UNIVERSIDADE DE LISBOA

FACULDADE DE MEDICINA VETERINÁRIA

FACTORS INFLUENCING AFFILIATIVE AND STEREOTYPICAL BEHAVIOUR EXPRESSION IN EIGHT COUPLES OF SCARLET MACAW (*Ara macao*) FROM A CONSERVATION BREEDING CENTRE IN COSTA RICA

FRANCISCA SEGURADO CARREIRA

ORIENTADOR:
Doutor João Nestor das Chagas e Silva
TUTORA:
Dra. Isabel Hagnauer

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Fatores que Influenciam a Expressão de Comportamentos Afiliativos e Estereotipados em Oito Casais de Araras-Escarlate (*Ara macao*) num Centro de Reprodução para Conservação na Costa Rica.

Resumo

A Arara-Escarlate (*Ara macao*) é uma espécie ameaçada que habita as florestas tropicais/subtropicais do Novo Mundo. Forma grupos sociais complexos, compostos por vários casais monogâmicos que se reproduzem sazonalmente em cavidades arbóreas. A perda de *habitat*, a caça furtiva para comercialização e a perseguição como praga agrícola estão entre as ameaças da espécie na América Central. Inúmeros projetos de conservação *in situ* e *ex situ* foram implementados, incluindo a criação em cativeiro para reintrodução na natureza. As condições ambientais em cativeiro devem permitir a exibição de comportamentos naturais, uma vez que estes promovem o bem-estar geral e a reprodução.

Este estudo visou identificar fatores que podem influenciar positiva ou negativamente a expressão de comportamentos afiliativos e estereotipados em cativeiro. O comportamento de oito casais num centro de reprodução em Alajuela, Costa Rica, foi mensurado utilizando amostragem intervalada, registo de ocorrências importantes entre os intervalos, e a sua localização espacial, com a ajuda da aplicação *ZooMonitor*. Para testar esta influência, tanto os "activity-budgets", como as diferenças entre casais e o momento da observação (presença de sombra suficiente; altura do dia; duração da união do casal; tempo passado desde a última refeição) foram avaliados.

Os comportamentos de conforto representaram a maioria das observações. Todos os casais exibiram comportamentos afiliativos, enquanto que raramente foram observados os agonísticos. Casais com menos sombra exibiram mais comportamentos afiliativos, e as araras revelaram-se mais suscetíveis de exibir comportamentos afiliativos e estereotipados, à tarde. Pressupõem-se a influência negativa do plano alimentar no bem-estar da população, pois quanto mais tempo passou desde a sua alimentação estas exibiram significativamente mais comportamentos estereotipados, e menos comportamentos afiliativos.

Algumas recomendações podem ser feitas para aprimorar o ambiente cativo dos animais observados, como práticas de enriquecimento ambiental alimentar adaptadas às necessidades da população, em associação com a preparação de alimentos fora do recinto e, o desenvolvimento de dietas específicas, contribuindo para a melhoria do seu bem-estar. Assim, a identificação e correção de fatores ambientais negativos pode promover o bem-estar e a taxa de sucesso reprodutivo em programas de reprodução, com implicações relevantes para a conservação da espécie como um todo.

Palavras-chave: Arara-escarlate; monitorização comportamental; fatores ambientais; reprodução para conservação; bem-estar.

Factors Influencing Affiliative and Stereotypical Behaviour Expression in Eight Couples of Scarlet Macaws (*Ara macao*) from a Conservation Breeding Centre in Costa Rica

Abstract

The Scarlet Macaw (*Ara macao*) is a threatened species that inhabits tropical/subtropical forests in the New World Tropics. It forms complex social groups, consisting of several monogamous couples that reproduce seasonally in existing tree cavities. Habitat loss, poaching for pet trade, and persecution as an agricultural pest are among some of the threats the species faces in Central America. Several *in situ* and *ex situ* conservation efforts have been employed, including captive breeding for subsequent reintroduction into the wild. Captive environmental conditions must allow the display of natural behaviours, as this promotes general welfare and reproductive efforts.

This study aimed to identify factors that may positively or negatively influence the expression of both affiliative and stereotypical behaviours in captivity. The behaviour of eight couples from a breeding centre in Alajuela, Costa Rica, was measured using interval-sampling, all-occurrence recording within the intervals, and a space use function with the help of the ZooMonitor app. To test this influence, both activity-budgets and differences between couples and time of observation (presence of sufficient shade; day time; length of time the couple had been together; time since last meal) were evaluated.

Comfort behaviours accounted for the majority of observations. All couples exhibited social affiliative behaviours, while social agonistic ones were rarely observed. Couples with less shade exhibited more social affiliative behaviours, and the macaws were more likely to engage in affiliative and stereotypical behaviours in the afternoon. The negative influence of the feeding schedule in the welfare of the population is presumed, as the more time passed since they were fed, significantly stereotypic behaviours were exhibited, and fewer affiliative ones.

Some recommendations can be made to refine captive conditions of the animals under observation, like foraging enrichment practices tailored to the macaw population's needs, associated with food preparation away from enclosures and the development of species-specific diets, contributing to improve their welfare. Therefore, the identification and correction of negative environmental factors may promote welfare and breeding success rates in breeding programmes, having important implications for the conservation of the species as a whole.

Key-words: Scarlet macaw; behavioural monitoring; environmental factors; conservation breeding; welfare.

Fatores que Influenciam a Expressão de Comportamentos Afiliativos e Estereotipados em Oito Casais de Araras-Escarlate (*Ara macao*) num Centro de Reprodução para Conservação na Costa Rica.

Resumo Alargado

A Arara-Escarlate (*Ara macao*) é uma espécie ameaçada que habita as florestas tropicais/subtropicais do Novo Mundo, onde se alimentam de diversas espécies de plantas. Forma grupos sociais complexos, compostos por vários casais monogâmicos que se reproduzem sazonalmente em cavidades arbóreas onde põem 3 ou 4 ovos, no entanto criam apenas 1 ou dois juvenis até à maturidade. A perda de *habitat*, a caça furtiva para a comercialização ilegal e a perseguição como praga agrícola estão entre as ameaças da espécie na América Central. Foram implementadas, desde a década de 90, diversas leis pelo governo da Costa Rica de modo a proteger a vida selvagem e, também, iniciados inúmeros projetos de conservação *in situ* e *ex situ*, incluindo a criação em cativeiro para reintrodução na natureza.

As condições de cativeiro devem permitir a exibição de comportamentos naturais, de modo a reduzir as disparidades entre o animal e o seu ambiente, uma vez que estes promovem o bem-estar geral e a reprodução. Os principais fatores a ter em conta no ambiente de animais cativos desta espécie são o clima, a nutrição, as instalações, o ninho, a organização social e o controlo de doenças. Este estudo visou identificar quais os fatores que podem influenciar positiva ou negativamente a expressão de comportamentos afiliativos e estereotipados numa população de Araras Escarlate.

Oito casais de Araras Escarlate foram observados no Centro de Aves em Perigo de Extinção (CRAVE) do Rescate Wildlife Rescue Centre, na Costa Rica, de fevereiro a abril de 2022. As instalações do centro possuíam adaptações para quando os casais se encontravam fora da época reprodutiva, com aviários de grandes dimensões, bem como para o decorrer da mesma, com aviários mais pequenos ao redor dos anteriormente referidos. Os aviários para a época reprodutiva estavam equipados com caixa de nidificação, estação de alimentação e com alguns poleiros de madeira, longos o suficiente para que os casais pudessem ficar lado a lado. A alimentação mais substancial era fornecida todas as manhãs, por volta das 7h e, cinco vezes por semana, era também fornecido um enriquecimento alimentar por volta das 15h. Foram realizados vinte e quatro dias de observações presenciais, sendo que cada casal era observado por um período de 15 min, perfazendo um total de 2h, realizadas duas vezes por dia: das 6h30 às 8h30 e das 15h as 17h. O etograma criado com base nas características comportamentais da espécie, foi dividido em sete categorias (conforto, procura de alimento, movimentação, social afiliativo, comunicação, social agonístico e estereotipado) e introduzido na aplicação ZooMonitor, que auxiliou a recolha dos dados. O seu comportamento foi medido

utilizando diversos métodos: amostragem intervalada a 30s; registo de ocorrências importantes entre os intervalos; e a sua localização espacial.

Os fatores que rodeavam o ambiente cativo foram divididos em dois grupos: fatores base, ou seja, constantes ao longo do período observacional, e por isso não avaliados; fatores variáveis, que foram avaliados quanto a presença/ausência de comportamentos sociais afiliativos (considerando que comportamentos de conforto quando em proximidade, são comportamentos sociais afiliativos) e estereotipados. Pretendeu-se perceber a influência da sombra no bem-estar geral dos animais, ao comparar casais que tinham mais sombra na instalação daqueles que tinham menos. A influência da hora do dia nos padrões comportamentais da população foi avaliada, comparando as observações realizadas de manhã, com as da tarde. Comparou-se a expressão destes comportamentos também entre casais que tinham sido emparelhados em anos distintos. E, por último, a influência do plano alimentar foi analisada através da adição do intervalo temporal estimado desde que estes animais tinham sido alimentados pela última vez. Para esse efeito, a análise estatística foi realizada utilizando modelos mistos lineares generalizados.

Os resultados mostraram que a maioria dos fatores base estavam de acordo com a literatura de modo a garantir o bem-estar, e assim maximizar a reprodução. No entanto, perturbações encontradas ao redor das instalações como abutres que as sobrevoavam, obras que decorreram no período observacional e, mesmo, a presença do observador, podem ter contribuído para a expressão de comportamentos estereotipados, bem como inibido a cópula e/ou postura por parte dos casais observados. O facto de as instalações permitirem o contacto destes animais com o solo, pode ter influenciado negativamente a saúde dos mesmos.

O "activity-budget" dos casais mostrou que: os comportamentos de conforto representaram a maioria das observações em todos os casais, demonstrando inatividade excessiva por parte dos mesmos; o comportamento de procura de alimento, exibiu uma média total baixa de observações, quando em comparação com o que se verifica na natureza; todos exibiram comportamentos afiliativos, enquanto que raramente foram observados comportamentos agonísticos, pelo que estes provavelmente se encontravam corretamente emparelhados; os comportamentos estereotipados foram pouco frequentes, mas variaram muito entre casais.

A localização espacial recolhida mostrou que os casais C5 a C8 passaram ligeiramente mais tempo juntos do que separados na sua instalação, quando comparados aos casais C1 a C4. Os casais com menos sombra (C6 a C8) encontram-se incluídos nos casais que estiveram mais juntos na instalação. A análise deste fator mostrou que a presença de sombra exibiu um efeito negativo na expressão de comportamentos sociais afiliativos, contrariamente ao especulado inicialmente. Pelo que, a presença de menos sombra pode ter levado a que os casais estivessem mais juntos na instalação, na pequena sombra que tinham, e que

efetuassem mais comportamentos de conforto em proximidade, os quais são considerados afiliativos.

Não foi possível tirar conclusões significativas acerca do fator "anos em casal", muito provavelmente devido à amostra estudada ser relativamente pequena, e, também, pois a maioria dos casais tinha sido emparelhado no ano do estudo, existindo apenas um representante das restantes categorias estudadas.

Os animais observados exibiram mais comportamentos afiliativos e estereotipados durante o período da tarde, o que revelou como variações diurnas influenciaram os seus padrões comportamentais. Ao saber que as araras estão mais propensas a iniciar comportamentos de conforto após a alimentação, percebemos como o alimento substancial fornecido de manhã pode ter também influenciado a que existissem mais períodos de inatividade neste período.

Quanto mais tempo passou desde a sua última alimentação, estes animais exibiram significativamente mais comportamentos estereotipados e, significativamente menos comportamentos sociais afiliativos. Aliando o resultado da análise estatística com a pouca prevalência de comportamentos de procura de alimento, pressupõem-se a possível influência negativa do plano alimentar no bem-estar geral destes animais e, também, como o enriquecimento alimentar fornecido foi insuficiente para colmatar as necessidades desta população.

Algumas recomendações podem ser feitas para aprimorar o ambiente cativo dos animais observados como o estabelecimento de práticas de enriquecimento ambiental alimentar adaptadas às suas necessidades, como o fornecimento de comida inteira em vez de cortada ou cozida, em associação com um horário de alimentação variável que estimule a procura de alimento por toda a instalação. Também a preparação de alimentos fora do recinto onde se encontram as araras, de modo a evitar o *Food-Antecipatory-Activity* (FAA) desenvolvido através de estímulos sonoros que permitiam aos animais antecipar a sua alimentação. Finalmente, o desenvolvimento de uma dieta espécie-específica e adaptada às necessidades da época reprodutiva, de modo a promover ainda mais a saúde dos indivíduos.

A identificação e correção de fatores ambientais negativos num ambiente cativo, pode contribuir para melhorar o bem-estar e a taxa de sucesso reprodutivo em programas de reprodução para reintrodução, produzindo uma descendência mais saudável e bem sociabilizada que, à posteriori se irá melhor adaptar ao seu *habitat* após reintrodução. Deste modo, a sustentabilidade do programa de conservação é garantida, tendo implicações positivas para a conservação da espécie como um todo.

Palavras-chave: Arara-escarlate; monitorização comportamental; fatores ambientais; reprodução para conservação; bem-estar.

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A. m. cyanoptera – *Ara macao cyanoptera*

A. m. macao – *Ara macao macao*

ACOPAC – Central Pacific Conservation Area

ACOSA – Osa Peninsula Conservation Area

ACTH – Adrenocorticotropic hormone

AD – Anno Domini

AM – ante meridiem

AUC – Area Under the ROC Curve

C – Couple

Ca – Calcium

CITES – Conservation on International Trade in Endangered Species

cm – Centimetre

CRASSA – Centro de Recuperação de Animais Selvagens de Santo André

CRAVE – Centro de Reproducción de Aves en Peligro de Extinción

CRH – Corticotrophin-releasing hormone

Cu – Copper

D – Day

EU Ban – European Union Wildlife Ban

FAA – Food-anticipatory-activity

FDB – Feather Damaging Behaviour

FSH – Follicle-Stimulating Hormone

g – Gram

GLMM – Generalized Linear Mixed Model

GnRH – Gonadotropin-releasing Hormone

h – hour

ha – Hectare

HPA – Hypothalamic-pituitary-adrenal axis

HPG – Hypothalamic-pituitary-gonadal axis

IUCN – International Union for Conservation of Nature

K – Potassium

km – Kilometres

km/h – Kilometres per hour

km^2 – Square kilometre

LAPPA – La Asociación para la Protección de los Psitácidos

LH – Luteinizing Hormone

m – Metre

Mg – Magnesium
MINAE – Ministerio de Ambiente y Energía
NDF – Neutral Detergent Fibre
ºC – Celsius degree
P – Phosphorus
PGE 1 – Prostaglandin E1
PGF2α – Prostaglandin F2 alpha
PM – post meridiem
ppm – Parts per million
S – Sulphur
SINAC – Sistema Nacional de Áreas de Conservación
spp. – Species
USA – United States of America
USFWS – United States Fish and Wildlife Services
WBCA – Wild Bird Conservation Act
Zn – Zinc

1. Internship activities

During the sixth year of the Veterinary Medicine master's degree I did two curricular internships. Both were chosen with the same field of interest in mind: wildlife rehabilitation.

1.1. Centro de Recuperação de Animais Selvagens de Santo André

The first part of the curricular internship was accomplished at the Centro de Recuperação de Animais Selvagens de Santo André (CRASSA), in Vila Nova de Santo André, Portugal, from October 11th until December 15th 2021, amounting to a total of 344 hours.

CRASSA's purpose is to rehabilitate injured or weakened wild animals, to try to release them back into the wild. The number of animals received per year has been increasing, and is now around 400. The great majority of species it receives are birds, and the most frequent causes of admission are trauma, debility and orphaned.

Since this was the first time I came into contact with the work area, my main objective was to understand all the factors that influence rehabilitating a wild animal. Therefore, I could learn how to proceed in all the tasks required, and ultimately practice time and again, in order to perfect my approach to the daily routine of a veterinarian at a wildlife rehabilitation centre.

In the morning the interns were assigned to one of three possible duties: clinic, internment, and kitchen. While on clinic duty I conducted physical examinations, prepared and administered oral, subcutaneous, intravenous and intramuscular treatments, positioned the animal for X-ray and helped to interpret the results, collected blood samples, practiced how to make bandages with and without splints, did physical therapy sessions, cleaned, disinfected and patched wounds, and did cloacal and mouth swabs. While on internment duty I cleaned and prepared (adjusted to each species and diagnosis) the carriers where the debilitated animals were placed to restrict movement. Finally, while on kitchen duty I would prepare and distribute food appropriate to the species (considering its health state) on the internment and the outside facilities, clean and disinfect the outside facilities, and prepare the latter with adequate environmental enrichment for the species it was meant to host. Correct catch, restraint and handling of the animals was a crucial factor across all duties.

In the afternoon we engaged in other important activities, including: performance of necropsies to try to fully understand the underlying cause of death of the animals under the centre's care; completion of laboratory complementary diagnostic exams such as coprology, blood smear visualisation, cytology, study more about the cases at hand, and help with the centre's maintenance.

The species with which I came into contact more frequently in this centre were:

Birds:

- Yellow-legged gull (*Larus michahellis*);
- Lesser black-backed gull (*Larus fuscus*);
- White stork (*Ciconia ciconia*);
- Peregrine falcon (*Falco peregrinus*);
- Common kestrel (*Falco tinnunculus*);
- Red kite (*Milvus milvus*);
- Eurasian griffon vulture (*Gyps fulvus*);
- Little owl (*Athene noctua*);
- Tawny owl (*Strix aluco*);
- Eurasian eagle-owl (*Bubo bubo*);
- Eurasian hoopoe (*Upupa epops*);

Mammals:

- European hedgehog (*Erinaceus europaeus*);
- Red fox (*Vulpes vulpes*);

1.2. Rescate Wildlife Rescue Centre

The second part of the curricular internship was accomplished at Rescate Wildlife Rescue Centre, in La Garita, Alajuela, Costa Rica from February 2nd until March 27th 2022, amounting to a total of 210 hours.

The main objective of this internship was the collection of data for the development of the master's thesis. Furthermore, this centre was also chosen because it is the largest one in Costa Rica, the most biodiverse country in the world. It was a great opportunity to come into contact with a wide variety of species of mammals, birds and reptiles, and learn about their particular characteristics.

The day started at 6 am, so that the two-hour long morning behavioural observation sessions could capture the before and after feeding time periods, which coincided with the morning time when the macaws are most active. At 9 am the day at the hospital started, where several different activities were performed, such as: physical examination; preparation and administration of oral, subcutaneous, intravenous and intramuscular treatments; collection of blood samples; performance and interpretations of complementary diagnostic exams (coprology, blood count and biochemical analysis, X-ray, ultrasound); anaesthesia monitoring and observation of orthopaedic surgery; performance of necropsies in mammals and birds; preparation and distribution of species-appropriate feeding; professional training and discussion of clinical cases with a specialist in exotic and zoo animal medicine about birds, reptiles and amphibians. At 3 pm, the afternoon behavioural observation sessions began, which were also two-hours long. During this period, the macaws are also very active, as the temperature had already slightly declined, but the sun had not set yet.

During the internship, an additional project was undertaken with the macaws of the breeding centre, as the veterinary team needed to assess the parasitological status of these animals. Every week, coprological examinations were done on a three-day pool of faecal samples from four of the centre’s enclosures, by means of the Sheather’s concentrated sugar solution floatation method, with formalin. Eggs of *Ascaridia spp.* and *Capillaria spp.* were found in some of the enclosures. However, deworming with Fenbendazole was only advised if the animals showed any signs of illness, as both these parasites are common in wild animals, and generally are not the underlying cause of disease. Furthermore, all the enclosures were outdoor and the macaws had direct contact to the floor, where the eggs can survive for a long time, so reinfection is almost inevitable if they are to be kept under the same conditions.

Being emerged in another culture had its challenges but, overall, it was a great experience that allowed a broadening of horizons, and understand that there are many ways of doing things successfully. It also taught me to see things from various perspectives, in order to find the one that will lead to a better outcome.

