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Original Research Paper

## Behavioral instability in two African wild dogs in Aalborg Zoo

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### Abstract:

This study is based on two African wild dogs (AWD) (*Lycaon pictus*), a male and a female, in Aalborg Zoo, Denmark. The female has never raised any of her litters and all cubs have died soon after birth. Zoo staff hypothesized, that this could be due to several management factors including stress induced from the parking lot/road situated just outside the enclosure and the female's lack of experience with raising cubs. A willow fence was put up to isolate the dogs visually from the outside. The purpose of this study was to analyze if habitat enrichment and olfactory enrichment could affect the behavioral reaction patterns of the AWDs. Individual behavior patterns are an indicator of differences in personality and a measure of welfare. The study was divided into a control period, a habitat enrichment period and an olfactory enrichment period each consisting of 3 days with 8 hours of video recording per day. An ethogram, based on previous observations of the AWD and a pilot study, was composed, and all behaviors observed were categorized. Various analysis and plots showed that, although some differences in specific behaviors and behavioral reaction patterns were present, the AWDs did not have a clear difference in personality. A difference in behavioural instability was found between two dogs when introduced to the enrichments. Due to changes in activity, it was concluded that the male reacted more positively to the habitat enrichment and the female reacted more positively when introduced to the olfactory enrichment. Several other categories of behaviour were observed in this study but, due to a low degree of data saturation, only data for behaviours "locomotion" and "standing still" were considered saturated enough to be used for data analysis.

**Keywords:** *Lycaon pictus*, olfactory enrichment, captivity, zoo, visual isolation, personality, ethology, animal welfare, behavioural reaction patterns, behavioural ecology.

### Introduction

The African wild dog (AWD) (*Lycaon pictus*) is an endangered canid whose population size is continuing to decline (Van der Weyde et al. 2015; IUCN 2020). Historic data indicates that AWDs were formerly distributed throughout sub-Saharan Africa and were probably only absent from lowland rainforest and the driest deserts (IUCN 2020). African wild dogs are social and live as packs ranging from 2-27 adults and yearlings, though packs of 5-15 adults and yearlings are most common (Creel & Creel 2002; Van den Berghe et al. 2019). Within packs there is a dominance hierarchy among males and females. Only the dominant female is assured of breeding, and reproduction is largely monopolized by the dominant male (Creel & Creel 2002; Woodroffe et al. 2009). Lack of success in reproduction can be caused by a lone pair and furthermore can be a sign that conditions in captivity are not optimal (Gusset & Macdonald 2010; Yordy & Mossotti 2016).



Due to this hierarchy, many of the behaviours expressed within a pack of AWDs are dominance or submission related (Chen 2019; Van den Berghe et al. 2019). Dominant females are known to show aggression by forcing subordinates to the ground, biting or growling (Chen 2019). African wild dogs are obligately cooperative breeders where subordinates help rear cubs and an unaided pair virtually never raise offspring to independence in any population (Creel 2005; Van den Berghe et al. 2012). This social way of living implies a close bond between members of a pack, and behaviours such as play and heaping serve to strengthen bonds and help maintain their social structure (Chen 2019). Within larger packs, heaping is not random and arrangements will often occur based on age, gender and rank (De Villiers et al. 2003). AWD hunt small to medium-sized antelopes, but occasionally also prey as small as hares and as large as wildebeest. AWD can maintain 45 km per hour for 5 km, and its home range size is 560-3000 km<sup>2</sup> (Castelló 2018).

When examining animal welfare, zoos often assess both positive indicators as well as the absence of negative indicators. There is no specific definition of welfare, but the five freedoms and the five opportunities are often used as indicators (Maple & Perdue 2013; Miller et al. 2020). One way to improve welfare of animals in captivity is to examine their personality and differences in personality within a pack (Pertoldi et al. 2020a). One aspect of personality can be shown through an individual's behavioural reaction patterns, which is the individual's behaviour in a specific environment (Goursot et al. 2019; Pertoldi et al. 2020a, b). This implies that a difference in behavioural reaction pattern between the two AWDs is a sign of personality (Sih et al. 2015; Roche et al. 2016). Packs with greater diversity in personality, are supposed to have an evolutionary advantage, compared to packs with individuals who are more alike (Pertoldi et al. 2020a). In addition, Behavioural instability which is a proxy of the unpredictability of behaviour can be considered an advantage as it reduces vulnerability to predators that might decode predictable patterns of their prey (Biro & Adriaenssens 2013).

