Aeres University of Applied Sciences

Thesis 2021

‘The effect of adding white belted black-and-white ruffed lemurs on the social structure of ring-tailed lemurs’.

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'What is the effect of adding white belted black-and-white ruffed lemurs on the social structure of ring-tailed lemurs?'

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November 2021

Preface

This thesis titled ‘the effects of the presence of white belted black-and-white ruffed lemurs on the social structure of ring-tailed lemurs’, was written as a final study for my degree in Applied Biology at Aeres Hogeschool, Almere. The findings of this study are meant for AquaZoo, Aeres Hogeschool and anyone else who is interested in the results.

This research was initiated by AquaZoo, Leeuwarden, the research question was formulated by Wiene van de Bunte.

My appreciation goes out to AquaZoo for the opportunity to conduct my research. Secondly, Wiene van de Bunte for the coaching and valuable feedback during my research. Tom Huisman and Kenny Noorlander for their advice and feedback. In addition, I would like to thank all the animal caretakers for helping me with my observations by adjusting their schedules and providing information about the individuals. Also a big thank you to all the volunteers who kept visitors at a distance and thereby brought some calmness to the outdoor enclosure.

Laura Cöhrs

Almere, November 2021

Abstract

English

There are currently about 2220 individual ring-tailed lemurs *(Lemur catta)* living in Madagascar, which has caused the species to be red-listed as critically endangered since 2018. To prevent the species from going extinct conservation strategies have been established. Because ring-tailed lemurs have a complex social structure, it is extremely hard to start a breeding program, which involves adding a new individual to an already existing group. This makes it more difficult to keep a stable group of ring-tailed lemurs in captivity while participating in a breeding program to conserve the species.

AquaZoo is a Dutch zoo that, as part of a breeding program, houses a group of ring-tailed lemurs where dominance and aggression play a major role. They want to add two white belted black-and-white ruffed lemurs *(Varecia variegata subcincta)* to look at what happens at the still unknown social structure of the group, after adding new individuals. The goal of the research is to increase welfare by having more insight into the structure. Research shows that within groups of ring-tailed lemurs, where aggression and dominance play a role, this is reduced when another species is added. The main question of this research is as follows: "What is the effect of adding white belted black-and-white ruffed lemurs on the social structure of a group of ring-tailed lemurs?".

This study used continuous focus sampling. A total of 506 observations were conducted over the course of two months. Observations were made prior to the introduction of the white belted black-and-white ruffed lemurs and one month after.

The results of this study show that there is no significant difference ($p = 0.22$) in positive interaction before the introduction of the white belted black-and-white ruffed lemurs as after. Also, no significant difference ($p = 0.07$) was shown in negative interaction. After the introduction the same group of individuals is dominant, but at the bottom of the rank the individuals alternate. A difference in density score of before introduction (0.81) and after (0.88) was found. Thus, it is concluded that no significant difference was found in both positive and negative interactions, the group did become closer after the introduction and the dominance status continued to change. It is recommended to start research on the social structure when a zoo wants to participate in a breeding program. When introducing another species of prosimians it is recommended to create multiple foraging-/ sleeping areas.

Dutch

Er leven momenteel ongeveer 2220 individuele ringstaartmaki's *(Lemur catta)* op Madagaskar, waardoor de soort sinds 2018 op de rode lijst van ernstig bedreigde soorten staat. Om te voorkomen dat de soort uitsterft, kan er een fokprogramma worden opgesteld binnen dierentuinen. Omdat ringstaartmaki's een complexe sociale structuur hebben, is het echter extreem moeilijk om een fokprogramma op te starten, waarbij een nieuw individu wordt toegevoegd aan een al bestaande groep. Dit maakt het moeilijker om een stabiele groep ringstaartmaki's in gevangenschap te houden en tegelijkertijd deel te nemen aan een fokprogramma om de soort in stand te houden.

AquaZoo is een Nederlandse dierentuin die, als onderdeel van een fokprogramma, een groep ringstaartmaki's huisvest waarbij dominantie en agressie een grote rol spelen. AquaZoo wil twee gordelvari's *(Varecia variegata subcincta)* toevoegen om te kijken wat er gebeurt, in de nog onbekende sociale structuur van de groep, na het toevoegen van nieuwe individuen. Het doel van het onderzoek is het welzijn verhogen door meer inzicht te krijgen in de sociale structuur. Uit onderzoek blijkt dat binnen groepen ringstaartmaki's, waar agressie en dominantie een rol spelen, dit gedrag vermindert wanneer er een andere halfaapsoort wordt toegevoegd. De hoofdvraag van dit onderzoek luidt als volgt: "Wat is het effect van het toevoegen van gordelvari's op de sociale structuur van een groep ringstaartmaki's?".

Dit onderzoek maakte gebruik van continue focus sampling. In totaal werden er 506 observaties uitgevoerd, over een periode van twee maanden. Observaties werden uitgevoerd een maand voorafgaand aan de introductie van de gordelvari's en een maand erna.

De resultaten van het onderzoek tonen aan dat er geen significant verschil ($p = 0.22$) is in positieve interactie vóór de introductie van de gordelvari's als erna. Ook in negatieve interactie werd geen significant verschil ($p = 0.07$) aangetoond. Zowel voor als na de introductie is dezelfde groep individuen dominant, maar onderaan de rangorde wisselen de individuen elkaar af. Er werd een verschil in dichtheid gevonden van vóór de introductie (0.81) en erna (0.88). Er kan dus geconcludeerd worden dat er geen significant verschil werd gevonden in zowel positieve als negatieve interacties maar dat de groep wel hechter werd na de introductie en dat de dominantiestatus bleef veranderen. Het is aan te bevelen om onderzoek te doen naar de sociale structuur wanneer een dierentuin wil deelnemen aan een fokprogramma. Bij het introduceren van een andere halfaapsoort is het aan te bevelen om meerdere foerageer-/slaapplekken te creëren.

Contents

1. Introduction

Lemurs (*Lemuridae*), are prosimians which belong to the order of primates and are endemic to the island Madagascar (Rowe & Donohue, 2020). Research by LaFleur et al. (2016) shows that lemurs are the most endangered group of mammals in the world. 103 out of the 107 species of lemurs are currently seriously threatened in their chances of survival (IUCN, 2020; Reumer, 2020). This is partly because prosimians live only on Madagascar and partly because they face habitat loss, poaching and illegal trade (Taylor, 2009).

The ring-tailed lemur (*Lemur catta*) represents one of the species that faces these threats. In the last decades there has been a major population decedge in this species, especially in the areas where no protection was present (Kappeler, 1990). In the year 2000, this number of individual ring-tailed lemurs was estimated to be between 10,000 - 100,000 (Taylor, 2009). Whereas in 2020 there were approximately 2220 individual ring-tailed lemurs living in Madagascar (LaFleur et al., 2020). Since this decedge is a continuing trend, the ring-tailed lemur was listed as 'critically endangered' on the IUCN's Red List of Endangered Species in 2018 (IUCN RedList, 2021). To prevent the ring-tailed lemur from going completely extinct, conservation strategies have been put in place worldwide (Reuter, et al., 2016).

One of the strategies is to participate in the European Endangered species programme (EEP). A participation in the EEP involves everything in the form of breeding programs and welfare guideedges. These breeding programs are used to keep species in captivity that are completely extinct in the wild, species that can be reintroduced to the wild, or species that are seriously threatened in their existence as in the case of ring-tailed lemurs. Breeding programs follow guideedges required for captive breeding. The most important guideedges relate to genetic diversity and the avoidance of inbreeding within the species (Witzenberger & Hochkirch, 2011).