The species I came into contact at Rescate were:

Mammals:

- Two-toed sloth (*Choloepus hoffmanni*);
- Mantled howler (*Alouatta palliata*);
- Common opossum (*Didelphis marsupialis)*
- Panamanian white-faced capuchin (*Cebus imitator);*
- Central American squirrel monkey (*Saimiri oerstedii);*
- Geoffroy's spider monkey (*Ateles geoffroyi);*
- Mexican hairy dwarf porcupine (*Coendou mexicanus);*
- Variegated squirrel (*Sciurus variegatoides);*
- Jaguarondi (*Herpailurus yagouaroundi*);
- Ocelot (*Leopardus pardalis);*
- Coyote (*Canis latrans*);
- Grey fox (*Urocyon cinereoargenteus);*
- White-nosed coati (*Nasua narica);*
- Baird's tapir (*Tapirus bairdii);*
- Hooded skunk (*Mephitis macroura*);
- Long-tailed weasel (*Neogale frenata).*

Birds:

- Scarlet macaw (Ara macao);
- Great green macaw (Ara ambiguus);
- Black-bellied whistling-duck (*Dendrocygna autumnalis);*
- Plain chachalaca (*Ortalis vetula);*
- White-tipped dove (*Leptotila verreauxi);*
- Common nighthawk (*Chordeiles minor);*
- Common potoo (*Nyctibius griseus);*
- Purple gallinule (*Porphyrio martinicus);*
- Brown pelican (*Pelecanus occidentalis);*
- Common black hawk (*Buteogallus anthracinus);*
- Costa Rican pygmy-owl (*Glaucidium costaricanum*);
- Spectacled owl (*Pulsatrix perspicillata);*
- Collared aracari (*Pteroglossus torquatus);*
- Bat falcon (*Falco rufigularis);*
- Yellow-naped parrot (*Amazona auropalliata*).

Figure 1 – The release of a rehabilitated animal, the main purpose of wildlife rehabilitation (original photo).

2. Introduction

Scarlet macaws (*Ara macao*) are one of the most iconic bird species in the humid forests of Central and South America, renowned for their vibrant colours and social behaviour. Unfortunately, they face a number of threats in the wild, including habitat loss, poaching for the illegal pet trade, and persecution as agricultural pests. As a result, *in situ* and *ex situ* conservation efforts have taken place in their countries of origin, particularly in Costa Rica, including captive breeding programmes, with the aim of maintaining viable populations for potential reintroduction (Olah, Butchart et al. 2016).

However, their welfare in captivity can be compromised by a variety of factors, i.e. inappropriate housing, diet, and social interaction, that directly affect the expression of affiliative behaviour, i.e. allopreening, pair bond formation, and copulation (Reimer et al. 2016) and the development of stereotypical behaviour, which involves repetitive, and unvarying movements, and can be a sign of poor welfare in captive animals, therefore negatively influencing reproduction (Mellor et al. 2018). Thorough understanding of scarlet macaw behaviour is crucial to provide appropriate management practices that promote animal welfare and the success of the breeding programmes. Although there is a lot of scattered information regarding the impact of such factors in the genus *Ara*, no research has been conducted that proves their direct influence on the behaviour of this species (Mason 2010; Chmura et al. 2020).

The aim of this research is to identify factors that may be positively, or negatively influencing the expression of both affiliative and stereotypic behaviours, within a breeding programme. To that end, behavioural observation and measurement was conducted in eight couples of scarlet macaw in Costa Rica. It is hoped that some conclusions can be drawn, in order to help improve the welfare of captive macaws and contribute to optimise management practices of scarlet macaw breeding programmes for conservation purposes, which will aid in the increase of reintroduction success, by promoting learning of natural behaviours of the species by the offspring.

3. Literature Review

3.1. Taxonomy

Macaws, a group of long-tailed and brightly coloured New World parrots (Silva 2018), belong to the Kingdom *Animalia*, Phylum *Chordata*, Class *Aves*, Order *Psittaciformes* and Family *Psittacidae* (Linnaeus and Salvius 1758). The family *Psittacidae* includes all parrots, characterised as stocky, large headed birds, with a powerful, short, curved, articulated bill, a highly developed tongue and jaw musculature, and short legs (Josep et al. 1997). Currently the following Genus of macaws are recognised: *Anodorhynchus*, *Cyanopsitta*, *Orthopsittaca*, *Primolius*, *Diopsittaca* and *Ara* (Silva 2018). The scarlet macaw was first described by Linnaeus in 1758 under the binomial name *Psittacus macao*. Now it is one of the nine species included within the genus *Ara*, with the scientific name *Ara macao* (Collar et al. 2020). Its two subspecies are *Ara macao macao* and *Ara macao cyanoptera* (Wiedenfeld 1994).

3.2. Morphology

This bird measures between 81 and 91 cm in height and weight from 800 to 1200g, with an average wing and tail length of 41 and 53 cm, respectively. The subspecies *A. m. macao* is slightly smaller than *A. m. cyanoptera* (Wiedenfeld 1994; Silva 2019). Just like other *Psittacidae* it has a large head, in relation to its body, with a very strong hooked beak. Its feet are zygodactyl, therefore digits II and III face forwards, while digits I and IV face backwards (Harcourt-Brown 2005). Despite being typified by its mostly scarlet-red plumage, when identifying this species, many other features of its external anatomy are worth mentioning (Wiedenfeld 1994; Schmidt and Niell 2000) (Figure 2, 3 and 4):

- Yellow iris, lateral skin of head totally devoid of feathers and light flesh coloured, with bright bony upper mandible, black lower mandible and side of the bill darker at the base;
- Nape, mantle, chest, abdomen and flank scarlet red;
- Scapular and lesser secondary coverts scarlet red;
- Median and greater secondary coverts yellow, with partially blue (*A. m. cyanoptera*) or green (*A. m. macao*) tips;
- Alula, primary coverts and primary and secondary feathers blue;
- Back, rump and upper tail coverts bright blue
- Long and pointy tail feathers scarlet red with blue tips, with reverse side orange to brownish;
- Underwing coverts metallic red;
- Feet grey-brown with dark grey claws;

Figure 2 – Scarlet Macaw (original photo).

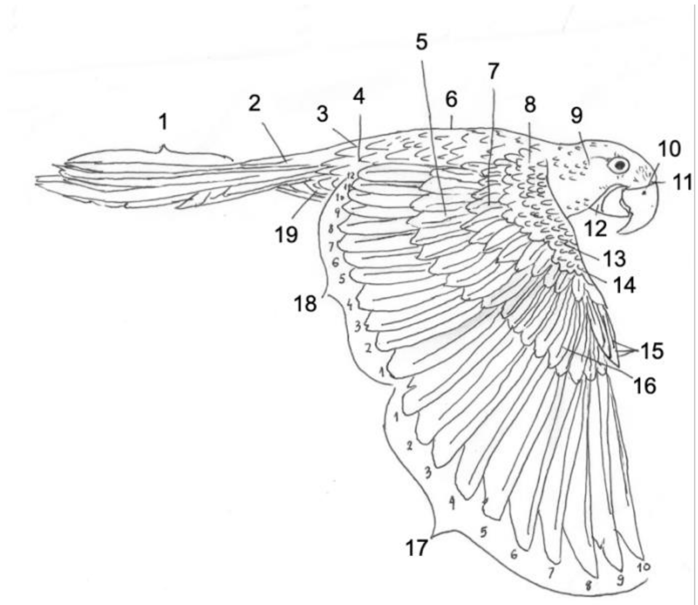


Figure 3 – Topography of a macaw adapted from Abramson et al. (1996). Legend: 1 – tail feathers; 2 – upper tail coverts; 3 – rump; 4 – tertiaries; 5 – dorsal greater secondary coverts; 6 – back; 7 – median wing coverts; 8 – scapular region; 9 – auriculars; 10 – nostril; 11 – cere; 12 – bare facial skin; 13 – dorsal lesser wing coverts; 14 – wrist; 15 – alular quills; 16 – dorsal greater primary coverts; 17 – ten primary flight feathers; 18 – twelve secondary flight feathers; 19 – under tail coverts.

3.3. Reproductive anatomy and physiology

3.3.1. The female

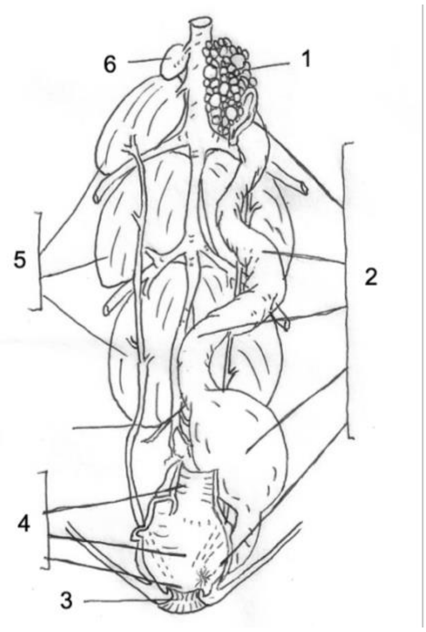


Figure 4 – Anatomy of the female reproductive tract adapted from Abramson et al. (1996). Legend: 1 – ovary; 2 – oviduct; 3 – vent; 4 – cloaca; 5 – kidney; 6 – vestigial ovary.

The process of bird flight requires a reduction in body weight, which is achieved in part by the laying of an externally incubated egg (Pollock and Orosz 2002). Moreover, in the majority of avian species, including the scarlet macaw, only the left side of the female reproductive tract is fully and functionally developed, consisting of, the left ovary and left oviduct (Figure 5) (Abramson et al. 1996; Pollock and Orosz 2002; Crosta et al. 2003; Harcourt-Brown 2005; Scagnelli and Tully 2017).

The ovary is located in the peritoneal cavity adjacent to the left abdominal air sac, attached to the body wall, caudolateral to the adrenal gland and cranial to the left kidney, in its cranial division (Abramson et al. 1996; Pollock and Orosz 2002; Harcourt-Brown 2005). This position is secured by the small mesovarium, the peritoneal lining which acts as its suspensory ligament (Pollock and Orosz 2002; Crosta et al. 2003; Scagnelli and Tully 2017). The blood supply enters the left ovary through the hilum coming from the left cranial renal artery, originating the ovario-oviductal artery, that also irrigates the oviduct. Venous drainage is shared between various ovarian veins that are directly connected to the caudal vena cava, through short segments (Pollock and Orosz 2002; Scagnelli and Tully 2017).

The female gonad can be divided into a central medulla and an outer cortex. The medulla consists of a neurovascular mesh that, with the aid of smooth muscle and interstitial cells, supports and nurtures each follicle. The cortex is made up of several obvious follicles, which will give the ovaries a different appearance depending on the phase they are in, since macaws are birds with a seasonal laying period, as will be addressed later on (Pollock and Orosz 2002). In the immature phase, the follicles are small giving the ovary a "brain-like" appearance (Abramson et al. 1996). The prenuptial acceleration phase happens at the start of follicular development, as each follicle reaches maturity at a different time a hierarchical pattern is shown, presenting an inhomogeneous colour. The culmination phase, simultaneous to egg-laying, means the enlarged follicles reach their most active stage of development. A yellow colour appears as the follicles are full of yolk proteins. To prevent immediate competition when passing into the oviduct, not all follicles reach maturity at the same time. Lastly, in the resting of refractory phase/nonbreeding season, there is a marked regression of the follicles meaning the ovary is quiescent and small (Pollock and Orosz 2002; Scagnelli and Tully 2017).

Each follicle contains a large primary oocyte surrounded by a multi-layered tissue and is suspended by a medullary stalk consisting of smooth muscle, blood vessels and nerves. The nerve supply has both adrenergic and cholinergic fibres, implying an important role in ovulation. The stigma or meridional band is where the follicular wall is split during ovulation, releasing a secondary oocyte which underwent a reduction division and is now haploid, to be captured by the oviduct. The surrounding tissue is known to have an endocrine role, allowing communication between the ovary and oviduct during the passage of each ovum (Pollock and Orosz 2002; Harcourt-Brown 2005). The sex linked chromosomes are carried by the females as each egg contains either a Z or W chromosome whereby combined with the male sperm

ZW is a female and ZZ is a male (Abramson et al. 1996). There is no post-ovulatory corpus luteum in birds (Pollock and Orosz 2002).

The oviduct is differentiated into five regions, associated with egg formation and development, as follows: infundibulum, magnum, isthmus, uterus or shell gland, and vagina (Abramson et al. 1996; Pollock and Orosz 2002; Crosta et al. 2003; Harcourt-Brown 2005; Scagnelli and Tully 2017). It is supported by a dorsal and a ventral ligament (Harcourt-Brown 2005). The basic structure of the wall resembles other tubular organs of the body, consisting of a mucosa, an underlying submucosa, external layers of smooth muscle and an epithelial peritoneum. Ciliated and glandular are the two predominant cell types found in the mucosa, which vary in number depending on the region's function. Multicellular tubular glands are also found in each region of the oviduct, except the vagina. Mucosal folds spiral along the oviduct to enable the rotation of each egg as it moves distally, propelled by the outer layer of smooth muscle and the cilia of the ciliated cells towards the cloaca (Pollock and Orosz 2002).

The proximal opening of the oviduct, the infundibulum, is responsible for capturing the ovulated ovum, an action facilitated by the conformation of the left abdominal air sac. Fertilisation of the oocyte by the spermatozoa is limited to a period of 15 minutes, since it must happen before the first layer of albumen surrounds it. Sperm is stored in the glandular grooves and tubular glands found in this portion, enabling a faster access to the oocyte. The yolk takes approximately one hour turning through the infundibulum, during which time a thin layer of dense albumen is deposited and twisted forming the chalazae (Pollock and Orosz 2002; Harcourt-Brown 2005; Scagnelli and Tully 2017).

In the magnum, the remnant of the albumen is added to the yolk over several hours, secreted by numerous tubular glands, which increase the thickness of the mucosal folds. This is the longer and most coiled portion of the oviduct (Abramson et al. 1996; Pollock and Orosz 2002; Harcourt-Brown 2005; Scagnelli and Tully 2017).

The isthmus is where the shell membranes (inner and outer) are formed, covering the egg while determining its final shape. It is shorter than the magnum and its mucosal folds are less prominent, reducing the time spent on this segment, which in chickens is about 75 minutes (Abramson et al. 1996; Pollock and Orosz 2002; Harcourt-Brown 2005; Scagnelli and Tully 2017).

The uterus, composed of two anatomically distinct portions, is cranially short with a reddened appearance and expands distally to form a pouch, also known as shell gland, that retains the egg through the entire process of egg shell formation. Apart from the longitudinal mucosal folds, distally there are also transverse folds in this region, resulting in lamellae with a leaf like appearance that flatten against the developing egg. Firstly, water and electrolytes are added to the albumen, which enlarges it to a plumped egg shape. The calcium carbonate and protein inner shell membrane is produced by extraction of large amounts of calcium from the bloodstream, with the need to release calcium stored in the long bones, presenting a white colouration in parrots. This membrane is then covered with a cuticle, outer shell membrane, which is water repellent, reduces evaporative loss and provides protection against microorganisms. The egg spends 80% of its time in the oviduct, in this region (Abramson et al. 1996; Pollock and Orosz 2002; Harcourt-Brown 2005; Scagnelli and Tully 2017).

The final part of the oviduct, the vagina, is the shortest but has the thickest muscular wall since its function is oviposition, providing conduit for the egg to pass from the oviduct through the cloaca and out the vent. The sperm storage in the vagina (vaginal sperm) is achieved due to the presence of tubular crypts or spermatic fossulae, which shows an important adaptation for rapid fertilisation. After copulation, part of the sperm is stored here and the rest is able to reach the infundibulum within a few minutes (Abramson et al. 1996; Pollock and Orosz 2002; Harcourt-Brown 2005; Scagnelli and Tully 2017). Usually the egg is described moving down the oviduct. However, due to its length and the size, it remains relatively static in the coelomic cavity while, by peristalsis the oviduct moves over it (Harcourt-Brown 2005). Scarlet macaws can lay 3 to 4 fertile eggs per breeding season (Silva 2019).

3.3.2. The male

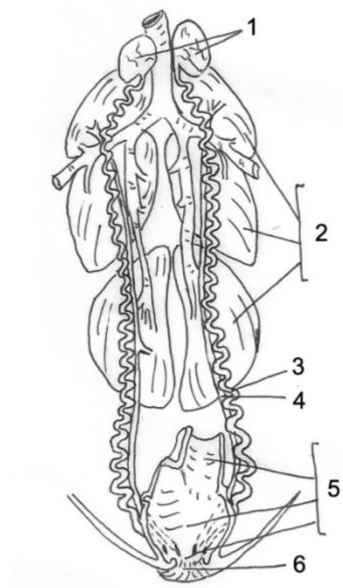


Figure 5 – Anatomy of the male reproductive tract adapted from Abramson et al. (1996). Legend: 1 – testes; 2 – kidney; 3 – ductus deferens; 4 – ureter; 5 – cloaca; 6 – vent.

A pair of testes, epididymis and ductus deferens constitutes the male bird reproductive tract (Figure 6). No accessory sex glands are present, unlike mammals, and both are hormonally active and functional, unlike their female counterparts (Abramson et al. 1996; Pollock and Orosz 2002; Crosta et al. 2003; Harcourt-Brown 2005; Scagnelli and Tully 2017).

The testes are located in the dorsal coelomic body cavity on either side of the vertebral column, next to each adrenal gland, between the caudal end of the lungs and cranial division of the kidneys, while partially surrounded by an abdominal air sac. The mesorchium is the mesentery ligament that suspends each testis into the coelomic cavity, serving also as conduit for nerves and blood vessels (Abramson et al. 1996; Pollock and Orosz 2002; Crosta et al. 2003; Harcourt-Brown 2005; Scagnelli and Tully 2017). The major share of blood entering the testis comes from the testicular artery, a branch of the cranial renal artery, and is drained by various short segments into the caudal vena cava (Crosta et al. 2003). In *Psittaciformes*, the right testis is smaller than the left one, due to germ cells migration during development, both presenting an oval to oblong shape with a melanistic colouration (Abramson et al. 1996; Crosta et al. 2003; Harcourt-Brown 2005; Scagnelli and Tully 2017).

The thin layer covering the testes, the tunica albuginea, gives their surface a smooth appearance, since they are not lobulated like in mammals (Pollock and Orosz 2002; Crosta et al. 2003; Scagnelli and Tully 2017). The inner part of the avian testis consists of thousands of seminiferous tubules, with a greater number of anastomoses than in mammalian species. Their mucosal lining is made up of a spermatogenetic epithelium or germ cells, supported by Sertoli cells. The interstitial, or Leydig cells, are found in between the tubules, their function will be addressed later on. Spermatogenesis occurs via three phases: multiplication of germ cells, which are then named spermatogonia; growth and enlargement of the latter, which turn into primary spermatocytes; the maturation phase includes the first division to form the secondary spermatocytes and the meiotic second division, which will then form spermatids. After each spermatid is developed into spermatozoa, they detach from the mucosal lining and are sent down the straight tubules, through the rete testis, towards the epididymis. This process takes less time in birds than in mammals (Pollock and Orosz 2002; Crosta et al. 2003).

Size variation of the testes is dependent on the phase of the reproductive cycle. In seasonal birds, like the scarlet macaw, the inactive testes are small with no obvious blood supply. Their dimension increases greatly with sexual activity exhibiting three phases of development: the regeneration phase when all the ductwork of the male reproductive tract regenerates; the acceleration one, characterised by the production of spermatozoa; finally, the culmination phase is when there is full development of the reproductive system (Pollock and Orosz 2002; Crosta et al. 2003; Harcourt-Brown 2005;).

The epididymis lies on the dorsomedial surface of the testis and is shorter than that of mammals. This region collects the spermatozoa from the rete testis by mean of efferent ductules, which enter along its whole length, leading them towards the ductus deferens (Pollock and Orosz 2002; Crosta et al. 2003; Scagnelli and Tully 2017).

