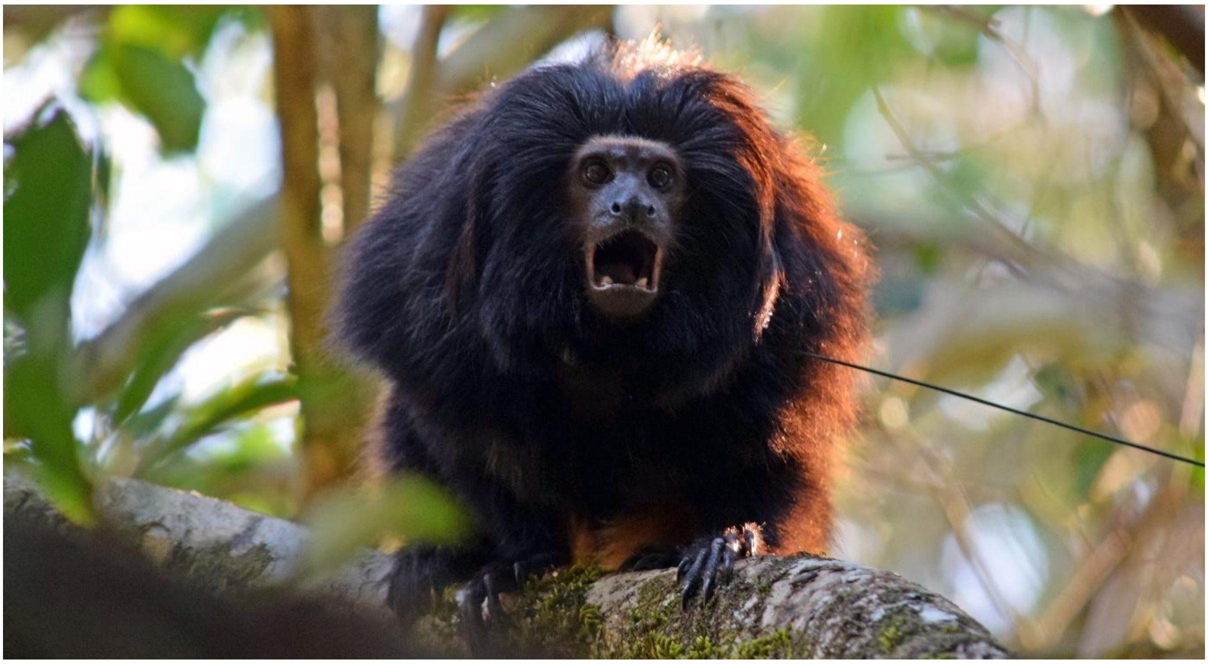

TESTING NEST BOX DESIGNS FOR TAMARINS

This studies evaluates which design of artificial nest box is the least accessible for predators of the tamarin and still most likely to be accepted as a suitable sleeping site for the black lion tamarin in the wild

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Leeuwarden, October 2017

Cover page photo:
Black lion tamarin in a semi-deciduous forest remand in Teodoro Sampaio, São Paulo State, Brazil. Santa Mônica Farm. Author: Miguelrangeljr. Taken on: 23 September 2014, extracted from:
https://commons.wikimedia.org/wiki/File:Black_lion_tamarin_Pontal_do_Paranapanema_3.jpg

Preface

This research report is part of a final thesis project for University of Applied sciences Van Hall Larenstein located in Leeuwarden, conducted by Judith Ahsmann. During this study different nest box designs were evaluated on preference and safety in captivity to be able to use boxes in a wild setting and create a suitable habitat for Black Lion Tamarins in Brazil.

I would like to use this moment to thank a few people who helped during the process of completing this report. At first I would like to thank Tine Griede and Hans Bezuijen for all the help and advising during the process of conducting this study. Also I would like to thank Dominic Wormell and Eluned Price for offering me the opportunity to conduct this research at Durrell Wildlife Conservation Trust and for providing all the necessary information and support needed to create this product. At last I would like to thank Frank Rietkerk and Warner Jens for giving me the opportunity to do some extra tests at Apenheul primate Park in the Netherlands. After all I really enjoyed conducting this study and I am very great full for all the support and positivity people around me gave me.

Judith Ahsmann,
October 2017, Leeuwarden

Summary

Durrell Wildlife Conservation Trust, located in Jersey, and IPÊ (Instituto de Pesquisas Ecológicas), located in São Paulo in Southern-Brazil, have been working together for years to save the critically endangered Black lion tamarin *(Leontopithecus chrysopygus).* The main concern for this species is that they live in 11 small fragmented populations (Kierulff, et al., 2008). Isolation of small populations due to habitat fragmentation is of big concern because this can result in small gene pools. Eventually this can lead to a decrease of the ability of the species to adapt to environmental changes or diseases (Frankham, 2005). To create a more viable situation, Durrell and IPÊ set up a project to reconnect these fragmented pieces of habitats by growing forest corridors (Durrell Wildlife Conservation Trust, 2016). The corridors are now 10 years old and previous assessment showed that enough food is available for the tamarins but no sleeping sites (Rezende, 2015). Since tamarin species are known to mainly sleep in hollow trees or tree cavities ((Franklin, et al., 2007); (Dietz & Peres, 1997)) and the forest has existed for only 10 years, no hollow tree or natural tree cavities are present yet. To create a more suitable habitat for the Black lion tamarins the idea of artificial sleeping sites developed. Nest boxes are successfully used as artificial nesting or sleeping sites for other species in the wild (Fidloczky, et al., 2014); (Libois, et al., 2012); (Katzner , et al., 2005) and for tamarins in captivity.

To create a nest box suitable for the wild, the box needed to be safe and used. Three features were considered to be important; shelves, tunnels and multiple entrance points. Since there are barely any black lion tamarins left in captivity, the closely related pied tamarin *(Saguinus bicolor)* was used to test nest box preference. Two experiments were conducted. The first one was a selection experiment in which was tested whether the tamarins would accept different types of nest boxes as a safe and comfortable sleeping site. Different nest box designs were presented and the choice of box to sleep in was recorded for 13 nights. This was done for all nest box designs. The second experiment was to test proximity to nest boxes, in which was investigated if tamarins spent more time in and around different nest box designs. To test the vulnerability of the nest box against possible predators, the different boxes were offered to ring-tailed coati's *(Nasua nasua)*, black crested macaques*(Macaca nigra)*, white headed capuchin *(Cebus capucinus)* and the yellow-breasted capuchin *(Cebus xanthosternos).* Observed was how much time it took the potential predators to obtain a novel food item presented inside different nest box designs.

In both the selection experiment as well as in the proximity experiments, boxes with shelves and tunnels were found to be most favourite. When testing nest boxes on safety, boxes with tunnels were most safe. None of the predators managed to obtain the novel food item from the box. Boxes with multiple entrance points were selected less. But giving the tamarins a nest box with multiple entrance points is the only possibility to give tamarins a possibility to flee if predators manage to get inside. A possibility to still use boxes with multiple entrance points and make them safe and favoured by tamarins would be to combine both those entrance points with tunnels. Since shelves were favoured by tamarins and safe from some predators, this feature could be implemented as well. But something to take into consideration is the group size of wild tamarins. By implementing a shelve into the design the space they have to sleep is reduced enormously. This might have an effect to the selection of boxes for big groups, since the space they get is not big enough. Another possibility to keep tamarins safe is to adjust the pop hole. Especially because of other potential predators present in the corridors. Large snakes and Tayra's*(Eira barbara)* are known to eat tamarins as well. Adjusting the shape and size of the pop hole to a minimum will decrease the chance of predation. A suggestion is to use a keyhole shaped pop hole. This design is already successfully used in mixed species exhibits in Apenheul primate park.

