Chronobiological Investigations on Przewalski Horse (*Equus ferus przewalskii*) and Red Deer (*Cervus elaphus*) under Quasi-Natural Conditions and Possible Approaches to Chronobiological Diagnosis of Stress

Chronobiologische Untersuchungen an Przewalskipferd (*Equus ferus przewalskii*) und Rothirsch (*Cervus elaphus*) unter naturnahen Bedingungen und Möglichkeiten der chronobiologischen Belastungsdiagnostik

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Jede Methode ist Rhythmus.

Novalis

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1. Einleitung

Es gibt kaum ein Tier auf dieser Welt, das sich den Auswirkungen menschlicher Aktivitäten entziehen kann. Abgesehen von teilweise unberechenbaren Einflüssen des Menschen auf Klima, Boden, Vegetation und Wasser, mit denen der Mensch indirekt auf Tiere und Pflanzen einwirkt, besteht ein breites Spektrum direkter Beziehungen zwischen dem Menschen und verschiedenen Tierarten. In diesen Beziehungen ist der Mensch aufgrund seiner erhöhten Handlungsfreiheit und seiner Einsicht in natürliche Zusammenhänge dem Tier gegenüber verantwortlich, es vor unnötigen schädigenden und Leiden verursachenden menschlichem Handeln zu schützen (SCHEIBE 1997). Um dieser Verantwortung gerecht zu werden, ist es unumgänglich, tierisches Leiden schnell zu erkennen und die Belastungsbedingungen dieser Tiere objektiv einzuschätzen. Dies gilt sowohl für landwirtschaftliche Nutztiere, als auch für Labor-, Haus-, Zoo- und Wildtiere.

Untersuchungen an Wildtieren unter natürlichen bzw. naturnahen Bedingungen sind besonders schwierig, da diese Tiere aufgrund ihrer versteckten Lebensweise, hohen Fluchtdistanz und Wehrhaftigkeit schwer handhabbar und die Umweltbedingungen in diesen Untersuchungen nicht normierbar sind. In den letzten Jahren wurde aber deutlich, daß es notwendig ist, gerade auch freilebende Wildtiere vor anthropogenen Störungen und belastenden Bedingungen zu schützen (INGOLD 1991, KIRKWOOD et al. 1996, COOPER 1998). Es ist demnach eine dringende wissenschaftliche Aufgabe und Herausforderung, geeignete Methoden zu entwickeln, mit denen Belastungsbedingungen bei freilebenden Wildtieren erkannt und beurteilt werden können.

Zur Untersuchung von Belastungsbedingungen dienen zum einen Messungen verschiedener physiologischer Parameter wie Herzfrequenz, Adrenalinspiegel oder Corticosteroidspiegel (STOTT 1981, BLACKSHAW 1986, JONES et al. 1990, WAGNER 1992). Physiologische Parameter lassen sich allerdings nur sehr bedingt am Wildtier im Freiland messen, da die Methoden zu stark auf das Tier rückwirken, geeignete Verfahren sehr kostenintensiv sind oder schlichtweg fehlen. Eine Alternative bieten Untersuchungen zum Verhalten der Tiere. Verhalten als Organismus-Umwelt-Beziehung auf der Grundlage eines Informationswechsels (TEMBROCK 1987) gibt Auskunft über den qualitativen Zustand der Organismus-Umwelt-Interaktion und damit auch über den inneren Status des Individuums. Da sich das Verhalten früher neuen Gegebenheiten anpaßt als physiologische Prozesse und anatomische Merkmale, können Belastungszustände für den Organismus durch Verhaltensveränderungen eher gekennzeichnet werden, als durch physiologische Veränderungen (SAMBRAUS 1978, SCHEIBE 1987). Mit den Verfahren der verhaltensorientierten Statusdiagnostik wird versucht, ein möglichst genaues Bild vom inneren Status eines Tieres zu erhalten. Dieser innere Status wird sowohl durch physiologische Prozesse (Brunst, Trächtigkeit, Krankheit), durch Störungen im sozialen Gefüge als auch durch andere Belastungsformen beeinflußt. Mittels einer langzeitigen individuellen Statusdiagnose können dann schon sehr frühzeitig Belastungsfaktoren für den Organismus erkannt werden.

Um den internen Status eines Individuums einzuschätzen, können einerseits rein quantitative Veränderungen im Verhalten gewertet werden und andererseits Zeitmuster (von langfristig und kontinuierlich gemessenen komplexen Verhaltensparametern) analysiert werden. Die Orientierung in der Zeit ist für jeden Organismus von herausragender Bedeutung. Es ist ein elementarer Anpassungsmechanismus, interne Zeitmuster mit äußeren Periodizitäten zu synchronisieren (ASCHOFF 1959, REMMERT 1969). Dabei ergeben sich sowohl eine zeitliche Abstimmung zwischen Organismus und Umweltperiodizitäten als auch eine hierarchische Funktionsabstimmung innerhalb des Organismus. Bedingungen, die das Individuum belasten, stören diese komplexen rhythmischen Kopplungen und verändern das artspezifische tagesperiodische Aktivitätsmuster (VEISSIER et al. 1989, BUCHHOLTZ 1993). Biorhythmische Analysen erfassen die zeitliche Abstimmung zwischen Organismus und Umweltperiodizitäten und die zeitliche Abstimmung innerhalb des Organismus. Unter belastungsneutralen Bedingungen stellen sich dabei vorzugsweise ganzzahlige Frequenzverhältnisse zwischen Aktivitäten verschiedener Funktionskreise des Organismus ein, die gemeinsam auf einen Zeitgeber (die externe 24-Stundenperiodik) abgestimmt sind. Demgegenüber zeigten die Aktivitäten von Tieren in Belastungssituationen verstärkt Periodika, die von der Zeitgeberfrequenz und ihren ganzzahligen Verhältnissen abweichen (SCHEIBE et al. 1974). Auf diesen Erkenntnissen basiert die biorhythmische Statusdiagnostik (SCHEIBE et al. 1978), mit der anhand der tages- und stundenrhythmischen Struktur langzeitig gemessener komplexer Funktionen der organismische Status eingeschätzt und (dem Menschen normalerweise unauffällige) Systemstörungen festgestellt werden können.

Um mit den Verfahren der biorhythmischen Statusdiagnostik Belastungsbedingungen bei Tieren erkennen zu können, sind langfristige, lückenlose Verhaltensaufzeichnungen mit festen Meßintervallen und gleichbleibender Meßgenauigkeit erforderlich. Die Methode zur Datenerfassung sollte dabei möglichst vielfältig einsetzbar, einfach zu handhaben und rückwirkungsfrei sein. Außerdem ist das Wissen um die Normalwerte einer Art unter natürlichen und so weit wie möglich stressfreien Bedingungen Voraussetzung, um ungewöhnliche Abweichungen von diagnostischer Relevanz erkennen zu können. Da sich Zeitmuster der Tagesrhythmik im Jahresverlauf ändern, kann eine solche Definition von artspezifischen Normwerten zudem nur jahreszeitbezogen erfolgen.

Ziel der vorliegenden Arbeit war es zum einen, verschiedene Methoden, die Verhaltensparameter an freibeweglichen Tieren automatisch erfassen können, zu erproben und direkt miteinander zu vergleichen. Es sollten Vor- und Nachteile der verschiedenen Methoden, deren Meßgenauigkeiten und mögliche Anwendungsgebiete aufgezeigt werden. Letztendlich sollte die Auswertung der Versuche Hinweise geben, inwieweit die verschiedenen Methoden bei einer langzeitigen Statusdiagnose am Wildtier geeignet sind und verwendet werden können. Dieser Teil der Arbeit ist im *Kapitel II "Darstellung und Vergleich verschiedener Meßsysteme"* behandelt. Das Speicher-Telemetrie-System ETHOSYS®, welches die motorische Aktivität und das Fressen an großen Herbivoren mißt, wird im *Abschnitt 2.1.* vorgestellt. Dieses System wurde in einem direkten Vergleich einem Funk-Telemetrie-System gegenübergestellt, daß die Herzfrequenz, die Körpertemperatur und die Aktivität an Rothirschen und Rehen erfaßt (*Abschnitt 2.2.*). Zwischen dem Kauschlagzähler APEC, der Fressen und Wiederkauen bei Milchvieh registriert, und ETHOSYS® wurde ein zweiter Vergleich durchgeführt (*Abschnitt 2.3.*).

Mit dem Speicher-Telemetrie-System ETHOSYS® wurden dann an zwei verschiedenen Tierarten Untersuchungen zum Zeitmuster der motorischen Aktivität und des Fressens über die Dauer eines Jahres durchgeführt. Diese werden in *Kapitel III "Chronobiologische Untersuchungen an zwei verschiedenen Herbivorenarten"* dargestellt. Um letztlich artspezifische Normwerte ableiten zu können, fanden die Untersuchungen unter möglichst ungestörten und naturnahen Bedingungen statt. Die Arten Przewalskipferd (*Equus ferus przewalskii* Poljakov) und Rothirsch (*Cervus elaphus* Linné) wurden bewußt gewählt, da es sich bei ihnen um zwei Herbivore handelt, die verschiedene Ernährungsstrategien verfolgen und sich daraus interessante vergleichende Aspekte ergeben. Der Rothirsch gehört in dem System der Wiederkäuer-Ernährungstypen zur Gruppe der Intermediärtypen (HOFMANN 1989), das Przewalskipferd ist dagegen kein Wiederkäuer und beim Typ des Rauhfutterfressers einzuordnen. *Abschnitt 3.1.* zeigt die Untersuchungen zum Przewalskipferd, *Abschnitt 3.2.* die zum Rothirsch.

Nach den methodischen und praktischen Aspekten meiner Arbeit stand die Frage, ob auf diesem Wege Belastungsbedingungen oder-situationen bei Wildtieren erkannt und bewertet werden können. Diesem Teil meiner Arbeit widmet sich *Kapitel IV "Methoden und Ergebnisse zur nicht-invasiven Statusdiagnose bei verschiedenen freibeweglichen Herbivorenarten"*. Hierbei wird auf Möglichkeiten und Ergebnisse der biorhythmischen Statusdiagnose bei verschiedenen Tierarten eingegangen (*Abschnitt 4.1.*). Ergänzend wurden in einem Versuch an gegatterten Rothirschen Ergebnisse aus der biorhythmischen Statusdiagnose (Verhaltensparameter) direkt mit denen aus Messungen der Cortisolmetabolitspiegel im Kot (physiologischer Parameter) verglichen (*Abschnitt 4.2.*).

Bei den hier beschriebenen Abschnitten dieser Dissertation handelt es sich immer um einzelne (bereits gedruckte bzw. eingereichte) Veröffentlichungen, die sich meist ihrerseits *in Einleitung, Methode, Ergebnisse, Diskussion* und *Literatur* gliedern und die ausschließlich gemeinschaftlich erbracht wurden. In den Titelblättern dieser Veröffentlichungen/Abschnitte sind jeweils der *eigene Anteil* an dieser Arbeit und nochmals das direkte *Teilziel* erklärt. Einigen *Abschnitten* ist ein *Anhang* nachgestellt, der Ergebnisse enthält, die in der Veröffentlichung keinen Raum fanden, mir im Bezug auf diese Dissertation aber wichtig erschienen. Im *Kapitel V "Generelle Zusammenfassung"* werden die wichtigsten Ergebnisse aller Teilarbeiten kurz in den Gesamtzusammenhang gebracht.

1. Introduction

There is hardly any animal in the world that is capable of evading the effects of human activity. Apart from partly unpredictable influences of man on climate, soil, vegetation and water through which man acts indirectly on animal and plant life, there is a wide range of direct relationships between man and various animal species. Man, on account of his wider freedom of action and insight into natural implications, has to bear responsibility for protecting animals from human action that might unnecessarily cause avoidable pain and suffering (SCHEIBE 1997). In order to live up to this responsibility, one has to realise without undue delay suffering of animals and make a proper, unbiased assessment of stressful conditions to which animals are exposed. This principle is equally valid for farm, laboratory, domestic, zoo and wild-living animals.

Studies on wild-living animals are extremely difficult to do under natural or quasi-natural conditions, as it is not easy to handle such animals because of their hidden way of life, long flight distance and defence capability. Hence, it is not possible to standardise the environmental conditions under which such studies may be undertaken. Nevertheless, experience obtained in recent years has clearly underlined the need for shielding also feral species against anthropogenic disturbance and stressors (INGOLD 1991, KIRKWOOD et al. 1996, COOPER 1998). It is, therefore, an important task of research and a scientific challenge to develop appropriate methods for identification and evaluation of stress conditions with relevance to wild-living animals.

Measurement of various physiological parameters, no doubt, proves helpful in stress-related investigations, for example, checks on heart rate as well as on adrenaline or corticosteroid levels (STOTT 1981, BLACKSHAW 1986, JONES et al. 1990, WAGNER 1992). However, there are several reasons for which physiological parameters are recordable only to a limited extent from feral animals under wildlife conditions. Appropriate procedures either are not available at all or are very expensive or may have a feedback impact on probands. Investigations of animal behaviour may offer an alternative. Behaviour, as an organism-environment relationship on the basis of exchange of information (TEMBROCK 1987), may be a source of information on the qualitative condition of organism-environment interaction and thus on the internal status of the individual. Conditions of stressful impact on the organism can be noticed and characterised sooner when looking at change of behaviour rather than change in physiology, since behavioural adaptation to changing conditions comes much earlier than physiological or anatomic adaptation (SAMBRAUS 1978, SCHEIBE 1987). Methods of behaviour-oriented status diagnosis are used in an attempt to obtain with greatest possible accuracy insight into the internal status of an animal. The internal status of an individual may depend on a number of conditions, including physiological processes, such as rut, pregnancy and illness, social disorder and other stress-related aspects of life. Long-term individual status diagnosis, consequently, may be an effective tool for very early detection of factors of stressful impact on the organism.

The internal, say, endogenic status of an individual may be judged from purely quantitative changes in behaviour as well as from time patterns, i.e. long-time, high-continuity complex analysis of behaviour parameters. Orientation in time has proved to be of outstanding significance to any organism. Synchronisation of endogenic time patterns with exogenic periodicities is an elementary mechanism of adaptation (ASCHOFF 1959, REMMERT 1969). This is a process which is accompanied by timing between the organism and environment-associated periodicities as well as by hierarchic functional fine-tuning within the organism. These complex rhythmic couplings are likely to be disturbed, and the species-specific pattern of daytime activity will be changed if the individual becomes exposed to conditions of stress (VEISSIER et al. 1989, BUCHHOLTZ 1993). Timing between organism and environmental periodicity and timing within the organism can be measured by means of biorhythmic analysis. Under stress-free (neutral) conditions, there will preferably be integral-number frequency relations among the activities of various functional circuits of the organism which are all tuned to one zeitgeber, i.e. exogenic 24-hour periodicity. The activities of animals exposed to stress situations, on the other hand, will increasingly exhibit periodicities that deviate from the zeitgeber frequency and its integral ratio (SCHEIBE et al. 1974). These findings provide the very basis for biorhythmic status diagnosis (SCHEIBE et al. 1978) by which the given organismic status can be evaluated against the background of daily and hourly rhythmicity of complex functions as measured over extended periods of time, a prerequisite for identification of system disorders (that would usually escape human attention).

To be successful in identifying stress conditions of animals, the procedures of biorhythmic status diagnosis will depend on long-time, gapless behaviour recording with constant measurement intervals and constant measuring accuracy. The method used for data acquisition should be of highly flexible applicability, easy to handle and without any impact on the animal. Knowledge of the normal values of the species reviewed is another prerequisite for success, i.e. values under natural and largely stress-free conditions. This is essential to realising deviations of diagnostic relevance. As time patterns of diurnal rhythmicity are subject to variation in the course of the year, such values of normal species-specific behaviour have to be defined with reference to seasons.

This study was conducted with the view to testing and comparing various methods for automatic measurement of behavioural parameters on free-ranging animals. Benefits and drawbacks of such methods were to be examined together with measuring accuracy and possible applications. The findings were to be evaluated to show the extent to which the methods were suitable for and applicable to long-time status diagnosis of wild-living animals. This part of the study is described in greater detail in *Chapter II* under the heading of *"Description and Comparison of Measuring Systems"*. The storage telemetry system ETHOSYS® for measurement of the locomotor and feeding activities of big herbivores is presented in *Sub-Chapter 2.1*. This system was directly compared (*Sub-Chapter 2.2*.) to a radio-telemetry system recording heart rate, body temperature as well as activity of red deer and roe deer. Another comparison was made (*Sub-Chapter 2.3*.) between ETHOSYS® and the APEC chew-beat counter by which eating and rumination of dairy cattle are recorded.

The storage telemetry system ETHOSYS® then was used on two different species to investigate time patterns of locomotor activity and feeding for a period of one year. These investigations are reported in *Chapter III* under the heading of *"Chronobiological Investigations on Two Herbivorous Species"*. They were conducted under widest possible undisturbed, quasi-natural conditions, as our interest was focused on normal species-specific values. The two species Przewalski horse (*Equus ferus przewalski* Polyakov) and red deer (*Cervus elaphus* Linné) were deliberately chosen, since they were two herbivores that pursue different feeding strategies and thus provide for interesting comparative aspects. Red deer belongs to the category of ruminant feeders (HOFMANN 1989) and thus is associated with the group of intermediate types, whereas Przewalski horse is no ruminant and comes up in the class of roughage eaters. The studies on Przewalski horse are reported in *Sub-Chapter 3.1*. and those on red deer in *Sub-Chapter 3.2*..

The question arose, against the background of the methodological and practical aspects of my study, whether this was an adequate approach to identifying and evaluating stress conditions and stress situations of wild-living animals. This aspect of my work is covered in *Chapter IV* under the heading of *"Methods and Results of Non-Invasive Status Diagnosis of Various Free-Ranging Herbivorous Species"*. Reference is made, in this context, to possible approaches to and results obtainable from biorhythmic status diagnosis of various animal species (*Sub-Chapter 4.1*.). In a complementary investigation on fenced red deer, results obtained from biorhythmic status diagnosis (behavioural parameters) were directly compared with findings recorded from measurement of cortisol metabolite levels in faeces (physiological parameters) (*Sub-Chapter 4.2*.).

The above sub-chapters of this doctoral thesis have been printed or submitted for separate publication. Most of them are made up of *Introduction, Methods, Results, Discussion* and *References*, and all of them are based on joint authorship. My *own contribution* and *specific sub-purpose* of work are clearly revealed in the cover pages of all publications / sub-chapters. Some of the *sub-chapters* have attached to them an *Annex* of results which I considered to be of relevance to this doctoral thesis, although no space had been available for them within the text body of the publications concerned. The most important results from all sub-chapters / sub-investigations are briefly put into context in *Chapter V* under the heading of *"General summary"*.

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2. Description and Comparison of Measuring Systems

2.1. ETHOSYS® - New System for Recording and Analysis of Behaviour of Free-Ranging Domestic Animals and Wildlife

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Eigenanteil**:**
- praktische Anpassung von ETHOSYS® an die Tierarten Przewalskipferd und Rothirsch:
  - Frequenz des Rupfens beim Grasen visuell ermittelt und die Größe des Frequenzfilters beim ETHOREC für die Arten Przewalskipferd und Rothirsch festgelegt,
  - Schutzumhüllungen und Befestigungen für die Meßhalsbänder angefertigt und erprobt,
  - Halsbänder angelegt und abgenommen
- Überprüfung der Funktionstüchtigkeit der Meßhalsbänder an Przewalskipferd und Rothirsch:
  - visuelle Paralleluntersuchungen durchgeführt,
  - Übereinstimmung zwischen den Beobachtungsdaten und den mit ETHOSYS® gemessenen Daten geprüft (Korrelationsberechnungen durchgeführt)
- Korrektur des Manuskriptes

Ziel der Arbeit:
Um die gestellten Gesamtziele zu erreichen, galt es, eine geeignete Methode zu entwickeln, die es am freibeweglichen Tier ermöglicht, über einen langen Zeitraum hinweg möglichst lückenlos Verhaltensparameter zu erfassen. Das grundsätzliche Konzept zu ETHOSYS®, die Erkennung der Verhaltensparameter und das Prinzip der Datenspeicherung und -übertragung betreffend, stammte aus dem Institut für Zoo- und Wildtierforschung Berlin (Forschungsgruppe Raum-Zeit-Orientierung). In Zusammenarbeit mit der IMF electronic GmbH (Frankfurt/Oder) erfolgte dann die technische Entwicklung und Optimierung von ETHOSYS®. Diese neue Methode sollte an verschiedenen Tierarten erprobt und in Hinblick auf Meßgenauigkeit, mögliche Fehlerquellen und Anwendungsgebiete beurteilt werden.

ABSTRACT
A storage telemetry system has been developed to monitor domestic animals and wildlife and has been tested under variable conditions on sheep, Przewalski horse and mouflon. It can be used for automatic recording of different patterns of behaviour, such as activity and feeding, and is based on advanced analysis of sensor-emitted signals. The system is made up of collars (ETHOREC) with sensors and electronic devices for behaviour recording, a central station (ETHOLINK) and software for data transmission and processing (ETHODAT). All components of the ETHOREC recording device are integrated in the collar. Long-time recording of behaviour through up to four different channels and in numerous animals at one and the same time are necessary elements to facilitate biorhythmic analysis of animals under free-ranging conditions.
The results obtained from this telemetry system were compared with visual observations on six sheep and four Przewalski horses. Parallel recordings were taken from four sheep, using a recorder for jaw movements. Locomotor activity usually was rated somewhat higher by observers, whereas feed uptake was rated lower. Higher feed uptake values were measured by means of the jaw movement recorder, though deviations thus measured varied less than those noticed by visual observations. All measured series exhibited significant correlations with control values. The system, consequently, was found to be more suitable for determination of diurnal patterns, change over time and relative comparison between behaviour levels than it actually was for measurement of absolute duration of a given behaviour.

Keywords: telemetry - diurnal patterns - activity - rhythms - feeding and nutrition

1. INTRODUCTION

Continuous measurement of animal behaviour may be useful in investigations on interaction between animal and environment. Change in living conditions usually entails behavioural adaptation. Such adaptation can be monitored by analysing both intensity and rhythmicity of behaviours, say, feeding and activity. General locomotor activity, being the final link in a long chain of internal processes, reflects both internal motivation and external stimuli and is characterised by continuous alternation between motion and rest (SZYMANZSKI 1920, ASCHOFF 1962).

General locomotor activity, according to this definition, circumscribes any recordable movement, independent of the animal's position, recumbent or standing, and thus describes any condition different from physical rest. It is expressed as the sum or duration of activities per time unit (GATTERMANN 1993).

Social and environmental stress in a herd of dairy cows on pasture was depicted by activity records, using mechanical vibracorders, for example, by ZEEB et al. (1971). Feeding behaviour is related to the general state of activity or inactivity and the nutritional state of the animal. Measurement of at least these two distinct behavioural patterns is desirable for diagnosis of behaviour in response to environmental effects and internal processes.

Grazing is the typical form of food uptake for the majority of free-ranging herbivores. It involves selection, prehension, mastication and swallowing, including the time spent on foraging (ARNOLD et al. 1978). This behaviour will be described in this paper as standing or walking with head down, whereas a series of prehensive bites will be defined as feeding or true grazing in a more restricted sense.

While variations in intensity of behaviour are easily detectable by short-time observation and sample collection, analysis of rhythm is not valid unless it is based on long-term uninterrupted data acquisition. Most types of harmonic analysis require long stretches of cycle recording (SOLLBERGER 1965). For example, a recording period of 312 hours would be required for differentiation between two rhythms of 24 or 26 hours in period length (MERCER 1965). Such demands can hardly be met by direct telemetry, since temporary disruption of data transmission cannot be totally ruled out. Direct telemetry for behaviour analysis is difficult to perform over long distances or if high numbers of animals are involved. It is difficult to affix several sensors to different parts of one animal body. Connections between sensors and transmitter are critical, and radio transmission of data may be disconnected by physical obstacles or long distance.

We have developed a telemetry system which eliminates some of the disadvantages of direct telemetry and provides an opportunity for research-related and routine observation of free-ranging domestic (farm) animals and wildlife. The process underlying the development of the system will be described in this report together with its specific value under current conditions and possible ways for further developments and applications in the future.

2. MATERIAL AND METHODS

2.1. Elements of the system and description of its functions

The priority objectives in system development were problem-free applicability to domestic and wild-living animals, absence of feedback to the animal, long-term recordability of at least two behavioural parameters (activity and feeding) and saving of results as equidistant time series to provide the pre-conditions needed for spectral analysis.

The ETHOSYS® system is made up of collars (ETHOREC), a central station (ETHOLINK) and software for laptop or PC (ETHODAT) (IMF 1993, SCHEIBE et al. 1993) [Fig. 1]. The collars can be affixed to larger free-ranging mammals (15 kg in body weight and more). Depicted in Fig. 2 is a Przewalski horse with a collar affixed, while application to mouflon is shown in Fig. 3. Several collars are managed from one central station. The software supplied with the system enables data transmission via the RS 232 interface from the central station to a PC or laptop.

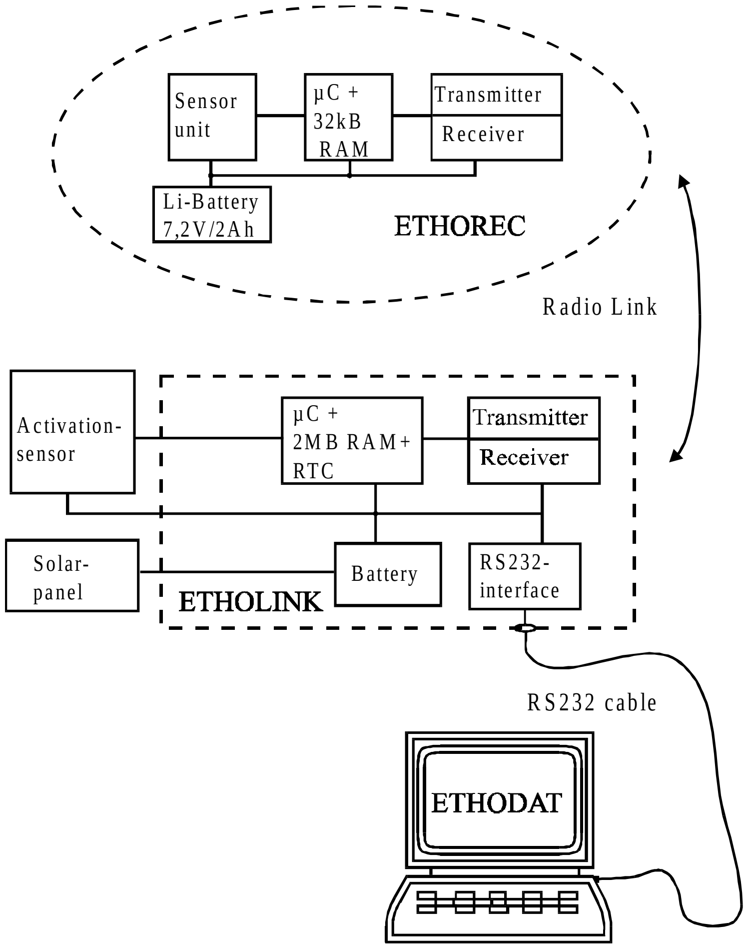

Fig. 1 Functional block diagram of ETHOSYS®. The ETHOREC registration device is designed as a collar. ETHOLINK is a self-contained central station. ETHODAT software can be used on a laptop or PC. ETHOLINK can communicate at a time with up to 16 ETHORECs.

Fig. 2 Przewalski horse wearing ETHOREC.

Fig. 3 Group of mouflon, one of them with ETHOREC attached.

The ETHOREC collar has attached to it sensors, data processing facilities for interpretation of signal patterns, a microcontroller with memory (RAM), a radio receiver and a transmitter for short-distance communication.

Every collar has two built-in sensors, one for acceleration and the other for position tracking of the animal's head (up or down). A piezoelectric element (Type PKS 41/MURATA), 2000 mg in mechanical load, is used as acceleration sensor. Species-specific and behaviour-specific angular positions of the collar may be identified either by means of a position detector (Type CW 1300-1/PEWATRON) or a Type NSW 1/GEMAC position detector with a reed switch. The raw data emitted by sensors are processed in a logic network. The output comes in four one-bit channels, each of them representing a different type of behaviour [Fig. 4].

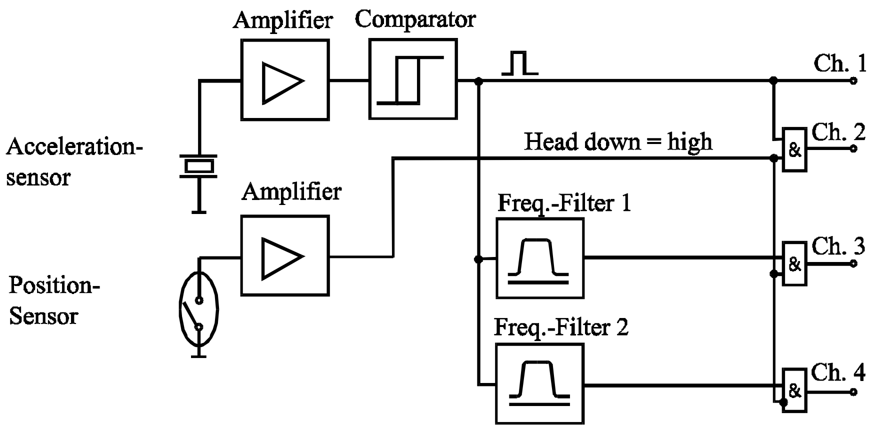

Fig. 4 Functional block diagram of the sensor unit of ETHOREC. Signals from acceleration sensor are taken up by Channel 1, with Channel 2 counting accelerations depending on state of position sensor. The other two channels contain information depending on accelerations which pass Frequency filters 1 and 2 as well as on state of position sensor.

Channel One is used to measure movements as an indicator of general locomotor activity. Hence, according to the definition by ASCHOFF (1962), any body movement is rated as an activity, no matter whether the animal is in standing or recumbent position.

