EFFECTS OF ENVIRONMENTAL ENRICHMENT ON THE BEHAVIOUR OF SCARLET IBIS,

EUDOCIMUS RUBER

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Resumo

O enriquecimento ambiental tem sido muito utilizado em zoos com o intuito de melhorar as condições de bem-estar animal, fornecendo estímulos que respondem às necessidades dos animais, permitindo comportamentos típicos da espécie. Neste estudo é abordado o efeito da implementação de diferentes tipos de enriquecimento num grupo de íbis-escarlate do Zoomarine, Algarve. Para tal, seguiu-se o desenho experimental do tipo ABA, consistindo numa primeira fase de *baseline* (apenas estudo comportamental), seguida de uma fase de estudo comportamental com enriquecimento (aplicando 4 tipos de enriquecimentos, físico, alimentar, sensorial e táctil, individualmente), e um pós-enriquecimento (estudo comportamental sem enriquecimento). No final procedeu-se a um estudo do comportamento com todos os enriquecimentos em simultâneo (pós-enriquecimento com enriquecimento). A comparação entre contextos revelou que, dos comportamentos analisados, existiu uma semelhança de 50% nos comportamentos exibidos no *baseline* e no pós-enriquecimento sem enriquecimento, o que sugere que após retirados os enriquecimentos os animais voltam aos seus comportamentos iniciais. Verificou-se ainda que cada tipo de enriquecimento teve um efeito diferente no tipo de comportamentos observados. O enriquecimento físico teve maior influência no *preening*, *exploration* e *foraging*; o nutricional na *maintenance* e *manipulation/play*; o sensorial no *attentive behaviour* e *resting*; e o enriquecimento táctil no *resting*, *exploration* e *stationary*. O comportamento exploratório surgiu associado à presença de enriquecimento físico, nutricional e táctil, sendo despertado apenas quando aplicado um estímulo novo. No entanto, devido à curta duração do estudo, torna-se também importante verificar se as conclusões obtidas não resultam apenas de um "efeito novidade".

Palavras-chave: enriquecimento ambiental; comportamento; íbis-escarlate; cativeiro

Abstract

Environmental enrichment has been increasingly used in zoos to improve animal welfare conditions, providing stimuli that respond to the needs of animals, allowing typical behaviours of a species. This study addresses the effect of implementing different types of enrichment in a group of scarlet ibises from Zoomarine, Algarve. For this purpose, the study followed an ABA-type behavioural experimental design, consisting of a first baseline phase (just behavioural study), followed by a phase with enrichment (applying 4 types of enrichment, physical, nutritional, sensory and tactile, individually), and a phase post-enrichment (without enrichment). In the end, a behavioural study with all enrichments simultaneously was also performed (post enrichment with enrichment). The comparison between contexts revealed that of the behaviours analysed, there was a 50% similarity in the behaviours exhibited in the baseline and in post-enrichment without enrichment, suggesting that after the enrichments removal, the animals returned to their initial behaviours. It was also found that each type of enrichment seemed to have a different effect on the type of behaviour observed. Physical enrichment had the greatest influence on preening, exploration and foraging; nutritional enrichment in maintenance and manipulation/play; sensory enrichment in attentive behaviour and resting; and tactile enrichment in resting, exploration and stationary. Exploratory behaviour was only associated with the presence of enrichment (physical, nutritional and tactile), being triggered when a new stimulus is applied. However, due to the short duration of the study, it is also important to verify whether the conclusions obtained are not just the result of a "novelty effect".

Key words: environmental enrichment; behaviour; scarlet ibis; captivity

Table of contents

List of tables

List of figures

1. Introduction

Animal welfare has been defined in different ways throughout the years, based either on health (biological function), natural way of living, or animals' feelings. For many animal welfare researchers, their view of animal welfare depends on the emphasis they put on each of these characteristics. However, none of these conceptions individually will relate to all ethical concerns about the quality of life of animals, therefore, the definition of animal welfare must reflect the whole set of ethical concerns that exist in society (Fraser et al., 1997). Thus, the concept of animal welfare has to encompass these different conceptions and reflect on what constitutes a good life for animals (Fraser et al., 2008). The concept of the Five Freedoms was developed in 1965 and is widely accepted, to describe when an animal is at an acceptable level of welfare. This includes freedom from hunger and thirst; freedom from discomfort; freedom from pain, injury and disease; freedom from fear and stress; freedom to express normal patterns of behaviour (Young, 2003). Later, the Five Domains model was proposed including not only five similar areas (nutrition, environment, health and behaviour; and affective experience domains – mental state) but also the positive and negative states that are associated with each area, which is used, not only but greatly in the zoo community (Binding, 2020; Mellor & Reid, 1994).

Although there is a perception of zoos only as entertainment institutions, over the years, zoos have increasingly focused on education and conservation, as well as animal welfare, as their goals (Carr & Cohen, 2011; Conway, 2003). Regarding conservation, its greatest contribution comes from *ex-situ* actions, which include education, captive breeding and wildlife management programs. Recently, the focus has been on *in situ* conservation, through programs for the rescue and recovery of endangered species, protection and restoration of habitats, and reintroduction programs (Tribe & Booth, 2015). As this reintroduction objective is not applied to all species in the zoo (only those threatened with extinction) (Keulartz, 2015), there is a concern to keep the captive populations healthy, ensuring their well-being. One of the ways zoos have been working on animal welfare is through environmental enrichment techniques (Binding et al., 2020).

Environmental enrichment is often used in zoos to improve the welfare of animals kept in captivity, promoting a better quality of life for these animals. Environmental enrichment consists of modifying the environment of animals in captivity for their benefit providing stimuli that respond to their needs and promote species-specific behaviours. In practice, it involves a set of original and creative techniques to keep captive animals occupied by increasing the range and diversity of behavioural opportunities and providing more stimulating environments (Shepherdson 1994; Shepherdson et al., 1998). Enrichment can be also rewarding for the individual, due to the benefits from the pleasure generated in the exploration (Inglis et al, 1997). Despite being highly used in captive animals, most of these studies focus on the implementation of enrichment in terrestrial mammals, with birds being a less studied group (King, 1993).

When a bird is moved to a captive environment, some important factors of its development are removed and/or substitute. Central aspects, such as what, when and where to eat and sleep, with whom to socialize, with whom and when to reproduce, are usually planned by the caretakers who are responsible for these animals. Sometimes this means that the food is always provided in the same way, every day, consequently extinguishing some behaviours typically associated with searching and hunting for food. Consequently, birds under human care can have their routines in a very predictable way and quite monotonous. For that reason, it is used environmental enrichment to change their routines, avoiding the same habitats for an extensive period (Martin, 1999). According to Bloomsmith et al. (1991), environmental enrichment can be divided into five types of enrichment: social, occupational, physical, sensory, and nutritional, each of which encompasses different categories. From these, the most common stimuli recommended in birds include vocalizations, natural or artificial objects in their space, providing food in different ways, and making changes in their substrates, by turning them over or creating small mounds or hills (AZA Charadriiformes Taxon Advisory Group, 2014). However, most zoos have focused on nutritional enrichment (Byrne, 2004).).

Scarlet ibis (*Eudocimus ruber*) is a colonial waterbird that can be found in the northern part of South America mostly on mangrove swamps and coastal lagoons. These birds live in large colonies frequently mixed with other species, namely, herons and spoonbills. They usually leave the roost in the morning to go feeding on the mud flats. During this time, the birds are often probing and pecking in the water to catch their prey. Before the sunset, the birds fly in large flocks to the roosting sites in V-formation (Ffrench & Haverschmidt, 1970).

Although one of the most attractive members of the family Threskiornithidade due to its colour, and some presence in captive environments, there are very few studies about this species in this context. In zoos, little behavioural research has been done about scarlet ibis. Antas (1979) focused on its breeding behaviour, such as Spil et al. (1985), who also made a small reference to their daily activity, but none of them focused on the general behaviour of this species. Thus, this study appears as one of the few attempts to investigate the normal behaviour of this species in a zoo, with the main goal of evaluating the effects of enrichment on the behaviour of scarlet ibis, and understanding which types of enrichment can contribute to the improvement of the animal welfare.

2. Methods

This study was developed at Zoomarine, a marine park in Guia, Algarve (Portugal), from November 2020 to March 2021.

Zoomarine opened to the public in 1991 and since then has established the sharing of knowledge and environmental education as their mission. Given its investment in these fields and its high zoological diversity, Zoomarine is a suitable park for the development of scientific studies.

Zoomarine has a wide variety of species including marine mammals; aquatic and terrestrial reptiles; tropical birds, birds of prey and water birds, and several species of fish. Birds are distributed in four different areas: ‘Rapinas’ (birds of prey), ‘Américas’, ‘Tropicais’ (with some tropical birds’ species) and ‘Magic Rainbow’ (with other tropical birds’ species).

This study was conducted in the ‘Américas’, a sizeable immersion habitat where people can walk through and find some species from South America. This habitat tries to recreate the natural environment of these species through the presence of four lakes (two larger and two smaller), a waterfall and several trees. There is also a walkway where people can walk and admire the animals with less disturbance.

The species chosen in this project was the scarlet ibis (*Eudocimus ruber*), which lives in this habitat with ten other different bird species and one reptile species. The current group of scarlet ibises have 16 individuals, with 11 adults, 4 offspring from 2020 and 1 juvenile from

2019. To facilitate data collection, the habitat was divided into 4 areas. The areas considered for this study are shown in Figure 1.

Figure 1. Américas habitat with the respective areas. A1 (Area 1) – large size lake area (exit side); A2 (Area 2) – medium-sized lake area (central zone); A3 (Area 3) – walkway; A4 (Area 4) – trees.

2.1. *Data Collection*

2.1.1. *Test phase*

In the first phase of the study, preliminary observations were made for two weeks from 16 to 27 November 2020 to create a general ethogram for this species (which was adapted from captive northern bald ibis). In the first two days, the *ad libitum* sampling method was used to acquire information about the observed behaviour of individuals and to get to know them. This phase also had the goal of selecting the sampling method that would be used in the rest of the study. The behaviours mentioned in the ethogram of Spiezio et al., 2018 for the Northern bald ibis were identified, and some other observed behaviours were added to this ethogram (Table 1). The remaining days were used as an adaptation period from the birds to the experimenter. As it was not possible to distinguish individuals from each other, the method chosen was scan and instantaneous sampling (Martin & Bateson, 1988), meaning that the behaviour of all individuals was recorded at a determined instant (in this case every 2 minutes).

Table 1. Ethogram for the scarlet ibis adapted from Spiezio et al., 2018. The "*" represents the behaviours that were observed in this data collection but were not described in the original ethogram.

Type of behaviour	Behaviour	Code	Description of behaviour
Solitary	Attentive behaviour	AB	Being vigilant, scanning the environment and listening or watching something in the surrounding.
	Preening	PR	Using the bill to straighten and clean their feathers; scratching their body (neck and head) using their feet; bathing and sunbathing (fanning the wings to warm up in the sun).
	Flight	FL	Flying or hopping in the habitat.
	Walking	WA	Walking around the habitat.
	Maintenance	MA	Eating the food provided in the feeding points around the habitat or provided by the caretakers in feeding sessions, and drinking.
	Manipulation/Play	MP	Manipulating food, twigs, rocks found in the habitat with the bill.
	Resting	RE	Standing on one or both legs, with the head turned back and tucked beneath the wings.
	Foraging	FO	Looking for food probing the lake, ground, tree branches, crevices, and other elements of the habitat with the bill. (Walking slowly and frequently make short pecks into the ground).
	Exploration*	EXP	Showing curiosity towards their environment (mostly associated with novelty).
	Stationary*	STA	Standing motionless on one or both legs with the head down and still
Social affiliative	Begging*	BE	Approaching an adult and rhythmically begs by bobbing the head and touching the adult with the bill (behaviour only exhibited by offspring).

	Preening others	PO	Tidying and cleaning the feathers of a conspecific with the bill.
	Other affiliative	OA	Greeting displays including head tossing, head rubbing, mutual bill shaking; observing conspecifics.
Social agonistic	Aggression	AG	Pecking towards approaching birds, hitting a conspecific with the bill or with the legs.
	Agonistic display	AD	Bill gaping, ruffling the feathers, moving or lunging toward conspecifics, touching slightly with the tip of the bill.
	Stealing*	STE	Grabbing a piece of food of another bird and sometimes pecking the bird.
Acoustic	Vocalizations*	VOC	Emission of vocalizations/sounds by the ibises

Note: Exploration was only observed in enrichment phases, in which the bird uses its bill to explore new parts of the lake it did not have access to, pulling algae at the bottom of the lake (physical enrichment at low tide), or to explore new items in the habitat (nutritional and tactile enrichment). Vocalizations only appeared after the sensory enrichment phase.

2.1.2. *Experimental design*

After the test phase, it was decided on the experimental design. The typical experimental design for environmental enrichment studies consists of an ABA design, where A represents the baseline condition and B the enrichment condition (Young, 2003). For this study it was also decided to include an additional phase of enrichment, to see what the enrichment devices could contribute to animals in terms of behaviour when present in the habitat simultaneously. As one of the objectives of the project was to understand which enrichment device can contribute to the improvement of animal welfare daily, this phase had the purpose to understand the behaviour of these animals in the presence of various stimuli. Therefore, the experimental design includes the baseline, enrichment (composed with each one of the types chosen), post enrichment without enrichment and post enrichment with enrichment (Table 2).

Table 2. Experimental design chronogram with dates for each phase in which behavioural data was collected and different stimuli was presented.

Phase	November	December										January							February								March										
	30	2	4	7	9	11	14	16	17	18	21	4	6	7	8	26	28	30	5	7	9	15	17	19	25	27	1	7	8	9	10	11	17	18	19	20	21
Baseline	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■																						
Physical enrichment																■	■	■																			
Nutritional enrichment																			■	■	■																
Sensorial enrichment																						■	■	■													
Tactile enrichment																									■	■	■										
Post enrichment without enrichment																												■	■	■	■	■					
Post enrichment with enrichment																																	■	■	■	■	■

2.1.2.1. *Baseline*

The baseline phase was carried out to observe and quantify the behaviour of the individuals throughout the day and to observe how they perform their daily routine. To study if there is any variation in the behaviour throughout the day, observations were carried out three times a day with one-hour duration: morning (from 10:00 am to 11:00 am), midday (from 1:30 pm to 2:30 pm) and afternoon (from 4:00 pm to 5:00 pm). At the beginning of each hour of observation, the temperature, the real feel, and atmospheric conditions (sunny, cloudy, rainy, storm, and windy) were registered (through weather.com). This phase lasted 15 days, from November 30th to January 8th (every two days during weekdays) with a total of 45 hours of observation.

In the behavioural observations, the number of groups and group size was also registered. In this study, it was decided that animals that were in the same area and were not separated by a barrier (i.e., area) were considered as groups (e.g., Area 3 is separated by Area 2 in the middle; concerning Area 4, some birds remained in the trees in Area 1 and others in the central trees in Area 2). The presence or absence of staff members and the occurrence of interaction with other species were also recorded.

The sampling method used in this phase was maintained throughout the following phases.

2.1.2.2. *Environmental enrichment*

Since information on environmental enrichment for this species is rare or even unknown, it was decided to select four different types of enrichment: physical, food, tactile and sensory. Due to the pandemic situation and security measures inside the park, this phase was initiated at the end of January and was performed with an interval of 5 days between the enrichments type, with 1 week for each enrichment type, and 9 hours of observation each (3 hours in the morning, 3 in the midday and 3 in the afternoon). For this phase, the interaction with the enrichment device was also documented.

Physical enrichment consisted of tidal simulation in the two larger lakes (Figure 2). Due to the trainers' logistics, it was not possible to recreate the tides as it happens in the coastal area near Zoomarine (Albufeira). It was then decided that during the morning the lake should simulate the low tide and, in the afternoon, the high tide. Thus, in the morning, 15 minutes before the beginning of the observations (9:45 am), the trainers enabled the system to decrease the amount of water in the lake (taking 1 hour to descend) reaching the low point at 10:45 am and so remained until the end of the morning data collection. As the lake was not very deep, to simulate a rising tide, it was decided that in the middle of the day, the depth of the lake would be intermediate, and only in the afternoon, the high tide was reached, to simulate the 6 hours difference between low and high tide. Thus, at the beginning of the afternoon's observations, the system was then turned on so the water in the lake could slowly increase. The lake took approximately half an hour to reach the maximum without overflowing. The simulation of these tides was only carried out on the days of the observations.

Figure 2. Tidal simulation in the two larger lakes in Area 1 (low tide) and Area 2 (intermediate tide), respectively (from left to right).

For nutritional enrichment, a simple device was developed, which consisted of a plastic mesh tube, where mussels were placed by the caretakers (Figure 3). This enrichment brought together two ideas in one: presenting the food in a different way and the introduction of a new type of food. In their daily routine, these ibises are typically fed twice a day, once in the morning and once in the afternoon. Their diet consists of both fish and meat, varying through the days.

In addition to these feeding sessions, a specific granulate is also provided for these birds, in dishes distributed in different areas of the habitat, for the birds to feed throughout the day. During this period of food enrichment, ibises were fed by caretakers only in the afternoon before the last observation period, to avoid an increase of daily calories in the animals' diet. A total of 6 devices were used, distributed in areas 1 and 2 (three in each area). In Area 1, one was placed on the ground and two were attached to the trunks over the lake, one being half-submerged in the water and the other above the water (Figure 4). In Area 2, two were placed on the ground at opposite points and one attached to the bamboo branches, being approximately at the height of the ibis (Figure 5). The devices were provided three times a day: morning, midday, and afternoon. The devices were placed just before the observation time began and removed after it ended.

Figure 3. Feeding enrichment for scarlet ibis with mussels in the interior.

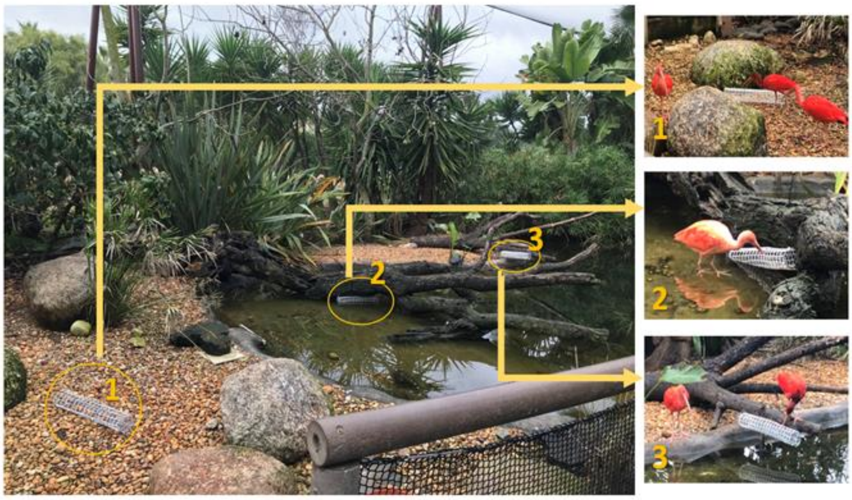

Figure 4. Feeding devices distributed by Area 1 (1 – ground; 2 – half-submerged in the water; 3 – above water).

