

SOS Rhino

# Sumatran rhino breeding program in Sepilok 2004 – 2005

Data collected and preliminary results



Tanjung

Gelugob

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## **1. INTRODUCTION:**

In 1985, the Rhino and Wildlife Conservation Committee (SRWCC) of Sabah established a local capture and breeding program. The capture program was activated in 1987 and began with the capture of rhinos that were exposed to poachers due to the loss of their habitat. A total of 10 rhinos were caught in Sabah between 1987 and 1995 but only eight of them survived. Seven of them were moved to the rhino breeding center in Sepilok, Sabah and one animal was equipped with a radio transmitter and released back into the wild (Bosi 1996).

In the year 2004 only two of the original seven rhino were still alive. The rhinos were under the care of the Sabah Wildlife Department (J.H.L.). SOS rhino is assisting the Sabah Wildlife Department with manpower and foreign expertise. The present report gives an overview of the management of the rhinos in Sepilok and it provides scientific data collected between 2004 and 2005, such as fecal hormones profiles, weight, food intake, body temperature, health of the rhinos.

## **2. ANIMALS, RHINO FACILITIES AND MANAGEMENT:**

### **2.1 Animals:**

Data was collected from two Sumatran rhinos (1.1) kept in the Rhino Breeding Centre in Sepilok, Sandakan, Sabah, Malaysia. Both rhinos were born in the wild. The male was caught on the 20th of July 1993 in the area of Lahad Datu, Sabah and the female was caught on the 17th of June 1994 in Sukau, Kinabatangan, Sabah. The estimated date of birth for the male was 1989 and for the female 1990.



Picture 1: The male Tanjung in the large outdoor enclosure



Picture 2: The female Gelugob in a mud wallow in the small outdoor enclosure.

## **2.2 Rhino enclosures:**

The facility consisted of three individual night stalls, and two outdoor enclosures. Each night stall had an indoor area with a water basin and an outside area with a cement wallow. The indoor area was covered by a corrugated iron roof; the outside area was open. In April 2004 however the outside area was covered by an opaque net in order to avoid that the animals are exposed to direct sunlight, especially when they are wallowing. The floor of the outside area of the night enclosures consisted out of soil. Soil is ideal for the feet of the rhinos but it was impossible to clean and to disinfect the enclosure. Therefore in April 2004, the floor of both enclosures was cemented (Picture 3). The outdoor enclosure consisted out of natural rainforest which was fenced with walls made from tropical hardwood (“belian”). The areas differed in size. The so called “breeding enclosure” was 2.5 acre or 0.01 square kilometers in size. The vegetation in this large enclosure can be characterized as a secondary rainforest consisting of a few tall trees and lots of bushes with a relatively dense canopy. Part of this enclosure was a visitor platform and a walkway for visitors but it was closed most of the time. The other outdoor enclosure was much smaller, approximately 0.5 acre in size. The vegetation in this small outdoor enclosure can be characterized as grass land, with a few bushes and trees. It did not provide a lot of cover and it had a noticeable inclination. Both enclosures consisted of a mud pool which the rhinos used for a mud bath. The night enclosures and the outdoor were built next to each other and the rhinos could see and hear each other through the wooden planks. The rhino facility had a chute for handling of the rhinos. The front and back part of the chute consist of iron poles that could be fully removed. The side parts consist of a movable iron grid which could be adjusted to the width of the animals (Picture 4).



Picture 3: Outside area of the night enclosure after renovation    Picture 4: Front of the chute with Gelugob inside.

### **2.3 Rhino management:**

Seven people were working in the rhino enclosures: Four staff from the Wildlife Department (James, Silih, Azri and Irwan) and 3 staff from SOS rhino (Dr. Petra, Justin, Benji). The work was scheduled as following: Two people went twice daily in the surrounding villages to collect suitable tree species as food for the rhinos. Three people stayed with the rhinos to clean the cages, to feed them and to collect the data. Two people were only part time working with the rhinos. They helped with different kinds of work like building in the enclosure, blood collection, treatment of the animals etc.

#### **2.3.1 Frequency of releasing the rhinos into the oversight enclosure:**

The rhinos were only released when they were healthy, and if there were enough people to monitor them. The male was usually released in the large outdoor enclosure and the female into the small.

In the year 2004, Tanjung spend 191 days of 232 days of observation in the outside enclosure (173 days in the large enclosure and 18 days in the small enclosure), and Gelugob spend 68 days of 233 days of observation in the outside enclosure (43 days in the small enclosure, 25 days in the large enclosure).

In the year 2005, the rhinos were seldom released in the outside enclosure. Reasons for that were health problems, risk of injury, problems with the enclosure and lack of people. Tanjung spend 62 days of 504 days of observation in the outside enclosure (= 1875 minutes) and Gelugob spend 29 days of 504 days of observation outside (= 586 minutes).

### **3. METHODS:**

The data and results for the year 2004 and 2005 will be presented as two separate reports as they were prepared as annual reports.

In the following the data and results for the year 2004 are presented.

#### **3.1 Sample collection in the year 2004:**

Data was collected from the 17<sup>th</sup> of March until the 31<sup>st</sup> of December 2004.

### **3.2 Blood collection:**

A total of 80 blood samples were collected in the year 2004, 52 samples from the female and 28 samples from the male (see Graph 1). The animals were trained to walk into a chute while blood was collected from the coccygeal vein of the tail. Blood collection was stopped if the rhino showed any signs of stress. The sample collection started on the 17<sup>th</sup> of March 2004 and finished on the 23<sup>rd</sup> of December 2004. During these 41 weeks of observation, we managed to collect one to three samples per week depending on the health condition of the animals and the success in collecting the sample (Graph 1). The blood samples were used for blood chemistry analyses.

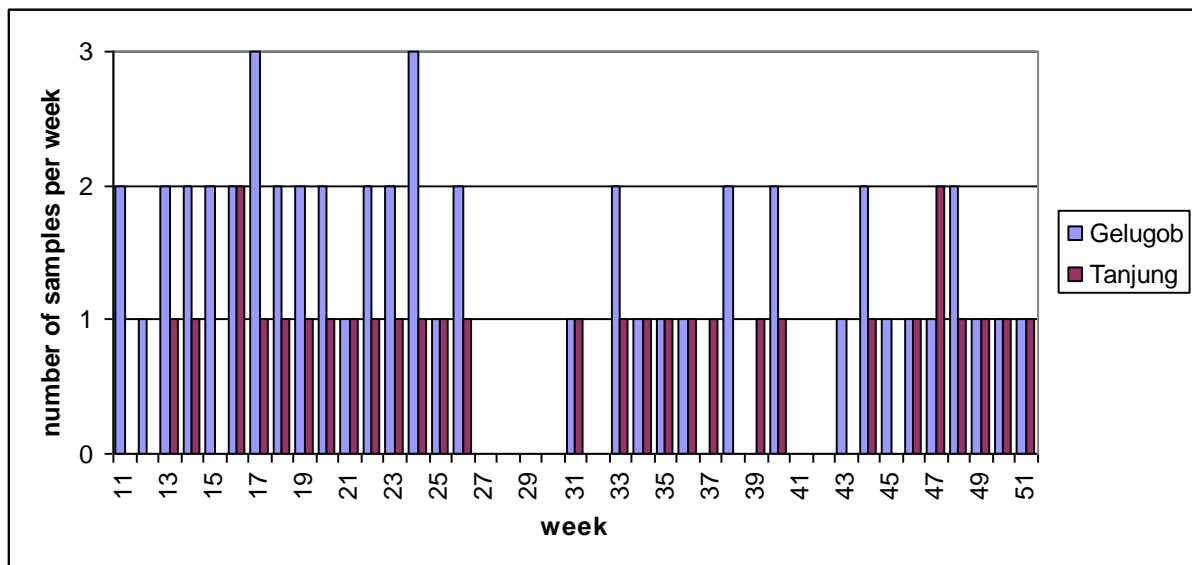


Figure 1: Number of blood samples collected from the male (Tanjung) and the female (Gelugob) in 2004.

The samples were collected in EDTA and serum tubes and sent on the same day to a local laboratory (Japlab, Sandakan, Sabah) for further analyses. The following blood parameters were analyzed: Haemoglobin, total white blood cell count (WBC), Neutrophils, Lymphocytes, Monocytes, Eosinophils (Eosin), Basophils (BAS), erythrocyte sedimentation rate (ESR), Platelets, UREA, creatinine, total bilirubin, total protein, albumin, globulin, Albumin/Globulin ratio, A/K Phosphatase, aspartate aminotransferase (SGOT), alanine aminotransferase (SGPT).



### **3.3 Fecal sample collection:**

A total of 595 fecal samples were collected from the male and female rhino in Sepilok, 301 samples from the female and 294 samples from the male (see Figure 2). The sample collection started on the 12<sup>th</sup> of March 2004 and ended on the 31<sup>st</sup> of December 2004.

