The behaviour and management of Przewalski's horses in semi-reserves

DISSERTATION ZUR ERLANGUNG DES
DOKTORGRADES DER NATURWISSENSCHAFTEN (DR. RER. NAT.)
DER FAKULTÄT FÜR BIOLOGIE UND VORKLINISCHE MEDIZIN
DER UNIVERSITÄT REGENSBURG

VORGELEGT VON

Riccarda Wolter
Hanau
Juni 2018

Promotionsgesuch eingereicht am:	05. Juni 2018
Die Arbeit wurde angeleitet von:	Prof. Dr. Konstanze Krüger

Unterschrift:

Manuscripts that contributed to this Thesis and Author's contribution

Chapter 1

Factors influencing grooming behaviour in wild living horses.
Riccarda Wolter & Konstanze Krüger (2015).
Current research in applied ethology 510, 242-248

Personal contribution:
Writing of the manuscript with correction of KK.

Chapter 2

Parameters for the analysis of social bonds: a case study in horses.
Riccarda Wolter, Volker Stefanski & Konstanze Krüger (2018).
Animals 8(11), 191; DOI: 10.3390/ani8110191

Personal contribution:
Planning the data recording together with KK; collection and preparation of data; analysis of data together with KK; writing of the manuscript with correction of VS and KK.

Chapter 3

The role of an alpha male in changing environmental conditions.
Riccarda Wolter, Norbert Pantel, Volker Stefanski, Erich Möstl, Konstanze Krüger (2014).
Physiology & Behavior 133, 236-243

Personal contribution:
Planning the data recording together with KK; collection of data; analysis of data together with EM, KK; writing of the manuscript with correction of NP, VS and KK.

Chapter 4

Comparison between the responses of two Przewalski's horse groups to semi-reserve management in different habitat types.
Riccarda Wolter, Norbert Pantel, Konstanze Krüger
Submitted to Basic and Applied Ecology

Personal contribution:
Planning the data recording together with KK; collection of data; analysis of data together with NP, KK; writing of the manuscript with correction of NP and KK.

Chapter 5

A case study on space, behaviour and well-being of horses (*Equus caballus*) in open stabling and open active stabling.
Daniela Töpfer, Riccarda Wolter, Konstanze Krüger (2014).
Current research in applied ethology 505, 252-254

Personal contribution:
Planning the data recording together with DT and KK; correction of the manuscript with KK.

TABLE OF CONTENTS

GENERAL INTRODUCTION

At the beginning of this general introduction, the animal species dealt with in this study, the Przewaslki's horse (*equus ferus przewalskii*), will be introduced through a short outline of its evolutionary history, the social structure and the differences and similarities between the Przewalski's horse and the domestic horse (*Equus caballus*). Furthermore, the role of the Przewalski's horse as a conservationist of semi-reserves will be introduced and I discuss the potential for stress levels to act as an indicator for the well-being of the horses. After that, my topic will be discussed in the light of behavioural ecology and sociobiology. The subsequent paragraph is devoted to an introduction of the aims of the study and will round up the general introduction.

The evolutionary history of the Przewalski's horse

By 55 million years ago, the first members of the horse family, the dog-sized *Hyracotherium,* were scampering through the forests that covered North America (Woodburne, 1982). For more than half their evolution, most horses remained small, forest browsers. But changing climate conditions allowed grasslands to expand, and about 20 million years ago, many new species rapidly evolved. Some became larger and had the familiar hooves and grazing diets that we associate with horses today. At various times during the approximately 4 million years since their first appearance, members of the genus *Equus* migrated in many different directions. Each of the many species had their own characteristic form and distinct habits. The surviving caballine horses are now reduced to two kinds – the domestic horse and the Przewalski's horse. The domestic horse population of today is a result of the interbreeding of many lines of wild horses from multiple regions (Flauger, 2010).

The very first visual account of the existence of Przewalski's type wild horse dates from about 20,000 years ago. Rock engravings, paintings and decorated tools dating from the late Gravettian culture to the late Magdalenian culture, consisting of 2,188 animal pictures were discovered in caves in western France, Italy and northern Spain; 610 of these were horse figures (Leroi-Gourhan, 1971). Many of them show clear characteristics features of the Przewalski's horse type, such as upright manes, the shape of the lower jaw and compact profile (Bouman and Bouman, 1994). The very first written accounts of Przewaslki's horses originate from Tibet (Zevegmid and Dawaa, 1973). For centuries nothing was heard of the species. Then around 1630, it was announced that an eminent Mongolian had presented a

Przewalski's horse as a gift to the emperor of Manchuria (Zevegmid and Dawaa, 1973). The first reports from a westerner of sightings of Przewalski's horses were from John Bell, a Scottish doctor in the service of Czar Peter the Great. His book in which the horses are mentioned was published in 1763 (Bouman and Bouman, 1994). Then for another one and a half centuries nothing more was heard about the horses. It was Colonel Nikolai Michailovich Przewaslki, who made mention of the wild horse again. He made an official announcement of his discovery of the Przewalki's horse in 1880 after returning from an expedition to central Asia. Less than 100 years later, in 1969 N. Dovchin was the last person to see a Przewalski'shorse, a stallion, in the wild (Bouman and Bouman, 1994). The animal was observed near the Tachyn-sharmountains in the western Gobi Desert on the margins of its former habitat (Bouman and Bouman, 1994; Wit and Bouman, 2006). Before extinction in the wild, the species was brought to Europe in the early 20th century by animal dealers such as Hagenbeck. From that time on the species has only survived in captivity. In 1945, only 31 horses remained in captivity. All existing animals can be traced back to 14 ancestors, mostly captured in the early twentieth century in Mongolia (Wit and Bouman, 2006). A species survival plan was set up in the USA in 1979, followed in 1986 by a European breeding programme (Bouman and Bouman, 1994). By the start of the 1990s, there were more than 1500 horses in captivity (King and Gurnell, 2005).

Consequently, many conservationists hail the Przewalski's horse as the best example of a species which has been successfully saved from extinction by breeding in captivity (Van Dierendonck et al., 1996). Nevertheless, the Przewalski's horse is still on the red list as "critically endangered". With the help of three internationally coordinated breeding programmes the population of the horses has increased to about 2000 animals (Goto et al., 2011). Since 1992, reintroduction projects have been started in China, Kazakhstan and Mongolia (King and Gurnell, 2005). Nowadays, the horses live in Zoos (Kolter and Zimmermann, 1988), in grazing projects or semireserves (Zimmermann, 2005; Steidele, 2011; Feh, 1988) or have been returned to the wild. In Europe, the number of horses living in semi-reserves has increased during recent years. Semi-reserves were created by the European Conservation Project for scientific research in preparation for reintroduction in the wilderness. They are defined as enclosures large enough to carry a group of Przewalski's horses throughout the year without any additional feeding. The semi-reserve offers diverse opportunities for significant scientific research (Budras et al. 2001). When talking about semi-nature reserves, one should keep in mind that not all projects are comparable with each other. While the semi-reserve Hortobágy in Hungary extends over 100.000 ha and is the biggest middle European steppe area, the reserves in Germany and other countries in most cases are confined by ecological restrictions and lack of space.

Social organization in horses

Before their disappearance from the wild, Przewalski’s horses are believed to have had a harem polygynous social organization (Kaszab, 1968; MacClintock, 1982), although no formal behavioural studies were made. Range establishment and area, as well as habitat use are discussed in Przewalski's horses (King, 2002). Re-introduced Przewalski's horse groups were reported to remain in separated home territories, i.e. the area through which groups regularly trek, in search for food, water, minerals and shelter (King and Gurnell, 2005). Harem groups usually consist of one to five stallions, several mares and their offspring (Feist and McCullough, 1975; Berger, 1977; Linklater, 2000). Family stallions have the exclusive mating rights with the mares in their harems (Klingel, 2012). Mares stay in their harem until death, while male offspring disperse from the natal groups at the age of about three or five. Before young stallions form their own harem, they live together with other stallions in bachelor groups (Flauger, 2010). Even in the absence of a stallion, harems are a stable group, indicating voluntary membership (Klingel, 2012). The size of a group's home range was dependent on the season. Scientists expect the territories of Przewalski's horse groups to overlap when the number of groups in a habitat increases (King, 2002; King and Gurnell, 2005)

In captivity the social system of Przewalski’s horses is determined, not by natural selection, but by those people who manage them in zoos and semi-reserves. Most commonly the animals are kept in harem groups consisting of one stallion and several mares and their juvenile offspring (Houpt and Boyd, 1994).

Differences and commonalities between the Przewalski’s horse and the domestic horse

Przewalski’s horses and domestic horses do not only present differences in their physical appearance and their behaviour, but varieties between both species are much more fundamental and can be even found in their genetic basis.

The chromosome number of the domestic horse is 2n = 64 and different races all have the same complement. The chromosomes of Przewalski’s horses are identical: 2n = 66, with more acrocentric and fewer metacentric elements than the chromosomes of the domestic horse

(Benirschke et al., 1965). The chromosomal difference between both horses was established to have occurred through a mechanism commonly observed in chromosomal evolution and specification, i.e., centromere-centromere fusion (Ryder, 1994). Consequently, the findings of Benirschke et al (1965) are significant for understanding of the relationship between Przewalski's horses and domestic horses. The existence of 2n = 66 chromosomes in Przewalski's horses confirms that these animals are more different from their domestic relatives which have only 2n = 64 chromosomes, than are any two breeds of domestic horse (Ryder, 1994). And even, if mixed offspring of Przewalski's horses and domestic horses were viable and reproduction feasible, cross-breeding must be avoided at all costs, because it would undeniably lead to the loss of the unique features of the endangered wild horse.

Phenotypical differences are for example the manes. Like zebras Przewalski's horses have short bristly manes which are dark, accentuated by lighter hairs. Contrary to the domestic horses Przewalski's horses shed their short-haired manes every summer, so they will not grow forelocks and long manes. The tail is about 90 cm long, with a longer dock and shorter hair than seen in domesticated horses. When compared to the domesticated horse, Przewalski's horse is more stockily built, with shorter legs. They typically weigh around 300 kilograms. The hooves of the Przewalski's horse are longer at the back and have significantly thicker sole horn than feral horses. This is beneficial as it improves the performance of the hooves.

Regarding the social behaviour, Christensen et al. (2002) reported that Przewalski's horses don't have evolved qualitative or quantitative differences in social behaviour when compared with central European horses. But in general, in its behaviour the Przewalski horse bears some resemblance to the zebra. Like the striped horse of the savanna, it cannot be handled, mounted or haltered. In most cases, when it needs to be transported or treated it has to be fully tranquilized.

The role of the Przewalski's horse as conservationist in semi-reserves

Przewalski's horse was last recorded in the wild in 1969. After their extinction in the wild many individuals were bred in captivity in zoos, but there are problems associated with long-term captive breeding of a species. Maintenance in artificial conditions accompanied by inbreeding can lead to a loss of genetic variability, development of genetic diseases, loss of seasonal reproduction, declines in fertility, increase in the mortality of young animals and other negative consequences (see Bouman and Bos, 1979; Volf, 1981). In 1980 the Foundation for the Preservation and Protection of the Przewalski Horse (FPPH) proposed a plan for the reintroduction into a wild reserve, based on the principle that the Przewalski’s horses cannot be released directly from zoos into the wild. An intermediate stay in semi-reserves for the Przewalski’s horse was discussed, accepted and later endorsed by the Symposium (Dathe, 1984). The first semi-reserves in western Europe were established by the Dutch couple Jan and Inge Bouman. Their dream was to buy Przewalski’s horses from Zoos, breed them and return their offspring to Mongolia (Bouman and Bouman, 1990). Zimmermann (2005) defined a semi reserve as an enclosed vegetation area on with a certain number of big herbivores, dependent on the biomass, can be pastured without additional feeding. Actually, this definition only partly reflects the important relevance of the semi reserves, as they not only serve as keeping and breeding stations, but also offer the possibility to observe in near-natural conditions.

Furthermore, a lot of conservation areas use big herbivores such as horses and cows to maintain open areas and to prevent those areas from scrub encroachment (Kolter et al., 1999). Such an extensive grazing allows the preservation of the biodiversity of the flora and fauna. Often small-scale biotopes arise, where new plant species and animal species can settle.

Additionally, the semi-reserves offer the opportunity to create concepts for environmental education. Nowadays, many Przewalski’s horse groups can be visited by school groups and other tourists which are interested in the horses as well as the flora and fauna of the respective semi-reserve. This offers a good opportunity to educate people about threatened animal and plant species and to inform them about the value of those species for the biodiversity.

Stress level as an indicator for the well-being of the horses

Although there are different studies about the behaviour of Przewalski’s horses in grazing projects (e.g. Redman and Goodwin, 1999; Christensen et al., 2002; Steidele, 2011), there are few studies (Budras et al., 2001) which investigate the well-being of horses and their reactions to different management regimes. Stress is a general term used to describe environmental factors that solicit adaptation mechanisms and a response to these challenges (Mormède et al., 2007). An organism can show two different responses to stress. Firstly, the HHN-System (hypophysis-hypothalamus adrenal cortex system) can be mobilized, which leads to an increased biosynthesis and the release of cortisol from the adrenal cortex. Secondly, the SAM-system (sympatho-adrenal medullar system) may release adrenalin and noradrenalin (Henry and Stephens, 1977). In the present study, I focused on the expression of the cortisol metabolites. In general, it should always be considered, that animals can experience both, a positive activation of the stress response systems (i.e. eustress) or environmental stimuli of an adverse nature (i.e. distress) (Buwalda et al., 2012). But it is known, that certain uncontrollable and/or unpredictable situations are likely to cause distress, which is expressed in physiological responses, such as decrease of immune functions, and changes in glucocorticoid as well as catecholamine secretion (Koolhaas et al., 2011). For example, it has been demonstrated, that limited space increases aggression rates in horses (Przewalski's horses: Boyd, 1991; domestic horses: Flauger and Krueger, 2013), but studies are lacking on whether the restriction of space would increase the stress hormone levels. Consequently, I investigated the stress level of the horses in the context of various management influences and other possible influencing factors e.g. the enclosure size, the hierarchy and the food supply.

Questions on the behavioural ecology and sociobiology of Przewalski's horses in semi-reserves

To date, many questions on the feeding behaviour and social behaviour of Przewalski's horses living in semi-reserves with limited available space and ecological restrictions remain to be answered. For example: Which habitats do the Przewalski's horses prefer and are their 4similarities in the preference between different horse groups? Is the activity budget in the semi-reserves comparable and what influencing factors can be found? Which of the factors: habitat, food supply, group composition, hierarchy and body condition, have the most influence on the stress hormone level of the animals? What is the group structure of the animals in semi-reserves? Is their social behaviour comparable to domesticated horses and what possibilities exist to analyse social bonds between group members?

These are some questions that will be asked within the outline of this thesis. Behavioural ecology provides a framework for answering these kinds of questions because it combines theories from evolution, ecology and behaviour (Krebs and Davis, 1997). The thesis combines the analysis of habitat use, activity, social behaviour and stress tolerance of groups of horses as well as the data of individual horses living in natural conditions in semi-reserves.

Earlier studies demonstrated that the horses have a territory size that seasonally adjusted (Roth, 2002). Berger et al. (1999) investigated their activity budget and found out, that their general activity is highest during summer and lowest during winter, while the feeding behaviour reaches its highest amount in autumn and is lowest during summer. However, actually there are only few studies which investigate the criteria influencing their habitat and feeding choice. For example, it is not known, if nutritional content alone is a deciding factor or how far other elements such as social or individual factors can play a role for the habitat use. It was to be expected that different observations have been made in different semi-reserves regarding their food preference and habitat choice (Scheibe et al., 1998).

Aims of thesis

First of all, my PhD thesis investigates the behaviour of Przewalski's horses living in natural conditions in semi-reserves and it compares the social behaviour as well as the behavioural ecology of different horse groups.

The first part of the study (**chapter 1**) is about the social behaviour and social bonds in horses. The understanding of the social structure and social behaviour is an important consideration when investigating the behaviour of the horses and judging their well-being. In common literature authors observe the number of mutual grooming events between individuals, to draw conclusions about the strength of the social bond between these individuals. To find out more about this behaviour, I started with a literature review on the mutual grooming of horses living in the wild.

Collecting the different literature and various analyses about grooming behaviour of horses, it quickly became clear that the large number of studies on social bonds differ greatly in their research design and interpretation of measurements. Therefore, I decided to investigate the comparability of mutual grooming and other parameters as measures of social bonds in feral horses (**chapter 2**). Furthermore, in this part of the study the social behaviour of different Przewalski's horse groups, in different semi-reserves, is compared to the social behaviour of domestic horses living in the wild.

In **chapter 3** the behaviour of a group of Przewalski'shorses is investigated while their environmental condition changes. In particular the function of the alpha-male was observed to find out more about his social role in a horse group. The background to this investigation is the fact that males are often removed from horse groups to breed with another group without consideration for the group composition or the individual social status of the animals. As alpha animals are needed to ensure group stability and decision making, I investigate the role of the alpha male. To this aim I compared the group's social structure and order of movement, as well as the animals' connectedness, activity budgets, and how factors such as social rank influenced the horses' behaviour. Through examination of the horses' faeces I also investigated the excretion of glucocorticoid metabolites (GCM), while at the same time exploring a new area as a parameter of glucocorticoid production. This data was collected both in an established area and later, during exploring of a new environment.

In **chapter 4** I focused on the general behaviour of Przewalski's horses in various semi-reserves and also on their behavioural and physiological responses to human management. I compared the horses' behavioural responses to different management influences, their habitat use, activity budget and their stress hormone excretions. The two observed horse groups lived in two semi-reserves with different habitats and different management regimes. One group consisted of male horses and lived in a pine forest with additional heathland. The other group consisted of female horses and a male horse which was integrated during the study. This group lived in a semi-reserve with an open character with sandy grassland, surrounded by various kinds of trees.

In the final chapter (**chapter 5**) I present a case study to space, behaviour and well-being of horses in open stables and open active stables. With the help of this study I wanted to find out more about the reactions of horses to different enclosure sizes and to be able to compare the reactions of domestic horses and Przewalski's horses to different husbandry systems. During this study the behaviour of 112 horses was observed in both open stables and open active stables. I address the questions of whether the aggression level and the affiliative behaviour of the horses is influenced by their husbandry system. Additionally, the number of diseases and injuries were recorded and a comparison of the injuries in connection with the different surroundings of the horses was carried out.

CHAPTER 1

Factors influencing grooming behaviour in wild living horses

Riccarda Wolter[ab]* & Konstanze Krueger[ab]

[a] University of Regensburg, Biologie 1, Universitätsstraße 31, 93053 Regensburg, Germany

[b] University of Applied Sciences Nuertingen, Faculty Agriculture, Economics and Management, Department Equine Management, Neckarsteige 6-10, 72622 Nuertingen, Germany

* Corresponding author

Published in Current research in applied ethology (2015) 510, 242-248

SUMMARY

Social grooming in Equids has been extensively investigated in recent years and several factors are known to influence grooming behaviour. Apart from seasonal conditions, there are also established social factors such as age, sex, hierarchy, group composition, and kinship. These factors can affect grooming frequencies and influence the choice of grooming partners. Moreover, it has been demonstrated that the group's aggression level can influence social behaviour and grooming intensity. So far it has not been confirmed whether individuals that show an increased grooming frequency also stand more often in close proximity to their grooming partners, as no clear correlation has been determined. This aspect needs urgent investigation, as both data sets are often used in combination when calculating social bonds.

Mutual grooming in horses

Mutual grooming is a behaviour which has been investigated predominantly in primate societies, and many studies have shown that coat care and the removal of ectoparasites and dead skin are not the only function. There have also been several studies on social grooming in equids showing that the forming and tightening of social bonds appear play a key role (Waring, 2003; Van Dierendonck, 2006). Moreover, the behaviour is applied in appeasement and conciliatory gestures (van Lawick-Goodall, 1968; Smuts et al., 1987; Aurelie et al., 1989; de Waal, 1989).

Besides social behaviour, there are some other factors which are influenced by social grooming, for example, grooming can lower the heart-rate, the blood pressure, and the cortisol level (Feh and de Mazières, 1993; McBride et al., 2004). Additionally, stress and boredom can be prevented by mutual grooming (Schino et al., 1988), and it is used as a kind of courtship behaviour (Hill, 1987; Seyfarthetal, 2001).

In most cases, social grooming is initiated by one individual approaching another and sniffing the body region which is later going to be groomed. This behaviour is typically shown at the neck, the withers and the spine (Boyd and Houpt, 1994).

While grooming in primates occurs very frequently and can be seen as a major part of the social life on which they spend about 20 % of their diurnal activity (Fedurek and Dunbar, 2009), in horses this behaviour is shown more rarely. A study by Boyd and Houpt (1994) demonstrated that Przewaslki’s horses (*Equus ferus przewalskii*) groom on average only once every two hours, but with great individual and seasonal variation. For example, some group members groomed once an hour, while others only groomed once in ten hours, and grooming frequency reaches its maximum in spring, when the animals start to lose their winter coat (Boyd and Houpt, 1994). In contrast, Kimura (1998), in a study on wild living horses (*Equus caballus*), found that the animals show their highest frequency of grooming during the summer months. If grooming frequency is considered to be 100 % during summer, the values declined to 14.4 % in winter, 25% in spring and 26.0% in autumn.

In addition to molting, weather and insect pressure can influence grooming frequency (Wells and von Goldschmidt-Rothschild, 1979), and a whole slew of social factors can affect grooming behaviour, e.g. new group members and the necessity to establish social bonds (Sigurjónsdóttir, 2003). Some of these social influences are specified below.

Age and Gender

A study on foals by Crowell-Davis et al. (1986) demonstrated that individual differences in grooming behaviour already exist at the age of only a few weeks, and these differences are connected to the age and gender of the foals. While fillies groomed every 1.2 hours, in colts the behaviour only occurred every 2.2 hours. Furthermore, it was observed that grooming frequency increases at the age of four weeks. The reason for this seems to be that foals stay close to their mothers, and in the first weeks grooming only takes place between dam and foal. Mutual grooming between foals starts around the fifth week when they start to establish contact with each other (Crowell-Davis et al., 1986). Overall, the wide individual differences observed in this study continued in adulthood (Abel, 2015). The choice of the grooming partner can also be influenced by the gender. Feh and de Mazières (1993) demonstrated that social grooming between mare-stallion pairs ($N = 72$, number of possible pairs = 16) occurs more often in spring compared with mare-mare-pairs ($N = 20$, number of possible pairs = 8; $\chi^2 = 5{,}57$, $df = 1$, $p < 0.02$). This difference was not observed in winter, and stallion-stallion-pairs didn't show mutual grooming at all. Sigurjónsdóttir (2003) demonstrated that in Icelandic horses the social structure is predominantly influenced by age and gender, with horses regrouping themselves according to these two factors. In the group of adult mares, as well as in the geldings and sub-adult animals, social grooming was observed regularly, and the horses preferred to groom partners of same age and gender ($N = 34$, $\tau_{rw} = 0.41$, $p < 0.001$).

Kinship

In Przewalski's horses, the degree of kinship between two animals has been shown to affect their frequency of mutual grooming. Boyd (1988) found that animals with close kinship to each other groom more often than animals with a more distant relationship. This was confirmed in a study on Icelandic horses, in which a positive correlation between the grooming frequency of a pair of horses and their relationship was also found (Sigurjónsdóttir, 2003).

Hierarchy

Apart from age, gender and relationship between the animals, hierarchy has also been investigated as a possible influence on grooming behaviour and choice of grooming partners. While Kimura (1998) did not find any significant correlation between animals' individual grooming frequencies and their social rank, nevertheless, it was shown that animals with a lower rank had a greater variety of grooming partners during summer. The choice of the grooming partner was negatively correlelated with the rank of the horse, both in summer and winter (r = -0.55, p < 0.05; r = -0.61, p < 0.05). However, Wells and von Glodschmidt-Rothschild (1979) found that Camargue horses preferred to spend time with animals of a similar social rank. This result was reaffirmed by Ellard and Crowell-Davis (1989). Unlike Kimura (1998), Sigurjónsdóttir (2003) found a positive correlation between rank and grooming frequency, with mares preferring to groom with other animals of a similar place in the hierarchy (N = 17, *Spearman's rho* = 0.154, p = 0.546), too.

Group composition

Most studies on social bonds in horses suggest that horses tend to have between 0 and 3 preferred partners (Tyler, 1972; Clutton-Brock et al., 1976; Monard et al., 1996; Kimura, 1998). Regarding group composition, a higher frequency of social grooming was found in groups without stallions. Moreover, in various studies on wild living horses and Przewalski's horses, it was observed that mares show more frequent mutual grooming with other mares of similar age when living in a group without a stallion (Tyler, 1972; Clutton-Brock et al., 1976; Sigurjónsdóttir, 2003), but preferred to groom with their 0-3 years old offspring when living in a harem group (Feist and McCullogh, 1976; Wells and Goldschmidt-Rothschild, 1979).

Aggressiveness

Feist and McCollough (1976), as well as Kolter and Zimmermann (1988), hypothesized that in some cases mutual grooming is used as a kind of appeasement gesture, and is applied to minimize aggression between the participants (Hogan et al., 1988; Keiper, 1988). Abel (2015) investigated whether stallions that show more affiliative behaviour have more mares with foals at foot, and therefore a higher rate of reproduction. Four harems of wild-living horses in

Italy were observed and the social index was calculated; this represented how many affiliative behaviours and agonistic behaviours were shown. The stallions in three groups showed more affiliative behaviours, and had a positive social index. In the fourth group, the stallion showed more agonistic than friendly behaviours and, interestingly, in this group the grooming index was much lower than in the other groups. This led to the conclusion that the behaviour of the stallions influenced the grooming behaviour of the mares.

Relation to the spatial structure of horse groups

The literature contains several studies on whether the grooming behaviour of animals has an influence on their spatial distribution, and different results have been found: while Cameron et al. (2009) found a correlation between spatial proximity and mutual grooming, Kimura (1998) came to the conclusion that the preferred neighbour of a horse is not necessarily their preferred grooming partner. This was confirmed in a study by Wells and von Goldschmidt-Rothschild (1979) on Camargue horses. They found that animals that often stood next to each other only rarely showed friendly interactions between them. The question of whether there is a relationship between the data on social grooming and on spatial proximity is especially important because both are used together to evaluate social bonds between individual animals. This method is commonly used, particularly in horses (Kimura, 1998; Heitor et al., 2006), as mutual grooming is rare compared to primates and the data are often not sufficient to yield significant results.

However, the combination of both behaviour patterns in the analysis of social bonds is questionable as, while mutual grooming requires deliberate action by both grooming partners, the spatial distribution can be influenced by one partner alone, who may even force the other horse to keep a certain distance, or to stay in close proximity. Therefore, a potential correlation between both parameters should be investigated to analyse the optimum way of calculating social bonds and to standardize the different existing methods in the future.

CHAPTER 2

Parameters for the analysis of social bonds: a case study in horses

Riccarda Wolter[ab]*, Volker Stefanski[c], Konstanze Krueger[ab]

[a] University of Regensburg, Biologie 1, Universitätsstraße 31, 93053 Regensburg, Germany

[b] University of Applied Sciences Nuertingen, Faculty Agriculture, Economics and Management, Department Equine Management, Neckarsteige 6-10, 72622 Nuertingen, Germany

[c] Institute of Animal Husbandry and Animal Breeding, University of Hohenheim, Garbenstr. 17, 70593 Stuttgart, Germany

* Corresponding author

Results published in a reviewed version of the manuscript in Animals (2018) 8(11), 191

ABSTRACT

In animal behaviour literature, there is a large number of studies on social bonds in primates, but social bonds are rather under-studied in many other taxa. The studies show substantial variation in the research design and the interpretation of measurements. Mutual grooming is used most often to evaluate social bonds, but as it occurs comparatively infrequent in many species, measurements of spatial proximity are commonly used additionally. In a social species which grooms rather seldom, the horse, we investigated social behaviour and spatial proximity. We observed 146 feral horses, five groups of Przewalski's horses and six groups of feral horses. We asked whether the individual animals' frequencies of grooming, friendly approach and close proximity with group members were affected by environmental and individual factors. Moreover, we asked if counts of affiliative approach were a suitable replacement for spatial proximity and would allow measuring social bonds from directed behaviour counts rather than from the undirected spatial distribution of animals. Grooming appeared to be a robust behavioural parameter, as its frequency was affected only by the animals' sex, while the friendly approaches and the spatial proximity were affected by various but different factors. Our results show a weak correspondence between the animals' absolute frequencies and their pairwise evolvement in mutual grooming and spatial proximity, and a stronger correspondence between the frequency and pairwise exchange of mutual grooming and friendly approaches. We therefore consider a combination of proactive behaviour counts to be a reliable approach to the analysis of social bonds in horses.

Keywords: feral horses; social bonds; mutual grooming; spatial proximity

INTRODUCTION

In many mammals, individuals prefer to affiliate with a small subset of available group members (Altmann, 1951). The formation of the resulting social bonds has been investigated mostly in primate societies (Silk et al., 2003; Langergraber et al., 2009; Mitani, 2009) but also in dolphins (Frère et al., 2010), rodents (Beery and Kaufer, 2015), ungulates (Cameron et al., 2009), and birds (Emery et al., 2007). The formation of social bonds is considered to promote fitness, as it can increase the bonded animals' reproductive success (feral horses: Cameron et al., 2009; Macaques: Schülke et al., 2010) and their offspring survival, e.g. in Baboons (Silk et al., 2003) and feral horses (Cameron et al., 2009). Furthermore, mutual grooming has been claimed to serve as appeasement behaviour (Feist and McCullough, 1976; Kolter and Zimmermann, 1988; Roubová et al., 2015), which may reduce aggression between group members (Hogan et al., 1988; Keiper, 1988; Frank, 2009).

However, the best method for measuring social bonds is still not clearly defined across mammalian species and is performed in many different ways. The measurement of the type, frequency and duration of friendly (affiliative) social behaviour can be useful. In many species it is common practice to use the frequency of mutual grooming between particular individuals (Silk et al., 2006). But, especially when mutual grooming behaviour is rare and does not allow for a robust analysis of social bonds, some studies combine the grooming data with other measures, such as spatial proximity (Kimura, 1998).

In this regard, the feral horse appears to be a good model species for discussing the evaluation of social bonds, as horses form stable social bonds (Berger, 1977; Keiper, 1986; Linklater et al., 1999; Heitor and Vicente, 2010) and their mutual grooming frequencies are low and show individual and seasonal variation. As in primates (Silk et al., 2003), mutual grooming is used to assess social relationships. In mutual grooming, one horse approaches another and begins sniffing or nuzzling the approached horse along the dorsal surface of the body from the neck, over the withers and the back, to the rump (Boyd and Houpt, 1994). Some horses groom mutually at least once an hour, while others in the group may only participate once every ten hours and some do not groom at all (Boyd and Houpt, 1994). For all horses, the frequency of grooming peaks in the spring when winter coats are shed (Boyd and Houpt, 1994) and participants receive coat care in areas of the body which are difficult to reach by self-grooming (Mazak, 1961; Feist and McCullough, 1976).

