AN INVESTIGATION INTO THE SOCIAL BEHAVIOURS AND REPRODUCTIVE SUCCESS OF A CAPTIVE POPULATION OF THE BLACK-FRONTED PIPING GUAN (*ABURRIA JACUTINGA*)

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UNIVERSIDADE ESTADUAL DO NORTE FLUMINENSE – UENF
CAMPOS DOS GOYTACAZES – RJ
DEZEMBRO 2023

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Dissertação apresentada ao Centro de Biociências e Biotecnologia da Universidade Estadual do Norte Fluminense Darcy Ribeiro – UENF, como parte das exigências para obtenção do título de Mestra em Ecologia e Recursos Naturais.

Orientador: Professor Dr. Carlos Ramón Ruiz-Miranda

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Abstract

The Black-fronted Piping Guan (*Aburria jacutinga*) is a cracid species native to the Atlantic Rainforest of Brazil, Paraguay and Argentina. While it was once an abundant, illegal poaching and habitat loss have led to significant population declines, with the species declared extinct or presumed extinct in various Brazilian states. Action plans initiated to assist in recovery of the species face challenges due to limited knowledge and low reproductive success in captivity. The project aimed to identify sexual behaviours and indicators of reproductive success in a captive population of *A. jacutinga* held at the Sector of Ethology applied to the Reintroduction and Conservation of Wild Animals (SERCAS) breeding facility at the State University of Northern Rio de Janeiro (UENF). Behavioural observations were conducted on 15 breeding pairs from August 2022 to February 2023. Wilcoxon rank-sum tests were used to analyse differences in the frequencies of behaviours between successful and unsuccessful breeding pairs. Behaviours associated with reproductive success were exhibited frequently in enclosures housing successful breeding pairs. These behaviours include *male offers item to female* ($p = .01$), *male mounts female* ($p = .02$) and *head nodding* (males: $p = .02$; females: $p = .049$), *male chases female* ($p = .002$) and *male wing display* ($p = .005$). Observing these behaviours within 6 days of introducing a breeding pair is indicative of the future reproductive success within that pair. Breeding pairs that actively engage with their environment and exhibit mutual interaction are more likely to produce fertile eggs. Behaviours of individuals in breeding pairs were not influenced by egg laying, but higher levels of social interactions and reproductive success was observed in naturalistic, over non-naturalistic, enclosures. Eggs laid in larger, more naturalistic enclosures had a higher fertility rate than those laid in smaller enclosures with limited light and vegetation (Pearson's chi-squared test, $p = .0004$). Recommendations have been outlined for future research into the reproductive behaviours and mating strategies of this species. Information gained through this study will allow for captive management facilities to manage breeding pairs more efficiently, encouraging successful reproduction and subsequently producing more offspring to be released into the Atlantic Rainforest as per the goals outlined in action plans to restore this endangered species.

Resumo estendido

A jacutinga (*Aburria jacutinga*) é uma espécie de cracídeo nativa da Mata Atlântica do Brasil, Paraguai e Argentina. A caça ilegal e a perda de habitat resultaram em declínios populacionais, levando à extinção declarada ou presumida em vários estados brasileiros. Em 2010, a Sociedade para a Conservação das Aves Brasileiras (SAVE Brasil) fundou o Projeto Jacutinga, um programa que investe na recuperação de *A. jacutinga* por meio do apoio à criação em cativeiro, reintrodução de indivíduos criados em cativeiro na Mata Atlântica e monitoramento das populações liberadas. Embora o Projeto Jacutinga tenha alcançado algum sucesso, com mais de 50 indivíduos reintroduzidos na floresta na cidade de São Francisco Xavier, mais liberações são necessárias para que o projeto atinja seu objetivo de recuperação da espécie. Uma análise de viabilidade populacional do local em São Francisco Xavier mostrou que a reintrodução sustentável da espécie requer a liberação de 20 indivíduos por ano durante 3 anos, e uma população cativa de pelo menos 250 indivíduos é necessária para viabilizar isso. Atualmente, no entanto, estima-se que a população cativa no Brasil seja de 200 indivíduos, e muitos deles não estão envolvidos ou não são candidatos adequados para programas de reprodução e liberação. O Setor de Etologia aplicada à Reintrodução e Conservação de Animais Silvestres (SERCAS) da Universidade Estadual do Norte Fluminense - Darcy Ribeiro (UENF) tem criado e fornecido candidatos para liberação ao Projeto Jacutinga desde o início de sua colaboração em 2017. No entanto, com uma taxa de fertilidade de 29,01%, é improvável que, sem intervenção, o SERCAS possa fornecer um número adequado de descendentes ao Projeto Jacutinga no futuro. Como as informações sobre a espécie são escassas e pouco se sabe sobre seus padrões de acasalamento ou comportamentos reprodutivos, é vital observar os pares ao longo de sua temporada reprodutiva para obter insights e compreensão sobre os comportamentos reprodutivos e como o manejo em cativeiro pode ser adaptado para atender melhor aos requisitos de *A. jacutinga* e aumentar seu sucesso reprodutivo.

Os objetivos deste projeto foram identificar comportamentos sociais dentro da população cativa e identificar comportamentos correlacionados ao sucesso reprodutivo, além de verificar se a presença desses comportamentos pode servir como um indicador confiável do sucesso reprodutivo de um par reprodutivo. O projeto também investigou se os comportamentos ligados ao sucesso reprodutivo aumentaram em frequência durante o período de postura de ovos de 6 dias e estudou os efeitos de variáveis secundárias, como tamanho e estilo do recinto, no sucesso

reprodutivo. Um etograma foi criado adaptando informações sobre comportamentos sociais da espécie, baseando-se em literatura relevante. Observações focais foram realizadas em pares reprodutores. No total, 15 pares foram observados por 44 horas ao longo de 35 dias, ou até que a fêmea tivesse completado uma ninhada. Os dados sobre a ocorrência e frequência dos comportamentos foram analisados juntamente com as taxas de fertilidade dos ovos para determinar as relações entre comportamentos e sucesso reprodutivo. Dados históricos também foram analisados para explorar a relação entre sucesso reprodutivo e variáveis, incluindo tamanho e estilo do recinto. A análise de dados exigiu o uso de testes não paramétricos devido ao tamanho amostral limitado e à natureza distorcida dos dados, conforme evidenciado pelos resultados dos testes de Shapiro-Wilk. A Análise de Similaridade (ANOSIM) foi escolhida como método de análise não paramétrica multivariada. A análise bivariada foi conduzida com testes de Wilcoxon de posto assinado, Wilcoxon de postos sinalizados, qui-quadrado de Pearson e testes exatos de Fisher, quando apropriado ($p < 0,05$).

Este estudo produziu três conjuntos principais de resultados. Primeiro, há evidências sólidas de uma temporada de reprodução específica, de agosto a janeiro, durante a qual a frequência de certos comportamentos observados entre pares reprodutores cativos bem-sucedidos e malsucedidos de *A. jacutinga* diferiu significativamente. Comportamentos reprodutivos foram identificados e podem ser utilizados como indicadores da probabilidade de sucesso reprodutivo de um par reprodutivo nesta instalação de criação em cativeiro. A ausência desses comportamentos após uma semana da introdução de um par reprodutivo indica uma baixa probabilidade de sucesso reprodutivo e fornece uma justificativa para separar machos de fêmeas. A postura de ovos não alterou a frequência de comportamentos sociais e reprodutivos de pares reprodutores. Segundo, há evidências de escolha de parceiros pelas fêmeas, demonstrada por uma aparente evasão aos avanços dos machos, às vezes resultando em danos ou lesões. O risco de lesões à fêmea foi aparente nos primeiros dias após a introdução de um par reprodutivo. Recomendações para a separação de pares reprodutores com incompatibilidade percebida foram delineadas. Outras evidências de escolha de parceiros são mostradas em dados históricos, que destacam que alguns pares reprodutores se reproduzem de maneira mais consistente e bem-sucedida do que outros. Terceiro, recintos naturalísticos aumentam a probabilidade de reprodução bem-sucedida nesta

população cativa. Comportamentos reprodutivos identificados foram amplamente observados em recintos maiores e mais naturalísticos. Ovos postos nesses recintos têm taxas de fertilidade mais altas do que os postos em recintos menores, com pouca luz e vegetação. As informações obtidas neste estudo permitirão que as instalações de manejo em cativeiro gerenciem pares reprodutores de maneira mais eficiente e aumentem o número de reproduções bem-sucedidas por estação reprodutiva. Os resultados demonstram a importância de recintos maiores e mais naturalísticos na reprodução bem-sucedida desta população cativa de *A. jacutinga*. A implementação de vegetação nativa e recintos maiores na instalação de criação em cativeiro incentivará a reprodução bem-sucedida de pares reprodutores, produzindo subsequentemente mais descendentes para serem liberados na Mata Atlântica de São Francisco Xavier, conforme as diretrizes do Projeto Jacutinga.

1 Introduction

1.1 *Species Introduction*

The Black-fronted Piping Guan (*Aburria jacutinga*), commonly referred to in Brazil as the jacutinga, is a cracid species endemic to the Atlantic Rainforest and found in Brazil, Paraguay and Argentina (BirdLife International, 2018). *A. jacutinga* is a medium-sized bird, weighing ~1.5kg (Bernardo *et al*., 2011; Galetti *et al*., 1997), and individuals are monomorphic. Adults can be identified by a white crest on the head, a deep blue and red coloured wattle and a striking blue beak and ring around the eye (Figure 1c).

Figure 1. Captive *A. jacutinga* at the breeding facility at the State University of Northern Rio de Janeiro (UENF) at various life stages: A) three newly hatched chicks in an incubator, B) two juvenile chicks in an enclosure and C) a fully grown adult male.

A. jacutinga is often found in forested areas, in pairs or small groups (ICMBio, 2018; Rubim and Bernardo, 2008). Observations of the species in both lowland and high-altitude forests suggest potential altitudinal migration in some populations (Galetti, 1997; Silveira, 2006). In drier habitats, it is often found near streams or rivers (Benstead *et al*., 1998; Bodrati and Cockle, 2006; Galetti *et al*., 1997), where it may be observed feeding on insects and small molluscs. However, the diet of *A. jacutinga* is primarily frugivorous, consisting of fruits, seeds and grains (Galetti *et al*., 1997; ICMBio, 2018). *A. jacutinga* is reliant on various plant species throughout its distribution for both food and shelter (Galetti *et al*., 1997), favouring areas rich in palm trees such as *Euterpe edulis* and *Syagrus romanzoffiana* (Bernardo and Clay, 2006; Galetti *et al*., 1997; ICMBio, 2018).

A. jacutinga has historically assumed significant socio-ecological importance. As an active agent of seed predation and subsequent dispersal, it encourages the regeneration of threatened tropical forests (Galetti *et al*., 1997). Further to this, it serves as a reliable indicator of general forest quality and ecosystem health; populations can be censused with relative ease and are highly sensitive to predation and habitat loss (Brooks and Strahl, 2000; Sánchez-Alonso *et al*., 2002), both factors recognised as primary threats to the species that have led to population declines. Consequently, *A. jacutinga* has been classified by the IUCN as globally endangered (BirdLife International, 2018).

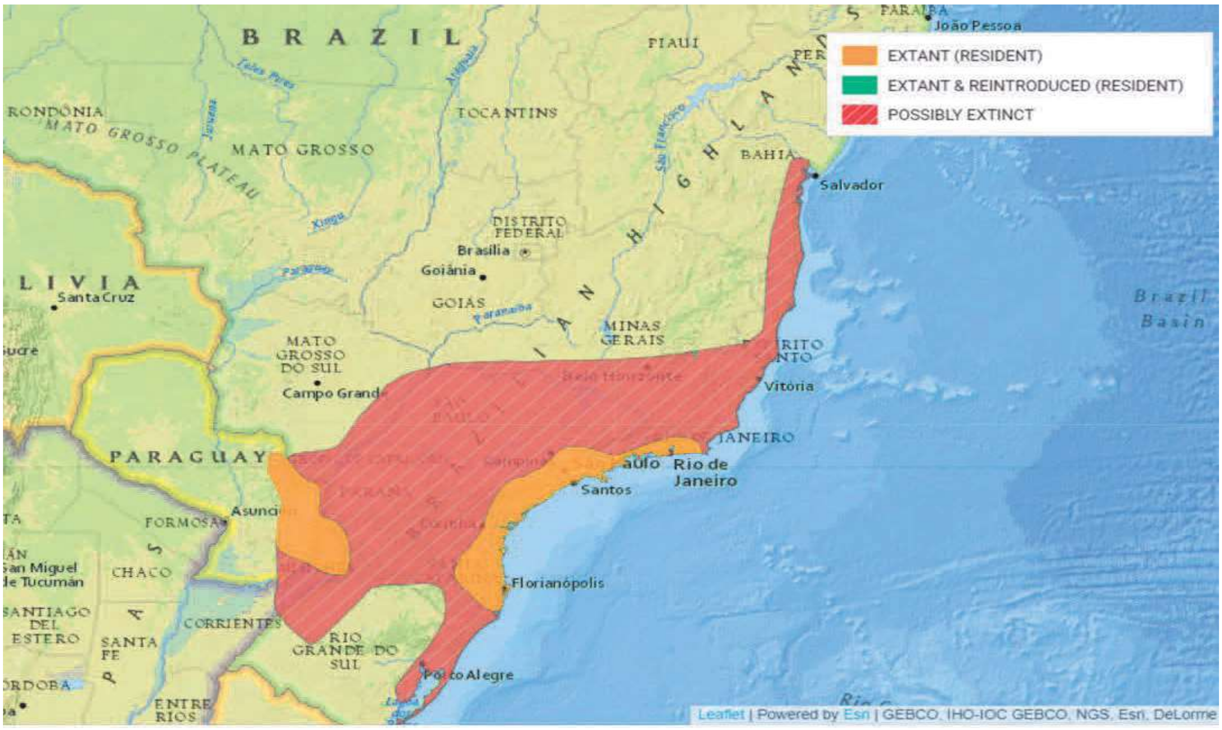

Figure 2. Spatial data detailing the distribution of *A. jacutinga* by BirdLife International and Handbook of the Birds of the World (2018).

Illegal poaching (Bernardo *et al*., 2011; Bodrati and Cockle, 2006) and habitat loss, facilitated primarily through the illegal extraction of palm plants (Bernardo and Clay, 2006; ICMBio, 2008), have resulted in significant and ongoing population declines in *A. jacutinga*. The estimated global population ranges from 1,500 to 7,000 individuals (BirdLife International, 2018; ICMBio, 2008). While it was “formerly one of the most abundant game bird cracids in the Atlantic Forest of Brazil" (Galetti *et al.,* 1997), the current Brazilian population size is estimated to be 2,500 (ICMBio, 2018), with remaining populations restricted to coastal areas in the south-east (Figure 2). The species is now extinct in the states of Bahia and Espírito Santo and is considered probably extinct in Rio de Janeiro (ICMBio, 2018). Despite various action plans emphasising the growing necessity for the conservation of this species (Brooks and Strahl, 2004; Bernardo and Clay, 2006; ICMBio, 2008), the population trend of *A. jacutinga* continues to decline (BirdLife International, 2018).

1.2 *Species Conservation*

The National Galliformes Action Plan (ICMBio, 2008) supported recommendations from Bernardo and Clay (2006) which stressed the necessity of conservation interventions to recover and protect wild populations of *A. jacutinga*. In 2010, the Society for the Conservation of Brazilian Birds (SAVE Brasil) founded the 'Programme for the Conservation of Game Birds in the Atlantic Forest: Reintroduction and Monitoring of Jacutingas', also referred to as Project Jacutinga. During its initial phase (2010–2013), the programme focused on Brazil's Serra do Mar region and confirmed the need for population reinforcement (Tassoni, 2022). Since 2014, Project Jacutinga has focused on the recovery of the species through supporting captive breeding, reintroducing captive bred individuals and monitoring released populations (SAVE Brasil, n.d.). Since the first release in 2016, over 50 individuals have been reintroduced to forested areas in the town of São Francisco Xavier, situated in the district of São José dos Campos, São Paulo (SAVE Brasil, n.d.). Some individuals released are still being monitored today, and in previous years they have been observed nesting and laying eggs in the wild (Souza *et al*., 2020).

A Population Viability Analysis (PVA) conducted for the release site in São Francisco Xavier suggests that the sustainable reintroduction of *A. jacutinga* requires the annual release of 20 individuals for 3 years (Phalan *et al*., 2020). An estimated captive population of 250 individuals is required to meet this target, but the current captive population in Brazil is ~200 (Phalan *et al*., 2020). This figure includes individuals that are not involved in, or are unsuitable candidates for, breeding and release programmes, indicating the necessity to bolster the captive population.

The success of Project Jacutinga relies significantly on the collaborative efforts of various captive breeding facilities across Brazil, which provide suitable individuals for release into the wild. These facilities include the Parque das Aves (Bird Park) in Paraná, the Brazilian Association of Zoos and Aquariums (AZAB) and the breeding facility at the Sector of Ethology applied to the Reintroduction and Conservation of Wild Animals (SERCAS) at the State University of Northern Rio de Janeiro (UENF).

SERCAS is a research centre dedicated to elaborating management methods and reproduction techniques of wild animals, specifically endangered species. Since 2017, SERCAS has partnered with Project Jacutinga. It now manages a captive population of *A. jacutinga* and conducts behavioural and reproductive studies to improve captive reproduction success. While this collaboration has observed success, as each breeding season produces fertile eggs, many breeding pairs do not lay eggs and the fertility rate of eggs laid at SERCAS stands at 29.01%.

1.3 *Captive Breeding*

Captive breeding has been implemented for decades as a tool to aid species recovery (D'Elia, 2010; Farquharson *et al*., 2018) and is actively recommended by the IUCN as a conservation action (CBSG, 2017). Despite this, many species face difficulties in achieving successful reproduction in captivity.

While factors such as the age and health status of captive individuals may influence productivity, the captive environment itself can also affect reproductive success. Hormonal fluctuations observed between captive and wild individuals are associated with captive breeding success (Dickens and Bentley, 2014; Jensen *et al*., 2019) and in a variety of species, wild-born individuals exhibit higher productivity levels than those born in captivity (Farquharson *et al*., 2018). Enclosure size (Ali, 2016) and design (Flanagan *et al*., 2020; Mirande *et al*., 1997) and the ability of an individual to choose their breeding partner (Asa *et al*., 2011; Massa *et al*., 1996) are ways in which the captive environment can influence reproductive success. Inadequate husbandry can also contribute to low rates of captive productivity (Ralls and Ballou, 2013; Snyder *et al*., 1996); this may be a consequence of poor species-specific knowledge or an inability to adequately recreate natural habitats or diets (van Heezik *et al*., 2005). In the case of *A. jacutinga*, a lack of comprehensive species knowledge is a barrier to understanding the low levels of reproductive success observed. This limited understanding also poses a potential obstacle to providing appropriate management techniques and establishing conditions that may result in higher levels of reproductive success.

To achieve the number of individuals required for successful reintroduction and recovery of the species as outlined in the PVA (Phalan *et al*., 2020), captive breeding centres must increase the number of viable offspring produced each breeding season. It is increasingly apparent that behavioural studies can offer invaluable insights that can aid conservation efforts and enhance levels of ex-situ reproductive success (Greggor *et al*., 2016; Swaisgood, 2016). Given the limited study and scarce literature on this species, further behavioural research into *A. jacutinga* is crucial for developing a deeper understanding of the reproductive behaviours and mating systems within this species to inform captive breeding centres, subsequently enhancing their productivity and the overall success of the reintroduction programme.

2 Objectives

The primary goal of this project is to enhance the general understanding of the individual and social behaviours exhibited by a captive population of *A. jacutinga* throughout their breeding season. This research aims to provide valuable information to support managers of captive breeding facilities in implementing species-specific husbandry practices. This, in turn, is expected to improve the reproductive success of captive populations and contribute to achieving the goals and targets outlined by Project Jacutinga.

More precisely, this project aims to accomplish the following:

1) Identify social behaviours within this captive population that are associated with successful reproduction.
2) Determine whether the presence or absence of such behaviours in the initial 6 days after introducing breeding pairs can serve as an indicator of their likelihood of reproductive success.
3) Investigate whether behaviours linked to reproductive success increase in frequency during the 6-day egg laying period.
4) Explore the effects of secondary variables, such as enclosure size and design, on reproductive success.

3 Methodology

3.1 *Experimental Design*

The project was conducted at SERCAS during the breeding season of *A. jacutinga*. Behavioural observations commenced in August 2022 and terminated in February 2023. The project utilised 8 of the 20 enclosures at the breeding facility (4x 8m^2 and 4x 16m^2) (Figure 3). 8 females and 4 males were selected from the captive population to take part in the study (Table 1). Breeding pairs were arranged to mitigate the potential negative impacts of inbreeding and genetic drift.

Figure 3. One of the large enclosures at SERCAS used to house *A. jacutinga* throughout this study with dimensions shown in metres. Constructing a partition wall in the middle of a large enclosure divides it into 2 small enclosures.

The project comprised 2 distinct stages, each containing 2 periods of 35 days (Table 2), during which breeding pairs were introduced and their behaviours observed. Stage 1 of the project utilised the large, naturalistic enclosures and stage 2 utilised the smaller, non-naturalistic enclosures. In both stages, females were assigned to an enclosure, remaining there for the entire reproductive season, while males were introduced to and housed with them for the 35-day observational period.

