

**Ecological, demographic and genetic  
constraints on the conservation of the globally  
endangered Lear's Macaw**



**PhD Thesis  
Erica Pacífico  
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## **Ecological, demographic and genetic constraints on the conservation of the globally endangered Lear's Macaw**

Thesis for the degree of doctor of philosophy  
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CERTIFICA:

Que los trabajos de investigación desarrollados bajo mi dirección en la Memoria de Tesis Doctoral "*Ecological, demographic and genetic constraints on the conservation of the globally endangered Lear's Macaw*" cumplen con los requisitos de calidad, originalidad y rigor científico y son aptos para ser presentados por la Licenciada en Biología, Especialista en Manejo de Vida Silvestre, Master en Ciencias (Zoología) Erica Pacífico, ante el Tribunal que en su día se designe, para aspirar al Grado de Doctora en Ciencias por la Universidad Pablo de Olavide.

Y para que así conste, y en cumplimiento de las disposiciones legales vigentes, extiendo el presente certificado a 04 de Abril de 2020.

Firma Director y Tutor:

A handwritten signature in blue ink, consisting of a stylized, cursive script that appears to be 'J. L. Tella'.

Dr. José Luís Tella

*"Empatia é conseguir emergir no mundo subjetivo do outro e de participar na sua experiência, na extensão em que a comunicação verbal ou não verbal o permite. É a capacidade de se colocar verdadeiramente no lugar do outro, de ver o mundo como ele o vê".*

Carl Rogers

*To my big old friend Chico  
and to my new little friend Sammy  
for the dedication, patience and  
partnership along my professional  
path.*

*To the lovely people (Sertanejas  
and Sertanejos) who share the  
Lear's Macaw home range and  
care about it.*

*To the many inspirational  
colleagues doing nature conservation  
work worldwide.*



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# Abstract

The Lear's Macaw (*Anodorhynchus leari*) is endemic to the Caatinga biome (tropical dry forest in NE Brazil) and is listed as globally Endangered in the IUCN Red List. This species uses sandstone cliffs for breeding and roosting communally and feeds mostly on fruits of the Licuri Palm (*Syagrus coronata*). The whole global population is concentrated in two close localities, probably as the outcome of a large population decrease and range contraction in the past decades, but is currently sharply increasing. If the population does not expand geographically, the continuous increase could result in the saturation of environmental resources and generate negative density-dependent effects. We used a multidisciplinary research approach to investigate the population demography, potential genetic constraints and threats that may affect the conservation, recovery and expansion of this species. In Section 1 we provide the first estimates of breeding population size and the main breeding parameters for the species, through quantification and monitoring of active nests between 2009 and 2010 in the two breeding sites known until then, and estimated that c. 80% of the global population is constituted by non-breeding individuals. In Section 2 we estimate the recent population trend by employing a detection-based analysis, developing a binomial - negative binomial N-mixture model to estimate population size from replicated roost counts done by the Brazilian Environment Agency (ICMBio) from 2001 to 2014. Results suggest that population size is much larger than previously thought and that it increased 333% in that period. We also make recommendations for an improved design for population monitoring programs. Considering the past demographic history, potential genetic constraints in the remaining, although recently increasing population, are a concern. Therefore, in Section 3, we describe species-specific microsatellite primers developed from wild individual samples. Fifteen unlinked loci resulted informative for individual identification of related wild nestlings and could be applied for population genetics research. Then, in Section 4, we selected polymorphic microsatellites to genotype non-invasive samples (molted

feathers) and estimate genetic diversity and effective population size across the current distribution range of the species. We found molted feather sample repetition (i.e. multiple feathers from single individuals), moderate levels of genetic diversity, and no evidence of strong inbreeding in any locality. Results also showed a wide genetic admixture among all localities and overall differentiation was low. The average adult sex-ratio based on non-invasive sampling is male-biased (0.61), underscoring the need for further research on the primary and secondary sex-ratios of the immature population. In Section 5, we describe our research on the current and historic distribution of the species, looking for the causes of local extinctions and the current threats that the population could be facing in an expansion process. By combining local knowledge (112 interviews) with field surveys, we located two recolonized breeding areas, a new communal roost, and another six areas from where the species disappeared. We also compiled past and current threats, assessed habitat traits in occupied and unoccupied areas, and finally developed habitat suitability models for the Lear's Macaw and its main food resource (Licuri Palm). The overlap of these two models allowed identifying optimal areas for the range expansion of Lear's Macaws, where future conservation actions should be concentrated. Given that the presence of invasive Africanized honey bees (*Apis cf. mellifera*) was identified as a potential threat to Lear's Macaws, in Section 6 we assessed the competition for cliff cavities used by nesting Lear's Macaws and honey bees. We recorded > 100 honeybee hives in the Lear's Macaw breeding sites, with a higher infestation in areas recently recolonized by the macaws. We treated hives with permethrin and, when feasible, we removed the comb and applied an insecticide (fipronil) to deter honeybee recolonization in the cavity. Our experimental hive treatments were effective, allowing nest recruitment and local population increase of Lear's Macaws. We recommend intensive and continued Africanized honeybee hive eradication to enhance habitat restoration and facilitating Lear's expansion into historical areas. The data generated by this thesis will be decisive for the design of management strategies aimed at the conservation of this endangered species.

# Resumen

El guacamayo de Lear (*Anodorhynchus leari*) es endémico del bioma Caatinga (según la Lista Roja de la IUCN). Esta especie utiliza acantilados de arenisca para reproducirse y como dormitorios, y se alimenta principalmente de los frutos de la palma licuri (*Syagrus coronata*). Toda la población mundial se concentra en dos localidades cercanas, probablemente como resultado de una gran disminución de su población y de su distribución en las últimas décadas, pero actualmente está aumentando de manera considerable. Si la población no se expande geográficamente, el aumento continuo podría resultar en la saturación de los recursos ambientales y generar efectos negativos dependientes de la densidad. En esta tesis utilizamos un enfoque de investigación multidisciplinar para estudiar la demografía de la población, las potenciales restricciones genéticas y las amenazas que pueden afectar a la conservación, recuperación y expansión de esta especie. En la Sección 1 aportamos las primeras estimaciones del tamaño de la población reproductora y los principales parámetros reproductivos de la especie, a través de la cuantificación y seguimiento de nidos activos entre 2009 y 2010 en los dos sitios de reproducción conocidos hasta entonces, y estimamos que cerca del 80% de la población mundial está formada por individuos no reproductores. En la Sección 2 estudiamos la tendencia reciente de la población empleando un análisis basado en la detección, desarrollando un modelo binomial negativo mixto para estimar el tamaño de la población a partir de los conteos replicados de los guacamayos en sus dormitorios realizados por el gobierno brasileño de 2001 a 2014. Los resultados sugieren que el tamaño de la población es mucho mayor de lo que se pensaba y que aumentó un 333% en ese período, lo que permite ofrecer recomendaciones para un mejor diseño de programas de seguimiento de la población. Teniendo en cuenta la historia demográfica pasada, nos preocupamos por las restricciones genéticas en la población restante, aunque en aumento reciente. Por ello, en la Sección 3, describimos quince microsatélites específicos para esta especie, desarrollados a partir de muestras de silvestres y que son informativos para la identificación individual y podrían aplicarse a la

investigación genética de poblaciones. A continuación, en la Sección 4, seleccionamos ocho microsatélites polimórficos para genotipar muestras no invasivas (plumas mudadas) y así poder estimar la diversidad genética y el tamaño efectivo de la población. Encontramos repetición del muestreo de plumas mudadas, niveles moderados de diversidad genética, y ninguna evidencia de endogamia significativa en ninguna localidad. Los resultados también mostraron una amplia mezcla genética entre todas las localidades y la diferenciación general fue baja. La proporción promedio de sexos en los adultos, basada en muestreo no invasivo, muestra un sesgo hacia los machos (0,61), lo que hace recomendable más investigación sobre la proporción de sexos primaria y secundaria de la población inmadura. En la Sección 5 describimos la distribución actual e histórica de la especie, buscando las causas de las extinciones locales y las amenazas reales que la población podría enfrentar en un proceso de expansión. Al combinar el conocimiento local (112 entrevistas) con trabajo de campo, localizamos dos áreas de reproducción recolonizadas, un nuevo dormitorio comunitario y otras seis áreas donde desapareció la especie. También recopilamos amenazas pasadas y actuales, evaluamos características del hábitat en áreas ocupadas y no ocupadas, y finalmente desarrollamos modelos de idoneidad de hábitat para el guacamayo de Lear y su principal recurso alimenticio (palma licuri). La conjunción de estos dos modelos permitió identificar áreas óptimas para la expansión geográfica del guacamayo de Lear, en las que se deberían concentrar las futuras acciones de conservación. Dado que la presencia de abejas melíferas africanas (*Apis cf. mellifera*) invasoras fue identificada como una amenaza potencial para los guacamayos de Lear, en la Sección 6 evaluamos la competencia por las cavidades de acantilados utilizadas para anidar por los guacamayos y estas abejas. Registramos más de 100 colmenas de abejas en los lugares de reproducción del guacamayo de Lear, con una mayor infestación en áreas recientemente recolonizadas por los guacamayos. Tratamos las colmenas con permetrina y, cuando fue posible, retiramos el panal y aplicamos un insecticida (fipronil) para evitar la recolonización de las abejas en la cavidad. Nuestros tratamientos experimentales fueron efectivos, permitiendo el reclutamiento de nidos y el

aumento de la población local de guacamayos. Recomendamos la erradicación intensiva y continua de las colmenas de abejas africanas para mejorar la restauración del hábitat y facilitar la expansión del guacamayo de Lear en áreas históricas. Los datos generados por esta tesis serán decisivos para el diseño de estrategias de manejo dirigidas a la conservación de esta especie en peligro de extinción.

# 1 Thesis Structure Presentation



This thesis was written based on six research manuscripts (sections), with the aim of submitting them to international scientific journals in the areas of biodiversity conservation, ecology, population genetics and wildlife management. The manuscripts were written in close collaboration with researchers from Brazil, Spain and the US, including senior and young researchers, PhD students and field biologists, as well as wildlife managers from the Brazilian Environmental Agency, all of them engaged with research and conservation of the Lear's Macaw (see note in the Introduction of each section).

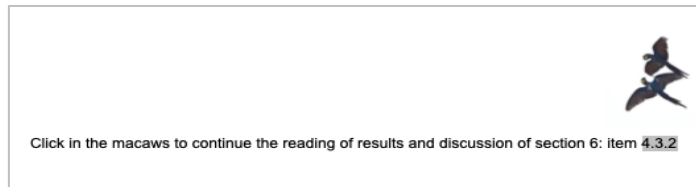
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The thesis is structured in three themes topics reflect and group the main objectives: (I) Lear's Macaw demography (Section 1: Breeding to non-breeding population ratio and breeding performance, and Section 2: Estimating population size and growth with a heterogeneous long-term census: population trends); (II) Population genetics (Section 3: Isolation and characterization of 15 new specific microsatellite markers, and Section 4: Population status assessment from non-invasive genetic samples); and (III) Threats to the species (Section 5: Past and current threats of the Lear's Macaw: implications for distribution and population expansion, and Section 6: Experimental removal of invasive Africanized honey bees to avoid competition for nest cavities of the endangered Lear's Macaw).

Attending to the thesis structure required by the Universidad Pablo de Olavide for the inclusion of unpublished material, this document begins with a general Introduction that includes background information about the central questions and the study species (Background), the main goals of this research,

and the specific goals to test the hypotheses posed, followed by the grouping of Introductions, Material and Methods, Results and Discussions of each section (manuscript). Finally, I close the thesis with a list of general conclusions of all sections together, followed by the list of tables, the list of figures and the literature cited.

To allow the continuous reading of each manuscript (section) in its regular format (for screen reading in PDF format), I provide a link (at the flying macaws) that redirect to the items following each section in the end of each Introduction, Materials and Methods, and Results and Discussion, as exemplified below:



I would like to emphasize that, throughout the five years of the development of this research, preliminary results were presented and discussed in several international conferences, which I list in the **Appendix 1**. I have also made these preliminary results available for the Lear’s Macaw Conservation Action Plan and the Lear’s Macaw international captive breeding program, participating as a specialist in the meetings organized annually by the Brazilian environmental agency (CEMAVE-ICMBio), thus assisting in the prioritization of the conservation strategies for this species and its habitat. Moreover, the fieldwork efforts made during this research also generate data for other studies regarding parrot ecology and conservation. I list the resulting publications in **Appendix 2**. I have published the section 1 in the Bird Conservation International journal in 2014 (**Appendix 3**) and submitted for review section 2 to The Condor Ornithological Applications in 2019 (**Appendix 4**), section 6 to Pest Management Science in 2019 and section 3 (**Appendix 5**).

## 2 Introduction



## 2.1 BACKGROUND

### 2.1.1 The role of ecology research applied to the conservation of small populations

The knowledge on population demographic processes is fundamental for the management of endangered species. However, little is still known about basic aspects of the biology, ecology and population dynamics of many species, thus making difficult the assessment of their conservation status. This situation applies for Neotropical Brazilian birds and especially for parrot species (Marini and Garcia 2005, Piacentini et al. 2015, BirdLife International 2019).

According to the IUCN Red List (BirdLife International 2019), only 30% of the 88 parrot species that occur in Brazil have been the focus of some biology and ecology studies, while 24 species are categorized as globally threatened and 60% show a decreasing population trend. These trends are mostly obtained from knowledge of experts rather than from population dynamics information, since only three species are the subject of large scale population monitoring programs (Piacentini et al. 2015, BirdLife International 2019). Controversially, 51% of the parrot species are considered “Least Concern”, despite the fact that basic information on their population ecology is lacking, and several parrot species endemic to Brazil have a small or unknown population size, most of them suffering the effects of habitat loss and poaching pressure for the illegal pet market (Tella and Hiraldo 2014, Pires et al. 2016). This constitutes a concerning association of threats that may lead to the decline of their populations (Allendorf et al. 2008, Olah et al. 2016, Berkunsky et al. 2017).

Under the above circumstances, in the absence of appropriate information, many conservation actions are executed intuitively or hastily, including population size estimates and evaluations of conservation status of threatened species (Robertson et al. 2006, Sanderson 2006, Katzner et al. 2011). Well-intentioned management strategies may not have the expected effects for the benefit of the

populations (Martinez-Abraín and Oro 2013) and can even generate negative effects in the medium and long-term, especially when the ecological and evolutionary attributes of these species are poorly known (Tella 2001).

Research applied to the conservation of wildlife is globally biased, and information is lacking for the regions where it is more needed (Wilson et al. 2016). Moreover, the uncertain information about conservation status of species could add risks to the improvement of knowledge, since funders for research would frequently prioritize to invest in research on Endangered species, being the Least Concern species neglected (Butchart and Bird 2010)

According to Caughley (1994), field research is needed to understand the dynamics of small populations and generate data that will support conservation actions to revert their population declines. Molecular biology tools can also help these population studies (Moore and Kukuk 2002), and when correlated to environmental and demographic variables can show precious information about the evolutionary and ecological traits and conservation threats of the species (Manel et al. 2003). Molecular markers, such as microsatellites and recently single nucleotide polymorphisms (SNPs), can be used for estimating demographic parameters such as population size, genetic structure and diversity, or the presence of genetic bottlenecks, as well as to answer questions related to the breeding behavior of the species (Schlötterer and Pemberton 1994; Schlötterer 2004, Miyaki and Alves 2006). Another important molecular tool is chromosomal sexing (Griffiths et al. 1998), that allows investigating the sex ratio in natural populations. Biases in the sex ratio of juveniles or adults may increase the probability of extinction of small populations through inverse *density-dependence Allee effects* (Courchamp et al. 1999). Thereby, the diagnosis of the sex ratio can be decisive for the design of management strategies aimed to the conservation of endangered species (Stephens and Surtttherland 1999, Robertson et al. 2006). In addition, ecological niche or distribution models can predict the distribution of the species in the landscape, using presence and absence or abundance data from the localities where the species is known to occur (Gillespie et al. 2008).

### 2.1.2 Small populations and vulnerability to extinction

It is known that small populations often have a reduced reproductive in the short-term and survival capacity, and that in the long-term they suffer a decreased ability to react to environmental changes, thereby increasing the risk of extinction (Frankham et al. 2008). A common consequence of population declines is the erosion of genetic diversity, which compromises population viability (Frankham 2005). Effective population size is a key factor influencing the evolutionary potential of a species over the medium to long-term (Frankham 2005; *Figure 1*). Populations with fewer than 500 individuals may be especially vulnerable to extinction, and one of the conservation concerns in long-lived species with small or isolated populations is that they may also suffer inbreeding depression (Frankham et al. 2012, Frankham 2005, 2015).

Deleterious effects of inbreeding depression as a consequence of the erosion of genetic diversity can increase the vulnerability of populations to anthropogenic impacts and environmental perturbations, and also to stochastic events (Mills and Smose 1994, Brook et al. 2002).

On the other hand, genetic drift also occurs in small populations, where infrequently occurring alleles face a greater chance of being lost. Once it begins, genetic drift will continue until the involved allele is either lost by a population or is the only allele present at a particular gene locus within a population. Both possibilities decrease the genetic diversity of a population. Genetic drift is common after a population experiences a population bottleneck. A population bottleneck arises when a significant number of individuals in a population die or are otherwise prevented from breeding, resulting in a drastic decrease in the size of the population. Genetic drift can result in the loss of rare alleles, and can decrease the size of the gene pool.

A species with a broad distribution rarely has the same genetic structure over its entire range, and a rapid population growth tends to lead to the retention of new mutations (Lynch et al. 1995, Land et al. 1995, Gazave et al. 2013). Thus,

population expansion is also important for the maintenance of genetic diversity levels (Frankham et al. 2005, Biere et al. 2012).

Considering all the above-mentioned aspects, if a small population has an acute decrease in a short period of time, their genetic attributes may be involved; *Figure 1*). Consequently, it is necessary to incorporate genetic diversity assessment to the management programs designed for the recovery and conservation of populations that present such a demographic history (Bell et al. 2019).

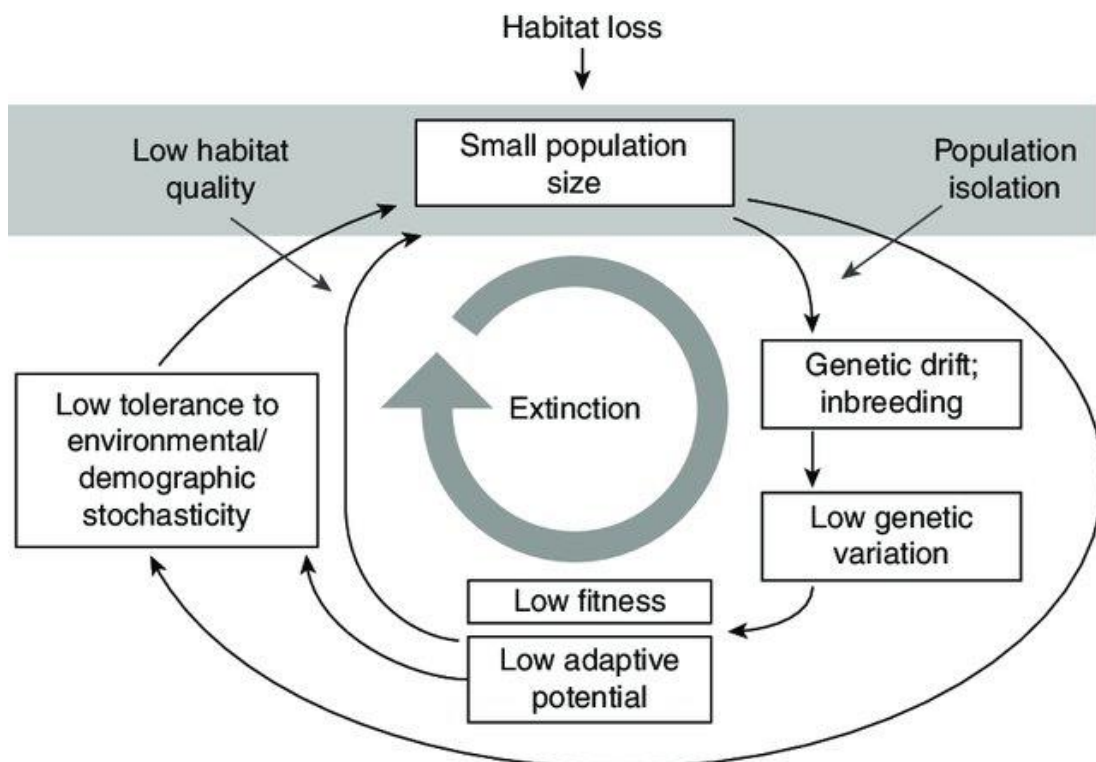


Figure 1. Small populations and vulnerability to extinction according Frankham et al. (2005), from Biere et al. (2012)

### 2.1.3 Lear's Macaw as a study case: questions and concerns

The Lear's Macaw (*Anodorhynchus leari*) wild population was discovered in 1979 in the state of Bahia (BA), being endemic to the *Caatinga* biome (Brazilian tropical dry forests). This population was considered to be declining, with an estimated global population size ranging between 60 and 200 individuals (Yamashita 1987). Several conservation issues have been diagnosed for the species after its discovery in the wild, such as the illegal trade for the pet market, the intense hunting activities in the area, and habitat loss affecting the Licuri Palm (*Syagrus coronata*), which fruits constitute the bulk of the diet of Lear's Macaws (Sick and Teixeira 1980, Yamashita et al. 1987).

The maximum lifespan in captivity of Lear's Macaw has been estimated in 45 years (Young et al. 2012). It is a large-bodied parrot (~75cm, ~800g, Forshw 2017) with high mobility (estimated by field observation of banded birds in ~45 km/daily from communal roosts to feeding sites) (Pacífico et al. 2018). Based on long-term observations of the breeding behavior and knowledge about phylogenetically related macaws, we expect for this species a late age of sexual maturity (~6 years), a monogamous mating system, and a high fidelity of breeding pairs to their nests-cavities, exclusively located in deep cavities at sandstone cliffs in the intermittent rivers areas (Pacífico 2011).

Two protected areas are safeguarding the Lear's Macaw nests: the Canudos Biological Station (CBS), created in 1993 by the NGO *Fundação Biodiversitas*, and the *Serra Branca* Environmental Protection Area from Brazilian Government Agency (IBAMA), created in 2001 in the south of the *Raso da Catarina* Ecological Station (RCES). The *Area de Proteção Ambiental da Serra Branca* (APA Serra Branca) overlaps the *Serra Branca* Farm (a private land used to access the RCES). Intensive education and enforcement work was done in these areas to reduce nest poaching and persecution, allowing that the population increased in the last decade (ICMBio 2012). Therefore, based in roost

counts it is assumed that the actual population has increased in the location where it is concentrated, the *Raso da Catarina* ecoregion, increasing from < 200 individuals in 2000 to > 1,200 in 2014 at least (ICMBio, 2012). Thus, the global conservation status of the species was recently downlisted by the IUCN Red from Critically Endangered to Endangered (BirdLife International 2019).

Despite of this population growth, there is another population 240 km far from the known breeding and roosting sites at *Raso da Catarina* Ecoregion that hold 36 individuals in 1994 but was reduced next year to only two individuals, and no further monitoring or detailed information was taken about it. ICMBio (2012) also affirms that the species has disappeared from all other unprotected areas with historic accounts of the occurrence of roosting sites.

The *Caatinga* biome is intensely threatened by agriculture, farming, overgrazing, illegal charcoal production, poverty and droughts, which cause an accelerated desertification (Leal et al. 2005, Oliveira et al. 2012). In this scenario, just 1% of the natural *Caatinga* landscape is legally protected, and there is a large lack of scientific knowledge on this biome (Milles et al. 2006, Santos et al. 2011).

Additionally, local farmers in the Raso da Catarina have been reported predation of their corn crops by Lear's Macaws, thus exposing the species to be killed by shot by the farmers (Brandt and Machado 1990, Santos-Neto and Gomes 2007). The species was reclassified by IUCN from "Critically Endangered" to "Endangered" due to the recent population increase in the Raso da Catarina Ecoregion (BirdLife International 2019). The fact that the roosting and breeding sites are restricted to two close areas (c. 37 km apart) indicate that there is a single population, and add concerns on the long-term persistence of the species.

The fact that the main roosting and breeding sites of Lear's Macaws are restricted to two close areas (c. 37 km apart) indicates that there is a single population, thus adding concerns on the long-term persistence of the species. This spatially concentrated population may suggest two alternative scenarios: (1) there are other suitable areas, but the long-term process of population reduction before the 80's ended on the current spatially-restricted population, or (2) there

is no more appropriate habitat available for the species. In any case, we must consider the possibility that the population crossed a genetic bottleneck, and current loss of genetic diversity and endogamy could compromise their long-term viability. Thus, we must consider their vulnerability both to environmental and demographic stochasticity (*Figure 2*).

In the second scenario, there is the possibility that the Licuri Palm population is not large enough and sufficiently productive across seasons to support the trophic requirements of the Lear's Macaw out of its current distribution, even inducing the species to look for alternative food, such as corn crops. Generally, corn is less nutritive than wild fruits and seeds, and therefore it is expected that its use may affect the health, the breeding success and the sex ratio of Lear's Macaw (Derrickson and Snyder 1992). Additionally, local farmers in the *Raso da Catarina* reported predation of their corn crops by Lear's Macaws, thus exposing the species to be shot by farmers (Brandt and Machado 1990, Santos-Neto and Gomes 2007).

Breeding of Lear's Macaw has been exclusively recorded in deep natural cavities in sandstone cliffs (Pacífico 2011). Long-term studies have shown that large macaws are faithful to their nests, breeding for several years in the same cavities (Guedes 1993, 2009), and then the availability of nesting cavities can be limiting their populations (Ball et al. 1999). On the other hand, the breeding performance of Lear's Macaw could be related to the Licuri Palm fructification. However, the distances traveled by macaws between breeding and foraging sites are not well known (Brandt and Machado 1990). Thus, it is unknown if the extent and distribution of foraging sites can limit the Lear's Macaw distribution.

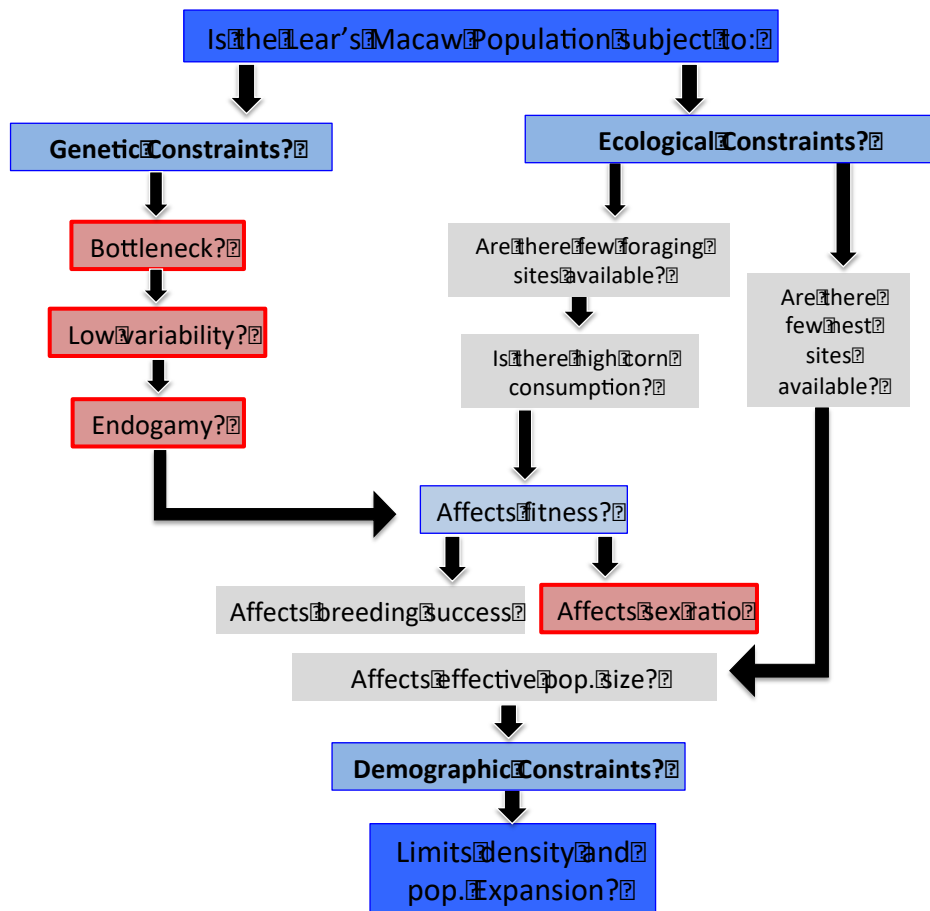


Figure 2. Flow-chart with the scenarios and questions that concerns the Lear's Macaw population.

Studies related to habitat availability, population genetics and sex ratio are considered of high priority according to the National Action Plan for the Lear's Macaw Conservation (ICMbio 2012). The following hypotheses can be tested to provide meaningful data to support conservation actions: Are the breeding success and the distribution of the population the Lear's Macaw constrained by habitat availability? Is the species genetically constrained? There is a sex bias in the population?

This thesis is justified by the need to assess the conservation problems mentioned above through a multi-disciplinary approach, including aspects of demography, population genetics and ecological modeling to generate scientific data to support the conservation of the globally endangered Lear's Macaw.

## 2.2 GOALS

The objective of this thesis is to investigate the environmental conditions, demographic traits and conservation threats of the remaining population of the Lear's Macaw. This research benefits from long term and intensive fieldwork efforts, combined with tools such as ecological modeling and molecular analyses. All data generated will be used to evaluate the potential of the studied population to increase and expand. My final goal is to increase our understanding of the Lear's Macaw population dynamics to support the application of public and private resources toward the conservation planning and actions for this globally endangered species.

### 2.2.1 Hypotheses and Specific Goals

The general objective has been addressed through the following specific goals and supporting hypotheses:

Hypothesis 1. The increasing Lear's Macaw population is spatially concentrated and needs to expand geographically.

- Specific goal 1: To estimate the number of breeding pairs and main breeding parameters to better understand the population demography. (Section 1)
- Specific goal 2: To estimate population size and growth over the last decade by applying a detection-based analysis (N-mixture model) on the replicated count data of the global population of macaws gathering at roosts in order to improve inferences about population trend and to make recommendations for more robust monitoring methods. (Section 2)

Hypothesis 2. The Lear's Macaw population is genetically constrained due its demography history.

- Specific goal 3: To develop a molecular toolkit with a set of specific microsatellite markers optimized for their use in non-invasive samples (e.g. molted feathers), to support population genetic research and the development of a genetic monitoring program for the integrated conservation management of the species. (Section 3)
- Specific goal 4: To assess the genetic population status of the species in the wild, based on individual genotypes obtained from molted feathers systematically collected from nests and all known roosting areas, allowing estimating genetic diversity and population size for each subpopulation and the genetic structure across the study area. (Section 4)

Hypothesis 3. The current and historical ranges of the Lear's Macaw are degraded due to anthropogenic disturbance. Foraging, nesting and roosting areas need restoration to allow population expansion and conservation.

- Specific goal 5: To to increase our knowledge on the historic and current distribution of the Lear's Macaw and on the past and current threats for the species, finally modeling the suitability of habitats and its main food resource (Licuri Palm) to better make predictions on the potential expansion of the species (Section 5)
- Specific goal 6. Investigate the extent of Africanized honey bee occurrence in the breeding cliffs of Lear's Macaws and the potential competition for nesting sites; and verify if the elimination of honey bee hives from cavities would increase nest availability and favor recruitment of macaw nesting pairs, allowing the macaw breeding population growth and expand to historical areas of occurrence. (Section 6)

## 2.3 INTRODUCTION: DEMOGRAPHY HISTORY

### 2.3.1 Section 1 - Breeding to non-breeding population ratio and breeding performance of the globally Endangered Lear's Macaw: conservation and monitoring implications<sup>1</sup>

The Lear's Macaw *Anodorhynchus leari* is endemic to the 'Caatinga' biome and considered as globally "Endangered" by IUCN (BirdLife International 2019). Its distribution is restricted to a small area in the Northeast of Bahia state, Brazil. Most known activities of the species are concentrated in two protected areas, Raso da Catarina Ecological Station (RCES) and Canudos Biological Station (CBS), where the whole population nests and roosts communally in the same cliff areas (Menezes et al. 2006). However, the birds perform daily movements from these sites to forage in neighboring unprotected areas (Brandt and Machado 1990, Santos-Neto and Camandaroba 2008, Silva-Neto et al. 2012).

The organizations CEMAVE/ICMBio and Biodiversitas Foundation have assessed changes in the population size of Lear's Macaw through the post-breeding monitoring of the two communal roosts since 1998, with standardized annual censuses conducted since 2004 (ICMBio 2012). In recent years, a population increase has been observed and although the long-term population increase could be partially explained by a more consistent monitoring effort, there is a consensus that the species is recovering in numbers of macaws counted in the roosting cliffs in the last decades. Due to these increases in overall population size, BirdLife International (2019) downgraded the threat category of the species from "Critically Endangered" (CR) to "Endangered" (EN) in the 2009 IUCN Red List of Threatened Species, based on the estimate of more than 250 mature

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<sup>1</sup> This paper was writing in collaboration with the following authors: Erica C. Pacífico, Eduardo A. Barbosa, Thiago Filadelfo, Kleber G. Oliveira, Luís F. Silveira And José L. Tella; and published in Bird Conservation International in March 2014.

individuals capable of reproduction (excluding those that will not produce new recruits).

Estimating the number of mature individuals is challenging for many species from which accurate population biology information is not available. Therefore, this number is often obtained by applying an assumed proportion of individuals that are mature to the estimated whole population size, an approach that often leads to gross overestimates of number of mature individuals (IUCN 2013). Especially in the case of long-lived species with deferred maturity, as in Lear's Macaw (Young et al. 2012), both the numbers of mature individuals and the breeding fraction may be much smaller than the non-breeding part of the population (Kenward et al. 2000, Negro 2011). There is however a marked scarcity of information on breeding to non-breeding ratios in birds, which may undermine the design of proper conservation actions, since these population fractions are often exposed to different threats related to their different use of space and resources (Penteriani et al. 2011).

The overall population size is reasonably well known for the Lear's Macaw. However, there is no information on the proportion of breeding birds, and the fact that sub-adults may form pairs and behave like nesting birds for a number of years before they actually breed might difficult its estimation. In the same way, most aspects of the breeding biology of the species are virtually unknown in the wild (Juniper and Parr 2010). The study of the reproductive success of Lear's Macaws results thus essential for a better design of conservation actions (BirdLife International 2019). The estimation of breeding parameters would help to understand the population ecology of the species (e.g., Carrete et al. 2006a), to better assess the threats the species is facing to, and for predicting population growth and extinction risk in the long-term through population viability analyses (Oro et al. 2008).

Given the importance of knowing the proportion of the population that is breeding and its breeding success, the Management Plan for the Conservation of Lear's Macaw considers the assessment of its breeding population size and breeding parameters as high priorities (ICMBio 2012). Therefore, the aim of this study was to estimate the number of breeding pairs and main breeding parameters for a better knowledge of the population ecology, conservation and monitoring needs of the species.



[Click on the macaws to continue the reading of materials and methods of section 1: 3.2.1](#)

### **2.3.2 Section 2 - Estimating population size and growth with a heterogeneous long-term census: population trends of the endangered Lear's Macaw<sup>2</sup>**

At least 437 species from 30 families of birds, including corvids, waterfowl, seabirds, raptors, vultures, herons, parrots, shorebirds and passerines, roost communally for all or part of the year (Beauchamp 1999). Communal roosts may reduce thermoregulatory demands, decrease predation risks, and serve a social and information function that increases foraging efficiency or mate acquisition (e.g., Chapman et al. 1989, Beauchamp 1999, Blanco and Tella 1999, Wright et al. 2003, Dwyer et al. 2018). Roosts also provide an opportunity for monitoring the size of bird populations (Berg and Angel 2006, Blanco et al. 2014). Counting the number of individuals arriving at, departing from, or attending roosts may produce an index of abundance as a proxy measurement for population size and provide reliable information on seasonal and annual changes of abundance and habitat utilization (Buckland et al. 2008, Conklin et al. 2008, Lambertucci 2010). Roost counts have often been used to monitor species that are widely dispersed during the day but concentrate in a few locations in the evening (e.g. Fuller and Mosher 1981, Bibby et al. 2000, Dénes et al. 2017).

Roost surveys, however, provide a number of analytical challenges for estimating population size and trend (Casagrande and Beissinger 1997, Dénes et al. 2017). The sizes and numbers of roosts can vary daily and seasonally (Lambertucci 2010, Seixas and Mourão 2018). Roosts can be difficult to detect, particularly in forest habitat (Gilardi and Munn 1998, Lee and Marsden 2012). In addition, roost locations may change frequently (Casagrande and Beissinger 1997). Moreover, detecting and counting individuals leaving from or arriving to roosts may be difficult when flock sizes are large, reducing the accuracy of counts

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<sup>2</sup> This manuscript was written in collaboration with the following authors: Erica C. Pacífico; A. Eduardo A. Barbosa; Francisco V. Dénes, Thiago Filadelfo, Antonio E. B. A. de Sousa, Andreza C. A. do Amaral, João L. X. do Nascimento, José L. Tella; Steven R. Beissinger; and submitted to *The Condor Ornithological Applications* in November of 2019.

(Dénes et al. 2017). Nevertheless, recent analytical advances have been used to overcome some of these challenges, particularly problems associated with detectability (e.g. Royle 2004, Kéry et al. 2005, Kéry 2008, Piepho and Oguto 2002, Schmidt et al. 2019). The ability to explicitly model detection probability as a function of covariates, while estimating abundance, has increased our understanding of the detection process, which in turn may improve sampling protocols and survey techniques for estimating abundance (Dénes et al. 2015).

The Lear's Macaw (*Anodorhynchus leari*) is a globally endangered parrot (Birdlife International 2018) that usually roosts communally in sandstone cliffs (Figure 3 A, B). Roost counts done by the National Center for Bird Conservation and Research (CEMAVE-ICMBio, Brazil) indicate a continuously growing population since the first counts were made in the 1980's (ICMBio 2012). Population increases of the Lear's Macaw may be the result of national and international, private and government investments in local conservation and public policy (Barbosa and Tella 2019).

The recent increase in macaws numbers and number of roosting cliffs used prompted the down-listing of the conservation status of the Lear's Macaw from Critically Endangered to Endangered (BirdLife International 2019). However, these population estimates were based on annual averages of morning and evening counts of macaws conducted at two well-known communal roosting areas (Yamashita 1987, Nascimento et al. 2001, Menezes et al. 2006). These averages did not account for seasonal variation in roost numbers that can result from pairs leaving roosts to attend their nests during breeding season (Gnam and Burchsted 1991, Cougill and Marsden 2004). Moreover, at dawn macaws tend to leave roosts in groups of sizes exceeding dozens individuals (Figure 3 C), while in the evening they commonly return to roosts in smaller groups or in pairs, often in the dark after sunset (Figure 3 D; Menezes et al. 2006). Both behaviors make it difficult to produce accurate estimates of the number of birds attending roosts.

The goal of our study is to estimate population size and growth over the last decade for the Lear's Macaw by applying a detection-based analysis, the N-mixture model (Royle 2004), to the replicated counts of the global population of

macaws at roosts. Recognizing that abundance estimates from N-mixture models can be sensitive to violations of the choice of distributional assumptions and sparse data (Joseph et al. 2009, Barker et al. 2018), we explore multiple formulations of mixture models and evaluate goodness of fit, treating our results as relative abundance indices rather than estimates of absolute abundance. Our motivations are to improve inferences of population trend and to make recommendations for more robust monitoring methods. Better estimates of the population size and trend of the Lear's Macaw will increase the efficient application of limited public resources in Brazil for research on this endangered species and planning for its conservation.

[Click on the macaws to continue the reading of materials and methods of section 2: 3.2.2](#)



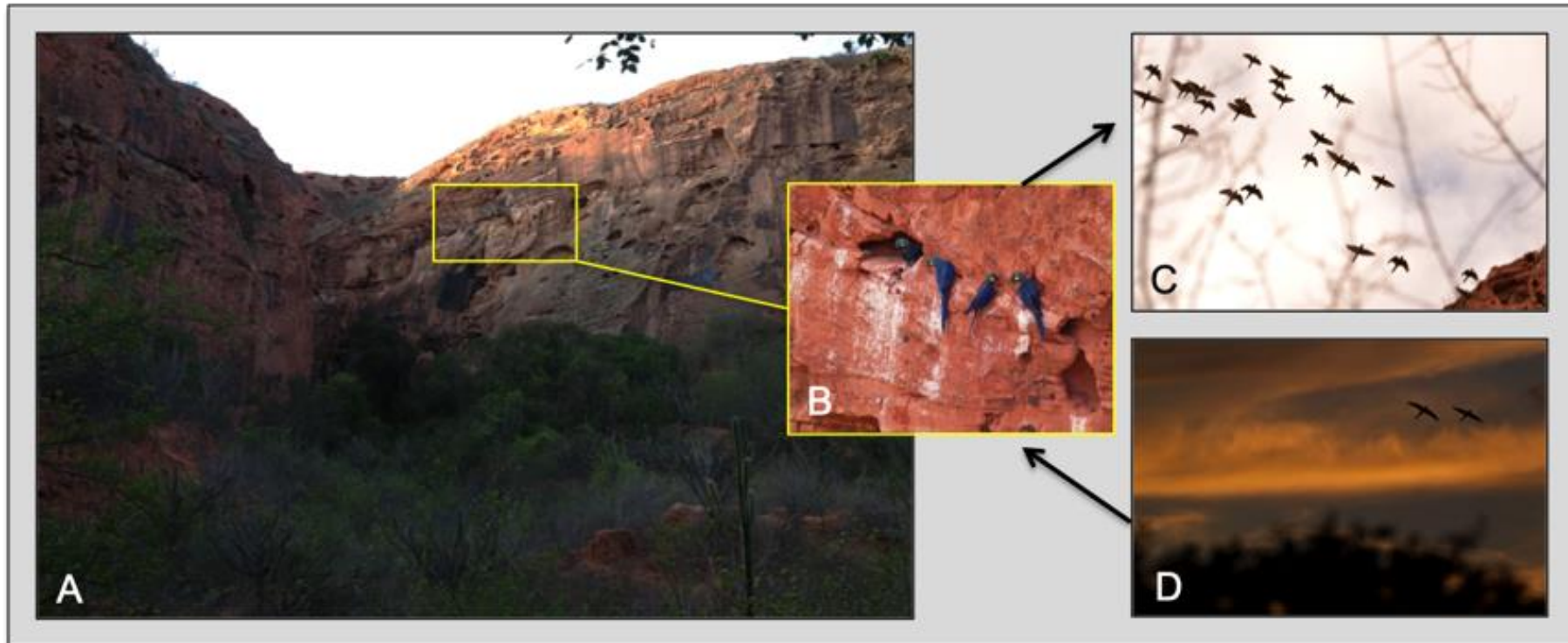


Figure 3. The collective roosting behavior of Lear's Macaws on sandstone cliffs in Toca Velha, Canudos Biological Station, Canudos, BA, Brazil (A) and macaws and their feces on the cliffs provide evidence for locating the communal roosts (B). Macaws leaving roosts in larger groups in the early morning at dawn (C), and returning to roosts in smaller groups or in pairs the evening, often-in dark conditions after sunset (D). Photo taken at Canudos Biological Station roosting (Biodiversitas Foundation) provided by Patrick I. Pina (A), João M. Rosa (B and D), Erica C. Pacifico (C)

## 2.4 INTRODUCTION: POPULATION GENETICS

### 2.4.1 Section 3 - Isolation and characterization of 15 new specific microsatellite markers for the Lear's Macaw<sup>3</sup>

Wild populations of parrots are threatened by habitat loss due to multiple anthropogenic impacts like overgrazing, deforestation, introduction of alien species, or disturbance of nesting and roosting sites (Berkunski et al. 2017). These impacts, along with the persistence of poaching, affect their ecological relationships and breeding capacity, potentially causing dramatic population declines (Olah et al. 2016a, Forshaw 2017, Heinsohn et al. 2015). Such declines can in turn cause depletions of genetic diversity, which can expose relict populations to the detrimental consequences of inbreeding and genetic drift (Frankhan 2005). In the case of endemic species with small distribution ranges, genetically impoverished populations may ultimately rely on demography rescue management to persist (Raisin et al. 2012, Holderegger et al. 2019).

Assessing demographic parameters such as effective population sizes, genetic diversity and inbreeding levels in natural populations of parrots is paramount for their conservation. However, estimating these parameters in wild populations is challenging, especially in dense tropical forests and other habitats where parrot tissue sampling is difficult (Olah et al. 2016b Rivera-Ortiz et al. 2017). Another challenge to obtain such genetic information is the availability of specific molecular markers such as microsatellites (Presti et al 2011, Presti and Wasko 2014). This is the case of the globally endangered Lear's Macaw, which presents a relict population with an reduced distribution range (BirdLife International, 2019). An additional population, isolated from the main nucleus and considered as functionally extinct, is requiring urgent management actions for its persistence (Filadelfo and Pacífico 2017). In this scenario, information on genetic parameters from both wild and captive populations

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<sup>3</sup> This manuscript was writing in collaboration with the following authors: Erica C. Pacífico, Gregorio Sánchez-Montes, Cristina Y. Miyaki, José L. Tella

is necessary to plan population management actions and to support the captive breeding program (Frankham 2008; Holderegger et al. 2019).

Recently, Jan and Fumagalli (2016) published primer sets of microsatellites for seven endangered parrot species, including 16 markers for *A. leari*. The present study complements this molecular toolkit with an additional set of 15 microsatellite markers specific for *A. leari*, which we tested on DNA samples from blood and feathers of wild macaws in a 3-step optimization procedure. Our new set, together with previous markers, supports the development of a population genetic research and a monitoring program to be applied for the integrated conservation management of this emblematic species.

[Click on the macaws to continue the reading of materials and methods of section 3: 3.3.1](#)



## 2.4.2 Section 4 – Population status assessment from non-invasive genetic samples<sup>4</sup>

Macaws are long-living Neotropical birds whose natural populations are threatened by multiple anthropogenic impacts (Berkunski 2017). In the last decades, many parrot species have experienced extreme reductions in their ranges of distribution caused by destruction and degradation of their habitats (Marsden and Royle 2015). Furthermore, persistent poaching has dramatically reduced wild populations and led to generalized declines, which are often overlooked in species with large ranges and with conservation status categorized as ‘least concern’ by IUCN (eg. *Ara chloropterus*, *Primolius maracana*) (Bodrati et al. 2006, Nunes et al. 2007, BirdLife International 2019).

A common consequence of such population declines is the erosion of genetic diversity, which compromises population viability (Frankhan 2005). The effective population size is a key demographic parameter informing about the evolutionary potential of a species over the medium to long term (Frankham 2005). Populations with effective sizes <500 may be especially vulnerable to extinction, and one of the conservation concerns in long-lived species with small or isolated populations is that they may be also subject to *inbreeding depression* (Frankham et al. 2012, Frankham 2005, 2015). Deleterious effects of *inbreeding depression* can increase the vulnerability of populations to anthropogenic impacts and environmental perturbations, and also to stochastic events (Mills and Smose 1994, Brook et al. 2002). Consequently, population status assessments based on genetic diversity characterization are urgently required to guide the conservation management programs of small populations affected by demographic declines (Frankham 2008, Frankhan et al. 2011, Bell et al. 2019), and the recovery of post-bottlenecked populations must be supervised (Lynch et al. 1995, Land et al. 1995, Gazave et al.

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<sup>4</sup> This manuscript was writing in collaboration with the following authors: Erica C. Pacífico, Gregorio Sánchez-Montes, Thiago Filadelfo, Fernanda R. Paschotto, Fernando Hiraldo Cano, Jose Antonio Godoy, Cristina Y. Miyaki and Jose L. Tella

2013).

One such case is the endangered Lear's Macaw (*Anodorhynchus leari*), endemic to the *Caatinga* dry forest in north-central Bahia state, Brazil. The species was thought to be extinct in the wild until a small group of macaws was located in 1978 in the *Raso da Catarina* (RASO) Ecoregion (Sick et al. 1989, Yamashita 1987). In other less intensely investigated areas, Lear's Macaws have apparently disappeared (e.g. *Baixa do Chico*, *Serra da Borracha*, *Serra da Canabrava*) or population has suffered such strong declines that no roosting or nesting locations have been found in the recent years (e.g. *Boqueirão da Onça*) (see below and Section 5). The putatively single relict population of *A. leari* probably shrunk following a long-term decline and has since remained concentrated in a few close roosting areas (Nascimento et al 2001). After intensive conservation efforts, this population has experienced an outstanding demographic recovery, and annual counts performed by CEMAVE-ICMBio (Brazilian Environmental Agency) have registered a 10-fold increase in population size, rising from about two hundred birds in 2001 to more than two-thousand individuals estimated in 2014 (See Section 2). The recovery of this relict population and the establishment of a solid conservation program motivated the reassessment of its IUCN category from Critically Endangered to 'Endangered' in 2009 (IUCN 2013, Barbosa and Tella 2019).

Additional minor breeding nuclei of *A. leari* may have, however, remained overlooked. According to local farmers, the *Boqueirão da Onça* (BDO) area historically retained more than 100 individuals, until bird traffickers systematically persecuted the macaws in their roosts. While RASO population was increasing, in the past 10 years, just two individuals were observed at BDO, feeding at *Cercadinho* Farm, and their nocturnal roosting site was not located. No evidence was found to suggest that BDO macaws were once in contact with the RASO population, so both nuclei might correspond to two different breeding units (see Section 5). This is an example of the important knowledge gaps that remain regarding the actual number of populations of *A. leari*, their connectivity and the magnitude of recent declines or extinctions across the specie's range. Many of these gaps will be appropriately addressed with the inclusion of genetic tools to the demographic research.

In the case of *A. leari*, like other parrots, it is key to develop protocols for

genetic diversity characterization from non-invasive tissue samples, to avoid stressful capture and manipulation of individuals. Also, Neotropical parrots in general have high mobility and live in poorly accessible habitats like cavities in cliffs and the canopy of deep rain forest. Therefore, non-invasive sampling protocols are essential to obtain sufficient and representative samples (like molted feathers) across all the age-classes of the population (e.g. breeders and non-breeders, in addition to nestlings which were more accessible to capture before fledging) (Presti et al. 2013, Olah et al. 2016b, Pacífico et al. 2018).

Considering the documented demographic history of *A. leari* in the last decades, characterized by strong population size fluctuations and recent expansion from RASO to other historical and new areas (see section 5), we hypothesize that the current *A. leari* population may suffer from genetic impoverishment as a result of a severe bottleneck. Therefore, the present work aims to assess the genetic status of the species in the wild, based on individual genotypes obtained from molted feathers systematically collected from nests and from all known roosting areas. We used specific microsatellites to genotype non-invasive samples (molted feathers) collected from localities across the entire range of distribution of *A. leari* in the RASO Ecoregion. Based on these data, we were able to 1) identify unique individuals and exclude repeated molted feather sampling, 2) estimate the genetic diversity and effective population size for each locality and the entire dataset and 3) assess the genetic structure across the study area. We also obtained preliminary insights about the sex ratio of the population for further detailed investigation. This research represents a first step towards a protocol for a genetic monitoring program of the population of this globally endangered species in the wild.

[Click in the macaws to continue the reading of materials and methods of section 4: item 3.3.2](#)



## 2.5 INTRODUCTION: THREATS

### 2.5.1 Section 5 - Past and current threats of the Lear's Macaw: implications for distribution and population expansion<sup>5</sup>

Very little is known about the ecology and conservation status of many species with a restricted occurrence in the world, and this especially applies to Neotropical parrots (Berkunsky *et al.* 2017). As a good example, 60.2% of the 88 species of parrots that occur in Brazil show decreasing populations trends and, controversially, 51% are categorized as of Least Concern by the IUCN Red List (BirdLife International 2017). Moreover, basic information on the biology, ecology, and/or accurate distribution ranges is lacking for most of these species (BirdLife International 2017). Populations are certainly suffering the effects of habitat loss and poaching pressure for the illegal local markets (Tella and Hiraldo 2014, Pires *et al.* 2016), but the actual contribution of these threats on population declines have not been evaluated for most of the species (Alves *et al.* 2013, Olah *et al.* 2016a, BirdLife International 2017).

In the last 20 years, two enigmatic and threatened parrot species, Spix's macaw (*Cyanopsitta spixii*) and Lear's Macaw (*Anodorhynchus leari*), endemic to the Caatinga Biome, northeastern Brazil had contrasting conservation outcomes. Field studies and conservation efforts were not sufficient to save the remaining individuals of the Spix's macaw (Juniper and Yamashita 1990, 1991), which is believed to be extinct in the wild, and much remains unknown about its range of distribution, natural history and ecological relationships (Mattos *et al.* 2005). On the other hand, active conservation efforts and long-term monitoring greatly increased the understanding of the basic biology and conservation needs of the Lear's Macaw (Nascimento *et al.* 2001; Pacifico *et al.* 2014, Barbosa and Tella 2019). Although the species is recovering in numbers - model predictions indicate that between 2004 and 2014 the Lear's Macaw population tripled in size from 665 to 2217 individuals (see section 2) -

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<sup>5</sup> This manuscript was writing in collaboration with the following authors: Erica C. Pacifico, Thiago Filadelfo, Mariana de Carvalho, Fernanda R. Paschotto, A. Eduardo A. Barbosa, Steven R. Beissinger, Fernando Hiraldo-Cano, Jose L. Tella, Francisco V. Denes

it is categorized as globally Endangered (BirdLife International 2017) and detailed information about the past and current threats that might persist and their effects (e.g. relative intensity of the threats, or how threats affect population dynamics and expansion) is still lacking.

The loss of food resources due to deforestation and overgrazing is considered one of the main threats for this species (BirdLife International 2017). Particularly, the reduction of Licuri Palm (*Syagrus coronata* Mart. Becc.) patches, whose fruits are key in the diet of the Lear's Macaw (Brandt and Machado 1998) taking also into account the poor habitat quality conditions (e.g. ecosystem degradation, habitat shifts and alteration by fire suppression, small-holder ranching and farming) (Andrade et al. 2015). Additionally, Lear's Macaws are occasionally persecuted and killed in corn plantations due to their crop damages (Manso 2014). Consumption of corn supposedly occurs when Licuri Palm fruits are scarce, but there is no evidence to support this information and no investigation on the seasonality of food resources and availability of foraging habitats has been conducted to date (ICMBio 2012). Consequently, the potential detrimental effects of low food availability on recruitment and population viability of Lear's Macaws remain to be assessed.

Another conservation concern for the Lear's Macaw is that, since the discovery of its occurrence in the wild in 1978 (Sick and Teixeira 1980), only two well-defined subpopulations, separated by 37 km, were known and monitored at Raso da Catarina ecoregion (RASO). Both subpopulations are in sites that hold sandstone cliffs used for communal breeding and roosting. The regular annual bird counts conducted by the Brazilian government in these two localities suggested the displacement of individuals between them, thus constituting a single small population (Menezes et al. 2006, Pacífico et al. 2014, see Section 2).

Another group of Lear's Macaws was found in August 1994 in Boqueirão da Onça (BDO) (Munn 1995) 230km west to the RASO population, in a different ecoregion called *Depressão Sertaneja Meridional*, characterized by higher-altitude dry forests (Veloso 2002). This group of c. 30 macaws was never seen again, and one year later only two individuals were observed (Dorivaldo pers. com. 2019). This population was never monitored, but it was considered as part of the same population found in RASO (Develey *in litt.* pud BirdLife International 2019). However, there is no

evidence to support this assumption, and thus two clear isolated areas for the range distribution of the species are considered (ICMBio, 2012).

Two federal protected areas (*Parque Nacional do Boqueirão da Onça*, since 2019, and *Estação Ecológica do Raso da Catarina*, since 1960), and a protected private propriety (*Estação Biológica de Canudos*, since 1990) overlap the range distribution of the species. However, the degree of protection in these areas and their surroundings is variable considering the anthropogenic activities that cause long term ecological impacts (e.g. free-range cattle, wildlife illegal hunting, bird poaching), which are strongly associated to the absence of regular surveillance and control (Silva et al. 2017, Shulz et al. 2018, Antongiovanni et al. 2018).

The increase in the Lear's Macaw remaining population in RASO is associated to the conservation efforts made in the pass 20 years, especially due to the protection of the principals breeding and roosting sites (Barbosa and Tella 2019). However, it remains unclear which specific conservation action, or combination of actions, were the most effective in contributing to the population recovery (Barbosa and Tella 2019). The persistent anthropogenic activities within the distribution range of the species add concerns about potential habitat constrictions that could increase vulnerability to environmental and demographic stochasticity in the remaining population (Caughley 1994), being also counterproductive for the conservation efforts made in the last decades to recover it.

In this study, we aimed to increase our understanding of the historic and current distribution of Lear's Macaw. We assess whether the increasing population in RASO could expand geographically or its expansion is constrained by the saturation of environmental resources, derived from persistent threats. We hypothesized that the extant subpopulations (in RASO and BDO) were inter-connected within a significantly larger distribution range. We expected that Lear's Macaw population originally was a metapopulation with many nucleus and that these nuclei were progressively isolated and became extinct during a population decline process. In the same line of reasoning, the increasing population in RASO should expand geographically, occupying new areas or historical areas where the species was locally extinct. We thus expected to find newly occupied breeding areas distant to RASO. However, such a geographic expansion could be constrained by current anthropogenic activities that

can directly or indirectly (e.g., limiting food resources such as Licuri Palm availability) affect the species.

With the above hypotheses in mind, we searched through the current and hypothetical historic distribution of the species to identify habitat conditions and persistent threats that could affect its remaining distribution range and limit its expansion. Specifically, our goals were to: 1) identify former local Lear's Macaw populations and its causes of extinction, through interviewing elderly people, 2) search for potential newly-occupied areas, 3) verify the habitat conditions at the current distribution of the species and historical areas, including potential areas for population expansion, 4) compile and assess the intensity of current threats for the species, and 5) build habitat suitability models for the Lear's Macaw and the Licuri Palm. Altogether, our results contribute for a better understanding of the potential future expansion of Lear's Macaws and the current threats that could constraint it, and to guide conservation actions for the recovery of this endangered species.

Click on the macaws to continue the reading of materials and methods of section 5: 3.4.1



## 2.5.2 Section 6 - Experimental removal of invasive Africanized honey bees to avoid competition for nest cavities of the endangered Lear's Macaw<sup>6</sup>

The Africanized honey bee (AHB) is a hybrid of two subspecies, the African honey bee (*Apis mellifera scutellata*) and the European honey bee (EHB, *Apis mellifera mellifera*) (Harrison et al. 2006). Since its initial introduction in São Paulo, Brazil in 1956, the AHB spread quickly north throughout South America, Central America and the Southwestern United States, becoming one of the most successfully invasive insects worldwide (Michener 1973, Kent 1989) AHB were brought into Brazil because they would be better adapted to the tropical climate than the temperate EHB; they are also more aggressive, reproduce faster, and utilize a wider range of nest sites compared to EHB (Collins et al. 1982, Winston 1992, De Jong 1996).

The aggressive behavior of AHB often bring them into conflict with humans in urban and rural areas, and their uncontrolled spread in natural areas can negatively affect wildlife (Oldroyd et al. 1994, Pereira and Chaud-Netto 2005). Competition between invasive AHB and native bees has resulted in population declines of the latter in their natural ecosystems worldwide (Dupont et al. 2004, Lindstrom et al. 2016, Valido et al. 2019) even driving some species to local extinctions (Torné-Noguera et al. 2016, Henry and Rodet 2018). Moreover, invasive AHB can build their hives in tree holes used as nesting sites by native species, including vertebrates (Coelho and Sullivan 1994, Veiga et al. 2013).

It is recognized that nest site availability might constrain breeding populations and reproductive success in cavity nesting birds Newton (1994), Sánchez et al. (2007), Cockle et al. (2010) although more evidence is needed to the understanding of this limitation in natural landscapes (Wiebe 2011). Parrots (Order Psittaciformes) are mostly obligate cavity-nesting species and many species are threatened with extinction, especially in the Neotropics (Olah et al. 2016a, Forshaw 2017). The

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<sup>6</sup> This manuscript was writing in collaboration with the following authors: Erica C. Pacífico, Caroline A. Efstathion, Thiago Filadelfo, Robert Horsburgh, Roberta C. Alves, Fernanda R. Paschotto, Francisco V. Denes, James Gilardi and José L. Tella; and was submitted to Pest Management Science in September of 2019.

provision of artificial nest-boxes is widely used as a conservation tool to increase nest-site availability. However, there has been little evidence systematically recorded on the effectiveness of artificial nests on the recruitment and productivity of the target species. Moreover, there are concerns about their frequent use by invasive species like AHB and predators (Berkunsky et al. 2017). The presence of invasive alien species in nests is one of the threats recognized for Neotropical parrots (Lindenmayer et al. 2009). Some actions to prevent the occupation of nest-boxes by AHB have been assessed to increase recruitment of an endangered parrot in the Atlantic rain forest (Efstathion et al. 2015). However, the extent of nest site competition between AHB and parrots has not been well studied (Vaughan et al. 2003, Kilpp et al. 2014, Bonaparte and Cockle 2017).

Here, we focus on nest-site competition between AHB and a globally endangered large parrot species (75 cm length), the Lear's Macaw *Anodorhynchus leari* (hereafter "macaw"). This species is endemic to the Brazilian Caatinga dry forest biome where the proliferation of exotic species such as AHB, in combination with other chronic and acute disturbances (e.g deforestation and overgrazing), is causing the disruption of critical ecological services and the loss of suitable habitats for macaws (Silva et al. 2017, Schulz et al. 2018, BirdLife Internacional 2019) The area of occurrence in the wild of this macaw species was discovered in 1978 and had an estimated global population smaller than 200 individuals at that time (Sick and Teixeira 1980, Yamashita 1987) It is believed that poaching reduced the species' occurrence to two small breeding areas 37km apart, resulting in a single population (Pacífico et al. 2014) This population has experienced a substantial increase in the last few decades, reaching at least 1200 individuals in 2014, due to the protection of nesting and roosting sites combined with other conservation actions (Barbosa and Tella 2019). These macaws nest exclusively in deep natural cavities in sandstone cliffs that provide adequate room, protection from predators and lower daily variation in the internal temperature and humidity (Pacífico 2011). A single cliff with many cavities may hold several breeding pairs (Pacífico 2011). However, only 20% of the population attempts to breed in a typical year and we suspected that nest site availability may limit population growth unless the species is able to recolonize historical areas where they have become locally extinct (Barbosa and Tella 2019).

During our long-term monitoring of breeding macaws, we observed the occurrence of AHB hives in their nesting cliffs, and through local farmers we learned that macaws used to breed previously in cavities that are currently occupied by AHB. Our aim was to investigate the extent of AHB occurrence in the breeding cliffs of macaws and the potential competition for nesting sites. We hypothesized that AHB compete with macaws for nesting cavities, and predicted that many of the cavities occupied by AHB may be suitable for the macaws to breed in. We further predicted that the elimination of AHB from cavities would increase nest availability and favor recruitment of macaw nesting pairs. Specifically, we (1) quantified the presence of AHB hives in the macaw core and historical areas, recently recolonized breeding sites; (2) explored differences in the location of cavities used by AHB and macaws, looking for signals of interspecific competition; (3) experimentally managed AHB (through the treatment and removal of hives) to assess nest recruitment and breeding success by macaws in cavities previously occupied by AHBs; and (5) discuss the challenges of AHB eradication as an urgent tool for allowing the population growth and expansion of macaws.

[Click on the macaws to continue the reading of materials and methods of section 6: 3.4.2](#)



### 3 Materials and Methods



### 3.1 STUDY AREA: LEAR'S MACAW RANGE

This study occurs over the entire distribution area of the Lear's Macaw in north-central Bahia state (BA), Brazil, considering both historic and recent records of the species, including those made during fieldwork. The estimated<sup>7</sup> range by IUCN redlist is considering two areas: Raso da Catarina and the Depressão Sertaneja Meridional ecoregions of the Caatinga dry forest biome (Velloso et al. 2002, ICMBio 2012, Birdlife Internacional 2019) The locality are composed of sedimentary rock, characterized by the alternation of calcareous sandstone outcrops and delimited by intermittent streams (Oliveira and Chaves 2010) Figure 4).

The altitude varies respectively between 380-550, to 600 to 1100 meters above sea level and temperatures range from 15 to 47° C. The climate is classified as Semi-Arid (Köppen-Geiger) with irregular and torrential rainfalls that average in 450-650 mm from December through July (Silva and Souza 2018). It is estimated that there are 37 foraging sites used by the Lear's Macaw (Santos-Neto and Camandoroba 2008) spread out in small patches of Licuri Palm (*Figure 5*).

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<sup>7</sup> The range of the macaws in the *Depressão Sertaneja Meridional* ecoregion is overestimated by IUCN (Birdlife Internacional 2017). The estimation was mistakenly based in the efforts of researchers from CEMAVE-ICMBIO in the search for the macaws interviewing local villagers to collect historical records, and is not based in historical records of the macaws.

