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RESEARCH IN ZOOS: HOW TO START, HOW TO AVOID THE MOST COMMON MISTAKES!

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Introduction

The situation of research projects in zoos quite often is characterized by several seemingly formidable obstacles. Some of them can be overcome by proper planning and hypothesis – driven approaches for often there are solutions due to recent developments in methodology. Many colleagues in and outside of the zoo community are not aware of these developments, which quite often leads to unnecessary friction and often embarrassing experiences. The present paper intents to address only two rather general aspects of proper planning in zoo-related projects i.a. hypothesis-finding and statistics. The reason for this is that many zoo projects specifically show deficiencies in these areas, and also that many colleagues outside the zoo research community tend to criticise zoo research specifically on the basis of these aspects. It is not my intention to address the specific planning aspects of specific areas (e.g. genetics, endocrinology, behaviour). These can be more easily obtained from laboratory textbooks, and from the methods sections of relevant papers.
Recently, BIAZA's Research Committee has started to publish a number of Zoo Research Guidelines on their website, and it is strongly recommended to refer to these in addition.

Hypothesis-finding and conceptualization

Zoo biology as a whole is a branch of conservation biology, an area that intends to integrate as many aspects of biological sciences and beyond. The underlying theme as in all biology, is adaptation and an understanding of adaptive processes as well as constraints upon these, can lead to better planning and execution of all types of zoo and conservation work (Hodges et all, 1995, Kaumanns & Gansloßer 1995). This approach, however, has to be specified in individual projects in order to avoid a fault that U. Reyer once called the 'little do we know about-approach' ('Wenig ist bekannt über-Ansatz') because many papers still draw their major justification by stating, in the introduction that this or that phenomenon has not yet been described, or quantified. It is a necessary precondition for scientific work to describe phenomena in an exact, objective and if possible quantitative way. But pure description, be it of anatomical structures, behavioural movements or DNA sequences, is not yet science!
Biological sciences aim at a better understanding of the phenomena of living organisms within the framework of a scientific approach. This means that general principles of scientific thinking and theory formation (e.g. Occam's principles of parsimony) have to be followed. However, in dealing with biological phenomena, we have to consider two additional characteristics that are specific for biological systems, namely the fact that biological systems are organised in a hierarchical way, often with synergistic interactions resulting in the whole being more than the sum of its parts and the historical dimension of all biological phenomena. In order to address these complexities in a testable and thus scientifically sound as well as well-focused way, Nico Tinbergen (1963) suggested the categorisation of biological research questions into four areas, each of which representing a different angle of approach, and being complementary to the others. Tinbergen stressed that only by

addressing all four and finding answers to all four can be claiming to understand a biological phenomenon.

The famous four questions are:

- phylogenetic history: where does this phenomenon come from in evolutionary time, how about the situation in ancestral or related species. This phylogenetic approach has clearly profited from methodological improvements of the comparative method in recent years, including its application in behavioural ecology (Harvey & Pagel, 1991).
- evolutionary function: what is the fitness benefit of a given trait, how does it relate to overall reproductive success of its bearer, in comparison to other conspecifics of the same generation?

  This is an area where zoo research can help a lot to develop and conduct life history-related research projects by analysing stud book data and other institutional records.
- physiological function: how does this trait work in terms of hormonal and neural control, external stimuli, biochemical, biomechanical and other mechanism, what is the influence of climate change on the process of living organisms etc. Again, zoo animals, being often kept and bred outside their natural range, could serve as models to develop hypothesis on the influence of climate change on all sorts of bodily functions etc.
- ontogenetic development: how does a given trait development in the individual path from zygote to post-reproductive senility elopmental biology in general has long since overcome the old nature-nurture dichotomy and by studying the closely related interactions between genome, extragenomic traditions and current external conditions (which all can be more closely controlled or at least documented in zoos) again zoo animals can be regarded as models for an understanding of developmental aspects in a broader context.

Once a phenomenon worth studying has been identified, be it from preliminary observations or literature it is important first to decide from which of the four angles the research project should be conducted - and then stay rigorously by within this angle. A mixing of causal and functional arguments is frequently encountered in the interpretation of descriptive but also sometimes analytical studies. An example: When asking about the benefit of infanticide for a male lion killing his predecessor's offspring, it is not allowed to argue that this is a consequence of stress after forceful take-over of a new pride, because the question relates to Tinbergen's second, the answer to the third category.

Careful observations and analysis of existing literature, as Innis (2006) has clearly demonstrated in her thesis on the value of bibliometry for zoo research, can lead to precisely formulated hypothesises and, based on these, specific predictions. It is important to check, during this stage, whether one's predictions are actually precise and unequivocal enough to be testable under the given conditions. Then, variables have to be defined, and again, it is necessary to clearly outline which are the independent and which are the dependant ones. Do visitor numbers influence the animals' activities, or do active animals attract more visitors? At this stage at the latest, it is urgent to consider statistical procedures, because specific tests often require a certain data structure.

After defining all variables, and carefully formulating, sampling and recording rules (what sort of data do we collect how) these should be written down and circulated to all people involved, including keepers, curators, vets etc. And they should be told that, if they have any objections to the way the project is to be conducted now, and only now, is the time to speak up or be forever silent (and do what the project requires). Many university colleagues are frustrated by the fact that zoo management accepts a research project that is later torpedoed by the lower levels, and many keepers are frustrated because they are never asked before student or researcher starts with data collection!

In planning zoo research projects WAZA has formulated and published Ethical Guidelines (Anon. 2005). As long as these are followed, there is no limit to what sort of research is being planned in zoo.

Statistics

Zoo and conservation biology research often suffer from a lot of statistical difficulties, leading to data sets that are "dirty", chaotic and "muddled".

Traditional statisticians often use this as a pretext to deny our research its scientific soundness. In general, the most-often voiced objections concern sample sizes data niveau, zoo data being (nominal/or ordinal data) or the unclear relations of several sets of dependent and independent variables. However, there are solutions to each of these statistical obstacles. The first generally accepted solution is to rely on non-parametric statistics. Zoo data (but also field data on rare animals) only rarely meet the preconditions of large statistically relevant sample sizes (> 15), normally distributed data, and clearly defined measurements on interval data niveau. If they do, traditional statistics can easily be applied.

Small sample sizes, however, need other approaches. Randomisation tests that generate their p-value from the data set itself instead of an assumed theoretical distribution are very powerful tools for small samples, comparing animals before and after treatment, comparing members of both sexes, etc. Single-case and small-case studies can be designed in an ABA/ABACA, etc. format, subjecting the same individuals to baseline and treatment and in "severe cases" of small samples (i. e. ≤ 5), results can be compared later by application of agglutination tests.

Another common problem in zoo (but also in field) studies of rare species is the fact that several dependent variables have one common underlying cause. This can be addressed by G-tests or their derivatives and tests for autocorrelation before using more than one data point per animal (called pseudoreplication, a common mistake in many biological disciplines).

Finally, in designing multi-centred studies that compare data from several zoos, e. g. studbook participants, the application of multivariate means is recommended.

Several caveats however, still have to be voiced, in order to avoid common statistical pitfalls. One is the problem of pseudo replication and, related to it the pooling error.

Measuring the same animal repeatedly in the same situations does not increase n, it only may increase exactness of the measurement.

And before combining data from different subsets strictly speaking, they have to be tested for statistical differences. Only data subsets that do not reject the null hypothesis of no statistical difference are allowed to be pooled. Other possibilities for statistically overcoming the pooling error exist, such as nested ANOVAs, but again require statistical competence and planning in advance of the project.

Another common mistake, even in analyses from high-quality labs, is a disregard of the problems of multiple tests. As soon as your data are subjected to more than one test, e.g. to answer several research questions, the results p-values have to be corrected, e. g. by application of Bonferroni procedure or post-hoc tests have to be used which include such a correction themselves.

Conclusions

Milinsky (1997) has outlined "seven deadly sins" in the study of behaviour. However, just as with Tinbergen's questions, these are not restricted to behavioural research and are quite often found in other zoo and conservation biology studies as well. In short, these seven sins are

- injustified conclusion from non-experimental i. e. correlational data

- pseudo replication by using data that are not independent
- time and sequence efforts in treatments, e. g. by disturbing the animals more and more often (or habituating them more and more!) during the course of the project
- observer bias
- animals not suitably accustomed to test procedures
- use of unsuitable controls
- attempts to "prove" the null hypothesis, specifically with small samples. Not detecting a difference is not the same as demonstrating that no difference exists!

Finally, one last remark: This paper only covers aspects of research design and procedure. It does not address the prejudice about zoo animals behaving abnormally because of an artificial environment. This is a criticism directed at zoos in general, which has been addressed in other contexts at least regarding behavioural studies (e. g. Carlstead 1996, Engel 1999).

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A PRELIMINARY ANALYSIS OF THE HABITAT REQUIREMENTS OF ADERS' DUIKER IN THE ARABUKO-SOKOKE FOREST, KENYA, AS DERIVED FROM INDIRECT FIELD SIGNS AND CAMERA-TRAP RECORDS.

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Abstract

The Whitley Wildlife Conservation Trust has been investigating the distribution and habitat requirements of Aders' duiker Cephalophus adersi (Thomas 1918) since 2002. This critically endangered antelope is only known to occur in two coastal forests in Kenya and also on Zanzibar. Recorded sightings, indirect duiker signs and camera traps have been used to find out more about this elusive species. In 2005, duiker signs (pellet piles and tracks) were quantified along with sightings of Aders' duiker in 40 plots throughout the Arabuko-Sokoke Forest, Kenya. These were related to vegetation measures including canopy cover, visibility and plant species along with measures of human and elephant disturbance. This revealed positive relationships between duiker signs and low visibility and high number of duiker food plants. A negative relationship was found between duiker signs and elephant disturbance. In 2006, 10 camera traps were set up within the forest. During a three month sampling period 10 pictures of Aders' duiker were taken along with another 16 animal species. These results are beginning to build a picture of the habitat requirements for Aders' duiker and the success of the camera trapping suggests that this technique may be valuable for surveying forests for this species and other elusive animals.

Keywords: forest antelope, Aders' duiker, Cephalophus adersi, habitat use, conservation

Introduction

Aders' duiker Cephalophus adersi is restricted to a very few fragmented forests in Zanzibar and on the coast of mainland Kenya and has been referred to as one of Africa's most threatened antelope (East 1999). This species is endemic to the coastal forests of eastern Africa, an internationally recognised hotspot for exceptional biodiversity and species endemism (Mittermeier et al. 2005).

On Zanzibar, Aders' duiker has undergone a dramatic decline in response to hunting and habitat loss. The population size has been estimated at approximately 5,000 in 1982 (Swai 1983), 1,400 in 1995 (Williams et al. 1996) and 614 ± 46 at the last survey in 1999 (Kanga & Mwinyi 1999). The species is now probably restricted to three forests on Unguja Island (Williams et al. 1996) although small numbers have been translocated to offshore islets as a conservation measure (Mwinyi & Wiesner 2003, Mwinyi personal communication).

As recently as 1982, Aders' duiker was described as widespread in forest and woodland along the Kenyan coast north of Mombassa almost up to the Somali border (Kingdon 1982). By the mid-

1990s the species was thought to be restricted to one site, the Arabuko-Sokoke Forest, and to be extremely rare. Sightings reported during research by Erustus Kanga from 1999 confirmed the continued existence of Aders' duiker in Kenya (Kanga 2002a). A drive-count survey in 2002, funded by Whitley Wildlife Conservation Trust, failed to capture any individuals although the species was observed during fieldwork (Kanga 2002b). Since that time infrequent sightings continue to be recorded by researchers and forest guides. In 2004, Aders' duiker was upgraded in the IUCN Red List from Endangered to Critically Endangered (Finnie 2004).

Knowledge of the ecological requirements and distribution of threatened species is crucial to their conservation. However, duikers are difficult to study due to their secretive behaviour and because they are often found in dense vegetation (Bowkett et al 2006). This situation is exacerbated when the study species occurs at low density as with Aders' duiker. The Whitley Wildlife Conservation Trust (Paignton Zoo Environmental Park) has funded and undertaken research and surveys for Aders' duiker since 2002. This paper presents the results of our investigations into the habitat requirements of Aders' duiker based on sightings and indirect field signs. However, recognising the limitations of these methods we also assess the potential of camera-trapping to record and monitor Aders' duiker in Kenyan coastal forests.

Methods

Study site

The Arabuko-Sokoke Forest (ASF) is situated in Kilifi and Malindi Districts, Kenya (39°50`E, 3°20`S). At c. 416 km2 this forest is the largest remaining block of tropical coastal forest in east Africa. The forest vegetation has been classified into four main habitat types dictated by local soil conditions: Cynometra forest, Cynometra thicket, Brachystegia woodland and mixed forest. However, these habitats can overlap and show significant internal variation. Other small antelope species found in the ASF include Harvey's duiker Cephalophus harveyi (Thomas 1893), blue duiker C. monticola (Thunberg 1789), grey or bush duiker Sylvicapra grimmia (Linneaus 1758) and suni Neotragus moschatus (Von Dueben 1846).

Analysis of habitat requirements

Vegetation and other habitat variables were measured at 40 locations throughout the ASF during May to July 2005 (see Table 1). Thirty-two locations were selected by random stratified sampling according to the percentage cover of the three main vegetation types (combining the two Cynometra habitats) and accessibility. A further 8 locations were selected due to their proximity to recent (since 1999) records of Aders' duiker. The diet of Aders' duiker has not been studied in any detail, however, Kanga (2002a) lists 18 plant species identified from the stomach contents of one individual and these were treated as "Aders' duiker food plants" in our analysis. Nomenclature of plants followed Robertson and Luke (1993).

Duiker habitat use was quantified by counting dung pellet piles and antelope paths (identified by footprints) up to 1 m either side of a 100 m x 100 m square transect. We were unable to identify dung or footprints to species and so these indirect measures are used as a proxy for habitat use by all small antelope species rather than Aders' duiker specifically. This transect was also used to record signs of human disturbance (cut trees and paths) and elephant disturbance (clearings and paths).

Table 1. Habitat variables measured in nested plots at 40 locations within the Arabuko-Sokoke Forest, Kenya. Asterisks mark variables retained for statistical tests following non-metric multidimensional scaling (Schrodt 2005).

Plot	Variable
10 x 10 m	1. Tree height (DBH ≥ 5 cm)
	2. Tree DBH
	2. Canopy cover (%)*
	3. Visibility (%)*
4 x 4 m	4. Sapling height (DBH < 5 cm and ≥ 1 m height)
2 x 2 m	5. Abundance of vascular plant species (< 1 m height)
100 x 100 m (transe	6. Elephant disturbance*
	7. Human disturbance*
	8. Duiker dung piles*
	9. Antelope paths*
Across plots	10. Species richness of Aders' duiker food plant
	11. Abundance of Aders' duiker food plants*

Relationships between and among duiker and habitat variables were explored by Schrodt (2005) using non-metric multidimensional scaling using the programme PC-ORD Version 4.27 (McCune and Mefford 1999). Relationships with habitat variables were quantified for Aders' duiker presence or absence using point-biserial correlation analysis with MS-Excel (following Kent & Coker 2003) and for indirect duiker sign with Spearman rank correlation. Due to many variables not being normally distributed, differences between plots with and without Aders' duiker sightings and between different habitat types were tested for with non-parametric tests using SPSS v.16.0 (SPSS Inc., Chicago, IL, USA).

Camera-trapping

Heat and motion, infra-red triggered camera-traps (DeerCam) were trialed in the ASF during September to December 2006 with the aim of assessing the potential of this method for recording Aders' duiker by comparing camera 'capture' rate with that of other species. Cameras were set to take pictures 24 hours a day on 200 ASA colour print film, with a 1-min delay between exposures. The date and time of each exposure were shown on the film. Ten cameras were kept in the field for a mean of 62.6 days. Camera-trap locations (n = 14) were selected in areas with confirmed Aders' duiker sightings (mostly in and around the central area designated as a strict nature reserve) and were either located within the habitat plots established in 2005 or along adjacent vehicle tracks. For any series of photographs of the same species the first picture was considered an independent capture event but subsequent pictures were excluded up to a maximum period of 1 hour. This was a compromise between scoring the same individual multiple times and missing individuals, following Bowkett et al (2008). Camera-trap capture rate was calculated across all locations as the number of independent photographs of a species per trap-day (expressed as a percentage).

Results

Habitat requirements

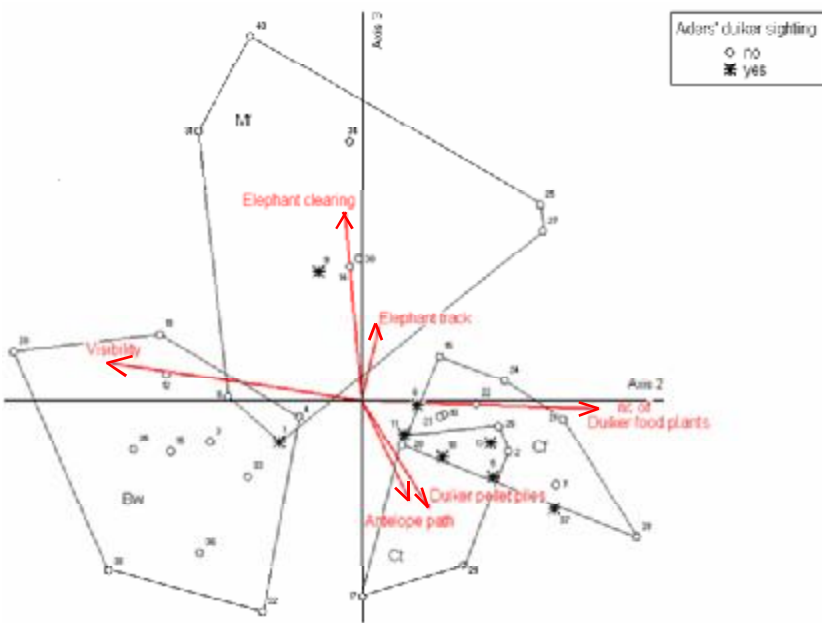

Figure 1. Non-metric multidimensional scaling bi-plot for 40 habitat plots in the Arabuko-Sokoke Forest, Kenya (Schrodt 2005). Those plots situated near Aders' duiker sightings are marked with an asterisk. Polygons enclose plots from the four major habitat types: Bw = Brachystegia woodland, Mf = Mixed forest, Cf = Cynometra forest, Ct = Cynometra thicket. Selected environmental/disturbance variables are overlaid (length of arrows is proportional to the importance of variables). Abundance of Aders' duiker food plants is labeled "nr. of Duiker food plants".

Table 2. Correlation coefficients for selected habitat variables with presence/absence of Aders' duiker (point-biserial) and with duiker field signs (Spearman rank) from 40 plots in the Arabuko-Sokoke Forest, Kenya. * = P < 0.05, ** = P < 0.01, *** = P <0.001

Habitat variable	Aders' duiker presence (1) absence (0) (rb)	Duiker field sign (dung and paths) (rs)
Visibility	-0.05	-0.57***
Canopy cover	0.22	-0.13
Elephant disturbance	-0.20	-0.43**
Human disturbance	-0.22	-0.16
Abundance of Aders' duiker foo plants	0.11	0.39*

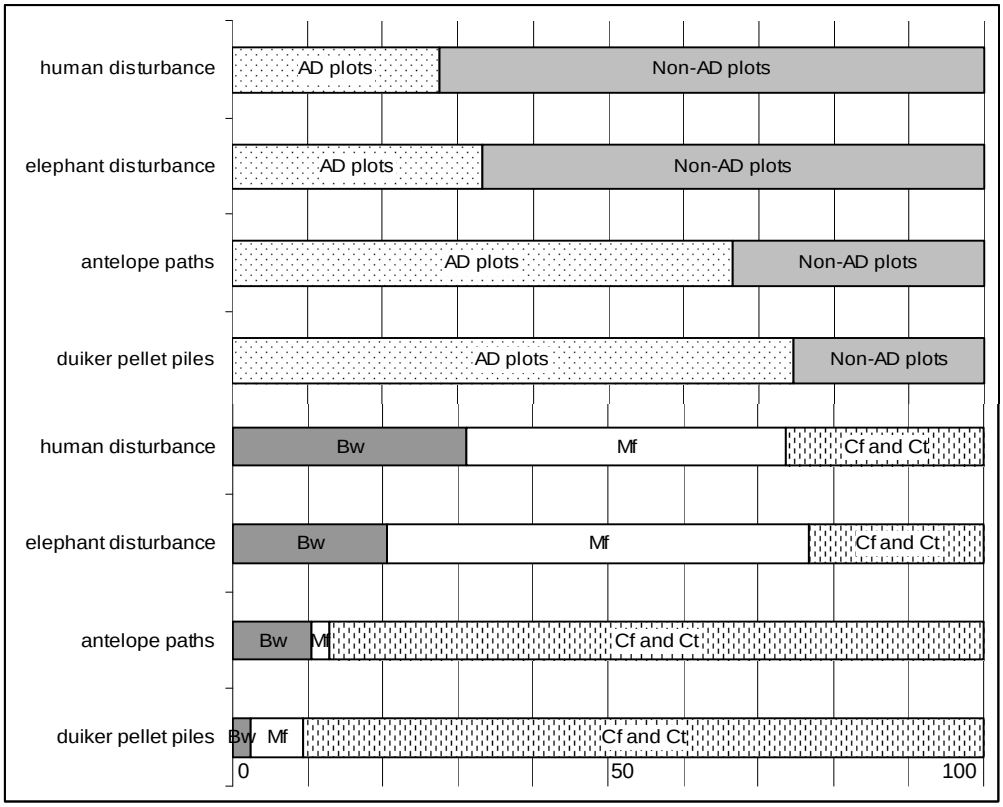

Figure 2. Graphical summary of the percentage of plots in which duiker field signs and human and elephant disturbance were recorded within the Arabuko-Sokoke Forest, Kenya. AD plots = with recent Aders' duiker sighting (post 1999), Non-AD plots = without recent sighting, Bw = Brachystegia woodland, Mf = Mixed forest, Cf and Ct = Cynometra forest and thicket.

Non-metric multidimensional scaling (NMS) was carried out by Schrodt (2005). After-the-fact coefficient of determination revealed that the three NMS axes explained 84.6% of the variation in the data (14.1%, 43.9% and 26.7% respectively). Several variables were found to be redundant in that their inclusion in the NMS analysis did not add to the variation explained by the data. These variables were therefore dropped from further analysis (Table 1). Examination of the NMS bi-plot (axes 2 and 3, explaining 70.6% of the data) showing habitat plots with recent Aders' duiker sightings shows that these plots are found mostly within Cynometra habitats and positively associated with duiker field signs and food plant abundance but negatively associated with high visibility and elephant disturbance (Figure 1).

The correlation analysis showed that duiker field sign was negatively related to visibility and elephant disturbance and positively related to the abundance of Aders' duiker food plants. The relationships between these habitat factors and the presence/absence of Aders' duiker followed the same directions but did not reach significance (Table 2).

The number of duiker dung piles was significantly greater in plots with Aders' duiker sightings (Mann Whitney test: U = 69, P < 0.05) and nearly so for antelope paths (U = 76.5, P = 0.062). There was also a difference in duiker field sign across habitat types (Kruskal Wallis test: dung piles ^{C} 2 = 17.12, P < 0.001; antelope paths ^{C} 2 = 15.88, P < 0.001) Canopy cover, visibility and elephant disturbance did not differ significantly between plots with and without Aders' sightings but did differ across habitat types (canopy ^{C} 2 = 8.05, P < 0.018; visibility ^{C} 2 = 16.24, P < 0.001; elephant disturbance ^{C} 2 = 10.11, P < 0.006). There was no significant difference in human disturbance across the conditions tested. A summary of plots containing duiker and disturbance signs is illustrated in Figure 2.

Camera-trapping

Camera-traps recorded 17 species from a total of 280 photographs. Five species of antelope were photographed and antelope species had the two highest camera-trap capture rates (suni and Harvey's duiker). Aders' duiker, with 10 independent capture events, had a capture rate comparable to that of other small antelope and similar to other rarely seen species such as aardvark (Table 3).

Table 3. Camera-trap capture rates (independent capture events per trap-day x 100) for mammal and bird species recorded from 14 locations in the Arabuko-Sokoke Forest, Kenya. Species listed in descending camera-trap rate order. Number of camera-trap locations in parentheses.