Figure 5. Feeding devices distributed by Area 2 (1 and 3 – ground; 2 – bamboo branches).

For sensory enrichment, it was decided to introduce vocalizations of scarlet ibis, namely calls. These sounds have been removed from xeno-canto.org website. A 10-second call was chosen and then transformed in a 1-hour loop, with 5 random pauses. This sound was only presented at the time of observations, to be possible to observe the animals' reaction. For this purpose, a sound column was placed in a central position of the habitat (Figure 6).

Figure 6. Sound column in the habitat (left) and its central position on the map (right).

Tactile enrichment consisted of 6 brushes distributed in areas 1 and 2 (three in each area). In Area 1, two were placed on the ground and one in the trunk. In Area 2 all three were placed in the ground (Figure 7). These brushes were placed just in the three observations time and removed at the end.


Figure 7. Tactile enrichment in Area 1 (left) and in Area 2 (right).

2.1.2.3. *Post enrichment*

The post enrichment consisted of two phases: the post enrichment without enrichment and the post enrichment with enrichment.

Before the post enrichment without enrichment, a 5 days pause in observations occurred. In this phase, the conditions were similar to the ones in the baseline and the same table of observations was used. This phase lasted for 5 consecutive days, followed by another 5 days pause without observations.

In post enrichment with enrichment phase, all the enrichments were applied simultaneously. Contrary to the enrichment phase, in this phase, the enrichments (when applied) remained in the habitat throughout all day, in its used placements. The tactile enrichment was placed approximately 15 minutes before the morning observation such as the column, and the nutritional enrichment was assembled just before the first observation of the day. To the tide simulation, this was similar to the first enrichment, with low tide in the mornings and high tide in afternoons. In the case of feeders' devices, these were only refilled with mussels at the time of observations. In the case of the sound column for the sensory enrichment, it was only placed in the habitat moments before each observation time due to battery charging requirements. At the end of the day all the enrichments were retrieved from the habitat to sanitize and placed in position the following day. This phase also lasted for 5 consecutive days.

Note: The data regarding all observations were recorded in different registration tables, available in Annex B.

2.2. *Data Analysis*

To avoid giving different significance weight to behaviours that had a very high or low number of occurrences, all observed behaviours were summed and averaged, resulting in 3060.5 behaviours. Behaviours that presented less than 10% of this value in occurrences, meaning less than 306 occurrences, were discarded from the analyses.

To perform the behaviours comparisons, a non-parametric test, the Kruskal-Wallis 1-way ANOVA test with multiple comparisons, all pairwise, was performed (since the data did not meet the assumptions of normality and homogeneity of variances). To study the differences regarding the phases, it was used a post hoc Wilcoxon Matched Pairs test.

The first comparison analysis was a general comparison between the different contexts, i.e., baseline, enrichment, post enrichment without enrichment and post enrichment with enrichment. The behaviours that presented significant differences between contexts, were then compared through the different phases of the study (to verify where occurred the differences), where the types of enrichment were separated, thus comparing the baseline, physical enrichment, nutritional enrichment, sensory enrichment, tactile enrichment, post enrichment without enrichment and post enrichment with enrichment

Concerning the activity budgets of ibises, the percentages of each exhibited behaviour in the different phases were used. The activity budgets were compiled into a single graphic to facilitate the comparison between phases. A chi-square test by Monte Carlo simulation was also used to predict which behaviours were observed more than expected in each of the phases.

Then, the different periods of the day (morning, midday and afternoon) and weather conditions (sunny, cloudy, rainy, windy/cloudy and storm) were also compared to understand if the behaviours varied depending on the time of day and weather.

Finally, the behaviours between the different areas presented in Figure 1 were also compared.

All the statistical analyses were performed using the IBM SPSS Statistics 27 software (IBM Inc.).

3. Results

This section will start with a comparison of ibises' behaviours across contexts (baselines and enrichments). Then, the differences observed between phases (type of enrichment applied) will be verified, to understand the differences obtained previously. Finally, comparisons of the behaviour of the ibises will be made regarding the time of day, weather, and area. Only behaviours that had at least 306 occurrences in total were selected (i.e., attentive behaviour, preening, flight, walking, maintenance, manipulation/play, resting, exploration, foraging and stationary).

3.1. *Comparisons between contexts*

The behaviours that significantly differed between contexts on the Kruskal Wallis test were: attentive behaviour ($H(3) = 150.775$, $p < 0.001$), preening ($H(3) = 110.876$, $p < 0.001$), walking ($H(3) = 344.501$, $p < 0.001$), maintenance ($H(3) = 399.855$, $p < 0.001$), manipulation/play ($H(3) = 30.175$, $p < 0.001$), resting ($H(3) = 24.553$, $p < 0.001$), exploration ($H(3) = 795.553$, $p < 0.001$), foraging ($H(3) = 187.008$, $p < 0.001$), and stationary ($H(3) = 83.667$, $p < 0.001$).

Walking, maintenance and foraging behaviours showed significant differences between all pairs of contexts (Figure 8, 9 and 10).

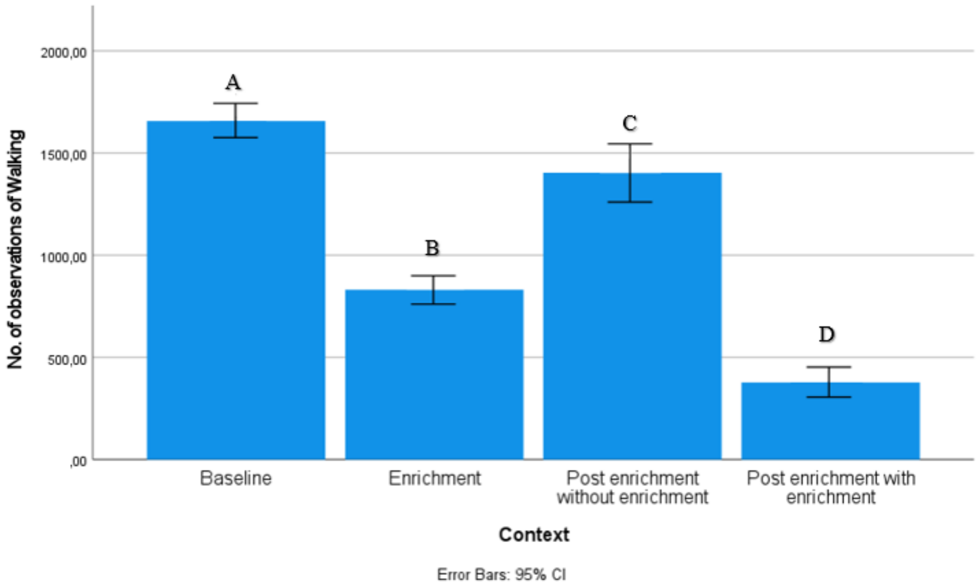

Figure 8. A comparison of the number of times walking was observed in each context.

Conditions with the same letter are not significantly different at the 0.05 level.

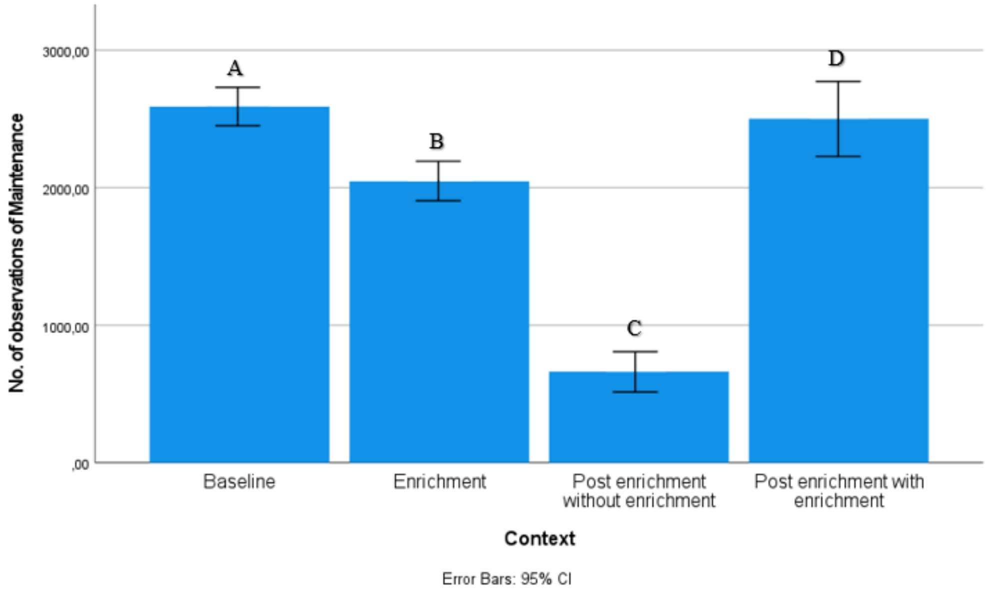

Figure 9. A comparison of the number of times maintenance was observed in each context. Conditions with the same letter are not significantly different at the 0.05 level.

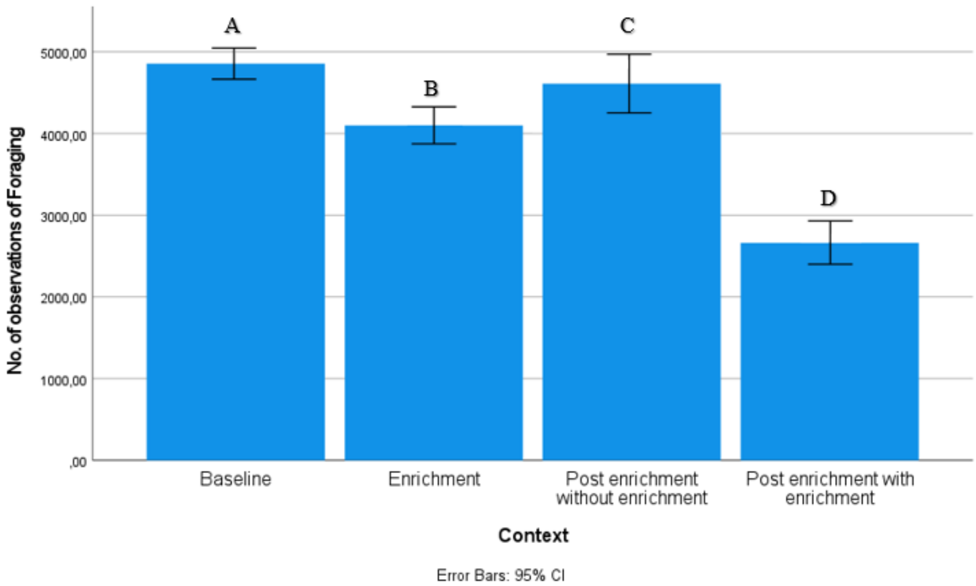

Figure 10. A comparison of the number of times foraging was observed in each context. Conditions with the same letter are not significantly different at the 0.05 level.

Attentive behaviour differed significantly in all comparisons (Baseline vs. Enrichment, $p < 0.001$; Baseline vs. Post enrichment with enrichment, $p = 0.002$; Enrichment vs. Post enrichment without and with enrichment, $p < 0.001$; Post enrichment without enrichment vs. Post enrichment with enrichment, $p < 0.001$), except between baseline and post enrichment without enrichment (p = 0.270), showing an increase in the contexts where the enrichments were present (Figure 11).

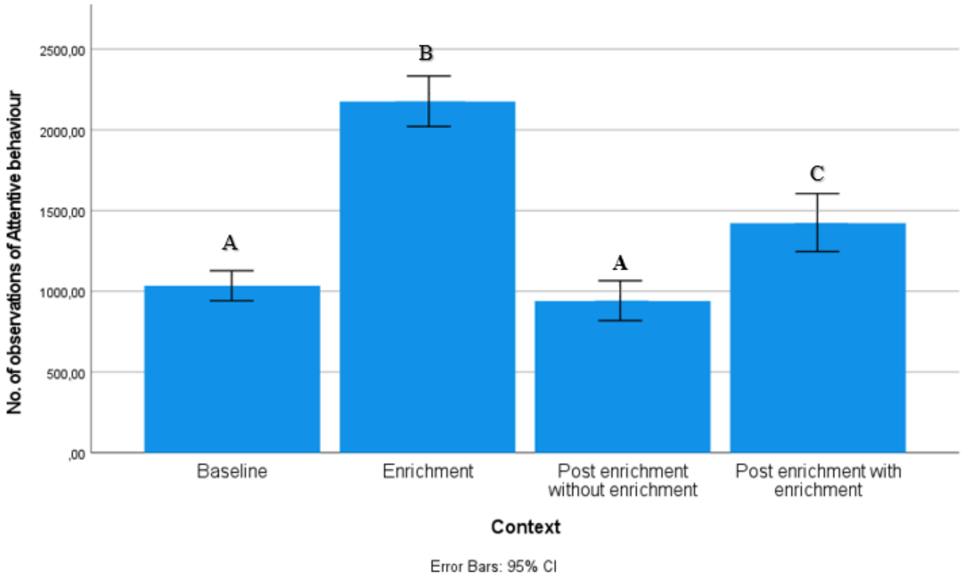

Figure 11. A comparison of the number of times attentive behaviour was observed in each context. Conditions with the same letter are not significantly different at the 0.05 level.

Preening showed significant differences between all pairs of contexts (Baseline vs. Post enrichment without and with enrichment, $p < 0.001$; Enrichment vs. Post enrichment without and with enrichment, $p < 0.001$; Post enrichment without enrichment vs. Post enrichment with enrichment, p = 0.014) with a gradual increase across contexts, with exception of the pair baseline and enrichment that showed no significant differences (p = 0.958) (Figure 12).

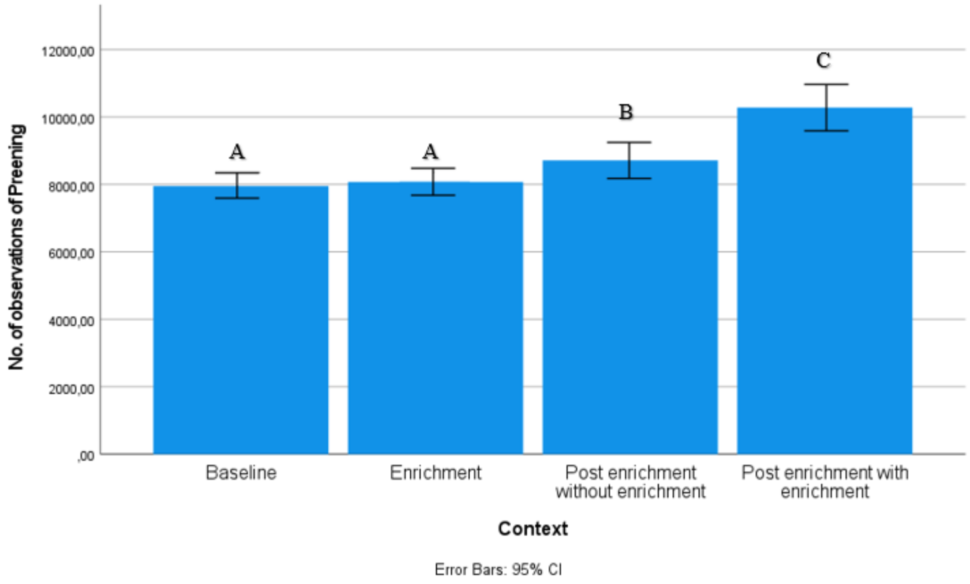

Figure 12. A comparison of the number of times preening was observed in each context. Conditions with the same letter are not significantly different at the 0.05 level.

Manipulation/Play showed significant differences between baseline and enrichment ($p < 0.001$), baseline and post enrichment without enrichment ($p < 0.001$), enrichment and post enrichment with enrichment ($p = 0.004$) and between both post enrichments ($p < 0.001$). It was similar between baseline and post enrichment with enrichment ($p = 0.326$) and between enrichment and post enrichment without enrichment (0.093) (Figure 13).

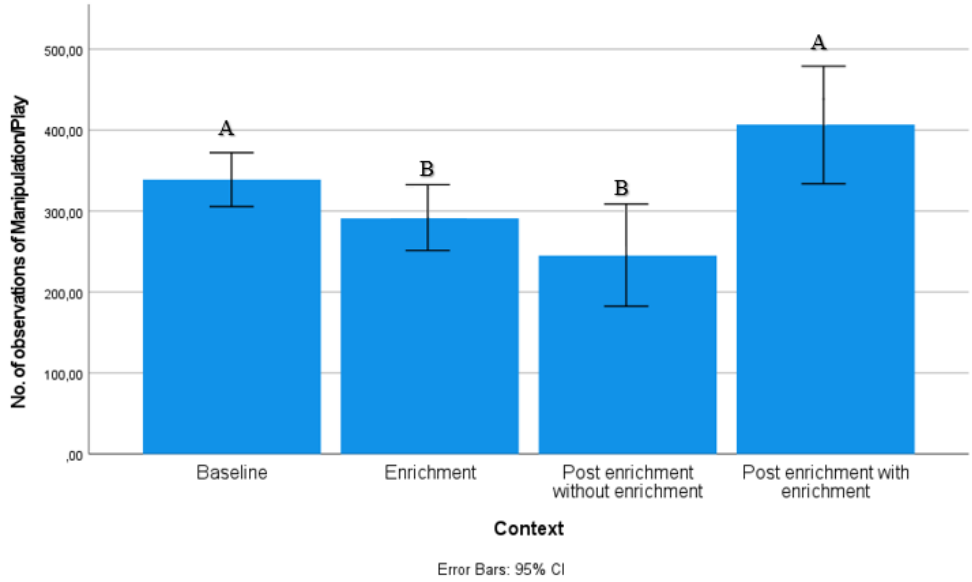

Figure 13. A comparison of the number of times manipulation/play was observed in each context. Conditions with the same letter are not significantly different at the 0.05 level.

