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Improving the welfare of the Amur leopards (*Panthera pardus orientalis*) at Nordens Ark (Sweden)

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ABSTRACT

Since recent years, people do not only want to prevent animal cruelty anymore, but also want to improve their welfare. Therefore, zoo institutions are under pressure to assure the wellbeing of their animals. And even though they work very hard to achieve this, it is not an easy job. Carnivores, most notably solitary felids, are amongst the most difficult species for whom to develop a proper environment. Previous studies conducted on the two Amur leopards currently living at *Nordens Ark* recorded signs of stress and pacing. However, no previous individual assessment had been carried out to evaluate their welfare and find out the possible reasons behind their behavior. The aim of this study was (1) to evaluate and improve the welfare of the two Amur leopards currently living at *Nordens Ark*, and (2) to find out if making changes in their environment could be a way to improve their welfare. To this end, a behavioral study was carried out during different periods of time on both individuals, while being exposed to four changes in their environment concerning training, increased zookeeper presence, increased environmental enrichment and visual barriers. Overall, these changes seem to have had a positive effect on both leopards. However, they still showed signs of poor welfare and more effort needs to be put to ensure their best welfare quality. Emphasis has been given to provide varying enrichment and training, and increasing presence of keepers. Moreover, individual differences due to different personalities should be taken into consideration.

INTRODUCTION

Animal welfare has been studied for decades now. Only recently, zoo animal welfare has gained more importance in research (Butterworth *et al*., 2011; Kagan *et al*., 2015). The public has started to care more about the needs of captive animals, and its awareness of animal welfare is increasing (Lyniuk, 2011; Whitham *and* Wielebnowski, 2013; Kagan *et al.*, 2015). People do not only want to prevent animal cruelty anymore, but also want to improve their welfare (Whitham *and* Wielebnowski, 2013). Therefore, zoo institutions are under pressure to assure the wellbeing of their animals. And even though they work very hard to achieve this, it is not an easy job (De Rouck *et al*., 2005).

But what is animal welfare and how can it be assessed? Animal welfare has many different approaches, and even though much effort has been put to find the perfect definition of it, the scientific concept of animal welfare is still developing. However, it is known that animal welfare is multifaceted, based on the health, physiology, behavior and reproduction/production of an animal (Keeling *et al.,* 2011). One common definition of animal welfare is the one given by the Five Freedoms definition, which defines good welfare as freedom: (i) from hunger and thirst; (ii) from discomfort, (iii) from pain, injury or disease; (iv) from fear and distress; and (v) to express normal behavior (FAWC, 2009). In the past, welfare assessment studies were focused on resource-based measures (RBMs) only, such as the quantity of food given to the animals or how big the enclosure is. However, animal welfare researchers are starting to use outcome-based measures (OBMs) in combination with RBMs, such as behavior or physical condition. It is believed that OBMs can provide better indicators of welfare since animal welfare is a characteristic of the individual animal and not just of the system it is kept in (Butterworth *et al.*, 2011).

Even though these welfare assessments have been mainly carried out on farm animals, zoo animal welfare studies based on OBMs are increasing as well (Butterworth *et al.*, 2011). Some species are proven to adapt perfectly to a life in captivity, and some people might argue that they live better than in the wild (free of predators, available food, shelter) (Mason, 2010). However, this is not the case for all zoo animals. Carnivores, most notably solitary felids, are amongst the most difficult species for whom to develop a proper environment due to their natural hunting behaviors, spatial requirements and quick habituation to novel conditions (Pitsko, 2003). Providing adequate welfare to these species is extremely complicated (Clubb *and* Mason, 2007). One of the most common problems captive felids face is stereotypic behaviors, especially pacing (Clubb *and* Vickery, 2006). Even though such behaviors might be the result of past experiences and a practical coping mechanism, their presence should be considered as a sign of non-optimal welfare (Mason, 2010).

This is the case of the two Amur leopards (*Panthera pardus orientalis*) currently living at *Nordens Ark*, a Swedish zoo located in the west coast. Apelqvist (2014) and Ahlrot (2016) both stated that the two individuals showed signs of stress and pacing during their studies. In a previous study (Stolt, 2012) conducted when another male was living there but still the same female, stated that both individuals

showed signs of stress. It is important to keep in mind that welfare is a characteristic of the individual animal (Kagan *et al.,* 2015). Each animal is unique, with its single perspectives, needs and preferences. Therefore, welfare should be assessed at the level of the individual (Whitham *and* Wielebnowski, 2013). Furthermore, while assessing the welfare of an animal, the presence of good welfare indicators should be considered as important as the absence of poor welfare indicators to determinate that an animal has good welfare (Keeling *et al.,* 2011). A list of diminished and enhanced welfare indicators in big cats can be found in Biolatti *et al.* (2016). However, no previous individual assessment has been carried out to evaluate the welfare of the two Amur leopards in *Nordens Ark* and find out the possible reasons behind their behavior.

Amur leopards (*Panthera pardus orientalis*), also known as Far Eastern leopards, are one of the rarest big cats in the world (Wang *et al*., 2017). What differentiates them from the other leopards is their adaptability to severe climate conditions such as snow and extreme cold (Uphyrkina *et al.*, 2002). They are also one of the world's most endangered species, listed as "critically endangered" by the IUCN, and in risk of immediate extinction (Wang *et al*., 2017). There are only around 80 individuals left in the wild, with a total area of distribution of approximately 4000km^2 in southwestern Primorsky Krai of Russia and Jilin and Heilongjian provinces in China (Land of the Leopard, 2015). Much effort has been put into the conservation of this animal and zoos play an important role in this matter. *Nordens Ark* is one of them. Therefore, it is crucial to ensure a good welfare to the two Amur leopards of this zoo. Effort should be made to improve their welfare, helping in the conservation of this species and avoiding its extinction.

Aim of the study

This study has two main aims: (1) to evaluate and improve the welfare of the two Amur leopards currently living at *Nordens Ark*, and (2) to find out if making changes in their environment could be a way to improve their welfare. To this end, a behavioral study was carried out during different periods of time on both individuals, while being exposed to four changes in their environment concerning training, increased zookeeper presence, increased environmental enrichment and visual barriers. Thus, the following research questions were addressed:

- *Which was the animal welfare state of both Amur leopards in Nordens Ark in the beginning of the study? And during the study? And in the end of the study?*
- *Did the combination of changes implemented in the environment have any effect on the welfare status of the Amur leopards in short term? If so, did it improve or worsen the welfare?*
- *Did the combination of changes implemented in the environment have any effect on the welfare status of the Amur leopards in long term? If so, did it improve or worsen the welfare?*
- *Can Nordens Ark improve the welfare of their individuals? If so, what could they do?*

MATERIAL AND METHODS

Animals

The two Amur leopards living at *Nordens Ark* during the year 2017 were used in this study [Figure 1]. The female, Bira, was born in 2009 at *Högholmen Zoo* in Helsinki (Finland). She arrived at *Nordens Ark* in the spring of 2010. The male, Denzel, was also born in 2009 at *Parc zoologique et botanique de Mulhouse* in Mulhouse (France). He was moved to *Yorkshire Wildlife Park* (England) in 2011. In 2014, he arrived to *Nordens Ark*.

Figure 1. Amur leopards from Nordens Ark. *Images of the two Amur leopards that were living in Nordens Ark during 2017 used in this study. On the left, the male Denzel; on the right, the female Bira.* Photo: Slof M., 2017

The enclosures

The leopards were not allowed to breed as they were categorized as a not proper breeding match. This is why the individuals were kept separately all the time in two different, side by side, enclosures, in sight of one another [Figure 2]. Denzel's enclosure (670m^2) was bigger than Bira's (540m^2). Both of them had an enriched environment with trees, varied vegetation, rocks, logs, climbing sites, wooden planks, small houses and other spots to hide. Both enclosures had a mesh fence as walls and roof, besides the central front part of Denzel's, which was made of glass. Visitors could not go around the entire enclosures but only stand by the front part [see Figure 2].

Both enclosures had a feeding cage [see 4 and 5 in Figure 2] that could be isolated from the rest of the enclosure, in which the animals had to stay in when the keepers had to go inside to clean, put enrichment and food.

Urials (*Ovis orientalis vignei*) were kept in an enclosure in front of them and were within the visual range of both Denzel and Bira. On the left side of Denzel's enclosure, there was an enclosure with Snow leopards (*Panthera uncia*).

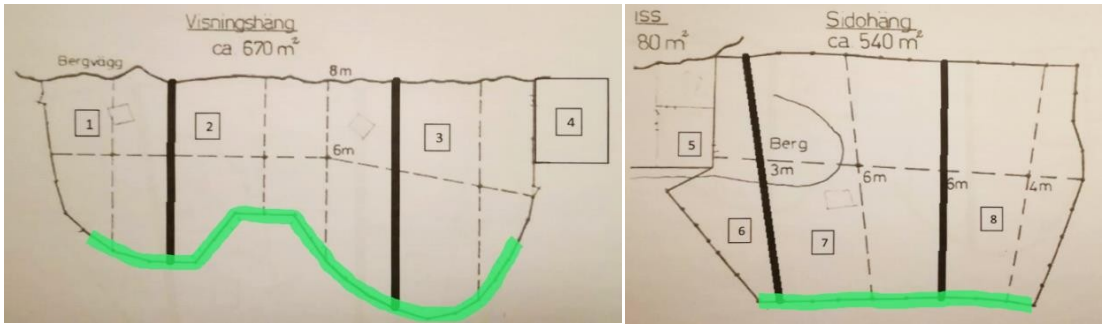

Figure 2. Amur leopard enclosures of Nordens Ark. *Left illustration shows Denzel's enclosure, right illustration shows Bira's enclosure. The two individuals were kept separately in side by side enclosures, in sight of one another. The areas where visitors could be are marked in light green. Feeding cages are indicated with number 4 and 5.*

Feeding, enrichment and training routines

Two zookeepers were in charge of taking care of the Amur leopards. Both leopards were fed 4-5 days a week, usually between 7:30 and 9:00, even though some later feedings occurred as well. The feeding routine was as follows: the keeper would call the animal and lead it to the feeding cage, where it would stay locked until the keeper was done. To keep the leopard calm and occupied while the caretaker was in the enclosure, and decrease so possible aggressiveness towards the people working in the area, the zookeeper would give small pieces of meat (such as ribs) with tweezers through the fence. While the leopard was busy eating, the zoo caretaker would enter the enclosure and leave the food for that day somewhere in the pen. The way of displaying the food was different every time and given in an enriched way: for example, ribs hidden in a box with holes [see Figure 3A], piece of horse meat tied to a heavy log [see Figure 3B] or quails hanging from trees. The leopards were fed with a variety of prey meat such as horse, roe deer, rooster, rabbit, guinea pig, and quail.

