



**Understanding the effects of conservation management,
inbreeding and diet on reproductive fitness in a
threatened bird species**

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reproductive fitness in a threatened bird species

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Abstract

Understanding the factors that influence reproductive success is crucial for effective conservation of threatened species. Inbreeding depression poses a significant threat to small populations, with its effects often varying across life stages and population contexts. Similarly, diet composition can substantially impact reproductive output, particularly in degraded habitats where natural food resources may be limited. While supplementary feeding can help overcome resource limitations, its broader effects on host and population health, including host-microbe relationships, remain poorly understood. Recent methodological advances now enable detailed examination of these factors: lethal equivalents can provide standardised measures of inbreeding effects, DNA metabarcoding can reveal unprecedented resolution of dietary patterns, stable isotope analysis can quantify supplementary food consumption at the individual level, and genome-resolved metagenomics can characterise gut microbial communities. Together, these approaches offer new opportunities to examine how genetic and dietary factors influence population recovery.

The Mauritius parakeet (*Alexandrinus eques*) provides an ideal study system for investigating these dynamics, having recovered from 20 individuals in the 1980s to several hundred birds across two populations (North and South) with different management intensities. Using long-term breeding data (2000-2020), we found significant inbreeding effects on hatching but not fledging success, with lethal equivalents differing between populations (North: 2.40, South: 4.39). DNA metabarcoding revealed 83 plant taxa in the diet, with higher native species richness in the North population despite historical habitat degradation. Stable isotope analysis

showed that supplementary food constituted a significant portion of the diet and boosted reproductive success, though higher consumption in the North (84.2%) compared to the South (29.4%) did not translate to significantly higher reproductive success. Additionally, an endemic plant species was a significant positive predictor of reproductive success in the South, whilst supplementary food species were positive predictors in the North. Preliminary genome-resolved metagenomic analysis revealed population-specific differences in gut microbiota composition, with higher microbial diversity in the South and a potential relationship between supplementary feeding and reduced microbiome diversity.

This research reveals population-specific patterns in how dietary composition and genetic factors influence reproductive success in a threatened species. The populations showed contrasting relationships between diet and breeding output: supplementary food was crucial in the North, while endemic plant consumption was more important in the South. Despite higher lethal equivalents, the South population maintained higher reproductive success. These findings demonstrate how genetic and dietary factors can interact differently across populations, highlighting the importance of comprehensive monitoring and locally adapted conservation strategies.

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Chapter 1 Introduction

Small populations face complex challenges that can accelerate their decline toward extinction (Peters, 1987; Wissel et al., 1994; Nordstrom et al., 2023). In recovering populations, key demographic parameters such as reproductive success, survival, recruitment, and dispersal drive population dynamics (Lack, 1955; Newton, 1998), with these parameters often influenced by interacting threats such as inbreeding depression and limited food availability (Newton, 1998; Krebs, 2002; Newton, 2007).

Recent methodological advances have enhanced our ability to understand how these threats influence demographic parameters. Standardised measures of genetic load through lethal equivalents enable comparisons of inbreeding effects across species and life stages (Nietlisbach et al., 2019). DNA metabarcoding provides unprecedented resolution of dietary patterns and resource use (Pompanon et al., 2012), while stable isotope analysis allows individual-level measurement of supplementary feeding use (Robb et al., 2011). Genome-resolved metagenomics now enable detailed characterisation of gut microbial communities and their metabolic functions, allowing study of how gut microbiota composition and function may influence host health and population recovery (West et al., 2019; Setubal, 2021; Bodawatta et al., 2022). Together, these approaches offer opportunities to examine how genetic factors, diet composition, and conservation interventions like supplementary feeding combine to influence population recovery.

These interactions are particularly crucial for island-endemic species, which have evolved in isolation and may be especially vulnerable to anthropogenic threats

(Frankham, 1997; Dwyer et al., 2021; Matthews et al., 2024). Small, island-endemic populations frequently face combined pressures from historical bottlenecks, habitat degradation, and disrupted ecological relationships. These populations are valuable systems for studying how inbreeding depression manifests, and how dietary adaptations and management interventions can support their recovery in a modified landscape (Matthews et al., 2024).

Once reduced to less than 20 individuals in the 1980s, the Mauritius parakeet's recovery and long-term monitoring provide a unique opportunity to understand the factors affecting reproductive success in a recovering threatened species. This thesis investigates how inbreeding depression, diet composition, and supplementary feeding influence reproductive success in this once critically endangered parrot (IUCN, 2019). Additionally, it provides the first characterisation of gut microbiota composition and function in this species.

1.1 Inbreeding depression

Small populations face two distinct genetic threats: the gradual erosion through drift of genetic variation necessary for adaptive evolution, and the accumulation of deleterious mutations as selection becomes less effective in the declining population. While both processes tend to be gradual, inbreeding can act swiftly to reduce fitness through increased homozygosity, posing a significant threat particularly given ongoing global fragmentation and population declines (Frankham & Ralls, 1998; Angeloni et al., 2011; Neaves et al., 2015; Stoffel et al., 2021).

The manifestation of inbreeding depression - reduced fitness resulting from the expression of deleterious recessive alleles - can significantly impact population viability

and recovery potential (Frankham, 2010; Hedrick & Garcia-Dorado, 2016). This effect occurs because inbreeding increases homozygosity, potentially exposing previously masked deleterious recessive alleles that reduce fitness (Keller & Waller, 2002). The severity of inbreeding depression typically increases with population isolation and decline, as mating between close relatives becomes more likely, gene flow is reduced, and genetic drift – leading to the fixation of deleterious alleles (Wang et al., 1999; Keller & Waller, 2002; Mathur & DeWoody, 2021). However, expression of inbreeding depression can vary substantially across different life stages, with effects often depending on environmental conditions (Wissel et al., 1994; Frankham & Ralls, 1998; Angeloni et al., 2011).

1.1.1 Quantification of inbreeding depression

Studies combining pedigree data with fitness traits have enhanced our ability to detect inbreeding depression in wild populations (Keller & Waller, 2002). Quantifying inbreeding depression typically involves either pedigree-based approaches, which calculate inbreeding coefficients (F) from known family relationships, or molecular methods that use genetic markers to assess relatedness between breeding pairs (Townsend & Jamieson, 2013). While these methods can produce similar results, their application depends on data availability - pedigrees may be incomplete in wild populations, while genetic approaches require adequate funding and sampling (Townsend & Jamieson, 2013).

In recent years, the characterisation of inbreeding load through lethal equivalents has provided standardised measures for comparing inbreeding depression between species (Nietlisbach et al., 2019; Bertorelle et al., 2022) and across different life stages (Grueber

et al., 2010; Nietlisbach et al., 2019). Lethal equivalents quantify the genetic load carried by a population - essentially measuring the number of lethal or severely deleterious alleles that would cause death if homozygous. When populations become small and inbred, increased homozygosity leads to the expression of this load (Caballero et al., 2017; Kyriazis et al., 2020), which manifests as reduced survival, compromised immune function, and lower reproductive fitness (Spielman et al., 2004a, 2004b; Brekke et al., 2010; White et al., 2015).

1.1.2 Variation in inbreeding effects on different life stages

Studies have shown that inbreeding and inbreeding depression are more prevalent in wild populations than previously realised (Keller & Waller, 2002; Frankham, 2010). This is evident across many fitness traits – inbreeding significantly affects birth weight and early survival (Coltman et al., 1998; Coulson et al., 1998, 1999), reproductive output (Miller, 1994; Madsen et al., 1999; Van De Castele, 2003), and resistance to environmental stress (Miller, 1994; Fox & Reed, 2011). These effects can directly impact population persistence (Keller & Waller, 2002), making it a key concern in conservation.

The severity of inbreeding depression can also vary substantially between life history stages (Huisman et al., 2016; Nietlisbach et al., 2019; Trask et al., 2021). For example, inbreeding may impact hatching and fledging success differently (Van De Castele, 2003), with studies across bird species revealing complex patterns. In the Guam kingfisher, Trask et al. (2021) found high inbreeding depression in hatch success (8.43 lethal equivalents) but no significant effects on first- and second-year survival. Similar life stage-specific effects have been documented in other threatened birds: the New Zealand hihi showed 1.03 lethal equivalents for male offspring survival (Brekke et al.,

2010), while Hawaiian crows exhibited 6.9 lethal equivalents for survival to 2 years (Hoeck et al., 2015).

These variations in inbreeding effects across life stages have important implications for conservation management. Reductions in hatch success can indicate issues with fertility and embryo development (Heber & Briskie, 2010), while fledging success might reflect the effectiveness of parental care (Szulkin et al., 2007). Understanding where inbreeding effects are strongest can help target conservation interventions more effectively and improve predictions of population viability (Keller & Waller, 2002; Pierson et al., 2015).

1.2 The role of diet in conservation

Understanding dietary patterns (Pompanon et al., 2012; Hoenig et al., 2022) and their impact on reproductive success (Tollington et al., 2019; Goldberg et al., 2020; Shutt et al., 2020) has become increasingly important in conservation biology, particularly for small populations where optimising breeding success is critical for recovery. Diet analysis can offer valuable insights into resource use and help evaluate the effectiveness of conservation interventions, such as supplementary feeding (Tollington et al., 2019). This understanding is especially important when managing populations in degraded habitats where natural food resources may be limited.

Supplementary feeding has emerged as a widely used conservation tool across various taxa, typically implemented when natural resources are limited, or where managers aim to enhance reproductive output (Ewen et al., 2015; Murray et al., 2016). In avian species, supplementary feeding can improve reproductive success through several mechanisms: enhancing female condition, leading to increased egg production, improving chick growth rates, and increasing fledging success (Robb et al., 2008; Harrison et al., 2010;

Ruffino et al., 2014). In addition to direct nutritional benefits, supplementary feeding can boost individual resistance to disease and parasites (Knutie, 2020) and can serve as a practical method for administering medications or vaccines when necessary (Murray et al., 2016). This technique has been crucial in many conservation successes, including the recovery of the New Zealand Kakapo (*Strigops habroptilus*) (Elliott et al., 2001; Clout et al., 2002), the California Condor (*Gymnogyps californianus*) (Walters et al., 2010), and the New Zealand hihi (*Notiomystis cincta*) (Castro et al., 2003; Doerr et al., 2017).

However, supplementary feeding programmes require careful monitoring due to potential unintended consequences. For example, supplementary feeding can disproportionately benefit certain individuals within populations (Tollington et al., 2019), potentially resulting in skewed sex ratios or imbalanced age structures (Clout et al., 2002; Robb et al., 2008). The clustering of individuals at feeding stations may accelerate the transmission of diseases (Robb et al., 2008; Murray et al., 2016) and increase stress levels in some species (Shochat et al., 2004; Faeth et al., 2005; Forristal et al., 2012). Furthermore, supplementary feeding areas can provide easy access for predators, thereby heightening predation risk for certain species. A significant concern is the inadvertent support of non-target species, particularly invasive ones (Galbraith et al., 2015), which can exacerbate existing conservation challenges.

Behavioural changes resulting from supplementary feeding present a different set of challenges. Such changes can have detrimental effects on population structure and may reduce individual hunting or foraging behaviours (Robb et al., 2008). This raises the risk of species becoming reliant on artificial resources, and a concomitant requirement for continued investment in management (Ferrière et al., 2021). Consequently, assessing

the effectiveness of supplementary feeding requires comprehensive monitoring strategies to identify and address these potential negative impacts.

Diet analysis serves as a valuable tool to aid assessment of the impacts of supplementary food provision, which is important for conservation projects with limited funding that must be directed to provide maximum benefit (Ewen et al., 2015). However, accurately quantifying the benefits of supplementary feeding requires careful monitoring of both the consumption of supplementary food and its relationship with reproductive parameters. Historically, assessing the effectiveness of supplementary feeding has posed challenges, particularly in measuring actual food consumption by individuals. Traditional methods, such as weighing the food removed from feeders, can be misleading, providing only population-level data rather than insights at the individual level (Robb et al., 2008; Hoenig et al., 2022).

Recent advances in techniques such as stable isotope analysis (SIA) have improved our ability to quantify individual supplementary food consumption and its relationship with breeding success (Robb et al., 2011; Tollington et al., 2019). Similarly, DNA metabarcoding enables detailed analysis of natural diet composition with unprecedented taxonomic resolution, potentially identifying the occurrence of individual species in the diet (Moorhouse-Gann et al., 2018; Ando et al., 2020). These methods can enhance our understanding of how specific aspects of diet at an individual-level can impact fitness, and can reveal individual- and population-level foraging patterns (Goldberg et al., 2020).

1.3 Stable isotope analysis

Supplementary feeding programs are critical to many conservation efforts, and stable isotope analysis (SIA) provides a robust method for evaluating their effectiveness (Robb et al., 2011). SIA has been utilised in dietary analysis of animal diets for decades, with recent advancements enabling increased taxonomic resolution (Crawford et al., 2008; Robb et al., 2008; Resano-Mayor et al., 2014). This method is particularly valuable for quantifying the contribution of supplementary food to wildlife diets, as anthropogenic food sources often have distinct isotopic signatures compared to natural foods (Hobson, 1999; Inger & Bearhop, 2008). A key advantage of SIA is its ability to provide temporally integrated dietary information through analysis of different tissue types: bone may indicate diet over the course of a year, hair over a few months, muscle over several weeks, and blood plasma over several days (Fry, 2006; Inger & Bearhop, 2008; Boecklen et al., 2011).

The technique relies on variations in naturally occurring isotopes - chemical elements with varying numbers of neutrons. For biologically important elements (Hydrogen, Carbon, Nitrogen, Oxygen, and Sulphur), the lightest stable isotope is typically the most abundant. The process of isotopic fractionation, where isotope ratios change during chemical reactions and diffusion, creates distinct isotopic 'signatures' in different organisms and habitats. Using mass spectrometry, the ratio of heavy to light isotopes can be measured and expressed in delta (δ) notation as parts per thousand (‰), standardised against international reference samples (Hobson, 1999; Inger & Bearhop, 2008). Most commonly, stable isotopes of nitrogen and carbon are used in diet analysis (Nielsen et al., 2018).

Continuous-flow isotope-ratio mass spectrometry has enabled faster, more cost-effective stable isotope analysis in dietary studies (Layman et al., 2007; Inger & Bearhop, 2008; Traugott et al., 2013). Further, the development of Bayesian isotopic mixing models has significantly enhanced our ability to estimate diet composition and account for uncertainty in model estimates (Parnell et al., 2010). These models can incorporate multiple dietary sources and account for various factors influencing isotopic signatures. Studies applying SIA to assess supplementary feeding have revealed important insights for conservation management. In garden birds, SIA enabled quantification of individual variation in supplementary food use, showing that artificial food contributed between 6% and 26% of individual diets – variation that would have been impossible to detect through traditional observation methods (Robb et al., 2011). Similar approaches have helped assess resource use in threatened species, from quantifying vultures' use of landfill sites (Tauler-Amettler et al., 2018), to evaluating dietary shifts and their impact on reproductive fitness in seabirds (Kowalczyk et al., 2019).

1.3.1 Limitations and methodological considerations

Successful implementation of different methods requires careful consideration of several factors. The isotopic composition of potential diet items must be well-characterised, requiring prior knowledge of the target species' diet. Uncertainty can arise when isotopic signatures of potential food items have not been characterised, or when there is insufficient variation in stable isotope ratios between dietary sources (Inger and Bearhop, 2008). It is also important to include trophic fractionation factors, which help to account for variation in tissue turnover rates (Hobson & Clark, 1992; Hobson, 1999; Bearhop et al., 2002).

These constraints often necessitate combining SIA with other dietary analysis methods to provide comprehensive understanding of species' diets and the effectiveness of supplementary feeding programmes. Despite these challenges, stable isotope analysis remains a valuable tool for understanding wildlife diets and evaluating conservation interventions like supplementary feeding.

1.4 DNA metabarcoding

Before recent developments in molecular analysis, dietary studies typically relied on direct observation of feeding behaviours or morphological examination of faeces by eye or under microscope (Alonso et al., 2015; Egeter et al., 2015; Nielsen et al., 2018). While these traditional methods provide valuable insights, they are limited by the ability to observe cryptic feeding events, require high levels of taxonomic expertise, and can be biased toward species with identifiable indigestible parts (Davison et al., 2002).

DNA metabarcoding has revolutionised dietary analysis by combining DNA-based taxonomic identification with high-throughput sequencing, providing a rapid, high-resolution method for analysing mixed-species samples (Taberlet et al., 2012). This technique employs universal primers to PCR-amplify short DNA barcodes from mixed species samples, which are then sequenced using high-throughput platforms, generating thousands to millions of sequence reads (De Sousa et al., 2019; Ruppert et al., 2019). A key advantage is the ability to detect DNA from species that may not be visually identifiable due to digestion, while providing higher taxonomic resolution than stable isotope analysis and enabling the processing of multiple samples simultaneously.

1.4.1 Technical workflow and implementation

DNA metabarcoding typically follows several key steps from sample collection through to taxonomic assignment (**Figure 1.1**). The choice of DNA barcode regions is crucial and varies by target taxa. For animals, the standard barcode is typically the 658 bp region in the mitochondrial cytochrome c oxidase 1 (CO1) gene (Hebert et al., 2004; Kress & Erickson, 2008; Kress et al., 2015). Plant identification often requires multiple regions, including plastid regions (rbcL and matK), the non-coding spacer trnH-psbA, and the internal transcribed spacer (ITS) region (Chase et al., 2007; CBOL, 2009; Yao et al., 2010; Moorhouse-Gann et al., 2018). Primers must be carefully selected to amplify degraded DNA from faecal or gut samples, maintain consistency within species but variability between species, and include highly conserved flanking regions for universal primer development (Moorhouse-Gann et al., 2018; Ando et al., 2020).

The Illumina MiSeq platform has become the standard for metabarcoding studies due to its high sequencing depth, accuracy, competitive cost, and established bioinformatic pipelines (Coissac et al., 2012). The bioinformatic process involves sequence merging and quality filtering, removal of primers and adaptors, elimination of short reads and singletons, and clustering of sequences into Operational Taxonomic Units (OTUs) or Amplicon Sequence Variants (ASVs) (Chiarello et al., 2022; Jeske & Gallert, 2022; Fasolo et al., 2024). The choice between ASVs and OTUs can significantly influence diversity measures – recent developments in ASV identification offer potentially higher resolution than traditional OTU clustering (Callahan et al., 2016; Chiarello et al., 2022).

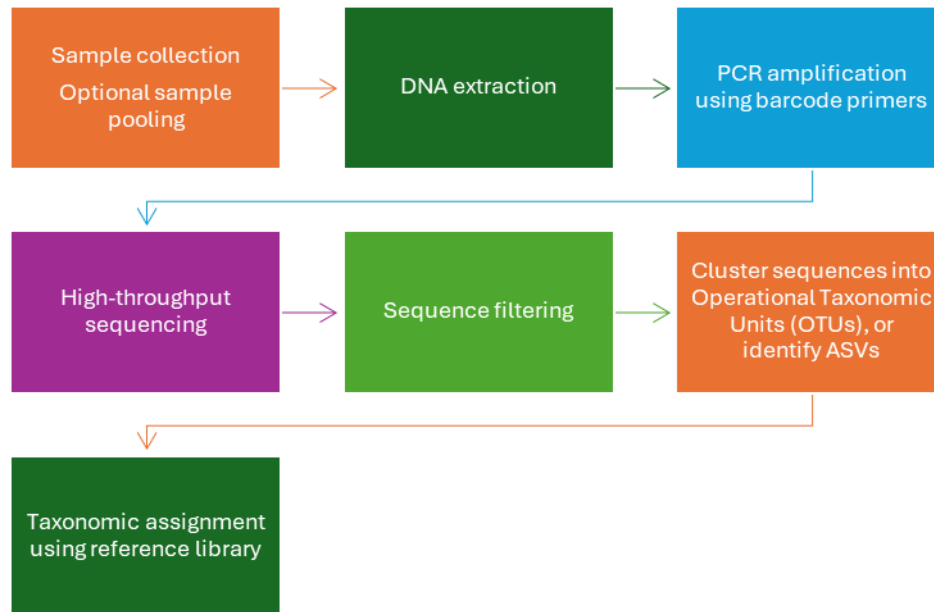


Figure 1.1. A typical DNA metabarcoding workflow from sample collection to taxonomic assignment.

1.4.2 Limitations

The success of metabarcoding relies heavily on comprehensive reference libraries. While platforms like the International Barcode of Life Database (Ratnasingham & Hebert, 2007) provide extensive resources, study-specific libraries often need to be developed when working with poorly characterised taxa or ecosystems (Moorhouse-Gann et al., 2018). Incomplete reference libraries may result in unassigned sequence reads. This limitation highlights the importance of developing and maintaining comprehensive reference databases for accurate taxonomic assignment (Fontes et al., 2021).

Beyond reference library challenges, quantitative interpretation of metabarcoding data presents additional complexities. While relative read abundance has been suggested as a proxy for relative biomass in samples, several factors can influence this relationship, including biological biases during digestion, technical biases during PCR amplification,

variation in DNA preservation, and differences in tissue density between food items (Lamb et al., 2019). To address these limitations, correction factors can be estimated through the analysis of mock communities with known species compositions (Thomas et al., 2016; Lamb et al., 2019). Many studies instead rely on frequency of occurrence across multiple samples to infer dietary importance (Goldberg et al., 2020).

1.5 The role of the gut microbiome in conservation

The gut microbiome plays crucial roles in host health and fitness, influencing digestion, immune function, and disease resistance (McFall-Ngai et al., 2013; West et al., 2019). Understanding host-microbe relationships has become increasingly important in conservation biology, particularly for threatened species facing environmental changes that may disrupt these relationships (McKenney et al., 2018; Bodawatta et al., 2022).

Recent advances in genome-resolved metagenomics have enhanced our ability to characterise microbial communities and their functions. While previous studies relied on 16S rRNA sequencing for taxonomic profiling, metagenomic approaches now enable direct identification of functional genes and metabolic pathways (Frioux et al., 2020; Setubal, 2021; Leonard et al., 2024). This has revealed how habitat modification and conservation interventions can influence gut microbiota composition and function (Amato et al., 2013; San Juan et al., 2020).

Studies of threatened birds have demonstrated important links between gut microbiota and conservation management. Research on the kakapo found that supplementary feeding significantly affected gut microbiome composition (West et al., 2022), while studies of house sparrows showed that captivity and commercial food diets reduced

microbial diversity (Florkowski et al., 2023). These findings suggest that conservation interventions may have unintended consequences for host-microbe interactions.

The relationship between gut microbiota and host health appears particularly important in recovering populations. Threatened species generally show lower bacterial diversity compared to non-threatened species (Huang et al., 2022), potentially affecting their ability to adapt to environmental changes. Understanding these relationships can inform conservation strategies - for instance, studies of the eastern black rhinoceros found links between gut microbiota composition and breeding success (Antwis et al., 2019).

This integration of microbiome research into conservation biology reflects growing recognition that host-microbe relationships may influence population recovery and persistence. As analytical techniques continue to advance, incorporating microbiome analysis into threatened species monitoring could provide new insights for conservation management.

1.6 The Mauritius parakeet as a study system

1.6.1 Historical decline and recovery

The Mauritius parakeet (*Alexandrinus eques*), also known as the Echo parakeet, is a medium-sized (130-210g) parrot endemic to Mauritius and the only extant native Mascarene parrot (Hume, 2007). The species experienced a severe decline beginning in the seventeenth century, reaching a critical low of approximately 20 individuals by the late 1980s, making it the world's rarest parrot (Jones, 1987; Thorsen & Jones, 1998). This decline stemmed from extensive habitat destruction, with native Mauritian forests reduced to just 4% of their original extent (**Figure 1.2**) (Maggs, 2016), alongside other threats including invasive predators (Black rats, *Rattus rattus*, and Crab-eating

macaques, *Macaca fascicularis*) and competition from introduced species for breeding sites (Thorsen & Jones, 1998; Jones & Merton, 2012).

Conservation efforts began in the early 1970s, intensifying in 1987 through collaboration between the Mauritian Wildlife Foundation and the National Parks and Conservation Service. Key interventions included establishing the Black River Gorges National Park, controlling invasive predators, providing artificial nest boxes and supplementary food, and releasing 129 captive-bred individuals between 1997-2005. These efforts proved highly successful, with the population growing to 143 breeding pairs by 2019 (MWF, 2020).

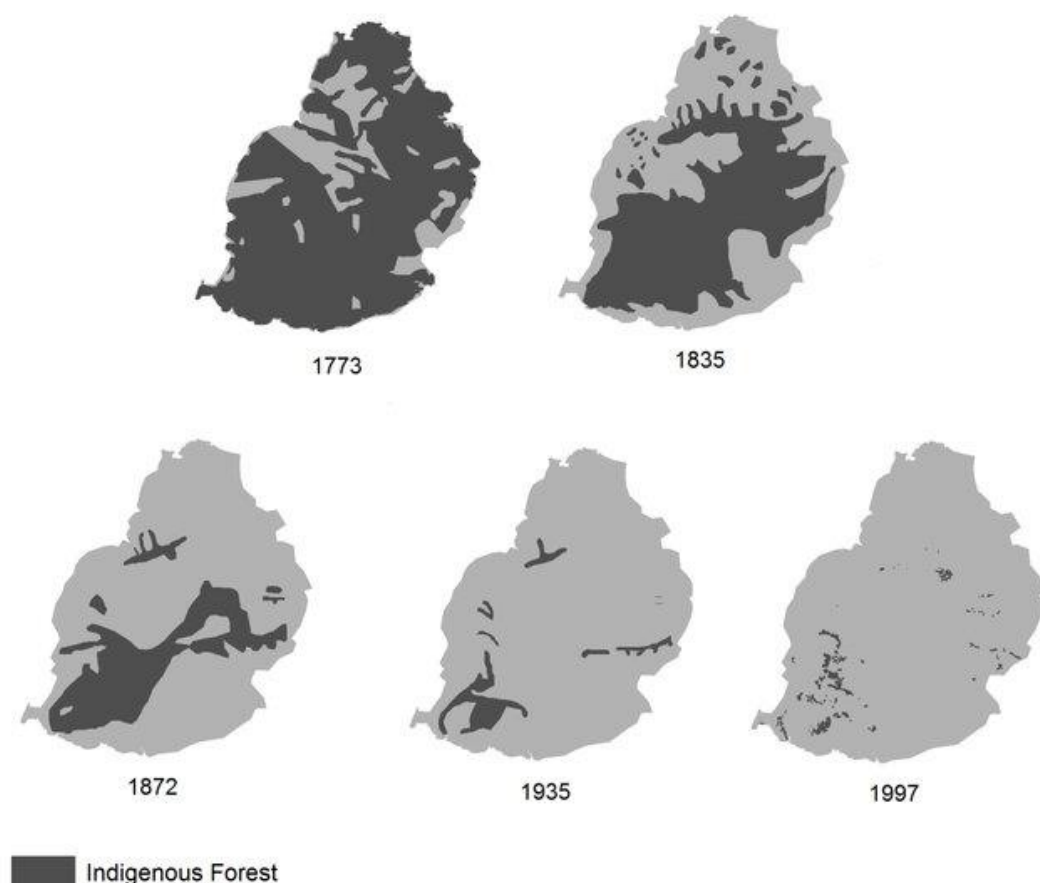


Figure 1.2. Illustration of the deforestation of native forest on Mauritius from 1773 to 1997 (illustration by Maggs, 2016).

1.6.2 Biology and Current Distribution

The species exhibits monogamous pair bonding and typically begins breeding at two years of age. The breeding season extends from October to February. Females lay clutches of one to four eggs and incubate for approximately 25 days while males provide food. After hatching, females remain with chicks for up to two weeks before joining males in foraging duties (Young, 1987; Jones and Duffy, 1993). Natural diet consists primarily of fruits, bark, flowers, and leaves throughout their range (Young, 1987).