This study is based on two AWDs in Aalborg Zoo (Denmark). Especially the reproduction has been a challenge for the dominant female since she has never been able to care for any of her litters and all her cubs have died soon after birth. This study seeks to investigate animal welfare, by looking at personality amongst AWDs in Aalborg Zoo. Differences in personality can be assessed based on how the AWDs react to changes in their environment. We used enrichments to change the environment. Studies have shown that olfactory enrichment from prey animals can lead to an increase in activity and display a variety of behaviours and potentially reduce stress among AWDs (Rafacz & Santymire 2014). Therefore, this study uses olfactory enrichment via scents from a West African dwarf goat (*Capra aegagrus hircus*). A change to the enclosure was planned during this study and its effect is also assessed as a form of habitat enrichment. On the basis of this, it is expected that the two kinds of enrichments will impact the behavioural reaction patterns and thereby contribute to better welfare amongst AWDs in Aalborg Zoo.

## **Methods**

### ***Subjects***

This study examines two AWDs, born and raised in captivity: A female, Giza (6 years old), and a male, Chobe (11 years old). Since the female has never been able to care for any of her litters, the zookeepers suspect that stress induced by increased visibility of the enclosure from outside the zoo, was part of the cause. Another cause could be she was born and raised in Aalborg Zoo without getting any experience of raising litters before her own were born.

### ***Procedures***

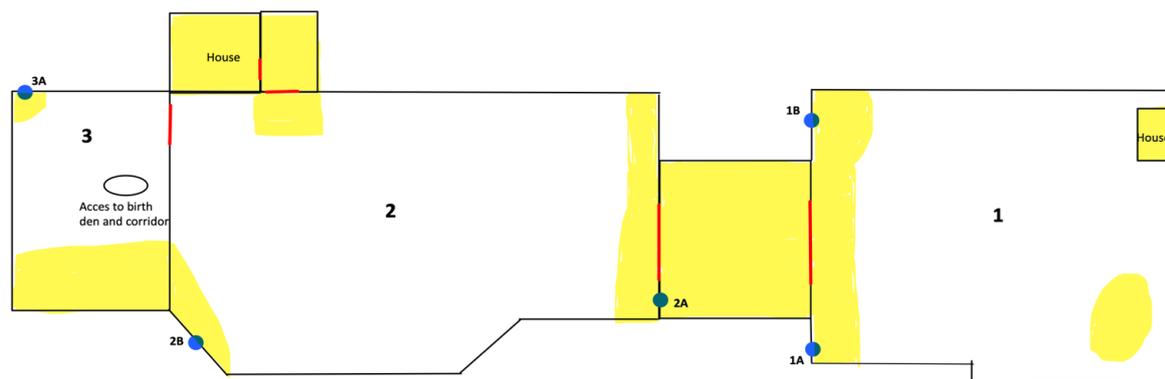
The two AWDs at Aalborg Zoo were observed during three periods: Initially, a control period was recorded. Immediately after the woven willow fence was established, a second period was recorded. A final period was recorded where the AWDs were exposed to olfactory enrichment. These three observation periods were designated "control", "habitat enrichment" and "olfactory enrichment", respectively.

Since AWDs are diurnal (Rasmussen 1999), the animals were recorded from 9 am to 5 pm. Due to daylight saving time, the recording period was changed to 8 am to 4 pm from October 25<sup>th</sup> and onward.

The control period was filmed from October 18<sup>th</sup> until October 20<sup>th</sup> 2020. The observation period with habitat enrichment was filmed from October 31<sup>st</sup> until November 2<sup>nd</sup>. The observation period with olfactory enrichment was filmed on November 5<sup>th</sup>, 7<sup>th</sup> and 9<sup>th</sup>. Each observation period had one day where the AWDs were fed. During the study, it was unknown that Giza was pregnant until she gave birth on November 12<sup>th</sup>.

### ***Recordings: cameras and placement***

For the recording of the AWDs behaviour, five cameras of the type: “Kitvision Venture 4K Action Camera”, were used (see Figure 1). Placement of the cameras was determined in order to limit the number of blind spots. Therefore, the cameras covered most of the outdoor area, but due to trees and other visual obstructions, it wasn't possible to eliminate all of the blind spots (approximately 10% of the area). The house which the AWDs had access to from enclosure 2 along with the area between enclosures 1 and 2 were major blind spots. Aalborg Zoo granted access to recordings from the birth den and corridor, which are underground areas, that the AWDs can access from enclosure 3.



**Figure 1:** Sketch of the entire outdoors enclosure of the AWDs in Aalborg Zoo. The enclosure is split into three parts designated: 1,2 and 3. Passageways between enclosures are marked with red. Cameras are marked with blue dots and designated: 1A, 1B, 2A, 2B and 3A. Blind spots are marked with yellow (approximately 10% of the area). The oval marks the access to the underground area designated: birth den and corridor.

### ***Enrichments***

Two types of enrichments were used in this study. A habitat enrichment, consisting of the woven willow fence, which was put up by Aalborg Zoo. The second type was olfactory enrichment consisting of faeces and hide from West African dwarf goats. Faeces (approximately 200 grams) was spread in enclosure 1 and hide was rubbed on trees, stumps and rocks in the same enclosure. The AWD were exposed to these olfactory enrichments every other day, alternating between faeces and hide.

