

# Effects of visual perception of humans on lions (*Panthera leo*) and tigers (*Panthera tigris*) in a zoo

**Author: Karsten I. van Dam**

Supervisors: Christian Tudorache

Hans de Iongh

Model Journal: Zoo Biology

In recent years, zoos pay more attention to animal welfare. The welfare of captive animals has therefore been the subject of many studies. These include habitat-enrichment- as well as feeding-enrichment strategies. This study is about another possibility to reduce stress: limiting the perception by lions and tigers of the human public. This was done with a Natural fence. Lions were found to stand still in their enclosure for a significantly longer period of time while this Natural Fence was partly removed. For the tigers, three behavior types turned out to be significantly different across the three phases tested. Also, cortisol levels were determined using hair samples. For each of the animals tested, cortisol levels were higher when part of the fence was removed, compared to baseline levels, although this difference was not significant.

## INTRODUCTION

The lion is a social species. Prides often consist of related females and their offspring, together with 1 or a few males (Spong, *et al.*, 2002). Each pride displays group hunting, and has their own permanent territory, which is defended against intruders. Prides have between 1 and 21 adult females and their dependent offspring, along with 1 to 9 adult males (Schaller, 1972; Mosser & Packer, 2009). Even though a pride is a fission-fusion social group, a pride is a stable social unit (Mosser & Packer, 2009).

Pride males often fight with other (prideless) males who are trying to take over the pride, or with males from within their own pride over dominance. When the challenger(s) succeed, they may kill all the cubs present in the pride, in order to get the females on heat as fast as possible. This way, the new pride males can mate with the females in the pride themselves. The former pride males, will have suffered severe injuries, or can be killed (Mosser & Packer, 2009).

Tigers have a different social structure compared to lions. The tiger is a solitary species, only coming together with other conspecifics during the mating season (Gour, *et al.*, 2013). However, like lions, tigers are territorial. Several studies on Amur (Siberian) tigers (*Panthera tigris altaica*) show that home-ranges of these tigers are exclusive (Matyushkin, 1978; Salkina, 1993). Another

study however, suggests home-ranges of Amur tigers overlap considerably (Bragin, 1986).

In zoos, lions are kept in relatively small groups. In this study, the groups consisted of one male with one or two females. The tigers were kept in similar groups, also one male with one or two females. Since tigers are solitary in the wild, this is an unnatural situation, which in turn can lead to higher stress levels.

Apart from behavioral responses to stress, there are also physiological responses. The most important of these responses is an increase in the production of stress-hormones (cortisol) by the hypothalamic–pituitary–adrenal (HPA) axis (Sindi *et al.*, 2014).

This was also seen in a study of Chosy *et al.* (2014), when they compared fecal glucocorticoid metabolite concentrations of 4 different species of felines. They found higher concentrations during a period their exhibit was under construction, compared to their baseline levels.

In wild Amur tigers, Naidenko *et al.* (2011) found there are seasonal differences in HPA activity, with the highest activity from November to January. In captive Amur tigers however, they did not find this seasonal change. They hypothesize that this difference can be explained by unfavorable conditions in the wild during these months, like low temperatures and a thick layer of snow.

A season when conditions are not optimal, fights with conspecifics, or with other species,

and infanticide are all contributors to stress. The last one however, can also be the other way around. Unfortunately, infanticide of cubs is sometimes observed in zoos or other breeding facilities, where lack of food or major fights are not an issue.

The killing of cubs by their own mother, like it happened for instance in Gaia Zoo (Kerkrade, the Netherlands) and in Planckendael (Mechelen, Belgium) can be attributed to stress (GaiaZoo webpage, 2014; Algemeen Dagblad, 2009).

In recent years, zoos pay more attention to animal welfare. To minimize stress and improve animal welfare, zoos around the world are experimenting with different enrichment strategies for carnivores and other captive animals to reduce stress and to promote mating. Examples of this can be found in the more natural looking environments in the cages, compared to some decades ago with rock formations, plants, hide-outs, and ponds (Quirke, O'Riordan and Zuur, 2012; Quirke and O'Riordan, 2011; McPhee, 2002). Zoos are also experimenting with different feeding-enrichment strategies. These include experiments with the feeding times, where the animals have to be at the right place at the right time, performing the right action, to mimic hunting and hunting success in the wild (Quirke and O'Riordan, 2011; Burgener, Gusset and Schmid, 2008; McPhee, 2002).

The enrichment strategies described in these publications show different degrees of effect. There may be another way to decrease stress in zoo animals: limiting the visibility of the human public and thus reducing direct contact with humans.

This can be done by placing a Natural Fence over the outer fences of the enclosures (Fig. 1). The idea is that due to this natural fence the animals become less perceptive of the people watching them all the time, and therefore stress may decrease.

During this study, parts of the existing natural fence were removed, giving the animals a better view of the people watching them. After two weeks, the fence was placed back to its original state.

The aim of my study was to analyze the

influence of the Natural Fence around enclosures with large felines (lions and tigers), by looking at both behavioral traits as well as cortisol levels.

I define the following research questions: 1) Are there any differences in behavior of the cats between the three phases (*Before*, *During* and *After* removal of the fence) and 2) Are there differences in cortisol levels before the fence was removed compared to when the fence was removed for two weeks (but before the fence was placed back)?

At the same time, cortisol was analyzed using a new technique, using hair. Cortisol is able to passively diffuse into growing hair cells from the capillary network surrounding the hair follicles. When the cells become keratinized and dehydrated, the cortisol is incorporated in the hair (Thieme and Sachs, 2007). To my knowledge, hairs have never before been used to determine cortisol levels in big felines.

I hypothesized that in the *During* phase in any case more stereotypic pacing would be seen, compared to the other phases. Also, I expected to see higher cortisol levels in the *During* phase, than in the *Before* phase.

## METHODS

### Study Location

Since October 26<sup>th</sup> 2011, Landgoed Hoenderdaell, a private zoo in Anna Paulowna (Noord-Holland, the Netherlands) has started a program to provide homes for discarded circus felines.