Currently, Mauritius parakeets inhabit the Black River Gorges National Park, divided between two distinct areas: 'Grand Gorges' in the North and 'Bel Ombre' in the South. Despite being separated by only 1.8 km, these populations show minimal natural dispersal between them (Raisin et al., 2012; Tollington et al., 2013). The populations occupy areas of differing habitat quality, with five Conservation Management Areas (CMAs) in the North and three in the South. These protected areas, ranging from 0.03 to 0.23 km² in size, are fenced to facilitate invasive plant removal and habitat restoration.

1.6.3 Current Management and Challenges

A key management component is supplementary feeding, with commercial parrot food provided at designated stations - daily in the North and weekly in the South. Mean distances to feeders differ substantially between populations (North: 1.15 km; South: 2.37 km). A significant setback occurred in 2005 with an outbreak of Psittacine Beak and Feather Disease (PBFD), necessitating enhanced biosecurity measures around feeding stations and modified nest management protocols (Kundu et al., 2012; Fogell et al., 2021).

1.6.4 Previous research and context

Long-term monitoring has enabled extensive research on various aspects of the species' recovery. Genetic studies have tracked population structure over time, showing initial genetic differentiation between populations, followed by homogenisation during intensive management, and subsequent re-emergence of genetic differences (Raisin et al., 2012; Tollington et al., 2013). Research has demonstrated short-term negative impacts of PBFD on breeding success, particularly affecting pairs using supplemental feeding stations (Tollington et al., 2015). Studies have also shown that supplementary feeding can improve reproductive success, with birds nesting closer to feeding stations incorporating more supplementary food in their diet (Tollington et al., 2019).

The species' recovery represents one of conservation's most successful interventions for a critically endangered parrot, leading to its downlisting from Endangered to Vulnerable on the IUCN Red List in 2019. Current management focuses on balancing population growth with disease risk through biosecurity protocols, supplementary feeding, and ongoing monitoring of breeding success and population health.

The Mauritius parakeet thus provides an ideal model system for understanding the challenges and opportunities in small population conservation. The species' history of having experienced a severe bottleneck followed by intensive management, offers unique opportunities to study inbreeding effects across different life stages. Its distribution across two geographically distinct populations with varying levels of habitat quality enables investigation of how environment influences diet composition and reproductive success. Additionally, the long-term supplementary feeding program, combined with detailed breeding records and genetic data, allows examination of how

artificial food provision affects breeding success in a threatened species. Together, these characteristics make the Mauritius parakeet particularly valuable for studying the complex interactions between genetics, diet, and reproductive success that are crucial for managing small populations.

1.7 Thesis aims and structure

This thesis investigates the factors influencing reproductive success in the Mauritius parakeet, focusing on three key aspects: inbreeding depression, diet composition, and supplementary feeding effectiveness. By combining genetic analysis, DNA metabarcoding, and stable isotope analysis with long-term breeding data, this research aims to provide insights that can inform conservation management of small populations.

The specific objectives of each research chapter are:

Chapter 2 examines the manifestation of inbreeding depression across different life stages in the recovering population. Using two decades of breeding data (2000-2020) and detailed pedigree information from 376 breeding pairs, this chapter:

- Quantifies inbreeding effects on two different life stages – hatching and fledging success
- Calculates and compares lethal equivalents between the North and South populations

Chapter 3 explores the effectiveness of supplementary feeding using stable isotope analysis. By analysing feather samples from 381 individuals across three breeding seasons alongside breeding records, this chapter:

- Quantifies individual variation in supplementary food consumption
- Examines spatial patterns in supplementary food use
- Investigates relationships between supplementary feeding and reproductive success

Chapter 4 investigates dietary patterns and their impacts on reproductive success using DNA metabarcoding. Through analysis of faecal samples collected across three breeding seasons, this chapter:

- Characterises dietary diversity and composition across populations with different management conditions
- Evaluates relationships between diet composition and reproductive success
- Assesses the influence of Conservation Management Areas on diet composition

Chapter 5 uses genome-resolved metagenomics to perform the first characterisation of gut microbiota in the Mauritius parakeet. Through analysis of faecal samples from both populations, this chapter:

- Reconstructs metagenome-assembled genomes to examine taxonomic composition and functional potential of gut microbial communities
- Investigates differences in microbiome diversity and function between populations
- Examines potential relationships between gut microbiota composition and supplementary feeding practices

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Chapter 2 Life-stage specific inbreeding depression in the Mauritius parakeet

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2.1 Abstract

Inbreeding depression poses a significant threat to small populations, with its effects often varying across life stages and population contexts. We examined inbreeding depression in Mauritius parakeets (*Alexandrinus eques*), which underwent a severe bottleneck to just 20 individuals in the 1980s. Using two decades of breeding data (2000-2020) and detailed pedigree information from 345 breeding pairs across two distinct populations, we quantified inbreeding effects on hatching and fledging success. We revealed strong life stage-specific inbreeding depression, with significant negative effects on hatching success but not fledging success. Lethal equivalents for hatching success differed between populations (North: 2.40, South: 4.39), despite higher overall reproductive success in the South. The absence of detectable inbreeding effects on fledging success could reflect masking effects of current management practices or historical purging of deleterious alleles affecting this life stage. Both populations showed elevated hatching failure rates (30.2% North, 21.2% South) compared to non-inbred bird populations, consistent with patterns observed in other bottlenecked island species. Our findings demonstrate how inbreeding effects can manifest differently across life stages and populations, providing crucial data for future population viability analyses and informing ongoing conservation management of this and other recovering island species.

2.2 Introduction

Inbreeding depression poses a growing threat to biodiversity as habitat fragmentation and population declines force many species into small, isolated populations (Frankham & Ralls, 1998; O’Grady et al., 2006; Willoughby et al., 2015). Understanding and quantifying genetic threats is increasingly critical for effective conservation management, yet studies quantifying direct fitness consequences of inbreeding remain relatively rare (O’Grady et al., 2006; Pierson et al., 2015; Armstrong et al., 2021).

The manifestation of inbreeding depression through deleterious recessive alleles can significantly impact population viability and recovery potential (Frankham, 2010; Hedrick & Garcia-Dorado, 2016). Birds provide particularly well-documented examples, with long-term studies revealing how inbreeding affects different components of fitness across life stages (Szulkin et al., 2007; Keller et al., 2008; Harrison et al., 2019).

Island species are valuable systems for studying inbreeding effects due to their restricted distributions and limited gene flow (Frankham, 1997, 1998; Ando, 2019; Dwyer et al., 2021). Many island endemics have experienced severe population bottlenecks, leading to increased inbreeding rates and loss of genetic diversity (Frankham, 1998; Jamieson, 2011; Leroy et al., 2021; Gautschi et al., 2024). However, fitness consequences of inbreeding in these populations often remain poorly understood, particularly across different life stages (Grueber et al., 2010; Pierson et al., 2015; Nietlisbach et al., 2019; Trask et al., 2021).

Lethal equivalents (LE) have emerged as a standardised measure for comparing inbreeding depression across species and systems (Nietlisbach et al., 2019; Trask et al., 2021; Bertorelle et al., 2022). Several studies document considerable variation in lethal

equivalents across life stages in birds, from 8.43 for hatch success in sihek (Trask et al., 2021), to 0.6 in New Zealand hihi chick survival (Brekke et al., 2010), to 6.9 for chick survival in Hawaiian crows (Hoeck et al., 2015). Many of these studies highlight how inbreeding effects can vary markedly across different life stages - for instance, while the sihek showed high inbreeding depression in hatch success, no significant effects were detected in first- and second-year survival (Trask et al., 2021).

The Mauritius parakeet (*Alexandrinus eques*) presents an opportunity to examine inbreeding depression in an island-endemic bird. Having declined to just 20 individuals in the 1980s (Thorsen & Jones, 1998), the species has recovered to several hundred birds following intensive conservation efforts (MWF, 2020). This recovery has been accompanied by extensive monitoring, providing two decades of detailed reproductive data (Tollington et al., 2015) and a genetically-confirmed pedigree (Raisin et al., 2012). No previous study has examined whether inbreeding depression affects reproductive success in this species.

The species is managed across two distinct populations with very low gene flow between them (Raisin et al., 2012; Tollington et al., 2013). Given documented differences in reproductive success between these populations (Tollington et al., 2019), we hypothesised that inbreeding depression might also vary between them.

This study aims to: (1) Quantify how inbreeding depression manifests across different life stages (hatch and fledge success); (2) Compare inbreeding effects between two geographically distinct populations under different management regimes; (3) calculate and compare lethal equivalents between populations to provide standardised measures for future population viability analyses.

We hypothesise that: (1) Inbreeding effects will be stronger at earlier life stages (hatch success) compared to later stages (fledge success), following patterns observed in other bird species; (2) inbreeding depression will differ between populations due to varying habitat conditions and management intensity; (3) lethal equivalents will be comparable to those found in other bottlenecked island bird populations.

2.3 Methods

2.3.1 Study species

The Mauritius parakeet is a medium-sized, arboreal parrot endemic to Mauritius and the only extant native Mascarene parrot (Hume, 2007). The species experienced a severe decline beginning in the seventeenth century, reaching a low of approximately 20 individuals by the late 1980s, making it the world's rarest parrot (Jones and Duffy, 1993).

Conservation efforts began in the early 1970s, with key interventions including establishing the Black River Gorges National Park, controlling invasive predators, and providing artificial nest boxes and supplementary food. This intensive management initially led to increased genetic homogenisation between populations through captive breeding, brood manipulation and translocations (Raisin et al., 2012). However, following an outbreak of beak and feather disease virus (BFDV) (Kundu et al., 2012; Fogell et al., 2019) management intensity was reduced and genetic differentiation between the two populations persists (Tollington et al., 2013). Conservation efforts proved highly successful overall, with the population growing to 143 breeding pairs by 2019 (MWF, 2020).

Mauritius parakeets exhibit a monogamous mating structure and have an adult lifespan of up to 21 years (MWF, 2020). Individuals typically reach sexual maturity at two years of

age and form breeding pairs with high nest site fidelity (Jones and Duffy, 1993). Pairs breed annually from September to February, with females laying up to four eggs per clutch and incubating for approximately 25 days while males provide food. After hatching, females remain with chicks for up to two weeks before beginning to leave the nest to forage (Young, 1987; Duffy, 1993). Chicks fledge at approximately 53-63 days old (Thorsen & Jones, 1998) and remain with parents for up to three months (Young, 1987). Natural diet consists primarily of fruits, bark, flowers, and leaves throughout their range (Young, 1987). While naturally cavity-nesters, most pairs now use artificial nest boxes (MWF, 2020).

Currently, the population inhabits the Black River Gorges National Park (**Figure 2.1**), divided between two distinct areas in the North and South of the park. Despite being separated by only 1.8 km, these populations show minimal natural dispersal between them (Raisin et al., 2012; Tollington et al., 2013). The populations occupy areas of varying habitat quality, with five Conservation Management Areas (CMAs) in the North and three in the South. These protected areas, ranging from 0.06 to 0.23 km² in size, are fenced to facilitate invasive plant removal and habitat restoration.

A key management component is supplementary feeding, with commercial parrot food provided at feeding stations – daily in the North and weekly in the South. Mean distances to feeders differ between the populations (North: 1.15 km; South: 2.37 km). The two populations have different levels of reproductive success (Tollington et al., 2019), different proportional consumption of supplementary food (Tollington et al., 2019), and different responses to infection by BFDV (Tollington et al., 2019).

2.3.2 Ethics statement

Sampling methods were approved by the University of Kent Research Ethics Advisory Group. All samples were collected by trained field staff at the Mauritian Wildlife Foundation and the National Parks and Conservation Service of the Government of Mauritius, as part of the ongoing conservation management and monitoring programme for the Mauritius parakeet (NPCS permit: NP-46/3V5). Samples were transported from Mauritius to UK under CITES Import Permit 598437/01 and Export Permit MU210193 and APHA Import Permit ITIMP22.0256. Field staff were trained in safe collection of blood samples and minimising bird-handling time to reduce stress on the birds.

2.3.3 Genetic data and calculation of inbreeding coefficients

Pedigree inbreeding coefficients, F , were calculated using the package ‘pedigree’ (Coster, 2010) in R (R Core Team, 2021) to process the Mauritius parakeet pedigree, which covered breeding attempts from 1991 to 2020. The kinship coefficient for a breeding pair is equivalent to the inbreeding coefficient, F , of their offspring.

A microsatellite genotype dataset produced in previous studies comprising 897 individuals using samples collected between 1993 and 2011 (Raisin et al., 2009; Tollington et al., 2013) and genotyped using a suite of 16 microsatellite markers was extended to include an additional 99 individuals that were recorded as breeding adults between 2011 and 2017. This molecular analysis brought the total individual genotypes to 996, spanning 1993 to 2017. The genotype dataset was used alongside the pedigree to validate the identity of observed breeding pairs and to fill in gaps in the studbook using the software Cervus (Marshall et al., 1998; Kalinowski et al., 2007). Parentage assignments by Cervus were validated individually for plausibility using field sighting

records. This procedure verified observed pairings and led to 9 newly assigned dams and 21 sires. The updated studbook was used for all subsequent calculation of individual inbreeding coefficients and pair kinship coefficients.

2.3.4 Life history and reproductive fitness data

Breeding data consisted of individual breeding attempts spanning 21 breeding seasons from 2000 to 2020 ($N_{\text{broods}}=2116$). Each breeding attempt details the sire and dam identified at the nest site by their unique ring combinations, year of the attempt, nest site location, number of eggs laid, number of chicks hatched, and number of chicks fledged by the end of the breeding attempt. This data was combined with potential predictors of hatch and fledge success: population, dam and sire individual inbreeding coefficients, distance to feeding station, and pair kinship.

Breeding pairs may make a second breeding attempt in the same season if the first clutch fails; for consistency, we analysed only the first breeding attempt in a season. Breeding pairs with missing information, for example with only one parent identified, were excluded from analysis. In total, 1293 first clutch broods with both parents identified and full breeding data (eggs, number of chicks, and number of chicks fledged) were included, featuring 376 unique breeding pairs and corresponding lifetime reproductive parameters. Following methods outlined in the 'pedigree' package (Coster, 2010), analyses were restricted to breeding pairs with at least two generations of ancestry in the pedigree to ensure reliable kinship estimates, reducing the dataset to 345 breeding pairs.

For each unique breeding pair, two binomial response variables were constructed to facilitate analysis with binomial GLMMs: (i) lifetime hatch success and (ii) lifetime fledge success. For lifetime hatch success, the binomial numerator was the number of

successes (i.e. the number of hatchlings) and the denominator was the number of failures (i.e. the number of eggs that did not hatch). For lifetime fledge success, the binomial numerator was the number of fledglings, and the binomial denominator was the number of chicks that did not fledge.

2.3.5 Statistical analysis

Generalised linear mixed models (GLMMs) were fitted using the package 'lme4' (Bates et al., 2015) with a binomial error structure and logit-link function. Models examined the effects of population (North or South), pair kinship (equivalent to offspring F), distance to nearest feeding station (km), Dam F, Sire F, presence of nest site within a CMA, and interactions between population and each covariate. Dam and sire individual inbreeding coefficients were included in addition to pair kinship as parent and offspring inbreeding levels do not necessarily correlate (Falconer & Mackay, 2009; Reid & Keller, 2009; Hoeck et al., 2015). To account for the non-independence of having the same individuals appearing in multiple breeding pairs, Pair ID was included as a random effect.

Prior to analyses, data exploration was performed and variance inflation factors (VIFs) calculated following Zuur et al. (2009). All predictors were standardised to avoid potential biases associated with multicollinearity (Cade, 2015). Model selection and averaging were performed using the 'MuMIn' package in R (Bartoń, 2023), employing an information-theoretic approach based on AICc and restricting the final model set to those within $\Delta AICc < 7$ (Burnham & Anderson, 2004; Burnham et al., 2011). Model-averaged parameter estimates, standard errors, and 95% confidence intervals were calculated. Significance of model-averaged coefficients was assessed using 95%

confidence intervals, and we calculated AICc-weighted t-statistics for each model-averaged coefficient as measures of relative variable importance (Cade, 2015).

2.3.6 Calculation of lethal equivalents

Following the identification of significant population differences in both hatch success ($p < 0.0001$) and fledge success ($p < 0.05$), separate population-specific models were fitted. These models were used alongside Equation 1, following methods of Grueber et al. (2010), to calculate lethal equivalents. Briefly, conditional survival estimates S_0 , at $F=0$, and S_f , at $F=0.25$ were calculated. These estimates were then used in the equation for inbreeding load to calculate lethal equivalents ($LE = 2B$) (Morton et al., 1956; Grueber et al., 2011).

$$\textbf{Equation 1: } B = -\ln(S_f / S_0) / f$$

B = inbreeding load

S_f = predicted survival at $F=0.25$

S_0 = predicted survival at $F=0$

2.4 Results

2.4.1 Inbreeding and lifetime fitness

Analysis included 345 breeding pairs (240 North, 105 South). Both populations showed substantial hatching failure, with mean hatching success significantly lower in the North (69.8%) compared to the South (78.8%; Wilcoxon rank-sum test, $W=15839$, $p < 0.0001$). Fledging success followed a similar pattern, being lower in the North (76.7%) than the South (80.9%; Wilcoxon rank-sum test, $W=14221$, $p < 0.05$) (**Figure 2.2**).

Pair kinship strongly predicted lifetime hatching success (estimate = -0.57, 95% CI: -0.84 to -0.30), with higher kinship associated with reduced hatching success (**Table 2.1**). Population also significantly influenced hatching success, being lower in the North (estimate = -0.61, 95% CI: -0.85 to -0.38). Distance to feeder negatively impacted hatching success (estimate = -0.18, 95% CI: -0.26 to -0.10). No other predictors or their interactions with population were significant, as indicated by confidence intervals overlapping zero.

Distance to feeder strongly predicted lifetime fledging success (estimate = -0.77, 95% CI: -1.17 to -0.36), with greater distances associated with reduced fledging success (**Table 2.2**). Unexpectedly, female inbreeding coefficient (Dam F) positively influenced fledging success (estimate = 0.46, 95% CI: 0.09 to 0.88). Population had a significant effect, with lower success in the North (estimate = -0.60, 95% CI: -1.09 to -0.13). No other predictors or their interactions with population were significant.

2.4.2 Lethal equivalents

Population-specific calculations revealed substantial differences in lethal equivalents between life stages and populations. With hatching success, the North population had 2.4 lethal equivalents (95% CI: 1.44 to 3.54) while the South showed nearly double that at 4.39 (95% CI: 0.66 to 12.54). In contrast, lethal equivalents based on fledging success were not significantly different from zero in either population (North: 0.51, 95% CI: -1.01 to 0.68; South: 0.65, 95% CI: -0.79 to 6.76)

2.5 Discussion

Our study revealed strong life stage-specific inbreeding depression in the Mauritius parakeet, with pronounced effects on lifetime hatching but not fledging success,

contributing to a growing body of research showing variable inbreeding depression across different life stages (Grueber et al., 2010; Huisman et al., 2016; Nietlisbach et al., 2019; Trask et al., 2021). This pattern aligns with evidence from other threatened birds where genetic load manifests most strongly in early life stages (Hemmings et al., 2012; White et al., 2015).

2.5.1 Life stage-specific inbreeding depression

The high hatching failure rates we observed (30.2% North, 21.2% South) exceed typical rates of 10% in non-inbred bird populations (Koenig, 1982; Briskie & Mackintosh, 2004). These rates mirror other bottlenecked populations (Briskie & Mackintosh, 2004) - for example, takahe show 31% egg failure rates (Jamieson & Ryan, 2000). A recent meta-analysis found average hatching failures of 16.8% across threatened birds compared to 12.4% in non-threatened species (Marshall et al., 2023).

The strong effect on hatch success, but not fledge success, suggests that genetic load is primarily impacting early developmental processes, a pattern recognised across diverse taxa (Heber & Briskie, 2010; Hemmings et al., 2012). In birds, hatching failure is a common manifestation of inbreeding depression (Koenig, 1982; Briskie & Mackintosh, 2004; Heber & Briskie, 2010).

The absence of detectable inbreeding effects on fledging success could have multiple explanations. Supplementary feeding boosts productivity in this species (Tollington et al., 2019) and may mask genetic effects, supported by our finding that increased distance from feeding stations reduces fledging success. Environmental stress typically amplifies genetic effects (Marr et al., 2006; Fox & Reed, 2011). Food supplementation could be reducing environmental stress and therefore masking underlying genetic load. This

masking effect has implications for conservation management, as populations appearing viable under supplemented conditions may have inbreeding load that could manifest under more challenging conditions (Hedrick & Garcia-Dorado, 2016).

Our finding of a positive relationship between female inbreeding coefficient and fledging success was unexpected, as it contradicts typical patterns in birds where maternal inbreeding reduces offspring fitness, as demonstrated in song sparrows and Seychelles warblers (Richardson et al., 2004; Marr et al., 2006). Similar findings emerged in Chatham Island black robins, where highly inbred mothers had improved offspring survival when paired with closely related males (Weiser et al., 2016). This pattern could reflect selective survival creating a robust breeding population of inbred females, or unmeasured correlations with habitat quality and mate choice.

One potential explanation is that inbred females who survived to breeding age may carry a lower genetic load (Keller & Waller, 2002; Weiser et al., 2016), though this would require validation through simulations (Pierson et al., 2015; Hedrick & Garcia-Dorado, 2016). While historical purging of deleterious alleles affecting post-hatching survival is possible (Hedrick & Garcia-Dorado, 2016; Caballero et al., 2017), this would be expected to affect the entire population rather than just inbred females (Dussex et al., 2023).

2.5.2 Lethal equivalents

The lethal equivalents we detected for hatch success (2.40-4.39) exceed values in some island birds like New Zealand hihi (1.03 LEs; Brekke et al., 2010) but fall below highly bottlenecked species like the Guam kingfisher (8.43 LEs; Trask et al., 2021). These moderate values suggest some population resilience despite the severe historical bottleneck.

However, our estimates likely represent only a portion of total inbreeding load. We calculated lethal equivalents for hatch and fledge success, but further life stages may have additional genetic load. Studies across taxa have demonstrated how measuring effects at single life stages may substantially underestimate total genetic burden. In song sparrows, 1.4 LEs were found for early survival compared to 24.6 LEs for total fitness (Keller, 1998). In great tits, 0.4 LEs were identified for hatching success and 2.1 for cumulative effects from egg stage to recruitment into the breeding population (Szulkin et al., 2007).

The implications of these lethal equivalent values for population viability require careful consideration. Previous research has shown that incorporating lethal equivalents into population viability analyses can significantly alter predictions of extinction risk (O'Grady et al., 2006; Trask et al., 2021).

2.5.3 Population differences

The contrasting patterns between the two populations – higher lethal equivalents but higher reproductive success in the South – suggest that factors beyond inbreeding are influencing reproductive success. The North shows higher population density (109 versus 52 breeding pairs), more frequent supplemental feeding, and higher artificial nest box density (MWF, 2020). The North also has a higher occurrence of invasive and non-native plants (Lorence & Sussman, 1986; Page & D'Argent, 1997; Florens et al., 2016). However, this population also has a higher number of CMAs (five versus three in the South) and DNA metabarcoding revealed a higher occurrence of native species in the diet of birds in the North (see Chapter 4).

Complex interactions between population density and habitat quality could explain the differences between populations. In Mauritius kestrels, juvenile survival rates remained stable until a threshold population density was reached, after which it declined (Nicoll et al., 2003). Studies across multiple bird species have demonstrated density-dependent effects on reproductive success having time-delayed responses (Paradis et al., 2002), further complicating our understanding of these relationships.

2.5.4 Conservation implications and future research

Our findings highlight the need for targeted monitoring and intervention strategies. Conservation programs should implement life stage-specific monitoring to identify where inbreeding effects manifest most strongly (Grueber et al., 2010; Trask et al., 2021). Early life stages require particular attention, where genetic load often shows strongest effects (Hemmings et al., 2012; White et al., 2015).

Such monitoring can inform the development of stage-specific interventions, such as artificial incubation for populations with high hatching failure, or targeted genetic rescue methods (Hedrick & Garcia-Dorado, 2016). Long-term population viability analyses incorporating our measured lethal equivalents, instead of the standard of 3.14 (Lacy, 2000), could be valuable to predict future population trajectories and evaluate management scenarios for each Mauritius parakeet population.

The differential expression of inbreeding depression between populations demonstrates the importance of population-specific management approaches. Genetic load can vary substantially between populations due to differences in population size, migration rates, and demographic history (Whitlock, 2002; Theodorou & Couvet, 2006). Conservation management strategies should consider local population dynamics, degree of isolation

(especially in populations where habitat fragmentation may be creating or reinforcing population structure), and historical bottleneck effects (Hedrick & Garcia-Dorado, 2016; Frankham, 2015).

Further research should investigate inbreeding effects on other life stages – while our study focused on hatching and fledging success, genetic load may also impact first-year survival, adult survival, age at first breeding, and lifetime reproductive success (Keller, 1998; Szulkin et al., 2007; Trask et al., 2021). Quantifying lethal equivalents across additional life stages would provide a more complete picture of inbreeding depression in this species and allow more accurate population viability modelling. Additionally, detailed assessment of management practices is warranted, particularly the role of supplementary feeding in potentially masking inbreeding effects.

2.6 Conclusion

This first documentation of inbreeding depression in the Mauritius parakeet reveals important life-stage specific effects that have direct implications for conservation management. The significant impact on hatch success, but not fledging success, suggests that management efforts should focus on supporting successful hatching. While detected lethal equivalents indicate substantial genetic load, their moderate values relative to other bottlenecked species suggest that the population has maintained some resilience despite its severe historical bottleneck. The estimated lethal equivalents may be used in future population viability analyses to help inform the ongoing conservation management of this recovering species.

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2.9 Tables and Figures

Table 2.1. Model-averaged coefficients for lifetime hatch success, with standard errors (SE), 95% confidence intervals, and variable t-statistics (absolute, ratio, and variance). Predictors are ordered by weighted t-statistics as a measure of relative variable importance (Cade, 2015; Robinson et al., 2017). Coefficient estimates where confidence intervals do not cross zero are in bold.

Variable	Estimate	SE	2.50%	97.50%	t.abs	t.ratio	var.t
(Intercept)	1.27	0.09	1.1	1.44	-	-	-
Kinship	-0.57	0.14	-0.84	-0.3	4.27	0.97	0.04
Pop (North)	-0.91	0.22	-1.35	-0.47	2.11	0.48	0.22
Distance to feeder	-0.34	0.17	-0.67	-0.04	1.95	0.44	0.13
Pop:Kinship	0.5	0.4	-0.02	1.3	1.31	0.29	0.25
SireF	0.39	0.36	-0.08	1.15	1.22	0.27	0.45
Pop:SireF	-1.25	1.14	-3.62	-0.03	1.19	0.26	0.49
Pop:Distance to feeder	0.54	0.47	-0.03	1.51	1.12	0.25	0.37
DamF	-0.03	0.09	-0.4	0.18	0.26	0.06	0.11
Within CMA	-0.07	0.25	-1.14	0.59	0.2	0.05	0.17
Pop:DamF	0.05	0.2	-0.19	1.27	0.2	0.04	0.19
CMA:Pop	0.22	0.78	-0.53	4.77	0.17	0.04	0.18

Table 2.2. Model-averaged coefficients for lifetime fledge success, with standard errors (*SE*), 95% confidence intervals, and variable *t*-statistics (absolute, ratio, and variance). Predictors are ordered by weighted *t*-statistics as a measure of relative variable importance (Cade, 2015; Robinson et al., 2017). Coefficient estimates where confidence intervals do not cross zero are in bold.