Resting increased in the context of post enrichment without enrichment, and therefore showed significant differences between this context and the baseline ($p < 0.001$), enrichment ($p < 0.001$), and post enrichment with enrichment ($p < 0.001$) contexts, showing no differences between the other pairs of contexts ($p > 0.05$) (Figure 14).

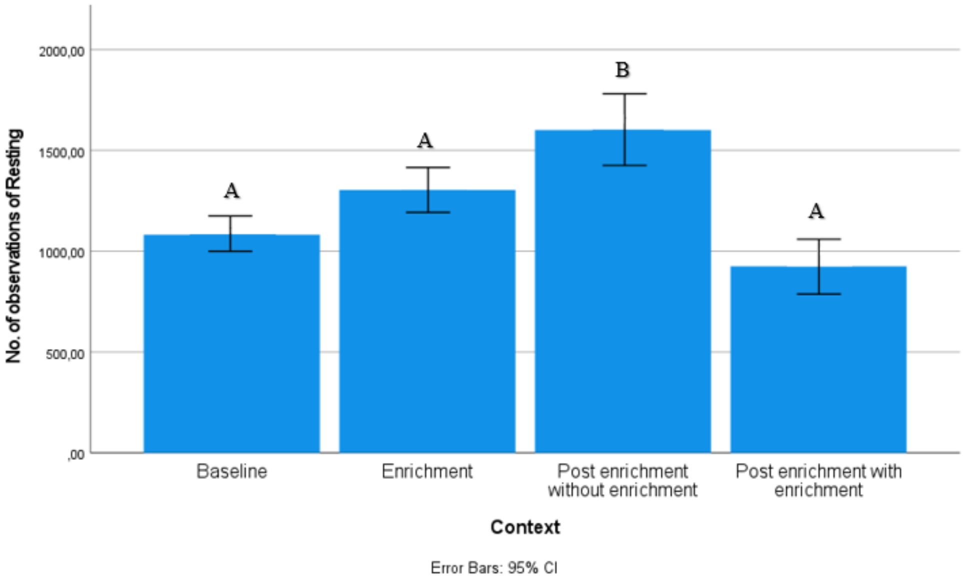

Figure 14. A comparison of the number of times resting was observed in each context. Conditions with the same letter are not significantly different at the 0.05 level.

Exploration remained equal between baseline and post enrichment without enrichment ($p = 0.936$), showing differences between the other comparisons (Baseline vs. Enrichment; Baseline vs. Post enrichment with enrichment, $p < 0.001$; Enrichment vs. Post enrichment without and with enrichment, $p < 0.001$; Post enrichment without enrichment vs. Post enrichment with enrichment, $p < 0.001$), with an increase in the post enrichment with enrichment context (Figure 15).

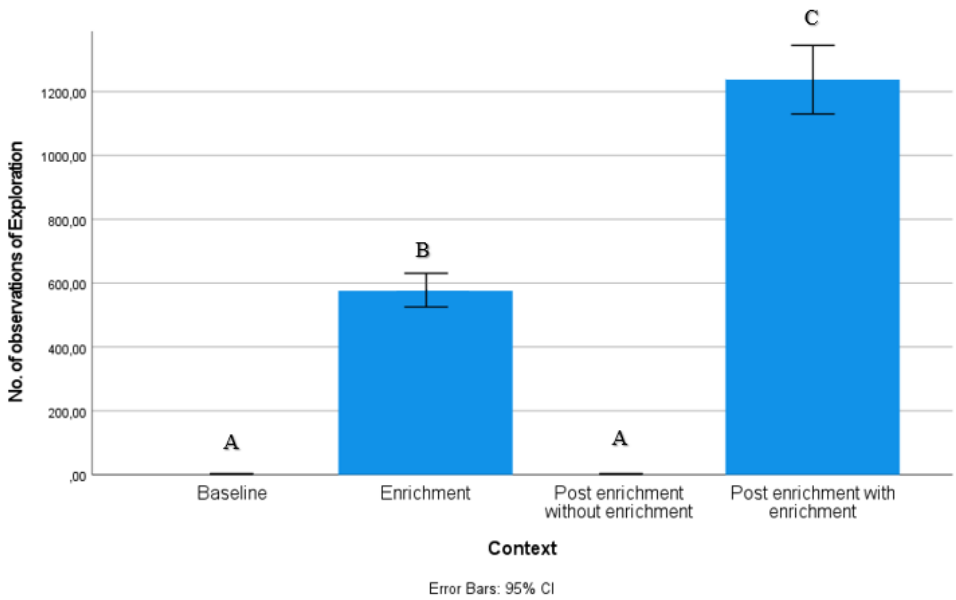

Figure 15. A comparison of the number of times exploration was observed in each context. Conditions with the same letter are not significantly different at the 0.05 level.

Stationary differed significantly in all comparisons where the enrichment was present (Baseline vs. Enrichment, $p < 0.001$; Baseline vs. Post enrichment with enrichment, $p < 0.001$; Enrichment vs. Post enrichment without enrichment, $p = 0.001$; Enrichment vs. Post enrichment with enrichment, $p < 0.001$; Post enrichment without enrichment vs. Post enrichment with enrichment, $p < 0.001$), except between baseline and post enrichment without enrichment ($p = 0.415$), showing a decrease in both enrichment and post enrichment with enrichment (Figure 16).

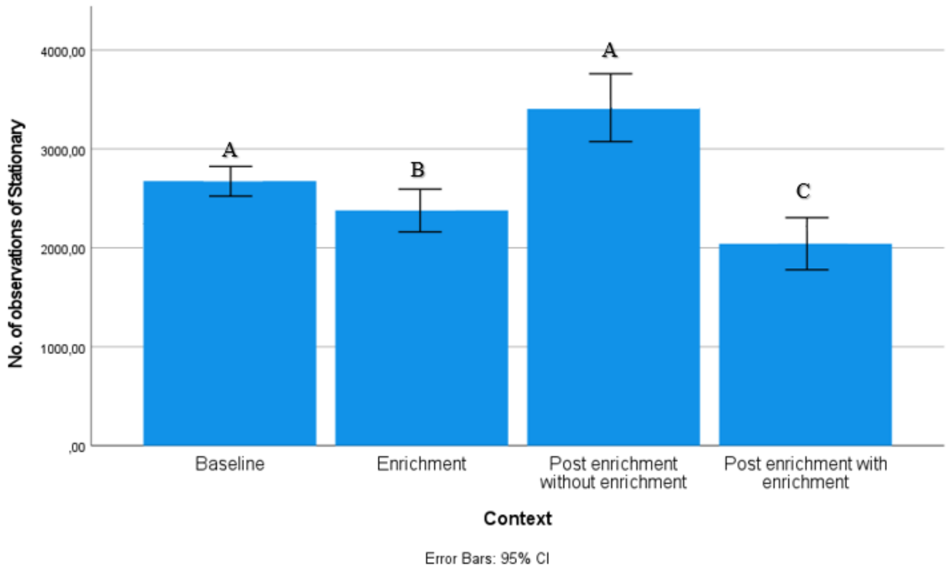

Figure 16. A comparison of the number of times stationary was observed in each context. Conditions with the same letter are not significantly different at the 0.05 level.

3.2. *Comparisons between phases (different types of enrichments applied)*

Through the activity budgets, it is possible to compare the percentages of each behaviour in the different phases (type of enrichment applied) (Figure 17).

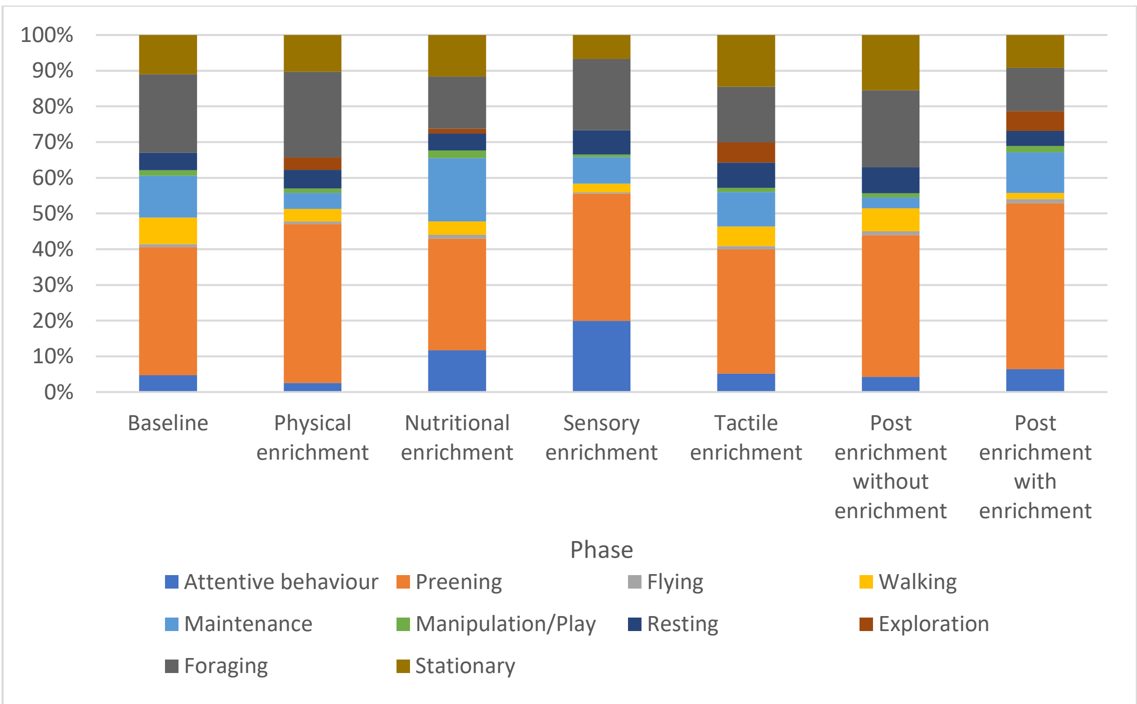

Figure 17. Comparison of the activities budgets of ibises in the different phases throughout the study.

It was observed an increase of attentive behaviour occurrences in phases correspondent to the nutritional (11.58%) and sensory (19.71%) enrichments compared to the other phases (baseline – 4.64%; physical enrichment – 2.55%; tactile enrichment – 5.15%; post enrichment without enrichment – 4.21%; post enrichment with enrichment – 6.37%).

Preening seems constant in all phases, being the behaviour with the highest occurrence.

Concerning the flying behaviour, it showed little variation in the different types of phases, being the behaviour with the lowest occurrence.

Walking was higher at baseline (7.42%), tactile enrichment (5.42%) and post enrichment without enrichment (6.30%).

Maintenance increased when nutritional enrichment (17.52%) was applied compared to all the other phases (baseline – 11.60%; physical enrichment – 4.44%; sensory enrichment – 7.41%; tactile enrichment – 9.66%; post enrichment without enrichment – 3.03%; post enrichment with enrichment – 11.21%).

Manipulation/play and resting showed no change between the different phases.

Exploration, although in lower occurrence, was observed in the presence of the physical (3.45%), nutritional (1.45%) and tactile enrichment (5.53%), and the phase with all the enrichments applied, the post enrichment with enrichment (5.55%).

Foraging decreased in the presence of nutritional (14.40%), sensory (19.78%) and tactile (15.59%) enrichment, and when presented all the enrichments (11.92%), while in physical enrichment it showed a slight increase (23.72%) compared to the baseline (21.76%). However, it was similar at baseline (21.76%) and post enrichment without enrichment (21.24%).

The group of ibises was more stationary in baseline (10.90%), tactile enrichment (14.38%) and post enrichment without enrichment (15.29%) phases.

Regarding statistical analysis, through the Wilcoxon Matched Pairs post hoc, it was possible to observe where the differences related to the contexts were found, when analysing the phases. Therefore, only the behaviours that obtained significant differences between contexts were put for this analysis.

It was observed that the behaviours that previously showed differences in all contexts, walking, maintenance, and foraging, also showed significant differences in almost all phases (Figures 18, 19, and 20). Walking decreased when presented all enrichments, having higher occurrence in baseline and post enrichment without enrichment (Figure 18). Maintenance suffered a decrease in post enrichment without enrichment and an increase in the nutritional enrichment phase (Figure 19). Foraging had a higher occurrence in physical enrichment and lower in the presence of all enrichments (Figure 20).

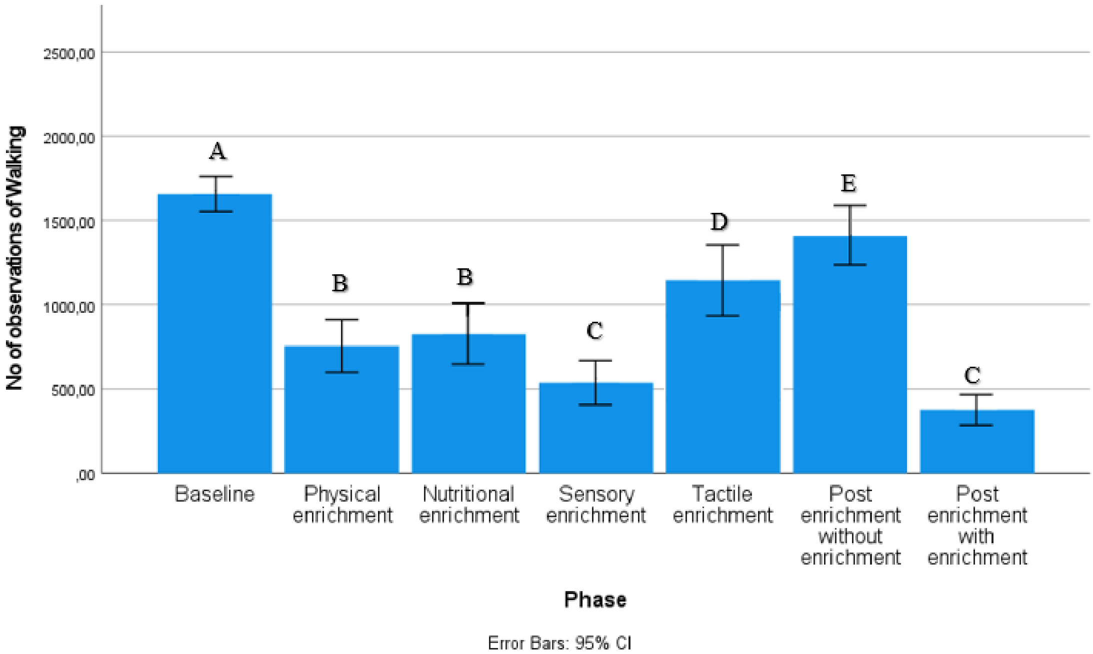

Figure 18. A comparison of the number of times walking was observed in each phase. Conditions with the same letter are not significantly different at the 0.05 level.

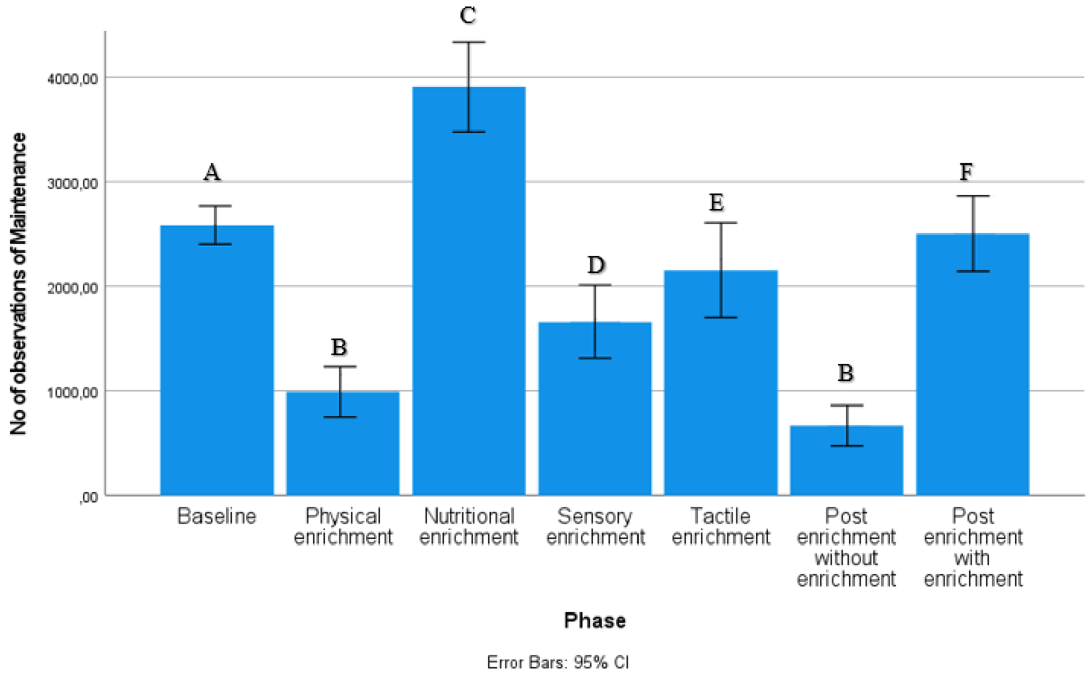

Figure 19. A comparison of the number of times maintenance was observed in each phase. Conditions with the same letter are not significantly different at the 0.05 level.

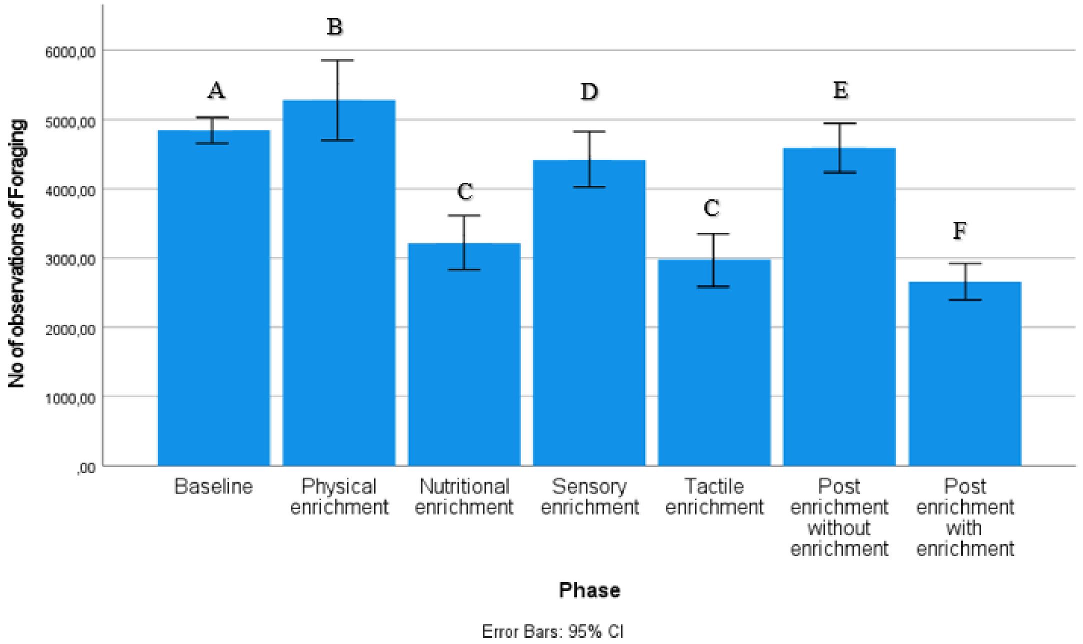

Figure 20. A comparison of the number of times foraging was observed in each phase. Conditions with the same letter are not significantly different at the 0.05 level.