This foundation, called *Stichting Leeuw*, has a building in the center of the zoo, allowing people to see the cats as they visit the zoo. Along the outer enclosures of these cages is a path of about one and a half meters in width for the caretakers (Fig. 1). Next to this path is a path for the visitors of Landgoed Hoenderdaell, which lies about 80 centimeters lower than the caretakers' path. These paths are also separated from each other by another fence. This last fence is a "Natural Fence", meaning that the wire mesh of the fence is covered with strips of bark, over which common ivy (*Hedera helix*) is growing. Visitors can see the animals through

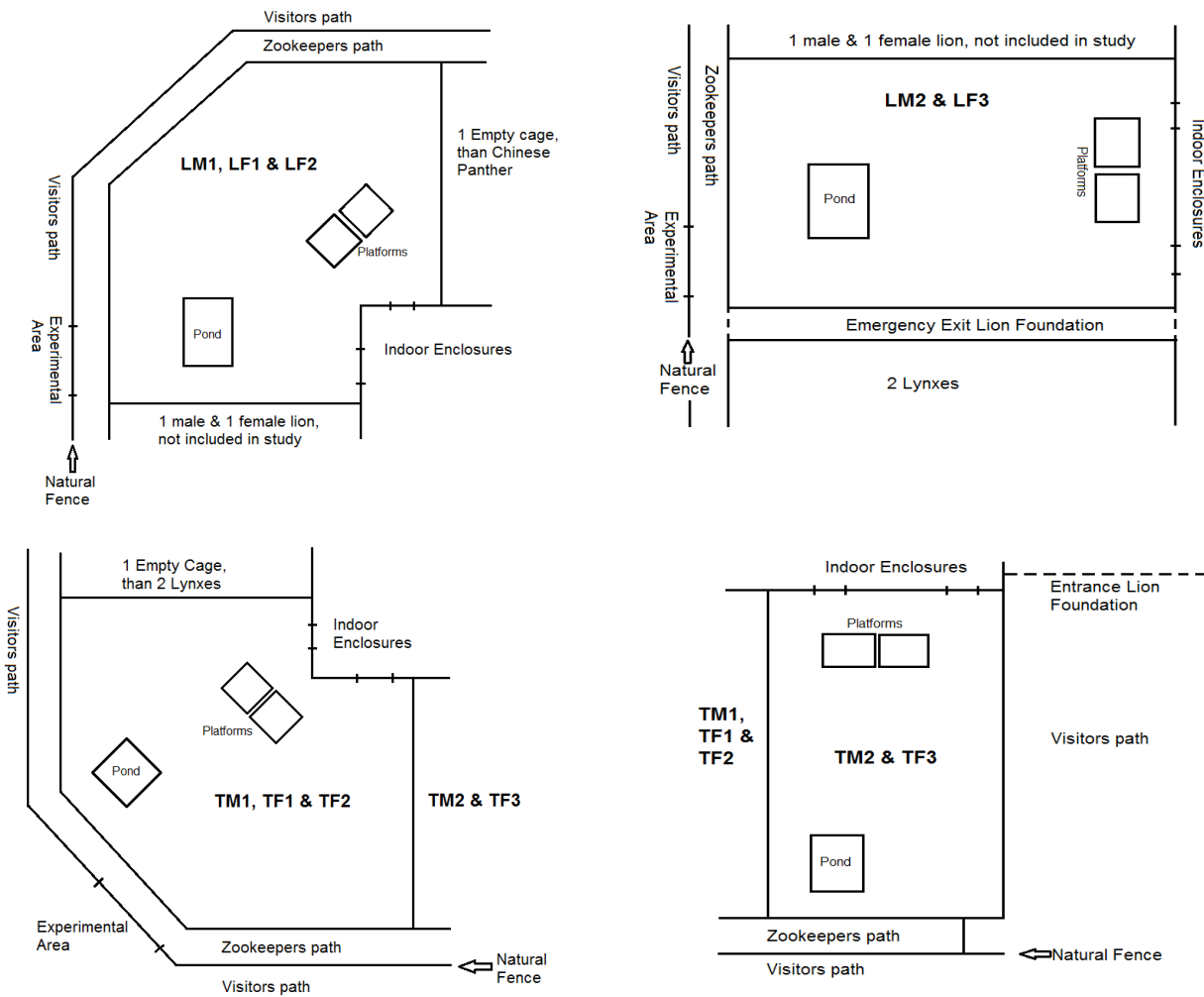


Fig. 1. The set-up of the experiment. From the indoor enclosures to the outer fence is about 18 meters. All cages are orientated with North to the right.

Top Left: A schematic map of the cage of male lion 1 and female lions 1 and 2.

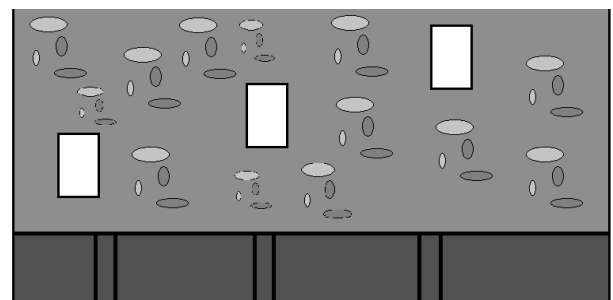
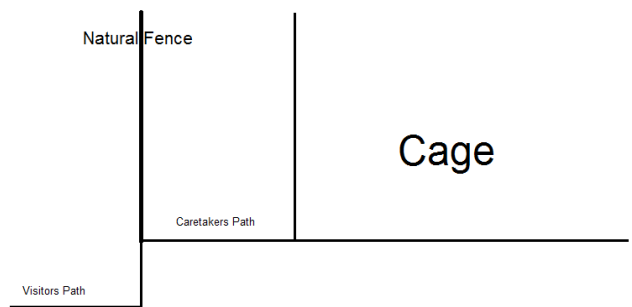
Top Right: A schematic map of the cage of male lion 2 and female lion 3.

Center Left: A schematic map of the cage of male tiger 1 and female tigers 1 and 2.

Center Right: A schematic map of the cage of male tiger 2 and female tiger 3.

Right: The side view of the cages

Bottom Right: The view of the visitors on the Natural fence, with the holes to look through.



**TABLE 1. Behavioral observations.** In this table the different types of behavior are shown. The first column is the code used in the ethogram, with its meaning in the second column and characteristics in the third (adapted from Quirke and O’Riordan, 2011).