Datasets for the analysis of social bonds in horses are commonly enlarged by measurements of spatial proximity, for example when horses are grazing and resting (Kimura, 1998; Sigurjónsdóttir et al., 2003; Heitor et al., 2006). Authors differ in their methods for measuring spatial proximity, with concern of the distance between two individuals and the timing of observation intervals (Crowell-Davis et al., 1986; Van Dierendonck et al., 1995; Christensen et al., 2002; Cameron et al., 2009). To sum up, the distance of two body lengths appears to be used most often (Van Dierendonck et al., 1995; Christensen et al., 2002; Cameron et al., 2009) and the sampling time interval of ten minutes appears to guarantee the independence of samples for calculating spatial proximity in horses, as horses show a mean latency of changing the spatial distribution of group members every 8 minutes (Christensen et al., 2002).

Besides of spatial proximity, mutual affiliative approaches were considered to be a good additional parameter (Cameron et al., 2009; Schneider and Krueger, 2012) for measuring social bonds. Mutual approaches were categorized as affiliative or “friendly” and were considered to reveal the desire for friendly interaction and proximity to other animals (Silk et al., 2003; Cameron et al., 2009) when a receiver responds by reciprocating an approach or behaves neutral, i.e. without challenging or fleeing the approaching animal (Schneider and Krueger, 2012).

The aim of this study was to explore the most reliable parameter for the analysis of social bonds in a species which grooms seldom, such as the horse. We compared the frequency of mutual grooming, friendly approaches and of close proximity with group members for each individual and in pairs of horses, which we obtained by observing five groups of Przewalski’s horses and six groups of feral horses living under semi-wild conditions.

Even though Przewalski’s horses and domestic horses (*Equus caballus*) were considered to show similar social behaviour (Christensen et al., 2002), we tested whether the frequencies of social behaviour and spatial proximities are equal in the Przewalski’s and the feral horses, because of evolutionary, genetic and environmental differences in the test horses of the present study. Przewalski’s horses were separated from other horse populations 117,000 years ago and differ in their chromosome numbers from other horses (Goto et al., 2011). Furthermore, most of the observed Przewalski’s horses were born in zoos whereas the feral horses were born in the wild.

Social behaviour frequencies can also be affected by the aggression level within a group (Hogan et al., 1988; Crofoot et al., 2011), and by the social rank of individual animals (patas monkeys: Nakagawa, 1992; horses: Kimura, 1998; Heitor and Vicente, 2010). Moreover, the gender, the horse breed, the group size, the group composition, the management regime and the individual itself were shown to affect social behaviour in horses in previous studies (Tyler, 1972; Clutton-Brock et al., 1976; Ellard and Crowell-Davis, 1989; Wells and Goldschmidt-Rothschild, 1979; Feh and de Mazières, 1993; Sigurjónsdóttir et al., 2003; Wolter and Krueger, 2015).

Therefore, we addressed the following questions: (1) Are the individual animals' frequencies of grooming, friendly approach and close proximity with group members affected by the horses' breeds (Przewalski's horse or feral horse), their aggressiveness, their social rank, the individuals themselves, the sexes, the group sizes, the group type (harem, mare group, bachelor group) and the management regimes? (2) Do the frequencies of grooming, friendly approach and close proximity with group members correspond to each other in individual animals? (3) Are there, and if so, how strong are, correlations between the frequency of mutual grooming, the frequency of staying in close proximity, and the frequency of friendly approaches in pairs of horses?

MATERIALS AND METHODS

Animals

We observed 146 feral horses, five groups of Przewalski's horses (*Equus ferus przewalskii,* N = 37) and six groups of feral horses (*Equus ferus caballus,* N = 109). The first group ("Group P-1") of the Przewalski's horses was a bachelor group consisting of five male animals, living in the Stadtwald Augsburg, Germany, which is part of a large municipal forest. The horses' ages ranged between 1.5 and 6 years. The second group ("Group P-2") was a mare group consisting of seven females, living in the semi-reserve Campo Pond in Hanau, Germany. The horses' ages ranged between 4 and 22 years. During the observation period, this mare group was changed into a harem group, when three of the seven mares were removed and a stallion was introduced. The group was observed two years later, as a harem group ("Group P-3"). During this observation, one male yearling lived in the group as well as the harem stallion. The fourth group ("Group P-4") was a mare group, consisting of nine mares, which lived in the semi-reserve Hohe Warte in Gießen, Germany. In this group, three of the mares originated from the group in Hanau (Group P-2), and now lived together with six further animals. The horses' age ranged between 1 and 14 years. The last group ("Group P-5") was a harem group located in the semi-reserve Ludwigsthal at the Bavarian forest, Germany and was composed of six mares, one stallion and two yearlings. The age of the horses ranged between 1 and 27. All horses were individually identified by their brands and colouration (Table A1-A5), well known to the park rangers, and registered at the European Conservation Breeding Program (Europäisches Erhaltungszuchtprogramm, EEP). Their social history was comparable: they were all born in small harem groups in zoo housing. Foals were excluded from the evaluations, because of the special status of foals in social horse groups (Araba and Corwell-Davis, 1994).

The horses at the semi-reserves fed on the natural vegetation in the areas, but some groups received additional hay in the winter. The bachelor group lived in a 15ha pine forest in winter, with an additional 15 ha of heath land open to them from October until March. The area of the group in Campo Pond consisted of 50 ha of poor sand grassland and with adjacent woods. The group in Hohe Warte lived on a 23ha mesophilic pasture, the group in Ludwigsthal lived on 15 ha, which comprised 12 ha grassland and 3 ha mixed forest.

In addition to the Przewalski’s horse groups, we observed the behaviour of 109 feral horses, living in six different social groups (Table A6). These were among about 300 feral horses, named ‘Cavalli di Esperia’. The observed population roams freely in the Abruzzi Mountains near Frosinone in Italy. The horses’ age ranged between 1 and 28 years according to previous studies (Schneider and Krueger 2012; Krueger et al. 2014), but precise ages were only known for half of the animals. All groups were composed of several females, their offspring and one stallion and changed in group composition for about 15% each year. All animals fed on the natural vegetation in the area. Additionally, they received hay in harsh winters, and water in the summer.

Freeze brands (i.e. white numbers, dorsal at the animals’ torso), hot brands, colorations and body proportions were used to identify the horses individually. The feral horses were observed yearly for previous studies and the majority of the animals were individually known to the research group since 2008.

Data sampling

For two groups (Group P-1 and Group P-2) data were collected over one year, at the rate of 15 hours each month. All other groups were observed for 15 hours in total (during one month). The data of the first two groups were divided by twelve to facilitate the comparison between groups. Data were collected from September 2012 until August 2013 for group P-1 and P-2 and in April and May 2015 for the groups P-3, P-4 and P-5. The feral horses in Italy, groups F-6, F-7, F-8 and F-9, were observed in May 2010 and the groups F-10 and F11 in June 2014. The observations were distributed evenly over 8 daylight hours and were spread over different consecutive days. It was not possible to record data blindly, because our study involved focal animals in the field.

The observers stayed about 20 – 200 m away from the animals, depending on the horses' spatial distribution. Binoculars were used to support identifications, if needed. As the horses were accustomed to people, their behaviour was not influenced by the observers' presence.

The comparatively small Przewalski's horse groups were observed by one person, who recorded the behaviour on paper. Group P-5 was observed by two persons, with one person observing the group at a time.

The large feral horse groups were observed by eight persons in total. Four persons observed a particular group at a time and split up into pairs. The two observers cooperated in collecting information and animal identities, and while A was writing, B continued observing. After the observation, an inter-observer reliability test was confirmed between the two observer pairs for each feral horse group. The median Spearman correlation coefficient for the inter observer reliability in the six groups was $r_s = 0.89$ (Min = 0.76, Max = 0.98).

Affiliative and agonistic behaviour (for further definition see below) was collected by continuous recording ad libitum (Martin and Bateson, 1986). Additionally, the spatial proximity of the animals (see below) was documented by scan sampling (Martin and Bateson, 1986) every ten minutes. We collected the frequency of behaviour and staying in close proximity to group members each individual animal showed within its group. Furthermore, we evaluated how often affiliative behaviour was displayed and animals stayed in close proximity within each pair of animals per group.

Affiliative Behaviour

a) Mutual grooming

Mutual grooming was defined as two horses standing beside each other, usually head-to-shoulder or head-to-tail, and grooming each other's neck, mane, rump, or tail by gentle nipping, nuzzling, or rubbing (McDonnell and Haviland, 1995).

b) Affiliative approaches

Affiliative approaches were counted when animals approached and stayed within one body length of an approached horse, when an approach elicited an affiliative reaction (an approach of the approached animal) or a neutral reaction of the approached horse (the approached animal stayed in close proximity). Approaches which resulted in grooming were not considered, so that the friendly approach and grooming data were mutually exclusive.

Agonistic behaviour

We counted agonistic behaviour of the horses, such as threats to bite or to kick, bites, kicks, chases, retreats and approaches which elicited a retreat of the approached horse (McDonnell and Haviland, 1995; McDonnell, 2003).

Social rank

The analysis of the horses' social rank was done as described in Krueger et al. (2014). We calculated the social rank of each animal with the help of the average dominance index (ADI) from their agonistic encounters as follows. The dominance index per pair of individuals, w_{ij} is the frequency an individual won against a certain opponent divided by the frequency of agonistic encounters between the pair, thus $w_{ij} = x_{ij} / (x_{ij} + x_{ji})$. Wins were counted for the initiator of an encounter when an approached or challenged animal retreated for one step or more. We excluded a pair from the analysis, if the two individuals were not involved in an encounter. The average dominance index of an individual is the average of all its dominance indices with all its interaction partners, thus $1/N\ \Sigma\ j\ w_{ij}$. The ADI can range between 0 and 1. Therefore, a higher value indicates a higher rank in the hierarchy (Hemelrijk et al., 2005).

Not only the type of agonistic behaviour, but also the reaction of the receiver is decisive in counting wins and losses. For example, an animal may respond by retreating, both, when it is being kicked and approached. In both cases the receiver loses and the initiator wins. This method enables all agonistic behaviour types to be used, irrespective of their frequency, and provides the largest possible sample size for the rank evaluation (Hemelrijk et al., 2005). We chose the ADI for its reliability and computational simplicity. Simulations showed that the ADI can deal with missing data between pairs of animals and still provides more reliable results then comparable dominance assessment methods (Hemelrijk et al., 2005).

Spatial proximity

A graph of the position of all group members was drawn every ten minutes during observation periods to determine the proximity of the observed individuals (Krueger et al., 2014). This interval was chosen according to the study of Wells and Feh (1976; cited in Feh, 1988), which shows that in grazing horses the probability of having the same individual in close proximity drops significantly after 8 min. Only two distance categories of spatial proximity were considered for evaluating close proximity: (I) body contact and (II) within two horse-lengths.

Statistical analysis

The statistical analysis and the figures were done with the R-Project statistical environment (R Development Core Team 2015). The tables were drawn with Excel 2007. Some of the data were not normally distributed (Shapiro-Wilk Test). Therefor we applied non-parametric tests. We applied GLMM General Linear Mixed Models for comparisons between the main variables and to investigate potentially influencing factors on the frequency of grooming behaviour, the friendly approaches and the spatial proximity (i.e. fixed factors: gender, group, and Przewalski’s versus feral horse, random factors: aggression levels, group sizes, and rank; Farine and Whitehead, 2015). All GLMs were randomized with 1000 permutations and data are only listened if the p-value differs significantly from the random value. Pairwise behaviour and proximity data were correlated with the Kendall's rank correlation tau test. To compare the correlation coefficients results, we used Wilcoxon signed rank tests. Multiple testing was corrected with a Sequential Bonferroni Procedure (Holm, 1979). All tests were two-tailed and the significance level was set at 0.05.

RESULTS

Effects on the frequencies of grooming, approach and close proximity of individual animals

We investigated factors which may have influenced the individual animals’ frequencies of being involved in the behaviour grooming, friendly approach and of being in close proximity with group members, such as the horse breed (Przewalski’s horse or feral horse), the aggression ratio, the social rank, the individuals themselves, the sex, the group size, the group type (harem, mare group, bachelor group) and the groups with their particular management regimes. An overview on the composition of the various horse groups is provided by table 1.

Table 1

Overview on the composition of the horse groups

Name of the Group	Group size	Horse breed	Gender distribution [m, f]	Group type	Average age [years]	Mean aggression ratio
P-1	5	Przewalski’s	5,0	Bachelor group	2.6	2
P-2	7	Przewalski’s	0,7	Mare group	8.7	5
P-3	6	Przewalski’s	2,4	Harem	8.5	2
P-4	9	Przewalski’s	0,9	Mare Group	6.2	11
P-5	9	Przewalski’s	2,7	Harem	10.4	40
F-6	23	Feral horses	3,20	Harem	N.A.	24
F-7	10	Feral horses	3,7	Harem	N.A.	25
F-8	12	Feral horses	3,9	Harem	N.A.	25
F-9	19	Feral horses	3,16	Harem	N.A.	21
F-10	26	Feral horses	7,19	Harem	N.A.	18
F-11	19	Feral horses	4,15	Harem	N.A.	20

The frequency of the individual animals’ grooming (Figure 1a) with group members was influenced only by their sex (GLM: $N = 146$, $t = -3.06$, $p = 0.003$), with stallions showing a significantly higher number of mutual grooming than mares. All the other factors did not affect grooming frequencies (GLM: $N = 146$, all $p > 0.05$).

Comparable to grooming, the individual stallions were more often involved in friendly approaches (Figure 1b) to group members than the individual mares (GLM: $N = 146$, $t = 5.21$, $p < 0.001$), and the smaller the group size was, the more approaches were shown per animal within the group (GLM: $N = 146$, $t = -3.54$, $p < 0.001$). Furthermore, the individual animals displayed more friendly approaches towards group members when they showed more aggressive behaviour (GLM: $N = 146$, $t = 4.03$, $p < 0.001$). Other factors did not have any effect on the frequency of friendly approaches (GLM: $N = 146$, all $p > 0.05$)

In contrast to the frequency of friendly approaches, the frequency of the individual animals staying in close proximity to other group members (Figure 1c) was lower if they showed more

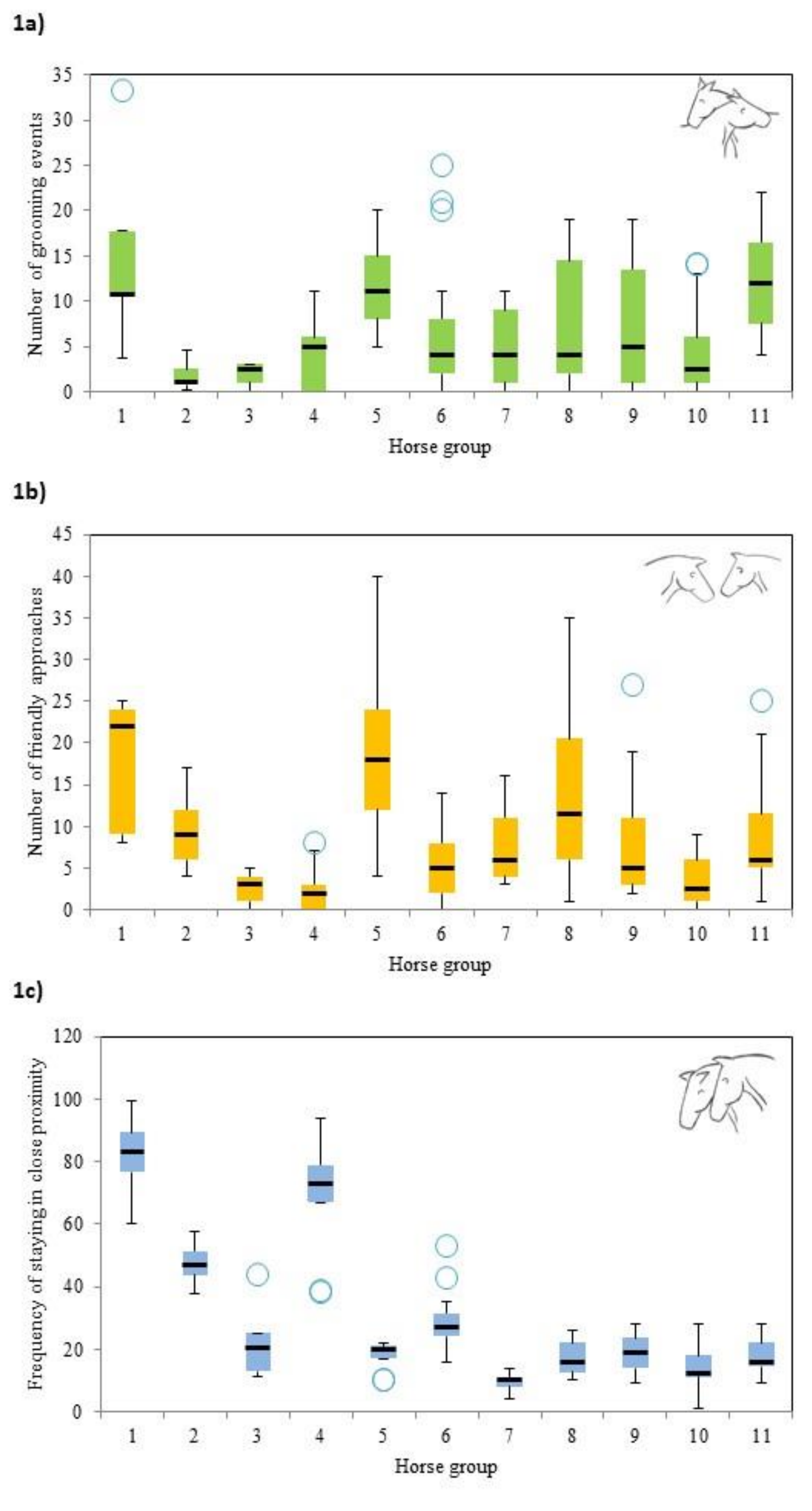


Figure 1. Frequencies of grooming events (a), frequencies of friendly approaches (b) and frequencies of standing in close proximity (c) per horse group. The boxes show 50% of the data, the upper and lower whisker 25% of the data each and the circles visualise outliers. The horizontal lines in the boxes demonstrate the median

aggressive behaviour (GLM: $N = 146$, $t = 2.02$, $p = 0.05$). However, similar to friendly approaches, the horses showed a higher frequency of spatial proximity with group members the smaller the group size was (GLM: $N = 146$, $t = 2.08$, $p = 0.04$). In harem groups, the frequency of close proximity was higher compared to the mare groups and the bachelor group (GLM: $N = 146$, $t = -11.92$, $p < 0.001$). Other factors did not show any effect on the animals' frequency of staying in close proximity with group members (GLM: $N = 146$, all $p > 0.05$).

Behaviour frequencies of group changing animals

We analysed whether animals which switched groups within the observation period altered their social behaviour. Mares which changed from staying in a mare group (Group P-2) to a harem group (Group P-3), did not alter their individual grooming frequency as well as their individual number of approaches and their frequency of being in close proximity to another horse (Grooming: GLM: $N = 4$, $t = -0.08$, $p = 0.94$; Friendly approaches: GLM: $N = 4$, $t = -1.11$, $p = 0.38$; Spatial proximity: GLM: $N = 4$, $t = 0.32$, $p = 0.78$). Moreover, three mares joined a new group in a different semi-reserve (P-2 and P-4). They also did not alter their individual grooming and approach frequencies as well as the frequency of being in close proximity (Grooming: GLM: $N = 3$, $t = 0.58$, $p = 0.67$; Friendly approaches: GLM: $N = 3$, $t = 1.39$, $p = 0.4$; Spatial proximity: GLM: $N = 3$, $t = -0.1$, $p = 0.94$).

Relationship between frequencies of grooming, approach and close proximity for individual animals

As none of the factors affected all three of the observed frequencies, the animals' grooming, approach and being in close proximity, we continued with comparing the frequencies without considering the factors. The individual animals' grooming frequencies were in line with their frequencies of approaching other group members (GLM: $N = 146$, $t = 3.39$, $p < 0.001$), but did not correspond to the animals being in close proximity to group members (GLM: $N = 146$, $t = -0.03$, $p = 0.98$). When comparing grooming, approach and close proximity frequencies of individual animals together, they did not correspond to each other (GLM: $N = 146$, $t = 0.54$, $p = 0.59$).

Correlations between grooming, approach and close proximity in pairs of animals

As social bonds are commonly assessed by analysing data for pairs of animals within their particular group, we continued to compare pairs of animals group wise for the correlation of their numbers of grooming and approach and the frequency the particular pairs stayed in close proximity. Again, factors will not be considered, as none of them affect all three of the parameters.

Correlations between mutual grooming and friendly approaches

We investigated whether pairs of horses which showed a high grooming frequency, also showed a high number of friendly approaches, and found one group with a strong, two groups with weak and two groups with no significant correlation within the five Przewalski's horse groups (Kendall's rank correlation tau test: "Group P-1": $N = 5$, $\tau = 0.119$, $p = 0.65$; "Group P-2": $N = 8$, $\tau = 0.202$, $p = 0.27$; "Group P-3": $N = 6$, $\tau = 0.511$, $p = 0.03$; "Group P-4": $N = 9$, $\tau = 0.555$, $p < 0.001$; "Group P-5": $N = 9$, $\tau = 0.573$, $p = 0.04$). In the Italian horse groups, the correlations between the frequencies of friendly approaches and mutual grooming were strong (Kendall's rank correlation tau test: "Group F-6": $N = 23$, $\tau = 0.266$, $p = 0.008$; "Group F-7": $N = 10$, $\tau = 0.484$, $p < 0.001$; "Group F-8": $N = 12$, $\tau = 0.645$, $p < 0.001$; "Group F-9": $N = 19$, $\tau = 0.532$, $p < 0.001$; "Group F-10": $N = 26$, $\tau = 0.558$, $p < 0.001$; "Group F-11": $N = 19$, $\tau = 0.623$, $p < 0.001$).

Correlations between the number of mutual grooming and the spatial proximity

In four out of five Przewalski's horse groups the horse dyads which groomed often, didn't show a high frequency of staying in close proximity (Kendall's rank correlation tau test, 4 out of 5 'P' groups: $p > 0.05$). Only in group P-5, the horses which groomed each other were also in close proximity significantly often (Kendall's rank correlation tau test: "Group P-5": $N = 9$, $\tau = 0.632$, $p = 0.01$).

However, in the feral horses, four out of six groups showed a weak and one group a strong correlation between mutual grooming and being in close proximity (Kendall's rank correlation tau test: "Group F-6": $N = 23$, $\tau = 0.262$, $p = 0.004$; "Group F-7": $N = 10$, $\tau = 0.179$, $p = 0.17$; "Group F-8": $N = 12$, $\tau = 0.239$, $p = 0.03$; "Group F-9": $N = 19$,

$\tau = 0.157$, $p = 0.02$; “Group F-10”: $N = 26$, $\tau = 0.157$, $p = 0.003$; “Group F-11”: $N = 19$, $\tau = 0.253$, $p < 0.001$).

Correlations between spatial proximity and friendly approaches

We also calculated the correlations between the frequency of the animal pairs being in close proximity and their number of friendly approaches. In four Przewalski’s horse groups, the correlation coefficients were low, only group P-5 showed a high correlation coefficient (Kendall’s rank correlation tau test: “Group P-1”: $N = 5$, $\tau = 0.205$, $p = 0.42$; “Group P-2”: $N = 8$, $\tau = 0.035$, $p = 0.83$; “Group P-3”: $N = 6$, $\tau = 0.289$, $p = 0.18$; “Group P-4”: $N = 9$, $\tau = 0.233$, $p = 0.08$; “Group P-5”: $N = 9$, $\tau = 0.72$, $p < 0.001$). In the feral horse groups, the correlation coefficients were non-significant in one group and low in five groups (Kendall’s rank correlation tau test: “Group F-6”: $N = 23$, $\tau = 0.286$, $p < 0.001$; “Group F-7”: $N = 10$, $\tau = 0.096$, $p = 0.45$; “Group F-8”: $N = 12$, $\tau = 0.238$, $p = 0.02$; “Group F-9”: $N = 19$, $\tau = 0.182$, $p = 0.007$; “Group F-10”: $N = 26$, $\tau = 0.119$, $p = 0.02$; “Group F-11”: $N = 19$, $\tau = 0.3$, $p = 0.01$).

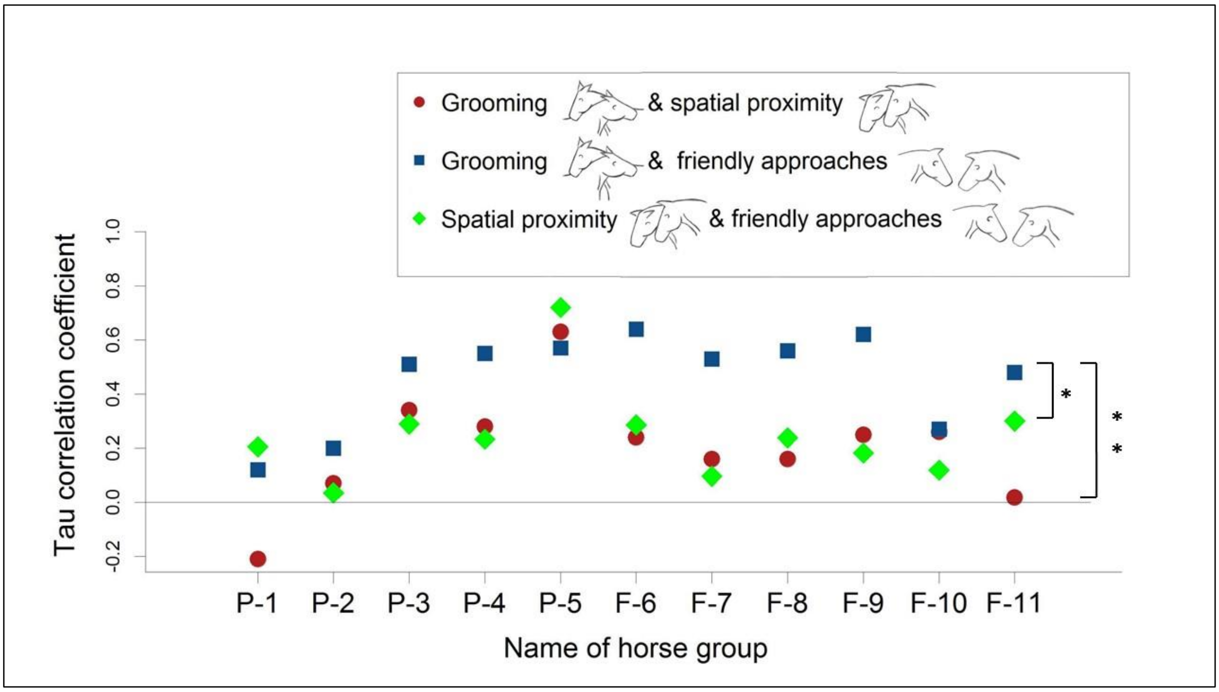


Figure 2. Correlations between the frequencies of grooming, friendly approaches and being in spatial proximity within pairs of horses per group. Correlations were best between the frequency of pairwise grooming and friendly approaches. Significant differences are given with * for p < 0.05 and ** for p < 0.01. The brackets connect two rows of the particular symbol types

Comparison between correlations of mutual grooming, friendly approaches, and spatial proximity in pairs of animals

When comparing the results of the pairwise correlation analyses for all groups (Figure 2), the correlation coefficients between mutual grooming and friendly approaches in pairs of animals were higher than the correlation coefficients between mutual grooming and the frequency of being in close proximity (Wilcoxon signed rank test: $N = 11$, $v = 64$, $p = 0.003$; significant after Sequential Bonferroni Correction) and higher than the correlation coefficients between the frequency of being in close proximity and the number of friendly approaches (Wilcoxon signed rank test: $N = 11$, $v = 60$, $p = 0.01$; significant after Sequential Bonferroni Correction). Correlations coefficients of the mutual grooming and the frequency of being in close proximity and the correlation between the friendly approaches and the frequency of being in close proximity did not differ (Wilcoxon signed rank test: $N = 11$, $v = 33$, $p > 0.05$).

DISCUSSION

Animals which displayed high frequencies of mutual grooming were often involved in friendly approaches but not necessarily in close proximity to group members. Also, in pairs of animals, mutual grooming exchanges correlated best with the exchange of friendly approaches but less with being in close proximity with particular group members. Both, for the overall frequencies of behaviour being involved in and the pairwise behaviour exchange, mutual grooming and friendly approaches did not correspond to close proximity with group members very well.

The value of grooming, affiliative approaches and spatial proximity for the measurement of social bonds

As expected, grooming appeared to be a robust, affiliative, social behaviour parameter, as its frequency was affected only by the animals’ sex in the present study (as in Sigurjónsdóttir et al., 2003), while the friendly approaches and the spatial proximity were affected by various but different factors. Especially, the level of the animals’ aggressiveness appeared to be important for evaluating additional parameters for measuring social bonds, as aggressive animals participated in friendly approaches frequently, but had a reduced spatial proximity with group members. This may indicate a proactive, social function of friendly approaches which aggressive animals may use to balance their social relationships (Hogan et al., 1988; Keiper, 1988; Frank, 2009) and an appeasement of group members towards aggressive animals (Feist and McCullough, 1976; Kolter and Zimmermann, 1988; Roubová et al., 2015) as discussed for the function of social bonds. Not only grooming, but also friendly approaches may serve the formation or stability of social bonds. Animals may exchange individual information about bonded and potentially bonded animals after approaching each other (Lim and Young, 2006). Furthermore, friendly approaches may be used to protect social bonds, as Schneider and Krueger (2012) showed that high ranking female horses approach socially bonded group members to intervene into grooming encounters with other group members.

Spacing behaviour can demonstrate social bonding to some degree, as horses which stay close to each other are more likely to interact (Christensen et al., 2002). However, spatial proximity can strongly be influenced by environmental factors, such as temperature (Tyler, 1972) and insect pressure (Rutberg, 1987), and by aggressive animals avoiding others and being avoided by others, as in the present study. Furthermore, spatial proximity may be enforced by high ranking group members, with the lower ranking animal rather enduring than wishing for closeness (Barton, 1993) and may not necessarily demonstrate preferences between particular pairs of animals (Wells and von Goldschmidt-Rothschild, 1979). All of these aspects suggest either a functional independence or a difference between the friendly approaches and the spatial proximity.

