(Spurenlesen als Erhebungsmöglichkeit zur Feststellung von verhaltensbiologischen und physiologischen Parameter bei freilebenden Wölfen (Canis lupus))

Diplomarbeit

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Table of Contents

Zussammenfassung	3
Abstract	4
1.Introduction	5
2.Methods.	7
3.Results	18
4.Disscussion	40
5.Conclusion	45
6.Acknowledgements	48
7.References	49
8. Appendix A: Methods of age estimation of wolf tracks in the snow	53
9.Appendix B Home range	55
10.Appendix C: Absence and presence of the pack in the study area	56
11.Appendix D: Hormone Levels in the wolves of the Herberstein animal park	59
12.Appendix E: Data collection in a foreign land – what you need to do	62
13.Appendix F: Lebenslauf	64

Zusammenfassung

Spurenlesen ist eine nicht-invasive Methode, mit deren Hilfe man verhaltensbiologische und physiologische Daten von freilebenden Säugetieren feststellen und diese mit Informationen über Ernährung und Genetik der einzelnen Individuen vergleichen kann. Wölfe (Canis lupus) sind eine seltene und sehr schwer zu untersuchende Art, die Jagd auf Paarhufer macht. In dieser Studie wurden die Spuren von Wölfen im Schnee in Alberta, Kanada, gelesen. Die Fährten dieser Tiere wurden verfolgt, die Exkremente eingesammelt und ihr Verhalten eingeschätzt. Die Ergebnisse wurden mit einem tragbaren GPS (Globales Positionssystem) aufgezeichnet und in digitale Karten eingetragen. Die zurückgelegten Entfernungen wurden zu 651 50-Meter-Intervallen zusammengefasst. Während der Arbeit im Feld, wurden mehrere Parameter für jede Einheit aufgenommen. Der Wolfskot wurde aufgesammelt, um später in Wien, Österreich, endokrinologische Analysen für die Exkretionsprodukte von Kortison und Testosteron durchzuführen. Die Kortisonkonzentrationen wurden als Methode verwendet, um den Grad an "Stress" festzustellen, dem das zu untersuchende Tieres vor kurzem ausgesetzt war. Die Androgenkonzentrationen gaben Aufschluss über das Geschlecht, den Fortpflanzungszustand, und agonistische Interaktionen von einzelnen Individuen. Um die Felddaten zu validieren und um den Stoffwechsel der Hormone nach der Exkretion zu kontrollieren, wurden weitere Kotproben von gefangenen Tieren in Österreich gesammelt. Eine Analyse über den Stoffwechselfortschritt zeigte keine allgemeinen Veränderungsmuster. Jedoch war der Datensatz, der analysiert wurde, zu klein um detaillierte Vergleiche mit der Physiologie der Wölfe in Kanada anzustellen. Über das Verhalten wurde herausgefunden, dass die Wölfe hintereinander über die Hügel und Kämme wanderten. Das dabei verwendete Habitat war bewaldet und damit sehr charakteristisch für Kleinhirsche. Wenn die Wölfe sich am Talboden bewegten, spalteten sie sich oft auf und begannen Elchspuren durch das Gebüsch und über offene Flächen zu verfolgen. Die Wölfe schienen sich entlang bekannten und möglicherweise häufig benutzen Korridoren innerhalb ihres Territoriums zu bewegen. Sie verfolgten selten die Spuren von Pferden, und diese schienen auch nicht Teil ihrer Ernährung im Winter zu sein. Eine hauptsächliche Nahrungsquelle stammte von Berglöwenrissen. Die Jagdstrategie der Wölfe änderte sich von Tag zu Tag nach neuerlichem Schneefall. Innerhalb einer Woche wechselte ihr Interesse von Kleinhirschen auf Elche und wieder zurück. Obwohl die Elchspuren intensiv verfolgt wurden, bestimmten Kleinhirsche den Hauptteil ihrer Ernährung. Die Studie bietet Beweise dafür, dass Wölfe hauptsächlich ihren Sehsinn für das Verfolgen von Spuren verwenden. Es kann geschlussfolgert werden, das Spurenlesen mehr qualitative Informationen über Wölfe liefern kann als Radiotelemetrie. Mehr Information über nicht-invasive endokrinologische und physiologische Techniken wird jedoch benötigt, bevor man diese Methode aktiv für Wolfsstudien einsetzten kann, was sicherlich von Interesse für den Naturschutz wäre.

Abstract

Tracking is a non-invasive technique that allows to assess behavioral and physiological data of free living mammals and compare it with individual nutritional and genetic information. Wolves (Canis lupus) are a rare and elusive species that prey on ungulates. In this study wolves were snow tracked in Alberta, Canada. To do this, the animals' trails were followed, excrements were collected and the behavior estimated. These results were recorded on a handheld GPS (Global Positioning System) and entered onto digital maps. The distances traveled during tracking were summed in 651 50-meterintervals. Several parameters were determined for each of these units in the field. Wolf feces were collected for endocrine analysis of cortisol and testosterone excretion products in Vienna, Austria. Cortisol concentrations were used as a method to estimate the level of "stress" the animal had recently been exposed to. Androgen levels reflected the sex, reproductive condition, and agonistic interactions of an individual. In order to validate the field data, and control for post excretion metabolism of the hormones, other fecal samples were collected from captive animals in Austria. An analysis of the metabolic progression did not show any general pattern of change. The data set analyzed was, however, to small to allow detailed comparisons with the physiology of the wolves in Canada. Behaviorally it was found that wolves travel in single file over hills and ridges. The habitat used was woody terrain most characteristic for deer. As the wolves moved into the bottom of valleys they split and began to trail moose through the brush and open field. Movement appeared to occur along well known and perhaps frequently used corridors within the wolves' home range. They did not follow the trails of horses, and they did not appear to be part of their diet. A major nutritional source was food scavenged from cougar. The wolves' hunting strategies changed from day to day after new snowfall: With in a week they shifted their interest from deer to moose and back to deer. Although moose were trailed intensely, deer were the major part of their diet. The study gives evidence that wolves primarily use their vision for following prey trails. It can be concluded that tracking can provide more qualitative information about wolves than radio telemetry. More information is however needed about noninvasive endocrine and physiological techniques before the method can actively be employed in studies on wolves, which would be of conservational interest.

1. Introduction

The gray wolf (Canis lupus) is a large carnivore that once was distributed all over the northern hemisphere. Its present range has been reduced to areas of low human density. The largest wolf populations can be found in Alaska, Canada, Russia and the eastern states of Europe. In nature wolves live in packs of 5 to 8 individuals on average (in extreme cases there can be packs as small as 2 up to 20; Okarma 1997). In general a pack (a band of wolves) is a family group of up to five generations (Mech 2000). They are large predators with large daily aliquots of movement (an average of 7.1 to 24 km of point to point distance per day) (Ooosenbrug 1982), and 18,54 km of average movement with many extreme days of 50 to 70 km (Priklonsky 1985). In most cases the nutritional needs are the major cause of movement (Theuerkauf 2003). Wolves can feed on almost any kind of animal but their main prey spectrum ranges from beavers to large herbivores (Mech 1970). With their high degree of sociality and well developed hunting strategies (Okarma 1997) wolves are able to prey on larger animals. One constraint is the limitation of feeding at a kill site and the necessary transport of meat over long distances to their offspring (Mech 1970). Wolves are very adaptable to environmental conditions for instance coping with road densities of <0.58km/km² (Thiel 1985) or <1.42km/km² (Merrill 2000). These adaptations are expressed as changes in their behavior (like hunting and foraging strategies), morphology (compare southern and northern subspecies in Freund 1999 and skull dimension changes between mountain and non mountain wolves in Hell (1982) and physiology. Behavior (Theuerkauf 2003) and physiology wolf phenotypes can also be changed by human activity (Creel 2002) and social status of individuals within the pack (Sands 2004).

Wolves are therefor an excellent model organism to study dynamic interactions between environmental recourses, the animals using them and their behavior. Wolves are however difficult to locate and observe over long periods of time. The size and shape of their home ranges can change with prey availability (Messier 1985; Everett 2003). They are very elusive and often avoid humans. They can travel long distances and are active mostly in twilight and at night (Bloch 2002). For these reasons it has been very difficult to get reliable information about the behavior, physiology and condition of wolves.

Although direct observations are difficult, it is possible to obtain information about animals in winter by following the tracks of animals in the snow. In this way home ranges (Ballard 1998) and activity (Theuerkauf 2002 and 2003) has been monitored. The initial advantage over telemetry was information about pack cohesion and hunting styles, which was assessed with tracking. Musiani (1998) was able to estimate travel speed of wolves with telemetry but his results showed that the actual travel route could only be documented by tracking. So tracking is nescessary as a prerequisite for interpretation of telemetry data. Although the collection of tracking data requires a larger time investment than telemetry (after trapping and installing the gear on the animals neck) it can produce reliable information on the direction of travel and distance covered (Kluth 1998; Elbroch 2003; Peham and

Young, per. comm.). It is also possible to estimate the age of the tracks, to determine the number of wolves in the group as well as their sex ratio (body position while urination and angle and height of urine tracks in snow). Lastly track analyses can shed light on the animals ages and reproductive conditions (e.g. blood in the urine of the female and mating beds; Kluth 1998; Elbroch 2003; Peham and Young, per. comm.).

Mech (1970) has done a lot of aerial surveys using life sightings and aerial tracking. The drawback of the approach was that the remains the wolves left behind as they travel (e.g. feces, urine, kills) could be collected on ground. These remains are important sources of information. A number of research groups have carried out dietary analysis of the wolf feces (e.g. Floyd 1978; Weaver 1993; Marquard-Petersen 1998; Spaulding 2000). Kill sites have also been investigated (Huggard 1993a and 1993b) and some studies have expanded the site investigations to include sent-marking behavior (Peters 1975). Feces and urine can also serve as sources for physiological data. Wolves like other animals secrete endocrine metabolites in their feces that can be measured in a laboratory. This, for instance, has been shown for the adrenal steroid cortisol by Creel (2002) and Sands (2004).

All these facts lead me to propose the following questions with regard to tracking and its use in filed studies of wolves:

1. Behavioral assessments:

- a) What information can be assessed by following wolf tracks concerning
 - the hunting or foraging behavior?
 - the cohesive of the pack and its consistency in different habitats?
 - social interactions with in a group?
 - the size of the home range?
 - Lastly, it was important to determine what kind of data could be obtained by analyzing the tracking sessions and environmental data with digital mapping and statistics?

b) What information can be found by comparing event markers like kills, feces, or resting places of wolves with tracking data?

2. Physiological assessments:

- Can adrenal and gonadal hormones be measured in feces and how can the concentration values be interpreted?
- Can the hormone concentrations be linked with the behavioral assessments?

To answer these questions free-living wolves were snow-tracked in Canada in winter. Data were analyzed with regard to predefined behavioral and environmental parameters. Fecal samples were collected parallel to tracking. Endocrine metabolites were measured in the samples. Validation procedures for the assay were carried out on captive individuals in Austria.

2. Methods

A) Overview

Tracking is one of the non-invasive techniques you can use to obtain information on free ranging mammals. Using this method one follows the imprints of the paws or hooves (tracks) of the animals in snow, dirt, sand or mud. As one travels along the wolf trails (the line of tracks the animal made while traveling) one tries to read the signs and patterns that can be seen on the surface and collect the needed data. Because tracking is a science and an art in itself, I prepared for the study by reading mammal tracking books (Elbroch 2003; Rezendes 1999), and participating in three separate tracking courses with renowned trackers in Estland (Das Wissen der Wildnis Homepage [Hp]), Idaho (Wilderness Awareness School Hp; The Shikari Tracking Guild Hp) and Alberta (A Naturalist's World Hp. These courses were 7, 14 and 2 days in length.

The experience of other trackers had shown that the only reasonable way to find wolves (Figure 1) and their tracks (Figure 2) was to gain knowledge of their general distribution through conversation with researchers, hunters/trappers and inhabitants of the area.



Figure 1. A gray wolf (*Canis lupus*) in profile. (Picture source: http://www.helsinki.fi/~mjkoskel/other/wolves.htm/)

Figure 2 shows a front (F) and a hind (H) track of a gray wolf with measurements of 10x8,5 cm and 9x7 cm without claws, respectively (Elbroch 2003).

Thereafter one has to drive along roads in the area, and make transects (straight line travel) of target areas with a truck, snowmobile/ATV or on skis, snowshoes or foot. Strategic points have to be checked in detail for tracks. These areas include geographical bottlenecks and types of habitats where large numbers of species live.

A good accessible matrix of small roads within the area is a good starting point to obtain continuous tracking data from an individual pack.

Each day of tracking began with an overview of what had happened on the road matrix in the hours or days since my last visit. Tracks were identified, aged and assigned a location with a handheld GPS (Global Positioning System) device and moved on until I had completed the roads of the matrix I planed to survey on these days (normally it was not possible to drive all the kilometers and do the tracking in one day). If there were no wolf tracks, I made a transect or investigated the rest of the road matrix. When there were tracks I wanted to follow, the first choice was based on age in order to obtain the freshest fecal samples. Fore-tracking (following the trail in the direction the animal traveled) was only done when the tracks were older than 10 hours. In most cases animals were fore-tracked, although sometimes I back-tracked them or did both. Tracking was primarily done on foot (with winter gear), and sometimes with the truck in the case the wolves stayed on the road. On foot the speed one can travel with depends on snow, tracking and gear conditions, and was in most cases 0,5 to 3 km per hour of tracking. With a vehicle the speed was up to 15 km/h.

One aim was to obtain physiological data. Non-invasive methods were used for collection. For this study it was not important, whether I fore- or back-tracked because the hormones in the feces do have an estimated 23 hours delay (Palme 2001). That means if there was a raising in the hormone concentrations in a wolf, one would find these changes in the feces that the wolf would defecate 20 to 26 hours after the event. Since the animals normally travel longer distances per day than it was possible to follow on foot, a correlation of any hormone concentration with any event from the tracking sessions was not feasible.

B) The Study Areas

Tracking mammals can be done in snow, mud or sand, but only snow is adequate because the other two substrates are to difficult and to time-consuming to get continuous data from. For the questions set out in the introduction, it was necessary to look at study areas with various kinds of terrain and habitats, varying human densities and a good snow cover over the whole winter. These can be found in southern Alberta, Canada, west of Calgary, around the Rocky Mountains. Alberta has a wolf population of about 2400 (Musiani 2004) with a higher abundance in the north perhaps because of the tourist load in the south. Although there are many national parks estimates of wolf numbers in the parks are low and decreasing (Callaghan, per .comm.). After a period of preparation in the areas, the data-collection was conducted from the end of December 2003 to the end of February 2004. The Central Rockies Wolf Project (CRWP) and Fish and Wildlife Canmore allowed me to work in these areas. CRWP, Banff National Park (BNP) and their associated researchers provided logistical support for data and material collection. The following three wolf packs (areas) were chosen for the study: Fairholme, Waiparous and Highwood. These differed in terms of interactions with humans. They were

in medium, low and very low human densitive areas, respectively. Beyond this interesting possibility, other factors affected the areas feasibility, so in the end only the Waiparous pack met all the criteria necessary for a tracking study.

The Fairholme pack was situated in the Bow Valley and the associated valleys in the Rocky Mountains. One third of the estimated home range of the current year was in Banff National Park. The towns Banff and Canmore are in this area with more than 25 000 residents, and a lot of tourists who use the park and the recreation areas. More than 20 people were looking for the Fairholme Pack over the whole winter but we found no evidence of its presence in the assumed home range. The area was accessible enough with roads but the wolves were not found.

The Highwood pack was situated in the Rocky Mountains and had 6 or 7 members. Highway 40 passes through the area and is closed in winter and spring. The estimated home range contains the Highwood Valley and associated valleys, which are nearly all part of the provincial park. No tourists or weekenders are allowed to be there when the road is closed. In Highwood the snow conditions in 2003/04 were very not amenable to tracking: In December the snow cover was thin and dispersed, so it was not possible to track them. A month later, there was too much snow to use the access road properly. There was a lack of continuous snow cover and the accessibility of the terrain was poor.