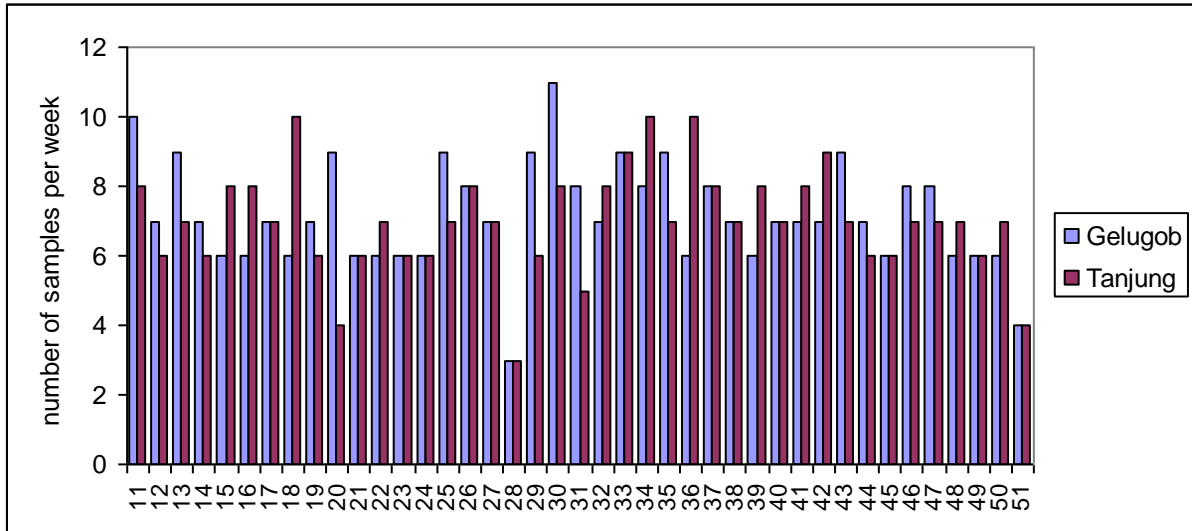


Figure 2: Number of fecal samples collected from the male (Tanjung) and the female (Gelugob) in 2004

Fecal samples were either collected from the rectum of the animal, from the wallow, or from the ground of the night enclosure. The samples collected from the water and from the ground of the enclosure were approximately half a day old. They were collected in the morning before cleaning the enclosure. Fresh fecal samples were collected from the rectum of the female, which took place in the morning. The feces were collected in a plastic bag, mixed and different parts of the dung were placed into a plastic tube. The tube was stored at  $-20^{\circ}\text{C}$  until processing. The fecal samples were shipped in Styrofoam to ensure that they stayed frozen during transportation.

#### **3.3.1 Fecal hormone analysis:**

The fecal hormone analyses were conducted by Assistant Prof. Dr. Schwarzenberger. The samples were analyzed in two different trials: all fecal samples collected between March 2004 and August 2004 were analyzed at once, the second trial contained fecal samples collected between August 2004 and March 2005.

Fecal samples were extracted and analyzed using an enzyme-immunoassays for immunoreactive total estrogen metabolites as described previously for the Indian rhinoceros

(Schwarzenberger et al, 2000); the group-specific antibody used was produced against oestradiol-17 $\beta$ -OH 17-HS:BSA.

### **3.4. Behavior observations:**

The behavior of the male and female was monitored six days per week, starting on the 29<sup>th</sup> of March 2004 and finishing on the 31<sup>st</sup> of December 2004. The first month was used to train the people in behavior observations and the data are not included in the analysis. This resulted in a total of 189 days of observation (from the 1<sup>st</sup> of May until the 31<sup>st</sup> of December 04).. Each rhino was monitored 6 times per day (3 times each in the morning and 3 times in the afternoon), starting from 9:15 in the morning and finishing at five in the afternoon. Each animal was observed for 15 minutes per hour. The time was measured with a stop watch giving a signal at the end of each observation period. The total time of observation was 483.25 hours. The male and female were observed almost the same length of time, Tanjung: 244.75 hours and Gelugob: or 238.5 hours. A total of 13 behavior categories were monitored. The behavior measured and the description of the behavior is given in Table 1.

Table 1: Behavior categories measured in 2004, the method used to describe the behavior (frequency/duration) and the description of the behavior is given.

<b>behavior event</b>	<b>what was measured</b>	<b>description of the event</b>
<b>feeding</b>	Frequency of feeding events	A new feeding event started when the animal walked more then 3 steps
<b>Lying</b>	The duration the animal was lying on the ground	Lying started when the animal was lying down and stopped when it got up
<b>wallowing</b>	The duration the animal was lying in the wallow	
<b>vocalization</b>	Frequency of single vocals	Each vocal was counted. Frequency modulated vocals were counted as one vocal. A new vocal started after a few seconds of silence.
<b>urinating</b>	Frequency of urination	The whole urination process was counted as a singular event it lasted until no fluid was emitted anymore
<b>urinating with hind leg</b>	Frequency of urination in combination with scratching of the hind leg	The whole urination process in combination with scratching of the hind leg was counted as a singular event it lasted until no fluid was emitted anymore
<b>defecating</b>	Frequency of defecation	The whole defecating process was counted as a singular event
<b>walking</b>	Frequency on initiation of walking	The event "walking" started when the animal walked more than 3 steps. It stopped when the animal was standing for more than 2 seconds.
<b>drinking</b>	Frequency of drinking	The event "drinking" stopped when the animal started to walk.
<b>comfort horn rubbing on trees</b>	Frequency of rubbing with the horn against an object	The event stopped when the animal started to walk

<b>comfort</b>	Frequency of rubbing with the whole body against an object	The event stopped when the animal started to walk
<b>contact between male and female</b>	The duration of direct contact between male and female	The "contact" started when the animal were in direct contact and it stopped when the animals were detached
<b>Erection</b>	The duration of the erection was measured	The event started when the penis was fully erected and stopped when the penis was retracted

In this report the behavior category “erection” has been analyzed only. The frequency of erection was correlated with fecal hormone analysis.

### **3.4.1 Video observation:**

Video observation started in October 2004. The behavior was recorded on two days per week. A total of 750 minutes were recorded, 570 minutes of Gelugob and 180 minutes of Tanjung. The video observation will help to consolidate findings made by direct observation and hopefully help to uncover obscure behavior. So far the data have not been analyzed.

### **3.5 Food intake:**

The food was collected twice daily at farms in the surrounding of Sepilok. The rhinos were fed 4 times daily and the amount and the type of food given were recorded every time. The left over from the previous day were weight the next day to calculate the amount of food eaten by the rhino.

The rhinos had daily access to a saltlick from AKZO NOBEL SALT. The composition of the saltlick is given in Table 2.

Table 2: Mineral composition of the salt lick provided daily to the rhinos.

<b>Minerals</b>	<b>concentration</b>
<b>NaCL</b>	> 97 %
<b>Na</b>	38.5 %
<b>Magnesium</b>	2000 mg/kg
<b>Manganese</b>	830 mg/kg
<b>Zinc</b>	810 mg/kg
<b>Copper</b>	220 mg/kg
<b>Iodine</b>	100 mg/kg
<b>Cobalt</b>	18 mg/kg
<b>Selenium</b>	10 mg/kg

### **3.6 Weight of the rhinos:**

The scale was broken and the rhinos were only weight twice per year, in January 2004 and in December 04.

### **3.7 Body temperature:**

The temperature was measured daily, except on Sundays starting on the 17<sup>th</sup> of November 2004 until the 30<sup>th</sup> of December. It was measured with an electronic thermometer in the rectum of the animal.

### **3.8 Ultrasound analysis:**

In May 2004 Dr. Nan Schaffer conducted ultrasound examinations of the male and female rhino. The female was examined on the 5<sup>th</sup>, 10<sup>th</sup> and 11<sup>th</sup> of May and on the 5<sup>th</sup> and 7<sup>th</sup> and 8<sup>th</sup> of November 04. The male was examined on the 6<sup>th</sup> and on the 10<sup>th</sup> of May only. The ultrasound machine used for the investigation was a Hitachi EUB-905.

### **3.9. Semen collection:**

Semen collection was attempted 11 times; 4 times in March, 3 times in April, 1 time in May, 2 times in August and 1 time in September. For semen collection his penis was covered with some lubricant and a plastic glove. He was scratche on the back which he liked very much and he got a penile massage as well as a rectal palpation. He usually got an erection after a few minutes but often there was no ejaculation. The whole procedure could last for 45 minutes depending on the reaction of the male. The fluid collected was spun for 10 minutes in a centrifuge at maximum speed and analyzed under the microscope.

