Behavioural Cost Minimisation and Minimal Invasive Blood-Sampling in Meerkats (*S. suricatta, Herpestidae*)

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Für Andi und meine Eltern

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List of Abbreviations

CO_2	carbon dioxide
D_2O	deuterated water, or doubly labelled water
DLW	doubly labelled water
EE	energy expenditure
^1H	hydrogen
^2H	D = deuterium
H_2O	water
k_D	deuterium washout rate
kJ	kiloJoule
k_O	heavy oxygen washout rate
kV	kiloVolt
min	minute
µl	microliter
ml	mililiter
n	sample size
N	body water pool
N_D	body water pool derived by deuterium dilution
N_O	body water pool derived by heavy oxygen dilution
^{16}O	oxygen
^{18}O	heavy oxygen
T_A	ambient temperature
T_B	body temperature
T_{LC}	lower critical temperature
TNZ	thermoneutral zone
T_{UC}	upper critical temperature
rCO_2	CO_2 production rate
RQ	respiratory quotient
W	Watt

ABSTRACT

Meerkats (*S. suricatta*) are social mongooses inhabiting semiarid habitats in southern Africa. They are physiologically desert-adapted, but also utilise a high proportion of behavioural thermoregulation. This study investigated how thermoregulation by behaviour can aid to minimise the physiological costs of thermoregulation. In a first part, behavioural observations derived from 230 observation hours at the "Kalahari Meerkat Project" in South Africa were correlated to ambient factors such as temperature, radiation, humidity and wind speed.

It could be shown that temperature had the most significant influence on the distribution of the behavioural data. Especially the meerkats' upper and lower critical temperatures, literature data obtained in the lab, could aid at explaining the proportions of time spent in sun, shadow and below, as well as the proportion of times spent with thermoregulatory behaviours (contact lying, sunbathing and piloerection). The influence of radiation added on the effect of temperature, as the animals utilised solar radiation to heat up at low ambient temperatures (T_A) and avoided it at high T_A. Influences of humidity and wind speed were of minor importance. It is evident, that meerkats utilise a high proportion of behaviour to minimise energetic costs. Extrapolating from physiological literature data, it can be assumed that this minimization can amount up to 18% by a single behaviour.

In a second part, a minimal invasive method of blood sampling was presented for zoo animals, also suitable for wild animals. For the fist time, this study could sample blood using tsetse *G. brevipalpis* in zoo animals on an outside enclosure. Insects were attached to the zoo animals in a box on a collar. These blood samples could be used to obtain data on energy expenditure with the "Doubly-Labelled -Water" (DLW) method and data on hormone levels. In contrast to previous works, study animals were not restricted in their mobility and could move freely during the blood sampling period. As insects were attached to the meerkats with a box, this technique allowed to derive blood samples at defined points of time, although the use may be limited to species habituated to close human presence. For the first time, this thesis presents a dipteran species that can be used as a tool for minimally invasive blood sampling, unlike previous works, that have been working with South American reduviid species. When planning to work in a South American environment, it was shown that these

commonly used reduviids can easily be sterilized with ionising radiation to minimise potential risks to the environment. It could be presented that not only reduviids posses beneficial properties as blood-sampling tools, but except for the obtained blood volumes, tsetse seem to be even more applicable as they were more reliable (in terms of feeding motivation) and less temperature-sensitive, both important criteria when working in the field.

Introducing a so far unimplemented, African blood parasitic species to obtain minimal invasive blood samples, a long term goal derived from this work could be to find suitable insect candidates in every environment where field studies could benefit from minimally invasive blood sampling.

ZUSAMMENFASSUNG

Erdmännchen (*S. suricatta*) sind tagaktive, gruppenlebende Mungoartige die in semiariden Habitaten Südafrikas leben. Physiologisch weisen sie einige deutliche Anpassungen an die Wüste auf, nutzen aber auch einen großen Anteil an Verhalten für die Thermoregulation. Diese Studie hat untersucht inwieweit die physiologischen Kosten der Thermoregulation durch Verhalten minimiert werden können. Im ersten Teil wurden Verhaltensbeobachtungen aus 230 Beobachtungsstunden am "Kalahari Meerkat Project" in Südafrika mit Umgebungsfaktoren wie Außentemperatur, Radiation, Luftfeuchtigkeit und Wind korreliert.

Es konnte gezeigt werden, dass Temperatur den bedeutendsten Einfluss auf die Verteilung der Daten hatte. Besonders die untere und obere kritische Temperatur der Tiere, Literaturwerte die im Labor gemessen wurden, waren besonders wichtig um zu erklären, wie viel Zeit in der Sonne, im Schatten und in den Höhlen verbracht wurde, aber auch wie viel Zeit mit thermoregulations-assoziierten Verhaltensweisen wie Liegen im Flächenkontakt, Sonnenbaden und Piloerektion verbracht wurde. Der Einfluss von Radiation baute auf dem der Temperatur auf, da die Tiere Sonnenstrahlung bei kühleren Temperaturen nutzen, um sich aufzuheizen und Sonnenstrahlung vermieden, wenn es warm war. Luftfeuchtigkeit und Wind schienen nur einen geringen Einfluss auf das Verhalten der Tiere auszuüben. Es wurde deutlich, das Verhalten einen wichtigen Anteil an der Minimierung der energetischen Kosten hat. Wenn man aus Literaturdaten extrapoliert kann man errechnen, dass schon eine einzelne Verhaltensweise die Energieausgaben um 18 % verringern kann.

Im zweiten Teil wurde eine minimal invasive Methode der Blutentnahme an Zootieren präsentiert, die sich auch für den Einsatz an Wildtieren eignet. Zum ersten Mal wurden mit Tsetse-Fliegen *G. brevipalpis* Blutproben von Zootieren im Außengehege entnommen. Die Insekten waren in einer Box eingeschlossen, die mittels eines Halsbandes an den Tieren angebracht wurde. Mit diesen Blutproben konnte der Energiestoffwechsel mit der Doubly-Labelled-Water (DLW) Methode, aber auch Hormontiter bestimmt werden.

Anders als bei bisherigen Arbeiten wurden die Tiere dabei nicht in ihrer Mobilität beschränkt und konnten sich währen der Blutentnahme frei bewegen. Da die Insekten mit Hilfe einer Box an der Tieren befestigt waren, können mit dieser Methode Blutproben zu einem genau

bestimmbaren Zeitpunkt entnommen werden, obwohl das Anbringen der Box wohl eine gewisse Habituation der Wildtiere an den Menschen voraussetzt.

Zum ersten Mal wurden in dieser Arbeit Dipteren als Werkzeug zur minimal invasiven Blutentnahme benutzt, vorherige Arbeiten hatten sich auf den Gebrauch zweier südamerikansicher Reduviiden-Arten beschränkt. Für den Einsatz der Technik auf dem südamerikanischen Kontinent konnte gezeigt werden, dass diese Reduviiden sehr einfach durch Röntgenstrahlung sterilisiert werden können, um potentielle Gefahren für die Umwelt zu vermeiden. Es konnte außerdem gezeigt werden, dass außer den momentan beliebten Reduviiden auch die hier benutzen Tse-tse Fliegen besonders vorteilhafte Eigenschaften besitzen, um die als Werkzeuge zur Blutentnahme einzusetzen. Bis auf die relativ geringe Blutmenge, die entnommen werden kann, erscheinen sie sogar besser für die Methode geeignet: Sie sind im Hinblick auf die Häufigkeit der Probenentnahme zuverlässiger und erscheinen weniger empfindlich im Bezug auf kühle Außentemperaturen, beides wichtige Vorteile beim Einsatz in freier Wildbahn.

Eine bisher nicht für diese Technik benutze, afrikanische Blutparasitenart wurde in dieser Arbeit vorgestellt um minimal invasiv Blutproben von Wildtieren zu entnehmen. Ein aus dieser Arbeit abgeleitetes Langzeitziel könnte daher sein, für jedes Habitat heimische Insekten mit ähnlich vorteilhaften Eigenschaften zu finden. Der Einsatz minimal invasiver Blutproben ist für die Freilandforschung von großem Interesse und um potentielle Gefahren für Ökosysteme auszuschließen empfiehlt es sich, heimische Insekten zu benutzen.

1 INTRODUCTION

Keeping body temperature constant is an energetically costly attribute of the avian and mammalian metabolism. The constantly high body temperature of endotherms allows them to uncouple their activity from ambient temperatures, but the high costs must be complied with a higher energy intake and/or energy conservation by distinct adaptations of behaviour and physiology (Bennett & Ruben 1979).

An animal's daily energy intake is mainly limited by the availability and digestibility of food. Vertebrate and invertebrate food of carnivores differs markedly in its energetic content. Invertebrate food is characterized by lower available energy (McNab 1989). Meerkats (Order: Carnivora; Family: Herpestidae; Species: *S. suricatta*) are mainly insectivorous (around 80% of a suricat's diet), but small vertebrates such as snakes (approx. 10%) or arachnids, as well as eggs and plant matter complete the diet. They forage regularly for these food items, digging in soil and grass and overturning rocks (Doolan & Macdonald 1996). The way of preying the food further reduces the density of available energy (McNab 1989), as searching and feeding on invertebrates on the ground also implicates sand and soil intake. Considering food availability it is necessary to add that prey abundance is highly dependent on climatic factors and can therefore vary significantly.

Meerkats inhabit portions of southern Africa, extending from the south west arid biotic zone and eastward into neighbouring southern savannah and grassland areas. These areas include the majority of the southern tip of Africa up to approximately 17 degrees South latitude. *S. suricatta* inhabits the driest and most open habitat of all mongooses (Estes 1992). It is particularly associated with "firm-to-hard calcareous ground such as that occurring around alkaline pans and watercourses" (Estes 1992). These regions, typical semiarid deserts, are characterised by extraordinarily low humidity, cloudiness, and high temperature fluctuations between day and night (up to 30 °C), erratic rainfall patterns and substantial fluctuations in productivity of flora and fauna (McKechnie & Lovegroove 2000; Schulz 2008; Doolan & Macdonald 1996). These authors state that such environmental conditions are likely to be important in the selection for physiological traits that conserve energy. Accordingly, meerkats have a significantly reduced basal metabolic rate (BMR) within the thermoneutral zone (TNZ). The fact that the TNZ also is distinctly narrow (lower critical temperature T_{LC} = 30 °C and

upper critical temperature T_{UC} = 33 °C), combined with a high thermal conductance and a remarkable capacity for heat dissipation via evaporation supports the conclusion that meerkats are excellent thermoregulators in a hot environment, adapted to desert-conditions (Muller & Lojewski 1986). Under cold conditions though, which occur regularly in Kalahari nights, meerkats have to use a high proportion of behavioural thermoregulation, in order to avoid the high energetic costs of physiological thermoregulation. Especially, as a small animal's energy household generally is more costly, as the proportion of surface and volume is less favourable in small bodies. Such thermoregulation by behaviour includes sunbathing during the day, (Ewer 1963), the avoidance of exposure to extreme ambient temperatures and precipitation, as well as huddling to minimize the proportion of surface exposed to a cold environment (Gilbert et al. 2006; Savory & MacLeod 1980).

The intent of the study is to examine the contribution of behavioural thermoregulation in minimising the physiological costs of thermoregulation and its impact on the animals' energy budget. To measure physiological costs and energy expenditures, the application of heavy isotopes is the method of choice. A new minimally invasive blood-sampling method is presented to recover the isotopes by using blood sucking insects. First results compare the applicability of different insect species. This investigation may provide alternative routes to regular invasive physiological methods in free-ranging animals. By testing a so far unimplemented, blood parasitic species endemic to Africa this study may aid at avoiding the risk of importing invaders and thus reduce potential ecological impacts in natural environments.

1.1 Objectives of this study

In a first part, behavioural observations examine which behaviours are utilised, to which extent they are used and how they are influenced by abiotic factors such as ambient temperature, radiation, humidity and wind-speed.

- The proportions of time spent in sun, shadow and below are examined for their correlation with the above mentioned abiotic factors.
- The effect of abiotic factors on thermoregulatory behaviours such as contact lying, piloerection and sunbathing is examined
- In conclusion, the proportion of time devoted for behavioural thermoregulation is surveyed in the context of a general activity budget.

In a second part, a minimal invasive method of blood sampling in wild animals is presented and first data on energy expenditure (EE) are described. Different options of obtaining data on EE minimally invasive are compared.

- The validity of saliva sampling as an alternative to blood sampling is discussed.
- A minimally invasive method of blood sampling using blood-sucking parasites is demonstrated.
- Different species of blood-sucking parasites are compared for their applicability in this method.
- The new minimally invasive blood sampling method is tested to obtain data of EE

1.2 Backgrounds on Meerkat Behaviour and Physiology

1.2.1 Meerkat Cooperation and Group Living

Meerkats are highly social and live in packs consisting of up to 3 familial groups with a total of 2-40 individuals (Clutton-Brock et al. 2001; Van Staaden 1994). Being „highly social" is attributed to the animals because there are distinct behavioural patterns that cost the individual energy while they act for the benefit of the entire group. These "cooperative behaviours" are:

"Social digging": this includes the cleaning of dens and sleeping burrows from sand, and creating new burrows. Suricate dens tend to be extensive and complex. Meerkats dig and extend tunnels when the ground is soft after rain, but usually they occupy existing burrows, especially ones prepared by ground squirrels *Xerus inauris* (Estes 1992). Helpers of both sexes contribute about the same extent to this cooperative behaviour (Clutton-Brock et al. 2002). Factors other than sex affecting the contributions to the cooperative behaviours are body weight (helpers younger than a year contribute more when heavier) and daily weight gain (helpers older than a year contribute more when gaining more weight per day), respectively (Clutton-Brock et al., 2002).

"Raised guarding" or sentinel behaviour: while a group of meerkats is foraging, a member of the group poses as a look-out, watching for predators and other danger. The sentinel alarms the group with distinctive alarm calls. Sentinel rotation occurs throughout the day among different members of the pack (Manser et al. 2001). Contributions to raised guarding increase with body weight to a greater extent in males than in females.

"Pup feeding": helpers, i.e. (usually) nonbreeding individuals, contribute to rearing young by feeding pups with invertebrates and small vertebrates (Russell et al. 2003). Cubs are fed from

an age of about 30 days on, when they begin to forage with the group, up to 90 days, when the young forage on their own. A higher helper/pup ratio (within larger groups) equals a higher rate of daily weight gain per pup resulting in a higher probability of juvenile survival (Clutton-Brock et al. 2001).

"Babysitting": the breeding female provides the pups with milk. The breeding male may take an active role in parental care by guarding the young. The rest of the pack act as helpers either by allolactating and therefore additionally nourishing the young (Clutton-Brock et al. 2001) or by guarding the den as babysitters. The babysitting by helpers allows the nursing female to forage, thus maintaining a sufficient milk supply (as her metabolism significantly increases during lactation (Scantlebury et al. 2002)). Both sexes contribute to babysitting, but the amount of contribution varies with the helper's sex. Females, especially heavy females, contribute more to the care of the young (i.e. babysitting, pup feeding and allolactation) than males (Russell et al. 2002)

"Allolactation": helping females may also lactate to the dominant female's pups, most commonly when they have lost their own litter, although they also appear to lactate spontaneously (Scantlebury et al. 2002). The author showed that allolactating females decreased in mass, while the other categories of animals (dominant lactating females and non lactating subordinate females) did not. This mass loss was equivalent to an energy deficit of 264 kJ/d, or 35 g milk/d. He concluded that this is potentially enough to support one extra pup per allolactator.

Female helpers generally increase their contributions in rearing young when growing older and/or heavier, whereas males increase their contributions to raised guarding (Clutton-Brock et al. 2000). Among meerkats, females are philopatric, i.e. the females remain and, improbable though possible, may breed as subordinates in their natal group (unless subordinate females are expelled by the dominant female). Males rather leave their natal group and found new groups with (expulsed) female parties, or join other groups as subordinates. Thus sex differences in cooperative behaviour appear to be associated to female philopatry (Russell et al. 2002) The males' lower contributions in rearing the young may be related to the fact that virtually all males disperse from their natal group and breed in other groups. They will gain less from increments in group size than females since they will breed in other groups.(Clutton-Brock et al. 2000). Generally, mortality decreases with increasing group size (Clutton-Brock et al. 1999). This means, by investing energy into the augmentation of the group, a helper increases its own chances of survival, and thus directly benefits from helping.

1.2.2 Behavioural Thermoregulation and Cost Minimization

Mammals are able to maintain a constant body temperature (T_B) by varying behaviour, insulation, and heat production. No matter what combination of methods an animal employs, it maintains an appropriate internal T_B by regulating heat exchange with the environment (Phillips 1992). The metabolic responses of an animal to variations in ambient temperature (T_A) in order to maintain their T_B cost energy. Behavioural adaptations are therefore a common mechanism to reduce the costs of thermoregulation.

Body temperature is established as a balance between heat input and heat loss. Heat input occurs through heat transfer with the environment or from obligatory or regulatory thermogenesis. Heat transfer, either loss or gain, between the animal and its environment can occur via conduction, convection, radiation and evaporation/condensation, or:

$S = M \pm E \pm R \pm C \pm K$ (Equation 1)

where: S = net rate of heat storage in body; M = metabolic heat production (always +); E, R, C, and K = net rates of heat transfer by evaporation, radiation, convection and conduction, respectively (Gordon 1994)

The rate of heat transfer for each mode is proportional to the surface area and, except for evaporation/conduction, proportional to the temperature gradient between the animal and the environment (Frappell & Cummings 2008).

According to Equation 1, an animal can employ several behavioural strategies to avoid the high physiological costs of thermoregulation:

- covering from radiation at high T_A (i.e. moving into shade), sheltering from wind and precipitation at low T_A as well as general avoidance of weather extremes (moving into burrows, crevices and the like, i.e. the usage of more suitable microhabitats)
- decrease locomotor activity in hot environments to reduce metabolic heat production, or increase activity in cold environments
- facilitating heat loss at high T_A by conducting heat to a cooler surface (contact lying)
- sunbathing at low T_A to utilise radiative energy to elevate T_B
- erecting hair (piloerection) at low T_A to increase insulation through an air layer that minimizes heat loss by convection and radiation
- using close body contact to conspecifics at low T_A to decrease body surface and thus minimise heat loss (huddling)

1.2.3 Thermoregulation, Thermoneutral Zone, Lower and Upper Critical Temperatures

Thermoregulation is an excellent example of a negative feedback regulatory system (Gordon 1994). Motor outputs for thermoregulation are derived from autonomic and behavioural processes and are activated to correct for deviations in skin and core temperature (Figure 1.1). The preoptic area and anterior hypothalamus (POAH) is a main site for the integration of thermoregulatory signals in the central nervous system (CNS). The POAH receives inputs from cutaneous thermal receptors via the spinothalamic and trigeminal afferent systems. Also within the POAH and other parts of the CNS are thermal receptors that detect minute changes in the temperature of the CNS. Depending on the severity of the thermal insult, a rise in core and/or skin temperature will lead to one or more adaptations like peripheral vasodilation, evaporative water loss, reduced metabolic rate or behavioural selection of a cooler T_A. Contrarily, a reduction in core and/or skin temperature results in the activation of one or more heat producing/conserving responses, including shivering and nonshivering thermogenesis, peripheral vasoconstriction and behavioural selection of a warmer T_A.

Shivering becomes evident by fast muscular contractions of antagonistic skeletal muscles that release heat because ATP is hydrolysed through the activity of the muscle, but without movement (Eckert 2002).

Only two animal tissues are specialized for nonshivering thermogenesis: brown adipose tissue in small eutherian mammals and cranial heater tissue in billfishes and the butterfly mackerel. Brown adipose tissue contains uncoupling protein 1 (UCP 1) that permits futile cycling of the mitochondrial electron transport chain to produce heat without ATP synthesis and degradation. Fish cranial heater tissue have lost their myofibrillar contractile apparatus and participate in futile cycling of Ca^{2+} between the cytoplasm and the sarcoplasmic reticulum, which is mediated in the ryanodine receptor by Ca^{2+}-ATPase. (Frappell & Cummings 2008)

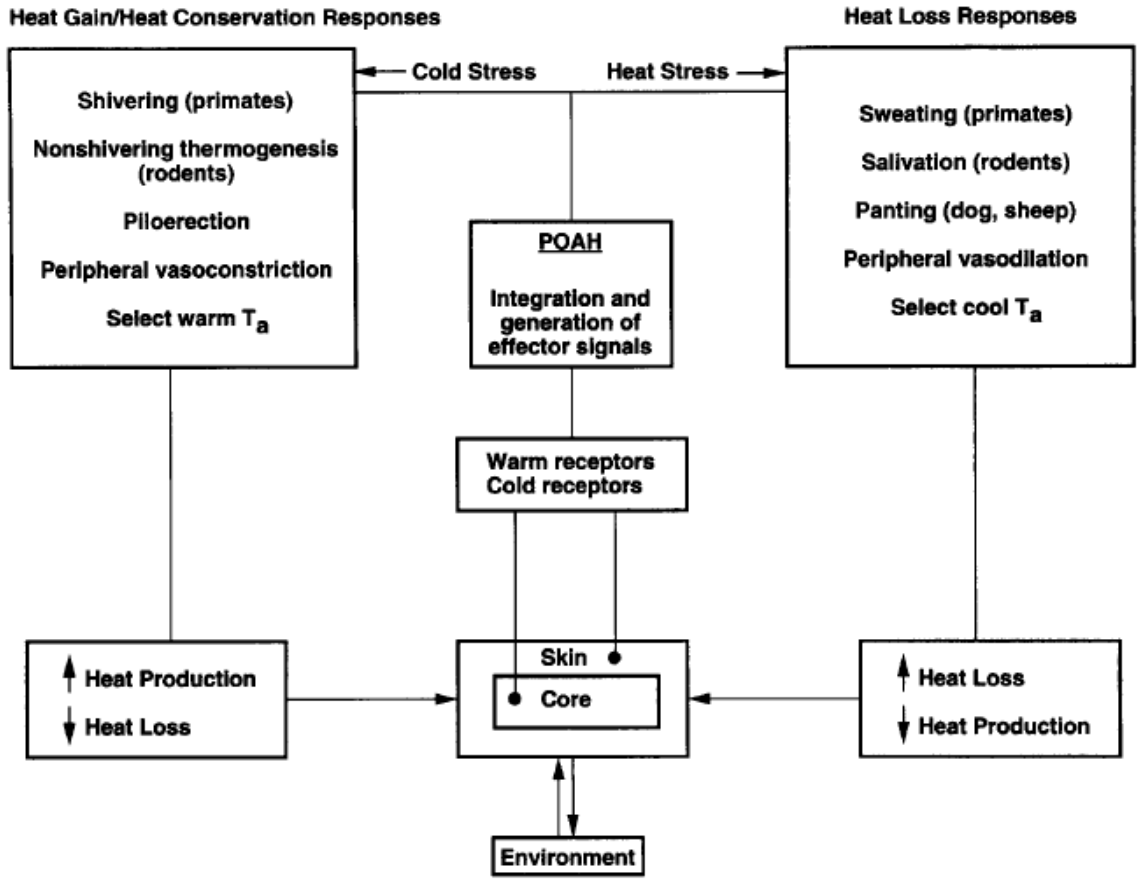


Figure 1.1: Block diagram showing the principal components of thermal homeostasis in mammals. Taken from Gordon (1994)

Note that some thermoregulatory effectors are unique to a particular taxa. For example, an increase in evaporative water loss is mediated through one of three principal mechanisms: sweating (e.g. primates, horse, camel); panting (e.g., dog, sheep); grooming of saliva onto the fur (e.g. many rodents). In rodents heat production via the activation of nonshivering thermogenesis is a key mechanism, especially during the adaptation to cold temperatures. On the other hand, humans and other large mammals have little ability for nonshivering thermogenesis and rely on shivering as the main source of heat production during cold exposure.

A figure demonstrating heat production and core temperature as a function of T_A is a useful way to summarize the general characteristics of thermoregulatory responses (Figure 1.2). The thermoneutral zone (TNZ) is a range of T_A's where metabolic rate is basal and core temperature is regulated with moderate shifts in dry heat loss via the control of skin blood flow. When T_A exceeds the upper limit of the TNZ, metabolic rate rises as a result of increases in respiratory rate, heart rate, motor activity and a direct effect from the elevated tissue temperature on cellular respiration. The T_A, at which metabolic rate increases above basal levels or where active increases in evaporative water loss begin is defined as the upper

critical temperature (T_{UC}). When T_A drops below the lower limit of the TNZ, peripheral blood flow is restricted to minimum levels and the animal must increase metabolic rate to maintain a balance between heat production and heat loss. The T_A at which metabolic rate increases above basal levels is defined as the lower critical temperature (T_{LC}). Laboratory rodents including the mouse, gerbil, golden hamster and rat have T_{LC}'s and T_{UC}'s of approximately 28 °C and 32 °C, respectively. Rats tend to have lower T_{UC}'s compared to the smaller rodent species (Gordon 1993).

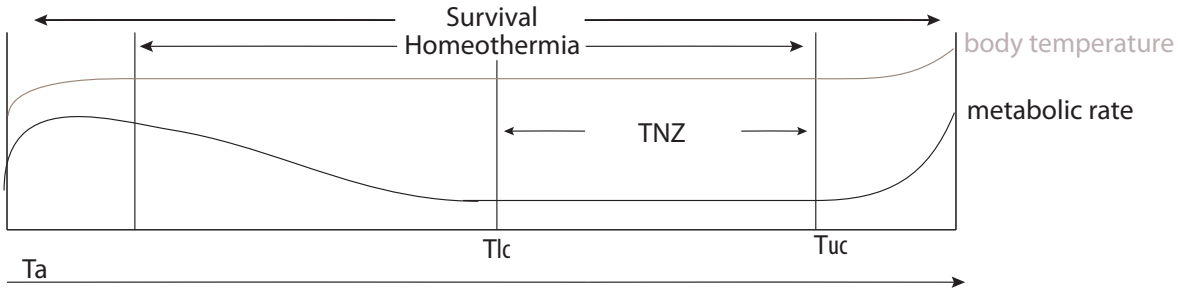


Figure 1.2: Survival-, homeothermian-, and thermoneutral zone
T_A = ambient temperature; T_{LC} = lower and T_{UC} = upper critical temperature, TNZ = thermoneutral zone (redrawn after Bianca, 1971).

1.2.4 Physiological Desert Adaptations of Meerkats

Meerkats have a high T_{LC} at 30 °C and a T_{UC} at 33 °C, representing a narrow TNZ. Their Basal Metabolic Rate (BMR) is decreased markedly (42 % below the mass-specific standard), and their total evaporative water loss (TEWL) at T_A up to 40 °C is sufficient to dissipate the entire metabolic heat production, i.e. they display a remarkable capacity for heat dissipation via evaporation. Together with their high thermal conductance, (18 % above mass-specific standard), this shows that they are physiologically adapted to desert-conditions, conditions of low food supply and high environmental heat load. (Muller & Lojewski 1986)

1.3 Backgrounds on Methodology

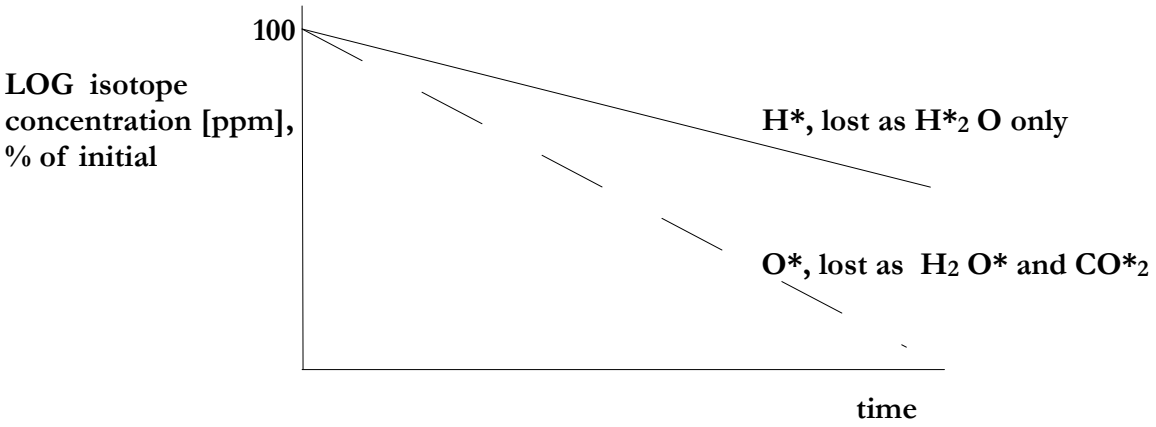
1.3.1 Doubly-Labelled-Water (DLW)

With the DLW-technique, metabolic rates of individual animals can be measured in their natural environment, outside of a laboratory (McNab 1989)

DLW is water containing enriched levels of stable isotopes of hydrogen and oxygen. These isotopes are administered to the animal's body water pool by intraperitoneal injection. In an animal whose body water has been enriched with a hydrogen isotope (H*), the hydrogen isotope concentration declines exponentially with time. H* is lost from the animal in water evaporated from lungs and skin as well as in water voided in urine, faeces, and glandular secretions. In an animal whose body water is enriched with a O*, the concentration of the

oxygen isotope [O*] also declines exponentially through time, but the slope for O* washout as a function of time is steeper than the slope for H* washout. H* is lost in form of water, as well as O*, but O* is also lost as CO_2. The washout of the heavy isotopes' concentrations (H* and O*) in body water are determined by taking samples of body fluids at two distinctive time points. Concentrations of the isotopes in these samples are determined via mass spectrometry. The difference of these two washout rates is then used to calculate energy expenditure (EE).