Waiparous is an area in the Foothills east of the Rocky Mountains and is still part of Canada's Western Boreal Forest (51°16′ - 51°27′ N, 114°38′ - 115°01′ W). In Alberta the temperature ranges in summer (June to August) from 15° C to 23° C and in winter (November to February) from -8° C to -25° C. Precipitation in the Foothills Region are 55 to 60 cm annually (Travel Alberta Hp). In general snow fall starts in January and ends in May. This year it started in the end of October and in November it had 50 cm of snow, which is a hard winter for southern Alberta (in some areas of British Columbia there can be as much as 300 cm of snow). The three wolves were assumed to be in the Waiparous pack. Their home range contained ranches and forestry areas. There were a lot of cows and horses in this area that were guarded in areas surrounded by fences. There are a few hundred inhabitants in this area. The time I spent in each of the three areas was varied however only Waiparous turned out to be accessible enough with adequate snow cover. It has a structured road matrix, which made it possible to monitor the wolf movements continuously. Therefore 26 days of data collection were done there of which 18 days included tracking wolves.

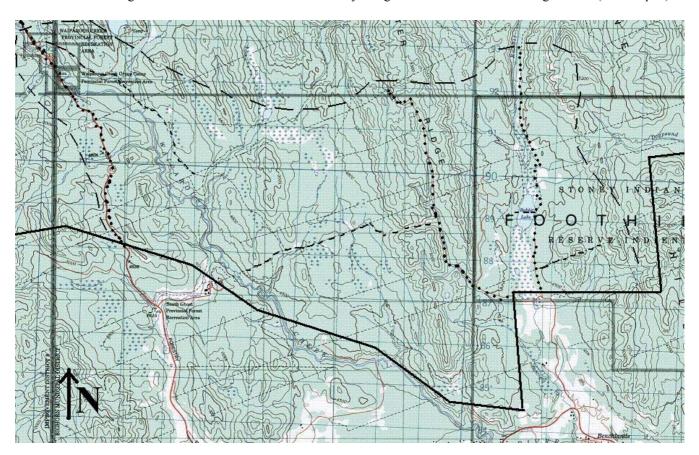


Figure 3 A section of Waiparous, the area were the data collection took place (line with long dashes). The thick lines indicate the southern boundary of home range of the Waiparous pack. The roads of the road matrix are indicated as dotted lines. The short dashed lines are the transects made on foot. The scale is 1:50,000, one side of a square is 1 km in length.

The flora of Waiparous/the Foothills is dominated by trembling aspen (*Populus tremuloides*), lodgepole pine (*Pinus contorta* var. *latifolia*) and black spruce (*Picea mariana*) on the ridges and the hillsides, and by brush, swamps and small lakes, rivers and creeks on the valleys. The mammalian fauna was dominated by white-tailed deer (*Odocoileus virginianus*), mule deer (*Odocoileus emionus*) and moose (*Alces alces*), horses and cows. Grizzly bear (*Ursus arctos*), wolf (*Canis lupus occidentalis*), lynx (*Lynx canadensis*) and cougar (*Puma concolor*) were present, together with a number of weasel species and a few lose Native American dogs.

The study area is owned by Native Americans (a part of the Stoney Indian Reserve in the middle and east of the map, including the Rabbit Lake), a rancher (with his cows, in the south), a trapper (with his horses, in the north), and the Government of Alberta (with the forestry in the west with a camping ground in the north). The roads of the search matrix were two dirt roads and highway 40 in the west. All of them run from south to north with distances of 2 to 7 km in between. In total, there were 18 km of road in the study area (Figure 3). The area was about 80 km² in size, with a human density of ten people within in the home range and one hundred outside to the south. The roads were checked at least once a week to give a general overview. This was not always possible with the concomitant work on the other two packs, and inclement weather.

In the study area 45 km of transects were made that did not overlap. In these transects the following predators were noted: 3 wolves, 1 cougar, 1 lynx, 4 foxes, 3 dogs. In addition the following potential prey were present in winter: 55 deer, 8 wild horses, 10 moose, and 28 domestic horses. These numbers were estimated by counting tracks in fresh snow that looked different from the next in shape and behavior. There were a lot of cows but they were well fenced and on the edge of the assumed wolf home range. (In summer they normally are herded into the woods. Only in 4 cases of killed cows wolves preying on cows had been reported over the last year at the ranch in the study area (Butters, per. comm.). The horses were kept in fenced areas in the core of the wolf home range, but the fences were lose, the doors open or passable and they were not otherwise contained. Surprisingly, only the fawns seem to have been preyed upon in summer (Burton, per. comm.). There are several "wild" horses in the west of the area in the forestry area, which escaped a few decades ago.

C) Data Collection and Analyses

a) Behavioral Parameters from Tracks

This section contains the definition, description and collection method for each behavioral parameter used.

In the field a handheld GPS from Garmin. For viewing the data on the computer aerial photos and topographical maps (1:50 000) were combined with the mapping software Arcview GIS 3.2 (and OziExplorer). A number of pictures from a digital camera (Kodak Easyshare cx4230) served as references. Observation and drawings were also recorded in a notebook. Tracking was not done on days with snowfall or the day after. Wildlife needs more than 12 hours to leave enough tracks so that it is feasible to track again (Callaghan, per. comm.). For the analyses, the stretch of kilometers from the tracking sessions was divided up into 50-meter sections. These 50 meters were estimated in the field, the parameters of this section were noted, and then the numbers of sections and their individual parameters were corrected according to measurements on a digital map. These measurements referred to the GPS data of the tracking sessions that were laid over the map.

The software Cybertracker (Cybertracker and American Cybertacker Hp) was tested but was found to be inadequate because it was not compatible with Arcview. If some improvements would be done in terms of compatibility and easier use, it would have been the first choice instrument to assess the parameters. The palmtop (used for the Cybertracker software), the GPS device and the digital camera worked properly at minus 20° C with and at minus 30° C without wind.

The following parameters refer to the wolf tracks themselves and the environment the wolves moved in or interacted with. All the parameters mentioned below were applied to each section. The categories within the parameters were exclusive.

Trail description and behavior:

- Cohesion: Cohesion of the pack was quantified on a three-point scale in categories. This was taken as an indicator of hunting or foraging strategies. The distances were "near" (0 to 50 m) and "far" (50 m and more). "Joined" was defined when the wolves traveled single file, where as "split" was defined when the trails of the individual wolves did not overlap. If they were near split in a section and near joined in one section, it was counted as near split. If they were far split where they were near in a section as well, it counted as far split.
 - 1: "Near joined" meant that the wolf trails did overlap.
 - 2: "Near split" meant that they ran beside each other within 50 m.
 - 3: "Far split" meant that the trails were more than 50m apart.
- Gait: In the same manner a three-point scale was defined to describe how fast the animals were traveling (Figure 4). In sections where more than one category occurred the one differing from "trot" was taken, because this is the baseline behavior of wolves as is indicated below. If "lower" and "faster" occurred in one segment, the most common one in terms of meters was chosen. Only urination and defecation were excluded because they happened in the flow of an animal's movement, it only changed speed. "Trot" was a gait (style of movement like walk and gallop), which is defined as baseline behavior for canids (John Young, per. comm.). This gait shows little stress or discomfort (Elbroch 2003). As the baseline gait changes (the animal's gait pattern gets "slower" or "faster", one sees side steps out of the line of travel that indicate a head shift), the animal becomes aware of internal or external events such as the urge to rest, urinate and defecate or movement, noise and odor of an other creature.
 - 1: "slower than trot"
 - 2: "trot"
 - 3: "faster than trot"
- Events: Changes in the gait pattern recorded as indications of complex behaviors.
 - 1: "hunting" the wolves were stalking or pursuing prey.
 - 2: "not interactive" the wolves directed their behavior towards inanimate things (e.g. investing a kill site, ground-scratching, sniffing, etc.).
 - 3: "interactive" the wolves directed their behavior towards each other, a traceable communication (e.g. short social interactions, group ceremonies [Mech 1970], possible mating activities, etc.).
- *Rest, urine and feces*: The locations and numbers of resting places, fecal and urine depositions were noted.

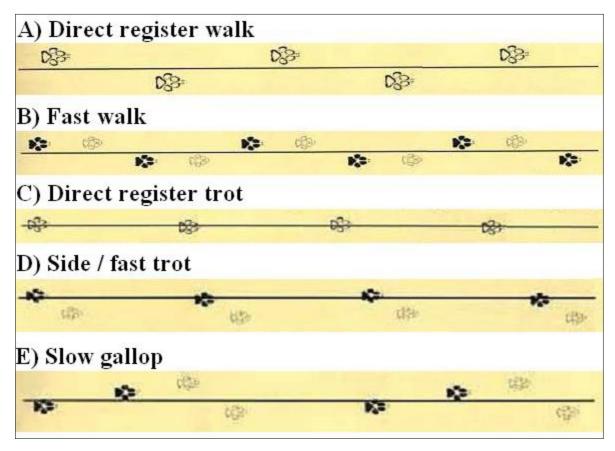


Figure 4 shows five examples what gaits of canids can look like. Those five are the most common I saw in the field. The black paw prints represent the front and the transparent the hind paws. The patterns gain speed from A to E: The direct register walk (A) is slowest and is used when traveling up steep slopes or when the animal is uncomfortable and aware (and there would be many side steps out of the straight line of travel which is symbolized with the straight line). This gait changes to a fast walk (B) as the wolf is looking and sniffing for some clues on the ground, and can shift into a direct register trot (C) which expresses comfort and awareness. This gait is used when animals travel long distances. This gait is called direct register because the hind paw hits the ground where the front paw met it before. If the animal wants to travel faster or its uncertainty increases because of a danger from behind it uses the side trot (D) where it shifts its hip to one side and the hind paws are imprinted in front of the front paws. In this gait it is easy to look back and forth without stopping. Finally in the gallop (E) all four feet of the animal leave the ground. That is different from the trot or the walk where the animal lifts two (the diagonal ones) feet or one foot at a time, respectively. The pictures and the technical words were taken from Elbroch (2003).

Track description:

- *Penetration-depth*: Penetration of the wolves into the snow was measured every 50 m (see snow-depth below).
- *Track age*: After locating sets of tracks, their age was estimated. If there were tracks of more than one individual on a trail, the ages and patterns were compared to determine which belonged together, the order in which they were produced and who many animals had traveled together (for more details about aging view Appendix A). It was not always possible to estimated the correct age.

The older the track and the longer ago the last snowfall was, the more difficult it was to make an accurate estimate. The following general categories were defined for aging tracks.

- 1: "less than 10 hours old" (in vulgo "last night")
- 2: "10 to 36 hours old" (in vulgo "1 day old")
- 3: "36 to 60 hours old" (in vulgo "2 days old")
- 4: "60 to 110 hours old" (in vulgo "3 to 4 days old")
- 5: "110 to 180 hours old" (in vulgo "5 to 7 days or one week old")
- 6: "180 to 350 hours old" (in vulgo "two weeks old")
- 7: "more than 350 hours old" (in vulgo "more than two weeks old")

Environmental descriptions:

- Last snow: Last snow fall was defined to describe the ending time of the last snow fall that covered 50% of the area (rough estimation) with a layer of fresh snow. Even only 3 mm of snow were enough for good tracking conditions. The categories were the same as in the one above.
- Snow-depth: Snow-depth was measured every 50m. Deer fawns, deer adults and wolves may be hindered at a snow-depth more than 40 cm (Huggard 1993a; Mech 1970). This was not a problem in the present study because only in fewer than 1% of the cases the snow was deeper than 40 cm. The scale that was measured in had spaces of 5 cm (0, 5, 10, 15 cm, etc.).
- Snow quality: A three-point scale was defined for the quality of snow. The hardness of snow was roughly estimated.
 - 1: "soft" the snow allowed to sink into it with ease.
 - 2: "light crust" the snow-surface offered little resistance before it broke under foot load.
 - 3: "hard crust" the snow-surface offered resistance under foot load but it did not break in all cases of wolf foot load (wolves are lighter than humans).
- Tracking quality: Tracking quality was measured with a three-point scale. It indicated the ease and difficulty to track under certain conditions.
 - 1: "poor" the tracks were not easy to read especially on hard snow, in wind blow areas or with aged tracks. It took a lot of time to follow them and not lose them.
 - 2: "fair" the tracks were made under softer snow and weather conditions than above and the tracks were not that aged or blown. It was possible to follow them but not impossible to lose them.
 - 3: "good" the tracks were made under soft snow conditions, were easy to read and follow.

- *Level*: Ground level changes were noted for comparison with forward locomotion. Categories here were "up", "straight" or "down" over the 50-meter segment or the most common one in terms of meters, if two or three were present.
 - 1: "up" means an angle of more than 15 degree of gradient.
 - 2: "straight" means an angle of less than 15 degree up or down.
 - 3: "down" means an angle of more than minus 15 degree of gradient.
- *Vegetation*: Five categories of vegetation types were used:
 - 1: "open" was swamp, ice or grass land and shrubs up to a height of 50 cm.
 - 2: "brush land" was tight or lose brush with a height of 0,5 to 3 m.
 - 3: "open forest" was loosely spaced trees with no undergrowth.
 - 4: "forest" was trees with undergrowth.
 - 5: "mixed vegetation" indicated that the wolf tracks were simultaneously found in different vegetation types.
- Path: For the paths the wolves traveled on five categories were found.
 - 1: "on road" the wolves were traveling on a road.
 - 2: "trailing" the wolves were trailing an animal's tracks for longer than 150 m.
 - 3: "on trails" the wolves changed from traveling on one wildlife trail to the next within 150 m.
 - 4: "free" the wolves moved freely in the landscape without a path.
- *Deer, moose, horse, prey and cougar trails*: Every trail of deer, moose, horse and all potential prey animals together, and cougar that was met and followed or crossed by the wolves was documented.
- Road crossing: Every road that was met and followed or crossed by the wolves was documented.
- The highest and lowest temperature of the day the track was made were taken from my weather journals. I did the same for the speed of wind (rough estimate) but it always was at low speed so it did not change the relative temperature for the wolves, and therefore was ruled out.

Dietary analysis:

Half of every feces I encountered in Waiparous was bagged, and analyzed for diet in the laboratory.
 I only took half of each because the wolves use there feces for marking and orientation causes (Asa 1985; Callaghan and Petrucci-Fonseca, per. comm.). The treatment of the feces and the analysis of hair was done according to the methods described in Kennedy (1981).

b) Physiological Parameters:

This section describes the methods used to collect and analyze the fecal samples of wolves. The samples were analyzed at the Department of Ethology (Institute of Zoology, University of Vienna) and at the Department of Biochemistry (Veterinarian University of Vienna).

Two hormones were of interest: cortisol and testosterone. Adrenal glucocorticoid production and cortisol concentrations in blood increase when mammals are in conflict situations or stressed (Morton 1995). High chronic stress of adrenal activity can lead to changes in the physiology and behavior and produce changes in an animal's susceptibility to infectious diseases (Sapolsky 1992). Cortisol can thus reflect an animal's condition and hence be a parameter of conservational importance.

Testosterone is a gonadal secretion product associated with both the expression of conflict and sexuality in male and female mammals. The data are interesting in comparison with adrenal activity as the two secretion patterns may differ depending on an animals sex, social status or level of environmental stress.

Field collection:

Samples were collected on seven focus packs by myself and some fellow researchers (see Acknowledgments). In addition, a number of samples were collected from captive individuals in Herberstein, Austria, to aid validation of the assays.

- In Canada: Wolf feces were collected if they were fresh (hours old) or frozen (outside temperature subzero) and if it had not been lying in the full sun. 0,5 to 3 grams of feces were put into a vial filled with methanol to store it for the hormone analysis. Samples were frozen until they were transported to Vienna.
- In Austria: In the animal park of Herberstein wolf feces were collected from a cage with 30 timber wolves (the same subspecies as in the study area). A timeline experiment was done to determine changes in hormonal concentrations in terms of time and ambient temperature. Samples were homogenized (in a plastic bag for several minutes) and allocated into 16 micro centrifugal tubes. Half of them were stored at room temperature the other half in the fridge. After ½, 1, 2, 4, 8, 12, 24, 36 hours they were frozen, and transported to Vienna. The tubes were sealed air-tight during storage outside of the freezer. Rupert Palme has suggested that the bacteria that disassemble the hormones in the feces are anaerobe ones (per. comm.).