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1. Introduction

Durrell Wildlife Conservation Trust, located in Jersey, and IPÊ (Instituto de Pesquisas Ecológicas), located in São Paulo in Southern-Brazil, have been working together for years to save the critically endangered black lion tamarin *(Leontopithecus chrysopygus).* black lion tamarins are considered to be the rarest and least known monkeys among the New World Primates. This species is part of the Callitrichidea family which are all small, squirrel sized animals (see figure 1) (Snowdon , et al., 1986). The black lion tamarin was expected to be extinct, until a small population was rediscovered in Morro Diablo in 1970 (Albernaz, 1997). Since this moment Durrell and IPÊ have been working together to conserve this species by using captive breeding and reintroduction (Durrell Wildlife Conservation Trust, 2016). These projects led to an estimated total population of 1000 adult individuals. Nowadays the main concern for this species is that they live in 11 fragmented populations around São Paulo, and only one is considered to be viable on long-term (Kierulff, et al., 2008). This population is positioned in Morro do Diablo State Park and is estimated at 820 adult individuals. The other individuals are divided over 10 small parts of fragmented forests (IUCN, 2016). Isolation of small populations due to habitat fragmentation is of big concern because this can result in small gene pools. A small gene pool can cause loss of genetic diversity, heterozygosity and polymorphism due to genetic drift or inbreeding (Frankham, 2005). Eventually this can lead to a decrease of the ability of the species to adapt to environmental changes or diseases (Frankham, 2005). To create a more viable situation, Durrell and IPÊ set up a project to connect these habitats to stimulate genetic exchange between populations (Durrell Wildlife Conservation Trust, 2016). This project started with the growing of more than 10km natural forest corridors between Morro do Diabo State Park and Pontal do Paranapanema. These corridors are now 10 years old. Assessment of these corridors show that approximately 50% of the planted trees offer food resources for the tamarins (Rezende, 2015). Besides the availability of food, sleeping sites are considered to be a limiting resource for the presence of certain species in the area (Luthermann, et al., 2010). Since tamarin species are known to mainly sleep in hollow trees or tree cavities ((Franklin, et al., 2007); (Dietz & Peres, 1997)) and the forest has existed for only 10 years, no hollow tree or natural tree cavities are present yet. This results in a lack of sleeping sites and therefore the forest corridors are not considered to be suitable for the black lion tamarins yet. (See figure 2)

FIGURE 1: BLACK LION TAMARIN (*LEONTOPITHECUS CHRYSOPYGUS*). PHOTOGRAPHER: ALAN HILL, 2013

FIGURE 2: LEFT; LIVING AREA. RIGHT; DETAILED MAP OF LIVING AREA, MARKED PATH ARE THE GROWN FOREST CORRIDORS

Durrell Wildlife Conservation Trust and IPÊ set up a conservation action plan for black lion tamarins. Part of this plan is to make the forest corridors more viable for black lion tamarins. To create a viable habitat more sleeping sites should be available which resulted in the idea of creating artificial sleeping sites. The use of nest boxes has been a successful habitat restoration tool for a wide range of bird species (Fidloczky, et al., 2014); (Libois, et al., 2012); (Katzner , et al., 2005) but is not commonly used for mammal conservation. A former study on the use of nest boxes for small arboreal cavity dependent mammals in Australia shows that nest boxes could be used as a successful habitat restoration tool as long as species specific boxes are used (Goldingay, et al., 2015). Because of the experiences with successful use of nest boxes for a wide range of tamarin species in captivity it is expected that nest boxes also could be used in a wild situation (D. Wormell, September 2016, personal communication).

For the use of nest boxes in a wild situation it is important to know which features are important for tamarin species. For the captive situation there are multiple examples of successful use of artificial nest boxes. These examples and the frequency in which the nest boxes are used by the tamarins provides important information of which features are relevant for the design. According to the EAZA (European Association Zoos and Aquaria) best practice guidelines for Callitirichids, for a family groups, at least one nest box should be offered in the inside enclosure. These nest boxes are, most of the time, simple boxes with one entrance hole of approximately 10cm square with a slide to capture the animals inside if needed (Bairrão Ruivo & Stevenson, 2015). The boxes are used as sleeping site and animals will retire to these boxes every evening after the lights turn down (Caine, 1987). nest boxes made of plywood proven to be suitable for tamarins in a captive situation (Bairrão Ruivo & Stevenson, 2015). Experimental studies to tamarins in captivity indicate that tamarins will select the nest boxes located in areas with the highest concealment levels (Caine, et al., 1992); (Heyman, 1995). Even though captivity might suggest what could be relevant for a tamarin in selecting and using a nest box, the situation in the wild is different and might require another design. Captivity lacks environmental threats, like predation and climate. Andersen,(1998) describes predation, lack of comfort and hygiene as the main threats from the wild to influence sleeping site selection in tamarins.

Black lion tamarins are a perfect prey for possible predators due to their size. Tamarins are most vulnerable during the night because of reduced predator detection and flight ability (Franklin, et al., 2007). One of the most pertinent strategies to gain protection is to select sleeping sites which predators cannot enter. But also the detectability of the sleeping sites and facilitating detection of the approach of a predators take part in the selection process (Anderson , 1998). Considering comfort of sleeping sites, it is found that all kind of tamarin species select tree hollows and cavities ((Franklin, et al., 2007) (Dietz & Peres, 1997)). It is possible to design a nest box which is less accessible for predators. However it is very hard to predict whether the tamarins will accept these nest boxes as a safe and comfortable sleeping site. Before these boxes are provided to the black lion tamarins in the wild there is a need to know how likely it is that they are indeed safe and will be accepted in the wild.

The safety from predators is much depending on the possibility of entrance for the predators. The main predators are capuchin monkeys *(Cebus apella.)*, tayra's *(Eira Barbara)* coati's *(Nasua nasua)* and large snakes (Franklin, et al., 2007). Capuchin monkeys are known for complex object manipulation when food is involved, they have a wide variability of tools they use to gather food (Eduardo & Izar, 2008). Coatis are known to be mainly omnivorous but their diet does mainly exists out of fruit and invertebrates. Occasionally they are able to catch a small vertebrate, mainly frogs and small mammals (Hirsch, 2009). They are found to collect black lion tamarins from sleeping sites which they could easily access (Franklin, et al., 2007). To make it harder for coatis and capuchin monkeys to get into the nest box a long entrance point, in the form of a tunnel could be relevant to keep capuchin monkeys out. Also adding a shelve inside of the nest box, creating an extra compartment, could be useful. Considering predation of snakes an entrance tunnel is not relevant. To give the tamarins a possibility to flee an extra entrance/exit point could be implemented. However these additions to the nest box might not only affect the accessibility for predators, but also the appreciation, resulting in use, of the tamarins.