Variable	Estimate	SE	2.50%	97.50%	t.abs	t.ratio	var.t
(Intercept)	1.54	0.1	1.35	1.73	-	-	-
Distance to feeder	-0.77	0.21	-1.17	-0.36	3.7	1	0.06
DamF	0.46	0.22	0.09	0.88	2.26	0.61	0.03
Pop (North)	-0.6	0.25	-1.09	-0.13	1.47	0.39	0.9
Pop:Kinship	0.08	0.24	-0.27	1.3	0.23	0.06	0.21
Pop:SireF	-0.13	0.42	-2.26	0.56	0.21	0.06	0.18
SireF	0.01	0.13	-0.42	0.46	0.2	0.05	0.04
Within CMA	0.03	0.18	-0.51	0.74	0.18	0.05	0.04
Pop:DamF	0.06	0.26	-0.65	1.17	0.15	0.04	0.05
Pop:Distance to feeder	-0.06	0.28	-1.26	0.74	0.14	0.04	0.04
Kinship	-0.02	0.1	-0.38	0.26	0.14	0.04	0.03
CMA:Pop	0.03	0.36	-2.08	3.22	0.04	0.01	0.01

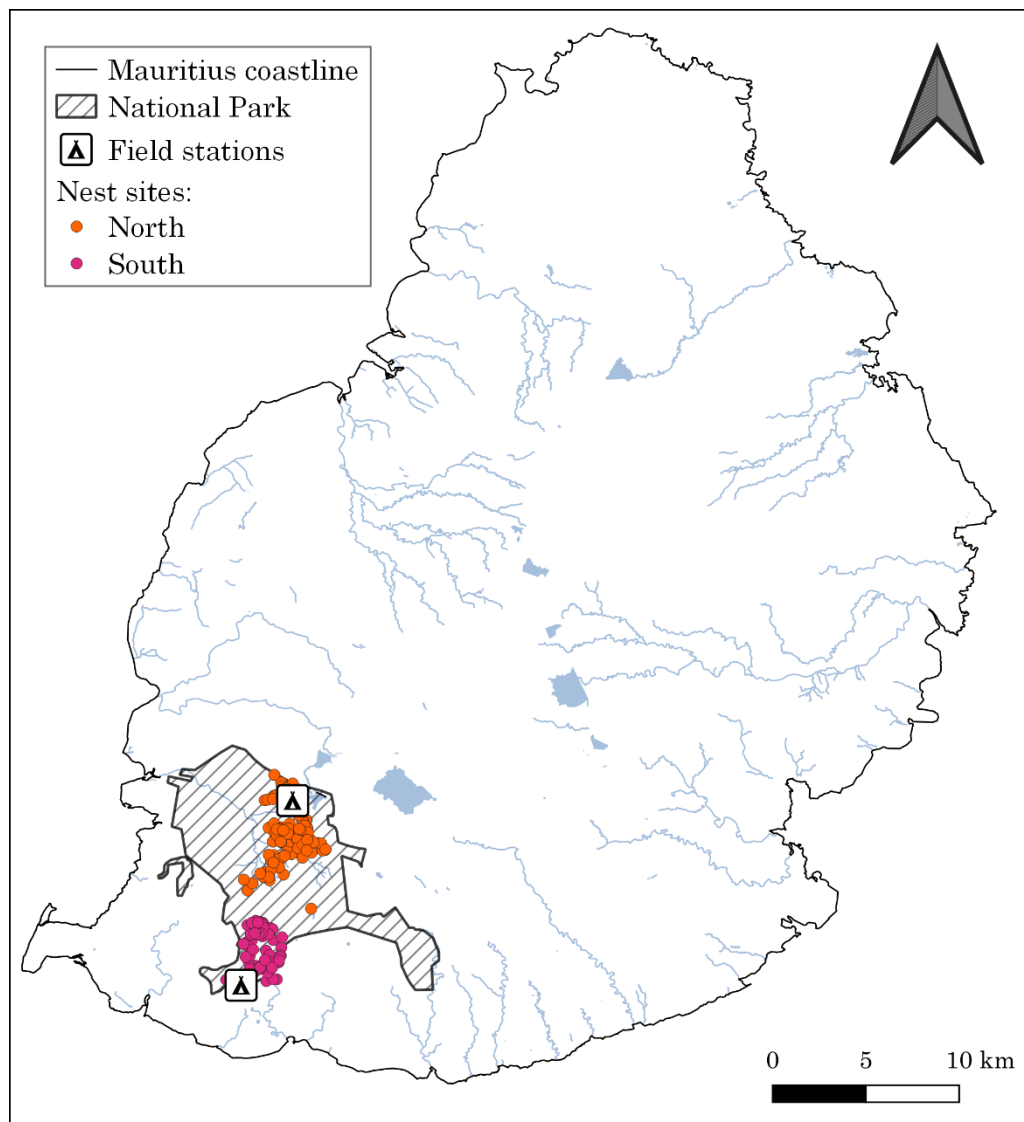


Figure 2.1. Distribution of monitored nest sites of the Mauritius parakeet in the Black River Gorges National Park.

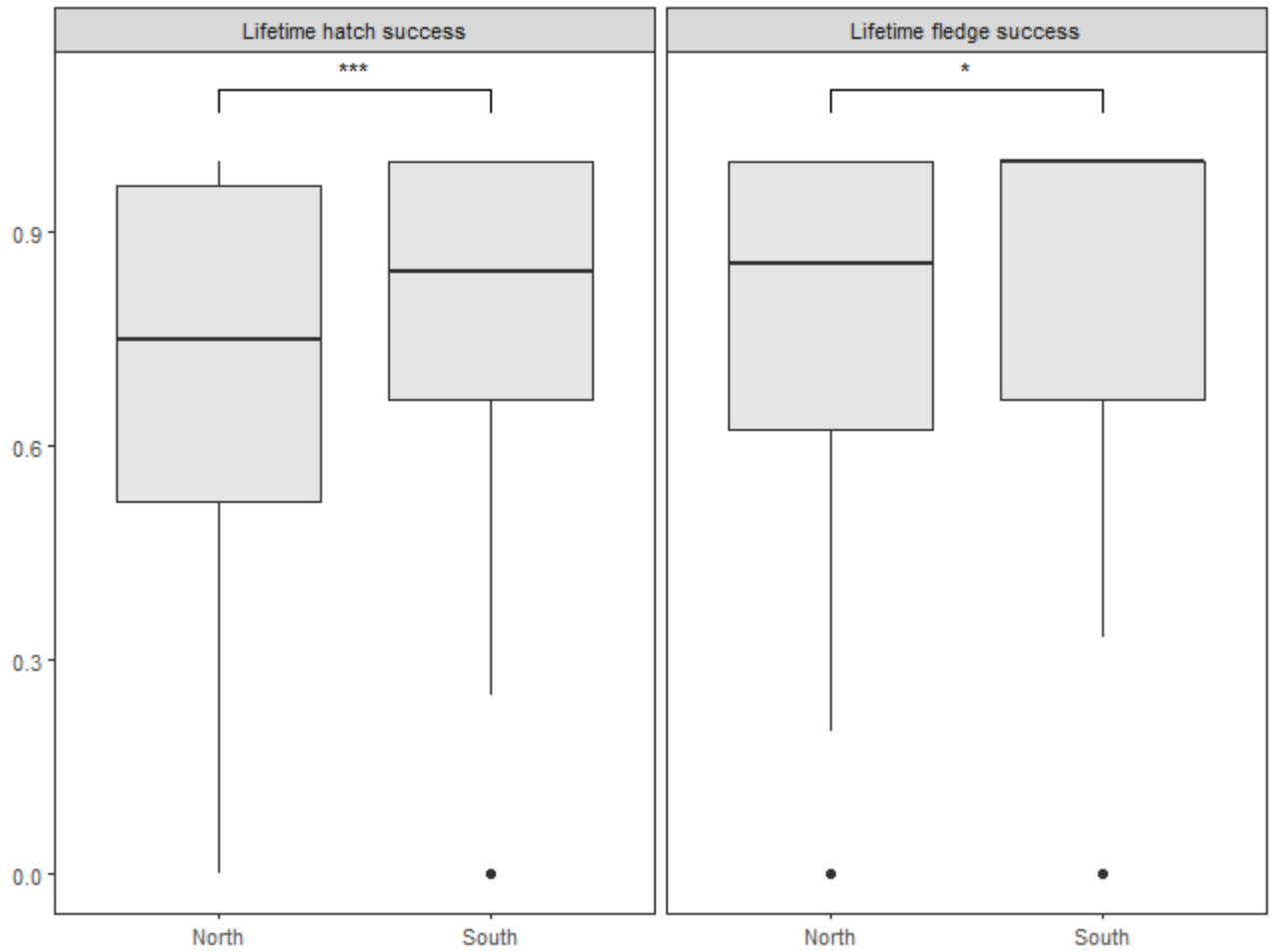


Figure 2.2. Lifetime hatch success and lifetime fledge success in the North and South populations.

Lifetime hatch and fledge success were significantly higher in the South (Wilcox test, $p < 0.0001$ and $p < 0.05$ respectively).

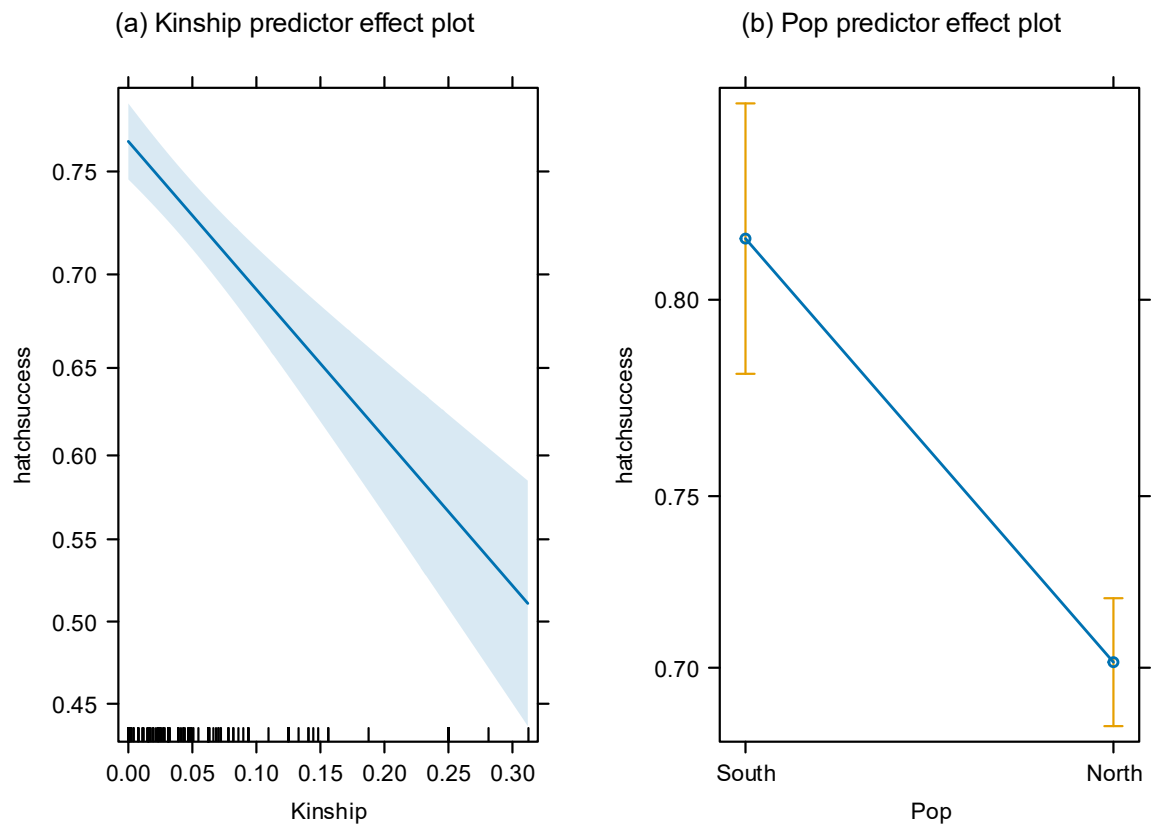


Figure 2.3. Effect plots showing predicted hatch success in relation to (a) pair kinship coefficient and (b) population in Mauritius parakeets, derived from a binomial GLM. Shaded areas and error bars represent 95% confidence intervals. The short vertical lines at the bottom of panel (a) show the distribution of kinship values in the dataset.

Chapter 3 Supplementary food use and reproductive success in the Mauritius parakeet

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3.1 Abstract

Supplementary feeding is a widely used conservation tool, yet its long-term impacts on wildlife populations remain debated. We investigated the effects of supplementary feeding on reproductive success in the endangered Mauritius parakeet (*Alexandrinus eques*) using stable isotope analysis of feather samples from 381 individuals across three breeding seasons (2014, 2020, and 2021) and two spatially distinct populations, alongside long-term breeding and beak and feather disease virus (BFDV) data. Supplementary food constituted a significant portion of parakeet diets (mean 66.9%), with higher consumption in the North population (84.2%) compared to the South (29.4%). While supplementary food consumption positively predicted reproductive success across both populations, the North population did not show significantly higher reproductive output despite greater supplementary food use, suggesting interaction with local environmental factors. Proximity to feeding stations strongly predicted supplementary food consumption but not reproductive success. We observed a weak positive but non-significant relationship between BFDV infection status and supplementary food consumption. Our results highlight a complex relationship between supplementary feeding and reproductive success in Mauritius parakeets. This study contributes to the understanding of supplementary feeding as a conservation tool and provides insights for managing endangered species.

3.2 Introduction

Supplementary feeding is widely used in conservation to support threatened populations, with notable successes in avian recovery programs like for the kakapo (Clout & Craig 1995) and California condor (Walters et al., 2010). However, its effects on wildlife populations can be complex and sometimes detrimental (Blanco et al., 2011; Martínez-Abraín & Oro, 2013; Ewen et al., 2015). While supplementary feeding can enhance survival and breeding success (González et al., 2006; Schoech et al., 2008), potential negative consequences include altered foraging behaviours, resource dependency, increased disease transmission, and disrupted natural selection (Jones & Reynolds, 2008; Robb et al., 2008; Blanco et al., 2011; Milner et al., 2014).

Understanding individual variation in supplementary food use presents a major challenge. Traditional methods often fail to capture actual consumption patterns – for example, weighing food removed from feeders can be misleading (Robb et al., 2011). Stable isotope analysis provides more accurate measurement of individual dietary patterns, over longer timescales than are possible using other traditional techniques like visual analysis of faecal or gut content (Inger & Bearhop, 2008; Hoenig et al., 2022). When combined with reproductive data, this approach can reveal how individual dietary choices influence fitness outcomes (Tollington et al., 2019).

The Mauritius parakeet (*Alexandrinus eques*) provides an ideal study system for investigating the relationship between supplementary feeding and reproductive success. This species has a well-documented history of conservation management, long-term individual-based breeding data, and established supplementary feeding program (Tollington et al., 2019; MWF, 2020). Previous research has successfully applied SIA to

quantify supplementary food uptake in breeding adults and its effects on reproductive success within a single breeding season in this species (Tollington et al., 2019). The population's distribution across areas with varying levels of habitat quality and management intensity creates a natural experiment to examine how environmental context influences the effects of supplementary feeding.

Using stable isotope analysis combined with long-term breeding data, we aim to: (1) examine how proximity to feeding stations influences supplementary food consumption across two populations with different feeding regimes, (2) investigate whether higher supplementary food consumption leads to increased reproductive success, and (3) assess the influence of Conservation Management Areas (CMAs), which are fenced areas actively managed to control invasive plant species, on the extent of reliance on supplementary feeding, providing insights into how habitat quality may affect the utilisation of supplementary food resources.

We hypothesise that: (1) supplementary food consumption will decrease with increasing distance from feeding stations, with this pattern being more pronounced in the North population where food is provided daily, (2) breeding pairs consuming higher proportions of supplementary food will produce more fledglings per breeding attempt, and (3) breeding pairs nesting within CMAs will show lower reliance on supplementary food due to better availability of natural food resources in these areas.

Our findings will contribute to the broader understanding of supplementary feeding as a conservation tool, offering insights that can be applied to the management of endangered species elsewhere. While our study focuses on the Mauritius parakeet, the methodological approach and ecological principles investigated have wide-ranging

implications for conservation biology and wildlife management across threatened taxa and ecosystems.

3.3 Methods

3.3.1 Ethics statement

Sampling methods were approved by the University of Kent Research Ethics Advisory Group. All samples were collected under a research permit from the National Parks and Conservation Service of Mauritius (NPCS permit: NP 46/3V5). Feather samples were exported from Mauritius and imported to the UK under CITES permits (Export permits: MU 220195, MU 220125; Import permit: 598437/02) and APHA Import Permit ITIMP21.0404. All samples were collected by trained field staff at the Mauritian Wildlife Foundation (MWF) as part of the ongoing conservation management and monitoring programme for the Mauritius parakeet. Field staff followed established protocols for safe collection of feather samples, taking only small (5 mm²) sections of primary feathers from 45-day old chicks during routine nest checks to minimise handling time and reduce stress on the birds.

3.3.2 Study system

The Mauritius parakeet (*Alexandrinus eques*) is an endemic species of parrot restricted to the Black River Gorges National Park in Mauritius, an island in the Indian Ocean (20°23'S, 57°23'E). This intensively studied species has been the subject of a successful, long-term conservation recovery program (Jones & Merton, 2012; Raisin et al., 2012; Tollington et al., 2015).

Mauritius parakeets form long-lasting monogamous pairs, typically breeding from their second year. They produce a single annual clutch of one to four eggs. The species' diet is supplemented with commercial parrot food provided year-round at purpose-built feeders (one in each population), with daily provision in the North and weekly provision in the South. Mean distances to feeders differ substantially between populations (South: 2.37 km; North: 1.15 km). Whilst evidence shows that supplementary feeding increases fledging success (Tollington et al., 2019), it is not known whether there is spatio-temporal variation in SF consumption and reproductive fitness.

The population comprises two distinct populations: 'Grand Gorges' in the North and 'Bel Ombre' in the South (**Figure 3.1**), separated by approximately 1.8 km. Despite their proximity, there is little evidence of natural dispersal between these populations (Raisin et al., 2012; Tollington et al., 2013). The North population is larger, with 94 known active nest sites compared to 48 in the South population (2020/2021 breeding season; MWF, 2021).

Reproductive performance has been closely monitored in these populations since the late 1990s. In 2005, there was an outbreak of beak and feather disease virus (BFDV) in the Mauritius parakeet population (Kundu et al., 2012). This outbreak temporarily decreased reproductive fitness, particularly affecting hatch success among pairs that used supplementary food (Tollington et al., 2015, 2019). However, BFDV prevalence (determined via PCR) was not higher among individuals that used supplementary food (compared to non-fed individuals).

Many of these breeding pairs nest within Conservation Management Areas (CMAs); fenced forest areas within the National Park that are managed intensively to control

predators and remove invasive plant species. There are four CMAs within the North population, and one within the South population. CMA size varies from 0.06 km² to 0.23 km². Given the well-established perimeters of CMAs and their known history of habitat restoration/quality, sampling could opportunistically be performed on portions of the population that nested inside and outside CMAs.

3.3.3 Sample collection

Feather samples were collected during routine nest checks across three breeding seasons (2014, 2020, and 2021) as part of the Mauritian Wildlife Foundation's ongoing monitoring program. During each check, small (5 mm²) sections were clipped from the primary feathers of 45-day old chicks. Additional feather samples were opportunistically collected when naturally shed feathers were found inside nest boxes during these checks.

Our dataset comprised 381 feather samples collected across the three breeding seasons. In the 2020 breeding season, we collected 97 feathers from 55 broods, and in 2021, we collected 103 feathers from 57 broods. To examine temporal changes in supplementary food consumption, we integrated isotopic data from 181 feathers collected during the 2014 breeding season (originally analysed by Tollington et al., 2019). Sampling was conducted across both CMA and non-CMA areas, with 57 samples collected from nests inside CMAs and 324 samples from nests outside CMAs. The majority of samples were collected from chicks, with nine samples collected from adult birds.

3.3.4 Stable isotope analysis of samples

Feather samples were processed for stable isotope analysis at the NERC Life Sciences Mass Spectrometry Facility, East-Kilbride, using a Costech ECS 4010 interfaced with a ThermoFisher Scientific Delta XP Plus IRMS. Samples were analysed via continuous-flow mass spectrometry in order to derive the ratios of the stable isotopes of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$). Replicate analyses of gelatine and two isotopically distinct alanine internal reference materials suggested that the reproducibility was 0.152‰ for $\delta^{15}\text{N}$ and 0.157‰ for $\delta^{13}\text{C}$.

Stable isotope values for plants known to be important food sources for the Mauritius parakeet (Jones et al., 2013; MWF, 2020), as well as supplementary pellets (KayteeExact Parrot Pellets; Kaytee Products Inc.) provided by MWF, were obtained from a previous study (Tollington et al., 2019). Since the supplementary pellets consist mainly of maize (*Zea* spp.), which has a C_4 carbon fixation strategy, the $\delta^{13}\text{C}$ isotope analyses revealed distinct isotopic signatures between these pellets and the upland tropical vegetation which have a C_3 carbon fixation strategy, providing an opportunity to quantify proportion of supplementary food intake alongside that of plant species in the diet of individual parakeets.

3.3.5 Isotope modelling

We used Bayesian stable isotope mixing models (SIMMs) implemented in the R package MixSIAR (Stock et al., 2018) to estimate the relative contributions of supplementary food and natural vegetation to each bird's diet. MixSIAR estimates source proportions while

accounting for uncertainty in isotopic signatures of food sources, trophic discrimination factors, and consumer isotope values.

Trophic discrimination factors of +2.16‰ for $\delta^{13}\text{C}$ and +3.84‰ for $\delta^{15}\text{N}$ were applied to correct for isotopic enrichment between food sources and feather tissue, following previous work on this species (Tollington et al., 2019) and broader studies of discrimination factors (Caut et al., 2009). These factors account for the systematic enrichment that occurs during the incorporation of dietary nutrients into consumer tissues.

We constructed a series of candidate models to identify the most important predictors of dietary source proportions, following methods of Stock et al. (2018). Models included different combinations of: population (North/South), based on previous evidence of spatially independent populations (Raisin et al., 2012; Tollington et al., 2013), breeding year (2014, 2020, 2021), distance of nest-site to the nearest feeding station (km), and age (adult/chick). Model selection was performed using the widely applicable information criterion (WAIC) and approximate leave-one-out cross-validation (LOO) (Vehtari et al., 2017). The relative support for each model was evaluated using LOO and Akaike weights, which estimate the probability that a given model provides the best predictions for new data (Burnham & Anderson, 2004). For subsequent analyses of breeding success, we used the mean predicted proportion of supplementary food consumption per sample as derived from the best-fitting mixing model.

3.3.6 Predictors of supplementary food consumption

Prior to analysis, individual proportional SF consumption values were arc-sin square-root transformed to improve normality (Crawley, 2012) and used as the response variable. We constructed linear mixed-effects models using transformed SF consumption as the response variable. Fixed effects included distance to the nearest feeding station, population (North or South), year (2014, 2020, 2021), and whether the nest site was inside or outside of a CMA. We also included interactions between population and all other fixed effects to test whether the effects of distance, year, and CMA varied between populations. To account for the non-independence of samples from siblings and repeated sampling of nest sites across years, we included Brood ID (a unique identifier combining nest site and year) as a random effect.

3.3.7 Brood-level variation in dietary composition

To check the similarity in dietary composition among siblings, we conducted intraclass correlation analyses using the transformed supplementary food values. We fitted a linear mixed-effects model using the 'lme4' package (Bates et al., 2015), with transformed supplementary food values as the response variable and Brood ID (a unique identifier combining nest site and year) as a random effect. The model structure was specified as: $\text{lmer}(\text{SF} \sim 1 + (1|\text{Brood_ID}))$. Intraclass correlation coefficients and their confidence intervals were calculated using the 'performance' package (Lüdtke et al., 2021) to assess the proportion of variance explained by brood membership.

3.3.8 Supplementary food consumption and reproductive success

We used brood-level proportional supplementary food consumption to represent parental diet at each nest, allowing us to analyse how supplementary feeding influences reproductive success. Following Tollington et al. (2019), we measured reproductive success as the number of birds fledged per breeding attempt. This measure served as the response variable in a linear mixed-effects model examining the relationship between supplementary food use and breeding output. The main predictor variable was the proportional consumption of supplementary food. Additional fixed effects included population (North or South), breeding year (2014, 2020, 2021), and whether the nest site was inside or outside of a CMA. We included interactions between population and all other fixed effects to test whether the effects of supplementary food use, year, and CMA varied between populations. While BFDV infection status may influence reproductive success, we did not include this as a predictor as data were only available for a subset of breeding pairs, which would have restricted our dataset and reduced statistical power.

3.3.9 Supplementary food consumption and BFDV

As part of ongoing disease surveillance by the Mauritian Wildlife Foundation and the University of Kent, blood samples from Mauritius parakeet chicks are routinely tested for beak and feather disease virus (BFDV) using PCR assays (Kundu et al., 2012; Fogell et al., 2019). We coded BFDV infection at the brood level as a binary variable (1 = at least one chick tested positive; 0 = no chicks tested positive) because sampling coverage varied among nests, preventing analysis at the individual chick level. This variation in sampling arose from both missing samples and failed tests. To examine the relationship between

supplementary food consumption and BFDV prevalence, we conducted a correlation test between brood-level supplementary food use and BFDV status.

3.3.10 Statistical analysis

For both analyses (predictors of supplementary food consumption and effects on reproductive success), we fitted initial models containing all possible predictors using the 'lme4' package in R (Bates et al., 2015). We then used an information-theoretic approach for model selection and averaging, implemented in the 'MuMIn' package (Bartoń, 2023). Models were ranked by their AICc values, and we retained all models with $\Delta\text{AICc} < 7$ in the final model set. From these models, we calculated model-averaged parameter estimates with standard errors and 95% confidence intervals. Variable importance was assessed using weighted t-statistics (absolute, ratio, and variance; Cade, 2015), and significance of model-averaged coefficients was determined using 95% confidence intervals.

3.4 Results

3.4.1 Stable isotope values

Analysis of 381 feather samples revealed distinct isotopic signatures between the two populations. Raw values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ from all feather samples showed distinct clustering by source type and, for feather samples, by population (**Figure 3.2**). Supplementary food had higher isotopic values ($\delta^{13}\text{C}$: $-19\text{‰} \pm 1\text{‰}$; $\delta^{15}\text{N}$: $7\text{‰} \pm 1.5\text{‰}$) compared to natural vegetation ($\delta^{13}\text{C}$: $-28\text{‰} \pm 3\text{‰}$; $\delta^{15}\text{N}$: $2.8\text{‰} \pm 3\text{‰}$). Feathers from the North population had values ranging from $\delta^{13}\text{C}$: -22‰ to -18‰ and $\delta^{15}\text{N}$: 3‰ to 7‰ , while samples from the South population had values ranging from $\delta^{13}\text{C}$: -26‰ to $-$

20‰ and $\delta^{15}\text{N}$: 2‰ to 5‰. The North population showed isotopic values closer to those of supplementary food, while samples from the South population had values intermediate between supplementary food and natural vegetation, suggesting different patterns of food resource use between the two populations.

3.4.2 SIMM results

The best-fitting SIMM included population (North/South) and distance to nearest feeder as predictors of supplemental food consumption (see **Table 3.1**). The number of predictors was constrained to two due to the number of dietary sources in the models (Stock et al., 2018). The predicted proportional supplementary food consumption from this model varied widely among individuals, ranging from 5.3% to 97.7% (mean = 66.9%). Consumption differed markedly between populations, with mean values of 84.2% in the North population and 29.4% in the South population. The proportion of supplementary food in the diet decreased with increasing distance from feeding stations in both populations (**Figure 3.3**).

3.4.3 Predictors of supplementary food consumption

The best-fitting model (Marginal $R^2 = 0.72$; Conditional $R^2 = 0.99$) revealed that population, distance to feeder, and year significantly predicted supplementary food consumption (**Table 3.2**). The North population showed consistently higher supplementary food consumption compared to the South, with this population difference becoming more pronounced in 2020 and 2021 compared to 2014. Supplementary food consumption decreased with increasing distance from feeders (**Figure 3.4**). Whether nests were inside or outside CMAs showed no significant effects on supplementary food consumption.

3.4.4 Brood-level variation in dietary composition

Intraclass correlations on proportional contribution of SF showed a high correlation coefficient among siblings and low within-brood variation (ICC = 0.989 [0.985, 0.992], variance within = 0.001, variance among = 0.1). This indicates that the diets of siblings are indistinguishable from one another, and so brood-level proportional consumption of SF was used for subsequent analyses to infer diet of each breeding pair.

