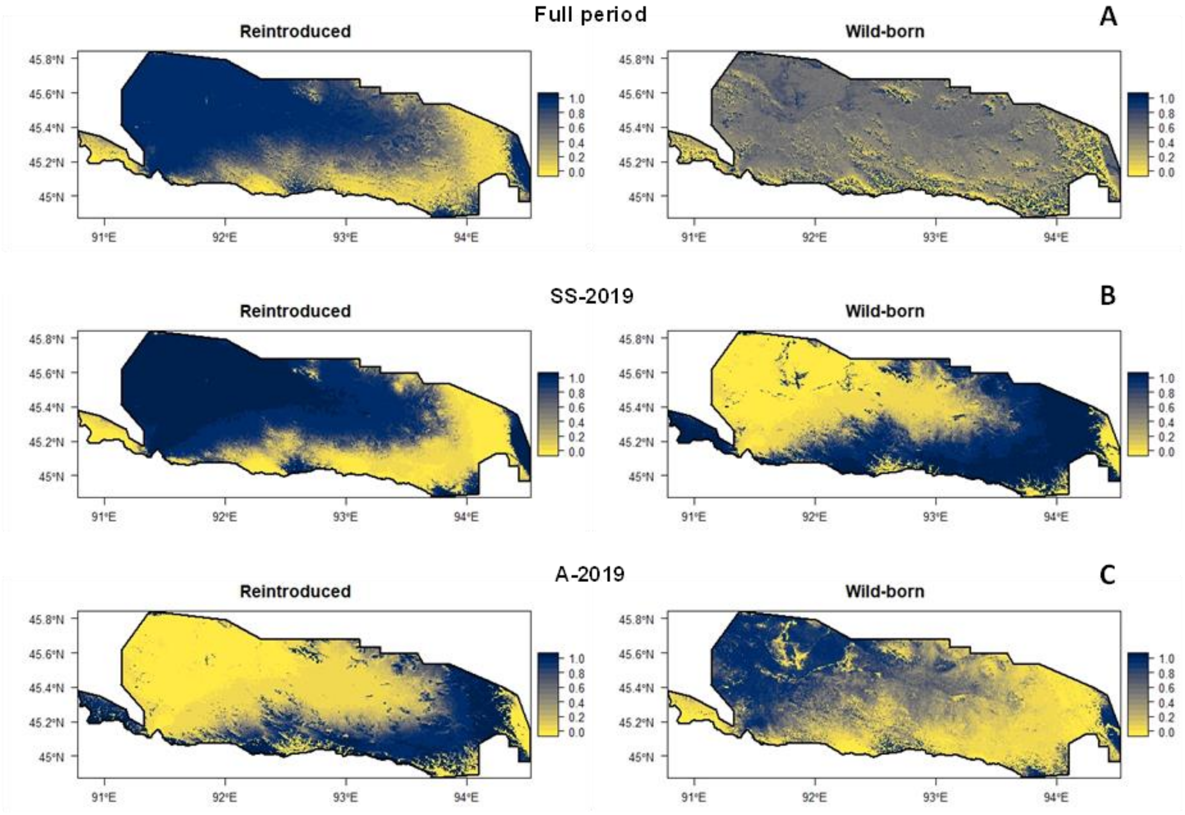

Figure 5.5. Probability map obtained by logistic regression analyses for Feeding (close to 1) and Resting (close to 0) for the GOBI study area at 100 m squares for: A) Full (Spring/Summer and Autumn 2019); B) SS-2019; and C) A-2019.

Table 5.4. Statistical comparison of spatial projections from Reintroduced and Wild-born (Origin) models based on the response variable (‘feeding’) within the different periods (Full, Spring/Summer 2019 (SS-2019) and Autumn 2019 (A-2019)) and between SS-2019 and A-2019. The proportion of the predicted feeding area is shown in brackets (1 pixel = 10000 m2 = 1 ha).

Period	**Origin**	**Fuzzy Kappa**	**Spearman's ρ**	**Area (ha)**			
				Partial	**Overlap**	**Nonoverlap**	**Total**
Full	Reintroduced	0.34	0.38	627892 (23.99%)	1160884 (44.36%)	570820 (21.81%)	2616868
	Wild-born			257272 (9.83%)			
SS-2019	Reintroduced	-0.63	-0.94	1353698 (51.73%)	415738 (15.88%)	16943 (0.64%)	
	Wild-born			830489 (31.73%)			
A-2019	Reintroduced	-0.52	-0.82	832237 (31.8%)	45135 (1.72%)	691291 (26.41%)	
	Wild-born			1048205 (40.05%)			
//	//	//		//	//	//	
SS-2019 vs. A-2019	Reintroduced	-0.61	-0.93	760071 (29.04%)	87361 (3.33%)	117301 (4.48%)	
				1652135 (63.13%)			
	Wild-born	-0.68	-0.9	1032487 (39.45%)	338154 (12.92%)	60853 (2.32%)	
				1185374 (45.29%)			

5.5. Discussion

5.5.1. Origin and experience of the harems

After release, Przewalski's horses require an extensive adaptation period as they do not present the typical behavioural pattern of wild individuals and their pattern shifts towards the “normal one” during the first one to two years (Bernátková et al., 2022; Boyd & Bandi, 2002; Scheibe et al., 1996). This fact suggests that the habitat used by horses could also differ according to their origin (natural/captive) and experience in the area. However, other significant factors might force Przewalski’s horses to select different areas for resting (and to leave the feeding area), such as a high presence of blood-sucking insects (Blank, 2020). Ungulates frequently move to specific landscape features to find high and unvegetated sites with reduced temperatures and higher wind speeds to avoid insects. Such areas are typically not suitable for feeding behaviour (Blank, 2020). Another reason for switching habitats might be simply the intention to reduce the risk of parasite infection. It is typical for ungulates to avoid areas with high faeces load for foraging (Coulson et al., 2018)

To test the habitat selection hypothesis, we compared the predicted areas preferred for ‘feeding’ and ‘resting’ between intra-annual (2019) selected periods (Full, Spring/Summer, Autumn), and between groups of different origins (Reintroduced and Wild-born). Our results showed clear differences between the areas predicted for ‘feeding’ and ‘resting’ between seasonal periods and for Reintroduced and Wild-born groups of Przewalski's horses. For Wild-born horses, the probability of ‘feeding’ increased (and the probability of ‘resting’ decreased) with higher NDVI maximum values during the Spring/Summer 2019 period; however, we observed the opposite trend for Reintroduced groups. Wild-born groups also used more area for ‘feeding’ than Reintroduced groups. As described in Kaczensky et al. (Kaczensky, Oyunsaikhan, et al., 2008), it is typical for Przewalski's horses in the GGBSPA to show a strong preference for highly productive plant communities. We assume that the selected Reintroduced groups were learning how to use the habitat properly being in the phase of exploring the area; therefore, their behaviour differed from the typical pattern (Boyd & Bandi, 2002; Scheibe et al., 1996) as they were not experienced enough to choose the most productive areas for ‘feeding’ and were ‘feeding’ on various places. Wild-born groups, on the other hand, fed predominantly on selected, more productive areas.

5.5.2. Inter-group hierarchy

Another reason for the selection of less productive areas for ‘feeding’ might be caused by the hierarchy between the observed horse groups, as the existence of the intergroup hierarchy in horses was previously suggested by Hirata (Hirata, 2022) and Maeda et al. (Maeda et al., 2021). The range values of NDVImx for Wild-born groups were much wider than the ones for Reintroduced groups in the Full and Spring/Summer 2019 periods, and the NDVImx values were much lower in Reintroduced groups than in the Wild-born ones. This suggests that the Wild-born groups can use higher-quality vegetation areas for ‘feeding. The Reintroduced groups might be not only inexperienced but also subordinate to Wild-born groups. This inter-group hierarchy often exists in multilevel societies, which have been described in many species, both human and animals, including horses (Grueter et al., 2017, 2020; Hirata, 2022; Maeda et al., 2021; Whitehead et al., 2012; Wittemyer et al., 2005). Miller and Dennisto (Miller & Dennisto, 2010) observed inter-group dominance in a herd of wild horses; during the dry season, higher-ranking groups accessed a water source whenever they desired, while lower-ranking groups had to wait for many hours until the area was no longer “under the control” of dominant groups. We assume that preferred ‘feeding’ areas in the GGBSPA might be under the control of “dominant harems”, as the GGBSPA is on the edge of Przewalski's horses’ original habitat (Gao X et al., 1989) and is characterized by very harsh environmental conditions (International Takhi Group, 2021; Kaczensky, Enkhsaikhan, et al., 2008) and areas with good quality vegetation are relatively sparse there.

Group size might also play an important role as larger groups typically have a more dominant position in the inter-group hierarchy (Maeda et al., 2021). The reintroduced horse groups of GGBSPA typically consist of 4 – 6 animals as they are formed by the newly released mares and one wild stallion. This might also cause a subordinate status for these groups.

5.5.3. Human-wildlife conflict and ‘feeding’ areas

Stable isotope analyses revealed that before they went extinct in the wild, Przewalski's horses transitioned from grazing in summer to a mixed grass-browse diet in winter. On the contrary, the reintroduced Przewalski's horses graze all year round (Kaczensky et al., 2017). Nevertheless, all equids are typical grazers who browse only when they have no other options (Mendoza & Palmqvist, 2008; Schoenecker et al., 2016). Kaczensky et al. (Kaczensky et al., 2017) suggested that the difference in diet between historic and reintroduced Przewalski's horses could be explained as a move from a historically inadequate “mixed diet” to a current more optimal “graze diet” due to better socio-cultural perceptions of the species and reduction of anthropogenic pressure, especially hunting. Before the extinction, Przewalski's horses were forced to feed out of their preferred feeding areas, which were widely used by herders, who also hunted the horses. However, Kaczensky et al. (Kaczensky et al., 2017) also pointed out that with the growth in human, livestock and Przewalski's horse numbers, human-wildlife conflict is expected to increase again as Przewalski's horse distribution was already reported to be negatively influenced by the presence of herders in the GGBSPA in 2007 (Kaczensky et al., 2007). Moreover, competition with livestock and increasing land use pressure have been cited as one of the main causes of the species’ extinction in its natural habitat (King et al., 2015)

During our research, we observed an apparent shift between the areas preferred for ‘feeding’ and ‘resting’ in Wild-born and Reintroduced groups, both over the selected periods and between groups of different origins. We assume that the switch between areas used by selected groups of Przewalski's horses was caused by human and livestock activity in the GGBSPA (Kaczensky et al., 2007): seasonal Przewalski's horse distribution in the GGBSPA was negatively linked to herder presence. Herding activity has traditionally been seasonal in the area, and most herders leave the GGBSPA in spring and return in autumn (Kaczensky et al., 2007; Suttie J.M. & Reynolds S.G., 2003). It has been detected previously that Przewalski’s horse range overlaps primarily with intermediate camps utilized by herders in spring and fall (Michler LM et al., 2021). Therefore, the selected Wild-born Przewalski's horse groups were able to use the most suitable areas for ‘feeding’ during spring (as they were among the dominant groups of the area), when herders were leaving the area (or left already) and were forced to move to less preferred habitats in autumn when herders were coming back to their winter camps. The Reintroduced groups were not able to use the areas with good fodder quality in spring, as they were subordinate to wild and bigger harems (Miller & Dennisto, 2010). In autumn, however, they were able to use the preferred areas as the wild harems were probably suspicious of humans and livestock and thus were forced to feed in suboptimal habitats because herder camps are typically placed in grass-dominated areas with good quality fodder (Burnik Šturm et al., 2017). The reintroduced groups were able to share these areas with humans, and their livestock as their fear of humans was most probably still reduced due to captive breeding (Ralls K. & Ballou J.D., 2013).

5.5.4. Implications for conservation

Studying the behaviour and ecology of animals is a crucial aspect of long-term monitoring of reintroduced populations (IUCN, 2013), as the response of animals to the environment is vital for their survival (Macdonald, 2016; Snijders et al., 2017). It has been stressed by the International Union for Conservation of Nature’s Red List of Threatened Species that a single population management approach for Przewalski's horses should be developed, and an action plan is needed for this species in Mongolia. Moreover, for the reintroduced Przewalski's horse populations, the small limited spatial distribution is one of the two primary threats to their survival, together with the small population size (King et al., 2015). In this study, we found out that there is an important link between the remotely sensed variables informing about the topography and vegetation quality and the seasonal habitat preference of Reintroduced and Wild-born Przewalski’s horses. These findings might assist in the successful management of the species and in the selection of future reintroduction sites, and therefore in further conservation of the species (Regos et al., 2022).

5.6. Conclusion

When choosing future reintroduction sites for Przewalski's horses, habitat suitability should be considered as a key factor, and a sufficient number of areas with good quality feed should be present. However, the importance of anthropogenic pressure should not be underestimated. Furthermore, the group size of the reintroduced groups should be considered, as it influences the inter-group hierarchy, and large groups usually have a more dominant position. Holding animals for a longer time in the acclimatization fence and releasing the group after a few years when it has more members or transportation of more animals in the same year (having more of them at the same time in the acclimatization period) might be beneficial for the success of the reintroduced animals and conservation of the species.

We finally highlight the spatial and temporal utility of descriptors related to vegetation and topography derived from remote sensing data at a high spatial resolution to detect habitat preferences by Przewalski's horses. The combination of analyses from behavioural data and ENMs is a valuable technique for understanding the spatial patterns and ecological processes connected to differences in the origins of Przewalski's horses and intra-annual variations. However, additional modelling approaches, as well as more robust seasonality-related satellite-derived products (e.g., ecosystem functioning attributes; EFAs; Regos et al., 2022), may provide new outputs on horses' habitat selection. Our method may offer an objective ecological and behavioural background for developing conservation strategies for this endangered species and therefore becomes a cost-effective and powerful Earth observation system to forecast wild-born and captive-born (reintroduced) horses' preferences for habitat selection at fine spatial and temporal scales. Thus, assisting decision-making and wildlife management protocols.

5.7. References

Arenas-Castro, S., Regos, A., Gonçalves, J. F., Alcaraz-Segura, D., & Honrado, J. (2019). Remotely Sensed Variables of Ecosystem Functioning Support Robust Predictions of Abundance Patterns for Rare Species. Remote Sensing, 11(18), 2086. https://doi.org/10.3390/rs11182086

Bahloul, K., Pereladova, O. B., Soldatova, N., Fisenko, G., Sidorenko, E., & Sempéré, A. J. (2001). Social organization and dispersion of introduced kulans (*Equus hemionus kulan*) and Przewalski horses (*Equus przewalski*) in the Bukhara Reserve, Uzbekistan. Journal of Arid Environments, 47(3), 309–323. https://doi.org/10.1006/JARE.2000.0714

Battin, J. (2004). When Good Animals Love Bad Habitats: Ecological Traps and the Conservation of Animal Populations. Conservation Biology, 18(6), 1482–1491. https://doi.org/10.1111/j.1523-1739.2004.00417.x

Bernátková, A., Oyunsaikhan, G., Šimek, J., Komárková, M., Bobek, M., & Ceacero, F. (2022). Influence of weather on the behaviour of reintroduced Przewalski's horses in the Great Gobi B Strictly Protected Area (Mongolia): implications for conservation. BMC Zoology, 7(1), 32. https://doi.org/10.1186/s40850-022-00130-z

Blank, D. A. (2020). Insect-repelling behavior in goitered gazelles: responses to biting fly attack. European Journal of Wildlife Research, 66(3), 43. https://doi.org/10.1007/s10344-020-01382-1

Boyd, L., & Bandi, N. (2002). Reintroduction of takhi, Equus ferus przewalskii, to Hustai National Park, Mongolia: time budget and synchrony of activity pre- and post-release. Applied Animal Behaviour Science, 78(2–4), 87–102. https://doi.org/10.1016/S0168-1591(02)00088-6

Burnham KP, & Anderson DR. (2002). Model Selection and Multimodel Inference: A Practical Information-Theoretical Approach. Springer-Verlag.

Burnik Šturm, M., Ganbaatar, O., Voigt, C. C., & Kaczensky, P. (2017). Sequential stable isotope analysis reveals differences in dietary history of three sympatric equid species in the Mongolian Gobi. Journal of Applied Ecology, 54(4), 1110–1119. https://doi.org/10.1111/1365-2664.12825

Coulson, G., Cripps, J. K., Garnick, S., Bristow, V., & Beveridge, I. (2018). Parasite insight: assessing fitness costs, infection risks and foraging benefits relating to gastrointestinal nematodes in wild mammalian herbivores. Philosophical Transactions of the Royal Society B: Biological Sciences, 373(1751), 20170197. https://doi.org/10.1098/rstb.2017.0197

Duncan, P. (1983). Determinants of the Use of Habitat by Horses in a Mediterranean Wetland. The Journal of Animal Ecology, 52(1), 93. https://doi.org/10.2307/4590

Duncan, P., Foose, T. J., Gordon, I. J., Gakahu, C. G., & Lloyd, M. (1990). Comparative nutrient extraction from forages by grazing bovids and equids: a test of the nutritional model of equid/bovid competition and coexistence. Oecologia, 84(3), 411–418. https://doi.org/10.1007/BF00329768

Gao X, Gu J, & Zhou J. (1989). The change on the distribution area of the wild horse in the modern times. Arid Zone Res. 1989;2(10)., 2(10).

Gorelick, N., Hancher, M., Dixon, M., Ilyushchenko, S., Thau, D., & Moore, R. (2017). Google Earth Engine: Planetary-scale geospatial analysis for everyone. Remote Sensing of Environment, 202, 18–27. https://doi.org/10.1016/j.rse.2017.06.031

Grueter, C. C., Qi, X., Li, B., & Li, M. (2017). Multilevel societies. Current Biology, 27(18), R984–R986. https://doi.org/10.1016/j.cub.2017.06.063

Grueter, C. C., Qi, X., Zinner, D., Bergman, T., Li, M., Xiang, Z., Zhu, P., Migliano, A. B., Miller, A., Krützen, M., Fischer, J., Rubenstein, D. I., Vidya, T. N. C., Li, B., Cantor, M., & Swedell, L. (2020). Multilevel Organisation of Animal Sociality. Trends in Ecology & Evolution, 35(9), 834–847. https://doi.org/10.1016/j.tree.2020.05.003

Guisan, A., & Zimmermann, N. E. (2000). Predictive habitat distribution models in ecology. Ecological Modelling, 135(2–3), 147–186. https://doi.org/10.1016/S0304-3800(00)00354-9

Hagen-Zanker, A. (2009). An improved Fuzzy Kappa statistic that accounts for spatial autocorrelation. International Journal of Geographical Information Science, 23(1), 61–73. https://doi.org/10.1080/13658810802570317

Hirata, S. (2022). Studying feral horse behavior from the sky. Artificial Life and Robotics, 27(2), 196–203. https://doi.org/10.1007/s10015-022-00746-x

Hu, J., & Jiang, Z. (2010). Predicting the potential distribution of the endangered Przewalski’s gazelle. Journal of Zoology, 282(1), 54–63. https://doi.org/10.1111/j.1469-7998.2010.00715.x

Illius, A., & Gordon, W. (1993). Diet Selection in Mammalian Herbivores: Constraints and Tactics. In R. Hughes (Ed.), Diet selection. An interdisciplinary approach to foraging behaviour (pp. 157–181). Blackwell Scientific Publications.

International Takhi Group. (2021). Save the Wild Horse. https://savethewildhorse.org/en/

IUCN. (2013). Guidelines for reintroductions and other conservation translocations (IUCN Species Survival Commission, Ed.).

Kaczensky, P., Burnik Šturm, M., Sablin, M. v., Voigt, C. C., Smith, S., Ganbaatar, O., Balint, B., Walzer, C., & Spasskaya, N. N. (2017). Stable isotopes reveal diet shift from pre-extinction to reintroduced Przewalski's horses. Scientific Reports, 7(1), 5950. https://doi.org/10.1038/s41598-017-05329-6

Kaczensky, P., Enkhsaihan, N., Ganbaatar, O., & Walzer, C. (2007). Identification of herder-wild equid conflicts in the Great Gobi B Strictly Protected Area in SW Mongolia. https://www.researchgate.net/publication/259602525_Identification_of_Herder-Wild_Equid_Conflicts_in_the_Great_Gobi_B_Strictly_Protected_Area_in_SW_Mongolia

Kaczensky, P., Enkhsaikhan, N., Ganbaatar, O., & Walzer, C. (2008). The Great Gobi B Strictly Protected Area in Mongolia - refuge or sink for wolves *Canis lupus* in the Gobi. Wildlife Biology, 14(4), 444–456. https://doi.org/10.2981/0909-6396-14.4.444

Kaczensky, P., Ganbataar, O., Altansukh, N., Enkhsaikhan, N., Stauffer, C., & Walzer, C. (2011). The Danger of Having All Your Eggs in One Basket—Winter Crash of the Re-Introduced Przewalski's Horses in the Mongolian Gobi. PLoS ONE, 6(12), e28057. https://doi.org/10.1371/journal.pone.0028057

Kaczensky, P., Oyunsaikhan, G., von Wehrden, H., & Walzer, C. (2008). Resource selection by sympatric wild equids in the Mongolian Gobi. Journal of Applied Ecology, 45(6), 1762–1769. https://doi.org/10.1111/j.1365-2664.2008.01565.x

Kerekes, V., Ozogány, K., Sándor, I., Vegvari, Z., Czető, C., Nyírő, B., Szabados, T., Széles, L., & Barta, Z. (2019). Analysis of habitat use, activity, and body condition scores of Przewalski's horses in Hortobagy national park, Hungary. Nature Conservation Research, 4(Suppl.2). https://doi.org/10.24189/ncr.2019.029

King, S. R. B., Boyd, L., Zimmermann, W., & Kendall, B. E. (2015, June 23). *Equus ferus* (errata version published in 2016). https://www.iucnredlist.org/species/41763/97204950

Kuemmerle, T., Perzanowski, K., Chaskovskyy, O., Ostapowicz, K., Halada, L., Bashta, A.-T., Kruhlov, I., Hostert, P., Waller, D. M., & Radeloff, V. C. (2010). European Bison habitat in the Carpathian Mountains. Biological Conservation, 143(4), 908–916. https://doi.org/10.1016/j.biocon.2009.12.038

Kuemmerle, T., Radeloff, V. C., Perzanowski, K., Kozlo, P., Sipko, T., Khoyetskyy, P., Bashta, A.-T., Chikurova, E., Parnikoza, I., Baskin, L., Angelstam, P., & Waller, D. M. (2011). Predicting potential European bison habitat across its former range. Ecological Applications, 21(3), 830–843. https://doi.org/10.1890/10-0073.1

Łopucki, R., Klich, D., & Gielarek, S. (2017). Do terrestrial animals avoid areas close to turbines in functioning wind farms in agricultural landscapes? Environmental Monitoring and Assessment, 189(7), 343. https://doi.org/10.1007/s10661-017-6018-z

Macdonald, D. W. (2016). Animal behaviour and its role in carnivore conservation: examples of seven deadly threats. Animal Behaviour, 120, 197–209. https://doi.org/10.1016/j.anbehav.2016.06.013

Maeda, T., Ochi, S., Ringhofer, M., Sosa, S., Sueur, C., Hirata, S., & Yamamoto, S. (2021). Aerial drone observations identified a multilevel society in feral horses. Scientific Reports, 11(1), 71. https://doi.org/10.1038/s41598-020-79790-1

McDonnell, S. M., & Haviland, J. C. S. (1995). Agonistic ethogram of the equid bachelor band. Applied Animal Behaviour Science, 43(3), 147–188. https://doi.org/10.1016/0168-1591(94)00550-X

McDonnell, S. M., & Poulin, A. (2002). Equid play ethogram. Applied Animal Behaviour Science, 78(2–4), 263–290. https://doi.org/10.1016/S0168-1591(02)00112-0

Mendoza, M., & Palmqvist, P. (2008). Hypsodonty in ungulates: an adaptation for grass consumption or for foraging in open habitat? Journal of Zoology, 274(2), 134–142. https://doi.org/10.1111/j.1469-7998.2007.00365.x

Michler LM, Kaczensky P, Ganbaatar O, & Treydte AC. (2021). On the Move–Do Domestic and Wild Ungulate Species Distributions Overlap in the Mongolian Gobi. In National Organizing Committee (Ed.), Proceedings of 2021 IGC/IRC Congress. Kenya Agricultural and Livestock Research Organization.

Miller, R., & Dennisto, R. H. n. (2010). Interband Dominance in Feral Horses. Zeitschrift Für Tierpsychologie, 51(1), 41–47. https://doi.org/10.1111/j.1439-0310.1979.tb00670.x

Moorhouse, T. P., Gelling, M., & Macdonald, D. W. (2009). Effects of habitat quality upon reintroduction success in water voles: Evidence from a replicated experiment. Biological Conservation, 142(1), 53–60. https://doi.org/10.1016/j.biocon.2008.09.023

Morueta-Holme, N., Fløjgaard, C., & Svenning, J.-C. (2010). Climate Change Risks and Conservation Implications for a Threatened Small-Range Mammal Species. PLoS ONE, 5(4), e10360. https://doi.org/10.1371/journal.pone.0010360

Mysterud, A., & Ims, R. A. (1998). Functional responses in habitat use: Availability influences relative use in trade-off situations. Ecology, 79(4), 1435–1441. https://doi.org/10.1890/0012-9658(1998)079[1435:FRIHUA]2.0.CO;2

National Statistic Office of Mongolia. (2021). National Statistic Office of Mongolia. 1212.Mn. https://www.1212.mn/default.aspx?fbclid=IwAR0BEXeCvXQhyRjP_kUAmDcNmEHTl40knH-nMilphtaOsOvy2UZLDZAr7x8//

Ralls K., & Ballou J.D. (2013). Encyclopedia of Biodiversity. Captive Breeding and Reintroduction (Levin S.A., Ed.; 2nd ed.). MA: Academic Press.

Rantanen, E. M., Buner, F., Riordan, P., Sotherton, N., & Macdonald, D. W. (2010). Habitat preferences and survival in wildlife reintroductions: an ecological trap in reintroduced grey partridges. Journal of Applied Ecology, 47(6), 1357–1364. https://doi.org/10.1111/j.1365-2664.2010.01867.x

Regos, A., Gonçalves, J., Arenas-Castro, S., Alcaraz-Segura, D., Guisan, A., & Honrado, J. P. (2022). Mainstreaming remotely sensed ecosystem functioning in ecological niche models. Remote Sensing in Ecology and Conservation, 8(4), 431–447. https://doi.org/10.1002/rse2.255

Rubenstein, D. (2011). Family Equidae (horses and relatives). Handbook of Mammals of the World. (R. A. Mittermeier & D. E. Wilson, Eds.; 2nd ed., Vol. 2). Lynx Edicions.

Scheibe, K., Lange B, Lange V, Eichhorn E, Scheibe A, & Streich J. (1996). Przewalskipferde in einem Semireservat–Verhaltensuntersuchungen zur Vorbereitung einer Auswilderung. Current Research in Applied Ethology, 137–136.

Schoenecker, K. A. , King, S. R. B. , Nordquist, M. K. , Nandintsetseg, D. &, & Cao, Q. (2016). Habitat and diet of equids. Wild Equids—Ecology, Management, and Conservation (J. I. , Ransom & P. Kaczensky, Eds.). Johns Hopkins University Press.

Sillero, N. (2011). What does ecological modelling model? A proposed classification of ecological niche models based on their underlying methods. Ecological Modelling, 222(8), 1343–1346. https://doi.org/10.1016/j.ecolmodel.2011.01.018

Sillero, N., Arenas-Castro, S., Enriquez-Urzelai, U., Vale, C. G., Sousa-Guedes, D., Martínez-Freiría, F., Real, R., & Barbosa, A. M. (2021). Want to model a species niche? A step-by-step guideline on correlative ecological niche modelling. Ecological Modelling, 456, 109671. https://doi.org/10.1016/j.ecolmodel.2021.109671

Snijders, L., Blumstein, D. T., Stanley, C. R., & Franks, D. W. (2017). Animal Social Network Theory Can Help Wildlife Conservation. Trends in Ecology & Evolution, 32(8), 567–577. https://doi.org/10.1016/j.tree.2017.05.005

Suttie J.M., & Reynolds S.G. (2003). Transhumant Grazing Systems in Temperate Asia (Suttie J.M. & Reynolds S.G., Eds.; Vol. 31). Food and Agriculture Organization of the United Nations.

Tweed, E. J., Foster, J. T., Woodworth, B. L., Oesterle, P., Kuehler, C., Lieberman, A. A., Powers, A. T., Whitaker, K., Monahan, W. B., Kellerman, J., & Telfer, T. (2003). Survival, dispersal, and home-range establishment of reintroduced captive-bred puaiohi, *Myadestes palmeri*. Biological Conservation, 111(1), 1–9. https://doi.org/10.1016/S0006-3207(02)00175-1

Visser, H., & de Nijs, T. (2006). The Map Comparison Kit. Environmental Modelling & Software, 21(3), 346–358. https://doi.org/10.1016/j.envsoft.2004.11.013

Wakefield, S., Knowles, J., Zimmermann, W., & van Dierendonck, M. (2002). Status and action plan for the Przewalski's Horse (*Equus ferus przewalskii*). Equids: Zebras, Asses and Horses Status Survey and Conservation Action Plan. (P. D. Moehlman, Ed.). H. Charlesworth & Co Ltd., Huddersfield, UK.