In body water:



The O* in injected H_2O* comes into isotopic equilibrium with oxygen in CO_2 (dissolved in body fluids) very rapidly, because of the presence of the carbonic anhydrase, which catalyses the reversible reaction forming carbonic acid from:

$$H_2O^* + CO_2 \leftrightarrow H_2CO^*_3 \leftrightarrow H_2O^* + CO^*_2$$

Thus, an estimate of the rate of production of CO_2 is possible from the washout rates (the slopes) of labelled hydrogen and oxygen, introduced simultaneously into the body.

At its best, isotope elimination techniques provide estimates of energy expenditure that have comparable accuracy to the standard laboratory techniques of indirect and direct calorimetry. (Speakman 1997)

1.3.2 Using Blood Sucking Parasites to Obtain Minimal Invasive Blood Samples

To be able to measure EE with DLW, two samples of body fluids are necessary. These can either be saliva, urine or blood. Typically, blood samples give the most reliable results on the concentration of heavy isotopes in body water. Obtaining blood samples from wild animals can be very invasive, as the test animals need to be caught and anaesthetized twice. In small mammals such as meerkats this implies severe health risks of the test animals on the one hand

and, on the other hand also potentially falsifies results as metabolic rates can be increased through stressful handling techniques. Thus, developing a minimally invasive technique of drawing blood samples in wild animals was an important goal of this thesis.

Using reduviid bugs as living syringes has become popular among zoos recently. Von Helversen and Reyer (1984), Schaub and Voigt et al. (2003, 2004, 2005 and 2006) used this technique to acquire blood from animals as small as 10 g body mass. Blood collection is especially difficult in these small-sized mammals because veins are too small for a conventional needle. Voigt et al. reported a potential for contamination of the blood sample with bug haemolymph or intestinal liquids, which was not a hindrance for DLW experiments as DLW is a ratio of two washout rates, and thus a small dilution will not alter the calculation of EE (Voigt et al. 2003; 2005). In other validation studies the suitability of bugs was tested for use in endocrinological studies. (Voigt et al. 2004) compared levels of steroid hormones, (progesterone, testosterone, and cortisol) in blood samples that were taken from the same individual with the conventional technique, and through bug feeding (fourth larval instar of *D. maximus*). In contrast to Voigt et al. (2003 and 2004) the authors took blood from the crop of the animals, thus avoiding contamination with haemolymph. Comparisons revealed no significant differences in hormone concentrations related to blood sampling method. Blood hormone concentrations remained unbiased even after 8 h within the bugs' intestinal tract, and based on hydrocorticosterone levels, the authors determined that blood collection through bug feeding caused less stress to the focus animal than the conventional needle and syringe method (Voigt et al. 2006). Thus, the use of reduviid bugs has been demonstrated to be suitable for the DLW-technique.

Obligate haemophagy in different insect species has evolved to specific adaptations which will be discussed comparatively in the following. Firstly, a general introduction summarizes the problems involved with blood consumption. After that, the specific adaptations to these problems of the species used in this study are listed.

Stimulus detection: External factors effecting the readiness of an insect to probe include vibration, surface texture (skin, hair, feather thickness), carbon dioxide and other odour levels, visual stimuli, contact-chemical stimuli and heat and moisture levels. Of these, heat is an important stimulant in many insects.

Anti-coagulants: Blood-sucking insects release blood from the circulatory system of their hosts by use of their mouthparts. They then take their blood-meal either from a pool that forms on the surface of the skin, or sometimes directly from the blood vessel. It is vitally important for the insect that the blood remains in a liquid form until feeding is complete. Should the blood coagulate, not only will the insect be unable to complete the blood meal, but its mouthparts will be blocked by the forming clot. Given the possibility of this unpleasant and potentially fatal event, it is not surprising that the saliva of most blood-sucking insects contains anti-coagulants. Consistent with the polyphyletic origins of blood-feeding in insects, it has been shown that different insects produce different coagulins that act at various points in the coagulation cascade.

Minimizing weight after feeding: The size of the blood meal is affected by a range of factors including ambient temperature, insect age, mating status, stage of the gonotrophic cycle. The gonotrophic cycle duration may then be defined as the time interval between two consecutive blood-meals, or the time interval between two consecutive acts of egg-laying (Lardeux et al. 2008), previous feeding history, and source of the blood meal (Lehane 2005). Most ectoparasites take up large blood meals (nymphal stages of hemipterans may as much as ten times their unfed body weight). These large blood-meals certainly impair the mobility of the insect, increasing the short-term chances of fatal incidents. The main benefit from taking up very large blood meals is probably the minimization of the number of visits that the parasite must pay to the host. Parasites have adapted their morphology and physiology to minimize the risks involved in taking such large meals, as it imposes considerable mechanical stress on the storage zone of the gut and the abdominal wall. The midgut storage regions of blood-feeders are capable of considerable stretching to accommodate the blood meal. There is some evidence that *R. prolixus* may be plasticized in response to feeding, the elasticity of the abdominal wall being switched on and off in response to the blood meal (Bennet-Clark 1963). The disadvantages of taking up very large blood meals are overcome by the fact that about 80 percent of the blood meal is water. Most of this water is not required by the insects, and they posses very efficient physiological systems for its rapid excretion, thereby reducing their weight and restoring their mobility. To achieve this, the meal is held in a distinct region of the midgut where the epithelium is adapted for rapid water transfer. In triatomine bugs and tsetse-

flies, water movement across this epithelium is linked to a ouabain-sensitive Na^+-K^+-ATPase located in the basal membranes of the epithelium with chloride as the counter-ion (Gooding 1975).

Hemiptera: Reduviidae

Reduviids, especially the subfamily *Triatominae*, are mainly blood feeders, and feed on a variety of vertebrates, including humans, and many are intimately associated with the habitual resting sites or nests of birds, mammals and other animals (Lehane 2005). Most triatomines (125 spp.) are confined to the Americas.

Triatomine bugs are obligate haematophages. Most feed at night and have an almost painless bite. Early instar nymphs may take up to 12 times their unfed body weight in blood, while adult bugs rarely take three times their unfed body weight. They posses a distinctly elongated head, bearing two prominent eyes, and four-segmented antennae laterally inserted on the head. As in bedbugs, the non-feeding insect folds the straight, three-segmented elongated rostrum (proboscis) under the head. These mouthparts are swung forwards in front of the head for feeding.

Hemiptera: Reduviidae: Rhodnius prolixus

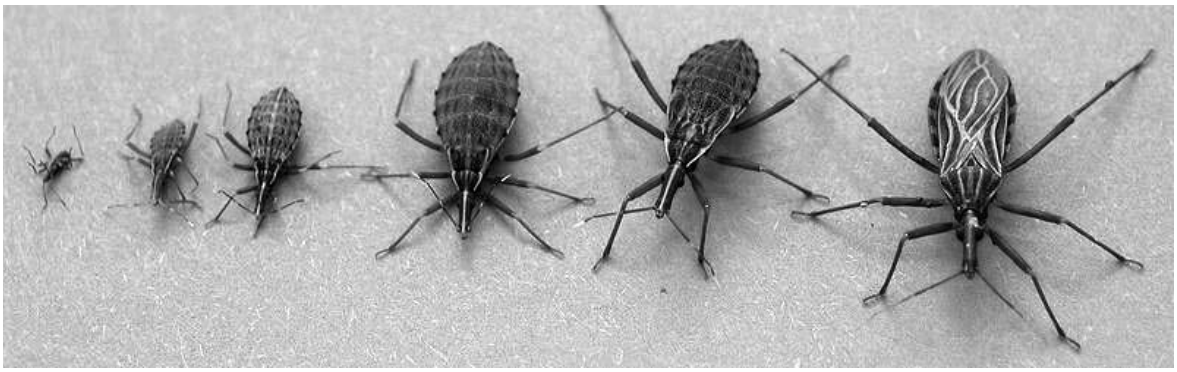


Figure 1.3: Larval stages 1-5 and adult stage of Rhodnius prolixus.
This image was published under the general public license GPL.

R. prolixus has become closely associated to humans' domestic and peridomestic environment. They are vectors of *Trypanosoma cruzi*, the causative agent of South American trypanosomiasis, or Chagas' disease. This is a zoonosis which, in its sylvatic cycle, is transmitted between a variety of animals, mainly rodents and marsupials, by sylvatic *triatominae*.

Stimulus detection: *R. prolixus* responds to temperature gradients between the host and the environment (convective heat). They will attempt to probe the inside of a glass container warmed on the outside by hands. In these insects, the heat receptors are restricted to the antennae (Schmitz et al. 2000)

Anti-coagulants: The salivary anti-coagulin of *R. prolixus*, Prolixin S, disrupts the coagulation cascade by preventing factor IX (Isawa et al. 2000). Removing salivary glands could

experimentally demonstrate though that *R. prolixus* can still successfully feed from a live rabbit, although feeding is slowed down (Lehane 2005).

Minimizing weight after feeding: Their very efficient pump works by generating an osmotic gradient across the epithelium, pulling out water passively. It is possible that the pump in *Rhodnius* is switched on by the same diuretic hormone, released from the mesothoraric ganglion in response to the blood meal, which stimulates a 1000-fold increase in fluid secretion from the malpighian tubes. These systems are so efficient that most of the fluid in the very large blood meals of *R. prolixus* is discarded within four hours of ingestion (Pereira et al. 1998).

The red blood cells in *R. prolixus* are lysed by haemolysin, produced in the anterior storage region of the midgut where no proteolytic digestion of the blood takes place (Azambuja et al. 1983).

Hemiptera: Reduviidae: Dipetalogaster maximus/maxima

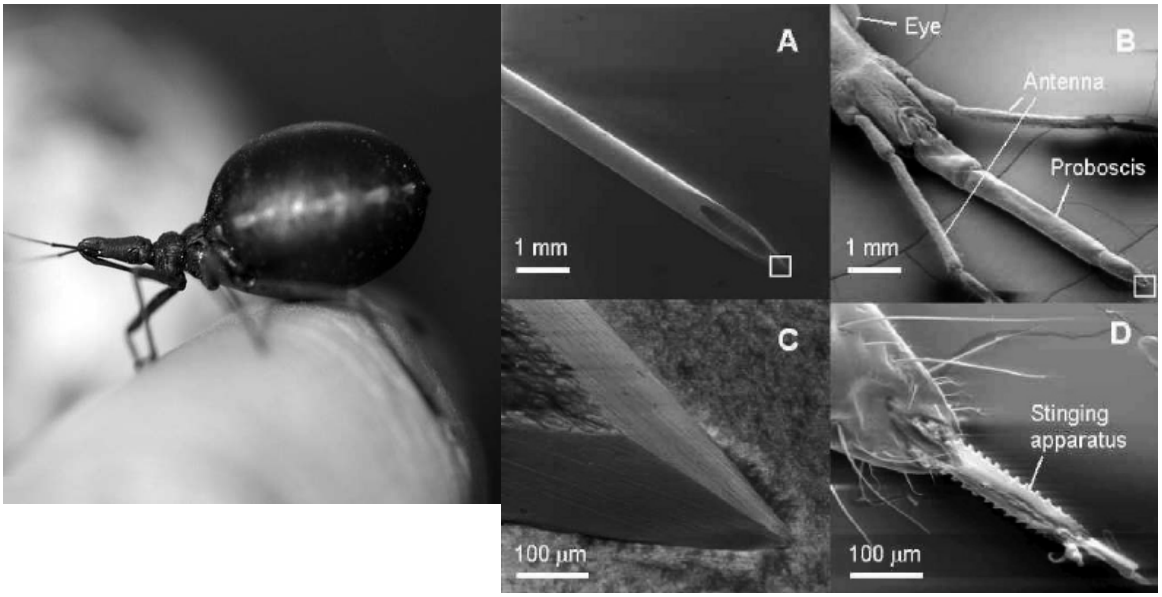


Figure 1.4: Dipetalogaster maximus on a human finger.
A-D: Comparison of the size of a conventional needle inserted into skin and the mouthparts of Dipetalogaster maximus. Pictures A-D taken from Voigt et al (2006).

Except for its size, the gross anatomy and physiology of this species is virtually identical to that of *Rhodnius*. *Dipetalogaster* is the largest reduviid known (Ryckman and Ryckman, 1967). Adults measuring 4 cm in length and each instar being about 6 times larger than the corresponding one in *Rhodnius* (Nijhout 1984). Despite their size, the stinging apparatus of these *triatominae* punctures the skin of the host in a much smaller spot than a conventional needle. Only the saw-blade-like tip of the proboscis shown in Figure 1.4 D is inserted into the host, whereas the whole diameter of the needle as seen in Figure 1.4 A is inserted into the skin when using a conventional blood sampling method.

Diptera: Glossinidae: Glossina spec.

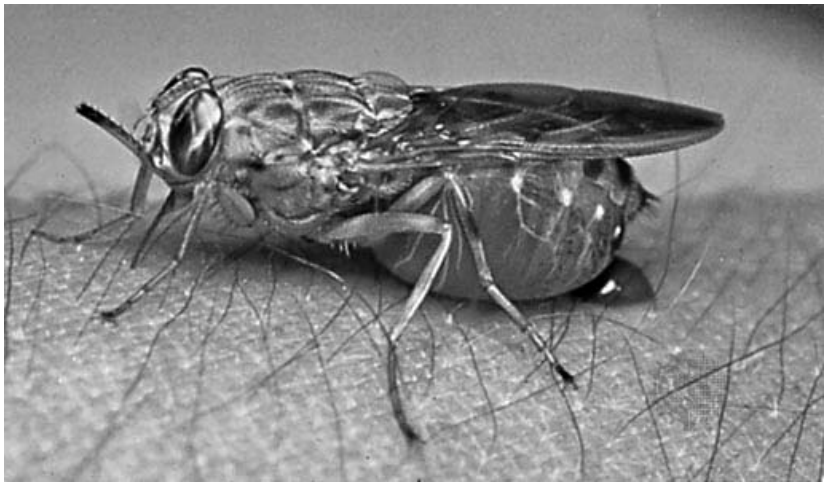


Figure 1.5: Glossina brevipalpis, sitting on human skin.
Picture: Anthony Bannister, from http://www.britannica.com

Stimulus detection: In tsetse flies, heat receptors are found on both the antennae and the prothoracic leg tarsi (Reinouts Van Haga & Mitchell 1975). Using these receptors, the fly can monitor substrate temperature and, provided there is a temperature differential between the substrate and the air, probing may be initiated. Elongated probing bouts could be induced *in vitro* by adding uric acid on the substrate, a component of human sweat, indicating that this odour excites taste neurons in the legs of the tsetse. (Van Der Goes Van Naters et al. 1998) could thus show that especially the combined stimulation of chemo- and thermoreceptors elicits a prolonged biting response. Using uric acid, feeding bouts more than doubled in length.

Anti-coagulants: *G. morsitans* uses an anti-thrombin in its saliva. But like *R. prolixus*, experimentally removing the salivary glands of the insects did not keep them from successfully completing a number of blood-meals on a host, although clots did form in their mouthparts (Lehane 2005). This suggests that although anti-coagulants seem to have an important role in protecting the fly from potentially harmful blood clots, anti-coagulation is not the only significance of saliva.

Minimizing weight after feeding: Taking up large quantities of blood does effect the mobility of the fly. Flight speed of *G. swynnertoni* decreases from 15 to 3-4 miles per hour after feeding, with the fly often only capable of a downward glide away from the host (Lehane 2005). The abdominal wall is provided with stretch receptors to prevent overdistension. This is neatly shown in female tsetse-flies, which retain the developing larva inside the abdomen until the larva is fully mature. As the larva grows, the size of blood meal diminishes so that the abdomen never exceeds a certain volume (Lehane 2005). Systems to lose the additional weight gained by the blood meal rapidly are so efficient that tsetse-flies can shed about 40 percent of

the weight in the first 30 minutes following feeding (Gee 1975).

Erythrocytes are haemolysed in tsetse in the posterior, digestive region of the midgut (Evans & Gooding 2002).

Hemiptera: Cimicidae: Cimex lectularius

Figure 1.6: Cimex lectularius, the common bed bug.
Source: http://www.entomology.ksu.edu

All members of the family *Cimicidae* feed on blood (Lehane 2005). Cimicids are now found throughout the world and are particularly well represented on the northern hemisphere. The majority are parasites on bats and/or birds, although a minority of species also feeds on larger mammals. The latter include the two species usually known as bedbugs, *Cimex lectularius* and *C. hemipterus*, which normally feed on humans. *C. lectularius* has been carried to all corners of the world in people's belongings, but is encountered most often in temperate regions. Bedbugs bite at night, and heavy infestations can disturb the sleeping human. Some individuals develop marked responses to bedbug bites that may include oedema, inflammation and an erythema at the site of the bite. A role for the transmission of diseases is yet to be proven, although the laboratory bedbugs have been shown to excrete hepatitis-B surface antigen for up to six weeks after an infected meal (Ogston & London 1980). Cimicids are also important economic pests of poultry.

Adult bedbugs are typical cimicids, being wingless, dorsoventrally flattened, brownish insects about 4-7 mm in length. Viewed from above they have a rather oval shape (Figure 1.6). The short rudimentary wings, or hemelytra, are clearly seen and well-developed eyes are clearly visible. When these insects are not feeding, the mouthparts are folded beneath the head and thorax. During feeding they are swung forwards in front of the head.

Nymphs and adults of both sexes feed on blood. As is normal in insects living entirely on blood, cimicids have symbiotic micro-organisms that provide supplementary nutrition. In bedbugs, these are housed in a mycetome sited in the abdomen. Bedbugs are unusual in

having two different symbiotic organisms in their mycetome.

Stimulus detection: Bedbugs feed mainly at night. During the feeding period of about ten minutes, they take two to five times their own body weight in blood from their hosts. They are only drawn to warm objects (37 °C) when they are less than 5 cm away, but react on a temperature difference (convective heat) of only 1-2 °C from the host to the environment (Lehane 2005). *Cimex lectularius* produce chemical haemolysins in their salivary glands.

Mating in bedbugs is very unusual. The male penetrates the cuticle of the female and deposits his sperm into the organ of Berlese (the organ where the female stores the sperm). The sperm eventually reaches the ovary by migrating through the haemolymph to the base of the oviducts, where they ascend to reach the unfertilized egg. The female lays about eight eggs a week, and may produce 100 or more during her lifespan. The eggs are cemented into cracks and crevices, in which the adults congregate and hide between feeding forays, and they hatch in about 7-10 days. There are five nymphal instars, each of which requires one to two blood meals before moulting into the next stage. Under good conditions the egg-to-adult period may be as short as five weeks, but bedbugs are characterised by a marked ability to withstand starvation- in the laboratory adult bugs may go as long as 18 months between meals. Consequently, adult lifespans and the length of time spent in each nymphal instar are particularly variable in these insects.

1.3.3 The "sterile insect technique" (SIT)

Sterile insect technique is a method of biological control, whereby millions of sterile individuals are released to eradicate the potentially harmful species in a certain environment. The released sex are normally males as it is the female that causes the damage, usually by laying eggs in the crop, or, in the case of mosquitoes, taking a bloodmeal from humans and thus spreading pests. The sterile males compete with the wild males for females. If a female mates with a sterile male then it will have no offspring, thus reducing the next generation's population. Repeated release of insects can eventually wipe out a population, though it is often more useful to consider controlling the population rather than eradicating it.

Insects are mostly sterilized with radiation, which might weaken the newly sterilized insects, if doses are not correctly applied, making them less able to compete with wild males. However, other sterilization techniques are under development which would not affect the insects' ability to mate. The technique has successfully been used to eradicate the Screw-worm fly (*Cochliomyia hominivorax*) in areas of North America.

The International Atomic Agency's Joint Programme has been developing the SIT against the most important tsetse species throughout Africa over several decades. Recent developments on membrane feeding, pupal sex-separation and semi-automated rearing could considerably increase production capacity and decrease sterile male production costs. For further information, consult the IAEA at: http://www-naweb.iaea.org/nafa/ipc/tsetse-flies.html.

Following examples and dosage recommendations of the SIT, insects used in this thesis were infertilised using ionizing radiation as a precaution against bastardization of fauna.

2 Animals, Materials and Methods

2.1 Animals and Their Environments:

2.1.1 Zoo animals

Table 2.1: House name, chip number, date of birth (D.O.B.) and sex of the zoo meerkat groups. (D) denotes dominance. Numbers in brackets represent numbers in Tables 3.1 and 3.4, as well as Figure 3.12.

MAIN ENCLOSURE Cologne			
Name	**Chip**	**D.O.B.**	**Sex**
Whithney (D)	9357	03.08.04	F
Percy	D3D6	10.10.99	M
Stevie (D)	886079	27.08.05	M
Jim	7838	21.09.06	M
Franz	0084	03.02.07	M
Ferdinand	7994	03.02.07	M
Michael	5338	03.02.07	M
Mark (7)	9238	22.04.07	M
Ace (8)	816079	22.04.07	M
Cass	6076	22.04.07	M
Benji (5)	4015	27.08.07	M

Name	**Chip**	**D.O.B.**	**Sex**
Eloy	6740	27.08.07	M
Basti	1453	27.08.07	M
Jack	4905	24.01.08	M
Martie	2669	11.04.08	F
Nathan	7834	11.04.08	M
Per	5028	28.06.08	M
Marie(6)	4160	28.06.08	F
SMALL ENCLOSURE Cologne			
Mario (4)	D-521C	08.09.96	M
Adam (1)	7392	10.02.03	M
Bob (2)	-	10.02.03	M
Buddy (3)	-	10.02.03	M

Animals in other Zoos:

Tierpark Fauna, Solingen, Germany			
Label	**Chip**	**Year of Birth**	**Sex**
LS (9)	-	2006	F
MB (10)	-	2006	F
RS (11)	-	2006	M
O (12)	-	2006	F

Toni's Zoo, Bertiswil – Rothenburg, Luzern, Switzerland			
Name	**Chip**	**Date of Birth**	**Sex**
Robbie	1337	15.05.02	M
Tina	2824	22.04.03	F
Bono	-	02.10.03	M
Larry	2759	02.10.03	M

2.1.2 Animals Kalahari Meerkat Project:

Table 2.2: Name, code and date of birth (D.O.B.) of the three observed groups (Lazuli, Aztecs, Elveera) at the "Kalahari Meerkat Project".
(D) denotes dominance. "Code" = meerkat project individual code, also denotes for the individuals sex: 2nd letter = group initial where individual was born, e.g. L for Lazuli, 3rd letter = sex (M/F).

Lazuli		
Name	**Code**	**D.O.B**
Aretha (D)	VLF094	22.02.04
J. Alfred Prufrock (D)	VLM105	22.02.05
Young	VLF111	12.03.05
Thundercat	VLM114	30.09.05
Bernard	VLM119	24.04.06
Caroline	VLF123	24.04.06
Shaka Zulu	VLM124	24.04.06
Bash	VLM126	02.10.06
Bosh	VLM127	02.10.06

Aztecs		
Name	**Code**	**D.O.B.**
Monkulus (D)	VWF063	22.02.04
Zaphod (D)	VVM032	09.12.98
Logan	VWM073	16.09.04
Alonzo Mourning	VWM100	11.11.05
Orinoco	VWM101	11.11.05
Burdock	VWF115	25.01.-01.02.07
Squig	VWF118	25.01.-01.02.07

Elveera		
Name	**Code**	**D.O.B.**
Jo Jo Hello (D)	VEF079	24.11.02
Teabag (D)	VYM113	08.07.04
Biltong	VYM114	08.07.04
Ash	VEM108	06.09.05
Gijima	VEM118	19.03.06
Mr. Scruff	VEM122	12.08.06
Beebop	VEF124	03.01.07
Rocksteady	VEM125	03.01.07

2.1.3 Zoo enclosures

Enclosure Cologne Zoo

The current Cologne meerkat enclosure was built in 2003. It offers large space (481 m²) compared to the previous enclosure (about 20 m²). Furthermore, several attributes ensure the animals' needs are complied with: five heat lamps, attached to umbrellas, are distributed over the enclosure. These provide the necessary heat in about 10 % of the total area and thus allow spatial choices, especially during wintertime.

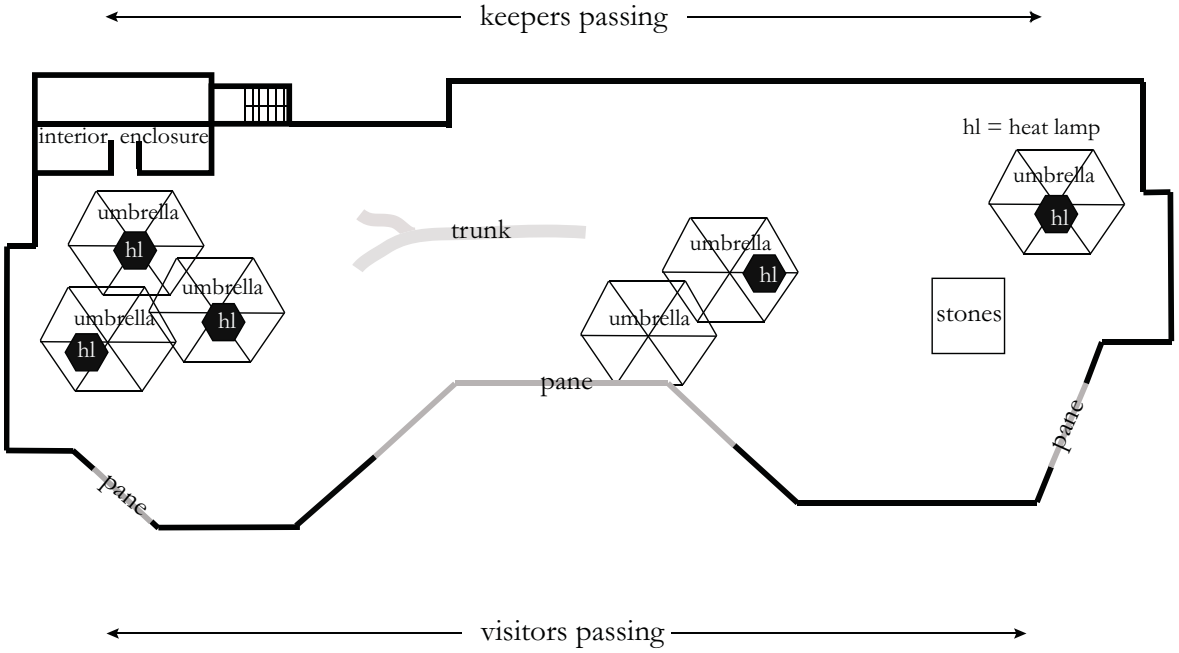


Figure 2.1: Enclosure map Cologne Zoo.