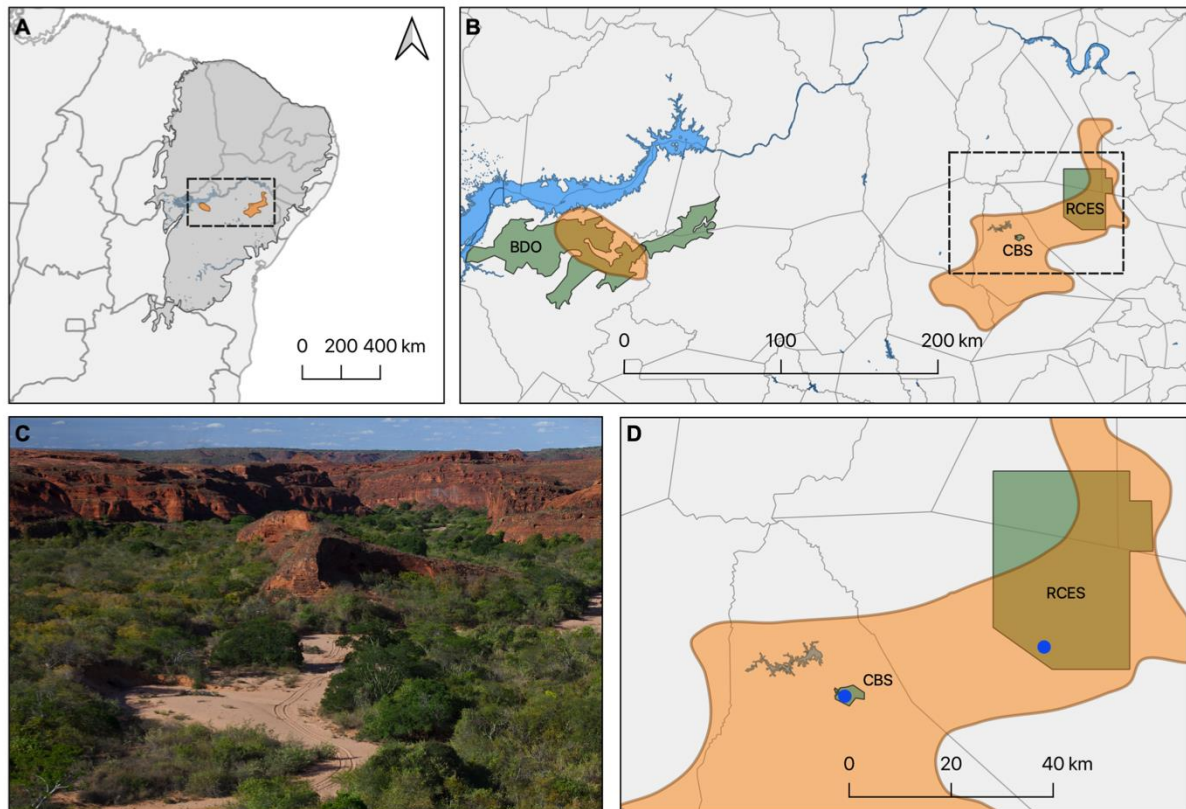


Figure 4. (A) Tropical dry-forest domain (Caatinga Biome) in north-east Brazil. (B) Estimated range by IUCN (BirdLife International 2019) based on ICMBio (2006, 2012) in north-central Bahia state (BA), in the ecoregions of Caatinga proposed by Velloso et al. (2002). Integrally protected areas that overlap the species records are show in green shape: Left to the right: Parque Nacional do Boqueirão da Onça (federal park are created in 2019); Estação Biologica de Canudos (private land since 1992); Estação Ecológica do Raso da Catarina (federal reserve created in 1967). (C) Field area landscape, with sedimentary rock, characterized by the alternation of calcareous sandstone outcrops and delimited by intermittent streams, were the macaws use to roost and breed (CBS – *Toca Velha* – *Fundação Biodiversitas*), photo by João Marcos Rosa); (D) Core occurrence area in the Raso da Catarina Ecoregion were CBS and RCES are located, and the nesting and roost sites sites of the Lear's Macaw were regularly monitored by conservation programs.



Figure 5. Patches of Licuri Palm (*Syagrus coronata*) Matt. in the study area (Canudos, BA). Photo by Manuel de la Riva.

## 3.2 MATERIALS AND METHODS: DEMOGRAPHY HISTORY

### 3.2.1 Section 1 - Breeding to non-breeding population ratio and breeding performance of the globally Endangered Lear's Macaw: conservation and monitoring implications

#### 3.2.1.1 Study area

Surveys were conducted at the two breeding sites known for the species (Figure 4D): Raso da Catarina Ecological Station (RCES; 09°52'S, 38°38'W and Canudos Biological Station (CBS; 09°57' S, 38°59'W;), known as Serra Branca and Toca Velha, respectively. Both are composed of sedimentary rock, characterized by the alternation of calcareous sandstone outcrops and delimited by intermittent streams (Oliveira and Chaves 2010). The areas are inserted in the Caatinga biome in the ecoregion of Raso da Catarina, where elevation reaches 800 meters in altitude and temperature varies between 15 and 45° C. Climate is semi-arid, rainfall being torrential and irregular, with annual averages around 650 mm concentrated between December and July (Velloso et al. 2002).

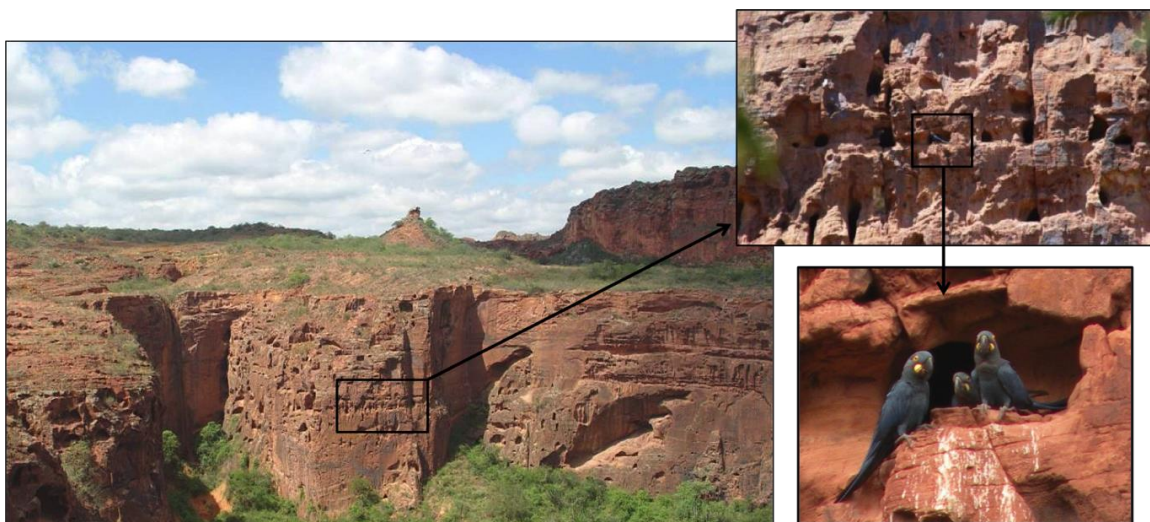


Figure 6. Cliffs with cavities where the macaws used to breed in *Toca Velha* (local name) in the Canudos Biological Station (CBS), private area from Biodiversitas Foundation, at Canudos municipality, Bahia.

### 3.2.1.2 Nest identification

The Lear's Macaw breeds exclusively in pre-existing cavities of calcareous sandstone cliffs existing at the two protected areas, where a number of potential nesting cavities are available for the species (*Figure 6*). According to Hart (1992) breeding activities start in mid-October with the exploration of cavities, and last until April-June when the last chicks leave their nests (Amaral et al. 2005). Nests searches were conducted by walking along the intermittent rivers located in the base of the cliffs looking for potential nest cavities and its exploration by macaw pairs (Renton and Brightsmith 2009). In order to identify the cavities actually occupied by breeding pairs for nesting (i.e., active nests), direct observation was undertaken for an average of 12 hr/day during three consecutive days at each reproductive site, two times a month, from early January to late June during two breeding seasons (2009 and 2010). January is the month in which active nests are more easily identified since breeding Lear's Macaws remain for longer periods inside their nests, coinciding with a breeding phenology period which spans from egg laying to the care of very young chicks (Pacífico 2011). Breeding sites at RCES were monitored by E.A.B. and K.O., while E.C.P. and T.F., together with field assistants, monitored the breeding sites at CBS. The criteria used to identify active nests (i.e., cavities where a pair initiated the reproduction) relied on the activity of birds, following Schneider et al. (2006) and Renton and Brightsmith (2009): (1) Time that the pair remained in the entrance of the cavity; (2) time that one of the individuals remained inside the cavity in the absence of its mate; and (3) observations of mate-feedings in the entrance of the cavity. The cliffs were photographed to aid locating both potential and active nest sites in each breeding season. Observations were conducted from distant points (> 100 m) to avoid disturbance (Schneider et al. 2006). This observation protocol allowed us to estimate the breeding population size and the breeding parameters of a subsample of nests (see below).

### 3.2.1.3 *Breeding parameters*

Breeding parameters were obtained from those nests (focal nests) for which visibility from distant points allowed determining their breeding output (number of fledglings observed in the nest entrance at the last breeding stage) by observations (Renton and Brightsmith 2009). The number and distribution of nests varied slightly between 2009 and 2010. Therefore, 34 focal nests (24 at RCES and 10 at CBS) were monitored to estimate breeding parameters in 2009, while 41 focal nests were monitored in 2010 (29 at RCES and 12 at CBS). At CBS we were also able to determine the breeding output by combining observations with direct nest inspections of all focal nests, using vertical descending techniques and climbing the sandstone cliffs three to five times until chicks were close to fledge. Direct inspections of the nests confirmed egg-laying in all active nests determined through observations at distance, it did not cause breeding failures, and provided the same estimations of breeding parameters as those obtained through observations at distance (Pacífico 2011). Therefore, we are confident the observation at distance of the activities of mated pairs and fledglings provided reliable estimators of breeding parameters. The observation protocol used for nest identification was extended to assess breeding output, but with increased efforts between March and June (ca. 6 hours of observation/researcher/day) coinciding with the period in which nestlings (between the 12<sup>th</sup> and 15<sup>th</sup> weeks after hatching) are first sighted at the entrance of nest cavities (*Figure 7* and *Figure 8*), where they further spend most of the daylight hours until they are able to fly (Pacífico 2011). During this period nestlings were easily identified, as they have smaller and paler lappets bordering the lower mandible than adults (Brandt and Machado 1990, Juniper and Parr 2010), they frequently solicit food to their parents, making distinct vocalizations, and take sunbaths and exercise their wings in preparation for flight (E. C. Pacífico, pers. observ.).

We defined breeding success as the percentage of pairs producing at least one fledgling, brood size as the average number of fledglings per successful pair, and productivity as the average number of fledglings per pair that attempt to breed (i.e., that occupied a nest).

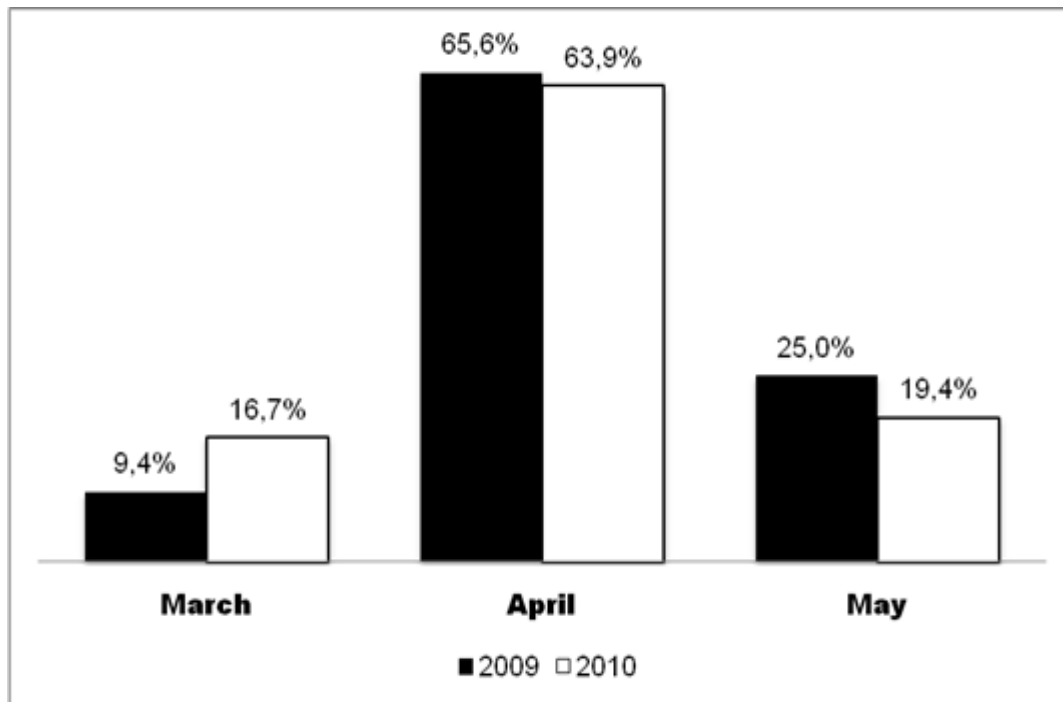


Figure 7. Percentage of full-grown nestling Lear's Macaws observed between March and May at the entrance of the nests cavities.

#### 3.2.1.4 Breeding Population size

The number of breeding pairs in the population was estimated as the total number of nests occupied in the 2010 breeding season, pooling focal (confirmed) and probable nests. Probable nests are those where it was possible to observe intense activity of macaw pairs throughout the entire breeding season, but that were difficult to monitor from the observation points, thus hindering the determination of breeding parameters. (i.e., those with poor visibility from which breeding output were not monitored).

#### 3.2.1.5 Statistical analyses

Differences in breeding parameters between breeding sites (CBS and RCES) and years (2009 and 2010) were assessed through Generalized Linear Models, fitting site, year and their interaction as fixed effects. The binomial distribution and logit link

function were used to analyze breeding success using nesting attempts as sampling units (0: unsuccessful, 1: successful), while the Poisson distribution and log link function were used for productivity (number of fledglings: 0-3) and brood size (number of fledglings: 1-3). All analyses were performed using SPSS 15.0.



Figure 8. A macaw pair and their nestling (between the 12th and 15th weeks after hatching) sighted at the entrance of nest cavities. Photo by João Marcos Rosa.

[Click on the macaws to continue the reading of results of section 1: 4.1.1](#)



### **3.2.2 Section 2 - Estimating population size and growth with a heterogeneous long-term census: the population trends of the endangered Lear's Macaw**

#### *3.2.2.1 Focal Species*

Since the rediscovery of the Lear's Macaw in the wild in 1978 (Sick et al. 1987), the breeding and roosting records of the species are concentrated in two areas in the Raso da Catarina Ecoregion that are 37 km apart: (1) Toca Velha, within a private conservation unit called Canudos Biological Station (CBS), and (2) the Serra Branca Environmental Protection Area, connected to the Raso da Catarina Ecological Station (RCES). These two areas likely comprise a single macaw population, given the relatively short distance between them (Pacífico et al. 2014), the species' high mobility, and records of tagged and tracked individuals using both areas (Pacífico et al. 2018). The Lear's Macaw is locally extinct in other (hereafter, historic) roosting and breeding areas (Menezes et al. 2006). An isolated macaw group was found 230 km west of the Raso da Catarina Ecoregion (Menezes et al. 2006), but contained only two remaining wild individuals and is now functionally extinct (Filadelfo and Pacífico 2017).

#### *3.2.2.2 Count dataset*

We first explored the raw data from the Lear's Macaw monitoring program performed by CEMAVE-ICMBio to clarify the sampling design. Counts were from 2001-2014 (except no data available for 2007) at known roosting cliffs located in the CBS and the RCES. As described by Nascimento et al. (2001) and Menezes et al. (2006), the sampling design used in the 2001 and 2003 counts was different from what was employed in other years. According to Nascimento et al. (2001), 3 days of counts in 6 roost locations were done simultaneously (two in each morning and two in each evening) during the late breeding period (May-June). The same roosting sites were sampled quarterly in 2002, and monthly in 2003 and 2004 (Menezes et al. 2006).

After 2004, counts were performed simultaneously for two days at 10 roosts, but the frequency of counts per month or year was not described. Counts of the individuals arriving or leaving the roosts were done twice a day. The morning counts were performed from 05:30 to 06:30, and the afternoon counts were done from 17:20 to 18:20. One to three field technicians and volunteers were placed at each roost site at a distance of 30-300m from the roosts (Barbosa et al. 2012) (See dataset from CEMAVE - ICMBio in **Appendix 6**).

### 3.2.2.3 Modeling approach

We assume the roost counts were from a single macaw population. Roost site-specific counts were not available for this study. Instead, we were able to obtain a population count for each morning (AM) or evening (PM) survey from the sum of the counts at each roost site. We only used counts during the non-breeding period from June after most fledglings have left their nests to November before egg laying commences (Pacífico et al. 2014) to model the abundance monthly from 2004 to 2014. We excluded counts obtained during the breeding period because breeding pairs of parrots change behavioral patterns and roost in their nests instead of at the surveyed roost sites (Cougill and Marsden 2004, Warburton and Perrin 2005). Moreover, yearling juveniles do not roost communally before June when the breeding season is likely to terminate in a normal breeding year (Pacífico et al. 2014).

We modeled abundance using a modified version of the binomial N-mixture model (Royle 2004). This type of hierarchical model combines a binomial GLM (general linear model) for the observed counts, and a standard Poisson or negative binomial model for the expected abundance. Our version of the model substituted replication in time (i.e., each site was sampled over multiple months, with repeated visits within months), assuming that the population was constant within months, for sample replication in space (i.e., visits to multiple sites).

We can write the hierarchical structure of the binomial–Poisson mixture model as follows:

$$\text{eqn 1: } N_{ik} \sim \text{Poisson}(\lambda_{ik})$$

$$\text{eqn 2: } Y_{ikj} \sim \text{Binomial}(N_{ik}, p_{ikj}),$$

where  $N_{ik}$  is the abundance of individuals at month  $i$  of year  $k$ ,  $\lambda_{ik}$  is the rate parameter of the Poisson distribution (the expected abundance) in the same period,  $Y_{ikj}$  is the number of individuals observed during each count  $j$ , and  $p_{ikj}$  is the probability of detecting an individual. In the binomial–negative binomial mixture model, eqn 1 is replaced by:

$$\text{eqn 3 } N_{ik} \sim \text{neg. binomial}(\lambda_{ik}, \alpha),$$

where  $\alpha$  is the dispersion parameter (a smaller  $\alpha$  implies higher variance).  $\lambda_{ik}$  and  $p_{ikj}$  can be modelled as a function of covariates with  $\log(\lambda_{ik}) = \mathbf{b}_0 + \mathbf{b}_1 \mathbf{x}_{ik}$  and  $\text{logit}(p_{ikj}) = \mathbf{c}_0 + \mathbf{c}_1 \mathbf{z}_{ikj}$ .

To evaluate how population size varied across months and years, we compared models that included  $\lambda_{ik}$  covariates: (1) month (June to November) as a numeric variable (i.e. 6-11) using a 1st and 2nd order polynomial, and alternatively as a factor; and (2) year as a numeric variable (2004-2014) for a linear effect, and alternatively as 2nd and 3rd order polynomials to allow potential increasing and decreasing abundance throughout the years. We also included a model with an interaction between months (treated as a factor) and years to test whether the effect of month varied among years. Moreover, to assess how time of survey influenced detection probability, models included a covariate indicating whether a count occurred in the morning or evening (AM/PM).

Preliminary analysis revealed that binomial-Poisson mixture models failed to converge due to high overdispersion. This can often occur when large count values are more frequent than expected with a Poisson distribution that has only one free parameter [ $\lambda$  in eqn 1] that describes both the mean and the variance. Thus, we next fit models using the binomial-negative-binomial mixture distribution, which allows the variance to differ from the mean by including a dispersion parameter ( $\alpha$ , eqn 3). None of these models experienced problems with infinite estimates of population size, which can occur with negative binomial models (Kéry 2018, Knappe et al. 2018).

Models were ranked using the Akaike's information criterion (AIC). We assessed goodness-of-fit with parametric bootstrapping by simulating datasets based

upon the fitted model, refitting the model, and evaluating the sum of the squared residuals for each simulation (Kéry and Royle, 2016). We then compared this sampling distribution to the observed sum of the squared residuals. Statistical analyses were done in R (R Development Core Team 2016) using package unmarked (Fiske et al. 2013) (See R script in **Appendix 7**).

[Click on the macaws to continue the reading of results of section 2: item 4.1.2](#)



### 3.3 MATERIALS AND METHODS: POPULATION GENETICS

#### 3.3.1 Section 3 - Isolation and characterization of 15 new specific microsatellite markers for the Lear's Macaw

##### 3.3.1.1 Biological Sampling

Tissue samples of wild *A. leari* individuals were obtained from five localities representing the species' entire range of distribution (Figure 9 A). Blood samples (0.1 ml) were collected from the wing brachial vein of 15 nestlings > 30 days old (Table 1), captured according to Pacífico et al. (2014) in two breeding localities: 1) Toca Velha in the Estação Biológica de Canudos and 2) Barreiras in a private land (Figure 9 B - red dots). Additionally, molted wing and tail feathers were collected following Gebhardt et al. (2009), in three locations: 1) roosting sites of Serra Branca in the Estação Ecológica do Raso da Catarina; 2) roosting site of Baixa do Chico in the Terra Indígena do Brejo do Brugo, and 3) foraging site of the farmland Cercadinho in the surroundings of the Parque Nacional do Boqueirão da Onça (Table 1, Figure 9 B – blue dots).

Blood samples were stored in 100% ethanol, while molted feathers were stored in craft paper envelopes; all samples were frozen at -18°C. For DNA extraction, blood and feather samples were digested in 100 mM NaCl, 50 mM Tris pH 7.5, 50 mM EDTA pH 8.0, 1% SDS and 25µl of proteinase K (20 mg/ml) at 55 °C for 8 hours. Genomic DNA was purified using a customized silica-based method in an automatic robotic system (Freedom EVO 100, from TECAN), using a homemade magnetic bead-based DNA purification system.

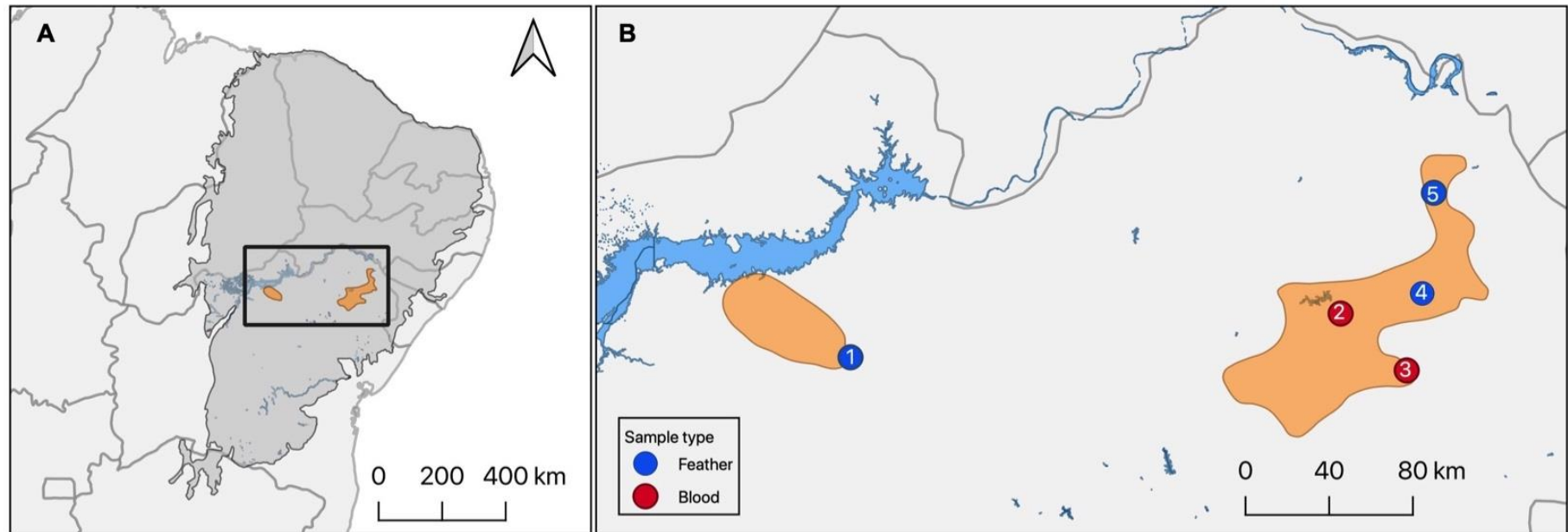


Figure 9. Study area: A. Geographical extension of the Caatinga Dry Forest Biome (dark grey) in Northeast Brazil. B. Location of the five sampling localities sampled (1 to 5, see Table 1) where the remaining population of *A. leari* aggregates in roosting sites, in the central-north of Bahia State, Brazil. Map obtained using QGIS v.3.8. (QGIS Development Team, 2019)

Table 1. Samples of *Anodorhynchus leari* used in the three phases of the study: sample identification code, locality (numbers in concordance with Fig. 1), geographical coordinates (latitude and longitude), sample tissue type, month and year of collection. \*Sample field number; blood samples are deposited at Museu de Zoologia da Universidade de São Paulo (MZUSP), DNA samples extracted from feathers are stored at Laboratorio de Ecología Molecular (LEM-EBD) and DNA samples from blood are also stored at Laboratório de Genética e Evolução Molecular de Aves (LGEMA-IBUSP) (pending number).

Genetic sampling	Sample	Locality (n.)	latitude	longitude	type	Month	year
Library enrichment	MZUSP 85891	Toca Velha (2)	S 9° 57.233	W 38° 59.588	blood	April	2008
Primers selection and optimization 1	LEM-EBD-adn254*	Cercadinho (1)	S 10° 08.266'	W 41° 08.080'	feather	May	2014
	183, F1-N02*	Toca Velha (2)	S 9° 57.233	W 38° 59.588	blood	April	2015
	181, F1-N55*	Barreiras (3)	S 9° 25.732'	W 38° 35.478'	blood	April	2015
	LEM-EBD-adn255*	Serra Branca (4)	S 9° 52.296'	W 38° 37.976'	feather	May	2014
	LEM-EBD-adn256*	Baixa do Chico (5)	S 9° 27.804'	W 38° 36.768'	feather	Jul	2014
Polymorphic primers selection and optimization 2; Individual identification	003, F1-N12*	Toca Velha (2)	S 9° 56.867'	W 38° 58.701'	blood	April	2008
	026, F1-N23*	Toca Velha (2)	S 9° 57.407'	W 38° 59.949'	blood	April	2009
	030, F2-N27*	Toca Velha (2)	S 9° 56.770'	W 38° 58.696'	blood	April	2009
	073, F1-N14*	Toca Velha (2)	S 9° 56.867'	W 38° 58.701'	blood	March	2011
	086, F1-N07*	Toca Velha (2)	S 9° 57.299'	W 38° 59.540'	blood	April	2011
	093, F2-N37*	Toca Velha (2)	S 9° 57.407'	W 38° 59.949'	blood	April	2011
	099, F1-N03*	Toca Velha (2)	S 9° 57.407'	W 38° 59.949'	blood	February	2012
	112, F2-N02*	Toca Velha (2)	S 9° 57.407'	W 38° 59.949'	blood	April	2012
	153, F2-N24*	Toca Velha (2)	S 9° 57.407'	W 38° 59.949'	blood	February	2014
	155, F2-N13*	Toca Velha (2)	S 9° 56.867'	W 38° 58.701'	blood	April	2014
	165, F1-N38*	Toca Velha (2)	S 9° 57.407'	W 38° 59.949'	blood	April	2014
166, F1-N26*	Toca Velha (2)	S 9° 57.407'	W 38° 59.949'	blood	April	2014	

### 3.3.1.2 *Microsatellites Isolation and Characterization steps*

Microsatellites were isolated and characterized in 3 steps (Figure 10). First, a microsatellite-enriched library (motifs: AGG, AAG, ACAT and ATCT) was obtained using DNA of one individual (Table 1) and Nextera XT DNA kit (Illumina) at the AllGenetics and Biology SL. Lab. Sequencing was performed on an Illumina MiSeq platform (PE300) and 5,606,286 paired end reads were processed in Geneious 8.1.5. using customized scripts (Untergrasser et al. 2012). A total of 633 primer pairs were designed using Primer3 in Geneious 8.1.5 (**Appendix 8**).

In a second step, we selected a subset of 62 microsatellites presenting more than seven motif repeats to test amplification on DNA samples from five individuals, one from each locality (Figure 10; Table 1). PCRs were composed by forward and reverse primers and either an M13-tail (5'GGA AAC AGC TAT GAC CAT) labeled with HEX dye, or an oligonucleotide CAG-tail (5'CAG TCG GGC GTC ATC) labeled with the FAM dye, to allow multiplexing primer pairs (up to four) and increase cost-efficiency of runs (Schuelke 2000).

The total volume of each PCR was 12.5 $\mu$ L, containing 1  $\mu$ L of DNA (10 ng/ $\mu$ L), 6.25  $\mu$ L of the Qiagen Type-it® Master Mix for Microsatellite PCR, 1.25  $\mu$ L of primer mix and 4  $\mu$ L of PCR-grade water. The optimal PCR protocol consisted of an initial denaturation step at 95 °C for 5 min, followed by 30 cycles at 95 °C for 30s, 56 °C for 90s and 72 °C for 30s; 8 cycles of 95 °C for 30s, 52 °C for 90s and 72 °C for 30s; and a final extension step at 68 °C for 30 min. PCR products were genotyped on an ABI 3130XL sequencer with the GeneScan 500 LIZ size standard (Applied Biosystems). Alleles were scored using Geneious 8.1.7 and reviewed in GENEMAPPER® 5.0 (Applied Biosystems™). From the 62 tested microsatellites, 31 were monomorphic and nine presented null alleles, thus rendering 22 polymorphic microsatellites, ranging from two to four alleles (**Appendix 9**).

In the third step, the 22 markers were tested on 12 blood samples from wild nestlings (Table 1). The same protocol and PCR conditions described above were used, except for the annealing temperature (between 56 °C and 63 °C).

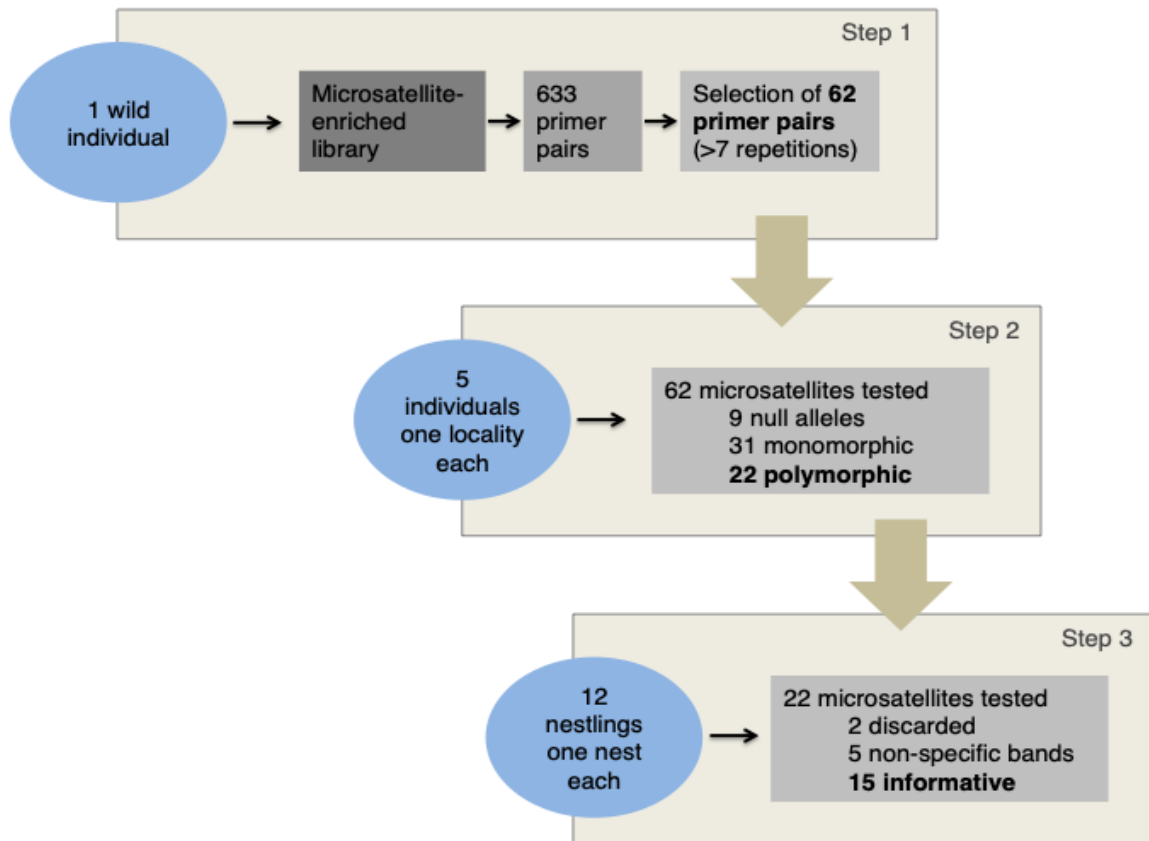


Figure 10. Flow-chart of the three-step isolation and characterization of specific microsatellites for the Lear's Macaw. First step: the microsatellite enriched library was obtained using the DNA sample of a single wild individual, 633 microsatellite primer pairs were tested on 5 individuals from 5 localities; nine primer pairs presented null alleles, 31 resulted in monomorphic, and 22 in polymorphic microsatellites. Third step: the polymorphic primer pairs were tested on 12 nestlings from different nests from the same locality; five primers produced low quality profiles with many stutter bands, two were discarded due low signal, and 15 were informative.

We used GIMLET® 1.3.2 (Valière 2002) to calculate the number of alleles ( $A$ ), observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosities, and polymorphism information content (PIC) in the final set of 15 loci and to estimate the probability of individual identification (PI) both accounting or not for the possibility of presence of siblings in the sample (PI sibs) for each marker. We tested linkage disequilibrium and deviance from Hardy-Weinberg proportions using GENEPOP 4.7.0 (Rousset 2008) in R program (R Development Core Team 2016), applying the sequential Bonferroni correction (Rice 1989).



### 3.3.2 Section 4 – Lear’s Macaw population status assessment from non-invasive genetic samples

#### 3.3.2.1 Study area and field sampling

The study area comprised the entire range of distribution of *A. leari* in north-central Bahia state (BA), Brazil, in the *Caatinga* dry forest biome (Velloso et al. 2002) (Figure 11 A). The landscape is dominated by a floristic variety of tree and shrub vegetation called *Reconcavo*, with high shrub vegetation endemism and cliffs characterized by alternating sandstone and limestone outcrops that are delimited by intermittent rivers (Silva and Souza 2018, Fernandez et al. 2020).

We sampled six different localities where the study species aggregates to roost, in two Ecoregions (Velloso et al. 2002): 1) In the *Raso da Catarina* Ecoregion (RASO) we sampled (a) the *Estação Biológica de Canudos (Toca Velha)*, (b) the *Estação Ecológica do Raso da Catarina (Serra Branca)*, (c) the *Terra Indígena Brejo do Burgo (Baixa do Chico)*, (d) the *Barreiras* farm and (e) the *Barra do Tanque* village (private rural areas). These localities are situated between 37 to 57 km apart (see Figure 11 B); 2) In the *Depressão Sertaneja Meridional* Ecoregion (f) we sampled the feeding site of *Cercadinho* farm in the *Boqueirão da Onça* area (BDO), 240km to the west of the RASO Ecoregion (see Lear’s Macaw range in Figure 9 B).

Samples collected from RASO were successfully genotyped, but samples collected from the isolated macaw population from BDO, did not have enough DNA material for genotyping proves and repetitions, probably because of fragmentation related to the preservation condition of the tissue of the molted feathers.

All sampling localities correspond to Lear’s Macaw roosting areas, which are mainly located in sedimentary cliff walls. We recorded one to six roosting cliff walls in each locality, except for one nocturnal roost in trees at one small fragment of degraded *Caatinga* dry forest (See Section 5). In the roosting sites at RASO, we observed approximately 20 to 200 macaws in each collective roosting cliff, but the number of macaws might vary seasonally within cliffs and within localities throughout the year. Macaws often also rest in large *Caatinga* trees during the day, and such trees were sources of molted feathers in *Barreiras* farm and the BDO. Nests were exclusively

recorded at sandstone deep natural cavities, mostly in the same cliffs where roosts occur (Figure 11 C and E).

We performed active searching under each roosting cliff collecting primary molted feathers on the ground (Figure 11 D and F) (Gebhardt et al. 2009). Most sampling (72%) was done during the breeding season (December to April) from 2009 to 2016. Among the breeding areas identified for the species, only one (*Toca Velha*) was accessible for collecting biological material from nests and nestlings. Molted feathers were actively collected inside the nests (21 molted feathers from 16 nests), during the annual breeding monitoring activities of the species from 2009-2016, and while we were capturing nestlings for banding, we also collected blood samples from them, following Pacífico (2011) (see **Appendix 10**).

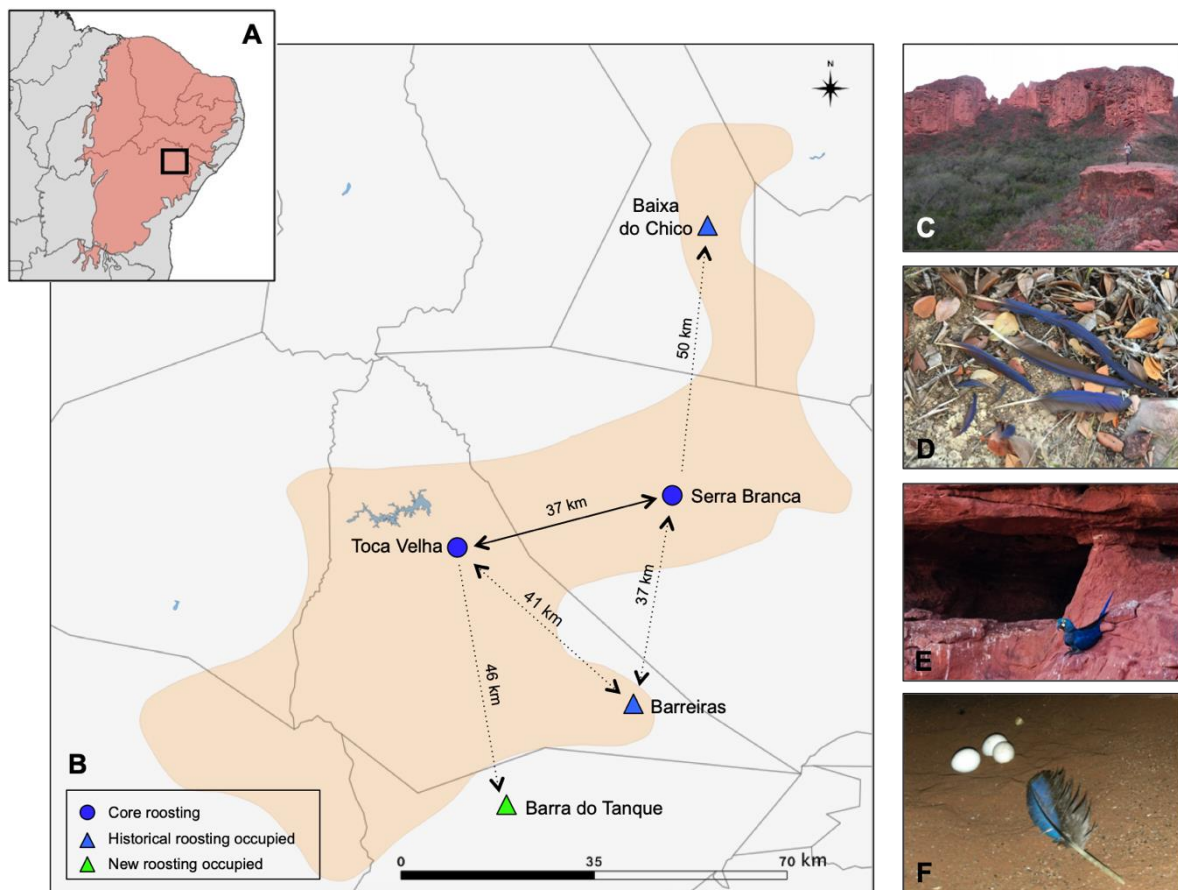


Figure 11. A. Study area in the north of Bahia state in the Caatinga biome (pink), northeast Brazil. B. Distribution range of Lear's Macaw at Raso da Catarina ecoregion (BirdLife International, 2018; orange shape) and sampling localities. Blue dots represent core roosting localities and triangles represent areas of recent expansion of the population (green: roost on trees; blue: roosts in sandstone cliffs). Arrows represent Lear's Macaw potential flow based on field observations (distance in km). C. Roosting cliffs (Barreiras). D. Molted feathers found on the ground at the bottom of each roosting site (Photos by

M. Fernanda L. da Silva). E. Lear's Macaw in the nest entrance (Photo by Cristine Prates). F. Molted feathers found on the nest ground during breeding activities monitoring (Photo by Erica Pacifico).

### 3.3.2.2 DNA extraction, sex determination and genotyping

Following Presti et al. (2013) we cut both the tip of the calamus and the umbilicus clot of each feather (*Figure 12*), and we preferred the use of the clot as a source of sample but we used the tip when the clot was in not good condition (e.g. dirty, damaged). Each sample was then digested overnight at 56° C in a solution of 315 µl of digestion buffer (100 mM NaCl; 50 mM Tris pH 7.5; 50 mM EDTA pH 8.0; SDS 1%) and 25 µl of proteinase K at 20 mg/ml). After digestion, a genomic DNA isolation protocol was implemented with a robotic Freedom EVO platform (Tecan).

To select the DNA samples for genotyping, we first performed sex determination as a filter of DNA amplification quality using standardized sexing primers M5-P8, recommended for low quality material (Bantock et al. 2008). Then we combined eight species-specific polymorphic microsatellite markers in a single multiplex panel to genotype all DNA samples using dye-labelled primers (*Table 2*; Schuelke 2000). Total PCR volume was 13.5 µl, including 6.25 µl of Type-it mastermix (Qiagen), 4.25 µl of primer mix (composed by 0.25 µl of a mix of 10 µM forward-F + reverse-R primers of markers *Ale176*, *Alea20*, *Alea23*, *Alea4*, *Alea5* and *Ale606*, respectively, 0.5 µl of a mix of 10 µM F+R primers of marker *Ale281* and 1 µl of a mix of 10 µM F+R primers of marker *Alea28*, 1 µl of RNase free water and 2 µl of template DNA (Pacifico et al. *in prep* (B) see Section 3, Jan and Fumagali 2016). Thermocycling conditions consisted on initial denaturation (5 min at 95 °C) followed by 30 cycles of denaturation (30 s at 95°C), annealing (90 s at 62 °C) and extension (30 s at 72 °C), with a final extension step of 30 min at 60 °C. All samples were amplified by duplicate, and fragment sizes of PCR products were analyzed with an ABI Prism 3730 sequencer. Alleles were scored manually using GeneMapper v4.0 (Applied Biosystems).

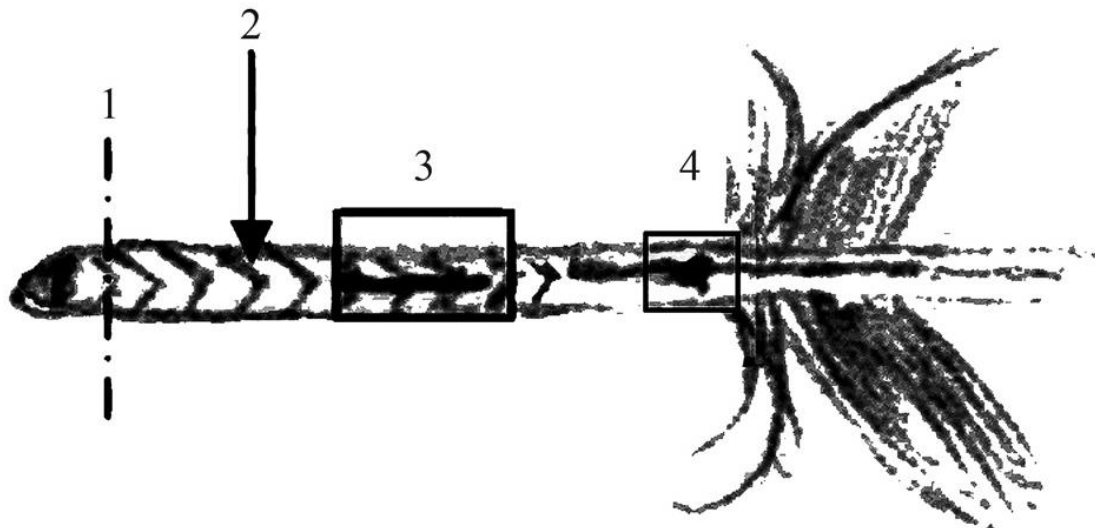


Figure 12 - General view of a typical flight feather: four different areas of the calamus that contain DNA (1) tip, (2) inner membrane, (3) blood clot outside the umbilicus, and (4) umbilicus clot. From Presti et al. (2013) modified from Horváth et al. (2005).

Table 2. Panel of eight microsatellite markers used for genotyping DNA samples of *A. leari*. The marker name, labelling dye, total number of alleles registered in our dataset (N. Alleles), allele size range and the number of localities showing evidence of significant departures from Hardy-Weinberg equilibrium (HWE) are shown for each marker along with the source paper.

Marker	Dye	#Alleles	Size range	HWE	Source
AI 176	NED	4	134-154	0	Pacífico et al. <i>in prep</i>
AI 020	PET	5	190-206	0	Jan and Fumegalli, 2016
AI 023	VIC	7	201-221	0	Jan and Fumegalli, 2016
AI 028	6-FAM	8	219-251	0	Jan and Fumegalli, 2016
AI 281	PET	5	102-130	1	Pacífico et al. <i>in prep</i>
AI 004	6-FAM	5	139-175	0	Jan and Fumegalli, 2016
AI 005	VIC	6	135-155	0	Jan and Fumegalli, 2016
AI 606	6-FAM	2	087-089	0	Pacífico et al. <i>in prep</i>

### 3.3.2.3 Individual identification, sex ratio estimation and genetic analyses

Given that feather samples were obtained from the ground and not directly from individuals, we first screened all genotypes to identify samples corresponding to the same individuals. We used *GenAlEx* v6.5b3 (Peakall and Smouse 2006) to test for multilocus matches among all samples (**Appendix 10**). We then removed duplicate individuals to generate a dataset of unique genotypes for each locality.

Sex ratio (SR) was estimated according to Ancona et al. (2017) where “*the proportion of males in the population was used as general measure (SR = Nmales/(Nmales+Nfemales)), thus results in values between 0 and 1 reflect the relative abundances of males and females in the population*”. Here we consider only unique individuals identified by genotyping, from feathers collected in the roosting sites that might include young juveniles and adults birds (breeders and senescent birds) from the sampled population, excluding the nestlings (for which we collected blood samples).

Genetic diversity was characterized for each locality and for the complete dataset by estimating allelic richness (*AR*), observed ( $H_0$ ) and expected heterozygosity ( $H_E$ ), the number of private alleles (*PA*) and the inbreeding coefficient  $F_{IS}$  using *GenAlEx* v6.5b3. We used *Genepop* (Raymond and Rousset 1995) to test for departures from Hardy-Weinberg proportions and for linkage disequilibrium among all markers applying the sequential Bonferroni correction to penalize for multiple comparisons (Rice 1989) and to estimate overall and pairwise differentiation ( $F_{ST}$ ) between sampling localities.

We used *Colony* (Jones and Wang 2010) to estimate the effective population size for each locality and for the complete dataset. All samples included in each analysis (either a single locality or the complete dataset) were combined as an overall *offspring* sample. We run eight replicates for each analyses, including two replicates for each combination of *sibship size prior* (either no prior or a weak prior for both paternal and maternal sibship sizes = 1, see Sánchez-Montes et al. (2017)) and inbreeding parameter (either accounting for the possibility of inbreeding in the population or not). All analyses were performed with *very long* run and *very high* precision settings (except for the complete dataset, which was performed with *long* run and *high* precision settings due to its computational complexity), and accounting for the possibility of polygamy in both sexes.

Finally, we explored the genetic structure across the sampling localities using *structure* (Pritchard et al. 2000). We implemented ten replicates for each possible number of clusters ( $K$ ) from one to ten in correlated allele frequencies analyses (Falush et al 2003) setting  $10^6$  burn-in and  $10^6$  post-burn-in iterations. The likelihood of the different  $K$ -values was inspected using the original (Pritchard et al. 2000) and the Delta (Evanno et al. 2005) methods in STRUCTURE HARVESTER (Earl and vonHoldt 2012). Results were summarized with clumpak (Kopelman et al. 2015).

[Click on the macaws to continue the reading of results of section 4: item 4.2.2](#)



## 3.4 MATERIALS AND METHODS: THREATS

### 3.4.1 Section 5 – Past and current threats of the Lear’s Macaw: implications for distribution and population expansion

#### 3.4.1.1 Study area

The area surveyed was mostly within the *Caatinga* biome, but we also explored the ecotone to the *Caatinga* and the adjacent *Cerrado* biome. The *Caatinga* is the semiarid hinterland of northeastern Brazil that presents a mosaic of different physiognomies spanning a broad range of woody plant densities (Silva et al. 2017). It is characterized by a high inter-annual variability in rainfall, with droughts that can last for years. These long droughts impose severe conditions to people living there and promote unique adaptations by the local biota (Andrade et al. 2017). The proportion of the natural *Caatinga* dry forest that is currently legally protected is negligible and there is a considerable lack of scientific knowledge to support social-environmental conservation initiatives (Leal 2005, Miles et al. 2006). This biome is intensively threatened by agriculture, farming, illegal charcoal production and poverty. Moreover, seasonal droughts have caused accelerated desertification process (Salvaterra 2017, Silva 2017, Schulz et al. 2019).

The *Cerrado* biome is the semiarid hinterland from central Brazil composed of a mosaic of different habitat types that range from open vegetation areas to dense forests, and is recognized as a global biodiversity hotspot (Myers et al. 2000). It has been suffering of intensive agriculture pressure as shown by an exponential habitat loss due to the mechanization of culture practices (e.g. soy) and cattle husbandry (Silva et al. 2017).

Core areas were defined as those situated in the *Raso da Catarina* ecoregion (RASO) in which >99% of the species population is concentrated and that have been monitored since 2001: *Toca Velha*, a private conservation unit called *Estação Biológica de Canudos*; and *Area de Proteção Ambiental da Serra Branca*, which overlaps the *Serra Branca* Farm (a private land used to access the federal/governmental environmental conservation unit called the *Estação Ecológica do Raso da Catarina*).

### 3.4.1.2 Road-side surveys

To obtain *in situ* information on the habitat conservation status for the Lear's Macaw and occurrence of other parrot species and livestock abundance, we designed 84 transects in pre-selected unpaved roads based on the available satellite images (Google Maps 2015) across all the study area, following methods described by Tella et al. (2013) and Dénes et al. (2015, 2017). We conducted multiple field surveys, mostly at the end of the breeding season (Pacífico et al. 2014), from April to August 2014, February to May 2015 and April-May 2016, covering the potential extent of the geographic range estimated for the species by IUCN (Bird Life International 2017) (Figure 13 A). Along the surveys we categorized the habitat and its conservation status (see Table 3) as natural conserved, natural degraded, natural degraded mixed with agriculture, and urban (including urban and rural human settlements), by observing the general phytophysognomy (vegetation predominance) as arboreal, shrubby, herbaceous, rock or otherwise clear of vegetation. We defined a transect segment in which the habitat type remained constant as a sample unit, so that changes in habitat type along the transect (e.g. natural conserved habitat into rural settlement means two different units). Within each sample unit, we recorded the presence/absence of Licuri Palm patches, and the anthropogenic land use, such as the presence of agriculture, pastures, or recent fires. We also counted free-ranging livestock (number of cattle and goats), given they may cause overgrazing and alter overall habitat quality within each sample unit (Ribeiro et al. 2015, Schulz et al. 2016, 2019). Additionally, we recorded parrot richness by auditory (vocalization) and direct (visual) observations, considering that the richness of parrot species, other than the Lear's Macaw may serve as potential indicators of habitat quality for the latter (Dénes et al. 2015, 2017).

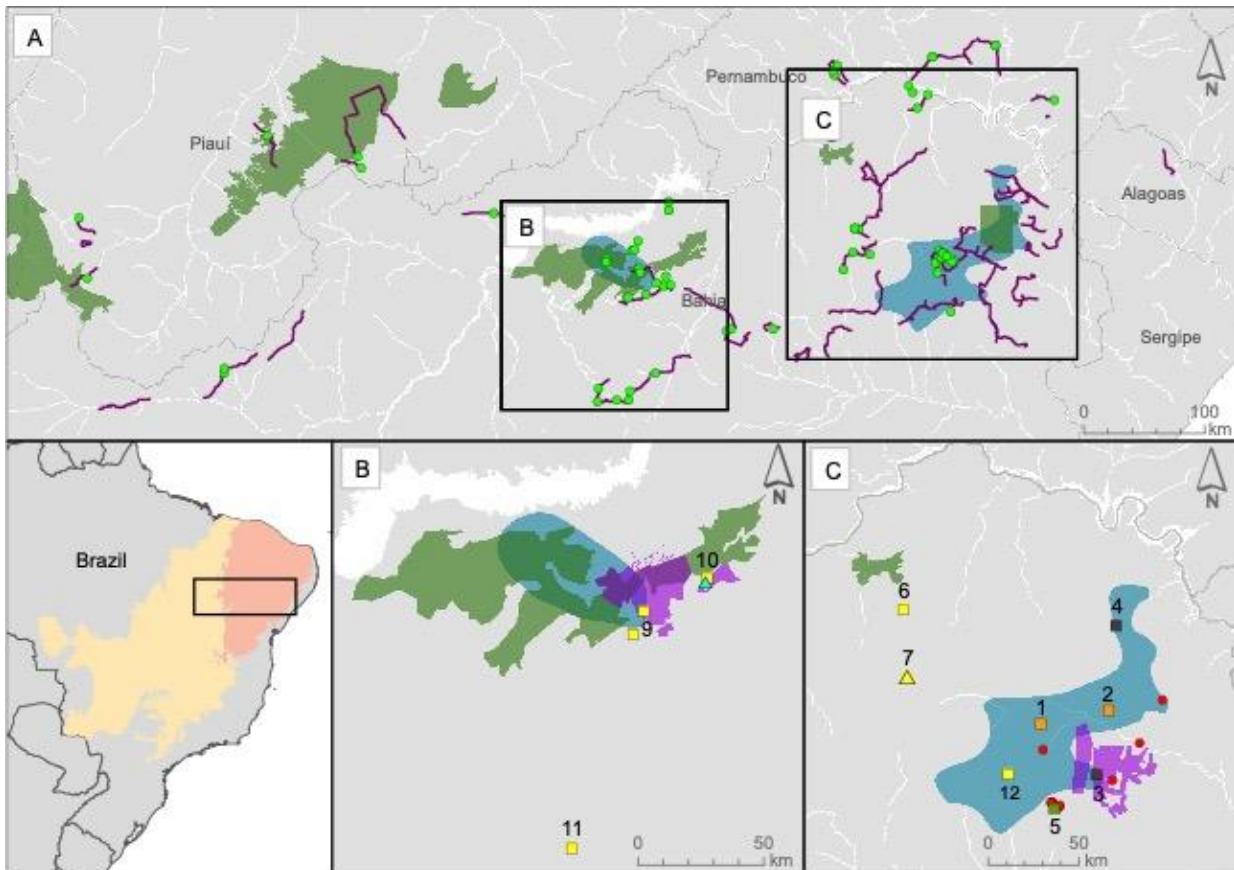


Figure 13 A. Study area in northeast Brazil, encompassing the potential occurrence area of Lear's Macaw (blue ranges) according to IUCN Red-list (Birdlife International 2013) and federal protected areas (green ranges). The areas explored through roadside surveys (purple lines) where the interviews were performed (green dots). B. (9-11) Historical sites (yellow squares) in the protected areas of National Park of Boqueirão da Onça (green shape). C. Core area where Lear's Macaw population is concentrated (blue range) in the Raso da Catarina ecoregion, with the previously known roosting sites in (1) Canudos Biological Station (EBC) and (2) Raso da Catarina Ecological Station (ESEC), and the recently occupied areas (green square) in the (5) Barra do Tanque, (3) Barreiras (both private areas, not protected) and (4) Baixa do Chico Community at the Indigenous land of Brejo do Burgo – Pankararés. Purple areas show those where wind farms are expected to be installed. Red dots are Lear's Macaw electrocution records in power lines recently observed.

Table 3 Categories used to describe the conservation status of habitats during the roadside surveys and criteria used to define them.

Habitat Category	Conservation Status	Field description	Vegetation
Natural	Conserved	Mostly with no evidence of recent anthropogenic use or transformation, fire, or deforestation in advanced process of regeneration (secondary vegetation). Typical Caatinga or Cerrado dry-forest with wood trees (high to medium size) and succulents. Patches of “campo limpo” or “campo rupestre” are not predominant. Licuri Palm patches embedded in natural vegetation.	Arboreal, shrubby and herbaceous, barren lands
	Degraded	Evidence of recent anthropogenic use or transformation with fire or deforestation. Typical Caatinga or Cerrado deforested. Licuri Palm patches surrounded by cleared herbaceous vegetation or abandoned pasture.	Arboreal, shrubby and herbaceous, barren lands
Natural degraded	Degraded mixed with agriculture	Typical Caatinga or Cerrado deforested with recent and active anthropogenic use (e.g fire, agriculture, pasture). Mostly small-sized pastures and crops for subsistence. Isolated Licuri Palms on pastures. <i>Prosopis</i> , <i>Anacardium</i> , <i>Agave</i> and other not seasonal cultivations can occur.	Herbaceous vegetation combined with less frequent arboreal and shrub vegetation
Not Natural	Rural settlement (agro-pastoral)	Extensive agricultural areas with predominance of crops and pastures, mostly associated with small villages and farms. Absence of natural vegetation except some Licuri Palms mixed with cultivation or exotic trees, especially in pastures.	Absent or just herbaceous vegetation, less frequently combined with shrub vegetation
	Urban	Cities or bigger villages, paved roads connecting them. Irregular dumps	Absent

### 3.4.1.3 Local knowledge

We performed interviews with local elderly people in the small villages found along transects and displacements. We target one to three people in each village, who was born before the 50's and have worked as a small farmer or related field activities and through that had contact with local wildlife (Garcia-Afonso et al. 2019). Using the snowball method of interview we started informal guided interviews (Service et al. 2014) to collect three groups of information: 1) personal information (age, gender, professional activity, and years living in the locality, 2) knowledge of local parrot species (e.g., common names, their use as pets, natural feeding, roosting or breeding behavior), and 3) if they demonstrated knowledge on parrots, information on local threats to the parrots that they mentioned (eg. deforestation, nest poaching and trapping for pets, hunting for food, invasive species) and population trends, including the local extinction of some species. These interviews, together with our field surveys, led us to get data about the past range distribution of Lear's macaws, the extinction of local populations, and the past and current threats for this and other parrot species (Lopes et al. 2018)

### 3.4.1.4 Habitat quality modeling

We employed a generalized linear modelling framework (GLM) to assess the potential correlations between a set of explanatory variables including Lear's macaw occurrence and anthropogenic activities (see below), and the richness of other local parrots species ( $R$ ) and the occurrence of the Licuri Palm. For parrot species richness models, the response variable was the number of parrot species - excluding *A. leari* - in each sample unit, and we used a *zero-inflated Poisson* (ZIP) GLM due to excessive zero observations of this variable. For Licuri Palm occurrence ( $\psi_{\text{Licuri Palm}}$ ) models, the response was a binary variable indicating whether Licuri Palms occurred in each sample unit, using a binomial distribution and logistic link function.

We used *in situ*-measured explanatory variables to assess the potential effects of various anthropogenic activities that may lead to habitat degradation, as follows: 1) cattle abundance and 2) goat abundance, as proxies of grazing pressure, 3)

presence/absence of pastures and 4) presence/absence of crops, representing the conversion of Caatinga vegetation and 5) presence/absence of burned vegetation, evidencing the use of fire as a common agricultural practice for clearing vegetation. We included a dichotomous variable 6) indicating whether the sample unit was within core or recently occupied areas (i.e. where the Lear's macaw is currently present) or within areas with historical records or without records (where the Lear's macaw is currently absent). We also included a variable to control for the effect of 7) ecoregion and 8) an offset indicating the length of the sample units to account for their variable sizes.

To better understand the factors influencing the conservation of potential Lear's macaw habitat, we also modelled the probability that sample units would have one of the four habitat conservation status (from natural to urban), using a multinomial log-linear approach (Venables and Ripley 2002). This model allowed us to evaluate how the response variable ( $\psi_{\text{status}}$ ) varied as a function of the following predictors: cattle and goat abundance, presence of Licuri Palm, presence of burned vegetation and ecoregion. We also included a sample unit length as an offset as described above.

We would like to emphasize that the purpose of our multiple models was not to provide a rigorous analysis for estimating the richness of parrot species, or the probabilities of occurrence of Licuri Palms or different habitat types. Instead, our goal was to evaluate the relative importance of the potential effects of a suite of anthropogenic activities, ecoregions and areas of Lear's macaw occurrence, on the different response variables. We evaluated such relative effects using analysis of deviance with likelihood ratio tests (multinomial log-linear model for habitat type) or Wald  $\chi^2$  tests (remaining models), and illustrate their effects with plots of model predictions for different covariate values.

Statistical analyses were done in R (R Development Core Team 2019) with packages *MASS* (Venables and Ripley 2002), *psci* (Jackman 2017), *nnet* (Venables and Ripley 2002), *bbmle* (Bolker and R Development Core Team 2017), and *glm.predict* (Schlegel 2019). Figures were made using R packages *ggplot2* (Wickham 2016), *gridExtra* (Auguie 2017), and ArcGIS (ESRI 2013).

### 3.4.1.5 Habitat suitability modelling

We used the Maximum Entropy algorithm - MaxEnt (Phillips et al., 2006) to identify the habitat suitability of Lear's macaw and Licuri Palm, due to the high performance and less sensitivity to possible geographical positioning errors (Hijmans and Graham 2006; Fourcade et al. 2014). For this, we compiled the occurrence of Lear's macaw based on our field data and from ICMBio (2012). We select 107 geographical coordinates, distributed throughout the species' core areas (77 records), expansion areas (20 records) and historical locations (10 records). For Licuri Palm, we compiled records of occurrence from a recent revision of the genus *Syagrus* (Noblick et al. 2017).

To choose the environmental variables for modelling, we first downloaded the nineteen bioclimatic variables from WorldClim – Global Climate Data, from 1970 to 2000 (Hijmans et al. 2005) and one of Human Foot Print from 1993 to 2009 (Venter et al. 2016). All the environmental variables were restricted to Brazil in ASCII grid format, World Geodetic System 1984 (WGS-84), and with 30 arc-seconds resolution (~ 1 km). To avoid overparameterization with redundant variables, we removed the strongly correlated ones (Pearson's  $R > 0.7$ ) (Dormann et al. 2007) and a subset of uncorrelated environmental variables was selected for Lear's macaw (n=8) and for Licuri Palm (n=9).

The models were generated by separating randomly the presence records into training (70% of the records) and test (30% of the records) with 1000 repetitions. We used cross-validation procedure to test the models. We evaluated the model performance, comparing to random predictions by analyzing the Receiver Operating Characteristics (ROC). The significance of the ROC plot is quantified using the Area Under the Curve (henceforth AUC) (Fielding and Bell 1997). AUC provides a single measure of the model's performance, regardless of any threshold rule (Phillips et al. 2006). Models with  $AUC \geq 0.75$  are considered potentially useful for species distribution modelling (Elith 2002).

We obtained the overlap between the habitat suitability maps (intersection) obtained for the Licuri Palm and Lear's macaw, extracting the potential occurrence

area for the macaw falling within the potential occurrence area for the palm. In this case we used a more conservative cut short (10 percentile training presence), to suggest areas more suitable for both species and thus that deserve more intensive conservation efforts.



[Click on the macaws to continue the reading of results of section 5: item 4.3.1](#)

### **3.4.2 Section 6 - Experimental removal of invasive Africanized honey bees to avoid competition for nest cavities of the endangered Lear's Macaw**

#### *3.4.2.1 Study area*

This study was conducted at Raso da Catarina ecoregion within the Caatinga biome, in central-north of Bahia State, northeast Brazil (Figure 14). (Velloso et al. 2002) The Caatinga is a semiarid (Köppen-Geiger), remote and undeveloped area, and refers mostly to a seasonally tropical dry forest, characterized by a mosaic of different vegetation physiognomies spanning a broad range of woody plant densities (Queiroz et al. 2017). The yearly mean temperature ranges from 25 to 30 °C. (Bonaparte and Cockle 2017). Annual rainfall varies greatly throughout the year with frequent droughts, and the number of dry months increases from the edges to the core of this region, with some areas experiencing periods of 7–10 months without rainfall (Andrade et al. 2017). Cretaceous sandstone canyons along intermittent rivers with natural and deep cavities formed by water erosion characterize the study area. (Oliveira and Chaves 2010). These cavities are where macaws and two other parrot species (Blue-crowned Parakeet, *Thectocercus acuticaudatus* and Turquoise-fronted Parrot, *Amazona aestiva*) find refuge to roost and breed (Pacífico 2011).