If at least one signal from the acceleration sensor is picked up for an interval of one second, this second is defined as an interval of activity. Linked to Channel Two is the logic connection between general activity and the "head down" signal from the position sensor. If these definitions are true, one second is counted as "activity with head down".

Two digital frequency filters, corresponding to two additional channels, are programmable for more specific interpretation. They can be used to define impulse intervals between 125 ms and 2000 ms for interpretation of signals emitted from the acceleration sensor. The user has to define rhythm and variability of the specific behaviour which is to be identified. Frequency filters are programmed along with collar manufacture. For example, a specific pattern of movements with head down can be identified as feeding. Variable lengths of intervals between two head movements are shown in Fig. 5 for grazing sheep and Przewalski horses, as identified by direct watching. Frequency filters were accordingly set to fit in with our experiments to identify feeding, settings being 250 ms to 750 ms for sheep and mouflon or 375 ms to 1375 ms for Przewalski horse, as may be seen from Fig. 5.

Channel Four is available for identification of specific movements with head up which may reflect rumination, as an example, or for distinction between different step rates, but this channel is not operational as yet.

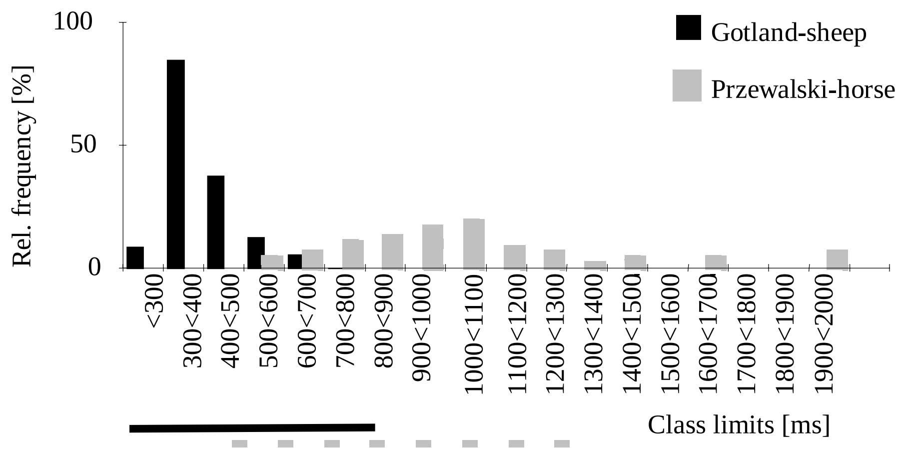

Fig. 5 Frequency distribution of observed intervals between head movements of Przewalski horses and sheep while grazing. Ranges of frequency filters for determination of feeding are marked below as a solid line for sheep and dotted line for Przewalski horse.

Each of the four channels is sampled once in a second by a microcontroller. If one of the possible logical decisions applies, the controller will count one up in the register concerned. On completion of a programmed interval, which may be up to 60 minutes, the counted total will be saved as the variable of this interval, and counting will start all over again. The variable measured for each of the time intervals may vary between zero and 100% or the number of seconds of the measured interval.

Summarised results are stored in a 32k RAM. Memory capacity is sufficient for 2047 data sets. With one-hour intervals of analysis, a collar can function independently of the central station without any loss of data for 85 days. Even with five-minute intervals of analysis, its operational capability will be one week. Earlier data are overwritten , so that most recent results will be available at all times.

Power is supplied to the collar from two 3.6V/2Ah Li-batteries, sufficient for one year of recording.

The transmitter is activated as soon as a signal from ETHOLINK is identified by the radio receiver. The collar identification code then will be emitted, followed by transmission of data from the memory. Complete reception of a data set is acknowledged by a separate signal, and only then will the memory be cleared to provide space for future entries.

The central station (ETHOLINK) is made up of a transmitter, a receiver and a microcontroller. Power is supplied from an accumulator which is recharged by means of a solar panel. The station can be activated by a passive infrared sensor, a light barrier or any kind of external switch. It will then emit a key signal, and in case of response from a collar data transmission will start from that collar to the station. The microcontroller has to organise data transmission and to save data in animal-specific files. With the technology available at present, up to 16 different collars can be managed from one station. Data transmission is possible from distances of 10 to 15 metres. The station is weatherproof and can work several months without human controlling. Its memory capacity of 2 MB RAM is sufficient for 57,000 data sets and can be easily enlarged.

The ETHODAT programme is data-linked through the RS 232 interface to the microcontroller of the ETHOLINK station. Once a PC or laptop is connected to the station, all files are separately transferred and organised in dBase format data files on a monthly basis. Listed in each of the files are the date, time of day and length of behaviour in seconds in the given interval for the four channels.

The programme provides for three preliminary forms of graphic display. For example, "results hourly" is a function by which results are displayed as percentage of behaviour time per hour for the four channels from one animal. Each of the graphs shows the results of two consecutive days. By changing from day to day, the time pattern of an animal can be tracked day after day. "Daily means" is the function to display daily mean values for the four channels. A summary graph also displays day-to-day data. The mean daily pattern over one month is displayed by an "hourly means" function. For more advanced analysis, data can be transformed to ASCII format.

Versions:
The logic network must be programmed, and frequency filters need to be set to species and research of interest. Versions have so far been designed for sheep (*Ovis aries*), mouflon (*Ovis ammon musimon*), cattle (*Bos taurus*), alpaca (*Lama pacos*), horse (*Equus przewalskii*), roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*).

A radio collar for direct transmission of the original sensor signals is available and can be used in preliminary studies on other species and for identification of new typical signal patterns correlated to other behaviours. Its signals can be acoustically displayed on the sound channel of a video camera in parallel to data recording from the animal. Comparability of behaviour and sensor signal patterns is thus ensured in a simply way. Such recordings may be helpful in adjusting frequency filters according to behaviour patterns of interest.

A simple monitoring receiver is available for (optional) visualisation of radio transmission from ETHOREC to ETHOLINK. Functions of ETHOREC and ETHOLINK are signalled by LEDs. Correct functioning of the system can be checked and verified from some distance.

Automatic data transmission may not be essential when it comes to tamed and well adapted animals. A version for direct data transmission to a laptop is available for this form of experimental application. For data transmission, receiver and laptop should be positioned close to the collar (5-10 m). Data transmission is organised by ETHODAT in the same way as from ETHOLINK.

2.2. Experimental application and testing
Correlations between behaviours observed and recorded in the field and ETHOSYS® measurements were tested in six sheep (two Soay sheep and four merino) and four Przewalski horses.

Visual observations were conducted by means of a laptop and Observer 2.0, a behaviour recording system. The system was configured to measure the behavioural categories of activity, activity with head down and feeding in time intervals identical to those of the ETHOREC-collar wearing animal under observation. Only one animal was tracked and continuously watched during a time interval. Recording was started by the observer at the beginning of an ETHOREC recording interval. After a series of sample intervals had been recorded from several animals, observation ended at the end of an ETHOREC recording interval.

Measurement and observation intervals were 15 minutes in nine cases. One earlier ETHOREC model, 30 minutes in measurement interval, was tested on one Przewalski horse. Observation intervals were irregularly distributed over five days, with attention being given to the need for coverage of different times of the day. Observation series for each of the animals included intervals of high, medium and low activity and feed uptake and thus represented full natural variability.

We also analysed correspondence of ETHOREC results with data obtained from APEC, an established data logger for jaw movements in sheep (BROUILLETTE et al. 1993). Either instrument was used on the same three sheep (Limousine) on an experimental pasture in Theix (INRA Clermont-Ferrand). The data obtained from ETHOREC for activity with head down and grazing were summarised on an hour-to-hour basis and were compared to the per-hour totals of APEC-recorded feeding time.
A total of sixty-eight hours was analysed from these animals (21, 22, and 25 hours, respectively).

Corresponding measurement and observation series were separately handled for each of the animals involved.

Pearson's correlation coefficients were calculated for recorded data versus field observations. Paired t-tests were performed to check differences between measurements provided by ETHOSYS®, on the one hand, and those obtained from observation, on the other. For each animal, both correlations and t-tests were separately calculated.

As opposed to the ETHOSYS® - observation comparison, the ETHOSYS® - APEC measurements showed significant autocorrelations and could not be treated as independent entities. Therefore, the correlation coefficients were calculated and tested by Bartletts method (LIENERT 1978). For the same reason, we disclaimed a statistical test of the mean differences. Only activity with head down and feeding were correlated to the APEC data, considering that APEC was a system for recording of jaw movements.

3. RESULTS

The data logger version of the telemetry system was repeatedly used in research on three alpacas, eight Przewalski horses and six sheep, while the radio-transmitting version was applied to two alpacas, nine sheep, four red deer, twelve Przewalski horses and four mouflons. We had an uninterrupted supply of data sets for more than six months from each of the animals involved. Apart from few broken neck hairs of some animals, there were no problems at all, and collars always were easily affixed.

All correlation coefficients between ETHOREC measurements and field observations were significant, as may be seen from Table 1. They differed from each other, depending on individuals and behaviours. Only six out of twenty correlations were less than 0.9. In one of the horses (horse 4), activity with head down and feeding could not be measured because of sensor malfunction.

In six of ten samples, no statistical difference was detected between activity intensities measured, on the one hand, and those observed in the field, on the other. In the remaining four samples, deviations between measurement and watching were between 6% and 20%. Observed activity data in these four cases were higher than data obtained by ETHOSYS®.

Significance was established for five of nine differences between observation and measurement of feeding. Feeding time was underestimated in one case and was overestimated in four cases. In four of nine samples, differences were found to exist between activity with head down and feeding as observed in the field. It was underestimated in one case and overestimated in three.

Tab. 1 Comparison of observed data with related ETHOSYS® data.

Animal	Inter-vals	Inter-val length [min]	ACTIVITY: correlation	FEEDING: correlation	ACT./H.D.: correlation	ACTIVITY: mean difference ETHO.-obs [% int.lgt]	FEEDING: mean difference ETHO.-obs [% int.lgt]	ACT./H.D.: mean difference ETHO.-obs [% int.lgt]
Horse 1	20	15	0.83*	0.68*	0.59*	-13.2*	3.9	22.8*
Horse 2	15	15	0.97*	0.96*	0.98*	-3.7	-16.7*	4.2
Horse 3	14	15	0.80*	0.90*	0.93*	-5.3	-8.2	9.4*
Horse 4	19	30	0.89*			-20.3*		
Sheep 1	16	15	0.97*	0.95*	0.95*	-2.0	19.1*	2.4
Sheep 2	14	15	0.99*	0.97*	0.98*	-2.2	22.2*	3.8
Sheep 3	13	15	0.98*	0.92*	0.99*	-2.4	8.3*	-0.5
Sheep 4	16	15	1.00*	0.95*	1.00*	-2.3	15.4*	3.0*
Soay 1	12	15	0.93*	0.85*	0.97*	-12.9*	-4.3	-11.1*
Soay 2	7	15	0.99*	0.96*	1.00*	-6.0*	-1.6	-0.9

(* $p \leq 0.05$)

Tab. 2 Comparison of related APEC and ETHOSYS® data

Animal	Intervals	Interval length [min]	FEEDING: correlation	ACTIVITY/ HEAD DOWN: correlation	FEEDING: mean difference ETHO. - APEC [% int.lgt]	ACTIVITY/H. D.: mean difference ETHO. - APEC [% int.lgt]
Sheep 1	21	60	0.96*	0.99*	-16.9	-13.9
Sheep 2	22	60	0.97*	0.98*	-12.8	-6.9
Sheep 3	25	60	0.99*	0.99*	-12.1	-5.3

(* $p \leq 0.05$)
The mean differences were not tested for significance (see Section 2.2.).

Correlations were always significant and very close between APEC and ETHOSYS® [Table 2].

An example of original data from either system in Fig. 6 illustrates close agreement among measured results, especially almost congruence of variations. An example of the original display is depicted in Fig. 7, with results obtained from a free-living mouflon. The screen copy from the ETHODAT display shows three channels for two consecutive days. General activity, activity with head down and grazing are closely correlated to each other but differ from each other by intensity. The average intensity of activity amounts to 40.5% and thus is about 10% higher than that for feeding. This difference accounts for movement and locomotion without feeding. On the first day, the animal started its activity with a grazing period at dawn, about 6 a.m. Activity and feeding were largely reduced between 10 and 11 a.m. Two relatively short peaks followed at noon and in the afternoon, with a main period of activity later in the evening. It was extended for some hours into the night and did not decline before midnight. Another early-morning peak in activity was displayed by the animal on the next day. It then stayed fairly inactive through the daylight hours and resumed higher activity in the evening. The behavioural pattern of those two days was characterised by high intensity of nocturnal activity, a phenomenon typical of this population.

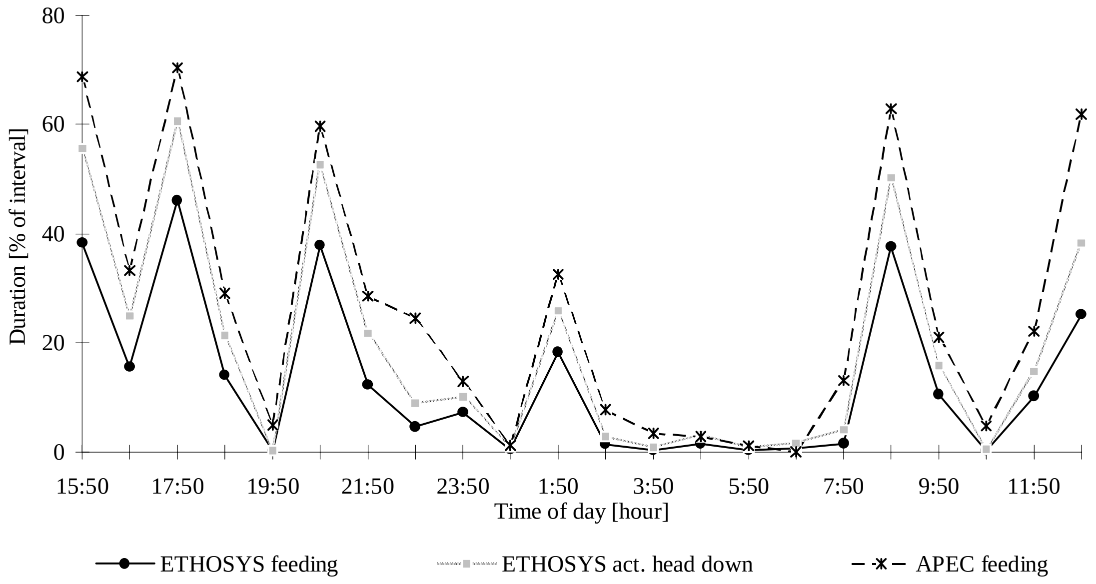

Fig. 6 Comparison of records by APEC and ETHOSYS® on sheep (breed: Limousin).

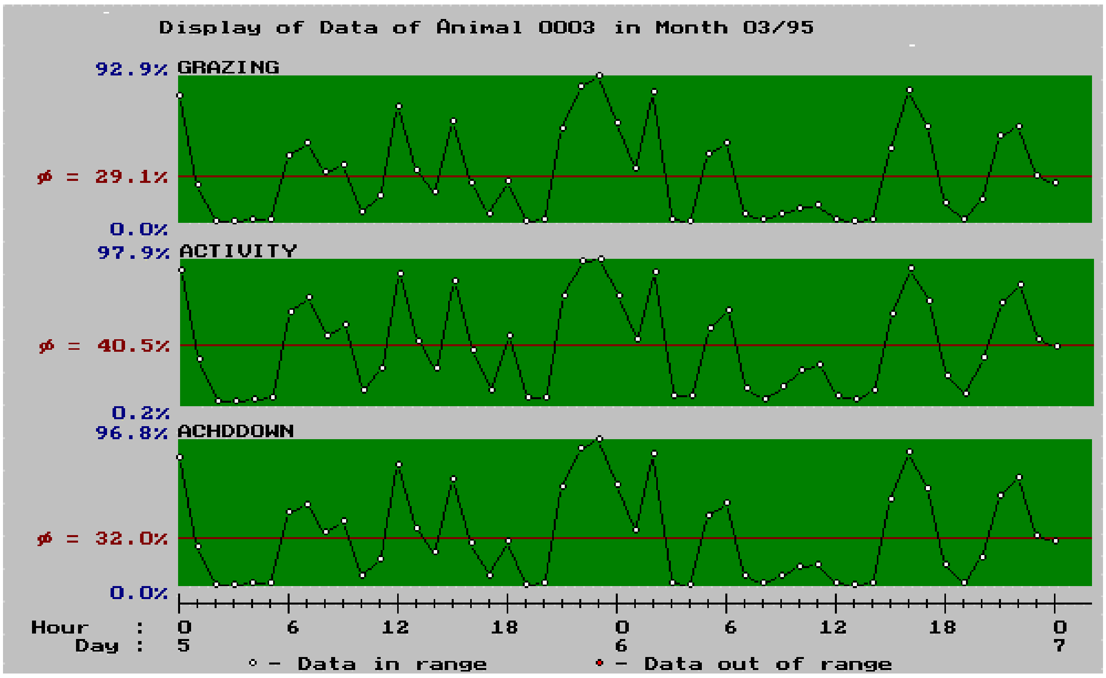

Fig. 7 Screen copy of original data display by ETHODAT for free-ranging mouflon. Top: grazing; bottom: activity with head down (ACHDOWN); centre: activity.

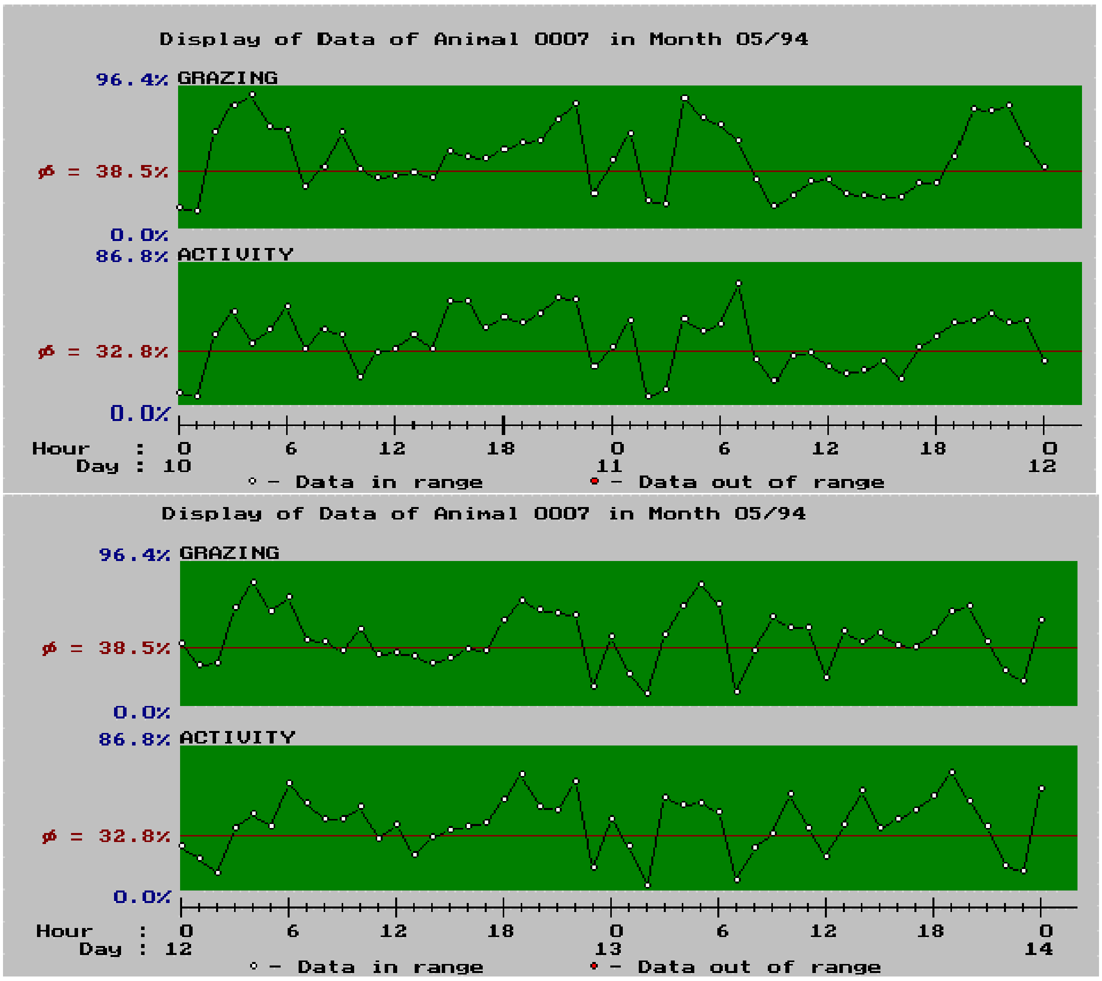

Fig. 8 ETHODAT displays of activity patterns of free-ranging Przewalski horse before and after introduction to neighbouring paddock of three newcomers of Scottish highland cattle. On 11th day, activity is reduced between 8 a.m. and 7 p.m. because of observation and surveillance behaviour but is resumed two days later (13th day).

Depicted in Fig. 8 is an example of recordings obtained from a Przewalski horse through four days in May 1994. The normal daily pattern on the tenth day was characterised by an activity and grazing peak in the early morning, moderate activity throughout the day and, again, high activity and grazing in the afternoon and evening. In late evening and even more past midnight, the per-hour activity total nearly dropped to zero. In the morning of the eleventh day, three Scottish highland cattle were introduced to the neighbouring paddock which had been unoccupied before. The horse stood quiet, watched the newcomers and reduced feeding activity during the daylight hours, as may be seen from the graph. It resumed its original behaviour pattern after Day 13. These are good examples to demonstrate the sensitivity of continuous behaviour analysis and the importance of adequate background information for correct interpretation.

4. DISCUSSION

Data loggers are well known and are used for several purposes in animal science and wildlife research. Evidence has been provided of their capabilities in examples reported by FRANCIS-SMITH et al. (1982), AHRENS et al. (1985), BECHET et al. (1989), BRUN et al. (1990), GILL (1991), MATSUI et al. (1992) and MATSUI (1994). All these data loggers were one-channel models with fairly simple sensory devices and without any signal interpretation. For recording specific behaviours, they had to be tightly affixed to defined parts of the body (FRANCIS-SMITH et al. 1982) or sensory devices had to be fastened separately from the data logger (AHRENS et al. 1985, BECHET et al. 1989, BRUN et al. 1990, MATSUI 1994).

A counter for jaw movements and identification of grazing and rumination, as developed by CHAMBERS et al. (1981), used a microswitch attached to a chin cord, an accelerometer and a mercury switch mounted on a headstall. A control and recording box was fixed to the back of the animal or behind the head. Interpretation of the sensor signals enabled distinction between grazing, plucking and rumination. Acceleration of head movements, associated with biting, was found to be characteristic of plucking movements, with some difference between sheep and cattle. Such accelerations are used also in ETHOSYS® to characterise feeding, and there is an additional option to judge the time pattern of these movements.

Instruments which are designed for measurement of more than one behavioural category usually need to be furnished with several sensors that must be fixed to different parts of the body, according to a system developed by RUTTER et al. (in press). Results of high accuracy may be obtained from such instruments, though their use is restricted to properly adapted animals. For data transmission, data loggers have to be removed from the animal, which is quite a simple procedure with some species of tamed and well adapted animals. Animals occasionally had to be handled for different purposes. Effects of such handling were not significant, though significance cannot be ruled out altogether.

Use of incorporated telemetry devices, such as those developed for game species by SCHOBER et al. (1982), seems to be the only way to avoid connecting wires, external sensors and aerials. This, however, requires a fairly extensive surgical procedure and, consequently, is an approach that is limited to few animals and to special fields of research, and it does not even eliminate the drawbacks of direct telemetry.

Very simple telemetry devices, as an alternative, are quite often used for radio location and activity recording, say, of deer (GREEN et al. 1990, CARRANZA et al. 1991, PALOMARES et al. 1991). A radio beacon is involved, its pulse frequency depending on the position of a mercury switch integrated in the radio collar. Changes in pulse frequency are indicative of activity but hardly enable identification of other behaviours. These signals sometimes are used to identify grazing by analysing patterns of pulse frequency change. In that context, an activity with head down is interpreted as feeding and roughly corresponds to the "activity with head down" function in ETHOSYS®. The signal amplitude also may occasionally be used as an additional source of information to identify activity (CEDERLUND et al. 1980). These procedures all depend on high-stability radio transmission and proper interpretation of the information received.

The GPS-1000 remote tracking system so far is the most advanced commercial development in wildlife telemetry. It includes a data logger and a radio link to a data-receiving station (RODGERS et al.

1994, RODGERS et al. 1996). GPS (acronym standing for Global Positioning System) provides information on locations and on the current activity state of the animal, all for storage. This information is based on signals of a dual-axis motion sensor. Numbers of events are counted for observation windows from ten minutes to three hours. With each GPS fix, typically done eight times/day, the latest result is stored together with the GPS data. The values merely provide limited information on behaviour and cannot be used for biorhythmic analysis.

Implantable devices were designed for measurement of activity and body temperature in domestic animals, especially swine. Results obtained from such devices may be saved in the memory of a microcontroller, following the transponder principle, with a transmission range of 50 cm (PUERS et al. 1995), or external repeater collars are used for continuous transmission within a range of 20 m (CATS et al. 1995). The purpose is identical with that of ETHOSYS®, acquisition of information with relevance to animal welfare or information on the current reproductive status in group housing systems or outdoor pig farming.

Our system provides for discrimination among several categories of behaviour and is based on electronic signal interpretation inside a collar. The results generally obtainable from that procedure are limited in accuracy, and the procedure has to be adjusted by observation to species and research objectives. Identification is restricted to behaviours which can be described in terms of acceleration, time pattern of acceleration and angular position. General activity versus resting, feeding or certain types of locomotion are some typical examples.

The amount of locomotor activity is generally underestimated by ETHOSYS®, in comparison to interpretation by observers. This may be attributable to more or less close connection of a collar to the body of the animal. Also, an acceleration sensor does not respond to slow movements. Finally, there always are differences among individuals, especially among horses. The Przewalski horses used in this study were not tamed, and application of the collar was not a simple procedure in all cases. Collars, consequently, were not too closely affixed, and their tightness differed slightly by individuals. This may have had an impact on results. Collar fixation in an almost identical manner was much easier with sheep. Notwithstanding deviations in level, shapes of activity curves were correctly recorded in all cases, and changes in activity levels over days or months could be adequately monitored.

Differences in collar fixation have even stronger effects on identification of movements. Nevertheless, except for one horse, close correlations were found to exist between recorded and observed behaviours. This type of behaviour may be identified by an observer even from a somewhat greater distance. There may be difficulties in places covered by high vegetation, also because under such circumstances animals may preferentially use the tips of grasses.

While feeding proper is well correlated to visually observed values, it can be overestimated by a human observer who has to watch animals under difficult site conditions, such as high vegetation or long distance. The same category may be overestimated by ETHOSYS® if the typical rhythm with head down is exhibited by the animal while moving on the ground without feeding. Underestimation of feeding by automatic identification, i.e. compared to visual observation, was attributable to several causes. Identification of specific intervals would depend on a minimal sequence of at least two or three typical movements. Isolated singular bites with long intervals in between cannot be properly identified by this method. There will always be some intervals that will lag behind the limits of the method, as may be seen from Fig. 5. A wider range tends to be accompanied by growing sensitivity but at the expense of lower specificity. For definition of typical intervals, a compromise will have to be made between these two aspects.
A field observer may not be able to realise from a distance whether an animal really bites or manipulate the food in its mouth or is just moving its head over the ground. Observations of that kind quite often are incorrectly interpreted as feeding. The "activity with head down" definition quite often reflects more clearly an activity which an observer would interpret as "grazing". The definition of feeding, as a sub-pattern of head movement, should be used for additional information and should be critically considered together with "activity with head down".

The above problems are reflected in a comparison between APEC and ETHOSYS®. Variations over time are nearly identical with each other, as is demonstrated by high correlations. "Activity with head down", under these circumstances, was in closer agreement with the level of feeding as recorded by APEC. APEC and ETHOSYS® were applied to three free-ranging sheep in another comparative study by BLANC et al. (1995). Over 304 hours of recording, correlations from 0.81 to 0.93 were found to exist between the feeding parameters of ETHOSYS® and APEC, while correlations of 0.9 and 0.95 were established for "activity with head down" (ETHOSYS®) and feeding (APEC). All ETHOSYS®-measured feeding parameters were lower, whereas all time-related characteristics were identical. In this study, "activity with head down" (ETHOSYS®) also was closer to the level of feeding as recorded by APEC.

It is our assumption that the basic logic and structure of ETHOSYS® can be used not only for identification of activity and feeding but for other behaviours as well. Rumination, for example, can be described as a certain movement pattern with head up. Yet, only a low level of movements is transferred on a collar, which makes identification difficult. There are several locomotor patterns, such as trot or gallop, which may be described as species-specific frequencies of movement with head up. If advantage was taken of the technology now available, distinction should be possible on the basis of appropriate frequency definitions. The fourth channel actually is earmarked for such applications.

ETHOSYS® has the major advantage of being based on one specific collar with all sensory and signal processing devices built into it and with no need for connecting wires and distributed sensors. The risk of irritating the animal or of technical disorders thus is minimised. This is of great importance for application to wild or untamed domestic animals, last but not least, under the aspect of animal welfare. Furthermore, on account of its data reduction to an equidistant time series of continuous measurement, the time of unaided or unsupervised operation without any loss of data is much longer than that of many other data logging devices.

The system is highly suitable for research on biological rhythms, since in such research knowledge of the time at which high or low behaviour intensities occur is more important than precise knowledge of the current intensity level.

The importance to domestic animals of research on biological rhythm has been expounded, for example, by LEFCOURT (1990), while TESTER et al. (1990) underlined the importance of activity to wild animals. Such research depends on the availability of suitable methods, and we strongly feel that major demands on studies into biological rhythm are met by ETHOSYS®.