The population of ring-tailed lemurs within Europe is spread across 25 countries within 308 zoos. The lemur groups consist of an average of fifteen animals making approximately 4.600 captive ring-tailed lemurs within Europe (ZIMS, 2021). To prevent ring-tailed lemurs, in captivity, from reproducing within the group with relatives, male ring-tailed lemurs can be castrated (Boulet, et al., 2009). The female ring-tailed lemurs can have contraception administered in the form of an implant (Grebe et al., 2019). Another important guideedge is animal welfare. Although these guideedges are designed with great care (Shire, 2012), the welfare of species that live in complex social groups must be monitored (Palagi et al., 2004).

Ring-tailed Lemurs are known not only for their iconic appearance and olfactory communication (Evans, 2019) but also for their relationships, the large socially complex groups they live in, and their social behavior. They have the highest social complexity among prosimians (Kulahci et al., 2018). Groups of ring-tailed lemurs usually consist out 15-25 individuals or at least 6 adult females. The density of a group of ring-tailed lemurs involves 1-6 individuals per hectare. The more food present in the area the higher the number of individuals per hectare (Wilson & Hanlon, 2010). Within a group of ring-tailed lemurs, social relationships are highly maintained. The most important factor in this process is grooming each other. Mutual grooming plays a major role in finding and strengthening a relationship between two or more individuals. Mainly family related individuals engage in mutual grooming (Schino & Alessandrini, 2018). Females within a group of ring-tailed lemurs become dominant over males when reaching puberty (Cavigelli et a., 2003). The males, at sexual maturity, leave the group to join another group and mate with them. In a group of ring-tailed lemurs living in captivity, such as a zoo, there is less emigration and immigration among ring-tailed lemurs. Logically, the only exchange of individuals occurs when zoos choose to do so. When a new ring-tailed lemur or any other prosimian is introduced into a group, one does not yet know how that introduction will

affect the social structure of the pre-existing group. This makes it more difficult to be able to create and maintain a stable group of ring-tailed lemurs in captivity and balance the well-being of the group (Fontani et al., 2020).

The successful coexistence of two species of prosimians depends on the social structure that, in this case the group of ring-tailed lemurs, holds (Fenn, 2015). Several studies have shown that ring-tailed lemurs can co-exist with at least nine other species of prosimians in captivity (Ferrie et al., 2013). Including the black-and-white ruffed lemur *(Varecia variegata),* red ruffed lemur *(Varecia variegata rubra)* and the white belted black-and-white ruffed lemur *(Varecia variegata subcincta)* (Taylor, 2009). Currently, at the present time, it is more common to see ring-tailed lemurs being merged with several other species of prosimians in Dutch zoos (EAZA, 2021). Thus, although ring-tailed lemurs are highly complex social animals and hardly encounter other lemur species in the wild, they seem to tolerate other species' presence within Dutch zoos (Fichtel et al., 2017).

AquaZoo is one of the zoos in the Netherlands that has a group of ring-tailed lemurs. The zoo's goal is to introduce two white belted black-and-white ruffed lemurs to the group of ring-tailed lemurs. However, the social structure of the group of ring-tailed lemurs is largely unknown. Therefore, the purpose of this study is to discover the social structure of the group of ring-tailed lemurs that is housed in AquaZoo. Within the social structure, we speak of three types of relationships. The negative relationship consists of a relationship where there is aggression or fear between individuals. The positive relationship consists of individuals who interact with each other in the form of grooming, resting and social play. The last type of relationship concerns family.

Through more insight on the social structure of the group, the welfare of the ring-tailed lemurs can be increased (Kilconye, 2000). In fact, within the group of ring-tailed lemurs at AquaZoo, there are two individuals who are being attacked by and excluded from the group. If there is high dominance among certain individuals this behavior could be reduced by adding new individuals/species to the group (Spiezio et a., 2017). To put this to the test, two white belted black-and-white ruffed lemurs will be introduced slowly to the group during the course of this study. The study will then look at how the white belted black-and-white ruffed lemurs presence may affect the social structure of the ring-tailed lemurs. So the goal of this study is to add another species to the group of ring-tailed lemurs in the hope that this will reduce the attacks on the two individuals. If so, this method could be used in other groups of ring-tailed lemurs where aggressive behavior and dominance plays a major role.

The main question is as follows; 'What is the effect of adding white belted black-and-white ruffed lemurs on the social structure of a group of ring-tailed lemurs?'

The sub-questions are concerned with relationships within the group of ring-tailed lemurs at AquaZoo and are as follows:

- 'How strong are the relationships among the group members in terms of betweenness, eigenvector and closeness, before adding white belted black-and-white ruffed lemurs?'
    'Which individuals are the most dominant before adding white belted black-and-white ruffed lemurs?'

- 'How strong are the relationships among the group members in terms of betweenness, eigenvector and closeness, after adding white belted black-and-white ruffed lemurs?'

- 'Which relationships, with the focus on negative dominance, have noticeably changed in the group of ring-tailed lemurs after the addition of the white belted black-and-white ruffed lemurs at AquaZoo?'

Previous research by Taylor (2009) and Villers & Lent (1993) has shown that multiple species of lemurs can be successfully housed together without aggression. Aggression occurred only when foraging and sleeping sites had to be shared with other species. Dominant behavior of ruffed lemurs decreased when they were housed together with a group of ring-tailed lemurs. No aggressive behavior was observed within the group of ring-tailed lemurs afterwards.

It is expected that during this study the same will happen and aggressive behavior within the group of ring-tailed lemurs will decrease. The hypothesis is that the presence of the white belted black-and-white ruffed lemur will have a positive effect, on the social structure of the ring-tailed lemurs. The longer the presence of the white belted black-and-white ruffed lemurs, the closer the group of ring-tailed lemurs will become. If this introduction is successful in improving the social interactions, further introductions may be used to improve animal welfare for lemurs in the breeding programme.

2. Methods

This study took place in 2021 during the period of August through October.

2.1 Research population.

During this study, observations took place within a group of ring-tailed lemurs which are housed at AquaZoo, Leeuwarden. The population consists of 11 ring-tailed lemurs of which 5 are males and 6 are females (Table 1). The group consists of 1 parental pair, 3 mothers and their offspring, see Appendix I for the family structure.

Table 1. Group composition during the study period.

Species	individual	Gender	Date of birth
Lemur catta	Frankie	male	19/03/2007
Lemur catta	Lena	female	31/03/2011
Lemur catta	Zara	female	04/10/2011
Lemur catta	Ambony	female	31/03/2012
Lemur catta	Mia	female	15/04/2016
Lemur catta	Bokita	female	02/03/2017
Lemur catta	Luana	female	29/09/2018
Lemur catta	Carl	male	24/03/2019
Lemur catta	Nala	male	24/03/2019
Lemur catta	Lee	male	26/03/2019
Lemur catta	Yaro	male	27/03/2019
Varecia variegata subcincta	Aramis	male	26/04/2004
Varecia variegata subcincta	Babakao	male	02/04/2011

2.2 Enclosures

The enclosure of the ring-tailed lemurs at Aquazoo Leeuwarden consists of 2 parts that are arranged as nature-like as possible. This means that there are no artificial materials present in the enclosures, except for ropes. There is an indoor and outdoor stay that are connected by means of 4 panels. These panels can be opened and closed from the inside and are connected to a wooden bridge that allows the ring-tailed lemurs to move across the water. During the observations, the indoor enclosure was closed from 09:00 - 16:00h. After 16.00h, the enclosure was opened for the night. During weather conditions such as heavy rainfall, the indoor enclosure was also opened during the day.