### ***Ethogram***

An ethogram (Table 1) was developed and used to categorize the AWDs' individual behaviour. The ethogram was customized to the AWD couple and the relationship between the two individuals. To further customize the ethogram to the examined individuals, a pilot study was conducted, where the AWDs' behaviour, over the course of one day, was noted. This method should ensure that any other behaviour wasn't missing, as well as which behaviors in the ethogram needed further specification. In addition to the species-specific behaviours that were already a part of the ethogram, the category “standing still” was added after the pilot study.

To account for the blind spots, data entries for "locomotion" were merged if an individual was observed immediately entering/leaving one camera's point of view to another. Furthermore, if an individual was out of sight for less than 10 seconds, returning, the entry was continued.

**Table 1:** Ethogram of the AWD's behaviours with indicators for each behaviour.

<u>Dominant and Submissive interaction</u>	
Dominance aggression	<b>Indicators</b>
	Ears flat (Chen 2019)
	Body low to ground (Chen 2019)
	Biting (Chen 2019)
	Food acquisition (One dog takes food from another dog) (Chen 2019)
Two or more indicators must be present at the same time in order to categorize behaviour as dominance aggression.	
Submission	<b>Indicators</b>
	Lowered head (Rafacz & Santymire 2014)
	Nose to ground (Rafacz & Santymire 2014)
	Rolling on to side (Rafacz & Santymire 2014)
	Two or more indicators must be present at the same time in order to categorize behaviour as submission. Often shown in cohesion with dominant behaviour in alpha female.
<u>Play</u>	
Play	<b>Indicators</b>
	Bow: A dog would stretch out its front legs while lowering its chest and keeping its rear end raised (Chen 2019)
	Parallel running: Individuals run flank to flank with each other (Chen 2019)
	Wrestle: Standing on hind legs, front legs on other dog, usually silent and with open mouth (Chen 2019)
<u>Resting</u>	
Heaping	<b>Indicators</b>
	Two or more dogs lie together in proximity while resting (Chen 2019) Note: if within 1 meter of each other, note as heaping; if more than 1 m, categorize as resting alone
Resting alone	<b>Indicators</b> Body in contact with the ground (Rafacz & Santymire 2014)
Standing still	<b>Indicators</b> Noted if individual is standing still for 10 seconds or more, this can be interrupted by brief locomotor activity (3 seconds or less).
<u>Active behaviour</u>	
Locomotion	<b>Indicators</b>
	Walk forward (Rafacz & Santymire 2014) Trot forward (Rafacz & Santymire 2014)
Pace	<b>Indicators</b> Walking back and forth over the same, small area (Rafacz & Santymire 2014)
Dig	<b>Indicators</b> Scratching ground with one or both front paws to make a depression (Rafacz & Santymire 2014)
<u>Miscellaneous</u>	
Defense mode	<b>Indicators</b>
	Ears forward (Chen 2019)
	Hackles high (Chen 2019)
	All dogs united (Chen 2019) Two or more indicators must be present at the same time in order to categorize behaviour as Defense Mode.
Engaging with enrichment	<b>Indicators</b> Engaging with goat-scent (Sniffing)
Other/individual unrecognized	<b>Indicators</b> Any behaviour not otherwise categorized.

### ***Reliability analysis***

The means of all the cumulated data entries were plotted in a graph to see whether the relevant data sets were saturated enough, meaning that no major changes to the mean of the data entry length occurred, when introducing more corresponding data (Saunters et al. 2018). The behaviours: dominance aggression, submission, dig, pacing and defense mode were omitted for further analysis as there were none or an insufficient number of entries.

Histograms were constructed in order to determine if data was normally distributed. Data entries three seconds or shorter were removed from the “locomotion” data sets, as this was considered transitional behaviour, and were therefore misleading for the data analysis. For “locomotion” and “standing still”, standard intervals are 5 seconds long, and a category consisting of all entries 100 seconds or longer is defined as outliers (Appendix A, Appendix B). For “resting alone” and “heaping”, the standard interval is 40 seconds. A category was included to account for outliers here as well, defined as all data entries lasting 700 seconds or longer (Appendix A, Appendix B).

### ***Behaviour analysis***

When attempting to determine the personality of the AWDs, different plots and tests were utilized. Firstly, percentile plots were constructed for each individual’s behaviour to decide how these were expressed throughout a day.

Line plots for the median of data entry length, standard deviation, kurtosis and skewness for “locomotion” and “standing still” were made for the male and female for control period to habitat enrichment period and from habitat enrichment period to olfactory enrichment period. To determine if the enrichments had an impact on the behavioural reaction patterns, differences in slope coefficients for the male and female were examined using Chi-squared tests, to determine if the differences were significant ( $P < 0.05$ ).

