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The Effect of Human Activity on the Welfare of the African Elephant (*Loxodonta africana*) in Namibia

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Preface

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Abstract

The African elephant (*Loxodonta africana*) is vital in several African ecosystems, accentuating the importance of conserving them. However, conservation efforts are constantly complicated due to human population growth. Anthropogenic disturbances has been linked with elevated stress levels in animals, which in turn is associated with decreased welfare and fitness. The present study seeks to replicate a previously conducted study of elephants in Tanzania, with the goal of evaluating the current Namibian elephant population and increasing the knowledge of what anthropogenic disturbances are affecting, if at all, the welfare of elephants. The study was done as a comparative study between areas with high (outside protected area) and low (inside protected area) human interference, using the non-invasive method of measuring the concentration of faecal glucocorticoid metabolites as a measure of stress. During 32 days in the field, a total of 90 dung samples were collected and analysed. The results supported the hypothesis with a significantly higher stress level recorded outside the protected national park, Etosha (ENP), compared to inside ENP. Further statistical analyses showed that the only variable explaining this variation significantly was area (inside vs. outside). The findings suggest that anthropogenic disturbances are a contributing factor, elevating stress levels in elephants residing in non-protected areas, potentially affecting their welfare. Human-elephant conflicts (HEC) seem to be an important underlying cause, hence emphasizing the importance of seeking to prevent and minimize HEC in future conservation work. The low physiological stress levels measured inside ENP further demonstrate the importance of protected areas for conservation purposes.

Abstrakt

Den afrikanske elefanten (*Loxodonta africana*) er sentral i flere afrikanske økosystem, noe som er med på å understreke viktigheten av å bevare den. Bevaringsarbeid kompliseres imidlertid stadig på grunn av menneskelig befolkningsvekst. Menneskeskapte forstyrrelser har blitt assosiert med økt stressnivå hos dyr, noe som videre er assosiert med nedsatt velferd og fitness. Denne studien ønsker å reprodusere en tidligere gjennomført studie på elefanter i Tanzania for å evaluere Namibias elefantpopulasjon og for å øke kunnskapen om hvilke antropogene forstyrrelser som påvirker velferd hos elefanter. Studien ble gjennomført som et komparativt studie mellom områder med høy (utenfor beskyttet område) og lav (innenfor beskyttet område) menneskelige påvirkning. Dette ved hjelp av å måle glukokortikoid-metabolitter i avføring som et mål på elefantenes stressnivå. I løpet av 32 dager i felten ble totalt 90 prøver samlet inn og analysert. Resultatene støttet hypotesen med et signifikant høyere stressnivå hos elefanter utenfor den beskyttede nasjonalparken, Etosha (ENP), i forhold til innsiden av ENP. Ytterligere statistiske analyser viste at den eneste variabelen som forklarte denne variasjonen signifikant, var område (innside kontra utside). Resultatene tyder på at menneskelige forstyrrelser er med på å øke stressnivåene hos elefanter utenfor beskyttede områder og dermed også potensielt påvirker deres velferd. Konflikter mellom elefanter og mennesker (HEC) ser ut til å være en viktig underliggende årsak. Dette understreker viktigheten av å jobbe for å hindre og minimere HEC i framtidig bevaringsarbeid. De lave stressnivåene målt på innsiden av ENP demonstrerer videre hvor viktig beskyttede områder er i arbeidet med å bevare dyreliv.

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1 Introduction

The conservation of biodiversity is constantly facing increased challenges as the world human population continues to grow, demanding more space and resources, causing habitat destruction and fragmentation. As human activity intensifies, so does the interaction with wildlife, typically increasing competition and conflicts. Hence, the importance of understanding how and to what extent this affects wildlife is steadily increasing.

Historically, humans have recognized the need to protect both nature and wildlife from the detrimental effects inflicted by themselves. During the last century this has resulted in an increasing tendency of the establishment of various types of protected areas in an attempt to minimize anthropogenic disturbances and provide a refuge for wildlife. However, protected areas are often too small to cater an elephant's demand of a large home range, inevitably compelling elephants to utilize human dominated landscapes. The edges of reserves are consistently prone to conflicts due to the proximity of an extensive wildlife, increasing the tendency of property damage incidents, livestock depredation, disease transmission etc. In addition, converting areas for conservation measures typically mean evicting humans from their previous settlements and denying them access to previously accessible resources. This has consequently led to increasing negative attitudes towards wildlife (Sarker 2010).

Frid and Dill (2002) looked at various research investigating the impact of anthropogenic disturbances on animal behaviour and found that these disturbances are often managed the same way as the risk of getting predated. The disturbance is perceived as a threat and the animal consequently responds by trying to alter its behaviour in order to manage the situation. However, in some circumstances altering the behaviour is not possible or enough, prolonging the following stress response mechanism, thus resulting in chronic stress. Chronic stress is associated with a range of potential negative consequences to an animal such as changes in immune competence, reproduction, metabolism and behaviour

(Cabezas et al. 2007; Engelmann et al. 2004; Jachowski et al. 2013a; Jachowski et al. 2012; Jachowski et al. 2013b; Korte et al. 2005; Millspaugh & Washburn 2004; Moberg & Mench 2000; Munck et al. 1984; Romero 2004; Von Holst 1998), hence contributing to the lowering of its welfare and fitness.

A significant factor complicating today's efforts of conserving the African elephant (*Loxodonta africana*) is the consistent on-going Human-elephant conflicts (HEC). The International Union for Conservation of Nature – African Elephant Specialist Group (AfESG) defines HEC as 'Any human elephant interaction which results in negative effects on human social, economic, or cultural life, on elephant conservation or on the environment'. HECs typically cause negative human attitudes towards elephants and have the potential to affect the welfare and fitness of an animal, both impeding conservational efforts (Sarker 2010). As a part of HEC, illegal hunting for ivory and meat causes the most detrimental effect on elephants in Africa. It has also been shown that the risk of getting hunted is related to elevated stress levels in elephants (Burke et al. 2008). Due to high poverty and the potential economic benefits, poaching is, despite the potential costs of being caught, a big problem throughout Africa. In Namibia however, the "Monitoring the Illegal Killing of Elephants" (MIKE), has counted relatively small numbers of illegally killed elephants in recent years (CITES 2013). According to MET (2009), the main sources of HEC in Namibia is competition over water resources in the north-west and crop-raiding in the north-east. CITES (2000) also reported an increase in elephants recorded as wounded, indicating an increase in reluctance towards elephants where people tended to be more inclined to resorting to extreme measures in order to deter elephants. HEC is considered one of five priority issues when it comes to the conservation of the African elephant, and it is therefore desirable to develop measures in order to improve co-existence between humans and elephants.

The level of human interference has also been shown to affect the distribution of elephants (Chase & Griffin 2009; Graham et al. 2009; Wittemyer et al. 2007). Barnes (1983) found that there was higher density of elephants inside the national park of Ruaha-Rungwa, and speculated that this might be due to the high density of humans surrounding the park, indicating that the elephants try to avoid coming into contact with humans. Graham et al. (2009) and Wittemyer et al. (2007) monitored collared elephants and found that they preferred areas with low human activity. Additionally, several studies have found evidence that elephants tend to find mechanisms to avoid contact with humans, such as using the cover of darkness when moving into areas to raid crops as well as varying their speed of movement relative to the risk of coming into contact with humans (Douglas-Hamilton et al. 2005;

Galanti et al. 2006; Graham et al. 2009; Sitati et al. 2003; Wittemyer et al. 2007).

Researching the effect of anthropogenic disturbances on the welfare of animals has progressively been recognized as an important element in conservation biology. However, observing an actual effect on fitness from anthropogenic disturbances in such a long-lived and slowly reproducing species as the elephant is difficult. The potential of temporally linking the disturbance to a physiological effect, such as elevated stress levels, is therefore highly valuable.

1.1   Animal Welfare and Stress

Animals are adapted to their habitats and predictable situations in everyday life through physiological, morphological and behavioural alterations. When exposed to unpredictable situations the endocrine and metabolic status of the animal may change, which in turn can affect the animals welfare (Möstl & Palme 2002). The definition of welfare is highly debated and is not necessarily easily implemented in research. However, a potential indicator of welfare is the absence or presence of stress in the animal (Broom & Johnson 1993).

Stress can be defined as a symptom of an animal being exposed to a hostile environment. This stimulus is called a stressor and can lead to a displaced homeostasis. The corresponding defence reaction is called a stress response. A stress response triggers a range of complex reactions in the nervous system, in which glucocorticoids (GC) and catecholamine hormones, such as adrenaline, are secreted by the adrenal glands. These hormones cause an increase in heart rate and energy level and allow the organism to mobilize all its resources in order to cope with the situation, hence potentially improving its chances of survival in a fight or flight-situation. Everything that is not essential for survival, such as digestion, growth and reproduction, is shut off (Möstl & Palme 2002; Von Holst 1998).

A stress response serves to re-establish homeostasis (Bomholt et al. 2004; De Kloet et al. 1998; Engelmann et al. 2004; O'Connor et al. 2000), and is adaptive in short-term, acute stress situations. However, a stress response can become highly maladaptive in long-term, chronic stress situations. During short-term stress, the feedback mechanism needed in order to restore homeostasis, operates efficiently and the homeostasis rapidly returns to normal. During long-term stress on the other hand, feedback signals are weak and the system remains activated for longer periods (Romero 2004), consequently leading to a wide range of potentially negative consequences to the animal. Typical consequences related to chronic

stress and prolonged periods of high cortisol concentrations are disruptive effects on behaviour, physiology and the immune system, potentially affecting an animal's fitness, population performance and resistance to diseases (Engelmann et al. 2004; Korte et al. 2005; Millspaugh & Washburn 2004; Munck et al. 1984; O'Connor et al. 2000; Romero 2004; Seeley et al. 2007; Von Holst 1998)

Stress is not inherently bad however. GCs are also released as a response to situations that are not necessarily considered stressful, such as courtship, copulation and when hunting etc. (Broom & Johnson 1993).