What distinguishes the ductus deferens from the ureters is a tortuous or zigzag appearance along its entire length, which is more pronounced in seasonally active birds. It broadens distally, upon contact with the urodeum on the dorsal wall of the cloaca, forming the ejaculatory papilla or seminal glomus, which is believed to have a role in sperm storage as its temperature can be 6ºC below body temperature, an important factor for sperm viability (Pollock and Orosz 2002; Crosta et al. 2003; Harcourt-Brown 2005; Scagnelli and Tully 2017). Macaws, like other *Psittaciformes*, do not have a phallus. Copulation occurs through eversion of the cloacal wall, the urodeum, which guarantees the transfer of semen into the everted vaginal orifice (Abramson et al. 1996; Crosta et al. 2003; Scagnelli and Tully 2017).

3.4. The endocrine system

The reproductive system is directly influenced by endocrine and behavioural components, which are led by the hypothalamus–pituitary–gonadal axis (HPG) in response to environmental triggers and internal factors (Crosta et al. 2003).

The hypothalamus is responsible for the secretion of a gonadotropin-releasing hormone (GnRH) that through portal blood vessels is transported to the anterior pituitary gland or adenohypophysis. GnRH has control over the secretion of the luteinizing hormone (LH) a gonadotropin produced by the pituitary gland (Pollock and Orosz 2002; Crosta et al. 2003; Ritchie and Pilny 2008). LH release is also stimulated by progesterone and other non-physiological doses of corticosterone and testosterone (Pollock and Orosz 2002). The follicle-stimulating hormone (FSH) is also produced by the pituitary but the influence of GnRH in its secretion is not yet fully understood (Pollock and Orosz 2002; Crosta et al. 2003). Pulsatile GnRH exposure leads to an increase in gonadotropin production, while a constant exposure will decrease its output. Both gonadotropins have a direct effect in gonadal function, stimulating the production and consequent release of gonadal steroids. Upon return to the central nervous system, via the bloodstream, they will exert a negative feedback on the production and release of GnRH (Pollock and Orosz 2002; Crosta et al. 2003). Only when conditions are appropriate for breeding will the HPG axis stimulate activation of the ovary and testes (Sant 2006).

3.4.1. Female reproductive regulation

There are seventeen different hormones involved in the ovulatory cycle of female birds (Crosta et al. 2003). During the pre-ovulatory phase there is a general upturn in hormonal activity, starting three weeks before ovulation with a marked rise of LH levels. Gonadotropins have a direct effect on steroid hormone production by pre-ovulatory follicles, although FSH may also have some influence on the production and maintenance of pre-ovulatory follicles and on the regulation of follicular atresia. Thecal interstitial cells of small pre-ovulatory follicles secrete oestrogens and androgens, which stimulate oviduct growth, development of oviductal glands, synthesis of vitellogenin, albumin and other oviductal proteins. Certain behaviours, such as increased food intake, nest-building, courtship and brood patch development, are also linked to higher oestrogen levels. High oestrogen concentrations, will prepare granulosa cells, of the large pre-ovulatory follicles, to secrete progesterone and prostaglandins, also leading to osteomyelosclerosis, a marrow ossification of the long bones linked to egg shell production, and hypercalcemia, due to the production of calcium-binding proteins (vitellogenin and albumin) (Pollock and Orosz 2002).

Progesterone levels begin to increase about one week previous to ovulation, reaching a peak 6 to 8 hours prior to this process. Rise in progesterone is required for the LH surge, which also occurs 6 to 8 hours before ovulation, an interaction in a positive feedback system that leads. Oestrogen levels fall during this time which, combined with the rise in progesterone levels, might play a more important role than absolute concentrations of this hormone. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is released 15 to 60 minutes before ovulation, causing uterine contractions. Ovulation is induced by the LH surge that causes the smooth muscle of the follicular stalk to contract, splitting the stigma in the process. In birds, the follicle regresses and is absorbed quickly, not originating either a corpus luteum nor corpus haemorrhagic (Pollock and Orosz 2002).

Oviposition is a complex process resulting in expulsion of the egg by relaxation of the abdominal and vaginal muscles and the utero-vaginal sphincter, while the uterus contracts. $PGF_{2\alpha}$ acts alongside vasotocin in stimulation of the myometrial contractions, while prostaglandin E (PGE 1) profoundly relaxes the sphincter (Pollock and Orosz 2002). Arginine vasotocin (avian antidiuretic hormone) concentrations are highest during oviposition, also appears to stimulate uterine contractions (Pollock and Orosz 2002; Ritchie and Pilny 2008). Prolactin influences incubation and parental behaviours. In *Psittaciformes* incubation starts while the clutch is being formed, since the interval between each oviposition is approximately 48 hours, therefore prolactin levels increase slowly, taking longer to reach the peak that signals the cessation of oviposition (Pollock and Orosz 2002).

3.4.2. Male reproductive regulation

Increasing concentration of gonadotropins in the bloodstream leads to testicular hypertrophy, tubular maturation and also an enlargement of the ductus deferens, all these characterise a sexually active male bird. LH acts directly in the interstitial cells, or Leydig cells, stimulating their growth and maturation. These cells contain cholesterol-rich lipid droplets which act as steroid hormone precursors, such as testosterone, their main product (Pollock and Orosz 2002; Crosta et al. 2003;). The Sertoli cells respond mostly to FSH and also produce steroid hormones in smaller quantities. Testosterone stimulates spermatogenesis, the growth of the epididymis and maturation of tubules, reaching its peak concentrations during the breeding season, decreasing later when the male provides parental care for the chicks, time when progesterone levels are gradually increasing. In captivity testosterone levels are often much lower than in free-ranging animals (Pollock and Orosz 2002). Male sexual behaviour, such as vocalisation and aggression, that are related to the establishment of territory or nest site, is expressed by the presence of low concentrations of estradiol, a hormone resulting from the conversion of testosterone by the enzyme aromatase in the central nervous system (Pollock and Orosz 2002).

3.4.3. Stress response

The endocrine stress response is a crucial physiological mechanism that enables animals to adapt to environmental challenges and cope with stressful situations (Allgayer and Cziulik 2007). Birds, like mammals, possess a complex system of endocrine stress responses led by the hypothalamic-pituitary-adrenal axis (HPA). Adverse situations (social interactions, environmental changes, and handling) trigger hypothalamic release of corticotrophin-releasing hormone (CRH), stimulating the pituitary release of adrenocorticotropic hormone (ACTH), which culminates in the adrenal secretion of glucocorticoids (Dickens and Bentley 2014). Physiological and behavioural responses to glucocorticoids include glucose mobilisation, increased lipogenesis and fat deposition, reduction of reproductive effort, escape response, and increase in locomotor activity, foraging and food intake. This mechanism allows an appropriate redirection of energy from noncritical expenditure (e.g. reproduction) towards self-maintenance or survival (Breuner 2011). Glucocorticoid release, in response to routinely considered stressful situations (sexual courtship, copulation, hunting and egg laying) is desirable. Nonetheless, chronic or intermittent activation of the stress response can have adverse consequences such as decreased individual fitness due to immunosuppression and tissue atrophy; behavioural changes, also known as stereotypies and alterations in the HPG, causing a decline in sex steroid concentrations, which causes reproductive suppression with consequent reproductive impairment (Pizzutto et al. 2009).

3.5. Distribution and Habitat

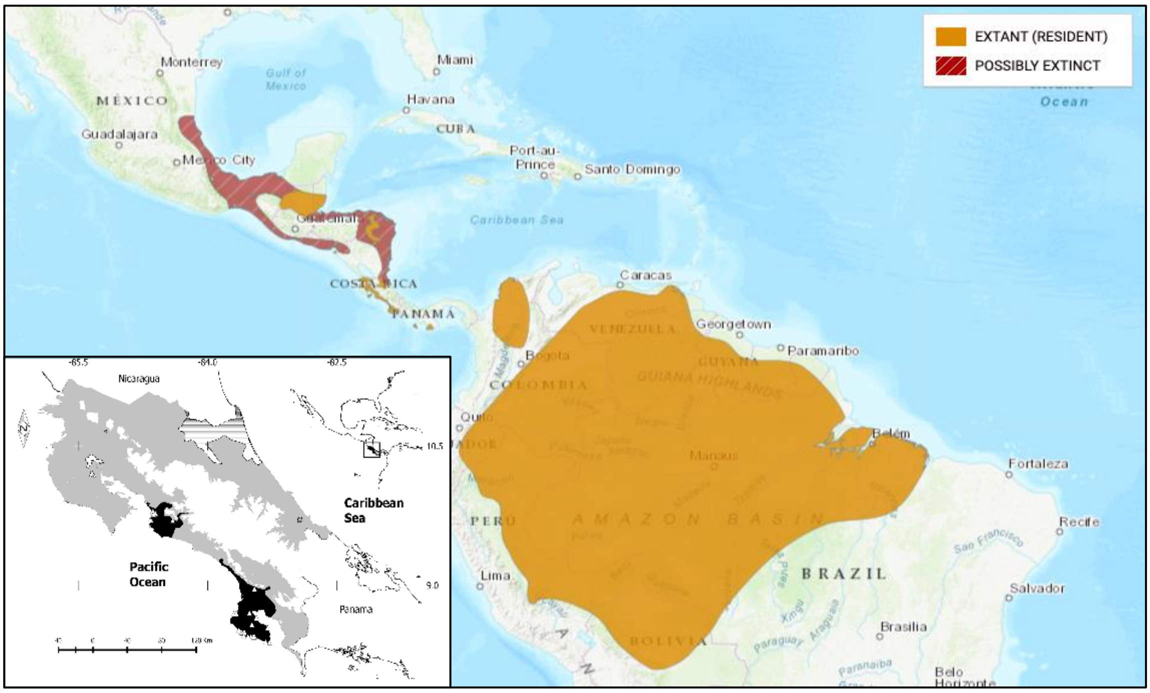


Figure 6 – Map of the geographic range of the scarlet macaw (*Ara macao*) worldwide according to BirdLife International (2016) and in Costa Rica, where black denotes current and grey historical distribution (adapted from Monge et al. 2016).

This species has a natural distribution throughout the New World Tropics, where it inhabits mostly subtropical/tropical (moist lowland; dry; mangrove vegetation above high tide level) forests, open woodlands, moist savannahs and occasionally adjacent agricultural fields, from sea level up to 1000 m above it (Silva 2018; Collar et al. 2020; IUCN 2022). Each subspecies has a distinct distribution, while *A. m. cyanoptera* ranges from northern Costa Rica as far north as south-eastern Mexico, *A. m. macao* occurs through Costa Rica, to Central Bolivia and Mato Grosso, Brazil (Figure 7) (Silva 2018; Collar et al. 2020). Converging in the Río San Juan area, the Central America boundary between the two is a central mountain range in Costa Rica, with *A. m. cyanoptera* found on the Caribbean (eastern) slope and *A. m. macao* on the Pacific (western) slope (Schmidt et al. 2019). The term scarlet macaw will be used throughout the dissertation, to designate *A. m. macao*, the most common subspecies in Costa Rica.

In the past, Costa Rica was mainly covered by tropical rainforests, enabling wildlife to spread throughout the whole country (Myers and Vaughan 2004). Today, its landscape is predominantly agricultural with limited stretches of forest (<40 ha), which are part of protected areas (Sanchez 2018; Myers and Vaughan 2004). A warm (mean annual temperature 25º-30ºC) and humid (2.5-3.3 m annual precipitation) climate typifies the country, marked by two distinct annual seasons: the dry (January-April) and the rainy (May-December) (Myers and Vaughan 2004). The scarlet macaw used to inhabit about 85% (42.500 km^2) of Costa Rica, inside its dry and wet forests from sea level until 1.500 m of elevation (Marineros and Vaughan 1996; Vaughan 2012). By 1993, with only 20% (9.100 km^2) of the habitat left within protected areas, wild scarlet macaws could only be found on the Pacific side of the country: in the Osa Peninsula Conservation Area (ACOSA) with 200 to 400 individuals, in the Central Pacific Conservation Area (ACOPAC) with 200 to 250 individuals (Figure 8), and in Palo Verde and Barra Honda National Park with 15 individuals (Marineros and Vaughan 1996).

Between 1995 and 2000, the population started to grow, with the help of conservation strategies applied (Vaughan, Nemeth, et al. 2003). Recent evaluations show that the ACOPAC population is now constituted of about 600 individuals, covering a wider range, and that the ACOSA has more than 1000 individuals (Vaughan 2019). The total world population is around 20.000 to 50.000 individuals (USFWS 2019).

Figure 7 – Map of the conservation areas across Costa Rica (SINAC 2023).
Legend: 1 - ACOPAC; 2 - ACOSA.

3.6. Ecology

The scarlet macaw lifespan is around 40 to 50 years in the wild, which may double in captivity, with a generation length, as in “the average age of parents of the current cohort”, of about 8.5 years (Silva 2019; Bird et al. 2020). The species is known to feed on approximately 25 families, and 126 species of plants including native seeds, fruits, leaf shoots, flowers, bark and, sometimes, insects (Vaughan et al. 2006; Collar et al. 2020; VKM et al. 2020). Seasonal movements might be observed in search for food (Collar et al. 2020).

Breeding or nesting season varies with latitude, rainy seasons and fruit availability in its distribution areas, but is usually between December and May in Costa Rica, with some onset variation (Guittar et al. 2009; Vaughan et al. 2009; Dear et al. 2010). Scarlet macaws are obligate secondary cavity nesters, laying their eggs inside natural cavities that are either natural holes (i.e., broken tree limb) or made by another bird such as large woodpeckers (i.e., primary excavator), and sometimes they even excavate their own cavities in soft snags (Collar et al. 2020). The species prefers deep hollows with large internal diameters (improves hatching success) and large entrances, in emergent trees with isolated canopies close to their conspecifics (anti-predator benefits) and to fruiting trees (Britt et al. 2014; Olah et al. 2014). The species will often nest in the cavities that previously proved successful (Renton and Brightsmith 2009). Their breeding success is dependent upon the availability and quality of nesting sites (Vaughan, Nemeth, et al. 2003).

Only 20% of the wild population may breed in a given year (Iñigo-Elias 1996), and as asynchronous egg layers a breeding pair can produce up to three or four eggs per clutch, with 8 hours to 7 day intervals, and is considered successful if one, two, or rarely, three young fledge the nest (Myers and Vaughan 2004; Vaughan 2019). Although three eggs hatch on average (mean clutch size is 2.7), in general only one or two chicks survive until fledging, mainly due to purposeful starvation of the youngest siblings (Vigo et al. 2011; Olah et al. 2014; Vigo-Trauco et al. 2021). Breeding failure usually happens during the incubation period, although egg replacement has been observed, if they are lost due to predation or by accident (Allgayer and Cziulik 2007; Olah e al. 2014). Eggs hatch 25 to 28 days after laying and younglings fledge the nest after approximately 75 to 85 days' post hatch (Vaughan et al. 2009; Vaughan 2019).

3.7. Behaviour

Wild individuals possess physical and behavioural adaptations that suit their complex everyday activities, including capturing and gathering food, avoiding predators, finding or creating shelter, and the choosing of mates in order to successfully reproduce. Regarding the scarlet macaw, the research done on the breeding ecology of the wild populations for conservation efforts is the starting point to fully understand their natural behaviour (Myers and Vaughan 2004).

3.7.1. Social organisation

Outside the breeding season, scarlet macaws are generally seen flying in bonded pairs or small family groups with one or two fledglings (Wilson 2006; Tynes 2010). However, they form large roosting flocks by night, engaging in social activities and resting. These split up into groups of 20 to 30 individuals at feeding trees by day, in order to minimise intraspecific competition for food, as has been observed in a mangrove reserve in Costa Rica between 7-9 am and 3-5 pm (Marineros and Vaughan 1996; Renton 2000; Seibert and Sung 2010). Flock formation is a behaviour endorsed by unstable food sources which is crucial to: increase foraging efficiency (individuals benefit from collective knowledge); help detect and avoid predators, which reduces predation pressure and strengthens territorial defence; and improve reproductive success by allowing access to partners (Seibert 2006; Seibert and Sung 2010).

The arrangement of flock members is not random because complex relationships are maintained between them, which can be measured by observation of spatial patterns and proximity (Seibert 2006).

The basis of a flock's complex social organisation is the establishment of dominance relationships, with display of assertive behaviour toward subordinates, who passively retreat based on the outcome of previous interactions. This reduces the occurrence of competitive conflicts, leading to more stable and predictable interactions (Seibert 2006; Seibert and Sung 2010; Tynes 2010). Highest in the hierarchy are the older and more aggressive breeding males, followed by the mature females paired with these males when these are close, otherwise they end up below all other adults (Tynes 2010). These individuals obtain priority access to food resources, roosting sites, nests, and mating opportunities (Seibert and Sung 2010). The scarlet macaw is socially monogamous and the bond between the pair is essential, not just to ensure reproductive success, but to inform the place that pair has in the social hierarchy (Andrews 2022). Weak bonds are formed among small social groups of immature females that move, feed and socialise together. On the other hand, the bonds between immature males of similar strength and social standing are quite strong, but end up weakening as they mature and start challenging each other in an aggressive way (Tynes 2010).

3.7.2. Foraging

In the wild, as opportunistic foragers, a parrot's diet is dependent upon its surrounding environment. Foraging, a very demanding high-energy activity, can amount to 70% of the waking day, beginning immediately after sunrise until sunset. Flying on average at 56 km/h, scarlet macaws travel tens of km a day in search for feeding sites, engaging in food search, selection and manipulation behaviours upon arrival (Renton 2000; Meehan and Mench 2006). The species relies on olfactory cues for food location and navigation within the habitat (Graham et al. 2006). Feeding is silent to avoid drawing attention of competitors and predators to the site (Meehan and Mench 2006; Seibert and Sung 2010).

3.7.3. Communication

As complex communicators, psittacine birds use vision, posture and vocalisation to interact with conspecifics (Wilson 2006). Vocalisation is the most diverse form of communication when it comes to parrots, as there are numerous calls containing meanings that no human can entirely understand. Examples reported in the literature include: flight calls to keep flocks together while flying; contact calls to locate flock members; alarm calls to alert other members of the presence of predators or other dangers; calls to inform food has been located; and other vocalisations to specify social relationships or potential mates (Seibert 2006; Tynes 2010). During vocal development, juveniles learn to communicate with other flock members through vocal mimicry of their parents' calls. This innate characteristic can be used to avoid predators or simulate their presence by mimicking their calls. Besides, psittacine birds possess vocal plasticity, for they can learn new vocalisations during their lifetime. Visual communication is achieved through signals (a behaviour that benefits the sender by influencing the recipients' behaviour) and displays (ritualised signal with a specific message), which are presented as a mixture of body and feather movements. Posture is relevant during courtship and agonistic interactions, which will be addressed later on (Seibert and Sung 2010).

3.7.4. Comfort

Comfort behaviours take up the major part of a 24-hour day. As previously mentioned, parrot flocks sleep and rest as a group in a roosting area, that is distinct from the feeding area where the day is spent. When nesting, the roosting area is near or at the nest site. The sleeping position of a parrot is fairly horizontal, while its lower body is in close contact with the perch. Before falling asleep it fluffs up its feathers and there is a decrease in blinking and eye movement. The eyes close only once the bird is asleep and it may tuck its head under the scapular feathers (Bergman and Reinisch 2006).

Time spent grooming comes second to foraging, while there is light, and is more commonly observed after the morning and afternoon meals, and right after returning to the roosting area. There are several grooming behaviours that serve different purposes. Beak preening helps to maintain feathers in good condition to enable flight, as well as thermoregulation, waterproofing, camouflage and communication. The beak is also used to clean feet and legs. Their flexibility enables the feet to "preen" the head, a behaviour known as scratching. Stretching normally follows a period of rest, before engaging in another activity, extending each leg and wing on the same side backwards and simultaneously. Other grooming behaviours include yawning, and beak rubbing and grinding (Bergman and Reinisch 2006).

3.7.5. Agonistic

Agonistic behaviour within the framework of social interactions can be either aggressive or submissive, a pattern of communication that functions to terminate the aggressive encounter, that avoids direct combat (Seibert 2006). Higher ranking individuals assert their dominance through aggressive behaviour such as turn threat (turn abruptly toward opponent, neck and head extended), beak gape (open beak toward opponent), peck threat (pecking without making contact), wing flapping, rushing (running towards opponent) and flight approach (flying towards opponent). Whereas the submissive will crouch, fluff their feathers, wag their heads, lift one foot, or completely avoid assertive flock members (Seibert 2006; Tynes 2010).