## **Results**

### ***Histograms***

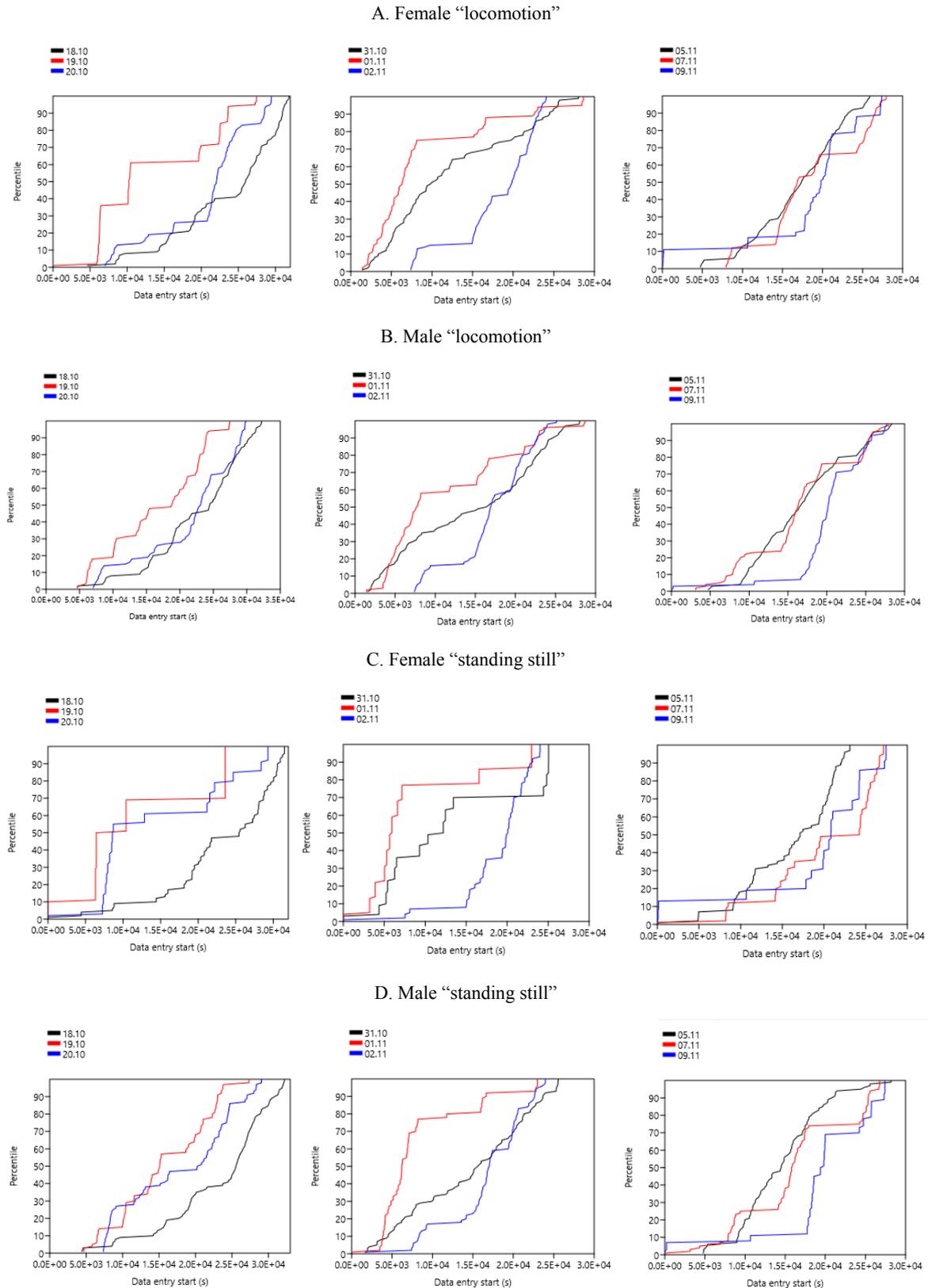
For each period, there has been plotted histograms for four categories: “locomotion”, “standing still”, “resting alone” and “heaping” (Appendix A, Appendix B). Plotted histograms were not normally distributed, and all skewed to the right in different degrees. Due to lack of normal distribution in all histograms, it was decided to use medians instead of means when plotting for slope coefficients (Gottschalk et al. 2020). As the central limit theorem (Upton & Cook 2014) states, data sets with a large sample size (30+) are also approximately normally distributed, this skewness can partially be attributed to fragmentation of data entries.

### ***Cumulated means of data entries***

The means of the cumulated data entry lengths of all behaviours, for both individuals, were plotted. Common behaviour such as “locomotion” and “standing still” showed clear saturation in the graphs (Appendix C, Appendix D). The rest of the categories all showed lack of data saturation (Appendix C, Appendix D).

