The welfare of ostriches, emus, rheas and marabou storks in mixed-species enclosures in zoos in the Netherlands

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A behavioural study of ostriches, emus, rheas and marabou storks in zoos in the Netherlands to get a better insight in their welfare in mixed-species enclosures

Keywords: behaviour, emu, feeding, health, housing, marabou stork, mixed-species enclosure, ostrich, rhea, welfare

RESEARCH REPORT

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Preface

The knowledge of the welfare of zoo animals has advanced greatly in the last years thanks to the study of their needs and how they can be compromised in captivity. More and more researches have been addressed to increase the welfare of animals in captivity. We are proud and honoured that we were given the opportunity to conduct such a research to indicate the welfare of ostriches, emus, rheas and marabou storks in zoos in the Netherlands.

During our bachelor education Wildlife Management, this research was conducted for our final thesis. Hereby we would like to thank the four anonymous zoos in the Netherlands for giving us the trust and time to conduct this research. Our special thanks goes to Amy Plowman, director of the Field Conservation and Research Department of the Whitley Wildlife Conservation Trust, for giving us the opportunity to do this research; and to our supervisors Tine Griede and Marcella Dobbelaar of The University of Applied Sciences Van Hall Larenstein, for coaching us during this project.

Leeuwarden, July 2014

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Summary

Mixed-species enclosures are becoming an increasingly popular form of housing species in zoological parks. The ratites ostrich, emu and rhea are an example of species being kept in mixed-species enclosures, most often with species such as giraffes, zebras, antelopes species or other bird species such as marabou stork. Although mixed-species enclosures provide a source of enrichment for all species involved, large ratites have been kept and bred with a varying degree of success in mixed-species enclosures. No European guidelines for the housing of ratites and storks in mixed-species enclosures have been established yet and this absence of guidelines results in different forms of housing and husbandry. The EAZA Ratite TAG and EAZA Ciconiiformes and Phoenicopteriformes TAG are concerned about the welfare of ostriches, emus, rheas and marabou storks in such mixed-species enclosures. Therefore, the Field Conservation and Research Department of the Whitley Wildlife Conservation Trust in Paignton, United Kingdom, commissioned a project. The project consists of at least three researches in three European countries. The aim of this research is to have a better insight of the welfare of ostriches, emus, rheas and marabou storks in mixed-species enclosures in the Netherlands.

To investigate the welfare of these four species in zoos in the Netherlands, behavioural observations were conducted in seven mixed-species enclosures in four zoos. The research focused on behaviour, behaviour in relation to feeding, behaviour in relation to housing and behaviour in relation to health. For the behavioural observations the methods instantaneous scan sampling, behavioural sampling and ‘nearest neighbour distance sampling’ were used. All recorded data were entered into IBM SPSS Statistics 21.

Data were analysed using a descriptive test, the chi-square test and the Spread of Participation Index (SPI). Results showed that just a few different types of behaviour occurred during the scan samples. Most interactions with both conspecifics and other species were neutral, followed by aggressive and friendly interactions. With regard to nearest neighbour distance, it turned out that conspecifics spent more time close to each other than to other species. Only in one enclosure simultaneous feeding occurred, in all other enclosures the bird species get fed in their own enclosure (inside enclosure), separated from the other species to prevent mutual competition. Bird species housed in a mixed-species enclosure with only one other species made the most use of the entire enclosure compared to bird species that were housed in a mixed-species enclosure with more than one other species. In several enclosures the birds were sexual active, although no breeding behaviour occurred during the observations. All individuals were free from injuries and diseases.

It cannot be concluded whether mixed-species enclosures in general are positive or negative for ratite or stork welfare. The welfare seems to depend on individuals, species and enclosure design.

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1 Introduction

Currently, mixed-species or multi-species enclosures are becoming an increasingly popular form of housing species in zoological parks (Chaiwan and Plowman, 2013). According to Hosey *et al* (2009) the benefit of creating mixed-species enclosures in zoos are considered to include the provision of: dynamic social stimulation; a more efficient use of enclosure space as different species are able to maximize the use; a higher-quality educational experience for visitors (Hosey *et al*., 2009). More often primates, ungulates and birds are involved in these associations (Chaiwan and Plowman, 2013). Ratites, also known as flightless birds (Williams, 2013), are an example of bird species being kept in mixed-species enclosures (Thomas and Maruska, 1996). These ratites are the ostrich (*Struthio camelus*) (Thomas and Maruska, 1996), the largest of living birds with a height between 1.75 and 2.75 meter (Williams, 2013); the rhea (*Rhea americana*) (Thomas and Maruska, 1996) which is the largest bird on the American continent and looks quite similar to the ostrich, although it is much smaller with a maximum height of 1.5 meter (Carro *et al*., 2011); and the emu (*Dromaius novaehollandiae*) (Thomas and Maruska, 1996), which is the second largest living bird of the world (Patodkar *et al*., 2009) as well as being the largest bird originating from Australia with a height between 1.5 and 2 meter (Williams, 2013). These species are being kept in enclosures with mammals such as giraffes (*Giraffa camelopardalis*), zebras (*Equus burchellii*), rhinoceros (*Rhinocerotidae*) and antelopes species (Thomas and Maruska, 1996). Sometimes they are also housed with other bird species such as the marabou stork (*Leptoptilos crumeniferus)* (Thomas and Maruska, 1996), which is a wading bird and one of the largest of the storks with a height of approximately 1.5 meter (Kahl, 1996).

Although mixed-species enclosures provide a source of enrichment for all species involved (Chaiwan and Plowman, 2013), Rees (2011) illustrated that large ratites have been kept and bred with a varying degree of success in mixed-species enclosures. Potential health problems are associated with mixed-species enclosures and include injuries as a result of interspecific aggression, transmission of disease between species and nutritional problems (Chaiwan and Plowman, 2013). Due to these occurring welfare problems, the EAZA Ratite TAG, established in 2008 (EAZA TAG, 2011), is concerned with the welfare of ostriches, emus and rheas in mixed-species enclosures, in collaboration with the EAZA Ciconiiformes and Phoenicopteriformes TAG, who is concerned about the welfare of marabou storks in these mixed-species enclosures. No European guidelines for the housing of ratites in mixed-species enclosures have been established yet and the absence of guidelines results in different forms of housing and husbandry (Gregson, pers. comm., 2014). As an EAZA TAG focuses on a specific group of animals and is responsible for their husbandry and management guidelines (EAZA, 2014), the EAZA Ratite TAG therefore requires a better insight of the welfare of ostriches, emus, rheas and marabou storks in mixed-species enclosures.