## **4. RESULTS 2004:**

### **4.1 Blood parameters**

The concentration of blood parameters established for Tanjung and Gelugob are given in Table 5 (see Appendix). A comparison of the median concentration of the blood parameters of the rhinos in Sepilok with the values given for Sumatran rhinos by Robin Radcliffe (Radcliffe et al 2004) reveals lower median concentration in hemoglobin, total white blood cell count

and globulin in the rhinos from Sepilok (Table 3). The comparison reveals higher median concentration in the parameter bilirubin in Sepilok compared to the values given in the literature (Table 3).

Table 3: Median concentration of blood parameters measured for the male and female Sumatran rhino in Sepilok compared with values for Sumatran rhinos given by Radcliffe.

		values Radcliffe	male median	female median	all values Sepilok median	difference Sepilok/Radcliffe
<b>Haemoglobin</b>	g/dl	13-14,1	9,65	10,05	9,85	-3,65
<b>PCV (hematocrit)</b>	%	34 - 45				
<b>Total W.B.C.</b>	/cmm	7800 - 11000	5700	8350	7025	- 800
<b>Neut.</b>	%		59	62,5	60,75	
<b>Lymph.</b>	%		41	35,5	38,25	
<b>Mono.</b>	%		0	2	1	
<b>Eosin</b>	%		0	0	0	
<b>BAS</b>	%		0	0	0	
<b>ESR</b>	mm/hr		7,5	11,5	9,5	
<b>Platelets</b>	/cmm		183000	203000	193000	
<b>UREA</b>	mmol		5,8	1,85	3,825	
<b>Sodium</b>	mmol/l	130 ± 5				
<b>Potassium</b>	mmol/l	4,5 ± 0,5				
<b>Chloride</b>	mmol/l	95 ± 5				
<b>Creatinine</b>	umol/l	133 ± 44 *	105	84,5	94,75	
<b>Total Bilirubin</b>	umol/l	5 ± 3 *	12	10	11	+ 3
<b>Total Protein</b>	g/l	75 ± 10 *	72	72	72	
<b>Albumin</b>	g/l	30 ± 10 *	40	38,5	39,25	
<b>Globulin</b>	g/l	50 ± 10 *	32	34	33	- 7
<b>A/G Ratio</b>			1,3	1,1	1,2	
<b>A/K Phosphatase</b>	U/L		89	77	83	
<b>SGOT</b>	U/l		18	14	16	
<b>SGPT</b>	U/l		25	14	19,5	
<b>Calcium</b>	mg/dl	12,5 ± 1				

\* values from Radcliffe have been converted

## **4.2 Fecal hormone concentration:**

The median estrogen metabolite concentration differs between fecal samples of the female collected between March and August 2004 (median 1.15 ng/g feces, IQR = 0.45, n = 72) and samples collected between August 2004 and March 2005 (0.63 ng/g feces, IQR = 0.20,

n = 78). The same applies for the median progesterone metabolite concentration (median<sub>March till August 2004</sub> 44 ng/g feces, IQR = 41, n = 72; median<sub>August till March 2005</sub>: 96 ng/g feces, IQR = 195, n = 78) and the median pregnandiol metabolite concentration (median<sub>March till August 2004</sub>: 37 ng/g feces, IQR = 34, n = 72; median<sub>August till March 2005</sub>: 66 ng/g feces, IQR = 55.5, n = 78) measured in the feces of the female Sumatran rhino. The fecal samples collected from the male Sumatran rhinos also differ in the median androsteron metabolite concentration between the two periods (median<sub>March till August 2004</sub> = 107 ng/g feces IQR = 86, n = 61; median<sub>August and March 2005</sub> 95 ng/g feces IQR = 59, n = 65). The data were analyzed separately for each sampling period due to the differences in median concentrations.

#### **4.2.1 Fecal hormone profile of the female:**

The female rhino was cycling irregularly (Figure 3). She developed two to three cycles at the beginning of the sampling period (12<sup>th</sup> of March until 16<sup>th</sup> of July 04) but at the end of the period (16<sup>th</sup> of July – 13<sup>th</sup> of August) there was no pattern detectable anymore. The time interval between successive pregnandiol peaks was 46 days, 41 days and 32 days.

During the second sampling period, from the 19<sup>th</sup> of August 2004 until the 1<sup>st</sup> of March 2005 there was no cycle detectable (Figure 4).

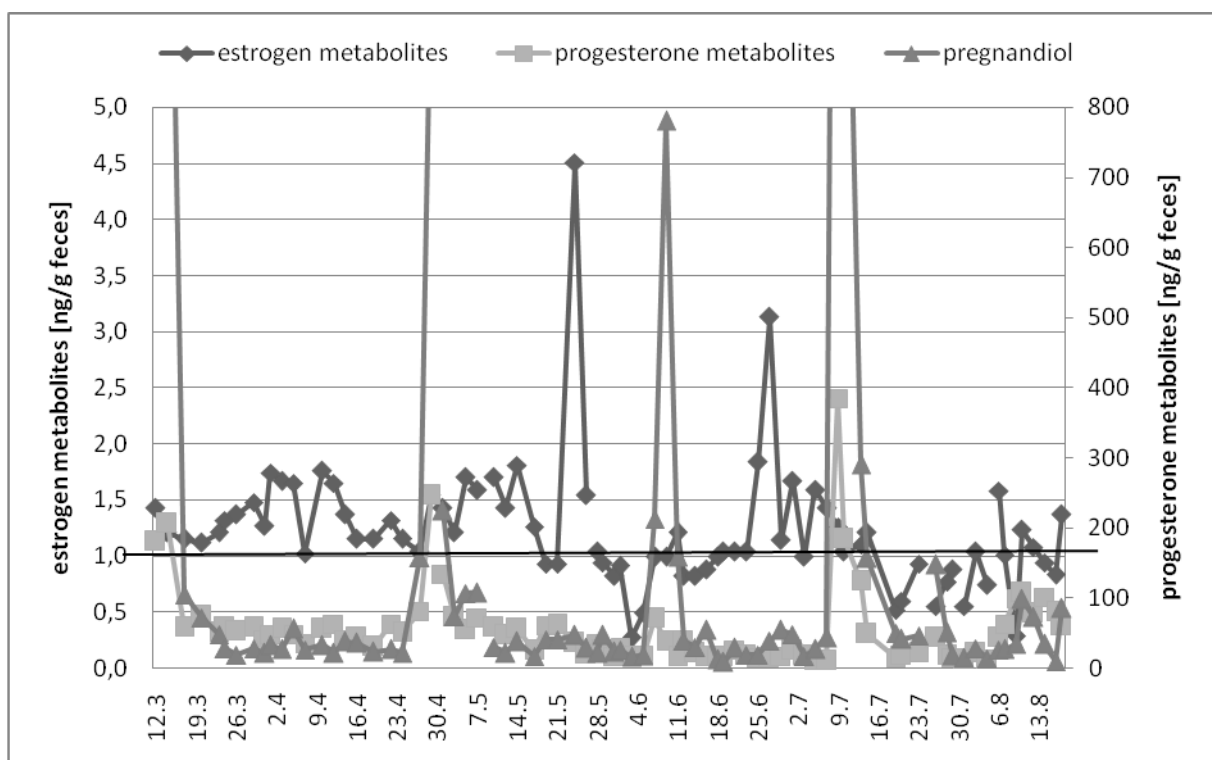


Figure 3: Progesterone, estrogen metabolite and pregnandiol metabolite concentrations of the female Sumatran rhino for the interval 12<sup>th</sup> of March 2004 until the 17<sup>th</sup> of August 2004. The horizontal line indicates median estrogen metabolite concentration.

