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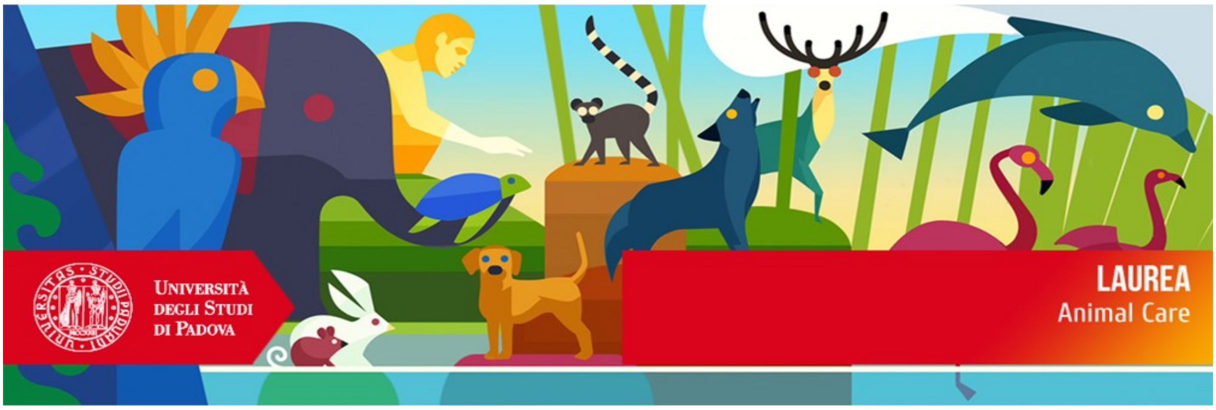

Effects of environmental enrichment on the behaviour of captive great green macaws (*Ara ambiguus*)

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Abstract

The great green macaw is a species threatened by extinction and classified as critically endangered by the IUCN. Thus, ex-situ maintenance plays a significant role in the conservation of this species and can be considered an insurance against extinction. Ex-situ conservation programmes are required to ensure high standards of animal welfare increasing in this way the probability of successful reintroductions. Captive animals are predisposed to chronic stress because of the lack of environmental stimuli, in their absence, the exhibition of natural behaviours is impaired and abnormal behaviour and stereotypes may arise, hence compromising their well-being. High levels of welfare can be achieved through environmental enrichment, an essential management tool that helps to improve the quality of life of captive animals stimulating the expression of typical normal behaviour and reducing behaviours indicative of dysfunction. This study aims to evaluate the effects of environmental enrichment techniques on the behaviour of ten captive great green macaws (*Ara ambiguus*), held at Rescate Wildlife Rescue Center, Costa Rica. The study consisted of two experimental phases: baseline, where no objects were introduced in the enclosure and enrichment phase, where four types of enrichment items were tested: feeding, cognitive, physical and sensory. Parrots' behaviours were recorded 5 days per week, for a total of 5 weeks (one week for the baseline and four for the enrichment). Scan sampling and instantaneous recording (every 15 seconds) were used. The results showed a significant decrease in Rest and a significant increase in Locomotion and Feeding. These findings have traditionally been correlated with improved animal welfare. However, macaws did not show interaction with physical and sensory enrichment, suggesting that is likely they had a preference towards food items, provided by feeding and cognitive enrichment, but also neophobia towards novelty could be the cause. Moreover, "Pecking objects" showed an increase during the enrichment phase. Further investigation is needed to clarify these issues. It was not possible to evaluate the effects of environmental enrichment on abnormal behaviours and stereotypes since they were recorded in neither of the two phases, anyway, is very likely that they were underestimated. Overall, this study demonstrates that environmental enrichment has the potential to recover normal behaviours and thus improving the quality of life of macaws.

1. Introduction

1.1. The biology of great green macaw

The subject of this study is the great green macaw (*Ara ambiguus*), also known as Buffon's macaw (Figure 1.1). This species belongs to the Psittacidae family and is the second-largest parrot in the world with its 85-90 cm in length and 1.3 kg in weight. As the name suggests the plumage is mainly green, the lime-green of the upper wings shades into blue at the flight feathers. Red-orange central tail feathers fade into greenish-yellow with light turquoise towards tips. In adults, the bare facial area is patched with black lines, red in old specimens. Above the huge grey beak, a bright red frontal patch is found (Boyd, 2014).

Macaws do not display external sexual dimorphism between males and females, therefore when sex needs to be identified, DNA testing is required (Helsen et al., 2015).

In captivity their lifespan ranges between 50-60 years, it becomes mature at 5-7 years old and the breeding age is up to 30-35 years.

The breeding season in Costa Rica starts in February and ends in June, in Ecuador is from June to November. This species is monogamous and mates usually remain bonded for their entire life. They nest in large natural cavities of mature trees, in particular mountain almond trees (*Dipteryx panamensis*), at heights corresponding to the crown of the tree (Monge et al., 2012). The female lays a clutch of 2-4 eggs that hatch after 26 days of incubation, performed presumably by the female while the male searches for food and feeds the incubating partner. Hatchlings are blind and naked and they exclusively rely on their parents' care. Both feed their chicks every 2-4 hours until the fledglings can reach feeding trees following adults. Juvenile birds remain with the family for a considerable period, at the beginning of the next breeding season they gradually leave the family nucleus but they stay in proximity with conspecifics (Boyd, 2014).

Great green macaws are found in pairs especially during the breeding season, throughout the non-breeding season they can also form small groups of 8 to 15 individuals that disperse in search of food. They migrate from lowland Atlantic forests to elevated volcanic regions of Costa Rica and Nicaragua's dry forests. This seasonal migration is correlated with the termination of fructification of *D. panamensis*. Indeed in Costa Rica, this plant is not only the major nesting tree but, moreover, its fruits comprise an important component of the great green macaw's nutrition, accounting for 85% of its diet when fruiting reaches its peak (van

Roosmalen, 2018). With the scarcity of these fruits, from April to August, these birds feed on *Sacoglottis trichogyna* (Chun and Christensen, 2008).

Figure 1.1

Two specimens of wild great green macaw around the forest. Picture by Ara Manzanillo (2020).

1.2. Distribution and conservation status

Two allopatric subspecies of *Ara ambiguus* are recognized: *Ara ambiguus ambiguus* (Bechstein, 1811) and *Ara ambiguus guayaquilensis* (Chapman, 1925) (https://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=714033#null).

The first one occurs in fragmented populations established in the humid tropical lowland forests of Central America, predominantly the Caribbean side from Honduras through Nicaragua, Costa Rica, Panama, where is found also along the Pacific side, to north-western Colombia. The *A. a. guayaquilensis* occurs in dry deciduous forests in western Ecuador where it is split into two isolated populations, one in the Cordillera de Chongón-Colonche and the other in the north on the borders with Colombia (Fig. 1) (Boyd, 2014).