3.4.5 Effect of supplementary food on reproductive success

The averaged model revealed that supplementary food consumption positively predicted the number of fledglings produced per breeding attempt (**Table 3.3**). The 2021 breeding season showed significantly reduced reproductive success compared to 2014. Population showed no significant influence on reproductive success. Other factors including breeding year 2020 and whether nests were inside or outside CMAs showed no significant effects on the number of fledglings produced.

3.4.6 Supplementary feeding and BFDV

Analysis of disease prevalence (available for 169 of 175 broods) revealed a weak positive but non-significant relationship between BFDV infection status and supplementary food consumption (cor = 0.14, p=0.06, 95% CI: -0.01 to 0.29).

3.5 Discussion

Our study resulted in three key findings about supplementary feeding in the Mauritius parakeet. First, as predicted, supplementary food consumption strongly decreased with distance from feeding stations, with higher overall consumption in the North population where food was provided daily (84.2%) compared to weekly provision in the South (29.4%). Second, while supplementary food consumption positively predicted fledgling numbers, suggesting nutritional benefits, this relationship was not proportional to consumption levels between populations. Third, contrary to our prediction, Conservation Management Areas showed no significant effect on supplementary food use.

3.5.1 Spatial pattern of supplementary food use

Distance from feeding stations was the strongest predictor of supplementary food consumption, demonstrating a clear spatial effect on resource use. This is in line with previous study of a single breeding season (Tollington et al., 2019). Birds nesting closer to feeding stations incorporated substantially more supplementary food in their diet, with this effect being particularly pronounced in the North population where daily food provision led to mean consumption of 84.2% compared to 29.4% in the South where food was provided weekly. This striking difference in consumption patterns suggests that feeding frequency, combined with spatial accessibility, strongly influences resource use. The decline in supplementary food consumption with increasing distance from feeders indicates that the benefits of supplementary feeding may be spatially limited, potentially creating heterogeneity in this resource availability across the population. These findings align with broader evidence that supplementary feeding can create localised areas of

high resource use (Murray et al., 2016), with important implications for feeding station placement in conservation programs.

Additionally, we found that population differences in supplementary food use became more pronounced in more recent years, as indicated by significant population:year interactions in 2020 and 2021. This temporal pattern suggests potential long-term changes in feeding behaviour that could require further investigation.

3.5.2 Supplementary feeding and reproductive success

Our analysis demonstrates that supplementary food consumption positively predicts the number of fledglings produced per breeding attempt, supporting the use of supplementary feeding as a conservation tool. The positive relationship between supplementary food consumption and number of fledglings produced aligns with previous research on this species (Tollington et al., 2019) and broader findings across endangered bird populations (González et al., 2006; Schoech et al., 2008; Doerr et al., 2017; Tollington et al., 2019).

However, the relationship between supplementary food and reproductive success shows unexpected complexity in the Mauritius parakeet - despite markedly higher supplementary food consumption in the North population, we did not observe proportionally higher reproductive output compared to the South. The South population appears to maintain effective reproduction with lower supplementary food use, potentially through more efficient utilisation of natural food resources, or may not require as much supplementary food due to better natural resources being available.

The more pronounced effect of supplementary food in our North population thus may reflect poorer natural food resources in this range, which would align with Ruffino et al.'s (2014) meta-analysis showing that supplementary feeding had stronger effects on reproductive success in habitats with limited natural food availability. However, direct measures of habitat quality between the two populations would be needed to confirm this pattern.

The higher breeding pair density in the North population may create density-dependent effects that influence reproductive success through increased competition for other resources affecting breeding output (Arcese & Smith, 1988; Paradis et al., 2002; Dhondt, 2012). This density effect might explain why increased supplementary food consumption does not translate into proportionally higher reproductive output in the North population.

3.5.3 Habitat quality and supplementary feeding

Contrary to our prediction, breeding pairs nesting within Conservation Management Areas showed similar levels of supplementary food consumption to those nesting outside these actively managed habitats. This unexpected finding challenges assumptions about how habitat quality influences supplementary food use and supports findings in other species where supplementary feeding benefits persisted regardless of habitat quality (Doerr et al., 2017). Several mechanisms might explain this pattern: (1) the scale of habitat management in CMAs may be insufficient to affect foraging decisions, (2) supplementary food may provide nutritional benefits not readily available from natural food sources, even in high-quality habitat, or (3) behavioural preferences for reliable food sources may override natural foraging opportunities close to feeders. These results

suggest that habitat quality alone may not be sufficient to reduce reliance on supplementary feeding once it has been established.

3.5.4 Distance to feeders and BFDV

Our study found a weak positive but non-significant relationship between BFDV infection status and supplementary food consumption. The potential for feeding stations to act as disease transmission hotspots remains a concern (Murray et al., 2016; Fogell et al., 2019), particularly given the higher use of supplementary food in the North population. This lack of a strong relationship between supplementary feeding and BFDV infection may be explained by our measure of BFDV infection (inferred from single blood tests of 45-day old chicks) not adequately capturing BFDV prevalence. Our study also could not consider any long-term effects of BFDV, such as on post-fledging survival and future reproductive success in subsequent generations.

The relationship between supplementary feeding and disease transmission is complex (Tollington et al., 2015; Wilcoxon et al., 2015). While some studies have found increased pathogen transmission at feeding stations (Murray et al., 2016; Lawson et al., 2018), others have shown that well-fed individuals may better tolerate infection due to improved condition (Kaneko et al., 2005; Wilcoxon et al., 2015). Our findings suggest the need for continued disease surveillance, particularly given the historical impact of BFDV in this population (Kundu et al., 2012).

3.5.5 Conservation implications

Our findings have important implications for supplementary feeding as a conservation tool. The provision of supplementary food must balance potential benefits against risks

such as increased disease transmission (Blanco et al., 2011) and resource sustainability (Chauvenet et al., 2012).

The contrasting patterns between our two populations raise important questions about supplementary feeding objectives. If the primary aim is to increase reproductive fitness through fledgling production, our results suggest that lower supplementary food consumption (as seen in the South) may be more effective than higher consumption. This counterintuitive finding could reflect several underlying mechanisms. The North population's higher dependence on supplementary food might result in reduced consumption of natural foods that provide essential nutrients. Alternatively, supplementary feeding might be supporting the breeding attempts of less fit individuals, potentially occupying nest sites that could otherwise be used by more productive pairs. This explanation would align with findings from Tollington et al. (2019) that older females received a disproportionate boost to reproductive success compared to younger females.

Evidence from multiple species suggests that modifying the spatial and temporal distribution of supplementary food can help mitigate some risks while maintaining benefits (Murray et al., 2016). For example, dispersing food over larger areas and periodically moving feeding locations could help reduce density effects while still providing nutritional support. Our finding that the South population maintains reproductive success with lower supplementary food use suggests such modified approaches might be viable.

These findings contribute to the growing understanding of supplementary feeding as a conservation tool whose effectiveness depends on multiple interacting factors (Ewen et

al., 2015). While supplementary feeding has played a crucial role in preventing extinction of this species (Jones & Merton, 2012), our results suggest opportunities for refining this management approach based on population-specific responses.

Future research should focus on understanding the mechanisms driving population differences in resource use efficiency and investigating potential long-term effects of varying supplementary feeding regimes. This would help us to understand whether modified feeding strategies could achieve similar conservation benefits while minimising risks and costs.

3.6 Conclusions

Our findings have important implications for supplementary feeding as a conservation tool. The contrasting patterns between populations suggest that maximising food provision may not be necessary for maintaining reproductive output. The South population's success with lower supplementary food consumption indicates opportunities for optimising feeding strategies while minimising risks such as disease transmission and density-dependent effects. Evidence from multiple species suggests that modifying the spatial and temporal distribution of supplementary food can help mitigate these risks while maintaining benefits. Such modifications could include dispersing feed over larger areas or periodically moving feeding locations to reduce density effects while still providing nutritional support.

3.7 Acknowledgements

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3.9 Tables and Figures

Table 3.1. Comparison of mixing models fit using MixSIAR on the feather stable isotope data with supplementary food and natural plant food sources, following methods of Stock et al. (2018). dLOOic is the difference in LOOic between each model and the model with lowest LOOic. The “Pop + dist_hop” model had the lowest LOOic and received 100% of the Akaike weight, indicating a 100% probability it is the best model.

Model	LOOic	SE (LOOic)	dLOOic	SE (dLOOic)	Weight	xi.C	xi.N
Pop + dist_hop	456	57.5	0		1	0.2	0.1
Pop + Season	517.2	58.3	61.2	30.5	0	0.2	0.1
Pop	529	60.6	73	27	0	0.2	0.1
Pop+Age	529.3	60.6	73.3	27	0	0.2	0.1
dist_hop	604.7	51.6	148.7	32.8	0	0.2	0.1
Season	792.1	47.1	336.1	36.6	0	0.3	0.1
Null	793.9	47	337.9	36.4	0	0.3	0.1
Age	794.2	47	338.2	36.4	0	0.3	0.1

Table 3.2. Model-averaged coefficients, standard errors (*SE*), confidence intervals, and variable *t*-statistics (absolute, ratio, and variance) from GLMM to predict SF use. Predictors are ordered by weighted *t*-statistics as a measure of relative variable importance (Cade, 2015; Robinson et al., 2016).

Coefficient estimates where confidence intervals do not cross zero are in bold.

Parameter	Estimate	SE	p-value	CI 2.5%	CI 97.5%	t abs	t ratio	var t
(Intercept)	0.64	0.02	<0.001	0.61	0.68	-	-	-
dist_hop	-0.21	0.03	<0.001	-0.26	-0.16	194.97	0.99	0.03
Pop (North)	0.33	0.04	<0.001	0.26	0.41	194.79	0.99	0.60
Year2020	-0.06	0.03	0.032	-0.11	-0.005	193.78	0.99	0.02
Year2021	0.03	0.03	0.229	-0.02	0.08	193.78	0.99	0.02
Cma	-0.05	0.04	0.284	-0.14	0.01	141.58	0.72	2144.53
Pop:dist_hop	-0.07	0.07	0.300	-0.21	0.005	130.10	0.66	2830.28
cma:Pop	0.09	0.11	0.418	-0.001	0.35	98.69	0.50	4849.52
Pop:Year2020	0.23	0.05	<0.001	0.12	0.33	193.96	0.99	0.02
Pop:Year2021	0.23	0.05	<0.001	0.13	0.33	193.96	0.99	0.02
Note: The year variable's t.abs, t.ratio, and var.t values are shared between Year2020 and Year2021								

Table 3.3. Model-averaged coefficients, standard errors (SE), confidence intervals, and t-statistics (absolute, ratio, and variance) for predictors of the number of fledglings produced per brood. Weighted t-statistics are given as a measure of relative importance of each covariate. Variables for which the confidence intervals do not cross zero are shown in bold.

Parameter	Estimate	SE	p-value	CI 2.5%	CI 97.5%	t abs	t ratio	var t
(Intercept)	2.18	0.11	<0.001	1.97	2.39	-	-	-
Year2021	-0.55	0.16	<0.001	-0.87	-0.23	3.33	0.99	0.01
SF_brood	0.73	0.26	0.005	0.23	1.24	2.62	0.78	0.20
Pop (North)	-0.40	0.30	0.181	-0.98	0.19	1.49	0.44	0.53
Year2020	-0.25	0.17	0.141	-0.58	0.08	3.33	0.99	0.01
Pop:Year2021	-0.47	0.45	0.298	-1.41	-0.07	1.07	0.31	0.58
Pop:Year2020	-0.29	0.36	0.419	-1.17	0.24	1.07	0.31	0.58
Cma	-0.15	0.20	0.454	-0.65	0.10	0.65	0.19	0.25
Pop:SF_brood	0.03	0.18	0.881	-0.73	1.27	0.14	0.04	0.05

*Note: The year variable's t.abs, t.ratio, and var.t values are shared between Year.2020 and Year.2021

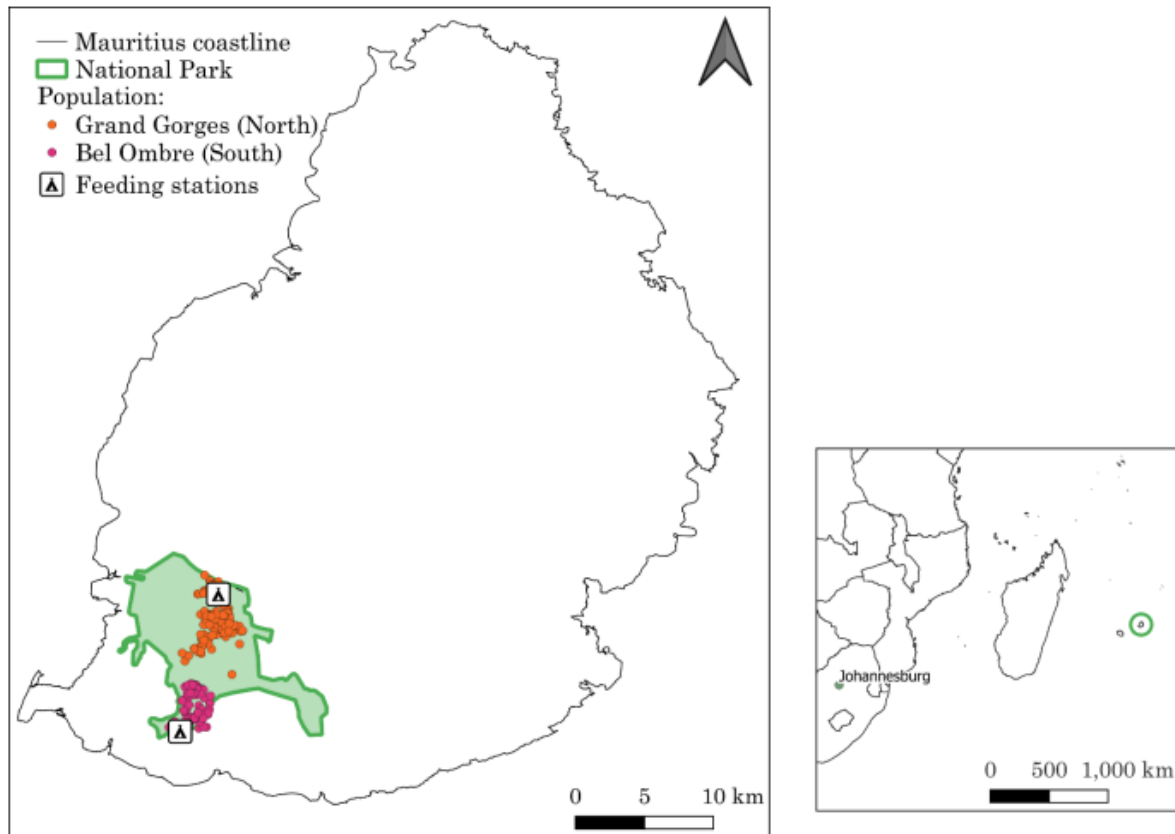


Figure 3.1. Nest site and feeding station locations in each population within the Black River Gorges National Park (left), and location of Mauritius in relation to South Africa (right).

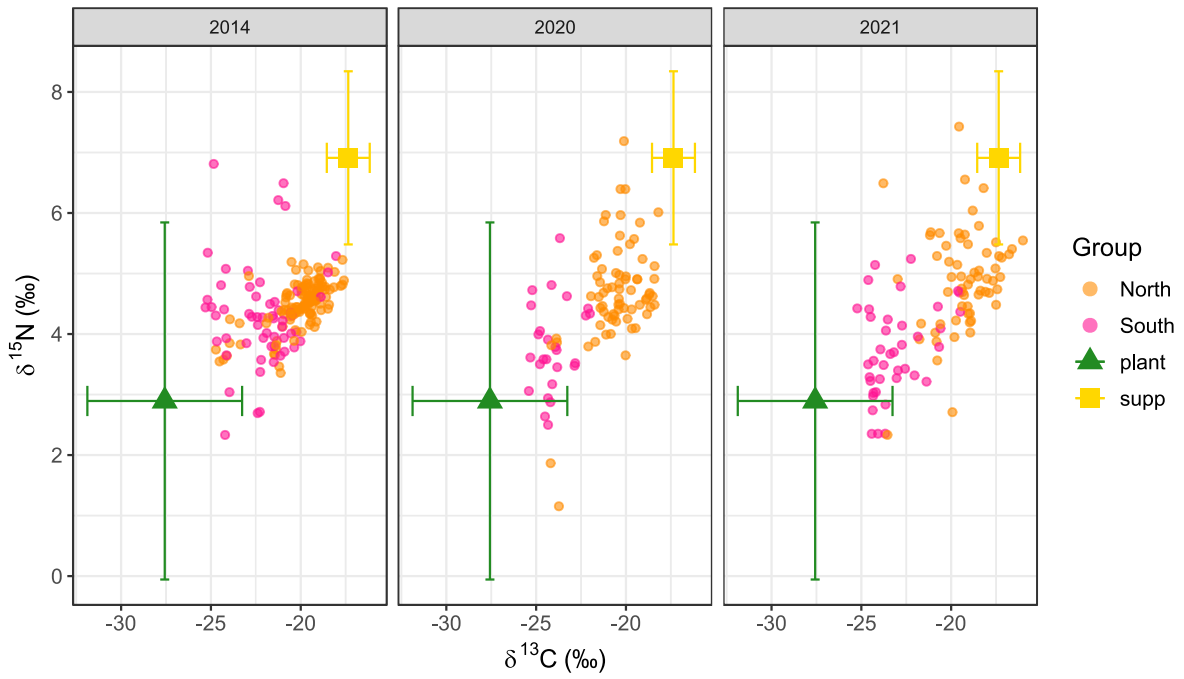


Figure 3.2. Isospace plot of mean ($\pm SD$) raw isotopic values from dietary sources (plants and supplementary food) and from feather samples from individuals in the North (GG) and South (BO) populations collected during breeding seasons 2014-2015, 2020-2021, and 2021-2022.

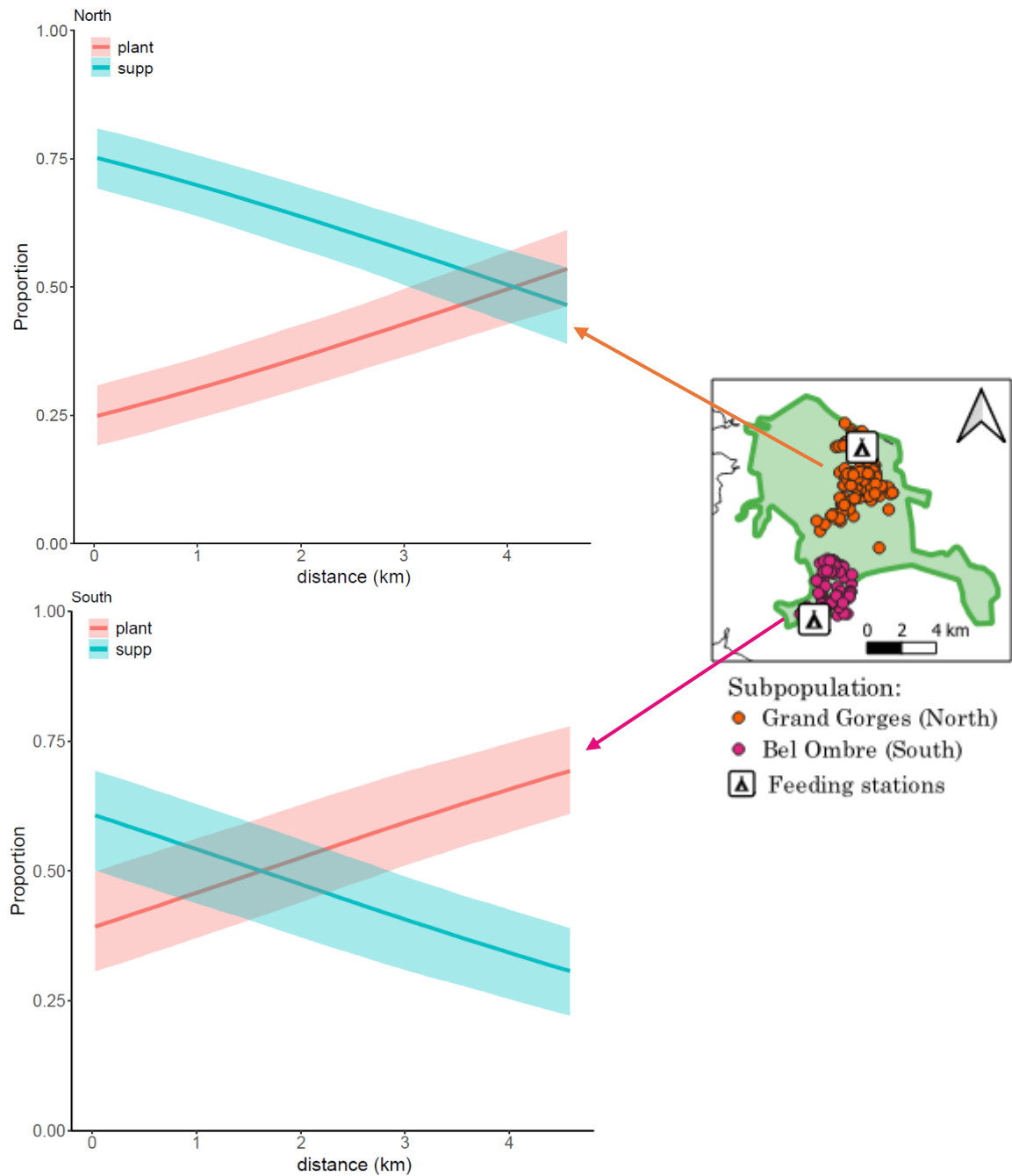


Figure 3.3. SIMM prediction for the change in consumption of different dietary sources with increasing distance to the nearest feeding station for the North population (top) and the South population (bottom). Nest sites for each population across the Black River Gorges National Park (right).

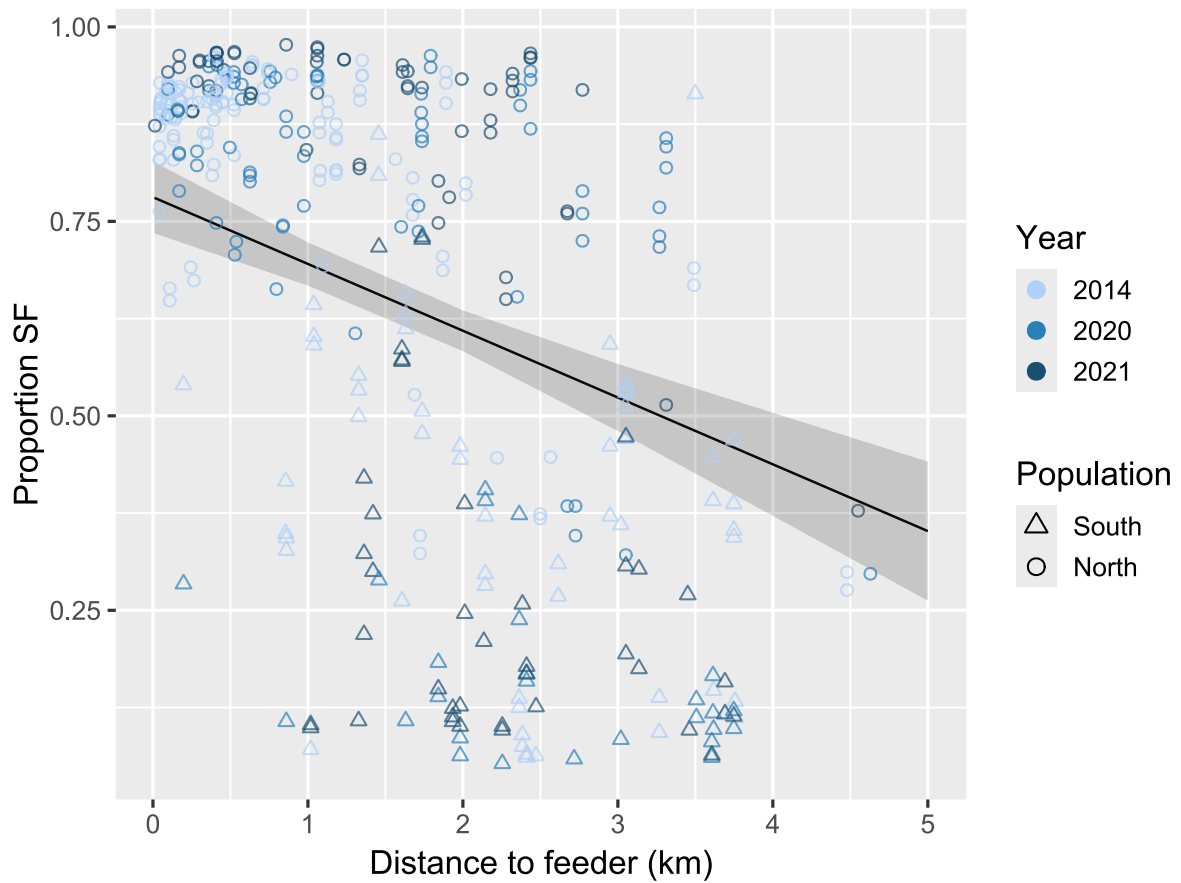


Figure 3.4. Variation in proportional SF consumption with distance to feeding station (km), with colours indicating the population and breeding year for each individual point ($R^2 = 0.40$).

Chapter 4 Dietary preferences and reproductive success of the Mauritius parakeet

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4.1 Abstract

Populations of threatened species frequently need to survive in non-optimal landscapes comprising degraded, altered and restored habitats. Such habitat mosaics may influence individual diets, potentially impacting fitness. We investigated dietary variation in the Mauritius parakeet (*Alexandrinus eques*), a vulnerable species with two populations spatially separated across areas of differing habitat quality. DNA metabarcoding identified 83 plant taxa with significant differences between North and South populations. The North population had significantly higher native species richness (4.7 vs 1.9 species per sample) despite historical declines in native vegetation across both ranges. Birds nesting within Conservation Management Areas - fenced sections where invasive plants have been removed - had higher overall dietary diversity but not higher native species consumption compared to those outside these areas. The populations had contrasting relationships between diet composition and reproductive success: in the North, consumption of supplementary food positively correlated with fledgling numbers, while in the South, consumption of endemic *Gaertnera* sp. had positive associations with reproductive success and two non-native species were negatively associated with fledgling numbers. These contrasting effects on reproductive success occurred despite the higher native plant diversity in the North population's diet, suggesting complex relationships between diet composition and breeding outcomes in different habitat contexts. This demonstrates how populations can develop distinct dietary strategies within modified habitats and highlights the importance of locally tailored conservation approaches.

4.2 Introduction

Habitat modification through loss, fragmentation, and human activities profoundly impacts ecosystems worldwide, leading to the decline of native species (Haddad et al., 2015; Vergara-Tabares et al., 2020). As landscapes transform, species face novel ecological pressures affecting their access to key habitat resources including food. This can manifest through both direct habitat alteration and non-native species proliferation (Traveset & Richardson, 2014; Schirmel et al., 2016; Bartley et al., 2019), with invasive plants particularly impacting herbivore diet composition (Atwood et al., 2020). While some species exhibit dietary flexibility in response to these changes (Wong & Candolin, 2015), the extent to which dietary adjustments meet the needs of the species varies considerably depending on the species in question and the changes occurring in their habitat (Bartley et al., 2019).

Conservation managers employ multiple strategies to address these challenges, from habitat restoration and invasive species control to supplementary feeding programmes (Baider & Florens, 2011; Blanco et al., 2011; Murray et al., 2016). While these interventions can support species' nutritional needs (Harrison et al., 2010; Murray et al., 2016), their interactions with natural foraging behaviour remain poorly understood. Supplementary feeding may alter foraging patterns or mask the importance of native food resources (Robb et al., 2008; Harrison et al., 2010; Blanco et al., 2011; Oro et al., 2013), making it important to understand how conservation interventions might influence foraging behaviour and fitness outcomes. This is particularly important for birds, where food quality and availability impact parental provisioning to nestlings (Tremblay et al., 2005; Wilkin et al., 2009) and can directly influence reproductive success through egg

production, chick growth, and fledging survival (Martin, 1987; Koutsos et al., 2001; Reynolds et al., 2003; Brightsmith et al., 2010).