1.2 Physiological Steps of a Stress Response

During a physiological stress response (illustrated in Figure 1), the sympathetic nervous system is stimulated, and the hypothalamic–pituitary–adrenal (HPA) system is activated (Sapolsky et al. 2000). The central and autonomic nervous system stimulates the secretion of corticotrophin-releasing hormones (CRH) from the hypothalamus, where it is passed through the hypothalamic-hypophyseal portal system to the anterior pituitary gland. This triggers synthesis, cleavage and secretion of adrenocorticotropic hormone (ACTH) (De Kloet et al. 1998). ACTH travels through the blood stream and further stimulates the adrenal cortex to release glucocorticoids (O'Connor et al. 2000; Seeley et al. 2007). GCs are then secreted to the liver where it is metabolized and further excreted either through the kidneys as urine, or as faeces via the bile into the gut where it undergoes bacterial metabolism (Palme 2005).

During stress, GC concentration will exceed the basal level (Seeley et al. 2007) and reach its highest peak in the blood about 15-30 minutes after being exposed to a stressor (De Kloet et al. 2005; De Kloet et al. 1998). In order to regain homeostasis, a negative feedback mechanism will secrete cortisol, inhibiting the hypothalamus and anterior pituitary gland, reducing ACTH secretion, hence inhibiting further HPA activation. During an acute stressor this mechanism is relatively quick, and the stress-induced activation of the system is terminated, hence the GC concentration return to its basal level again within 60-90 minutes (De Kloet et al. 2005). During chronic stress the feedback signals are weakened and the level of ACTH and GC remains elevated, allowing those systems activated by acute stressors to continue for extended periods, consequently disrupting homeostasis (Mendoza et al. 2000; Seeley et al. 2007).
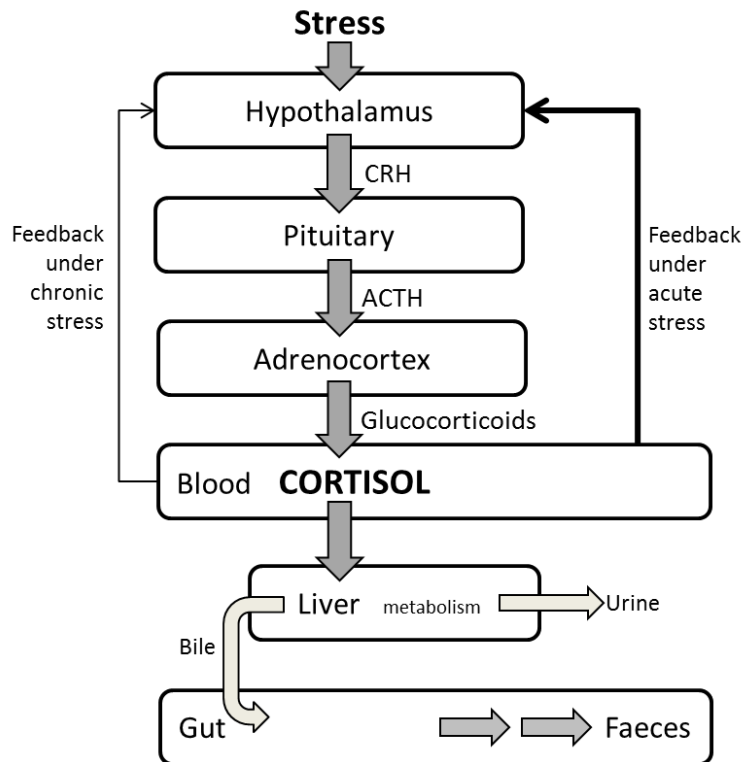
Figure 1: Illustration of the physiological mechanisms during a stress response; from the exposure of a stressor to the excretion of glucocorticoid metabolites through the faeces. The feedback arrows demonstrate how strong the feedback signal is under acute and chronic stress - Thick arrow indicating strong feedback and thin arrow indicating weak feedback. Based on Möstl and Palme (2002) and Boonstra et al. (1998).

1.3   Sampling Methods

The effects of a stressor can be observed by looking at behavioural alterations, though this tends to be more subjective and hence allow room for misinterpretation (Rushen 2000; Weary et al. 2006). Measuring the actual physiological stress response is therefore a more accurate assessment method. Commonly, the secretion of the hormone glucocorticoid (i.e., cortisol and corticosterone) is used as a measure of stress (Palme 2005; Wasser et al. 2000; Wingfield et al. 1994).

Taking blood samples as a way of measuring stress level in wild life has long been the most common method of assessing this kind of data. There are several advantages using this method; one being that it allows for a simultaneous collection of a range of different blood components, in which one can make a more comprehensive evaluation of the overall state of

the animal (e.g. Clinchy et al. 2004; Sheriff et al. 2010, 2011; Trumble & Castellini 2002). However, blood samples only measure hormone concentration at a single point in time, meaning that stochastic events that occur in temporal proximity to the blood sampling may affect the results, making it unrepresentative when measuring long-term stress level. Blood sampling in it self often requires handling or confinement of the animal, potentially initiating a stress response. Hence, the final observed stress levels are likely to reflect the stress induced by the sampling procedure itself, affecting a "true baseline" stress profile (Von Holst 1998). Due to the invasive nature of blood sampling the method is not always easily implemented in the field and in many cases it is less safe for the researcher. This is especially true for the handling of elephants. Choosing a non-invasive method, both for the sake of the animal and the researcher is therefore in many cases desired.

Several alternating techniques have been developed, many of which are gaining popularity in wildlife research; e.g. determining corticoid (metabolite) concentration in urine and/or faeces (Beerda et al. 1996; Hay & Mormede 1998; Morrow et al. 2002; Rehnus et al. 2009; Sheriff et al. 2009; Tingvold et al. 2013), saliva (Beerda et al. 1996; Cooper et al. 1989) and milk (Verkerk et al. 1998), or measuring GC concentration in hair and feathers (Bortolotti et al. 2009; Van den hauwe et al. 2005). Which method is best suited depends on the nature of the study and the species being studied.

Faecal sampling offers the advantage that it is non-invasive, preventing biases induced by increases in GC due to handling the animal. Furthermore, faecal glucocorticoid metabolite (FGM) levels reflect an integrated level of GCs over a species-specific time period (depending on defecation frequency), rather than a certain point in time. Hence, FGM levels are not susceptible to possible short-term fluctuations in GC level due to normal pulsatile secretion of GC. It therefore most likely reflects a more accurate assessment of chronic stress (Harper & Austad 2000; Millspaugh & Washburn 2004), hence providing a more compound picture of the animals' overall welfare.

In elephants, FGM concentration reflects the average stress level from the last two to three days. Radio metabolism studies have found that the maximum peak of metabolite excretion is correlated with the digestive transit time (Palme et al. 1996). In elephants this delay has been measured to range between 30-50 hours (Ganswindt et al. 2003; Wasser et al. 2000). Several conducted ACTH-tests have demonstrated that the changes observed in FGM concentration are correlated to the respective changes of steroid concentration in the blood, both in elephants (Foley et al. 2001; Wasser et al. 2000) and other species (Creel et al. 2002; Huber et al. 2003).

However, there are several factors that may affect the general potential of using GC concentration as a measure of stress. Such factors include seasonal and daily fluctuations in glucocorticoid excretion (Creel et al. 2002; Von Holst 1998), reproductive status (Gobush et al. 2008; Rasmussen et al. 2008), diet (Millspaugh & Washburn 2004; Woolley et al. 2009), gender (Huber et al. 2003; Touma et al. 2003) and food availability (Huber et al. 2003; Viljoen et al. 2008). Studies have also shown that environmental conditions (Rehnus et al. 2009; Washburn & Millspaugh 2002) and age of the sample (Lexen et al. 2008; Möstl et al. 1999) might influence FGM concentration. Hence, all of these factors should be taken into account when working with stress and GC analyses (Millspaugh & Washburn 2004).

1.4　Aim of Study

This study was conducted on request from the Ministry of Environment and Tourism in Namibia in response to a similar study conducted in Tanzania by Tingvold et al. (2013), which gained a great deal of attention when published. Tingvold found that elephants in Tanzania had a higher level of stress hormones outside the Serengeti national park compared to the inside, indicating lower welfare and a range of possible negative consequences to the elephants, potentially contributing to lower fitness. The elevated stress level was found to correlate with the level of human interference. Investigating stress related to human disturbance in Namibia is especially interesting considering that it is a country with far lower human population density and elephant poaching incidents compared to Tanzania.

The study was done as a comparative study between ENP and carefully selected areas characterized by human interference, measuring stress level as an indicator of welfare, using the non-invasive method of measuring FGM. The aim of this study was to evaluate how interactions with humans and rural communities might affect stress levels in the African elephant in Namibia. Acquiring this knowledge is beneficial in assessing whether or not the present conservational methods are in fact effective or if they might need to be changed. It can provide valuable information and clues to what human induced factors are causing and/or contributing to these elevated stress levels and hence give valuable key information in the development and improvement of today's conservation strategies for elephants all over Africa.

The hypothesis tested is that elephants living in an area with high probability of coming into confrontational contact with humans sustain higher levels of physiological stress compared to elephants living in areas with low human interference. An inherent assumption

is that high physiological stress indicates a negative physiological condition and thus also lowered welfare and potentially lowered fitness for the elephant.

2   Materials and Methods

The fieldwork was conducted in the period May to August 2014, and was done as a collaboration study between two master students from NTNU (Iris Ringstad and Louis Hunninck).

During ten weeks in Namibia, a total of 32 days were spent in the field looking for and collecting dung samples from elephants. During this period, there were approximately 260 unique elephant sightings. Of 32 field days, 14 days were spent inside ENP, where a total of 56 dung samples were successfully collected. The total number of unique elephant sightings inside ENP was $\geq$ 194; hence approximately 29% of the elephants observed were sampled. No field days were spent without any collected samples inside the park.