Figure 3. Examples of food display for the Amur leopards in Nordens Ark. *Keepers presented the food in different and enriched ways. (A) Box with holes with hidden ribs inside* (Photo: Slof M, 2017)*; (B) Piece of meat tied up to a log* (Photo: Stolt J, 2012).

Different types of enrichment were given to the leopards as well. Some examples are spices (such as garlic or paprika powder, cardamom, curry, cinnamon and ginger), peppermint oil, honey, cardboard boxes and tubes, straw, hay, valerian and blood.

Concerning the training routines, one of the leopards' keepers and another trainer were the ones responsible to train both animals. It consisted in short training session (around 10min) where different behaviors were trained, such as laying still, follow, gape, being touched and target. The sessions were carried out by one trainer at a time (once or twice a day), which was standing right outside the enclosure, being able to be in contact with the animal if needed but not having to be inside [see Figure 4].

Figure 4. Training session. *The trainer Emma and Bira during a training session in Nordens Ark.* Photo: Slof M, 2017

Training was mainly used as a form of enrichment, to stimulate the individuals and give them learning opportunities, which has been proven to be very beneficial for them (Melfi, 2013). Furthermore, it also helped the animals to be less anxious or stressed during the presence of humans, and easier for the keepers and veterinarians to handle them (e.g. during husbandry or medical health checks).

Study design

The study consisted of continuous focal sampling behavioral observations, as described by Martin *and* Bateson (2007), while being exposed to four changes in their environment concerning training, increased zookeeper presence, increased environmental enrichment and visual barriers. All observations were made *in situ* by the same observer, standing outside the enclosures, at the visitors' path. Behaviors were recorded every time they were performed (as frequencies) and if simultaneous behaviors occurred, both were recorded. Only the duration of the behaviors pacing and panting were recorded. The number of visitors was also registered. The ethogram defining the behaviors observed during the data collection is presented in Table 1, supported by the universal ethogram for the Felidae from Stanton *et al*. (2015).

***Table 1. Ethogram**. Definition of each behavior used during the behavioral observations of the two Amur leopards in NA.*

Behavior	*Definition*
Laying	Body on the ground in a prone position. Head resting or not on the substrate or thoracic limbs.
Sitting	Upright position. Hip, pelvic limbs and forelegs resting on the floor. Hind legs flexed, while front legs are extended and straight.
Walking/Running	Displacement on some surface, using the 4 locomotor members and at least one touching the ground.
Jumping	Leaping from one place to another, with none of the locomotive members touching the ground.
Eating	Ingesting food, chewing and swallowing it.
Drinking	Taking liquid into mouth lapping it up with the tongue.
Pacing	Moving from one side to another repeatedly, following the same path over and over again. Two identical routes back and forward must be performed at least.
Panting	Breathing with short, quick breaths with mouth open or closed. The tongue sticking out or not. Torso is distinctly moving faster than during normal breathing.
Head-rolling	Pelvic legs resting on the floor, forelegs suspended in the air or against a vertical surface, and head tossing in a circular motion.
Staring outside	The four legs are touching the ground, not displacement, and the head is focused on one specific point for more than 5 seconds.
Vocalizing	The individual utters a sound or call from its throat and mouth, while standing, sitting or walking.
Not visible	Individual not visible for the observer.
Other	Any other behavior that does not fit in the previous behaviors.

Zookeepers started their activities in the park around 07:15. The park opened at 10:00 and closed at 17:00 (or 19:00 during high season), around the same time the keepers stopped working. To be able to see the behaviors of the leopards before, during and after keepers' activities and visitor hours, the daily schedule detailed in Table 2 was elaborated. Each individual was observed for 3 hours a day (morning, midday and afternoon), in sequences of two consecutive days.

These observations were carried out during three different periods of time to see the effect of the changes in both short and long term: *Period 1* - before changes, *Period 2* - immediately after changes (short term effect), and *Period 3* - some weeks after changes (long term effect). Each period lasted for 10 days (5 sequences of two consecutive days), thus a total of 30 hours of observations per animal. Altogether sums up to 30 days of observations, 90 hours per individual.

Table 2. Daily schedule of the observations. *Two consecutive days sequences, 3 hours of observations per animal (morning, midday and afternoon). In Day 1, observations started with the male and in Day 2 observations started with the female.*

	DAY 1	DAY 2
07:00 – 08:00	Denzel	Bira
08:30 – 09:30	Bira	Denzel
11:00 – 12:00	Denzel	Bira
12:30 – 13:30	Bira	Denzel
16:00 – 17:00 alt. 18:00 – 19:00*	Denzel	Bira
17:30 – 18:30 alt. 19:30 – 20:30*	Bira	Denzel

*Depending on the opening hours of the park.

The changes were implemented the day after *Period 1* had ended. *Period 2* started the following day, and *Period 3* started 6 weeks after the second period ended. During the weeks between *Period 2* and *Period 3* the changes continued being implemented.

Changes

The following list details the changes conducted in the study:

1. Training: Each individual was trained 5 days a week.
2. Increased keeper presence: Keepers had to go to the enclosures of the leopards every day, even when it was not a feeding day.
3. Increased environmental enrichment: Keepers had to give different enrichment every day. Food had to always be presented in an enriched way as well.
4. Visual barriers: To keep the visitors farther away from Denzel, a wooden wall and bamboo plants were put in the most visitor-exposed parts of the male enclosure. No visual barriers were constructed in Bira's enclosure.

Due to time limitation in the study, changes were all implemented at the same time.

Data handling

All data collected was summarized and processed in Microsoft Excel 2010. Observed behaviors were grouped as follows: Inactive Behaviors (*Laying* and *Sitting*), Active Behaviors (*Walking*, *Running* and *Jumping*), Stereotypic Behaviors (*Pacing*, *Panting*, *Head-rolling* and *Staring outside*), Feeding Behaviors (*Eating* and *Drinking*), Vocal Behaviors (*Vocalizing*), Not Visible Behaviors (*Not Visible*) and Other Behaviors (*Other*). The abbreviations Dz and Bi were used for Denzel and Bira, respectively.

The behaviors *Head-rolling* and *Staring Outside* were repetitive, unvarying and apparently functionless, thus they were considered as abnormal behaviors (as suggested by Mason *et al.,* 2007), and therefore categorized in the behavior group of Stereotypic Behaviors.

Activity budgets for each behavior from each individual during each period were calculated. The total frequency and the total frequency depending on the time of the observation (morning, midday or afternoon) were calculated for each individual and period for both Active and Stereotypic Behaviors. For *Pacing* and *Panting*, the total duration (in minutes) and the total duration (in minutes) depending on the time of the observation were also calculated. For *Pacing*, the means of the duration (in minutes) for each hour with their standard errors were calculated as well. Moreover, the effect of training was analyzed: first, the frequencies of every observation day of each behavior were calculated depending on if they had had training that day or not. Afterwards, frequency means from all day frequencies of the same behavior were calculated. Then, they were sorted as Diminished Welfare Indicators (DWI) or Enhanced Welfare Indicators (EWI) based on Biolatti *et al*. (2016) and plotted. Additionally, the effect of visitors was analyzed. The mean values of the number of visitors in front of the enclosures of each observation time frame were calculated. The number of visitors from every hour of observation against the respective pacing frequency and duration (in minutes) were plotted. No further statistical analysis was carried out due to data not being independent.

RESULTS

Activity budgets

Activity budgets for Denzel and Bira are summarized in Figure 5 and Figure 6, respectively. For Denzel, the most frequent behavior in *Period 1* was Stereotypic Behaviors, followed by Active and Inactive Behaviors. There was an increase over periods of Active Behaviors and Vocal Behaviors. On the other hand, Stereotypic Behaviors decreased over periods. The Feeding Behaviors, Not Visible and Other stayed practically constant during the three periods.

For Bira, the most frequent behavior in *Period 1* was also Stereotypic Behaviors, followed by Inactive and Active Behaviors. There was an increase over periods of Active Behaviors. On the other hand, Stereotypic Behaviors decreased in *Period 2*, but in *Period 3* it increased again, although not to the same level as in *Period 1*. Oppositely, the following behaviors increased in *Period 2* but decreased again in *Period 3,* although not as much as in *Period 1*: Inactive Behaviors, Vocal Behaviors and Other. Not Visible and Feeding Behaviors stayed practically constant during the three periods.