The Field Conservation and Research Department of the Whitley Wildlife Conservation Trust (WWCT) (Paignton, United Kingdom), therefore carried out this project that looks at behaviour, interaction (the way in which animals act to, react on and influence each other) and spacing use of the ratites and marabou stork housed in mixed-species enclosures in various zoos, to investigate possible welfare effects of mixed species and thus to determine whether mixed-species enclosures are beneficial for

the bird species. This project needs to provide data whereon European husbandry and management guidelines can be based.

The project consists of similar researches in at least three different European countries. The first study was carried out in zoos in the United Kingdom (UK) in 2013 by Chaiwan and Plowman. The study in the UK used instantaneous scan sampling and behavioural sampling to record behaviour, especially the interactions of the species, the nearest neighbour distance (NND) and the enclosure use. This research, which will be the second study, will be conducted in four zoos in the Netherlands and will be additional to the study in the UK. Therefore the same methods of research will be used. After this research, the Field Conservation and Research Department of WWCT plans to carry out a third research in a third European country to add extra information.

All these studies need to investigate the animal welfare of the bird species ostrich, emu, rhea and marabou stork in mixed-species enclosures. Principles to evaluate animal welfare, according to the Welfare Quality Project, are (1) behaviour, (2) feeding, (3) housing and (4) health (Blokhuis, 2008).

(1) As this research needs to be additional and/or comparable to the study in the UK, the same welfare indicators for behaviour will be used, concluded into different categories: activity related behaviours (i.e. walking, running, preening and waltzing), social behaviours (i.e. vocalization, threatening and submission), sexual behaviour (i.e. clucking, kanteling and copulation), abnormal behaviour (i.e. pacing, feather-picking and aggression) and interaction (friendly, neutral and aggressive). (Chaiwan and Plowman, 2013)

    These behaviour types are investigated as possible welfare indicators, as social interactions or as general activities for these species (Plowman, pers. comm., 2014).

    Behaviour is the main topic to investigate the welfare in this study, with frequency variation being relevant.

(2) To ensure good feeding in mixed-species enclosures it is important to see whether there is simultaneous feeding (feeding of more than one species at the same time) and how this influences the interaction with conspecifics and other species. Simultaneous feeding is common when animals are kept together. Dominance hierarchies can create differential access to supplementary feed and the limited space in which food is put out can increase the tendency. (Avent, 2008)

(3) According to the Welfare Quality Project, good housing means comfort around resting, thermal comfort and ease of movement (Blokhuis, 2008). It is important so see whether the bird species (are able to) make use of the entire enclosure that is provided (Chaiwan and Plowman, 2013).

(4) Good health means the absence of injuries and diseases (Blokhuis, 2008). As this study will focus on behaviour, health is disregarded unless certain injuries or diseases clearly have a relationship with the frequency of behaviours or occurrence of interactions. Next to this, to investigate the welfare of the bird species, the life history and events of the individual birds in the enclosures could have a relation with occurrence of interactions (Dawkins, 1990).

Aim of research

To have a better insight of the welfare of ostriches, emus, rheas and marabou storks in mixed-species enclosures in zoos in the Netherlands.

Main research question

How is the welfare of ostriches, emus, rheas and marabou storks in mixed-species enclosures in zoos in the Netherlands?

Sub research questions

Behaviour

1. What is the frequency of types of behaviour shown in the ostrich, emu, rhea and marabou stork in the enclosure?
2. What are the percentages of friendly, neutral and aggressive interactions of the individual ostrich, emu, rhea and marabou stork with conspecifics?
3. What are the percentages of friendly, neutral and aggressive interactions of the individual ostrich, emu, rhea and marabou stork with each of the other species?
4. What are the percentages of the different nearest neighbour distances between the individual ostrich, emu, rhea and marabou stork and each of the other species?

Feeding

5. How does feeding in the enclosure influence the interaction between the individual ostrich, emu, rhea and marabou stork and each of the other species?

Housing

6. How do the individual ostrich, emu, rhea and marabou stork and the other species in the enclosure make use of the enclosure?

Health

7. What is the relationship between the individual life history and events of each ostrich, emu, rhea and marabou stork and the frequency of friendly, neutral and aggressive interactions with each of the other species?

2 Methods and materials

2.1 Research design

The research was a qualitative research where data were collected, analysed and interpreted by observing the research population (Anderson, 2006). This qualitative research was designed to gather information about the welfare of the ostrich, emu, rhea and marabou stork in mixed-species enclosures in zoos in the Netherlands. As this research will be additional to the similar previous research in the UK, and future research will be conducted to add information to the project, the same methods of behavioural research were necessary to complete a research based on more data. Figure 1 displays the research design of the qualitative research.