Outside ENP, 18 days were spent in the field and a total of 39 dung samples were collected. During the fieldwork four duplicates were discovered, which left a final count of 35 unique samples. The total number of unique elephant sightings during this period was $\geq$ 65 individuals, hence dung samples from approximately 54% of the elephants seen outside ENP was collected. On six of the field days outside ENP, no samples were collected.

A total of 95 dung samples were successfully collected and analysed during the fieldwork (see Appendix I for further details). As mentioned, four discovered duplicates were removed. An additional individual was removed due to its obviously bad physical state and its corresponding high FGM concentration (see section 3.1). Thus, a total of 90 samples (geographical distribution illustrated in Figure 2) were used in the statistical analyses of which 39 were males, 44 were females, and 7 were not sexed. When comparing stress level between females and males, the seven samples where sex was not known, were removed.

2.1   Study Area

Namibia covers a land area of 825 615 km^2, and has a population of just over 2.1 million people (UNFPA 2011). The study area in Namibia included three areas inside national parks, Etosha (ENP), as well as four areas in the Kunene region, outside ENP.

ENP is located in the north-western part of Namibia and covers 22,750 km^2. The park is home to more than 100 mammal and 300 bird species, and is famous for its huge dry salt lake called the Pan, which covers around 4800 km^2. This lake will only fill with water after severe rains during the rainy season, usually from January to March. Once filled up, it usually only stay wet for a few days. Inside the lake there is almost no vegetation, while the near surroundings consist mostly of grasslands. Most of the park however, consists of dry savannah and woodland. The park boundaries around ENP are fully fenced, making migration between the park and the surroundings close to impossible. On occasions though, elephants break down the surrounding fences, allowing for some migration to happen. Within the park there are fenced in areas where tourists and park staff live and traffic in and out of the park is regulated. Hence, human interference is low and poaching is rare. According to CITES, no elephants have been hunted illegally within ENP for three decades (CITES 2000, 2013).

Inside ENP, three study areas were selected in order to control for any possible effect of precipitation on stress level; Otjovasandu in the west, Okaukuejo in the central part and Namutoni in the east (see Figure 2). Mean annual precipitation vary from 350mm in the western region to 500mm in the eastern region. The four areas in the Kunene region are located on the western and southern side of ENP (see Figure 2) and were chosen due to evident human activity. The areas included; the edges of Khorixas, an area dominated by livestock rearing activities; the Ugab river catchment area, an ephemeral river characterized by both livestock and tourism activity; the Hoanib river catchment area, an ephemeral river system with potential sources of disturbance from livestock farming and tourists; Purros, where the main forms of land use is livestock farming, followed by tourism activity.

The study was conducted during the dry season as previous studies have shown that the lack of rain makes the elephants reduce their home ranges, typically residing closer to ephemeral rivers and/or artificial water points where they can more easily find food and water (Chase & Griffin 2009; Leggett 2006). Thus, locating elephants are easier. During the rainy season elephants tend to disperse more, utilizing bigger ranges.
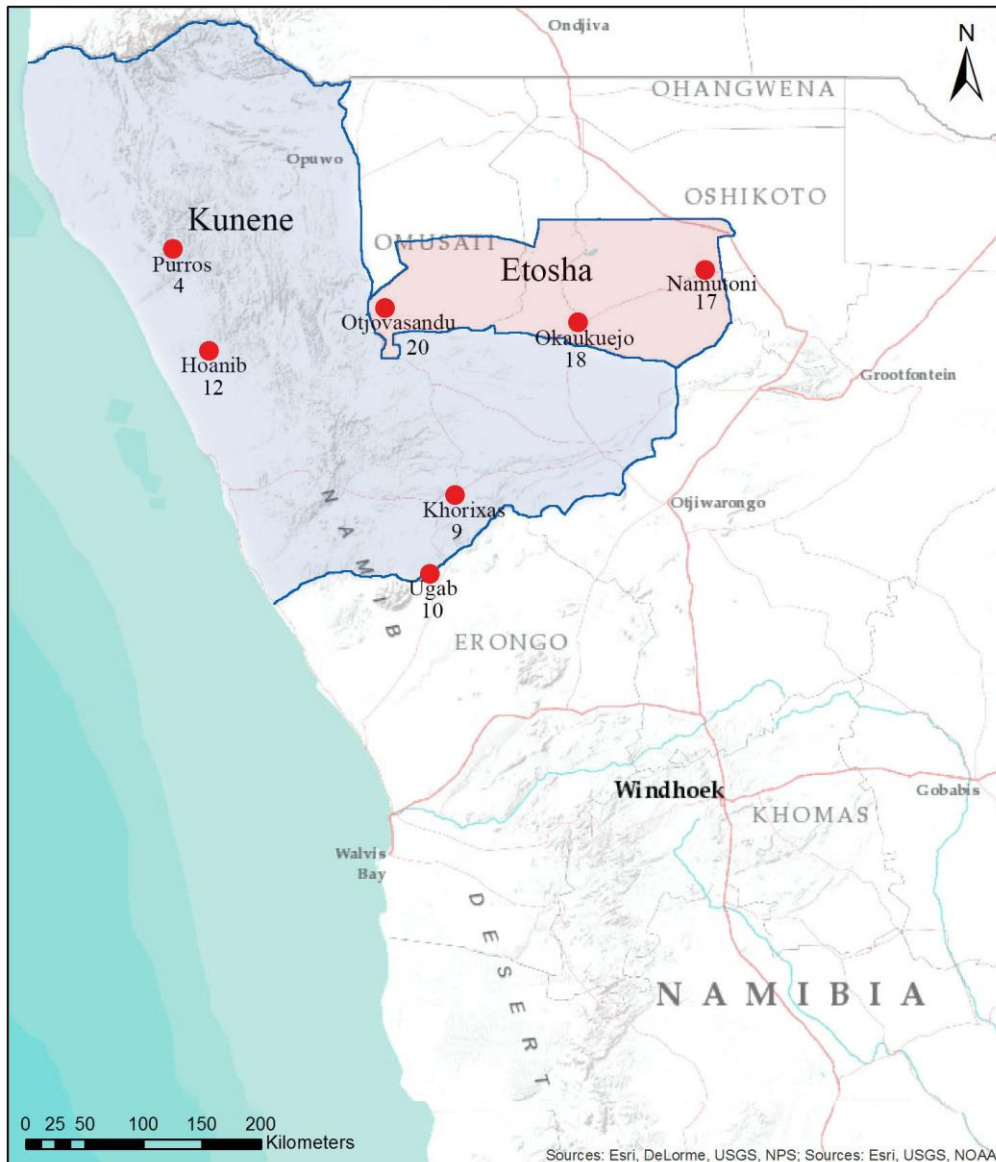
Figure 2: Map showing the location of all seven areas selected for the field work in Namibia; Inside Etosha National Park (ENP): Otjovasandu, Okaukuejo and Namutoni; Outside ENP, in the Kunene region: Purros, Hoanib, Khorixas and Ugab. Numbers indicate the number of samples collected in the respective areas.

2.2 Study Species

The African elephant is the world's biggest terrestrial animal alive today, weighing up to 7,500 kg and reaching as high as 4 meter tall at the shoulders (Houck et al. 2001). The elephants that roam across the Namibian desert landscape have adapted to the arid conditions and typically grow taller than other populations of African elephants. Due to mineral deficiencies they also tend to have shorter tusks. In Namibia the elephants only inhabit the

northern region of the country, ranging over an area of 146 900 km^2, 18% of Namibia's total land coverage (Blanc et al. 2007). Today they are only known to come as far south as the Ugab River. In 2012, the number of elephants in Namibia was estimated to be just over 20,000. In Etosha national park the estimate was about 3,400, whilst the Kunene region had an estimate of 300 (IUCN 2013).

Elephants live in dynamic social systems where related adult female elephants form matriarchal family units in which they care for their offspring (Houck et al. 2001). The family units are lead by the eldest, most experienced and dominant female, and the whole group usually contain from 2-30 individuals. Adult males are compelled to leave their natal units at around 12-15 years of age, and most of them will live solitary the rest of their lives, though some males can be found living in smaller bull herds. Elephants are in need of huge home ranges and the desert dwelling elephants of Namibia have some of the largest home ranges ever recorded, with ranges as large as 12800 km^2 (Leggett 2006).

Depending on location and context, elephants are both considered ecosystem engineers (Haynes 2012) and/or keystone species (Caro & Girling 2010), making them vital in many African ecosystems. For instance, their way of living is important in structuring the landscape; Pulling down trees and breaking up thorny bushes helps create grassland of witch other species are dependent (Primack 2010).

2.3   Group Identification

Elephants were located using the method recommended by Moss (1996); Inside ENP with the help from park rangers; outside ENP local rangers would assist with the search as well as acquiring information from local people along the road. Information about waterholes, both artificial and natural, proved especially valuable and a large portion of the samples were collected near water points. When coming across fresh dung and/or footprints, following the tracks would always be attempted if possible by car. In the Khorixas region, access to GPS coordinates from three different collared elephants; two males and one female belonging to a family group, made it possible to track these individuals. Locating all three animals was attempted, however, only the family group was successfully found.

Observations and collection of data was mostly done from the car, though in the Ugab river area, rocky hills surrounding the dried out riverbed was also climbed in order to get a better observation point. When driving, roads were primarily used, though on some occasions off-roading was also necessary in order to follow the elephants.