### ***Percentile plots***

Percentile plots were constructed for every day for “locomotion” and “standing still” and was done for both individuals (Figure 2 A-D). The lower number of data entries in some data sets lead to some of the plots’ lines developing less smoothly. There can be seen an apparent correlation in only a few plots (etc. female “locomotion” olfactory enrichment period; male “locomotion” control period).



**Figure 2 (A-D):** Percentile plots of male and female “locomotion” and “standing still” throughout the day. The x-axis represents when a data entry has begun in seconds, where 0 is 8 am. The y-axis represents the percentile of the amount of data entries. The x-axis varies with 3,600 seconds (one hour) in plots from October 31<sup>st</sup> to November 09<sup>th</sup>, due to daylight saving time.

### Slope coefficients

It was determined whether the slope coefficient of median, standard deviation, kurtosis and skewness was significantly different when comparing the two individuals (Table 2). The slope coefficient of the median for “locomotion” from the habitat enrichment period to the olfactory enrichment period was significantly different ( $P=0.02335$ , Slope coefficient-male=2, Slope coefficient-female=24.5). The standard deviation for “locomotion” was also significantly different, both from the control period to the habitat enrichment period ( $P=0.0093$ , Slope coefficient-male=6.44, Slope coefficient-female=-8.12) and from the habitat enrichment period to the olfactory enrichment period ( $P=0.00001$ , Slope coefficient-male=-25.91, Slope coefficient-female=35.83). For “standing still” there was a significant difference ( $P=0.00001$ , Slope coefficient-male=-25.91, Slope coefficient-female=35.83) between the two individuals in kurtosis from control period to habitat enrichment period ( $P=0.000013$ , Slope coefficient-male=-23.66, Slope coefficient-female=15.73) and from habitat enrichment period to olfactory enrichment period ( $P=0.00167$ , Slope coefficient-male=3.8, Slope coefficient-female=-17.11).

**Table 2:** Slope coefficients for the line plots for median, standard deviation kurtosis and skewness for “locomotion” and “standing still” from control to habitat enrichment period and from habitat enrichment to olfactory enrichment period for the male and female. Significant difference between the male and female slope coefficient was calculated using Chi-squared test. An “X” in the category “P-value<0.05” indicates a significant difference.

Control to habitat enrichment (locomotion)					
	Slope coefficient male	Slope coefficient female	Expected value	P-value	P-value> 0.05
Median	2.50	3.00	2.75	0.899	X
Std. deviation	6.44	-8.12	-0.84	0.0093	
Kurtosis	-32.89	-23.70	-28.29	0.8159	X
Skewness	-2.22	-2.71	-2.46	0.8879	X
Habitat enrichment to olfactory enrichment (locomotion)					
	Slope coefficient male	Slope coefficient female	Expected value	P-value	P-value> 0.05
Median	2.00	24.50	13.25	0.02335	
Std. deviation	-25.91	35.83	4.96	0.00001	
Kurtosis	11.52	1.73	6.62	0.12194	X
Skewness	0.98	0.20	0.59	0.2225	X
Control to habitat enrichment (Standing still)					
	Slope coefficient male	Slope coefficient female	Expected value	P-value	P-value> 0.05
Median	2.00	-1.5	0.25	0.2627	X
Std. deviation	5.85	-1.30	2.28	0.0778	X
Kurtosis	-23.66	15.73	-3.97	0.000013	
Skewness	-2.12	1.78	-0.17	0.2318	X
Habitat enrichment to olfactory enrichment (Standing still)					
	Slope coefficient male	Slope coefficient female	Expected value	P-value	P-value> 0.05
Median	-7.50	-1.00	-4.25	0.09476	X
Std. deviation	-12.12	-4.91	-8.51	0.5090	X
Kurtosis	3.80	-17.11	-6.65	0.00167	
Skewness	0.63	-2.14	-0.75	0.3151	X

## Discussion

### Discussion of data reliability

After observing the individuals, it was noticed that for categories “resting alone” and “heaping”, there could be differentiated between sleep-related behaviour (all data entries 700 seconds or longer) and rest-related behaviour (all data entries shorter than 700 seconds). This leads to a misrepresentation in these two categories but during data analysis, the histograms were modified with the previously mentioned differentiation to reduce the effects this had on the histograms. To sum up, the categories

“locomotion” and “standing still” are representative as data has been sufficiently saturated (Saunters et al. 2018). More data entries would not affect the means of the cumulated data entries in a major way. However, for “resting alone” and “heaping”, satisfactory data (Saunters et al. 2018) saturation was rarely achieved. Due to this, more entries would likely alter the means of the cumulated data entries along with other statistical values (e.g., median, kurtosis, std. deviation).