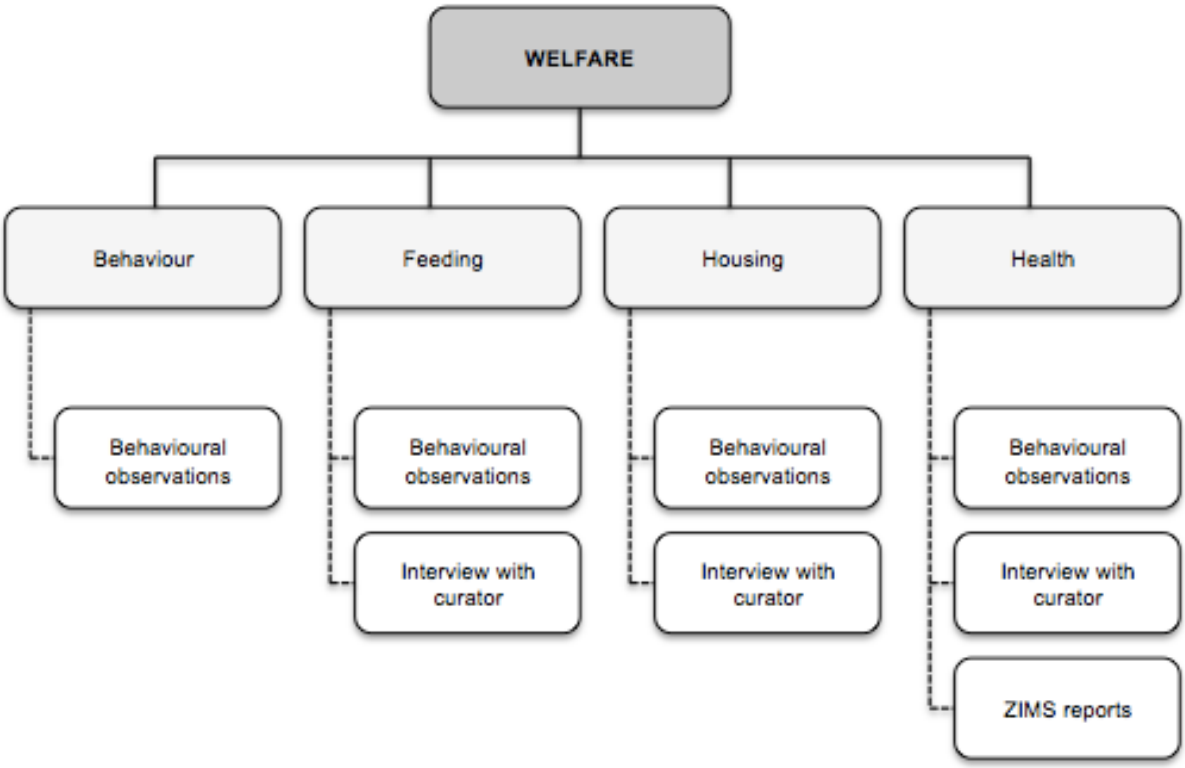

Figure 1: Research design

To assess the welfare of the ostrich, emu, rhea and marabou stork in the Netherlands, the research looked into behaviour, behaviour in relation to feeding, behaviour in relation to housing and behaviour in relation to health.

Behaviour was investigated by behavioural observations: instantaneous scan sampling and behavioural sampling (2.3 Data collection and materials). Also behaviour in relation to feeding, housing and health was investigated through these behavioural observations. In addition to that the ZIMS reports of the animals were studied and a small interview was held with the curator or keeper.

2.2 Research population and study site

The research on ostrich, emu, rhea and marabou stork was carried out in seven enclosures in four zoological parks in the Netherlands (Table 1).

Table 1: The research population

Enclosure	Species	M.F.U	Mixed-species (number)
Enclosure 1	Ostrich	1.2.0	Giraffe (7), Zebra (6), Rhinoceros (4), Waterbuck (3), Wildebeest (3), Red river hog (2), Crowned crane (4), Guineafowl (9)
Enclosure 2	Emu	1.1.0	Kangaroo (17)
Enclosure 3	Ostrich	1.1.0	Marabou stork (4), Crowned crane (2)
	Marabou stork	2.2.0	Ostrich (2), Crowned crane (2)
Enclosure 4	Marabou stork	4.4.0	Rüppels vulture (2), White-headed vulture (2), White-backed vulture (2), Hooded vulture (3), Crowned crane (2), Hamerkop stork (2), Black kite (2)
Enclosure 5	Rhea	1.1.0	Vicuña (3)
Enclosure 6	Ostrich	0.2.0	Giraffe (8), Zebra (8), Guineafowl (20)
Enclosure 7	Rhea	1.1.0	Wallaby (3)

2.3 Data collection and materials

Data were collected five hours a day (depending on zoo opening times from 09.00h or 10.00h) and two days per enclosure, equal to the study in the UK. Figure 2 gives a schedule of the observations.

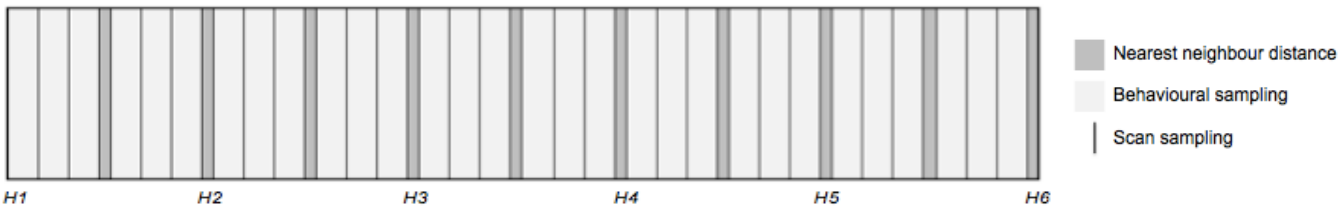

Figure 2: Planning for behavioural observations. Each hour (H) was divided six times, ten minutes each. Every ten minutes scan sampling was done. Every half hour nearest neighbour distance was measured. In between, behavioural sampling was done when interactions occurred.

A preliminary observation was conducted in enclosure 1 and enclosure 2. It was found that the two observers were observing equally, and because there were two observers it was possible to do scan sampling and behavioural sampling, or scan sampling and nearest neighbour distance at the same time (Figure 2).

2.3.1 Data collection of behaviour

To gather information about behaviour; frequency of types of behaviour, interactions and the nearest neighbour distance, different types of sampling were used. During the observations the observers were standing outside, in the visitor area of the enclosure, to minimise disturbance.

Frequency of types of behaviour

Instantaneous scan sampling means the whole group of subjects were scanned rapidly at regular intervals (Bateson and Martin, 2007) of ten minutes and the behaviour of each individual/species at that instant was recorded in approximately one minute (Figure 2). Scan sampling included potential 27 types of behaviour; additional to these types of behaviour 'out of sight' was included (Table 2). Appendix I gives the data collection sheet that was used for scan sampling.

Table 2: Ethogram for ostrich, emu, rhea and marabou stork.

Behaviour type	Abbreviation	Explanation
Activity related behaviours		
Walking	W	Slowly/quietly moving forward
Running	R	Fast moving forward
Standing/sitting	Ss	Standing or sitting on one place, no movement
Feeding/drinking	Fd	As stated
Preening	Pr	Side of the bill repeatedly rubbed over body feathers
Waltzing	Wa	Twirl around. Sick birds rarely twirl
Sleeping/resting	Sr	Standing or lying down
Thermoregulatory	Th	Bird will open-mouth breath and open their wings or bend down covering their legs with wings
Trembling	Tr	Resembles shivering
Body shaking	Bs	Shaking the body for short moment
Leg stretching	Ls	Stretch the legs
Rolling head rubs	Rh	Rubbing the head and bill at the base of the tail
Scratching	Sc	One foot raised without the wing lifting and scratching with toe while the head is lowered
Anxiety stretch	As	Wings fully spread and body erect
Social behaviour		
Threat	T	Typical threatening posture: stand tall with tail erect, hissing, wings slightly open, feathers puffed up (around neck)
Vocalization	V	Distress calls and booming, similar to that of a pigeon. Produced by mature males to establish territorial boundaries
Submission	S	Run away from an aggressive animal or drop to the ground without defending
Forward clattering threat	Fct	Fast bill clattering, whilst titling body up and down
Sexual behaviour		
Clucking and fluttering	Cf	Rapidly opening and closing her beak, flutter wings by dropping them low and forward, vibrating in sequence