Endocrine analysis:

The fecal samples from Canada were dried, dry-weighed and then the vials with the feces were refilled with 10 ml methanol (90 percent). For the Austrian samples, after thawing 0,5 g of feces of the

Diplomarbeit von Wolfram Jaschke 2004:

The Use of Tracking in the Assessment of Behavioral and Physiological Parameters in Free-living Wolves (Canis lupus)

Herberstein wolves were treated with 5 ml of methanol. The dry-weight was measured in each sample and used as a basis for concentration. A comparison of the results from both groups demonstrated that the analyses were comparable.

The feces in the methanol were centrifuged at 1500 rpm for 15 min. After that 1 ml of the liquid was used for cortisol and testosterone samples. For the cortisol analyses an ether extraction under a N₂-stream was done because the levels were at the lower end of the standard curve. Thereafter all samples were treated with assay buffer. The enzyme immunoassay (EIA) procedures and the antibody/antigen characteristics of the assay are described in Palme (1994 and 1997) and Schwarzenberger (1996). Two different EIA were used for cortisol and testosterone.

D) Statistical Analysis

a) Behavioral Parameters from Tracks

All sections were analyzed in SPSS 8.0. Spearman correlation (R) were used. The percentages in the results refer to the sum of the 651 sections as 100%, otherwise the number of cases (n) is written in brackets.

All tables were constructed in Microsoft Excel 2000. For the home range estimation I used fixed-kernel estimations. This was done with the mapping software Arcview GIS 3.2 together with the figures.

b) Physiological Parameters:

All tables and figures were constructed in Microsoft Excel 2000. The values were analyzed in SPSS 8.0. Descriptive analyses (χ^2 -test after Pearson) and Spearman correlations (R) were conducted.

3. Results

A) Behavioral assessments:

The following is an overview on the 26 tracking sessions that were done on Waiparous pack on 18 separate days (Table 1). Trails that do not belong together in time belong to different tracking sessions.

Tracking session	Date	Estimated date	e # of wolves	km/session	Wolf km /session
1	20.12.2003	13.12.2003	3	0,15	0,45
2	23.12.2003	17.12.2003	3	1,05	3,15
3	23.12.2003	16.12.2003	2	0,40	0,80
4	29.12.2003	17.12.2003	3	6,85	20,55
5	30.12.2003	30.12.2003	3	1,75	5,25
6	07.01.2004	05.01.2004	3	2,80	8,40
7	07.01.2004	04.01.2004	2	1,40	2,80
8	07.01.2004	03.01.2004	2	1,50	3,00
9	15.01.2004	08.01.2004	1	1,85	1,85
10	16.01.2004	09.01.2004	1	3,75	3,75
11	17.01.2004	15.01.2004	1	0,75	0,75
12	17.01.2004	16.01.2004	3	0,50	1,50
13	17.01.2004	15.01.2004	2	1,20	2,40
14	23.01.2004	16.01.2004	3	0,80	2,40
15	23.01.2004	16.01.2004	3	4,65	13,95
16	02.02.2004	31.01.2004	3	5,35	16,05
17	03.02.2004	31.01.2004	3	3,10	9,30
18	04.02.2004	31.01.2004	3	1,25	3,75
19	06.02.2004	01.02.2004	3	7,15	21,45
20	18.02.2004	14.02.2004	2	0,70	1,40
21	18.02.2004	16.02.2004	2	3,30	6,60
22	19.02.2004	15.02.2004	2	1,55	3,10
23	20.02.2004	19.02.2004	3	0,65	1,95
24	21.02.2004	20.02.2004	2	2,35	4,70
25	23.02.2004	22.02.2004	3	0,45	1,35
26	23.02.2004	22.02.2004	2	0,40	0,80
26 sessions	18 days	19 days	2,42 (mean)	55,7 km	141,5 km

Table 1. Between Dec 20th and Feb 23rd 26 tracking sessions were conducted in the study area on 18 separate days. The date of the track generation ("estimated date") was estimated as described in Appendix A. For each session the table shows the number of wolves ("# of wolves") that were followed during the whole session, "km/session" indicates how many km the author had traveled. "Wolf km/session" represents the km the wolves traveled and is basically the number of wolves times km/session.

For each session the number of wolves, the km that I walked along wolf trails and the km the wolves traveled in sum are listed. The average values per session were 2,42 wolves, 55,7 km and 141,5 km, respectively. The tracking sessions represent parts of the animals' movement on 19 separate days.

Some of the tracking sessions were found to be unusable for comparative analyses for several reasons: In the sessions 9, 10 and 11 only one wolf was present and so it was not possible to determine the cohesion of the pack. These sessions were then excluded from analysis.

The sessions 1, 2, 3, 4, 7 and 20 were also excluded because the tracks quality was poor. This made estimates of traveling speed and the cohesiveness difficult and unreliable.

In 15 and 22 the wolves were traveling on a road (like in 4, 9 and 10). In all cases, the road had also been used by humans and the wolves' tracks disturbed. These sessions were then also excluded from analysis.

The remaining 15 sessions (5, 6, 8, 12, 13, 14, 16, 17, 18, 19, 21, 23, 24, 25 and 26) were used for the analysis and are shown on the aerial photo in Figure 5. They were conducted on 11 separate days, were 32,6 km in length. In these sessions, there was an average of 2,67 wolves and a total of 88,9 km wolf trails. The data represented parts of the animals' movement on 12 separate days.

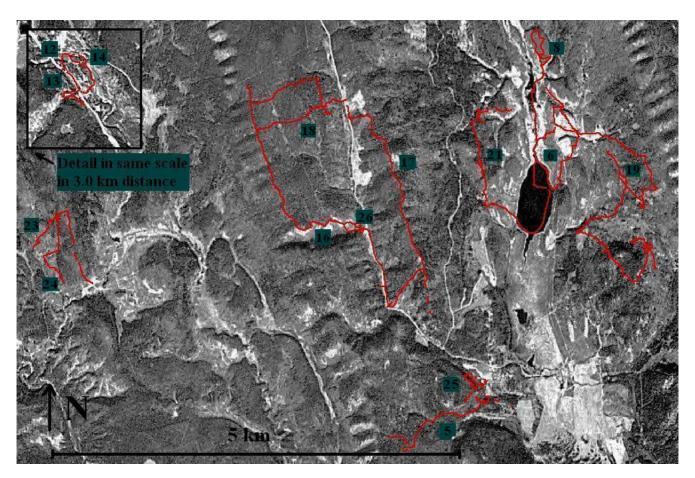


Figure 5. 15 of 26 tracking sessions (compare Table 1) are indicated as red lines and are labeled with the corresponding session number. Only these 15 were used in the analysis of the tracking sessions. In the upper left-hand corner of the aerial photo there is a detail of an area that is 3.0 km WNW of the location shown, both in the same scale.

The descriptive statistics of the 50-m-sections for the 15 sessions are presented in Table 2.

Cohesion			Gait		
Concion	occurrence	percent	Guit	occurrence	percent
near joined	405	62,2	slower	17	2,6
near split	148	22,7	trot	619	95,1
far split	98	15,1	faster	15	2,3
sum	651	100	sum	651	100
Sum	031	100	Sum	031	100
Event			Rest		
Zvene	occurrence	percent	11050	occurrence	percent
0	630	96,8	no	643	98,8
hunting	8	1,2	yes	8	1,2
not interact	7	1,1	sum	651	100
interactive	6	1,1	34111	031	100
sum	651	100			
Sum	031	100	Feces		
Urine			reces	occurrence	percent
Offic	occurrence	percent	0	643	98,8
0	584	89,7	1	8	1,2
1	62	9,5		651	100
2	5	0,8	sum	031	100
	651	100			
sum	031	100	Chary donth		
Donatuation d	anth		Snow-depth	oggurranga.	naraant
Penetration-d	_	naraant	0 to 5	occurrence 85	percent
0 to 5	occurrence 472	percent	6 to 10	216	13,1
		72,5			33,2
6 to 10	123	18,8	11 to 15	146	22,4
11 to 15	49	7,5	16 to 20	103	15,8
16 to 20	1	0,2	21 to 25	60	9,2
21 to 25	5	0,8	26 to 30	32	4,9
26 to 30	1	0,2	31 to 35	5	0,8
sum	651	100	36 to 40	3	0,5
G 114			41 to 45	1	0,2
Snow quality			sum	651	100
	occurrence	percent			
soft	364	55,9	Tracking qua	lity	
light crust	202	31,0		occurrence	percent
hard crust	85	13,1	poor	123	18,9
sum	651	100	fair	177	27,2
			good	351	53,9
Last snow			sum	651	100
	occurrence	percent			
1 day	107	16,4	Vegetation		
2 days	62	9,5		occurrence	percent
3 to 4 days	111	17,1	open	156	24
5 to 7 days	143	22,0	brush land	58	8,9
1 week	50	7,7	woods	265	40,7
2 weeks	178	27,3	mixed	20	3,1
sum	651	100	open forest	152	23,3
			sum	651	100
Path					
	occurrence	percent	Level		
on road	58	8,9		occurrence	percent
trailing	88	13,5	uphill	85	13
on trials	446	68,5	straight	488	75
free	59	9,1	downhill	78	12
sum	651	100	sum	651	100
~			2 4111		

Cougar trails			Moose trails		
	occurrence	percent		occurrence	percent
0	542	83,3	0	576	88,5
1	109	16,7	1	75	11,5
sum	651	100	sum	651	100
Deer trails			Horse trails		
	occurrence	percent		occurrence	percent
0	524	80,5	0	636	97,7
1	120	18,4	1	4	0,6
2	5	0,8	2	0	0.0
3	2	0,3	3	4	0,6
sum	651	100	>3	7	1,1
			sum	651	100
Prey trails					
	occurrence	percent	Road crossing	5	
0	401	61,6		occurrence	percent
1	198	30,4	no	632	97,08
2	38	5,8	yes	19	2,92
>2	14	2,3	sum	651	100
sum	651	100			

Table 2. The numbers and the percentages of the parameters involved in the final analysis are listed in this table. The sum of the 50-m-plots is 651. Numbers represent the occurrences in all sessions and the percentages of the total.

In Table 2 the parameters are listed. They were applied to of the 50-m sections (n=651) that are parts from the tracking sessions. The following contains a description how the individual parameters appeared to covary. The same sequence is used as in methods. Only the relevant data are shown. Where it is not indicated the percentages in the results refer to the sum of the 651 sections as 100%. All percentages were made on the bases of 651 and of selected cases. Within these percentages a comparison of the parameters was done. If the ratio was more than 1,4 or less than 0,71 times the data were reported. This represents the extremes in the cooccurrence in the different parameters.

Trail description and behavior:

• Cohesion, Trail Characteristics and Behavior: The wolf trails were near joined, near split and far split in 62,2%, 22,7% and 15,1%.

Trail Characteristics:

- In all the 651 cases the wolves were in the open in 24%, in brush land in 8,9%, in the open forests in 23,3%, in the woods in 40,7% and in mixed vegetation types in 3,1%.

They were joined near, in 405 cases. Of these 24,2% were in the open, 7,9% in brush land, 22,7% in the open forest, 45,2% in the woods and 0% in mixed vegetation types.

They were split near, in 148 of the cases. Of these 23,6% were in the open, 9,5% in brush land, 30,4% in open forest and 36,5% in the woods and 0% in mixed vegetation types.

Split far was in 98 cases. In 23,5% of these, the wolves were in the open, in 12,2% in brush land, in 15,3% in the open forest, and in 28,6% in the woods and in 20,4% in mixed vegetation.

This means that the wolves split far more often in mixed vegetation types (6.6 times) and less often in open forest (0,67 times) and woods (0,70 times).

General Behavior

- In all the 651 cases the wolves were on the road, trailing, on wildlife trails and in free movement in 8,9%, 13,5%, 68,5% and 9,1%.

In the joined near cases (n=405), 9,1% were on a road, 7,9% they trailed prey, 78,3% they traveled on wildlife trails and 4,7% free movement was found.

In split near, in 3,4% of these cases (n=148) they traveled on a road, in 14,9% they trailed prey, in 64,9% they traveled on wildlife trails and in 16,9% free movement was present.

In split far (n=98), 16,3% of these cases were on a road, in 14,9% they trailed prey, in 34,7% they traveled on wildlife trails and in 33,7% they were in free movement.

This means that the wolves joined near less often while they trailed prey (0,59 times). They split near more often in free movement (1,9 times) and less often on the road (0,38 times). They split far more often in free movement (3,7 times) and on the road (1,8 times) and less often on wildlife trails (0,51 times).

- In all the 651 cases the wolf trails were near joined, near split and far split in 62,2%, 22,7% and 15,1%.

Roads were crossed in 19 cases. Of these 57,9% they were joined near, 10,5% split near and 31,6% split far.

This means that the wolves crossed the roads more often as they were split far (2,1 times) and less often split near (0,46 times).

Gait and Landscape

- The wolves were slower than trot, trotting and faster than trot in 2,6%, 95,1% and 2,3% of the sections, respectively.

In the joined near cases (n=405), 1% of the occurrences were with movement slower than trot, 98,3% they were trotting and 0,7% faster than trot.

In split near (n=148), 4,7% of these cases were slower than trot, 91,2% trotting and in 4,1% faster.

In split far (n=98), 6,1% of these cases were slower than trot, 87,8% trotting and in 6,1% faster. This means that the wolves joined near less often as they moved slower (0,39 times) and faster (0,30 times) than trot. They split near more often as they moved slower (1,8 times) and faster (1,8 times) than trot. They split far more often as they moved slower (2,4 times) and faster (2,7 times) than trot.

- In all the 651 cases the wolves went up, down and straight on 13%, 12% and 75% of the sections.

In joined near, 15,3% of these cases (n=405) were found with downhill movement, 15,8% with uphill and 68,9% straight.

In split near (n=148), in 8,8% of these cases were downhill, 10,1% uphill and 81,1% straight.

In split far (n=98), 3,1% were downhill, in 6,1% uphill and 90,8% straight.

This means that the wolves split near less often in downhill (0,68 times). They split far less often in downhill (0,24 times) and uphill (0,51 times) movement.

Urination and Prey Trails

- In all the 651 cases the wolf trails were near joined, near split and far split in 62,2%, 22,7% and 15,1%.

They urinated once or twice in 67 cases. Of these they were joined and slit near, and split far in 71,6%, 17,9% and 10,5%, respectively.

This means that the wolves urinated less often as they were split near and far (0,70 times).

- In all the 651 cases the wolf trails were near joined, near split and far split in 62,2%, 22,7% and 15,1%.

Deer trails were present in 127 cases, when the wolves were joined near 66,1% of these cases, split near 16,5% and split far 17,3%.

Moose trails were present in 75 cases, when the wolves were joined near 34,7% of the cases, split near 21,3% and split far 44%.

Horse trails were present in 15 cases, when the wolves were joined near 60%, split near 40% and split far 0%.

Cougar trails were present in 109 cases, when the wolves were joined near in 82,6%, split near 16,5% and split far 0,9%.

This means that moose trails were present more often as the wolves were split far (2,5 times) and less often as they were joined near (0,56 times). The horse trails were present more often as the wolves were split near (1,8 times) and never as they were split far (0,00 times). That means that cougar trails were present less often as they were far (0,05 times).

Tracking Quality and Snow Characteristics

- In all the 651 cases the tracking quality was poor in 18,9%, fair in 27,2% and good in 53,9%. In joined near, for 15,6% of the cases the tracking quality was poor, in 25,7% fair and in 58,8% good.

In split near, 31,1% of the cases had poor tracking quality, 20,9% fair and 48% good. In split far, 14,3% of the cases were poor, 42,9% fair and 42,9% good.

This means that the wolves split near more often in cases with poor tracking quality (1,7 times). They split far more often in cases with fair tracking quality (1,6 times).

- In all the 651 cases the snow was soft in 55,9%, it had a light crust in 31% and it had a hard crust in 13,1%.

In joined near (n=405), the snow was soft in 59,3% of the cases, it had a light crust in 31,4% and a hard crust in 9,4%.

In split near (n=148), the snow was soft in 42,6% of the cases, in 35,8% there was a light crust and in 21,6% a hard crust.

In split far (n=98), the snow was soft in 62,2% of the cases, light crust was present in 22,4% and hard crust in 15,3%.

This means that the wolves split near more often when the snow had a hard crust (1,7 times).