Figure 1.2
Distribution of the two subspecies of Ara ambiguus.
BirdLife International and Handbook of the Birds of the World (2016) 2014. Ara ambiguus. The IUCN Red List of Threatened Species. Version 2021-3

As reported by BirdLife International (2020), the global population accounts for at least 525 mature individuals, this number is alarming and aggravated by the fact that the trend is in continuous decline: 80-99% over three generations. Given that, is not surprising that the IUCN (International Union for Conservation of Nature) Red List has declared the great green macaw as "critically endangered" in 2020. The subspecies *A. a. guayaquilensis* was incorporated in the Red List of birds of Ecuador under the heading "critically endangered" already in 2002 (Cornejo, 2015) and the current population is estimated to be 50-70 individuals.

This precarious condition is justified by anthropogenic actions. The major factors responsible for the decline of the species are deforestation, habitat loss, and degradation. Costa Rica, where my study was conducted, has experienced one of the biggest deforestation rates throughout the 20th century. It started by occasional logging with the Spanish colonization in the 1500s and it increased at high rates until the 1970s when the peak was reached (Chun and Christensen, 2008) reducing the historical range up to ~90% (BirdLife International, 2020). Natural resources were exploited to meet political, social and demographic pressures, Costa Rica's population grew at fast rates after World War II achieving its apex in the 1960s, this finally led to forest clearing to obtain space for agriculture, cattle-ranching, plantations of coffee and monocultures of crops such as bananas, pineapples, oil palm and sugar canes (Chun and Christensen, 2008).

Another important threat that has contributed to the decline of the great green macaw and its habitat is the unsustainable cutting of *Dipteryx panamensis*, due to the high commercial value of the wood, this leads to the loss of nesting sites. Costa Rica decided to ban the logging of almond trees in 2008 to protect *A. ambiguus* that relies upon this tree for its survival (Fraixedas et al., 2014).

This species is facing other threats such as illegal pet and feather trade despite being included in Appendix I and II of CITES (Convention on Trade in Endangered Species of Wild Fauna and Flora).

Besides the control of the trade through CITES, other measures for the protection of *A. ambiguous* have been implemented throughout the last decades. Wildlife awareness has increased and this species is now the subject of several programmes and conservation plans, not only in Central America but also in Ecuador.

The Macaw Recovery Network (MRN) (https://macawrecoverynetwork.org/#) founded in 2018, tries to save from the edge of extinction the great green (primary focus) and scarlet macaw, and the yellow-naped amazon parrot. To accomplish this, the network operates on a large scale across Costa Rica, grouping teams of international experts that focus on implementing the best techniques in conservation. MRN carries out the monitoring and the rescuing of the species, it protects areas for those parrots that are reintroduced and it runs one of the world's most important breeding programs. Census of the species is fundamental to understand the dynamics of the population, its size and how to direct conservation efforts. In October 2021 teams of experts working for MNR conducted a population census in the northeaster of Costa Rica, 340 individuals were counted, this is the largest record of the species ever documented. It indicates that the population has grown since the 90's decade.

Many other entities support the conservation of the great green macaw trying to increase its population through rescue, captive breeding, release, and forest restoration. Examples are Ara Manzanillo that has founded new populations of great greens through a "soft release" protocol, assisted breeding program and, in collaboration with the Ministry for the Environment of Costa Rica, has developed a community outreach program; or Fundación Jocotoco in Ecuador that runs a reintroduction program for its endemic subspecies (https://www.parrots.org/projects/great-green-macaw/).

A key point in the conservation of the great green macaw is the protection of its habitat and resources, this turns out to be a strategy that benefits a myriad of other species inhabiting the same lowland wet forests. For this reason, the great green macaw can be considered an "umbrella species" that deserves conservation priority since its strong influence on the fauna and flora of its habitat (Chassot and Arias, 2012).

A big achievement has been officially reached in March 2001 with the establishment of the San Juan–La Selva (SJLS) Biological Corridor situated in Costa Rica. With its 246,608 hectares, this system of biological corridors links together forested areas within a fragmented landscape from southern Nicaragua to Costa Rica's Central Volcanic Range reserve (McClearn, 2011). This is the result of an alliance between 22 organizations, among which is present the Great Green Macaw Research and Conservation Project. Indeed, one of the purposes of this corridor is the protection and conservation of the great green macaw within its natural ecosystem, and in addition, it strives to create the Mesoamerican Biological Corridor (MBC), an international initiative that would connect endangered habitats from southern Mexico to Panama to preserve biodiversity and promote sustainable development. The core protected area of the SJLS Biological Corridor is the Maquenque Mixed National Wildlife Refuge which extends for 54,000 hectares. Located to the south of the Nicaraguan border, with its high density of *D. panamensis,* is home to approximately 200 great greens. This transboundary conservation process also promotes the integration of local communities with the purpose of increasing awareness towards the preservation of the great green macaw's habitat (Chassot and Arias, 2012; Chun and Christensen, 2008).

1.3 Captive breeding and environmental enrichment

As previously mentioned, captive breeding is another aspect of species conservation efforts. Depending on the circumstances, removing threats such as deforestation may prove insufficient in saving endangered species from extinction. In these cases, *in situ* conservation is flanked by captive breeding, a process that consists in breeding animals that are threatened by extinction in captivity, with the aim of reintroducing them back into the wild once there is a sufficient and suitable natural environment or when species threats in the wild are minimized (Wakchaure and Ganguly, 2016).

A major objective in captive breeding is to avoid inbreeding and loss of genetic variation, that in small populations leads to detrimental effects. It's essential to create self-sustaining captive populations that resemble, from a behavioural and genetic point of view, their counterparts in the wild, as consequence, preservation of the maximum genetic variability plays a key role in the continuity of the species in a long-term vision. The reintroduction of endangered and captive individuals in the wild contributes to their survival by increasing the population numbers and minimizing genetic drift and inbreeding (Wakchaure and Ganguly, 2016).

Many problems can arise with captive breeding, N.J. Collar (2000) claims that this method does not contribute efficiently to the conservation of threatened parrots due to numerous obstacles related to husbandry, domestication, reintroduction, disease spread from captive to wild parrots, human interactions and last, but not least, the costs. Anyway, he sustains also that when a species is near the brink of extinction, nothing should prevent from trying all possible ways to save it. When it comes to reintroduction, hand-raised parrots must be able to avoid predators, obtain food by themselves, form social groups with conspecifics and reproduce for the continuity of the species. This is possible only if they develop these skills during captivity. In a recent study, Brightsmith and co-workers (2005) demonstrate that hand-raised psittacines can be successfully reintroduced in the wild. In this experiment three groups of scarlet macaws (*Ara macao*) hand-raised were released in forested areas in three different sites, two in Costa Rica and one in Peru. Results were quite surprising compared to other studies, in fact, the overall rate of survival was 89% per year. This can be explained by an optimal rearing strategy that minimized post-weaning human contact and favoured social skills development between conspecifics, but also by a low-density of predators in the release sites since captive-raised parrots lack antipredator skills. Thanks to the ability to socialize, birds formed core flocks and this proved to be crucial for their survival, they also paired with wild mates and bred successfully in Peru. Finally, this work shows that ex-pets macaws were inappropriate candidates since they showed maladaptive behaviours.