Whitehead, H., Antunes, R., Gero, S., Wong, S. N. P., Engelhaupt, D., & Rendell, L. (2012). Multilevel Societies of Female Sperm Whales (*Physeter macrocephalus*) in the Atlantic and Pacific: Why Are They So Different? International Journal of Primatology, 33(5), 1142–1164. https://doi.org/10.1007/s10764-012-9598-z

Wittemyer, G., Douglas-Hamilton, I., & Getz, W. M. (2005). The socioecology of elephants: analysis of the processes creating multitiered social structures. Animal Behaviour, 69(6), 1357–1371. https://doi.org/10.1016/J.ANBEHAV.2004.08.018

Zhang, Y., Cao, Q. S., Rubenstein, D. I., Zang, S., Songer, M., Leimgruber, P., Chu, H., Cao, J., Li, K., & Hu, D. (2015). Water Use Patterns of Sympatric Przewalski's Horse and Khulan: Interspecific Comparison Reveals Niche Differences. PLOS ONE, 10(7), e0132094. https://doi.org/10.1371/journal.pone.0132094

ZIMS for Studbook for Przewalski's horse, & Šimek J. (2022, November 22). ZIMS for Studbook for Przewalski's horse. Species360 Zoological Information Management System. 2022. http://zims.Species360.org

CHAPTER 6

Social networks of reintroduced Przewalski's Horses in the Great Gobi B Strictly Protected Area (Mongolia)

Wild-born stallion and a reintroduced mare grooming in the Great Gobi B Strictly Protected Area, Mongolia.

(Photo by Anna Bernátková)

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6.1. Abstract

Analysing social networks is a powerful tool for understanding the ecology of social species. While most studies focus on the role of each group member, few compare groups with different characteristics. The current population of Przewalski's horses (*Equus ferus przewalskii*) at the Great Gobi B Strictly Protected Area (Mongolia) includes groups of wild-born and captive-bred individuals with different experiences with the area (acclimatizing, long-term reintroduced and wild-born), therefore serving as an ideal natural behavioural lab. We filmed 11 groups for 141.5 hours in summer 2018 (July), late spring 2019 (May, June), and autumn 2019 (September, October). Affiliative and agonistic interactions were recorded, and social networks were created. We tested the influence of origin, experience, season, sex, age, relative time belonging to the group, relatedness, and dominance rank on different network indices at the individual and group levels. We found that groups with greater experience in the area are generally better connected than members of the newly formed groups. However, these strong networks were created by wild-born individuals with very low interaction rates. On the contrary, inexperienced groups composed of captive-bred individuals displayed many interactions but created weak social networks. The results show a trend of behavioural transition from acclimatizing through long-term reintroduced to wild-born groups, supporting that the newly-formed groups of released Przewalski's horses need time to display the typical social behaviour patterns of wild-born individuals. Long-term monitoring of released Przewalski's horses is recommended to promote success of this reintroduction program.

Keywords: Przewalski's horses, reintroduction, social behaviour, soft-release

6.2. Introduction

Studying the social behaviour and networks of large social ungulates is a crucial aspect of understanding their adaptation to the environment, and is vital to their survival (Knight 2001; Conradt et al. 2009; Bousquet 2011; Macdonald 2015; Snijders et al. 2017). Long-term monitoring assessing the adaptation of the animals to the new conditions (Sarrazin and Barbault 1996; Seddon 1999; Fischer and Lindenmayer 2000) and creating socially functional populations (Goldenberg et al. 2019) is especially important for reintroduced populations (Berger-Tal et al. 2011; IUCN/SSC 2013; Nelson 2014). The adaptability of animals will be affected by their origin (captive-breeding), reintroduction technique (commonly through soft-release methods), familiarity (Shier 2006, Moseby et al. 2020), and life experiences, which significantly influence social behaviour and reintroduction success (Berger-Tal et al. 2011). Animals captured from wild populations (i.e., wild-born individuals) fare better after release than those reared in captivity (Fischer & Lindenmayer 2000; Teixeira et al. 2007; Dickens, Delehanty & Romero 2009). Captivity might also promote the development of behavioural characteristics (such as higher aggressiveness or boldness) that may jeopardise their survival (Baker et al. 1998; Bremner-Harrison et al. 2004; Kelley et al. 2005). Personality and social behaviour within the group can also strongly impact reintroduction success (Gusset et al. 2010; Gaudioso et al. 2011).

The Przewalski's horse was listed as Extinct in the wild in 1996 and as Endangered since 2011 thanks to ongoing captive breeding and reintroduction efforts (King et al. 2015). As described by Scheibe et al. (1997) and Pereladova et al. (1999), the behaviour of reintroduced Przewalski's horses differs from the typical pattern of wild conspecifics in the first year after release. Just after this period, horses show a similar yearly activity budget to that observed among wild Przewalski's horses. Przewalski's horses live in non-territorial stable family groups (harems) composed of one (or more) adult stallions, several mares, and their offspring (Boyd et al. 2016). Females' and males' priorities are the main determinants of group cohesiveness in horses: females prioritise consistent access to feed, water, and a low risk of intraspecific harassment (Rubenstein 1994; Nowzari et al. 2013; Schoenecker et al. 2016), while males prioritise the proximity to females and a reduced probability of costly conflicts (Berger 1986; Rubenstein 1994). A social arrangement based on reasonably satisfying the needs of both sexes is the key to forming stable social and breeding groups (harems) in Przewalski's horses (Berger 1986; Rubenstein 1994; Berger 1999).

Once a group is created, individual interactions determine the social structure or social network (Hinde 1976). These interactions can differ greatly based on the age, sex, reproductive state, personality, and life history of each individual, affecting the general structure of the group (Hinde 1976; Lehner 1996; Whitehead 2008; van Dierendonck et al. 2009). Relatedness is another important factor; it enhances proximity tolerance and promotes an increase in the number of affiliative interactions (Berger 1986), including grooming, playing, approaching another conspecific, practising head resting or head rubbing, and olfactory investigation (McDonnell & Haviland 1995; McDonnell & Poulin 2002). On the other hand, interactions that typically encourage or maintain increased distance between individuals are known as agonistic interactions (Feh 1988), which are often divided into aggressive and defensive actions (Feh 1988, Rubenstein 1994; Cozzi et al. 2010) and are commonly used to define the social hierarchy (Miller and Denniston 1979; Feh 1999). Head threats, bite threats, kick threats, biting, and kicking are examples of basic agonistic behaviours (McDonnell & Haviland 1995; McDonnell & Poulin 2002). A specific example of agonistic interaction in horses is herding. It is a behaviour presented by a dominant stallion to maintain a harem and to direct its movement (King 2002; King et al. 2016).

The previously described social behaviours can be used to characterise the agonistic and affiliative social network at the group level, providing specific metrics for each individual involved (Whitehead 2008). This allows an investigation of the links between individual traits and their connections in populations where all the members are known and easily identified (Croft et al. 2008). Przewalski's horses have complex but relatively stable social networks; moreover, the current Great Gobi B population in Mongolia is composed of wild-born and captive-bred individuals with varied life experiences, all of which can be individually recognised. For these reasons, they serve as an ideal model species for analysing social behaviour and how it affects the adaptation of the animals during the reintroduction process, which is the primary goal of this study.

Several previous studies on the social behaviour of horses focused primarily on the relationships among group members (Ebhardt 1957; Blakeslee 1974; Wells & Goldschmidt-Rothschild 1979; Keiper & Sambraus 1986; Keiper 1988; van Dierendonck et al. 1995; Berger 1999; Vervaecke et al. 2007; Bourjade et al. 2009a). However, there has not been any attempt to study groups made up of individuals with different origins (reintroduced vs. wild-born) nor to study social network differences among groups with different origins (acclimatizing vs. long-term reintroduced vs. wild-born). Such studies are surprisingly few for wild, free-roaming horses (translocated vs. born in the wild) and missing for wild-living Przewalski's horses (acclimatizing vs. long-term reintroduced vs. wild-born).

Numerous studies stressed that understanding ungulate adaptation to their environment through different aspects, including social behaviour, is essential for their survival (Knight 2001; Conradt et al. 2009; Bousquet 2011; Macdonald 2015; Snijders et al. 2017). And the lack of information on origin, as one of the crucial factors (Berger-Tal et al. 2011) to be analysed, became apparent. Therefore, we analysed the impact of origin and experience on social metrics, including interaction rate, social relationships and structure and thus, on the formation of stable social networks in Przewalski's horses. Along with the origin of the horses, we also tested the influence of other common factors on social network characteristics, including sex, age, relative time belonging to the group, relatedness, and dominance rank as potential sources of variability. We hypothesised that the social network measures are affected by individual traits including origin (captive breeding vs. wild-born) or the previous experience of each individual in the studied group. More specifically, we hypothesise that a higher number of interactions, but weaker networks may exist between the horses at the beginning of the reintroduction process compared to lower interaction rates and strong social networks in wild-born horses. At the group level, we expect changes in the group metrics with more experience, which commonly means a reduction in the metrics´ values, meaning that the roles of each individual in the group are well-defined.

6.3. Materials and Methods

6.3.1. Study area

The Great Gobi B Strictly Protected Area GGBSPA (established in 1975, 45°00'-26'N – 91°29'-49'E) is a part of the Great Gobi Biosphere Reserve. Since 2019, it encompasses ≈18 000 km^2 of desert steppe and desert habitat (≈9 000 km^2 before the enlargement, ITG International Takhi Group, 2021). This protected area in SW Mongolia is a reintroduction site for Przewalski's horse and an important refuge for several other endangered species (Kaczensky et al. 2004; Kaczensky et al. 2008). Despite its protected area status, the GGBSPA is used by about 130 families with close to 70 000 heads of livestock, mainly in winter and during spring and fall migration (National Statistic Office of Mongolia 2021).

The climate of the GGBSPA is continental and very dry. The temperatures differ significantly during the day and night and between the seasons (cold season mean monthly temperatures vary from − 20°C to 4°C between October and April; warm season mean monthly temperatures vary from 14°C to 19°C between May and September; Michler et al. 2022). The altitude ranges from 1 100 to 2 900 masl (ITG International Takhi Group 2021). The GGBSPA is situated between the Altai Mountains and the national border with China. The precipitation is within the typical range of a semi-desert climate; the annual average precipitation is 100 mm with a differentiated peak in summer (>80%; June–August). Snow cover typically lasts for around 100 days. Rain and snowfall substantially over in both time and space (Burnik Šturm et al. 2017). Drought, pronounced temperature variation, and enormous annual differences are characteristic of the habitat (ITG International Takhi Group 2021). The Great Gobi B SPA belongs to the central Asian plant geographical area. This region has shallow and poorly developed soils and is dominated by semidesert and desert-steppe vegetative cover with arid-adapted plants; the most typical are *Haloxylon ammodendron, Ephedra przewalskii, Reaumuria soongarica, Anabasis brevifolia, Stipa spp., Artemisia spp. and Ajania spp*. Plants with higher nutritional quality are frequently found in pastures with limited forage availability (Michler et al. 2022).

6.3.2. Data collection

Data were collected in three different seasons: summer 2018 (10.07.2018 – 22.07.2018), late spring 2019 (19.5.2019 – 23.06.2019) and autumn 2019 (10.09.2019 – 01.10.2019). In the summer of 2018, three groups were observed (Azaa, Tsetsen and Mares18) involving 29 individuals. In spring 2019, 4 groups were observed (Azaa, Tsetsen, Hustai1 and Mares19) involving 34 individuals. In autumn 2019, 4 groups were observed (Azaa, Tsetsen, Hustai2 and Tanan) involving 35 individuals. Specific information on the groups and individuals and their different origins (wild-born; long-term reintroduced; acclimatizing) is described in Table 6.1 and in the Supplementary Material. The season was defined in consultation with rangers of GGBSPA and local inhabitants (the herders move from winter to summer camps in spring and back in autumn). Two of the observed groups consisted of wild-born horses (born in the GGBSPA), and two consisted of reintroduced horses. The long-term reintroduced and acclimatizing horses originally come from various breeding centres in Europe and are transported to the Czech Republic into a breeding facility area from which target mares are selected and transported to the GGBSPA; part of the observation of the reintroduced groups was conducted in soft-release acclimatisation enclosures (at this point, the horses were in the GGBSPA from 2 days up to 3 months after the transport from Europe). The acclimatisation enclosures are ≈500 m from the GGBSPA administration building and field station. There are four interconnected rectangle-shaped enclosures of 1.20 km^2, 0.33 km^2, 0.23 km^2, and 0.20 km^2, respectively, with natural steppe vegetation and a natural stream with water. The enclosures are fenced with 2 m high wire mesh. The smallest enclosure has a shelter consisting of three perpendicular walls with a roof. The wild-born groups were always observed in the wild (Supplementary Material). The harems were located during daily monitoring. Once one of the target harems was located, the harem was approached and filmed from 150m to 800m using a 4K Panasonic VX1 video camera with a tripod using the zoom function. One harem was filmed at a time, and all recordings were made by the same researcher (AB). Each group and each individual was identified by the principal researcher based on the physical feature information provided by the GGBSPA administration. Before the data collection started, the GGBSPA administration official trained the researcher to distinguish each horse. To prevent any disturbance of the horses, the least human-shy wild-born harems were selected in cooperation with the local rangers. Thanks to the rangers' knowledge of each group, a sufficient distance could be maintained so as not to distract the horses or to avoid triggering flight responses. Enclosed horses were also filmed. An equivalent distance could be kept for the enclosed horses thanks to the extensive size of the enclosures. However, these individuals were acclimated to human presence and management at the time of our data collection. The video recordings were made daily across the whole study period (in Summer, Spring, and Autumn, respectively), and each observation day was dedicated to two groups. Time from morning to afternoon or from midday to evening was typically spent with each group. When possible, the recorded groups were changed every day, and an equal percentage of the morning-afternoon/midday-evening period was spent with each group to collect comparable data regarding environmental conditions and hours of observation. In the summer of 2018, 45 hours of recordings were collected from 3 groups. In late spring 2019, 108 hours of videos were recorded from 4 groups. In autumn 2019, 88.5 hours of videos were recorded from 4 groups. The recording time was similarly distributed among all studied groups (details of recording time distributed across groups are provided in Table 6.1). Winter monitoring was not feasible due to low accessibility to the area (frozen paths), the technical impossibility of recording the groups due to the low temperatures.

Table 6.1. Composition, experience, stability, and reproductive information of the Przewalski's horses groups studied in 2018 and 2019. Stability index indicates how similar was a group respect the previous observation period. Detailed information about the individuals composing each group is shown as Supplementary Material.

Group	Season/Year*	Observation hours	Group experience**	Individuals	Number of breeding mares	Stability index***	Age of foals (d)****
Azaa	Summer/18	18.5	wild-born	1 dominant ♂, 9 ♀, 3 ♂, 4 foals	6	0.86	46
Mares18	Summer/18	17.5	acclimatizing	4 ♀	4	0.00	-
Tsetsen	Summer/18	11.2	wild-born	1 dominant ♂, 4 ♀, 3 ♂	3	0.00	-
Azaa	Spring/19	30.0	wild-born	1 dominant ♂, 8 ♀, 2 ♂, 3 foals	5	0.83	26
Hustai1†	Spring/19	25.5	long-term reintroduced	1 dominant ♂, 4 ♀	4	0.00	-
Mares19	Spring/19	21.0	acclimatizing	3 ♀	3	0.00	-
Tsetsen	Spring/19	31.5	wild-born	1 dominant ♂, 5 ♀, 3 ♂, 3 foals	4	0.75	28
Azaa	Autumn/19	22.5	wild-born	1 dominant ♂, 6 ♀, 2 ♂, 5 foals	5	0.75	100
Tanan	Autumn/19	21.0	long-term reintroduced	1 dominant ♂, 4 ♀	4	0.00	-
Hustai2	Autumn/19	20.0	acclimatizing	1 dominant ♂, 3 ♀	3	0.00	-
Tsetsen	Autumn/19	25.0	wild-born	1 dominant ♂, 5 ♀, 3 ♂, 3 foals	4	0.75	128

Only animals older than one year (inclusive of) were used in our research. Foals are shown in the table to illustrate the general information on the studied groups.

*Summer/18 period accounts for July 7th – July 22nd, 2018, Spring/19 accounts for May 19th – June 23rd 2019 and Autumn/19 accounts for September 10th – October 10th 2019.

**Group experience: acclimatizing (from 2 days up to 3 months after the transport from Europe, fenced area); long-term reintroduced (after one year acclimatisation period, released); wild-born (born in the wild). One mare in the wild-born Azaa harem was born in captivity in Europe and reintroduced to the GGBSPA in 2004.

***Stability index was counted as the inter-yearly changes in the number of breeding mares (in %; the number of mares present in the group during the previous and the observation year, divided by the number of mares present in one or another year). Zeros represent groups which existed for less than one year.

****Average foals' age (in days) at the end of each selected observation.

[†] Hustai1 was created after Hustai stallion joined Mares18.

6.3.3. Data processing

Behavioural Observation Research Interactive Software BORIS 7.12.2 (Friard and Gamba 2016) was used for event logging, video coding and recording. An ethogram (Table 6.2) based on published material for horses (McDonnell and Haviland 1995; McDonnell and Poulin 2002) and consultation with the GGBSPA administration was used to categorise the behaviours. The ethogram consisted of two categories: agonistic (-Agonistic) and affiliative (-Affiliative). Other behavioural categories (feeding, lead and follow, locomotion, resting, and others) were also recorded but not used for this study. Data from BORIS was transferred to Excel. These data were further analysed in SOCPROG 2.9 for MATLAB2018B (analysis of data on the social structure; Whitehead 2015) and DomiCalc (tools for dominance analysis; de Silva et al. 2017). In DomiCalc, only agonistic interactions were analysed. All the other social network measures were calculated in SOCPROG separately for agonistic and affiliative behaviours.

Table 6.2. Ethogram of the behaviours recorded within this study, partially based on McDonnell and Haviland 1995; McDonnel and Poullin 2002).

Category	**Behaviour**	**Definition**
Affiliative	Approach	Movement of a horse to initiate a behaviour towards another horse.
	Play	The behaviour appears to have no immediate use or function for the animal, involving a sense of pleasure. Various behaviours presented while playing with other individual/s.
	Grooming	Two members standing beside one another, usually head-to-shoulder or head-to-tail, grooming (each) other's neck, mane, rump, or tail by gentle nipping, nuzzling or rubbing.
	Head resting	The horse is placing its head on the other horse's body.
	Head rubbing	The horse is rubbing its head on the other horse's body.

	Olfactory investigation	The olfactory investigation involves sniffing various parts of another horse's head and/or body. They are considered affiliative if followed by another affiliative behaviour.
Agonistic	Arched neck threat	Neck tightly flexed with the muzzle drawn toward the chest. Arched neck threats are observed during close aggressive encounters and ritualised interactions.
	Bite threat	No contact is made. The neck is stretched and ears pinned back as the head swings toward the target horse, warning to maintain distance.
	Bite	Opening and rapid closing of the jaws with the teeth grasping another horse. The ears are pinned, and lips retracted.
	Chase	One horse pursuing another, usually at a gallop. The chaser typically pins the ears, exposes the teeth, and bites at the pursued horse's rump and tail. The horse being chased may kick out defensively with both rear legs.
	Ears back	Ears pressed caudally against the head and neck. They were typically associated with intense aggressive interaction.
	Fight	Various behaviours are associated with fighting, not a single aggressive movement. More than one aggressive attempt must be present, including striking, rearing, mounting, lunge, levade, and repeated biting/kicking.
	Head bump	In two horses: a quick lateral toss of the head that forcefully contacts the head and neck of another horse. Usually, the eyes remain closed and the ears forward.
	Herding	Combination of a threat (usually bite) and ears laid back with forward locomotion, apparently directing the movement of another horse.
	Interference	Disruption of combat of other horses by moving between the fighting individuals, pushing, attacking, or simply

	approaching the combatants. One or more horses may simultaneously interfere with an encounter.
Kick threat	Similar to a kick but without sufficient extension or force to make contact with the target. The hind leg(s) lifts slightly off the ground and under the body in tense "readiness".
Kick	One or both hind legs lift off the ground and extend towards another horse, with apparent intent to make contact.
Push	Pressing of the head, neck, shoulder, chest, body or rump against another in an apparent attempt to displace the target horse.
Retreat	A movement that maintains or increases an individual's distance from an approaching horse or a horse initiating some behaviour. The head is usually low, and the ears are turned back. The retreat can be at any gait, even very slow and with little movement from the initiator.
Snapping	Moving the lower jaw up and down in a chewing motion. A sucking sound may be made. Typically, the head and neck ate extended, with the ears relaxed and oriented back or laterally.
Threat	Giving the general appearance of a warning to maintain distance. Threats are typically not directed toward the particular part of the body of another horse.
Olfactory investigation	Involves sniffing various parts of another horse's head and/or body. Considered agonistic if followed by another agonistic behaviour.

6.3.4. Social network measures for individuals

Social network analysis conducted in SOCPROG allowed us to calculate the following measures: Strength, Eigenvector Centrality, Reach, Clustering Coefficient and

Affinity. Social network diagrams for agonistic (Figure 6.1) and affiliative (Figure 6.2) interactions were also prepared in SOCPROG.

Strength is the sum of association indices of any individual with all other individuals. It is counted as the sum of associations minus one. High Strength indicates that an individual strongly associates with others (Whitehead 2008).

Eigenvector Centrality measures how well an individual is associated with others and how well they are associated. Thus, to have high Eigenvector Centrality, an individual will have relatively strong associations with other individuals who have relatively strong associations (Whitehead 2008).

Reach of an individual is a measure of indirect connectedness, so the behaviour of A towards B may influence the behaviour of B towards C (Whitehead 2008).

The Clustering Coefficient is a measure of how well the associates of an individual are themselves associated. If all associates of an individual are linked, then the Clustering Coefficient of that individual is 1.0; if none are, it is 0.0. Clustering coefficients are high in societies containing tight, closed, homogeneous social units and lower in strict territorial societies. The Clustering Coefficient used in the SOCPROG version utilised in this research is the one of Holme et al. (2007), which states that if A is strongly connected to B and C, then B and C are also likely to have a strong connection.

Affinity is a measure of the Strength of its associates, weighted by the association index between them. Thus, an individual with high Affinity has relatively high associations with individuals with high Strength (Whitehead 2008).

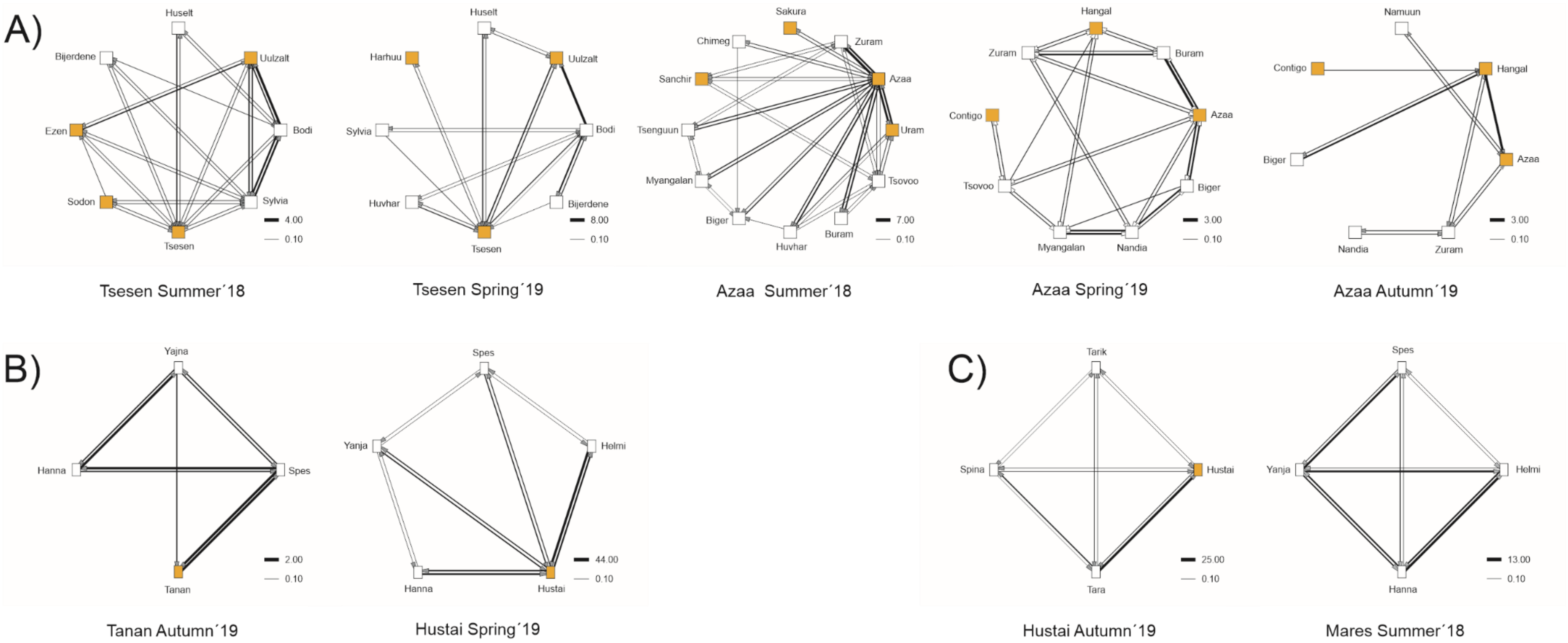

Figure 6.1. Social networks of the agonistic interactions within adult Przewalski's horses' group members. Females are indicated by white, and males by orange colour. All the studied groups are shown grouped as wild-born (A), long-term reintroduced (B), and acclimatizing groups (C). The thickness of each dyadic interaction indicates its strength.

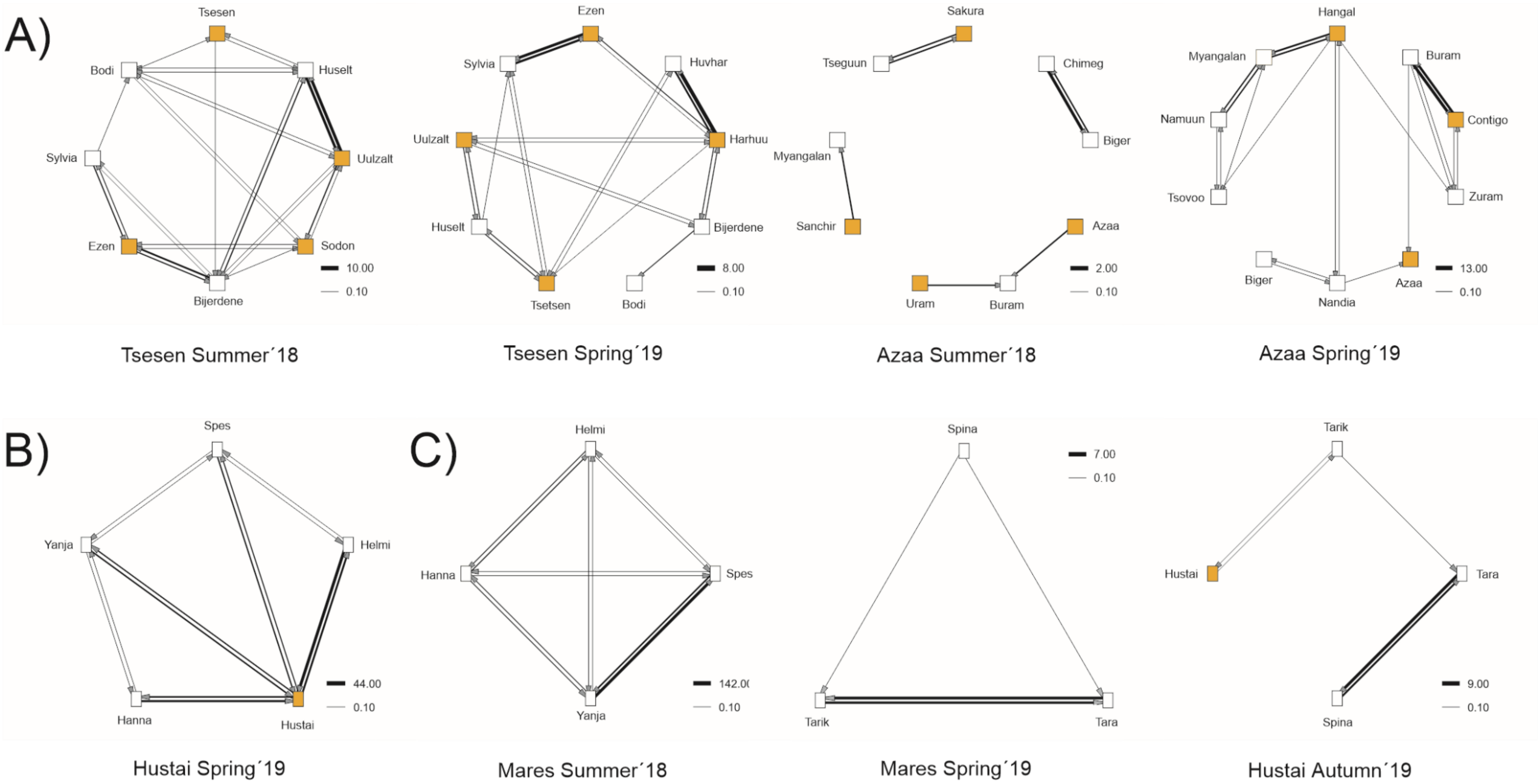

Figure 6.2. Social network of affiliative interactions within adult Przewalski's horses' group members. Females are indicated by white, and males by orange colour. All the studied groups are shown grouped as wild-born (A), long-term reintroduced (B), and acclimatizing groups (C). The thickness of each dyadic interaction indicates its strength.

