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Diurnal and ultradian rhythms of behaviour in a mare group of Przewalski horse (*Equus ferus przewalskii*), measured through one year under semi-reserve conditions

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Abstract

Investigations were conducted on four horses from a group of 12 Przewalski mares raised in different zoos and kept in a 44-ha enclosure under semi-natural conditions. Activity and feeding were continuously measured every second and were saved every 15 min by the storage-telemetry system ETHOSYS, from June 1995 to July 1996. Body mass of the horses was regularly recorded. Daily and monthly mean values, power spectra and DFC (as a measure for stability of rhythms synchronised with circadiurnal period) for activity and feeding were calculated. The general pattern of activity and feeding over the year was closely related to sunrise and sunset. Feeding accounted for 40% of total activity in summer and 62% in spring (all-year average being 52%). The level of activity was lowest in winter; whereas feeding was lowest in summer. The time budget for feeding reflected both feeding conditions and the annual pattern of body condition. Greatest activity occurred during daylight hours. Only on hot summer days, activity at night was higher than during daylight hours. Spectral analysis of activity and feeding in Przewalski horse showed a time pattern which was characterised by 24-h rhythmicity, but also by ultradian components with period lengths between 4.8 and 12 h, i.e., an activity pattern of up to five strong bouts per day. Annual variation in the pattern of power spectra was not high during the year. Results are discussed in connection with horse feeding strategy. Analysing the time structure of long-term and continuously measured activity and feeding could be a useful method to follow the

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general living conditions, especially the nutritional situation and to detect stressful conditions.

Keywords: Horse; Przewalski horse; Circadian rhythm; Ultradian rhythm; Feeding and nutrition; Telemetry; Stress

1. Introduction

Conservation of endangered species in zoos and other protected areas so far has saved several species from extinction. Strategically planned collection, preferentially concentrating on 'flagship species', is part of modern conservation (Hutchins et al., 1995). Breeding in captivity, on the other hand, poses many problems, and reintroduction of a species into its former habitat from captive-bred stock is not a simple procedure (Snyder et al., 1996). During the re-adaptation and transition from protected to natural conditions, diagnostic tools may be of importance to monitor the general state of free-ranging individuals. Accurate analysis of time budgets and rhythmic patterns of long-term and continuously measured complex behaviours (such as activity and feeding) may be such a tool. All physiological and behavioural rhythms of a healthy animal are synchronised in a meaningful way to both the time structure of the environment as well as to each other (Aschoff, 1958). Many sub-functions are included in most complex functions, such as activity and feeding, which are thus modulated by distinct biological time patterns (for instance, reproduction or digestion). Since the circadian rhythm is the most influential factor, all the other rhythms are more or less related to it. Behavioural rhythms play a major role in the ecological relations of a species and are part of its evolutionary adaptation (Aschoff, 1958). High selection pressure may change the diurnal activity pattern of a species, and nutritional chains may depend on activity rhythms (Remmert, 1969). Biological rhythms express adaptation to the annual change of the photoperiod. They also provide information on physical parameters, such as the nutritional state, social status or stress. Hence, they are tools to describe the situation of individuals and groups of animals (Tester and Figala, 1990).

Activity rhythms can be continuously recorded, and convenient analysis, for example, should indicate critical conditions. Knowledge of the normal values for a species under natural but maximally stress-free conditions is a precondition for identification of unusual deviations of diagnostic importance.

The Przewalski horse (*Equus ferus przewalskii*) is one of the ungulate species that, by being kept in zoos, has been saved from extinction. Being an attractive large mammal, it is a 'flagship species' for conservation projects. Reintroduction into its former habitat is accompanied by many difficulties and is a great challenge (van Dierendonck et al., 1996). Even if environmental conditions and organisation are optimal, the animals may have problems with natural conditions due to lack of experience, lack of acclimatisation or unsuitable physiological or morphological characteristics. The responsibility of humans for protecting animals from unnecessary suffering, pain and distress requires before reintroduction to identify and exclude individuals that are found unable to cope with natural conditions.

It was important, against this background, to analyse the basic behaviour parameters, activity and feeding of Przewalski horses in nature-like conditions at annual, daily and ultradian levels. It was our aim to describe the normal, species-specific variation of behaviour throughout the year and to demonstrate possibilities for detection of unusual or stressful conditions. Such information may, as well, provide basic comparative data relevant to the domestic horse. The methods used (long-time registration of behavioural parameters, analysis of behavioural rhythms in order to identify stress) could play an important role in the realisation of rutting seasons, illness or stress both in wild animals or extensively kept animals.

2. Material and methods

2.1. Animals

The study was conducted on a herd of the Schorfheide/Liebenthal semi-reserve (Fig. 1). They were born in different zoos and preserves and were selected for this semi-reserve and thus, for potential renaturalisation under the European Preservation-Breeding Programme for the Przewalski Horse (EPP). All were well-adapted to the semi-reserve conditions and were in good physical condition throughout the study period. Under natural conditions, a leading stallion keeps the herd together, guards and defends it against dangers and competitors and fertilises the mares. In a mare herd under wildlife conditions, a mare took the position of leading stallion and maintained it even after the herd was joined by stallions (Klimov, 1990). In our herd, the most experienced mare, Alina, exhibited typical stallion behaviour. A stable social hierarchy prevailed among the other mares during the investigation. Scheibe et al. (1997) provided information about the social structure of this herd from 1992 to 1995.