Besides the selection of the nest box it could be interesting to investigate time spent around the nest box during the time of selection. The time spent around a nest box might be an indicator for preference. For both selection of boxes and behavioural changes it is possible to test in a captive situation. Also the possibility for predators to reach tamarins inside the nest box could be tested in captivity. This could provide relevant information to use for the implementation of nest boxes in the wild.

There are not many black lion tamarins kept in captivity outside of Brazil. However closely related tamarin species are kept in many zoos in Europe. These species use similar sleeping sites in the wild (Vidal & Cintra, 2006); (Rezende, 2015). In Durrell Wildlife Conservation 7 groups of pied tamarins *(Saguinus bicolor)* are kept in neighbouring comparable exhibits, offering an excellent situation for choice experiments.

Coati's are kept at Durrell Wildlife Conservation Trust and are natural predators of black lion tamarins. This Durrell population is, therefore perfect to test if predators can get inside the box. Besides coati's, capuchin monkeys are the main predators of black lion tamarins in the wild. Since capuchin monkeys are not kept at Durrell Wildlife Conservation Trust, the captive group of Sulawesi black-crested macaques*(Macaca nigra)* was used to test predator accessibility of the nest box. This species does show very similar behaviours, considering feeding patterns and tool use, as the capuchin monkeys and is quite famous for their strength and ability to break things in captivity (O'Brien & Kinnaird, 1997). To see the effect of capuchin monkeys two different species are used located at Primatepark Apenheul in the Netherlands. This were white headed capuchin monkeys *(Cebus capucinus)* and the golden-bellied capuchin*(Cebus xanthosternos).*

This studies aims to know which design of artificial nest box is the least accessible for predators of the tamarin and still most likely to be accepted as a suitable sleeping site for the black lion tamarin in the wild.

To achieve that the following question has to be answered:

''What is the ultimate design of a suitable artificial sleeping site, tested in captivity, to implement as a habitat restoration tool in the forest corridors for black lion tamarins.''

To answer this research question several sub-questions are formed:

1) *What is the influence of multiple entrance/exit point of the nest box on the selection of nest boxes in captive tamarins*
2) *What is the influence of entrance tunnels on the selection of nest boxes in captive tamarins*
3) *What is the influence of the implementation of a shelve inside the nest box on the selection of nest boxes in captive tamarins*
4) *What is the influence of multiple entrance/exit points on the behaviour around the nest box in captive tamarins*
5) *What is the influence of entrance tunnels on the behaviour around the nest box in captive tamarins*
6) *What is the influence of the implementation of a shelve inside the nest box on the behaviour around the nest box in captive tamarins*
7) *What is the influence of the implementation of entrance tunnels to the nest box on the safety of the tamarins from predators*
8) *What is the influence of multiple entrance/exit point of the nest box on the safety of the tamarins from predators*
9) *What is the influence of the implementation of a shelve inside the nest box on the safety of the tamarins from predators*

2. Material and methods

2.1. Study design

To answer the research questions a quantitative research was conducted (Baarda en de Goede, 2006). To test nest box selection and changes in nest box related behaviours, different nest box designs were developed. Characteristics which were considered to be important were: the number of entrance points: one or two, implementation of entrance tunnels and the presence of a shelve in the box (Wormell & Price, pers. comm., September 2016).

To verify the nest boxes provide a safe sleeping site, the boxes were designed in a way which was considered to be predator proof. To test if the boxes did keep predators out a case study is conducted. In this study the box was offered inside enclosures of potential predators, to see to see if they were able to intrude and how long it takes them.

2.2. Materials

For this study, nest boxes with different characteristics were used. One with a entrance tunnel (see figure 2), a box with a shelve (see figure 3) a box with multiple entrance points (see figure 4) and a normal empty nest box. The box was designed in a way that the basis was the same during the whole experiment. Only the specific characteristics important for the experiment were adjustable. The boxes will be coded as is shown in table 1.

TABLE 1 NEST BOX CODES

Code	Nest box
NB-T	Nest box with tunnels
NB-S	Nest box with shelve
NB-E	Nest box with multiple entrances
NB-N	Normal, empty nest box

Cameras were used to prevent that the presence of humans would influence the behaviours. In total 20 cameras were used.

FIGURE 3 NEST BOX WITH TUNNEL

FIGURE 4 NEST BOX WITH SHELVE

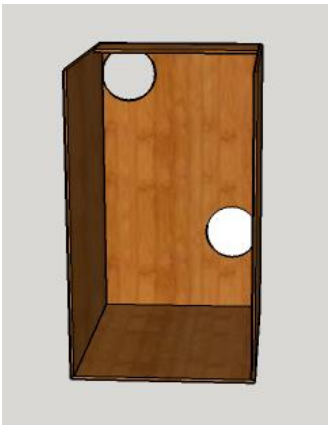

FIGURE 5 NEST BOX WITH MULTIPLE ENTRANCE POINTS

2.3. Study population

Nr.	Enclosure	Group composition
1	G7	1.1.0*
2	G12	1.1.0
3	G4/5	0.2.0
4	F2	4.0.0
5	OS4	1.1.0
6	0S7	1.1.0
7	SCA/B	3.3.0
***Notation: male.female.unknown**		

TABLE 2 RESEARCH POPULATION PIED TAMARINS

The research population consisted of 20 pied tamarins divided over 7 groups (see table 1). All these groups were used in nest box preference tests and behavioural observations.

For the anti-predation tests four different species are used. The first species was the Sulawesi crested macaque *(Macaca nigra).* The population existed of seven individuals in one multi male- multi female group. The second species was the coati *(Nasua nasua).* This population existed out of three individuals, all females. Both these groups were situated at Durrell Wildlife Park in Jersey. Also, tests were done at Apenheul Primate Park in the Netherlands with the white headed capuchin *(Cebus capucinus)* and the golden-bellied capuchin*(Cebus xanthosternos).*

2.4. Study site

Tests on nest box selection(sub-questions 1 to 3) and nest box behaviours (sub-question 4 to 6) are done in the inside enclosures of captive Pied tamarins and a group of Golden lion tamarins.

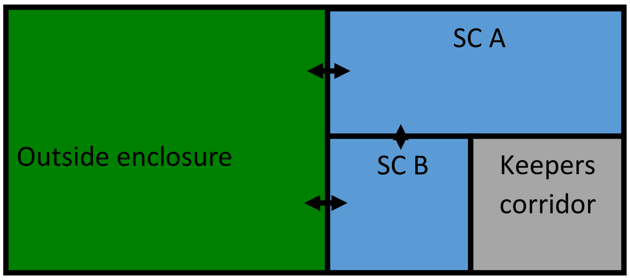

FIGURE 6: SC ENCLOSURE DESIGN

The enclosures of the pied tamarins were all based on the same principles (see figure 6, 7 and 8). The buildings existed of multiple enclosures. In all these enclosures tamarin species were housed. In the inside enclosures, there was no visible contact with neighbouring individuals. All the individuals did have access to an outside enclosure during the day. All observations were done in February, March and the tamarins were locked inside during nights.