Table 1. Details of the sex, date of birth and origin of the captive individuals housed at SERCAS involved in this project. M= male, F = female.

Jacutinga ID	Sex	Date of birth	Origin
J022	M	08/10/2016	Criadouro COMFAUNA
J043	F	20/06/2016	Criadouro COMFAUNA
J080P	M	26/09/2013	São Francisco Xavier
J080L	F	29/09/2012	São Francisco Xavier
J081P	M	30/07/2014	TROPICUS
J082P	M	15/12/2014	TROPICUS
J083P	F	12/10/2016	Criadouro COMFAUNA
J083L	F	28/01/2016	Criadouro COMFAUNA
J084	F	29/09/2014	TROPICUS
J082	F	17/12/2012	TROPICUS
J002	F	01/12/2017	SERCAS
NVFem	F	Unknown	PETRÓPOLIS

To investigate the relationship between social behaviours and reproductive success, observing both successful and unsuccessful reproduction was necessary. Data from previous breeding seasons at SERCAS show patterns of reproductive success among certain breeding pairs. It was hypothesised that this pattern would persist, enabling the comparison of behaviours and social interactions in both successful and unsuccessful breeding pairs, which served as precedent for the experimental design of this project.

In stage 1a, the 4 females were separated into two groups: group A and group B. Group A females were paired with males with whom they had previously achieved reproductive success. In contrast, group B females were paired with males with whom they had not experienced successful reproduction. These pairs remained in the same enclosure for 35 days, during which behavioural observations were conducted. Subsequently, the males were removed, and the females were given a rest period of 7 days before being introduced to their next breeding partner. During stage 1b, the second 35-day period, group A females were paired with a male with whom they had yet to achieve reproductive success. Group B females were paired with a male they had previously reproduced with successfully. In stage 2b, only 1 female (J082) had a history of previous reproductive success and was consequently paired with her previously successful partner (J022). The remaining males were assigned randomly to the enclosures with other females throughout stage 2 of the project.

Table 2. The experimental design of the project showing the division of male and female *A. jacutinga* into different breeding pairs and enclosures for observations. Enclosures housing breeding pairs with previous reproductive success are marked with an asterisk. Date = The date a breeding pair were first introduced.

Stage 1a				**Stage 1b**			
Date	**Enclosure**	**Female**	**Male**	**Date**	**Enclosure**	**Female**	**Male**
01/08	2-3*	J083P	J081P	07/09	2-3	J083P	J022
04/08	4-5	J080L	J080P	09/09	4-5*	J080L	J082P
08/08	14-15*	J083L	J022	09/09	14-15	J083L	J080P
11/08	12-13	J043	J082P	01/09	12-13*	J043	J081P
Stage 2a				**Stage 2b**			
Date	**Enclosure**	**Female**	**Male**	**Date**	**Enclosure**	**Female**	**Male**
10/11	6	J084	J022	27/12	6	J084	J080P
14/11	20	NVFem	J080	30/01	7	NVFem	J081P
17/11	16	J002	J081P	04/01	16	J002	J082P
21/11	7	J082	J082P	04/01	20*	J082	J022

Removal of the male from the female's enclosure occurred under one of three conditions. Firstly, if the 35-day observational period concluded without the female laying eggs, the male was removed to prepare for the next stage of observations. Secondly, if the female had laid three eggs, which is the typical clutch size in this captive population, the male was removed to alleviate stress on the female and prevent potential damage to the eggs. Finally, severe or prolonged aggression exhibited by either individual toward the other resulted in the male being removed from the enclosure to ensure the health and safety of both members of the breeding pair. In previous breeding seasons at SERCAS, male resource guarding has hindered female foraging, and excessive pecking of a female's cloaca by a male resulted in fatal injuries.

Data on the occurrence and frequency of behaviours were analysed alongside the fertility rates of laid eggs to determine relationships between certain behaviours and reproductive success. In addition, historical data were analysed to explore the relationship between confounding variables, such as enclosure size and style, and reproductive success.

3.2 *Egg Incubation and Fertility*

Throughout this project, the aim was for eggs to remain in the nest of the enclosure in which they were laid, with incubation and rearing by the female. However, some females spent extended periods away from the nest. In such instances, eggs were removed and kept in an incubator (Premium Ecológica, Chocadeira, IP130) at 38°C and 65% humidity. Egg fertility was assessed by candling with a cold light ovoscope (Premium Ecológica, Ovoscópio P, 030801).

3.3 *Behavioural Observations*

Behavioural observations were conducted using a focal sampling method and an ethogram explicitly designed for this project (Appendix A: Table 4-7). This ethogram was adapted from Rivera (2016) and Robbi (2020) and incorporated information on the reproductive behaviours of *A. jacutinga* described in the literature (del Hoyo *et al.,* 2020b; de Souza *et al.*, 2020). Each enclosure was observed for 60 minutes, with 30 minutes focusing on each individual in the breeding pair. Observations were divided equally between morning (07:00-12:00) and afternoon (12:00-17:00) sessions, with details recorded on paper and later uploaded to a digital spreadsheet for further analysis. Observations were divided into 3 phases:

- Phase 1: Individuals were observed for 1 hour per day (30 minutes each in the morning and afternoon) for the first 6 days of the observational period. This was undertaken to ascertain whether the behaviours and interactions exhibited by individuals in a breeding pair could indicate the likelihood of future reproductive success in that pair.

- Phase 2: After the first 6 days, individuals were observed for 4 hours per week, or 8 hours per week for each breeding pair. This continued until the end of the 35-day period or until an egg was produced.
- Phase 3: If the female produced an egg, observations returned to the previous frequency of 1 hour per day per individual. This took place from the day the first egg in the clutch was discovered for 6 days until the third and final egg was laid.

3.4 *Data Analysis*

Analysis of data and creation of graphs was carried out using R Studio statistical software (v4.3.1; R Core Team, 2023). Due to a combination of low sample size and skewed data, as identified by Shapiro-Wilk tests, non-parametric tests were utilised throughout data analysis. Analysis of Similarities (ANOSIM) was selected as a method of multivariate non-parametric analysis and calculated using the vegan R package (v2.6.4; Oksanen *et al*., 2022). Bivariate analysis was conducted with Wilcoxon signed-rank, Wilcoxon rank-sum, Pearson's chi-squared and Fisher's exact tests where appropriate ($p<0.05$).

3.4.1 *Behavioural Analysis*

Due to variations in the number of observed hours between enclosures, attributable to the absence of egg-laying in some breeding pairs, average values of the count data of behaviours were calculated per hour observed. Behavioural data were separated for analysis into event and state behaviours, the former including social interactions, individual behaviours and vocalisations. Results have been divided considering the objectives of this project; data from phases 1-3 analysed the overall changes in behaviour throughout the study, phase 1 was analysed individually to determine if behaviours in the first week of observation are indicative of future reproductive success and data collected during phases 2 and 3 were analysed to identify any differences in the frequency of behaviours before and after the egg laying process began.

ANOSIM was calculated to determine similarities in the frequency of behaviours between pairs which did or did not produce fertile eggs. Wilcoxon rank-sum tests were performed to further analyse each behaviour individually and determine whether its frequency differed significantly between pairs that did or did not produce fertile eggs.

Wilcoxon signed-rank tests were used to determine whether the frequency of behaviours differed before and after the first egg was laid.

To create activity budgets, behaviours were grouped and analysed using a Wilcoxon rank-sum test; foraging at and away from the feeder were grouped into 'foraging', locomotive states of walking, running and flying were grouped into 'locomotion' and vigilant and resting vigilant were combined into 'active'. Prior to grouping, behaviours were also analysed using Wilcoxon rank-sum to explore relationships between time spent in state behaviours and reproductive success.

Data from enclosure 12-13 in stage 1a of observations were incomplete as the male was removed from the enclosure after exhibiting elevated levels of resource guarding and aggression directed towards the female. Data for enclosure 12-13(a) has been excluded from the analyses.

3.4.2 *Enclosure Style*

To assess the potential influence of the captive environment on reproductive success, the frequency of behaviours was tested against the style of enclosure in which breeding pairs were housed. Enclosures have been categorised as naturalistic or non-naturalistic, with naturalistic enclosures being larger, having an open-style roof, and containing more vegetation compared to non-naturalistic enclosures, which were smaller and had a covered roof and limited vegetation. Wilcoxon rank-sum tests were used to determine differences in the frequency of behaviours in naturalistic and non-naturalistic enclosures.

3.4.3 *Egg Fertility*

Historical data from eggs laid in the observed breeding season (2022-2023) was combined with historical data (2017-2022) to analyse trends in egg status (broken, fertile or infertile). Pearson's chi-squared and Fisher's exact tests were used where appropriate to analyse egg status alongside enclosure size (large or small), roof type (open or covered), breeding pair and both the breeding season and month in which the first egg in a clutch was laid.

The decision to analyse enclosure size and roof style separately in the historical data analysis, while testing against naturalistic or non-naturalistic enclosures in the data collected for this study, was made due to a historical lack of uniformity in enclosure style. In previous years, some larger enclosures featured open roofs while small enclosures were covered. Construction work has since been completed to leave the enclosures as they are in the current study.

151 eggs have been laid at SERCAS since the breeding programme began in 2017. However, some of these eggs were laid by females who were not in contact with a male, and therefore, it is not possible that they were fertile. These eggs and their data have been removed from the analysis, resulting in 131 eggs being included in the final analysis.

4 Results

In the observed breeding season, eggs were laid in 4 enclosures (Table 3) and of these enclosures, 3 produced fertile eggs. Outside of the project, a further 6 eggs were laid and have been included in analysis of egg hatches, fertility rates and confounding variables. Data detailing count data of behaviours can be seen in Appendix B (Table 8-10).

Table 3. Results detailing the number of eggs laid per breeding pair, the fertility rate of the clutch. Days = the duration between the introduction of a breeding pair and the day the first egg of a clutch was laid.

Enclosure	Male ID	Female ID	Eggs	Fertile (%)	Days
2-3a	J081P	J083P	3	33.33	27
4-5b	J082P	J080L	3	33.33	20
14-15a	J022	J083L	3	66.66	20
14-15b	J080P	J083L	3	0	23

4.1 *Behaviours Observed Throughout the Study (Phases 1-3)*

4.1.1 *Social Interactions*

Results of the multivariate analysis showed no significant difference in the frequency of social interactions between enclosures which did or did not produce fertile eggs (ANOSIM, $p = .21$)

Behaviours associated with successful reproduction were identified using Wilcoxon rank-sum tests (Figure 4). In enclosures in which fertile eggs were produced, *male offers item to female* ($p = .01$, $r = 0.68$), *female approaches male* ($p = .03$, $r = 0.6$), and *male mounts female* ($p = .02$, $r = 0.63$) were more frequently observed than in enclosures in which fertile eggs were not produced.

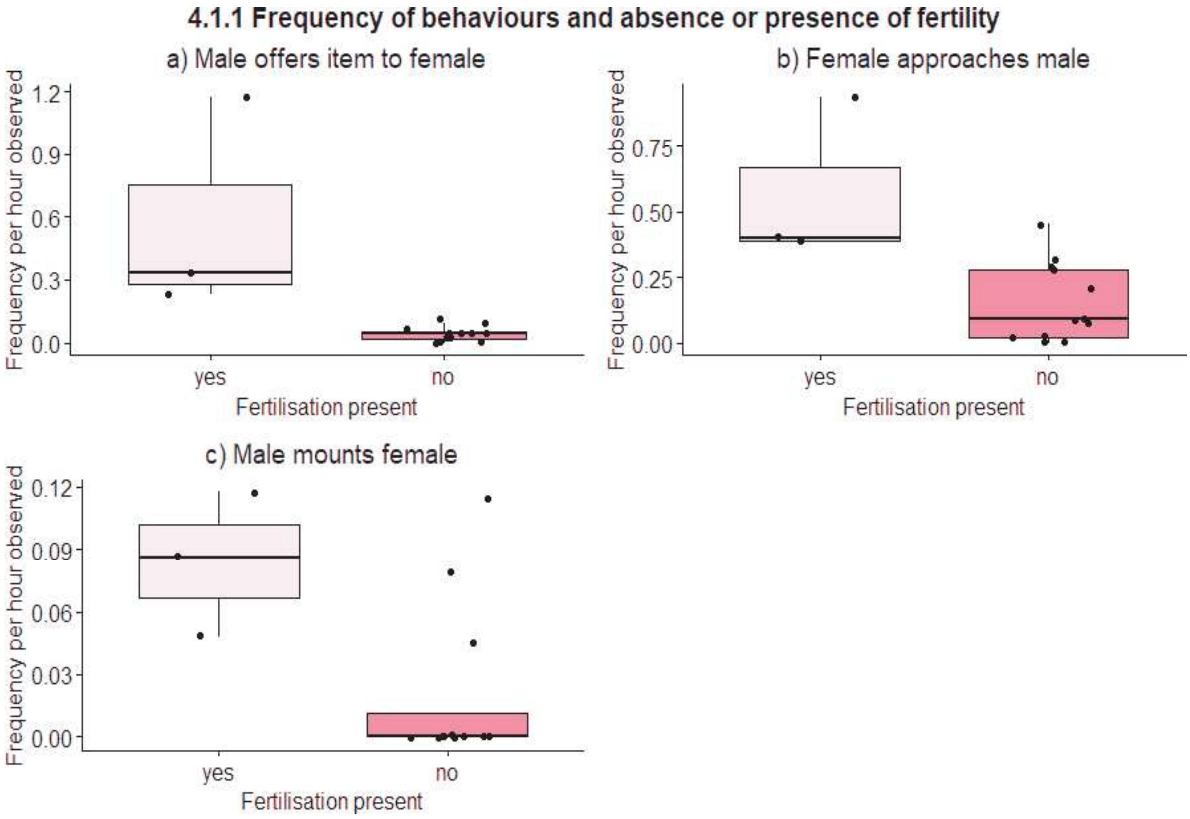

Figure 4. Boxplots detailing the frequencies of social interactions in enclosures during all phases of the observational period. Points detail the frequency of occurrences for each breeding pair.

4.1.2 *Individual Behaviours*

Results of the multivariate analysis showed no significant difference in the frequency of individual behaviours between enclosures which did or did not produce fertile eggs (ANOSIM, p = .21).

Behaviours associated with successful reproduction were identified using Wilcoxon rank-sum tests (Figure 5). *Nodding* (♂: p = .02, *r* = 0.6; ♀: p = .049, *r* = 0.53), *descending whistle (♂)* (p = .04, *r* = 0.56) and *nodding vocalisation (♂)* (p = .01, *r* = 0.68) were more frequent in enclosures in which fertile eggs were laid than in enclosures which did not produce fertile eggs.

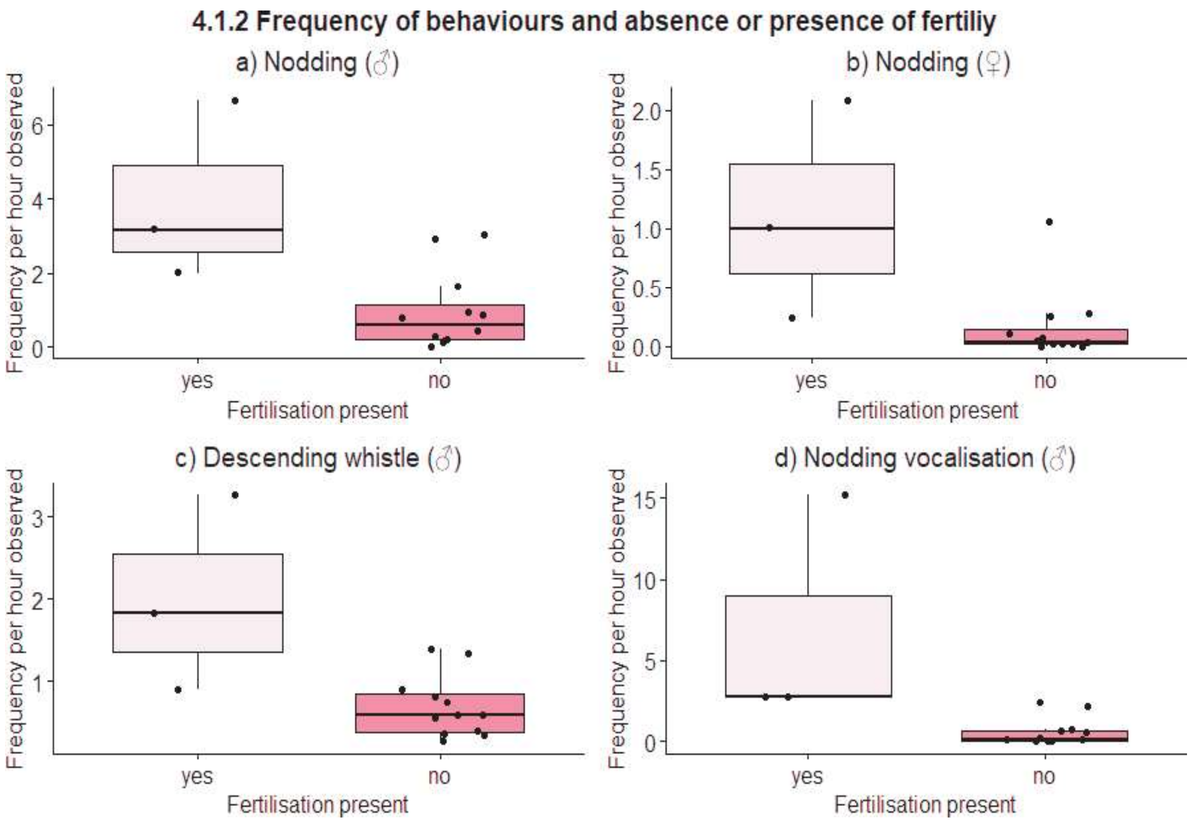

Figure 5. Boxplots detailing the frequencies of individual behaviours in enclosures during all phases of the observational period. Points detail the frequency of occurrences for each breeding pair.

4.1.3 *Activity Budgets*

Behaviours associated with reproductive success were identified using Wilcoxon rank-sum tests. In enclosures in which fertile eggs were produced, females spent less time in the *active* state (p = .03, r = 0.56) and more time *foraging* (p = .02, r = 0.6) than those in enclosures which did not produce fertile eggs (Figure 6). Male activity did not differ significantly between those enclosures which did or did not produce fertile eggs.

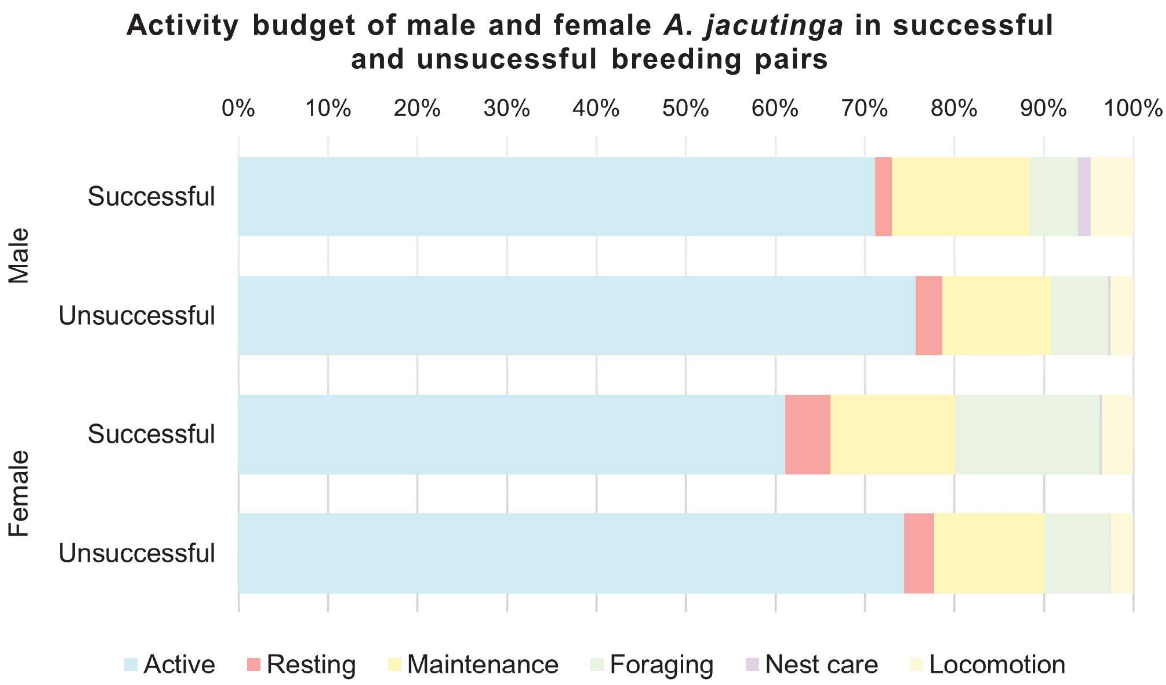

Figure 6. Time activity budget of male and female *A. jacutinga* in enclosures in successful and unsuccessful breeding pairs.