The umbrellas are special constructions that are joined to an drainage system, in order to collect rain water and avoid puddles on the surface (Figure 2.1). The ground of the enclosure is a custom built loam/sand mixture, very similar to Kalahari ground. This has two advantages: the consistence is hard enough to allow digging and this mixture has very distinct thermal properties. According to (Van Staaden 1994), temperatures in the underground burrows are moderate as the soil radiates the absorbed heat with an 8-hour lag. Hence, burrows are coolest during the day and warmest during the night.

Enclosure Tierpark Fauna:

The principal design of this enclosure is very similar to that of Cologne zoo, as the ground is also made of a sand-loam mixture that allows the meerkat to dig lasting burrows. There is one heated, raised position on the outside enclosure. The overall size is considerably smaller than in Cologne (as is the numbers of meerkats housed there). A heated inside enclosure provides a sheltered environment that is regularly used during the wintertime. Unlike Cologne, the inside enclosure also has a window that allows the meerkats to scan the sky while being inside.

2.1.4 Kalahari Meerkat Project

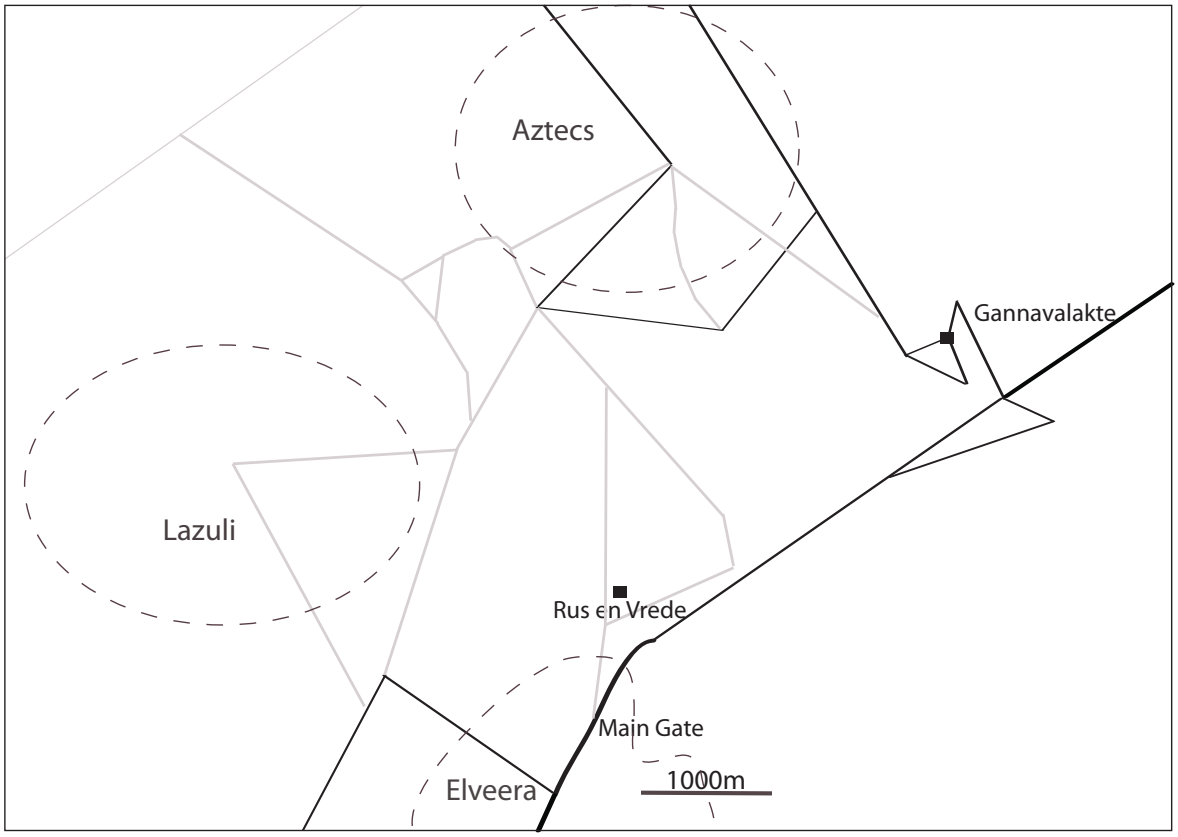


Figure 2.2: Map of the main "Kalahari Meerkat Project"-area with both farm houses (Rus en Vrede and Ganavalakte). Dashed lines indicate the territory of the respective meerkat group between February and March 2008. Permanent lines indicate roads, while bigger roads are drawn thicker than smaller roads.

The "Kalahari Meerkat Project" is a field project situated near Van Zylsrus, South Africa. The area consists of sparsely vegetated sand dunes and river terraces, on either side of the dry Kuruman riverbed. Data were collected from three habituated meerkat groups with comparable sizes that occupied sizes from 2-8 km^2. Animals were habituated to the researchers, visited daily, individually marked and at least one member of each group had a radio collar that could be found via telemetry. Consequently, it was possible to locate a target animal at a desired time.

2.2 Methods

2.2.1 Standard Procedures

Characterization of Individuals - Marking

After the meerkats had been identified by scanning their transponders, the animals' fur was regularly highlighted with black hair dye in distinct positions to facilitate individual recognition (e.g. "right shoulder").

Weighing

A scale (Sartorius TE 4100; capacity 4 kg, resolution 1 g, precision ± 2 g) was placed on a balanced position within the enclosure or in the field. Meerkats were trained to step on the scale by means of positive enforcement training, so weight could be determined for each individual meerkat. This procedure was repeated each morning and evening in order to determine daily weight gain and overnight weight loss.

Ambient Temperature, Humidity, Wind and Radiation

During behavioural observation ambient temperature (T_A), humidity, wind and total solar radiation were measured every 15 minutes. T_A and humidity were probed using a thermo-hygrometer (Dewpoint Pro, Carl Roth GmbH + Co. KG, Karlsruhe, Germany; scale: -40 °C - +70 °C, resolution 0.1 °C, precision 0.5 °C), wind was measured using an anemometer (Windmaster 2, Kaindl electronic, Rohrbach, Germany; resolution 0.1, precision ± 4 %). Radiation was measured using a global radiation analyser (Mac Solar, Solar Cosa Berlin; scale: 0 – 1400 W/m^2, precision ± 2 %). All measurements were obtained at about 20 cm above ground within the animals' microclimate.

The Mac Solar is able to detect global radiation, i.e. direct solar radiation as well as diffuse atmospheric radiation using a photovoltaic cell. Hereby, the biggest amount of energy is contained in the infra-red part of the spectrum, as most ultraviolet radiation is absorbed by the atmosphere. Thus, it is important to measure global radiation as every energetic beam reaching an animal can possibly contribute to its thermal load.

Radiation was measured directed upwards, downwards and from both sides at meerkat height while being orientated to the South.

Temperature was measured while the thermal sensor was shielded from direct solar radiation, as this would falsify the T_A measurements. This means, usually the sensor was placed below a shrub or small bush.

2.2.2 Behavioural Data:

All behavioural data was observed by means of scan sampling, with instantaneous sampling recording-rules (Martin & Bateson 2007). Scan interval was one minute. Scans were recoded using a Toshiba Pocket PC e350 with Microsoft© Pocket Excell software.

Behaviour was classified in mutually exclusive categories (for extensive explanation of these categories please check the ethogramm in APPENDIX):

Thermoregulatory relevant behaviours and physiological reactions:
sunbathing, huddling, contact lying, stay in shade, stay below, (piloerection, shivering, panting)
The three bracketed physiological reactions were recorded additionally and are not mutually exclusive with the other behaviours.

Other behviour: out, lying, allogrooming, low sitting, high sitting, eating, low standing, high standing, move, foraging, run, playfight, mob, frenzy, fight, climbing

Helping behaviour: babysitting, guarding, pupfeed, digging

2.2.3 Physiological Data

Doubly-Labelled-Water

Isotopes Used

Validation experiments for the minimal invasive application of the DLW-technique were conducted with either deuterated water D_2O (>99 APE Deuterium, Sigma Aldrich, Taufkirchen, Germany) or doubly-labelled-water $D_2{}^{18}O$ in a high enrichment mixture (about 65% ^{18}O and 35% D), purchased from Prof. John Speakman's "energetics research lab" in Aberdeen, Scotland.

Sample Analysis

DLW samples were either blood samples heat sealed in glass capillaries, or saliva samples soaked on a cotton bud, also heat-sealed into a glass capillary for storage and sending. Samples were analysed by the energetics research lab in Aberdeen, Scotland.
In the lab, capillaries were broken and each sample (blood and saliva) was distilled in a vacuum line, and the distillate was trapped in a glass tube. Part of the distillate was heat-sealed in glass microcapillary tubes. An internal lab standard is used in the isotope ratio mass spectrometer (IRMS; Micromass OPTIMA machine), with which the distilled samples are run in the same batch. The IRMS measures the proportion of heavy isotopes against the proportion of the light (abundant) isotopes in the sample against a standard sample. As each laboratory uses its own standard, these standards need to be calibrated previously against a range of International Atomic Energy Agency standards (SMOW/SLAP standard; SMOW = standard mean ocean water, SLAP = standard light Arctic precipitate).
The usual gas for mass spectrometric determination of deuterium abundance is pure

hydrogen gas (H_2). The usual gas employed for mass spectrometric evaluation of ^{18}O enrichment is CO_2.

DLW and Saliva - In Vivo Experiments: Comparability of isotope levels in blood and saliva samples

To determine whether it is possible to obtain energy expenditure (EE) data with saliva samples using DLW, saliva isotope concentration was compared to blood isotope concentration *in vivo*. Both fluids were sampled simultaneously after injecting D_2O intraperitoneally in four male meerkats in Cologne zoo, that had been kept in a separate enclosure for husbandry reasons. Meerkats were trained beforehand to chew on the cotton bud to guarantee a sufficient amount of saliva.

The cotton buds used were kindly provided by Ebelin Cosmetics, Karlsruhe, Germany and originated form the same lot to avoid possible background isotope fluctuation in the cotton buds. Cotton is an organic material and thus contains a background concentration of heavy hydrogen (= deuterium) and oxygen isotopes. If the cotton in the bud originates from different plantations, it could contain varying isotope backgrounds. Thus, it was important to use cotton buds with equal cotton composition.

Day I: meerkats were anaesthetized with 0,08 ml/kg ketamine hydrochloride and 0,06 ml/kg domitor. Anaesthetization was conducted by Dr med. vet. Christian Detmer, experiments were filed to, and affirmed by, the Cologne district administration as animal experiments under reference number: 50.203.2-K.

After taking a blood sample from the jugular vein to estimate background isotope enrichments of ^{2}H and ^{18}O isotope background, meerkats were injected intraperitoneally with deuterium only, as heavy oxygen is very expensive and the administration of one isotope is sufficient to test for differences in concentration in two body fluids. After one hour, both blood and saliva samples were taken. Meerkats were then given an antidote anaesthetic ("Antisedan").

Day III and VI: meerkats were anaesthetised again and the respective blood and saliva samples were taken. Anaesthesia and blood withdrawals were again conducted by Dr. med. vet. Detmer, after successful sample drawing meerkats were given an antidote anaesthetic ("Antisedan").

DLW and Saliva - In Vitro Experiments: Effect of cotton contact on the deuterium concentration in the samples

To test for a possible influence of the cotton on isotope concentration in the sample, different concentrations of deuterated water (1 : 2000; 1 : 4000; 1 : 8000) were either sealed in capillaries directly, or soaked on a cotton bud and sealed. All samples were analyzed for ratios of ^{2}H : ^{1}H using isotope-ratio mass spectrometry. Concentrations used are in the range of

physiological concentrations.

DLW and Saliva - In Vitro Experiments: Effect of cotton contact time on the deuterium concentration in the sample

To test if contact time also had an effect on the isotope concentration in the samples, deuterated water was soaked on a cotton bud while contact time – i.e. time before the sample was extracted from the cotton by centrifugation – varied (1 h, 24 h, 120 h, 168 h; n = 2, deuterated water was diluted 1 : 2000).

DLW and Saliva - In Vivo Experiments: Oral isotope administration

To test whether isotopes can be administered orally instead of intraperitoneally, both heavy isotopes (^{2}H and ^{18}O) were given separately to the same test animals. One Isotope was administered orally, i.e. the meerkats drank it from a syringe, while the other isotope was injected intraperitoneally (0.3 ml) without anaesthesia 30 min later. Time delay was chosen as oral administration implies the necessity for water resorption first. Saliva was then collected on cotton buds, kindly provided by Ebelin cosmetics, Karlsruhe, Germany.

According to German animal protection act, medical/research procedures under anaesthesia should only be performed if the pain without induces more damage to the animal's health than the anaesthesia itself. Here, we decided that a single intraperitoneal injection implies less pain and risks than prior anaesthesia. Intraperitoneal injections were performend by Dr. med. vet. Olaf Behlert. These experiments were filed to, and affirmed by the Cologne district administration as animal experiments under reference number: 50.203.2-K.

Multiple saliva samples - to control for isotope concentration in the sample - were taken daily in the consecutive days.

DLW and Minimal Invasive Blood Sampling: Bug container design

In order to obtain blood samples without anaesthesia, different types of blood sucking insects were administered into collars worn by the meerkats. As the major goal of these experiments was to avoid stress for the test animals, they were trained to wear collars prior to the experiments. Collars enclosed the insects, while one side was made of a gauze where the insects could sting through.

DLW and Minimal Invasive Blood Sampling: Collar training

By means of positive enforcement training, meerkats were guided to tolerate collars around their neck and abdomen, i.e. meerkats received small rewards when they tolerated to be touched at the neck and wear the experimental collar. During experiments, meerkats were observed for behavioural abnormalities (e.g. increased locomotor activity) and these

observations were analysed separately after the experiments were finished.

DLW and Minimal Invasive Blood Sampling: Ionising Radiation as a Method of Sterilisation

Ionising radiation is currently the method of choice for rendering insects reproductively sterile (Dyck et al. 2005). A variety of radiation sources can be used, here X-rays were chosen to ionise the reproductively fertile insects and thus sterilise them to avoid bastardization of fauna in the unlikely event of escape. An X-ray radiator could be provided by the institute of developmental biology in Cologne. Such a device produces X-rays when a beam of electrons strikes material with a high atomic number, such as tungsten. X-rays, like gamma rays, are electromagnetic radiation. Radiation generated in this manner (by the rapid deceleration of a charged particle) is also known as "Bremsstrahlung". "Bremsstrahlung" (braking radiation) has a broad energy spectrum with a maximal equal to the energy of the incident electrons.

The absorbed dose used to induce sterility is of prime importance to guarantee a sufficient level of sterility, while ensuring that the sterilized insects are still in good shape and can be used to draw blood samples.

Therefore, 4 groups of *Rhodnius prolixus* (n = 20 per group, instars 2 and 3) were radiated with either 80 Gy (typical dose for adult reduviids following (Dyck et al. 2005), 40, 20 and 0 Gy (control group; Gy = [J/kg]). Insects were placed in the 95 % confidence belt under the radiation source. Effectively absorbed doses in three groups are summarized in Table 2.3. Sterilisation time was 40, 20 and 10 min in the three groups. An 1 mm copper plate was used as filtering material (depending of the material and thickness of the material, either high- or low energy beams are filtered; here an 1 mm copper plate filtered for low-energy radiation that maximizes the impact on DNA while at the same time minimises the impact on the cells). Voltage was 120 kV in all groups. Insects were kindly provided by Dr. Christian Voigt, Institute for Zoo and Wildlife research (IZW), Berlin, Germany. Insects were sent back to the IZW after irradiation and fed over the next six months there.

Table 2.3: Specifications of the sterilisation experiment with Rhodnius prolixus:
"dosage": X-ray dose per group; "95% confidence belt": depending on the position under the tube (radiation source), different amounts of radiation reach the sample, within this belt, 95 % of the emitted radiation reaches the sample; "sterilisation time": time the samples were placed under the tube; "distance to radiation source": depending on the distance, different amount of radiation can reach the sample, here the nearest possible setting was chosen; "filter": choosing a low energetic metal like copper maximises the impact of the radiation on the DNA (sterilisation!) while minimising the radiation impact on the cell; "voltage": the induced voltage affects penetration depth, as the maximum of the emitted radiation is influenced through voltage.

Dosage	Effectively absorbed radiation (placed in 95 % confidence belt)	Sterilisation time	Distance to radiation source (tube)	Filter	Voltage
80 Gy	75-80 Gy	40 min	25 cm	1 mm copper plate	120 kV
40 Gy	38-40 Gy	20 min	25 cm	1 mm copper plate	120 kV
20 Gy	19-20 Gy	10 min	25 cm	1 mm copper plate	120 kV

DLW and Minimal Invasive Blood Sampling: Measuring EE with minimal invasive blood sampling

On day one, the animals were weighed (± 1.0 g Sartorius balance). Isotope background enrichment in the animals was obtained by samples of tap water, as this is a good approximation on the isotope levels and minimises the impact on the test animals. Afterwards, a known mass of DLW water was administered intraperitoneally, 0.6 ml kg^{-1} body weight; injections were performed by Drs. med. vet. Olaf Behlert and Christian Detmer). Syringes were weighed before and after administration (± 0.0001 g, Sartorius balance) to calculate the mass of DLW injected. Blood samples were taken after one hour to estimate initial isotope enrichments using the blood-sucking parasites (*R.prolixus* and *D. maximus,* kindly provided by Prof Schaub, Bochum University, Germany and purchased from Dr. Christian Voigt, IZW, Berlin, Germany as well as *G. brevipalpis,* kindly provided by Dr. Udo Feldmann, International Atomic Agency, Vienna).

After 40 min (reduviids) or 3-4 min (tsetse) the meerkats were released from the insects, and the blood samples drawn from the insects using a syringe (tsetse flies were killed directly after the blood meal in diethylether, reduviids usually survive the puncture).

Final blood samples were taken after 2-3 days in adults to estimate the isotope elimination rates.

Experiments were filed to, and affirmed by the North Rhine-Westphalia State Environment Agency (LUA NRW; File number 8.87-50.10.45.08.187).

Measuring hormone levels with minimal invasive blood sampling

To test whether samples obtained minimally invasive with tsetse can also used to determine hormone levels, *G. morsitans* were fed *in vitro* from an artificial food source. *G. morsitans* was kindly provided by Dr. Peter Takac, from the Institute of Zoology in Bratislava, Slovakia.

Food source was sheep blood, heated to 37 °C, obtained from a butchery. Blood was covered with a silicon membrane (kindly provided by the IAEA who had developed these membranes to feed their colonies).

Flies were fed 1-2 days after eclosion. The membrane had been treated with a 0.15 mM aqueous solution of uric acid prior to fly feeding. Flies were placed on the food source in transparent cylinder of 10 cm hight and 4 cm diameter, whose ground was made of a gauze allowing the flies to feed.

Flies were allowed to feed on average 5.5 ± 1.3 min. Due to the transparent nature of the cylinders it could be determined at eyesight when flies were feeding, so feeding time was varied depending on the initiation of feeding.

At each sampling point, blood was removed from the food source using a syringe. These samples act as controls for the blood samples obtained with the flies, to determine a possible influence of the insects digestion in hormone levels (here cortisol) in the samples.

On average, meals of 7.5 ± 2.2 flies were pooled to obtain a sample volume of about 90 - 100 µl blood. Samples were centrifuged at 4600 rpm for 15 min, then 40 µl of the supernatant were transferred to a second Eppendorf tube and stored at -20 °C.

Samples were analysed by MLM Medical Labs, Moenchengladbach, Germany.

The supernatant was defrosted and buffered using 160 µl of Advia Centaur Multi-Diluent 3 dilution buffer (Siemens, Erlangen; Germany). Hormone levels were analysed with the ADVIA Centaur system (Siemens, Erlangen, Germany).

The ADVIA Centaur cortisol assay is a competitive immunoassay employing direct chemiluminescent technology that derives its name from the coupling of the cortisol to acridiniumester, a chemiluminescent dye, that dissociates to at contact to acids and subsequently emits light at 430 nm wavelength.

The sensitivity of this system it between 0.2 - 75 µg/dl and 5.5 - 2069 mmol/l cortisol respectively.

2.3 Timetable Observations/Experiments

2.3.1 Preliminary Observations

To test the feasibility of close behavioural observations as well as possibility to obtain minimal invasive physiological data in the field, one month of preliminary observation was conducted in **April 2006**. One main goal was to finish an ethogram of all relevant behaviours.

2.3.2 Behavioural Observations:

Behavioural data was collected between **November 2007 and February 2008** at the "Kalahari Meerkat Project", South Africa. Three groups were observed for a total of 230 observation hours.

2.3.3 Physiological Data:

DLW and saliva:	**June 2006 (analysis finished by 03/07)**
DLW and minimal invasive blood samples:	**June 2007 - April 2009 (analysis finished by 07/09)**
Sterilisation experiments *Rhodnius prolixus*:	**August 2007 (bugs observed until 02/08)**
Parasites and hormone level analyses:	**September 2009 (analysis 09/09)**

2.4 Analysis

2.4.1 Behavioural data:

Figure 3.1: Hourly averages of the time spent in sun, shade and below of the three observed meerkat groups. The averages are calculated as proportions of all scans (n = 60 scans per hour, times the number of animals per group). Here, 228 observation hours were used for the calculation of the data. In total, the number of scans amounts to: "Elveera"(76h*60*10a) + "Lazuli" (81h*60*9a) + "Aztects" (71h*60*7a) = 45600 + 43740 + 29820 = 119160.

Figure 3.2: Correlations of time spent in sun in relation to T_A and average solar radiation. n = 147 observation hours, as these represent the number of observation hours where full data on T_A, radiation, humidity and wind-speed were available and weather was not cloudy. These are: "Elveera" (46h*60*10a) + "Lazuli" (66h*60*9a) + "Aztects" (35h*60*7a) = 27600 + 35640 + 14700 = 77940 scans.

Figure 3.3: Correlations of time spent in shade in relation to T_A and average solar radiation. n = 184 observation hours, as these represent the number of observation hours where full data on T_A, radiation, humidity and windspeed were available. These are:
"Elveera" (64h*60*10a) + "Lazuli" (75h*60*9a) + "Aztects" (45h*60*7a) = 38400 + 40500 + 18900 = 97800 scans.

Figure 3.4: Correlations of time spent below in relation to T_A and average solar radiation. n = 85 observation hours, as these represent the number of observation hours where full data on T_A, radiation, humidity and windspeed were available, and T_A is above 32 °C. Hours with lower T_A represent mainly observation hours in the mornings and evenings. These were excluded from the calculation, as time of day exhibits a confounding effect on the distribution of the data here. During the mornings and evenings, meerkats use their burrows for sleeping purposes, independent of abiotic influences like T_A and radiation. This amounts to a total number of:

"Elveera" (33h*60*10a) + "Lazuli" (28h*60*9a) + "Aztects" (24h*60*7a) = 19800 + 15120 + 10080 = 45000 scans.

Figure 3.5: Correlation of ambient temperature and average solar radiation. n = 184 hours. Hourly averages of T_A and radiation, derived from 4 values taken every 15 min, were used for the calculation.

Figures 3.6 - 3.8: Correlations of time spent with contact lying, piloerection and sunbathing in relation to T_A and average solar radiation. n = 184 observation hours, as these represent the number of observation hours where full data on T_A, radiation, humidity and wind speed were available. These are:

"Elveera" (64h*60*10a) + "Lazuli" (75h*60*9a) + "Aztects" (45h*60*7a) = 38400 + 40500 + 18900 = 97800 scans.

Figure 3.9: Correlations of time spent foraging with T_A and radiation, as well as the proportion of time spent foraging plotted against daytime. n = 184 observation hours, representing 97800 scans.

Figure 3.10: Activity budget of all observation hours (n = 228, 97800 scans). Percentages were calculated as proportions, i.e. numbers of scans on the respective behaviour divided by the total number of scans.

2.4.2 Physiological data: Calculation of energy expenditure from isotopic enrichments

Enrichments of deuterium and oxygen that were analysed in the samples need to be converted into rates of energy expenditure. Firstly, the body water pool (N) must thus be calculated from the dilution space, i.e. from the concentration in the injectate the amount of body water can be derived by the degree of dilution in the sample.

N can be calculated by ^{18}O (N_O) dilution and ^{2}H = D (N_D) dilution. N_O was used as an estimate of body water because it is closer to the estimate of body water by desiccation. ^{2}H molecules are smaller than ^{18}O molecules and are therefore more mobile and more likely to exchange with other molecules in the body, thereby giving a lower estimate of body water.

N_O and N_D are calculated using both the plateau method (when the initial sample is taken during the plateau phase – i.e. after equilibrium of the isotopes within the body water pool) and the intercept method (this method back-extrapolates to the injection time and does not depend on taking a sample within the equilibrium period). Figure 2.3 illustrates these two possibilities:

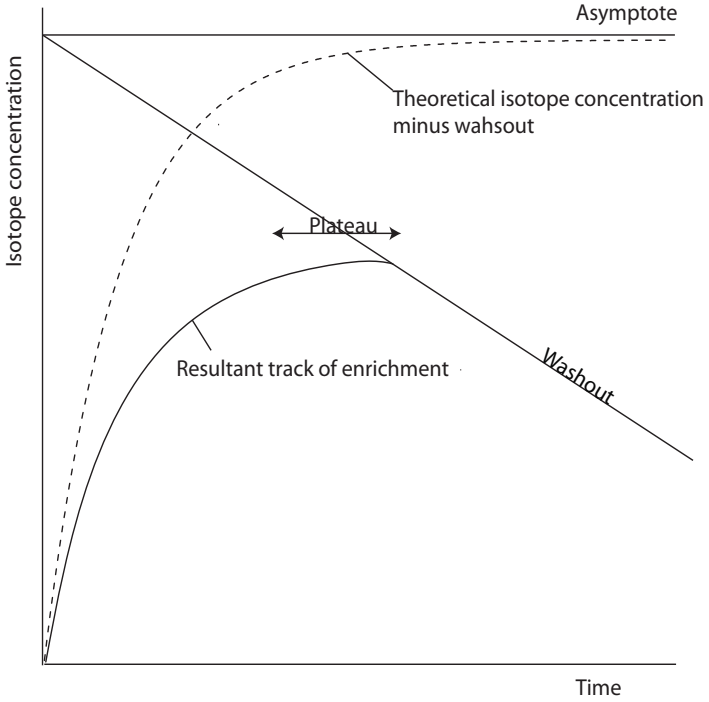


Figure 2.3: Isotope enrichment and washout.
Anticipated time tracks for the intensity of isotope concentration in the body when the isotopes are slowly infused, and when the water content in the body is also being continually turned over by an inflow and an outflow. Please note that if the washout curve is being extrapolated back to the time 0 it crosses the ordinate axis at the asymptote. Redrawn after Speakman (1997).

The dashed line indicates the theoretical time line of isotope concentration in the body, if there was no washout. The isotopes are accumulating after administration and reach a maximum concentration close to the asymptote. But if the washout is taken into account (since all the time the isotopes are spreading into the body pool, some are being lost in evaporated water, urine and CO_2 and these are replaced continuously by unlabelled metabolic water), isotope concentration reaches a maximum at the "plateau" and not close to the asymptote, and then follows the washout line. This indicates that after administering the isotopes one cannot wait indefinitely to take the initial sample, for the actual "plateau" intensity exists only for a brief period. Another possibility than measuring the initial isotope concentration at the plateau directly is extrapolating it back to the time of isotope entry from the washout curve. This becomes evident as the washout line crosses the y-axis exactly on the point of the true asymptote (intercept method).

The four N-values (i.e. N_D and N_O both from plateau and intercept method) should be quite close to one another, although N_D should be higher than N_O, and plateau estimates are

generally higher than intercept estimates. Here, the intercept method is of bigger importance as with this method, the samples didn't needn't be taken at exactly the time point, when isotopes are in the plateau phase, i.e. at highest concentration in the body.

As evaporative water loss might influence the isotope concentration in the samples, the rate of carbon dioxide production (rCO_2) was calculated with the following equation, recommended by Speakman (= equation 7.17 in (Speakman 1997)), controlling for 25% evaporative water loss to total water flux at 37 °C body temperature:

$$rCO_2 = (N/2.078)*(k_O-k_D)-0.0062*k_D*N \qquad = \text{EST 9}$$

with N representing the size of the body water pool, k_O and k_D representing the washout rates (k) of oxygen-18 and deuterium.