Kanteling	K	Bird drops to his hocks, and fans both wings forward and backward while hitting his head on each side of his spine
Copulation	Co	Takes 30 to 60 seconds
Abnormal behaviour		
Pacing	Pa	Walking back and forth on the same area
Feather picking	Fp	Aggressively peck feathers from the back/tail of penmates
Toe and face pecking	Tfp	Excessive toe and face pecking
Behavioural stargazing	Bs	Continually lift head up and back to the extent that is eventually touches his spine
Pica	Pi	Ingestion of faeces
Aggression	Agg	Intensity pursue and attempt to kick another animal
Extra		
Out of sight	Oos	Not in sight during the scan sample so type of behaviour is unknown

Interaction

Behavioural sampling was used to record all occurrences of interspecific and intra-specific interactions (Bateson and Martin, 2007). For the category interaction, it was recorded whether it was a friendly, neutral or aggressive interaction (Table 3). In addition to the type of interaction, the actor and recipient were recorded. For the ostrich, emu, rhea and marabou stork it was recorded which individual was the actor or recipient, for the other species only the species was recorded. Behavioural sampling was used whenever interactions occurred during observation hours (Figure 2). Appendix II gives the data collection sheet that was used for behavioural sampling.

Table 3: Types of interaction.

Interaction type	Explanation
Friendly	
Play	Actor plays with member(s) of the other species or attempts to elicit play or attempts to join in intraspecific play
Curious approach	Actor moves toward member(s) of other species at a slow pace and does not display any aggressive behaviour, but shows interest in other individual or initiates interaction
Curious approach	Recipient moves to 1 meter of actor
Neutral	
Moving together	Individuals of both species travel in the same

	direction in close proximity (1m), include foraging or exploration behaviours
Close proximately	Actor moves to 1 meter of individual(s) of other species but shows no interest in interacting and does not touch
Unclear	An interaction occurs but it is difficult to discern the type of interaction
Vigilant-ignore	Recipient appears aware of behaviour of the actor but does not move from area
Moving away	Recipient retreats from actor and the area they were previously occupying. Includes moving short distances away or leaving
No reaction	Recipient does not respond to the behaviour of the actor
Aggressive	
Threat display	Actor engages in non-vocal aggressive behaviours toward member(s) of other species
Vocalization	Actor makes a call towards another individual
Vocalization	Recipient faces the actor and makes a call
Segregate into species group	Members of the two species move toward their own species members to form a cluster
Aggression	Recipient behaves in an agonistic way toward the actor, moving into closer proximity and making threatening displays toward another individual

Nearest neighbour distance

The nearest neighbour distance was recorded every thirty minutes. For each individual ostrich, emu, rhea or marabou stork it was measured whether the individual was within a distance of <1 meter, >1-5 meter, >5-10 meters or >10-50 meters of an individual of the same species or another species in general (no individual). Appendix III gives the data collection sheet that was used for sampling the NND.

2.3.2 Data collection of behaviour in relation to feeding

Feeding

To see whether feeding had an effect on behaviour, an interview with the curator/keeper was used to collect data about feeding: when the feeding times were, how many times a day the animals get fed, whether there was (always) simultaneous or not and whether the feeding was given on the same time (but on separate areas).

2.3.3 Data collection of behaviour in relation to housing

Enclosure use

While scan sampling the behaviour types, the enclosure use was recorded as well. The enclosures were divided into sections that were based on location and habitat. The location of the type of behaviour, was also noted. In addition, data about housing (whether the mixed-species enclosure was always a mixed-species enclosure or whether the species were separated by night, by season etc.) were collected through an interview with the curator/keeper.

2.3.4 Data collection of behaviour in relation to health

Breeding behaviour

ZIMS reports and a small interview with the curator/keeper was used to gather information about the life history of the ostrich, emu, rhea and marabou stork individuals in the zoos. During scan sampling and behavioural sampling, it became clear that it was impossible to distinguish all individuals and thus to see the relationship between the life history indicators and the behaviour. Therefore, only the relationship between breeding behaviour and type of interactions were observed.

2.4 Data preparation and analysis

2.4.1 Data preparation

All recorded data were entered into Microsoft Excel 2010. Behaviour types with no or low occurrences were combined: waltzing, trembling, body shaking, wing expanding, leg stretching, rolling head rubs, scratching and anxiety stretch became ‘other activity behaviour’. Threat, vocalization, submission and forward clattering threat became ‘social behaviour’. Clucking and fluttering, kanteling and copulation became ‘sexual behaviour’. Feather picking, toe and face pecking, behavioural stargazing, pica and aggression became ‘other aggressive behaviour’. After that the data were entered into IBM SPSS Statistics 21.

2.4.2 Data analysis of behaviour

Frequency of types of behaviour

To analyse the frequency of types of behaviour, 27 types of behaviour were included in the data collection sheet following the ethogram. Behaviour types with low occurrences were combined with similar behaviours. The descriptive analysis in IBM SPSS was used.

Interaction

In total, the friendly, neutral and aggressive interactions between all possible species-species combinations were calculated. The percentage of each interaction was calculated and to determine whether this was significant, the chi-squared test in IBM SPSS was used.

Nearest neighbour distance

The percentages of the total amount of each nearest neighbour distance category were calculated and to determine whether the found distances were significant, the chi-squared test in IBM SPSS was used.

2.4.3 Data analysis of behaviour in relation to feeding

Feeding

To analyse whether (simultaneous) feeding influences the interactions between the ostrich, emu, rhea and marabou stork and each of the other species in the enclosure, the total number of friendly, neutral and aggressive interactions were counted during feeding.