Results obtained from application of the method to alpaca proved helpful in relating grazing activity to pasture quality or activity rhythms to health or sickness or social position of the animal thus tracked. Parturition, for example, was predicted several hours to one day in advance by checking on feeding behaviour and patterns of activity (SCHEIBE et al. 1991). Lambing time of free-ranging mouflon was determined with high accuracy without any need for additional observation (LANGBEIN et al. 1995). By evaluating ETHOSYS®-generated activity data, we determined the period of time needed by Przewalski horses for adaptation to semi-natural conditions (BERGER et al. 1995 b), and we also succeeded in detection of social stress in a group of farmed red deer (BERGER et al. 1995 a).

Significant advantages are offered by the principle of storage telemetry for research on numerous species of free-ranging animals. As no continuous radio transmission is required, animals may take advantage of an unlimited range, and the only condition is that they should return more or less regularly at fairly long intervals to certain predetermined places, such as a salt lick or water source. The same principle has proved helpful in simultaneous management of several animals for investigation of differences among individuals or species or studies into specific breed behaviours or social interactions.

The system, on account of its specific potentials, can be effectively used in scientific research as well as in routine observation of high-value individuals or flocks under extensive rearing conditions.

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ANNEX / ANHANG

Tab. Ergebnisse der Korrelationsberechnung zwischen mit ETHOSYS®-gemessenen Daten und direkten Beobachtungsdaten bei 4 gegatterten Rothirschen

		correlation coefficients (n=20)			
		collar specially for red deer / data transmission by radio		collar not specially for red deer / data transmission by connection with PC	
measured (collar)	: observed (real)	red deer 2	red deer 3	red deer 4	red deer 5
general activity	: general activity	0.81**	0.72**	0.95***	0.54*
feeding (plucking)	: feeding (plucking)	0.77**	0.86**	0.52*	0.08
activity with head down	: activity with head down without plucking	-0.02	0.58*	0.46*	0.45*
activity with head down	: activity with head down and with plucking	0.82**	0.87**	0.99***	0.99***
rumination	: rumination	-0.22	0.24	-0.17	-0.36
rumination	: general activity with head held up	0.76**	0.37	-0.28	0.11
rumination	: general activity	0.08	0.41	0.33	0.35
		interval = 15min		interval = 30min	

critical value (2-tail. 0.05): +/- 0.4426

Diese Tabelle zeigt zusätzlich die Ergebnisse der Korrelationsberechnung zwischen den mit ETHOSYS® gemessenen Daten und den direkten Beobachtungsdaten bei 4 gegatterten Rothirschen. Generell wurden die Aktivität und die Aktivität mit gesenktem Kopf signifikant richtig erkannt. Nach dem Einstellen des Meßhalsbandes auf die Rupffrequenz des Rothirsches erhöht sich die Genauigkeit der Daten des Fressens, so daß auch das Fressen signifikant richtig erkannt wurde. Wiederkauen wurde von den Meßhalsbändern nicht erkannt.

2. Description and Comparison of Measuring Systems

2.2. Comparison of Two Telemetric Methods for Measuring Behavioural Parameters

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Eigenanteil:
- praktische Anpassung von ETHOSYS® an die Tierart Rothirsch
- ETHOLINK-Station in dem Versuchsgehege aufgebaut und Halsbänder angelegt
- Funktionstüchtigkeit von ETHOSYS® während der Untersuchung überprüft:
    - visuelle Paralleluntersuchungen durchgeführt,
    - Übereinstimmung zwischen den Beobachtungsdaten und den mit ETHOSYS® gemessenen Daten geprüft (Korrelationsberechnungen durchgeführt)
- gesamte Auswertung und Berechnung zum Vergleich der mit ETHOSYS® und mit VIENNA erfaßten Daten
- Manuskript verfaßt (Korrektur durch Mitautoren) und Grafiken erstellt
- Manuskript in's Englische übersetzt (Korrektur durch Dolmetscher)

Ziel der Arbeit:
Diese Arbeit entstand aus einer Zusammenarbeit zwischen der Forschungsgruppe Raum-Zeit-Orientierung des Institut für Zoo- und Wildtierforschung in Berlin und der biotelemetrischen Arbeitsgruppe des Institut für Wildtierkunde und Ökologie in Wien. Ziel der Arbeit war es, das in Berlin entwickelte nicht-invasive Speicher-Telemetrie-System ETHOSYS® (das die Aktivität und das Fressen bei großen Herbivoren mißt) mit dem in Wien entwickelten invasiven Funk-Telemetrie-System VIENNA (das die Herzfrequenz, Körpertemperatur und Aktivität bei Rehen und Rothirschen erfaßt) in einem Versuch direkt miteinander zu vergleichen. Beide Meßsysteme waren unabhängig voneinander entwickelt worden, um - verschiedenen Konzepten folgend - Belastungen an frei beweglichen (Wild)Tieren erkennen und bewerten zu können. Vor- und Nachteile, Meßgenauigkeit, und mögliche Anwendungsgebiete der beiden Systeme sollten einander gegenübergestellt werden. Tierschützerischen Interessen folgend war zu prüfen, ob gegebenenfalls das invasive Meßsystem durch die nicht-invasive Methode ersetzt werden kann.

ABSTRACT
Two different telemetry systems are presented and the results concerning their efficiency in providing activity and feeding data are compared. The first one is the non-invasive storage telemetry system ETHOSYS® for continuous registration of activity and feeding on free ranging or grazing mammals developed by the Institute for Zoo Biology and Wildlife Research in Berlin. The second one is the invasive, radio telemetry system VIENNA for measuring heart rate, body temperature and activity on free ranging roe deer and red deer developed by the Research Institute of Wildlife Ecology in Vienna. Both systems are suitable for long-term applications and are designed to minimise effects on animal behaviour. These two systems were used at the same time on one red deer (*Cervus elaphus*) for nine months under seminatural conditions. The activity patterns measured by the system from Vienna correlated well with the results of ETHOSYS®. In contrast to VIENNA the storage telemetry system ETHOSYS® provides uninterrupted time series of fundamental behaviours due to its data storage technology. Both systems show their pros and cons and offer different research possibilities.

1. INTRODUCTION

Presently game never lives in a real natural environment. Human influence is manifold, but fairly different in type and intensity. It may be a major stressor or prevent the animals from their spacial and temporal ecological niche. Telemetric measurements of behaviour and physiological data are of high importance to analyse these influences. Two systems, developed independently in Vienna and Berlin, following different concepts but both able to measure animal activity, are compared in a study on red deer (*Cervus elaphus*).

2. MATERIALS AND METHODS

The storage telemetry system ETHOSYS® (SCHEIBE et al. 1995, SCHLEUSNER 1995) is made up of collars (ETHOREC), a central station (ETHOLINK) and software for laptop or PC (ETHODAT). The collars can be affixed to larger free-ranging mammals (15 kg in body weight and more). The ethorecorder contains a sensor which detects movements with an adjustable sensitivity. It is coupled to a timing circuit and a second sensor which detects the angular position of the head. So, identification of some behavioural elements of the animal carrying the collar is possible. The ethorecorder notes all movements of the animal which are passed on to the collar as "global activity". When the position sensor detects the head held down and the movement sensor records a species-specific rhythm of head movements, the storage telemetry system identifies these actions additionally as "feeding". The sensors are polled every second and the results are compiled. The data are summed-up and stored as functions of time in predetermined periodic intervals (mostly 15 minutes, half-hour or one hour intervals) in a data logger. The storage capacity is 2047 data sets. When the memory of the collar is full the initial data are overwritten, with the latest results always being available. The power supply of a collar is sufficient for at least one year of recording. The telemetry system is equipped with a short-range radio data link for transmission of data from the collar to the ETHOLINK station. ETHOLINK saves the data in animal specific files of up to 16 animals. They can be transferred to a laptop or PC via a RS-232 interface.

The program ETHODAT enables graphic display and transformation of data to the ASCII format for more advanced biorhythmical analysis such as autocorrelation function and power spectral analysis. The "Degrees of Functional Couplings" (DFC) are used for comparison of the rhythmic structure (SCHEIBE et al. 1978). DFC's are defined as the percentage of the total power of harmonic significant components to the total significant power of the spectrum. We define all components of a power spectrum which are harmonic to the external circadian zeitgeber (for instance 3, 4, 4.8, 6, 8, or 12 hours period length) as harmonic periods. For this type of analysis, uninterrupted data sets of approximately 10 days are necessary. DFC's were found to be high in well adapted, healthy and undisturbed individuals, but lowered during adaptation periods, sickness or social interactions.

The transmitting subunit of the radio telemetry system VIENNA (SCHOBER et al. 1995) consists of an implantable pulse-modulated heart rate and temperature transmitter (100 kHz) using pulse-interval modulation (for QRS detection and temperature) and pulse-duration modulation (for discrimination between heart beat pulses and temperature pulses) as well as an extracorporal repeater collar (150 MHz) which adds some activity (head up/down) information to the heart beat pulses by pulse-duration modulation.

The receiving subunit is a battery-powered, microprocessor-controlled rf receiving and data pre-processing system with a built-in storage capability (SCHOBER 1986). Data are pre-processed on a minute basis, storing various characteristic heart rates, body temperature, activity and reliability data. A RS232 connection from the receiving unit to a PC or Laptop and an optional software package offers a program for short-term studies of heart rate on a beat-to-beat basis (SCHOBER et al. 1989).

For detection of animal's activity, when "active" was defined as any behaviour except of that when the animal is lying ("inactive"), the mean heart rate, the head-down time (sec), the number of head-up/down changes and the number of rf signal strength variations, all on a minute basis, have proven to be sufficient. An algorithm for activity detection in red deer was established experimentally which is shown in table 1, with HRmean being the daily average of the heart rate of the specific animal. After applying this algorithm, short data gaps (caused by animals coming out of receiving range) up to 2 minutes between two "inactive" periods or up to 9 minutes between two "active" periods are set to "inactive" or "active", respectively. The same "filtering" technique is applied to short "active" periods between two "inactive" periods and vice versa. The reason for applying the "filtering" algorithm was, that we found "active" periods shorter than 3 minutes and "inactive" periods shorter than 10 minutes to be very improbable in red deer.

Tab. 1 Algorithm for activity detection from radiotelemetric data of the system VIENNA.

Head-up/down [number/minute]	Signal strength variations [number/minute]	Heart rate [beats/minute]	Inactive	Active
0	≤ 10	$HR \leq \overline{HR} + 40\%$	*	
0	≤ 10	$HR > \overline{HR} + 40\%$		*
0	> 10	$HR \leq \overline{HR} - 15\%$	*	
0	> 10	$HR > \overline{HR} - 15\%$		*
1	≤ 5	$HR \leq \overline{HR}$	*	
1	≤ 5	$HR > \overline{HR}$		*
1	> 5	$HR \leq \overline{HR} - 15\%$	*	
1	> 5	$HR > \overline{HR} - 15\%$		*
≥ 2	any	any		*

$\overline{HR}$ = daily mean heart rate of the individual

These two systems were applied during the same study on one red deer in an enclosure of about 40 ha. Measurements recorded by ETHOSYS® were compared with simultaneous visual observations. Data of the system VIENNA were compared with data of simultaneous video recordings of one animal which was separated together with a second one in a special enclosure of about 2 ha. To compare the two systems the data of both were aggregated in units of 15 minutes and the amount of activity was calculated.

3. RESULTS

For ETHOSYS® the correlation coefficient of measured and observed activity with a total of 26 periods of 15 minutes was 0.56. Correlation coefficients of investigations on red deer measured before with the same system in research enclosures in France were between 0.72 and 0.95. The low correlation coefficient in this investigation we attribute to the difficult visual observational conditions due to the richly structured enclosure and shy game.

For system VIENNA the correlation coefficient of measured and observed activity with a total of 708 periods of 15 minutes of video observation was 0.90. The mean portion of time, classified wrong (active/inactive or vice versa) during 10 days of observation was 7.6%.

The storage telemetry system ETHOSYS® with a measure interval of 15 minutes worked about 10 months. The system VIENNA with a measure interval of 1 minute worked about 6 months. Both systems were started in April 1995. For 93 days the two telemetry systems worked simultaneously on the same animal. In spite of the differing definition of activity the data of ETHOSYS® and of the system VIENNA showed a correlation coefficient of 0.62.

This relatively low correlation coefficient was due to different types of activity measured by the two systems. ETHOSYS® registers movements while the system VIENNA counts any behaviour different from lying. In spite of this the main structure of measured activity shows high similarity between the two measuring systems.

Apart from this there are short gaps in the dataline of VIENNA as a result of disturbances of data transmission and three big gaps in the dataline of ETHOSYS®. Only one big gap of more than 20 days in the dataline of VIENNA was a result of technical failure. Thereafter, 2290 short gaps with an average duration of 10.4 data sets (about 10 minutes) were registrated in the dataline of the system VIENNA. The three gaps in the dataline of ETHOSYS® with an average duration of 901 data sets (about 9 days) occurred at the time of the greatest availability of natural food, therefore the deer did not come to the feeding place near the central station for more than 21 days. After this time the initial data were overwritten and the gaps came about.

The longest continuously measured period was 3496 one minute data sets (about 2.5 days) by the system VIENNA and 9663 15 minutes data sets (about 100 days) by ETHOSYS®.

Tab. 2 Comparison of important features of the storage telemetry system ETHOSYS® and the radio telemetry system VIENNA.

	ETHOSYS®	VIENNA
Application	external, for routine and experiments	surgical, restricted to experimental situations
Influence on animals	minimal	after rehabilitation minimal
Parameters	activity, grazing (other in prep.)	activity, heart rate, body temperature, location possible
Activity definition	any movement	animal standing
Transmission	data storage, short-range rf-link	direct rf-transmission, continously
Advantage	28 days of uninterrupted data guaranteed	actual status available, location possible
Disadvantage	possible gaps if animal does not return to central station, no location possible	restricted to receiving range, short-time interruptions possible
Accuracy	medium	high
Original data format	totals for intervals, resolution prepro-grammed, analysis simply	beat per beat registration, high resolution but analysis expensive
Main performance	stress indication based on long time effects, biorhythmic analysis	stress indication based on short time reactions, measurement of ampli-tudes and time structure

4. DISCUSSION

In contrast to ETHOSYS® the data transmission of the system VIENNA is not bound on a central receiving station to which animals must approach. Moreover, radio location is possible. The disadvantages of this system are the severe surgical procedure, the one-way implants and non-predictable gaps in the data in situations when animals get out of receiving range. Moreover the telemetry system VIENNA provides important physiological data especially for the assessment of short-time reactions as well as for studies of the energy metabolism.

In contrast, ETHOSYS® provides uninterrupted time series of fundamental behavioural parameters due to its storage technology. These can be used for biorhythmic analysis. ETHOSYS® provides the opportunity to assess long adaptation processes and general living conditions. A localisation is not possible. The data transmission is bound to a central receiving station, which may be a disadvantage for the application on non-territorial or free-ranging animals.

Both systems are suitable for long-term registration of activity patterns.

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ANNEX / ANHANG

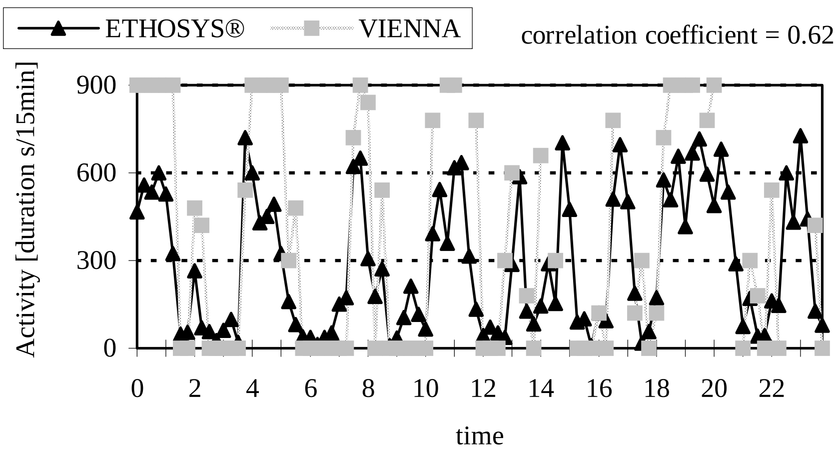

Abb. Direkter Vergleich der Originaldaten eines Tages, die jeweils mit ETHOSYS® bzw. VIENNA gemessen wurden (Rothirschkuh „Bela“, 15. Mai 1995)

Die zusätzliche Abbildung gibt am Beispiel der Originaldaten eines Untersuchungstages einen Eindruck über die Übereinstimmung der mit ETHOSYS® und VIENNA gemessenen Daten. ETHOSYS® zeigt durchgängige Datenreihen, wogegen bei VIENNA mehrere kurze Datenausfälle fast an jedem Untersuchungstag auftraten.

Tab. Überblick über die Datenausfälle bei der Untersuchung

	ETHOSYS® [1 data set = 15 min]		VIENNA [1 data set = 1 min]	
	month/year	gaps [%]	month/year	gaps [%]
red deer "Bela"	04/95 start: 11.4.95	0.0	04/95 start: 24.4.95	2.9
	05/95	0.0	05/95	15.2
	06/95	27.7	06/95	41.4
	07/95	41.4	07/95	60.2
	08/95	1.4	08/95	1.7
	09/95	7.4	09/95 end: 11.9.95	1.7
	10/95	14.1		
	11/95	0.0		
	12/95	0.0		
	01/96 end: 18.1.96	0.0		
period of measurement	26653 datasets =277 days		201408 datasets = 139 days	
longest period of continuous registration	9663 datasets = 100 days		3496 datasets	
the average duration of continuous registration	5987 datasets = 62 days		63.5 data sets	
number of gaps	3		2290	
the average duration of gaps	901 datasets = 9 days		10.4 datasets	

Die Tabelle gibt einen Überblick über die Datenausfälle bei den beiden Meßsystemen während der Untersuchung. Bei VIENNA traten Lücken im Datensatz auf, wenn sich das Tier außerhalb des Empfangsbereiches bzw. im Funkschatten aufhielt. Bei ETHOSYS® kam es zum Datenverlust, wenn das Tier 21 Tage lang nicht an der Lockfutterstelle (an der die Zentralstation ETHOLINK installiert war) erschien. Dies geschah vor allem in Zeiten, in denen das Futterangebot in dem 40ha großen Gehege sehr gut war, wohingegen in den Wintermonaten die Lockfutterstelle regelmäßig besucht wurde und die Datenerfassung bei ETHOSYS® lückenlos erfolgte.

2. Description and Comparison of Measuring Systems

2.3. Comparison of Two Automatic Methods for Measuring Grazing Behaviour

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published in Ann. Zootech. (1995) 44, Suppl. 235.

Eigenanteil:
- ETHOLINK-Station in dem Versuchsgehege aufgebaut
- Funktionstüchtigkeit von ETHOSYS® während der Untersuchung überprüft
- gemeinsam mit dem Hauptautor das Manuskript (in Englisch) verfaßt

Ziel der Arbeit:
Diese Arbeit entstand aus einer Zusammenarbeit zwischen dem Institut National de la Recherche Agronomique in Theix und dem Institut für Zoo- und Wildtierforschung in Berlin. Ziel der Arbeit war es, das in Berlin entwickelte Speicher-Telemetrie-System ETHOSYS® (das Aktivität, Fressen und Wiederkauen bei großen Herbivoren erfaßt, indem es die Bewegungen und die Stellung des Tierkopfes mißt und interpretiert) mit dem in Frankreich entwickelten Kauschlagzähler APEC (der Fressen, Wiederkauen und Ruhen bei Milchvieh erkennt, indem er Kieferbewegungen mißt und interpretiert) in einem direkten Versuch miteinander zu vergleichen. Es sollte geprüft werden, inwieweit die Verhaltensparameter Fressen und Wiederkauen, die von beiden Systemen - allerdings auf unterschiedlichem Wege - registriert werden, einander entsprechen. Dies geschah von französischer Seite aus mit dem Ziel, gegebenenfalls in späteren Untersuchungen zum Futteraufnahmeverhalten bei Herbivoren das leichter handhabbare ETHOSYS® der bis dahin genutzten französischen Methode APEC vorzuziehen. Von deutscher Seite bestand das Interesse, die mit ETHOSYS® indirekt gemessenen Verhaltensdaten zum Fressen und Wiederkauen mit direkt gemessenen Daten abzusichern bzw. dadurch das Meßsystem zu korrigieren.

Visual observation of grazing behaviour is time consuming and not easy during night. So, automatic recording systems of grazing activities are interesting insofar as they allow to collect information during long periods and night.
We compared two of these systems. The first one, APEC, was developed by the INRA station, Theix. It measures, with a good reliability, eating and rumination by recording jaw movements (BROUILLETTE et al. 1993, JDS 76, suppl. 1, 405). APEC includes a pneumatic transducer, fixed under the jaw, and a data logger. All jaw movements create pressure variations which are detected every 2.5 seconds by an electronic transducer which converts pneumatic impulses to binary notation. Data stored are collected after handling the animal and connecting the APEC with a computer. Then, values are categorised into eating, ruminating and resting by an interpretative software program. The second system, ETHOSYS®, was developed by the Institute for Zoo Biology and Wildlife Research, Berlin. A collar realises an indirect measurement of the activities through two sensors. One measures acceleration, the second the head position. Thus, activities are identified on the basis of animal movements, head position and rhythms of neck movements. Logic functions, included into the collar, interpret the signal patterns and categorise them into eating, ruminating, activity with head down and total activity. Interpretations are registered each second by a microcontroller which summarises the results in programmed intervals from 1 to 60 minutes and stores them in a memory. Data collection is automatic as data files can be sent to a receiving station by radio telemetry.
To compare these two systems we equipped 3 grazing ewes with the same combination of one APEC and one ETHOSYS® collar, whose sampling interval was 5 minutes. Data were collected for total periods going from 90 h to 116 h. For each of these 3 recording periods, we compared the average per hour of time spent in one activity and we calculated from these averages the correlation coefficients between: (A) Eating measured by APEC and by ETHOSYS®, (B) Eating measured by APEC and activity head down measured by ETHOSYS®, (C) Ruminating measured by APEC and by ETHOSYS®.

Ewe	Recording period [hour]	Correlation coefficient			Eating by ETHOSYS® [% eating by APEC]	Activity head down by ETHOSYS® [% eating by APEC]	Ruminating by ETHOSYS® [% ruminating by APEC]
		A	B	C			
1	90	0.91	0.95	0.66	56	105	61
2	98	0.81	0.90	0.56	44	111	106
3	116	0.93	0.94	0.80	55	99	58

The measurement of grazing activities obtained from the two methods are highly correlated, particularly concerning eating [Table]. Correlations for rumination differ between the recording periods but are higher than 0.5.
Considering APEC as the reference we observe that the activity called „head down“ by ETHOSYS® gives a better estimation of eating than the activity called „eating“, which certainly concerns only biting.
In conclusion, ETHOSYS® gives a good estimation of eating through the „activity head down“. As for rumination, APEC is more precise as it realises direct records of jaw movements. The disadvantages of APEC are that it is time consuming in application and its storage capacity is limited to 6 days. Although it realises an indirect measurement of behaviour ETHOSYS® is interesting specially for the study of free ranging herbivores thanks to its simple application, the automatic data transfer and its capacity to work independently for a year.

3. Chronobiological Investigations on Two Herbivorous Species

3.1. Diurnal and Ultradian Rhythms of Behaviour in a Mare Group of Przewalski Horse (*Equus ferus przewalskii*), Measured Through One Year under Semireserve Conditions

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Eigenanteil:
- praktische Anpassung von ETHOSYS® an die Tierart Przewalskipferd
- Funktionstüchtigkeit von ETHOSYS® während der Untersuchungen überprüft
- gesamte Auswertung und Berechnung der erfaßten Daten
- Manuskript verfaßt (Korrektur durch Mitautoren) und Grafiken erstellt
- Manuskript in's Englische übersetzt (Korrektur durch Dolmetscher)

Ziel der Arbeit:
Vorversuche hatten gezeigt, daß mit ETHOSYS® eine geeignete Methode zur langfristigen, lückenlosen Erfassung von Verhaltensparametern an frei beweglichen Tieren gegeben ist. Dieses Meßsystem wurde an Przewalskipferden in einem Semireservat eingesetzt, um einerseits die Fähigkeiten dieses Systems unter naturnahen Bedingungen in der Praxis unter Beweis zu stellen, aber vor allem um Möglichkeiten der biorhythmischen Analyse solch langfristig erfaßter Daten aufzuzeigen. Vor dem Hintergrund der geplanten Auswilderung dieser Tiere war zu prüfen, ob der Einsatz dieser Meß- und Analysemethoden zur routinemäßigen Überwachung des Belastungs- und Gesundheitszustandes bei diesen Tieren (im Freiland) beitragen kann. Um überhaupt Belastungssituationen bei diesen Tieren erkennen und bewerten zu können, sind artspezifische Grundwerte unerläßlich. Die Arbeit zielte dabei vornehmlich auf die Erfassung der Grundwerte des Verhaltens von Przewalskipferden und fand daher unter weitgehend ungestörten, naturnahen Bedingungen statt.

ABSTRACT
Investigations were conducted on four horses from a group of twelve Przewalski mares raised in different zoos and kept in a 44-ha enclosure under seminatural conditions. Activity and feeding were continuously measured every second and were saved every 15 minutes 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-hour rhythmicity, but also by ultradian components with period lengths between 4.8 and 12 hours, 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 general living conditions, especially the nutritional situation and to detect stressful conditions.

Keywords: horse - Przewalski horse - circadian rhythm - ultradian rhythm – activity - feeding and nutrition – telemetry - stress diagnosis

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 thus are 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 et al. 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 semireserve [Fig. 1]. They were born in different zoos and preserves and were selected for this semireserve and thus for potential renaturalisation under the European Preservation-Breeding Programme for the Przewalski Horse (EPP). All were well adapted to the semireserve conditions and 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.
Clear judgeable hierarchy conflicts between mares were registered in regular visual observations of the herd (see also Chapter 2.3.). The individual index of dominance, according to BOWEN et al. (1978) varying between +1 (absolutely dominant) and -1 (completely subdominant), was calculated for each of the animals from victories and defeats.

Fig. 1 The Przewalski horse mare herd of Schorfheide/Liebenthal semireserve.

Name	Sex	Birthday	Birth place	Stud book number	Date of arrival in semi-reserve	Index of dominance
Alina	female	21/12/88	Cologne	1789	08/04/92	1.00
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	-0.90
Lulu	female	04/08/92	Cologne		28/05/93	-1.00

Index of dominance = (victories - defeats) / (victories + defeats)

2.2. Location

Semireserves 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 semireserve 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*, *Cirsium vulgare*, *Dactylis glomerata*, *Lolium perenne*, *Trifolium 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 semireserve.

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 300g. 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, lying or standing.

A series of prehensive 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 minutes, 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 minutes). The internal battery of ETHOREC is sufficient for about one 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 hours, measuring interval: 15 minutes) and in monthly intervals for the total herd (duration: 24 hours, measuring interval: 10 minutes).

2.4. Data analysis

Data collected by ETHOSYS® from four animals (Duma, Spirre, Mada and Mida) over at least one year were used for this analysis. In total, a data volume of 1,498 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 then were tested for annual variation by the Fried-

man 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 7 consecutive days, with a delay of one 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 one 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 et al. 1976, SCHEIBE et al. 1999). This 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)) (1). Harmonic ultradian periods are defined as periods which were synchronised with the external circadian zeitgeber in relation to an integral number (that means 24 hours divided by 1, 2, 3 etc. gives harmonic periods)

$$DFC\ [\%] = \frac{SI(harm) * 100}{SI(total)} \qquad (1)$$

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).

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.

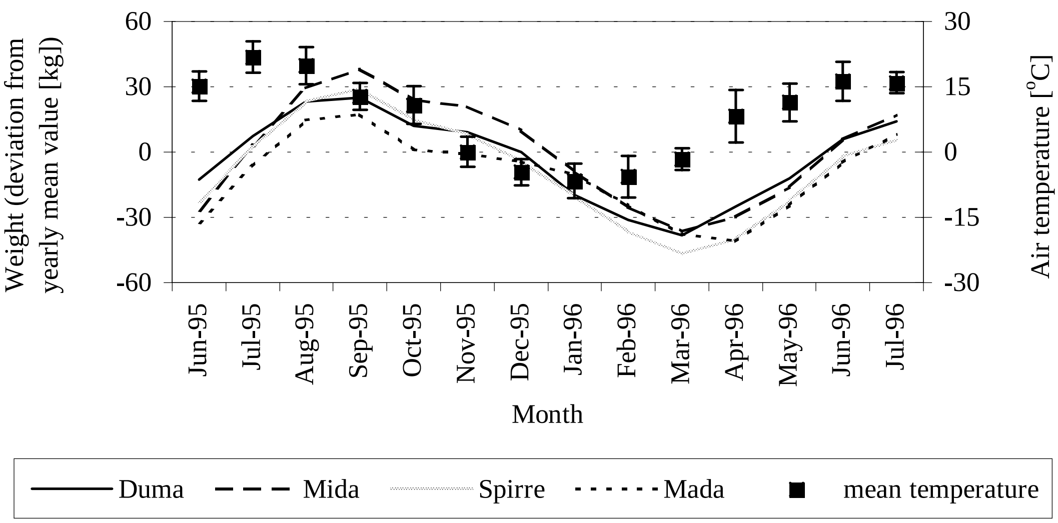


Fig. 2 Annual variation of body mass of four Przewalski horses under semireserve conditions (monthly mean values) and annual variation of air temperature (monthly mean values calculated from daily mean values and standard deviation).