### ***Comparing the predictability in personalities***

Due to the significant difference in standard deviation for “locomotion” (Table 2), it can be determined that the male's expression of this behaviour became more unstable (unpredictable), compared to the female, after the transition to the habitat enrichment period. The female, however, displayed more behavioural instability in "locomotion" after the transition to the olfactory enrichment period. Furthermore, an increase in activity for “locomotion”, can be determined for the female from the habitat enrichment period to the olfactory enrichment period due to difference in the slope coefficient for the median in this period. It indicates that the male has responded more positively on the habitat enrichment while the female has responded more positively on the olfactory enrichment. This difference could be because the female is born in the enclosure thus used to it, while the male came from a much larger British enclosure with more hiding areas. In a study from 2014, faeces from AWDs' natural prey was used as enrichment to great success, in order to raise “locomotion” levels. In addition to this, it was found that this type of enrichment also increased the frequency of positive social behaviour in packs, more specifically submission and dominance related behaviours (Rafacz & Santymire 2014).

The female displayed a higher level of behavioural instability for “standing still” from the control period to the habitat enrichment period, compared to the male (Table 2). However, the male's expression of "standing still" was determined to be behavioural instable from the habitat enrichment period to the olfactory enrichment period (Table 2). Whether the increase in behavioural instability for “standing still” is an indicator of successful enrichment is uncertain, as the behaviour not only includes an inactive form of resting while standing, but also an observant response to the AWDs' immediate surroundings.

When comparing the significant difference of the two behaviours, the individuals show unpredictability in both enrichment types but during different periods. Rafacz et al. (2016) suggest a possible link between individual responses to enrichment and either social structure or individual variance. As mentioned earlier, behavioural instability in animal behaviour is an advantage in the wild and might relate to the success of reintroduction of possible future generations of AWDs (Biro & Adriaenssens 2013).

### ***Behavioural tendencies***

A significant difference in the personality between the AWDs cannot be assumed due to only 5 of the 16 analysis of the slope coefficients showing a significant difference (Table 2). There still were differences in the reaction patterns as previously mentioned. The lack of significant differences can be attributed to the fact that a behaviour, like “locomotion”, often was executed simultaneously, as the AWDs spent much of the time in close proximity. It was often observed that if one individual would begin a certain behaviour and the other AWD would immediately mirror this. An extension of this can be seen in the percentile plots. All plots with a larger number of data entries, more closely aligned with each other (Figure 2). This supports the previous claims about a lack in difference of personality for the individuals.

It was also observed that some behaviours were more prone to be displayed by one specific individual and not the other, and others were completely exclusive. For example, the female AWD was the only to display “pacing”, and, with her being the dominant female, “dominance aggression” was also exclusive to her, while “submission” was exclusive to the male. However, the behaviours were not displayed in a large enough quantity for data saturation to be satisfactory (Saunters et al. 2018). If the observational periods were extended by a significant amount, data saturation would improve and therefore any tendencies not visible before due to lack of data, would be more apparent.

### *Assessing welfare among the AWDs*

When attempting to evaluate welfare among the two AWDs, the freedom/opportunity to express species-specific behaviour is particularly noticeable (Miller et al. 2020). With the AWDs primarily expressing “locomotion” and “standing still”, the variation in behavioural pattern is low and thus indicating lack of welfare (Maple & Perdue 2013). In addition, the lack of success in reproduction can be a sign of the individual not being able to express species-specific behaviour, which can indicate lack of animal welfare (Gusset & Macdonald 2010; Yordy & Mossotti 2016). When looking at personality, there are signs of difference in personality between individuals based on significant difference in behavioural reaction patterns, which can indicate animal welfare (Allard et al. 2019).

### **Conclusion**

This study sought to examine personality among two AWDs in Aalborg Zoo. Signs of personality were found, but exclusively for “locomotion” and “standing still”, data was sufficiently saturated. Regarding unpredictability in personality, both types of enrichment implied more behavioural instability in “locomotion and “standing still” for both individuals. The male responded more positively on the habitat enrichment whereas the female responded more positively on the olfactory enrichment. Signs of welfare in the form of indications of personality are present, but indications of lacking welfare occur as well. The small pack and very little space in the enclosure compared to that of wild living AWDs may induce stress with the consequence of reduced behavioural expression and measurable personality.