Name	Sex	Birthday	Birth place	Stud book number	Date of arrival in semireserve	Index of dominance
Alina	female	21/12/88	Cologne	1789	08/04/92	1
Sprille	female	15/04/90	Springe	4523	08/04/92	0.38
Ashnai	female	15/04/91	Cologne	4587	08/04/92	-0.08
Spirre	female	27/04/91	Springe	4680	08/04/92	-0.66
Sirena	female	13/05/91	Munich	4634	13/05/92	0.18
Nomin	female	18/05/91	Cologne	4588	08/04/92	0.12
Barbarina	female	23/05/91	Munich	4636	13/05/92	0.35
Bulgania	female	30/06/91	Duisburg	4579	13/05/92	-0.33
Mada	female	10/10/91	Nürnberg	4651	28/07/92	-0.56
Mida	female	30/11/91	Nürnberg	4557	28/07/92	-0.63
Duma	female	24/05/92	Duisburg		28/05/93	-0.9
Lulu	female	04/08/92	Cologne		28/05/93	-1

index of dominance victories defeats victories defeats

Fig. 1. The Przewalski horse mare herd of Schorfheide/Liebenthal semi-reserve.

Clear judgeable hierarchy conflicts between mares were registered in regular visual observations of the herd (see also Section 2.3). The individual index of dominance, according to Bowen and Brooks (1978) varying between +1 (absolutely dominant) and −1 (completely subdominant), was calculated for each of the animals from victories and defeats.

2.2. Location

Semi-reserves were created by the European Conservation Project for scientific research in preparation for reintroduction. They are defined as enclosures large enough to carry a certain group of Przewalski horses without a need for additional feeding throughout the year, though provisions are made for interventions that may be required for veterinary care (Zimmermann, 1997).

The Schorfheide/Liebenthal semi-reserve is situated in a forest area about 70 km north of Berlin. It is an enclosure of 0.42 km^2, with a large fenced meadow and some small plots of woodland (pine and oak). In 1990, a seed mixture was sown on this abundant field (mainly *Lolium perenne*, *Trifolium repens*). The area, up to 1994, carried a mosaic of different herb communities (above all *Urtica dioica*, *Artemisia* sp., *Cirsium arvense*, *C. vulgare*, *Dactylis glomerata*, *L. perenne*, *T. repens*, *Bromus* sp.), a development greatly attributable to horse grazing. A freely accessible watering place is connected to an automatic recording unit for registration of date, time, identification of individuals and body mass. Close to this water source are salt licks and a weather station (Digitar from 3465 Diablo Ave., Hayward, CA 94545, USA). The weather station records and saves in half-hour intervals all important weather parameters. Four raised hides at different points ensure good overseeability of this hilly semi-reserve.

2.3. Recording activity and feeding

General locomotive activity and feeding were recorded by a storage telemetry system ETHOSYS (Scheibe et al., 1998). The system consists of collars named ETHOREC (containing a measurement system, a microcontroller with memory, receiver and transmitter), a central station (ETHOLINK) and software for data transmission on a PC and graphic display (ETHODAT). An ETHOREC for horses weighs about 300 g. The sensor system of ETHOREC identifies all movements of the animal, which are passed on to the collar.

In one recording channel, general locomotor activity (following the definition of Aschoff, 1962) is recorded as the result of all movements, independent of the animal's position, whether lying or standing.

A series of prehensile bites with time intervals between 375 and 1375 ms is defined as feeding. When the head of the animal is held down and is moved in this characteristic pattern of feeding, ETHOREC identifies these actions additionally as 'feeding' on a second recording channel.

Each second, the sensors of collars were requested, results are summed up, and at the end of an analysis interval of 30 or 15 min, they are saved in the internal memory. The process of counting is then initiated again. The resulting time series are automatically transferred by radio from the collar to the central station (ETHOLINK), as soon as an animal is identified by a passive infrared detector within transmitting range. Once the data are transmitted the memory of ETHOREC is cleared for further records. The 32k RAM memory capacity of ETHOREC is sufficient for 2047 data sets (corresponding to 21 days with a saving interval of 15 min). The internal battery of ETHOREC is sufficient for about 1 year.

Previous investigations (Berger, 1993) have shown that the behaviour parameters of activity and feeding were correctly identified by collars. Visual observations on social structure, choice of food and space–time–behaviour of the herd were undertaken to ensure proper interpretation of ETHOREC data and to obtain further information about the horses' way of life. They were undertaken in 14-day intervals for all individuals (duration: 8 h, measuring interval: 15 min) and in monthly intervals for the total herd (duration: 24 h, measuring interval: 10 min).

2.4. Data analysis

Data collected by ETHOSYS from four animals (Duma, Spirre, Mada and Mida) over at least 1 year were used for this analysis. In total, a data volume of 1498 consecutive animal days was analysed.