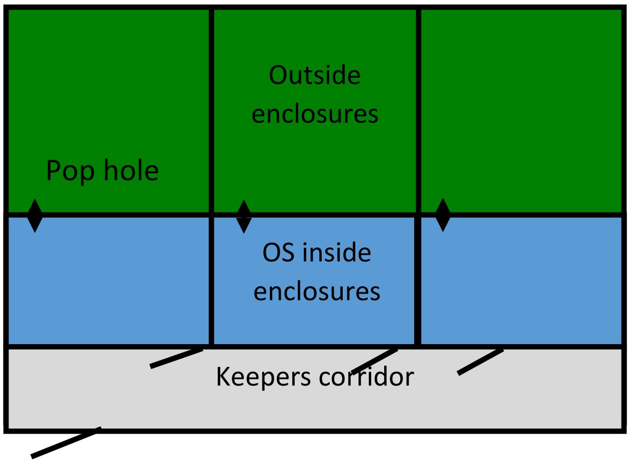

FIGURE 7: OS/G ENCLOSURE DESIGN

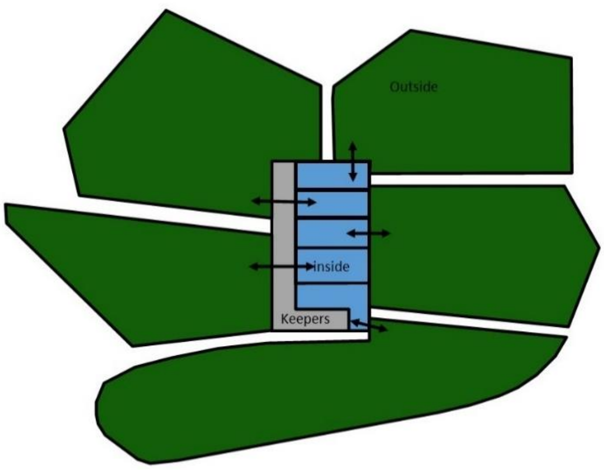

FIGURE 8: F ENCLOSURE DESIGN

All the inside enclosures contained shaving substrate and similar furniture; wooden perching's, ropes, and a satellite cage. Since all the individuals are kept in the same Zoo, bias caused by different husbandry methods, as well as environmental factors is minimized.

For the anti-predation tests the inside enclosure of coatis was used. The coatis shared their inside enclosure with howler monkeys. Former tests with howler monkeys and nest boxes show that they are not really interested in the boxes (Wormell & Price, pers. comm., September 2016).

The macaque enclosure had an inside enclosure with three different compartments. Besides the inside enclosure there was an outside cage and a big naturalistic outside enclosure. From the keepers corridor there was a good overview over this outside cage. The nest box was placed in the outside cage.

For both the White headed capuchin as the yellow bellied capuchin the outside enclosures were used to place the nest box. Both the outside enclosure existed of an island with all kind of furniture in it, to climb and play around. The nest boxes were screwed to a wooden pole.

2.5. Data collection

Nest box selection experiment

During the nest box selection experiment two types of boxes were placed in the inside enclosures of all 7 groups of pied tamarins. In total 14 boxes were used for this experiment. One of the two boxes present was a normal nest box. This meant it had the same size as the other box but no special features. This nest box will be coded as NB-N. The other box had one of the tested features. The tested features were a nest box with tunnel (NB-T), a nest box with shelve (NB-S) and a nest box with multiple entrance points (NB-E). This meant that the individuals had to choose between those two available boxes every evening.

During the whole experiment the same boxes were used to prevent that placing a new, complete clean box, could bias the results. Before the observations started both boxes were presented to the test groups. This because their current nest box needed to be removed but removing their old nest box without a period of overlap might be too stressful for them. Therefore the study started with three days habituation period in which both boxes were presented as an NB-N design. To prevent bias due to complete new boxes, the boxes were built in a way that they could be adapted to the current stage of the study. Opposite of each nest box a camera was placed to record all the behaviours. The cameras were triggered by motion and therefore the bias due to human presence was minimized. Observations were done during 13 days for every different nest box type. The types of next boxes offered in the different phases differed per group preventing bias due to environmental factors(see table 3). To divide the boxes over groups the Latin square method was used. The groups were selected by random selection.

TABLE 3 TIME SCHEDULE NEST BOX SELECTION EXPERIMENT

Nr.	Enclosure	First 13 days	Second 13 days	Third 13 days
1	G7	NB-S	NB-T	NB-E
2	G12	NB-E	NB-S	NB-T
3	G4/5	NB-S	NB-T	NB-E
4	F2	BN-E	NB-S	NB-T
5	OS4	NB-S	NB-E	NB-T
6	0S7	NB-T	NB-E	NB-S
7	SCA/B	NB-T	NB-E	NB-S

During this study the selection of box was observed. In the evening the cameras recorded which box the animals chose to sleep. The cameras were left on during the entire night to see if any events took place in which animals switched from box. In the morning, after the animals woke up as normal, the cameras were turned off and memory cards were collected to transfer the information to the computer. In the afternoon all the memory cards were been put back in the cameras and the cameras were turned on.

The observations were be done in three different settings and resulted in 91 observations for pied tamarins, repeated in all different settings.

Time spent around nest boxes

To assess how much time is spent around different types of nest boxes, observations were done during the last hour before retirement. During this experiment two types of nest boxes were present in the enclosure. Opposite of these boxes cameras will be placed. To see which parts of the enclosure the tamarins use more often, the enclosure will be divided in different sections:

- **Section 1.1: Inside nest box type 1**
- **Section 1.2: Inside nest box type 2**
  Individuals are noted as inside nest box when their whole body is inside of the box except for the tail. If the tail is still hanging out of the box the individual will still be seen as inside.

- **Section 2.1: touching nest box type 1**
- **Section 2.2: touching nest box type 2**
  Touching will described as purposely touching the box. This could be: touching the box with their hands, sitting on the box, rubbing body to the box, standing half in box, scent marking box.

- **Section 3.1: Close to nest box type 1**
- **Section 3.2.: Close to nest box type 2**
  Individuals will be noted down as close to the box when they are observed with their full body within a 50 cm circle around the box. Except for the tail. The 50 cm circle will be marked on surrounding perching's to make it easy to observe.

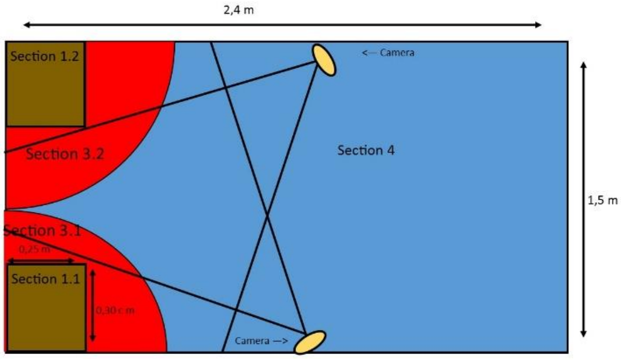

FIGURE 9 SECTION DIVISION INSIDE ENCLOSURE NEST-BOX INTERACTION EXPERIMENT

- **Section 4: Not close to any of the boxes**(see figure 9).
  If the individual is not found in any of the sections described above it will be noted down as not close to any of the boxes.