Comparison between Przewalski’s horses and feral horses

In the present study, we did not find significant differences in the frequencies of mutual grooming, friendly approaches, and spatial proximity between the Przewalski’s horses and the feral horses. Our findings support the notion of Christensen et al. (2002) of Przewalski’s horses not having evolved qualitative or quantitative differences in social behaviour compared to central European horses, from which they separated 117,000 years ago (Goto et al., 2011).

Robustness of social behaviour

Horses which were observed in different group compositions did not alter their frequencies of grooming and friendly approaches. This is according to reports of strong and stable affiliative relationships in horses (Waring, 2003; Ostner and Schülke, 2014). Furthermore, social relationships (i.e. demonstrated by the frequency of mutual grooming and friendly approach) appeared to be stronger in smaller rather than in larger groups, as it was claimed for social groups in general (Pusey and Packer, 2003). However, a follow up study is needed to investigate whether, in addition to friendly approaches, further proactive behaviour, such as grazing and resting together, could be combined with the mutual grooming data to analyze social bonds in feral horses more precisely. Further individual factors (e.g. reproductive status and age, Sigurjónsdóttir et al. ,2003), which were missing for most of the observed horses in the present study, may also be considered. Moreover, evaluations on the effect of environmental factors such as the season, food availability and parasite load are urgently needed, but were not the focus of this study.

CONCLUSION

Proactive behaviour, such as friendly approaches, are a good alternative to spatial proximity data to gain further information on the strength, direction and quality of social bonds between individuals in horses. We would expect the correlations between friendly approaches and mutual grooming to be robust for the majority of horse groups, as this was the case for all the groups of the present study, even though the groups varied in their composition, individual parameters, and habitats.

Acknowledgements

We would like to thank the owners of the Przewalski's horses, the Landschaftspflegeverband Stadt Augsburg e.V., the Bundesforst and the Nationalparkverwaltung Bayrischer Wald for the permission to observe the animals. Moreover, we would like to thank Maria and Amerigo Nota for providing us with test horses in Italy, Mirjam Miorin-Bellermann, Lena Abel, Ann-Cathrin Grudacker, Kristina Hinz, Gabriela Schmid, Felix Käfer, Serena Sennet, Tamara Willig and Florian Oberparleiter for help with the horse observations and data recordings, as well as Knut Krueger for help with statistics. Furthermore, we would like to thank Juergen Heinze, Norbert Pantel and Birgit Flauger for discussing the topic and Kate Farmer for language corrections.

CHAPTER 3

The role of an alpha animal in changing environmental conditions

Riccarda Wolter[ab]*, Norbert Pantel[c], Volker Stefanski[d], Erich Möstl[e], Konstanze Krueger[ab]

[a] University of Regensburg, Biologie 1, Universitätsstraße 31, 93053 Regensburg, Germany

[b] University of Applied Sciences Nuertingen, Faculty Agriculture, Economics and Management, Department Equine Management, Neckarsteige 6-10, 72622 Nuertingen, Germany

[c] Landschaftspflegeverband Stadt Augsburg e.V., Dr.-Ziegenspeck-Weg 10, 86161 Augsburg, Germany

[d] Institute of Animal Husbandry and Animal Breeding, University of Hohenheim, Garbenstr. 17, 70593 Stuttgart, Germany

[e] Institute of Medical Biochemistry, Department of Biomedical Sciences/Biochemistry, University of Veterinary Medicine, Veterinär-Platz 1, 1210 Vienna, Austria

* Corresponding author

Published in Physiology & Behavior (2014) 133, 236-243

ABSTRACT

The maintenance and development of conservation areas by grazing of large herbivores, such as Przewalski's horses is common practice. Several nature conservation areas house male bachelor groups of this species. When males are needed for breeding they are removed from the groups, often without considering group compositions and individual social positions. However, alpha animals are needed for ensuring group stability and decision making in potentially dangerous situations in several species. To investigate the role of the alpha male in a bachelor group, we observed the behaviour of five Przewalski's horse males during the enlargement of their enclosure. We analyzed the group's social structure and movement orders, as well as the animals' connectedness, activity budgets, and whether they moved with preferred group members and how factors such as social rank influenced the horses' behaviour. We also investigated the excretion of glucocorticoid metabolites (GCM) via faeces of the horses while exploring a new area as a parameter of glucocorticoid production. Our results show that the alpha male is important for a bachelor group in changing environmental conditions. The alpha male had the highest level of connectedness within the group. When exploring the new environment, its position in the group changed from previously being the last to being the first. Furthermore, the whole group behaviour changed when exploring the new area. The stallions showed reduced resting behaviour, increased feeding and did not stay close to each other. We found that the excretion of glucocorticoid metabolites of most horses rose only marginally during the first days on the new area while only the alpha male showed a significant increased amount of glucocorticoid production during the first day of the enclosure enlargement.

Keywords: alpha male, horse, *Equus ferus przewalskii,* bachelor group, group structure

INTRODUCTION

The maintenance and development of conservation areas by grazing of large herbivores, such as horses, red deer, cattle or others is frequently practiced (Bullock and Pakeman, 1996; Brockway and Lewis, 2003). Aside of the grazing, these so called semi-reserves offer space for keeping species with space requirements that are difficult to meet in limited zoo housing, such as the Przewalski's horse (*Equus ferus przewalskii*) (Tilson et al., 1988). Especially Przewalski's horse males need lots of space for avoiding conflict with other stallions as they tend to show more aggressive behaviour than domestic horses (Feh, 1988; Van Dierendonck et al., 1996; Kolter and Zimmermann, 2001; Christensen et al., 2002). Their aggression level is particularly high when they are kept in small enclosures, which is one of the most common management problems in several species (horses: Skiff, 1982; Hogan et al., 1988; chimpanzee; Bloomsmith et al., 1994; hens: Hughes and Wood-Gush, 1977). Other factors which may affect the aggression level of Przewalski's stallions are group size, age and the presence of other equids in close vicinity (Kolter and Zimmermann, 2001; Puschmann, 2007).

However, the management of male horses in bachelor groups is very important (Tilson et al., 1988) for providing breeding stallions for species conservation programs, such as the European endangered species programme (EEP) (Kolter and Zimmermann, 2001). The training of agonistic behaviour and development of physical stamina in bachelor groups have been shown to be useful for adequate social development and for the males' future reproductive success in many species (canids: Bekoff, 1974; squirrel monkeys: Biben, 1998; rats: Pellis and Pellis, 2006) for example, when obtaining and maintaining a future harem in horses (Klingel, 1980; Berger, 1986; Berger, 1987; McDonnell and Haviland, 1995). Activities such as playing, fighting and mounting were assumed to improve the colt's social skills allowing appropriate interaction when paired with mares (Boyd and Houpt, 1994).

Debate remains about the importance of a stable social organization of managed groups. When males are needed for breeding they are often removed from the groups without considering group compositions and individual social positions (Krueger et al., 2014). Some authors propose to remove the alpha male and move him to a mare group, as, in unmanaged horse groups, the most dominant male is most likely to become a harem stallion (Zharkikh and Andersen, 2009). It has been argued that replacements in bachelor groups are in line with frequent group changes of unstable bachelor groups in the wild (Puschmann, 2007). However, in several species alpha animals are needed for reducing the aggression level within the group, for ensuring group stability, for group leading, and for decision making in potentially dangerous situations (primates: Henzi et al., 1998; Aureli and de Waal, 2000; Flack et al., 2005; Flack et al., 2006; King et al., 2008; hyenas: Zabel et al., 1992; Smith et al., 2010; deer: Jennings et al., 2009; oryx: Walther, 1991; horses: Feist and McCullough, 1976).

Before being extinct in the wild, Przewalski's horses are believed to have formed non-territorial harem bands and bachelor groups similar to feral horses (Berger, 1977). Range establishment and area, as well as habitat use are discussed in Przewalski's horses. Re-introduced Przewalski's horse groups were reported to remain in separated home ranges, i.e. the area through which groups trek regularly, in search for food, water, minerals and shelter. The size of a group's home range was dependent on the season. Scientists expect home ranges of Przewalski's horse groups to overlap when the number of groups in a habitat increases (King, 2002; King and Gurnell, 2005).

Studies in feral horses indicate that the social organization and the behaviour of equids have remained relatively unaffected by domestication (Tyler, 1972; Feist and McCullough, 1976; Waring, 1983). Bachelor groups include young males which live separate from the natal band and older males who do not hold an own harem (Feist and McCullough, 1976; McDonnell and Haviland, 1995; Khalil and Kaseda, 1998). Usually, small male groups show linear hierarchies (Grzimek, 1949; Berger, 1977; Houpt and Keiper, 1982). In breeding groups, movement initiation correlates with social rank, so that dominants are usually followed by subordinates (African Buffalo: Prins, 1995; cattle: Šárová et al. 2007; Šárová et al. 2010; pigeons: Nagy et al., 2010; hyenas: Holekamp et al., 2000; feral dogs: Bonanni et al., 2010; horses: (Tyler, 1972; Heitor et al., 2006). However, in bachelor groups, the alpha male usually is the last animal in the line, like harem stallions which herd their mares from behind (Feist JD, McCullough, 1976; Tilson et al., 1988). Herding is used to maintain group cohesion when other groups approach or some of the group members separate from the group (Kolter and Zimmermann, 2001). Feral dogs (Bonanni et al., 2010) and primates (Byrne et al., 1990; King et al., 2008) prefer to move with particular group members, which is not the case for feral horse harems. It remains to be investigated, whether Przewalski's horse males align with certain animals when moving.

The social rank is considered to affect the animal's stress level. In some species, high-ranking males show higher stress hormone levels (glucocorticoids) than lower-ranking males (apes: Gesquiere et al., 2011; wolves: Sands and Creel, 2004; Muller and Wrangham, 2004). In a natural population of savannah baboons (Gesquiere et al., 2011) and in wolves (Sands and Creel, 2004), the highest-ranking male even had the highest glucocorticoid concentration. In contrast, higher ranking red deer had lower glucocorticoid levels than lower ranking ones

(Balfanz, 2005). A recent study suggests that stress levels in female domestic horses are independent from rank (study in press). The finding correlates with the suggestion of environmental changes having a clear impact on the stress level of individual animals (Wiepkema and Koolhaas, 1993), as reported for dogs (Sigg and Tobler, 1986; Beerda et al., 1999) and mice (Van Loo et al., 2004).

In this study, we analyzed the behaviour and glucocorticoid level of five semi-wild Przewalski's horse bachelors when their enclosure was enlarged. We analysed the horses' social ranks by applying an Average Dominance Index (ADI) because of its reliability and computational simplicity (Hemelrijk et al., 2005). We observed the activity budgets of the individual horses and furthermore, we recorded the movement orders, and the distances between the animals by drawing spatial distribution graphs. Moreover, we analysed the group structure and the connectedness of the horses in affiliative interactions.

The study addresses the following questions: (1) What is the group structure and how are animals connected in the bachelor group? (2) Does the horses' behaviour change while they are exploring a new, unknown area? (a) Does then movement order change? (b) Do males preferentially move with certain group members? (c) Do they align with the preferred animals when exploring the enlarged enclosure? (d) Do males show an enhanced glucocorticoid production while exploring a new area and are there any differences between group members?

MATERIALS AND METHODS

Animals

We investigated a group of five Przewalski's horse males (*Equus ferus przewalskii*) living in the Stadtwald Augsburg, Germany, which is part of a large municipal forest. The horses' ages ranged between 1.5 and 6 years with a median of 2.6 years. All horses were individually identified by their brands and colouration (Table 2). Their social history was comparable, as they were all born into small harem groups in zoo housing. They were separated from their natal group when they were between 1 and 2 years old and integrated into the bachelor group at the Stadtwald Augsburg. The horses fed on the natural vegetation in the area, additional food was not provided. The housing area consisted of a 15ha pine forest, which was enlarged by 15ha of heathland at October 22nd 2012. Both areas were separated by a fence with two gates.

Table 2

Individual horse data

Studbook-No.	Name	born	living in Augsburg since	ADI	social rank
4839	Marlon	20.07.2006	06-2007	0.950	1
5648	Solongo	07.06.2010	09-2011	0.516	2
5665	Xaran	05.07.2010	09-2011	0.467	3
5732	Vinter	13.01.2011	06-2012	0.243	4
5639	Kalmoek	25.05.2010	01-2012	0.083	5

Data sampling

For social rank evaluations behavioural data was collected by continuous recording for 15 hours ad libitum (Martin and Bateson, 1986) in September 2012. Moreover, at the pine forest in September 2012 (18th – 21st) and after entering the new area (the heathland) in October 2012 (23rd – 26th) the animals' activity budget, the movement order in the group, the movement directions, and the distances between the animals were documented, both for 16 hours by scan sampling (Martin and Bateson, 1986) every five minutes. These observations were distributed evenly over 8 daylight hours and were spread over 4 consecutive days.

During the enlargement day at October 22nd, the activity budget, the movement order and direction, as well as the distances between the animals were noted by scan sampling every minute for three hours, from the time point when the horses passed the gates. During all observation days in October we collected faecal samples of all horses for measuring glucocorticoid metabolites. The observer stood about 20 m away from the animals, depending on their spatial distribution. Due to the fact that the horses are accustomed to people, their behaviour was not influenced by the observer's presence.

Social rank

We counted agonistic behaviours of the group members, such as threats to bite or to kick, bites, kicks, chases, retreats and approaches in agonistic contexts (McDonnell and Haviland, 1995; McDonnell, 2003) The social rank of each horse was calculated with the average dominance index (ADI) from their agonistic encounters as follows. The dominance index per pair of individuals, w_{ij} is the number of times an individual won against a certain opponent divided by the total number of agonistic encounters between the pair, thus $w_{ij} = x_{ij} / (x_{ij} + x$

$_{ji}$). Wins were counted for the initiator of an encounter when an approached or challenged animal retreated for one step or more. If two individuals were not involved in an encounter with each other, this pair was excluded from the analysis. The average dominance index of an individual is the average of all its dominance indices with all its interaction partners, thus $1/N \Sigma j w_{ij}$. The ADI ranges between 0 and 1, a higher value indicates a higher dominance rank in the group (Hemelrijk et al., 2005).

The reaction of the receiver, not the type of agonistic behaviour, is decisive for counting wins and losses. For example, an animal may respond by retreating whether it is being kicked or approached. In both cases the receiver loses and the initiator wins. This method enables all agonistic behaviour types to be used, irrespective of their frequency, and provides the largest possible sample size for the dominance calculation (Hemelrijk et al., 2005). We have chosen the ADI for its reliability and computational simplicity. Studies show that the ADI can deal with missing data between pairs of animals and still provides more reliable results then comparable dominance assessment methods (Hemelrijk et al., 2005).

Activity budget

The activity of the animals was calculated from the following activity patterns (Boyd et al., 1988):

1. Resting (standing approximately motionless, with relaxed facial muscles, ears turned sideways and dropped eyelids; or in a lying position)
2. Standing awake (eyes are wide opened and ears are turned forward, horse is intent)
3. Feeding (walking in search for food and ingestive behaviour)
4. Locomotion (locomotory movements to or from an area)
5. Other (all other activity pattern as e.g. agonistic behaviour, affiliative behaviour, drinking, self-grooming)

For the data analysis, the time budget for all observation periods per animal was calculated to analyse if the horses differed from each other in their activity. Furthermore, the activity budget for the whole group was calculated for each of the three sampling periods before, during and after the enlargement. We calculated the averages of the percentage of activity pattern frequencies for all individuals (Figure 4). As the number of scans differed between the three observation periods (before and after enlargement the scan was taken every 5 minutes, during the enlargement period every minute), we tested whether different observation frequencies effected the results, but found no difference between taking every scan and only every fifth scan of the enlargement periods for the analysis. Therefore, analyses were run with the largest possible sample size (before and after enlargement: every 5 minutes, during the enlargement period every minute).

Nearest neighbours, connectedness and group structure

For analysing whether the animals preferentially move with certain group members and in a particular distance to their group members each horse's nearest neighbour and the distances to the other animals were recorded at each data sampling. We measured the distance between the head of a particular horse (A) to any body part of a second horse (B) and distinguished between 4 distance categories (Figure 5):

1. Distance from horse's head to the next horse < 0.5 m
2. Distance from horse's head to the next horse = 0.5 - 2 m
3. Distance from horse's head to the next horse = 2 -5 m
4. Distance from horse's head to the next horse = 5 - 10 m

To analyse the group structure and each horse's connectedness within the group, we applied network analysis (Croft et al., 2008). We measured the connectedness among group members and analyzed the connectedness index for each individual by dividing the number of affiliative interactions of each individual through the total number of affiliative interactions that all animals exchanged among each other.

To analyse the group structure, we investigated whether horses are organized in small-world networks in which all group members interact directly and regularly with each other (Watts and Strogatz, 1988). Small-world networks can be measured by analysing a group's clustering coefficient and the average path length from the agonistic and affiliative interactions among group members. The clustering coefficient gives the proportion of group mates that interact with each other, which ranges from 1 for agonistic and affiliative interactions with all animals to 0 for no interactions. The average path length gives a value for whether animals pass on their information directly to each other, with the value 1 for direct information transfer and greater than 1 when information is passed via other animals to reach animals not directly connected to the information provider. In small-world networks the clustering coefficient and the average path length of a group approach the value 1 (Watts and Strogatz, 1988).

Movement order

For analysing the movement order, we drew spatial distribution graphs of the animals and the direction of movement for each horse at every data sampling.

A movement was recorded when one or more animals moved ≥ 3m away from their group members' previous position towards unoccupied space. The animals may either have moved a) from the periphery of the group, b) from the centre of the group, passed other group members and then moved into "unoccupied space", or, c) if the group was scattered, they moved towards unoccupied space within the groups range. A movement could either be accomplished in a single continuous bout that was observed for ≤ 10s, or in multiple bouts, with in-between stops of ≤ 2s, that were observed for ≤ 30s.

The movement order was recorded:

i) when at least 2 animals moved into the same direction when distribution graphs were drawn,

ii) when the animals moved into the same direction for approximately 50% of the distance or more and,

iii) when the animals moved together at an angle of approximately 15° or less to the movement direction.

Movements of stallions towards each other for the exchange of social behaviours were not counted. From the distribution graphs we calculated the percentages of positions during movements for each particular horse (Figure 6 and Figure 7). The data does not allow for distinguishing leadership for the animals as we did not document movement initiations. Generally, movements in Przewalski's horses can be initiated by departing front animals or by herding from behind (Bourjade, 2009).

Faecal cortisol metabolites

Faecal samples of all horses were collected during the enlargement day and the following observation days in October 2012.

We picked up the samples with unused one-way gloves, stored them in glasses and kept them on ice until freezing. The time until freezing did not take longer than 3 hours. Faecal samples were processed with the method described in Flauger et al. (2010). We extracted cortisol metabolites from the horse faeces as described for faecal GCM extraction in ruminants (Palme and Möstl, 1996). In brief, 0.5 g faeces of an individual sample plus 1 ml water and 4 ml methanol were vortexed for 30 minutes. The methanolic suspension was centrifuged, a small part of the supernatant diluted in assay buffer and directly analysed by an enzyme immunoassay directed against 11-oxoaetiocholanolone-17-CMO coupled to bovine serum albumin. Interassay coefficients of variance for the assay were 13.3% and 15.7% (n = 9). Values represent percentage variance for high and low-quality controls. The enzyme immunoassay was validated for equine faecal glucocorticoid metabolites with an ACTH Challenge Test (Flauger et al., 2010).

Horses excrete GCMs in faeces with a delay of about 24 hours compared to plasma (Palme and Möstl, 1996; Möstl et al., 1999). Therefore, GCMs values were compared to the observations that were made 24 hours before (Figure 8). For example, the values of the GCMs that were assigned to the enlargement day were extracted from the faecal samples that were defecated the day after the enlargement day and the values assigned to the period before the enlargement were extracted from the faecal samples defecated in the morning of the enlargement day.

Data analysis

For statistical analysis we used the R-Project statistical environment (R Development Core Team 2011). To compare datasets with low sample sizes we used non-parametric tests. Friedman-exact tests were applied to compare dependent datasets. For the analysis of contingency tables, we used Fisher's-exact tests. Correlations were calculated with the Kendall-tau test. We used chi-square exact tests for likelihood equations in metric datasets. An ANOVA was used for comparing the five horses in their deviations of cortisol metabolite excretions between observations. All tests were two-tailed and the significance level was set at 0.05.

RESULTS

Social rank

The social organization came close to a linear hierarchy, with the oldest male Marlon in the top position. Both stallions that came to Augsburg after Marlon had arrived, in 2011, ranked at the positions 2 and 3 and the two males that came to Augsburg last, in 2012, filled the rank 4 and 5. When comparing the interactions of pairs of animals, only the alpha male was dominant over all others. In one case a lower ranking horse dominated a group mate with a higher dominance index (Figure 3). Between the two horses Vinter and Kalmoek no interactions were observed. Kalmoek was placed at the last position, as is ADI value was lower than the ADI of Vinter. The lower ADI was due to the fact that Kalmoek had to retreat from the horses in position 1 and 3 more often than Vinter.

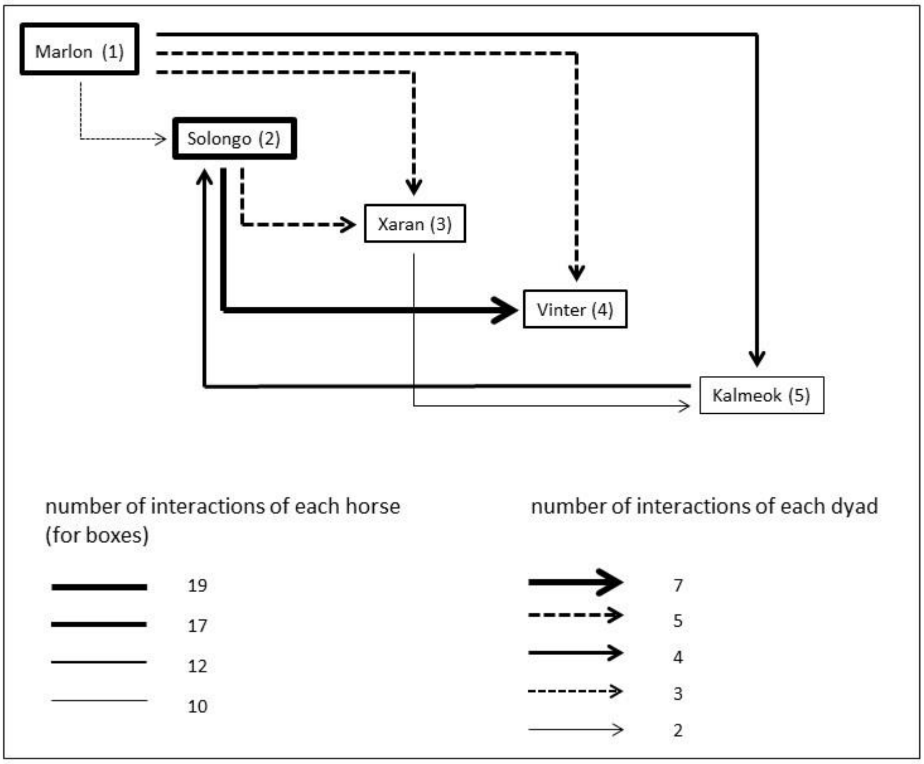


Figure 3. Agonistic sociogramm of the males. The direction of the arrows indicates which horses were dominant over the others. The different diameters of the boxes indicate the interaction proportions of each horse and the different diameters of the arrows indicate the interaction proportions of each dyad

Activity budget

All activity patterns were shown from all animals during our data sampling, but with different frequencies. Before the enclosure was enlarged, the observed animals spent 42% of their time feeding, 22% standing awake, 11% resting, 15% in locomotion, and 10 % for other behaviours (Figure 4). At the day of enclosure enlargement and during the observations of the following days, the males showed reduced resting behaviour (Friedman-exact test: $N = 5$, $\chi^2_2 = 9.5$, $p = 0.009$) and reduced standing (Friedman-exact test: $N = 5$, $\chi^2_2 = 9.58$, $p = 0.008$). In contrast, the proportion of feeding behaviour increased during the enclosure enlargement (Friedman-exact test: $N = 5$, $\chi^2_2 = 8.44$, $p = 0.015$). While the proportion of locomotion didn't change, other behaviour pattern were only shown before and after the enclosure enlargement, not during the enlargement period (Friedman-exact test: $N = 5$, $\chi^2_2 = 10$, $p = 0.007$).

Comparing the activity budgets off all horses during the day of enclosure enlargement, we found that all horses showed almost the same proportions off all behavioural categories. Only Marlon, the alpha male, showed more locomotion and standing awake than other group members, but the difference between the group members was not significant (Friedman-exact test: "locomotion": $N = 5$, $\chi^2_2 = 7.12$, $p = 0.125$; "standing awake": $N = 5$, $\chi^2_2 = 5.21$, $p = 0.579$).

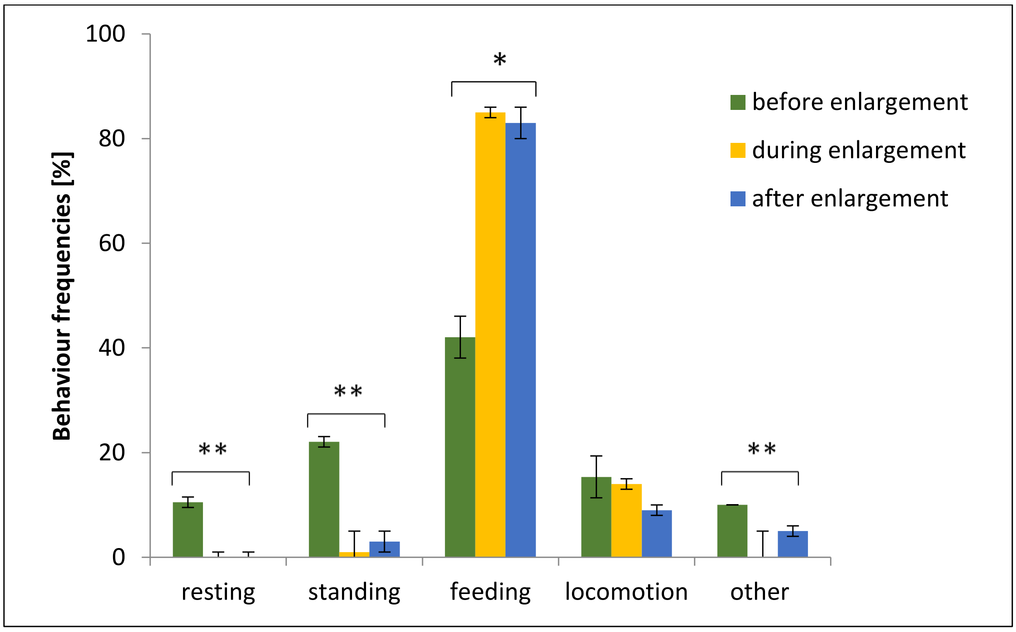


Figure 4. Percentage of behaviours displayed during the three observation periods. Homochromatic bars add to 100%. The bars depict the mean of the particular behaviour data from the 5 stallions and the whiskers show the standard deviation. Significant differences are given with * for $p < 0.05$, with ** for $p < 0.01$ and with *** for $p < 0.001$

Nearest neighbours, connectedness and group structure

All distance categories were found between all pairs during the observations before and after the enlargement. During the enclosure enlargement the smallest distance category of < 0.5 meters wasn't shown by the stallions (Figure 5), in contrast to the preceding and following days (Chi²-test: $\chi^2_2 = 38.1$, $p < 0.001$). Instead, the distance category of 0.5 – 2 meters occurred more often during the enclosure enlargement period (Chi²-test: $\chi^2_2 = 6.059$, $p = 0.048$).

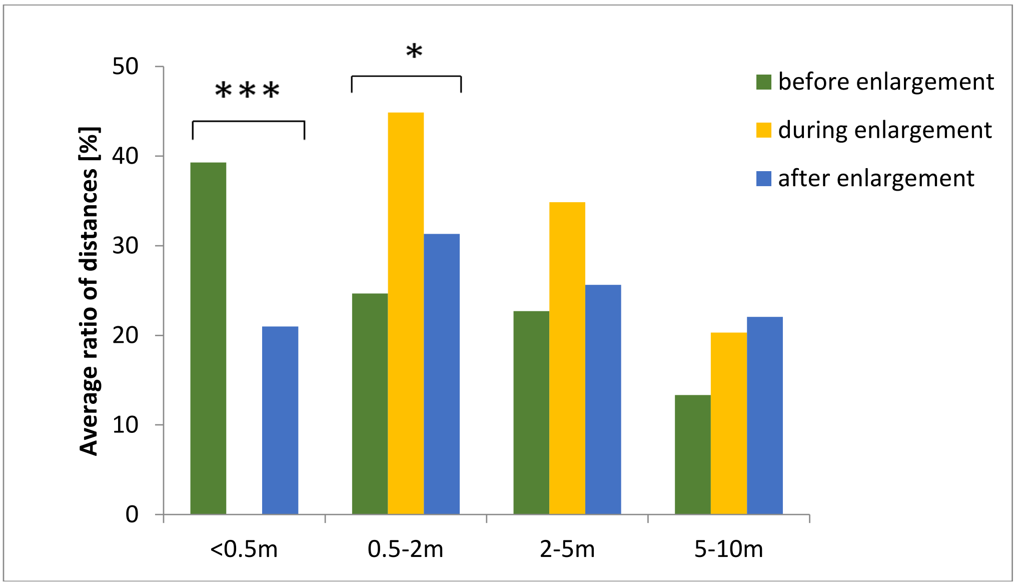


Figure 5. Percentage of distance categories during the three observation periods. The columns show the different distance categories during each observation. During the enlargement horses were never < 0.5m apart from each other. Significant differences are given with * for p < 0.05, with ** for p < 0.01 and with *** for p < 0.001

All horses interacted with all group members directly. The clustering coefficient of the group and the average path length for information transfer was 1 ($N = 5$), which implies, that information was transferred directly (clustering coefficient) and not via other group members (average path length). Therefore, the bachelor group can be considered to be organized in a small-world network.

The individuals differed in their degree of affiliative behaviour exchange. The connectedness between group mates, demonstrated by the degree of affiliative behaviour exchange, gradually decreased from the alpha male Marlon, with the highest connectedness to Xaran with the lowest connectedness ($N_{grooming} = 31$, $N_{animals} = 5$, conn. Index: “Marlon” = 0.77, “Solongo” = 0.52, “Vinter” = 0.42, “Kalmeok” = 0.23 “Xaran” = 0.06). The animals' degree of connectedness did no correlate with their social rank in the group (Kendall-tau test: $\tau = 0.599$, $p = 0.221$).