- In all the 651 cases the snow depth ranged from 0 to 10 cm in 46,3%, from 11 to 20 cm in 38,2% and from 21 to 45 cm 15,5%.

In joined near, the snow was 0 to 10 cm deep in 48,9% of the cases, 11 to 20 cm in 39,3% and 21 to 45 cm in 11,9%.

In split near, the snow was 0 to 10 cm deep in 48,6% of the cases, 11 to 20 cm in 35,8% and 21 to 45 cm in 15,5%.

In split far, the snow was 0 to 10 cm deep in 31,6% of the cases, 11 to 20 cm in 37,8% and 21 to 45 cm in 30,6%.

That means that the wolves split far more often when the snow was 21 to 45 cm deep (2,0 times) and less in 0 to 10 cm deep snow (0,68 times).

- In all the 651 cases the wolves penetrated less than 5 cm into the snow in 72,5% of the sections, in 18,9% it was 6 to 10 cm and in 8,6% it was 11 to 30 cm.

In joined near, 74,6% of the cases (n=405) showed track penetration of 0 to 5 cm, 21% 6 to 10 cm and 4,4% 11 to 30 cm.

In split near, 79,1% of the cases (n=148) showed track penetration of 0 to 5 cm, 14,2% 6 to 10 cm and 6,8% 11 to 30 cm.

In split far, 54,1% of the cases (n=98) showed track penetration of 0 to 5 cm, 17,3% 6 to 10 cm and 28,6% 11 to 30 cm.

That means that the wolves joined near less often when the penetration showed 11 to 33 cm (0,51 times). They split far more often when the track penetration showed 0 to 5 cm (3,3 times).

- The last snow was 1, 2, 3 to 4, 5 to 7 days and 1 and 2 weeks before the session on 16,4%, 9,5%, 17,1%, 22% and 7,7% and 27,3% of the 651 occasions.

In joined near (n=405) the last snow had occurred 1 day before in 21,7%, 2 days in 12,1%, 3 to 4 days in 19%, 5 to 7 days in 18,8%, 1 week in 5,9% and 2 weeks in 22,5%.

In split near (n=148) the last snow had fallen 1 day before in 4,1%, 2 days in 8,1%, 3 to 4 days in 20,9%, 5 to 7 ago in 23,6%, 1 week in 2% and 2 weeks in 41,2%.

In split far (n=98) the last snow was 1 day before in 13,3%, 2 days in 1%, 3 to 4 days in 3,1%, 5 to 7 days in 32,7%, 1 week in 23,5% and 2 weeks in 26,5%.

This means that the wolves split near more often 2 weeks after the last snow fell (1,5 times) and less often 1 day after (0,25 times) and 1 week after (0,26 times). They split far more often 5 to 7 days (1,5 times) and 1 week (3,1 times) after the last snow fell and less often 2 days (0,11 times) and 3 to 4 days (0,18 times).

- *Gait*: The wolves were slower than trot, trotting and faster than trot in 2,6%, 95,1% and 2,3% of the sections, respectively.
 - In all the 651 cases the wolves were in the open in 24%, in brush land in 8,9%, in the open forests in 23,3%, in the woods in 40,7% and in mixed vegetation types in 3,1%.
 - Among the slower than trot occurrences (n=17) 29,4%, were in the open, 11,8%, in open forest, 23,5%, in brush land, 35,3% in woods and 0% in mixed vegetation.

The wolves trotting cases (n=619) were 23,4%, in the open, 8,9%, in brush land, 23,6% in open forest, 40,9% in woods and 3,2% in mixed vegetation.

Faster than trot cases (n=15) were 40% in the open, 6,7% in brush land, 13,3% in open forest, 40% in woods and 0% in mixed vegetation.

That means that the wolves were faster than trot more often in the open (1,7 times) and less often in the open forest (0,57 times). They were never faster (0,0 times) or slower (0,0 times) than trot while moving freely.

- In all 651 cases the wolves went up, straight and down on 13%, 75% and 12% of the sections. The wolves were slower than trot (n=17) 11,8% uphill, in 82,4% straight and 5,9% downhill. The wolves were trotting (n=619) 13,4% uphill, 74,5% straight and 12,1% downhill.
- The wolves were faster than trot (n=15) 0% uphill, 86,7% straight and 13,3% downhill.
- That means that the wolves were slower than trot less often as they went downhill (0,49 times). They never were faster than trot while moving uphill (0,0 times).
- In all the 651 cases the tracking quality was poor in 18,9%, fair in 27,2% and good in 53,9%. For the slower than trot cases (n=17) tracking quality was poor in 41,2%, fair in 35,3% and good in 23,5%.

For the trotting cases (n=619) tracking quality was poor in 18,7%, fair in 27,3% and good in 54%.

For the faster than trot cases (n=15) tracking quality was poor in 0%, fair in 13,3% and good in 86,7%.

That means that the wolves were slower than trot more often with poor tracking quality (2,2 times) and less often with good tracking quality (0,44 times). They were faster than trot more often in good tracking quality (1,6 times) and never in poor tracking quality (0,0 times).

- In all the 651 cases the snow was soft in 55,9%, it had a light crust in 31% and it had a hard crust in 13,1%.

Slower than trot (n=17) cases were found in soft snow 23,5% of the time, with a light crust 23,5% and hard crust 52,9%.

Trotting cases (n=619) were found in soft snow 56,2%, light crust 31,7% and hard crust 12,1%. Faster than trot cases (n=15) were found in soft snow 80% of the time, with a light crust 13,3% and hard crust 6,7%.

That means that the wolves were slower than trot more often on snow with a hard crust (4,0 times) and less often in snow with a light crust or soft snow (0,42 times). They were faster than trot more often soft snow (1,4 times) and less often on snow with a light (0,43 times) and a hard (0,51 times) crust.

- *Events*: 21 events were recorded.
 - There was one hunting event over 8 sectors where the wolves were split far (50%), and then joined near for (50%). 87,5% of the sectors were found in open forest and 12,5% in the open. The wolves were moving freely (100%).
 - The not-interactive events (n=6) happened in 50% in the open and 50% in open forest. Similarly, 50% were on wildlife trails and 50% while moving freely (50%).
 - For the interactive events (n=6), the same percentages were found.
- *Rest*: The wolves rested 8 times. This occurred in the open (50%), in brush land (12,5%) and in woods (37,5%).
- *Urine*: On 67 occasions the single urinations were found and on 5 occasions two were in close proximity. The urination was found to be done by adult males in 6,7% of the cases, adult females in 6,7% and juveniles in 1,3% (the number of wolves was three). In the remainder of the cases it was uncertain what the sex or age of the animal was, 85,3% of the cases.
 - In all the 651 cases the wolves were in the open in 24%, in brush land in 8,9%, in the open forests in 23,3%, in the woods in 40,7% and in mixed vegetation types in 3,1%.
 - Of the urinations (n=67) 25,4% were in the open, 3% in brush land, 23,9% in open forest, 46,3% in woods and 1,5% in mixed vegetation.
 - This means that the wolves urinated less often in brush land (0,34 times) and mixed vegetation (0,48 times).
 - In all the 651 cases the wolves were on the road, trailing, on wildlife trails and in free movement in 8,9%, 13,5%, 68,5% and 9,1%.
 - Of the urinations (n=67) 7,5% were on the road, 10,4% while trailing, 76,1% on wildlife trails and 6% while moving freely.

This means that the wolves urinated less often while moving freely (0,66 times).

- In all 651 cases the wolves went up, straight and down on 13%, 75% and 12% of the sections. The wolves urinated as they traveled up, straight and down on 20,9%, 71,6% and 7,5. This means that the wolves urinated more often when they went up (1,6 times) and less often down (0,63 times).
- In all 651 cases, deer trails were present in 19,5%, moose trails in 11,5%, horse trails in 2,3% and cougar trails in 16,7%.
- The wolves urinated (n=67) when deer trails were present in 28,4%, moose in 10,7%, horse in 0% and cougar in 29,9%.
- This means that the wolves urinated more often when deer (1,5 times) and cougar trails (1,8 times) were present and never when horse trails were present (0,0 times).
- In all the 651 cases the tracking quality was poor in 18,9%, fair in 27,2% and good in 53,9%. The wolves urinated (n=67) when tracking quality was poor in 9%, fair in 23,9% and good in 67,2% of the cases.

This means that wolves urinated less when tracking quality was poor (0,48 times).

• *Feces*: On 8 occasion defectaion was found. This was seen in the open (25%), in open forest (62,5%) and in woods (12,5%). It did not happen on trails of deer or moose or horse, but it was often found on cougar trails (25%). Only in one section urine and feces were present together.

Track description:

• *Penetration-depth*: The wolves penetrated less than 5 cm into the snow in 72,5% of the sections, in 18,9% it was 6 to 10 cm and in 8,6 it was 11 to 30 cm. The reason for that is that the wolves often traveled on the trails of other animals, and therefore the snow had already been compressed. The penetration-depth correlated with the snow-depth (R=0,53, p<0,001).

Environmental descriptions:

- Last snow: The last snow was 1, 2, 3 to 4, 5 to 7 days and 1 and 2 weeks before the session on 16,4%, 9,5%, 17,1%, 22% and 7,7% and 27,3% of the occasions. Last snow correlated significantly with snow quality (R=0,629, p<0,001). That meant got the longer ago the snowfall, the harder the snow. It also correlated significantly with tracking quality (R=0,61, p<0,001).
 - The wolves went up, straight and down on 13%, 75% and 12% of the sections.

 As the last snow fall was 1 day ago (n=107) the wolves went uphill in 21,1%, straight in 55,1% and downhill in 23,4%.

As the last snow fall was 2 days ago (n=62) the wolves went uphill in 22,6%, straight in 61,3% and downhill in 16,1%.

As the last snow fall was 3 to 4 day ago (n=111) the wolves went uphill in 13,5%, straight in 79,3% and downhill in 7,2%.

As the last snow fall was 5 to 7 day ago (n=143) the wolves went uphill in 9,1%, straight in 88,8% and downhill in 2,1%.

As the last snow fall was longer than a week ago (n=50) the wolves went uphill in 8%, straight in 86% and downhill in 6%.

As the last snow fall was longer than 2 weeks ago (n=178) the wolves went uphill in 9%, straight in 74,7% and downhill in 16,3%.

This means that the wolves went uphill and downhill more often the younger the snow was (1,6 times and more), less often after the 4th day, but with 2 weeks of no snowfall the wolves start to go more downhill again.

- The last snow was 1, 2, 3 to 4, 5 to 7 days and 1 and 2 weeks before the session on 16,4%, 9,5%, 17,1%, 22% and 7,7% and 27,3% of the occasions.

Deer trails were present (n=107) the last snowfall was 1 day ago, 2 days ago, 3 to 4 day ago, 5 to 7 day ago, longer than a week ago and longer than 2 weeks ago in 24,4%, 18,9%, 2,4%, 22%, 3,9% and 28,3%, respectively.

Moose trails were present (n=75) the last snow fall was 1 day ago, 2 days ago, 3 to 4 day ago, 5 to 7 day ago, longer than a week ago and longer than 2 weeks ago in 0%, 0%, 30,7%, 45,3%, 8% and 16%, respectively.

This means that deer trails were present more often in the first two days after snowfall (1,5 times and more) and less 3 and 4 days after (0,14 times) and after the first week (0,51 times). Moose trails were present more often 3 to 7 days after the last snow fell (1,8 times and more), less after the second week (0,59 times) and never on the first two days after (0,0 times).

- *Snow-depth*: The snow depth ranged from 0 to 10 cm in 46,3%, from 11 to 20 cm in 38,2%, from 21 to 40 cm 15,3% and from 41 to 45 cm in 0,2%. The ground was always covered with more than one cm of snow (100%).
 - In all 651 cases the snow depth ranged from 0 to 10 cm in 46,3%, from 11 to 20 cm in 38,2% and from 21 to 40 cm 15,5%.

As deer trails were present (n=127) the snow was 0 to 10 cm in 61,4%, 11 to 20 cm in 31,5% and 21 to 40 cm deep in 7,1%.

As moose trails were present (n=75) the snow was 0 to 10 cm in 5,3%, 11 to 20 cm in 40% and 21 to 40 cm deep in 54,7%.

As horse trails were present (n=15) the snow was 0 to 10 cm in 0%, 11 to 20 cm in 20% and 21 to 40 cm deep in 80%.

As cougar trails were present (n=109) the snow was 0 to 10 cm in 85,3%, 11 to 20 cm in 12,8% and 21 to 40 cm deep in 1,8%.

This means that deer trails were present more often in shallower snow (1,3 times) and less in deeper snow (0,46 times). Moose trails were present more often in deeper snow (3,5 times) and less in shallower snow (0,11 times). Horse trails were present more often in deeper snow (5,2 times) and never in shallower snow (0,00 times). Cougar trails were present more often in shallower snow (1,8 times) and less in deeper snow (0,12 times).

- Tracking quality: The tracking quality was poor in 18,9%, fair in 27,2% and good in 53,9%.
 - In all 651 cases the wolves were in the open in 24%, in brush land in 8,9%, in the open forests in 23,3%, in the woods in 40,7% and in mixed vegetation types in 3,1%.

The tracking quality was poor (n=123) in the open in 24,4%, in the brush land in 5,7%, in the open forest in 52,8%, in the woods in 14,6% and in mixed vegetation in 2,4%.

The tracking quality was fair (n=177) in the open in 35%, in the brush land in 20,9%, in the open forest in 12,4%, in the woods in 23,7% and in mixed vegetation in 7,9%.

The tracking quality was good (n=351) in the open in 18,2%, in the brush land in 4%, in the open forest in 18,5%, in the woods in 58,4% and in mixed vegetation in 0,9%.

This means that the tracking quality was poor more often in the open forest (2,3 times) and less often in brush lands (0,64 times) and the woods (0,36 times). It was fair more often in the open (2,3 times), in brush lands (2,4 times) and mixed vegetations (2,6 times) and less often in the woods (0,58 times). It was good more often in the woods (1,4 times) and less in the other vegetation types.

- Snow quality: In 55,9% the snow was soft, in 31% it had a light crust and in 13,1% it had a hard crust. Snow quality correlates negatively with tracking quality (R=0,82, p<0,001). That means the harder the snow gets the harder it becomes to track, which seems obvious and is known by practitioner.
 - In all 651 cases the wolves were in the open in 24%, in brush land in 8,9%, in the open forests in 23,3%, in the woods in 40,7% and in mixed vegetation types in 3,1%.

The snow was soft (n=364) in the open in 17,6%, in the brush land in 5,5%, in the open forest in 19,5%, in the woods in 56% and in mixed vegetation in 1,4%.

The snow had a light crust (n=202) in the open in 34,7%, in the brush land in 16,8%, in the open forest in 22,3%, in the woods in 21,3% and in mixed vegetation in 5%.

The snow had a hard crust (n=85) in the open in 25,9%, in the brush land in 4,7%, in the open forest in 42,4%, in the woods in 21,2% and in mixed vegetation in 5,9%.

This means that the snow was softer more often in the woods and less often in the other vegetation types. It had a light crust more often in the open (2,0 times), in brush lands (1,9 times)

and mixed vegetations (1,6 times) and less often in the woods (0,52 times). It had a hard crust more often in the open forest (1,8 times) and in the mixed vegetations (1,9 times) and less often in the woods (0,52 times).

- Level: The wolves went up, straight and down on 13%, 75% and 12% of the sections.
 - In all 651 cases the wolves were in the open in 24%, in brush land in 8,9%, in the open forests in 23,3%, in the woods in 40,7% and in mixed vegetation types in 3,1%.

The wolves went uphill (n=85) in the open in 7,1%, in the brush land in 2,4%, in the open forest in 29,4%, in the woods in 60% and in mixed vegetation in 1,2%.

The wolves straight (n=488) in the open in 28,9%, in the brush land in 10,5%, in the open forest in 21,1%, in the woods in 36,1% and in mixed vegetation in 3,5%.

The wolves downhill (n=78) in the open in 11,5%, in the brush land in 6,4%, in the open forest in 30,8%, in the woods in 48,7% and in mixed vegetation in 2,6%.

This means that the wolves went up more often in the woods (1,5 times) and less often in the open (0,30 times), brush land (0,27 times) and mixed vegetations (0,39 times). They went down less often in the open (0,48 times).