More precisely, in enclosures in which fertile eggs were present, less time was spent in *resting vigilance* (♂: p = .004, *r* = 0.67; ♀: p = .03, *r* = 0.56), and increased time in *vigilance* (♂: p = .009, *r* = 0.63; ♀: p = .031, *r* = 0.56), *foraging away from the feeder* (♂: p = .004, *r* = 0.67; ♀: p = .031, *r* = 0.56), *running* (♂: p = .011, *r* = 0.67; ♀: p = .011, *r* = 0.67) and *flying (♂)* (p = .031, *r* = 0.56) than in enclosures with an absence of fertile eggs (Figure 7-8).

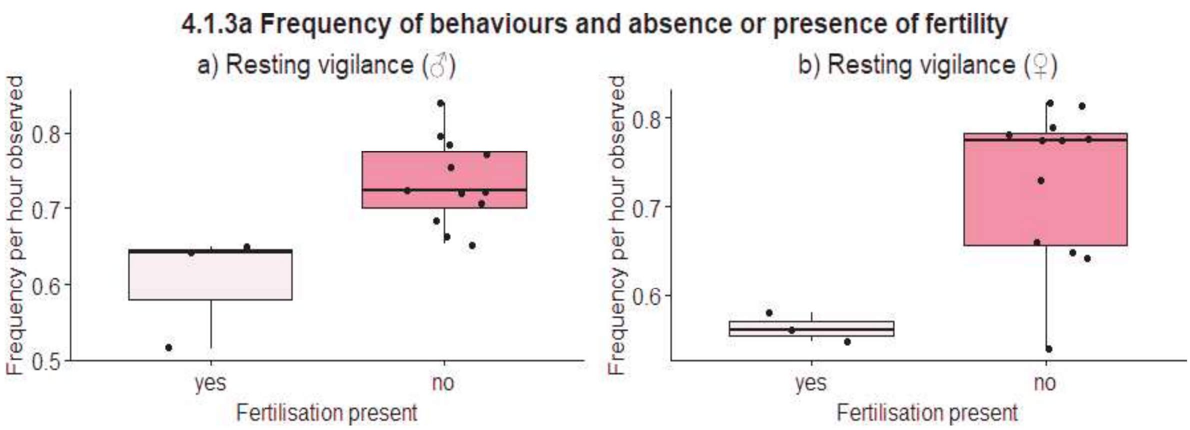

Figure 7. Boxplots detailing the frequencies of state behaviours in enclosures which did and did not lay fertile eggs during all phases of the observational period. Points detail the frequency of occurrences for each breeding pair.

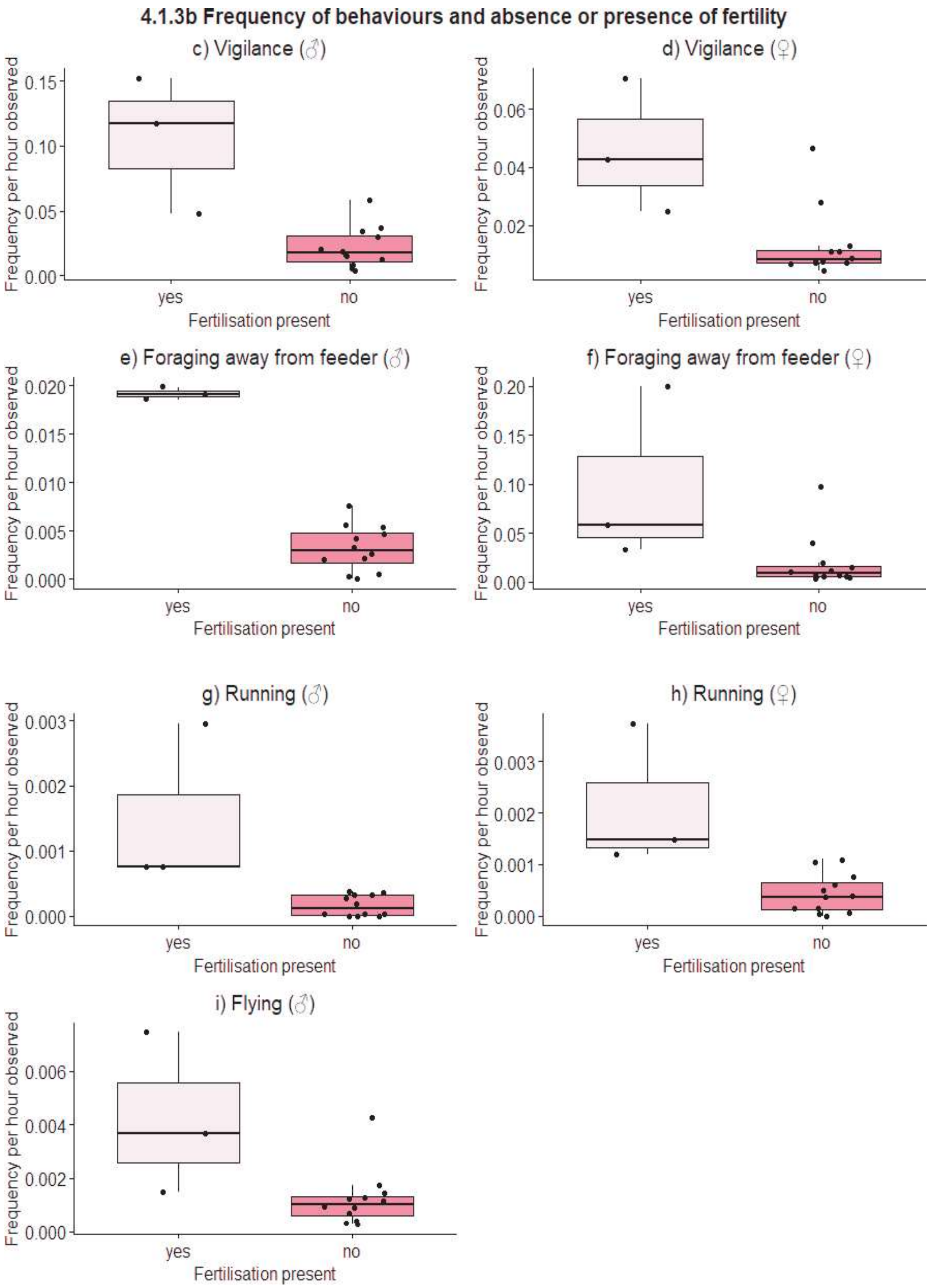

Figure 8. Boxplots detailing the frequencies of social interactions in enclosures which did and did not lay fertile eggs during all phases of the observational period. Points detail the frequency of occurrences for each breeding pair.

***4.2** Behaviours Observed in the First 6 Days of the Study (Phase 1)*

***4.2.1** Social Interactions*

Results of the multivariate analysis showed a significant difference in the frequency of social interactions observed between enclosures in which fertile eggs were or were not produced (ANOSIM, p = .01, *r* = 0.32).

Behaviours associated with successful reproduction were identified using Wilcoxon rank-sum tests (Figure 9). *Female offers item to male* (p = .03, *r* = 0.59), *female approaches male* (p = .04, *r* = 0.55) and *male chases female* (p = .002, *r* = .84) were more frequently observed in enclosures in which fertile eggs were produced than in those with an absence of fertile eggs.

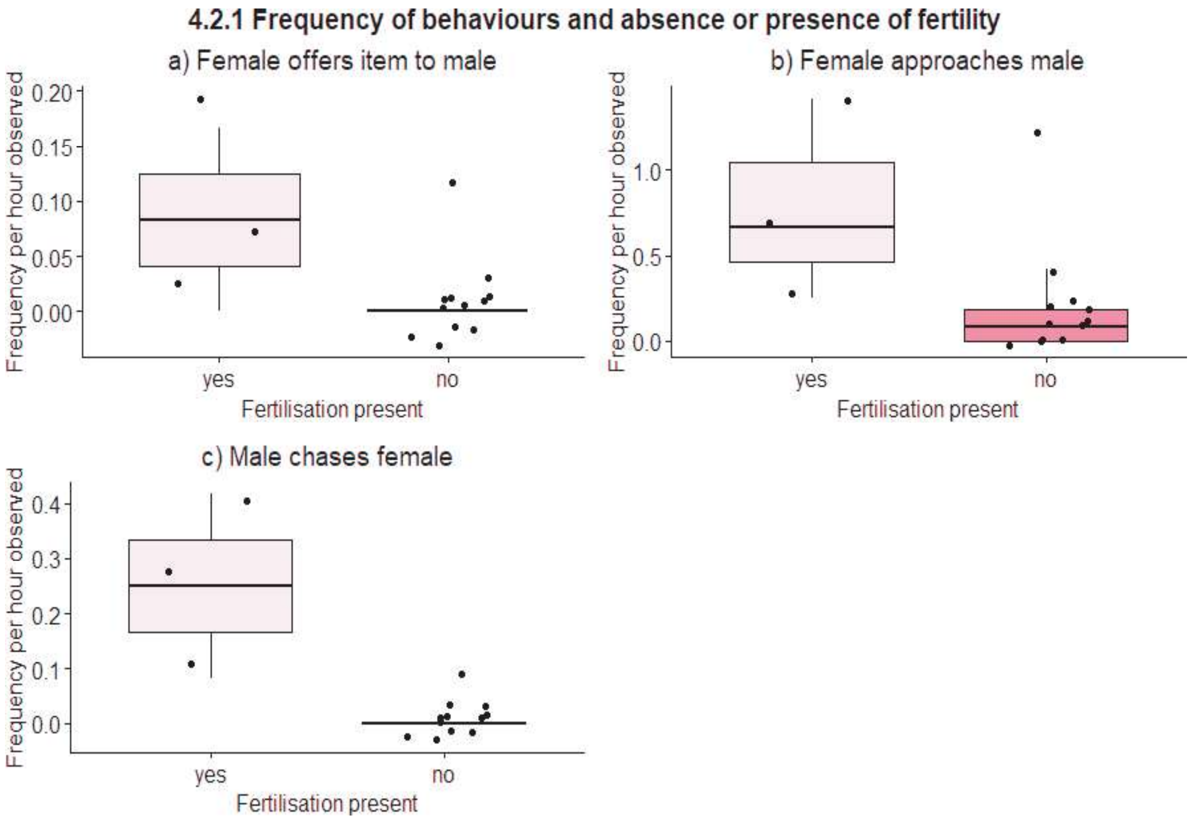

Figure 9. Boxplots detailing the frequencies of social interactions during the first six days of observations in enclosures which did and did not produce fertile eggs. Points detail the number of occurrences for each breeding pair.

4.2.2 Individual Behaviours

Results of the multivariate analysis showed no significant difference in the frequency of individual behaviours between enclosures in which fertile eggs were or were not produced (ANOSIM, p = .56).

Behaviours associated with successful reproduction were identified using Wilcoxon rank-sum tests (Figure 10). In enclosures in which fertile eggs were produced, *wing display (♂)* (p = .005, *r* = 0.76), *nodding (♂)* (p = .02, *r* = 0.64) *nodding vocalisation (♀)* (p = .02, *r* = 0.61) and *warble (♀)* (p = .04, *r* = 0.56) were more frequently observed than in enclosures in which no fertile eggs were laid.

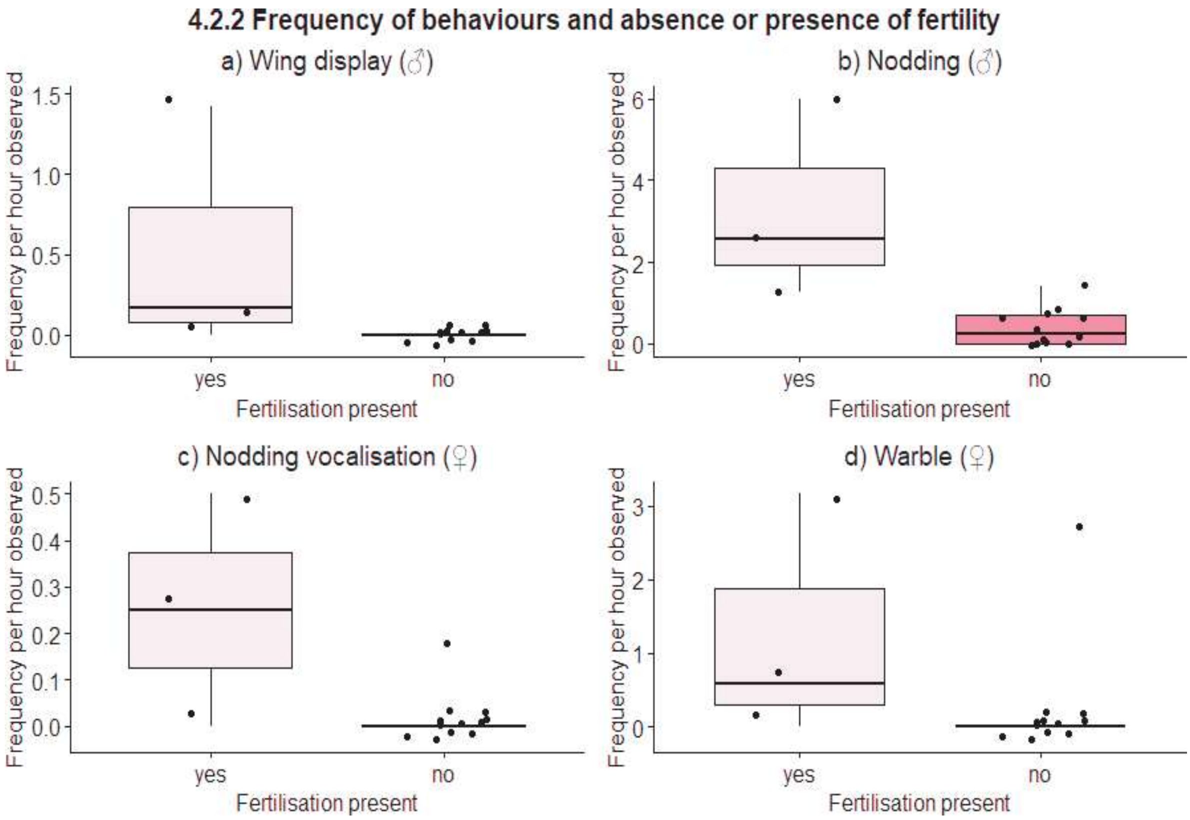

Figure 10. Boxplots detailing the frequencies of individual behaviours during the first six days of observations in enclosures which did and did not produce fertile eggs. Points detail the number of occurrences for each breeding pair.

4.2.3 Activity Budgets

Behaviours associated with successful reproduction were identified using Wilcoxon rank-sum tests. In the first six days after introducing a breeding pair, females in enclosures which went on to produce fertile eggs engaged in *nest care* more than females in enclosures which did not produce fertile eggs (p = .018, *r* = 0.63) (Figure 11). Male behaviours did not differ between enclosures which did and did not reproduce successfully.

More precisely, in enclosures in which fertile eggs were produced, males spent less time in *resting vigilance* (p = .009, *r* = 0.63) and engaged more in *vigilance* (p = .018, *r* = 0.6) and *flying* (p = .036, *r* = 0.56) than in enclosures in which fertile eggs were not produced. Female *nest care* (p = .018, *r* = 0.631) was significantly higher in enclosures in which fertile eggs were produced (Figure 12).

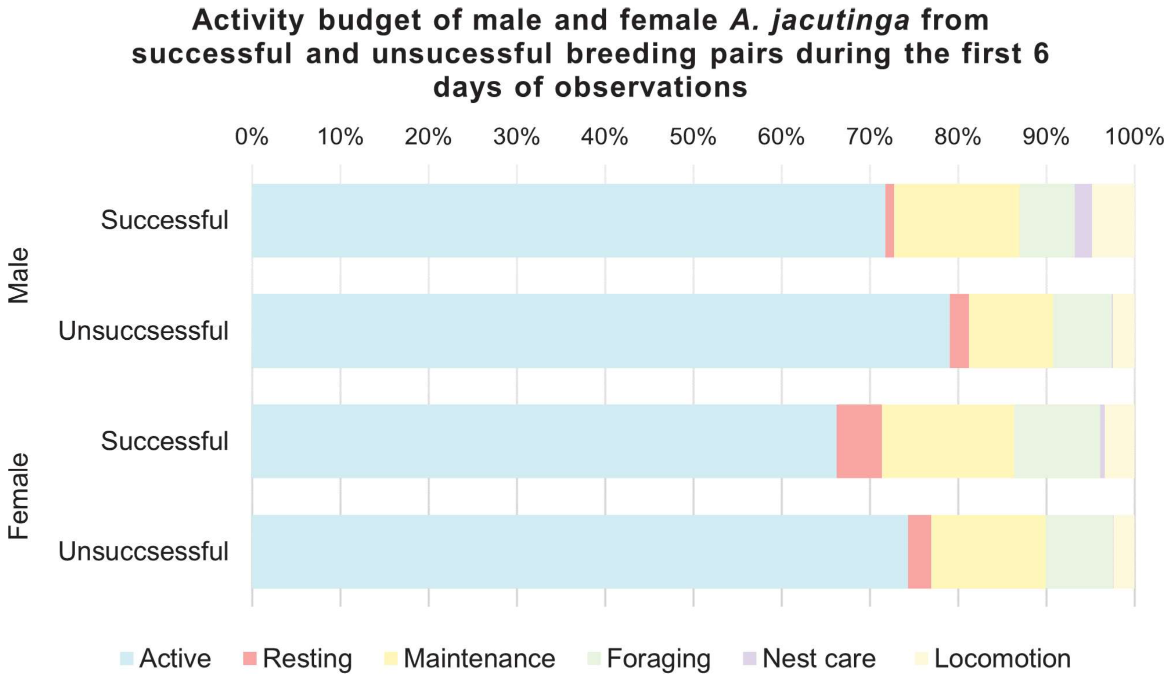

Figure 11. Time activity budget of male and female *A. jacutinga* in the first 6 days of the observational period who were successful or unsuccessful in reproduction.

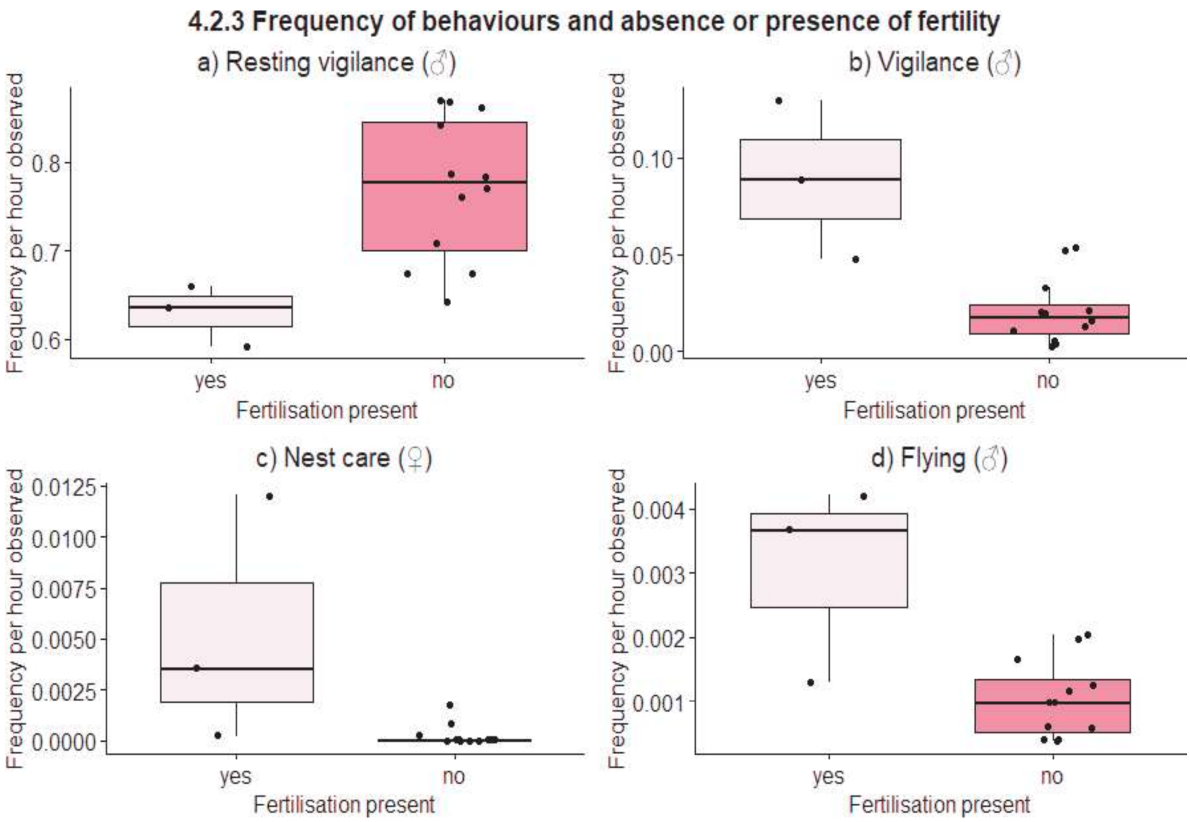

Figure 12. Boxplots detailing the frequencies of state behaviours during the first six days of observations in enclosures which did and did not lay fertile eggs. Points detail the number of occurrences for each breeding pair.