The Mauritius parakeet (*Alexandrinus eques*), an island-endemic parrot, provides an ideal opportunity to examine questions about diet, supplementary feeding and reproductive success in a modified landscape. Since the 16th century, Mauritius has experienced extensive forest modification, with many areas now dominated by invasive species due to historical introductions and land-use practices (Hammond et al., 2015). Within this altered landscape, the Mauritius parakeet persists in two distinct areas of the Black River Gorges National Park. The North population inhabits areas dominated by invasive *Psidium cattleianum* and *Ligustrum robustum* (Virah-Sawmy et al., 2009), but also some of Mauritius' best preserved native forest fragments. The South population's range similarly includes CMAs, as well as areas dominated by non-native *Syzygium jambos* (Virah-Sawmy et al., 2009). Historical data revealed significant degradation across both population ranges – in one study area within the North population range, native tree density declined by 70% between 1939 and 1999, with substantial reductions in species richness (Virah-Sawmy et al., 2009). While small remnants of forests with high native content still exist (Hammond et al., 2015), both Mauritius parakeet populations must navigate a complex mosaic of degraded and restored habitat.

Conservation management varies between populations through two main approaches. First, Conservation Management Areas (CMAs) have been established in both ranges - these are fenced sections where invasive plants are removed to promote native species recovery (MWF, 2020). Second, supplementary feeding is provided at different intensities: daily in the North and weekly in the South. Previous research has shown that

Mauritius parakeets nesting closer to supplementary feeding stations incorporate more supplementary food in their diet, which correlates with improved reproductive success (Tollington et al., 2019; **Chapter 3**). However, a detailed understanding of how habitat quality and management through CMAs affects dietary breadth and whether supplementary feeding influences foraging patterns for natural foods is lacking.

Understanding these relationships requires detailed dietary analysis, which has become possible with advances in DNA metabarcoding. DNA metabarcoding enables dietary analysis with a higher level of taxonomic resolution than previously possible through traditional methods (Pompanon et al., 2012; Ando et al., 2020). The advantages of this method have been demonstrated by recent avian diet studies (e.g. Shutt et al., 2021; Tang et al., 2022; Schumm et al., 2023; Stenhouse et al., 2023; Fablet et al., 2024). For example, metabarcoding of blue tit faecal samples revealed detailed information on plant and invertebrate diet components across different seasons and habitats (Shutt et al., 2021).

Using DNA metabarcoding of faecal samples, this study investigates dietary patterns and their impacts on reproductive success across different levels of habitat modification and management intensity. We specifically examine: (1) how dietary diversity varies between populations with different habitat compositions, (2) whether dietary composition differs between nests under different management conditions (inside vs outside CMAs), (3) whether supplementary feeding is related to the diversity and composition of plants in the diet, and (4) how dietary composition relates to reproductive success. We hypothesise that: (1) dietary composition will differ significantly between North and South populations reflecting potential differences in resource availability between the

two areas, (2) parakeets that breed within CMAs will show greater diversity of native plant species in their diet compared to those outside of CMAs, (3) access to supplementary food may either facilitate more extensive foraging for diverse native foods by reducing time constraints or reduce dietary diversity through increased reliance on artificial food sources, and (4) consumption of native plant species will positively correlate with reproductive success.

4.3 Methods

4.3.1 Ethics statement

Sampling methods were approved by the Research Ethics Committee of the School of Anthropology and Conservation, University of Kent. All samples were collected alongside trained field staff at the Mauritian Wildlife Foundation (MWF) and the National Parks and Conservation Service (NPCS) of the Government of Mauritius, as part of the ongoing conservation management and monitoring programme for the Mauritius parakeet (NPCS permit: NP-46/3V5). Samples were transported from Mauritius to the UK under APHA Import Permit ITIMP21.0404. Field staff were trained in safe collection of faecal samples and minimising bird-handling time to reduce stress on the birds.

4.3.2 Study species

The Mauritius parakeet (*Alexandrinus eques*) inhabits forested areas of the Black River Gorges National Park on Mauritius (20°23'S, 57°23'E), across two populations: 'Grand Gorges' in the North and 'Bel Ombre' in the South. Despite being separated by only 1.8 km, these groups show minimal natural dispersal between them (Raisin et al., 2012; Tollington et al., 2013; **Figure 4.1**) owing to a high plateau and fragmented habitat. The North population contains five CMAs, while the South population has three. These

protected areas range from 0.06 to 0.23 km² in size (MWF, 2020). Fences surrounding CMAs do not prevent birds from foraging outside of the CMA.

Mauritius parakeets exhibit monogamous pair bonding and typically commence breeding at two years of age. The breeding season extends from October to February, with females laying clutches of one to four eggs. Females incubate for approximately 25 days while males provide food (Young, 1987; Jones and Duffy, 1993). After hatching, females remain with chicks for up to two weeks before joining males in foraging duties (Young, 1987; Duffy, 1993). Chicks typically fledge between February and March (Thorsen & Jones, 1998), remaining dependent on parental care for up to three months post-fledging (Young, 1987).

Natural diet consists primarily of fruits, bark, flowers, and leaves throughout their range (Young, 1987). To supplement this diet, commercial parrot food is provided at designated feeding stations, with different provisioning schedules between populations. This supplementary feeding has been linked to improved fledging success (Tollington et al., 2019), though variation in natural diet and its influence on nestling provisioning remains poorly understood. Supplementary food is provided daily in the North and once per week in the South, and feeders are typically empty by the time they are refilled in both locations.

4.3.3 Plant DNA reference library

We compiled a DNA barcode reference library specific to Mauritian plants targeting the Internal Transcribed Spacer 2 (ITS2) region using S2F and S3R markers (Chen et al., 2010). Further details on the reference library construction and validation are provided in the supplementary information. The library was constructed using multiple sources:

1. Sequencing data from Mauritian plant samples collected on mainland Mauritius, primarily from arboretums managed by the National Parks and Conservation Service (NPCS); the National Park; and other conservation areas with assistance from Mauritian Wildlife Foundation (MWF) and Ebony Forest. Of the known 691 plant species on Mauritius (Baider et al. 2010), plant sampling was prioritised based on known occurrence of a plant species in Mauritius parakeet diet (from prior direct observations by MWF), endemism to Mauritius or the Mascarene Islands, and whether any barcodes from a species were already available on the NCBI GenBank database (Sayers et al., 2022).
2. Sequencing data from plant samples acquired from an herbarium collection at Kew Gardens, London, UK.
3. ITS2 sequences downloaded from the NCBI GenBank database, including some generated in a prior study by Moorhouse-Gann et al. (2018) which focused on plants from Round Island and Ile Aux Aigrettes, two small islets off the coast of Mauritius.

Library construction was completed at the NERC Environmental Omics Facility (NEOF), with initial lab work (DNA extraction and library preparation) taking place at the University

of Sheffield, and library sequencing taking place at the University of Liverpool. Species assignment was verified for each sequence by BLASTn searches against all other sequences in the library and against the NCBI GenBank database. If the sequence's top bit score matched exclusively to a single species, the sequence was identified to species level. In cases where the top bit score was identical for multiple species, but all belonged to the same genus, the identification was assigned to the genus level. When the top bit score was the same for multiple genera within a single family, it was assigned to family level. Finally, if the sequence's top bit score matched to multiple families, it was classified as unknown, as no specific taxonomic assignment could be made. This BLASTn approach to verify the species of each sample was chosen due to its widespread use in DNA metabarcoding studies for assigning sequences to taxa and assessing taxonomic resolution, as demonstrated in prior research (García-Robledo et al., 2013; Hawkins et al., 2015; Ando et al., 2020).

4.3.4 Faecal sampling

Faecal samples were collected from Mauritius parakeet chicks at approximately 45-days old during routine nest site visits with support from MWF field staff. At this stage, both male and female parent typically forage and feed chicks, so the samples should reflect combined parental diet (Young, 1987; Duffy, 1993). A total of 164 samples were collected during the breeding season (October to February) across three years (2020-2022) from the North (n=119) and South (n=45) populations of the Black River Gorges National Park. Of these, 30 samples were collected from within CMAs and 134 from outside CMAs, with all CMA samples coming from the North population.

4.3.5 DNA extraction and sequencing

Dried faecal samples were first homogenised using a sterilised metal bead and TissueLyser (Qiagen, Manchester, UK). DNA was then extracted from at least 10 mg of homogenised faecal sample using the QIAamp DNA Stool Mini Kit (Qiagen, Manchester, UK), following the manufacturer's protocol. To control for potential contamination, one DNA extraction negative was included in each extraction session.

The first PCR amplification was carried out in 20 µl reaction volumes containing 10 µl of Qiagen Multiplex PCR Master Mix, 2 µl each of UniPlantF and UniplantR primers (Moorhouse-Gann et al., 2018), 5 µl ddH₂O, and 1 µl of DNA template (at 10ng/µl). The PCR program consisted of an initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 30 sec, 56°C for 30 sec, and 72°C for 1 min, with a final extension step of 72°C for 10 min. PCR products were visualised on 1% agarose gel to check for contamination and amplification success. Successfully amplified products were then purified using Promega Pronex beads (Promega Corporation, Madison, USA) to remove residual reagents.

A second PCR was performed to add indexed Illumina adapters to the amplicons from each sample. These reactions were carried out in 20 µl volumes containing 10 µl of Meridian Bioscience MyTaq HS mix (Meridian Bioscience, Cincinnati, USA), 1 µl each of the forward and reverse primers (at 10 µM), 1 µl sterile ddH₂O, and 8 µl of template from the first PCR. To verify the successful addition of Illumina adapters, products from both the first and second PCR were analysed on an Agilent Tapestation with a D1000 ScreenTape (Agilent Technologies, Waldbronn).

For library preparation, amplicons were combined into pools of up to 24 samples at equal concentrations. These sample pools were purified with Promega Pronex beads and run on a TapeStation to check for primer dimers. Quantification was performed using qPCR on an Applied Biosystems QuantStudio 12K Flex (Applied Biosystems, Foster City, USA). The sample pools were then combined into one final pool at a concentration of 4 nM. The exact concentration of this final pool was determined by repeating the qPCR before sequencing.

Sequencing was performed at the Centre for Genomic Research (University of Liverpool, UK) on an Illumina MiSeq platform (Illumina, San Diego, USA) using 250 bp paired end reads.

4.3.6 Bioinformatics

Raw sequencing reads were processed using the DADA2 pipeline (Callahan et al., 2016) implemented in R. This pipeline was used to filter and de-noise the raw sequencing data, resulting in the inference of amplicon sequence variants (ASVs) without the need for OTU clustering. Taxonomic assignment was initially performed using our custom reference library for Mauritian plants. For ASVs that could not be assigned taxonomy using this reference library, a BLASTn search against the GenBank database (Sayers et al., 2022) was conducted to identify taxonomic affiliations based on sequence similarity, with a minimum percent identity threshold of 97%.

This pipeline generated 2699 ASVs from all faecal samples, with 1899 (70.4%) being assigned to at least Family level. The 800 ASVs that could not be identified to at least Family level were removed from the analysis. The remaining 1899 ASVs were collapsed into 98 unique taxa based on their taxonomic assignments. To mitigate potential

incorrect inferences in downstream analyses, contaminant taxa originating from sources such as reagents and laboratory environments were identified and removed from the analysis. We used the 'decontam' package (Davis et al., 2018) in R to remove contaminants based on their prevalence in true samples and negative controls.

Following the recommendations of Drake et al. (2022) and Tercel et al. (2022) additional steps were taken to refine the contaminant removal process. These steps included the removal of taxa with very low abundance (fewer than 10 reads) and the subtraction of the maximum reads observed for a taxon within a negative control sample from all true samples. These measures were implemented to further enhance the accuracy and reliability of the dataset by minimising the influence of contaminating sequences and sequencing artifacts.

To ensure that the removal of unassignable ASVs and the collapse from ASVs to taxa did not bias the alpha and beta diversity metrics, the methods described below to calculate alpha and beta diversities were repeated using ASVs rather than their assigned taxa – the results were not significantly different, so we continued the analysis using assigned taxa as this is more relevant to the application of our results in understanding the diet of the Mauritius parakeet.

4.3.7 Native and non-native species in diet

To assess the composition of native and non-native plant species in the diet, the plant species were categorised into two groups: native and non-native plant taxa (POWO, 2025). The species richness of native and non-native plants in the diet were compared within each Mauritius parakeet population. Because of non-normal distribution of the species richness data, a Wilcoxon rank-sum test was performed to evaluate significant

differences between the richness of native, non-native, and SF-derived species (those known to occur within supplementary food pellets) in each population.

4.3.8 Alpha and beta diversity

Alpha and beta diversity measures were calculated for each sample, and compared between populations, and between samples within CMAs and outside of CMAs. To account for varying sequencing depths across samples, which ranged from less than 1,000 to over 145,000 reads (see **Figure 4.2**), species abundance data were first rarefied to a read depth of 10,000 using the ‘vegan’ package (Oksanen et al., 2022). This read depth was chosen as a compromise between retaining samples and preserving plant species diversity, and at this read depth all rarefaction slopes were close to zero (mean: 0.000015, range: 0 to 0.0002) indicating adequate coverage.

The ‘phyloseq’ package (McMurdie & Holmes, 2013) was employed to calculate and visualise alpha diversity using two metrics: "Observed" (species richness) and "Shannon" (Shannon diversity index) (Shannon, 1948). The alpha diversity metrics were plotted to visualise the distribution of richness and diversity across samples. Differences in alpha diversity values between groups were tested for significance using paired Wilcoxon rank-sum tests (also known as Mann-Whitney U tests).

To facilitate the beta diversity analysis, which compares diet composition across samples, ordination analyses were performed on the rarefied species abundance data using Jaccard dissimilarity distances. Jaccard dissimilarity was selected as it is specifically designed for binary presence/absence data and focuses on compositional differences between samples rather than species abundances (Anderson et al., 2006). Principal Coordinates Analysis (PCoA) was used to visualise sample relationships and

quantify variance explained by each axis. Additionally, Non-metric Multidimensional Scaling (NMDS) was performed to assess ordination robustness via stress values. Both analyses were conducted using the 'ordinate' function from the 'phyloseq' package.

To assess the significance of differences in diet composition between groups, a permutational multivariate analysis of variance (PERMANOVA) was performed. First, a Jaccard dissimilarity distance matrix was calculated based on the rarefied species abundance data using the distance function from the 'phyloseq' package. This distance matrix was then used as the response variable in a PERMANOVA, which was conducted using the `adonis2` function from the `vegan` package. CMA (inside or outside) and population (North or South) were used as explanatory variables in this analysis. To account for the nested structure of the data, permutations were conducted within each stratum defined by the combination of levels of these factors.

4.3.9 Predictors of plant species richness in diet

Factors influencing plant diversity in the diet were analysed using Generalised Linear Mixed Models (GLMMs) to account for multiple samples from the same brood (N samples = 149, N broods = 114). Three response variables were examined: total species richness excluding supplementary food (SF) species, Shannon diversity, and native species richness. For each response variable, we tested the effects of population, CMA (inside/outside), distance to nearest CMA (in km), distance to supplementary feeding station (km), year (2020-2022), and presence of SF-derived species. Distance to nearest CMA was calculated as the direct distance in kilometers from each nest site to the center of the nearest CMA.

Models were fitted using the 'glmmTMB' package (Brooks et al., 2017) with negative binomial distribution for count data (total and native species richness) due to overdispersion, and Gaussian distribution for Shannon diversity. All models included brood ID as a random effect to account for non-independence of samples from the same brood. The significance of model coefficients was assessed using 95% confidence intervals.

4.3.10 Diet composition and reproductive success

Diet composition was assessed based on the presence or absence of plant species in samples from each brood, serving as a proxy for parental diet. Reproductive success was measured as the number of fledglings per brood.

To analyse the relationship between diet composition and reproductive success, we performed GLMs using package 'glmmTMB' with gaussian distribution (after confirming normal distribution of fledgling numbers), with the number of fledglings per brood ($N_{\text{broods}}=96$ after data filtering to remove missing values from number of fledglings) as the response variable. Plant species were included as predictors in the model based on their frequency in the samples - we selected 14 plant species (**Table 4.1**) that were present in at least 20 samples and identified at least to the genus level, ensuring a robust dataset for analysis.

The initial model included all selected plant species, population, CMA, year, and distance to supplementary feeding station as fixed effects. Given the demonstrated differences in diet between populations, separate models were additionally performed for each population.

For each model, we performed model selection and averaging using the 'MuMIn' package (Bartoń, 2023). Our approach employed an information-theoretic framework based on the Akaike Information Criterion corrected for small sample sizes (AICc). We restricted our final model set to those with $\Delta\text{AICc} < 7$ before model averaging (Bolker et al., 2009; Burnham, Anderson, & Huyvaert, 2011).

From this set of top models, we calculated model-averaged parameter estimates, standard errors, and 95% confidence intervals. The significance of model-averaged coefficients was assessed using these 95% confidence intervals. To evaluate the relative importance of variables, we calculated weighted t-statistics following Cade (2015). This approach provided three metrics: absolute t-values indicating the strength of effects, t-ratios showing the relative importance of each predictor compared to the strongest predictor in each model, and t-variances reflecting the consistency of effects across models. Together, these metrics allowed us to quantify the relative influence of each predictor on reproductive success while accounting for model uncertainty.

4.4 Results

4.4.1 ITS2 library

In total, 560 plant tissue samples were collected representing 217 plant species. Sequencing was successful for 320 out of 560 samples, with sequencing failure typically associated with plants from 19 plant families, including Ebenaceae, Phyllanthaceae, and Celastraceae. Among the 30 herbarium samples provided by Kew Gardens, sequencing was successful for only seven samples. The 320 successful sequences represented 71% of plant species sampled. Through species assignment tests, 71% of sequences were assigned to species, 88% to genus (i.e. where a reference species was not available and

only one sequence of a species was available, it could only be confirmed to be correct at genus level), and 95% to family.

Following the species assignment tests, 296 of 320 sequences were compiled into the reference library (see **Supplementary Table 1**). The 296 sequences represent 147 plant species – 132 native to Mauritius (including 83 species endemic to Mauritius) and 15 introduced. In addition, 371 barcodes from 143 additional species were downloaded from GenBank, which included 14 endemic to Mauritius, 6 endemic to the Mascarenes, 29 native to Mauritius, and 58 introduced species. These additions increased the total number of species in the library to 290. This comprises approximately 42% of the 691 plant species known to be in Mauritius (Baider et al. 2010).

4.4.2 Faecal DNA extraction & sequencing

A total of 218 DNA extractions were attempted from the 190 Mauritius parakeet samples, of which 207 (94.9%) were successfully sequenced. The successful sequences represent samples collected from 156 individuals across three breeding seasons (2020 to 2022) and from two populations (see **Table 4.2**).

4.4.3 Diet composition

Of the 290 species in our ITS2 reference library, 83 taxa (29%) were detected in faecal samples. For previously documented parakeet food sources, 11 of 38 known species (29%) were detected at species level, while 19 of 38 (50%) were detected at genus level. By population, 74 taxa were detected in the North population and 43 in the South (**Table 4.3**). The 83 taxa represent 9.8% of the 691 plant species known to occur in Mauritius (Baider et al., 2010).

The identified taxa consisted of 44 plant families and 75 genera. Of the 83 taxa, 68 were identified to species level, 76 to genus and 83 to family level; 21 were categorised as endemic, 22 as native, 29 as non-native, and 3 as 'SF-derived' (known ingredient in supplementary food), or "NA" for taxa identified only to genus or family level, where that genus or family had multiple species that belonged to different categories. **Figures 4.3** and **4.4** indicate how these taxa occur in breeding pair diet across populations and between pairs breeding within and outside of CMAs, respectively.

The plant species with the highest prevalence across all samples was *Gaertnera sp.* (endemic, previously observed in diet), present in 79 samples – 61 in the North, 18 in the South (which translates as 53.5% of all samples in the North, and 42.8% of all samples in the South). There are approximately twelve species of *Gaertnera* known in Mauritius and all are endemic (Atkinson et al., 2007). The second most common species was *Triticum Urartu* (non-native, present in supplementary food). *T. urartu* was identified in 77 samples – 66 in the North (57.8% of samples), and 11 in the South (26.2% of samples). The next most common taxa, in order of prevalence, were *Tabernaemontana persicariifolia* (native, previously observed in diet), *Olea lancea* (native, previously observed in diet), *Tridax procumbens* (non-native, novel diet component), *Labourdonnasia revoluta* (endemic, previously observed in diet), *Avena sativa* (non-native, supplementary food), and *Labourdonnasia calophylloides* (endemic, previously observed in diet).

The most common species in the North was *Triticum Urartu* (non-native, supplementary food), followed by *Olea lancea* (native, previously observed in diet) and *Tabernaemontana persicariifolia* (native, previously observed in diet). In the South, the

most common species was an unknown species in the Asteraceae family, followed by *Tridax procumbens* (non-native, novel diet component), *Gaertnera sp.* (endemic, previously observed in diet) and *Syzygium jambos* (non-native, novel diet component).

Out of 38 plant species previously known to be in the Mauritius parakeet diet (see **Table 4.4**), 29 were present in the prepared ITS2 barcode library. Of the 29 present in the library, 11 species (37.9%) were detected in faecal samples and 19 species (65.5%) had their genus detected in faecal samples.

4.4.4 Native vs non-native species in the diet

Native species richness was significantly higher than non-native species richness in the North (Wilcoxon rank-sum test, $W = 9954.5$, $n = 113$, $p < 0.001$), with means of 4.7 ± 0.3 and 2.1 ± 0.2 species per sample, respectively. In the South, there was no significant difference between the native and non-native species richness (Wilcoxon rank sum test, $W = 907.5$, $n = 43$, $p > 0.05$), with means of 1.9 ± 0.2 and 1.7 ± 0.2 species per sample. There was no significant difference in native species richness between samples within CMAs ($n = 29$) and outside of CMAs (Wilcoxon rank-sum test, $W = 1431.5$, $p > 0.05$). The mean number of native, non-native and SF-derived plant species per sample in each population are shown in **Table 4.5**.

4.4.5 Alpha & beta diversity

Alpha diversity was significantly higher in the North than the South for both Observed (Wilcoxon rank-sum test, $W = 1487.5$, $p < 0.001$) and Shannon (Wilcoxon rank-sum test, $W = 1113$, $p < 0.001$) measures of diversity (**Figure 4.5**). Species richness within CMAs was significantly higher than outside of CMAs Species richness within CMAs was significantly

higher than outside of CMAs (Wilcoxon rank-sum test, Observed $W = 1035.5$, $p < 0.01$; Shannon $W = 1004$, $p < 0.001$).

Beta diversity analysis indicated significant differences in diet composition between the North and South (PERMANOVA, $F=8.77$, $R^2=0.057$, $p=0.001$), and between samples within and outside CMAs (PERMANOVA, $F=2.71$, $R^2=0.018$, $p=0.004$). PCoA ordination showed that the first two axes explained 23.8% and 12.8% of variation respectively (36.6% combined), with the remaining 63.4% distributed across additional axes. NMDS analysis achieved a stress value of 0.187, indicating usable representation of the data relationships in two dimensions (Dexter & Bollens, 2018). PCoA plots illustrating dietary composition differences among samples are shown in **Figure 4.6**.

4.4.6 Predictors of plant species richness in diet

The models revealed significant effects of population and supplementary food presence on plant species diversity in Mauritius parakeet diet (**Table 4.6**). Model selection based on AIC indicated a single top model for each response variable, with no competing models within 7 AIC units.

Birds in the North population showed significantly higher total species richness (excluding SF species) compared to the South, with the North population consuming 35% more total plant species and 77% more native species than the South. The presence of supplementary food in the diet was also positively associated with total species richness. Neither CMA presence, distance to nearest CMA, distance to feeding station, nor breeding season had significant effects on total species richness.

Shannon diversity showed similar patterns, with higher diversity in the North population and when supplementary food was present. The strongest effect on both Observed species richness and Shannon diversity was supplementary food presence, followed by population effects, as indicated by their relative t-values.

For native species richness specifically, the North population exhibited substantially higher richness compared to the South. The presence of supplementary food was also positively associated with native species richness. As with the other diversity measures, CMA presence, distance to nearest CMA, distance to feeding station, and breeding season showed no significant effects on native species richness.

Across all three response variables, population and supplementary food presence consistently emerged as the strongest predictors based on t-statistics, while other environmental and management factors had minimal influence on dietary diversity.

4.4.7 Plant species & reproductive success

The full averaged model examining factors influencing the number of fledglings per brood revealed several significant relationships (**Table 4.7**). The presence of *Gaertnera sp.* was positively associated with fledgling numbers, while *Labourdonnaisia calophylloides* and *Homalanthus sp.* showed negative associations (**Figure 4.7**). Breeding season also had a negative effect on fledgling numbers. Other factors including population, distance to feeder, and CMA status showed no significant effects (see **Table 4.7**).

Analysis of the populations separately revealed distinct patterns (**Tables 4.8 and 4.9**). In the North population, breeding season showed a negative effect on fledgling numbers, *Labourdonnaisia calophylloides* was negatively associated with fledgling success, while

Triticum urartu (a wheat species present in supplementary food) showed a positive association. In the South population, *Gaertnera* sp. was positively associated with fledgling numbers, while both *Ligustrum robustum* and *Homalanthus* sp. showed negative associations. Unlike the North population, the South showed no significant seasonal effect on fledgling numbers.

4.5 Discussion

This study is the first to utilise DNA metabarcoding to characterise the Mauritius parakeet diet, and to relate diet to reproductive success in this species. Our findings revealed distinct patterns in Mauritius parakeet diet across the two populations, particularly higher native plant diversity in the North (4.7 vs 1.9 species per sample) despite a landscape dominated by invasive plants such as guava (*Psidium cattleianum*) (Florens et al., 2016) outside of CMAs. The populations showed contrasting relationships between diet and reproductive success: species associated with supplementary feeding enhanced reproduction in the North, while endemic *Gaertnera* sp. consumption positively influenced fledgling numbers in the South, suggesting population-specific adaptations to local conditions.

4.5.1 Dietary breadth

Our DNA metabarcoding approach substantially expanded our understanding of Mauritius parakeet dietary breadth. While previous field observations had documented 38 plant species in their diet – 29 of which were included in our DNA reference library – we identified 83 distinct taxa, with 68 resolved to species level. Interestingly, only 11 species (or 19 at genera) overlapped between field observations and our molecular analysis, suggesting that traditional observational methods may significantly

underestimate dietary diversity. This pattern aligns with findings from Goldberg et al. (2020) who similarly found DNA metabarcoding revealed considerably more dietary components than previous methodologies in ground squirrels. The limited overlap between field observations and molecular detection (11 species) could indicate several things that visual observations may bias towards more conspicuous feeding behaviours or larger food items, or that some feeding occurs in locations or at times that make direct observation difficult.

This expanded dietary profile has important implications for understanding the species' ecological flexibility and potential resilience to habitat modification. With more than twice as many food plants identified than previously known, Mauritius parakeets appear to have a broader dietary niche than earlier assumed. This expanded dietary breadth may help explain their ability to persist in modified landscapes, particularly in the North where we found unexpectedly high native plant diversity in the diet despite habitat degradation. Additionally, the identification of previously unknown food plants provides new targets for habitat management and restoration efforts.

An unexpected finding was the relatively low consumption of dominant invasive plants. At a site within the North population range, Virah-Sawmy et al. (2009) documented that *Psidium cattleianum* alone comprised 57% of all individual plants. While both *Psidium cattleianum* and tree privet (*Ligustrum robustum*) are highly invasive and widespread across the North population's range, these species appeared infrequently in our dietary samples. Of 156 total faecal samples analysed, *P. cattleianum* was detected in only 21 samples (19 North, 2 South) and *L. robustum* in 24 samples (20 North, 4 South). This suggests that parakeets may be actively selecting against these abundant invasive

species in favour of less common native foods, though further research incorporating systematic vegetation surveys would be needed to confirm this pattern of dietary selection.