6.3.5. Characteristics of the group social network

The group social network characteristics were calculated only for affiliative interactions and included Community division by modularity (Q) and Centralisation index (CI). Community division by modularity was calculated in SOCPROG. The Centralisation index was counted based on the formula provided by Ramos et al. (2019).

Community division by modularity (Q)

Association indexes are often high among individuals belonging to the same cluster and low among individuals belonging to different clusters. Community division by modularity shows that group's partition into communities. A modularity value of more than 0.3 means a strong group division (Newman 2004; Whitehead 2009).

Centralisation index (CI)

It shows if and how much a network is controlled by a single or a few individuals. Centralisation measures show a network's tendency for one or a few nodes to be more central than others (Sueur et al. 2011; Griffin and Nunn 2012; Ramos et al. 2019).

Interaction rates

Interaction rates were calculated for all interactions and each interaction category (agonistic and affiliative) for each season. Rates of interactions were counted as the sum of all interactions of a given animal in a given season divided by the total number of observation hours.

6.3.6. Social and ecological factors

Social and ecological factors used in the analyses included: season, group, sex, age, origin, relative time belonging to the group, relatedness and dominance rank. Season, group, sex, age and origin (wild-born *vs*. captive-breed) of all individuals are described in the Supplementary Material. Only individuals over one year of age were used in our study.

Relative time belonging to the group was counted as the time in years that a given individual belonged to a group divided by the average time belonging to the group for all the group members. This relative measure was chosen over the absolute time of an animal belonging to the group to standardise the data among groups with different experiences, but also for statistical reasons (see the *Data analysis* section). The demographic information for each group and each individual was provided from the GGBSPA administration database.

Kinship was analysed in the PMx package, which provides kinship parameters to assess relative genetic uniqueness and degree of relatedness (Ballou et at. 2020). The kinship data is determined by observation of all the harems kept in the GGBSPA administration database and the *Studbook of the Przewalski horse* (Prague Zoo, https://przwhorse.zoopraha.cz/). The data is based on observation, *i.e.*, the maternal relatedness is assumed by the mother/foal interaction, and the paternal relatedness is assumed by the stallion associations at the time of conception. A relatedness index was calculated for each animal as the average relatedness with all the other group mates.

Dominance rank for each member of a group was calculated with the formula: $CBI=(B+\sum b+1)/(L+\sum l+1)$ where B represents the number of individuals that the member defeated in one or more interactions, $\sum b$ represents the total number of individuals (excluding the member) that those represented in B defeated, L represents the number of individuals by which the member was defeated, and $\sum l$ represents the total number of individuals (excluding the member) by which those represented in L were defeated. One is added to the numerator and the denominator in the equation because some group members might not have been observed winning or losing an interaction (Clutton-Brock et al. 1979, Clutton-Brock et al. 1982). An animal is considered defeated after showing a submissive response to displays of aggression or direct physical violence behaviour aimed towards the animals by another group member (Tai et al. 2022). This approach was used for each aggression-submission bout, respectively. The behaviours considered submissive during our study are described in the ethogram (Table 6.2) and include Retreat and Snapping.

6.3.7. Data analysis

All analyses were conducted in IBM® SPSS® Statistics 28 (IBM, Armonk, New York). Data inspection and normality tests were conducted, and further analyses were designed according to the characteristics of the studied variables.

A set of generalised linear mixed models (GLMMs) were designed to test the influence of the selected social and ecological fixed factors (season, group, sex, age, origin, relative time belonging to the group, relatedness index, and dominance rank) on the social network measures of the studied individuals. A first GLMM focused on understanding the factors affecting the rate of all interactions among studied horses. The second set of GLMMs focused on understanding the factors affecting the rate and social network measures of agonistic interactions of the studied horses. The last set of GLMMs was designed to test the factors affecting the rate and social network measures of affiliative interactions of all studied horses. Multicollinearity was tested through the Variance Inflation Index, which showed adequate values (VIF < 5) for the selected factors. The total time belonging to the group, parity (as a metric of reproductive experience) and group size had been previously excluded due to VIF values over 10. The data structure was determined by season as subject and group as a repeated measure, with ID as a random factor. Gamma response was used in all cases except for Eigenvector Centrality-Affiliative, where an Inverse Gaussian response was used. Satterthwaite approximation and robust estimation of fixed effects and coefficients were used to deal with potential violations of the model assumptions (unequal sample size in the different groups). To discard non-significant variables, the traditional stepwise backward selection procedure was used: the model was repeatedly run, excluding the less significant variable from the previous model until a final model with only significant variables was obtained. Corrected Akaike Information Criterion was used to finally select the best model, specifically when models with marginally significant variables were involved.

Median tests were used for comparing the studied behavioural indices at the group level among seasons and groups with different experiences (horses transported from Europe kept in soft-release enclosures; free-ranging reintroduced horses; free-ranging wild-born horses). We refer to the groups in soft-release enclosures as "acclimatizing", to free-ranging reintroduced ones as "long-term reintroduced", and to free-ranging wild-born groups as "wild-born".

6.4. Results

The interaction rate of all interactions decreased significantly with origin (lower in wild horses, F=3.758, p=0.057, β=-0.510) relative time belonging to the group (F=13.864, p <0.001, β=-0.515) and dominance rank (F=12.685, p <0.001, β=-0.153).

The rate of agonistic interaction decreased significantly with relatedness and dominance rank. Strength-Agonistic decreased with relatedness and dominance rank (Table 6.3). Eigenvector Centrality-Agonistic was lower for individuals with higher social rank. Reach-Agonistic decreased with age, relatedness and dominance rank. Clustering Coefficient-Agonistic was lower in wild individuals (Figure 6.3) and also decreased with age. Affinity-Agonistic decreased significantlywith a longer time belonging to the group and a higher degree of kinship.

The rate of affiliative interactions and Strength-Affiliative significantly decreased with relative time belonging to the group and dominance rank and were lower in wild-born horses (Table 6.4; Figure 6.3). Eigenvector Centrality-Affiliative was lower for individuals with higher social rank, in wild-born individuals (Figure 6.3) and those belonging to the group for longer, but increased with age and relatedness and was higher in females. Reach-Affiliative decreased with time and dominance rank and was lower in wild-born animals (Figure 6.3). Clustering Coefficient-Affiliative was lower in animals with a higher degree of kinship and increased with age. Affinity-Affiliative showed significant differences in origin, relative time belonging to the group and dominance rank, being lower in wild-born animals (Figure 6.3), in those with a longer time belonging to the group and in dominant animals.

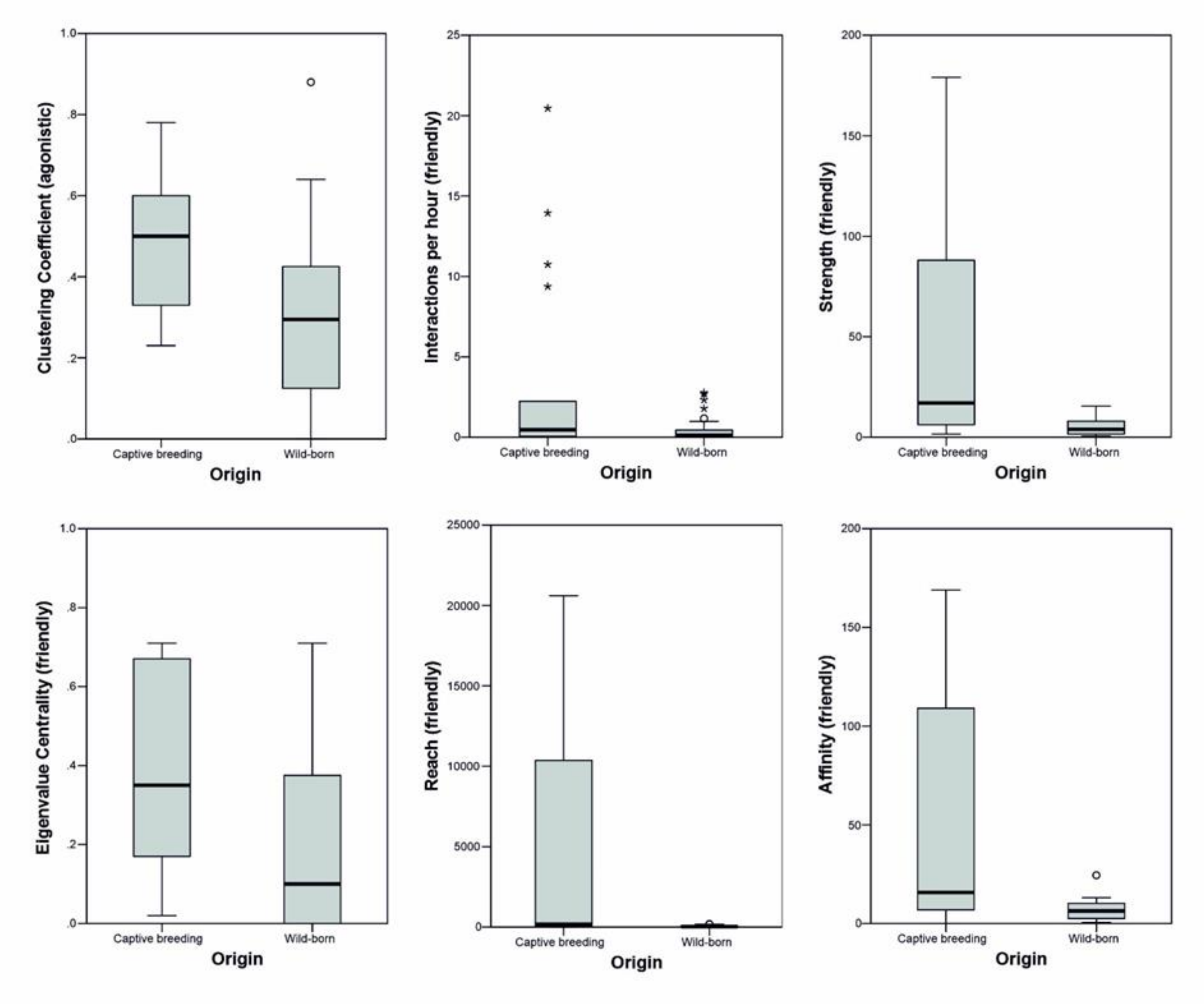

Figure 6.3. Differences in agonistic (top-left figure) and affiliative (rest of the figures) interaction rates and social network measures in the studied Przewalski's horses according to their origin (captive-breeding *vs*. wild-born).

Table 6.3. General linear mixed models (GLMMs) assessing the factors affecting the rate and social network measures of agonistic interactions of all studied horses.

Factor	**Interaction rate**	**Strength**	**Eigenvector Centrality**	**Reach**	**Clustering Coefficient**	**Affinity**
Sex	ns	ns	ns	ns	ns	ns
Age	ns	ns	ns	F=5.586, p=0.022, β=-0.075	F=6.645, p=0.013, β=-0.038	ns
Origin*	ns	ns	ns	ns	F=9.127, p=0.004, β=-0.445	ns
Time in herd	ns	ns	ns	ns	ns	F=7.797, p=0.007, β=-0.313
Relatedness	F=10.833, p=0.002, β=-4.840	F=14.021, p<0.001, β=-5.225	ns	F=72.966, p<0.001, β=-13.330	ns	F=39.447, p<0.001, β=-6.063
Rank	F=7.218, p=0.009, β=-0.127	F=6.394, p=0.014, β=-0.113	F=16.767, p<0.001, β=-0.094	F=6.744, p=0.012, β=-0.147	ns	ns

ns = not significant

*Captive-breeding origin was used as category of reference. Therefore, positive β means higher values in wild animals compared with reintroduced ones.

At the group level, social network measures and characteristics of the social network are shown in Table 5. These measures were generally stable since median tests detected a low influence of season and group experience. Nevertheless, certain differences were detected. Eigenvector Centrality-Agonistic was higher for groups with high (wild-born, 0.470) and medium experience (long-term reintroduced, 0.455) compared to the groups with low experience (acclimatizing, 0.304; M=9.000, p=0.011). The same pattern was evident for Eigenvector Centrality-Affiliative (M=5.760, p=0.056), being higher in groups with high experience (0.457) compared to medium (0.350) and low experience (0.334).

Table 6.4. General linear mixed models (GLMMs) assessing the factors affecting the rate and social network measures of affiliative interactions of all studied horses.

Factor	Interaction rate	Strength	Eigenvector Centrality	Reach	Clustering coefficient	Affinity
Sex*	ns	ns	F=10.282, p=0.003, β=0.086	ns	ns	ns
Age	ns	ns	F=7.682, p=0.009, β=0.016	ns	F=4.008, p=0.054, β=0.06	ns
Origin**	F=6.028, p=0.017, β=-0.953	F=14.348, p<0.001, β=-1.459	F=5.211, p=0.029, β=-0.340	F=49.953, p<0.001, β=-3.457	ns	F=18.103, p<0.001, β=-1.366
Time in herd	F=18.413, p<0.001, β=-0.966	F=19.626, p<0.001, β=-1.041	F=12.197, p=0.001, β=-0.306	F=27.657, p<0.001, β=-1.516	ns	F=11.252, p=0.002, β=-0.633
Relatedness	ns	ns	F=13.842, p<0.001, β=3.100	ns	F=8.673, p=0.006, β=-3.732	ns
Rank	F=4.364, p=0.042, β=-0.128	F=4.475, p=0.047, β=-0.119	F=12.184, p=0.001, β=-0.530	F=8.625, p=0.005, β=-0.214	ns	F=11.115, p=0.002, β=-0.159

ns = not significant

*Male sex was used as category of reference. Therefore, positive β means higher values in females compared with males.

**Captive-breeding origin was used as category of reference. Therefore, positive β means higher values in wild animals compared with reintroduced ones.

Table 6.5. Social network measures and characteristics of the group social network at group level for all the groups studied.

Period	**Group**	**Measure**	**Strength**	**Eigenvector Centrality**	**Reach**	**Clustering Coefficient**	**Q***	**Cl(%)****
Summer/18	Azaa	Agonistic	7.67	0.25	124.17	0.34		
		Affiliative	0.89	0.16	0.94	0.00	0.84	65.56
Summer/18	Tsetsen	Agonistic	5.50	0.32	37.56	0.36		
		Affiliative	9.00	0.27	103.62	0.17	0.38	44.58
Summer/18	Mares18	Agonistic	18.50	0.49	355.13	0.59		
		Affiliative	119.25	0.48	15621.25	0.29	0.38	36.84
Spring/19	Azaa	Agonistic	4.33	0.31	21.50	0.21		
		Affiliative	7.50	0.17	80.90	0.15	0.67	62.79
Spring/19	Tsetsen	Agonistic	6.38	0.31	60.00	0.45		
		Affiliative	5.55	0.28	37.94	0.05	0.48	46.00
Spring/19	Hustai1	Agonistic	43.20	0.42	2795.20	0.49		
		Affiliative	14.80	0.35	307.70	0.23	0.47	53.70
Spring/19	Mares19	Agonistic	-	-	-	-		
		Affiliative	4.33	0.51	24.33	0.39	0.50	37.78
Autumn/19	Azaa	Agonistic	2.14	0.33	6.57	0.24		
		Affiliative	-	0.79	7.77	4.40	-	46.8
Autumn/19	Tsetsen	Agonistic	-	-	-	-		
		Affiliative	-	-	-	-	-	-
Autumn/19	Tanan	Agonistic	3.25	0.49	11.13	0.46		

		Affiliative	-	-	-	-	-	-
Autumn/19	Hustai2	Agonistic	16.00	0.45	316.25	0.42		
		Affiliative	5.00	0.38	34.25	-	0.56	53.04

Dashes indicate measures that couldn´t be calculated due to low number of interactions performed by the horses at each herd and period.

* Community division by modularity. ** Centralization index.

6.5. Discussion

Studies reporting differences in social network metrics between groups are rare, and even more when these groups vary in terms of their experience in the context of a reintroduction program (wild-born *vs*. acclimatizing *vs*. long-term reintroduced groups). Most previous studies of the social behaviour of horses focused primarily on the relationships among group members but not on the comparison of different groups of different origins (Ebhardt 1957; Blakeslee 1974; Wells and Goldschmidt-Rothschild 1979; Keiper and Sambraus 1986; Keiper 1988; van Dierendonck et al. 1995; Berger 1999; Vervaecke et al. 2007; Bourjade et al. 2009a). The rate of interactions and Strength are highly connected, and the models showing the variables affecting them were remarkably similar; thus, these two measures are discussed together.

The results confirmed our hypothesis: horses' origin (captive-breeding *vs*. wild-born) strongly influences the social network at agonistic (Table 6.3) and affiliative (Table 6.4) levels. For agonistic interactions, origin affected the Clustering Coefficient-Agonistic, which was lower in wild-born individuals (*i.e.*, the network density in terms of aggressiveness was lower for wild-born horses of our study). This result agrees with previous studies revealing that in stable groups, the hierarchy between horses is set, and the knowledge of the dominance status of group mates makes new challenges unnecessary (Berger 1977; Wells & Goldschmidt-Rothschild 1979; Heitor et al. 2006). One limitation of the study is that only wild-born animals were observed exclusively in free-ranging conditions, while reintroduced individuals were observed both in enclosures and free-ranging. The lower Clustering Coefficient-Agonistic in wild animals in our study is in line with previous studies suggesting that horses in enclosed areas display higher aggression rates (Keiper & Receveur 1992).

Origin strongly affected most social network metrics based on affiliative interactions (Table 6.4). The lower Strength-Affiliative and rate of affiliative interactions observed in wild-born horses confirm previous observations by Feh and Carton de Graumont (1995), who stated that new groups display more affiliative interactions than stable groups of horses. Weaker affiliative connections and network densities were also found in wild-born animals compared to the captive-bred ones (lower Eigenvector Centrality-Affiliative, Reach-Affiliative and Affinity-Affiliative). These results indicate the existence of well-established roles among the group members for wild-born horses, not needing continuous reinforcement. Some of our captive-bred individuals were observed shortly after transportation from Europe. Thus the higher occurrence of affiliative interactions and Strength-Affiliative (as well as higher Eigenvector Centrality-Affiliative, Reach-Affiliative, and Affinity-Affiliative) among them could also be interpreted as a stress-reducing behaviour (Hogan et al. 1988). A low indirect connectedness was found in wild-born horses (low Affinity-Friend); in other words, lower "behavioural contagion", which is also typical for stable groups. Altogether, the results suggest that forming complex stable relationships requires time; thus, such a process may be essential for the long-term success of the reintroduction program (Gusset et al. 2010; Gaudioso et al. 2011).

Beyond the horses´ origin, other factors affected the social networks for agonistic and affiliative interactions. The Strength-Agonistic and the rate of agonistic interaction significantly decreased with dominance rank and kinship (Table 6.3). Various studies have reported opposite results: regardless of their weight, height, sex, or time belonging to the group, aggressive horses attain higher ranks than passive horses (fenced: Ebhardt 1957; free-ranging: Blakeslee 1974; Keiper and Receveur 1992). However, finding of other studies align with our results, suggesting that the most aggressive horses are not usually the most dominant (Berger 1986; Feh 1988; van Dierendonck et al. 1995; Bourjade et al. 2009b). We interpret reduced aggression in high-ranking animals in our study is caused by the fact that once the hierarchy is formed (stable harems), it is maintained without the necessity of continuous aggressive interactions (Berger 1977; Wells and Goldschmidt-Rothschild 1979).

Regarding kinship, low aggression between relatives has been observed in other social ungulates like red deer (Ceacero et al. 2007). In feral horses, Heitor et al. (2006) found no effect of kinship on aggressive behaviour, and Boyd and Houpt (1994) stated that closely-related Przewalski's horses are typically less aggressive towards each other than towards more distantly related individuals. Moreover, high-ranking horses did not have strong, aggressive associations with individuals who have strong associations themselves. Results thus suggest that the affiliative bonds among relatives become essential in harsh environments for reducing stress (Hogan et al. 1988).

Clustering Coefficient-Agonistic decreased with age, and Reach-Agonistic decreased with age, relatedness and dominance rank, meaning that older animals, those with relatives in the group, and high-ranked ones show low indirect connections regarding agonistic behaviours, again typical of stable groups (Table 6.3). The horse's social position in the group is highly correlated with its age (Houpt et al. 1978, Keiper and Sambraus 1986, Keiper and Receveur 1992, Linklater et al. 1999), which may also explain these results. Animals belonging to the group for a longer time and animals with a higher degree of kinship were less likely to be aggressive towards very aggressive individuals (lower Affinity-Agonistic; Table 6.3).

Strength-Affiliative significantly decreased with relative time spent in the group and higher dominance rank. Older animals, more closely-related animals and females had stronger associations with individuals who also have strong associations (higher Eigenvector Centrality-Friend; Table 6.4). Friends of related and older horses were more likely to be friends also with each other. Less dominant animals were more likely to associate with individuals who have strong associations. Previous studies found no link between time belonging to a group and the occurrence of allogrooming, nor between affiliative interactions and dominance rank (CluttonBrock et al. 1976; King and Gurnell 2019). However, a negative correlation between affiliative behaviours and a horse's dominance rank was reported by Keiper and Receveur (1992). It has been proven that affiliative behaviour, especially allogrooming, might be costly for the donor (tooth attrition, loss of water in saliva, reduction in feeding, or reduction in vigilant behaviour; Mooring and Hart 1995; Henzi and Barrett 1999). For this reason, it could be exchanged for other social benefits, including tolerance from more dominant individuals or maintaining social bonds (Henzi and Barrett 1999; Gumert 2007; Schino 2007). In severe environmental conditions such as in the Mongolian Gobi, group living is highly connected to the fitness of equids and may even be vital for their survival (Zhang et al. 2015, Gersick et al. 2017). Therefore, the negative correlation of dominance rank and affiliative interactions among horses in our study might be driven by the "trade-off hypothesis", indicating that subordinates exchange allogrooming (and other affiliative behaviours) to be tolerated in the group and develop and preserve social bonds with more dominant horses. Indeed, adult horses show little allogrooming rate (Crowell-Davis et al. 1986). Another explanation might be connected to the need for appeasement or reconciliation as in group living horses, where affiliative reunions often serve as mechanisms of non-dispersive conflict resolution (Cozzi et al. 2010). In this case, reconciliation would be directed by subordinate towards more dominant horses, which might use grooming (or other affiliative interactions) to appease higher-ranked animals (Keiper 1988).

Clustering Coefficient-Affiliative decreased with kinship, but Eigenvector Centrality-Affiliative increased with a higher degree of relatedness in affiliative interactions in our study. To have high Eigenvector Centrality, individuals have relatively strong associations with others who also have relatively strong associations (Whitehead 2008). This result is in line with the study of Heitor et al. (2006), who found that kinship significantly affects the affiliative behaviours of horses; bonds are reciprocal and more robust among horses with higher relatedness and with Van Dierendonck et al. (2004), who observed a positive correlation between kinship and allogrooming. We assume that this result might be caused by the fact that horses do not choose their friends based on relatedness but also a close acquaintance or knowledge of other group members (Monard et al. 1996; Linklater 2000).

Sex did not influence the majority of the network measures of our study. It only affected Eigenvector Centrality-Affiliative, which was higher in females. Keiper and Receveur (1992) also found that females initiated most allogrooming events in a group of Przewalski's horses in a semi-reserve. We assume that affiliative behaviour between mares in our study provides harem cohesiveness independent of the stallion, as described by Crowell-Davis et al. (1986).

Contradictory to previous research, we observed no effect of seasons on the social network measures of our study. Previous studies detected the season's influence on affiliative and agonistic interactions. King and Gurnell (2019) found prevalence of affiliative interactions in the spring season, and Tyler (1972) and Kimura (1998) observed the most associative interactions in the summer. It is noted that winter monitoring was not possible due to low accessibility to the area (frozen paths) and the technical impossibility of recording the herds due to the low temperatures. For this reason, we emphasise a need for further research concerning all seasons (including winter).

At the group level, we detected no influence of experience on the affiliative and agonistic interaction rates of the studied groups, and the social network measures were relatively stable. Nonetheless, experience influenced Eigenvector Centrality-Agonistic, indicating high aggressiveness towards group mates who are also highly aggressive. It was higher in groups with high (wild-born) and medium (long-term reintroduced) experience compared to the groups with low experience (acclimatizing). Similarly, Eigenvector Centrality-Affiliative indicating strong associations with highly associative group mates was marginally higher in groups with high experience compared to medium and low experience. It is exciting to interpret these results in connection with the results obtained at the individual level: while wild-born groups have well-defined social networks (see Figures 1 and 2) and strong association indices, wild-born individuals have weak ones. In other words, members of wild-born harems do not need to display many agonistic or affiliative behaviours to maintain a group with strong social metrics. On the contrary, groups composed of on captive-born individuals are highly interactive both in terms of agonistic and especially affiliative interactions.

It should be noted that three groups (Mares18, Mares19, and Hustai2) were observed exclusively in their enclosures. Moreover, the Mares19 group was observed just three days after transportation to Mongolia. Even if the enclosures are large enough to allow the animals not to be disturbed by human presence, the behaviour of these horses could have been influenced by these factors (transportation, semi-captivity). Similarly, more extended recording of more groups would have yielded more robust results; thus, extended monitoring of different types of groups is strongly recommended.

In summary, all the individual and group-level results indicate that experienced groups composed of wild-born individuals are more strongly socially connected than newly formed groups based on reintroduced individuals. The transition from the soft-released to the wild-born groups clearly shows more robust networks with lower interaction rates in line with previous studies describing the noticeable acclimatisation period during which released horses gradually switch to the behavioural patterns of wild horses (Scheibe et al. 1997; Boyd and Bandi 2002). Indeed, our previous results in these same groups also show that wild-born harems are better adapted to deal with changing weather conditions than reintroduced ones (Bernatková et al. 2022). As the population of Przewalski's horses in Mongolia grows, understanding its behaviour becomes increasingly vital and may aid in improving the reintroduction program. Przewalski's horses not only require an acclimatisation period in the enclosure to adapt to the local environmental conditions but also to form social relationships through a high rate of interactions during this period. Long-term monitoring of the behavioural ecology of released Przewalski's horses released to novel habitats is thus essential to ensure a successful reintroduction.

6.6. References

Baker A. 2015. Personality and its role in the fitness and life history of Przewalski’s horses *Equus ferus przewalskii* at Le Villaret, France. MSc. Nottingham Trent University, Nottingham, UK.

Barber JA, Crowell-Davis SL. 1994. Maternal behavior of Belgian (*Equus caballus*) mares. Appl Anim Behav Sci. 41: 161–189. doi: https://doi.org/10.1016/0168-1591(94)90021-3

Berger A. 1999. Diurnal and ultradian rhythms of behaviour in a mare group of Przewalski horse (*Equus ferus przewalskii*), measured through one year under semi-reserve conditions. Appl Anim Behav Sci. 64: 1–17.

Berger J. 1977. Organizational systems and dominance in feral horses in the Grand Canyon. Behav Ecol Sociobiol. 2: 131–143.

Berger, J., 1986. Wild horses of the Great Basin. Chicago, USA: University of Chicago Press.

Berger J, Cunningham C. 1987. Influence of familiarity on frequency of inbreeding in wild horses. Evolution 41: 229-31.

Berger A. Scheibe, Eichhorn KM, Scheibe K, Streich J. (1999). Diurnal and ultradian rhythms of behaviour in a mare group of Przewalski horse (*Equus ferus przewalskii*), measured through one year under semireserve conditions. Appl Anim Behav Sci 64, 1-17.

Berger-Tal O, Polak T, Oron A, Lubin Y, Kotler BP, Saltz D. 2011. Integrating animal behavior and conservation biology: a conceptual framework. Behav Ecol. 22: 236–239. doi: https://doi.org/10.1093/beheco/arq224

Bernátková A, Oyunsaikhan G, Šimek J, Komárková M, Bobek M, Ceacero F. 2022. Influence of weather on the behaviour of reintroduced Przewalski’s horses in the Great Gobi B Strictly Protected Area (Mongolia): implications for conservation. BMC Zool. 7(1):1-17.

Bernstein, I.S. and L. G. Sharpe (1966): Social roles in a rhesus monkey group. Behaviour. 26: 91-104

Blakeslee, J. 1974. Mother-young relationships and related behavior among free-ranging Appaloosa horses. MSc. Idaho State University, Pocatello, 113 pp.

Bourjade M, Tatin L, King SRB, Feh C. 2009b. Early reproductive success, preceding bachelor ranks and their behavioural correlates in young Przewalski's stallions. Ethol Ecol Evol. 21:1–14. doi: https://doi.org/10.1080/08927014.2009.9522507

Bourjade M, Thierry B, Maumy M, Petit O. 2009a. Decision-making in Przewalski Horses (*Equus ferus przewalskii*) is driven by the ecological contexts of collective movements. Ethology. 115:321–330. doi: https://doi.org/10.1111/j.1439-0310.2009.01614.x

Bousquet, C. A. H. 2011. Group decision-making in meerkats (*Suricata suricatta*). Ph.D. University of Zurich, Zurich, Switzerland.

Boyd LE, Houpt KA. 1994. Przewalski's Horse: The History and Biology of an Endangered Species. State University of New York Press, Albany.

Boyd L, Bandi N. 2002. Reintroduction of takhi, *Equus ferus przewalskii*, to hustai National Park, mongolia: Time Budget and Synchrony of activity pre- and post-release. Appl Anim Behav Sci. 78:87–102. doi: https://doi.org/10.1016/s0168-1591(02)00088-6

Boyd, L. E. Scorolli, A. Nowzari, H. and Bouskila, A. 2016. Social organization of wild equids. *In*: Ransom, J. I. and Kaczensky, P. eds. Wild Equids: ecology, management, and conservation. Baltimore, Maryland, USA: John Hopkins University Press, 7–22.