4.3 *Behaviours of Successful Breeding Pairs*

Differences in the frequencies of behaviours before and after egg laying were analysed using Wilcoxon signed-rank tests. No significant difference was identified in the frequency of social interactions, individual behaviours, or state behaviours before and after egg laying (Appendix C: Table 11-22),

4.4 *Enclosure Style (Phases 1-3)*

4.4.1 *Social Interactions*

Results of the multivariate analysis including data from phases 1, 2 and 3 showed a significant difference in social interactions of individuals housed enclosures of different styles (ANOSIM, p = .01, *r* = 0.2).

Associations between enclosure style and frequency of behaviours were identified using Wilcoxon rank-sum tests. In naturalistic enclosures, *male pecks female* (p = .02, *r* = 0.61), *female approaches male* (p = .03, *r* = 0.59), *male chases female* (p

= .02, *r* = 0.64) and *male mounts female* (p = .002, *r* = .81) were more frequently exhibited than in non-naturalistic enclosures (Figure 13).

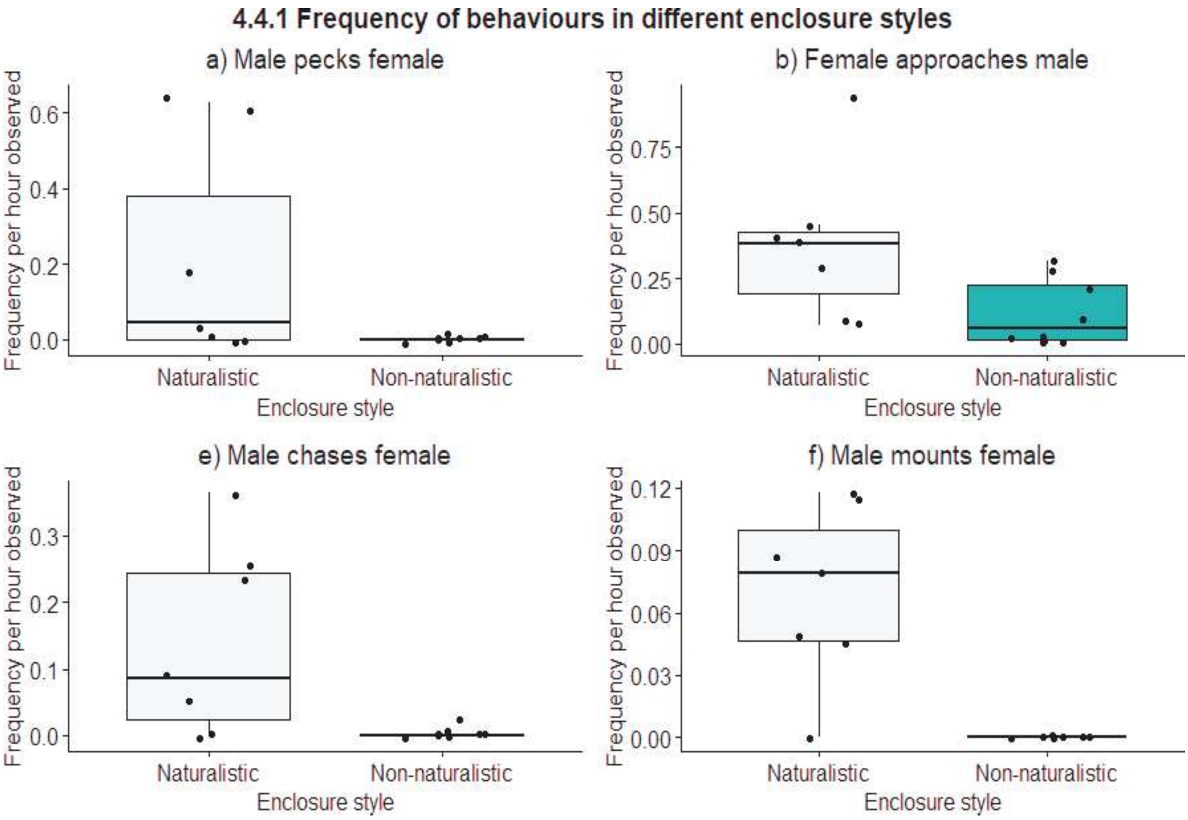

Figure 13. Boxplots detailing the frequencies of social interactions in enclosures of different styles. Points detail the number of occurrences for each breeding pair.

4.4.2 *Individual Behaviours*

Results of the multivariate analysis showed a significant difference in individual behaviours of birds housed enclosures of different styles (ANOSIM, p = .03, *r* = 0.17).

Associations between enclosure style and frequency of behaviours were identified using Wilcoxon rank-sum tests. *Raising crest (♀)* (p = .049, *r* = 0.52), *warning (♀)* (p = .003, *r* = 0.794) and *warning vocalisation (♀)* (p = .01, *r* = 0.67) were significantly more frequent in naturalistic enclosures (Figure 14).

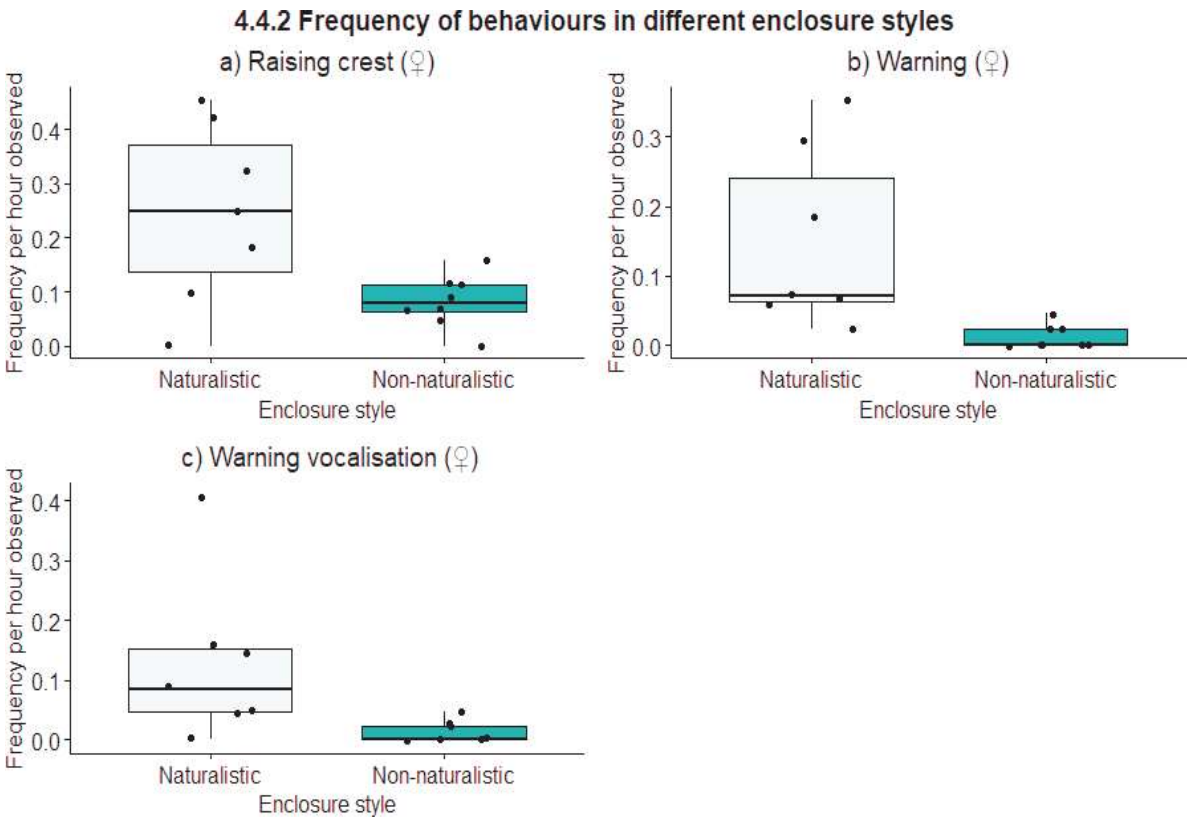

Figure 14. Boxplots detailing the frequencies of individual behaviours in enclosures of different styles. Points detail the number of occurrences for each breeding pair.

***4.4.3** Activity Budgets*

Associations between enclosure style and frequency of behaviours were identified using Wilcoxon rank-sum tests. *Maintenance (♀)* (p = .02, *r* = 0.6) and *nest care (♀)* (p = .02, *r* = 0.63), were more frequently observed in naturalistic enclosures than non-naturalistic enclosures (Figure 15).

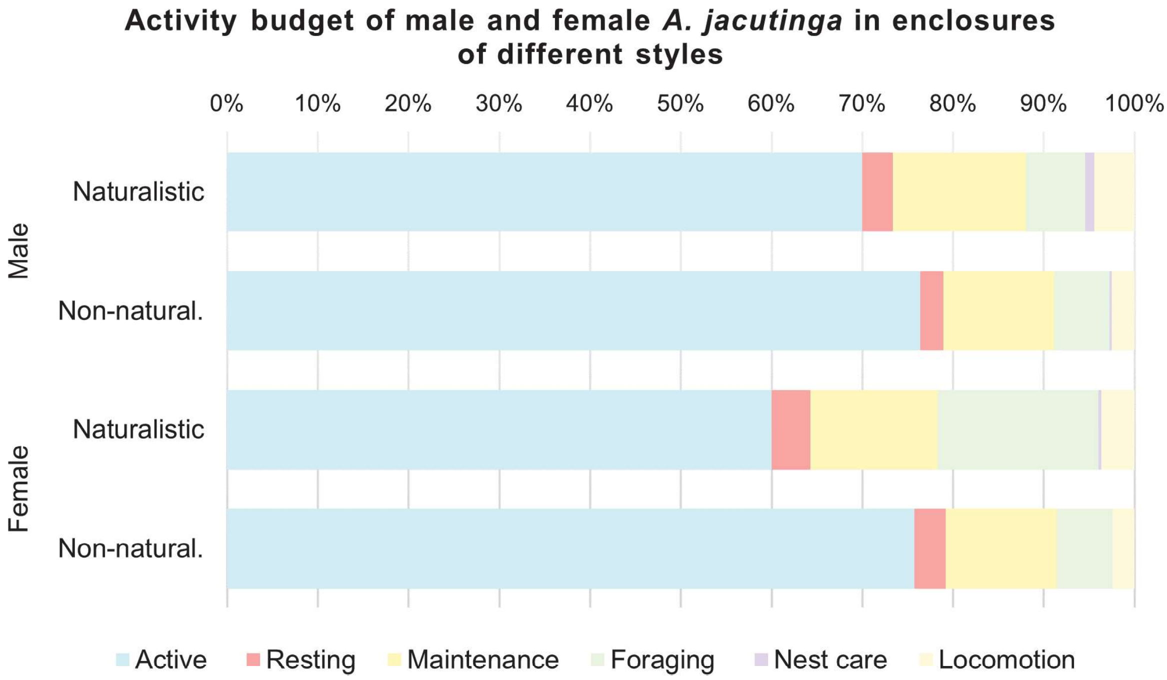

Figure 15. Time activity budget of male and female *A. jacutinga* in naturalistic and non-naturalistic enclosures using data collected throughout the entire observational period.

More precisely, in naturalistic enclosures, *resting vigilance* was exhibited less frequently (♂: $p = .001$, $r = 0.78$; ♀: $p = .0003$, $r = 0.84$) in naturalistic enclosures than non-naturalistic enclosures. *Vigilance* (♂: $p = .04$, $r = 0.53$; ♀: $p = .0003$, $r = 0.83$), *maintenance (♀)* ($p = .02$, $r = 0.6$), *foraging away from the feeder (♂)* ($p = .02$, $r = 0.59$) *nest care (♀)* ($p = .02$, $r = 0.63$) and *running* (♂: $p = .006$, $r = 0.72$; ♀: $p = .006$, $r = 0.72$) were exhibited more frequently in naturalistic enclosures (Figure 16-17).

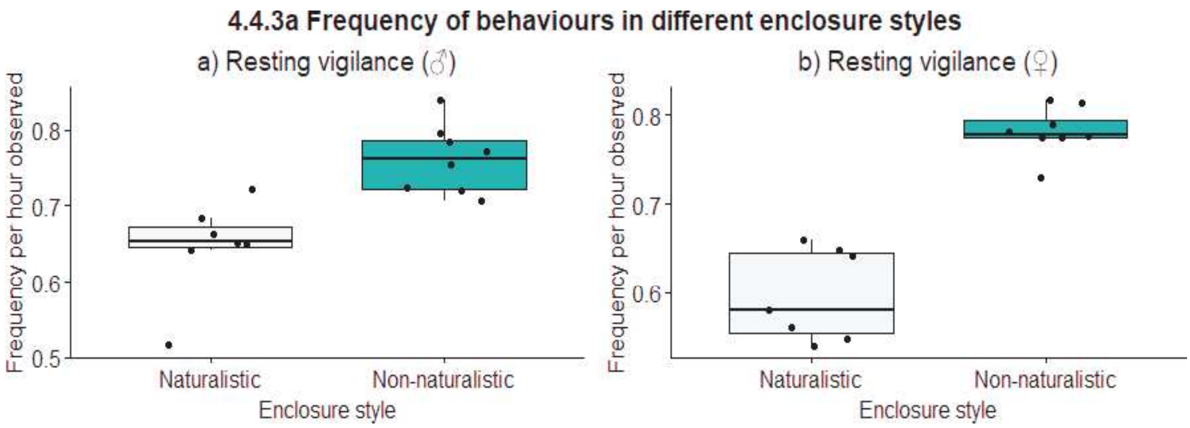

Figure 16. Boxplots detailing the frequencies of state behaviours in enclosures of different styles. Points detail the number of occurrences for each breeding pair observed.

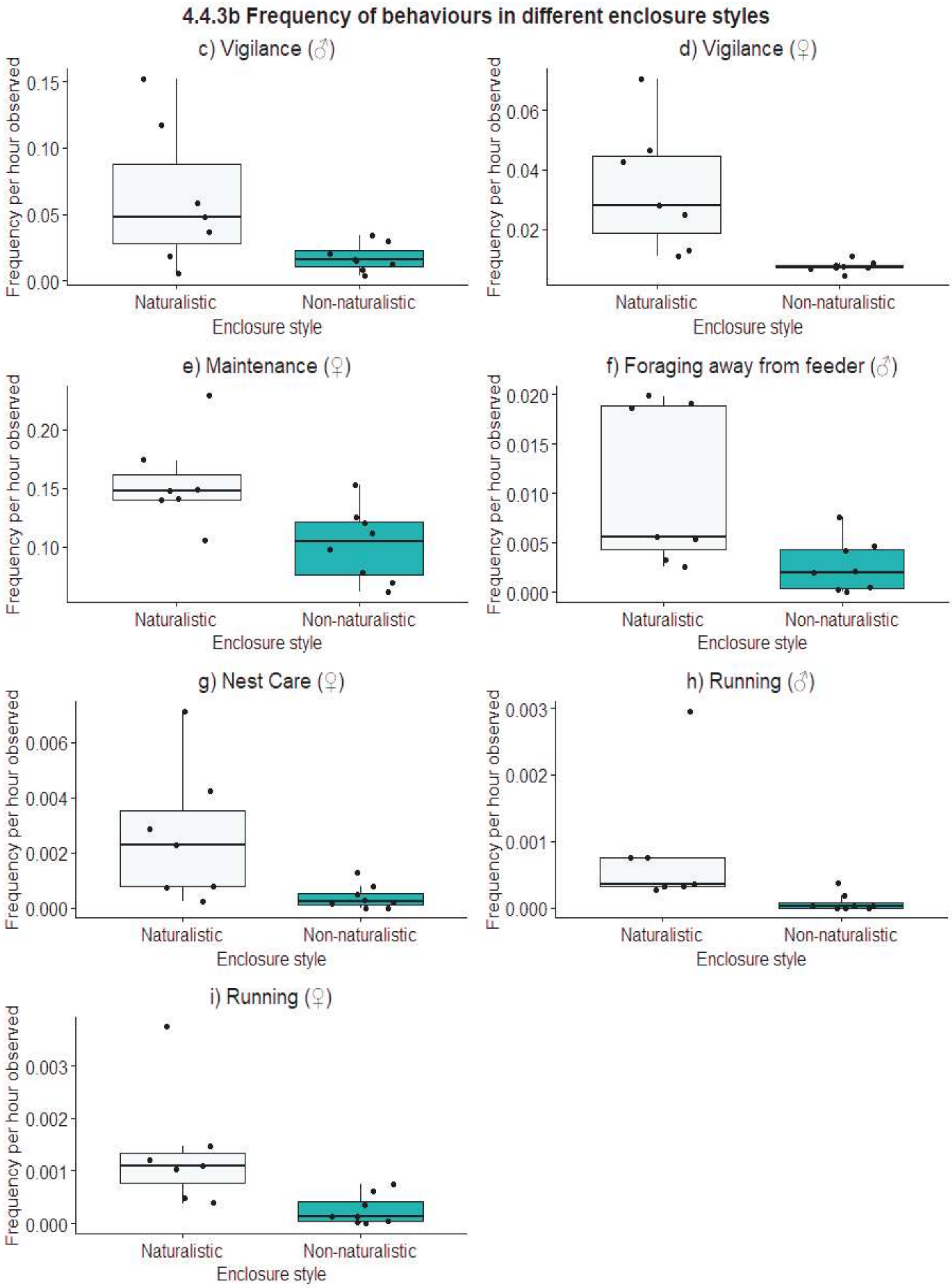

Figure 17. Boxplots detailing the frequencies of state behaviours in enclosures of different styles. Points detail the number of occurrences for each breeding pair observed.

***4.5** Confounding Variables*

There was a significant association identified between egg status and enclosure size (Pearson's chi-squared: $X^2(2, N=131) = 15.536$, $p = .0004$). The overall fertility rate of large enclosures (40.74%) was higher than that of small enclosures (10%) (Figure 18), while the overall fertility rate of all eggs laid at the SERCAS facility is 29.01%. The residuals provide further evidence of this; large enclosures were found to have a significantly positive association with fertile eggs, and a negative association with infertile eggs. For small enclosures the reverse is true, as they were found to be positively associated with infertile eggs and negatively with fertile eggs.

Differences in egg status were positively associated with enclosure roof style, (Pearson's chi-squared: $X^2(2, N=131) = 7.936$, $p = .018$). The fertility rate of enclosures with covered roofs was 22.54% and in open-roofed enclosures, 36.67% (Figure 19). Similarly, the infertility rate of enclosures was 67.61% and 43.33% for covered and open-roofed enclosures, respectively. Analysis of residuals shows that open-roofed enclosures have a strong positive association with fertile eggs and a negative association with infertile eggs. Covered enclosures have a strong positive association with infertile eggs and a negative association with fertile eggs.

There is a significant association between breeding season and egg status (Fisher's exact, $p = .002$). The breeding seasons with the highest levels of reproductive success were 2018-2019 and 2021-2022, in which 40% and 34.15% of eggs laid, respectively, were fertile. Breeding seasons 2017-2018 and 2019-2020 experienced the lowest rates of success, with fertility rates of 21.7% and 13.04% respectively (Figure 20).

There is a statistically significant association between egg status and the month in which eggs were laid (Fisher's exact, $p=0.005$), with a fertility rate of 0% in February, March and July (Figure 21). The months with the highest levels of fertility were December (56%) and January (100%).

There is a difference in the status of eggs laid by different breeding pairs (Fisher's exact, $p= 0.0000001$), with the most successful breeding pairs being identified as those with the ID 3B, 3C and 3D (Figure 22). 7 breeding pairs (1A, 2B, 4A, 4C, 4D, 6F and 7F) experienced no reproductive success.

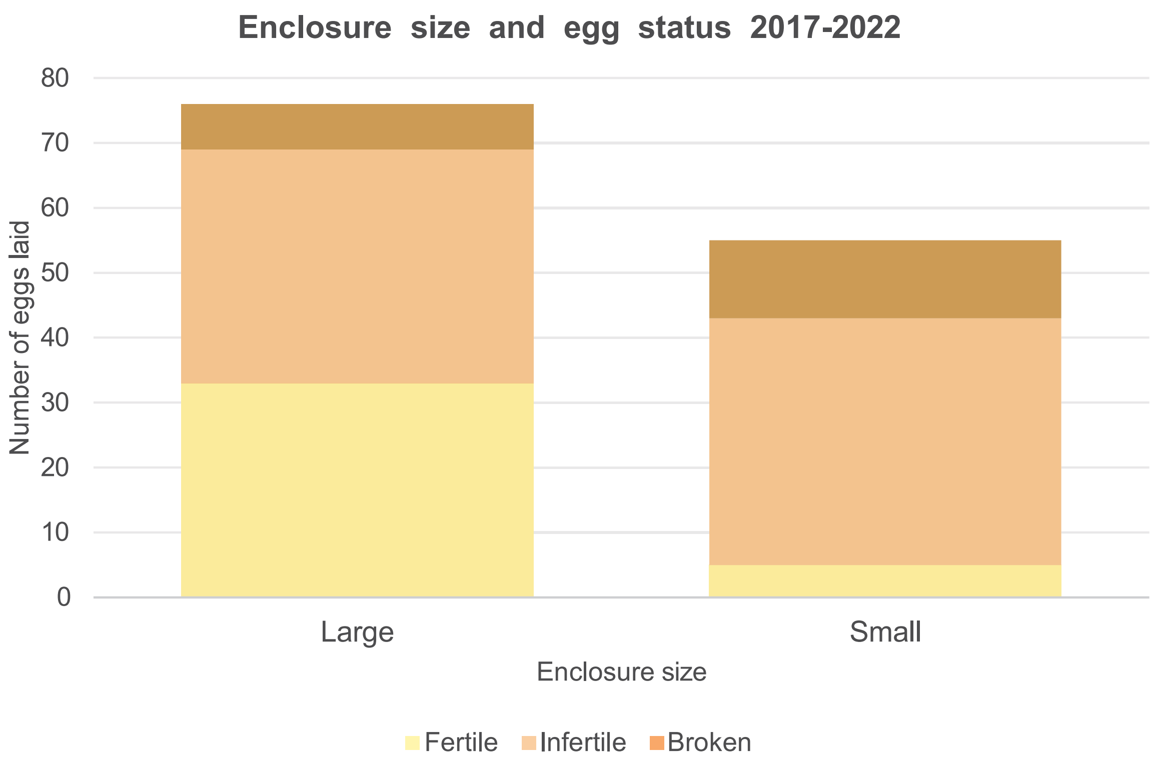

Figure 18. Bar chart detailing the status of eggs (fertile, infertile or broken) laid at SERCAS in large and small enclosures.