3.7.6. Affiliative

Parrot affiliative behaviours, that arise from activation of the HPG axis and pair bond formation, consist of: allopreening, close proximity, nest seeking and preparation, reduced aggression, courtship feeding or allofeeding, courtship and copulation (Sant 2006; Seibert 2006; Spoon 2006).

Allopreening happens "when an individual uses its beak to groom another" (Seibert 2006, p.46) and is believed to be the key mechanism for strengthening the pair bond and other preferred social relationships (Seibert 2006; Seibert and Sung 2010; Picard et al. 2019). Both allopreening and close physical proximity indicate that the birds have affiliative social bonds (Picard et al. 2019). The bird requesting allopreening lowers its head showcasing the neck to be preened first in scarlet macaws, this activity may involve the head, neck, wings, and tail regions (Bergman and Reinisch 2006; Seibert 2006). It is still unclear whether head preening and mutual preening are related to stronger and more valuable relationships (Picard et al. 2019), but Silva (2018) described that in macaws, mutual preening of the vent area was more commonly observed between pairs during the breeding season.

Courtship feeding or allofeeding is "the regurgitation of food by one individual to another excluding the provisioning of a mate during incubation or brooding and the feeding of chicks" (Spoon 2006, p. 69). The female crouches down, lowers her head, ruffles the feathers, and vocalises in order to request feeding from the male, who in turn displays head bobbing, grasps her beak and regurgitates (Seibert 2006). This behaviour, driven by increased levels of testosterone, is related with courtship display and copulation in species where the female alone incubates the eggs, due to the caloric demand of egg laying and chick feeding. Moreover, it may function to strengthen and maintain the bond (Millam 1999; Sant 2006; Seibert 2006; Spoon 2006; Seibert and Sung 2010).

In macaws, courtship by the male includes a mixture of simple moves such as hopping, bowing, strutting, or tail wagging, while blushing on their facial skin and contracting the pupils (Sant 2006; Silva 2018). When the female is receptive, she moves forward and waves her tail, signalling the mate (Wilson 2006). Copulation is done from the side, whereby the male mounts the female with one foot placed on the perch, which allows contact between their cloacae until ejaculation. This lasts about a minute and is hormonally timed with ovulation to achieve internal fertilisation (Sant 2006).

The monogamous pair bond formation is a very common adaptation of tropical and subtropical psittacine species. It allows reproduction to occur as soon as the environmental cues permit, without requiring complex courtship rituals and grants the advantage and mutual benefit of task division, when preparing to hatch and rear young (Sant 2006). Exhibition of the affiliative behaviours described can be observed throughout the year, but intensify toward and during the breeding season conveying sexual signals, a powerful mechanism to time reproductive efforts through trigger of the hormonal cascade (Renton 2000; Sant 2006; Wilson 2006). Aggressive behaviours in defence or support of the mate are also commonly observed in bonded pairs (Seibert and Sung 2010).

3.7.7. Parental

After finding and preparing an individual nest, eggs are laid inside and incubated by the female while the male forages for food, assuming a bi-parental care strategy from the start. Scarlet macaws greatly defend their nests from predators and can fight with conspecifics to demonstrate power over them (Wilson 2006; Vigo-Trauco 2020). Hatchlings are altricial, which is defined as "born naked, blind, weak, and helpless" by Seibert and Sung (2010, p. 3). During this stage they depend on the parents to stay warm, so the male provides food for both the female and the new-borns, spending up to 20% of the time preening and feeding his young (Myers and Vaughan 2004; Seibert and Sung 2010). Vigo-Trauco (2020) found that in scarlet macaw pairs, even though the female is the major nestling care provider, the male gradually and significantly increased the care given. Chicks are fed an average of 3.6 times per hour, both during the day and at night (a macaw can store food in the crop for over 7 hours) (Vigo-Trauco 2020). As hatchlings grow, both adults spend more time away from the nest to look for food, since they can already thermo-regulate on their own, attaining adult size and plumage prior to fledgling ((Renton 2000; Olah et al. 2014; Collar et al. 2020).

3.7.8. Juvenile

Scarlet macaw fledglings spend around 70-80% of time alone and resting. Their only interactions are with the parents and usually they stay within a 1 km radius from the nest. After leaving the nest, they learn the locations and seasonal patterns of food resources, at a landscape scale, by following their parents. Several weeks are spent gaining muscle coordination (playing with small sticks and leaves and with other fledglings), flight abilities and adjusting to social interactions (Myers and Vaughan 2004). They remain with the parents for up to one year, until the pair begins to nest again, although it has been recorded that on some occasions the pair will not nest again until the second year (Collar et al. 2020). During development, social interactions become more diverse and complex for fledglings cease to interact solely with their parents and clutch mates, and are integrated into larger groups (Meehan and Mench 2006). Young psittacine birds have been observed to engage in different forms of group play, including beak wrestling, pushing each other with their feet or chasing each other on foot or in flight (Seibert and Sung 2010). Macaws do not reach sexual maturity until three or four years of age (Collar et al. 2020).

3.8. History

Close interaction between humans and macaws dates back to 1100 AD. To the New World Indians parrots were of great importance, demonstrated by the incorporation of their feathers into ceremonial masks, as well as their names into song and their association with divinities. The birds, already kept in captivity, were also used as items of exchange, in between islands and tribes. These animals were first introduced to Europe when Christopher Columbus returned from the Caribbean islands, in 1493. What followed was the beginning of commercial trade in the neotropical parrots, as their beauty and colours had sparked curiosity within the European nobility (Silva 2018).

For centuries, all the commercialised birds were trapped in the wild, crated and sent to foreign ports. It was not until the late 20th century that the rearing of exotic species augmented the wild imports. Trade reached its peak in the 1970s and 1980s, the United States of America (USA) being the largest importer of wild birds. During the 1970s a total of 7.5 million birds were traded annually worldwide (Silva 2018). The appeal behind wanting a pet parrot was originally for their colourful feathers, temperament, in particular the ability to imitate the human voice. Nowadays, people who own them increasingly recognize how complex, intelligent and highly social these birds are (Harcourt-Brown 2005).

The calling from parrot conservationists, for prioritisation of the reduction in trade of wild parrots to help protect natural habitat, received legislative support in 1992 with the passing of the Wild Bird Conservation Act (WBCA) and in 2007 with the European Wild Bird Trade Ban (EU ban), prohibiting the import of wild-caught birds into the European Union countries, which banned imports of the Convention on International Trade in Endangered Species (CITES) listed parrots to the US (Wright et al. 2001; Silva 2018; Cardador et al. 2019). Consequently, trade in wild birds shrunk exponentially but never to a halt, as South America, Southeast Asia, and the Middle East continue to play major and increasing roles in the legal and illegal trade of wild parrots (Berkunsky et al. 2017; Silva 2018). Conservation of these species, including the scarlet macaw, has been gaining importance since.

3.9. Conservation

The family *Psittacidae* has more endangered species than any other bird family, as 28 to 30% of the 398 known species worldwide and 37% of 155 neotropical species are classified as threatened (Brightsmith and White 2012; Olah et al. 2014; Berkunsky et al. 2017). According to the International Union for Conservation of Nature (IUCN) Red List, 56% of all species are in decline, 35% are stable and only 9% are increasing (Olah, Butchart, et al. 2016).

Ara macao is listed as Least Concern in the IUCN Red List (BirdLife International 2022), as it is the macaw with the widest distribution of all 17 species (Mexico to Brazil) (Marineros and Vaughan 1996; Vaughan, Nemeth, et al. 2003). However, it is considered threatened in some countries of Central America, meaning ''any species which is likely to become an endangered species within the foreseeable future throughout all or a significant portion of its range'' (USFWS 2019, p.6308), as is the case of Costa Rica where it is included in the Lista Oficial de Especies en peligro de extinción y con poblaciones reducidas y amenazadas (Official List of Endangered Species and with Reduced and Threatened Populations). It has also been classified as threatened (Appendix I) under CITES since 1985 (CITES 2022) because of the effect that trading has on its natural populations. The scarlet macaw has been identified as a species with very unique functional characteristics (e.g. longevity, fecundity, size, etc.), whose functions in the ecosystem are irreplaceable should it become extinct. It is therefore a species worthy of further conservation concern, despite its widespread dispersion and commonality (Kosman et al. 2019).

3.9.1. Main threats

The main threats faced by neotropical parrots derive directly from their appeal as pets, loathe for impacts caused on agriculture and rapid expansion of the human population in Central American countries (Guatemala; Belize; Honduras; El Salvador; Nicaragua; Costa Rica and Panama), whose economic development depends on the rich and renewable natural resources. These comprise their capture in the wild (poaching) to supply the demand for the pet trade, their persecution for food or crop protection in emergent agricultural regions, that ultimately lead to accelerated and extensive natural habitat destruction rates, including deforestation, erosion and contamination (Marineros and Vaughan 1996; Wright et al. 2001; Allgayer and Cziulik 2007; White et al. 2012; Olah, Butchart, et al. 2016). Certain characteristics of some parrot species, such as low reproductive rates, yearly and small clutch size, low chick and fledging survival, late reproductive maturity and restrictive nesting requirements, result in low juvenile recruitment and end up decreasing their ability to recover from anthropogenic disturbances, as is the case of the scarlet macaw (Wright et al. 2001).

There is a deficient quantification of the parrot illegal international trade, since 60% of them die before ever being exported. Meaning that between 1991 and 1996, a total of 1.2 million parrots being exported, does not reflect the actual number of poached birds. The motivation behind this activity comes from market demand, large profits connected to the pet industry and, more often than not, the rural poverty found in many of the countries with wild parrot populations (Wright et al. 2001). In 2000, Drews demonstrated that in Costa Rica the high demand in local trade for pet parrots (around 25.000 to 35.000 every year) resulted in considerable amounts of wildlife removal from natural environments, which is likely to exceed the pressure generated by the international trade of these animals. Poaching was found a major cause for low recruitment rates, and therefore low reproductive success, in the ACOPAC scarlet macaw population, where 64% of nests were at high risk of poaching (Marineros and Vaughan 1996; Vaughan, Nemeth, et al. 2003). Dear et al. (2010) found that inside the ACOSA, approximately 25 to 50 scarlet macaw chicks were poached each year, even though this activity was already illegal, because such activities are difficult to monitor and enforce with limited funds and supervision. Berkunsky et al. (2017) showed that globally, the capture of wild parrots for the local pet trade was still the threat most closely associated with decreasing population trends.

The primary cause of forest loss in Costa Rica is conversion to agriculture (crop and pasture), although other land uses have also contributed, such as infrastructure, logging, fires, oil and gas extraction and mining. Particularly, road construction is an important driver of deforestation, as it provides access to previously remote areas and allows further expansion of these activities. Costa Rica's rate of deforestation was some of the highest in the world, as the country's forest cover declined from 67% in 1940, to 17 to 20% by 1983. Even though total forest had an increase over the past 25 years (1990– 2015), some level of deforestation still occurs in parts of the country (USFWS 2019).

Deforestation and forest degradation pose a threat to the scarlet macaw by directly eliminating the species' tropical forest habitat, through removal of large forest sections with the trees that support the species' essential needs for nesting, roosting and, food, that fragment the landscape and reduce and isolate populations (USFWS 2019). As obligate secondary cavity-nesters, with specific larger size requirements, nest cavity scarcity becomes a major contributor to population decline, leading to competition for the existing ones with other conspecifics and other avian species, such as the chestnut-mandibled toucan (*Ramphastos swainsonii*), the barred forest falcon (*Micrastur semitorquatus*), and the yellow-naped amazon (*Amazona auropalliata*). Africanized honey bees (*Apis mellifera scutellata)* and ants are also reported to be a serious competitor with scarlet macaws for nest cavities (Marineros and Vaughan 1996; Vaughan, Nemeth, et al. 2003; Renton and Brightsmith 2009; Olah et al. 2014). Scarlet macaws can colonise partially cleared and cultivated landscapes, if the landscape provides dietary requirements, and maintains enough large trees (USFWS 2019). However, in this habitat, they become more obvious and accessible to potential poachers, hunters and farmers, while feeding on crops. As a result, agricultural frontiers may become ecological traps (Berkunsky et al. 2017).

3.9.2. Conservation strategies

Seeing how problematic these threats were, the Costa Rican government took action. By 1985, 27% of its national territory had been consolidated into protected national parks, forest reserves and indigenous reserves. During the 1990's, noticing how wildlands continued to be threatened by the nearby communities, the Ministerio de Ambiente e Energía (Ministry of Environment and Energy - MINAE) created the Sistema Nacional de Áreas de Conservación (National System of Conservation Areas - SINAC) to manage the former by promoting the preservation, restoration and protection of their ecological equilibrium and biodiversity (Vaughan 2012; Sanchez 2018) and new laws protecting forests and mechanisms of payment for ecosystem services were implemented. Costa Rica was the only country in Mesoamerica to experience a positive change in forest cover from 1990 to 2015, gaining 192.000 ha of total forest area, with an annual rate of approximately 7.700 ha (USFWS 2019).The Ley de Conservación de Vida Silvestre (Wildlife Conservation Law nº7317) was implemented in 1992 and included a Reglamento para la Tenencia en Cautiverio (Regulation for Captive Possession), which regulates permits for hunting, possession, commercialisation and release of wildlife (Takahashi 2006). The country evolved to be considered a global leader in biodiversity conservation and education, even though the latter is taking more time to fully develop (Vaughan, Gack, et al. 2003).

Scarlet macaw conservation actions in Costa Rica started in 1990, when The Regional Wildlife Management Program for Mesoamerica and the Caribbean (RWMPMC) developed a long term research project with the ACOPAC population, aimed at improving the species conservation status through the collection of economic, sociological and ecological information necessary to develop innovative management techniques. Although ecotourism was already increasing exponentially in Costa Rica, lack of understanding, limited restrictions and the guides' desire to showcase wildlife species in order to please their customers, had researchers questioning its compatibility to conservation efforts. The only Costa Ricans who directly benefited from ecotourism were hotel, restaurant, gas station and store owners. It was clear that without financial benefit, as well as involvement in conservation of the local human communities, the scarlet macaw populations were likely to severely decline leading to possible extinction (Marineros and Vaughan 1996). Seeking to avoid that outcome, socioeconomic and ecological programmes were established, with the help of the LAPPA association (La Asociación para la Protección de los Psitácidos), enabling the increase in ecological knowledge, the identification of institutions, organisations and researchers working with the species, the use of *in situ* management techniques to recover an endangered population and the exchange of this information through a number of scientific publications, to contribute for the conservation of other endangered scarlet macaw populations throughout Central America (Marineros and Vaughan 1996; Vaughan 2006a; Vaughan 2019).

Socioeconomic management measures, since 1996, included wildlife yearly environmental education programmes for local communities in schools (practical information, visits to see macaws, raising funds, activities to do at home to reach the parents), for local park guides (training on legislation and rules within the reserve, basic biology and survival issues of the species to protect and teach visitors), for visiting public (biology taught by park guides, visitor centre installation) and also encourage information exchange to increase public knowledge and highlight conservation relevance (courses for local guides, arts and crafts workshops, tourist shop set up by locals, nest protection in private farms, dissuade poachers, planting important trees for macaw life cycle, participation of hotel and tour agencies in conservation and habitat protection), in order to provide jobs for locals and inspire action for natural resource management. The synergic relationship between all profitable elements involved in ecotourism proved to be the only solution for local community development (Marineros and Vaughan 1996; Vaughan, Gack, et al. 2003; Vaughan, Nemeth, et al. 2003; Vaughan 2019). Several environmental education programmes have been employed around the country since. However, their success alludes towards the implementation of such programmes in the school curriculum, while ensuring a more long-term duration and selected outdoor activities that illustrate what is taught (Vaughan, Gack, et al. 2003; Vaughan 2019). The environmental course was taught until 2018, a lot of educational talks and presentations were held by LAPPA, many articles made it to newspapers, websites and local magazines, which helped to raise awareness to the issue (Vaughan 2019).

Ecological research in Costa Rica featured long-term studies on scarlet macaw range (Marineros and Vaughan 1995; Dear et al. 2010), demography (Vaughan et al. 2005; Monge et al. 2016), nest site selection and nesting success (Vaughan et al. 2003b), nest characteristics (Guittar et al. 2009), breeding behaviour (Vaughan et al. 2003b), frequency of parental nest visitation (Vaughan et al. 2009); post fledging period (Myers and Vaughan 2004), diet (Vaughan et al. 2006; Hamm et al. 2020), population genetic viability (Nader et al. 1999; Monge et al. 2016). The Tambopata Macaw Project in Peru, where scarlet macaws are still abundant, provided ideal circumstances for the understanding of the species ecology, which can be applied to other locations where the population is declining (Olah et al. 2014). Studies on nesting success and conservation techniques (artificial nest boxes) (Nycander et al. 1995), cavity selectivity (Renton and Brightsmith 2009), artificial nest acceptance and nest selectivity (Olah et al. 2014), parasite prevalence (Olah et al. 2013), species genetics (Olah et al. 2015; Olah, Heinsohn, et al. 2016), blood biochemical values (Peruffo et al. 2016), and movement recording with satellite telemetry (Brightsmith et al. 2021) were done over a 25-year period. Recently they expanded their actions and started working alongside conservation programmes in Costa Rica, where they partnered with Rescate Wildlife Rescue Centre and are applying the knowledge gained in reintroduction efforts. Other studies concerning conservation efforts have been done in Guatemala/Belize (Britt et al. 2014; García-Anleu et al. 2017) and Mexico (Figueras 2014; Escalante-Pliego et al. 2022).

The closure of high poaching risk nests, with provision of quality artificial ones replicating scarlet macaw ecology, near natural nest sites, has been used and proved successful as a management strategy to increase the number of active pairs (through monitoring and application of necessary adaptations), where nest cavity scarcity is a major limiting factor. Close proximity between nests eases their protection during the whole breeding season and post fledging dependence period, by lowering human resource costs and facilitating research, environmental education and ecotourism (Vaughan, Nemeth, et al. 2003; Myers and Vaughan 2004; Guittar et al. 2009; Vaughan et al. 2009). Artificial nest boxes have continued to be used in low hollow availability areas, to support reintroduction and translocation and to enhance ecotourism by increasing nesting bird numbers, in large enough numbers to facilitate statistical interference in Peru (Olah et al. 2014), Guatemala and Belize (Britt et al. 2014), Mexico (Estrada 2014) and Costa Rica. Population monitoring played a big part when evaluating the success of conservation management techniques (Vaughan et al.

2005). Without that, the initial decreased state of the population would not have been acknowledged (Vaughan 2012). Habitat wise, park rangers had an important role in habitat preservation and protection (Vaughan et al. 2005), and a private entity turned out to be a major stakeholder in reforestation efforts creating forestry nurseries to plant 3000 macaw feeding and nesting trees per year in their properties (Vaughan, Nemeth, et al. 2003; Vaughan 2019).

By 2006, the ACOPAC programme had acquired sufficient funding, had published a great number of scientific studies, local communities showed a bigger consciousness level, money from ecotourism was benefiting the local communities, the macaw population increased and poaching was reduced to a minimum, demonstrating how successful a long-term programme can be (Vaughan 2006a).

In the ACOSA, conservation efforts quickly followed the work started in the ACOPAC. The population started to increase between 2000 and 2010 due to protection by MINAE, which involved growth in sustainable ecotourism that benefited local communities, environmental education in the area, habitat that provided more forest availability for food and nests and introduction of exotic flora used by macaws for feeding. But continued population monitoring, securing long-term economic sustainability and environmental education programmes, are necessary actions in order to continue to protect the species (Dear et al. 2010).

Funding for conservation research was and is continuously at fault, which has led conservation projects around Mesoamerica to seek out funding from ecotourism, promoting paid volunteer programmes that result in a mutually beneficial relationship: environmental education and nature experience for the volunteer in exchange for labour (Brightsmith et al. 2008).