Movement order

Moving in front position was shown by all five horses. But the proportion of walking in front of the group changed for each horse between September, on the well-known area, and October, on the new area (Figure 6) (Chi²-test: „known area“: $\chi^2_2 = 17.200$, $p = 0.002$; “new area”: $\chi^2_2 = 87.700$, $p < 0.001$). The alpha-male Marlon showed a significant higher frequency of walking in front of the group in the new area (Fisher-exact test: $p < 0.001$), whereas Solongo and Xaran passed significantly less time in the first position at the new habitat (Fisher-exact test: “Solongo”: $p < 0.001$, “Xaran”: $p < 0.001$).

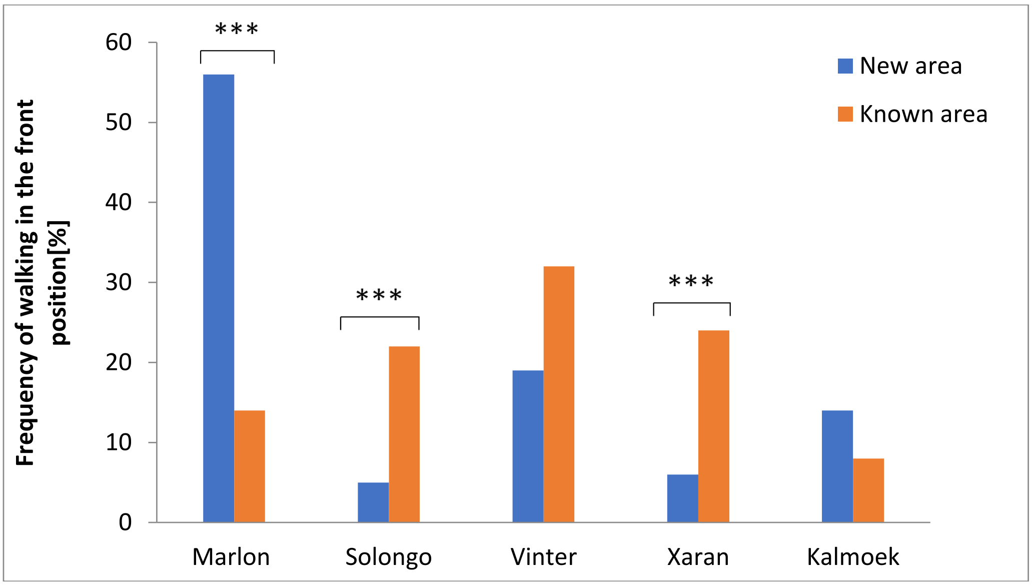


Figure 6. Percentage of walking in the front position for each male. The two graphs show the percentages of being in the front position at the old, known area and during the exploration of the new area. Significant differences are given with * for p < 0.05, ** for p < 0.01 and *** for p < 0.001

We compared the percentage of each animal of walking in the different positions in September and found an unbalanced distribution for all horses, as all males preferred some positions and avoided walking in others. Vinter walked mainly in the first position (Chi²-test: $\chi^2_4 = 29.3$, $p < 0.001$) and Solongo in the second position (Chi²-test: $\chi^2_4 = 12.65$, $p = 0.013$). Xaran showed the most balanced distribution with the highest value in the fourth position (Chi²-test: $\chi^2_4 = 10.7$, $p = 0.03$), as well as Kalmoek, that preferred also the fourth position (Chi²-test: $\chi^2_4 = 25.2$, $p < 0.001$). The alpha male spent most time in the last position walking behind all other stallions (Chi²-test: $\chi^2_4 = 128.7$, $p < 0.001$). The favoured walking position did not correlate with the social rank of the animals (Kendall-tau test: $\tau = -0.316$, $p = 0.613$).

As the number of walking in the front position between the different areas changed for some individuals, we evaluated how often the individuals were in the back of the group (Figure 7). Similar to the results of walking in the front position, we found that the frequency of the alpha male changed significantly between test situations, as he had a higher frequency of walking in the back of the group on the known area (Fisher-exact test: “Marlon”: $p < 0.001$). All other stallions showed a higher rate of walking in the back of the group on the new area (Fisher-exact test: “Solongo”: $p < 0.01$, “Vinter”: $p < 0.001$, “Xaran”: $p < 0.001$, “Kalmoek”: $p < 0.01$).

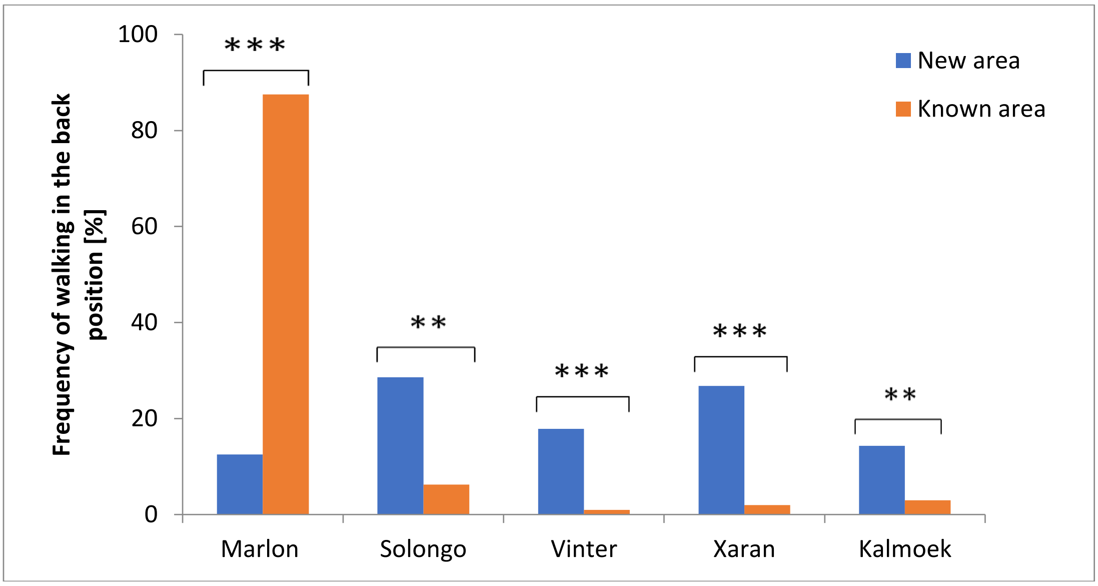


Figure 7. Percentage of walking in the back position for each male. The two graphs show the percentages of being in the last position at the old, known area and during the exploration of the new area. Significant differences are given with * for p < 0.05, ** for p < 0.01 and *** for p < 0.001

Faecel cortisol metabolites

The glucocorticoid metabolites were analyzed before the horses entered the new area and on the first three days at the new area. We compared the values of all animals for the four consecutive days and found that only two horses showed significant deviations from the cortisol metabolite values of their group mates (Chi²-test: „Marlon“: $N = 4$, $\chi^2_3 = 11.529$, $p = 0.009$; „Kalmoek“: $N = 4$, $\chi^2_3 = 10.227$, $p = 0.017$). The alpha male had the lowest GCM values before the enclosure enlargement (Figure 8), but the value did not differ significantly from the GCM values of the other animals. He showed a higher faecal cortisol metabolite increase than any other group member from the day before to the day of the enlargement (ANOVA: $F1,5 = 9.522$, $p = 0.05$) and stronger decrease from the day of the enlargement to the first day after the enclosure was enlarged (ANOVA: $F1,5 = 33.23$, $p = 0.01$). The GCM's of all the other group members increased only marginally and not significantly.

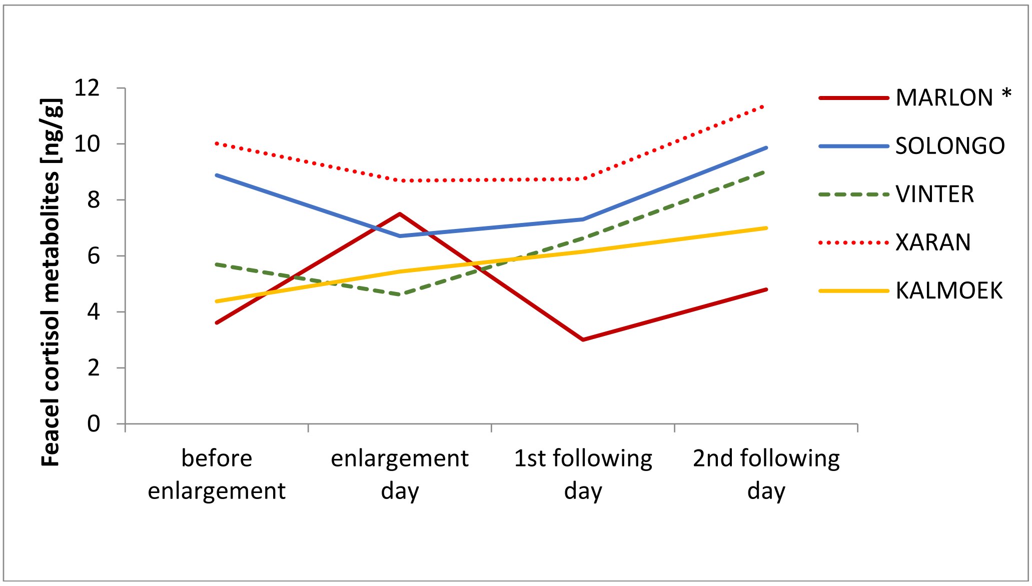


Figure 8. Faecal cortisol metabolites of the stallions. The x-axis lists the different days on which faecal samples were collected. The star indicates the alpha male

DISCUSSION

In the present study, the alpha male took the initiative during the enclosure enlargement and changed his walking position from the last to the front position. We found that the alpha male had a higher increase of cortisol metabolites and a higher ratio of locomotion and standing awake than any other group member during the enlargement day. Additionally, the alpha animal had the highest level of social connectedness within the group, which supports the thesis of his great importance for the group (Walther, 1991).

The hierarchy was nearly linear

The hierarchy in the observed bachelor group was linear when the ADI values were taken into account. When comparing the outcome of interactions for the possible dyads, we found that one male with a lower ADI value dominated a stallion with a higher ADI value. This supports other studies which suggest linear hierarchies to be common in bachelor groups of horses (Feist, 1971; Tyler, 1972; Tilson et al., 1988; Zharkikh and Andersen, 2009). Especially for small groups, with up to 9 horses, strong linear hierarchies were reported (Houpt et al., 1978). The inconsistency in the hierarchy of the present group may be caused by the fact that 4 of the 5 stallions were immature and at almost similar age. Consistent with other studies (Grzimek, 1949; Tyler, 1972; Waring, 1983), we found the oldest animal to be at the top position of the hierarchy.

Feeding behaviour increased and resting behaviour diminished during enlargement

The time the observed Przewalski's horses spent feeding before their enclosure was enlarged is in line with the report of Boyd et al. (1988). However, when the enclosure was enlarged by an area with – compared to the forest enclosure - rich vegetation the horses' feeding duration increased to its double. The time the horses spent standing awake before the enclosure was enlarged is comparable to the study of Boyd et al. (1988), but we observed less resting behaviour, including standing resting and laying down laterally at their sides and sternally at their chests. The difference of observed resting behaviour may be a seasonal deviation as Boyd et al. (1988) observed Przewalski's horses during the summer. During the enclosure enlargement and in the following days resting and standing awake were hardly ever shown as the horses were busy with feeding. Other influencing factors as the weather, the harassment of blood sucking insects and the body condition of the horses seemed not to affect their behaviour, as all of these factors stayed almost the same during the study. Only the mean temperature was lower in October than in September. The most influencing fact that affected the behaviour of the horses appeared to be the diet in the different biotopes, as there was a high offer of old grass on the enlargement area. This aspect will be analyzed and presented in detail in a follow up publication.

In the present study the horses' level of locomotion is comparable to the study of Popolow (1984), in which Przewalski's horses spent 13% of their time with locomotion. Altogether, the horses were more active during the enlargement period, but the level of locomotion didn't change between observation situations, as movements that were done during grazing were assigned to the feeding behaviour. This fits our observations that the horses did not travel longer distances on the new area.

Other behaviours as drinking, self-grooming, mutual grooming or playing were shown as well before and after the enlargement, but not during the first inspection of the new area.

The time budgets of all 5 stallions during the enlargement were almost alike. Only the alpha male showed a higher level of locomotion and standing awake. This supports the thesis that he took the initiative and controlled the situation, as it has been reported about alpha animals in other species (primates: Kitchen et al., 2005; King et al., 2008; dogs: Bonanni et al., 2010).

The movement position of the alpha male changed from the last to the first position

Normally, the alpha male moves in the last position of the group and herds the group from behind (Berger, 1977). This typical behaviour was shown before the enlargement quite often, as the alpha male spent most of the time walking in the back of the group. When the enclosure was enlarged, the alpha stallion walked in the front position most often. The other group members took over the front position almost equally often but seldom.

In line with other studies in horses (Bourjade et al., 2009) and other species (Lamprecht, 1992; Leca et al., 2003; Mirabet et al., 2008) we found a variable distribution of taking the front position when moving and a variable movement order.

Nearest neighbor frequencies changed on the new area

In theoretical approaches, one of the main characteristics of the social behaviour of grazing animals is how they distribute themselves across the landscape (Sibbald et al., 2005), as the frequency of spatial closeness may serve as quantitative indicator for associations between group members (Wasilewski, 2003). Distances between grazing animals are also affected by the risk of predation, as most sheep will flock together in response to a fearful stimulus (Krause, 1994). Within groups of sheep, some individuals tend to stay closer to their neighbours than others (Lynch et al., 1985), which could be due to differences in the fear of predators and/or differences in social motivation.

Christensen et al. (2002) found that Przewalski’s horses tend to show a closer contact to each other as domestic horses, as the study group did not spread out on large pasture available, but grouped up tightly. Moreover, the horses maintained a significantly shorter distance to their 1st and 2nd direct neighbours, than did domestic stallions. Other authors report about environmental influences on group spacing behaviour in feral ponies, for example the temperature (Tyler, 1972) and fly harassment (Rutberg, 1987). These influences should not have been affecting our observed horse group. The mean temperature in September 2012 was 13°C, whether in October 2012 the temperature had a mean value of 7,84°C. As it is reported, that horses tend to stay closer to each other during cold temperatures, our findings were opposite to this. For that reason, the area seems to have had a greater impact to the distribution than the temperature had. The number of insect attacks was very low as well during September as in October.

In our study we found that during the enclosure enlargement, the Przewalski’s horses did not stay in a distance smaller than 0.5 meters to each other but spread more widely over the new area as on the older enclosure. This may have been caused by the fact that they did not show resting behaviour during the enlargement for which horses usually stay closer to each other (Duncan and Vigne, 1979). Moreover, the spatial distribution of the group may have been caused by a vegetation change (Dwyer and Lawrence, 1999) from pine forest to grassland. The new area seems not to have frightened the horses, as there is no indication that they were staying closer to each other.

All horses interacted directly. The interactions were affiliative as well as agonistic and most pairs showed both interaction ways. For all pairs we found at least one kind of both alternative interaction possibilities. The horses in the present study can be considered to be organized in small world networks (Watts and Strogatz, 1998; Croft et al., 2008) in which most members interact with each other directly with similar frequencies. This group structure provides the basis for equal information transfer. But the number of affiliative interactions with other group members differed for the individuals. The alpha male exchanged affiliative behaviour with all group mates, whereas one male, Xaran, interacted affiliatively with only one other individual and showed a very small number of grooming events so that he had the smallest connectedness index.

The animals were not stressed by their new environment

Stress is a general term used to describe environmental factors solicitating adaptation mechanisms and the response to these challenges (Mormède et al., 2007). An organism can show two different responses to stress. Firstly, the HHN-System (hypophysis-hypothalamus adrenal cortex system) can be mobilized, which leads to an increased biosynthesis and the release of cortisol from the adrenal cortex. Secondly, the SAM-system (sympatho-adrenal medullar system) may release adrenalin and noradrenalin (Henry and Stephens, 1977). In the present study the catecholamines, adrenalin and noradrenalin, were not analyzed. We focused on the expression of the cortisol metabolites. In our study the animals responded to the challenges of their environment with only a marginally increase of glucocorticoid metabolites (GCMs).

It remains debatable, whether the animals of the present study experienced a positive activation of the stress response systems (i.e. eustress) or environmental stimuli of aversive nature (i.e. distress) (Buwalda et al., 2012). Especially uncontrollable and / or unpredictable situations are likely to cause distress, which is expressed in physiological responses, such as depressions of immune functions, and changes in glucocorticoid as well as catecholamine secretion (Koolhaas et al., 2011).

For analyzing, whether horses perceive environment enlargements as an uncontrollable or unpredictable situation catecholamin and immune system reactions should be controlled in a corresponding, follow up study.

However, on the enlargement day, the alpha male showed the highest increase of excreted GCMs. A reason for that could have been the change of his spatial position in the group. He was in the front position during most group movements and therefore behaved in an unusual way, as the alpha stallion normally walks behind his group (Feist and McCullough, 1976; Tilson et al., 1988).

Before the enlargement period, the alpha male had the lowest GCM value. This is in contrast to other studies which report that the highest-ranking males exhibit higher stress hormone levels (Gesquiere et al., 2011) but supports the thesis of environmental changes having a clear impact on the stress level of individual animals (Wiepkema and Koolhaas, 1993).

The reader should keep in mind, that it is a case study with some limitations because of the small sample size. Nevertheless, most of the results are very well comparable to many other studies (Waring, 1983; Popolow, 1984; Boyd et al., 1988; Tilson et al., 1988; Bourjade et al., 2009; Zharkikh and Andersen, 2009), but for a generalization of the results, the study should be expanded to a larger population.

CONCLUSION

We found that the alpha male appears to have an outstanding position in the group. During the enlargement period, especially the alpha stallion showed a higher frequency of walking in the front position and had a higher stress hormone level as his group members. Moreover, he had the highest value of connectedness in the group. In general, the horses didn’t seem to be very stressed when they explored the new area, as their cortisol metabolites increased only marginally. A follow up study is needed for evaluating how fast the alpha position will be replaced when the alpha animal is removed from the group. Moreover, it should be investigated, if and to what degree younger stallions learn from the alpha male and how important he is for the group stability, for reducing the aggression level and for decision making.

Acknowledgements

We would like to thank the owner of the Przewalski's horses, the Zoo of Augsburg and the Landschaftspflegeverband Stadt Augsburg e.V. for the permission to observe the animals and Knut Krüger for help with the "R"-environment as well as Jürgen Heinze and Birgit Flauger for helpful discussions.

CHAPTER 4

Comparison between the responses of two Przewalski's horse groups to semi–reserve management in different habitat types

Riccarda Wolter[ab]*, Norbert Pantel[c], Konstanze Krueger[ab]

[a] University of Regensburg, Biologie 1, Universitätsstraße 31, 93053 Regensburg, Germany

[b] University of Applied Sciences Nuertingen, Faculty Agriculture, Economics and Management, Department Equine Management, Neckarsteige 6-10, 72622 Nuertingen, Germany

[c] Landschaftspflegeverband Stadt Augsburg e.V., Dr.-Ziegenspeck-Weg 10, 86161 Augsburg, Germany

* Corresponding author

Manuscript submitted to Basic and Applied Ecology

ABSTRACT

Over the last twenty years, numerous studies on grazing projects with Przewalski's horses have been undertaken. However, studies on their behavioural and physiological responses to human management in different habitat types are needed. We observed two Przewalski's horse groups in two semi-reserves with different habitats and management regimes. Group 1 was a bachelor group of male horses living in a pine forest with additional heathland. Group 2 was a harem of female horses and one male, living in an open sand grassland semi-reserve, surrounded by different kinds of trees; this was comparable to the habitat in which the species evolved. We investigated the horses' behavioural responses to different management regimes, their habitat use, their activity budget, and their stress hormone excretions (faecal cortisol metabolites, GCM) to evaluate possible stress responses to the management regime, and to social and environmental factors. Our results indicate a preference for open grassland and seasonal variation in the activity budget in relation to food availability in both groups of horses. However, individual parameters, such as the animals' position in the hierarchy, had a greater effect on the stress hormone concentrations than the type of habitat, the food supply, or changes in the group composition. But, the highest stress response was caused by space reduction in both groups, even though they were expected to be accustomed to space limitations from previous zoo housing.

Keywords: Przewalski's horses; grazing projects; habitat use; stress hormones; body condition

INTRODUCTION

The value of herbivores for biodiversity and the development of central European landscapes have been well discussed by nature protection associations, especially in the 1990s (Bunzel-Drüke, 1997, 2008). Since then, numerous grazing projects with Przewalski's horses and other large herbivores have been implemented (Riecken et al., 2001; Anders et al., 2004; Sonnenburg et al., 2004; Zimmermann, 2005). The areas grazed by Przewalski's horsesinclude endangered habitat types, which suffer from massive changes in land use, sand harvesting, agricultural changes, and constant nitrogen input from the air. Their number and dimensions decreased severely during the last fifty years. In many places, only small areas of the endangered habitats remain, with the result that many of their inhabitants from flora and fauna are considered as endangered, red listed species. Previous investigations and reports verify that extensive permanent grazing maintains and promotes light habitat structures and, therefore, has positive effects on the biodiversity and the soil quality (Riecken, 2001; Zimmermann, 2005; Bunzel-Drücke, 2008; Krueger, 2012). Large grazers, such as the Przewalski's horse, create a mosaic of micro habitats and rich structured biotopes which conserve or even increase biodiversity (Riecken et al., 2001; Zimmermann, 2005; Bunzel-Drüke, 2008).

Przewalski's horses feed under almost continuous and slow locomotion, and about 17 hours per day are dedicated to searching for food and ingestion (Schach, 1994). Their browsing, rolling, and kicking behaviours break up dense vegetation and their faeces fertilize large areas (Roth 2002). Moreover, the horses maintain open areas by chewing young tree shoots and feeding on the undergrowth and they support the maintenance of ecosystems which are adapted to extensive grazing by endemic domestic and wild animals (Zimmermann et al., 1998; Woodfine, 2003).

Przewalski's horses graze a wide range of forage crops but during the vegetation period show a preference for areas of high green phyto mass density (Duncan, 1983). Nevertheless, if food is scarce in winter they will feed on old grass and bushes in winter (Roth, 2002). Earlier studies on the habitat use of horses demonstrate that horses prefer open grasslands, but their use of particular areas for feeding and resting may vary throughout the year and depend on the vegetation and food supply (Fraser and Gordon, 1997; Kolter et al., 1999; Roth, 2002; Woodfine, 2003; Steidele, 2011). During hot summer days, the horses visit wind-exposed areas with sparse vegetation (Kolter et al., 1999) or the cool shade in forests (Berger et al., 2006).

The activity of Przewalski’s horses reaches its highest level in summer and its lowest in winter (Berger et al., 1999; Arnold et al., 2006). The horses spend most of their time feeding in autumn and spring, but less so in the summer (Berger et al., 1999). Resting behaviour depends to a large extent on the availability of food: it is longer when there is plenty of food (Bogner and Grauvogl, 1984; Duncan, 1992; Boyd and Houpt, 1994; Van Dierendonck et al., 1996; Berger et al., 1999; Woodfine, 2003) and almost eliminated when there is insufficient food (Hechler, 1971; Zeitler-Feicht, 2008).

Therefore, a lack of food availability may induce stress (Fraser 1974), but stress may also be caused by environmental factors, such as unfavourable ambient temperatures, unsuitable housing conditions, and insufficient space (Fraser, 1974), as well as by individual aspects, such as the social rank (domestic horses: Flauger, 2010; red deer: Balfanz et al., 2005), the gender (Touma and Palme, 2005), or the animal’s individual body condition (domestic horses: Kimball 2008; badgers: George et al., 2014; Weddell seals: Bartsh et al., 1992).

Although there are different studies about the behaviour of Przewalski’s horses in grazing projects (e.g. Redman and Goodwin, 1999; Christensen et al., 2002; Steidele, 2011; Wolter et al., 2014), only a few (Budras et al., 2001; Wolter et al., 2014) address the wellbeing of the horses and their reactions to nature conservation management regimes. Limited space, for example, has been shown to increase aggression rates in horses (Przewalski’s horses: Boyd, 1991; domestic horses: Flauger and Krueger, 2013), but studies are lacking on whether the restriction of space would cause stress demonstrated by increases in the stress hormone levels. Moreover, studies are lacking on whether keeping Przewalski’s horses in certain habitat types would cause stress. The Przewalski’s horse is a typical steppe herbivore (Van Dierendonck and Wallis de Vries, 1996), accustomed to dry, open landscapes. Not all semi-reserves offer these habitat types.

The aim of this study was to compare two groups of Przewalski’s horses to assess whether their habitat use, their activity budget, and their stress status is affected by the habitat types and management regimes of the grazing projects they are kept in. We therefore observed the habitat choice and the behaviour of the two Przewalski’s horse groups for one year. One group lived in a semi-reserve with mostly dry, sandy, nutrient-poor grassland, steppe vegetation, and some forest. The other group was restricted to an open pine forest during the summer and had access to open grassland during the winter. We analysed the horses’ reactions to additional food supply and to changes in group composition (Flauger, 2010). In addition, we analysed individual parameters such as the social rank and the body condition of the animals.

The study addresses the following questions: (1) Which of the available habitat types do Przewalski's horses prefer, and are there similarities in the preference between the two groups? (2) Is the activity budget of the two groups comparable and how does it change over the year? (3) How do the horses respond to various management activities, and are the responses comparable between both groups? (4) Is the welfare of the horses, as demonstrated by their stress hormone level, affected by their habitat and the possibility of choosing between various habitat types, by the food supply, by the group composition, by the hierarchy, or by the individual body condition?

MATERIALS AND METHODS

The semi-reserves

Semi-reserve "Stadtwald Augsburg"

The nature reserve Stadtwald Augsburg includes the largest connected area of light pine forests on river gravel on the German part of the river Lech. In 2007 the Landcare Association Augsburg started a grazing project with Przewalski's horses. The pastured area for the horses consisted of a 15 ha pine forest year round, with an additional 15 ha of heathland from October to April (Figure A1). The forest and the heathland were separated by a fence with two gates. The pine forest includes different types of habitat: a heathland area with transition into pine forest, a cleared area which was cut down in 2012, two areas with spruces, four areas of light pine forest, and two areas of thick pine forest with a denser distribution of trees. The heathland area is distinguished by two types of vegetation: semi-dry grassland and tall oat grass meadow.

Semi-reserve "Campo Pond"

Campo Pond was a military compound of the US-army from 1945 until 2008. During that period, the use of tanks and other heavy military vehicles created a special biotope with different habitats, such as sand, neglected grassland, steppe vegetation, and forests. In 2009, the first Przewalski's horses arrived to occupy these habitats for landscape conservation. The 50h area consisted of two equally sized enclosures, connected by a permanently open corridor (Figure A2). Both areas were open sand grassland, surrounded by different kinds of forest.

Animals

We observed two groups of Przewalski's horses (*Equus ferus przewalskii*). The first group was a bachelor band living in the Stadtwald Augsburg, Germany, comprising five male horses. The horses' ages ranged between 1.5 and 6 years, with a median of 2.6 years. The second group consisted of seven females, living at the semi-reserve Campo Pond in Hanau, Germany. The horses' ages ranged between 4 and 22 years, with a median of 8.3 years. In the July of the observation period, three of the seven mares were removed and a stallion was introduced, changing it to a harem group. All horses were individually identified by their brands and colouration (see Appendix Table A1 & A2). Both groups fed on the natural vegetation in their respective areas, but the group at Campo Pond received additional hay during the winter months.

Data collection

We collected data for both groups from September 2012 until August 2013. We observed each group for 16 hours every month, with these hours distributed evenly over the daylight hours from 8.30 a.m. to 4.30 p.m. Observations were spread overconsecutive days for each group, and each group was observed for 192 hours in total.

For social rank evaluations, behavioural data were collected by continuous recording each month for 16 hours ad libitum (Martin and Barteson, 1986). We collected several faecal samples for all horses every month (see below) to measure glucocorticoid metabolites, and we defined each horse's body condition index (Rudman and Keiper, 1991). To calculate activity budgets, the behaviour of the individual animals (see below) was documented by scan sampling (Martin and Barteson, 1986) every five minutes. The scan sampling was integrated into the 16 hours of ad libitum sampling for each group.

The observer stood about 20 m away from the animals, depending on their spatial distribution. The horses are accustomed to people, and their behaviour did not appear to be influenced by the observers' presence. A habituation phase of about 5-10 minutes at the beginning of each observation period was conducteduntil the animals ignored the person.

Habitat use and GPS location

During each scan, the GPS location of the group was recorded by an observer standing in the middle of the group, using a Garmin eTrex venture HC and, we wrote down the main vegetation type surrounding each animal in a radius of approximately half a meter (Table 3 & Table 4). As the variety of habitats in both areas was interlocked at small-scale, we decided to focus on the main vegetation types, which could clearly be distinguished.

Table 3

Vegetation types of Augsburg in order of their size

Type of vegetation	Size [ha]
Light pine forest	6.04
Tall oat grass meadow	4.09
Thick pine forest	3.94
Spruces	3.23
Semi-dry grassland	2.34
Clearance area	1.87
Heathland + Pine forest	1.02

Table 4

Vegetation types of Campo Pond in order of their size

Type of vegetation	Size [ha]
Open grassland	25.3
Coniferous forest	8.13
Deciduous forest	6.88
Mixed forest	3.05
Bushes	2.76
Others	2.16
Pioneer forest & cleared corridors	1.72

The horses' habitat choice was defined as the proportion of a habitat visited by the animals, relative to proportion of the habitat in the semi-reserve. This was quantified by calculating a selectivity index (S_i) for each vegetation composition using Jacobs' modification (Jacobs 1974) of Ivlev's selectivity index (Ivlev, 1961) as follows: $S_i = (c_i - a_i) / (c_i + a_i -$

$2c_ia_i$), where c_i is the proportion (between 0 and 1) of horses observed on habitat_i and a_i the proportion (between 0 and 1) of habitat_i in the study area. We took into account that the availability of areas differed between summer and winter for the animals in Augsburg in the calculation. We adapted a_i for each month. The selectivity index S_i varies from –1.0 to + 1.0, with –1.0 meaning complete avoidance, 0 meaning no active choice and +1.0 meaning complete choice of a particular habitat. The Jacobs' index was chosen for its low sensitivity to variations in the relative abundance of vegetation compositions, enabling the ranking of abundant and rare habitat types according to their acceptance from the animals. The habitat preference can be assigned to five categories: Strong avoidance ($-1 < S_i < -0.5$), moderate avoidance ($-0.5 < S_i < -0.25$), neutrality ($-0.25 < S_i < 0.25$), moderate preference ($0.25 < S_i < 0.5$) and strong preference ($0.5 < S_i < 1$).