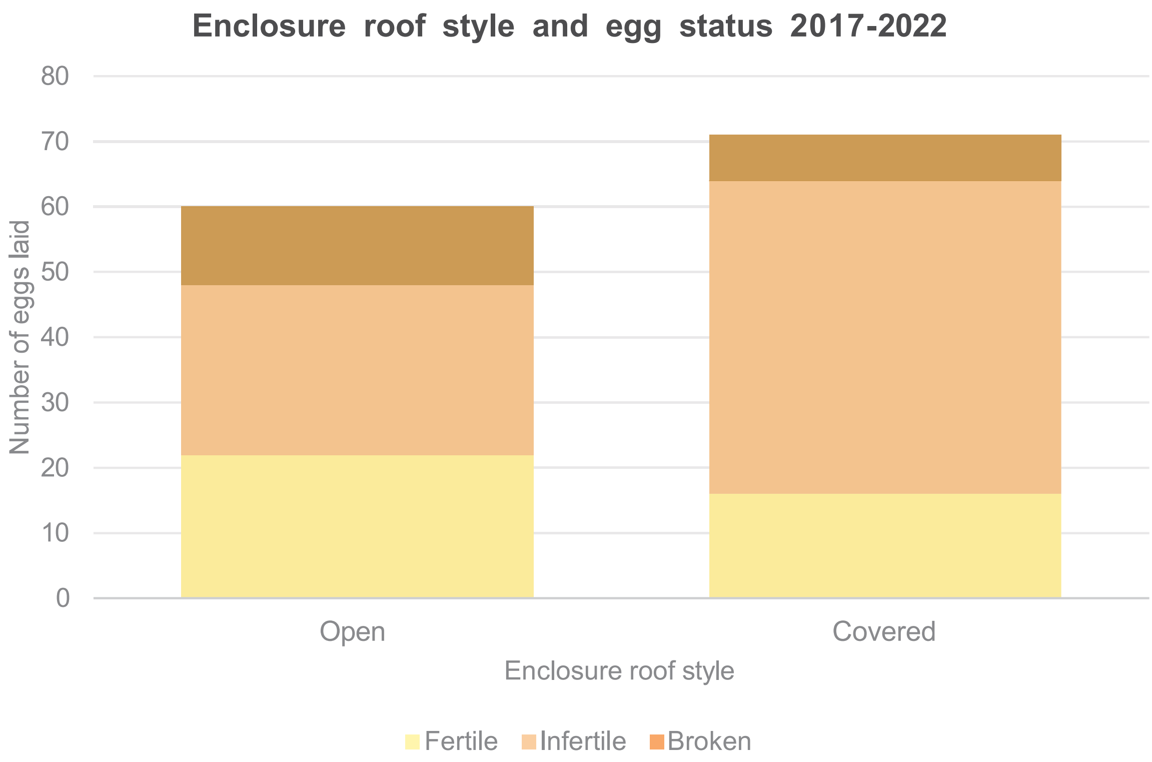

Figure 19. Bar chart detailing the status of eggs (fertile, infertile or broken) laid at SERCAS in enclosures with open and covered roofs.

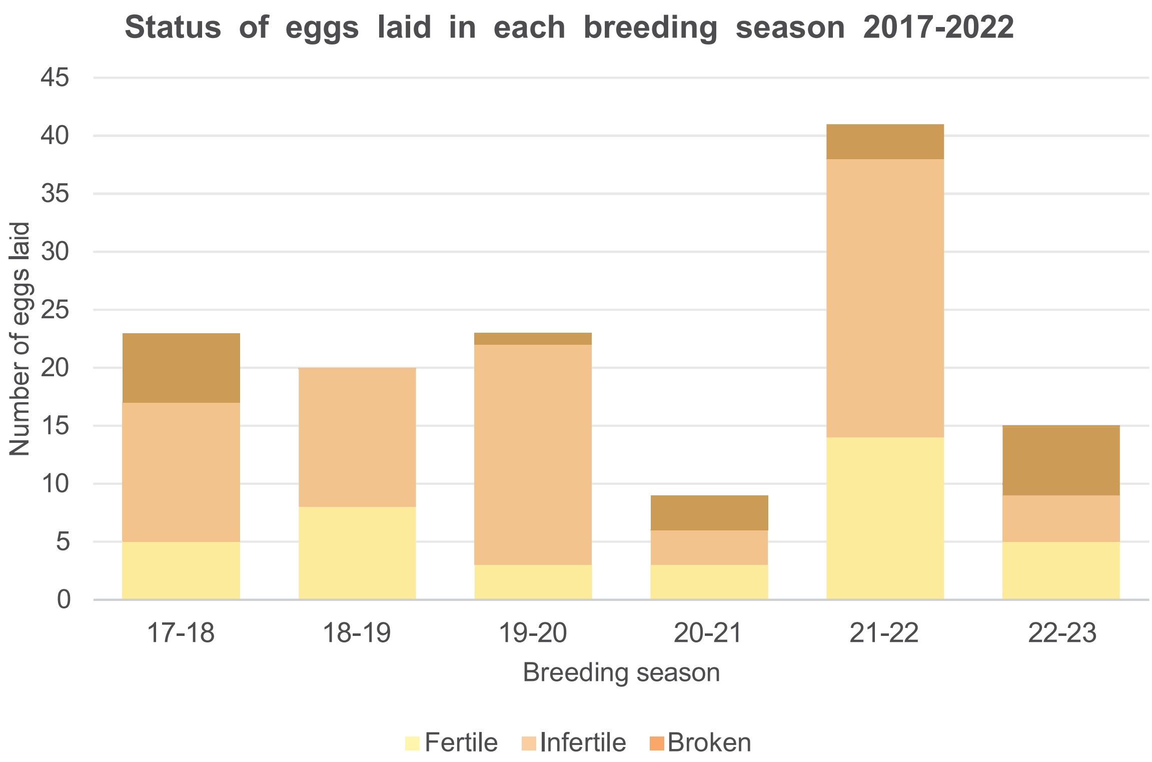

Figure 20. Bar chart detailing the status of eggs (fertile, infertile or broken) throughout each breeding season at SERCAS.

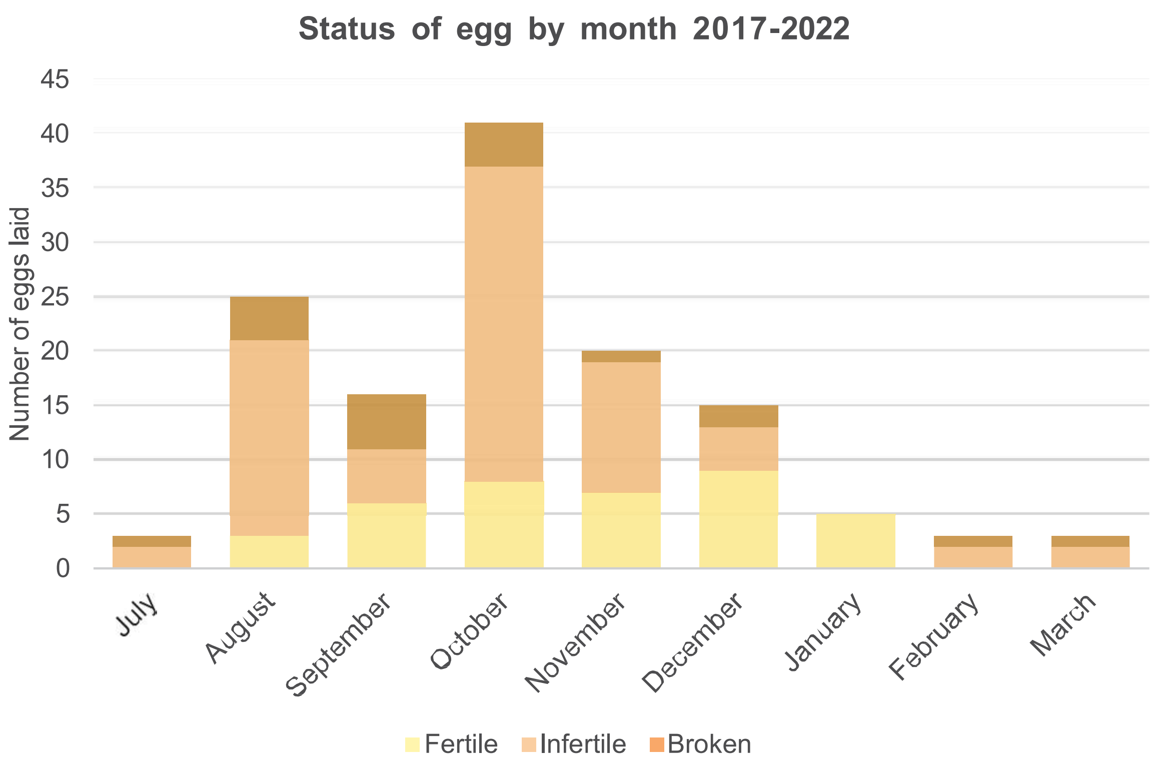

Figure 21. Bar chart detailing the status of eggs (fertile, infertile or broken) laid at SERCAS in different months.

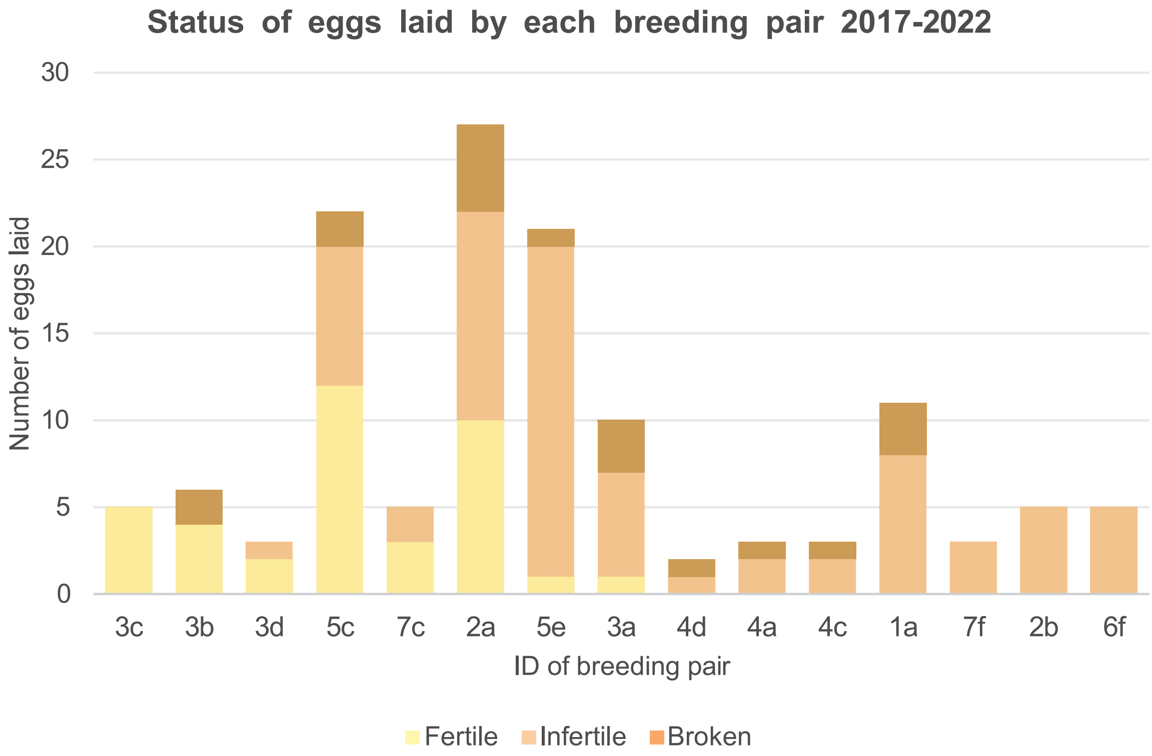

Figure 22. Bar chart detailing the status of eggs (fertile, infertile or broken) laid at SERCAS by each breeding pair.

5 Discussion

This study yielded three main sets of results. Firstly, there is strong evidence of a specific breeding season, from August to January, during which the frequency of certain behaviours observed between successful and unsuccessful captive breeding pairs of *A. jacutinga* differed significantly. Reproductive behaviours have been identified and can be utilised as indicators of the likelihood of reproductive success for a breeding pair in this captive breeding facility. After one week of introducing a breeding pair, the absence of such behaviours indicates a low likelihood of reproductive success and provides a rationale for separating males from females. Egg laying did not alter the frequency of social and reproductive behaviours in breeding pairs.

Secondly, there is evidence of mate choice by females, demonstrated through an apparent evasiveness to advances from males, sometimes resulting in harm or injury. The risk of such injury to the female was apparent during the first few days after introducing a breeding pair. Recommendations for the separation of breeding pairs with perceived incompatibility have been outlined. Further evidence of mate choice is apparent in historical data, highlighting that certain breeding pairs reproduce more consistently and successfully than others.

Thirdly, naturalistic enclosures increase the likelihood of successful reproduction in this captive population. Reproductive behaviours identified were primarily exhibited in larger and more naturalistic enclosures. Eggs laid in such enclosures have higher fertility rates than those laid in smaller enclosures with limited light and vegetation.

5.1 *Reproductive Behaviours*

Reproductive behaviours identified through this study were categorised as such due to their association with successful reproduction in breeding pairs. My results expand upon and present differences from previous ethograms of *A. jacutinga* (de Souza *et al.,* 2020; Rivera, 2016; Robbi, 2020).

The *descending whistle*, emitted exclusively by males, was associated with successful reproduction in *A. jacutinga*. Similar vocalisations are documented across the Cracidae family (Baldo and Mennill, 2011; del Hoyo and Kirwan, 2020; del Hoyo *et al*., 2020a; Maira, 2009), serving various functions such as alarm calls (Baldo and Mennill, 2011) or, as observed in the Yellow-knobbed Curassow (Crax daubentoni), intrasexual warnings to rival males (Buchholz, 1995). The descending whistle in *A. jacutinga* may be an example of an intrasexual signal. It was frequently exhibited alongside agonistic, territorial behaviours between males in adjacent enclosures, characterised by vigilance, raised crests and warning vocalisations. The descending whistle was also exhibited alongside reproductive behaviours such as *wing display* and *nodding*, highlighting the importance of the descending whistle in reproduction, though further investigation is necessary to determine the primary function of the vocalisation.

Male offers item to female was frequently exhibited in successful breeding pairs and was associated with reproductive success. This may be an example of courtship feeding, a ritual well-documented in gallinaceous (Stokes and Williams, 1971) and cracid (Frank-Hoeflich *et al*., 2007; González-García *et al*., 2017; Sick, 1970) species. Courtship feeding involves the male offering food to the female, often accompanied by vocalisations or other behaviours relevant to courtship, and encourages interactions between the breeding pair, providing males the opportunity to pursue females (Stokes and Williams, 1971). During my observations, males frequently exhibited *nodding* and *nodding vocalisation* while offering items to females. Occasionally, females reciprocated and exhibited *nodding*, and at times *nodding vocalisation*, in return and approached the male. *Female approaches male* was also associated with reproductive success, highlighting the significance of interaction between a breeding pair as a facilitator of successful reproduction. In response to this behaviour, the male often initiated a chase, occasionally leading to mounting the female.

There was also an association between *male mounts female* and reproductive success in a breeding pair. Accounts of copulation in *A. jacutinga* describe “the male, with wings partially extended, chasing the female to corner her on a substrate" (de Souza *et al.*, 2020). From my observations, two distinct behaviours are evident: *male chases female* and *male mounts female*. Although chases often preceded mounting and apparent copulation, not all chases resulted in mounting. Often, the male ceased pursuit before the female stopped fleeing. Contrastingly, mounting occurred on several occasions in the absence of a chase. In enclosure 14-15, female J083L allowed both breeding partners to mount her without any prior chase, illustrating the justification for distinguishing between *chasing* and *mounting* or copulatory behaviours.

Similar distinctions have been made between the behaviours *nodding* (♂♀) and *nodding vocalisation* (♂), both of which were associated with reproductive success and often exhibited alongside other reproductive behaviours. While de Souza *et al.,* (2020) describe the courtship behaviour of the "nodding call" in *A. jacutinga*, in my observations *nodding* and *nodding vocalisation* were not mutually exclusive. In contrast to reports suggesting that females engage in *nodding* exclusively without vocalisation (de Souza *et al*., 2020), I observed that females exhibit *nodding vocalisation*, though less frequently than males. Furthermore, female nodding vocalisations were notably quieter and of longer duration than those emitted by males. This distinction highlights potential differences in the functions of nodding and nodding vocalisations between males and females throughout the breeding season. However, it remains possible that these behaviours serve a specific function when performed together as a ‘nodding call’.

5.1.1 *Indicators of Future Reproductive Success*

During the initial 6 days following the introduction of breeding pairs, high frequencies of reproductive behaviours were observed in pairs that subsequently produced fertile eggs. The presence of such behaviours indicates a high likelihood of future reproductive success in newly introduced breeding pairs. In contrast, an absence or low frequency of such behaviours suggests that future reproductive success is unlikely.

An association was identified between successful reproduction and the behaviour *female offers items to male.* This is distinct from courtship feeding, in which males offer items to females (Smith, 1980). Allofeeding, documented in various avian species, may serve to establish dominance (Kalishov *et al*., 2005; Kemp and Kemp, 1980) or build and reinforce social bonds (Craig, 1988). Alternatively, allofeeding may result from altered behaviour due to the “artificial conditions” implemented by a captive environment (Lack, 1940).

Alongside this, the *warble* vocalisation emitted exclusively by females was associated with reproductive success. This vocalisation was primarily observed following instances of females being chased or mounted by a male. Notably, female J043 in enclosure 12-13a emitted the warbling sound at high frequencies after increased aggression from a breeding partner.

The *wing display*, performed exclusively by males, was exhibited at high frequencies in enclosures housing successful breeding pairs and was found to be associated with reproductive success. This behaviour resembles ‘wing clapping’, a territorial defence documented in various species within the family Tetraonidae, including the spruce grouse (*Canachites canadensis*), Franklin’s spruce grouse (*C. c. franklinii*) and the dusky grouse (*Dendragapus obscurus*) (Blackford, 1958; Johnsgard, 2016; Schroeder *et al.*, 2021; Schroeder and Boag, 1989). The heightened frequency of this behaviour following male transfer into new enclosures with breeding females suggests this may be a display of territoriality or dominance.

Similarly*, male chases female* was exhibited at high frequencies in the first six days after pair introductions. This behaviour, which often preceded mounting of the female and typically lasted from ~5 to 90 seconds, may serve as a courtship behaviour or territory defence mechanism (Gowaty and Buschhaus, 1998; Johnsgard, 2016). While these behaviours are associated with reproductive success, a deeper understanding of their precise functions, particularly determining whether they are indicators of dominance or components of courtship rituals, warrants further investigation.

***5.1.2** Activity Budgets of Successful Breeding Pairs*

Analysis of activity budgets revealed associations between certain behaviours and reproductive success. Although they cannot strictly be categorised as reproductive behaviours, prolonged engagement in these state behaviours suggests a high likelihood of future reproductive success.

Vigilance (♂♀) was more frequently exhibited in successful, rather than unsuccessful, breeding pairs and was therefore associated with reproductive success. Males demonstrated vigilance while engaging in territorial and agonistic interactions with neighbouring males in adjacent enclosures, alongside during pauses in behaviours such as wing display, chasing and mounting. Similarly, females exhibited vigilance alongside territoriality, following a chase, or while a male was engaging in displays or nodding behaviours.

Foraging away from the feeder (♂♀) was also more frequently exhibited by successful breeding pairs. This behaviour in females may be a response to nutrient depletion, as female birds typically increase foraging rates in the reproductive period to ensure replenishment of the essential micro and macronutrients required for egg production (Perrins, 2008; Reynolds and Perrins, 2010). High frequencies of foraging in males may be attributed to increased time spent foraging for items to offer to females. Alternatively, increased foraging in males may share a relationship with the mating dance, which comprises several behaviours, including walking in small circles and foraging. The mating dance was recorded on two occasions throughout my observations; however, males frequently walked in a circular pattern and pecked at the substrate almost ritualistically. It is possible that males were carrying out the individual components of the mating dance and that the captive environment has modified the expression of this behaviour. However, further research is needed to explore the relationship between the captive environment and the expression of social behaviours.