Regarding the attentive behaviour, it was verified that this was lower in physical enrichment, and higher in nutritional and sensory enrichments, and therefore the significant differences were related to these phases. The significant differences were observed between: baseline and physical enrichment ($p = 0.009$), baseline and nutritional enrichment ($p < 0.001$), baseline and sensory enrichment ($p < 0.001$), and baseline and post enrichment with enrichment ($p < 0.001$); physical enrichment and nutritional enrichment ($p < 0.001$), physical enrichment and sensory enrichment ($p < 0.001$), physical enrichment and tactile enrichment ($p < 0.001$), physical enrichment and post enrichment without enrichment ($p < 0.001$), and physical enrichment and post enrichment with enrichment ($p < 0.001$); nutritional enrichment and sensory enrichment ($p < 0.001$), nutritional enrichment and tactile enrichment ($p < 0.001$), nutritional enrichment and post enrichment without enrichment ($p < 0.001$), and nutritional enrichment and post enrichment with enrichment ($p < 0.001$); sensory enrichment and tactile enrichment ($p < 0.001$), sensory enrichment and post enrichment without enrichment ($p < 0.001$), and sensory enrichment and post enrichment with enrichment ($p < 0.001$); tactile enrichment and post enrichment with enrichment ($p = 0.005$); and between both post enrichments ($p < 0.001$); showing no differences between the other phases. There was also an increase from baseline to sensory enrichment and a decrease in the last 3 phases (Figure 21).

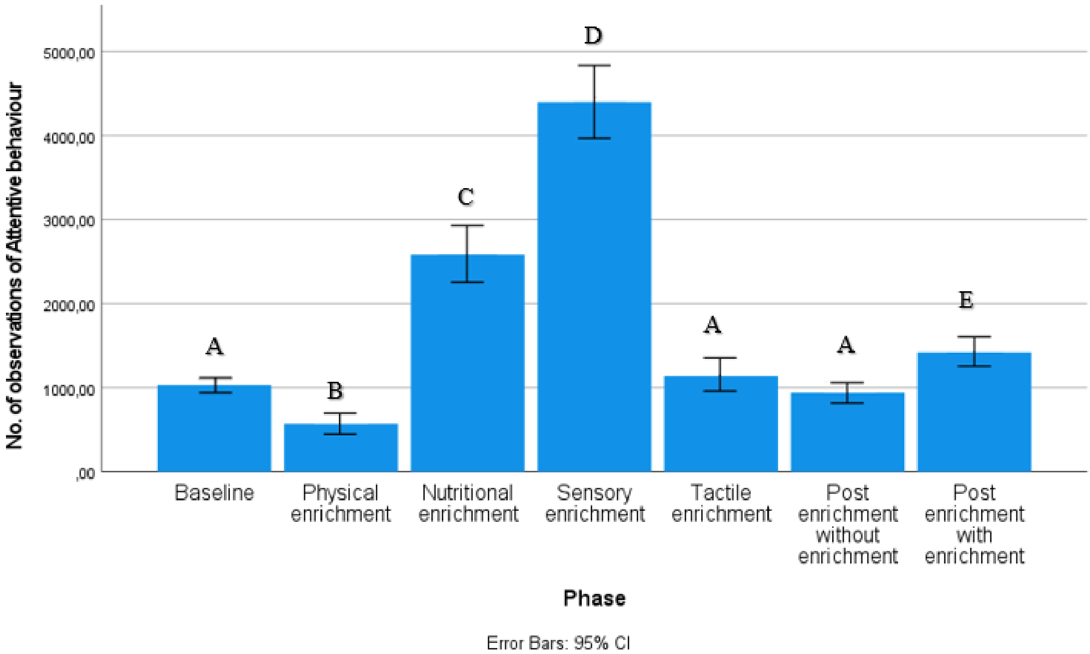

Figure 21. A comparison of the number of times attentive behaviour was observed in each phase. Conditions with the same letter are not significantly different at the 0.05 level.

Concerning the preening behaviour, despite having registered a gradual increase across contexts, a more detailed analysis reveals that it has suffered a decrease in nutritional enrichment, gradually increasing thereafter. The significant differences were then obtained in the comparisons between: baseline and physical enrichment (p = 0.014), baseline and nutritional enrichment (p < 0.001), baseline and post enrichment without enrichment (p < 0.001), and baseline and post enrichment with enrichment (p < 0.001); physical enrichment and nutritional enrichment (p < 0.001), physical enrichment and sensory enrichment (p < 0.001), physical enrichment and tactile enrichment (p < 0.001), and physical and post enrichment without enrichment (p < 0.001); nutritional enrichment and sensory enrichment (p < 0.001), nutritional enrichment and tactile enrichment (p < 0.001), nutritional enrichment and post enrichment without enrichment (p < 0.001), and nutritional enrichment and post enrichment with enrichment (p < 0.001); sensory enrichment and post enrichment without enrichment (p = 0.003), and sensory enrichment and post enrichment with enrichment (p < 0.001); tactile enrichment and post enrichment without enrichment (p < 0.001), and tactile enrichment and post enrichment with enrichment (p < 0.001); and between both post enrichments (p = 0.005) (Figure 22).

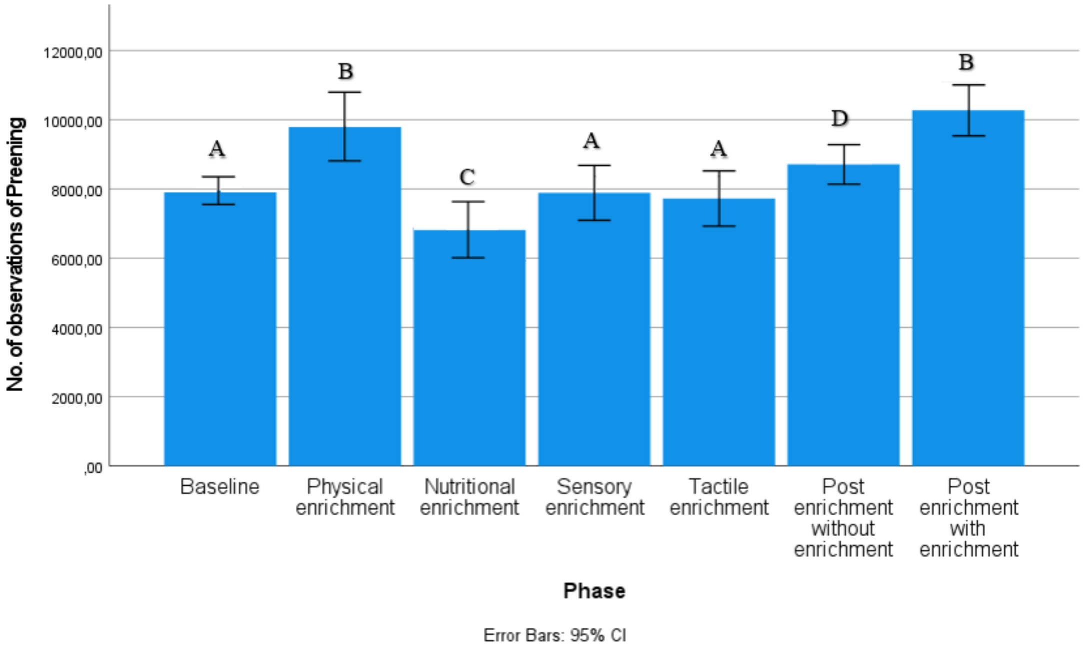

Figure 22. A comparison of the number of times preening was observed in each phase. Conditions with the same letter are not significantly different at the 0.05 level.

Manipulation/Play was presented higher occurrence in nutritional enrichment and lower in sensory enrichment. Thus, it was verified significant differences between: baseline and nutritional enrichment (p = 0.027), baseline and sensory enrichment (p < 0.001), baseline and tactile enrichment (p = 0.010), and baseline and post enrichment without enrichment (p < 0.001); physical enrichment and nutritional enrichment (p = 0.007), and physical enrichment and sensory enrichment (p = 0.004); nutritional enrichment and sensory enrichment (p < 0.001), nutritional enrichment and tactile enrichment (p = 0.009), nutritional enrichment and post enrichment without enrichment (p = 0.014), nutritional enrichment and post enrichment with enrichment (p = 0.011); sensory enrichment and tactile enrichment (p = 0.019); sensory enrichment and post enrichment without enrichment (p = 0.016), and sensory enrichment and post enrichment with enrichment (p = 0.005); tactile enrichment and post enrichment with enrichment (p = 0.003); and between both post enrichments (p = 0.008). It was also verified that manipulation/play presented some variation between baseline, physical enrichment, and nutritional enrichment, with an increase in nutritional enrichment, and a slight decrease in physical enrichment, then decreasing abruptly in the sensory enrichment phase, then registering an increase until the last phase of the study (Figure 23).

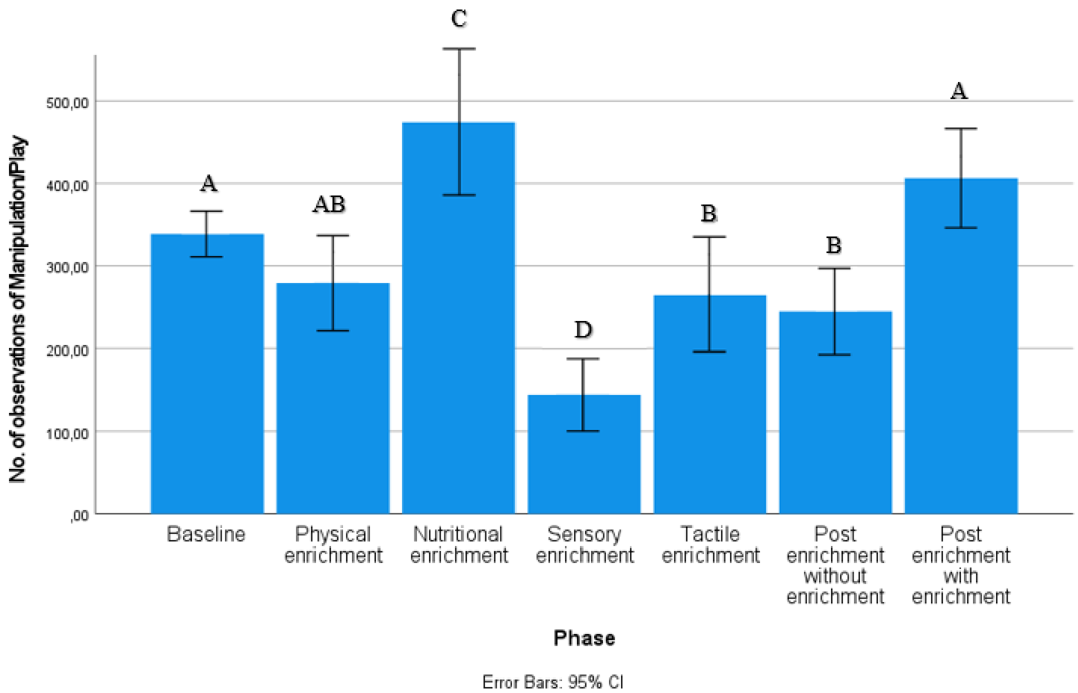

Figure 23. A comparison of the number of times manipulation/play was observed in each phase. Conditions with the same letter are not significantly different at the 0.05 level.

Comparing the resting behaviour in more detail between the different phases, it was verified an increase of this behaviour in the sensory and tactile enrichments and post enrichment without enrichment, and it was lower in the last phase, with all enrichments. It was observed significant differences between: baseline and sensory enrichment (p < 0.001), baseline and tactile enrichment (p < 0.001), and baseline and post enrichment without enrichment (p < 0.001); physical enrichment and sensory enrichment (p < 0.001), physical enrichment and tactile enrichment (p < 0.001), physical enrichment and post enrichment without enrichment (p

= 0.007), and physical enrichment and post enrichment with enrichment (p < 0.001); nutritional enrichment and sensory enrichment (p < 0.001), nutritional enrichment and tactile enrichment (p < 0.001), nutritional enrichment and post enrichment without enrichment (p < 0.001), and nutritional enrichment and post enrichment with enrichment (p = 0.001); sensory enrichment and post enrichment with enrichment (p < 0.001); tactile enrichment and post enrichment with enrichment (p < 0.001); and between both post enrichments (p < 0.001); thus verifying a higher occurrence in post enrichment without enrichment, and a decrease in nutritional enrichment and in post enrichment with enrichment (Figure 24).

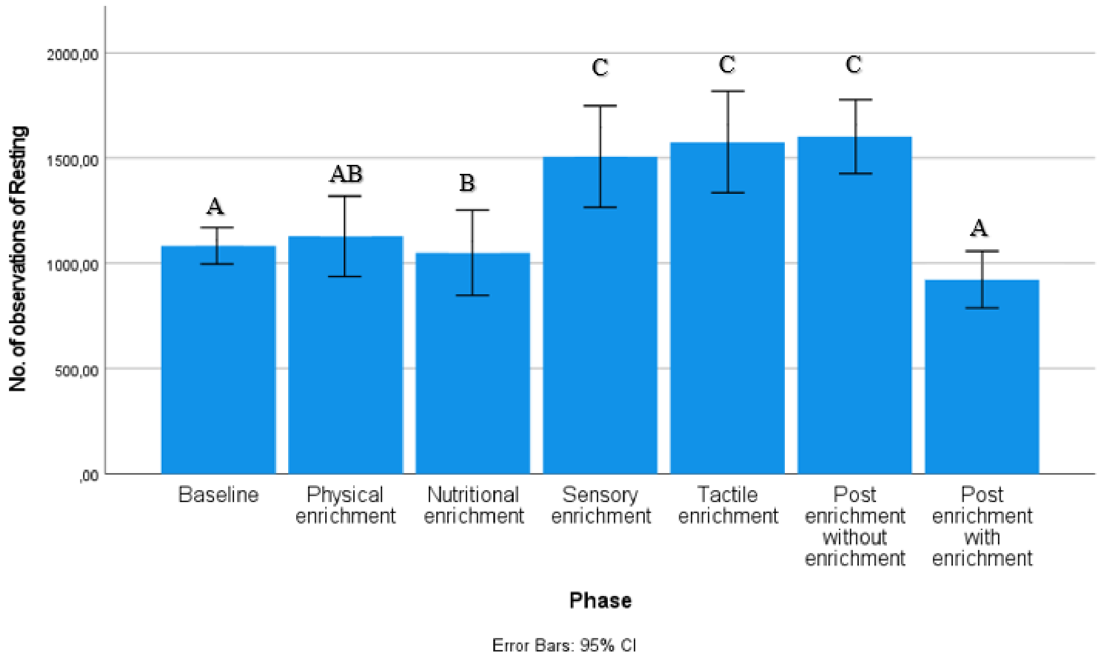

Figure 24. A comparison of the number of times resting was observed in each phase. Conditions with the same letter are not significantly different at the 0.05 level.

Exploration only occurs in the presence of physical, nutritional, and tactile enrichments, and in the presence of all. The significant differences were obtained between: baseline and physical enrichment (p < 0.001), baseline and nutritional enrichment (p < 0.001), baseline and tactile enrichment (p < 0.001), and baseline and post enrichment with enrichment (p < 0.001); physical enrichment and nutritional enrichment (p < 0.001), physical enrichment and sensory enrichment (p < 0.001), physical enrichment and tactile enrichment (p = 0.003), physical and post enrichment without enrichment (p < 0.001), and physical enrichment and post enrichment with enrichment (p = 0.001); nutritional enrichment and sensory enrichment (p < 0.001), nutritional enrichment and tactile enrichment (p < 0.001), nutritional enrichment and post enrichment without enrichment (p < 0.001), and nutritional enrichment and post enrichment with enrichment ($p < 0.001$); sensory enrichment and tactile enrichment ($p < 0.001$), and sensory enrichment and post enrichment with enrichment ($p < 0.001$); tactile enrichment post enrichment without enrichment ($p < 0.001$); and between both post enrichments ($p < 0.001$); registering a decrease between physical and sensory enrichment, and an increase in tactile and post enrichment with enrichment. (Figure 25).

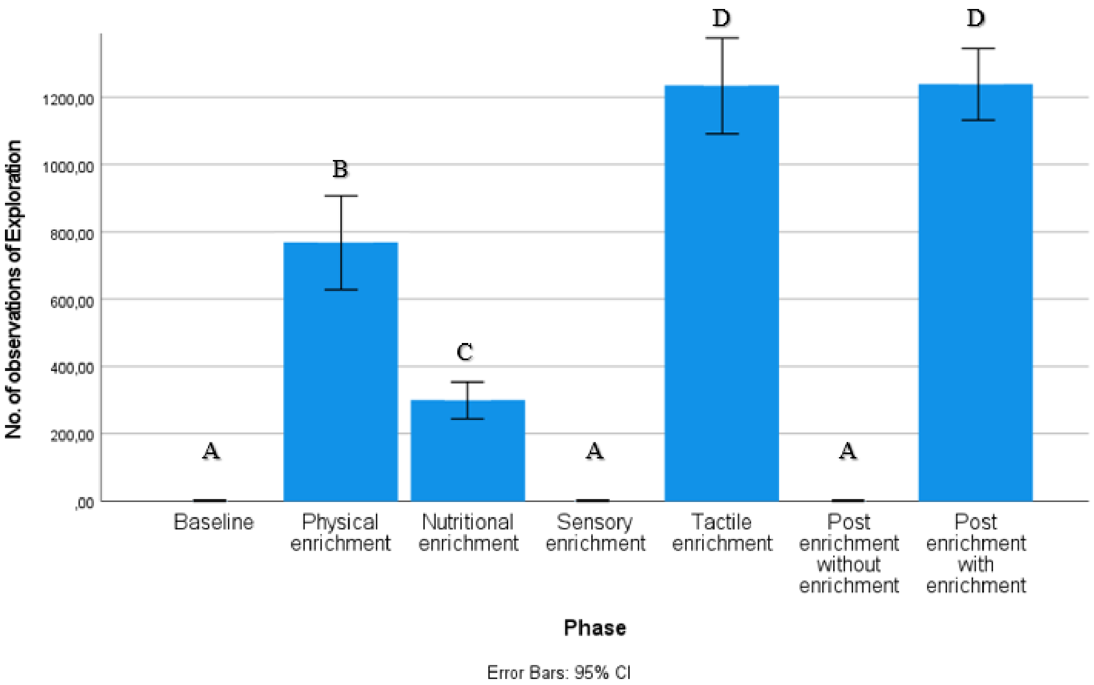

Figure 25. A comparison of the number of times exploration was observed in each phase. Conditions with the same letter are not significantly different at the 0.05 level.