The difference between SP, WA and PA is that WA stands for walking around in the cage, for instance to go to the pond for a drink, or lying down somewhere else. PA is pacing up and down like SP, but with the difference that there is an obvious reason. This can be that the vehicle that transports the meat for the animals just passed, or people are standing on the zookeepers path giving the animals some direct attention. SP is pacing up and down without any obvious reason.

Behavior	Description	Characteristics
SP	Stereotypic Pasing	Head lower than shoulders, no obvious reason, repetition of movement
SS	Standing Still	Self Explanatory
LU	Lying with Head Up	Self Explanatory
LD	Lying with Head Down	Self Explanatory
LP	Lying with Head on Paws	Self Explanatory
IC	Interaction	Either friendly or aggressive
SU	Sitting upright	Self Explanatory
PA	Pacing	Not SP. Head above shoulders. Often obvious reason, like food-cart passing
WA	Walking	Walking around (most of the) cage
LC	Licking/cleaning	Self Explanatory

holes in this fence of bark and ivy, with only the wire mesh of the fence between them and the caretakers' path. The base of the cage is also higher than the visitors' path. The idea of this is that it will let the animals feel they are in control. The five lions were, like the 5 tigers, distributed over two cages. One cage contained one male and one female, and the other contained one male and two females (Fig. 1).

### Behavioral Observations

For this study, five Bengal tigers (*Panthera tigris tigris*) and five African lions (*Panthera leo leo*) were observed. Tigers and lions were treated as separate groups during the entire study. After 2 weeks of observations, part of the Natural Fence in front of the cages was removed for 2 weeks. After these 2 weeks, the fence was placed back, and the observations continued for another 2 weeks. For the behavioral observations, I used an ethogram (Table 1) adapted from Quirke and O’Riordan, (2011). For 15 minutes during the morning between 09:00h (opening of the park) to 11:00h I observed the behavior of the individuals in each cage (four cages in total). The order of the cages was randomized. I repeated the observations from 12h to 13:30h and from 14:00h to 16:00h. This way, each cage, and therefore each individual, was observed for 45 minutes each day, 225 minutes a week.

### Cortisol Measurements

Cortisol levels were determined using hair. Hair samples were collected from the indoor enclosures. These enclosures were cleaned every day, and the cats cleaned themselves regularly. Hairs collected from the indoor enclosures are therefore either of that day, or the night before.

To extract this cortisol, methodologies of Accorsi *et al.* (2008) and Koren *et al.* (2002) were used. Accorsi *et al.* (2008) modified their methodology from Koren *et al.* (2002).

A sample of 10 mg of hair was cut into pieces of about 1 to 3 mm in length using fine scissors. 1,5 ml of pure methanol was then added to the hair. This was incubated at +50°C, while gently shaking. After 18h, the liquid was separated from the hairs using a filter. Using an air-stream suction hood at 37°C, the liquid was then evaporated to dryness. Then, 0.6 ml of phosphate-buffered saline (PBS) 0.05 M, pH 7.5 was added to dissolve the residue. Using a Human Saliva Cortisol ELISA kit, the amount of cortisol was obtained, using the protocol provided by the kit.

### Statistical Analysis

First, I tested the data for normality using the D’Agostino & Pearson Omnibus Normality Test (significance level for every test used was  $P < 0.05$ ).

Second, differences between males and females were tested using a Mann-Whitney Test on the average time each sex spent on every behavior type.

Then, differences in the time of day the observations were made were tested using the Friedman Test (nonparametric variant of the Repeated Measures Test) on the average time spent on every behavior per animal per time of day. Time of day was sorted into three groups: morning (from 09:00h to 11:00h), early afternoon (12:00h to 13:30h) and later in the afternoon (14:00h to 16:00h).

Using the Friedman Test, I compared the period of fence removal (*During*) with the other two periods (*Before* and *After* the fence was removed). For the Post-Hoc Test, a Dunn's Multiple Comparisons Test was used.

The difference between the phases was also determined using another test. For this test, it is first important to know that only those observation were included of which there was no difference between the individuals of the same sex. For the difference within sexes, a Kruskal-Wallis Test was used for the females (N=3). For the males I used a Mann-Whitney Test (N=2).

Then, I used a Kruskal-Wallis Test to determine differences between the phases a second time. Here as well, the Dunn's Multiple Comparisons Test was used as the Post-Hoc Test.

## RESULTS

### Behavior

For both lions and tiger, non of the behavior types showed a normal distribution for all three phases. Therefore nonparametric tests were used.

When male and female lions were compared across all three phases, no significant differences were found for any of the behavior types. As a result, males and females could be put together, making an N of 5 instead of 3 (females) and 2 (males).

When looking at the time of the day different observations were made, also no significant difference were found between observations made in the morning, those made in the early afternoon and those made later in the afternoon.

Because there is no difference between the time of the day the observations were made, nor a difference between males and females, all observation periods could be put together.

When testing for differences between the phases using a Friedman Test, there is a significant difference in behavior type SS across the phases ( $P=0.0123$ ;  $N=5$ ). A Post-Hoc Test confirms this difference to be only between the *During* and *After* phase. When looking at Fig. 2, the *During* phase has a much higher average number of seconds compared to both the *Before* and *After* phase. However, only the difference between

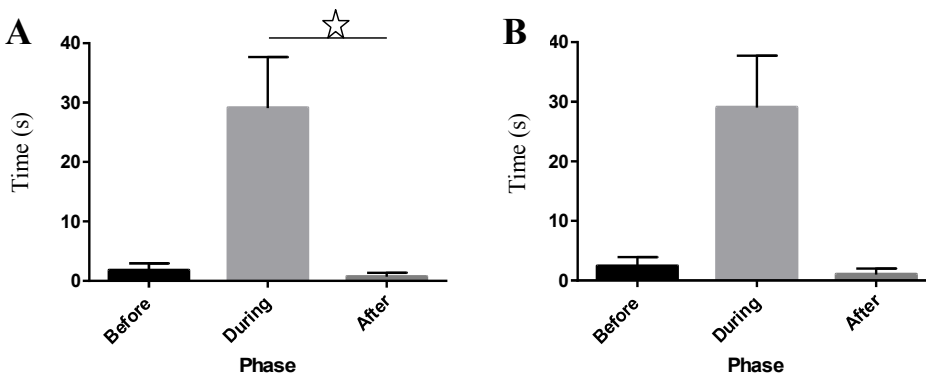


Fig. 2. Differences the phases for behavior type SS for the lions. The X-axes shows the phase, the Y-axes the mean of the seconds a specific behavior (in this case SS) occurs in 15 minutes of observation, shown with the Standard Error of the Mean.