Nevertheless, there is still some controversy surrounding captive breeding programs. On the one hand, captivity may be considered ideal because it ensures freedom from predation, hunger, and extreme weather conditions; on the other hand, a controlled environment is sterile, the space is limited, it is predictable and behavioural opportunities are limited due to lack of resources promoting mental and physical stimulation (Collar, 2000; de Almeida et al., 2018; Miglioli and Vasconcellos, 2021; de Andrade and de Azevedo, 2011). Therefore, as Meehan and Mench sustain in the "Manual of parrot behavior" (2006), life in captivity is a paradox, because while trying to reduce environmental pressures, stress arises since expression of normal behaviour is prevented or reduced, creating in this way a mismatch between the macaw and its environment. Consequently, it is fundamental to know the behavioural repertoire of parrots in the wild and how it differs in captive conditions in order to understand how captivity might affect their welfare. However, there are hardly any studies based on behavioural observations of wild macaws. What is well known is that in the wild there is a constant engagement of psittacines with their environment, in particular, they spend a great portion of their time in foraging and locomotion. They travel in search of food among different feeding areas and once arrived they are involved in the selection, manipulation and consumption of food. Moreover, since their hatching, they are engaged in social interactions that diversify and increase in complexity as they grow up. In contrast, captivity does not offer all these opportunities, for instance, activity budget related to foraging behaviour is greatly reduced, food is provided at regular intervals, already selected and peeled, locomotion is constrained by enclosure's design and in most cases, they are not allowed to establish social groups (C. Meehan and Mench, 2006).

Anyway, a wild environment cannot be re-created in captive conditions, but environments that do not offer animals proper stimuli may compromise their welfare and reproduction, leading to low quality of life. Besides helping in maintaining genetic variability, captive breeding programs must ensure high welfare to animals to increase the probability of successful reintroductions (Greggor et al., 2018; Mason et al., 2007). Animal welfare can be defined as the maintenance of good psychological and physical health of animals by satisfying their needs (Young, 2003). Negative indicators of animal welfare are correlated with an increased incidence of health problems, fearfulness and chronic stress (high glucocorticoids levels) that results in immune system suppression, poor reproductive performance, poor growth and development of abnormal repetitive behaviours (ARBs) (de Almeida et al., 2018; Greggor et al., 2018).

ARBs are commonly displayed by captive wild animals, including parrots, in zoos and similar situations. The development of ARBs can be triggered by internal factors such as dietary deficiencies and hormones or by external factors like isolation from conspecifics and impoverished environments that prevent the animal from the exhibition of highly motivated behaviours (Mellor et al., 2018). Abnormal behaviours recorded for captive-reared parrots are self-mutilation, feather plucking, exaggerated aggression and stereotypes (Azevedo et al., 2016; de Almeida et al., 2018; C. L. Meehan et al., 2003). Stereotypes are defined as repetitive and functionless behaviour patterns that are influenced by the captive environment (Mason et al., 2007). Examples of stereotypic behaviours in psittacines are pacing on the perch, enclosure walls or floor, twirling or side-to-side movements of the head or body, cage bar and/or wall chewing (Mellor et al., 2018).

Because of the evidence that barren captive conditions can induce an impairment of physiological and behavioural development and concurrently compromise well-being, the concept of environmental enrichment comes into play. Environmental enrichment is a set of techniques that aims to enhance the physical and psychological well-being of animals retained in captivity by providing stimuli that meet their species-specific needs (Young, 2003). Environmental enrichment is used as a strategy for mitigating frequencies of ARBs, increasing behavioural diversity, increasing the number or range of normal behaviour patterns, enhancing positive utilization of the environments and the ability to cope with challenges in a more normal way (Young, 2003). This becomes crucial especially when animals are part of reintroduction programmes because it helps them build up skills to survive in the wild (C. Meehan and Mench, 2006; de Andrade and de Azevedo, 2011).

Several studies on different species reported positive effects of environmental enrichment on the expression of reproductive behaviours, such as increased survival and number of offspring, fertility and copulation (Fisch et al., 2017; Mellor et al., 2018; Wafer et al., 2016). This aspect is of relevance also for captive programs that aim at achieving breeding.

Environmental enrichment techniques incorporate everything from items to social company. Five major types of enrichment have been identified: social, occupational or cognitive, physical, sensory and nutritional or feeding (Young, 2003).

2 Aim of the study

The aim of the current study was to assess the behavioural response of 5 pairs of great green macaws in captivity to different types of environmental enrichment, namely cognitive, feeding, physical, and sensory in comparison to previous conditions in which they were not enriched.
The rationale was to test different enrichments having the prerequisite characteristics of being easily available at low price, low time consuming and as such insertable in the husbandry routine, guaranteeing the safety of the animals. This in particular considering that environmental enrichment, a consolidated strategy for improving the well-being of captive animals, is under-represented in the scientific literature of avian species.

3 Materials and methods

3.1 Location

This study was conducted at Rescate Wildlife Rescue Center, a wildlife rehabilitation facility located in La Garita, in the province of Alajuela, Costa Rica. With its 36 acres of natural tropical forest, the centre is subdivided into 3 main areas:

- A Lifetime Care Sanctuary that is home to 800 non-releasable wild animals.
- A Wildlife Rehabilitation Center and veterinary clinic.
- An Endangered Wildlife Breeding Center that hosts scarlet macaw (*Ara macao*) and great green macaw (*Ara ambiguus*) specimens, whose offspring are released in the wild, contributing in this way to the conservation of their species. Visitors are not allowed to enter here.

3.2 Animal husbandry and housing

The study was performed on 10 great green macaws (A*ra ambiguus*) grouped in pairs, one male and one female, housed in five enclosures in the Breeding center. The age is known just for two of them, these were born in Rescate Wildlife Rescue Center, one in 1996 and one in 2006, more data are shown in Table 3.1.

During the whole experiment the birds were fed every day, once in the morning at approximately 6:00 am and once in the afternoon around 2:00 pm, water was provided *ad libitum*. They received the same diet and each couple shared the food bowl. Breakfast consisted of a mush of rice, fruits, beans, eggs, bread, dog food and some additive nutrients, in this way they could not select the ingredients; at lunch they were fed with chopped seasonal fruits and vegetables, sunflower seeds or palm oil nuts, two pieces of each type of food were offered to each pair to avoid conflict. The macaws were kept in outdoor enclosures measuring 400 cm (length) x 198 cm (width) x 292 cm (height), the walls consisted of wire mesh with a mesh diameter of 3 mm and a mesh opening of 8 cm. Each enclosure was provided with a wood nest box measuring 117 cm (length) x 47 cm (width) x 42 cm (height), positioned under a roof. The floor was made of soil and some plants. There were four or five perches per enclosure.