Stationary decreased in physical, sensory and post enrichment with enrichment enrichments, so the significant differences obtained seem to be related to these phases. Thus, there were significant differences between: baseline and physical enrichment ($p = 0.018$), baseline and sensory enrichment ($p < 0.001$), baseline and tactile enrichment ($p < 0.001$), and baseline and post enrichment with enrichment ($p < 0.001$); physical enrichment and nutritional enrichment ($p < 0.001$), physical enrichment and sensory enrichment ($p < 0.001$), physical enrichment and tactile enrichment ($p < 0.001$), physical and post enrichment without enrichment ($p = 0.001$), and physical enrichment and post enrichment with enrichment ($p = 0.001$); nutritional enrichment and sensory enrichment ($p < 0.001$), nutritional enrichment and tactile enrichment ($p < 0.001$), nutritional enrichment and post enrichment without enrichment ($p < 0.001$), and nutritional enrichment and post enrichment with enrichment ($p < 0.001$); sensory enrichment and tactile enrichment ($p < 0.001$), sensory enrichment and post enrichment without enrichment ($p < 0.001$), and sensory enrichment and post enrichment with enrichment (p < 0.001); tactile enrichment and post enrichment with enrichment (p < 0.001); and between both post enrichments (p < 0.001); thus, it was verified that some decreases occur until the sensory enrichment phase (physical and sensory enrichment), and then it increases again in the following phases (tactile enrichment and post enrichment without enrichment), decreasing in the last one (Figure 26).

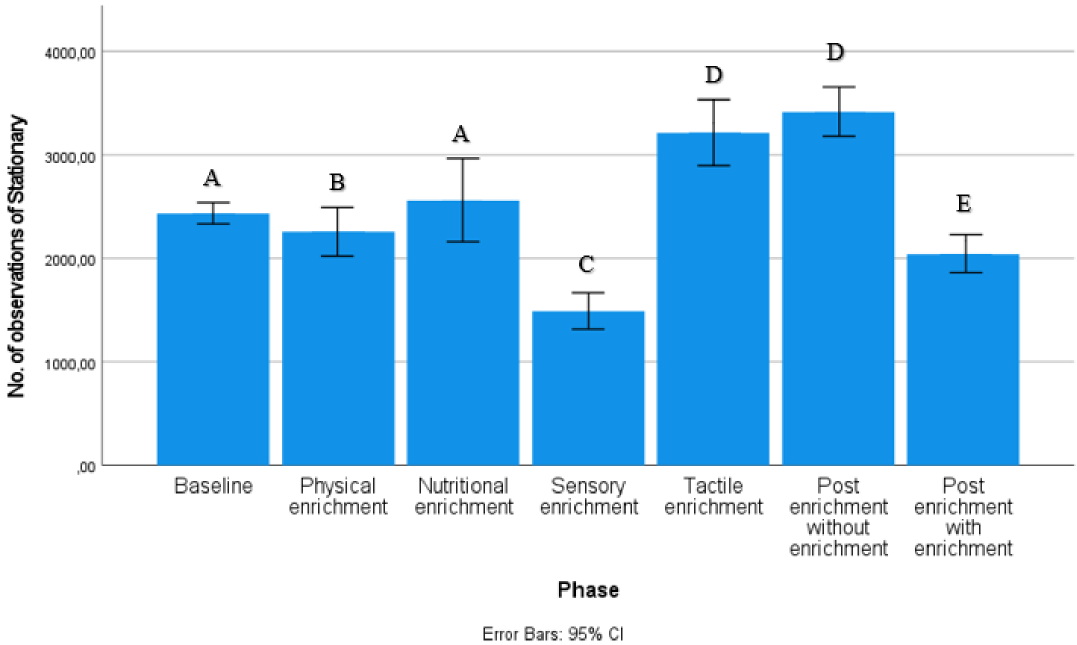

Figure 26. A comparison of the number of times stationary was observed in each phase. Conditions with the same letter are not significantly different at the 0.05 level.

3.3. *Comparison between phases – Chi-square test by Monte Carlo simulation*

There were significant differences in the behaviours exhibited by ibises depending on the phase of the study (χ^2 (54) = 5960.981, p < 0.001). Table 3 shows the behaviours that happened more than expected, by the Monte Carlo simulation, in the different phases of the study.

Table 3. The number of occurrences of each behaviour in each phase. The bold values represent the behaviours that appeared more than expected and were considered significant by the Z-score of Chi-Square test (z-score > 1.96).

Behaviour	**Phase**						
	Baseline	Physical enrichment	Nutritional enrichment	Sensory enrichment	Tactile enrichment	Post enrichment without enrichment	Post enrichment with enrichment
Attentive behaviour	1035	114	**517**	**880**	230	314	475
Preening	7954	**1958**	1378	1578	1545	**2912**	**3434**
Flight	171	32	49	15	41	**85**	**86**
Walking	**1657**	151	165	107	242	**470**	126
Maintenance	**2590**	198	**782**	331	431	226	**836**
Manipulation/Play	339	56	**95**	29	53	82	**136**
Resting	1082	226	210	**301**	**315**	**535**	309
Exploration	0	**154**	60	0	**247**	0	**414**
Foraging	**4856**	**1059**	643	883	696	**1584**	889
Stationary	2432	451	513	298	**642**	**1140**	682

Here is possible to verify that attentive behaviour increased in nutritional and sensory enrichment. Walking appears more than expected when there is no enrichment (baseline and post enrichment without enrichment). It is also observed that, although exploration is only present in the presence of physical, nutritional, tactile enrichment and with all enrichments, it occurred more than expected in the physical, tactile and post enrichment with enrichment phases. Foraging occurs more than expected in the baseline, physical enrichment and post enrichment without enrichment.

3.4. *Comparisons between time of day*

In general, the period of the day also had influence in the behaviour of ibises, in particular: attentive behaviour ($H(2) = 8.341$, $p = 0.015$), preening ($H(2) = 145.362$, $p < 0.001$), flight ($H(2) = 36.857$, $p < 0.001$), walking ($H(2) = 232.259$, $p < 0.001$), maintenance ($H(2) = 295.886$, $p < 0.001$), manipulation/play ($H(2) = 127.855$, $p < 0.001$), resting ($H(2) = 609.687$,

$p < 0.001$), exploration ($H(2) = 139.837$, $p = 0.000$), foraging ($H(2) = 805.473$, $p = 0.000$), and stationary ($H(2) = 278.732$, $p = 0.000$).

In general, more active behaviours (such as walking, maintenance, exploration and foraging) were observed in the morning and midday periods, compared to the afternoon, when there was an increase in preening, resting and stationary behaviours (Figures 27, 28 and 29).

Note: The results of Kruskal-Wallis pairwise test for time of day are available in Annex C1.

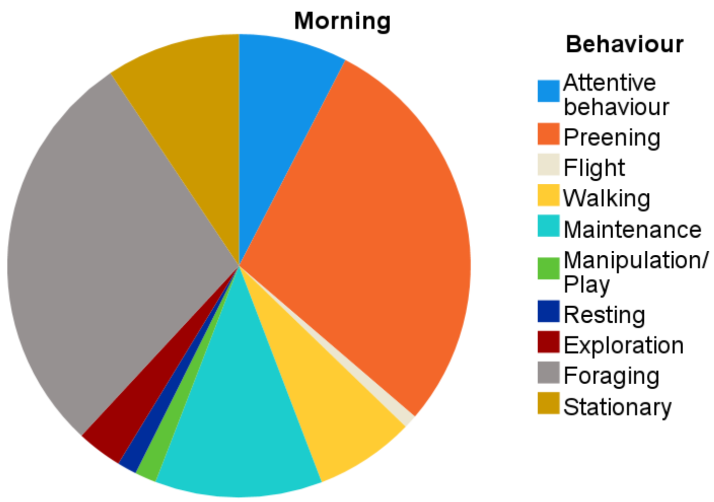

Figure 28. Proportion of behaviours exhibited in the morning period.

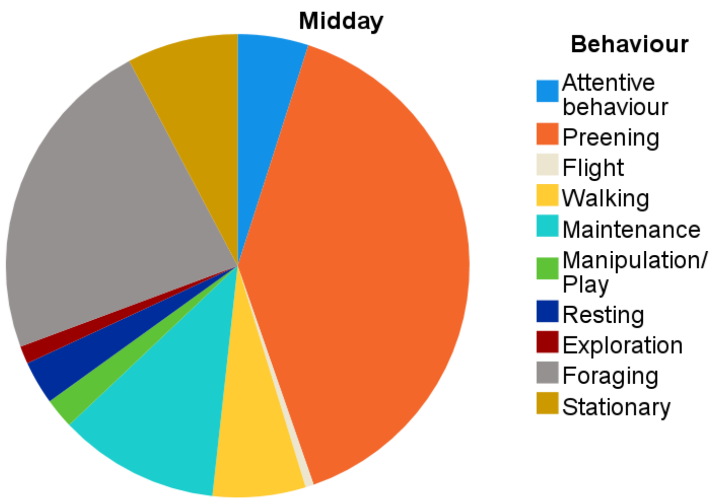

Figure 27. Proportion of behaviours exhibited in the midday period.

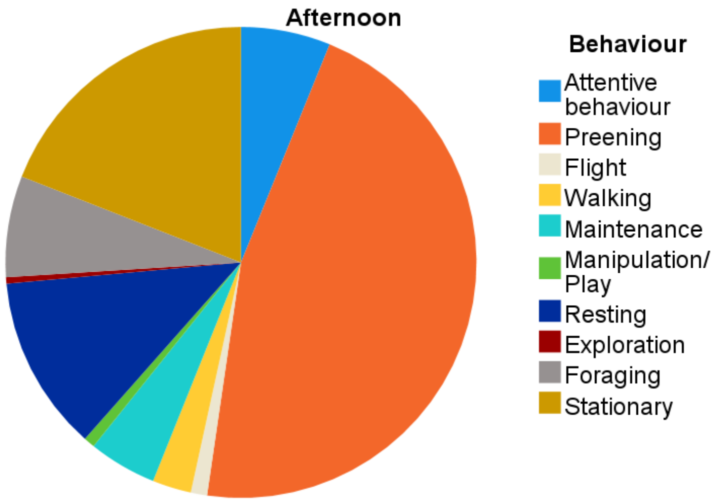

Figure 29. Proportion of behaviours exhibited in the afternoon period.

3.5. *Comparisons between weather conditions*

As the study, despite its short duration, was subject to different atmospheric conditions, an analysis was made to understand if these changes had any effect on the ibises' behaviours. Among the most representative behaviours, there were significant differences in: attentive behaviour ($H(4) = 112.900$, $p < 0.001$), preening ($H(4) = 73.875$, $p < 0.001$), walking ($H(4) = 185.884$, $p < 0.001$), maintenance ($H(4) = 165.082$, $p < 0.001$), manipulation/play ($H(4) = 35.795$, $p < 0.001$), resting ($H(4) = 16.263$, $p = 0.003$), exploration ($H(4) = 139.484$, $p < 0.001$), foraging ($H(4) = 194.748$, $p < 0.001$), and stationary ($H(4) = 73.364$, $p < 0.001$).

It was verified then that the weather influences the behaviour of the ibises, being in general more active in periods without rain and wind (rainy, windy/cloudy and storm conditions).

In sunny and cloudy conditions, the behaviour of the ibises was similar (Figures 31 and 32). In rainy and windy conditions (Figures 33 and 34), it was found that the foraging behaviour (one of the behaviours with the highest percentage in the activities budget) suffered a marked decrease, compared to sunny and cloudy conditions. It was also found that under stormy conditions (Figure 35), the ibises are completely inactive, only exhibiting attentive, preening, resting and stationary behaviours.

Note: The results of Kruskal-Wallis pairwise test for weather conditions are available in Annex C2.

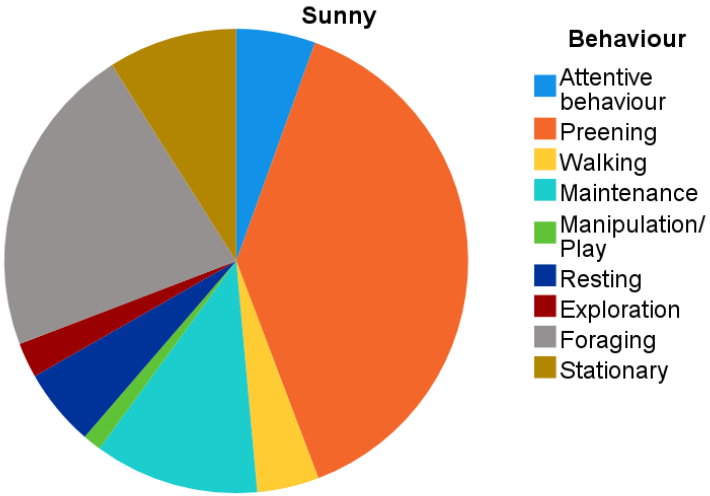

Figure 32. Proportion of behaviours exhibited in sunny conditions.

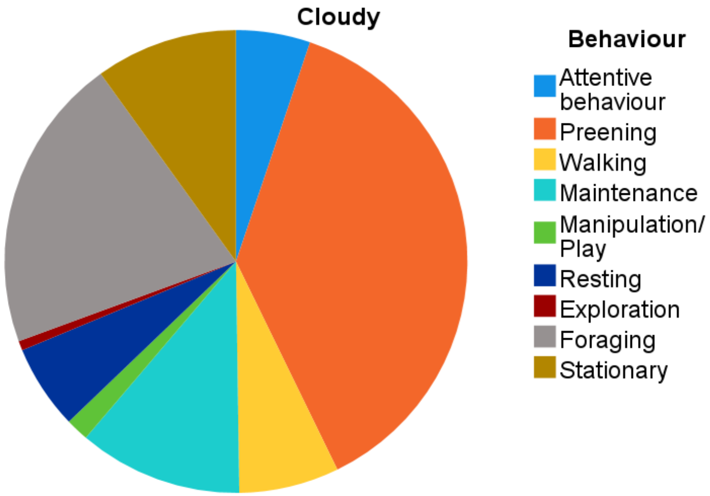

Figure 31. Proportion of behaviours exhibited in cloudy conditions.

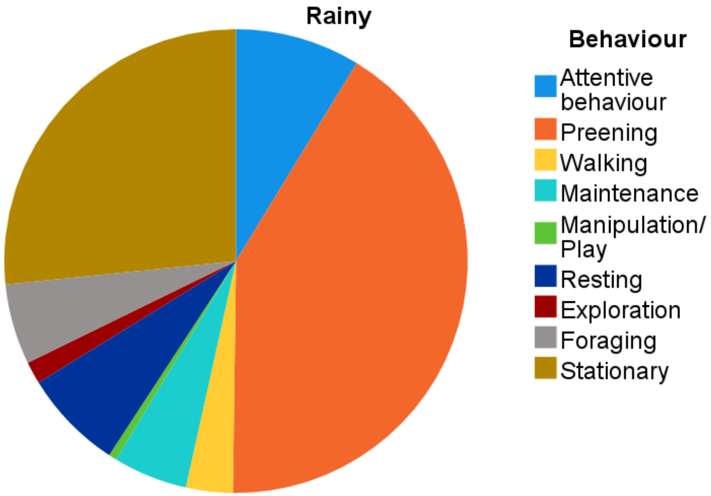

Figure 30. Proportion of behaviours exhibited in rainy conditions.

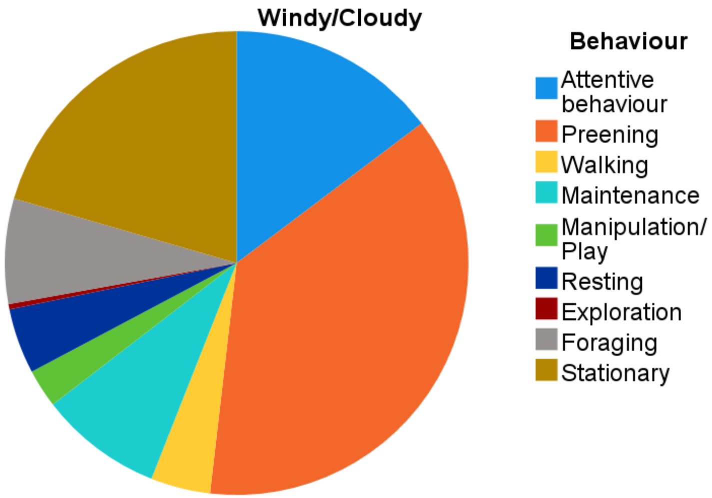

Figure 33. Proportion of behaviours exhibited in windy/cloudy conditions.

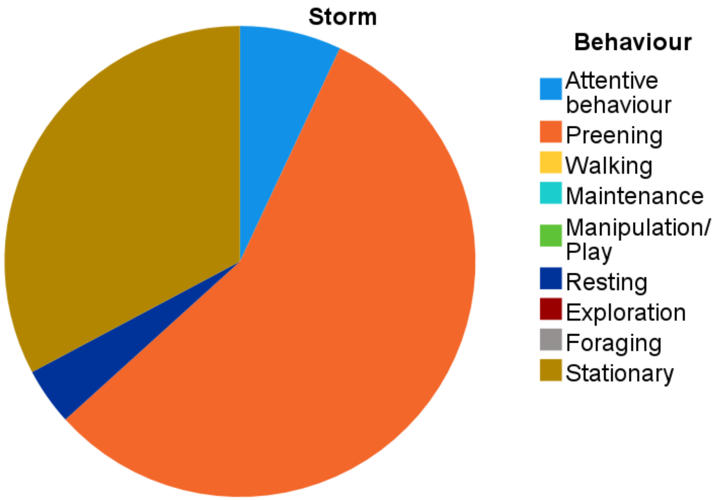

Figure 34. Proportion of behaviours exhibited in stormy conditions.