In breeding pairs which achieved reproductive success, running (♂♀) and flying (♂) were exhibited at high frequencies. This may be attributed to increased engagement in reproductive behaviours that require locomotion, including female approaches male and male chases female. Males in successful breeding pairs often exhibited a behaviour resembling the wing display. However, it was not recorded as such due to an absence of the characteristic sound associated with the behaviour. In this flight display, males would traverse a perch in a vigilant state and pause, emitting the descending whistle, before briefly taking flight to a nearby perch, with flight typically lasting ~1-2 seconds. This behaviour was repeated several times and likely contributed to the elevated levels of flying observed in successful breeding pairs.

Female nest care was associated with reproductive success. While there was no association between male nest care and successful reproduction, I observed males in both successful and unsuccessful breeding pairs spend time in and around the nest, exhibiting *nodding, nodding vocalisation* and *descending whistle*. These findings are consistent with reports which describe females as the primary sex responsible for nest care and maintenance (de Souza *et al*., 2020; del Hoyo *et al*., 2020b).

Resting vigilance was notably more prevalent in enclosures where reproductive success was absent. The high frequency of this behaviour suggests low levels of mutual interaction and active engagement with the environment, indicating that successful reproduction in this species is facilitated by increased social interaction.

5.2 *Evidence of Female Mate Choice*

The findings of this study present evidence of intersexual selection within *A. jacutinga*; the existence of sexual signals such as the male wing display and descending whistle suggest some degree of female choice is present within the species and that females may choose mates based on their preferences concerning these characteristics.

Females demonstrated evasiveness towards male advances in various scenarios. Despite males persisting with nodding, displays, or attempts to offer food or other items, females did not always reciprocate by nodding, accepting offered items, or approaching the male. Active rejection of male advances was evidenced in females fleeing from chases. On some occasions, however, the female ceased fleeing and allowed the male to mount and attempt copulation. This may be an example of direct choice (Orbach, 2019; Wiley and Poston, 1996) if the female allowed copulation after assessing the male and his chase.

Additionally, the act of a female fleeing from an approaching male frequently stimulated chases in adjacent enclosures which contained breeding pairs. Avoidance of pursuit by her breeding partner may be attributed to female awareness of a potential, and perhaps preferred, mate in an adjacent enclosure. However, while fleeing serves as a means for females to exercise mate choice, the confines of an enclosure limit the ability of the female to truly escape the pursuit of the male. Consequently, the decision to permit mounting and attempted copulation by the male may be influenced by the constraints of the captive environment.

All reproductive success observed during this study was achieved by breeding pairs with a history of successful reproduction. Analysis of historical data has identified patterns of reproductive success among breeding pairs; certain breeding pairs consistently achieved higher rates of reproductive success than others. Among the pairs which have historically achieved the highest fertility rates (>60%), 3 include the male J081. While this may suggest that J081 is a capable, fertile and preferable mate, when paired with female J080L they achieved a fertility rate of 10%. As the female J080L has achieved higher levels of reproductive success with other males, the concept of mate choice or preference must be considered. Both individuals have demonstrated reproductive success with other partners, suggesting factors such as limited pair bonding or preference for other partners may contribute to low fertility rates when paired together.

Familiarity and previous reproductive success with a breeding partner may influence mate choice in birds (Beguin *et al*., 2013; Senar *et al*., 2013). However, given the role of SERCAS as a conservation facility aimed at increasing species population size, successful breeding pairs are typically housed together each breeding season to maximise offspring production. These highly successful breeding pairs may develop stronger bonds over time due to their recurrent pairing and prolonged periods of cohabitation.

5.3 *Enclosure Style Influences Reproductive Success*

Several behaviours were exhibited at high frequencies in naturalistic enclosures when compared to non-naturalistic enclosures. This suggests that the captive environment influences the behaviour of captive individuals.

Raising crest (♀), *warning* (♀), and *warning vocalisation* (♀) were frequently exhibited in naturalistic enclosures. Females exhibited these behaviours in two distinct scenarios. Firstly, females exhibited such behaviours upon being approached or chased by males, potentially as an agonistic response to unwanted pursuit and displays of reproductive behaviours. Secondly, these behaviours were exhibited towards humans passing in front of enclosures to access other areas of the research centre. These behaviours are identical to those concerning territoriality in males (de Souza *et al*., 2020), suggesting that females in this captive population engage in agonistic territory defence.

Maintenance (♀) was associated with naturalistic enclosures; females housed in naturalistic enclosures spent significant time engaging in grooming and maintenance behaviours. While birds may naturally spend considerable amounts of time engaging in self-maintenance and grooming, particularly in environments free of risk or immediate threat (Delius, 1988; Kozak *et al*., 2019, displacement preening may be a response to stressful situations (Kozak *et al*., 2019; Spruijt *et al*., 1992), such as being unable to escape the advancement of potentially unwanted mates. Further investigations into this behaviour in captive *A. jacutinga* may provide valuable insights into the determinants contributing to high frequencies of self-maintenance.

Male pecks female was associated with naturalistic enclosures. This may be attributed to a potential relationship between pecking and reproduction; while pecking itself was not associated with reproductive success, it was observed almost exclusively alongside chasing and mounting behaviour. Male aggression towards females has been described in *A. jacutinga* (de Souza *et al.,* 2020) and other cracid species, including the White-winged Guan (*Penelope albipennis*) (Angulo Pratolongo, 2020) and the Alagos curassow (*Mitu mitu*) (de Avelar Azeredo and Simpson, 2014), indicating that it may be a typical feature of courtship within this species.

Several behaviours identified as reproductive behaviours through this study were associated with naturalistic enclosures, including males chasing and mounting females and the behaviours *female approaches male*, *running* (♂♀) and *flying* (♂). As these behaviours require or are associated with some form of locomotion, and naturalistic enclosures are larger than non-naturalistic enclosures, the increased space may facilitate the sexual and social behaviours in this captive population. The effects of increased space on activity levels are documented in other species, including in captive populations of the Humboldt penguin (*Spheniscus humboldti*) (Marshall *et al*., 2016), Domestic fowl (*Gallus gallus domesticus*) (Mallapur *et al*., 2009) and the Japanese quail (*Coturnix japonica*) (Galef *et al*., 2006).

5.3.1 *Fertility Rates Influenced by Enclosure Design*

Analysis of historical data shows that the fertility rates of eggs laid in large and open-roofed enclosures were higher than those laid in small and covered enclosures. This, coupled with the fact that egg-laying in this study was exclusively observed in naturalistic enclosures, suggests enclosure design may influence reproductive success.

Enclosure size is directly related to reproductive success in various captive avian species including the Black stork, (*Ciconia nigra*) (King, 1994) and the Indian Peafowl (*Pavo cristatus*), which also experiences increased egg fertility and average chick weight in larger enclosures (Ali, 2016). The captive population of *A. jacutinga* may experience increased fertility and reproduction rates in large enclosures due to their increased social activity, as evidenced in this observational study. However, reaching definitive conclusions is challenged due to confounding variables arising from the lack of uniformity in historical enclosure design at the SERAS breeding facility.

Further research is necessary to strengthen evidence of the relationship between egg fertility and both enclosure size and roof type. Additionally, the study's design, which involved housing historically successful breeders in large enclosures and less successful breeders in small enclosures, may introduce bias. The higher fertility rate of eggs observed in large enclosures may result from successful breeders being housed in such enclosures rather than the size or roof style of the enclosure itself. Future studies may reverse the design of this project, housing unsuccessful breeders in large or open-roofed enclosures, and vice versa, to test the relationship between enclosure size and egg fertility.

5.4 *Confounding Variables*

The results of the historical data analysis showed significant variation in reproductive success both throughout breeding seasons and between months within breeding seasons. These findings offer valuable insights that can inform captive management strategies to maximise offspring production.

Variation in egg status was observed across breeding seasons, with fewer clutches recorded during the 2020-2021 and 2022-2023 breeding seasons. These declines coincided with periods of disruption to the captive population. In 2020-2021, construction work was undertaken at the SERCAS facility to remove the covered roof of the large enclosures. During the 2022-2023 breeding season, males were frequently relocated between enclosures as per the design of this study, contrasting with previous years when males often remained with females throughout the breeding season unless aggression levels necessitated separation. These scenarios likely caused increased stress to individuals in breeding pairs, which has the potential to disrupt the reproductive potential of captive species (Carlstead and Shepherdson, 1994; Griffith *et al*., 2017; Morgan and Tromborg, 2007) and may account for the diminished egg production and reproductive success observed.

The analysis also revealed distinct patterns in months with high reproductive success, with December and January exhibiting the highest fertility rates. Conversely, low levels of egg-laying and fertility rates of 0% were observed in July, February and March. This pattern aligns with reports that the breeding season of *A. jacutinga* spans from August to January (Galetti *et al*., 1997; del Hoyo *et al*., 2020b; de Souza *et al*., 2020). While December and January are, historically, the most successful months for reproduction at SERCAS, this study did not observe reproductive success within these months. This discrepancy could be attributed to a potential bias, as the females with the highest levels of historic reproductive success were utilised in stage 1 of the project from August to October, while less successful breeders were utilised in stage 2 from November to February. Contrarily, females are consistently housed in the same enclosures throughout the year, outside of the breeding season. This suggests that the increased levels of reproductive success observed in larger and open-roofed enclosures are due to a female's prolonged exposure to a more suitable environment. Conversely, females housed in the less optimal conditions of the smaller enclosures may experience decreased reproductive success. This highlights the importance of further research into the relationship between enclosure size and design and reproductive success.

5.5 *Focus Areas for Future Research*

This study has identified reproductive behaviours in this captive population of *A. jacutinga* and highlighted the relationship between enclosure style and reproductive success. However, further research is required to develop a comprehensive understanding of this species and establish captive management techniques that will aid in increasing reproductive success.

Firstly, it is crucial to carry out physical examinations of the sexual organs and hormone levels of both males and females within the captive population to identify any limitations to the ability of individuals to reproduce. Without this, the suggestion that some individuals are incapable of successful reproduction, thus influencing trends in reproductive success, cannot be dismissed.

Investigation into the functions of several behaviours, particularly the sexual displays of males, is needed. For example, the wing display and descending whistle were associated with reproductive success, but further research may assist in determining whether these serve as inter- or intrasexual signals. Additionally, behavioural research may aim to better describe the process of copulation in *A. jacutinga* or explore the relationship between and reproduction and aggressive behaviours, such as pecking and chasing, which this study found to be associated with reproductive success. Further exploration of these characteristics will enhance our comprehension of the reproductive dynamics of the species.

Territoriality can also be tested through minor adjustments to the captive environment. Given that reproductive success in captive territorial species is influenced by visual access to conspecifics (Flanagan *et al.*, 2020; Mirande *et al.*, 1997), implementing a curtain to block visual access to neighbouring breeding pairs may influence not only reproductive success but also the exhibition of agonistic and territorial defence behaviours. This intervention could provide valuable insights into the role of territoriality in the behaviour of *A. jacutinga*, supplying information that may assist captive breeding programmes and increase the suitability of management techniques.

Exploring the mate choice or preference of females is essential to generate an understanding of the as-yet-unconfirmed mating system in this species and further develop knowledge of reproductive behaviours. Subsequent studies may introduce a third male to breeding pairs and observe interactions within the group to investigate female mate choice. However, this is not currently recommended at SERCAS facility due to the restricted enclosure size. The implementation of larger and more naturalistic environments is strongly recommended; this would be advantageous not only for the reproductive success of the captive population, given the observed relationship between enclosure style and egg fertility rates, but for the efficacy of future experiments aiming to investigate mating systems, sociality and other aspects of *A. jacutinga* behaviour.

5.6 *Recommendations for the Captive Breeding of A. jacutinga*

The following recommendations are suggested for the successful management of this captive *A. jacutinga* population. Implementing these measures may enhance reproductive success and contribute to the conservation goals outlined by Project Jacutinga.

1) *Introduce breeding pairs in August:* House males and females separately outside of the breeding season, with males transferred into female enclosures in early August.
2) *Conduct behavioural observations:* Assess pair compatibility and likelihood of successful reproduction over at least 6 days with a focus on behaviours highlighted in this study: a) nodding (and nodding vocalisations), b) offering items to conspecifics, c) wing display, d) male chases female and e) male mounts female. Additionally, note prolonged engagement in flight (♂), nest care (♀), foraging (♂♀) or approaching conspecifics (♂♀). These behaviours indicate potential reproductive success, while their absence after six days suggests a low likelihood. In such cases, consider removing the male and introducing a new mate to the original female.
3) *Assess level of aggression:* Monitor male aggression, including resource guarding of nest space or food. Remove males from the enclosure upon observing excessive pecking that removes feathers from the female or females being denied access to the feeding station for over 24 hours. Allow the female recovery time before introducing a new potential breeding partner.
4) *Remove male after laying:* Males must be removed from the enclosure after the female has laid the final egg in her clutch to prevent stress to the female or damage to the eggs.
5) *Monitor incubation:* Monitor females and their incubation of the eggs. The female should begin incubating after the third egg in the clutch is laid. If the female spends extended periods away from the nest, assess the egg fertility through candling and incubate artificially if necessary.

6 Conclusion

The objectives outlined by this project have been successfully met, as reproductive behaviours within this captive population of *A. jacutinga* have been identified and can be utilised as reliable indicators of future reproductive success for breeding pairs. Results have also highlighted the effect of enclosure size and design on reproductive success in this captive population. Insights gained from this research will assist captive breeding facilities in implementing more suitable and effective management practices for captive *A. jacutinga* during the breeding season. Specifically, the recommendation to employ larger and more naturalistic enclosures, coupled with the practice of observing breeding pairs during the initial 6-day period post-introduction to assess reproductive behaviours, is expected to enhance the success rates of this captive breeding programme. These adjustments to current practices are expected to yield a higher number of offspring per season, thereby increasing the number of individuals suitable for controlled release into the Atlantic Rainforest in São Francisco Xavier. This will provide valuable support to Project Jacutinga in its goals to protect and recover this endangered species.

7 References

Ali, Z., (2016). Effect of spacing on reproductive performance of Indian Peafowl (Pavo cristatus) in captivity. *Pakistan Journal of Science*, 68(4).

Asa, C.S., Traylor-Holzer, K., and Lacy, R.C., (2011) Can conservation breeding programs be improved by incorporating mate choice? *International Zoo Yearbook*, 45: 203–212.

de Avelar Azeredo, R.M. and Simpson, J.G.P., (2014). Rearing the extinct in the wild Alagoas curassow Pauxi mitu for future reintroduction programmes. *International Zoo Yearbook,* 48(1): 29-38.

Baldo, S. and Mennill, D.J., (2011). Vocal behavior of Great Curassows, a vulnerable Neotropical bird. *Journal of Field Ornithology*, 82(3): 249-258.

Beguin, N., Leboucher, G., Bruckert, L. and Kreutzer, M., (2006). Mate preferences in female canaries (Serinus canaria) within a breeding season. *Acta ethologica*, 9(2): 65-70.

Bernardo, C.S.S., Rubim, P., Bueno, R.S., Begotti, R.A., Meirelles, F., Donatti, C.I., Denzin, C., Steffler, C.E., Marques, R.M., Boyendorp, R.S., Gobbo, S.K. and Galetti, M., (2011). Density estimates of the Black-Fronted Piping guan in the Brazilian Atlantic rainforest. *The Wilson Journal of Ornithology* 123(4): 690-698.

Bernardo, C.S.S., and Clay, R.P., (2006). Black-fronted Piping-guan (Aburria jacutinga). *In:* Brooks, D.M., (ed), *Conserving Cracids: the most Threatened Family of Birds in the Americas*. Houston: Museum of Natural Science. p.52-56.

BirdLife International, (2018). Pipile jacutinga (Global assessment). The IUCN Red List of Threatened Species 2018: e.T22678429A132049346.

BirdLife International and Handbook of the Birds of the World, (2018). Pipile jacutinga (spatial data). The IUCN Red List of Threatened Species. Version 2021-3. https://www.iucnredlist.org.

Blackford, J.L., (1958). Territoriality and breeding behaviour of a population of blue grouse in Montana. *The Condor*, 60(3): 145-158.

Bodrati, A. and Cockle, K., (2006). Habitat, distribution, and conservation of Atlantic Forest birds in Argentina: notes on nine rare or threatened species. *Ornitología Neotropical*, 17: 243-258.

Brooks, D.M. and Strahl, S.D., (2000). *Curassows, guans and chachalacas: Status survey and conservation action plan for cracids 2000-20004.* Gland, Switzerland and Cambridge, UK: IUCN.

Buchholz, R., (1995). The descending whistle display and female visitation rates in the yellow-knobbed curassow, Crax daubentoni, in Venezuela. *Ornitologia Neotropical, 6: 27-36.*

Buchwalder, T. and Huber-Eicher, B., (2004). Effect of increased floor space on aggressive behaviour in male turkeys (Meleagris gallopavo). *Applied Animal Behaviour Science*, 89(3-4): 207–214.

Bussolini, L.T., Crates, R., Magrath, M.J. and Stojanovic, D., (2023). Identifying factors affecting captive breeding success in a critically endangered species. *Emu-Austral Ornithology*, p.1-9.

Carlstead, K. and Shepherdson, D., (1994). Effects of environmental enrichment on reproduction. *Zoo Biology,* 12(5): 447-458.

CBSG, (2017). IUCN Red List captive breeding recommendations, http://www.cbsg.org/iucn-red-list-captive-breeding-recommendations.

Chico Mendes Institute for Biodiversity Conservation (ICMBio), (2008). Plano de ação nacional para a conservação dos Galliformes ameaçados de extinção (aracuãs, jacus, jacutingas, mutuns e urus). Série Espécies Ameaçadas 6. [National action plan for the conservation of endangered Galliformes (aracuãs, jacus, jacutingas, curassows and urus). Endangered Species Series 6].

Craig, A.J., (1988). Allofeeding and dominance interactions in the cooperatively breeding pied starling. *Animal Behaviour*, 36(4): 1251-1253

D'Elia, J., (2010). Evolution of avian conservation breeding with insights for addressing the current extinction crisis. *Journal of Fish and Wildlife Management,* 1(2): 189-210.

Delius, K., (1988). Delius, J., 1988. Preening and associated comfort behaviour in birds. *Annals of the New York Academy of Sciences*, 525: 40-55.

Dickens, M.J. and Bentley, G.E., (2014). Stress, captivity, and reproduction in a wild bird species. *Hormones and Behavior*, 66(4): 685-693.

Farquharson, K.A., Hogg, C.J. and Grueber, C.E., (2018). A meta-analysis of birth-origin effects on reproduction in diverse captive environments. *Nature Communications,* 9(1): 1055.

Flanagan, A.M., Rutz, C., Farabaugh, S., Greggor, A.L., Masuda, B. and Swaisgood, R.R., (2020). Inter-aviary distance and visual access influence conservation breeding outcomes in a territorial, endangered bird. *Biological Conservation*, 242.

Frank-Hoeflich, K., Silveira, L.F., Estudillo-López, J., García-Koch, A.M., Ongay-Larios, L. and Piñero, D., (2007). Increased taxon and character sampling reveals novel intergeneric relationships in the Cracidae (Aves: Galliformes). *Journal of Zoological Systematics and Evolutionary Research*, 45(3): 242-254.

Galef Jr., B.G., Watkins, S.J. and Salehi, P., (2006). Effects of enclosure size on sexual behaviour of Japanese quail (Coturnix japonica). *Journal of Comparative Psychology*, 120(4): 433.

Galetti, M., Martuscelli, P., Olmos, F. and Aleixo, A., (1997). Ecology and conservation of the Jacutinga Pipile jacutinga in the Atlantic Forest of Brazil. *Biological Conservation,* 82: 31-39.

González-García, F., Santana-C, E., Barbudo, P.D.J., Rico-Gray, V. and Moliner, V.U., (2017). Diet and feeding behaviour of the Horned Guan (Oreophasis derbianus) in Mexico. *The Wilson Journal of Ornithology*, 129(4): 771-782.

Gowaty, P.A. and Buschhaus, N., (1998). Ultimate causation of aggressive and forced copulation in birds: Female resistance, the CODE hypothesis, and social monogamy. *American Zoologist*, 38(1): 207-225.

Greggor, A.L., Berger-Tal, O., Blumstein, D.T., Angeloni, L., Bessa-Gomes, C., Blackwell, B.F., St Clair, C.C., Crooks, K., de Silva, S., Fernández-Juricic, E. and Goldenberg, S.Z., (2016). Research priorities from animal behaviour for maximising conservation progress. *Trends in Ecology & Evolution*, 31(12): 953-964.