Species	Camera-trap capture rate	Species	Camera-trap captu rate
Suni		Syke's monkey	
Neotragus moschatus	10.70 (7)	Cercopithecus mitis	0.96 (3)
Harvey's duiker		Bushbuck	
Cephalophus harveyi	3.99 (6)	Tragelaphus scripta	0.80 (3)
African civet		Caracal	
Civettictis civetta	2.08 (3)	Caracal caracal	0.48 (3)
Yellow baboon		Crested porcupine	
Papio cynocephalus	1.76 (4)	Hystrix cristata	0.48 (3)
Four-toed sengi		Blue duiker	
Petrodromus tetradactylus	1.76 (1)	Cephalophus monticola	0.32 (1)
Aders' duiker		Honey badger	
Cephalophus adersi	1.44 (1)	Mellivora capensis	0.32 (1)
Aardvark		African elephant	
Orycteropus afer	1.12 (3)	Loxodonta africana	0.16 (1)
Golden-rumped sengi		Squirrel spp.	
Rhynchocyon chrysopygus	1.12 (1)		0.16 (1)
Kenya crested guineafowl			
Guttera pucherani	1.12 (3)		

Discussion

Data exploration using multi-dimensional scaling highlighted potential relationships between the presence of Aders' duiker and various habitat factors likely to be important to small forest antelope. Duikers were associated with areas of low visibility, typical of Cynometra vegetation, perhaps to avoid detection by predators. Aders' duiker presence and general duiker field sign were associated with the abundance of Aders' duiker food plants indicating that these species may be useful for identifying duiker habitat. However, this must be treated with caution as food plants were identified based on the stomach contents of a single individual. Interestingly, there was no relationship between duiker variables and human disturbance despite the negative effects of subsistence hunting on duiker populations close to villages documented elsewhere (Noss 1999, Nielsen 2006).

However, there was a lack of statistical support for the factors affecting Aders' duiker presence, in part due to the small sample size. There were no significant correlations between Aders' duiker presence and other variables and only one variable, duiker dung piles, was found to be significantly different between plots with and without Aders' duiker sightings (Table 3).

Our results for Aders' duiker highlight the difficulty of studying rare, cryptic species for which there is a lack of data. Using informal sightings to record a species' presence or absence takes no account of sampling effort or differences in observer reliability. In this case, Aders' duiker sightings were rare and may have been biased towards the area around the strict nature reserve, as this is the area most often visited by tourist guides and forest staff. Duiker dung piles and paths are more easily quantified measures of habitat use but are of limited value when they do not differentiate between species.

Camera-trapping provides species-specific records for distribution or ecological studies and has been shown to have a greater detection efficiency than other duiker survey methods (Bowkett et al. 2006). As reported in Anon. (2007), these are probably the first ever photographs of this species in the wild (although Franziska Schrodt obtained a blurred photograph with a handheld camera in 2005). The camera capture rate for Aders' duiker was lower than that of the more frequently encountered small antelope species found in the study area as expected for a rare species. However, the trap-rate was within the overall range of trap rates for antelope species recorded here and in Bowkett et al. (2008). Despite this success, photographs of Aders' duiker were limited to one camera location alongside a vehicle track and so could not be related to habitat variables. Obtaining sufficient camera-trap records to quantify habitat selection for Aders' duiker is likely to require more intensive sampling.

This study suggests several habitat factors of potential importance to the future conservation management of this highly threatened species. More robust data-sets are required to investigate these factors and camera-traps are a potential tool for obtaining these. Following our initial results in the ASF, we plan to use camera-traps to survey coastal forests where Aders' duiker has not been recorded or is believed to be extinct. A reliable sighting from Dodori Forest (c. 250 km north of ASF) was reported in 2004 (Andanje & Wacher 2004) raising the possibility that viable Aders' duiker populations may persist on the mainland outside of the Arabuko-Sokoke Forest.

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INFRARED THERMOGRAPHY - STUDIES OF THE GIRAFFE SKIN

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Abstract

The technique of infrared thermography is a rather new non-invasive diagnostic tool used for distant diagnosis of pregnancies as well as for the localisation of inflammatory processes in zoo and wildlife research. During routine check-ups at Zoo Frankfurt, infrared thermographical observations showed that giraffes emitted heat non-uniformly from their surface. The thermographic pattern correlated with fur colour and was qualitatively independent of the level of incident solar radiation. These observations allowed to test the validity of hypotheses concerning the biological meaning of the fur colour of giraffes for their thermoregulation. Further applications of infrared thermography in zoo and wildlife research are critically discussed.

Keywords

fur pattern, Giraffa camelopardalis rothschildi, non-invasive monitoring, surface temperature, thermoregulation

Introduction

Every object with a temperature above absolute zero emits infrared light as an exponential function of its temperature (Bowling 1967). Homoeothermic endothermic animals emit heat radiation when the environmental temperature is lower than their body temperature. Recording of this infrared radiation can be used to determine the skin temperature - contact free over a distance of several meters.

Thermographic cameras convert this radiation into a digital colour image, which shows the amplitude and spatial distribution of surface temperature. The measuring range can be optimised for the actual measuring situation by means of a reference scale, which is fed into computer software that also corrects the data according to environmental temperature, humidity and object distance.

Here I show thermographic data of the surface temperature of the giraffe Giraffa camelopardalis rothschildi. These data match the results of histological studies of the various colour areas and are discussed with respect to a putative thermoregulatory function of fur colour.

Material and Methods

The experimental animals were specimens of Rothschild Giraffes (Giraffa camelopardalis rothschildi) of Zoo Leipzig. They were observed inside their stable and in the outside pen under various climatic conditions.

Temperature measurements were performed by two different types of thermographic cameras (THV 570 and ThermaCAMTM PM 695 PAL; Flir Systems). A computer program (ThermaCAMTM Reporter 2000 Professional; Flir Systems) was used to adjust the colour scale of

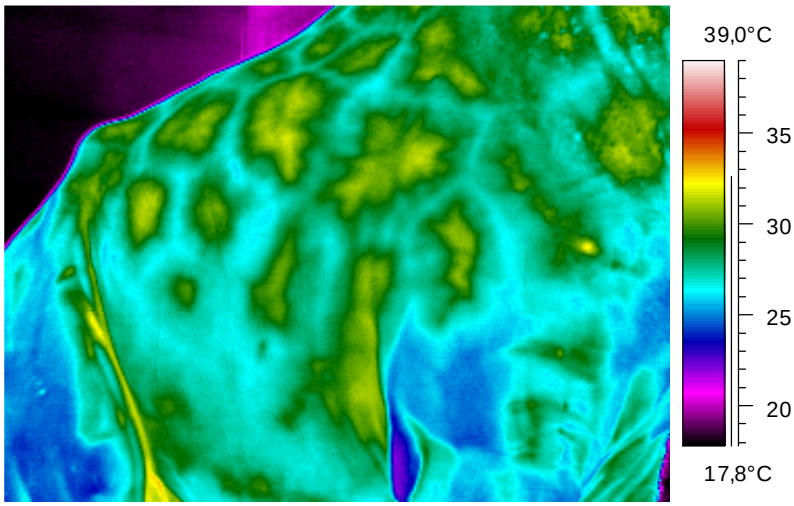

Figure 1 Thermographic image of a giraffe bull Max inside the stable. The pattern of heat emission matches the fur coat colour. (Right side colour scale shows temperature adjusted to actual climatic conditions; the black bar between the colour bar and the scale indicates the chosen temperature range of the recording)

Figure 2 Thermographic image of a giraffe bull Max inside the stable. The pattern of heat emission matches the fur coat colour. (Right side colour scale shows temperature adjusted to actual climatic conditions; the black bar between the colour bar and the scale indicates the chosen temperature range of the recording). The presence of stable bars distorts the measurements.

the thermographic image to the actual situation and also for post processing to recalculate the influence of environmental temperature and humidity as well as variations in object distance, position and its relative size.
Histological studies of skin biopsies followed conventional techniques to differentiate the dermal layers and to determine their thickness.

Results

The histological sections through the black, brown and white areas of the fur coat indicated that the black areas had a significantly lower hair density than white or brown areas. In addition, the thickness of the epidermis underneath areas of black fur (10,4 µm) was on average significantly thinner than underneath areas of white or brown fur.
Thermographic data of the average surface temperature of the different fur areas showed temperature difference of up to 3,7° C between the darkest and lightest areas. The distribution exactly matches the visible colour pattern of the fur and the histological studies of the underlying skin. The feet, lower limbs and the tail have temperatures close to the environmental temperature, while the surface temperature of the giraffe body is more than 10oC (light fur areas) to 15°C (black areas) higher. Additionally, relative heat emission is recorded as highest over the belly.

Heat transmission increases with higher average environmental temperature. In particular, black fur areas showed significantly higher heat emission than white and brown fur areas. White fur areas showed least heat transmission. Black fur-areas have a shorter way of conduction to accomplish heat transmission. The average temperature difference measured between white and black fur areas was 1,1° C inside the stables and 1,6°C in the outside penAn indication of putative false measurement may be recorded is illustrated in Figure 2. This shows the striking effect on the thermographic pattern of bars lying between the camera and both the experimental animal and external heat sources; these bars clearly may modulate the apparent surface temperature. The position of the animal with respect to the lens is relevant, as those body parts closer to the camera appear warmer than those that are more distant.

Discussion

These pilot studies with infrared thermocameras, accompanied by histological analyses, have shown that coat colour fur patterns in giraffes may play a role in thermoregulatory processes. The pattern of surface temperatures match the differences in fur coat colour areas. The fur spots themselves can be understood as thermographic windows; heat transmission by convection takes place more easily from areas of darker fur;, Areas of lighter coloured fur show better insulation.

These results suggest there may be a similar biological influence of the fur patterns in other animals. An example is the striped pattern found in Zebras (Benesch and Hilsberg 2006). These are often hypothesized either as camouflage adaptations to the structure of their environment or for individual pattern recognition. Infrared-thermography offers the opportunity to study thermoregulation of such species under quasi natural conditions, as no direct contact to the skin surface is necessary.

Infrared thermography has also been applied to the diagnosis of inflammations, as the skin surface temperature is locally increased in these areas. The effect of medical treatment and the dynamics of the healing process can be recorded. Other applications of thermography include the monitoring of pregnancy without stress. For each of these applications, knowledge of the “normal” pattern of surface temperature of an animal is essential (Kaspari 2004). The reactivity of the skin temperature

in lower or higher environmental temperature and humidity, especially under extreme conditions, are also likely to help with the design of “furniture” in a pen such that it matches the preferred temperature range.

In summary, these observations on giraffes indicate that the method of infrared thermography can be used, not only as a diagnostic tool in veterinarian medicine, but as a method for research in wild animal physiology.

Acknowledgments

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NON-INVASIVE MONITORING OF HORMONES AS A RESEARCH TOOL IN ZOO AND WILD ANIMALS

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Introduction

Steroid hormones (sex steroids and glucocorticoids) are of great scientific interest because they are largely involved in virtually all body functions including the regulation of reproduction, development, stress reactions and the expression of behavior. Thus, understanding the factors that influence endocrine function is the key to maximize reproductive success and animal welfare.

Hormones are the basis of reproduction. Gonadotropin releasing hormone (GnRH) is the hormone on top of the hierarchy controlling reproduction. It is produced by the hypothalamus, a gland located at the base of the brain. GnRH controls reproduction through two subworkers: follicle stimulating hormone (FSH) and luteinizing hormone (LH). These two hormones, known as gonadotropins, are produced by the pituitary gland. When levels of GnRH rise in blood, the pituitary responds by increasing its release of FSH and LH, which are then free to act in their ultimate targets, the ovary and the testes. LH and FSH promote ovulation and stimulate secretion of the sex hormones estradiol (an estrogen) and progesterone (a gestagen) from the ovaries and testosterone (an androgen) from the testes. While many hormones interact during reproduction, estradiol, progesterone and testosterone are most well-known, all belonging to the class of steroid hormones.

The hypothalamic-pituitary-adrenocortical (HPA) axis plays a central role during stress. Stressors stimulate the hypothalamic release of corticotropin-releasing hormone (CRH) inducing the secretion of ACTH from the pituitary and as a consequence that of corticosteroids (corticosterone and/or cortisol) from the adrenal cortex (Mormede et al., 2007). It is generally thought that, if severe enough, chronically high levels of glucocorticoids may decrease fitness, for instance, by causing immunosuppression and atrophy of tissues (Munck et al., 1984), and may lead to the suppression of reproductive performance (for review see Wingfield and Sapolski, 2003).

Steroid hormone measurements (usually in blood samples collected over periods of several days) provide essential information to understand sequence and duration of the reproductive cycle, time of ovulation in relation to mating, and the course of hormone secretion during pregnancy. The quantification of cortisol in blood samples provides valuable information about an animal's adrenocortical activity and has been used as a parameter of stress.

Traditionally, hormones are measured in blood samples that provide a measure of circulating plasma steroid concentrations. The main drawback of blood collection is the necessity of catching and handling animals, which is not always feasible or desired. Stress caused by catching and restraining before sampling, together with venipuncture might activate the HPA axis and thus confound the effects of the experimental treatment. Repeated blood sampling might also induce anticipatory stress reactions of the animals involved. In pigs cortisol concentration consistently increased during the time–course and the increase was seen after 5 min of stress (Rosochacki et al., 2000). In chipmunks glucocorticoids in blood increased above basal level during the 30 min after capture and initial handling stress (Kenagy et al., 2000), whereas in captive wild dogs cortisol concentrations increased from 10 – 20 min after darting (De Villiers, 1995). In addition, plasma

hormone levels reflect the hormonal status of an individual at a certain point in time. If the pattern of hormone release has a strong pulsatory component, a single blood sample may not be sufficient to determine the endocrine status of an individual. (Mormede et al., 2007)

Therefore invasively collected blood samples are inappropriate when long term studies based on repeated samples are needed to monitor reproductive activity and stress events in zoo animals with the exception of animals that were adapted of being sampled.

Natural steroid hormones have very short half-lives (few minutes). They are extensively metabolized mainly in the liver and are excreted via the bile into the gut and via the kidney into the urine (Palme et al., 2005). These metabolites are measured by non-invasive methods to assess an animal's endocrine status. Unlike blood sampling, the collection of excreta does not require special skills. Typically, samples can be collected with great ease and multiple samples can be obtained from one individual, even in the field. Sampling usually does not conflict with animal welfare considerations, and does not require special permits. In addition, steroid metabolites measured in feces and urine represent pooled fractions of excreted hormones, providing an integrated measure of steroid levels over a longer period of time.

Urine and feces contain steroids that have been eliminated from the circulation. Therefore, steroid amounts analysed in feces reflect an event a certain time ago. The species variation in the delay of fecal hormone excretion must be considered, resulting in time lags between 1 and more than 24 hrs. Knowledge of those delay times of fecal excretion is crucial for the experimental setup, because these times will determine sampling intervals, primarily if acute stress events should be monitored (Palme et al., 2005).

Due to bacterial enzymes, fecal steroids may undergo further metabolism and thus are not stable. Therefore the best option is to collect a sample shortly after defecation and to freeze immediately (Khan et al., 2002). Especially in the field, where direct freezing is difficult an alternative preservation method are the elimination of fecal moisture by drying (Pettitt et al., 2007) or the addition of ethanol (Terio et al., 2002).

Biological validation of non-invasive hormone measurements

Because metabolism and excretion of steroids differ significantly between species (Palme et al., 2005; Schwarzenberger, 2007) these non-invasive methods must be validated for each species before application (Palme, 2005). In this regard one of the most important aspects is the physiological validation of the technique (Touma and Palme, 2005). This can be done by pharmacological induction of physiological changes in circulating hormone levels and evaluation whether these changes are reflected in measured concentrations of fecal steroid metabolites afterwards. In this respect, the most widely used approach is the stimulation of adrenocortical activity with ACTH (ACTH challenge test) and the testosterone release with GnRH. Ideally, fecal samples have to be collected frequently a certain time before and after the challenge. To study female reproduction samples collected throughout a cycle or a pregnancy are necessary to establish an optimal assay for monitoring follicular and luteal activities. One experiment is shown in figure 1 demonstrating the pharmacological stimulation of adrenal activity of a roe deer. The administration of ACTH to one animal clearly increased fecal glucocorticoid metabolites. Following the ACTH injection, fecal metabolite concentrations peaked after 10 hrs reflecting an approximately 5 fold

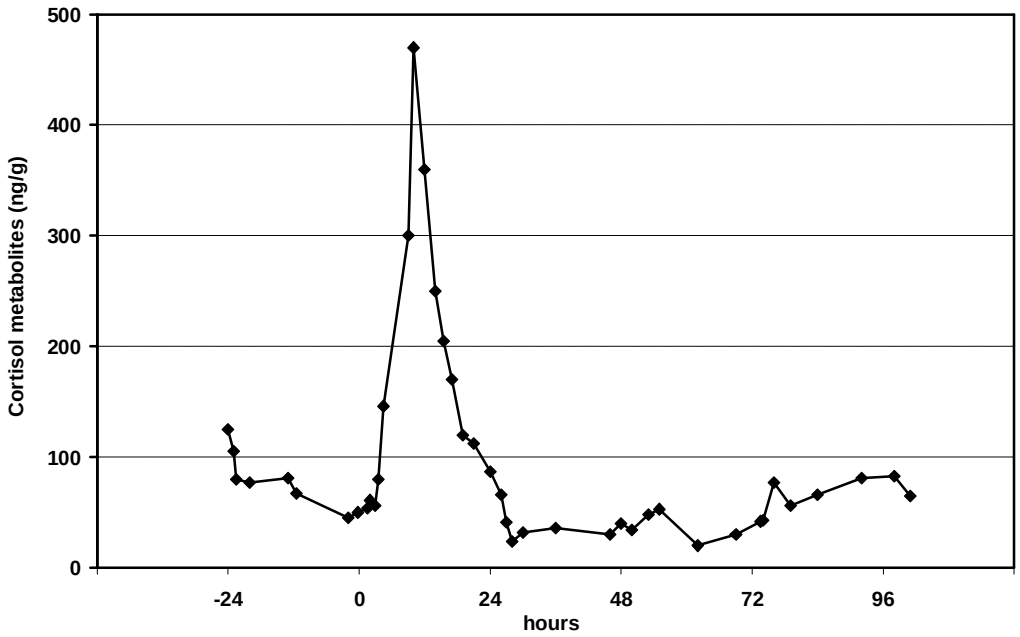

Figure 1. Stimulation of adrenal activity after administration of ACTH to one male roe deer. Fecal samples were collected for five days from all defecations starting on the day before treatment. The samples were analysed for cortisol metabolites (from Dehnhard et al., 2001).

increase and returned below basal levels within 24 hrs after treatment. This result suggests, that the measurement of fecal glucocorticoid metabolites clearly reflect adrenal activity and is appropriate to monitor stressful events. Due to considerable individual variations, both in basal and ACTH induced levels of fecal metabolites several individuals (of both sexes) should be used for physiological validation. In addition, other factors such as age and season might be interacting covariates (Touma and Palme, 2005). In a follow-up experiment capture, veterinary treatment and transportation of animals were used as experimental stress resulting in a 7.5fold increase of fecal metabolites 12 hrs after the onset of the stressful situation (figure 2).

Characterisation of hormone metabolites

Usually the chemical identity of the fecal and urinary metabolites is unknown but they might be characterized and probably identified with the help of a radiometabolism experiment. After injection of 3H (tritiated) labelled hormones, the excreted metabolites of a particular steroid can be analysed by high-performance liquid chromatography (HPLC). Figure 3 shows the distribution of radiolabelled metabolites separated on a non-polar (reversed phase) column based on differences in their polarity. The extract of male lynx feces is composed of several radiolabeled metabolites.

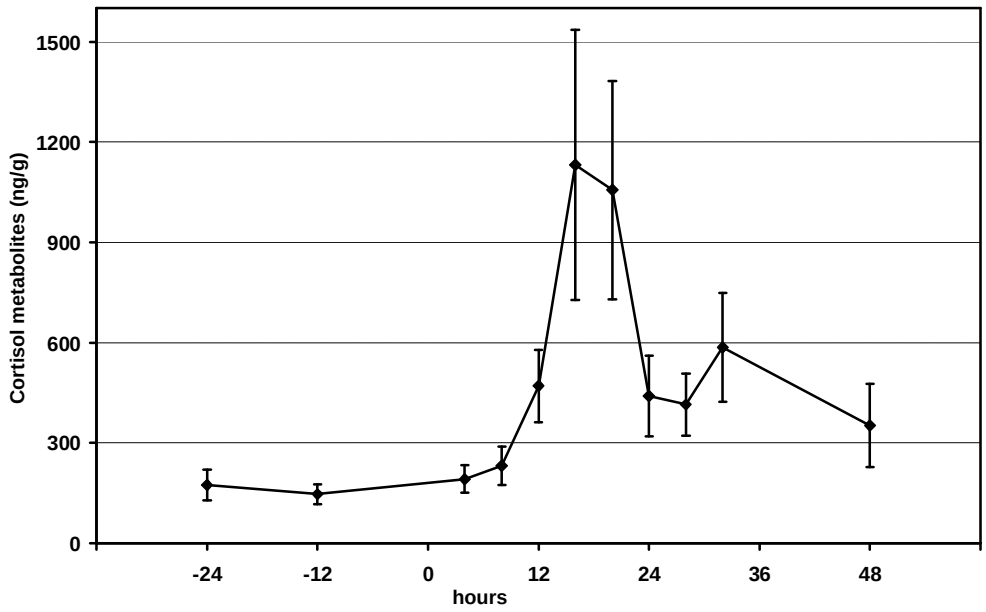

Figure 2. Fecal cortisol metabolite concentrations (mean + SEM) of four roe deers after 15 minutes of moving the animals out of their enclosure, individual manual restraint, loading into transportation crates (two animals per crate) which were fixed onto a pickup truck (20 minutes), followed by an 2-hour-drive with two interruptions of 15 minutes. Ten minutes after re-arrival at the station, the animals were set free into their enclosure. As the time and frequency of defecation differed, all values were allocated into time frames of 4 hrs (from Dehnhard et al., 2001).

The majority was detected in fractions 14–18, 21–23, 29–32, 45–48 and 61–62. Two minor radioactive peaks co-eluted with testosterone and dihydrotestosterone (DHT) at fraction 36 and 45, respectively, suggesting that the circulating hormone itself is no longer present at all or present only in minor amounts. What is measured in feces are metabolites of the original hormone referred as hormone metabolites (Palme et al., 2005; Schwarzenberger, 2007). Because antibodies for these metabolites are usually not available, researchers use commercial or in-house made antibodies for the original hormone or similar compounds, hoping that these antibodies cross-react with one or several of the hormone metabolites. To further characterize fecal metabolites, HPLC immunograms should be performed. After chromatographic separation, the presence of immunoreactive metabolites in collected fractions is determined with different assays demonstrating that metabolites of the hormone are indeed detected and which metabolites are measured by the applied immunoassays.

To achieve final identification of metabolites, the introduction of other, highly sophisticated analytical techniques, like mass spectrometric characterization using liquid chromatography mass spectrometry (LCMS) and a are necessary (Hauser et al., 2008). All together such validation steps are essential until an assay can be used for routine monitoring of gonadal and adrenal steroids in

captive and free-ranging animal populations to investigate e.g. hormone–behavior relationships, endocrine regulation of reproduction, and questions regarding animal welfare

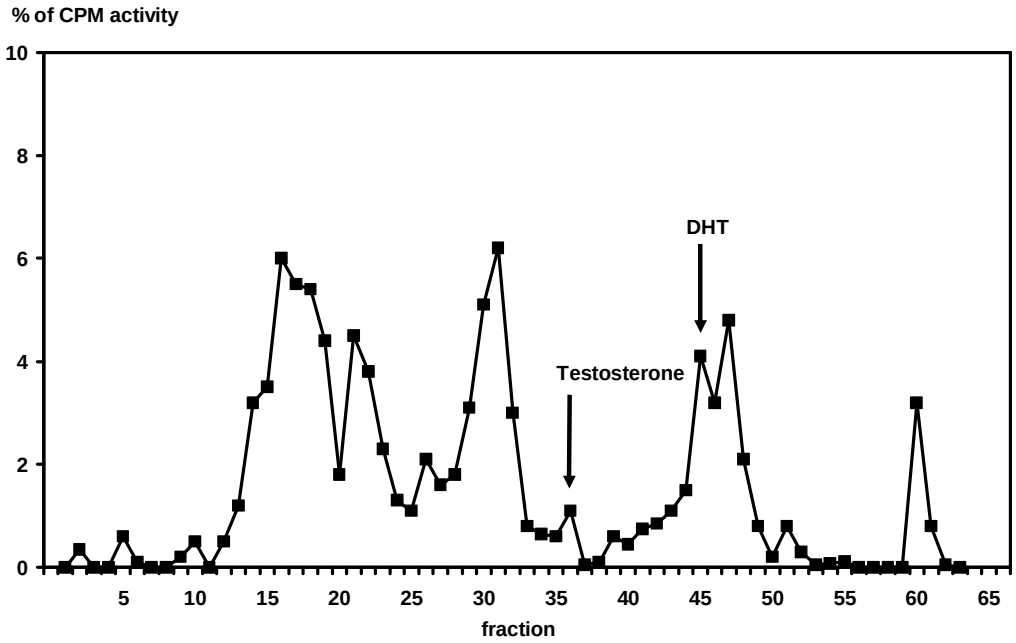

Figure 3. Testosterone radiometabolism study in a male Eurasian lynx. A fecal extract was subjected to HPLC separation and radioactivity (CPM) was measured in each fraction. Arrows indicate the elution positions of testosterone and dihydrotestosterone (DHT).
From Jewgenow et al., 2006

Monitoring luteal activity in females

Attempts to facilitate or improve reproductive efficiency in the management of rare animals often fail, in part, because necessary basic reproductive/endocrine information is unavailable. Therefore the method of choice is to use a non-invasive method for the longitudinal monitoring of ovarian activity in non-tractable animal species. An example is shown in figure 4. A female Spectacled bear was continuously mated by the male. This contrasts the reported breeding season of April to June in South America. To analyse gonadal steroids urinary and fecal samples were collected throughout the entire year. Urine analysis in the Spectacled bear was ineffective in demonstrating estrogenic activity in this female. The estrogen concentrations fluctuated between 0.1 and 0.8 ng/mL (figure 4). There was not any estrogen peak related to the observed mating. An ultrasound examination on October 16 confirmed an existing pregnancy and the date of implantation was estimated to be three weeks before (based on experiences from Brown bears). On November 25, within the predicted period of parturition, urine contaminated with blood was collected, however, no bear cub was detected suggesting that parturition and fetophagia had occurred. Fecal gestagen analyses failed to monitor pregnancy in this species. Alternatively urine

samples were subjected to gestagen analyses. On October 20, a distinct pregnancy related progesterone elevation was observed followed by a drop to basal levels just prior to parturition.