3.9.3. Captive breeding and reintroductions

Most aviculturists, other than economic advantages for themselves, saw in captive breeding an opportunity to provide a genetically viable stock, which would thereafter allow the reintroduction of these species back into their natural habitat, for conservation purposes (Abramson 1991). However, when reintroduction programmes first came into use, some researchers showed reservations concerning captive breeding as a conservation tool, since the majority of reintroduced individuals were lost only a few days following release and lacked survival skills. Furthermore, these programmes needed to include a large number of individuals, were an expensive activity, reliant on worker skills, none or few had been successful and could end up having harmful impacts on the native population. Therefore, these were considered a last management alternative, after active and passive management were exhausted (Marineros and Vaughan 1996; Snyder et al. 1996; Wright et al. 2000; Brightsmith et al. 2005; Vaughan 2006b). This can be true especially in Central America, where experience, scientific knowledge and economic resources are insufficient to deepen field research (Vaughan 2006b).

The positive aspects of reintroductions include easy management of a closed population and the educational potential it creates (Forbes 2006; Vaughan 2006b). But negative aspects often overlap these, as much can go wrong: lack of fundamental elements within the habitat (food, water, shelter); maladaptation of reintroduced individuals; deficient predator avoidance; subclinical disease development; dispersion outside release site; loss of wild behaviours in captivity (reduced breeding success; disrupted social organisation; human imprinting); endogamous or exogamous decline (Myers and Vaughan 2004; Vaughan 2006b). By studying the parent-fledgling relationship of scarlet macaws, Myers and Vaughan (2004) outlined the challenges faced in *ex situ* programmes by highlighting the importance of natural recruitment (prolonged parental care, experiment with physical environment at landscape scale, learn locations and seasonal patterns of food resources and gradually adjust to social relationships). Without dismissing their potential to improve the status of this species in Costa Rica, they concluded that *ex situ* programmes should focus on the re-establishment of scarlet macaw populations in parts of their historic range, where conservation value if successful is great, while risks to existing populations are small.

These results aided in the development of rearing (reduced contact with humans and maximised contact with other macaws) and release (soft release techniques that allow young macaws to gradually explore their new ranges in cohesive social groups) practices that more closely mimicked natural development (Myers and Vaughan 2004). When breeding in captivity, it is important to make sure the species is able to reproduce, there is active disease control, genetic and ethological impairment is avoided, there is sufficient financial and logistical support and that project continuity and administrative compromise is secured (Herrera et al. 2001; Takahashi 2006; Vaughan 2006b).

The pioneering scarlet macaw reintroduction programme in Costa Rica and Peru was successful due to prior research among those involved and post-release monitoring, being that reintroduction success is measured by more than 50% first year survival and successful breeding by released birds (Brightsmith et al. 2005; White et al. 2012). Conclusions reached had great impact on further studies done with reintroduction for this species, showing that captive breeding and reintroduction can be used to re-establish psittacines in areas where they have disappeared (Brightsmith et al. 2005).

By 2010, several reintroduction programmes had been active and successful throughout the country (Dear et al. 2010). But Dear et al. (2010) posed an important research question, if the ACOSA wild population has not naturally recolonized the reintroduction sites being used, maybe the correspondent habitat is lacking something essential for population survival. White et al. (2012) then described general recommendations to successfully undergo parrot reintroduction efforts based on previous publications: correction of original causes of extirpation or endangerment (habitat loss, hunting, chick harvesting); guarantee physical condition and health of released birds; use of captive parent-reared and non-imprinted confiscated birds for this purpose; thorough evaluation of potential release sites (guarantee quality habitat, within historic range and with low predation risk, avoid areas with viable populations of wild conspecifics); release of sufficient numbers of birds to promote flock cohesion; pre-release conditioning in soft release enclosures (flight training, socialisation, local acclimatisation, experience with local foods); predator averting training; allocation of adequate resources for monitoring success; post release supplementary feeding encourage social interactions and site fidelity. Guidelines for reintroductions and other conservation translocations were also published by the IUCN in 2013.

Moreover, throughout Central America (Honduras, El Salvador, Mexico and Costa Rica) scarlet macaw reintroductions have become a common practice (Estrada 2014; Figueras 2014). The programme developed in Mexico, one of the most complex and successful to date, with 92% of first year survival rate, may come to serve as a reference point for future macaw reintroduction attempts (Estrada 2014). In total, hundreds of scarlet macaws have been released into the wild in Costa Rica. Most projects conduct environmental education at a local level, attracting additional media attention at a local and national level. As a result, each reintroduction project educates the public about the importance of scarlet macaws and of conservation and the environment in general (USFWS 2019). However, since 1997, at least 700 birds of the species have been released in at least 12 sites in Costa Rica with little monitoring or control. These flawed programmes should be discouraged (Vaughan 2019).

3.9.4. Future prospects

In order to overcome the main challenges still faced by reintroduction programmes (flock cohesion; avoid premature dispersal; rapid reproduction onset post release), new and innovative techniques are being studied to improve success rates (White et al. 2021; Woodman et al. 2021).

As previously mentioned, the youngest nestlings of a brood are more likely to suffer impaired growth and development, or stunting, leading to high mortality rates when fledged (Myers and Vaughan 2004). Vigo-Trauco et al. (2021) instead of hand-rearing, used foster parenting to overcome this limiting factor, with the help of age/developmental stage matching among foster and resident chicks, supplemental feeding in the transitional phase and veterinary care if necessary, which proved successful. No chick was rejected, similar feeding rates to resident chicks and growth were observed and almost 90% of all foster chicks fledged. The fledging success per available nest increased from 23% to 43%, and chick death by starvation decreased from 35% to 6%, which demonstrates that this is a viable *in situ* conservation strategy to be applied in other areas in the future.

The future poses some new challenges to the conservation of the scarlet macaw, and probably the biggest of all is climate change. Impacts in ecotourism and the communities it supports are yet to be evaluated, and how this will affect the species behaviour, feeding and nesting habits. This highlights the need for continued research, monitoring and *in situ* management of populations. Environmental education in schools and with community talks should be continued, and include the whole range expansion of the species. The results of this initiative with the scarlet macaw can provide guidelines for the recovery of endangered wildlife populations in Central America and other continents (Vaughan 2019).

3.10. Conservation breeding

Several parrot species, including the scarlet macaw, are commonly kept in captivity, either in private houses, zoos or breeding facilities, but there is a general lack of knowledge about the natural variability between the many species, that inevitably hampers the provision of adequate social and physical environments which ensure welfare and successful reproduction (Spoon 2006; Andrews 2022). Wild animals interact with a dynamic, complex and mostly unpredictable environment (Reimer et al. 2016). However, in captivity the majority of their needs are fulfilled without direct involvement (Meehan and Mench 2006; Reimer et al. 2016). In conservation breeding special attention should be given to this matter, as individuals must be able to survive in the wild, after reintroduction (Greggor et al. 2018).

3.10.1. General welfare

Greggor et al. (2018, p. 1) defined welfare conditions as the ones where “animals are well nourished, safe, lack pain, fear, and distress, and have the ability to develop and express species-typical relationships, behaviours, and cognitive abilities”, based on the Opportunities to Thrive belief. One must understand the biology and ecology of a species in order to judge objectively how an inappropriate captive environment might be affecting natural behaviour expression and functioning, reproductive success, genetic composition and general welfare, so that appropriate management changes can be made (Myers and Vaughan 2004; Meehan and Mench 2006; Rose and Riley 2019; Ramos-Güivas et al. 2021; Andrews 2022). Captive macaws of the genus *Ara*, as one of the most intelligent parrot species, are prone to suffer from poor captive welfare (Mason 2010; Mellor et al. 2021). They frequently experience significant limitations in their ability to perform natural innate behaviours such as: foraging; social interaction; and locomotion. The inhibition of innate behaviours can give rise to a disparity between the parrot and its surroundings (Meehan and Mench 2006). If this disparity is substantial it might lead to the manifestation of abnormal behaviours, and have negative impacts on welfare and reproductive success (Reimer et al. 2016; Miglioli and Vasconcellos 2021).

Abnormal or stereotypic behaviours are repetitive movements that have no apparent purpose or function, induced by frustration and poor welfare (Meehan and Mench 2006; Reimer et al. 2016). Naturally, species/individual intrinsic factors will also determine the development of such behaviours (Meehan and Mench 2006), as demonstrated by Mellor et al. (2021) that found smaller-brained species most likely to express poor welfare through apathy, in contrast with larger-brained species (i.e., the scarlet macaw). Over time, these can vary in form and frequency if conditions remain the same, becoming increasingly difficult to reverse (Meehan and Mench 2006; Mellor et al. 2018), to the point of being present even if current welfare is adequate (Mellor et al. 2018). Although stereotypies manifest in a variety of forms, they can be categorised into three distinct groups: oral stereotypies (i.e., sham chewing, bar biting, feather damaging, head moving), locomotor stereotypies (i.e., route tracing, pacing, repetitive body movements, hanging), and object-directed stereotypies (i.e., repetitive manipulation of objects) (Meehan and Mench 2006). Understanding the underlying cause of these behaviours will allow improvements within the animal's environment, which can slow down their development in a gradual way (Meehan et al. 2004; Mellor et al. 2018).

Although the genus *Ara* has inherently low reproductive rates (Brightsmith and White 2012), stressful environments that promote long term elevated glucocorticoid levels (chronic stress) will negatively impact reproductive efforts (i.e., reduced copulation effort) by downregulation (Hemmings et al. 2012; Vidal et al. 2019; Ramos-Güivas et al. 2021). Notwithstanding, the mere presence of low glucocorticoid concentrations cannot be regarded as an exclusive welfare indicator. Instead, it should be supplemented by the assessment of additional parameters, such as breeding success and/or the absence of stereotypical behaviours, which are commonly exhibited by this group of avian species, as noted by Vidal et al. (2019).

In the absence of the requisite environmental stimuli that regulate the species' reproductive physiology and behaviour in a positive manner (changes in day length, temperature, or rainfall, the presence of nesting sites, and the presence of potential mates), there will be a general lack of reproductive effort, as the HPG axis remains dormant (Sant 2006; Allgayer and Cziulik 2007; Ubuka et al. 2013). Environmental cues need to provide perfect conditions that enable a balanced physiological well-being, for successful breeding to occur (Pollock and Orosz 2002; Crosta et al. 2003). So, it is reasonable to presume that preserving birds in an environment that closely emulates their native habitat would be the most advantageous approach for promoting their well-being and averting diseases (Rasidi and Cornejo 2021).

Acknowledging that replicating the natural habitat within a captive setting is unfeasible, it is necessary to identify the key environmental factors essential for the birds' normal behavioural development, followed by developing pragmatic approaches to integrate these elements within the captive context (Meehan and Mench 2006). The primary challenges to the welfare of captive parrots can be classified into three categories, namely husbandry (i.e., inadequate nutrition, unsanitary conditions, insufficient veterinary care, and insufficient opportunities for bathing), environment (i.e., limited living space, poorly spaced bars, improperly sized perches, unstimulating living conditions, social isolation), and necessary human interactions. Mellor et al. (2021) noted that larger brained species should be granted extra welfare protection in captive environments. It is extremely challenging to offer animals a sense of choice and control in captivity, a subject that has gathered the attention of many scientists and led to the emergence of animal welfare science, in order to deal with these ethical considerations (Meehan and Mench 2006; Rose and Riley 2019).

Environmental enrichment, that takes into account the species' behaviour and ecology, as well as the individual's age and history, is a technique used that can substantially improve welfare and breeding output, as has been done in several highly intelligent macaw species (Azevedo et al. 2016; Reimer et al. 2016; de Almeida et al. 2018; Checon et al. 2020; Miglioli and Vasconcellos 2021). A variety of enrichment options, such as nutritional, occupational, physical, sensory stimulation, and social interaction, can be utilised, designed, and constructed to cater to the diverse needs of parrots. The expression of foraging, locomotion, and social behaviours, rather than abnormal ones can demonstrate its success (Meehan and Mench 2006; Andrews 2022). Notwithstanding, de Almeida et al. (2018) points out that certain issues such as optimal duration of enrichment, aversion to novel stimuli, as well as their preferences for specific enrichment items and overall management practices, require further study to effectively promote welfare.

Endangered bird species are harder to breed in captivity than their non-endangered close relatives, probably because they are much less adaptable to new environments (Mason 2010). Management practices that keep captive wild animals in good welfare conditions are essential for species long term conservation goals, as reproductive efforts are more likely to succeed (Spoon 2006; Greggor et al. 2018). Ex-pet parrots are also frequently surrendered to conservation breeding centres for a variety of reasons, which can have impacts on social structure and reproductive outcome (Andrews 2022). Therefore, environmental enrichment programs are important to stimulate the recovery of natural behaviour of macaws kept in *ex situ* conditions (Checon et al. 2020).

3.10.2. Appropriate climate

Photoreceptors located in the pineal gland of avian species are able to detect light stimuli either autonomously or via visual pathways (Sant 2006). This fundamental mechanism regulates their circadian rhythms, which in turn impact vital physiological processes such as body temperature regulation, hormonal secretion, metabolic activities, and reproductive functions, therefore mediating seasonal breeding (Sant 2006; Wilson 2006).

However, the environmental stimuli for breeding highly depend on the habitat and intrinsic characteristics of the species, for example: the ability to survive and reproduce in arid regions is significantly influenced by the limiting factor of water; temperature effect on reproductive initiation is variable, low temperatures delay, and high temperatures accelerate gonadal regression; in moderate tropical climates, environmental factors can periodically impact food supplies and habitat; and in tropical and subtropical areas various mechanisms have developed to signal the onset of breeding season, such as responding to the cessation or arrival of seasonal rains, since food abundance is highly dependent upon it (Sant 2006; Hau et al. 2008; Chmura et al. 2020; Raisi and Cornejo 2021). Chmura et al. (2020) concluded that non-photic environmental information can modify gonadal growth, lay date and gonadal regression, even in seasonal breeders that are thought to rely primarily on photoperiod. In the case of the scarlet macaw, cessation of seasonal rains and slight photoperiod increase play a supportive role as breeding stimuli, but do not ensure it (Wilson 2006; Hau et al. 2008; Vaughan et al. 2009). Brightsmith and White (2012) concluded that psittacine ecology is expected to be profoundly affected by slight changes in global climate patterns.

3.10.3. Nutritional requirements

The presence of nutrient deficiencies and excesses in a bird's diet can instigate a multitude of pathologies, such as: defects in reproduction, embryonic development, and the growth and maturation of offspring; heightened susceptibility to illness; behavioural abnormalities; and eventually, high mortality rates. Accurately ascertaining the nutrient requirements of a given species entails a thorough understanding of its natural feeding behaviours, along with the use of calculated and theoretical nutrient requisites. Once these requirements are identified, suitable diets and husbandry practices may be selected to satisfy them all (Matson and Koutsos 2006; Allgayer and Cziulik 2007).

Cornejo et al. (2012) evaluated the nutritional composition of crop contents of free-living scarlet macaw chicks in Peru: neutral detergent fibre (NDF) 42.8%; Ash 7.15%; Crude fat 19%; Crude protein 17.3%; P 0.34%; K 0.92%; Ca 0.88%; Mg 0.29%; Cu 14.4 ppm; Zn 39.8 ppm; S 0.31%. Silva (2018) noted that larger species, such as most macaws, require a higher degree of fat, so nuts are an important addition to the diet, particularly when nesting and rearing young, meaning that insufficient fat can also deter breeding. Knowledge of specific gustatory preferences, aversions and specific needs, can facilitate diet formulation by maximising palatability and feed acceptance, as well as effective supplementation (i.e., providing a supplemental calcium source to egg-laying birds) (Matson and Koutsos 2006). Chmura et al. (2020) found that food supplementation can advance gonadal development and lay, stimulating reproduction. The crucial role of foraging activities in the natural behavioural repertoire of wild macaws, results in a heightened motivation to seek out and obtain food, which is hard to meet in captive environments, where pelleted or seed diets are poured into bowls, ultimately contributing to the development of abnormal behaviours due to stress (Meehan and Mench 2006; Seibert and Sung 2010). Providing foraging enrichment, defined as "opportunities to work in order to locate, access and consume food items" (Meehan and

Mench 2006, p. 311), may enhance the animal's feeding experience and reduce oral stereotypic behaviours [i.e., feather damaging behaviour (FDB)], and connected frustrated foraging behaviours (Meehan et al. 2004; Matson and Koutsos 2006; Mason 2010).

It has been demonstrated that macaws often work for food, even when the same items are readily accessible (Rozek and Millam 2011). Various feeding enrichment techniques have proved successful in the reduction of abnormal behaviours, as well as general welfare improvement in captive parrot species. Bassett and Buchanan-Smith (2007), by combining theory with empirical results, concluded that unpredictable feeding schedules and elimination of unreliable signals relating to feeding (i.e., sounds of food preparation long before food is handed out), promoted both exploratory behaviour and memory performance, while reducing food- anticipatory activity (FAA). Watters et al. (2019) then tested automated feeding devices using variable schedules in the golden conure (*Guaruba guarouba*), which successfully reversed FDB and reduced stereotypic behaviours, since explorative behaviour was encouraged. Moreover, James et al. (2021) found that blue and gold macaws (*Ara ararauna*) devoted a notably greater amount of time to activities such as feeding, manipulating food, allofeeding, and allopreening, and lesser amount of time vocalising and resting, when fed whole foods instead of chopped. This study concluded that whole foods represent a beneficial form of foraging enrichment for macaws, with a positive influence on pair bonding, reduction in aggression, a decreased risk of bacterial contamination and desiccation of the food, and general welfare, while also being a time saver to the keepers. Other studies in captive parrots have also found that the provision of more naturalistic foods and foraging enrichments are essential for promoting general welfare and, consequently, reproductive success (Mellor et al. 2021; Miglioli and Vasconcellos 2021).

3.10.4. Housing conditions

Captivity often severely restricts parrots' locomotor capabilities, primarily due to limitations imposed by their cage environment, which significantly curtails their ability to fly and engage in other forms of locomotion. This might lead to the development of some forms of stereotypies (Meehan et al. 2004; Meehan and Mench 2006).

When designing the enclosures, it is important to consider both the natural behaviour and health of the birds, as many species, including the scarlet macaw, that solve complex problems while exploring their habitat, are highly intelligent (Wilson 2006; Andrews 2022). Contact with the ground, besides bearing health risks, is not essential to arboreal species (Wilson 2006). Enclosure size for large macaw pairs should be no smaller than 3 m long, 2.1 m wide, and 2.1 m high (Wilson 2006; Silva 2018). For part of the year, however, they ought to be placed in a large aviary at least 15 m long, with adequate congregation locations (i.e., a long perch) in order to promote socialisation of the group (Silva 2018; Raisi and Cornejo 2021). Both enclosure types need to have certain structural elements such as perches positioned above the keeper's height, naturalistic vegetation, and allow flight, which not only enables the performance of all locomotion patterns characteristic of the species, but also helps them feel safe and secure in their environment (Wilson 2006; Raisi and Cornejo 2021). Macaws adapt better to wooden perches, since they allow chewing and good sitting positions. One common form of physical enrichment consists in changing the enclosure layout by adding new perches or moving those that exist around (Andrews 2022).

In breeding centres dedicated to conservation there should be a clear separation between individuals that are to be used in future reintroductions and the previously captive ones, that have been humanised and are used to direct contact with people (Allgayer and Cziulik 2007). Disturbances such as close predators, unfamiliar sounds or people might inhibit the pair from copulating or laying, as well as precipitate abnormal behaviours, and should therefore be avoided or minimised (Wilson 2006; Allgayer and Cziulik 2007). In order to monitor birds without causing disturbances, a recommended method is camera installation (Silva 2018).

3.10.5. Nesting

In wild psittacine birds, sexual hormone secretion is stimulated by the finding, and preparation of a cavity, which further stimulates copulation and oviposition to occur (Sant 2006). Thus, nest box provision is the single most important stimulus to induce reproductive development when attempting to breed these species, even though their efficacy may be dependent on individual experience (Martin and Romagnano 2006; Wilson 2006). Nest box preference in size, shape, construction material, location, and interior light intensity varies depending on the species involved (Martin and Romagnano 2006).