Griffith, S.C., Crino, O.L., Andrew, S.C., Nomano, F.Y., Adkins-Regan, E., Alonso-Alvarez, C., Bailey, I.E., Bittner, S.S., Bolton, P.E., Boner, W. and Boogert, N., (2017). Variation in reproductive success across captive populations: methodological differences, potential biases and opportunities. *Ethology*, 123(1): 1-29.

van Heezik, Y., Lei, P., Maloney, R. and Sancha, E., (2005). Captive breeding for reintroduction: influence of management practices and biological factors on survival of captive kaki (black stilt). *Zoo Biology: Published in affiliation with the American Zoo and Aquarium Association,* 24(5): 459-474.

del Hoyo, J., and Kirwan, G.M., (2020). Yellow-knobbed Curassow (Crax daubentoni), version 1.0. *In:* Del Hoyo, J., Elliott, A., Sargatal, J., Christie, D.A. and de Juana, E., (eds.) (2020) *Birds of the World.* Ithica, New York, USA: Cornell lab of Ornithology. doi.org/10.2173/bow.yekcur1.01

del Hoyo, J., Kirwan, G.M. and Christie, D.A., (2020a). Wattled Curassow (Crax globulosa), version 1.0. *In:* Del Hoyo, J., Elliott, A., Sargatal, J., Christie, D.A. and de Juana, E., (eds.) (2020) *Birds of the World.* Ithica, New York, USA: Cornell lab of Ornithology. doi.org/10.2173/bow.watcur1.01

del Hoyo, J., Kirwan, G.M. and Sharpe, C.J., (2020b). Black-fronted Piping-Guan (Pipile jacutinga) version 1.0. *In:* Del Hoyo, J., Elliott, A., Sargatal, J., Christie, D.A. and de Juana, E., (eds.) (2020) *Birds of the World.* Ithica, New York, USA: Cornell lab of Ornithology. doi.org/10.2173/bow.bfpgua1.01

Jensen, T., Jamieson, S.E., Castro, I., Gartrell, B., Cockrem, J.F. and Durrant, B., (2019). Serum prolactin and testosterone levels in captive and wild brown kiwi (Apteryx mantelli) during the prebreeding, breeding, and incubation periods. *Zoo Biology*, 38(3): 316-320.

Johnsgard, P.A., (2016). *The North American grouse: Their biology and behaviour.* Electronic ed. Lincoln: University of Nebraska.

Kalishov, A., Zahavi, A. and Zahavi, A., (2005). Allofeeding in Arabian babblers (Turdoides squamiceps). *Journal of Ornithology,* 146: 141-150.

Kemp, A.C., (1995). Aspects of the breeding biology and behaviour of the Secretarybird Sagittarius serpentarius near Pretoria, South Africa. *Ostrich,* 66(203): 61-68.

Kemp, A.C. and Kemp, M.I., (1980). The biology of the Southern ground hornbill Bucorvus leadbeateri (Vigors) (Aves: Bucerotidae). *Annals of the Transvaal Museum,* 32(4): 65-100.

King, C.E., (1994). Black stork (Ciconia nigra) management in Europe. *International Zoo Yearbook,* 33(1): 49-54.

Kozak, A., Rozempolska-Rucinska, I., Kasperek, K. and Bownik, A., (2019). Level of stress in relation to emotional reactivity of hens. *Italian Journal of Animal Science*, 18: 1251-1258.

Lack, D., (1940). Courtship feeding in birds. *The Auk,* 87: 169-178.

Maira, L.S.L., (2009). Status and ecology of the Wattled Curassow (Crax globulosa Spix, 1825) in the low Caquetá River, Colombian Amazon. MA Dissertation, Ås, Norwegian University of life Sciences.

Mallapur, A., Miller, C., Christman, M.C. and Estevez, I., (2009). Short-term and long-term movement patterns in confined environments by domestic fowl: Influence of group size and enclosure size. *Applied Animal Behaviour Science*, 117(1-2): 28-34.

Marshall, A.R., Deere, N.J., Little, H.A., Snipp, R., Goulder, J. and Mayer-Clarke, S., (2016). Husbandry and enclosure influences on penguin behavior and conservation breeding. *Zoo Biology*, *35*(5): 385-397.

Martin, K., Robb, L.A., Wilson, S., and Braun, C.E., (2020). Whitle-tailed Ptarmigan (Lagopus leucura), version 1.0. *In:* Rodewald, P.G. (ed.) (2020) *Birds of the World.* Ithica, New York, USA: Cornell Lab of Ornithology. doi.org/10.2173/bow.whtpta1.01

Mason, G.J., (2010). Species differences in responses to captivity: stress, welfare and the comparative method. *Trends in Ecology & Evolution*, 25(12): 713-721

Massa, R., Galanti, V. and Bottoni, L., (1996). Mate choice and reproductive success in the domesticated budgerigar, Melopsittacus undulatus. *Italian Journal of Zoology,* 63(3): 243-246.

McCowan, L.S.C., Rollins, L.A., & Griffith, S.C., (2014). Personality in captivity: More exploratory males reproduce better in an aviary population. *Behavioural Processes*, 107: 150–157.

McKinney, F., Sorenson, L.G., and Hart, M., (1990). Multiple functions of courtship displays in dabbling ducks (Anatini). *The Auk*, 188-191.

Miglioli, A. and da Silva Vasconcellos, A., (2021). Can behavioural management improve behaviour and reproduction in captive blue-and-yellow macaws (Ara ararauna)? *Applied Animal Behaviour Science*, 241.

Mirande, C.M., Carpenter, J.W. and Burke, A.M., (1997). The effect of disturbance on the reproduction and management of captive cranes. *In*: Urbanek, R.P., Stahlecker, D.W., (eds.) (1997) *Proceedings of the Seventh North American Crane Workshop*. Mississippi: North American Crane Working Group. p.56-61.

Montgomerie, R. and Holder, K., (2020). Rock Ptarmigan (Lagopus muta), version 1.0. *In:* Billerman, S.M., Keeney, B.K., Rodewald, P.G. and Schulenberg, T.S., (eds.) (2020) *Birds of the World*. Ithica, New York, USA: Cornell Lab of Ornithology. https://doi.org/10.2173/bow.rocpta1.01

Morgan, K.N. and Tromborg, C.T., (2007). Sources of stress in captivity. *Applied Animal Behaviour Science*, 102(3-4): 262-302.

Nuechterlein, G.L. and Storer, R.W., (1982). The pair-formation displays of the western grebe. *The Condor*, 84(4): 351-369

Orbach, D.N., (2019). Sexual strategies: Male and female mating tactics. *In:* Würsig, B., (ed,), *Ethology and Behavioural Ecology of Odontocetes*. Switzerland: Springer. P.75-93.

Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., O'Hara, R., Solymos, P., Stevens, M., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., Evangelista, H., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M., Lahti, L., McGlinn, D., Ouellette, M., Ribeiro Cunha, E., Smith, T., Stier, A., Ter Braak, C., Weedon, J., (2022). _vegan: Community Ecology Package_R package version 2.6-4. https://CRAN.R-project.org/package=vegan

Perrins, C.M., (2008). Eggs, egg formation and the timing of breeding. *Ibis*, 138(1): 2-15.

Phalan, B., Barbosa, A. E. A., Bernardo, C. S. S., Bosso, P., Chaves, F. G., Corrêa, L. L. C., Croukamp, C., Develey, P., Fernandes, K. C., Girão, W., Gomes, A. R. F., Gomes, M., Gonsioroski, G., Leus, K., Nunes, F., Olmos, F., Pina, P. I., Rocha, F. L., Ruiz-Miranda, C., Scherer, P., Silva, M., Sousa, A. E. B. A., Tassoni, A., Traylor-Holzer, K., Ubaid, F. & Valtuille, T., (2020). *Ex situ assessment for integrated conservation planning for Galliformes and Tinamiformes in Brazil.* Foz do Iguaçu, Paraná: IUCN SSC Conservation Planning Specialist Group.

R Core Team, (2023). *R: A Language and Environment for Statistical Computing.* R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Ralls, K. and Ballou, J.D., (2013) Captive breeding and reintroduction. *In:* Levin, S.A. (ed.), *Encyclopedia of Biodiversity*, 2.ed, Volume 1. Waltham, Massachusetts, USA: Academic Press.

Redies, H., (2013). Observations on White-browned Guan Penelope jacucaca in north-east Brazil. *Cotinga*, 35: 63–70.

Reynolds, S.J., Mänd, R. and Tilgar, V., (2004). Calcium supplementation of breeding birds: directions for future research. *Ibis*, *146*(4): 601-614.

Reynolds, S.J. and Perrins, C.M., (2010). Dietary calcium availability and reproduction in birds. *Current Ornithology,* 17: 31-74.

Ribeiro, M.C., Metzger, J.P., Martensen, A.C., Ponzoni, F.J. and Hirota, M., (2009). The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. *Biological Conservation*, 142(6): 1141-1153

Rivera, D.N., (2016). *Uso de etograma na conservação da Jacutingas - Aburria jacutinga (Spix, 1825) (Galiformes: Cracidae): Comportamento antipredatório e avaliação de dieta como subsídio para a criação e soltura.* MA Dissertation, São Paulo, The Federal University of São Carlos. [Translation: Use of ethograms in the conservation of Jacutingas - Aburria jacutinga (Spix, 1825) (Galiformes: Cracidae): Anti-predatory behaviour and diet assessment as a subsidy for breeding and release.]

Robbi, B., (2020). O papel da Fundação Parque Zoológico de São Paulo na conservação de espécies ameaçadas de extinção: o caso da Aburria jacutinga. MA Dissertation, São Paulo, The Federal University of São Carlos. [Translation: The role of the São Paulo Zoological Park Foundation in the conservation of endangered species: the case of Aburria jacutinga.]

Rubim, P. and Bernardo, C.S.S., (2008). Distribution and status of Jacutinga (Aburria jacutinga) at Estaçao Ecológica Juréia-Itatins, Atlantic rainforest, Brazil. *Bulletin of the Cracid Specialist Group*, 25: 18-23.

SAVE Brasil, (n.d.a). *Projeto jacutinga*. [Online] Available at: *http://savebrasil.org.br/projeto-jacutinga/*

Sánchez-Alonso, C., Oliveras, I. and Martín, M., (2002). Density estimates of guans (Aves: Cracidae): Pipile jacutinga and Penelope obscura. *In:* Mateos, E. Guix, J.C. Serra, A. and Pisciotta, K., (eds.), *Censuses of vertebrates in a Brazilian Atlantic rainforest area: the Paranapiacaba fragment.* University of Barcelona, Spain: Animal Biodiversity Resource Center.

Schroeder, M.A. and Boag, D.A., (1989). Evaluation of a density index for territorial male spruce grouse. *The Journal of Wildlife Management,* 53(2): 475-478.

Schroeder, M.A., Blomberg, E.J., Boag, D. A., Pyle, P. and Patten, M. A., (2021). Spruce Grouse (Canachites canadensis), version 1.1. *In*: Rodewald, P.G., (ed), *Birds of the World*. Ithaca: Cornell Lab of Ornithology.

Senar, J.C., Mateos-Gonzalez, F., Uribe, F. and Arroyo, L., (2013). Familiarity adds to attractiveness in matters of siskin mate choice. *Proceedings of the Royal Society B: Biological Sciences,* 280(1773): 20132361.

Sick, H., (1970). Notes on brazilian Cracidae. *The Condor*, 72(1): 106-108.

Snyder, N.F., Derrickson, S.R., Beissinger, S.R., Wiley, J.W., Smith, T.B., Toone, W.D. and Miller, B., (1996). Limitations of captive breeding in endangered species recovery. *Conservation biology*, 10(2): 338-348.

de Souza, L. D. C. D., Pereira, A. T., Azevedo, C. S. D., São Bernardo, C. S., Develey, P. F. and Ruiz Miranda, C. R., (2020). Reproductive and agonistic behaviours of black-fronted piping guans candidates for release and reintroduced (Galliformes: Cracidae) in the Brazilian Atlantic Forest. *Oecologia Australis*, 24(4): 802.

Spruijt, B.M., Van Hooff, J.A. and Gispen, W.H., (1992). Ethology and neurobiology of grooming behavior. *Physiological reviews*, 72(3): 825-852.

Stokes, A.W., and Williams, H.W., (1971). Courtship feeding in gallinaceous birds. *The Auk,* 88(3): 543-559.

Swaisgood, R.R., (2007). Current status and future directions of applied behavioural research for animal welfare and conservation. *Applied Animal Behaviour Science*, 102: 139-162.

Tassoni. A., (2022). *Jacutingas de volta à Mata Atlântica - Oeco.* [Online] *https://oeco.org.br/analises/jacutingas-de-volta-a-mata-atlantica/* [Translation: Jacutingas return to the Atlantic Forest]

Toledo-Lima, G.S., de Oliveira, T.M., Macario, P., de Oliveira, D.V. and Pichorim, M., (2013). Notes on reproductive biology of two species of cracids in northeastern Brazil. *The Wilson Journal of Ornithology*, *125*(3): 665-669.

de la Torre, G., de Bastiani, E., Fratoni, R. and Freitas, F., (2020). Breeding records of Black-Fronted Piping Guan Aburria jacutinga in coastal Paraná, southern Brazil. *Contiga*, 42:126-128.

Wiley, R.H. and Poston, J., (1996). Perspective: indirect mate choice, competition for mates, and coevolution of the sexes. Evolution, 50(4), pp.1371-1381.

8 Appendices

8.1 Appendix A: Ethograms

Table 4. Ethogram detailing the state behaviours observed throughout this project.

Behaviour ID	Behaviour	Description
V	Vigilant	Stationary; standing or sitting. The neck is fully extended. May be moving head from side to side, looking around in various directions. Animal may be pacing.
R	Resting	Sitting or standing with neck reclined and eyes closed or in a state of opening and closing repeatedly.
RV	Resting vigilant	Stationary; standing or sitting. The neck can be anywhere between fully reclined and extended. May be moving head from side to side, looking around in various directions
FF	Foraging at feeder	At the feeder in the enclosure, using the beak to eat/drink food/water in the bowls provided. Can also be eating in the general area food that may have fallen from the bowl provided.
FA	Foraging away from feeder	Away from the feeder, the animal is using the beak to consume items or peck/forage around the general area. Can be without consumption of any material, i.e. moving objects/substrate around with the beak.
M	Maintenance	General self-maintenance/grooming: Using the feet to scratch other areas of the body, using the beak to clean the feathers/feet/other body parts
LW	Locomotion (walking)	Using the legs to walk in an apparent calm manner or at a slow pace.

LR	Locomotion (running)	Using the legs to run and moving at a quick pace.
LF	Locomotion (flying)	Using the wings in locomotion from one area to another
NC	Nest care	In the nest, moving or arranging nest material around with the feet or beak.
NV	Not visible	Animal is out of sight of the researcher and unable to be observed.

Table 5. Ethogram detailing the social behaviours observed throughout this project.

Behaviour ID	Behaviour	Description
OFF	Offer item	Picking up an item with the beak or feet and offering it to another individual. The item is accepted.
OFF.N	Offer item (not accepted)	Picking up an item with the beak or feet and offering it to another individual. The item is ignored or not accepted.
APP	Approach	Approaching another animal in any form of locomotion (walk/run/fly).
MT	Mount	Male mounting female: she appears receptive (lifts/moves tail to the side to allow for interaction of cloacas) or unreceptive (keeps tail firmly positioned downward). Often seen after chase behaviour and can be accompanied by various vocalisations or pecking behaviour.
PK	Peck	Using the beak to peck another individual. Includes removal/plucking of feathers.
CH	Chase	Following in pursuit of a fleeing individual. Can occur via any form of locomotion (walking/running/flying).
NEUT	Neutral	Being in the vicinity of another individual without any of the above behaviours present (a neutral state) with less than ~30cm between the two individuals.

Table 6. Ethogram detailing the individual event behaviours observed throughout this project, excluding vocalisations.

Behaviour ID	Behaviour	Description
WRN	Warning	Full extension of the neck and raising the crest on the head. Animal can be standing or pacing from one area to the other. Often accompanied by *Warning* vocalisation.
CR	Raising crest	Raising the crest on the head, without full extension of the neck. Not accompanied by vocalisation.
WF	Wing flap	Extending of the wings, opening and closing in a flap several times while animal is standing or in walking/running locomotion.
WDs	Wing display	Specific clapping sound of the wings emitted while animal is in flight, identified by the sound created through the behaviour - similar to clapping together of hands. So far observed exclusively in males.
TF	Tail fan	Extension/fanning of the tail feathers. Tail can be raised slightly in an upwards position.
TS	Tail shake	Shaking of the tail and feathers.
NO	Shaking head ‘no’	Shaking the head specifically from side to side, as if saying 'no' - often many times quickly in succession.
YES	Nodding head ‘yes’	Nodding the head as if to say 'yes' - often many times quickly in succession.

BS	Body shake	Shaking the body, wings and tail (and sometimes head) all together several times in quick succession.
HS	Head shake	Shaking/flicking the head the head up and down or side to side.
WS	Wing shake	Opening the wings and shaking while standing or in walking locomotion.
PC	Pace	Pacing (while walking or running) from one area to another repeatedly with short pauses between.
YWN	Yawn	Opening the beak very wide for a short period as if yawning.
MtD	Mating Dance	Potential reproductive dance - animal paces slightly/walks on the spot/in circles with a stomping motion by the feet. Can be accompanied by raised/fanned tail and foraging on the ground

Table 7. Ethogram detailing the vocalisations observed throughout this project.

Behaviour ID	Behaviour	Description
C	Call	A singular, sharp, high pitched sound.
WDESC	Whistle descending	A long note which begins high in pitch and gradually descends. So far observed exclusively in males.
WASC	Whistle ascending	A series of shorter whistles which begin at a lower pitch and increase noticeably in pitch and volume with each whistle. There can be 3-5 whistles in succession.
WCH	Whistle/chirp	A long note which may increase slightly in pitch but is often continuous. Often a very quiet sound.
HK	Honk	A harsh, honking sound.
WRN	Warning	The sound observed when individuals are performing 'warning' behaviour. A series of chirps and honks.
YES	Nodding vocalisation	A combination of chirps and whistles heard when males are exhibiting 'nodding' behaviour. Or, in females, exclusively chirps.
SC	Screech	A high-pitched vocalisation often exhibited in times of distress, such as when bird is being handled by researchers.
WRB	Warble	A warbling cry, varying in pitch. Exhibited so far only by females and in times of distress, following chase/mount or excessive aggression from males.

***8.2** Appendix B: Results of Observations*

Enc. = enclosure ID, ID = couple ID, Style = enclosure style (where N = naturalistic and Nn = Non-naturalistic), Hrs = hours each breeding pair were observed and Fert = whether this pair produced fertile eggs. Behaviour names correspond to the ID field of the ethogram tables (Appendix A), with M or F preceding the behaviour ID to identify male or female behaviours.

Table 8. Table showing observational count data for social behaviours throughout the observational period.

					Behaviours										
Enc.	**ID**	**Style**	**Hrs**	**Fert**	**M_OFF**	**F_OFF**	**M_OFF.N**	**F_OFF.N**	**M_PK**	**F_PK**	**M_APP**	**F_APP**	**M_CH**	**M_MT**	**NEUT**
23a	3b	N	42	Yes	14	4	0	0	7	0	20	16	2	2	14
45a	4a	N	44	No	0	0	0	0	2	0	12	20	0	0	24
1415a	5c	N	35	Yes	8	0	0	0	22	0	12	14	3	3	2
23b	5b	N	44	No	2	0	0	0	0	0	34	4	16	2	2
45b	2a	N	34	Yes	40	2	0	2	0	0	13	32	8	4	5
1213b	3d	N	44	No	4	0	0	0	26	0	20	3	11	5	1
1415b	4c	N	38	No	1	1	0	0	0	0	4	11	0	3	5
6a	5f	Nn	44	No	0	1	0	0	0	0	2	4	0	0	8
7a	2e	Nn	44	No	0	0	0	0	0	0	2	0	0	0	1
16a	3g	Nn	44	No	5	0	0	0	0	0	8	1	0	0	8
20a	4h	Nn	44	No	1	0	1	0	0	0	7	14	0	0	22
6b	4f	Nn	44	No	2	1	0	0	0	0	9	12	0	0	8
7b	5e	Nn	44	No	2	0	2	1	0	2	19	9	0	0	10
16b	2g	Nn	44	No	2	0	0	0	0	0	19	0	1	0	2
20b	3h	Nn	44	No	3	2	0	1	0	0	24	1	0	0	14

Table 9. Table showing observational count data for individual behaviours throughout the observational period.

					Behaviours												
Enc.	**ID**	**Style**	**Hrs**	**Fert**	**M_PC**	**F_PC**	**M_CR**	**F_CR**	**M_WRN**	**F_WRN**	**M_WF**	**F_WF**	**M_WS**	**F_WS**	**M_WD**	**M_YES**	**F_YES**
23a	3b	N	42	Yes	7	41	14	4	7	3	10	9	0	0	0	84	10
45a	4a	N	44	No	21	33	7	20	0	13	17	5	0	1	0	1	2
1415a	5c	N	35	Yes	68	48	4	0	4	2	15	9	0	0	17	111	35
23b	5b	N	44	No	78	15	25	11	19	3	11	0	0	0	5	42	1
45b	2a	N	34	Yes	29	10	16	11	3	12	5	3	0	0	13	227	71
1213b	3d	N	44	No	0	18	20	8	0	1	2	1	0	1	0	37	1
1415b	4c	N	38	No	72	63	5	16	2	7	8	10	0	0	0	4	40
6a	5f	Nn	44	No	27	48	9	0	6	0	7	1	0	0	7	18	0
7a	2e	Nn	44	No	11	15	11	2	3	0	5	7	0	1	0	128	3
16a	3g	Nn	44	No	0	4	21	3	8	0	5	4	0	0	0	12	0
20a	4h	Nn	44	No	66	8	3	4	0	2	5	5	0	0	0	9	1
6b	4f	Nn	44	No	53	99	3	5	2	1	22	4	0	0	0	6	11
7b	5e	Nn	44	No	15	189	24	7	6	0	12	11	0	0	0	133	12
16b	2g	Nn	44	No	19	94	21	5	15	1	14	8	0	0	3	72	1
20b	3h	Nn	44	No	6	0	26	3	8	0	6	7	0	0	0	34	5

Table 5 cont. Table showing observational count data for individual behaviours throughout the observational period.