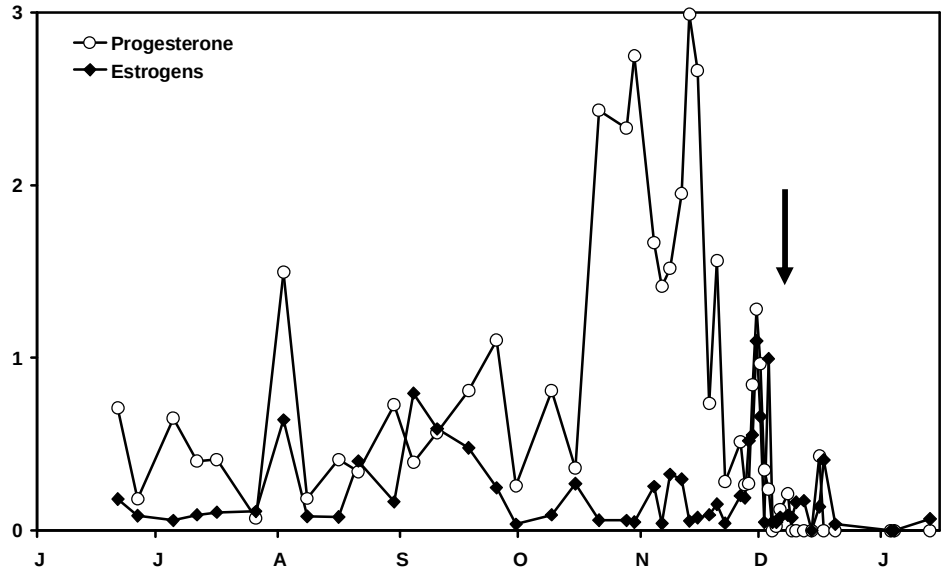

Figure 4. Urinary estrogen and progesterone metabolite profiles in a female Spectacled bear throughout 2002. The arrow indicates the day of assumed parturition indicated by blood contaminated urine (from Dehnhard et. al., 2006)

This example shows that non-invasive monitoring of urinary gestagens is an appropriate tool to monitor pregnancy in the Spectacled bear. Contrary to urine, fecal gestagen analyses failed to monitor luteal activity possibly due to the lack of an adequate antibody.

Monitoring testicular activity in males

Similar non-invasive approaches are needed for males to provide comparable informations regarding how various factors influence testicular function. Such knowledge is essential for planning assisted reproductive techniques. Because steroid hormone metabolite excretion occurs mainly via feces in felids (Brown et al., 1994), we developed a method for measuring fecal testosterone metabolites in the Eurasian lynx.

Figure 5 presents means + SEM determined with a testosterone assay in four captive Eurasian males. In all four males, similar pattern of fecal testosterone metabolite concentration was obtained with highest concentrations during the breeding season (March–April) and low values in January/February were found. However, only in male one this difference was significant ($p<0.05$).

Closing remarks

In summary, measuring steroid hormone metabolites in urinary and fecal samples has become a powerful non-invasive tool that provides important information about an animal's endocrine status

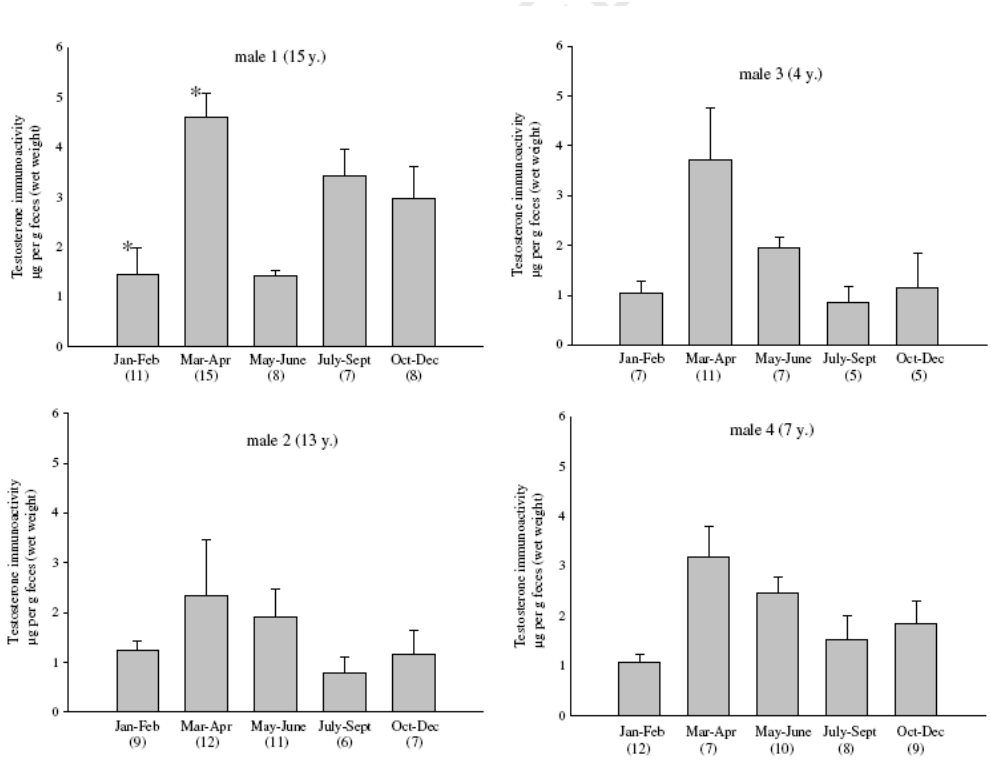

Figure 5. Fecal testosterone metabolites determined in four captive Eurasian males. Means + SEM are presented bimonthly for the period before and after breeding season (March–April) and quarterly for the rest of the year. From Jewgenow et al., 2006

The monitoring of hormonal activity by means of hormone metabolite analysis offers several advantages and has been successfully applied to various species of mammals and birds. Because the sampling is completely non-invasive, the animal's behavior and endocrine state as well as physiological functions, like the circadian hormone rhythms, are not affected by stress responses associated with capture, restraint, or blood sampling. The non-invasiveness and superficial ease of the method are certainly appealing to many investigators. This is reflected in the increasing amount of publications that are based on non-invasive hormone measurements. Emphasis must be placed on the establishment and analytical validation of such non-invasive methods. It is mandatory to evaluate the immunoassays used for urinary and fecal hormone metabolites for each species under investigation. A physiological and biological validation is absolutely essential. Without such validations, measurements of excreted hormone patterns can mean anything and, in the worst case, may have nothing to do with the hormone in question.

Non-invasive techniques have tremendous potential for diverse investigations in laboratory, farm, zoo, and wild animals. In the near future established routine methods might be transferred to scientifically ambitious zoos equipped with their own lab as a basis to monitor reproductive and adrenal activity to support reproductive management and evaluation of animal welfare.

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SPECIFIC CHARACTERISTICS IN THE BEHAVIOUR OF THE SUBORDINATED FEMALE OF AN ORANG-UTAN GROUP IN ZOO

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Abstract
Behaviour observations in zoo can contribute to improvements of the welfare of captive animals. An important aspect when working on apes can be the consideration of the individual traits of the animals. For the present study the orang-utan group at Frankfurt Zoo (1,3 adults and 2,1 youngsters) was observed for three months having regard to chronobiological aspects. At the time of this study, the group comprised seven orang-utans: one adult male, three females and three infants. The subordinated female was hand reared. Her activity pattern shows specific characteristics in comparison to the other adults. She apparently avoids her conspecifics by means of shifting activity phases to evening hours. Even during sleeping time she keeps off the others, which are cuddled in their 'nest' in a big box aloft: she builds her night nest on the ground. Furthermore she alone aims at direct visitor contact in order to play or begging for fodder. The most outstanding remarkable individual behaviour is intensive regurgitation and reingestion. Especially with evenings' feed she sometimes keeps herself busy up to one and a half hour. The results illustrate among other things, that one should take into account the individual traits of the animals when considering environmental enrichment.

Key words: Orang-utan, Subordinated female, Hand rearing, Individual differences, Regurgitation, Reingestion, Begging, phase delay, Sleeping behaviour

Introduction
The keeping of primates is challenging, as there are big differences in the mode of life between free-living and captive animals. Maybe the biggest discrepancy is that apes spend bulk time searching for food and ingestion; orang-utans spend approximately 50 – 60 % of their daily activity foraging (Van Noordwijk and van Schaik 2005, Galdikas 1988). In captivity however, most part of the day they have „leisure time". Besides, orang-utans in the wild live more or less as loners, rather than in closely related groups; this somehow indifferent status is called „semisolitary" (Galdikas 1995; Utami et al. 1997) or can be described as a „loose community" (Singleton and van Schaik 2002). The contrast of the different situations regarding life of wild and captive orang-utans, arises a couple of questions. During a study in Frankfurt Zoo two of these questions shall be discussed:

- Are there individual differences in the behaviour or in the activity pattern of the orang-utans?
- Does the close cohabiting of orang-utans affect the activity-pattern of individual animals? (with reference to Mackinnon 1974). In the following I want to present some results of the study with special focus on the subordinated female and the special differences to the other adult orang-utans in the group. Although the sample size is small, findings were obtained. Every orang-utan (at least these in Frankfurt Zoo) shows individual characteristics in their behaviour. Nevertheless more future observations in other zoos should be done to substantiate these findings and to have more comparisons.

Studied animals and methods

At the time of this study, the group comprised seven orang-utans: the adult male Charly and the dominant female Djambi (both born in the wild in the late 1950ties), the female Rosa (born in 1989) with two youngsters (five years old and the baby, who was younger than a half year at the time of the observation) and the subordinated female Sirih (born in 1992) with a two year old infant. This subordinated female had to be raised by a human family, where she lived for several months (Schmidt and Schmidt 1996).

The enclosure was made up of six rooms, three indoors and three outdoors, equipped with poles, chains, ropes, tree trunks, resting shelves, hammocks, water tanks and a seesaw. In one of the indoor rooms the animals were able to retreat into a large box aloft. With few exceptions the orang-utans were able to choose their whereabouts freely. Actually a modern enclosure is being build up. Direct, protected contact to the keepers took place during the feeding of mash in the morning and fruit juice in the afternoon as well as during 'handling training' (Golinowska et al. 2002).

Data were collected by direct observation over periods of about four hours each. For every individual its place, activity and social contact to other orang-utans or to humans were recorded every full minute . If an animal was not visible, this was noted for that minute. Also keeper's activity, weather, number of visitors and other events were recorded. All observations have been made between 07.00h (begin of keeper's work) and 22.00h. To avoid disturbing the animals at night, no nocturnal observations have been conducted. Therefore the emphasis of this study was placed on the daytime. This can also be justified with the diurnal activity of orang-utans (Mackinnon 1974; Sommer and Ammann 1998; Wich et al. 1999) and the aims of the study.
Overall thirteen behaviour categories have been recorded; among others: Sleeping or dozing, resting, observing, nest-building, food intake, drinking, and social contact.

A total of 245 hours on 61 days between April and August 2005 have been analysed, consisting of 14707 data sets per individual. To analyse and to recognise time pattern the behaviour is diagrammed in a so called chronoethogramme, which has been calculated in Clocklab (Actimetrics, USA). In a chronoethogramme you can recognise not only what the animal had done, you can also recognise at what time of the day it did. In the ideal case you have 24-hours-observations. But by direct observation, this is impossible to reach.

Results and Discussion

In many aspects the subordinated female Sirih showed special characteristics. This is obviously connected with the fact, that she was orphaned and had to be raised by a human family over a period of several months (see above). Thus she acquired not only a special relation and a special approach to humans, but also it could have an effect on her relation to her conspecifics (hierarchy, only marginal resistance in conflicts etc.) - see Kaumanns et al. (2004) for suggestions.

Phase delay in evening hours

None of the observed orang-utans slept as frequently between 7.00h and 18.00h as Sirih (16.4 % compared to approx. 10 %; fig. 1). She regularly rested in the midday hours (between 11.00h and 14.00h; fig. 2). But in the evening hours (between 18.00h and 21.00h) she was considerably longer active in the enclosure (mostly in the outdoor enclosure) than the other adults. She was engaged with manipulation of things, social contact with the infants, or begging visitors for fodder (see below). The other adults climbed into the large box aloft around 18.00h. They left it only occasionally to fetch some food or nesting material. Even though they did not sleep immediately, they retreated in the evening hours. The resting behaviour in the evening of the male and the two

dominant females resembles the one of free-living orang-utans ("last recorded nesting time was 18.40h (Borneo)";Mackinnon 1974: 46). In contrast, the subordinated female is over two hours longer active together with her daughter and often also with the five year old youngster (mostly in the outdoor enclosure). Her social rank permits the conclusion, that she avoids the other females in temporal manner, because she cannot avoid them in spatial manner. The two dominant females have "free run of the cage" (Nadler and Tilford 1977: 303), Sirih often has to sidestep them. Conflict situations are additionally stressful for her. But in evening hours Sirih has the possibility to do what she likes - undisturbed and stress-free. In return for this she slept more frequently in the midday hours than her conspecifics. Her resting time corresponded again with the daily resting time of wild orang-utans (Mackinnon 1974).

In the enclosure there was a large box aloft with limited inside-visibility for visitors. For this reason the stay in this box can be interpreted as a retreat, even though the animals do other things than resting or sleeping. In comparison to the other adult orang-utans the subordinated female Sirih spent much less time hidden in that box (fig. 3); only for short periods in the mornings she was to be seen in the box after the others had left (fig.4). The male and the two dominant females spent the night in the big box aloft, but Sirih built her night nest on the floor, where she slept together with her daughter. The male Charly mostly built his night nest in one of the entries of the box, so he could be observed. That is why he was less frequently "hidden in the box" than was the case with the two dominant females (fig. 3).

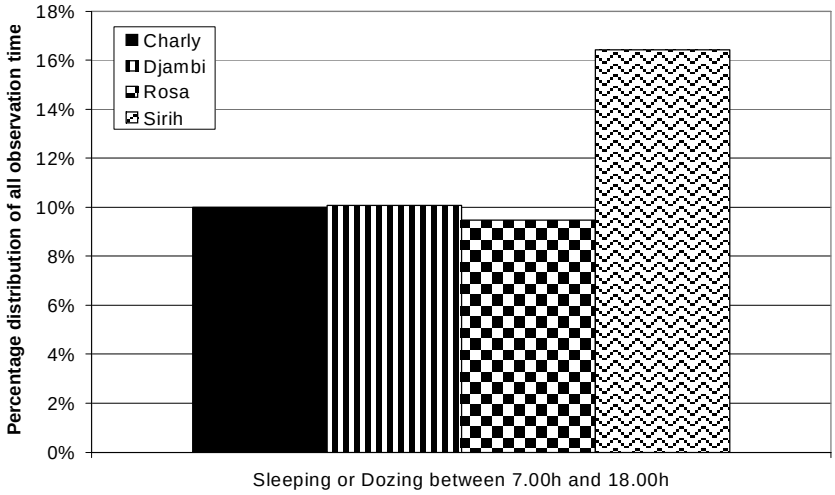

Figure 1. Sleeping behaviour at daytime. During the day the subordinated female Sirih slept at most.

One simple explanation could be that the box was too small for more than three adult orang-utans. Another explanation is that the box is seized by the dominant male and females, so that there is no more room for the subordinated female Sirih. In regard to the sleeping or resting place she had to avoid in spatial manner. The second explanation is supported by the fact that it could never be noticed that Sirih retreated into the box for resting behaviour

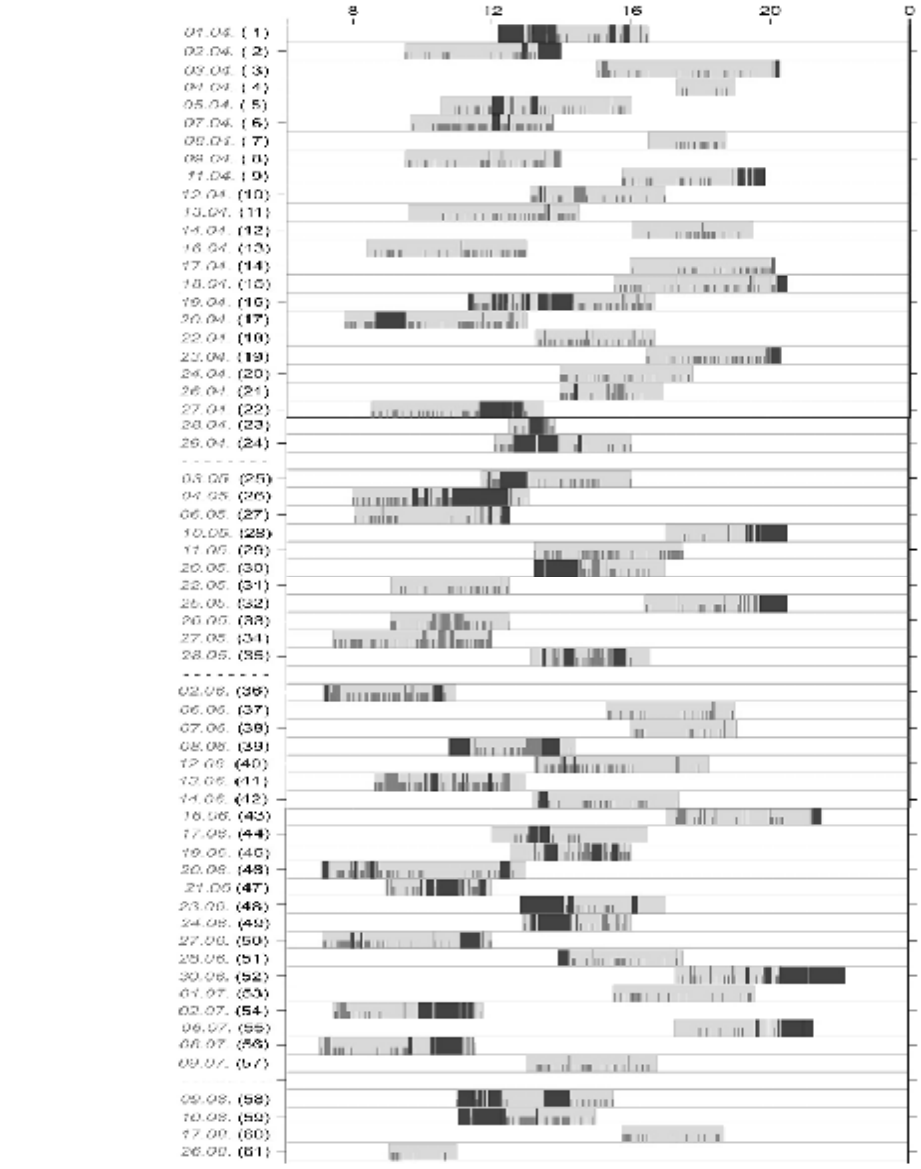

Figure 2 - Inactive behaviour of the subordinated female Sirih. Discontinuous chronoethogramme (single plot); light grey back: observation time; black: sleeping or dozing; dark grey high: resting; dark grey low: sitting and looking. Sirih regularly slept in the midday hours. In the evenings she began to sleep between 19.30 and 21.00

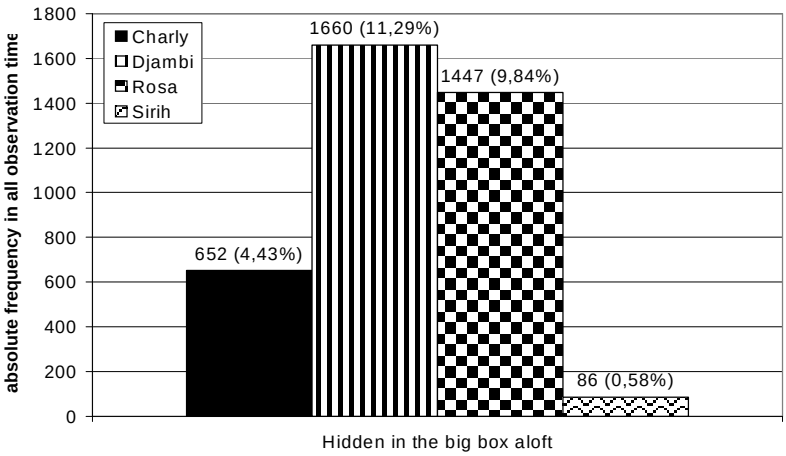

Figure 3. Hidden in the big box aloft. Only those minutes are illustrated, in which the animals were completely hidden and observation was impossible. The subordinated female Sirih was only a few times hidden in the box. The male Charly built his night nest in the entrance of the box, so he was not so often hidden as the two dominant females.

Contacting visitors and begging for fodder

The subordinated female is the only one, who played with the visitors (making grimaces, teasing visitors with water etc.). She furthermore is the only one, who aimed at direct contact to visitors in order to quite succesfully begging for fodder (fig. 5). By throwing little sticks to visitors she caught their attention and initiated an interaction with them. Cook and Hosey (1995) described similar behaviour of chimpanzees. Sirih succeeded in sometimes being "rewarded" by getting back not only the sticks, but even something to eat. Thus she achieved to get among other things prezzels, tulips, a cap, once some scotch, icecream and several packages of buiscuits. Mainly, this behaviour was observed in the evening hours, when a lot of visitors still were in the zoo, and sometimes in afternoon hours just before feeding time. Living in close contact to a human family in her infancy (Schmidt and Schmidt 1996), she very likely learned to use certain gestures for communication with humans. Being a young orang-utan child, this time in the human family must have been formative for her (Kaumanns et al. 2004), which is not meant in a way, she had necessarily learnt begging; but because of her relation to humans she easily can be in contact to visitors because she learnt how to. Besides the opportunity to interact with the visitors could even be enriching (Cook and Hosey 1995). Regarding to Cook and Hosey (1995) begging is particularly used "by subordinated chimpanzees and appears to be a food-soliciting gesture". This seems to apply to Sirih.

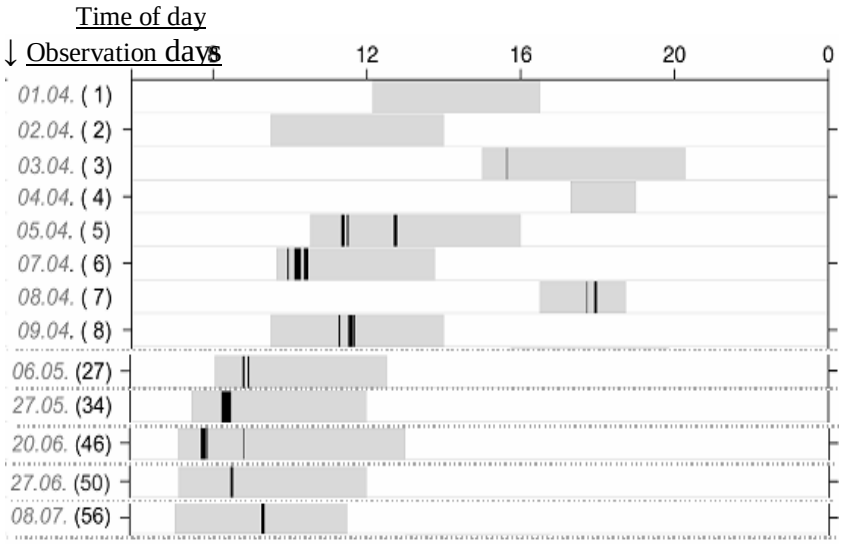

Figure 4. Hidden in the box - the subordinated female Sirih. Discontinuous chronoethogramme (single plot); *light grey*: observation time; *grey dotted lines*: marking non-shown observation days; *black*: hidden in the box. Only on some days - and then only in the morning hours - Sirih was inside the box. She never slept there.