Figure 1. 'outdoor enclosure'.

The outdoor enclosure of the ring-tailed lemurs is located at the beginning of the walking route in Aquazoo, see Figure 1. This is an unfenced area that visitors can walk through during opening hours (09:00 - 17:00). Half of the area consists of bushes and there are 4 large trees present. The other half of the area consists of grasslands. The vegetation that grows there is mainly elderberries, nettles, a variety of grasses, willows and Hazel. In the middle of the area is a small hill and there is a rope hanging from the south to the north which the ring-tailed lemurs use to move around. The entire outdoor enclosure is approximately 1200 m².

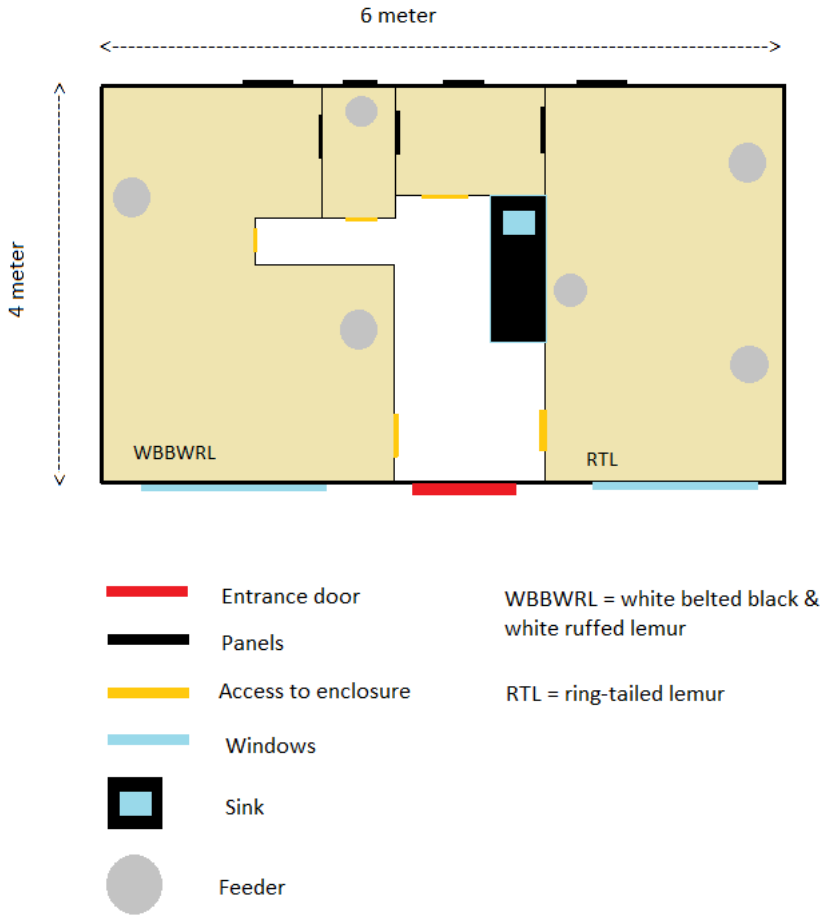

Figure 2. 'Indoor enclosure'.

The indoor enclosure (Figure 2) of the ring-tailed lemurs is located next to the entrance door of the outdoor area. The enclosure consists of 4 smaller enclosures connected by panels. There is a sink and all the enclosures can be entered separately. The indoor enclosure has a total area of approximately 24 m². There are climbing structures present and the floor is covered with sawdust.

2.2 Data collection

2.2.1 Observations

Based on research by Shire (2012) and Laméris (2021) an ethogram and protocol have been created (Appendix II, III). During the first 4 hours of observation, behaviors that were not observed such as "interaction with human" were being removed from the ethogram.

The 11 ring-tailed lemurs were identified prior to the observations by the chip in their necks. This created a file where each ring-tailed lemur was described regarding their external characteristics. For example, the condition of the coat, facial colors, body size, sex and unchangeable features such as a split ear. (Appendix IV).

The data was collected through continuous focal samples lasting 15 minutes each (Appendix III). During continuous focal sampling, all social behaviors were noted, as were the identity of the recipient as well as the duration of the behavior. All observations were conducted on a random order basis by using a random number generator. In this way, the order of observations varies by individual.

All individuals were observed a total of 46 times. This results in a total of 253 observations before and 253 observations after the introduction. This data is then transferred into several excel sheets and from excel into the program SPSS (Statistical Package for the Social Sciences). In SPSS a paired t-test has been carried out to reproduce the results (Appendix VI).

2.3 Data Analysis

2.3.1 Relationships

To process the data regarding the relationships between individual ring-tailed lemurs, a sociogram was used. A social network analysis can be used to determine the relationship and influence a ring-tailed lemur has on the group and other individuals within that group (Molinaro, 2021). A sociogram consists of nodes and edges between them. The nodes represent the individuals and the edges between the nodes represent the relationships between the individuals. A sociogram thus represents the social structure at a glance (Kulachi et al., 2017). By using a sociogram, key roles of individuals can be revealed. With the use of package 'Igraph' in the program R (version 3.4.1), network density and centrality measures such as; degree, eigenvector, betweenness and closeness were calculated and visualized (Luthe, Wyss & Schuckert, 2012).

2.3.1.2 Network density

To determine the density score of the group, a formula was used where the number of connections an individual has is divided by the total number of connections an individual can make. The number of connections is based on positive interactions and gives a score between 0 and 1, the higher the number the higher the density of the group, the closer the group.

2.3.1.3 Centrality measures

Degree is the number of direct relationships that an individual has within the group. For example, this group consists of 11 individuals, which makes 10 the highest number of degree (Molinaro, 2021).

The eigenvector is a number between 0 and 1 which indicates how much influence an individual has on the group. The higher the number the more influence. The eigenvector is based on the connections each individual has with other high influence (dominant) individuals (Bonacich, 2007).

Betweenness is based on edges in the sociogram (Figure 3) that run between individuals. The more edges passing through an individual, the more information is passed to other individuals. The higher the betweenness score the more important the individual is to the group.

Closeness, like the eigenvector, gives a number between 0 and 1 showing the shortest edges in the sociogram. The higher the number the more short connections with all other individuals there are. (Kasper & Voelkl, 2009).

2.3.2 Hierarchy

To determine the position of the individuals regarding dominance, a dominance matrix was made. The matrix was formed based on two forms of interactions that ring-tailed lemurs can exhibit with each other. These were aggressive and submissive interactions. Aggressive interactions included the behaviors; "barking," "crowding," "chasing," "biting," and "fighting". Submissive interactions included

the behaviors; “avoid” and “being chased away”. During observations all behaviors have been noted in combination with the individual performing the behavior and the individual who was receiving the behavior. The higher the score the more dominance was observed and the more negative interactions the individual won from another individual. The formula that was used was;

The proportion of wins between individual 1 (i) and individual 2 (j) (Pij). The times that i defeats j (ij) is then divided by the total number of interactions between i and j (nij). This gives the formula Pij= ij/nij. The second part of the formula is as follows; David's score = w + w2 - l - l2.
W represents the sum for all Pij values of i. W2 represents all w values and is then divided by the Pij values with which i interacted. l represents the sum for all Pji values of j. l2 represents all l values which are then divided by Pji values (Gammell et al., 2003) (Appendix V).

3. Results

In this next chapter, the results of this research will be treated by sub-question.

3.1 Relationships before adding white belted black-and-white ruffed lemurs

During the observations, 357 positive interactions were observed in the month prior to the introduction of the white belted black-and-white ruffed lemurs. This resulted in an average of 17.8 positive interactions per observation day.