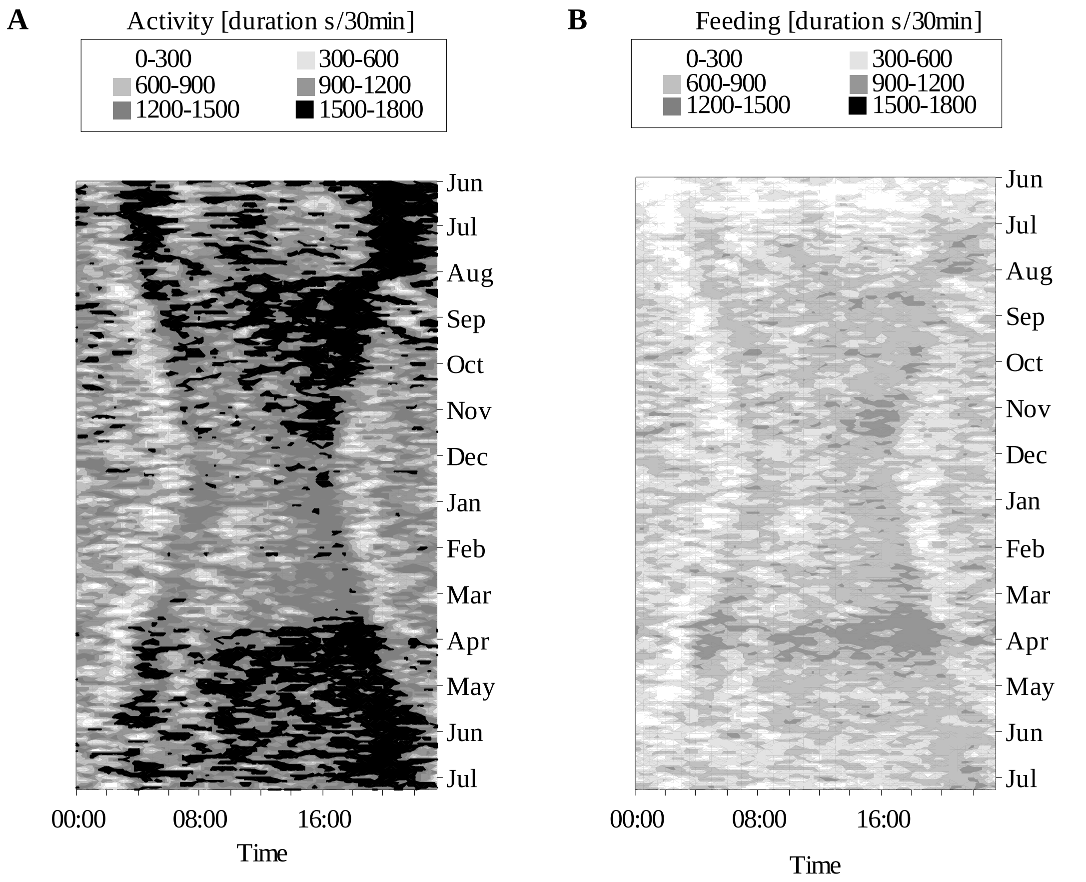


Fig. 3 (A) Activity pattern during one year (mean values of four Przewalski horses).
(B) Feeding pattern during one year (mean values of four Przewalski horses).

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 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 normally is 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 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.

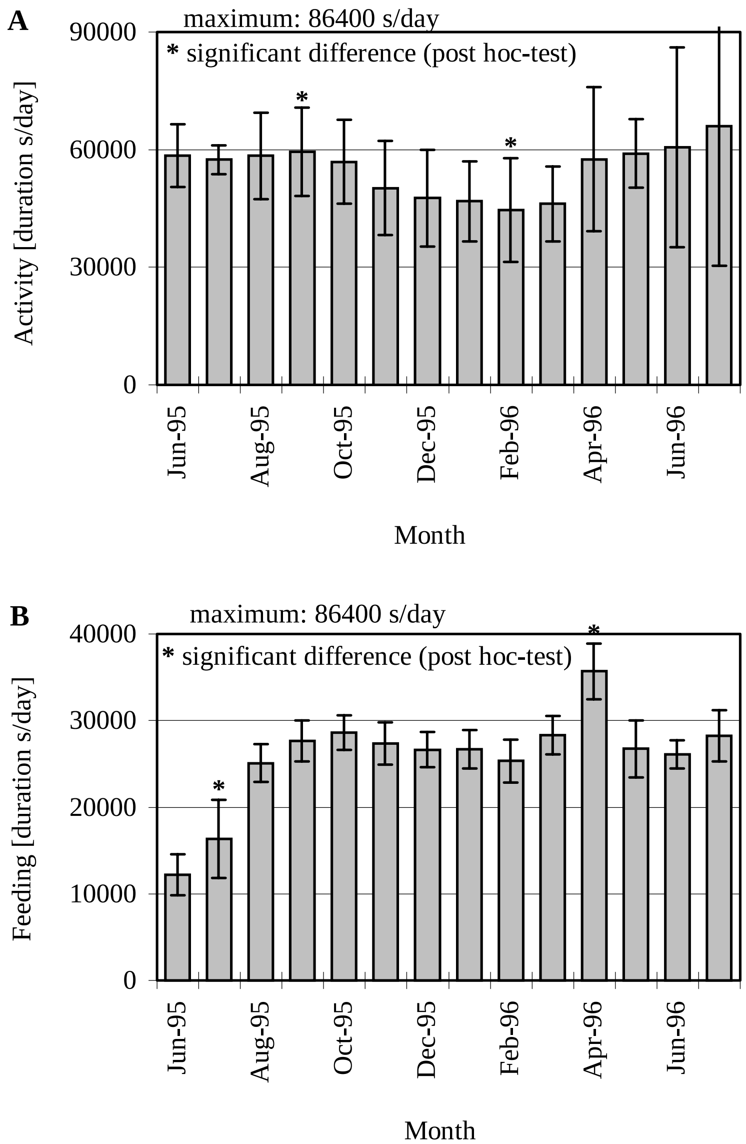


Fig. 4 (A) Annual variation of daily total activity (monthly mean values and standard deviation).
(B) Annual variation of daily total feeding (monthly mean values and standard deviation). All data represent the mean value of four Przewalski horses.

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 night-time 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 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 seven-day period (A). Detected in this example were the 24 hours period, a significant harmonic period of 12 hours length and a significant nonharmonic period of 4.1 hours 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 methods) is shown (B). No systematic variation was obvious during the year for the ultradian or the

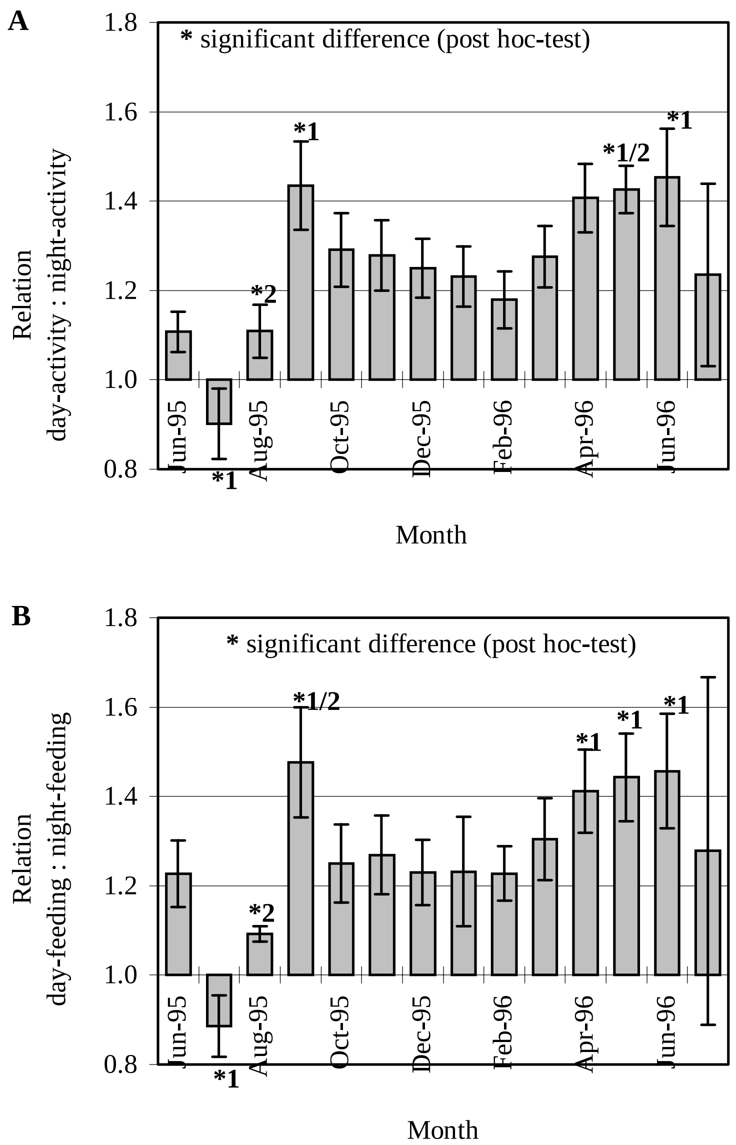


Fig. 5 (A) Annual variation of relationship between activity at daylight and activity at night (monthly mean values and standard deviation).
(B) Annual variation of relationship between feeding at daylight and feeding at night. (monthly mean values and standard deviation). All data represent the mean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

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-hour-period (14.0%) and 6-hour-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, as well as between December 1995 and 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.

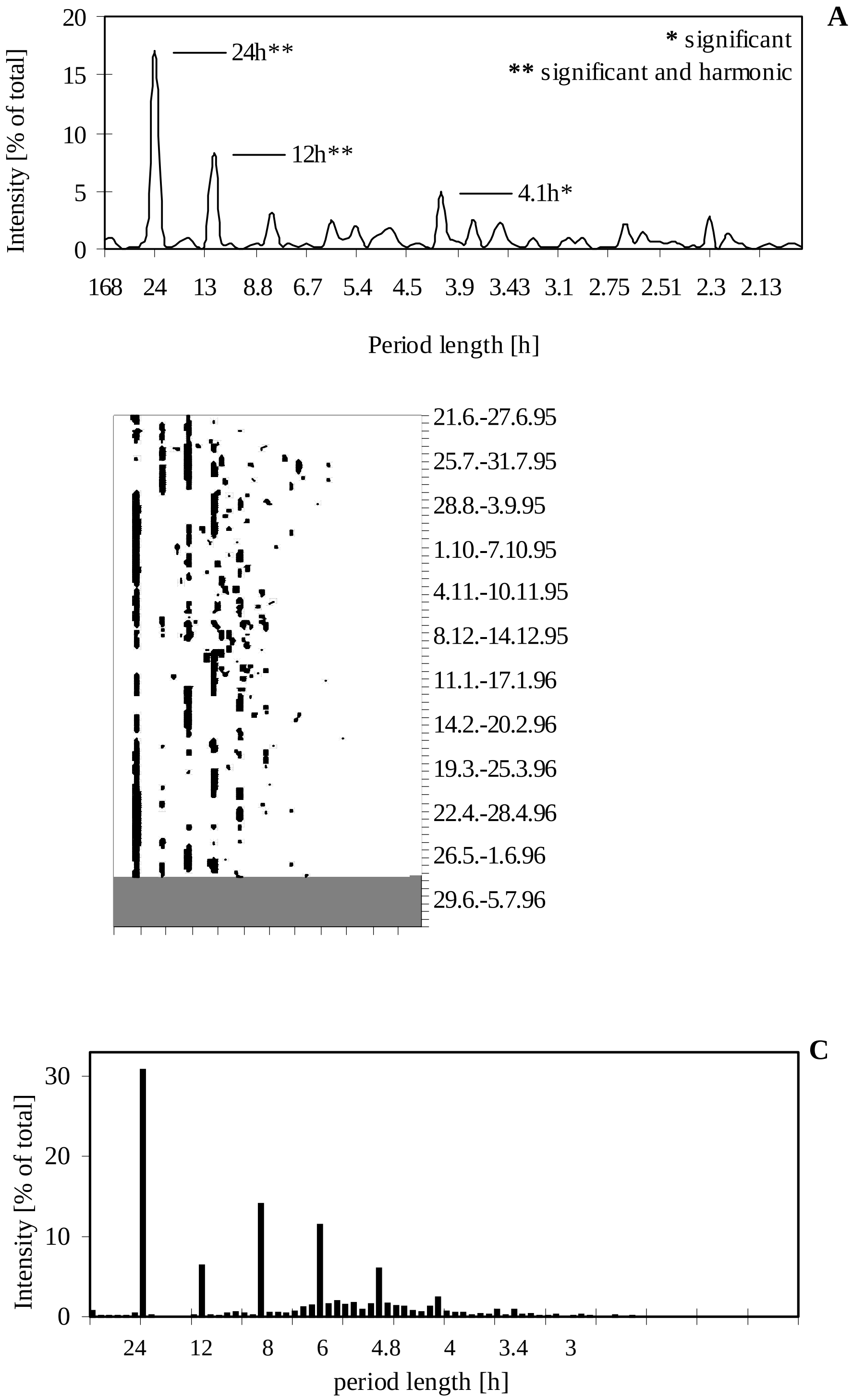
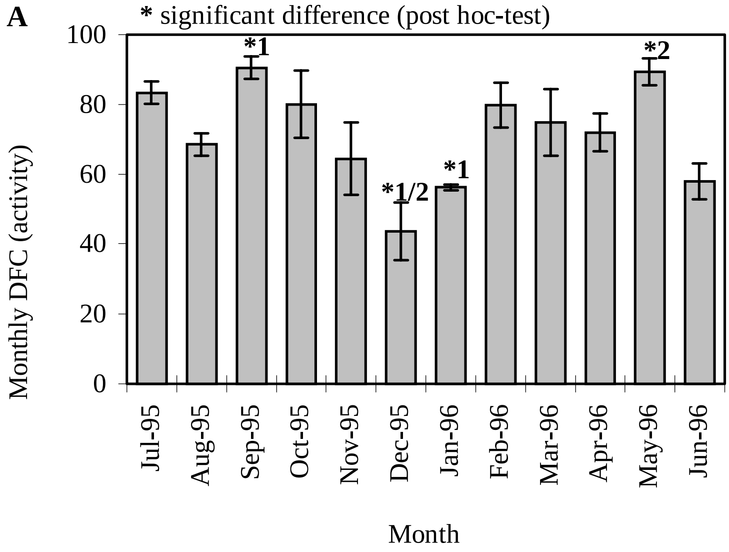


Fig. 6 (A) Typical power spectrum of activity calculated over a period of 7 successive days (Przewalski horse "Duma", April 18 to 24, 1996).
(B) Annual variation of existence of significant periods in power spectra of activity of Przewalski horse "Duma".
(C) Total intensity of significant periods in power spectra of activity of Przewalski horse "Duma", added up over the whole year and depicted in percent of the total.

Time series were recorded from one animal during its stay in the zoo, its transportation from zoo to the semireserve and in the subsequent period in the semireserve, and DFCs of activity are depicted as an example of adaptation to seminatural 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%).



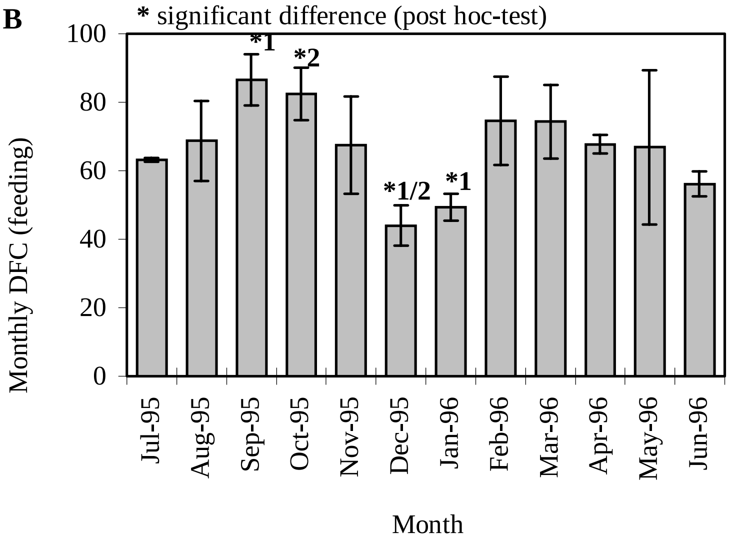


Fig. 7 (A) monthly DFC for activity during one year (mean values and standard deviations of four Przewalski horses).
(B) monthly DFC for feeding during one year (mean values and standard deviations of four Przewalski horses).

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 et al. 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 et al. 1980, KASEDA 1983, ARNOLD 1984, BOGNER et al. 1984, DUNCAN 1985, MAYES et al. 1986, FRASER 1992). However, the diagnostic value of continuous activity records has been demonstrated for domestic horses (by GILL) as early as in 1991.

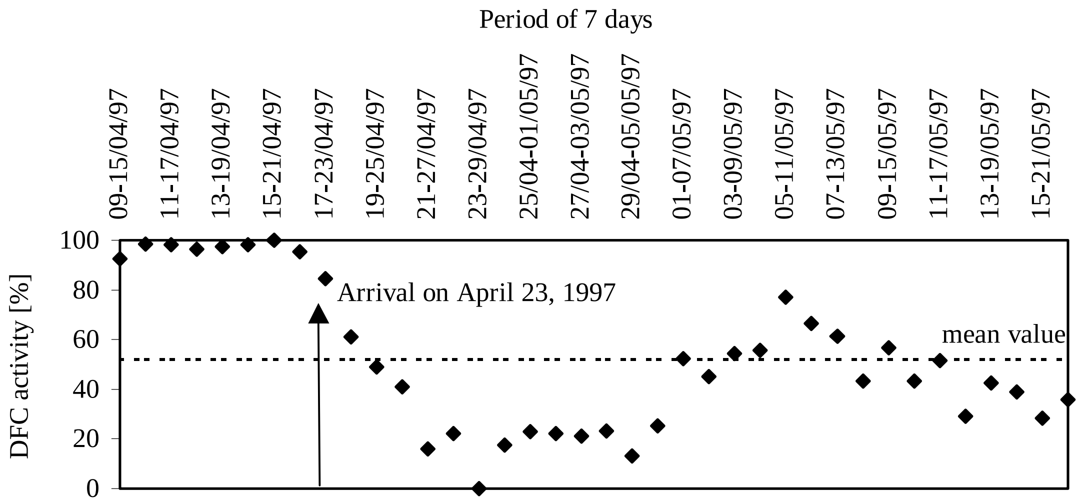


Fig. 8 DFCs of activity of Przewalski horse "Medi", from April 9 to May 22, 1997 (calculated over overlapping periods of seven days with a delay of one day). On April 23, "Medi" was transferred from a zoo to the semireserve.

The results present a typical year in this mare herd under seminatural 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 two and a half 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 semireserve 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% +/- 15.7%) was higher in winter (48.4% +/- 15.4%) than in summer (30.7% +/- 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 et al. 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-hour-day were always timed at dusk and dawn (BUBENIK 1961, KEIPER et al. 1980, KASEDA 1983, MAYES et al. 1986, BOYD et al. 1988). Long interruption of grazing activity was observed at noon in the months of summer (RUBINSTEIN 1981, MAYES et al. 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 et al. 1986, BOYD et al. 1988). In the course of the night, grazing activity of horses is lower and resting behaviour increases (KEIPER et al. 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, activity and feeding at night were 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 one a half year was reported by VAN DIERENDONK 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-hour period. Conspicuously high contributions of ultradian harmonic periods, between 12- and 4.8-hours in lengths, were found in the power spectra of horses. These ultradian periods generated 2 to 5 activity bouts per day. Ultradian nonharmonic periods between 6.7- and 4-hours 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 et al. (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 "Degrees of Functional Coupling" (as an objective parameter of co-ordination 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 et al. 1974), ewes (SINZ et al. 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 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 still was 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 does not exist until now. Drastically decreased DFC during adaptation to seminatural conditions [see Fig. 8] was one of several accidentally found one-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, continuos behaviour records should be applied to identify and prevent risk situations of individuals and groups.

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3. Chronobiological Investigations on Two Herbivorous Species

3.2. Diurnal and Ultradian Rhythms in Red Deer Behaviour
- One-Year Measurements under Quasi-Natural Conditions -

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Eigenanteil:
- praktische Anpassung von ETHOSYS® an die Tierart Rothirsch
- ETHOLINK-Station in den Versuchsgehegen aufgebaut und Halsbänder angelegt
- Funktionstüchtigkeit von ETHOSYS® während der Untersuchungen überprüft
    - visuelle Paralleluntersuchungen durchgeführt,
    - Übereinstimmung zwischen den Beobachtungsdaten und den mit ETHOSYS® gemessenen Daten geprüft (Korrelationsberechnungen durchgeführt)
- gesamte Auswertung und Berechnung der erfaßten Daten
- Manuskript verfaßt (Korrektur durch Mitautoren) und Grafiken erstellt
- Manuskript in’s Englische übersetzt (Korrektur durch Dolmetscher)

Ziel der Arbeit:
Diese Arbeit entstand aus der Zusammenarbeit des Institut für Zoo- und Wildtierforschung Berlin, des Institut National de la Recherche Agronomique in Theix und des Institut für Wildtierkunde und Ökologie in Wien. Ziel war es, Möglichkeiten zur biorhythmischen Analyse langfristig erfaßter Daten an Rothirschen unter naturnahen Bedingungen darzustellen. Vor dem Hintergrund der Wald-Wild-Management-Diskussion war zu prüfen, ob der Einsatz solcher Meß- und Analysemethoden zur routinemäßigen Überwachung des Belastungs- und Gesundheitszustandes bei diesen Tieren (im Freiland) beitragen kann. Die Untersuchung, die unter weitgehend ungestörten, naturnahen Bedingungen stattfand, zielte dabei vornehmlich auf die Erfassung artspezifischer, biorhythmischer Grundwerte des Verhaltens, deren Kenntnis notwendig ist, um überhaupt Belastungssituationen bei Tieren bemerken und bewerten zu können. Vergleichende Betrachtungen zu der bewußt ähnlich angelegten Arbeit an den Przewalskipferden sollten zusätzliche interessante Hinweise auf die artspezifische biorhythmische Anpassung an Umweltbedingungen erbringen.

ABSTRACT
The studies described in this paper were conducted on two red deer hinds in a quasi-natural 37-hectare enclosure in Slovakia between April 1995 and January 1996. One stag and one red deer hind were studied in a two-hectare experimental enclosure of INRA Theix from July 1997 through May 1998. Activity and feeding were recorded by means of the storage-telemetry-system ETHOSYS®. Daily and monthly mean values as well as power spectra were calculated for activity and feeding.
The general pattern of activity and feeding over the year was closely related to light-dark change. Feeding accounted for 36 % of total activity in autumn and 44 % in winter (all-year average being 41 %). Levels of activity and feeding were lowest in winter and highest in summer. Greatest activity was observed in daylight hours and gradually shifted to dark hours in summer. Spectral analysis of red deer activity and feeding revealed a time pattern in which the intensity of ultradian components, between 4.8 and 12 hours in period length, quite often was higher than that of the circadian rhythm. Time patterns of activity and feeding moved into higher frequency ranges during periods of better food supply. In such periods, there were up to eight strong activity bouts per day (three-hour rhythm). The results are discussed in connection with the feeding strategy of red deer.
Analysis of the time pattern of long-term high-continuity measurement of behavioural parameters, such as activity and feeding, with reference to structural measures (Degrees of Functional Coupling - DFC) proved to be an appropriate approach to the follow-up of general living conditions and identification of stress conditions.

Keywords: red deer - diurnal rhythm - ultradian rhythm - activity - feeding and nutrition - telemetry - stress diagnosis

1. INTRODUCTION

Red deer (*Cervus elaphus* Linné), a big herbivore, is a major component of the sylvan ecosystem. This species is interwoven with the evolutionarily grown complex network of space and time requirements of all species of the biotope. Yet, free-ranging wild-living animals, including red deer, are put under growing pressure by man with regard to their space and time requirements. In addition to frequently unpredictable human influence on climate, soil, vegetation and water, i.e. indirect action on fauna and flora, man has come into direct competition to animals (by turning landscapes into built-up areas or by farming, forestry, hunting and nature-oriented tourism). Anthropogenic interference has been leading to significant changes in wildlife behaviour, including reduced rumination and feed intake in concomitance with increased activity, vigilance and flight behaviour (CEDERNA 1985, SKOGLAND et al. 1988, HUMPHRIES et al. 1989, SCHNIDRIG et al. 1991) and even general deterioration of health, physical condition and reproduction (SQUIBB et al. 1986). Forest and field damage is on a strongly rising trend, which actually worsens the conflict situation especially at the expense of red deer and roe deer (GUTHÖRL 1994, SCHÜTZ 1994). Man, by virtue of his greater freedom of action on nature, should accept responsibility for defusing such problems (and avoiding unnecessary suffering of animals) and, in this context, should give proper attention to both human interest in the use of nature and claims of wildlife on their environment. Neglect of wildlife demands on the environment has been identified as a cause of suffering (SCHEIBE 1997). Knowledge of species and their environmental needs, including time patterns (i.e. environmental requirements in time), therefore, is a prerequisite for unbiased assessment of stress conditions to which an animal or group of animals may be exposed and is, at the same time, a step towards reasonable forest and wildlife management.

Red deer is one of the most frequently investigated wild-living animals in Europe, and there is widespread discussion on cervid activity patterns (for instance CRAIGHEAD et al. 1973, GEORGII 1981, 1983, FISCHER 1985, RISENHOOVER 1986, BEIER et al. 1990, GREEN et al. 1990). The conclusion may be derived from those discussions that time patterns of deer are of high variability, depending on a wide range of different factors. OZOGA et al. (1970) found that while environmental conditions could partially suppress or emphasise activity patterns, they were not capable of generating basically new structures. Hence, highly accurate structural analysis of behaviour rhythms of red deer might provide information on time requirements of the species. The normal values that are typical of a given

species under natural conditions with minimised stress should be known to identify unusual deviations of diagnostic relevance. This study was conducted for the purpose of describing all-year normal, species-specific variations in red deer behaviour rhythms and elucidating ways to recognise unusual or stressful conditions.

2. MATERIAL AND METHODS

2.1. Animals

Studies were conducted on four red deer from two different experimental enclosures. Sex, age, site of enclosure and measuring period may be seen from Fig. 1. All animals were in good physical condition and were integrated in natural herd union, with each of them being linked to another sub-group. The young male was still in his growth phase throughout the study period (mean weight: 118.5 kg). The females (mean weight: 112 kg) had completed growth and were involved in the natural reproduction cycle.

Number of deer	1	2	3	4
Sex	female	female	female	male
Place of enclosure	Velcize	Velcize	INRA-Theix	INRA-Theix
Year of birth	1991	1991	1990	1996
Measuring period:				
Start	11/04/95	18/05/95	18/07/97	18/07/97
End	18/01/96	07/01/96	09/06/98	19/05/98
Portion of measured data [%]:				
Jan	100	100	100	100
Feb			100	100
Mar			100	100
Apr	100		100	100
May	100	47.4	99.9	100
Jun	72.3	15.1	100	
Jul	58.6	16.4	100	100
Aug	98.6	52.4	100	100
Sep	92.6	0	61.3	100
Oct	85.9	53.2	0	55.7
Nov	100	100	55.5	100
Dec	100	94.3	98	97.9
Total duration of measurement [days]	277	116	269	290
Average duration of continuous registration [days]	62	19	67	97
(range)	(28 to 100 days)	(3 to 51 days)	(21 to 146 days)	(60 to 146 days)
Number of gaps in data registration	3	5	3	2
Average duration of gaps [days]	9	23	19	8
(range)	(6 to 13 days)	(2 to 55 days)	(1hour to 56 days)	(16 hours to 14 days)

Fig. 1 Overview of red deer investigated as well as duration and success of behaviour study, using the storage-telemetry-system ETHOSYS® on the animals involved.

2.2. Enclosures

The experimental enclosure of Velcize is part of a larger enclosure for breeding and testing of fallow deer (*Dama dama*). It is highly structured and 37 hectares in size. Crop fields account for 40%, meadows for 24% and forest with a brook for 36%. It is a mixed forest made up of wainscot oak and acacia (*Prunus spinosa, Robinia pseudoacazia)* and is interspersed by trenches up to five metres in depth. A catch fence is located in the southern part of the enclosure where a bait feeding place is filled if an animal has to be captured.

The experimental enclosures of INRA Theix are about two hectares in size and are made up of high-nutrient meadows on mountain slopes with a brook and scattered groups of trees. Daily per-capita rations of 500g of alfalfa (*Medicago* sp.) and 200g of barley (*Hordeum* sp.) were additionally fed to the animals, from August 11, 1997, through December 24, 1997. Thereafter, from December 24, 1997, through May 1, 1998, 300g/die of barley were added for each of the females and 500g/die for the males. Hay was additionally offered *ad libitum* between September 15, 1997, and April 1, 1998. No additional rations were fed any longer after May 1, 1998. Animals were driven for easier management into a drop-floor catch system which ensured non-invasive handling.

Either investigation area was characterised by continental climate, with high-snow and cold winter seasons and hot, dry summers. The mean temperature in Velcize was 9.4°C during the period of investigation (minimum: -12.6°C, maximum: 31.2°C). The area was under a complete snow cover for 40 days in the winter season of 1995/96. The mean temperature of Theix for the period of investigation was 8.8°C (minimum: -10.1°C, maximum: 30.5°C). The area was under a complete snow blanket for 24 days in the winter season of 1997/98.

2.3. Recording of activity and feeding

General locomotor activity and feeding were recorded by means of the storage-telemetry-system ETHOSYS® (SCHEIBE et al. 1998). Measuring collars were applied to the animals. The approximate weight of a collar for red deer was 250g. A two-channel measuring system within the collar could recognise any movement of the animal which was transmitted to the collar. "General" locomotor activity (according to the definition by ASCHOFF 1962) resulting from all movements independent of the animal's position, lying or standing, was recorded by one of the channels. When the collar-bearing animal held its head downward and moved it in a way characteristic of red deer feeding (plucking of bites at frequencies between 375ms and 1,250ms), this movement was additionally recorded as "feeding" by the other channel.