For these observations the same groups were used as for the nest box selection experiment. All groups are observed three times during every different experiment setup. This will resulted in a total of 24 observations per different situation. The observations were done on a daily basis using camera recordings. The cameras recorded a view of the boxes and of the section described as ''Close to nest box''. Cameras were turned on between 15.00 and 15.30 to make sure the last hour before retirement was recorded.

During this experiment data is collected on time spent in the different sections described above. For the last hour before retirement is observed how many individuals were found in the different sections every minute. This is done four times for all the groups during for each different nest box type.

Anti-predation experiment

To test if the designed nest box is safe for the tamarins an anti-predation test was conducted. In this experiment three different types of predators were used: coati's, Sulawesi black-crested macaques and capuchin monkeys. Both the coati's and the macaques were studied in Durrell Wildlife Park in Jersey. The capuchin monkeys were studied in Primate park Apenheul situated in the Netherlands. For the coatis a nest box was attached to a hard wired fence in the inside enclosure. The keepers corridor is constructed out of this material and by attaching the box to this fence, observers will have good visibility on the activities. From the public site the entrance points of the nest boxes were visible. In the macaque enclosure the box was attached in the outside cage to the fence located at the keepers corridor.

TABLE 4 TIME SCHEDULE ANTI-PREDATION EXPERIMENT

Wk	Group	Nest box	Number of obs.
1	Coatis Macaques Capuchin	NB-N	4 coati 4 macaque 8 Capuchin
2	Coatis Macaques Capuchin	NB-S	4 coati 4 macaque 8 capuchin
3	Coatis Macaques Capuchin	NB-T	4 coati 4 macaque 8 capuchin
4	Coatis macaques Capuchin	NB- E	4 coati 4 macaque 8 capuchin
Total number of observations:			**16 per species**

Inside of the boxes a treat will be offered. The requirements of this treat are; it must be a food item they really desire and it must not be easy to grab too mimic a tamarin prey. The treat will be put in a cardboard cup and attached to a bungee to make it hard for the coatis and macaques to grab the treat. For the macaques peanut butter was used and for the coati's dog biscuits or morio worms. Both these treats were not part of the daily diet which made them very desirable by these species. For both capuchin monkeys different kinds of fruits and nuts were used. What kind of fruit was used depended on the diet of that specific day. If apples were part of the morning feed another fruit item was chosen to make the capuchin monkeys really want to have the treat.

During the experiment the whole group was present in the enclosure. Although, for all the species only one individual at the time investigated the box. Most of the times this was the most dominant individual. The individuals lower in rank were chased away by this individual.

For this test one box at the time was available in the enclosure. This box will be the same as used for the tamarin experiments and also adjustable for different situations. A certain design of the box will be placed in the enclosure with the treat inside it. For an hour the groups will be observed. The time it takes in minutes to get the treat will be recorded. If the predators were not able to get the treat after 60 minutes the observation stops and will be repeated the day after. Nevertheless the box will stay in the enclosure to see if they might figure out how it works over time. After four days of observations the box will be adapted to the next design (see table 4).

2.6. Data analysis

To analyse the data collected during this study the statistical program IBM SPSS statistics will be used. The collected data will be implemented in this program. To analyse the results mixed models were used.

To analyse nest box selection a generalized linear mixed model (GLMM) was build. In this model, group was added as a subject to correct for the differences between groups. The dataset was aggregated to a binomial data set in which the number of times the tamarins selected the special nest box was counted of the total amount of times they had to make a decision between boxes. The number of times the new box was selected was the dependent variable. The nest box type present in the enclosure and the order in which the box was offered were implemented as fixed factors. To compare the significant differences the post hoc Sidak test was used. Using this binomial data set the probability of selection of different box types was calculated.

Enclosure use by tamarins was analysed by using a linear mixed model (LMM) with an random intercept for group. In this model the dependent variable was time spent in a certain section in the enclosure. The independent variables were different nest box types, nest boxes selected overnight and the interaction between those two variables. The distribution of % of time spent in certain sections of the enclosure was tested on normality and if needed transformed using Arsin squire-root. To compare significant effects between variables the Sidak post-hoc test was used.

The safety from predators was evaluated by conducting a general liner model (GLM) in which time they spent on entering the box was the dependent variable. The fixed factors were the box type, the species and the number of days the box was present in the box. To compare significant differences a pairwise comparison was made by using the post-hoc Sidak test.

3. Results

3.1. Nest box selection in tamarins

To test whether the tamarins would accept different types of nest boxes as a safe and comfortable sleeping site, a normal familiar nest box and a special nest box were presented in the enclosure. Seven groups of tamarins had to make the decision to spend the night in the normal nest box or in the special nest box. The choice of selection was recorded for 13 nights. After 13 nights the special box was replaced by another special nest box design and the experiment was repeated. This was done with all three special nest box types (see figure 2, 3 and 4). So in total all seven groups were observed for 13 nights which resulted in 91 recordings per nest box type. The special nest boxes were boxes with a shelve, a tunnel or multiple entrance points. Every group had access to all special boxes over three periods of 13 nights. To prevent that the order of offering different designs would influence the choice made by the tamarins, the order in which the boxes were presented differed (see table 3).

To analyse these numbers the dataset was aggregated to a binominal dataset in which the total numbers of selecting a special box was counted. Using this information the estimated experimental probability was calculated with Generalized linear mixed models (see formula). The total number of events were the number of times a group selected a special nest box. The total number of trials was 13.

$$experimental\ probability = \frac{Number\ of\ events\ occuring}{Total\ number\ of\ trials}$$

The Generalized linear mixed model was also used to correct for the different group effects. The probability that a tunnel would be selected is highest of all with 0.87. shelves came second with .63 and at last the probability that shelves would be selected was lowest 0.004 (Figure 10). Although these number differ the probability that a special box would be selected was not significant related to the design available (GLMM: *F=2.890* , *P=0.087*).

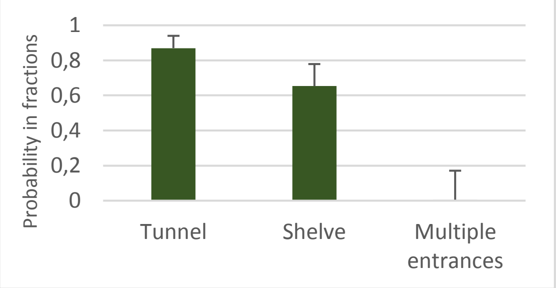

FIGURE 10: PROBABILITY OF SELECTION OF SPECIAL BOXES. DATA ARE PRESENTED AS MEAN± SEM (N=91).

TABLE 5: PROBABILITY OF SELECTION OF SPECIAL BOXES IN DIFFERENT ORDER

	Nest box	Probability of selection
Order 1	Shelve	0,225
	Tunnel	0,951
	Entrance	0
Order 2	Tunnel	1
	Entrance	0,077
	Shelve	0,923
Order 3	Entrance	0,719
	Shelve	0,635
	Tunnel	0,369

As mentioned before, the order in which the special nest boxes tamarins were offered to the tamarins differed per group. This was done to prevent that the order would influence the selection behaviour of the tamarins. Analysis revealed that the order in which boxes were offered to tamarins had a significant effect on the probability of selection of different nest box designs (see table 5)(GLMM:$F_{(2,14)}$=9,043, P<0,05).