3.1.1 Influence, betweenness and closeness score before the introduction

To determine the relationships between individuals in the group of ring-tailed lemurs, the degree, eigenvector, betweenness and closeness, for each individual, were calculated in R. Table 2 shows that each individual has a degree of at least 7 or higher. Bokita, Lee, Nala and Zara have the least number of direct relationships, where Luana and Yaro have the highest number of direct relationships.

Bokita has the highest eigenvector while Luana has the lowest eigenvector.
Carl and Lee have the highest betweenness score, where Luana has the lowest.

The closeness scores are close together, no individual stands out.

Table 2. The centrality values per individual based on the time spent on positive behaviors, before the introduction of the white belted black-and-white ruffed lemurs, during the research. Blue for male and pink for female.

Name	Age	Degree	Eigenvector	Betweenness	Closeness
Ambony	9	9	0.51	0.56	0.09
Bokita	4	7	1.00	0.49	0.08
Carl	2	9	0.77	1.18	0.10
Frankie	14	8	0.46	0.32	0.08
Lena	10	8	0.74	0.38	0.09
Lee	2	7	0.78	1.15	0.08
Luana	3	10	0.44	0.09	0.10
Mia	5	8	0.53	0.24	0.09
Nala	2	7	0.48	0.24	0.08
Yaro	2	10	0.72	0.54	0.09
Zara	10	7	0.80	0.74	0.10

Figure 3 shows the sociogram of the group before the white belted black-and-white ruffed lemurs were introduced. The relationship between Bokita and Lena is the strongest (Figure 3). Luana has strong relationships with Ambony and Mia and also maintains weak relationships with all other individuals in the group. Frankie has an average number of relationships but they are all weak.

3.1.2 Density score before the introduction

The density of the group of ring-tailed lemurs, in the month prior to the introduction of the white belted black-and-white lemurs, is 0.81.

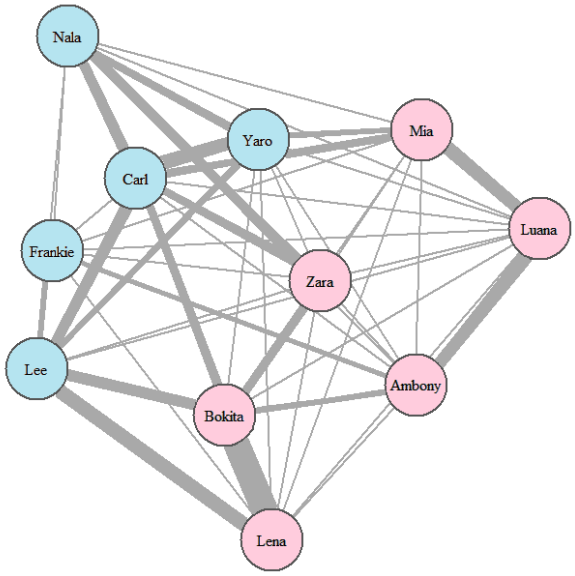

Figure 3. Sociogram based on positive interactions observed during the research, before the introduction of the white belted black-and-white ruffed lemurs. The nodes represent the individuals indicated in blue, male and pink for female. The edges between the nodes represent the strength of the relationship between them.

3.1.3 Dominance hierarchy before the introduction

Based on the David's score (Table 3), the dominance hierarchy within the group was calculated (appendix VI). As shown in Table 3, 3 out of the 6 females are high in dominance. The males are on average in the ranking.

Zara is the most dominant individual and Bokita is the least dominant individual, she ranks lowest among all individuals. The oldest individual (Frankie) is low in ranking but differs only slightly from the score of Carl who is higher in ranking, this is also the case for Ambony and Yaro.

Table 3. Dominance hierarchy normalized David's score per individual based on negative behavior observed, before the introduction of the white belted black-and-white ruffed lemurs, during the behavioral study. The higher the score the more dominance. Blue stands for male and pink stands for female.

Rank	Name	Age	NorDS
1	Zara	10	2.50
2	Mia	5	2.40
3	Luana	3	2.16
4	Lee	2	1.05
5	Yaro	2	0.30
6	Ambony	9	0.29
7	Carl	2	-0.10
8	Frankie	14	-0.11
9	Nala	2	-0.44
10	Lena	10	-2.14
11	Bokita	4	-2.30

3.2 Positive interactions after adding white belted black-and-white ruffed lemurs

In the month after the introduction of the white belted black-and-white ruffed lemurs, 409 positive interactions were observed between individuals, with an average of 20.4 interactions per day.

The difference in positive interactions, in the group of ring-tailed lemurs, before (M = 32.45; SD = 8.89) and after (M = 37.18; SD = 7.87) the introduction of the white belted black-and-white ruffed lemurs was not significant (Paired samples t-test; p = 0.22).

3.2.1 Influence, betweenness and closeness after the introduction

Each individual has a degree of at least 8 or higher (table 4). Bokita, Carl, Lena, Mia, and Yaro have the least number of direct relationships with other individuals. Lee, Nala, and Zara have direct relationships with all other individuals.

Bokita is the only individual with an eigenvector of 1. Zara has the lowest eigenvector.

Lee is the most important passer of information for the group, given the betweenness.
The least important individuals are Lena and Zara. With a score of 0, they have no contribution in the group in terms of passing on information.

Looking at closeness the numbers are again very close to each other, no individual stands out.

Table 4. The centrality values per individual based on the time spent on positive behaviors, after the introduction of the white belted black-and-white ruffed lemurs, during the research. Blue for male and pink for female.

Name	Age	Degree	Eigenvector	Betweenness	Closeness
Ambony	9	9	0.88	0.18	0.10
Bokita	4	8	1.00	0.17	0.09
Carl	2	8	0.57	0.14	0.08
Frankie	14	9	0.59	0.04	0.09
Lena	10	8	0.85	0.00	0.08
Lee	2	10	0.92	1.08	0.10
Luana	3	9	0.99	0.07	0.10
Mia	5	8	0.79	0.25	0.10
Nala	2	10	0.81	0.89	0.10
Yaro	2	8	0.60	0.14	0.10
Zara	10	10	0.45	0.00	0.10

Sociographic analysis show that the relationship between Bokita and Lena is again the strongest but differs little from the relationship between Lee and Lena (figure 4). The relationship between Frankie and Ambony is also strong. Followed by the relationship between Mia and Luana and the relationship between Mia and Ambony. Zara has a direct relationship with all the other individuals but they are all weak. Lee also has a direct relationship with all the other individuals of which four involve a strong relationship.

3.2.2 Density score after the introduction

The density score of the group of ring-tailed lemurs, after the introduction of the white belted black-and-white ruffed lemurs, is 0.88.

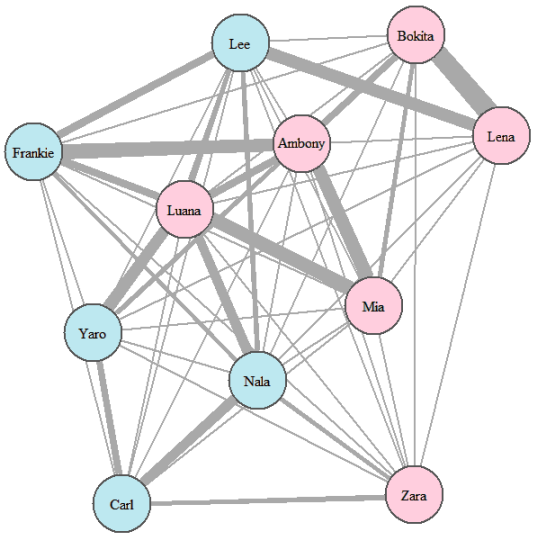

Figure 4. Sociogram based on positive interactions observed during the research, after the introduction of the white belted black-and-white ruffed lemurs. The nodes represent the individuals indicated in blue, male and pink for female. The edges between the nodes represent the strength of the relationship between them.