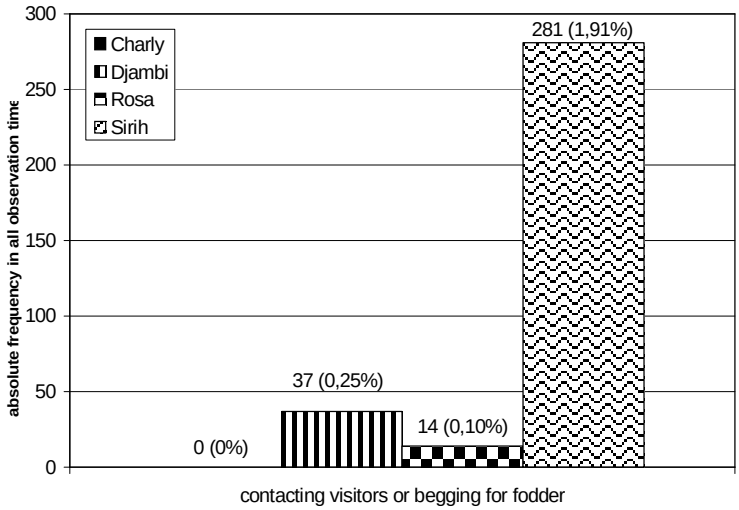

Figure 5. Contacting visitors or begging for fodder. The male Charly was never seen by contacting visitors or begging. The subordinated female Sirih showed begging behaviour most of all.

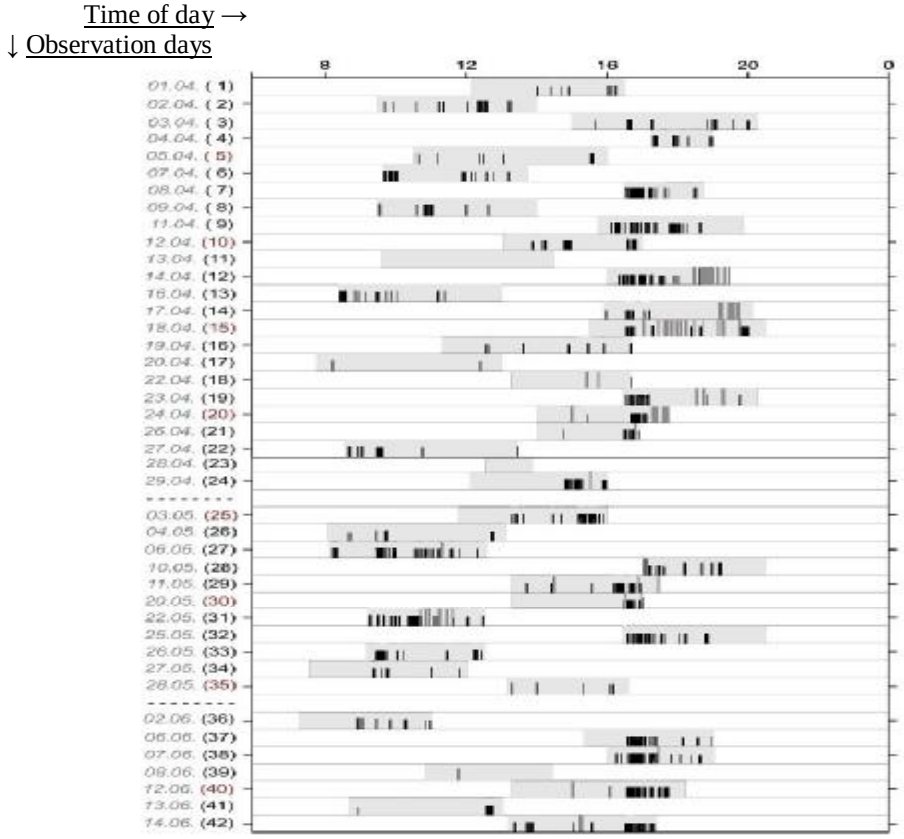

Figure 6. R/R-behaviour of the subordinated female Sirih. Discontinuous chronoethogramme (single plot); *light grey*: observation time; *half high black*: feeding; *high grey*: regurgitation and reingestion. In the last 20 observation days R/R-behaviour was not observed, so these days are not illustrated. On some days R/R-behaviour was recorded in the evening hours after feeding, e.g. observation day 12, 14 and 15.

Regurgitation and Reingestion (R/R)

A further distinctive feature of Sirih is the regurgitation and reingestion of food. On some days R/R-behaviour was recorded in the evening hours after feeding (fig. 6), mostly being an intensive activity. Remarkably, it sometimes took more than an hour from feeding time to the beginning of R/R-behaviour. At this time the keepers had not been in the houses anymore. Therefore they were not aware of that phenomenon.No other orang-utan showed this behaviour in a "passionate" intensity like Sirih (all in all 137 minutes). Mostly she lied down on her waist making a circle with her arms and regurgitated in the middle. Regurgitation is accompanied by her chewing wood wool. Her daughter Jahe and the youngster Galdikas often begged for the regurgitated food and for the chewed wood wool. It cannot be excluded, that the other females also regurgitated; but if they did, it was very inconspicuous.

For the R/R-behaviour there are several explanations possible. One is that R/R-behaviour is an interesting activity. The following observation gives a good example: Once (fig. 6, Observation day 12) there was a container in the enclosure, in which she regurgitated. She swung the regurgitated food, soaked wood wool in it and so on. Than she reingested and regurgitated again. This activity lasted for about an hour. Her daughter was engaged too and fed the regurgitated food, too. Especially with this container it seemed to be an interesting activity. Sirih gave the impression of elevated concentration: she devised again and again a new possibility to get the regurgitated food out of the container.

On other recording days (fig. 6, Observation day 14, 15, 20, 29) there seemed to be a „relative lack of food". At the beginning of observation time at noon the vegetables had been eaten up, which was unusual for this time of the day; the evening food (fruits) were eaten up in relatively short time (once in 20 minutes). During those evenings Sirih showed R/R-behaviour. As the subordinated female she had to suffer strongest of "relative lack of food". Perhaps she ate too fast, swallowing bits too big.

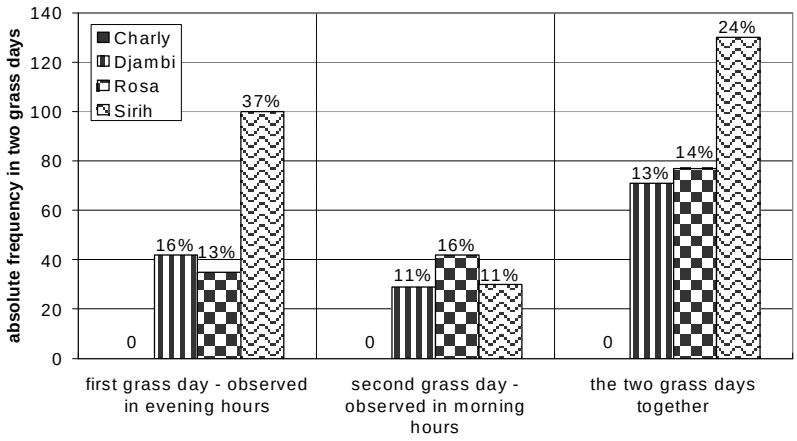

Figure 7. Special food day - feeding grass. Grass feeding was in addition to normal feeding. First of all Sirih was interested in grass respectively in the grass seeds. The other females fed less as Sirih, the male had never been observed in contact with the grass.

Considering that free-living orang-utans spend 50 – 60 % of their time with food intake (Van Noordwijk and van Schaik 2005; Galdikas 1988), the R/R could be a possibility to elongate the ingestion and a way of keeping oneself busy (Maple 1980). Therefore, enrichment and increased activity regarding food could reduce this behaviour (Zizzo et al. 2006). Proposals for food enrichment have been elaborated by Golinowska et al. (2000). On days with special food (like a huge pile of grass) R/R-behaviour have not been observed. But it should be noted that not every animal responds in the same intensity to a certain enrichment (fig. 7). First of all Sirih was interested in grass respectively in the grass seeds. The other females fed less than Sirih, the male had never been observed in contact with the grass (fig. 7).

Conclusions

The subordinated female showed a lot of special characteristics in her behaviour compared to her conspecifics. Her activity pattern has a phase delay in the evening hours to avoid the dominant females. She begs visitors for fodder; an intensive R/R-behaviour was observed. The reasons could be her raising up not by her mother and conspecifics and her being the subordinated female, what means stress, especially in cramped environment.

The behaviour of the adult orang-utans shows how important possibilities of retreat are for the animals. In addition the behaviour of the subordinated female indicates, that there should be several possibilities of retreat in one enclosure, which can be used independently. The individual characteristics of the animals should be considered by changes in feeding and feeding enrichment, because not every individual reacts in the same way to feeding enrichment. Following studies in other zoos could substantiate these findings.

One of the limitations of these study is the small simple size. One further limitation certainly is the irregular distribution of the observation time: the most observations happened between 10:00 a.m. and 17:00 p.m., whereas the early morning hours and the late evening hours were underrepresented.

Acknowledgements

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THE MORPHOLOGY OF THE TONGUE OF THE GIRAFFE (Giraffa camelopardalis)

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Abstract

The morphology of the tongue in an adult giraffe and a fetus in the last third of gestation was observed macroscopically. The tongues had a strongly elongated body of the tongue with a triangular sharpened apex. A distinct part of the tongue in the posterior part of the tongue was the lingual prominence.

On the dorsal surface of the tongue of the adult animal and the fetus two types of lingual gustatory papillae were well-distinguished: fungiform papillae and vallate papillae. Filiform and conical papillae were observed among mechanical papillae on the surface of the tongues.

Fungiform papillae formed accumulations on the apex of the tongue, but on the anterior and middle part of the body of the tongue they were uniformly scattered among the filiform papillae. The conical papillae were distributed on the anterior part and lateral surfaces of the lingual prominence. In the adult animal they had a well-developed keratinized processes, whereas in the fetus these processes were small or absent. On the posterolateral sides of the lingual prominence 8-10 vallate papillae were present. The root of the tongue in the giraffe was smooth, without lingual papillae.

The morphology of the tongue and the arrangement of lingual papillae on the dorsal surface of the tongue in the giraffe resemble morphological features observed in ruminants. The shape of the tongue and the distribution of lingual papillae in the giraffe fetus were similar to those in the adult animal.

Keywords: Morphology, Tongue, Lingual papillae, Giraffe

Introduction

As it is reported in the results of studies on herbivorous mammals the morphology of the tongue and the microstructure of the mucosa on the dorsal surface of the tongue is strongly influenced by the type of food and feeding behavior (Iwasaki, 2002).

So far macro- and microscopic observations were made mainly in ruminants, such as cattle, the goat, Barbary sheep, lambs, Indian muntjacs, roe deer, black buck (Antilope cervicapra) and Formosan serow (Steflik et al. 1983, Atoji et al. 1998, Kumar et al. 1998, Emura et al. 1999, Emura et al. 2000, Tadajlli and Pazhoomand 2004, Zheng and Kobayashi 2006, Jackowiak et al. 2007).

In these animals the special part of the tongue, which plays an important function in the mastication of food is the lingual prominence, also called the lingual torus. These studies focused on the microstructure of mechanical filiform and conical or lentiform papillae, which can have a thick layer of well-keratinized epithelium or special types of keratinized processes. The object of our research was a herbivorous giraffe. Observations of feeding of these animals show a great mobility of the tongue, including the ability to protrude the tongue or manipulate it while gathering plant food, e.g. picking leaves.

The aim of our study was to describe the morphology of the tongue in the giraffe and characterize the distribution and types of lingual papillae on the surface of the tongue.

Material and methods

The tongues of an adult 8–year-old giraffe and a fetus from last third of gestation obtained from the Zoological Garden in Poznań were used in the study. The dissected tongues were cleaned in saline, fixed in 10% neutral formalin, measured and documented with a digital camera.

Results and Discussion

The tongues of the adult giraffe and its fetus had an elongated body with a pointed, pigmented apex. The length of the pigmented area measured from the apex of the tongue in the giraffe fetus was about 5 cm and in the adult animal it was ca. 15 cm (Fig.1,2)

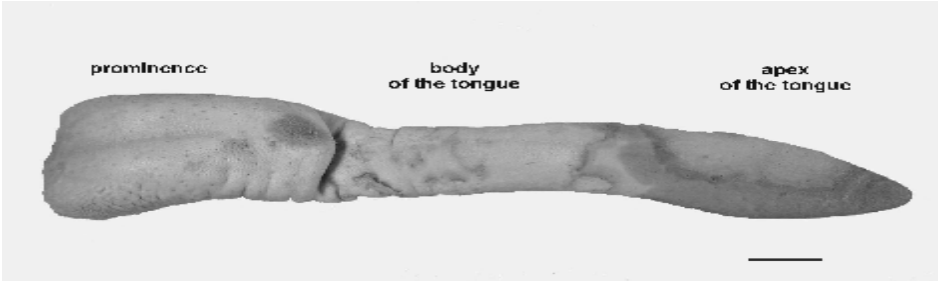

Figure 1. The dorsal surface of the tongue of the adult giraffe. Bar = 4 cm.

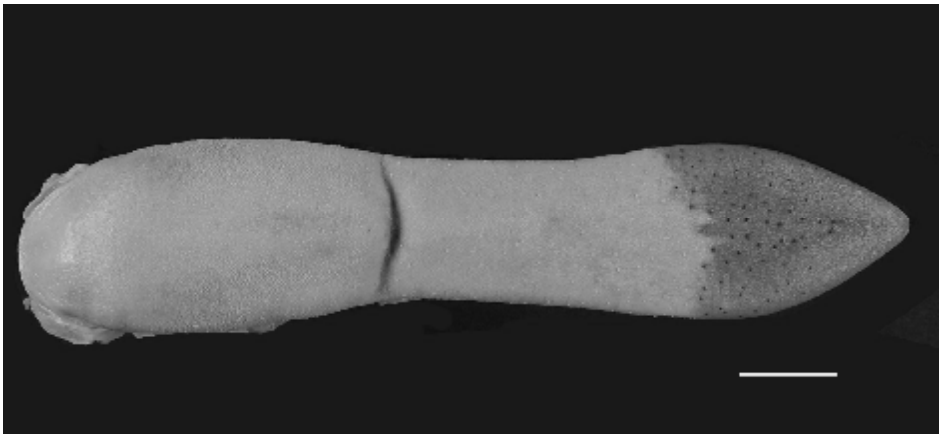

Figure 2. The dorsal surface of the tongue of the giraffe fetus in the last trimester of prenatal development. Bar = 2 cm.

The total length of the tongue of the adult giraffe was approx. 43 cm, while the total length of the tongue of the fetus was approx. 17 cm. The width of the tongue in the adult giraffe measured at the apex and the body of the tongue was 5.7 cm and 4.5 cm, respectively. In the fetus the width of the tongue apex was 3.5 cm, while that of the body was 2.5 cm.

The length of the free part of the tongue, i.e. a part of the tongue participating in the manipulation of collected food, was measured. It was found that the distance between the apex and the attachment of the lingual frenulum in the fetus was 7.5 cm, while in the adult giraffe it was as much as 19.5 cm.

The characteristic structure on the posterior part of the tongue was an elevation of the lingual muscle called the lingual prominence (Fig. 1,2,4).

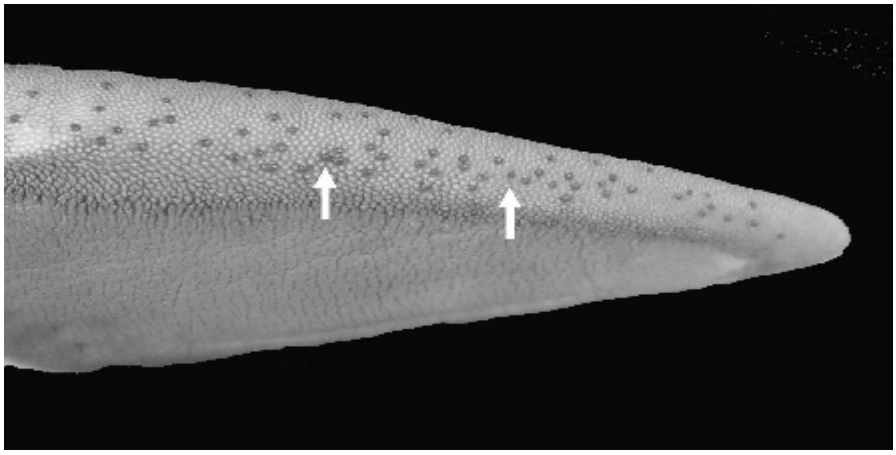

Figure 3. A lateral view on the apex of the tongue in the giraffe fetus. Note numerous dark fungiform papillae on the border of the tongue (arrows).

The length of the lingual prominence of the adult giraffe was approx. 17 cm, and the width was about 8 cm. The length of the lingual prominence in the fetus was approx. 7.5 cm, whereas its width was about 4 cm.

It results from a comparison of the dimensions of the tongue in the fetus and the adult giraffe that the length and the width of the tongue in the analyzed areas of the tongue still increases in the pre- and postnatal development by ca. 1.8 - 2.5 times.

Macroscopic observations of the rough, hard surface of the apex, body and on the lingual prominence of the tongue in the adult and fetal giraffes allowed us to distinguish two types of mechanical papillae and two types of gustatory papillae. The ventral surface of the tongue was smooth and devoid of lingual papillae.

Mechanical papillae in the giraffe were represented by filiform papillae and conical papillae. The short well-keratinized filiform papillae covered the entire dorsal surface of the apex and body, and they were also observed on the lateral parts of the lingual prominence. Conical papillae were distributed mainly on the anterior border and the lateral part of the prominence (Fig.4). These papillae had triangular, short, keratinized processes. On the median line of the prominence and on

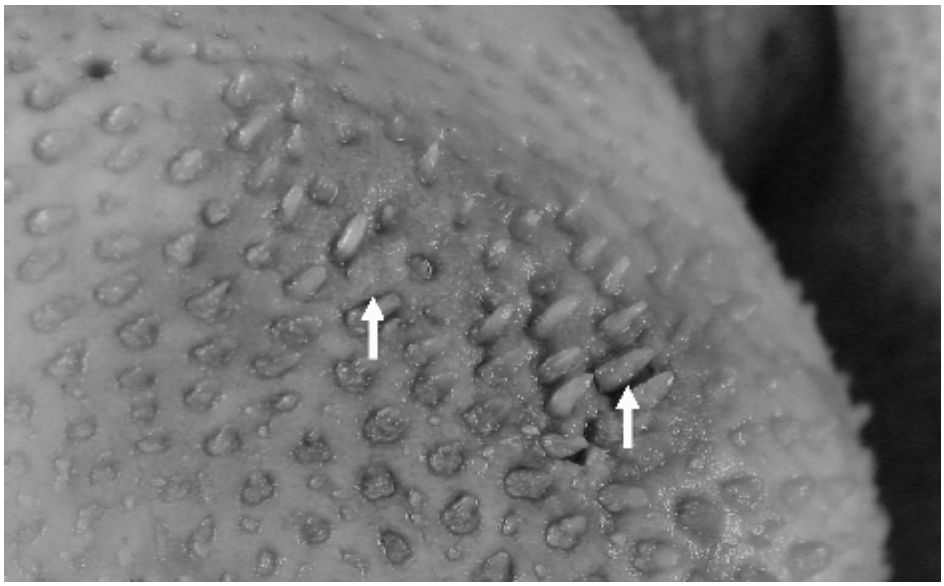

Figure 4. The surface of the anterior part of the lingual prominence in the adult giraffe. Arrows show keratinized conical papillae

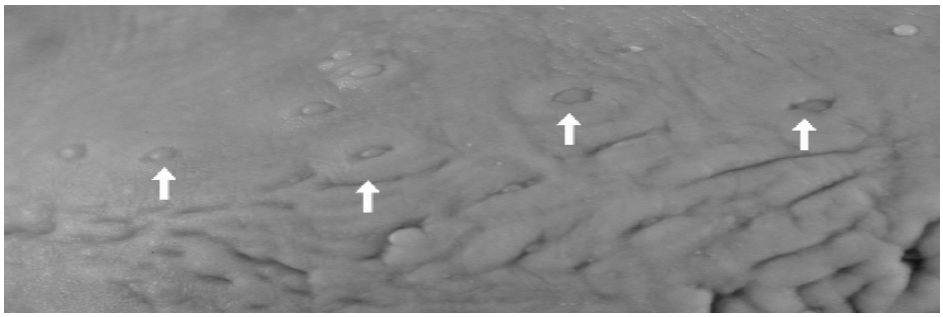

Figure 5. Posterolateral surface of the lingual prominence in the adult giraffe. Arrows mark vallate papillae

the root of the tongue conical papillae underwent a reduction and took the shape of short, keratinized elevations of the epithelium.

The surface of the root of the tongue at the pharynx was smooth and only the openings of lingual glands were present (Fig. 1,2). The distribution of small filiform papillae and conical papillae in the giraffe was similar to that in many species of artiodactyls, which eat soft parts of plants. Such a distribution helps in the transport of food and mastication in the posterior part of the tongue before food is swallowed by the animals. The so-called lentiform papillae were not observed on the prominence of the tongue in the giraffe.

The most numerous gustatory papillae in the giraffe were fungiform papillae. They created peculiar dense accumulations on the tip of the tongue and along the border of the apex of the tongue (Fig. 2,3). Such an arrangement of papillae is a species-specific trait. Due to considerable manipulative ability of this free part of the tongue during the collection of food such an arrangement of gustatory papillae facilitates preliminary gustatory selection of consumed food.

Fungiform papillae on the body and on the entire lingual prominence were uniformly distributed between well-keratinized mechanical papillae. Fungiform papillae on the lingual prominence were bigger than those on the body of the tongue. Several authors reported the presence of large fungiform papillae (about 2-3 times larger) on the lingual prominence near rows of vallate papillae (Yoshimura et al. 2000, Yamaguchi et al. 2002, Zheng et al. 2006, Jackowiak et al. 2007).

The second type of gustatory papillae were vallate papillae situated on both posterolateral surfaces of the lingual prominence (Fig.5). The number of vallate papillae on each side was 8 - 10. The body of the each vallate papilla was surrounded by a continuous furrow and a flat wall of the papilla. Previous studies on herbivorous mammals showed that the number and shape of gustatory vallate papillae is species-specific. Vallate papillae in ruminants are situated in one or two rows, or grouped irregularly along the posterolateral border of the lingual prominence. The total number of vallate papillae is species-specific. In the mountain goat there are 6 - 10 vallate papillae, in the Bactrian camel there are 6 - 8, in the roe deer 24 -28, in the black buck 30,in the Japanese serow there are 24, while in the kudu there are as many as 51 (Emura et al. 1999, Yamaguchi et al. 2002, Jackowiak, 2007, Jackowiak et al. 2007).

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REPRODUCTION OF WHITE RHINOCEROS (CERATOTHERIUM SIMUM) AND BLACK RHINOCEROS (DICEROS BICORNIS) IN CAPTIVITY

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Abstract

Although populations are on a stable level (Foose, 2006), however, this is not only due to the reproduction of the animals kept in captivity, but in case of white rhinos the yearly animals imported from the wild are important factors influencing the growth rate. Between years 1976-1982, 1984-1988, 1998-2002 three peaks in the number of births.
In total 744 birth , the most successful year was 1979 when 43 white rhinoceroses (26 males, 17 females) were born.
Maximum numbers of offspring of white rhinoceros occurred between the 8th and 15th and in the 18th year of mother life. Generally the number of births decreased with age, The highest number of births among black rhinos occurred between the 7th and the 15th year of mother life
Among 202 females whose pregnancies were recorded in studbooks, 39.1% were pregnant only once, whereas 7.9% were pregnant at least 10 times till the beginning of 2005. Among 443 females imported from the wild, only 156 were pregnant until 2005. Seventeen females were pregnant already during transport, which means they had been conceived in the wild. Among 301 males imported from the wild, only 92 took part in reproduction successfully until 2005.). The average amount of pregnancies per one female in captivity and in the wild was respectively 0.6 i 0.8. F= 3.861, p= 0.233.
In captivity females of both species had their first offspring 2-3 years later than those in the wild.