For comparative reasons, rCO_2 is also shown following Lifson &McClintock (1966), an equation often used in older literature to derive the production of CO_2. (Lifson & McClintock 1966, equation 35):

$$r\,CO_2 = N/2.08\,(k_O-k_D)-0.015\,k_{2D}N \qquad = \text{EST 1}$$

The washout rates were calculated using the following equation:

$$k= [\log(c_I-c_B)-(\log(c_F-c_B)]/t\,,$$

with c_B representing the basal isotope enrichment (atom %), c_I the initial isotope enrichment (atom %), c_F the final isotope enrichment (atom %) and t the time that elapsed between the initial and final sample.

The rate of carbon dioxide production was converted average daily energy expenditure between initial and final sample (EE; kJ/d), following (Speakman, 1997; equation 8.3):

$$EE = rCO_2\,(1.106+[3.941/RQ]),$$

with RQ equalling the respiratory quotient that was 0.8 (= ratio between carbon dioxide produced and oxygen consumed). After Speakman (1997) assuming an RQ of 0.8 rather than measuring it introduces errors less than 5 % into the calculation of energy expenditure.

2.4.3 Statistical Analyses and Programs Used

Statistics:

Correlations were statistically examined using Spearmann rank correlations due to the non-normal distribution of the behavioural data (Figures 3.2 - 3.4 and 3.6 – 3.8 and 3.10).

Correlation of T_A and solar radiation (Figure 3.5) was tested using a Pearson correlation (data was distributed normally).

Comparisons of means were statistically tested using Kruskal-Wallis statistics, due to the non-normal distribution of the behavioural data (Figure 3.9).

Data were fit using linear regressions (Figure 3.2 and 3.5).

Variances of the slopes of the normalized (log) blood and saliva washout rates were compared using F-tests (Figure 3.11).

Programs:

Data was observed using Microsoft Pocket Excel, and transferred using Microsoft Active Sync.

Analysis was performed using Excel 2007, Open Office 3.1 and Graph Pad Prism 5, graphs were created using Graph Pad Prism 5 and Adobe Illustrator (in Creative Suite 4).

Statistical tests (Spearmann correlation, Pearson correlation, Kruskal-Wallis-test, linear regression) were performed in Graph Pad Prism 5.

3 RESULTS

3.1 Behavioural Data

Meerkats are small, physiologically heat-adapted (Muller & Lojewski 1986) desert animals. As a small animal's energy household is generally costly, a high amount of behavioural cost minimization can be expected. Thermoregulatory cost minimization by behaviour includes exposure to energy sources such as radiation at low ambient temperatures (T_A), avoidance of extreme ambient temperatures, high radiation levels and wetness.

Time of day is one first approach to integrate abiotic ambient factors, influencing the occurrence and distribution of different behaviours. Thus, Figure 3.1 compares the percentages of times spent in sun, shadow and below ground in the animals' dens of the three meerkats groups observed at the Kalahari meerkat project. In the mornings, at low T_A and

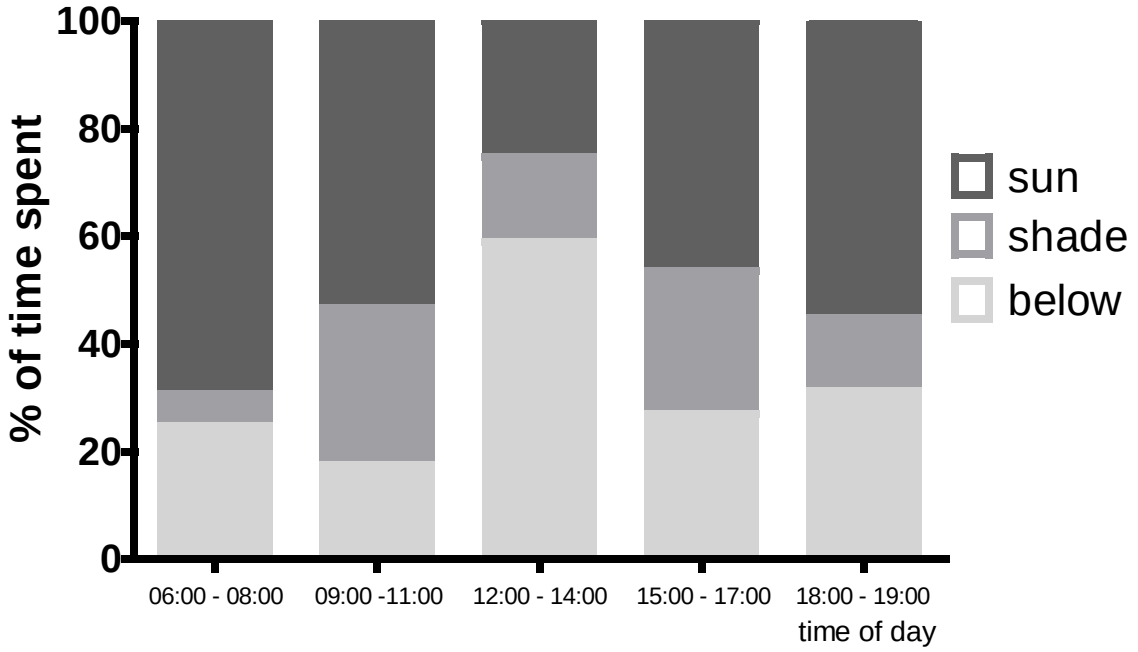


Figure 3.1: Effect of day time on the proportion of time spent in sun, shadow and below ground.
Proportions were calculated from 228 observation hours (119160 scans), observed at three groups at the "Kalahari Meerkat Project". Times of day are: 06:00 – 08:00, 09:00 – 11:00, 12:00 – 14:00, 15:00 – 17:00 and 18:00 – 19:00. All scans where weather conditions were cloudy were excluded from the calculation.

radiation levels, 69.3 % of the time was spent in the sun. Only 6 % was spent in the shade, and 24.7 % below. (06:00 – 08:00).

Later, as temperatures and radiation levels increased, time spent in the shadow increases to 29.2 %, and time below decreases to 17.5 % (09:00 - 11:00). At noon, when temperature and radiation were at their maximum, most time was spent below and only comparably little time was spent in the sun and the shade (25.2 % and 15.9 %). In the afternoons, with decreasing temperatures and radiation levels, time was evenly allocated between sun (46.4 %) and shade or below ground (26.6 and 27 %; 15:00 – 17:00). In the evenings, the highest proportion of time was passed in the sun (55.2 %), and the least in the shade (13.7 %).

The probabilities of residence in the sun, shadow and below are mutually exclusive as is shown in Figure 3.1. The sum of these categories adds up to 100 % of time. Please bear this in mind when examining Figures 3.2 to 3.4, where the effect of ambient temperatures and solar radiation on the probabilities of residence in sun, shadow and below is explained in more detail. If the meerkats spent 0 % in sun, they could have spent this time in either shadow or below – i.e. 100 % in shadow, or 50 % in shadow and 50 % below, or any other possible combination.

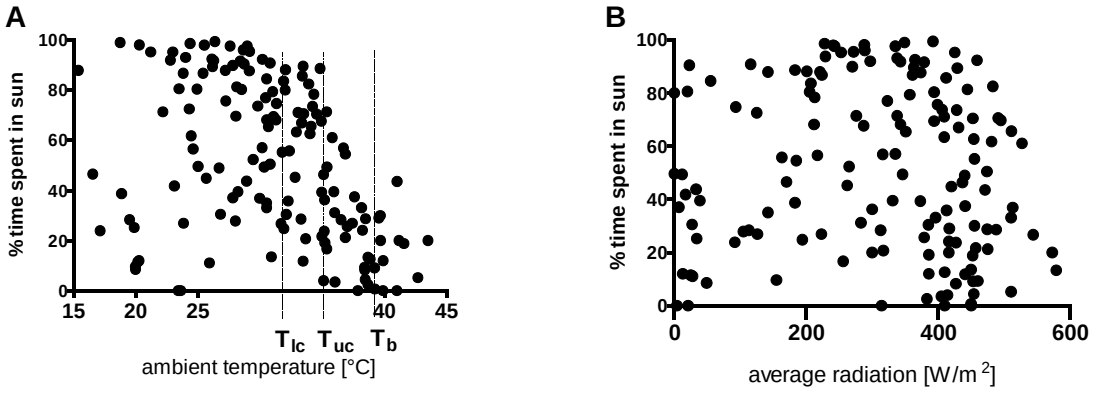


Figure 3.2: Effect of T_A and radiation on time spent in sun.
(A) In relation to ambient temperature and (B) in relation to average global solar radiation. Units are °C and W/m². Correlation to ambient temperature is highly significant. (Spearmann rank correlation, $p < 0.0001$), correlation to radiation is not significant. Proportions were calculated from 147 observation hours (77940 scans) observed at three meerkat groups (hours with cloudy weather conditions were excluded from the calculation).

When comparing the effect of T_A and solar radiation on the time spent in sun (Figure 3.2), it becomes clear that below the meerkats TNZ (below 30 °C), T_A does not seem to influence the distribution of the data. Above 30 °C though, the proportion of time spent in sun rapidly decreases with increasing T_A. This correlation is highly significant (Spearman rank correlation, $p < 0.0001$). When splitting the data to below and above 30 °C (which represents the animals' T_{LC}) correlations are not significant below 30 °C, but highly significant above 30 °C ($p < 0.0001$). This is also represented by the slopes and their respective standard deviations of

the regression lines in the two temperature intervals. Under 30 °C: y = 2.4 ± 1.1 x + 2.8 ± 27.2, whereas above 30 °C y = -5.1 ± 0.7 x + 223.4 ± 23.4 (linear regressions). The slope of the regression line above 30 °C is (steeply) negative, which implies that with increasing temperatures, leaving areas with sunshine became more and more important. Interestingly, time spent in sun could not be correlated significantly to radiation (Spearmann rank correlation, data n.s.).

When plotting time spent in the shade against ambient temperatures T_A, it is obvious that residence time in the shadow increased with increasing temperatures. At temperatures higher than 30 °C, proportions increase to 60 % and higher (Figure 3.3 A). At temperatures above 37 °C, which corresponds to the animals' core body temperature (T_B), less time was spent in the shade except for a few outliers. This corresponds to the percentages of time spent below ground in relation to T_A (Figure 3.4 A). Above the upper critical temperature T_{uc}, values increase up to 80 %, but only at T_A higher than the animals' T_B, i.e. above 37 °C, more than 80 % of the time was spent below. This means below 37 °C, time was spent in either shade or below ground, but above 37 °C, most of time was spent below.

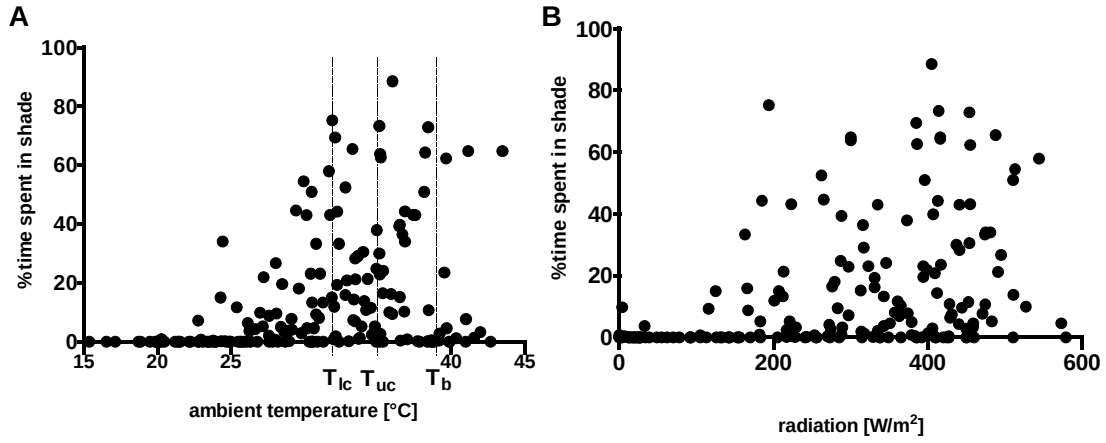


Figure 3.3: Effect of T_A and radiation on time spent in shade.
(A) In relation to ambient temperature and (B) in relation to average global solar radiation. Units are °C and W/m². Both correlation are highly significant (Spearmann rank correlation, p < 0,0001). Proportions were calculated from 184 observation hours (97800 scans) observed at three meerkat groups.

Correlations of time spent below to T_A and radiation are only shown for T_A higher than 32 °C (Figure 3.4 A), as lower temperatures occurred mainly in the mornings and evenings (Figure 3.4 C). This has a confounding effect on the distribution of the data, as at these times of day the burrows are mainly used for sleeping purposes independently of abiotic influences.

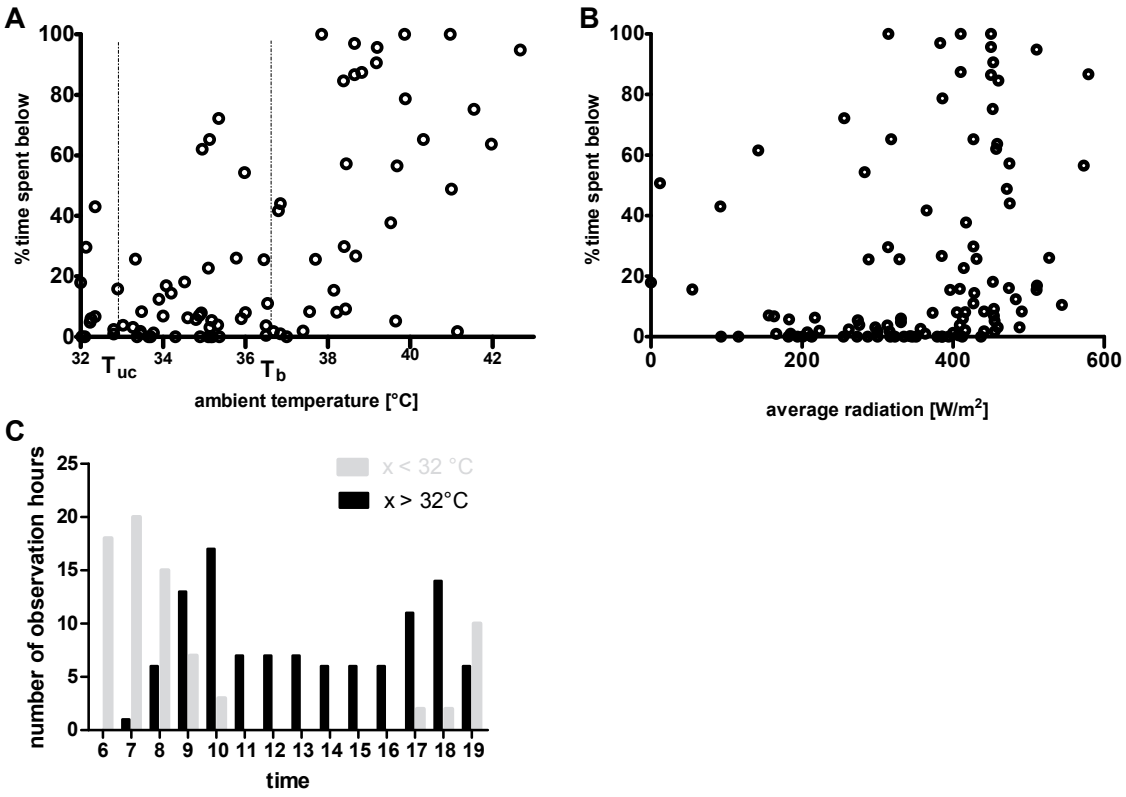


Figure 3.4: Effect of T_A and radiation on time spent below ground at T_A above 32 °C.
(A) In relation to ambient temperature and (B) in relation to average global solar radiation. Units are °C and W/m². Both correlations are highly significant (Spearmann rank correlation, p < 0,0001).(C) Temperature values smaller than 32 °C were excluded from the calculation as these represent observation hours in the mornings and evenings. Time of day has a confounding effect on the data here, as these are the times meerkats sleep in their burrows, independent of T_A or radiation. Proportions were calculated from 85 observation hours (45000 scans) observed at three meerkat groups.

Apart from temperature, possibly important ambient factors for the distribution of the data are global solar radiation, humidity and wind speed, as all can have a huge impact on an animal's thermoregulation. Figures 3.3 B to 3.4 B show that correlations of radiation, here

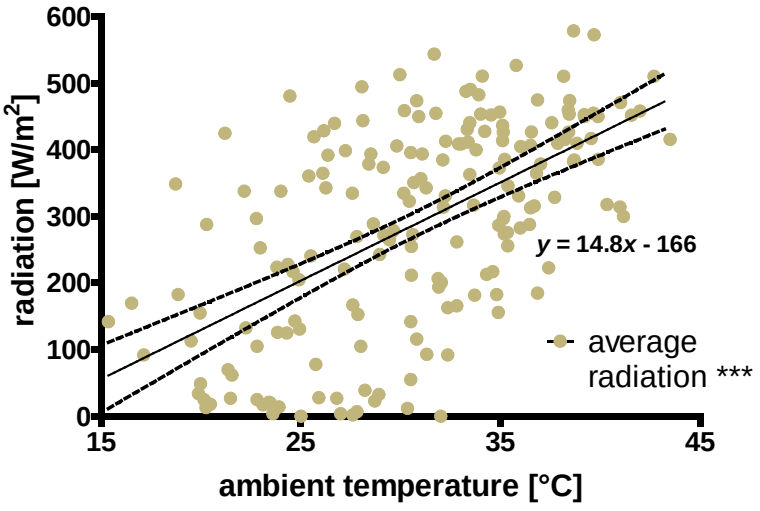


Figure 3.5: Correlation of ambient temperature and average global solar radiation.
Fit = linear regression fit, with 95 % confidence interval. This correlation is highly significant (Pearson correlation, p < 0,0001; n = 184 observation hours).

average global solar radiation, display a similar image to correlations on T_A. Both correlations are also highly significant, similar to the correlations of time spent in shade and below with T_A. This is not surprising, as solar radiation and ambient temperature are interdependent on another (Figure 3.5). Thus both might influence the distribution of the behavioural data shown in Figures 3.3, 3.4 and 3.6 to 3.8.

Interestingly, temperatures measured in the field seem to correspond to the - laboratory obtained - temperatures T_{LC} and T_{UC}, as these together with T_B explain large parts of the distribution of the data. Statistically, this was confirmed as time spent in sun, shade and below (Figures 3.2 - 3.4), but also times spent contact lying (Figure 3.6), with erected hair (piloerection, Figure 3.7) and sunbathing (Figure 3.8) are significantly correlated with ambient temperature and sometimes, radiation. Humidity was only correlated to the time spent in shade (data not shown), not to time spent in sun or below. Correlations to wind speed were not significant (data not shown).

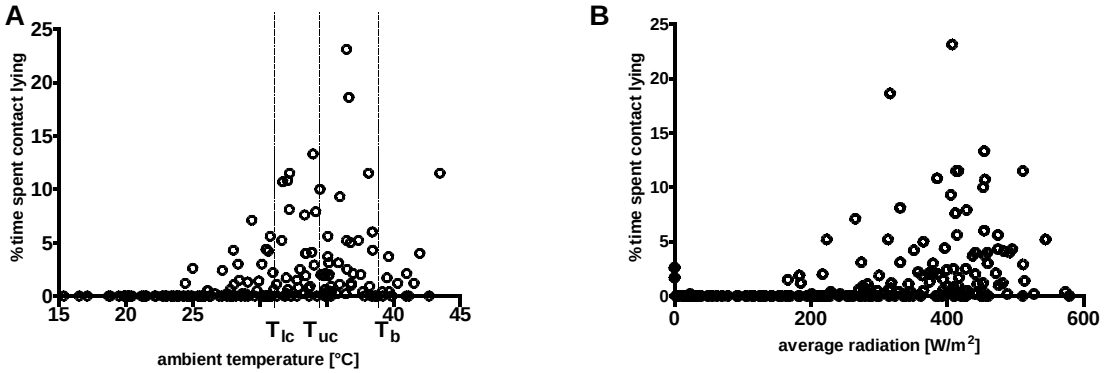


Figure 3.6: Effect of T_A and radiation on time spent contact lying.
(A) In relation to ambient temperature and (B) in relation to average global solar radiation. Units are °C and W/m². Both correlations are highly significant (Spearmann rank correlation, $p < 0{,}0001$). Proportions were calculated from 184 observation hours (97800 scans) observed at three meerkat groups.

Other than leaving sunny areas to retreat into shade or below, there are several behaviours that can actively aid thermoregulation, and thus minimize physiological costs. Contact lying is a good example, as it aids to conduct heat to a (cooler) surface. When the proportions of time spent contact lying are correlated to ambient temperature (Figure 3.6), it becomes obvious that this behaviour increases above 30 °C (up to 13 %), while highest values (23 %) were reached above 33 °C, the animals upper critical temperature. Above T_B percentages seem to decrease again. This matches the finding that above 37 °C, when most time was spent below ground (Figure 3.4).

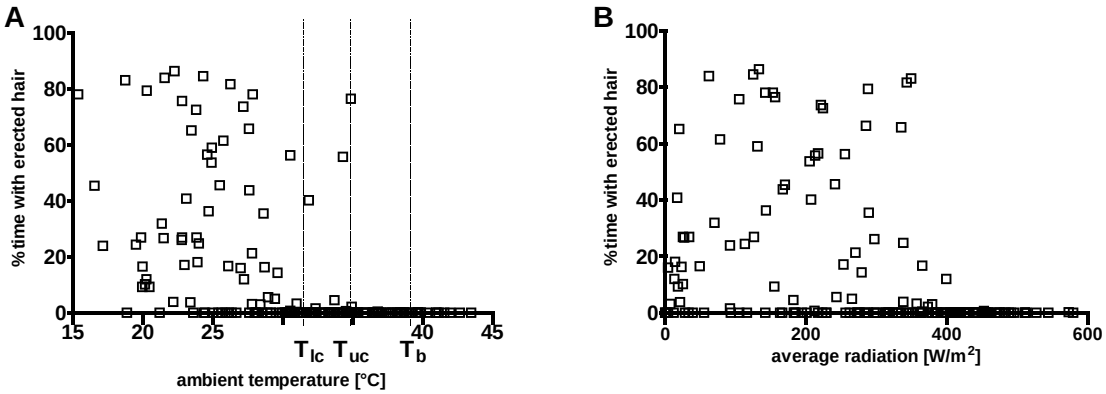


Figure 3.7: Effect of T_A and radiation on the proportion of time while hair was erected.
(A) In relation to ambient temperature and (B) in relation to average global solar radiation. Units are °C and W/m². Both correlations are highly significant (Spearmann rank correlation, p < 0,0001). Proportions were calculated from 184 observation hours (97800 scans) observed at three meerkat groups.

Another powerful means to minimise physiological costs is piloerection (Figure 3.7). This behaviour can be displayed in thermoregulatory context or in the context of approach to (foreign) objects or individuals. In a thermoregulatory context, it increases insulation by creating a layer of air around the body that counteracts radiative and convective heat loss. When plotting piloerection against T_A, 30 °C appears to be a sharp border again below which piloerection is regularly used (up to 86 % the time was spent with erected hair), and above which the proportions suddenly drop to zero. This means, meerkats use piloerection right up to their T_{LC} to avoid heat loss. While piloerection is mainly displayed below 30 °C, there are still few incidents when this behaviour occurred at higher T_A, but never above their T_{UC}.

Regarding the proportions spent sunbathing (Figure 3.8), it is obvious that this behaviour is also shown only below the T_{LC}. But, unlike piloerection, it is displayed only up to 27 °C, well below the T_A when hair was still erected. Sunbathing is a behaviour that actively uses solar energy to heat the body of the animal, other than piloerection, that prevents the body from losing too much internal metabolic heat.

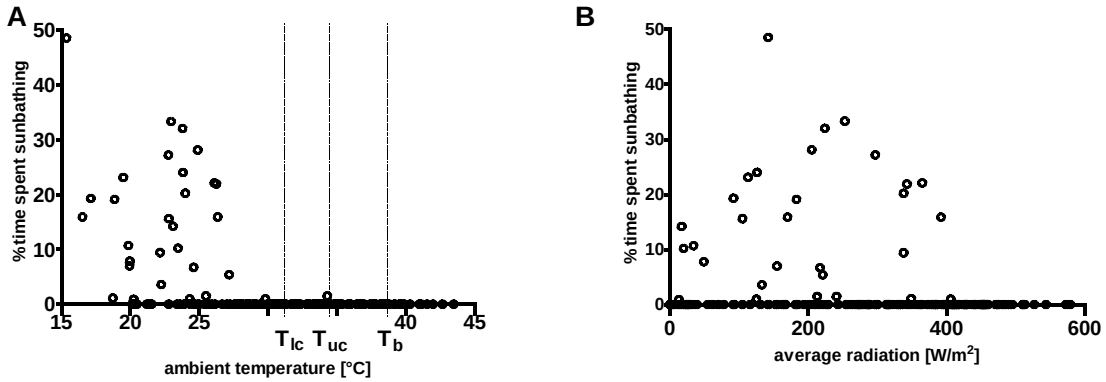


Figure 3.8: Effect of T_A and radiation on time spent sunbathing.
(A) In relation to ambient temperature and (B) in relation to average global solar radiation. Units are °C and W/m². Both correlations are highly significant (Spearmann rank correlation, p < 0,0001). Proportions were calculated from 184 observation hours (97800 scans) observed at three meerkat groups.

The total proportion of behaviours in the context of thermoregulation (below, contact lying, sunbathing, huddling) amounts up to 28.7 % of the total time, of which "below" accounts for 24.6 % (compare Figure 3.9). As the time spent below was not only influenced by thermogulatory needs, but "being below" also meant the the animals could be sleeping, this value was slightly corrected and hours between 06:00 and 08:00 am, as well as the last hour in the evenings (19:00) were excluded from the calculation. This corrects the time spent below to 23.4 % and the proportion of behaviours in the context of thermoregulation to 27.5 %. These are only the mutually exclusive behavioural categories, whereas spending time in sun, shade or with piloerection are not mutually exclusive with e.g. foraging, it is not possible to calculate the exact proportion of these strategies in the activity budget.

If using the proportions of time spent in sun below 30 °C, time spent in the shade and below above 30 °C and dividing it through the total amount of scans, time spent on behavioural thermoregulation by site selection amounts to as much as 52.2 %!

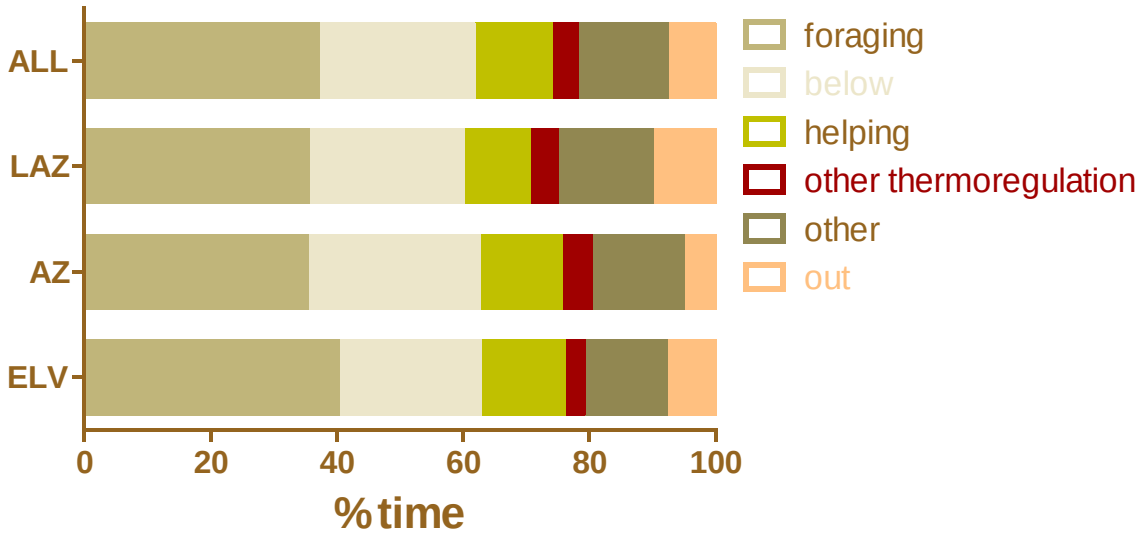


Figure 3.9: Activity budget of the three meerkat groups in South Africa.
"ALL": average of the three groups, "LAZ": Lazuli, "AZ": Aztecs,"ELV": Elveera. Behavioural categories as defined (see ethogram), except for: "helping": guarding, digging, babysitting, pup feed; "other thermoregulation": contact lying, sunbathing and huddling; "other": lying, allogrooming, low sit, high sit, low stand, high stand, move, run, climb, eating, play, foraging competition, mobbing, fight, frenzy, IGE; n = 228 observation hours (119160 scans). Means do not differ statistically between the groups (Kruskal-Wallis statistic, n.s.).