Most of the macaw nests are concentrated in the two protected areas of the Raso da Catarina: Toca Velha (Canudos Biological Station, a private area) and Raso da Catarina Ecological Station (ESEC Raso da Catarina, a governmental conservation unit). However, there are historical records of macaws nesting at sites surrounding these two areas, and these local breeding populations have experienced abrupt declines or even disappeared in the 1980's (Figure 14). (IBAMA 2012)

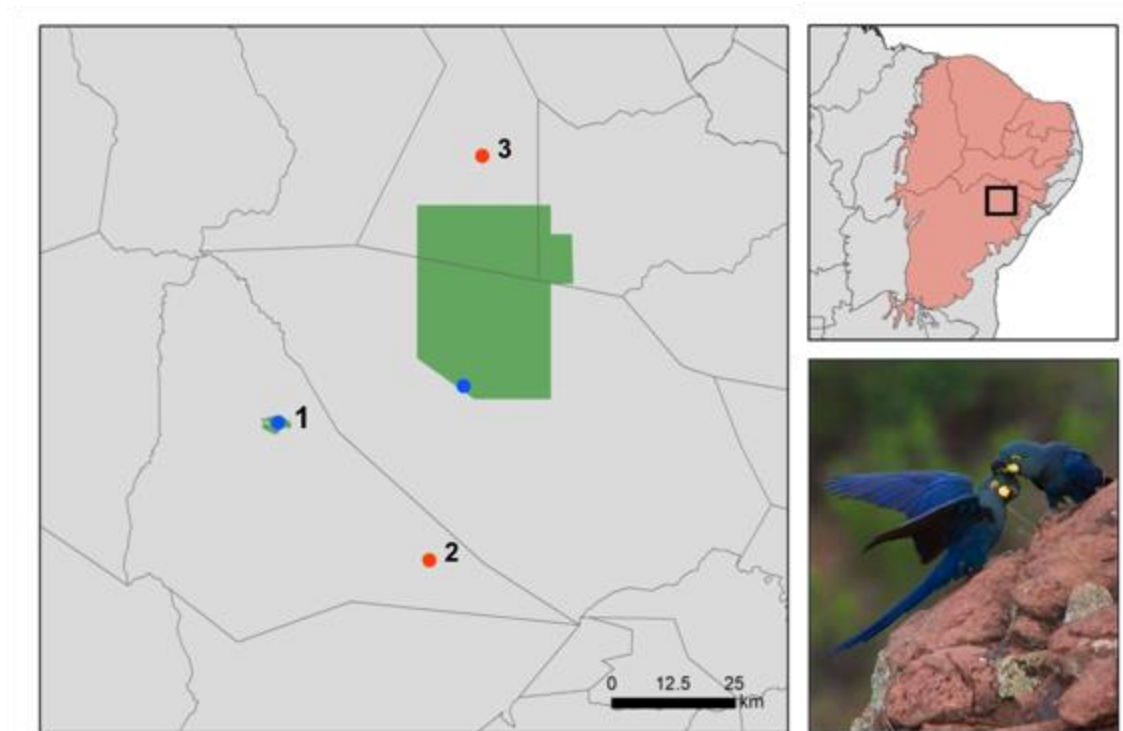


Figure 14. Distribution of the Caatinga biome (pink area in top-right map) in Northeastern Brazil and location of the study area (main map), indicating the historic (orange dots) and core (blue dots) breeding areas of the Lear's Macaw: 1) Toca Velha (Canudos Biological Station), 2) Barreiras (Private rural land), 3) Baixa do Chico community (Terra Indígena Brejo do Burgo). Protected areas are in green; from left to right: Canudos Biological Station (Fundação Biodiversitas) and Estação Ecológica do Raso da Catarina (ICMBio). Lear's Macaw pair photo by João Marcos Rosa.

#### 3.4.2.2 Survey of macaw nesting sites, nest-site recruitment and breeding success

We surveyed three locations as indicated in Figure 14: (1), Toca Velha, which is considered the “core area”, where we monitored breeding macaws since 2008; and two historical sites recolonized by macaws at least since 2014: Barreiras, private land in Euclides da Cunha and Canudos municipalities, and Baixa do Chico, in the Terra Indígena Brejo do Burgo, an indigenous reserve of the Pankararés ethnicity within the Rodelas and Nova Gloria municipalities.

We systematically used the same methodology to identify cavities used by macaws as nest sites and monitor their breeding success across a long-term monitoring scheme (Pacífico et al. 2014) Briefly, we identified potential breeding sites by observing cliffs at a distance (using binoculars 10x40mm and telescopes 20-60x80mm) to avoid disturbance, between 6-10AM during the periods of mating, egg

laying and incubation (February-March). Using snapshot sampling, we recorded for each five minutes the presence or absence of macaws in the entrance of each cavity. We considered as active nests *those* cavities where we recorded the presence of a macaw pair for three consecutive days. Breeding success was determined by accessing each active nest twice to record the presence of eggs and nestlings, and a breeding attempt was considered successful when at least one nestling was observed at the end of its development stage (Pacífico et al. 2014). We measured the height above the bottom of the cliff of each active macaw nest using a laser range-finder (6x20mm, 6.0°). For this study, the core area was annually surveyed from 2010 to 2018. Surveys of the historical areas began in 2016 and lasted until 2018.

#### 3.4.2.3 *Survey of Africanized honey bees hives*

A first quantification of AHB hives was performed just in the core area during the 2010 breeding season of macaws. Thereafter, in 2016 we conducted a three-week long survey to quantify the total number of hives in both the core and historical areas. A cavity was considered to have a hive if we could see comb or propolis (the resin produced by the bees that is associated with hive activity) around the entrance. Hives were also defined as active or non-active by the direct observation of the activity of bees. Setting up sugar water feeding stations to attract AHB complemented direct observations. This allowed us to note the “bee lining” flight path, after they had gorged on sugar water, following them back to their nesting cavity, helping to locate less obvious hive locations. (Visscher and Seeley (1989) Bee lining observations were made in the morning (5:30-7AM). We also measured the height above the bottom of the cliff of each cavity occupied by AHB as done for macaw nests.

#### 3.4.2.4 *Experimental treatment of Africanized honey bees hives*

Previous studies have shown that permethrin application prevents AHB from colonizing bird nest boxes; it is a chemical that is safe for birds, has low toxicity to vertebrates and is not persistent in the environment (Efstathionet al. 2015). Therefore, we used permethrin to eliminate active AHB hives. Approximately half of the hives

were treated, the rest being considered as a control for the experiment (see Results). To avoid AHB attacks, the best strategy was to approach the active hives wearing beekeeping suits and using beekeeping smokers. Using a crossbow (Barnett Raptor FX), we shot each active hive (Figure 15 A) with at least 0.1ml (concentration 1ul/ml) of permethrin in powder form. This powder was contained in a 3ml glass vial attached to the tip of the arrow (Figure 15 B). The powder spreads out on the hive upon impact, killing most of the bees within a couple of hours. One to nine shots were necessary depending on hive size, but in most cases (44 out of 52 hives) two to three shots were enough to spread the powder and cause AHB colony disturbance and death. In the following days we observed each hive shot to check for AHB activity, and we immediately noticed a considerable number of dead AHB on the ground, right below the treated hive. The next day we rappelled down the cliffs, cleared out the cavity entrance, removed the combs using a mattock-pick (Figure 15 C), and then we spread Fipronil-based Formilix® (concentration 2,5%-w/w) - a hydrophobic pesticide belonging to the phenylpyrazole group - in the cavity entrance as a long term deterrence to AHB reoccupation (Henry et al. 2012). The hives removed were impressively large, in some cases fully obstructing the cavity entrance and growing towards the interior of the cavity (mean diameter of 8 hives measured at the entrance of the cavity = 83.93cm, range: 26.5 – 110cm). Not all poisoned hives could be later removed, as some cavities were inaccessible by rappelling (see results and discussion). These treatments always took place after the breeding season of the macaws to avoid disturbance.

A pilot treatment was first performed in three hives at the core area in 2010-2011. Then we replicated this successful experience by designing and conducting the main experiment in 2016, including both the core and the historical areas.

#### 3.4.2.5 Statistical analysis

We used Yate's corrected Chi-square tests to compare the proportion of cavities occupied by macaws and AHB hives in historical and core areas, the proportion of treated and non-treated cavities later recruited by macaws, and the proportion of successfully breeding macaw pairs. Differences between the height of

cavities used by AHB and macaws and sites (historical and core areas) were tested using a generalized linear model (with a normal distribution and identity link function) in R program (R Development Core Team 2016; see script in **Appendix 13**)

Click on the macaws to continue the reading of results of section 6: item 4.3.2.1



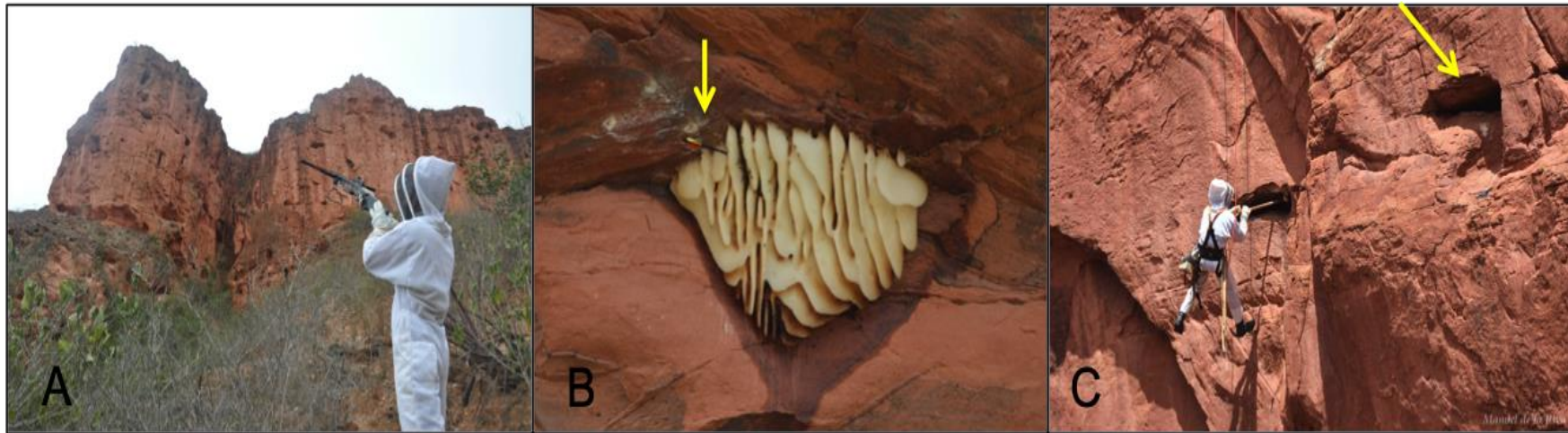


Figure 15. A. Africanized honey bee hive treatment using cross-bow for shooting the pesticide. Photo by EC Pacífico. B. Arrow fixed in the hive after shooting. Photo by RA Cunha. C. Removal of a previously treated hive close to a Lear's Macaw nest (yellow arrow). Photo by Manuel de la Riva

## 4 Results



## 4.1 RESULTS: DEMOGRAPHY HISTORY

### 4.1.1 Section 1 – Breeding to non-breeding population ratio and breeding performance of the globally Endangered Lear’s Macaw: conservation and monitoring implications

#### 4.1.1.1 Breeding parameters

Overall breeding success reached 80% of the breeding attempts ( $N = 75$ ) recorded in focal nests. Productivity averaged 1.33 (+ 0.86 SD) fledglings per breeding attempt ( $N = 75$ , *Figure 16*), while brood size averaged 1.67 (+ 0.60 SD) fledglings per successful nest ( $N = 60$ ). Successful nests fledged two chicks (53.3%) one chick (40%) or three chicks (6.7%).

Breeding success did not differ between sites (Ward’s  $\chi^2_1 = 0.069$ ,  $P = 0.79$ ) but was higher in 2010 (estimated marginal mean: 88% + 0.05 SE, Ward’s 95% IC: 0.78 – 0.98) than in 2009 (estimated marginal mean: 71% + 0.08 SE, Ward’s 95% IC: 0.55 – 0.88, Ward’s  $\chi^2_1 = 3.27$ ,  $P = 0.07$ ; *Figure 16*). Similarly, productivity did not differ between sites (Ward’s  $\chi^2_1 = 0.64$ ,  $P = 0.42$ ) but was higher in 2010 (estimated marginal mean: 1.55 + 0.14 SE) than in 2009 (estimated marginal mean: 1.15 + 0.14 SE) (Ward’s  $\chi^2_1 = 3.79$ ,  $P = 0.05$ ). There was no significant interactions site x year for breeding success (Ward’s  $\chi^2_1 = 0.87$ ,  $P = 0.35$ ) nor productivity (Ward’s  $\chi^2_1 = 1.13$ ,  $P = 0.29$ ). Brood size, however, did not vary between sites (Ward’s  $\chi^2_1 = 1.14$ ,  $P = 0.28$ ) nor years (Ward’s  $\chi^2_1 = 0.54$ ,  $P = 0.46$ , interaction site x year Ward’s  $\chi^2_1 = 0.21$ ,  $P = 0.65$ ; *Figure 17*).

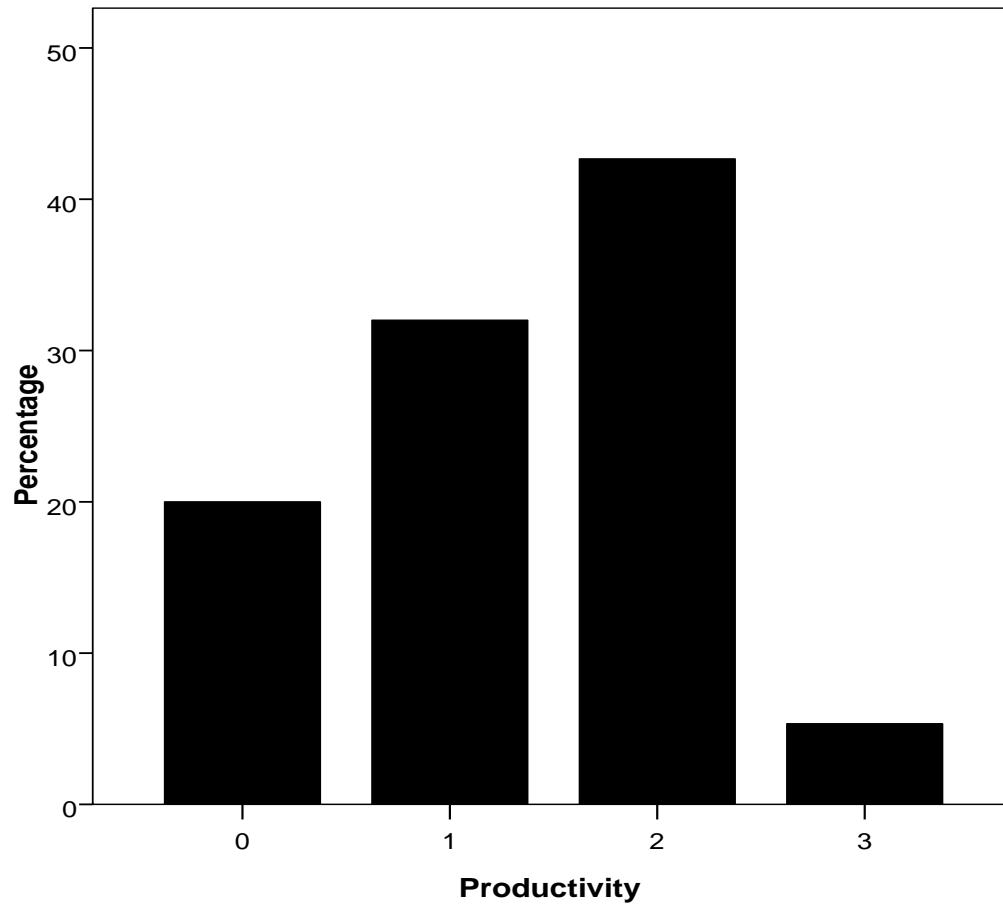


Figure 16. Productivity (number of fledglings per breeding attempt, N = 75) of Lear's Macaws in 2009-2010. Raw data is depicted as the percentage of cases with 0 to 3 fledglings.

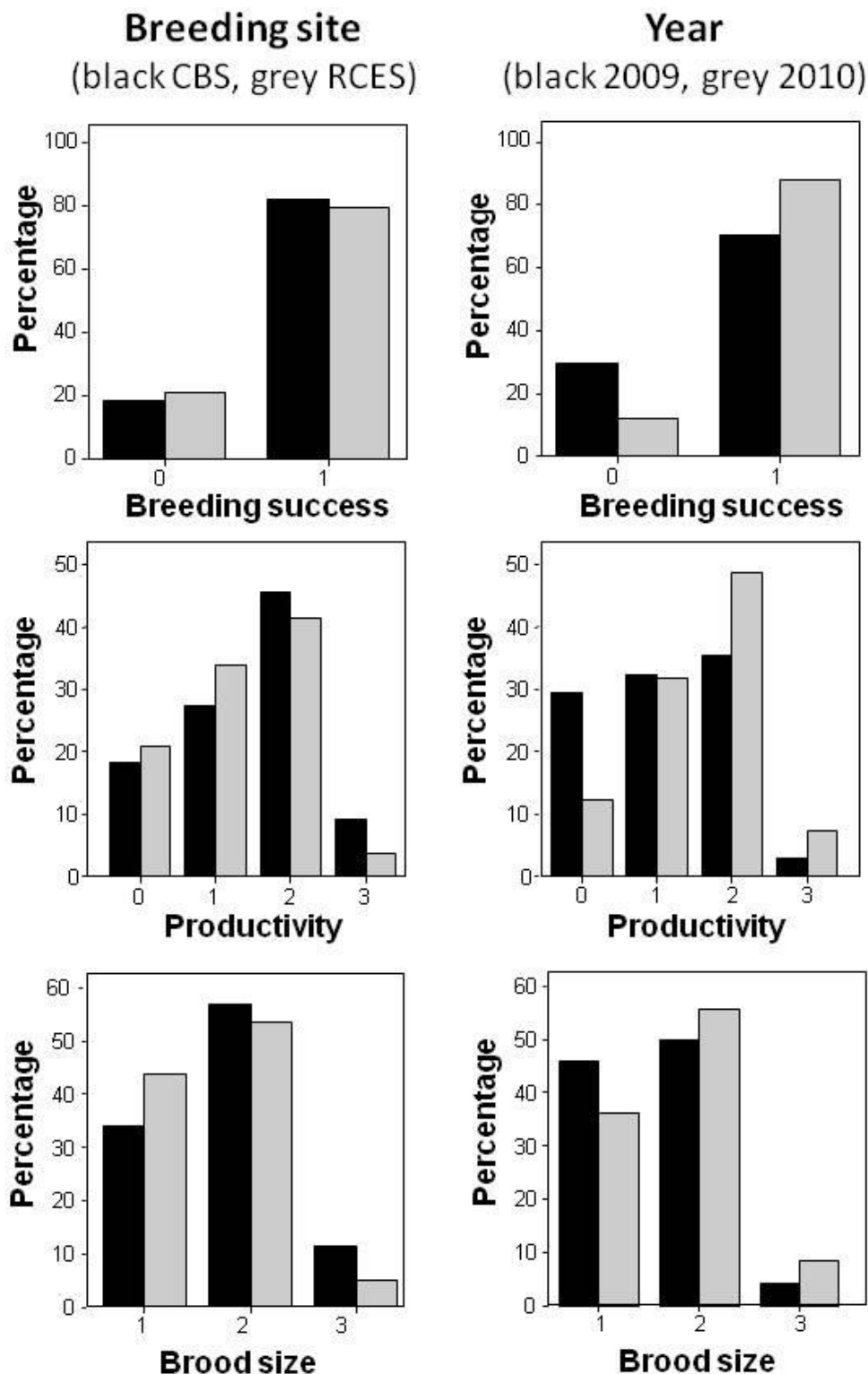


Figure 17. Breeding success (percentage of successful -0- and unsuccessful -1- nests), productivity (percentage of nests raising 0-3 fledglings), and brood size (percentage of successful nests raising 1-3 fledglings) of Lear's Macaws in relation to breeding site and year.

#### 4.1.1.2 *Breeding population size*

In 2010, 20 probable nests were recorded at CBS and another 53 at RCES but could not be properly monitored because of their difficult visibility. These 73 probable nests together with the 41 monitored focal nests leads to the estimate of 114 breeding pairs (i.e., 228 breeding individuals). ICMBio (2012) censused a total of 1,125 Lear's Macaws in 2010. Therefore, the 228 breeding individuals represented 20.3% of the population.

[Click on the macaws to continue the reading of the discussion of section 1: item 5.1.1](#)



## 4.1.2 Section 2 – Estimating population size and growth with a heterogeneous long-term census: the population trends of the endangered Lear’s Macaw

### 4.1.2.1 Heterogeneity of Lear’s Macaw roost count surveys

Roost counts of Lear’s Macaws from 2001 to 2014 exhibited high inter-annual heterogeneity in field sampling effort. Of a total of 165 counts, there were 3 to 20 counts per year (Figure 18). After accounting for variation among years, the number of counts per month did not vary considerably (Figure 19;  $\chi^2 = 0.03$ , df (year) = 12, df (month) = 10,  $P = 1$ ). However, the number of months sampled annually varied significantly from one to ten (Figure 2B;  $\chi^2 = 29.38$ , df = 13,  $P = 0.005$ ). Roost count frequency did not differ by time of day (75 AM counts and 73 PM counts), although information on time of count was missing for the early years (2001 to 2003). Most counts (70.3%) were done during the non-breeding season (June to November) especially after 2008 (92.5% of the counts) (Figure 18 and Figure 19). These measures of sampling effort varied between years but not linearly with year. Only total counts by roosting areas (CBS or RCES) were available for analysis.

The number of macaws counted at roosts varied by the time of the day. Significantly fewer birds were counted in evening than morning (mean<sub>morning</sub> = 1072.07, mean<sub>evening</sub> = 841.96, Welch two-sample  $t = 2.72$ , df = 96.87,  $P < 0.01$ ; Figure 20). This effect was probably due to the lower visibility of the macaws when arriving at sunset or darkness to roosts. Time of day was included as a detection covariate for subsequent models of macaw abundance.

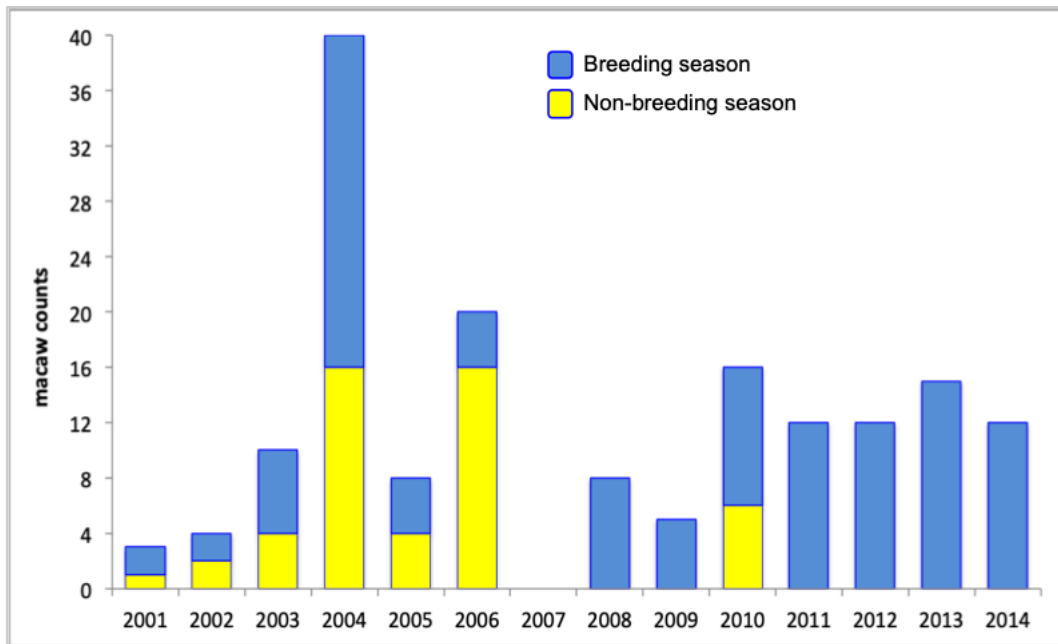


Figure 18. Variation in the roost survey efforts of the global population of the Lear’s Macaw. Number of counts per year.

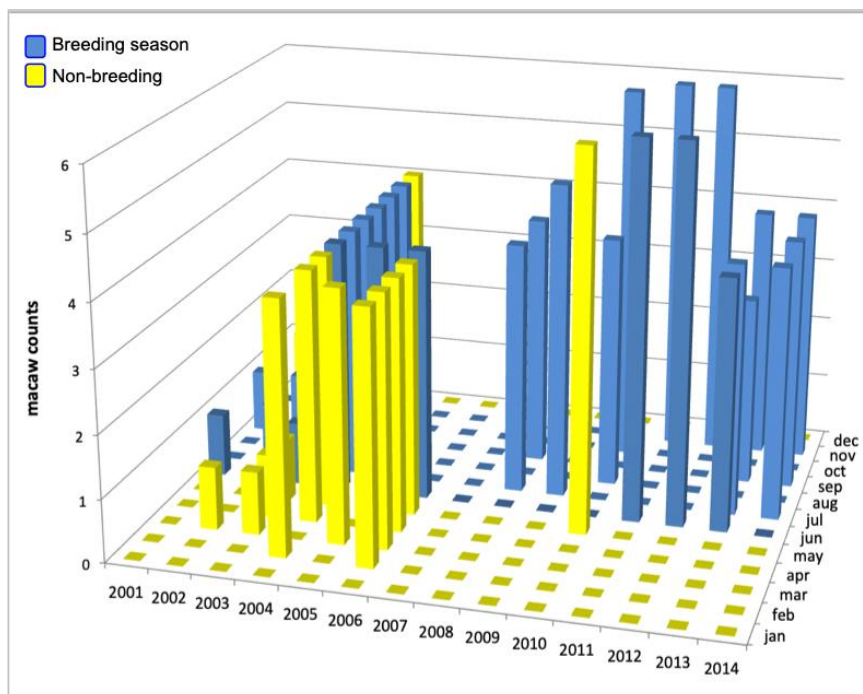


Figure 19 Variation in the roost survey efforts of the global population of the Lear’s Macaw, number of counts done by year and by month. Lighter bars represent counts done during the breeding season (December to May) and darker bars represent counts done during the non-breeding season (June to November).

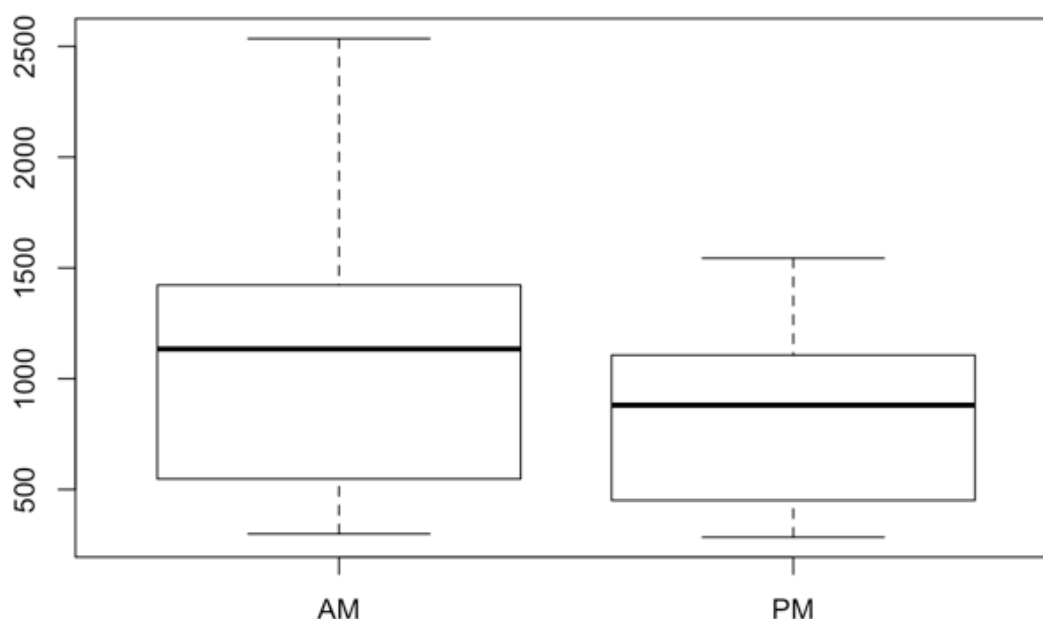


Figure 20. Variation in the number of Lear's Macaw individuals counted in morning (AM) and afternoon (PM) during the non-breeding period (July to November).

#### 4.1.2.2 Modeling Lear's Macaw population size and growth with roost counts

The best abundance model included month as a linear effect and year as a quadratic effect (*Table 4*, *Table 5*). The model with these effects and detection varying by time of day had a  $\Delta\text{AIC} > 4.7$  and an AIC weight of 0.85. The sum of squared residuals from parametric bootstrapping indicated that model fit was adequate ( $P = 0.22$ ; *Figure 21*). Detection probability differed greatly by time of the day, with a significantly higher probability for morning surveys ( $P = 0.600$ , 95%CI = 0.600-0.613) than evening surveys ( $P = 0.490$ , 95%CI = 0.485-0.496). Model predictions found that monthly abundance decreased by  $\sim 13\%$  from June to November (*Figure 22*), after taking into account between-year variation and imperfect detection.

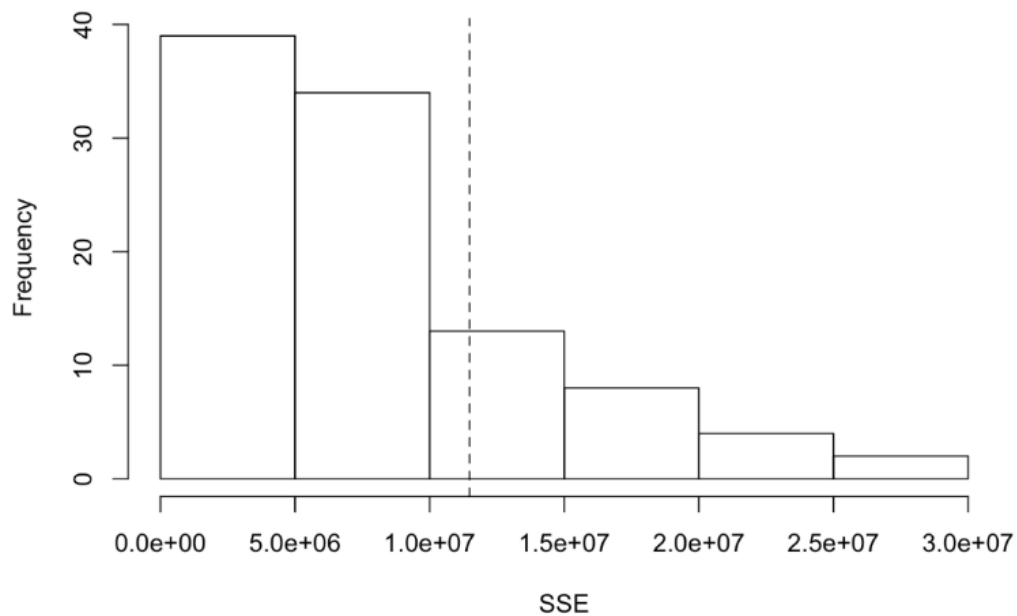


Figure 21. Parametric bootstrap of sum of squared residuals (SSE) for the best-ranked model. Observed residual variance (vertical dashed line) is within the distribution of expected residual variance (histogram bars)

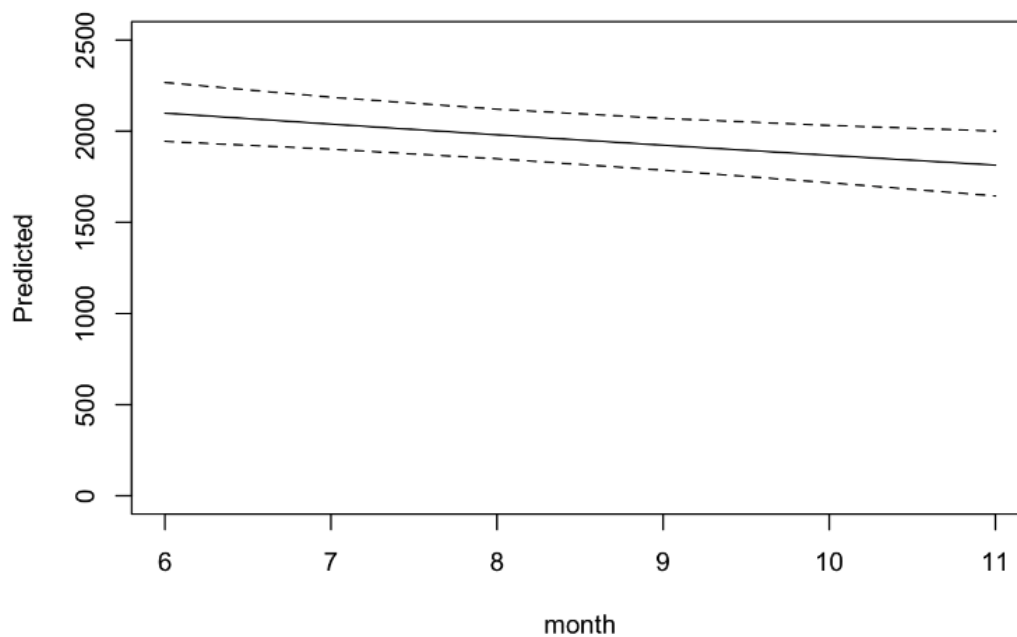


Figure 22. Abundance of Lear's Macaws for June-November of year 2010 predicted by the best-ranked model with 95% confidence intervals (dashed lines).

Model predictions indicate that between 2004 and 2014 the Lear's Macaw population tripled in size (mean = 333.37%; 95% CI: 275.62-403.22) from 665 to 2217 individuals in 2014 (95% CI: 2016.10 – 2437.88). When the few counts obtained in 2001 and 2003 were included in the model, the population size increase reached 755% (95% CI: 669.3-851.7) between 2001 and 2014 (Figure 23). Annual estimates of modeled population size were greater than annual averages based on the raw count data used by CEMAVE-ICMBio (Table 6). The ratio of the annual modeled estimate of population size to the annual count average ranged from 1.17 in 2013 to 2.03 in 2006. Model-estimated population sizes for each year were closer to the maximum number of macaws counted each year, especially in the first three years of the study when population was less than 600 individuals. In 2002, 2003 and 2013, the maximum counts were larger than the modeled population size estimate but within the 95% CI. For 2007, when counts were not performed, the model estimated 1220 macaws. In 2009, when the global population was thought to exceed 900 macaws and supported downlisting the conservation status of the species, the model estimated a population size of 1626 macaws (Table 6).

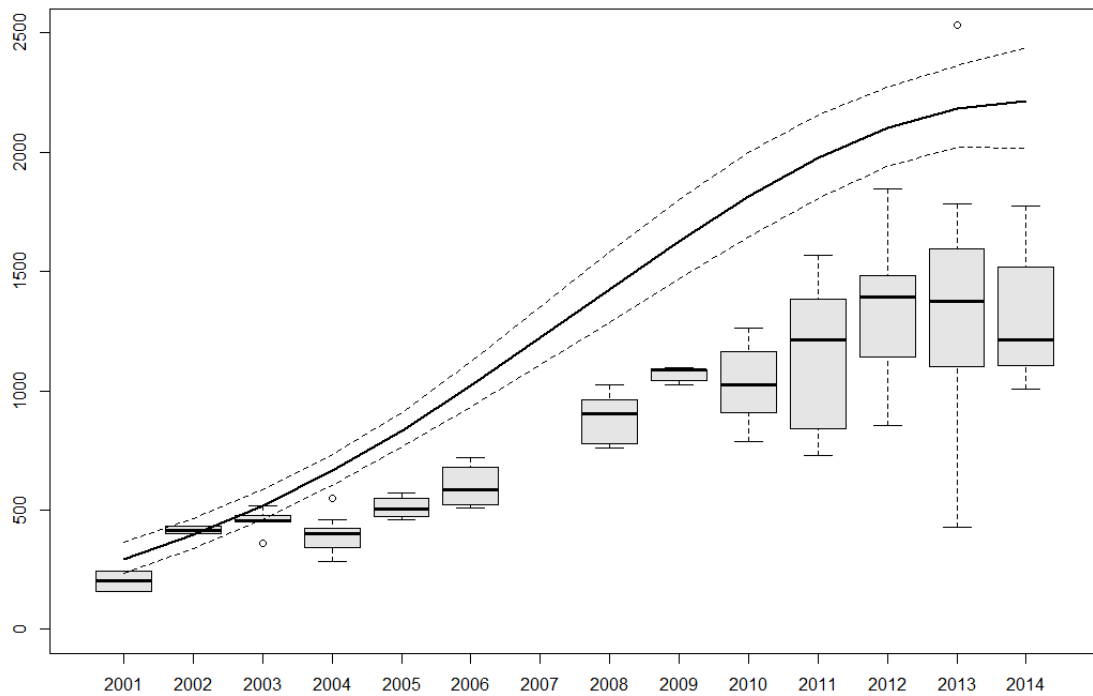


Figure 23. Abundance of Lear's Macaws predicted by the best-ranked model for 2001-2014 (black line) with 95% confidence intervals (dashed lines). Boxplots are the counts for respective years using the month of November as reference. Note differences between predictions and counts, particularly after 2004.

Table 4. Model selection results for binomial - negative binomial N-mixture models to estimate population size of Lear's Macaws from roost counts.  $\lambda$  is abundance and  $p$  is detection probability.  $\lambda(.)$  represents null model for abundance; month<sub>numeric</sub> is month (July-November) as a numeric linear effect (6-11); month<sub>numeric<sup>2</sup></sub> is month as a 2<sup>nd</sup> order polynomial; month<sub>factor</sub> is month as a factor; year is a numeric linear effect; year<sup>2</sup> is year as a 2<sup>nd</sup> order polynomial; year<sup>3</sup> is year as a 3<sup>rd</sup> order polynomial; survey time is a factor indicating whether counts occurred in the morning or the afternoon; \* indicates covariate interaction. For each model, the number of parameters estimated (K), its AIC score, the difference between AIC and the best model ( $\Delta$ AIC), and the model's AIC weight (AIC<sub>w</sub>) are displayed.

Model	K	AIC	$\Delta$ AIC	AIC <sub>w</sub>
$\lambda(\text{month}_{\text{numeric}} + \text{year}^2); p(\text{survey time})$	7	5973.62	0	0.85
$\lambda(\text{month}_{\text{factor}} + \text{year}^2); p(\text{survey time})$	11	5978.33	4.71	0.08
$\lambda(\text{month}_{\text{numeric}^2} + \text{year}^2); p(\text{survey time})$	8	5978.73	5.11	0.06
$\lambda(\text{month}_{\text{numeric}} + \text{year}); p(\text{survey time})$	6	5992.79	19.17	0
$\lambda(.); p(\text{survey time})$	4	6042.74	69.12	0
$\lambda(\text{month}_{\text{factor}} * \text{year}^2); p(\text{survey time})$	21	6102.42	128.8	0
$\lambda(\text{month}_{\text{numeric}} + \text{year}^3); p(\text{survey time})$	8	7516.44	1542.82	0

Table 5. Parameter estimates from the best-ranked abundance model of Lear's Macaws in Table 3.  $\lambda$  is abundance,  $p$  is detection probability, and  $\alpha$  is overdispersion for negative binomial parameter of  $p$ . Time of day references PM relative to AM counts.

Parameter	Estimate	SE	95%CI
<b><math>\lambda</math></b>			
Intercept	5.684	0.181	5.329 – 6.040
Month <sub>numeric</sub>	-0.029	0.01	-0.050 – -0.008
Year	0.331	0.038	0.255 – 0.407
Year <sup>2</sup>	-0.011	0.002	-0.016 – -0.007
<b><math>p</math></b>			
Intercept	0.436	0.013	0.409 – 0.463
<i>Time of day</i> <sub>PM</sub>	-0.472	0.010	-0.492 – -0.453
<b><math>\alpha</math></b>	4.82	0.365	4.102 – 5.531

Table 6. Comparison of the average number of Lear's Macaws and the maximum number of macaws counted, which were used to estimate population size by CEMAVE-ICMBio, to the annual estimate of population size and its 95 % confidence intervals derived the best-ranked model in Table 1. The ratio of underestimation for each year based on the average and the maximum number of birds counted. \*Year when the conservation status was down-listed by IUCN Red List.

Year	Mean count	Maximum count	Modeled estimate	Modeled 95% CI	Ratio Model to Mean	Ratio Model to Maximum
2001	228	280	293.64	236.72 - 364.24	1.29	1.05
<b>2002</b>	<b>332</b>	<b>431</b>	<b>394.75</b>	336.12 - 463.60	1.19	0.92
2003	442	529	518.39	459.90 – 584.31	1.17	0.98
<b>2004</b>	<b>389</b>	<b>548</b>	<b>665.02</b>	604.60 – 731.48	1.71	1.21
2005	444	570	833.39	763.20 – 910.04	1.88	1.46
<b>2006</b>	<b>502</b>	<b>652</b>	<b>1020.25</b>	930.66 – 1118.46	2.03	1.56
2007	NA	NA	1220	1105.40 – 1346.74	NA	NA
<b>2008</b>	<b>883</b>	<b>969</b>	<b>1425.4</b>	1285.58 – 1580.42	1.61	1.47
2009*	1068	1097	1626.71	1467.31 – 1803.42	1.52	1.48
<b>2010</b>	<b>1125</b>	<b>1433</b>	<b>1813.52</b>	1644.38 – 2000.05	1.61	1.27
2011	1049	1569	1975.03	1807.38 – 2158.22	1.88	1.26
<b>2012</b>	<b>1262</b>	<b>1845</b>	<b>2101.18</b>	1940.98 – 2274.60	1.66	1.14
2013	1331	2535	2183.7	2019.57 – 2361.17	1.64	0.86
<b>2014</b>	<b>1299</b>	<b>1773</b>	<b>2216.98</b>	2016.10 – 2437.88	1.71	1.25

Click on the macaws to continue the reading of the discussion of section 2: item 5.1.2



## 4.2 RESULTS: POPULATION GENETICS

### 4.2.1 Section 3 - Isolation and characterization of 15 new specific microsatellite markers for the Lear's Macaw

After DNA fragment size genotyping, 15 loci produced unambiguous allele peaks (one to five alleles per locus in this final step and seven (Ale292, Ale421, Ale514, Ale624, Ale324, Ale500 and Ale624) did not show clear assignable peaks or had a weak signal, rendering them inefficient for future studies with degraded DNA samples (such as molted feathers). Only two of the 15 assignable markers failed to amplify more than two samples (Ale504: 3 samples and Ale361: 4 samples), and one of them (Ale361) was monomorphic (Table 7).

The genetic diversity values obtained for each of the final 15 microsatellites using computer analysis (described in the materials and methods). Most of the selected markers showed moderate levels of polymorphism, except for one monomorphic locus and two biallelic loci showing low heterozygosity and PIC values. With the observed levels of genetic variation the combination of the two most polymorphic loci (i.e., the product of their PI values) was sufficient for individual identification below a 0.05 alpha error threshold. The combination of the four most polymorphic loci was necessary to reach the same level of individual identification when accounting for the possible presence of siblings in the sample (product of PI sibs indices). None of the markers showed evidence of linkage disequilibrium or deviance from Hardy-Weinberg equilibrium after applying the sequential Bonferroni correction (Table 7).

Table 7. Characterization of 15 microsatellite loci for the Lear's Macaw (*Anodorhynchus leari*). N: Number of successfully amplified samples, A: observed number of alleles, HO: observed heterozygosity, HE: expected heterozygosity, PIC: polymorphism information content, PI: probability of individual identification. PI sibs: probability of individual identification when accounting for the possibility of siblings in the sample.

Locus	Forward primer sequence	Reverse primer sequence	Repeated motif	Annealing temp. (°C)	N	Allele size range (bp)	A	H <sub>O</sub>	H <sub>E</sub>	PIC	PI	PI sibs
<i>Ale066</i>	GAAAGCCTGCCATGCCAAAG	TCTTGCACCTCCCTGAACCC	(AC)16	56	12	174-178	3	0.917	0.692	0.589	0.153	0.465
<i>Ale103</i>	TGGAACGCAATGGAGGACAG	GGCTGTCTGCTCTTTGATGC	(AC)12	59	12	189-193	2	0.083	0.083	0.077	0.811	0.923
<i>Ale176</i>	AACAGCTTCCTCAGTGTGGG	TCCTTCCTCCCTCTTCCACT	(AAGGC)9	59	12	153-174	4	0.750	0.743	0.663	0.084	0.427
<i>Ale281</i>	GCATAGGAATCCAACATCGGC	TTGCTCTGGGATGCTGCTTC	(AG)24	57	12	115-145	4	0.583	0.736	0.649	0.101	0.433
<i>Ale309</i>	TGAGGTTCCAAGAGACTCTCCT	TTCAGATCCTTGGCCCAGTG	(AC)9	57	11	144-146	2	0.091	0.091	0.083	0.796	0.916
<i>Ale327</i>	GTGATCTGGTGTGAGGACCA	GACAGTCTGCTTCACTCCAGA	(AC)10	59	12	170-182	4	0.750	0.746	0.663	0.090	0.426
<i>Ale361</i>	ACAGGGACTTGAATGTAGCAGT	ATGATGGTGTGATCCCAGCC	(AC)7	56	8	175	1	0.000	0.000	0.000	1.000	1.000
<i>Ale363</i>	TGGGAGGGTTTATTGGTTGGG	TGTGGGATTGGCCACTATAGG	(AC)12	59	12	106-130	3	0.417	0.540	0.420	0.294	0.574
<i>Ale409</i>	GGTTTCAGTCCTCCCTTGACT	TCCCTTCTTCTCCCATTCCC	(AG)10	57	12	124-126	2	0.583	0.518	0.373	0.364	0.596
<i>Ale418</i>	CGCTTGCATGTA CTCTTACCC	TCAAGGGCAGTTGTGGGAG	(AC)16	56	12	203-224	3	0.417	0.453	0.369	0.315	0.629
<i>Ale504</i>	GCTTCTTAGCCATGCAACCC	ACAAAGCCAACTTCTACAACAA	(AT)7	59	9	148-150	2	0.556	0.425	0.321	0.386	0.659
<i>Ale517</i>	TTGTAGTAAGTGTGGGTAAAGCC	CATGGAGCTCTGGGCAGAAA	(ACT)16	56	11	155-167	4	0.545	0.515	0.451	0.206	0.579
<i>Ale529</i>	TGATAAGCAGAGGAGGAATCCT	AGATGGAGGTGCTGTGAGAC	(AC)16	59	12	128-150	5	0.667	0.710	0.628	0.101	0.448
<i>Ale606</i>	AATCCCAACTCAACAGGGTTGT	CTTTCTACCTTCTCAAACACCCT	(AC)11	59	11	104-106	2	0.364	0.312	0.253	0.464	0.736
<i>Ale628</i>	AGAGGGAATGTCTCTTCCATT	TGCTAGATAACATACACAGCCAG	(AC)9	56	11	135-147	3	0.545	0.450	0.385	0.280	0.628

Click on the macaws to continue the reading of the discussion of section 3: item 5.2.1



## 4.2.2 Section 4 – Lear’s Macaw population status assessment from non-invasive genetic samples

### 4.2.2.1 Non-invasive biological sampling and individual identification

We collected a total of 1,189 samples and could isolate DNA from 52.48% of molted feathers. Successful DNA amplification by genotyping was possible in 83% of selected DNA samples for sex determination ( $n = 165$ ). We genotyped a total of 147 molted feather samples, and after genotyping we found that 15.64% ( $n = 23$ ) of the samples were replicates.

The percentage of genotyped individuals in relation to the number of individuals estimated by roosting counts of the macaws in each sampled area varied considerably (see roosting counts methods and numbers in Section 2, Appendix 6). Among the samples from the areas where the macaws have recently expanded (*Barreiras*, *Barra do Tanque* and *Baixa do Chico*), those from *Barreiras* represented a larger proportion of the local population (70%; 21 individuals identified from ~30 individuals counted at roosts), and thus genetic sampling can be considered representative. On the other hand, in the *Baixa do Chico* locality representation amounted to 31.25% (25 individuals were identified from ~ 80 individuals counted at roosts), and in the *Barra do Tanque* area, only 6% of the estimated local population was genotyped (9 individuals identified from ~150 individuals estimated). In the core areas of *Toca Velha* (Canudos) and *Serra Branca* (Jeremoabo), representation was, respectively, 8.13% (~750 estimated population, 61 samples) and 0.9% (9 individuals identified from ~1000 estimated population). We also sampled molted feathers ( $n = 3$ ) from a roosting site disconnected from the *Raso da Catarina* Ecoregion population, at *Cercadinho* Farm at *Boqueirão da Onça* area, but unfortunately, after sexing and microsatellite testing, the amount and quality of DNA was not sufficient for genotyping (see *Table 8*).

All the genotyped samples (including molted feathers and blood from nestlings,  $n = 10$ ) showed concordant allele assignments between both genotyping replicates (**Appendix 10**).

Multiple feather samples were identified in eleven individuals from *Toca Velha* (three males, eight females), one from *Serra Branca* (undetermined sex), six from *Baixa Chico* (four males and two females) and three from *Barreiras* (two males, one female), represented by two or more feather samples each. Among them, one female from *Toca Velha* and one male from *Barreiras* were represented by four and three feather samples, respectively. All matched genotypes in *Serra Branca* and *Barreiras* were from samples collected in the same year (2014), whereas matches in *Toca Velha* and *Baixa Chico* were collected with up to three (2010-2013) or two (2014-2016) years difference, respectively. No matches were found in *Barra Tanque*, nor among samples obtained in different localities.

Table 8. Aproximated estimated number of individuals (Nest), total numbers of molted feathers collected in the field (Ncol), selected for DNA extraction (Next), with molecular sexing consistent results (Nsex) and successfully genotyped (Ngen), number of unique individuals identified after genotyping (Nindiv) and estimation of adult sex ratio (SR) considering unique individuals (i.e. excluding the repeated individuals identified in each locality).

Locality	Nest	Ncol	Next	Nsex	Ngen	Nindiv	SR
Toca Velha	750	438	260	62	62	51	0.68
Serra Branca	1000	203	96	10	12	9	1.00
Barreiras	30	95	65	25	27	21	0.62
Baixa do Chico	80	269	100	32	31	25	0.56
Barra do Tanque	150	181	100	26	9	8	0.51
Cercadinho	2	3	3	2	0	NA	NA
Overall	2012	1189	624	165	137	114	0.61

#### 4.2.2.2 Genetic Diversity, Population size and genetic differentiation

All localities, as well as the pooled dataset, showed moderate levels of genetic diversity, with Toca Velha presenting the highest estimates (Table 9). No evidence of consistent departure from Hardy-Weinberg genotypic proportions or linkage disequilibrium between any pair of markers was found in any of the localities after applying the Bonferroni correction. No evidence of strong inbreeding (*i.e.*, high values of  $F_S$ ) was found in any locality (Table 9).

Estimates of  $N_e$  for the total dataset were consistent among replicates, indicating an overall low population size in the range of 49 to 53. Estimates of  $N_e$  for the different localities were also low and variable among replicates, mainly depending on the use or not of the sibship size prior. While *Serra Branca* and *Barra do Tanque* showed up to a 6-fold difference in  $N_e$  estimates mediated by the use or not of a weak sibship size prior, and unrealistic 95% confidence intervals in some cases, *Toca Velha* showed the most consistent estimates, with  $N_e$  in the range of 22 to 30. *Barreiras* and *Baixa do Chico* showed estimates with informative 95% confidence intervals, but a 2-fold difference in estimates depending on the use or not of the sibship size prior (Table 9).

Overall genetic differentiation was low (overall  $F_{ST} = 0.014$ ), with *Barra do Tanque* and *Toca Velha* showing the highest pairwise differentiation ( $F_{ST} = 0.042$ ). Low  $F_{ST}$  values were in concordance with results of *structure*; although the most likely value of  $K$  was 2 according to both the original and Delta methods, the five localities showed wide genetic admixture (Table 9; Figure 24).

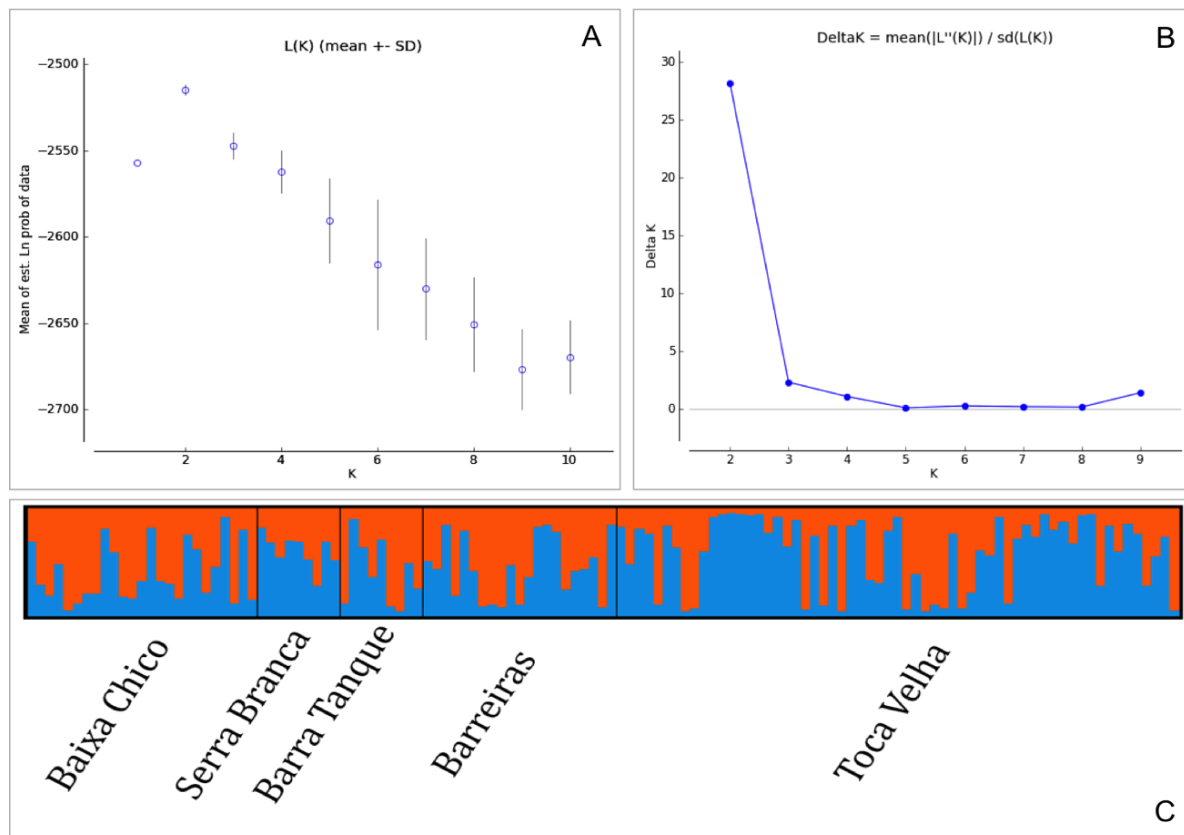


Figure 24. Genetic structure among the five sample localities: Likelihood of the different numbers of clusters (K) following A) the original and B) the Delta K methods. C) Individual assignment plot showing the probability of assignment of each individual to either one of the K = 2 predefined clusters.

Table 9. Sample size ( $N$ ), average estimates (and standard errors) of genetic diversity indexes and effective population size ( $N_e$ ) with 95% confidence intervals (95% CI) for each locality and for the complete dataset.  $AR$ : allelic richness,  $H_o$ : observed heterozygosity,  $H_E$ : expected heterozygosity,  $PA$ : number of private alleles (only for separated localities). Sib. prior: use or not of the sibship size prior = 1 in  $N_e$  analyses.

Locality	$N$	$AR$	$H_o$	$H_E$	$PA$	$F_{IS}$	Sib. prior	Inbreeding	$N_e$ (95% CI)
<b>Toca Velha</b>	61	5.33 (0.55)	0.72 (0.07)	0.67 (0.06)	0	-0.05 (0.02)	No	No	29 (18-51)
								No	30 (19-52)
								Yes	28 (17-48)
<b>Toca Velha</b>	61	5.33 (0.55)	0.72 (0.07)	0.67 (0.06)	0	-0.05 (0.02)	Weak	Yes	30 (19-51)
								No	24 (15-45)
								Yes	22 (13-40)
<b>Serra Branca</b>	9	4.44 (0.34)	0.56 (0.06)	0.60 (0.05)	0	0.04 (0.08)	Weak	Yes	24 (15-45)
								No	22 (13-40)
								Yes	22 (13-40)
<b>Serra Branca</b>	9	4.44 (0.34)	0.56 (0.06)	0.60 (0.05)	0	0.04 (0.08)	No	No	48 (16-Inf)
								Yes	48 (15-Inf)
								Yes	29 (12-Inf)
<b>Baixa Chico</b>	25	4.89 (0.48)	0.67 (0.05)	0.66 (0.03)	0	-0.02 (0.04)	Weak	Yes	29 (12-Inf)
								No	9 (4-46)
								Yes	9 (3-45)
<b>Baixa Chico</b>	25	4.89 (0.48)	0.67 (0.05)	0.66 (0.03)	0	-0.02 (0.04)	No	Yes	9 (4-46)
								No	9 (4-46)
								Yes	9 (4-64)
<b>Barreiras</b>	21	5.33 (0.55)	0.61 (0.06)	0.63 (0.05)	1	0.04 (0.03)	Weak	No	32 (18-64)
								Yes	32 (18-66)
								Yes	32 (17-64)
<b>Barreiras</b>	21	5.33 (0.55)	0.61 (0.06)	0.63 (0.05)	1	0.04 (0.03)	No	Yes	32 (18-61)
								No	14 (7-30)
								Yes	14 (8-31)
<b>Barra Tanque</b>	9	4.00 (0.33)	0.64 (0.07)	0.59 (0.05)	0	-0.08 (0.06)	Weak	Yes	14 (7-30)
								No	14 (7-30)
								Yes	14 (8-31)
<b>Barra Tanque</b>	9	4.00 (0.33)	0.64 (0.07)	0.59 (0.05)	0	-0.08 (0.06)	No	No	26 (14-59)
								Yes	26 (14-57)
								Yes	26 (14-58)
<b>Barra Tanque</b>	9	4.00 (0.33)	0.64 (0.07)	0.59 (0.05)	0	-0.08 (0.06)	Weak	No	26 (14-59)
								Yes	14 (7-30)
								No	14 (7-33)

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							No	Yes	14 (7-30)
								Yes	14 (7-33)
								No	29 (11-Inf)
								No	29 (12-Inf)
							No	Yes	29 (11-Inf)
							Weak	Yes	29 (10-Inf)
								No	5 (2-20)
								No	5 (2-20)
							Weak	Yes	5 (2-20)
								Yes	5 (2-20)
<b>Total</b>	125	4.80 (0.21)	0.64 (0.03)	0.63 (0.02)	-	-0.01 (0.02)	No	No	54 (38-80)
								No	
							No	Yes	49 (34-73)
							Weak	Yes	
								No	50 (34-75)
								No	56 (39-85)
							Weak	Yes	50 (34-75)
								Yes	56 (39-85)

#### 4.2.2.3 Sex ratio estimation

Overall sex ratio was 0.61 ( $n = 114$ ), indicating a general male bias. This bias was extreme in *Barra do Tanque*, where all identified individuals were males ( $\sim$ SR = 1.00,  $n = 8$ ). In *Serra Branca* ( $n = 9$ ) the proportion of males to females was close to 1:1 (SR = 0.56), while in BDO all collected feathers corresponded to females, according to molecular sexing. However, we could not genotype these samples, so we still cannot state that the two individuals observed in the field are females (*Table 8*).

Click on the macaws to continue the reading of the discussion of section 4: item 5.2.2



## 4.3 RESULTS: THREATS

### 4.3.1 Section 5 - Past and current threats of the Lear's Macaw: implications for distribution and population expansion

#### 4.3.1.1 Local knowledge and threats to Lear's Macaws

We contacted 144 people along transects and displacements in the surveyed area from 29 municipalities (green dots in *Figure 13 A*) from which we could interview 112. Most interviews (90.3%) were done within the potential distribution range of the Lear's Macaw estimated by IUCN. From the total people 72% were men, 66% were older than 60 years, 63.9% used to be a farmer, and 80,5% were born and always lived in the same locality. Most of the interviewees (69.4%) knew the Lear's Macaw and 46.05% of them indicated that the species is common in their locality, while 27.63% indicated it is rare, 22.36% indicated its presence in the past and further local extinction, and 3.94% admitted lacking knowledge about the conservation status of the species.

Among interviewees (n=112), 55.3% identified trapping and nest poaching as a threat for parrots species, while 32.1% identified deforestation, and 32.1% identified hunting. Other threats mentioned included shot at crops (16.1%), competition with Africanized honey bees (15.2%), seasonal acute droughts (13.4), fire (11.6%), disturbance in communal roosts (10.7%), overgrazing (6.2%), roads (3.6%), and collision at power lines - electrocutions (1.8%).

Macaw fatalities due to electrocutions by power line collisions were reported by three interviewees, at feeding sites close to the *Barra do Tanque* (2 reports) and *Serra Branca* (1 report) roosting sites (Table 2). Additional electrocutions at power lines were later reported to us by the local community mostly in the feeding sites (i.e. not from interviews; *Figure 25 C*). In total, we compiled eleven electrocutions from six localities in the RASO area (*Figure 13 C*): *Matinha* (1 electrocution) and *Brejo Grande*

(1) in *Jeremoabo* municipality; *Barra do Tanque* (2) and *Juazeiro* Farm (3) in *Euclides da Cunha* municipality, and *Malhador da Jurema* (1) and the *Serra Branca* village (1) in *Canudos* municipality. In addition, while conducting the road surveys, we identified two locations with power lines (village *Rosário* and *Juazeiro* farm; *Figure 25 A* and *B*) where the macaws (one to 25 individuals) were observed perching during the day, close to feeding areas with *Licuri* Palm patches and arboreal *Caatinga* forest. We observed the macaws and noticed that the macaws used the power lines to rest (i.e. resting during the day) and play with each other.

Presence and prospection of wind farms was seen as a threat by interviewees (2.7%) due to habitat degradation, depletion of food resources (deforestation), and human disturbance during wind farm implementation and operation. However, evidence of collision risk of macaws at wind turbines was still not reported by interviewees, and we not performed systematic field observations to verify it.

The bulk of local knowledge, together with our field data collected during roadside transects (see below), helped to compile the occurrence of threats in different localities within the current and past distribution of Lear's Macaws (*Table 10*). The most pervasive threat identified was hunting (in ten of the 11 main localities), followed by deforestation (9 localities), overgrazing and fire (8 localities) and nest poaching (7 localities), evidencing that direct mortality (including nestling removal), food suppression and habitat loss are all relevant impacts for the species. Among the localities surveyed, *Barreiras*, *Baixa do Chico* and *Serra do Massacará* ranked highest in number of threats (seven threats each).

Table 10 Ranking of importance of the main threats found for the Lear's Macaw in the current or past localities occupied by the species, combining local knowledge and field observations.

Threats	Impacts	Toca Velha	Serra Branca	Barreiras	Baixa do Chico	Barra do Tanque	S. Borracha	S. Canabrava*	Boq. da Onça Cercadinho*	Boq. da Onça Delfina	Gruta dos Brejões	Serra do Massacarã	Ranking
Human disturbance at roosts	Habitat Loss			X	X	X				X		X	<b>6</b>
Invasive Africanized honey bees		X	UNK	X	X		UNK			UNK	X	X	<b>6</b>
Overgrazing	Habitat Loss	X	X	X	X	X	X	X				X	<b>8</b>
Deforestation	Food suppression			X	X	X	X	X	X	X		X	<b>9</b>
Fire					X	X	X	X	X	X		X	<b>8</b>
Wind farms	Habitat Loss								X	X			
	Food suppression			X									<b>3</b>
	Mortality increase												
Macaw hunting					X			X	X	X		X	<b>5</b>
Hunting		X	X	X	X	UNK	X	X	X	X	X	X	<b>10</b>
Gun shot at crops	Mortality increase		X			X		UNK			UNK	X	<b>3</b>
Parrot nest poaching		X	UNK	X	X		X			X	X	X	<b>7</b>
Power line collision				X			X						<b>2</b>
<b>Total threats by area</b>		<b>4</b>	<b>4</b>	<b>7</b>	<b>7</b>	<b>4</b>	<b>6</b>	<b>4</b>	<b>3</b>	<b>6</b>	<b>6</b>	<b>7</b>	



Figure 25 A. Lear's Macaw observed using the power lines during foraging in the Licuri Palm patches. B. Macaws perching in the power lines structures (Photo by Thiago Filadelfo). C. Dead Lear's Macaw found electrocuted under a power line (Photo by Marlene Reis).

#### 4.3.1.2 Review of Lear's Macaw breeding and roosting locations

##### 4.3.1.2.1 Core areas

The main remaining population is concentrated in two protected areas in RASO: *Toca Velha* and *Serra Branca* (Figure 13 C, locations 1 and 2 respectively). This population was discovered in 1978 and has been monitored since 2001 (Nascimento et al. 2001). There are three roosting cliffs and six breeding cliffs at *Toca Velha* and five roosting cliffs and four breeding cliffs at *Serra Branca*. Roost counts in these two areas from 2001 to 2014 were used to predict the population trend, and we estimated that population tripled in size (mean = 333.37%) in 10 years (from 665 to 2217 individuals) (see section 2). This population growth may reflect the beneficial outcome of the restricted access to these protected areas, excluding hunters and nest poachers, both of which were identified as key threats to the species (Table 1). However, we observed activities such as goat and cattle grazing, despite their being in protected areas, which would likely have detrimental effects to the vegetation and thus habitat quality for the macaws, particularly in terms of food resources. Moreover, the presence of livestock and their keepers near roosts and nests disturb the macaws, especially cattle that were observed in the bottom of roosting cliffs. Livestock keepers were also observed in both areas indicating that access restriction is compromised.

##### 4.3.1.2.2 Recently occupied areas

We surveyed areas historically used by the macaws as the *Baixa do Chico* village - Brejo do Burgo Pankararés Indigenous Land, in the south of *Glória* municipality, situated 50 Km north of the *Serra Branca* the nearest roosting area. According to local residents, large numbers of the species gathered and bred in this locality in the past (c. 1980), but were extirpated due to hunting and illegal capture of nestlings for the pet trade. Macaws recolonized the area in January 2014 (location 4 in the Figure 13 C). We observed about 50 individuals roosting in May of 2014 (end of the breeding season) and according to locals interviewed at least two cavities in the cliffs were used for nesting by the species in the past (c. 1980). We repeated surveys in the following years and observed an increasing number of roosting macaws (84 individuals by 2016). We also confirmed breeding attempts in the indicated historical nests, with a minimum of three breeding pairs in 2016 (see section 6).