2.4.4 Data analysis of behaviour in relation to housing

Enclosure use

The Spread of Participation Index (SPI) was used to calculate enclosure use. The knowledge through this method is useful in situations in zoos where it is desirable to maximise the enclosure use by the animals and also to evaluate the effects of the enclosure use on the behaviour of the animals and on the management actions. The formula to calculate the enclosure use:

$$SPI = \frac{\sum(F_e - F_o)}{2(N - F_{emin})}$$

In this formula F_o is the observed frequency of observations in one section, F_e is the expected frequency of observations in one section, which is based on the size of the section. This assumes an equal use of the whole enclosure where the sub of the absolute value of (F_o-F_e) is calculated for each site or substrate. N is the total number of observations. F_{emin} is the expected frequency of the observations in the smallest section. The resultant index ranges between 0 and 1: values towards 0 represent a maximum enclosure use and values close to 1 indicate preferential use of one section within the enclosure. (Plowman, 2003) The SPI was calculated for each species per enclosure to compare enclosure use.

2.4.5 Data analysis of behaviour in relation to health

Breeding behaviour

As it was impossible to distinguish each individual due to unreadable rings or no rings at all, it was not possible to measure the relationship between life history indicators and the behaviour. Therefore there was only looked into whether breeding behaviour occurred or not. These results were analysed in a descriptive way.

3 Results

Data were collected five hours a day with two days per enclosure, so a total amount of 70 hours of observations. The four principles that were measured to indicate the welfare were behaviour, feeding, housing and health.

3.1 Behaviour

With regard to behaviour the frequency of types of behaviour, the interactions with both conspecifics and other species and the nearest neighbour distances were recorded.

Frequency of types of behaviour

During 70 hours of observation, 420 scan samples were taken and 1498 instances of behaviour were recorded for the focal bird species.

The behaviour types that could be recorded were investigated as possible welfare factors, as social interactions or as general activities. Out of 27 types of behaviour that might be showed, the ostrich performed only six types of behaviour (N = 423), the emu performed four types of behaviour (N = 120), the rhea performed five types of behaviour (N = 240) and the marabou stork performed six types of behaviour (N = 715). All species performed walking, standing/sitting (especially the marabou stork with 54%), feeding/drinking (especially the emu with 52%) and preening behaviour, and except for the emu all species performed sleeping/resting behaviour, especially the rhea (26%) and marabou stork (21%). Only the marabou storks showed thermoregulation behaviour (3%) and only ostriches showed abnormal behaviour: pacing behaviour (19%). This pacing behaviour occurred in enclosure 1 and 6, where the ostriches are housed with rhinoceros, waterbuck, wildebeest, crowned crane, red river hog and/or giraffe, zebra, guineafowl. During the scan samples, no sexual, social or abnormal (other than pacing behaviour for ostriches) behaviours occurred. (Figure 3)

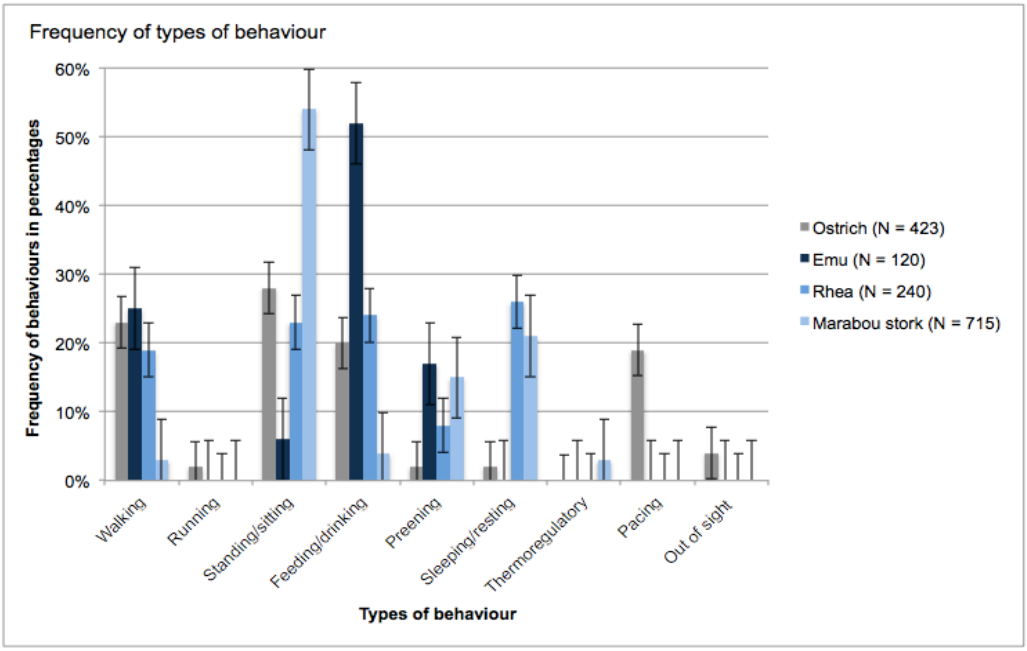

Figure 3: The frequency of types of behaviour of the ostrich, emu, rhea and marabou storks, displayed with error bars.

Interaction with conspecifics

Every interaction that occurred between conspecifics in mixed-species enclosures was recorded within 70 hours of observation. In total 278 interactions between conspecifics were recorded. The interactions that occur with conspecifics are important and might influence welfare.

In marabou storks aggressive interactions were most frequent with a number of 71 interactions, followed by 36 neutral interactions and 19 friendly interactions (X^2_{84} = 119.010, N = 126, P = 0.007). Of the aggressive interactions that occurred between the marabou storks, 57 of these interactions happened when being fed by a keeper. Only friendly interactions between marabou storks occurred between individuals of different sexes (Figure 4). Ostriches only displayed neutral interactions with conspecifics: no friendly or aggressive interactions occurred. For the other species no significant difference in occurrence of type of interactions were found.