Register of great green macaws					
Cage	**Identification code**	**Sex**	**Origin**	**Date of entry**	**Date of birth**
V1	zoovae 1 150	F	Tortuguero	27/09/2018	NA
V1	zooave 1 189	M	NA*	04/04/2017	NA
V4	ZA 9 81	F	Rescate wildlife rescue center		2006
V4	ZA 9 59	M	Zona Norte	29/05/2006	NA
V6	zooave 1 143	F	NA	NA	NA
V6	ZA 9 84	M	Rescate wildlife rescue center	NA	NA
V9	zooave 1060	F	Donated	1999	NA
V9	ZA 9 74	M	Rescate wildlife rescue center		1996
V2	ZA 1 110	F	Tortuguero	13/02/2018	NA
V2	zooave 1 199	M	Guápiles	05/06/2017	NA

Table 3.1

Great green macaw's register.

* Not Available

3.3 Experimental procedure

The setup I used bears a close resemblance to the one used by other similar experiments with captive macaws (Azevedo et al., 2016; Miglioli and Vasconcellos, 2021; de Andrade and de Azevedo, 2011).

The study was divided into three phases:

I. Preliminary: observations of animal behaviour without enrichment implementation to assess the behaviours performed and adjust the ethogram developed in previous studies (Azevedo et al., 2016; Miglioli and Vasconcellos, 2021).
II. Baseline: observations of animal behaviour without interventions and based on the definitive ethogram.
III. Enrichment: observations of animal behaviour with the introduction of four types of enrichment (Figure):

a. Feeding Enrichment (FE): introduction of two sticks with banana slices.
   The sticks were placed once a day, every morning, right before I started to observe the bird couple assigned to feeding enrichment. Despite the additional food provided by this enrichment, macaws continued to be fed as usual, without reducing the food ratio.
b. Sensory Enrichment (SE): introduction of two sets of keys hung on the mesh of the enclosure.
   This item was placed once a week on the first day of observations and removed on the fifth day at the end of the observation sessions.
c. Physical Enrichment (PE): introduction of two swings hung on the roof of the enclosure. A branch constituted the seat where the bird could perch on, the two iron-support rods were decorated with multicolour wood blocks and cardboard pieces.
   This item was placed once a week on the first day of observations and removed on the fifth day at the end of the observation sessions.
d. Cognitive Enrichment (CE): introduction of two paper rolls filled with straw and bread pieces, they were hung on the mesh of the enclosure.
   The paper rolls were placed once a day, every morning, right before I started to observe the bird couple assigned to cognitive enrichment.

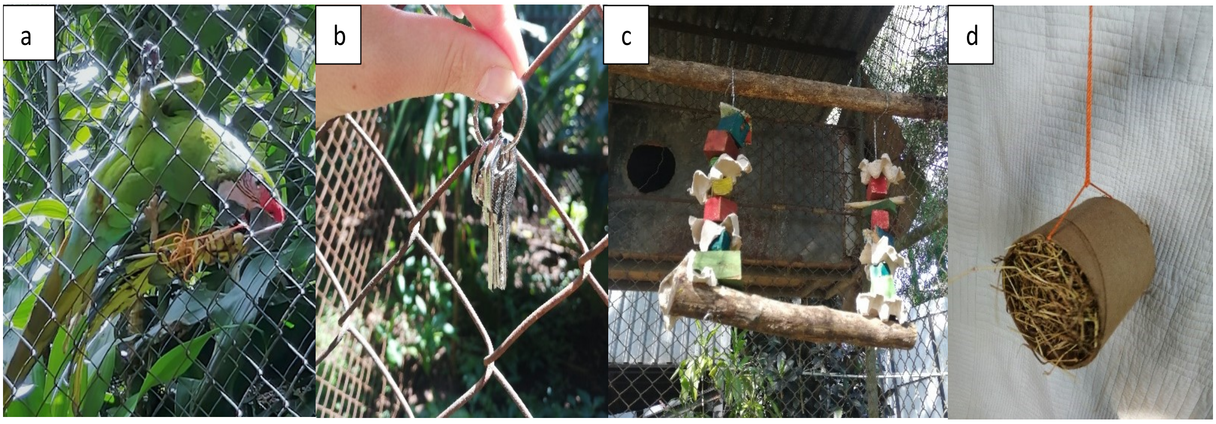


Figure 3.1

Environmental enrichment items tested during enrichment phase.
a) stick with banana slices; b) set of keys; c) wood swing; d) paper rolls filled with straw and bread pieces.

3.4 Animal observations

Behavioural data collection consisted of two sessions of 15 minutes each per pair of macaws, totalling 30 minutes of observations per cage in a day.

Focal sampling and instantaneous recording (every 30 seconds) were applied to the preliminary stage, the same type of recording was maintained for baseline and enrichment stages, instead, the focal sampling was replaced with scan sampling. 12,5 hours were totalled for the baseline phase, 50 hours for the enrichment phase.

The first session started at 8 a.m., the second at 11 a.m., this timetable, adjusted after the preliminary phase, was ideal because there was a high probability of rain in the afternoon, and it did not overlap with feeding time.

The observation sequence of the cages was the same among the two sessions, but different each day and repeated every week.

Preliminary and baseline data collection was performed five consecutive days each, the enrichment phase consisted of four weeks (Table 3.2), five days of observations, from Saturday to Wednesday, and 2 days of interval, Thursday and Friday, between one treatment and the other to avoid possible consequences from the previous treatment to the next one.

Behavioural recordings were based on an ethogram of the species (Table 3.3) resulting from consultation of scientific literature (Azevedo et al., 2016; Miglioli and Vasconcellos, 2021; de Almeida et al., 2018) and 15 hours of preliminary observations.

Date	Cage	E. Type *	Date	Cage	E. Type	Date	Cage	E. Type	Date	Cage	E. Type
17-19 August	V1	FE	21-25 August	V1	SE	28-1 September	V1	CE	4-8 September	V1	PE
17-19 August	V2	SE	21-25 August	V2	FE	28-1 September	V2	PE	4-8 September	V2	CE
17-19 August	V4	CE	21-25 August	V4	PE	28-1 September	V4	FE	4-8 September	V4	SE
17-19 August	V6	PE	21-25 August	V6	CE	28-1 September	V6	SE	4-8 September	V6	FE
17-19 August	V9	FE	21-25 August	V9	SE	28-1 September	V9	CE	4-8 September	V9	PE

Table 3.2

Experimental scheme of the enrichment phase developed for great green macaw (*Ara ambiguus*). The table is subdivided into 4 sections that illustrate the 4 types of enrichment assigned to each cage every week for a total of 4 weeks of the treatment.