The collar sensors were read in one-second intervals. Readings were summed up and were stored in an internal memory at the end of a 15-minute analysis interval, whereafter the process of counting was recommenced. As soon as an animal was identified by means of a passive infrared detector within the transmission range of a central station, the generated time series were automatically radioed from the collar to that central station which was installed in a position preferred by the animal.

Once the data have been transmitted, the memory is cleared for more recordings. The RAM memory capacity of the collar is 32k, sufficient for 2,047 data records (21 days of measurement). Integrated battery life is good for about one year.

Investigations were conducted beforehand on fenced red deer (at least 20 memory units per collar, equivalent to five hours) to make sure that the behaviour parameters of activity and feeding were correctly identified by the collars.

2.4. Data analysis

An overview of the period of time and success of data recording from the four test animals is given in Table 1. All data series, as recorded from each of the animals for activity and feeding, were analysed in separate steps.

Daily phases of activity and feeding were selected for each of the animals. Phases from which no activity was recorded were defined as rest phases. Original data recorded from all four animals were averaged within defined periods of time and were presented in plotter graph format in an attempt to realise a basic pattern of behaviour parameters throughout the year.

Daily totals were calculated for activity and feeding (A). The percentage of activity in daylight hours was related to that in dark hours for each day (B). The daylight hours, i.e. the time from sunrise to sunset, were derived from Ahnert's astronomic table (BURKHARDT et al. 1994). The (daily) A and B parameters for any given month were summarised for each of the animals and were averaged. Those calculated monthly values were tested for their annual variation, using Friedman's test, and were subsequently verified by means of multiple pairwise comparison (DANIEL 1990). In the context of multiple comparison, we did not present p-values for each individual comparison but only for their significance.

Those initial macroscopic analyses were followed by closer structural investigation of each of the time series. The original data series were checked for their relative rhythmic components. All data files were subdivided into data sets for seven consecutive days, with a delay by one day between every two data sets. From each of those partially overlapping data files, autocorrelation functions were calculated, and power spectra were derived from the latter. The periods of those power spectra were tested for significance (ANDEL 1984), which gave the significant periodic components of the original data series. A three-dimensional graph was used to define the extent to which these components were distributed among high and low ranges of the annual frequency spectrum. The three-dimensional graph was as follows: x-axis = period length, y-axis = year subdivided into successive seven-day segments, z-axis = intensity of significant period of power spectrum.

The values of these significant periods are used to calculate the "Degree of Functional Coupling" (DFC) (SINZ et al. 1976, SCHEIBE et al. 1999) which expresses the relationship between the total intensities of significant harmonic periods, [SI(harm)], and of all significant periods, [SI(total)] (1). Harmonic periods, in this context, are defined as periods which are synchronised by an integral-number relationship with the circadian zeitgeber (i.e. 24 hours divided by 1, 2, 3 etc. gives harmonic periods).

$$\text{DFC [\%]} = \frac{\text{SI (harm)} * 100}{\text{SI (total)}} \qquad (1)$$

DFCs varied between 100% (i.e. internal synchronisation within the organism and between organism and environment) and 0% (i.e. desynchronisation). The resulting DFCs (calculated from the above power spectra over seven days) were continuously mapped for each of the animals throughout the year and were compared with points in time at which unusual external influences occurred.

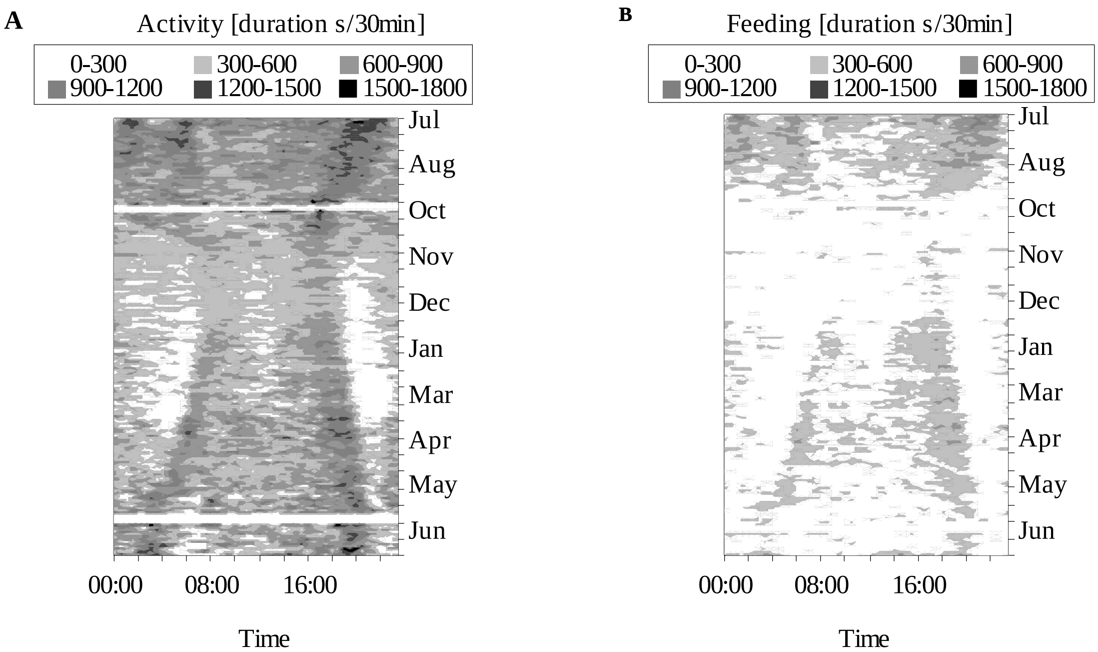


Fig. 2 (A) Activity pattern for one year (averaged for all animals).
(B) Feeding pattern for one year (averaged for all animals).

3. RESULTS

The general distribution of activity (A) and feeding (B) was averaged for all red deer involved for the whole year and may be seen from Fig. 2. Activity is characterised by a polyphasic daily pattern throughout the year. Clearly visible are a linkage between two main peaks to annually varying sunrise and sunset times as well as the major rest phase during the first part of the night, just after sunset. Certain activity phases are discernible during the night, most of them close to midnight. Floating seasonal changes in activity and feeding levels are clearly visible, as well.

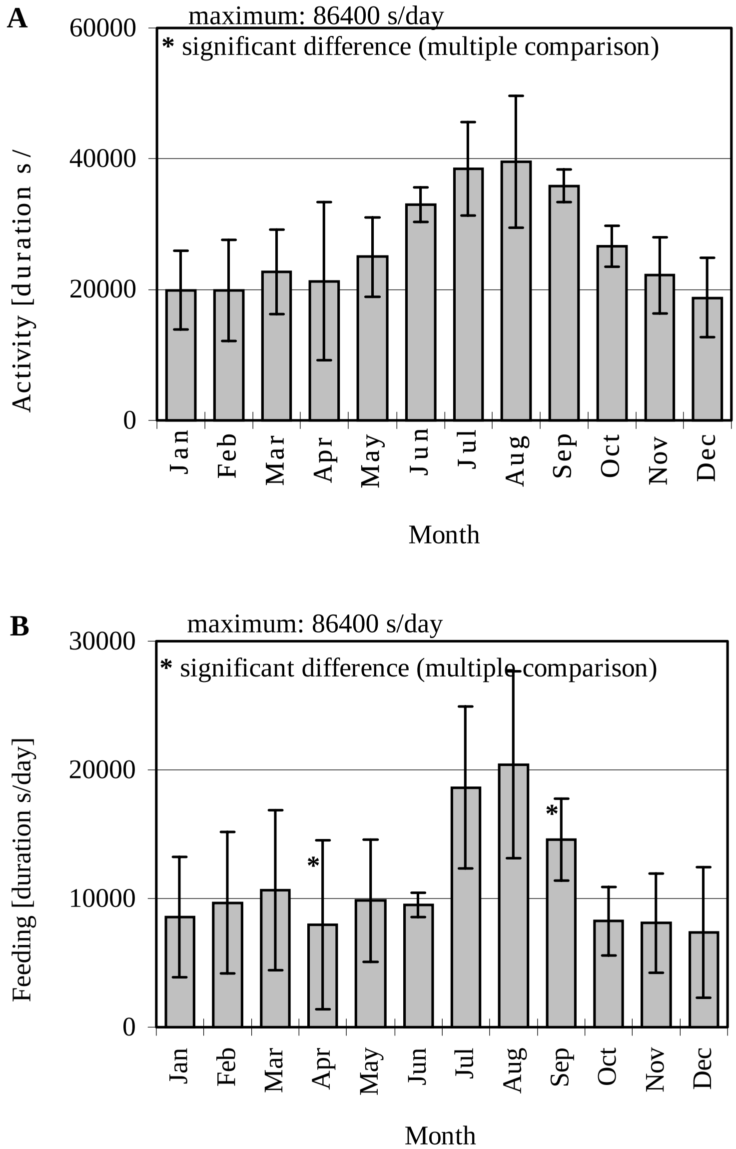
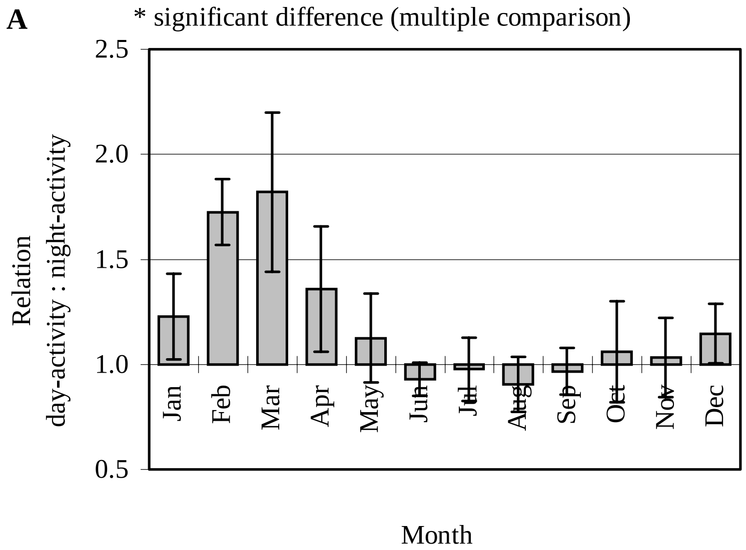


Fig. 3 (A) Annual variation of daily total activities (monthly mean values and standard deviation.
(B) Annual variation of daily total feeding activities (monthly mean values and standard deviation); all data representing mean value of all red deer.

Significant variations over the months were recorded from daily totals of activity and feeding, using the Friedman test for activity, with $n = 4$, $p = 0.03$, and for feeding, with $n = 4$, $p = 0.03$ [Fig. 3]. Activity was reduced in the winter season and was high in summer, with floating transitional periods in between. Maximum and minimum values of the two behavioural parameters did not deviate from each other in the course of the year, although feeding accounted for 36 % of all red deer activity in autumn and for 44 % in winter (annual average: 41 % ± 9 %). Average daily rest periods were 75 % in winter and 55 % in summer (annual average: 66 %). Multiple comparison did not reveal any significant difference of either behavioural parameter between July and December.



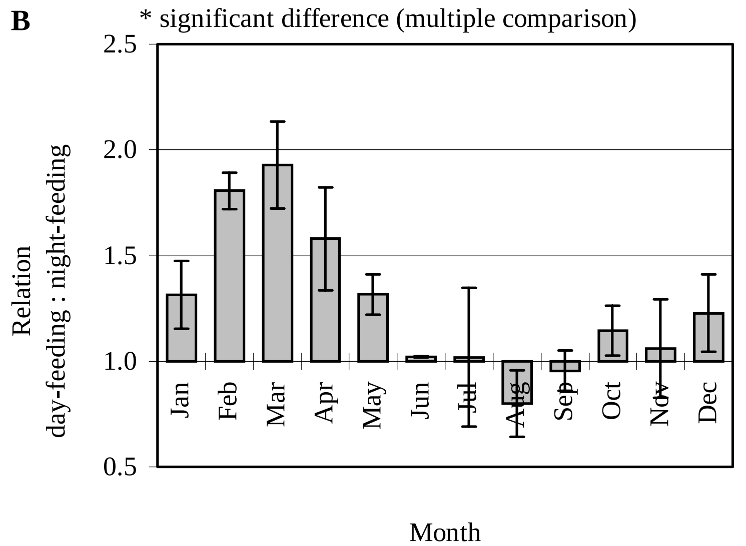


Fig. 4 (A) Annual variation in percentual relationship between daytime and overnight activities (monthly mean values and standard deviation).
(B) Annual variation in percentual relationship between daytime and overnight feeding activities (monthly mean values and standard deviation); all data representing mean value of all red deer.

No significant variation from month to month was recorded from the ratio between the percentage of daytime activity/feeding and that of overnight activity/feeding, as may be seen from Fig. 4 (activity: n = 4, p = 0.08; feeding: n = 4, p = 0.07), though daytime activity and feeding were higher (without significance) than overnight activity and feeding from February to April. A trend to higher overnight activity of red deer was observed from June through September. Transitions, however, were fluent between those phases.

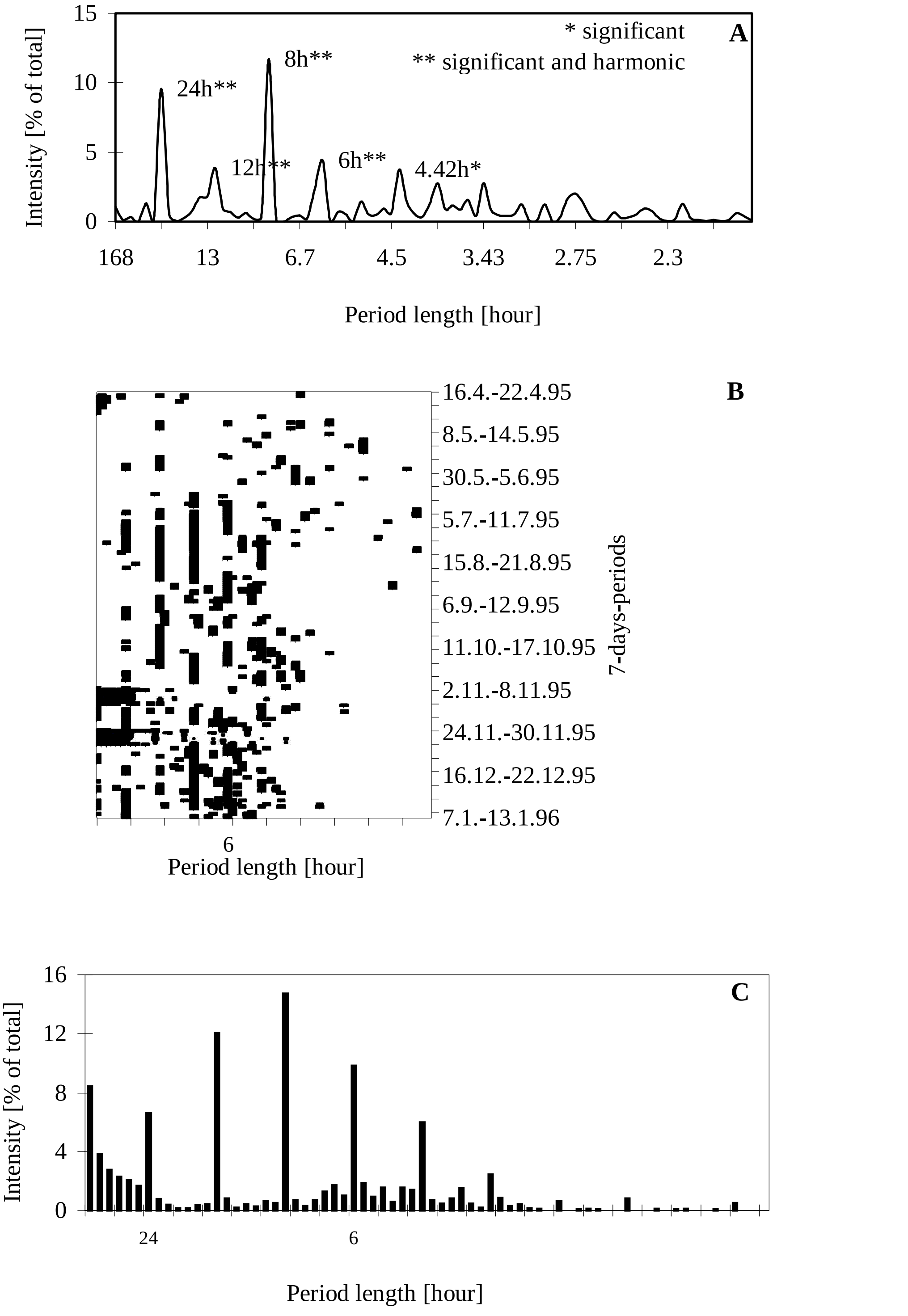


Fig. 5 (A) Typical power spectrum of activity (red deer No. 1, calculated for days from July 9 through July 15, 1995).
(B) Occurrence of significant periods in power spectrum of activity of red deer No. 1 from April 1995 through December 1996.
(C) Overall intensity summed up from each of the significant periods through one whole year in activity power spectrum of red deer No. 1, in percent relative to total of all significant periods.

Depicted in Fig. 5 is a typical power spectrum of activity of red deer No. 1, calculated for a seven-day period (A). The typical 24-hour period was found together with significant harmonic periods of twelve, eight and six hours and a significant non-harmonic period of 4.42 hours in length. A three-dimensional format, as described under the heading of "Material and methods", was used with reference to the example of this individual to show the typical distribution of all significant activity periods over the entire period of measurement (B). The time pattern of ultradian components, in this context, was found to be shifted towards lower frequencies during the winter season. The intensities of each of the significant periods were summed up for one year and were standardised (as percentage of the total of intensities of all significant periods throughout one year) and, finally, are presented in the form of a power spectrum (C). The eight-hour period was found to be the strongest of all periods (14.7% relative to the total). It was followed by the twelve-hour period (12 %) and the six-hour period (9.8 %). Only then followed the circadian period (6.6 %). The surprisingly high percentage of infradian periods was attributable to three short phases (each of them with seven consecutive power spectra) within the entire period of investigations, as may be seen from Part B of the figure. The animal was immobilised for two hours in the first phase (April 1995), while other animals were chased for immobilisation within the enclosure in the two remaining phases (both in November 1995).

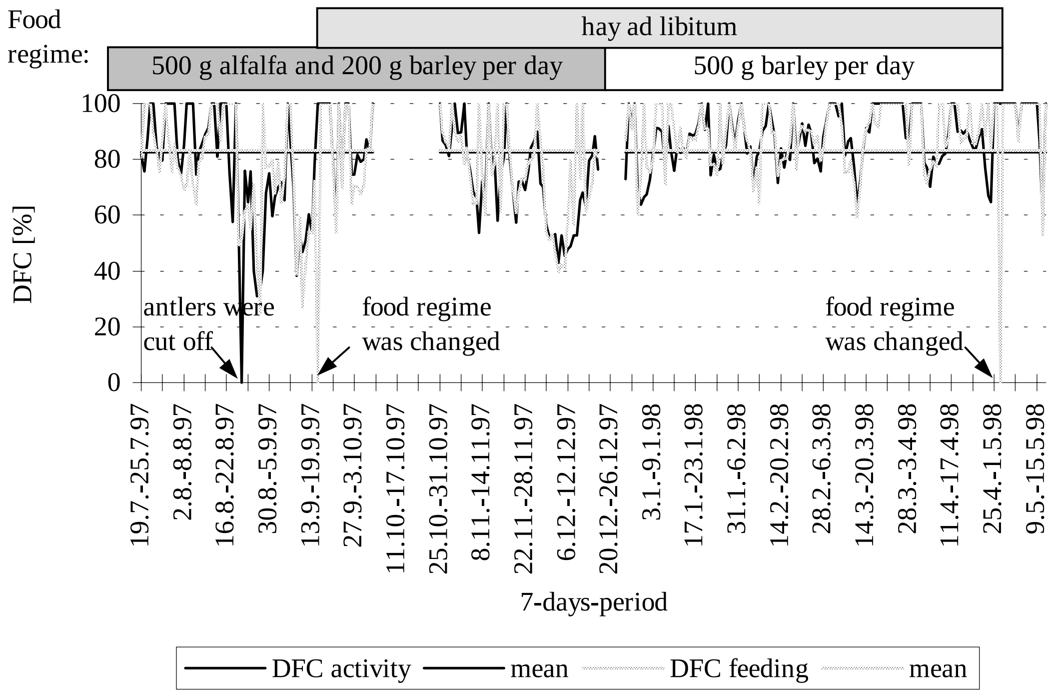


Fig. 6 DFCs for activity and feeding of red deer No. 4, from July 19, 1997, through May 18, 1998 (calculated by overlapping seven-day periods with delay of one day); antlers were cut off on August 21; food regimes were changed on September 15, December 24 (gap in data record) and May 1.

The DFCs for activity and feeding of red deer No. 4 for the entire period of measurement are given in Fig. 6. DFC mean values were 82.5 % for activity and 83.2 % for feeding. The DFC for activity dropped to 0% on August 21 (the day on which the animal's antlers were sawn off). The DFC for feeding dropped to 0% on September 15 and May 1 (days on which feeding regimes were changed).

4. DISCUSSION

While issues relating to land use play a much greater role in the literature, numerous authors have given as well attention to the time pattern of activity of free-ranging cervids. Subjects thus covered include visual observations of hand-reared or kept deer species (COLLINS et al. 1978, BUBENIK 1984, CEDERNA et al. 1985, BÜTZLER 1987) as well as long-time studies with interpretation of data obtained from discontinuous radiotelemetry (for instance CRAIGHEAD et al. 1973, GEORGII 1981 and 1983, FISCHER 1985, BEIER et al. 1990, GREEN et al. 1990). All cases in which sufficient data where available showed a bimodal daily rhythm with activity peaks around dusk and dawn. Also discernible are annual variations in the activity rhythm and its dependence on a diversity of other factors.

FISCHER (1985) reported annual variation in total daytime activity between a minimum of 31 % (without hunting) and 41 % (with hunting) in winter and a maximum of 58 % in summer. Seasonal variation in total daytime activity was equally described by other authors who found minimum winter values of 44 % (GEORGII 1984), 29 % (CRAIGHEAD et al. 1973) and 40 % (BEIER et al. 1990) and maximum summer values of 63 % (GEORGII 1984), 60 % (CRAIGHEAD et al. 1973) or in autumn of 65 % (BEIER et al. 1990). Total daytime activities measured in this study (25 % in winter and 45 % in summer), basically, are in line with the above ratios but are clearly lower in absolute figures. Direct comparability of activity values, in general, is difficult, as different authors have used different definitions in their investigations for behavioural parameters, animal species, measuring methods and measuring intervals. For example, methods used by several authors enabled differentiation merely between lying and not lying. The term of activity, in such cases, was assigned to any behaviour exhibited by an animal when it was not in lying position. ETHOSYS®, on the other hand, rated any second without movement as non-active. Besides, our investigations were conducted under conditions of minimised disturbance which may be another reason for our very low activity data.

There are substantial discrepancies among literature data on the percentual contribution of foraging to total daytime activity. CRAIGHEAD et al. (1973) reported 69 % in summer and 92 % in winter for elk (*Cervus canadensis*), while COLLINS et al. (1978) quoted 89 % for the same species. Other values were 95 % for white-tailed deer (*Odocoileus virginianus*) (BEIER et al. 1990) and 97 % for Alaska moose (*Alces alces*) (RISENHOOVER 1986). In studies on red deer (*Cervus elaphus*) undertaken by BUBENIK (1984), the average contribution made to daytime activity by foraging ranged from 12.5 % to 30.8 % (three to five hours). In those studies, foraging was associated to selection and intake of food, whereas in our ETHOSYS® study intake of food was the only behavioural component recorded (and defined as feeding), so that lower values had to be expected. In our study, the contribution of feeding to total daytime activity throughout a year amounted to 41 %, with a minimum in June (28.9 %) and a maximum in August (51.6 %). The amount of pure feeding per day ranged between 8.5 % (December) and 23.6 % (August), the annual average being 12.9 %. These values fit in comfortably with the data reported by other authors, the more as no complete agreement could be expected anyway, since the contribution made to total activity by foraging/feeding is likely to vary along with the mode of nutrition and administration of feed.

Seasonal change in behaviour of deer is not simply a response to seasonal variation of weather conditions. Annual activity patterns rather reflect variations in metabolic energy consumption and season-related differences in quantity and energy quality of food available (MOEN et al. 1977). Being an intermediate type, red deer is capable of readjusting its physiology and behaviour and is thus properly adapted to changing feeding conditions (HOFMANN 1989). Red deer, in response to decrease in food availability and substantive decline in endogenous energy reserves, tends to increase food intake to make up for lower nutrient levels. That is why the contribution made by feeding activity to overall activity (anyway very low) is at its highest in autumn and winter. Red deer, on the other hand, responds to growing availability of appropriate food by selection of more easily (and thus faster) digestible plants or parts of them. This seems to offer an explanation for shifting of the time pattern of activity and feeding to lower frequencies in periods of abundant food supply. Hence, the annual variance measured for the power spectra of feeding (and thus the fine structure of this behaviour parameter) is indicative of a general strategy of red deer as an intermediate type. Longer active and passive periods in winter, as compared to those in summer, were as well recorded by GEORGII et al. (1978) and CEDERLUND (1981). The "rumen fill theory" was suggested as an explanation by CEDERLUND (1981). In summer food is available in higher quantity than in winter. The rumen is filled up sooner, and the animal will lie down sooner for rumination. Also in summer, the high-energy, easily digestible food is more effectively converted. The rumen is emptied sooner, and the animal will resume foraging.

The ratio between daytime and overnight activities of red deer, furthermore, is interpreted by GEORGII et al. (1978) as a measure of stress from exposure to disturbance. Activity tends to be shifted to the night hours if animals have no way out for evasion of disturbance during the day. BÜTZLER (1987) noticed predominance of daytime activity in the absence of anthropogenic disturbance. The animals involved in our study were exposed to little disturbance all the time (with very rare exceptions). We found, however, that red deer, generally day-active in winter, shifted activities to the night hours in summer. This is in contradiction to the findings reported by GEORGII et al. (1978) and BÜTZLER

(1987) according to which such behavioural change was induced by human interference. It is our working hypothesis that higher overnight activity in summer was attributable to higher ambient temperatures during the day. Daytime molestation by flying insects may have been another contributory factor to overnight activity. In the literature, activity patterns with regard to this particular aspect were shown to be highly variable. Elk (*Cervus canadensis*) investigated by LIEB (1981), just like red deer in our study, was more strongly day-active in winter as compared to summer, whereas elk studied by GREEN et al. (1990) was primarily night-active in winter. There was, however, general and unambiguous agreement in all studies, including our own, on a close relationship between activity/feeding and dusk/dawn hours.

Automatic data acquisition provided long-term, high-continuity and no-impact behaviour information which was of constant accuracy throughout the entire period of measurement. Those data, on account of power spectra for the behaviours of activity and feeding, provided insight into the complex rhythmic structure of red deer behaviour.

The power spectra for activity and feeding revealed a surprisingly high contribution of ultradian harmonic periods between twelve and three hours in period length. Such ultradian periods generated two to eight activity bouts daily. Those periods usually were higher in intensity than circadian rhythmicity. The external zeitgeber period (change of light and darkness) is biologically reflected in change of sleep and wakefulness or active and passive behaviours of animals. In addition, daytime behaviour of animals (ruminants, such as red deer in particular) was found to depend additionally on feed intake, digestion, rumination and other important organic functions with periods shorter than 24 hours. In the healthy animal, there are meaningful correlations between physiological and behaviour-related rhythms as well as between both of them, on the one hand, and environmental periodicities, on the other (ASCHOFF 1962). Most of the complex behaviour functions (such as activity and feeding) are clearly integrated into biological time patterns and are modulated by the latter. They maintain a more or less clearly defined relationship with circadian rhythm, the most important of all rhythms. It was in this context that we quite often obtained from red deer highly unambiguous rhythms from the power spectra of activity and feeding, and those rhythms, after all, exhibited an integral-number relationship with the 24-hour period. Unfavourable conditions, such as illness or stress, consequently should result in an impairment of that time pattern of internal harmony.

The Degrees of Functional Coupling (DFC), being a measure of harmony between various organic functions as well as between the latter and the external circadian zeitgeber, enabled assessment of the organismic status (SCHEIBE et al. 1999, SINZ et al. 1976). In our experience, long-time decrease of DFCs below 60 % (coupled with sudden and strong change in activity levels) seems to be indicative of deviations from standard conditions. Fig. 6 shows, by the example of red deer No. 4 from the experimental enclosures of INRA Theix, that certain external factors led to impairment of the activity pattern and - in this case - even to short-time total collapse of internal synchronisation. Sudden drop of DFCs to minimum values, despite very high DFCs through the remaining months of the study period, was in all three cases attributable to sudden human interference with the environment of the animals. It seems to be noteworthy, in this context, that each of the DFCs declined within the functional cycle that was directly affected by the disturbance. For example, change of food entailed minimum values of feeding DFCs but was of no impact upon activity DFCs. All DFC drops exclusively occurred when the values measured on the "stress day" were for the first time included in DFC calculation, whereas no drops were recordable any more from the subsequent six DFCs in which the same values were included as well.

For animal No. 1 in the experimental enclosure of Velcize, the significant periods of the power spectrum for activity (used in calculating DFCs) are plotted over the period of investigation in Fig. 5B. There were three phases which, conspicuously, were strongly characterised by the presence of infradian periods. Here again, the activity pattern was severely impaired, with DFCs dropping to minimum values for either behavioural parameter. These phases, too, may be interpreted as response to severe anthropogenic interference. In the first of these three phases (April 1995), the animal was immobilised for two hours, while in the other two phases (both in November 1995) human activities occurred within the enclosure, affecting this animal though unrelated to it. DFCs dropped to minimum levels on all these occasions (along with seven successive values) whenever the values measured on the stress day were included into chronobiological calculations. On balance, the DFCs of INRA Theix animals were clearly above those recorded from the animals of the Velcize experimental enclosure. The conditions of INRA Theix were less irritative and were more properly controlled and safer than those of Velcize.