Keywords: reproduction, white rhinoceros, Ceratotherium simum, black rhinoceros, Diceros bicornis

Introduction

Nowadays, the two species of African rhinoceros, black rhinoceros (Diceros bicornis) and white rhinoceros (Ceratotherium simum) are the most numerous representatives of their family: more than 3724 and around 14,542 living individuals, respectively (Internet 1). In comparison, Asian species are much less numerous: Indian rhinoceros (Rhinoceros unicornis) - 2,400 individuals, Sumatran rhinoceros (Dicerorhinus sumatrensis) - 300, Javan Rhinoceros (Rhinoceros sondaicus) - 70 (Internet 1). Despite a huge demand for their horns, mainly on the Asian market, there is an increasing tendency in the number of the animals. This is due to successful in situ conservation. Effective preservation of rhinos' natural habitats should be accompanied by their self-sufficient breeding in captivity, in zoos and safari parks. This form of preservation should aim to collect a diversified gene bank of both species, in case if the catastrophe like the slaughter of African rhinos that happened in second half of XX century was to repeat. The role of zoos can be fulfilled only

when breeding of rhinoceroses is coordinated not only at the country or continent level, but between all the institutions keeping black or white rhinoceroses.
In 1966 dr Heinz-Georg Klos, the manager of the zoo in West Berlin, started to collect the data to the studbooks of the both African species of rhinos. In 1970 the books were published for the first time, and since then updated consecutively. Since 1981 the data has been published as the "International Studbook of African Rhinoceroses". Since 1995 Andreas Ochs has become the new global studbook keeper (Rookmaaker, 1998). The last issue of studbooks for both African rhino species was published in 2005.

The aim of the study was to investigate some trends in breeding as well as the current condition of the animals kept in captivity, basing on the studbooks of the White and Black Rhinos (Ochs, 2005a, 2005b).

Material and methods

The material to this investigation were the newest studbooks of white and black rhinoceros (Ochs 2005a, 2005b). However, older issues were used in cases of mistakes or the lack of data. In the 2005 issues, the chronological lists of animals reported until 1st January 2005 were published. They were organized by the date of the report, and not by the year of appearing in the collection of a particular zoo or other institution cooperating with the studbooks coordinator (Ochs, 2005a, 2005b). Each animal in the studbook is described by its own number, sex, parents, the date and place of birth, the dates and places of all its transfers, the current status and, if the animal has died, the date and the reason for death. Also the names, the number of offspring and the information which generation the animal represents (accepting that all the rhinos captived in the wild are the zero generation) are gathered. Basing on this information, the following has been established:

- Countries and places from which the zero generation animals originate
- The number of the animals that started the population
- The number of the offspring born
- The schedule of births during the year
- The average age of a male/female when having the first/ last calf
- The age and sex structure of the existing population

In the last issues of the studbooks of both species data concerning 1475 southern white (Ceratotherium simum simum), 28 northern white (C.s.cottoni), 773 eastern ecotype of black (Diceros bicornis michaeli), and 151 south-central ecotype of black (D.b.minor) rhinoceros was published. In this publication there is information about all the abortions/miscarriages and stillbirths confirmed.

Specimens born and reported in the first months of 2005 are included in the studbooks. The data concerning them was useful to elaborate the schedule of births during the year, establish the level of mortality and to find out main reasons for deaths in the group of animals till the third year of life.
In order to compare the parameters of reproduction of white rhinoceros females born in the wild (P) and born in captivity (F1, F2, F3), one way ANOVA was applied. The criteria of choosing the females were: the number of pregnancies of each female and her age, the lower limit being 4th year of life (reaching the reproduction age). Before performing the calculation, it was checked if analysed samples satisfyed the assumptions of the analysis of variance (both samples should be approximately normally distributed with equal variances). The variances in both samples proved to be significantly different, therefore an additional analysis of the data was necessary.

Results

The first white rhinoceros (southern subspecies) was born in the Pretoria Zoological Garden on 8th June 1967. Between years 1976-1982, 1984-1988, 1998-2002 three peaks in the number of births were reported (Fig. 1). First of them was a result of a large import of white rhinoceroses in years 1970-74. The females which then arrived to the Pretoria Zoological Garden gave births to the first time in captivity. The two other peaks resulted from parturitions by the females imported in the years 1976-1978 and 1998-1999. Also the males that reached their sexual maturity and the parturitions of females imported before 1976 were important factors influencing the high number of births.

In total 744 calves (399 males, 339 females, 6 unknown) were born in all the institutions cooperating with the studbook coordinator. The most successful year was 1979 when 43 white rhinoceroses (26 males, 17 females) were born.

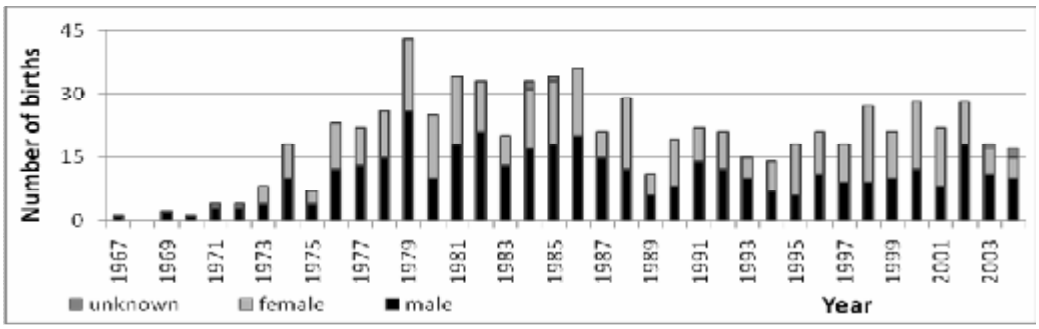

Figure 1. The number of white rhinoceroses born in captivity in successive years.

The first calf of black rhinoceros (eastern ecotype) was born in the Chicago Brookfield Zoo on 7th October 1941. Since 1960, almost every year the births were reported (Fig. 2). Increase in the number of births can be explained by the fact that the females from the respective generations reached their sexual maturity. In total, 504 black rhinoceroses (238 males, 251 females, 15 unknown) were born in all the institutions. The most successful year was 1997, when 24 calves (12 males, 10 females, 2 unknown) were born.

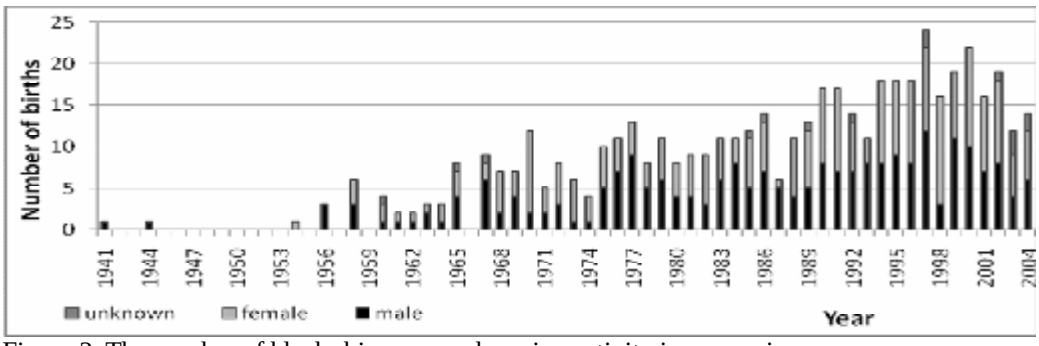

Figure 2. The number of black rhinoceroses born in captivity in successive years.

Parturitions among rhinoceroses kept in captivity took place all over the year, with a peak from September to January (Fig. 3). Average number of parturitions per month was 62,2 for white rhinos and 42,1 for black rhinoceros. For animals living in the wild a peak of calving was observed from March to July (Owen-Smith,1975).

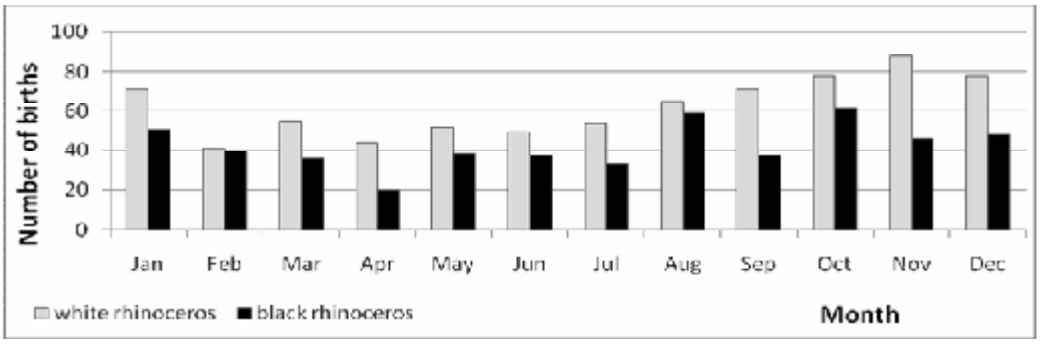

Figure 3. Distribution of white/black rhinoceroses' births during the year.

This is because, although rhinos reproduce un-seasonally, the geographical position of an institution and connected with this climate and seasons of the year influence the time of births. The animals exhibited in traditional zoos are often mated from spring till the late autumn, because the weather during winter could increase the risk of getting hurt during the courtship or copulation.

Maximum numbers of deliveries among white rhinoceros were recorded between the 8th and 15th and in the 18th year of mother life (Fig. 4). The high number of births in the 10th year of female life was a consequence of a large number of primiparae's deliveries at this age and successive childbirths of females that had given birth earlier (Fig. 4).

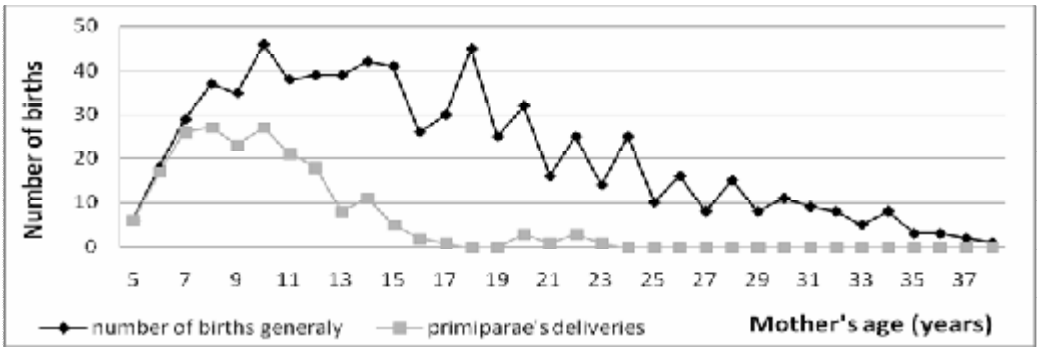

Figure. 4.Number of births of both northern and southern white rhinoceroses in relation to mother's age.

Although generally the number of births decreased with age, it was observed that between the 18th and 30th year of a female life, every two years the number of births increased. The highest level of primiparae's deliveries is observed between the 7th and the 11th year of life.

The highest number of births among black rhinos occurred between the 7th and the15th year of mother life (Fig. 5). In the 9th year of female life the maximum of deliveries was recorded

(analogously to white rhinoceroses). A peak of primiparae's deliveries took place between the 7th and the 9th year of mother life.

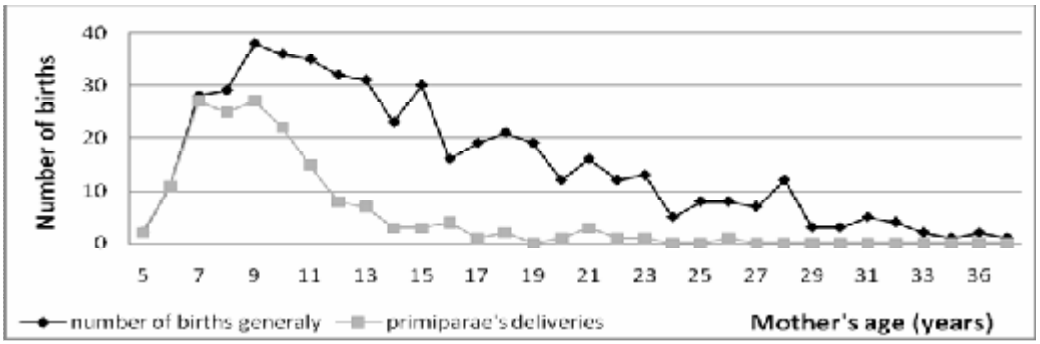

Figure 5. Number of births of black rhinoceroses (eastern and south-central ecotypes pooled) in relation to mother's age.

Among 202 females whose pregnancies were recorded in studbooks, 39.1% (79 specimens) were pregnant only once, whereas 7.9% (16 animals) were pregnant at least 10 times till the beginning of 2005 (Fig. 6). Among 443 females imported from the wild, only 156 were pregnant until 2005. Seventeen females were pregnant already during transport, which means they had been conceived in the wild.

Among 301 males imported from the wild, only 92 took part in reproduction successfully until 2005.

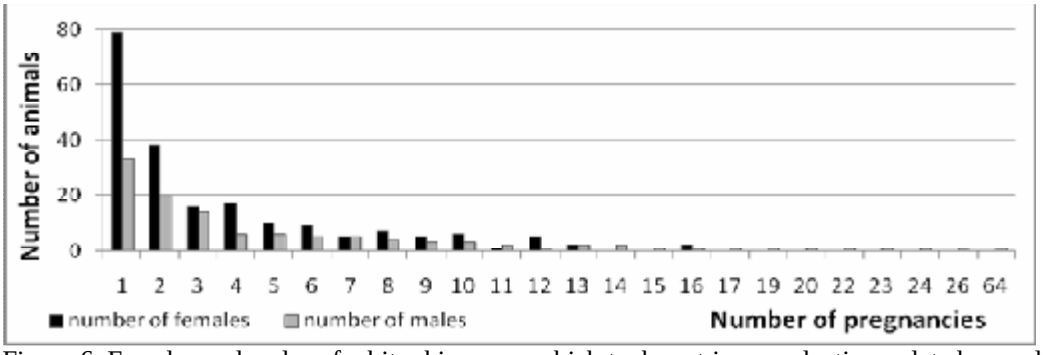

Figure 6. Females and males of white rhinoceros which took part in reproduction, related according to the number of pregnancies produced by them. The number of all pregnancies recorded in a studbook regardless to the result (life parturition, stillbirth, abortion) are included. For males "number of pregnancies" means the number of conceptions.

The ANOVA analysis showed that the assumption of the low limit of the females' age was insufficient, as the analysed sample of females born in the wild consisted of 165 females (almost 40% of the sample) at the age over 32, which combined with the lack of such animals in the sample consisting of specimens born in captivity could significantly distort the results of the test (overstate the amount of pregnancies per one female born in the wild). The average amount of pregnancies per

one female in captivity and in the wild was respectively 0.6 i 0.8. The one way analysis of variance gave the following results: F= 3.861, p= 0.233.

The p-value is significantly higher than the assumed level of significance ($p < 0.05$), which tells that there is no ground for rejecting the zero hypothesis (equality of average values in both groups).

Both the female record holders and the most successful male came from San Diego Wild Animals Park where the high number of pregnancies was a result of a male constant presence in the large female herd. There were 28,45% - 33 males that took part in reproduction successfully only once until 2005 of all 116 males that took part in reproduction. Twenty males (17,24%) conceived females at least 10 times (Fig. 6).

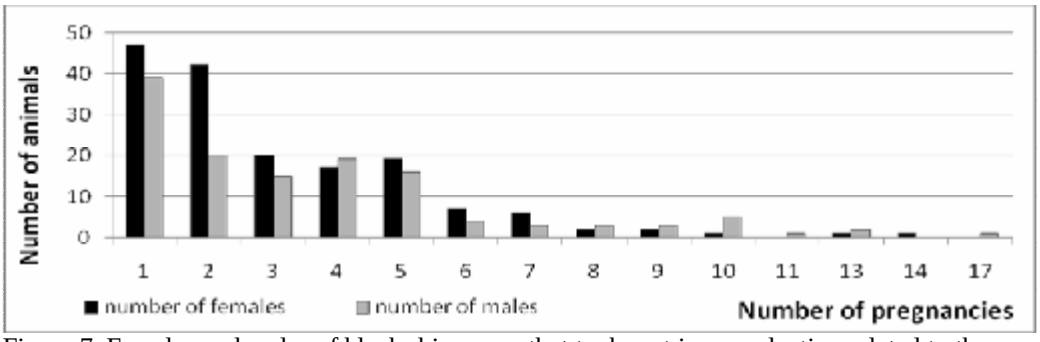

Figure 7. Females and males of black rhinoceros that took part in reproduction related to the number of pregnancies produced by them.

Fourty seven females (28.5%) whose pregnancy was recorded in a studbook until 2005 were pregnant only once (Fig. 7). Only 3 females (1.8%) were pregnant at least 10 times.

Fourty seven males (29.8%) which took part in reproduction successfully until 2005, conceived females only once. Nine males (6.9%) succeeded at least 10 times.

Among 202 females imported from the wild only 95 were pregnant until 2005. Among 184 males imported from the wild only 73 took part in reproduction successfully.

A female that reached the highest number of births (14) lived in the San Francisco Zoological Garden. Seventeen most successful males and a female having been pregnant 13 times lived in the Hiroshima Zoological Garden.

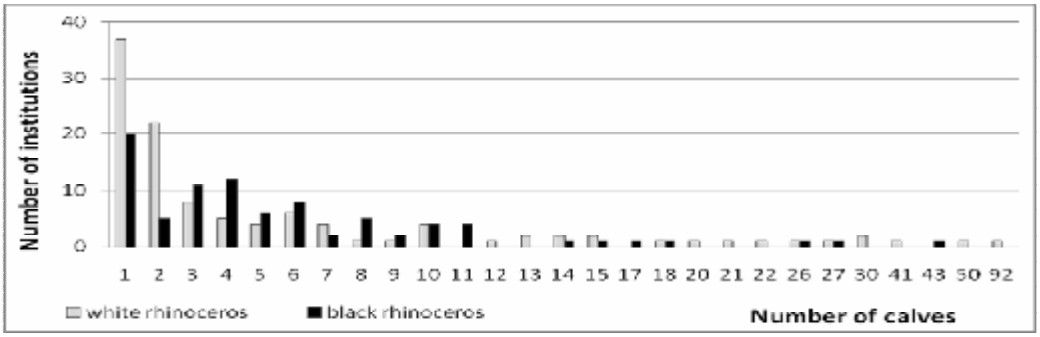

Figure 8. Institutions ordered according to the number of white or black rhinoceroses' births.

In total, 744 white rhinoceroses were born in 111 institutions between 1967 and 2004 (Fig. 8). An average number of calves in one institution, where at least one white rhinoceros was born, was 6.7 during 37 years (1967-2004). The institutions where the largest number of calves were born are: the San Diego Wild Animals Park (92 animals), The Safari Park London- Whipsawed (50) and the Pretoria-Lichtenberg Game Breeding Centre (41).

In 504 black rhinoceroses were born in 86 institutions between 1941 and 2004. The average number of calves born (only the institutions where at least one black rhinoceros was born) was 5.9 during 63 years. The highest amount of calves was born in the Lewa Wildlife Conservancy in Kenia (43), the Port Lympne Zoological Garden (27) and in the Dvur Kralove Zoological Garden (26).

Discusion

Rookmaaker, in his book about the history of breeding rhinoceroses in captivity, (Rookmaaker, 1998) questions whether proceeding on keeping south white rhino studbook is justifiable. He suggests reducing the number of those animals in zoos and safari parks. The money and space saved thanks to these solutions could be used to breed black and Asian rhinos. Rookmaaker (1998) supports his view with the fact that the southern white rhinos are not on the verge of extinction, contrary to the other species of rhinoceroses, even with hunting for them being allowed (South Africa, Namibia).

In our opinion Rookmaaker is right to some extend, however zoos should still keep a healthy, self-sufficient and genetically diverse population of the southern white rhinoceros.

The data is satisfactory because the populations are on a stable level (Foose, 2006), however unfortunately, this is not only due to the reproduction of the animals kept in captivity, but in case of white rhinos the yearly animals imported from the wild are important factors influencing the growth rate.

The low number of captive white rhinos population founders was due to inadequate method of exposition: young animals (male/-s and female/-s) were imported together and grew up also together, which had an evil influence on their breeding behaviour and female oestrus. Close investigation will find out other reasons for problems connected with breeding white rhinos.

What is more these results are not satisfactory comparing to the record of animals from the metapopulations of wild rhinoceroses living in Africa. In the years 2001 - 2003 the number of southern white rhinos increased by 13.5% every two years (Emslie, 2004). In Suazi yearly growth of population during 10 years was 9.4% and the number of animals increased from 27 to 61 (Reilly et al. 2004). The growth of black rhinos population was 5.2%, which lets us to believe that the number of these animals will be increasing, providing that the level of the population is stable (Emslie, 2004).

In captivity females of both species had their first offspring 2-3 years later than those in the wild (9.5 year old females in captivity versus 6-7 year old in the wild). What should be noticed is the connection between a big number of animals imported from the wild by one zoo and a big number of birth in the succeeding year (Whipsnade Zoo, San Diego WAP). Every significant increase in the number of births was a result of an increased import of rhinoceroses in preceeding years.

Unsatisfactory is still the fact that untill the beginning of 2005 many females of both African rhino species which have been bred in captivity were pregnant only once. History shows us that the situation in African countries is unstable and that it is easy to destroy even the most numerous populations of animals during short periods of time (Emslie and Brooks, 1999). Therefore, establishments dealing with breeding rhinoceroses in captivity should be able to have (if such a

need appears) a number of animals sufficient to the possible further reintroduction of the species in places where they would be killed.

As far as the idea of switching money and space in zoos and safari parks to breeding more endangered species (like black or Indian rhinos), this could be introduced by the substitution of southern white rhinos in the institutions that cannot afford keeping more than three animals. Also extended promoting the problem among the new institutions, stressing how important these species are for the nature preservation and how the institutions would be interesting for visitors, when breeding the rhinoceroses. It is still true that challenge for the institutions keeping rhinos in captivity is implying and developing biotechnological methods in reproduction not only of rhinoceroses but all the endangered species (Western, 1984).

The first successful insemination of a southern white rhino female was possible in 2004 (Internet 2). Before, the method of collecting sperm from both African species and from indian rhino had been applied (Internet 3). The newest achievement was collecting ovum cells from the black rhino female. This was done in Western Plains Zoo, Australia, in order to develop the method of in vitro fertilisation (Internet 4).

This actions are to give a chance to rare and endangered species (northern white, javan or sumatran rhino) to be saved from the fate of western black rhino subspecies.

In the case of white rhinos, a suitable structure of the stable herd, including the age and number of females, needs to be established as this factor may have a crucial influence on the reproduction. These aspects of white rhinoceros breeding need close investigation. In the case of black rhinos a big mortality rate not only of young but also adult specimens remains the unsolved problem.

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Internet 2: http://www.sosrhino.org/news/rhinonews083005a.php

Internet 3: http://www.sosrhino.org/research/res_rrp_semencollection.php

Internet 4: http://www.sosrhino.org/news/rhinonews101106.php

ENRICHMENT OF MANED WOLVES (chrysocyon brachyurus) AT FRANKFURT ZOO

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Abstract:
Maned wolves are crepuscular and nocturnal animals, who are rarely seen in action by visitors in zoos due to their lifestyle. In order to change this, enrichment measures were carried out during opening hours with the aim of extending the activity of the animals during daylight. Several enrichment measures, namely hidden food, buried food, hanging food, food in pipes, live food, objects on a rope, odour tracks of cinnamon and lions' excrement, were offered to the five maned wolves (1.1 with 3.0 pups) in order to observe their reactions. By means of chronoethological studies we could clearly recognise that the animals differently reacted to each measure. Movable or moving objects were far more interesting for the animals than odour tests or static objects. Objects thrown into the water tank increased activity most.
It must be stated that compulsory enrichment did not lead to a significant increase of their activity and the animals kept up their usual activity patterns.
In future studies new enrichment measures focusing on movable objects should be developed in order to improve the maned wolves' activity during opening hours.

Keywords: activity pattern, chronoethology, longtime video recording, evaluation of enrichment measures

Introduction:
Maned wolves are canids which live in many zoos all over Europe. Their behaviour is execptionally interesting and visitors like to observe them. But these animals are crepuscular and nocturnal (Dietz 1984), and,
due to their lifestyle in zoos, they are rarely seen in action during opening hours. Only at feeding times all animals are active in daylight. The rest of the day the visitors see maned wolves laying in a corner of their enclosure, or the animals cannot be seen anywhere.
Is it possible to shift the animals' activity into daylight and opening hours by enrichment programs? This paper critically evaluates different methods of enrichment by long term video recording and direct observation.

Material and Method:
In this study a maned wolf couple with three weaned pups (three months old) were observed in Frankfurt Zoo. Therefore four cameras, which covered nearly the whole outdoor enclosure, were installed. The data were collected by the means of a timelapse recorder. Within data interpretation once a minute animal behaviour was noticed. Several enrichment measures, namely feeding out of time (before or after normal feeding time), buried food, hanging food, food in pipes, alive food, objects on a rope (Fig. 01), odour tracks of cinnamon and lions' excrement, were offered to the maned wolves in order to observe their reactions. The different measures were given daily over a bout of one month.