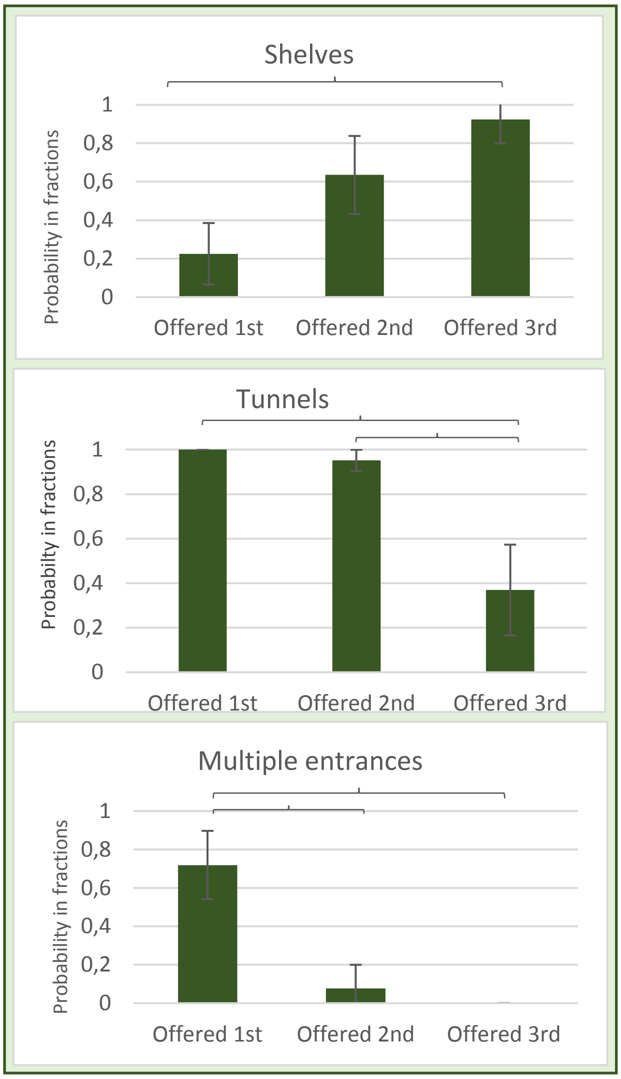

FIGURE 11: PROBABILITY OF SELECTION OF SPECIAL BOXES WHEN OFFERED IN DIFFERENT (N=91). DATA REPRESENT MEAN± SEM. HANGER REPRESENTS A SIGNIFICANT DIFFERENCE ((P<0,05).(Method of hanger: *(Anderson, et al., 2014)*

When a nest box with a shelve was offered in the first period, the probability that this box would be selected was 0,225. While when a box was offered in the third period the probability of selection significantly increased to 0.923 ($T_{(12)}$=3,455, *P*=0,005).

Boxes with tunnels were significantly more selected when offered first ($T_{(12)}$=-3,096, *P*=0,009) or second period ($T_{(12)}$=-2,782, *P*=0,017). In both cases selection of the special nest box was above 0.9. The probability that boxes with tunnels were selected if presented as third decreased to 0.369.

Boxes with multiple entrance points were most selected if offered first (compared to offered second: ($T_{(12)}$=2.968, *P*=0.012), compared to offered third: ($T_{(12)}$=4.039, *P*=0.002). The probability that this box would be selected in the first period is 0.719. While the probability that this box would be selected if offered second period is 0.123. And when offered in the third period the probability is 0 (see figure 11).

3.2. Enclosure use

The same set up as for the selection experiment was used to test proximity to different special nest boxes. A normal nest box and a special nest box were presented in the enclosure. Seven groups of tamarins had access to those boxes for 13 nights. After 13 nights the special box was replaced by another special design. During those 13 nights every group of tamarins was observed for four times to research proximity. This resulted in 28 observations per nest box type (7 groups * 4 observations). During these observations was recorded in which section of the enclosure the different individuals were present for every minute of the last hour before retirement. This was done by using motion activated cameras which were programmed to take a picture every minute. The recordings were used to calculate the % of time they spent in and around different nest box types. During these recordings tamarins were observed to be either inside of a nest box or not visible. Since the aim was to research the proximity to nest boxes the time inside of nest boxes was considered to be most valuable and the other data was left out.

When boxes with shelves were present in the enclosure more time was spent inside the box with shelve(36%) as in normal nest boxes (12%). If boxes with tunnels were present, the time spent in those boxes with tunnels was 47%, while the time spent in normal boxes was 12%. The percentage of time spent inside boxes with multiple entrance points was 4%, while the time spent inside normal nest boxes was 45%. The approximate time spent in different nest boxes is significantly related to the availability of different nest box designs (*LMM:* $F_{(2,68)}$=47.38, *P*<0.001). (figure 12).

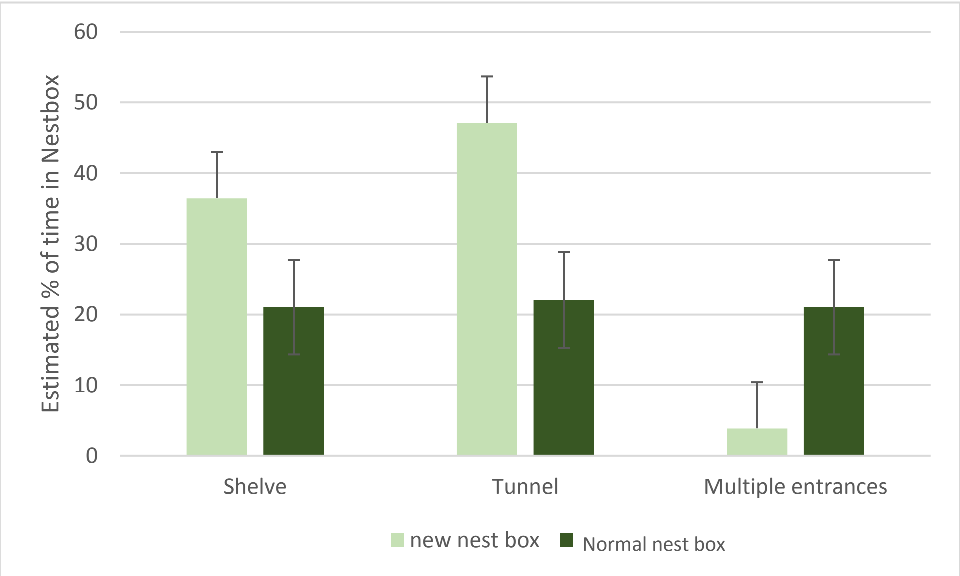

FIGURE 12: MEAN TIME SPENT INSIDE NEST BOX WHEN DIFFERENT NEST BOX TYPES WERE PRESENT. DATA REPRESENT *mean± SEM* **(N=28).**

As described in chapter 3.1. both shelves and tunnels were the nest boxes which were selected most and now the results show that most time is spent inside of these nest boxes. To see if there is any relation between those factors the interaction between selection and time spent inside boxes was added to the model(LMM: $F_{(2,64)}$=*5,453, P=.007*).