Another subpopulation (location 3 in the Figure 13 C) was verified in the site known as *Barreiras*, an uninhabited rural area, in the south of the *Canudos* municipality, situated 40km southeast and 37 km south of the *Toca Velha* and *Serra Branca* subpopulations respectively. This site was previously considered as a foraging area occasionally frequented by macaws from the two known subpopulations. The fact that macaws have been regularly observed foraging in the *Licuri Palm* patches in this area (Santos and

Camandoraba 2008) suggests that it is a feeding site. Moreover, the macaws generally arrive in the area in groups of c. 15-35 individuals around 8-9:00AM, further suggesting that they roost far from this locality, as macaws often leave known roosts at dawn, i.e. 5-6:00 AM). However, we recorded 22 macaws in May 2014 and 19 in April 2016 roosting in two different cliffs in each occasion, located at 3 and 5 km respectively, and arriving at the licuri patches around 6:00AM in both instances. Moreover, observations in the located roosting sites made in July and September (2014 and 2016) suggested that the macaws were not roosting in the area. That could indicate that the macaws roosted in the area only during the breeding season, or that the small number of macaws could make them more difficult to detect if they switch to other unknown roosting sites.

Farmers and honey-hunters informed us in 2014 that the species always used to breed there, indicating at least five nests. We confirmed breeding activities in this area in the following years, with at least five breeding pairs in 2016. The intense and frequent disturbances caused by honey hunters, competition for nest cavities with Africanized honey bees, and active nest poaching by people compromised there the roosting and nesting activities of the macaws, thus limiting the permanent use of this area by the species (see Section 6).

The same individuals recorded foraging in the Licuri Palm patches and in the roosting sites in the cliffs of *Barreiras* were also frequently observed using as a diurnal roost at a *braúna* tree (*Schinopsis brasiliensis* Engler) (~ height 16m, DBH - 1.17m) during the hottest time of the day (aprox. 10 am to 3 pm), indicating that large trees are also key for the species. Accordingly, we discovered a new nocturnal communal roost using trees instead of cliffs (location 5 in *Figure 13 C*) thanks to interviews and surveys done in May 2016 at *Barra do Tanque* village, within a private farm called *Baixa de Canudos*, located in *Euclides da Cunha* municipality. A considerable number of individuals (147-157) were found roosting in a small fragment of degraded vegetation with a patch of native trees, with two aroeiras (*Myracrodruon urundeuva* Alemão - mean height 12m and mean DBH - 1.12m) and three *braúnas* (mean height 11.33m and mean DBH - 1.09m) used as roosts. No evidence of breeding activity has been found to date, and the nocturnal roosts are shared with black (*Coragyps atratus*) and turkey vultures (*Cathartes aura*).

#### 4.3.1.2.3 Historical areas

Interviews lead us to identify areas where Lear's Macaws were locally extirpated. We obtained information on the areas of historical macaw occurrence, potential extinction causes and roosting and feeding sites and behaviour. These areas include (see locations in *Figure 1*): *Serra da Borracha*, identified as a roosting and breeding site (location 6), *Serra da Cana Brava*, likely a foraging site of macaws from *Serra da Borracha* (location 7), *Boqueirão da Onça* specifically at *Serra do Talhado - Parque Eólico Delfina* (location 10), likely the roosting and breeding site of the macaws found in location 9, the foraging site of *Cercadinho Village* (both in the surroundings of the *Boqueirão da Onça* National

Park, location 10, Figure 1 B), where we confirmed the existence of a functionally extinct population with only two non-breeding adults remaining (based on observations between 2012 and 2018), *Gruta dos Brejões*, reported as a roosting and breeding site for a small group (ca. 30) of macaws (location 11), and Serra do Massacará (Terra Indígena Massacará, location 12), reported as an occurrence area with no additional information on its use by macaws (i.e. foraging or roosting/breeding).

#### 4.3.1.3 Lear's Macaw habitat

##### 4.3.1.3.1 Anthropogenic activity impacts

We analyzed data from 71 transects (1989.54km) from the two main regions that correspond to the Lear's Macaw potential occurrence, including the two current ranges defined for the species: RASO (1329.81km surveyed in 54 transects) and BDO (659.73km surveyed in 17 transects). From the total of km surveyed, 28.22% were categorized as natural habitat with no evidence of anthropogenic activity, but the majority (53.54%) was categorized as natural degraded habitats (23.66%) or mixed with agriculture (29.88%), while non-natural habitats (i.e. rural settlement or agro-pastoral) covered 18.22% of the km surveyed. Urban areas, with a negligible length in km surveyed relative to the other habitats in the study area, were not analyzed.

We recorded the presence of multiple agricultural crop types in the surveyed area. Most of them corresponded to seasonal subsistence cultures (e.g. *Opuntia*, *Zea mays*, *Vigna*, *Ricinus*, *Manihot*, *sugarcane*), while there were some patches of more permanent cultivation with arboreal vegetation structure (e.g. *Prosopis juliflora*, *Agave sisalana*, *Anacardium occidentale*, *Musa*, *Mangifera*, *Cocos* and *Eucaliptus*). We also observed evidence of non-natural fires, recent or old, in the form of burned natural vegetation and crops (e.g. sugarcane is often burned before harvesting). Other anthropogenic land uses and disturbances observed during surveys include irregular garbage dumps, and maintained and abandoned pastures.

Potential overgrazing was assessed with counts of free-range cattle (2454 encounters, with 8749 individuals counted) and goats (153 encounters, 4875 individuals) and their inclusion as covariates in the multinomial log-linear model for the probability of occurrence of the different habitat types. The predictions from this model indicated that increasing numbers of cattle were negatively correlated with the occurrence of natural habitat, were uncorrelated with degraded natural habitat and degraded habitat mixed with agriculture, and positively correlated with agro-pastoral habitats (Table 11, Figure 26 A, C, E and G). The abundance of goats, however, was negatively correlated to the occurrence of agro-pastoral habitat, was positively correlated with degraded natural habitat and uncorrelated to natural habitat and degraded natural habitat mixed with agriculture (Table 11, Figure 26 B, D, F and H). The estimated effects of cattle and goat abundance were similar among the different Lear's Macaw occurrence areas (Table 11, Figure 26). No clear

differences in the above-mentioned effects were observed among the different Lear's Macaw occurrence ecoregions (*Table 11, Figure 27*).

Table 11 Results for the habitat conservation status occurrence model (multinomial GLM). The response, habitat type, is a categorical variable with four levels: conserved habitat, degraded habitat, degraded habitat mixed with agriculture; and rural settlement (agro-pastoral) habitat. We used conserved habitat as baseline, and provide coefficient estimates for each of the other categories. Categorical covariate baseline levels are as follows: Licuri Palm - absent; fire - absent; ecoregion - BDO; Lear's Macaw occurrence - no record. Cattle and goat are count variables.

Multinomial model coefficients (logit link)				
Degraded habitat	Estimate	Std. Error	z value	Pr(> z )
(Intercept)	-0.591	0.275	-2.145	0.032
cattle	0.020	0.013	1.604	0.109
goat	0.006	0.005	1.222	0.222
Licuri Palm present	-0.736	0.422	-1.742	0.082
fire present	15.012	0.328	45.704	< 0.000
<b>ecoregion RASO</b>	<b>0.413</b>	<b>0.312</b>	<b>1.323</b>	<b>0.186</b>
LM occurrence core	0.281	0.304	0.925	0.355
LM occurrence historic	0.380	0.424	0.895	0.371
LM occurrence recent	0.089	0.483	0.185	0.854
Degraded habitat mixed with agriculture				
(Intercept)	-0.083	0.233	-0.357	0.721
cattle	0.020	0.012	1.737	0.082
goat	0.002	0.005	0.394	0.694
Licuri Palm present	0.895	0.299	2.992	0.003
fire present	13.556	0.383	35.415	< 0.000
<b>ecoregion RASO</b>	<b>0.279</b>	<b>0.270</b>	<b>1.030</b>	<b>0.303</b>
LM occurrence core	-0.062	0.280	-0.221	0.825
LM occurrence historic	0.066	0.384	0.172	0.863
LM occurrence recent	0.417	0.416	1.003	0.316
Rural Settlement (Agro-pastoral)				
(Intercept)	0.298	0.232	1.285	0.199
cattle	0.024	0.012	2.076	0.038
goat	-0.012	0.007	-1.710	0.087
Licuri Palm present	-0.935	0.386	-2.422	0.015
fire present	13.066	0.437	29.901	< 0.000
<b>ecoregion RASO</b>	<b>0.084</b>	<b>0.275</b>	<b>0.304</b>	<b>0.761</b>
LM occurrence core	-0.043	0.288	-0.148	0.882
LM occurrence historic	0.475	0.361	1.314	0.189
LM occurrence recent	0.202	0.427	0.475	0.635

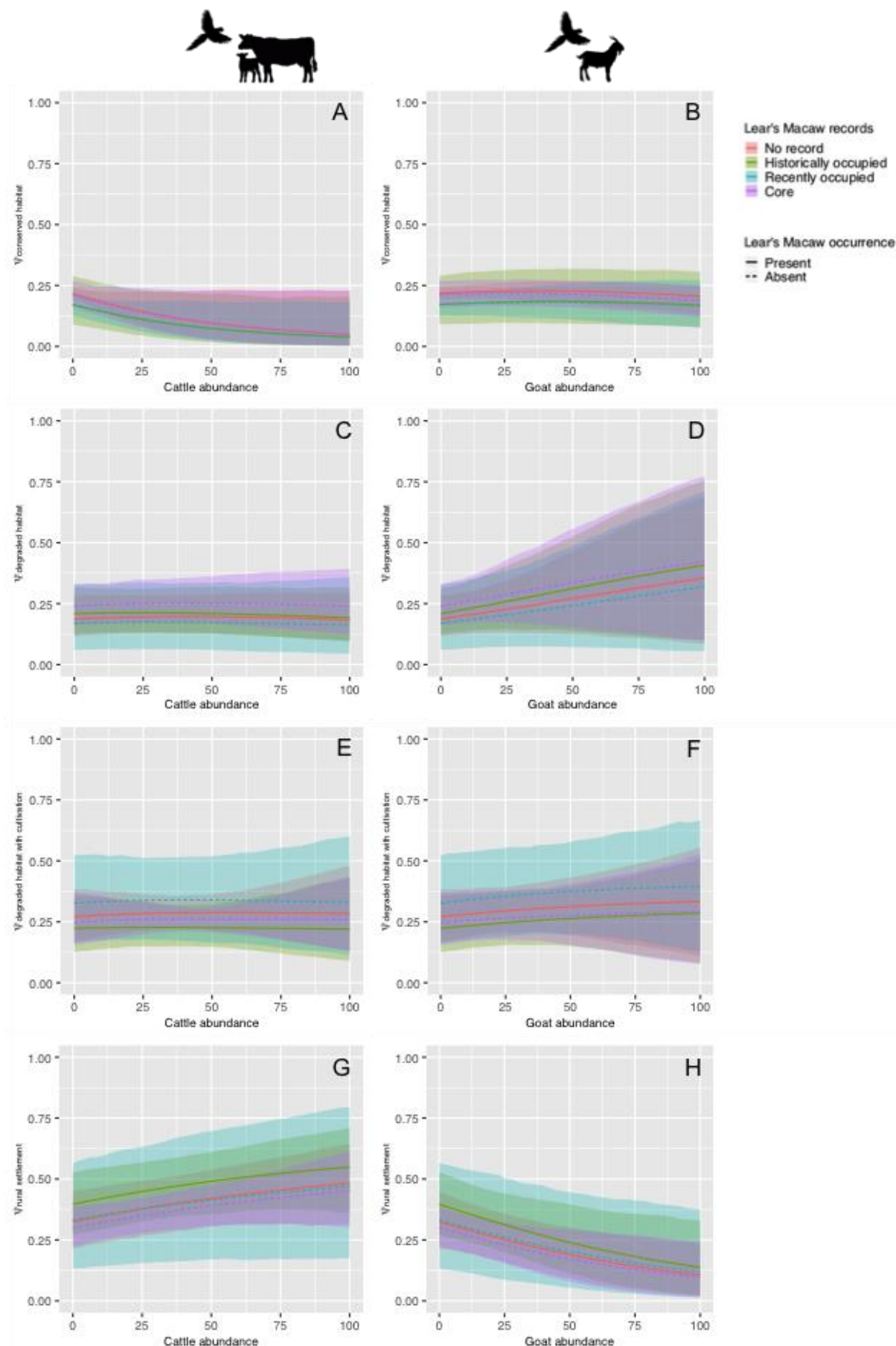


Figure 26 Model predictions with 95% CI for probability of occurrence ( $\Psi$ ) of the different habitat conservation status as a function of overgrazing estimated through cattle and goat abundance, for areas where the Lear's Macaw is present (core or recently occupied areas) or absent (areas with historical records or without records). Additional covariates in the model (i.e. other than the ones shown in each plot) were set to the following baseline values to generate  $\Psi$  predictions: cattle abundance (for plots A, C, E and G) = 0; goat abundance (for plots B, D, F and H) = 0; Licuri Palms absent; no fire; region: Raso da Catarina.

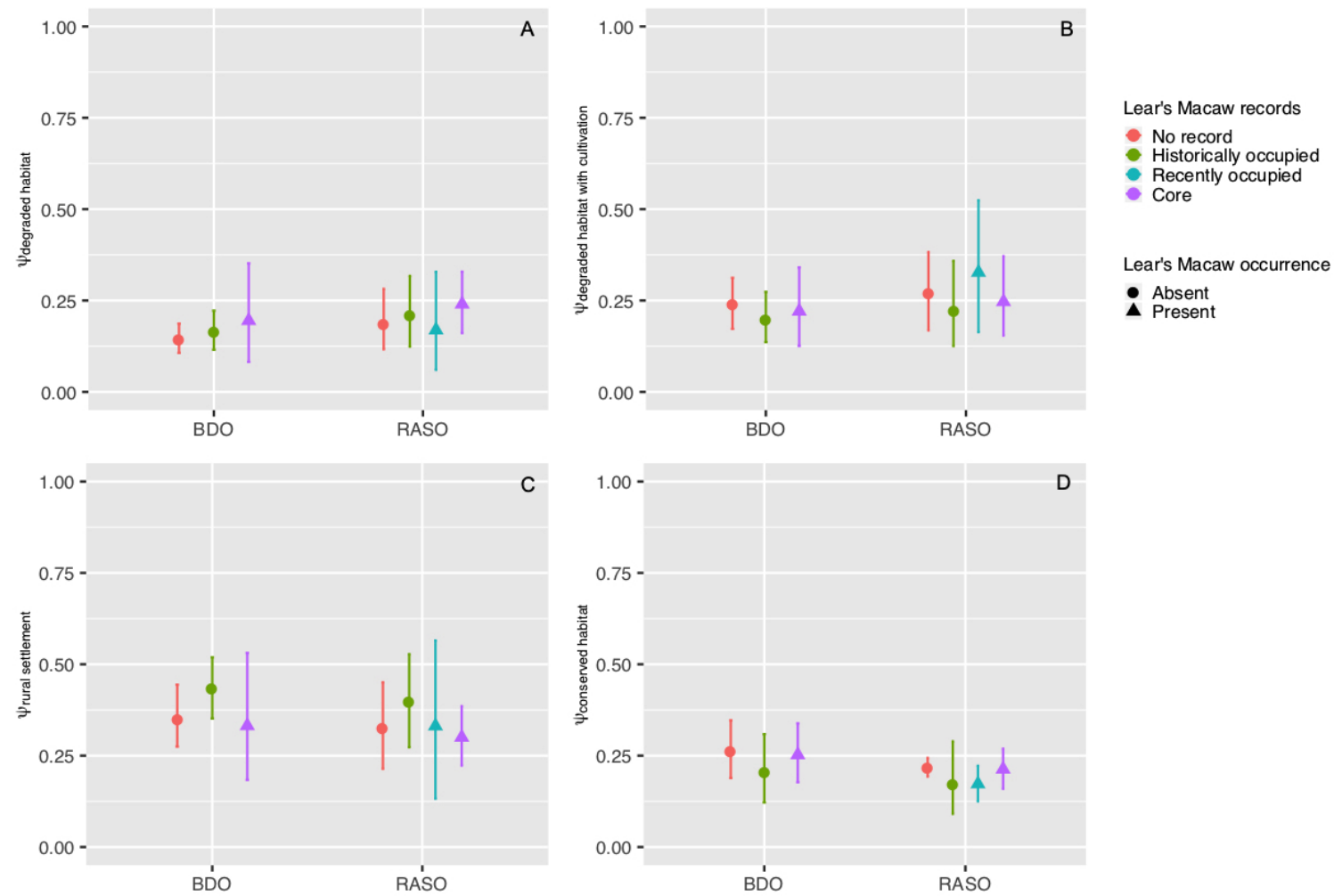


Figure 27 Model predictions with 95% CI for probability of occurrence ( $\Psi$ ) of the different habitat types as a function of the different studied regions (Raso da Catarina and Boqueirão da Onça), for areas where the Lear's Macaw is present (core or recently occupied areas) or absent (areas with historical records only or without records). Additional covariates in the model (i.e. other than the ones shown in each plot) were set to the following baseline values to generate  $\Psi$  predictions: cattle abundance = 0; goat abundance = 0; Licuri Palm absent; no fire; region: Raso da Catarina.

We recorded 787 Licuri Palm patches, of which 270 (34%) were associated with ranching areas, 199 (25%) with agricultural areas, and 3% had evidence of fire use. Regarding the associations of Licuri Palm with the different conservation status habitat types, the predictions from the multinomial log-linear model indicate that its occurrence is negatively correlated with degraded habitat (*Table 11*) and rural settlements (*Table 11*), positively correlated with degraded habitat mixed with agriculture (*Table 11*), and had no clear correlation with conserved habitat (*Table 11*). Predictions from the binomial GLM indicate that the probability of occurrence of Licuri Palm patches is uncorrelated to the abundance of cattle or goats (*Table 12, Figure 28 A and B*), but is positively correlated with the presence of pastures, cultivation and fires (*Table 12, Figure 28 C, D and E*). Licuri patches also have a higher probability of occurrence in BDO than in RASO (*Table 12, Figure 28 F*). Occurrence of Licuri Palm patches is significantly higher in core areas and significantly lower in historical areas compared to areas recently occupied and those not occupied by Lear's Macaws (*Table 12*).

Table 12 Results for the Licuri Palm occurrence model (binomial GLM). Categorical covariate baseline levels are as follows: ecoregion - BDO; ranching - absent; agriculture - absent; fire - absent; Lear's Macaw occurrence - no record. Cattle and goat are count variables.

Model coefficients (binomial with logit link):				
	Estimate	Std. Error	z value	Pr(> z )
(Intercept)	-1.690	0.242	-6.993	< 0.000
ecoregion RASO	-1.518	0.289	-5.25	< 0.000
Cattle	-0.001	0.007	-0.179	0.858
Goat	-0.013	0.008	-1.572	0.116
ranching present	0.832	0.259	3.216	0.001
agriculture present	1.000	0.265	3.771	< 0.000
fire present	1.046	0.604	1.733	0.083
LM occurrence core	0.922	0.299	3.084	0.002
LM occurrence historic	-1.404	0.459	-3.061	0.002
LM occurrence recent	0.110	0.506	0.218	0.827

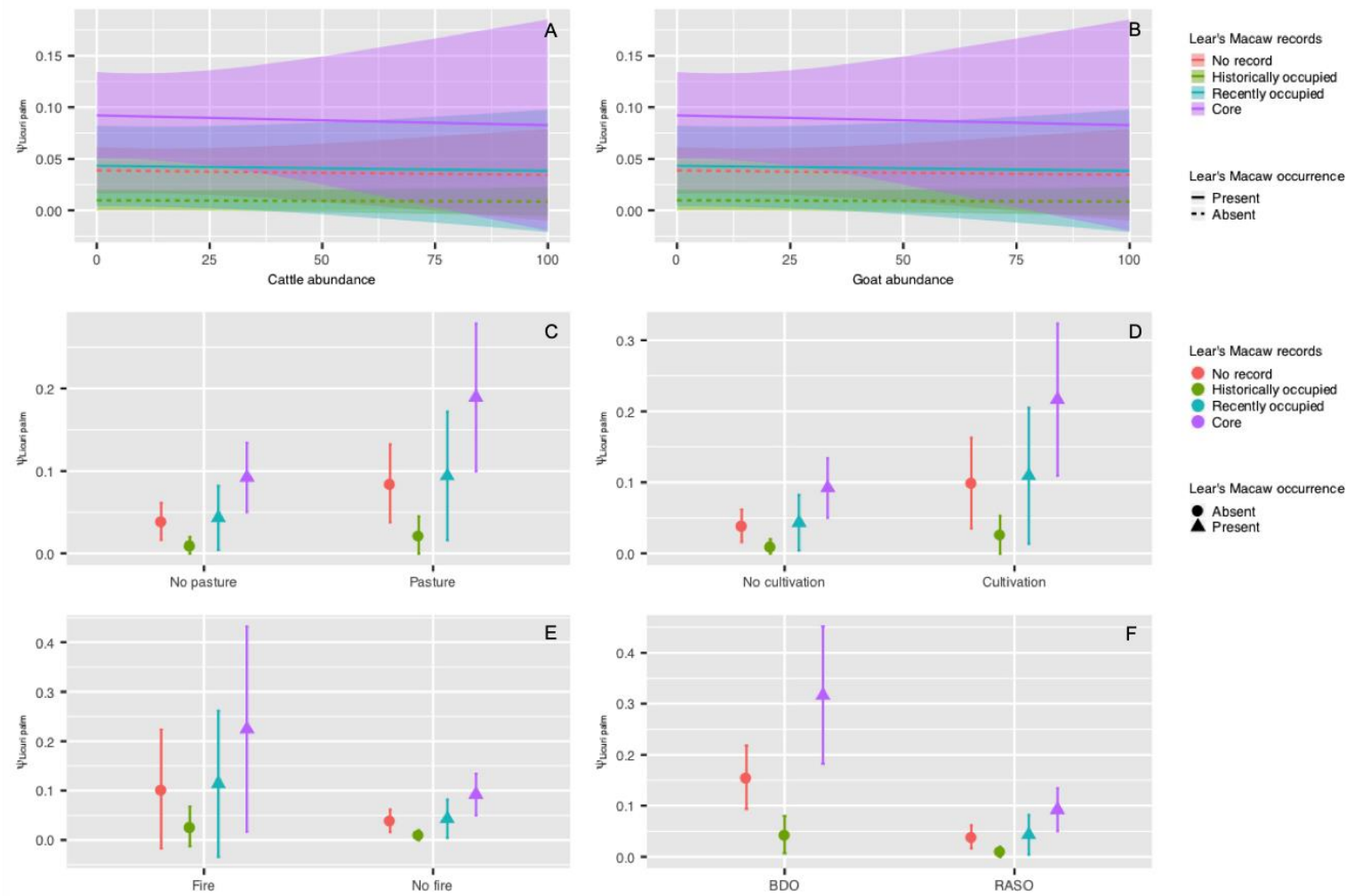


Figure 28 Model prediction with 95% CI for probability of Licuri Palm presence ( $\Psi_{\text{Licuri Palm}}$ ) as a function of the abundance of cattle (A) and goats (B), presence of pasture (C), presence of cultivation (D), presence of fire (E) and the different regions (F), for areas where the Lear's Macaw is present (core or recently occupied areas) or absent (areas with historical records only or without records). Additional covariates in the model (i.e. other than the ones shown in each plot) were set to the following baseline values to generate  $\Psi$  predictions: cattle abundance = 0; goat abundance = 0; no pasture; no cultivation; no fire; region: Raso da Catarina.

#### 4.3.1.3.2 Correlations with parrot richness

We encountered six parrot species resident of the *Caatinga* area surveyed: Lear's Macaw (*Anodorhynchus leari*), blue-winged macaw (*Primolius maracana*), turquoise-fronted parrot (*Amazona aestiva*), blue-crowned parakeet (*Thectocercus acuticaudatus*), blue-winged parrotlet (*Forpus xanthopterygius*), and cactus parakeet (*Eupsittula cactorum*) (Figure 29).

Predictions from the (ZIP-GLM) model for richness of parrots other than *A. leari* show no clear correlation with cattle and goat abundance (Table 13, Figure 30 A and B). However there was a lower richness in areas with the presence of cultivation and fires (Table 13, Figure 30 C-E), while a nearly significant effect suggested higher parrot richness in BDO than in RASO (Table 13, Figure 30 F). Moreover, parrot richness was higher in the areas where the Lear's Macaw is currently present (core and recently occupied areas) than in the areas where it is currently absent (areas with historical records or without records).



Figure 29 Parrot species recorded in the *Caatinga* during road surveys. Up from left to right: blue-winged parrotlet (*Forpus xanthopterygius*), blue-crowned parakeet (*Thectocercus acuticaudatus*) and cactus parakeet (*Eupsittula cactorum*), and below from left to right turquoise-fronted parrot (*Amazona aestiva*), blue-winged macaw (*Primolius maracana*) and Lear's Macaw (*Anodorhynchus leari*).

Table 13 Results for the parrot richness zero-inflated Poisson (ZIP) GLM model. Categorical covariate baseline levels are as follows: ecoregion - BDO; ranching - absent; agriculture - absent; fire - absent; Lear's Macaw (LM) occurrence - no record. Cattle and goat are count variables.

Count model coefficients (Poisson with log link):				
	Estimate	Std. Error	z value	Pr(> z )
(Intercept)	-2.619	0.228	-11.508	< 0.000
ecoregion RASO	-0.451	0.235	-1.919	0.055
Cattle	0.005	0.005	1.127	0.260
Goat	-0.002	0.004	-0.633	0.527
ranching present	-0.345	0.243	-1.419	0.156
Cultivation present	-2.051	0.270	-7.597	< 0.000
fire present	-2.223	0.455	-4.888	< 0.000
LM occurrence core	2.091	0.193	10.824	< 0.000
LM occurrence historic	-0.551	0.256	-2.155	0.031
LM occurrence recent	2.033	0.321	6.34	< 0.000
Zero-inflation model coefficients (binomial with logit link):				
(Intercept)	-3.680	0.647	-5.689	< 0.000

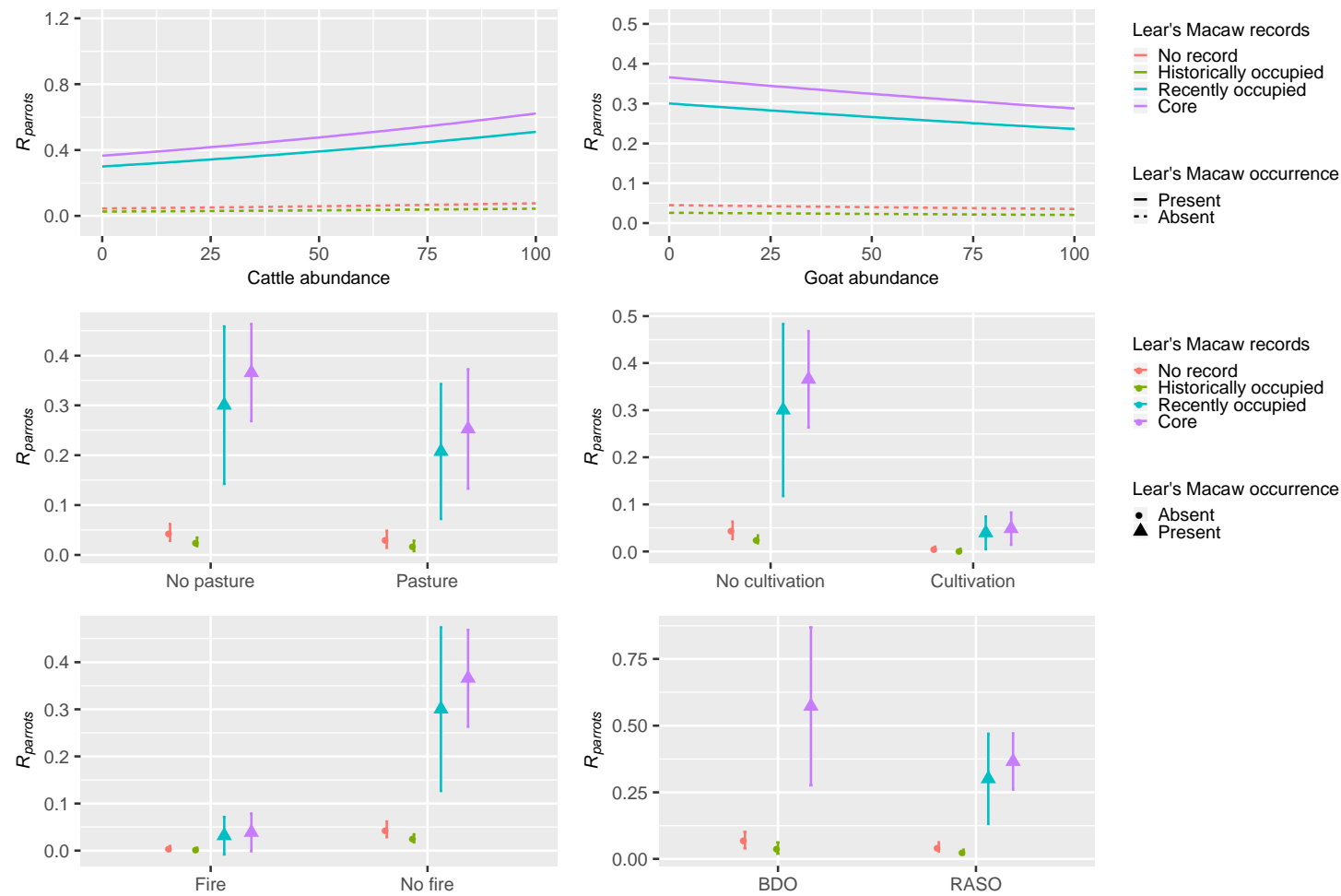


Figure 30 Model prediction with 95% CI for parrot richness ( $R_{parrots}$ ) as a function of the abundance of cattle (A) and goats (B), presence of pasture (C), presence of cultivation (D), presence of fire (E) and the two ecoregions (F), for areas where the Lear's Macaw is present (core or recently occupied areas) or absent (areas with historical records or without records). Additional covariates in the model (i.e. other than the ones shown in each plot) were set to the following baseline values to generate  $\Psi$  predictions: cattle abundance = 0; goat abundance = 0; no pasture; no cultivation; no fire; region: Raso da Catarina.

#### 4.3.1.3.3 Geographic range of suitable habitat

The variables that most contributed for modeling the habitat suitability for the Lear's Macaw were Precipitation of Wettest Month, Temperature Annual Range, Temperature Seasonality and Annual Precipitation (**Appendix 11**), while for modeling Licuri Palm habitat suitability were Temperature Annual Range, Temperature Seasonality and Human Foot Print (**Appendix 12**).

The model for the Lear's Macaw was statistically significant ( $p < 0.01$ ) and had a good performance identifying suitable areas for the species (AUC =  $0.996 \pm 0.001$  SD). The maximum training sensitivity plus specificity logistic threshold was 0.215, and the training omission was 0.057. For the Licuri Palm the model was also statistically significant ( $p < 0.01$ ) and had a good performance identifying suitable areas for the species (AUC =  $0.983 \pm 0.004$  SD). The maximum training sensitivity plus specificity logistic threshold was 0.070, and the training omission was 0.020.

The Figure 8A shows results of the model estimating higher suitability for the Lear's Macaw in the *Raso da Catarina* ecoregion (Figure 31 A), where most Lear's Macaw records occur. Beyond that area, the model predicted high habitat suitability to the northeast and southwest. There are also a few suitable areas further west in the *Depressão Sertaneja Meridional* ecoregion including *Boqueirão da Onça* area which suggest a potential habitat connection between BDO and RASO. Our Licuri Palm model shows a higher habitat suitability for the species from the coastal regions of *Alagoas*, *Sergipe* and *Bahia* states toward the interior of the states of *Paraíba* and *Pernambuco* (Figure 31 B). These models projections illustrate a wider potential range for the Licuri Palm than for the Lear's Macaw. Therefore, the spatial overlap between the two species' projections mostly resemble the high suitability areas estimated for the Lear's Macaw. Remarkably, most of the overlapped area shows a medium to low habitat suitability (Figure 31 C).

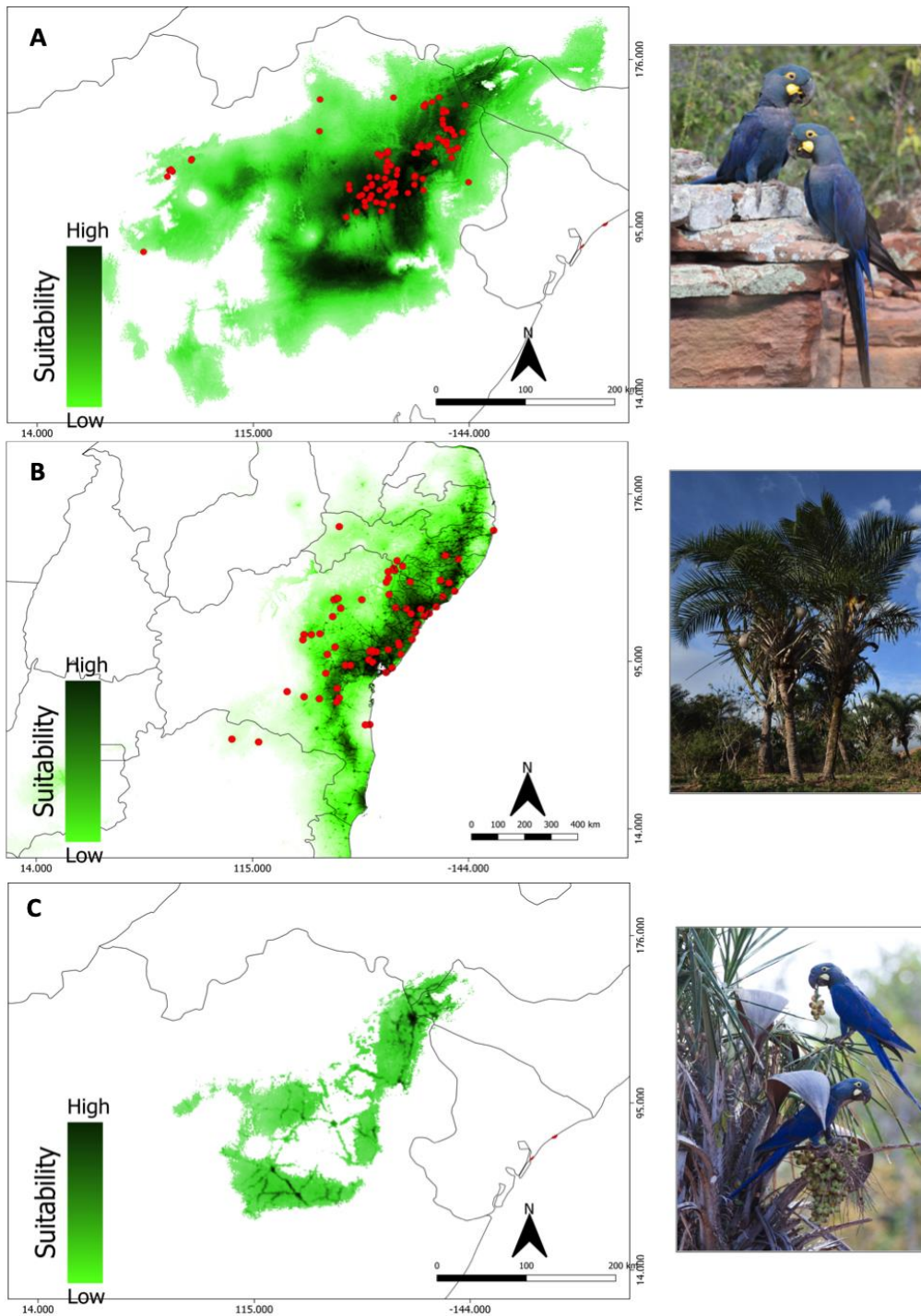


Figure 31 Projections of the habitat suitability models based and A) Licuri Palm records (red dots), and B) Lear’s Macaw records (red dots). C) Overlap of habitat suitability predictions between both species.

Click on the macaws to continue the reading of the discussion of section 5: item 5.3.1



### 4.3.2 Section 6 - Experimental removal of invasive Africanized honey bees to avoid competition for nest cavities of the endangered Lear's Macaw

#### 4.3.2.1 Location and abundance of Africanized honey bees hives and macaw nests

We recorded seven active Africanized honey bees (AHB) hives and 22 macaw nests during our first survey conducted in the core area in 2010. Our larger-scale survey conducted in 2016, when we were able to survey most of the macaw's nesting cliffs (12 out of 14) both in the core and historic breeding sites, offered a better picture of the distribution and abundance of AHB. A total of 99 AHB hives were recorded, but unevenly distributed between core ( $n = 4$ , three were removed in 2010-2011 and not reoccupied) and the historical areas ( $n = 95$ ) (Table 14). The proportion of inactive hives (50%) was similar among sites (Table 14). It is important to note, however, that non-active hives also obstruct the entrance of cavities potentially preventing their use by macaws (see data set in the **Appendix 14**) Comparing core and historical areas, macaw nest abundance was inversely proportional to the abundance of AHB hives (Yates' corrected  $\chi^2 = 90.78$ ,  $df = 1$ ,  $p < 0.0001$ ) and to the abundance of active hives (Yates' corrected  $\chi^2 = 49.65$ ,  $df = 1$ ,  $p < 0.0001$ ) (Figure 32). There was 0.1 and 10.5 AHB hives per macaw nest in the core and historic areas respectively, with an extreme case where we recorded 18.7 AHB hives per macaw nest in a historic nesting site (Barreiras, Table 14).

The height of cavities used by AHB and macaws ranged between 2 and 60 m above the ground level of the cliffs. Macaws used cavities at lower heights than AHB (estimate + SE for macaws:  $- 7.65 + 3.07$ ,  $t = - 2.49$ ,  $p = 0.014$ ), while controlling for differences in cavity height between core and historical areas ( $t = - 7.48$ ,  $p < 0.001$ ) and the potential interaction between species and areas ( $t = 1.46$ ,  $p = 0.15$ ) (Model' adjusted R-squared = 0.53,  $F = 43.76$ ,  $p < 0.0001$ ).

Table 14. Nesting cliffs surveyed, number of macaw nests and number of AHB hives in the core area in 2010 and both core and historic areas surveyed in 2016. The number of observed and treated hives (number of active hives in brackets) is indicated for each survey, as well as the treatment category: control (not treated), only poisoned, and poisoned and combs further removed (with the number of cavities later occupied by macaws in brackets). The total number of cavities recruited by macaws is also shown (with the number of successful breeding events in brackets).

Area	location	Year	cliffs	macaw nests	AHB hives (active)		AHB hive treatment (recruitment)			Recruitments (breeding success)
					observed	treated	Control	only poisoned	poisoned + removed	
<b>Core</b>	Toca Velha	2010	4	22	7 (7)	3 (3)	4 (0)		3 (3)	3 (1)
		2016	4	40	4 (4)	3 (3)	1 (0)	2 (0)	1(0)	0
<b>Historic</b>	Barreiras	2016	3	5	56 (26)	31 (22)	25 (0)	16 (2)	15 (1)	3 (2)
		2016								
	Baixa do Chico		3	4	39 (18)	15 (10)	24 (0)	15 (2)		2 (1)
<b>Total</b>					<b>102 (51)</b>	<b>52 (38)</b>	<b>50 (0)</b>	<b>33 (4)</b>	<b>19 (4)</b>	<b>8 (4)</b>

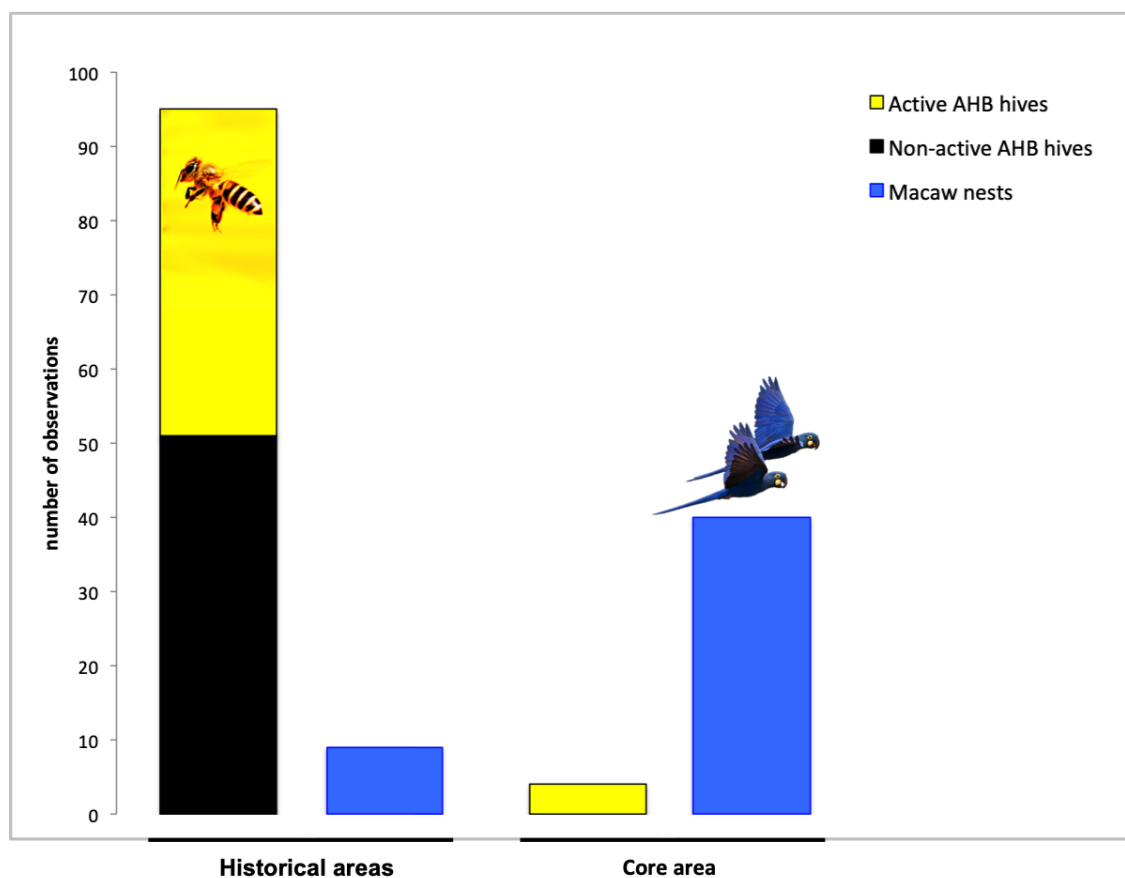


Figure 32. Number of Lear's Macaw nests and Africanized honey bee hives recorded in the core and historical nesting areas of the species in 2016. Honey bee image adapted by photo from Hans Benn, <https://pixabay.com/pt/photos/abelha-inseto-girassol-amarelo-1948684>. Lear's Macaw pair image adapted from photo by João Marcos Rosa.

#### 4.3.2.2 Africanized honey bees hive treatment and its effectiveness for nesting macaw recruitment

All three cavities occupied by AHB hives and treated during our 2010 pilot experiment in the core area were occupied by nesting macaws in the subsequent years, 2011-2012 (Table 14, Figure 33). Another five AHB-treated cavities were recruited by nesting macaws (in 2017-2018) after our larger-scale experiment conducted in 2016 (Table 14). Overall, 15.4% of the treated AHB-cavities ( $n = 52$ ) were successfully occupied later by macaws while none of the non-treated ones ( $n =$

50) were occupied (Yates' corrected  $\chi^2= 6.35$ ,  $df = 1$ ,  $p=0.0117$ ), thus demonstrating the effectiveness of the experimental treatment of AHB hives. Although not statistically significant (Yates' corrected  $\chi^2= 0.21$ ,  $df = 1$ ,  $p = 0.64$ ) due to the low sample size, the proportion of cavities recruited by macaws that were treated and removed (21%,  $n=19$ ) was almost twice as high compared to hives which were only treated (12.1%,  $n = 33$ ). Interestingly, the five cavities identified as a macaw nests up to 30 years ago by local farmers became occupied by macaws after our AHB hive treatment. Although low sample sizes preclude statistical analysis, the eight AHB-treated cavities occupied by macaws were higher off the ground (mean = 15.75 m) than those treated but not recruited (mean = 11.79 m). During the period 2016-2018, when we monitored both core and historical areas, seven empty cavities (i.e., not occupied previously by AHB) were occupied by macaws (all of them in the core area) while five pairs occupied AHB-treated cavities (all of them in the historical areas). Thus, the experimental treatment of AHB hives was associated with an increase in recruitment of new breeding macaw pairs by 71.4 %. The breeding success of newly recruited pairs in AHB-treated cavities (Table 14) was similar to that of pairs recruited in empty cavities (Yates' corrected  $\chi^2 = 0.53$ ,  $df=1$ ,  $p = 0.57$ ).



Figure 33. Africanized honey bee hive obstructing a cavity (A) in the Toca Velha locality (core area) in 2010. The same cavity is occupied by a pair of Lear's Macaws after treatment, in February 2011 (B), which bred successfully was confirmed by the observation of a Lear's Macaw fledgling in late April 2011 (C). Photos by EC Pacifico

#### 4.3.2.3 Evidence of illegal parrot poaching

We found that AHB hives are opportunistically exploited by local people for honey-hunting. We observed that honey-hunting mostly occurred at night and during the breeding season of macaws. This activity, involves human camps and people walking up and above the macaw breeding cliffs. To reach the hives people built permanent handmade stick ladders and scaffolds (Figure 34), 39 of them were found in Barreiras and 11 in Baixa do Chico, none in Toca Velha. Some ladders give direct access to the nests of macaws (Figure 34) and other popular parrot species (e.g. Blue-fronted amazon parrot, *Amazona aestiva*) evidencing honey-hunting and parrot poaching could occur simultaneously.

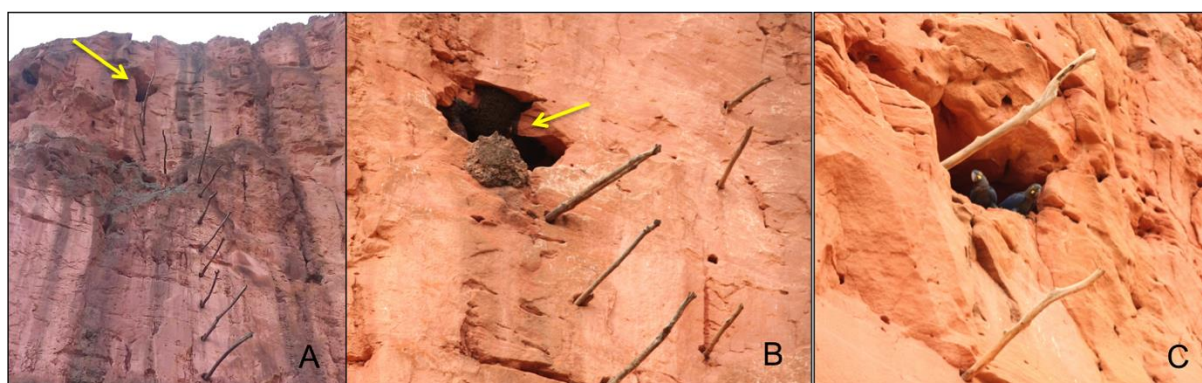


Figure 34. Poaching ladders used by locals to reach the Africanized honeybee hives (B – under yellow arrow). Ladders are accessing directly to active Lear's Macaw nests in the historical breeding area (A, C). Photos by T Filadelfo

Click on the macaws to continue the reading of the discussion of section 6: item 5.3.2



## 5 Discussion



## 5.1 DISCUSSION: DEMOGRAPHY HISTORY

### 5.1.1 Section 1 – Breeding to non-breeding population ratio and breeding performance of the globally Endangered Lear’s Macaw: conservation and monitoring implications

#### 5.1.1.1 Breeding parameters

The breeding success of Lear’s Macaw (80%) was much higher than in three species of the genus *Ara* (48%, Blue-and-yellow *Ara ararauna*, Green-winged *A. chloropterus*, and Scarlet Macaw *A. macao*) in lowland Amazonian forests (Renton and Brightsmith 2009) but only slightly higher than that of the Blue-and-Yellow Macaw (72%) in Cerrado savannah (Bianchi 1998). Two different estimates of reproductive success, based on nest occupation (51%) and egg-laying records (74%) of the Hyacinth Macaw (*Anodorhynchus hyacinthinus*) in northern Pantanal (Antas et al. 2010), yielded lower values as well, a result similar to that of Guedes (1993, 2009) in southern Pantanal. Differences among species may be partially related to the sampling size or different methodologies applied (observations of nest occupation versus egg-laying recording though direct nest inspections). In the Lear’s Macaw, however, breeding parameter estimates were consistent when obtained by nest inspections and observations at distance (Pacífico 2011).

The average productivity (1.33) and brood size (1.67) of Lear’s Macaw indicate that each breeding pair normally produces one to two chicks, contrasting with its congener Hyacinth Macaw that usually rears only one chick (Guedes 1993, 2009). In other macaw species of genus *Ara*, however, successful broods of two or even three chicks are not rare, but average productivity (0.6 – 0.94) is also smaller than in the Lear’s Macaw (Bianchi 1998, Bravo and Brightsmith 2006, Renton and Brightsmith 2009).

The species cited above, with the exception of Lear's Macaw, nest mostly in tree holes. Given the higher breeding parameters of Lear's Macaws, it is worth questioning whether nest substrate (tree holes vs. cliff cavities) may play a role in the breeding success of the species. Future studies of cliff nesting *Ara* macaws would be useful for addressing this issue. The availability of tree-holes is known to be a limiting factor in the density of bird populations (Cockle et al. 2010). Forest removal, logging and natural or human-made fires diminish cavity availability, especially for large macaws (Bravo and Brightsmith 2006), and this limited availability may increase processes of competition. Nest losses due to interspecific competition compromise the reproductive success of Hyacinth Macaws (Guedes 2009, Antas et al. 2010), while the main cause of breeding failure seems to be clutch predation in this large macaw species (Pizo et al. 2008, Antas et al. 2010). The colonial cliff-nesting behavior of Lear's Macaws, however, could reduce predation risk as has been suggested for the cliff-nesting burrowing parrot *Cyanoliseus patagonus* (Masello and Quillfeldt 2002). This hypothesis could be further tested by comparing breeding parameters of some macaw species which breed both in tree-holes and cliffs (Rojas et al. 2012), and could add insight into the evolutionary transition in the use of nesting substrates by parrots (Brightsmith 2005).

Breeding parameters of Lear's Macaws did not vary between the two breeding sites. Renton and Brightsmith (2009) also did not find variations in productivity among breeding sites of three large macaw species. However, both breeding success and productivity were somewhat larger in 2010 than in 2009. These differences could be related to seasonal and inter-year variability in food resources for the species. Santos-Neto and Camandaroba (2008) were able to map the 37 biggest patches of Licuri Palm tree *Syagrus coronata*, which offer the main food item to Lear's Macaws (Brandt and Machado 1999), around breeding sites. The average distance from breeding sites to these Licuri Palm patches was 49.5 km for CBS and 45.9 km for RCES (Santos-Neto and Camandaroba 2008). Moreover, palm patches are small and highly degraded by humans and goats and show a marked fruit seasonality influenced by rainfall (Rocha 2009). The dependence of Lear's Macaws on Licuri nuts is not strict since at least five other wild fruits are part of its diet during the breeding season, and macaws regularly consume maize perhaps as a response of the scarcity of wild fruits

(Brandt and Machado 1990, Silva-Neto et al. 2012). In fact, the 'Caatinga' dry forest has been continuously devastated and its conservation status has received little attention by Brazilian governments (Leal et al. 2005). Further studies of the spatial and temporal availability of food resources, related to rainfall regimes, are therefore needed for a better understanding of the variability in breeding parameters and the conservation problems faced by the species.

#### 5.1.1.2 Breeding population size

Non-breeding population fractions are often cryptic and more difficult to estimate than their breeding counterparts since the later are attached to breeding sites and are easier to monitor (Penteriani et al. 2011). In the case of Lear's Macaw, however, both breeding and non-breeding groups use the same cliffs for roosting, thus making both parts of the population equally easy to monitor but increases the possibility of inflating breeding estimates based on total counts of individuals. Moreover, individuals close to maturity could mate and prospect nest cavities before reproducing, which could introduce an important error in the breeding population estimate (BirdLife International 2019).

Renton and Brightsmith (2009) observed that 25% of nests inspected by *Ara macaw* during the breeding season did not result in active nests. However, the combination of observations at distance with nest inspections of focal nests indicated that the survey methodology used to identify breeding pairs of Lear's Macaws was appropriate. Using our estimate of 114 pairs breeding in 2010, the proportion of breeding individuals was about 20 % of the whole population in 2010. This is similar to the proportion estimated for healthy populations of several macaw species (10-20 %; Munn 1992) and for the whole population of the globally endangered Red-fronted Macaw *Ara rubrogenys* (16-33 %, Tella et al. 2013). However, it is lower than in other long-lived species with deferred sexual maturity like the Common Buzzard *Buteo buteo* (40%, Kenward et al. 2000), the Red-billed Chough *Pyrrhocorax pyrrhocorax* (40 – 72 %, Blanco et al. 2009), the Bearded Vulture *Gypaetus barbatus* (56 %,

Gómez de Segura et al. 2012), the Egyptian Vulture *Neophron percnopterus* (ca. 45 %, J.A. Donázar pers. comm.) and 18 seabird species (30 - 73 %, Warham 1996).

#### 5.1.1.3 Conservation and monitoring implications

A recent increase in breeding numbers of Lear's Macaws may be logically inferred from the positive population trend of the species recorded in recent decades (BirdLife International 2019). However, there are several reasons to do not blindly assume past or future linear relationships between breeding and overall population size. On the one hand, an overall population increase could result from conservation actions (BirdLife International 2019) that could significantly increase breeding output and adult survival without increasing the number of breeding pairs. On the other hand, breeding numbers of hole-nesting parrots can be limited by the quantity and quality of nesting sites (Cockle et al. 2010), thus breaking the assumed direct relationship between number of individuals and number of breeding pairs. This could explain the lower breeding to non-breeding population ratios in parrots compared with other long-lived species.

Currently, nearly all Lear's Macaws are concentrated at two breeding/roosting sites separated by just 38km, with the observed increase in numbers of macaws roosting in CBS in parallel to a decrease of macaws roosting at RCES strongly suggesting that individuals moving between these sites belong to a single population (Menezes et al. 2006). Moreover, a small group located in 1995 in an unprotected area between the Campo Formoso and Sento Sé municipalities in Bahia, 230 km to the west (BirdLife International 2019), seems to have been nearly extirpated with only two individuals located in 2012, probably due to trapping for illegal trade (Filadelfo and Pacífico 2017; see section 5). The reduction to a single population not only makes the species more vulnerable to stochastic processes but also to crowding effects when facing nesting habitat limitations. If the Lear's Macaw population does not expand to distant, potential nesting sites, it is expected that the breeding population size will not increase after exceeding the carrying capacity (in terms of nest-site availability) despite of further increases in overall population size. Moreover, the

percentage of breeding Puerto Rican parrots *Amazona vittata* decreased with a temporal increase of the total population size in absence of nest-site limitation or skewed sex ratios, arguing potential social factors to explain this pattern (Beissinger et al. 2008). In this sense, Blas et al. (2011) experimentally demonstrated that non-breeders of the long-lived black kite *Milvus migrans* were physiologically capable of reproducing but were socially subordinated to breeders, as shown by their adrenocortical response to stress. Finally, the proximity of communal roosts gathering non-breeding individuals to nesting sites may contribute to supply mate losses (Blanco and Tella 1999) and buffer local extinction processes (Carrete et al. 2007). However, the spatial overlap of breeders and non-breeders in isolated populations of birds may also reduce their population growth through density-dependent processes. Negative effects can arise when non-breeders compete for resources with breeders or interfere with their breeding activities. This seems to be the case of the Bearded Vulture, where the crowding resulting from a population increase and overlap of breeding and non-breeding population fractions over decades resulted in a density-dependent breeding depression (Carrete et al. 2006a) and a change in the mating system to polygamy that reduced the potential effective population size (Carrete et al. 2006b). A similar density-dependent situation could apply for an island population of Red-billed Choughs, showing the by far lowest breeding to non-breeding population rate known for this species (Blanco et al. 2009). In the case of Lear's Macaws, the large non-breeding population overlaps foraging areas with breeders and such a food competition could compromise their breeding condition and success, especially in years of food shortage if the population continues to grow. Moreover, interference of non-breeders with breeding activities could also increase in an overcrowding situation, thus further reducing breeding performance (Renton 2004, Carrete et al. 2006a).

The above uncertainties on the future population projection of the Lear's Macaw call for the necessity of new monitoring and conservation efforts. Further monitoring must focus on the breeding fraction of the population and its breeding parameters, rather than solely on overall population size as done so far, to properly assess changes in population dynamics, the life-stage related threats and the conservation

status of the species in the long term. There is also the need for investigating the annual rates of juvenile and adult survival which, together with population and breeding parameter estimates, will allow the creation of a population viability models (PVA) that ultimately would set the conservation status and conservation action priorities for the species. This would require mark-resighting work and, ideally, tagging birds for remote tracking, which would add valuable information on the causes and rates of mortality that indeed can vary between the different population fractions (Oro et al. 2008, Grande et al. 2009). Remote tracking would also result essential to determine the range movements and use of space by breeding and non-breeding individuals in relation to the spatial and seasonal changes in food resources (Tanferna et al. 2013), as well as to investigate whether non-breeders could prospect distant, potential but still unknown nesting areas for the species. This would help to delineate protected spaces, covering the most important foraging areas, and planning the geographical expansion of the species, which could require direct intervention in the case the species would not be able to naturally disperse. In other case, the species could end in a unique overcrowned population suffering from density-dependent negative effects in a near future.

## 5.1.2 Section 2 – Estimating population size and growth with a heterogeneous long-term census: the population trends of the endangered Lear’s Macaw

### 5.1.2.1 Estimation of Population Size from Roost Counts

Estimates of population size and its uncertainty provides essential information for tracking population trends, understanding the factors affecting population growth, and projecting the viability of threatened populations (Shaffer 1981, Caughley 1994, Beissinger and McCullough 2002). Such estimates are also needed for evaluating the success of conservation initiatives and planning for population recovery (Butchart et al. 2010, Ethier and Nudds 2015). Roost counts provide an efficient way to monitor population size of species that are widely dispersed during the day but concentrate in a few locations in the evening.

Roost counts, when combined with N-mixture models, proved useful for estimate trend of Lear’s Macaw population in a robust fashion. The main challenges associated with the roost counts for estimating abundance and population size of parrots include potential variation in detectability between counts conducted at different times of day, daily and seasonal variation in the number and size of roosts, and variation in roost site locations (Casagrande and Beissinger 1997, Dénes et al. 2017). The modeling approach used a version of the N-mixture model to analyze roost count data. It allowed us to account for the effects of imperfect detection on morning and evening counts (e.g., Table 4), and to quantify the effects of seasonal variation in roost size during the survey period (*Figure 20*). To the best of our knowledge, the location and number of roosts did not change within years during our study period; if it had, a modification of Casagrande and Beissinger’s (1997) estimator using variation in roost size and the number of roosts could be used. Thus, the modeling approach we applied to the Lear’s Macaw roost counts should produce robust results and could be used to estimate population size from similarly heterogeneous data of other bird species with comparable roosting behavior.

Our results indicate that the size of Lear's Macaw population had been underestimated annually by the analytical methods used by CEMAVE-ICMBio to estimate population size (Figure 23, Table 6). Imperfect detection of individuals is likely to be an important cause of these differences in population estimation (Thompson 2002). The population estimates of CEMAVE-ICMBio averaged morning and afternoon counts. However, afternoon counts of macaws had a lower detection probability (Table 4 and Table 5), which may be due to low visibility since sunset is ~17:25 in this latitude, with little variation throughout the year. The inclusion of afternoon counts without accounting for imperfect detection negatively biased the population estimate and is likely the main cause of underestimation of Lear's Macaw population size in the CEMAVE-ICMBio estimate. The low probability of detection for individuals in afternoon counts resulted in higher abundance estimates using the N-mixture models than the CEMAVE-ICMBio counts, even when considering the maximum number of birds counted (except for 2013). The official population size estimate by CEMAVE-ICMBio assumed that all the individuals were counted, but it may best be considered a conservative estimate given the detectability issues discussed above. Our results demonstrate that modeling not only improved the population size estimates, but also that the maximum number of macaws counted each year is a better index of population size than the average of the counts in the year.

Our application of a version of the N-mixture model was a first attempt to account for the factors affecting imperfect detection for roost counts of Lear's Macaws. We envision multiple ways to improve future analysis to account for more sources of heterogeneity in roost counts. Due to site-level aggregation of the Lear's Macaws roost counts provided to us (six roosts in ESEC and four roosts in CBS), we were unable to address variation in detection and abundance among roosts and how those differences might have varied throughout the year. Neither could we assess the effects of other factors that could potentially affect detectability, such as variation among observers and distance from the observer and the roost site (Dénes et al. 2015). Accounting for these sources of variation could improve precision and accuracy of the estimates of Lear's Macaw population size.

### 5.1.2.2 Design of Monitoring and Conservation Programs for the Lear's Macaw

There has been no increase in the monitoring efforts over the past decade while the Lear's Macaw population has expanded. Despite the monitoring design shortcomings, we were able to verify strong population growth over the last decade (333.37%). We also noted a slight decline in the estimated population size within each year from June to November (*Figure 22*). This decrease could be a result of mortality, particularly of recently fledged individuals. On the other hand, it may also suggest the existence of unknown roosting sites to which individuals, possibly young macaws, may have dispersed. Menezes et al. (2006) suggested the potential for unknown roost sites after an abrupt decline in the counts in December 2002 and August 2003. With the recovery of the Lear's Macaw population, however, new permanent or temporary roosts may exist in unknown locations or at historical roost sites no longer monitored (Blanco and Tella 1999, Blanco et al. 2014).

We make five recommendations to guide future monitoring of the Lear's Macaw population. First, active searches for roost sites should be made at least once a year before macaw counts are conducted. This may help to identify critical areas of population expansion. Adding new roost sites to the monitoring effort will yield more accurate estimates of population size and trend. Second, it would be greatly beneficial to report the results of each individual roost count to permit analysis that will allow better understanding of the seasonality of population fluctuations (e.g. Seixas and Mourão 2018). Third, the breeding period should be excluded from the annual effort to count roosts, since one or both parents remain in the nest at night from December (onset of incubation) to April-June (feeding of nestlings and fledglings). Concentrating roost surveys after the breeding period every year, or every two years, is the best option to avoid population underestimation. Fourth, increasing the number of morning counts would yield more reliable results and be more cost-effective than conducting afternoon counts. Fifth, estimates of the annual nesting population size based on counts of the number of macaw pairs nesting in cliffs (Pacífico et al. 2014) could also provide reliable, complementary information on population trend that could be used

in integrated population models (Zipkin et al. 2019) and provide information on the potential of recolonization of historical breeding areas.

Our results indicate that the Lear's Macaw population is much larger than previously thought, and suggest that it could continue to recover in future years. Expanding the capacity of this population to grow should be a major conservation concern and the next step for planning of management actions. Conservation actions should focus on areas of potential population expansion, and implementing environmental education activities and environmental laws in these areas, as demonstrated by Barbosa and Tella (2019) that have been done in the core nesting and roosting area. Protection of the roosting and breeding sites in the expansion areas is needed to avoid poaching, which led to decline of the species in the past. Habitat conservation is lacking in the rural areas used by Lear's Macaw for foraging. Knowledge of key foraging locations is needed to identify important locations for conserving for food resources to support population expansion. The Caatinga forest is poorly conserved, with decades of habitat alteration and overgrazing. Most of the Licuri Palms, *Syagrus coronata* (MART. (BECC.) Arecacea), the Lear's Macaw main food source, occur on private propriety and have been exploited by local communities (Andrade et al. 2015, Schulz et al. 2018). Although the population increase exhibited by the Lear's Macaw over the past decade is extremely encouraging, much remains to be done to secure the future of this species.

## 5.2 DISCUSSION: POPULATION GENETICS

### 5.2.1 Section 3 - Isolation and characterization of 15 new specific microsatellite markers for the Lear's Macaw

We characterized a set of 15 microsatellite markers showing adequate amplification in both invasive (blood) and non-invasive (molted feather) samples from wild Lear's Macaw individuals. These microsatellites showed low to moderate levels of polymorphism in related nestlings, but the combination of two to four most informative loci, depending on the presence of highly related individuals in the sample, was sufficient for reliable individual identification.

Although informative, our markers showed lower levels of polymorphism than the set developed by Jan and Fumagalli (2016). We attribute this to the fact that our results represent a sample from the wild population, while Jan and Fumagalli (2016) used samples from a captive population. Given the species' demographic history and its association with the pet trade (ICMBio 2012), the captive population may include lineages that are now extinct in the wild, and in this case the captive population could retain a higher genetic diversity than the current remaining wild population. This hypothesis should be further investigated with analyses of larger samples from both captive and remaining wild populations.

The main purpose for developing these new molecular tools is to assist in the conservation of the Lear's Macaw. Using a higher proportion of the markers or the complete set is expected to provide the required power of resolution for pedigree inference of wild and captive individuals, and if the genetic structure of the wild population is evaluated, the microsatellite markers could also help to identify the geographic origin of individuals recently seized, assist illegal trade persecution and allow the identification of vulnerable breeding sites that need protection (Presti et al. 2015, Ferreira et al. 2015, Almeida et al. 2019).

The microsatellites developed in this study can be used for assessing the genetic diversity of captive and wild populations, estimating effective population sizes

of natural colonies and detecting demographic bottlenecks, which are paramount goals in the Lear's Macaw conservation action plan (ICMBio 2012). This information is urgently required to support adequate conservation management programs for this endangered species (Presti et al. 2011). Moreover, application of our microsatellites to identify individuals may assist research on the Lear's Macaw ecology, including the verification of nest fidelity among years, addressing sex ratios sampling bias (i.e. exclusion of replicated samples from molted feathers), and dispersal (through genetic tagging) (Olah et al. 2017, Brouwer and Griffith 2019, Heinsohn et al. 2019). Altogether, conservation programs for the Lear's Macaws will benefit from pilot studies combining both sets of markers to test their performance for the specific goals to be addressed, and so design cost-effective monitoring protocols.