4.5.2 Population differences

The striking difference in diet composition between North and South populations suggests strong spatial variation in foraging patterns. Despite historical vegetation surveys showing higher non-native species dominance in the North (Lorence & Sussman, 1986; Virah-Sawmy et al., 2009; Florens et al., 2012), the North population had higher native species richness per sample (4.7 vs 1.9 species). This unexpected finding is particularly notable given that at one reserve within the North population range, native tree density declined by 70% between 1939 and 1999, with mean species richness declining from 27.9 to 15.1 species (Virah-Sawmy et al., 2009).

Several mechanisms might explain this higher dietary diversity in the North, where the population inhabits a complex mosaic ranging from heavily invaded areas to five weeded CMAs. While our statistical models did not show a significant effect of CMA presence on diet composition, birds may be selectively foraging for native plants within CMAs while nesting outside them, or targeting scattered native resources rather than abundant invasives like *Psidium cattleianum*. The spatial configuration of CMAs across the landscape could facilitate access to diverse foraging opportunities, while high levels of habitat degradation may force birds to utilise a broader range of food sources, similar to dietary shifts observed in other species facing habitat changes (Varner & Dearing, 2014; Birnie-Gauvin et al., 2017; Kowalczyk et al., 2019; Lei et al., 2019). The heterogeneous landscape could promote dietary diversity through its mix of native remnants and non-

native species (Tscharntke et al., 2005; Fischer & Lindenmayer, 2007; Abbas et al., 2011; Chetcuti et al., 2020).

The more frequent supplementary feeding in the North may further enable this diverse foraging strategy by providing reliable energy for exploring and exploiting scattered resources (Harrison et al., 2010; Murray et al., 2016). In contrast, the South population's lower native plant diversity likely reflects both less frequent supplementary feeding, which may encourage reliance on specific native species like *Gaertnera* sp., and their different habitat composition, where large areas are dominated by *Syzygium jambos* (Virah-Sawmy et al., 2009), present in 17 out of 45 samples from this population.

4.5.3 Diet composition and reproductive success

Our hypothesis that native plant consumption would positively correlate with reproductive success showed population-specific support. In the North, supplementary food consumption (*Triticum urartu*) showed a positive association with fledgling numbers (Estimate = 0.57, 95% CI: 0.10 to 1.05), while the native *Labourdonnaisia calophylloides* had a negative effect (Estimate = -0.46, 95% CI: -0.88 to -0.04). This suggests that supplementary feeding may play a particularly important role in supporting reproduction in the North. This aligns with broader patterns in avian species where diet quality directly influences reproductive success through egg production, chick growth, and fledgling survival (Martin, 1987; Reynolds et al., 2003; Koutsos et al., 2001; Brightsmith et al., 2010), and with previous studies finding that supplementary food boosts reproduction in birds (González et al., 2006; Tollington et al., 2019).

In contrast, the South population showed a strong positive association between the endemic *Gaertnera* sp. (Estimate = 0.79, 95% CI: 0.38 to 1.20) and fledgling numbers,

while two non-native species - *Homalanthus* sp. (Estimate = -0.73, 95% CI: -1.34 to -0.12) and *Ligustrum robustum* (Estimate = -1.17, 95% CI: -1.83 to -0.51) - were negatively associated with reproductive success. The lack of a significant effect of breeding season in the South, contrary to the North, further emphasises how differently these populations respond to environmental and dietary factors. These population-specific relationships between diet and reproductive success likely reflect differences in habitat composition and management intensity, with the North population showing enhanced reproductive success from supplementary feeding despite their higher native species consumption, while in the South reproductive success was linked to specific native food resources like *Gaertnera* sp.

4.5.4 Conservation management areas

While we predicted CMAs would increase native species consumption through invasive plant removal (MWF, 2020), our findings revealed more nuanced patterns. Initial Wilcoxon tests suggested that CMAs increased overall plant species diversity in the parakeet diet, but did not specifically enhance native species consumption. However, when examined in more complex models that included both presence within CMAs and distance to nearest CMA, along with other predictors like supplementary feeding and population effects, neither measure of CMA influence significantly predicted species richness or native species consumption. Similarly, neither CMA metric predicted fledging success in either population-specific models or models of the entire population.

This unexpected result may reflect the way parakeets use the landscape - since their foraging ranges extend beyond CMA boundaries, birds may be accessing native plant resources across the habitat mosaic regardless of their nesting location relative to CMAs.

The five CMAs in the North and three in the South may serve as important reservoirs of native plant diversity, but their influence on bird diet could be more complex than our spatial metrics could detect. Rather than shifting consumption solely toward native species, CMAs may be effectively increasing the total diversity of food resources available to parakeets across the broader landscape.

The different spatial arrangement of CMAs between populations - five in the North versus three in the South - may contribute to the observed differences in dietary diversity between populations, even if our measures of CMA influence do not emerge as significant predictors in our models. CMAs may function as resource hubs within the habitat mosaic, facilitating access to native plants in ways that are not captured by simple distance metrics or nesting inside/outside categorisation. Understanding these landscape-level effects would require additional research into parakeet movement patterns and resource use across different spatial scales.

4.5.5 Limitations and future directions

While DNA metabarcoding provided unprecedented resolution of dietary composition, several methodological limitations should be considered. First, the relationship between sequence read abundance and actual biomass consumption remains uncertain, limiting our ability to quantify the relative importance of different food items (Pompanon et al., 2012; Thomas et al., 2016; Lamb et al., 2019). Additionally, dietary studies using DNA metabarcoding rely upon construction of a DNA reference library, which can be difficult to achieve fully. Our incomplete plant DNA reference library, particularly the inability to sequence certain key species like *Diospyros* sp., suggests that our dietary analysis may underestimate true diversity, a common limitation in metabarcoding studies (Pompanon

et al., 2012; Ando et al., 2020). The development of improved genetic markers or inclusion of a panel of different markers targeting different regions of DNA (Goldberg et al., 2020), for poorly-sequenced plant families would enhance future dietary analyses and potentially reveal additional important food resources.

A critical gap in our current understanding is the relationship between plant availability and dietary selection across the complex habitat mosaics inhabited by both populations. Without recent comprehensive surveys of plant availability and abundance across these complex landscapes, we cannot determine whether dietary patterns reflect food preferences or simply resource availability. For instance, the higher native plant diversity in the North population's diet could reflect either active selection of native species or greater accessibility of these resources through the spatial arrangement of CMAs and other habitat features. This distinction is important for understanding foraging behaviour and informing habitat management decisions.

Future studies should incorporate systematic vegetation surveys across both the managed and unmanaged portions of each population's range, along with detailed movement data to understand how birds navigate these habitat mosaics. Understanding foraging patterns at multiple spatial scales - from individual CMAs to the broader landscape - would help clarify how habitat configuration influences resource use. Additionally, investigation of seasonal variation in diet composition and its relationship with reproductive timing would provide valuable insights into resource requirements during different life stages.

4.5.6 Conclusion

This study suggests complex and population-specific dietary adaptations of Mauritius parakeets across a heterogeneous landscape. The DNA metabarcoding approach substantially expanded our understanding of their dietary breadth, identifying 83 taxa compared to the 38 species previously known from field observations. This enhanced resolution enabled detection of unexpected patterns, particularly higher native plant diversity in the North population's diet despite historical declines in native species density.

The population-specific nature of these dietary patterns highlights the importance of local adaptation in conservation management. While both populations inhabit complex mosaics of native and non-native vegetation, they show distinct foraging strategies. The North population maintains a more diverse diet with a mean of 4.7 native species per sample compared to 1.9 in the South. The positive association between supplementary food-related species and reproductive success in the North, contrasted with the importance of endemic *Gaertnera* sp. in the South, demonstrates how populations can develop distinct strategies for success within different habitat configurations. While CMAs did not show clear direct effects on diet composition or reproductive success, they may play more complex roles in the landscape than our analyses could detect.

These findings have important implications for conservation management of Mauritius parakeets and other species in heterogeneous landscapes. The population-specific relationships between diet and reproductive success suggest that management strategies should be tailored to local conditions rather than applying uniform approaches across populations. In the North, maintaining supplementary feeding appears crucial for

reproductive success, while in the South, protecting and potentially expanding populations of key native species like *Gaertnera* sp. may be more beneficial.

Future research priorities include developing improved genetic markers to improve the DNA reference library for Mauritian plants, comprehensive habitat surveys to understand resource availability across the different landscape elements, and longer-term studies to examine the relationship between diet and long-term reproductive success. Understanding how birds utilise different elements of their habitat mosaic will be crucial for refining conservation strategies and ensuring long-term population persistence.

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4.8 Tables and Figures

Table 4.1. Plant species included in the analysis

Family	Genus	Species
Rubiaceae	<i>Gaertnera</i>	<i>Gaertnera</i> sp.
Poaceae	<i>Triticum</i>	<i>Triticum</i> <i>Urartu</i>
Apocynaceae	<i>Tabernaemontana</i>	<i>Tabernaemontana persicariifolia</i>
Oleaceae	<i>Olea</i>	<i>Olea lancea</i>
Poaceae	<i>Avena</i>	<i>Avena sativa</i>
Sapotaceae	<i>Labourdonnaisia</i>	<i>Labourdonnaisia revoluta</i>
Sapotaceae	<i>Labourdonnaisia</i>	<i>Labourdonnaisia calophylloides</i>
Celastraceae	<i>Elaeodendron</i>	<i>Elaeodendron orientale</i>
Euphorbiaceae	<i>Homalanthus</i>	<i>Homalanthus</i> sp.
Sapotaceae	<i>Sideroxylon</i>	<i>Sideroxylon puberulum</i>
Euphorbiaceae	<i>Hancea</i>	<i>Hancea integrifolia</i>
Oleaceae	<i>Ligustrum</i>	<i>Ligustrum robustum</i>
Myrtaceae	<i>Syzygium</i>	<i>Syzygium jambos</i>
Myrtaceae	<i>Psidium</i>	<i>Psidium cattleianum</i>

Table 4.2. Faecal DNA sequencing sample sizes.

Season	North	South	Total
2020	49	16	65
2021	46	18	64
2022	19	8	27
Total	114	42	156

Table 4.3. Plant taxa identified in the Mauritius parakeet diet, with the number of samples the species was identified in in the North, South, and total population. Distribution is given as endemic, native, non-native, or SF (known ingredient of supplementary food). Plant species identified in only one sample were excluded. Where the species was only identified to family level, the genus and species are replaced by “NA”.

Family (and common family name)	Species scientific name	North	South	Total	Distribution
Amaranthaceae (Amaranth family)	<i>Amaranthus tortuosus</i>	15	1	16	Non-native
Apocynaceae (Dogbane family)	<i>Tabernaemontana persicariifolia</i>	64	12	76	Native
Asteraceae (Daisy family)	<i>Tridax procumbens</i>	43	29	72	Non-native
	<i>Asteraceae_NA_NA</i>	26	35	61	Unknown
	<i>Erigeron canadensis</i>	11	1	12	Non-native
	<i>Distephanus populifolius</i>	7	0	7	Endemic
	<i>Helichrysum mauritianum</i>	2	0	2	Endemic
Bignoniaceae (Bignonias or trumpet vines family)	<i>Tabebuia pallida</i>	0	5	5	Non-native
Boraginaceae (Borage family)	<i>Varronia curassavica</i>	3	3	6	Non-native
Burseraceae (Torchwoods family)	<i>Protium obtusifolium</i>	1	1	2	Endemic
Celastraceae (Spindle tree family)	<i>Elaeodendron orientale</i>	28	11	39	Native
	<i>Pleurostylia leucocarpa</i>	3	2	5	Endemic
Chrysobalanaceae (Cocoplum family)	<i>Grangeria borbonica</i>	7	1	8	Native
Cucurbitaceae (Gourd family)	<i>Cucurbitaceae_NA_NA</i>	1	1	2	Unknown
Ebenaceae (Ebony family)	<i>Diospyros tessellaria</i>	5	0	5	Endemic
Euphorbiaceae (Spurge family)	<i>Homalanthus sp.</i>	33	5	38	Non-native
	<i>Hancea integrifolia</i>	18	7	25	Native
	<i>Stillingia lineata</i>	9	0	9	Native
	<i>Euphorbia pyrifolia</i>	1	3	4	Endemic
Fabaceae (Pea or legume family)	<i>Pongamia pinnata</i>	1	1	2	Non-native
Hypericaceae (St. John’s wort family)	<i>Hypericaceae_NA_NA</i>	5	0	5	Unknown
Lauraceae (Laurel family)	<i>Litsea glutinosa</i>	1	3	4	Non-native
Malpighiaceae (Barbados cherry family)	<i>Hiptage sp.</i>	8	2	10	Non-native
Malvaceae (Mallow family)	<i>Sida pusilla</i>	2	0	2	Native
Melastomataceae (Melastome family)	<i>Warneckea trinervis</i>	5	2	7	Endemic
Monimiaceae (Monimia family)	<i>Tambourissa cordifolia</i>	4	0	4	Endemic
	<i>Tambourissa peltate</i>	2	0	2	Endemic
Moraceae (Mulberry or fig family)	<i>Ficus reflexa</i>	18	0	18	Native
	<i>Ficus rubra</i>	2	0	2	Native
Myrtaceae (Myrtle family)	<i>Syzygium jambos</i>	5	17	22	Non-native
	<i>Psidium cattleyanum</i>	19	2	21	Non-native

Family (and common family name)	Species scientific name	North	South	Total	Distribution
	<i>Eugenia lucida</i>	3	1	4	Endemic
Oleaceae (Olive family)	<i>Olea lancea</i>	65	9	74	Native
	<i>Ligustrum robustum</i>	20	4	24	Non-native
Passifloraceae (Passionflower family)	<i>Passiflora suberosa</i>	2	0	2	Non-native
Phyllanthaceae (Leaf-flower family)	<i>Margaritaria anomala</i>	2	0	2	Native
Poaceae (Grass family)	<i>Triticum Urartu</i>	66	11	77	SF
	<i>Avena sativa</i>	62	9	71	SF
	<i>Cenchrus echinatus</i>	7	1	8	Non-native
	<i>Stenotaphrum dimidiatum</i>	4	3	7	Native
Rhamnaceae (Buckthorn family)	<i>Scutia myrtina</i>	5	0	5	Native
Rosaceae (Rose family)	<i>Rubus rosifolius</i>	5	0	5	Non-native
Rubiaceae (Coffee or madder family)	<i>Gaertnera sp.</i>	61	18	79	Endemic
	<i>Bremeria landia</i>	7	0	7	Endemic
	<i>Fernelia buxifolia</i>	2	0	2	Endemic
	<i>Rubiaceae_NA_NA</i>	2	0	2	Unknown
	<i>Pyrostria viburnoides</i>	0	2	2	Endemic
Rutaceae (Rue or citrus family)	<i>Triphasia trifolia</i>	3	0	3	Non-native
Sapindaceae (Soapberry family)	<i>Molinaea alternifolia</i>	14	3	17	Native
Sapotaceae (Sapote family)	<i>Labourdonnaisia revoluta</i>	54	8	62	Endemic
	<i>Labourdonnaisia calophylloides</i>	45	3	48	Native
	<i>Sideroxylon puberulum</i>	25	4	29	Endemic
	<i>Sideroxylon grandiflorum</i>	13	1	14	Endemic
Solanaceae (Nightshade or potato family)	<i>Solanum nigrum/americanum</i>	0	5	5	Non-native
	<i>Solanum lycopersicum</i>	1	1	2	Non-native
Stilbaceae (Stillbe family)	<i>Nuxia verticillate</i>	2	0	2	Native
Thymelaeaceae (Mezereon family)	<i>Wikstroemia indica</i>	6	0	6	Non-native

Table 4.4. Plant species previously observed in the Mauritius parakeet diet (MWF, 2020). Numbers in parentheses indicate the total number of faecal samples in which the species or genus was detected by DNA metabarcoding of faecal samples.

Family (common name)	Plant species	Species in DNA library	Species detected in faecal samples	Genus detected in faecal samples
Apocynaceae (Dogbane family)	Tabernaemontana persicariifolia	Y	Y (76)	Y (76)
Burseraceae (Torchwood family)	Canarium paniculatum	Y	N	N
	Protium obtusifolium	Y	Y (2)	Y (2)
Calophyllaceae (Calophyllum family)	Calophyllum eputamen	Y	N	N
	Calophyllum parviflorum	Y	N	N
	Calophyllum tacamahaca	N	N	N
Celastraceae (Spindle tree family)	Elaeodendron orientale	Y	Y (39)	Y (39)
	Pleurostyliia leucocarpa	Y	N	Y (5)
Connaraceae (Connarus family)	Cnestis polyphylla	Y	N	N
Ebenaceae (Ebony family)	Diospyros egrettarum	Y	N	Y (5)
	Diospyros melanida	N	N	Y (5)
	Diospyros revaughanii	Y	N	Y (5)
	Diospyros tessellaria	Y	Y (5)	Y (5)
Euphorbiaceae (Spurge family)	Hancea integrifolia	Y	Y (25)	Y (25)
	Stillingia lineata	Y	Y (9)	Y (9)
Lauraceae (Laurel family)	Litsea monopetala	N	N	Y (4)
Myrtaceae (Myrtle family)	Eugenia bojeri	Y	N	Y (4)
	Eugenia lucida	N	Y (4)	Y (4)
	Eugenia orbiculata	N	Y (1)	Y (4)
	Eugenia pollicina	N	N	Y (4)
	Psidium cattleianum	Y	Y (21)	Y (21)
	Syzygium glomeratum	Y	N	Y (22)
	Syzygium coriaceum	Y	N	Y (22)
Oleaceae (Olive family)	Ligustrum robustum	Y	Y (24)	Y (24)
	Olea lancea	Y	Y (74)	Y (74)
Ochnaceae (Ochna family)	Ochna mauritiana	Y	N	N
Phyllanthaceae (Leaf-flower family)	Securinega durissima	N	N	N
Polypodiaceae (Polypody family)	Phymatodes scolopendria	Y	N	N
Rubiaceae (Coffee/madder family)	Gaertnera psychotrioides	Y	N	Y (79)
Sapotaceae (Sapote family)	Labourdonnaisia calophylloides	Y	Y (48)	Y (62)
	Labourdonnaisia glauca	N	N	Y (62)

Family (common name)	Plant species	Species in DNA library	Species detected in faecal samples	Genus detected in faecal samples
	Labourdonnaisia revoluta	Y	Y (62)	Y (62)
	Mimusops erythroxyton	Y	N	N
	Mimusops balata	Y	N	N
	Sideroxyton cinereum	N	N	Y (29)
	Sideroxyton grandiflorum	Y	Y (14)	Y (29)
	Sideroxyton puberulum	Y	Y (29)	Y (29)
	Nuxia verticillata	Y	Y (2)	Y (2)
Stilbaceae (Stilbe family)				

Table 4.5. Mean number of native, non-native, supplementary food-derived (SF), unknown, and total plant species identified per sample by population.

Population	Native	Non-native	Unknown	SF	Total
North	4.7	2.1	0.4	1.1	8.3
South	1.9	1.7	0.9	0.4	4.9

Table 4.6. Model coefficients and statistics for predictors of plant diversity measures in Mauritius parakeet diet. Results shown are from Generalised Linear Mixed Models with brood ID as random effect. Includes model coefficient estimates, standard errors (SE), p-values, 95% confidence intervals, and t-statistics (absolute t-value, t-ratio relative to maximum t-value, and variance in t-values) indicating relative predictor importance. Negative binomial models were used for species counts (Observed and Native species) and Gaussian model for Shannon diversity. Variables for which 95% confidence intervals do not cross zero are shown in bold.

Response variable	Parameter	Estimate	SE	p-value	CI 2.5%	CI 97.5%	t abs	t ratio	var t
Observed (excluding SF species)	Intercept	1.34	0.18	<0.001	0.99	1.69	7.52	1	0
	SF presence	0.4	0.09	<0.001	0.22	0.58	4.32	1	0
	Pop North	0.36	0.11	0.001	0.14	0.57	3.2	1	0
	Season 2021	-0.12	0.1	0.215	-0.32	0.07	1.24	0.71	0
	Season 2022	-0.21	0.12	0.08	-0.45	0.03	1.75	0.71	0
	Nearest feeder	-0.01	0.09	0.9	-0.18	0.16	0.13	1	0
	Nest in CMA	0.06	0.12	0.608	-0.18	0.31	0.51	1	0
	Nearest CMA	0.06	0.12	0.625	-0.17	0.29	0.49	1	0
Shannon	Intercept	1.01	0.19	<0.001	0.64	1.38	5.3	1	0
	SF presence	0.64	0.1	<0.001	0.44	0.84	6.35	1	0
	Pop North	0.44	0.12	<0.001	0.21	0.67	3.7	1	0
	Season 2021	-0.04	0.11	0.735	-0.26	0.18	0.34	0.29	0
	Season 2022	-0.16	0.14	0.236	-0.43	0.1	1.19	0.29	0
	Nearest feeder	0.05	0.1	0.601	-0.15	0.25	0.52	1	0
	Nest in CMA	0.13	0.15	0.39	-0.16	0.41	0.86	1	0
	Nearest CMA	0	0.13	0.996	-0.26	0.26	0.01	1	0
Native Species	Intercept	1.52	0.79	0.054	-0.03	3.08	1.93	1	0
	Pop North	2.31	0.49	<0.001	1.34	3.27	4.69	1	0
	SF presence	1.49	0.42	<0.001	0.66	2.32	3.51	1	0
	Season 2021	-0.63	0.46	0.175	-1.53	0.28	1.36	1	0
	Season 2022	-0.71	0.56	0.21	-1.81	0.4	1.25	1	0
	Nearest feeder	-0.12	0.42	0.776	-0.94	0.7	0.28	1	0
	Nest in CMA	0.22	0.6	0.712	-0.96	1.41	0.37	1	0
	Nearest CMA	0.36	0.55	0.51	-0.71	1.43	0.66	1	0

Table 4.7. Model-averaged coefficients estimates, standard errors (SE), 95% confidence intervals, and t-statistics (absolute, ratio, and variance) for predictors of the number of fledglings produced per brood. Weighted t-statistics are given as a measure of relative importance of each covariate. Variables for which 95% confidence intervals do not cross zero are shown in bold.

Variable (Full model)	Estimate	SE	p-value	CI 2.5%	CI 97.5%	t_abs	t_ratio	var.t
Intercept	2.39	0.21	<0.001	1.97	2.80	11.25	NA	NA
Gaertnera sp.	0.50	0.19	0.01	0.14	0.87	2.58	0.93	0.14
Labourdonnaisia calophylloides	-0.49	0.20	0.01	-0.88	-0.10	2.47	0.90	0.08
Season	-0.57	0.20	0.01	-0.97	-0.17	2.41	0.88	0.26
Homalanthus sp.	-0.45	0.21	0.03	-0.85	-0.06	2.26	0.83	0.10
CMA	-0.23	0.28	0.41	-0.90	0.09	1.58	0.61	0.10
Triticum urartu	0.07	0.16	0.67	-0.15	0.68	1.31	0.54	0.17
Elaeodendron orientale	-0.10	0.17	0.57	-0.64	0.11	1.25	0.44	0.12
Sideroxylon puberulum	0.05	0.14	0.71	-0.16	0.64	1.25	0.48	0.06
Hancea integrifolia	-0.03	0.13	0.79	-0.72	0.24	1.00	0.39	0.10
Ligustrum robustum	-0.01	0.06	0.91	-0.57	0.32	0.76	0.30	0.10
Olea lancea	0.02	0.08	0.85	-0.24	0.56	0.65	0.26	0.17
Distance to feeder	0.00	0.03	0.91	-0.15	0.24	0.61	0.23	0.11
Syzygium jambos	0.01	0.09	0.87	-0.31	0.68	0.60	0.22	0.03
Avena sativa	0.01	0.07	0.87	-0.27	0.55	0.57	0.21	0.15
Labourdonnaisia revoluta	0.00	0.04	0.99	-0.39	0.37	0.37	0.15	0.08
Pop North	0.05	0.21	0.83	-0.37	0.46	0.36	0.14	0.09
Tabernaemontana persicariifolia	0.00	0.04	1.00	-0.35	0.35	0.21	0.08	0.04
Psidium cattleianum	0.00	0.05	0.99	-0.51	0.48	0.16	0.06	0.02

Table 4.8. Model-averaged coefficients estimates, standard errors (SE), 95% confidence intervals, and t-statistics for predictors of fledgling numbers in the North population. Variables for which 95% confidence intervals do not cross zero are shown in bold.

Variable (North model)	Estimate	SE	p-value	CI 2.5%	CI 97.5%	t_abs	t_ratio	var.t
(Intercept)	650	285	0.03	82	1219	-	-	-
Season	-0.32	0.14	0.03	-0.60	-0.04	2.37	0.94	0.15
Labourdonnaisia calophylloides	-0.46	0.21	0.03	-0.88	-0.04	1.71	0.66	0.21
Triticum urartu	0.57	0.24	0.02	0.10	1.05	1.50	0.60	0.42
Homalanthus sp.	-0.17	0.24	0.49	-0.82	0.08	0.92	0.36	0.38
CMA	-0.36	0.33	0.28	-1.07	0.06	0.73	0.30	0.41
Sideroxylon puberulum	0.08	0.18	0.66	-0.14	0.77	0.62	0.24	0.31
Syzygium jambos	0.15	0.33	0.64	-0.24	1.37	0.34	0.14	0.19

Table 4.9. Model-averaged coefficients estimates, standard errors (SE), 95% confidence intervals, and t-statistics for predictors of fledgling numbers in the South population. Variables for which 95% confidence intervals do not cross zero are shown in bold.

Variable (South model)	Estimate	SE	p-value	CI 2.5%	CI 97.5%	t_abs	t_ratio	var.t
(Intercept)	88.76	212.66	0.69	-351.37	528.88	-	-	-
Gaertnera sp.	0.79	0.20	0.00	0.38	1.20	3.61	0.94	0.19
Ligustrum robustum	-1.17	0.32	0.00	-1.83	-0.51	2.89	0.75	0.71
Homalanthus sp.	-0.73	0.29	0.02	-1.34	-0.12	1.85	0.48	0.31
Tabernaemontana persicariifolia	-0.07	0.18	0.70	-0.85	0.22	0.78	0.21	1.01
Season	-0.04	0.11	0.70	-0.26	0.18	0.43	0.12	0.04
Hancea integrifolia	-0.06	0.17	0.74	-0.88	0.27	0.37	0.11	0.29

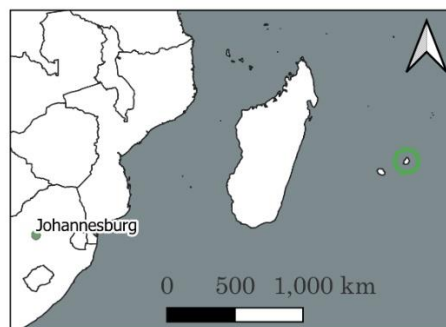


Figure 4.1. Distribution of the Mauritius parakeet population across the North and South of the Black

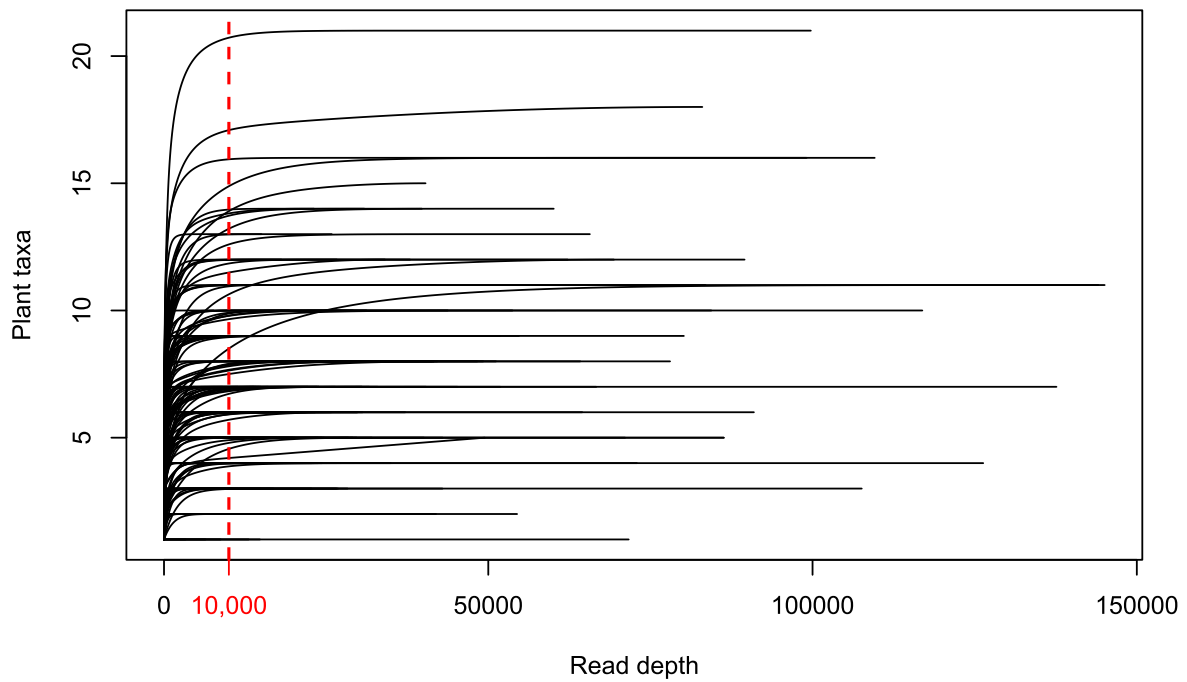


Figure 4.2. Rarefaction curves showing accumulation of plant taxa with increasing sequencing depth. Each curve represents an individual faecal sample, reaching an asymptote when additional sequencing depth does not yield new plant taxa. Analysis of curve slopes at 10,000 reads (mean slope = 0.00015) indicates adequate sequencing depth for detecting plant diversity.