Comparing the time allocated to certain behaviours in Figure 3.9, it is obvious that most time was attributed to foraging. It would be assumed, as meerkats attribute almost 40 % of their time to this behaviour that its occurrence, implying activity, could be correlated to abiotic factors. Interestingly, the proportion that was spent foraging could neither be correlated to T_A or radiation (Figure 3.10), humidity nor wind speed (Spearmann rank correlation n.s., data for

the latter two not shown). It appears that proportions of time spent foraging decreased above the T_B, as is in accordance with the fact that above 37 °C, meerkats spent most of their time below. Unfortunately, as foraging and time spent in sun or shade are not mutually exclusive, the proportion of foraging time in sun or shade cannot be determined from these data. As foraging made up almost 40 % of the total time and thus much of the data presented in Figures 3.2 – 3.4 refers to foraging (total time spent in sun, shadow and below). This means it can be assumed that with increasing temperatures, shady feeding sites were selected.

Figure 3.10 C shows that at noon, when temperature and radiation levels where at their maximum, most time was spent below, as levels of foraging that do not seem to vary with daytime otherwise, evidently decrease at noon.

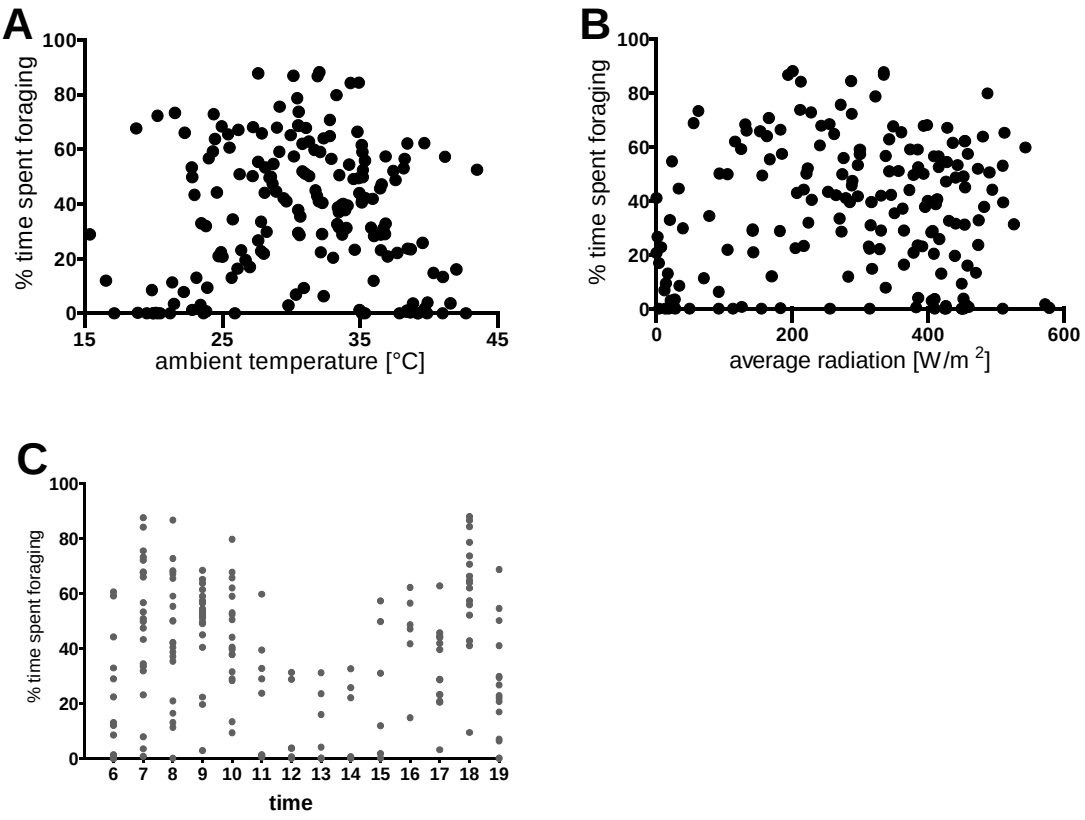


Figure 3.10: Proportion of time spent foraging in relation to (A) T_a, (B) average radiation and (C) time of day. The percentage of time spent foraging could not be significantly correlated to temperature, radiation, relative humidity or wind speed (Spearmann rank correlation, n.s.). n = 184 observation hours (97800 scans).

Summarizing, it is obvious that thermoregulation by behaviour is important in meerkats and accounts for more than 27,5 % of their time budget. Only foraging took up more time (36.9), while helping behaviour made up only 12,2 % of the total activity budget (1.8 % guarding, 2.2 % digging, 8.0 % babysitting, 0.1 % pupfeed). Together, foraging, thermoregulation by behaviour (the activity budget proportion of 27.5 %) and helping make up almost 80 % of the

total activity budget.

Thermoregulatory behaviours could all be correlated to T_A and, to a lesser degree, radiation. It is obvious that these behaviours - avoiding temperature extremes, utilizing or avoiding thermal radiation - are an important part of the animals' behavioural repertoire. To find out to which extent such behaviours make up a substantial amount of the animals' energy budget though, it is necessary to measure their energy expenditure.

3.2 Physiological Data

Measuring energy expenditure in the field is complicated. As methods of direct and indirect calorimetry cannot be used in natural habitats, the only realistic possibility to obtain data on energy expenditure of animals in their natural habitat is using isotopes that act as a marker on metabolic CO_2-production, which can be transformed into rates of energy expenditure (EE). This usually means working with doubly-labelled-water (DLW). This technique requires two samples of body fluids of an animal, and prior administration of heavy water that is labelled both with deuterium (heavy hydrogen) and heavy oxygen (hence the name). Usually, the isotopes are administered via an intraperitoneal injection, and the first sample of a body fluid (blood, saliva or urine) is taken at the time point of maximal isotope enrichment in the body of the animal (which, in small mammals is usually after about one hour). Typically, the body fluid is blood which is sampled when the study animal is caught and anaesthetised. The second sample must be obtained after a few days, which usually implies a second anaesthesia of the test animal. Between these two samples the rates of EE are measured.

Anaesthetising small mammals twice in short time periods can be a substantial risk to the test animals condition, and also implies the hazard of falsifying EE data as the stress forced on the animals can alter rates of EE.

An important aim of this thesis thus was to establish a minimal invasive approach to DLW. To avoid stress forced on the test animals, and possible impact of this stress on the animals' metabolism, it was decided to sample body fluids minimally invasive. Urine appeared not applicable, as picking up urine samples from the ground (after urination of the test animal) usually includes picking up soil with the sample. Unfortunately, the soil seems to contain some background humidity that falsifies the isotope dilutions in the urine (personal comment Dr. Mike Scantlebury). Saliva on the on the hand could easily be probed from meerkats and thus appeared to be the most promising alternative to conventional blood sampling.

3.2.1 DLW and Saliva - *In Vivo* Experiments: Comparability of isotope levels in blood and saliva samples

A first series of tests involved checking whether levels of heavy isotopes in saliva were the same to those in blood (blood was sampled conventionally from 4 anaesthetized animals from the jugular vein). Isotopes were administered intraperitoneally, and both fluids were sampled at the same time. One problem of this experiment was whether the amount of saliva that could be collected from the meerkats would be enough for sample analysis. For DLW analysis a minimum amount of 50 µl of fluid is necessary. After testing different methods to collect saliva directly, without an absorbing material (for instance using a small spatula that was softly inserted into the animals' mouths), I found that it was impossible to obtain the required amounts and decided to use an absorbing material that the test animals could chew on. By the time when the experiments were conducted (07.08-12.08.2006) literature data suggested that cotton was the material used most commonly in order to collect saliva samples, thus cotton was used as absorbing material. Blood samples were drawn from the jugular vein.

Table 3.1: Comparison of deuterium levels in blood and saliva samples obtained at similar time points in four male adult meerkats.
"Ppm": parts per million; "difference": difference between blood and saliva ppm values ; "BG":(isotopic) background samples, later subtracted from the ppm values probed on day one, three and six; "day I": initial sample, with the highest isotope concentration, usually sampled after the isotopes have distributed equally in the body water pool (about one hour after isotope administration); "day III": samples were taken 48 h after the initial sample, "day VI": samples were taken 120 h after the initial samples.

DAY	ANIMAL	BLOOD ppm	SALIVA ppm	DIFF ppm	In % %		BLOOD ppm	SALIVA ppm	DIFF ppm	In % %
			absolute values					**minus BG**		
BG	1	151.53	155.29	-3.76	**-2.48**					
DAY I	1	573.30	541.45	31.85	**5.56**	DAY I	421.80	386.20	35.60	**8.40**
DAY III	1	483.13	447.12	36.01	**7.45**	DAY III	331.60	291.80	39.80	**12.00**
DAY VI	1	362.07	335.00	27.07	**7.48**	DAY VI	210.50	179.70	30.80	**14.60**
BG	2	151.20	152.22	-1.02	**-0.67**					
DAY I	2	665.72	608.38	57.34	**8.61**	DAY I	514.50	456.20	58.40	**11.30**
DAY III	2	525.31	489.37	35.94	**6.84**	DAY III	374.10	337.20	37.00	**9.90**
DAY VI	2	375.04	350.37	24.67	**6.58**	DAY VI	223.80	198.20	25.70	**11.50**
BG	3	151.79	154.91	-3.12	**-2.06**					
DAY I	3	643.48	593.49	49.99	**7.77**	DAY I	491.70	438.60	53.10	**10.80**
DAY III	3	562.14	519.89	42.25	**7.52**	DAY III	410.40	365.00	45.40	**11.10**
DAY VI	3	433.40	388.34	45.06	**10.40**	DAY VI	281.60	233.40	48.20	**17.10**
BG	4	150.95	150.81	0.14	**0.09**					
DAY I	4	560.75	554.20	6.55	**1.17**	DAY I	409.80	403.40	6.40	**1.60**
DAY III	4	467.72	398.00	69.72	**14.91**	DAY III	316.80	247.20	69.60	**22.00**
DAY VI	4	357.22	321.37	35.85	**10.04**	DAY VI	206.30	170.60	35.70	**17.30**

Fortunately, all cotton buds contained enough amount of saliva to analyse the samples. Table 3.1 compares isotopic enrichment of both blood and saliva samples in the four test animals (*in vivo*) on days one, three and six.

Both body water samples were drawn at near time points, i.e. to be able to use saliva as a surrogate for blood samples, values found in blood and saliva should be on a comparable level. Please note that the percentile difference between deuterium levels in blood and saliva varies between 0.9 % and 14.91 %. Within each test animal, levels varied between 2.48% and 7.48% (Animal 1), 0.67% and 6.58 % (2), 2.06% and 10.4% (3) and 0.09% and 14.91% (4). Deuterium enrichment was always lower in saliva samples than in the respective blood samples, except for three background samples (animals 1, 2 and 3).

As it is necessary to draw two samples of a body fluid to be able to calculate energy expenditure (EE) with DLW, the observed variation is higher than shown in this experiment conducted with only one isotope.

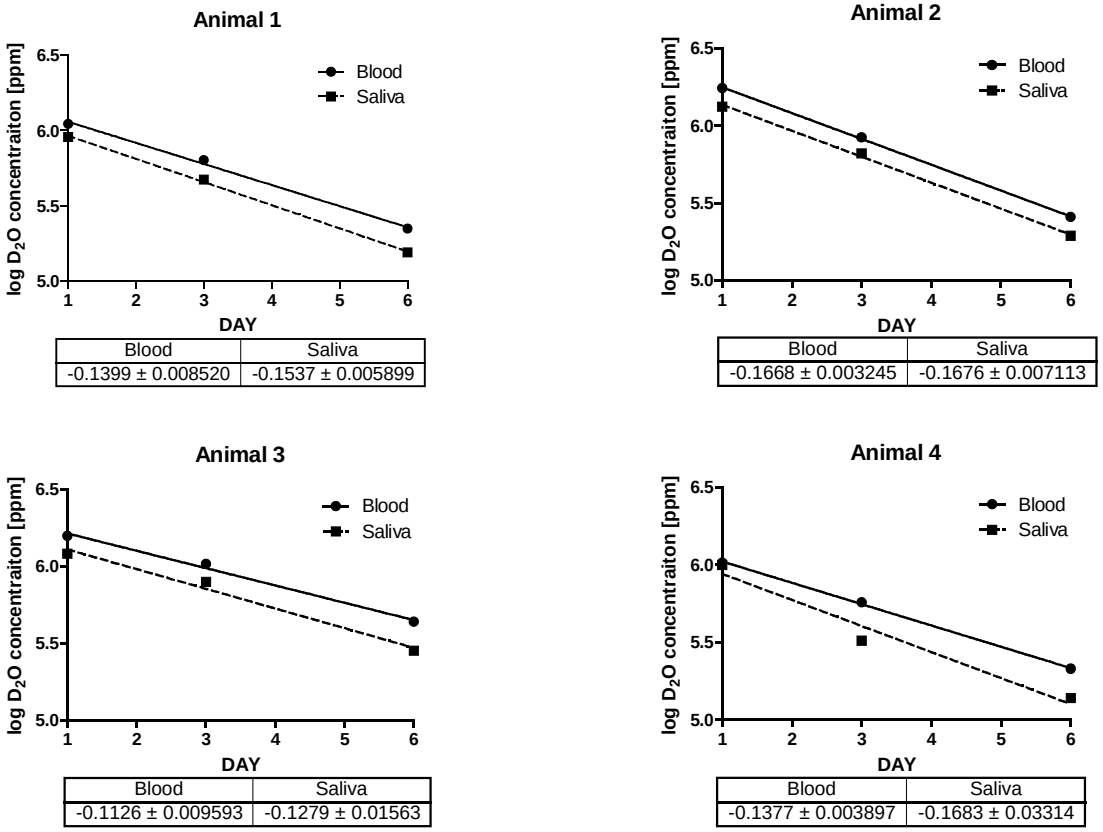


Figure 3.11: Comparison of deuterium levels in blood and saliva samples obtained at similar time points in four male adult meerkats.

Please note that background isotope enrichments have been subtracted from the enrichments of day one, three and six. Below the x-axis slopes for blood and saliva washout rates are shown."Ppm": parts per million. Slopes of blood and saliva washout rates are not significantly different in all four animals (F-test, n.s.).

Figure 3.11 illustrates logarithms of the deuterium enrichments in blood and saliva (compare Table 3.1), where the slopes of the lines indicate the washout rates of the isotopes in the body fluids. Slopes look similar and are not statistically different from each other in all four animals (F-test, n.s.). Maximum difference between washout rates of blood and saliva is 21 % in animal four though.

As deuterium concentrations are always lower in saliva than in blood, either the absorbing material of the saliva sample – cotton – induces changes in the deuterium concentration in the saliva samples, or the deuterium concentration is lower in saliva itself.

3.2.2 DLW and Saliva - In Vitro Experiments: Effect of cotton contact on the deuterium concentration in the samples

To test whether the absorbing material had a confounding influence on isotope concentrations in the samples, an *in vitro* experiment compared samples of isotopic dilutions that had been sealed directly to those which had been absorbed on cotton first. All samples were heat-sealed into glass capillaries (deuterium enrichment in the samples is up to 2000 times above background, hence the diffusion pressure is very high and it is necessary to seal such probes in glass to inhibit isotopic exchange with the environment). It is obvious that isotope concentrations are always lower in the samples that had cotton contact before.

Interestingly, the difference appears to increase with increasing isotope concentration. At the 1 : 8000 dilution, the percentile difference is 2.33, whereas at the 1 : 2000 dilution it increased to 2.56.

Table 3.2: Influence of cotton contact on deuterium concentration in the sample.
"D_2O without cotton contact": the samples had been sealed in glass capillaries directly; "D_2O with cotton contact ": samples had been soaked on a cotton bud first and were then sealed in glass capillaries; "ppm": parts per million. Please note that the percentile difference increases with increasing deuterium concentration.

	D_2O without cotton contact [ppm]	D_2O with cotton contact [ppm]	Difference [ppm]	Percentage [%]
Dilution				
1 in 8000	276.67	270.21	6.46	2.33
1 in 4000	398.14	388.32	9.82	2.47
1 in 2000	654.69	637.95	16.74	2.56

3.2.3 DLW and Saliva - In Vitro Experiments: Effect of cotton contact time on the deuterium concentration in the samples

To test whether the time the sample was exposed to the cotton also had a confounding effect on the concentration in the sample, samples where soaked on cotton buds with varying contact time (1 h - 168 h). Table 3.3 summarizes these results. Unlike the differing isotope concentrations, the time exposed to the absorbing material does not seem to influence the

deuterium concentration in the sample, as mean deuterium concentrations do not deviate with different contact times.

Please note that, especially in the *in vitro* part of these experiments, sample size is rather small. This can be attributed to the fact that DLW-analysis is very expensive. For further explanation please consult the respective DISCUSSION section.

Table 3.3: Influence of contact time to cotton on the Deuterium concentration.
Deuterated water was soaked on a cotton bud while contact time varied from 1 hour to 168 hours; n = 2; "mean": mean value; "sd": standard deviation; "ppm": parts per million.

Time on cotton bud	**Mean [ppm]**	sd [ppm]
1 hour	**670.64**	1.91
24 hours	**669.85**	0.30
120 hours	**674.47**	0.50
168 hours	**673.12**	0.40

Summarizing, this means that using saliva samples collected on cotton buds are always underestimated (*in vitro* and *in vivo*), and can introduce errors of up to 21 % in the isotope washout rates (animal 4). This error can very likely be attributed to the absorbing material, cotton, as *in vitro* testing suggested. Thus this approach of minimal invasive sampling was not pursued further, another but a different idea involving minimal invasive blood sampling was followed.

3.2.4 DLW and Saliva - *In Vivo* Experiments: Oral Isotope Administration

Unfortunately the data on oral isotope administration were too bad to analyse (body water values obtained with these samples was way off the expected range) and the only sample that provided data for analysis showed that the isotope enrichment in the sample after oral administration was less than half of the expected value. This (one) sample thus indicated that much of the administered isotope was not incorporated into the body. Because of these dramatically bad results, no further attempts of administering the isotopes orally were made, especially since the common method of intraperitoneal isotope administration has a low impact and does not require anaesthesia but just one (quick) capture and injection of the test animal.

3.2.5 DLW and Minimal Invasive Blood Sampling: Bug Container Design

As using saliva as a substitute for blood samples has proven inadequate, using blood-sucking insects as "living syringes" seemed a promising way to obtain to withdraw blood samples minimally invasive. Literature data and personal communication with the respective authors

suggested that South-American reduviid bugs appeared to be ideal candidates and provided the necessary "skills" like taking up large quantities of blood (up to 5 ml in adult *D. maximus*), not ingesting the blood meal too soon as had been previously tested by e.g. by Voigt et al (2003). These reduviid bugs need up to 40 minutes for their blood meal, the sample, from the meerkats. Thus, one of the first requirements to use these insects was to design a container that allowed safe attachment of the bugs on the insectivorous meerkats and also allowed the bugs to sting.

The container enclosing the blood-sucking parasites needed to fulfil two requirements: save the insect from being detected, and possibly eaten by the meerkats, as well as a meshed side that allowed the insect to sting and take up the blood from the meerkat. To meet with these requirements, old photo boxes where cut open and a gaze was attached to the box. This design proved to be sufficiently strong to protect the bug from predation by the meerkat, at the same time allowing the insect to move and find the best spot to acquire the blood.

Figure 3.12: Bug container design.
(A) Meerkat in "Tonis's Zoo", Luzern, Switzerland wearing a collar with a reduviid bug. (B) Detail of the container enclosing the bug, please note that the box is made of gauze on one side, where the bug (here R. *prolixus) can feed.*

Figure 3.12 shows a meerkat wearing a collar at Tonis's Zoo in Luzern, Switzerland, where first experiments to test the feasibility to use reduviid bugs on meerkats had been tested in June 2007. The box firmly encloses the insects while at the same time allowing them to feed on the meerkats. The box was clipped over a collar, that was closed with a Velcro strip, to ensure easy and quick attachment and detachment on the meerkats.

To be able to attach this container on the meerkats, the animals needed to be trained prior to the experiments to ensure that attaching the collar with the container does not induce any stress on the test animals, as well as to generally enable handling the (priorly untrained) meerkats.

3.2.6 DLW and Minimal Invasive Blood Sampling: Collar Training

Figure 3.13: Positive enforcement training in free-ranging meerkats.
(A) + (B) Previously untrained meerkats at the "Kalahari Meerkat Project", Van Zylsrus, South Africa. (A) The animal wearing the collar is guarding for predators, and (B) the animal is grooming himself, a typical comfort behaviour.

An important step towards the feasibility of this minimal invasive method was to train the meerkats to wear collars containing the parasitic insects without any impact on their natural behaviour. Both meerkats in the European zoos and at the "Kalahari meerkat project" were not used to training of this kind before. Typically, "positive enforcement training" is the best approach to train wild animals to learn certain behaviours or tasks. This means, the test animal is, in a first step, becoming familiar with being rewarded if it displays a certain behaviour and in a second step then trained to display this behaviour whenever needed. In this manner, meerkats received tiny amounts of reward (crumbled egg, cat milk or water) whenever they tolerated wearing the experimental collars. Initial experiments with a small sample size (n = 4) at Toni's Zoo, Switzerland indicated, that it was possible to train the meerkats within 3-4 days, so that blood samples could successfully be obtained without indication of an impact on their natural behaviour.

Figure 3.13 A shows a meerkat in the Kalahari guarding for predators while wearing the collar, 3.13 B shows a meerkat that was grooming his fur. Grooming is a typical comfort behaviour, while generally comfort behaviours only occur in a relaxed atmosphere.

This means, the collar training has been proven successful in zoos as well as in the field, at free-ranging meerkats, as the animals quickly learned to tolerate the collar and showed no signs of altered behaviour while wearing the collar.

3.2.7 DLW and Minimal Invasive Blood Sampling: Weight Training

Purpose of obtaining weight data in this study was to calculate overnight weight loss and daily weight gain data of the observed study animals. These parameters can give valuable additional

information when collecting data on energy expenditure, as they show integrated effects of nutritional energy uptake, exposure to ambient conditions, cost minimization by behaviour and physiological adaptations.

Usually, study animals at the "Kalahari meerkat project" are very well habituated and weights of individuals can be collected twice, sometimes even three times a day. Typically, when working with living animals generally and free-ranging animals particularly, many confounding variables can minimise the success of collecting such data.

Unfortunately, just prior to the time of my main observation period on the "Kalahari meerkat project", there had been severe problems with the study population. Instead of 12 groups with up to 50 individuals each, only 6 group with 6-8 individuals were available for research.

Some of the groups had just re-established and habituation was poor compared to regular standards. Unfortunately, this greatly affected the amount of weight data that could be obtained.

Of about 2500 weight points (morning an evening weights in three months in groups of average 8 animals) that should have been obtained, only a little over 1000 points could be collected. As these data points were not always consecutive, (e.g. morning weight in individual A but no evening weight for this individual), only about 22% of the daily-weight-gain and overnight-weight-loss data could be calculated (full weight data are shown in APPENDIX).

Unfortunately, this was not enough data for successful correlations to ambient conditions or meerkat activity and thus conclusions on the meerkats ' energy expenditure.

3.2.8 Ionising Radiation as a Method of Sterilisation

After successfully establishing a training method to guarantee that the collar would not influence the meerkats' natural behaviour, it was necessary to process some "ecological safety parameters" when handling introduced (not native) insect species in a natural environment, outside of a laboratory. Reduviid bugs originate from South America, and it would be dangerous to handle them in the South African Kalahari desert without ensuring that they cannot spread in this environment.

Thus, following the "sterile insect technique" (Dyck et al. 2005), ionising radiation was used to sterilize the bugs, i.e. develop a protocol for the used reduviid species that guarantees they cannot produce fertile offspring.

Three groups of second and third instar *Rhodnius prolixus* (n = 20 per group) were radiated with different doses of X-rays (20, 40 and 80 Gray) and together with an unradiated control group, subsequently observed for the next six months. Parameters of interest thus were:

a) survival rates and general "welfare" in each group:

The dosage of ionising radiation is a critical parameter as, on the one hand, a main goal of the experiment was to sterilise the insects, and on the other hand the bugs are further used as "living syringes". This means, it was important that the dosage was enough to guarantee sterilisation, but was not too high and would induce X-ray sickness in the insects or reduce survival rates too much

b) possible production of (fertile) eggs:

Typically, if the dose is applied correctly, the animals might still lay eggs, but these will not develop any further. The parameter observed here thus was not if there are eggs produced at all, but if these eggs would develop and hatch.

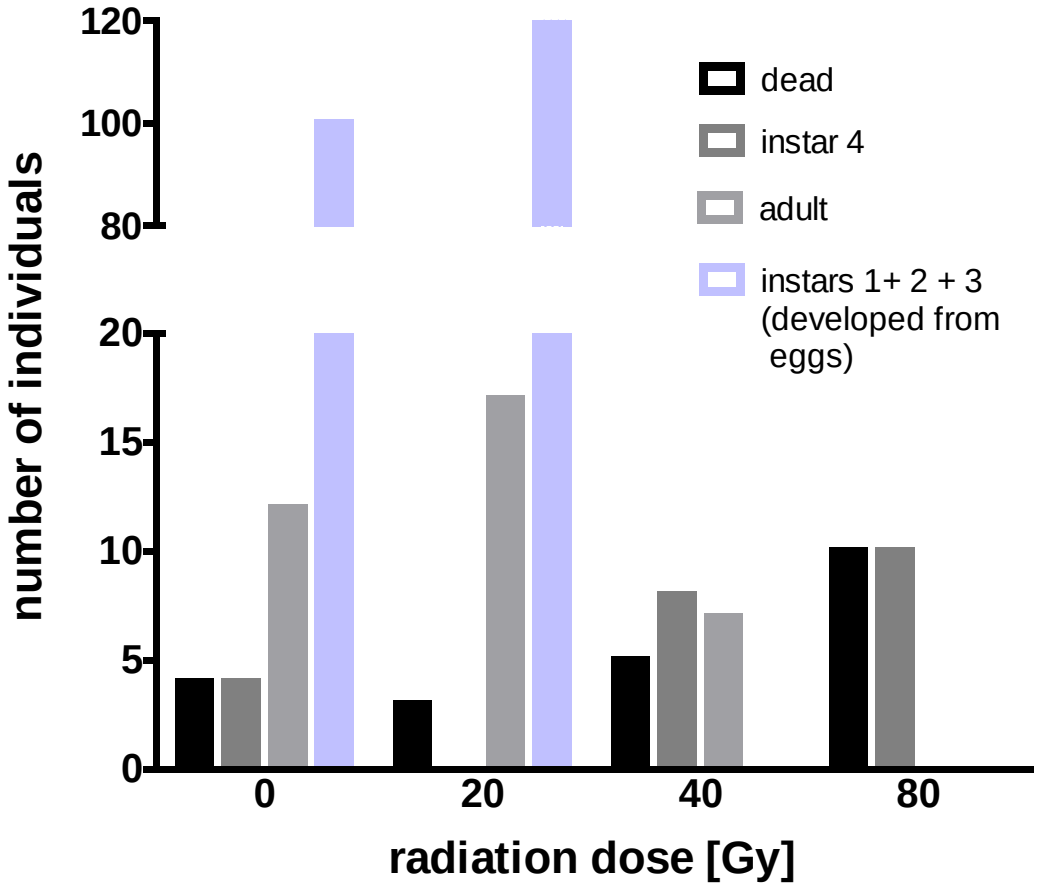


Figure 3.14: Effect of different doses of radiation on the development of four groups on reduviid bugs (R. prolixus)

n = 20 per group, test animals were in instars 3 and 4 (of five). 0 Gy: control group; 20 Gy – 80 Gy: test groups I, II and III; "dead": number of dead individuals within 6 months of after-radiation-observation; "adult": number of individuals that have hatched into the adult stage; "instar 4": number of individuals that have hatched into the fourth instar (of five); "instars 123": as indicated by the numbers (up to 120 in a group of originally 20 individuals), these are animals hatched from eggs laid AFTER the radiation experiment.