After locating one or more elephants, a set of baseline data was collected in accordance to Moss (1996), with some alterations (see Appendix II). First off, date, time, GPS coordinates, habitat type, weather and temperature were recorded. Next step was observing and counting the number of individuals present. As the surroundings could be rather complex with bushes and trees, counting the exact number of individuals could sometimes be difficult. This was further complicated if the herd was on the move. In these circumstances the count was recorded as X+, where "X" represents the actual number of observed elephants and "+" indicate that there might be more unspotted individuals belonging to the herd. At waterholes, where several herds would often mingle together, the full count of individuals was noted as one family group unless the groups were clearly separated. Furthermore, observing and noting group dynamics was done, categorizing individuals into calves, juveniles, sub-adults and adults, and determining their sex (especially of sub-adults and adults). However, this was not always possible in bushy terrains due to poor visibility. If possible, pictures were taken of the whole herd together. Very characteristic individuals were particularly noted in order to increase recognition of the herd.

2.4   Elephant Identification

After noting the initial group data, the next step was collecting individual data. When defecation was observed, the time was noted and pictures taken in order to be able to locate the correct droppings afterwards. Given as a percentage, the size of the respective elephant was estimated relative to the biggest female in the herd – this is usually the female matriarch. The biggest female would always be set to a size of 100%. Males on the other hand, were set to a size of up to 200%.

In order to increase recognition of individuals and avoid duplicate samples, pictures were taken of all elephants defecating as far as was possible. The code of the pictures was always noted. Easily recognizable characteristics were noted to further increase identification, typically including descriptions or drawings of features such as; ears - wear, tear and holes; tail - length, hairs; tusks – length and general size, curvature, wear and tear, etc. Individuals would also be compared to other easy recognizable individuals in the herd. On a few occasions faecal samples were collected where it was not possible to properly identify the elephant defecating. These samples were only used in tests where the unknown parameters were unnecessary.

In order to determine the sex of each elephant, looking for typical sexually dimorphic traits was done according to Moss (1996). Primarily this meant looking for genitalia, breasts and mammalian glands. In situations where this was difficult because of thick vegetation, inconvenient angles etc., looking at head shape and general body shape and size was done to determine sex (see Figure 3). A female elephant in general has a more angular head profile, whereas a male elephant skull is much more rounded due to more muscle mass around the skull bone. These muscles help the males hold up their considerable bigger and heavier skull and tusks. Their tusks are usually much thicker and hence more tapered towards the end, whereas the females' tusks are usually thinner and more uniform. The area between the eyes also tends to be broader in males compared to females. Determining the sex of younger individuals was often more difficult compared to older individuals, but was always attempted in defecating individuals.
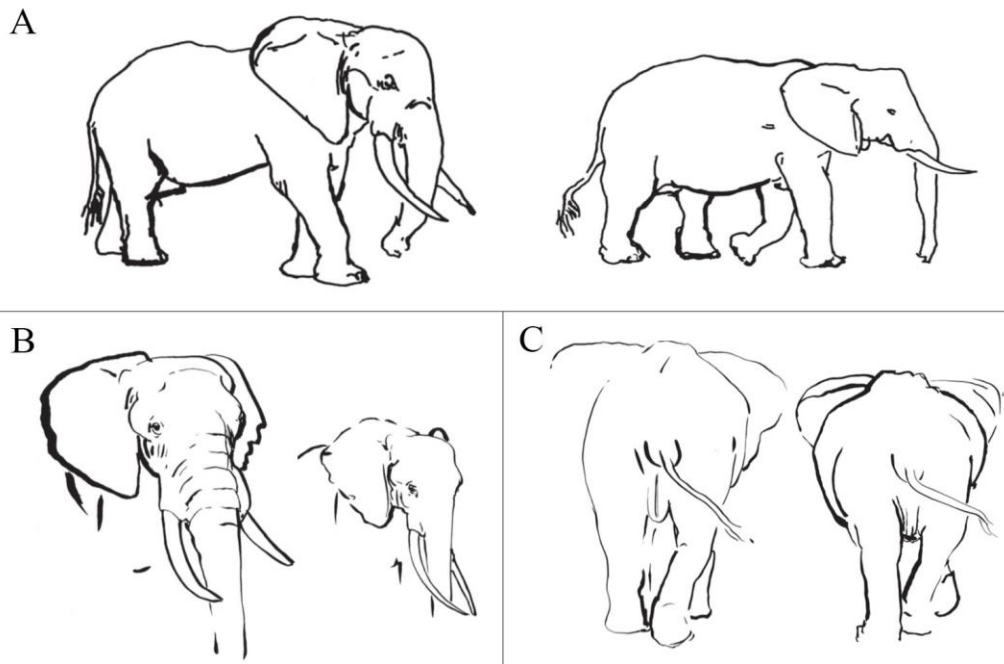
Figure 3: Illustration showing sexual dimorphism in elephants. Male to the left and female to the right; A. Difference in body- and head shape from a profile view; B. Difference in head shape, tusks and eyes; C. Difference in body shape from behind, with an underbelly, genital view (Moss 1996).

2.5 Field Processing of Faecal Samples

In order to minimize any potential environmental effect on the glucocorticoids in the faeces, collection would happen as soon as possible after defecation. The timespan from defecation to collection was always noted and never exceeded 2 hours. However, on a few occasions faecal samples were collected where time of defecation was not known. This was done due to the obvious freshness of the bolis when they were found. Due to the knowledge obtained on the elephants in the respective areas where this was conducted, chances of getting duplicates were non-existent.

The samples were collected using disposable gloves and plastic knifes, peeling of the outer layer with mucus. Bigger undigested materials were avoided. As there is a possibility of corticosteroids and their metabolites to be unevenly distributed in the faeces, the collection was done from three to four bolis. This was done in order to get a result that would reflect the most accurate average content of glucocorticoid in the faeces. The dung was transferred to 50 mL sample tubes and placed in a portable freezer ($\approx$ -18 °C), connected to the car, within 15 minutes after collection. The sample tubes were transferred to a fixed freezer ($\approx$ -18 °C) in the office of Etosha Ecological institute once this was possible. As there could be up to seven days in between each time the samples were transferred to the fixed freezer, the portable freezer was hooked up to an external power source during the night in order to ensure enough power for the freezer throughout the night. All samples were kept frozen until the extraction process.

However, due to a couple of incidents where connecting the freezer to an external power source during the night was impossible, the car battery was drained, leaving the freezer with no electricity in a period of up to six hours. Thus, some samples got partly defrosted. However, as the temperature was still low in the freezer when electricity was provided again, it was found that the incident most likely had little to no effect on the concentration of glucocorticoid metabolites in the faeces. In order to control for possible biases due to this event, a small experiment was conducted in the lab in order to test for the effects of defrosting. Though not included in this study, the results gave no clear indications that the experienced defrosting had any effect on the FGM level in the respective samples.

2.6   Extraction of Faecal Glucocorticoid Metabolites

The extraction process took place after all 95 samples had been collected, within 60 days after the first collection. It was conducted in the lab at the Etosha ecological institute in Okaukuejo, ENP. The procedures were conducted according to Palme (2005) and Palme et al. (2013).

The sample tubes (typically eight at a time) were taken out of the freezer and left to defrost for about half an hour, whereas the dung was taken out of the tube and further defrosted while getting thoroughly mixed by gloved hands for 5-10 minutes. Obvious pieces of undigested materials were taken out and 0.5 grams ($\pm$ 0.02 grams) of faeces were weighed and transferred into marked 15 mL centrifuge tubes. The exact weight was always noted in order to be able to correct for this during the final hormone analysis. To each sample, 5 mL of 80% methanol was added and the mixture was hand vortexed for 1 minute. The vortexed samples were then centrifuged for 20 minutes at 1500 rpm to separate the supernatant from the faecal material. From the separated supernatant, 0.5 mL was transferred into two marked, 1.5 mL vials (0.5mL for each vial). The vials were left uncapped and put in a fume hood in order to dry. The drying process took a maximum of two days. After the samples were fully dried, the vials were capped and stored in room temperature for further laboratory processing and analysis.

2.7   Enzyme Immunoassay

The hormone analysis was performed within four months after the first collection took place, and within 60 days after the extraction process. The samples were analysed at the University of Veterinary Medicine in Vienna, Austria, by Associate Professor Dr. Rupert Palme.

The enzyme immunoassay (EIA), a method specially developed for GC metabolites, was conducted according to the EIA protocol developed at the Department of Biomedicine at the University of Veterinary Medicine in Vienna (Palme 2014). In order to reflect the elephants stress level, the cortisol metabolite 11-oxoaetiocholanolone, earlier validated for African elephants (Ganswindt et al. 2003; Stead et al. 2000; Viljoen et al. 2008), was used in the EIA process. This method detects glucocorticoid metabolites with a 5β-3a-ol-11-one structure. See Crowther (2008) for an illustration of the EIA-procedure. Solutions referred to in the description below are listed in Appendix III.

Before use, micro titer plates (MTP), consisting of 96 wells, pre-coated with protein A (Sigma P-7837), were washed three times with a washing solution before it was blotted dry with paper towels. Caution was taken not to touch the underside of the plate. Assay buffer for nonspecific binding (NSB) and zero binding (0), standards (4.1), pool X and Y (PX, PY) was dispensed and sampled into earlier prepared MTP (see example of arrangement in Appendix IV), 0.05 mL of each. 0.01 mL from each sample (11-oxoaetiocholanolone) was added with 0.04 mL assay buffer (Sigma T-1503) in order to dispense a total of 0.05 mL. Further, 0.1 mL of biotin-labelled steroid was dispensed into each well using a multi-pipette, followed by 0.1 mL of antibody solution. The MTPs were then covered with parafilm and a dust cover (Nunc 264623) and mildly shaken overnight at 4°C.

Next, the incubated MTP was decanted and washed in cold (4°C) washing solution four times. 0.25 mL of enzyme solution was then added to each well, whereas the covered plate was incubated on an MTP-shaker at 4°C for 45 minutes. Again, the MTP was decanted and washed four times in cold (4°C) washing solution. 0.25 mL of substrate solution was further dispensed into each well, whereas the MTP was covered and incubated at 4°C in the dark for another 45 minutes. Lastly, 0.05 mL of stop reagent was added to each well.