Briard L, Dorn C, Petit O. 2015. Personality and affinities play a key role in the organisation of collective movements in a group of domestic horses. Ethology. 121:888–902. doi: https://doi.org/10.1111/eth.12402

Burnik Šturm M, Ganbaatar O, Voigt CC, Kaczensky P. 2017. First field-based observations of δ 2H and δ 18O values of event-based precipitation, rivers and other water bodies in the Dzungarian Gobi, SW Mongolia. Isot Environ Healt S. 53(2):157-71. https://doi.org/10.1080/10256016.2016.1231184

Ceacero F, Landete-Castillejos T, García AJ, Estévez JA, Gallego L. 2007. Kinship discrimination and effects on social rank and aggressiveness levels in Iberian red deer hinds. Ethology. 113(12):1133-1140.

Clutton Brock TH, Greenwood PJ, Powell RP. 1976. Ranks and relationships in Highland ponies and Highland cattle. Z Tierpsychol. 41:202-216.

Clutton-Brock TH, Albon SD, Gibson RM, Guinness FE. 1979. The logical stag: adaptive aspects of fighting in red deer (*Cervus elaphus* L.). Anim Behav. 27: 211–225.

Clutton-Brock TH, Guinness FE, Albon SD. 1982. Red Deer: Behavior and Ecology of Two Sexes. University of Chicago Press, Chicago,

Conradt L, Krause J, Couzin ID, Roper TJ. 2009. "leading according to need" in self-organizing groups. Am Natural. 173:304–312. doi: https://doi.org/10.1086/596532

Cozzi A, Sighieri C, Gazzano A, et al .2010. Post-conflict friendly reunion in a permanent group of Horses (*Equus caballus*). Behav Processes. 85:185–190. doi: https://doi.org/10.1016/j.beproc.2010.07.007

Croft DP, James R, and Krause J. 2008. Exploring Animal Social Networks. Princeton, USA: Princeton University Press, New Jersey.

Crowell-Davis SL, Houpt KA, Carini CM. 1986. Allogrooming and nearest-neighbour relationships among foals of *Equus caballus*. Appl Anim Behav Sci 15: 113–123. doi: https://doi.org/10.1016/0168-1591(86)90057-2.

Duncan P, Boy V, Monard A-M. 1996. The proximate mechanisms of natal dispersal in female horses. Behaviour 133:1095–1124. doi: https://doi.org/10.1163/156853996x00611

Ebhardt, H. 1957. Drei unterschiedliche Verhaltensweisen von Islandpferden in norddeutschen Freigehegen. Saugetierkd. Mitt. 5:113-117.

Ellis L. 1995. Dominance and reproductive success among nonhuman animals: a cross-species comparison. Ethol Sociobiol 16:257–333

Estep DQ, Crowell-Davis SL, Earl-Costello S-A, Beatey SA. 1993. Changes in the social behaviour of drafthorse (*Equus caballus*) mares coincident with foaling. Appl Anim Behav Sci. 35:199–213. doi: https://doi.org/10.1016/0168-1591(93)90137-e.

Feh C. 1988. Social behavior and relationships of Przewalski horses in Dutch semi-reserves. Appl Anim Behav Sci. 21:71–87.

Feh, C. 1990. Long-term paternity data in relation to different aspects of rank for Camargue stallions, *Equus caballus*. Anim Behav. 40:995–996.

Feh C, de Grammont SC. 1995. A Natural Herd of Przewalski Horses on the Causse Méjan: Technical and Scientific Report (1993–1995). Association pour le Cheval de Przewalski—TAKH, Le Villaret, Meyrueis.

Feh C 1999. Alliances and reproductive success in Camargue stallions. Anim Behav. 57: 705–713. doi: https://doi.org/10.1006/anbe.1998.1009.

Feh C, de Mazières J. 1993. Grooming at a preferred site reduces heart rate in horses. Anim Behav. 46:1191–1194. doi: https://doi.org/10.1006/anbe.1993.1309.

Feist JD. 1971. Behavior of feral horses in the Pryor Mountain Wild Horse Range. Master's thesis, University of Michigan, Ann Arbor, Michigan.

Feist, J. D. and McCullough, D. R. 1976. Behavior patterns and communication in feral horses. Z. Tierpsych. 41:337–371.

Fischer J, Lindenmayer DB. 2000. An assessment of the published results of animal relocations. Biol Cons. 96:1–11. doi: https://doi.org/10.1016/s0006-3207(00)00048-3

Fischhoff IR, Sundaresan SR, Cordingley J, Rubenstein D. 2007. Habitat use and movements of Plains Zebra (*Equus Burchelli*) in response to predation danger from Lions. Behav Ecol 18:725–729. doi: https://doi.org/10.1093/beheco/arm036

Flack JC, Girvan M, de Waal FB, Krakauer DC. 2006. Policing stabilizes construction of social niches in primates. Nature 439:426–429. doi: https://doi.org/10.1038/nature04326

Gersick AS, Rubenstein DI. 2017. Physiology modulates social flexibility and collective behaviour in equids and other large ungulates. Philos. Trans. R. Soc. Lond., B, Biol. Sci. 372:20160241. doi: https://doi.org/10.1098/rstb.2016.0241

Griffin RH, Nunn CL. 2011. Community structure and the spread of infectious disease in Primate Social Networks. Evol Ecol 26:779–800. doi: https://dx.doi.org/10.1007/s10682-011-9526-2

Goldenberg SZ, Owen MA, Brown JL, Wittemyer G, Oo ZM, Leimgruber P. 2019. Increasing conservation translocation success by building social functionality in released populations. Global Ecol Cons. 18:e00604.

Gumert M.D. 2007. Payment for sex in a macaque mating market. Anim Behav. 74: 1655–1667. doi: https://dx.doi.org/10.1016/j.anbehav.2007.03.009

Heitor F, Oom Mdo, Vicente L. 2006. Social relationships in a herd of Sorraia Horses. Behav Process. 73:170–177. doi: https://dx.doi.org/10.1016/j.beproc.2006.05.004

Henzi S. Barrett L. 1999. The value of grooming to female primates. Primates 40: 47–59. doi: https://dx.doi.org/10.1007/bf02557701

Hinde, R. A. 1976. Interactions, relationships, and social-structure. Man. 11: 1–17.

Holme P, Min Park S, Kim BJ, Edling CR. 2007. Korean university life in a network perspective: Dynamics of a large affiliation network. Phys. A: Stat. Mech. Appl. 373:821–830. doi: https://dx.doi.org/10.1016/j.physa.2006.04.066

Hogan E, Houpt K, Sweeney K. 1988. The effect of enclosure size on social interactions and daily activity patterns of the captive Asiatic Wild Horse (*Equus przewalskii*). Appl. Anim. Behav. Sci. 21: 47-168.

Houpt KA, Law K, Martinisi V. 1978. Dominance hierarchies in domestic horses. Appl Anim Ethol 4:273–283

Houpt K, Keiper R. 1982. The position of the stallion in the equine dominance hierarchy of feral and domestic ponies. J. Anim. Sci. 54: 945-950.

Houpt KA, Boyd L 1994. Social behavior. In LE Boyd, KA Houpt (Eds), Przewalski’s Horse. State University of New York Press, Albany, New York, pp 229–254.

IUCN/SSC, 2013. Guidelines for reintroductions and other conservation translocations. IUCN Species Survival Commission. Gland, Switzerland.

Keiper, R. and Sambraus, H. 1986. The stability of equine dominance hierarchies and the effects of kinship, proximity, and foaling status on hierarchy rank. Appl. Anim. Behav. Sci. 16: 121-130.

Keiper, R. 1988. Social interactions of the Przewalski horse herd at the Munich Zoo. Appl. Anim. Behav. Sci. 21: 89-97.

Keiper R. Receveur H. 1992. Social interactions of free ranging Przewalski horses in semi-reserves in the Netherlands. Appl Anim Behav Sci 33(4): 303– 318. doi: https://dx.doi.org/10.1016/s0168-1591(05)80068-1

Kimura R. 1998. Mutual grooming and preferred associate relationships in a band of free-ranging horses. Appl Anim Behav Sci. 59:265–276. doi: https://dx.doi.org/10.1016/s0168-1591(97)00129-9

King SRB. 2002. Behavioural ecology of Przewalski horses (*Equus przewalskii*) reintroduced to Hustai National Park, Mongolia. Ph.D. Queen Mary, University of London, London, UK.

King SRB, Asa C, Pluhacek J, Houpt KA, Ransom JI. 2016. Behavior of horses, zebras, and asses. In: Ransom, J. I. and Kaczensky, P. eds. Wild Equids: ecology, management, and conservation. Baltimore, Maryland, USA: John Hopkins University Press, 23–40.

King SRB, Boyd LE, Zimmermann W, Kendall BE. 2015. *Equus ferus*. The IUCN Red List of Threatened Species. Available from: http://dx.doi.org/10.2305/IUCN.UK.2015-2.RLTS.T41763A45172856.en.

King SRB. Gurnell J. 2019. Associative behaviour in Przewalski's horses reintroduced into Mongolia. Nat Cons Res 4: 1–9. doi: https://dx.doi.org/10.24189/ncr.2019.023

Knight J. 2001. If they could talk to the animals. Nature. 414:246–247. doi: https://dx.doi.org/10.1038/35104708

Krueger K, Heinze J. 2008. Horse sense: Social status of Horses (*Equus caballus*) affects their likelihood of copying other horses' behavior. Anim Cogn 11:431–439. doi: https://dx.doi.org/10.1007/s10071-007-0133-0

Krueger K, Flauger B, Farmer K, Hemelrijk C. 2014. Movement initiation in groups of feral horses. Behav Process 103:91–101. doi: https://dx.doi.org/10.1016/j.beproc.2013.10.007

Lehner, P. N. 1996. Handbook of ethological methods. 2nd ed. Cambridge, UK: Cambridge University Press.

Linklater WL. 2000. Adaptive explanation in socio-ecology: Lessons from the Equidae. Biol Rev 75:1–20. doi: https://dx.doi.org/10.1111/j.1469-185x.1999.tb00039.x

Linklater WL, Cameron EZ, Minot EO, Stafford KJ. 1999. Stallion harassment and the mating system of horses. Anim Behav 58:295–306. doi: https://dx.doi.org/10.1006/anbe.1999.1155

List C. 2004. Democracy in animal groups: A political science perspective. Trend Ecol Evol 19:168–169. doi: https://dx.doi.org/10.1016/j.tree.2004.02.004

Lusseau D. 2006. Evidence for social role in a Dolphin Social Network. Evol Ecol 21:357–366. doi: https://dx.doi.org/10.1007/s10682-006-9105-0

Lusseau D, Conradt L. 2009. The emergence of unshared consensus decisions in Bottlenose Dolphins. Behav Ecol Sociobiol. 63:1067–1077. doi: https://dx.doi.org/10.1007/s00265-009-0740-7

Macdonald DW. 2016. Animal behaviour and its role in carnivore conservation: Examples of seven deadly threats. Anim Behav 120:197–209. doi: https://dx.doi.org/10.1016/j.anbehav.2016.06.013

McDonnell SM, Haviland JC. 1995. Agonistic ethogram of the equid bachelor band. Appl Anim Behav Sci. 43:147-88.

McDonnell SM, Poulin A. 2002. Equid play Ethogram. Appl Anim Behav Sci 78:263–290. doi: https://dx.doi.org/10.1016/s0168-1591(02)00112-0

Menzel EW. 1973. Leadership and communication in young chimpanzees. Menzel EW (Ed.), Precultural Primate Behaviour, Karger, Basel. pp. 192-225

Miller R, Denniston R. H. 1979. Interband dominance in feral horses. Z. Tierpsychologie. 51: 41–47.

Michler LM, Kaczensky P, Ploechl JF, Batsukh D, Baumgartner SA, Battogtokh B, Treydte AC. 2022. Moving Toward the Greener Side: Environmental Aspects Guiding Pastoral Mobility and Impacting Vegetation in the Dzungarian Gobi, Mongolia. Rangeland Ecol Manag 83:149-60. https://doi.org/10.1016/j.rama.2022.03.006

Moehlman, P. D. 1998. Behavioral patterns and communication in feral asses (*Equus africanus*). Appl Anim Beh Sci. 60: 125–169.

Moehlman PD, King SRB, Kebede F. 2016. Status and conservation of threatened equids. In: Ransom, J. I. and Kaczensky, P. eds. Wild Equids: ecology, management, and conservation. Baltimore, Maryland, USA: John Hopkins University Press, 167–186.

Monard AM, Duncan P. 1996. Consequences of natal dispersal in female horses. Anim Behav 52: 565-79.

Mooring MS, Hart B.L. 1995. Costs of allogrooming in impala: Distraction from vigilance. Anim Behav 49: 1414–1416. doi: https://dx.doi.org/10.1006/anbe.1995.0175

Moseby KE, Blumstein DT, Letnic M, West R. 2020. Choice or opportunity: are post-release social groupings influenced by familiarity or reintroduction protocols? Oryx. 54(2): 215-221.

Newman MEJ. 2006. Modularity and community structure in networks. Proc. Natl. Acad. Sci. 103, 8577–8582. doi: https://doi.org/10.1073/pnas.0601602103 .

Nowzari H, Hemami M, Karami M, et al. 2013. Habitat use by the Persian onager, *Equus hemionus onager* (Perissodactyla: Equidae) in Qatrouyeh National Park, Fars, Iran. J Nat History 47:2795–2814. doi: https://dx.doi.org/10.1080/00222933.2013.802040

Ozogány K. Vicsek T. 2015. Modeling the Emergence of Modular Leadership Hierarchy During the Collective Motion of Herds Made of Harems. J Stat Phys. 158:628–646. doi: https://doi.org/10.1007/s10955-014-1131-7

Pereladova OB, Sempéeré AJ, Soldatova NV, Dutov VU, Fisenko G, Flint VE. 1999. Przewalski's horse—adaptation to semi-wild life in desert conditions. Oryx. 33:47-58.

Pyritz LW, Fichtel C, Huchard E, Kappeler PM. 2013. Determinants and outcomes of decision-making, group coordination and social interactions during a foraging experiment in a wild primate. PLoS ONE. doi: https://dx.doi.org/10.1371/journal.pone.0053144

Reebs SG. 2000. Can a minority of informed leaders determine the foraging movements of a fish shoal? Anim Behav 59:403–409. doi: https://dx.doi.org/10.1006/anbe.1999.1314

Roberts JM, Browning BA. 1998. Proximity and threats in Highland Ponies. Soc. Networks. 20:3.

Rubenstein DI. 1989. Life history and social organization in arid adapted ungulates. J. Arid Environ. 17:145–56.

Rubenstein DI. 1994. The ecology of female social behaviour in horses, zebras and asses. In: Jarman, P. and Rossiter, A. eds. Animal Societies: individuals, interactions, and organization. Kyoto, Japan: Kyoto University Press, 13–28.

Ramos A, Manizan L, Rodriguez E, et al. 20.19. The social network structure of a semi-free roaming European bison herd (*Bison bonasus*). Behav Process. 158:97–105. doi: https://dx.doi.org/10.1016/j.beproc.2018.11.005

Rowell TE. 1974. The concept of social dominance. Behav. Biol. 11:131-154

Sarrazin F, Barbault R. 1996. Reintroduction: Challenges and lessons for basic ecology. Trends in Ecology & Evolution. 11:474–478. doi: https://dx.doi.org/10.1016/0169-5347(96)20092-8

Scheibe KM, Lange B, Lange V, Eichhorn E, Scheibe A, Streich J. 1997. Przewalskipferde in einem Semireservat–Verhaltensuntersuchungen zur Vorbereitung einer Auswilderung. Curr. Res. Appl. Ethol. 376:120–131.

Schino G. 2007. Grooming and agonistic support: a meta-analysis of primate reciprocal altruism. Behavioural Ecology. 18: 115–120. doi: https://doi.org/10.1093/beheco/arl045

Schoenecker KA, King SRB, Nordquist MK, Nandintsetseg D, Cao Q. 2016. Habitat and diet of equids. In: Wild Equids: ecology, management, and conservation. Baltimore, Maryland, USA: John Hopkins University Press, 41–57.

Schneider G, Krueger K. 2012. Third-party interventions keep social partners from exchanging affiliative interactions with others. Anim Behav 83:377–387. doi: https://dx.doi.org/10.1016/j.anbehav.2011.11.007

Seddon PJ. 1999. Persistence without intervention: assessing success in wildlife reintroductions. Trends in Ecology & Evolution, 14:50-53

Shier DM. 2006. Effect of family support on the success of translocated black-tailed prairie dogs. Cons Biol. 20(6):1780-1790.

de Silva S, Schmid V, Wittemyer G. 2016. Fission–fusion processes weaken dominance networks of female Asian elephants in a productive habitat. Behav Ecol 28:243–252. doi: https://dx.doi.org/10.1093/beheco/arw153

Snijders L, Blumstein DT, Stanley CR, Franks DW (2017) Animal Social Network Theory can help wildlife conservation. Trends in Ecology & Evolution 32:567–577. doi: https://dx.doi.org/10.1016/j.tree.2017.05.005

Souris A-C, Kaczensky P, Julliard R, Walzer C. 2007. Time budget, behavioral synchrony- and body score development of a newly released Przewalski's horse group *Equus ferus przewalskii*, in the Great Gobi B Strictly Protected Area in SW Mongolia. Appl Anim Behav Sci 107:307–321. doi: https://dx.doi.org/10.1016/j.applanim.2006.09.023

Sueur C, Petit O, De Marco A, et al. 2011. A comparative network analysis of social style in macaques. Anim Behav 82:845–852. doi: https://dx.doi.org/10.1016/j.anbehav.2011.07.020

Tai Y-M, Chen M-M, Zhang Y-H, Ma A-X, Wang H, Wang X. 2022. Social Rank or Social Bonds: Which one Facilitates Coalition Formation in Male Tibetan Macaques? Biology 11:1269. doi: https://doi.org/10.3390/biology11091269

Tang-Martinez Z. 2001. The mechanisms of kin discrimination and the evolution of kin recognition in vertebrates: A critical re-evaluation. Behav Processes 53:21–40. doi: https://dx.doi.org/10.1016/s0376-6357(00)00148-0

van Dierendonck MC, de Vries H, Schilder MBH. 1995. An analysis of dominance, its behavioural parameters and possible determinants in a herd of Icelandic horses in captivity. Net J Zoo. 45:362–385. doi: https://dx.doi.org/10.1163/156854295x00366

van Dierendonck MC, Wallis de Vries MF. 1996. Ungulate reintroductions: Experiences with the takhi or Przewalski horse (*Equus ferus przewalskii*) in Mongolia. Conservation Biology 10:728–740.

van Dierendonck MC, Sigurjónsdóttir H, Colenbrander B, Thorhallsdóttir AG. 2004. Differences in social behaviour between late pregnant, post-partum and barren mares in a herd of Icelandic horses. Appl Anim Behav Sci 89:283–297. doi: https://dx.doi.org/10.1016/j.applanim.2004.06.010

van Dierendonck MC, de Vries H, Schilder MBH Colenbrander, Thorhallsdóttir B, Sigurjónsdóttir H. 2009. Interventions in social behaviour in a herd of mares and geldings. Appl Anim Behav Sci. 116:67–73. doi: https://dx.doi.org/10.1016/j.applanim.2008.07.003

Vervaecke, H. Stevens, J.M.G. Vandemoortele, H. et al. 2007. Aggression and dominance in matched groups of subadult Icelandic horses (*Equus caballus*). J Ethol 25: 239–248. doi: https://doi.org/10.1007/s10164-006-0019-7

Waring G. 2003. Horse behavior, 2nd edn. Noyes, Park Ridge, 375 pp

Wells S, von Goldschmidt-Rothschild B. 1979. Social behaviors and relationships in a herd of Camargue horses. Z. Tierpsychol. 49: 363-380.

Whitehead, H. 2008. Analyzing animal societies: quantitative methods for vertebrate social analysis. Chicago, USA: The Unviersity of Chicago Press.

Whitehead, H. 2009. SOCPROG programs: analysing animal social structures. Behav. Ecol. Sociobiol. 63, 765–778. https://doi.org/10.1007/s00265-008-0697-y.

Wolff AM, Hausberger N. 1997. Le Scolan Experimental tests to assess emotionality in horses Behav. Process. 40: 209-221

Zhang Y, Cao QS, Rubenstein DI, Zang S, Songer M, Leimgruber P, Chu H, Cao J, Li K, Hu D. 2015. Water use patterns of sympatric przewalski's horse and Khulan: Interspecific comparison reveals niche differences. PloS one. doi: https://dx.doi.org/10.1371/journal.pone.0132094

CHAPTER 7

Novel observation of play behaviour between a harem holder and a bachelor group of Przewalski's horses in the wild

Dominant stallion (left) guarding his grazing mares (right) in the Great Gobi B Strictly Protected Area, Mongolia.

(Photo by Anna Bernátková)

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7.1. Abstract

Przewalski's horses live in stable nonterritorial families (harem) of one or more harem stallions, several mares and their offspring. The harem stallion typically behaves aggressively towards any male intruder approaching the harem. Play behaviour is frequently observed among the group members in horses. For stallions, the most common, well-known cases of play behaviour are found between members of bachelor groups (groups of young stallions) and between the harem stallion and his offspring. The play between the harem stallion and members of the bachelor groups is, on the other hand, seemingly much rarer as a description of such events in the literature is anecdotal. In this note, we present our observation conducted in the Great Gobi B Strictly Protected Area (Mongolia) and describe the play behaviour between a Przewalski's horse harem stallion and members of a bachelor group. The observation was done as a part of broader research during which selected Przewalski's horses' groups were located by binoculars in daily monitoring routines and filmed from a close distance (from 150 m to 800 m). Behavioural Observation Research Interactive Software (BORIS) was used to extract data from the video recordings. The observation described in this note lasted for ∼ 180 minutes, during which the horses engaged in three separate bouts of play and repeated inter-group association. During the whole study (241,5 hours of video recordings), this was the only observation including inter-group interactions. We observed two other events during which two harems approached each other (but never merged). Our observation is the first video recording of such an event and raises the question of how prevalent this behaviour is in the Przewalski's horses' restored natural populations. We conclude that this behaviour is unusual or has not been sufficiently studied in wild or feral harems. Further technological advances may help reveal more information on this topic.

Keywords: *Equus ferus przewalskii,* Gobi, stallion, social behaviour, Mongolia

7.2. Introduction

Since the earliest systematic studies of behaviour, the subject of why animals play has been a topic of discussion (Darwin, 1871; Groos, 1911). Play is mainly perceived as a tool by which young animals acquire skills (*e.g.*, fighting, mating, social behaviour) necessary for their adult life (Martin & Caro, 1985; Paquette, 1994). However, the fact that play is observed in many species after sexual maturity suggests that it may be relevant for animals throughout their lifetime (Asensio et al., 2022; Beckel, 1991; Bond & Diamond, 2003).

Adult-only social play is typically observed among group mates and is mainly described as a mechanism for fostering social networks and encouraging collaboration among individuals. Adult play may provide a social bonding purpose similar to grooming, allowing animals to socialise. In other words, it enables animals to create and sustain social connections as well as restrain anger, lower tension, and enhance tolerance under challenging situations (Baldwin & Baldwin, 1973; Enomoto, 1990; Ferguson & Frankis, 2001; Martin & Caro, 1985; Merrick, 1977; Norscia & Palagi, 2011; Palagi et al., 2006).

In horses, play is most common and noticeable in foals and yearlings, although it also occurs in adult individuals (McDonnell & Poulin, 2002). Play is also frequently observed among the members of the horse bachelor groups (Feh, 2005), including Przewalski's horses (Christensen et al., 2002). Play behaviour is believed to be essential in developing specific social skills (*e.g.*, fighting) in young males compared to young females (Crowell-Davis et al., 1987; Rho et al., 2007). Indeed, it has been observed that colts (male foals) interact more actively with the harem stallion (their father) than fillies (female foals; Crowell-Davis et al., 1987; Šandlová et al., 2020). However, in adult stallions with their harem, observations of play with other mature stallions are very scarce (Berger, 1986; McDonnell & Poulin, 2002).

Przewalski's horses (*Equus ferus przewalskii*) live in stable, nonterritorial family units called harems (Bourjade et al., 2009) and exhibit female defence polygyny. The harems consist of one or more harem stallions (males), several mares (females), and their young progeny (King et al., 2015; Rubenstein, 2011). When a colt or filly reaches reproductive age, typically between the ages of one and four, they separate from their family. While colts usually join a bachelor group until they can form their own harem, fillies typically join another stable family unit (Bahloul et al., 2001; L. Boyd & Bandi, 2002; International Takhi Group, 2021; King et al., 2015). Mature stallions form a new harem by taking over an existing harem or joining females dispersed from other harems. These stallions become harem holders and protect the mares and their offspring from other stallions and predators (Boyd et al. 2016). To guard the harem successfully, the stallion typically stays more vigilant, feeds less, moves more and shows less behavioural synchrony than other harem members (King et al., 2016; Souris et al., 2007). As mares are generally protected from stallion harassment by the group structure, they are often in better condition and their reproductive success is higher than in mares not bonded to a breeding group (Linklater et al., 1999). It was also hypothesised that affiliative interactions between mares and other mares or stallions might boost reproductive success by minimising harassment (Cameron et al., 2009; Linklater et al., 1999). Cases of two or more stallions defending the same harem have been reported (Linklater et al., 2013). Previously, it was suggested that these stallion alliances improve the ability of stallions to defend their mutual family group from other stallions (Boyd et al., 2016) and might increase the foal survival rate in their group (Feh, 1999). However, removal experiments showed that the cooperative hypotheses do not appear to explain the existence of multi-stallion groups (Linklater et al., 2013).

It has been described that in free-roaming populations of horses, most stallions know each other and their relative fighting ability (Rubenstein & Hack, 1992), and most encounters result in ritualised behaviours (Sigurjonsdottir et al., 2012). In this note, we provide a recording and description of the play behaviour between Przewalski's horse harem stallion and members of a bachelor group in the Great Gobi B Strictly Protected Area (Mongolia) to raise the question of how prevalent and important this behaviour is in the Przewalski's horses' restored natural populations and to encourage future research on this topic.

7.3. Methods and results

This observation was conducted in July 2018 in the Great Gobi B Strictly Protected Area (GGBSPA), Mongolia, during broader research fieldwork (July 2018, May-June 2019, and September-October 2019) focusing on the behavioural ecology of Przewalski's horses. In the summer of 2018, two wild-born harems involving 21 adult horses were recorded. In spring 2019, three groups were recorded (two wild-born harems and one harem consisting of long-term reintroduced mares and a wild-born stallion) involving 25 adult horses. In autumn 2019, four groups were recorded (two wild-born harems and two harems consisting of long-term reintroduced mares and wild-born stallions) involving 27 adult horses (Bernátková et al., 2022).

During the whole study (241,5 hours of recordings), we observed only one inter-group interaction (the one described in this short note). Once, two harems were observed feeding close to each other (∼ 50m) but never merged or interacted (one of these harems was Tsetsen, the one also involved in the observation described here). Two harems approaching each other (neither of which was Tsetsen) was also observed just once, but both groups stopped ∼ 300m from each other except for the harem stallions, who kept approaching each other, stopped ∼ 50m from each other and returned to their group.

The GGBSPA, in SW Mongolia, is a reintroduction site for Przewalski's horse and an important refuge for several other endangered species (International Takhi Group, 2021; Kaczensky et al., 2008, 2011). Since 2019, it has encompassed ≈18 000 km^2 of desert steppe and desert habitat (≈9 000 km^2 before the enlargement, International Takhi Group, 2021). At the time of the research, there were 349 Przewalski’s horses in 24 harems and 3–5 bachelor groups. The structure and number of the bachelor groups changes frequently. During this research, the GGBSPA population comprised a maximum of 200 females and 149 males (GGBSPA administration, personal communication). Fieldwork consisted of locating Przewalski's horses' groups with binoculars during daily monitoring routines. Once one of the target groups was localised, it was approached and filmed from a close distance without disturbing them (from 150 m to 800 m) by a 4K Panasonic VX1 video camera with a tripod. The ethogram consisting of 5 categories (feeding, locomotion, resting, social and other) was used to categorise observed behaviours. Play behaviour was included in the social category (Bernátková et al., 2022). Behavioural Observation Research Interactive Software (BORIS) was used for event logging, coding, and observation of the captured videos (Friard & Gamba, 2016).

Play between harem stallions and members of bachelor groups has never been observed in the population of the GGBSPA (GGBSPA administration, personal communication). While observing one of the selected groups (wild-born harem called Tsetsen harem), we noticed the non-agonistic social behaviour of the harem stallion towards a bachelor group which approached his harem. All the videos referred to in the following text are available in Appendix 3 of this thesis and will be available at the acta ethologica journal website, once this article will be published online. The Tsetsen harem consisted of 8 individuals: 1 harem stallion (Tsetsen, seven-years-old), three adult mares (two-, five- and six-years-old), 2 two-year-old males, 1 one-year-old male and 1 one-year-old female. The GGBSPA administration confirmed that none of the mares was in oestrus during the observation. Tsetsen was a harem holder for the first time in his life, and the harem was relatively new (it was formed just two months before our observation). All the breeding mares remained there until our following fieldwork one year later. Tsetsen established his harem by taking over half of the mares from his father's harem, in which all the mares of our study had been together since 2016. The bachelor group consisted of 5 stallions of various ages (3 three-year-old stallions, one four-year-old stallion and one seven-year-old stallion). The GGBSPA administration confirmed that the two oldest bachelor stallions were cousins (their mothers were sisters) of Tsetsen, and had previously been together with Tsetsen in a bachelor group. However, each of these stallions had been born into a different harem (GGBSPA administration, personal communication).