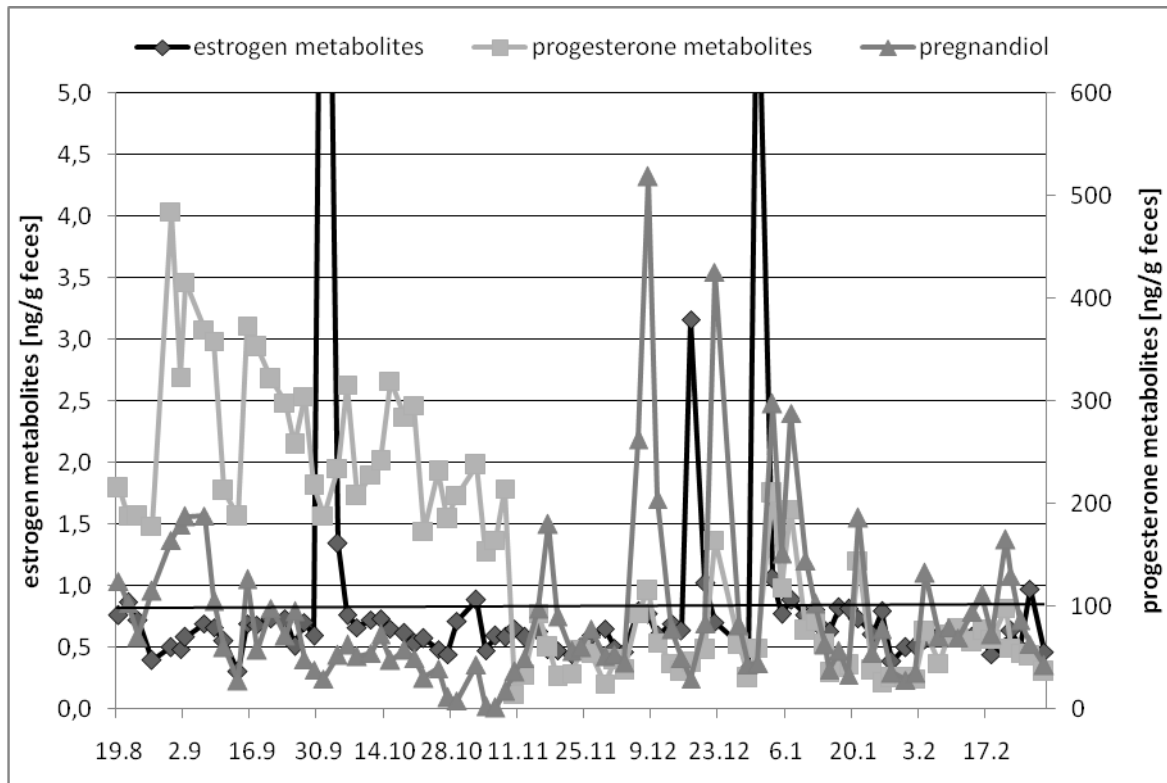


Figure 4: Progesterone, estrogen metabolite and pregnandiol metabolite concentrations of the female Sumatran rhino for the interval 19<sup>th</sup> of August 2004 until 1<sup>st</sup> of March 2005. The horizontal line indicates median estrogen metabolite concentration.

#### **4.2.2 Fecal hormone profile of the male**

The epi-androsterone and the estrogen metabolite profile of the male Sumatran rhino have a similar progression. Peak estrogen metabolite concentrations correlate with peak epi - androsterone concentration, except for a few exceptions (beginning of June 04, beginning of August 04, Figure 5).

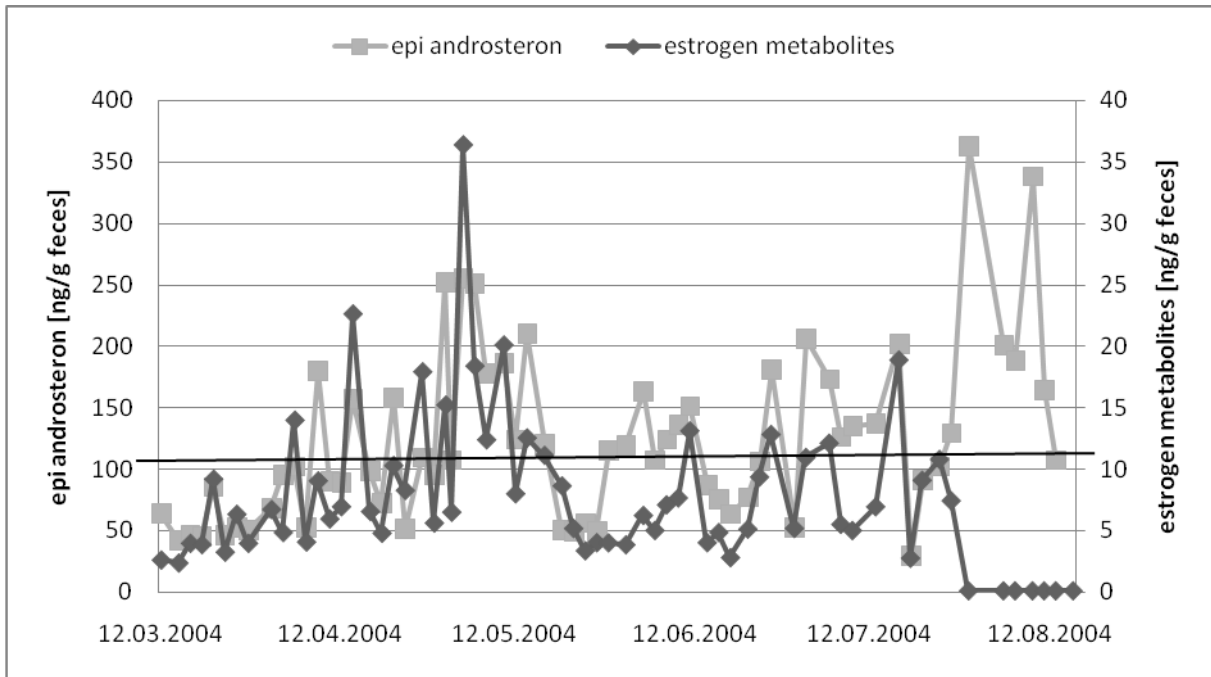


Figure 5: Epi-androsteron and estrogen metabolite profile of the male Sumatran rhino established for the period from the 12<sup>th</sup> of March until the 16<sup>th</sup> of August 04. The horizontal lines indicated median epi-androsteron concentrations.

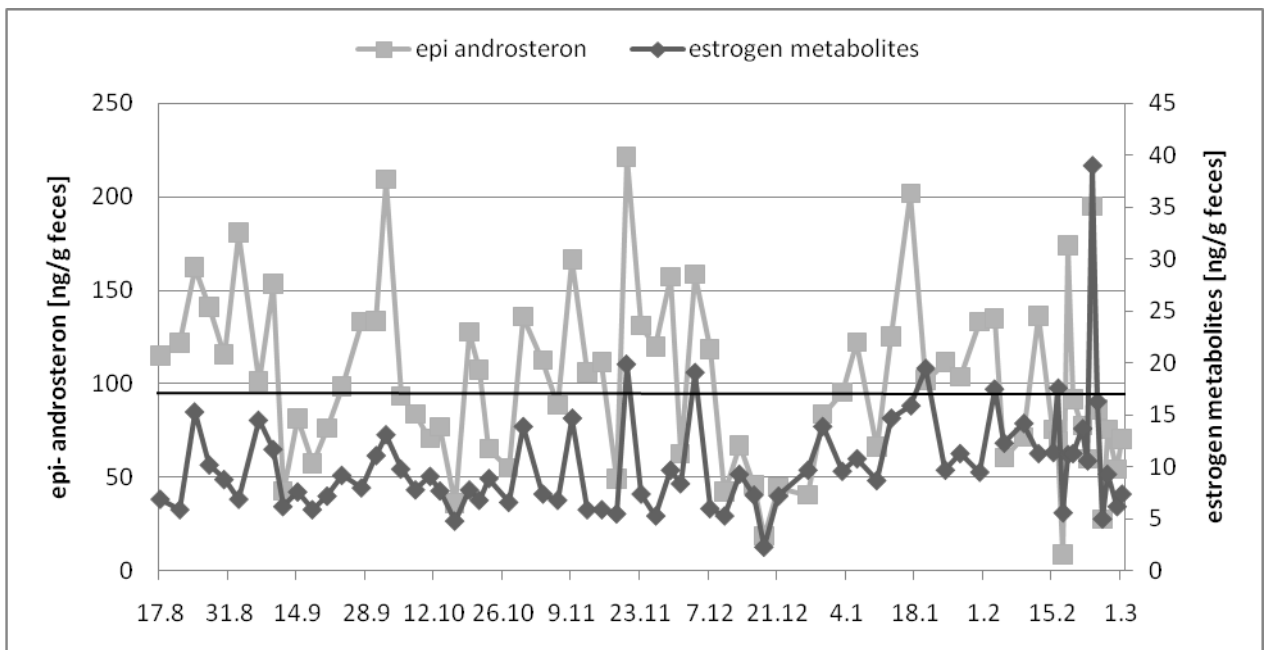


Figure 6: Epi-androsteron and estrogen metabolite profile of the male Sumatran rhino established for the period from the 16<sup>th</sup> of August 04 until the 1<sup>st</sup> of March 05.

### **4.3 Sexual behavior:**

The male had frequent erections during the observation period March until November but no erection in December 04. The highest frequency of erection was observed in July. In this month the male had on 33 % of all days of an erection.