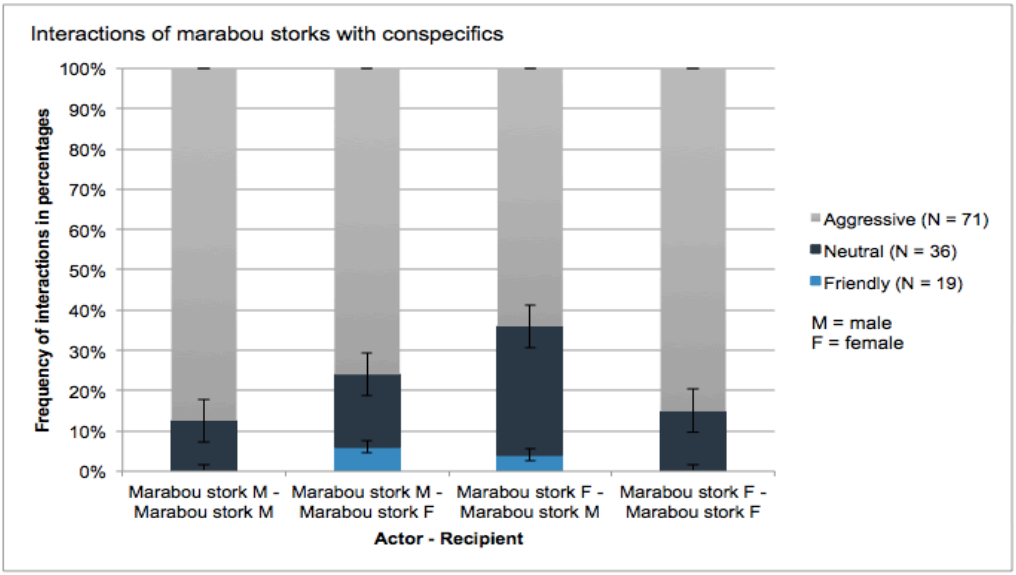

Figure 4: Significant interactions of marabou storks with conspecifics, displayed with error bars.

Interaction with other species

To assess the impact of interactions with other species in the enclosure on welfare in 70 hours of observation, 823 interactions were recorded. Of these interactions, 718 interactions were neutral, 79 interactions were aggressive and 26 interactions were friendly. The focal bird species were the actors of 79% of the total interactions, with 571 neutral interactions, followed by 66 aggressive interactions and 16 friendly interactions.

In ostriches, 94.5% of the interactions were neutral, followed by 3.5% of the interactions being aggressive and 2% of the interactions being friendly (X^2_{10} =30.876, N = 398, P = 0.001). The number of neutral interactions (N = 376) occurred the most, although there is a difference in aggressive and friendly interactions for males and females: the female ostriches performed friendly interactions only towards rhinoceroses and aggressive interactions only towards waterbucks. Towards other species, the female ostriches interact neutral. On the other hand, male ostriches performed aggressive interactions towards giraffes and zebras, and neutral interactions towards other species. Crowned cranes, giraffes and rhinoceroses performed aggressive interactions towards all ostriches equally. For the combination rhinoceros with ostrich, aggressive interactions were only performed by the female rhinoceroses; friendly interactions came from the male rhinoceros. (Figure 5)

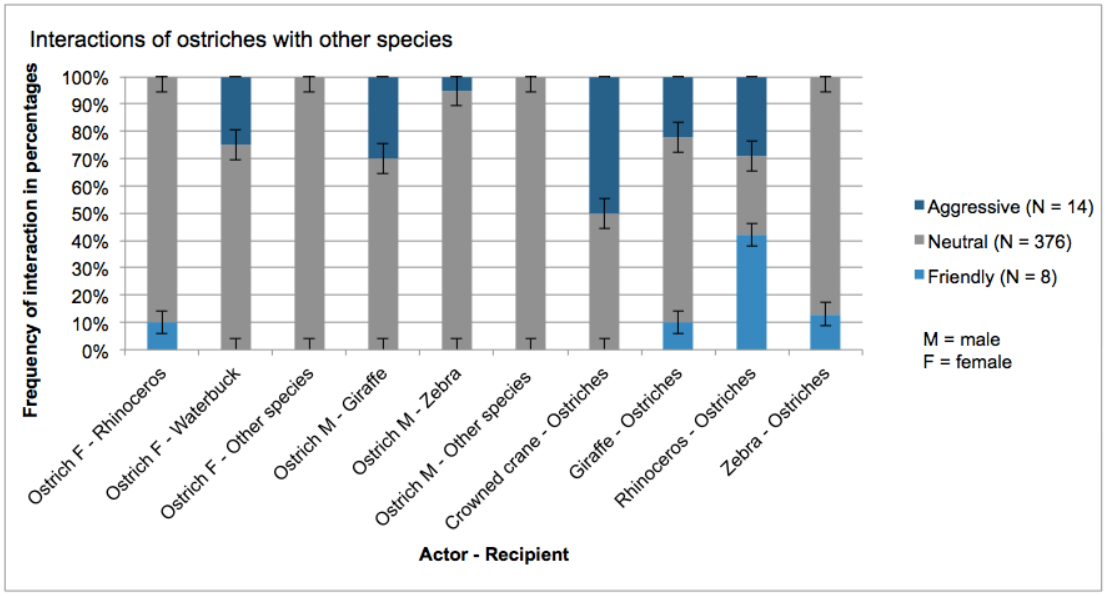

Figure 5: Significant interactions of ostriches with all other species in the enclosure, displayed with error bars.

In emus, in 89% of the interactions that occurred the emu (male and female equally) was the actor and the kangaroo was the recipient. Of all interactions, 82% were neutral interactions, 12% were aggressive interactions and 6% were friendly interactions (X^2_4 = 17.173, N = 139, P = 0.02).
In rheas all interactions towards other species (wallabies) were neutral (X^2_3 = 32.000, N = 32, P < 0.001), with almost no interactions from the male (N = 2).
For marabou storks, no significant differences in occurrence of type of interactions were found.

Nearest neighbour distance

The distance of how close conspecifics and other species were in the enclosure was observed with 70 hours of observations and 140 nearest neighbour distances were recorded
All emu, rhea and marabou stork conspecifics spent over 30% of the observed time less than a meter away from each other, except for the ostrich which was only in close proximity with conspecifics 13% of the observed time.

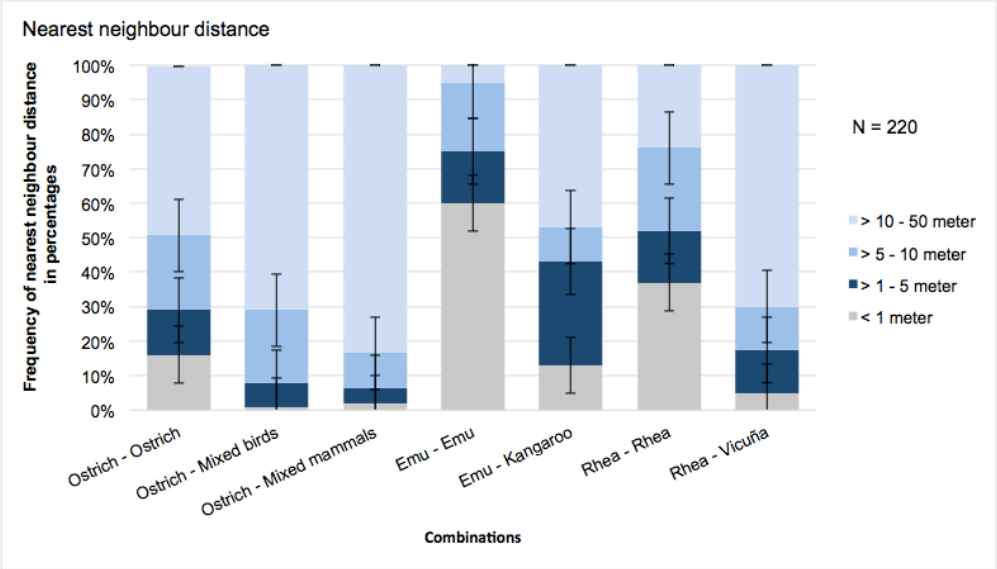

Figure 6: Nearest neighbour distance of ostriches, emus and rheas, displayed with error bars.