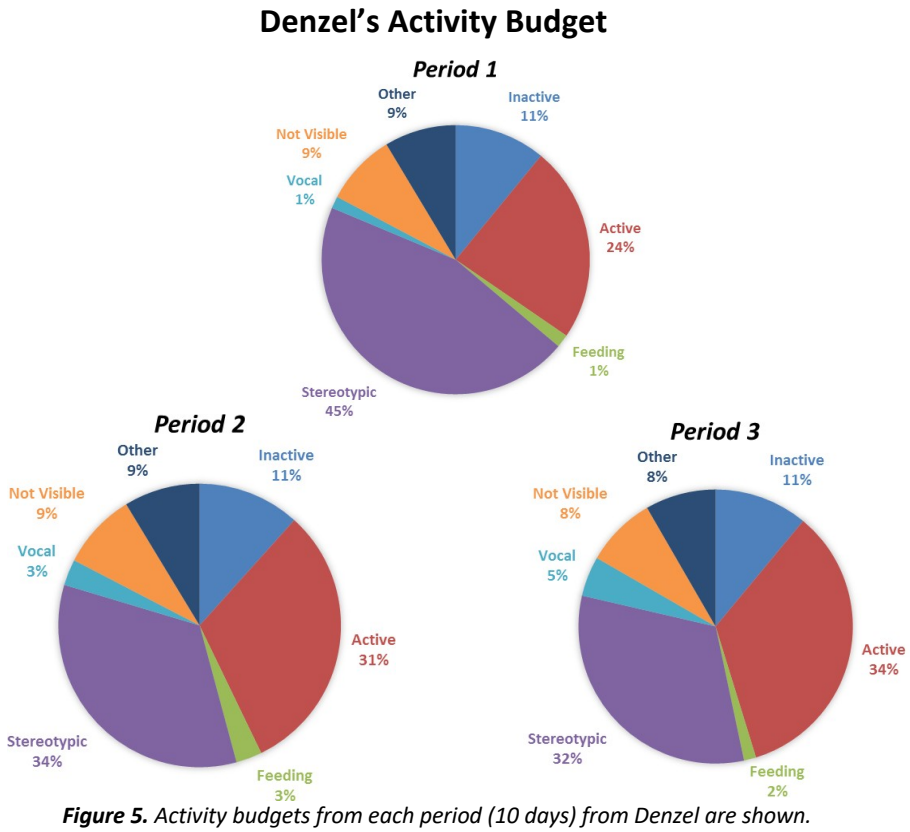

Figure 5. *Activity budgets from each period (10 days) from Denzel are shown.*

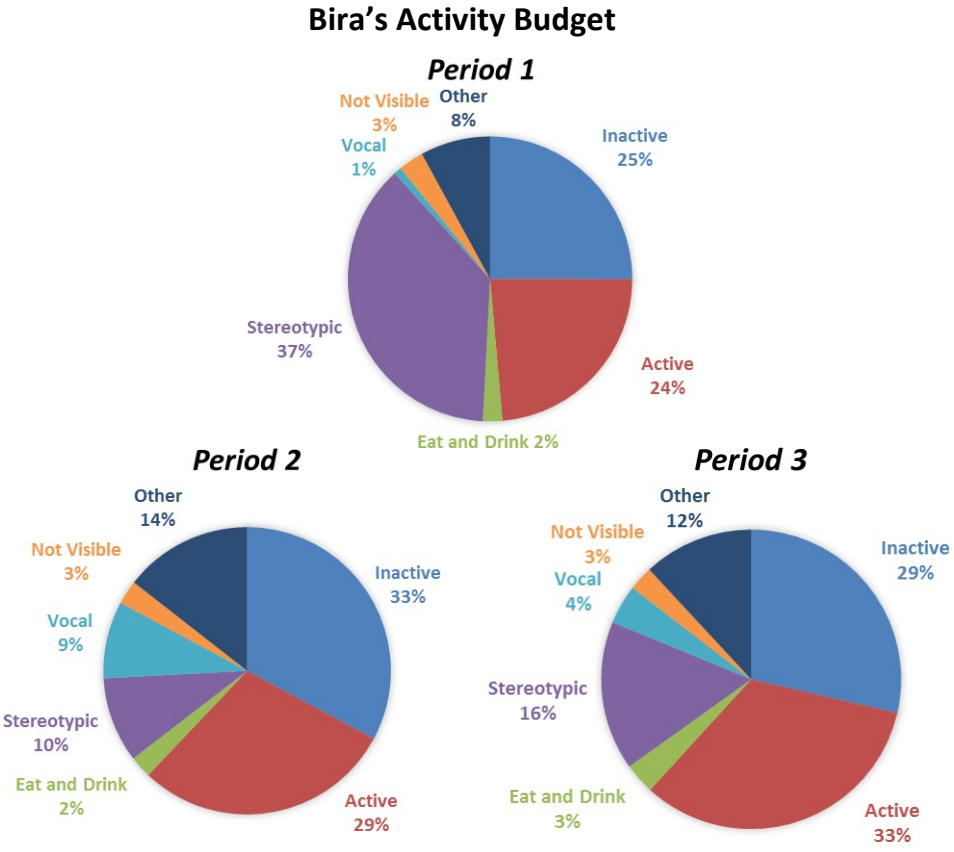

Figure 6. *Activity budgets from each period (10 days) from Bira are shown.*

Frequencies and duration of behaviors

The behaviors that showed a change over periods in Figure 5 and Figure 6 (*Pacing*, *Panting*, *Head-rolling*, *Staring outside* and Active Behaviors*)* were studied into more detail.

Pacing

Table 3 shows the means of the duration of Denzel's and Bira's pacing for each hour, during the three periods. The highest mean of pacing in an hour was during 11:00-12:00 in *Period 1* for Denzel and during 07:00-08:00 in *Period 3* for Bira. The lowest value was during the two afternoon observation hours of *Period 1* and the first afternoon observation hour from *Period 3* for Denzel, and during 19:30-20:30 from *Period 3* for Bira. There is a clear decrease in the duration of pacing during the second observation hour among all periods, in both Bira and Denzel. Denzel's mean duration decreases considerably over periods, contrarily to Bira which shows an unclear pattern.

Table 3. *The means and their standard errors of the duration (in minutes) of pacing for each hour during the three periods for each individual are shown.*

DENZEL	PERIOD 1	PERIOD 2	PERIOD 3
07:00 – 08:00	11.08 ± 4.79	9.77 ± 2.45	4.18 ± 1.39
08:30-09:30	9.39 ± 6.21	3.93 ± 2.04	1.58 ± 0.63
11:00-12:00	14.20 ± 5.45	5.47 ± 2.08	1.52 ± 1.26
12:30-13:30	5.20 ± 5.20	2.73 ± 2.49	1.30 ± 0.94
16:00-17:00 / 18:00 -19:00	0 ± 0	0.07 ± 0.07	0 ± 0
17:30 – 18:30 / 19:30 – 20:30	0 ± 0	0.20 ± 0.20	3.37 ± 2.07

BIRA	PERIOD 1	PERIOD 2	PERIOD 3
07:00 – 08:00	14.28 ± 5.98	3.22 ± 2.58	19.60 ± 6.19
08:30-09:30	6.75 ± 3.25	6.60 ± 4.4	2.00 ± 1,04
11:00-12:00	9.20 ± 6.62	0.35 ± 0.35	10.60 ± 6.54
12:30-13:30	10.00 ± 4.82	1.00 ± 1	7.20 ± 4.8
16:00-17:00 / 18:00 -19:00	8.80 ± 5.62	1.27 ± 0.91	9.80 ± 4.99
17:30 – 18:30 / 19:30 – 20:30	10.20 ± 5.14	0 ± 0	2.40 ± 2.40

Table 4 shows the means of the duration of Denzel's and Bira's pacing depending on the keepers' activity (before, with and after keepers went to the enclosure), during the three periods. There's a clear decrease of pacing when the keepers are around, especially in *Period 2* for Denzel and *Period 3* for Bira. During *Period 1* both Bira's and Denzel's pacing increased after the keepers had left, contrary to the other periods for Denzel and during *Period 2* for Bira.

Table 4. *The means and their standard errors of the duration (in minutes) of pacing depending on the keeper's activity during the three periods for each individual are shown.*

DENZEL	PERIOD 1	PERIOD 2	PERIOD 3
Before Keepers	11.08 ± 4.79	9.77 ± 2.45	4.18 ± 1.39
With Keepers	9.39 ± 6.21	3.93 ± 2.04	1.58 ± 0.63
After keepers	4.85 ± 2.18	1.69 ± 0.9	1.24 ± 0.66

BIRA	PERIOD 1	PERIOD 2	PERIOD 3
Before Keepers	14.28 ± 5.98	3.22 ± 2.58	19.60 ± 6.19
With Keepers	6.75 ± 3.25	6.60 ± 4.4	2.00 ± 1.04
After keepers	9.55 ± 2.57	0.66 ± 0.34	7.50 ± 2.37

Denzel paced the most during *Period 2* and similar during the other two periods [see Figure 7a]. However, the highest duration of pacing happened during *Period 1*, and decreased over periods [see Figure 7b]. Thus, the frequency did not change but the duration decreased considerably. On the other side, Bira paced the most during *Period 1*, the least in *Period 2* and increased a bit again in *Period 3* [see Figure 7c]. Concerning the duration of her pacing, a similar pattern was drawn, having the highest duration of pacing in *Period 1*, the lowest in *Period* 2 and an increase in *Period 3* [see Figure 7d].

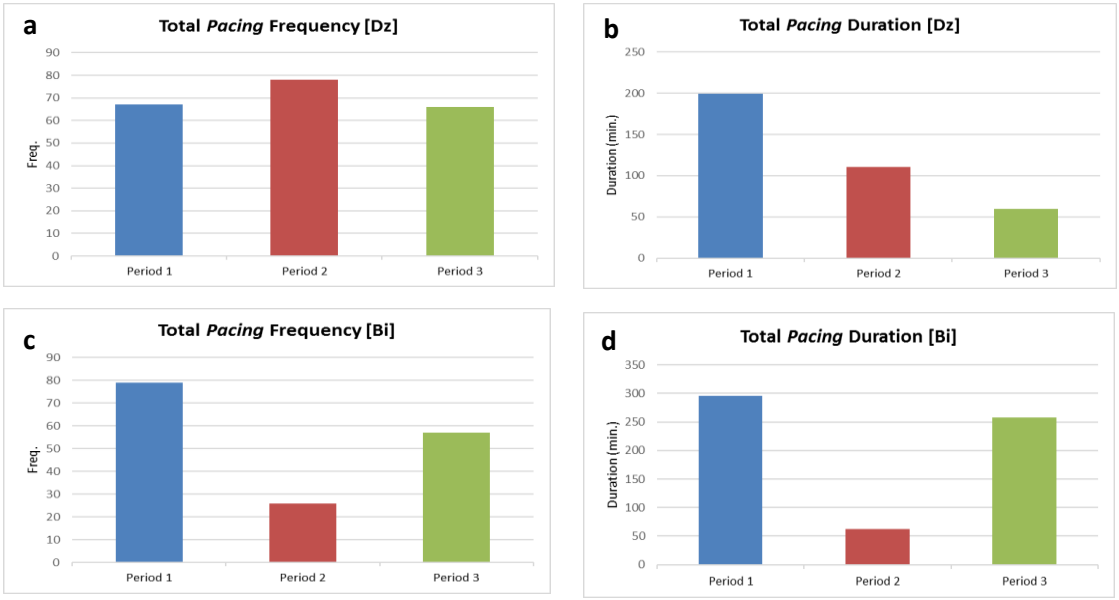

Figure 7. *Left charts (a and c) show the total pacing frequency of each individual during each period (10 days). Right charts (b and d) show the total duration (in minutes) of pacing for each individual during each period (10 days).*

Concerning the total duration of pacing depending on the time of day (morning, midday or afternoon observations), the following results were obtained [see Figure 8]: the time of day when Denzel paced the most was during the mornings of *Period 1*, and the least during the afternoons of *Period 1*. In both mornings and middays, there was a decrease over periods. On the contrary, there was an increase over periods in the afternoons. Bira paced the most during the mornings of *Period 3* and the least during the afternoons of *Period 2*. Bira paced the least during *Period 2*, and quite similar during *Period 1* and *Period 3*.