## 5.2.2 Section 4 – Lear’s Macaw population status assessment from non-invasive genetic samples

### 5.2.2.1 Genetic patterns and demographic history of Lear’s Macaw

Considering the demographic declines documented in the Lear’s Macaw in the last century (section 2), we expected that the surviving population might show severely reduced genetic diversity, which would suggest a compromised viability in the long-term (Frankham et al. 2015). Fortunately, our results show no evidence of such extreme genetic impoverishment in the studied population, thus highlighting that *A. leari* still harbors a moderately diverse genetic pool in the wild. In contrast, effective size is alarmingly low. The maintenance of moderate levels of genetic diversity with low number of individuals in the wild could indicate that this population was never much bigger than it is nowadays or, alternatively, genetic rescue mechanisms may have effectively counteracted the demographic bottleneck. At any rate, decided efforts should be targeted to reduce anthropogenic pressure over this wild population, so to ensure its safe consolidation.

Excluding commissioned poaching for pet trade to collectors (Herrera and Hennessey 2007, Barbosa and Tella 2019), no evidence exists of poaching of the Lear’s Macaw for local use as a pet (see Section 5). The captive Lear’s Macaw population has a small size ( $n=166$  individuals by 2018) and is composed of 60% captive born birds, while the remainder are wild birds that were rescued injured, and seized/confiscated birds, victims of illegal trafficking. This indicates that the species is rare in captivity. Few breeding pairs are available for matching, leading to low success rates in the captive breeding program, thus only three institutions, parrot breeders specialists, have successfully bred this species by 2018. It is important to highlight that the number of captive birds does not include institutions that house macaws with suspicious and/or undeclared origin (*i.e.* that might have been bought from wildlife dealers or irregular trade between bird collectors) (Scientific Authority CITES, personal communication).

Based on these evidences, we suggest that this species might have already been rare in the wild when wildlife illegal traffic intensified in the 1970's. The maintaining of the genetic diversity with low number of individuals in the wild could also indicate that this population was never much bigger than it is nowadays. Thus, the population was recently harvested and reduced to the remaining population, and this population decline was so recent that did not reflect in genetic diversity.

We have information of recent extinction in localities across the specie's distribution range (see section 5) and that important threats have been overlooked (e.g. Power Line collisions and Africanized honey-bees nest competition) (see section 5 and 6) and it is also necessary to understand how climate in the past centuries - long or frequent droughts – may have contributed to the population dynamics of *A. leari* (e.g Moura et al. 2018).

In *Barreiras* locality we identified roost and nest intense disturbance as motivation for the macaws to abandon or frequently change their roost location and breeding failure (see section 6). We concern about the low effective size of this breeding population and the vulnerability of this site to persist as a genetic unit. Results of genetic structure assessment suggest a sustained connectivity across the study area. Non-systematic records of parrots marked in *Toca Velha* and re-sight at *Barreiras* further confirm individual dispersal between this two localities (Pacífico et al. 2018). Low genetic differentiation may imply relatively high gene flow throughout the study area. Maintaining habitat corridors facilitating this natural connectivity is crucial for the integrity of the gene pool of the wild population, and should therefore be a conservation priority (Frankham et al. 2008).

None of the breeders genotyped by feathers collected in the nests ( $n = 25$ ) were among the genotyped feathers collected in the ground at roost cliffs bottom in the same locality ( $n = 41$ ). All mentioned samples were collected during breeding season, which supports that breeders are likely to roost inside the nests during breeding season and not collectively at roost. However more extensive research on parentage analysis and information on molting season are needed to confirm this hypothesis.

### 5.2.2.2 Genetic management and monitoring implications

Low genetic diversity is a major concern in the management of small/engendered populations, due to inbreeding-related threats (Frankham et al. 2014). The moderate genetic diversity of the wild population of the Lear's Macaw facilitates management actions for the recovery of this endangered species in areas of acute decline. These actions should be targeted to increase the effective size of the wild breeding units. The cornerstone of the conservation plan should address the main factors threatening the natural recovery of this population: the loss of food resources and habitat quality, human-macaw conflicts and wildlife international trafficking (nest poaching). Such threats may increase with the numeric and geographic expansion (including recolonization of unprotected areas) of Lear's Macaw in the wild and should therefore be prioritized over other concerns such as genetic enrichment via captive breeding and release.

The captive Lear's Macaw stock is indeed relatively small ( $n = 166$  individuals by 2018) and is 60% composed of captive born birds. The remaining 40% corresponds to either rescued wild injured birds or seized/confiscated birds, victims of illegal trafficking. Few breeding pairs are available for matching, leading to low success rates in the captive breeding program. As a consequence, only three institutions, parrot breeder specialists, have successfully bred this species by 2018. It is important to highlight that the number of captive birds does not include institutions that house macaws with suspicious and/or undeclared origin (*i.e.* that might have been bought from wildlife dealers or irregular trade between bird collectors). Despite the knowledge of the existence of such irregular Lear's Macaw keeping in a few breeder institutions, no initiative has been taken to retrieve and repatriate the macaws to Brazil to increase the captive breeding stock. (Cornejo 2018)

Genetic management of Lear's Macaw should also focus on minimizing of inbreeding, particularly for the captive population and the wild sub-populations from the *Barreiras* and *Boqueirão da Onça* areas, more susceptible due to their smaller size. Because the number of breeding captive birds is small and few individuals are

suitable for release due humanized behavior (hand-raised birds) (Cornejo 2018), the reinforcement of the above-mentioned declined wild populations using captive born birds as main source, at this instance, could be counter-productive for the stabelishment of a genetically diverse captive population.

#### *5.2.2.3 Future directions: covering knowledge gaps and new hypothesis*

This first genetic assessment of the wild *A. leari* population illustrates that currently available molecular markers provide sufficient genetic information to study the demography of Lear's Macaw from non-invasive tissue samples. Investing in genotyping of DNA samples from molted feathers from nests and its nestlings through time will allow filling critical knowledge gaps about the mating system and breeding success of this species in its natural habitats, including behavioural components like extra pair paternity rate and nest fidelity patterns.

Genotyping the isolated macaws from the functionally extinct population of the *Boqueirão da Onça* is necessary to complete the demographic history of Lear's Macaw and assist the identification of the origin of the confiscated birds that make up a portion of the captive breeding population. Possible biases in the sex ratio of the wild population should also be widely investigated to infer its implications for the population expansion.

## 5.3 DISCUSSION: THREATS

### 5.3.1 Section 5 - Past and current threats of the Lear's Macaw: implications for distribution and population expansion

Local knowledge has been widely recognized as a valuable resource to gain information on current and past ecological conditions or conservation threats for wildlife (Nazarea 2006). The interviews conducted at local communities were especially useful to gather information about the historic distribution of the Lear's Macaw, its conservation threats and causes of extinction. Another useful outcome of the interviews was the indirect outreach to the local community, which may have increased their awareness to the Lear's Macaw and its conservation. Perhaps as a consequence, subsequent to the interviews we received voluntary reports about incidents with macaws, in particular regarding electrocuted individuals and nest poaching.

The low number of Lear's Macaws recorded ( $n = 44$  encounters) through the roadside surveys prevented us from directly assessing, with modeling approaches, the impacts of anthropogenic disturbances and habitat changes on their occurrence or abundance. This was the motivation for assessing whether parrot richness might serve as an adequate proxy. Our findings indicate that parrot richness is a good indicator of Lear's Macaw presence, inasmuch as the Lear's Macaw habitat quality is reflected in our classification of the species' occurrence areas, measurement of livestock densities and presence of cultivations and fires. We however recognize the limitation of such a proxy, and suggest that it should be used conservatively, for example to assist in the ranking of different areas that might be suitable for the species as foraging sites.

Interviews and road surveys allowed us to list and rank the multiple threats occurring in the different areas studied. Among these, hunting and deforestation are the most geographically widespread. The interaction between humans and nature in the Caatinga, likely including hunting and small-scale slash-and-burn agriculture, began in the Late Pleistocene–Holocene, when the first populations arrived to the

region (Silva et al. 2017). Subsequent colonization by the Portuguese from their settlements along the coast introduced livestock in the region, first in vast ranches along rivers, followed by expansion in other areas once settlers learned it was possible to use the native vegetation to feed their cattle and as timber for construction and to generate the energy they needed (Silva et al. 2017).

Our results show that these activities, ancient as they may be, remain widespread and relevant anthropogenic threats to the Lear's Macaw. We found that the majority of roadside habitats surveyed had evidence of some kind of anthropogenic impact, a pattern generally present throughout the *Caatinga* (Silva et al. 2017). Cultivation practices also follow the general pattern in the *Caatinga* (Silva et al. 2017). Most of the semiarid *Caatinga*, however, is not suitable for agriculture and has been used for livestock and fuel wood production (Sá Barretto Sampaio et al. 2017). We found that cattle and goat densities are distributed differently in relation to habitat, with cattle density positively correlated with the degree of habitat anthropization while goats are more abundant in degraded natural habitat and less so in agro-pastoral habitat. This corroborates the assertion that, in the study area, cattle are likely concentrated in land dedicated to extensive ranching (i.e. pastures), while goats are predominantly free-ranging in natural habitats causing overgrazing and habitat deterioration (Melo 2017, Sá Barretto Sampaio et al, 2017).

Free-ranging livestock are often assumed to represent a risk to natural environments by promoting changes in the structure and function of ecosystems (Carmel and Kadmon 1999; Schulz et al. 2016; Dénes et al. 2018). In the *Caatinga*, grazing by goats resulted in a reduction of soil carbon at shallow depths (Schulz et al. 2016), while high abundance of both cattle and goats was shown to have a negative effect on plant communities and their taxonomic composition, with a stronger negative impact on seedling and sapling diversities (Ribeiro et al. 2016). Melo (2017) found that goats preferred successional areas over natural vegetation, using areas with less vegetation in a proportion higher than available in the landscape and similarly avoiding mature forest with denser vegetation, which is in line with our findings. This might suggest that, rather than being an acute disturbance directly forcing the degradation of natural *Caatinga* vegetation, goats most likely act as a barrier to forest regeneration (Melo 2017). In the context of the habitat quality and population dynamics of the Lear's

Macaw, this suggests that free-ranging domestic goats similarly represent a hurdle to the macaw's population expansion through dampening of habitat carrying capacity, primarily by preventing the recovery of potential foraging sites. Conversion of natural vegetation into cattle pastures, on the other hand, likely represents an acute disturbance that may result in degradation of habitat quality for the Lear's Macaw habitat, although under certain circumstances pastures may retain some potential as foraging sites (see below).

Based on our road survey results, anthropogenic land uses (i.e. ranching and cultivation) and the disturbance caused by fire do not represent a barrier to the occurrence of Licuri Palm patches. We emphasize, however, that this may not apply to the plant's regeneration, as our survey methodology did not allow us to assess seedling and sapling occurrence or abundance. Tella et al (2019) verified high recruitment of Licuri Palm plants in degraded habitats, but did not specifically test for livestock density effects on that recruitment in those habitats. Further investigations of patterns affecting the occurrence and regeneration of Licuri Palm are thus warranted to confirm our results. Nevertheless, given the importance of the Licuri Palm as a food resource for the Lear's Macaw (Brandt and Machado 1990), our findings indicate that pastures, cultivated land and even burned vegetation may possess some potential as foraging sites for the macaw, conditional on their retaining of Licuri Palm patches. Importantly, if Licuri Palm recruitment in such patches is impeded, which again remains to be disproved, this potential will likely not persist in the long-term.

Hunting was practiced by the first inhabitants of the Caatinga (Melo 2004) and has continued over the years, notwithstanding changes in the laws regulating these activities, which have culminated in the prohibition in all of Brazil (Alves et al. 2009). As shown in our threats ranking, however, the practice continues to occur extensively. Yet little attention has been given to hunting as a threat to Lear's Macaws (ICMbio 2012). One of the most fundamental uses of game animals in the region is to meet nutritional needs, as bushmeat constitutes an important protein source for several rural and urban communities, especially during prolonged drought periods when livestock herds die from hunger and thirst (Alves et al. 2012, Mendonça et al. 2016). Birds are the second group of preferred vertebrates targeted for hunting in the

Caatinga (after large mammals), and this pressure seems to have increased in recent years, possibly due to the decline of the preferred species (Albuquerque et al. 2017). In general, birds are relatively more abundant and easily found and hunted than mammals, and they can be captured with a variety of hunting techniques, including non-selective traps (Albuquerque et al. 2017). The bird species most often hunted as a protein source by locals in the Caatinga are from the Columbidae, Tinamidae, Anatidae and Cracidae families (Albuquerque et al. 2017). In addition, macaw species in general are often hunted for their large and colorful feathers (Berkunsky et al. 2014). Our findings suggest that hunting may be a widespread threat to Lear's Macaws, and merits further investigations.

Our findings indicate that nest poaching remains a high-ranking threat, as recognized for other Neotropical parrot species (Tella and Hiraldo 2014, Berkunsky et al 2017). In the last fifty years (since approx. 1970), the illegal international trade has specifically targeted Lear's Macaws, with several cases of birds confiscated by the authorities (Barbosa and Tella 2019). However, no evidence exists of poaching of the Lear's Macaw for local use as a pet, contrarily to what is commonly observed for other local parrot species (e.g. *Amazona aestiva*, *Eupsittula cactorum*, *Primolius maracana*) (Authors unpubl. data). Therefore, the international trade can be regarded as a key threat to the wild population of Lear's Macaw. Nest poaching represents a serious threat via direct exploitation of individuals from the poached locality, but it may also have an additional impact by increasing the risk of extinction of the surrounding population. We are unaware of the exact number of extinct populations that may have been connected to the current surviving population. However, we have reports of recent extinction in at least six localities across the species' distribution range. We believe that only the poaching control policies in the core areas, especially access restriction and vigilance to protect the nests and roosting sites of *Serra Branca* and *Toca Velha*, and environmental conservation actions performed in the last 20 years (Barbosa and Tella 2019), prevented stronger demographic collapse. Although those core areas are well protected from humans, free ranging goats still prevent the environment of being renewed.

Other threats such as the introduction of nest competitors (e.g. invasive Africanized honey-bees), human disturbances at roosts, persecution and killing when

feeding on crops, wind-farm collisions, and electrocutions at powerlines should not be overlooked, despite their lower ranking. As much as the prospection of wind farms in the Lear's Macaw range, more than habitat loss due to implementation of wind farms, wind turbines can seriously impact population dynamics in long lived birds that aggregate in roosts and could limit the movement of individuals and expansion of the population (Carrete et al. 2009, 2012).

The wind energy sector in Brazil has expanded quickly over the last decade and this growth is expected to continue in the forthcoming years (Pinto et al. 2017). The impacts on birds are well known in other countries (Drewitt and Langston 2006, Fox and Petersen 2019) but can only be speculated here. More research will be need to understand Lear's macaw interactions with wind turbines to better predict mortality risks and develop strategies to avoid and minimize them. For now, there are wind farms already operating in the surrounds of Boqueirão da Onça site and under construction in Canudos site, both very close to foraging and breeding/roosting areas.

The situation of the *Barreiras* locality, one of the new roosting and nesting localities found (locality 3, *Figure 13*), illustrates how different threats can synergize to impact the Lear's Macaw. In contrast to Toca Velha and Serra Branca, these areas are not protected nor regularly monitored, besides wind farm prospection, invasive Africanized honey bees have usurped Lear's Macaw nest cavities and, accompanying the bees, honey hunters use hand-made ladders to access cliff cavities and often combine honey extraction with parrot poaching (see Section 6). In May 2015, during the interviews, one honey-hunter told us that he had been offered ten thousand Brazilian Reais (c. U\$3,300 at the time) to poach Lear's Macaw nestlings in the site. Not surprisingly, we later found that two illegally traded nestlings aged ca. 45 days old had been seized in Portugal, in March of the same year. The intense and frequent disturbances caused by honey hunters, competition for nest cavities with Africanized honey bees, and active nest poaching compromised the roosting and nesting activities of macaws, thus limiting the permanent use of this site by the species and compromising its local population growth (see Section 6).

Locations of macaw electrocution were concentrated in the foraging area of *Euclides da Cunha* municipality, in the RASO ecoregion, and may function as an ecological trap. A way to reduce the attractiveness of such a trap is to eliminate or

manipulate the environmental cues animals use to assess the quality of the habitat, resource, or situation (Hale and Swearer 2016, Robertson and Blumstein 2019). In the case of Lear's Macaw electrocution at power lines, we observed that the need to forage in anthropogenic areas makes the availability of perching sites limited, since large woody trees are rare due to deforestation. Thus, power lines play a role as a perches to the macaws while they approach to the feeding areas. Most of the reports of macaws killed by electrocution and shot at crops we compiled were located in the surroundings of *Euclides da Cunha* municipality, in the RASO ecoregion, where we discovered one roosting site in trees. That Lear's Macaw roost in trees is a new finding, as the species was known to only use sandstone cliffs to roost. From all Lear's Macaw's roosts, this is the only one located in a degraded habitat, a patch of typical Caatinga woody trees surrounded by pasture and agriculture.

In the face of the global change scenario, it is also necessary to understand how climate shapes the distribution of the species (Walther et al. 2002), and how may have contributed to the population dynamics of the Lear's Macaw and its current rarity, as droughts and rain (Albuquerque et al. 2012) interfere with food resources availability (vegetation phenology) and potentially influence Lear's Macaw fitness, recruitment and breeding success (e.g Moura et al. 2018). Information on Lear's Macaw geographical occurrence or density is based on isolated field records. However the *Caatinga* forests, as other Neotropical landscapes are difficult to access and inaccessible areas have had limited survey efforts and unbiased data is rarely available, thus modelling of habitat suitability should also consider accessibility (Raxworthy et al. 2003, Collen et al. 2008, Cardador et al. 2017)

Our findings indicate that Human Foot Print variable shows no contribution for modeling habitat suitability of the Lear's Macaw. However, the majority of the impacts that compose HFP are of little importance (i.e. high extension of urban areas, paved roads, night lights, railways and waterways) across the occurrence area of the species, with the exception of ranching and agriculture. We suggest that the lack of native vegetation (deforestation is not considered in the HFP) could be a more determinant factor of habitat suitability. Large trees are also key for the species conservation since roosting and diurnal resting sites were also confirmed in trees, and trees other than the Licuri Palm also offer food resources to the species (author

unpublished data). Moreover, changes in the native *Caatinga* vegetation are particularly responsible of changes in precipitation (Salvatierra et al. 2017).

We show that there is higher Licuri Palm habitat suitability toward the coast. But the area of potential occurrence of macaws falls under the potential area of occurrence of Licuri Palm. Through the intersection of both areas shown in *Figure 31 C* we identified the areas of higher suitability for both species, and we suggest that they must be prioritized for conservation actions and efforts. Nevertheless, areas outside this intersection may also be relevant due to the their potential role they play in the conservation of the *Caatinga* in general. This is the case of the BDO region. Here, the two non-breeding macaws living isolated for 25 years represent a functionally extinct population. Yet this is an Important Bird Area and priority for *Caatinga* conservation due to their endemic and threatened plant and animal species, including the largest population of jaguars (*Panthera onca*) in the biome, so much that it became a national park in 2019. Given how official protection can be beneficial for the species, as exemplified by the nesting sites in *Toca Velha* and *Serra Branca*, we believe the BDO site should not be disregarded. Efforts are already underway to establish in the area an experimental Lear's Macaw release and monitoring of captive-born immatures, as part of an ongoing population reinforcement program (Authors unpublished). An interaction between the remaining macaws and released individuals could be highly beneficial for the establishment of the latter, and potentially for the recovery of this population.

Effective conservation demands an accurate understanding of the distribution range, habitat and threats of endangered species (Katzner et al. 2011). Sensitive to the context of the *Caatinga* region and its local people, here we increase this understanding with an assessment of the habitat status of the current and historic distribution ranges of the Lear's Macaw, an evaluation of the current threats and likely causes of local extinctions, and the identification of optimal areas for the range expansion of the species. Our findings will be decisive for the design of efficient strategies to diminish/neutralize threats, improve habitat quality and recover locally extinct populations of the Lear's Macaw.

### 5.3.2 Section 6 - Experimental removal of invasive Africanized honey bees to avoid competition for nest cavities of the endangered Lear's Macaw

#### 5.3.2.1 Evidence of competition between invasive Africanized honey bees and macaws for nest sites

There is substantial and widespread evidence that AHB compete with a variety of parrot species for their tree-nesting cavities and artificial nest-boxes often placed to increase nest-site availability by conservation programs (Lindenmayer et al. 2009, Efstathion et al. 2015, Vaughan et al. 2003, Kilpp et al. 2014, Bonaparte and Cockle 2017). The aggressive behavior of AHB facilitates their occupation of nests and could even cause the death of parrots using the same cavities. In fact, AHB might have contributed to the global extinction of the Spix's macaw (*Cyanopsitta spixii*), which formerly also inhabited the Caatinga biome, Juniper and Yamashita (1990) since one of the last females which bred in the wild was found killed by AHB inside the nest, together with her nestlings (Lima L, 2015, pers. comm.). AHB could also be associated with the population decline to local extinction of Lear's Macaws during the 1980's. The locals first noticed AHB moving into the area and the associated increase of attacks to people and animals in the late 1960's, which is in line with the documented expansion of AHB into Northeast Brazil (Michener 1973). However, the potential impact of AHB on macaws has, until now, never been assessed nor considered a threat (BirdLife Internacional 2018).

To our knowledge, our 2016 survey constitutes the first assessment of the potential impact of AHBs on birds at a population scale. Moreover, our survey covered most of the global breeding distribution of the macaw. Our results show a high infestation of the macaw nesting cliffs by AHB, especially in the historical sites where AHB hives outnumber macaw nests by one order of magnitude. The fact that local farmers reported AHB occupying cavities that were once used by macaws to nest, and that macaws reoccupied those cavities as soon as the AHB were removed,

provides clear evidence of interspecific competition between AHB and macaws. AHB occupancy of cavities ideal for macaws may also lead to a higher macaw intraspecific competition among macaws for the few available cavities. In fact, during our surveys of breeding macaws we recorded two pairs trying to nest in the same cavity, which resulted in both pairs failing to breed and the death of one of the adults during the fights. On the other hand, the fact that macaw nests were sited at significantly lower heights than AHB hives, and that the recruitment of AHB-treated cavities by macaws tended to occur at higher off the ground among those available, suggests that the infestation by AHB is forcing macaws to nest at lower heights. These lower cavities can be suboptimal for macaws, since predation risk, human disturbance and poaching (see below) may increase and cause breeding failures, as the nests are more accessible.

#### *5.3.2.2 Effectiveness of Africanized honey bees treatment for the recovery of the macaw population*

Permethrin was previously used to deter the AHB occupancy of artificial nest-boxes placed in trees to facilitate the reproduction of an endangered parrot species. (Efstathion et al. 2015) Here, we show the effectiveness of shooting permethrin into hives sited in high cliffs, resulting in the death of the bees in all cases. This technique, combined with the removal of hive combs after the treatment whenever possible, resulted in the subsequent occupation of AHB-treated cavities by macaws.

Our study system is unavoidably constrained to small sample sizes due to the global scarcity of macaw breeding pairs. Despite that fact, our experimental design and population monitoring survey allowed us to statistically demonstrate the effectiveness of the AHB treatment. Recruitment of new breeding macaw pairs occurred in 15% of the AHB-treated cavities but in none of the untreated ones. Perhaps a surprising result is that AHB cavities that were only treated were also selected by macaws, although at a lower rate than those that were treated and removed, while none of the AHB inactive hives left as a control (i.e., unmanaged)

were selected by macaws. A possible explanation is that the application of permethrin acted as a repellent and discouraged further reoccupation of hives as it is typical of AHB (Efstathion et al. 2015), and thus macaws could excavate the combs with their strong bills allowing access to the cavity without being attacked by the aggressive AHBs.

The recruitment of eight new breeding pairs might seem trivial, but it is highly relevant for this macaw species conservation. In fact, our AHB treatment experiment allowed for the increase of newly recruited pairs by >70%. Moreover, after decades of conservation efforts the global population has increased, but it is still mainly concentrated in the core areas (Barbosa and Tella 2019, ICMBio 2012). Unless the species can recolonize former occupied habitat, nest site availability is expected to become a limiting factor for this growing population, slowing population growth and breeding performance through density-dependent effects (Pacífico et al 2014). In this sense, our results show that nest-site availability is constrained by AHB infestation in the two historic areas recently recolonized, and that a 44 % increase in the breeding population of macaws in these areas (growing from 9 to 14 pairs) was only possible after our AHB treatment.

### *5.3.2.3 Indirect positive effects of Africanized honey bees treatment*

The recovery of the Lear's Macaw, once close to extinction, was probably possible mostly thanks to the protection of their nesting and roosting cliffs in the core area, thus avoiding disturbance and nest poaching for wildlife trade. (Barbosa and Tella 2019)

However, we found here that historical areas are highly infested by AHB and that hives are opportunistically exploited by local people for honey-hunting. We observed that honey-hunting mostly occurred at night, when temperatures drop and thus AHB are slower and less defensive (Collins et al. 1982) and during the breeding season of macaws. This activity, involving human camps and movements around the cliffs, noise, and the use of flashlights, causes disturbance to roosting macaws and may affect their breeding success. Moreover, people built permanent handmade stick

ladders and scaffolds to reach the hives that are used by locals and outsiders for honey-hunting. However, honey-hunting is masking parrot poaching, as some ladders give direct access to the nests of macaws and Blue-fronted amazon parrot. Therefore, the elimination of AHB hives, and associated ladders, would substantially reduce disturbance and poaching pressure on macaw nests.

Apart from indirect effects of AHB removal on macaws and other cliff-nesting parrots, there are others regarding biodiversity. Invasive honey bees have negative impacts on the diversity and density of native bee species. There are approximately 200 species of native bees (“meliponas”) present in the Caatinga biome that are important for pollinizing a variety of plant species (Lindstrom et al. 2016, Henry and Rodet 2018, Alaux et al. 2019, Zanella 2000, Zanella and Martins 2003). Honey from the melipona is traditionally collected by the local population and is important for medicinal and cultural uses (Alves and Alves 2011). It is feared that people are losing the tradition and knowledge of harvesting honey from melipona as they switch to collect honey from the more abundant, non-native AHB (Carvalho et al. 2018). Therefore, AHB treatment may also contribute to the conservation of both biodiversity and cultural heritage in the Caatinga.

#### *5.3.2.4 Challenges of Africanized honey bees hives removal*

The experimental design of hive eradication was affected by the cliff accessibility using rappelling techniques and by the high number of AHB hives and their aggressive behavior. We were constantly chased and attacked during the surveys. Because of that, we found that the use of a crossbow was very effective for poisoning the hives. Using this technique, we could reach a large number of hives in a few fieldwork days (up to 16 hives were shot in just one day). The weakening of the honey bee colony was confirmed the next day after being shot (many dead bees were observed straight below the hive), allowing us to have a safer approach to the infested cliffs. The physical hive removal after treatment was much more risky and time demanding, and thus only three to five hives could be removed per day. Moreover, a

large proportion of treated hives could not be removed given their inaccessibility, even for our team, who were highly trained on safe rappelling techniques.

Although macaws also recruited cavities that had been treated but had not had the comb removed, we believe that the combination of crossbow shooting and removal of the comb is the best management strategy for avoiding AHB reoccupation for longer term. In fact, the three cavities treated with both methods in 2010-2011 were not re-infested by 2018. The hive removal would 1) encourage the use of the cavity by the macaws, as some hives can be too large and deep for the macaws to dig and clean out, thus preventing or delaying their breeding attempt, and 2) will better prevent reoccupation by AHBs, due to removal of the attractive pheromones (Efstathion et al. 2015).

We expect that the application of Fipronil insecticide after comb removal will repel AHBs and make re-infestation of the cavity unlikely (Henry et al. 2012, Kairo et al. 2016, Zaluski et al. 2017). However, the use of insecticides may be controversial since they could poison non-target organisms (Zavaleta et al. 2001). We recorded a variety of species inhabiting cavities (ants, beetles, native bees, tarantulas, geckos, rats, bats and their ectoparasites), and therefore recommend further investigations on the effects of insecticides on this community, including the application of alternative insecticides, before widespread use to minimize these potential impacts.

Once the AHB invasion was identified as an ecological issue (Dupont 2004, Lindstrom et al. 2016) massive efforts were put in place to prevent their spread and to eradicate them (Henry and Rodet 2018, Schneider et al. 2004). However, these attempts often failed, mostly because local control and reduction of colonies are not attainable through sustained eradication efforts in the area of interest (Taylor et al. 2007). Uncertified or informal beekeeping for commercial honey production remains a potential threat to the environment due to the establishment and spread of the AHB through uncontrolled swarm division that often occurs when apiaries are not properly managed (Kent 1989, Torné-Noguera et al. 2016) as it seems the case in our study area. Therefore, AHB monitoring and eradication should be maintained in the long term to avoid re-infestation of the macaw nesting cliffs. The methodology we have tested here could be complemented with placing bee trap boxes with pheromone

lures in the proximity of cliffs. This method was shown to be efficient at attracting AHBs that would otherwise re-colonize parrot nests (Efstathion et al. 2015).

Management strategies for invasive species should be combined with environmental-social awareness to avoid social and ecological mismatches (Beever et al. 2019). Further study is needed to determine the actual socio-economic benefits of honey hunting, beekeeping and the use of both the AHB and melipona by the locals in our study areas, as AHB eradication could receive opposition by this small sector of the society. On the other hand, the risk of AHB attacks on children and goats was mentioned to us as a concern for the indigenous community, and villagers demonstrated an interest for AHB eradication. The engagement of beekeepers, landowners and community leaders on the eradication of invasive AHB are indispensable. This process has the potential to bring environmental awareness about local biodiversity and demonstrate how conservation is compatible with traditional honey hunting practices.

## 6 General Conclusions



1. Breeding parameters of Lear's Macaws did not differ between the two main breeding subpopulations.
2. Further studies of the spatial and temporal availability of food resources, related to rainfall regimes, are needed for a better understanding of the annual variability in breeding parameters and the conservation problems faced by the species.
3. About 80% of the global population is constituted by non-breeding individuals, which can include juveniles, immatures, senescent and mature individuals with suppressed reproduction.
4. Our reanalysis of roost counts accounting for imperfect detectability indicate that the Lear's Macaw population is much larger than previously thought, that has increased > 300% over a decade, and that it could continue to grow in future years.
5. The reduction to a single population not only makes the species more vulnerable to stochastic processes but also to crowding effects when facing nesting and foraging habitat limitations.
6. If the Lear's Macaw population does not expand to distant, potential nesting sites, it is expected that the breeding population size will not increase after exceeding the carrying capacity, potentially arising negative density-dependent processes that could revert its current recovery.
7. The above uncertainties on the future population projection of the Lear's Macaw call for the necessity of new monitoring and conservation efforts. Further monitoring must focus on the breeding fraction of the population and its breeding parameters, rather than solely on overall population size as done so far, to properly assess changes in population dynamics, the life-stage related threats and the conservation status of the species in the long term.
8. The 15 species-specific microsatellite markers developed can be applied for individual identification of Lear's Macaws and represent a key complementary research tool to investigate population genetics of this species.
9. Genetic assessment from Non-invasive sampling resulted in a small genetic sample size than expected due to the low quality of DNA extracted. However using eight species-specific polymorphic microsatellite markers we were able to genotype more than hundred samples and identify unique individuals.

10. Genetic analysis from material collected in five localities in the Raso da Catarina area show no evidence of low genetic diversity in the studied population.
11. The fact that there remains a moderate genetic diversity among the individuals from the increasing population of Raso da Catarina, favors management actions for the recovery of this endangered species in areas of acute decline, such as translocation.
12. Based in this sampling we observed tendency to male bias (0.65) indicating that sex ratio must be widely investigated across the primary and secondary productivity to infer its implications for the population continuous increasing and expansion.
13. We set a framework to incorporate genetic information into integrative management for the conservation of this globally endangered species.
14. Fieldwork combined with interviews to elderly people allowed to locate historical sites occupied by Lear's Macaws and two recolonized breeding sites, as well as to identify past and current threats for the species.
15. We verified that the distribution of the species is constrained by the reduction of environmental resources, derived from persistent threats such as hunting, deforestation and poaching. We also identified new, previously overlooked, threats such as power line eletrocutions and wind farms that merit further investigation as potential ecological traps.
16. Our results increase the historic and current distribution of Lear's Macaw. The extant subpopulations (in RASO and BDO) were inter-connected within a significantly larger distribution range. The increasing population in RASO shows evidence of geographic expansion, occupying new areas or historical areas where the species was locally extinct. However these areas are in poor environmental conditions that hinder the establishment of new subpopulation.
17. Invasive Africanized honey bees (AHB) are competing for nesting sites with Lear's Macaws, in particular limiting nest-site availability in those breeding sites recently recolonized by this species.
18. Our experimental treatment of AHB hives proved to be successful at allowing Lear's Macaws to occupy previously used and new cliff nesting cavities and to

increase their breeding population size in the short-term. We recommend maintaining this management scheme, with some improvements, and to apply it to other identified historical breeding to allow the range expansion and recovery of this species.

19. It is crucial to develop a social-environmental working plan involving public policies and local communities, and balancing the need for AHB beekeeping activities while protecting biodiversity in these highly sensitive habitats.

# 7 Appendices

## Appendix 1. List of presentations in International Conferences

- Pacífico EC, Filadelfo T, Tella JL (2018) Improving external marking techniques for bird identification as an alternative for monitoring not recapturable wild macaws. Psittaciformes Reintroduction Workshop (PRW). International Ornithological Congress, Vancouver, Canadá. August, 2018
- Pacífico EC. Current Lear's Macaw research and conservation actions. Speaker at the IX International Parrot Convention, Loro Parque Foundation, Santa Cruz de Tenerife, Spain. September 2018.
- Filadelfo T, Pacífico EC. An urgent strategy to recover a functionally extinct population of the endangered Lear's Macaw and to rescue its historical home range. Oral presentation at the 28th International Congress for Conservation Biology (ICCB 2017). Cartagena, Colombia. August 2017.
- Pacífico EC, Efstathion C, Filadelfo T, Horsburgh R, Cunha R. Highlighting the invasive Africanized-honey bees as an increasing threat to the endangered Lear's Macaw. Oral presentation at the 28th International Congress for Conservation Biology (ICCB 2017). Cartagena, Colombia. August 2017.
- Pacífico EC, Barbosa AEA, Filadelfo T, Carvalho GM, Hiraldo F, Tella JL. The discovery of two threatened breeding subpopulations of the Endangered Lear's Macaw. Poster Presentation at the 9th Annual Parrots International Symposium. Campo Grande MS, Brazil. November 2015.
- Silva MFL, Pacífico EC, Filadelfo T, Dénes, FV. Exploratory analysis of the neonatal development patterns of the endangered Lear's Macaw, *Anodorhynchus leari*, in the wild. Poster Presentation at the 9th Annual Parrots International Symposium. Campo Grande MS, Brazil. November 2015.
- Pacífico EC. The Emerging Threats to the Lear's Macaw Hidden in the Unknown Caatinga Dry-Forest. Speaker at the 9th Annual Parrots International Symposium. Campo Grande MS, Brasil. Noember, 2015.
- Pacífico EC, Filadelfo T, Ribeiro T, Dénes FV, Tella J, Miyaki CY. On the sex ratio of the globally endangered Lear's Macaw (*Anodorhynchus leari*). Oral presentation at the X Neotropical Ornithological Congress and XXII Congresso Brasileiro de Ornitologia. Manaus, AM, Brazil. July 2015.

## Appendix 2. List of publications in collaboration

Tella, J.L.; Hiraldo, F.; **Pacífico E. C.**, Díaz-Luque, J. A.; Dénes, F. V.; Fontoura, F. M.; Guedes, N.; G. Blanco. Conserving the Diversity of Ecological Interactions: The Role of Two Threatened Macaw Species as Legitimate Dispersers of “Megafaunal” Fruits. **Diversity** 2020, 12, 45. <https://doi.org/10.3390/d12020045>

Sebastián-González, E.; Hiraldo, F.; Blanco, G.; Hernández-Brito, D.; Romero-Vidal, P.; Carrete, M.; Gómez-Llanos, E.; **Pacífico, E. C.**; Díaz-Luque, J. A.; Dénes, F. V.; Tella, J. L. *The extent, frequency and ecological functions of food wasting by parrots*. **Scientific Reports**, v. 9, p. 15280, 2019. <http://dx.doi.org/10.1038/s41598-019-51430-3>

Berkunsky, I. Quillfeldt, P. Brightsmith, D.J. Abbud, M.C. Aguilar, J.M.R.E. Alemán-Zelaya, U. Aramburú, R.M. Arce Arias, A. Balas Mcnab, R. Balsby, T.J.S. Barredo Barberena, J.M. Beissinger, S.R. Rosales, M. Berg, K.S. Bianchi, C.A. Blanco, E. Bodrati, A. Bonilla-Ruz, C. Botero-Delgadillo, E. Canavelli, S.B. Caparroz, R. Cepeda, R.E. Chassot, O. Cinta-Magallón, C. Cockle, K.L., Daniele, G. De Araujo, C.B. De Barbosa, A.E. De Moura, L.N. Del Castillo, H. Díaz, S. Díaz-Luque, J.A. Douglas, L. Figueroa Rodríguez, A. García-Anleu, R.A. Gilardi, J.D. Grilli, P.G. Guix, J.C. Hernández, M. Hernández-Muñoz, A. Hiraldo, F. Horstman, E. Ibarra Portillo, R. Isacch, J.P. Jiménez, J.E. Joyner, L. Juarez, M. Kacoliris, F.P. Kanaan, V.T. Klemann-Júnior, L. Latta, S.C. Lee, A.T.K. Lesterhuis, A. Lezama-López, M. Lugarini, C. Marateo, G. Marinelli, C.B. Martínez, J. McCreynolds, M.S. Mejia Urbina, C.R. Monge-Arias, G. Monterrubio-Rico, T.C. Nunes, A.P. Nunes, Fdp Olaciregui, C. Ortega-Arguelles, J. **Pacífico, E.** Pagano, L. Politi, N. Ponce-Santizo, G. Portillo Reyes, H.O. Prestes, N.P. Presti, F. Renton, K. Reyes-Macedo, G. Ringler, E. Rivera, L. Rodríguez-Ferraro, A. Rojas-Valverde, A.M. Rojas-Llanos, R.E. Rubio-Rocha, Y.G. Saidenberg, A.B.S. Salinas-Melgoza, A. Sanz, V. Schaefer, H.M. Scherer-Neto, P. Seixas, G.H.F. Serafini, P. Silveira, L.F. Sipinski, E.A.B. Somenzari, M. Susanibar, D. Tella, J.L. Torres-Sovero, C. Trofino-Falasco, C. Vargas-Rodríguez, R. Vázquez-Reyes, L.D. White, T.H. Williams, S. Zarza, R. Masello, J.F. *Current threats faced by Neotropical parrot populations*. **Biological Conservation**, v. 214, p. 278-287, **2017**. <http://dx.doi.org/10.1016/j.biocon.2017.08.016>

Blanco, Guillermo; Bravo, Carolina; **Pacífico, Erica C.**; Chamorro, Daniel; Speziale, Karina L.; Lambertucci, Sergio A.; Hiraldo, Fernando; Tella, José L. *Internal seed dispersal by parrots: an overview of a neglected mutualism*. **PeerJ**, v. 4, p. e1688, **2016**. <http://dx.doi.org/10.7717/peerj.1688>

Tella, José L.; Baños-Villalba, Adrián; Hernández-Brito, Dailos; Rojas, Abraham; **Pacífico, Erica**; Díaz-Luque, José A.; Carrete, Martina; Blanco, Guillermo; Hiraldo, Fernando. *Parrots as overlooked seed dispersers*. **Frontiers in Ecology and the Environment**, v. 13, p. 338-339, **2015**. <http://dx.doi.org/10.1890/1540-9295-13.6.338>

## Appendix 3. Publication of Section 1

Bird Conservation International, page 1 of 11. © BirdLife International, 2013  
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### Breeding to non-breeding population ratio and breeding performance of the globally Endangered Lear's Macaw *Anodorhynchus leari*: conservation and monitoring implications

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#### Summary

Lear's Macaw *Anodorhynchus leari* is currently listed as "Endangered" by IUCN. Although it only breeds on cliffs at two protected sites in Bahia State, Brazil, there is no accurate information on population parameters such as the number of breeding pairs and their breeding performance. Between 2009 and 2010, we sought to quantify, for the first time, breeding population size and the main breeding parameters for the species in the two known breeding sites, by quantifying the number of active nests and monitoring 75 breeding attempts. Overall, 80% of the breeding attempts were successful with 1.33 ( $\pm$  0.86 SD) fledglings/breeding attempt (productivity) and 1.67 ( $\pm$  0.60 SD) fledglings/successful nest (brood size). Breeding success and productivity were higher in 2010, while brood size did not vary between years and breeding sites. By adding 73 estimated nests to the 41 nests monitored, 228 individuals were estimated to be reproductively active in 2010, representing c.20% of the population (1,125 individuals). Given that the species is confined to a single population, further population increases could provoke overcrowding and negative density-dependent effects if it does not expand geographically. Therefore, long-term population monitoring focusing on the fraction of the population that is actually breeding and its breeding performance, rather than solely on the whole population size, is important for a better understanding of the population dynamics and conservation of this species.

#### Resumo

A arara-azul-de-lear *Anodorhynchus leari* é atualmente classificada como "Em Perigo" de extinção pela IUCN. Sabe-se que a espécie nidifica em paredões de arenito localizados em duas áreas protegidas no Estado da Bahia, mas não há informações precisas sobre parâmetros populacionais como número de pares reprodutivos e seu desempenho. O objetivo deste estudo foi obter as primeiras estimativas do tamanho da população reprodutiva e os principais parâmetros reprodutivos para a espécie, através da quantificação de ninhos ativos e do monitoramento de 75 tentativas reprodutivas entre os anos de 2009 e 2010, nos dois sítios reprodutivos conhecidos. O sucesso reprodutivo atingiu 80% das tentativas, com 1.33 ( $\pm$  0.86 SD) filhotes/tentativa de reprodução (produtividade) e 1.67 ( $\pm$  0.60 SD) filhotes/ninhos com sucesso reprodutivo (tamanho da ninhada). Houve maior sucesso reprodutivo e produtividade em 2010, enquanto o tamanho da ninhada não variou entre os anos e os sítios reprodutivos. Ao adicionar 73 prováveis ninhos aos 41 ninhos monitorados, estimou-se 228 indivíduos ativos reprodutivamente em 2010, representando c.20% da população (1,125 indivíduos). Considerando que a espécie está limitada a uma única população, um aumento constante da mesma poderá resultar na saturação dos recursos ambientais e gerar efeitos dependentes da densidade negativos caso não haja expansão geográfica. Portanto, para entender melhor a dinâmica da população e os problemas de conservação desta espécie, é importante um monitoramento populacional em longo prazo com foco na parcela reprodutiva da população e no seu desempenho reprodutivo, ao invés de apenas no número total de indivíduos da população.

## Introduction

Lear's Macaw *Anodorhynchus leari* is endemic to the 'Caatinga' biome and considered as globally "Endangered" by IUCN (BirdLife International 2012). Its distribution is restricted to a small area in the north-east of Bahia state, Brazil and is concentrated in two protected areas, Raso da Catarina Ecological Station (RCES) and Canudos Biological Station (CBS), where the whole population nests and roosts communally on sandstone cliffs (Menezes *et al.* 2006). However, the birds perform daily movements from these sites to forage in neighboring unprotected areas (Brandt and Machado 1990, Santos-Neto and Camandaroba 2008, Silva-Neto *et al.* 2012). The organizations CEMAVE/ICMBio and Biodiversitas Foundation have assessed changes in the population size of Lear's Macaw through the post-breeding monitoring of the two communal roosts since 1998, with standardised annual censuses conducted since 2004 (IBAMA 2006). In recent years, a population increase from 570 in 2004 (IBAMA 2006) to 1,125 individuals in 2010 has been observed (Lugarini *et al.* 2012). Although the long-term population increase is partially explained by a higher monitoring effort, there is a consensus that the species has been recovering in numbers over the past few decades (BirdLife International 2012). Due to these increases in overall population size, BirdLife International (2012) downgraded the threat category of the species from "Critically Endangered" (CR) to "Endangered" (EN) in the 2009 IUCN Red List of Threatened Species, based on the estimate of more than 250 mature individuals capable of reproduction (excluding those that will not produce new recruits; IUCN 2013).

Estimating the number of mature breeding individuals is challenging for many species for which accurate population biology information is not available. Therefore, this number is often obtained by applying an assumed proportion of individuals that are mature to the estimated whole population size, an approach that often leads to gross overestimates of number of mature individuals (IUCN 2013). Especially in the case of long-lived species with deferred maturity, as in Lear's Macaw (Young *et al.* 2012), both the number of mature individuals and the breeding fraction may be much smaller than the non-breeding part of the population (Kenward *et al.* 2000, Negro 2011). There is however a marked scarcity of information on breeding to non-breeding ratios in birds, which may undermine the design of proper conservation strategies, since these population fractions are often exposed to different threats related to their different use of space and resources (Penteriani *et al.* 2011).

The overall population size of Lear's Macaw is reasonably well known. However, there is no information on the proportion of breeding birds, and the fact that sub-adults may form pairs and behave like nesting birds for a number of years before they actually breed makes this estimation difficult (BirdLife International 2012). In the same way, most aspects of the breeding biology of the species are virtually unknown in the wild (Juniper and Parr 2010). A study of the reproductive success of Lear's Macaw is therefore essential to design effective conservation actions (BirdLife International 2012) and to understand the population ecology of the species (e.g. Carrete *et al.* 2006a). This will allow a better assessment of the threats the species is facing and allows prediction of population growth and extinction risk in the long-term through population viability analyses (Oro *et al.* 2008).

Given the importance of knowing the proportion of the population that is breeding and its breeding success, the Management Plan for the Conservation of Lear's Macaw considers the assessment of its breeding population size and breeding parameters as high priorities (IBAMA 2006, Lugarini *et al.* 2012). Therefore, the aim of this study was to estimate the number of breeding pairs and main breeding parameters for a better knowledge of the population ecology, conservation and monitoring needs of the species.

## Methods

### Study area

Surveys were conducted at the two breeding sites known for the species: Raso da Catarina Ecological Station (RCES; 09°52'S, 38°38'W), and Canudos Biological Station (CBS; 09°57' S, 38°59'W),

*Breeding population and breeding performance of Lear's Macaw*

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known as Serra Branca and Toca Velha, respectively. Both are composed of sedimentary rock, characterised by the alternation of calcareous sandstone outcrops and delimited by intermittent streams (Oliveira and Chaves 2010). The areas are inserted in the Caatinga biome in the ecoregion of Raso da Catarina, where elevation reaches 800 m and temperature varies between 15 and 45°C. Climate is semi-arid, rainfall being torrential and irregular, with annual averages between 450–650 mm concentrated between December and July (Velloso *et al.* 2002).

*Nest identification*

Lear's Macaw breeds exclusively in pre-existing cavities of calcareous sandstone cliffs. Breeding activities start in September–October with the exploration of cavities and last until April when the last chicks leave their nests (IBAMA 2006). Nest searches were conducted by walking along the intermittent rivers located at the base of the cliffs looking for potential nest cavities and their exploration by macaw pairs (Renton and Brightsmith 2009). In order to identify the cavities actually occupied by breeding pairs for nesting (i.e. active nests), direct observation was undertaken for an average of 12 hr/day at each site, during three consecutive days, twice a month, from early January to late June during two breeding seasons (2009 and 2010). Nests are most easily identified in January as this coincides with Lear's Macaws remaining for longer periods inside their nests as they sit on eggs and brood very young chicks (Pacífico 2011). Breeding sites at RCES were monitored by E.A.B. and K.O., while E.C.P. and T.F., together with field assistants, monitored the breeding sites at CBS. Similarly to Schneider *et al.* (2006) and Renton and Brightsmith (2009), active nests were identified based on continued observation of the following behaviour for three consecutive days: (1) the pair remained inside or in the entrance of the cavity; (2) in the absence of the mate, one of the individuals remained inside the cavity; and (3) mate-feeding was performed in the entrance of the cavity. The cliffs were photographed to aid location of both potential and active nest sites in each breeding season. Observations were conducted from distant points (> 100 m) to avoid disturbance (Schneider *et al.* 2006). This observation protocol allowed us to estimate the breeding population size and the breeding parameters of a subsample of nests (see below).

*Breeding parameters*

Breeding parameters were obtained from those nests (focal nests) where it was possible to see the number of fledglings observed in the nest entrance (Renton and Brightsmith 2009). The number and distribution of nests varied slightly between 2009 and 2010. Therefore, 34 focal nests (24 at RCES and 10 at CBS) were monitored to estimate breeding parameters in 2009, while 41 focal nests were monitored in 2010 (29 at RCES and 12 at CBS). At CBS we were also able to determine the breeding output by combining observations with direct nest inspections of all focal nests, using abseiling techniques in the sandstone cliffs, three to five times until chicks were close to fledge. These additional inspections confirmed that the breeding parameters obtained by observation were valid (Pacífico 2011). The observation protocol used for nest identification was extended to assess breeding output, but with increased efforts between March and June (c.6 hr of observation/researcher/day) coinciding with the period in which nestlings are first sighted at the entrance of nest cavities (between the 12<sup>th</sup> and 15<sup>th</sup> weeks after hatching). This is a good metric to determine breeding success as nestlings they tend to spend most of the daylight hours at the entrance until they are able to fly (Pacífico 2011). During this period nestlings were easily identified, as they have smaller and paler lappets bordering the lower mandible than adults (Brandt and Machado 1990, Juniper and Parr 2010). We defined breeding success as the percentage of pairs producing at least one fledgling, brood size as the average number of fledglings per successful pair, and productivity as the average number of fledglings per pair that attempt to breed (i.e. that occupied a nest).

*Breeding population size*

The number of breeding pairs in the population was estimated as the total number of nests occupied in the 2010 breeding season, pooling focal (confirmed) and probable nests. Probable nests were defined as those nests where intense activity by macaw pairs was observed throughout the entire breeding season, but given the difficulty of monitoring them from suitable observation points, it was not possible to determine breeding parameters.

*Statistical analyses*

Differences in breeding parameters between breeding sites (CBS and RCES) and years (2009 and 2010) were assessed through Generalized Linear Models, fitting site, year and their interaction as fixed effects. The binomial distribution and logit link function were used to analyse breeding success using nesting attempts as sampling units (0: unsuccessful, 1: successful), while the Poisson distribution and log link function were used for productivity (number of fledglings: 0–3) and brood size (number of fledglings: 1–3). Estimated marginal means (i.e. the mean response for each factor level adjusted for any other variables included in the model) were provided for significant effects. All analyses were performed using SPSS 15.0.

**Results***Breeding parameters*

As expected, fully-grown Lear's Macaw nestlings were first observed at the entrance of their nests from March to May, showing a peak in April (65.6% in 2009 and 63.9% in 2010, Figure 1), indicating that most nestlings fledged during this month. Overall breeding success was 80% of the breeding attempts ( $n = 75$ ) recorded in focal nests. Productivity averaged  $1.33 \pm 0.86$  SD fledglings per breeding attempt ( $n = 75$ , Figure 2), while brood size averaged  $1.67 \pm 0.60$  SD fledglings per successful nest ( $n = 60$ ). Successful nests fledged two chicks (53.3%) one chick (40%) or three chicks (6.7%).

Breeding success did not differ between sites (Wald's  $\chi^2_1 = 0.069$ ,  $P = 0.79$ ) but was higher in 2010 (estimated marginal mean:  $88\% \pm 0.05$  SE, Wald's 95% CI: 0.78–0.98) than in 2009 (estimated marginal mean:  $71\% \pm 0.08$  SE, Wald's 95% CI: 0.55–0.88, Wald's  $\chi^2_1 = 3.27$ ,  $P = 0.07$ ; Figure 3). Similarly, productivity did not differ between sites (Wald's  $\chi^2_1 = 0.64$ ,  $P = 0.42$ ) but was higher in 2010 (estimated marginal mean:  $1.55 \pm 0.14$  SE) than in 2009 (estimated marginal mean:  $1.15 \pm 0.14$  SE) (Wald's  $\chi^2_1 = 3.79$ ,  $P = 0.05$ ). There was no significant interaction of site x year for breeding success (Wald's  $\chi^2_1 = 0.87$ ,  $P = 0.35$ ) nor productivity (Wald's  $\chi^2_1 = 1.13$ ,  $P = 0.29$ ). Brood size also did not vary between sites (Wald's  $\chi^2_1 = 1.14$ ,  $P = 0.28$ ) and between years (Wald's  $\chi^2_1 = 0.54$ ,  $P = 0.46$ , interaction site x year Wald's  $\chi^2_1 = 0.21$ ,  $P = 0.65$ ; Figure 3).

*Breeding population size*

In 2010, 20 probable nests were recorded at CBS and another 53 at RCES but could not be properly monitored because of the difficult visibility. These 73 probable nests together with the 41 monitored focal nests leads to the estimate of 114 breeding pairs (228 breeding individuals). Lugarini *et al.* (2012) censused a total of 1,125 Lear's Macaws in 2010. Therefore, the 228 breeding individuals represented 20.3% of the population.

**Discussion***Breeding parameters*

The breeding success of Lear's Macaw (80%) was much higher than in three species of the genus *Ara* (48%, Blue-and-yellow *Ara ararauna*, Green-winged *A. chloropterus*, and Scarlet Macaw

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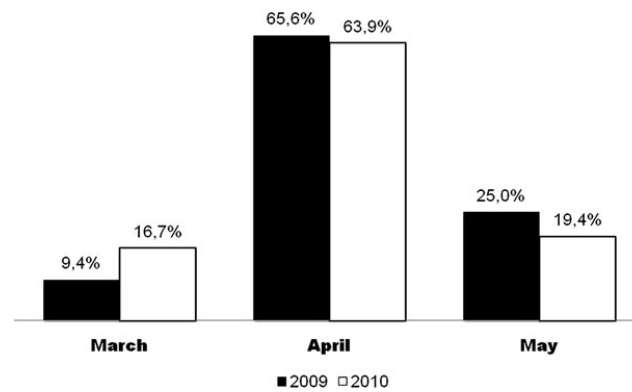


Figure 1. Percentage of full-grown nestling Lear's Macaws observed between March and May at the entrance of the nest cavities.

*A. macao* in lowland Amazonian forests (Renton and Brightsmith 2009) but only slightly higher than that of the Blue-and-yellow Macaw (72%) in Cerrado savannah (Bianchi 1998). Estimates of reproductive success of the Hyacinth Macaw *Anodorhynchus hyacinthinus* in the northern (Antas *et al.* 2010) and southern Pantanal (Guedes 2009) based on egg-laying records also yielded lower values (74% and 73%, respectively). Differences between species may be partially related to the sampling size or different methodologies applied (observations of nest occupation versus egg-laying recording though direct nest inspections). In Lear's Macaw, however, breeding parameter estimates were consistent when obtained by nest inspections and observations at distance (Pacífico 2011).

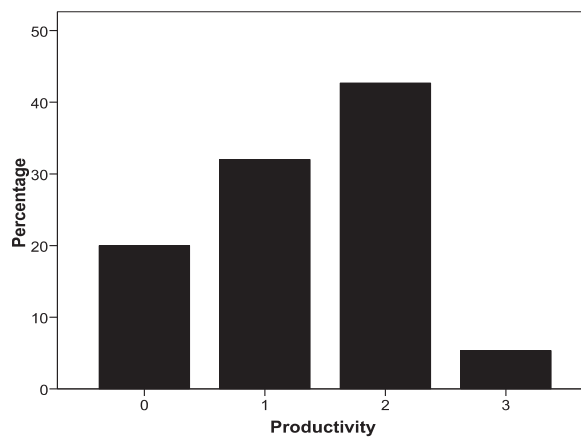


Figure 2. Productivity (number of fledglings per breeding attempt,  $n = 75$ ) of Lear's Macaws in 2009–2010. Raw data are depicted as the percentage of cases with 0–3 fledglings.

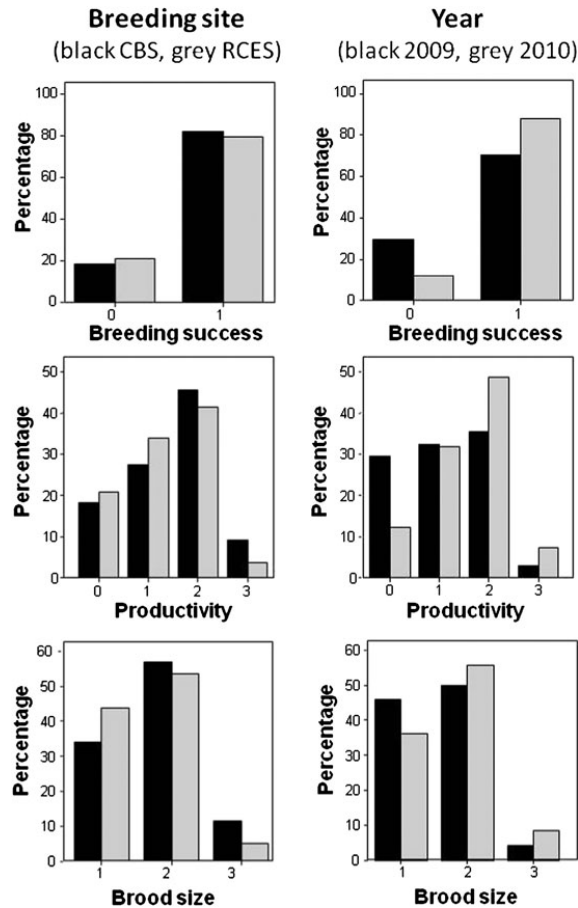


Figure 3. Breeding success (percentage of successful -0- and unsuccessful -1- nests), productivity (percentage of nests raising 0-3 fledglings), and brood size (percentage of successful nests raising 1-3 fledglings) of Lear's Macaws in relation to breeding site and year.

The average productivity (1.33) and brood size (1.67) of Lear's Macaw indicate that each breeding pair normally produces 1-2 chicks, contrasting with its congener Hyacinth Macaw that usually rears only one chick (Guedes 1993, 2009). In other macaw species of genus *Ara*, however, successful broods of two or even three chicks are not rare, but average productivity (0.6-0.94) is also smaller than in Lear's Macaw (Bianchi 1998, Bravo and Brightsmith 2006, Renton and Brightsmith 2009).

The species cited above, with the exception of Lear's Macaw, nest mostly in tree holes. Given the higher breeding parameters of Lear's Macaws, it is worth questioning whether nest substrate (tree holes vs. cliff cavities) may play a role in the breeding success of the species. Future studies

of cliff nesting *Ara* macaws would be useful in addressing this issue. The availability of tree-holes is known to be a limiting factor in the density of parrot populations (Cockle *et al.* 2010). Forest removal, logging and natural or human-made fires diminish cavity availability, especially for large macaws (Bravo and Brightsmith 2006), and this limited availability may increase competition. Nest losses due to interspecific competition compromise the reproductive success of Hyacinth Macaws (Guedes 2009, Antas *et al.* 2010), while the main cause of breeding failure seems to be clutch predation in this large macaw species (Pizo *et al.* 2008, Antas *et al.* 2010). The colonial cliff-nesting behavior of Lear's Macaws, however, could reduce predation risk as has been suggested for the cliff-nesting Burrowing Parrot *Cyanoliseus patagonus* (Masello and Quillfeldt 2002). This hypothesis could be further tested by comparing breeding parameters of some macaw species which breed both in tree-holes and cliffs (Abramson *et al.* 1995, Rojas *et al.* 2013), and could add insight into the evolutionary transition in the use of nesting substrates by parrots (Brightsmith 2005).

Breeding parameters of Lear's Macaw did not vary between the two breeding sites. Renton and Brightsmith (2009) also did not find variations in productivity among breeding sites of three large macaw species. However, both breeding success and productivity were somewhat larger in 2010 than in 2009. These differences could be related to seasonal and inter-year variability in food resources for the species. Santos-Neto and Camandaroba (2008) were able to map the 37 biggest patches of licuri palm tree *Syagrus coronata*, which provide the main food item of Lear's Macaw (Brandt and Machado 1990) around breeding sites. The average distance from breeding sites to these licuri palm patches was 49.5 km for CBS and 45.9 km for RCES (Santos-Neto and Camandaroba 2008). Moreover, palm patches are small and highly degraded by humans and goats and show a marked fruit seasonality influenced by rainfall (Rocha 2009). Lear's Macaws are not strictly dependent on licuri nuts as at least five other wild fruits are part of its diet during the breeding season, and macaws regularly consume maize perhaps as a response to the scarcity of wild fruits (Brandt and Machado 1990, Silva-Neto *et al.* 2012). In fact, the Caatinga dry forest has been continuously devastated and its conservation status has received little attention by Brazilian governments (Leal *et al.* 2005). Further studies of the spatial and temporal availability of food resources, related to rainfall regimes, are therefore needed for a better understanding of the variability in breeding parameters and the conservation problems faced by the species.

#### Breeding population size

Non-breeding population fractions are often cryptic and more difficult to estimate than their breeding counterparts since the later are attached to breeding sites and are easier to monitor (Penteriani *et al.* 2011). In the case of Lear's Macaw, however, both breeding and non-breeding groups use the same cliffs for roosting, thus making both parts of the population equally easy to monitor but increases the possibility of inflating breeding estimates based on total counts of individuals. Moreover, individuals close to maturity could mate and prospect nest cavities before reproducing, which could introduce an important error in the breeding population estimate (BirdLife International 2012). Renton and Brightsmith (2009) observed that 25% of nest cavities inspected by *Ara* macaws during the breeding season did not result in active nests. However, the combination of observations at distance with nest inspections of focal nests indicated that the survey methodology used to identify breeding pairs of Lear's Macaws was appropriate. Using our estimate of 114 pairs breeding in 2010, the proportion of breeding individuals was about 20% of the whole population in 2010. This is similar to the proportion estimated for healthy populations of several *Ara* species (10–20%; Munn 1992) and for the whole population of the globally Endangered Red-fronted Macaw *Ara rubrogenys* (16–33%, Tella *et al.* in press.). However, it is lower than in other long-lived species with deferred sexual maturity like the Common Buzzard *Buteo buteo* (40%; Kenward *et al.* 2000), Red-billed Chough *Pyrrhocorax pyrrhocorax* (40–72%; Blanco *et al.* 2009), Bearded Vulture *Gypaetus barbatus* (56%; Gómez de Segura *et al.* 2012), Egyptian Vulture *Neophron percnopterus* (c.45%; J.A. Donázar pers. comm.) and 18 seabird species (30–73%; Warham 1996).

*Conservation and monitoring implications*

A recent increase in breeding numbers of Lear's Macaws may be logically inferred from the positive population trend of the species recorded in recent decades (BirdLife International 2012). However, there are several reasons for not blindly assuming past or future linear relationships between breeding and overall population size. An overall population increase could result from conservation actions (BirdLife International 2012) that could significantly increase breeding output and adult survival without increasing the number of breeding pairs. Breeding numbers of hole-nesting parrots can be limited by the quantity and quality of nesting sites (Cockle *et al.* 2010), thus breaking the assumed direct relationship between number of individuals and number of breeding pairs. This could explain the lower breeding to non-breeding population ratios in macaws compared with other long-lived species (see above).

Currently, nearly all Lear's Macaws are concentrated at two breeding/roosting sites separated by just 38 km and there is a strong suggestion that individuals moving between these sites belong to a single population (Menezes *et al.* 2006). A small group located in 1995 in an unprotected area between the Campo Formoso and Sento Sé municipalities in Bahia, 230 km to the west (BirdLife International 2012), seems to have been nearly extirpated with only two individuals located in 2012, probably due to trapping for illegal trade (ICMBio unpubl. data). The reduction to a single population not only makes the species more vulnerable to stochastic processes but also to crowding effects when facing nesting habitat limitations. If the Lear's Macaw population does not expand to distant, potential nesting sites, it is likely that the breeding population size will not increase after exceeding the carrying capacity in terms of nest-site availability despite further increases in overall population size. Other social factors may also limit the number breeding as, for example, the percentage of breeding Puerto Rican parrots *Amazona vittata* decreased with an increase in the total population size in absence of nest-site limitation or skewed sex ratios (Beissinger *et al.* 2008). On a more positive note, the proximity of communal roosts of non-breeding individuals to nesting sites may contribute to supply mate losses (Blanco and Tella 1999) and buffer local extinction processes (Carrete *et al.* 2007). However, the spatial overlap of breeders and non-breeders in isolated populations of birds may also reduce their population growth through density-dependent processes. Negative effects can arise when non-breeders compete for resources with breeders or interfere with their breeding activities (Carrete *et al.* 2006a, 2006b, Blanco *et al.* 2009). In the case of Lear's Macaw, the large non-breeding population competes with breeders in foraging areas and this could compromise their breeding condition and success, especially in years of food scarcity. Moreover, interference of non-breeders with breeding activities could also increase in an overcrowding situation, thus further reducing breeding performance (Renton 2004, Carrete *et al.* 2006a).

The above uncertainties on future population projections for Lear's Macaw call for the necessity of new monitoring and conservation efforts. Further monitoring must focus on the breeding fraction of the population and its breeding parameters, rather than solely on overall population size, as done so far, to fully assess changes in population dynamics, threats related to life-stage and the conservation status of the species in the long term. There is also the need to investigate the annual rates of juvenile and adult survival which, together with population and breeding parameter estimates, will allow the creation of population viability models (PVA) that ultimately would determine the conservation status and conservation action priorities for the species. This would require capture-mark-resighting work and, ideally, tagging birds for remote tracking, which would add valuable information on the causes and rates of mortality that can vary between the different population fractions (Oro *et al.* 2008, Grande *et al.* 2009). Remote tracking would also provide data essential to determine the range movements by breeding and non-breeding individuals in relation to the spatial and seasonal changes in food resources (Tanferna *et al.* 2013), as well as to investigate whether non-breeders could prospect distant, potential but still unknown nesting areas for the species. This would help to delineate protected areas, covering the most important foraging areas, and planning the geographical expansion of the species. If the species is not able to disperse naturally, it could result in an overcrowded population suffering from negative density-dependent effects in a relatively short time frame.