3.2.3 Dominance hierarchy after the introduction

A pattern can be seen in the ranking of the females. Again they are most dominant while the males rank on average.

Luana has a very high dominance score compared to the other high ranking females. Carl has a very low score and Mia and Zara have a score very close to each other.

Table 5. Dominance hierarchy normalized David's score per individual based on negative behavior observed during the behavioral study, after the introduction of the white belted black-and-white ruffed lemurs. The higher the score the more dominance. Blue stands for male and pink stands for female.

Rank	Name	Age	NorDS
1	Luana	3	2.96
2	Ambony	9	1.81
3	Mia	5	1.3
4	Zara	10	1.2
5	Frankie	14	1.01
6	Yaro	2	0.33
7	Nala	2	0.24
8	Bokita	4	-0.94
9	Lee	2	-1.55
10	Lena	10	-2.23
11	Carl	2	-4.05

3.3. Changes in negative dominance after adding white belted black-and-white ruffed lemurs

During the observations, 37 negative interactions were observed in the month prior to the introduction of the white belted black-and-white ruffed lemurs. This gave an average of 1.8 negative interactions per observation day. In the month after the introduction, 72 negative interactions were observed between individuals. Which gave an average of 3.6 interactions per day. This difference in negative interactions, in the group of ring-tailed lemurs, before (M = 3.45; SD = 4.55) and after (M = 6.63; SD = 7.07) the introduction of the white belted black-and-white ruffed lemurs was, however, not significant (Paired samples t-test; p = 0.07).

3.3.1 Sociogram of the negative interactions

The sociogram (Figure 5) shows that Carl did not have any negative interaction with the other individuals prior to the introduction. Zara was the most dominant in the month prior to introduction (Table 3) and had the most negative interactions with the other individuals. Bokita received the most negative interactions but did not herself inflict negative interactions on other individuals. The most negative interactions occurred one-way from Mia to Bokita, Zara to Bokita, and Luana to Bokita.

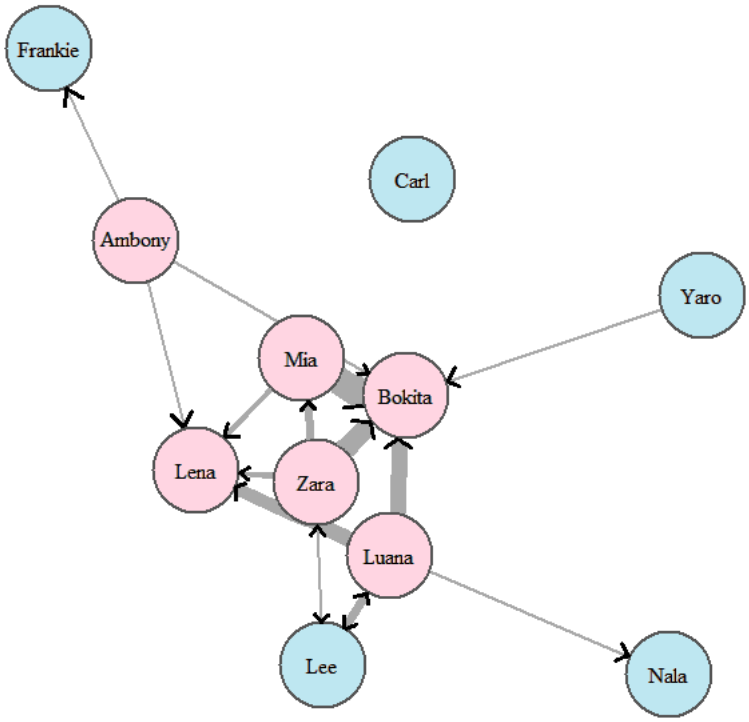

Figure 5. Sociogram based on negative interactions observed during the research, before the introduction of the white belted black-and-white ruffed lemurs. The nodes represent the individuals indicated in blue, male and pink for female. The edges between the nodes represent the strength of the negative interactions between both individuals.

Figure 6 shows the sociogram of negative interaction among individuals after the introduction of the white belted black-and-white ruffed lemurs. Yaro did not have any negative interactions with other individuals during that month. Luana was the most dominant in this month (Table 5) and inflicted the most negative interactions on other individuals. Carl was the least dominant individual in this month but does not receive the most negative interactions. Bokita, as in the month before the introduction, receives a lot of negative interaction but does have negative interactions with Lena and Frankie herself. Most negative interactions occurred one-way between Luana and Lena.

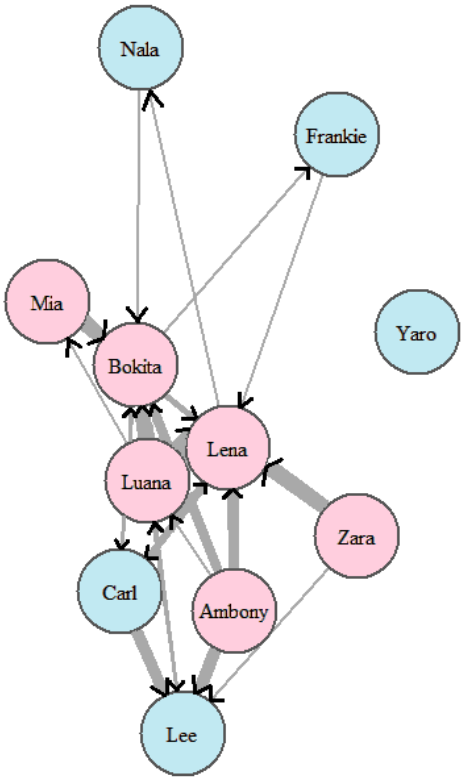

Figure 6. Sociogram based on negative interactions observed during the research, after the introduction of the white belted black-and-white ruffed lemurs. The nodes represent the individuals indicated in blue, male and pink for female. The edges between the nodes represent the strength of the negative interactions between both individuals.

4. Discussion

The purpose of this research is to determine if introducing another lemur species can have a positive effect on the social structure of a group of ring-tailed lemurs.

4.1 Changes in positive interactions

The results show an increase in positive interactions by 14% between the month before and after the introduction (table 4). Specifically, the density score of the group was higher (0.88) after the introduction than in the month before (0.81). This is consistent with what Spiezio et al. (2017) described.

Although an increase by 14% was found in positive interactions. The positive interactions from before and after the introduction was not significant (Paired samples t-test; $p = 0.22$). This is also the case for the observed negative interactions, since there was not a significant (Paired samples t-test; $p = 0.07$) difference found. Unexpectedly there was an increase in negative interactions of 23% the month before and after the introduction. This increase may be due to the fact that the females who were lower in ranking started having more negative interactions with other individuals in the group. Markus (2020) explained that even if females are low in ranking they are still dominant over the males. This can be seen by Bokita and Lena (Figure 6) who started showing dominant behavior at the younger male Carl.

In the month before the introduction, Bokita has by far the highest eigenvector score while she is the lowest ranking individual (Table 2). This can be explained by the negative interactions (Figure 5). The sociographic analysis shows that Bokita is the individual upon whom the dominant individual inflicts their negative interactions. This was an expected result since Kittler & Dietzel (2016) described that dominant females mostly focus on other females within the group. With all the focus the eigenvector will be higher since the individual will have more contact with high influence individuals.