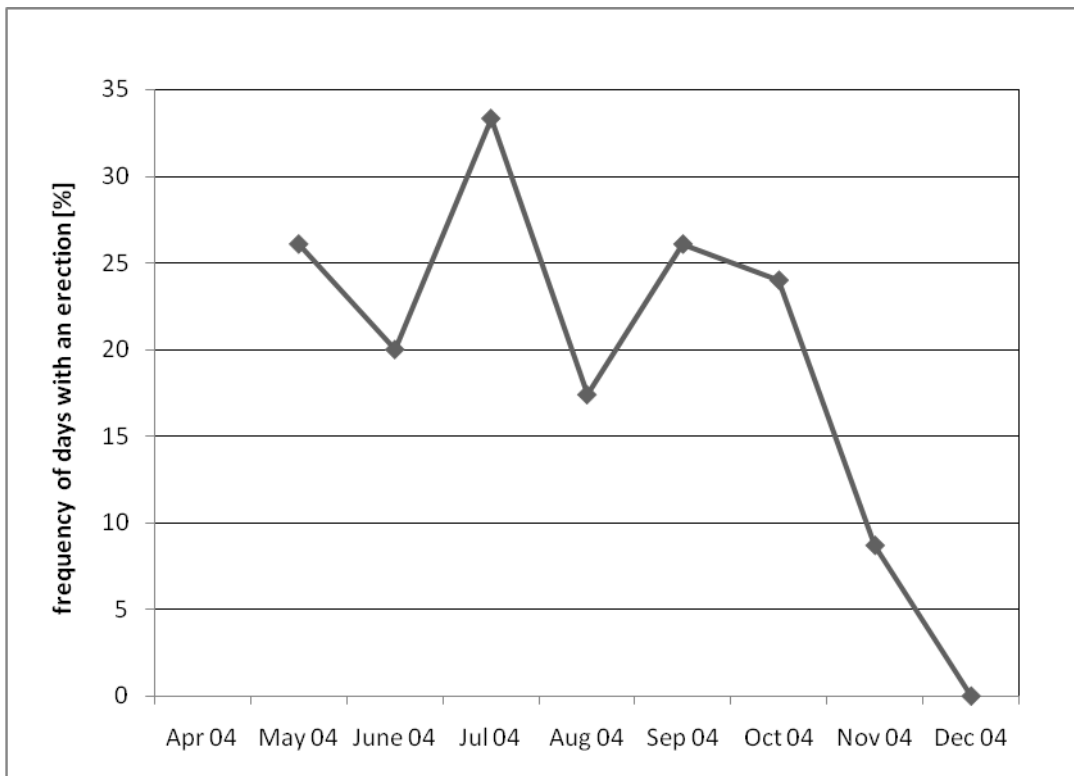


Figure 7: The days of erection in relation to the days of observation for the observation period May 04 until December 04.

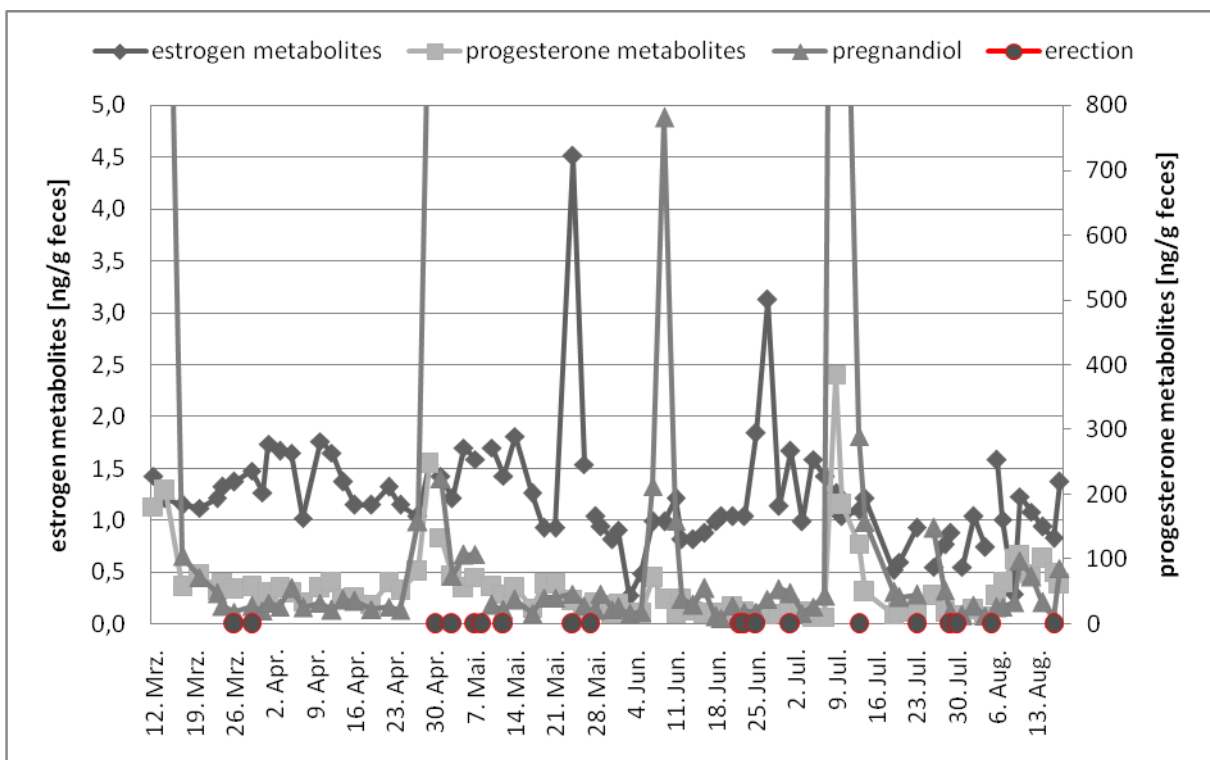


Figure 8: The hormone profile of the female Sumatran rhino in relation to the days of erection of the male rhino.

#### **4.4 Food:**

The food given to the rhinos consisted of leaves and fruits. A total of 36 different tree species (the most frequent fed species are shown in Figure ??) were fed in the year 2004 and four different types of fruit (bananas, sugar cane, rambutan and watermelon). The principal food (68 % of the total amount of food) fed to the rhinos consisted of 3 tree species and 1 fruit only: Tapai apai (*Timonius flavescens*), Daun Nangka (*Ficus lepicarpa*) Kulimpapa (*Vitex spec.*), and bananas (*Musa spec.*).

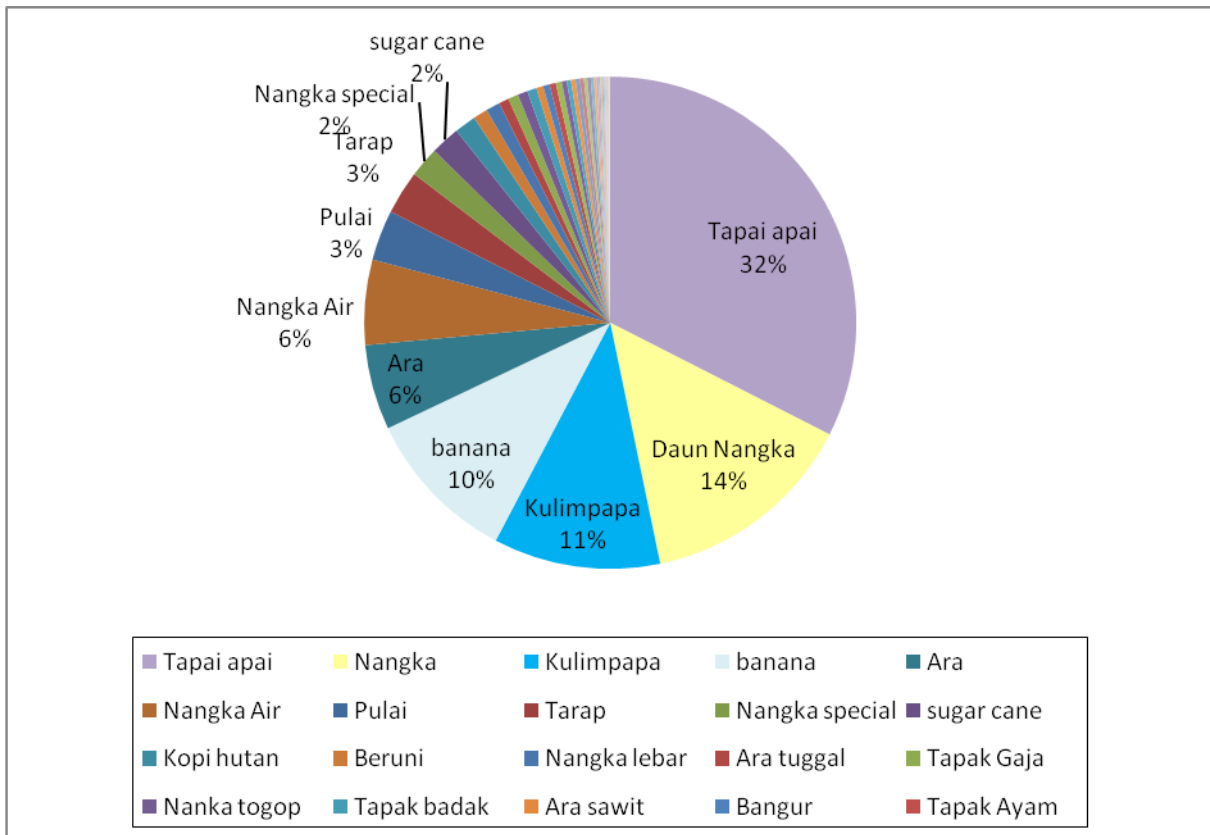


Figure 9: Percentage of tree and fruit species fed to the female Sumatran rhino in the year 2004. The graph includes all species fed to the rhino but only the 20 most frequently fed species are listed.