Each of the data series of activity and of feeding was subjected to several steps of analysis.

(A) General parameters were computed from original data and were examined for monthly variation. Daily activity phases were selected. If no activity was noticed, it was defined as resting. Mean daily total activity per month was computed for each of the animals. The relationship of activity for hours of natural light to activity for hours of darkness was calculated for each of the animals and months. The time between sunrise and sunset, as the daily light period, was taken from Ahnert's astronomic table (Burkhardt et al., 1994). These monthly values were then tested for annual variation by the Friedman test and, subsequently, the multiple comparisons post-hoc test (Daniel, 1990). In case of multiple comparisons, we do not show *p*-values for the individual comparisons but only their significance.

(B) Original data series were examined for their relative amounts of rhythmic components. All data files were subdivided into data sets for seven consecutive days, with a delay of 1 day between the data sets. From these partially overlapping data files, the autocorrelation functions were computed, and the power spectra were calculated from the latter. The periods of the power spectra were tested for significance (Andel, 1984), which gave the significant periodic components of the original data series. As appropriate statistical methods are lacking, evaluation of annual variation of significant periods of power spectrum was possible only by description of three-dimensional figures (x-axis = period length, y-axis = year, subdivided into successive 7-day segments with a delay of 1 day between them, z-axis = intensity of significant period of power spectrum).

(C) The values of these significant periods are used to calculate the 'Degree of Functional Coupling' (DFC) (Sinz and Scheibe, 1976; Scheibe et al., 1999). This

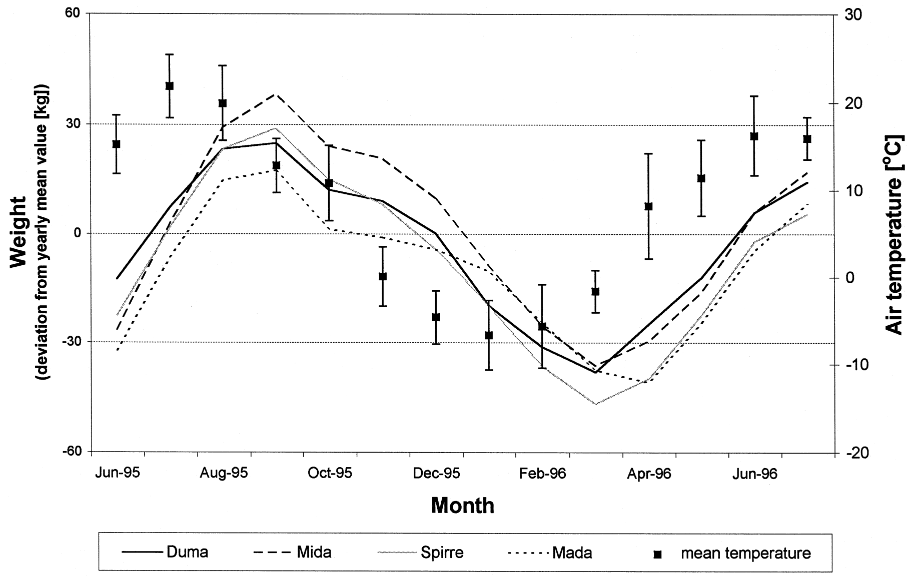

Fig. 2. Annual variation of body mass of four Przewalski horses under semi-reserve conditions (monthly mean values) and annual variation of air temperature (monthly mean values calculated from daily mean values and standard deviation).

expresses the relationship of the total of intensity of significant harmonic periods (SI(harm)) with the total of intensity of all significant periods (SI(total)) (Eq. (1)).

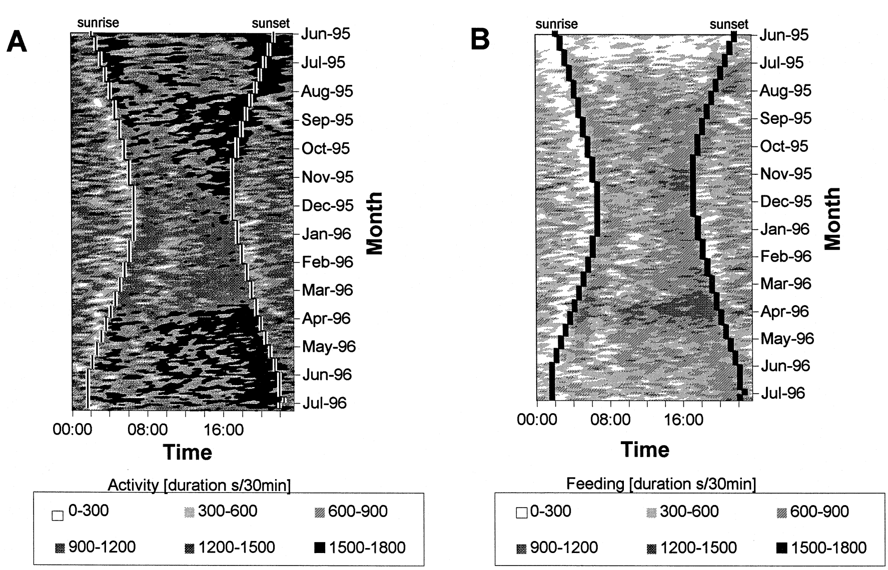

Fig. 3. (A) Activity pattern during 1 year (mean values of four Przewalski horses). (B) Feeding pattern during 1 year (mean values of four Przewalski horses).