For macaws, the nest should be around 60-90 cm long and 30-35 cm squared, for large nests allow the male to stay inside with the female while she incubates the eggs (Silva 2018). These birds have been proved to prefer horizontal nest boxes in captivity, which allows mating to occur inside, and later on, hatchlings to spread out in order to better tolerate sudden temperature rises (Martin and Romagnano 2006; Wilson 2006; Silva 2018). Although different materials have been used to craft artificial nest boxes for macaws (wood box; tin; barrel), wooden boxes are usually preferred (Wilson 2006; Silva 2018). Silva (2018) described a technique that involves the attachment of bark to both the exterior and interior walls of a nest, thereby stimulating the natural behaviour of chewing and presenting the pair with the ability to regulate the size of the nest. These should be placed above 2 m high, away from the food and general traffic, in order to minimise disruptions which helps transmit safety to the pair. Direct visual contact to other nesting conspecifics is important in species, such as the scarlet macaw, where individuals of the group nest in the same area (Allgayer and Cziulik 2007). Considering the protective nature of macaws, it is recommended that nests be positioned externally to their enclosures, with the entrance facing inward, as well as the installation of a door capable of obstructing the pair's access to the nest during external inspections. The inside of the nest needs to be dark (Wilson 2006; Silva 2018).

The provision of nesting substrate, prepared with tree trunk chips and wood shavings, is essential to mimic nest preparation that can be chewed into the proper consistency (Abramson et al. 1995; Wilson 2006). This method can potentially serve as a form of stimulation for the pair, while also reducing incidents of mate aggression in some species. The initial preoccupation of the male in preparing the nesting cavity may provide the female with more time to reach optimal breeding condition (Wilson 2006).

3.10.6. Social structure and breeding behaviour

Environments that do not allow, or stimulate social interactions and sexual behaviour expression, such as courtship, copulation, nest building, egg incubation, and rearing of offspring, which are critical behavioural needs, are more prone to lead to stereotypies that consume the individual to the exclusion of breeding (Meehan and Mench 2006; Greggor et al. 2018). Some of the resulting abnormal behaviours may prejudice effective reproduction, such as eggs destruction, nest abandonment, aggression towards mate or chicks, and stereotypies that are all-consuming and prevent individuals from engaging in breeding activities (Wilson 2006). Therefore, besides what has been previously mentioned, careful consideration of the species' mating systems and social needs within their life stage is required, in order for breeding to properly occur, a main indicator of positive welfare (Wilson 2006; Andrews 2022).

To obtain breeding pairs, mature individuals of equal male and female numbers can be released simultaneously in a large enclosure at the onset of breeding season, which will encourage natural pairing to occur, as opposed to forcing a bond between two individuals (Allgayer and Cziulik 2007; Silva 2018). By allowing self-selection, more compatible pairs are formed, increasing fertility, hatching probability, parental care coordination, and subsequent rearing of young, hence reproductive success (Spoon et al. 2006; Silva 2018). To know which individuals have started to form a bond, direct observation or the use of cameras (conservation breeding) can be employed (Silva 2018), to look for typical behaviours of mutual interest: staying close together, face touching, allopreening, allofeeding, and courtship behaviour (Sant 2006; Allgayer and Cziulik 2007; Silva 2018). These behaviours enhance the pair bond and trigger reproductive activity due to hormonal flow (Sant 2006). When opting for collective pairing enclosures, the probability of homosexual pair formation increases, since mutual interest can be seen in same sex birds (Allgayer and Cziulik 2007; Silva 2018). Sexing is an important part of reproductive management and can be done in various different ways: laparoscopy is a surgical procedure that allows the visualisation of the gonads and sex determination; DNA examination is performed through a blood sample sent to a molecular biology laboratory for identification; and karyotyping, which is a cytogenetic technique that allows the identification of the sex chromosome pairs, ZZ for males and ZW for females, from the pulp of the feathers (Abramson et al., 1995).

Open nests can be either placed inside the big enclosure from the start, or bonded pairs can be separated into their own enclosures. Nesting pairs vocalise more and display aggressive behaviour while protecting the nest (i.e., wing opening and lunging), while visits to the nest become more and more frequent (Allgayer and Cziulik 2007; Silva 2018). In monogamous species, as is the case of the scarlet macaw, long-term pairs show higher reproductive success, for increased familiarity between individuals will enhance parental care coordination (Seibert and Sung 2010). After the breeding season, the practice of placing multiple pairs together in a large flight enclosure is becoming widespread. This allows birds to interact as a flock for the rest of the year, a form of social enrichment, and has proved to increase breeding success in the subsequent breeding season (Silva 2018).

Hand rearing is a risk factor for abnormal behaviour development, reduced welfare and poor mating success for adult birds, since the first two weeks of a parrot's life are essential for normal sexual behaviour development, as well as conspecific socialisation (Schmid et al. 2006; Wilson 2006; Williams et al. 2017; Mellor et al. 2018). In conservation breeding, reproduction in the wild of the offspring is the main goal, so parent rearing is mandatory, which is effective in the rear of social and well balanced younglings, in addition to the welfare benefits that it has on the parents (Schmid et al. 2006; Greggor et al. 2018). It also prevents changes in behavioural phenotype of the population, which would limit the success of future reintroductions (Rose and Riley 2019). When fledglings reach independence, housing them with other juveniles of the same species is recommended to allow flock socialisation, and for them to acquire species-specific behavioural patterns (Schmid et al. 2006; Wilson 2006). The compatibility of the group may change as they reach sexual maturity, leading to aggressiveness, in which case group changes should be made and said individuals separated (Meehan and Mench 2006).

3.10.7. Disease control

Appropriate nutrition, husbandry, and management are crucial to maintain the health of a captive flock (Silva 2018; Rasidi and Cornejo 2021). As said before, improper conditions can result in abnormal behaviour due to stress, which reduces the immune system and makes animals more susceptive to disease, injury, and even death (Meehan and Mench 2006; Rasidi and Cornejo 2021). Visible cues including activity patterns and/or body and feather condition, help keepers determine the general welfare of the individuals (Andrews 2022). Moreover, subclinical diseases can lead to breeding failure by deterring birds from breeding, the passing of the disease onto younglings through the egg or production of infertile eggs (Silva 2018). In usual practice, the use of as little pharmaceutical therapy as possible, is favoured (Rasidi and Cornejo 2021). So, the use of vitamin complexes and antibiotics as stimulants for mating is not advised (Allgayer and Cziulik 2007). Therefore, it is essential to establish a preventive health program, with annual veterinary check-ups, to monitor disease and detect potential issues, before they affect the welfare of the entire group (Rasidi and Cornejo 2021).

3.10.8. Summary

Psychological well-being is of great importance to induce breeding, and as this chapter has led on, in addition to appropriate nutrition, housing and nesting, the establishment of a well thought out environmental enrichment program can significantly improve quality of life for captive birds (Meehan and Mench 2006). A mixture of foraging, physical and social enrichment is optimal, and has been shown to produce several positive effects, including the reduction of undesirable behaviours such as screaming, feather picking, and stereotypy, an increase in physical activity and playful behaviour, a decrease in fear responses to novelty, and an improvement in reproductive success. Importantly, these benefits have been observed without significantly increasing the risk of illness or injury (Rose and Riley 2019). Furthermore, cooperation across research groups and breeding facilities can help build husbandry knowledge and create more comprehensive management plans (Andrews 2022). And as Greggor et al. (2018, p.5) concluded, “the more we learn about the unique species under our care, the more we can provide them with opportunities to thrive.”

4. Experimental Work

4.1. Introduction

Most of the available information on the effects of environmental conditions in scarlet macaws originates largely from general knowledge of expert breeders, rather than scientific publications. Therefore, there are still many open opportunities for further research on this topic. The utilisation of quantitative methods to evaluate animal welfare is a valuable tool that can aid in the refinement and advancement of animal husbandry practices, leading to the mitigation of any signs of impaired welfare or deviation from the natural behavioural patterns of the species under consideration (Rose and Riley 2019).

4.2. Material and Methods

4.2.1. Location

The Centro de Reproducción de Aves en Peligro de Extinción (CRAVE), located in La Garita, Alajuela (10°00′44″N 84°16′32″W), is active since the mid-1990s (Rescate Wildlife Rescue Centre 2022; Benavides and Janik 2008) and has breeding programmes for the two different species of macaws native to Costa Rica: the scarlet macaw (*Ara macao*) and the great green macaw (*Ara ambiguus*). This study focuses on the scarlet macaw breeding programme, which is now well established as it has been running since 1998 (Benavides and Janik 2008), giving the collected data more reliability. The rescued macaws of Costa Rican origin, eligible for the breeding programme, either must be confiscated from illegal captivity or deemed unable to thrive in the wild.

4.2.2. Housing and husbandry

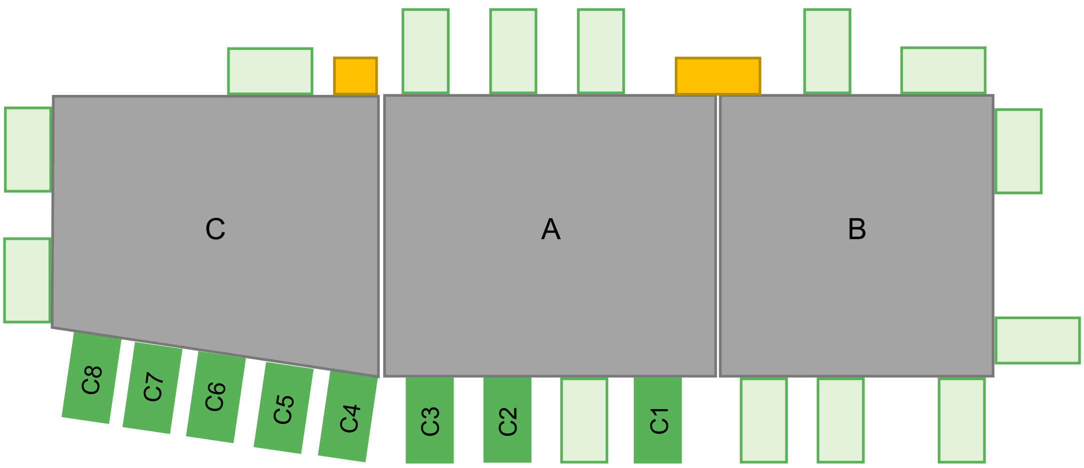


Figure 8 – Scheme of the enclosures at CRAVE, as seen from above. A, B and C are the enclosures for the breeding pairs. In green are the nesting enclosures for the breeding season. In yellow are the entrance doors into all enclosures.

The all-outdoor enclosures of the breeding centre can be sorted into three categories: pairing, breeding and retiring. Upon arrival, the macaws are placed in the pairing enclosures where, through socialising with their conspecifics, breeding pairs are formed. Once the pairing occurs, sexing is done by a DNA test from blood sampling, to confirm they are made up of a male and a female. Once confirmed, they are moved into the breeding enclosures, as per letters A, B and C (Figure 9). When breeding season arrives, each pair is transferred to the surrounding smaller enclosures, that have doors facing the bigger ones in order to prevent escape. When the pairs do not breed for three or more consecutive years, they are considered non-breeding pairs and are moved to the retiring enclosures. Within the retiring enclosures, sometimes new pairs are formed and start to breed again. In that case, the new pairs are moved back into the breeding enclosures.

Eight breeding couples of scarlet macaws were studied: C1; C2; C3; C4; C5; C6; C7; C8. All these couples had been placed in the smaller breeding enclosures only a week before the start of observations, as these had just been built. Their years as a couple varied: C3 was paired in 2021 (1 year as couple); C4 was paired in 2017 (5 years as couple); C5 was paired in 2020 (2 years as couple); C1, C2, C6, C7 and C8 were newly paired. All previously paired couples had laid eggs and raised offspring.

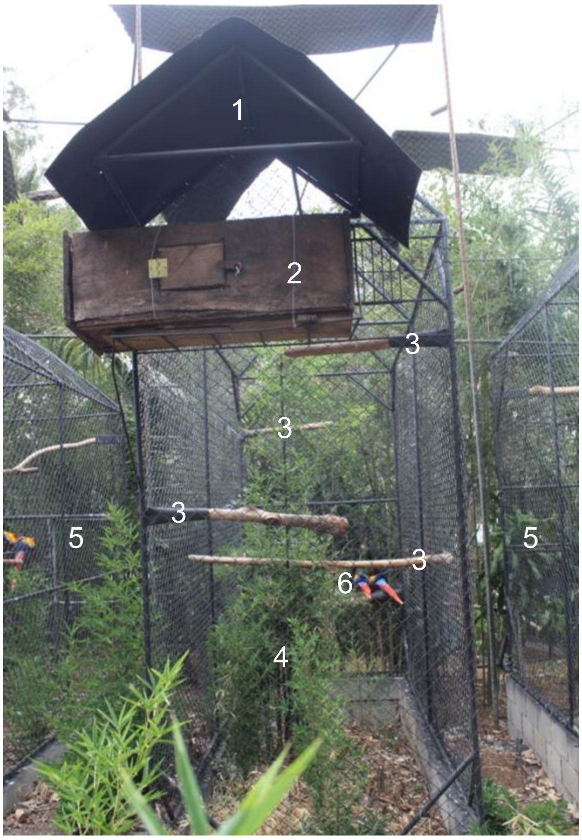


Figure 9 – Outside enclosures (original photo).
1 - metal plate; 2 - nest box; 3 - perch;
4 - bamboo plants; 5 - next-door enclosures; 6- couple feeding at the feeding station.

Figure 10 – Feeding stations, where the thickness of the wire mesh can be assessed (original photo). Red – feeding station; green – bowl placed in feeding station; yellow – perch stand

All the enclosures measured approximately 1.5 m wide by 3 m long, varying only in height, which was between 3 and 4 m. The walls were made out of wire mesh, strong and thick enough to withstand the force of the macaw's beak, there were at least four wooden perches per enclosure, long enough for the couple to stand next to each other (Figure 10), and a feeding station that enables the provision of food and water without entering the enclosures (Figure 11). Wood nest boxes measuring 47 cm wide x 117 cm long x 42 cm high, horizontally positioned under a metal plate, were a part of each enclosure to provide the macaws with favourable conditions for breeding (Figure 10). Each nest had a circular entrance, with a diameter of 15 cm, on the left or right side. Wood shavings and pieces of hard wood were placed inside the nest, to stimulate macaws to prepare the nest. The ground consisted of dry soil with variable sized plants (i.e., bamboo) (Figure 10) and rocks. There were trees covering the enclosures of couples C1, C2, C3, C4 and C5. However, C6, C7 and C8 only had shade under the metal plates that covered the nests.

There were two disturbances near the macaw enclosures throughout the observational period worth mentioning: the construction of new enclosures for other birds on the breeding centre; the fact that vultures oftentimes flew closely over the premises, which led the macaws to raise alarm calls.

The macaws were fed the same diet and were fed usually twice a day, in the morning around 7 am and in the afternoon around 3 pm, except on Thursdays and Fridays, when only the morning meal was provided due to staff rest. The morning meal consisted of a mixture of boiled food (rice, beans, carrots, squash, cucumber, cabbage, beetroot and soya beans), crushed fruits (banana, papaya and melon), dog food, calcium and Pecutrin (a mineral supplement with vitamin A, D3 and E). This way the macaws could not select what they ate, ensuring that their diet was well balanced. After placing the mixture in the bowl, a boiled egg and crushed crackers were added on top before distribution, each bowl being shared by the couple. For the afternoon meal, two pieces of five types of fruit, which varied every day, and a handful of sunflower seeds were placed in each bowl (Figure 11). This contributed to environmental enrichment by allowing the couple to select their preferred food. Water was available *ad libitum* inside another bowl, and was replaced every time they were fed.

Figure 11 – Scheme of food preparation for the afternoon meal (original photos).

4.2.3. ZooMonitor

The ZooMonitor app, developed by the Lincoln Park Zoo, allows the recording of animal behaviour (Ross et al 2016). Designing a project in this platform starts with setting the duration of observation sessions and adding focal subjects. The selection of the sampling method will enable the recording of behaviours in different ways: interval sampling will record at pre-set time intervals; all-occurrences sampling will record the onset of each behaviour; continuous sampling will record the duration of each behaviour. To add the specific behaviours relevant to the project, one or more channels have to be created, with a different sampling method. A space use function for interval sampling is also available, in which the exact position of the bird within the enclosure can be registered and associated with any specific behaviour that is being observed (Table 1).

After creating a project, several sessions of behaviour can be recorded using the app on a mobile device. When starting a new session, it is required to choose the subjects under observation. While the session is running, the user only needs to select the behaviour and position of the individuals under observation, in each time interval. This tool not only facilitates data collection, but also its further analysis. The app automatically transfers all the information into an excel file database, ready for export, where a more in-depth manual analysis can be conducted.

4.2.4. Behavioural observations

The project concerned a total of 24 focal subjects, as each couple was divided into a male (Couple 1 M), a female (Couple 1 F) and the both together (C1). This meant 16 individual birds plus 8 couplings of the same birds. The study was conducted over a six-week period, between February and April 2022, for a total of 24 days. Twice a day, between 6:30 and 8:30 am and then between 3 and 5 pm, on-site behavioural observations of the eight breeding pairs were recorded. Each couple was recorded for 15 minutes at a time, with a daily total of 30 minutes. Every day (D), a different couple was recorded first, following the order of their numbering (Example: D1: C1-C2-C3... / D2: C2-C3-C4… / D8: C8-C1... / D17: C1-C2...). This amounted to 3 rounds of observations, so that couples were observed at several time periods more than once, giving reliability to the collected data.

In the app, twenty-one behaviours were registered and grouped in seven distinct categories: comfort, movement, foraging, communication, social affiliative, social agonistic and stereotypical (Table 1). All behaviours were sampled at 30 second intervals, meaning that every 30 seconds a behaviour was scanned. An all-occurrences sampling channel was created for short period behaviours, that happened within the 30 second interval, and were relevant to the research. A record of space use data was also done to determine whether the birds spent more time apart or next to each other.

Table 1 – Ethogram used on the project made in the ZooMonitor app, adapted from Cornejo et al. (2005), Silva (2019) and Miglioli and Vasconcellos (2021).