- *Vegetation*: The wolves were in the open in 24%, in brush land in 8,9%, in the open forests in 23,3%, in the woods in 40,7% and in mixed vegetation types in 3,1%.
 - In all 651 cases the snow depth ranged from 0 to 10 cm in 46,3%, from 11 to 20 cm in 38,2% and from 21 to 45 cm 15,5%.

In the open (n=156) the snow was 0 to 10 cm in 49,4%, 11 to 20 cm in 28,8% and 21 to 45 in 21,8% deep.

In the brush land (n=58) the snow was 0 to 10 cm in 22,4%, 11 to 20 cm in 58,6% and 21 to 45 in 19% deep.

In the open forest (n=152) the snow was 0 to 10 cm in 57,2%, 11 to 20 cm in 36,8% and 21 to 45 in 5,9% deep.

In the woods (n=265) the snow was 0 to 10 cm in 43,8%, 11 to 20 cm in 40,4% and 21 to 45 in 15,8% deep.

In mixed vegetation (n=20) the snow was 0 to 10 cm in 40%, 11 to 20 cm in 35% and 21 to 45 in 25% deep.

This means that the snow was shallower in the open forest (0,36 times) and deeper in brush land and mixed vegetations (1,6 times).

- *Path*: The wolves were on the road, trailing, on wildlife trails and in free movement in 8,9%, 13,5%, 68,5% and 9,1%.
 - The wolves went up, straight and down on 13%, 75% and 12% of the sections.

As they traveled on the road (n=58), they traveled uphill in 5,2%, straight in 93,1% and downhill in 1,7%.

As they were trailing (n=88), they traveled uphill in 2,3%, straight in 87,5% and downhill in 10,2%.

As they traveled on wildlife trails (n=446), they traveled uphill in 16,8%, straight in 68,4% and downhill in 14,8%.

As they moved freely (n=59), they traveled uphill in 8,5%, straight in 88,1% and downhill in 3,4%.

This means that roads were present less often as the wolves went up and down (0,4 times and less). As they were trailing prey, they went up less often (0,18 times). As they moved freely, they went up and down less often (0,65 times and less).

- In all 651 cases the wolves were on the road, trailing, on wildlife trails and in free movement in 8,9%, 13,5%, 68,5% and 9,1%.

Deer trails was present (n=127) while traveling on the road, trailing prey traveling, on wildlife trails and moving freely in 0,8%, 19,7%, 79,5% and 0% of the cases, respectively.

Moose trails were present (n=75) while traveling on the road, trailing prey traveling, on wildlife trails and moving freely in 2,7%, 76%, 5,3% and 16%, respectively.

Horse trails were present (n=15) while traveling on the road, trailing prey traveling, on wildlife trails and moving freely in 0%, 26,7%, 40% and 33,3%, respectively.

Cougar trails was present (n=109) while traveling on the road, trailing prey traveling, on wildlife trails and moving freely in 0%, 2,8%, 91,7% and 5,5% of the cases, respectively.

This means that deer trails were present more often while trailing (1,5 times) and less often on the road (0,09 times). Moose were present more often while trailing (3,9 times) and free movement (1,8 times) and less often on the road (0,08 times) and on wildlife trails (0,30 times). Horse trails were present more often on trailing (2,0 times) and free movement (3,7 times) and less often on wild life trails (0,58 times) and never on the road (0,0 times). Cougar trails were present less often while trailing (0,21 times) and free movement (0,60 times) and never on the road (0,00 times).

- In all 651 cases the wolves were in the open in 24%, in brush land in 8,9%, in the open forests in 23,3%, in the woods in 40,7% and in mixed vegetation types in 3,1%.

As they traveled on the road (n=58), they traveled in the open in 74,1%, in brush land in 8,6%, in open forest in 17,2%, in the woods in 0% and in mixed vegetation in 0%.

As they were trailing (n=88), they traveled in the open in 25%, in brush land in 9,1%, in open forest in 9,1%, in the woods in 42% and in mixed vegetation in 14,8%.

As they traveled on wildlife trails (n=446), they traveled in the open in 14,6%, in brush land in 9,2%, in open forest in 26,9%, in the woods in 47,8% and in mixed vegetation in 1,6%.

As they moved freely (n=59), they traveled in the open in 44,1%, in brush land in 6,8%, in open forest in 23,7%, in the woods in 25,4% and in mixed vegetation in 0%.

This means that the wolves traveled on the road more often in the open (3,1 times) and never in the woods (0,00 times) and mixed vegetations (0,00 times). They trailed potential prey more often in mixed vegetations (4,8 times) and less often in open forests (0,39 times). They traveled on wildlife trails less often the open (0,61 times) and in mixed vegetations (0,52 times). They moved freely more often in the open (1,8 times), less often in the woods (0,62 times) and never in mixed vegetations (0,00 times).

- *Deer trails*: In 80,5% of the sections there were no deer tracks, in 18,4% there was one and in 1,2% there was more than one deer trail.
 - In all 651 cases the wolves went up, straight and down on 13%, 75% and 12% of the sections. Deer trails were present (n=127) when the wolves traveled uphill in 19,7%, straight in 66,1% and downhill in 14,2%.

This means that deer trails were present more often uphill (1,5 times).

- *Moose trails*: In 88,5% of the sections there were no moose tracks, in 11,5% there was one moose trail.
 - In all 651 cases the wolves went up, straight and down on 13%, 75% and 12% of the sections. Moose trails were present (n=75) when the wolves traveled uphill in 0%, straight in 93,3% and downhill in 6,7%.

This means that moose trails were present more often straight, less often downhill (0,56 times) and never uphill (0,00 times).

- *Horse trails*: In 97,7% of the sections there were no horse tracks, in 0,6% there was one and in 1,7% there was more than one horse trail. In 15 sections horse trails had been present.
 - In all 651 cases the wolves went up, straight and down on 13%, 75% and 12% of the sections. Horse trails were present (n=15) when the wolves traveled uphill in 0%, straight in 100% and downhill in 0%.

This means that horse trails were always present straight, and never down or uphill (0,00 times).

- *Cougar trails*: In 83,3% of the sections there were no cougar tracks, in 16,7% there was one cougar trail.
 - In all 651 cases the wolves went up, straight and down on 13%, 75% and 12% of the sections. Moose trails were present (n=75) when the wolves traveled uphill in 25,7%, straight in 55% and downhill in 19,3%.

This means that cougar trails were present more often uphill (2,0 times) and downhill (1,6 times).

- Road crossing: There were 19 road crossings in this study.
 - In all 651 cases the wolves were on the road, trailing, on wildlife trails and in free movement in 8,9%, 13,5%, 68,5% and 9,1%.

In 31,6% of the road crossing cases (n=19) the wolves traveled on the road, in 0% were trailing, in 47,4% traveled on wildlife trails and in 21,1% moved freely.

This means that the wolves crossed the road more often as they were traveling on the road (they left or entered road travel) (3,6 times) and moved freely (2,3 times) and never while trailing (0,0 times).

- *Wolf number*: As there were only two wolves (n=174) they were joined near in 65,3%, split near in 28,3% and split far in 6,9%. It correlates significantly with tracking quality (R=0,648, p0,001), snow quality (R=-0,638, p0,001) and last snow (R=-0,536, p0,001). That means as the wolves were only two, the tracking quality is poorer, the snow is harder and the last snowfall is longer ago.
 - In all 651 cases the wolf trails were near joined, near split and far split in 62,2%, 22,7% and 15,1%.

As there were two wolves (n=174) they were joined near in 64,9%, split near in 28,2% and split far in 6,9%.

As there were three wolves (n=477) they were joined near in 61,2%, split near in 20,8% and split far in 18%.

That means as the wolves were two in number, they split far less often (0,46 times).

- In all 651 cases the wolves were slower than trot, trotting and faster than trot in 2,6%, 95,1% and 2,3% of the sections, respectively.

As there were two wolves (n=174) they were slower in 1,7%, trotting in 98,3% and faster in 0%. As there were three wolves (n=477) they were slower in 2,9%, trotting in 93,9% and faster in 3,1%.

This means that two wolves less often moved slower (0,65 times) and never faster (0,00 times).

- In all 651 cases wolves were present as two and three individuals in 26,7% and in 73,3%. The wolves urinated (n=67) as there were two and three wolves in 10,4% and 89,6%, respectively.

This means that the wolves urinated less often as they were two in number (0,39 times).

Diet analysis from feces

An analysis with the hair of the feces from Canada (n=24) was conducted. Table 3 shows the date/age of the sample and the species recognizable within it. Species recognition was difficult. The color of the hair and their appearance with and without a microscope was compared, and with hair that had been found in the field from moose, horse and deer.

The dates of the 24 feces ranged from October 2003 (they were not older than the first snowfall at the end of October, the feces were on snow or only partly covered) to the mid of February 2004. They represent three months. Twelve of the samples were over one month old. The deer represented the major part of the diet (71%). Moose were found in only 8% and the non-ungulate species in 21% of the samples.

Age	moose	deer	horse	other mammals
0 to 1 week	2	1	0	0
2 to 4 weeks	0	7	0	2
1 to 3 months	0	9	0	3
Sum	2	17	0	5

Table 3 The results of the 24 fecal samples that were used for the diet analysis. There are three feces age classes in which three species and one group of species (all other mammals) are contained. There were not any avian remains. The sums of the age classes were representative for the time span of the study. The samples reach from February 18th 2004 back into November or October 2003.

Home range analysis

For home range analysis 30 to 100 locations are necessary to make accurate home range size estimations for one season (Girard 2002). 42 locations from the whole pack from a quarter season were used that were at least 24 hours apart (Appendix B). For this analysis the locations had to be 24 hours apart.

The data was taken from the Waiparous pack. None of the sessions were excluded. The tracking information collected suggested that the wolves stayed within the home range on 24 of the 76 days (Appendix C). Random urine spots of the tracking session were considered for the analysis that had at least an age difference of 20 hours. Additionally a location for each of the old scats (that were used for the diet analysis) was taken, but only if I had confidence that they differed 24 hours (in 6 cases I was not). A number of 42 locations were taken.

Although it is clear (Appendix C) that the information was biased by roads and transects and that the study area of about 80 km² may not represent the actual home range of the pack which may vary between 200 to 400 km² for 3 wolves (Okarma 1997), it still represents the usage of these 80 km².

B) Physiological assessments with feces:

In Canada, 69 fecal samples were collected from southern Alberta of which only 4 came from the study area.

In Herberstein 8 samples were collected (2 female, 6 male) and were sub sampled several times (107 sub samples) and stored at different temperatures as indicated in Methods.

The different compositions (percent of hair content, color and purity of the feces) of the feces from Herberstein and Canada were compared for parameter correlations and group differences (χ^2 -test and correlation after Spearman) but no significant difference or correlation was found. The concentrations of cortisol and androgen metabolites were also compared. Different but not correlating values were found for these adrenal and gonadal metabolites. Two different assay analysis techniques were used. No significant correlations were found between the two parameters in either of the assay analyses. In order to compare concentrations between the sample sets, I defined classes of the concentrations for each assay techniques A and B which are listed in Table 4. These data give an overview of how the hormone concentrations from Herberstein and Canada differed and what similarities they had. These two sets consist of 7 classes, respectively. The values of the categories for each hormone was can be seen in the Figures 6-9.

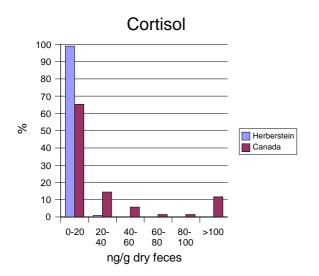
For Figure	For assays	Class 1	Class 2	Class 3	Class 4	Class 5	Class 6	Class 7
6 7	Cortisol A Androgen A		20-40 200-400	40-60 400-600	60-80 600-800	80-100 800-1000	>100 >1000	
8 9			,				108 to 324 1080 to 3240	

Table 4. Two assay analyses groups for fecal androgen and cortisol metabolites. Results in each assay system were divided up into six and seven classes of concentrations The first assay system set was constructed through adding 20 and 200 for cortisol and testosterone, respectively, to the former class value. The second set was constructed through multiplying the last value of the former class with 3. The values are given in ng/g dry feces.

The Figures 6 and 7, 8 and 9 show that the hormone levels both androgens and corticosteroids in the Canadian animals were higher then those from captive animals in the Herberstein. In addition these differences were found in both assay analyses. In Figure 6 99% of the Herberstein sample values are found in the first (lowest) class of cortisol concentrations whereas only 65% of the Canadian samples. Of the latter 14% are in the second and 11% in the last (highest) class.

In Figure 7 one can see that there were similar distributions of classes for the Canadian samples but this time for the androgen concentrations 58%, 13% and 14% were found and 80% of the captive samples in the lowest concentration class.

In Figure 8 for cortisol there are 20%, 32% and 39% of the Herberstein samples in the first, second and third classes where as there are 10%, 38%, 30%, 10% and 10% distributions for Canadian samples in the second to the sixth class.



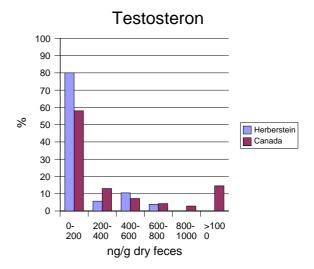
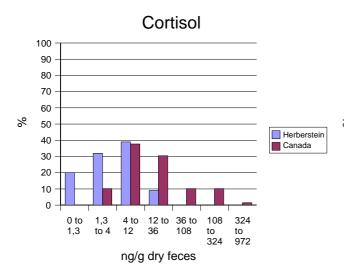
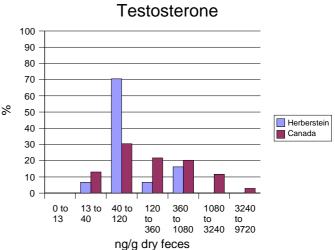


Figure 6 shows the concentration of cortisol from Figure 7 shows the concentration of androgens assay system A in the dry wolf feces of from assay system A in the dry wolf feces of Herberstein (light) and Canada (dark) with the use Herberstein (light) and Canada (dark) with the use of the first set of categories mentioned in the text. of the first set of categories mentioned in the text. The values of the samples are expressed in percent. The values of the samples are expressed in

percent.





values of the samples are expressed in percent.

Figure 8 shows the concentration of cortisol from Figure 9 shows the concentration of androgens assay system B in the dry wolf feces of Herberstein from assay system B in the dry wolf feces of (light) and Canada (dark) with the use of the Herberstein (light) and Canada (dark) with the use second set of categories mentioned in the text. The of the second set of categories mentioned in the text. The values of the samples are expressed in percent.

Diplomarbeit von Wolfram Jaschke 2004:

The Use of Tracking in the Assessment of Behavioral and Physiological Parameters in Free-living Wolves (Canis lupus)

In Figure 9 again there is a similarity in the distribution of endocrine concentrations in the Canadian samples (13%, 30%, 22%, 20% and 12%) for androgens. In the captive Herberstein samples, the values appeared to differ. 70% and 16% were found in the second and the fourth category.

On average the Canadian and the Herberstein samples had 7 and 3,7 times higher values, respectively. The pure values of the hormone concentrations, their means and their standard deviation are listed in Table 5 and 6 for cortisol as well Table 7 and 8 for testosterone, respectively.

For further information about the individual samples see the graphs in Appendix D. As mentioned before we had expected the concentrations of the metabolites to change with the age or storage conditions of the samples but they do not show that phenomenon. The first and the last value did not differ significantly.

For the sub samples (the different time points) of each sample set of Herberstein a mean and a standard deviation (SD) were calculated with the values of Table 5 and 7 and entered into the Tables 6 and 8. That was done for the sub samples with that were frozen 15 min after defectaion as well, and for the warm and the cold stored ones from the "age" of 1 hour plus.

From the data of table 6 and 8 it is clear that the hormone concentrations did vary but there was no regularities to be seen in the patterns of change.