In order to measure the absorbance, an automatic MTP reader with reference filter of 620 nm and measuring filter of 450 nm was used. A standard curve (Appendix V) was used in order to determine conversion from absorbance (B/B0) to steroid concentration (pg/well). Further, in order to account for the dry weight differences in each sample, as well as converting the results from ng to pg, the following equation (1) was used:

$$\frac{ng\ (steroid)}{g(faeces)} = \frac{pg\ (pr\ well)\ \times\ extraction\ volume\ \times\ dilution\ factor}{faecal\ weight\ \times\ sample\ volume\ \times\ 1000} \qquad (1)$$

Extraction volume represents the mixed volume of the aqueous organic solvent for the extraction (µL) and the faecal weight (mg). Sample volume represents the volume that was transferred to the EIA (in µL), which was then multiplied by 1000 in order to convert the results from pg to ng.

2.8　Statistical Analyses

All statistical analyses were performed using SPSS (IBM Statistics, Version 22.0.). Four of the collected samples were obtained from the same individual at different times. These were eventually found to be duplicates after watching pictures from previous visits. Hence, using random selection, one of the samples was chosen for the further analyses. There was also a line of dung from a running elephant (six bolis), which was collected as two samples in the field; these were most likely from the same individual (FGM concentration of 82 ng/g and 84 ng/g) and the mean from these two samples were therefore calculated for the statistical analysis.

In order to normalize the data, the FGM concentrations were log transformed before the statistical analyses. One-way ANOVA (analysis of variance) and Tukey's *posthoc* tests were used in order to compare variance of hormone concentrations between individuals. Model effects were; gender, area, group size and body size. A linear regression model with FGM level as dependent variable and with area and body size as independent variables was performed as a final test. Significance level was set to $p \leq 0,05$.

3 Results

3.1 Distribution of Data

Table 1 provides an overview over the number of days spent in the field, the number of unique elephant sightings and the number of collected sample during the fieldwork. From all collected samples, the faecal glucocorticoid concentrations ranged from 10 ng/g to 200 ng/g throughout the whole sample pool, with a mean of 61 ng/g ($\pm$ 35.17 SD, N = 89). The sample with the overall highest FGM concentration of 200 ng/g belonged to a very old female individual (for an extensive overview over all samples and their respective FGM values, see Appendix I). Her back was obviously swayed and she would always arrive way behind her herd at the waterhole. Because of her visibly bad state and the biasing effect of keeping her values in the sample pool, it was decided to leave her sample out throughout the statistical analysis (see section 3.3 for the effect of removing her from the analyses).

Table 1: An overview of the number of days in the field, the number of unique elephant sightings and the number of collected samples, both inside and outside Etosha National park (ENP).

	Inside ENP	Outside ENP	Total
Days in the field	14	18	32
Unique elephant encounters	194	65	259
Sampled elephants	56	34	90

3.2 Precipitation

No correlation was found between annual rainfall and stress level in the three different areas inside ENP (log transformed values: ANOVA; F = 0.623, df = 2 and 52, p = 0.54).

3.3   Area

Before removing the obviously old and deviating individual from the sample pool, there was already a statistically significant difference in mean FGM concentration between individuals inside and outside of ENP, (log transformed values: ANOVA; $F = 12.53$, $df = 1$ and 89, $p < 0.001$). However, by removing her from the test, the results became even more significant. In addition, removing this sample reduced the variation of the samples from inside ENP, (log transformed values: ANOVA; $F = 15.6$, $df = 1$ and 88, $p < 0.0001$; Figure 4). Recorded mean values were 48.1 ng/g (±18.1 SD, $N = 55$) inside ENP and 77.2 ng/g (±40.6 SD, $N = 35$) outside ENP.
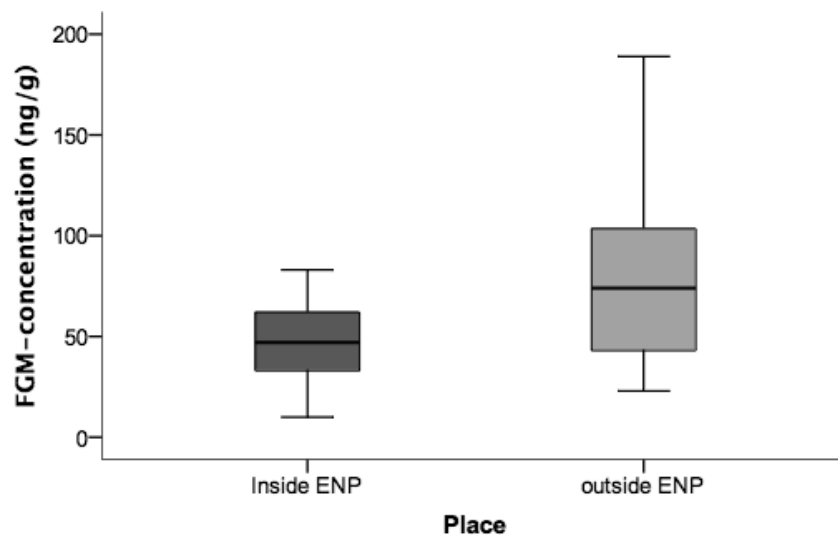
Figure 4: Mean faecal glucocorticoid metabolite (FGM) concentration in elephants residing inside Etosha National Park (ENP) compared to elephants residing outside ENP.

By further dividing into the seven originally selected areas, three inside ENP and four outside ENP, a statistically significant difference between the areas was found (log transformed values: ANOVA; $F = 3.35$, $df = 6$ and 83, $p = 0.005$; Figure 5). The highest FGM concentrations were found outside ENP, in Purros and Ugab. The lowest FGM concentrations were found inside ENP, in Otjovasandu and Okaukuejo. Tukey's posthoc tests showed no statistically significant difference between the areas inside ENP, nor did it show any significance between the areas outside ENP. This validates the combination of the three areas inside ENP to one category (inside ENP), as well as the four areas outside ENP to a second category (outside ENP).
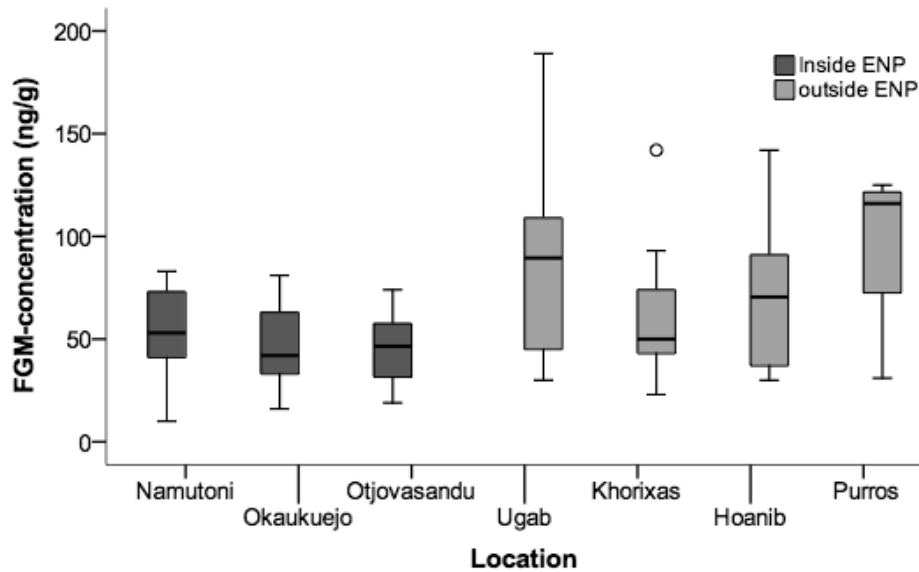
Figure 5: Mean faecal glucocorticoid metabolite (FGM) concentration grouped according to sampling location. Inside Etosha National Park (Namutoni, Okaukuejo and Otjovasandu) coloured in dark grey and outside ENP (Ugab, Khorixas, Hoanib and Purros) coloured in light grey.

3.4 Gender

There was a statistically significant difference in stress levels between males and females (log transformed values: ANOVA; F = 3.98, df = 1 and 81, p = 0.049). Males had a significantly lower FGM level than females; with non-log transformed mean values; 54.0 ng/g (±31.8 SD, N = 39) and 65.3 ng/g (±33.9 SD, N = 44), respectively. Looking only at the males inside and outside ENP, there was a statistically significant difference in stress level between the two areas, with a lower level inside ENP (log transformed values: ANOVA; F = 8.14, df = 1 and 37, p = 0.007; Figure 6). Females had a statistically significant lower level inside ENP as compared to the outside as well (log transformed values: ANOVA; F = 6.85, df = 1 and 42, p = 0.012; Figure 6).
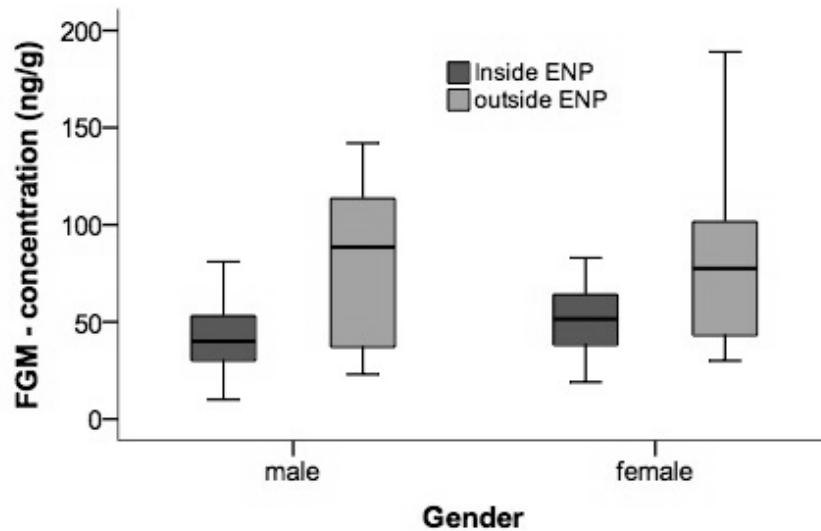
Figure 6: Mean faecal glucocorticoid metabolite (FGM) concentration observed in males and females. Dark bars show samples from elephants sampled inside Etosha National Park, while light bars show elephants sampled outside ENP.