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## Appendix 4 Submission proof of research manuscript presented in Section 2

The Condor: Ornithological Applications  
 Improving inference on population trend from heterogeneous roost counts of the  
 endangered Lear's Macaw (*Anodorhynchus leari*, Psittacidae)  
 --Manuscript Draft--

Manuscript Number:	
Full Title:	Improving inference on population trend from heterogeneous roost counts of the endangered Lear's Macaw ( <i>Anodorhynchus leari</i> , Psittacidae)
Short Title:	Improving inference on population trend from roost counts
Article Type:	Research Article
Keywords:	roosting site counts, population size, population trend, N-mixture models, parrot conservation
Corresponding Author:	Erica Cristina Pacifico, MSc. PhD Student Estacion Biologica de Donana CSIC Sevilla, Sevilla SPAIN
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Abstract:	Roost counts may be an effective way to estimate population size for birds that roost communally at night but are widespread during the day. However, the size and number of roosts can vary daily and seasonally, and roosts can change location. Detecting and counting individuals on roosts may be difficult when visibility is poor or flock sizes are large. We employ a detection-based analysis by developing a binomial - negative binomial N-mixture model to estimate population size from roost counts. We apply it to replicated counts done by CEMAVE-ICMBIO of the Lear's Macaw at roosts from 2001-2014. We found high inter-annual heterogeneity of roost counts due to variability in monthly field sampling effort, but the number of counts per month at roost did not vary considerably. However, significantly fewer birds were counted in evening than morning counts. The best N-mixture model included abundance with month as a linear effect and year as quadratic effect, and detection varying by time of day. Predicted detection was higher for macaws in morning than afternoon counts (AM; PM: ). Macaw abundance at roosts declined within years ~13% (June-November) after taking into account between-year variation and imperfect detection. Population size estimates increased by 333% between 2004 and 2014 (2217 individuals, 95% CI = 275.62 – 403.22). The ratio of the annual modeled estimate of population size to the average

	annual count ranged from 1.17 in 2013 to 2.03 in 2006. The annual modeled estimates of population size were closer to the maximum number of macaws counted at roosts each year. We discuss the issues related to the estimation of population size from roost counts that can be addressed by the judicious use of N-mixture models. We also make recommendations for the design of monitoring program for the Lear's Macaw as its population continues to recover.
Additional Information:	
Question	Response
Have you submitted this article to this journal previously?	No
All accepted Research Articles, Perspectives, Commentaries, and Reviews are published with a foreign language abstract (in addition to the English abstract). Which language would you prefer your abstract be translated into?  If there is another language you would prefer for your abstract, choose "Other" and provide that foreign language abstract with your submission.	Portuguese
Suggested Reviewers:	<p>Stuart J. Marsden, PhD Professor, Manchester Metropolitan University s.marsden@mmu.ac.uk recent papers published in the field, and has developed demographic studies with parrots in the neotropics</p> <p>Stuart H.M. Butchart, PhD researcher, University of Cambridge stuart.butchart@birdlife.org Chief Scientist at BirdLife International, develop BirdLife's global scientific data, research and priority-setting. Has experience on endangered species and on inferences on the conservation status of the species</p> <p>Guilherme Mourão, PhD researcher, Empresa Brasileira de Pesquisa Agropecuaria guilherme.mourao@embrapa.br Recent publication on parrot roosting counts</p>

## Appendix 5. Submission proof of research manuscript presented in Section 6

Pest Management Science

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**Experimental removal of invasive Africanized honey bees to avoid competition for nest cavities of the endangered Lear's Macaw**

Journal:	<i>Pest Management Science</i>
Manuscript ID	PM-19-0842
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	04-Sep-2019
Complete List of Authors:	Pacífico, Erica; Estación Biológica de Doñana, Conservation Biology; Grupo de Pesquisa e Conservação da arara-azul-de-lear, Lear's macaw research and conservation group Efstathion, Caroline; AVIANPEC, Avian Preservation and Education Conservancy Filadelfo, Thiago; Grupo de Pesquisa e Conservação da arara-azul-de-lear, Lear's macaw research and conservation group Horsburgh, Robert; AVIANPEC, Avian Preservation and Education Conservancy Alves, Roberta; Grupo de Pesquisa e Conservação da arara-azul-de-lear, Lear's macaw research and conservation group Paschotto, Fernanda; Grupo de Pesquisa e Conservação da arara-azul-de-lear, Lear's macaw research and conservation group Denes, Francisco; University of Alberta Faculty of Agricultural Life and Environmental Sciences, Department of Biological Sciences Gilardi, James; World Parrot Trust, Parrot Conservation Tella, José; Estación Biológica de Doñana, Conservation Biology
Key Words:	Biological invasions, Africanized honey bees, Lear's Macaw, nest-site competition, Caatinga, invasive species eradication, permethrin, fipronil

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<http://mc.manuscriptcentral.com/pm-wiley>

**Appendix 6. Database of macaws roost counts between 2001 and 2014 (Nascimento et al 2001, Menezes et al 2006, ICMBio 2012), collected and provided by CEMAVE-ICMBio for the analysis presented in the section 2.**

<b>census number</b>	<b>ref</b>	<b>time</b>	<b>year</b>	<b>month</b>	<b>date</b>	<b>count</b>	<b>locality</b>
1	Cont. 1	NA	2001	6	1/6/01	246	Canudos e Jeremoabo
2	Cont. 1	NA	2001	9	20/9/01	157	Canudos e Jeremoabo
3	Cont. 1	NA	2002	7	21/7/02	431	Canudos e Jeremoabo
4	Cont. 1	NA	2002	9	22/9/02	400	Canudos e Jeremoabo
"5 e 11"	Cont. 1	NA	2003	6	29/6/03	455	Canudos e Jeremoabo
"6 e 12"	Cont. 1	NA	2003	7	27/7/03	479	Canudos e Jeremoabo
"7 e 13"	Cont. 1	NA	2003	8	24/8/03	359	Canudos e Jeremoabo
"8 e 14"	Cont. 1	NA	2003	9	21/9/03	517	Canudos e Jeremoabo
"9 e 15"	Cont. 1	NA	2003	10	NA	451	Canudos e Jeremoabo
"10 e 16"	Cont. 1	NA	2003	11	NA	450	Canudos e Jeremoabo
"17 e 18"	Cont. 1	PM	2004	6	NA	367	Canudos e Jeremoabo
"19 e 20"	Cont. 2	AM	2004	6	NA	300	Canudos e Jeremoabo
"21 e 22"	Cont. 3	PM	2004	6	NA	405	Canudos e Jeremoabo
"23 e 24"	Cont. 4	AM	2004	6	NA	420	Canudos e Jeremoabo
"25 e 26"	Cont. 1	PM	2004	7	NA	304	Canudos e Jeremoabo
"27 e 28"	Cont. 2	AM	2004	7	NA	420	Canudos e Jeremoabo
"29 e 30"	Cont. 3	PM	2004	7	NA	285	Canudos e Jeremoabo
"31 e 32"	Cont. 4	AM	2004	7	NA	382	Canudos e Jeremoabo
"33 e 34"	Cont. 1	PM	2004	8	NA	307	Canudos e Jeremoabo
"35 e 36"	Cont. 2	AM	2004	8	NA	393	Canudos e Jeremoabo
"37 e 38"	Cont. 3	PM	2004	8	NA	377	Canudos e Jeremoabo
"39 e 40"	Cont. 4	AM	2004	8	NA	353	Canudos e Jeremoabo
"41 e 42"	Cont. 1	PM	2004	9	NA	412	Canudos e Jeremoabo
"43 e 44"	Cont. 2	AM	2004	9	NA	429	Canudos e Jeremoabo
"45 e 46"	Cont. 3	PM	2004	9	NA	413	Canudos e Jeremoabo

"47 e 48"	Cont. 4	AM	2004	9	NA	459	Canudos e Jeremoabo
"49 e 50"	Cont. 1	PM	2004	10	NA	441	Canudos e Jeremoabo
"51 e 52"	Cont. 2	AM	2004	10	NA	412	Canudos e Jeremoabo
"53 e 54"	Cont. 3	PM	2004	10	NA	427	Canudos e Jeremoabo
"55 e 56"	Cont. 4	AM	2004	10	NA	548	Canudos e Jeremoabo
"57 e 58"	Cont. 1	PM	2004	11	NA	333	Canudos e Jeremoabo
59 e 60"	Cont. 2	AM	2004	11	NA	384	Canudos e Jeremoabo
"61 e 62"	Cont. 3	PM	2004	11	NA	297	Canudos e Jeremoabo
"63 e 64"	Cont. 4	AM	2004	11	NA	439	Canudos e Jeremoabo
"65 e 66"	Cont. 1	PM	2005	6	15/6/05	461	Canudos e Jeremoabo
"67 e 68"	Cont. 2	AM	2005	6	16/6/05	570	Canudos e Jeremoabo
"69 e 70"	Cont. 3	PM	2005	6	16/6/05	481	Canudos e Jeremoabo
"71 e 71"	Cont. 4	AM	2005	6	17/6/05	524	Canudos e Jeremoabo
"73 e 77"	Cont. 1	PM	2006	6	17/6/06	720	Canudos e Jeremoabo
"74 e 78"	Cont. 2	AM	2006	6	18/6/06	636	Canudos e Jeremoabo
"75 e 79"	Cont. 3	PM	2006	6	18/6/06	536	Canudos e Jeremoabo
"76 e 80"	Cont. 4	AM	2006	6	19/6/06	509	Canudos e Jeremoabo
"81 e 82"	Cont. 1	PM	2008	7	11/7/08	969	Canudos e Jeremoabo
"82 e 90"	Cont. 2	AM	2008	7	12/7/08	1024	Canudos e Jeremoabo
"83 e 91"	Cont. 3	PM	2008	7	12/7/08	902	Canudos e Jeremoabo
"84 e 92"	Cont. 4	AM	2008	7	13/7/08	952	Canudos e Jeremoabo
"85 e 93"	Cont. 1	PM	2008	9	26/9/08	762	Canudos e Jeremoabo
"86 e 94"	Cont. 2	AM	2008	9	27/7/08	790	Canudos e Jeremoabo
"87 e 95"	Cont. 3	PM	2008	9	27/7/08	907	Canudos e Jeremoabo
"88 e 96"	Cont. 4	AM	2008	9	28/7/08	761	Canudos e Jeremoabo
"97 e 102"	Cont. 1	AM	2009	7	14/7/09	1087	Canudos e Jeremoabo
"98 e 103"	Cont. 2	AM	2009	7	15/7/09	1089	Canudos e Jeremoabo
"99 e 104"	Cont. 3	PM	2009	7	15/7/09	1024	Canudos e Jeremoabo
"100 e 105"	Cont. 4	AM	2009	7	16/7/09	1097	Canudos e Jeremoabo
"101 e 106"	Cont. 5	PM	2009	7	16/7/09	1042	Canudos e Jeremoabo
"107 e 117"	Cont. 1	PM	2010	8	3/8/10	998	Canudos e Jeremoabo

"108 e 118"	Cont. 2	AM	2010	8	4/8/10	1052	Canudos e Jeremoabo
"109 e 119"	Cont. 3	PM	2010	8	4/8/10	1262	Canudos e Jeremoabo
"110 e 120"	Cont. 4	AM	2010	8	5/8/10	1126	Canudos e Jeremoabo
"111 e 121"	Cont. 1	PM	2010	10	5/10/10	787	Canudos e Jeremoabo
"112 e 122"	Cont. 2	AM	2010	10	6/10/10	943	Canudos e Jeremoabo
"113 e 123"	Cont. 3	PM	2010	10	6/10/10	815	Canudos e Jeremoabo
"114 e 124"	Cont. 4	AM	2010	10	7/10/10	1205	Canudos e Jeremoabo
"115 e 125"	Cont. 5	PM	2010	10	7/10/10	907	Canudos e Jeremoabo
"116 e 126"	Cont. 6	AM	2010	10	8/10/10	1165	Canudos e Jeremoabo
"127 e 139"	Cont. 1	PM	2011	6	14/6/11	728	Canudos e Jeremoabo
"128 e 140"	Cont. 2	AM	2011	6	15/6/11	1451	Canudos e Jeremoabo
"129 e 141"	Cont. 3	PM	2011	6	15/6/11	1401	Canudos e Jeremoabo
"130 e 142"	Cont. 4	AM	2011	6	16/6/11	1247	Canudos e Jeremoabo
"131 e 143"	Cont. 5	PM	2011	6	16/6/11	1273	Canudos e Jeremoabo
"132 e 144"	Cont. 6	AM	2011	6	17/6/11	1569	Canudos e Jeremoabo
"133 e 145"	Cont. 1	PM	2011	11	22/11/11	805	Canudos e Jeremoabo
"134 e 146"	Cont. 2	AM	2011	11	23/11/11	1178	Canudos e Jeremoabo
"135 e 147"	Cont. 3	PM	2011	11	23/11/11	824	Canudos e Jeremoabo
"136 e 148"	Cont. 4	AM	2011	11	24/11/11	1091	Canudos e Jeremoabo
"137 e 149"	Cont. 5	PM	2011	11	24/11/11	859	Canudos e Jeremoabo
"138 e 150"	Cont. 6	AM	2011	11	25/11/11	1364	Canudos e Jeremoabo
"151 e 163"	Cont. 1	PM	2012	6	13/6/12	1407	Canudos e Jeremoabo
"152 e 164"	Cont. 2	AM	2012	6	14/6/12	1845	Canudos e Jeremoabo
"153 e 165"	Cont. 3	PM	2012	6	14/6/12	1397	Canudos e Jeremoabo
"154 e 166"	Cont. 4	AM	2012	6	15/6/12	1423	Canudos e Jeremoabo
"155 e 167"	Cont. 5	PM	2012	6	15/6/12	1207	Canudos e Jeremoabo
"156 e 168"	Cont. 6	AM	2012	6	16/6/12	1569	Canudos e Jeremoabo
"157 e 169"	Cont. 1	PM	2012	11	6/11/12	856	Canudos e Jeremoabo
"158 e 170"	Cont. 2	AM	2012	11	7/11/12	1388	Canudos e Jeremoabo
"159 e 171"	Cont. 3	PM	2012	11	7/11/12	993	Canudos e Jeremoabo
"160 e 172"	Cont. 4	AM	2012	11	8/11/12	1152	Canudos e Jeremoabo

"161 e 173"	Cont. 5	PM	2012	11	8/11/12	1135	Canudos e Jeremoabo
"162 e 174"	Cont. 6	AM	2012	11	9/11/12	1541	Canudos e Jeremoabo
"175 e 190"	Cont. 1	PM	2013	6	6/6/13	1064	Canudos e Jeremoabo
"176 e 191"	Cont. 2	AM	2013	6	7/6/13	1415	Canudos e Jeremoabo
"177 e 192"	Cont. 3	PM	2013	6	7/6/13	1377	Canudos e Jeremoabo
"178 e 193"	Cont. 4	AM	2013	6	8/6/13	1643	Canudos e Jeremoabo
"179 e 194"	Cont. 1	PM	2013	7	5/7/13	1397	Canudos e Jeremoabo
"180 e 195"	Cont. 2	AM	2013	7	6/7/13	1716	Canudos e Jeremoabo
"181 e 196"	Cont. 3	PM	2013	7	6/7/13	1544	Canudos e Jeremoabo
"182 e 197"	Cont. 4	AM	2013	7	7/7/13	1786	Canudos e Jeremoabo
"183 e 198"	Cont. 1	AM	2013	9	7/9/13	1218	Canudos e Jeremoabo
"184 e 199"	Cont. 2	PM	2013	9	7/9/13	910	Canudos e Jeremoabo
"185 e 200"	Cont. 3	AM	2013	9	8/9/13	2535	Canudos e Jeremoabo
"186 e 201"	Cont. 1	PM	2013	11	8/11/13	559	Canudos e Jeremoabo
"187 e 202"	Cont. 2	AM	2013	11	9/11/13	1143	Canudos e Jeremoabo
"188 e 203"	Cont. 3	PM	2013	11	9/11/13	428	Canudos e Jeremoabo
"189 e 204"	Cont. 4	AM	2013	11	10/11/13	1235	Canudos e Jeremoabo
"205, 2017, 229, 241"	Cont. 1	PM	2014	7	25/7/14	1096	Canudos, Jeremoabo, Barreiras, Baixa do Chico
"206, 218, 230, 242"	Cont. 2	AM	2014	7	26/7/14	1577	Canudos, Jeremoabo, Barreiras, Baixa do Chico
"207, 219, 231, 243"	Cont. 3	PM	2014	7	26/7/14	1141	Canudos, Jeremoabo, Barreiras, Baixa do Chico
"208, 220, 232, 244"	Cont. 4	AM	2014	7	27/7/14	1467	Canudos, Jeremoabo, Barreiras, Baixa do Chico
"209, 221, 233, 245"	Cont. 1	PM	2014	9	19/9/14	1006	Canudos, Jeremoabo, Barreiras, Baixa do Chico
"210, 222, 234, 246"	Cont. 2	AM	2014	9	20/9/14	1575	Canudos, Jeremoabo, Barreiras, Baixa do Chico
"211, 223, 235, 247"	Cont. 3	PM	2014	9	20/9/14	1232	Canudos, Jeremoabo, Barreiras, Baixa do Chico
"212, 224, 236, 248"	Cont. 4	AM	2014	9	21/9/14	1773	Canudos, Jeremoabo, Barreiras, Baixa do Chico
"213, 225, 237, 249"	Cont. 1	PM	2014	11	21/11/14	1117	Canudos, Jeremoabo, Barreiras, Baixa do Chico
"214, 226, 238, 250"	Cont. 2	AM	2014	11	22/11/14	1195	Canudos, Jeremoabo, Barreiras, Baixa do Chico
"215, 227, 239, 251"	Cont. 3	PM	2014	11	22/11/14	1085	Canudos, Jeremoabo, Barreiras, Baixa do Chico
"216, 228, 240, 252"	Cont. 4	AM	2014	11	23/11/14	1327	Canudos, Jeremoabo, Barreiras, Baixa do Chico

## Appendix 7. R Code developed for the analysis for the population trends from Section 2, Materials and Methods.

```

# Load data, prepare for unmarked ####
library(readr)
library(knitr)
library(kableExtra)
library(unmarked)
library(nmixgoF)

path<- #set path for 'supplement_dataset.csv' file

matrix<- read_csv(path,col_types = cols(date = col_date(format = "%Y-%m-%d")))

# a function to get mid day for month and convert to julian day, to use to get the julian date of surveys with known month but unknown day
mid.month.day <- function(month){
  day <- paste(month,"-", "15", sep="")
  day <- strptime(day,"%m-%d")
  day <- as.integer(floor(julian(day, origin="2016-12-31")))
  return(day)
}

matrix$julian <- NA
matrix$julian[which(is.na(matrix$date)==T)] <- format(matrix$date[which(is.na(matrix$date)==T)], "%j")
matrix$mid.month.day[which(is.na(matrix$date)==T)] <- mid.month.day(matrix$month[which(is.na(matrix$date)==T)])
matrix <- as.data.frame(matrix)
matrix <- matrix[,c(3:5,7,9)]
kable(matrix,digits = 6, caption="Census dataset", format = "html") %>%
  kable_styling(bootstrap_options = c("striped","hover","condensed"), full_width = FALSE)

# Some exploratory plots and tests: ###
boxplot(count~time, data=matrix[11:116,])
testdf<-matrix[11:116,c(1,4)]
testdf$time<-as.factor(testdf$time)
t.test(count~time, data=testdf)
boxplot(count~year, data=matrix[11:116,])

# Format data for 'unmarked' ####
## observation data
sites <- unique(matrix[,c(2,3)])
rownames(sites) <- seq(length=nrow(sites))

y <- matrix(NA,34,6)
times <- matrix(NA,34,6)
months <- years <- rep(NA,34)

for (i in 1:34){
  counts <- matrix[which(matrix$year==sites[i,1]&matrix$month==sites[i,2]),4]
  time <- matrix[which(matrix$year==sites[i,1]&matrix$month==sites[i,2]),1]
  month <- matrix[which(matrix$year==sites[i,1]&matrix$month==sites[i,2]),3]
  year <- matrix[which(matrix$year==sites[i,1]&matrix$month==sites[i,2]),2]
  for(t in 1:length(counts)){
    y[i,t] <- counts[t]
    times[i,t] <- time[t]
    months[i] <- month[t]
    years[i] <- year[t]
  }
}
df <- as.data.frame(cbind(months,years,y))
colnames(df) <- c("Month","Year","1","2","3","4","5","6")
kable(df, caption="Counts for each surveyed month", format = "html") %>%
  kable_styling(bootstrap_options = c("striped","hover","condensed"), full_width = FALSE)
kable(cbind(df[,1:2],times), caption="Time of day of counts", format = "html") %>%
  kable_styling(bootstrap_options = c("striped","hover","condensed"), full_width = FALSE)
obs.covs <- list(times=times)

site.covs <- data.frame(year=sites[[1]]-2000,year2=(sites[[1]]-2000)^2,year3=(sites[[1]]-2000)^3 ,month=sites[[2]],month2=sites[[2]]^2,fmonth=as.factor(sites[[2]]))
y <- y[11:34,]
#y[20,3]<-NA #removing this outlier (see table)
obs.covs$times<- obs.covs$times[11:34,]
site.covs <- site.covs[11:34,]
umf1<- unmarkedFramePCount(y=y,siteCovs = site.covs,obsCovs = obs.covs)
summary(umf1)

# Fit models ###
# Note: only negative-binomial models converge

#Null (state level)
#m01 <- pcount(~times-1,umf1,mixture = "P")# does not converge
#m02 <- pcount(~times-1,umf1,mixture = "NB")
#Linear year
#m03 <- pcount(~times-year+month,umf1,mixture = "P",se=FALSE )
#m04 <- pcount(~times-year+month,umf1,mixture = "P",starts=coef(m1)) # does not converge
#m05 <- pcount(~times-year+month,umf1,mixture = "NB",se=FALSE )
#m06 <- pcount(~times-year+month,umf1,mixture = "NB",starts=coef(m05))
# Quadratic year
#m07 <- pcount(~times-year+year2+month,umf1,mixture = "P",se=FALSE )
#m08 <- pcount(~times-year+year2+month,umf1,mixture = "P",starts=coef(m07))# does not converge
#m09 <- pcount(~times-year+year2+month,umf1,mixture = "NB",se=FALSE )
#m10 <- pcount(~times-year+year2+month,umf1,mixture = "NB",starts=coef(m09))
# Cubic year
#m11 <- pcount(~times-year+year2+year3+month,umf1,mixture = "P",se=FALSE )
#m12 <- pcount(~times-year+year2+year3+month,umf1,mixture = "P",starts=coef(m11))# does not converge
#m13 <- pcount(~times-year+year2+year3+month,umf1,mixture = "NB",se=FALSE )
#m14 <- pcount(~times-year+year2+year3+month,umf1,mixture = "NB",starts=coef(m13))
# Quadratic month and year effects
#m15 <- pcount(~times-year+year2+month+month2,umf1,mixture = "NB",se=FALSE)
#m16 <- pcount(~times-year+year2+month+month2,umf1,mixture = "NB",starts=coef(m15))
# Month effect as a factor, and include interaction with years
#m17 <- pcount(~times-year+year2+fmonth,umf1,mixture = "NB",se=FALSE )
#m18 <- pcount(~times-year+year2+fmonth,umf1,mixture = "NB",starts=coef(m17))
#m19 <- pcount(~times-fmonth*(year+year2),umf1,mixture = "NB",se=FALSE )
#m20 <- pcount(~times-fmonth*(year+year2),umf1,mixture = "NB",starts=coef(m19))

flist <- fitList(times_Null=m02,times_monthyear=m06,times_monthyear2=m10,times_monthyear3=m14,month2year2=m16,fmonthyear2=m18)
modSel(flist)

# Best model includes a linear effect of 'month' (as numeric variable) and 'year' as a 2nd order polynomial for abundance

# Model results ###
# Model summary and CIs
summary(m10)
round(cconfint(m10,type="det"),3)
round(cconfint(m10,type="state"),3)
round(cconfint(m10,type="alpha"),3)

# Predicted detection probabilities (AM/PM)
newDataDet <- data.frame(times=factor(c("AM","PM")))
pred<- predict(m10,type = 'det', newdata = newDataDet, appendData=TRUE)
pred[,c(5,1:4)]

```

```

# Abundance predictions
newData <- data.frame(year = 10, year2=100, month = 6:11)
pre1 <- predict(m10, type = 'state', newdata = newData, appendData=TRUE)
plot(Predicted-month,data=pre1, main= "Predicted abundances (2010) with 95%CI", type="line",ylim=c(0,2500))
lines(lower-month,data=pre1, main= "Predicted abundances, November", type="line",lty=2)
lines(upper-month,data=pre1, main= "Predicted abundances, November", type="line",lty=2)
newData2 <- data.frame(year = 4:14, year2=(4:14)^2, month = 11)
pre2 <- predict(m10, type = 'state', newdata = newData2, appendData=TRUE)
plot(Predicted-year,data=pre2, main= "Predicted abundances (Nov) with 95%CI", type="line",ylim=c(0,2500))
lines(lower-year,data=pre2, main= "Predicted abundances, November", type="line",lty=2)
lines(upper-year,data=pre2, main= "Predicted abundances, November", type="line",lty=2)

# Model goodness-of-fit
#Parametric bootstrap of sum of squared residuals
pb <- parboot(m10, nsim=100, report=1, seed=333)

print(pb)
plot(pb, main="")

# Population trend ###
newData3 <- data.frame(year = c(1:14), year2=c(1:14)^2, month = 11)
pre3 <- predict(m10, type = 'state', newdata = newData3, appendData=TRUE)
trend1 <- round(pre3[14,c(1,3,4)]/pre3[4,c(1,3,4)]*100,2)
trend1
trend2 <- round(pre3[14,c(1,3,4)]/pre3[1,c(1,3,4)]*100,2)
trend2

matrix2<-rbind(rbind(matrix,c("AM",2007,0,0,0)),c("AM",2007,0,0,0))
matrix2$count<-as.numeric(matrix2$count)
b1<-boxplot(count~year, data=matrix2,col=c(rep("grey90",6),"white",rep("grey90",7)),border=c(rep(1,6),"white",rep(1,7)),ylim=c(0,2500))
b1
lines(Predicted-year,data=pre3, main= "Predicted abundances (Nov) with 95%CI", type="line",ylim=c(0,2500),lwd=2)
lines(lower-year,data=pre3, type="line",lty=2)
lines(upper-year,data=pre3, type="line",lty=2)

newDat <- data.frame(Year=c(2001:2006,2008:2014),`Median census`=b1$stats[3,c(1:6,8:14)],Estimate=pre3[c(1:6,8:14),1],Ratio=pre3[c(1:6,8:14),1]/b1$stats[3,c(1:6,8:14)])

kable(newDat,digits = 2, caption="Census and Estimates", format = "html") %>%
  kable_styling(bootstrap_options = c("striped","hover","condensed"), full_width = FALSE)

```

**Appendix 8. Characterization of 633 primer pairs for microsatellites. T<sub>m</sub>: melting temperature in oC; Amplicon size in base pairs; Motif: repeat unit sequence; Repeat number: number of repeats observed; Selected primers: 0 - not selected for further testing, 1 - selected.**

Locus	Forward primer	Reverse primer	Forward primer T <sub>m</sub>	Reverse primer T <sub>m</sub>	Amplicon size	Motif	Repeat number	Selected primers
Ale001	AGAATCGTGGTGTGAGGCTG	AAGGTGAAGGGCAGATGAGC	60.037	60.035	151	AGC	5	0
Ale002	TCTGCCAGCTCTGAGTTGTG	GCAAGCTGGCACCACCTTTAC	59.966	60.039	211	AGC	5	0
Ale003	AGTGGGCAGGATGAAAGGTG	CGAAGCAAGGGTAGGAAGCA	59.961	60.036	168	AC	6	0
Ale004	TCAGTGCAGCACAGAGGAAG	AGTCCCACCTTCCTCCTCTC	59.966	59.958	105	AG	5	0
Ale005	AGCACCAGCAGCATTGTTTG	TGTGCGTGTTACACCTCA	59.967	59.894	119	AGG	5	0
Ale006	TACCTTCCTCCTGCCCTACC	TGCAGCTCTCATCTTCAGGC	60.031	60.108	97	AG	5	0
Ale007	CCACACTGTCACTCCATCCC	GGGATAGACAGACAGCGTGG	60.036	59.897	97	AG	5	0
Ale008	CCTCCCTCGCTTCTTCCTTG	GGATGGGTGGGAAGCATGAA	60.108	60.033	217	AC	5	0
Ale009	CCCTGGCAGGAATTAGCACA	GTCCTGGGTGTATGTCTGGC	60.034	60.108	132	AAT	5	0
Ale010	GTGCCCTTCTTAGAGCCAGG	GCTGCTGTGGAGCTGAGTTA	60.107	60.037	171	AGG	5	0
Ale011	CCTCCATGCCTCACACTGAG	TCCCAAGCACTCACCATCAC	60.108	59.963	244	AC	5	0
Ale012	GCAGGCTGAGTACTTGCTGA	CTTCCCTTAGCGGACCTGTG	60.037	60.108	102	ACAGG	5	0
Ale013	AGCGTCAGTGCTCCTTTACC	GGCTAGCTGCAGTGCTTAGT	60.037	60.108	101	AC	6	0
Ale014	ACAGCTATTCCCAATGGCCC	ACAGTGGCCATACATGCCAA	60.106	59.961	110	AC	5	0
Ale015	GATTGTGGCCAACCTGGAGGA	ACACGCCATAGAGACAAGGC	59.962	60.108	115	ACC	5	0

Ale016	CTGTCACCTTGTCTGTGGCT	ACCAGGATCCCGAAGTCTCA	59.893	59.959	99	AC	5	0
Ale017	CTGACACCTCTGGAGCCTTG	TCTCAAGGGTGGCTAGGACA	60.037	59.884	92	AG	5	0
Ale018	ATCAATACACGCTGCAACGC	CAGTACCAGCCGAAGTCCAG	59.903	60.109	92	AG	5	0
Ale019	GGGCTCAGATGCAGAATGGT	TCTGTCAGGAGGGATCCGAG	60.107	60.106	96	AC	5	0
Ale020	GCCCAAAGCAAACAAAGCCT	CAGCCTGTTGTCAGCTGAGA	60.179	59.966	382	AG	8	1
Ale021	TGCCTTAGCCACCATGCTAC	GCTGCCTAGGAATGCAGTGA	60.107	60.108	125	AC	5	0
Ale022	TATGGCGTCATGCTCTGCAA	GCTCAGCACCAAAGTATGC	60.108	60.109	111	AC	5	0
Ale023	TCCCGCTCCTCAGTAGTGAA	CCCATAGGAGTGGCCGAAAG	59.961	60.179	107	AT	5	0
Ale024	CCCAGCTATGGTTCCCACTC	TACGCTGCGTTCTTAGTGCA	59.819	60.039	97	AGG	6	0
Ale025	GAAAGTTTCCATGCGGCAGG	TCAGGCTGCAAACCCATTCT	60.109	59.887	138	AG	5	0
Ale026	CTGAGGAGCACTGCTGAGAG	GATTAGCATGACAGGCCGGA	59.826	59.893	108	AC	5	0
Ale027	TGCCTTGCTCCGGTTAATGT	CAGTTTGGCTGGGCTAAAGC	59.962	59.755	267	AT	5	0
Ale028	GGCCTGGTAATCTGTTGGCT	ACATCCGTGTGCCATGTTCA	60.034	60.251	113	AG	5	0
Ale029	GCTGAGGAGGAACTTGCTGT	GACAGGAGACTTCAGCTGGG	59.964	59.751	164	AG	5	0
Ale030	TCATGAGGCAGCTGAATGGG	TCCAATCCACCTCCTCCAA	60.107	60.179	97	AC	5	0
Ale031	TCCCGCTCAAGCATAACAGTG	CATGGCTCACTTCCTAGGCC	60.108	60.179	160	ATC	6	0
Ale032	CCTGCTTCCTTGCAAGCATG	AAGAACTGCTGCGAACTGGT	60.109	60.179	90	AGG	5	0
Ale033	TTCCTTACCTGTTTGCGCCT	AGCATGAACAGGGTTGTGCT	59.89	60.179	111	AT	9	1
Ale034	TGGTGTCCCTAGATGGTGCT	CACTGTTGTCCGATTCCCGA	60.253	60.038	233	AG	5	0
Ale035	GGACAGGGAGATCGAGGGTA	TGCAGGTTGCTCTGGTTGAA	59.815	60.107	106	AC	5	0
Ale036	TGTCTTTGTCCGTCTGGCTC	CACGGACACAGAAAGCACAC	59.966	59.695	155	AC	6	0

Ale037	TCCGCGCAGAGTTAGTCATC	CTTCTGTGGGCTTCCTGCTT	59.899	60.251	126	ATC	5	0
Ale038	CCACTCACGGCATCAACAGA	CTGGAAGTGCATCCTCAGCA	60.321	60.036	154	AT	5	0
Ale039	GTCCTTTGTCCTGGCATGGA	GGCTTCTGCTTGCAAATGGT	59.962	59.681	98	AC	6	0
Ale040	AACCTGGAGAGGCTGAAGGA	GAATCACCCATCACCCCTCGG	60.179	60.179	161	AG	5	0
Ale041	TGGTTTAAATGCCGCCCTCA	TTCTGCACAGGCTTTCCACA	60.251	60.107	159	AT	7	1
Ale042	GGCATCTGCATTCCCATTGC	GCAGCATTCTCACAAGGTGC	60.25	60.109	99	AG	5	0
Ale043	AGCCAGCAGGACTTCCTAGT	TATTAGCTGGGCCGTTGTGG	60.252	60.107	95	AG	5	0
Ale044	ACTGCTGCTCATCTCCCTCT	CACCTCACAAGGACAGGGAC	60.325	59.965	93	ACC	5	0
Ale045	TCCTCTCCTCTCGTCTCCAC	GAGCTGGTAGGAAAGGCTGG	59.746	60.107	91	AGAGG	8	1
Ale046	CGAGTACGGATCCATGACGG	G TTCAGAGGCAGGTGTGGAG	60.04	60.321	121	AT	6	0
Ale047	TTCCCAAATCACCTTCCCGG	GAGATGTTCTTGCAGGGCCA	59.961	60.323	102	AC	5	0
Ale048	AAGGCAGAGTGGTGCATGAA	CACTAGTGGCAGCCTCACAT	59.889	59.749	105	AG	5	0
Ale049	AACCAGCATCAATGGCCTCA	GCCACTTGCTGGAAGGATCA	59.96	60.323	106	AC	5	0
Ale050	GCCTCAAGACTGGAGGCTTT	ATCAAGCATGAGGGCCTGTT	59.963	59.666	93	AC	5	0
Ale051	GGGCTGGAAGTAGACGATCC	CCAGTCCTGTTGAGAGGCAG	59.61	60.037	100	AT	5	0
Ale052	GGAGATGTTCCAGCAGTGGG	AAGTGAGTGAGCACCCATGG	60.394	59.963	96	AC	5	0
Ale053	CTTTGTGAGTCCCTGCCTCC	AAGTGAGGTTGCTGACAGGG	60.322	59.891	111	AC	5	0
Ale054	CACCACATGTCTTCGCTTGC	TGCTCTCCGAGAACCAAAGG	60.11	59.678	105	AC	5	0
Ale055	TTTCCACTGAGCTGGTCTGC	AAGCCAAGGATAAGCGAGGG	60.25	59.819	139	AC	5	0
Ale056	TATCTTTCTGCCAGCGCCTT	CCCAAAGTCCACCGTGTCT	59.747	60.179	134	AC	6	0
Ale057	CTGCTCCAGAAGTCCCTTGG	TGCCTCAGTTTCCCTGACTG	60.036	59.602	98	AC	5	0

Ale058	GGCCTCTAATAGCCTGTGGG	GTGTCCTGTAGTGCCCTGC	59.602	60.037	155	AC	5	0
Ale059	CAGAGGAGTGAAAGCGTGGT	ACAAGTCTGGTGGTCATGGG	59.966	59.598	135	AT	6	0
Ale060	TACACATTGCCACCACCTC	CCTTCCACTCCCTCCCTAGG	59.962	60.399	90	AG	5	0
Ale061	CACGTTCCCTCTGGCTCTGTT	TCTTCTGGCCATGGCAAAGA	59.966	59.595	223	AC	5	0
Ale062	GTCGCTGATGGAGGGAAGTG	TTTGCCCAACTGCATCTTGC	60.461	59.966	91	AG	5	0
Ale063	ACCACAGTGGGAGCTGTTTG	TGAGGAATGAAGCCCTGCAG	60.466	60.035	107	AC	5	0
Ale064	GAACTACGTGGCTGACTGGG	AACAGTGATGCTCAAGGGCA	60.39	59.889	95	AC	5	0
Ale065	CTCATGAGGCCACCCGTTAG	GCACTTAGCCCAGCTGACTT	60.179	60.322	115	AC	5	0
Ale066	GAAAGCCTGCCATGCCAAAG	TCTTGCCTTCCCTGAACCC	60.391	59.89	161	AC	16	1
Ale067	CTCCTCTGCACTGGTTCCTG	CCCTAGGCACCATGCTTCTG	60.037	60.465	131	AC	5	0
Ale068	TTCCACGTTACCATGACGCA	TTGGTTGAGACGAAGGCAGT	59.966	59.532	102	AC	5	0
Ale069	GGCTCTGAGACAGTGACCAC	CTACCCTAGCTTTGCCAGGC	60.038	60.465	145	AG	5	0
Ale070	GATCTAGGCTGAGGAGGGCA	TGAGAGGCAGCAAAGCATGA	60.471	59.963	224	AC	6	0
Ale071	TGAGGCCGGGATGGAAATTT	AGCTGCCATTCAGTAGAGCC	59.665	59.82	107	AG	6	0
Ale072	AGGTGGAAGGCTAGAAGGGA	TGGCTAAGACATGGAGCTGC	59.582	60.108	142	ACC	5	0
Ale073	GACTTGCTGCCATGTAGTGC	CAGGGACGCTCATCTGAGTC	59.551	59.898	104	AC	7	1
Ale074	GTAACTTTGCTAGCGTGCCC	GCTGGAGCAAACAATCAGCC	59.553	60.109	122	AGG	5	0
Ale075	GCTGCATTCGTTTGCCACAT	GTGTAGCCCAGGAATGCGTA	60.389	59.823	197	AC	6	0
Ale076	GCCGAGTGCTGCTTCTCATA	GCTCCTTCCGCTCATTTGGA	60.179	60.393	150	AC	5	0
Ale077	AAGTGCCTGGAGTGATGCAG	TGTTAGATGCAGGGAGGTGC	60.322	59.747	122	AGG	5	0
Ale078	TGAGGCACTGTTCCCTTTGT	ACATCGTGCAGTGCAGAAGA	59.449	59.966	126	AG	5	0

Ale079	CAAGGGCAGTGTGACTCCAT	TGGTTTGTGAGGCAGGTCT	59.963	59.449	113	AAT	5	0
Ale080	CAGGACAGTGACAGAGGCAG	GCATGCCGGCTAACTAGAGG	60.038	60.601	277	AG	5	0
Ale081	CTGAAGGCAACCAAGCAAGG	ATCGGACAGCTTTGCAGACT	59.684	59.677	128	AG	6	0
Ale082	TCTCATGCTCTGCCAGTGAG	GGGCGTGATATGCTGTGGTA	59.464	59.894	108	AG	5	0
Ale083	CACTGCTTCTCTGGAGCTGA	ACATGCAACGTACAGGCTGA	59.393	59.965	124	AC	5	0
Ale084	GCACTGTTCCACCTCAACCT	GTCTCCTCATGCCAGACTCC	60.179	59.536	148	AT	5	0
Ale085	CAGCCCTTTCTGTCTGTGCT	GCCACAGCGATTAGTGGGAA	60.251	60.392	99	AG	5	0
Ale086	ACTGAGCTCTGTCTTTGGGC	CAAGGACCTGGGCTGTTTTCAG	59.964	60.607	161	AC	5	0
Ale087	GTCGCTCCCTTCTTGTCTGA	GCTATTTGACATCCGCAGCG	59.396	60.042	298	AG	5	0
Ale088	TATGCTCTTTCGCAGGCTGA	GAACAATGCAGTCTGCCAGC	59.463	60.109	91	AG	5	0
Ale089	GGCTTGTTGAGTCATGCAGC	ATGCTCTGGATTCGTCAGCA	60.109	59.461	242	AGC	5	0
Ale090	TCAGCATGAGCCAGCAGTAC	ATCACATCCCTCAGCCTGTG	60.108	59.455	97	AC	5	0
Ale091	TATCCTGGTGGCCCTCCAAT	GGGTGAAGGATGAAGCAGGT	60.328	59.67	90	AG	5	0
Ale092	CCCTGTAGGCCTGACACATG	CTCCAAACACAGCACAGAGC	60.108	59.41	197	AC	5	0
Ale093	CCTGCTGTGCTGCCTGTATA	CTGTTTAGCAAGCCTGGCTG	59.821	59.475	94	ATCC	5	0
Ale094	ACACTGCCTTTCTTTGTGCG	AATCGGGATGCAAAGCCAGT	59.618	60.324	120	AG	7	1
Ale095	AGGGCTCAGGATACTCCC	CAGACTTGGCAGCAAACAGG	60.399	59.687	112	AC	5	0
Ale096	GGGTAGCACAACCACAAGCT	CATGGCTGCCTCATCTCAGT	60.537	59.819	92	AG	5	0
Ale097	CAAGGAGCTCACTGTAGCCA	GGCAGGGTGTGAGAAGTTGA	59.39	59.891	110	AC	6	0
Ale098	CCTCTTGAGACAGCCACCTG	AGTCCTCACACTGCTTCCAG	60.037	59.316	123	AT	6	0
Ale099	TGGCCATGCTGAGATACCAG	ACAGTCTCCGAGTCTGCTCA	59.529	60.251	121	AC	5	0

Ale100	CTCCAGGCTCACAGGAAACT	CTCTGATGCTGCTGTGGGAA	59.31	60.036	208	AG	5	0
Ale101	AGGTGCTGCTCTCAATTCCC	TGCAGATGGGCTTCACTCTT	60.035	59.305	202	AC	5	0
Ale102	AGCCTGTGCCTTGAACAGAA	ACCCTGAATACCTGCCACCT	59.817	60.55	97	AC	5	0
Ale103	TGGAACGCAATGGAGGACAG	GGCTGTCTGCTCTTTGATGC	60.322	59.549	170	AC	12	1
Ale104	CACCGCCTCATTAGCAAAGC	CGCAACCAACATCTGGGTTT	59.901	59.327	98	AC	6	0
Ale105	TATTTGTTTGGCGCTGGAGC	GGGAAGCATCAGAGCCAGAA	59.474	59.746	118	AG	5	0
Ale106	GGGCTGTGAGATGGCTCTAC	CTGTTCTGCTCAGCCCTTCC	59.894	60.676	139	AGC	6	0
Ale107	CCTGAAACAGCACATGCAGC	TTTCCTGCCTTGGGTGCTTT	60.388	60.398	130	AC	5	0
Ale108	TATTCGTTGTTGGGTCCTCG	GATCAGCCAGGGTGCTACAA	59.467	59.747	155	AC	5	0
Ale109	AAGTGTTCAAGCCATCCGTGT	GTCTCAGGGCACAAGAGACA	59.891	59.318	90	AC	6	0
Ale110	TTTCTCCTGCCACAGCTAGC	CAGAGGATGCTGCAGAGGAT	60.036	59.24	139	AG	5	0
Ale111	AGGAAACTGCAAGCCTTGGAA	TGCTTACAGAGGGCGTGATT	59.814	59.387	93	AGC	5	0
Ale112	AAGGGAATCACATCGGTGCT	CAGATGGGCATGGGAAGGAG	59.379	60.179	115	AG	5	0
Ale113	GCTTCTACCTCCTGCCTGTC	GCCTAGCAGCAAATCTGAGC	59.823	59.338	93	AT	5	0
Ale114	CAAACCTGAACCCAGGCAGTG	TTGCTGTCTCCATCTCGTCG	59.33	59.829	115	AC	22	1
Ale115	GGTGGGTCCTGCTACAGAC	GGACTGACATGCTTCAAGGC	60.037	59.193	284	AC	9	1
Ale116	GCCTGCATTGTCATGTAGCC	GACCTGAGCAGATGAGGCAC	59.617	60.462	110	AG	5	0
Ale117	CTGTTTCCAGCACACGGTGA	CAGCAAGAAGAGCCTGGGAA	60.814	59.963	93	AGC	6	0
Ale118	AGGAAAGGCACCCTCAAAGC	GCAACTCAGGTGGCAACTTC	60.541	59.688	151	AT	5	0
Ale119	CCCTGCTTTCTCCCTGCTTT	GTACCACAGACACCCAGCAC	60.252	60.603	102	AC	6	0
Ale120	CTCTTTCCGCCCTCCTTTGG	ACACAGAAACCTTCCAGCCC	60.678	60.179	238	AC	6	0

Ale121	TAGGGTTCTGATCCAGCACG	GCACAGATCCCAACTCCACA	59.176	59.963	108	AG	5	0
Ale122	CCCTTCGGATACAGGCAGTT	CTCCAATAACCTCGTGCCA	59.458	59.68	103	AC	5	0
Ale123	TGGACCTCCAGCACTCAGAA	CCACCATGACCTTCAGCTCC	60.472	60.394	125	AC	5	0
Ale124	AAACTTGCGTCCCCTGACT	GCACCCAAGTCCTCCTACAA	59.82	59.309	110	AAT	5	0
Ale125	GGAATCCCTGAGGCACAAA	TCGATGCTTATTGGAGCCCA	59.96	59.163	97	AC	5	0
Ale126	CCTCTCAGGTTCCACTGTC	CGCATCTGCAATGTAGGAGC	60.321	59.415	188	AC	7	1
Ale127	CTGCATGTGTATGTGCGTGC	CACATCCTGCAGCAAAGCAC	60.522	60.388	204	AC	5	0
Ale128	CTGCTGTTGCTGCAGTTCTT	ACTCAGCTCCCTCCCATGAA	59.333	60.253	125	AC	6	0
Ale129	CCTGAGGTGAATGCAAAGCC	GCCACAGTTCAGGCTAGTC	59.471	60.392	118	AC	5	0
Ale130	GAATACTGCGCCCAACCAAC	ACCTGGCTGATTTGTCCAGT	59.83	59.226	174	AC	5	0
Ale131	AGGCTAGAGGGTGTGGAAGA	GGGAACTGTTGCTGGAAGCT	59.586	60.538	115	AG	5	0
Ale132	ACACAGACAGCTTGCCAAGT	ACAGCAGCACAGCAATCTTC	60.107	59.12	100	AC	6	0
Ale133	GTGAACCAGGAGTCCAGCAG	CGTGGCTCCGCTCATCTATG	60.321	60.666	192	ACC	5	0
Ale134	GCAGCTTGAAATGGAAGCA	TCAGCTCGACAACACATCCG	59.395	60.389	110	AG	5	0
Ale135	TGTCTCACCAGGCTGATGTG	TGTTCTGCCAGGCACTATGC	59.677	60.678	247	AC	5	0
Ale136	GCAGCAAGGGAGAAAGCTTT	GGCCTTGTGGTGCAATGAAG	59.035	60.038	99	AC	5	0
Ale137	AGATCACAATCTCCTCCGCC	TGAGGAGGTGAATGAGCTGC	59.244	59.749	91	AC	5	0
Ale138	GAGCATGGGAAGGCCTTGAG	TGCACAAGGAAACCTGGACA	60.751	59.743	103	AC	6	0
Ale139	AGCCACACCATTCACCTTG	TCAGCTGCCAGTACAAACCA	60.54	59.528	119	AC	5	0
Ale140	GGACAGGACAAGGCAGGTC	CCCTGCTCAGAAGGACAGTG	60.003	60.037	96	AC	5	0
Ale141	CCCACAGAGACCTGCACAG	GCCTCTCAGAACCACAGGTC	60.004	60.037	95	AG	5	0

Ale142	AGCCAGGACAATTCTCCCTTG	TGCACAGGCTACTAGGCAAC	59.995	60.036	152	AG	5	0
Ale143	CCTGTGAGCACTCAGGTGG	TTCAGTCATCCCAACAGCCC	60.004	59.962	93	AG	7	1
Ale144	CCGCATGATCAGTCAGCCTT	AGTCATTGTCCCAGTGCACG	60.463	60.604	179	AG	5	0
Ale145	CCTCCATCATGGTGTTTGGC	CACCTCAGGAAACGCCTCAA	59.18	60.25	114	AT	6	0
Ale146	ATGGAGAGCTTAGCTGCAGG	GCAATGGGCAGCAAGTTACA	59.531	59.397	98	AG	5	0
Ale147	GGTGCTTGCCAAGACCAGAA	AACCCTACATCTGCTGCAGG	60.823	59.746	163	AT	5	0
Ale148	GGTGAGGTGGCTGAACTTCT	GGTGTCCTACGTCAAAGGT	59.602	59.317	90	AC	10	1
Ale149	TCCACACTGGAGAAGGCACA	CACCCAAACTTGCTGACACG	61.054	59.971	100	AC	5	0
Ale150	TGCTGAAGAGGTCGTTGGAG	CCCTCTTGGAGTTGACACCA	59.682	59.235	116	AG	6	0
Ale151	GGGAATAACAGCCCTGCACA	TGGGTGACATCGAGGTTTGT	60.323	59.238	98	AC	5	0
Ale152	CACGCTGTGACCCTCCTAAG	TCTCACCAGCAGGGATGAGG	60.109	60.98	92	AC	5	0
Ale153	GTATGAGCTGGCCCTCATCT	TCAATGGCACGGGATTGACA	58.945	59.963	109	AGG	5	0
Ale154	TAGAGCGCTTGCACAATCAC	TAGCCCAAGTGTGCATCCAG	58.921	60.035	94	AC	6	0
Ale155	TCTTCCGCTCAACTGACGAC	TCTGCAAGTCCAGTCTTAGGA	60.039	59.923	189	AG	6	0
Ale156	GCTTCCCATCACTGTGGGAA	AGTCGGTACCATGGTGGGA	59.962	59.92	122	ACC	6	0
Ale157	AAAGCAGTGCAGCTGTTTGC	CCTGCAGTGTCTTTGAAGGC	60.529	59.405	116	AC	5	0
Ale158	GCTACAAGTCCCCTGCTCC	GTGAGGCTGGATCACTGCTG	60.392	60.744	100	AC	5	0
Ale159	GGCACCCTGCATGTTTACA	TGTTCTGAGACCCAGGAGCT	59.042	60.179	109	AC	7	1
Ale160	GAACCTCTGCCAGTAGCTCA	GTGAACTGAAATGCCAGGGC	59.102	59.755	167	AC	5	0
Ale161	TCTGTGGGCATGATAGGGTG	GTACGTGACTGGAGGCACTT	59.161	59.683	125	AC	5	0
Ale162	TAGCTTGGTTGAGGTCTGGG	GGACAGCATCTGACCAGGAG	59.016	59.822	92	AAG	5	0

Ale163	TCTAAACCCAGCCAGAACGT	TTTGCTTTCCTTCCCTCCC	58.947	59.886	95	AAAC	5	0
Ale164	GAGTGACGAGTGACGACCTC	CTGCAGCACAAGCAAAGGG	59.834	60.007	97	AG	5	0
Ale165	CTCAGTAGCTGCACCAGTCC	GGCTTAAGTTCACAGTGCTGC	60.108	60.069	108	ATCC	5	0
Ale166	TCAAGCTGCGTATCCCATCC	TCAGTCCCTGTCTTGTGAAGC	59.893	59.929	139	AG	5	0
Ale167	TGCATGTGGGAAGCTGGAAT	TGCAGGAATCCCAGTATCCC	59.96	58.86	95	AC	5	0
Ale168	TTGCTGTGCCACTCCTTCAA	TGCATGTTAGCACCCCTTCACT	60.107	59.926	118	AC	5	0
Ale169	TTGCCACTACTTTGTGCGTG	TTGGAAGACTGCTGTGCTGG	59.34	60.536	96	AC	6	0
Ale170	ATCTGAAGGTCTCTGGCCCA	TGGAGCCTTTCAGGACTCAG	60.253	59.02	92	AG	6	0
Ale171	TACTCCGAGTGTTCCTCCAGC	AGAAGCTCATGGGAATGCCA	59.393	59.373	140	AG	5	0
Ale172	ATTGGTGTGGCCACAGTCA	CCAGGTCAGTTTGTACAGGA	60.106	59.859	139	AC	5	0
Ale173	GGCAAGGGAGAAGAACCACA	TGTTGCCTTAGTGTCTGTGCT	59.89	59.859	239	AC	5	0
Ale174	CTTTGCACTTCAACGCCTCC	CCAGACCTATTTCCCATGCCA	60.04	59.785	126	AC	5	0
Ale175	CTCTGTGACCCTCAGCAGC	GGGAAATACAGAGCCCGACC	60.079	60.179	293	AC	5	0
Ale176	AACAGCTTCCTCAGTGTGGG	TCCTTCCTTCCCTCTTCCACT	59.891	59.848	144	AAGGC	9	1
Ale177	TCACCAAAGCAAGCAGTAGC	CACAGTCTCATGCTGGACGT	58.762	60.038	124	AT	6	0
Ale178	GCTTCAGCCCTCTGTGTTCT	GCCTCAGTCACAGAACAAAGC	59.964	59.735	121	AC	5	0
Ale179	CCACATGTGTCTGAGGACCA	TGCATTACTGGGCAGTGCTA	59.313	59.382	107	AC	5	0
Ale180	GCCTGTCTCCTAGCCTTTGA	AAGGGCAGAATTGGGTGCGAA	59.095	59.596	94	AG	6	0
Ale181	ACACCAGCTACCAAGGAGGA	TGTAGTGCCTGGGAAAGTGT	60.179	58.863	122	AGC	5	0
Ale182	TTCTTGGCACCTGAAGAGCC	CCATCAGTGCCAGGTGAG	60.251	60.078	101	AC	5	0
Ale183	GCAGTGAACACTTTGCTGCT	AAGCAAATCACTGCCTGTGG	59.617	59.036	123	AC	5	0

Ale184	GGGCAACGGGTTTAAACTGA	CCTTGAACACTGCCAGGGAT	58.679	59.961	137	AC	5	0
Ale185	CACCTCCTGATTCAGCTGCA	AGCAGAGGGTCAGATCTGGC	60.036	61.337	311	AC	5	0
Ale186	GCATCCAAAGAAACACGCTGA	GGTACAGACACATCCAGGGC	59.733	60.108	96	AC	5	0
Ale187	GTAGAGCTGAGCCTGCAGAC	TGCAACATGGCAGAGGTAACA	60.179	60.203	112	AG	5	0
Ale188	AATAAACAGCCGTGTTGCC	CGTGACGCTGGAAGAGTTTC	59.399	59.215	115	AGC	5	0
Ale189	AGCACTGTTGGTCTGGTTTCA	AGAGCAGCTCAGCACAATCA	60.065	59.676	191	AG	6	0
Ale190	GGGTTGTTCTCCTGTGCTCA	AGAGATGAGGCTTGTGCATGT	59.891	59.719	136	AC	5	0
Ale191	CTCCTTCCAGGGTTTGATGCT	TGTACAAGGCAGGAACCCAG	59.995	59.6	141	AT	5	0
Ale192	GTAGGCAGCCATAGGGAAGC	CCTCAAACCTGAGCTCTTGC	60.251	58.836	108	AC	5	0
Ale193	ACTCTGTGCAGTGTGGATGT	TGCAGCGGTAAGTCATTGGG	59.239	60.676	93	AG	5	0
Ale194	CCAACAGAAGGGAGGATGTGCG	ACCCAATCTGCATCACCCCTG	60.406	60.034	100	AAAT	5	0
Ale195	TGACCACCTCTGTCTGTTAC	GTTACGGGTGCAGGAGACAA	59.587	59.966	150	AG	5	0
Ale196	CTGTAGACGGGACATGGCAC	AATCCACTCTGTGACCTGGG	60.46	59.013	113	AG	5	0
Ale197	GGCCTTGGCACCAAATGAAA	ACCTTGTGCCTCTTGTTTCCA	59.604	60.064	109	AC	5	0
Ale198	ATACGGTTGTCGCTGCTTCT	CTGGGCTACAGCTGTCTGG	59.753	59.78	241	AC	6	0
Ale199	CCCTCTTGAGTGCACAGATGT	CATCCAGAGGGTCATGGGAC	59.997	59.528	122	AC	5	0
Ale200	ATGCTCTGTGGTGGGAATCC	CCTGCAGGAGGTAAGCCAG	59.743	59.777	103	AC	6	0
Ale201	CCAGTTGGAGGCACTTGGAT	GTAGGTGAGCAAAGAGGTGC	59.961	58.556	106	AC	5	0
Ale202	CTCCTGTCTGAGCCACAACC	TCTGTTTCAGGTGCTGCAGC	60.321	61.168	106	AC	5	0
Ale203	TCAGAGTACTTGGCTTCGCT	AATCCACCTTGAGCAGCGT	58.742	60.251	245	AC	5	0
Ale204	GACCAAATCGTAGCCGCTA	AGGTAGCAGTGATATGTGCC	59.896	59.582	92	AC	5	0

Ale205	GGAGCATCTTCATGGACCTCA	ACTGAAAGGAGCGTGACCAG	59.512	59.966	101	AG	5	0
Ale206	AGCAGCTCTTTGATAGCACGA	GATTGTGGAGGGAAGCAGCA	59.794	60.323	97	AT	5	0
Ale207	AGGTCTGTGCAAAGGGTTACA	GGTACCCTGAAGGCACTGTC	59.507	60.036	116	AC	5	0
Ale208	CCATCGAGGAGGCAGTCAAA	GTGCAAGTGCTGGCAGAAG	59.75	59.715	98	AG	6	0
Ale209	ATGATTGCAGGAGTGGGCTG	TTGTCCATGCTGACAGACACA	60.395	59.859	238	AC	5	0
Ale210	GCCCTGTTCCCTCTGTGTTT	TTGATGACATGGTCCCTGGT	60.179	58.629	107	AC	5	0
Ale211	AGCTGATGCCTTGTTCCTCA	TGTGCATACAGCCACCACA	59.887	59.546	90	AC	5	0
Ale212	TCCATCTGCTCAAAGCTCCA	TCAGAGGCTTTGCTGTGAGC	59.013	60.605	93	AC	5	0
Ale213	AGCCCAGCTGGAGATTTCTC	CGGTCCCTATGGGCACACTA	59.454	61.046	106	AC	5	0
Ale214	GGTCGGGCTCAGCATAGTC	CTGCAGGATCAAGAGGAGCG	59.932	60.53	122	AC	5	0
Ale215	TCAGTTGAGGTCCTAGGTCGA	TACTATCCGGGACAGCACCT	59.647	59.739	94	AAG	5	0
Ale216	TCTTCCTCACAGAGCTGATGC	CACTCAGCGCTGCATTGATT	59.793	59.549	266	AC	5	0
Ale217	GCACCTCTCCATTTGGTAGGG	CAGTGTGCGTCTTCCAAGA	60.409	60.25	90	AC	5	0
Ale218	GGCAGCATGTAACATCTGTGC	GCCTTGCTACTGACTCGCAT	60.202	60.461	93	AC	6	0
Ale219	GGGTATGAACAGGCCTTGAGA	TGCATATGGTCTGGCTGTGG	59.44	60.107	109	AC	5	0
Ale220	AAATCTCCACAGGCTGGTCT	AGTGCTGGCGAGTGTAAGTG	58.634	60.32	101	AG	7	1
Ale221	TCATCAGCACACCCATCTGC	CCGTGTTGGGTCAGAGGAG	60.393	59.707	96	AC	5	0
Ale222	AAACGTTGCAGAGCTGTCTC	AGAACACTGCCATGGTCCAA	58.779	59.522	104	AC	5	0
Ale223	TTCCATGCTTTCAGGGTCGG	GGTGCCGCTTCTTGATGA	60.322	60.377	90	AC	6	0
Ale224	GCTGTTCCCTCCATACTGCT	GCAGCAGTTTCCTTAGTGGG	59.456	58.831	151	AC	5	0
Ale225	AGCATGCATCACACTCAGCA	TGCACAACCTCACCCCTTGGC	60.322	61.391	240	AC	5	0

Ale226	ACCCAAGATGCCATCACCAT	GCACTGCTGCTTCTTGCTG	59.367	60.081	93	AC	5	0
Ale227	GCGAATGGGAAGAGAAGTCG	CTTTGTCCTTGCGCTCATGC	58.718	60.456	190	AC	5	0
Ale228	ACACGGATCGATCTTCACGG	ACACGGTCTAGCGTTTATGC	59.899	58.363	313	AC	5	0
Ale229	CCTGGTGGATGCATTTACAGC	CCTGGGCCACATAAGTCTTCA	59.541	59.718	135	AC	5	0
Ale230	CTGCACAGTGCTCCAGGTAC	TCAGCAACGTATCCCTATGGC	60.672	59.93	135	AG	5	0
Ale231	CTCCTGGCACCTTCTGAAA	AGACACAAGGAGAGGAAACCG	59.598	59.655	140	AG	6	0
Ale232	ATTCAGCATTTCCAGGGCCT	ACTGATTGAAGGGTCACTGCA	59.663	59.579	152	AG	7	1
Ale233	TGGCAACGGTGATGTACTGA	CTGTTCTGCGCCAGGATCTG	59.318	61.088	98	AC	5	0
Ale234	GTGCAGGGAACCTTCACACTTC	ACTGACACCCAGAACACAGC	59.396	60.179	120	AC	5	0
Ale235	AGGTGTGCATGGTCGTATGG	TGGCACACGTCCCTGTGTAA	60.108	61.687	139	AC	5	0
Ale236	TGTGGTGCCCAACGTGTATT	AGGTTGTATGCTCACGTGTCT	60.179	59.381	132	AAAT	5	0
Ale237	GGACAACACGCACGTACAGG	TGCTGCACTCTGCTCTACTG	61.554	59.754	99	AC	5	0
Ale238	AGCTGTGACTTAACTCTGGCA	CCGAGGAGTGTGACCATGAC	59.306	60.109	105	ACC	5	0
Ale239	GCACATAACCAGTGGGATCTCT	CGTGCACTGAAGCAGCAATT	59.235	60.04	119	ATC	6	0
Ale240	AGTCTCCTTATGGTGGCACTG	GCACCTTCCACTAGACCAGG	59.443	59.749	141	AC	5	0
Ale241	GGTGTCTCCTGCTGGGAAG	CCGTAGAGCGTCAGAGTGTT	59.702	59.478	133	AC	5	0
Ale242	TCTCCTCAACACCAACTGCA	AGGGAGAGCTTGAAAGTCCC	59.163	59.012	105	AC	5	0
Ale243	CTCGAATCTCACCCATGGCT	ACTCCTTTAAGCAGCTGGGA	59.533	58.638	120	AC	8	1
Ale244	ACCTGCCTCAGCTACTATGC	CTCTGAAAGCCCTGTTCCCTT	59.244	59.925	94	AC	5	0
Ale245	CGAGTCCCTGTACAGCAGC	GCCAAGCCTCCATGCCTTTA	60.154	60.684	98	AT	5	0
Ale246	CCTTCCGTCCAGGTAGCTAG	GAGAGGAGCATTGCGAGTGG	58.966	60.809	90	AC	5	0

Ale247	AGCTTAATGAGTTGCCGCTC	ACCAGTGCAAATCCACAGGA	58.626	59.522	125	AC	6	0
Ale248	TTTGCCACTGGAGGGACAG	AGAGCTACCTGTGCTGCAAA	59.544	59.602	92	AC	5	0
Ale249	GGTTGAGGCTACACCCTGTC	GCACCAGCACAGATAAACCT	60.036	58.174	104	AC	5	0
Ale250	TGGAGAGGAGATGCAAGAGTG	GTTCCCTGGGAGCAGAGACC	59.17	60.036	105	AG	5	0
Ale251	GCTGGAGGCTGGCAGAATAT	AACCTCAAATGCTGCACAACA	59.89	59.244	108	AC	5	0
Ale252	GTCAGGGACCTACAGCTTGAC	GGCACAAGCACAGAAGTGGA	60.067	60.819	95	AT	5	0
Ale253	TGCTTTCGAGTCTTCCCTGTT	ACAGCTGTGAGACAAGCCTT	59.584	59.528	126	AG	5	0
Ale254	CTGCTTGCCCATAGAACCCT	CTCAGTGCACCGACACCAG	59.743	60.666	100	AC	5	0
Ale255	GCTCTTGCAAAGGAGAGGGA	GCATGATAACGGCCCTCCC	59.674	60.601	107	AC	5	0
Ale256	GCTCTGTGTTGGTGGGTGAA	GGGCTGTGTATTTGCTGGAAG	60.465	59.526	100	AGC	5	0
Ale257	AGTGGACTAGATACTCGCCA	TGCGATGTAGGATGACCCAG	59.785	59.247	95	AC	5	0
Ale258	CAGCTTCTCTTGCAACTTGT	TTGATCACAGGCTGGTGCAT	59.048	59.961	107	AC	5	0
Ale259	GGAAATCCCAGTCGTCCAGG	GGCCTCTTTAGCCCGCATC	60.108	60.894	111	AGG	5	0
Ale260	TGCAGCTCTTCATGGGTCATT	AGCATGCGGACTAGAAACACA	59.995	59.998	124	AG	5	0
Ale261	GTTGGCCAGGGAGCTAGAAG	TGTGGTTAGACATTGGCACC	60.107	58.096	135	ATC	5	0
Ale262	TGAGTTTGTGGTGGTTCCC	GCAGTCTGCACCCAAATGAC	58.23	59.758	115	AC	5	0
Ale263	GCTGAACGCCATGAGAAGCA	GCAAACACTGCCTCGGTATC	61.301	59.273	257	AGC	6	0
Ale264	TCACCTCTGGCTCTGAGTG	GTGCTCACTGGCAATATGCTG	59.028	59.935	157	AAT	5	0
Ale265	CCACTGAACAGCACCATGC	TGGGAATGCACCTGGACTTT	59.417	59.518	90	AC	5	0
Ale266	GCCCGTGAGAGCCAAACT	CCTCCGTGTGCAACTCTTCT	59.967	59.966	105	AC	5	0
Ale267	GGGCAGCTAAGAGTTCCTCC	TCCTGACTCACTGCTTAGGTG	59.821	59.104	109	AC	5	0