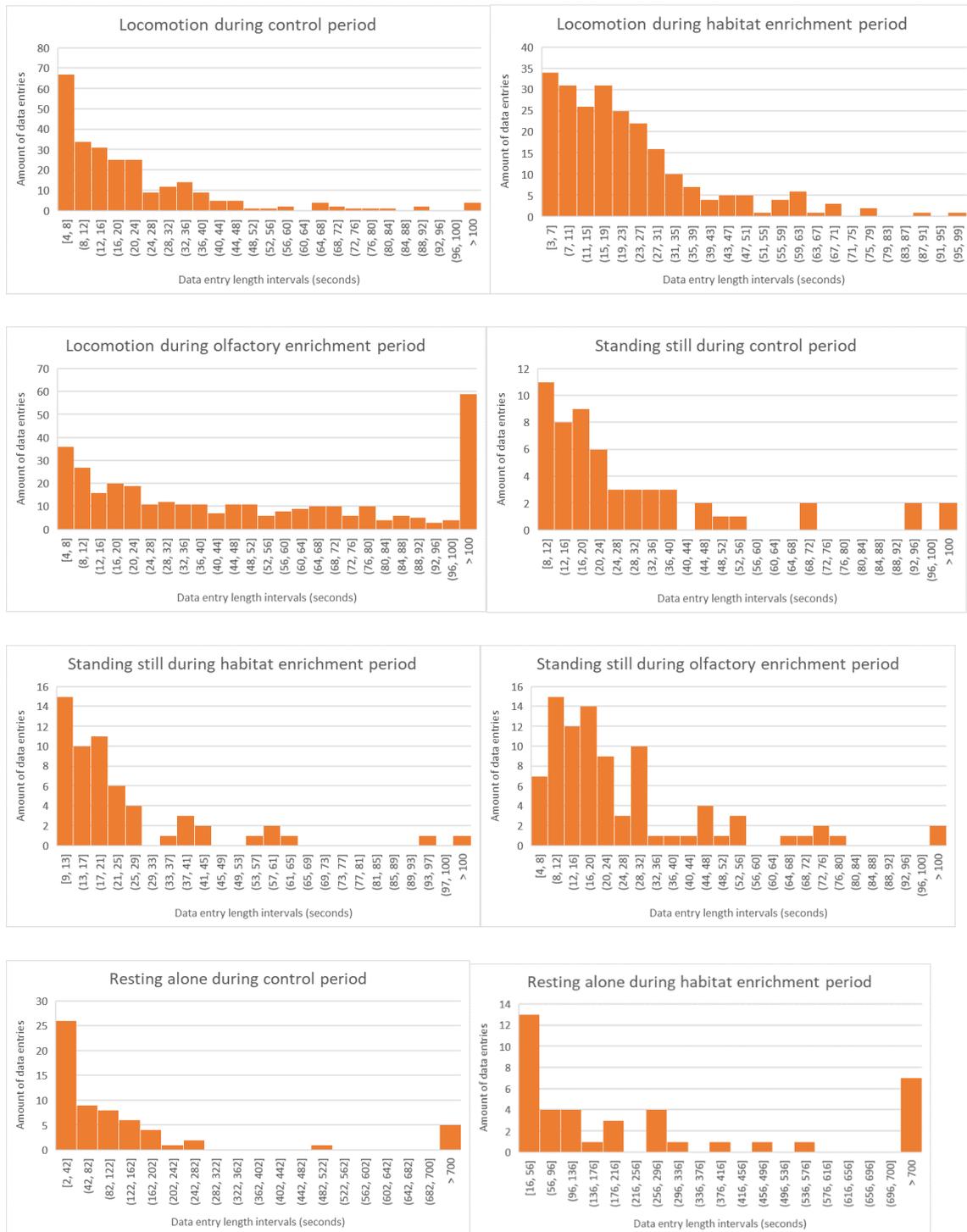
The use of the behavioural instability concept has been shown to be quite useful for behavioural studies and can give indications of the degree of animal welfare. Most importantly the measurements of behavioural instability can be compared across zoological gardens and will allow a quantitative comparisons (Linder et al. 2020, Pertoldi et al. 2020a,b).