TD .	Cortisol Metabolites (ng /g dry feces)											
x hou	perature ırs	0,25	0,25	0,25	0,5	1	2	4	8	12	24	36
1	Cold					4,51	2,52	3,63	8,05	8,89	4,00	6,32
	Warm	3,61	5,38	1,91	1,99	1,89	1,71	1,89	2,08	2,25	1,90	1,95
2	Cold					11,76	10,63	9,24				
	Warm	3,83	7,94	10,32	11,04	9,71	10,33	8,67				
3	Cold					18,13						
	Warm	7,47	10,34	11,46	29,21							
4	Cold					13,03	15,21	13,63	19,96			
	Warm		11,09	11,19		15,13	23,60	17,00				
5	Cold					1,24	1,15	1,20	0,98	0,71	1,47	0,82
	Warm	3,24	1,46	2,04	0,76	1,70	0,98				0,81	0,73
6	Cold					6,78	6,91	7,86	8,14	7,76		
	Warm	5,48	6,26	7,27		7,84	8,64	4,18	5,07	6,66		
7	Cold					1,79	1,03		1,57	1,19	1,40	1,96
	Warm	1,62	1,65	1,49		2,29	1,62		2,99	1,85	1,20	1,93
8	Cold					7,83	4,87	8,94	2,62	1,16	0,95	2,40
	Warm	2,62	0,56	0,68			4,64		3,97	8,04	0,60	1,22

Table 5 The cortisol concentrations in fecal samples of Herberstein wolves. Samples (n=8) were stored for the given time (upper row) in either cold or room temperature (warm). After that they were frozen. There are no initial values for the cold temperature because the sub samples were prepared and stored outside of the fridge. For four of the samples it was not possible to get a value within the first 0,5h. For other samples it was not possible to get values beyond 1h because of the small amounts of material. Some values are missing or off the standard curve, these 7 of the 107 values were excluded.

Cortisol Metabolites Statistics (ng / g dry feces)									
Sample	Mean (all)	SD	Mean (beg)	SD	Mean (cold)	SD	Mean (warm)	SD	
1	3,58	±1,75	3,63	±1,16	5,42	±2,00	1,95	±0,12	
2	9,34	$\pm 1,54$	7,36	$\pm 2,36$	10,54	$\pm 0,87$	9,57	$\pm 0,60$	
3	11,85	$\pm 3,14$	9,76	±1,53	18,13	± 0	-	-	
4	15,54	$\pm 3,10$	11,14	$\pm 0,05$	15,46	$\pm 2,25$	18,58	$\pm 3,35$	
5	1,29	$\pm 0,46$	2,25	$\pm 0,66$	1,08	$\pm 0,21$	1,05	$\pm 0,32$	
6	6,83	$\pm 1,01$	6,34	$\pm 0,62$	7,49	$\pm 0,52$	6,48	$\pm 1,48$	
7	1,97	$\pm 0,67$	1,59	± 0.07	1,49	$\pm 0,28$	1,98	$\pm 0,44$	
8	3,41	±2,38	1,29	±0,89	4,11	±2,66	3,70	±0,23	

Table 6. For all time points (all) of each sample a mean and a SD were calculated for the cortisol metabolite concentrations in the Herberstein samples (left). The same was done for the first three time points (beg for beginning), for all the cold stored (cold) and all the warm stored (warm) time points of each sample.

m				Andr	ogen Mo	etabolite	es (ng/g o	dry fece	s)			
x hou	perature ırs	0,25	0,25	0,25	0,5	1	2	4	8	12	24	36
1	Cold					85,25	74,94	74,25	62,33	82,04	54,54	51,33
	Warm	60,96	61,65	35,29	58,67	52,48	64,17	52,71	62,33	68,06	54,31	46,52
2	Cold					78,75	116,5	63,5				
	Warm	95,25	86	80,75	119,75	77,5	72,25	61,5				
3	Cold											
	Warm	424,56	431,54	618,75	434,71	323,65						
4	Cold					27,74	34,43	32,76	30,37			
	Warm	75,33	58,11	58,59		42,57	40,65	88,24	30,13			
5	Cold					100,52	99,76	115,5	67,9	107,91	86,86	88,76
	Warm	97,67	89,71	85,53	49,12	123,84	107,72				103,55	92,36
6	Cold					58,14	48,71	43,61	59,71	75,43		
	Warm	61,29	95,07	38,89		49,5	68,36	48,71	87,21	45,57		
7	Cold					720,5	385,79	477,71	328,95	318,74	353,57	365,36
	Warm	553,67	703,21	541,88		619,4	588,24		451,79	563,1	502,07	441,31
8	Cold					71,82	94,47	72,79	100,78	55,49	119,71	158,85
	Warm	73,93	87,35	92,21		86,22	96,57	184,09	77,97	46,1	60,34	89,62

Table 7 The androgen metabolite concentrations in Herberstein wolf samples. Samples (n=8) were stored for the given time (upper row) in either cold or room temperature (warm). After that they were frozen. There are no initial values for the cold temperature because the samples were prepared and stored outside of the fridge. For four of the samples it was not possible to get a value at 0,5h. For other samples it was not possible to get values after 1h because of the lack of material. A few values were either missing or off the standard curve. For these reasons 2 of the 107 measurements were excluded.

			Testoste	rone (ng /	g dry feces)			
Sample	Mean (all)	SD	Mean (beg)	SD	Mean (cold)	SD	Mean (warm)	SD
1	61,21	±9,34	52,63	±11,56	69,24	±11,29	57,23	±6,54
2	85,18	±15,36	87,33	$\pm 5,\!28$	86,25	±20,17	70,42	±5,94
3	446,64	$\pm 68,84$	491,62	$\pm 84,76$	-	-	323,65	± 0
4	47,17	$\pm 16,65$	64,01	$\pm 7,55$	31,33	$\pm 2,27$	50,40	$\pm 18,92$
5	94,45	$\pm 13,45$	90,97	$\pm 4,47$	95,32	±12,12	106,87	$\pm 8,91$
6	60,02	±13,43 ±104,3	65,08	±19,99	57,12	±8,77	59,87	±14,33
7	494,71	0	599,59	$\pm 69,09$	421,52	$\pm 101,\!48$	527,65	$\pm 62,60$
8	92,25	±23,64	84,50	±7,05	96,27	±25,86	91,56	±27,87

Table 8. For all time points (all) of each sample a mean and a SD were calculated for the androgen metabolite concentration in Herberstein samples (in the left). The sam was done was done for the first three sub samples (beg for beginning), for all the cold stored (cold) and the warm stored one (warm) time points of each sample.

4. Disscussion

A) Behavioral assessments:

Behavioral data has been analyzed for three free-living wolves in Alberta, Canada. The animals were snow-tracked over a time period of two months. Aerial photos and mapping software were used to show where the wolves had been traveling. It was possible to show how long the animals stayed in a certain section of the area through the tracking information that had been collected. The collected tracking data from all sessions was broken up into 50-m sections (n=651). In these sections several parameters were looked at to get a reflection of the animals' behavior. A diet analysis was conducted to shed light on the feeding habits of the wolves and a home range size estimation was done within the study area.

It was found that several parameters found in tracks can be used to assess information about the behavior of wolves. This is information that cannot be obtained through radio telemetry or direct observation.

A lot of time was spent in an effort to obtain information on other packs. Although there were no concrete behavioral results the effort demonstrated how important it is to have a well structured matrix of small roads to assess continuous data from tracking one wolf pack. Although it was not possible to check the whole matrix every week, I found the wolf tracks on nearly every day (18 days of wolf tracking of 25 days of data collection). There was evidence that the wolves stayed 24 days in the area. On 12 of these days they were near the lake. On 28 days they were probably not in the area or not moving. Although the results of the tracking sessions in sum has been only 56 km, the data suggest that there is a lot more potential in the method of tracking. It is interesting that the wolves spent half of the time at the lake, which is rich structured with various types of vegetation. The home range size estimation supports that the wolves used that area intensively, as well as the camping ground in the west north-west of the area. The terrain there is also very suitable for moose.

A lot of the data point toward the fact that the wolves were hunting moose in the surroundings of the lake (which is moose habitat). The wolves changed from a single file travel mode into a splitting mode, especially in the areas of brush land, level ground and deep snow. They appeared to have traveled single file through the open forests and woods over the ridges of the hills, which is supported by Kunkel 2001 where they traveled in shallower snow and encountered more deer like in this study. There where the land became flat, open and brushy they trailed moose over longer distances, and I once tracked a hunting event in an open forest area, which still is suitable for moose (e.g. good for cover) but they were trailed in the woods as well. That does not mean the wolves would have neglected the deer (if we look at the results of the diet analysis the opposite is the case and they trailed deer as well but not that often as with moose) but it seems that they more often traveled on wildlife trails through the hilly areas and, if they would have seen movement, they would have rushed for it

(wolf hunting strategy while traveling; Young, per. comm.). Kunkel (2001) suggested that wolves have to surprise deer to hunt successfully and that they chase prey preferably downhill. In this study I only found evidence of trailing the deer downhill and not uphill. There were few encounters with moose while the wolves traveled on wildlife trails. They only occurred while they trailed and moved freely. In Kunkel (2000) moose kill sites were at lower elevations and hiding-cover levels was lower at kill versus control sites. That seems to be comparable with the data set presented here. In this area the wolves trailed moose in the open or brush land. One impression I had from the trailing data was that the wolves seemed to be aware of relevant hunting characteristics in the their own territory. They knew where there were areas of deep snow, spots to observe hunting grounds, locations where they could check for food. On the same spot where the wolves scavenged a dead horse I found an older horse skull, so it seems that location has been used earlier. Nonetheless, it was not hard to find the place with all the ravens and magpies near the carrion.

For some days I had the impression that the wolves were affected by a lack of nutrition. I had found no kills or feces for a long time and the feces found contained large amounts of earth and grass. On the other hand on Isle Royale, Peterson (1977) found that winter movements varied from year to year and the average travel distances between kills ranged from 18,5 to 54,1 km/kill. Actually three times I had the impression the wolves shifted their behavior from hunting to scavenging. In these cases they traveled the ridges of the hills where there was evidence of cougar presence and the wolves checked two cougar kill sites. On the other occasions the wolves were found to scavenge a dead wild horse killed by a cougar. The third was a uncertain death of a mule deer which looked like a cougar kill. They never split near or far while following cougar tracks.

Cohesion was an important behavior which could be assessed well in tracks. The wolves crossed roads more often in far split mode. In one case there appeared to be specific behaviors associated with the crossing. The wolves slowed down, walked over the road (with side steps which indicates head shift) and then sped up fast on the other side. In the split far mode they often changed speed and moved freely or they were trailing and kept moving straight. And they moved more from one vegetation type to the next, which per se may increase the chance to meet prey (edge effect). They traveled a lot more on wildlife trails and only in ten percent of the cases on the road, which should also improve chances to meet prey. This supports the thought that they are not simply traveling from one prey area to the next but are constantly on the hunt while moving.

Urine and feces are thought to be scent marks (Asa 1985) and territorial boundary markers (Peters 1975). But they do not seem to serve as boundary markers in all or many cases as described in a research proposal by Krizan (1994), where he cited a personal communication with Paquet. Paquet stated that urine is used for orientation. It seems to be interesting that the wolves urinated a lot in the presence of deer and cougar trails and in soft snow. Those three situations occur often together in the woods. They also urinated more often when joined near, which again happened in the woods more often than elsewhere. This was rare in the brush land. In the brush the wolves trailed moose, and

urination never occurred while trailing. It occurred more often when the snow-depth was low and the wolves were joined near (this was found in the woods). They never urinated in presence of horse trails. They also did not trail horses very often (and this with moose trails present) or meet them. The wolves never split far on horse trails as they did with moose. So one might assume they were not interested in horses as prey in winter.

Another impression I had was that the wolves stayed in areas with snow and tried to stay out of areas without snow (evidence for that is the snow-depth in each plot versus the observation of the melting snow). I suggest that olfactory cues are stronger on snow and do not lose the odor as fast as on bare ground. Another point is that it is easier to follow visual input of snow tracks with odor cues than to trail smell alone. The visual input allows one to increase speed (which they did in soft snow and when tracking quality was good and decreased it when it was poor) especially in the open and the woods. Smell can be blown away or vanish somewhere and they would have to keep their noses on the ground and the eyes more down than a top predator might like which slows down the more fainted the smell is or the trail gets worse to vision-track. That is supported by the fact that the animals split near as tracking quality decreased and hardness of the snow increased (which indicates worse tracking quality). The visual hunting is also supported by the fact that I found two different ridges on which the wolves laid down to observe the lake and the surrounding swamps that lay beneath them. They stayed in single file more often when the snow was soft and split as it got harder (to read) and the chance of prey encounter increased (or disturbance in the case of deer, see above). In the first week after snowfall the wolves split more as the week progressed. Thereafter, no pattern was recognizable. In the beginning of that week they would often shift from hills to flat or downhill stretches. This may mean that the wolves encountered more deer after the snowfall and that in time they then shifted to moose. After about a week, however they shifted back to deer. The explanation would be that after snow fall the snow depth is lower under trees and the snow cover is smooth. Outside of the forest, the snow stays fresh longer and may provide more information. In the forest the snow falls down from branches after some days and disrupts the surface. In Mech (1970) there are descriptions of wolves smelling moose over a kilometer in good wind in winter, but when the wind is bad they can not perceive them even a hundred meters away. That is possible in large open lands. But in well-structured habitat vision may be at least as important as is shown in this study.

On occasions where the wolves become interactive or non-interactive the surroundings were suitable: it was open or open forest with level ground. That would support the theory that hunting animals are and have to be always aware of their surroundings and are still perceptive of what is happening around them while being interactive.

The correlation of snow-depth and penetration-depth seems to be obvious, and also the correlation of snow and tracking quality with the time of the last snowfall. This also applies for tracking quality and snow quality. They match and either of the parameters can be used to predict the other with certainty. What is rather confusing is the correlation of wolf number with tracking and snow quality as well as

last snowfall. This connection infers that the worse the conditions to trail prey visually, the more often the animals split for a longer period of time like large packs do in summer (Mech 1970).

Priklonsky (1985) and Musiani (1998) stated that single wolves seem to travel slower than wolves in groups of 3 or more. I had two tracking sessions where a single wolf galloped or side trotted nearly the whole way down the roads. The road travel could be an advantage but the wolf even galloped up a slope and then along the slope through the open forest. The animal did not scent-mark at all which suggested that it may have been the yearling and that it had been separated from its parents and hence was looking for them.

It is known that juvenile animals show more exploratory behavior. With this urge it is not surprising that in nearly all cases the speed and splitting increased with the juvenile present. The urination increased when the juvenile stayed in the group. The juvenile appeared to leave (that is what I suggest) the group again when the snow got hard (after they had eaten; I suggest as well that new snow increases the hunting success within the next 7 days dramatically although I can not prove it).

0.23 to 0.37 moose/km² seem to be the low and the high in moose densities in Quebec (Messier 1985). Deer densities can be 0 to 5 to 20 to 45 deer/km² in Minnesota (Nelson 1981). It is possible that there are more than 15 times this density of deer in the study area. If one uses those indices or if I use my ratings there would be about 5 to 7 times more individual deer on the transects I made but there were only 127 plots with deer and 75 plots with moose presence which would shift the interest of the wolves onto moose.

Although the feces I collected in the field might be as little as 18% or 9% of the feces produced over a period of 4 months the data indicates that deer was the preferred prey in the diet of the wolves in winter. Moose were trailed but 8 times more deer were eaten (or probably about 7 times more, if one looks at the biomass-indices and the results in Floyd 1978) it seems to be a contradiction. But it is also known that in wolf-moose scenario on the Isle Royale, Mech (1970) found that only 6 of 131 wolf-moose contacts the wolves killed the prey (5%). That indicates 1 of 20 moose met is killed but how many do they have to trail to be able to hunt one? It seems obvious that if there are 5 to 15 times more deer abundant (and have a higher reproduction rate), and deer are more vulnerable than moose (through their more fragile body), more deer will be taken. This was found by Kunkel in their study (1999) which is indicated through the diet analysis in this study.

Actually little is known about the interactive and not-interactive behavior of the wolves (Bloch 2002). Although in this study it was possible to determine the age and gender of the individuals through tracking cues it could be a lot more difficult to do the same if there were more wolves in a pack. It helps to know the history of the pack, know how many pups there should be from a howling census in summer (Harrington 1982; Fuller 1988). Direct observations can reveal a lot of valuable information as well but in most cases is probably not as valuable as tracking in determination of age and gender.