The measurement and analysis methods, when applied to free-ranging red deer, might be useful for identification of serious irritations resulting from human activity and, consequently, might contribute to unbiased discussion of issues of relevance to forest and game management. Yet, combination of ETHOSYS® with a location system will be indispensable to ensure recovery of measuring collars and thus obtain also data on land use by the animals concerned.

Additional studies will have to be conducted on responses to various forms of stressors (e.g. heat, pregnancy, birth, injury, lack of resources etc.) to define species-specific standards of behaviour-determining demands on the environment. Collection of power spectra from other herbivorous species, over one whole year, might be of interest for comparative studies.

5. SUMMARY

Long-time automatic acquisition of behavioural rhythms of locomotor activity and feeding together with high-accuracy structural analysis of the data recorded proved helpful in obtaining information on time requirements of red deer. Factors relating to the external environment were shown together with fixed main patterns as well as extremely variable components in the activity pattern. Irritations caused by human interference were identified by means of rhythmic analysis. Key data obtained under largely low-stress quasi-natural conditions might be used, complementary to recordings from less controllable areas, to identify and prevent risk situations of adverse effect on individuals or groups, which - just as in the case of red deer - might help to accomplish meaningful forest and game management.

ACKNOWLEDGEMENT

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ANNEX / ANHANG

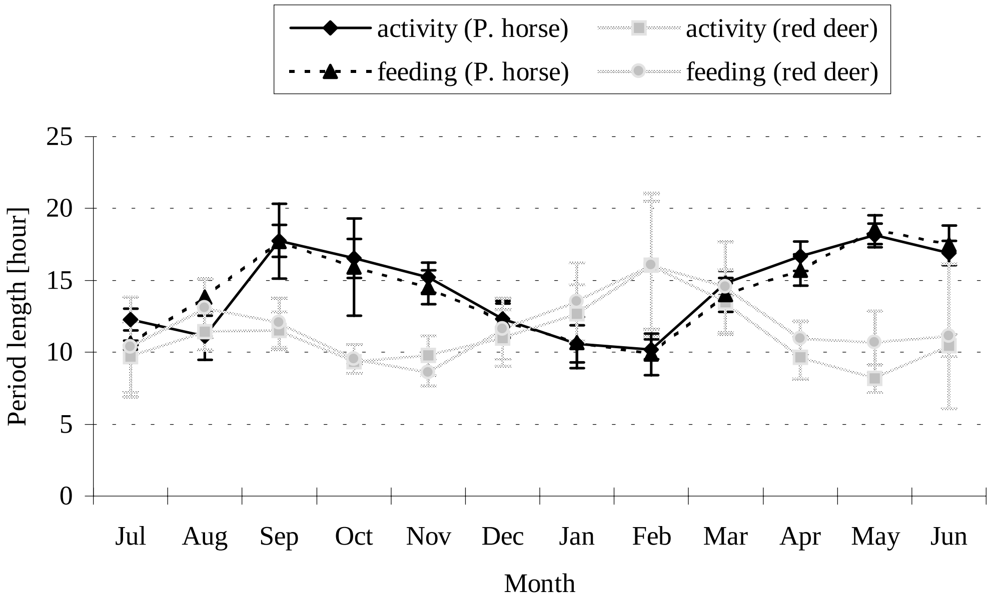

Abb. Gewichtete Mittel der Intensitäten der signifikanten harmonischen Perioden aus den Leistungsspektren, die aus den Daten eines Monats berechnet wurden.

Diese Abbildung stellt die jährliche Variation der signifikanten Perioden der Aktivität und des Fressens beim Przewalskipferd und beim Rothirsch (jeweils über 4 Tiere gemittelt) vergleichend dar. Dabei wurde das gewichtete Mittel von den Intensitäten der signifikanten harmonischen Periodenlängen ermittelt, die in den Leistungsspektren auftraten, zu deren Berechnung die Originaldaten eines ganzen Monats verwendet wurden.

Die hohe Standardabweichung bei den Rothirschen im Monat Februar ist dabei auf geschlechtsspezifische Unterschiede zurückzuführen. So lag das gewichtete Mittel der signifikanten Perioden der Aktivität und des Fressens bei dem männlichen Rothirsch im Februar bei einer Periodenlänge von 20 Stunden, bei den Weibchen lag dieser Wert bei rund 11 Stunden. Wie schon im *Abschnitt 3.2.* diskutiert, verschieben die Rothirsche das Zeitmuster der Aktivität und des Fressens in den Wintermonaten bis in das Frühjahr hinein in Bereiche niedrigerer Frequenzen (16-h Periodenlänge). Dagegen verlegen die Przewalskipferde das Zeitmuster der beiden Verhaltensparameter in den niederfrequenten Bereich (bis 18-h Periodenlänge) zu Zeiten guten Futterangebotes (Frühjahr und Herbst). Die Abstände zwischen den einzelnen Fress- und Aktivitätsperioden sind also – wie es schon im *Abschnitt 3.1.* beschrieben und diskutiert wurde – in den Wintermonaten (mit schlechtem Futterangebot) kürzer.

4. Methods and Results of Non-Invasive Status Diagnosis of Various Free-Ranging Herbivorous Species

4.1. Comparative Analysis of Ultradian and Circadian Behavioural Rhythms for Diagnosis of Biorhythmic State of Animals

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Eigenanteil:
- praktische Anpassung von ETHOSYS® an die Tierarten Przewalskipferd und Rothirsch
- die Funktionstüchtigkeit der Meßhalsbänder bei den Untersuchungen an Przewalskipferd und Rothirsch überprüft:
  - visuelle Paralleluntersuchungen durchgeführt,
  - Übereinstimmung zwischen den Beobachtungsdaten und den mit ETHOSYS® gemessenen Daten geprüft (Korrelationsberechnungen durchgeführt)
- Sozial- und Vigilanzverhalten bei Przewalskipferd und Rothirsch beobachtet und ausgewertet
- die mit ETHOSYS® erfaßten Daten bei Przewalskipferd und Rothirsch biorhythmisch ausgewertet
- das Manuskriptes korrigiert und alle Grafiken zum Przewalskipferd und Rothirsch erstellt

Ziel der Arbeit:
Die theoretischen Hintergünde, Methoden und Anwendungsmöglichkeiten der biorhythmischen Statusdiagnose sollten anhand von Beispielen vorgestellt und diskutiert werden. Die vorgestellten Ergebnisse an verschiedenen Tierarten sollten auf den artübergreifenden Charakter der Untersuchungen hinweisen, das breite Spektrum der Untersuchungsbedingungen verdeutlichen und verschiedene Einsatzmöglichkeiten erkennen lassen.

ABSTRACT
A chronobiological procedure has been developed for evaluation of living conditions, behaviour and internal state of free-ranging animals. It is based on continuous recordings of activity and feeding with subsequent comparison of levels, daily patterns as well as daily and ultradian rhythms. Telemetric observations were carried out on alpaca, sheep, Przewalski horse, roe deer, red deer and mouflon under various conditions. The time patterns of the different species were analysed macroscopically and by autocorrelation function and power spectral analysis. A stable ultradian structure of behaviour was obvious especially for ruminants. A more unstable, adaptive time pattern was found in Przewalski horses. Activity as a multi- purpose behaviour was generally more variable than feeding which in most cases was of clearly rhythmic and harmonic structure. Degrees of Functional Couplings (DFCs) were used for comparison of rhythmic structure. DFCs express the percentage of the circadian component and harmonic ultradian components in relation to all rhythmic components of a spectrum. They were found to be high in well adapted, healthy and undisturbed individuals but were lowered during periods of adaptation, sickness or social interactions.

Keywords: ultradian rhythm - circadian rhythm – adaptation – stress – alpaca – sheep - Przewalski horse - roe deer - red deer - mouflon

1. INTRODUCTION

Behaviour is a strategy of the organism to overcome exogenous disturbances and stabilise the endogenous milieu (TEMBROCK 1987, HAFEZ 1968). It can be regarded as a correcting element in the controlled system of organism and environment.

General locomotor activity, the final link in a long chain of endogenous processes, reflects both endogenous motivation and exogenous cues and varies continually between motion and rest (SZYMANSKI 1920, ASCHOFF 1962). The circadian rhythm of activity can be interpreted as a programmed control, tuning the organism as a whole to periodic change of environmental conditions. In herbivores, oscillation of feeding and non-feeding (or rumination in ruminants) represents the regulatory function of a particular behaviour. It ensures a more or less continuos flow of nutrients through the digestive tract, providing energy for the organism. Close and well defined meaningful coordination between time structures of different physiological and behavioural functions seems to be characteristic of the healthy organism. Fixed phase relations were described in humans, for example, between body temperature, activity and excretion of minerals (ASCHOFF et al. 1967). To describe such a correlation between different physiological and behavioural parameters, ASCHOFF used the term "internal synchronisation" (ASCHOFF 1969).

External stressors act as disturbances on this regulatory system. A changing internal state will influence the present value of the system. Both may change the intensity of behaviours, and also the time structure of behaviour. Direct measurement of stress (e.g. by endocrinological parameters in blood samples) is not practicable in free-ranging animals. Side-effects of sampling may be stronger than the effect of the original stressor. However, weak stressors, quite often of long duration, are characteristic of the environmental conditions of these animals. Wild animals, just as domestic species may suffer from man-induced influences or diseases. Wild animals do not display their suffering, but conceal it as far as possible, in order not to attract predators or to lose protection by their social group. Nevertheless, long-term effects may become manifest in the frequency and time structure of behaviour. As simple behavioural parameters, such as activity, can be conveniently recorded by telemetry, it should be possible to investigate such stressors by following biological rhythms. The importance and value of biological rhythm research on wild animals was demonstrated, for example, by TESTER et al. (1989).

There are several standard procedures which are frequently applied to analysis of biological rhythms. Cosinor, autocorrelation function and power spectral analysis are some of them. It is a question how the information extracted by these methods from original data series can be used to analyse adaptive processes in free-ranging animals.

Several years ago, we developed a procedure for quantification of the harmonic frequency structure of behavioural rhythms in sheep (SINZ et al. 1976). Based on telemetric long-term measurements of activity and feeding, it was now applied to time series from alpacas, Przewalski horses, red deer and mouflons under various conditions. It is the purpose of this report to describe the basic idea and the procedure, to give some examples of application and discuss the potential benefit of general application.

2. MATERIAL AND METHODS

2.1. Animals

A study was conducted on behavioural rhythms in a group of five alpacas (*Lama guanicoe f. pacos*) kept in Tierpark Berlin- Friedrichsfelde. The animals (one stallion and four females) lived on a pasture of 3,700 m^2 but were kept in stables overnight. Food was provided daily in the stable in the evening. Additional feed was offered on the pasture, especially in winter. We analysed 17 activity records from each of these animals on several occasions.

We monitored the behaviour of Przewalski horses in a semireserve of 42 ha north of Berlin. Twelve zoo-born mares lived in that area, uncontrolled and without additional feeding or any other human influence. We took activity records in irregular intervals over five years. One complete annual record from one animal was evaluated for this report.

Red deer were investigated in two research enclosures. A group of four females was studied for ten days on an experimental pasture of 3,800 m² in Theix (INRA Clermont-Ferrand, France). While the nutritional demands of the animals were covered by the pasture, a small ration of concentrates was additionally offered once a day. Two female red deer were subjects of comparative research on a near-natural site of 40 ha in Velcice (Slovakia), in co-operation with the Institute of Wildlife Research and Ecology (Vienna). These animals lived in a herd of 16. Hay was additionally offered *ad libitum* but was rarely accepted.

Behaviour recordings were obtained from free-ranging mouflons on an island north of Berlin. The animals were members of a population of approximately 120 individuals, inhabitating an area of 700 ha. They were accustomed to a salt lick and to a feeding place where small amounts of food were provided for attraction. It was here that the central station of the data acquisition system (see below) was installed. Results were recorded from five animals throughout a period of five months and were evaluated to give a total overview of 400 animal-days.

Activity and feeding of a male roe deer were recorded in an experimental enclosure of 900m^2 over a period of four months. Characteristic samples were chosen from this animal for comparison.
Efforts were made in all studies to obtain additional information on social conditions, animal state and external influences.

2.2. Data acquisition

Versions of the storage telemetry system ETHOSYS® were applied to all animals. The system enables continuos automatic recording of locomotor activity and feeding in a four-channel data logger in the form of an animal collar. A collar has two built-in sensors, one for acceleration and the other for position tracking of the animal's head (up or down). If at least one signal from the acceleration sensor is picked up for an interval of one second, this second is counted as "activity". Linked to a second channel is the logic connection between general activity and the "head down" signal from the position sensor. If these definitions are true, one second is counted as "activity with head down". A digital frequency filter is used for more specific interpretation. The specific pattern of movements with head down can be additionally used for correct identification of grazing, and this behaviour is recorded by another channel. The results of those continuos recordings are accumulated for intervals of 15 or 30 minutes. They are then saved in a data file which represents a time series with equidistant intervals.

This data file can be automatically transmitted to a central station via a radio link and thereafter to a PC, or can be directly transmitted to a computer via the RS 232 interface (SCHEIBE et al. 1998). Versions without radio link were used on alpacas (a prototype version with an analysis interval of one hour) and temporarily also on Przewalski horses. Uninterrupted data sets were analysed for 7 to 10 days in all cases.

2.3. Data analysis

The measure to be defined should describe the degree of synchronisation between internal rhythms and the external 24-h-period. This makes sense for time periods containing not too many subsequent days. Therefore, we subdivided the data series (activity or feed uptake) into consecutive or overlapping samples of 7 to 10 days which were equal in length for each application. The "Degree of Functional Coupling" (DFC) then was calculated for each of the samples. The DFC is computed in three steps: First, the periodogram is calculated, consisting of the periodogram ordinates for all Fourier frequencies $\omega = 2\pi j/n$, j=1,...,q with q=n/2 (n even) or q=n/2-1 (n odd), with n being the number of data points in the sample. Second, the periodogram ordinates (which are basically variance components assigned to cyclic fluctuations containing n/j data points per cycle, j=1...,q) are tested for statistical significance (test according to R.A. Fisher, see ANDEL 1984). Finally, the DFC is defined as

$$DFC = 100 * \frac{SP(harm)}{SP(total)} \qquad (1)$$

SP(total) denotes the sum of all significant periodogram ordinates (i.e., the variance assigned to significant periods). SP(harm) denotes the sum of those periodogram ordinates which are significant and harmonic to the circadian period. A period is regarded to be harmonic, if day length is a multiple of period length (expressed as time interval). According to this definition, period lengths of 24, 12, 8, 4.8, 4, 3.3, 3, ... hours are harmonic. DFC obviously describes that percentage of cyclic behaviour components in the sample (in terms of variance) which is synchronised with the circadian rhythm. The calculations were performed by a self - written program.

The DFC measure defined above is intraparametric; it applies to a time series sample of a single parameter. This concept can be generalised involving more than one parameter. In case DFC(Pi) = 100*SPi(harm)/SPi(total) , i=1,2 are the DFCs for two parameters P1, P2 based on two samples which were simultaneously recruited from the same animal and at the same time intervals. The "multiple DFC" involving both parameters is then defined as

$$DFC\ (P1, P2) = 100 * \frac{SP1(harm) + SP2(harm)}{SP1(total) + SP2(total)} \qquad (2)$$

This definition presumes standardisations in such a way that Spi(total), i=1,2 are expressed as ratios between the sum of significant periodogram ordinates and the sum of all periodogram ordinates. The multiple DFC is a weighted mean of the single DFCs because of

$$DFC\ (P1, P2) = \frac{100}{SP1(total) + SP2(total)} * (SP1(total) * DFC(P1) + SP2(total) * DFC(P2)) \qquad (3)$$

The multiple DFC can also be defined for more than two parameters. It characterises the total synchronisation of the parameter set considered with the circadian rhythm.

DFCs were statistically analysed for random samples taken from alpacas, mouflons and one Przewalski horse. A two-factorial ANOVA (factor one: individual, factor two: stressing or normal situation) was applied to the alpaca data. For mouflon, occurrence of the lowest DFCs in the week of parturition was tested against distribution by chance, using the binominal test (BORTZ et al. 1990). A series of DFCs for 56 consecutive weeks was calculated from the activity data of a Przewalski horse. Periods of shooting activity in the surroundings were separated from periods when this did not occur. The values in these different periods did not show autocorrelation and were treated as independent values. The Mann-Whitney U-test was used to test the differences of DFCs between these periods. As the observations on red deer were based on a limited sample, results were not statistically analysed.

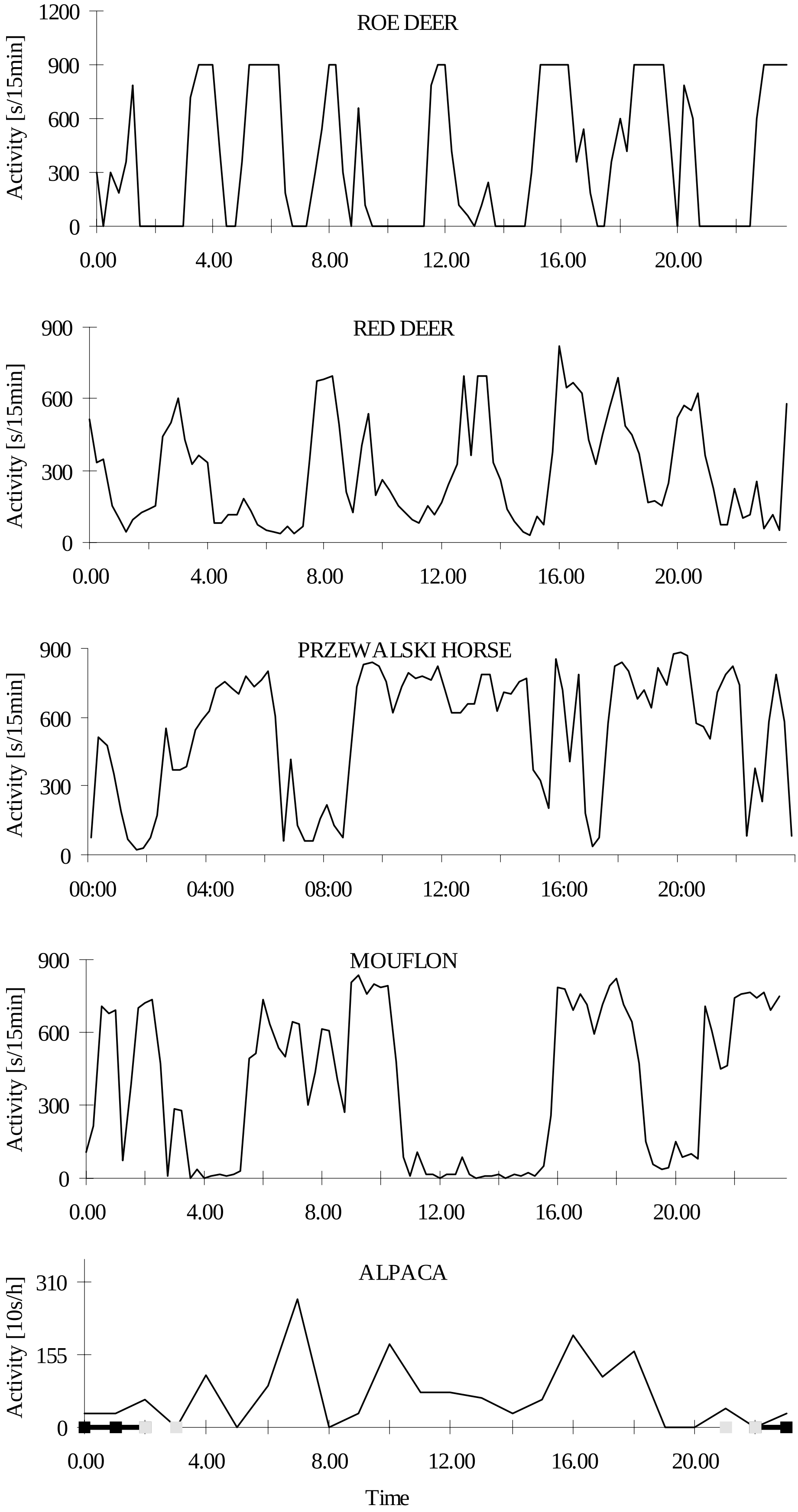

Fig. 1 Characteristic activity patterns of different species at the same time of the year; original values for one day each, measured by storage telemetry on free-ranging animals; lengths of dusk, dawn and darkness are depicted in bottom graph.

3. RESULTS

Activity and feeding in ruminants but also in non-ruminating herbivores are dominated by ultradian rhythms. Originals of daily records from individuals of different species are depicted as examples in Fig. 1. A daily rhythm is identifiable only by modulation of peak level, peak frequency or peak duration. Species-specific differences in activity patterns are also visible and are characterised by a high number of short activity peaks in roe deer, a lower number of peaks in red deer and mouflon and very long peaks in Przewalski horse. The number of activity peaks in alpaca is similar to that in horse, but peak duration is much shorter.

An overview of long-term organisation of activity and feed uptake is given in Fig. 2 by an example of Przewalski horse. The influence of sunrise and sunset on the level of activity is very clearly visible, and so is the varying structure of ultradian components. Annual change in feed uptake is obvious and differs moderately from the annual pattern of activity. Feeding intensity is low during summer, higher in fall and reaches its maximum in early spring, to drop again in late spring and to stay low in summer.

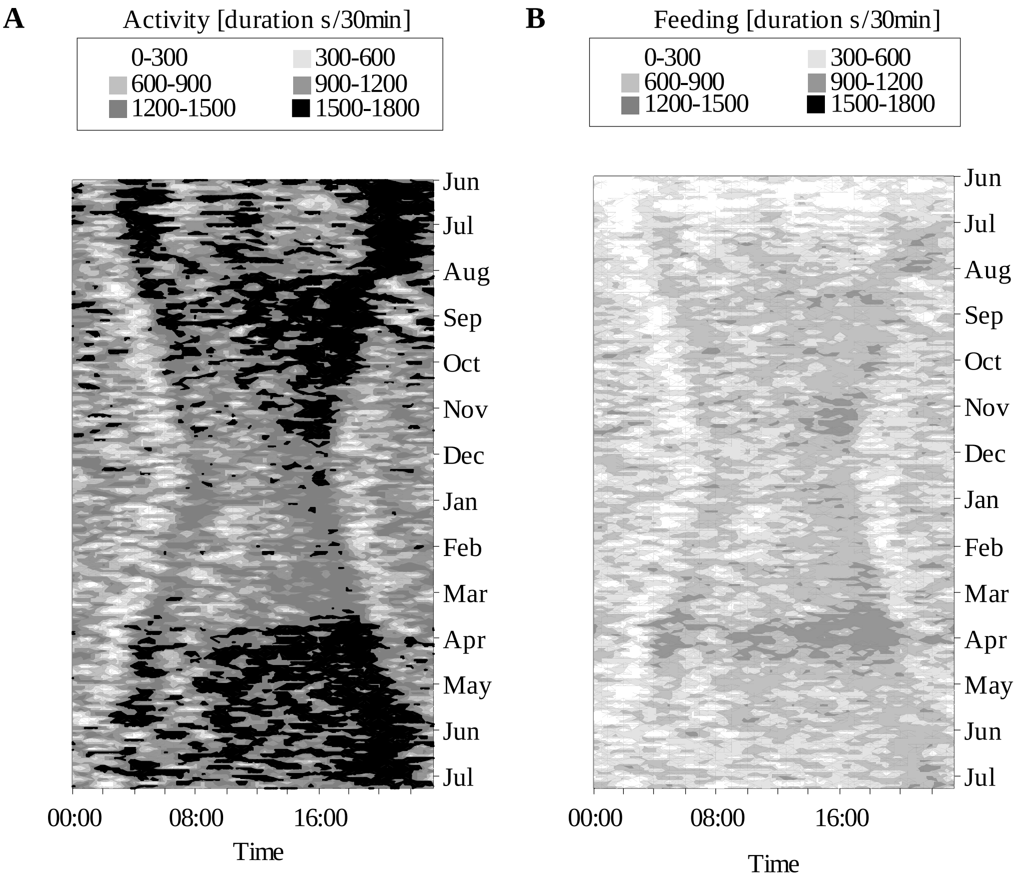

Fig. 2 Ultradian, diurnal and seasonal patterns of activity and feeding of a free-ranging Przewalski horse.

The further analytical procedure is illustrated in Fig. 3. An original activity record from a free-ranging Przewalski horse is displayed for 10 days (top), with the autocorrelation function of these data given in the centre. The power spectrum is shown in the lowermost graph. Periods of significance are marked. The activity structure is a complex one and is dominated by the 8-hour period. There is a second ultradian component, a 4.8-hour period, that plays a significant role in the power spectrum. The 24-hour periodicity comes out quite clearly, though its spectral power is somewhat lower than that of the 8-hour component. The DFC for this sample is 100%, since only harmonic components (24h, 8h, 4.8h) could be identified.

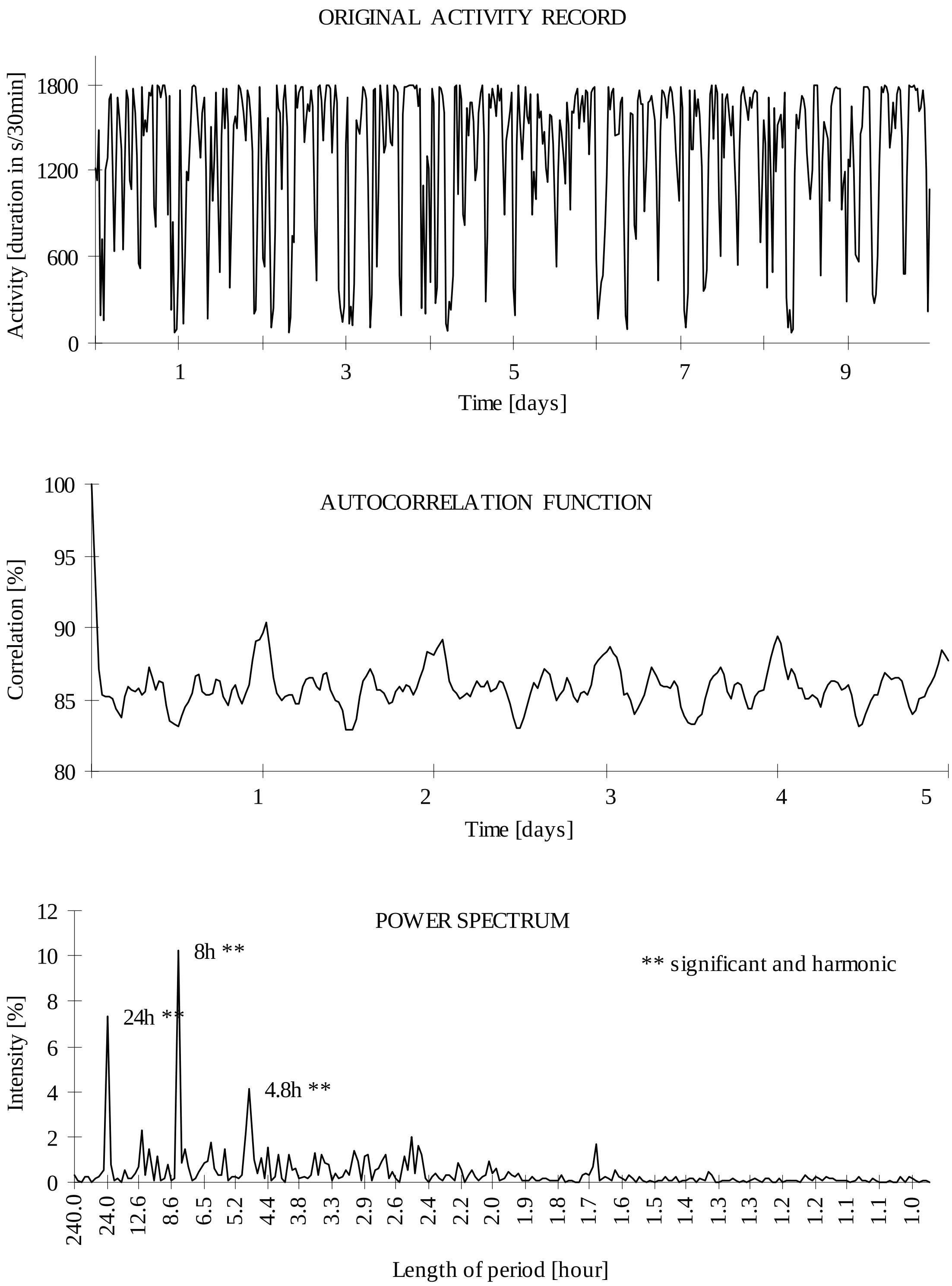

Fig. 3 Time series of activity, autocorrelation function and power spectrum recorded from a Przewalski horse for a series of 10 days.

Normal (multiple) DFC values of activity and feeding were higher than 90 % in alpaca [Table 1]. Intraparametric DFCs for activity normally were higher than 80 %, while most values for feeding were equal or close to 100 %. In an accidentally hurt animal, the intraparametric DFC for activity was as low as 28 %, but the DFC for feeding stayed unchanged, resulting in a multiple DFC of 57 %. The alpha female of the group was quite frequently engaged in social interactions and busy in care of all young animals of the group. Her DFCs at any time were somewhat lower than those of all other animals. Samples were taken from two females until just before parturition. DFCs were lowered also in these cases. The lowered DFCs in the sample from the hurt animal and before parturition were statistically different from all other values (p = 0.001), whereas differences between individuals could not be found.

Varying DFCs for activity, with a mean value of 85.5 %, were characteristic of a Przewalski horse for up to two years from introduction to the semireserve. There was a high frequency of portions of non-harmonic ultradian components. Somewhat higher DFCs occurred after two years (mean value of 90.3%), and a more stable and harmonious activity pattern was visible. The DFCs for feeding stayed as low as 76 % throughout the whole time of recording. Resulting (multiple) DFCs were 80.9 % for the first two years and 83.6 % thereafter.