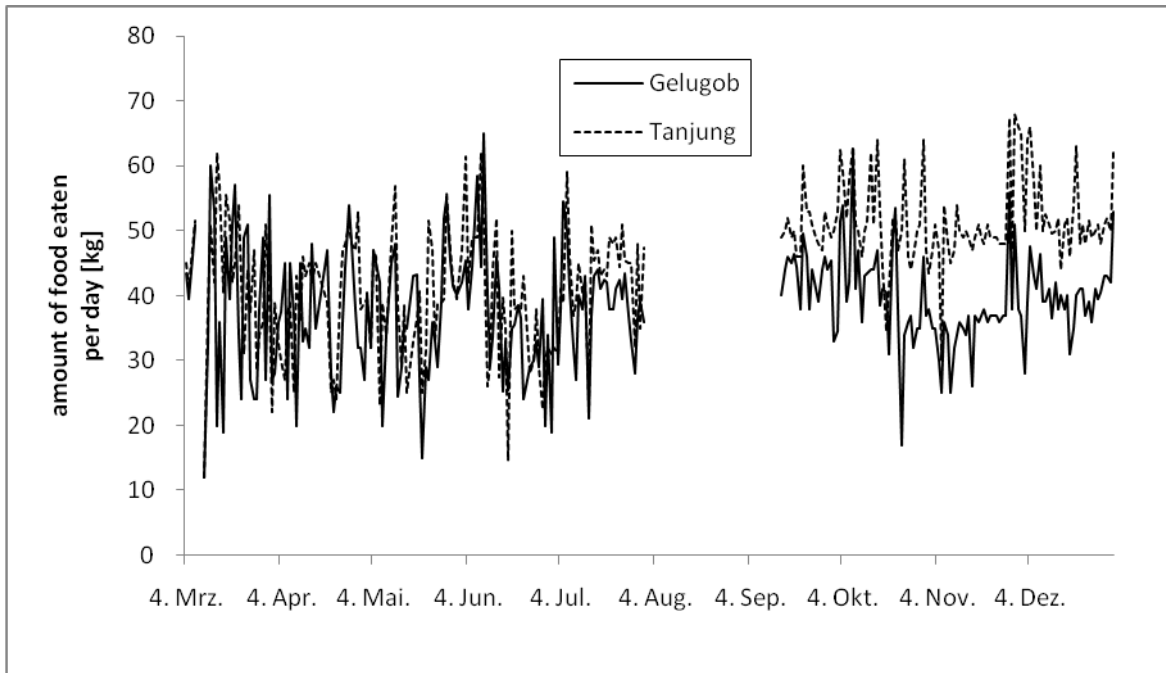


Figure 10: Daily amount of food eaten (leaves and fruits) by the male Tanjung and the female Gelugob in the year 2004.

The female consumed an amount of 38 kg daily (median IQR = 10 kg) and the male consumed a median amount of 47 kg of food daily (IQR = 12 kg).

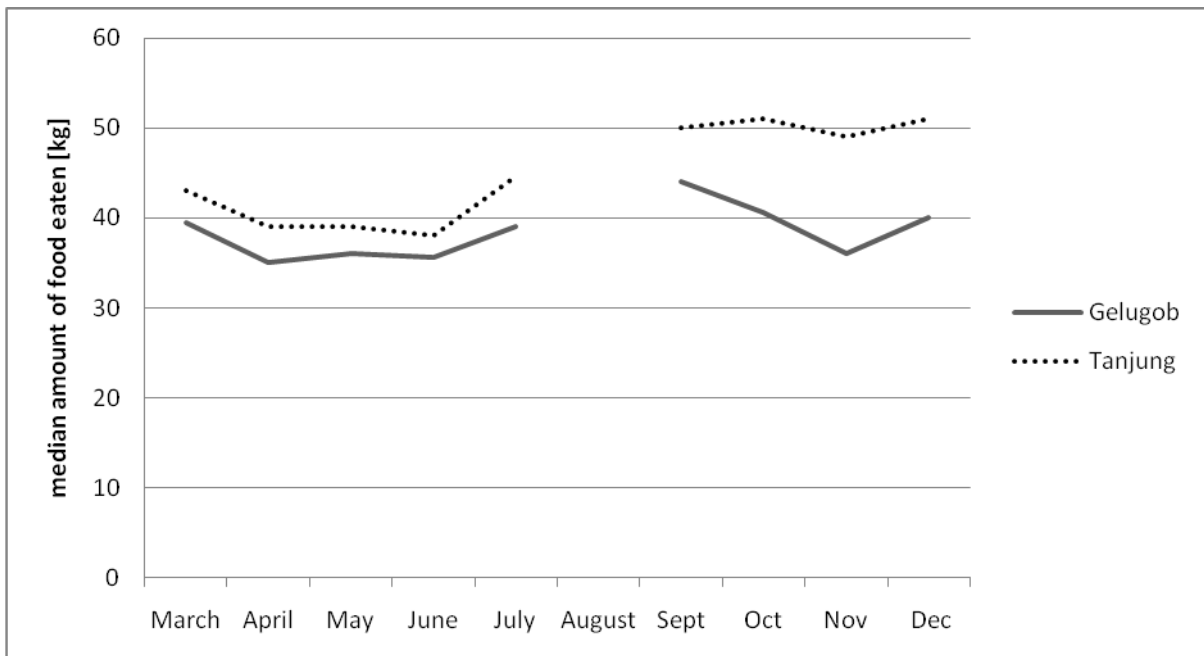


Figure 11: Median amount of food eaten by Tanjung and Gelugob between March and December 2004.

At the beginning of the observation period, in March 2004, the rhinos were fed large amounts of bananas (200 kg per month). We reduced the amount of bananas and increased the amount

of leaves fed to the rhinos in the year 2004 (Figure 12, Figure 13) in order to achieve a well-balanced food which resembles more the natural diet.

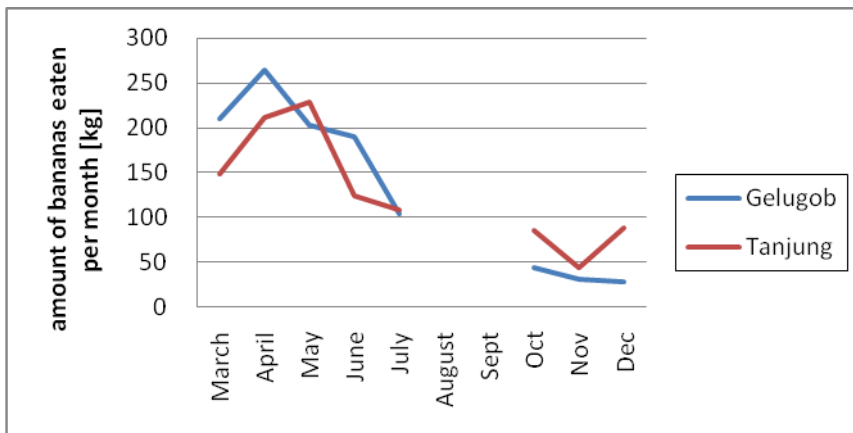


Figure 12: Total amount of bananas eaten each month. There were no data available for August and only half a month of data available for September 04.