Ale268	ACAGGACTGGATTCTGGGTTTC	GCTTCTACCGACTGTGGCTT	59.96	60.037	92	AG	6	0
Ale269	GGCAGCAGTTCGGTACACT	ACAGCCAATAGTTCTGCCACA	60.005	59.926	128	AC	6	0
Ale270	CTGATCCCATAAACCACCTGC	AGTGTGCAAGGCTCCGTAA	58.027	59.892	290	AAT	6	0
Ale271	GCTGAGGGTGCTCTGGTG	GGGTGTGCGATTGTTGTCAC	60.047	60.04	240	AG	5	0
Ale272	GACCAGACACATCCACACCT	ACTGTTACTCAAGCGTGGA	59.313	59.589	92	AC	5	0
Ale273	GGCGCTGATGACCTCGAGTA	CTCTGAGCCGTCCATCTCTG	61.726	59.615	90	AGG	6	0
Ale274	GCTTAGCTTGAGACCATGTGG	AAAGTCATCCCTGCTGCCAA	58.987	59.887	127	AG	5	0
Ale275	CTGCTACATGCGCATGCTTT	AGCAGAGTCATAAAGGACGCAA	59.9	60.028	113	AG	5	0
Ale276	CTCATAGGATGGCTGCCTCG	GCATCCTTTCCTTCCACTCC	60.038	58.905	103	AG	5	0
Ale277	CCAGCCTGACCAAGCAGTG	CACGTCATGCCAGGATGTA	60.97	59.822	105	AC	20	1
Ale278	ACACTTGCTCACTTCGTGCT	TCCCAAACAGGACAGAGACTC	60.179	59.029	96	AC	5	0
Ale279	GCACCAGACCATGAGAACTCT	CTGTGGCCTGGACATTCAAC	59.722	59.116	157	AAG	5	0
Ale280	GCAAACCTGCCTCTTACCATCAC	TGTGGCCTAGGTGCAAAGAG	59.838	59.963	238	AC	5	0
Ale281	GCATAGGAATCCAACATCGGC	TTGCTCTGGGATGCTGCTTC	59.463	60.679	115	AG	24	1
Ale282	GTGGGTGGTTAAGCTCTTCTGA	AGCACTGAAACAGCTCACCA	59.961	59.819	101	AC	5	0
Ale283	GGATTGGGAGGATGCTTAGC	TCTCTGCCTGGAAGTGAAGC	58.097	59.677	140	AC	5	0
Ale284	CCATCTGCACCTCAGTCTGG	GAGGCAGCTCACCACGAG	60.108	60.126	129	AC	5	0
Ale285	TCTGAAGGCTTTGGGTGGAA	GCCACCTTCAGCTGATTACC	59.15	58.616	151	AG	5	0
Ale286	GCAAGTAGAGGGCTGAAAGGG	AACCCTTACCTGGCAAACCA	60.68	59.441	98	AG	5	0
Ale287	CTTACAGTTGCTGCAGACTTGG	CACCAGGCTTTGTACGTTG	59.775	59.971	91	AC	5	0
Ale288	GTCCTGTCCTCTCCTTTCCT	GAACTGGGTCCGGTAGGGTA	58.054	60.325	93	AGAGG	7	1

Ale289	CTGGAGATACGCAGCCCTC	CAGCACTGGGAAGGAGAGGA	59.634	60.907	91	AC	5	0
Ale290	TTTCGCTGCTATCATGGGCA	CACTAGAGGAGCAGTGACT	60.107	58.833	109	AGC	5	0
Ale291	AGCGTGTACAACATCCCAGC	CATCAGTGTGCTGCTGCAAACA	60.674	59.395	110	AC	5	0
Ale292	AGTTCATCCAGTTCTGACACCC	CAGCATTCAAGGCCAGGTTG	59.961	59.754	98	AC	7	1
Ale293	GCGGAGAGCGTACTATAGGC	CCCCTGGTATGACAGATCCA	59.831	58.881	91	AC	6	0
Ale294	GCTGTAGGCTGCTGATTGAT	AAGCTGGGCAGAACATGGAG	58.035	60.323	149	AC	5	0
Ale295	AGCCTCACTGACTTGCATCG	CCAAAGTTCTGGCATCTGGT	60.391	58.085	128	AGC	5	0
Ale296	TGTTAGTACAGCTGCTCAGAGC	CAACTCTGCCCACTGCATG	60.094	59.76	98	AC	5	0
Ale297	TCTGTGTCCATCCAAACGCA	GACAAGCACTTTGGTATGGGTG	59.892	59.771	273	AC	5	0
Ale298	AGGACGGCATTCTACTCTCC	TGTGTCTTATGGTACGCTCACC	59.749	60.094	97	AG	5	0
Ale299	AGCCCTTTCCTAGGCAATGT	ACAGGGACAGATTTTCAGTGGG	58.997	59.648	135	AAAG	5	0
Ale300	ACCTCCACCTGCAATTGTA	CCTTGCTTCACCCAACAGTG	59.295	59.33	120	AG	5	0
Ale301	CCTCAAGGCCGCTGTGTTA	ATCCTGAACAGCTGCTGCC	60.004	60.378	99	AGC	5	0
Ale302	TGCGTGCCAGAGATAAGTCA	TGTGGCAACTGTCTCCTTCT	59.105	59.51	90	AC	5	0
Ale303	CCTCCTGCACCCTTACTCCT	AGGTTGCTTCTGTCTTCAGT	60.619	59.229	136	AG	5	0
Ale304	GTCTTGACCCTGGCAGGC	GTGACACGCAAACATGACCC	60.36	60.04	96	AC	5	0
Ale305	GCACTGGATTTGATAGCTTGCC	TCCATGGGCTGTTGCTTTCA	60.224	60.179	115	AC	5	0
Ale306	TGCATTCACATCCACACGTG	TGAGGAGTGCAGTGCCAAA	59.126	59.469	100	AC	9	1
Ale307	GGTCCTTTCCAACCCTAACTGT	GTTTGCAGGCTCACAAGTCC	59.892	59.688	142	AT	6	0
Ale308	AAAGCAAGCAGACAAGGGAAA	CTTGCAAGGTCAGGCCCTAA	58.612	59.962	95	AC	5	0
Ale309	TGAGGTTCCAAGAGACTCTCCT	TTCAGATCCTTGGCCCAGTG	59.889	59.671	127	AC	9	1

Ale310	GTCAAGGACAGGGATCGAGA	AGGGAGCAACATCTGAACGA	58.525	59.023	91	AG	5	0
Ale311	GAGGCTGCTCTGTGACCAAT	CAGTCTGTTGATGTTAGGCAGC	60.036	59.582	141	AGGC	6	0
Ale312	TGTGCTGCTGACAGGGTAAC	AGCAGCTCCAGACTGGGT	60.25	60.204	92	AG	5	0
Ale313	GCAGGAGCACATGAGATTTCA	ATCATCCTCATGGCCCTCCT	58.637	60.104	107	AG	6	0
Ale314	TCTTTGCATTCAACAGCAGCC	ACAGACAGCAGAGGTTTGACA	60	59.514	112	AGC	5	0
Ale315	CTGTTTCACTCAGGGAACGGA	ACTTGAGCCATTGGAGAACCA	59.93	59.575	101	AC	5	0
Ale316	AGCTTAGCTGGAGTCACATCA	ATGTTTGCAAGGCTTGTCCG	58.817	59.685	94	AG	6	0
Ale317	TGGCAGTTCCTCACAATGGC	ATTGTCCCTGAGTTCTGTCCC	60.895	59.369	140	AT	5	0
Ale318	TCCTAGGACAACATCAGGCAC	TGTGCTCTTCACCCTCTCAG	59.445	59.028	102	AC	5	0
Ale319	ATGGAAACCAAGCACAGAGC	TGGCTAGATCCAAACTGCCT	58.749	58.711	96	AAAG	6	0
Ale320	GCAAAGCAAACACTACAGGAGGG	CCCTCTGCTCCCTTCTCCT	59.458	59.997	94	AG	5	0
Ale321	CCCAGCAGGAATTGAACTTTCC	ACGCAGTGAAAGAGGCATCT	59.768	59.677	134	AT	7	1
Ale322	TGTGGGTTTGGGTTCTGATTCA	GAGCATGCACCTCAGCTGAT	60.092	60.464	166	AC	5	0
Ale323	GGCTATCAGGAGCAGTGCAA	TGTGGTCTGGCCTGGGAA	60.108	60.45	92	AC	5	0
Ale324	GATGCGATGGGATGCAGTGA	CCTTGCATCGCATTCTGATCTG	60.534	60.032	140	AGATG	8	1
Ale325	ACTGTACAGGTCCCTTGGTGC	TCAGAAACACCTGACAAGCTCA	59.603	59.83	252	AG	5	0
Ale326	CTCCACACTCTACAGAGCCC	GTGGTATGCAGCCCTGGTG	59.179	60.75	90	AG	5	0
Ale327	GTGATCTGGTGTGAGGACCA	GACAGTCTGCTTCACTCCAGA	59.023	59.384	155	AC	10	1
Ale328	GGCACCTGATGGTAGATAAGGG	AGGGAGAGGGTAGGAGGGAA	59.961	60.558	120	AC	5	0
Ale329	TGTTGGAACGCTTAACTGGAG	GGGCCCTTGTCAGACAGGAAA	58.51	59.89	95	AC	5	0
Ale330	CCTGGGCAAGGCATCTGGTA	GCACTCAGCTGATGCCTTGA	61.925	60.676	102	AC	5	0

Ale331	GATTACTGGTTGCTGCTGTCA	TCCTTGTGCAAGACCCACAG	58.573	60.179	144	AC	6	0
Ale332	CACCATGCTCCGATCCACC	CCGAGAGCAAGCCTGATGTG	60.525	61.088	94	AC	6	0
Ale333	GGGACTGGAATAAACACCGGT	CAGATCTCCATAACCGTCCTG	59.996	59.384	145	AG	5	0
Ale334	GCCTGGATTCCCATTCAAAGC	ACTGGAAGGCTGCTATGAGAC	59.862	59.516	189	AC	5	0
Ale335	GGTGAGGTGACAGCACTGTT	TGTGCCTAAGAGCTCATCTCA	60.179	58.539	157	AAT	5	0
Ale336	TGCCTGACAACACTCATTAGCT	CCACAAGCACGACCTCACAT	59.961	60.604	91	AC	5	0
Ale337	CCTGTGTATGCCTTCAGTGGT	TGATTCTCCACTTGGGCTCT	59.996	58.339	91	AC	5	0
Ale338	AGCCCAACTTATGCATGCAA	CCCAGTATCGTCAGAGCTGT	58.441	58.893	100	AC	6	0
Ale339	ATTCCAAGCGGCCATCACA	GAAGAACGCGGAGGTTGCT	60.001	60.668	140	AGG	5	0
Ale340	CTGCCAAAGAAGCCACCATT	TTGAATGGCAGGTGCTTTGA	59.029	58.298	95	AAAT	5	0
Ale341	GCATCCATCTGTAGGACGCT	GCATTATCATCAGGCAGTTGGC	59.609	60.288	105	AG	6	0
Ale342	ACCTACTCCAGTGTCTGTCAC	GATACACTCACCTGGGCAGC	58.76	60.463	101	AC	5	0
Ale343	ACCACAGAAGGGCTTCAGC	CAGGTTTGCAGGATGGGAAA	59.926	58.369	91	AG	5	0
Ale344	TGAAGCTGCTCATCCATGATGT	ACTATCAAGGCAGCTGCAGT	60.093	59.383	91	AC	5	0
Ale345	CTGTTAGCCTTTGCCTGTGG	CCCTTTCAGCACCAGCATT	59.116	58.162	102	AG	5	0
Ale346	GGCCAAAGATCCATTTGCTGA	TGTCGGTAAAGCCCAGTAGG	59.173	59.1	102	AC	5	0
Ale347	GGGTAGTCCAACGCAGAAAGA	GCAATGGGATACTGCGACAAA	59.998	59.257	289	AC	6	0
Ale348	CCGGGTGGTTGGAACATACAT	GTCCACTATGTCTGGCGTAAGA	59.673	59.574	285	AC	5	0
Ale349	CAGCACATGCAGGGATCTGT	CACCTCCTGCAGTGCTGG	60.394	60.36	94	AGG	5	0
Ale350	GCCTAAGACAGCATCTGACCA	TCAGTTGAGGTCTGACATCGG	59.792	59.454	90	AAG	6	0
Ale351	GCTGCTTCTCTGCTGTGACT	CACCGCACTGCTCTGGAG	60.321	60.434	124	AAGG	5	0

Ale352	CCCTGGCAGCAAAGAAATGC	ACCTTCGCTAACCTTTCTGTGA	60.391	59.632	103	AC	5	0
Ale353	CCCGCTCCTTGTAATCGGAG	CCGGGATGTGCGAGGAAC	60.249	60.512	103	AC	5	0
Ale354	TGTTGCTTAGGCCGTAAATGAC	GTGTTCAAGGAGCATCGGGA	59.255	60.036	90	AT	5	0
Ale355	TGACCACGTGGAGGGTTTG	TCCTTGCTATGCAGAAACCCT	59.853	59.365	97	AC	6	0
Ale356	TCCTTACCCTCCAAGGAAAGG	CATGTCTGGTCAGCAAAGGC	58.725	59.475	127	AG	5	0
Ale357	CCAACCACTGGCCATAGAGA	GCTCTAGATAGCCCAGTGAGG	59.088	59.108	127	AC	9	1
Ale358	CTGTACTCGCTGGCATGGAG	AGAGGCCACAAAGGGAATACT	60.529	58.721	91	AG	6	0
Ale359	CTGCAGCCGGGTGTTGTTT	CACCCTCCAGACAGACACTG	61.489	59.68	184	AC	7	1
Ale360	CAAGCAGAAGGTTAGCTTGGG	GGTCTGGCACGTGATGTCA	59.185	60.005	392	AAAC	5	0
Ale361	ACAGGGACTTGAATGTAGCAGT	ATGATGGTGTGATCCCAGCC	59.36	59.816	160	AC	7	1
Ale362	TGTCCAGAGCAACAAGAGCAT	ATGTTGGCTCATGACCATGG	59.927	58.228	135	AG	7	1
Ale363	TGGGAGGGTTTATTGGTTGGG	TGTGGGATTGGCCACTATAGG	59.922	59.226	97	AC	12	1
Ale364	TGTAAATGCCAGCGGTAACC	CCTTCACTGAATGCCAGATGC	58.545	59.596	153	AC	5	0
Ale365	AGACTCCAAATTCCACAGCCT	TGGCAGGCCAAATTGGAGT	59.293	59.844	91	AC	5	0
Ale366	TGGAATCTGGTCCGCGTAAG	TGTGGTATCCGTATCCTGTCAC	59.825	59.308	258	AG	5	0
Ale367	CAGAACAGAGGAAGTTGGTGC	AGCATTACATCTTGCTGGGA	59.124	59.995	90	AG	5	0
Ale368	CTTTGCCAGGCTAGCTGACT	ATTAACCTTAGGGCAGATGCG	60.036	58.149	157	ACC	5	0
Ale369	CTTTGTTACCGATGGGCTGC	GAATTCGCCGTGCCAGGA	59.548	60.436	94	AGG	5	0
Ale370	TGTTTACCTGTGGCAAGAAGG	AGGAGCACTGCCTGAATGTG	58.418	60.322	93	AC	5	0
Ale371	CAGGGACCAAGCCGCAAA	GATATGTTCTTTGGCGGCGG	60.597	59.692	97	AG	5	0
Ale372	AGCCTACTGTCTACGAGCATAAC	GTTCTGACACGCCCTTCTGA	59.12	59.966	96	AG	5	0

Ale373	AACAGGCAGGTGTTTCTCGT	GGGCATTTAGCTCCTTTCCTT	59.82	58.26	99	AAACT	5	0
Ale374	CCTGCACATCCCTCACCC	AAAGAGGTGCAGAGGAGTGG	59.727	59.31	94	AC	5	0
Ale375	AGCCTGTGAAGTCTGGTGAC	AGGTCATGTTGCTCTGAGATGT	59.605	59.429	192	AG	5	0
Ale376	TGGCAACTAAAGCATCAGGAGA	GCAGAGGCCTATGCAATTGG	59.694	59.328	268	ATC	5	0
Ale377	AACTGACTGCCAGCTGAAGC	TGGGTAAACACAGGAAGCAAGT	60.888	60.092	102	AGG	5	0
Ale378	GATGTGGATGAGTGCGCAGA	CAAACCACAGGGCTGGCA	60.461	60.521	153	AC	5	0
Ale379	AGAGGAGACAAGCCGCTCTA	ATCAAACCACAAGACCAGCTT	60.034	58.051	131	AAC	5	0
Ale380	ACTACTCCCAGCGTACATTCT	CCGTTGACCAGCACAGAAGA	58.264	60.25	100	AT	5	0
Ale381	ATAATTGCCACTTGCCAATGC	TGCCCCGTATAGGTTTGCCAG	58.091	60.107	195	AC	5	0
Ale382	TCAGCTGTGCTTGGTATCAGT	GCAGCATTAGAGTGTCAACGG	59.375	59.605	111	AC	9	1
Ale383	CTCAACCCATGGGAGTTGCA	GTTGTTGTGGTTTAAGCCCAGC	60.251	60.802	140	AG	6	0
Ale384	AGAGCATGTTTGGGAACTGGA	TGCAATCACATTTTCAGCCCA	59.575	58.37	105	AG	6	0
Ale385	CAGAGTGACGCTTATTTCCA	GTGCTGAAGGCCGTTTGATG	58.027	60.11	96	AT	5	0
Ale386	AACCTCCAGCATTCCTCAGC	CAGTGTTATGGCTCTGTGACTCT	60.035	60.056	95	AT	5	0
Ale387	CTGTTGGATGTAGCAGGCCA	TGCACGCTTGGATGGCTG	60.035	61.058	91	AG	5	0
Ale388	TGGTGAGATGAGATCAACCCA	AGCAGAAGGAGAATCAGGGC	58.45	59.454	106	AC	5	0
Ale389	TGCACTGAGCTGAGTGACAA	TCCTCACAGAGTAACCCTGTTG	59.535	59.367	98	AC	5	0
Ale390	ACCTCATTCATGTGCACAGTT	TCCAGAAGGGAGGCATGAGT	58.132	60.253	180	AC	5	0
Ale391	AAACCTGGGACCTTTGCCAC	TCAGAGGAAACATGCCCTTCTT	60.759	59.623	90	AGG	5	0
Ale392	TCAGAGCATTGTGACCAGCA	ACAGCAGCTGTTCAATCTTAGC	59.602	59.254	93	AC	5	0
Ale393	GTGCGGCTTTACCTGAACAAG	ACCCACACTCACAGTAACACT	60.069	58.884	95	AC	6	0

Ale394	GAGGAAGAGCCCTGCGTC	GGCCAGACTCAACATCACTCA	59.814	59.998	92	AG	5	0
Ale395	CAGGGAAAGCAGGGAAGAGG	CTGTGCTGTGGTGGCCA	60.035	59.842	115	AC	5	0
Ale396	AGCACTTGTGTAGGTTGGAGA	AGTCACAGGTTGTCCCAGAC	59.562	59.241	224	AC	5	0
Ale397	CAGCCAGTAGGGAGTGCAAT	GTGCTAGACCCTTGCTCTATCT	59.746	59.038	157	AG	5	0
Ale398	ACCAGGTATCCAGTGACAACA	CGAGGGTTTGAGCTTGCTGT	58.665	60.886	183	AGC	13	1
Ale399	GTTCTGGTGAGGCGATGTGA	AAGCTATCTCACTCCTGTTGGAC	60.037	59.803	90	AGG	5	0
Ale400	ACCTGTAACCTGACTTGTGGC	CCATTCCATCCCATTGCATTCC	60.203	59.962	114	AATGG	6	0
Ale401	CCAAGTTCTCCTGCTTATCCC	CTCTGTGGACTTACGGTGCA	58.076	59.683	98	AT	8	1
Ale402	AACCACCCACTCCCTCAGTA	GAAAGGGCCTGGCAGCAG	59.807	61.067	96	AC	5	0
Ale403	TTTCCTTCTTCCCTGGGCC	GGCCAGACAGTGTAATTTGTGA	59.886	58.854	95	AC	5	0
Ale404	AGGCCATGGGAATAACAAGGT	ACACGAGTGTCTCCATCAGT	59.354	58.378	92	AC	5	0
Ale405	GTCCTGGGTTGCTGCTCAT	TGAAATCTCCATCTGGGTGGT	60.002	58.722	93	AC	5	0
Ale406	TCATCTGTGGTGGAGCCCAT	TCAAGTCTGTCTCCTGAACCAC	60.916	59.634	276	AC	5	0
Ale407	GCAATGTGCACGTAAAGCCT	GGTTAGGGTTAGGGTTACGGAT	59.759	58.959	126	AACTCT	5	0
Ale408	TGCCCTATTCATGAGAGTGACA	CACTTGGTGTGGTGTAGCCA	58.893	60.179	91	AC	5	0
Ale409	GGTTTCAGTCCTCCCTTGACT	TCCCTTCTTCTCCCATTCCC	59.3	58.404	110	AG	10	1
Ale410	TCAACCATGCACTGCCATCC	AAACTTTGCTGCGACGCC	60.967	59.669	99	AC	5	0
Ale411	ACTAGTTGTGAAAGCAGTGTGC	CCTGGTACTCTGGCAGCCTA	59.386	60.688	117	AC	6	0
Ale412	AGCCCTATTTCACTGTTGTGGT	TCAGCTCTGGACAGTCTCACT	59.892	60.203	314	ACC	6	0
Ale413	AATGGCGCATTCAACTCCTG	ACCAGTGGTGTCTCAGAGTT	59.187	59.494	90	AT	5	0
Ale414	GCAGAGCTAGATGTGGTGGG	ACAGCTCAGTTGTATTCTCCCT	60.179	58.823	155	AC	5	0

Ale415	TGCTAACAGAGTGCTTGACAG	CAGCCTGGTTCTCACCCCTTG	58.241	60.607	153	AGC	5	0
Ale416	TTACTGTGCATCCTGCCC	GCGTTCTTCAGACATTAGAGTGC	60.322	59.939	101	AC	5	0
Ale417	AATGCCATCAACCCAAACACC	GGAGAGCCTGGGAGGTGA	59.653	59.96	140	AGG	5	0
Ale418	CGCTTGCATGTACTCTTACCC	TCAAGGGCAGTTGTGGGAG	59.066	59.544	196	AC	16	1
Ale419	CACATGCACATTAGTCAACCCA	GCCATGCAGATCATCAGCAG	59.181	59.406	103	ATCC	5	0
Ale420	ACTCCAACAGCAGCCCTTAC	GGTCTGGCTGGGCTTTGC	59.963	61.377	115	AC	5	0
Ale421	TCTTAAGGTCCTTTCCAACCCT	CGGAGCAGAGTGAGCGTTAT	58.667	59.899	177	AC	7	1
Ale422	TGGACGAAGATCTCTGCAACT	TGCTTGTGCAGGATCAAATGG	59.104	59.453	99	AG	5	0
Ale423	GGGCTGCAAGAAGGTGAGAA	ACCATGTGGAAATTATGTGGTGC	60.251	59.805	91	AC	6	0
Ale424	GCTCACAACATGCTGACCAC	GACTAAACCCACAAGTGAAGCA	59.761	58.789	90	AG	6	0
Ale425	CCCTTTCCTTACGTGGCTCC	TCCTTTCTTCCTAGCTTTGCTGT	60.393	59.927	345	AG	5	0
Ale426	GACATTGAGGTGCTGGAGC	GTGGAAGGACTGTGGGAGC	58.524	60.003	93	AG	5	0
Ale427	TTCAGCTTGAGAGGAGCCAC	AGGGCGGAGATGCATGC	59.677	59.843	90	AC	5	0
Ale428	GAAGACGGACGCTCTGTAGC	CCAGAACAATGGACCAACTTGT	60.524	59.042	139	AT	5	0
Ale429	GGCCTGTTATTGCGAGCAG	ACCTGTGAAGAACGTTCTGGA	59.276	59.239	119	AGC	5	0
Ale430	GCCAGATGCCTTACCCATGT	CAACAATCCTCTGCCATAACCTG	60.107	59.621	91	AC	6	0
Ale431	TCAGTATTCTTCTCCAGCAGGG	CGTGAGCACCATTACATCC	59.231	59.271	103	AAC	5	0
Ale432	TGCCACATCTGAGTACTCCCT	TGCATCTGGACACAGTGATTGT	60.273	60.225	226	AC	5	0
Ale433	TGACCTAGTTTGTGTCGAGTGG	TGCCAGCATGTCTCATGATCT	59.965	59.511	93	AG	6	0
Ale434	AAGTCCTACCTTTCCACTGCC	GCATTTCCAGAAGTGCATGAGC	59.998	58.473	190	AC	5	0
Ale435	GCAATGCATGGGCTTCGTG	GCTCTGTATCTCTTCTCTGCC	60.518	59.964	90	ACTAG	6	0

Ale436	TGGATTGGCAGCTAACACAGT	CGTTACAGGGAGAAACAAGCA	59.926	58.51	132	AC	6	0
Ale437	AGCACTTCTGTGAGTGTTTAGGA	TTCCCTCAAGTGCCAGGTTG	59.612	60.179	182	AG	6	0
Ale438	GCATCTGCAAAGAGATAACCACT	TCCTGTCTCCATGCAACACC	58.46	59.963	102	AGG	5	0
Ale439	TGCATGATACTTGCATGGAACA	TACTGCATTCTCCTTGGCCC	58.646	59.744	123	AG	5	0
Ale440	GACGCAATTCCTCACTCACA	CCTCAGTGCAAGCAGAATGAC	58.857	59.532	98	AG	5	0
Ale441	CTGGGCAACTGACTGTGTCA	GGGCTTGTGATAGTAAAGAGGGA	60.179	59.545	104	AC	5	0
Ale442	CCTGTCAGTGTCTACGTCTCA	GAGTATCCTGAACCAGCAGCT	58.848	59.516	99	AC	6	0
Ale443	CCAGCTCGTGGTCCTGTTAT	TGTGAAGACACTCCTAGATGGG	59.463	58.9	113	AT	6	0
Ale444	CTGTGGGTGCACATAGCTGT	GTGCCCACACTGCCCTC	60.322	60.341	90	AC	7	1
Ale445	TGCCTCTGAAGTACCAGATGG	TCTCTTCAGCATGTCTCTGG	59.166	59.17	142	AG	6	0
Ale446	CCCTGGGTTTAAAGCTGATGC	GGCAGAGATCAGCCGGTG	59.522	60.203	92	AGG	7	1
Ale447	TCCAGCACCATGCCAGC	CCCTTGCTGCCTCCTCTTTC	60.009	60.679	190	AC	5	0
Ale448	TGTATTGCCTGTGGGAAGCA	ACACTTGACAAGGTGCATTTCT	59.596	58.709	102	AC	5	0
Ale449	AGCATTACCACGTATACAGCCT	TCCAAAGGCCCTGCAGAATA	59.301	59.001	139	AC	5	0
Ale450	GGGTTACCTGATGCTTCCAGT	AGCTTATCCATCAGCTCCCT	59.718	58.583	95	AG	5	0
Ale451	TCGTGGTTTAAAGCCCAGCC	GTGCTACGGTTAAGACTTGCA	60.303	58.59	90	AG	5	0
Ale452	AGATCGGTACCTGCTTAGTGC	TGGATTTACTGTGACACCCTCC	59.591	59.695	94	AC	5	0
Ale453	TCTGTACCTGGGAGTGCGAT	ACTACATGGCAGTATTTGGGTCT	60.324	59.476	141	AAT	7	1
Ale454	GTGGACTAGATACTCGCCCAT	GTGTAGGATGACCCGGAGATC	58.76	59.386	90	AC	5	0
Ale455	ACACTGACATGCATACCAACC	AGTTCTCCAGGAAACCACAGG	58.564	59.578	90	AC	5	0
Ale456	GAAGGGCAGGATAGGGTTGG	GCCCTGTCCTATCCTATATTTCC	59.816	59.288	107	AGAGG	5	0

Ale457	TGTTCTAGTGTCTGTGCTGAC	GTCTTCTATGCTTGATGGCGG	59.964	59.13	294	AT	8	1
Ale458	AGTCTGGGCTAACTCACCTC	AAGGCAGCCCTCTCATCCC	58.437	61.382	91	AGC	5	0
Ale459	GCTCACGCCTCCGATACAC	ACGCGTGGTGCATCACAT	60.589	60.359	90	AC	5	0
Ale460	GCATGGCTTGTCTCACAGTG	ACCATTGCCAGGATGTTCTTT	60.069	58.107	92	AG	5	0
Ale461	CATGCGATTCCACATGAGCA	ACCAAGGACAGCTTTCACATT	58.981	58.051	112	AC	5	0
Ale462	AGGCACCCAATATCCTCTCAA	CCGTAAATCTGGGCTCAGTCT	58.508	59.517	161	AGC	7	1
Ale463	TGTAGGTCCTTTCCAGCTAACT	GATGGGACGGGACAGGTTAG	58.482	59.536	90	AGGAT	5	0
Ale464	TCAGAATGCACCCACATTTGG	GCAGCAATGATGTCACTCTTCC	59.106	59.903	96	AT	5	0
Ale465	GGGCACATGCACGTGTAA	TACCCTGCCTCTGCTACTGA	58.338	59.665	90	AC	5	0
Ale466	ACCAGCAATCCATAAGAACCTGT	CACTGACAATGCACCACACTG	59.989	60.002	147	AG	5	0
Ale467	CCGCTCTCCTCTCAGCATT	AGGTTAGTCCTTGCCAATTTGT	59.822	58.155	201	AC	5	0
Ale468	TGTGTTCCATGCTCAGATTTGT	GTGAACAGCAGAAGGGCCTT	58.511	60.538	124	AT	5	0
Ale469	TGCAATGCTTTAATTGTCACCC	TTTCTGTCCACAGCTGGCAT	58.076	59.889	130	AC	5	0
Ale470	AACTCAGAGTCTGAATGGATGC	TTCCAGGTGTGCTGACAGTG	58.129	60.179	90	AG	5	0
Ale471	TCACCAGGACCAGGGCC	GTCGTGGATCAAGCAGGAGG	60.604	60.461	90	AG	5	0
Ale472	CGCTTATGTCACTGCTCTGTAC	CTACCACAAGCCGAGGGAC	59.145	59.782	142	AC	5	0
Ale473	ACTCAACAGAGGCTTCATTCTCA	CGGATGCCTCTGGTCATGTA	59.674	59.247	105	AC	5	0
Ale474	GAAGGCCTGGGACACCC	GGAAAGGAGTGCACATCCCT	59.247	59.67	118	AG	5	0
Ale475	GTTCCCGTGTGCACCACAT	TGTAGGGTCCCAGCTGCA	60.897	60.204	126	AGGATG	9	1
Ale476	CCTCCTCGGACAGCGGA	CCACACGATGGCCAAGACAT	60.425	60.678	90	AC	6	0
Ale477	GCCGCACTGTGTTATTTATTGC	AGGTAAACATCCTGAGGCACC	59.148	59.718	93	AC	7	1

Ale478	GGGTCTGTTGGCTCGCTATTA	GGGTCAGCAATTCAGAGACAT	59.862	58.003	114	AG	5	0
Ale479	TGGACAAGAAGGAGAATCAGCAA	CCAAAGCCAATCTTTCCCTGC	59.927	60.067	100	AC	5	0
Ale480	TGTCTGTCTCCTTGCAGAGC	GCACTACACCGCCAGGC	59.68	60.825	93	AGC	5	0
Ale481	CTAGGGAGTAGGTCGCTGGA	TGAACCACAGAAGTGTCTAGGAA	59.817	59.035	95	AG	5	0
Ale482	TGAAGTAGCATTGCATTACGGA	GTACCCTGGGCTGCATGAAA	58.132	60.323	103	AC	5	0
Ale483	TGTGTTCTGTACCTGCATGAAAG	CAGGGCTGGTGTCCACTTAA	59.185	59.6	91	AC	5	0
Ale484	GCACCTATGTCATAGTCTGAGGG	TGAAAGGTCAAGGGCTGTTCA	59.994	59.786	92	AC	5	0
Ale485	TGCTAACTCTAGGAGCTTAGGC	ACACCCTAGACTGCTGTCAG	59.04	58.739	146	AC	5	0
Ale486	TGGCTTCTGCTCTCCATTATTTTC	GCCAGCCCCTAGACAACAT	58.798	60.035	122	AAG	5	0
Ale487	GGTAGGACAGGGTAGGGCA	TCCTATGCTACCCTACCCTATCC	59.997	59.729	91	AGAGG	5	0
Ale488	CTGCCC GCGTGACAGATC	GCAACTCTTTCCACAACACTGCA	60.888	59.595	140	AG	5	0
Ale489	CATAGGAACGGTTTCATCCTTGT	CATTCCTAGGCTGGTGCACA	58.738	60.035	107	AC	5	0
Ale490	TGGAGGACAAGTTGCCTCAT	TCAGGCAGCTGATCTATGATGA	58.933	58.763	139	AG	5	0
Ale491	CAGTCTGGCAAACATTCAGCC	GCCAAAGTTCACATGTAATGGC	60.068	58.747	105	AT	6	0
Ale492	GGCATCCCATACCTCTTAGCA	AGCTTGATT CAGAACCACTCT	59.3	59.358	100	AC	6	0
Ale493	GGGACACATAGGGAACATGGG	TGCCCATGTTAACACGTTTGTA	60.134	58.788	90	AC	5	0
Ale494	CTCAGGTCGGGCTGGGTAT	AGACCACTACTGCTACAACCA	60.762	58.395	104	AAT	5	0
Ale495	ACATGAGTTGTTACGTCCTGGG	CTGAGAATGGTGTGTTTCAGG	60.289	58.918	108	AC	6	0
Ale496	GCACAGAGCCCTCATTTGAG	AGTGAAACCAGGACAACAGAAC	58.907	58.721	96	AC	5	0
Ale497	ACCATACCAAAGGAAATGTCAGG	CTTACAGCATGGCCCTGGAA	58.656	60.034	125	AG	5	0
Ale498	TTGGGTAGCCTGATTGTTTCCA	ATGCGCACTGGCTTTGGA	59.891	60.282	340	AG	5	0

Ale499	AGAGGCCCTTTCATTGTCTTGT	GCCACTTCAAACCTCTGTTCTGC	59.891	60.287	96	AC	5	0
Ale500	GGGACTGATCATGCTTGTCC	GTGCTGCAGCGTAACGGA	58.327	60.737	99	ATCC	10	1
Ale501	GCACAATAGTACTGCTCTGGG	TTTGGTTTGCCAGACCAAGT	58.445	58.141	95	AC	5	0
Ale502	AGCAGTGCTGAGGATGTCAA	ACTGAACGAATGCCACTAAAGAC	59.311	59.254	112	AT	6	0
Ale503	GTGCTGGTTATGCCTGGTGT	GATGACGATACACAGTGAAAGGG	60.609	59.134	90	AC	5	0
Ale504	GCTTCTTAGCCATGCAACCC	ACAAAGCCAACCTTCTACAACAA	59.543	58.979	131	AT	7	1
Ale505	GTTGGGATCAAAGGGAAATGTGA	GGGTCACTGTGCAGGTCTAA	59.168	59.315	192	AC	5	0
Ale506	GTCATCACAATTCCTGCCTCA	ACTGCTTGTCTCATTGGCATG	58.285	59.183	98	AC	6	0
Ale507	TCAATTCACATTAGCGTGCTTGT	CAGGTTCTGCTGACAGGCA	59.498	59.928	96	AC	5	0
Ale508	CAAGGCTGCACAAATGGCTG	TGTTAACATAAGGAGCAGTGGG	60.669	58.05	96	AGC	5	0
Ale509	CCTGGTGCTGACCCAGG	GGGATGTGATGCTACTGCGG	59.252	60.882	390	ATC	6	0
Ale510	AGCAGAAGTTACATGGGAACGT	CACTGAACGGGCTGCACA	59.961	60.591	90	AG	5	0
Ale511	AGCATGAAAGCTAGGCCTGG	AGAGCCTTCTGGAAATGAATCTG	60.107	58.475	99	AG	5	0
Ale512	TCAGCAAACGGTTCTTCAATAGC	TGGTCTTCCAGAGCCCTCT	59.812	59.528	254	AG	5	0
Ale513	GCCTGAAGCCACTGGACTG	ATGGTGCACTGTGCCAGC	60.674	60.988	91	AC	5	0
Ale514	TCCAAGAGTCACAAGAATGCCT	GCACAACCTTGCCCTTCTAAACC	59.627	60.288	175	ATC	7	1
Ale515	GGGATTCAGAGTCTTTCACACG	AGGTCACACCATTGATCCCT	59.003	58.326	105	AC	5	0
Ale516	TGTAATGGTTGGACTCGGAAAG	AGGAGGCAGTCTTTCATTGG	58.329	59.995	140	AG	6	0
Ale517	TTGTAGTAAGTGTGGGTAAAGCC	CATGGAGCTCTGGGCAGAAA	58.356	60.035	140	ATC	16	1
Ale518	CCTCTAAGGAGTGCAAGTTGAAT	TGCAGCCTTTGTCTGACACA	58.416	60.107	95	AG	5	0
Ale519	TGGTGAGACTAAGAAAGAGACGA	TGCGTACCTTACAGGCCCTA	58.604	60.325	342	AC	6	0

Ale520	CCTGTGCCCTGTGTTGTAA	AGTAACAGAACACAGGGTTTGGT	58.314	60.054	90	AG	5	0
Ale521	AACACAAGACCACTACTGCTAC	GGCTGGGTATAGGTGGTGAG	58.009	59.241	90	AAT	5	0
Ale522	GGAGAATGGGACCAGGAAGTG	GCTGAAAGCAGAATCTCACATGA	60.065	59.312	91	AC	5	0
Ale523	CCCACAGGGTGTGCCTG	GGGTTGGAAGCTGATCCCA	59.925	59.309	140	AC	5	0
Ale524	TCATTGCCAGAAGATGCTTACTT	ACCCACTGCTCATATGGCTG	58.407	59.818	152	AG	5	0
Ale525	TCAGAAGGTAGGAAAGGAAAGCA	CAAGTTGGCCAAAGCAACTGT	59.349	60.135	148	AG	5	0
Ale526	CTTTGGTCCTGTCCCGGAG	GCCATGCCAGGATGACAC	59.703	58.482	90	ATCC	5	0
Ale527	AACACAGATTACTGCTGCTACCA	GTCGGGCTTGGTATTAGCTGG	59.992	60.81	90	AAT	5	0
Ale528	TCGCGGGAGGTCACTTTAAA	CCTGATCTAGTACTCCATGCTGA	59.319	58.852	383	AG	5	0
Ale529	TGATAAGCAGAGGAGGAATCCT	AGATGGAGGTGCTGTGAGAC	58.066	59.099	113	AC	16	1
Ale530	CTCTCAGTGTTACCTGCATTGC	TGTGCAGACTCGACTGATACT	59.582	58.563	90	AC	8	1
Ale531	TGCACGGGTACATTCACAT	AATACTCTCAGGTATGTGGGACA	60.251	58.379	97	AG	5	0
Ale532	AGGGAATTCAGTTCTGAAGGAGT	CCAAGAACCTCTGCTCCTCA	59.087	59.02	113	AC	5	0
Ale533	CCCTTTGAATGCAGCCTTCT	TGTAATCCCAGTGGCTTTGATG	58.446	58.645	104	AC	5	0
Ale534	TAGGGCATGTGCATGTTCGAC	GGGACAGAAAGATTACTCAGGTG	60.462	58.491	241	AC	5	0
Ale535	ATGGCATGTGAGAAGTGGTTT	CACATGCTGGCTTGTAGAATGG	58.12	59.901	97	AG	5	0
Ale536	ACAGAGTTTCAGCCAATAGTCCT	ATCTGAGGTAGTGCATGAGGC	59.415	59.584	90	AG	7	1
Ale537	GCAATGGAACCACAGCATGT	CAGGATTCAAAGGACAGAAAGGT	59.395	58.598	156	AG	5	0
Ale538	TGCTATAGCTTGTCTCTGCAGA	TGGACTGCACTCATAACTGACC	58.973	60.028	102	AT	5	0
Ale539	GGGCTGATGGCATCCTCTTT	GTTAGGATATGGATGTGAGCACA	60.106	58.039	110	AC	5	0
Ale540	TCTGTCTTAGATGCTGAGCTTGT	GCCTTTGGTGTGATTGGT	59.487	58.439	99	ACC	6	0

Ale541	ACTGGATTGGTAAGCACAGCT	CCTCTACACAGTTCTGTATGCC	59.648	58.213	121	AC	6	0
Ale542	TCCTTCGCCCTTAACATCAGA	ACTCTGGATGGTTGTTTCTCCT	58.815	59.018	91	AT	5	0
Ale543	TGGCAACATAAAGCTATCCAGC	TTCTGCTGACGTGCTGTGC	59.048	61.247	92	AG	6	0
Ale544	AGACATCCAACATTGCCTTCAT	TGCTCTAGTGATGGACTGCA	58.295	58.439	97	AG	5	0
Ale545	CACCGATCGGCTGCTCC	TTCTACCAGCGCTGCAC	60.257	60.047	103	AATG	5	0
Ale546	TGGGTTATTTATGCTGCTCACCT	ACATGCTGCTCTGGGCTC	60.054	59.729	90	AC	5	0
Ale547	CTATCTCCTTGGGCTGCCTC	ATCTCTATCTGACTTCTTGCAGC	59.604	58.052	91	AC	5	0
Ale548	CCAGGATATTGTGACTGCAATGT	GCATCCTGCAGCTAACCAAT	59.05	58.603	97	AATGG	5	0
Ale549	AACCATGTCCAAATACTTCTGCT	GGGAAAGTGGACGTACACCT	58.333	59.317	91	AG	7	1
Ale550	TGGGTTTATTAATGCACATGGC	CCACAAGCACTGAACAGCTC	58.236	59.41	143	AG	5	0
Ale551	TGACTGGATAGGAAAGGAAAGGA	CCCTACACTACCCTGCCCT	58.565	59.997	98	AGGAT	7	1
Ale552	TCTTCAGCTGGGTCCTGTGA	ATGCAAGGATGTATAGGAAAGGG	60.472	58.009	94	AC	8	1
Ale553	TGAAGGCACAAGAGCAATCC	GGGTACATGTTGGACTTGATGA	58.462	58.05	110	AC	6	0
Ale554	GCCATGCCCAAATACAGCAA	GCTATTAGCTGGGAAGTCTGAAT	59.464	58.033	122	AG	5	0
Ale555	TTCCATACAGAGGCAGCCA	TTCACAGTCTGTTTGGCTACTTT	59.009	58.477	105	AT	6	0
Ale556	CCCAGACCCTCTGTGAAGGA	ATGGATTATTGCAGTATGACCGT	60.545	58.027	143	AGC	7	1
Ale557	ACCCACCTTCTCTTAGAATGGT	GAGTCAGCGCTCCACCAAC	58.468	61.03	99	AC	5	0
Ale558	CCTGCCCTGGAGCAAAGG	CCTGCCATGGCTCCCAG	60.362	59.756	104	AG	6	0
Ale559	CGGAATAGTGCAATGGAGTGTG	AGCAGGTTTACTTCGATGTCTCA	59.647	59.742	113	AC	5	0
Ale560	ACGTTGGTACATTTATGCTGGT	TTCACTGATGGAGCTCTGGA	58.318	58.057	342	AC	6	0
Ale561	ACCTCTTACAGATTTGCTCACT	GTTGGTCCATCAGTCTTCTCCT	59.928	59.431	138	AC	5	0

Ale562	TTTCAGTCTTCTCATGGTAAGCC	GTGCGCTAGACCTGTTGAAG	58.42	58.928	101	AC	5	0
Ale563	CATGGCAGAACAACAAGGCT	GGTTTGTACTCAGTCTTCCTT	59.036	58.295	93	AC	6	0
Ale564	TCAGAACACAAAGTCGCTTCC	TCAATGGACTATGCCTGAATCCA	58.79	59.541	91	AT	5	0
Ale565	CCCCTGACAGATGTCTTGTTT	GTGCTGGGTCAACGGGTT	58.52	60.203	142	AG	5	0
Ale566	ACATTCACCCTATGGAAAGCAA	ACTGTATGTGTATGCGCACG	58.584	58.723	141	AC	5	0
Ale567	GGTGCCATGTACCCGGTG	GCGCCATCACTACGGG	60.438	60.257	184	AGC	6	0
Ale568	CCATCATACTTGCTCCCGA	ATCTCCCTAATGTTTAAGCCTCG	59.241	58.038	136	AC	5	0
Ale569	TTATCAGTGAACCATAGGGACCT	GGTGGTGATACCGAGACAGTC	58.369	59.865	94	AG	5	0
Ale570	TCTTTCCCAGTGAACTTTGTCA	GAGTGTACTGCTCAGGCTAGC	58.405	60.202	99	AG	5	0
Ale571	AGAGTTGGGTCATTTATTCACGG	TGCAGGATTTAGCAAGTTGCC	58.738	59.454	114	AT	5	0
Ale572	TTCTGAAAGCTGCCATGCTG	TCTGTGAGAACAAGAACTCTCC	59.114	58.05	90	AC	5	0
Ale573	CTGTGAGACAAGAAGGACCTTAC	TCCCATTGGAGCCAAGTGC	58.439	60.304	98	AC	5	0
Ale574	GGGATCTACTCTGCTTGCAAG	AATGACTATTTGCTCTTGCGTGC	58.441	60.427	190	AC	5	0
Ale575	CCTTTCCAGTATTTCCACTAGCC	GGTAACTGCTCTGGGAACAC	58.8	58.19	92	AG	5	0
Ale576	TTTCAACCTTCCCTTGATCCAAT	CCCATTAGAAAGTGTGCACC	58.249	59.729	102	AG	5	0
Ale577	ACTGCTCACTAGATCAGGTGC	GCACCATGTGAATACATTATGCC	59.52	58.451	95	AG	5	0
Ale578	CAAGTGGTGAGCAATTGTATCCT	AGGTATAACACAAACCACCGCT	58.988	59.961	91	ACC	7	1
Ale579	AGAGACAGTAAAGTGAAGGTGGT	CGTGATGGAAGAGACCTCTCA	59.032	58.905	155	AC	5	0
Ale580	CATCTTGTGTATGACCTTGCGA	AGAGCTCAGCAGGATTAACCT	58.738	59.157	95	AC	5	0
Ale581	GGAGAGGCTGAGGAAGTTGA	TTCCCATCCTACCAATTGTCTTC	58.729	58.145	90	AC	5	0
Ale582	CAAAGACTGGACACCAAACACA	GTATGCAACCTGCTTCACATTG	59.248	58.495	111	AT	5	0

Ale583	AAAGAAGGCTATCTGCGTGTA	CGTCCATTTCAAGCAAGTGC	58.424	58.31	129	AC	5	0
Ale584	AGCTCCAAGATACTGCCTACTG	ACCTCATGCATCTATATGGCACT	59.302	59.414	138	AC	6	0
Ale585	GGCTCCGCGGTGTATGAG	AAAGCCGTGGCCCAAGC	60.28	61.005	193	AC	5	0
Ale586	GCAAGGCTAGCTTATTCTCGGA	TGTATGTAGACAAGGAGAAGGCA	60.224	58.906	119	AT	5	0
Ale587	TGAGCATCAGGAAATACTTCGTC	TTCCGTCCATGATGTCTTCCA	58.566	59.094	390	AT	5	0
Ale588	AAGCTGTAAACTGTCTCTTCTCC	GCAGCTTAGAAACCTGACGTG	58.111	59.539	93	AC	5	0
Ale589	AGATGCGTGCTGTGCCAT	GGATGCGCTGCCTGCTC	60.046	61.306	143	AC	14	1
Ale590	GGTTATAGAGGTGGATGCTCCT	ACAATGGAGATGGAGGATTTCCC	58.756	60.117	95	ACC	5	0
Ale591	GCTCCAGTCAGCAGAAGATG	AAGTTACAGTACTGCACAATGGT	58.346	58.285	106	AG	6	0
Ale592	ACAAACTTGTTTGAACCTGGGC	TCTCCTTCATGCATCATTCCCT	58.405	59.218	186	AC	5	0
Ale593	GCTGGTGGCTGCAGACA	GGTCCCTGCCGTCCTG	59.927	59.682	107	AGC	5	0
Ale594	GGCAAGATCCATGAGCAAGTC	ATCCTTTGTGAAGACCAGAAACA	59.325	58.208	190	AC	5	0
Ale595	TGTGGTACATTTGTAGTGTGGAG	CATTTATGCAGCTGCCAATGC	58.363	59.135	96	AC	5	0
Ale596	AGTGTCTGTTTGTCTTAGCTG	CTGCCTGCAAGGAGCTCC	58.87	60.438	236	AG	5	0
Ale597	GGAGCTTCCGTGCAGGG	CTGCGCGGTGGAAGGAG	60.094	60.499	90	ATCC	6	0
Ale598	AATGTGGCAATATCCTTGGTCA	TGGTACTGGACTTCAGAGTGTG	58.02	59.372	102	AC	5	0
Ale599	AAATCCCTGTGAACATCCTGC	AATCTGTGGAAGAGATAACGTGC	58.55	58.815	140	AC	5	0
Ale600	GTACAAAGGGACATGTGAGTACA	CCCTTTGCACATACATGTCCC	58.11	59.249	93	AC	5	0
Ale601	TCCTCATGCATCCTTGTGTCT	GCATCTTTCATACAGCTTTCTGC	59.693	58.65	144	AG	5	0
Ale602	ACAGGCTGAAATGACATTTGCC	TCTCTAAGTTGTCTTTGGCCAAA	60.029	58.212	157	AAC	5	0
Ale603	TCTGGTATCCTTTGGAATGGCC	TTGGATGAGAATTTCACTGGGTT	60.092	58.26	91	AC	5	0

Ale604	TTGTGGATGTATAGTGCAGTGTT	ATCAGAGCCAATGGTTTGCA	58.095	58.073	99	AT	5	0
Ale605	GCCGGGTGAGTGGTGATG	TGAATGAGGCTTTGACAGAGTTT	60.437	58.536	100	AGG	5	0
Ale606	AATCCCAACTCAACAGGGTTGT	CTTTCTACCTTCTCAAACACCCT	60.091	58.026	90	AC	11	1
Ale607	TCCCTTCCAAGTGAAATAGCCT	GACCCAGATAGAGTTCTCCTGAA	59.08	58.718	113	ACTAG	5	0
Ale608	CTCTGATTTCTGATGCGAGTTCA	GGATGGGAATGGATTTGAAGCA	58.821	58.967	125	AG	5	0
Ale609	ACCTGATGGTTGTATGTGAGATG	TCGTATGATCCATTCAGCCTGC	58.226	60.549	91	AC	5	0
Ale610	GCGAATAAAGGTAAGCAAACAGC	AAAGGAAATATAAGCGGGAGGC	59.148	58.513	97	AC	5	0
Ale611	ACAGTTTGTGTCTGCCTTAGGA	GCGTCAGATGTGTATAAGAGACA	59.562	58.078	137	AC	5	0
Ale612	ACCCAGTCTACTTCCAAAGAAAG	AAGGAGGGATTGATAGCCACA	58.026	58.508	165	AC	5	0
Ale613	TTGACAGCTAATTGTGGACTTGG	CCCAAGTGTGCGTGTGC	59.18	59.291	90	AC	5	0
Ale614	GGGATGTGCTCCAGTCCC	CCCTCACCATAAACATACCTACC	59.408	58.031	102	AT	5	0
Ale615	TGAGTAAAGGAAATACAAGCGGG	CAAACAGCGCTGGGTGC	58.741	59.694	91	AC	5	0
Ale616	AATGTCAGTGATAGGTTGTCAGC	TTAGGAGCTATGGATTGCCTGA	58.743	58.683	90	AG	5	0
Ale617	GGTGATGTGAGAATAACTGGGAA	ACTTTCTTGGTCTGACCTCAA	58.156	59.222	140	AC	5	0
Ale618	AGCTTGAGTTCACATCAGTTTGG	GCTGATGCTGATGATTGAGATGA	59.433	58.934	117	AC	5	0
Ale619	GTCTCAGTGCTTACTGAAGTCCT	AGAAGTTCAGTTCACAACGAGTT	59.743	58.495	300	AG	5	0
Ale620	TTGTAGTTATGGCGGCGATAATG	TTTGTTTACTATTGGGCTGGGAC	59.256	58.916	240	AG	5	0
Ale621	TGATGGTTCTGAAGAAAGATGGG	TTCTCTGTTGCTTCCAAATCTGC	58.407	59.746	90	AC	17	1
Ale622	GCACAAGTTCTTGCATTGAAGG	CCATGTAAGTTTGCTTAACCACC	58.945	58.195	118	AT	6	0
Ale623	GTTTCCTACTCTAGCAGTTGGGT	ATTTACATACACCACATCTGGCA	59.739	58.146	91	AT	5	0
Ale624	CCCTACCCTACCCAATAGTATCC	GCATAGGATAGGAAAGGATCGGA	58.64	59.23	102	AGAGG	9	1

Ale625	ACGTTGTTCCAATAGATGACCAA	CTAGTATAATCCACGTGGGCAGA	58.352	59.427	90	AC	5	0
Ale626	TGCTGCTTCAAATTAAGGATGA	CGATCCTAACAAGAAGAACCACC	58.665	59.067	90	AC	5	0
Ale627	GCGGGCATGCAGGAGTG	TCCCGCTTGCATGCACG	61.232	61.147	90	AC	6	0
Ale628	AGAGGGAATGTCTCTTCCCATT	TGCTAGATAACATACACAGCCAG	58.531	58.051	122	AC	9	1
Ale629	CCTGTGGGAAGGTATTTAAAGCA	ACCATTAATGCAACTCCCAAAGT	58.659	58.84	139	AC	5	0
Ale630	TGTTCAAACCACAAAGTTCAGTG	GGTGACCAAGTTTGAGTCATCT	58.196	58.255	143	AC	6	0
Ale631	GTTGTAATGCAGTGTGGTTTGAC	CCAGGAGTATTACAGTTCAGAGC	58.958	58.312	91	AG	6	0
Ale632	ATTGTGTATGACATTGCTCTTGC	CAGGGTGAGTCAATAGAAGACG	58.01	58.225	90	AAC	5	0
Ale633	TCCACTTACCAGTATATCACCGT	TGTACTIONCAGCAATTGTGATACC	58.46	58.487	90	AG	6	0

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Appendix 9. Results of amplification with 62 microsatellites primers tested on samples from five individuals from different localities. The first 22 microsatellites primers were polymorphic and selected for further tests.

Loci	Forward primer	Reverse primer	Forward primer Tm	Reverse primer Tm	Motif	Repeats unit	Reverse primer tail	Fluoresc. label	(1) Cercadinho	(2) Toca Velha	(3) Barreiras	(4) Serra Branca	(5) Baixa Chico	n. allele	Primer Alleles size
<i>Ale066</i>	GAAAGCCTGCCA TGCCAAAG	TCTTGCACTTCCC TGAACCC	60.391	59.890	AC	16	CAG	FAM	171-173	177-177	173-173	NA	175-177	4	171-177
<i>Ale327</i>	GTGATCTGGTGT GAGGACCA	GACAGTCTGCTT CACTCCAGA	59.023	59.384	AC	10	CAG	FAM	NA	169-169	169-169	155-155	175-177	4	155-177
<i>Ale517</i>	TTGTAGTAAGTGT GGGTAAAGCC	CATGGAGCTCTG GGCAGAAA	58.356	60.035	ATC	16	M13	HEX	NA	153-156	156-162	NA	156-159	4	153-162
<i>Ale176</i>	AACAGCTTCCTCA GTGTGGG	TCCTTCCTCCCT CTTCCACT	59.891	59.848	AAGGC	9	M13	HEX	NA	156-156	151-156	151-151	161-161	3	151-161
<i>Ale529</i>	TGATAAGCAGAG GAGGAATCCT	AGATGGAGGTGC TGTGAGAC	58.066	59.099	AC	16	M13	HEX	NA	135-135	131-131	NA	135-139	3	131-139
<i>Ale628</i>	AGAGGGAATGTC TCTTCCCAT	TGCTAGATAACAT ACACAGCCAG	58.531	58.051	AC	9	CAG	FAM	NA	134-146	134-146	NA	140-146	3	134-146
<i>Ale103</i>	TGGAACGCAATG GAGGACAG	GGCTGTCTGCTC TTTGATGC	60.322	59.549	AC	12	M13	HEX	NA	189-189	189-189	NA	189-193	2	189-193
<i>Ale281</i>	GCATAGGAATCCA ACATCGGC	TTGCTCTGGGAT GCTGCTTC	59.463	60.679	AG	24	CAG	FAM	112-112	NA	130-130	130-130	112-130	2	112-130
<i>Ale292</i>	AGTTCATCCAGT CTGACACCC	CAGCATTCAAGG CCAGGTTG	59.961	59.754	AC	7	CAG	FAM	105-107	105-107	105-107	105-107	105-107	2	105-107
<i>Ale309</i>	TGAGGTTCCAAGA GACTCTCCT	TTCAGATCCTTG GCCAGTG	59.889	59.671	AC	9	M13	HEX	NA	144-146	144-146	NA	144-144	2	144-146
<i>Ale324</i>	GATGCGATGGGA TGCAGTGA	CCTTGCATCGCA TTCTGATCTG	60.534	60.032	AGATG	8	M13	HEX	NA	140-140	140-140	NA	135-140	2	135-140
<i>Ale361</i>	ACAGGGACTTGA ATGTAGCAGT	ATGATGGTGTGA TCCCAGCC	59.360	59.816	AC	7	CAG	FAM	NA	175-175	175-175	173-173	175-175	2	173-175
<i>Ale363</i>	TGGGAGGGTTTAT TGGTTGGG	TGTGGGATTGGC CACTATAGG	59.922	59.226	AC	12	CAG	FAM	NA	105-129	105-105	NA	129-129	2	105-129
<i>Ale409</i>	GGTTTCAGTCCTC CCTTGACT	TCCCTTCTTCTCC CATTCCC	59.300	58.404	AG	10	CAG	FAM	NA	123-123	123-123	115-123	123-123	2	115-123
<i>Ale418</i>	CGCTTGCATGTAC TCTTACCC	TCAAGGGCAGTT GTGGGAG	59.066	59.544	AC	16	CAG	FAM	NA	191-191	202-202	NA	202-202	2	191-202
<i>Ale421</i>	TCTTAAGTCCCTT TCCAACCCT	CGGAGCAGAGTG AGCGTTAT	58.667	59.899	AC	7	CAG	FAM	NA	152-152	152-152	162-162	152-152	2	152-162
<i>Ale500</i>	GGGACTGATCAT GCTTGTCC	GTGCTGCAGCGT AACGGA	58.327	60.737	ATCC	10	CAG	FAM	80-80	NA	NA	104-104	NA	2	80-104

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<i>Ale504</i>	GCTTCTTAGCCAT GCAACCC	ACAAAGCCAAC TCCTACAACAA	59.543	58.979	AT	7	M13	HEX	NA	147-149	147-147	147-147	147-147	2	147-149
<i>Ale514</i>	TCCAAGAGTCACA AGAATGCCT	GCACAACTTGCC CTTCTAAACC	59.627	60.288	ATC	7	M13	HEX	NA	192-195	192-195	192-192	192-195	2	192-195
<i>Ale589</i>	AGATGCGTGCTG TGCCAT	GGATGCGCTGCC TGCTC	60.046	61.306	AC	14	CAG	FAM	NA	157-157	149-149	NA	149-157	2	149-157
<i>Ale606</i>	AATCCCAACTCAA CAGGGTTGT	CTTTCTACCTTCT CAAACACCCCT	60.091	58.026	AC	11	CAG	FAM	102-102	100-102	100-102	102-102	100-102	2	100-102
<i>Ale624</i>	CCCTACCCTACCC AATAGTATCC	GCATAGGATAGG AAAGGATCGGA	58.640	59.230	AGAGG	9	M13	HEX	83-83	83-83	83-88	88-88	88-88	2	83-88
<i>Ale020</i>	GCCCAAAGCAAA CAAAGCCT	CAGCCTGTTGTC AGCTGAGA	60.179	59.966	AG	8	M13	HEX	NA	404	404	NA	NA	1	404
<i>Ale033</i>	TTCCTTACCTGTT TGCGCCT	AGCATGAACAGG GTTGTGCT	59.890	60.179	AT	9	CAG	FAM	NA	123	123	NA	123	1	123
<i>Ale041</i>	TGGTTTAAATGCC GCCCTCA	TTCTGCACAGGC TTTCCACA	60.251	60.107	AT	7	M13	HEX	NA	176	176	176	176	1	176
<i>Ale045</i>	TCCTCTCCTCTCG TCTCCAC	GAGCTGGTAGGA AAGGCTGG	59.746	60.107	AGAGG	8	CAG	FAM	NA	108	NA	NA	NA	1	108
<i>Ale073</i>	GACTTGCTGCCAT GTAGTGC	CAGGGACGCTCA TCTGAGTC	59.551	59.898	AC	7	CAG	FAM	115	115	115	115	115	1	115
<i>Ale094</i>	ACACTGCCTTTCT TTGTGCG	AATCGGGATGCA AAGCCAGT	59.618	60.324	AG	7	CAG	FAM	NA	133	133	133	133	1	113
<i>Ale115</i>	GGTGGGTCACTG CTACAGAC	GGACTGACATGC TTCAAGGC	60.037	59.193	AC	9	M13	HEX	NA	299	299	299	299	1	299
<i>Ale143</i>	CCTGTGAGCACT CAGGTGG	TTCAGTCATCCCA ACAGCCC	60.004	59.962	AG	7	M13	HEX	106	NA	106	106	106	1	106
<i>Ale148</i>	GGTGAGGTGGCT GAACTTCT	GGTGTCCTACG TCAAAGGT	59.602	59.317	AC	10	CAG	FAM	NA	NA	NA	80	NA	1	80
<i>Ale159</i>	GGCACCCTGCA TGTTTACA	TGTCTGAGACC CAGGAGCT	59.042	60.179	AC	7	M13	HEX	125	125	125	125	125	1	125
<i>Ale232</i>	ATTCAGCATTTC AGGGCCT	ACTGATTGAAGG GTCACTGCA	59.663	59.579	AG	7	CAG	FAM	NA	168	168	NA	168	1	168
<i>Ale243</i>	CTCGAATCTCACC CATGGCT	ACTCCTTTAAGCA GCTGGGA	59.533	58.638	AC	8	CAG	FAM	133	133	133	133	133	1	133
<i>Ale277</i>	CCAGCCTGACCA AGCAGTG	CACGTCATGCCC AGGATGTA	60.970	59.822	AC	20	CAG	FAM	83	83	83	83	83	1	83
<i>Ale288</i>	GTCCTGTCCTCTC CTTTCCT	GAACCTGGGTCGG GTAGGGTA	58.054	60.325	AGAGG	7	M13	HEX	85	NA	NA	NA	NA	1	85
<i>Ale306</i>	TGCATTACATCC ACACGTG	TGAGGAGTGCAG TGCCAAA	59.126	59.469	AC	9	M13	HEX	NA	NA	NA	NA	114	1	114
<i>Ale321</i>	CCCAGCAGGAAT TGAACCTTCC	ACGCAGTGAAAG AGGCATCT	59.768	59.677	AT	7	CAG	FAM	NA	NA	147	NA	147	1	147

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<i>Ale357</i>	CCAACCACTGGC GCTCTAGATAGC CATAGAGA CCAAGTGAGG	59.088	59.108	AC	9	M13	HEX	NA	141	141	NA	141	1	141
<i>Ale359</i>	CTGCAGCCGGGT CACCCTCCAGAC GTTGTTT AGACACTG	61.489	59.680	AC	7	M13	HEX	NA	201	201	201	201	1	201
<i>Ale382</i>	TCAGCTGTGCTTG GCAGCATTAGAG GTATCAGT TGCAACGG	59.375	59.605	AC	9	M13	HEX	125	125	125	125	125	1	125
<i>Ale398</i>	ACCAGGTATCCA CGAGGGTTTGAG GTGACAACA CTGCTGT	58.665	60.886	AGC	13	M13	HEX	NA	203	203	NA	203	1	203
<i>Ale444</i>	CTGTGGGTGCAC GTGCCCACTG ATAGCTGT CCCTC	60.322	60.341	AC	7	CAG	FAM	NA	99	99	99	NA	1	99
<i>Ale446</i>	CCCTGGGTTTAAA GGCAGAGATCAG GCTGATGC CCGGTG	59.522	60.203	AGG	7	M13	HEX	104	104	104	104	104	1	104
<i>Ale453</i>	TCTGTACCTGGGA ACTACATGGCAG GTGCGAT TATTTGGGTCT	60.324	59.476	AAT	7	M13	HEX	NA	156	156	NA	156	1	156
<i>Ale475</i>	GTTCCCGTGTGC TGTAGGTCCCA ACCACAT GCTGCA	60.897	60.204	AGGATG	9	M13	HEX	NA	143	143	NA	143	1	143
<i>Ale477</i>	GCCGCACTGTGT AGGTAACATCC TATTTATTGC TGAGGCACC	59.148	59.718	AC	7	M13	HEX	NA	105	105	NA	105	1	105
<i>Ale530</i>	CTCTCAGTGTTAC TGTGCAGACTCG CTGCATTGC ACTGATACT	59.582	58.563	AC	8	CAG	FAM	NA	102	102	NA	102	1	102
<i>Ale536</i>	ACAGAGTTTCAGC ATCTGAGGTAGT CAATAGTCTT GCATGAGGC	59.415	59.584	AG	7	CAG	FAM	NA	102	102	NA	102	1	102
<i>Ale549</i>	AACCATGTCCAAA GGGAAAGTGGAC TACTTCTGCT GTACACCT	58.333	59.317	AG	7	M13	HEX	105	105	105	105	105	1	105
<i>Ale552</i>	TCTTCAGCTGGGT ATGCAAGGATGT CCTGTGA ATAGGAAAGGG	60.472	58.009	AC	8	M13	HEX	105	105	105	105	105	1	105
<i>Ale556</i>	CCCAGACCCTCT ATGGATTATTGCA GTGAAGGA GTATGACCGT	60.545	58.027	AGC	7	M13	HEX	158	158	158	158	158	1	158
<i>Ale578</i>	CAAGTGGTGAGC AGGTATAACACA AATTGTATCCT AACCAACCGCT	58.988	59.961	ACC	7	CAG	FAM	97	97	97	97	97	1	97
<i>Ale114</i>	CAAACCTGAACCCA TTGCTGTCTCCAT GGCAGTG CTCGTCG	59.330	59.829	AC	22	M13	HEX	NA	NA	NA	NA	NA	NA	NA
<i>Ale126</i>	CCTCTCAGGTTCC CGCATCTGCAAT ACACTGC GTAGGAGC	60.321	59.415	AC	7	M13	HEX	NA	NA	NA	NA	NA	NA	NA
<i>Ale220</i>	AAATCTCCACAGGAGTGTGGCGAG CTGGTCT TGTAACCTG	58.634	60.320	AG	7	CAG	FAM	NA	NA	NA	NA	NA	NA	NA
<i>Ale362</i>	TGTCCAGAGCAA ATGTTGGCTCAT CAAGAGCAT GACCATGG	59.927	58.228	AG	7	M13	HEX	NA	NA	NA	NA	NA	NA	NA
<i>Ale401</i>	CCAAGTTCTCCTG CTCTGTGGACTT CTTATCCC ACGGTGCA	58.076	59.683	AT	8	CAG	FAM	NA	NA	NA	NA	NA	NA	NA
<i>Ale457</i>	TGTTCTAGTGTCC GTCTTCTATGCTT TGTGCTGAC GATGGCGG	59.964	59.130	AT	8	M13	HEX	NA	NA	NA	NA	NA	NA	NA