The observation started on 18.07.2018 at 11.29; there were no water points or prime forage areas at a close distance (at least 5km from the observation point). Two other harems were observed from our position (~ 1km from the observation point). No interaction between them and the Tsetsen harem or between them and the bachelor group was observed. At the beginning of the observation, all members of the Tsetsen harem were resting. The bachelor group started approaching the Tsetsen harem and stopped relatively close to the Tsetsen harem (~ 40m). Members of the Tsetsen harem became alert, looking toward the approaching bachelor group. After approximately 30 seconds, the harem stallion Tsetsen moved a bit forward (~ 5m) in front of his harem in the direction of the bachelor group, standing in an alert position facing the bachelors. All the other members of the Tsetsen harem stood close to each other in a relaxed position, and none seemed to be alert anymore. After approximately 1 minute, Tsetsen started running towards the bachelors, joined them, and the horses started to investigate each other mutually. The rest of Tsetsen's harem remained resting, paying almost no interest in the interactions between Tsetsen and the bachelors (Video 1, Appendix 4). After approximately 1 minute, Tsetsen returned to his harem, and both groups stood approximately 20m from each other. Eighteen minutes after our observation started, Tsetsen joined the bachelor group again. We observed mutual investigation and play behaviour (muzzle sniffing, slight rearing, head resting) between him and the stallions of the bachelor group. No direct aggression was observed (Video 2, Appendix 4). Twenty minutes after our observation started, one two-year-old stallion from the Tsetsen harem approached the bachelor group and Tsetsen. The rest of the harem followed him. Both groups merged momentarily, but after a few mutual aggressive encounters started by the harem mares (kick threats, kicking), they split. At this moment, Tsetsen herded his harem and then chased the bachelors away. However, 25 minutes after the start of our observation, the Tsetsen harem and bachelor group merged again, and no aggressive behaviour, herding or any other behaviour aiming to prevent this from the Tsetsen side was observed this time (Video 3, Appendix 4). We observed both groups merging and splitting away multiple times. During these events, Tsetsen mostly remained closer to the bachelor group than to his harem. The 2-year-old stallion from the Tsetsen harem was also observed joining the bachelor group and Tsetsen in the mutual investigation and play behaviour (Video 4, Appendix 4). Approximately 50 minutes after the start of our observation, the oldest mare of the Tsetsen harem led the harem away from the bachelors. Tsetsen followed his harem; however, he stopped in the middle between his harem and the bachelor group when his harem stopped. The two-year-old stallion from the Tsetsen harem did not follow the harem but remained with the bachelor group and rested with them while the Tsetsen harem returned to Tsetsen. Afterwards, both groups (Tsetsen with his harem and the bachelor group with the two-year-old stallion) rested approximately 80m apart. Approximately 95 minutes after the start of our observation, the bachelor group with the two-year-old stallion from the Tsetsen harem started running towards the Tsetsen harem. At first, Tsetsen tried to chase the bachelors away from his harem and the two-year-old stallion, but after a moment, he stopped, showed no more aggression and moved back towards his harem, followed by the two-year-old stallion from his harem and the bachelors. Both groups merged again. When the groups split (~ 10 – 15m apart), Tsetsen remained with the bachelors, and the two-year-old stallion remained with the Tsetsen harem. Again, play behaviour was performed (nipping, slight rearing) between Tsetsen and three members of the bachelor group (Video 5, Appendix 4). Approximately 104 minutes after the start of our observation, the two-year-old stallion from the Tsetsen harem moved towards the bachelor group (and Tsetsen). He was followed by the Tsetsen harem, except for the oldest mare and her one-year-old daughter. Both groups merged again, and we observed aggressive mutual encounters between the mares of the Tsetsen harem and the members of the bachelor group, started by a kick from the two-year-old mare from the Tsetsen harem towards the centre of the merged groups. This lasted approximately one minute, and all the members of the Tsetsen harem moved back to join the oldest mare and her daughter, except for Tsetsen. He was standing with the bachelors, pawing the ground. Afterwards, he started moving towards his harem and again stopped between the two groups (Video 6, Appendix 4). Both groups merged again after Tsetsen started moving back towards the bachelor groups (~ 108 minutes after the start of our observation) and was followed by his harem (Video 7, Appendix 4). After approximately 3 minutes, Tsetsen herded his harem to make the harem move a bit far from the bachelor group (~ 5m). One of the adult mares kicked Tsetsen (Video 8, Appendix 4), the whole Tsetsen harem moved towards the bachelor group, and both groups merged again, with no more aggression observed (Video 9, Appendix 4). Approximately 114 minutes after the start of our observation, the oldest mare of the Tsetsen harem started leading the whole harem away from the bachelors. The Tsetsen harem stopped approximately 20m from the bachelor groups. We observed both groups standing and resting. Approximately 118 minutes after the start of our observation, two members of the bachelor group started to walk away slowly. In contrast, the rest of the bachelor group (3 horses) remained. Both groups were resting apart from each other (~ 20-30m), and no more interactions were observed between the two groups (Video 10, Appendix 4). We remained in the place until all the members of the bachelor group left the area and went out of sight (~ 180 minutes after the start of our observation). No more interactions between the Tsetsen harem and the bachelor groups were observed by us or by the rangers after this event.

As a context for this observation, we observed 429 play interactions among 9 harems during the entire study period, and just 22 of them (5%) involved the harem holder stallion. Three of the 9 harems were composed exclusively of the harem holder and mares (no other stallions were in the harem). The 6 other harems that included other stallions further than the harem holder displayed 274 play behaviours, 11 of them (4%) involving the harem holder stallion and just one play interaction was observed involving the harem holder (not Tsetsen) and another stallion at the harem.

7.4. Discussion

This appears to be the first evidence showing the harem stallion (Przewalski's horse harem holder) tolerating his group to "spend time" with a group of bachelors and playing himself with the bachelor stallions. Numerous studies describing the play behaviour of young males and members of bachelor groups have been published (*e.g.*, Christensen et al. 2002; McDonnell & Haviland, 1995; Zharkikh & Andersen, 2009). The presence and importance of play between the harem stallion and his foals have also been emphasised (Feh, 2005; Šandlová et al., 2020; Wells & Goldschmidt-Rothschild, 2010). However, the subject of harem holders possibly playing with members of a bachelor group solely appears in the literature as short mentions supplementing other topics (King et al., 2016; McDonnell & Poulin, 2002). Only Berger (1986) described the play between harem stallions and bachelor groups of feral horses in more detail. However, he claimed that this behaviour is a type of parental investment. The bachelors who played with the stallion were usually either sons of the stallion or grew up in his harem (they were foals when he took over the harem). The play between them happened 18 months after the bachelors left the stallions' harem. After this time, he probably did not perceive them as sons anymore and did not tolerate their presence in the harem (Berger, 1986). On the contrary, the bachelors in our observation were not sons of the harem stallion and did not grow up in his harem. Despite this fact, they were not only tolerated by the stallion, but they also engaged in mutual play.

We observed a young stallion (7 years old) who became a harem holder for the first time in his life. Tsetsen established his harem by taking over half of the mares from his father's harem. One year after the described observation (in 2019), we recorded that one three-year-old stallion in the Tsetsen harem (the then two year old described interacting with the bachelor group) was repeatedly mating with mares of the harem (*i.e.*, both Tsetsen and this stallion were mating with the mares during this breeding season). Thus, we conclude that those two stallions formed an alliance that year (Boyd et al., 2016).

The harem stallion Tsetsen was probably not the father of any of the foals in the GGBSPA at the time of our observation (GGBSPA administration, personal communication). Therefore, we conclude that the play behaviour between him and the bachelor stallions should not be attributed to parental investment, as suggested by Berger (1986). However, it is important to state that due to the population's size and type (wild horses) and the area, the reproduction records of the GGBSPA are based on direct observations (GGBSPA administration, personal communication). Berger (1986) also described that familiarity, not the actual genetic relatedness, is the probable cause of harem stallion tolerance and play behaviour with bachelors. It has been proven that horses have a good memory (Hanggi & Ingersoll, 2009; Lansade et al., 2020) and that familiarity, not relatedness, is the main factor in social relations between adult horses (Mendonça et al., 2021). Bachelor groups are generally less stable, and both intra- and inter-group play behaviour is prevalent (Feh, 2005; King et al., 2016, 2022; McDonnell & Poulin, 2002; Zharkikh & Andersen, 2009). The GGBSPA administration confirmed that Tsetsen was in the same bachelor group as the two oldest stallions from the bachelor group, so they know each other very well. Therefore, he might have been able to assess their fighting ability (Rubenstein & Hack, 1992) and not perceive them as competitors (Rubenstein, 1994). He probably also knew the other three stallions from the bachelor groups as in feral and wild horse populations; all the bachelors typically know each other (Feh, 2005; Rubenstein & Hack, 1992; Sigurjonsdottir et al., 2012).

Furthermore, no sexual behaviour was observed between the members of the bachelor group and harem mares, and the aggressive behaviours between them were most probably caused solely by the increased proximity. For this reason, Tsetsen could use this opportunity to maintain social bonds with members of the bachelor group and possibly also to practice and improve his fighting skills, as frequently observed in bachelor groups (King et al., 2016, 2022; McDonnell & Poulin, 2002; Zharkikh & Andersen, 2009). Another reason for Tsetsen's tolerance and "friendliness" might be that he was an inexperienced harem holder (Boyd et al., 2016) and had limited control over his harem (Linklater et al., 2013), as he seemingly formed an alliance with a three-year-old stallion (his half-brother) from his harem one year after this observation was conducted (Boyd et al., 2016).

In summary, our observation appears to be the first video record of a harem holder playing with sexually mature bachelors and tolerating their presence close to his harem. Although play behaviour is generally characteristic of immature animals, its presence in adults has also been revealed, and many of its functions are probably yet to be discovered. Our observation raises the question of how common this behaviour is in the re-established natural populations of Przewalski's horses. Short events are challenging to monitor in the wild, but the development of technology (*e.g.*, unmanned aerial vehicles; Schad &

Fischer, 2022) may help reveal the extent to which play between adult stallions occurs and its importance in inter-group relationships. The new technology might be beneficial in vast areas with large populations of wild horses, where most of the groups are too suspicious of human presence to be observed in person, such as the population in the GGBSPA or populations of free-roaming horses in Australia and the USA.

7.5. References

Asensio, N., Zandonà, E., Dunn, J. C., & Cristóbal-Azkarate, J. (2022). Socioecological correlates of social play in adult mantled howler monkeys. *Animal Behaviour*, *186*, 219–229. https://doi.org/10.1016/j.anbehav.2022.01.017

Bahloul, K., Pereladova, O. B., Soldatova, N., Fisenko, G., Sidorenko, E., & Sempéré, A. J. (2001). Social organisation and dispersion of introduced kulans (*Equus hemionus kulan*) and Przewalski horses (*Equus przewalski*) in the Bukhara Reserve, Uzbekistan. *Journal of Arid Environments*, *47*(3), 309–323. https://doi.org/10.1006/JARE.2000.0714

Baldwin, J. D., & Baldwin, J. I. (1973). The role of play in social organisation: Comparative observations on squirrel monkeys (Saimiri). *Primates*, *14*(4), 369–381. https://doi.org/10.1007/BF01731358

Beckel, A. L. (1991). Wrestling Play in Adult River Otters, *Lutra canadensis*. *Journal of Mammalogy*, *72*(2), 386–390. https://doi.org/10.2307/1382111

Berger, J. (1986). *Wild horses of the Great Basin: social competition and population size.* University of Chicago Press.

Bernátková, A., Oyunsaikhan, G., Šimek, J., Komárková, M., Bobek, M., & Ceacero, F. (2022). Influence of weather on the behaviour of reintroduced Przewalski's horses in the Great Gobi B Strictly Protected Area (Mongolia): implications for conservation. *BMC Zoology*, *7*(1), 32. https://doi.org/10.1186/s40850-022-00130-z

Bond, A., & Diamond, J. (2003). A Comparative Analysis of Social Play in Birds. *Behaviour*, *140*(8–9), 1091–1115. https://doi.org/10.1163/156853903322589650

Boyd, L., & Bandi, N. (2002). Reintroduction of takhi, *Equus ferus przewalskii*, to Hustai National Park, Mongolia: time budget and synchrony of activity pre- and post-release. *Applied Animal Behaviour Science*, *78*(2–4), 87–102. https://doi.org/10.1016/S0168-1591(02)00088-6

Boyd, L. E., Scorolli, A., Nowzari, H., & Bouskila, A. (2016). Social organisation of wild equids. In J. I. Ransom & P., Kaczensky (Eds.), *Wild Equids: ecology, management, and conservation.* (pp. 7–22). John Hopkins University Press.

Christensen, J. W., Zharkikh, T., Ladewig, J., & Yasinetskaya, N. (2002). Social behaviour in stallion groups (*Equus przewalskii* and *Equus caballus*) kept under natural and domestic conditions. *Applied Animal Behaviour Science*, *76*(1), 11–20. https://doi.org/10.1016/S0168-1591(01)00208-8

Crowell-Davis, S. L., Houpt, K. A., & Kane, L. (1987a). Play development in Welsh pony (*Equus caballus*) foals. *Applied Animal Behaviour Science*, *18*(2), 119–131. https://doi.org/10.1016/0168-1591(87)90186-9

Darwin, C. (1871). *The descent of man.* Appleton.

Enomoto, T. (1990). Social play and sexual behavior of the bonobo (*Pan paniscus*) with special reference to flexibility. *Primates*, *31*(4), 469–480. https://doi.org/10.1007/BF02382531

Feh, C. (1999). Alliances and reproductive success in Camargue stallions. *Animal Behaviour*, *57*(3), 705–713. https://doi.org/10.1006/anbe.1998.1009

Feh C. (2005). Relationships and communication in socially natural horse herds. In D. Mills & S. M. McDonnell (Eds.), *The Domestic Horse: The Evolution, Development and Management of its Behaviour*, (pp. 83–93). Cambridge University Press.

Ferguson, E., & Frankis, J. (2001). Sex and Sexual Orientation. *Journal of Homosexuality*, *41*(2), 119–143. https://doi.org/10.1300/J082v41n02_07

Friard, O., & Gamba, M. (2016). BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods in Ecology and Evolution*, *7*(11), 1325–1330. https://doi.org/10.1111/2041-210X.12584

Groos, Karl. (1911). *The play of animals*. Appleton.

Hanggi, E. B., & Ingersoll, J. F. (2009). Long-term memory for categories and concepts in horses (*Equus caballus*). *Animal Cognition*, *12*(3), 451–462. https://doi.org/10.1007/s10071-008-0205-9

International Takhi Group. (2021). *Save the Wild Horse*. https://savethewildhorse.org/en/

Kaczensky, P., Enkhsaikhan, N., Ganbaatar, O., & Walzer, C. (2008). The Great Gobi B Strictly Protected Area in Mongolia - refuge or sink for wolves *Canis lupus* in the Gobi. *Wildlife Biology*, *14*(4), 444–456. https://doi.org/10.2981/0909-6396-14.4.444

Kaczensky, P., Ganbataar, O., Altansukh, N., Enkhsaikhan, N., Stauffer, C., & Walzer, C. (2011). The Danger of Having All Your Eggs in One Basket—Winter Crash of the Re-Introduced Przewalski's Horses in the Mongolian Gobi. *PLoS ONE*, *6*(12), e28057. https://doi.org/10.1371/journal.pone.0028057

King, S. R. B., Boyd, L., Zimmermann, W., & Kendall, B. E. (2015). *Equus ferus (errata version published in 2016)*. https://www.iucnredlist.org/species/41763/97204950

King, S. R. B., Asa, C., Pluhacek, J., Houpt, K. A., & Ransom, J. I. (2016). Behavior of horses, zebras, and asses. In J. I. Ransom & P. Kaczensky (Eds.), *Wild Equids: ecology, management, and conservation* (pp. 23–40). John Hopkins University Press.

King, S. R. B., Schoenecker, K. A., & Cole, M. J. (2022). Effect of adult male sterilisation on the behavior and social associations of a feral polygynous ungulate: the horse. *Applied Animal Behaviour Science*, *249*, 105598. https://doi.org/10.1016/j.applanim.2022.105598

Lansade, L., Colson, V., Parias, C., Trösch, M., Reigner, F., & Calandreau, L. (2020). Female horses spontaneously identify a photograph of their keeper, last seen six months previously. *Scientific Reports*, *10*(1), 6302. https://doi.org/10.1038/s41598-020-62940-w

Martin, P., & Caro, T. M. (1985). *On the Functions of Play and Its Role in Behavioral Development* (pp. 59–103). https://doi.org/10.1016/S0065-3454(08)60487-8

McDonnell, S. M., & Poulin, A. (2002). Equid play ethogram. *Applied Animal Behaviour Science*, *78*(2–4), 263–290. https://doi.org/10.1016/S0168-1591(02)00112-0

Mendonça, R. S., Pinto, P., Inoue, S., Ringhofer, M., Godinho, R., & Hirata, S. (2021). Social determinants of affiliation and cohesion in a population of feral horses. *Applied Animal Behaviour Science*, *245*, 105496. https://doi.org/10.1016/j.applanim.2021.105496

Merrick, N. J. (1977). Social grooming and play behavior of a captive group of chimpanzees. *Primates*, *18*(1), 215–224. https://doi.org/10.1007/BF02382960

Norscia, I., & Palagi, E. (2011). When play is a family business: adult play, hierarchy, and possible stress reduction in common marmosets. *Primates*, *52*(2), 101–104. https://doi.org/10.1007/s10329-010-0228-0

Palagi, E., Paoli, T., & Tarli, S. B. (2006). Short-Term Benefits of Play Behavior and Conflict Prevention in Pan paniscus. *International Journal of Primatology*, *27*(5), 1257–1270. https://doi.org/10.1007/s10764-006-9071-y

Paquette, D. (1994). Fighting and playfighting in captive adolescent chimpanzees. *Aggressive Behavior*, *20*(1), 49–65. https://doi.org/10.1002/1098-2337(1994)20:1<49::AID-AB2480200107>3.0.CO;2-C

Rho, J. R., Srygley, R. B., & Choe, J. C. (2007). Sex Preferences in Jeju Pony Foals (*Equus caballus*) for Mutual Grooming and Play-Fighting Behaviors. *Zoological Science*, *24*(8), 769–773. https://doi.org/10.2108/zsj.24.769

Rubenstein, D. (2011). *Family Equidae (horses and relatives). Handbook of Mammals of the World.* (R. A. Mittermeier & D. E. Wilson, Eds.; 2nd ed., Vol. 2). Lynx Edicions.

Rubenstein, D. I. (1994). *The ecology of female social behaviour in horses, zebras and asses.* (P. Jarman & A. Rossiter, Eds.). University Press.

Rubenstein D I., & Hack, M. A. (1992). Horse signals: The sounds and scents of fury. *Evolutionary Ecology*, *6*(3), 254–260. https://doi.org/10.1007/BF02214165

Šandlová, K., Komárková, M., & Ceacero, F. (2020). Daddy, daddy cool: stallion–foal relationships in a socially-natural herd of Exmoor ponies. *Animal Cognition*, *23*(4), 781–793. https://doi.org/10.1007/s10071-020-01388-x

Schad, L., & Fischer, J. (2022). Opportunities and risks in the use of drones for studying animal behaviour. *Methods in Ecology and Evolution*. https://doi.org/10.1111/2041-210X.13922

Sigurjonsdottir, H., Thorhallsdottir, A. G., Hafthorsdottir, H. M., & Granquist, S. M. (2012). The Behaviour of Stallions in a Semiferal Herd in Iceland: Time Budgets, Home Ranges, and Interactions. *International Journal of Zoology*, *2012*, 1–7. https://doi.org/10.1155/2012/162982

Souris, A.-C., Kaczensky, P., Julliard, R., & Walzer, C. (2007). Time budget-, behavioral synchrony- and body score development of a newly released Przewalski's horse group *Equus ferus przewalskii*, in the Great Gobi B strictly protected area in SW Mongolia. *Applied Animal Behaviour Science*, *107*(3–4), 307–321. https://doi.org/10.1016/j.applanim.2006.09.023

Wells, S. M., & Goldschmidt-Rothschild, B. (2010). Social Behaviour and Relationships in a Herd of Camargue Horses. *Zeitschrift Für Tierpsychologie*, *49*(4), 363–380. https://doi.org/10.1111/j.1439-0310.1979.tb00299.x

Zharkikh, T. L., & Andersen, L. (2009). Behaviour of Bachelor Males of the Przewalski Horse (*Equus ferus przewalskii*) at the Reserve Askania Nova. *Der Zoologische Garten*, *78*(5–6), 282–299. https://doi.org/10.1016/j.zoolgart.2009.10.005

CHAPTER 8

8. General discussion

With the constant growth in the human population and increasing pressure on the remaining natural areas, conservation biology has grown in importance over the last decades (Lindenmayer & Hunter, 2010; Robinson, 2006). As early as 1992, the Earth Summit in Rio de Janeiro formed the Convention on Biological Diversity and the Global Environmental Facility, and important steps in biodiversity conservation have been taken since. However, in order to at least preserve what remains from the natural world, more immense steps must be taken further (Robinson, 2006).

Reintroduction has become one of the most important tools for endangered species conservation, and sometimes it is the only remaining option (Armstrong & Seddon, 2008; Seddon et al., 2007). Nonetheless, it has numerous limitations. The original habitat of the species might be lacking or severely disturbed, relevant information on the species' behaviour and ecology before extinction is often deficient and effective post-release monitoring might be challenging due to the elusive or apprehensive behaviour of the reintroduced species (IUCN, 2013; Wallach et al., 2007). Due to the lack of suitable habitat and relevant information, some species, including Przewalski's horse, have been reintroduced to the edge of their native area (Gao et al., 1989; Wakefield et al., 2002; Wallach et al., 2007). Nonetheless, the case of Przewalski's horse is widely accepted as an example of successful species reintroduction (King et al., 2015; King, 2005). Numerous studies focused on the horses have been done since the reintroduction started (Turghan et al., 2022). However, as the population of Przewalski's horses grows, understanding its behaviour also becomes increasingly vital. There is a scarcity of data immediately after transportation, after release and for wild-born individuals, which is exacerbated by the fact that only a few individuals are transported from Europe each year (and even during some years, there has been no transportation). In this thesis, we focused on analysing the possible influence of the origin and experience of the Przewalski's horses (after transportation, after release and in wild-born individuals) on their behaviour, and how these differences might affect the reintroduction process.

The results of the study show that origin and experience influence Przewalski's horse behaviour in different contexts, including their response to weather changes, the social behaviour of groups and individuals and their habitat preferences. Furthermore, the effect of other social and environmental factors on horse behaviour was also revealed during our research.

We found out that weather patterns influence the behaviour of Przewalski’s horses. Feeding increased significantly in cloudy and windy conditions, and it was more frequent in autumn than in spring and summer. Temperature and cloudiness positively explained locomotion, which was greater in the summer than in the spring and autumn. Resting behaviour decreased with altitude and cloudiness, and group dispersion reduced during resting. Higher temperatures were associated with increased social interactions, which were more common in summer compared to spring and autumn. Most importantly, we found differences in the exhibition of behaviours across the selected harems when we grouped them according to their origin and experience. We revealed that after approximately 1-year, horses adapt to novel conditions and display the typical behavioural pattern of wild-born Przewalski’s horses, supporting the result of Scheibe et al. (1996). However, we discovered certain dissimilarities in the groups of comparable origin and experience. These were most probably due to the inexperience of the harem holder (Klimov, 1988) and the enclosement of some groups (Hogan et al., 1988; Keiper & Receveur, 1992).

To test whether there are differences in habitat selection between reintroduced and wild-born groups of horses, we compared predicted feeding and resting areas for these groups of horses between different selected periods. During the spring/summer season, the probability of feeding increased with better feed quality (higher NDVI maximum values) in the wild-born horses. This result is in line with previous research stating that Przewalski's horses frequently exhibit a high predilection for more productive vegetation in the GGBSPA (Kaczensky et al., 2008). However, we observed the opposite tendency for reintroduced groups. In addition, wild-born groups utilised more area for foraging than the reintroduced ones. We assume that this was due to the adaptation period and learning process of reintroduced horses (Boyd & Bandi, 2002; Scheibe et al., 1996) and, therefore, failure in the selection of the “best feeding areas”. Another reason might be the higher hierarchical position of more experienced, dominant (Hirata, 2022; Maeda et al.,

2021) and larger (Maeda et al., 2021) wild-born groups, which have better chances of using the favoured areas (Miller & Dennisto, 2010). Nevertheless, we observed a shift in the preferred feeding areas between the wild-born and reintroduced groups of Przewalski's horses in the autumn period, when herders started coming back to the GGBSPA. This was most probably due to the fact that while wild-born horses are shy of human-livestock presence (Kaczensky et al., 2007), while the reintroduced individuals are still used to it (Ralls & Ballou, 2013) and could share the best feeding areas with livestock (Burnik Šturm et al., 2017).

In order to effectively assess determinants of the social behaviour of Przewalski’s horses, we did not only include origin and experience in the analysis but also considered other variables (season, sex, age, relative time belonging to the group, relatedness, and dominance rank). We discovered less aggression in our study's high-ranking animals. This finding is consistent with prior research indicating that once the hierarchy is established, it is typically maintained without the need for ongoing agonistic interaction (Berger, 1977; Wells & Goldschmidt-Rothschild, 2010). As described in Boyd and Houpt (1994), we also observed lower aggression among related animals and in older animals (Keiper & Receveur, 1992).

Many factors contributed considerably to the social network metrics of friendly interactions. In accordance with previous research, the number of these interactions fell significantly as the relative time spent in the group and dominance rank increased (Keiper & Receveur, 1992). The rate of friendly interactions was also lower in older, more related animals (Heitor et al., 2006), and females (Keiper & Receveur, 1992). However, friends of related and older animals were more likely to be friends with each other. Less dominating animals were more likely to associate with very associative animals.

Foremost, our findings showed a significant impact of origin and experience on the formation of stable social networks at both the group and individual levels. We noticed a distinct shift from recently reintroduced through long-term reintroduced to wild-born Przewalski’s horses with a high number of interactions and weaker networks at the beginning of the reintroduction process to much lower interaction rate and strong social networks in wild-born horses.

Moreover, during our research, we made a video that appears to be the first recording of a harem holder playing with sexually mature bachelors and tolerating their presence in his harem. The subject of harem holders possibly playing with members of a bachelor group solely appears in the literature as short mentions supplementing other topics (King et al., 2016; McDonnell & Poulin, 2002). Only Berger (1986) described the play between dominant stallions and bachelor groups of feral horses in more detail. Our observation raises the question of how common and important this behaviour is in the re-established natural populations of Przewalski's horses.

Understanding animal ecology and behaviour represents an essential element in species conservation (Blumstein & Fernández-Juricic, 2004). It is a valuable approach that might be used to monitor reintroduced populations and evaluate reintroduction success (Berger-Tal et al., 2011; IUCN, 2013). Captive breeding and reintroduction of animals necessitate an understanding of behavioural aspects such as mate selection, social structure, and how the environment influences behaviour. Because captive animals frequently lack opportunities to develop natural behavioural patterns or reactions to environmental factors during critical learning stages, captive breeding and reintroductions may fail (IUCN, 2013; Shumway, 1999). Animals must acquire a range of behaviours that are hard to imitate in captivity, such as intraspecies relationships, where to obtain food, where to rest safely, and how to avoid predators, and ethology might bring relevant insights into these mechanisms (Shumway, 1999). If conservation behaviour is involved in conservation steps too late (or not at all), conservation programs may suffer unnecessary setbacks and lose resources, or even the subject animals (Blumstein & Fernández-Juricic, 2004). Fortunately, behavioural research is being recognised and involved in conservation studies and programmes progressively. However, much larger steps and cooperation between various scientific fields and experts are needed in order to incorporate behaviour conservation more effectively. For instance, using open-source geographic information system data to extend the information on broader specimen localities or geographical ranges would be highly beneficial. Applying behavioural traits as modifiers can strengthen explanatory power in conservation evaluations and predictive models (Tobias & Pigot, 2019). Our research outputs highlight the importance of behavioural studies in species reintroduction. We found that the behaviour of reintroduced and wild-born Przewalski‘s horses differs and that there is a conversion in behaviour from recently reintroduced through long-term reintroduced towards wild-born horses. Our results imply that reintroduced Przewalski‘s horses do not only require sufficient time to adapt to the novel habitat and weather conditions but also to develop strong and complex relationships and to break their bonds with humans. The International Union for Conservation of Nature's (IUCN) Red List of Threatened Species has emphasised the need for an action plan and a uniform population management strategy for Przewalski's horses in Mongolia (King et al., 2015). In order to assist these actions and provide comprehensive knowledge, future research should focus on the ecology of the species, considering the differences between animals of different origins, as these factors might have a strong influence on the behaviour of Przewalski's horses and, therefore, on reintroduction success. According to the IUCN's Reintroduction and Invasive Species Specialist Groups, it is desirable to monitor the behaviour of translocated individuals and use that information to assess how well the translocation is going; and to propose possible changes and further steps to support successful management. However, the data must be comparable and should be obtained from similar natural populations (IUCN, 2013). For this reason, we suggest that, within the realm of possibility, all future studies on Przewalski's horses consider animals of different origins.