In ostriches, 15% of the time was spent less than a meter apart (X^2_{75} – 50.208, N = 60, P < 0.01; X^2_{18} = 50.208, N = 40, P < 0.01; X^2_{60} = 266.164, N = 40, P = < 0.01) respectively. Ostriches seemed not to react when giraffes, rhinoceroses, crowned cranes and red river hogs were less than a meter away. Emus spent 60% of the time less than a meter apart and generally spent approximately half of their time more than 10 meters away from the other species (X^2_6 = 20.206, N = 40, P = 0.03). Rheas spent 37% of the time less than a meter from one another and kept a meter or more distance between themselves and the other species in the enclosure (X^2_6 = 20.872, N = 40, P = 0.02). (Figure 6) It is remarkable that the marabou storks also spent the majority of the time less than a meter apart and kept more than a meter away from other species in the enclosure. Though none of the nearest neighbour distances concerning the marabou storks were significant.

3.2 Behaviour in relation to feeding

Feeding

In mixed-species enclosures simultaneous feeding might influence the interaction with conspecifics and other species. It was found by the interviews that separately feeding in the enclosures was more common than simultaneous feeding: only in enclosure 7, wherein rheas are housed with wallabies, simultaneous feeding occurred. This happened inside and before opening times, therefore it cannot be said whether simultaneous feeding influenced the frequency and type of interactions.

The marabou storks that were housed with different bird-species (enclosure 4) were the only individuals that were fed during opening times and so during the observations. This happened outside in the presence of the other species. Interactions with other species did not take place, as the keeper fed the marabou storks by hand. Interactions with conspecifics did take place during this moment of feeding: 80.5% of the aggressive interactions occurred during feeding (N = 71). No friendly or neutral interactions occurred among conspecifics during feeding. (Table 4)

Table 4: Place, type and time of feeding per enclosure.

Enclosure	Place of feeding	Type of feeding	Time of feeding
Enclosure 1	Inside	Separate	After opening times
Enclosure 2	Inside	Separate	After opening times
Enclosure 3	Inside	Separate	Before and after opening times
Enclosure 4	Outside	Separate	During opening times, around 12.00 o'clock
Enclosure 5	Inside	Separate	Before opening times
Enclosure 6	Inside	Separate	After opening times
Enclosure 7	Inside/outside	Simultaneous	Before opening times

3.3 Behaviour in relation to housing

Enclosure use

The Spread of Participation Index (SPI) was used to measure the enclosure use of all species in the mixed-species enclosures. During 70 hours of observing, the location where the focal bird species were located during the performance of a behaviour type was recorded 2960 times.

It was found that the focal bird species made the most use of the enclosure when they were housed with only one other species in the enclosure (rhea and emu). The rheas housed with three vicuñas, made the most use of the enclosure (SPI = 0.21).

It was also found that when ostriches and marabou storks were housed together, they do not make as a good use of the enclosure as when they are housed with other mammal and/or bird species. In general the mammals housed with ostriches made the most use of the enclosure. (Figure 7)

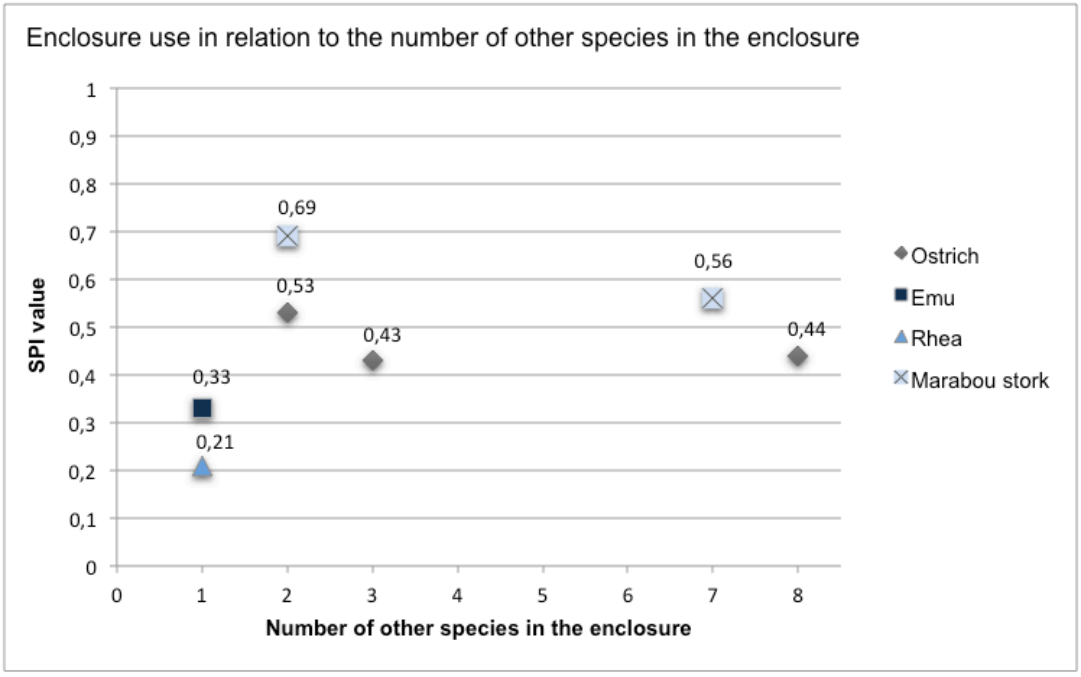

Figure 7: Enclosure use of ostriches, emus, rheas and marabou storks in relation to the number of other species in the enclosure.