* Enrichment Type

Behavioural categories and observed behaviours	Acronym	Description
Rest		
Inactive	IN	The macaw is inactive, with either eye open or closed, stationary, perched.
Maintenance		
Preening	PR	The macaw cleans and arranges its own feathers with the beak
Rubbing	RUB	The macaw rubs its beak against the perch
Locomotion		
Flying	FLY	The macaw flies through the enclosure.
Walking on the ground	WG	The macaw walks on the ground of the enclosure
Walking on the wire bars	WB	The macaw climbs on the wire bars of the enclosure using the beak and/ or feet
Walking on the perch	WP	The macaw walks on the perch of the enclosure
Foraging		
Feeding	FED	The macaw licks, pecks, or ingests food or water from the feeder.
Interacting with other objects		
Pecking objects from the enclosure	PECK	The macaw pecks, damaging nests, chews wood from the perches, the enclosure walls or any object except enrichment.
Interaction with enrichment		
Interacting with enrichment	IE	The macaw pecks, manipulates or picks up enrichment items
Affiliative interaction		
Positive social interaction	PSI	Feeding another individual, or any type of conspecific friendly interaction (beak touches)
Allopreening	ALP	Being cleaned or cleaning the feathers of another individual
Agonistic behaviour		
Aggressive social interaction	ASI	The macaw physically assaults another bird or steals food from another individual
Stereotypes		
Pacing	PAC	The macaw walks back and forth on the perch or on the wire mesh repetitively and with no apparent reason.
Beak-hitting	BH	The macaw hits or scratches the beak repeatedly on the wire mesh repeatedly
Head-moving	HM	The macaw moves its head back and forth and/or from side to side repeatedly
Wire-chewing	WC	Chewing on the enclosure wire bar with the bar held in the beak while hanging on it
Hanging	HG	The macaw hangs from the ceiling, in a horizontal or vertical position, trembling, or with fast and short movements of the wings
Not visible		
Macaw not visible	MNT	
Activity not visible	ANT	The macaw's activity is not visible for the observer even if the bird is visible
Others		
Other behaviours	OTH	Other behaviours not listed above. Ex: defecating, sneezing, scratching, vocalizing etc.

Table 3.3
Ethogram developed for great green macaw (*ara ambiguus*), based on five days of preliminary observations and adapted from scientific literature (Azevedo et al., 2016; Miglioli and Vasconcellos, 2021; de Almeida et al., 2018).

4 Data management and statistical analysis

All data gathered during the behavioural observations and those obtained through the anamnesis and animal records were insert in Excel spreadsheets. The experimental unit was the pair of great green macaws housed in a cage. Behavioural data recorded using the scan sampling technique were transformed from absolute frequencies of scans into percentages by dividing the number of times a parrot was observed to perform a given behaviour by the total number of scans observed in the corresponding session. Data were then submitted to descriptive analysis. Behavioural prevalence were submitted to statistical analysis using a mixed model with PROC MIXED of SAS (SAS Institute Inc.) considering the fixed effect of the type of environmental enrichment and the effect of the cage as random. Pairwise comparisons of lease square means of behavioural prevalence among enrichments and the basal observations where tested using the Bonferroni test. Statistical significance was set for values of $P < 0.05$.

5 Results and discussion

Results of the current study refer to data regarding 5 pairs of great green macaws observed during 25 days of behavioural observations, 5 days for the baseline phase and 20 days for the enrichment phase.

Results are expressed as prevalence (percentage of scans) in which a parrot per cage was observed in each behaviour listed in the ethogram.

As Table 4.1 highlights, a very surprising result emerging from the data is that some behaviours were not performed by the macaws even if expected by the ethogram and by similar studies on captive psittacines (Assis et al., 2016; Azevedo et al., 2016; de Almeida et al., 2018; de Andrade and de Azevedo, 2011; Miglioli and Vasconcellos, 2021), these behaviours were those classified under the category Stereotypes: "Pacing", "Beak-hitting", "Head-moving", "Wire chewing" and "Hanging". On first impression, the fact that behaviours indicative of dysfunction were not recorded could appear a positive indicator of animal welfare, but the most appropriate explanation for this situation is that stereotypes were probably underestimated because the observation time was not sufficient to detect them. In addition, they are difficult to recognize without previous training and experience. Continuous behavioural observations or video recordings could be a useful tool to document and assess abnormal behaviours (C. Meehan and Mench, 2006).

Exhibition of "Aggressive social interaction" showed very low frequency and was only recorded when cognitive and feeding enrichment items were offered (Table 4.1), the underlying cause could be food competition, since both the items provided contained food, or hierarchy within the couple (de Andrade and de Azevedo, 2011). To overcome the competition is sufficient to provide enough enrichment items for both to allow simultaneous access (Young, 2003). However, in this study, the frequency of agonistic interactions was too low to trigger concern about the couples that showed this behaviour (Collar, 2000).

Other behaviours that were not displayed or with a prevalence lower than 1.2% were "Flying", "Walking on the ground", "Macaw not visible" and "Activity not visible".

Behaviour	Basal	Type of enrichment			
	No enrichment	Cognitive	Feeding	Physical	Sensory
INACTIVE	68.1 ± 31.7	40.0 ± 33.8	41.9 ± 33.6	50.3 ± 38.0	50.2 ± 35.7
PREENING	11.1 ± 19.3	12.4 ± 13.3	13.1 ± 17.6	11.4 ± 16.4	18.1 ± 21.4
RUBBING	0.6 ± 1.6	0.7 ± 1.5	1.2 ± 2.2	0.5 ± 1.1	0.8 ± 1.5
FLYING	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.5
WALKING on the GROUND	0.0 ± 0.0	0.1 ± 1.0	0.4 ± 1.8	0.2 ± 0.7	0.0 ± 0.2
WALKING on the wire BARS	3.0 ± 4.1	4.6 ± 6.2	8.6 ± 11.3	3.7 ± 5.0	2.7 ± 5.2
WALKING on the PERCH	0.9 ± 2.0	1.8 ± 2.8	2.7 ± 3.2	1.4 ± 1.9	1.2 ± 1.9
FEEDING	1.7 ± 5.5	3.9 ± 11.5	1.5 ± 5.3	7.6 ± 15.8	3.4 ± 8.8
PECKING objects from the enclosure	1.6 ± 3.2	5.4 ± 9.6	6.3 ± 8.4	5.4 ± 9.4	3.5 ± 7.1
INTERACTING with ENRICHMENT	0.0 ± 0.0	8.9 ± 20.1	12.5 ± 17.9	0.1 ± 0.7	0.0 ± 0.2
POSITIVE SOCIAL INTERACTION	0.3 ± 1.1	0.8 ± 2.0	0.7 ± 1.7	0.5 ± 1.9	0.4 ± 1.1
ALLOPREENING	10.6 ± 22.7	17.7 ± 27.5	7.5 ± 16.5	16.9 ± 24.8	17.2 ± 25.7
AGGRESSIVE SOCIAL INTERACTION	0.0 ± 0.0	0.5 ± 2.3	0.2 ± 1.0	0.0 ± 0.0	0.0 ± 0.0
PACING	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
BEAK-HITTING	0.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
HEAD-MOVING	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
WIRE-CHEWING	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
HANGING	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MACAW NOT VISIBLE	1.2 ± 7.1	1.3 ± 7.2	1.5 ± 7.0	0.9 ± 4.2	0.9 ± 3.3
ACTIVITY NOT VISIBLE	0.6 ± 2.5	0.1 ± 0.3	0.5 ± 3.1	0.3 ± 1.5	0.1 ± 0.6
OTHER behaviours	0,4 ± 0.8	1,9 ± 3.0	1,5 ± 1.8	1,0 ± 1.5	1,3 ± 1.9

Table 4.1

Descriptive statistics of percentages of scans (mean ± SD) in which a parrot per cage was observed engaged in each behaviour during basal behavioural observations and the enrichment phase according to the type of enrichment.