CHAPTER 9

9. General conclusions

The last Przewalski's horse was spotted in the Great Gobi B Strictly Protected Area in Mongolia in 1969, and it was declared extinct in the wild in 1996. The species was categorized as Endangered in 2011 as a result of ongoing reintroduction efforts (King et al., 2015). It is a flagship species that could be utilized to promote the preservation of its whole habitat (A. Berger et al., 1999). Because Przewalski's horse was never researched in the wild before extinction, only anecdotal reports of its original ecology, genetics, social organization, and behaviour are known (Mohr, 1972). Therefore, all available information on the species in human care and in reintroduction initiatives is critical for recognizing its requirements. The objective of the research on the reintroduced individuals and populations should involve the creation of a basic reference dataset to provide scientists and authorities with effective means for long-term monitoring and management of reintroduction projects worldwide (King et al., 2015).

In the reintroduction sites, there is typically a mixture of reintroduced and wild-born horses (Boyd & Bandi, 2002; International Takhi Group, 2021; Turghan et al., 2022) and origin and experience of the individuals might influence their behaviour (Battin, 2004; Rantanen et al., 2010). Despite the fact that Przewalski's horses have been widely studied since the species' restoration in the wild, information on the impact of origin on their behaviour is lacking. The current thesis investigated the influence of weather, determinants of social behaviour and habitat selection, considering the different origins of Przewalski's horses and taking into account the possible effect of seasonal changes. In this thesis, the origin of the horses was proven to be one of the most important factors influencing the behaviour of Przewalski´s horses. In general, wild-born horses of our study displayed better weather adaption, more complex social networks, and a selection of feeding locations with higher-quality vegetation when compared to reintroduced horses. The need to acclimate to novel conditions was most probably the cause of altering the behavioural pattern and habitat utilization of recently reintroduced individuals, and these horses also require sufficient time to form stable social relationships. The findings support the hypothesis of horses' gradual behavioural adaption to their new surroundings, which won't likely be totally complete until the second generation arrives. These findings might support the successful conservation of the species and upcoming reintroduction programmes. We suggest that, if possible, future studies should include Przewalski´s horses´ individuals and/or groups of different origins. We also suggest that further research might concentrate on captive breeding practices and how they affect individual adaptation to reintroduction.

CHAPTER 10

10. General references

Armstrong, D., & Seddon, P. (2008). Directions in reintroduction biology. Trends in Ecology & Evolution, 23(1), 20–25. https://doi.org/10.1016/j.tree.2007.10.003

Baguette, M., Blanchet, S., Legrand, D., Stevens, V. M., & Turlure, C. (2013). Individual dispersal, landscape connectivity and ecological networks. Biological Reviews, 88(2), 310–326. https://doi.org/10.1111/brv.12000

Bainbridge, I. (2014). PRACTITIONER'S PERSPECTIVE: How can ecologists make conservation policy more evidence based? Ideas and examples from a devolved perspective. Journal of Applied Ecology, 51(5), 1153–1158. https://doi.org/10.1111/1365-2664.12294

Banes, G. L., Galdikas, B. M. F., & Vigilant, L. (2016). Reintroduction of confiscated and displaced mammals risks outbreeding and introgression in natural populations, as evidenced by orang-utans of divergent subspecies. Scientific Reports, 6(1), 22026. https://doi.org/10.1038/srep22026

Battin, J. (2004). When Good Animals Love Bad Habitats: Ecological Traps and the Conservation of Animal Populations. Conservation Biology, 18(6), 1482–1491. https://doi.org/10.1111/j.1523-1739.2004.00417.x

Berger, A., Scheibe, K.-M., Eichhorn, K., Scheibe, A., & Streich, J. (1999). Diurnal and ultradian rhythms of behaviour in a mare group of Przewalski horse (*Equus ferus przewalskii*), measured through one year under semi-reserve conditions. Applied Animal Behaviour Science, 64(1), 1–17. https://doi.org/10.1016/S0168-1591(99)00026-X

Berger, J. (1977). Organizational systems and dominance in feral horses in the Grand Canyon. Behavioral Ecology and Sociobiology, 2(2), 131–146. https://doi.org/10.1007/BF00361898

Berger-Tal, O., Blumstein, D. T., & Swaisgood, R. R. (2020). Conservation translocations: a review of common difficulties and promising directions. Animal Conservation, 23(2), 121–131. https://doi.org/10.1111/acv.12534

Berger-Tal, O., Polak, T., Oron, A., Lubin, Y., Kotler, B. P., & Saltz, D. (2011). Integrating animal behavior and conservation biology: a conceptual framework. Behavioral Ecology, 22(2), 236–239. https://doi.org/10.1093/beheco/arq224

Berger-Tal, O., & Saltz, D. (2014a). Using the movement patterns of reintroduced animals to improve reintroduction success. Current Zoology, 60(4), 515–526. https://doi.org/10.1093/czoolo/60.4.515

Berger-Tal, O., & Saltz, D. (2014b). Using the movement patterns of reintroduced animals to improve reintroduction success. Current Zoology, 60(4), 515–526. https://doi.org/10.1093/czoolo/60.4.515

Bernátková, A., Oyunsaikhan, G., Šimek, J., Komárková, M., Bobek, M., & Ceacero, F. (2022). Influence of weather on the behaviour of reintroduced Przewalski's horses in the Great Gobi B Strictly Protected Area (Mongolia): implications for conservation. BMC Zoology, 7(1), 32. https://doi.org/10.1186/s40850-022-00130-z

Blumstein, D. T., Letnic, M., & Moseby, K. E. (2019). In situ predator conditioning of naive prey prior to reintroduction. Philosophical Transactions of the Royal Society B: Biological Sciences, 374(1781), 20180058. https://doi.org/10.1098/rstb.2018.0058

Bowling, A. T., Zimmermann, W., Ryder, O., Penado, C., Peto, S., Chemnick, L., Yasinetskaya, N., & Zharkikh, T. (2003). Genetic variation in Przewalski's horses, with special focus on the last wild caught mare, 231 Orlitza III. Cytogenetic and Genome Research, 102(1–4), 226–234. https://doi.org/10.1159/000075754

Boyd, L., & Bandi, N. (2002). Reintroduction of takhi, *Equus ferus przewalskii*, to Hustai National Park, Mongolia: time budget and synchrony of activity pre- and post-release. Applied Animal Behaviour Science, 78(2–4), 87–102. https://doi.org/10.1016/S0168-1591(02)00088-6

Boyd, L. E. (1988). Time budgets of adult Przewalski horses: Effects of sex, reproductive status and enclosure. Applied Animal Behaviour Science, 21(1–2), 19–39. https://doi.org/10.1016/0168-1591(88)90099-8

Boyd, L. E. (1991). The behaviour of Przewalski's horses and its importance to their management. Applied Animal Behaviour Science, 29(1–4), 301–318. https://doi.org/10.1016/0168-1591(91)90256-W

Boyd, L. E., Carbonaro, D. A., & Houpt, K. A. (1988). The 24-hour time budget of Przewalski horses. Applied Animal Behaviour Science, 21(1–2), 5–17. https://doi.org/10.1016/0168-1591(88)90098-6

Boyd, L., & Houpt, KA. (1994). Przewalski's Horse: The History and Biology of an Endangered 576 Species. State University of New York Press.

Burnik Šturm, M., Ganbaatar, O., Voigt, C. C., & Kaczensky, P. (2017). Sequential stable isotope analysis reveals differences in dietary history of three sympatric equid species in the Mongolian Gobi. Journal of Applied Ecology, 54(4), 1110–1119. https://doi.org/10.1111/1365-2664.12825

Caro, T. (2007). Behavior and conservation: a bridge too far? Trends in Ecology & Evolution, 22(8), 394–400. https://doi.org/10.1016/j.tree.2007.06.003

Cassini, M. H. (1999). Importancia de la etología en la Conservación. Etología, 7, 69–75.

Caughley, G., & Gunn, A. (1996). Conservation biology in theory and practice. Blackwell Science.

Challender, D. W. S., & MacMillan, D. C. (2014). Poaching is more than an Enforcement Problem. Conservation Letters, 7(5), 484–494. https://doi.org/10.1111/conl.12082

Der Sarkissian, C., Ermini, L., Schubert, M., Yang, M. A., Librado, P., Fumagalli, M., Jónsson, H., Bar-Gal, G. K., Albrechtsen, A., Vieira, F. G., Petersen, B., Ginolhac, A., Seguin-Orlando, A., Magnussen, K., Fages, A., Gamba, C., Lorente-Galdos, B., Polani, S., Steiner, C., … Orlando, L. (2015). Evolutionary Genomics and Conservation of the Endangered Przewalski's Horse. Current Biology, 25(19), 2577–2583. https://doi.org/10.1016/j.cub.2015.08.032

Fischer, J., & Lindenmayer, D. B. (2000). An assessment of the published results of animal relocations. Biological Conservation, 96(1), 1–11. https://doi.org/10.1016/S0006-3207(00)00048-3

Gao X, Gu J, & Zhou J. (1989). The change on the distribution area of the wild horse in the modern times. Arid Zone Res. 2(10).

Gaunitz, C., Fages, A., Hanghøj, K., Albrechtsen, A., Khan, N., Schubert, M., Seguin-Orlando, A., Owens, I. J., Felkel, S., Bignon-Lau, O., de Barros Damgaard, P., Mittnik, A., Mohaseb, A. F., Davoudi, H., Alquraishi, S., Alfarhan, A. H., Al-

Rasheid, K. A. S., Crubézy, E., Benecke, N., … Orlando, L. (2018). Ancient genomes revisit the ancestry of domestic and Przewalski's horses. Science, 360(6384), 111–114. https://doi.org/10.1126/science.aao3297

Heitor, F., Oom, M. do M., & Vicente, L. (2006). Social relationships in a herd of Sorraia horses. Behavioural Processes, 73(2), 170–177. https://doi.org/10.1016/j.beproc.2006.05.004

Hirata, S. (2022). Studying feral horse behavior from the sky. Artificial Life and Robotics, 27(2), 196–203. https://doi.org/10.1007/s10015-022-00746-x

Hogan, E. S., Houpt, K. A., & Sweeney, K. (1988). The effect of enclosure size on social interactions and daily activity patterns of the captive Asiatic wild horse (*Equus przewalskii*). Applied Animal Behaviour Science, 21(1–2), 147–168. https://doi.org/10.1016/0168-1591(88)90105-0

International Takhi Group. (2021). Save the Wild Horse. https://savethewildhorse.org/en/

IUCN. (2013). Guidelines for reintroductions and other conservation translocations (IUCN Species Survival Commission, Ed.).

Jiang, Z., & Zong, H. (2019). Reintroduction Of The Przewalski's Horse In China: Status Quo And Outlook. Nature Conservation Research, 4(Suppl.2). https://doi.org/10.24189/ncr.2019.045

Kaczensky, P., Enkhsaihan, N., Ganbaatar, O., & Walzer, C. (2007). Identification of herder-wild equid conflicts in the Great Gobi B Strictly Protected Area in SW Mongolia. https://www.researchgate.net/publication/259602525_Identification_of_Herder-Wild_Equid_Conflicts_in_the_Great_Gobi_B_Strictly_Protected_Area_in_SW_Mongolia

Kaczensky, P., Ganbataar, O., Altansukh, N., Enkhsaikhan, N., Stauffer, C., & Walzer, C. (2011). The Danger of Having All Your Eggs in One Basket—Winter Crash of the Re-Introduced Przewalski's Horses in the Mongolian Gobi. PLoS ONE, 6(12), e28057. https://doi.org/10.1371/journal.pone.0028057

Kaczensky, P., Oyunsaikhan, G., von Wehrden, H., & Walzer, C. (2008). Resource selection by sympatric wild equids in the Mongolian Gobi. Journal of Applied Ecology, 45(6), 1762–1769. https://doi.org/10.1111/j.1365-2664.2008.01565.x

Kajiwara, I., Yoshihara, Y., & Sato, S. (2016). A preliminarily assessment of landscape factors affecting habitat use by Przewalski horses and habitat evaluation in Hustai National Park, Mongolia. Mammalian Biology, 81(3), 340–344. https://doi.org/10.1016/j.mambio.2016.02.009

Keane, A., Jones, J. P. G., Edwards-Jones, G., & Milner-Gulland, E. J. (2008). The sleeping policeman: understanding issues of enforcement and compliance in conservation. Animal Conservation, 11(2), 75–82. https://doi.org/10.1111/j.1469-1795.2008.00170.x

Keiper, R., & Receveur, H. (1992). Social interactions of free-ranging Przewalski horses in semi-reserves in the Netherlands. Applied Animal Behaviour Science, 33(4), 303–318. https://doi.org/10.1016/S0168-1591(05)80068-1

King, S. R. B., & Gurnell, J. (2005). Habitat use and spatial dynamics of takhi introduced to Hustai National Park, Mongolia. Biological Conservation, 124(2), 277–290. https://doi.org/10.1016/j.biocon.2005.01.034

King, S. R. B. (2005). Proceedings of the Mongolian Biodiversity Databank Workshop: Assessing the Conservation Status of Mongolian Mammals and Fishes: I - Results and Outputs of the Workshop. Mongolian Journal of Biological Sciences, 3(2). https://doi.org/10.22353/mjbs.2005.03.08

King, S. R. B., Asa, C., Pluhacek, J., Houpt, K. A., & Ransom, J. I. (2016). Behavior of horses, zebras, and asses. In J. I. Ransom & P. Kaczensky (Eds.), Wild Equids: ecology, management, and conservation (pp. 23–40). John Hopkins University Press,.

King, S. R. B., Boyd, L., Zimmermann, W., & Kendall, B. E. (2015, June 23). *Equus ferus* (errata version published in 2016). https://www.iucnredlist.org/species/41763/97204950

King, S. R. B., & Gurnell, J. (2010). Effects of fly disturbance on the behaviour of a population of reintroduced Przewalski horses (*Equus ferus przewalskii*) in Mongolia.

Applied Animal Behaviour Science, 125(1–2), 22–29. https://doi.org/10.1016/j.applanim.2010.03.006

Klimov, V. V. (1988). Spatial-ethological organization of the herd of Przewalski horses (*Equus przewalskii*) in Askania-Nova. Applied Animal Behaviour Science, 21(1–2), 99–115. https://doi.org/10.1016/0168-1591(88)90103-7

le Flohic, G., Motsch, P., DeNys, H., Childs, S., Courage, A., & King, T. (2015). Behavioural Ecology and Group Cohesion of Juvenile Western Lowland Gorillas (Gorilla g. gorilla) during Rehabilitation in the Batéké Plateaux National Park, Gabon. PLOS ONE, 10(3), e0119609. https://doi.org/10.1371/journal.pone.0119609

Lindenmayer, D., & Hunter, M. (2010). Some Guiding Concepts for Conservation Biology. Conservation Biology, 24(6), 1459–1468. https://doi.org/10.1111/j.1523-1739.2010.01544.x

Liu, G., Shafer, A. B. A., Zimmermann, W., Hu, D., Wang, W., Chu, H., Cao, J., & Zhao, C. (2014). Evaluating the reintroduction project of Przewalski's horse in China using genetic and pedigree data. Biological Conservation, 171, 288–298. https://doi.org/10.1016/j.biocon.2013.11.022

MacDonald, D. W. (1990). Behavioral studies as a tool for vertebrate conservation. Fourth International Symposium on Wildlife Conservation, 129–133.

Maeda, T., Ochi, S., Ringhofer, M., Sosa, S., Sueur, C., Hirata, S., & Yamamoto, S. (2021). Aerial drone observations identified a multilevel society in feral horses. Scientific Reports, 11(1), 71. https://doi.org/10.1038/s41598-020-79790-1

Miller, R., & Dennisto, R. H. (2010). Interband Dominance in Feral Horses. Zeitschrift Für Tierpsychologie, 51(1), 41–47. https://doi.org/10.1111/j.1439-0310.1979.tb00670.x

Mohr E. (1972). The Asiatic wild horse (Volf J, Ed.; 2nd ed.). J. A. Allen & Co.

Monaghan, P. (1993). Studying animal behaviour: what's the use? Etología, 3, 79–93.

Mongolian Takhi Strategy and Plan Work Group. (1993). Recommendations for Mongolia's Takhi Strategy and Plan. Mongolian Government, Ministry of Nature and Environment.

Moorhouse, T. P., Gelling, M., & Macdonald, D. W. (2009). Effects of habitat quality upon reintroduction success in water voles: Evidence from a replicated experiment. Biological Conservation, 142(1), 53–60. https://doi.org/10.1016/j.biocon.2008.09.023

Ralls K., & Ballou J.D. (2013). Encyclopedia of Biodiversity. Captive Breeding and Reintroduction (Levin S.A., Ed.; 2nd ed.). MA: Academic Press.

Rantanen, E. M., Buner, F., Riordan, P., Sotherton, N., & Macdonald, D. W. (2010). Habitat preferences and survival in wildlife reintroductions: an ecological trap in reintroduced grey partridges. Journal of Applied Ecology, 47(6), 1357–1364. https://doi.org/10.1111/j.1365-2664.2010.01867.x

Robert, N., Walzer, C., Rüegg, S. R., Kaczensky, P., Ganbaatar, O., & Stauffer, C. (2005). Pathologic Findings In Reintroduced Przewalski's Horses (*Equus Caballus Przewalskii*) In Southwestern Mongolia. Journal of Zoo and Wildlife Medicine, 36(2), 273–285. https://doi.org/10.1638/03-035.1

Robinson, JG. (2006). Conservation Biology and Real-World Conservation. Conservation Biology, 20(3), 658–669. https://doi.org/10.1111/j.1523-1739.2006.00469.x

Scheibe, K., Lange, B., Lange, V., Eichhorn, E., Scheibe, A., & Streich, J. (1996). Przewalskipferde in einem Semireservat–Verhaltensuntersuchungen zur Vorbereitung einer Auswilderung. Current Research in Applied Ethology, 137–136.

Schonewald-Cox, C., Chambers, S., MacBryde, B., & Thomas, L. (1983). Genetics and Conservation: A Reference for Managing Wild Animal and Plant Populations (C. Schoenwald-Cox, S.M. Chambers, B. MacBryde, & W.L. Thomas, Eds.; 1st ed.). Benjamin/Cummings.

Seddon, P. J., Armstrong, D. P., & Maloney, R. F. (2007). Developing the Science of Reintroduction Biology. Conservation Biology, 21(2), 303–312. https://doi.org/10.1111/j.1523-1739.2006.00627.x

Snijders, L., Blumstein, D. T., Stanley, C. R., & Franks, D. W. (2017). Animal Social Network Theory Can Help Wildlife Conservation. Trends in Ecology & Evolution, 32(8), 567–577. https://doi.org/10.1016/j.tree.2017.05.005

Soulé, M. E., & Wilcox, B. A. (1980). Conservation biology: an evolutionary-ecological perspective. Sinauer Associates.

Souris, A. C., Kaczensky, P., Julliard, R., & Walzer, C. (2007). Time budget-, behavioral synchrony- and body score development of a newly released Przewalski's horse group *Equus ferus przewalskii*, in the Great Gobi B strictly protected area in SW Mongolia. Applied Animal Behaviour Science, 107(3–4), 307–321. https://doi.org/10.1016/j.applanim.2006.09.023

Tobias, J. A., & Pigot, A. L. (2019). Integrating behaviour and ecology into global biodiversity conservation strategies. Philosophical Transactions of the Royal Society B: Biological Sciences, 374(1781), 20190012. https://doi.org/10.1098/rstb.2019.0012

Turghan, M. A., Jiang, Z., & Niu, Z. (2022). An Update on Status and Conservation of the Przewalski's Horse (*Equus ferus przewalskii*): Captive Breeding and Reintroduction Projects. Animals, 12(22), 3158. https://doi.org/10.3390/ani12223158

van Dierendonck, M. C., & Wallis de Vries, M. F. (1996). Ungulate Reintroductions: Experiences with the Takhi or Przewalski Horse (*Equus ferus przewalskii*) in Mongolia. Conservation Biology, 10(3), 728–740. https://doi.org/10.1046/j.1523-1739.1996.10030728.x

Wakefield, S., Knowles, J., Zimmermann, W., & van Dierendonck, M. (2002). Status and action plan for the Przewalski's Horse (*Equus ferus przewalskii*). Equids: Zebras, Asses and Horses Status Survey and Conservation Action Plan. (P. D. Moehlman, Ed.). H. Charlesworth & Co Ltd., Huddersfield, UK.

Wallach, A. D., Inbar, M., Scantlebury, M., Speakman, J. R., & Shanas, U. (2007). Water requirements as a bottleneck in the reintroduction of European roe deer to the southern edge of its range. Canadian Journal of Zoology, 85(11), 1182–1192. https://doi.org/10.1139/Z07-098

Wells, S. M., & Goldschmidt-Rothschild, B. (2010). Social Behaviour and Relationships in a Herd of Camargue Horses. Zeitschrift Für Tierpsychologie, 49(4), 363–380. https://doi.org/10.1111/j.1439-0310.1979.tb00299.x

Wolf C. M., Garland, T., & Griffith, B. (1998). Predictors of avian and mammalian translocation success: reanalysis with phylogenetically independent contrasts. Biological Conservation, 86(2), 243–255. https://doi.org/10.1016/S0006-3207(97)00179-1

Xia, C., Cao, J., Zhang, H., Gao, X., Yang, W., & Blank, D. (2014). Reintroduction of Przewalski's horse (*Equus ferus przewalskii*) in Xinjiang, China: The status and experience. Biological Conservation, 177, 142–147. https://doi.org/10.1016/j.biocon.2014.06.021

Zhang, Y., Cao, Q. S., Rubenstein, D. I., Zang, S., Songer, M., Leimgruber, P., Chu, H., Cao, J., Li, K., & Hu, D. (2015). Water Use Patterns of Sympatric Przewalski's Horse and Khulan: Interspecific Comparison Reveals Niche Differences. PLOS ONE, 10(7), e0132094. https://doi.org/10.1371/journal.pone.0132094

ZIMS for Studbook for Przewalski's horse, & Šimek J. (2022). ZIMS for Studbook for Przewalski's horse. Species360 Zoological Information Management System. 2022. http://zims.Species360.org

CHAPTER 11

11. Appendices

Appendix 1	Supplementary material to Chapter 4
Appendix 2	Supplementary material to Chapter 5
Appendix 3	Supplementary material to Chapter 6
Appendix 4	Supplementary material to Chapter 7

Appendix 1

Supplementary material for Chapter 4: Influence of weather on the behaviour of reintroduced Przewalski´s Horses in the Great Gobi B Strictly Protected Area (Mongolia). Implications for conservation.

Table S4.1. Pearson´s correlations among all weather variables measured during the research.

	TR (True)	WS (m/s)	CW (m/s)	HW (m/s)	TP (°C)	WC (°C)	RH (%)	HI (°C)	DP (°C)	WB (°C)	BP (mb)	AL (m)	DA (m)	Clouds (location)	Clouds (whole)
Dispersion	0.002	0.027	0.027	0.049*	-0.120***	-0.119***	-0.041	-0.132***	-0.103***	-0.179***	0.310***	-0.309***	-0.228***	-0.015	-0.042
TR (True)		0.294***	0.341***	0.271***	0.120***	0.099***	0.120**	0.151***	0.214***	0.255***	-0.251***	0.252***	0.212***	0.130***	0.226***
WS (m/s)			0.878***	0.112***	0.095***	0.039	-0.083**	0.097***	-0.001	0.054*	-0.170***	0.170***	0.145***	0.114***	0.130***
CW (m/s)				0.154***	0.085**	0.031	-0.006	0.098***	0.072**	0.106***	-0.201***	0.201***	0.153***	0.151***	0.167***
HW (m/s)					0.001	-0.012	0.077**	0.020	0.108***	0.092***	-0.095***	0.095***	0.046	0.065**	0.138***
TP (°C)						0.995***	-0.516**	0.989***	-0.134***	0.642***	-0.249***	0.249***	0.919***	-0.076**	0.068**
WC (°C)							-0.501**	.984***	-0.121***	0.652***	-0.235***	0.234***	0.910***	-0.081**	0.065**
RH (%)								-0.402***	0.824***	0.301***	0.031	-0.030	-0.382***	0.347***	0.284***
HI (°C)									-0.013	0.730***	-0.265***	0.265***	0.924***	-0.034	0.106***
DP (°C)										0.632**	-0.155**	0.155**	0.012	0.236**	0.243**
WB (°C)											-0.267**	0.268**	0.683**	0.186**	0.311**
BP (mb)												-1.000**	-0.606**	0.034	-0.044
AL (m)													0.606**	-0.033	0.046
DA (m)														-0.056*	0.096**
Clouds (location)															0.782**

Significance is indicated as *, ** and *** for 0.05, 0.01 and 0.001 levels, respectively.

MG (mag) = magnetic heading; TR (True) = true heading; WS (m/s) = windspeed; CW (m/s) = crosswind calculation; HW (m/s) = headwind/tailwind; TP (°C) = temperature; WC (°C) = windchill; RH (%) = relative humidity; HI (°C) = heat stress index; DP (°C) = dewpoint temperature; WB (°C) = wet bulb temperature; BP (mb) = barometric pressure; AL (m) = altitude; DA (m) = density altitude; clouds (location) = % of cloud cover in the place of observation; clouds (whole) = % of cloud cover in the observable surrounding.

Table S4.2. Pearson´s correlations among all the weather variables and the percentage representation of each studied behavioural categories.

	Feeding	Locomotion	Other	Resting	Social
Dispersion	0.190***	-0.055*	-0.065**	-0.058*	-0.049
TR (True)	-0.045	0.051*	-0.109***	0.076**	0.018
WS (m/s)	-0.053*	0.014	-0.087**	0.087***	0.028
CW (m/s)	-0.003	0.010	-0.106***	0.075**	0.001
HW (m/s)	-0.016	0.009	-0.024	0.038	-0.019
TP (°C)	-0.182***	0.108***	0.007	0.023	0.099***
WC (°C)	-0.180***	0.103***	0.024	0.016	0.092***
RH (%)	0.078**	0.008	-0.023	-0.038	-0.037
HI (°C)	-0.181***	0.119***	0.001	0.019	0.097***
DP (°C)	-0.001	0.061*	0.014	-0.046	-0.013
WB (°C)	-0.152**	0.130**	0.017	-0.005	0.063*
BP (mb)	0.130**	-0.054*	-0.074**	0.003	-0.045
AL (m)	-0.129**	0.054*	0.074**	-0.003	0.045
DA (m)	-0.205**	0.115**	0.037	0.016	0.099**
clouds place	0.094**	-0.014	-0.071**	-0.042	0.027
clouds whole	0.028	0.007	-0.061*	-0.016	0.047

Significance is indicated as *, ** and *** for 0.05, 0.01 and 0.001 levels, respectively.

MG (mag) = magnetic heading; TR (True) = true heading; WS (m/s) = windspeed; CW (m/s) = crosswind calculation; HW (m/s) = headwind/tailwind; TP (°C) = temperature; WC (°C) = windchill; RH (%) = relative humidity; HI (°C) = heat stress index; DP (°C) = dewpoint temperature; WB (°C) = wet bulb temperature; BP (mb) = barometric pressure; AL (m) = altitude; DA (m) = density altitude; clouds (location) = % of cloud cover in the place of observation; clouds (whole) = % of cloud cover in the observable surrounding.

Appendix 2

Supplementary material for Chapter 5: Habitat use differs in released and wild-born Przewalski's Horses of the Great Gobi B Strictly Protected Area (Mongolia)

Table SM5.1. Frequency of the two response variables (Feeding and Resting) within the Origin groups (Reintroduced vs. Wild-born) and the three different time periods (Full, Spring/Summer 2019 (SS-2019) and Autumn 2019 (A-2019)).

Period	**Origin**	**Response variable**	
		Feeding	**Resting**
Full	Reintroduced	132	127
	Wild-born	283	312
SS-2019	Reintroduced	90	60
	Wild-born	121	212
A-2019	Reintroduced	42	67
	Wild-born	162	100

Table SM5.2. Range values (minimum and maximum) of predictors per time periods (Full, Spring/Summer 2019 - SS-2019 and Autumn 2019 - A-2019).

	Predictors						
Period	**ALOSchi**	**ALOSdem**	**ALOSldfs**	**ALOSmtpi**	**LCdcl**	**LCdclpro**	**NDVImx**
Full	0 - 254	1027 - 3314	11 - 42	-179 - 226	20 - 90	0 - 100	0.03 - 0.84
SS-2019	idem	idem	idem	idem	idem	idem	-0.46 - 0.73
A-2019	idem	idem	idem	idem	idem	idem	-0.33 - 0.68

Table SM5.3. Regression coefficients of standardised predictors and their confidence intervals (within brackets). Model performance based on the Akaike Information Criterion value (AIC), the Nagelkerke pseudo-R^2 and the Accuracy is also shown. For comparison, a baseline 'null model' containing a single intercept term was used (AIC in brackets). Significance values in AIC cells correspond to an ANOVA test between null models and logistic models. Feeding was considered the base class for each model. RE = Reintroduced; WB = Wild-born; Spring/Summer 2019 (SS-2019); Autumn 2019 (A-2019).