Harmonic ultradian periods are defined as periods which were synchronised with the external circadian zeitgeber in relation to an integral number (that means 24 h divided by 1, 2, 3, etc., gives harmonic periods)

$$\mathrm{DFC}[\%] = \mathrm{SI(harm)}^{*}100/\mathrm{SI(total)} \tag{1}$$

The DFC varied between 100% (internal synchronisation of organism and between organism and environment) and 0% (desynchronisation). DFC results (calculated from power over 7 days, as explained above) were continuously mapped over the year for each of the animals. Monthly DFCs (calculated over a full month) of each animal were computed and tested for annual variation (see above).

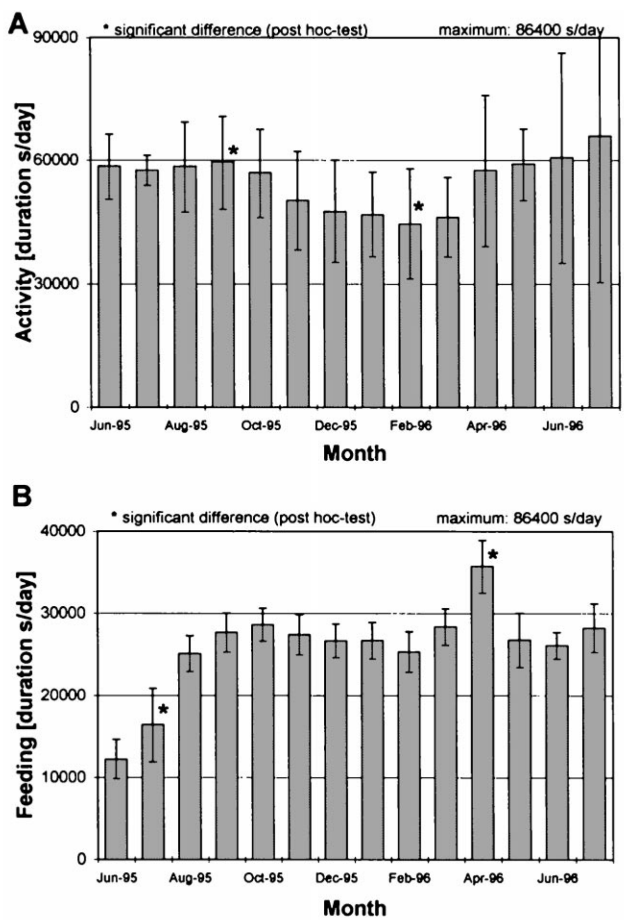

Fig. 4. (A) Annual variation of daily total activity (monthly mean values and standard deviation). (B) Annual variation of daily total feeding (monthly mean values and standard deviation). All data represent the mean value of four Przewalski horses.

3. Results

Fig. 2 shows the annual variation of air temperature and of body mass of the four horses under investigation. The four body mass curves reach their maximum in September. The highest temperatures were measured in July. Although temperature is at its lowest in January, body mass reaches its lowest point in March.

Fig. 3 demonstrates the mean annual pattern of activity (A) and feeding (B) for all four animals. Activity shows a polyphasic pattern during the day. The ultradian and daily pattern is highly variable, but the links between the two main peaks and the variation of sunrise, as well as sunset throughout the year are most clearly visible, as is the main pause in the second part of the night just before sunrise. There are several

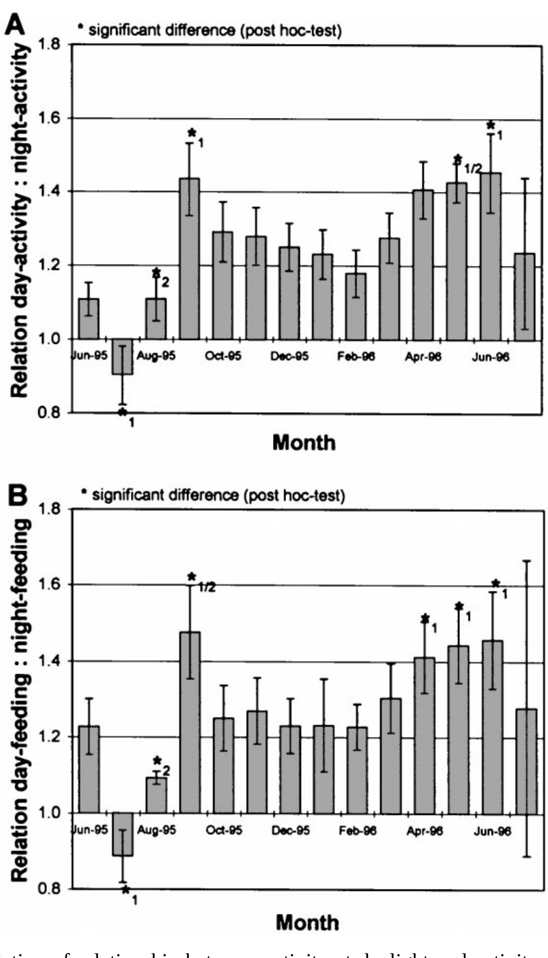

Fig. 5. (A) Annual variation of relationship between activity at daylight and activity at night (monthly mean values and standard deviation). (B) Annual variation of relationship between feeding at daylight and feeding at night (monthly mean values and standard deviation). All data represent the mean value of four Przewalski horses.