3.5   Body Size

There was no statistically significant difference between recorded FGM concentrations and body size (log transformed values: ANOVA; $F = 1.31$, df = 3 and 86, $p = 0.276$).

3.6   Group Size

A statistically significant difference between group size and stress level was recorded (log transformed values: ANOVA; $F = 2.90$, df = 3 and 82, $p = 0.04$; Figure 7). Tukey's posthoc test showed that groups of 1 individual (all male bulls) had significantly lower FGM concentration compared to both groups of 2-5 individuals and groups of 6-10 individuals.
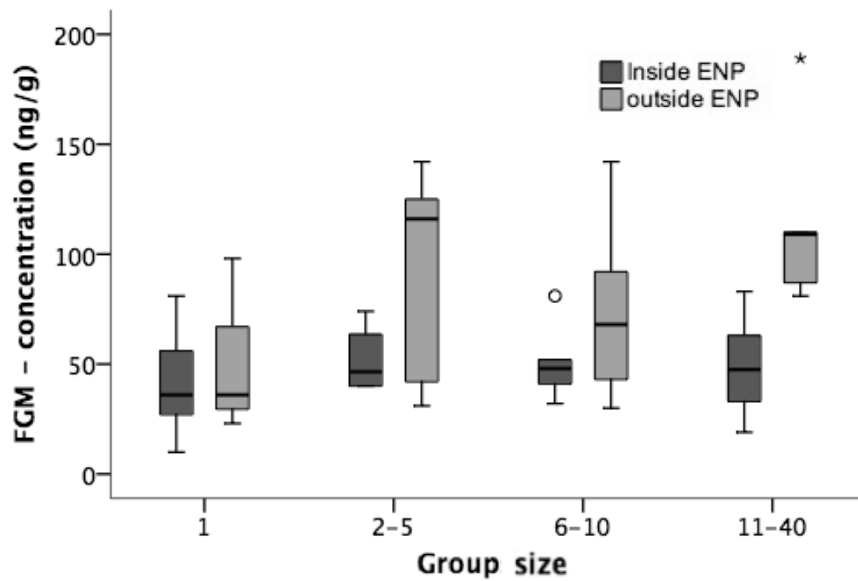
Figure 7: Mean faecal glucocorticoid metabolite (FGM) concentration observed in different elephant group sizes, ranging from 1 to 40 individuals. Dark bars represent data from samples collected inside Etosha National Park (ENP), whilst light bars represent samples collected from outside ENP.

3.7 Multivariate linear regression

A linear regression analysis with log transformed values of stress level as the dependent variable and place (inside/outside), gender and group size as independent variables was significant ($r^2 = 0.207$, F = 6.71, df = 3 and 77, p < 0.0001). However, the only independent variable explaining this variation significantly was area (inside/outside) (t = 3.76, p < 0.0001), while gender (t = 1.08, p = 0.282), and group size (t = 0.59, p = 0.554) were non-significant.

4 Discussion

In the present study, recorded data on FGM concentrations in elephants inside and outside the protected national park, Etosha, clearly show differences in stress level between these two areas. A range of possible stress factors were tested in order to determine whether or not they contributed to the elevated FGM concentration recorded in elephants outside ENP. The results particularly demonstrate a difference in anthropogenic disturbances, either directly or indirectly affecting the observed differences in stress levels.

4.1 Stress Levels Inside Etosha National Park

The low level of stress inside ENP compared to the outside indicates an overall higher welfare and further demonstrates the importance of protected areas. The similar levels of FGM inside ENP indicate that the elephants are not affected by the slight difference in tourist density between the three areas. This might suggest an increased tolerance or even habituation towards humans and/or cars, probably as a response to a prolonged period of exposure to non-threatening behaviour from tourists and staff visiting the park (for a review on research demonstrating this effect, see Grissom and Bhatnagar (2009)). For instance, tourists are not allowed to go out of their car and can only visit the park between sunrise and sunset.

4.2 Elephant Abundance

The number of elephant encounters during the field work (Table 1), as well as results from aerial counts (IUCN 2013) show a huge difference in abundance and density of elephants inside ENP and the Kunene region. In theory, this could demonstrate a preference towards settling in areas with less anthropogenic disturbances. This is similar to the findings of a range of different researchers who typically observed higher elephant abundance inside

protected areas compared to the outside (Blake et al. 2007; Graham et al. 2009; Tingvold et al. 2013; Wittemyer et al. 2007). Remis and Kpanou (2011) found a similar pattern in elephants in the Dzanga-Sangha Reserve, Central African Republic, where elephant abundance increased further away from human settlement. The findings are further reinforced by studies that found elephants to move faster through unprotected areas compared to protected ones (Douglas-Hamilton et al. 2005; Galanti et al. 2006; Sitati et al. 2003).

An important factor counteracting this claim is the fenced border surrounding ENP, which in theory prevents elephants from choosing to reside inside or outside ENP. However, according to Acting Chief Warden, Shayne Kotting (personal communication), these fences are regularly broken by elephants, sometimes several times during a week. The actual breakthrough seems to happen from the inside. Most fences are repaired within a week, concurrently leaving the border permeable until fixed, hence allowing for the possibility of migration across the border. However, even if some elephants successfully cross the border, the number of elephant's leaving/entering ENP under these circumstances is not known and is unlikely to account for the observed differences in elephant density.

4.3   Gender

At first glance, the results indicate a significant difference in stress level between males and females. This is somewhat similar to the results from Tingvold et al. (2013), where males showed a generally lower FGM concentration than females. However, the differences in Tingvold's study were non-significant. Elevated stress level in females could reflect the added social responsibility of adult females living in a herd (taking care of juveniles and calves), as well as potential added nutritional needs of pregnant and/or lactating females (Barnes 1983). Even though there was no correlation between stress level and body size (data not presented in this paper), the highest mean FGM concentration was found in elephants with body size 95-100%. This size category mostly consists of adult females, further building on the theory about adult females possibly being more stressed due to their social responsibility in a herd. Greater social responsibility was also argued to be the cause of higher stress hormone levels in adult females compared to weaned calves in the study by Woolley et al. (2009).

There was a statistically significant difference between groups size and stress level, with solitary individuals having the lowest mean FGM concentration, both statistically different from groups of 2-5 and 6-10 individuals. All groups of 1 individual were male

individuals; hence the results might demonstrate the opposite of what is observed in adult females, namely the solitary nature of males in which they only have to care for themselves. Woolley et al. (2009) however, found the opposite, namely higher stress in male elephants compared to females.

However, a linear regression analysis showed no significant effect, neither in relation to sex, nor group size. Only place (inside/outside) was significant, indicating that these two factors are not contributing to the overall higher stress level recorded outside compared to inside the protected area.

4.4 Protected Areas Compared to Unprotected Areas

The overall higher stress levels of the elephants residing outside ENP suggest a lower welfare and hence demonstrate a potential effect of reduced fitness in elephants residing in unprotected areas.

4.4.1 Precipitation Level and Water Availability

The difference in geographical localization of the samples makes the corresponding change in annual precipitation level a potential factor affecting stress levels. The difference in annual rainfall is a major factor affecting the access to water resources, which is of great importance to the elephants in the arid landscape of Namibia. Elephants in Namibia are, during the dry season, prone to serious droughts and thus also drought-related mortalities, especially the younger individuals (Lindeque & Archibald 1991; Lindeque & Lindeque 1991).

As a way of controlling for this factor, three different study areas were chosen inside ENP, differing in longitude, stretching from east to west. Potentially perceived threats were expected to be equally low within all three areas, thus anticipating corresponding low stress levels. The results showed a slight difference between the three areas, but were non-significant and did not correlate with annual rainfall. In theory this rules out precipitation level as a contributing factor to elevated stress.

Several studies have found stress levels to correlate with the dry and wet season, with heightened levels during the dry season (Foley et al. 2001; Viljoen et al. 2008; Woolley et al. 2009). This indicates that the lack of rain and hence availability of water and forage, is an important contributing factor of elevated stress. However, Woolley et al. (2009) found no difference in stress level between dry and wet season in the Pilanesberg National Park (PNP)

in South Africa. He argues that even though PNP has differences in forage quality and quantity over the season, water is not equally restricted during the dry season. These findings underpin the extensive importance of water availability. Hence, it might be that the continuous access to water through a high density of artificial water points within ENP eases the difference between the wet and dry season. During the dry season, water availability on the outside consists of a smaller amount of artificial water points for wildlife, remaining pools of water from the ephemeral river and water points made for the human settlements. Hence the difference from wet to dry season might be much more evident on the outside compared to the inside of ENP.

Lack of water resources compels the elephants to reside closer to ephemeral rivers and/or artificial water points (Chase & Griffin 2009; Leggett 2006). This typically means residing closer to human settlements, which increases the probability of everyday encounters and hence HEC. Elephants may also be more inclined to exploit water points primarily made for the human settlement, consequently leading to increased HEC. Conflicts over water is also what is observed and recognized as the main reason for HEC in the Kunene region (CITES 2000; MET 2009).

When conducting the fieldwork in Khorixas, a local MET employee stated that local people sometimes tried to prevent elephants from stealing their water by reducing the water level in their water well, enabling elephants to reach it. The elephants typically respond by stealing water directly from their houses, inflicting huge damages. Thus, an important job for the local MET employees is to educate the local people, encouraging sharing their water resources as opposed to hindering access for the elephants. This information suggests negative attitudes towards elephants, which is further emphasized by the findings from CITES (2000), who reported an increase in badly injured elephants due to people using severe deterring methods to frighten them from water resources etc., consequently resulting in increased HEC.