Home range estimation by tracking will never be as accurate as it can be with telemetry. It always will be road biased and biased to the possibility to intrude into the landscape. If there is not a real extended

road matrix, tracking will not be the method you want to choose for large mammals. Although it can be useful to determine corridors the animals chose to cross a certain area.

B) Physiological assessments:

Wolf feces was collected in Alberta, Canada (in Waiparous in the field), and in Austria (in the animal park of Herberstein. They were analyzed for the hormone concentrations of cortisol and testosterone.

The results of this study suggest that the disintegration of hormones in the feces of wolves in winter is much slower than we had thought. Alternatively one might say that we were not able to measure the hormones correctly or exactly enough to get distinct differences between hormone concentrations. The field samples seem to have higher concentrations (4 to 7 times) of gonadal and arenal hormones. This is a phenomenon often seen in comparisons of field and captive data. In this case, however it could also be related to differing treatment and storage of the feces.

The procedure to extract hormones from feces used by Creel (2002) and Sands (2004) was different from the one in this study. The results can be compared relativity to each other in terms of low to high cortisol concentrations, which is about 800 to 1900 ng glucocorticoids/g dry feces for Creel's, and 500 to 2000 ng cortisol/g dry feces for Sands's. In this study I found 1 to 125 ng cortisol/g dry feces, which is incomparably low. Both of them cold-dried the feces and boiled them in ethanol, which I did not do. Sands showed that intense snowmobile activity is creating high levels of glucocortisoteroid in wolf feces (about 100% more than wolves in a control area). Creel showed that high-ranking wolves were more stressed (about 50% more) than lower ranking but that there is no difference in respect of gender. Though ungulates can have daily concentration fluctuations of a factor of five in the feces (Palme, per. comm.) it seems that this is not the case in wolves. There might be fluctuations but they must be in about 20% of a mean value for a day.

Since we know that canids have 23 hours (Palme 2001) or 20 to 28 hours (Schatz 2001) delay to show changes hormone concentrations in the feces (the gastrointestinal passage time for food) it was not possible to relate the values to the tracking data.

The values for the cortisol and testosterone concentrations of Herberstein do not show a pattern at all in terms of how long does it take at which temperature that the hormones are disintegrated and the concentrations drop. The concentrations did increase in some cases and in others they decreased only to increase again. This is a problem because we know that they are deterring over time. With this study we do not know if it is equal for the concentration values to collect a fresh feces in winter or a 3 days old feces in summer. We do not know if the values are reliable, and so we can not tell, if the feces from Canada have correct values and if so how could they be interpreted.

Because the number of the Herberstein feces is too small I was not able to examine differences based on sex, age or social status of the wolf.

5. Conclusions

The behavioral assessments of this study demonstrate the following points:

Researchers could use the results of this study to predict the occurrence of wolves with in their home range in respect of snowfall: Within the first three days (if the temperature is near zero or above possibly only 2 days) the wolves seem to be in deer habitat: on the ridges and slopes of the hills within the forest (traveling single file). From the 3rd to 7th day post snowfall, they are assumed to be trailing moose in the valley bottoms in the brushes and in the open (in areas with deeper snow; and in split mode). After that they seem to shift back to a more random prey encounter.

Wolves seem trail mainly moose in the valley bottoms in split mode, try to encounter deer while traveling over hillsides and ridges and prefer hunting per vision before smell.

Wolves seem to be cautious in the presence of cougar trails, and view this species as competitors (over deer) which is supported by their frequency of urine scent-marking, and scavenge its kills.

They do not seem to include horses or wild horses in to their prey in winter but scavenge sites of dead horses if they encounter them.

Wolf corridors can be viewed through home range size estimation but will be always road and transect biased.

The physiological assessments reveal that there is much less we know about hormones than we thought: Methods of hormone analysis should be validated, tested and reviewed as much as the disintegration-time of hormones. But if the results are correct, they suggest that wolves have higher cortisol and testosterone concentrations in nature. These concentrations deter in such a slow rate that the method of collecting feces for hormonal analysis would be feasible in summer in a country like Portugal. This would have a high impact on conservational research on large carnivores.

Further research with refined concepts is needed:

1. We need to know the range of the hormone concentrations in the wolf feces and how fast they deter. Personal communications with Dittami showed that the hormones are expected to be disintegrated within a few hours at room temperature, where as this study suggests that there is not much change in the hormone concentrations after 36 hours at room temperature. Wolf feces should be sampled as in Herberstein and a timeline should be determined for the disintegration of the hormones in the wolf feces. This time the feces should be treated as they would occur in nature, aerobe. To gain knowledge of the hormone concentration maxima in the feces wolves should be experimentally treated with ACTH (adrenocorticotrophic hormone) like in white-tailed deer (Millspaugh 2002). With this the animals would produce an intensive adrenal reaction. In that way one can be sure that the animal had been stressed. To improve the methods one should conduct a HPLC (high-performance liquid chromatography) like in the study of Wasser (2000), which is also the opinion of Palme for this case (per. comm.). With that one could determine, if there were a better hormone

- or hormone metabolite to measure stress in wolves. The levels of cortisol metabolites in the wolf feces of this study were quite low.
- 2. One need also determine which method of hormone analysis is best and most accessible for the field biologists, in order to implement a wide spread and reproducible method to assess stress levels of wolves. This would be very valuable for conservation. Wolf packs in high and in low human densities could be compared (with the same prey species present) with large scale animal counts for prey population densities. The difference between freezing the samples or storing them in methanol or ethanol should be determined. It also should be determined if the original or the dried feces are more representative and what difference there are between feces with differing percentages of hair, dirt, fat, protein and moisture. Lastly one should examine how much influence fasting has upon the hormone concentrations in the first feces after the re-feeding.
- 3. A way should be found to tell whether wolves suffer from low nutrition in the field, possibly through component analyses of feces and urine.
- 4. A study area with more frequent snowfalls and the same variation of habitats should be found. The pack should consist of at least three or better five but not much more individuals because it could be difficult to keep an eye on that many wolves, if you are alone. The manpower of the research team number should be increased from one to three. At least two people with two trucks should be present as well as a real good road matrix. The study parameters should be refined and the animals back-tracked or better fore-tracked if at least one individual of the pack is radio collared. This should be very positive for the time budget of the researchers. The road matrix should be investigated more regularly and the wolf trails should be tracked more intense, but still with the necessary respect for the animals to not urge them out of their comfort zone. The study should be conducted year round to determine if there is a way to track in summer. In my opinion one can predict the movement of the wolves better after one gets to know the home range of the wolves (best with GPS-collars and telemetry), the corridors they use for traveling, the areas they mainly use for hunting and the behavior of the individual and the pack. Possibly it can be shown how they react to the smell of there own excrements, whether they are attracted or repelled. This could indicate if they use their urine and feces for territoriality reasons only or for setting waypoints to know, if they have been there recently or not. Maybe in time research may be able to recognize the mechanisms used in orientation, navigation and hunting.
- 5. A big improvement would be an additional person who walks straight random transects at the same time through the study area as the other team members to assesses the parameters for control sections. This would reveal more details about the hunting strategies of following certain animals, about the wolves' habitat use and more about the connection of snow and tracking quality with the habitats and the usage of the wolves in respect of these.
- 6. The whole study should be conducted for several packs in different areas (flat land, hilly, mountainous) with the same spectrum of ungulate prey (which should be possible for deer and

Diplomarbeit von Wolfram Jaschke 2004:

The Use of Tracking in the Assessment of Behavioral and Physiological Parameters in Free-living Wolves (Canis lupus)

moose) to assess further information about habitat use of the wolf in connection with habitat use of the prey animals.

- 7. Urine should be included into the hormonal analysis because it is easier to access than feces and easy as well to collect frozen or mingled in the snow, respectively. But before that experiments with all the unknown methods should be conducted until they are reliable enough to use them on field data.
- 8. Diet analysis should be done as well to correlate the findings to the tracking data like in this study. So we would know how much time they spend on the heels of animals mostly and which prey is eaten finally.
- 9. The determined hormone concentrations should be related to events that were recorded in the field. In that way a continuous tracking session (one starts where he/she stopped the day before) of back or fore-tacking should give the best information about behavior and physiology. But this depends on the information about the disintegration ratio of the hormones and the outside temperature.

The methods of tracking and non-invasive hormone analysis contain a lot of future potentials especially for wildlife conservation, as well as for behavioral and physiological science.

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Diplomarbeit von Wolfram Jaschke 2004:

The Use of Tracking in the Assessment of Behavioral and Physiological Parameters in Free-living Wolves (Canis lupus)

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A naturalist's world http://www.tracknature.com/

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Das Wissen der Wildnis: http://www.wildniswissen.de/

The Shikari Tracking Guild: http://www.shikari.org/

Travel Alberta http://www1.travelalberta.com/content/weather/

Wilderness Awareness School: http://www.natureoutlet.com/

D) Personal Communications:

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Dittami, J., head of the Department of Ethology (Institute of Zoology, University of Vienna).

Palme, R., professor at the Department of Biochemistry (Veterinarian University of Vienna).

Paquet, P.C., founder of the CRWP, adjunct professor at the University of Calgary.

Peham, W., founder of the nature and wilderness school Das Wissen der Wildnis.

Young, J., founder of the Wilderness Awareness School and The Shikari Tracking Guild.

8. Appendix A: Methods of age estimation of wolf tracks in the snow

When it comes to age animal tracks several components that influence the appearance of the track need to be looked at. The tracker has to estimate the age of the track with his knowledge of weather (time and intensity of snowfalls, rain, sun shine hours per day, wind, temperature, snow depth, snow pack), with the knowledge of the condition of the surface (in this case snow) over the last few days in the sun and in the shade, on the hill side and in the valley bottom and the knowledge how tracks of known age look like on this very day (because he examined tracks of know age and his own tracks he made the days before) and with the appearance of the track itself and the surroundings of the track, in the case of snow: Is there new snow in the track, how dry was the snow when the track was made versus now and the days before? How hard is the surface and how hard are the edges, the walls and the bottom of the track and how do they look like? How big are the snow crystals in the track and out side? Where and how much are the edges and the walls molten and where are objects which could produce shade (trees, shrubs, plants, rocks, hills, mountains) around the track? Are there conifer needles or parts or debris in the track and if, where in the track? Were there new tracks made over older tracks and how do they differ? And the location of the track (elevation, valley, hill side, level of ground and the movement of the snow crystals if not on level ground. Is it on a road or wildlife trail?) and through experiment (How do his own tracks with his shoes and hands look like in the snow beside the track?) and through trailing (means following the tracks) and comparing the tracks he follows with the components mentioned above.

Since aging is one of the hardest challenges in tracking, the estimated age can only be as accurate as the tracker's tracking ability and his knowledge of weather and the area as well as his knowledge of the animals he follows.

Because aging is that complex, the following pictures of wolf prints can only be examples of how an aged track looks like. Each picture has a few descriptions which try to describe the most impacting components mentioned above. Temperature ranges are given for the lowest and the highest temperature between the event of imprint and the event of photography (pictures from personal archive):

- 1: A few hours old, no sun (clouds), -8 to 4° C, made after fresh snow (3 mm) on a small plowed road.
- 2: One or two days old, about 20 hours of sun but -22 to -5° C, on a valley bottom, light wind.
- 3: About 5 days old, more than 20 hours of sun, very molten, -2 to +13° C, pine needles in track, last snow fall before 12 days.
- 4: About 5 days old, some hours of wind, more than 20 hours of sun, -20 to 0° C, debris in the track, not around.
- 5: About a week old, long hours of wind on a small lake, not much hours of sun, 0 to $+9^{\circ}$ C, the wind blew the track out, tracks were made at about $+1^{\circ}$ C because they were made when the surface water of the lake was freezing, so it was in the night.
- 6: About a week old (known age, saw the trail 1 week before), not much wind, a lot of sun, 0 to +7° C.



9. Appendix B Home range

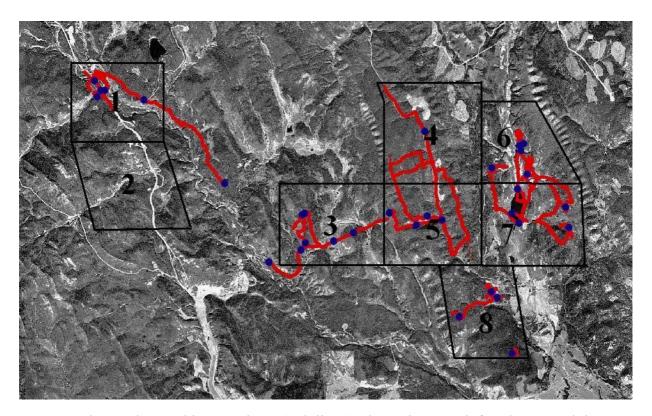


Figure B1 shows the tracking sessions (red lines), the points used for the Kernel home range estimation (blue) and the segments used for determination where and when the wolves were present (compare Appendix C).

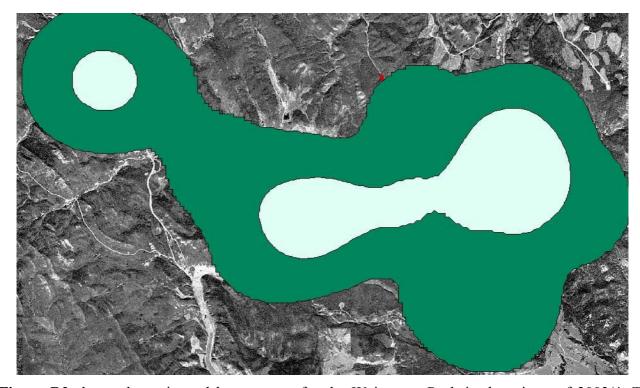


Figure B2 shows the estimated home range for the Waiparous Pack in the winter of 2003/4. This shape was produced with a Kernel home range estimation in Arcview GIS 3.2. The white areas contain 50% of the points that were chosen for the analysis, the green area contains 95% of them.

10. Appendix C: Absence and presence of the pack in the study area

In Table C1 it is shown show that I chose 8 sections (Figure B1) in the study area in which nearly all track sightings were included. It was not possible to monitor the wolves in all the segments everyday for a lack of time in a day, the lack of possibility during my presence in the other packs areas and during snowfalls and the day after. Within the mentioned 74 days I was able to find evidence that the wolves had been within all the segments on 24 days, on 16 days I was relatively sure that they were not with in the segments and on 34 days I could not tell if they had been there or not. I divided the days into 5 groups of each 15 days and found that the wolves used the lake and the area in a radius of 2 km in 4 of the 5 groups.