activity peaks also during the night, most of them of lower intensity and shorter than during the day. The ultradian rhythm originates mainly from feeding activity which accounts for approximately 40% of total activity time in summer and 62% in spring. During winter and autumn, 55% of total activity was feeding. The amount of daily rest is normally 48% in winter and 30% in summer (annual average: 36%). Also evident from this figure is the (occasionally leapwise) change of levels of activity and feeding over the year. Sometimes, there are changes in intensity and in the relationship between day and night activities within a few days in both parameters.

The daily levels of activity and feeding (Fig. 4) varied significantly over the months (Friedman test for activity, $n = 4$, p-value $= 0.0002$; Friedman test for feeding, $n = 4$, p-value $= 0.02$). The post-hoc test showed a significant difference in activity between September 1995 and February 1996. Decreased activity in winter and a high standard

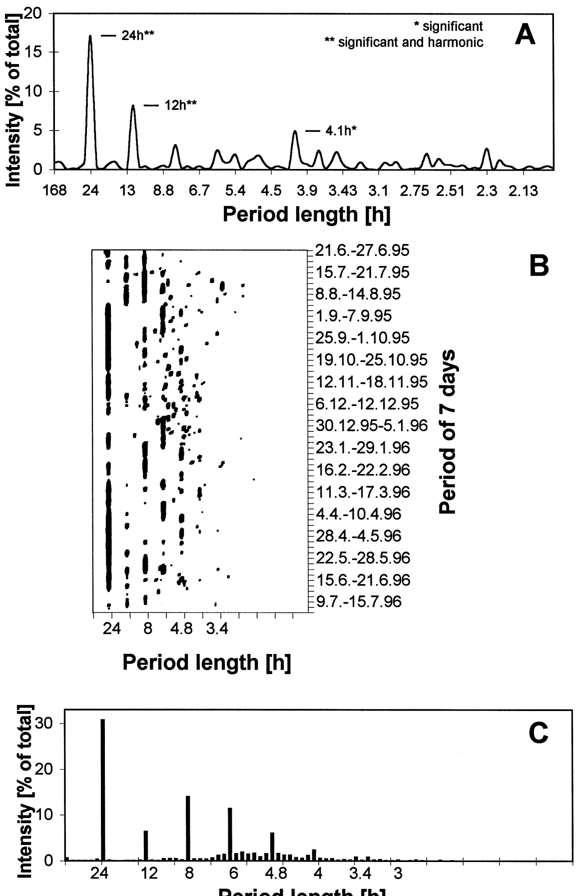

Fig. 6. (A) Typical power spectrum of activity calculated over a period of seven successive days (Przewalski horse ‘Duma’, April 18 to 24, 1996). (B) Annual variation of existence of significant periods in power spectra of activity of Przewalski horse ‘Duma’. (C) Total intensity of significant periods in power spectra of activity of Przewalski horse ‘Duma’, added up over the whole year and depicted in percentage of the total.

deviation of activity in July 1996 were recordable, as well. Feeding varied significantly between July 1995 and April 1996. Feeding was at its highest level in Spring 1996 (especially in April) but was unexpectedly low in summer (June/July 1995). This illustration also shows the levels of activity and feeding to be unrelated to each other; maxima and minima of both parameters deviated from each other.

Activity and feeding were not evenly distributed over the hours of daylight and darkness (Fig. 5). The Friedman test results showed that the ratio of daytime activity/feeding to nighttime activity/feeding was not equally distributed ($n = 4$, p-values $= 0.001$). There were significant differences in activity between July 1995 and September 1995/May 1996/June 1996 as well as between August 1995 and May 1996. Feeding varied significantly between July 1995 and September 1995/April 1996/May 1996/June 1996 as well as between August 1995 and September 1995. July 1995 was