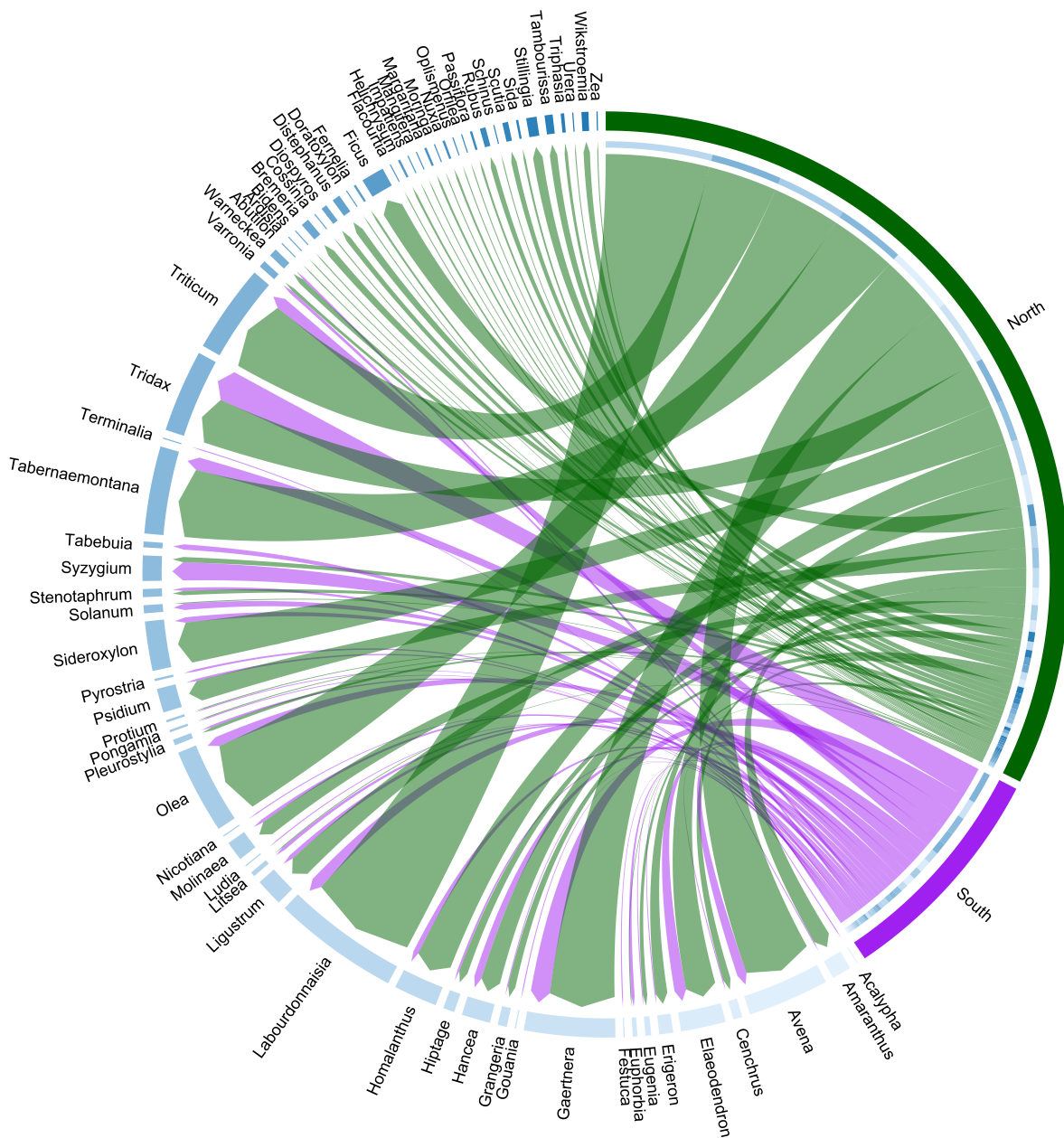


Figure 4.3. Distribution of plant genera found in the diet of Mauritian parakeets in North (green) and South (purple) populations. Chords connecting the population arcs to genus arcs indicate the presence of a plant genus in that population. The width of each chord is proportional to the frequency of the plant genus in that population. Genera with wider connections to a population indicate a stronger presence or preference, while those connected to both populations suggest common plant resources between the North and South.

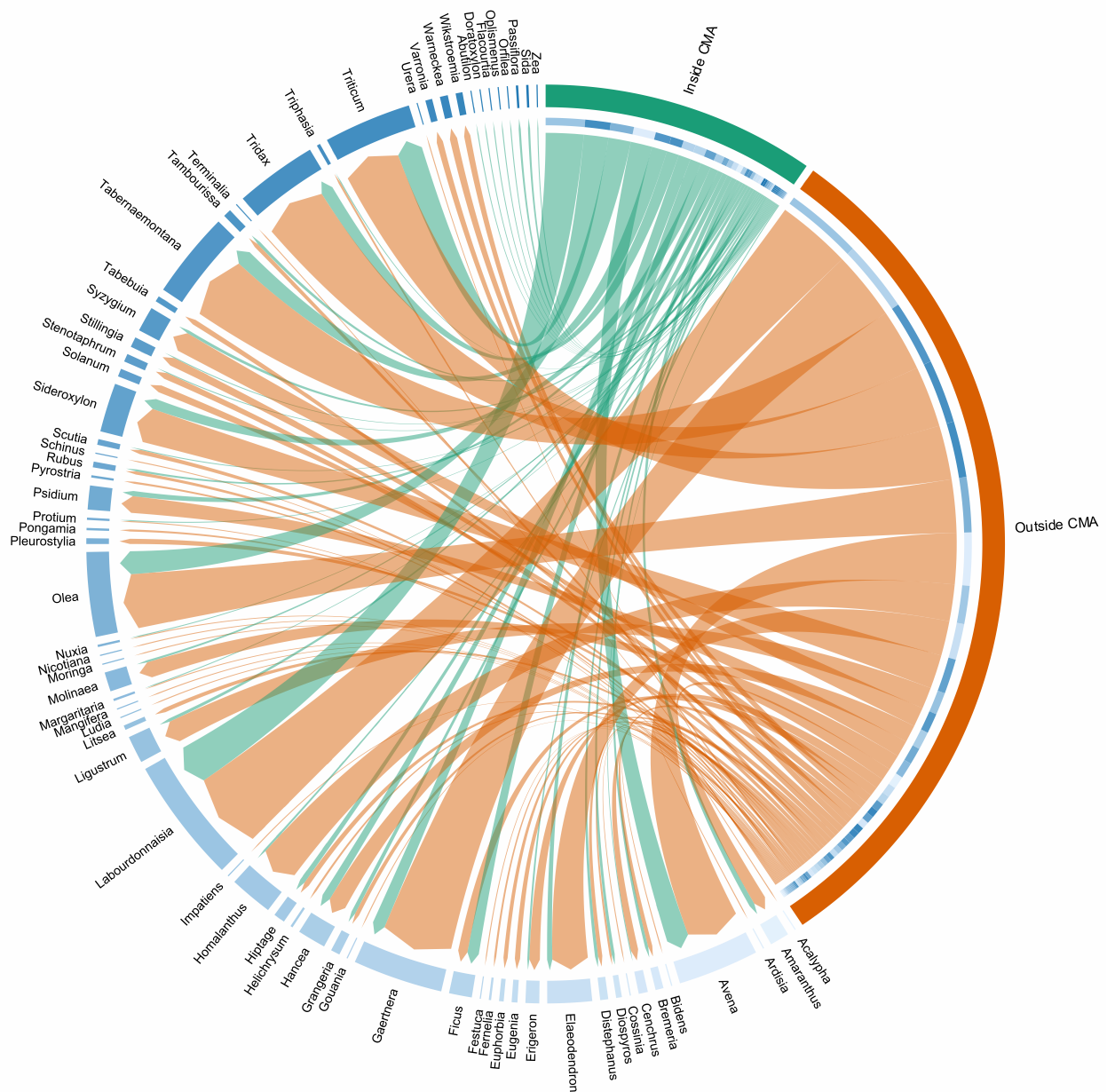


Figure 4.4. Distribution of plant genera found in the diet of Mauritius parakeets breeding inside and outside of CMAs. Chords connecting the inside/outside CMA arcs to genus arcs indicate the presence of a plant genus within that group. The width of each chord is proportional to the frequency of the plant genus in that group. Genera with wider connections to a population indicate a stronger presence or preference, while those connected to both populations suggest common plant resources between both groups.

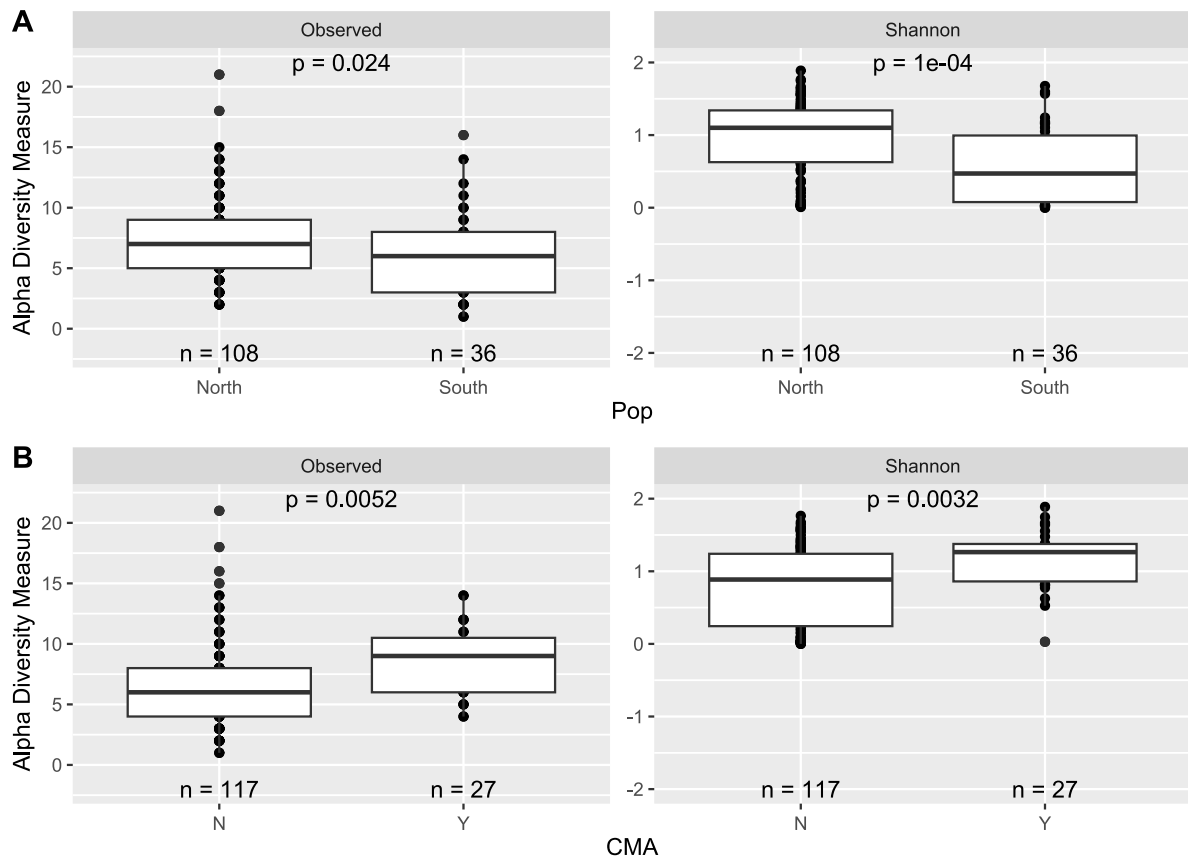


Figure 4.5. Alpha diversity of plant species in Mauritius parakeet faecal samples. **A)** Comparison of observed species richness and Shannon diversity between North (n=108) and South (n=36) populations. **B)** Differences in alpha diversity metrics between nests located outside (N, n=117) and inside (Y, n=27) Conservation Management Areas (CMA). Displayed p-values were calculated using Wilcoxon rank-sum tests.

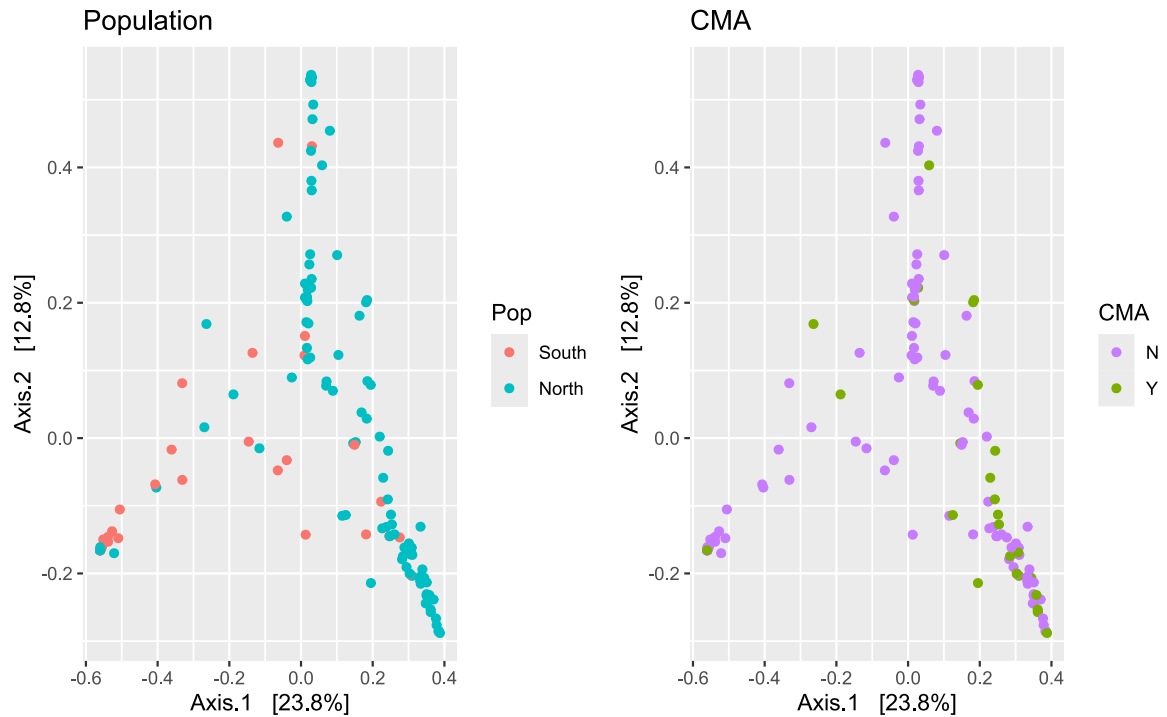


Figure 4.6. Principal Coordinates Analysis (PCoA) plots illustrating dietary composition differences among samples. Left panel shows samples color-coded by population (South: red, North: teal). Right panel shows the same samples color-coded by presence inside (Y: green) or outside (N: purple) Conservation Management Areas (CMAs). Each point represents an individual sample. Axis 1 explains 23.8% of the variation, while Axis 2 explains 12.8%. Closer points indicate more similar diets, while distant points represent more divergent dietary compositions. Jaccard dissimilarity was used to quantify compositional differences based on species presence/absence.

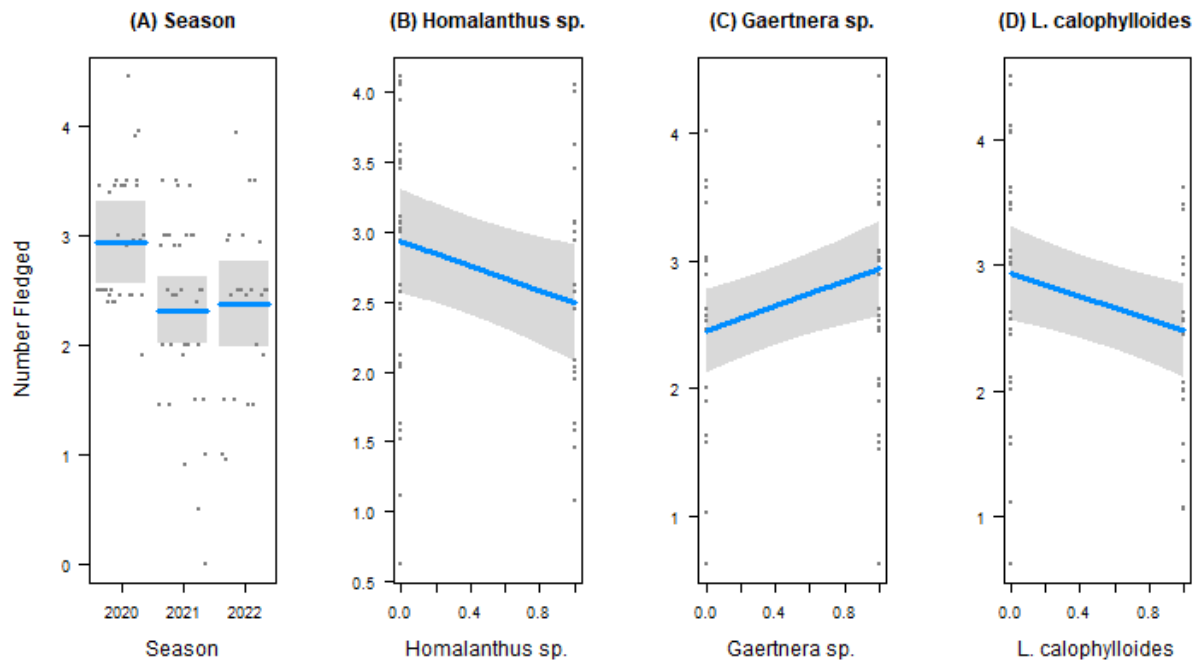


Figure 4.7. Partial residual plots showing the significant predictors of fledging success in our model.

The plots show the relationship between number of fledglings and (A) breeding season, showing a significant decline over time ($p = 0.004$ for 2021, $p = 0.018$ for 2022); (B) presence of *Homalanthus* sp. in the diet, showing a negative relationship ($p = 0.027$); (C) presence of *Gaertnera* sp. in the diet, showing a positive relationship ($p = 0.006$); and (D) presence of *L. calophylloides* in the diet, showing a negative relationship ($p = 0.012$). Blue lines represent model predictions and grey shading represents 95% confidence intervals. Points show partial residuals.

4.9 Supplementary Information

4.9.1 Background: ITS2 barcode library

DNA barcoding has emerged as a powerful tool for species identification and biodiversity assessment (Moritz & Cicero, 2004; DeSalle & Goldstein, 2019), particularly in regions with high levels of endemism such as Mauritius (Florens, 2013). The potential of the ITS2 region as a universal DNA barcode for plants has been demonstrated in several studies (Chen et al., 2010; Yao et al., 2010; Moorhouse-Gann et al., 2018; Al-Juhani, 2019).

Mauritius has a rich diversity of plant species (Atkinson et al., 2007; Florens et al., 2012), many of which are endemic and face threats from habitat loss and invasive species (Baider & Florens, 2011; Florens et al., 2012). Establishing a comprehensive DNA barcode library for Mauritian plants is crucial for conservation and management efforts, as well as for facilitating taxonomic research and ecological studies.

4.9.2 Methods: ITS2 barcode library

A comprehensive list of plants present in Mauritius was curated by consulting multiple sources. The primary references included the Moorhouse-Gann et al. (2018) study on offshore Mauritius islands, the Kew Gardens online database, a plant list curated by the IUCN SSC Mascarene Islands Plant Specialist Group, and a plant guide published by Mauritian Wildlife Foundation (Atkinson et al. 2007). Through this compilation process, plants were categorised according to their range status as endemic, native, or introduced, with additional notation of species observed in Mauritius parakeet foraging behaviours.

Plant tissue samples were collected systematically from multiple individuals representing each identified plant species at every site, with a target minimum of three individuals per species where feasible. All collected samples underwent preservation through drying over silica gel. Field identification was conducted with substantial expert support, drawing from multiple authoritative sources. This included sampling from trees previously identified and tagged by the Mauritian Wildlife Foundation during routine plant phenology monitoring, collection from maintained Arboretums or native plant nurseries, and supervised sampling by experts from NPCS, the Mauritius Forestry Service, MWF, and Ebony Forest. Species verification was strengthened through comprehensive documentation including photographic evidence and GPS coordinates where possible.

DNA extraction was performed using the Qiagen DNeasy Plant Pro kit following the manufacturer's protocol. The complete second internal transcribed spacer of nuclear ribosomal DNA (ITS2) and partial 5.8S and 26S sequences were amplified using primers S2F and S3R as described in Chen et al. (2010).

PCR reactions were conducted in 10 µl volumes, comprising 2µl DNA, 4µl multiplex PCR mix, 1 µl of each primer, and 2 µl of low TE. The thermal cycling protocol consisted of initial denaturation at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 56°C for 30 seconds, and extension at 72°C for 1 minute, concluding with a final extension at 72°C for 10 minutes.

The sequencing approach evolved during the study. Initial sequencing was performed bidirectionally via Sanger sequencing by GeneWiz, with manual contig construction and consensus sequence creation in Geneious Prime. To address potential contamination issues, the protocol was later modified to utilise Illumina MiSeq sequencing at the Centre

for Genomic Research (University of Liverpool, UK), employing 250 bp paired-end reads. Raw sequences underwent filtering following the DADA2 pipeline, with manual verification of dominant sequences through BLAST searches.

To ensure accurate species assignments and mitigate the potential for contamination or misidentification during tissue sampling, all obtained sequences were subjected to a BLASTn search against the custom reference library. A species assignment was accepted as correct when the query sequence from a sample matched with, and had the highest Bit-Score with, another sample sequence of the same species.

Single-sequence species underwent a BLASTn comparison against the custom database to ensure they did not closely match any other species present on the islands, and against the GenBank nucleotide database to check for matches to at least the same genus or family.

This BLASTn approach to verify the species of each sample was chosen due to its widespread use in DNA metabarcoding studies for assigning sequences to taxa and assessing taxonomic resolution, as demonstrated in prior research (García-Robledo et al., 2013; Hawkins et al., 2015; Ando et al., 2020).

4.9.3 Results: ITS2 barcode library

In total, 560 plant tissue samples were collected, representing 217 distinct plant species. However, for 121 of these species, obtaining samples from three individuals was not possible due to their rarity or difficulty in locating and identifying them.

Sequencing was successful for 57% of the collected samples (320 out of 560). Sequencing failures were mostly observed within specific plant families; for instance, 19

families exhibited either zero or very low sequencing success rates (<30%). Examples include Ebenaceae (7% success rate out of 29 samples), Phyllanthaceae (0% success rate out of 8 samples), and Celastraceae (20% success rate out of 10 samples). Among the 30 herbarium samples provided by Kew Gardens, sequencing was successful for only 7 samples (23.4%).

Overall, sequences were obtained for at least one individual within 70.9% of the sampled plant species (154 out of 217). The 320 sequences comprised the final compilation of a reference library, which was subsequently used for species verification.

To maximise the reference library and facilitate dietary studies focusing on Mauritian species, additional sequences were sourced from GenBank and a prior study on Mauritian plant barcoding conducted by Moorhouse-Gann et al. (2018).

71.4% of sequences were assigned to species, 87.6% to genus (i.e. where a reference species was not available and only one sequence of a species was available, it could only be confirmed to be correct at genus level), and 95.4% to family.

Five sequences, including three of which only had one sample, were a close match to taxa from an incorrect genus and were therefore removed from the reference library as they were likely the result of a misidentification or contamination. Of the remaining species with only one sample, none were a close match with any other species in the reference library, and Blastn searches of the GenBank nucleotide database supported that the samples had been taken from the correct genus. These species were therefore included in the final reference library.

Several species matched very closely to other species of the same genus (99-100% pairwise identity), within the following genera: *Syzygium*, *Coffea*, *Cynanchum*, *Dombeya*, *Ruizia*, *Trochetia*, *Tambourissa*, *Psiadia*, *Hibiscus*, *Labourdonnaisia*, and *Erythroxylum* (excluding *E. hypericifolium*). These species were kept in the library as it could not be determined whether the close matches were due to incorrect species identification or minimal intraspecific variation within the ITS2 region for species of that genus.

Following the species assignment tests, 296 of 320 sequences generated in this study were compiled into a reference library (Table A1.1) and will be uploaded to GenBank for wider use. The 296 sequences represent 147 plant species – 132 native to Mauritius (including 83 species endemic to Mauritius) and 15 introduced.