All the individuals in this group maintain a low closeness score, despite the fact that they are family members of each other. While Carl and Nala are twins, Carl has the lowest closeness score of all the other members in the group. Sauther & Sussman (1993) explained in their study that ring-tailed lemurs that form a family, in this case even being twins, have closer ties to each other than individuals unrelated to them. Although this was not the case for the family of Zara, Carl and Nala, it was an expected result since Markus (2020) reported in his study that females will spend less time grooming and bonding with her offspring if they are male.

4.2 Changes in dominance hierarchy

Ambony, mother of one family, was not the most dominant female before the introduction and her offspring were spread over the ranking. In the month after, Ambony made a big move up in the ranking. Now that Ambony is higher in ranking, her offspring; Luana, Mia, Frankie and Yaro follow her almost back-to-back. This was expected since Bergstrom & Fedigan (2010) described that when females get higher in ranking, her offspring will also be higher in ranking. But for Zara and her offspring, this was the other way around. Based on observations of a group of ring-tailed lemurs, Koyama et al. (2005) mentioned that even if the mother is longer in ranking than her offspring, she will still be dominant over them.

It should be said that during this research all females were on contraception. The use of contraception can affect the testosterone levels. This means that high levels of this particular hormone can cause more dominant behavior (Gould & Ziegler, 2007).

4.3 Changes in negative interactions

Young males tend to be more active and show more positive and negative behavior, within the group than the other ring-tailed lemurs do. What is interesting about the sociogram of negative interactions is that Carl did not have any negative interactions with the other individuals before the introduction, (Figure 5) but he does have a negative score on the dominance ranking. This could be caused by the fact that even if males don't have any interaction, they are low in ranking compared to the females (Koyama et al., 2005). In the month after the introduction Yaro has no negative interactions with other individuals, he does have a positive score on the dominance ranking. Research by Koyama et al. (2005) says that this higher position in ranking could be caused by the fact that Yaro is part of the most dominant family.

4.1.1 Limitations

The group of ring-tailed lemurs was observed in a non-controlled environment for only a certain short period of time (8 weeks). There were several aspects that may have played a role in the mainly aggressive behavior the ring-tailed lemurs displayed towards each other and the white belted black-and-white ruffed lemurs.

The second part of this research started on the first day that the ring-tailed lemurs and the white belted black-and-white ruffed lemurs were placed together in the outdoor enclosure. Especially during the first week, the white belted black-and-white ruffed lemurs displayed dominant behavior towards the ring-tailed lemurs. During the last three weeks, this behavior changed into being more submissive during the observations. The ring-tailed lemurs behaved submissively and hesitantly during the first week after the introduction. During the last three weeks, they showed more interest in the white belted black-and-white ruffed lemurs. Lee, Yaro, and Luana in particular were displaying aggressive behavior towards the white belted black-and-white ruffed lemurs. Because no longer period was maintained between getting to know each other, the environment, and the observations, one cannot rule out the possibility that after the full four weeks of observation, different behaviors were shown by both species. Such as the expression of more or less aggression and dominance.

Another important detail is that the ring-tailed lemurs are housed in a walk-through area. Visitors are allowed to enter the area during the day. However, it is forbidden to feed or touch the ring-tailed lemurs (and the white belted black-and-white ruffed lemurs). However, this frequently occurred and it cannot be ruled out that certain aggressive behavior is based on the actions of visitors.

During the study, both Nala and Frankie had to be medically examined several times and were taken out of the group for a period of time. This too could have led to aggressive behavior when the individuals returned to the group, which may have influenced the results of the study (Markus, 2020).

5. Conclusions

In order to provide more clarity on the addition of another lemur species to a group of ring-tailed lemurs and thereby be engaged in the welfare of both species, the following main question was answered; 'What is the effect of adding white belted black-and-white ruffed lemurs on the social structure of a group of ring-tailed lemurs?' In order to answer the main question, four sub-questions have been answered.

- *'How strong are the relationships among the group members, in terms of betweenness, eigenvector and closeness, before adding white belted black-and-white ruffed lemurs?'*
  'Which individuals are the most dominant before adding white belted black-and-white ruffed lemurs?'

The social network of the group of ring-tailed lemurs, prior to the introduction of the white belted black-and-white ruffed lemurs, is not fully saturated but has a network density of 0.81. On a scale of 0 to 1, this score means that the group was very close in the month before the introduction. The bonds between mother and offspring are strong and this is especially apparent between Lena and Bokita, the two ring-tailed lemurs who are at the bottom of the ranks. The largest family within this group consists of five individuals. Looking at the centrality measures, it can be seen that the degree starts at 7, which means that each individual, outside of their own family, has at least two direct relationships. There is no individual with a score of 0 on the categories; eigenvector, betweenness and closeness. Because of this it can be stated that each individual has a role within the group. Thus, it can be stated the relationships within the group, before the introduction of the white belted black-and-white ruffed lemur is strong.

The most dominant individuals, prior to the introduction of the white belted black-and-white ruffed lemurs are all female; Zara, Mia and Luana.

- *'How strong are the relationships among the group members, in terms of betweenness, eigenvector and closeness, after adding white belted black-and-white ruffed lemurs?'*

After the introduction of the white belted black-and-white ruffed lemurs, the network density was measured at 0.88, which means that the group got even more closer than the month before. The bonds between family members were also stronger. In contrast to the month before (2 individuals), in the month after 3 individuals have a degree of 10. All ring-tailed lemurs have a degree of at least 8 in the month after. Because of this it can be stated that the relationships within the group of ring-tailed lemurs are strong.

However, there is no significant difference in the positive interactions before (Paired samples t-test; $p = 0.07$) and after (Paired samples t-test; $p = 0.22$) the introduction of the white belted black-and-white ruffed lemurs.

- *'Which relationships, with the focus on negative dominance, have noticeably changed in the group of ring-tailed lemurs after the addition of the white belted black-and-white ruffed lemurs at AquaZoo?'*

Most of the negative interactions come from females. In this case; Zara and Mia. They had the most negative interaction with Bokita. In month two Zara had no negative interaction with Bokita at all and the negative interaction between Mia and Bokita has become less. This means that their relationship has changed after the introduction.

Although there are more positive interactions after the introduction of the white belted black-and-white ruffed lemurs and also the bonds between the ring-tailed lemurs in the group have become stronger, the difference in both positive and negative interaction between the two months is not significant. Since scores on relationships between group members are not independent observations, the interpretation of these test results is somewhat contentious. There is, however, a clear difference in the density of the group that can be demonstrated with the density score. In either way, the group of ring-tailed lemurs has become closer. In the month after the introduction, more negative interactions were observed than in the month after, from the individuals low in the dominance ranking. Regarding this, it can be explained that these individuals (Bokita and Lena) also dared to engage in the negative interaction and thus got higher in the dominance ranking. This behavior contributes to a group in which almost every individual has an equal role.

5.1 Recommendations

For increasing well-being within a group of ring-tailed lemurs, a recommendation, on a short-term basis can be, that when closing in the ring-tailed lemurs, the families are maintained together. This research showed that the young individuals tend to feel unease whenever they are separated by their family. To prevent the individuals from showing aggressive behavior towards other individuals, the families should be kept together.

When one wants to introduce another prosimian to a group of ring-tailed lemurs it is recommended to have separate foraging and sleeping/resting areas for both species. During this research the white belted black-and-white ruffed lemur had a hard timing moving in to their indoor enclosure whenever the ring-tailed lemurs blocked their door. Having multiple foraging and sleeping/resting areas around the outdoor enclosure could mean that there is less territorial behavior.