Behavioural categories	Description
COMFORT	
Resting	inactive with eyes closed or not involved in any activity
Preening	auto feather maintenance, scratching, beak rubbing against objects and stretching
MOVEMENT	
Locomotion	moving some part of the body without locomotion, flying, walking or climbing using the beak and/ or feet
FORAGING	
Feeding	licking, pecking or ingesting food or water
COMMUNICATION	
Vocalizing	any vocal emission
SOCIAL AFFILIATIVE	
Allopreening	mutual feather maintenance
Allofeeding	mutual feeding between two birds
Face Touching	mutual touching of the beak or face when approaching
Courtship	male approaches female while showing physical abilities
Copula	attempts to copulate and actual copulation
Inside Nest	one or both of the birds are inside the nest
Positive Affiliation	approach of one bird without consequent distancing of the other
Other positive Interactions	positive interactions not listed above
SOCIAL AGONISTIC	
Physical Assault	attacking with beak, claws or wings
Negative Affiliation	flying/walking away, threatening, repelling or intimidating
Stealing Food	taking food from other individual
Physical Assault While Feeding	attacking with beak, claws or wings while feeding
STEREOTYPICAL	
Pacing	walking back and forth on the perch or on the wire mesh
Head Moving	moving the head back and forth and/or from side to side repeatedly
Hanging	hanging from the ceiling, in a horizontal or vertical position, trembling, or with fast and short movements of the wings
Repetitive Body Movement	moving the body up and down, from side to side, or in a circular motion

4.2.5. Data processing, characterisation and statistical analysis

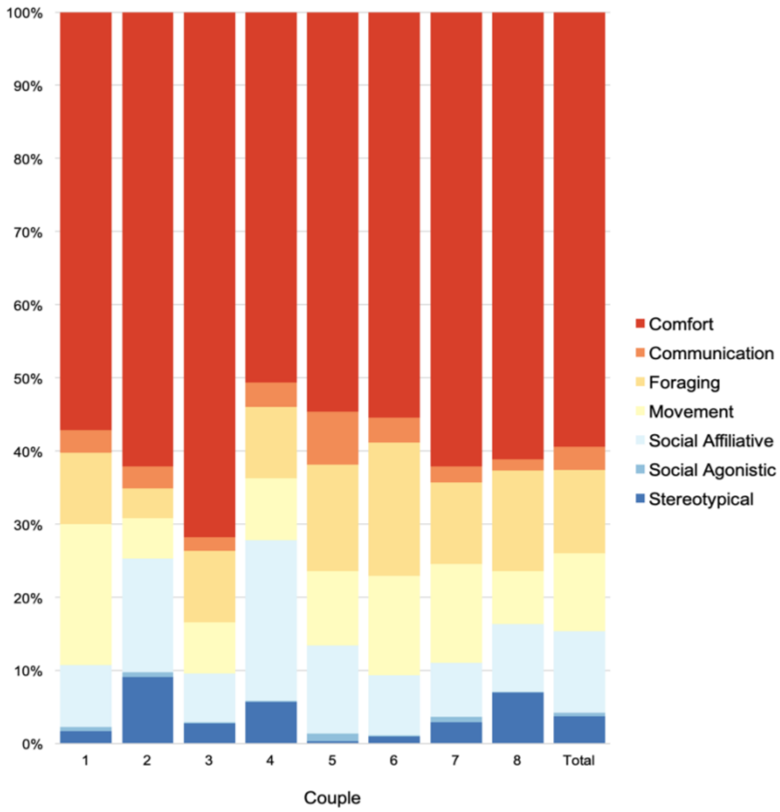
Data processing was done in Excel 2016 (Microsoft Corporation 2016). After downloading the data base from ZooMonitor, new columns were created in order to include further relevant information that would be needed for visualisation and statistical analysis of the data. These included: day numbering ("Day"); time of day in each observation ("AM PM"); couple numbering ("Couple"); how long had the couple been paired ("Years as Couple"); whether or not the couples had enough shade in the enclosures ("Shade"); whether or not the individuals were standing next to each other ("Close Together"); the category in which the behaviour was inserted ("Behaviour Category"); the presence/absence of stereotypical behaviour ("Stereotypic Behaviour"); the presence/absence of affiliative behaviour ("Affiliative Behaviour"); whether or not they were fed in the afternoon ("Afternoon meal"); at what time they were fed ("Time of Feeding"); how long ago were they fed ("Time Since Feeding"); time intervals since their last meal ("Hours Since Feeding"), which could either be 0-2h (immediately after morning/afternoon meals), 8-10h (long after morning meal and before afternoon meal), 16-18h (before morning meal and fed the previous afternoon), or 22-24h (before morning meal and not fed the previous afternoon). Data characterisation was also done with Excel 2016 (Microsoft Corporation 2016), with the use of pivot tables to create tables and graphs.

The statistical analysis was conducted with the R software v. 4.2.2 (R Core Team 2022). Generalized Linear Mixed Models (GLMM) were used to ascertain what factors influenced the exhibition of affiliative, and stereotypical behaviours. Since both response variables were binary (presence/absence of affiliative/stereotypical behaviour), a binomial distribution and a logistic link function were applied within the models. The independent variables considered were "Shade", "Years as Couple", "AM PM", and "Hours Since Feeding". Given that the eight different couples observed in the study and the day in which the observation took place cannot be considered independent variables, "Couple" and the "Day" were considered random factors in the models, in order to reflect the repeated measurements aspect of the project design. Furthermore, the quality of the models was evaluated using the Area Under de ROC Curve (AUC), which measures the model's ability to differentiate between the presence (Yes) and absence (No) of the binary variables. Its value varies between 0.5 (equal to a random classification) and 1 (perfect distinction of the model into Yes and No). The models were achieved using the lme4 v. 1.1-29 package (Bates et al. 2022), and the AUC using the presence/absence package (2008).

4.3. Results

4.3.1. Activity-budget

Graph 1 – The mean frequency (percentages) in which the couples were observed expressing each behaviour category, in interval sampling.

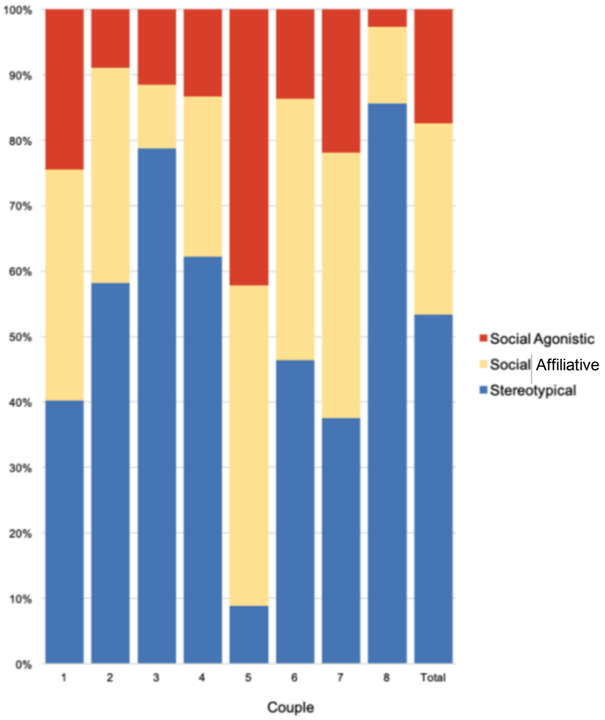


The total number of entries for this study was 21.585, with each representing one of the behaviours exhibited by the female, the male, or the couple together while socializing. The interval-sampling channel had 20.746 entries, which amounted to 96.1% of all entries. The activity-budget for each couple is depicted on Graph 1. All categories are represented in smaller or larger amounts in each couple. Overall, the couples were mainly observed exhibiting "Comfort" behaviours (59.4%). "Foraging" (11.4%), "Social Affiliative" (11.2%), and "Movement" (10.6%) had similar frequencies of observation. "Stereotypical" (3.8%) and "Communication" (3.2%) came next, while "Social Agonistic" (0.4%) was the category least observed. However, there are some important differences between the couples worth mentioning. C3 was observed considerably more often in the "Comfort" category (71.8%), in which C4 was observed less often than all other couples (50.6%). In the "Foraging" category C2 had the lowest frequency (4.1%), and C6 the highest (18.2%), both differing considerably from the overall score. The couple that exhibited more "Social Affiliative" behaviour was C4 (22%), followed by C2 (15.6%). This category was scarcely displayed by C3, amounting only to 6.7% of their activity budget. The major representative of the "Stereotypical" category was C2 with 9.1%, whereas C5 was the minor with only 0.3%.

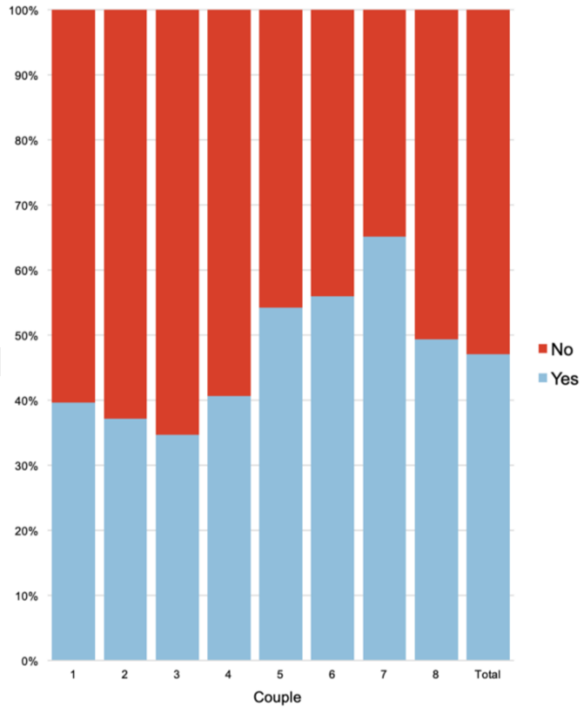
Table 2 – Differences on the counts of all-occurrence event recording per couple observed.

Couple	1	2	3	4	5	6	7	8	Total
Count of all-occurrence events	102	67	174	45	102	110	128	111	839

Graph 2 – The mean frequency (percentages) in which the couples were observed displaying relevant all-occurrence behaviours.



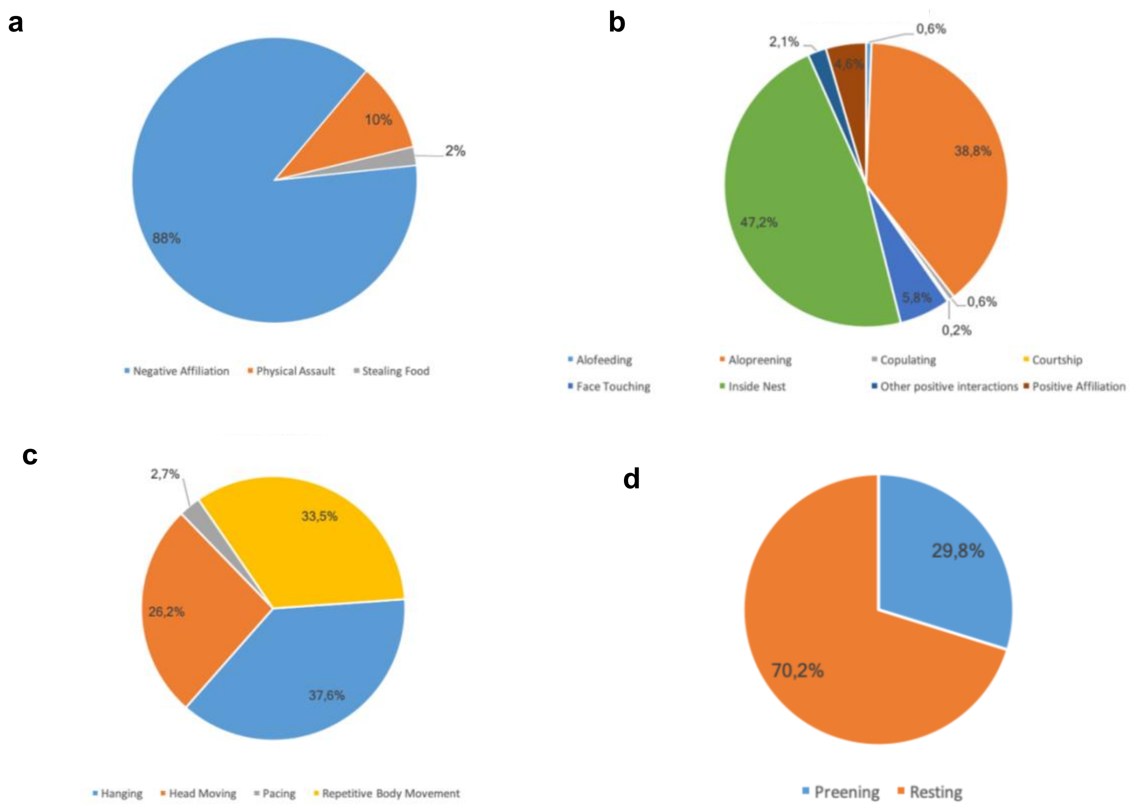
Graph 3 – The mean frequency (percentages) in which the couples were observed either close together (Yes) or apart (No).



The all-occurrence sampling channel only represented 3.9% (839 entries) of the total observations, and its usage varied in between the couples, as it is shown in Table 2, with C3 as the couple in which it was applied more often and C4 the least often. The mean frequencies exhibited of each category per couple, are depicted on Graph 2. Overall, the category "Stereotypical" was the most represented (53.4%), in contrast to the category "Social Agonistic", which was the least represented (17.4%). C3 and C8 were the couples that exhibited more "Stereotypical" behaviour within the 30s intervals, with 78.7% and 85.6% of their all-occurrence sampling count, respectively. C5 only displayed 8.8% of its all-occurrence sampling count, within this category. The couple that exhibited more "Social Affiliative" behaviour within the 30s intervals was C5, with 49% of its all-occurrence sampling count, and the one that exhibited less was C3 with only 9.8%.

Overall, the couples were as much seen together (47.1%) (Figure 13c) and apart (52.9%). However, C7 was seen together (65.2%) considerably more often than apart (34.8%), in contrast to C3, which was mostly seen apart (65.3%) than together (34.7%).

Graph 4 – The mean frequency (percentages) each behaviour was observed within the four different categories that included more than one behaviour.



Legend: a - Social Agonistic; b - Social Affiliative; c - Stereotypical; d - Comfort.

After characterisation of the different categories, since four of them included several distinct behaviours, it is important to acknowledge what behaviours were observed the most. In all categories, all the behaviours were observed at least once. Within the “Social Agonistic” category (Graph 4a), the most prevalent behaviour was “Negative Affiliation”, with great distinction from the others. The most frequent behaviours of the category “Social Affiliative” (Graph 4b) were “Inside Nest” and “Allopreening” (Figure 13b). The “Stereotypical” category (Graph 4c) had three of the four behaviours it included almost evenly distributed: “Repetitive Body Movement”, “Hanging” (Figure 13a), and “Head Moving”. Finally, the category “Comfort” (Graph 4d) had as a most frequent behaviour, “Resting” (Figure 13d).

Figure 12 – Macaws displaying some of the above mentioned behaviours (original photos). Legend: a - Hanging; b - Allopreening; c - Standing close together; d - Resting.

4.3.2. Factors affecting affiliative and stereotypical behaviour

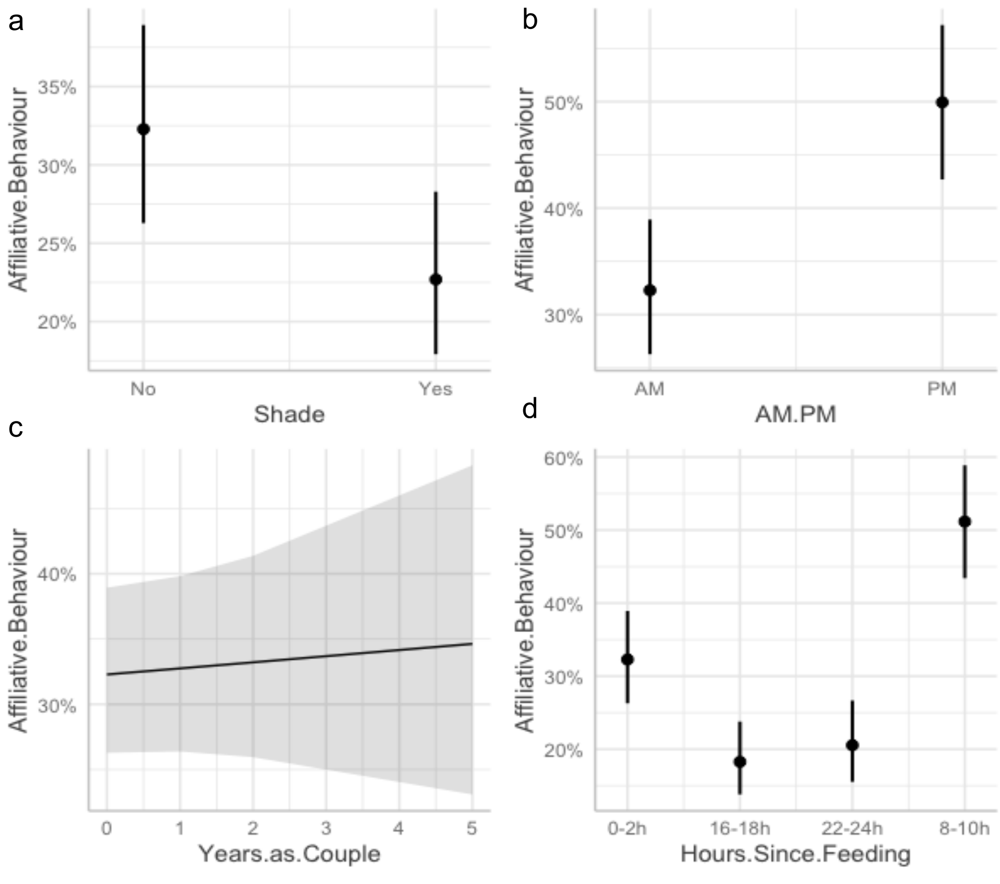
Table 3 – Affiliative behaviour GLMM containing the output of estimate of regression coefficients (Est.) and respective 95% confidence intervals (2,5% and 97,5%), the standard errors (Std. Error), the z-values and correspondent levels of significance (p-value= Pr (>|z|)) for the fixed effects.

	Est.	2,5%	97,5%	Std. Error	z-value	p-value
(Intercept)	-0.74073	-1.03110000	-0.4503521	0.14815	-5.000	5.74e-07 ***
Shade (Yes)	-0.48514	-0.81932014	-0.1509677	0.17050	-2.845	0.00444 **
AM PM (PM)	0.73849	0.66633525	0.8106514	0.03682	20.059	< 2e-16 ***
Years as Couple	0.02106	-0.07647963	0.1186095	0.04977	0.423	0.67211
Hours Since Feeding (16-18h)	-0.75731	-0.93407013	-0.5805500	0.09019	-8.397	< 2e-16 ***
Hours Since Feeding (22-24h)	-0.61173	-0.80365561	-0.4198090	0.09792	-6.247	4.18e-10 ***
Hours Since Feeding (8-10h)	0.78753	0.67218954	0.9028649	0.05885	13.383	< 2e-16 ***
Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1						

The response variable "Affiliative Behaviour" used in the model is a sum between all the behaviours included in the "Social Affiliative" category, and the ones belonging to the "Comfort" category exhibited when the couple was standing together.

The couples that had more shade ("Shade" – Yes) exhibited significantly less affiliative behaviour (Est. = -0.48514; z=-2.845; p < 0.01) than the ones that had less shade ("Shade" – No) (Table 3; Graph 5a). Moreover, when the macaws had not been fed for over 16h ("Hours Since Feeding" – 16-18h and 22-24h), the expression of affiliative behaviours was significantly inferior (Est.= -0.75731; z=-8.397; p<0.001 and Est.= -0.61173; z=-6.247; p<0.001, respectively) than when they had been fed recently ("Hours Since Feeding" – 0-2h) (Table 3; Graph 5d). On the other hand, if they had been fed in the morning, but had not received the afternoon meal yet ("Hours Since Feeding" – 8-10h), their affiliative behaviour expression significantly increased (Est.= 0.78753; z=13.383; p<0.001) (Table 3; Graph 5d), which is in line with the fact that within the afternoon ("AM PM" – PM), the display of affiliative behaviour among couples also had a significant increase (Est.= 0.73849; z=20.059; p<0.001) (Table 3; Graph 5b). The couple's years together had no significant impact of affiliative behaviour display (Table 3; Graph 5c).

Graph 5 – Partial dependence plots characterizing the effect of each of the tested variables, on the response variable (extent of the presence of "Affiliative Behaviour").



Legend: a - Effect of shade absence/presence; b - Effect of morning and afternoon time periods; c - Effect of years as a couple; d - Effect of hours spent without being fed.