3.6. *Comparisons between areas*

The imaginary separation of space by areas was made just to facilitate data collection. Also, it was expected that behaviours will change depending if the bird was on the ground or in the trees. Here, the significant differences were obtained in: attentive behaviour ($H(3) = 577.293$, $p < 0.001$), preening ($H(3) = 2832.306$, $p < 0.001$), flight ($H(3) = 534.693$, $p < 0.001$), walking ($H(3) = 838.277$, $p < 0.001$), maintenance ($H(3) = 2972.147$, $p < 0.001$), manipulation/play ($H(3) = 297.167$, $p < 0.001$), resting ($H(3) = 2094.498$, $p < 0.001$), exploration ($H(3) = 472.985$, $p < 0.001$), foraging ($H(3) = 2801.363$, $p < 0.001$), and stationary ($H(3) = 1975.062$, $p < 0.001$).

It was verified that in the trees (Area 4) the predominant behaviours were preening, resting and stationary (Figure 38). On the ground, this is where there is greater activity, as it is in these places that they feed and move through the habitat. The similarity between Area 1 and Area 2, makes the behaviours almost similar between the two, however, regarding maintenance, there was a clear preference for Area 1 (Figures 35 and 36). In Area 3, the walkway, the predominant behaviours were walking and foraging (Figure 37).

Note: The results of Kruskal-Wallis pairwise test for the areas are available in Annex C3.

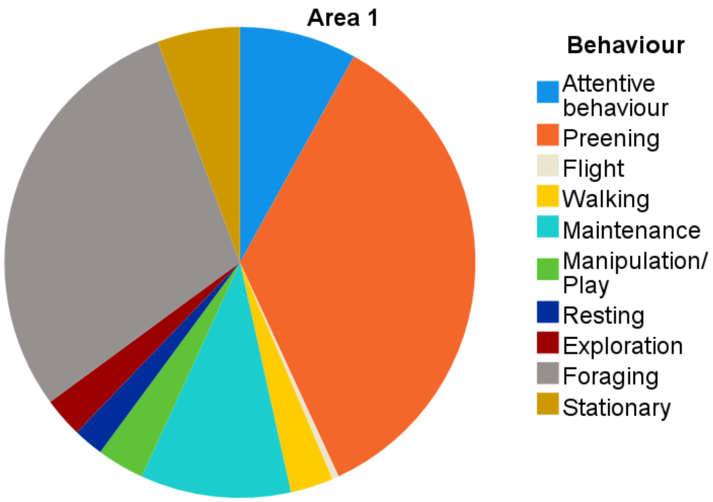

Figure 36. Proportion of behaviours exhibited in Area 1.

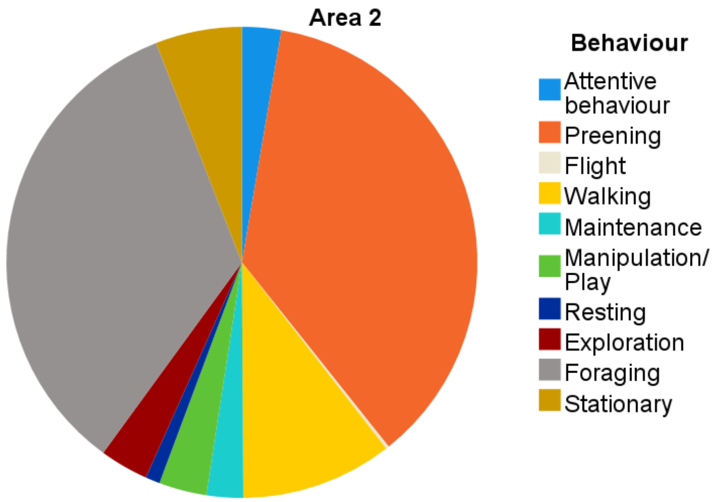

Figure 35. Proportion of behaviours exhibited in Area 2.

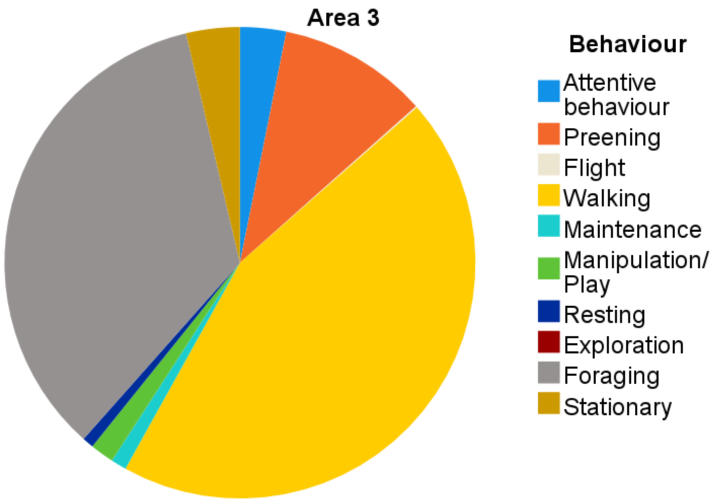

Figure 37. Proportion of behaviours exhibited in Area 3.

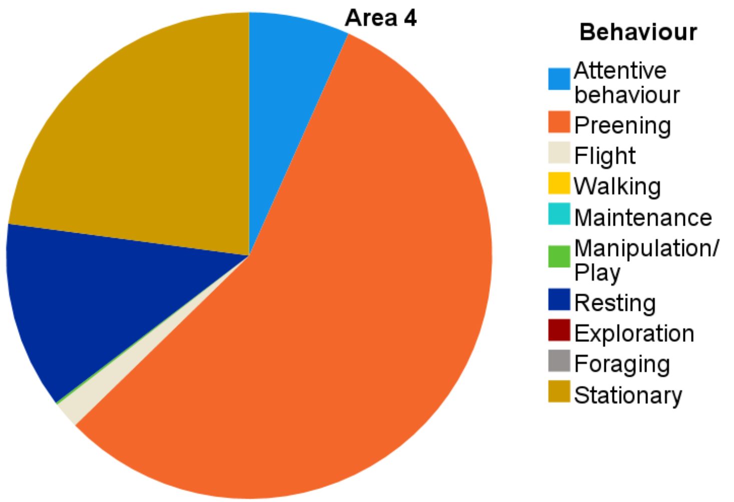

Figure 38. Proportion of behaviours exhibited in Area 4.

4. Discussion

To analyse the effects of environmental enrichment on the behaviour of aquatic birds in captivity, a study was conducted at Zoomarine, Guia, in the 'Americas' habitat. This habitat is characterized by an environment that tries to recreate a typical forest in southern America, where several bird species are present. The species chosen for this study was the scarlet ibis, with a total of 16 individuals. Studies with environmental enrichment with captive birds are less common than other groups of animals, for example, mammals (King, 1993), increasing the importance of studies like the present one to increase the knowledge and understand which enrichment designs/materials may affect or benefit these birds in this context.

For this project, four types of enrichment were applied to verify their effect on the behaviour of the ibises: physical, nutritional, sensory, and tactile enrichment.

To understand the behaviours exhibited by the scarlet ibis in a captive environment, it was used an ethogram of another species of ibis, the Northern bald ibis (Spiezo et al., 2018), and adapted to the one in the present study. Stereotyped behaviours described for some captive birds, such as feather pulling or constantly grooming (Breed & Sanchez, 2010), were not observed during data collection and so, were not included in the ethogram. This may be a result of a more naturalistic environment for these specimens at Zoomarine, with more space to perform their behaviours and some characteristics that are similar to their natural habitat and intra-species interactions.

In the present study, the purpose of enrichment was to increase the different stimuli presented in the environment that may cause changes in the behavioural patterns of these animals, allowing the display of a higher number of natural behaviours (Young, 2003).

4.1. *Comparisons between contexts*

The environmental enrichment techniques applied in this study showed an effect on some behaviours exhibited by scarlet ibises, namely the solitary behaviours, as attentive behaviour, preening, walking, maintenance, manipulation/play, resting, exploration, foraging and stationary. From these, walking, maintenance and foraging behaviours exhibited significant differences in all comparisons, not providing information about the true effect of the enrichment. For these behaviours, it was not possible to evaluate if the behaviour differences were happening due to enrichment or not. Only the behaviours that showed similarities in some comparisons and differences in others were selected, to understand the origin of these events as behaviours that showed significant differences between all contexts would not have a clear explanation. These three behaviours: walking, maintenance and foraging, should be better studied in future projects to understand the relation between the enrichment and baseline, so it can be possible to adapt and improve the habitats in zoological institutions.

In a comparison between contexts, it would be expected that baseline and post enrichment without enrichment contexts would have a similar response in terms of behaviours since the latter is often considered a second baseline (Ings et al., 1997; Shepherdson et al., 1993; Young, 2003). However, here it was found that this was not always the case, verifying that 50% of the analysed behaviours showed significant differences between the two contexts.

Of the behaviours analysed, it was verified that attentive behaviour and exploration remained the same in both contexts (baseline and post enrichment without enrichment), which was expected as a result of a predictable environment, meaning that these animals have no reason to be alert or searching for new stimuli, individuals or predators, as opposed to adding something new to their environment (enrichment phases), that stimulates their curiosity. Exploration is a behaviour of curiosity, elicited in the presence of new, novel, or different stimulus (Welker, 1956), and hence is associated with the presence of enrichment. The fact that the stationary behaviour does not differ in both, seems to reveal that after the removal of the stimuli, the animals return to their normal activity level, following the prediction of some studies, in which after the enrichment period, the animals return to their previous patterns, exhibiting more rest behaviours such as sleeping and resting (Shepherdson et al., 1993; Young, 2003). Similar results were obtained by Vargas-Ashby & Pankhurst (2007), that noticed that stationary behaviour of Northern bald ibis significantly decreased in the presence of feeding enrichment, increasing active behaviours, such as walking, flying and foraging.

In the case of preening, this was a behaviour that remained equal in baseline and enrichment, and then showed a linear increase across contexts, and hence across time, which may suggest an independent factor causing the differences (Young, 2003). In this case, the increase in photoperiod from the enrichment phases to the post-enrichment phases (January, February, and March), although not very accentuated, may have influenced this behaviour, since, in natural environments, ibises tend to be more active in periods of lesser light during the day, early in the morning and before nightfall due to foraging and feeding (Ffrench &

Haverschmidt, 1970), not having this need in captivity, since the food is delivered at specific times and earlier than sunset. Therefore, the increase in light during the day can make the birds more likely to preen themselves, taking advantage of the light to keep warm. In addition, ibis typically tends to feed before 7 am and after 5 pm (Spil et al., 1985), a condition that in a zoological institution it may not be possible to apply as the schedules and routines are dependent on the caretakers.

It was also verified that the manipulation/play behaviour remained the same between baseline and post enrichment with enrichment contexts and between enrichment and post enrichment without enrichment contexts. The similarity between the two pairs, although contradictory at the first glance, may be explained by the fact that in the presence of several types of enrichment the ibises are more susceptible to explore than manipulate or play, as none of the enrichment devices introduced was designed to be played with.

One unanticipated result was the fact that there were no significant differences in resting between baseline and both enrichment and post enrichment with enrichment. It would be expected that there was a decrease in resting in these two enrichment contexts (Shepherdson et al., 1993; Young, 2003), which happened, but not in enough number to be significant. Further work is necessary to understand what variables may be acting on resting in these birds.

4.2. *Comparisons between phases*

Comparing the different phases of the study it was observed that the differences obtained in attentive behaviour were related to physical, nutritional, and sensory enrichment. It was observed that attentive behaviour was displayed in lower numbers in the physical enrichment perhaps since the tidal simulation is not something that will alert them. However, in terms of nutritional and sensory enrichment, it is the opposite, with an increase in this behaviour. This increase in nutritional enrichment may be related to the fact that the food provided in this enrichment is different from their usual diet, which calls for greater interest and attention, thus also leading to immediate consumption after its presentation. In a study with elephants, it was also verified that changing a small portion of their diet resulted in an immediate response, and therefore shows that there is greater interest/attention to new items in the diet (Stoinski et al., 2000).

Regarding the preening behaviour, it was observed that the most significant differences were related to the nutritional enrichment with fewer behavioural displays in this phase. These differences were to be expected, since, in the presence of this type of enrichment, behaviours associated with feeding increased, and some inactive behaviours, such as preening and resting, decrease, as described previously (Shepherdson et al., 1993; Vargas-Ashby & Pankhurst, 2007).

Exploration did not show differences between baseline and sensory enrichment; and between sensory enrichment and post without enrichment, which was expected, since the sounds themselves did generate a more exploratory behaviour by the ibises, but rather greater attention to exploring from whom/where the sound comes (AZA Charadriiformes Taxon Advisory Group, 2014). At the same time, this behaviour did not show differences between tactile enrichment and post enrichment with enrichment, thus suggesting that tactile enrichment may have a greater influence on ibises in terms of exploration display.

The significant differences obtained in stationary behaviour comparisons were related to physical and sensory enrichments, and post enrichment with enrichment. In these phases, there was a decrease in this behaviour. In physical enrichment, this behaviour has decreased by the displacement to the water to explore and forage in the lakes. In the case of sensory enrichment, this decrease may be related to the increase in attentive behaviour. However, this behaviour also turns out to be more inactive (because the birds were standing still when they were alert), so it cannot be inferred that this enrichment influences the level of activity of the birds. In the final phase of enrichment, with all the enrichment, there is a marked decrease in this behaviour, which reveals that the presence of several types of enrichment simultaneously leads to a higher level of activity in the birds.

4.3. *Effect of each enrichment*

The context of enrichment aimed to verify the effect that each enrichment has on the behaviour of ibises (i.e., an evaluation of behaviours directed to the type of enrichment). Analysing the effect of each type of enrichment in the behaviour of ibises it was possible to observe that they seem to influence their behaviour in different ways. Physical enrichment (i.e., tidal simulation) had a greater effect on preening, exploration, and foraging. The descent of the tide allowed the exploration of the lake, as they were able to access the deepest part of it, and also made some pieces of food from the feeding period more visible, also increasing foraging. Also, these behaviours are within the expected trend for behaviours elicited by tidal simulation, which are normally foraging, probing, walking, and wading (AZA Charadriiformes Taxon Advisory Group, 2014).

As expected, nutritional enrichment caused an increase in eating (maintenance) (Vargas-Ashby & Pankhurst, 2007), but also in manipulation/play. As this type of enrichment was provided in the form of a "cage", the animals had to manipulate the device to reach the food, increasing the engagement of the animals towards the device. This increase in feeding behaviour can also be seen as an increase in the number of times the ibises move to feed autonomously. In addition to feeding sessions, various dishes with a specific granulate distributed throughout the habitat were also available, but with only occasional displacements to these dishes, and eating more in the presence of the trainers. However, in this study, autonomous feeding and feeding in the presence of trainers were not taken into account, so this distinction is suggested as a suggestion variable for future works to determine whether an increase in autonomous feeding occurs in the presence of enrichment.

Sensory enrichment caused an increase in attentive behaviour. Normally, providing birds vocalizations aims to elicit vocalizations and search behaviours in birds (AZA Charadriiformes Taxon Advisory Group, 2014). Here, it was observed that the sounds caught the attention of the ibises maybe because of the feeling of a bigger group, since these calls may, for example, function to maintain group cohesion (e.g., through attraction to foraging sites) (Suzuki et al., 2017), and hence they were more attentive and looking for the presence of more birds. Despite not being present in the analyses as they were not of a representative value, it was observed that ibises started to vocalize more after this type of enrichment, which may suggest some interest or curiosity from the birds, however, more studies are needed to learn the relation between this enrichment and these birds.

Tactile enrichment had a particular effect on resting, exploration, and stationary behaviours. As it is something completely different from what these birds were used to, there is a greater tendency to explore what is new (Welker, 1956). However, it was also observed that, in the presence of this type of enrichment, the resting and stationary behaviours were greater than expected, which may indicate that, despite being something new and different, it eventually may cause habituation (i.e., “response decrement as a result of repeated stimulation” (Harris, 1943)) and the “novelty effect” quickly disappears. For example, the presentation of olfactory enrichment in captive black-footed cats led to an increase in the percentage of time spent in active behaviours, such as moving and exploration, but the effect quickly decreased by the third day of exposure, causing habituation to the stimuli. One way to avoid habituation to the tactile enrichment, in this study the brushes, maybe providing it occasionally as a way to renew interest in this stimulus, as it will again increase the ibises' curiosity and hence their exploratory behaviour (Wells & Egli, 2004).

The pause of 5 days between each phase, in addition to being mandatory due to pandemic conditions, was also important to counteract the cumulative effects of enrichment. Continuous presentation of stimuli may result in overstimulated birds before the new enrichment device because they have already been subjected to a previous one, which can lead to hyperactivity, stress or even less exploration of new stimuli, as has already been observed in mice (Christakis et al., 2012). Thus, this pause allows the birds to return to their normal behaviour before the next stimuli presentation.

The last phase had as its main goal to understand how the animals reacted to all stimuli at the same time. Furthermore, it allows us to see if there is a good reaction to the presence of various stimuli since these are intended to continue to be applied in the habitat by the caretakers. Thus, it was found that in this phase there was a more similar proportion of the different behaviours, apart from preening, which increased, revealing a greater balance between the behaviours. However, it should be noted that although the provision of several stimuli simultaneously can produce strong results, does not allow the distinction of the effect of each one (Swaisgood & Shepherdson, 2006), hence, initially, the individual stimuli were provided.

4.4. *Effects of time of day, weather condition, and area*

Regarding the activity budget for this species, little is known on how behaviours are displayed, both in terms of duration and frequency. However, it was described in the natural environment, that these birds tend to forage in flocks early in the morning and before nightfall, being more inactive between these periods (Ffrench & Haverschmidt, 1970). Similar results were obtained in captive animals by Spil et al. (1985), however, they did not explore which other behaviours were also displayed, focusing only on foraging, and resting. In the present study, it was also verified that the behaviours displayed in the highest numbers were both foraging and preening, however, regarding the time of day, it was found that they had more active behaviours in the morning and midday periods, and less active in the afternoon, which contradicts the mentioned studies. These differences may be due to the temporal proximity between morning and midday periods, and hence the similarity of behaviours. The afternoon period was chosen to fit the trainers' schedule and routines and may not represent a true afternoon period as it was from 4:00 pm to 5:00 pm and the sunset was between 5:05 pm and 5:45 pm until January, and between 5:55 pm and 6:45 pm, from February to March, expecting more activity around these hours, and therefore have resulted in greater inactivity in this period considered as afternoon. Moreover, at this time, animals were already fed and may be preparing for the night. These differences may also result from the fact that the behaviour of animals in captivity may differ from those exhibited in the natural environment, as a result of a more predictable environment (Martin, 1999).