As can be seen in figure 13, in all cases most time was spent inside of the nest box which was selected to sleep in. If a normal nest box was selected, in all cases, less than 2%. While the time spent in boxes with tunnels was 78%, time spent in boxes with shelves was 30% and time spent inside of boxes with multiple entrance points was 55%.

When new nest boxes were selected to sleep in most time was spent inside of new nest boxes. When the special box was a box with shelve was present 58% if the time was spent inside this box. 54% of the time was spent inside boxes with tunnels and 15% of the time was spent inside of boxes with multiple entrance points. Most time, 8%, was spent inside normal nest boxes when a special box with multiple entrance points is present. 6% of the time was spent in normal boxes when boxes with tunnels were present and 1% of the time when boxes with shelves were present (figure 13).

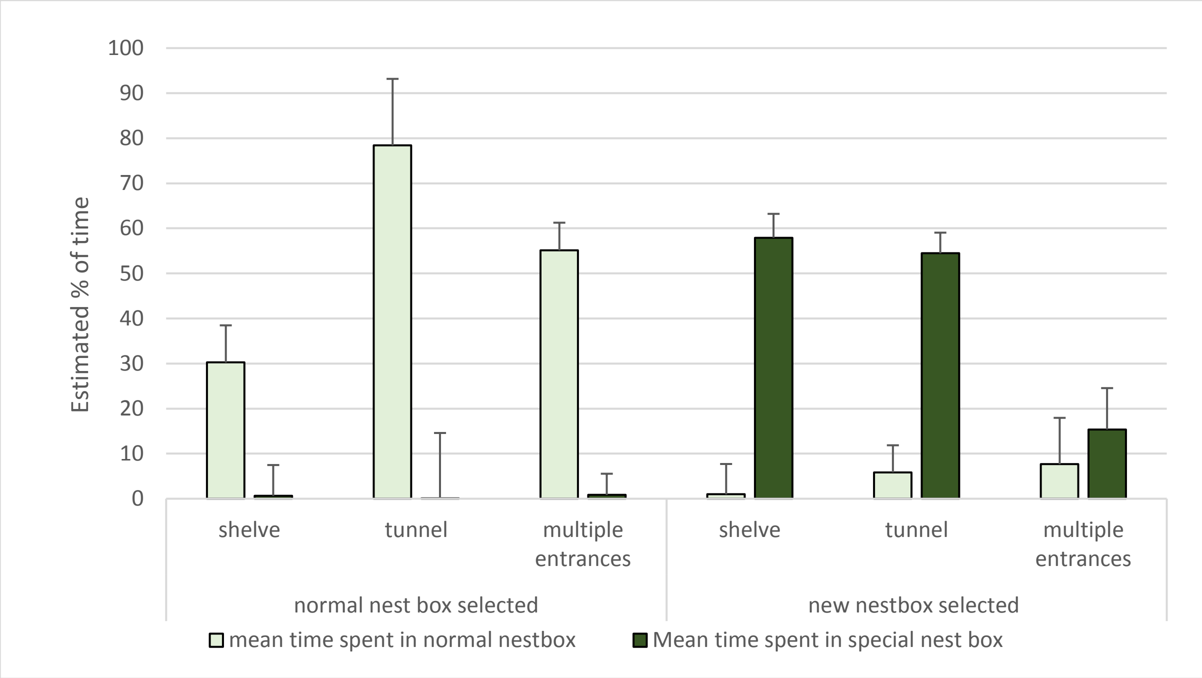

FIGURE 13: MEAN TIME SPENT INSIDE NEW/NORMAL NEST BOXES COMPARED TO BOX SELECTED TO SLEEP IN. DATA REPRESENT MEAN± SEM (N=28)

3.3. Anti-predation experiment

To test the vulnerability of the nest box against possible predators, one nest box was placed in the enclosures of ring-tailed coati's, black crested macaques, white headed capuchin and the yellow-breasted capuchin. For every species four days of observations were done, this resulted in a total of 16 observations per nest box type. At the start of every observation a novel food item was offered inside the nest box to see how much time it took the predators for to successfully enter the box. The observation period was 60 minutes. After four days the nest box design was changed and the experiment was repeated. This was done for all the special nest boxes and the normal nest box.

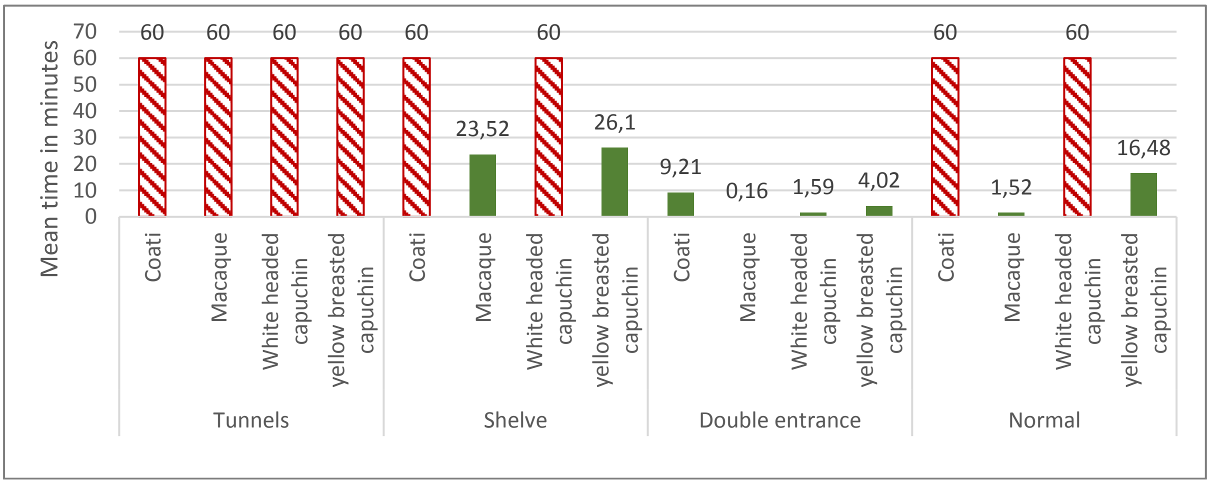

FIGURE 14: MEAN TIME SPENT IN MINUTES ON ENTERING DIFFERENT NEST BOX TYPES BY DIFFERENT SPECIES. DATA REPRESENT MEAN TIME IN MINUTES AND SECONDS. RED ARCED BARS REPRESENT BOXES WHICH WERE NOT SUCCESSFULLY ENTERED.

Not all nest box types were successfully entered by the predators. Nest boxes with tunnels were never successfully entered. And nest boxes with shelves and normal nest boxes were only successfully entered by macaques and yellow breasted capuchin monkeys. Boxes with multiple entrance points were successfully entered by all the species(Figure 14). The time it took the predators to successfully enter a box is significantly related to nest box design (*GLM: F=4.029, P=.029*).

Only looking at the successful attempts, it took predators on average more time to enter a nest box with a shelve (25 minutes) than a box with multiple entrance points (4 minutes) (Sidak: *P=.025*)(Figure 15).