A: Friedman Test. Even though there is a big difference between the *Before* and *During* phase, only the difference between the *During* and the *After* phase is significant when using a Friedman Test between the phases.

B: Kruskal-Wallis Test. Although this behavior did not show any significant differences between the phases when using the Kruskal-Wallis Test, this graph looks similar to the graph of the same behavior when using the Friedman Test.

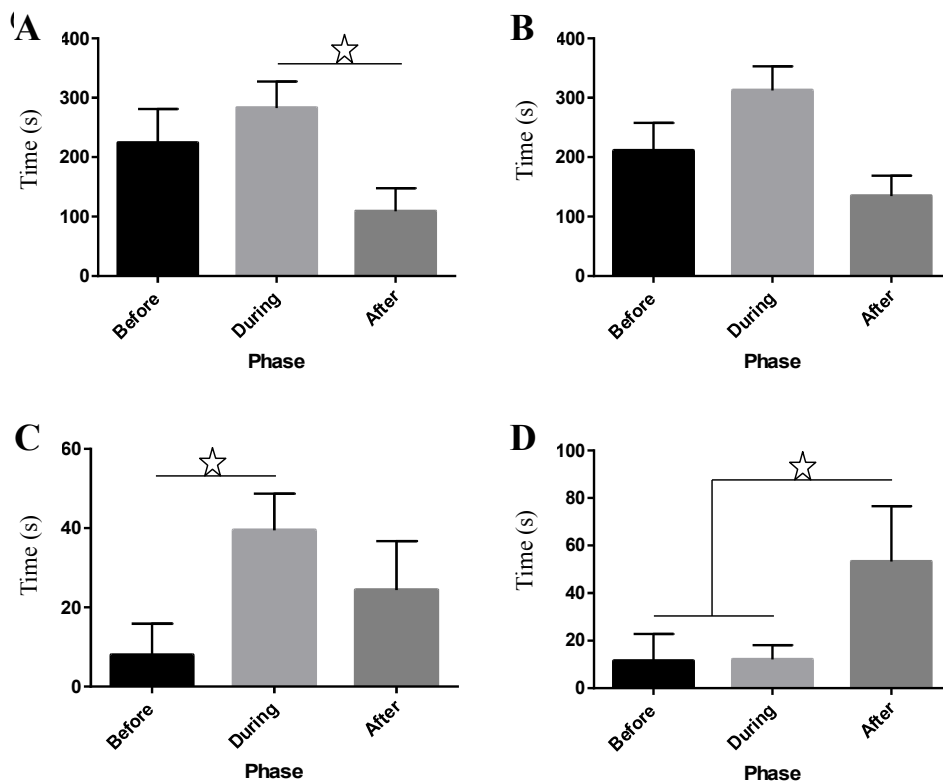


Fig. 3. Differences the phases for behavior type LU, WA and PA for the tigers. The X-axes show the phases, the Y-axes the mean of the seconds a specific behavior (WA, LU and PA) occur in 15 minutes of observation, shown with the Standard Error of the Mean.

- A: Using the Friedman Test on behavior type LU. There is a significant difference between the *During* and *After* phase.  
 B: Using the Kruskal-Wallis Test on behavior type LU. There is an overall significant difference between the phases.  
 C: Using the Kruskal-Wallis Test on behavior type WA. Phases *Before* and *During* are significantly different from each other.  
 D: Using the Kruskal-Wallis Test on behavior type PA. Phases *Before* and *During* differ significantly from the *After* phase.

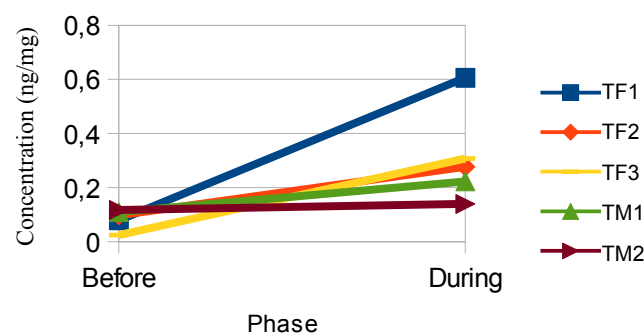


Fig. 4. Cortisol levels of the tigers. This Graph shows the cortisol concentrations (in ng/mg hair) of the individual animals determined from hair samples taken just before the fence was partly removed (*Before*), and just before the fence was put back (*During*). As we can see, the cortisol levels in the *During* period are higher for each cat, compared with the period before the fence was removed. The female tigers are TF1, TF2 and TF3. The male tigers are TM1 and TM2. Male lion Tristan also showed an increase of 232% in cortisol concentration when comparing *During* with *Before*. But since this was the only lion cortisol data was available for, this lion is left out of the graph.

*During* and *After* is significant.

There were a few behavior types where there were differences between the individuals of the same sex. For the females, there was an overall significant difference between the individuals in behavior type LC in the *During* phase (see appendix, p. 14). The two males were different in behavior type LU, also in the *During* phase (see appendix, p. 15).

Next, a Kruskal-Wallis Test was used to determine the differences in behavior types across the phases. Again, none of the data were used where the individuals of the same sex showed a difference. This means that e.g. for behavior type LU in the *During* phase, only the observations of the females were included. No significant results for any of the behavior types were found.

However, behavior type SS shows the same pattern as the graph made when using the Friedman Test (Fig. 2), with also a higher average number of seconds in the *During* phase, compared to the other phases.

Like with the lions, no difference was found in any of the behavior types observed in the tigers, and therefore males (N=2) and females (N=3) can be put together to get an N of 5 tigers.

There was no significant difference when comparing the observations made during different times of the day, making me able to put all observation periods together.