Tab. 1 Degrees of Functional Couplings in alpacas; extraordinary situations are marked with asterisks indicating values that deviate with significance from all others.

Animal	DFC multiple [%]	Activity DFC intrapar [%]	Feeding DFC intrapar [%]	Remarks
1	92.7	82.0	100	
	91.0	85.7	100	
	95.0	91.3	100	
	57.3	27.6	100	hurt ***
	81.0	80.5	81.5	
	96.9	93.9	100	
	72.4	60.1	80.1	parturition ***
2	91.7	88.1	94.1	
	80.5	78.5	82.4	
	88.7	88.6	88.7	
	96.3	96.0	96.5	
	51.6	39.7	61.2	parturition ***
	91.9	94.3	89.5	
3	75.4	86.1	30.6	alpha-female
	84.4	66.9	100	
	63.8	55.3	83.4	
	62.4	32.5	84.4	

Seasonal variation of DFCs from the same animal as displayed in Fig. 2 is shown in Fig. 4. During that time, the individual had a medium social status in the group. DFCs varied around a medium level but, on three occasions, were reduced with significance (p = 0.0005) to or close to zero. The first two phases of reduced DFCs were related to hunting activity (November and December) in the surrounding area. The third phase of reduced DFCs was in June and coincided with the start of shooting at a firing range just opened in a distance of 1 km. Firing practice started with heavy and noisy weapons, but later the noise level was reduced by about 10 decibel, since smaller calibres were used and additional noise-insulating measures taken.

In red deer (Theix experiment), individual DFCs were related to the social structure of the experimental group [Table 2]. The alpha female succeeded in maintaining an undisturbed activity pattern, resulting in high DFCs. In sub-dominant animals, the (intraparametric) DFC for activity was more strongly affected than the (intraparametric) DFC for feeding, but in the animal of the lowest rank, time structure of feeding was affected as well. This resulted in a (multiple) DFC as low as 28 %.

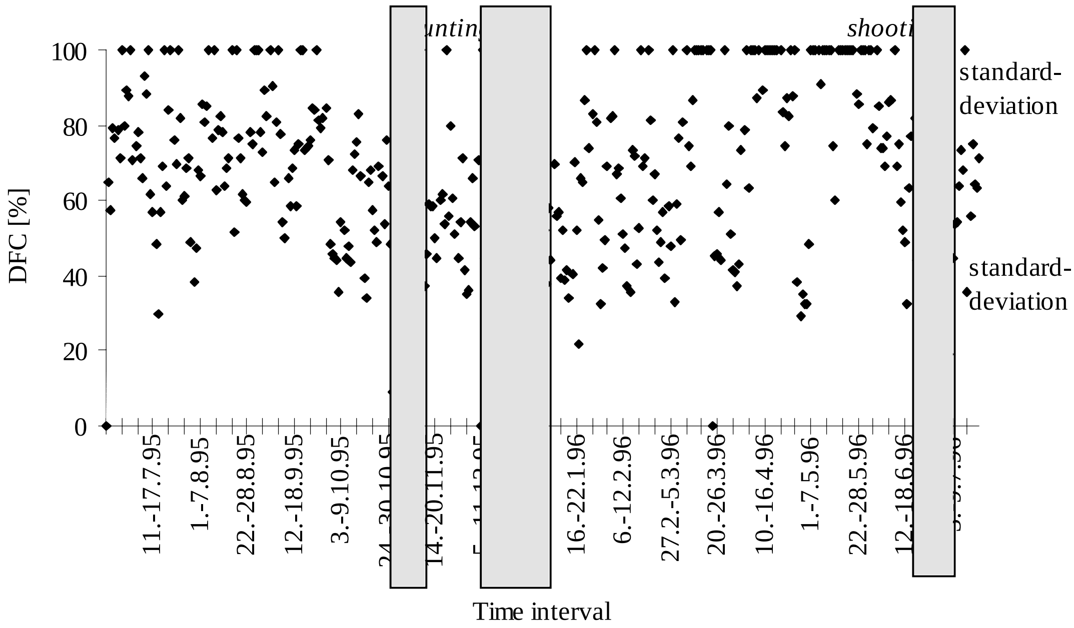

Fig. 4 Degrees of Functional Couplings (DFC) from a Przewalski horse; period of hunting in surrounding area highlighted together with shooting in nearby firing range.

In red deer (Theix experiment), individual DFCs were related to the social structure of the experimental group [Table 2]. The alpha female succeeded in maintaining an undisturbed activity pattern, resulting in high DFCs. In sub-dominant animals, the (intraparametric) DFC for activity was more strongly affected than the (intraparametric) DFC for feeding, but in the animal of the lowest rank, time structure of feeding was affected as well. This resulted in a (multiple) DFC as low as 28%.

Tab. 2 Social rank and DFCs in a group of red deer.

	DFC intrapar (Activity)	DFC intrapar (Feeding)	DFC multiple
dominant (alpha)	88.1	100.0	94.1
subdominant	61.2	66.3	63.8
subdominant (omega)	55.9	0.0	28.0

A free-ranging animal in a large enclosure (Velcice experiment) showed fairly low (intraparametric) DFCs in activity (46.4 %) throughout the period of recording. This individual was not fully integrated in the social structure of the herd, and more or less persistent social stress of unknown intensity may be assumed. Nevertheless, DFCs between 80 % and 95 % were recorded in some periods. For three periods, DFCs were extremely low (< 20 %). In the first of these periods, the animal was immobilised for an experimental procedure of one hour and subsequently was set free, but the procedure was seen to have after-effects for a considerably extended period of time. Various human activities inside the enclosure (but not directly affecting this animal) were reported for the two other periods of lowered DFCs.

The DFCs from free-ranging mouflon normally were between 70 % and 100 % throughout the year. It was only during the peripartal period that they were lowered for a short period of time [Fig. 5], resulting from a general change in the time structure of activity and spatial isolation as well as from social isolation of mother and lamb. Values recovered soon, when mothers, together with their lambs, rejoined the social group. The lowest DFCs were always found in the week of parturition, a pattern which was found to differ with significance (p = 0.0006) from random distribution.

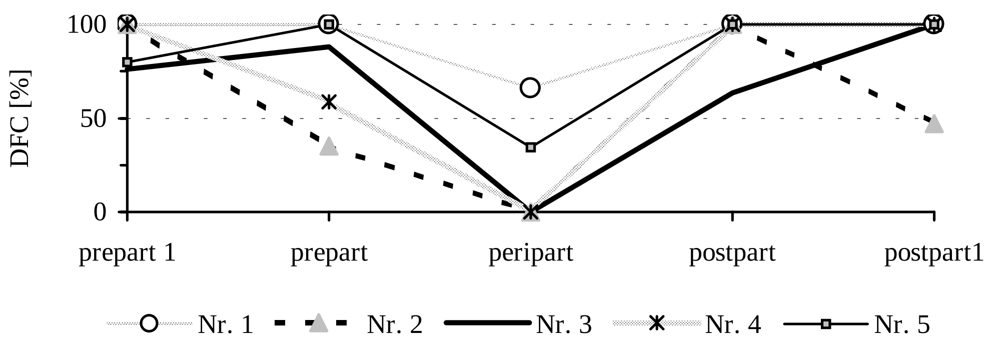

Fig. 5 Degrees of Functional Couplings (DFC) from free-ranging mouflons before, during and after parturition.

4. DISCUSSION

Rhythms of physiological functions and related behaviours are essential components of the ecological relations between an organism and its environment. The ecological niche had been defined also regarding the dimension of time (ASCHOFF 1954; REMMERT 1969). Human activities in natural ecosystems, for example, may change the living conditions of animals (SCHULER 1994). The temporal structure of the behaviour of wild animals, consequently, will reflect the individual's reaction to these circumstances.

There are many of external conditions or internal processes by which the temporal organisation of an organism may be adversely affected. The heart rate of undisturbed tree shrews shows a clear diurnal pattern with ultradian components. Social stress altered this pattern not only in level but also in frequency structure (STÖHR 1986, HOLST 1994). The number of activity bouts per day in calves declined after transport and introduction into a new environment. Together with the time spent standing or moving, the activity pattern could be used to describe the process of adaptation (VEISSIER et al. 1989). The postoperative relationship between circadian and ultradian rhythms of core temperature in sheep was investigated by MOHR et al. (1992). The time pattern of the core temperature was changed by surgical treatment, and during the healing process normal phase relations were restored.

Impairment of phase relations, changes in frequency structure, loss of rhythmicity or reduction of amplitude are regarded as signs of adaptation, disease or premortal state. In such conditions, single functional systems react to stressors independently from the general synchronised time hierarchy of the organism. The lack of internal synchronisation itself can be interpreted as a stressful situation (MOTOHASHI 1987, VEISSIER et al. 1989, DAIMON et al. 1992, MINORS et al. 1996, BICAKOVA-ROCHER et al. 1996). Repetition of daily routines is an expression of hierarchic and harmonic temporal co-ordination between several physiological and behavioural systems and the Zeitgeber and characteristic for the healthy, unimpaired and stress-free organism. Numerous observers have described more or less identical time patterns of behaviour from day to day in stress-free conditions (MICHAEL 1970, MAYES et al. 1986, BUCHENAUER et al. 1988).

Long-term recording without affecting the subjects of investigation is a prerequisite for investigation of behavioural rhythms. This is difficult to perform on animals that are free-ranging in a natural environment. Analytical methods of biorhythm research, therefore, have rarely been applied to investigations of environmental relationships of free-ranging domestic animals or wildlife. However, the importance of biological rhythm research for domestic animals has been demonstrated, for example, by LEFCOURT (1990) and SCHEIBE (1991), while TESTER et al. (1990) described the importance of activity analysis in wild animals. It is very obvious that continuous activity records over extended periods of time and with high resolution, as demonstrated in Fig. 2, hold a wide variety of information which is really utilised only in rare cases. At least three distinct fundamental processes became visible: a daily rhythm, the influence of sunrise and sunset, and an ultradian component related to feeding behaviour. These processes not only differ from each other but also are of seasonal variability.
Ultradian rhythms are major components of the time series observed. They occur in many physiological and behavioural functions of lower and higher animals, including man, and modulate circadian

rhythms in a complex manner (STUPFEL et al. 1990, HALLE et al. 1994). The circadian and ultradian time structure of biological processes is not limited to pineal and pituitary hormones but includes as well other physiological aspects, such as glucose regulation and insulin secretion. The circadian rhythm of activity and sleep has bearings on this complex endocrine temporal organisation and, in turn, is affected by the latter (VAN CAUTER 1990). Ultradian rhythms of oxygen consumption or carbon dioxide emission correspond to ultradian rhythms of activity (RÜBSAMEN et al. 1981, LLOYD et al. 1991). In domestic cattle, the time structure of body temperature and activity both show ultradian and circadian components. Circadian as well as ultradian components were also visible in prolactin, but cortisol seemed to be solely determined by several ultradian components (LEFCOURT 1990). Peripheral concentrations of growth hormone for cows exhibited sinusoidal circadian rhythms and an ultradian rhythm with a period of approximately 80 minutes (LEFCOURT et al. 1995).

Activity includes all behavioural patterns, from orientation to higher locomotor activity, e.g. for escape. For herbivores, the ultradian component of the activity cycle depends primarily on feeding and related behaviours. The species-specific pattern of feed uptake and digestion strongly determines all other activities (HAFEZ 1975, ARNOLD et al. 1978, PRATES et al. 1995). The different ruminant species are characterised by their selectivity for plant cell contents (concentrate selectors, e.g. roe deer) or their ability to use forage rich in plant cell walls (cellulose, roughage eaters, e.g. mouflon, HOFMANN 1989). Concentrate selectors exhibit short cycles of feeding and rumination, whereas a greater period length of the ultradian rhythm of food uptake is characteristic of roughage eaters [Fig. 1]. The function of the ruminant digestive system depends on a regular pattern of feeding, rumination and digestion. Horses as non- ruminant roughage eaters seem not to depend as much as ruminants on a regular pattern of uptake and digestion. They are always ready to react very strongly to disturbance and are always ready to run away from apparent danger. Hence, normal DFCs may be moderately lower than those of ruminant species. Being specialised grazers, horses have longer feeding periods, for example, than red deer. Alpaca, a ruminant species, seems to depend on regular rotation of feeding and rest and also on a well defined daily routine. As these animals lived on pasture only during the daylight hours and were kept in individual stables at night, their daily activity pattern reflected this influence.

A complete power spectrum displays the whole range of ultradian and circadian components (theoretically demonstrable by time series), depending only on sample interval and length of time series. The question arises how this information can be used to evaluate the current state of an organism. For example, reconstitution of rhythms of body weight, food and water consumption and locomotor activity of rats after phase shift of the Zeitgeber was followed up by means of spectral analysis under laboratory conditions (MINEMATSU et al. 1995).

DFCs were developed to concentrate in one single value all the information of a spectral analysis on diurnal and ultradian rhythms (SINZ et al. 1976). They express the correlation between the frequency structure of one or several time series from one individual and the Zeitgeber frequency. Following ASCHOFF´s model of internal synchronisation, they can be described as presentation of both external and internal synchronisation. These DFCs differ from the original definition, which was oriented to phase angle relations, as they are based on frequency relations. The value of multiple DFCs rises with the number of organismic functions recorded in parallel, but it is because of the integrative function of activity that even this behavioural parameter alone provides for a good insight into the current relationship between external Zeitgeber and internal rhythms. Our results suggest, at least for the herbivorous species investigated, that an unambiguous hierarchic frequency structure exists for activity as well as between activity and feeding. A harmonic frequency relation between the ultradian component (which is primarily but not exclusively feeding) and the daily pattern of active versus inactive state of the organism reflects successful adaptation to the primary timer. High DFCs were recordable under such conditions, most of them coming close to 80 %. DFCs were found to be decreased in a diversity of situations.
Our observations show, that ultradian rhythms are more affected than the circadian component by external disturbances and changing internal states, and DFCs are lowered in very different situations. They all have in common the tendency of forcing the organism to become adapted to new and more or less annoying situations, be it social isolation (SCHEIBE et al. 1974), social stress as described by V.HOLST (1994), adaptation to thermal discomfort (LANGBEIN et al. 1993) or structure of vegetation (MITLÖHNER 1996).

In our experiments, we found DFCs reduced by conditions which may be described as stress. Clear signs of irritation, attributable to accidental injury, were exhibited by a wounded alpaca for several days. In this context, decrease of DFCs in the alpha female of the alpaca group showed that even a dominant individual may be strained. Other stressors, such as a new, unknown environment or noise reduced DFCs also in Przewalski horses. Shooting is a type of noise of a highly particular nature. We observed, on several occasions after shots were fired, flight, restlessness, and high vigilance in the group of Przewalski horses. This was in fair agreement with results reported by HERBOLD et al. (1992) who found in roe deer and red deer that out of all the acoustic disturbances tested shots triggered the highest heart rate reaction. In the experimental group of red deer, the alpha female obviously suppressed the subdominant individuals and accomplished her own preferred activity structure. The activity structure of one of the free-ranging individuals was affected for an extended period of time by immobilisation and human activities inside the large enclosure. The generally low DFCs in this particular individual probably reflected its insufficient integration into the social system of the group.

Parturition is an event of greatest importance to a female organism. The process of parturition itself is not without risk to the mother and is accompanied by a decisive change to her daily routine. Parturition proper is triggered by rise in the corticosteroid level and resulting increase in placental production of estrogens. Physiology and behaviour are substantively affected by the need for care of the young, lactation, and change in energy requirements. These endocrinological and behavioural changes began to take effect some days or hours before parturition and were characterised by lowered DFCs in both alpaca and mouflon.

It is our conclusion that in the species investigated DFCs are high under normal, undisturbed conditions but may just as well be reduced by external stressors or endogenous functional changes.
The criterion allows for quantitative comparison between different situations and can be used to describe the gravity of a stressor. It is over a certain range independent of the overall level of activity or the sensitivity of a sampling procedure. Hence, comparison is possible among DFCs obtained by means of different measuring devices.
As this parameter can be automatically determined, it can be used as a routine for scanning procedures. Large Data sets can be reduced to one single value, describing the rhythmic structure of the whole time series. DFCs can be used in statistical analysis. More or less subjective description of time patterns or even of power spectra can be replaced by objective measures.

Independent of different species-specific rhythmic patterns, DFC is one approach to quantitative assessment of this pattern. The assumption can be made that its application may provide meaningful results from time series in many species and various environments and may be useful for description of states in man and animals.

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4. Methods and Results of Non-Invasive Status Diagnosis of Various Free-Ranging Herbivorous Species

4.2. Stress Diagnosis by Non-Invasive Methods on Fenced Red Deer

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Eigenanteil:
- praktische Anpassung von ETHOSYS® an die Tierart Rothirsch
- ETHOLINK-Station in dem Versuchsgehege aufgebaut
- Halsbänder angelegt und abgenommen
- Funktionstüchtigkeit von ETHOSYS® während der Untersuchung überprüft:
  - visuelle Paralleluntersuchungen durchgeführt,
  - Übereinstimmung zwischen den Beobachtungsdaten und den mit ETHOSYS® gemessenen Daten geprüft (Korrelationsberechnungen durchgeführt)
- Sozial- und Vigilanzverhalten der Tiere beobachtet und ausgewertet
- Kotproben (jedem Tier zugeordnet) gesammelt
- Tiere gewogen und umgetrieben
- die mit ETHOSYS® erfaßten Daten biorhythmisch ausgewertet
- Manuskript in Englisch verfaßt (Korrektur durch Mitautoren) und Grafiken erstellt

Ziel der Arbeit:
Diese Arbeit entstand in einer Zusammenarbeit des Institut für Zoo- und Wildtierkunde Berlin und des Institut National de la Recherche Agronomique. Zwei Methoden der nicht-invasiven Erkennung von Streß sollten in einem experimentellen Gehegeversuch an Rothirschen getestet und direkt miteinander verglichen werden. Die Methode der biorhythmischen Statusdiagnose stützt sich dabei auf die Bewertung von Verhaltensdaten und wurde in Vorversuchen am Rothirsch und anderen Tierarten schon angewandt. Bei der Analyse des Cortisolspiegels im Kot bilden physiologische Daten die Grundlage, diese Methode ist bei anderen Arten schon etabliert, beim Rothirsch dagegen noch in der Entwicklung.

ABSTRACT
Two different non-invasive methods to detect stress conditions were tested on red deer. The first one is capable recognising stress conditions through impairment of rhythmic synchronisation between organism and its environment. The Degrees of Functional Coupling (DFC) served as measure of rhythmic synchronisation between organism and environment. Based on three investigations, it was clearly shown that decreasing DFCs were associated with different stress conditions (social stress, human interference). In one experiment, cortisol metabolite levels were additionally determined in faeces to prove the practicability of such determination as an indicator of stress. Measurement of cortisol metabolite levels in faces revealed a tendency towards higher cortisol metabolite levels during an unexpected phase of external human disturbance. An effect of disturbed social conditions remained questionable.

Keywords: red deer – stress – telemetry – biorhythms - cortisol

1. INTRODUCTION

Game, nowadays, does not live in a real natural environment. Human influence is manifold, but is fairly different in type and intensity. It may be a major stressor or keep the animals out of their spatial and temporal ecological niche. This, of course, applies even more strongly to animals under keeping conditions. The aim of our experiments was to elucidate the effect of different kinds of stressors on red deer. Therefore it was important to find non-invasive and simple methods which did not affect the animals and promised to be of general applicability.

One established method is the analysis of biorhythms, particularly rhythms of long-term and continuously measured behavioural parameters. Behavioural rhythms have developed by way of evolution and are relating to the environmental conditions of the animal. All rhythms of an healthy organism are synchronised in a meaningful way both to the time structure of its environment and to each other. Stress conditions and disease may result from interference with such rhythms, their internal and external harmony. However, assessment of biorhythms calls for continuous recording of behavioural parameters over long periods of time.

Concentrations of glucocorticoids in blood plasma had been widely used to evaluate stress responses in mammals. Measuring of faecal cortisol metabolites as an indicator of stress offers the advantage of a simple sample technique which omits stressful blood sampling by venipuncture. A recently established non invasive method for determination of faecal cortisol metabolites, consequently, seemed promising to assess adrenal activity in red deer.

2. MATERIAL AND METHODS

The storage-telemetry-system ETHOSYS® (SCHEIBE et al. 1998) for continuous registration of activity and feeding on free ranging or grazing mammals was developed for the purpose of the experiments. With ETHOSYS®, animals are equipped with a neck collar for indirect measurement of activities by use of acceleration and angular position sensors. Different behaviours thus are identified on the basis of animal movements, head position and rhythms of head movements. Logic functions, included in the collar, interpret the signal patterns and categorise them into feeding, activity with head down and total activity. Interpretations are registered in one-second intervals by a microcontroller which summarises the results in self-programmed intervals and saves them in a memory. Data collection is automatic, as the information can be sent by short-range radio to the receiving station installed on places, preferred by the animals.

These data can be chronobiologically analysed. At first, the mean daily total activity was computed for each of the animals. Secondly, the power spectrum of autocorrelation function was calculated. This was followed by determination of the Degree of Functional Coupling (DFC) - a measure of rhythmic synchronisation between organism and environment (SCHEIBE et al. 1978). DFC expresses the percentage of significant harmonic components in relation to all significant components of the power spectrum. A period is regarded to be harmonic if day length is a multiple of period length (e.g.

24-, 8-, 6- or 4.8-hour periods). DFC can vary between 100% (when organism and environment are perfectly synchronised) and 0% (for total desynchronisation). In previous studies, DFC was shown to be a good indicator of stress in several species, including sheep, alpaca and horse (SCHEIBE et al. in press.).

A first investigation was carried out to test ETHOSYS® on red deer, with involvement of four hinds of the same age. These deer were kept in the same enclosure for 10 days. During this period, social behaviour was observed. At the beginning of the experiment, the weight of each animal was recorded.

In a second investigation, ETHOSYS® and the radio-telemetry system for measuring heart rate and activity, developed in VIENNA (SCHOBER et al. 1995), were simultaneously applied for nine months on the same red deer kept under nature-like conditions in an enclosure of about 40 ha.

The third investigation on a total of 25 one year-old stags was conducted to observe DFC during stepwise increase of social stress. In the experimental group, social instability was created in four phases by stepwise increasing of group size and by exchanging individuals [Fig. 1]. In each phase, the group was held constant for 14 days. In the control group, contemporaneously kept in a neighbouring enclosure, no deer were added or exchanged. In the third phase, groups were accidentally disturbed by nearby mowing operations. Social behaviour of both groups and vigilance behaviour of every animal were daily observed. Weights of all animals were recorded at the beginning and end of experiment.

The third investigation [see Fig. 1] additionally served to test a second non-invasive method for detection of stress conditions. To characterise adrenal activity in red deer, a recently established method for determination of faecal 11,17-dioxoandrostones (a group of cortisol metabolites; PALME et al. 1997) was performed on faecal samples collected from each of the animals on days 3 and 10 during each of the experimental phases. Correlations between cortisol metabolite levels and behavioural parameters were tested by variance analysis.

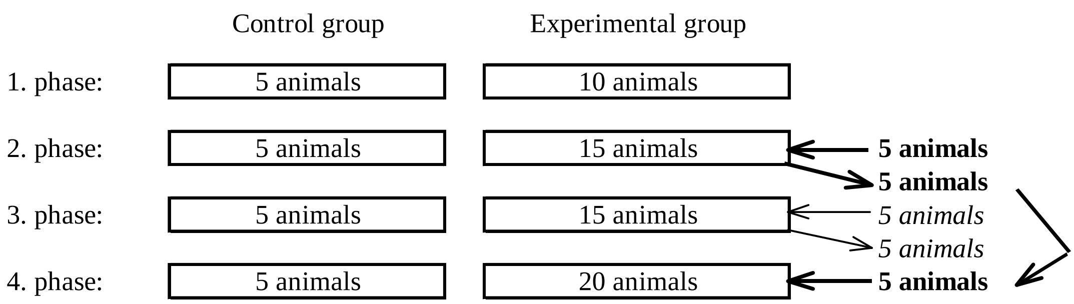

Fig. 1 Set up of the third experiment, stepwise increase of social stress.

3. RESULTS AND DISCUSSION

A unambiguous relationship between weight of animal, index of dominance and DFC [Tab. 1] was found in the first investigation. The heaviest animal occupied the highest rank and showed the highest DFC for activity and feeding. Its DFC suggests that there was no indication of stress on this individual. Animal No. 4 had the lowest weight and lowest rank in the hierarchy. During daily conflicts, this animal was most frequently attacked by others and was prevented from grazing, ruminating or resting. These social interactions resulted in extremely low DFC. Social stress caused by other animals led to complete desynchronisation between feeding and circadian zeitgebers.

In the second investigation, the main structure of measured activity showed high similarity between the two different measuring systems (BERGER et al. 1997) and decreasing DFCs during periods of stress of different origin [Fig. 2]. After short immobilisation of the animal, the DFC dropped to a low level and was only slowly re-established. Low DFCs were observed also during two phases in November, when other animals were captured after hunting in the enclosure.

In the third investigation, a significant difference of DFC (feeding) between the experimental and control groups was found to exist only in the first phase [Tab. 2]. No such significant differences were revealed in any of the other phases. Accordingly, we could not affirm social stress from social instability and rising group size. Comparison between phases showed significant changes in either group [Tab. 3]. DFCs in either group were clearly decreased in the third phase compared to the other three phases [Tab. 4]. A stress reaction, consequently, was established only in the third phase. This was certainly due to exterior disturbance (mowing operations in a neighbouring enclosure).

Tab. 1 Weight, index of dominance and DFC in the group of four hinds (first investigation).

Animal	Weight [kg]	Index of dominance*	DFC [%]	
			Activity	Feeding
red deer 1	118.88	1	88.06	100.0
red deer 2	115.4	-0.19	/	/
red deer 3	111.87	-0.2	61.20	66.3
red deer 4	99.4	-1	55.86	0.0

* Index of dominance = (victories - defeats) / (victories + defeats)

Tab. 5 shows means, standard deviations and range of cortisol metabolite levels measured on days 3 and 10 in each phase. A significant difference between experimental and control groups could be found only in the third phase [Tab. 2]. This primarily was due to elevation of metabolite concentrations on day 10, after exchange of five individuals, and thus may not have reflected an effect of increased social instability. A stress-related increase in adrenal activity should occur immediately after introduction of new animals and should lead to elevation in faecal cortisol metabolites within 24 hours, as was recently demonstrated for roe deer (DEHNHARD pers. comm.). Our sampling regime on days 3 and 10 of each phase may have been unsuitable for identification of treatment-dependent changes of adrenal activity.

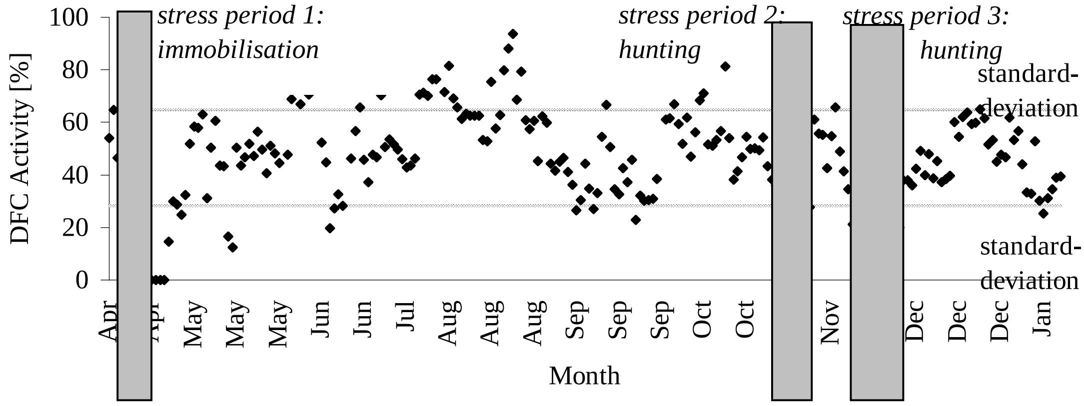

Fig. 2 DFCs (activity) of one hind from April 1995 to January 1996 (second investigation), periods of stress by human interference are indicated.

Comparison between phases showed significant changes for cortisol metabolites in either group [Tab. 3]. This was due to higher cortisol metabolite levels in phase three and may have resulted from external disturbance reported rather than from treatment.

Tab. 2 Comparison between control and experimental groups (variance analysis) in the third experiment.

	Cortisol	DFC Activity	DFC Feeding
1. Phase	/	/	*
2. Phase	/	/	/
3. Phase	*	/	/
4. Phase	/	/	/

* significant different between control and experimental group

Tab. 3 Comparison between phases (variance analysis) in the third experiment.

	Cortisol	DFC Activity	DFC Feeding
Experimental group	*	*	*
Control group	*	*	*

* significant different between phases

Tab. 4 DFCs of activity and feeding for each animal in different phases (third experiment).

DFC Activity [%]

Phases	Control group						Experimental group										
	1	2	3	4	5	Mean	1	2	3	4	5	6	7	8	9	10	Mean
1	95	100	93	100	100	97.7	100	100	100	100	100	82	100	100	100	90	97.2
2	82	85	66	90	86	81.6	100	100		100	95	92	74	100	92	95	94.2
3	73	71	76	65	71	71.3	72	60		68	55	64	66	63	64	68	64.5
4	89	82	100	100	100	94	78	79		93	92	86	100	87	88	94	88.4

DFC Feeding [%]

Phases	Control group						Experimental group										
	1	2	3	4	5	Mean	1	2	3	4	5	6	7	8	9	10	Mean
1	94	100	92	86	100	94.3	0	100	63	100	84	70	100	100	100	86	80.3
2	83	94	92	100	100	93.9	100	100	86	100	95	90	73	100	86	100	92.9
3	73	100	71	64	91	79.7	61	44	55	61	78	68	77	53	58	67	62.2
4	93	100	100	100	100	98.5	87	84	86	86	86	86	100	100	86	82	88.3

Despite the small number of data, the results revealed certain trends. We obtained a negative relationship between DFC (of activity or feeding) and cortisol metabolite levels and a positive relationship between daily totals (of activity and feeding) and cortisol metabolite levels. Among behavioural parameters, rising vigilance indicated rising stress, and a high rank in social hierarchy sometimes was related to stress. We think, that the need to defend their high rank quite frequently may have contributed to this result. In comparison with the index of dominance, vigilance appears to be the best indicator of stress.