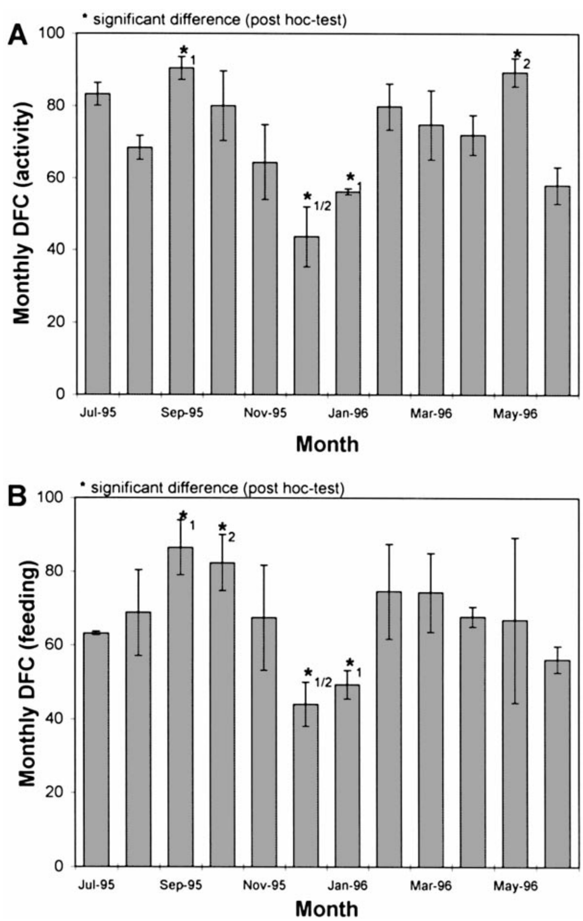

Fig. 7. (A) Monthly DFC for activity during 1 year (mean values and standard deviations of four Przewalski horses). (B) Monthly DFC for feeding during 1 year (mean values and standard deviations of four Przewalski horses).

the only month with higher activity and feeding at night than activity and feeding at day. In all other months, daytime activity and feeding were higher than those at night. The standard deviation in July 1996 again was extremely high.

Fig. 6 shows a typical power spectrum for activity of one animal during a 7-day period (A). Detected in this example were the 24-h period, a significant harmonic period of 12-h length and a significant nonharmonic period of 4.1-h length. The typical occurrence of all significant periods of activity over the year, using the example of one animal in a three-dimensional figure (as described in Section 2) is shown (B). No systematic variation was obvious during the year for the ultradian or the 24-h component. These diverse (significant) intensities of different periods were summed up over the year and were depicted in a standardised way as percentage relative to the total (C). The strongest period was the circadian (30.8% of total), followed by the 8-h period (14.0%) and 6-h period (11.4%).

Fig. 7 displays the monthly DFCs of activity (A) and feeding (B) of all animals over one full year. The DFCs (calculated over a month) varied in general, but were especially low during Winter and Summer 1996. There was a significant annual variation of monthly DFCs (Friedman test for activity, $n = 4$, p-value $= 0.0007$; Friedman test for feeding, $n = 4$, $p = 0.0067$). Monthly DFCs of activity were significantly different from each other between December 1995 and September 1995/January 1996/May 1996. The same significance was exhibited by monthly DFCs of feeding between September 1995 and December 1995/January 1996, as well as between October 1995 and December 1995.

Time series were recorded from one animal during its stay in the zoo, its transportation from zoo to the semi-reserve and in the subsequent period in the semi-reserve, and

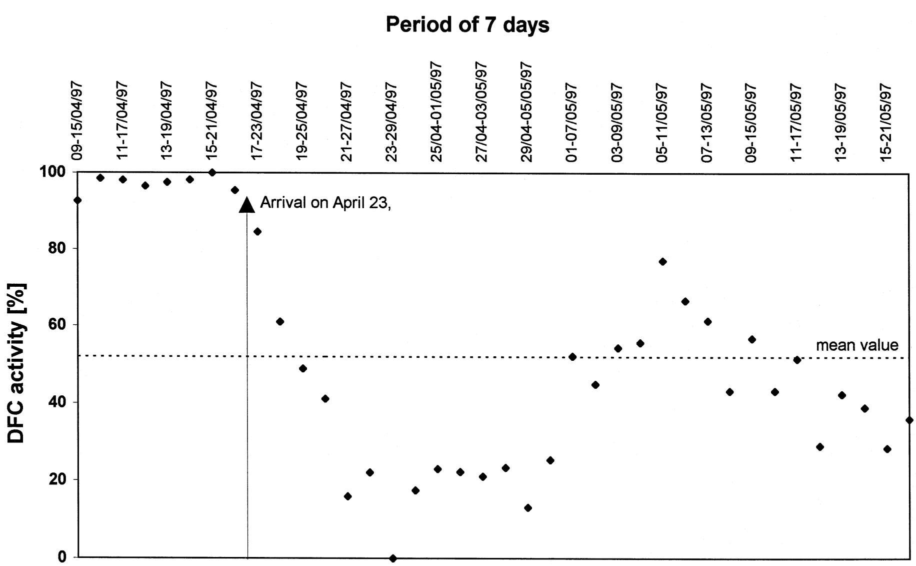

Fig. 8. DFCs of activity of Przewalski horse 'Medi', from April 9 to May 22, 1997 (calculated over overlapping periods of 7 days with a delay of 1 day). On April 23, 'Medi' was transferred from a zoo to the semi-reserve.

DFCs of activity are depicted as an example of adaptation to semi-natural conditions (Fig. 8). DFCs were very high during the zoo period, drastically lower during the period of adaptation, and slowly recovered to mean values which, however, were even clearly lower than the mean DFC of activity for the total herd during the same period of time (80.6%).