Activity budget

The activity budget of the animals was calculated from the following activity patterns (Boyd et al., 1988):

1. Resting (standing with minimal motion, relaxed facial muscles, ears turned sideways and dropped eyelids, or in a lying position)
2. Standing awake (eyes are wide open and ears turned forward, horse is attentive)
3. Feeding (walking in search for food, ingestive behaviour)
4. Locomotion (movements to or from an area)
5. Other (all other activity pattern, such as social behaviour, drinking and comfort behaviour)

The time budget per observation period per animal was calculated to analyze whether the horses differed from each other in their activity. Furthermore, the activity budget for the whole group was calculated from the observations of each month. We calculated the averages percentages of activity pattern frequencies for all individuals.

Social rank

We counted agonistic behaviours of group members, such as threats to bite or to kick, bites, kicks, chases, and retreats and approaches in agonistic contexts (McDonnell and Haviland, 1995; McDonnell, 2003). The reaction of the receiver was decisive for counting wins and losses, not the type of agonistic behaviour. For example, an animal may respond by retreating whether it is being kicked or approached. In both cases the receiver "lost" and the initiator "won". This method enables all agonistic behaviour types to be used, irrespective of their frequency, and provides the largest possible sample size for the dominance calculation (Hemelrijk et al., 2005). The social rank of each horse was calculated with the average dominance index (ADI) from their agonistic encounters as follows. The dominance index per pair of individuals w_{ij} is the number of times an individual won against a certain opponent divided by the total number of agonistic encounters between the pair, thus $w_{ij} = x_{ij} / (x_{ij} + x_{ji})$. Wins were counted for the initiator of an encounter when an approached or challenged animal retreated for one step or more. If two individuals were not involved in an encounter with each other, this pair was excluded from the analysis. The average dominance index of an individual is the average of all its dominance indices with all its interaction partners; thus, $1 / N\Sigma j w_{ij}$. The ADI ranges between 0 and 1; a higher value indicates a higher dominance rank in the group (Hemelrijk et al., 2005). We choose the ADI for its reliability and computational simplicity. Studies show that the ADI can deal with a lack of data between pairs of animals and still provides reliable results (Hemelrijk et al., 2005).

Faecal cortisol metabolites

A minimum of two fresh faecal samples were collected from each horse each month. Samples were collected at the same time (with a maximum deviation of two hours) on two consecutive days for all horses in each observation month. We picked up the samples with unused single use examination gloves, stored them in glass containers, and kept them on ice until freezing. The time until freezing was less than 3 hours. Faecal samples were processed using the method described by Flauger et al. (2010). We extracted cortisol metabolites from the horse faeces as described for faecal GCM extraction in ruminants (Palme and Möstl, 1996). In brief, 0.5 g faeces of an individual sample, plus 1 ml water, and 4 ml methanol were vortexed for 30 min. The methanolic suspension was centrifuged and a small part of the supernatant was diluted in assay buffer and directly analysed by an enzyme immunoassay directed against 11 oxoaetiocholanolone-17-CMO coupled to bovine serum albumin. Interassay coefficients of variance for the assay were 13.3% and 15.7% (n = 9). Values represent percentage variance for high and low-quality controls. The enzyme immunoassay was validated for equine faecal glucocorticoid metabolites with an ACTH Challenge Test (Flauger et al., 2010). Horses excrete GCMs in faeces with a delay of about 24 h compared to plasma (Palme and Möstl, 1996; Möstl et al., 1999). Therefore, GCMs values were compared with the observations that were made 24 h before.

Body condition score

The body condition of the horses was assessed monthly using a 5 point visual scale with half point gradations, from 1 for obese to 5 for emaciated horses (Rudman and Keiper, 1991). The body condition of a horse can be scored best where tissue covers the bones: at the vertebrae, the rump, the hips, the shoulders, and the neck. For example, the more prominent the hip bones of a horse, the worse its body condition.

Temperature

We analyzed the temperature of both semi-reserves by scan sampling every half an hour with a Technoline EA-3010.

Compliance with ethical standards

No permission from the German animal welfare board was required for the field observation of the horses' social behaviour. The owner of the horses and the administrations of the observation areas were informed about the observations and permitted the non-invasive data collection.

Statistical analysis

The statistical analysis and the figures were done with the R-Project statistical environment (R Development Core Team 2015). Some of the data were not normally distributed (Shapiro Wilk Test). Therefore, we applied non-parametric tests. We applied generalized linear models (GLMs) to analyze general effects on the habitat use and the level of glucocorticoid metabolites. All GLMs were randomized with 1000 permutations and data are listed only if the p-values differed significantly from the random value. Wilcoxon signed rank tests and Friedman-exact tests were applied to compare activity budgets of the animals during different seasons. We compared the frequencies of behavioural patterns between both horse groups and the glucocorticoid levels within the groups between different months by applying Mann-Whitney U tests. Multiple testing was corrected with a Sequential Bonferroni Procedure (Holm, 1979). Low sample sizes were analyzed with exact procedures. All tests were two-tailed and the results of the statistical tests were considered significant at $p < 0.05$.

RESULTS

Habitat choice

From October to March, seven habitats types were accessible to the animals at the semi-reserve in Augsburg, when the fence between the two enclosure sections was open and both types of heathland, as well as the clearance area, were available. During that time, the horses showed a preference for the tall oat grass meadow (Jacobs' index = 0.5) over all other kinds of habitat (Figure 9). Both heathland areas together accounted for between 70% and 97% of time spent on the habitats according to season (83% on average).

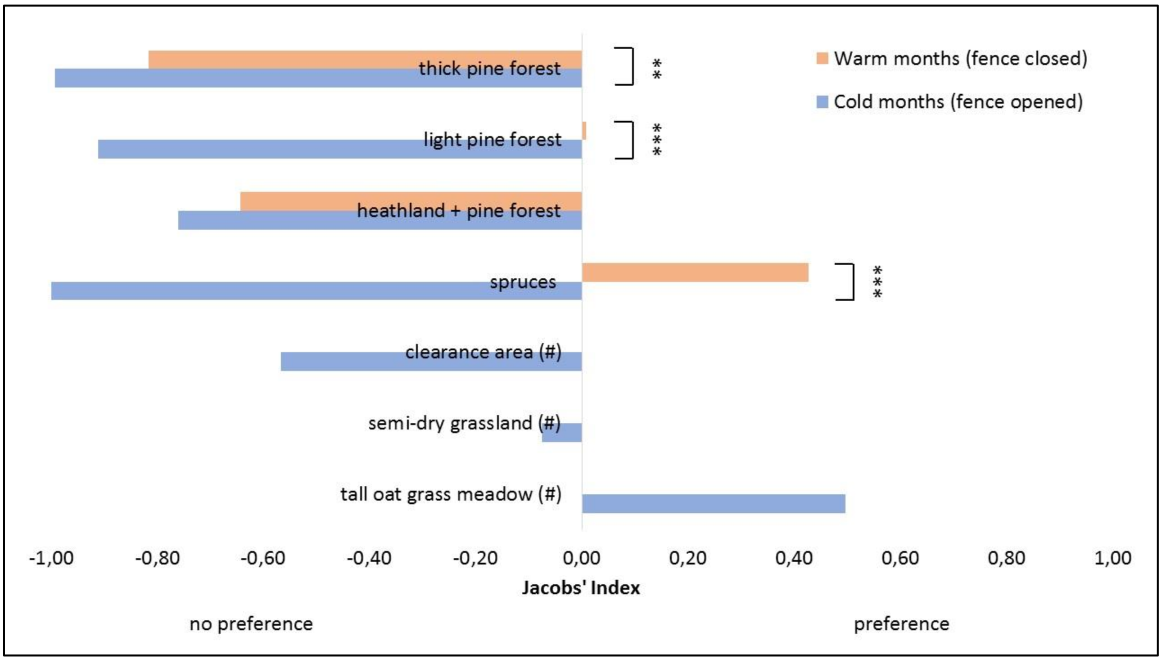


Figure 9. Annual habitat choice in the semi-reserve Augsburg (Jacobs' index). Habitats which were accessible to the horses only from October until March are marked in the figure with (#). Significant differences are given with * for $p < 0.05$, with ** for $p < 0.01$ and with *** for $p < 0.001$

When the fence was closed in September, as well as from April to August, and the horses had the option to choose between four different types of habitats (the light and the thick pine forest, the spruces and the combination of pine forest with heathland), we found a preference for the spruces (Jacobs' index = 0.43), neutrality for the light pine forest (Jacobs' index = 0.01) and avoidance for both other types of habitat (Jacobs' index "heathland + pine forest" = -0.64, Jacobs' index "thick pine forest" = -0.82; Figure 9).

Comparing the Jacob's index of the various types of habitat for all months with each other, differences could only be found for the spruces (GLM: N = 12, t = - 2.672, P = 0.023), meaning the habitat use of this vegetation type showed the highest variation over the year. The horses did not visit the areas covered with spruces in winter and the percentage of visits increased up to 68% in summer. Consequently, the Jacobs' index varied from -1 in the winter to 0.79 in the summer (Table 5). All other vegetation types showed no significant variation of habitat use during the time they were accessible to the horses (GLM: N = 12, all P > 0.05).

Table 5

Average Jacobs' indexes (Si) for the main habitat in each season for the group in Augsburg. Bold characters are for selected food items, i.e. mean – s.e.m. > 0. Standards are for avoided items, i.e. mean + s.e.m. < 0

Mean (s.e.m.)	autumn	winter	spring	summer
light pine forest	-0.38 (0.23)	-1.00 (0.00)	-0.31 (0.24)	-0.11 (0.01)
tall oat grass meadow	**0.15 (0.09)**	**0.81 (0.06)**	**0.24 (0.00)**	n.a.
thick pine forest	-0.89 (0.09)	-1.00 (0.00)	-0.87 (0.08)	-0.86 (0.10)
spruces	-0.57 (0.43)	-1.00 (0.00)	-0.36 (0.28)	**0.79 (0.04)**
semi-dry grassland	**0.12 (0.05)**	-0.25 (0.14)	**0.09 (0.00)**	n.a.
clearance area	-0.10 (0.02)	-0.98 (0.02)	-0.27 (0.00)	n.a.
heathlind + pineforest	-0.77 (0.05)	-0.86 (0.11)	-0.18 (0.14)	-1.00 (0)

The group at the semi-reserve Campo Pond showed a preference for the open grassland (Jacobs' Index = 0.65), while all other vegetation types were avoided all through the whole year (Figure 10).

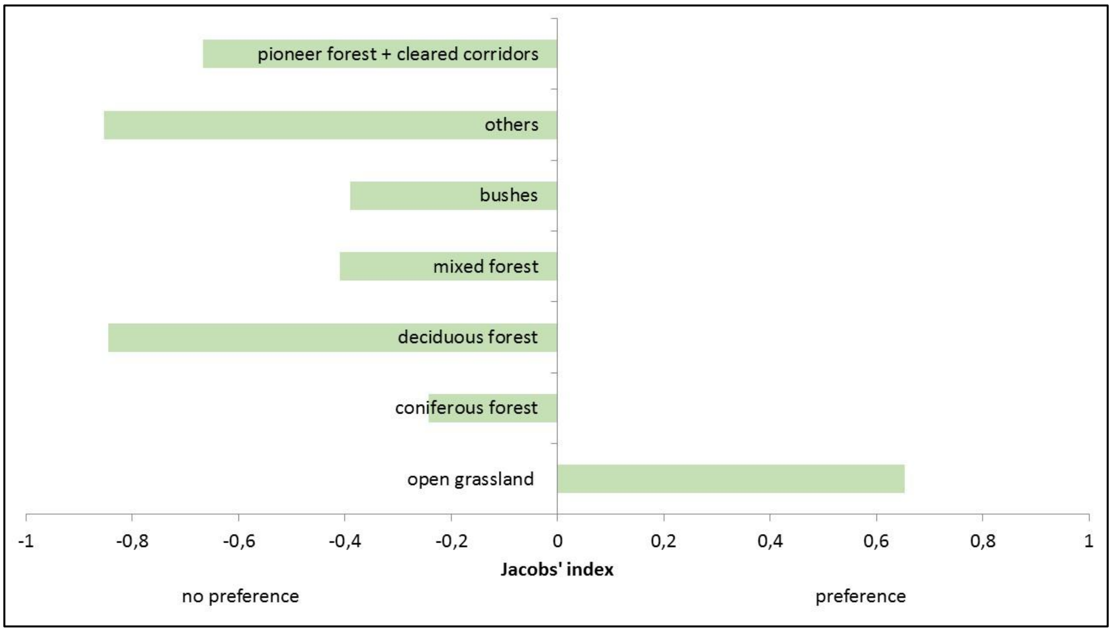


Figure 10. Annual habitat choice at the semi-reserve Campo Pond (Jacobs' index). The habitats were accessible during the whole year. No differences were found for the choice of habitats with particular vegetation types over the observation months

The open grassland accounted for between 74% and 100% of time spent on the habitat according to season (83% on average). We found no differences between the habitat use and the various vegetation types in the different observation months (GLM: $N = 12$, all $p > 0.05$; Table 6).

Table 6

Average Jacobs' indexes (Si) for the main habitat in each season for the semi-reserve at Campo Pond. Bold characters are for selected food items, i.e. mean – s.e.m.> 0. Standards are for avoided items, i.e. mean + s.e.m.<0

Mean (s.e.m.)	autumn	winter	spring	summer
open grassland	**0.48 (0.18)**	**0.48 (0.22)**	**0.87 (0.09)**	**0.78 (0.17)**
coniferous forest	-0.61 (0.29)	-0.36 (0.32)	**0.87 (0.10)**	-0.87 (0.12)
deciduous forest	-0.74 (0.14)	-1.00(0.00)	-0.81 (0.19)	-0.83 (0.17)
mixed forest	-0.23 (0.20)	-0.23 (0.15)	-0.60 (0.24)	-0.58 (0.42)
bushes	**0.21 (0.20)**	-0.71 (0.20)	-0.69 (0.15)	-0.36 (0.30)
others	-0.85 (0.15)	-0.90 (0.06)	-1.00 (0.00)	-0.66 (0.18)
pioneer forest + cleared corridors	-0.37 (0.3)	-0.45 (0.28)	-0.86 (0.14)	-0.99 (0.01)

Activity budget

We compared the frequencies of the behaviours during each season between the horses in Augsburg and at Campo Pond. The horses did not differ in their frequency of resting, standing, feeding and locomotion (Mann-Whitney U test: $N_1 = 60$, $N_2 = 80$, "Resting": $p > 0.05$; "Standing": $p > 0.05$; "Feeding": $p > 0.05$; "Locomotion": $p > 0.05$). However, we found a higher frequency for other behaviour pattern among the horses in Augsburg compared to the group at Campo Pond (Mann-Whitney U test: $N_1 = 60$, $N_2 = 80$, $p < 0.001$).

In Augsburg, all activity patterns were shown by all animals during our data sampling, but with different frequencies (Figure 11). The horses rested least often in autumn and most often in summer (Friedman-exact test: $N = 60$, $\chi^2_3 = 33.72$, $p < 0.001$). They fed more often in winter than in the other observation seasons (Friedman-exact test: $N = 60$, $\chi^2_3 = 33$, $p < 0.001$). In contrast the animals stood awake less often (Friedman-exact test: $N = 60$, $\chi^2_3 = 19.25$, $p < 0.001$), and showed less locomotion (Friedman-exact test: $N = 60$, $\chi^2_3 = 26.15$, $p < 0.001$) and other behaviour patterns (Friedman-exact test: $N = 60$, $\chi^2_3 = 17.73$, $p < 0.001$) during winter compared to the other seasons. Pairwise comparisons for the behaviour frequencies between the seasons are given in the Appendix (Table A7).

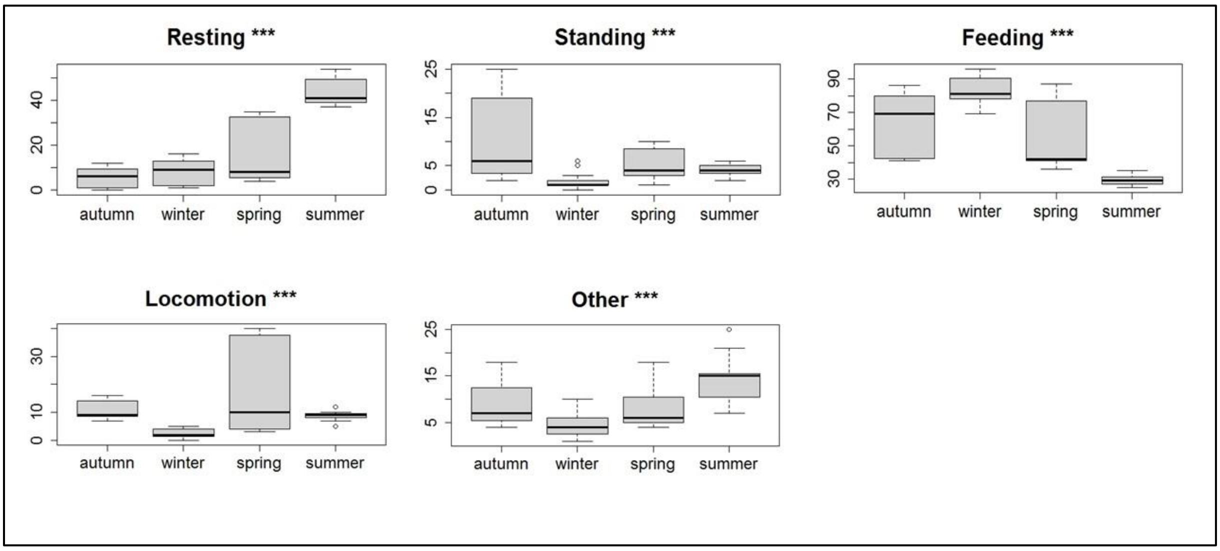


Figure 11. Behavior frequencies displayed at the semi-reserve Augsburg during the four seasons. In case the particular behaviors differed in their frequencies during the seasons, significances are given with * for p < 0.05, with ** for p < 0.01 and with *** for p < 0.001 for each behaviour

As in Augsburg, at the semi-reserve Campo Pond all activity patterns were shown from all animals during our data sampling, but some of them with different frequencies (Figure 12).

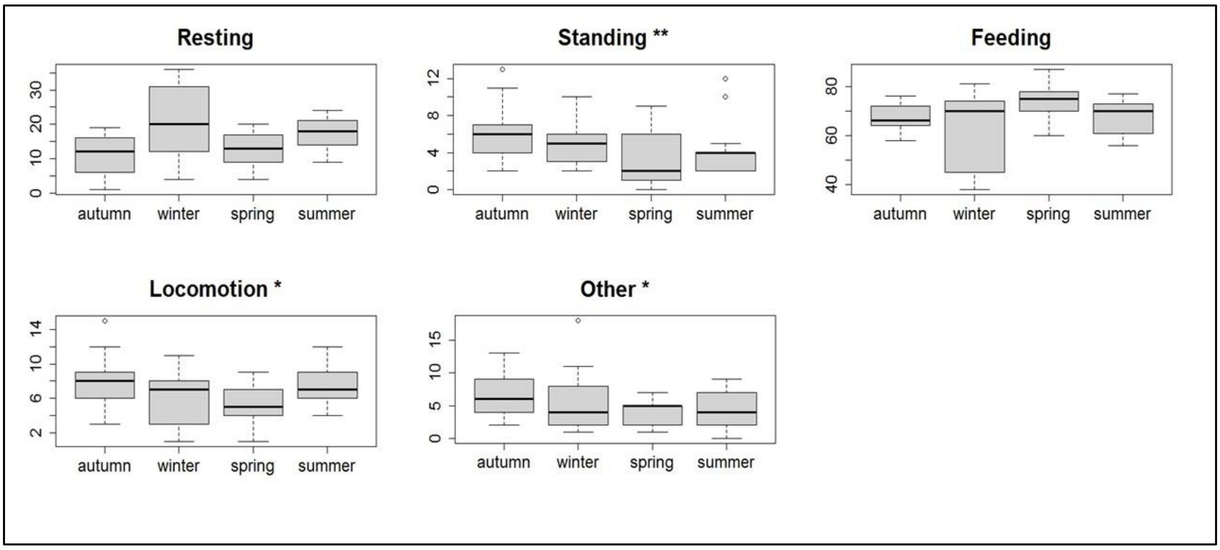


Figure 12. Behavior frequencies displayed at the semi-reserve Campo Pond during the four seasons. In case the particular behaviors differed in their frequencies during the seasons, significances are given with * for p < 0.05, with ** for p < 0.01 and with *** for p < 0.001 for each behavior

Resting behaviour (Friedman-exact test: $N = 80$, $\chi^2_3 = 3.8$, $p > 0.05$) and the frequency of feeding (Friedman-exact test: $N = 80$, $\chi^2_3 = 5.32$, $p > 0.05$) was constant over the year. The animals' locomotion (Friedman-exact test: $N = 80$, $\chi^2_3 = 10.14$, $p = 0.017$), standing awake (Friedman-exact test: $N = 80$, $\chi^2_3 = 14.69$, $p = 0.002$) and other behaviours (Friedman-exact test: $N = 80$, $\chi^2_3 = 11.12$, $p = 0.011$) were shown with the highest frequency in autumn and with the lowest frequency in spring. Again, pairwise comparisons between the behaviour frequencies of the seasons are given in the Appendix (Table A8).

Faecal cortisol metabolites

Both groups showed individual variation in the level of stress hormone metabolites per month ("Augsburg": GLM: $N = 60$, $t = 1.976$, $p = 0.049$; "Campo Pond": GLM: $N = 78$, $t = 2.705$, $p < 0.008$; Figure 13), but there was no difference in the animals' stress hormone levels between the groups over the whole year (GLM: $N = 138$, $t = -1.481$, $p = 0.144$).

The horses in Augsburg had higher cortisol metabolite levels the lower they were in social rank (GLM: $N = 60$, $t = 2.992$, $p = 0.004$). The highest-ranking stallion showed the lowest concentration of cortisol metabolites during the whole year (see Figure 13: the lowest line refers to the alpha male Marlon). In contrast, the horses at Campo Pond showed no correlation between stress hormones and social rank (GLM: $N = 80$, $t = 1.478$, $p = 0.143$).

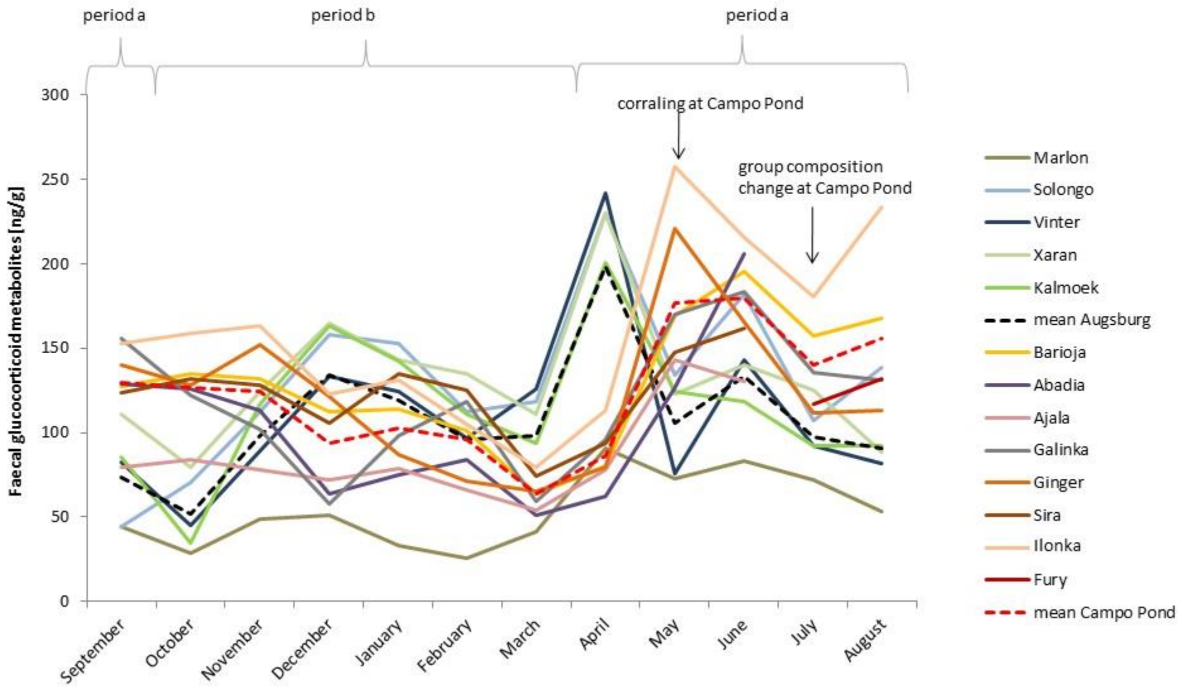


Figure 13. Faecal cortisol metabolites of both horse groups. The x-axis lists the different month. The dashed lines represent the mean values for both horse groups. The brackets over the a-axis represent the two observation months for Augsburg. During period a, the horses only had access to the pine forest and during period b, they could graze the whole area. The arrows indicate the corralling of the mares after the deworming in May and the change of group composition at Campo Pond in July when 3 mares were taken out of the group and the stallion arrived

Influence of management factors on the stress hormones

The horse group in Augsburg showed no difference in the level of glucocorticoid metabolites during the months spent in the pine forest (April – September) and when they had the possibility to choose between forest and heathland (GLM: $N = 60$, $t = -1.363$, $p = 0.178$). Only in April, immediately after the fence was closed and the horses restricting to the pine forest, were the horses' stress hormone levels significantly higher than during the rest of the year (GLM: $N = 60$, $t = -5.144$, $p < 0.001$).

Similarly, the group at Campo Pond showed an enhanced glucocorticoid level when they were restricted to a small enclosure with a size of approximately 1013 m^2 for 48 hours after the animals had been dewormed in May (GLM: $N = 78$, $t = 3.67$, $p < 0.001$). The change of the group composition during July, when three mares left the group and a stallion added to change the group into a harem, caused no significant increase of glucocorticoid levels (GLM:

N = 78, *t* = 1.142, *p* = 0.257). However, the feeding of additional hay during winter months may have reduced stress in the group at Campo Pond, as glucocorticoid metabolite levels were significant lower during the months with additional feeding than during the months without feeding (GLM: *N* =78; *t* = -2.386, *p* = 0.019).

Influence of body condition and temperature on the glucocorticoid hormone values

The mean body condition index of the horses in Augsburg was 2.41 and the mean index of the horses at Campo Pond was 3.00. The horses in Augsburg showed no significant correlation between the body condition index of the individual animals and their individual quantity of glucocorticoid metabolites (GLM: *N* = 60, *t* = 0.314, *p* = 0.755), whereas the horses at Campo Pond had a negative correlation, with the animals in a better body condition (with a lower body condition index) having higher cortisol metabolite values (GLM: *N* = 80, *t* = -6.54, *p* < 0.001).

DISCUSSION

Habitat choice

The habitat use of the horses in Augsburg was influenced by the management procedures of the semi-reserve. The horses in Augsburg only preferred to stay in the spruces when they were restricted to the pine forest from April to October and preferred the tall oat grass meadow when they had free access to all habitat types from October to March. Previous studies (e.g. Steidele, 2011) showed that Przewalski's horses prefer heathland and open grassland year round, and woodland is mostly avoided. Ihle (1984) and Jaworowska (1976) mentioned that horses visit exposed, windy hills or hill slopes if possible, and only a few individuals prefer to rest in the shade of single trees or in the woods.

The group at Campo Pond showed typical resting behaviour for wild equids (Heintzelmann-Gröngröft, 1984) and rested at the open grassland. Rather than protecting themselves against the heat during the hot summer, they choose open habitats with low insect pressure (Zeitler-Feicht, 2008). This observation matches the behaviour of Przewalski's horse mares from other studies, which rested at preferred habitats for food intake (Kolter et al., 1999). They preferred short types of grass and avoided long grasses (Kolter et al., 1999), which corresponds to their need for free sight, and space for flight (Pirkelmann, 1991). Both groups constantly visited the same resting places, as reported for other Przewalski's horses (Van Dierendonck, 1996).

As the heathland in Augsburg consists of two habitat categories, it is noteworthy that the tall oat grass meadow was frequented more often than the semi-dry grassland. The horses' choice may have been due to the superior nutrient supply at the tall oat grass meadow, or to its location. The tall oat grass meadow was located at the outer area of the enclosure and the horses may have preferred to graze along the fence (Schaupp, 2008).

Activity

The activity pattern of the horse groups was also affected by the management. During the winter, the horses in Augsburg spent most time feeding, when they had additional access to the heathland areas with plenty of grass. At the same time, the horses at Campo Pond had only a little grass and lost body mass, and this may have reduced their activities to save energy, i.e. a walking hibernation processes, a common response to reduced ambient temperatures (Scheibe and Streich, 2003, Arnold et al., 2004, Brinkmann et al., 2014). The decrease in body condition of Przewalski's horses during winter is typical, and independent of the sex (Kuntz et al., 2006). In spring, the horses at Campo Pond may have found the newly grown grass tasty and increased their duration of feeding. In Augsburg the horses showed the most resting behaviour during the hot summer months. This result is comparable to other studies which demonstrated that the length of the resting period depends on the temperature (Ihle, 1984). At Campo Pond the group showed enhanced resting behaviour during summer and winter. The resting in winter may have gone along with reduced basic metabolic rates, i.e. walking hibernation.

Stress responses

The impact of the management was also reflected by the animals' stress responses. The group at Campo Pond showed the highest excretion of stress hormones in May, when they were corralled for 48 hours in a small enclosure after being dewormed. Similarly, the group in Augsburg had the highest stress hormone concentration in April, when they were fenced into the pine forest. The high stress hormone concentration is supported by the observation that, directly after fencing, the horses spent most of their time walking along the fence, trying to get back on the heathland. In May, stress hormones went down to baseline again, even though the animals had to stay in the forest.

In general, the two groups did not differ in their stress hormone levels. Whether they grazed in the forest or on the grassland did not affect their stress response. Furthermore, neither the age, nor the group composition, or the changes in group composition at Campo Pond, when a stallion was integrated and when three mares left the group, caused stress in the horses. Horses are accustomed to changing group compositions, as, in nature, they live in a fission-fusion society with frequent departures and arrivals of group members (Keiper et al., 1985).