Weather conditions can influence the behaviour of birds (e.g. Boyer & MacDougall-Shackleton, 2020; Grubb, 1977, Mainwaring et al., 2021). Here it was verified that the weather influenced the behaviour of the ibises, being, in general, more active in periods without rain and wind. Normally, in windy conditions, birds tend to forage less and are therefore more inactive than in sunny conditions (Grubb, 1977). Rain also can affect the hunting effort and foraging success in birds, as a result of their prey's decreased activity (Dawson & Bortolotti, 2000; Mainwaring et al., 2021). Here, although there are no prey animals, it was found that the ibises were also more inactive in the presence of rain, not engaging in foraging behaviour.

Regarding the area, it was verified that in the trees the predominant behaviours were preening, resting and stationary, which was expected since here, it is the resting area for the animals (Ffrench & Haverschmidt, 1970). On the ground, this is where there is greater activity, as it is in these places that they feed and move through the habitat.

4.5. *Conclusions and recommendations to future studies*

In short, this study demonstrates that the use of these enrichments may promote changes in the behaviours displayed by ibises. It is important to emphasize that environmental enrichment has the function of providing the promotion of new stimuli, which must be appealing and stimulating according to the needs of the animals (Shepherdson et al., 1998). However, a systematic rotation and re-evaluation of enrichment plans are also essential, either by the way or frequency in which they are provided, or even by providing different enrichment types, to avoid possible habituation. Thus, environmental enrichment must be a dynamic process that responds to the needs of animals. In the present study, nutritional enrichment is the easiest to cause behavioural changes, as it will always have some impact on feeding behaviour and could be something beneficial to the animals at a nutritional level (Swaisgood &

Shepherdson, 2006). At the same time, the physical and tactile enrichment also showed interesting results, causing a more exploratory behaviour in these animals, which is why enrichments that cause behavioural changes concerning their environment (by creating more stimulating environments) should also be favoured to cause greater exploration of the environment in which they live. On the other hand, among the enrichments applied, perhaps the sensory enrichment, the vocalizations, is the one of less interest, since it only arouses a greater degree of attention, and therefore not something that is ideal with these animals in such context. However, the appearance of vocalizations after displaying this stimulus should be a factor for further study to understand whether it was a consequence of this stimulus or a result of temporal change since the breeding season starts in mid-spring (Ffrench & Haverschmidt, 1970; Hughes & Owen, 1988). Animals may have started to exhibit more social behaviours (vocalizing to each other) as spring approached. One way to understand this will be through the provision of vocalizations at a different period long before the breeding season, such as in winter, to check whether the number of vocalizations occurs in the same frequency or even if there are differences between sounds' parameters and characteristics.

The short duration of the study may influence the conclusions obtained, so as a future direction, longer duration studies are suggested to better understand the cumulative effect of enrichment on behaviour. Thus, it is possible to see if the observed effects result only from a "novelty effect" and then disappear (which appeared to be the case with tactile enrichment) or if they remain.

Nevertheless, it should be noted that just with behavioural analysis it is not possible to effectively assess whether there was an improvement in welfare related to enrichment. Here only the behaviour of the animals was evaluated, verifying that behavioural changes occur in the presence of enrichment, which is not necessarily an improvement in welfare. The analysis of physiological indicators can provide more data on welfare, and by combining the behavioural data, it can result in a better welfare assessment (Maple & Perdue, 2012). Furthermore, animal welfare refers to the state of an individual, being measured at the individual level (Broom, 1986, 1991), not the group level. In this study, behavioural effects were assessed at the group level as identification of the animals was not possible, and so it is another factor that does not allow us to conclude whether there was an effect on the welfare of the individuals. Thus, future work that intends to evaluate the effect of environmental enrichment on these birds should focus on individuals.

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6. Annexes

A. State of the art

The science of animal welfare

The definition of animal welfare is relatively recent and appeared in the 1960s due to the concern expressed about the conditions in which farm animals were treated. Since then, has been expanded to other captive animals in different environments such as laboratories, domestic, zoos and aquariums (Broom, 2011). One of the major difficulties of animal welfare science is the definition of animal welfare itself. Numerous definitions are differentiated by the emphasis given to different characteristics. Some scientists consider the health and functioning of animals as one of the main criteria to assess animal welfare, focusing on whether there are diseases or injuries and their ability to function biologically within limits. For others, natural behaviour and the natural way of living is emphasized, that is, providing opportunities for the animals to perform their natural behaviour and have natural elements in their environment. Other cases are emphasized the affective states of animals, that is, the feelings of the animal about itself and its surrounding environment (Fraser, 2008; Young, 2003). All these definitions are valid as well as their combination, however, the focus should be on its goals, instead of its definition, which is: to maintain the animal in good physical and psychological health (Young, 2003).

One of the first concepts to ensure that animals experience an acceptable level of welfare is the Five Freedoms developed by the UK's Farm Animal Welfare Council in 1979. This concept refers that animals in captivity should have: freedom from thirst and hunger (through the provision of food and water), freedom from discomfort (by providing an appropriate environment), freedom from pain, injury and disease (by providing animal health care), freedom from fear and distress (by providing protection, through proper handling and treatment) and freedom to express normal behaviours (by providing proper facilities and areas big enough so animals can exhibit their specific behaviours, like jumps and runs). These principles are recognized worldwide as a reference for assessing welfare. Concerning the first four freedoms, their absence results in physical and psychological problems, so they are used as indicators of animal welfare (Maple & Perdue, 2012; Young, 2003). However, the last freedom, to express normal behaviours, it's more difficult to correlate with good or bad behaviour as a stand-alone measure and sometimes justify as an indicator of animal welfare, since it doesn't result in physical improvements that can be measurable, and also because the non-performance of some wild behaviours doesn't compromise the animal welfare, as the fact of doesn't exist predation risk in captivity, so the animals don't need to stay alert for predators (Veasey et al., 1996; Young, 2003). Although, behavioural assessment is vital to investigate the welfare of animals in captivity when combined with other methods (Hill & Broom, 2009). Yet, the increase in scientific knowledge suggests that instead of focusing on whether all these freedoms are guaranteed, attention should be given to whether animals have "a life worth living". For this to happen, it's necessary to minimize their negative experiences, providing opportunities to have positive experiences, which are rewarding for the animals (Mellor, 2016). The model that includes all these areas and positive and negative states is known as the Five Domains model (that includes physical/functional domains – nutrition, environment, health and behaviour; and affective experience domains – mental state) (Figure 39), which is used, not only but greatly in the zoo community. This Five Domains model (Figures 40, 41, 42 and 43) is a redefinition of the Five Freedoms, that emphasizes the role of positive and negative experiences in the animal's welfare status, instead of the absence of something (Binding, 2020; Mellor & Reid, 1994).

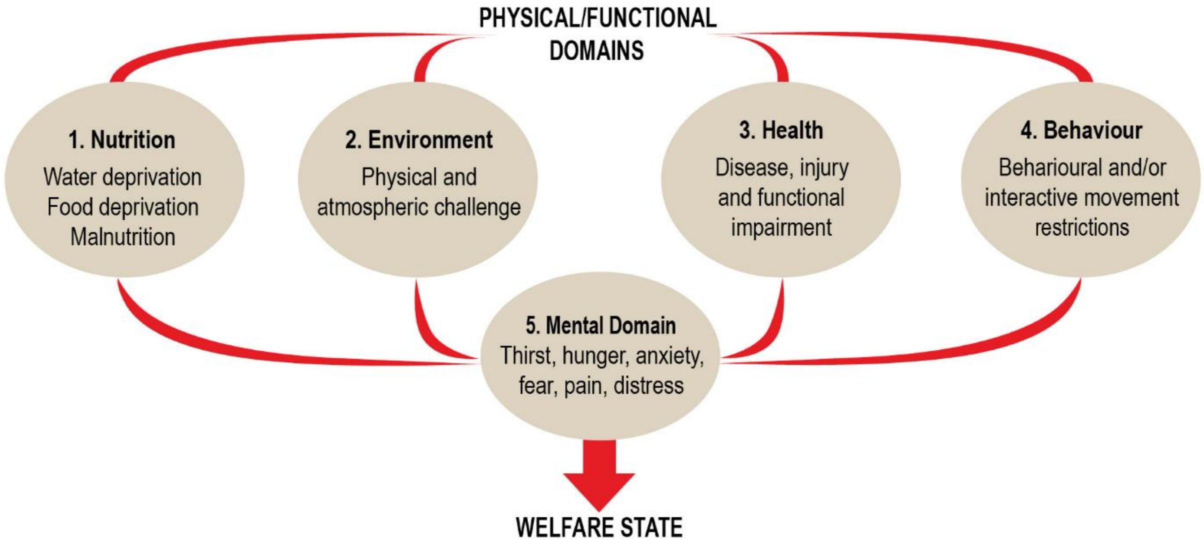


Figure 39. Schematic representation of the basic structure of the 1994 Five Domains model: Physical/Functional Domains (1- Nutrition, 2- Environment, 3- Health, 4- Behaviour) and Affective Experience Domains (5- Mental State) (retrieved from Mellor, 2020).

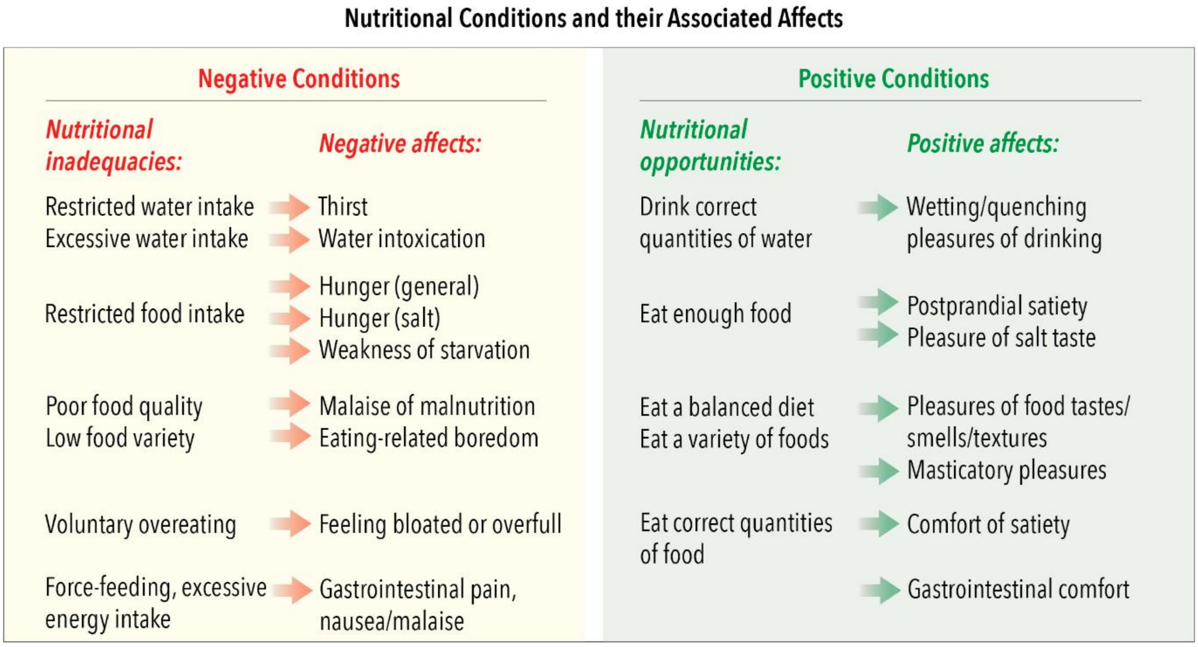


Figure 40. Domain 1: Nutritional conditions and their associated affects (retrieved from Mellor et al., 2020).

Physical Environmental Conditions and their Associated Affects

Negative Conditions		Positive Conditions	
Unavoidable physical conditions:	***Negative affects - forms of discomfort:***	***Enhanced physical conditions:***	***Positive affects - forms of comfort:***
Close confinement; overcrowding	*Physical:* general stiffness, muscle tension	Space for spontaneous locomotion	*Physical* comfort
Unsuitable substrate, wet/soiled ground	*Physical:* musculoskeletal pain, skin irritation	Suitable substrate, well-drained ground	*Physical* comfort
Air pollutants: NH_3, CO_2, dust, smoke	*Respiratory:* breathlessness, air passage irritation/pain	Fresh air dissipates contaminants	*Respiratory* comfort
Aversive odours	*Olfactory:* revulsion at foul or repellent odours	Foul smells dissipated by fresh air & good hygiene	*Olfactory* comfort
Thermal extremes	*Thermal:* chilling, dampness, overheating	Effective shelter and shade available	*Thermal* comfort
Loud or otherwise unpleasant noise	*Auditory:* impaired hearing or ear pain	Effective noise control measures are in place	*Auditory* comfort
Light: inappropriate intensity	*Visual:* eye strain due to flashing, glare or darkness	Light intensity kept at tolerable levels	*Visual* comfort
Monotony: ambient, physical, lighting	Malaise from unnatural constancy	Within-day environmental variability maintained	Congenial variety and predictability
Unpredictable events	Anxiety, fear, hypervigilance	Predictability achieved by established routines	Relaxation-based ease and calmness
Physical limits on rest and sleep	Exhaustion	Conditions conducive to rest and sleep	Well rested

Figure 41. Domain 2: Physical environmental conditions and their associated affects (retrieved from Mellor et al., 2020).

Health Conditions and their Associated Affects

Negative Conditions		Positive Conditions	
Presence of:	***Negative affects:***	***Minimal or no:***	***Positive affects:***
Injury: acute, chronic, husbandry mutilations *Disease:* acute, chronic	Pain (many types), breathlessness, debility, weakness, sickness, malaise, nausea, dizziness	Injury	*Comfort* of good health and functional capacity
		Disease	*Comfort* of good health and functional capacity
Functional impairment: due to limb amputation, other therapies; genetic, lung, heart, vascular, kidney, gut, neural, or other problems		Functional impairment	*Comfort* of good health and functional capacity
Obesity or leanness: physical and metabolic consequences	Affects of being too fat or thin, and of metabolic and pathophysiological sequelae	Extreme body condition scores	*Comfort* of good health and functional capacity
Poisons	Many affects due to mode of action	Poisoning	*Comfort* of good health and functional capacity
Poor physical fitness, muscle de-conditioning	Physical weakness and exhaustion	Poor fitness (fitness level good)	Vitality of fitness and pleasurably vigorous exercise

Figure 42. Domain 3: Health conditions and their associated affects (retrieved from Mellor et al., 2020).

Behavioural Interactions and their Associated Affects

INTERACTIONS WITH THE ENVIRONMENT

Exercise of 'agency' is impeded:	*Negative affects:*	*Exercise of 'agency' is promoted*	*Positive affects:*
Invariant, barren, confined environment (ambient, physical, biotic)	Boredom, helplessness Depression, withdrawal	Varied, novel environment	Interested, pleasantly occupied
Inescapable sensory impositions Choices markedly restricted Environment-focussed activity constrained Foraging drive impeded	Various combinations: startled by unexpected events, neophobia, hypervigilance, anger, frustration, negative cognitive bias	Congenial sensory inputs	Likes novelty, post-inhibitory rebound
		Available engaging choices	Calm, in control
		Free movement	Engaged by activity
		Exploration, foraging	Energised, focussed

INTERACTIONS WITH OTHER ANIMALS

Animal-to-animal interactive activity constrained	Loneliness, depression Yearning for company	Bonding/reaffirming bonds	Affectionate sociability
		Rearing young	Maternal, paternal or group rewards
	Thwarted desire to play	Playing	Excitation/playfulness
	Sexual frustration	Sexual activity	Sexually gratified
	Thwarted hunting drive	Hunting	Alert engagement, highly stimulated
Significant threats Limits on threat avoidance, escape or defensive activity	Anger, anxiety, fear, panic, insecurity, neophobia	Absence of threats Using refuges, retreat or defensive attack	Secure, protected, confident
Limitations on sleep/rest	Exhaustion	Sleep/rest sufficient	Energised, refreshed; post-inhibitory rebound

INTERACTIONS WITH HUMANS

Negative human attributes and behaviour:	*Animal behaviours and negative affects:*	*Positive human attributes and behaviour:*	*Animal behaviours and positive affects:*
Attitude: uncertain, fearful, indifferent, insensitive, impatient, oppressive, belligerent, domineering, callous, cruel, vindictive *Voice:* hesitant, angry, loud, shouting *Aptitude:* inexperienced, unskilled, untrained, unqualified	*Behaviours (e.g.):* long flight distance, hypervigilant, attack/ fight, hyper-reactive, escape avoidance, freezing, cowering, appeasing, withdrawn, non-compliant	*Attitude:* confident, caring, sensitive, patient, kind, empathetic *Voice:* confident, calm, clear, encouraging, pleasantly rhythmic *Aptitude:* experienced, skilled, trained, qualified	*Behaviours:* short flight distance, calm alertness, at ease with imposed hands-off or hands-on contact, compliantly responsive, explores novel events, seeks contact, variably bonded with humans
Handling/controlling: erratic, rough (slap, hit, kick, grab, poke, beat, whip); excessively forceful, violent; punishment-focussed; more negative pressure than is needed for training objective	*Affects:* anxiety, fear, panic, terror, neophobia; insecurity, confusion, uncertainty, persistent unease; helplessness; pain from injuries; negative cognitive bias	*Handling/controlling:* skillful, gentle (stroke, touch, push, guide); firm, temperate, restrained; reward-focussed; mimics allo-grooming by conspecifics; using subtle pressure cues, secondary reinforcers and timely release of aversive stimuli	*Affects:* calm, confident, at ease, feels in control; enjoys variety; finds being bonded with humans rewarding

Figure 43. Domain 4: Behavioural interactions and their associated affects (retrieved from Mellor et al., 2020).

This model emphasizes the subjective experiences of animals (i.e., their affects), recognizing that specific physiological mechanisms interact with the animals' affective states, with negative affects compromising welfare and positive affects increasing welfare (Mellor et al., 2020).

In the context of captivity, animals are subject to different conditions from which species naturally evolved (Martin, 1999). Several factors compromise the animal's welfare, such as confinement to limited space and easy access to food, which can cause monotony for animals, and sometimes lead to the expression of abnormal behaviours, with potential consequences both physical and psychological. For example, the inability to express natural behaviour for birds in captivity can lead to an obsessive-compulsive disorder, such as pulling off their feathers, or grooming, causing physical damage (Breed & Sanchez, 2010; Martin, 1999).