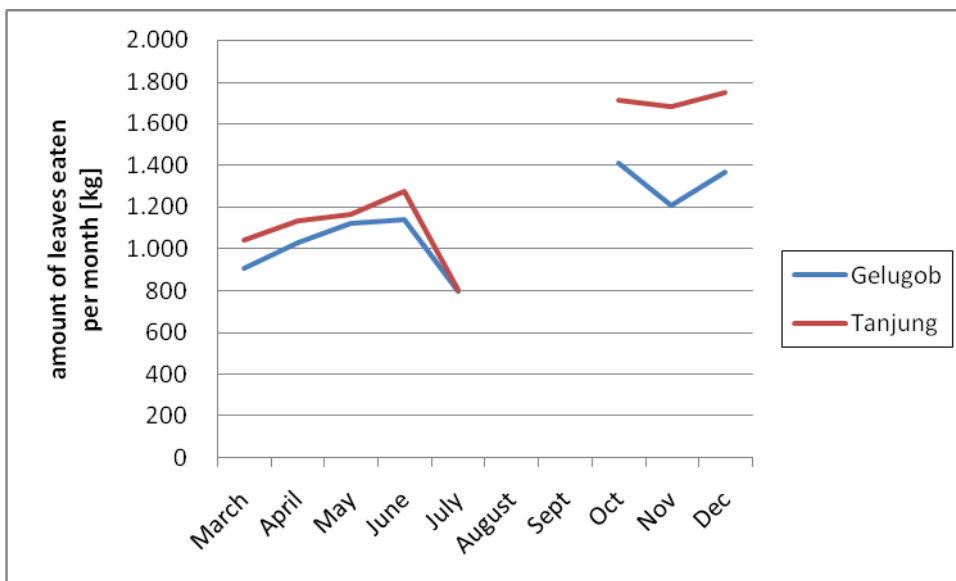
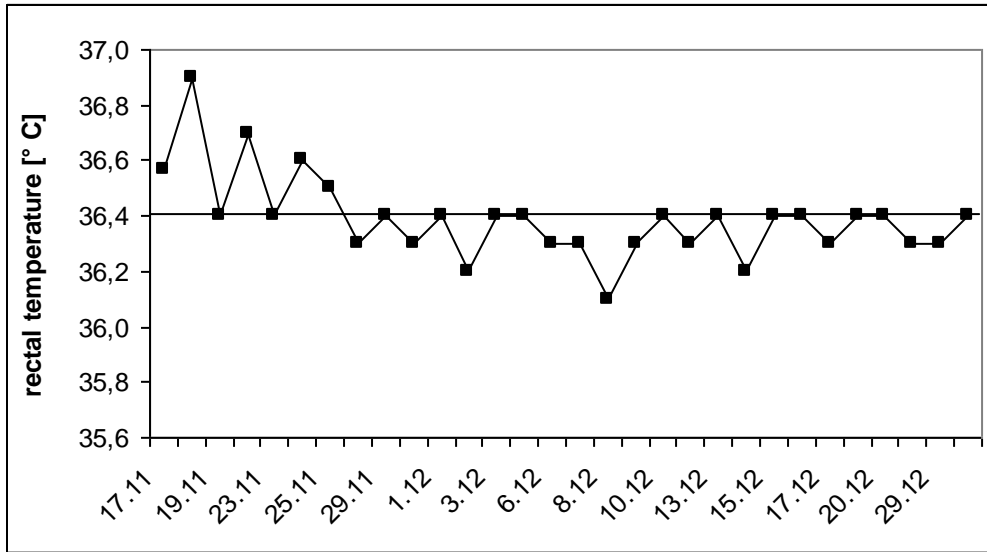


Figure 13: Total amount of leaves eaten per month. There were no data available for August and only half a month of data available for September 04.

#### **4.5 Weight of the rhinos:**

The average weight of the female was 544 kg the average weight of the male was 541 kg (n = 2). Both rhinos lost around twenty kilogram of weight between January 2004 (Gel: 558 kg; Tanj = 554 kg) and December 2004 (Gel: 530 kg; Tanj: 528 kg).

## 4.6 Rectal temperature:



Graph 14: Morning rectal temperature of the female (G). The line indicates the median temperature.

The rectal temperature of the female was on average 36,4 °C (median  $\pm$  0,1 IQR).

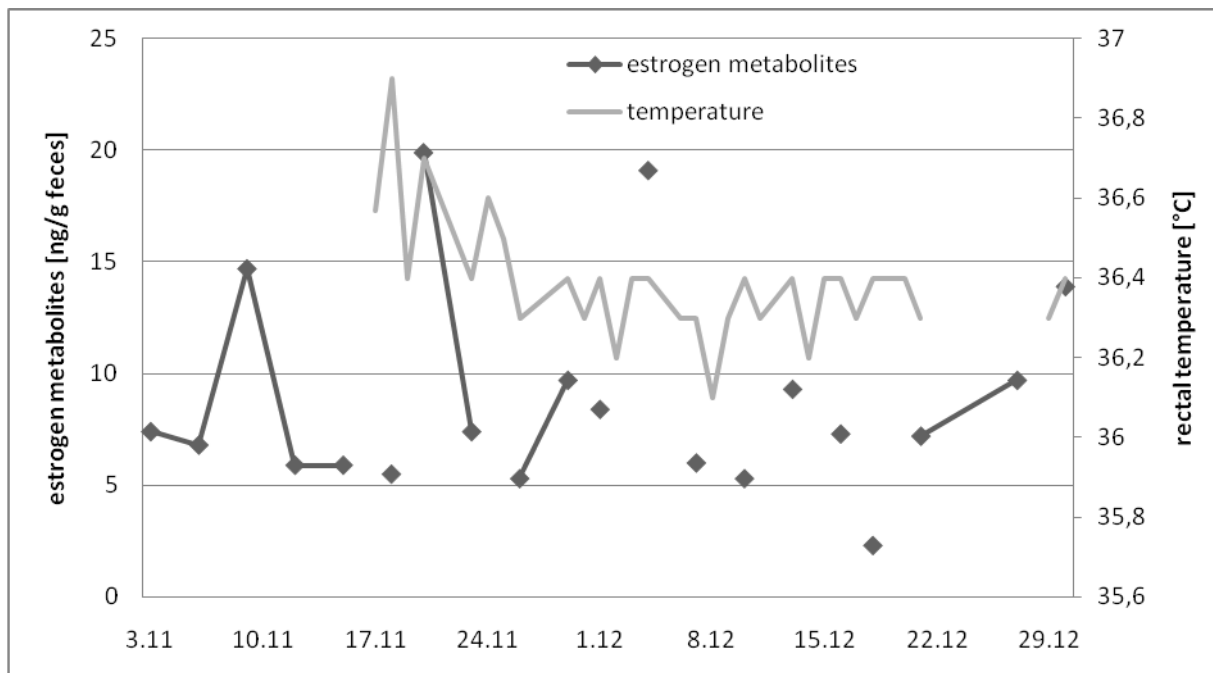


Figure 15: Estrogen metabolites and rectal temperature of the female rhino in the time between the 3rd of November and the 31st of December 04.

There is no correlation between estrogen metabolites and the rectal temperature. An elevated rectal temperature was measured during the first peak in estrogen metabolite concentration, on the 20<sup>th</sup> of November, but not during the second peak on the 4<sup>th</sup> of December 04.

#### **4.7 Ultrasound analysis:**

The ultrasound analysis revealed that Gelugob's reproductive track was still considerably healthy for her age. No tumor or cyst could be found. The right ovaries were very small. The evaluation of Tanjung revealed that he was in pretty good condition for his age.

#### **4.8 Semen collection:**

Fluid was collected on almost all semen collection trials but only on the 5<sup>th</sup> of May 2004 two sperms were found by Dr. Nan Schaffer.

#### **4.9. Health of the rhinos:**

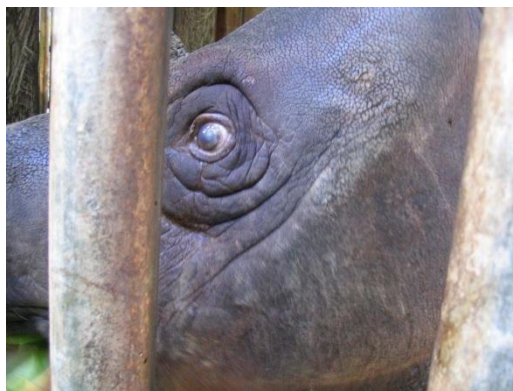
##### **4.9.1 Health of Gelugob:**

In April 2004 the female broke off her front horn. She stuck her horn under the lock of her door and instead of putting her head down to remove it she pulled and panicked and broke off the horn. The wound was bleeding a little bit but it healed very well after treatment with an antiseptic cream. It is likely that the female got stressed by the construction work in the neighboring enclosure and that she panicked in a situation which was routine for her due to the stress.

On the 8<sup>th</sup> of July 2004 the female developed eye opacity. Her left eye was covered with a white film and the eye ball was surrounded by a red circle (see Picture 5), possibly a general conjunctivitis. Some lacrimation could be observed in the affected eye. Thereupon the eye was treated 3 times daily with Optrex.



Picture 5: Gelugob's left eye on the 9<sup>th</sup> of July 2004.