3.4 Behaviour in relation to health

Breeding behaviour

As no injuries and diseases were present and it was not possible to measure the relationship between life history indicators and the behaviour, it was only investigated whether breeding behaviour occurred. By interviews it was found that, due to surplus problems, ostriches, emus and rheas were not allowed to breed. Breeding behaviour occurred among these species, but eggs had been removed.

As no surplus problems occurred among marabou storks, these species were allowed to breed. In enclosure 4, two chicks were present. Parents were not identified, so a relation between breeding behaviour and the frequency and type of interaction could not be made. In enclosure 3, the marabou storks did not perform any breeding behaviour during the observations or before. (Table 5)

Table 5: Breeding behaviour of the ratites and storks in the enclosures.

Enclosure	Past breeding	Current breeding	Additional information
Enclosure 1	Yes	Yes	Eggs will be removed.
Enclosure 2	Yes	Yes	Eggs will be removed.
Enclosure 3	No	No	Reason why ostriches and marabou storks are not breeding is unknown.
Enclosure 4	Yes	Yes	Two youngsters during the observations.
Enclosure 5	Yes	Yes	Eggs will be removed.
Enclosure 6	No	No	Two female ostriches so breeding is not possible.
Enclosure 7	Yes	No	Eggs will be removed.

4 Discussion and conclusion

It cannot be concluded whether mixed-species enclosures are positive or negative for ratite or stork welfare. There were positive outcomes concerning the welfare of the focal bird species, but also points of concern were determined. Therefore, the welfare seems to depend on individuals, species and enclosure design. An important conclusion is the great similarity between this research, conducted in the Netherlands, and the previous research by Chaiwan and Plowman (2013), conducted in the UK.

Whether animals will adapt well in captivity and perform natural behaviours is of great importance for their welfare (Bishop *et al*., 2013). All focal bird species showed comparable types of behaviour with those observed in the previous study of Chaiwan and Plowman (2013) and with those observed in in-situ researches (Blache and Martin, 1999; Davies, 2002; Deeming *et al*., 1999; Kahl, 1966; Williams *et al*., 1993). However, in the observed ostriches pacing behaviour occurred, which is part of abnormal behaviour. Although in the study of Chaiwan and Plowman (2013), no stereotypic or abnormal behaviour was seen. Also in the wild, pacing behaviour was not observed (Deeming *et al*., 1999; Williams *et al*., 1993).

For rheas, it is interesting to see they performed little to no preening when housed with wallabies, while Raikow (1968) stated rheas should pause for a brief period of time to preen. Adaptation problems might be an explanation for this (Hosey *et al*., 2009) as the rheas had been recently moved to this enclosure since the previous mixed-species enclosure with the tapirs caused a lot of stress among the rheas (Snijders, pers. comm., 2014).

For all focal bird species, interactions with both conspecifics and other species occurred. Unlike in the previous study by Chaiwan and Plowman (2013) where friendly interactions occurred the most, neutral interactions were most frequent in this study, followed by aggressive interactions and friendly interactions occurred the least. Neutral interactions being most frequent, simply indicates no interest in one another when approaching, which might be a sign that the individuals are used to live in the same enclosure (Chaiwan and Plowman, 2013). Marabou storks showed the highest amount of aggressive acts, especially among conspecifics. Veasey and Hammer (2010) stated that intraspecific aggression has a higher chance of occurring than interspecific aggression, due to the fact that competition for resources is more intense among conspecifics as they need to compete directly for food, shelters and mates. However, it is interesting to see that this species in the UK, during the same time of the year, displayed mostly neutral and friendly interactions (Chaiwan and Plowman, 2013).

Conspecifics spent more than 30% of the observed time close to each other. The focal bird species spent most of the observed time a greater distance (more than 10 meters away) from the other species in the enclosure than from their conspecifics. In-situ researches also showed ignoring, tolerating and avoiding behaviour towards other species when looking at interspecific behaviours (Williams *et al*., 1993). The study of Chaiwan and Plowman (2013) showed comparable outcomes. Only for the rhea, it stated that other species in the enclosure were actively maintaining avoidance behaviour towards rheas, while in this study the avoidance behaviour was on the part of the rheas.

While aggressive interactions occurred the least among conspecifics, they occurred the most among marabou storks during separate feeding. Probably due to the fact that competition for resources such

as food is intense (Veasey and Hammer, 2010), and because of the small area where the food was provided, which led to a none-comfortable approaching at the feed station (Avent, 2008; Smith, 2000). Apart from this enclosure, separate feeding was common in the other enclosures as well. According to the keepers and curators, separate feeding was done to prevent competition. McLeod (2002) also recommends separate feeding in mixed-species enclosure to reduce mutual competition. However, in the enclosure where rheas were housed with wallabies, simultaneous feeding occurred. According to the keepers, within this mixed-species combination simultaneous feeding worked out well.

In all mixed-species enclosures, the focal bird species had the availability of their own inside enclosure, which ensured the ability of comfort around resting (Blokhuis, 2008). However, the ostriches in enclosure 1 and the emus in enclosure 2 only had access to this inside enclosure before and after opening times and therefore did not have the choice to enter their inside enclosure. Apart from the comfort around resting, the enclosure use was determined by using SPI. These results indicate that lower welfare in enclosures occurs with several other species, and better welfare in enclosures occurs with only one other species. However, it is not sure whether this is the case: many zoo animals do not use all of the space available to them. This might indicate that the enclosure is not as well designed as it could be, or that there are objects or habitat types within the enclosure to which the animals are attracted or trying to avoid (Chaiwan and Plowman, 2013).

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Appendix I - Instantaneous scan sampling sheet

Date: *Time:* *Weather:*

Observers name: *Species:* *Other variables:*

Scan sam.	Activity behaviour														Social beh.			Sexual beh.			Abnormal behaviour							
	W	R	Ss	Fd	Pr	Wa	Sr	Th	Bs	We	Ls	Sc	St	Rhr	T	V	S	Cf	K	Co	Fp	Tfp	Bs	Pi	Agg	As	Fct	Oos
Location																												
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NOTES

Appendix II - Behaviour sampling sheet

Actor	A1:	A2:	A3:	A4:	A5:
Friendly					
Neutral					
Aggressive					
Recipient	R1:	R2:	R3:	R4:	R5:
Friendly					
Neutral					
Aggressive					

Appendix III - Nearest neighbour distance sheet

Date: Time: Weather Other variables:

Species-species	<1	> 1 - 5	> 5 - 10	> 10 - 50	Notes