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<i>Ale462</i>	AGGCACCCAATAT CCGTAAATCTGG CCTCTCAA GCTCAGTCT	58.508	59.517	AGC	7	M13	HEX	NA	NA	NA	NA	NA	NA	NA
<i>Ale551</i>	TGACTGGATAGG CCCTACACTACC AAAGGAAAGGA CTGCCCT	58.565	59.997	AGGAT	7	M13	HEX	NA	NA	NA	NA	NA	NA	NA
<i>Ale621</i>	TGATGGTTCTGAA TTCTCTGTTGCTT GAAAGATGGG CCAAATCTGC	58.407	59.746	AC	17	CAG	FAM	NA	NA	NA	NA	NA	NA	NA

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**Appendix 10. Genotype of DNA from molted primary feathers of *Anodorhynchus leari* collected in the five localities of study, and from blood samples collect in the breeding site of Toca Velha. For each sample, information is shown about the specific site of collection and the habitat type, roost or nest (in the case of nests, the nest ID is included), the sex of the individual (based on molecular analyses) and its multilocus genotype based on eight polymorphic microsatellites. 'Ind': indetermined sex. 'NA': missing genotype. \*Samples excluded of the analysis due repetition.**

Sample type	Date	Locality	Site	Habitat	Sex	Genotype								
						<i>Aleari_176</i>	<i>Alea20</i>	<i>Alea23</i>	<i>Alea28</i>	<i>Aleari_281</i>	<i>Alea4</i>	<i>Alea5</i>	<i>Aleari_606</i>	
230	feather	2015-01	Toca Velha	TVelha A	Roost	Female	134-139	190-198	213-221	231-231	102-122	167-171	135-143	089-089
235	feather	2015-01	Toca Velha	TVelha A	Roost	Male	139-144	198-202	209-220	231-231	118-122	167-167	135-135	089-089
238	feather	2015-01	Toca Velha	TVelha A	Roost	Male	134-144	190-190	220-220	219-231	118-118	163-167	143-151	089-089
240	feather	2015-01	Toca Velha	TVelha A	Roost	Male	134-144	206-206	209-221	219-251	122-126	167-171	143-151	089-089
245	feather	2015-01	Toca Velha	TVelha A	Roost	Male	154-154	190-194	220-221	227-239	118-122	139-167	139-151	087-089
438	feather	2009-04	Toca Velha	TVelha B	Nest-N01	Male	134-139	190-206	217-220	231-235	102-118	139-167	135-147	087-089
442*	feather	2010-12	Toca Velha	TVelha B	Nest-N01	Female	134-134	194-198	213-220	219-235	118-126	163-167	143-147	089-089
407	feather	2014-02	Toca Velha	TVelha B	Nest-N02	Female	134-144	190-194	213-216	NA-NA	102-118	139-139	143-143	087-089
990	blood	2016-04	Toca Velha	TVelha B	Nest-N03	Female	139-144	206-206	220-221	219-227	118-122	167-167	143-143	089-089
1366	feather	2016-04	Toca Velha	TVelha B	Nest-N03	Female	139-139	198-206	213-220	219-219	102-122	167-167	143-143	089-089
431	feather	2013-04	Toca Velha	TVelha B	Nest-N23	Ind.	139-144	198-202	201-209	231-231	118-122	167-167	135-135	089-089
1357	feather	2016-03	Toca Velha	TVelha B	Nest-N23	Male	134-154	198-206	213-220	231-235	118-126	163-171	143-147	087-089
423	feather	2014-04	Toca Velha	TVelha B	Nest-N26	Male	134-154	198-202	213-216	219-239	118-122	163-167	143-155	087-089
419	feather	2014-04	Toca Velha	TVelha B	Nest-N44	Female	134-144	NA-NA	NA-NA	NA-NA	118-118	139-139	143-143	089-089
418	feather	2014-02	Toca Velha	TVelha B	Nest-N46	Male	144-154	190-194	217-220	219-231	102-118	139-139	135-143	087-089
405	feather	2014-02	Toca Velha	TVelha B	Nest-N47	Male	144-154	NA-NA	NA-NA	NA-NA	118-130	167-167	143-151	087-089
420	feather	2014-04	Toca Velha	TVelha B	Nest-N48	Male	144-154	194-202	217-221	231-247	118-122	163-167	143-143	089-089
415	feather	2014-02	Toca Velha	TVelha B	Nest-N49	Female	134-144	190-206	213-216	219-231	118-126	171-175	135-143	089-089
364	feather	2013-01	Toca Velha	TVelha B	Roost	Male	134-134	190-206	209-220	227-231	102-118	139-167	143-147	089-089
367	feather	2013-01	Toca Velha	TVelha B	Roost	Female	134-144	190-202	216-217	219-231	102-118	167-167	143-147	089-089
368	feather	2013-01	Toca Velha	TVelha B	Roost	Female	139-154	202-206	220-220	219-235	102-130	167-167	143-143	087-087
369*	feather	2013-01	Toca Velha	TVelha B	Roost	Female	139-154	202-206	220-220	219-235	102-130	167-167	143-143	087-087

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<b>749</b>	feather	2014-02	Toca Velha	TVelha B	Roost	Male	144-144	198-206	201-213	231-239	122-122	163-167	135-151	089-089
<b>750</b>	feather	2014-02	Toca Velha	TVelha B	Roost	Male	139-144	202-206	NA-NA	NA-NA	118-122	167-167	143-143	089-089
<b>752</b>	feather	2014-02	Toca Velha	TVelha B	Roost	Male	134-139	198-206	209-220	219-247	122-126	163-167	143-143	089-089
<b>753</b>	feather	2014-02	Toca Velha	TVelha B	Roost	Female	144-154	198-206	216-216	219-231	118-122	167-171	143-143	089-089
<b>754</b>	feather	2014-02	Toca Velha	TVelha B	Roost	Male	144-154	198-206	209-220	231-235	118-122	167-171	135-143	089-089
<b>757</b>	feather	2014-02	Toca Velha	TVelha B	Roost	Female	134-144	190-206	209-220	219-251	118-122	167-171	143-147	089-089
<b>1291</b>	feather	2016-04	Toca Velha	TVelha B	Roost	Female	139-139	190-202	213-220	231-235	118-122	139-171	143-143	089-089
<b>1303</b>	feather	2016-04	Toca Velha	TVelha B	Roost	Male	144-144	198-206	201-220	227-231	118-122	163-167	135-151	089-089
<b>1312</b>	feather	2016-04	Toca Velha	TVelha B	Roost	Female	139-154	194-198	201-220	219-251	102-122	167-171	135-139	089-089
<b>1316</b>	feather	2016-04	Toca Velha	TVelha B	Roost	Male	139-144	194-198	209-220	219-231	102-122	167-167	135-143	089-089
<b>1317</b>	feather	2016-04	Toca Velha	TVelha B	Roost	Male	134-139	190-206	213-216	219-231	118-122	167-167	135-135	089-089
<b>1318</b>	feather	2016-04	Toca Velha	TVelha B	Roost	Female	139-144	190-206	220-220	219-231	102-122	139-171	135-147	087-089
<b>1319</b>	feather	2016-04	Toca Velha	TVelha B	Roost	Female	139-154	202-206	216-220	219-239	102-130	139-167	139-151	087-089
1320*	feather	2016-04	Toca Velha	TVelha B	Roost	Female	139-154	202-206	216-220	219-239	102-130	139-167	139-151	087-089
<b>978</b>	blood	2016-03	Toca Velha	TVelha C	Nest-N34	Male	134-139	194-194	209-216	231-231	118-122	167-171	143-147	089-089
<b>1342</b>	feather	2016-03	Toca Velha	TVelha C	Nest-N34	Female	139-144	194-206	209-220	231-235	118-122	163-171	135-143	089-089
1343*	feather	2016-03	Toca Velha	TVelha C	Nest-N34	Female	139-144	194-206	209-220	231-235	118-122	163-171	135-143	089-089
313*	feather	2015-04	Toca Velha	TVelha C	Roost	Male	134-144	198-202	213-216	219-231	118-118	163-167	143-155	087-089
<b>317</b>	feather	2015-04	Toca Velha	TVelha C	Roost	Male	134-139	190-194	213-216	219-231	122-126	167-175	135-147	089-089
<b>318</b>	feather	2015-04	Toca Velha	TVelha C	Roost	Female	134-154	194-206	213-213	231-235	118-118	139-171	143-147	087-089
<b>321</b>	feather	2015-04	Toca Velha	TVelha C	Roost	Male	144-144	202-206	201-216	219-231	118-122	163-167	135-151	089-089
<b>322</b>	feather	2015-04	Toca Velha	TVelha C	Roost	Male	134-144	198-202	213-216	219-231	118-118	163-167	143-155	087-089
<b>326</b>	feather	2015-04	Toca Velha	TVelha C	Roost	Female	154-154	202-206	216-220	219-227	102-130	139-167	139-143	087-089
328*	feather	2015-04	Toca Velha	TVelha C	Roost	Female	139-144	194-206	209-220	231-235	118-122	163-171	135-143	089-089
<b>330</b>	feather	2015-04	Toca Velha	TVelha C	Roost	Female	144-154	198-206	220-220	231-231	122-126	167-167	135-143	089-089
<b>332</b>	feather	2015-04	Toca Velha	TVelha C	Roost	Male	134-144	202-206	209-220	219-247	102-122	167-171	143-143	089-089
<b>422</b>	feather	2014-04	Toca Velha	TVelha D	Nest-N12	Male	134-154	190-202	213-217	219-231	102-118	139-167	135-143	087-089
<b>128</b>	blood	2013-04	Toca Velha	TVelha D	Nest-N13	Male	139-144	202-202	220-220	231-247	122-126	167-167	143-155	089-089
<b>129</b>	blood	2013-04	Toca Velha	TVelha D	Nest-N13	Male	134-134	198-202	209-220	219-247	122-126	167-167	135-143	089-089
<b>154</b>	blood	2014-04	Toca Velha	TVelha D	Nest-N13	Female	134-144	198-206	209-209	231-247	122-126	167-167	135-143	089-089

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<b>155</b>	blood	2014-04	Toca Velha	TVelha D	Nest-N13	Male	134-134	202-202	209-220	219-247	122-122	167-167	135-143	089-089
<b>156</b>	blood	2014-04	Toca Velha	TVelha D	Nest-N13	Male	134-139	202-202	209-220	231-247	122-122	167-167	135-143	089-089
<b>186</b>	blood	2015-04	Toca Velha	TVelha D	Nest-N13	Male	134-134	198-202	209-220	219-231	122-122	167-167	143-155	089-089
<b>983</b>	blood	2016-03	Toca Velha	TVelha D	Nest-N13	Male	134-144	202-202	220-220	219-247	122-126	167-167	143-155	089-089
<b>984</b>	blood	2016-03	Toca Velha	TVelha D	Nest-N13	Male	134-144	198-202	209-220	219-247	122-122	167-167	135-143	089-089
<b>336B</b>	feather	2015-01	Toca Velha	TVelha D	Nest-N13	Male	139-154	206-206	216-220	227-231	118-130	167-167	139-143	087-087
<b>402</b>	feather	2013-01	Toca Velha	TVelha D	Nest-N14	Female	139-154	194-202	213-220	239-239	102-130	139-167	139-151	089-089
<b>399</b>	feather	2013-02	Toca Velha	TVelha D	Nest-N42	Female	134-139	198-202	213-220	231-239	118-118	167-167	143-151	089-089
400*	feather	2013-02	Toca Velha	TVelha D	Nest-N42	Female	134-139	198-202	213-220	231-239	118-118	167-167	143-151	089-089
<b>464</b>	feather	2015-04	Toca Velha	TVelha D	Nest-N59	Ind	134-139	194-206	213-220	231-231	118-122	167-171	135-147	089-089
<b>380</b>	feather	2013-04	Toca Velha	TVelha D	Roost	Female	134-134	198-202	213-216	219-231	118-122	167-167	143-151	089-089
<b>381</b>	feather	2013-04	Toca Velha	TVelha D	Roost	Female	134-144	NA-NA	209-220	219-231	118-122	167-167	143-155	089-089
<b>384</b>	feather	2013-04	Toca Velha	TVelha D	Roost	Female	134-134	194-198	213-220	219-235	118-126	163-167	143-147	089-089
<b>385</b>	feather	2013-04	Toca Velha	TVelha D	Roost	Male	154-154	202-202	216-216	227-231	118-130	139-167	135-139	089-089
<b>386</b>	feather	2013-04	Toca Velha	TVelha D	Roost	Female	134-144	190-206	220-220	219-219	102-118	163-167	143-155	089-089
387*	feather	2013-04	Toca Velha	TVelha D	Roost	Female	144-154	198-206	216-216	219-231	118-122	167-171	143-143	089-089
<b>391</b>	feather	2014-02	Toca Velha	TVelha D	Roost	Female	144-144	194-206	216-220	231-247	118-118	139-167	135-143	087-089
393*	feather	2014-02	Toca Velha	TVelha D	Roost	Male	134-154	190-202	213-217	219-231	102-118	139-167	135-143	087-089
395*	feather	2014-02	Toca Velha	TVelha D	Roost	Female	134-144	190-206	220-220	219-219	102-118	163-167	143-155	089-089
404*	feather	2014-02	Toca Velha	TVelha E	Nest-N27	Male	134-139	NA-NA	209-220	NA-NA	122-126	163-167	143-143	089-089
<b>687</b>	feather	2014-07	Serra Branca	SBranca A	Roost	Female	134-154	198-202	216-220	231-231	118-118	167-167	143-143	089-089
<b>927</b>	feather	2014-09	Serra Branca	SBranca A	Roost	Ind	139-139	198-202	221-221	235-247	118-122	167-171	143-147	087-089
<b>928</b>	feather	2014-09	Serra Branca	SBranca A	Roost	Male	139-144	206-206	213-221	NA-NA	118-122	167-171	143-143	089-089
<b>953</b>	feather	2014-09	Serra Branca	SBranca B	Roost	Male	144-154	206-206	220-220	NA-NA	118-118	163-167	143-143	089-089
<b>954</b>	feather	2014-09	Serra Branca	SBranca B	Roost	Male	134-154	198-206	213-216	NA-NA	118-118	167-167	143-143	087-089
<b>630</b>	feather	2014-06	Serra Branca	SBranca C	Roost	Male	134-144	190-206	220-220	219-231	122-122	167-175	143-151	089-089
<b>646</b>	feather	2014-06	Serra Branca	SBranca C	Roost	Female	134-144	206-206	220-220	231-231	126-126	139-167	143-143	089-089
<b>971</b>	feather	2014-09	Serra Branca	SBranca D	Roost	Female	139-144	194-202	209-220	231-239	122-122	163-167	135-139	089-089
972*	feather	2014-09	Serra Branca	SBranca D	Roost	Male	139-144	194-202	209-220	NA-NA	122-122	163-167	135-139	089-089
<b>976</b>	feather	2014-09	Serra Branca	SBranca D	Roost	Female	134-134	206-206	216-220	219-231	102-118	163-167	135-151	087-089

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<b>264</b>	feather	2014-11	Baixa Chico	BChico A	Roost	Male	144-154	190-202	209-220	231-235	118-122	139-167	135-143	087-087
<b>265</b>	feather	2014-11	Baixa Chico	BChico A	Roost	Male	144-154	202-202	201-220	NA-NA	102-118	139-167	135-143	087-089
<b>266</b>	feather	2014-11	Baixa Chico	BChico A	Roost	Female	134-144	198-206	220-220	231-231	122-126	167-171	143-143	087-087
<b>268</b>	feather	2014-11	Baixa Chico	BChico A	Roost	Female	139-144	206-206	213-216	NA-NA	122-126	167-171	147-155	089-089
<b>281</b>	feather	2015-02	Baixa Chico	BChico A	Roost	Male	139-144	190-206	220-220	219-231	102-102	167-167	135-147	089-089
<b>286</b>	feather	2015-02	Baixa Chico	BChico A	Roost	Male	139-144	190-190	216-220	231-231	102-122	163-167	143-143	089-089
<b>287</b>	feather	2015-02	Baixa Chico	BChico A	Roost	Male	144-154	190-198	216-220	231-239	118-118	167-167	143-143	087-089
<b>288</b>	feather	2015-02	Baixa Chico	BChico A	Roost	Male	144-154	194-206	216-220	231-231	102-118	139-167	135-143	089-089
<b>289</b>	feather	2015-02	Baixa Chico	BChico A	Roost	Male	139-144	202-206	217-220	227-231	102-122	139-167	143-155	089-089
<b>297</b>	feather	2015-02	Baixa Chico	BChico A	Roost	Female	144-144	198-198	220-220	219-231	102-118	167-167	135-155	087-089
<b>503</b>	feather	2014-07	Baixa Chico	BChico A	Roost	Female	134-134	190-206	209-220	219-231	118-118	139-167	143-151	087-089
507*	feather	2014-07	Baixa Chico	BChico A	Roost	Male	139-144	190-206	220-220	219-231	102-102	167-167	135-147	089-089
<b>508</b>	feather	2014-07	Baixa Chico	BChico A	Roost	Female	139-139	194-206	220-220	231-239	102-122	167-167	143-147	087-089
<b>509</b>	feather	2014-07	Baixa Chico	BChico A	Roost	Female	139-139	198-206	209-220	219-227	118-118	139-167	147-147	087-089
<b>512</b>	feather	2014-07	Baixa Chico	BChico A	Roost	Male	134-144	206-206	209-217	219-235	102-118	167-167	147-151	087-089
522*	feather	2014-07	Baixa Chico	BChico A	Roost	Male	134-144	206-206	209-217	219-235	102-118	167-167	147-151	087-089
<b>553</b>	feather	2014-05	Baixa Chico	BChico A	Roost	Male	154-154	202-202	213-220	239-239	118-126	167-167	143-143	089-089
<b>557</b>	feather	2014-05	Baixa Chico	BChico A	Roost	Male	134-139	198-206	209-220	235-239	118-122	167-167	143-143	089-089
558*	feather	2014-05	Baixa Chico	BChico A	Roost	Female	139-139	194-206	220-220	231-239	102-122	167-167	143-147	087-089
<b>566</b>	feather	2014-09	Baixa Chico	BChico A	Roost	Male	139-154	194-206	220-220	227-231	118-130	167-171	139-147	087-087
<b>880</b>	feather	2014-09	Baixa Chico	BChico A	Roost	Female	144-144	190-198	216-220	231-231	118-122	167-167	143-143	087-089
<b>1200</b>	feather	2016-05	Baixa Chico	BChico A	Roost	Female	134-144	206-206	217-221	219-227	118-122	167-171	143-151	087-087
<b>1201</b>	feather	2016-05	Baixa Chico	BChico A	Roost	Male	144-154	206-206	213-216	231-239	102-102	167-171	143-151	089-089
<b>1202</b>	feather	2016-05	Baixa Chico	BChico A	Roost	Male	144-154	NA-NA	216-221	227-231	102-122	163-171	143-143	089-089
<b>1203</b>	feather	2016-05	Baixa Chico	BChico A	Roost	Male	139-144	194-198	209-220	219-231	118-122	163-163	143-155	089-089
1204*	feather	2016-05	Baixa Chico	BChico A	Roost	Female	134-144	198-206	220-220	231-231	122-126	167-171	143-143	087-087
<b>1206</b>	feather	2016-05	Baixa Chico	BChico A	Roost	Male	154-154	202-206	NA-NA	NA-NA	118-122	167-167	143-147	087-087
1208*	feather	2016-05	Baixa Chico	BChico A	Roost	Male	139-154	194-206	220-220	227-231	118-130	167-171	139-147	087-087
<b>1223</b>	feather	2016-04	Baixa Chico	BChico A	Roost	Male	139-144	198-206	209-220	219-235	122-122	167-171	143-147	087-089
<b>1233</b>	feather	2016-04	Baixa Chico	BChico A	Roost	Male	139-144	194-206	213-220	231-239	118-118	139-167	143-143	089-089

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1239*	feather	2016-04	Baixa Chico	BChico A	Roost	Male	144-154	206-206	213-216	231-239	102-102	167-171	143-151	089-089
<b>772</b>	feather	2014-05	Barreiras	Barre A	Roost	Male	134-139	202-202	213-221	223-231	122-122	167-167	143-143	089-089
775*	feather	2014-05	Barreiras	Barre A	Roost	Male	NA-NA	NA-NA	209-220	NA-NA	118-122	139-167	143-143	087-089
779*	feather	2014-05	Barreiras	Barre A	Roost	Male	139-139	190-206	209-216	231-231	102-126	167-167	143-143	087-089
<b>780</b>	feather	2014-05	Barreiras	Barre A	Roost	Male	139-154	190-202	220-220	227-235	102-122	167-167	143-143	087-087
<b>783</b>	feather	2014-05	Barreiras	Barre A	Roost	Male	139-144	198-206	209-220	227-231	118-122	139-167	143-143	087-089
<b>787</b>	feather	2014-05	Barreiras	Barre A	Roost	Male	139-139	194-206	213-216	231-239	126-130	139-167	143-151	089-089
<b>794</b>	feather	2014-05	Barreiras	Barre A	Roost	Female	154-154	190-202	220-220	227-239	122-122	139-167	143-151	087-089
<b>796</b>	feather	2014-05	Barreiras	Barre A	Roost	Male	144-144	202-206	216-220	231-231	118-122	139-175	135-143	087-089
797*	feather	2014-05	Barreiras	Barre A	Roost	Male	NA-NA	198-206	209-209	235-247	118-122	167-171	143-147	087-089
<b>798</b>	feather	2014-05	Barreiras	Barre A	Roost	Male	139-139	190-190	217-220	231-239	102-102	167-171	143-143	089-089
800*	feather	2014-05	Barreiras	Barre A	Roost	Female	134-154	198-206	213-220	231-231	118-126	167-171	143-155	087-087
<b>807</b>	feather	2014-05	Barreiras	Barre A	Roost	Female	139-144	190-194	209-220	231-239	102-118	167-167	143-143	089-089
<b>808</b>	feather	2014-05	Barreiras	Barre A	Roost	Female	NA-NA	190-206	NA-NA	NA-NA	118-122	139-167	143-143	087-089
<b>810</b>	feather	2014-05	Barreiras	Barre A	Roost	Male	134-139	198-206	209-209	235-247	118-122	167-171	143-147	087-089
<b>816</b>	feather	2014-05	Barreiras	Barre A	Roost	Male	144-144	206-206	220-220	227-247	118-122	163-167	143-147	089-089
<b>817</b>	feather	2014-05	Barreiras	Barre A	Roost	Male	134-139	206-206	NA-NA	231-231	122-122	167-167	143-143	089-089
<b>819</b>	feather	2014-05	Barreiras	Barre A	Roost	Female	134-154	198-206	213-220	231-231	118-126	167-171	143-155	087-087
<b>825</b>	feather	2014-07	Barreiras	Barre A	Roost	Female	134-144	206-206	209-220	219-227	118-122	171-171	143-143	089-089
<b>829</b>	feather	2014-07	Barreiras	Barre A	Roost	Female	139-144	194-206	213-220	219-239	102-122	167-167	139-143	089-089
<b>831</b>	feather	2014-09	Barreiras	Barre A	Roost	Female	139-139	202-206	213-213	219-231	118-122	167-167	143-143	087-089
<b>832</b>	feather	2014-09	Barreiras	Barre A	Roost	Female	134-139	194-202	201-220	219-239	102-118	167-167	143-143	089-089
833*	feather	2014-09	Barreiras	Barre A	Roost	Male	139-144	NA-NA	209-220	NA-NA	118-122	139-167	143-143	087-089
<b>1191</b>	feather	2016-05	Barreiras	Barre A	Roost	Male	139-139	198-202	213-220	231-239	102-126	167-167	135-151	089-089
<b>1194</b>	feather	2016-05	Barreiras	Barre A	Roost	Male	134-144	202-206	216-217	219-231	118-118	139-139	143-143	087-087
<b>1196</b>	feather	2016-05	Barreiras	Barre A	Roost	Male	134-144	190-206	209-220	231-231	118-122	167-167	143-143	089-089
<b>1003</b>	feather	2016-04	Barra Tanque	BTanque A	Roost	Male	134-139	194-202	216-216	235-239	118-122	171-171	143-151	087-087
<b>1004</b>	feather	2016-04	Barra Tanque	BTanque A	Roost	Male	134-144	202-206	216-220	219-251	122-122	163-167	143-143	089-089
<b>1005</b>	feather	2016-04	Barra Tanque	BTanque A	Roost	Ind	144-144	198-206	216-220	NA-NA	102-118	167-171	143-143	089-089
<b>1007</b>	feather	2016-04	Barra Tanque	BTanque A	Roost	Male	144-154	206-206	220-221	219-231	102-122	167-167	143-143	087-089

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<b>1010</b>	feather	2016-04	Barra Tanque	BTanque A	Roost	Male	139-154	NA-NA	NA-NA	NA-NA	122-122	167-167	135-143	089-089
<b>1011</b>	feather	2016-04	Barra Tanque	BTanque A	Roost	Male	139-154	206-206	220-220	231-239	102-102	167-171	143-143	087-089
<b>1014</b>	feather	2016-04	Barra Tanque	BTanque A	Roost	Male	139-154	194-206	213-216	219-239	102-130	139-167	143-151	087-089
<b>1016</b>	feather	2016-04	Barra Tanque	BTanque A	Roost	Male	134-144	206-206	209-220	231-239	102-102	167-167	143-143	089-089
<b>1018</b>	feather	2016-04	Barra Tanque	BTanque A	Roost	Male	144-154	206-206	216-220	219-239	122-130	163-167	143-151	089-089

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**Appendix 11. Estimates of relative contributions of the environmental variables to the Maxent model for Lear's Macaw, *Anodorhynchus leari*.**

Variable	Percent contribution	Permutation importance
bio13_ Precipitation of Wettest Month	38.7	77
bio7_ Temperature Annual Range	30.9	22.5
bio4_ Temperature Seasonality	17.8	0.1
bio12_ Annual Precipitation	9.3	0.1
bio18_ Precipitation of Warmest Quarter	2.9	0.3
bio5_ Max Temperature of Warmest Month	0.2	0
bio15_ Precipitation Seasonality	0.1	0
HFP_ Human Foot Print	0	0

**Appendix 12. Estimates of relative contributions of the environmental variables to the Maxent model for Licuri Palm, *Syagrus coronata*.**

Variable	Percent contribution	Permutation importance
bio7_ Temperature Annual Range	43.8	29.7
bio4_ Temperature Seasonality	27.7	55.1
HPF_ Human Foot Print	21.9	3.1
bio18_ Precipitation of Warmest Quarter	4.3	8.3
bio15_ Precipitation Seasonality	1.6	0.6
bio14_ Precipitation of Driest Month	0.5	2.9
bio13_ Precipitation of Wettest Month	0.1	0.2
bio8_ Mean Temperature of Wettest Quarter	0	0
bio5_ Max Temperature of Warmest Month	0	0

## Appendix 13. Script R Code from section 6

```

## From Agresti(2007) p.39
# Chi-squared test for counts of macaw nests and bee hives between core and historic areas ###
M<- as.table(rbind(c(7,95),c(33,8)))
dimnames(M) <- list(Species = c("Abelhas", "Araras"),
                    Area = c("Core","Historic"))
(Xsq <- chisq.test(M))
(Xsq <- chisq.test(M)) # Prints test summary
Xsq$observed # observed counts (same as M)
Xsq$expected # expected counts under the null
Xsq$residuals # Pearson residuals
Xsq$stdres # standardized residuals

#####

# refazendo o Chi-squared test for counts of macaw nests and bee hives between core and historic areas com os numeros de ninhos
# corrigidos na TABELA 1 nova (considereei 8 ninhos na historical area e 40 ninhos na core area, tambem mudei o numero de
# colmeias na core area porque ?? havia manejado 3 colmeias, entao ficam s?? 4 colmeias ###
Mnovo<- as.table(rbind(c(4,95),c(40,8)))
structure(Mnovo)
dimnames(Mnovo) <- list(Species = c("Abelhas", "Araras"),
                        Area = c("Core","Historic"))
(Xsq <- chisq.test(Mnovo))
(Xsq <- chisq.test(Mnovo)) # Prints test summary
Xsq$observed # observed counts (same as Mnovo)
Xsq$expected # expected counts under the null
Xsq$residuals # Pearson residuals
Xsq$stdres # standardized residuals

#####

### LILA VEJA O TESTE CHI-QUADRADO PARA SUCESSO REPRODUTIVO AQUI!!! BJO!!!
# Chi-squared test for nest success (i.e. at least one fledgling) between LM and AHB/LM nests, for the years 2012, 2017 and
# 2018 ###
bees<- read.csv("~/Dropbox/A.leari-CHICO/bees_learns_data10.csv", sep=";", stringsAsFactors = F)

# criando uma nova tabela com 2 colunas, uma com o sucesso reprodutivo de cada ninho (0 ou 1), e a outra com a esp?cie (AHM/LM
# ou LM), apenas para as cavidades recrutadas
bees2<-as.data.frame(rbind( # esse comando coloca as colunas de produtividade dos diferentes anos como uma coluna s??, e coloca
# a segunda coluna com a esp?cie
  cbind(bees$prod_2012,bees$specie),
  cbind(bees$prod_2017,bees$specie),
  cbind(bees$prod_2018,bees$specie)
),stringsAsFactors = F)

colnames(bees2)<- c("nest.success","species") # atualizando os nomes dessas 2 colunas

bees2<-subset(bees2,species!="AHB") # esse comando elimina as cavidades que s??o apenas colm?ias

bees2$nest.success<-as.numeric(bees2$nest.success) #convertendo a coluna de produtividade em valores num?ricos. esse comando
# converte todos os valores n??o num?ricos em 'NA' (isso vai ser importante para eliminar as cavidades n??o recrutadas - ver
# abaixo)

bees2$nest.success[which(bees2$nest.success>1)]<-1 # esse comando converte todos os valores de produtividade que s??o >1 em 1,
# entao a coluna passa a ser nest success (com 0s e 1s)

bees2 # essa ?? a tabela

table(bees2) # esse comando faz uma "pivot table" (tabela de conting?ncia) da tabela bees2. Importante ?? que ela ignora os
# NAs, entao considera apenas os ninhos recrutados:
#species
#nest.success AHB/LM LM
#0 8 28
#1 4 38

4/(8+4) #=0.33 esse valor ?? a propor????o de ninhos com sucesso dentro os AHB/LM
38/(28+38) #=0.57 esse valor ?? a propor????o de ninhos com sucesso dentro os LM
# A princ?pio, a propor????o de ninhos com sucesso entre os LM ?? bem maior que os AHB/LM.

# Essa parte ?? o teste chi-quadrado:
M2<-table(bees2)
(Xsq2 <- chisq.test(M2)) # Prints test summary

# o resultado d?? um p=0.2169, ou seja, n??o h?? diferen?a significativa entre as propor????es

Xsq2$observed # observed counts (same as M)
Xsq2$expected # expected counts under the null
Xsq2$residuals # Pearson residuals
Xsq2$stdres # standardized residuals

```

```
#####
### contando s?? o sucesso e insucesso nas cavidades recrutadas em cada ano (2011, 2012, 2017, 2018)
#cavity.success AHB/LM LM
#0             4      1
#1             4      5

4/(4+4) #0.5 propor????o de ninhos com sucesso dentre os AHB/LM
5/(1+5) #0.83 propor????o de ninhos com sucesso dentre os LM

M3<-as.table(rbind(c(4,1),c(4,5)))
dimnames(M3) <- list(cavity.success = c("not.successfull", "successfull"),
                    treatment = c("managed", "natural"))
(Xsq3<- chisq.test(M3, correct = F))

Xsq3$observed
Xsq3$expected
Xsq3$residuals
Xsq3$stdres

#####

# GLMM for height between bee and macaw-occupied cavities ###
library(lme4)
library(bbmle)
library(merTools)
library(ggplot2)
library(gridExtra)

bees<- read.csv("~/Dropbox/A.Leari/bees_learns_data8.csv", sep=";")
summary(bees)

bees<-bees[-which(bees$specie=="AHB/LM"),]

bees$cliff_height_m<-bees$cliff_height_cm/100
bees$cavity_height_cm<-as.numeric(as.character(bees$cavity_height_cm))
bees$cavity_height_m<-bees$cavity_height_cm/100

m1<-lm(cavity_height_m~specie*locality,data=bees)
m2<-lm(cavity_height_m~specie*locality,data=bees)
m0<-lm(cavity_height_m~1,data=bees)

summary(m1)
summary(m2)

anova(m1)
anova(m2)
AICtab(m1,m0,m2)

newdf<-data.frame(specie=c("AHB", "LM", "AHB", "LM", "AHB", "LM"),locality=sort(rep(levels(bees$locality),2)))
pred1<-predict(m6,newdata=newdf,se.fit=T,type="response")

df2<-
data.frame(locality=newdf$locality,species=newdf$specie,mean=pred1$fit,upper=pred1$fit+1.96*pred1$se.fit,lower=pred1$fit-1.96*
pred1$se.fit)

p1<-ggplot(df2,aes(x=locality,y=mean,colour=species))
p1+geom_pointrange(aes(ymin = lower, ymax = upper),position = position_dodge(0.2))+xlab("Locality")+ylab("Height (m)")

cliff.height.bx<-subset(bees,locality=="Baixa do Chico")$cliff_height_m
cliff.height.ba<-subset(bees,locality=="Barreiras")$cliff_height_m
cliff.height.tv<-subset(bees,locality=="Toca Velha")$cliff_height_m

df3<-data.frame(
  locality=c(rep("Baixa do Chico",3),rep("Barreiras",3),rep("Toca Velha",3)),
  species=rep(c("AHB", "LM", "zCliff height"),3),
  mean=c(pred1$fit[1:2],mean(cliff.height.bx),
        pred1$fit[3:4],mean(cliff.height.ba),
        pred1$fit[5:6],mean(cliff.height.tv)),
  upper=c(pred1$fit[1:2]+1.96*pred1$se.fit[1:2],
        mean(cliff.height.bx)+sd(cliff.height.bx),
        pred1$fit[3:4]+1.96*pred1$se.fit[3:4],
        mean(cliff.height.ba)+sd(cliff.height.ba),
        pred1$fit[5:6]+1.96*pred1$se.fit[5:6],
        mean(cliff.height.tv)+sd(cliff.height.tv)),
  lower=c(pred1$fit[1:2]-1.96*pred1$se.fit[1:2],
        mean(cliff.height.bx)-sd(cliff.height.bx),
        pred1$fit[3:4]-1.96*pred1$se.fit[3:4],
        mean(cliff.height.ba)-sd(cliff.height.ba),
        pred1$fit[5:6]-1.96*pred1$se.fit[5:6],
```

```

mean(cliff.height.tv)-sd(cliff.height.tv))

p1<-ggplot(df3,aes(x=locality,y=mean,colour=species))
p2<-p1+geom_pointrange(aes(ymin = lower, ymax = upper),position = position_dodge(0.2))+xlab("Locality")+ylab("Height (m)")
+theme(legend.title = element_blank(),legend.position = c(0.95,0.75))+scale_color_manual(values=c("#FFCC33", "#3366CC",
"#FF6633"),labels = c("AHB", "LM", "Cliff"))+annotate("text",label="B",x=0.5,y=60,fontface = "bold")

p3<-ggplot(bees, aes(x=locality, y=cavity_height_m,colour=specie))
p4<-p3+geom_boxplot()+xlab("Locality")+ylab("Height (m)")+scale_color_manual(values=c("#FFCC33", "#3366CC"))+theme(legend.title
= element_blank(),legend.position = c(0.95,0.8))+annotate("text",label="A",x=0.5,y=60,fontface = "bold")
p5<-grid.arrange(p4,p2)

ggsave(filename = "/Users/franciscodenes/desktop/bees.png",plot=p5)

str(bees)

str(bees_learns_data10)

#####

# ANOVA for height cavities USED between SPECIE bee and macaw, LOCATION, SPECIE bee and macaw x LOCATION ###

bees<- read.csv("~/Dropbox/A.learni-CHICO/bees_learns_data8.csv", sep=";")
summary(bees)

str(bees)
# variavel tem que ser fator (specie) num??rica (height)?
# converter factor em numerico pra height

```

## Appendix 14. Dataset of Africanized honey bee survey and management.

identificador	cavity	specie	area	locality	cliff_n umber	cliff_na me	cliff_o rientation	cliff_hei ght_cm	cavity _heig ht_cm	year_A	recrui tment	year_L	apelido_ni nho	prod_20 10	prod_201 1	prod_201 2	prod_201 7	prod_20 18	year_hive
										HB_surv ey_local ity		M_recrui tment							year_hive _manage ment
AB prox. N15_Toca Velha_3	AB prox. N15	AHB	core	Toca Velha	3	Saco3	E	2750	1550	2009	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	2016
AB_Baixa do Chico_8	AB	AHB	historic	Baixa do Chico	8	ninhos_ estrada	E	3700	1000	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	2016
AB1_Baixa do Chico_10	AB1	AHB	historic	Baixa do Chico	10	baixa_fe chada	NE	600	800	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	2016
AB1_Barreiras_6	AB1	AHB	historic	Barreiras	6	ze_mari nho	NE	2000	NA	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	2016
AB10_Baixa do Chico_10	AB10	AHB	historic	Baixa do Chico	10	baixa_fe chada	SE	3000	1200	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	2016
AB10_Baixa do Chico_9	AB10	AHB	historic	Baixa do Chico	9	dormito rio_1	W	5600	300	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	not_mana ged
AB10_Barreiras_ 5	AB10	AHB	historic	Barreiras	5	saco_ab elhas	E	2850	1000	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	2016
AB10_Barreiras_ 6	AB10	AHB	historic	Barreiras	6	ze_mari nho	SE	2000	NA	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	not_mana ged
AB11_Baixa do Chico_10	AB11	AHB	historic	Baixa do Chico	10	baixa_fe chada	SE	3000	1100	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	not_mana ged
AB11_Baixa do Chico_9	AB11	AHB	historic	Baixa do Chico	9	dormito rio_1	W	5600	300	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	not_mana ged
AB11_Barreiras_ 5	AB11	AHB	historic	Barreiras	5	saco_ab elhas	E	2850	900	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	2016
AB11_Barreiras_ 6	AB11	AHB	historic	Barreiras	6	ze_mari nho	SE	2000	NA	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	not_mana ged
AB12_Baixa do Chico_10	AB12	AHB	historic	Baixa do Chico	10	baixa_fe chada	SE	3000	800	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	2016
AB12_Baixa do Chico_9	AB12	AHB	historic	Baixa do Chico	9	dormito rio_1	W	5600	2500	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	not_mana ged
AB12_Barreiras_ 5	AB12	AHB	historic	Barreiras	5	saco_ab elhas	N	2850	900	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	2016
AB12_Barreiras_ 6	AB12	AHB	historic	Barreiras	6	ze_mari nho	NE	2000	NA	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	not_mana ged
AB13_Baixa do Chico_10	AB13	AHB	historic	Baixa do Chico	10	baixa_fe chada	SE	3000	400	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	not_mana ged



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AB2_Baixa do Chico_10	AB2	AHB	historic	Baixa do Chico	10	baixa_fe chada	SE	3000	700	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	2016
AB2_Baixa do Chico_8	AB2	AHB	historic	Baixa do Chico	8	ninhos_ estrada	E	3700	1100	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	2016
AB2_Barreiras_5	AB2	AHB	historic	Barreiras	5	saco_ab elhas	N	2850	1000	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	2016
AB2_Barreiras_6	AB2	AHB	historic	Barreiras	6	ze_mari nho	E	2000	NA	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	not_mana ged
AB20_Baixa do Chico_10	AB20	AHB	historic	Baixa do Chico	10	baixa_fe chada	NE	3000	1900	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	not_mana ged
AB20_Barreiras_6	AB20	AHB	historic	Barreiras	6	ze_mari nho	E	2000	NA	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	not_mana ged
AB21_Baixa do Chico_10	AB21	AHB	historic	Baixa do Chico	10	baixa_fe chada	NE	3000	700	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	2016
AB21_Barreiras_5	AB21	AHB	historic	Barreiras	5	saco_ab elhas	E	2850	1800	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	2016
AB21_Barreiras_6	AB21	AHB	historic	Barreiras	6	ze_mari nho	E	2000	NA	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	not_mana ged
AB22_Baixa do Chico_10	AB22	AHB	historic	Baixa do Chico	10	baixa_fe chada	NE	3000	400	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	not_mana ged
AB22_Barreiras_5	AB22	AHB	historic	Barreiras	5	saco_ab elhas	E	2850	700	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	2016
AB22_Barreiras_6	AB22	AHB	historic	Barreiras	6	ze_mari nho	E	2000	NA	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	not_mana ged
AB23_Baixa do Chico_10	AB23	AHB	historic	Baixa do Chico	10	baixa_fe chada	NE	3000	1700	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	2016
AB23_Barreiras_5	AB23	AHB	historic	Barreiras	5	saco_ab elhas	E	2850	400	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	2016
AB23_Barreiras_6	AB23	AHB	historic	Barreiras	6	ze_mari nho	N	2000	NA	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	not_mana ged
AB24_Barreiras_5	AB24	AHB	historic	Barreiras	5	saco_ab elhas	E	2850	600	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	2016
AB24_Barreiras_6	AB24	AHB	historic	Barreiras	6	ze_mari nho	N	2000	NA	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	not_mana ged
AB25_Barreiras_5	AB25	AHB	historic	Barreiras	5	saco_ab elhas	E	2850	NA	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	not_mana ged
AB25_Barreiras_6	AB25	AHB	historic	Barreiras	6	ze_mari nho	N	2000	NA	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	not_mana ged
AB26_Barreiras_7	AB26	AHB	historic	Barreiras	7	tchau_ mainha	N	1450	400	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	not_mana ged
AB27_Barreiras_7	AB27	AHB	historic	Barreiras	7	tchau_ mainha	N	1450	500	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	2016
AB28_Barreiras_7	AB28	AHB	historic	Barreiras	7	tchau_ mainha	N	1450	500	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	2016



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AB8_Baixa do Chico_10	AB8	AHB	historic	Baixa do Chico	10	baixa_fe chada	SE	3000	2600	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	not_mana ged
AB8_Baixa do Chico_8	AB8	AHB	historic	Baixa do Chico	8	ninhos_ estrada	E	3700	900	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	2016
AB8_Barreiras_5	AB8	AHB	historic	Barreiras	5	saco_ab elhas	E	2850	1700	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	2016
AB8_Barreiras_6	AB8	AHB	historic	Barreiras	6	ze_mari nho	N	2000	NA	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	not_mana ged
AB9_Baixa do Chico_10	AB9	AHB	historic	Baixa do Chico	10	baixa_fe chada	NE	3000	500	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	not_mana ged
AB9_Baixa do Chico_9	AB9	AHB	historic	Baixa do Chico	9	dormito rio_1	W	5600	1000	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	not_mana ged
AB9_Barreiras_5	AB9	AHB	historic	Barreiras	5	saco_ab elhas	E	2850	1600	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	2016
AB9_Barreiras_6	AB9	AHB	historic	Barreiras	6	ze_mari nho	SE	2000	NA	2016	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	2016
saco2_Toca Velha_2	dormitorio saco2	AHB	core	Toca Velha	2	Saco2	E	2000	6000	2009	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	not_mana ged
judity_AB1_Toca Velha_4	judity da jatoba da judity_AB1	AHB	core	Toca Velha	4	judity_j udith	NW	2000	6000	2009	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	2016
judity_AB2_Toca Velha_4	judity da jatoba da judity_AB2	AHB	core	Toca Velha	4	judity_j udith	NW	2000	600	2009	0	not_recr uited	not_recrui ted	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	not_recr uited	2016
N01_Toca Velha_1	N01	LM	core	Toca Velha	1	Saco1	NW	4800	2900	2009	1	2008	lua	0	0	INACTIVE	0	INACTIV E	not_mana ged
N02_Toca Velha_1	N02	LM	core	Toca Velha	1	Saco1	E	5700	2100	2009	1	2008	pe	2	1	1	1	2	not_mana ged
N03_Toca Velha_1	N03	LM	core	Toca Velha	1	Saco1	E	5700	3500	2009	1	2008	reformado	2	2	2	0	1	not_mana ged
N06_Toca Velha_2	N06	LM	core	Toca Velha	2	Saco2	E	6000	2000	2009	1	2008	cachoeira_ s2	0	INACTIVE	0	INACTIVE	INACTIV E	not_mana ged
N07_Toca Velha_2	N07	LM	core	Toca Velha	2	Saco2	E	8000	2500	2009	1	2008	tunel_par ede_preta	1	1	1	1	2	not_mana ged
N12_Toca Velha_3	N12	LM	core	Toca Velha	3	Saco3	SW	5330	2850	2009	1	2008	1_s3	2	2	0	0	1	not_mana ged
N13_Toca Velha_3	N13	LM	core	Toca Velha	3	Saco3	SW	5330	2850	2009	1	2008	2_s3	3	2	0	0	2	not_mana ged
N14_Toca Velha_3	N14	LM	core	Toca Velha	3	Saco3	W	5330	2850	2009	1	2008	3_s3	1	2	0	0	0	not_mana ged
N15_Toca Velha_3	N15	LM	core	Toca Velha	3	Saco3	E	2750	1550	2009	1	2008	4_s3	1	1	NA	0	INACTIV E	not_mana ged
N23_Toca Velha_1	N23	AHB/LM	core	Toca Velha	1	Saco1	SW	6200	3600	2009	2	2011	fusca	BEEHIVE	0	0	0	0	2010

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N24_Toca Velha_1	N24	LM	core	Toca Velha	1	Saco1	E	5700	3350	2009	1	2009	loca	2	2	2	0	3	not_managed
N25_Toca Velha_1	N25	LM	core	Toca Velha	1	Saco1	E	5700	1950	2009	1	2009	dois_olhos	1	INACTIVE	INACTIVE	0	INACTIV E	not_managed
N26_Toca Velha_1	N26	LM	core	Toca Velha	1	Saco1	N	3450	2250	2009	1	2009	morcego	E	INACTIVE	INACTIVE	0	1	not_managed
N27_Toca Velha_11	N27	LM	core	Toca Velha	11	isolado1	E	6150	2950	2009	1	2009	macambir a	2	0	INACTIVE	INACTIVE	0	not_managed
N31_Toca Velha_1	N31	LM	core	Toca Velha	1	Saco1	E	5700	1300	2009	1	2010	cruz	1	INACTIVE	INACTIVE	2	2	not_managed
N32_Toca Velha_1	N32	AHB/LM	core	Toca Velha	1	Saco1	SW	4800	2900	2009	2	2012	quadrado	BEEHIVE	BEEHIVE	1	INACTIVE	0	2011
N34_Toca Velha_2	N34	LM	core	Toca Velha	2	Saco2	E	8000	3000	2009	1	2011	monoteta	not_recruited	0	INACTIVE	INACTIVE	INACTIV E	not_managed
N35_Toca Velha_1	N35	LM	core	Toca Velha	1	Saco1	N	4950	1750	2009	1	2012	cachoeira_2_baixo	not_recruited	not_recruited	NA	2	2	not_managed
N36_Toca Velha_1	N36	LM	core	Toca Velha	1	Saco1	E	5700	2400	2009	1	2011	duas_fendas	not_recruited	1	2	INACTIVE	0	not_managed
N37_Toca Velha_1	N37	LM	core	Toca Velha	1	Saco1	E	5700	1600	2009	1	2011	nave	not_recruited	2	0	INACTIVE	INACTIV E	not_managed
N38_Toca Velha_1	N38	LM	core	Toca Velha	1	Saco1	E	5700	3300	2009	1	2011	triangulo	not_recruited	2	2	INACTIVE	INACTIV E	not_managed
N39_Toca Velha_3	N39	AHB/LM	core	Toca Velha	3	Saco3	W	8200	2400	2009	2	2012	zequinha	BEEHIVE	BEEHIVE	0	INACTIVE	0	2011
N41_Toca Velha_1	N41	LM	core	Toca Velha	1	Saco1	E	5700	3050	2009	1	2013	gruta	not_recruited	not_recruited	not_recruited	INACTIVE	2	not_managed
N42_Toca Velha_3	N42	LM	core	Toca Velha	3	Saco3	W	6750	2600	2009	1	2013	olho_esq_preguica	not_recruited	not_recruited	not_recruited	INACTIVE	INACTIV E	not_managed
N43_Toca Velha_1	N43	LM	core	Toca Velha	1	Saco1	E	5700	2500	2009	1	2013	tromba_elfante	not_recruited	not_recruited	not_recruited	2	2	not_managed
N44_Toca Velha_1	N44	LM	core	Toca Velha	1	Saco1	E	5700	3150	2009	1	2014	pedrinha	not_recruited	not_recruited	not_recruited	1	2	not_managed
N46_Toca Velha_1	N46	LM	core	Toca Velha	1	Saco1	E	5700	3500	2009	1	2014	acima_dir_eita_reformado	not_recruited	not_recruited	not_recruited	1	2	not_managed
N47_Toca Velha_1	N47	LM	core	Toca Velha	1	Saco1	E	5700	3170	2009	1	2015	acima_gruta	not_recruited	not_recruited	not_recruited	1	1	not_managed
N48_Toca Velha_1	N48	LM	core	Toca Velha	1	Saco1	E	5700	2600	2009	1	2014	gina	not_recruited	not_recruited	not_recruited	INACTIVE	INACTIV E	not_managed
N49_Toca Velha_1	N49	LM	core	Toca Velha	1	Saco1	E	5700	2350	2009	1	2015	sombrancelha	not_recruited	not_recruited	not_recruited	1	0	not_managed
N51_Toca Velha_2	N51	LM	core	Toca Velha	2	Saco2	E	4250	2050	2009	1	2014	umbigo	not_recruited	not_recruited	not_recruited	0	0	not_managed
N52_Toca Velha_1	N52	LM	core	Toca Velha	1	Saco1	E	5700	2600	2009	1	2014	igreja	not_recruited	not_recruited	not_recruited	INACTIVE	1	not_managed

## APPENDICES

N55_Barreiras_6	N55	LM	historic	Barreiras	6	ze_mari nho	N	2000	1150	2009	1	2015	N1_ze_ma rinho	not_recr uited	not_recrui ted	not_recrui ted	0	0	not_mana ged
N56_Toca Velha_12	N56	LM	core	Toca Velha	12	isolado2	W	4720	3520	2009	1	2015	bilau	not_recr uited	not_recrui ted	not_recrui ted	0	0	not_mana ged
N57_Toca Velha_13	N57	LM	core	Toca Velha	13	isolado3	S	2000	1700	2009	1	2015	roca_zequ inha	not_recr uited	not_recrui ted	not_recrui ted	0	E	INACTIV not_mana ged
N58_Toca Velha_1	N58	LM	core	Toca Velha	1	Saco1	E	5700	1550	2009	1	2015	direita_cr uz	not_recr uited	not_recrui ted	not_recrui ted	1	2	not_mana ged
N60_Baixa do Chico_8	N60	LM	historic	Baixa do Chico	8	ninhos_ estrada	E	3700	1000	2009	1	2016	estrada_ju a	not_recr uited	not_recrui ted	not_recrui ted	0	1	not_mana ged
N62_Baixa do Chico_8	N62	LM	historic	Baixa do Chico	8	ninhos_ estrada	E	2340	700	2009	1	2016	ze_arara	not_recr uited	not_recrui ted	not_recrui ted	INACTIVE	NA	not_mana ged
N63_Toca Velha_1	N63	LM	core	Toca Velha	1	Saco1	E	5700	2600	2009	1	2016	abaixo_es q_gruta	not_recr uited	not_recrui ted	not_recrui ted	INACTIVE	E	INACTIV not_mana ged
N64_Toca Velha_1	N64	LM	core	Toca Velha	1	Saco1	E	5050	3100	2009	1	2016	monstrinh o_olhos	not_recr uited	not_recrui ted	not_recrui ted	INACTIVE	E	INACTIV not_mana ged
N65_Toca Velha_1	N65	LM	core	Toca Velha	1	Saco1	E	5700	2300	2009	1	2016	calcinha_p reta	not_recr uited	not_recrui ted	not_recrui ted	1	1	not_mana ged
N67_Toca Velha_1	N67	LM	core	Toca Velha	1	Saco1	W	6200	1550	2009	1	2016	direita_fus ca	not_recr uited	not_recrui ted	not_recrui ted	0	2	not_mana ged
N70_Toca Velha_1	N70	LM	core	Toca Velha	1	Saco1	E	5700	2650	2009	1	2016	abaixo_dir eita_pedri nha_boca _maior	not_recr uited	not_recrui ted	not_recrui ted	0	E	INACTIV not_mana ged
N74_Barreiras_7	N74	LM	historic	Barreiras	7	tchau_ mainha	N	1450	1250	2009	1	2015	tchau_tch au_mainh a	not_recr uited	not_recrui ted	not_recrui ted	INACTIVE	E	INACTIV not_mana ged
N76_Baixa do Chico_8	N76	AHB/LM	historic	Baixa do Chico	8	ninhos_ estrada	E	2600	1100	2016	2	2017	cupinzeiro _serrote_g ato	not_recr uited	BEEHIVE	BEEHIVE	0	E	INACTIV not_mana ged
N77_Baixa do Chico_9	N77	AHB/LM	historic	Baixa do Chico	9	dormito rio_1	W	5600	1600	2016	2	2017	direita_do rmitorio_n ovo	not_recr uited	BEEHIVE	BEEHIVE	1	E	INACTIV not_mana ged
N78_Toca Velha_1	N78	LM	core	Toca Velha	1	Saco1	E	5700	2150	2009	1	2018	coruja	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	1	not_mana ged
N79_Toca Velha_1	N79	LM	core	Toca Velha	1	Saco1	E	5050	2950	2009	1	2018	monstrinh o_boca	not_recr uited	not_recrui ted	not_recrui ted	not_recrui ted	1	not_mana ged
N80_Barreiras_5	N80	AHB/LM	historic	Barreiras	5	saco_ab elhas	N	2850	1300	2016	2	2018	parede_m eio_pa	not_recr uited	BEEHIVE	BEEHIVE	not_recrui ted	3	2016
N81_Barreiras_5	N81	AHB/LM	historic	Barreiras	5	saco_ab elhas	N	1500	1300	2016	2	2018	baixa_acei ro	not_recr uited	BEEHIVE	BEEHIVE	not_recrui ted	0	2016
N82_Barreiras_5	N82	AHB/LM	historic	Barreiras	5	saco_ab elhas	E	2250	1550	2016	2	2018	parede_es querda_cu eca	not_recr uited	BEEHIVE	BEEHIVE	not_recrui ted	2	2016

## Appendix 15. Permits (police documents) SISBIO.



Ministério do Meio Ambiente - MMA  
Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio  
Sistema de Autorização e Informação em Biodiversidade - SISBIO

## Autorização para atividades com finalidade científica

Número: 12763-5	Data da Emissão: 24/10/2013 21:46	Data para Revalidação*: 23/11/2014
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\* De acordo com o art. 33 da IN 154/2009, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.

## Dados do titular

Nome: Erica Cristina Pacifico de Assis	CPF: 220.210.158-66
Título do Projeto: Monitoramento da Biologia reprodutiva da arara-azul-de-lear <i>Anodorhynchus leari</i> (Aves: Psittacidae) na natureza	
Nome da Instituição: REITORIA DA UNIVERSIDADE DE SÃO PAULO	CNPJ: 63.025.530/0001-04

## Cronograma de atividades

#	Descrição da atividade	Início (mês/ano)	Fim (mês/ano)
1	Diagnóstico da área de estudo	02/2010	02/2010
2	Coleta de dados e marcação de aves	02/2010	12/2011
3	Análise e tabulação dos dados coletados	02/2010	12/2011
4	Cadastramento dos ninhos ativos	02/2010	12/2011
5	conclusão e apresentação dos resultados obtidos	12/2011	06/2012
6	Coleta de dados reprodutivos e marcação das aves na estação reprodutiva 2013	01/2013	07/2013
7	Coleta de dados reprodutivos e marcação das aves na estação reprodutiva 2014	01/2014	07/2014
8	Coleta de dados reprodutivos e marcação das aves na estação reprodutiva 2015	01/2015	07/2015

## Observações e ressalvas

1	As atividades de campo exercidas por pessoa natural ou jurídica estrangeira, em todo o território nacional, que impliquem o deslocamento de recursos humanos e materiais, tendo por objeto coletar dados, materiais, espécimes biológicos e minerais, peças integrantes da cultura nativa e cultura popular, presente e passada, obtidos por meio de recursos e técnicas que se destinem ao estudo, à difusão ou à pesquisa, estão sujeitas a autorização do Ministério de Ciência e Tecnologia.
2	Esta autorização NAO exime o pesquisador titular e os membros de sua equipe da necessidade de obter as anuências previstas em outros instrumentos legais, bem como do consentimento do responsável pela área, pública ou privada, onde será realizada a atividade, inclusive do órgão gestor de terra indígena (FUNAI), da unidade de conservação estadual, distrital ou municipal, ou do proprietário, arrendatário, posseiro ou morador de área dentro dos limites de unidade de conservação federal cujo processo de regularização fundiária encontra-se em curso.
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8	Em caso de pesquisa em UNIDADE DE CONSERVAÇÃO, o pesquisador titular desta autorização deverá contactar a administração da unidade a fim de CONFIRMAR AS DATAS das expedições, as condições para realização das coletas e de uso da infra-estrutura da unidade.
9	As atividades contempladas nesta autorização abrangem espécies brasileiras constantes de listas oficiais (de abrangência nacional, estadual ou municipal) de espécies ameaçadas de extinção, sobreexploradas ou ameaçadas de sobreexploração.

## Equipe

#	Nome	Função	CPF	Doc. Identidade	Nacionalidade
1	André Becker Simões Saldenberg	Pesquisador, auxiliar de coleta de dados	221.951.668-78	30404880 SSP-SP-SP	Brasileira
2	Luis Fábio Silveira	Pesquisador auxiliar	884.171.156-68	m5601877 SSP-MG-MG	Brasileira
3	Thiago Filadelfo Miranda	Pesquisador auxiliar, Anilhador Sênior	009.715.865-86	0838007945 SSP-BA	Brasileira

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Ministério do Meio Ambiente - MMA  
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 Sistema de Autorização e Informação em Biodiversidade - SISBIO

### Autorização para atividades com finalidade científica

Número: 12763-5	Data da Emissão: 24/10/2013 21:46	Data para Revalidação*: 23/11/2014
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#### Dados do titular

Nome: Erica Cristina Pacifico de Assis	CPF: 220.210.158-66
Título do Projeto: Monitoramento da Biologia reprodutiva da arara-azul-de-lear <i>Anodorhynchus leari</i> (Aves: Psittacidae) na natureza	
Nome da Instituição : REITORIA DA UNIVERSIDADE DE SÃO PAULO	CNPJ: 63.025.530/0001-04

#### Locais onde as atividades de campo serão executadas

#	Município	UF	Descrição do local	Tipo
1	CANUDOS	BA	RPPN - Reserva Biológica de Canudos	Fora de UC Federal

#### Atividades X Táxons

#	Atividade	Táxons
1	Captura de animais silvestres in situ	<i>Anodorhynchus leari</i>
2	Coleta/transporte de amostras biológicas in situ	<i>Anodorhynchus leari</i>
3	Marcação de animais silvestres in situ	<i>Anodorhynchus leari</i>

#### Material e métodos

1	Amostras biológicas (Aves)	Animal encontrado morto ou partes (carcaça)osso/pele, Fezes, Sangue, Ectoparasita, Penas
2	Método de captura/colela (Aves)	Captura manual
3	Método de marcação (Aves)	Microchip, Anilha de inox

#### Destino do material biológico coletado

#	Nome local destino	Tipo Destino
1	REITORIA DA UNIVERSIDADE DE SÃO PAULO	coleção
2	REITORIA DA UNIVERSIDADE DE SÃO PAULO	coleção
3	REITORIA DA UNIVERSIDADE DE SÃO PAULO	coleção


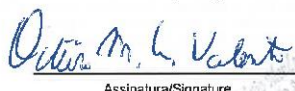
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## Appendix 16. Permits (police documents) CITES.

 <b>REPÚBLICA FEDERATIVA DO BRASIL</b> MINISTÉRIO DO MEIO AMBIENTE - MMA INSTITUTO BRASILEIRO DO MEIO AMBIENTE E DOS RECURSOS NATURAIS RENOVÁVEIS - IBAMA SGEM Tronco 2 - Ed. Sede - Caixa Postal nº 99970 - CEP 051-999 - Brasília-DF		 <b>CONVENÇÃO SOBRE O          COMÉRCIO INTERNACIONAL          DE ESPÉCIES DA FLORA          E FAUNA SILVESTRES          EM PERIGO DE EXTINÇÃO</b>		<b>CONVENTION ON          INTERNATIONAL TRADE          IN ENDANGERED SPECIES          OF WILD FLORA          AND FAUNA</b>		<b>1) Pág. Nº 1/2</b> <b>2) Data Emissão/Issuing Date: 30/12/2014</b> <b>3) Válido Até/Valid Until: 30/06/2015</b>	
<b>4) Licença nº/Permit n°:</b> <b>14BR016156/DF</b>		<b>6) Selo nº/Stamp n°:</b> <b>1237050</b>		<b>8) Controle/Check 1:</b> 1Y3QSPK1PIT3AUHD		<b>9) Autoridade Adm. Emitente/Issuing Management Authority</b>  Assinatura/Signature	
<b>5) Licença de/Permit for</b> <b>Exportação/Export</b>		<b>7) Selo/Stamp</b> <b>1237050</b>					
<b>10) Importador/Importer</b> Erica Cristina Pacifico de Assis Estacion Biológica de Doñana, CSIC, Avda. Americo Vespucio Sevilla - 41092 fone: - Espanha - ES				<b>11) Exportador(Re-exportador)/Exporter(Re-exporter)</b> Erica Cristina Pacifico de Assis Rua Oscar Freire, 2595 apt.52 SAO PAULO - 05409012 fone: 97433480 - ericapacifico81@gmail.com Brasil - BR			
<b>12) País Importador/Country of Import</b> Espanha - ES				<b>13) País Exportador(Re-exportador)/Country of Export(Re-export)</b> Brasil - BR			
<b>14) Objetivo da Operação/Purpose of the transaction</b> S - Científico/Fins científicos...							
<b>15) Condições Especiais/Special Conditions</b> For live animals, this permit or certificate is only valid if the transport conditions conform to the Guidelines for Transport and preparation for shipment of live wild animals and plants or, in the case of air transport, to the IATA Live Animals Regulations.							
A exportadora apresentou TRTM e esclareceu não haver acesso ao patrimônio genético pelo fato de a pesquisa se enquadrar nos itens I e II da Resolução CGEN 21/2006.							
<b>16) Dados do Transporte/Transportation Data</b> Local/Place: ALF/Al São Paulo Data Provável/Probable Date: 26/09/2014							
ESTA LICENÇA É VÁLIDA SOMENTE PARA UMA OPERAÇÃO/ THIS PERMIT OR CERTIFICATE IS ONLY VALID FOR ONE SHIPMENT.							
<b>17) Item</b>		<b>21) Anexo/Origem</b>		<b>18) Produto/Product</b>		<b>19) Quantidade-Unidade Medida/Quantity Unit</b>	
<b>20) Espécie: nome científico</b>		<b>21) Anexo/Origem</b>		<b>22) Descrição: Parte</b>		<b>23) Cód. País de Origem-Comprovante-Data</b>	
<b>Species: scientific name</b>		<b>Appendix/Source</b>		<b>Description: Part</b>		<b>Country of Origin-Permit-Date</b>	
<b>common name</b>				<b>Quantity-Unit-Mark</b>		<b>Country reexportation-Certificate-Date</b>	
17) I				18) SANGUE/BLOOD		19) -- 312,00 ML --	
20) 1. Anodomyrchus leari		21)		22) sangue/blood		23) - -	
Arara-azul-de-leari		I   W		312,00 ML -		24) - -	
Indigo-maracá							
17) II				18) PENAS/ FEATHER		19) -- 714,00 UN --	
20) 1. Anodomyrchus leari		21)		22) penas/ feather		23) - -	
Arara-azul-de-leari		I   W		714,00 UN -		24) - -	
Indigo-maracá							
17) III				18) DNA		19) -- 108,00 UN --	
1* Verificar/Verify: <a href="http://ibama.gov.br/cites/verificar">http://ibama.gov.br/cites/verificar</a> E-mail: <a href="mailto:cites.sede@ibama.gov.br">cites.sede@ibama.gov.br</a> 1ª Via - Original - Importador   Exportador - Brasil   Importer   Exporter - Brazil 2ª Via - Exportador   Importador - Estrangeiro   Exporter   Importer - Other Country 3ª Via - Aduana / Customs 4ª Via - IBAMA							

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