Session	Date	Seg 1	Seg 2	Seg 3	Seg4	Seg 5	Seg6	Seg7	Seg8	All Segments
3	13.12.2003	no	no	not	200	no	200	Mag	Mag	*****
3	13.12.2003	no	no	not	no	no	no	yes	yes	yes
	15.12.2003	no	no	not not	no	no	no	no	no	no
5	16.12.2003	no	no		no	no	no	no	no	no
4 u 6	17.12.2003	no no	no no	not not	no	no	no no	yes	no no	yes
4 4 0	18.12.2003	not	not	not	yes no	yes not	no	yes	no	yes not
	19.12.2003	not	not	not	no	not	no	no no	no	not
	20.12.2003	not	not	not	no	not	no	no	no	not
	21.12.2003	not	not	not	no	not	no	no	no	not
	22.12.2003	not	not	not	no	not	no	no	no	not
	23.12.2003	not	not	not	no	not	no	no	no	not
	24.12.2003	not	not	not	no	not	no	no	no	not
	25.12.2003	not	not	not	no	not	no	no	no	not
	26.12.2003	not	not	not	no	not	no	no	no	not
	27.12.2003	not	not	not	no	not	no	no	no	not
	28.12.2003	not	not	not	no	not	no	no	no	not
7	29.12.2003	not	not	not	no	not	no	no	yes	yes
7	30.12.2003	not	not	not	not	not	not	not	yes	yes
,	31.12.2003	not	not	not	not	not	not	not	not	not
	1.1.2004	no	no	no	not	no	no	no	not	no
	2.1.2004	no	no	no	not	no	no	no	not	no
10	3.1.2004	no	no	no	not	no	yes	yes	not	yes
9	4.1.2004	no	no	no	not	no	yes	yes	not	yes
8	5.1.2004	no	no	no	not	no	yes	yes	not	yes
	6.1.2004	no	no	no	not	no	not	not	not	not
	7.1.2004	no	no	no	not	no	not	not	not	not
11	8.1.2004	no	no	no	not	yes	not	not	not	yes
12	9.1.2004	no	no	yes	not	no	not	not	not	yes
	10.1.2004	no	no	not	not	not	not	not	not	not
	11.1.2004	no	no	not	not	not	not	not	not	not
	12.1.2004	no	no	not	not	not	not	not	not	not

Session	Date	Seg 1	Seg 2	Seg 3	Seg4	Seg 5	Seg6	Seg7	Seg8	All Segments
	13.1.2004	no	no	not	not	not	not	not	not	not
13	14.1.2004	yes	no	not	not	not	not	not	not	yes
13 u 15	15.1.2004	yes	no	not	not	not	not	not	not	yes
14,16,17	16.1.2004	yes	no	not	not	not	not	not	not	yes
	17.1.2004	no	no	not	not	not	not	not	not	not
	18.1.2004	no	no	not	not	not	not	not	not	not
	19.1.2004	no	no	not	not	not	not	not	not	not
	20.1.2004	no	no	not	not	not	not	not	not	not
	21.1.2004	no	no	not	not	not	not	not	not	not
	22.1.2004	no	no	not	not	not	not	not	not	not
	23.1.2004	no	no	not	not	not	not	not	not	not
	24.1.2004	not	not	not	not	not	not	not	not	not
	25.1.2004	not	not	not	not	not	not	not	not	not
	26.1.2004	not	not	not	not	not	not	not	not	not
	27.1.2004	not	not	not	not	not	not	not	not	not
	28.1.2004	not	not	not	not	not	not	not	not	not
	29.1.2004	not	not	not	not	not	not	not	not	not
	30.1.2004	not	not	not	not	not	not	not	not	not
19bis22	31.1.2004	not	not	no	yes	yes	no	yes	no	yes
22	1.2.2004	not	not	no	no	no	no	yes	no	yes
22	2.2.2004	not	not	no	no	no	no	yes	no	yes
	3.2.2004	not	not	no	no	no	no	no	no	no
	4.2.2004	not	not	no	no	no	no	no	no	no
	5.2.2004	not	not	no	no	no	no	no	no	no
	6.2.2004	not	not	no	no	no	no	no	no	no
	7.2.2004	not	not	no	no	no	no	no	no	no
	8.2.2004	not	not	no	no	no	no	no	no	no
	9.2.2004	not	not	no	no	no	no	no	no	no
	10.2.2004	not	not	no	no	no	no	no	no	no
	11.2.2004	not	not	no	no	no	no	no	no	no
	12.2.2004	not	not	no	no	no	no	no	no	no
23	13.2.2004	not	not	no	no	no	no	yes	no	yes
24	14.2.2004	not	not	no	no	no	no	yes	no	yes
25	15.2.2004	not	not	no	yes	yes	no	no	no	yes
24	16.2.2004	not	not	no	no	no	no	yes	no	yes
	17.2.2004	not	not	no	no	no	no	no	no	no
	18.2.2004	not	not	no	no	no	no	no	no	no
26	19.2.2004	not	not	yes	not	no	not	not	no	yes
26	20.2.2004	not	not	yes	not	no	not	not	no	yes
27	21.2.2004	not	not	yes	not	no	not	not	no	yes
28 u 29	22.2.2004	not	not	not	not	yes	not	not	yes	yes
	23.2.2004	not	not	not	not	no	not	not	no	not
	24.2.2004	not	not	not	not	no	not	not	no	not

Diplomarbeit von Wolfram Jaschke 2004:

The Use of Tracking in the Assessment of Behavioral and Physiological Parameters in Free-living Wolves (Canis lupus)

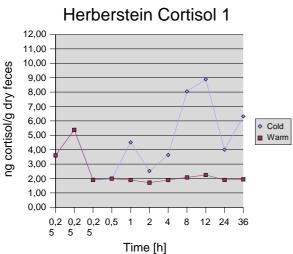
24 sessions	74 days	3 yes	0 yes	4 yes	3 yes	5 yes	3 yes	12 yes	4 yes	24 yes
		25 no	28 no	27 no	33 no	34 no	38 no	29 no	39 no	16 no
		46 not	46 not	43 not	38 not	35 not	33 not	43 not	31 not	34 not

Table C1. In this figure I listed the absence and presence of the wolf pack with in 74 days. "Seg" means segment. "Yes" means that there had been wolves in that segment on that day, "no" means that I was sure that they were not there on that day due to transects without wolf track sightings, and not means that I was not able to determine if they had been in that segment on that day. The most right column represents all segments together and I used a "yes" if there was a wolf track sighting that day, "no" if there was not, and "not" if I could not tell (which is the case if there are 4 or more "not" in a row.

11. Appendix D: Hormone levels in the wolves of the Herberstein animal park

The following figures show the hormone concentrations of cortisol and testosterone found in the wolves of Herberstein, Austria. We expected the hormone levels to drop because the anaerobe bacteria are disrupting the structure of the hormones, but they did not drop over time at room temperature or in the fridge, respectively.

On the x-axis one finds the hours that past since the feces had been deposited. The value on the y-axis is given in ng of the specific hormone per gram of dry feces. Each of the Figures D1 to D16 has a warm and a cold line. The warm line (dark) stands for the values at room temperature, the cold ones (light) for the ones at fridge temperature (compare Methods). The lines are broken sometimes due to the lack of data for 12 values and due to the fact that in 4 of the 8 cases it was not possible to take a sample at 0,5 hour after deposition. Compare the lines with the values shown in the Tables 4 and 5.



0.00 0,2 0,2 0,2 0,5 2 8 12 24 36 5 Time [h]

12,00

11,00

10,00

9,00

8.00

7.00

6,00

5,00

4,00

3,00

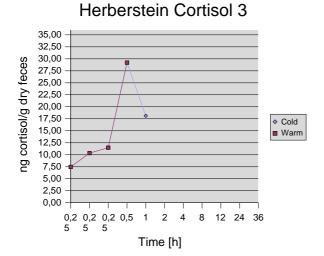
2,00

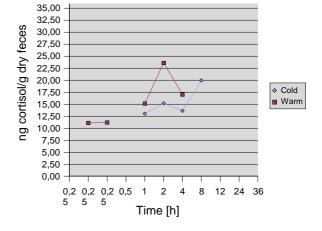
1.00

ng cortisol/g dry feces

Figure D1. Cortisol concentration of sample 1.

Figure D2. Cortisol concentration of sample 2.





Herberstein Cortisol 4

Herberstein Cortisol 2

Figure D3. Cortisol concentration of sample 3.

Figure D4. Cortisol concentration of sample 4.

♦ Cold

■ Warm

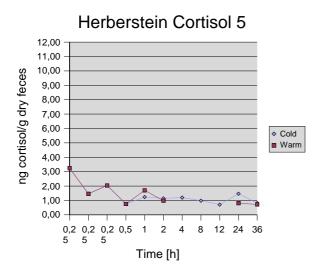


Figure D5. Cortisol concentration of sample 5.

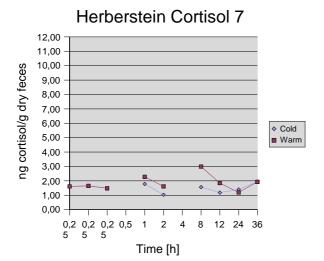


Figure D7. Cortisol concentration of sample 7.

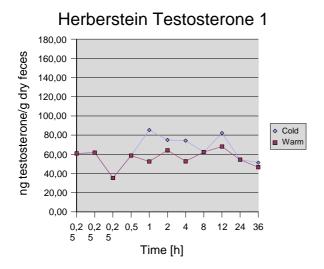


Figure D9. Testosterone concentration of sample 1.

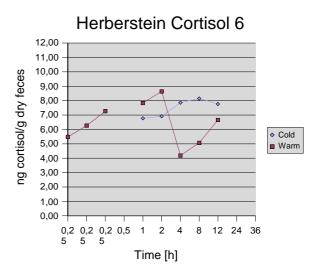


Figure D6. Cortisol concentration of sample 6.

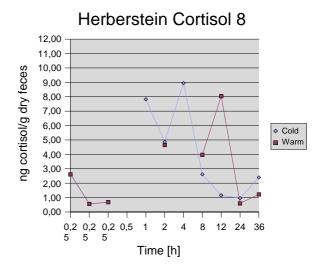


Figure D8. Cortisol concentration of sample 8.

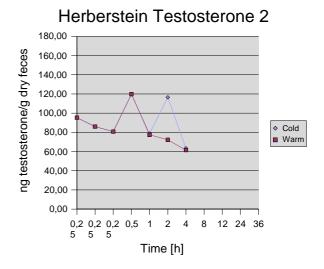


Figure D10. Testosterone concentration of sample 2.

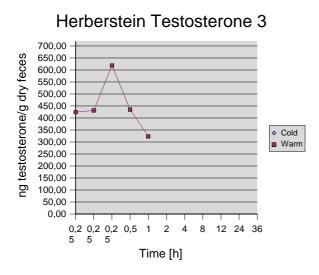


Figure D11. Testosterone concentration of sample 3.

Herberstein Testosterone 4 180,00 ng testosterone/g dry feces 160,00 140,00 120,00 100,00 ♦ Cold 80,00 60,00 40,00 20,00 0,00 0,2 0,2 0,2 0,5 8 12 24 36 5 5 5 Time [h]

Figure D12. Testosterone concentration of sample 4.

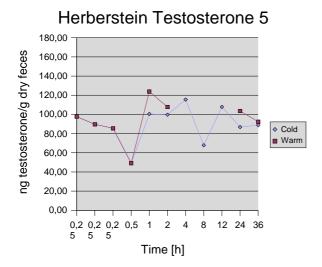


Figure D13. Testosterone concentration of sample 5.

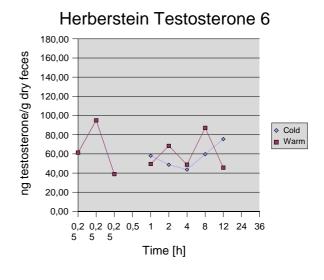


Figure D14. Testosterone concentration of sample 6.

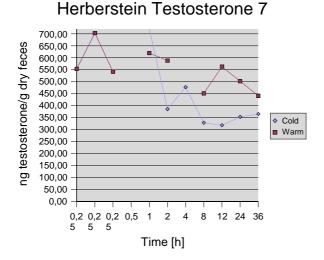


Figure D15. Testosterone concentration of sample 7.

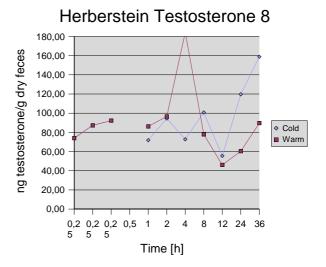


Figure D16. Testosterone concentration of sample 8.

12. Appendix E: Data collection in a foreign land – what you need to do

If you want to go to do research in a foreign country you have to think about several things, and they are getting more important, if you wish to study or conduct your field season(s) there.

The following steps are not very distinct from each other and might flow one into the other, but might be helpful as a guide to get everything sorted out.

a) First of all make a concept and a time plan of what you want to do in this land:

- What do you want to do? Which subject(s)/animal(s) do you want to study? Where do you want and where can you conduct my study/studies? What requirements have to be met so the study can be planed in what kind of terrain?
- How long does it take and how long do you want to stay? Do you need a visa for that time, where do you get that from (where is the Embassy in your country) and how long does it take to get it and what do you need for that?
- Whom do you want to work with? In most cases you need to have contact with a scientific group/organization (e.g. a university) or with a governmental department/organization: Where can you make contact with one of these, and do you need a letter of acknowledgment from them for the visa, your university and/or your sponsors/sponsoring departments?
- How much money will it cost and where could you get that from? Do you need a truck, an assistant, a room to stay a computer and/or laboratory space to work at? In most cases there are departments of the university or the government of your country who will agree to give you a stipend for your project: Where can you find them and what do you need to get the money from them? In most cases the people you are going to work with can supply you with goods, staff members or places to stay or work at: Whom do you have to ask for what and do they want money for it?

If you now know what you want to do, where and how long you can to stay, with whom you are going to work with, how much it will cost and where you will get the money from you can start with the next step.

b) Second phase: Preparations

- What kind of gear do you need and are you able to handle it? Do you need special skills to conduct your study, where and who long do you need to be trained?
- If you are working as a field biologist you are going to need a drivers license, an international drivers license and a criminal record check in case you are going to drive a truck from the

government. But what ever you think you are going to need, take those three with you That will save you a lot of troubles.

- If you can, test <u>all</u> your methods in your home country and be sure you can handle everything with ease and reproducibility, or plan to have enough time in the foreign country for that. Sometimes you might find that you do not know enough about a method or that there is not enough information available. Test them especially if they are not common methods everybody in the field is familiar with. Test them even if you think they will work. And be sure you have enough literature to support every single step you want to make. A real good concept with a really good literature support takes a lot of time but that is the case for visa and for sponsors in most cases. You can combine that.
- Make a second and very detailed concept with a good literature background after you have tested all your methods to the extend. This will save you a lot of trouble and time because in your home country you almost always have the better facilities or a better access to them then in the foreign country.
- Be aware that you can not transport every gear you need to into and out of the foreign country: This concerns laboratory liquids and personal food. Be also aware that if you want to send material per mail you must not send bigger gear than a certain length (e.g. skis have to be send per cargo and fetch from the airport). So make sure which gear you can transport how and which you want to buy in the foreign country and how you have to transport it all (e.g. methanol has to be transported with a separate dangerous goods cargo from Canada to Austria). The more you know about all these things the easier your project can be conducted.
- Make sure that the airport from which you want to leave the country again is the nearest to the last town or city you are in. As you are leaving the field for your home country again, there can be nothing more nerve-racking that going onto a hour-long bus ride to your airport.

List of contacts:

Austria	For financial support	Institute for Studies Grant Board (Studienbeihilfenstelle) and the Institute of International Relations in Vienna
	For import of animal parts	Ministry for Women and Health and the Airport Veterinarian in Vienna
Canada	For contact	The nearest University to your study area and the Department for Fish and Wildlife (Government)
	For export of animal parts	Federal Government with the CITES export permit and the Department for Fish and Wildlife
	For export rules	Airport security of the accordant airport
Further	Further information	Contact myself over the Department of Ethology (Institute of Zoology, University of Vienna)

13. Appendix F: Lebenslauf

Wolfram Jaschke, geboren am 31. Januar 1980 in Innsbruck.

1986 - 1990	Besuch der Volkschule in Innsbruck							
1990 - 1998	Besuch des Akademischen Gymnasiums Innsbruck, Abschluss mit AHS-Matura							
1998 - 2001	Erster Abschnitt in Biologie an der Universität Innsbruck, Abschluss mit erster Diplomprüfung							
2001 - 2003	Zweiter Abschnitt in Biologie (Zoologie, Wahlfach Anthropologie) an der Universität Wien							
2002	im September, 1 Monat Volontärsarbeit bei der Wolfszählung in Portugal							
	im Oktober, Besuch des 2. Internationalen Kanidenkongresses in Köln, Deutschland							
	im Winter, 3 Wochen Beobachtung von Wölfen im Schönbrunner Tiergarten im Rahmen einer universitären Veranstaltung							
2003	im Februar, 1 Woche Spurenlesekurs (Schwerpunkt: Wölfe) in Estland							
	Anfang des Sommersemester, Start der Diplomarbeit bei Prof. Dr. John Dittami							
	im August, 2 Wochen Spurenlesekurs (Schwerpunkt: Wölfe) in Idaho, USA							
	im September, Besuch des 2. Internationalen Weltwolfskongresses in Banff, Alberta, Kanada							
2003 - 2004	im Winter, Datensammlung für die Diplomarbeit in Alberta, Kanada							
2004	in Frühling und Sommer, Hormon- und Datenanalysen in Wien							
	im Mai, Vortrag zum Thema Wölfe (im Allgemeinen) in Kirchbichl vor den Jagdaufsehern des Bezirks Kufstein							
	im September, Abschluss des Biologiestudiums (Zoologie) mit der zweiten Diplomprüfung							