To counteract these welfare problems associated with the captive environment, environmental enrichment techniques are used to increase the quality of life of these animals.

Environmental enrichment

Environmental enrichment is a form of improving the welfare of captive animals by adding novelty and/or new stimuli into their environments (Newberry, 1995). According to Shepherdson et al. (1998), environmental enrichment “seeks to improve the quality of care of captive animals, providing stimuli for optimal psychological and physiological well-being”. In practice, it involves innovative and creative techniques to keep animals occupied by providing more stimulating and naturalistic environments. Thus, environmental enrichment can give animals more control over their lives, allowing highlight the appropriate behaviour of the species (Shepherdson, 1994; Shepherdson et al., 1998; Young, 2003). As with welfare, the emphasis on environmental enrichment must be focused on its goals. Thus, its goals consist in increasing behavioural diversity, reducing frequencies of abnormal behaviour, increasing normal behaviours patterns, increasing positive use of the environment and increasing the ability to cope with challenges (Young, 2003). The focus on these goals allows the development of efficient management practices for zoo animals, by creating richer and more stimulating environments (Maple & Perdue, 2012).

Successful environmental enrichment may lead to an increase in typical species behaviours and/or a decrease in abnormal and stereotyped behaviours, through the reduction of stress factors or stimulation of new behaviours (Mason et al., 2007; Young, 2003). However, environmental enrichment is not always successfully applied, leading to negative effects, since the introduction of new elements can lead to frustration (Mason et al., 2007). Thus, it is necessary to understand the reasons for the occurrence of certain behaviour and a careful evaluation of what types of enrichment are more beneficial for a specific species (Maple & Perdue, 2012).

According to Bloomsmith et al. (1991), environmental enrichment techniques can be divided into five major categories, depending on the factors handled: social, occupational, physical, sensory and nutritional.

Social enrichment may involve direct contact between individuals of the same or different species (human or non-human) or indirectly, through odour, visualization of other individuals or co-operative devices. This type of enrichment takes on particular relevance in social animals.

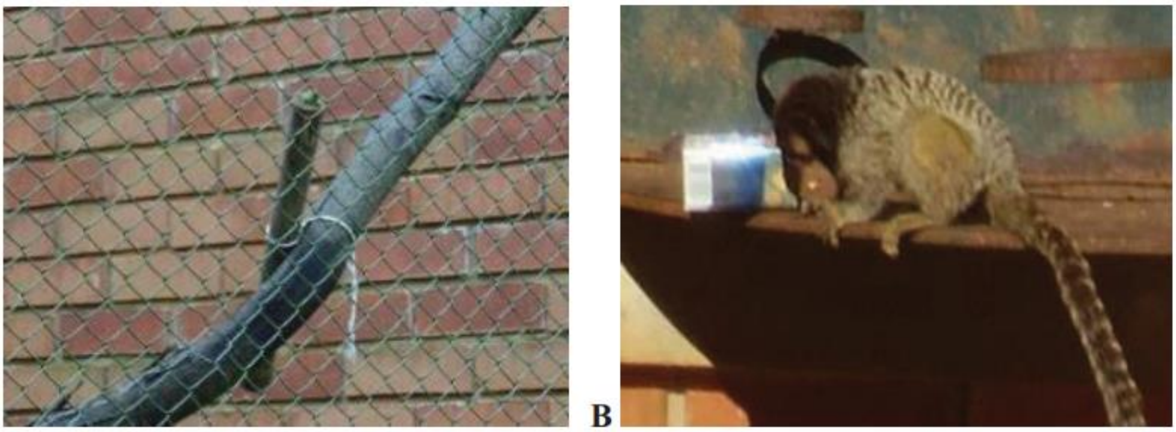
Occupational enrichment stimulates the ability to solve problems, freedom of choice and control over the environment by introducing challenges that can be psychological (like puzzles, that animals can manipulate and explore) or physical, promoting exercise (using mechanical devices) (Figure 44).

Figure 44. Example of an occupational enrichment provided to orangutangs at Hagenbeck Zoo in Hamburg, in which the animals have to manipulate food out of the box using sticks (retrieved from https://sg.news.yahoo.com/).

In the case of physical environmental enrichment, the changes occur at the physical area in which the animals live, by increasing its size and complexity or by adding permanent or temporary accessories (Figure 45).

Figure 45. An example of physical enrichment provided to blue-and-yellow macaws which consist of adding accessories to their enclosure, in this case, sisal rope (retrieved from de Almeida et al., 2018).

In sensory enrichment, the senses of animals are stimulated by introducing auditory, visual, taste, olfactory and/or tactile stimuli (e.g. videos, vocalizations, aromas, odours) (Figure 46).



A B

Figure 46. Example of sensory enrichment provided to a group of marmosets consisting in visual models (A- bamboo; B- cardboard boxes) where spices and herbs that release odour

were introduced, combining the use of the two senses, vision and smell, for the exploration these stimuli (retrieved from Borges et al., 2011).

Finally, nutritional enrichment includes changes not only in how the food is presented to the animals but also in the type of food that is provided (offering treats or varying to food that is not included in the normal diet) (Figure 47).

Figure 47. Example of feeding enrichment provided to waldrapp ibis consisting of a wire mesh tube suspended in a branch and attached to the ground, where the ibises had to pull food through the holes (retrieved from Vargas-Ashby & Pankhurst, 2007).

History of environmental enrichment and some examples

Environmental enrichment techniques are applied, at least since the beginning of the last century. This practice was already recognized and used by zookeepers and other animal caretakers even before receiving its name (Mellen & MacPhee, 2001).

In 1925, Yerkes published the first article recognizing the importance of environmental enrichment as a methodology to improve the well-being of captive animals. Later, Hediger (1950; 1969) identified the importance that physical and social environments, and diet, had on the welfare of animals. In the '60s, the knowledge of animals´ natural history begins to be used to include in the exhibit areas/habitats plans. In the '80s there was an increase in sharing the ideas for environmental enrichment between entities responsible for the care of animals. Before this date, many examples of environmental enrichment were unpublished (cited by Mellen & MacPhee, 2001). In the last decade, it was observed an increase of published articles resulted from the raised concern about animal well-being (de Azevedo et al., 2007).

Currently, most enrichment programs consist of providing the animals' enclosures new or bigger areas, and novel experiences that stimulate and may encourage species-typical behaviours (Martin, 1999). Most of the environmental enrichment studies are applied to terrestrial mammals, especially non-human primates and carnivores, and a lesser degree in birds (King, 1993). For example, the application of sensorial and feeding enrichment in marmoset (*Callithrix penicillata*), shows an increase in exploratory and social behaviours, and foraging, decreasing some observed stereotypic behaviours (Borges et al., 2011). Jacobsen et al. (2010) also verified that in presence of enrichment, the capuchin monkeys (*Cebus apella*) engaged in species-specific behaviours and exhibit behaviours similar to their natural counterparts. Although non-human primates and carnivores are more represented in enrichment studies (perhaps due to their close evolutionary relationship with humans), birds tend to be more represented in greater numbers in zoological institutions (King, 1993). Is also proved that birds are very intelligent animals, with the most known case of crows that use tools to resolve problems, dropping stones in a tube to release food (von Bayern et al., 2009). As a result, these animals are also subject to boredom as any other mammals, which may result in stereotyped or abnormal behaviours, like feather plucking and severe self-mutilation, because of the extremely monotonous and predictable routines, in captivity (King, 1993; Martin, 1999). The most common enrichment used in birds tends to be nutritional enrichment (Byrne, 2004). Studies by Lima et al., 2019 showed that this food-based environmental enrichment proved to be an effective tool for the greater rhea (*Rhea americana*), reducing the expression of abnormal behaviour like eating faeces and pacing, and increasing walking and foraging (Lima et al., 2019). The use of feeding enrichment on captive waldrapps (*Geronticus eremita*) also proved to be important in increasing the activity of these animals. Because of inactivity, these birds develop problems like bumblefoot. In the presence of enrichment, these animals became more active, exhibiting behaviours such as foraging, walking, and flying (Vargas-Ashby & Pankhurst, 2007).

Vital history of the scarlet ibis

The scarlet ibis (Figure 48) is a colonial waterbird that breeds in inland freshwater and coastal habitats in northeastern South America. It is a social species both during nesting and when feeding. In nature, they typically leave the communal roast in the morning and go feed in large aggregations. When the sunset approaches, the flocks begin to approach the roost, flying together until after sunset (Ffrench & Haverschmidt, 1970; Garcia, 2014; Hancock et al., 1992). Generally, they share their habitat with other species, namely herons and spoonbills (Ffrench & Haverschmidt, 1970). This close association with other wading birds can sometimes lead to intense interactions among individuals. For example, ibises tend to defend very aggressively their individual space, as a consequence of having their captured prey stolen by other birds, even though, they do the same for other species. Nevertheless, sharing the feeding areas also allows a better chance to "camouflage" from predators (hiding among the other birds) and find prey more easily (through the agitation in the water generated by various birds) (Hancock et al., 1992).

The breeding season usually initiates in mid-spring after the onset of heavy rains, with the pairing of breeding pairs (Ffrench & Haverschmidt, 1970; Hughes & Owen, 1988). It is believed that the choice of this period to start reproduction may have to do with the fact that the water level increases, and therefore more food becomes available, favouring the feeding and growth of the offspring. However, it can also be a result of the photoperiod change, with reproducing season starting as the days become longer (Garcia, 2014). Their breeding is usually gregarious and synchronous (i.e., a colonial and social breeding system), in which adults tend to visit the colony site in groups, roost there and then take collective flights. The males perform collective courtship displays and build the nests in trees at an altitude close to each other (possibly to reduce risks of predation), approximately at the same time, and then females respond, surrounding the male. In these close nests, occurs synchrony among pairs, culminating in the production of offspring of the same age. A few days after the completion of the nest the female has a 2-3 eggs clutch (Hughes & Owen, 1988; Olmos & Silva, 2001). The eggs are normally incubated for 23 days, and the hatchling leaves the nest after 23 days, but are still dependent on parents to feed them until their juvenile phase. Parental care then starts to decrease after the first month, and at the second month, the young's start to group and only mix with the adults in the following breeding season (Antas, 1979; Ffrench & Haverschmidt, 1970).

In the wild, ibises feed mainly on small crabs and molluscs, but they also feed on aquatic insects, small fishes and snails, and green algae (Ffrench & Haverschmidt, 1970). Their long bill allows them to forage for food in the shallow and muddy waters where they live (Garcia, 2014). They feed primarily by non-visual probing (with the bill) in water walking slowly, and sometimes pecking prey. When on dry soil surfaces, they generally feed visually pecking for prey (Hancock et al., 1992).

The world population of scarlet ibises is suspected to be in decline, due to human pressure through habitat destruction. Yet, because of their high number, this species does not approach the thresholds for the Vulnerable category under the red list, and it is evaluated as Least Concern (BirdLife International, 2016).

Figure 48. Scarlet ibis at Zoomarine, Algarve.

Scarlet ibis behavioural studies

Little is known regarding scarlet ibis behaviour and most of the studies were done in wild populations.

In the wild, Ffrench & Haverschmidt (1970) described the behaviour of scarlet ibis. They verified that ibises are very social animals, forming flocks up to 60 animals, with an average of 15 to 20 animals. Normally ibises fly in large flocks between different feeding and roosting sites. It is described that they leave the roosts in the early morning to go feed on the mudflats when the tide is low, or in the mangrove forests when the tide is high, and when the sun goes down, they fly to a communal roost. Olmos & Silva (2001) studied the breeding biology of the scarlet ibis in more detail. It was verified that nest-building and egg-laying were synchronous, and that nests are built close to each other to reduce predation risk.

In a captive environment, Antas (1979) also study the breeding behaviour. It was verified that an introduction of a new diet (natural items based as shrimp) increase breeding activity. Here, the nest building, brooding and care of young was shared by both sexes, but males were also responsible for the protection of the nest, eggs, and hatchling. Spil et al. (1985) did a more comprehensive study of the behaviour of captive scarlet ibises, studying both daily activity and breeding biology and social interactions. They verified that the daily activity was similar to the wild, with birds being more active in the early morning and before nightfall, foraging the ground. Relatively to breeding biology, they were able to observe three types of behaviour displayed in the courtship: snap display, dipping snap display and twig pulling. Also verified that both sexes participate in nest construction and in caring of offspring. Regarding social behaviour, it was verified a dominance hierarchy among adult birds, associated with the bill size and bird weight, with males being dominant over females.

Brief note about conservation in zoos

Zoological institutions have come a long way since the time they were considered just animal collection sites for the public to see and with little or no concern about animal rights and conservation (Carr & Cohen, 2011; Tribe & Booth, 2003). In the past years, zoos have experienced significant changes in their structure and functions, emphasizing their role in species conservation (Tribe & Booth, 2003). There was a shift to educating people about conservation in zoos through programs and activities, and also through the evolution of the design of the animals' habitats. This is intended to inspire people about the importance of preserving nature and changing attitudes (Mcelroy, 2015; Rabb, 1994). In addition to education, zoos have contributed to the conservation of biological diversity through the rescue, maintenance and restoration of certain taxa, namely large vertebrate threatened species. This is achieved through captive breeding programs, which aim to maintain a genetic reservoir through the genetic management of zoological collections, and function as a stock for declining populations in the wild, maintaining the genetic variability of a species (Rabb, 1994). Zoos have also been working on reintroduction programs through their collaborations and joined efforts to restore populations of endangered species in the wild. Therefore, when captive breeding for reintroduction is performed successfully, it can contribute to the conservation of endangered species (Tribe & Booth, 2003).

B. Registration tables

1. Baseline and Post enrichment without enrichment table

DAY Nº _______ TEMPERATURE _______ ATMOSPHERIC CONDITIONS _________________

DATE _______ REAL FEEL _______

HOUR	GROUP SIZE	BEHAVIOUR				OTHER SPECIES NEARBY	PRESENCE STAFF	OBSERVATIONS
		A1	A2	A3	A4			

2. Table for enrichments phases

DAY Nº ________ TEMPERATURE ________ ATMOSPHERIC CONDITIONS ____________________

DATE ________ REAL FEEL ________

HOUR	GROUP SIZE	BEHAVIOUR				INTERACTION ENVIRONMENTAL ENRICHMENT		OTHER SPECIES NEARBY	PRESENCE STAFF	OBSERVATIONS
		A1	A2	A3	A4	YES	NO			

3. Table for post enrichment with enrichment phase

DAY Nº _______ TEMPERATURE _______ ATMOSPHERIC CONDITIONS _________________

DATE _______ REAL FEEL _______

HOUR	GROUP SIZE	BEHAVIOUR				INTERACTION ENVIRONMENTAL ENRICHMENT											OTHER SPECIES NEARBY	PRESENCE STAFF	OBSERVATIONS
		A1	A2	A3	A4	TIDE			FOOD			SOUNDS		TACTILE					
						YES		NO	YES		NO	YES	NO	YES		NO			
						A1	A2		A1	A2				A1	A2				

C. Results of Kruskal-Wallis Pairwise Method for Time of day, Weather and Area

1. *Comparisons between time of day*

Table 4. Results of Kruskal-Wallis Pairwise Method for the time of day (only p-values).

Comparison	Attentive behaviour	Preening	Flight	Walking	Maintenance	Manipulation/ Play	Resting	Exploration	Foraging	Stationary
Morning vs. Midday	0.010*	< 0.001*	0.006*	0,791	0.165	<0.001*	< 0.001*	< 0.001*	0.776	0.863
Morning vs. Afternoon	0.887	0.000*	0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*
Midday vs. Afternoon	0.015*	0.813	<0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*

Note: "*****" represent the significant differences between time of day obtained in Kruskal-Wallis test; significance level = 0.05.

2. Comparisons between weather conditions

Table 5. Results of Kruskal-Wallis Pairwise Method for the weather (only p-values).

Comparison	Attentive behaviour	Preening	Walking	Maintenance	Manipulation/ Play	Resting	Exploration	Foraging	Stationary
Sunny vs. Cloudy	0.253	< 0.001*	< 0.001*	0.841	0.682	0.954	< 0.001*	0.826	0.802
Sunny vs. Rainy	0.001*	< 0.001*	< 0.001*	< 0.001*	0.026*	0.276	< 0.001*	< 0.001*	< 0.001*
Sunny vs. Windy/Cloudy	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	0.002*	< 0.001*	0.014*	0.039*
Sunny vs. Storm	< 0.001*	0.028*	< 0.001*	< 0.001*	0.031*	0.039*	0.002*	< 0.001*	0.026*
Cloudy vs. Rainy	< 0.001*	< 0.001*	< 0.001*	< 0.001*	0.001*	0.291	0.181	< 0.001*	< 0.001*
Cloudy vs. Windy/Cloudy	< 0.001*	0.004*	0.026*	0.458	0.522	0.034*	0.096	0.002*	< 0.001*
Cloudy vs. Storm	0.001*	< 0.001*	< 0.001*	< 0.001*	0.009*	0.022*	0.299	< 0.001*	0.001*
Rainy vs. Windy/Cloudy	0.001*	0.282	0.561	< 0.001*	< 0.001*	0.001*	0.053	0.634	< 0.001*
Rainy vs. Storm	< 0.001*	< 0.001*	0.023*	0.002*	0.180	0.036*	0.141	< 0.001*	0.002*

Windy/Cloudy vs. Storm	< 0.001*	< 0.001*	< 0.001*	< 0.001*	0.001*	0.012*	0.810	< 0.001*	0.004*

Note: "*" represent the significant differences between time of day obtained in Kruskal-Wallis test; significance level = 0.05.

3. Comparisons between areas

Table 6. Results of Kruskal-Wallis Pairwise Method for the areas (only p-values).

Comparison	Attentive behaviour	Preening	Flight	Walking	Maintenance	Manipulation/ Play	Resting	Exploration	Foraging	Stationary
Area 1 vs. Area 2	< 0.001*	0.072	0.002*	< 0.001*	< 0.001*	0.824	< 0.001*	0.916	0.090	0.964
Area 1 vs. Area 3	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*
Area 1 vs. Area 4	0.038*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*
Area 2 vs. Area 3	0.328	< 0.001*	0.270	0.001*	< 0.001*	< 0.001*	0.703	< 0.001*	< 0.001*	< 0.001*
Area 2 vs. Area 4	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*
Area 3 vs. Area 4	< 0.001*	< 0.001*	< 0.001*	< 0.001*	0.488	< 0.001*	< 0.001*	0.896	< 0.001*	< 0.001*

Note: "*" represent the significant differences between time of day obtained in Kruskal-Wallis test; significance level = 0.05.