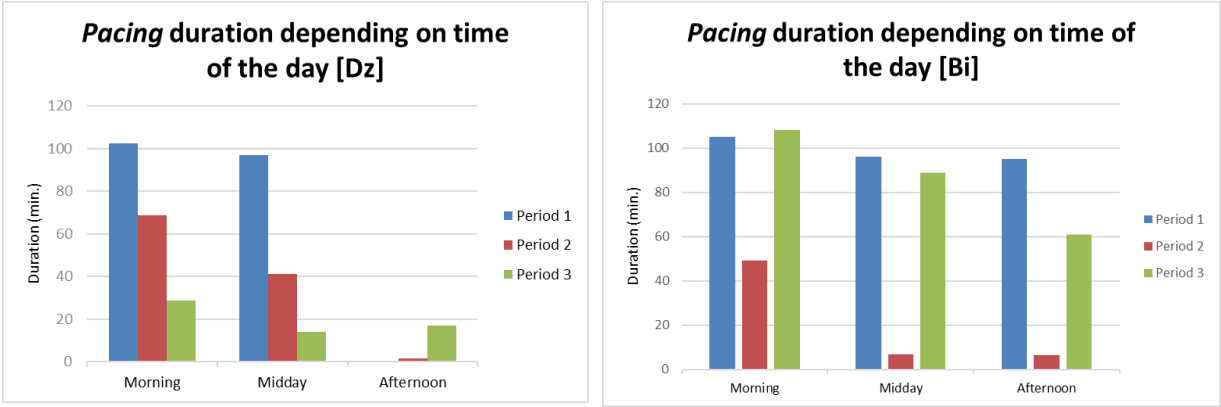

Figure 8. *The total duration of pacing (minutes) depending on the time of the observation (Morning, Midday or Afternoon) during each period (10 days) for each individual is shown.*

Panting

The frequency of panting increased with time for Denzel [see Figure 9a]. However, the highest duration of panting happened during *Period 2*, followed by *Period 3* and having the least duration during *Period 1* [see Figure 9b]. On the other side, Bira panted the most during *Period 1*, the least in *Period 2* and increased a bit again during *Period 3* [see Figure 9c]. Concerning the duration of her panting, a similar pattern was seen, having the highest duration of panting during *Period 1*, the lowest during *Period 2*, and an increase during *Period 3* [see Figure 9d].

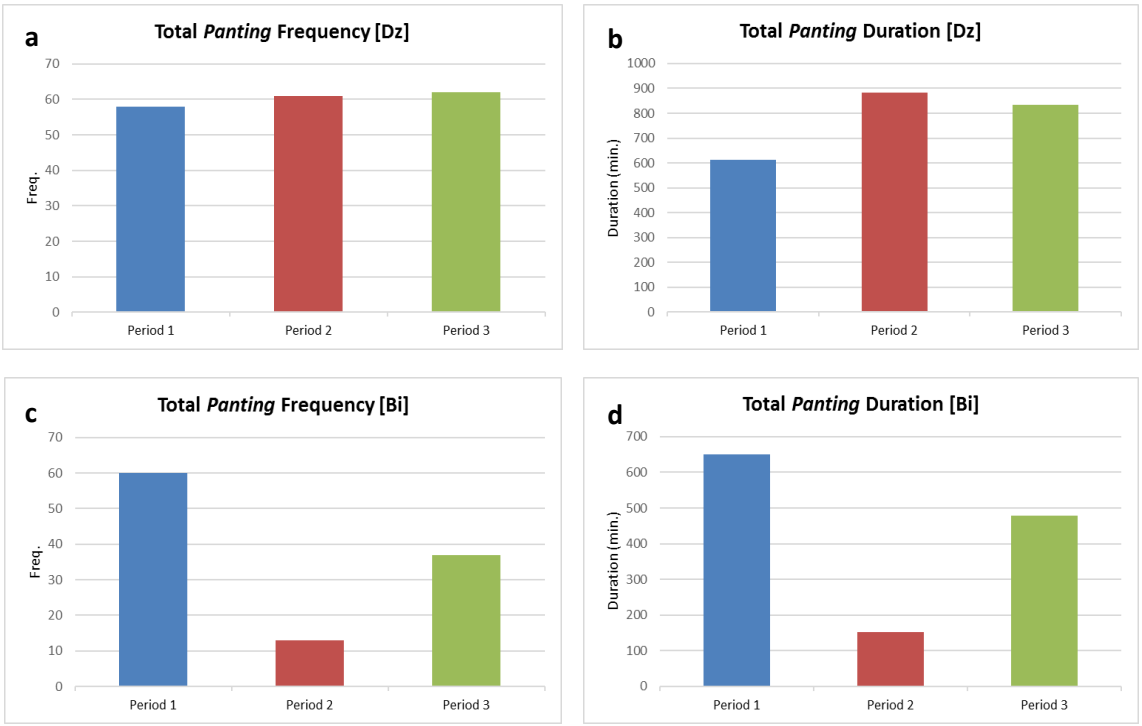

Figure 9. *Left charts (a and c) show the total panting frequency in each animal during each period of time (10 days). Right charts (b and d) show the total duration (in minutes) of panting for each individual during each period of time (10 days).*

Concerning the total duration of panting depending on the time of the day (morning, midday or afternoon observations), the following results were obtained: the time of the day when Denzel panted the most was during the mornings of *Period 2*, and the least during the afternoons of *Period 1* [Figure 10a]. Bira panted the most during the mornings of *Period 1* and the least during the afternoons of *Period 2* [Figure 10b].

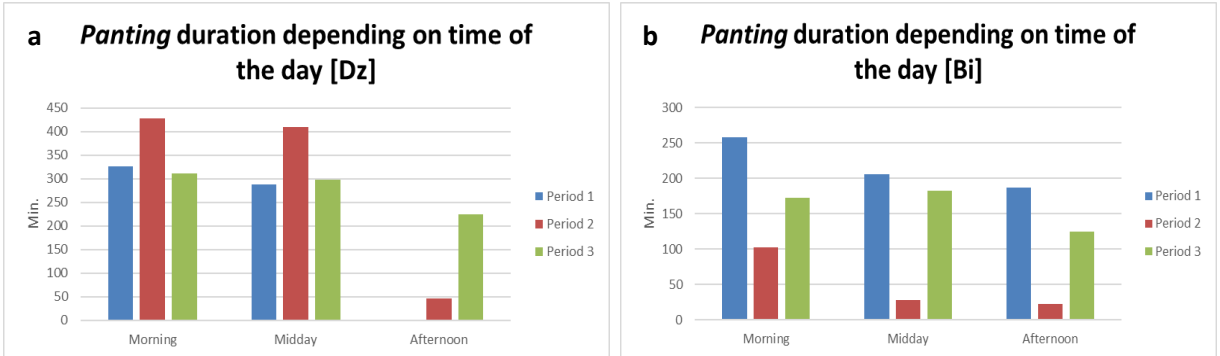

Figure 10. *The total duration of pacing (minutes) depending on the time of the observation (Morning, Midday or Afternoon) during each period (10 days) for each individual is shown.*

Head-rolling

Head-rolling was only seen in Denzel, no head rolls were observed in Bira. There was a clear increase in the frequency of head rolls over periods [see Figure 11a]. Denzel did more head rolls in the morning, decreasing during midday, and decreasing again during the afternoon. During the afternoons of *Period 1*, no head rolls were observed, but they were during *Period 2* and it increased during *Period 3* [see Figure 11b].

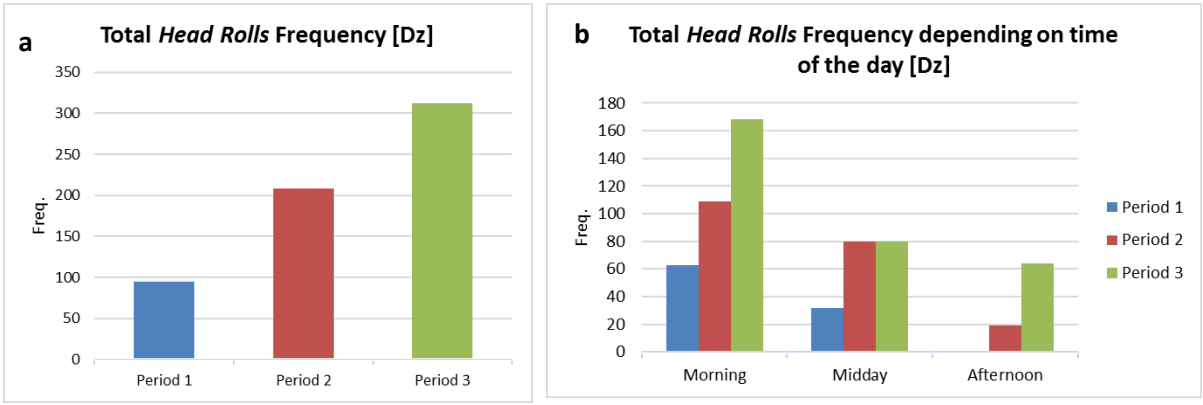

Figure 11. *On the left chart (a), the total frequency of Denzel's head rolls for each period (the total number of head rolls performed in 10 days) is shown. On the right chart (b), the frequency of the head rolls depending on the time of the day (number of head rolls performed in 10 days during morning, midday and afternoon) is shown.*

Staring outside

Concerning Denzel, there was a clear decrease in the frequency of this behavior over periods [see Figure 12a]. Denzel stared outside the most during the mornings of each period, decreasing during middays and decreasing again in the afternoons. During *Period 1* no staring outside was observed in the afternoon, however it was observed during *Period 2* and *Period 3* [see Figure 12b]. Concerning Bira, she stared outside the most during *Period 1* and it decreased considerably in *Period 2*, staying practically the same during *Period 3* [see Figure 12c]. The highest frequency of staring outside was during the afternoons of *Period 1* [see Figure 12d].