4. Discussion

Complete annual activity pattern of Przewalski horse in a natural environment has not been described, as yet. Information is available on the activity pattern in zoos (Bubenik, 1961), and the time budget has been observed in restricted periods on pasture (for example, by Boyd et al., 1988; Sauerland, 1992). Our own former telemetric records on the activity of Przewalski horse were restricted to sample periods during wintertime (Berger and Scheibe, 1994). Also, regarding free-ranging domestic horses and other equids, only general descriptions exist of annual and daily activity cycles, based on short sampling periods (Klingel, 1967; Kownacki et al., 1978; Schäfer, 1978; Keiper and Keenan, 1980; Kaseda, 1983; Arnold, 1984; Bogner and Grauvogl, 1984; Duncan, 1985; Mayes and Duncan, 1986; Fraser, 1992). However, the diagnostic value of continuous activity records has been demonstrated for domestic horses by Gill (1991).

The results present a typical year in this mare herd under semi-natural conditions:

-Spring: Body mass of horses is at its lowest. Average daily feeding is higher than at any other time during the year. Activity level is generally high.

-Summer: Body mass is increasing. Feeding levels drop to the minimum in the year. Activity levels are relatively high. During hot summer days, activity change to be higher at night than at daytime.

-Autumn: Body mass of horses is at its maximum. Activity and feeding levels are generally high.

-Winter: Body mass of horses is decreasing. Average of activity on one whole day is lower than at any other time of the year. Feeding level is generally high.

Activity and feeding behaviour of the same herd of Przewalski horses based on observational data collected in the course of 2.5 years were described by Scheibe et al. (1997). The observation period of Scheibe includes, in the first year, an adaptation process to nature-like conditions, but just after the first winter in the semi-reserve, these purely visual observations revealed a similar annual pattern as presented above.

In this investigation on automatically obtained data, the percentage of resting behaviour during the whole day (mean: 36.4% $\pm$ 15.7%) was higher in winter (48.4% $\pm$ 15.4%) than in summer (30.7% $\pm$ 29.6%). In the literature, the percentage of resting behaviour during the whole day ranges from 4.6% (Arnold, 1984) to 35.1% (Lobanov, 1983). Views of several authors differ from each other even more with regard to points in time of the main rest phases (see Lobanov, 1983; Boyd et al., 1988), and homogeneous statements cannot be derived from these investigations based on mere observation of the animals.

Literature statements about percentages of feeding behaviour per day partly differ from each other due to the various observation intervals and observation methods (for

Przewalski horse: 2.9–37.8% [Bubenik, 1961], 39.7% [Lobanov, 1983], 46.4 ± 5.9% [Boyd et al., 1988]; for Koniks: 55.3–69.6% [Kownacki et al., 1978]; for Camargue horses: 60–70% [Duncan, 1985], 51–63% [Mayes and Duncan, 1986]; for domestic horses: 17–67% [Arnold, 1984]). Data of this investigation showed the mean relative amount of real feeding time per day to be 29.8% ± 13%, with a minimum in June (14.1% ± 2.7%) and a maximum in April (41% ± 3.7%). The main periods of feeding during a 24-h day were always timed at dusk and dawn (Bubenik, 1961; Keiper and Keenan, 1980; Kaseda, 1983; Mayes and Duncan, 1986; Boyd et al., 1988). Long interruption of grazing activity was observed at noon in the months of summer (Rubinstein, 1981; Mayes and Duncan, 1986). In summer, horses increasingly shifted their search for food to the hours of night to avoid disturbance by flying insects and high temperatures (Kaseda, 1983; Duncan, 1985; Mayes and Duncan, 1986; Boyd et al., 1988). In the course of the night, grazing activity of horses is lower and resting behaviour increases (Keiper and Keenan, 1980). All these statements were confirmed by the results obtained from this investigation.

The results showed the level of activity on daytime to be higher than that at night, though Przewalski horses, nevertheless, were looking for food and water also at night. However, they clearly stood closer to each other and were more vigilant and reactive towards environmental influences than they would be on daytime. Only in July 1995, were activity and feeding at night higher than on daytime due to high temperatures and disturbance by flying insects. Intensity of activity and feeding vary independently from each other, although the time patterns of both parameters are nearly the same.

The activity budget of Przewalski horses after introduction to a Mongolian preserve in the course of 1.5 years was reported by van Dierendonck et al. (1996). These animals gave no clear pattern of behaviour during the first summer and winter. Only after the first winter season data of van Dierendonk indicates very well the variation of feeding conditions in the different seasons and roughly follows the pattern confirmed in our investigation. However, observations of van Dierendonk were made only in daylight, and more subtle differences could not be identified by that sampling procedure. The relationship between feeding time and activity, as recorded in the observation of van Dierendonk, may become more informative against the background of results in this investigation. Deviations from the annual pattern described there can be explained by the fairly long period of adaptation to the annual variation of climatic and, even more, nutritional conditions, as described also by Scheibe et al. (1997).

There clearly is a resting phase before sunrise, almost during the whole year. The subsequent activity peak is correlated with dawn, whereas no external zeitgeber is recognisable for the onset of this resting phase, approximately at the same time. This shows a complex interaction between a light-insensitive ultradian rhythm and the circadian rhythm, as described by Gerkema et al. (1993).