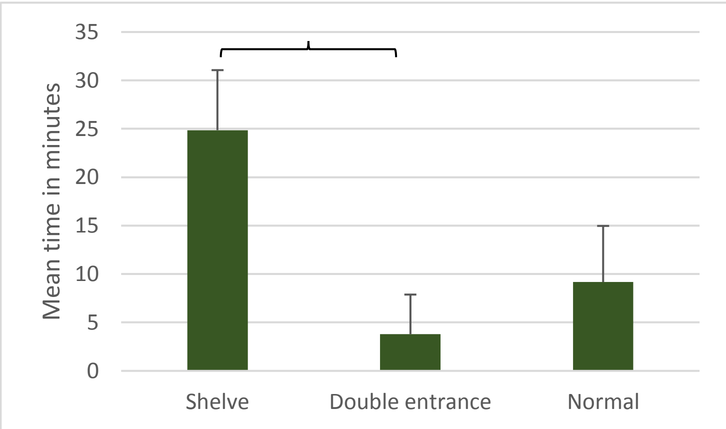

FIGURE 15: MEAN TIME SPENT IN MINUTES ON SUCCESSFULLY ENTEREING DIFFERENT NEST BOX TYPES. DATA REPRESENT MEAN± SEM. HANGER REPRESENTS SIGNIFICANT DIFFERENCE (p<.05)

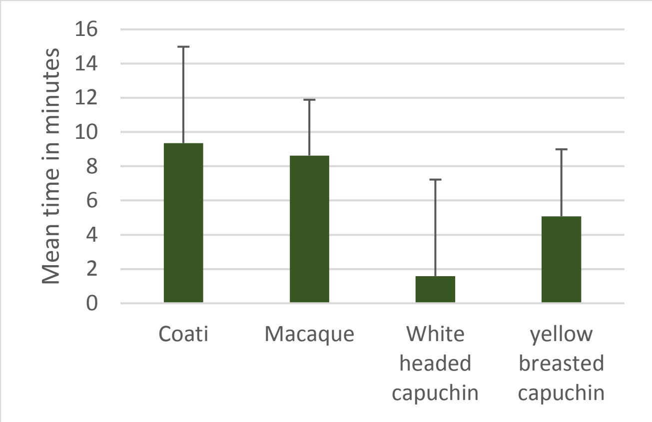

FIGURE 16: MEAN TIME SPENT IN MINUTES ON ENTERING NEST BOXES. DATA REPRESENT MEAN± SEM. HANGER REPRESENTS SIGNIFICANT DIFFERENCE (p<.05)

The time spent on successfully entering a nest box differed per species. White headed capuchin monkeys were fastest but this is probably because the only box they successfully encountered was the box with multiple entrance points. For the other boxes more time was needed. Although, no significant differences between species were observed.

4. Discussion & Conclusion

This research aimed to know which design of artificial nest box is the least accessible for predators and still most likely to be accepted as a suitable sleeping site for the black lion tamarins in the wild. By conducting three different experiments, more knowledge was gained about what is important for tamarin species and which features can keep them safe. The information can be divided in three categories which are; use of nest boxes, proximity to nest box and safety of nest boxes.

In both the selection experiment as well as in the proximity experiments, boxes with shelves and tunnels were found to be most favourite. When testing nest boxes on safety, boxes with tunnels were most safe. A reason for favouring boxes with shelves and tunnels could be because of the higher concealment levels of these boxes. Earlier studies show that tamarin species in captivity prefer concealed locations for their sleeping sites (Caine, et al., 1992) (Franklin, et al., 2007). Boxes with multiple entrance points were selected less. But giving the tamarins a nest box with multiple entrance points is the only possibility to give tamarins a possibility to flee if predators manage to get inside. The reason boxes with multiple entrance points are selected less might be because they feel more exposed. Tamarins in the wild try to find safe concealed sleeping sites (Franklin, et al., 2007). A possibility to still use boxes with multiple entrance points and make them safe and favoured by tamarins would be to combine both those entrance points with tunnels. In this way the boxes would still be safe concealed areas. Shelves could also be implemented to give tamarins an even more concealed feeling but might not be necessary.

Since shelves were favoured by tamarins, this is a feature which could be implemented as well. But something to take into consideration is the group size of wild tamarins. For this study most groups consisted out of two individuals per group. The average group size of a wild group of tamarins is 4,6 individuals and ranges from two till eight (Miller, et al., 2003). Even groups of 14 individuals are observed (Dominic Wormell, May 2016, Pers. Communication). By implementing a shelve into the design the space they have to sleep is reduced enormously. This might have an effect to the selection of boxes for big groups, since the space they get is not big enough.

An important reason for giving tamarins a double entrance points is that wild predators are more motivated as captive animals to find food (Veasey, et al., 1996). The probability that they would successfully enter a box is expected to be higher so giving tamarins a possibility to flee might increase their chance on survival. Not only primates are predators of tamarins. In this research primates were used to give an indication on the safety of the nest box but in the wild species like Tayras*(Eira barbara)* and snakes also hunt tamarins (Franklin, et al., 2007). The probability that these species are capable of entering a nest box is higher because of their size and biology. Snakes, for example, are expected to be capable of entering a 10 cm square pop hole. Another possibility to keep tamarins safe is to adjust the pop hole. When doing observations on nest box safety a key shaped pop hole was suggested(Figure 17). This design is already successfully used in mixed species exhibits in Apenheul primate park. Because of the shape, only tamarins are capable of getting through the pop hole (Arun Idoe,

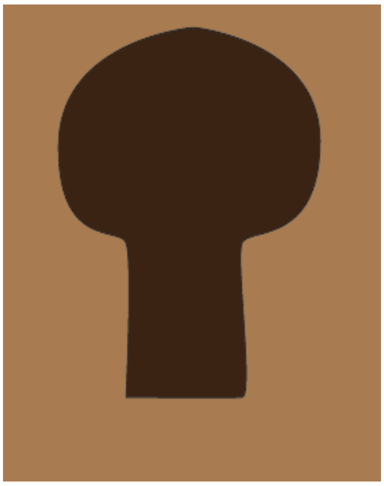

FIGURE 17: POPHOLE SHAPE USED IN APENHEUL PRIMATE PARK.

June 2017, pers. Communication). This will decrease the chance that predators will enter the nest box.

For this research, captive tamarins were used. There is a risk of making assumptions for wild tamarins based on behaviour of captive tamarins. Behaviour of animals in captivity might adapt to a captive situation over time. Studies done on behavioural changes in tamarins show that locomotor and foraging skills are less developed over time. These behavioural differences are caused by the effect of keeping animals in a captive situation where they don't have to forage or have the same space to move as wild individuals (Stoinski, et al., 2002). These behavioural differences might also influence the selection of sleeping sites. In captivity, tamarins are taught to use nest boxes overnight and therefore it is likely that they will accept certain nest box designs. Wild tamarins are not used to nest boxes and therefore it is less likely that wild tamarins will accept nest boxes. At the moment a trial is started with placing nest boxes in tamarin habitat to see if they use these boxes (Dominic Wormell, August 2017, pers. Communication) (Figure 17).

FIGURE 17: PLACING NEST BOX IN TAMARIN HABITAT IN SÃO PAULO, BRAZIL

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