Tab. 5 Mean, standard deviation and range of cortisol metabolite levels in control and experimental groups for different acts of sampling (third experiment).

Cortisol metabolite level [ng/g]

Phases	Day	Control group				Experimental group			
		mean	standard dev.	minimum	maximum	mean	standard dev.	minimum	maximum
1	3	11.3	7.5	3.1	24.6	30.1	18.9	10.5	79.9
	10	15.4	14.1	4.7	43.3	16.4	11.6	5.7	45.9
2	3	33.4	32.2	4.7	87.9	39	42.0	5.3	140
	10	24.5	4.1	18.5	29.7	16.9	14.9	3.4	53.1
3	3	21.3	5.5	12.7	29.6	29.4	12.7	11	46.6
	10	36	11.4	20.2	55.5	60.5	37.7	9.6	144
4	3	25.4	11.3	14.7	39.6	24.9	14.2	11	56.9
	10	39.9	14.3	20	60.7	36.8	19.9	11.3	76.3

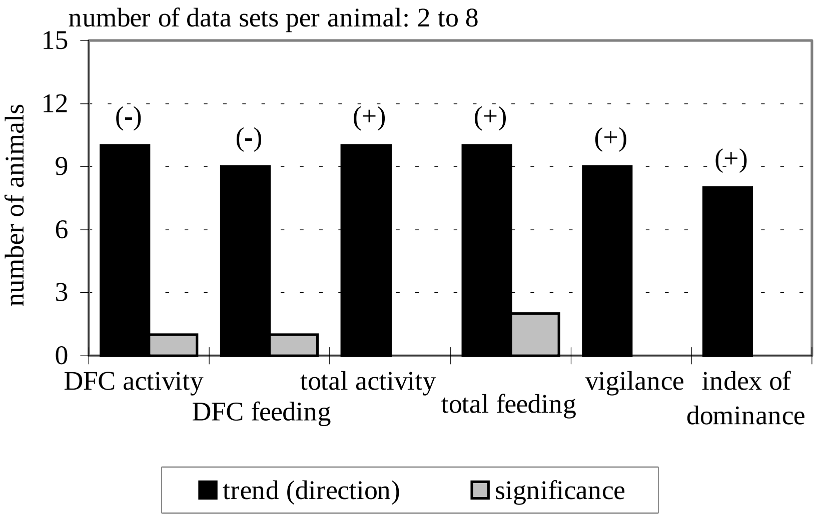

Fig. 3 summarises data obtained by correlating cortisol metabolite levels and behavioural parameters. Trends and significant correlations between cortisol metabolite level and behavioural parameters (third experiment).

CONCLUSION

A time structure of behavioural parameters which are in harmony with the circadian zeitgeber is characteristic of normal conditions. This clear rhythmic pattern of activity and feeding was disturbed during different stress periods. Therefore, analysing of time structure of long-term and continuously measured activity and feeding proved to be a useful method to detect stressful conditions. In case of cortisol metabolite levels in faeces, no significant effect could be related to the treatments. This might be attributable to an inadequate sampling regime on days 3 and 10 after treatment. Further investigations therefore should be carried out considering data from roe deer which showed increase of cortisol metabolites in faeces within 24 hours after application of stress.

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5. Generelle Zusammenfassung

Die Arbeit zielte darauf, Methoden und Erkenntnisse zur verhaltensorientierten (insbesondere der biorhythmischen) Statusdiagnose auszuarbeiten, um die Lebensbedingungen von Wildtieren möglichst objektiv einschätzen zu können. Die Schwierigkeiten lag dabei zunächst in der Methodik. Grundvoraussetzung der biorhythmischen Statusdiagnose ist es, biologische Rhythmen erfassen und erkennen zu können. Es sind also langfristige und vor allem lückenlose Aufzeichnungen von physiologischen oder Verhaltensparametern erforderlich, die feste Meßintervalle mit gleichbleibender Meßgenauigkeit aufweisen. Ohne technische Hilfsmittel sind solche Untersuchungen an Wildtieren, die oft scheu, nachtaktiv und in weiträumigem, unübersichtlichem Gelände leben, nicht durchführbar. Auch die Interpretation automatisch gewonnener Daten gestaltet sich schwierig, da das Wildtier von verschiedensten Umweltfaktoren (z.B. Klima, Nahrungsangebot, Sozialpartner) komplex beeinflußt wird, die sich wiederum oft der Untersuchung entziehen, da langfristige direkte Beobachtungen im Gelände mit zu hohen Rückwirkungen auf das Tier bzw. zu hohem Aufwand verbunden sind. So stand am Anfang meiner Arbeit ein Vergleich verschiedener Methoden, die sich zur langfristigen, automatischen Erfassung von Verhaltensparametern an freibeweglichen Tieren eignen. Es sollten Vor- und Nachteile der verschiedenen Methoden, deren Meßgenauigkeiten und mögliche Anwendungsgebiete aufgezeigt werden. Letztendlich sollte die Auswertung der Versuche Hinweise geben, inwieweit die verschiedenen Methoden bei einer langzeitigen Statusdiagnose am Wildtier geeignet sind und verwendet werden können. Bei den getesteten Methoden handelte es sich um das Speicher-Telemetrie-System ETHOSYS®, das Funk-Telemetrie-System VIENNA und den Kauschlagzähler APEC.

ETHOSYS® erfaßt lückenlos und langfristig die Aktivität und das Fressens an großen Herbivoren mittels extrakorporaler Meßhalsbänder. Ein Bewegungssensor mit nachgeschaltetem, einstellbaren Zeitfenster und ein Neigungsschalter in dem Halsband erfassen Bewegungen und die Stellung des Kopfes. Alle Bewegungen, die sich auf dieses Halsband übertragen werden als "Aktivität" definiert. Ist der Kopf des Halsbandträgers gesenkt und bewegt sich dabei in einem für die Nahrungsaufnahme charakteristischen Muster, wird dies zusätzlich als "Fressen" bewertet. Die gemessenen Daten werden in selbstbestimmten Intervallen im Halsband abgespeichert. Im Empfangsbereich einer Feststation werden die im Halsband gespeicherten Daten per Funk auf die Station übertragen und können unabhängig vom Tier dort entnommen werden.
VIENNA erfaßt die Herzfrequenz, die Körpertemperatur und die Aktivität bei Reh und Rothirsch. Bei dieser Methode wird subkutan ein Herzfrequenzsender implantiert, der ebenfalls subkutan mit zwei EKG-Elektroden im Brustbereich verbunden ist. Der chirurgische Eingriff ist durch die Anwendung eines Repeaterhalsbandes bei weitem nicht mehr so groß, wie bei der vormaligen Version der Wiener Methode, bei der Antenne und Neigungsschalter noch im Rücken und Nackenbereich implantiert wurden. Über dieses extrakorporale Repeaterhalsband mit Antenne werden dann die Herzfrequenz, die Körpertemperatur, die Kopfstellung des Tieres und die Empfängerfeldstärke funktelemetrisch abgerufen. Anhand von logischen Filtern wird dann auf die Gesamtaktivität des Tieres geschlossen. Der aktive Zustand war vor allem durch häufige Pegeländerungen und Kopfstellungswechsel und einen deutlichen Anstieg der mittleren Herzfrequenz gekennzeichnet.
APEC erfaßt Ruheverhalten, Fressen und Wiederkauen bei Milchvieh. Das Meßsystem wird unter dem Kiefer des Tieres befestigt. Kieferbewegungen werden dann über ein Luftkissen als Druckänderungen elektronisch registriert und gespeichert. Nachdem das Meßsystem dem Tier abgenommen wurde, können die gespeicherten Daten ausgelesen werden. Mit einem Software-Programm werden die Werte in Fressen, Wiederkauen und Ruhen klassifiziert.

Die jeweiligen Meßsysteme, die einander entsprechende Verhaltensparameter registrieren, wurden in zwei getrennten Untersuchungen gleichzeitig am selben Tier getestet und miteinander verglichen (für die Aktivität: ETHOSYS® und VIENNA, für Fressen: ETHOSYS® und APEC). Obwohl die Definitionen der einzelnen Verhaltensparameter voneinander abwichen, stimmten die ermittelten Daten in hohem Maße überein. Aus den Meßparametern, den Möglichkeiten zur Analyse der gewonnenen Daten, der Art der Anbringung der Meßsysteme am Tier, des technischen und personellen Aufwandes, der Datengenauigkeit und der Zuverlässigkeit des Datengewinns ergeben sich mögliche Einsatzfelder der jeweiligen Methoden. Die Ähnlichkeit der gleichzeitig mit verschiedenen Methoden gemessenen Daten führt also nicht zwangsläufig dazu, die getesteten Methoden in den jeweilig möglichen Einsatzgebieten beliebig miteinander austauschen zu können. Letztendlich bestimmend bei der Wahl der Methode ist die zu untersuchende Hypothese, das Forschungsziel der jeweiligen Untersuchung.

So erfaßt APEC die Verhaltensparameter Fressen und Wiederkauen äußerst präzise, kontinuierlich und mit festem Registrierintervall von 2,5 Sekunden. Untersuchungen zum Nahrungsverhalten bei verschiedenen Tierarten, die die Analyse der Zeitstruktur einschließen, sind damit gut möglich. Aufgrund der geringen Speicherkapazität von bis zu 6 Tagen und der zeitaufwendigen und komplizierten Befestigungsweise am Tier ist APEC für den Einsatz an Wildtieren und für langzeitige Untersuchungen allerdings ungeeignet.

Mit VIENNA lassen sich über lange Zeiträume hinweg sowohl physiologische als auch Verhaltensparameter mit sehr hoher Genauigkeit aktuell erfassen. Nachteilig sind die aufwendige Operation bei jedem Versuchstier und die häufigen kurzzeitigen Datenausfälle, die entstehen, wenn das Tier den Empfangsbereich verläßt oder sich im Funkschatten aufhält. Durch diese häufigen Lücken im Datensatz sind die Voraussetzungen für biorhythmische Analysen nur sehr bedingt gegeben. Gegen einen Einsatz an vielen Tieren oder in freiem Gelände spricht der hohe technische Aufwand bei dieser Methode. Trotzdem ist VIENNA gut geeignet, nicht sichtbare Erregung bei Tieren zu erkennen bzw. Reaktionen auf kurzzeitige Belastungen von Gehegetieren zu erforschen. Anhand der gewonnenen physiologischen Daten lassen sich dann auch Aussagen zu energetischen Bilanzen treffen.

ETHOSYS® zeichnet sich durch langfristige und vor allem lückenlose Datenerfassung mit einem festem Registrierintervall von 1 Sekunde aus. Die Länge eines lückenlosen Datensatzes ist allerdings davon abhängig, wie häufig und wie lange sich das Versuchstier in dem Empfangsbereich einer (auch tragbaren) Feststation aufhält (Die Mindestlänge beträgt 21 Tage bei einem 15-Minuten-Speicherintervall, die Maximallänge rund ein Jahr). Es ist damit sehr gut zur langfristigen biorhythmischen Statusüberwachung von Tieren geeignet. ETHOSYS® ist einfach und kostengünstig an verschiedenen Tierarten in ausnahmslos jedem Gelände zu handhaben. Für einen Einsatz an Tieren in freier Wildbahn ist die Kombination dieses Meßsystems mit einem Ortungssystem unumgänglich, damit die Datenübertragungen gewährleistet und die Meßhalsbänder wiedererlangt werden können.

Neben den methodischen Voraussetzungen ist das Wissen um die Lebensweise einer Art unter natürlichen und möglichst belastungsfreien Bedingungen in seiner jahreszeitlichen Varianz erforderlich, um ungewöhnliche Abweichungen von diagnostischer Relevanz erkennen zu können. Aus diesem Wissen lassen sich tierartbezogene Umweltansprüche ableiten. Mittels ETHOSYS® wurde am Przewalskipferd (*Equus ferus przewalskii*) unter Semireservatsbedingungen und an gegatterten Rothirschen (*Cervus elaphus*) das Zeitmuster der Aktivität und des Fressens über die Dauer eines Jahres untersucht. Von Juni 1995 bis Juli 1996 wurden an 4 Przewalskipferden Daten über insgesamt 1498 Tage erfaßt. Von April 1995 bis Januar 1996 und von Juli 1997 bis Mai 1998 wurden an jeweils 2 Rothirschen Daten über insgesamt 952 Tage gemessen. Diese Arbeit diente dazu, artspezifische Normwerte festzustellen und fand daher unter möglichst ungestörten und naturnahen Bedingungen statt.

Bei beiden Tierarten konnte trotz der geringen Tierzahl einheitlich ein artspezifisches Zeitmuster der gemessenen Verhaltensparameter über ein Jahr gezeigt werden. Dabei zeigten sich äußeren Umweltfaktoren gegenüber sowohl feste Grundmuster als auch äußerst variable Komponenten im Aktivitätsmuster beider Arten.

Beiden Arten gemeinsam war ein polyphasisches Tagesmuster über das gesamte Jahr mit Aktivitätsgipfeln um den Sonnenauf- und untergang. Jahreszeitliche Schwankungen, eine Circadianrhythmik und ultradiane Rhythmen bestimmten das Zeitmuster beider Verhaltensparameter bei beiden Arten. Im Sommer wies ein Wechsel von überwiegender Tagaktivität zu verstärkter Nachtaktivität bei beiden Tierarten auf Beeinflussung durch Hitze bzw. Fluginsekten hin.

Beim Przewalskipferd ist das Niveau der Aktivität und des Fressens im Jahresgang voneinander losgelöst, während die Aktivität im Sommer am höchsten und im Winter am niedrigsten war, wies das Fressen im Herbst und Frühjahr die höchsten Werte auf und hatte seine Minimalwerte im Sommer. Dagegen erreichen Aktivität und Fressen beim Rothirsch zur gleichen Zeit ihre Tiefstwerte (in den Wintermonaten) und Höchstwerte (in den Sommermonaten).

Jahreszeitliche Variationen des Zeitmusters (Tagesgesamtaktivität, Verhältnis Tagaktivität zu Nachtaktivität) erfolgten beim Przewalskipferd z.T. sprunghaft innerhalb einer Woche. Dabei wurde im Juli/August 1995 sprunghaft zwischen überwiegend tagaktiv und überwiegend nachtaktiv gewechselt, womöglich um hohen Lufttemperaturen und Insekten am Tage auszuweichen. Sobald im Frühjahr 1996 frische Pflanzen wuchsen und sich die Luft erwärmte, steigerten die Pferde ihre Aktivität und ihr Fressen sprunghaft. Beim Rothirsch hingegen verliefen jahreszeitliche Variationen des Zeitmusters stets fließend.

Artspezifische jahreszeitbezogene Veränderungen in der Ernährungsweise in Abhängigkeit von den Ernährungsbedingungen führten zu einer Variation der ultradianen Struktur der Aktivität und des Fressens. Bei beiden Arten waren die Zeitmuster der Aktivität und des Fressens von der 24-Stunden-Rhythmik und ultradianen Komponenten von 4,8- bis 12-Stunden Periodenlänge charakterisiert. Beim Rothirsch verschoben sich diese in Zeiten guten Futterangebotes in den höherfrequenten Bereich bis hin zur 3-Stunden-Rhythmik. Diese saisonale Varianz im Zeitmuster des Fressens ist bei den Pferden vergleichsweise weniger ausgeprägt als beim Rothirsch. Sie könnte damit auf die generelle Ernährungsstrategie der jeweiligen Tierart hinweisen. Pferde (als Rauhfutterfresser) decken ihren Nahrungsbedarf, indem sie ihre Nahrungsaufnahme steigern, um geringeren Nährstoffgehalt im Futter zu kompensieren. Dies bedeutet lediglich eine quantitative Änderung der Fressaktivität und geht nicht mit einer Umstellung des Verdauungsapparates oder einer Umstrukturierung der Verhaltensrhythmik einher, wie es bei den Rothirschen (als Intermediärtyp) der Fall ist. Intermediärtypen variieren dagegen zwischen zwei Ernährungstypen. Sie reagieren auf abnehmende Verfügbarkeit geeigneten Futters entweder damit, weniger Futter aufzunehmen und dafür leicht verdauliche Pflanzen oder Pflanzenteile zu selektieren (Konzentratselektierer) oder sie verbrauchen mehr Futter geringerer Qualität (Gras- und Raufutterfresser). So waren zu Zeiten sich ändernden Nahrungsangebotes beim Pferd vor allem starke (sprunghafte) quantitative Änderungen im Verhalten zu verzeichnen, wogegen die quantitativen Änderungen beim Rothirsch fließend verliefen und (im Vergleich zum Pferd) stärkere Änderungen in der Zeitstruktur des Verhaltens auftraten.

Verschiedene Belastungsbedingungen ließen sich bei beiden Tierarten durch die biorhythmische Analyse langfristig und lückenlos erfaßter Verhaltensdaten erkennen und bewerten.
Unter belastungsneutralen Umweltbedingungen stellen sich vorzugsweise ganzzahlige (harmonische) Frequenzverhältnisse zwischen Aktivitäten der verschiedenen Funktionskreise des Organismus ein. Diese sind gemeinsam auf den Grundrhythmus der externen 24-Stundenperiodik abgestimmt. Diese hierarchische Frequenzabstimmung des Organismus führt bei komplexen rhythmischen Funktionen (wie der Aktivität und dem Fressen) vorzugsweise zu Periodenlängen, die in einem ganzzahligen (harmonischen) Verhältnis zur 24-Stunden-Periode stehen. Die biorhythmische Überwachung der Aktivität und des Fressens erwies sich als geeignetes Mittel zur Einschätzung der organismischen Funktionslage bei verschiedenen Tierarten. Um Störreize unter zumeist naturnahen Bedingungen ermitteln und einschätzen zu können, dienten Leistungsbezogenen Kopplungsgrade (LKG), die ein Maß für die Harmonie zwischen internen Rhythmen und der externen 24-Stunden-Periode sind. Diese LKGs konnten den Einfluß verschiedener Belastungsbedingungen und -situationen auf die Zeitstruktur der Aktivität und des Fressens an verschiedenen Tierarten verdeutlichen (u.a. die Überführung eines Przewalskipferdes vom Zoo ins Semireservat, in der Nähe durchgeführte Jagden bei Przewalskipferden und Rothirschen, Futterumstellung und Geweihabschnitt bei gegatterten Rothirschen, Sozialstress bei Rothirschen und Alpakas, Ablammen bei Mufflons und Alpakas). Insgesamt verfügte der Verhaltensparameter Fressen über eine größere Stabilität gegenüber Störungen aus der Umwelt, die Aktivität ist Umwelteinflüssen gegenüber sensibler.

Das Speicher-Telemetrie-System ETHOSYS® konnte verschiedenen Tierarten angepaßt werden. Die alleinige automatische Registrierung von Verhaltensdaten ist allerdings nicht in der Lage, das Verhalten eines Tieres/einer Tiergruppe vollständig zu beschreiben. Um möglichst objektiv bewerten zu können, ist es unerläßlich, zusätzlich das Tier zu beobachten und Umweltfaktoren, die für das Tier bedeutungsvoll sind, zu erfassen.

5. General Summary

This study was conducted with the view to preparing methods and concepts for behaviour-oriented (especially biorhythmic) status diagnosis for the purpose of widest possible unbiased assessment of the living conditions of free-ranging wildlife. Initial problems were related to methodology. Biorhythmic status diagnosis primarily depends on the capability of measuring and recognising biological rhythms. This calls for long-term, gapless recording of those physiological or behavioural parameters which are characterised by regular measuring intervals and constant measuring accuracy. Without availability of technical aids, investigations of that kind cannot be conducted on wild-living animals which quite often are shy, night-active and have their habitat in large areas that are hardly overseeable. Interpretation of automatically acquired data proves to be difficult, as well, since wild-living animals are exposed to and affected by a complexity of environmental factors (e.g. climate, availability of food, social partners), with many of them escaping investigation, since long-time direct field observation would have too high an impact upon the animal or would be associated with impermissibly high expenses. That is why my study had to be started by a comparison of various methods which were thought to be potentially suitable for long-time automatic acquisition of behavioural parameters from free-ranging animals. Benefits and setbacks of various methods were to be elucidated together with their measuring accuracies and possible applications. Evaluation of our experiments and trials was to suggest the extent to which each of the methods was applicable to long-time status diagnosis of the wild-living animal. Three approaches were tested, in this context, the storage telemetry system ETHOSYS®, the radio telemetry VIENNA system and the APEC chew-beat counter.

ETHOSYS®, using extracorporal measuring collars, is capable of high-continuity, long-term recording of activity and feeding from big herbivores. A motion sensor, with a readjustable time window and tilt switch connected to it in the collar, scans movements and positions of the head. Any movement which is transmitted to the collar is defined as "activity". Downward bending of the head together with movement characteristic of food intake is additionally evaluated as "feeding". All data measured are saved in the collar in preselected intervals. When the animal is within the reception range of a central station, these data are radioed to that station from where they can be picked up independent of the animal's current position.

VIENNA can be used for recording of heart rate, body temperature and activity of roe deer and red deer. A heart rate transmitter is subcutaneously implanted and subcutaneously connected to two ECG electrodes in the thoracic region. A repeater collar is used in the new version of the surgical intervention which, consequently, is much less invasive than it used to be with the previous version of the VIENNA method for which an aerial and tilt switch had to be implanted into the dorsal and neck regions. It is this extracorporal repeater collar with aerial from which heart rate, body temperature, head posture and receiver field strength are selected and read by radio telemetry. Logic filters are used to draw conclusions as to the animal's overall activity. The active status was primarily characterised by frequent changes of level and head posture and clearly measurable rise in the mean heart rate.

APEC is capable of measuring rest behaviour, feeding and rumination in dairy cattle. The measuring system is attached in submandibular position to the animal. Jaw movements are electronically recorded through an air cushion as pressure changes and are saved. Data thus saved can be read after the measuring system has been dismantled from the animal. A software programme is available for classification of all values related to feeding, rumination and rest.

The two measuring systems for recording of behaviour parameters were separately tested on one and the same animal and were compared with each other (ETHOSYS® and VIENNA for activity; ETHOSYS® and APEC for feeding). While the definitions of the behaviour parameters deviated from one another, the data thus measured were in fair agreement with each other. The parameters that can be measured, the possibilities for analysis of data recorded, the mode of attachment of the systems to the animal, input in terms of hardware and personnel, data accuracy and high reliability of data acquisition are factors in support of possible applications of either method. The similarity of data measured by different methods does not necessarily support interchangeability of the two methods for one and the same application. The choice of methods actually has to depend on the hypothesis to be verified, that is the research purpose of the study at hand.

For example, APEC is capable of measuring the behaviour parameters of feeding and rumination with extremely high accuracy and continuity in fixed recording intervals of 2.5 seconds. This enables studies on feeding behaviours of various animal species, including analysis of time patterns. APEC, on the other hand, has a low memory capacity of up to six days, and its attachment to the animal is a time-consuming and complicated exercise. It, therefore, would not be suitable for use on wild-living animals nor for long-time investigations.

VIENNA is capable of up-to-date recording of both physiological and behavioural parameters over extended periods of time and with very high accuracy. Disadvantages include the need for tedious operation on each of the test animals and frequent short-time breakdown of data which may occur whenever the animal moves out of service area or stays in a silent zone (radio shadow). Hence, data records are likely to suffer many gaps which impair conditions for biorhythmic analysis. Also, the method is less suitable for application to large numbers of animals or use under outdoor conditions because of the need for high technical input. VIENNA, nevertheless, is suitable for recognition of otherwise invisible and thus concealed excitation or investigation of enclosure-kept animals and their response to short-time stress. Physiological data thus acquired might as well provide information on energy balances.

ETHOSYS® is characterised by a capability of long-time, gapless data acquisition with a constant recording interval of one second. The length of a gapless data record, however, will depend on how frequently and how long the test animal stays in the service area of a (perhaps portable) central station (minimum length being 21 days with 15-minute saving interval, appropriate maximum length being one year). Hence, the system is highly suitable for long-time biorhythmic status monitoring of animals. ETHOSYS® is easy to handle and of low-cost applicability to various animal species in any kind of natural environment. It has to be combined with a location system for use on animals under wildlife conditions to ensure proper data transmission and recovery of measuring collars.

These methodological prerequisites have to be supported by knowledge of the given species' lifestyle under natural, troublefree conditions in all seasons of the year in order to identify unusual deviations of diagnostic relevance. Such knowledge is essential, as species-related demands on the environment may be derived from it. The ETHOSYS® system was used on Przewalski horse (*Equus ferus przewalski*) under semi-reserve conditions and on enclosure-kept red deer (*Cervus elaphus*) in a one-year investigation of the time pattern of activity and feeding. Data collected on 1,498 days were recorded from four Przewalski horses from June 1995 through July 1996. Data collected on 952 days were recorded from two red deer each between April 1995 and January 1996 as well as between July 1997 and May 1998. This study was intended to provide species-specific standard values and, therefore, was conducted under more or less troublefree and quasi-natural conditions.

Notwithstanding the small number of animals involved, a species-specific time pattern of behaviour parameters was obtained from either species for one year. The activity of either species exhibited both rigid patterns as well as extremely variable components in response to external environment factors.

The two species had in common for the whole year a polyphasic daily pattern with activity peaks at sunrise and sunset. The two behaviour parameters of either species were characterised by seasonal variations, a circadian rhythm and ultradian rhythms. In either species, change from predominant daytime activity to increased overnight activity in summer suggested response to heat or to molestation by flying insects.

In Przewalski horse, annual activity and feeding levels were found to be separated from each other. Activity was at its highest in summer and lowest in winter, but feeding was at its highest in autumn and spring, with minimum values being recorded in summer. In red deer, on the other hand, both activity and feeding had their lowest (winter) and highest (summer) values at one and the same time.

In Przewalski horse, some of the seasonal variations in time patterns (total daily activity, relationship between daytime and overnight activities) occurred in a leapwise manner within one and the same week. In July/August 1995, there was a leapwise change from predominant daytime activity to predominant overnight activity, perhaps to avoid high ambient temperatures and insects. Horses drastically increased activity and feeding as soon as fresh plants began to emerge and air temperature went up in spring 1996. In red deer, on the other hand, all seasonal variations in time patterns took a course of gradual transition.

Species-specific season-related variations in nutrition, depending on nutritional conditions, led to variations in the ultradian structure of activity and feeding. Time patterns of activity and feeding in either species were characterised by 24-hour rhythmicity and by ultradian components of 4.8 to twelve hours in period length. In red deer, these periods were moved to higher frequencies up to three-hour rhythmicity at times of high food supply. Seasonal variance of time pattern for feeding was less strongly pronounced in horse as compared to red deer. It may thus be an indicator to species-specific general feeding strategies. Horses are roughage eaters and tend to satisfy their food demand by consuming higher quantities to make up for lower nutrient levels. This actually means nothing but a quantitative modification of feeding activity and is not accompanied by transformation of the digestive tract or restructuring of behavioural rhythm, as is the case with red deer, an intermediate type. Intermediate species vary between two types of feeding. Intermediate types tend to respond in two ways to reduced availability of appropriate food: either be reducing food consumption and selecting instead easily digestible plants or parts of them (selection for concentrates) or by consuming more food of lower quality (grass and roughage eaters). That is why in periods of changing food availability, horse primarily exhibited strong, leapwise quantitative change in behaviour, whereas quantitative change in red deer was of a gradual, transitional nature and was accompanied by stronger change (as compared to horse) in the time pattern of behaviour.

Various stress conditions of either species were recordable and could be evaluated by biorhythmic analysis of behaviour data that had been acquired in gapless condition over extended periods of time. Stress-neutral environmental conditions resulted primarily in integral-number (harmonic) frequency relations between activities of various functional cycles of the organism. These were jointly adjusted to the basic rhythm of external 24-hour periodicity. Such hierarchic frequency tuning of the organism, in the context of complex rhythmic functions (such as activity and feeding), primarily leads to period lengths which are in an integral-number (harmonic) relationship to the 24-hour period. Biorhythmic monitoring of activity and feeding proved to be an appropriate tool for assessment of organismic functionality in various animal species. Degrees of Functional Coupling (DFCs), a measure of harmony between internal rhythms and the external 24-hour period, were used to identify and evaluate irritative stimuli under usually quasi-natural conditions. Such DFCs helped to illustrate effects of various stress conditions and stress situations on time patterns of activity and feeding in various animal species (such as transport of a Przewalski horse from a zoological garden to our semi-reserve, hunting in short distance from positions of Przewalski horse and red deer, change in feeding regimes, removal of antlers from enclosure-kept red deer, social stress among red deer and alpaca individuals, lambing of mouflon and alpaca). The behaviour parameter of feeding, on balance, exhibited higher stability to environment-borne stress, while activity was of higher sensitivity to environmental effects.

The storage telemetry system ETHOSYS® was successfully adjusted to various animal species. Yet, the behaviour of one animal or a group of animals cannot be completely described by isolated automatic recording of behaviour data. For the purpose of unbiased assessment, it is indispensable to keep the animal under physical observation and to determine all environmental factors which may have a bearing on it.

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Selbständigkeitserklärung

Ich erkläre, daß ich die vorliegende Arbeit (entsprechend der genannten Verantwortlichkeit) selbständig und nur unter Verwendung der angegebenen Quellen und Hilfsmittel angefertigt habe. Den benutzten Werken wörtlich oder inhaltlich entnommene Stellen wurden als solche in der vorliegenden Arbeit kenntlich gemacht. Der Eigenanteil an den teilweise bereits veröffentlichten Arbeiten, die in dieser Dissertation zusammengestellt wurden, ist jeweils deutlich von mir dargestellt.

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