Predictors	***Full***		***SS-2019***			***A-2019***
	RE	**WB**	**RE**	**WB**	**RE**	**WB**
ALOSchi	0.06	**0.49***	0.19	-0.03	-0.03	0.41
	(-0.10 – 0.23)	**(-0.03 – 1.02)**	(-0.06 – 0.46)	(-0.84 – 0.77)	(-0.67 – 0.51)	(-0.51 – 1.37)
ALOSdem	**-0.46****	-0.05	**-1.15****	**1.05*****	0.89	-0.28
	(-0.88 – -0.05)	(-0.37 – 0.28)	**(-2.20 – -0.14)**	**(0.45 – 1.68)**	(-0.07 – 2.18)	(-0.92 – 0.35)
ALOSldfs	-0.23	0.07	**-0.72*****	0.19	0.24	0.15
	(-0.59 – 0.13)	(-0.14 – 0.28)	**(-1.28 – -0.20)**	(-0.12 – 0.51)	(-0.57 – 1.01)	(-0.2 – 0.51)
ALOSmtpi	**0.28****	0.29	-0.11	0.75	**0.95****	-0.03
	(0.04 – 0.53)	(-0.22 – 0.81)	(-0.48 – 0.24)	(-0.2 – 2.1)	**(0.24 – 1.81)**	(-1.36 – 1.33)
LCdcl	**-0.71****	0.2	-0.83	**-0.52***	0.3	0.23
	(-1.38 – -0.06)	(-0.05 – 0.46)	(-2.03 – 0.32)	**(-1.08 – 0.01)**	(-1.23 – 1.95)	(-0.17 – 0.63)
LCdclpro	0.44	-0.13	0.07	**0.66****	0.27	-0.06
	(-0.13 – 1.02)	(-0.41 – 0.14)	(-0.89 – 1.04)	**(0.12 – 1.23)**	(-0.97 – 1.53)	(-0.67 – 0.57)
NDVImx	-0.15	0.08	-0.29	**1.45*****	0.44	**-0.92****
	(-0.51 – 0.20)	(-0.09 – 0.27)	(-0.82 – 0.23)	**(0.77 – 2.19)**	(-0.36 – 1.29)	**(-1.83 – -0.22)**
Constant	**0.62***	**-0.21***	**1.69*****	-0.51	**-1.39***	**0.29***
	(-0.01 – 1.28)	**(-0.47 – 0.04)**	**(0.58 – 2.89)**	(-0.97 – 0.1)	**(-3.4 – 0.23)**	**(-0.22 – 0.8)**
AIC	347.62 (379.86)**	831.38 (866.28)***	203.35 (215.61)*	408.32 (461.53)**	122.82 (156.33)***	342.01 (369.22)***
pseudo-R^2	0.12	0.02	0.12	0.17	0.41	0.11
Accuracy (%)	66	54	65	66	72	63

Note: *p<0.1; **p<0.05; ***p<0.01

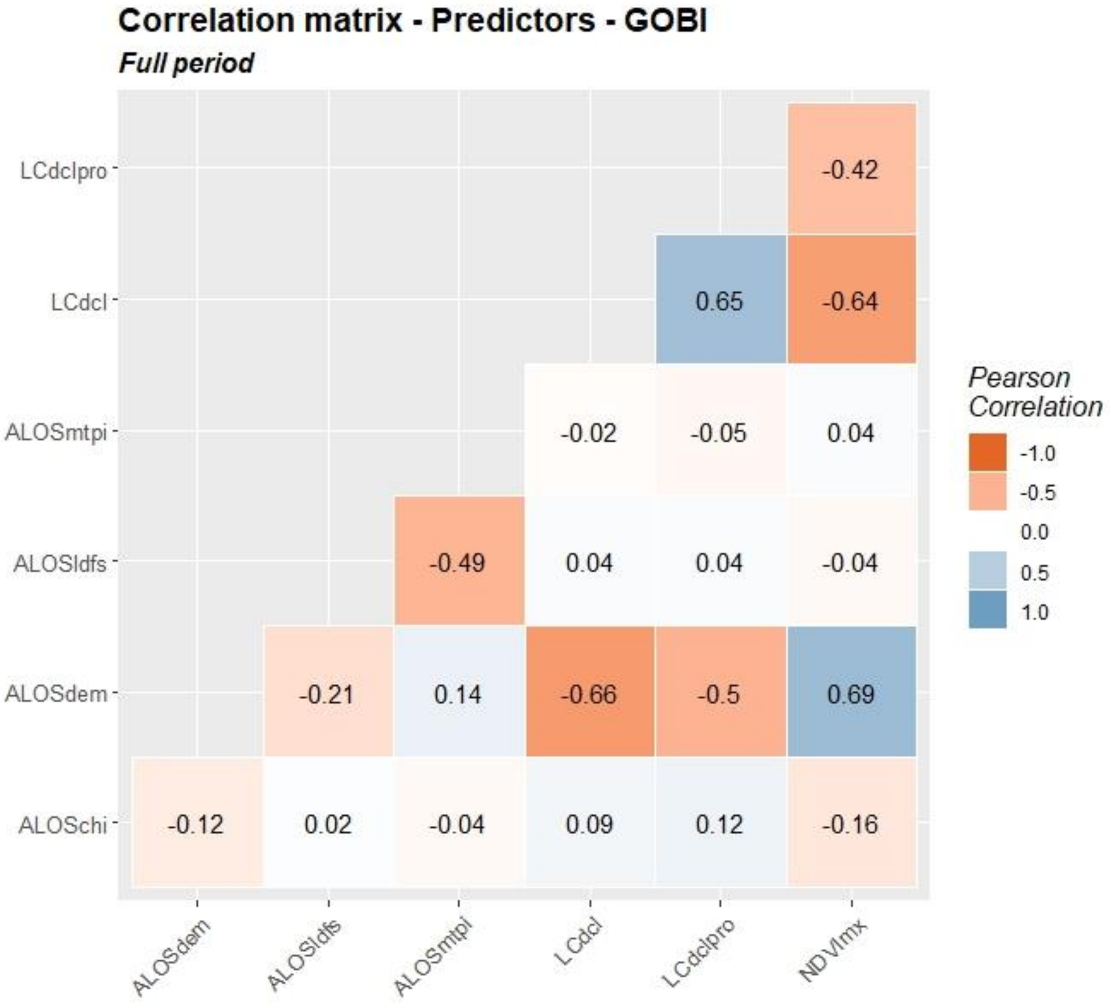

Figure SM5.1. Pair-wise correlation matrix among the selected descriptors used in model fitting for the Full period (Spring/Summer and Autumn 2019). Values represent the Pearson rank-order correlation coefficient (values scaled from -1 to 1). See acronyms in Table 2.

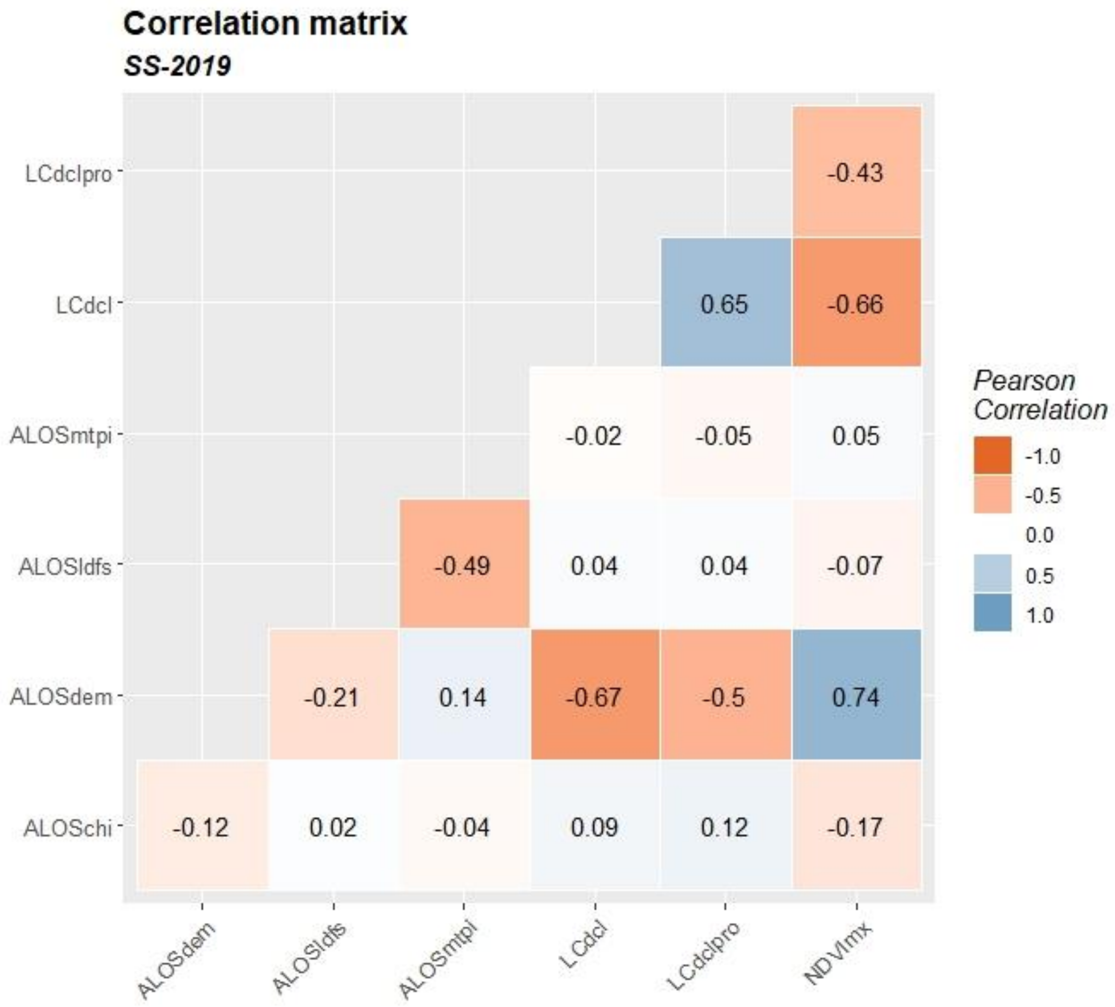

Figure SM5.2. Pair-wise correlation matrix among the selected descriptors used in model fitting for the Spring/Summer 2019 period. Values represent the Pearson rank-order correlation coefficient (values scaled from -1 to 1). See acronyms in Table 2.

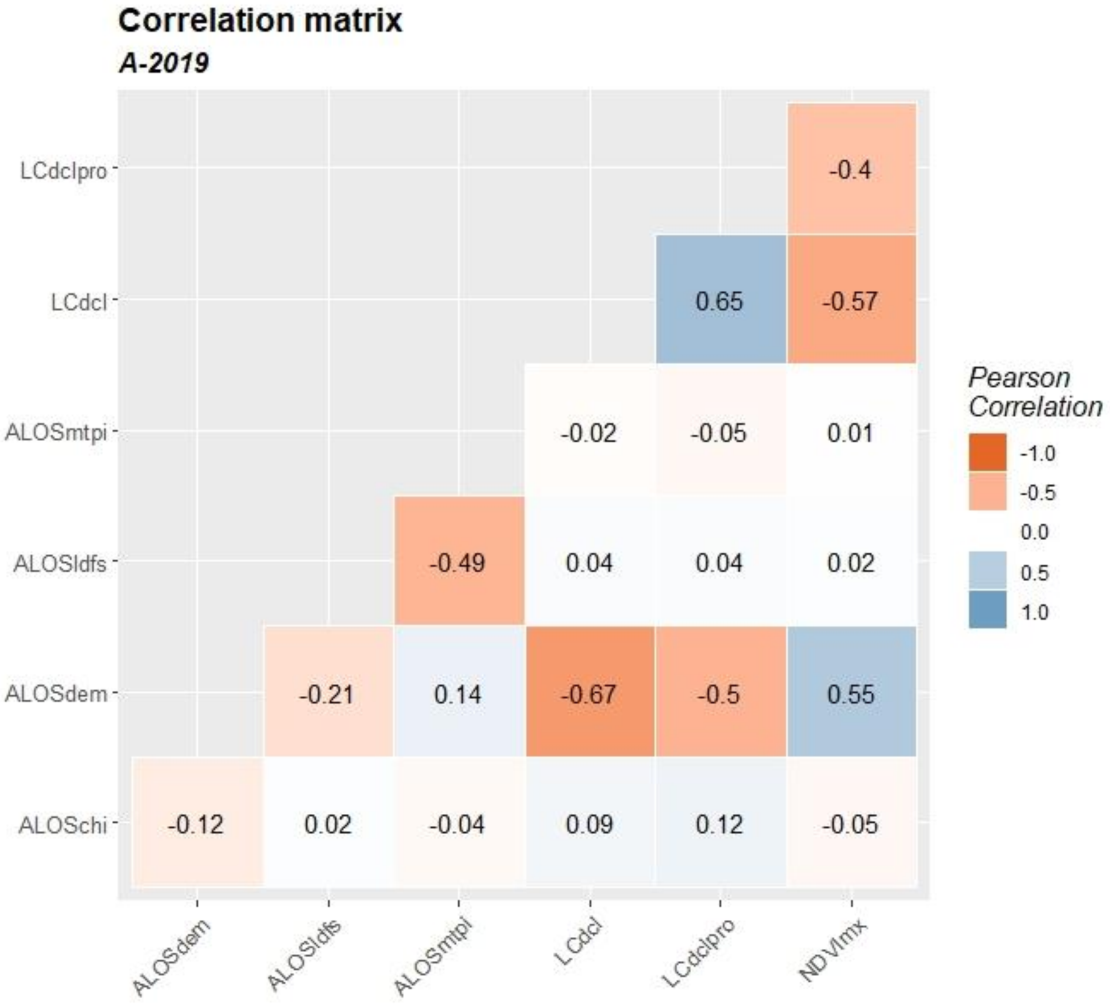

Figure SM5.3. Pair-wise correlation matrix among the selected descriptors used in model fitting for the Autumn 2019 period. Values represent the Pearson rank-order correlation coefficient (values scaled from -1 to 1). See acronyms in Table 2.

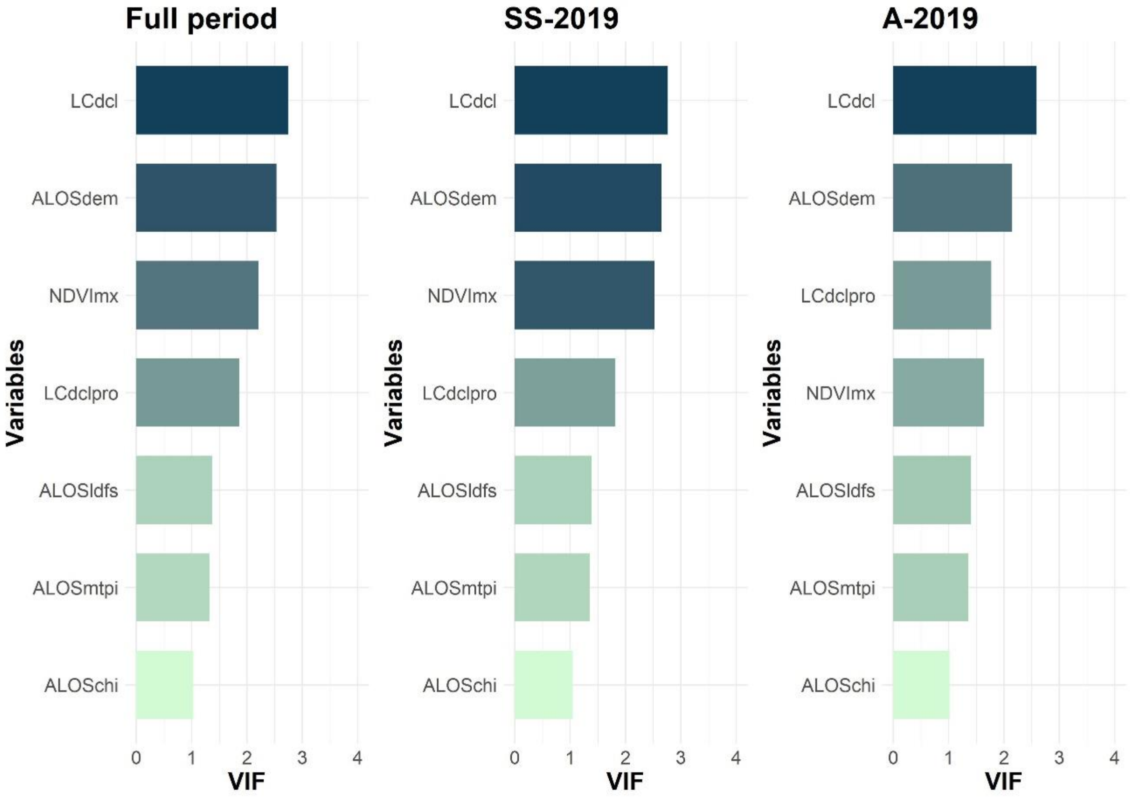

Figure SM5.4. Variance Inflation Factor (VIF) test between satellite-derived predictors for the Full, Spring/Summer (SS-2019) and Autumn 2019 (A-2019) periods.

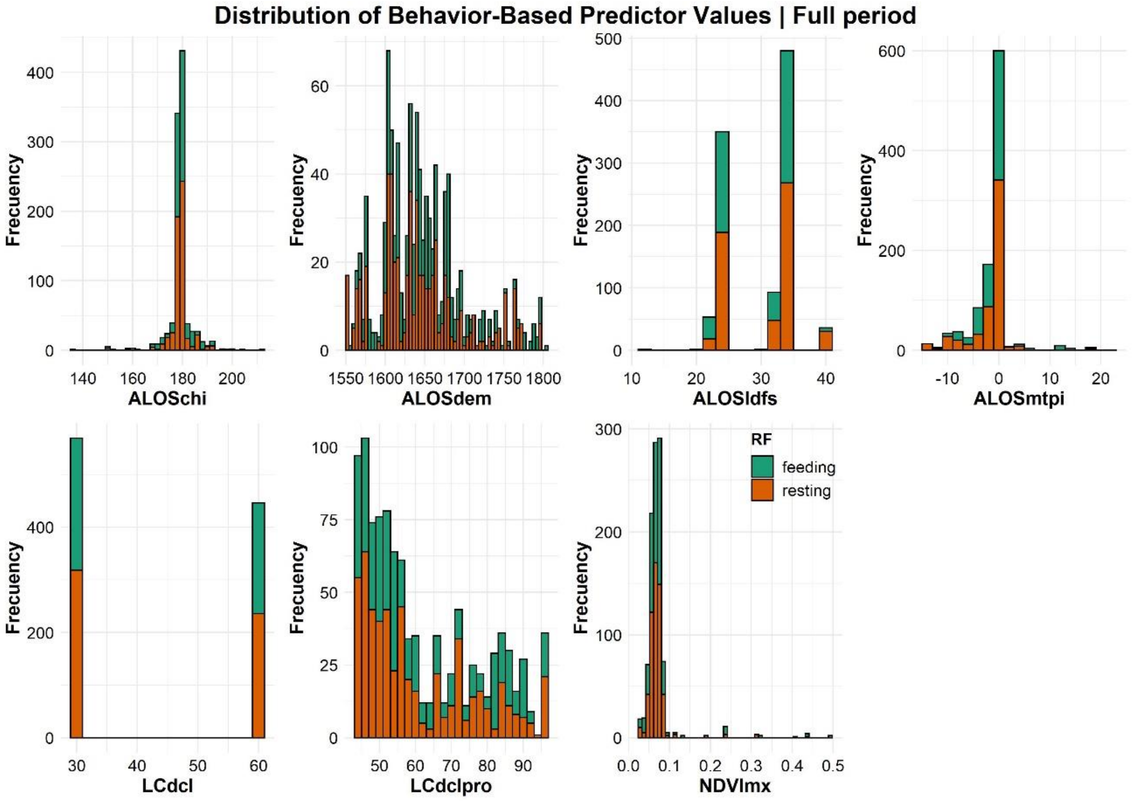

Figure SM5.5. Summary of the frequency data of the satellite-derived predictor values for the Full period (Spring/Summer and Autumn 2019).

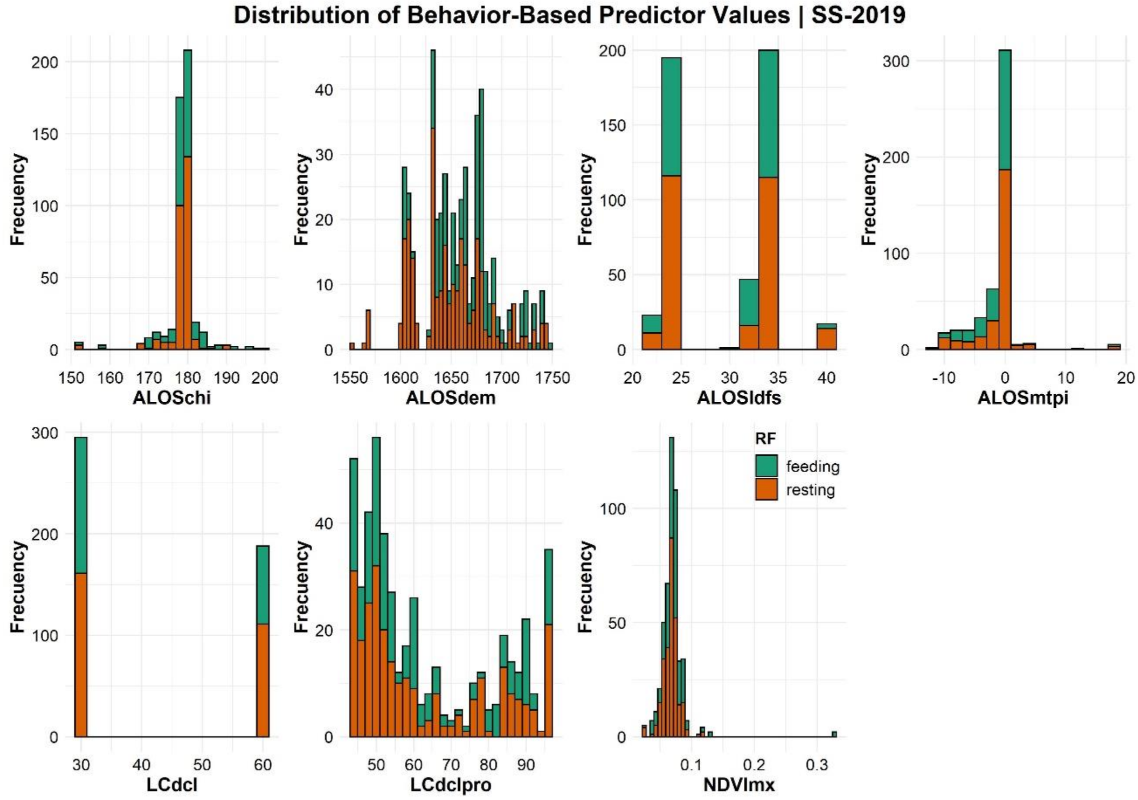

Figure SM5.6. Summary of the frequency data of the satellite-derived predictor values for the Spring/Summer 2019 period.

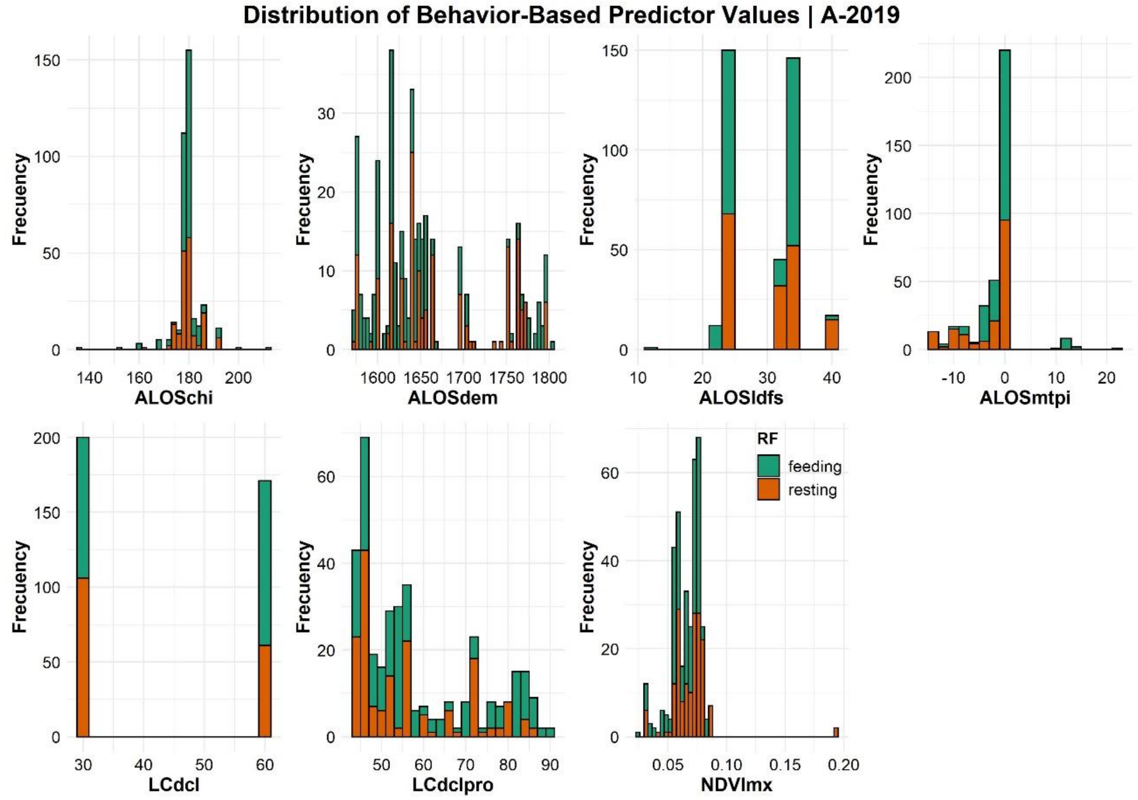

Figure SM5.7. Summary of the frequency data of the satellite-derived predictor values for the Autumn 2019 period.

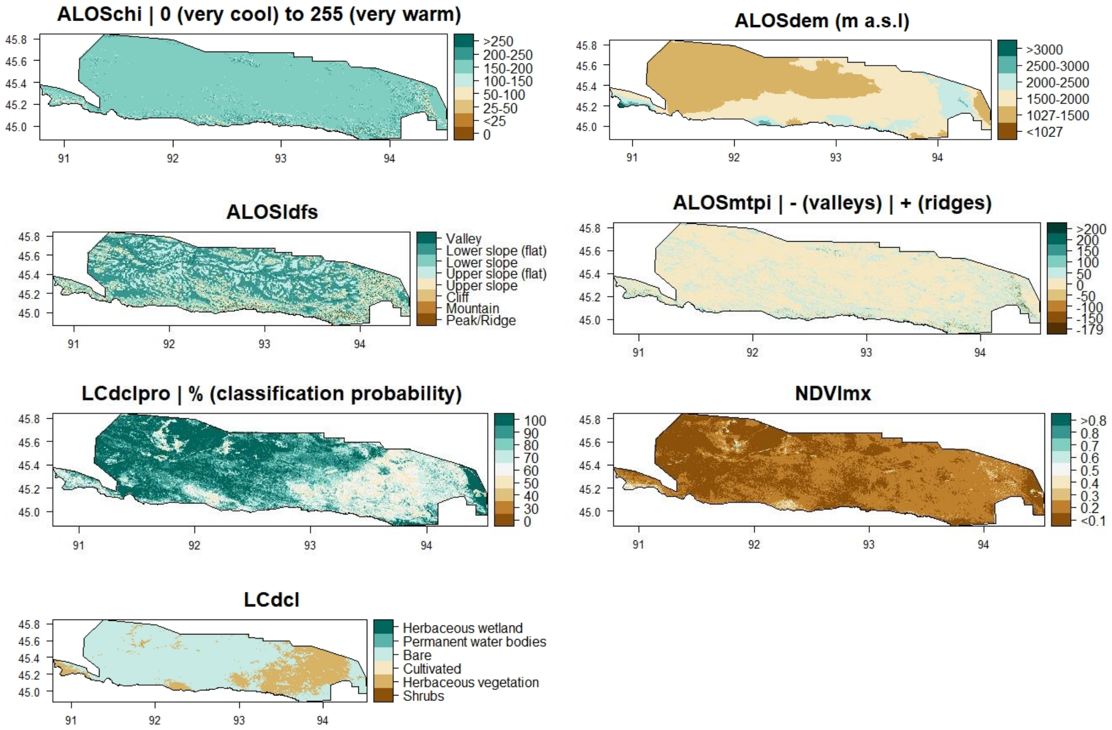

Figure SM5.8. Spatial projections of the satellite-derived remote sensing predictor variables for the GOBI study area at 100 m squares for the Full period (Spring/Summer and Autumn 2019).