Figure 3.14 combines the observations. Firstly, in the control group (0 Gy) 4 of 20 animals died, 12 reached the adult stage and another four were in the fourth instar after six months of observation. The adult animals had produced 100 eggs, which had hatched into larvae (instars 1, 2 and 3). Interestingly, in group I (20 Gy), only three animals had died and 17 had reached the adult stage. They had produced 120 eggs, that had hatched into instars 1,2 and 3. This finding might emphasise the importance of a proper statistical experimental design – or hint that this dosage is far too low to sterilise these insects.

In group II (40 Gy), mortality rate was only slightly raised (5 of 20), but only 7 animals had reached the adult stage, and 8 were still in the fourth instar. No fertile eggs were found in this group. This indicates, that at 40 Gy, animals still seem to be in good condition and sterilisation was successful. At 80 Gy (the dosage indicated in literature) mortality rate had raised drastically (10 of 20), and none of the insects had reached the adult stage.

Figure 3.15: Effect of ionizing radiation of the fertility of reduviid bugs.
Eggs collected from bugs (A) untreated and (B), (C) and (D) sterilized with ionizing radiation (dose: 80 Gray). (A) Unradiated control group, the foetus in the utter right egg is clearly visible. Picture taken 6 d after the ionization of the other group. Magnification: 25x (B) Eggs laid by radiated reduviid bugs, picture taken 6 d after ionization. No foetus visible; magnification 25x (C) Picture taken 13 d after radiation. Magnification 25x. (D) Picture taken 20 d after radiation. Magnification 25x

Summarizing these findings, 40 Gy seems to be the best radiation dose as animals are in good condition and sterile. Higher doses, as suggested by literature, only radically reduced the animals survival rates. Although the insects radiated here were not in an adult stage, but in instars 3 and 4. This might explain why literature suggests higher radiation levels than those found in this study.

Figure 3.15 shows eggs that had regularly been collected after ionization of the test groups, and within the control group. The radiation effect is clearly visible: Untreated reduviid bugs' eggs develop normally, the foetus is clearly detectable (Figure 3.15 A), whereas eggs laid by radiated bugs appear totally infertile, no foetus can be detected in the eggs. Pictures of the eggs were taken 6 (Figure 3.15 B), 13 (Figure 3.15 C) and 20 d (Figure 3.15 D) after radiation to monitor whether the development was just delayed.

3.2.9 Measuring EE with minimal invasive blood sampling

Despite providing data on the feasibility of blood sampling via reduviids and adding the protocol of the sterilisation experiments to the application to import the sterilised insects in the South-African Kalahari desert several months before the start of the study in the Kalahari, the import was declined just ten days prior to my flight date to South Africa, in spite of repeated attempts of contacting the people in charge and offering them more info and all my raw data. In those ten days, the only plausible alternative to the South-American reduviids were bed bugs (*Cimex lectularius*, *Heteroptera*) that were kindly provided by Bayer Crop science.
Although breeding them in the Kalahari was possible, the species seemed to react very sensitive to the harsh Kalahari environment, as all the individuals I took to the field for several hours (securely locked in the collars for the meerkats), died with the exception of four or five. Thus I was not able to measure any energy expenditures of free-ranging meerkats *in situ*.

Although not being able to apply the technique in the field in the course of this thesis, I decided to validate the technique with respect to proving its feasibility on free-ranging animals. In order to avoid further problems when importing non-native species I searched for an alternative native African insect that could be used in the field in future studies and tested its properties.
As African reduviids are insectivorous and not blood parasites, the most promising candidate of African alternatives to the *R. prolixus* and *D. maximus* appeared to be tsetse flies *Glossina spec.* (in terms of meal duration, meal volume and availability of the insects). *Glossina spec.* was

kindly provided by Dr. Udo Feldman of the International Atomic Agency in Vienna. Tsetse flies need 2-3 minutes for a meal as a opposed to 40 minutes in the reduviid bugs and take up volumes of up to 50 µl, rarely 60 µl (*G. brevipalpis*), which is less than in the reduviids, but sufficient for DLW analysis if several flies are used. At this point it was considered necessary to compare the different species of parasites - reduviids and flies - as potential blood sucking instruments. Thus, in a series of experiments the energy expenditures of meerkats measured with both South American and South African insects was compared in the groups at the Cologne Zoo and at the Tierpark Fauna in Solingen. The meerkat group in Cologne zoo was bigger and their behaviour was comparable to the field (Habicher, 2004), whereas the group in Solingen had the advantage of a better accessibility due to the smaller enclosure size.

Three attempts were made to measure EE with reduviids. The first experiment conducted in July 2008 had to be aborted as only four blood samples could be obtained from nine animals, while these were two times two samples from different animals. i.e. no data on EE could be obtained. In the second experiment in October 2008 only 2 very small samples could be obtained from 11 animals. In a third experiment in December 2008, no blood samples could be obtained from four animals.

Another three attempts were made using tsetse flies. In the first experiment in December 2008 eight blood samples could be obtained from four animals on two days. In the second experiment in January 2009, 7 blood samples could be obtained from four animals in two days. In the third experiment in April 2009 8 samples could be obtained from 8 animals on three days.

Table 3.4: Comparison of success rates between reduviid bugs and tsetse-flies.
"maximum blood intake": maximum amount of blood that could be obtained with one insect, "meal duration": amount of time necessary to draw a sample; "samples per trials": number of successful samples per trial number; "samples per animal": number of samples that could be obtained per meerkat; "calculation of EE possible": number of times when two samples from the same meerkat could be obtained and these samples could be analyzed to calculate EE.

	Reduviids	Tsetse flies
Maximum blood uptake	5 ml (adult *D. maximus*)	60 µl (*G. brevipalpis*)
Meal duration	40 minutes	2-3 minutes
Samples per trials	6 of 47	23 of 45
Samples per animal	6 of 24	23 of 16
Calculation of EE possible	0	9

Table 3.4 summaries the advantages and disadvantages of the different insects used in this study: While with reduviid bugs large quantities of blood can be obtained, their reliability is very poor. Only six samples in three experiments could be collected from 24 animals, and no two consecutive samples from the same test animals that are required to measure EE with DLW. While the amount of blood that can be sampled with tsetse flies is limited to an absolute maximum of 60 µl, they are very reliable and need only a very brief period of time to take up the blood meal, which increases the accuracy of the experiment as sampling time is so much shorter.

As tsetse-flies posses very powerful physiological adaptations to quickly excrete the water in the blood (compare 1.2.2 in INTRODUCTION) to regain their mobility, the probability that parameters in the sample/blood meal could be changed quicker than in the – rarely flying – reduviids, tsetse flies were killed in diethylether immediately after blood sampling.

Analysis results of the blood samples obtained with the tse-tse flies are summarized in Table 3.5. K_D and k_O, the washout rates of both heavy isotopes, are derived if the log of the isotopes enrichments is plotted against time. Slopes of the lines then represent the washout rates. After (Speakman 1997) the fundamental basis of the DLW technique is the evaluation of the divergence of the washout curves for the two isotopic labels. The closer together these lines are, the more likely it is that any slight error in evaluation of either curve will lead to errors in the predicted CO_2 production and hence energy expenditure. The closeness of these two lines is represented by the ratio of their gradients: the k_D/k_O ratio. The closer it is to 1 the greater the contribution of water to the total turnover of the oxygen isotope, and less precision one has in the resultant estimate. Typical values for small mammals are ratios between 1.2 and 1.6. Hence, it is obvious that in animals 6 (negative ratio), 11 (ratio too high) and 12 (ratio too high), values are not within range. In animals 5, 7, 8 9 and 10, values of this ratio are within the usual range.

Another indicator of preciseness is the dilution space N (estimates of body water), derived from the dilution rates of both isotopes (N_d and N_o). Typically, these values are between 60 – 70 % (pers comment Dr. Paula Redman, lab manager at Prof. Speakman's lab).

Dilution spaces of both isotopes can be calculated using two different methods: the "plateau" method, and the "intercept" method. The plateau method implies that the initial sample is taken at exactly the time point when isotope concentration in the body is highest, and thus that the isotope concentration in the initial sample represents the highest possible concentration at any time point.

Table 3.5: Results of DLW experiments using tse-tse G. brevipalpis as a method of blood withdrawal. "Animal" for further characterization please consult Table 2.1; "BM initial": body mass at isotope administration; "BM final": body mass at the end of experiment; "amout injected": $D_2{}^{18}O$ mass injected; "k_D": deuterium washout rate; k_O: heavy oxygen washout rate; "k_O/k_D": ratio of heavy oxygen to deuterium washout rates, an indicator of precision; "N_D": body water pool, i.e. a body water estimate, derived from deuterium dilution; "N_O": body water pool derived from heavy oxygen dilution, N_O and N_D are calculated using both the "plateau" method and the "intercept" method; DEE: daily energy expenditure; "EST 1": (Lifson & McClintock 1966) equation 35; "EST 9": (Speakman 1997), equation 7.17; both EST 1 and EST 9 can be derived following either "plateau" or "intercept" method.

Animal	BM initial	BM final	amount injected	k_D	k_O	k_O/k_D
5	1321	1333	0.8762	0.0098	0.0128	1.3005
6	909	920	0.5671	-0.0020	0.0006	-0.3010
7 (01/09)	1380	1378	0.8529	0.0053	0.0080	1.5049
7 (04/09)	1319	1384	0.8939	0.0055	0.0070	1.2783
8	1441	1464	0.8805	0.0068	0.0088	1.2946
9	1079	1137	0.6585	0.0031	0.0049	1.5648
10	1088	1041	0.6557	0.0052	0.0077	1.4859
11	1048	1065	0.6602	0.0013	0.0035	2.7292
12	978	985	0.6517	0.0002	0.0018	7.5181
	plateau	**plateau**		**intercept**	**intercept**	
Animal	N_D [% BM]	N_O [% BM]	N_D/N_O	N_D [% BM]	N_O [% BM]	N_D/N_O
5	83.82	77.49	*1.08*	83.00	76.51	*1.08*
6	121.25	111.48	*1.09*	121.49	111.41	*1.09*
7 (01/09)	93.35	81.14	*1.15*	93.17	80.90	*1.15*
7 (04/09)	86.03	79.25	*1.09*	85.64	78.78	*1.09*
8	67.72	63.41	*1.07*	67.48	63.12	*1.07*
9	82.83	78.70	*1.05*	82.70	78.52	*1.05*
10	69.65	64.71	*1.08*	69.51	64.52	*1.08*
11	80.71	77.05	*1.05*	80.66	76.93	*1.05*
12	80.01	77.76	*1.03*	80.00	77.70	*1.03*
	EST 1 Plateau	**EST 1 Intercept**	**EST 9 Plateau**	**EST 9 Intercept**	**problem**	
	DEE [kJ/day]	**DEE [kJ/day]**	**DEE [kJ/day]**	**DEE [kJ/day]**		
5	937.82	925.91	1002.57	989.83	high BWE	
6	negative k_O/k_D ratio	high BWE	negative k_O/k_D ratio	high BWE	negative k_O/k_D ratio	
7 (01/09)	975.98	973.11	1014.69	1011.71	high BWE	
7 (04/09)	501.85	498.91	539.57	536.41	high BWE	
8	565.13	562.53	605.01	602.22		
9	497.84	496.66	515.43	514.20	high BWE	
10	558.66	557.01	581.73	580.01		
11	k_O/k_D ratio too high	high BWE	k_O/k_D ratio too high	high BWE	k_O/k_D ratio too high	
12	k_O/k_D ratio too high	high BWE	k_O/k_D ratio too high	high BWE	k_O/k_D ratio too high	

The "intercept" method takes the washout curve of the isotopes into consideration and extrapolates it back to the time of isotope entry. It is an indirect method for measuring the initial intensity. Using this method, it is not necessary to time the initial sample to coincide exactly with the "plateau" period, the period of the highest possible isotope concentration in the body.

The only two animals that show a reasonable value for N are animals 8 and 10 (67.48 and 63.12 %; and 69.51 and 64.52 % respectively). The percentages of body water for animals 5, 6, 7 (both measurements) and 9 are out of the usual range.

With blood-sucking parasites sampling time cannot be timed as exactly as when anaesthetising a test animal and drawing the blood with a syringe. Therefore, values derived with the intercept method are probably preferable over those values derived with the plateau method.

Finally, daily energy expenditure DEE can be calculated using to different formulas: EST 1 following an older formula from (Lifson & McClintock 1966): $rCO_2 = N/2.08\ (k_O-k_D)-0.015\ k_{2d}N$ and EST 9 following (Speakman 1997): $= rCO_2 = (N/2.078)*(k_O-k_D)-0.0062*k_D*N$.

As in older literature DEE is usually calculated after EST 1, it is advisable to show both formulas for reasons of comparability. Both formulas use N to calculate DEE, i.e. there are four possible DEE values: EST 1 using N calculated with both plateau and intercept method, as well as EST 9 using N calculated with both plateau and intercept method. In this study, however, Est 9 following the intercept method is the most reasonable assumption for DEE, as the intercept method is independent of exact plateau timing, and EST 9 takes evaporative water loss into account. For the two animals where testing did not indicate abnormalities DEEs are: Animal 8 – 602.22 kJ/d and animal 10 – 580.01 kJ/d.

3.2.10 Other applications of the minimal invasive blood sampling method: measuring hormone levels

To determine if the minimally invasive blood sampling method can also be used to determine hormone levels in the blood samples, tsetse were fed from an artificial blood source in the laboratory. Unfortunately, *G brevipalpis* was not available as the IAEA's colony in Vienna had been moved to Pretoria, South Africa this summer. Thus *G. morsitans* were used for this experiment.

At each sampling instant, blood was also drawn from the food source with a syringe. These samples act as controls for the possible influence of the insects on the blood sample.

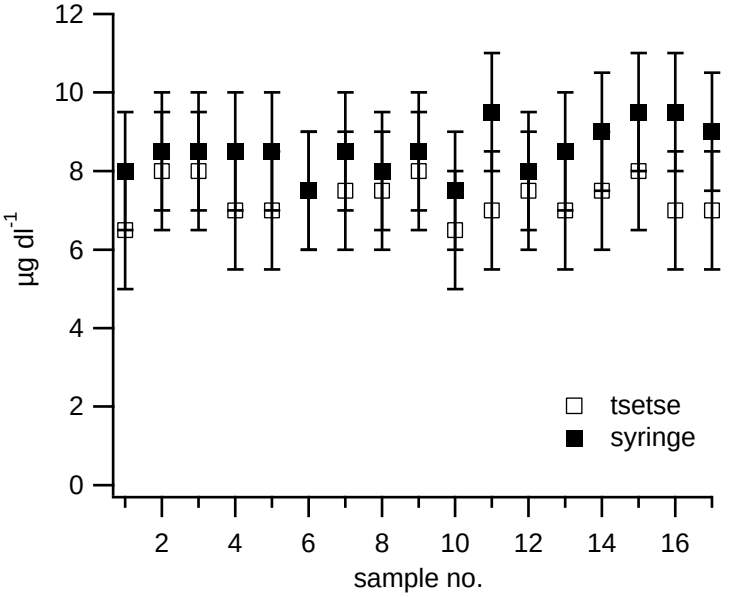


Figure 3.16: Differences in cortisol levels between blood sampled from a food source using syringes and tsetse flies, respectively
Precision of the assay was determined from duplicate analysis and was 1,5 µg/dl.

Figure 3.16 shown the differences between samples taken up with a syringes and with tsetse-flies. Interestingly, there appears to be a slight dilution in the fly-samples, as concentrations were minimally lower in these samples. The precision of the assay was determined by duplicate analysis of the samples that were taken up with syringes. Maximum measured error was 1.5 µg/dl, shown as error bars in Figure 3.16. This precision error of 1.5 µg/dl represents a relative difference of 18-20 %. The mean cortisol concentrations in both groups were 7.3 and 8.5 µg/dl, respectively. Assuming an error of 20 %, this gives a mean ± error of 7.3 ± 1.5 (flies) and 8.5 ± 1.7 (syringes) respectively. In spite of the slight dilution of the samples drawn via tsetse-flies, means of the two groups are thus not different.

To clarify this statistically, a normal distribution was generated using the "R"- function "rnorm", as data was not distributed normally (Kolmogorow-Smirnow-test). As all samples had been derived from the same food source, values were naturally not distributed normally. A t-test of the normalised data showed no significant difference between the two pools of samples.

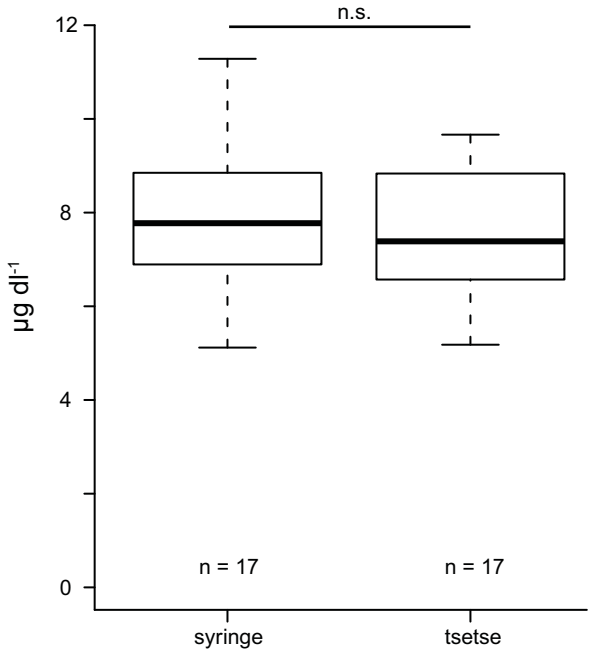


Figure 3.17: Comparison of cortisol levels in blood samples obtained via tsetse vs. conventional sampling. As data were not distributed normally (Kolmogorow-Smirnow-test), normal distribution were generated using R-statistical software. Distributions of cortisol levels were drawn defined by the mean and standard deviation as explained in the text. T-test showed no significant difference between both pools ($p = 0.44$).

4 DISCUSSION

4.1 Behavioural Thermoregulation

Influence of Temperature:

Ambient temperature affects the meerkats' behaviour considerably. With increasing T_A, time spent in sun decreased, whereas time spent in shade and below increased. Especially 30 °C, representing the lower critical temperature T_{LC}, seems to be an important border temperature as the residence proportions very suddenly alter here. Time spent in the sun was not significantly influenced by T_A below the T_{LC}, above it could be correlated highly significant. Proportions of time spent in shadow and below look very similar. Times spent in shade and below both increase significantly above the T_{LC}, but whereas time spent in shade appears to decrease slowly above the animals' upper critical temperature T_{UC}, time spent below still increases. If T_A further increase above the meerkats' (core) body temperature, T_B, time spent in shade further decreases whereas time spent below reaches maximal values of up to 100% (i.e. the entire group spent 100% of the time below). Energy and water loss minimization strategies can be used to explain this distribution: Above the thermoneutral zone TNZ, i.e. above 33 °C in meerkats, thermoregulation becomes costly in terms of energy. The small temperature gradient (37 °C in the animal and 33 °C T_A) can still be utilized to lose heat via convection and radiation, but to dissipate the entire metabolic heat, panting needs to be utilized, that costs energy and, water. Above 37 °C, there is no more temperature gradient to the environment, and the only means by which heat can be dissipated is via evaporative cooling, i.e. mainly panting in meerkats. These small carnivores also utilize another strategy in conditions of extreme ambient heat: Muller & Lojewski (1986) reported that meerkats rub their nose over their fur, thus wetting it with water that had formed through condensation in the vicinity of the nostrils. Neither panting nor rubbing the nose over the fur could be observed in the current study. But both panting and wetting fur cost energy and also water. It appears that, when the risk of water loss increases (above 37 °C), using the shade is no longer efficient and the meerkats make use of a more suitable microhabitat.

In the shade, T_A is still above T_B, and the animal still needs to utilize energy and water to cool down (no significant differences of T_A between sun and shade could be measured). The only advantage of being in the shade instead of the sun is avoiding further absorption of radiative

energy. Retreating below ground though means that extreme ambient temperatures can be avoided. Lynch (1980) summarized that daily air temperature variation can be as much as 18 °C in summer, while burrow temperature variation is only about 1 °C (22.6 - 23.2 °C). At the highest T_A measured in this thesis (45 °C) this implies that by retreating into the burrow systems, meerkats avoid thermal stress of more than 20 °C. Müller and Lojewski (1986) measured the meerkats' oxygen consumption in relation to T_A. If assuming a mean burrow temperature of 25 °C instead of 45 °C air temperature, this would save about 0.12 ml O_2 consumption per gram per hour (compare Figure 4.1). Assuming a mean weight of 800 g per meerkat, this would calculate to 96 ml O_2/h. Assuming a mean caloric equivalent of 20 kJ/l O_2, this means a meerkat would save 1.92 kJ/h, or 46 kJ/day which corresponds to a reduction of energy expenditure about 18%. Comparing this to the basal metabolic rate obtained by Müller & Lojewski (which is 6.1 kJ/h or 147 kJ/day) the reduction by retreating into the burrows at T_A of 45 °C makes up 30% of the meerkats' BMR, and hence represents a substantial amount of energy. As meerkats huddle at temperatures below their TNZ (personal observation in Zoos and in the field) to reduce heat loss by minimising the surface area exposed to the environment, it can assumed that they huddle when the group retreats to the burrows. This very probably further reduces the metabolic rates when they are in the burrows, and at burrow temperatures of 25 °C the animals are very likely in thermoneutrality. Thus, the amount of energy that is saved by retreating in the burrows is even larger than explained above.

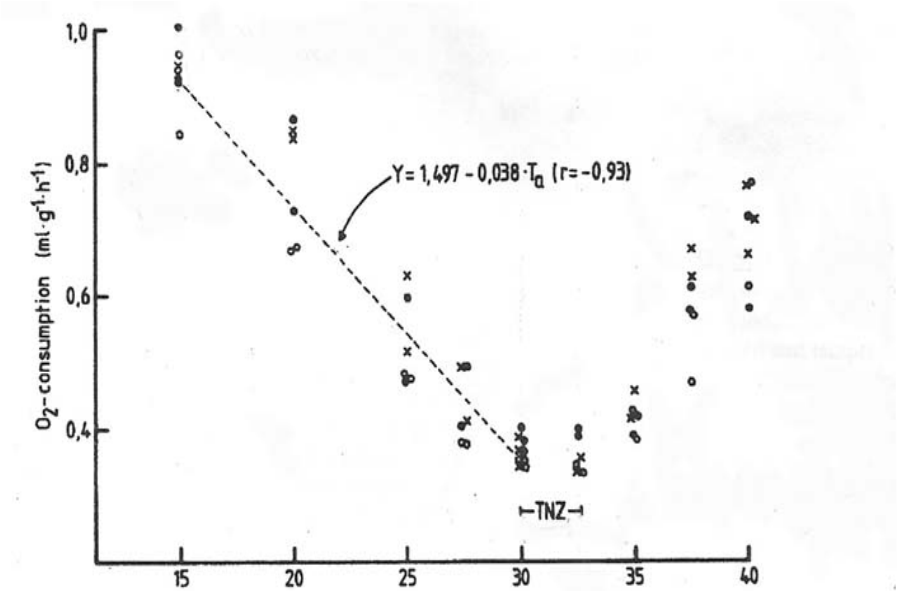


Figure 4.1: Oxygen consumption over the range of 15 °C to 40 °C.
*Taken from Müller & Lojewsky (1986). At 25 °C, a mean oxygen consumption of 0.56 [ml/g*h] was assumed, whereas at 40 °C, a mean of 0.68 [ml/g*h] was assumed for the calculation of energy savings by retreating to the burrows. This represents a reduction of metabolic rate of about 0.12 ml /g*h) or 1.92 kJ/h (18%) when taking advantage of the temperatures 20 °C below T_A in the burrows.*

The proportions of times spent contact lying, with piloerection and sunbathing could also all be correlated to T_A and can thus be regarded as behaviours that minimise energy expenditure. Contact lying facilitates conductive heat loss to a cooler surface (and thus reduces core temperatures) while sunbathing and piloerection either utilise solar energy to heat the body up and help to minimise heat loss by radiation and convection, respectively.

Accordingly, proportions of contact lying increased above 30 °C, while highest values were reached above 33 °C, and then the proportions decreased again (when the animals retreated into their burrows).

Sunbathing and the utilisation of piloerection were both used only below the TNZ, i.e. below 30 °C. But while piloerection prevents heat loss, and was thus used up to the animals' lower critical temperature, sunbathing actively utilises solar energy, and was only displayed below 27 °C, not closer to the TNZ. An explanation could be that sunbathing is primarily used in the mornings, after the meerkats got up from their burrows. Müller & Lojewski report that meerkats decrease their body temperature about 1 °C at night (thus saving about 10 % of their metabolic heat production, as the gradient to the environment decreases and thus less heat is lost. According to Lynch (1980) burrow temperatures never exceed 25 °C). In the mornings, they use solar energy to heat up and raise their T_B to usual levels. An alternative explanation is that sunbathing and foraging are mutually exclusive behaviours, while piloerection and foraging are not. This means, at lower T_A (of below 27 °C), meerkats use solar energy to actively heat their body, whereas between 27 °C and the T_{LC} at 30 °C, piloerection still reduces heat loss but allows the animals to forage.

In addition to the behavioural strategies explained above, Müller & Lojewski (1986) found that meerkats have a significantly raised thermal conductance (18% above mass specific level), a reduced BMR (42% below the mass specific level) and a remarkable capacity for heat dissipation via evaporation. A reduced BMR, raised conductance and high lower critical temperature are typical adaptations for desert-dwelling species (Haim & Izhaki 1995). These adaptations to conditions of high environmental heat load and intense radiation allow them to spend more time in the sun, as a low BMR helps to diminish the problems in a hot environment by reducing the contribution of internal heat production to the total heat load. A raised conductance allows to dissipate more metabolic heat, at least at T_A's below their body temperature.

This seems to be reflected by the high proportion of foraging on total activity, as their physiological adaptations to high environmental heat load, allow comparatively “cheap”

physiological thermoregulation. To further reduce their metabolic costs, they utilise a high proportion of behavioural thermoregulation.

While behavioural thermoregulation amounts to more than 27. 5% (compare 3.1 in RESULTS) of the total behaviour, foraging alone takes up 36.9% of the total time. Another 12.2 % of the time are being spent with helping behaviour. This means these three domains (thermoregulation, foraging and helping) account for almost 80% of the total meerkat time.

Influence of Solar Radiation:

Radiation appears to have an influence similarly important than ambient temperature, considering that correlations to time spent in shade, below and also contact lying, piloerection and sunbathing are highly significant. It is difficult though to differentiate between the influences of ambient temperature and radiation, especially as both are highly correlated, or intertwined. The ambient temperature values described above thus represent radiation data to a certain degree.