Table 1. Enrichment and its effect on activity. - = no reaction; + = little reaction; ++ = clear reaction; +++ = very strong reaction

Mode of Enrichment	EFFECT
Feeding out of time (earlier or later)	-
Buried food (mice and chicken)	+
Food in pipes (mice and chicken)	+
Locust in pipes	++
Odour track of cinnamon	-
Hanging food (chicken)	+
Alive fish in water tank	+++
Odour track of lions excrements	-
Objects on a rope	++

Figure 1. Maned wolf catches food from a rope

Results:

We could clearly recognise that the animals reacted differently to each measure (Table 1). Movable or moving objects were far more interesting for the animals than odour tests or static objects. Fish thrown into the water tank increased activity most. The young maned wolves reacted clearly more intensively than their parents (Fig 2).

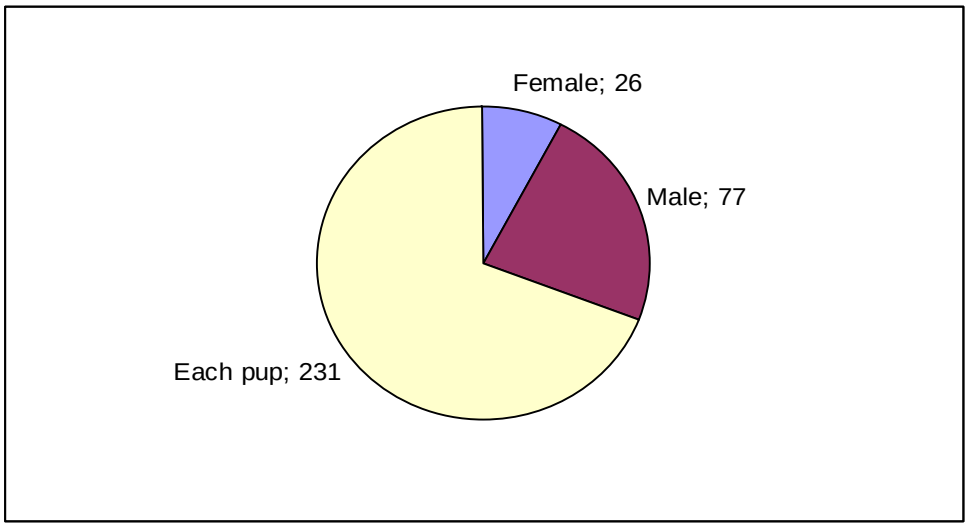

Figure 2. Total of minutes of activity to enrichment for each individual over the complete 26 observation days.

The interest in the enrichment did not last more than 15 to 20 minutes for all animals together. Only some objects, like locusts in a pipe or objects on an rope called up more interest. Alive fish increased activity most (Fig 3).

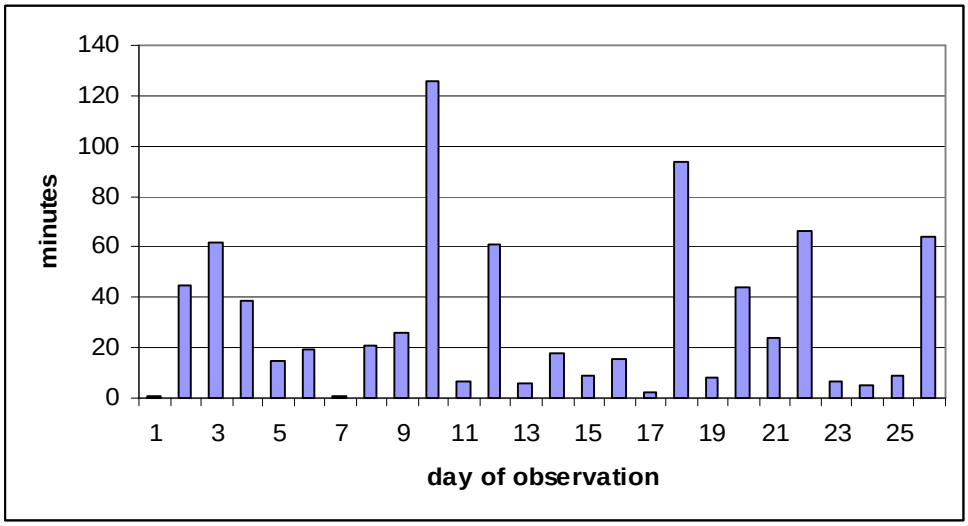

Figure 3. Reaction to entrichment for all 5 maned wolves together in minutes over the day.
day 10 = Alive fish in water tank; days 3,18 and 22 = Locust in pipes;
days 12 and 26 = Objects on a rope

Discussion:

The success of increasing the activity by means of enrichment was not convincing. The maned wolves just use the different programs for a short time, about ten to fifteen minutes. Only the use of live fish in the tank grows activity up to one hour. In other studies young maned wolves are reported to play with live food, too (Encke, Gandras and Bieniek 1970).

The reason for the ineffectiveness of odour tracks could be that we have used the wrong odours. In other surveys other flavours were used with more profit (Werle, Fletchall, Weinhardt & Westbrock 1995).

Ortner (1995) concluded that maned wolves need a long time to adapt to innovative enrichment. When they know them, they use them often and willingly.

Conclusions:

It can be concluded that compulsory enrichment like in our study does neither extend nor change the maned wolves' activity significantly. Movable objects as enrichment are most promising to increase the maned wolves' activity during opening hours.

Acknowledgments:

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WHAT'S IN A HOWL? A COMPARISON OF VOCAL PERFORMANCE IN CAPTIVE HOWLER MONKEYS

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Abstract.

The howler monkey genus (Alouatta sp.) is characterised by the production of loud call or howl vocalisations. Investigations into Alouatta howl calls have suggested territorial demarcation, inter-troop spacing, mate defence and attraction, group cohesion and resource location as possible functions of these vocalisations in the wild. In terms of the social function of these calls, the alpha male of Alouatta troops may use howl contests to assess opponents and rival males and perform howls as an alternative to physical conflict. Females are often a limiting resource for male howler species therefore transmitting information on the identification and rank of an individual may be beneficial during mate selection and defence.

The breeding rates of Alouatta species in the captive environment are low within European institutions and are limited in number and diversity of founders, making breeding from all pairs a priority. Behavioural and vocal data were collected on four captive groups of A. caraya; two family groups, and two single pairs and one wild group. This preliminary study investigates the influence of social housing situations in captive Alouatta caraya and its possible influence on behaviour patterns and breeding rates.

Key words: Howler monkeys, vocalisations, captivity, wild, behaviour.

Introduction.

The howler monkey genus (Alouatta) is among the largest of the New World primates (Crockett & Eisenberg, 1987), their location is spread across much of South America, including southern Brazil, Paraguay, Northern Argentina, Panama, Eastern Bolivia and Belize (Eisenberg and Redford, 1999; Rowe, 1996).

Howler social groups contain a variable number of male members. Groups may be comprised of a multi-male troop, a multi-female troop (usually containing four males and up to four females) or a bachelor male troop (Crockett & Eisenberg, 1987).

Howler species spend approximately 24% of their time feeding; 10-24% travelling and 60% of their time resting, and at night are inactive (Silver, Ostro, Yeager and Horwich, 1998; Altmann, 1959). Resting, feeding and vocalising are performed in a bimodal pattern through the day, early morning and late afternoon (Benton, 1976; Cornick and Markowitz, 2002; Baldwin and Baldwin, 1976)

The Alouatta genus is characterised by the production of loud roar or howl vocalisations (Whitehead, 1995, Jones, 1980; 1983). Investigations into Alouatta howl calls have suggested territorial demarcation (Carpenter, 1934; Altmann, 1959), inter-troop spacing (Altmann, 1959; Chivers, 1969; Baldwin and Baldwin, 1976; Whitehead, 1995; Cornick and Markowitz, 2002), mate defence and attraction (Kitchen, 2004; Sekulic, 1982), group cohesion (Carpenter, 1934;

Altmann, 1959; Jones, 1983; Bernstein, 1964; Collias and Southwick, 1952) and resource location (Cornick and Markowitz, 2002) as possible functions of these vocalisations in the wild.

Few studies have been conducted on captive howler monkeys as Alouatta species are known for their inability to adjust to captivity (Crandall, 1964; DuMond, 1967, as cited in Benton, 1976). This preliminary study investigates the influence of social housing situations and environmental stimuli on captive and wild Alouatta caraya behaviour patterns and vocal performances.

Materials and methods

Behavioural and vocal data were collected on five groups of A. caraya. Four captive groups; two family groups, two pairs and one group of wild howler monkeys were studied. The groups were classed into either 'family' groups; those containing an adult male, an adult female and a range of group members of differing age-sex classes, or a 'pair'; a study group containing only one adult male and one adult female. The data for all family and all pair groups were combined for each sex as behavioural patterns were found to be similar in the two classifications.

Instantaneous scan sampling of all state behaviours performed by the adult male and adult female of each group were conducted every 10 minutes (0800 to 1630 hours) for 10 days on the captive groups. Behavioural data on the wild group was collected every 10 minutes (0710 to 1730 hours) for two days using the same sampling method. The rates of howling, termed the vocal rate, (average number of times the adult males perform howl vocalisations each day) were calculated for each captive group using the following formula;

Rate of howling = total number of howls performed in 10 days

Data were analysed using Randomisation Tests, Design 6 (one-way small group repeated measures) (Todman and Dugard, 2001).

Results

Behavioural activity budgets

Results for male behaviour found that family housed males spent significantly less time locomoting ($p<0.05$) and resting alone ($p<0.00$) than pair housed males. Also, graphs suggested that family housed males spent more time resting socially than pair housed males (Figure 1), although randomisation tests were found not to be significant. Graphical trends were similar for captive female howler monkeys (Figure 2). Those housed in a family group were suggested to spend less time locomoting, resting alone and more time resting socially than those in a pair; however, results were not found to be significant.

Comparison between family groups in captivity were made from the graphs (Figures 1 and 2) as the wild data set were deemed too small for statistical analysis. Results suggest that captive groups rest more socially than those in the wild. Wild males were suggested to spend more time resting solitary than females, females spending more time resting socially. Females in the wild were suggested to spend less time resting alone than captive females.

The graphs (Figures 1 and 2) may suggest that both males and females in the wild group spent a larger percentage of time locomoting and out of sight than captive howlers; however, statistical analysis were not performed due to a small data set.

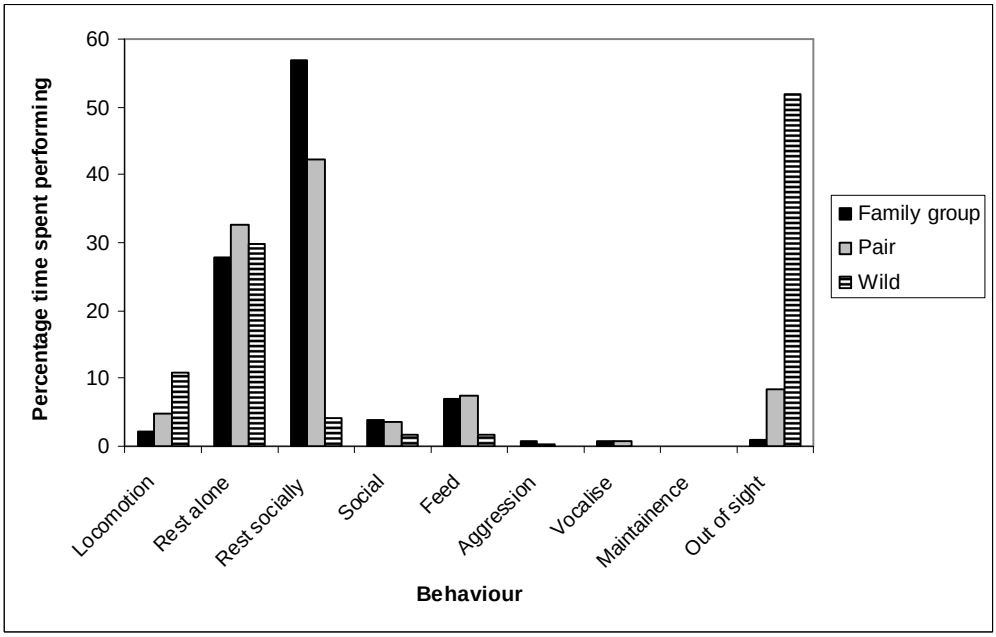

Figure 1. Male behavioural activity budgets.

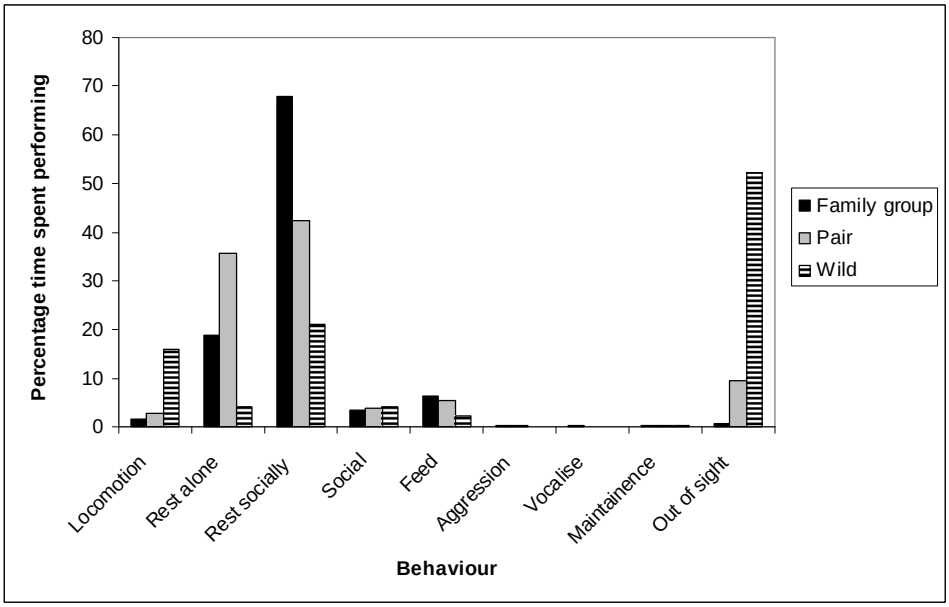

Figure 2. Female behavioural activity budgets.

Howling rates

Results for the range of howls and howling rates for adult males showed variation in the frequency of howl performance between captive groups (Tab. 1). The highest range of howls and rates of howling were performed by group 1, groups 2 and 3 performed similar rates; group 4 did not perform vocalisations during the study.

Table 1. Range of howls and vocal rates of the adult males in each study group.

Group number	Social group classification	Range of howls per day	Rate of howling per day
1	Family	1 to 9	3.5
2	Family	0 to 3	0.58
3	Pair	0 to1	0.5
4	Pair	0	0

Discussion

Howlers social groups commonly consist of one of two types; multi-male, multi-female groups usually containing four males and up to four females (Rowe, 1996), or bachelor groups of males (Crockett & Eisenberg, 1987). The higher percentage of time spent resting socially for both males and females in captive family groups may be explained by an increased opportunity to rest with other family members due to a larger number of individuals in the group.

Captive females which are group housed spent more time resting socially than those housed in pairs. High levels of social resting were also found in the females of the wild study group, such results may be due to similar reasons. The captive family groups contained young and infants which the females were currently nursing. In the wild, female group members have been reported to act both maternally and territorially, those who are not mothers themselves also caring for the offspring of the group (Crockett & Pope, 1993), thus spending a large proportion of time resting socially with their young.

Howler monkeys are highly territorial. Studies conducted in the wild suggest that male howlers defend a territorial home range when another group comes into visual contact (Collias and Southwick, 1952; Carpenter, 1934). Territorial defense is usually carried through by the performance of howl vocalisations (Altmann, 1959; Collias and Southwick, 1952; Carpenter, 1934; Southwick, 1962; Chivers, 1969; Bernstein, 1969; Drubble and Gautier, 1993; Horwich and Gebhard, 1983; Chiarello, 1995; Whitehead, 1987). Data showed that in the wild, adult males rest more alone, away from the main group. Preceding territorial vocal battles (Collias and Southwick, 1952), the adult males of a group may be suggested to display high levels of vigilance and thus, rest alone, away from the main group in order to actively defend the family from external threats.

The high percentage of time spent locomoting by wild individuals may result from increased opportunity to travel around the forest and the necessity of travel to locate food resources. Wild studies have suggested that howlers spend 10-24% of their day travelling (Silver, Ostro, Yeager and

Horwich, 1998). Wild studies report that howlers spend up to 60% of their time resting (Silver, Ostro, Yeager and Horwich, 1998) as howlers do not have a specialised digestive tract and must spend a large proportion of their day at rest to digest their food (Crockett & Eisenberg, 1987) which may explain the high percentage of out of sight data for the wild group may be assigned to resting.

In captivity, the howler monkey's food is provided each day as part of the husbandry routine, whereas wild counterparts must travel between food sites to feed which may be some distance apart. This may also be one of the reasons that there are such high levels of out of sight recordings in the wild group as they were travelling high in the trees and could not always be located by observers on the ground.

The Alouatta genus is characterised by the production of loud roar or howl vocalisations (Whitehead, 1995, Jones, 1980; 1983). For species that inhabit densely vegetated areas, natural selection has favoured the performance of vocal signals over large distances (Whitehead, 1989). The functions of wild howler monkey vocalisations include territoriality (Carpenter, 1934, Altmann, 1959), mate defence and attraction (Kitchen, 2004, Sekulic, 1982), group cohesion (Carpenter, 1934, Altmann, 1959, Jones, 1983, Bernstein, 1964; Collias and Southwick, 1952) and resource location (Cornick and Markowitz, 2002).

The vocalisations performed by captive howler groups, as they are not regularly exposed to the vocalisations of conspecific howler monkeys, it may be suggested, are performed in response to external factors and environmental influences. The group with the highest range of howls per day and overall howl rate, group 1, were housed adjacent to the main zoo vehicle access route through the zoo and were regularly stimulated by the low frequency sound of vehicles passing by. In the wild, howling has been noted to be performed in response to a range of external stimuli such as heavy rain, thunder, lightening, strong wind, firing of a weapon, aeroplane engines, predator attack, the cry of a disturbed bird, fallen young and at the sight of humans or dogs (Altmann, 1959; Baldwin and Baldwin, 1976; Bernstein, 1964; Carpenter, 1934; Chivers, 1969; Crockett and Eisenberg, 1987; Sekulic, 1983; Kitchen, 2004).

The males of both groups 1 and 2 were housed in their family groups therefore the males may have been vocalising as a form of group defense (Kitchen, 2004; Sekulic, 1982).groups 1 and 2 also performed higher howling ranges per day than the other study groups. It has also been reported that in the wild, alpha males howled more often and for longer durations if their troop contained offspring. In A. pigra, playback experiments supported findings into the assessment of inter-group fighting ability by both sexes in groups that contain an infant, (Kitchen, 2004; 2006). If groups which are performing vocal battles contained a higher number of males than the counter-calling group and therefore are more likely to win a vocal contest, adult males were observed to only howl if offspring were present, suggesting that male vocalisations are performed in group defence (Kitchen, 2004). Such vocal encounters may act as an alternative to physical aggression (Altmann, 1959; Carpenter, 1934; Cornick and Markowitz, 2002; de Cunha and Byrne, 2006).

Groups 2 and 3 performed similar vocal rates. As stated above, the male of group 2 may have been performing vocalisations as a form of mate defense. Group 3 were a pair housed next to a highly vocal group of Siamang gibbons (Symphalangus syndactylus) which may have acted as a vocal stimulus and encourged the male howler of the group to vocally respond to their calls.

Group 4 did not perform vocalisations during the study; this group were experiencing housing problems and may have been affected by a highly vocal group of monkeys close to the study group.

Conclusion

Preliminary results suggest that housing and husbandry may influence the behaviour of captive howler monkeys. The presence of infants or a breeding partner may encourage the adult females in captive groups to interact more with their offspring, the males of family housed groups performing more social behaviours than those housed in a pair.

The influence of housing and husbandry may be suggested to impact on the vocal behaviour of captive male howler monkeys. Males housed in a family group were found to vocalise more frequently, perhaps functioning in mate or group defense. Also, environmental stimuli such as nearby vocal primates or environmental stimuli were suggested to influence the performance of howl vocalisations and encourage howling bouts.

Comparisons of captive and wild groups may be difficult due to the small sample size of the wild data set in this study but data suggests that behavioural differences may be due to variations in habitat and ecological variables.

This study is part of an ongoing PhD project. In total, 12 groups will be studied where vocalisations are to be analysed in addition to the behavioural study to further investigate the influence of housing regimes on captive howler monkey behaviours.

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INFLUENCE OF FEEDING REGIME ON MORTALITY AND GROWTH RATE IN THERAPOSIDS SPIDERS (Araneae, Theraposidae)

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Abstract

Spiders of Theraposidae family belong to the section Orthognata, which consists of primitive, giant spiders with orthognatic arrangement of chelicerae. Theraposids inhabit naturally tropical regions of Africa and both Americas. In the accepted system seven subfamilies of these spiders are distinguished (Avicularinae, Eumenophoriinae, Grammostolinae, Ischnocolinae, Ornithoctoninae, Selenocosminae and Theraposinae). Because of environmental preferences these spiders are characterized by wide plasticity even in the same genus, e.g. *Brachypelma* lives both in Brazilian rainforests and in Mexican deserts.

Spiders like all Arthropods grow by leaps through moulting. Moulting is a "delicate" process depending on: abiotic environmental factors (such as temperature and humidity); the spider's own condition (like body damages, parasites, age and size, genetic component) and accessibility of food. The purpose of the research was verification of the frequency of feeding influences on basic parameters of spiders' post-embryonic development (such as growth rate, survival, moulting synchronicity). Experimental breeding consisted of eight genera of Theraposidae family. Developmental parameters were researched in all of them: Acanthoscurria, Brachypelma, Chromatopelma, Hysterocrates, Lasiodora Poecilotheria, Phormictopus, and Stromatopelma. Experimental sample was divided into 3 groups: (1) fed often, (2) fed rarely, (3) starvation. Analyses proved that a wide spectrum of features exists with feeding regimes accounting for variability. Evident changes were: decrease of body mass and size, decrease of moulting frequency, higher mortality.

Key words: spiders, Theraposids, development, feeding regime, mortality, survivorship, growth rate.

Introduction

Family Theraposidae is one of few families which belong to old section Orthognatha, which are characterized by: orthognatic arrangement of chelicerae; presence of respiratory organs as 2 pairs of lungs (book lungs) with complete lack of tracheae; heart structure with 3 – 4 pairs of ostias. Called Bird-eating-spiders are the largest spiders in the World (e.g. size of female *Theraposa blondi* can reache 20 cm, Smith 1994) and most long-lived (e.g. the female *Lasiodora parahybana* is able to live more than 20 years). They are characterized by quite primitive body structure (respiratory system, visual sense, reproductive organs) and simple pattern of courtship. The amazing hairiness of their body is the most characteristic trait of this family comprising the following types of hair: covering, sensory (tactile, trichobothrium), urticating – used for defense - (present only in some subfamilies) and modified hair (adhesive on extremities tarsus – scopulae, spines and club-shaped sensilla). Theraposids are usually tropical spiders, inhabiting naturally warm regions of Africa and America, preferring diversified

environments (e.g. within one genus *Brachypelma* there are species which occur both in the brazilian rainforest - *albopilosus* and the Mexican desert – *smithi*). Wide diversity can also be apparent in the life mode of these spiders. There are known two ground burrowing species (majority of Theraposidae, subfamilies Grammostolinae, Theraposinae, Eumenophoriinae, Ischnocolinae) and tree-dwelling species weaving thick cocoons between limbs and under the bark (Avicularinae, genus *Poecilotheria* from family Selenocosminae). Life mode affects the morphological structure of Theraposids: the largest species live on the ground; they are heavy and square-built, and in most cases they possess urticating hair (genus *Brachypelma*).There is an extraordinary subfamily among them – Ishnocolinae, represented by fast-running and jumping spiders. Tree-dwelling spiders are smaller and have abdomen relatively smaller to the rest of the body; they also have longer extremities which often possess on edges flattened hair used for jumping and short-distance gliding. Members of this group are usually more venomous than others (genus Poecilotheria has the strongest venom among Theraposidae).