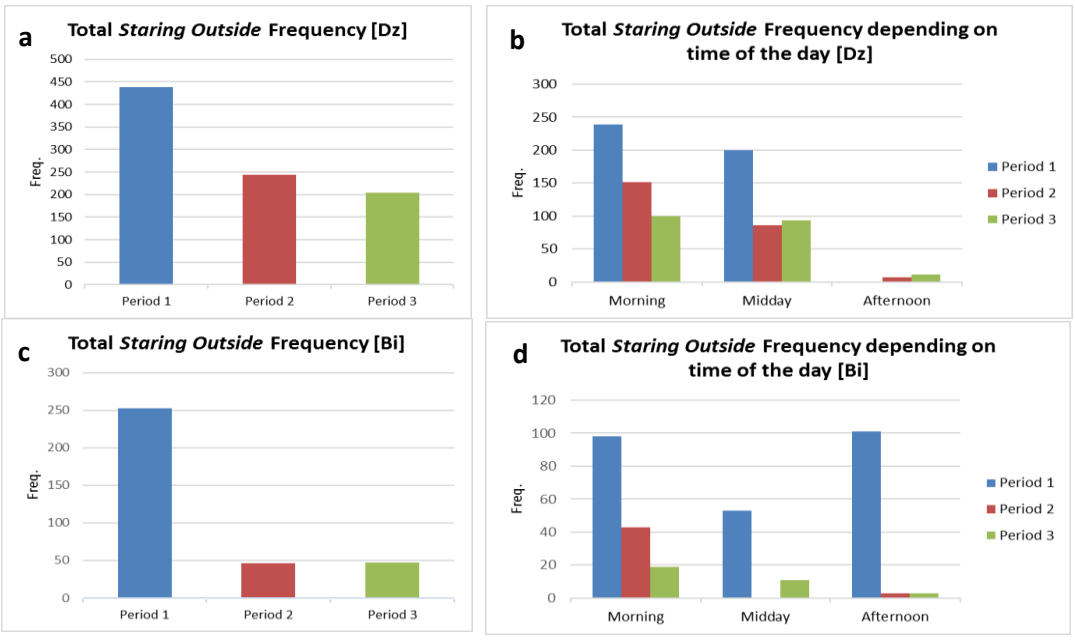

Figure 12. *Left charts (a and c) show the total staring outside frequency in each animal during each period (10 days). Right charts (b and d) show the frequency of staring outside depending on the time of the day.*

Active Behaviors

The behaviors in this group (*Walking, Running* and *Jumping*) were analyzed all together. Concerning Denzel, there was a clear increase in the frequency of these behaviors over periods [see Figure 13a]. Denzel performed Active Behaviors the most during the mornings of each period, decreasing during middays and decreasing again in the afternoons. During *Period 1* almost no Active Behaviors were observed in the afternoon, however they increased during *Period 2* and *Period 3* [see Figure 13b]. Concerning Bira, she performed Active Behaviors the most during *Period 2* and similarly during *Period 1* and *Period 3* [see Figure 13c]. Mornings were mainly the time of day when Active Behaviors were observed the most, followed by middays and afternoons [see Figure 13d].

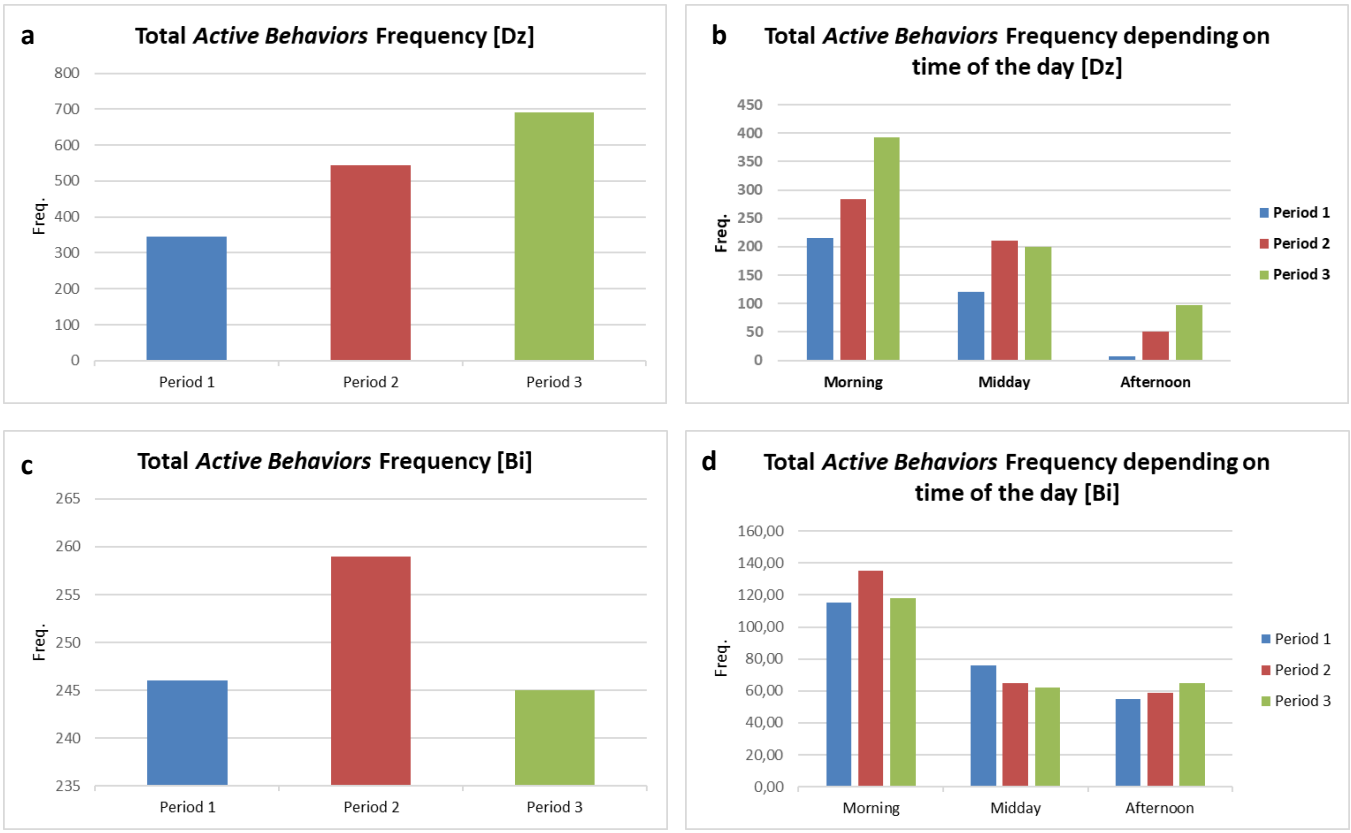

Figure 13. *Left charts (a and c) show the total Active Behaviors frequency in each animal during each period of time (10 days). Right charts (b and d) show the frequency of Active Behaviors depending on the time of the day.*

Training

To study if the presence and absence of training influenced the leopards' behaviors, all observed behaviors were sorted as Diminished Welfare Indicators (DWI) or Enhanced Welfare Indicators (EWI) [see Table 5], based on Biolatti *et al*. (2016). Vocalizations have been suggested as a marker of both diminished and enhanced welfare according to Boissy *et al*. (2007). In this case, Vocal Behaviors is considered as both a DWI and an EWI, because it was not possible to distinguish each type of vocalization during observations.

Table 5. *List of all observed behaviors sorted as Diminished Welfare Indicators (DWI) or Enhanced Welfare Indicators (EWI).*

Diminished Welfare Indicators (DWI)	Enhanced Welfare Indicators (EWI)
Pacing	Active Behaviors
Panting	Inactive Behaviors
Head-rolling	Feeding Behaviors
Staring outside	Vocal Behaviors
Vocal Behaviors	Not Visible
	Other Behaviors

For Denzel, all DWI mean frequencies tended to be lower on a training day, except for head-rolling, which increased on training days [see Figure 14a]. Concerning EWI, the frequency of the means of Active, Inactive and Feeding behaviors were higher when training. Other Behaviors was lower during training days and Vocal Behaviors had practically the same mean frequency [see Figure 14b]. For Bira, both DWI [Figure 14c] and EWI [Figure 14d] mean frequencies tended to be lower in training days.