For the first time, automatic acquisition of data gave us an insight into the complex rhythmic structure of behaviour by means of power spectral analysis of the parameters of activity and feeding in Przewalski horse.

The power spectra of activity clearly show the close link to the 24-h period. Conspicuously high contributions of ultradian harmonic periods, between 12 and 4.8 h in lengths, were found in the power spectra of horses. These ultradian periods generated two to five activity bouts per day. Ultradian nonharmonic periods between 6.7 and 4 h in length were important in the power spectra of horses only in certain phases. The general annual variation of significant periods does not show a clear annual pattern.

Common basic principles are visible when comparing the strategies of feeding of most free-ranging herbivores. They respond to decreasing availability of suitable food either by taking in smaller quantities and selecting only easily digestible plants or parts of plants (concentrate selector; e.g., roe deer), or by consuming more food of lower quality (grass and roughage eaters; e.g., cattle and sheep) (Hofmann, 1989). A third one varies between the two first, by changing its physiology and behaviour and by that being adapted to food conditions (intermediate type; e.g., red deer). Horses meet their nutritional demands by increasing their food intake to compensate for the lower nutrient content of the food (Fujikura et al., 1989; van Soest et al., 1995; van Wieren, 1995) and for that they are roughage eaters. This means only change in quantity of feeding and not change of digestion or transformation of behaviour rhythmic. Therefore, the low annual variance measured in the pattern of power spectrum (and, consequently, in fine structure of this behaviour parameter) of feeding is indicative of the general strategy of horses as roughage eaters.

The results of this investigation clearly show an increase of feeding activity relative to locomotor activity during the winter season. By concentrating all remaining activity on feeding, horses economise on energy in this strenuous time. In late February, the grass was only 2–3 cm. Video recording provided evidence to the effect that the specific plucking movements of the head for grazing occurred quicker and in shorter intervals than in summer. Apart from generally preferred feeding plants, mares now fed also on mugwort (*Artemisia* spp.), dry leaves, bark and ants. This was in agreement with findings reported by Mayes and Duncan (1986) according to which the originally high selection of food by horses dropped to zero when the supply of preferred feeding plants decreased below a certain threshold.

The ‘DFC’ (as an objective parameter of coordination of different organismic functions both with each other and with the external circadian zeitgeber) enabled assessment of the organismic state (Scheibe et al., 1999). This has been verified by investigations on lambs (Scheibe and Sinz, 1974), ewes (Sinz and Scheibe, 1976), cattle (Langbein, 1991) and alpacas (Ziller, 1991).

The investigation shows a generally low DFC of activity for all four horses in late autumn and early winter and in July 1996.

The low DFC level in late autumn and early winter could be attributed to a decline in nutritive supply as well as to low temperature and snow. Competition for food was observed only in periods of snow, when the horses defended places cleared from snow. On the other hand, lowered DFC coincided with the hunting period in the surrounding area. It ended at the end of the year and the DFC began to recover at the same time, while food supply was still decreasing.

The sudden decrease of DFC in July 1996 can be explained by the opening of a nearby shooting range at the same time. Visual observations repeatedly confirmed the intensive reaction of the horses to the sound of shooting (flight, high vigilance, interruption of resting and feeding periods). When noise levels on the shooting range were reduced, horse DFC increased. Observations on short-time reactions to different kinds of stressor on red deer and roe deer showed that heart rate reaction to shooting sounds was the greatest, as compared to all acoustic disturbances (Herbold et al., 1992).

Valid experiments about effects of stressors on DFCs on Przewalski horses do not exist until now. Drastically decreased DFC during adaptation to semi-natural conditions (see Fig. 8) was one of several accidentally found on–off observations. As our experience shows, long-term DFC below 60% associated with strong changes in activity levels, is indicative of deviation from standard conditions of Przewalski horses. The measuring and analysing methods may be of some use in the detection of serious stressful conditions during reintroduction of animals to the wild or during extensive keeping of animals. In this case, it would be useful to combine the measuring method ETHOSYS with a location system to receive data about the use of space by the animals.

Additional investigations including those on reactions to different forms of stressor (e.g., heat, pregnancy, birth, wounds, lack of resources and others), are necessary to define species-specific standards of behaviour-determining requirements of the environment. Further investigations might elucidate the extent to which the significant annual variations demonstrated in this investigation were determined by usual yearly rhythm. Collecting power spectra over a whole year of other herbivores would be of interest for comparative studies.

5. Conclusion

Continuous records of behavioural rhythms of locomotor activity and feeding can be applied to describing the adaptation of individuals to a natural environment. Changing relations between daytime and nocturnal activities are indicative of heat load and disturbance by insects. Stress due to human interference can be identified by rhythmic analysis. This form of analysis is also convenient to follow up individual adaptational processes, such as introduction into a new herd.

Basic data from nature-like but protected areas can be used for comparison to records under less controlled conditions. In such circumstances, continuous behaviour records should be applied to identify and prevent risk situations of individuals and groups.

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