As it is known all Arthropoda, including all spiders, are closed in hard chitinous armour, so they have a problem with systematically increasing body size. Only the abdomen is able to grow in such way, and the rest of body grows by leaps through systematic moulting. This process depends on: abiotic environmental factors (such as temperature, humidity); spiders' own condition (like body damage, presence of parasites, age and size, genetic component) and accessibility of food. During moulting all parts of the body maintain the same shape before moulting; only body size and proportion can change (this is true especially for early development stages). Also various sensory structures like sensory hair can be replaced. Moulting between larval and nymphal stages results in the most changes (Wurdak & Ramousse, 1984). Moulting of young spiders in initial phases can occur even every few days, in later life, the period of preparing old coat to remove becomes longer and longer. Spiders need definite numbers of instars (intermoult period – following Whitcomb 1978) to reach sexual maturity. This number is usually less in males' development than in females (Jackson 1978). Smaller species don't need as many instars as larger, longer-lived ones (Foelix 1996 according to Bonnet 1930. In laboratory breeding of spiders in constant abiotic conditions, researching the influence of various diets on development and morphological differentiation is quite simple. It has been repeatedly shown that the quantity and quality of food significantly influence many aspects of spiders' lives. Females of Bird-eating-spiders fed by poorly differentiated food weren't able to produce eggs (Baerg 1958); and spider lings of *Atypena formosana* showed the most survival in groups with the most diversified diet (Sigsgaard et al. 2001). Death in the *ecdysis* stage of moulting of individuals in laboratory breeding usually appears in poor feeding by mono-diet groups (Uetz et al. 1992). Various feeding regimes cause differences in growth rate and development of spiders. Growth rate is higher and duration of instars is shorter when spiders are on higher food level (Jackson 1978; Higgins, Rankin 2001; Sigsgaard et al. 2001; Li 2000). It was experimentally proved that mature females and males of Theraposidae (without differences) deprived of food absolutely but with availability of water, survived about 2 years (Baerg 1958). By way of compassion: with food available but with no water these spiders survived following 81 days and 7 months (Baerg 1958). Influence of feeding regime on life aspects of Theraposidae spiders have not been investigated yet.

The purpose of this research was to verify frequency of feeding influence on basic parameters of spider post-embryonic development (such as growth rate, survival, moulting synchronicity).
The null hypothesis assumed that greater frequency of feeding positively affects survival and growth rate of spiders. In this case it should be expected that individuals from well -fed group

will be characterized by the lowest rate of mortality and the fastest growth; opposed to this starved group of spiders will have the highest mortality rate, and the slowest growth. According to this assumption the experimental sample should decompose on 2 groups properly to feeding regime. Because Theraposids are long-lived spiders and they reach maturity after a couple of years, eg. in genus *Brachypelma* males after 7 – 8 years, and females after 9 – 10 (Locht et al. 1999) differences of longevity dependent on sex were not taken into consideration.

Materials and methods

Experimental breeding consisted of 10 genus of Theraposidae family. Developmental parameters were researched in all of them*: Acanthoscurria, Brachypelma, Chromatopelma, Hysterocrates, Lasiodora Poecilotheria, Phormictopus,* and *Stromatopelma*. Experimental sample was divided into 3 groups: (1) fed often, (2) fed rarely, (3) species was starved (fed very rarely, only to keep them alive).

Breeding conditions: all spiders were kept from early development stages (1-3 instar) in individual boxes. Temperature of breeding was constant: at day oscillated between 28^0C (summer) and 25^0C (winter); at night between 21^0C (summer) and 19^0C (winter). The main food for spiders were crickets in size appropriate to the spiders' age and size. Bigger and better-fed spiders sometimes ate other food instead of crickets, like one-day-old mice or larvae of flour beetle. Everything consumed was measured and tabulated to verify nutritional difference between individuals of the 3 feeding groups in following instars.

All statistical analyses were made using the following programs: SPSS12.0 for Windows, Statistica 7.0, and elements of packet S-plus. SD (standard deviation) and SE (standard error) has been used as the measure of differentiation. Every analysed variable has been verified in accordance with normal distribution (Shapiro – Wilka test has been used). If the variables significantly diverged from theoretical distribution, transformations were used (in or arcsin). Survival analyses have been used to find significant factors affecting survival rates. Tables of survival rates (see Stearns 1992) have been used as matrix for primary data. To compare survival rates between groups, the Gehan-Wilcoxon test was used.

Results

Survival in experimental group

Group first [1] contained 7 genera of Theraposids spiders. There was 11 species, and 52 individuals in this group. In the second group [2] was distinguished also 7 genera, 8 species and 21 individuals. Third group [3] contained 3 genera, 3 species, and 44 individuals. In [1] group spiders moulted mean (±SE) 3.6±0.1, range: 1 – 8, n = 299 times. The last in this group 8th moult obtained 13.4 % of individuals. In group with food deficiency [2] spiders molted mean (±SE) 3.3±0.2, range: 1 – 7, n = 60 times. 7th moult obtained 33.3% of spiders. In starving group [3] spiders moulted mean (±SE) 2.4±0.1, range: 1 – 6, n = 141 times. The last moult in this group – 6th obtained 6.8% of individuals. Respective proportion shows table 1. Proportion of survivorship in 3 groups shows table2.

Table 1 . Amount proportion of spiders in three experimental groups in following instars.

group	instar	% of observation	n
1	1	100.0	52
1	2	98.0	51
1	3	96.1	50
1	4	96.1	50
1	5	71.1	37
1	6	59.6	31
1	7	44.2	23
1	8	13.4	7
2	1	100.0	12
2	2	91.6	11
2	3	83.3	10
2	4	83.3	10
2	5	58.3	7
2	6	50.0	6
2	7	33.3	4
3	1	100.0	44
3	2	79.5	35
3	3	70.4	31
3	4	43.1	19
3	5	22.7	10
3	6	6.8	3

Table 2. Survival in 3 feeding groups.

Group	Start	Instar 1 [%]	Instar 2 [%]	Instar 3 [%]	Instar 4 [%]	Instar 5 [%]	Instar 6 [%]	Instar 7 [%]
1	100	100	98.0	96.1	96.1	71.1	59.6	44.2
2	100	100	91.6	83.3	83.3	58.3	50.0	33.3
3	100	100	79.5	70.4	43.1	22.7	6.8	-

Mean(±SE) duration of instar for [1] group amounted 72.0±4.3 days, variation range: 2 – 264, n = 299. The longest was 8th instar (mean = 272.1 days, n = 7) and 1st instar lasted the shortest time (mean = 38.6, n = 48). Differences between respective instars was significant (ANOVA Kruskall – Wallisa H7 = 70,0, $p < 0.0001$).

Mean(±SE) duration of instar for [2] group amounted 54.3±5.1 days, variation range was included in compartment 21 - 231, n = 58. 7th instar (last) was the longest (mean = 80.0±16.8 days, n = 4), the shortest time spiders spent in 1st instar (mean = 41.4±4.6, n = 10). Differences between respective instars was significant (ANOVA Kruskall – Wallisa H5 = 16.0, $p < 0.01$).

Mean(±SE) duration of instar for [3] group amounted 67.0±4.7 days, variation range: 20 -364, n = 141. The longest was 6th (last) instar (mean = 80.0±16.8 days, n = 4) and the 1st lasted the shortest (mean = 41.4±4.6 days, n = 10). Differences between respective instars was significant (ANOVA Kruskall – Wallis H5 = 65.9, p < 0.0001).

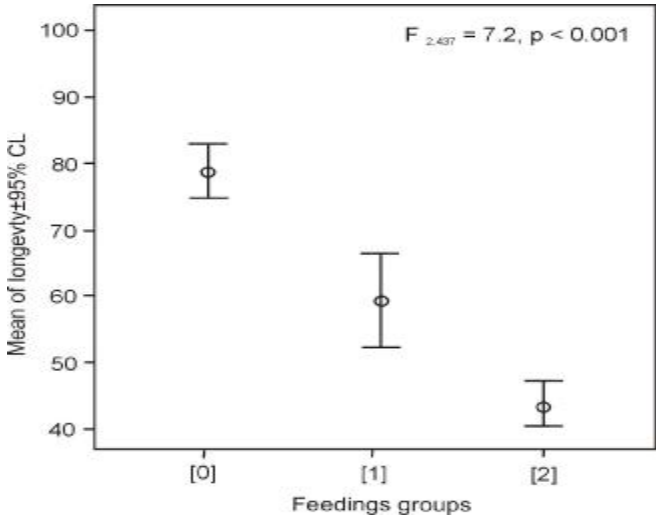

Figure1: mean[±95%CL] live longevity in 3 groups

Reasons of death in experimental group

In most cases direct reason of death was unknown (66.8 % death cases). Respective factors placed in table 2.

Individuals from [1] group was characterized by the longest mean survival (mean = 79.2±0.4 days). Spiders belonged to [2] and [3] groups lived proportional shorter, accordingly: mean = 59.7±0.9 for group [2]; mean = 52.1±2.5 for group [3].

Respective mean live longevities differed significantly (ANOVA F2,437=7.2, p < 0.001

Table 3 Out of experiment reasons of death.

Lp	Reason of death	N	%
1	Did not prepar to moulting	1	2.1
2	Incorrect moulting	2	4.2
3	Died after normal moulting	8	16.7
4	Injury of abdomen	1	2.1
5	Mildew	2	4.2
6	Mites	1	2.1
7	Unknown reason	33	66.8

Discussion

Both results achieved and previous papers (Sigsgaard et al. 2001, Li 2002, Higgins and Rankin 2001, Uetz et al. 2002, Jespersen and Toft 2003) showed great correlation of survival rates with food quantity and quality. It is obvious that spiders kept in optimal laboratory conditions are characterized by better survival in comparison with wild individuals, and this was noted in all developmental stages. Single factor, like different quality of food, introduced into the experiment can result in significant changes in survival rates of immature individuals, which are more sensitive to the factor. The biggest influence of nutrition on survival rates can be observed in spiderlings (2nd –5th instar); mortality was evidently higher for groups with a poor diet, and lower for well fed individuals. Growth rate was higher and intermoults periods were shorter for spiders on higher nutrition levels. In experiments among three groups with different feeding regimes the highest survival characterized the frequently fed group (1).

Lack of food inhibits growth rate: moulting frequency decreases, intermoult periods are longer and increase of body size in every instar is lower. Primary instars are usually shorter, following instars are going to be longer time after time (Jackson 1978, Downes 1987, Buddle 2000). This is true for all spider families and seems to be independent of environmental factors. In (1) group 59.6 % of individuals survived to 6th instar, in (2) group with food deficiency 50.0 % of individuals survived, and in the last (3) starving group only 6.8 %. It has to be said that generally spiders belonging to (1) group survived to the end of experiment and reached 8th instar. Spiders from (2) group reached 7th instar, and last group still stayed in a very long 6th instar. For the above reason, survival analyses have been made only for 6th instar. In early growth period the length of instar duration showed no significant differences between the three groups - first intermoult period in (1) group lasted mean 38.6 days, in (2) group 41.4 days, and in (3) group about 41.5 days. The influence of feeding regime on length of instar duration began to be evident in the second instar. It is hard to explain but against assumption and earlier results the clear observation was made that individuals from (2) group were moulting more frequently and spent less time in respective intermoult periods than individuals fed better (1 group). It was noted already after second moulting – in (1) group 3rd instar lasted mean 52.8 days, and in (2) group 40.6 days. In following instars this difference was more and more significant – 6th instar in (1) group lasted mean 92.8 days, same period for (2) group amount mean 78.5 days. This could suggest that well-fed spiders, which increase their sizes and body mass very fast, can lengthen intermoult periods in comparison to spiders fed rarely but not treated by nutritional stress. Spiders from (3) group and the lowest feeding level showed expected results, meaning that their moulting frequency was the lowest and they had the longest duration of following intermoult periods (6th instar took them mean 328.0 days). Theraposidae, as long-lived animals, reach maturity after a few years. Therefore to get reliable results, the experiments were made on juvenile individuals (to 8th instar). Among them only few completed moulting cycle and it was only males which matured earlier than females. Within the timeframe of observation, most individuals progressed to the 6th to 8th instars. By the 8th intermoult period, the moulting frequency decreases; mean length of 8th instar was 200.6 days.

Conclusions

-all groups of spiders analyzed are characterized by high sensibility to deficiency of food
-under constant breeding conditions group (3) wasn’t able to survive to fifth instar, in (1) group spiders started dieing after 2 instar
-individuals of group (1) and (2) have similar probability of death in a given instar.

-until the third instar all groups have similar growth rates; after, group (1) have the highest moulting frequency and group (3) have the lowest. In (3) group the fifth instar was significantly longer than others instars in this group.

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DIFFERENCE IN MATE CHOICE BETWEEN DALMATIAN PELICAN AND GREAT WHITE PELICAN AND IT'S IMPLICATION FOR HUSBANDRY

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Abstract

In captivity, similar as in situ, Dalmatian pelican (Pelecanus crispus) and Great white pelican (Pelecanus onocrotalus) share colonies. At Poznan Zoo, where sex proportion was skewed in reason of lack of males at both species, have appeared homosexual pairs of females (F-F-P), and interspecific pairs. At those interspecific pairs male was always Dalmatian pelican, and female Great white pelican. The explanation of this phenomenon is in the difference in mate choice: , Great white pelican looking actively partner, and Dalmatian pelican passively. Both sexes are prone to initiate mate choice in both species.

Keywords: zoo, mate choice, interspecific pairs, homosexual pairs, hybrids.

Introduction

Appearance of hybrids in zoos is common: P. onocrotalus and P. rufescens (Grummt 1984 after Hopkinson, 1926, 1939), P. erythrorhynchos and P. occidentalis (Grummt 1984 after Man, 1938; Gray, 1958), P. erythrorhynchos and P. onocrotalus (Grummt 1984 after Antonius 1933; Kloos, 1964, 1969a; Kloos,,1966a) P crispus and P rufescens (Grummt 1984 after Kloos 1966b, 1967, 1969a; Kloos, 1968, 1969b). However, there is no report of ,hybrids between P. cripus and P. onocrotalusd in zoo. In the wild those two species of pelicans occupy the same breeding grounds and sometimes share colonies, (Dementiev i Gladkov, 1951; Dolgušin, 1960; Hatzilacos, 1986; Romanov, 1987; del Hoyo et al. 1992; Linkov, 1994; Gordienko, 1994; Crivelli, 1997; Crivelli et al. 1998).The latter can influence mate choice between the two different species

Great white pelicans usually comes on breeding grounds for about 1- 2 weeks after Dalmatian pelicans, and later starts breeding (Lopatin & Sibgatullin, 1994, Romashova, 1994). This different in breeding time usually prevent appearance of hybrids, but appearance of strange chicks in Prespa (Greece), and genetic investigation at lake Aktas (Georgia- Turkey border), confirm appearance of few hybrids in situ (Alain Crivelli personal communication)

In captivity usually both species of pelican are kept together through the years and risk of hybridisation is much higher. Similar is at Poznan zoo where pelicans are kept together on island in a big pond.

The aim of the present study is to find the explanations of the occurrence of hybrids between Dalmatian and Great white pelicans at Poznan zoo.

Material and methods

The observations were conducted from 1994 till 2007 during the breeding season (over 1000 hours).

Dalmatian pelicans and Great white pelicans share the same exhibit maintained also as a breeding colony.

On average there were 11 males and 15 females of Dalmatian pelican, and 7 males and 11 females of Great white pelicans, and 5 hybrids. Pelicans were kept on a pond 4,39 ha large. There are two islands, but only one, overgrown with holy (Sambucus nigra) and different willow species (Salix sp.) was used for nesting.

Observations were made from south-eastern bank of the pond, using binocular 20/50.

Additionally information from ISIS (International Species Information System www.isis.org) database were used.

Calculations were conducted using the SPSS for Windows package and

STATISTICA 6.0 PL. All basic statistical analyses were applied according to the recommendations of Zar (1999).

Results

Identification criteria of hybrids

First problem in hybridization in pelicans is their identification. All hybrids at Poznan zoo had seen similar. First visible difference appears during development. Chick are dark (Similar as Great white pelican), but in age about 2.5 – 3 month, in contrary to Great white pelicans which appear white down, hybrids left dark. Much more visible as well in chicks as in adult birds is shape of bare skin around eyes and on the bill's base (fig 1).

Base was always similar to Dalmatian's pelican, and bare skin around eyes similar to Great white pelicans. This difference allows an easy identification.

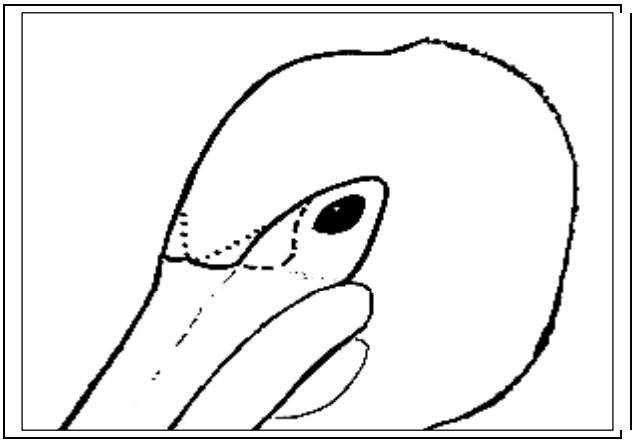

Figure 1. Difference in shape of bare skin around eyes and on the bill's base at hybrids. Spotted line show base of Pelecanus onocrotalus. Dash line show base at P. crispus.

Hybrid occurrence

Up to now Hybrids were recorded in three institutions Budapest (1 chick), Augsburg (3 chicks) and Poznan (5 Chicks). In the three zoos the father was always a Dalmatian pelican, and the mother a Great white pelican.

Occurrence of hybrids was expected at Augsburg zoo where there are 5 males without females of P. crispus, and unbalanced sex-ratio of Great white pelicans (from 2 males and 7 females in 2000year up to 5 males and 9 females in 2006 year).

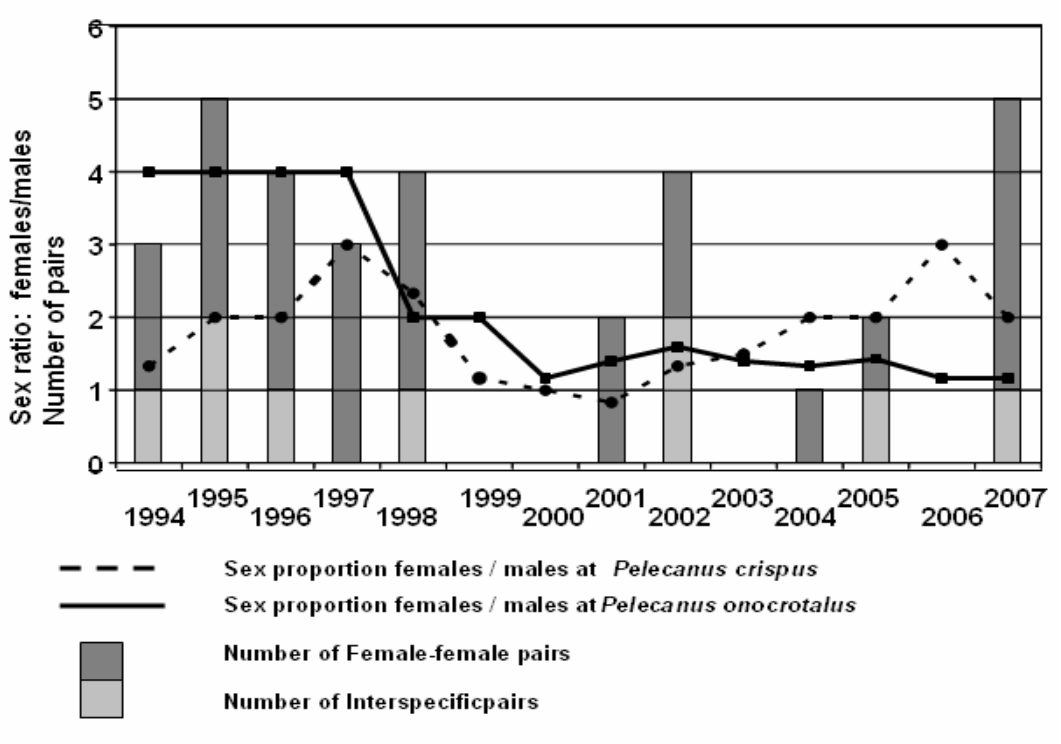

Figure 2. Different in sex proportion of Dalmatian, Great white pelican (Pelecanus crispus, P. onocrotalus), number of homosexual pairs of females and number of interspecificpairs.

The occurrence of hybrids at Budapest zoo was unexpected considering that sex ratio for both species was skewed for more males, unfortunately there were 10 individuals with unknown sex. At Poznan zoo for both species sex ratio was skewed for more females (see fig 2) Risk of hybridisation and Extra-pair paternity should be low due to mate guarding. From all eggs laid by homosexual females pairs only 12,9 % were fertilized, in contrary to 43,7% of fertilized eggs at interspecific pairs. This different is statistically significant, (א2 = 6,56 $p<0.01$), and is similar to monospecific sexual pairs. (56,2%).

In Poznan zoo interspecific pairs were created by 5 males of Dalmatian pelicans and 4 females of Great White Pelicans. Only one exception from this scheme was homosexual, interspecific pair of two females.

Interspecific pairs are not stable and usually birds stay together only for one clutch. Only one pair stay together for repeated clutch (taking eggs and repeating clutch was practised every year).

Typically the situation is that male of Dalmatian pelican was inexperienced (first clutch) on the other hand female of Great white pelican was usually experienced.

Obviously reason of interspecificpairs and hybrids appearance was a lack of males in both species of pelicans. At Poznan zoo appearance of homosexual females pairs and interspecificpairs were correlated with a biais sex-ratio toward females for both species rs=0,571; N=14; p=0,033 . Also statistically significant is correlation at Great white pelicans of sex disproportion and appearance of homosexual pairs rs=0,815; N=14; p<0,01.
On the other hand appearance of homosexual pairs of Dalmatian pelicans, and interspecificpairs wasn't statistically significant.

Difference in courtship of Dalmatian and great white pelican at Poznan zoo

Both species are social birds but Dalmatian pelican is bit more solitary, Dalmatian pelicans created pairs just before breeding; formed pairs were not stable and last only to the end of breeding season.
Very important for pair was nest, pelicans spent a lot of their activity on building it and nest were very solid, sometimes over one meter high.
If pelican loose their first clutch, pair rarely stayed on the nest together, and repeated breeding, but usually they left nest. Before renesting they might choose the same bird or another.
Both sexes were prone to initiate mate choice, usually by inflating pouch behaviour, this is manifestation of readiness to breeding and is kind of matrimonial offer.
If in colony there were bird ready to breed, it's immediately run and answerd by greeting behaviour, so for successful mating in a colony two birds must be ready to breed in the same time. This probability increases with number of pelicans in colony, and have implication for husbandry.
Dalmatian pelicans can breed even when if one pair is present and probability for breeding increase with number of pelicans, and only colonies with large number of birds breed regularly.

Different system of pair maiting occurs for Great white pelican. Pairs are much more stable, and they change partners rarely. They start breeding in given year with past year partner, additionally partners kept contact, by companion, or voice through the year.
Great white pelicans used as nest material everything what laid around nest and nests are less well done than those of Dalmatian pelican. After eggs loss they leave the nest always and the pair remained together.
Pair maiting is also different than for Dalmatian pelican. Similar as it was at Dalmatian pelican also both sexes were prone to initiate to create a new pair. At Poznan conditions unbalanced sex ratio was typical, and it could be reason why usually was observed that females starts pair maiting.
Female run to male and greeted him and escape trying to provoke male to follow her. This behaviour was repeated for many times, finally pair run to the colony.
Great white pelicans are much more social than Dalmatian pelicans. Social stimulation starts at 6-8 individuals and usually all formed a pair and breed.
Pairs are much more stable than Dalmatian, and usually change partner every second year

It seems that Great white pelicans understood Dalmatian pelican's signals.
Alone females of Great white pelicans run to Dalmatian pelican male, greeted him and escaped..

So the important factor in creating interspecific pairs is an unbalanced sex-ratio not in Dalmatian but in at Great white pelicans.
Identificationj of a nest with an interspecific pair is rather easy. Of course if male and female occupy simultaneously one nest or when female of Great white pelican sit at nest seems as Dalmatian one, because mostly male collect nest material.
At pelicans mate guarding exist, so risk of hybridisation in reason of extra pair copulation is rather low.

Discussion

The most probable reason of appearance homosexual pairs of females (F-F P) is lack of males (Shugart, 1980; Conover, 1983; Langrenade & Mousseau 1983; Conover & Hunt, 1984a, 1984b; Kovacs & Ryder, 1985; Lorek & Tryjanowski, 1993). This hypothesis was tested by Conover (1984), He indicated that experimental removing of males influenced on increasing of female-female pairs in larids colony. Up to now we haven't found evidence of homosexual pairs of pelicans in the wild. Only Kazakov et al. (1994) found example how two females laid eggs to one nest (it could be as well polygamy system). Nest with a high number of eggs (Conover, 1986) can be circumstances of appearance F-F P in situ. Numbering of eggs at Poznan Zoo showed that all clutch consisted from more than 2 eggs appeared in reason rolling eggs or F-F P (usually females from nest where there were 4 eggs loose one egg). Results obtained at Poznan Zoo confirm that homosexual maiting females pairs is due to an unbalanced sex ratio. This result should be treated with caution, because one very stable F-F P was counted every year. This pair was created by females after breakdown of polygamy system consisted of male and three females. (Ćwiertnia & Bereszyński, 2003).