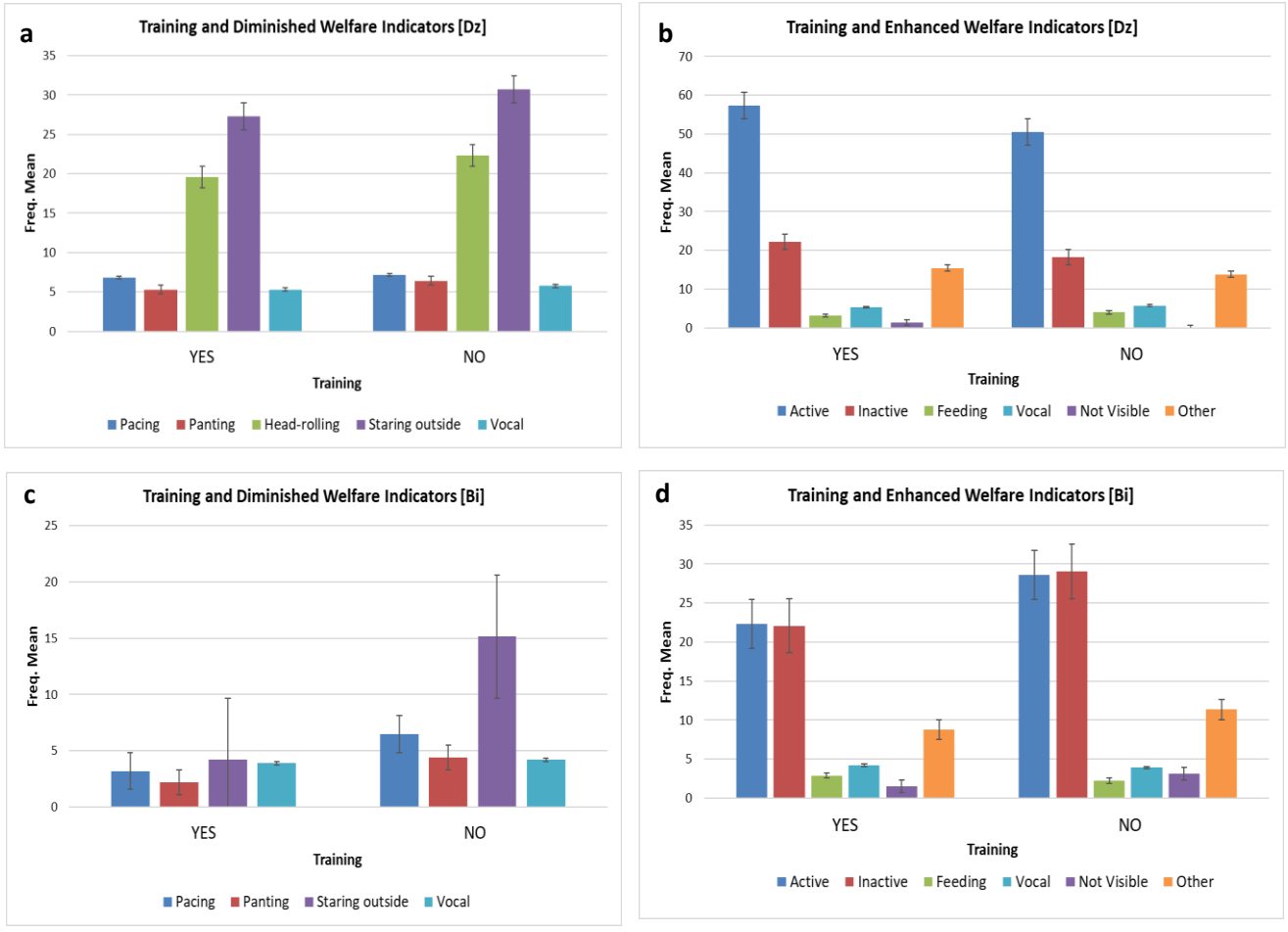

Figure 14. *The frequency means of each Diminished (a and c) and Enhanced (b and d) Welfare Indicators depending on if they had training or not are shown for both individuals.*

Enrichment

To study if the presence and absence of enrichment influenced the leopards' behavior, the same procedure as in Training was followed, using Diminished Welfare Indicators (DWI) or Enhanced Welfare Indicators (EWI) [see Table 5].

It seems that enrichment did not have a big influence on Denzel's behavior (both DWI and EWI). Nevertheless, head-rolling decreased the days he got enrichment and staring outside increased [see Figure 15a]. He was more active the days there was no enrichment in the enclosure [see Figure 15b]. For Bira, both DWI [Figure 15c] and EWI [Figure 15d] mean frequencies tended to be lower during the days she got enrichment.

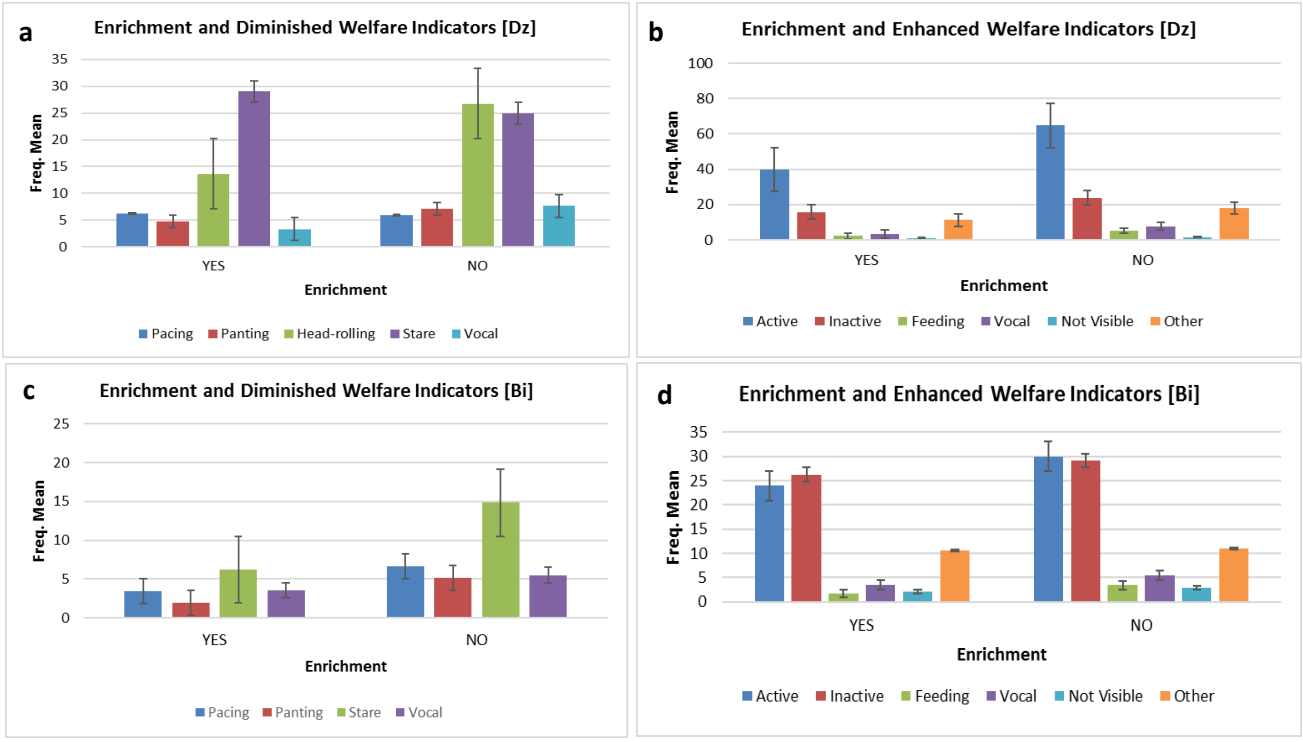

Figure 15. *The frequency means of each Diminished (a and c) and Enhanced (b and d) Welfare Indicators depending on if they had enrichment or not are shown for both individuals.*

Effect of visitors

The visitors opening hours of the park were from 10:00 – 17:00 during *Period 1* and *Period 3*, and from 10:00 – 19:00 during *Period 2*. The mean values of the number of visitors in front of the enclosures of each observation time frame are summarized in Table 6.

Table 6. *Mean values of the number of visitors in front of the enclosures of each observation time frame.*

	Denzel			Bira		
	Period 1	**Period 2**	**Period 3**	**Period 1**	**Period 2**	**Period 3**
11:00 – 12:00	154	150	22	74	190	40
12:30 – 13:30	126	290	56	145	322	52
16:00 – 17:00	31	*No obs.*	12	23	*No obs.*	13
18:00 – 19:00	*No obs.*	13	*No obs.*	*No obs.*	22	*No obs.*

The maximum number of visitors in an hour in front of Denzel's enclosure was **402** visitors, during 12:30 – 13:30 of *Period 2*. The maximum number of visitors in front of Bira's was **507** visitors, also during 12:30 – 13:30 of *Period 2*.

When comparing the number of visitors from every hour of observation against the respective pacing frequency and duration (in minutes), no direct relation was found for neither Denzel nor Bira [see Figure 16].

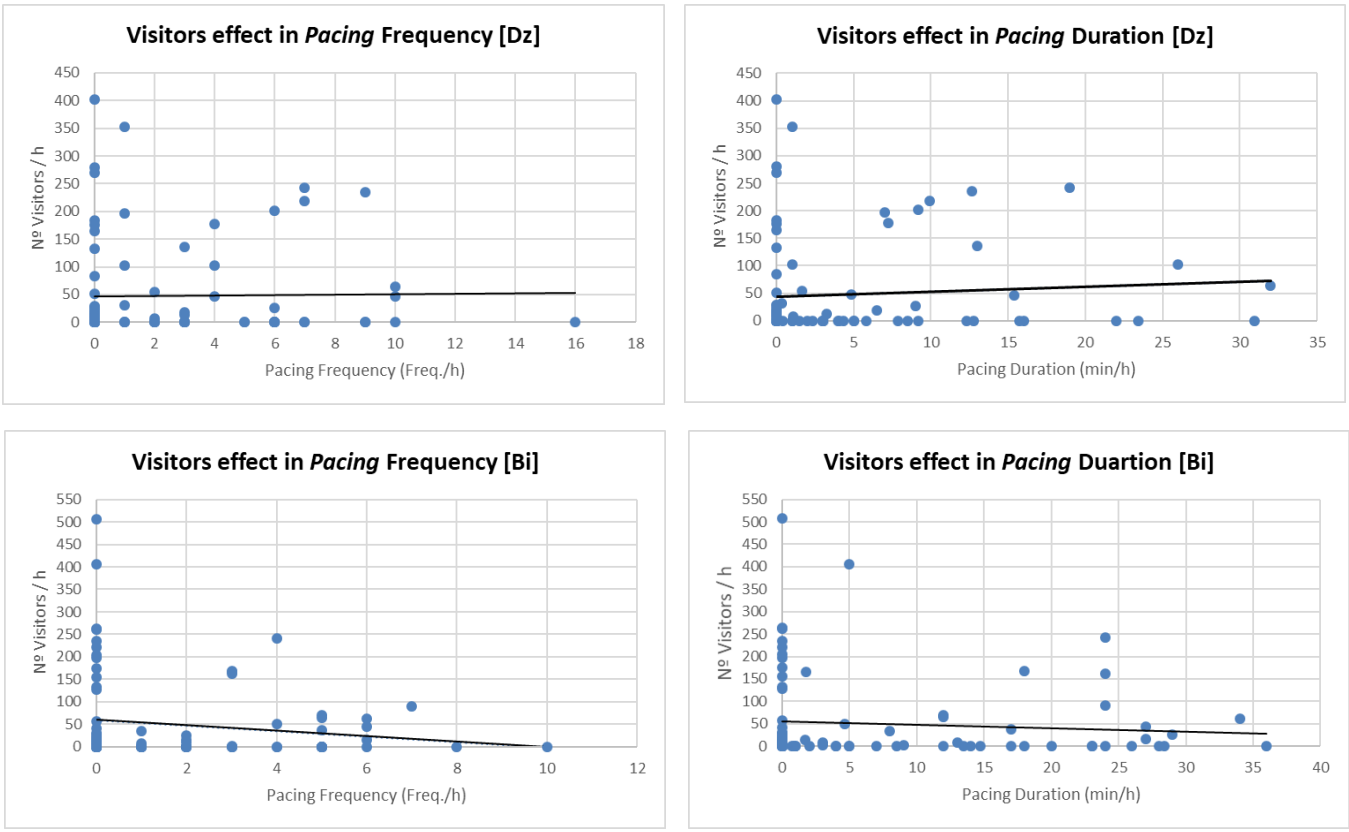

Figure 16. *Left plots show the effect of the visitors in the pacing frequency per hour in each animal. Right charts show the effect of the visitors in the pacing duration (min) per hour in each animal.*

Additionally, aggressive behaviors (showing teeth, ears back, growling and attacking) have been seen in both Bira and Denzel towards visitors when they were too close to the fence, especially if they were holding a phone or a camera.