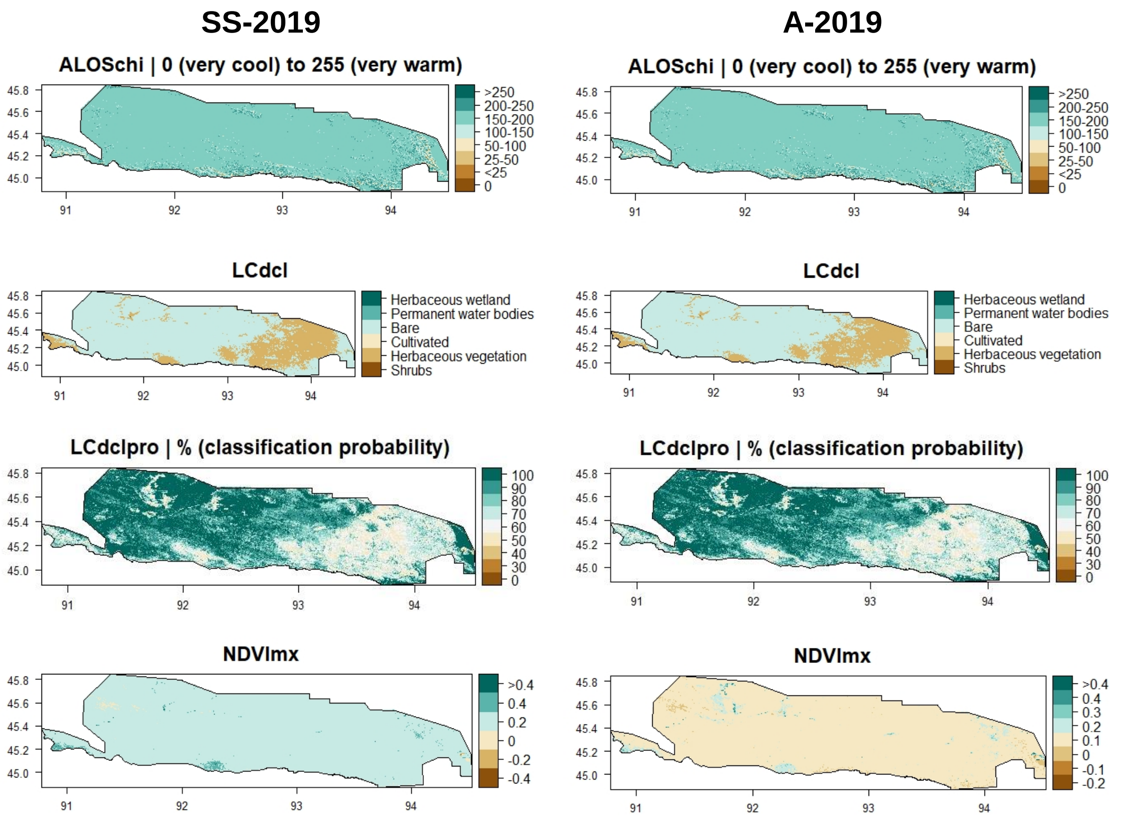

Figure SM5.9. Spatial projections of the satellite-derived remote sensing predictor variables for the GOBI study area at 100 m squares for the Spring/Summer (SS-2019) and Autumn (A-2019) 2019 periods.

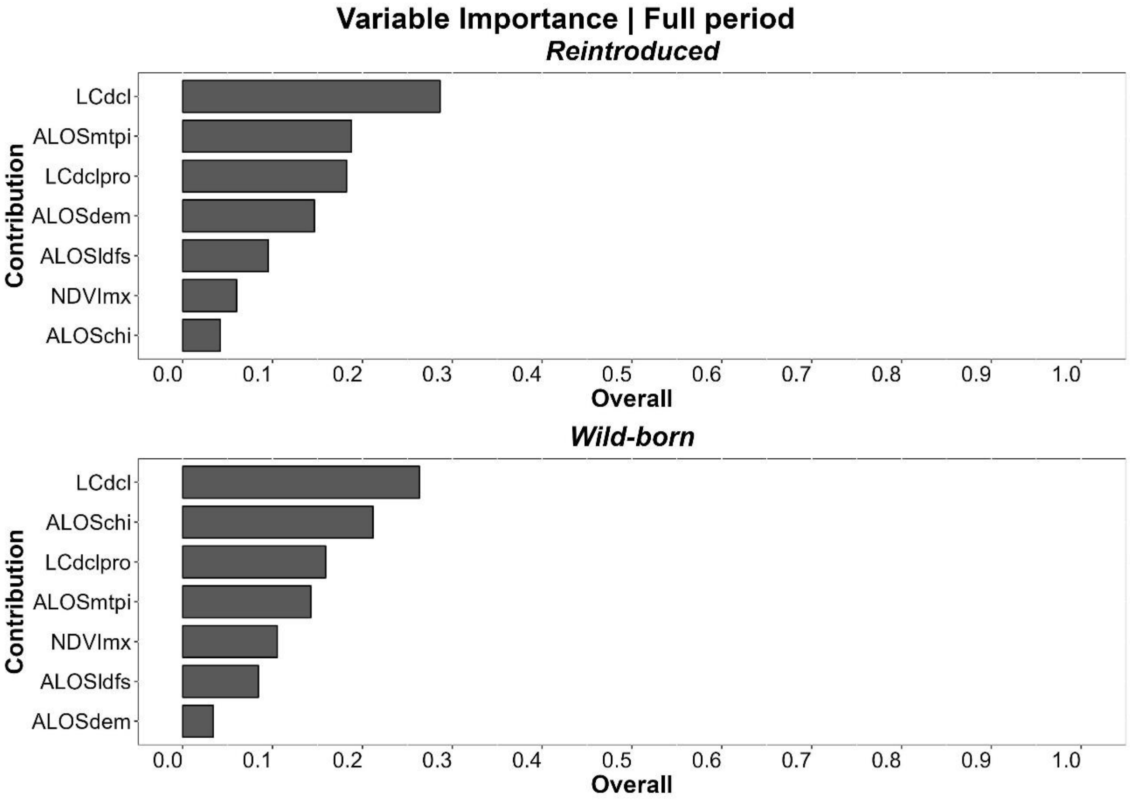

Figure SM5.10. Relative predictor contributions across models for the two horse classes (Reintroduced and Wild-born) and for the Full period (Spring/Summer and Autumn 2019). See Table 2 to check the codes and description of each predictor.

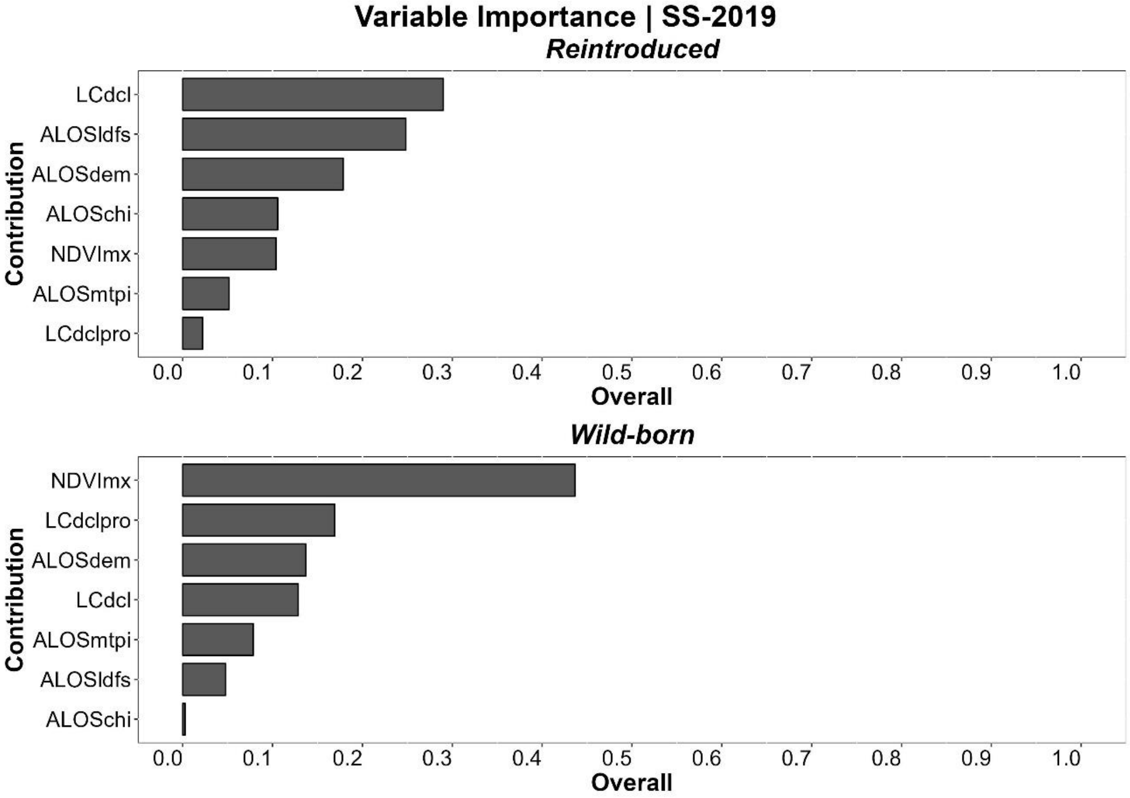

Figure SM5.11. Relative predictor contributions across models for the two horse classes (Reintroduced and Wild-born) and for the Spring/Summer 2019 period. See Table 2 to check the codes and description of each predictor.

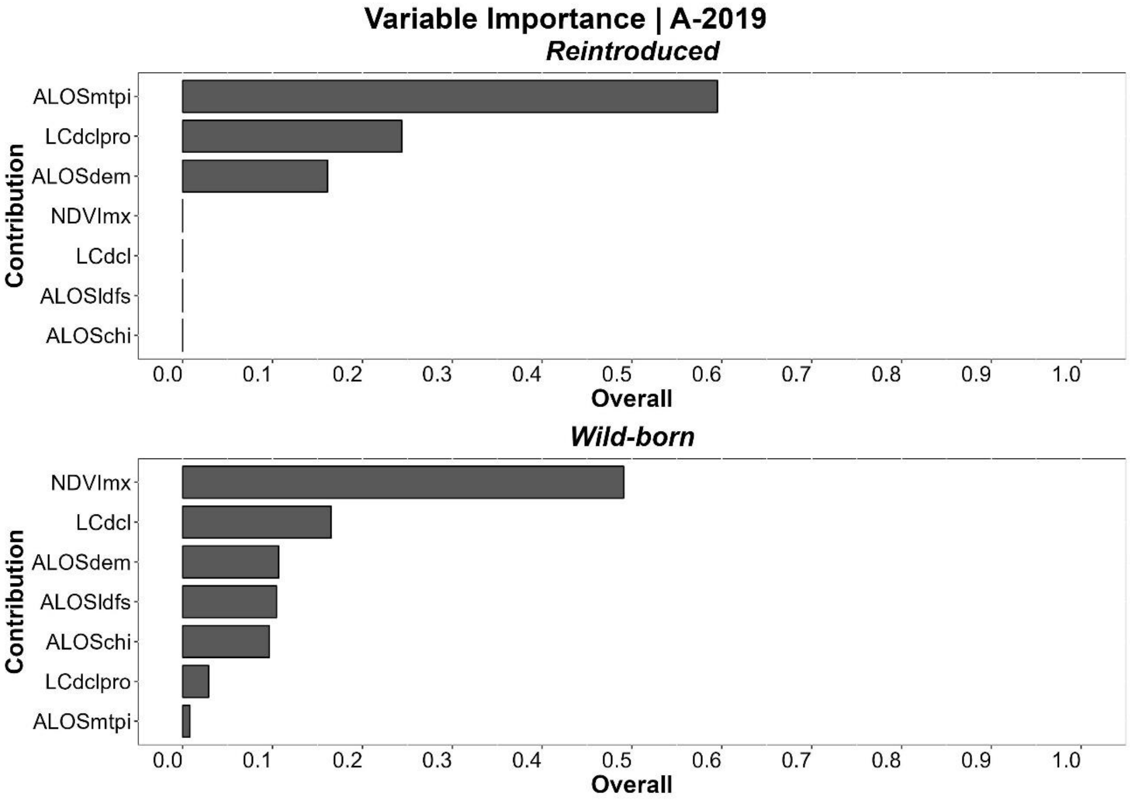

Figure SM5.12. Relative predictor contributions across models for the two horse classes (Reintroduced and Wild-born) and for the Autumn 2019 period. See Table 2 to check the codes and description of each predictor.

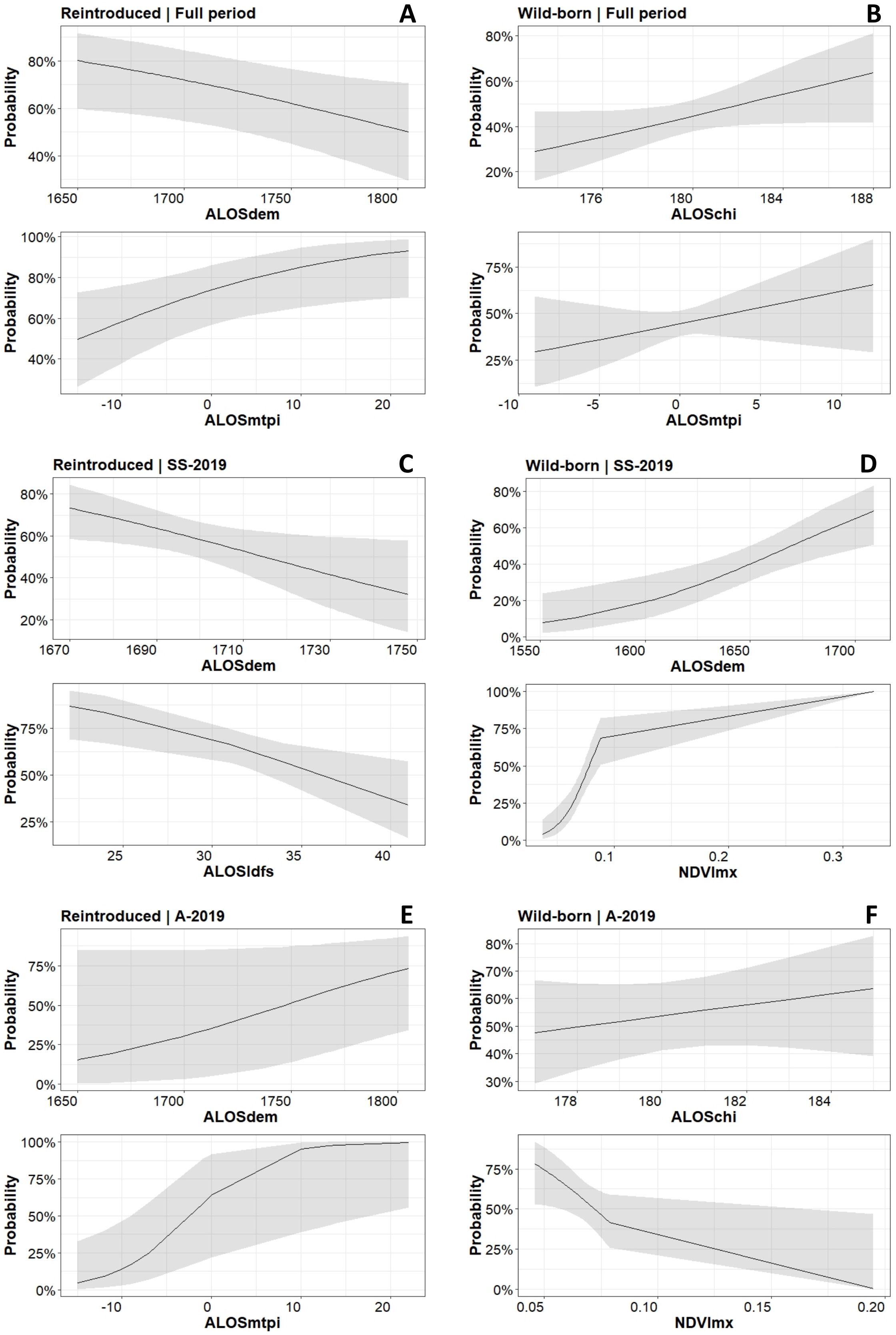

Figure SM5.13. Response curves of predicted 'feeding' against the most important satellite-derived predictor variables for: A-B) the Full period (Spring/Summer and Autumn 2019); C-D) the Spring/Summer 2019 period (SS-2019); and E-F) the Autumn 2019 period (A-2019).

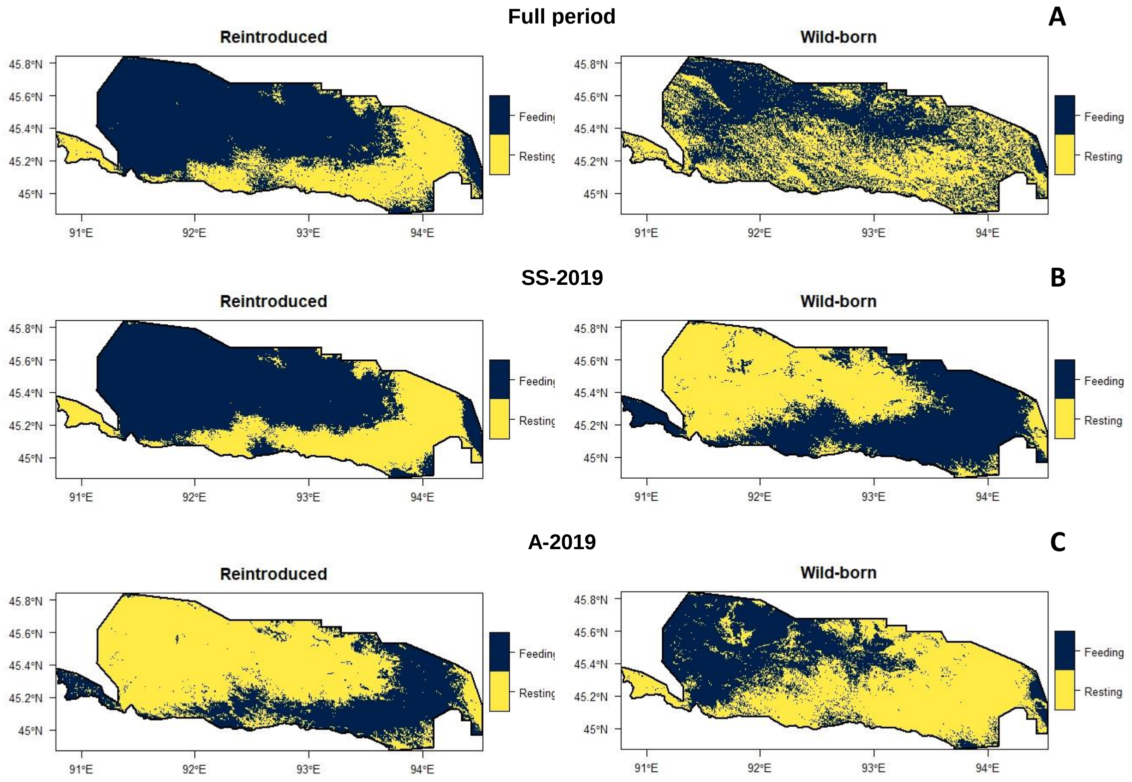

Figure SM5.14. Binary maps representing the spatial projections of preference areas for ‘feeding’ vs. ‘resting’ by the two types of horses (Reintroduced and Wild-born) in the GOBI study area at 100 m squares and for: A) Full (Spring/Summer and Autumn 2019); B) SS-2019; and C) A-2019.

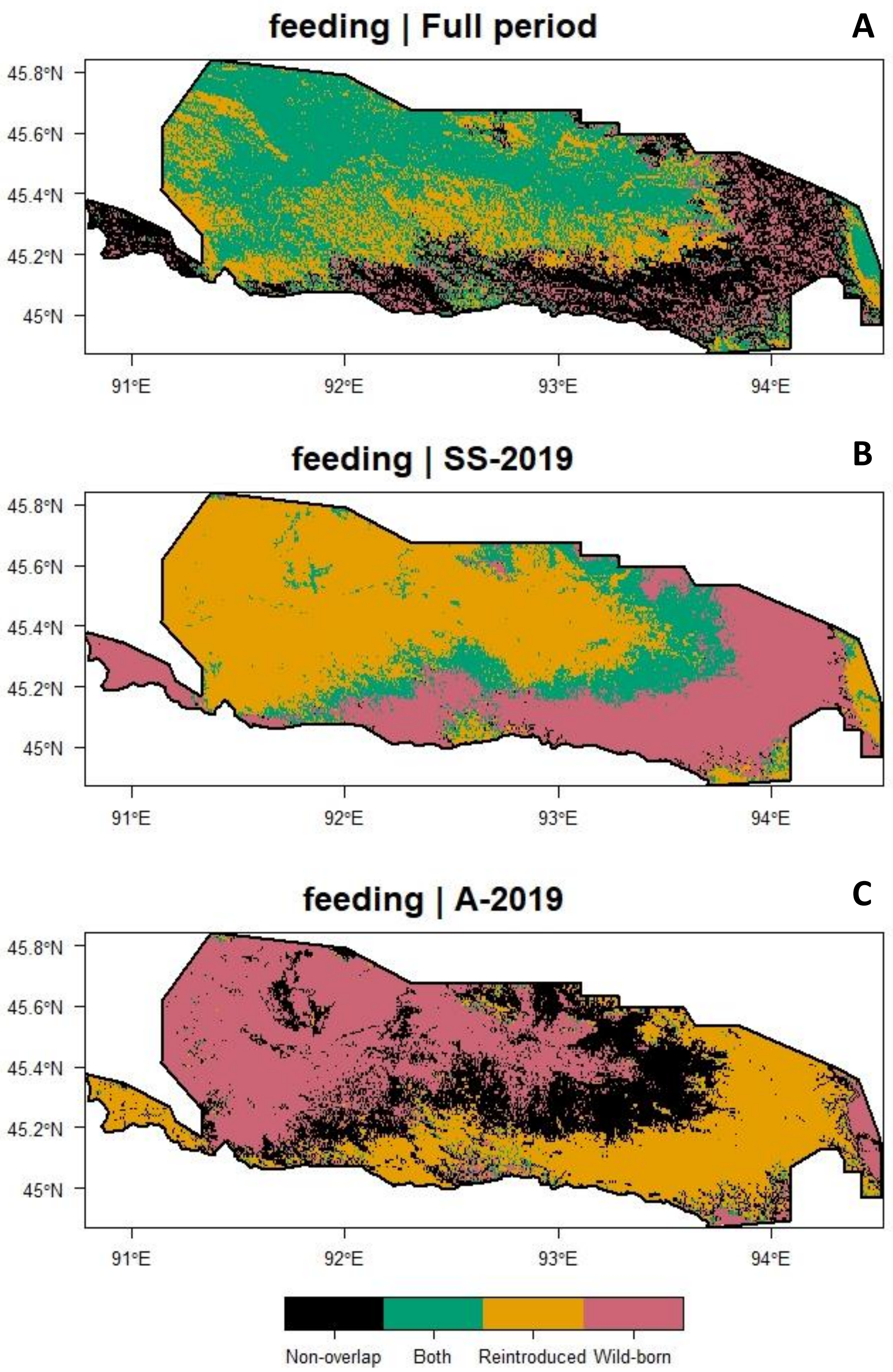

Figure SM5.15. Overlay maps of predicted 'feeding' behaviour for the GOBI study area per period: A) Full (Spring/Summer and Autumn 2019); B) SS-2019; and C) A-2019.

Appendix 3

Supplementary material for Chapter 6: Social behaviour of reintroduced Przewalski's Horses in the Great Gobi B Strictly Protected Area (Mongolia).

Studied animals

In July 2018, the initial data in the Great Gobi B SPA, Mongolia, was collected. Three easy-to-follow groups were recorded (wild- born Azaa, wild-born Tsetsen and acclimatizing Mares18). Azaa harem consisted of 17 individuals – 1 dominant stallion, 6 breeding mares, 2 two-year-old males, 1 one-year-old male, 3 one-year-old females, 2 male and 2 female foals. At the end of observation, the average age of foals was 46 days. All the members of Azaa harem, except for one breeding mare, were born in the wild – GGB SPA. Tsetsen harem consisted of 8 individuals – 1 dominant stallion, 3 breeding mares (one of them was only 2 years old), 2 two-year-old males, 1 one-year-old male and 1 one-year-old female. All the members of Tsetsen harem were born in the wild – GGB SPA. Group of Mares18 consisted of 4 mares – 3 three years old and 1 four years old. All mares were born ex-situ, transported to GGB SPA by Prague Zoo and released to the acclimatization fence on 20.6.2018. All four mares came from three European breeding facilities. Two of them were born in the Helsinki Zoo, one came from the Swiss Wildnispark Zürich Langeberg, and one came from the German Wisentgehege Springe. The four mares were observed in the acclimatization enclosure during this research period.

The data collection continued in late May - June summer 2019. Same groups as in 2018 and one other group were recorded (wild-born Azaa, wild-born Tsetsen, reintroduced Hustai1 – previously Mares18 – and acclimatizing Mares19). Azaa harem consisted of 14 individuals – 1 dominant stallion, 5 breeding mares, 2 two-year-old females, 2 one-year-old males, 1 one-year-old female, 2 male and 1 female foal. One two-year-old female left the group just at the beginning of our observation period – on 21.5.2019. At the end of observation, the average age of foals was 26 days. All the members of Azaa harem, except for one breeding mare, were born in the wild – GGB SPA.

Tsetsen harem consisted of 12 individuals – 1 dominant stallion, 4 breeding mares, 1 three years old male (which was observed breeding with mares in the harem several times), 1 two-year-old male, 1 two years old female, 1 one-year-old male and 3 female foals. At the end of observation, the average age of foals was 28 days. All the members of Tsetsen harem were born in the wild – GGB SPA.

Hustai1 consisted of the Mares18 and a stallion which joined them by getting into the acclimatization fence. This stallion was 7 years old and was born in Hustai Nuruu National Park and transported to GGB SPA 21.07.2016. Hustai and "his" mares were released from the acclimatization fence on 20.5.2019, immediately before our observation period. The Hustai1 harem, Azaa and Tsetsen harem were observed in the wild.

Mares19 consisted of 3 mares – 2 five years old and 1 six years old. All mares were born ex-situ, transported to GGB SPA by Prague Zoo and released to the acclimatization fence 19.6.2019, so just three days of recordings were collected. All three mares came from three European breeding facilities. Two of the three mares come from Prague Zoo, Czech Republic. One mare was born directly in Prague, one in the Prague Zoo breeding facility in Dolní Dobřejov. One mare was born in Springe, Germany. The three mares were observed in the acclimatization enclosure during this research period.

The last data collection was conducted in autumn 2019. Same groups as in summer 2019 were recorded (wild-born Azaa, wild-born Tsetsen, reintroduced Tanan (previously Mares18/Hustai1) and acclimatizing Hustai2 (previously Mares19). Azaa harem consisted of 14 individuals – 1 dominant stallion, 5 breeding mares, 2 one-year-old males, 1 one-year-old female, 3 male foals and 2 female foals. At the end of observation, the average age of foals was 100 days. All the members of Azaa harem, except for one breeding mare, were born in the wild – GGB SPA.

Tsetsen harem consisted of 12 individuals, the same as in summer 2019. The average age of foals (at the end of observation) was 128 days.

Tanan consisted of the mares from Hustai1 harem from summer 2019 and stallion Tanan, which joined them after he defeated Hustai stallion on 3.8.2019. This stallion was 8 years old and was born in GGB SPA 21.07.2016. The Tanan harem, Azaa and Tsetsen harem were observed in the wild.

Hustai2 consisted of the Mares19 and Hustai stallion, which joined them by getting into the acclimatization fence after losing his original harem to Tanan stallion. The three mares and Hustai stallion were observed in the acclimatization enclosure during this research period.

Appendix 4

Supplementary material for Chapter 7: Novel observation of play behaviour between a harem holder and a bachelor group of Przewalski's horses in the wild

Video S1:

https://czuvpraze-my.sharepoint.com/:v:/g/personal/ceacero_ftz_czu_cz/ETXJD9pDZ5NCk_HbLiv3N6YBkKiH0DGV-8jUVNeCEua8XA?e=rneken

Video S2:

https://czuvpraze-my.sharepoint.com/:v:/g/personal/ceacero_ftz_czu_cz/Eb9bvCTGKjxLgYVYi-33UsIBSWDYWBdcTwd83NisDR-TRA?e=ieMsV3

Video S3:

https://czuvpraze-my.sharepoint.com/:v:/g/personal/ceacero_ftz_czu_cz/Ed9IrzLvgkRLoF08XLKXuOkBBeNma0x5zljVBOFX0C47tA?e=77luxr

Video S4:

https://czuvpraze-my.sharepoint.com/:v:/g/personal/ceacero_ftz_czu_cz/EXtsRd3k1yZNsnXRgmhK184BrEuf44VP-rz8fdsChNCaoQ?e=9VgnBw¨

Video S5:

https://czuvpraze-my.sharepoint.com/:v:/g/personal/ceacero_ftz_czu_cz/EZRfsdEU2OpMmgA2OtEM7b0B8_Y_9Gs6nCvfRMGnH9xCDg?e=lFU39M

Video S6:

https://czuvpraze-my.sharepoint.com/:v:/g/personal/ceacero_ftz_czu_cz/EYZr8_EfRY5Om6tUqA4sEgABTu8S5g1KL5HM_RiHivDe2g?e=UGwyMT

Video S7:

https://czuvpraze-my.sharepoint.com/:v:/g/personal/ceacero_ftz_czu_cz/EZbGYjIlOWZGjYFRhUE1W3ABmkvFJ9KHQH2mhlz-jm8R3Q?e=zwr6Yn

Video S8:

https://czuvpraze-my.sharepoint.com/:v:/g/personal/ceacero_ftz_czu_cz/EbRkGbSReXFIjiGF-9zbLKgBOWTewDk6RKwI1cONQ7eJ2Q?e=MuTHtx

Video S9:

https://czuvpraze-my.sharepoint.com/:v:/g/personal/ceacero_ftz_czu_cz/EcClLsRFLvlEu-vTYqnVrHoBc1DDLcqRGKdZFJ91F_gk8Q?e=eqkNTh

Video S10:

https://czuvpraze-my.sharepoint.com/:v:/g/personal/ceacero_ftz_czu_cz/EQkfZDL3nqZBhPZCVYwPOtYBBDaeFGh5GYha6QP3IDZGkQ?e=Y9u6IW

Note: The videos will be also available at the web of the publisher, https://doi.org/10.1007/s10211-023-00421-9