It is also necessary to share more knowledge about the territorial and aggressive behavior of prosimians with the animal caretakers. This mainly involves the territorial behavior that the ring-tailed lemurs can display towards other prosimians, or specifically the white belted black-and-white ruffed lemurs, and how they then respond to it. During this research not all individuals focused themselves on the white belted black-and-white ruffed lemurs. If this behavior continues for a longer period of time, a separation in the group may occur. In the short term, enrichment for both species can be used to prevent this.

Another recommendation would be to start research on the group of ring-tailed lemurs and that way get a clear understanding of their social structure, when one wants to participate in a breeding program. With more knowledge about the social structure the right individual can be taking out of the group. Or, the most dominant individuals can be placed apart when a new individual is being introduced to the group.

5.1.1. Recommendations for AquaZoo

Currently, when the ring-tailed lemurs are closed in, Bokita and Lena are kept separate from the group because of Lena's low rank position. However, Lee also belongs to this family and the absence of Lena and Bokita causes him to feel unease. He then projects this agitation onto the other individuals in the group.

Also, a ring-tailed lemur should only in case of an emergency be absent from the group for more than 8 hours. This is because after 8 hours of Frankie's absence, due to a medical check out, it was found that signs of aggression were occurring from Frankie directed at the group and vice versa.

In the long term it is advisable to continue the research on the social structure of the group when one wants to make some changes, such as adding a new individual because of a breeding program. Once it

is clear which individual is the most dominant, it is easier to determine which individual should be used for breeding. Within this group I advise to choose an individual that has the least number of relatives at that time. The more relatives, the higher the chance of dominance. In this case, if Ambony would be used in the breeding program, this would mean that Ambony and her offspring, Mia, Luana and Yaro could display even more dominant behavior. Given that Bokita belongs to the family that is at the bottom of the rank, and being one of the younger females, Bokita would be the most obvious choice (If the breeding program allows).

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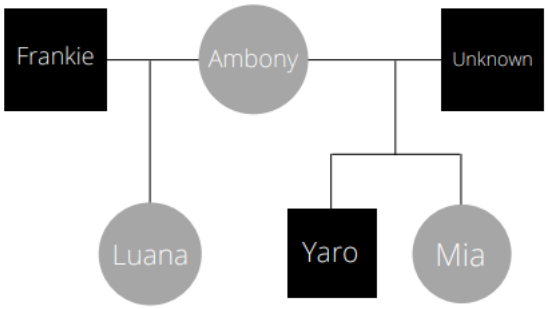
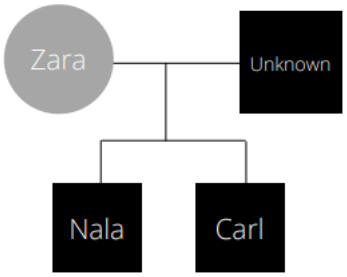
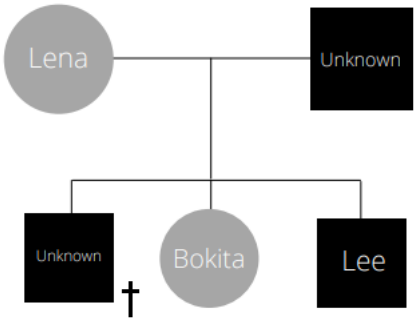
Appendix I; ‘Family structure ring-tailed lemurs’.







Appendix II. 'Ethogram'.

Solitary behavior

Behavior	abbreviation	definition	Type
Foraging	F	Individual searches for food through active search or inactive search, through visual search for food in the enclosure. Consuming or not consuming the food.	State
Grooming	G	Individual grooms itself by using its paws or teeth/mouth.	State
Sitting	SI	Individual is sitting upright, alert, head up and eyes open.	State
Vocal expression	VE	Individual makes a purring or growling sound. These occur in various contexts such as grooming behavior or when threatened.	Event
Exploring	EX	Licking, biting, or smelling objects within the enclosure, except for the food/drink containers.	State
Solitary play	SP	Individual interacting with or using an object on his own.	State
Locomotion	L	To locomote on one or more limbs by swinging, climbing or jumping.	State

Positive social behavior

Behavior	abbreviation	Definition	Type
Social foraging	SF	Individual searches for the same food or eats actively in the presence, at least 5 meters side by side of at least one other individual.	State
Social resting	SR	Individual rests with at least one other individual.	State
Grooming of others	GOO	Individual cleans a member of the group by using its paws or teeth/mouth.	State
Sexual Behavior	SB	Individual has sexual interaction with another individual.	State
Social play	SSP	Individual participates in running, holding, grabbing and biting without aggression with at least one other individual.	State

Negative social behavior

Behavior	abbreviation	Definition	Type
Repress	RP	Individual makes way for another individual.	Event
Chasing	C	Individual chases another individual. (Running).	Event
Hit	H	Individual violently touches another animal by using its paw.	Event
Avoiding	AV	Individual runs away from his spot as soon as another individual approaches.	Event
Biting	B	Individual attacks a member of the group bij using his teeth.	Event

Inactive behavior

Behavior	abbreviation	Definition	Type
Resting	R	Individual has its head down, eyes open.	State
Sun	SU	Individual moves toward the sunlight. The front of the body turned and open towards the sun.	State
Sleeping	S	Individual has it's head down, eyes closed.	State

Other behavior

Behavior	abbreviation	Definition	Type
Out of sight	OS	Individual is out of sight for the observer.	State
Marking	M	Individual uses its scent glands to mark surfaces with. Scent glands are located under the tail/arm and inner lower arm.	Event
Interaction with human	IH	Individual communicates or has some other form of interaction with visitors/caretakers.	Event

Appendix III. 'Protocol'.

FOCUS sampling

Individual; Date; Time; Weather;

	______	Time / Behavior	F	ZV	Z	L/R	VU	EX	SS	L	SF	SR	VZA	SG	SSP	V	J	SL	VM	BIJ	R	ZO	S	UZ	G
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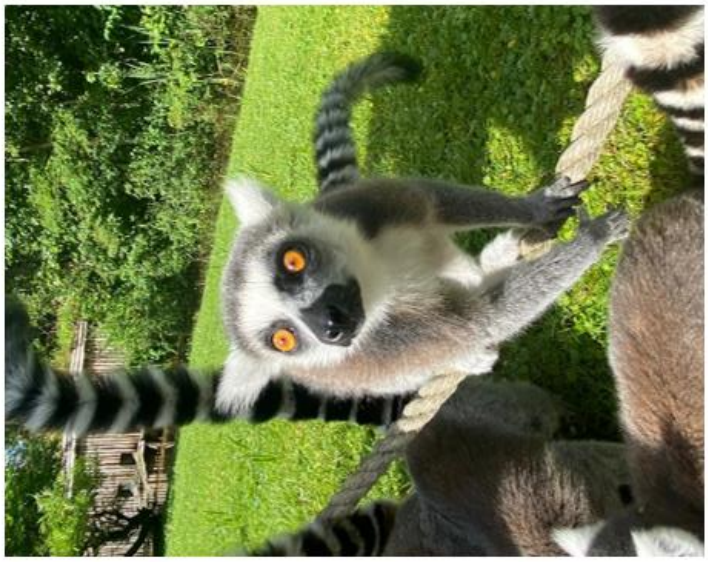
Appendix IV. ‘Identification map ring-tailed lemurs'.