Solar radiation influences the body as it is energy [W/m^2 i.e. $J/s*m^2$] that is absorbed and thus can be utilized to heat the body, or avoided to not further increase the body's heat load. Maximum radiation intensities that were measured during this study were 1300 [W/m^2]. This means, meerkats foraging in the sun would expose their dorsal areas alone (the areas directed upwards) to as much as 13 J/s (if assuming $0.01m^2$ for this area) that can potentially be absorbed. Absorption rates are naturally highly dependent on fur characteristics such as colour, fur length, fur density; skin colour and the like; body posture, body temperature and many more (e.g. Stelzner & Hausfater 1986; Rogowitz & Gessaman 1990; Bakken 1976). Nonetheless, if assuming all energy would be absorbed, this would add up to as much as 780 J/h, or 0.78 kJ/h on the dorsal areas alone. The overall radiation would accumulate to few kJoules per hour, a substantial amount for a small sized mammal (BMR in meerkats is approx. 150 kJ/d). Meerkats have a thermal conductance that is significantly above the mass specific rate. This implies, that they can dissipate metabolic heat easily at T_A below T_B. Above 37 °C though, environmental radiation is also absorbed to a high degree and must thus be avoided. This is reflected by the dependency of behaviours that utilize or avoid radiation: Behaviours that utilised radiative energy to heat up the body (sunbathing) were displayed strictly below the TNZ, whereas excess radiation absorption was avoided as illustrated by the increase in time spent in shade (above 30 °C) and below (above 37 °C) at T_A above the TNZ.

Summarizing, this means that radiation has an important influence on the meerkats' thermoregulatory behaviour, adding on the influence of temperature. It is interesting though, that the upper and lower critical temperatures, values obtained in the the laboratory, could best explain the distribution of the behavioural data obtained in the field. This means that these temperature values obtained in climatic cabinets were confirmed in the field. Thus, though statistical analyses suggested that radiation also has an important influence on the behaviour, it occurs that temperature seems to be the most significant influence (as in a climatic cabinets the only parameter is temperature, there is no additional radiation nor wind and humidity is constant).

Influence of Humidity and Wind Speed:

Humidity appeared to have an influence less important than temperature or radiation, as only the proportion of time spent in shadow could significantly be correlated to humidity (Spearmann rank correlation, data not shown). It need to be taken into account though, that humidity and T_A are also intertwined and this correlation could simply have been influenced by the temperature data.

Wind speed could not be successfully correlated to any category of the behavioural data (Spearmann rank correlations, data not shown).

Summary ethological data: Temperature had the most significant influence on the distribution of the behavioural data. Especially the upper and lower critical temperatures, literature data obtained in the lab, were of most significance to explain the proportions of time spent in sun, shadow and below, as well as the proportion of times spent with thermoregulatory behaviours (contact lying, sunbathing, and piloerection). The influence of radiation added on the effect of temperature, as the animals utilised solar radiation to heat up at low T_A and avoided it at high T_A. Influences of humidity and wind speed were of minor importance. It is evident, that meerkats utilise a high proportion of behaviour to minimise energetic costs. Extrapolating from physiological literature data, it can be assumed that up to 18 % of energy can be saved by a single behaviour. It is very difficult though to quantify the proportion of energy saved and it would be much preferable to measure the animals' energy expenditure in the field.

4.2 Validation of a minimal invasive approach to the DLW method

Measuring energy expenditures in the field would complete the behavioural data, as data on EE can allow more detailed - and more reliable insight on how much energy the animals effectively save by using behavioural cost minimization strategies. When measuring EE while at the same time observing behaviour, specific costs of single behaviours can be extrapolated statistically given abiotic factors are also measured at the same time (McNab 1989).

The DLW-technique requires two samples of body fluids of an animal (conventionally blood), and prior administration of both heavy isotopes. Blood is typically sampled from the jugular vein when the test animal is anaesthetized. Between these two samples the rates of EE are measured.

As anaesthetising small mammals twice in short time periods can be a substantial risk to the test animals' condition, and also implies the hazard of falsifying EE data as the stress forced on the animals can alter rates of EE, an important aim of this thesis thus was to establish a minimal invasive approach to DLW.

4.2.1 DLW and Saliva - *In Vivo* Experiments: Comparability of isotope levels in blood and saliva samples

A first experiment compared the isotopic enrichments in blood (conventionally drawn) and saliva sampled at the same time. Table 3.1 showed that the differences of deuterium concentration varied between 0.9 % and 14.91 %. In this experiment only deuterium was used to compare both body fluids as heavy oxygen is very expensive and one isotope suffices to compare the differences in the enrichment levels. Looking at the raw data, one would assume errors of about 20 %, introduced by using saliva instead of blood samples (difference of the deuterium washout rates in blood and saliva in animal 4). To obtain values of energy expenditure, the slopes of the washout rates are of central importance, i.e. the equation to calculate EE is as follows: $rCO_2 = (N/2)(k_O-k_D)$, where k_O and k_D are the washout rates of the heavy isotopes and N is the dilution space (body water pool), usually calculated with heavy oxygen dilution (compare 2.4.2 in ANIMALS, MATERIALS AND METHODS). Slopes appeared to be similar on a first sight (and are not significantly different in all four animals, F-test n.s.). The 21 % difference in animal four are assumed to calculate the total error according to the error propagation method:

$$\Delta rCO_2=\sqrt{(\Delta N/2(k_O-k_D))^2+(N/2\Delta k_O)^2+(N/2\Delta k_D)^2}$$

Assuming the same error of 21 % on N, k_D and k_O (as N is calculated with the intercept method here and is thus also dependent on the slope) the resulting error on rCO_2 (ΔrCO_2) can amount to as much as 220 % (k_D and k_O were assumed to be equal here)!

This potentially huge error, together with the fact that isotope concentration was always lower in saliva than in blood samples, indicates that either the absorbing material of the saliva sample – cotton – induces changes in the deuterium concentration, or that the concentration of deuterium is different between blood and saliva. This is not likely though, as Poppitt et al. (1994) reported that concentrations of both isotopes in saliva samples collected simultaneously to blood samples in tenrecs are very similar to those in the blood samples. To test to which degree the absorbing material influenced isotope concentration in the saliva sample, a series of *in vitro* experiments were conducted.

4.2.2 DLW and Saliva - *In Vitro* Experiments: Effect of cotton contact and cotton contact time on the deuterium concentration in the samples

The notable errors of deuterium concentrations in the saliva samples could possibly originate from an influence of the cotton on the concentrations in the samples. Interestingly, the differences of deuterium concentration in samples with or without cotton contact increased with the amount of deuterium in the sample. This could indicate that the cotton seems to interact with the isotope in the samples. The sample size in these experiments naturally was very small, due to the very high costs of isotope sample analyses, but there are two facts that can affirm this theory. Firstly, (Knauf 2005) found very similar deviations when comparing hormone levels in saliva taken with cotton with those from blood. The cotton seemed to have absorbed the oestrogen (personal communication). This is interesting, as isotopes and hormones are molecules of very different sizes and molecular composition, and the fact that one seems to interact with the cotton would not automatically lead to the assumption that the other does as well.

Secondly, the fact that the concentrations of deuterium were lower in the samples that had been in contact to cotton clarifies that this cannot be a result of fractionation, or evaporation. Isotopic fractionation refers to the fact that the compositions of water in its liquid phase and gaseous phase are different in terms of the ratio of heavy to abundant isotope. The fractionation coefficient of hydrogen (in gaseous water relative to liquid water) is 0.93, this means that gaseous phase of deuterium is lighter than the liquid phase, or phrased differently that more deuterium is in the liquid phase than in the gaseous phase. As the amount of deuterium in the samples that had been in cotton contact was always lower than in the samples without cotton contact and this difference increased with increasing deuterium concentration, this indicates that this effect could not have been caused by isotopic fractionation or evaporation in general. If this had been the case, the concentration of deuterium in the samples with cotton contact must have been higher than in the ones without.

Contact time did not seem to influence the deuterium concentration however, as no difference between samples in contact to cotton for different periods of time could be found.

Summarizing, this means that very probably the lower isotope concentrations in saliva samples relative to blood samples have been caused by the absorbing material used in this thesis, cotton, and that it is not advisable to sample saliva on cotton in order to determine concentrations of heavy isotopes .

As other absorbing materials would bear similar risks, and meerkats do not produce sufficient amounts of saliva without chewing on an absorbing material, taking saliva samples to obtain EE rates in meerkats was abandoned in favour of minimal-invasive blood sampling.

4.2.3 Measuring EE with minimal-invasive blood sampling

Using blood-sucking insects as a tool to withdraw minimal-invasive blood samples seemed a promising idea. Literature data and personal communication with the respective authors suggested that South-American reduviid bugs appeared to be ideal candidates in terms of blood volume uptake (up to 5 ml in adult *D. maximus*) and blood storage in their guts (Voigt et al. 2003). Reduviid bugs need up to 40 minutes to draw their blood meal from the meerkats. Thus, one of the first requirements to use these insects was to design a container that allowed safe attachment of the bugs on the meerkats (meerkats being insectivorous) and also allowed the bugs to feed inside the container.

Bug container design:

The container enclosing the blood-sucking parasites fulfilled the two necessary requirements: 1) protecting the insect from being predated by the meerkats and 2) allowing the insect to feed on the meerkats. With this design, blood samples could be obtained successfully on several trials using both *D maximus* and *R. prolixus*. The problems of blood uptake with these two species indicated under 3.2.9 in RESULTS could be attributed to temperature-related problems, as the insects had been used on outside enclosures at ambient temperatures below 20 °C. At the same temperatures though, tsetse could feed more easily. This can probably attributed to the fact that tsetse flies can actively raise their body temperature by shivering thermogenesis.

Summarizing, this means that the container design proved adequate and the problems involved in obtaining blood samples could more likely be attributed to the low temperatures than problems with the boxes themselves.

Meerkat collar training:

Meerkats could easily and quickly be habituated to the presence of a collar, and within few days no signs of behavioural disturbances could be detected. After an initial habituation period, the animals displayed their natural behaviour, often also comfort behaviour while

wearing the collars. After using the collars together with the insects though, some animals initially refused the collars and had to be habituated again, indicating a mild discomfort caused by the sting from the insects. After very few (1-2) habituation practices, i.e. meerkats were habituated to wear the collars without insects, meerkats displayed natural behaviour wearing the collar again. This implies that when the use of the insects is planned on a regular basis, a strict schedule of training without insects and experimenting with insects should be followed to avoid that test animals associate the collar with the (mildly discomforting) insect sting. After each blood sampling experiment, meerkats should again be briefly habituated to the collars (without insects).

Reduviid sterilisation:

Following the practises of the "sterile insects technique", it was very easy to sterilise the reduviid bugs using ionizing radiation (in this case X-rays). The three groups irradiated with 20, 40 and 80 Gy, respectively, and the test group had been observed for six months after sterilization. In test group one and the control group sterilisation was insufficient, as fertile eggs had been laid and developed into instars 1,2 and 3 in the six months following the sterilisation experiment. Group II and III were proven infertile, and as in group II (40 Gy) infertility was not accompanied by a significant reduction in overall fitness, the dosage of 80 Gy increased the mortality rate drastically (to 50 %).

Thus it appears that the best dosage to sterilize *R. prolixus* seem to be 40, or maybe 60 Gy when radiating adult reduviids to ensure total infertility (*R. prolixus* irradiated in this study were in instars 3 and 4).

The idea of sterilizing the South-American bugs was to use them in the field in South Africa, which had later been declined by the South African Department of Agriculture. Bastardization of fauna is a real threat in our globalised world and can pose a substantial risk to a habitat. So it is understandable that South-African authorities refused to issue the permit to use the foreign bugs in the field, even if these data suggest that it is very easy to guarantee infertility in the insects. Scientists working with reduviid bugs in foreign habitats such as European Zoos, particularly those in warmer regions, should thus seriously consider sterilising the insects prior to their use to minimise the risk that they to invade into Southern European habitats.

Comparison of different species to sample blood minimally invasive:

In this thesis, two species of reduviid bugs - *R. prolixus* and *D. maximus* – were used together with one species of tsetse, *G. brevipalpis* to draw minimally invasive blood samples. The two families of blood-sucking parasites each have advantages and disadvantages for this method:

Reduviidae

Reduviid bugs have been tested for their characteristics on the feasibility to use them as instruments for blood withdrawal before. The authors could prove that principally, they are suitable for DLW analysis (even though they found a small dilution of the blood samples in the 2003 paper, which does not matter for the analysis of DLW, as this represents a ratio of two different isotope washouts and the dilution is thus reduced. Moreover, this dilution could later, in the 2006 paper, be attributed to wrong handling of the insects). The bugs are able to ingest large amounts of blood per meal, up to 5 ml in *D. maximus* (personal communication Dr. Reiner Pospischil, Bayer Crop Science). In this study however, taking blood samples with the reduviid bugs proved to be very difficult

One potential problem appeared to be the strong motivation for motor activity in meerkats. Voigt et al. had used their bugs on fixed bats, and rabbits. As in this thesis, the aim was to reduce the impact on the test animals, fixing them was no option as it probably poses greater stress on them than anaesthesia. Literature data (Pereira et al. 1998) suggests that reduviid bugs are very sensitive to movement of host animals. The authors of this study found that 46% of *R. prolixus* were still unfed after 2 h exposure to unanaesthetized mice, while in the control group exposed to anaesthetized mice for 1 h, only 2.1 % were still unfed. Another finding was that bloodmeal size was significantly correlated to time of exposure to the hosts, indicating that the nymphs had repeatedly ingested small amounts of blood.

As exposure time or time for bloodmeal intake significantly lowers the precision of the DLW technique (as time of sampling has to be extrapolated), the consecutive ingestion of several small blood meals poses a real problem to the application of this method.

Another finding of this thesis was that reduviid bugs seem to be extremely temperature-dependent. Both species originate from a tropical climate and their ability to habituate to cooler temperatures seems to be limited. During this thesis, it was only once possible to obtain two blood samples from the same test animal on consecutive days using reduviid bugs (during the previous testing period in the Swiss zoo). During the main experimental period in Cologne and Solingen however, only 6 samples could be obtained from 47 trials. Ambient temperatures during these experiments varied from >20 °C in the first experiment, to about 2 °C in the last. Four of the six samples were obtained in the first experiment, and none in the last.

In Cologne zoo it was recently possible to obtain blood samples from giraffes in a heated inside-enclosure using *D. maximus* that were tested for sources of blue-tongue antibodies. 5 bugs had to be attached to the giraffe to obtain the sample (success rate thus was 1/5), but the consecutive sample volume was > 500 µl. This sample could successfully be analysed

Summarising, it is thus advisable to use reduviids when larger amount of blood are needed, temperatures can be controlled for and host animals can either be fixated minimally invasive (i.e. learning to hold still via training), or exhibit a lower locomotor activity than meerkats. An obligatory pre-requisite is the provision of sufficiently high temperatures of above 20 °C.

Glossinidae

Tsetse-flies are very limited in the amount of maximal blood intake. Maximum amounts in *G. brevipalpis* were 60 µl in few very good samples. These are of course very small sample sizes, but sample volume can be increased by using several insects at the same time (for which the collars were slightly altered to create more room for the insects). A great advantage of tsetse is that they appear to more reliable and less sensitive for interference and temperature. Out of 45 trails in this study, 23 samples could be obtained. Ambient temperatures during the experiments ranged from about 20 °C (sampling on the outside enclosure) to 2 °C (sampling in a heated inside enclosure, about 15 °C). Insects are naturally ectothermic and are thus immobile at low T_A, but as tsetse-flies are capable of shivering thermogenesis (Loli & Bicudo 2005), they can be used at lower T_A than the reduviids. The temperature-influenced range of activity in tsetse is roughly from 16°C to 35°C, with an optimum at 25 °C (Einer et al. 1971), whereas reduviids need a minimum temperature of above 20°C. One striking advantage of the flies over the reduviids is the very short ingestion period. This greatly reduces the (potential) impact on the test animals, facilitates handling procedures and, increases precision as the sampling period is significantly decreased. Although temperature induced problems reduced the amount of samples that could be obtained with the flies (experiments were conducted during winter, and even heating inside enclosures could not raise T_A above 15-18 °C, which is chilly for the insects), still half of the attempts were successful in terms of blood sample volume. Ambient temperatures were one major problem though, as several attempts had to be made to obtain the samples, as the feeding motivation of the flies was significantly reduced. This could add further possibilities for errors to the calculation of EE with DLW, as usually the initial sample should be obtained at an exact time point (which was not always possible). If an alternative calculation method for the maximal concentration of isotopes in the body is used though ("intercept method", compare below) this problem can be overcome.

As one important goal of this thesis was to develop a technique that enables minimal invasive blood sampling in the zoo and in the field, insects used should not pose any potential hazard to the environment where they are used. Tsetse flies are widely spread in sub-Saharan Africa, and the species used in this thesis, mainly *G. brevipalis* appears to live in South Africa (Kwa-

Zulu-Natal; Esterhuizen et al. 2005) although this is in contrast to historic records of the insect's distribution. If these insects are used in the field, this is not far away from the natural distribution and poses less risk on the habitat than introducing a species from another continent. More than that, *G. brevipalpis* were obtained from the International Atomic Agency in Vienna, who has moved its colonies to Pretoria, South Africa this summer. It will thus be no problem to obtain these insects in South Africa, and especially to obtain irradiated insects, incapable of reproduction. This ensures that the insects pose no risk whatsoever to the environment when used in the field.

Summarising, tsetse are limited in the amount of blood intake per fly. This can be difficult if larger amounts of blood are needed. Other than that, they are much more reliable in their biting response than reduviid bugs and have a dramatically shorter ingestion period, which is beneficial if samples must be obtained at precise points of time. Potential stress through handling or biting on the test animals is greatly reduced. Moreover, *Glossina spec.* can be obtained already reproductively sterile from the International Atomic Agency, which facilitates field experiments in respect to logistics, as field facilities rarely posses ionisation devices.

For the first time, a dipteran insect species was presented to successfully obtain minimally invasive blood samples. It could be shown that each species of dipterans and reduviids has different adaptations and characteristics, suitable for different scientific problems and, different habitats.

Problems involved with calculating EE using DLW :

Using DLW opens up a wide array of new situations in which data on EE can be obtained. Unfortunately, the technique is rather sensitive, and many different errors can influence the calculation of EE. At its best, the technique can provide estimates of EE that have comparable accuracy to the standard laboratory techniques of direct and indirect calorimetry. Because the biochemical basis of the technique is well understood today (Speakmann, 1997), the conditions in which it might not perform reliably can be recognized and therefore data should cautiously be interpreted.

After (Speakman 1997) the fundamental basis of the DLW technique is the evaluation of the divergence of the washout curves for the two isotopic labels. The closer together these lines are, the more likely it is that any slight error in evaluation of either curve will lead to errors in

the predicted CO_2 production and hence energy expenditure. The closeness of these two lines is represented by the ratio of their gradients: the k_O/k_D ratio. The closer it is to 1 the greater the contribution of water to the total turnover of the oxygen isotope, and less precision one has in the resultant estimate.

Typical values for small mammals are ratios between 1.2 and 1.6. Therefore, EE estimates of animals 6 (k_O/k_D negative), 11 ($k_O/k_D > 2$) and 12 ($k_O/k_D > 7$) cannot be considered correct and must be refused. It is hard to interpret what exactly has gone wrong with these data, as the values differ so greatly from the expected range. The most plausible explanation appears to be wrong sealing of the samples in glass capillaries, this means that either blood was accidentally burned while sealing and the measured isotope enrichments are artefacts created by isotopic fractionation, or the sample was not sealed entirely and could have exchanged isotopes with the environment. This is a mistake that happens rather often, especially to people inexperienced with the technique.

In animals 5, 7, 8 9 and 10, k_O/k_D values are within the usual range and can therefore be discussed further.

Another indicator of precision is the dilution space N (estimates of body water pool), derived from the dilution rates of both isotopes (N_d and N_o). Typically, these values are between 60 -70 %. Dilution spaces of both isotopes can be calculated using two different methods: the "plateau" method, and the "intercept" method.

Unfortunately, estimates of body water pool, calculated with both methods, are far away from the expected range in animals 5, 7 (both January and April values), as well as animal 9.

This can most likely be explained with leakage at the point of isotope entry. Another influence could be the high body mass of the animals, or, more precisely, their (potentially high) body fat content. As the two reasonable samples were derived from two of the heaviest animals though, this is less likely than the problem of correct administration.

Administering isotopes to the animal is a task that must be performed gently. If not all of the injected isotope spreads in the body water pool (some tiny drips might leak from the puncture without noticing) this introduces huge errors, as the body water pool is largely overestimated. As DLW experiments are very expensive and the funds for this study were restricted, the sample size of performed experiments was not very high and thus the high body water estimates may simply reflect a lack of routine, like the problems with the k_O/k_D values.

Still, two values of EE were could be obtained with this technique, proving the feasibility of this approach.

As explained in detail above, many possible errors can occur using this method. Two samples of the same test animal must be obtained at defined points of time. Isotope administration must be handled cautiously, as well as the sealing of the blood samples.

Thus it can be summarized that using blood-sucking parasites for blood sampling to obtain DLW data is about the most complex application for this method. It is possible, though the person planning the experiments should be aware that a very large sample size should be planned (to be able to use only those samples obtained at the rights points of time and with enough blood volume), isotope administration and sealing need quite some experience and grants should suffice to cope for the extra-costs derived from the larger sample size.

Other applications of the minimal invasive blood sampling method - measuring hormone levels:

Blood from an artificial food source was fed to tsetse *G. morsitans*, while at the same time samples were drawn via syringes to test if the blood-ingestion of the flies had any influence on the cortisol concentration in the sample.

Prior too feeding, the membrane that covered the blood was treated with a 0.15 aqueous solution of uric acid, following (Van Der Goes Van Naters et al. 1998**)** who found that this increases the length of the flies feeding bouts. In this study, this treatment increased feeding rates from about 50 % (i.e. 50 % of the flies did feed; preliminary lab experiment, data not shown) to slightly more than 70 % (this experiment). The above cited authors concluded that the combined stimulation of chemo- and thermoreceptors elicits a increased biting response In future studies, it will be interesting to determine if application of uric acid solution on non-sweating mammals can also raise feeding rates outside of the laboratory.

One problem of this application to the method are the relatively small blood volumes that can be obtained with tsetse-flies. Because of a logistic problem, *G. brevipalpis*, that take up up to 60 µl blood were not available when this experiment was conducted, so *G. morsitans* was used that takes up volumes of up to 25 µl. This is why blood of on average 7.5 ± 2.2 flies had to be pooled to obtain a sufficiently large amount of blood. This does not pose any problem in the lab, but could be potentially difficult in the field.

Means of the blood samples obtained with flies and without were 7.3 ± 1.5 and 6.8 ± 1.7 µg/dl cortisol, respectively. Although there appears to be a slight dilution, means are not different from each other. The dilution could potentially be attributed to:

1) Tsetse are parasites that feed on blood and thus digest their meal. Erythrocytes are haemolysed in tsetse in the posterior, digestive region of the midgut (Evans & Gooding 2002), and even though flies were killed fight after the blood-meals, some of them might have already digested small amounts of the meal, enough to account for the slight dilution effect found in this study.
2) Blood was drawn from the punctured abdomen of the (killed) flies using a microcapillary, before it was collected in an Eppendorf-tube and centrifuged. The capillary force might have been enough for a small haemolytic effect that would account for the slightly reduced cortisol concentration.

Both of these effect of their combination thus can explain the observed difference.

Statistical analysis of these data is somewhat challenging. As all samples were derived from the same food source to minimise potentially confounding factors, values obtained in this experiment were very similar and thus not distributed normally. To correct for this, data were normalised using the "rform" function in "R" statistical software. After normalisation, a there was no significant difference between the two sample pools (t-test, $p = 0.44$).

Method precision was tested with duplicate analysis of the "syringe"-samples. Maximum divergence of two analyses from the same sample was 1.5 µg/dl. This corresponds to a relative error of 18-20%, which is well within usual precision limits of this assay (personal communication PD Dr. Stephan Wnendt, Managing Director, MLM Medical Labs Moenchengladbach, Germany).

Although there is a slight trend to dilute the cortisol concentrations in the fly samples, means can be regarded equal, as the precision of this assay is limited.

Summary physiological data: For the fist time, this study presents DLW data obtained minimally invasive from zoo animals outside a laboratory. The only other work that used blood-sucking parasites to obtain DLW data was performed on fixed bats (Voigt et al. 2003; 2005). Here, the study animals were not restricted in their mobility and could move freely during the blood sampling period. The collar used to attach the insects to the meerkats also allows to derive blood samples at defined points of time, although the use is limited to species habituated to close human presence. Other than in previous works, blood samples were obtained using tsetse *G. brevipalis* here, as their motivation for food intake is more reliable than in the usually used reduviids *R. prolixus* and *D. maximus*. This has a very beneficial implication, as, for the first time, another blood-sucking parasite is presented as an instrument of blood sampling.

Reduviid bugs are South American insects and are thus difficult to use when planning to work in the field on other continents. Tsetse are of African origin and thus pose a distinctly smaller risk to African environments. The flies used in this thesis were obtained from the International Atomic Agency, which permits sterilization prior to the use in the field, and thus further minimises potential risks to the environment. When planning to work in a South American environment, it was shown that reduviids can also easily be sterilized with ionising radiation. It could be presented that not only reduviids posses beneficial properties to use them for blood sampling, as the insects used here are dipterans. A long term goal derived from this work would thus be to find suitable insect candidates in every environment where field studies could benefit from minimally invasive blood sampling.

OUTLOOK

The minimally invasive blood sampling method presented in this study can be used for a wide array of scientific questions like DLW, hormone analyses and general serological studies. The application of this method presented in this study allows to draw samples from free-ranging, specific individuals at distinct time points. This opens up some new perspectives like measuring very short-term changes in individual hormone levels and putting these in relation to e.g. social behaviours. As several consecutive blood samples can be collected from the same individual at one day, also interesting questions like circadian influences on stress and hormone levels could be answered in the field.

Generally, minimally invasive blood sampling has so far only been used in husbandry environments, such as zoos or rescue/breeding stations. Using a native, African, so far unimplemented insect in this thesis that can be obtained already reproductively infertile, this study is an important step towards the usage of this technique in the African field

A long-term goal of this project thus naturally is to find suitable native insects to be used on other continents to avoid the risks of importing invaders and thus reduce potential ecological impacts in natural environments.

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ERKLÄRUNG

Ich versichere, dass ich die von mir vorgelegte Dissertation selbständig angefertigt, die benutzen Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit – einschließlich Tabellen, Karten und Abbildungen –, die anderen Werken im Wortlaut oder dem Sinn nach entnommen sind, in jedem Einzelfall als Entlehnung kenntlich gemacht habe; dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; dass sie – abgesehen von unten angegebenen Teilpublikationen – noch nicht veröffentlicht worden ist sowie, dass ich eine solche Veröffentlichung vor Abschluss des Promotionsverfahrens nicht vornehmen werde. Die Bestimmungen der Promotionsordnung sind mir bekannt. Die von mir vorgelegte Dissertation ist von Prof. Dr. Gunther Nogge betreut worden.

Köln, den 29. September 2009

Curriculum Vitae

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Verheiratet

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2003 – 2004	Universität zu Köln, Zoologisches Institut und Zoo Köln, Diplom in Biologie, Gesamtnote: „sehr gut". Diplomarbeit unter der Betreuung von Herrn Prof. Gunther Nogge Thema: Enclosure use and social behaviour in meerkats, Note: „sehr gut"
1998 – 2003	Universitäten Freiburg, Marburg und Köln, Studium der Biologie. Hauptfach: Zoologie, erstes Nebenfach: Genetik, zweites Nebenfach: Biochemie
2000	Universität Freiburg, Vordiplom Biologie
1989 – 1998	Abitur am Albertus-Magnus-Gymnasium, Rottweil

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2005 – 2009	mehrfache Lehrassistenz verschiedener zoologischer Kurse sowie Betreuung von Praktikanten und Diplomanten im Kölner Zoo
2002 – 2003	Lehrassitentin: Laborkurs in Zoomorphologie für Erstsemester
2001	Studentische Hilfskraft an der Universität Karlsruhe, Institut für Informatik, SFB humanoide Roboter, AG Prof. Dillmann

Köln, den 29. 09.2009 Alexandra Habicher

APPENDIX

Ethogram

GENERAL BEHAVIOUR

Lying: L

An animal is lying on the ground, in a rolled-up posture. Contact with substrate is minimal.

Contact Lying: C

The animal lies on its belly or back on the ground with all extremities stretched forward and backward. High level of contact with substrate.

Huddling: H

A group of meerkats lye on top of each other, while the extremities are stretched out to maximize body/ surface contact.