					Behaviours												
Enc.	**ID**	**Style**	**Hrs**	**Fert**	**M_NO**	**F_NO**	**M_YWN**	**F_YWN**	**M_HS**	**F_HS**	**M_BS**	**F_BS**	**M_TS**	**F_TS**	**M_TF**	**F_TD**	**M_MtD**
23a	3b	N	42	Yes	43	78	79	5	79	30	10	19	0	0	0	0	0
45a	4a	N	44	No	104	85	3	4	19	7	1	7	0	0	0	0	0
1415a	5c	N	35	Yes	34	84	8	4	10	9	11	7	0	0	2	15	2
23b	5b	N	44	No	60	89	12	14	12	11	20	29	0	1	0	0	0
45b	2a	N	34	Yes	22	137	22	5	9	6	42	14	1	0	3	1	0
1213b	3d	N	44	No	3	48	37	7	63	6	12	13	2	0	0	0	0
1415b	4c	N	38	No	100	43	16	6	9	7	22	10	0	1	0	3	0
6a	5f	Nn	44	No	18	50	8	5	10	5	16	14	0	0	0	0	0
7a	2e	Nn	44	No	32	39	18	8	9	10	10	15	3	0	1	2	0
16a	3g	Nn	44	No	16	23	87	9	71	16	10	21	0	0	0	1	0
20a	4h	Nn	44	No	154	43	13	6	7	10	11	17	3	2	0	0	0
6b	4f	Nn	44	No	151	106	3	5	10	16	7	11	1	2	0	0	0
7b	5e	Nn	44	No	46	106	25	2	17	15	5	9	0	2	0	0	0
16b	2g	Nn	44	No	61	75	15	7	22	8	13	7	0	0	4	0	0
20b	3h	Nn	44	No	73	119	55	8	49	26	14	14	1	0	0	0	0

Table 10. Table showing observational count data for vocalisations throughout the observational period. Social behaviours are depicted as per the Behaviour ID outlined in the ethogram.

					Behaviours												
Enc.	**ID**	**Style**	**Hrs**	**Fert**	**M_WCH**	**F_ WCH**	**M_C**	**F_C**	**M_ASC**	**M_DSC**	**F_WRB**	**M_HK**	**F_HK**	**M_WRN**	**F_WRN**	**M_YES**	**F_YES**
23a	3b	N	42	Yes	548	1286	377	605	158	38	7	0	4	6	0	117	0
45a	4a	N	44	No	1524	1401	116	389	27	61	0	4	29	0	18	0	0
1415a	5c	N	35	Yes	816	2647	83	125	95	64	26	7	3	4	3	97	24
23b	5b	N	44	No	494	1002	179	757	209	59	0	20	0	13	2	32	0
45b	2a	N	34	Yes	1962	5761	415	660	412	111	160	19	15	94	5	516	24
1213b	3d	N	44	No	109	620	472	137	183	26	350	0	0	0	2	25	0
1415b	4c	N	38	No	417	4188	172	390	57	10	0	0	33	1	6	0	2
6a	5f	Nn	44	No	437	1065	92	564	101	17	46	6	0	8	0	6	0
7a	2e	Nn	44	No	1080	1028	366	341	22	24	0	10	2	5	0	107	5
16a	3g	Nn	44	No	69	1564	228	506	181	36	10	17	1	7	0	8	0
20a	4h	Nn	44	No	1124	1157	100	489	28	33	0	0	2	0	1	0	0
6b	4f	Nn	44	No	936	1371	120	357	6	16	0	0	0	2	1	0	0
7b	5e	Nn	44	No	924	1764	29	574	54	15	0	4	0	3	0	94	0
16b	2g	Nn	44	No	547	1670	347	474	61	26	0	6	14	13	2	27	0
20b	3h	Nn	44	No	467	1139	233	941	79	40	0	8	2	10	0	4	0

8.3 *Appendix C: Results of Analyses*

Table 11. Results of Wilcoxon rank-sum tests on the frequency of social interactions in enclosures which did and did not produce fertile eggs using data obtained from all phases of the observational period (*4.1.1 Social Interactions*).

Social Interactions	Presence of fertile egg		
	p	W	*r* effect size
Male offers item (Accepted)	**.01**	0	0.68
Female offers item (Accepted)	.12	8	0.42
Male offers item (Not accepted)	.54	21	0.19
Female offers item (Not accepted)	.47	14	0.21
Male pecks female	.08	8	0.48
Female pecks male	.74	19.5	0.13
Male approaches female	.43	12	0.22
Female approaches male	**.02**	2	0.6
Male chases female	.06	6	0.51
Male mounts female	**.02**	3	0.63
Neutral behaviours	.94	17	0.04

Table 12. Results of Wilcoxon rank-sum tests on the frequency of individual behaviours in enclosures which did and did not produce fertile eggs using data obtained from all phases of the observational period (*4.1.2 Individual Behaviours*).

Social Interactions	**Presence of fertile egg**		
	p	W	*r* effect size
Pace (♂)	.52	13	0.19
Pace (♀)	1	18	0
Raise crest (♂)	.72	21	0.11
Raise crest (♀)	.77	20.5	0.09
Warn (♂)	.83	16	0.07
Warn (♀)	.07	5	0.5
Wing flap (♂)	.52	13	0.19
Wing flap (♀)	.43	12	0.22
Wing display (♂)	.09	7.5	0.47
Mating dance (♂)	.07	12	0.52
Wing shake (♀)	.41	22.5	0.24
Nodding 'yes' (♂)	**.02**	2	0.6
Nodding 'yes' (♀)	**.049**	4	0.53
Shaking head 'no' (♂)	.35	25	0.26
Shaking head 'no' (♀)	.17	8	0.37
Yawn (♂)	.52	13	0.19
Yawn (♀)	.52	23	0.19
Head shake (♂)	.52	13	0.19
Head shake (♀)	.52	13	0.19
Body shake (♂)	.35	11	0.26
Body shake (♀)	.61	14	0.15
Tail shake (♂)	.81	20	0.08
Tail shake (♀)	.23	25.5	0.34
Tail fan (♂)	.12	9	0.43
Tail fan (♀)	.17	9.5	0.38
Whistle/Chirp (♂)	.17	8	0.37
Whistle/Chirp (♀)	.13	7	0.41
Call (♂)	.3	10	0.3
Call (♀)	.62	14	0.15
Ascending whistle (♂)	.1	6	0.45
Descending whistle (♂)	**.04**	3	0.56
Honk (♂)	.51	13	0.19
Honk (♀)	.16	8	0.38
Warning vocal. (♂)	.28	10	0.28
Warning vocal. (♀)	.55	13.5	0.17
Nodding vocal. (♂)	**.01**	0	0.68
Nodding vocal. (♀)	.05	7	0.53
Warble (♀)	.06	6	0.51

Table 13. Results of Wilcoxon rank-sum analyses on the frequency of state behaviours in enclosures which did and did not produce fertile eggs using data obtained from all phases of the observational period (*4.1.3 Activity Budgets*).

Social Interactions	**Presence of fertile egg**		
	p	W	*r* effect size
Resting Vigilant (♂)	**.004**	36	0.67
Resting Vigilant (♀)	**.03**	33	0.56
Resting (♂)	.63	22	0.15
Resting (♀)	.14	7	0.41
Vigilant (♂)	**.009**	1	0.63
Vigilant (♀)	**.03**	3	0.56
Maintenance (♂)	.23	9	0.33
Maintenance (♀)	.63	14	0.15
Foraging at feeder (♂)	.07	31	0.49
Foraging at feeder (♀)	.29	10	0.3
Foraging away from feeder (♂)	**.004**	0	0.67
Foraging away from feeder (♀)	**.03**	3	0.56
Nest care (♂)	.09	6	0.45
Nest care (♀)	.13	7	0.41
Locomotion: Walking (♂)	.1	6	0.45
Locomotion: Walking (♀)	.36	11	0.26
Locomotion: Running (♂)	**.01**	0	0.67
Locomotion: Running (♀)	**.01**	0	0.67
Locomotion: Flying (♂)	**.03**	3	0.56
Locomotion: Flying (♀)	.36	11	0.26

Table 14. Results of Wilcoxon rank-sum tests on the frequency of social interactions in enclosures which did and did not produce fertile eggs using data obtained from the first six days of the observational period (4.2.1 Social Interactions).

Social Interactions	**Presence of fertile egg**		
	p	W	*r* effect size
Male offers item (Accepted)	.05	7	0.53
Female offers item (Accepted)	**.03**	7	0.59
Male pecks female	.27	13	0.32
Male approaches female	.3	10.5	0.29
Female approaches male	**.04**	3.5	0.55
Male chases female	**.002**	0.5	0.84
Male mounts female	.27	13	0.32
Neutral behaviours	.66	14.5	0.13

Table 15. Results of Wilcoxon rank-sum tests on the frequency of social interactions in enclosures which did and did not produce fertile eggs using data obtained from the first six days of the observational period (*4.2.2 Individual Behaviours*).

Social Interactions	**Presence of fertile egg**		
	p	W	*r* effect size
Pace (♂)	.2	8	0.36
Pace (♀)	1	17.5	0.02
Raise crest (♂)	.42	24	0.23
Raise crest (♀)	1	18.5	0.02
Warn (♂)	.88	19.5	0.06
Warn (♀)	.23	11	0.34
Wing flap (♂)	.33	11	0.28
Wing flap (♀)	.76	15.5	0.1
Wing display (♂)	**.005**	6	0.76
Mating dance (♂)	.07	12	0.62
Wing shake (♀)	.54	21	0.19
Nodding 'yes' (♂)	**.02**	1	0.64
Nodding 'yes (♀)	.09	7.5	0.47
Shaking head 'no' (♂)	.94	19	0.04
Shaking head 'no' (♀)	.08	5.5	0.47
Yawn (♂)	.25	9.5	0.32
Yawn (♀)	.39	24	0.24
Head shake (♂)	.77	15.5	0.09
Head shake (♀)	.6	14	0.15
Body shake (♂)	.16	8	0.38
Body shake (♀)	.38	11.5	0.25
Tail shake (♂)	.74	19.5	0.13
Tail shake (♀)	.74	19.5	0.13
Tail fan (♂)	.27	13	0.32
Tail fan (♀)	.6	15	0.16
Whistle/Chirp (♂)	.13	7	0.41
Whistle/Chirp (♀)	.35	11	0.26
Call (♂)	.42	24	0.23
Call (♀)	.51	13	0.19
Ascending whistle (♂)	.08	5.5	0.47
Descending whistle (♂)	.15	7.5	0.39
Honk (♂)	1	18	0
Honk (♀)	1	18	0
Warning (♂)	1	17.5	0.02
Warning (♀)	.61	15	0.16
Nodding vocal.	.09	7.5	0.47
Nodding vocal.	**.02**	6.5	0.61
Warble (♀)	**.04**	7.5	0.56

Table 16. Results of Wilcoxon rank-sum analyses on the frequency of state behaviours observed during the first 6 days of the observational period (*4.2.3 Activity Budgets*).

Social Interactions	**Presence of fertile egg**		
	p	W	*r* effect size
Resting Vigilant (♂)	**.009**	35	0.63
Resting Vigilant (♀)	.29	26	0.3
Resting (♂)	.47	23.5	0.2
Resting (♀)	.29	10	0.3
Vigilant (♂)	**.02**	2	0.6
Vigilant (♀)	.18	8	0.37
Maintenance (♂)	.71	5	0.48
Maintenance (♀)	.29	10	.3
Foraging at feeder (♂)	.54	23	0.19
Foraging at feeder (♀)	.18	8	0.37
Foraging away from feeder (♂)	.12	7	0.42
Foraging away from feeder (♀)	.07	5	0.49
Nest care (♂)	.25	10	0.31
Nest care (♀)	**.02**	3	0.63
Locomotion: Walking (♂)	.23	9	0.34
Locomotion: Walking (♀)	.36	11	0.26
Locomotion: Running (♂)	.17	9.5	0.38
Locomotion: Running (♀)	.12	7	0.42
Locomotion: Flying (♂)	**.04**	3	0.56
Locomotion: Flying (♀)	.29	9	0.34

Table 17. Results of Wilcoxon signed-rank tests on the frequency of social interactions before and after egg laying in enclosures which produced fertile eggs using data obtained from phases 2 and 3 of the observational period (*4.3.1 Social Interactions*).

Social interaction	p
Male offers item (Accepted)	.05
Female offers item (Accepted)	.37
Female offers item (Not Accepted)	1
Male pecks female	.37
Male approaches female	1
Female approaches male	.25
Male chases female	1
Male mounts female	.42
Neutral behaviours	.25

Table 18. Results of Wilcoxon signed-rank tests on the frequency of individual behaviours before and after egg laying in enclosures which produced fertile eggs using data obtained from phases 2 and 3 of the observational period (*4.3.2 Individual Behaviours*).

Individual behaviour	**p**
Pace (♂)	1
Pace (♀)	.5
Raise crest (♂)	.75
Raise crest (♀)	.37
Warn (♂)	1
Warn (♀)	.37
Wing flap (♂)	1
Wing flap (♀)	1
Wing display (♂) (♂)	1
Nodding ‘yes’ (♂)	.5
Nodding ‘yes’ (♀)	.25
Shaking head ‘no’ (♂)	1
Shaking head ‘no’ (♀)	1
Yawn (♂)	.18
Yawn (♀)	.25
Head shake (♂)	.75
Head shake (♀)	.18
Body shake (♂)	1
Body shake (♀)	.75
Tail shake (♂)	1
Tail fan (♂)	1
Tail fan (♀)	.37
Whistle/Chirp (♂)	1
Whistle/Chirp (♀)	.5
Call (♂)	.25
Call (♀)	.25
Ascending whistle (♂)	.25
Descending whistle (♂)	.25
Honk (♂)	1
Honk (♀)	1
Warning (♂)	1
Warning (♀)	1
Nodding vocalisation (♂)	.5
Nodding vocalisation (♀)	.37
Warble (♀)	.37

Table 19. Results of Wilcoxon signed-rank analyses on the frequency of state behaviours before and after egg laying in enclosures which produced fertile eggs using data obtained from phases 2 and 3 of the observational period (*4.1.3 Activity Budgets*).

State behaviour	p
Resting vigilance (♂)	.25
Resting vigilance (♀)	.25
Resting (♂)	.75
Resting (♀)	.25
Vigilant (♂)	.25
Vigilant (♀)	.25
Maintenance (♂)	1
Maintenance (♀)	.25
Foraging at feeder (♂)	.25
Foraging at feeder (♀)	.25
Foraging away from feeder (♂)	.75
Foraging away from feeder (♀)	.25
Nest care (♂)	.25
Nest care (♀)	1
Locomotion: Walking (♂)	1
Locomotion: Walking (♀)	1
Locomotion: Running (♂)	1
Locomotion: Running (♀)	.75
Locomotion: Flying (♂)	1
Locomotion: Flying (♀)	.5

Table 20. Results of Wilcoxon rank-sum tests on the frequency of social interactions in enclosures of different styles (naturalistic and non-naturalistic) using data obtained from all phases of the observational period (*4.4.1 Social Interactions*).

Social Interactions	**Enclosure style**		
	p	W	*r* effect size
Male offers item (Accepted)	.2	39.5	0.35
Female offers item (Accepted)	.56	33	0.17
Male offers item (Not accepted)	.2	21	0.35
Female offers item (Not accepted)	.8	26	0.09
Male pecks female	**.02**	44	0.61
Female pecks male	.42	24.5	0.24
Male approaches female	.22	39	0.33
Female approaches male	**.03**	47.5	0.59
Male chases female	**.02**	47	0.64
Male mounts female	**.002**	52	0.81
Neutral behaviours	.6	23	.15

Table 21. Results of Wilcoxon rank-sum tests on the frequency of individual behaviours in enclosures of different styles (naturalistic and non-naturalistic) using data obtained from all phases of the observational period (*4.4.2 Individual Behaviours*).

Social Interactions	**Enclosure style**		
	p	W	*r* effect size
Pace (♂)	.3	37.58	0.29
Pace (♀)	1	28.5	0.02
Raise crest (♂)	.77	25	0.09
Raise crest (♀)	**.048**	45.5	0.52
Warn (♂)	.52	22	0.18
Warn (♀)	**.003**	54	0.79
Wing flap (♂)	.6	33	0.15
Wing flap (♀)	1	28	0
Wing display (♂)	.37	35	0.25
Mating dance (♂)	.35	32	0.28
Wing shake (♀)	.5	32.5	0.19
Nodding ‘yes’ (♂)	.69	32	0.12
Nodding ‘yes (♀)	.18	40	0.36
Shaking head ‘no’ (♂)	.69	24	0.12
Shaking head ‘no’ (♀)	.52	34	0.18
Yawn (♂)	1	28.5	0.02
Yawn (♀)	1	27.5	0.02
Head shake (♂)	.39	36	0.24
Head shake (♀)	.45	21	0.21
Body shake (♂)	.22	39	0.33
Body shake (♀)	.91	29.5	0.05
Tail shake (♂)	.47	22	0.2
Tail shake (♀)	.49	22.5	0.2
Tail fan (♂)	.94	29	0.04
Tail fan (♀)	.37	35	0.25
Whistle/Chirp (♂)	.6	33	0.15
Whistle/Chirp (♀)	.6	33	0.15
Call (♂)	.27	38	0.3
Call (♀)	.77	25	0.09
Ascending whistle (♂)	.05	45	0.51
Descending whistle (♂)	.08	43.5	0.46
Honk (♂)	1	27.5	0.02
Honk (♀)	.14	41	0.4
Warning vocal. (♂)	.82	25.5	0.08
Warning vocal. (♀)	**.01**	49.5	0.67
Nodding vocal. (♂)	.32	37	0.27
Nodding vocal. (♀)	.21	37	0.34
Warble (♀)	.21	38	0.34

Table 22. Results of Wilcoxon rank-sum tests on the frequency of state behaviours in enclosures of different styles (naturalistic and non-naturalistic) using data obtained from all phases of the observational period (*4.4.3 Activity Budgets*).

Social Interactions	**Enclosure style**		
	P	W	*r* effect size
Resting Vigilant (♂)	**.001**	2	0.78
Resting Vigilant (♀)	**.0003**	0	0.84
Resting (♂)	.09	43	0.45
Resting (♀)	.07	44	0.48
Vigilant (♂)	**.04**	46	0.54
Vigilant (♀)	**.0003**	56	0.84
Maintenance (♂)	.24	27	0.27
Maintenance (♀)	**.02**	48	0.6
Foraging at feeder (♂)	1	28	0
Foraging at feeder (♀)	.07	44	0.48
Foraging away from feeder (♂)	**.02**	48	0.6
Foraging away from feeder (♀)	.05	45	0.51
Nest care (♂)	.95	29	0
Nest care (♀)	**.02**	49	0.63
Locomotion: Walking (♂)	.07	44	0.48
Locomotion: Walking (♀)	.23	39	0.33
Locomotion: Running (♂)	**.006**	52	0.72
Locomotion: Running (♀)	**.006**	52	0.72
Locomotion: Flying (♂)	.23	39	0.33
Locomotion: Flying (♀)	.46	35	0.21

8.4 Appendix D: Further Supplementary Material

To analyse reproductive success at SERCAS, each breeding pair with a history of egg laying was assigned an ID number. Each male was assigned a number (MID) and each female assigned a letter (FID), which combined creates a Couple ID.

Table 23. Details on the reproductive history of breeding pairs at SERCAS (2017-2022), including fertility and infertility rates of the eggs produced by each pair. MID = Male ID, FID = Female ID, Clutches = The total number of clutches laid per breeding pair, Eggs = The total number of eggs laid per breeding pair.

Couple ID	Male	MID	Female	FID	Clutches	Eggs	Fertility (%)	Infertility (%)
3c	J081	3	J083L	c	2	5	100.00	0.00
3b	J081	3	J083P	b	2	6	66.67	0.00
3d	J081	3	J043	d	1	3	66.67	33.33
7c	J004	7	J083L	c	1	5	60.00	40.00
5c	J022	5	J083L	c	8	22	54.55	36.36
2a	J082	2	J080L	a	10	30	33.33	40.00
3a	J081	3	J080L	a	4	10	10.00	60.00
5e	J022	5	J082	e	10	21	4.76	90.48
1a	J083	1	J080L	a	4	11	0.00	72.73
2b	J082	2	J083P	b	2	5	0.00	100.00
4a	J080P	4	J080L	a	1	3	0.00	66.67
4c	J080P	4	J083L	c	1	3	0.00	66.67
4d	J080P	4	J043	d	1	2	0.00	50.00
6f	J009	6	J084	f	2	5	0.00	100.00
7f	J004	7	J084	f	1	3	0.00	100.00