Especially at Campo Pond, we observed seasonal effects on the values of glucocorticoid metabolites (Touma and Palme, 2005). Stress hormones were higher during warm months than during cold months, similar to another study in Przewalski's horses (Steidele, 2011) and a study in white-tailed deer (Taillon and Côté, 2008). In the course of the year, lower temperature goes along with low food supply, and it has been shown in other animals that the deprivation of food or a poor food quality may lead to an increase in cortisol (horses: Steidele, 2011; elephants: Foley et al., 2001; mule deers: Saltz and White, 1991). The stress hormones increased with the onset of food deprivation (Selye, 1976). Only when the horses at Campo Pond received additional hay, in February, did the level of stress hormones decrease again, as in the study of Saltz (1988).

Finally, the social rank of the animals also affected the cortisol values (Wolter et al., 2014), as has been found in red deer (Balfanz et al., 2005). The highest-ranking mare at Campo Pond had the lowest body condition and, on average, a lower glucocorticoid metabolite concentration. This was similar to the highest-ranking stallion of the bachelor group in Augsburg, who also had the lowest level of stress hormones in his group (see also: Wolter et al., 2014).

CONCLUSION

In both groups the highest increase in stress hormones was found when the animals were fenced in smaller areas. The food supply influences the stress level of the animals only to a certain degree, while individual parameters such as the position in the hierarchy seemed to be more important. In their habitat use, the horses preferred open habitats, such as the grassland, and when kept in a forest, the open pine wood.

Acknowledgements

We would like to thank the owners of the Przewalski's horses, the Zoos of Augsburg and Munich and the Bundesanstalt für Immobilienaufgaben for the permission to observe the animals. We would also like to thank Knut Krueger for help with the statistics, Kate Farmer for language corrections and Juergen Heinze, Volker Stefanski, Maria Hanauer and Birgit Flauger for discussing the topic.

A case study on space, behaviour and well-being of horses (*Equus caballus*) in open stabling and open active stabling

Daniela Töpfer[a*], Riccarda Wolter[ab], Konstanze Krueger[ab]

[a] University of Regensburg, Biologie 1, Universitätsstraße 31, 93053 Regensburg, Germany

[b] University of Applied Sciences Nuertingen, Faculty Agriculture, Economics and Management, Department Equine Management, Neckarsteige 6-10, 72622 Nuertingen, Germany

* Corresponding author

Published in Current research in applied ethology (2014) 505, 252-254

SUMMARY

The behaviour of 112 horses was observed in open stabling and open active stabling. The horses showed less agonistic behaviour outside the feeding area in open active stabling as there is more space for each horse. Also, affiliative behaviour increases as a function of more space per horse, while agonistic behaviour with a risk of injury decreases. These results are independent of the husbandry system. For this reason, stabling with a large area per horse is recommended. Diseases over the previous twelve months were recorded and show skin diseases in open stabling, and hoof abscesses in open active stabling. Regarding feeding in open active stabling, it was found that horses fed with hay controlled by transponders showed more agonistic behaviour compared to horses fed using transponders but with access to hay ad libitum. Therefore, transponder feeding in combination with ad libitum feeding is recommended. Open stabling has the advantage of synchronized feeding.

MATERIALS AND METHODS

62 of the 112 horses were housed in 5 different open active stables using the activity concepts of the companies HIT and Schauer, while the other 50 horses lived in open stables with runouts. The group sizes ranged between 7 and 17 horses. The horses were of mixed genders and aged between 1 and 30 years, encompassing various breeds, sizes and colours. Nine hours of observations were recorded in each location. These hours were distributed in blocks of three hours at various times of day over three days within one week. The entire observation time comprised 90 hours.

RESULTS

The average space for each horse was larger in the open active stables than in the open stabling with runout. Horses in the bigger open stables showed significantly less agonistic behaviour outside the feeding area than horses living in smaller open stables (Spearman rang correlation test, $N = 54$, $rs = -0.295$, $p = 0.02$). Regarding agonistic behaviour in open stabling during feeding, horses in stables with exclusive transponder feeding displayed highly significantly more agonistic behaviour in the feeding area than horses fed using transponders but with access to hay ad libitum (Wilcoxon test for independent samples, $N1 = 25$, $N2 = 29$, $W = 560$, $p < 0.001$).

Regardless of the husbandry system, affiliative behaviour was shown more often in stables with a more space available (Spearman rang correlation test, $N = 112$, $rs = 0.297$, $p = 0.0014$). Within the framework of the study, diseases of the previous twelve month were recorded. We found that skin diseases occurred more often in open stables, with Icelandic horses being the most vulnerable. 9 out of the 62 horses living in the five open active stables suffered from abscesses in their hooves, a condition not found in the open stables. Comparing both types of stables, we found the following: The 50 horses in the open stables rolled 30 times and played 50 times in total, while the 62 horses in the open active stables rolled 16 times and played 20 times in total. Agonistic behaviour with risk of injury was shown 98 times in the open stables and 69 times in the open active stables.

CONCLUSION

This case study demonstrates that horses show less agonistic behaviour and increased affiliative behaviour as a function of the amount of space per horse, regardless of the husbandry system. Affiliative behaviour promotes the well-being of horses, and agonistic behaviour increases the risk of injuries. For this reason, large spaces are advisable both for open active stables and for open stables with runout. Our data indicate that open active stables are preferable to open stables because of the larger areas.

When feeding roughage, the combination of a feeding system with transponders and access to hay ad libitum reduces agonistic behaviour in the feeding area compared to a transponder only feeding system.

Acknowledgements

We thank Schauer Agrotronic GmbH for the financial support of this case study.

GENERAL DISCUSSION AND PERSPECTIVES

In this final chapter, I discuss the social behaviour and options for investigating social bonds in horses. Thereafter, I present the potential of the Przewalski's horse in biodiversity conservation in semi-reserves. Additionally, I discuss stress level as a potential indicator for the well-being of horses. As my project is intended to provide suggestions for the management of Przewalski's horses in semi-reserves, I present some practical guidelines on how to reduce stress level and how to monitor the well-being of the horses. Finally, I discuss perspectives for further analysis and research.

How to investigate social bonds of horses

For my PhD project, I investigated social behaviour and social bonds in Przewalski's horses kept in semi-reserves and in semi-wild living feral horses. In many mammalian species, the strength of social bonds between two individuals is usually measured by counting the number of mutual grooming events they display in a specified time (Silk et al., 2006). As this measure is very reliable, it is frequently used to assess social relationships in primates (Silk et al., 2003), but also in many other species (e.g. lions: Matoba et al., 2013; ravens: Orlaith and Bugnyar 2010). Mutual grooming is believed to form and strengthen social bonds (Arnold and Grassia, 1982; Van Dierendonck et al., 2009; Waring, 2003) and to play a key role in the social life of animals. Nevertheless, in horses mutual grooming occurs comparatively infrequently (Boyd and Houpt, 1994). Some individuals mutually groom once an hour on average, some only a few times a day, and others even less frequently or not at all (Boyd and Houpt, 1994). Therefore, I compared the literature on further parameters to investigate social bonds in horses (chapter 1). Surprisingly, the authors described various ways of measuring social bonds in horses (Cameron et al., 2009; Van Dierendonck et al., 1995; Christensen et al. 2002; Crowell-Davis et al., 1986; Feh, 1988). Therefore, I decided to assemble the data from other studies, including mutual grooming (Silk et al., 2006), friendly approaches (Cameron et al., 2009; Schneider and Krueger, 2012) and spatial proximity (Kimura, 1998), and to evaluate these data for comparability and combinability (chapter 2).

The best practice appears to be to collect data on the frequencies of mutual grooming by continuous recording, as grooming frequencies differ between various species. In horses they are low and show both individual and seasonal variation (Boyd and Houpt, 1994). It should be kept in mind that the frequency and duration of mutual grooming could be influenced by external factors such as parasite loads, temperature and insect pressure (Wells and von Goldschmidt-Rothschild, 1979).

However, the studies that broadened their data sets by using measurements of spatial proximity, for example when horses are grazing and resting (Heitor et al., 2006; Kimura, 1998; Sigurjónsdóttir et al., 2003), show huge variations in the distance measured between two individuals and the observation intervals (Cameron et al., 2009; Christensen et al., 2002; Crowell-Davis et al., 1986; Van Dierendonck et al., 1995).

In contrast to grooming behaviour, for Przewalski’s horses kept in semi-reserves it is not possible for human observers to collect the data on spatial proximity by continuous sampling. Video analysis and individual recognition of animals from pictures is very difficult in Przewalski’s horses, as they are very hard to distinguish from each other. Moreover, the different habitat types the horses live in, including dense forests, make video observations very difficult, so the animals would need to be equipped with GPS collars. This would involve high risks because the animals would need to be immobilized for collars to be fitted, and most of the horses’ owners do not permit immobilization unless it is anesthesia on veterinary grounds. Uncoordinated movements, when animals are still dizzy while getting up, can result in injury (Walzer et al., 2006). In addition to all these reasons, continuous recording of spatial distributions is impossible because of the permanent rearrangement of group members. The appropriate recording method for the spatial proximity in Przewalksi’s horses therefore appears to be scan sampling with counting intervals of 8-10 minutes, which guarantees a certain independence of the samples (Christensen et al., 2002).

Another disadvantage of using spatial proximity is the definition of proximity itself. The distance of two body lengths is used most often (Cameron et al., 2009; Christensen et al., 2002; Van Dierendonck et al., 1995), but it is unclear whether horses would interpret this closeness as friendly and social, or whether friendly and social proximity begins nearer or further away. Combining spatial proximity measurements with established measures of animal welfare, such as heart rate (Kovács et al., 2014), heat rate variability (Kovács et al., 2014), stress parameters (Möstl and Palme, 2002), immune parameters (Passantino et al., 2010) and other behaviours (Veissier and Boissy, 2014), may provide information on the sensation horses feel when they are close to others. It remains to be seen whether pairs of horses vary in the distances at which they feel most comfortable, and whether this distance is affected by factors such as age, sex, breed, and relationship.

Besides spatial proximity, friendly approaches are also considered to be a good additional parameter to broaden social relationship analysis (Cameron et al., 2009; Schneider and

Krueger, 2012). The only disadvantage is the argument that mutual grooming requires a friendly approach, and that mutual grooming and friendly approach data could be considered duplicated because they overlap. However, this is also the case for mutual grooming and spatial proximity: to groom each other animals must stay in close proximity. Nevertheless, it is possible to collect independent data sets for friendly approaches and mutual grooming by only including approaches that are not followed by grooming in the approach data.

My findings support the hypothesis that the most reliable option for investigating social bonds in horses is the observation of a combination of proactive behaviours, in this case, mutual grooming and friendly approaches. These behaviours are characterised by the horse's initiative and are more strongly correlated than spatial proximity and grooming (see chapter 2). Grooming and approaches are both proactive behaviours, can be measured reliably using the same observation technique (continuous sampling), and can be observed easily in the field without the need for transponders.

Regarding chapter 2, a follow up study would be necessary to investigate whether other proactive behaviours, such as grazing and resting together, could be combined with the mutual grooming data to generate an even more reliable, comprehensive data set to analyze social bonds in horses more precisely.

The social behaviour of Przewalski's horses in semi-reserves and what we can learn from it

Five different groups of Przewalski's horses and the six harems of feral-living horses did not differ in their social behavior in the frequency of mutual grooming, friendly approaches, spatial proximity, or aggressions. This supports Christensen et al. (2002), who found social behaviour frequencies to be similar in Przewalski's and domestic horses. In chapter 3 of my thesis, I focus on the social behaviour of the stallions and the role of the alpha male when the home range is expanded into a novel area. It has been reported in several species that alpha animals reduce aggression level within the group, ensure group stability, and provide leadership and decision making in potentially dangerous situations (primates: Henzi et al., 1998; Aureli and de Waal, 2000; Flack et al., 2005a; Flack et al., 2005b; Flack et al., 2006; King et al., 2008; hyenas: Zabel et al., 1992; Smith et al., 2010; deer: Jennings et al., 1981; oryx: Walther, 1991 and horse: Feist and McCullough, 1976). In the group of male Przewalski's horses I also demonstrated the importance of the alpha male, showing that he has a special role in the group and that his behaviour changes completely in different environments.

When bachelor groups or harem groups move, the alpha male usually is the last animal in line, as in harems stallions herd their mares from behind (Tilson et al., 1988; Feist and McCullough, 1976). Herding maintains group cohesion when other groups approach or when some horses separate from their group. However, during the first period on the enlarged area, the alpha male of the group led the group from the front instead of herding from behind. Moreover, he was the only animal in the group to experience an increase in stress hormones, and the group had a higher connectedness in this situation. To sum up, the alpha male appears to have a clear and defined position in the group. The management of grazing projects should carefully consider the alpha animals' social role before removing them for breeding purposes. A follow up study could investigate how quickly the alpha position is reestablished when the alpha male is removed from the group. This would be especially interesting in groups with a similar composition to that in Augsburg, where all horses apart from the alpha male were immature. With such a group composition an alpha male is urgently needed for decision making and leadership.

Further studies may also investigate whether, and to what degree, young stallions learn from the alpha male, and how important an alpha animal is for group stability, reducing the aggression level, and decision making.

The potential of the Przewalski's horses for conservation in semi-reserves

In chapter 4, I focused on the habitat use of Przewalski's horses in semi-reserves. The results support the findings of earlier studies (Steidele, 2011) that Przewalski's horses prefer open areas with grassland and avoid forests if possible. However, in contrast to earlier studies, the bachelor group observed in the present study did not have a free choice of habitats throughout the year. In summer it was restricted to a pine forest, while the mare group at Campo Pond could roam on open land and forest areas during the whole year. Furthermore, I investigated possible preferences for certain types of forest in the bachelor group, as the pine forest in their enclosure offered various habitat types with differences in the density of the vegetation. In accordance with an earlier study (Roth, 2002) this group preferred open forest to thick pine forest. Moreover, they spent a lot of their resting time under the spruces in the northern part of their enclosure. Some horses may have preferred to rest in the shade of single trees, as described by Ihle (1984) and Jaworowska (1976). Moreover, the vegetation in the section containing the spruces was very poor compared to the rest of the enclosure. As the insect pressure is lower on wind exposed areas with poor vegetation, some horses prefer sparse areas during resting times (Keiper et al., 1980; Keiper and Berger; 1992; Duncan, 1992).

Furthermore, Przewalski's horses prefer to rest in areas which they also prefer for food intake (Kolter et al., 1999). This was consistent with my study, as the mare group at Campo Pond used the same areas for both, resting and feeding. The same result was found for the stallion group during the winter months, when the horses had the possibility to graze on the open area. A detailed overview about the monthly habitat use for both horse groups can be found in the appendix (Figure A3 and A4).

Stress evaluation of Przewalski's horses in semi-reserves and guidelines for semi-reserve management

The suitability of the semi-reserve in Augsburg for keeping Przewalski's horses is questionable, as its pine forest and the heathland area with long old grass are vegetation types avoided by Przewalki's horses if they have a choice (Kolter et al., 1999). I studied the stress levels the horses may be exposed to (Yarnell et al., 2015; Erber et al., 2013) by collecting faecal samples and measuring the glucocorticoid (stress hormone) concentration in the individual animals. This allowed me to compare the stress level in the different semi-reserves and evaluate the horses' reactions to different management interventions.

The literature presents plenty of information on stress responses in domestic horses to various influences and management interventions, e.g. changes in husbandry system (Erber et al., 2013), social separation during training in young horses (Hartmann et al., 2011), group transfers of mares (Cassandra et al., 2013), short-term isolation of mares (Strand et al., 2002) and general housing conditions (Visser et al., 2008). However, there are few studies on the stress responses of Przewalski's horses. Przewalski's horses are an endangered species and their survival is of great importance. Semi-reserves are increasingly being used for breeding as it is easier to control the birthrate and the genetics of the offspring than in the wild. As it is known that an elevated stress level has negative effects on the fertility of animals (Córdova-Izquierdo et al., 2014), their well-being should be of primary importance to zoos and reserve managers, and hazardous management interventions should be avoided whenever possible.

Therefore, I focused on whether management interventions cause a significant increase in faecal glucocorticoid, which is an indicator of high stress levels. In chapter 4, I demonstrated that being corralled had the strongest effect on stress levels. Both groups showed massive behavioural responses to being restricted to a small enclosure. They became restless and walked continuously along the fence, and the number of faecal glucocorticoid metabolites increased significantly. In contrast, changes in group composition did not significantly affect the stress level of the horses. Changes in group composition appear to be perceived as natural, common occurences. In nature, 80-90 % of maturing offspring leave their natal group (Berger, 1986), some mares switch harem groups, and young stallions leave their natal harem to establish their own harems or to join bachelor groups (Houpt and Boyd, 1994).

Interestingly, being released onto the steppe after one year in an enclosure did not cause an increase in stress hormones according to Souris et al. (2007). Horses being reintroduced into the wild may show lower aggression rates as more space is available (Flauger, 2010) and they can avoid horses of other groups. This is supported by Hogan et al. (1988), who found Przewalski’s horses to be more aggressive towards one another when moved from pasture to a small enclosure.

Furthermore, the mare group at Campo Pond showed different stress responses in different observation months, as in the study of Steidele (2011). The question remains whether these changes are triggered by changes in temperature or food supply, as these two variables usually change together. It has been shown in other animals that food deprivation may lead to increased cortisol (horses: Steidele, 2011; elephants: Foley et al., 2001; mule deers: Saltz and White, 1991), and stress hormones decreased when the horses received additional hay in the present study. It therefore seems that the food supply, or at least a combination of food supply and climatic conditions, influence stress levels.

Finally, individual factors also affected the cortisol values in my study. The highest-ranking mare at Campo Pond and the highest-ranking stallion of the bachelor group in Augsburg had the lowest glucocorticoid metabolite concentration when under regular management conditions.

Perspectives for Przewalski’s horse management

Regarding the management of Przewalski’s horses in semi-reserves, the results of the present study indicate that the horses should only be fenced in under exceptional circumstances. Supplementary feeding of older or weakened animals is highly recommended during months of low food supply, as increased stress could weaken the animals further (Kelley, 1980). Ad libitum feeding areas, which can only be entered by animals which need additional food, may be a good solution. Horses can then be fed selectively, can enter and leave the area of their free will, and are unlikely to be victims of food related aggressions (see also chapter 5).

In terms of well-being, naïve young horses that go from a zoo to a semi-reserve should have their body condition score especially closely monitored during times of short food supply, as they sometimes need to learn from older or more experienced horses which plants are edible (Krueger et al., 2014). The body condition can change quickly during cold months, so regular monitoring, at least once every two weeks, is necessary to ensure that changes in the well-being of the horses are recognized quickly.

Group composition and social bonds should always be taken into account when horses are taken out of groups, especially as the stability of groups and of the hierarchy can have a strong influence on the horses' aggression and stress levels (Sapolsky, 1992). Alpha-animals should not be removed from the groups if it can be avoided as they hold leadership positions and depending on the group composition they may not be replaced in the short run.

As the stress level and the aggression level increase with reduced space, semi-reserves need to offer as much space as possible, particularly when the horses split themselves in subgroups. Regarding the suitability of habitats, in chapter 4 my study indicates that horses prefer open areas with grass, but Przewalski's horses can be kept in densely wooded areas as long as sufficient food for all the horses is available, especially when the woods contain long, old grass.

SUMMARY

In recent years, Przewalski's horses have been increasingly kept in semi-reserves. However, there are only few studies on their behaviour and their ability to adapt to management interventions. In the main part of my dissertation, I focus on investigating the animals' behaviour in different semi-reserves with varying habitats and living spaces. In addition, I investigate the horses' behaviour during various management interventions and analyse the ensuing changes in stress levels. Another aspect of my dissertation is the study of social behaviour in Przewalski's horses. I investigate the parameters that should be used to demonstrate social bonds between individuals and assess which data provide the most meaningful results.

In the commentary to **chapter 1,** several studies investigating social bonds in horses are discussed. Comparing the various studies, it is striking that no homogeneous analyses or evaluation criteria exist. While some authors only consider social grooming, others include data on the spatial proximity of the individuals in their evaluations, and various definitions of proximity can also be found in the literature. Additionally, some authors use friendly approaches between individuals as a further parameter when investigating the social bonds.

Continuing with this theme, in **chapter 2** I investigate the social behaviour of the horses and compare various analysis methods. I show that proactive behaviour, such as friendly approaches, is a good alternative to spatial proximity when investigating social bonds between group members, and is also useful for expanding the often very small data sets of mutual grooming in horses. Comparing Przewalski's horses with wild living horses, I found no significant differences in the social behavior and the frequency of social interactions, regardless of group size, group composition, habitat, and individual parameters such as age and gender.

In **chapter 3,** I investigate the behaviour of a Przewalski's horse group when exploring a new area of their enclosure. Their behaviour changed, showing less resting and more feeding. Furthermore, the animals maintained greater distances from each other, and the alpha male, instead of herding the group from behind, led the group around the new area and walked in front of the other group members. Moreover, he showed a substantial increase in stress level during the first day.

A general comparison of the behaviour of the Przewalski's horses in different semi-reserves is provided in **chapter 4**. In it, the habitat choice of the animals and their reactions to various management interventions are investigated. It is shown that Przewalski's horses prefer

open grassland to dense woods, although keeping Przewalski's horses in a pine forest does not influence the animals' stress level. In contrast to habitat, food range, and changes in the group composition, which do not appear to change stress levels, individual factors, such as the hierarchy, influence the glucocorticoid level of the animals significantly. The largest increases in stress hormones were demonstrated when the horses were temporarily confined in smaller areas.

The importance of the available space is also discussed in **chapter 5,** where it is shown that horses show less aggressive behaviour when more space is provided. In contrast, the husbandry system does not influence the animals' aggression, but the way of feeding can additionally reduce agonistic behaviour, for example if food is offered ad libitum.

In summary, the results of this study provide indications for the optimization of keeping Przewalski's horses in semi-reserves. The animals can adapt themselves to the environment and thrive in habitats which do not correspond to their original steppe-like home. Nevertheless, the semi-reserves should provide sufficient grassland, as the horses prefer this type of habitat. General speaking, any types of habitat can only offer a suitable living space if the food range is sufficient for the number of horses. Otherwise, and especially during could winter months, supplementary feeding is necessary according to the body condition of the animals. This is particularly important for older, weakened, or very young animals, which are still adapting to life in the semi-reserve. Without sufficient food, stress hormones can increase and negatively influence the well-being of the horses. The same is true for management interventions: restricting the animals to small enclosures, for example, can adversely affect the horses' well-being and should be only done if absolutely necessary.

Targeted behaviour observations allow the animals that have a special meaning for the group to be identified, and these should not be taken out of the group unless it is unavoidable, as young and unexperienced horses orientate themselves on those animals. This is especially true for the alpha male in a bachelor group, as these groups are often composed of young horses and the alpha-male provides the necessary stability and experience. Social bonds between individuals can be investigated by observing friendly and proactive behaviour, and social grooming and friendly approaches yield suitable data for such analysis.

ZUSAMMENFASSUNG

Przewalski-Pferde werden in den letzten Jahren zunehmen in Semi-Reservaten gehalten. Dennoch gibt es nur wenige Studien über ihr Verhalten und ihre Anpassungsfähigkeit an unterschiedlichste Management-Eingriffe. Im Hauptteil meiner Doktorarbeit konzentrierte ich mich auf die Untersuchung und das Verhalten der Tiere in verschiedenen Semi-Reservaten mit unterschiedlichen Habitaten und Lebensräumen. Zudem untersuchte ich das Verhalten der Pferde während diverser Management-Eingriffe. Dabei wurde zugleich der Stresszustand der Tiere untersucht. Einen weiteren Aspekt meiner Doktorarbeit stellte die Untersuchung des Sozialverhaltens der Przewalski-Pferde dar. Hierbei analysierte ich, mit welchen Parametern sich soziale Banden zwischen Individuen am besten aufzeigen lassen können und welche Daten die aussagekräftigsten Ergebnisse liefern.

In der Literaturzusammenfassung von **Kapitel 1** werden verschiedene Studien diskutiert, die soziale Banden zwischen Pferden untersuchen. Beim Vergleich der diversen Studien wurde schnell deutlich, dass es hierbei keine einheitlichen Analyse- und Auswertungskriterien gibt. Während einige Autoren nur die gegenseitige Fellpflege berücksichtigen, beziehen andere Autoren räumliche Nähe der Individuen zueinander in ihre Auswertung mit ein. Doch auch hier gibt es elementare Unterschiede in der Literatur zur Definition von Nähe. Wieder andere Studien verwenden freundliche Annäherungen zwischen Individuen als zusätzlichen Datensatz für die Bestimmung ihrer sozialen Bande.

In **Kapitel 2** untersuchte ich aufbauend auf das vorangegangene Kapitel das Sozialverhalten der Pferde und verglich die unterschiedlichen Analysemethoden. Dabei zeigte ich, dass für die Bestimmung sozialer Banden zwischen Gruppenmitgliedern proaktive Verhaltensweisen, wie etwa freundliche Annäherungen, eine gute Alternative zur räumlichen Nähe darstellen, um damit die oft geringen Datensätze zur sozialen Fellpflege bei Pferden zu erweitern. Beim Vergleich von Przewalski-Pferden und verwilderten Hauspferden konnte kein Unterschied im Sozialverhalten und in der Frequenz sozialer Interaktionen festgestellt werden, unabhängig von der Gruppengröße, der Gruppenzusammensetzung, dem Lebensraum und individuellen Parametern wie Alter und Geschlecht.

In **Kapitel 3** untersuchte ich das Verhalten einer Przewalski-Pferd Gruppe beim Erkunden einer neuen Fläche. Das Verhalten der Tiere änderte sich beim Erkunden des unbekannten Gehegeabschnitts. Sie zeigten deutlich weniger Ruheverhalten und fraßen dagegen mehr als sonst. Zudem hielten die Tiere größere Abstände zueinander. Vor allem beim Alpha-Hengst wurden große Unterschiede im Verhalten festgestellt, statt die Herde wie sonst von hinten

anzutreiben, übernahm er auf der neuen Fläche die Führung und zeigte im Vergleich zu den anderen Tieren am Tag der Erweiterung einen starken Anstieg seines Stresslevels.

Einen allgemeinen Vergleich des Verhaltens von Przewalski-Pferden in unterschiedlichen Semireservaten liefert **Kapitel 4**. Darin wurden die Habitatnutzung der Tiere erforscht und ihre Reaktionen auf diverse Management-Eingriffe analysiert. Es bestätigte sich, dass Przewalski-Pferde offene Graslandflächen gegenüber dicht bewachsenen Wäldern bevorzugen. Dennoch geht die Haltung von Przewalski-Pferden in einem Kiefernwald nicht mit einem erhöhten Stresslevel der Tiere einher. Im Gegensatz zum Lebensraum, zum Futterangebot und zu Veränderungen in der Gruppenzusammensetzung beeinflussten individuelle Parameter wie die Hierarchie die Stresshormonkonzentration der Pferde deutlich stärker. Der deutlichste Anstieg der Stresshormonkonzentration erfolgte in beiden Gruppen, als diese zeitweise in kleineren Arealen eingesperrt wurden.

Mit den Reaktionen von Pferden auf das vorhandene Platzangebot beschäftigt sich auch **Kapitel 5**. Hier wurde demonstriert, dass Pferde weniger aggressive Verhaltensweisen zeigen, wenn ihnen mehr Platz zur Verfügung steht. Das Haltungssystem beeinflusst die Aggressivität der Tiere dagegen nicht, allerdings kann die Art der Fütterung agonistische Verhaltensweisen zusätzlich minimieren, beispielsweise indem Futter ad libitum angeboten wird.

Zusammenfassend liefern die Ergebnisse dieser Studie Hinweise für die Optimierung der Haltung von Przewalski-Pferden in Semireservaten. Die Tiere können sich gut an ihre Umgebung anpassen und kommen auch mit Habitaten zurecht, die ihrem ursprünglichen steppenähnlichen Lebensraum nicht entsprechen. Dennoch sollten in den Semireservaten ausreichend große, offene Graslandflächen zur Verfügung stehen, da die Tiere diese Bereiche bevorzugen. Generell gilt, dass alle Lebensraumtypen für die Pferde nur solange einen adäquaten Lebensraum bieten, solange die Futtermenge ausreichend für die Anzahl der Pferde ist. Andernfalls muss besonders während der kalten Wintermonate eine Zufütterung der Tiere erfolgen, über die jeweils in Abhängigkeit von der körperlichen Verfassung der Tiere entschieden werden muss. Dies gilt besonders für ältere, geschwächte oder sehr junge Tiere, die sich erst noch an das Leben in den Semireservaten gewöhnen müssen. Andernfalls kann es zu einem Anstieg der Stresshormone bei den Tieren kommen, die deren Wohlergehen negativ beeinflussen können. Gleiches gilt für Management-Eingriffe wie das Einsperren der Tiere, die das Wohlergehen der Pferde ebenfalls stark beeinflussen können und sollten nur in Ausnahmefällen stattfinden.

Anhand gezielter Beobachtungen des Verhaltens können Tiere identifiziert werden, die für die Gruppe eine besondere Bedeutung haben und nach Möglichkeit nicht aus der Gruppe entfernt werden sollten, da sich besonders junge und unerfahrene Pferde häufig an ihnen orientieren. Dies gilt vor allem für den Alpha-Hengst in einer Junggesellengruppe, die sich häufig aus jungen, unerfahrenen Pferden zusammensetzt. Der Alpha-Hengst sorgt in einer solchen Gruppe für die nötige Stabilität und Erfahrung und seine Rolle kann nur schwer von einem jungen Pferd übernommen werden. Freundschaften zwischen den Individuen lassen sich dabei anhand von Beobachtungen der freundlichen, proaktiven Verhaltensweisen analysieren. Dabei eignen sich vor allem die gegenseitige Fellpflege und freundliche Annäherungen, um Freundschaften zwischen Individuen zu analysieren.