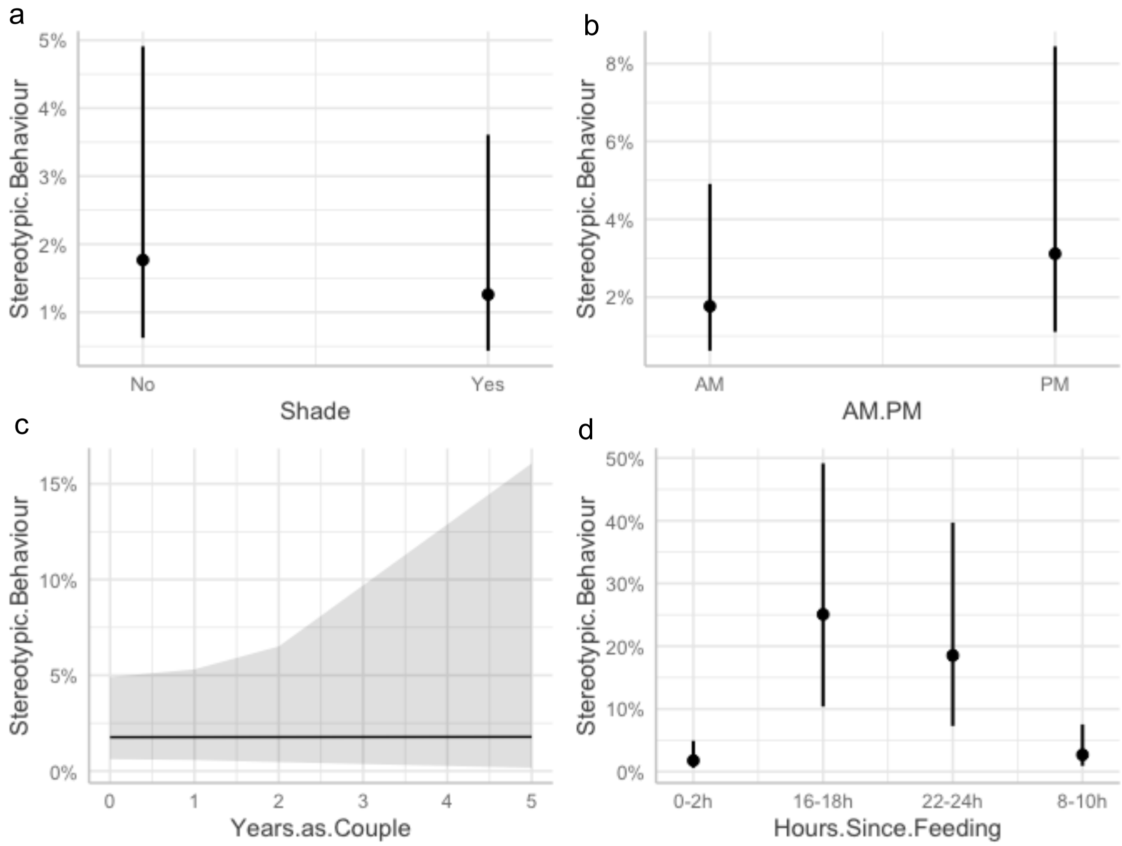
Table 4 – Stereotypical behaviour GLMM containing the output of estimate of regression coefficients (Estimate) and respective 95% confidence intervals (2,5% and 97,5%), the standard errors (Std. Error), the z-values and correspondent levels of significance (p-value= Pr(>|z|)) for the fixed effects.

	Est	2.5%	97.5%	Std. Error	z value	p-value
(Intercept)	-4.018294	-5.0727239	-2.9638633	0.537985	-7.469	8.07e-14 ***
Shade (Yes)	-0.343202	-1.7910044	1.1046009	0.738688	-0.465	0.642211
AM PM (PM)	0.580733	0.3852290	0.7762379	0.099749	5.822	5.82e-09 ***
Years as Couple	0.001999	-0.4192487	0.4232476	0.214926	0.009	0.992577
Hours Since Feeding (16-18h)	2.923637	2.6789833	3.1682906	0.124826	23.422	< 2e-16 ***
Hours Since Feeding (22-24h)	2.537040	2.2916116	2.7824675	0.125221	20.261	< 2e-16 ***
Hours Since Feeding (8-10h)	0.427511	0.1828397	0.6721830	0.124835	3.425	0.000616 ***
Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1						

Neither shade, nor the couple's years together had any influence on stereotypical behaviour expression. However, feeding times had a major impact on the display of stereotypical behaviours, as these were exhibited far more often when the macaws had not been fed for over 16h ("Hours Since Feeding" – 16-18h and 22-24h) (Est.=2.923637; z=23.422; p<0.001 and Est.=2.537040; z=20.261; p<0.001, respectively), than when they had been fed recently ("Hours Since Feeding" – 0-2h) (Table 4; Graph 6d). Even though the expression of stereotypical behaviours when they had been fed in the morning but had not received the afternoon meal yet ("Hours Since Feeding" – 8-10h), was also frequent, it was not as significant as the former (Est.= 0.427511; z=3.425; p<0.001) (Table 4; Graph 6d). Within the afternoon ("AM PM" – PM), the display of stereotypical behaviour among couples had also a significant increase (Est.=0.580733; z=5.822 p<0.001) (Table 4; Graph 6b).

Both models presented a moderately good AUC, which had a value of 0.7082351 for the "Affiliative Behaviour" model, and a value of 0.823456 for the "Stereotypical Behaviour" model. This evidence leads us to conclude that the findings that derived from these models are reliable.

Graph 6 – Partial dependence plots characterizing the effect of each of the tested variables on the response variable (extent of the presence of "Stereotypical Behaviour").



Legend: a - Effect of shade absence/presence; b - Effect of morning and afternoon time periods; c - Effect of years as a couple; d - Effect of hours spent without being fed.

4.4. Discussion

Reproductive success and welfare of captive scarlet macaws is highly dependent on the environmental conditions provided, and general management practices applied within each breeding programme. Behavioural observation and measurement is a commonly used tool in this setting, as it allows the quantitative and qualitative assessment of displayed behaviours, in relation to the circumstances prevailing at the time of measurement. The aim of this study is the identification of factors that might influence the expression of both affiliative and stereotypical behaviours, by applying this observational approach. In the context of a scarlet macaw breeding facility for conservation purposes, this will allow for the disclosing of the factors' positive/negative influence. So how exactly do these factors influence such behaviours?

The findings revealed variances between each couple's activity budgets. While stereotypic behaviours were generally infrequent in the interval sampling observations (Graph 1), there was considerable variability in their expression across the different couples (C5 0.7%

- C2 10.4%). Furthermore, the data indicated that the third most frequent behaviours in the interval samplings were the social affiliative, with allopreening and standing inside the nest being the prevailing behaviours (Graph 4b). However, these social affiliative behaviours also exhibited substantial variability between couples (C3 6.9% - C4 22%). Social agonistic behaviours were rarely observed (Graph 1 and 2). The amount of time macaws spent standing in proximity to each other also varied widely across the couples. Foraging activities were observed in a mere 11.4% of the overall interval observations, while comfort behaviours accounted for a substantial 59.4% (Graph 1). This result provides support for the notion that, among the population under investigation, the feeding schedule significantly influenced the incidence of stereotypical behaviours (Graph 6d). The data additionally supported the notion that in the afternoon, macaws were more likely to engage in affiliative and stereotypical behaviours (Graph 5b and 6b). Furthermore, it was found that having less shade was a contributing factor to the display of affiliative behaviour, which was contrary to the expectations that less shade would negatively affect breeding attempts.

Baseline factors

A lot of the factors that may influence reproduction and general welfare, such as climate, housing, nesting and general social surroundings, remained constant throughout the observational period and in between the couples, being considered part of the baseline of the study. In order to correctly interpret the findings, the adequacy of these environmental factors needs to be taken into account.

The observations took place between February and March 2022, coinciding with the mid breeding season, which is generally from December to May, in Costa Rica (Vaughan et al. 2009). Here, the cessation of seasonal rains and slight photoperiod increase, act as the first reproductive stimuli (Wilson 2006; Hau et al. 2008; Vaughan et al. 2009) and positively influence the general display of affiliative behaviours, which was considerably high. The fact that all the scarlet macaws were in their country of origin, makes the climatic characteristics of the breeding enclosures (photoperiod, temperature and humidity) appropriate for this species.

The housing characteristics of the centre were consistent with what was found in the literature. The size of the enclosures, measuring 1,5m x 3m x 3/4 m, was deemed appropriate for the species and was found to be in agreement with the ideal size for large macaws, measuring, 2,1 m x 3m x 2.1m (Wilson 2006; Silva 2018). While the width of the enclosures was slightly smaller than the recommended size for macaws, it did not appear to limit the locomotion capabilities of the birds, as flights were observed from one end of the enclosure to the other. The breeding enclosures provided for the macaws during the off-breeding season (Figure 9) were found to be at least 15 m long, which aligns with Silva's (2018) recommendation and is an effective way to overcome the restrictions smaller enclosures might impose on the birds' locomotor capabilities during the breeding season (Meehan and Mench 2006). The enclosures were equipped with natural tree branch perches, with at least two of them placed high above human height, and had vegetation inside while still allowing for flight. These features, as noted by Wilson (2006) and Raisi and Cornejo (2021), enable the performance of all locomotion patterns typical of scarlet macaws and provide a sense of safety and security for the birds. However, disturbances occurring in the breeding facilities, such as the presence of predators and noise from construction work in progress, as well as the observer's presence while collecting data, may have inhibited the macaw couples from copulating or laying and caused abnormal behaviour (Wilson 2006; Allgayer and Cziulik 2007). Additionally, the possibility of contact with the ground, might present a risk to the general health of the birds (Wilson 2006).

Regarding nesting conditions, the enclosures were equipped with wooden nest boxes, which are found to be the preferred material for macaws (Silva 2018) and a powerful stimulus for reproductive development (Wilson 2006). The dimensions of the nest boxes were 47cm x 117cm x 42cm, and they were oriented horizontally (Figure 10), providing sufficient space for both individuals to copulate and incubate as well as for hatchlings to spread out in case of temperature rises (Martin and Romagnano 2006; Wilson 2006; Silva 2018). To offer shade, a metal plate was used to cover the nest (Figure 10). Furthermore, a circular entrance was positioned on either the left or right side of the nest box to ensure that the interior remained dark, which is essential for breeding macaws (Wilson 2006; Silva 2018). To promote a sense of safety and security for the birds, all nests were placed at least 2 meters off the ground, on the opposite end of the feeding station, and in direct visual contact with the other couple's enclosures (Figure 10) (Allgayer and Cziulik 2007). Nest preparation is an essential reproductive stimulus, and to encourage this behaviour, tree trunk chips and wood shavings were provided. These materials allow the macaws to create a comfortable and suitable nesting environment (Wilson 2006).

The breeding programme commenced with the employment of natural pair bond formation, a technique that has been shown to enhance couples' compatibility (Silva 2018). At the onset of the breeding season, males and females were placed together in a large enclosure, allowing for general social interactions with conspecifics and the expression of sexual behaviour. To ensure that the bonded pairs consisted of one male and one female, a DNA test was conducted on all individuals displaying mutual interest behaviours. Only those individuals that were confirmed to be a male-female pair were included in the program and placed in smaller enclosures (Figure 10). The general social surroundings of the studied couples stimulated interactions between conspecifics, and expression of sexual behaviour, which may have contributed in some degree, to the prevention of stereotypical behaviours, ultimately leading to reproductive success and positive welfare (Meehan & Mench, 2006; Greggor et al., 2018; Andrews, 2022).

Differing factors

In contrast, distinct differences were evident either between observational circumstances or among the various couples, which could be specific to each couple or linked to their particular environment. This study aimed to investigate the potential influence of some of these variations on the exhibition of behaviours that are associated with welfare ("Social Affiliative") or the lack thereof ("Stereotypical") (Reimer et al. 2016).

The heterogeneity observed in the behavioural patterns of couples may be attributed to their diverse welfare backgrounds before being confiscated and handed to CRAVE. Specifically, factors such as the quality and duration of previous housing conditions, the presence of social companionship, and the availability of resources for exploration and stimulation may have influenced the animals' behavioural responses in the breeding centre. The fact that these origins and the individual age of macaws were unknown, made it impossible to include such differences in the analysis. The only activity-budget aspect that all eight couples had in common was that they spent the majority of their time exhibiting comfort type behaviours.

One could infer that, since stereotypical behaviours are common in the scarlet macaw, and tend to be harder to reverse the longer the animal remains under poor welfare conditions (to the point of being present even if the current welfare conditions are adequate) (Mellor et al. 2018), one or both individuals within the couples that exhibited this behaviour more often (C2, C8, C4), could have been kept under poorer past welfare conditions for longer periods of time. The fact that locomotor stereotypes are more represented (repetitive body movements and hanging), may indicate that some of the animals have had spatial restrictions (Meehan and Mench 2006).

The rarity of social agonistic behaviours, adding to the exhibition of affiliative behaviour all couples had towards each other in the present study, suggests that this population of macaws may have established a peaceful and cooperative social structure, which reinforces the fact that natural pairing is a good way to increase the compatibility between individuals (Silva 2018). The variations observed in affiliative behaviour frequency among the different couples contradict the idea that longer-term couples exhibit greater levels of social affiliation (Seibert and Sung 2010), as this factor was not found to be statistically significant (Table 3) even though the older couple was the one that exhibited affiliative behaviours more often (C4). A plausible explanation for this finding may be attributed to the fact that, with the exception of year zero, each longer-term macaw couple had bonded in distinct years, with only one representative couple per year.

Accurately ascertaining the nutrient requirements of a given species is a difficult task (Matson and Koutsos 2006), but is one of the most important factors to take into account within the captive setting. All the birds at Rescate Wildlife Rescue Centre were fed the same nutrient filled morning diet, the one described in the material and methods. Even though this is not ideal, none of the birds in the study displayed any visible signs of nutritional deficiencies or excesses. However, it is possible that the overall welfare of the macaws was more affected by the limited opportunities for foraging, because macaws were fed a mesh diet in a bowl. In their natural habitat, foraging can occupy up to 70% of their waking day (Renton 2000), yet foraging activities accounted for only 11.4% of the overall interval observations, while comfort behaviours represented over half of the observations (Graph 1). Of these last behaviours, preening was observed only 29.8% of the time, which deviates from the wild state where it is the most frequent behaviour after foraging (Bergman and Reinisch 2006). Additionally, macaws are not typically known to sleep extensively during the daytime. Thus, the feeding patterns of the breeding program appear to have had a negative impact on the couples, as evidenced by the significantly higher frequency of stereotypical behaviours and presence of FAA, preceding the morning feeding, regardless of whether they were fed in the afternoon or not (Graph 6d). Conversely, affiliative behaviours were significantly less frequent during these same time periods (Graph 5d). These findings are consistent with the literature, which suggests that the development of stereotypical behaviours in macaws is linked to their heightened motivation to forage for food (Meehan and Mench 2006; Seibert and Sung 2010). Therefore, the afternoon meal provided insufficient enrichment to satisfy their foraging needs.

The results indicating that macaws were more likely to engage in affiliative and stereotypical behaviours in the afternoon suggest that diurnal variations may influence their behavioural patterns. Given that the 8-10h since feeding time period was situated within the afternoon, it is reasonable to assume that it too exhibited a similar relationship. A plausible explanation for this finding may be that, after the morning feeding, birds were more inclined to engage in comfort behaviours, as noted by Bergman and Reinisch (2006), and hence were more active in the afternoon.

The results suggest that the availability of shade may have affected the frequency of couples standing close to each other, as the shade was restricted to a single perch, necessitating to maintain close proximity. Couples C6, C7, and C8, which did not have access to a large shade, exhibited a higher frequency of observations together compared to couples C1, C2, C3, and C4, which had access to shade, as evidenced by Graph 3. C5 was an exception to the other couples with shade, as they spent more time standing together. Unexpectedly, the presence of shade was found to decrease affiliative behaviours, contrary to initial expectations. This finding may be attributed to the inclusion of comfort behaviours while in close proximity, as an affiliative behaviour even though they are not directly socialising, as this was in accordance with the literature cited (Sant 2006; Seibert 2006; Spoon 2006).

The findings presented in the previous paragraphs can make a contribution to the field of animal welfare in captive breeding programs, particularly for the scarlet macaw species. By examining the behavioural patterns of captive macaws in response to environmental factors, this study shows how negatively these can impact the individuals, while highlighting the importance of considering the animals' natural behaviours and needs in captive settings

Recommendations

Although captive environments restrict animals' natural behaviour, proper management practices can still ensure their welfare. Overall, these environmental conditions were designed to maintain the birds' natural behaviour patterns as much as possible, while meeting the specific needs of breeding macaws. Thus, the methodology implemented in this breeding program prioritizes animal welfare and maximizes breeding success, which is vital for maintaining viable populations of macaws in captivity. A lot of the enrichments suggested by Andrews (2022) were applied: provision of nesting materials; social group managing to mimic natural age structures; allow pairs to incubate and rear their own young; allow mate choice; proper enclosure heights. However, there is always space for improvement, which was something the breeding centre had been working on that year, with the construction of the new enclosures, including the ones where the couples were observed.

This study provides a viable way to assess general welfare of captive individuals, by emphasising the factors that may be contributing for and against it, showing where improvements need to be made. It is recommended that species-specific diets be prioritized over generic mixes, which may not adequately meet the nutritional and behavioural requirements of the birds, potentially compromising their reproductive and overall health. Various approaches outlined in the literature can aid the breeding centre in enhancing their feeding enrichment and schedule. One strategy is to relocate the food preparation area further away from the enclosures to prevent the development of FAA and stereotypical behaviours, as not to allow the macaws to hear sounds of food being prepared. Additionally, the utilisation of a consistent auditory cue, such as a bell, before feeding can mend predictability and reduce stress, as suggested by Bassett and Buchanan-Smith (2007). The provision of whole foods instead of chopped or boiled, as evaluated by James et al. (2021), may also benefit both the macaws and staff, despite the potential challenges with food selection. Additionally, a variable feeding schedule that incorporates foraging opportunities throughout the whole enclosure, may significantly enhance the welfare of the macaws. In essence, enrichment practices should be tailored to the macaw population needs, taking into account their social structure and behaviours. It is important, however, to introduce changes gradually to minimize potential stressors on the animals, and proceed in such a way that couples have as little contact with humans as possible, as this is a conservation breeding programme. The observer could do the analysis from videos taken by cameras on the premises, so as not to disturb the animals. However, this is a measure that requires a large investment.

Further studies within this field could take this methodology and apply it to the most diverse captive settings and species, evaluating different factors of variation between the environmental conditions of individuals and among each of them, to study their impact on general welfare and/or reproduction. This methodology could also be used to study the impacts of different environmental enrichment techniques being applied, to groups of the same species.

5. Study limitations

The primary limitation of this study was the recent placement of the macaws in the described enclosures, resulting in a lack of full acclimatisation to the new environment (Silva 2018). This can potentially be an aversive event for them (Mellor et al. 2018), influencing the expression of affiliative and stereotypical behaviour. The lack of reproduction by any of the observed couples during that breeding season could be attributed to this element. Future studies should consider using acclimatized individuals to minimize these effects.

The unawareness of the exact origin of the macaws, limited the analysis of stereotypical behaviours. It would have been valuable to investigate how origin influenced these behaviours, and assess whether they were a result of the factors under study. The underrepresentation of some of the "Years as Couple" entries also prevented a comprehensive evaluation of its significance. Future studies should include the same number of couples in each different pairing year to facilitate valid comparisons.

Additionally, some observations had to be done in different days due to unforeseen events, but were considered as part of the same day, as observational order of the couples and the time of observation were maintained. The presence of incomplete sessions during data upload could not be rectified, and is represented in Table 5. While these limitations do not invalidate the findings of the study, they do highlight the importance of careful planning and execution in future research.

Table 5 – Variation on the portion of observations done per couple observed (without the All-Occurrence events), due to the incomplete nature of some of the sessions.

Couple	1	2	3	4	5	6	7	8	Total
Portion of observations (%)	12,62%	11,73%	13,01%	12,79%	11,88%	13,02%	12,47%	12,47%	100%

6. Conclusion

Based on the findings of the study, it can be concluded that the spent much of the day being inactive, limited foraging opportunities, not only failed to stimulate this behaviour, but had a significant impact on the behaviour display of the macaw couples, leading to the expression of stereotypical behaviours and reduced affiliative behaviours. The study also showed that the time of day had an influence on the behavioural patterns of the macaws, with afternoon periods being associated with increased affiliative and stereotypical behaviours. Finally, the presence of less shade significantly increased the expression of affiliative behaviours, which contradicted initial expectations.

In conclusion, the findings of this study provide valuable insights into the impact some practices can have on the welfare and behaviour of macaw couples in breeding programmes. The welfare of captive macaws can continuously be improved, resulting in happier and healthier individuals, who can almost display their full behaviour repertoire, as long as the negative factors are identified and corrected. These changes can also contribute to the long-term sustainability of the breeding programme by improving breeding success rates and reducing the need for medical interventions, due to poor health caused by inadequate feeding and poor enrichment practices. In addition, the production of healthy offspring and the provision of an environment that allows for their normal development is critical in promoting the socialisation process and subsequent adaptation to a wild setting once reintroduced. This, in turn, can contribute to the maintenance of genetic diversity and ultimately to the long-term survival of a species. Therefore, improving the welfare of captive animals, such as the macaws studied in this research, can have important implications not only for the individual animals themselves, but also for the conservation of the species as a whole.

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