Even though precipitation level appears to be insignificant to observed differences in stress level within ENP, either directly or indirectly through HEC, the quantity and frequency of available water is likely contributing to elevated stress levels observed in the Kunene elephants.

4.4.2 Poaching

A study by Burke et al. (2008) showed a correlation between hunting risk and elevated stress level. It is possible that this is also the case for the elephants roaming outside Etosha. Tingvold et al. (2013) concluded that long on-going hunting activity was a main reason for the observed elevated FGM level in the Tanzanian elephants. At present though, poaching is not a very common problem in Namibia. According to numbers by MIKE, 26 elephants were illegally killed in the Caprivi region from 2002 - 2011 (CITES 2013). In contrast, no elephants were recorded as illegally killed inside ENP or in the Kunene region during the same period. Historically however, there have been periods of severe declines in the Kunene elephant population due to intensive hunting and poaching etc. Before 1900 the estimated elephant population in the north-western region was between 2500-3500 (Viljoen 1987), this number was reduced to approximately 360 by 1983.

The reason for the low level of poaching incidents in Namibia is probably due to a combination of several factors. Two main factors are probably effective law enforcement (CITES 2000; Leggett et al. 2003) making it risky engaging in the illegal activity, as well as the increasing focus on conservation efforts since the early 1980s (Leggett et al. 2003), such as different types of community-based conservation efforts. Bandyopadhyay et al. (2004) for instance, concluded that Namibian households benefit from participating in local conservancies. When the local people recognize the elephant as a good source of income, making money of tourism etc., the tolerance generally increases, making it less desirable to engage in poaching activities.

Even though current incidents of poaching are low, suggesting that it is not a contributing factor to the observed elevated stress level in non-protected areas, it is hard to rule this factor completely out. As elephants are such long-lived species, one can speculate if previous historical events might still remain as a factor affecting the present elephant's perception of humans as threatening, hence elevating the physiological stress level when in proximity to humans. Another possibility could be that some current elephant populations in Namibia are previous residents from neighbouring country Angola, potentially being affected by the resent civil war and possibly high hunting/poaching levels in this region. However, little is known about the elephant population of Angola, as both surveys and research on the Angolan elephant populations are close to non-existent (Blanc et al. 2007), making this assertion highly speculative.

4.4.3 Tourism

Tourism is a potential stress factor both on the inside and on the outside of ENP. However, as previously discussed, it might seem like the elephants inside ENP have gained increased tolerance and maybe even habituated to cars and humans. This might not be the case in the elephants of the Kunene region. In the Ugab, Hoanib and Purros riverbed, there are limited rules to what tourists can do. Tourists are free to drive pretty much wherever they want, they can walk out of their cars and many tourists join night camps in the middle of the riverbed. As elephants frequently visit the riverbeds during the dry season due to the obvious water availability, the assumption is that tourist-elephant encounters happen quite frequently during this period. If elephants outside Etosha in general associate people as a potential threat because of other HECs, it is likely that tourists are perceived the same way, even though they do not pose any real danger to the elephants.

4.5   Previous and Future Studies

This is the second time such a study has been conducted with similar findings, emphasizing the importance of this kind of research. Tingvold et al. (2013) found a mean FGM concentration of 62.61 ng/g inside Serengeti National Park, compared to a mean FGM concentration of 115.72 ng/g on the outside (ANOVA F = 8.006, df = 3, p < 0.0001). These are overall higher levels compared to present findings from this study, which might reflect inherent differences between Namibia and Tanzania, i.e. the overall higher level of poaching incidents and higher population density in Tanzania. However, the recorded stress level might not be directly comparable due to the slight difference in the extraction process, using 80% methanol instead of 90% ethanol for the separation of supernatant and faecal matter.

4.5.1 Temperature

Though not presented in the previous results, when looking at the relation between collected temperature measurements and recorded FGM concentration, the observed results were non-significant. This is not very surprising as the temperature was only measured at the time of defecation. Considering the temperature varied greatly during a day (from 0ºC to 30ºC), it does not make sense to correlate a single point-in-time measure of temperature, with the measured stress level reflecting the potential effect of temperature 30-50 hours before the actual sample collection. Therefore, in order to test this properly, a possible option might be

to obtain continuous data on temperature. This could be especially valuable in order to detect how high or low the temperatures need to be in order to affect stress level.

4.5.2   Vegetation Cover

Though data on habitat was noted during the fieldwork, this was limited to the habitat type observed at the place of defecation. In other words, a thorough data set on vegetation cover, structure and composition was not properly provided during the study. Difference in diet, food availability and/or quality can therefore not be entirely ruled out as a contributing factor to observed variations in stress levels. For instance, in the Hoanib River, it was possible to observe, from the faeces, a diet consisting of more seeds (from the seedpods of the *Faidherbia albida* tree) compared to the other areas. What impact this might have on stress level, however, is unknown.

4.5.3   Understanding the Recorded FGM Concentrations

As mentioned by Millspaugh and Washburn (2004), an unresolved issue affecting the potential of FGM analysis for conservation purposes, is understanding at what concentrations the FGM level becomes damaging, as well as determining the actual timespan the heightened FGM levels need to subside in the body in order to affect negatively on fitness.

5 Conclusion

As hypothesised the findings presented in this paper suggest that anthropogenic disturbances are a contributing factor affecting stress levels in the Namibian elephant population outside ENP. To the elephants residing in non-protected areas, conflicts arising from human-elephant interactions seem to cause increasing FGM concentration, indicating chronic stress levels. Even though there might be additional factors contributing to higher physiological stress levels outside ENP, it seems that several of them first and foremost contribute to increasing incidents of HECs, hence indirectly increasing stress levels. Even in a country like Namibia, with low human population density, low levels of poaching, as well as promising conservational efforts at the community level, it seems like human activity negatively affect the welfare of elephants. Due to the many negative consequences related to chronic stress, seeking to improve welfare as a conservation measure is important.

The low physiological stress levels measured inside ENP, demonstrates the importance of protected areas. Due to the elephant's inherent need of large ranges, however, planning to conserve them solely within protected areas is in most cases unlikely to serve as an option. This emphasizes the importance of minimizing HEC and planning for human-elephant co-existence in future conservational work, as well as the importance of current and future establishments of protected areas.

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Appendix I – Data set

Data collected during fieldwork.

Sample no.	Area	Sex	Body size (%)	Group size	Temperature (°C)	FGM concentration	Log-transformed FGM concentration
1	Nam	Male	120	2	22	40	3,69
2	Nam	Male	130	2	22	53	3,97
3	Nam	Male	120	6	24	52	3,95
4	Nam	Male	140	6	24	41	3,71
5	Nam	Male	85	6	24	32	3,47
6	Nam	Male	160	1	22	10	2,30
7	Nam	Fem	100	5+	8	81	4,39
8	Nam	Male	110	1	15	73	4,29
9	Nam	Male	155	2	22	74	4,30
10	Nam	Fem	75	26+	23	61	4,11
11	Nam	Fem	95	26+	23	200 (removed)	-
12	Nam	Fem	100	26+	23	73	4,29
13	Nam	Fem	95	26+	23	54	3,99
14	Nam	Male	100	26+	23	27	3,30
15	Nam	Fem	95	26+	23	52	3,95
16	Nam	Male	140	26+	23	46	3,83
17	Nam	Fem	100	28+	28	83	4,42
18	Nam	-	40	28+	28	64	4,16
19	Oka	Male	150	1	24	16	2,77
20	Oka	Male	120	1	24	53	3,97
21	Oka	Male	100	3	22	40	3,69
22	Oka	-	60	34+	26	77	4,34
23	Oka	Fem	90	34+	26	48	3,87
24	Oka	Male	140	1	26	31	3,43
25	Oka	Fem	100	34+	26	43	3,76
26	Oka	Fem	100	5	26	31	3,43
27	Oka	-	-	5	26	45	3,81
28	Oka	Fem	100	5	26	63	4,14
29	Oka	-	-	5	26	38	3,64
30	Oka	Male	150	1	28	81	4,39
31	Oka	Fem	85	34+	27	33	3,50
32	Oka	Male	140	1	27	27	3,30

33	Oka	Male	170	1	26	36	3,58
34	Oka	Male	40	34+	24	41	3,71
35	Oka	Fem	95	34+	24	66	4,19
36	Oka	Male	40	34+	24	63	4,14
37	Otjo	Fem	95	26+	26	31	3,43
38	Otjo	Fem	95	26+	26	19	2,94
39	Otjo	Fem	95	26+	26	61	4,11
40	Otjo	Male	80	26+	26	28	3,33
41	Otjo	Male	95	26+	26	24	3,18
42	Otjo	Male	50	36+	25	34	3,53
43	Otjo	Fem	95	36+	25	32	3,47
44	Otjo	Male	40	36+	25	29	3,37
45	Otjo	Male	90	36+	25	73	4,29
46	Otjo	Fem	100	36+	25	47	3,85
47	Otjo	Fem	65	36+	25	51	3,93
48	Otjo	Male	50	36+	25	49	3,89
49	Otjo	Male	150	36+	25	38	3,64
50	Otjo	Male	150	1	26	56	4,03
51	Otjo	Fem	100	26+	25	65	4,17
52	Otjo	Fem	95	26+	25	33	3,50
53	Otjo	Fem	95	7	25	46	3,83
54	Otjo	Fem	95	10	24	50	3,91
55	Otjo	Fem	90	12	24	74	4,30
56	Otjo	Fem	70	12	24	59	4,08
57	Khor	Male	140	1	21	23	3,14
58	Khor	Fem	95	10	22	93	4,53
59	Khor	Fem	95	10	22	43	3,76
60	Khor	Fem	95	10	22	Dup (removed)	-
61	Khor	Fem	-	-	8	74	4,30
62	Khor	-	-	-	8	50	3,91
63	Khor	Fem	-	-	8	43	3,76
64	Khor	-	-	-	8	53	3,97
65	Khor	Male	130	2	20	42	3,74
66	Khor	Male	140	2	20	142	4,96
67	Ugab	Male	95	15	24	Dup (removed)	-
68	Ugab	Male	95	15	26	Dup (removed)	-
69	Ugab	Fem	95	15	26	87	4,47
70	Ugab	Male	95	15	26	Dup (removed)	-
71	Hoan	Male	130	1	30	98	4,58
72	Hoan	Male	80	7	15	82	4,41
73	Hoan	Fem	55	10	17	84	4,43
74	Hoan	Male	140	1	17	36	3,58
75	Hoan	Male	150	10	21	38	3,64
76	Hoan	Fem	95	10	21	68	4,22
77	Hoan	Fem	95	10	21	31	3,43
78	Hoan	Fem	70	10	21	51	3,93
79	Hoan	Fem	95	7	22	73	4,29
80	Hoan	Fem	55	7	22	30	3,40