PUBLICATIONS AND PRESENTATIONS

Publications

Töpfer D, Wolter R, Krüger K (2014) A case study on space, behaviour and well-being of horses (*Equus caballus*) in open stabling and open active stabling. Current research in applied ethology (KTBL-Schrift) 505, 252-254

Wolter R, Pantel N, Möstl E, Küger K (2014) Das Verhalten von Przewalski Pferden in Semireservaten. Konferenzband 1. Pferdetage Baden-Württemberg. Matthaes Medien, Stuttgart, S. 35

Wolter R, Pantel N, Stefanski V, Möstl E, Krüger K (2014) The Role of an alpha animal in changing environmental conditions. Conference Proceedings 10th International Equitation Science Conference 1, 100

Wolter R, Pantel N, Stefanski V, Möstl E, Krüger K (2014) The Role of an alpha animal in changing environmental conditions. Physiology & Behavior 133, 236-243

Wolter R, Krueger K (2015) Influencing factors on grooming behaviour in wild living horses. Current research in applied ethology (KTBL-Schrift) 510, 242-248

Wolter, R., & Krueger, K. (2015) The analysis of social bonds in feral horses. Proceedings of the 3rd International Equine Science Meeting 1, 24

Wolter R, Stefanski V, Krüger K (2018) Parameters for the analysis of social bonds in horses. Animals 8(11), 191

Manuscripts submitted

Wolter R, Pantel N, Krüger K. Comparison between the responses of two Przewalski’s horse groups to semi–reserve management in different habitat types. Submitted to Basic and Applied Ecology

Presentations at meetings and workshops

Wolter R, Pantel N, Stefanski V, Möstl E, Krüger K (2013) Die Rolle des Alpha-Hengstes in einer Przewalski Bachelor-Gruppe beim Erkunden einer neuen Fläche in einem Semi-Reservat. (Poster) 6th Göttinger Horse Days, Göttingen, Germany

Wolter R, Pantel N, Stefanski V, Möstl E, Krüger K (2014) Das Verhalten von Przewalski Pferden in Semireservaten. (Talk) 1st Horse Days Baden-Württemberg, Nürtingen, Germany

Wolter R, Pantel N, Stefanski V, Möstl E, Krüger K (2014) The behaviour of Przewalski's horses in semi-reserves. (Talk) 4th International Symposium of Przewalski's horses, Hanau, Germany

Wolter R, Pantel N, Stefanski V, Möstl E, Krüger K (2014) The Role of an alpha animal in changing environmental conditions. (Poster) 10th International Equitation Science Conference, Bredsten, Denmark

Töpfer D, Wolter R, Krüger K (2014) A case study to space, behavior and well-being of horses (*Equus caballus*) in open stables and open active stables (Poster) 46th International Meeting of Applied Ethology, Freiburg, Germany

Wolter R, Krüger K (2015) The analysis of social bonds in feral horses. (Talk) 3rd International Equine Science Meerting, Nürtingen, Germany

Wolter R, Krüger K (2015) Die Analyse sozialer Bindungen bei verwilderten Pferden. (Talk) 47th International Meeting of Applied Ethology, Freiburg, Germany

Wolter R, Pantel N, Krüger K (2016) The habitat use of Przewalski's horses in semi-reserves. (Talk) 6th International Symposium of Przewalski's horses, Hanau, Germany

Wolter R, Kürger K (2016) The analysis of social bonds in feral horses. (Poster) 8th European Conference of Behavioural Biology, Vienna, Austria

Wolter R (2018) Stressphysiologie und Analysemethoden. (Talk) Meeting „Stress Workshop", Nürtingen, Germany

INTERNATIONAL COOPERATION

- University of Applied Science Nürtingen-Geislingen, Faculty Agriculture, Economics and Management, Department Equine Management
- Prof. Dr. Volker Stefanski, University of Hohenheim, Institute of Animal Husbandry and Animal Breeding
- Prof. Dr. Rupert Palme, University of Veterinary Medicine, Vienna, Institute of Biochemistry, Department of Biomedical Sciences/Biochemistry
- Prof. Dr. Daniel Rubenstein, University of Princeton, Princeton Environmental Institute, Department Ecology and Evolutionary Biology
- Katharine Farmer, Centre for Social Learning & Cognitive Evolution, School of Psychology, University of St Andrews, St Andrews, Scotland
- Dr. Waltraut Zimmermann & Dr. Lydia Kolter, Zoo Köln
- Dr. Anne Berger, Leibniz Institute for Zoo and Wildlife Research
- Norbert Pantel, Landschaftspflegeverband Stadt Augsburg e.V.
- Tierpark München, Tiergarten Nürnberg, Zoo Augsburg
- Bundesanstalt für Immobilienaufgaben, Bundesforst
- Landschaftspflegeverband Mittelfranken
- Nationalparkverwaltung Bayrischer Wald

THANK YOU …

Prof. Dr. Konstanze Krüger
... for supervising my work
... for the support at all times
... for your encouragement whenever I needed it

Prof. Dr. Jürgen Heinze
... for giving me the opportunity to work in your group
... for agreeing to act as a mentor of my thesis

Prof. Dr. Volker Stefanski
... for giving me the opportunity to collaborate with you
... for agreeing to act as a mentor of my thesis

Knut Krüger
... for helping me with all sorts of statistical issues and all kinds of computer problems

Isabell Marr & Laureen Esch
... for your help with problems and good advices
... for our nice trips to Vienna, Freiburg & Göttingen

Kate Farmer & Susan Price
... for correcting my English and for improving my knowledge of the English language

The Owners of the Przewalski's horses, The Landschaftspflegeverband Stadt Augsburg e.V., the Bundesforst and the Nationalparkverwaltung Bayrischer Wald
... for allowing me to observe the animals

Maria and Amerigo Nota
... for providing me with test horses in Italy

Waltraut Zimmermann & Lydia Kolter
... providing me with literature and good advises

The Laboratory Group of Erich Möstl and Rupert Palme
... for doing the first analyses of the faecal samples

Andrea Dobler
... for allowing me to analyse the samples in your laboratory

Norbert Pantel
... for all advise during my studies and your helpfulness
... for nice evenings at the 3 Königinnen at Augsburg

Marion Beil
… … for giving me the possibility to store the faecal samples in your freezer and for good conservations

Birgit Flauger, Maria Hanauer & Verena Fröhlich
... for discussing the topic

Mirjam Miorin-Bellermann, Lena Abel, Ann-Cathrin Grudacker, Kristina Hinz, Gabriela Schmid, Felix Käfer, Serena Sennet, Tamara Willig and Florian Oberparleiter
… for your help with the horse observations and data recordings

Marco
… for your love and your support

My parents and my sister
… for your support during my entire life

Uni Nürtingen Förderung & Schauer Agrotronic GmbH
… for the financial support

Cooperation partners
… for your help

… to all the people I didn't mention by name but who helped me with conducting test series and with whom I discussed my work and got helpful comments and ideas

ERKLÄRUNG

Hiermit erkläre ich, die vorliegende Dissertation selbständig und ausschließlich unter der Verwendung der angegebenen Quellen und Hilfsmittel angefertigt zu haben. Diese Arbeit wurde bisher weder einer Prüfungsbehörde vorgelegt noch veröffentlicht.

Hanau, im Juni 2018

Riccarda Wolter

REFERENCES

Abel L (2015) Does a ‘smart’ stallion’s band contain more reproductive mares with foals at foot than the band of an ‘aggressive’ stallion? Bachelor Thesis, University of applied sciences Nürtingen-Geislingen, Nürtingen

Altmann M (1951) The study of behavior in a horse-mule group. Sociometry 14, 351-354

Anders K, Mrzljak J, Wallschläger D, Wiegleb G (2004) Handbuch Offenlandmanagement am Beispiel ehemaliger und in Nutzung befindlicher Truppenübungsplätze. Springer, Berlin

Araba BD, Crowell-Davis SL (1994) Dominance relationships and aggression of foals (*Equus caballus*). Appl Anim Behav Sci 41, 1-25

Arnold GW, Grassia A (1082) Ethogram of agonistic behaviour for thoroughbred horses (1982) Appl Anim Ethol 8, 5-25

Arnold W, Ruf T, Reimoser S, Tataruch F, Onderscheka K, Schober F (2004) Nocturnal hypometabolism as an overwintering strategy of red deer (*Cervus elaphus*). Am J Physiol Regul Integr Comp Physiol 286, 174–181

Arnold W, Ruf T, Kuntz R (2006) Seasonal adjustment of energy budget in a large wild mammal, the Przewalski horse (*Equus ferus przewalskii*) II. Energy expenditure. The J of Exp Biol 209, 4566-4573

Aureli F, van Schaik C, van Hooff J (1989) Functional aspects of reconciliation among captive long-tailed macaques (*Macaca fascicularis*). Am J Primatol 19, 39–51

Aureli F, de Waal FBM (2000) Natural conflict resolution. University of California Press, Berkley

Balfanz F, Beiglböck C, Huber S, Palme R, Arnold W (2005) Seasonal variation of cortisol secretion in free-ranging Red Deer. XXVIIth IUGB Congress, Hannover, 28.08.-03.09.2005

Balfanz F (2005) Quantifizierung der Stressbelastung beim Rothirsch: Auswirkung von Stoffwechselaktivität und sozialen Hierarchien. Abschlussbericht, Deutsche Wildtierstiftung

Barton RA (1993) Sociospatial mechanisms of feeding competition in female olive baboons, *Papio Anubis*. Animal Behav 46, 791-802

Bartsh SS, Johnston SD, Siniff DB (1992) Territorial behaviour and breeding frequency of male Weddell seals (*Leptonychotes weddellii*) in relation to age, size and concentrations of serum testosterone and cortisol. Can J of Zoology 70, 680-692

Beerda B, Schilder MB, van Hooff JA, de Vrie HW, Mol JA (1999) Chronic stress in dogs subjected to social and spatial restriction I. Behavioural Responses. Physiol Beha 66, 233-242.

Beery AK, Kaufer D (2015) Stress, social behavior, and resilience: Insights from rodents. Neurobiol Stress 1, 116-127

Bekoff M (1974) Social Play and Play-Soliciting by Infant Canids. Am Zool 14, 323-340.

Benirschke K, Malouf N, Low RJ, Heck H (1965) Chromosome complement: differences between *Equus caballus* and *Equus przewalski*, Poliakoff. Science 148, 382-383

Berger J (1977) Organizational systems and dominance in feral horses in the Grand Canyon. Behav Ecol Sociobiol 2, 131–146

Berger J (1987) Reproductive fates of dispersers in a harem-dwelling ungulate, wild-horses. In: Chepko- Sude D, Hapin Z (Eds) Mammalian Dispersal Patterns: The Effects of Social Structure on Population Genetecis. The university of Chicago Press, Chicago, pp. 41-54

Berger J (1986) Wild horses of the great basin. University of Chicago Press, Chicago

Berger A, Scheibe KM, 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. Appl Anim Behav Sci 64, 1–17

Berger A, Scheibe KM, Wollenweber K, Patan B, Schnitker P, Herrmann C, Budras KD (2006) Jahresrhythmik von Aktivität, Nahrungsaufnahme, Lebendmasse und Hufentwicklung bei Wild- und Hauspferden in naturnahen Lebensbedingungen. Cur Res Appl Ethol 448, 137–146

Biben M (1998) Squirrel monkey play fighting: making the case for a cognitive training function for play. In: Bekoff M, Byers JA (Eds) Animal Play. Evolutionary, Comparative, and Ecological Perspectives. Cambridge University Press, Cambridge, pp. 161-182

Bloomsmith MA, Laule GE, Alford PL, Thurston RH (1994) Using training to moderate chimpanzee aggression during feeding. Zoo biol 13, 557-366.

Bogner H, Grauvogl A (1984) Verhalten landwirtschaftlicher Nutztiere. Eugen Ulmer, Stuttgart

Bonanni R, Cafazzo S, Valsecchi P, Natoli E (2010) Effect of affiliative and agonistic relationships on leadership behaviour in free-ranging dogs. Anim Behav 79, 981–991

Bouman I, Bouman J (1990) Report on the semi-reserves project IUCN/WWF no. 3077. Proceedings of the 5th International Symposium on Przewalski’s Horse. Zool. Garten Leipzig 1, 184-190

Bouman I, Bouman J (1994) The history of Przewalski’s horse. 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, pp. 5-38

Bouman J, Bos H (1979) Two symptoms of inbreeding depression in Przewalski horses living in captivity. In: DeBoer LEM, Bouman J, Bouman I (Eds) Genetics and hereditary diseases of the Przewalski Horse. Foundation for the Preservation and Protection of the Przewalski Horse, Rotterdam, pp. 111-117

Bourjade M, Thierry B, Maumy M, Petit O (2009) Decision-making in Przewalski horses (*Equus ferus przewalskii*) is driven by the ecological contexts of collective movements. Ethol 115, 321- 330

Boyd L (1988) The behaviour of Przewalski’s horses. Dissertation, Cornell University, Ithaca

Boyd L (1991) The behaviour of Przewalski's horses and its importance to their management. Appl Anim Behav Sci 29, 301-318

Boyd L, Carbonaro DA, Houpt KA (1988) The 24-hour time budget of Przewalski horses. Appl Anim Behav Sci 21, 5–17

Boyd L, Houpt KA (1994) Przewalski's Horse. The history and biology of an endangered species, State University of New York Press, Albany

Boyd L, Houpt KA (1994) 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, pp. 195-227

Brinkmann L, Gerken M, Hambly C, Speakman, JR, Riek, A (2014) Saving energy during hard times: Energetic adaptations of Shetland pony mares. J Exp Biol 217, 4320-4327

Brockway DG, Lewis CE (2003) Influence of deer cattle grazing and timber harvest on plant species diversity in a longleaf pine bluestem ecosystem. Forest Ecol Manag 175, 49-69

Budras KD, Scheibe K, Patan B, Streich WJ, Kim K (2001) Laminitis in Przewalski horses kept in a semireserve. J Vet Sci 2, 1–7

Bullock JM, Pakeman RJ (1996) Grazing of lowland heath in England: Management methods and their effects on heathland vegetation. Biol Conserv 79, 1-13

Bunzel-Drüke M (1997) Großherbivore und Naturlandschaft. Schr.-R. f. Landschaftspfl. u. Natursch. 54, 109-128

Bunzel-Drüke M (2008) Wilde Weiden – Praxisleitfaden für Ganzjahresbeweidung in Naturschutz und Landschaftsentwicklung. Arbeitsgemeinschaft Biologischer Umweltschutz im Kreis Soeste. V., Bad Sassendorf-Lohne

Buwalda B, Scholte J, de Boer SF, Coppens CM, Koolhaas JM (2012) The acute glucocorticoid stress response does not diffenrentiate between rewarding and aversive social stimuli in rats. Horm Behav 61, 218-226

Byrne RW, Whiten A, Henzi SP (1990) Social relationships of mountain baboons: Leadership and affiliation in a non-female-bonded monkey. Am J Primatol 20, 313–329

Cameron EZ, Setsaas TH, Linklater WL (2009) Social bonds between unrelated females increase reproductive success in feral horses. P Natl Acad Sci USA 106, 13850-13853

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

Clutton-Brock TH, Greenwood PJ, Powell RP (1976) Ranks and relationships in Highland ponies and Highland Cows. Z Tierpsychol 41, 202–216

Córdova-Izquierdo A, Villa-Mancera A, Olivares Pérez J, Sánchez-Aparicio P (2014) Environmental stress effect on animal reproduction. Open J Anim Sci 4, 79-84

Crofoot MC, Rubenstein DI, Maiya AS, Berger-Wolf TY (2011) Aggression, grooming and group-level cooperation in white-faced capuchins (*Cebus capucinus)*: Insights from social networks. A J Primatol 73, 821–833

Croft DP, James R, Krause J (2008) Exploring animal social networks. Princeton University Press, Princeton

Crowell-Davis SL, Houpt KA, Carini CM (1986) Mutual grooming and nearest-neighbor relationships among foals of *Equus caballus*. Appl Anim Behav Sci 15, 113–123

Dathe H (1984) Conclusions and recommendations. 4th International Symposium on the Preservation of the Przewalki’s horse. Equus 2, 246-247

De Waal F (1989) Peacemaking among primates. Harvard University Press, Cambridge

Duncan P (1983) Determinants of the Use of Habitat by Horses in a Mediterranean Wetland. J of Anim Ecol 52, 93-109

Duncan P (1992) Horses and Grasses. The Nutritional Ecology of Equids and their Impact on the Camargue. Springer, New York

Duncan P, Vigne N (1979) The effects of group size in horses on the rate of attacks by blood-sucking flies. Anim Behav 17, 623-625

Dwyer CM, Lawrence AB (1999) Ewe–ewe and ewe–lamb behaviour in a hill and a low land breed of sheep: a study using embryo transfer. Appl Anim Behav Sci 61, 319–334

Ellard ME, Crowell-Davis SL (1989) Evaluating equine dominance in draft mares. Appl Anim Behav Sci 24, 55-75

Emery NJ, Seed AM, von Bayern AMP, Clayton NS (2007) Cognitive adaptations of social bonding in birds. Philos T Roy Soc B 362, 489–505

Erber R, Wulf M, Aurich J, Rose-Meierhöfer S, von Lewinski M, Möstl E, Aurich C (2013) Stress response of three-year-old horse mares to changes in husbandry system during initial equestrian training. J Equine Vet Sci 33, 1088-1094

Farine DR, Whitehead H (2015) Constructing, conducting and interpreting animal social network analysis. J Anim Ecol 84, 1144-1163

Fedurek P, Dunbar RIM (2009) What Does Mutual Grooming Tell Us About Why Chimpanzees Groom? Ethology 115, 566–575

Feh C (1988) Social behaviour and relationships of Prezewalski horses in Dutch semi-reserves. Appl Anim Behav Sci 21, 71–87

Feh C, de Mazières J (1993) Grooming at a preferred site reduces heart rate in horses. Anim Behav 46, 1191–1194

Feist JD (1971) Behavior of feral horses in the Pryor Mountain Wild Horse Range. Master's Thesis, University of Michigan, Michigan

Feist JD, McCullough DR (1975) Reproduction in feral horses. J Reprod Fertil, Supplement 23, 13-18

Feist JD, McCullough DR (1976) Behavior patterns and communication in feral horses. Z Tierpsychol 41, 337–371

Flack JC, de Waal FBM, Krakauer DC (2005) Social structure, robustness, and policing cost in a cognitively sophisticated species. Am Nat 165, 126–39

Flack JC, Girvan M, de Waal FBM, Krakauer DC (2006) Policing stabilizes construction of social niches in primates. Nature 439, 426–429

Flack JC, Krakauer DC, de Waal FBM (2005) Robustness mechanisms in primate societies: a perturbation study. P Roy Soc of Lond B Bio 272, 1091–1099

Flauger B (2010) The introduction of horses into new social groups with special regard to their stress level. Dissertation, University of Regensburg, Regensburg

Flauger B, Krueger K (2013) Aggression level and enclosure size in horses (*Equus caballus*). Pferdeheilkd 29, 495–504

Flauger B, Krueger K, Gerhards H, Möstl E (2010) Simplified method to measure glucocorticoid metabolites in faeces of horses. Vet Res Commun 34, 185-95

Foley CAH, Papageorge S, Wasser SK (2001) Noninvasive stress and reproductive measures of social and ecological pressures in free-ranging African elephants. Conserv Biol 15, 1134–1142

Frank R (2009) Impatient traders or contingent reciprocators? Evidence for the extended time course of grooming exchanges in baboons. Behav 146, 1123–1135

Fraser AF (1974) Farm animal behaviour. Bailliere Tindall, London

Fraser MD, Gordon IJ (1997) The diet of goats, red deer and South American camelids feeding on three contrasting Scottish upland vegetation communities. J Appl Ecol 34, 668–686

Fraser ON, Bugnyar T (2010) Do ravens show consolation? Responses to distressed others. Plus One e*10605*

Frère CH, Krützen M, Mann J, Connor RC, Bejder L, Sherwin WB (2010) Social and genetic interactions drive fitness variation in a free-living dolphin population. P Natl Acad Sci USA 107, 19949-19954

George SC, Smith TE, Mac Cana PSS, Coleman R, Montgomery WI (2014) Physiological stress in the Eurasian badger (*Meles meles*): Effects of host, disease and environment. Gen and Comp Endocrinol 200, 54-60

Gesquiere LR, Learn NH, Simao CM, Onyango PO, Alberts SC, Altman J (2011) Life at the Top: Rank and Stress in Wild Male Baboons. Science 333, 357-60

Goto H, Ryder OA, Fisher AR, Schultz B, Kosakovsky Pond SL, Nekrutenko A, Makova KD (2011) A massively parallel sequencing approach uncovers ancient origins and high genetic variability of endangered Przewalski's horses. Genome Biol Evol 3, 1096-1106

Grzimek B (1949) Rangordungsversuche mit Pferden. Z Tierpsychol 6, 455-64

Hartmann E, Christensen JW, Keeling LJ (2011) Training young horses to social separation: effect of a companion horse on training efficiency. Equine Vet J 43, 580-584

Hechler B (1971) Beitrag zur Ethologie des Islandpferdes. Dissertation, University of Gießen, Gießen

Heintzelmann-Gröngröft B (1984) Spezielle Ethologie – Pferd. In: Bogner H, Grauvogl A (Eds) Verhalten landwirtschaftlicher Nutztiere. Eugen Ulmer, Stuttgart, pp 87 – 147

Heitor F, do Mar Oom M, Vicente L (2006) Social relationships in a herd of Sorraia horses Part I. Correlates of social dominance and contexts of aggression. Behav Process 73, 170–177

Heitor F, Oom MM, Vicente L (2006) Social relationships in a herd of Sorraia horses Part II. Factors affecting affiliative relationships and sexual behaviours. Behav Process 73, 231–239

Heitor, F, Vicente L (2010) Affiliative relationships among Sorraia mares: influence of age, dominance, kinship and reproductive state. J Ethol 28, 133-140

Hemelrijk CK, Wantia J, Gygay L (2005) The construction of dominance order: comparing performance of five methods using an individual-based model. Behav 142, 1043-1064

Henry JP, Stephens PM (1977) Stress, health and the social environment; A sociobiologic approach to medicine. Springer-Verlag, Berlin

Henzi SP, Lycett JE, Weingrill T (1998) Mate guarding and risk assessment by male mountain baboons during inter-troop encounters. Anim Behav 55 1421–1428

Hill DA (1987) Social relationships between adult male and female rhesus macaques: I. Sexual consortships. Primates 28, 439–456

Hogan ES, Houpt KA, 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 21,147–168

Holekamp KE, Boydston EE, Smale L (2000) Group travel in social carnivores. Chicago University Press, Chicago

Holm S (1979) A simple sequentially rejective multiple test procedure. Scand J of Stat 6, 65-70

Houpt KA, Boyd L (1994) Social behaviour. 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, pp. 229-254

Houpt KA, 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, Law K, Martinisi V (1978) Dominance hierarchies in horses. Appl Anim Ethol 4, 273-283

Hughes BO, Wood-Gush DGM (1977) Agonistic behaviour in domestic hens: The influence of housing method and group size. Anim Behav 25, 1056-1162

Ihle P (1984) Ethologische Studie über den Tagesrhythmus von Pferden in Abhängigkeit von der Haltungsform. Dissertation, University of Gießen, Gießen

Ivlev VS (1961) Experimental ecology of the feeding of fishes. Yale University Press, New Haven

Jacobs J (1974) Quantitative measurement of food selection: A modification of the forage ratio and Ivlev's electivity index. Oecologia 14, 413-417

Jaworowska M (1976) Verhaltensbeobachtungen an primitiven Pferden, die in einem polnischen Waldschutzgebiet –in Freiheit lebend –erhalten werden. Säugetierkundl Mitt 24, 241-268

Jennings DJ, Carlin CM, Gammell MP (2009) A winner effect supports third-party intervention behaviour during fallow deer (*Dama dama*) fights. Anim Behav 77, 343–348

Kaszab Z (1968) Entgegnung auf die Bemerkungen von Jiri Volf bezüglich: „New Sighting of Przewalski horses?“. Mamm biol 33, 62-63

Keiper RR (1986) Social structure. The veterinary clinics of north america. Equine Pract 2, 465–484

Keiper RR (1988) Social interactions of the Przewalski horse (*Equus przewalskii* Poliakov, 1881) herd at the Munich Zoo. Appl Anim Behav Sci 21, 89–97

Keiper RR, Sambraus HH (1986) The stability of equine dominance hierarchies and the effects of kinship, proximity and foaling status on hierarchy rank. Anim Behav 16, 121-130

Kelley KW (1980) Stress and immune function: a bibliographic review. Ann Rech Vet 11, 445-478

Khalil AM, Kaseda Y (1998) Early experience affects developmental behaviour and timing of harem formation in Misaki horses. Appl Anim Behav Sci 59, 253-263

Kimball C (2008) Equine Body Condition Scoring. Vet Tech 29, 548-556

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

King AJ, Douglas CMS, Huchard E, Isaac NJB, Cowlishaw G (2008) Dominance and affiliation mediate despotism in a social primate. Curr Biol 18, 1833–1838

King SRB (2002) Home range and habitat use of free-ranging Przewalski horses at Hustai National Park, Mongolia. Appl Anim Behav Sci 78, 103-13

King SRB, Gurnell J (2005) Habitat use and spatial dynamics of takhi introduced to Hustai National Park, Mongolia. Biol Conserv 124, 277–290

Kitchen DM, Cheney DL, Seyfarth RM (2005) Male chacma baboons (*Papio hamadryas ursinus*) discriminate loud call contests between rivals of different relative ranks. Anim cogn 8, 1-6

Klingel H (1980) Die soziale Organisation freilebender Equiden. Equus 2, 128 – 131

Klingel (2012) Wild equids ecology and behaviour, distribution, social behaviour, and social organization. Proceedings of the 2nd International Equine Science Meeting 1, 7

Kolter L, Schach C, Weber T (1999) Habitat Use of Feral and Przewalski´s Horses. Natur- und Kulturlandschaft 3, 332-342

Kolter L, Zimmermann W (1988) Social behaviour of Przewalski horses (*Equus p. przewalskii*) in the Cologne Zoo and its consequences for management and housing. Appl Anim Behav Sci 21, 117–145

Kolter L, Zimmermann W (2001) The keeping of Przewalski’s horse bachelor groups for the EEP - Stallions living in enclosures and reserves. Z Kölner Zoos 44, 135-151

Koolhaas JM, Bartolomucci A, Buwalda B, de Boer SF, Flugge G, Korte SM, Meerlo P, Murison R, Olivier B, Palanza P, Richter-Levin G, Sgoifo A, Steimer T, Stiedl O, van Dijk G, Wohr M, Fuchs E (2011) Stress revisited: a critical evaluation of the stress concept. Neurosci Biobehav Rev 35, 1291-1301

Kovács L, Jurkovich V, Bakony M, Szenci O, Póti P, Tözsér J (2014) Welfare implication of measuring heart rate and heart rate variability in dairy cattle: literature review and conclusions for future research. Animal 8, 316-330

Krause J (1994) Differential fitness returns in relation to spatial position in groups. Biol Rev Camp Philos Sco 69, 187–206

Krebs JR, Davis NB (1997) Behavioural ecology. Blackwell Scientific Publication, Oxford

Krueger K, Flauger B, Farmer K, Hemelrijk C (2014) Movement initiation in groups of feral horses. Behav Process 103, 91–101

Krueger T (2012) Rinder- und Pferdebeweidung ist gut für die Natur. Junge Wiss 93, 28-35

Kuntz R, Kubalek C, Ruf T, Tataruch F, Arnold A (2006) Seasonal adjustment of energy budget in a large wild mammal, the Przewalski horse (*Equus ferus przewalskii*) I. Energy intake. J Exp Biol 209, 4557-4565

Lamprecht J (1992) Variable leadership in bar-headed geese (*Anser Indicus*): an analysis of pair and family departures. Behav 122, 105–119

Langergraber K, Mitani J, Vigilant L (2009) Kinship and social bonds in female chimpanzees (*Pan troglodytes*). Am J Primatol 71, 840–851

Leca J-B, Gunst N, Thierry B, Petit O (2003) Distributed leadership in semifree-ranging white-faced capuchin monkeys. Anim Behav 66, 1045–1052

Leroi-Gourhan A (1971) Préhistoire de l’art occidental. Éditions D’art Lucien Mazenod, Paris

Lim MM, Young LJ (2006) Neuropeptidergic regulation of affiliative behavior and social bonding in animals. Horm Behav 50, 506-517

Linklater WL, Cameron EZ, Monit EO, Stafford KJ (1999) Stallion harassment and the mating system of horses. Anim Behav 58, 295–306

Linklater WL (2000) Adaptive explanation in socio-ecology: lessons from the Equidae. Biol Rev Camb Philos Soc 75, 1-20

Lynch JJ, Woodgush DGM, Davies HJ (1985) Aggression and nearest neighbours in a flock of Scottish Blackface ewes. Biol Behav 10, 215–225

MacClintock D (1982) Where have all the horses gone? Anim Kingdom 85, 26-31

Martin P, Bateson P (1986) Measuring Behaviour - An introductory guide. Cambridge University Press, Cambridge

Matoba T, Kutsukake N, Hasegawa T (2013) Head rubbing and licking reinforce social bonds in a group of captive African lions, *Panthera leo*. Plos One 8, e*73044*

Mazak V (1961) Die Hautpflege bei dem Przewalski–Urwildpferd (*Equus przewalskii* Poliakow). Equus 1, 105–121

McBride SD, Hemmings A, Robinson K (2004): A preliminary study on the effect of massage to reduce stress in the horse. J Equine Vet Sci 24, 76–81

McDonnell SM (2003) The Equid Ethogram: A practical field guide to horse behavior. Eclipse Press, Lexington

McDonell SM, Haviland JCS (1995) Agonistic ethogram of equid bachelor band. Appl Anim Behav Sci 43, 147-188

Mirabet V, Fréon P, Lett C (2008) Factors affecting information transfer from knowledgeable to naive individuals in groups. Behav Ecol Sociobiol 63, 159–171

Mitani JC (2009) Male chimpanzees form enduring and equitable social bonds. Anim Behav 77, 633–640

Monard AM, Duncan P, Boy V (1996) The Proximate Mechanisms of Natal Dispersal in Female Horses. Behaviour 133, 1095-1124

Mormède P, Andanson S, Aupérin B, Beerda B, Guémené D, Malmkvist J, Manteca X, Manteuffel G, Prunet P, van Reenen CG, Richard S, Veissier I (2007) Exploration of the hypothalamic-pituitary-adrenal function as a tool to evaluate animal welfare. Physiol Behav 92, 317–39

Möstl E, Messmann S, Bagu E, Robia C, Palme R (1999) Measurement of glucocorticoid metabolite concentration in faeces of domestic livestock. J Vet Med A 46, 621-631

Möstl E, Palme R (2002) Hormones as indicators of stress. Domest Anim Endocrinol 23, 67-74

Muller MN, Wrangham RW (2004) Dominance, cortisol andstress in wild chimpanzees (*Pan troglodytes schweinfurthii*). Behav Ecol Sociobiol 55, 332–340

Nagy M, Akos Z, Biro D, Vicsek T (2010) Hierarchical group dynamics in pigeon flocks. Nature *464*, 890–893

Nakagawa N (1992) Distribution of affiliative behaviors among adult females within a group of wild patas monkeys in a nonmating, nonbirth season. Int J Primatol 13, 73-96