Behaviours that were not observed and thus showed a prevalence of 0 for the large majority of the data points (> 90% of the data) were removed from further statistical analysis.
Results of the multivariate analysis on the prevalence of behaviours are reported in Table 4.2. The behaviours that statistically differed ($p<0.05$) between baseline and types of enrichment were “Inactive”, “Walking on the wire bars”, “Walking on the wire perch”, “Feeding”, “Pecking objects from the enclosure”, “Interacting with enrichment” and “Other behaviours”. Whereas the analysis did not reveal any significant differences between baseline and types of enrichment for “Preening”, “Rubbing”, “Positive social interaction” and “Allopreening”.

Behaviour	Significance P-value
INACTIVE	0.0004
PREENING	0.3659
RUBBING	0.2687
WALKING on the wire BARS	0.0003
WALKING on the PERCH	0.0041
FEEDING	0.0393
PECKING objects from the enclosure	0.0147
INTERACTING with ENRICHMENT	<0.001
POSITIVE SOCIAL INTERACTION	0.5733
ALLOPREENING	0.1795
OTHER behaviours	0.0025

Table 4.2

Results of the multivariate analysis on the prevalence of behaviours observed in parrots expressed as percentages of scans in which a parrot per cage was observed engaged in each behaviour.

As shown in Figure 4.1, “Inactive” behaviour was the most exhibited behaviour during the baseline and enrichment phase, however, enrichment caused a reduction in the number of scans in which parrots were inactive. The decrease of inactivity correlates fairly well with previous findings in the literature about psittacines (Assis et al., 2016; Checon et al., 2020; de Andrade and de Azevedo, 2011; Miglioli and Vasconcellos, 2021; Reimer et al., 2016). Even if inactivity was reduced by all four types of enrichment, those types that differed statistically from the baseline phase were feeding and cognitive enrichment. One possible explanation is that since parrots were not at all interested in physical and sensory enrichment items, as I will discuss later, they spent more time resting when swings and sets of keys were offered to them.

A reduction of “Inactive” represents a positive response in the search for the well-being of captive great green macaws that are part of an environmental enrichment program (Checon et al., 2020; de Andrade and de Azevedo, 2011). Indeed, resting or inactivity behaviours, if performed with excess, could indicate that animals are stressed or frustrated, probably because of lack of environmental stimuli, leading to a state of boredom and idleness (Reimer et al., 2016). Prolonged inertia is one of the major problems in captive animals and could result in the development of overweight that negatively affects their welfare and even reproduction (Miglioli and Vasconcellos, 2021).

For what concerns the behavioural category “Interaction with enrichment”, the types of enrichment that statistically differed from the baseline stage were cognitive and feeding enrichment. As illustrated in Figure 4.1, macaws interacted more frequently with feeding than cognitive enrichment (12,4603% vs 8,9493%), although there is no statistically significant difference between them. As already mentioned, cognitive items were provided with food. This preference towards items with food and avoidance for those without it is likely to be associated with the degree of interest for enrichment items or the degree of neophobia. De Almeida et al. (2018) while testing different enrichment objects found that blue-and-yellow macaws did not interact with them to the same extent, in particular, the authors verified that birds interacted more with food items. Analogue results are documented in similar studies with parrots (C. L. Meehan et al., 2004; Miglioli and Vasconcellos, 2021; Reimer et al., 2016). These outcomes suggest that birds prefer those items with food because they are accustomed to and are part of their diet. Furthermore, psittacine birds are attracted by food because foraging is a natural behaviour that keeps them busy for hours in the wild (de Almeida et al., 2018). According to Simone-Freilicher and Rupley (2015) psitaccids’ preferences are influenced by colours, indeed they show attraction or aversion towards certain colour. This may affect the selction of enrichment items. Thus, assessing the preference is important to ensure better maintenance of birds in captive environments and better housing conditions.

Unlike other research carried out in this area, physical and sensory enrichment did not show a significant difference compared to the baseline phase, indeed the' frequency of interaction with them is near 0% for both. A satisfactory explanation for this may be that swings and sets of keys, since completely unfamiliar to macaws, elicited fear in birds. This is understandable since psittacids are prey animals and therefore they tend to be neophobic, indeed in the wild, they have a major probability of survival if they come close to new things with extreme prudence (de Almeida et al., 2018; Luescher, 2008). Fear can be caused by various stimuli and novelty is one of them. It has been demonstrated that birds and mammals reared in captive barren conditions show fearful reactions at a higher degree when exposed to novelty compared to those reared in enriched environments and thus experiencing novelty constantly (C. Meehan and Mench, 2006). Fox and Millam (2007) in their study with orange-winged Amazon parrots found that a frequent rotation of enrichment objects, called "high novelty", is more efficient in reducing neophobia than enrichment alone. However, they highlight that individual behavioural traits should be taken into consideration because they found that "high novelty" treatment caused an increase in fear in more fearful individuals. Moreover, in this study some items were perceived as more frightening, thus even the types of enrichments must be considered to minimize fear and stress.

Grate green macaws that were involved in this study were not used to receive enrichments as it was not part of the rescue center routine and this may be the reason why they showed an avoidance response and/or fear towards enrichment objects that did not contain food.

A possible solution if the parrots display fearfulness is to introduce the items gradually, initially the objects should be placed at a short distance outside the enclosure and as the parrots show curiosity about them or stop performing nervous and fearful behaviour, the enrichments can be approached slowly until they are inserted in the cage (Simone-Freilicher and Rupley, 2015).

Among the various enrichment items used by Reimer et al. (2016) in their experiment with captive macaws, swings were those that invoked the lowest interaction. Anyway, they state that even if macaws interacted significantly less with swings, this does not mean that this enrichment failed, actually, behavioural changes induced by it could be intense as their study demontrated. However, in my experiment physical and sensory enrichment did not result significantly different from the baseline phase in none of the displayed behaviours, thus, in my opinion, five days of exposure to distinct enrichment items were not sufficient to let macaws interact with them and consequently to induce behavioural changes, at least for what concerns physical and sensory objects.

It would be interesting to test other objects, for example, ropes, wood sticks, bamboo perches and dried leaves that have been found to be successful in other experiments (Azevedo et al., 2016; Checon et al., 2020; de Almeida et al., 2018; Miglioli and Vasconcellos, 2021; Reimer et al., 2016) with psitaccids where environmental enrichment was analysed.

In the literature I have not found studies about the effects of environmental enrichment on parrots that include sensory enrichment, hence comparisons with my result are not possible. Probably this is due to the fact that all enrichment items could be considered also as "sensory" since they stimulate eyesight, especially if coloured, taste, in particular nutritional enrichment, touch and smell, although scent enrichment has not been examined in psittacine birds (Simone-Freilicher and Rupley, 2015).