The Friedman Test showed an overall significant difference in the amount of time spent on behavior type LU ( $P=0.0394$ ,  $N=5$ ). A Post-Hoc Test showed the *During* and *After* phase differ significantly from each other (Fig. 3). For the males a Mann-Whitney Test showed that LU was significantly different between individuals in the *During* phase and LP in the *After* phase (see appendix, p. 17). For the females, significant differences were found between individuals in SP, LD and IC (see appendix, p. 16) using a Kruskal-Wallis Test.

Using the second test (Kruskal-Wallis), three behavior types showed significant differences across the phases for the tigers (again LU,  $P=0.037$ ,  $N=107$ ; WA,  $P=0.0456$ ,  $N=141$ ; PA,  $P=0.0092$ ,  $N=141$ ; Fig. 3).

LU has an overall significant difference, whereas a Dunn's Multiple Comparisons Test

shows the difference in WA can be found between the *Before* and *During* phase, and the difference in PA is between the *After* phase and the *Before* phase, as well as between the *After* phase and the *During* phase (Fig. 3).

### Cortisol

The cortisol concentrations of the five tigers as well as that of one male lion did not show significant differences between the end of the *Before* phase and the end of the *During* phase (Kruskal-Wallis Test).

However, all six animals showed an increase in cortisol concentration in the *During* phase, varying from 118% and 203% for the male tigers, to 283%, 765% and 1225% for the female tigers, and 232% for the male lion (Fig. 4).

### DISCUSSION

Although most behavior types did not show any significant differences across the phases, there were a few which were significantly different. However, the only behavior type that was found to be significantly different in both different tests was LU for the tigers, with LU highest in the *During* phase.

On the other hand, there were a few behavior types that had a very low P-value (like for the lions behavior type SP,  $P=0.0556$ ;  $N=5$  while using the Friedman Test), although not significant. This does indicate however that, when more animals are included in the experiment, these results could become significant. Though, when conducting an experiment in a zoo, it is not always possible to include more animals in a study.

Another individual observation I made during the phase when the fence was removed, was that one of the male tigers seemed to lie down in a corner, almost out of sight from the people standing in front of the hole in the Natural Fence. Before the fence was removed, and after it was placed back, this tiger did not lie down in that corner, but instead it lay down in the center of the enclosure.

Compared to other studies involving either feeding-enrichment or habitat-enrichment

(Burgener, Gusset and Schmid, 2008; McPhee, 2002; Quirke, O'Riordan and Zuur, 2012; Quirke and O'Riordan, 2011) this study found less differences in stress-levels across the phases. However, all differences found during this study are comparable with the results found in those other studies.

The Friedman Test used the number of animals as N. Since the number of animals in a zoo experiment is often very limited, like in this study, the Kruskal-Wallis Test was done as a second way of interpreting differences between the phases. Since there were no significant differences between the observations made on different times of the day, and no significant difference was found between males and females, I was able to use the number of observations as N, instead of the number of animals. With this larger N, I was able to find more significant differences, compared to using the actual number of animals as N.

Hairs turned out to be a very possible way of obtaining cortisol levels in an absolutely noninvasive way. The results were similar (although not significant) to the results Chosy *et al.* (2014) found while using feces. The only limit one might have is time. It takes a considerable amount of time to cut the hairs in small pieces. However, if the whole hair can be used for other studies, it would be much easier. Especially when a ball mill can be used.

The choice of using hair to determine cortisol concentrations in this study was a practical one. In future however, it will be important to know the growth speed of the hairs in these big cats. For this study, using these animals, this was not doable, due to the fact the animals would have to be sedated twice in a very short timespan. This in turn makes it an invasive method.

The initial idea for this project was to use feces, but this turned out to be hard to carry into effect for several reasons.

First, as the observations had started, it turned out to be practically impossible to determine the exact time the feces was dropped, which is essential since the metabolites of cortisol are volatile. And even when the exact time is known, it turned out that a lot of the cats all defecated at the same place in the cage, on top of the other, therefore making it difficult to

determine which cat was responsible for which part of the feces.

Another issue is the so-called ACTH challenge, necessary to serve as a standard in the feces cortisol metabolites test. Since the animals in this study are no pets or tamed animals, but potentially very dangerous, these animals have to be sedated, which makes the ACTH challenge unreliable.

Another possibility would have been saliva. Here again though, is the problem of how to obtain it.

Again, the animals would have needed to be sedated twice, and even then there is the possibility of contaminating the saliva samples with blood (Cook, 2012).

## CONCLUSIONS

Even though most behavior types did not differ between the phases, some significant differences were found for both the tigers and the lions.

Using hair turned out to be an effective way to determine cortisol levels in big cats. It is noninvasive, and hairs are easy to collect, in contrast to saliva, blood or feces.

The behavior combined with the differences in cortisol (although not significant, all animals tested showed an increase once the fence was removed) would make me suggest the fence works as it should. This Natural Fence does indeed provide a more relaxing living environment for the big cats.

## ACKNOWLEDGMENTS

I would like to thank Landgoed Hoenderdaell/Stichting Leeuw for providing the opportunity to study their animals for this project. Also, I would like to thank Christian Tudorache for his comments and help with the cortisol measurements and the statistics, and Hans de Iongh for his support and comments during this experiment.