Additional remarks

The behaviors observed and categorized as Other Behaviors were the following: *Smelling, Urine marking, Defecating, Peeing, Crouching, Licking, Scratching, Rolling, Rubbing* and *Playing*. There was a clear predominance of *Rolling*, *Crouching, Rubbing* and *Urine marking* during *Period 2* and *Period 3*.

DISCUSSION

The aim of this study was to evaluate and improve the welfare of the two Amur leopards currently living at *Nordens Ark*, exposing them to four changes in their environment concerning training, increased zookeeper presence, increased environmental enrichment and visual barriers. A discussion of the effect of these changes on each studied behavior is given below, as well as a discussion of the effect of the visitors and the importance of enrichment.

Activity budgets

Wild felines are quite active during the entire 24-hour cycle (Lyniuk, 2011). However, predators in captivity frequently show a higher frequency of resting behaviors due to under-stimulation (Lyniuk, 2011; Mallapur *and* Chellam, 2002; Sulser *et al.,* 2008). In this study, the most frequent behaviors for Denzel during *Period 1* were Stereotypic Behaviors, followed by Active Behaviors and Inactive Behaviors. Over periods, Inactive Behaviors stayed constant but Active Behaviors increased while Stereotypic Behaviors decreased. There is a clear exchange between these two last groups of behaviors. For Bira, the most frequent behaviors were also Stereotypic Behaviors, and very similar frequencies in Inactive and Active Behaviors. In *Period 3*, Active Behaviors were the most frequent behavior group followed closely by Inactive Behaviors, with much less Stereotypic Behaviors. This seems to be contrary to the usually higher frequency of resting behaviors in captive predators. However, the dominance in active behaviors are similar to the findings of a previous study of Transvaal lions (*Panthera leo krugeri*) and Amur leopards (Lyniuk, 2011). The higher activity in Denzel compared to Bira also coincides with Lyniuk (2011) and a study on Amur leopards (McWhorter, 2014), where all the males showed higher locomotion behaviors than the females. The Amur leopards in that last study, however, showed more resting behaviors than active behaviors in general.

The fact that both individuals ended up having Active Behaviors as the most frequent behavior group is a positive sign. Not only the absence of considered negative behaviors or poor welfare is important, but the presence of positive ones and good well-being as well (Boissy *et al*., 2007; Whitham *and* Wielebnowski, 2013). The decrease of Stereotypic Behaviors and increase of Active Behaviors over periods in both Denzel and Bira suggests that the changes in their environment had a positive effect.

There are reasons to think that during *Period 2* and part of *Period 3* Bira was in heat. Both Other and Vocal Behaviors increased after *Period 1*. The predominant behaviors in these two behavioral groups during the last two periods were *Rolling*, *Crouching, Rubbing*, *Urine marking* and *Vocalizing*, all considered indicators of estrus in leopards (Allwin *et al.*, 2016; Brown *et al.*, 1995; Heptner *et al.*, 1992; Owen *et al.,* 2010; Schmidt *et al*., 1993; Umapathy *et al.*, 2007). The indicators of estrus seemed to be more intense during *Period 2* than in *Period 3*. It was first hypothesized that the fact that Bira spent such an increasingly amount of time performing Inactive Behaviors and so little performing Stereotypic Behaviors during *Period 2* could be explained by her estrus state. However, according to previous studies (Allwin *et al.,* 2016; Palita *et al.,* 1996), female leopards in estrus are characterized by an increase

in general activity such as restlessness, pacing and locomotion, contrary to what Bira did. No references were found stating the contrary. Thus, more research concerning estrus behavior in captive leopards would be needed to support the hypothesis of a correlation between Bira's behavior during *Period 2* and her estrus state.

Frequencies and duration of behaviors

Pacing

Pacing is known to be one of the most typical stereotypies in captive felids (Breton *and* Barrot, 2014; Swaisgood *and* Shepherdson, 2005) and Bira and Denzel are no exception. Both individuals paced throughout all three periods.

A variability in the pacing routine of the two individuals can be seen between periods. There was a clear difference between the frequency and the duration of pacing in Denzel. Even though the frequency of pacing did not change significantly over periods, there was a strong decrease in duration. This can be considered a positive effect: pacing can be regarded as a coping mechanism to handle stress, thus every time an animal starts pacing means that something needs to be handled (Mason, 2006). We could then consider that the duration of pacing reflects how long they need to perform this behavior in order to cope with unsatisfied needs. The longer the time they need to handle this stressful situation, the longer the duration of the pacing will become. Hence, a decrease in its duration would mean less time needed to handle stress, which could be explained by a decrease in either the intensity or amount of stress the animal is faced to. If so, we could speculate that the changes in the environment helped decrease Denzel's stress.

Oppositely, Bira had the same pattern for both frequency and duration. As mentioned previously, Bira seemed to be in heat during *Period 2* and part of *Period 3*. According to previous studies (Allwin *et al.,* 2016; Palita *et al.,* 1996), felines in estrus are more prone to restlessness and pacing, which is contrary to Bira's behavior. This could mean that the changes in the environment in short term were very effective to her but lost effect in the longer term. Nevertheless, the estrus state should not be underestimated and it should be considered as an affecting factor to her behaviors during these periods anyways.

Looking at the duration of pacing depending on time of day, each individual has again its own pattern. Denzel paced the most during mornings and middays, and nothing or little time during afternoons (depending on the period). This is similar to the findings of McWhorter (2014) and Mallapur *and* Chellam (2002), which both reported strong peaks of activity in the morning and a decrease during the day, with another small peak in the afternoon. Bira, on the other hand, paced quite equally during the entire day. Denzel's pacing seems to be related to time of day, but not Bira's.

These pacing patterns could be related to the keepers' activity. Zookeepers have a very important role in the well-being of the animals and the quality of the keeper-animal interaction directly affects the individual (Hosey, 2008; Whitham *and* Wielebnowski, 2013). Previous studies show that pacing in

captive leopards increases before the presence of keepers (McWhorter, 2014; Mallapur *and* Chellam, 2002). Some studies also show that pacing and other abnormal behaviors can decrease during the time the keepers are in the enclosure, especially if they give attention to the animal (Baker, 2004; Whitham *and* Wielebnowski, 2013). For example, Mellen *et al*. (1998) showed a negative correlation between pacing and the amount of keeper interaction in small felids, and Wielebnowski *et al*. (2002) found lower concentrations of fecal glucocorticoid metabolites (used to assess stress response) when keepers spent more time interacting with clouded leopards.

Following this train of thought, the pacing routines of Bira and Denzel can be better understood. The keepers and the trainer would normally go to the enclosures between the first and second observation hours (07:30-09:30). Both Bira and Denzel showed a decrease in the duration of pacing from 08:30-09:30 (except in *Period 2* for Bira), which could be explained by the increased presence of the keepers. Within the same line of reasoning, the fact that Denzel had such a high pacing duration from 11:00-12:00 in *Period 1* but not in *Period 2* and *Period 3* could be explained by the total absence of keepers in the enclosure some days during the first period, making Denzel pace for a longer amount of time. We can see as well that Denzel paced the most during the first observation hour in *Period 3*, and quite equally during the rest of the day (except in the afternoon, which is discussed later). Taking into consideration that keepers had to go to the enclosure every day in this period, the fact that Denzel paced the most during the first observation hour could mean that he paced expectant for the keeper to come and once the keeper had come by the enclosure, he decreased his pacing considerably. Denzel's lower duration of pacing from 12:30-13:30 is similar to the findings of the study of Indian leopards from Mallapur *and* Chellam (2002), which recorded the calmest moment of the day at 12:00.

As already mentioned, Denzel spent all the afternoons of *Period 1* laying on a high rock at the back of the enclosure, mainly sleeping, not moving during the whole observation hours. However, he started pacing in *Period 2* and it even increased in *Period 3*. The reasons behind this are not clear and a more thorough study would be needed to better comprehend this pattern. Being active during the afternoon is not unsual *per se* as other studies of leopards show active behaviors in the afternoon (Mallapur *and* Chellam, 2002; McWhorter, 2014). However, the new presence of pacing might indicate that a new stressor has appeared during this time of day, which was not there before the changes were implemented. Further research would be needed to find this new stressor.

Panting

Both Bira and Denzel performed this behavior during pacing or after a sudden fright (e.g. by an abrupt noise). Contrarily to Denzel's pacing pattern, the panting increased in both frequency and duration over periods. Concerning the duration depending on time of day, the same pattern can be seen. However, Bira had the same pattern in both her pacing and pant. This illustrates that the changes in the environment affected differently each behavior and individual.

Bira's panting decrease in *Period 2* could be explained again by her estrus state, even though it does not go in accordance with the literature (Allwin *et al.,* 2016; Palita *et al.,* 1996).

Temperature can also have an effect on panting (Saxon, 2016). However, temperatures were similar during the three periods (World Temperatures, n.d.), so heat does not seem to be the reason behind this behavior in this case.

Head-rolling

There are not that many references that address head-rolling in felines. However, during a behavioral study conducted on two Amur leopards from the Jackson Zoological Park (McWhorter, 2014), a behavior with the exact same definition as the behavior *Head-rolling* seen in Denzel was recorded. The author categorized this behavior as an abnormal repetitive behavior as well. Similar behaviors have been recorded and related to pacing or other abnormal behaviors in a variety of zoo and laboratory animals, such as giant pandas (Liu *et al.,* 2017), black bears (Eliam *et al.,* 2015), rats (Hoshi *and* Ohtsuka, 2009), giraffes, llamas, brown and polar bears and some primates (Chutchawanjumrut, 2015). However, every study used a different name to what seems to be the same behavior (neck- twisting, head-tossing, head-rolling and head-twisting). A consensus on this behavior's name is necessary. Moreover, further studies in this area should be carried out to fully understand the purpose, triggers and meaning of this behavior.