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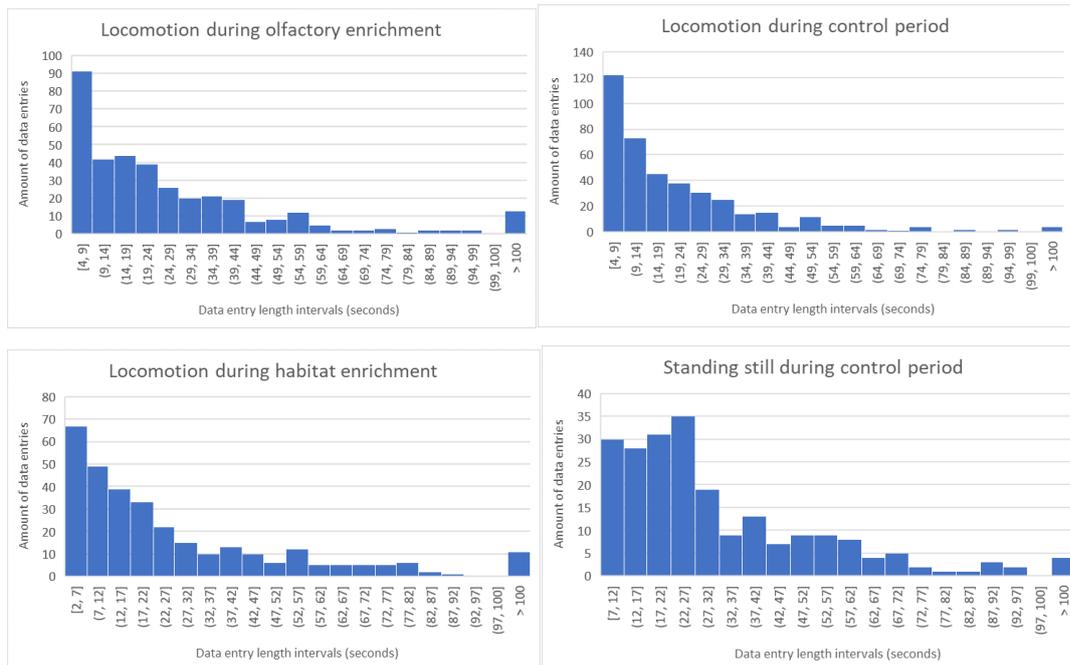
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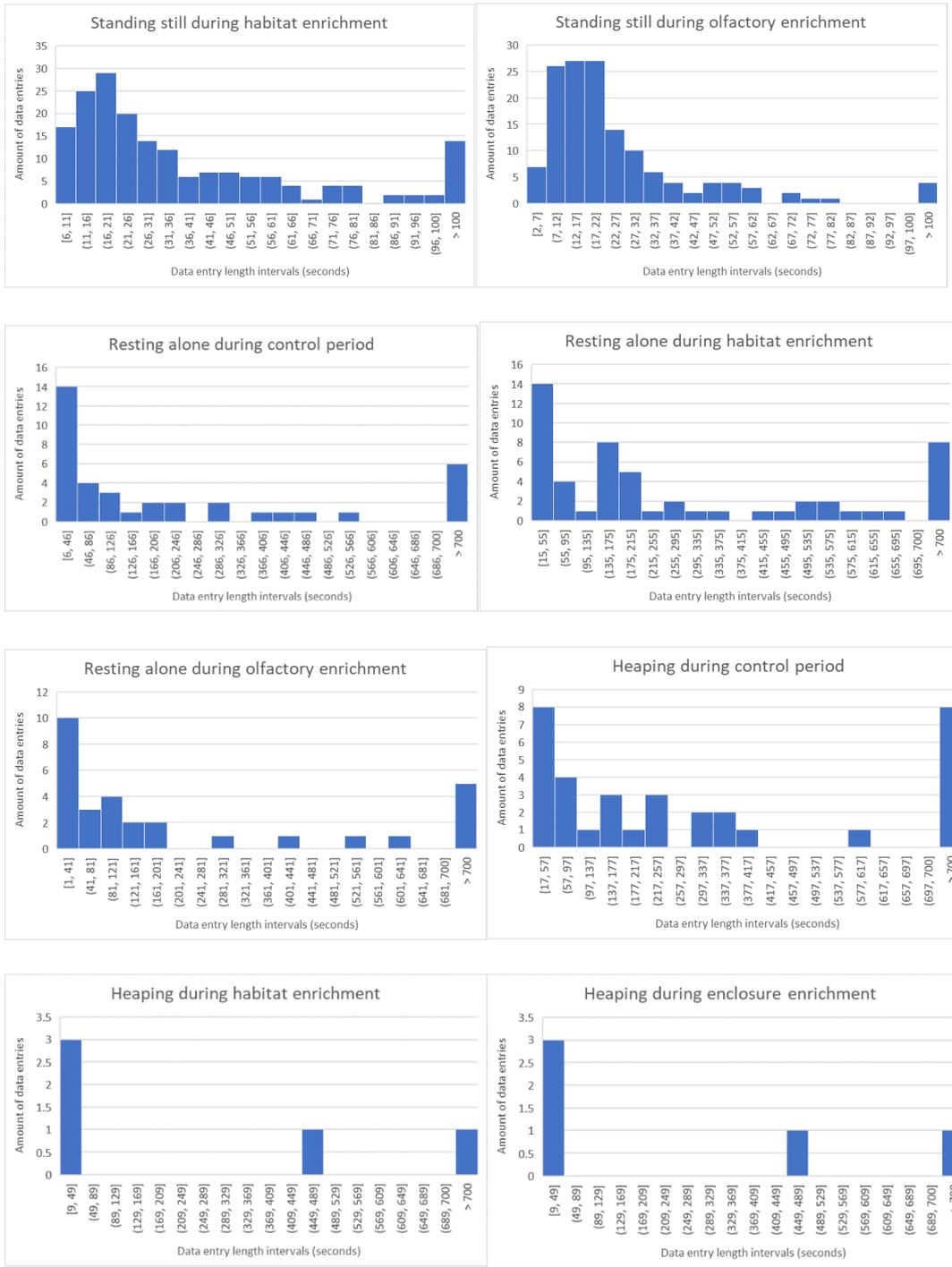
**Appendix A: Histograms female**





**Appendix B: Histograms male**





**Appendix C: Cumulated graphs female**



**Appendix D: Cumulated graphs male**