## REFERENCES

- Accorsi, P.A., Carloni, E., Valsecchi, P., Viggiani, R., Gamberoni, M., Tamanini, C., Seren, E. (2008). Cortisol Determination in Hair and Faeces from Domestic Cats and Dogs. *General and Comparative Endocrinology*, 155, p. 398-402
- Algemeen Dagblad: <http://www.ad.nl/ad/nl/5596/Planet/article/detail/2056889/2009/12/12/Zeldzame-leeuw-bijt-drie-welpen-dood.dhtml> (in Dutch)
- Bragin, A.P. (1986). Territorial Behavior and Possible Mechanisms of Regulation of the Population Density in the Amur Tiger (*Panthera tigris altaica*). *Zoological Journal*, 65, p. 272–282 (in Russian)
- Burgener, N., Gusset, M., Schmid, H. (2008). Frustrated Appetitive Foraging Behavior, Stereotypic Pacing, and Fecal Glucocorticoid Levels in Snow Leopards (*Uncia uncia*) in the Zurich Zoo. *Journal of Applied Animal Welfare Science*, 11, p. 74-83
- Chosy, J., Wilson, M., Santymire, R. (2014). Behavioral and Physiological Responses in Felids to Exhibit Construction. *Zoo Biology*, 33, p. 267–274
- Cook, N.J. (2012). Review: Minimally Invasive Sampling Media and the Measurement of Corticosteroids as Biomarkers of Stress in Animals. *Canadian Journal of Animal Science*, 92, p. 227-259
- GaiaZoo webpage: <http://www.gaiazoo.nl/zoo-is-gaia/nieuws/leeuwenwelpen-gaiazoo/> (in Dutch)
- Gour, D.S., Bhagavatula, J., Bhavanishankar, M., Reddy, P.A., Gupta, J.A., *et al.* (2013) Philopatry and Dispersal Patterns in Tiger (*Panthera tigris*). *PLoS ONE*, 8 (7): e66956. doi:10.1371/journal.pone.0066956
- Koren, L., Mokady, O., Karaskov, T., Klein, J., Koren, G., Geffen, E. (2002). A Novel Method Using Hair for Determining Hormonal Levels in Wildlife. *Animal Behaviour*, 63, p. 403-406
- Mason, G.J. (1991). Stereotypies: a Critical Review. *Animal Behaviour*, 41, p. 1015-1037
- Matyushkin, E.N. (1978). Characteristic Features of Distribution and Ecology of the Amur Tiger as a Geographical Form of a Species Adapted to an Extreme Environment. *International Tiger Symposium, Leipzig Zoological Garden*, 1, p. 23–32
- McPhee, M.E. (2002). Intact Carcasses as Enrichment for Large Felids: Effects on On- and Off-Exhibit. *Zoo Biology*, 21, p. 37-47
- Mosser, A., Packer, C. (2009). Group territoriality and the benefits of sociality in the African lion, *Panthera leo*. *Animal Behaviour*, 78, p. 359-370
- Naidenko, S.V., Ivanov, E.A., Lukarevskii, V.S., Hernandez-Blanco, J.A., Sorokin, P.A., Litvinov, M.N., Kotlyar, A.K., Rozhnov, V.V. (2011). Activity of the Hypothalamo–Pituitary–Adrenals Axis in the Siberian Tiger (*Panthera tigris altaica*) in Captivity and in The Wild, and Its Dynamics Throughout the Year. *Biology Bulletin*, 38(3), p. 301-305
- Quirke, T., O’Riordan, R.M. (2011). The effect of a randomised enrichment treatment schedule on the behaviour of cheetahs (*Acinonyx jubatus*). *Applied Animal Behaviour Science*, 135, p. 103-109
- Quirke, T., O’Riordan, R.M., Zuur, A. (2012). Factors Influencing the Prevalence of Stereotypical Behaviour in Captive Cheetahs (*Acinonyx jubatus*). *Applied Animal Behaviour Science*, 142, p. 189-197
- Salkina, G.P. (1993). Current Status of a Population of Amur Tigers in the South-East Sikhote-Alin. *Bulletin Moscovsky Obschectva Prirody Otdyel Biologiya*, 98, p. 45–53
- Schaller, G.B. (1972). The Serengeti Lion: A study of Predator–Prey Relations. *Chicago: The University of Chicago Press*
- Sindi, S., Fiocco, A.J., Juster, R.P., Lord, C., Pruessner, J., Lupien, S.J. (2014). Now You See It, Now You Don’t: Testing Environments Modulate the Association Between Hippocampal Volume and Cortisol Levels in Young and Older Adults. *Hippocampus*, 24, p. 1623-1632
- Spong, G., Stone, J., Creel, S., Björklund, M. (2002). Genetic Structure of Lions (*Panthera leo L.*) in the Selous Game Reserve: implications for the evolution of sociality. *Journal of Evolutionary Biology*, 15, p. 945-953
- Thieme, D., Sachs, H. (2007). Examination of a Long-Term Clozapine Administration by High Resolution Segmental Hair Analysis. *Forensic Science International*, 166, p. 110- 14

**APPENDIX**

In this appendix I have the results of all the statistical tests that were done for this project.

Lions: Testing for Normality, *Before* (D'Agostino & Pearson omnibus normality test)

Behavior	P	N	Mean	St.Dev	St.Error	Conclusion
SP	< 0,0001	23	34,35	98,3	20,5	****
SS	< 0,0001	23	1,739	5,762	1,201	****
WA	Not observed	23	0	0	0	Not observed
LU	0,185	23	330,9	308,6	64,34	NS
LD	0,0809	23	283,5	351,2	73,23	NS
LP	0,016	23	206,1	330,5	68,91	*
IC	< 0,0001	23	3,478	11,52	2,403	NS
SU	< 0,0001	23	32,61	156,4	32,61	****
PA	< 0,0001	23	4,783	22,94	4,783	****
LC	< 0,0001	23	2,609	12,51	2,609	****

Lions: Testing for Normality, *During* (D'Agostino & Pearson omnibus normality test)

Behavior	P	N	Mean	St.Dev	St.Error	Conclusion
SP	< 0,0001	111	31,17	103,1	9,786	****
SS	< 0,0001	111	29,1	90,26	8,567	****
WA	< 0,0001	111	4,234	29,86	2,835	****
LU	0,0094	66	256,1	291,6	35,9	**
LD	< 0,0001	111	382,3	377,3	35,81	****
LP	< 0,0001	111	99,82	216,4	20,54	****
IC	< 0,0001	111	0,5405	4,009	0,3805	****
SU	< 0,0001	111	18,09	115,5	11,01	****
PA	< 0,0001	111	26,22	112,3	10,66	****
LC	< 0,0001	45	7,111	33,35	4,971	****

Lions: Testing for Normality, *After* (D'Agostino & Pearson omnibus normality test)

Behavior	P	N	Mean	St.Dev	St.Error	Conclusion
SP	Not observed	29	0	0	0	Not observed
SS	< 0,0001	29	0,6897	3,714	0,6897	****
WA	< 0,0001	29	17,24	80,44	14,94	****
LU	0,1312	29	293,4	307,2	57,05	NS
LD	< 0,0001	29	458,6	381,3	70,81	****
LP	< 0,0001	29	111,7	220,9	41,01	****
IC	< 0,0001	29	6,207	23,21	4,31	NS
SU	< 0,0001	29	9,655	51,99	9,655	****
PA	Not observed	29	0	0	0	Not observed
LC	< 0,0001	29	2,414	13	2,414	****