We belief that the reason of appearance interspecificpairs is the same (lack of males). In all examples at such pairs male was Dalmatian pelican however female was great white pelican. Probably reason of those are different systems of pair bounding. If Dalmatian pelicans present signal about readiness to breeding (by inflating pouch behaviour) on the other hand great white pelican actively searching partner. This different in behaviour during maiting of interspecificpairs always should take advantage for great white pelican. We could expect that during lack of females, males of great white pelicans should bond with females of Dalmatian pelican. It is strange to observe that two interspecific pairs occurr during 2001 when sex-ratio of both species were balanced.

In situ hybrids appeared what showed genetic investigations (Alain Crivelli, pers. comm.) but are very rare. Probably different in migration on breeding grounds, and follow that different breeding season (Lopatin & Sibgatullin, 1994, Romashova, 1994) prevent that. Another explanation can be that Great white pelicans maiting occur during winter on the wintering grounds. Unfortunately this strategy can be risky, because European and African population of Great white pelican join together during winter. (Izhaki et al., 2002). Maybe this is reason why changing partner (in heterosexual pairs) at great white pelicans always occurred after first clutch of given year.

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THE INFLUENCE OF HUSBANDRY SCHEDULES ON THE ACTIVITY RHYTHM OF CAPTIVE KOALAS

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Abstract

A common problem in koala husbandry is their sensitivity to stress and the related fast loss of weight. Regular weighing is therefore used to control well-being in captive koalas, but if this interrupts resting times, it might pose a stressor itself. Resting times are endogenously controlled and can only be altered to a certain degree; therefore handling schedules should be matched to the activity pattern of an animal.

For this paper, we compared the 24 hour activity patterns over four weeks of one male and four female koalas at Taronga Zoo, Sydney, Australia, with little keeper contact, to the patterns of one male and one female koala at Vienna Zoo, Austria, with weighing once a day. Weighing at Vienna took place at (i) 1015 and (ii) 1600.

At Taronga Zoo, activity patterns were uniform, and showed a distinct daily pattern with little activity in the morning. At Vienna Zoo, patterns differed clearly between individuals. The male showed similarities to the koalas at Taronga Zoo, with longer resting periods in the morning, but the female displayed no distinct resting times.

To test if weighing interrupted the morning resting period at Vienna Zoo, weighing was shifted to 1600. The male responded immediately with a prolonged resting period in the morning, while weighing now fell into the afternoon activity period. The female, however, did not change her activity pattern within the next four weeks.

Based on these data we assume that weighing in the morning can be a disturbance for koalas, so the afternoon would be a better time for handling.

Introduction

While the koala is seen in almost every zoo or wildlife park in Australia, at present it is kept in only six European zoos. There are two major reasons for that. For once, koalas exclusively feed on certain species of eucalyptus, which are difficult and expensive to grow in the northern parts of Europe. The other reason is that koalas are generally regarded as very sensitive to stress. In Australian zoos, stress is considered to be the most common cause of death in captive koalas (Wood 1978).

Since eucalyptus is rich in toxins, koalas need to restrict their food intake and thus conserve energy; they rest for 18 – 22 hours per day (Lee & Martin 1988). Interruptions of resting times as well as handling are considered to be stressors for zoo koalas (Wood 1978). Signs of stress in koalas include loss of weight and fur, but health in koalas can deteriorate beyond recovery at an

alarming rate without the koala showing obvious signs of ill health or stress (Carrick et al. 1990; Gordon 1990; Zoological Society of San Diego 2001).

To monitor well-being in captive koalas, they are weighed on a regular basis. The husbandry guidelines for koalas in Europe and North America require daily weighing if possible, but also warn that this might stress the koala (Zoological Society of San Diego 2001). The impact of weighing might depend on the time of day. Often handling and feeding times in zoos are scheduled according to the keeper's and visitor's needs, not to the animal's. Gattermann and Weinandy (1997) have shown that handling of hamsters during their resting times results in a higher increase of heart rate than during their activity times. Resting times in animals are endogenously controlled and species specific. They are synchronised with the environment by periodical external signals, so-called zeitgebers. The most common zeitgeber is light, but in few cases food or noise can also act as a zeitgeber if light is missing. More often handling and food overlay or mask the endogenous rhythm and, in worst case, disturb resting times (Aschoff 1954).

Studies on free-ranging koalas have shown that they are mainly active during twilight and night, but activity during the afternoon and sometimes even in the morning has been reported too (Nagy and Martin 1985, Robbins and Russell 1978). Similar patterns have been observed in captive koalas in an Australian zoo: there was an extended resting period in the morning and a strong reaction on the introduction of fresh browse (Benesch 2009). To test the influence of weighing on the activity patterns of koalas, in this paper we (i) compare the activity patterns of these koalas, which are weighed about once a month and experience only little keeper contact to the patterns of two koalas from Vienna Zoo, where they are weighed daily in the morning, and (ii) based on the differences found shifted the weighing time into the afternoon to see if the patterns of these koalas change and if resting times were extended.

Material and method

Studied animals

At Taronga Zoo, Sydney, Australia (151°26'E, 33°84'S), one male and four female adult koalas have been observed for 28 days in summer 2003/04 (December to January). The koalas were kept as group in a fenced outdoor area of 113m², containing three 5m high dead trees and one living non-eucalyptus tree. The enclosure was partly roofed to provide shelter.

The keeper entered the enclosure daily between 0655 and 0730 hours for approximately 20 minutes for cleaning, and between 1400 and 1500 hours to remove old eucalyptus branches. Fresh browse was provided at 1530 hours. Koalas were caught in irregular intervals (between one and four months) for weighing. Aside of this, contact to the keeper was rare and brief.

At Tiergarten Schönbrunn (Vienna Zoo), Austria (48°12'N, 16°22'E), one male and one female adult koalas were observed for 28 day in summer 2004 (June to July). The koalas were kept separately in indoor enclosures, which were newly built according to the regulations of the San Diego Koala husbandry guidelines. The floor space of the enclosures was 5.5 m x 4 m. The floor was concrete with no natural substrate. Three 2.5 m vertical tree trunks were provided, which were connected as a triangle by two horizontal trunks in each direction. The enclosures were separated by a medium-high glass wall which allowed the koalas to see, hear and smell each other. In addition to artificial lights, natural light was provided through large skylights. Temperature and humidity have been constant at 22°C ± 1°C and 60% humidity.

Keeper routine was performed according to the San Diego Koala husbandry guidelines: Between 0800 and 0930, the enclosure was entered by the keeper for cleaning. Regularly, the

animals, especially the female, were picked up and carried around for a couple of minutes. After cleaning, a small amount of new browse was provided. At 1015, the animals were weighed on a stationary branch. Afterwards, additional browse was provided. If necessary, at approximately 1545 additional browse was introduced.

To assess the influence of weighing on the activity rhythm, it was shifted from 1015 to 1600 on 1 July 2005. Koalas have been observed two weeks prior and four weeks after the shift.

Data Collection and Processing

The enclosure in Sydney was equipped with four black/white, infrared sensitive CCD-cameras (AD-502A, lenses varied between 4 and 12 mm, Watec Comp.). At night, infrared light was provided by four self-made infrared lights (152 cascaded infrared diodes SFH 485, wavelength 880 nm, Siemens). Animals were continuously videographed by time-lapse recording (two pictures per second).

At Vienna Zoo, a black/white, infrared sensitive Bosch digital video observation system including cameras and infrared lights was used. Pictures were stored digitally and analysed using the picture archive programme BoVis 6.0 (Bosch 2002).

Feeding and locomotor activity of each individual animal was assessed in each five minute intervals. Feeding was recorded whenever a koala was seen feeding for at least 2 minutes per interval. Double plots of activity (48h time scale with every day repeated once) and activity profiles (mean counts per 5-minute interval on a 24h time scale) were calculated showing feeding and locomotor activity using a custom made computer program (© Tronje Krop, TU Darmstadt).

Results

Comparison between Taronga Zoo, Sydney and Vienna Zoo

As shown exemplary for the male and one female from Taronga Zoo, activity patterns were very regular between single days (Fig. 1a,b). Fig. 2 (top) additionally shows the similarity between individuals. There was a clear discrimination between night and day, with little activity in the morning. The koalas did not react on the keeper working in the enclosure at around 0700. At 1530, activity increased immediately after fresh browse had been introduced by the keeper. A second increase in feeding was seen at dusk. Feeding and locomotor activity was high during the night and ceased with dawn.

In Vienna, only the male showed a clear discrimination between day and night (Fig. 1c). Activity began in the late afternoon and was high throughout the night. It decreased during the first morning hours, but there was a slight increase around 0800, when the keeper entered for cleaning. After this, the male rested. Following the weighing at 1015, a short locomotion bout was seen almost daily. Fig. 2 (bottom) shows a clear activity peak interrupting a resting period. Feeding afterwards was only observed on some days, but was more regular in the afternoon.

In the female, there was no clear day-night discrimination (Fig. 1d). She displayed frequent activity bouts during most of the day and during the complete night. Activity ceased at dawn, but began again when the keeper entered for cleaning. During this time, contact to the keeper was regular and frequently induced by the koala. Fig. 2 (bottom) again shows low activity levels prior to the weighing at 1015, which was followed by locomotor activity and a short feeding bout. Afterwards the female usually rested for at least one hour.

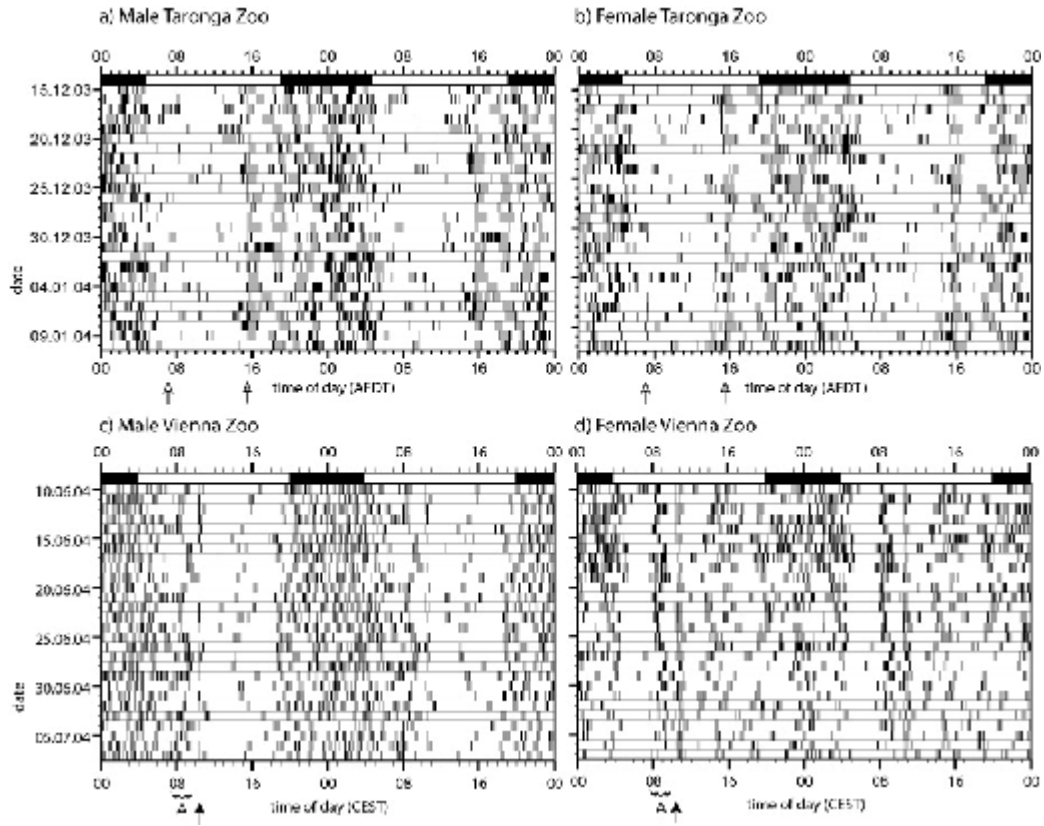

Figure 1. Activity pattern (double plot) of the male (a) and one female (b) at Taronga Zoo in summer 2003/04 and male (c) and female (d) at Vienna Zoo in summer 2004. Grey bars = feeding, black bars = locomotor activity. Dark bar on top indicates natural night. Arrows: white = cleaning of enclosure, grey = introduction of fresh browse, black = weighing followed by introduction of fresh browse.

Activity bouts in Vienna are generally shorter than in the Sydney koalas. Especially feeding bouts of the duration of the one following the food introduction at 1530 in Sydney were not observed.

Despite the dramatic differences between both zoos in the morning, activity, especially feeding, was higher after 1400. Activity further increased in both zoos towards sunset and remained high during the night.

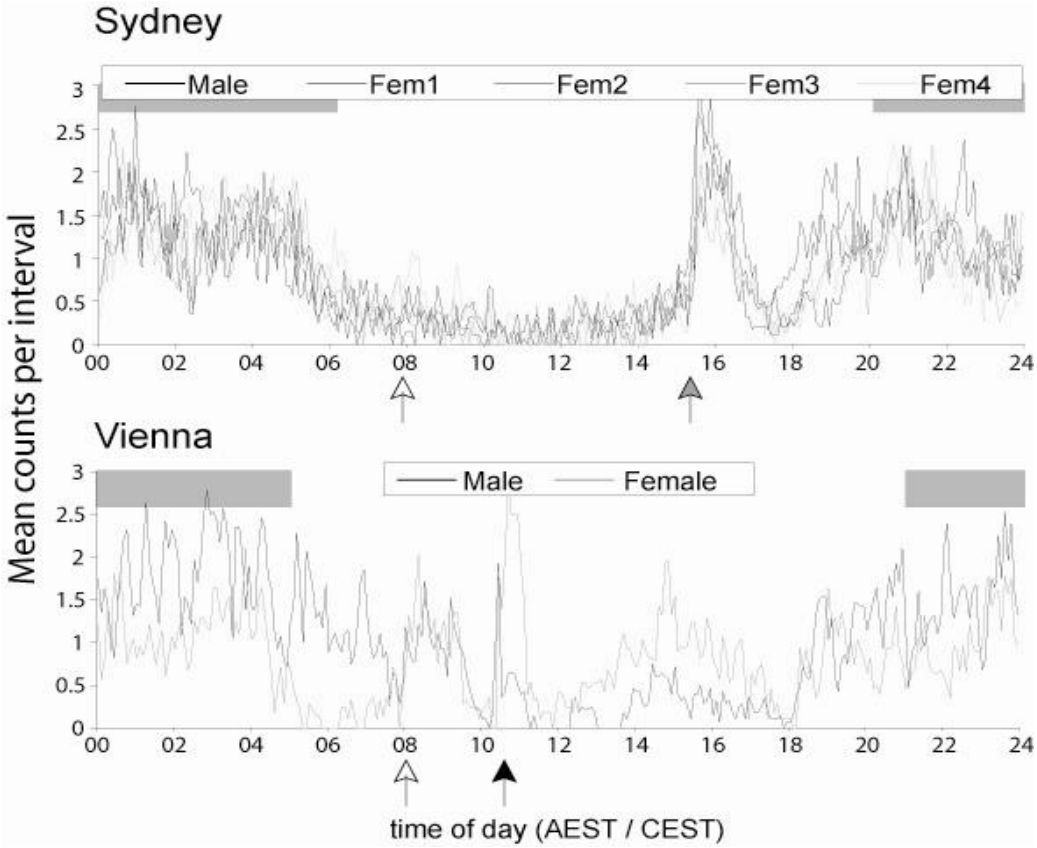

Figure 2. Average activity levels for the individual koalas in Sydney and Vienna over four weeks in summer. Grey bar on X-axis indicates natural night. Arrows: white = cleaning of enclosure, grey = introduction of fresh browse, black = weighing followed by introduction of fresh browse.

Shift of weighing time to 16:00

Based on the activity in the afternoon at Taronga Zoo and in the Viennese male, weighing and browse introduction at Vienna Zoo was shifted into the afternoon in June 2005. In the two weeks prior to the shift, the plots of the male Bilyarra were similar to the plots of the previous summer (Fig. 3a). There was no activity prior and after the weighing at 1015, but a regular short locomotor activity at 1015, resulting from a change of location after weighing. When weighing was shifted on 01 June, the male rested uninterrupted for seven hours before he started feeding in the afternoon from his own volition. After he was weighed, he immediately started to feed on the freshly introduced browse. From this day on, little activity was seen between 10:00 and 14:00, while during the complete observation, there were feeding bouts in the afternoon.

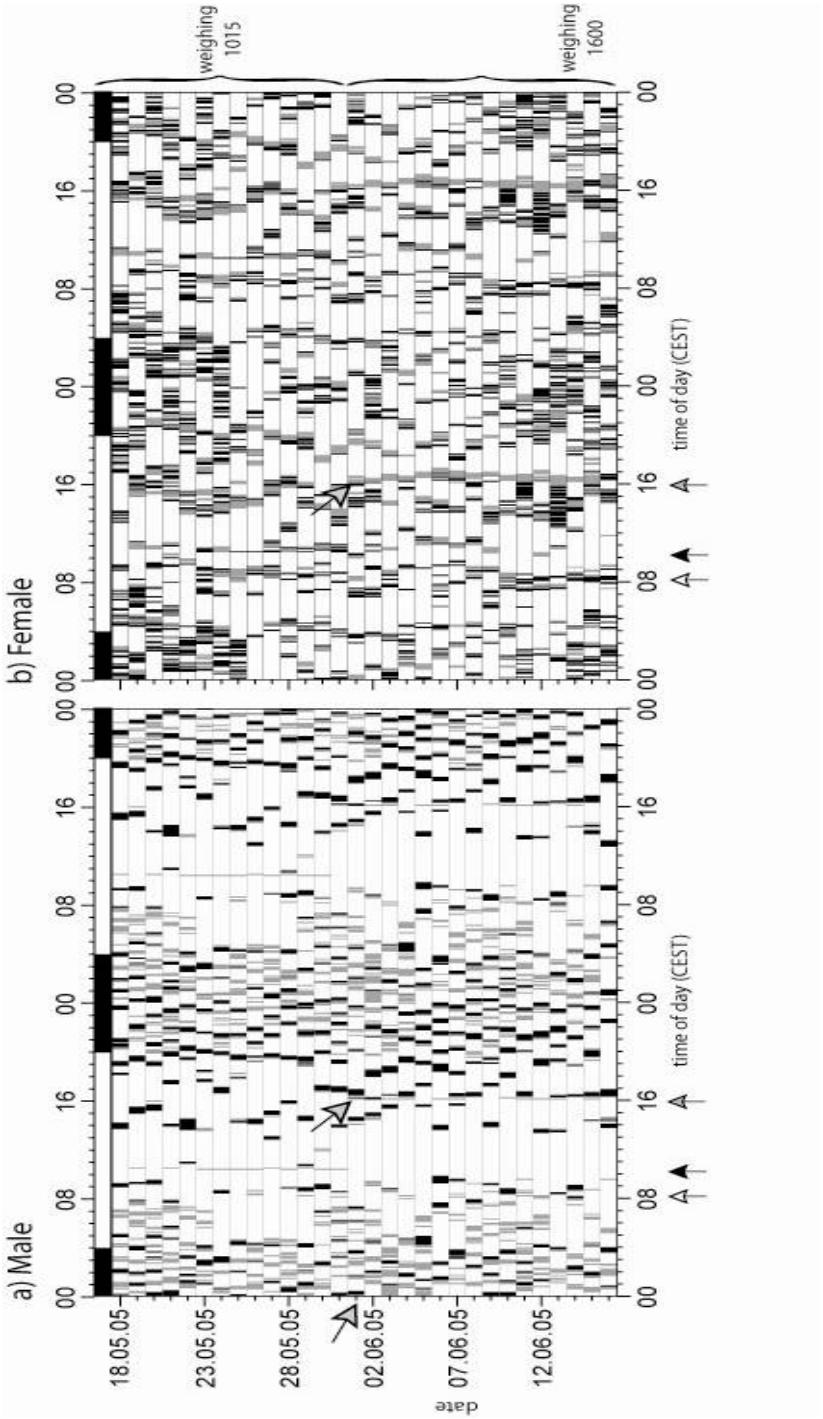

Figure 3. Activity pattern of male and female at Vienna Zoo in summer 2005. Daily weighing time has been shifted from 1015 to 1600 on 1 June (grey arrow). Grey bars = feeding, black bars = locomotor activity. Dark bar on top indicates natural night. Arrows: white = cleaning of enclosure, grey = introduction of fresh browse, black = weighing followed by introduction of fresh browse.

In the female, only little changes were visible (Fig. 3b). The plot shows activity bouts distributed over the complete 24 hours. Weighing was followed by some locomotor activity and feeding, but this activity bout was not longer than those during the remaining time. The morning bout was followed by a longer resting period. In the afternoon, frequent feeding and locomotor activity had been observed between resting periods. After the weighing time had been shifted to the afternoon, there were still feeding bouts and locomotor activity in the morning. These bouts became less frequent after 10 June, but there still was no prolonged resting period in the morning as had been observed in Sydney koalas and the Vienna male. The introduction of fresh browse after the weighing at 16:00 was followed by a band of feeding.

Discussion

The Sydney koalas displayed a strong uniformity in activity patterns between individuals. Long term observations have shown that this uniformity remained stable during the complete year (Benesch 2009). The activity periods during night and afternoon are similar to those observed in free-ranging koalas (Robbins & Russell 1978; Nagy & Martin 1985; Mitchell 1990). Feeding bouts in the morning have been rarely reported in the wild (Nagy & Martin 1985) and morning in Sydney is a pronounced time for resting. Nagy and Martin (1985) found that body temperature of free-ranging koalas was lowest in the morning. Hence, it is possible that the morning is the physiological resting time of koalas.

Both Vienna koalas rested for short periods in the morning, which were interrupted by the presence of the keeper and handling. In the female, the keeper triggered long activity bouts at both times. In the male, activity is triggered during the morning cleaning too. However, prior to weighing he was regularly asleep and usually showed only little locomotor activity and rarely fed afterwards. This leads to the assumption that handling in the morning interrupts the physiological resting times of the koalas.

In the afternoon, feeding activity was regular in both Vienna koalas as well as in the koalas at Taronga Zoo and in free-ranging koalas (Robbins & Russell 1978; Nagy & Martin 1985; Mitchell 1990). In Vienna, this activity was not triggered by fresh browse or keeper's presence. It seems that activity in the afternoon is induced by the endogenous rhythms of the koalas. Therefore, handling during the afternoon might be less disturbing than during the morning. To test this, weighing and second browse introduction has been shifted to 1600 in the afternoon in the second part of this study.

The two koalas reacted rather differently to the shift, which is not to surprising considering their individual differences in their activity patterns. In the two weeks prior to the change, the plots clearly showed that the male was interrupted in its resting time by the weighing. After weighing was shifted to the afternoon, the male started feeding prior to the weighing from his own volition. This shows that the male is much more receptive for food in the afternoon and is probably less disturbed by the handling. The immediate acceptance of the afternoon weighing indicates that weighing in the morning interrupted the endogenous activity rhythm of the male koala.

In the female the change was not as effective. There was little structure in the activity pattern during the first two weeks as had been observed in the previous year. Generally, locomotor activity was high during the study. Weighing was usually followed by some locomotor activity and a short feeding bout. In contrast to the male, there still was activity at the original weighing time after the shift and no extended resting period emerged. It seems that the female lacks an endogenous stimulus for extended resting in the morning. However, activity bouts were

generally less frequent in the morning. It might take longer than the four weeks of observation to manifest a change in activity pattern.

Conclusion and outlook

Data from two zoos and several free-range studies imply that the morning is the physiological resting time of koalas. Handling at this time would interrupt the resting period which can result in a decrease of well-being and health. In both koalas at Vienna Zoo, daily weighing at 1015 seemed to interrupt resting. A shift of weighing time to 1600 resulted immediately in a longer resting period in the morning and more activity in the afternoon in the male, supporting the assumption that weighing masks the endogenous rhythm.

However, in the female, there was no immediate change related to the shift. Her activity lacks a basic pattern similar to other observed koalas. It is possible that other factors, e.g. artificial lighting, have a stronger influence than handling. A control of her rhythms at a later time is advisable to test for long-term changes in the pattern.

The interruption of resting times is a common problem in zoo animals with long resting periods. It is tempting to use food or handling to induce activity and increase attractiveness for the visitors. In some species this might be necessary to ensure an adequate amount of activity for the captive animal. However, the time of handling must be considered and activity should be induced during the physiological activity times of an animal to ensure its well-being and health.

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