An increase in Locomotion ("Walking on the wire bars" and "Walking on the perch") was observed by offering to the macaws feeding enrichment, this was the only type of enrichment that showed significant difference compared to the baseline phase (Figure 4.1). There is a high probability that birds walked more when feeding enrichment was provided because they were attracted by the food items and wanted to reach them. An increase in Locomotion when environmental enrichment is offered has been reported also in other studies with psittacines (de Almeida et al., 2018; Melo et al., 2014; Pimenta et al., 2009) and may denote the success of the enrichment since activity enhances the quality of life and is positive for health (de Almeida et al., 2018).

Earlier studies with blue-and-yellow macaws (de Almeida et al., 2018; Miglioli and Vasconcellos, 2021), Lear's macaws (Azevedo et al., 2016) and cockatiels (Assis et al., 2016) recorded a significant decrease of "Pecking objects from the enclosure" from pre-enrichment to enrichment phase. Pecking objects or object destruction is considered an undesirable behaviour, not only because it involves a continuous replacement of ruined objects, but also because it could be a sign of boredom that manifests itself when environmental stimuli are not enough (Miglioli and Vasconcellos, 2021). Parrots possess high levels of cognition, indeed, even if their brains are smaller, they contain a higher number of neurons, nearly twice, compared to primate brains of the same mass, and with neuronal densities in the telencephalon that exceed those found in mammals. This justifies their high "cognitive power" and thus their intelligence (Olkowicz et al., 2016). Therefore, in the absence of cognitive stimuli, macaws are more predisposed to stress and boredom that lead them to object destruction as a way to overcome tediousness (Miglioli and Vasconcellos, 2021).

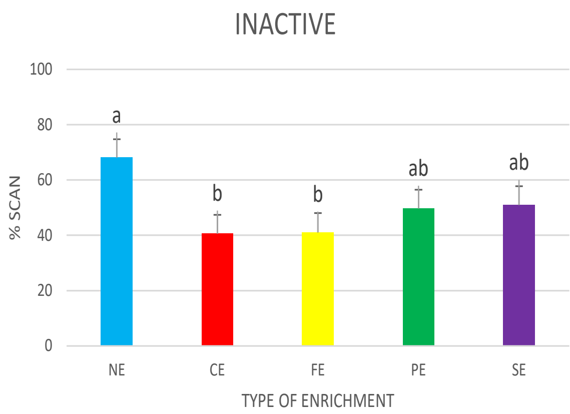
My results do not support their observation, actually, this behaviour increased from the baseline phase to the experimental phase when the four types of enrichment items were introduced, in particular, there was a significant difference during feeding and physical enrichment (Figure 4.1). There is no biological explanation for this rather contradictory result, but there are some possible justifications for this outcome. As already explained, one clarification could be that pecking the objects was a method to occupy time and reduce boredom, but this does not justify why the introduction of feeding enrichment, the one with which parrots have interacted more, induced such an increase. Biting is a common habit in nature and also in captivity because it helps the bird to wear its beak down and avoid overgrowth, therefore it is likely that enrichment items were not sufficient abrasive to accommodate this need (Assis et al., 2016; Azevedo et al., 2016). Anyway, further data collection would be needed to determine exactly how environmental enrichment affected this behaviour. Since comparison with literature seems contradictory, it would be interesting associate qualitative behavioural observation, such as QBA, to the quantitative ones, in order to clarify the dynamic style of expression of such behaviour.

Feeding or nutritional enrichment is often used in captive conditions to stimulate the exhibition of normal behaviours, in particular, to encourage foraging activity. Increasing the time spent feeding is one of the goals of environmental enrichment because it is associated with positive welfare effects and behavioural changes (van Zeeland et al., 2013). For example, it has been shown that foraging opportunities both prevent and decrease abnormal behaviours, like psychogenic feather picking, in young Amazon parrots (C. L. Meehan, Millam, et al., 2003).

As reported earlier, depending on the season and the species, wild psittacids spend 40% to 60% of their waking hours in foraging activity (Lightfoot and Nacewicz, 2006), they travel long distances to reach feeding sites and once arrived they are engaged in a range of behaviours including food selection and manipulation. Captive parrots are constrained by captive feeding methods, thereby they do not have the opportunity to express feeding behaviour as they would in the wild, not only they are not required to travel several miles, search and select their food, but moreover they have limited to no possibility to manipulate items to get the food (C. L. Meehan, Millam, et al., 2003; van Zeeland et al., 2013). The majority of the parrots living in captive conditions, especially those that are part of reintroduction programmes, are not considered domesticated since they are only a few generations out of the wild (C. Meehan and Mench, 2006). Hence, they preserve the instincts, needs and behaviour of their wild counterparts. If wild psittacids are highly encouraged in the performance of foraging behaviours, captive psittacine birds are also potentially highly encouraged to perform these behaviours (C. L. Meehan, Millam, et al., 2003). To prove their behavioural need to forage is the fact that captive parrots show contrafreeloading, which means that, despite the food being freely available, they prefer to obtain food through enrichment items (Azevedo et al., 2016; van Zeeland et al., 2013).

This lends support to previous findings in the literature, where food enrichment items promoted an increase in Foraging behaviour (Azevedo et al., 2016; Checon et al., 2020; de Andrade and de Azevedo, 2011; Miglioli and Vasconcellos, 2021). Contrary to expectations "Feeding" showed a significant difference just between physical enrichment and baseline phase in this study (Figure 4.1). One might think that feeding and cognitive enrichment were not efficient in increasing the foraging behaviour despite containing food. However, it is not possible to affirm this due to a methodological issue. In previously mentioned studies, under "Feeding" was included either the food presented as enrichment, either the one in the usual form, instead in my study "Feeding" referred only to the food presented in the common form in the feeder. Therefore, when macaws were eating the food provided by feeding and cognitive enrichment, this was not recorded under the Foraging category, but under the category Interaction with enrichment. Nevertheless, it is very likely that I would have found a significant increase in foraging behaviour if the same method of observation used in the mentioned studies had been applied also in this one.

The four types of environmental enrichment promoted an increase compared to the baseline phase in "Other behaviours", which revealed to be significant when cognitive and feeding enrichment were applied (Figure 4.1). My study corroborates previous results in the literature (Assis et al., 2016; de Andrade and de Azevedo, 2011) that give a reasonable explanation for this outcome. The authors sustain that the activity of scratching the body in psittacids is a sign of relaxation and tranquillity. Thus, according to this explanation, environmental enrichment had a positive influence on the well-being of great green macaws. Anyway, this category comprises also other behaviours apart from scratching, consequently more extensive research is needed to clarify which behaviours were performed and how these were affected by the enrichment.