Tigers: Testing for Normality, *Before* (D'Agostino & Pearson omnibus normality test)

Behavior	P	N	Mean	St.Dev	St.Error	Conclusion
SP	0,0315	29	256,9	360	66,85	*
SS	< 0,0001	29	26,21	77,94	14,47	****
WA	< 0,0001	29	7,931	42,71	7,931	****
LU	0,0987	29	210,7	252,1	46,82	NS
LD	0,0046	29	297,6	390,7	72,55	**
LP	0,0003	29	159,3	307,5	57,09	***
IC	< 0,0001	29	6,207	33,43	6,207	****
SU	< 0,0001	29	1,724	9,285	1,724	****
PA	< 0,0001	29	11,38	61,28	11,38	****
LC	< 0,0001	29	49,31	156	28,97	****

Tigers: Testing for Normality, *During* (D'Agostino & Pearson omnibus normality test)

Behavior	P	N	Mean	St.Dev	St.Error	Conclusion
SP	< 0,0001	34	139,7	280,8	48,16	****
SS	< 0,0001	87	24,83	65,18	6,988	****
WA	< 0,0001	87	39,43	86,5	9,274	****
LU	0,0063	53	312,3	296,1	40,68	**
LD	0,0517	34	283,8	339,8	58,27	NS
LP	< 0,0001	34	83,24	165,3	28,34	****
IC	< 0,0001	34	1,176	6,86	1,176	****
SU	< 0,0001	87	4,483	31,02	3,326	****
PA	< 0,0001	87	12,07	56,45	6,052	****
LC	< 0,0001	87	31,95	77,86	8,348	****

Tigers: Testing for Normality, *After* (D'Agostino & Pearson omnibus normality test)

Behavior	P	N	Mean	St.Dev	St.Error	Conclusion
SP	0,0278	25	310,8	349,5	69,89	*
SS	< 0,0001	25	24,4	48,83	9,765	****
WA	< 0,0001	25	24,4	61,65	12,33	****
LU	0,0057	25	134,4	172,3	34,46	**
LD	0,0656	25	263,2	367,3	73,46	NS
LP	0,0015	16	143,8	295,2	73,79	**
IC	< 0,0001	25	2,4	7,234	1,447	****
SU	< 0,0001	25	6	24,49	4,899	****
PA	< 0,0001	25	53,2	116,9	23,38	****
LC	< 0,0001	25	28,4	74,93	14,99	****

Lions: Differences between males and females (Mann-Whitney Test)

Behavior	P	Conclusion
SP	0,4	NS
SS	> 0,9999	NS
WA	> 0,9999	NS
LU	0,4	NS
LD	0,9	NS
LP	0,9	NS
IC	0,7	NS
SU	0,3	NS
PA	> 0,9999	NS
LC	0,3	NS

Tigers: Differences between males and females (Mann-Whitney Test)

Behavior	P	Conclusion
SP	0,4	NS
SS	0,1	NS
WA	0,2	NS
LU	0,9	NS
LD	0,1	NS
LP	0,5	NS
IC	> 0,9999	NS
SU	> 0,9999	NS
PA	0,9	NS
LC	0,9	NS

Lions: Differences in Times of the Day the observations were made (Friedman Test)

Behavior	P	N	Treatments	Conclusion
SP	0,3873	5	3	NS
SS	0,9537	5	3	NS
WA	0,0741	5	3	NS
LU	0,9537	5	3	NS
LD	0,9537	5	3	NS
LP	0,5216	5	3	NS
IC	0,5556	5	3	NS
SU	> 0,9999	5	3	NS
PA	0,1944	5	3	NS
LC	0,9815	5	3	NS

Tigers: Differences in Times of the Day the observations were made (Friedman Test)

Behavior	P	N	Treatments	Conclusion
SP	0,9537	5	3	NS
SS	> 0,9999	5	3	NS
WA	0,3673	5	3	NS
LU	> 0,9999	5	3	NS
LD	0,1821	5	3	NS
LP	0,1111	5	3	NS
IC	0,8426	5	3	NS
SU	> 0,9999	5	3	NS
PA	0,8426	5	3	NS
LC	0,3673	5	3	NS

Lions: Differences within the sexes, Females, *Before* (Kruskal-Wallis Test)

Behavior	P	N	Observations	Conclusion
SP	> 0,9999	3	14	NS
SS	0,5714	3	14	NS
WA	Not observed	3	14	NS
LU	0,8143	3	14	NS
LD	0,181	3	14	NS
LP	0,8078	3	14	NS
IC	> 0,9999	3	14	NS
SU	> 0,9999	3	14	NS
PA	Not observed	3	14	NS
LC	0,5714	3	14	NS

Lions: Differences within the sexes, Females, *During* (Kruskal-Wallis Test)

Behavior	P	N	Observations	Conclusion
SP	0,4311	3	66	NS
SS	0,4019	3	66	NS
WA	0,3748	3	66	NS
LU	0,5129	3	66	NS
LD	0,3326	3	66	NS
LP	0,0791	3	66	NS
IC	Not observed	3	66	NS
SU	0,6201	3	66	NS
PA	0,3016	3	66	NS
LC	0,0256	3	66	*

Lions: Differences within the sexes, Females, *After* (Kruskal-Wallis Test)

Behavior	P	N	Observations	Conclusion
SP	Not observed	3	16	NS
SS	0,5	3	16	NS
WA	0,5	3	16	NS
LU	0,9635	3	16	NS
LD	0,6749	3	16	NS
LP	0,8443	3	16	NS
IC	0,5	3	16	NS
SU	0,5	3	16	NS
PA	Not observed	3	16	NS
LC	0,5	3	16	NS

Lions: Differences within the sexes, Males, *Before* (Mann-Whitney Test)