B

81	Hoan	Fem	100	7	22	137	4,92
82	Hoan	Fem	95	7	22	142	4,96
83	Purr	Male	120	3	22	118	4,77
84	Purr	Fem	100	3	22	114	4,74
85	Purr	Male	140	3	22	125	4,83
86	Purr	Male	70	2	17	31	3,43
87	Ugab	Fem	95	15	28	189	5,24
88	Ugab	Male	95	15	28	109	4,69
89	Ugab	Fem	100	15	28	81	4,39
90	Ugab	Fem	95	15	28	110	4,70
91	Ugab	Fem	95	8	26	43	3,76
92	Ugab	Male	90	8	26	95	4,55
93	Ugab	Fem	95	8	26	30	3,40
94	Ugab	Fem	100	8	26	92	4,52
95	Ugab	-	-	8	26	45	3,81

C

Appendix II – Field Data Collection Sheet

Data sheet for the collection of information in the field.

Date: **Weather:**

Time: **Temperature:**

GPS coordinates: **Habitat:**

Total number of individuals:

Adults: Fem.:   Male: Unsexed:

Sub-adults: Fem.:   Male: Unsexed:

Juveniles: Fem.:   Male: Unsexed:

Calves: Fem.:   Male: Unsexed:

Sample no.	Sex	Size (%)	Time of collection	Picture no.	Special characteristics	Other comments

Appendix III – Buffers and solutions for the EIA procedure

Buffers and solutions, listed in the EIA-protocol by Palme (2014), used in the EIA procedure by Associate Prof. Dr. R. Palme at the University of Veterinary Medicine, Vienna, Austria.

1.1. Coating buffer

1.59 g Na_2CO_3 (Merck 106392 or Sigma S-7795)

2.93 g $NaHCO_3$ (Merck 106329 or Sigma S-6014)

> Dissolve and fill up to 1 l with DDW, adjust to pH 9.6 with (about 10 ml) HCl (1 mol/l).
> Filter through Sep-Pak® C18 (see 1.3.1.)

1.2. HCl (1 mol/l)

920 ml DDW + 80 ml 37% HCl (Merck 100317 or Sigma H-1758)

1.3. Assay buffer

2.42 g Trishydroxyaminomethane (Merck 108382 or Sigma T-1503), 20 mmol/l
17.9 g NaCl (Merck 106404 or Sigma S-9625), 0.3 mol/l)

1 g Bovine serum albumin (Sigma A-4503)

1 ml Tween 80 (Merck 822187 or Sigma P-8074)

> Dissolve and fill up to 1 l with DDW and adjust to pH 7.5 with (about 17 ml) HCl (1 mol/l)
> Filter through Sep-Pak® C18 (see 1.3.1.)

1.3.1. Filtration of buffer through Sep-Pak® C18

Sep-Pak® classic C18 cartridge (360 mg; Waters WAT051910)

> Rinse with 5 ml methanol (Merck 106009), followed by 10 ml DDW (done by hand with a syringe)
> Connect column to tubing of peristaltic pump (flow rate of 2 to 10 ml/min)
> Discard the first 10 ml of the filtrated buffer
> Collect buffer in clean bottle

1.4. "Second" coating buffer

3.146 g Trishydroxyaminomethane (see 1.3.)

23.3 g NaCl (Merck 106404 or Sigma S-9625)

13 g BSA (Sigma A-4503)

1.3 g Sodium azide (Merck 106688)

Dissolve and fill up to 1.3 l with DDW and adjust to pH 7.5 with (about 40 ml) HCl (1 mol/l)

Filter trough SEP-PAK C18 (see 1.3.1.)

1.5. Washing solution

0.5 ml Tween 20 (Merck 822184); add 2.5 l DDW

1.6. Substrate buffer for peroxidase

1.36 g Sodium acetate (Merck 6267) = 10 mmol/l

Dissolve and fill up to 1 l with DDW and adjust to pH 5.0 with (~8 ml) 5% citric acid (Merck 100244)

1.7. Enzyme solution for Streptavidin-reaction

30 ml assay buffer (see 1.3.)

+ 0.001 ml Streptavidin-POD-conjugate (=0.5 U; Roche 11 089 153 001, 500 U)

Mix on a magnetic stirrer a few minutes before use
(the working solution has to be prepared immediately before use!)

1.8. Substrate solution for peroxidase

30 ml of substrate buffer 1.6.

+ 0.5 ml 3,3',5,5'-Tetramethylbenzedine (0.4%) - Store in a dark bottle!

(0.4% = 0.4 g [Fluka 87748] in 100 **g*** Dimethylsulfoxide [Fluka 41641])

+ 0.1 ml H_2O_2 (0.6%; 0.3 ml H_2O_2 [35%, Merck 108600] + 17.5 ml DDW)

Mix gently on a magnetic stirrer a few minutes before use
(the working solution has to be prepared immediately before use!)
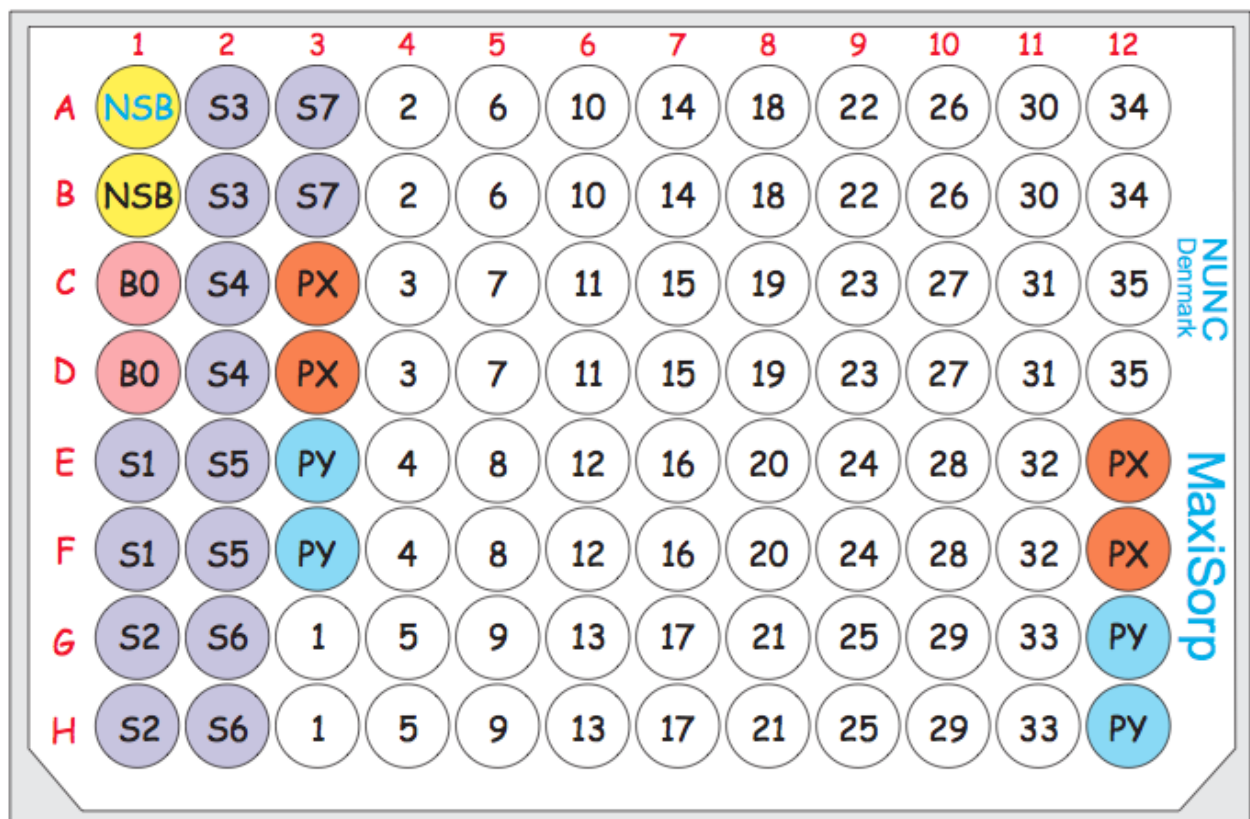
*not ml, as it is very viscous

1.9. Stop reagent: 2 mol/l H_2SO_4

900 ml DDW + 100 ml H_2SO_4 (95-97%; Merck 100731)

Appendix IV – Example of arrangement on MTP

An illustration of a possible arrangement of standards, pools and samples on the MTP during the EIA procedure, from Palme (2014).

Appendix V – Standard curve

With an automatic MTP reader, connected to a computer equipped with a special software for calculation, this standard curve was used in order to determine the conversion of absorbance (B/B0) to steroid concentration (pg/well), from Palme (2014).