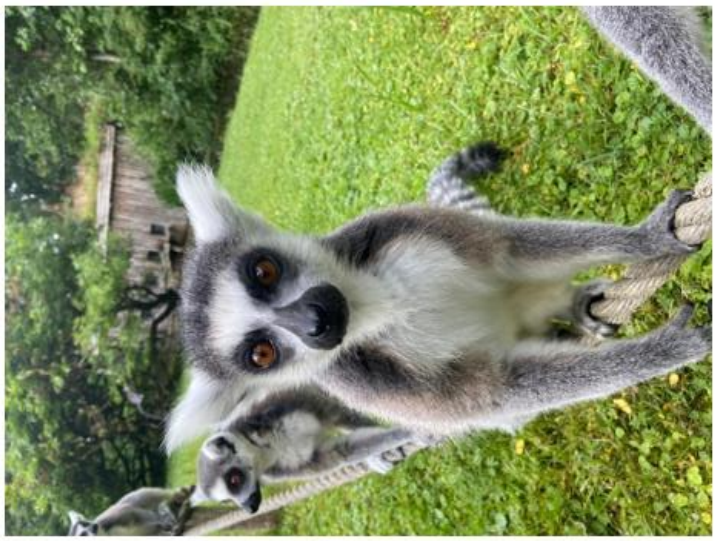


NALA

Geslacht: man
Geboortedatum: 24 maart 2019
Kenmerken:
Weinig zwart rond ogen. Korte neus.
Kleine oren. Klein van stuk.

AMBONY

Geslacht: vrouw
Geboortedatum: 31 maart 2012
Kenmerken:
Zwart vlekje rechterkant van neusbrug.
Brede oren, lange witte pluimen.



MIA

Geslacht: vrouw
Geboortedatum: 15 april 2016
Kenmerken:
Brede oren. Lange neus.
Lange witte pluimen aan de oren.
Zwarte ‘wallen’ onder ogen.

LEE

Geslacht: man
Geboortedatum: 26 maart 2019
Kenmerken:
Naar binnen geklapt oor.

YARO

Geslacht: man
Geboortedatum: 27 maart 2019
Kenmerken:
Geen tot weinig witte pluimen aan de oren.
Lange neus met lange zwarte strepen op neusbrug.

ZARA

Geslacht: vrouw
Geboortedatum: 4 oktober 2011
Kenmerken:
Smalle oren.
Lange witte pluimen aan oren.
Neuszwart raakt zwarte ooghoeken.

LENA

Geslacht: vrouw
Geboortedatum: 31 maart 2011
Kenmerken:
Wit/grijze borst, schouders. (Bruin mist).
Korte neus.

BOKITA

Geslacht: vrouw
Geboortedatum: 2 april 2017
Kenmerken:
Scheur in het rechteroor.

LUANA

Geslacht: vrouw
Geboortedatum: 29 september 2018
Kenmerken:
Korte witte pluimen aan brede oren.
Zwarte vacht bovenkant oog loopt in punt.

CARL

Geslacht: man
Geboortedatum: 24 maart 2019
Kenmerken:
Lange witte pluimen aan oren.
Bredere neus. Klein van stuk.
Grotere zwarte ooghoeken.

FRANKIE

Geslacht: man
Geboortedatum: 19 maart 2017
Kenmerken:
Alfa man. Groot van stuk.
Vergrote geurklieren in armen.
Hoektanden zichtbaar.
Geslachtorgaan zichtbaar.

Appendix V. 'David's score'.

Negative interactions before the introduction

	Lee	Yaro	Zara	Mia	Ambony	Nala	Lena	Bokita	Luana	Carl	Frankie
Lee		0	0,2	0	0	0	0	0	0,5	0	0
Yaro	0		0	0	0	0	0	0,15	0	0	0
Zara	0,12	0		0,33	0	0	0,4	0,25	0	0	0
Mia	0	0	0		0	0	0,5	0,73	0	0	0
Ambony	0	0	0	0		0	0,1	0,08	0	0	0,11
Nala	0	0	0	0	0		0	0	0	0,05	0
Lena	0	0	0	0	0	0		0	0	0	0
Bokita	0	0	0	0	0	0	0		0	0	0
Luana	0,66	0	0	0	0	0,5	0,6	0,8		0	0
Carl	0	0	0	0	0	0	0	0	0		0
Frankie	0	0	0	0	0	0	0	0	0	0	

Negative interactions after the introduction

	Lee	Yaro	Zara	Mia	Ambony	Nala	Lena	Bokita	Luana	Carl	Frankie
Lee		0	0	0	0	0	0	0	0,06	0	0
Yaro	0		0	0	0	0	0	0	0	0	0
Zara	0,14	0		0	0	0	0,5	0	0	0	0
Mia	0	0	0		0	0	0,28	0,4	0	0	0
Ambony	0,37	0	0	0		0	0,42	0,15	0,05	0	0
Nala	0	0	0	0	0		0	0,11	0	0	0
Lena	0	0	0	0	0	0,12		0	0	1	0
Bokita	0	0,33	0	0	0	0	0		0	1	0,33
Luana	0,12	0	0	0,09	0	0	0,91	0,73		0	0
Carl	0,36	0	0	0	0	0	0,37	1	0		0
Frankie	0	0	0	0	0	0	1	0	0	0	

David's score before the introduction

W	W2		L	L2		NOR DS
0,7	1,5	2,2	0,791	0,35	1,141	1,059
0,15	0,15	0,3	0	0	0	0,3
1,108	1,597	2,705	0,2	0	0,2	2,505
1,23	1,23	2,46	0,33	0,06	0,39	2,4
0,294	0	0,294	0	0	0	0,29
0,055	0	0,055	0,5	0	0,5	-0,44
0	0	0	1,6	0,54	2,14	-2,14
0	0	0	2,013	0,29	2,303	-2,303
2,566	0,489	3,055	0,5	0,39	0,89	2,165
0	0	0	0,055	0,05	0,105	-0,105
0	0	0	0,11	0	0,11	-0,11

David's score after the introduction

W	W2		L	L2		NOR DS
0,06	0,11	0,17	0,99	0,73	1,72	-1,55
0	0	0	0,33	0	0,33	0,33
0,64	0,56	1,2	0	0	0	1,2
0,68	0,75	1,43	0,09	0,009	0,099	1,3
0,99	0,82	1,81	0	0	0	1,81
0,11	0,18	0,29	0,12	0,41	0,53	-0,24
1,12	1,74	2,86	3,48	1,61	5,09	-2,23
1,66	2,06	3,72	2,39	2,27	4,66	-0,94
1,85	1,27	3,12	0,11	0,05	0,16	2,96
1,73	2,09	3,82	2	5,87	7,87	-4,05
1	1,12	2,12	0,33	0,78	1,11	1,01

Appendix VI. 'Paired T-test'.

positive interactions	individual	before	after	
	Ambony	23	35	
	Bokita	48	46	
	Carl	44	35	
	Frankie	23	21	
	Lee	40	34	
	Lena	32	37	
	Luana	25	52	
	Mia	27	39	
	Nala	21	44	
	Yaro	36	30	
	Zara	38	36	
				t test (unpaired)
	mean	32,45455	37,18182	0,222748
				t test (paired)
				0,227971
	standard deviation			
		8,897023	7,871908	

negative interactions	individual	before	after	
	Ambony	3	13	
	Bokita	0	2	
	Carl	0	9	
	Frankie	0	1	
	Lee	2	1	
	Lena	0	5	
	Luana	12	25	
	Mia	9	8	
	Nala	0	1	
	Yaro	1	0	
	Zara	11	8	
				t test (unpaired)
	mean	3,454545	6,636364	0,245899
				t test (paired)
				0,074806
	standard deviation			
		4,559977	7,074573	