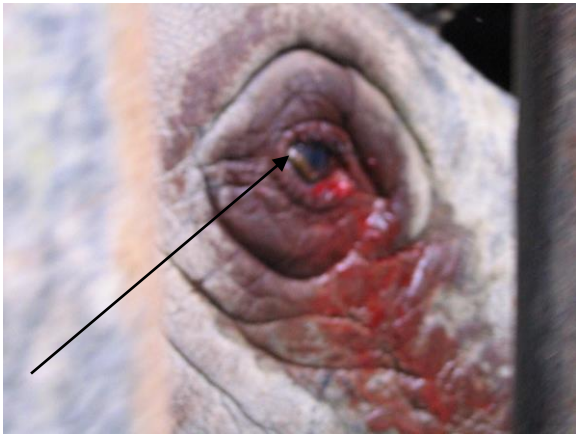
Picture 6: Gelugob's left eye on 14<sup>th</sup> of July

Optrex contains distilled water witch hazel BPC 13 % preserved with Benzalkonium chloride 0,005% buffered with Borox and Boric Acid. On the following day the eye was treated hourly throughout the night with 20 ml Optrex and 2 ml Spersadexoline. Spersadexoline is an antibiotic eye treatment that contains the active ingredients Chloramphenicol, Dexamethasone Sodium Phosphate and Tetrazolinehydrochloride. The treatment showed its effect within a few days. On the 14<sup>th</sup> of July, only 6 days after the beginning of the eye infection most of the white tissue was gone and only a small white dot was left on the eye (Picture 6).

Possible explanation for the development of the eye infection:

We did not observe how it happened. The cloudiness was noticed early in the morning. The day before, the female was released in the outside enclosure. During this time she stayed the whole day in the mud wallow. It is possible that she got something in her eye, like a leech or a stick or just mud. It is also possible that the reflection of the sun in the water of the wallow has caused an infection. A comparison of the eye problem in the rhino with problems occurring in horses I noticed that an uveitis also known as moon blindness developed in horses is very similar in appearance.

On the 6<sup>th</sup> of August 2004 we observed a leech in the right eye of the female rhino (Picture 7). The leech was possibly sitting on the conjunctiva of the eye. Eventually the leech fell off and the eye was bleeding. Within the next 1,5 month the eye developed a serious eye infection. Unfortunately, I haven't got any pictures or description of the progression of the eye infection until the 18<sup>th</sup> of October 2004 (Picture 8). On that day there was no iris or pupil visible anymore. The whole eye was whitish – pink. The female had obvious pain and was rubbing the eye regularly. From the 2<sup>nd</sup> of September until the 15<sup>th</sup> of September the eye was treated every two hours with Spersadaxoline (see description above) Continuous eye fluid samples were collected and send for drug sensitive tests. The results are given in the attachment (Table 6). The treatment protocol of the eye until the 4<sup>th</sup> of November is given in Table 4.



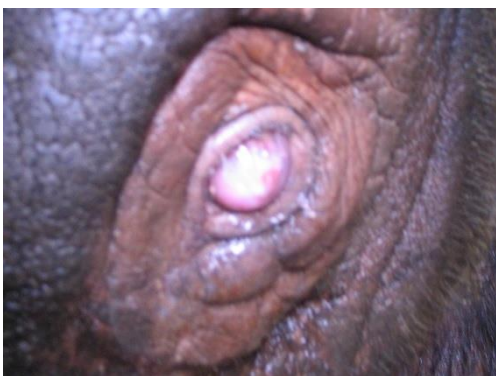
Picture 7: The right eye of the female rhino on the 6<sup>th</sup> of August 04. The arrow marks the leech.



Picture 8: The right eye of the female on the 18<sup>th</sup> of October 04.

From the 28th of October onwards the eye condition improved. The eye was less pink and less swollen and within the next weeks the eye healed but the female stayed blind.

The female also developed an eye infection on her left eye, possibly by cross infection. The eye was white and pink like the right eye (see Picture9) with a noticeable elevation in the center of the cornea. Both eyes were treated with the same medication as given in Table 4. The left eye did recovered faster than the right eye but the eyesight was lost as well.



Picture 9: Left eye of the female rhino on the 18<sup>th</sup> of October 04.



Picture10: Left eye on the 15<sup>th</sup> of November 04



Table 4: Treatment protocol of the right eye of the female rhino.

	Spersa-dexoline	Maxi-trol	Oxy-mycine	Terra-mycine	Nor-floxine	Optrex	Optigen	Genta-micine cream	Nor-floxine tablettts	Genta-micine injection
2.9. - 15.9.04	x									
16.9.04		x								
17.9. - 21.9.04			x	x						
21.9. - 28.9.04	x					x				
29.9. - 11.10					x	x				
12.10.04						x	X			
14.10.07							X	X		
15.10.20 04							X	X	x	
19- 21.10.04							X	X	x	x
22 - 30.10							X	X		
31. - 4.11								X		x

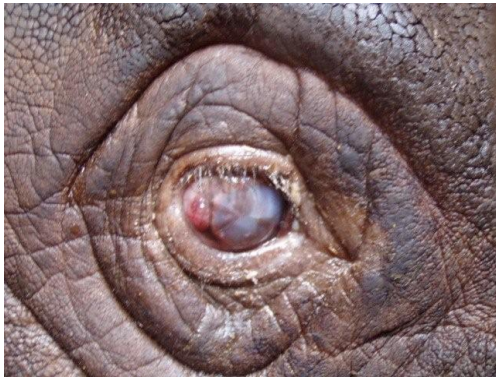
In December the treatment was changed according to recommendation by Dr. Janna Wynne, from Los Angeles Zoo. She suggested using a broad spectrum antibacterial coverage, combined with an antifungal cream.

The eyes were treated with Okacin containing Lomefloxacin 0,3 % (a difluorinated quinolone derivate) effective against gram positive and gram negative bacteria and Gyno Trosyd a Tioconazole broad spectrum antifungal which is fungicidal to yeast and other fungi and has activity against certain gram positive organism such as Staphylococcus and Streptococcus.

The treatment protocol was as following:

8:00 antifungal cream  
 9:00 Okacin eye drops  
 11:00 Okacin eye drops  
 14:00 Okacin eye drops  
 15:00 antifungal cream  
 16:00 okacin eye drops  
 18:00 antifungal cream

From the 7<sup>th</sup> of December until the 8<sup>th</sup> of January 2005 the eye was treated 3 times per day with Okacin and antifungal cream.



Picture 11: right eye of the female rhino on the 3<sup>rd</sup> of January 05.



Picture 12: left eye of the female rhino on the 3<sup>rd</sup> of January 05

#### **4.9.2 Health of Tanjung:**

In July 2004 the male broke off his horn but in his case nobody observed how it happened. The keepers saw in the morning that he was bleeding and that the horn was loose. The horn fell off the next day. The wound was treated with the antiseptic cream and it healed nicely.

#### **5. Appendix:**

Table 5: Blood parameters established for the male and female Sumatran rhino.

		<b>Gelugob</b>	<b>Tanjung</b>	<b>Gelugob</b>	<b>Gelugob</b>
		<b>5.11.04</b>	<b>27.11.05</b>	<b>27.11.04</b>	<b>8.12.04</b>
<b>haemoglobin</b>	g/dl	9,8	9,5	8,6	11,5
<b>hematocrit</b>	%				
<b>total W.B.C.</b>	/cmm	6500	4900	7300	9400
<b><u>differential counts:</u></b>					
<b>neut.</b>	%	61	57	69	56
<b>lymph.</b>	%	39	43	31	40
<b>mono.</b>	%	0	0	0	4
<b>eosin</b>	%	0	0	0	0
<b>BAS</b>	%		0	0	0
<b>ESR</b>	mm/hr	8	7	11	12
<b>platelets</b>	/cmm	193000	173000	272000	134000
<b>UREA</b>	mmol	10	1,6	1,7	2
<b>creatinine</b>	umol/l	96	114	93	76
<b>total Bilirubin</b>	umol/l	12	12	8	12
<b>total Protein</b>	g/l		72	69	75
<b>albumin</b>	g/l		40	37	40
<b>globulin</b>	g/l		32	33	35
<b>A/G Ratio</b>			1,3	1,1	1,1
<b>A/K phosphat.</b>	U/L		89	77	
<b>SGOT</b>	U/l	14	22	18	10
<b>SGPT</b>	U/l	22	28	14	14

Table 6: Results of the drug sensitive test from samples taken from Geulog with a cotton wool (swab) or by flushing the eye with sterile water.

		Staphylo- coccus spp	Strepto- coccus spp	Acineto- bacter 1 woffii	Gram neg bact	Candida	Coryne- bacterium	Chryse- bacterium meningoseticum
<b>18.11.</b>								
<b>left eye</b>	swab	x	x			x	x	
	flush				x	x		
<b>right eye</b>	swab	x				x		x
	flush					x		
<b>30.11.</b>								
<b>Left eye</b>	swab	x	x			x	x	
	flush	x		x		x		
<b>7.12.</b>								
<b>left eye</b>	swab		x					
	flush		x			x		x
<b>right eye</b>	swab							
	flush					x		x
<b>14.12.</b>								
<b>left eye</b>	swab	x	x					
	flush	x		x	x			
<b>right eye</b>	swab	x	x					
	flush	x		x	x			
<b>21.12.</b>								
<b>left eye</b>	swab	x	x					
	flush			x				
<b>right eye</b>	swab	x	x					
	flush	x			x			