According to the zookeeper I. Enggren (personal communication, 21 June, 2017), Denzel had training sessions in the previous zoo where he was housed. One of the trained behaviors was to stand on his hind-limbs and lean on the fence, similar to the head-rolling position. It could be that this learned behavior was not forgotten and he kept doing it in the new housing. This goes in accordance with both Swaisgood *and* Shepherdson (2005), and Mason (2006), who stated that stereotypies might not reflect the current condition of the animal, but the one from a prior housing. Furthermore, there was a clear increase of head rolls over periods. Following the same line of thought, it could be that the new implemented training reminded him of his previous training and it made him head-roll even more.

However, the head-rolls have been related to pacing (Chutchawanjumrut, 2015; Eliam *et al.,* 2015; Hoshi *and* Ohtsuka, 2009; Liu *et al.,* 2017; McWhorter, 2014). In Denzel's case, it seems that this behavior works as a mechanism to stop pacing, since he would end his pacing with a head-roll almost every time. In that case, an increase in this behavior should not be seen as something negative. Let's keep in mind that even though an abnormal behavior looks superficially functionless, it can actually be a coping mechanism for the animal to handle stress and its occurrence could be actually helping the individual (Mason, 2006; Whitham *and* Wielebnowski, 2013). On the other hand, these head-rolls might be due to a health issue (e.g. neurological disorder). A health check should be taken into consideration to rule out this possibility.

Staring outside

Macri and Patterson-Kane (2011) conducted a study on solitary and non-solitary snow leopards (*Panthera uncia*) in different zoos from the United Kingdom. On some of the solitary leopards, a *stand and observe* behavior was recorded described as "*Cat is standing, eyes are focused at a certain stimulus or looking about the enclosure. Ears attentive*", which fits with the *Staring Outside* behavior of this study. During the study conducted by Apelqvist (2012) in Nordens Ark, the same behavior was observed in both Bira and Denzel during their pacing.

Staring Outside seems to be related to pacing because both Bira and Denzel only performed it during their pacing routines and always at the same spots. Denzel would always stare outside when he was pacing at the right part of the enclosure, the nearest to Bira's. It was difficult to determine in which direction Denzel was really looking. It seemed that he was looking to the visitors' path, where the zookeepers would normally come from. This could mean that Denzel's pacing was triggered by the anticipation for a keeper to come or by the frustration of no keeper showing up. Furthermore, Denzel performed this behavior the most during mornings and middays, and almost none during afternoons. If the reason behind this behavior is related to zookeepers, the fact that the keepers had to increase their presence in the enclosure, spending more time with the animals, could have had an effect on this behavior: more keepers' presence reduced Denzel's frustration of keepers not showing up, and so *Staring outside* reduced.

On the other hand, Bira's reason behind this behavior seems to be different from Denzel's. Bira did not have such a clear decreasing pattern, and she performed the behavior more equally throughout the day, especially in *Period 1*. Bira's *Staring Outside* behavior seems to be related to the presence of other animals. She would stare outside at the back part of the enclosure, where there were no other enclosures, but where they might have been a wild animal living nearby who dragged her attention. She also showed interest in the urials from the enclosure in front of her while performing this behavior. Apelqvist (2012), also mentioned a high interest of Bira in the urials.

Active Behaviors

Results show that as Stereotypic Behaviors decreased, Active Behaviors increased in both Bira and Denzel. As already mentioned, resting behaviors are dominant in predators in captivity while active behaviors are dominant in the wild (Lyniuk, 2011; Mallapur *and* Chellam, 2002; Sulser *et al.,* 2008). The increase of Active Behaviors tells us that the implemented changes had a positive effect on both individuals. Their behavior at the end of the study corresponds better to the behavior of their wild conspecifics, likely caused by these changes.

Both leopards showed the highest activity during the mornings of all periods and a lower activity during the rest of the day. This goes in accordance with their nature of being active in the early mornings and late evenings, resting throughout the rest of the day (crepuscular animals) (Bailey, 1993; Santiapillai *et al*., 1982).

Training

Training animals is a tool that has been increasing during the last decade in zoos (Melfi, 2013). Research shows that it can be very enriching and beneficial for their welfare (Melfi, 2013; Westlund, 2014). It is thus not surprising to see that there was a decreased frequency in Diminished Welfare Indicators (DWI) the days of training in both Bira and Denzel and at least an increased frequency of Enhanced Welfare Indicators (EWI) in Denzel.

It is a bit confusing that Bira had both a decrease in EWI and DWI during training days. However, this can be explained by her decrease in activity in general during *Period 2*, and thus a decrease of both EWI and DWI during training days.

Considering that training had a positive effect on the welfare of both individuals, the zoo should continue training them. However, they should be aware that according to Melfi (2013), once a behavior is learnt, training that behavior is unlikely to be enriching anymore. Therefore, it is important to keep variability in the training plan to ensure the greatest benefit.

Enrichment

Studies show that enrichment can reduce the presence of abnormal behaviors considerably in zoo animals (Chutchawanjumrut, 2015; Damasceno *et al.*, 2017). However, it is not always easy to provide the adequate enrichment, especially in captive felids, due to the complex predation and exploratory behaviors they have (Damasceno *et al.*, 2017; Macri *and* Patterson-Kane, 2011). Variability is also key to ensure a proper enrichment and avoid habituation to new stimuli (Yu *et al.,* 2009).

Results show that enrichment did not have a lot of effect on both Bira's and Denzel's behavior. Most of the enrichment given by the zookeepers were odor-based (such as spices, peppermint oil, valerian and blood), straw and hay, and cardboard boxes and tubes. Yu *et al*. (2009) noted that leopards seem to lose interest in scents with time. This could explain the low effect of the enrichment on the individuals. Therefore, it is very important that the keepers take this into consideration and constantly change the odor they use. Furthermore, felines rely more on visual and auditory cues than olfactory ones during hunting (Macri *and* Patterson-Kane, 2011), thus odor should not be used alone and should be simultaneously given with other enrichments. Some proven effective enrichments for leopards according to literature are modifying food presentation, incorporating feeding boxes, bones and frozen treats, catnip and nutmeg (McWhorter, 2014), hay balls with cinnamon scent (Damasceno *et al.*, 2017) and puzzles (Lyniuk, 2011).

Effect of visitors

Visitors and their effect on zoo animals has been broadly studied the past few decades (e.g. Chamove *et al*., 1998; Cunningham, 2005; Davey, 2007; Hosey, 2000; Hosey, 2008; Hosey, 2013; Mallapur *and* Chellam, 2002; Mallapur *et al.,* 2005; Margulis *et al*., 2003; O'Donovan *et al.,* 1993; Sellinger *and* Ha, 2005). It seems that depending on the animal and zoo, this effect can be negative (Chamove *et al*., 1998; Davey, 2007; Sellinger *and* Ha, 2005), positive (Davey, 2007; Mallapur *and* Chellam, 2002; Sellinger *and* Ha, 2005) or neutral (Cunningham, 2005; Davey, 2007; Margulis *et al.,* 2003; O'Donovan *et al*., 1993). The results of this study show that visitors did not trigger pacing in neither Bira nor Denzel, which suggests that visitors do not have a negative effect on them, or at least not a visible one. This goes according with Hosey (2008), which stated that stereotypies such as pacing in felids do not seem to be affected by visitors. Nevertheless, both Bira and Denzel showed aggressive behaviors (showing teeth, ears back, growling and even attacking) towards visitors when they were too close to the fence, especially if they were holding a phone or a camera (e.g. trying to take a picture without the fence in the way). Special care should be given to this and some security measures should be put to avoid any possible future accidents, for both the visitors and the animals' safety (e.g. put a barrier so that visitors cannot get that close).

Design limitations

As mentioned before, the four changes were implemented together due to time limitation. Thus, the effect of every single change could not be studied separately, and they could only be studied as a group. However, this study was mainly meant as a pilot study, to see if making changes in the environment can improve the welfare of the two leopards. It is clear that it does. Therefore, future studies on Bira and Denzel should focus on which particular changes would improve their welfare best and implement them.

Ethical reflection

Zoos and the ethics behind their existence has been broadly discussed. Keeping animals in captivity just for human amusement might be ethically wrong. However, zoos do not only exist for that purpose. They play an important role in biodiversity and wildlife conservation too (Gray, 2015). Many species have been saved from extinction or brought back from it thanks to the efforts made in zoos, such as the Arabian Oryx (*Oryx leucoryx*), the Przewalski's Horse (*Equus ferus przewalskii*), the Panamanian Golden Frog (*Atelopus zeteki*) and the Golden Lion Tamarin (*Leontopithecus rosalia*) (Taronga Conservation Society Australia, n.d.). *Nordens ark* keeps animals for conservation and educational purposes, not visitors' amusement. Thus, keeping Amur leopards in this zoo does not seem ethically wrong, taking into consideration their population status. However, Bira and Denzel were not allowed to breed as they were not considered a good breeding match. Thus, one could consider that keeping them is not ethical in this case. Maybe *Nordens Ark* should contemplate to change the pairs with other individuals so that they could breed and help so in the conservation of the species. However, this should

be thought thoroughly, since this would mean exposing the animals to a lot of stress, mainly due to transportation and having to get used to a completely new environment.

Ethical issues do not have easy solutions, or not even a right or wrong solution. Nevertheless, it is important to focus on the bigger picture but never forget the individual.

Conclusions

Overall, the changes in the environment seemed to have had a positive effect on both leopards. In the beginning of the study, both animals had poor animal welfare, but it improved over periods. However, they still showed signs of poor welfare in both short and long term, so unfortunately both individuals still did not have an ideal welfare state at the end of the study. Thus, more effort needs to be put to ensure them the best welfare. The fact that these changes had a positive effect show that *Nordens Ark* can improve the welfare of these two individuals. It is vital to continue giving variability to the animals by providing them with both varying enrichment and training, and maintaining, or even increasing, the presence of keepers. Nevertheless, more research is needed to further understand their needs. An important matter to keep in mind is the individual differences that these two leopards showed. There is clear evidence that Bira and Denzel have different personalities and each one has their own patterns, thus what might work for Denzel, might not work for Bira, and *vice versa*.

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