INACTIVE
100
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b
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NE
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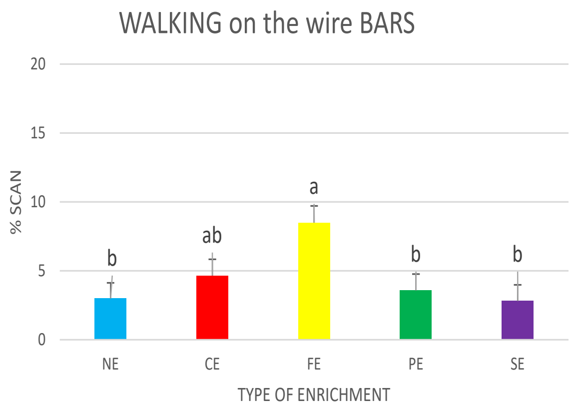
PE
SE
TYPE OF ENRICHMENT


WALKING on the wire BARS
20
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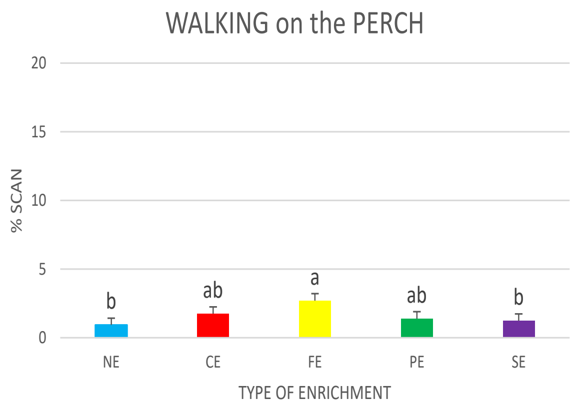
TYPE OF ENRICHMENT


WALKING on the PERCH
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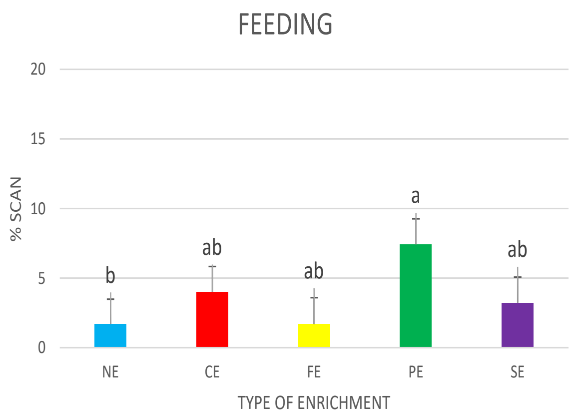
TYPE OF ENRICHMENT


FEEDING
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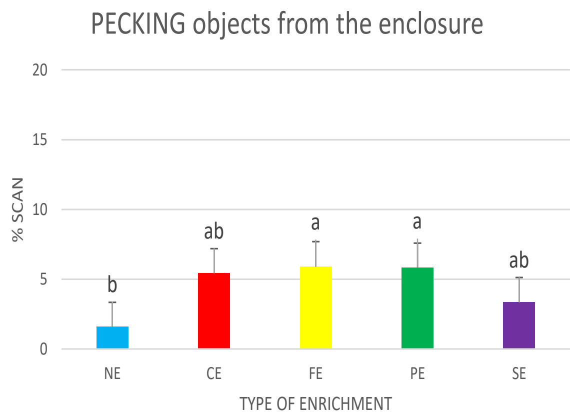
PECKING objects from the enclosure
20
15
10
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0
% SCAN
b
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a
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NE
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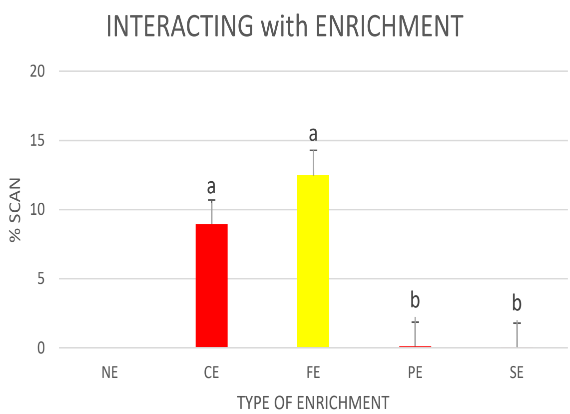
TYPE OF ENRICHMENT


INTERACTING with ENRICHMENT
20
15
10
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% SCAN
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b
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NE
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TYPE OF ENRICHMENT

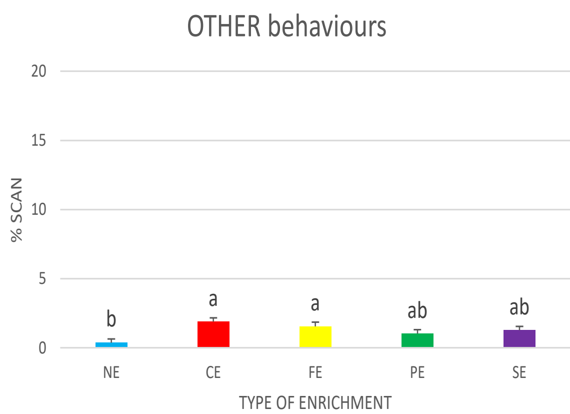
OTHER behaviours
20
15
10
5
0
% SCAN
b
a
a
ab
ab
NE
CE
FE
PE
SE
TYPE OF ENRICHMENT

Figure 4.1

Percentage of scans of those behaviours that were statistically different between the different types of enrichment in great green macaws (*Ara ambiguus*). Different letters indicate statistically significant differences ($p<0.05$). NE = no enrichment; CE = cognitive enrichment; FE = feeding enrichment; PE = physical enrichment; SE= sensory enrichment.

6 Study limitations

It is plausible that a number of limitations might have influenced the results obtained, thus data must be taken carefully. First of all, the time available for the collection of the data (six weeks) was limited and lower than previous similar studies on captive psittacids (Azevedo et al., 2016; Checon et al., 2020; de Almeida et al., 2018; Miglioli and Vasconcellos, 2021), thus it is very likely that this factor has had an impact on the results. It could be that a prolonged period of enrichment observations would have allowed to document interactions between macaws and physical and sensory enrichment.

Duration of observation sessions (15 minutes each) may have affected the results, prolonged behavioural sampling including afternoon sessions would be interesting to investigate.

The wet season constituted another limitation because it did not allow to record behavioural data in the afternoon.

The sample size (ten individuals) is not large, hence it might have represented another limitation since this does not permit a well-structured statistical analysis.

7 Conclusion

This paper has investigated the effects of environmental enrichment on captive green great macaws. Results obtained in this study provide evidence that environmental enrichment positively affected some behaviours: reduction of Rest and increase in Locomotion and Foraging. However, this study did not succeed in reducing pecking behaviour, that instead showed an increase, and in providing evidence of macaws' interaction with physical and sensory enrichment, since parrots interacted just with nutritional and cognitive enrichment objects. There are also some issues that need further investigation, such as neophobia, preference towards specific items, the underestimation of abnormal behaviours and the effects of environmental enrichment on "other behaviours" since it is a broad category.

Environmental enrichment program should be implemented on constant basis and experimentation and evaluation of additional enrichment items is suggested to effectively reduce the stress in captivity and promote natural behaviours.

Taken together, these findings highlight that environmental enrichment has the potential to improve the quality of life of psittacines and other captive birds.

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