Behavior	P	N	Observations	Conclusion
SP	Not observed	2	9	NS
SS	0,3333	2	9	NS
WA	Not observed	2	9	NS
LU	0,7738	2	9	NS
LD	0,2619	2	9	NS
LP	0,1667	2	9	NS
IC	> 0,9999	2	9	NS
SU	Not observed	2	9	NS
PA	0,3333	2	9	NS
LC	Not observed	2	9	NS

Lions: Differences within the sexes, Males, *During* (Mann-Whitney Test)

Behavior	P	N	Observations	Conclusion
SP	0,165	2	45	Ns
SS	0,9377	2	45	Ns
WA	Not observed	2	45	NS
LU	0,0387	2	45	*
LD	0,2468	2	45	NS
LP	0,5956	2	45	NS
IC	0,4667	2	45	NS
SU	0,7277	2	45	NS
PA	0,2121	2	45	NS
LC	> 0,9999	2	45	NS

Lions: Differences within the sexes, Males, *After* (Mann-Whitney Test)

Behavior	P	N	Observations	Conclusion
SP	Not observed	2	13	NS
SS	Not observed	2	13	NS
WA	0,3846	2	13	NS
LU	0,9029	2	13	NS
LD	0,2797	2	13	NS
LP	0,324	2	13	NS
IC	0,3846	2	13	NS
SU	Not observed	2	13	NS
PA	Not observed	2	13	NS
LC	Not observed	2	13	NS

Tigers: Differences within the sexes, Females, *Before* (Kruskal-Wallis Test)

Behavior	P	N	Observations	Conclusion
SP	0,9287	3	17	NS
SS	0,8284	3	17	NS
WA	> 0,9999	3	17	NS
LU	0,302	3	17	NS
LD	0,2747	3	17	NS
LP	0,2966	3	17	NS
IC	Not observed	3	17	NS
SU	0,5882	3	17	NS
PA	Not observed	3	17	NS
LC	0,7549	3	17	NS

Tigers: Differences within the sexes, Females, *During* (Kruskal-Wallis Test)

Behavior	P	N	Observations	Conclusion
SP	0,0005	3	53	***
SS	0,6173	3	53	NS
WA	0,4716	3	53	NS
LU	0,6375	3	53	NS
LD	0,0049	3	53	**
LP	0,3979	3	53	NS
IC	0,033	3	53	*
SU	0,4668	3	53	NS
PA	0,2814	3	53	NS
LC	0,5527	3	53	NS

Tigers: Differences within the sexes, Females, *After* (Kruskal-Wallis Test)

Behavior	P	N	Observations	Conclusion
SP	0,2887	3	16	NS
SS	0,4866	3	16	NS
WA	0,7858	3	16	NS
LU	0,6532	3	16	NS
LD	0,125	3	16	NS
LP	0,825	3	16	NS
IC	0,125	3	16	NS
SU	0,125	3	16	NS
PA	0,8519	3	16	NS
LC	0,125	3	16	NS



Tigers: Differences within the sexes, Males, *Before* (Mann-Whitney Test)

Behavior	P	N	Observations	Conclusion
SP	0,3106	2	12	NS
SS	0,4697	2	12	NS
WA	Not observed	2	12	NS
LU	0,3245	2	12	NS
LD	0,3434	2	12	NS
LP	0,4167	2	12	NS
IC	> 0,9999	2	12	NS
SU	Not observed	2	12	NS
PA	> 0,9999	2	12	NS
LC	> 0,9999	2	12	NS

Tigers: Differences within the sexes, Males, *During* (Mann-Whitney Test)

Behavior	P	N	Observations	Conclusion
SP	0,0682	2	34	NS
SS	0,5153	2	34	NS
WA	0,897	2	34	NS
LU	0,0134	2	34	*
LD	0,55	2	34	NS
LP	0,8906	2	34	NS
IC	0,4118	2	34	NS
SU	0,7504	2	34	NS
PA	0,1622	2	34	NS
LC	0,2584	2	34	NS

Tigers: Differences within the sexes, Males, *After* (Mann-Whitney Test)

Behavior	P	N	Observations	Conclusion
SP	> 0,9999	2	9	NS
SS	0,2222	2	9	NS
WA	Not observed	2	9	NS
LU	0,4444	2	9	NS
LD	0,8611	2	9	NS
LP	0,0278	2	9	*
IC	> 0,9999	2	9	NS
SU	> 0,9999	2	9	NS
PA	> 0,9999	2	9	NS
LC	> 0,9999	2	9	NS

Lions: Differences between the phases (Friedman Test)

Behavior	P	N	Treatments	Conclusion
SP	0,0556	5	3	NS
SS	0,0123	5	3	*
WA	0,5556	5	3	NS
LU	0,9537	5	3	NS
LD	0,9537	5	3	NS
LP	0,6914	5	3	NS
IC	0,7593	5	3	NS
SU	0,3086	5	3	NS
PA	0,1111	5	3	NS
LC	0,4444	5	3	NS

Lions: Differences between the phases (Kruskal-Wallis Test)

Behavior	P	Observations	Treatments	Conclusion
SP	0,1747	163	3	NS
SS	0,0945	163	3	NS
WA	0,4121	163	3	NS
LU	0,5025	118	3	NS
LD	0,269	163	3	NS
LP	0,1074	163	3	NS
IC	0,1571	163	3	NS
SU	0,9678	163	3	NS
PA	0,4395	163	3	NS
LC	0,9722	97	3	NS

Tigers: Differences between the phases (Friedman Test)

Behavior	P	N	Treatments	Conclusion
SP	> 0,9999	5	3	NS
SS	> 0,9999	5	3	NS
WA	0,1358	5	3	NS
LU	0,0394	5	3	*
LD	0,9537	5	3	NS
LP	0,6914	5	3	NS
IC	0,4383	5	3	NS
SU	0,5926	5	3	NS
PA	0,1728	5	3	NS
LC	> 0,9999	5	3	NS

Tigers: Differences between the phases (Kruskal-Wallis Test)

Behavior	P	Observations	Treatments	Conclusion
SP	0,1299	88	3	NS
SS	0,6866	141	3	NS
WA	0,0456	141	3	*
LU	0,037	107	3	*
LD	0,7977	88	3	NS
LP	0,902	79	3	NS
IC	0,3069	88	3	NS
SU	0,6044	141	3	NS
PA	0,0092	141	3	**
LC	0,8354	141	3	NS