Nuñez CMV, J, Adelman JS, Smith J, Gesuiere R, Rubenstein DI (2014) Linking social envorinment and stress physiology in feral mare (Equus caballus): Group transfer elevate fecal cortisol levels. Gen Comp Endocrinol 196, 26-33

Ostner J, Schülke O (2014) The evolution of social bonds in primate males. Behav 151, 871–906

Palme R, Möstl E (1996) Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. Proceedings, 1st International Symposium Physiology and Ethology of Wild and Zoo Animals, Berlin, p. 84

Passantino L, Passantino A, Ostillio A, Jirillo F, Russo C, Patruno R, Passantino GF (2010) Depression of cellular immunity correlated to stress: questions on welfare and protection in Italian trotters after running performance. Biomed Res 21, 341-348

Pellis SM, Pellis VC (2006) Play and the development of social engagement: A comparative perspective. In: Marshall PJ, Fox NA (Eds) The development of social engagement, Neurobiological perspectives. Oxford University Press, Oxford, pp. 247–274

Pirkelmann H (1991) Pferdehaltung. Eugen Ulmer, Stuttgart

Popolow C (1984) Time budgets of Equus przewalskii at the Bronx Zoo. Unpublished Under graduate research paper. Cornell University, Ithaca

Prins HH (1995) Ecology and Behaviour of the African Buffalo: Social Inequality and Decision Making. Springer, Dordrecht

Puschmann W (2007) Zootierhaltung, Tiere in menschlicher Obhut, Säugetiere. Harri Deutsch Verlag, Frankfurt

Pusey AE, Packer C (2003) The Ecology of relationships. In: Krebs JR, Davis NB (Eds) Behavioural ecology. Blackwell Scientific Publication, Oxford, pp. 254–283

Redman P, Goodwin D (1999) Eliminatory behaviour of a bachelor group of Przewalski horses in a semi reserve; comparison with the domestic horse. In: Harris P, Goodwin D, Green RE (Eds) The Role of the Horse in Europe. 31st International Congress of the International Society for Applied Ethology, Equine Vet J, Cambridgeshire, p 68

Riecken U, Schröder E, Fink P (2001) Halboffene Weidelandschaften und Wildnisgebiete als Ziele des Naturschutzes aus Bundessicht – Alternativen zum Erhalt und zur Pflege von Offenlandbiotopen. Natur- und Kulturlandschaft 4, 88-94

Roth F (2002) Entwicklung der räumlichen und sozialen Organisation von Przewalskipferden (*E. ferus przewalskii*) unter naturnahen Bedingungen im Pentezuggebiet (Hortóbagy Nationalpark, Ungarn). Dissertation, University of Cologne, Cologne

Roubová V, Konečná M, Šmilauer P, Wallner B (2015) Whom to groom and for what? Patterns of grooming in female Barbary Macaques *(Macaca sylvanus*). Plos One 10, e*0117298*

Rudman R, Keiper RR (1991) The body condition of feral ponies on Assateague Island. Equine Vet J 23, 453-456

Rutberg AT (1987) Horse fly harassment and the social behavior of feral ponies. Ethology 75, 145-154

Ryder OA (1994) Genetic studies of Przewalski’s horses and their impact on conservation. 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, pp. 75-92

Saltz D (1988) Urinary cortisol and urea nitrogen responses to winter stress in mule deer (*Odocoileushemionus*). Dissertation, Colorado State University, Colorado

Saltz D, White GC (1991) Urinary Cortisol and Urea Nitrogen Responses to Winter Stress in Mule Deer. J Wildl Manag 55, 1-16

Sands J, Creel S (2004) Social dominance, aggression and faecal glucocorticoid levels in a wild population of wolves, *Canis lupus*. Anim Behav 67, 387-396

Sapolsky R (1992) Cortisol concentrations and the social significance of rank instability among wild baboons. Psychoneuroendocrinology 17, 701-709

Šárová R, Spinka M, Panamá JLA (2007) Synchronization and leadership in switches between resting and activity in a beef cattle herd--A case study. Appl Anim Behav Sci 108, 327–331

Šárová R, Spinka M, Panamá JLA, Simecek P (2010) Graded leadership by dominant animals in a herd of female beef cattle on pasture. Anim Behav 79, 1037–1045

Schach C (1994) Physiologische und ökologische Aspekte der Ernährung von PrzewalskiPferden (*Equus p. przewalkii*) in einem neu angelegten Semi-Reservat. Diploma thesis, University of Cologne, Cologne

Schaupp A (2008) Habitatnutzung, Aktivitätsbudget und Futteraufnahmeverhalten beim Przewalski-Pferd in der Döberitzer Heide. Bachelor Thesis, University of Hildesheim/Holzminden/Göttingen, Hildesheim

Scheibe KM, Lange B, Sieling C, Scheibe A (1998) Przewalskipferde in einem Semireservat – Entwicklung von Ortspräferenzen und Einfluss auf Vegetationsstrukturen. Natur- und Kulturlandschaft 3, 348-353

Schino G, Scucchi S, Maestripieri D, Turillazzi PG (1988) Allogrooming as a tension-reduction mechanism: A behavioral approach. Am J Primatol 16, 43–50

Schneider G, Krueger K (2012) Third-party interventions keep social partners from exchanging affiliative interactions with others. Anim Behav 83, 377-387

Schülke O, Bhagavatula J, Vigilant L, Ostner J (2010) Social bonds enhance reproductive success in male Macaques. Curr Biol 20, 2207–2210

Seyfarth RM, Palombit RA, Cheney DL (2001) Female–female competition for male ‘friends’ in wild chacma baboons (*Papio cynocephalus ursinus*). Anim Behav 61, 1159–1171

Sibbald AM, Elstion DA, Smith DJF, Erhard HW (2005) A method for assessing the relative sociability of individuals within groups: an example with grazing sheep. Appl Anim Behav Sci 91, 57-73

Sigg H, Tobler I (1986) Motor activity of mongrel dogs under three different housing conditions. Z Versuchstierkd 28, 157-165

Sigurjónsdóttir H, Van Dierendonck MC, Snorrason S, Thórhallsdóttir AG (2003) Social relationships in a group of horses without a mature stallion. Behav 140, 783–804

Silk JB, Alberts SC, Altmann J (2003) Social bonds of female Baboons enhance infant survival. Science 302, 1231–1234

Silk JB, Altmann J, Alberts SC (2006) Social relationships among adult female baboons (*Papio cynocephalus*). II. Variation in the quality and stability of social bonds. *Behav Ecol Sociobiol* 61, 197-204

Skiff EM (1982) The effect of enclosure design on social interactions and daily activity patterns of the captive Asiatic wild horse (*Equus przewalskii*). Master’s Thesis, University of Minnesota, Minnesota

Smith JE, Van Horn RC, Powning KS, Cole AR, Graham KE, Memenis SK, Holekamp KE (2010) Evolutionary forces favoring intragroup coalitions among spotted hyenas and other animals. Behav Ecol 688, 284–303

Smuts B, Cheney D, Seyfarth R, Wrangham R, Struhsaker T (1987) Primate societies. University of Chicago Press, Chicago

Sonnenburg H, Gerken B, Wagner HG, Ebersbach H (2004) Das Hutewaldprojekt im Naturpark Solling-Vogler. LÖBF-Mitteilungen 4, 40-47

Souris AC, 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

Steidele N (2011) Monitoring a bachelor group of Przewalski horses throughout the course of a year with particular attention paid to sleep behaviour and rank order. Dissertation, University of Munich, Munich

Strand SC, Tiefenbacher S, Haskell M, Hosmer T, McDonnell SM, Freeman DA (2002). Behavior and physiologic responses of mares to short-term isolation. Appl Anim Behav Sci 78, 145-157

Taillon J, Côté SD (2008) Are fecal hormone levels linked to winter progression, diet quality, and social rank in young ungulates? An experiment with white-tailed deer (*Odocoileus virginianus*) fawns. Behav Ecol and Sociobiol 62, 1591-1600

Tilson RL, Binczik GA, Reindl NJ, Sweeny KA (1988) Buddies and bullies: social structure of a bachelor group of Przewalski horses. Appl Anim Behav Sci 21, 169-185

Touma C, Palme R (2005) Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of validation. Ann of the New York Academy of Sci 1046, 54-74

Tyler SJ (1972) The behaviour and social organisation of the new Forest ponies. Anim Behav Monogr 5, 85–196

Van Dierendonck, MC (2006) Social contact in horses: implications for human horse interactions. Dissertation, Universität Utrecht, Utrecht

Van Dierendonck MC, Bandi N, Batdorj D, Dugerlham S, Munkhtsog B (1996) Behavioural observations of reintroduced Takhi or Przewalski horses (*Equus ferus przewalskii*) in Mongolia. Appl Anim Behav Sci 50, 95-114

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. Neth J Zool 45, 362–385

Van Dierendonck MV, de Vries H, Schilder MBH, Colenbrander B, Þorhallsdóttir AG, Sigurjónsdóttir H (2009) Interventions in social behaviour in a herd of mares and geldings. Appl Anim Behav Sci 116, 67-73

Van Dierendonck, Wallis de Vries MF (1996) Ungulate reintroductions: experiences with the Takhi or Przewalski horse (*Equus ferus przewalskii*) in Mongolia. Conserv Biol 10, 728-740

Van Lawick-Goodall J (1968) The behavior of free living chimpanzees in the Gombe Stream Reserve. Animal Behavior Monographs 1, 161–311

Van Loo PLP, Van der Meer E, Kruitwagen CLJJ, Koolhaas JM, Van Zutphen LFM, Baumans V (2004) Long-term effects of husbandry procedures on stress-related parameters in male mice of two strains. Lab Anim 38, 169-177

Veissier I, Boissy A (2007) Stress and welfare: two complementary concepts that are instrinsically related to the animal’s point of view. Physol Behab 92, 429-433

Visser EK, Ellis AD, Van Reenen C (2008) The effect of two different housing conditions on the welfare of young horses stabled for the first time. Appl Anim Behav Sci 114, 521-533

Volf J (1981) Lebensauer de Przewalski's—Wildpferde (*Equus przewalski* Polf, 1881) in Gefangenschaft. Zool Garten, 51, 385-387

Walther FR (1991) On herding behavior. Appl Anim Behav Sci 29, 5–13

Walzer C, Kaczensky P, Ganbataar O, Lengger J, Enkhsaikhan N, Lkhagvasuren D (2006) Capture and anaesthesia of wild Mongolian equids - the Przewalski's horse (*E. ferrus przewalskii*) and the Khulan (*E. Hemionus*). Mongol J Biol Sci 4, 19-28

Waring GH (2003) Horse behavior. Noyes Publishing, Norwich

Waring GH (1983) Horse Behaviour. The Behavioural Traits and Adaptions if Domestic and Wild Horses, including Ponies. Noyes Publications, New Jersey

Wasilewski A (2003) 'Friendship' in ungulates? – Sociopositive relationships between non-related herd members of the same species. Dissertation, Philipps-Universität Marburg, Marburg

Watts DJ, Strogatz SH (1998) Collective dynamics of `small-world' networks. Nature 393, 440–442

Wells S, von Goldschmidt-Rotschild B (1979) Social behaviour and relationships in a herd of Camargue horses. Z Tierpsychol 49, 363-380

Wiepkema PR, Koolhaas JM (1993) Stress and animal welfare. Anim Welfare 2, 195-218

Wit P, Bouman I (2006) The tale of Przewalski's horses: Coming home to Mongolia. KNNV Publishing, Utrecht

Wolter R, Krueger K (2015) Influencing factors on grooming behaviour in wild living horses. Cur Res Appl Ethol 510, 242-248

Wolter R, Pantel N, Stefanski V, Möstl E, Krueger K (2014) The role of an alpha animal in changing environmental conditions. Physiol & Behav 133, 236-243

Woodburne MO (1982) A reappraisal of the systematics, biogeography, and evolution of fossil horses. Paleobiology 8, 315-327

Woodfine T (2003) The Eelmoor Marsh Przewalski's horse Project: The Complementary Management of an Endangered Equid and a British Nature Reserve. Dissertation, University of Kentuky, Kentuky

Yarnell K, Hall C, Royle C, Walker SL (2015) Domesticated horses differ in their behavioural and physiological responses to isolated and group housing. Physiol Behav 143, 51-57

Zabel CJ, Glickman SE, Frank LG, Woodmansee KB, Keppel G (1992) Coalition formation in a colony of prepubertal spotted hyaenas. In: Harcourt AH, de Waal FBM (Eds) Coalitions and Alliances in Humans and Other Animals. Oxford University Press, Oxford, pp. 113-135

Zeitler-Feicht M (2008) Handbuch Pferdeverhalten. Eugen Ulmer, Stuttgart

Zevegemid D, Dawaa N (1973) Die seltenen Großsäuger der Mongolischen Volksrepublik und ihr Schutz. Naturschutz u Landschaftsforsch Berlin 13, 87-106

Zharkikh TL, Andersen L (2009) Behaviour of Bachelor Males of the Przewalski Horse (*Equus ferus* 669 *przewalskii*) at the Reserve Askania Nova. Zool Garten 78, 282-299

Zimmermann W (2005) EEP Asiatic Equids Husbandry Guidelines. Zool Garten, Köln

Zimmermann W, Kolter L, Sandor I, Dukat S (1998) Przewalskipferde in der Hortobagy Puszta - ein Natur- und Artenschutzprojekt. Z Des Kölner Zoo 1, 37-55

APPENDIX

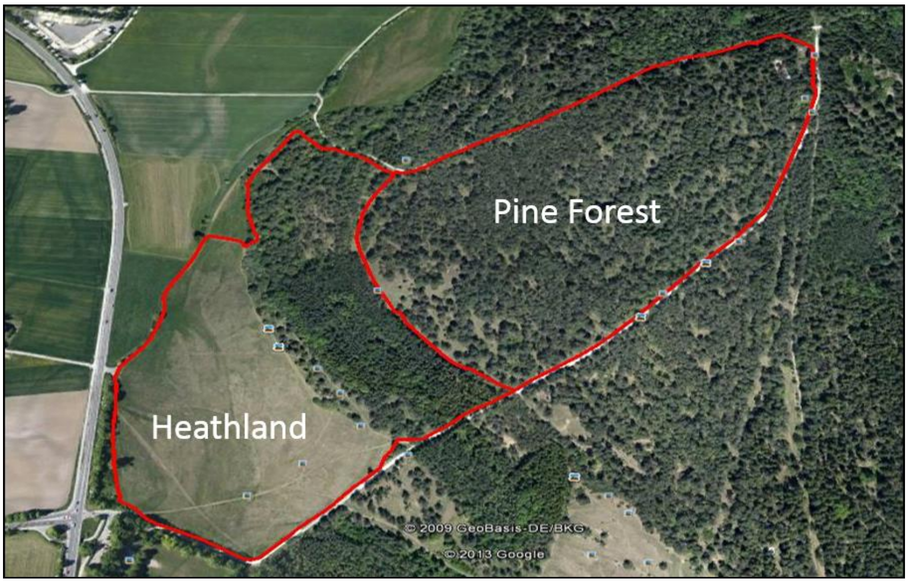
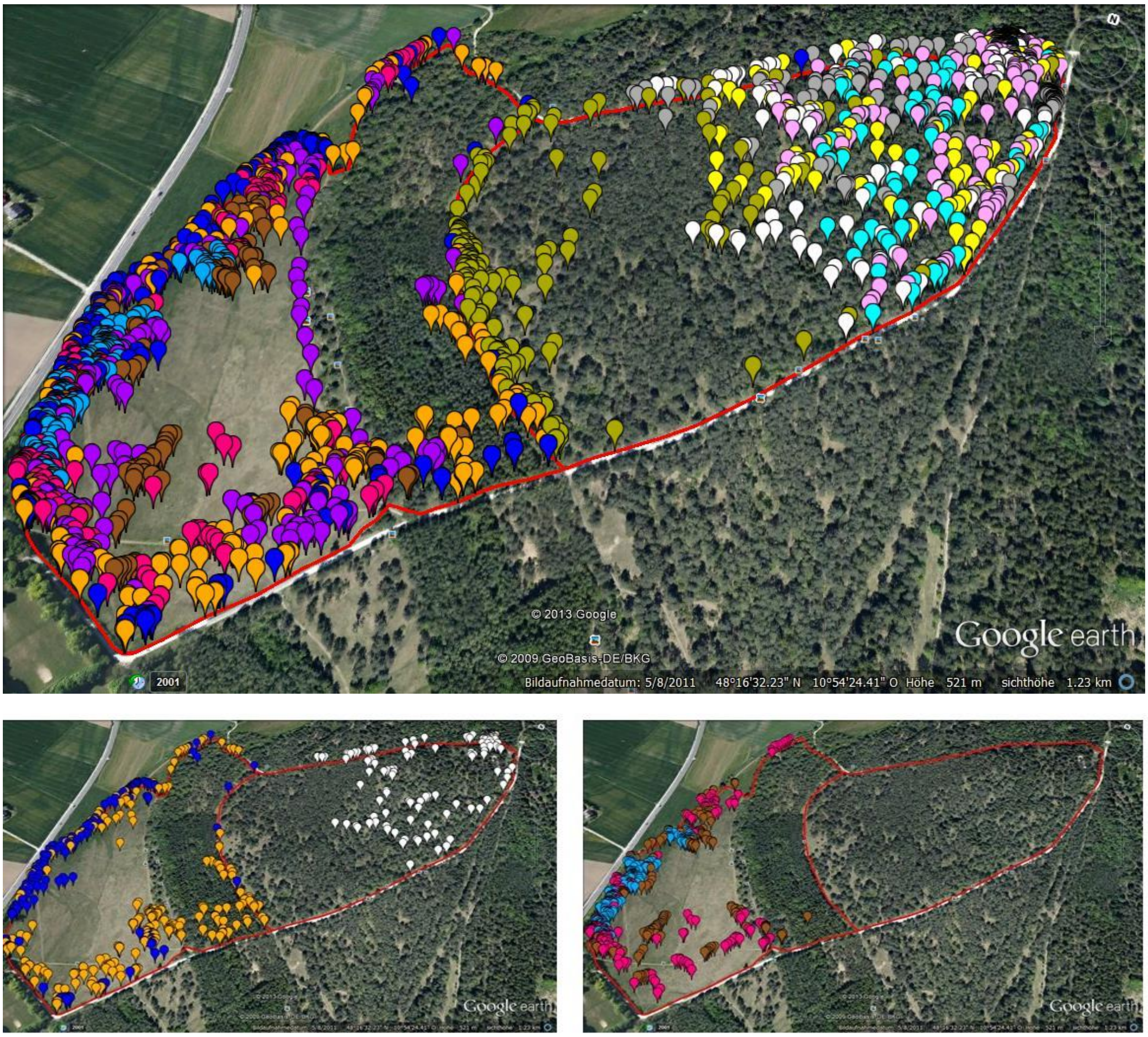


Figure A1. Aerial view with fence (red line) at Augsburg

Figure A2. Aerial view with fence (red line) at Campo Pond



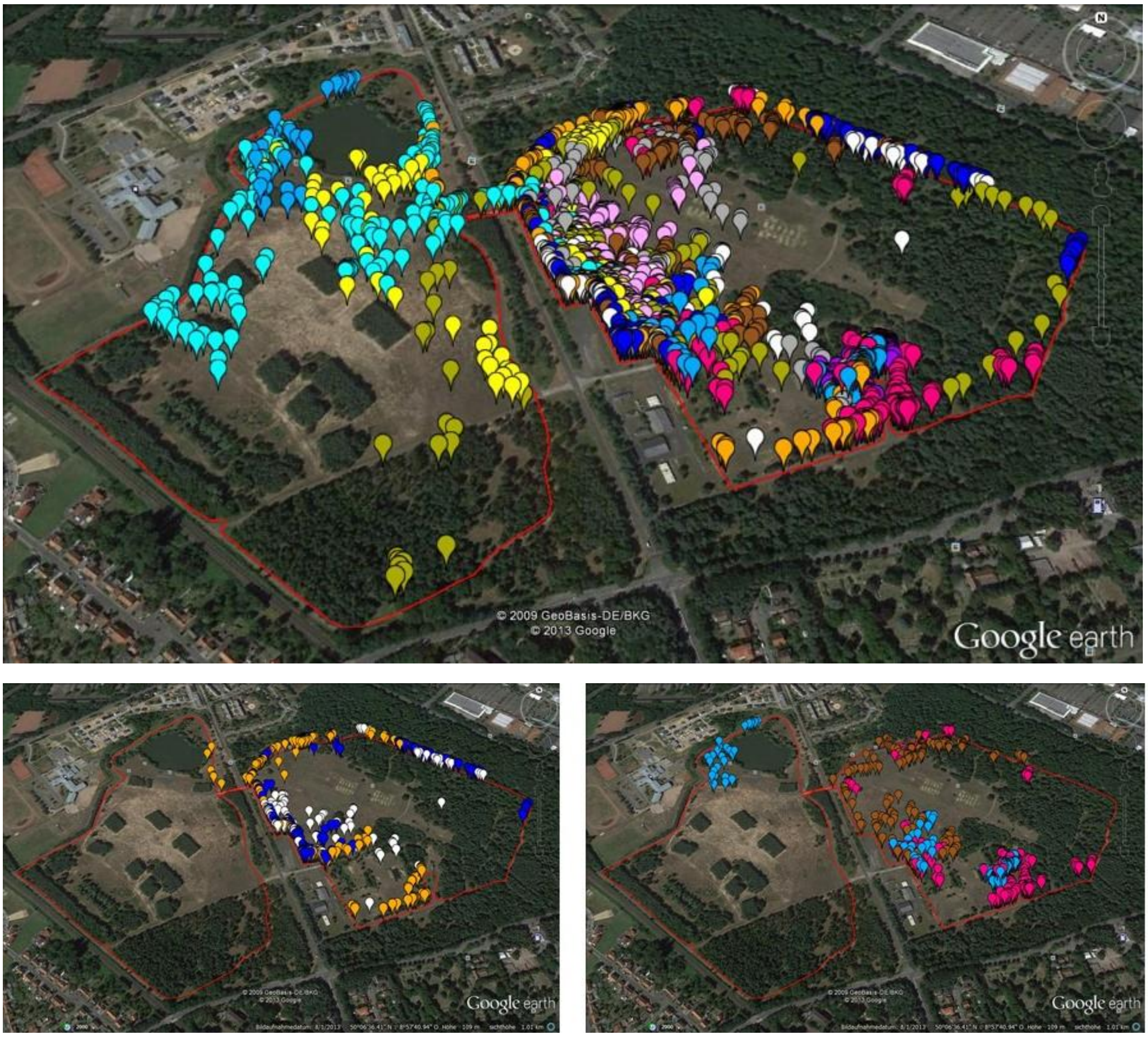
a) Autumn:
White = September
Orange = October
Dark blue = November

b) Winter:
Brown = December
Pink = January
Light blue = February

c) Spring:
Purple = March
Olive = April
Grey = May

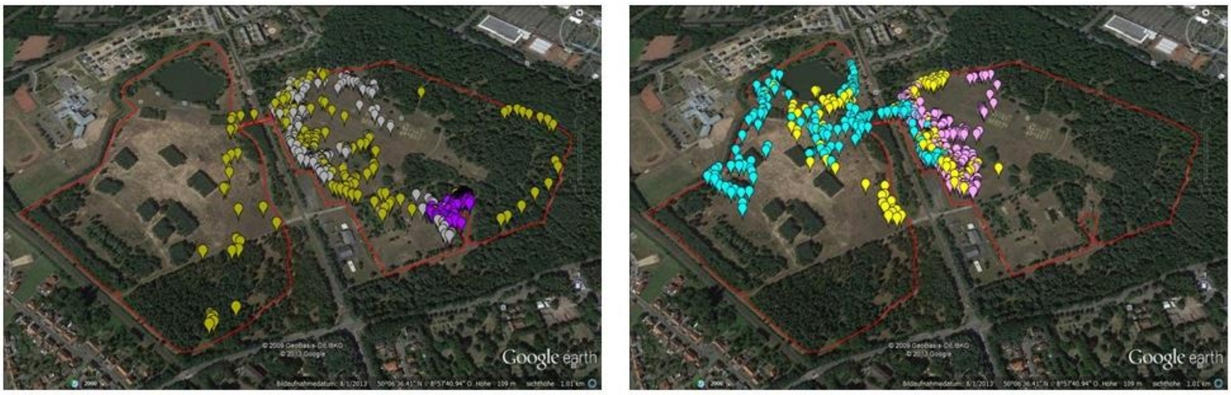
d) Summer:
Rose = June
Yellow = July
Turquoise = August

Figure A3. Aerial view with fence at Augsburg and with GPS points showing the position of the horse group during the whole year (above) and during various seasons: a) autumn, b) winter, c) spring and d) summer



a) Autumn:
White = September
Orange = October
Dark blue = November

b) Winter:
Brown = December
Pink = January
Light blue = February



c) Spring:
Purple = March
Olive = April
Grey = May

d) Summer:
Rose = June
Yellow = July
Turquoise = August

Figure A4. Aerial view with fence at Campo Pond and with GPS points showing the position of the horse group during the whole year (above) and during various seasons: a) autumn, b) winter, c) spring and d) summer

Table A1
Individual horse data of the group in Augsburg (P-1)

Studbook-No.	Name (sex)	Birthdate	Former location	Living in Augsburg since
4839	Marlon (m)	20.07.2006	Stuttgart (GER)	06-2007
5639	Kalmoek (m)	25.05.2010	Kerkrade (NED)	01-2012
5648	Solongo (m)	07.06.2010	Hunnebostrand (SWE)	09-2011
5665	Xaran (m)	05.07.2010	Hunnebostrand (SWE)	09-2011
5732	Vinter (m)	13.01.2011	Hunnebostrand (SWE)	06-2012

Table A2
Individual horse data of the group in Hanau (P-2)

Studbook-No.	Name (sex)	Birthdate	Former location	Living in Hanau since
2043	Barioja (f)	07.08.1990	Beigarten (GER)	09-2009
3406	Abadia (f)	06.06.2000	Nurnberg (GER)	09-2010
4747	Ginger (f)	20.05.2006	Munich (GER)	09-2010
4965	Fury (m)	14.05.2007	Sprakel (GER)	07-2013
5319	Sira (f)	28.05.2006	Nurnberg (GER)	09-2009
5326	Galinka (f)	16.09.2006	Munich (GER)	09-2010
5339	Ilonka (f)	31.08.2008	Ludwigsthal (GER)	09-2010
5345	Ajala (f)	30.04.2008	Nurnberg (GER)	09-2009

Table A3
Individual horse data of the group in Hanau (during second observation) (P-3)

Studbook-No.	Name (sex)	Birthdate	Former location	Living in Hanau since
2043	Barioja (f)	07.08.1990	Beigarten (GER)	09-2009
4747	Ginger (f)	20.05.2006	Munich (GER)	09-2010
4965	Fury (m)	14.05.2007	Sprakel (GER)	07-2013
5326	Galinka (f)	16.09.2006	Munich (GER)	09-2010
5339	Ilonka (f)	31.08.2008	Ludwigsthal (GER)	09-2010
6473	Oleg (m)	24.06.2014	_____	06-2014

Table A4
Individual horse data of the group in Gießen (P-4)

Studbook-No.	Name (sex)	Birthdate	Former location	Living in Gießen since
3406	Abadia (f)	06.06.2000	Nurnberg (GER)	07-2013
4579	Marie (f)	31.07.2005	Stuttgart (GER)	03-2012
5319	Sira (f)	28.05.2006	Nurnberg (GER)	07-2013
5345	Ajala(f)	30.04.2008	Nurnberg (GER)	07-2013
5374	Janine (f)	04.05.2009	Beigarten (GER)	03-2012
5448	Jana (f)	29.08.2009	Ludwigstahl (GER)	07-2012
5650	Krissi (f)	08.06.2010	Beigarten (GER)	03-2012
5668	Kati (f)	09.07.2010	Beigarten (GER)	03-2012
6175	Nele (f)	26.04.2013	_____	04-2013

Table A5
Individual horse data of the group in Ludwigsthal (P-5)

Studbook-No.	Name (sex)	Birthdate	Former location	Living in Ludwigsthal since
1691	Nadja (f)	06.05.1988	Munich (GER)	08-2005
2373	Holly (f)	24.06.1992	Munich (GER)	08-2005
2471	Calgary (f)	24.05.1993	Munich (GER)	08-2005
4543	Fumia (f)	13.06.2005	Munich (GER)	08-2005
5316	Aytan (m)	08.03.2006	Tennenlohe (GER)	07-2011
5736	Laika (f)	23.04.2011	_____	04-2011
5892	Leila (f)	09.07.2011	_____	07-2011
6447	Olga (f)	31.05.2014	_____	05-2014
6497	Otto (m)	31.10.2014	_____	10-2014

Table A6
Individual horse data of the feral horse groups from Italy

Name of the group	Number of individuals	Males	Females	Yearlings / Foals
Group F-6	23	1	18	4
Group F-7	10	1	5	4
Group F-8	12	1	7	4
Group F-9	19	1	14	4
Group F-10	26	1	13	12
Group F-11	19	1	11	7

Table A7
Results of the Wilcoxon signed rank test for the group in Augsburg. Results marked with a star are significant after Bonferroni correction

		Resting		Standing		Feeding		Locomotion		Other	
Season 1	**Season 2**	**V**	**P-value**	**V**	**P-value**	**V**	**P-value**	**V**	**P-value**	**V**	**P-value**
Autumn	Winter	13	0.008 *	120	0.001 *	0	0.000 *	120	0.001 *	97	0.006 *
Autumn	Spring	16.5	0.015	72.5	0.221	75.5	0.394	36	0.315	51	0.725
Autumn	Summer	0	0.001 *	96	0.044	120	0.001 *	89	0.105	19	0.038
Winter	Spring	28	0.074	10	0.008	105	0.011	1	0.001 *	19.5	0.023
Winter	Summer	120	0.000 *	101.5	0.002 *	120	0.000 *	120	0.001 *	120	0.001 *
Spring	Summer	120	0.001 *	41.5	0.306	120	0.001 *	44	0.378	104.5	0.012

Table A8
Results of the Wilcoxon signed rank test for the group at Campo Pond. Results marked with a star are significant after Bonferroni correction

		Standing		Locomotion		Other	
Season 1	**Season 2**	**V**	**P-value**	**V**	**P-value**	**V**	**P-value**
Autumn	Winter	127	0.421	144	0.150	112	0.257
Autumn	Spring	182	0.004 *	187	0.002 *	171.5	0.014
Autumn	Summer	122	0.033	68.5	1	140	0.003 *
Winter	Spring	176.5	0.001 *	123.5	0.5	130.5	0.349
Winter	Summer	117	0.058	58	0.392	117	0.058
Spring	Summer	68	1	16.5	0.005 *	67	0.669