Low sitting – incorporates: I

- Resting / lazy sitting (according to Estes 1992)

The animal sits, with the lower extremities stretched forward, the trunk being folded forward, while the head touches the ground in between the legs.

AND

- Sitting (Four Legs) / low sitting (Estes 1992):

The animal sits with the lower extremities on the ground, while the upper extremities touch the ground.

Both low sitting postures have a high level of contact with the substrate.

High sitting (Two Legs; Estes 1992): Y

The animal sits upright, with the lower extremities and backside on the ground, while the upper extremities are bent in front of the body.

Sunbathing: X

Posture as high sitting but belly is clearly directed towards the sunlight. This posture enables meerkats to absorb energy in form of solar radiation, used to rise body temperature in the mornings, after it had been lowered to 36°C during nighttime (Müller & Lojewski, 1986).

Low standing (four legs): S

An animal stands on the ground while all four extremities are on the ground. The backside does not have ground contact.

High standing (two legs, alert): T

The animal stands upright with toes and footpad touching the ground, while the upper extremities are bent in front of the body.

Move: M

The animal walks with moderate speed.

Running: R

The animal is running at a particularly higher speed than when moving.

Climbing: K

Moving vertically.

Foraging: F

An animal is moving across the floor, with ducked body and lowered tail, while it is digging the ground superficially.

Eating: E

Smaller pieces – chewing and swallowing. Bigger pieces are pushed on the floor with the upper extremities and the meerkat rips out pieces by biting and pulling the head upwards, while the teeth still grip the prey.

PLAY: P

Playing (object):

An animal touches an object or scratches it for a prolonged period.

AND

Playfight:

In opposite to "real" fighting the roles of the fighting animals change rapidly. "Fight" or "playfight" can both be accompanied by vocalization, while bouts of "playfighting" are shorter and playfighting is frequently interrupted.

COOPERATIVE BEHAVIOUR (FOLLOWING CLUTTON-BROCK)

Raised guarding: G

An animal is sitting (two or four legs) or standing on an at least 10 cm raised position and is watching the environment. This behaviour is accompanied by varying vocalization, that signalizes presence of a guard as well as various types of danger to the other group members.

In the zoo, also "Ground guarding":

Same posture and meaning as raised guarding, but the animal is guarding from the ground.

Social digging: D

S.d. is digging to establish or restore a burrow. The animal's body is halfway in the burrow-entrance, with the lower extremities opened widely, while the animal is digging the soil out through its legs.

Babysitting: B

A newborn litter typically rests in the dens until the cubs are about three weeks old. In this period, they are guarded and probably also warmed by a helper, an animal that stays with the cubs, while the rest of the group is foraging outside the burrow.

"Babysitting" typically is recorded when a helper is out of sight for a prolonged period, if there are cubs present.

Feeding young: J

A sub- or adult animal is carrying food in its mouth and puts its down in front of the cub.

AGONISTIC BEHAVIOUR

Foraging competition: V

Two or more animals fight over a food item. This behavior is often termed "hip slamming", as the animals push each other away with their hips and utter threat calls.

Mobbing : N

Typically, meerkats mob threating enemies such as snakes. Then the entire group approaches the thread, bites and chases it away. Is accompanied by threatening vocalization.

Fight: Q

Any combination of "threatening", "chasing" or "gripping", which is often
followed by a "bounding in place" of the two fighting animals. Meerkats also use a so called "backing attack"(Estes; 1991) offensively to threaten their opponent. Real fights are usually fierce and can involve deaths. Fighting either occurs when single or few females are expulsed from their natal group, usually subordinate females are expulsed by the dominant female. If so, the expulsed animals are fought by the entire group. Another possibility for fighting occurs when two foreign groups collide.

Threatening:

An animal is growling while head and tail are lowered.

Chasing:

Posture and vocalizations are the same as "threatening", but the chasing animal is running after the chased one.

Biting:

An animal is biting another one. This behaviour occurs during fights as well as play fights.

Pushing aside:

An animal pushes another animal aside.

Gripping:

An animal is gripping another one with the upper extremities.

OTHER BEHAVIOUR

Allogrooming: A

Mutual cleaning of fur, ears and mouth region with licking and smooth biting.

Side by side:

Two animals are accompanying each other with raised tails, while their sides might touch.

Licking/smelling genitals:

An animal is licking or smelling at the genitals of another one, which they might do mutually.

Grooming:

Animal A is cleaning B's fur, ears and mouth region with licking and smooth biting.

Touching the snout:

An animal is giving another one a short touch with the snout.

Cheek marking:

The animals are approaching and touching each other's cheeks, mutually marking themselves with the corresponding glands.

Lowcreep

A subdominant animal is approaching a dominant one in lowered posture.

Out: O

Frenzy: Z

A meerkat displays several agitated, fast movements in the context of marking behaviour. Usually "frenzy" occurs when a meerkat picks up marks left by other meerkat and then overmarks these trails with its own smell. A "frenzy" bout can last several minutes.

Below: U

The animal is below ground, while "below" is clearly distinguishable from "out".

IGE: W

Inter Group Encounter, two meerkat groups meet and fight.

RECORDED ADDITIONALLY

Piloerection: 2

An animal's fur is erected to build up an (additional) insulating air layer.

Panting: 3

Open mouthed, high frequency respiration. In meerkats, panting bouts are interrupted by deep breaths to avoid respiratory alkalosis (Müller & Lojewski, 1986).

Shivering: 4

Here, shivering thermogenesis (SH). SH becomes evident by fast muscular contractions of antagonistic skeletal muscles that release heat because ATP is hydrolysed through the activity of the muscle but without movement

Weight Data Field

AZTECS	Animal/Date	VWF063	VVM032	VWM073	VWM100	VWM101	VWF115	VWF118
MW	24.12.2007							
MW	26.12.2007							
MW	27.12.2007	1008	790		682	750	709	630
MW	28.12.2007							
MW	29.12.2007							
MW	30.12.2007							
MW	31.12.2007	801	846	760		718	699	628
MW	01.01.2008							
MW	02.01.2008	804	826		634	696		630
MW	03.01.2008		853		637	703	566	655
MW	04.01.2008							
MW	05.01.2008	767	841		636	691	562	638
MW	06.01.2008							
MW	07.01.2008							
MW	08.01.2008	791	850				590	673
MW	09.01.2008	783	878			699	582	
MW	10.01.2008	798	875	739		694	576	675
MW	11.01.2008	778	865	698	610	694		676
MW	12.01.2008							
MW	13.01.2008							
MW	14.01.2008							
MW	15.01.2008							
MW	16.01.2008	787			828	747	584	706
MW	17.01.2008	761					580	698
MW	18.01.2008							
MW	19.01.2008							
MW	20.01.2008							
MW	21.01.2008	764		706	613	667	571	
MW	22.01.2008	778	847	698	632	691		
MW	23.01.2008	752					555	
MW	24.01.2008							
MW	25.01.2008	719	843	704	606		563	
MW	26.01.2008	729	840	712	594	671	571	532
MW	27.01.2008	713	841	718	556	684	569	
MW	28.01.2008							
MW	29.01.2008							
MW	30.01.2008	713	832	714	620	671	575	534
MW	31.01.2008	724	845		623		582	532
MW	01.02.2008	711			621	686		537
MW	02.02.2008							
MW	03.02.2008							
MW	04.02.2008	713	823		621	676	577	522
MW	05.02.2008							
MW	06.02.2008							
MW	07.02.2008							

MW	08.02.2008	718	809	742	620		569	
MW	09.02.2008	740	822	771			585	545
MW	10.02.2008							
MW	11.02.2008							
MW	12.02.2008	767	822	729	630	692	580	562
MW	13.02.2008	756	835	739	623	683	601	534
MW	14.02.2008	739	814	719	607	666	568	512
MW	15.02.2008	742	806	715	595	690	581	512
MW	16.02.2008	730	815	717	601	690	587	510
MW	17.02.2008							
MW	18.02.2008	727	837	711	667		571	513
MW	19.02.2008							
MW	20.02.2008	714	799	692	592	695	565	497
MW	21.02.2008							
MW	22.02.2008	768	796		596	675	572	521
LW	24.12.2007							
LW	26.12.2007							
LW	27.12.2007							
LW	28.12.2007							
LW	29.12.2007							
LW	30.12.2007							
LW	31.12.2007	842	857	786		725	722	653
LW	01.01.2008							
LW	02.01.2008	838	840	757	665	712		652
LW	03.01.2008	803	867	673			636	666
LW	04.01.2008							
LW	05.01.2008	822	867			704		662
LW	06.01.2008							
LW	07.01.2008							
LW	08.01.2008	846	877		662			697
LW	09.01.2008	823	914	753		703	607	685
LW	10.01.2008	817	882			709	618	681
LW	11.01.2008	822	885		633	720		
LW	12.01.2008							
LW	13.01.2008							
LW	14.01.2008							
LW	15.01.2008							
LW	16.01.2008							
LW	17.01.2008	790	880		621		574	
LW	18.01.2008							
LW	19.01.2008							
LW	20.01.2008							
LW	21.01.2008	804	860	730	642	711	605	
LW	22.01.2008	821	856			728		
LW	23.01.2008	783	856					
LW	24.01.2008							
LW	25.01.2008							

LW	26.01.2008	763	854	738		668	624	551
LW	27.01.2008	704	824		589	688	569	524
LW	28.01.2008							
LW	29.01.2008							
LW	30.01.2008	753	855	748	640	706	597	578
LW	31.01.2008	753	864	755	635		633	572
LW	01.02.2008	765	857		651	714	633	
LW	02.02.2008							
LW	03.02.2008							
LW	04.02.2008	747	854	780	623		607	
LW	05.02.2008							
LW	06.02.2008							
LW	07.02.2008							
LW	08.02.2008							
LW	09.02.2008							
LW	10.02.2008							
LW	11.02.2008							
LW	12.02.2008	785	843	752	635	690	611	577
LW	13.02.2008	781	848	759	635		623	559
LW	14.02.2008	751	872	723	614	696	614	551
LW	15.02.2008				611	690	591	
LW	16.02.2008	737	812	720	600	703	580	515
LW	17.02.2008							
LW	18.02.2008	725	833	713	613	671	582	528
LW	19.02.2008							
LW	20.02.2008							
LW	21.02.2008							
LW	22.02.2008	744	824		613		606	546
EW	24.12.2007	1010	843	781	677	742	719	623
EW	26.12.2007							
EW	27.12.2007				695			629
EW	28.12.2007							
EW	29.12.2007	1050	858	810	691	753	761	669
EW	30.12.2007							
EW	31.12.2007	848	864	791	630	729		664
EW	01.01.2008							
EW	02.01.2008	823				716	616	658
EW	03.01.2008	809	852					
EW	04.01.2008	823		752		713	624	668
EW	05.01.2008	787				621		651
EW	06.01.2008	831	873				623	
EW	07.01.2008	856	882			719		702
EW	08.01.2008	849	900			741	601	696
EW	09.01.2008	855	880	759	640			695
EW	10.01.2008							
EW	11.01.2008	795	857				592	705
EW	12.01.2008							

EW	13.01.2008							
EW	14.01.2008					728	677	761
EW	15.01.2008							
EW	16.01.2008							
EW	17.01.2008							
EW	18.01.2008							
EW	19.01.2008							
EW	20.01.2008	830						
EW	21.01.2008	837			640	705		
EW	22.01.2008	786	859		646		587	
EW	23.01.2008	764	881		664			
EW	24.01.2008	781	869	733	646	718	650	561
EW	25.01.2008	778	827	704	599	693	616	585
EW	26.01.2008							
EW	27.01.2008	755	852		643	719		544
EW	28.01.2008	715	851	730	647	713	586	549
EW	29.01.2008	776	869	745	656	659	605	587
EW	30.01.2008	775	852		677	727	652	554
EW	31.01.2008	779	854		650			581
EW	01.02.2008							
EW	02.02.2008	764	822		650			565
EW	03.02.2008	774	835		661	708		558
EW	04.02.2008	757	866	765	639	684	600	567
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EW	06.02.2008							
EW	07.02.2008							
EW	08.02.2008							
EW	09.02.2008	806	845		650	714	621	585
EW	10.02.2008							
EW	11.02.2008	819	856	762	668	722	634	627
EW	12.02.2008	760	841		649	708	623	566
EW	13.02.2008	759	823	728	632	695	598	562
EW	14.02.2008	749	820	732	619	715	607	545
EW	15.02.2008	747	824	726	618	692	600	529
EW	16.02.2008	729	806					523
EW	17.02.2008	748	866	736	700	630	606	549
EW	18.02.2008	740	851	715	607	703	587	608
EW	19.02.2008	720	804			675		513
EW	20.02.2008							
EW	21.02.2008							
EW	22.02.2008	771	816			695		

Elveera	Animal/Date	VEF079	VEM108	VYM113	VYM114	VEM118	VEF119	VEF120	VEM122	VEF124	VEM125
MW	24.12.2007	1042	702	766	736	677			585	533	553
MW	26.12.2007										
MW	27.12.2007										
MW	28.12.2007		717		766	706			603	551	573
MW	29.12.2007		697	796	769	698				543	567
MW	30.12.2007										
MW	31.12.2007	1072	704			692			596	561	573
MW	01.01.2008										
MW	02.01.2008	1078	692	769	742	678			596	572	575
MW	03.01.2008		688	767	727				576	546	559
MW	04.01.2008	1092	693	765	710	657			569	545	549
MW	05.01.2008	1107	671		723	668			570	550	540
MW	06.01.2008										
MW	07.01.2008	1095	687			660			581	548	557
MW	08.01.2008		705			667			584		543
MW	09.01.2008	904	680			664			585		561
MW	10.01.2008	857	663			645				550	555
MW	11.01.2008		663	744		652			565		563
MW	12.01.2008										
MW	13.01.2008		649			646				539	549
MW	14.01.2008	822	662						549	536	551
MW	15.01.2008										
MW	16.01.2008	861	699	768	706	647				553	546
MW	17.01.2008		658			667			542	542	558
MW	18.01.2008	815	662		691	629			648	545	
MW	19.01.2008										
MW	20.01.2008	812	654		694	661		656	558		563
MW	21.01.2008		655			656			554	544	
MW	22.01.2008	775	645	680	647	588			543	517	556
MW	23.01.2008										
MW	24.01.2008		624		640	628			511		524
MW	25.01.2008		623			647	586		533		
MW	26.01.2008	781	625			637	601		533	531	
MW	27.01.2008										
MW	28.01.2008										
MW	29.01.2008										
MW	30.01.2008	782	648		661	648			543		
MW	31.01.2008	815	681			664	676		556		
MW	01.02.2008		669			660			580		590
MW	02.02.2008										
MW	03.02.2008										
MW	04.02.2008										
MW	05.02.2008										
MW	06.02.2008	776	696		713	690		703	596	555	627
MW	07.02.2008										
MW	08.02.2008	769	670		696	654		677		526	526
MW	09.02.2008		676			647	663	681		527	

MW	10.02.2008										
MW	11.02.2008	746	661		680	634	616			528	565
MW	12.02.2008										
MW	13.02.2008	652	633			632		669	550	508	540
MW	14.02.2008										
MW	15.02.2008	772	650	681	689	639	626		572	523	561
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MW	17.02.2008										
MW	18.02.2008	755	653	699	667	630		677	546	521	
MW	19.02.2008										
MW	20.02.2008										
MW	21.02.2008										
MW	22.02.2008										
LW	24.12.2007	1056	710	786	755	692			596	546	569
LW	26.12.2007										
LW	27.12.2007										
LW	28.12.2007	1098		808		730				570	591
LW	29.12.2007	1061	700	782	737	685			595	563	565
LW	30.12.2007										
LW	31.12.2007	1095	726	801		718			625	585	597
LW	01.01.2008										
LW	02.01.2008	1087	698	778	735				602	568	573
LW	03.01.2008	1094	703	776	733	646				545	560
LW	04.01.2008	1007	681	786	744				589	569	584
LW	05.01.2008	1124	689	771	738	675			583	560	561
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LW	07.01.2008	1124	709	776	725	669			584	560	568
LW	08.01.2008		720	813	748	707			616	562	599
LW	09.01.2008										
LW	10.01.2008	861	675		703	658				557	552
LW	11.01.2008	867	688	768		658			587		562
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LW	13.01.2008	895	704		718	701				584	602
LW	14.01.2008										
LW	15.01.2008	913	676		712	600				586	
LW	16.01.2008		694		730	680				590	590
LW	17.01.2008	876	664	765							583
LW	18.01.2008										
LW	19.01.2008										
LW	20.01.2008					687			589		597
LW	21.01.2008					680		697	568	554	
LW	22.01.2008	812				679		677	557	530	568
LW	23.01.2008										
LW	24.01.2008			733			585		548	514	
LW	25.01.2008		647		677	666	627	705	566	547	
LW	26.01.2008	805	671	762	676	674	636	705	579	508	
LW	27.01.2008										
LW	28.01.2008										

LW	29.01.2008										
LW	30.01.2008										
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LW	01.02.2008	808	681	751		669	673	752		561	573
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LW	03.02.2008										
LW	04.02.2008										
LW	05.02.2008										
LW	06.02.2008	784			701	677		718		551	621
LW	07.02.2008										
LW	08.02.2008	800	663		702	656	638		577	543	581
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LW	10.02.2008										
LW	11.02.2008	773	672		684	647	628		569	551	579
LW	12.02.2008										
LW	13.02.2008		658	721	669	639			585	540	587
LW	14.02.2008										
LW	15.02.2008	782	664	727	682	645	634	690	544	526	575
LW	16.02.2008										
LW	17.02.2008										
LW	18.02.2008										
LW	19.02.2008										
LW	20.02.2008										
LW	21.02.2008										
LW	22.02.2008										
EW	24.12.2007										
EW	26.12.2007										
EW	27.12.2007		734			732			629	581	593
EW	28.12.2007	1103	713		775	699			599	554	583
EW	29.12.2007	1069	717		748	683			579	579	557
EW	30.12.2007	1098	723	762	756	703			624	592	594
EW	31.12.2007		716		755					568	599
EW	01.01.2008										
EW	02.01.2008	1089	701	772	742				583	554	565
EW	03.01.2008										
EW	04.01.2008	1116	677	776	735	682			589	572	568
EW	05.01.2008										
EW	06.01.2008	1112	710	787	745	674			599	567	583
EW	07.01.2008	1131	722	776	739	691			609	576	588
EW	08.01.2008										
EW	09.01.2008		680	771		652			592	557	562
EW	10.01.2008										
EW	11.01.2008		687			670			563	555	561
EW	12.01.2008										
EW	13.01.2008										
EW	14.01.2008					694					
EW	15.01.2008	934	719			664			606		
EW	16.01.2008										

EW	17.01.2008										
EW	18.01.2008		708			658					
EW	19.01.2008	661						680	606		603
EW	20.01.2008		662								
EW	21.01.2008										
EW	22.01.2008										
EW	23.01.2008										
EW	24.01.2008	835	629	761	694	680	595				586
EW	25.01.2008	813	667	756		684	638	684	565	562	561
EW	26.01.2008										
EW	27.01.2008						657		539		
EW	28.01.2008		710								
EW	29.01.2008										
EW	30.01.2008	869	703		720	681	713	720	570	577	585
EW	31.01.2008	885			723	662	690	620	770	611	583
EW	01.02.2008										
EW	02.02.2008					676			590		
EW	03.02.2008										
EW	04.02.2008	848			731	687	682	736			
EW	05.02.2008	852	741			700	674	731	636	581	605
EW	06.02.2008	836			729	693	720	724	613	565	636
EW	07.02.2008										
EW	08.02.2008										
EW	09.02.2008	808	695		712	687	662	719	581	583	589
EW	10.02.2008	694				646			585	544	582
EW	11.02.2008										
EW	12.02.2008	765	640	698	655	638	621	688	566	523	550
EW	13.02.2008	784	676	722	693	653	636	690	584	553	587
EW	14.02.2008	790	677	712	692	648	644	701	578	540	585
EW	15.02.2008										
EW	16.02.2008										
EW	17.02.2008	779	715	682	668	652	650	699	546	489	573
EW	18.02.2008	797	700	730	663	650	654	699	570	544	581
EW	19.02.2008										
EW	20.02.2008										
EW	21.02.2008	789	652		666	663	641	696	563	540	569
EW	22.02.2008										

Lazuli	Animal/Date	VLF094	VLM105	VLF111	VLM114	VLM119	VLF123	VLM124	VLM126	VLM127
MW	24.12.2007	834	866	962	842		639	672	620	
MW	26.12.2007	844	851	965						624
MW	27.12.2007	858	863							
MW	28.12.2007	870	886	1023		692	699		679	643
MW	29.12.2007	849	875	1007		686	706			672
MW	30.12.2007									
MW	31.12.2007	858	916			687	705		676	692
MW	01.01.2008									
MW	02.01.2008	851	872	1012	844	693	699		678	663
MW	03.01.2008									
MW	04.01.2008	818	876	1001			679	699		645
MW	05.01.2008	826	877	786	812	668		704	671	642
MW	06.01.2008									
MW	07.01.2008	813	881	783			666			
MW	08.01.2008	846	896	795		682	680		664	639
MW	09.01.2008									
MW	10.01.2008	842	890	797		678	688		694	651
MW	11.01.2008	864	899	802		666	676		660	658
MW	12.01.2008	854	889	784		671	675		681	643
MW	13.01.2008									
MW	14.01.2008	832	871				672		651	633
MW	15.01.2008									
MW	16.01.2008	889	899	842		671		684	668	632
MW	17.01.2008									
MW	18.01.2008	850	855			679		704	655	640
MW	19.01.2008									
MW	20.01.2008									
MW	21.01.2008	850	846			689			674	645
MW	22.01.2008									
MW	23.01.2008									
MW	24.01.2008	824	843	795		656	675	691	668	647
MW	25.01.2008	806	848	770		668	636	660	681	608
MW	26.01.2008	826	856	764			662		684	
MW	27.01.2008									
MW	28.01.2008									
MW	29.01.2008	858	860	763		679	696	711	661	621
MW	30.01.2008	854	857	768			681	684	688	638
MW	31.01.2008									
MW	01.02.2008	833	820	763	764	648	673		673	604
MW	02.02.2008	826	809	727	641	667	661	593	667	
MW	03.02.2008									
MW	04.02.2008									
MW	05.02.2008	876	823	767	791		718	680	659	619
MW	06.02.2008	893	828	769	783	655	728		663	629
MW	07.02.2008									
MW	08.02.2008	888	818	730			721		650	619
MW	09.02.2008	887	818	752		668	755		661	622

MW	10.02.2008									
MW	11.02.2008	909	828	731	764		770		679	632
MW	12.02.2008	903	812	729			772	672	655	629
MW	13.02.2008									
MW	14.02.2008									
MW	15.02.2008	904	789	716	766	638	778	660	644	608
MW	16.02.2008	933	814		748	632	787	671	670	609
MW	17.02.2008		793	737		630	788		638	596
MW	18.02.2008									
MW	19.02.2008									
MW	20.02.2008									
MW	21.02.2008									
MW	22.02.2008									
LW	24.12.2007	863	886	960	847		671	700	646	
LW	26.12.2007	865	862							645
LW	27.12.2007	860	875			694	686			655
LW	28.12.2007	874	897	1028						674
LW	29.12.2007	854	876	1027	851	693	721		684	674
LW	30.12.2007									
LW	31.12.2007	900	931		895	728	733	745	708	701
LW	01.01.2008									
LW	02.01.2008	877	906	1045	871	707	714	749	698	690
LW	03.01.2008									
LW	04.01.2008	836	900	1013	854	686	717	745	689	671
LW	05.01.2008	863	891		834	682			653	652
LW	06.01.2008									
LW	07.01.2008	863	886				960		649	
LW	08.01.2008	870	908			700		689	710	662
LW	09.01.2008									
LW	10.01.2008	869	922							660
LW	11.01.2008									
LW	12.01.2008	890	913			687	699			
LW	13.01.2008									
LW	14.01.2008	872	897			685				
LW	15.01.2008									
LW	16.01.2008	894	924			706	725			
LW	17.01.2008									
LW	18.01.2008									
LW	19.01.2008									
LW	20.01.2008									
LW	21.01.2008		877							
LW	22.01.2008									
LW	23.01.2008									
LW	24.01.2008	859	885	845		691			710	663
LW	25.01.2008									
LW	26.01.2008	841	857	842	796	718	679			634
LW	27.01.2008									
LW	28.01.2008									

LW	29.01.2008									
LW	30.01.2008	890	893	838			723	718	694	
LW	31.01.2008									
LW	01.02.2008	847	854		781	665	694	693	685	656
LW	02.02.2008	838				648			679	591
LW	03.02.2008									
LW	04.02.2008									
LW	05.02.2008	914	868	826	809	652		704	674	615
LW	06.02.2008	892	838	810	788	677	747	717	671	628
LW	07.02.2008									
LW	08.02.2008	905	827	795	789	670	758	685	727	629
LW	09.02.2008	910	832	783	794	678	760	699		646
LW	10.02.2008									
LW	11.02.2008									
LW	12.02.2008	971	888	801	794	674	801	697		647
LW	13.02.2008									
LW	14.02.2008									
LW	15.02.2008	945	810	774		680	808	682	662	635
LW	16.02.2008	941	815	750		664	821	702	646	614
LW	17.02.2008									
LW	18.02.2008									
LW	19.02.2008									
LW	20.02.2008									
LW	21.02.2008		808		760	654			660	
LW	22.02.2008									
EW	24.12.2007	838	869	998		664	684		638	625
EW	26.12.2007		896							
EW	27.12.2007	905	919	1062		723	738		714	693
EW	28.12.2007		891							
EW	29.12.2007									
EW	30.12.2007	886	919	1066			733	706	715	700
EW	31.12.2007	877	910	1057		722	728	760		717
EW	01.01.2008	872	889	1040		707	718	740	696	683
EW	02.01.2008									
EW	03.01.2008	845	893	1036	846	685	697	710	703	673
EW	04.01.2008		883	1024	819	672	690	707	686	643
EW	05.01.2008			801				733	677	652
EW	06.01.2008		874							622
EW	07.01.2008	864	892		843	713				
EW	08.01.2008	883			874				706	
EW	09.01.2008	863	909						740	
EW	10.01.2008									
EW	11.01.2008									
EW	12.01.2008									
EW	13.01.2008									
EW	14.01.2008	939	685							
EW	15.01.2008									
EW	16.01.2008									

EW	17.01.2008									
EW	18.01.2008	847	874					707	712	643
EW	19.01.2008									
EW	20.01.2008	895	887			743				688
EW	21.01.2008									
EW	22.01.2008				848		712			
EW	23.01.2008	832	861	858		678			680	
EW	24.01.2008	867	887	883	806	704	644	660	684	625
EW	25.01.2008	852	862	831	789	726	679	693	691	611
EW	26.01.2008	865	899	888	814	703	699	724		666
EW	27.01.2008	874	882	869	843	682	703	746	691	655
EW	28.01.2008									
EW	29.01.2008	877	890	842	822	671	722		690	668
EW	30.01.2008		877	844	772	677	682		677	607
EW	31.01.2008	851	848	833	761	660	703	685	676	630
EW	01.02.2008									
EW	02.02.2008									
EW	03.02.2008	891	850	828	806	734	734	712	685	636
EW	04.02.2008	913	852	858	806	665	740	708	662	640
EW	05.02.2008	907	845	840	805		748	739	677	665
EW	06.02.2008									
EW	07.02.2008									
EW	08.02.2008	928	864	832	861	688	783	719	693	
EW	09.02.2008	916	842	797	794	681	777	696		656
EW	10.02.2008	919	821	775	769	679	781	684		650
EW	11.02.2008	939	870	834		673	822	692		665
EW	12.02.2008	968	866	798	812	652	796	690	684	651
EW	13.02.2008	937	863	775	777	666	792		670	649
EW	14.02.2008									
EW	15.02.2008	956	826	801	769	648	811	696	678	622
EW	16.02.2008									
EW	17.02.2008									
EW	18.02.2008	964	812	785	791	682	849	695	680	
EW	19.02.2008									
EW	20.02.2008									
EW	21.02.2008	1007	827	808			688	840	670	619
EW	22.02.2008	974	806	767	776	676	828	696	653	621