Supplementary Table 1: Plant species list with sequencing and species assignment result

Order	Family	Binomial name	Status	Sequenced	Species assigned	Genus assigned	Family assigned	In library
Malpighiales	Euphorbiaceae	<i>Acalypha integrifolia</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Arecales	Arecaceae	<i>Acanthophoenix rubra</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Caryophyllales	Amaranthaceae	<i>Ourea congesta</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Ericales	Ericaceae	<i>Agarista salicifolia</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Fabales	Fabaceae	<i>Albizia vauhanii</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Asparagales	Asphodelaceae	<i>Aloe purpurea</i>	Endemic	TRUE	FALSE	FALSE	TRUE	TRUE
Asparagales	Orchidaceae	<i>Angraecum cadetii</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Malpighiales	Phyllanthaceae	<i>Antidesma madagascariense</i>	Native	FALSE	FALSE	FALSE	FALSE	FALSE
Gentianales	Rubiaceae	<i>Antirhea borbonica</i>	Native	FALSE	FALSE	FALSE	FALSE	FALSE
Crossosomatales	Aphloiaceae	<i>Aphloia theiformis</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Icacinales	Icacinaceae	<i>Apodytes dimidiata</i>	Native	FALSE	FALSE	FALSE	FALSE	FALSE
Ericales	Primulaceae	<i>Ardisia crenata</i>	Introduced	TRUE	TRUE	TRUE	TRUE	TRUE
Lamiales	Acanthaceae	<i>Barleria observatrix</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Cucurbitales	Begoniaceae	<i>Begonia salaziensis</i>	Native	TRUE	FALSE	TRUE	TRUE	TRUE
Gentianales	Rubiaceae	<i>Bertiera zaluzania</i>	Endemic	TRUE	FALSE	FALSE	TRUE	TRUE
Gentianales	Rubiaceae	<i>Bremeria landia</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Myrtales	Myrtaceae	<i>Melaleuca citrina</i>	Introduced	TRUE	TRUE	TRUE	TRUE	TRUE
Malpighiales	Clusiaceae	<i>Calophyllum eputamen</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Malpighiales	Clusiaceae	<i>Calophyllum tacamahaca</i>	Native	FALSE	FALSE	FALSE	FALSE	FALSE
Sapindales	Burseraceae	<i>Canarium paniculatum</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Malpighiales	Salicaceae	<i>Casearia thwaitesii</i>	Endemic	TRUE	FALSE	FALSE	TRUE	TRUE
Celastrales	Celastraceae	<i>Elaeodendron orientale</i>	Native	FALSE	FALSE	FALSE	FALSE	FALSE
Apiales	Apiaceae	<i>Centella asiatica</i>	Introduced	TRUE	TRUE	TRUE	TRUE	TRUE
Gentianales	Rubiaceae	<i>Chassalia boryana</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Gentianales	Rubiaceae	<i>Chassalia sp</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE

Malpighiales	Euphorbiaceae	<i>Claoxylon linostachys</i>	Endemic	TRUE	FALSE	FALSE	TRUE	TRUE
Lamiales	Lamiaceae	<i>Volkameria heterophylla</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Myrtales	Melastomataceae	<i>Miconia crenata</i>	Introduced	TRUE	TRUE	TRUE	TRUE	TRUE
Oxalidales	Connaraceae	<i>Cnestis polyphylla</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Gentianales	Rubiaceae	<i>Coffea macrocarpa</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Gentianales	Rubiaceae	<i>Coffea mauritiana</i>	Native	TRUE	FALSE	TRUE	TRUE	TRUE
Gentianales	Rubiaceae	<i>Coffea myrtifolia</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Lamiales	Bignoniaceae	<i>Colea coleii</i>	Endemic	TRUE	FALSE	FALSE	TRUE	TRUE
Gentianales	Rubiaceae	<i>Coptosperma borbonicum</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Gentianales	Rubiaceae	<i>Coptosperma cymosum</i>	Endemic	TRUE	FALSE	TRUE	TRUE	TRUE
Sapindales	Sapindaceae	<i>Cossinia pinnata</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Asparagales	Amaryllidaceae	<i>Crinum mauritianum</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Malpighiales	Euphorbiaceae	<i>Croton grangerioides</i>	Endemic	TRUE	FALSE	TRUE	TRUE	TRUE
Asparagales	Orchidaceae	<i>Cryptopus elatus</i>	Native	TRUE	FALSE	FALSE	TRUE	TRUE
Cyatheales	Cyatheaceae	<i>Alsophila borbonica</i>	Native	FALSE	FALSE	FALSE	FALSE	FALSE
Gentianales	Apocynaceae	<i>Cynanchum glomeratum</i>	Endemic	TRUE	FALSE	TRUE	TRUE	TRUE
Gentianales	Apocynaceae	<i>Cynanchum scopulosum</i>	Endemic	TRUE	FALSE	TRUE	TRUE	TRUE
Vitales	Vitaceae	<i>Cyphostemma mappia</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Lamiales	Acanthaceae	<i>Dicliptera falcata</i>	Endemic	TRUE	FALSE	TRUE	TRUE	TRUE
Arecales	Arecaceae	<i>Dictyosperma album</i> var. <i>album</i>	Native	FALSE	FALSE	FALSE	FALSE	FALSE
Arecales	Arecaceae	<i>Dictyosperma album</i> var. <i>conjugatum</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Ericales	Ebenaceae	<i>Diospyros boutoniana</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Ericales	Ebenaceae	<i>Diospyros chrysophyllos</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Ericales	Ebenaceae	<i>Diospyros egrettarum</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Ericales	Ebenaceae	<i>Diospyros leucomelas</i>	Endemic	TRUE	FALSE	TRUE	TRUE	TRUE
Ericales	Ebenaceae	<i>Diospyros melanida</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Ericales	Ebenaceae	<i>Diospyros nodosa</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Ericales	Ebenaceae	<i>Diospyros revaughanii</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE

Ericales	Ebenaceae	<i>Diospyros tessellaria</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Asterales	Asteraceae	<i>Distephanus populifolius</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Sapindales	Sapindaceae	<i>Dodonaea viscosa</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Malvales	Malvaceae	<i>Dombeya acutangula</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Malvales	Malvaceae	<i>Dombeya acutangula var. rosea</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Malvales	Malvaceae	<i>Ruizia ferruginea</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Malvales	Malvaceae	<i>Dombeya sp</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Sapindales	Sapindaceae	<i>Doratoxylon apetalum</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Asparagales	Asparagaceae	<i>Dracaena concinna</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Asparagales	Asparagaceae	<i>Dracaena floribunda</i>	Endemic	TRUE	FALSE	FALSE	FALSE	FALSE
Asparagales	Asparagaceae	<i>Dracaena reflexa</i>	Native	TRUE	FALSE	TRUE	TRUE	TRUE
Oxalidales	Elaeocarpaceae	<i>Elaeocarpus bojeri</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Malpighiales	Achariaceae	<i>Erythrospermum monticola</i>	Endemic	TRUE	FALSE	FALSE	FALSE	FALSE
Malpighiales	Erythroxylaceae	<i>Erythroxylum hypericifolium</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Malpighiales	Erythroxylaceae	<i>Erythroxylum laurifolium</i>	Endemic	TRUE	FALSE	TRUE	TRUE	TRUE
Malpighiales	Erythroxylaceae	<i>Erythroxylum macrocarpum</i>	Endemic	TRUE	FALSE	TRUE	TRUE	TRUE
Malpighiales	Erythroxylaceae	<i>Erythroxylum sideroxyloides</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Myrtales	Myrtaceae	<i>Eugenia elliptica</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Myrtales	Myrtaceae	<i>Eugenia lucida</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Myrtales	Myrtaceae	<i>Eugenia orbiculata</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Myrtales	Myrtaceae	<i>Eugenia pollicina</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Malpighiales	Euphorbiaceae	<i>Euphorbia pyrifolia</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Gentianales	Rubiaceae	<i>Fernelia buxifolia</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Gentianales	Rubiaceae	<i>Fernelia decipiens</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Rosales	Moraceae	<i>Ficus mauritiana</i>	Native	FALSE	FALSE	FALSE	FALSE	FALSE
Rosales	Moraceae	<i>Ficus reflexa</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Ericales	Lecythidaceae	<i>Foetidia mauritiana</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Asparagales	Asparagaceae	<i>Furcraea foetida</i>	Introduced	TRUE	FALSE	FALSE	FALSE	FALSE
Gentianales	Rubiaceae	<i>Gaertnera psychotrioides</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE

Gentianales	Rubiaceae	<i>Gaertnera rotundifolia</i>	Endemic	TRUE	FALSE	FALSE	TRUE	TRUE
Fabales	Fabaceae	<i>Gagnebina pterocarpa</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Rosales	Rhamnaceae	<i>Gouania tiliifolia</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Malpighiales	Chrysobalanaceae	<i>Grangeria borbonica</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Celastrales	Celastraceae	<i>Gymnosporia pyria</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Malpighiales	Euphorbiaceae	<i>Hancea integrifolia</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Asparagales	Orchidaceae	<i>Hederorkis scandens</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Zingiberales	Zingiberaceae	<i>Hedychium coronarium</i>	Introduced	FALSE	FALSE	FALSE	FALSE	FALSE
Asterales	Asteraceae	<i>Helichrysum mauritianum</i>	Endemic	TRUE	FALSE	TRUE	TRUE	TRUE
Laurales	Hernandiaceae	<i>Hernandia nymphaeifolia</i>	Native	TRUE	FALSE	TRUE	TRUE	TRUE
Malvales	Malvaceae	<i>Hibiscus boryanus</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Malvales	Malvaceae	<i>Hibiscus columnaris</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Malvales	Malvaceae	<i>Hibiscus fragilis</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Malvales	Malvaceae	<i>Hibiscus genevei</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Malvales	Malvaceae	<i>Hibiscus liliiflorus</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Malpighiales	Malpighiaceae	<i>Hiptage benghalensis</i>	Introduced	FALSE	FALSE	FALSE	FALSE	FALSE
Malpighiales	Euphorbiaceae	<i>Homalanthus populifolius</i>	Introduced	TRUE	FALSE	FALSE	FALSE	FALSE
Malpighiales	Salicaceae	<i>Homalium integrifolium</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Malpighiales	Linaceae	<i>Hugonia serrata</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Arecales	Arecaceae	<i>Hyophorbe lagenicaulis</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Arecales	Arecaceae	<i>Hyophorbe vaughanii</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Gentianales	Rubiaceae	<i>Ixora borboniae</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Gentianales	Rubiaceae	<i>Ixora parviflora</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Ericales	Sapotaceae	<i>Labourdonnaisia calophylloides</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Ericales	Sapotaceae	<i>Labourdonnaisia glauca</i>	Endemic	TRUE	FALSE	TRUE	TRUE	TRUE
Ericales	Sapotaceae	<i>Labourdonnaisia revoluta</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Arecales	Arecaceae	<i>Latania loddigesii</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Arecales	Arecaceae	<i>Latania verschaffeltii</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Malpighiales	Euphorbiaceae	<i>Orfilea neraudiana</i>	Endemic	TRUE	FALSE	FALSE	TRUE	TRUE

Vitales	Vitaceae	<i>Leea guineensis</i>	Native	FALSE	FALSE	FALSE	FALSE	FALSE
Lamiales	Oleaceae	<i>Ligustrum robustum</i>	Introduced	TRUE	TRUE	TRUE	TRUE	TRUE
Laurales	Lauraceae	<i>Litsea glutinosa</i>	Introduced	TRUE	TRUE	TRUE	TRUE	TRUE
Laurales	Lauraceae	<i>Litsea monopetala</i>	Introduced	TRUE	FALSE	FALSE	FALSE	FALSE
Malpighiales	Salicaceae	<i>Ludia mauritiana</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Sapindales	Anacardiaceae	<i>Mangifera indica</i>	Introduced	TRUE	TRUE	TRUE	TRUE	TRUE
Sapindales	Rutaceae	<i>Melicope chapelieri</i>	Endemic	TRUE	FALSE	TRUE	TRUE	TRUE
Myrtales	Melastomataceae	<i>Memecylon ovatifolium</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Ericales	Sapotaceae	<i>Mimusops erythroxylon</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Ericales	Sapotaceae	<i>Mimusops petiolaris</i>	Endemic	TRUE	FALSE	FALSE	TRUE	TRUE
Sapindales	Sapindaceae	<i>Molinaea alternifolia</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Sapindales	Sapindaceae	<i>Molinaea laevis</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Myrtales	Myrtaceae	<i>Eugenia kanakana</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Gentianales	Rubiaceae	<i>Morinda citrifolia</i>	Introduced	TRUE	TRUE	TRUE	TRUE	TRUE
Gentianales	Rubiaceae	<i>Ixora parviflora var. parviflora</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Polypodiales	Polypodiaceae	<i>Nephrolepis cordifolia</i>	Native	FALSE	FALSE	FALSE	FALSE	FALSE
Asterales	Campanulaceae	<i>Nesocodon mauritanus</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Lamiales	Stilbaceae	<i>Nuxia verticillata</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Malpighiales	Ochnaceae	<i>Ochna mauritiana</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Laurales	Lauraceae	<i>Ocotea mascarena</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Santalales	Olacaceae	<i>Olax psittacorum</i>	Native	FALSE	FALSE	FALSE	FALSE	FALSE
Lamiales	Oleaceae	<i>Olea lancea</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Pandanales	Pandanaceae	<i>Pandanus carmichaelii</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Pandanales	Pandanaceae	<i>Pandanus eydouxia</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Pandanales	Pandanaceae	<i>Pandanus microcarpus</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Pandanales	Pandanaceae	<i>Pandanus palustris</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Pandanales	Pandanaceae	<i>Pandanus rigidifolius</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Pandanales	Pandanaceae	<i>Pandanus sphaeroideus</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Pandanales	Pandanaceae	<i>Pandanus utilis</i>	Native	TRUE	FALSE	FALSE	FALSE	FALSE
Pandanales	Pandanaceae	<i>Pandanus vandermeeschii</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE

Malpighiales	Passifloraceae	<i>Passiflora suberosa</i>	Introduced	TRUE	TRUE	TRUE	TRUE	TRUE
Rosales	Urticaceae	<i>Pilea laevicaulis</i>	Endemic	TRUE	FALSE	TRUE	TRUE	TRUE
Pinales	Pinaceae	<i>Pinus elliottii</i>	Introduced	TRUE	TRUE	TRUE	TRUE	TRUE
Caryophyllales	Nyctaginaceae	<i>Pisonia costata</i>	Endemic	TRUE	FALSE	TRUE	TRUE	TRUE
Apiales	Pittosporaceae	<i>Pittosporum balfourii</i>	Native	FALSE	FALSE	FALSE	FALSE	FALSE
Apiales	Pittosporaceae	<i>Pittosporum ferrugineum</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Apiales	Pittosporaceae	<i>Pittosporum senecia</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Asparagales	Orchidaceae	<i>Platylepis occulta</i>	Native	FALSE	FALSE	FALSE	FALSE	FALSE
Celastrales	Celastraceae	<i>Pleurostylia leucocarpa</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Apiales	Araliaceae	<i>Polyscias dichrostachya</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Apiales	Araliaceae	<i>Polyscias gracilis</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Apiales	Araliaceae	<i>Polyscias maraisiana</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Apiales	Araliaceae	<i>Polyscias mauritiana</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Apiales	Araliaceae	<i>Polyscias sp.</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Sapindales	Anacardiaceae	<i>Poupartia pubescens</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Rosales	Urticaceae	<i>Pouzolzia laevigata</i>	Native	TRUE	FALSE	TRUE	TRUE	TRUE
Lamiales	Lamiaceae	<i>Premna serratifolia</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Sapindales	Burseraceae	<i>Protium obtusifolium</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Asterales	Asteraceae	<i>Psiadia arguta</i>	Endemic	TRUE	FALSE	TRUE	TRUE	TRUE
Asterales	Asteraceae	<i>Psiadia cataractae</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Asterales	Asteraceae	<i>Psiadia lithospermifolia</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Asterales	Asteraceae	<i>Psiadia mauritiana</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Asterales	Asteraceae	<i>Psiadia penninervia</i>	Endemic	TRUE	FALSE	TRUE	TRUE	TRUE
Asterales	Asteraceae	<i>Psiadia terebinthina</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Myrtales	Myrtaceae	<i>Psidium cattleianum</i>	Introduced	FALSE	FALSE	FALSE	FALSE	FALSE
Gentianales	Rubiaceae	<i>Pyrostria viburnoides</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Gentianales	Rubiaceae	<i>Ramosmania rodriguesii</i>	Native	FALSE	FALSE	FALSE	FALSE	FALSE
Zingiberales	Strelitziaceae	<i>Ravenala madagascariensis</i>	Introduced	TRUE	FALSE	FALSE	FALSE	FALSE
Caryophyllales	Cactaceae	<i>Rhipsalis baccifera</i>	Native	TRUE	FALSE	FALSE	TRUE	TRUE
Asterales	Rousseaceae	<i>Roussea simplex</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE

Rosales	Rosaceae	<i>Rubus rosifolius</i>	Introduced	TRUE	FALSE	TRUE	TRUE	TRUE
Malvales	Malvaceae	<i>Ruizia blackburniana</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Rosales	Rhamnaceae	<i>Scutia myrtina</i>	Native	FALSE	FALSE	FALSE	FALSE	FALSE
Malpighiales	Phyllanthaceae	<i>Securinea durissima</i>	Native	FALSE	FALSE	FALSE	FALSE	FALSE
Asterales	Asteraceae	<i>Senecio lamarckianus</i>	Endemic	TRUE	FALSE	TRUE	TRUE	TRUE
Ericales	Sapotaceae	<i>Sideroxylon cinereum</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Ericales	Sapotaceae	<i>Sideroxylon grandiflorum</i>	Endemic	TRUE	FALSE	TRUE	TRUE	TRUE
Ericales	Sapotaceae	<i>Sideroxylon puberulum</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Liliales	Smilacaceae	<i>Smilax anceps</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Lamiales	Verbenaceae	<i>Stachytarpheta jamaicensis</i>	Introduced	TRUE	TRUE	TRUE	TRUE	TRUE
Malpighiales	Euphorbiaceae	<i>Stillingia lineata</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Myrtales	Myrtaceae	<i>Syzygium commersonii</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Myrtales	Myrtaceae	<i>Syzygium contractum</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Myrtales	Myrtaceae	<i>Syzygium coriaceum</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Myrtales	Myrtaceae	<i>Syzygium cymosum</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Myrtales	Myrtaceae	<i>Syzygium glomeratum</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Myrtales	Myrtaceae	<i>Syzygium guehoi</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Myrtales	Myrtaceae	<i>Syzygium jambos</i>	Introduced	TRUE	TRUE	TRUE	TRUE	TRUE
Myrtales	Myrtaceae	<i>Syzygium latifolium</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Myrtales	Myrtaceae	<i>Syzygium mamillatum</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Myrtales	Myrtaceae	<i>Syzygium mauritianum</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Myrtales	Myrtaceae	<i>Syzygium pyneei</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Lamiales	Bignoniaceae	<i>Tabebuia pallida</i>	Introduced	TRUE	TRUE	TRUE	TRUE	TRUE
Gentianales	Apocynaceae	<i>Tabernaemontana persicariifolia</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Malvales	Malvaceae	<i>Talipariti tiliaceum</i>	Native	FALSE	FALSE	FALSE	FALSE	FALSE
Laurales	Monimiaceae	<i>Tambourissa cordifolia</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Laurales	Monimiaceae	<i>Tambourissa peltata</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Laurales	Monimiaceae	<i>Tambourissa quadrifida</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Laurales	Monimiaceae	<i>Tambourissa sp.</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE

Laurales	Monimiaceae	<i>Tambourissa tau</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Lamiales	Bignoniaceae	<i>Tecoma stans</i>	Introduced	FALSE	FALSE	FALSE	FALSE	FALSE
Arecales	Arecaceae	<i>Tectiphiala ferox</i>	Endemic	FALSE	FALSE	FALSE	FALSE	FALSE
Myrtales	Combretaceae	<i>Terminalia bentzoë</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Myrtales	Lythraceae	<i>Tetrataxis salicifolia</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Malvales	Malvaceae	<i>Thespesia populnea</i>	Native	TRUE	FALSE	FALSE	TRUE	TRUE
Myrtales	Melastomataceae	<i>Pleroma vimineum</i>	Introduced	TRUE	TRUE	TRUE	TRUE	TRUE
Sapindales	Rutaceae	<i>Zanthoxylum asiaticum</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Malvales	Malvaceae	<i>Ruizia boutoniana</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Malvales	Malvaceae	<i>Ruizia parviflora</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Malvales	Malvaceae	<i>Ruizia triflora</i>	Endemic	TRUE	FALSE	TRUE	TRUE	TRUE
Sapindales	Meliaceae	<i>Turraea thouarsiana</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Malvales	Malvaceae	<i>Urena lobata</i>	Native	TRUE	TRUE	TRUE	TRUE	TRUE
Sapindales	Rutaceae	<i>Vepris lanceolata</i>	Native	TRUE	FALSE	TRUE	TRUE	TRUE
Poales	Poaceae	<i>Chrysopogon argutus</i>	Native	TRUE	FALSE	FALSE	TRUE	TRUE
Myrtales	Melastomataceae	<i>Warneckea trinervis</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE
Sapindales	Rutaceae	<i>Zanthoxylum heterophyllum</i>	Endemic	TRUE	TRUE	TRUE	TRUE	TRUE

4.9.4 Summary: ITS2 Barcode Library

This study presents the first comprehensive ITS2 DNA barcode library for mainland Mauritian plants, significantly advancing our capacity to study and conserve the island's unique flora. While previous studies have constructed DNA barcode libraries for dietary studies (Valentini et al. 2009; Gebremedhin et al. 2016), including work on Mauritian offshore islands (Round Island and Ile Aux Aigrettes) (Moorhouse-Gann et al., 2018), our work represents the first attempt to barcode the mainland flora, particularly covering the complete range of plant species utilised by endemic birds such as the Mauritius parakeet and pink pigeon.

The sequencing success rate of 57% highlights both the potential and challenges of DNA barcoding in tropical island systems. While this rate is lower than some previous studies, it is comparable to other work in tropical regions and reflects the particular challenges of working with Mauritian flora, especially in families such as Ebenaceae and Pandanaceae. These challenges suggest the need for optimisation of extraction and amplification protocols for recalcitrant plant families.

Our species assignment rates (71.4% to species level, 87.6% to genus, and 95.4% to family) validate DNA barcoding as an effective tool for plant identification in Mauritius (Kress and Erickson, 2008). These resolution levels are particularly significant given Mauritius' high endemism rate and the presence of many closely related species. The inclusion of native, endemic, and introduced species enhances the library's utility for various applications, from biodiversity assessment to biosecurity efforts (Hajibabaei et al., 2007; Aylagas et al., 2016; Hardulak et al., 2020; Ghosh-Harihar et al., 2021).

Comprehensive DNA barcode libraries tailored to specific regions can substantially improve taxonomic resolution in DNA metabarcoding dietary studies (Hofreiter et al., 2000; Taberlet et al., 2007; García-Robledo et al., 2013). Our library will particularly benefit studies of trophic interactions in Mauritius' remaining forest remnants, where most endemic species are managed. The ability to accurately identify plant species from environmental samples will enhance both ecological research and conservation management.

Future development of this resource should focus on three key areas. First, expanding the library to include currently unrepresented species, particularly those that proved challenging to sequence. Second, trialling alternative ITS2 primers to improve sequencing success for problematic families. Third, evaluating the potential benefits of a multi-locus approach incorporating additional markers such as *rbcL* and *matK*. Many studies suggest that a multi-locus approach might be essential for species-level discrimination (Hebert et al., 2004; Chase et al., 2007; Kress & Erickson, 2007; Erickson et al., 2008; Kane & Cronk, 2008; Lahaye et al., 2008; Kane et al., 2012), though findings from the Consortium for the Barcode of Life (CBOL Plant Working Group, 2009) indicate that additional loci may not substantially improve species-level resolution. This suggests that future efforts should be directed toward optimising existing protocols as well as expanding marker sets.

Chapter 5 Preliminary study into the gut microbiome of the Mauritius parakeet

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5.1 Abstract

This study presents the first metagenomic analysis of gut microbiota in the threatened Mauritius parakeet (*Alexandrinus eques*), examining differences between two populations and investigating relationships with supplementary feeding practices. Using genome-resolved metagenomics to analyse faecal samples, we reconstructed 212 metagenome-assembled genomes (MAGs) and identified significant differences in both taxonomic composition and functional potential between North and South populations. The South population exhibited consistently higher alpha diversity, though certain metabolic functions showed contrasting patterns. Although not statistically significant, our analysis suggested a consistent positive trend between gut microbiome alpha diversity and distance from supplementary feeding stations across both populations. With increasing distance from feeders, we observed significantly decreased abundance of vitamin biosynthesis ($p = 0.007$), antibiotic degradation ($p = 0.003$), and toxin biosynthesis ($p = 0.01$) functions. Age-related differences in microbiota composition were detected in the North population but not the South, with microbiome diversity being lower in adults than in chicks. Our findings suggest that management interventions such as supplementary feeding may have complex and potentially unexpected effects on host-microbe interactions. While preliminary due to limited sample sizes, this study demonstrates how detailed characterisation of gut microbiota using genome-resolved metagenomics can provide insights relevant to conservation management of threatened species.

5.2 Introduction

Avian gut microbiomes have significant roles in nutrient digestion, immune system development, disease resistance, and behaviour (Capunitan et al., 2020; Alberdi et al., 2022; Bodawatta et al., 2022; Wu et al., 2022). Studies in wild birds have revealed complex relationships between gut microbiota, life history, and environmental factors, particularly diet and foraging strategies. Research in light-bellied Brent geese has shown how foraging strategies can influence microbiome diversity and stability (Jones et al., 2023), while studies in great tits found age-dependent variation in gut microbiota associated with life history and productivity (Somers et al., 2023). Recent research in collared flycatchers has demonstrated direct links between gut microbiota and fitness, finding associations between gut microbiome diversity and reproductive success (Martta et al., 2024).

These relationships between diet and gut microbiota are particularly relevant for conservation management of threatened birds, where supplementary feeding is widely used but may impact the composition and functional capabilities of the gut microbiome (McKenney et al., 2018; Greene et al., 2019; Knutie, 2020; Dodd & Grueber, 2021). Studies on the kakapo (*Strigops habroptilus*) found that supplementary feeding significantly affected gut microbiome composition (West et al., 2022b), with an early study revealing a low-diversity microbiome amongst chicks (Waite et al., 2012). Similarly, research on house sparrows (*Passer domesticus*) showed reduced diversity and an altered functional profile in the microbiome following brief periods of captivity and a restricted diet of commercial seed mix (Florkowski et al., 2023).

The Mauritius parakeet, *Alexandrinus eques*, provides an ideal study system for assessing the influence of supplementary feeding on gut microbiome composition. This threatened endemic parrot has been subject to intensive conservation management since the 1980s (Thorsen & Jones, 1998; Jones & Merton, 2012; MWF, 2020), including long-term supplementary feeding. The species now occupies two distinct populations that receive different levels of management intervention – daily supplementary feeding in the North population and weekly feeding in the South. Previous research has shown that supplementary feeding significantly influences both diet composition and reproductive success in this species (Chapter 3; Tollington et al., 2019), with birds in the North population showing higher proportional consumption of supplementary food (84.2%) compared to the South (29.4%). Despite lower supplementary food consumption, the South population maintains higher reproductive success (Chapters 2-4).

To understand how supplementary feeding might influence gut microbiota in this system, both population-level patterns and specific functional traits should be considered. Alpha diversity (diversity within individual samples) often indicates microbiome stability and resilience, with higher diversity typically providing multiple pathways for essential functions like nutrient processing and pathogen resistance (Tuddenham & Sears, 2015; Kohl et al., 2016; Sun et al., 2022). Beta diversity analysis can reveal how factors like diet and habitat influence microbial communities between populations or conditions (Kohl et al., 2014; Knutie, 2020; Liukkonen et al., 2024). However, the specific composition and functional traits of the microbiome can be more important than diversity metrics alone (Dodd & Grueber, 2021; Worsley et al., 2021; Hernández et al., 2024), particularly when

assessing relationships between gut microbiota and fitness outcomes in threatened species (Martta et al., 2024).

This study is first to characterise the gut microbiome of the Mauritius parakeet and to explore how it may be influenced by the provision of supplementary food. The aims of this study are to:

- (i) Characterise the gut microbiome of the Mauritius parakeet.
- (ii) Assess alpha and beta diversity of the microbiome and its relationship with population, age (adult or chick), and supplementary feeding.
- (iii) Assess functional differences in the microbiome between the two populations.

5.3 Methods

5.3.1 Study system and supplementary feeding

The study was conducted across two populations of Mauritius parakeet (see **Figure 5.1**) that receive different supplementary feeding regimes. The North population receives daily supplementary feeding, while the South population is fed weekly. Supplementary food consists of commercial parrot pellets (Kaytee Exact), containing processed grains (ground corn, wheat, and oat groats), protein sources (corn gluten meal, dried egg, soybean meal), and multiple additives including preservatives, vitamins (A, B12, D3, E, K), minerals, amino acids (L-lysine, DL-methionine), probiotics (*Bacillus licheniformis*, *B. subtilis*), and prebiotics (fructooligosaccharide) (see Supplementary Table 1 for full ingredient list).

5.3.2 Sample collection

A total of 36 faecal samples were collected from Mauritius parakeets, with 10 samples from the South population (n=2 adults, n=8 chicks) and 26 from the North (n=6 adults, n=20 chicks) (**Figure 5.1**). Assistance in site access and bird handling was provided by the Mauritian Wildlife Foundation (MWF). Sample collection was authorised for research purposes by the National Parks and Conservation Service of Mauritius (NPCS). Samples were collected from approximately 45-day old chicks in their nests during routine health checks.

Samples were preserved in a buffer provided by the Earth Hologenome Initiative (EHI) (Leonard et al., 2024) and stored in a -20°C freezer in Mauritius before being transferred to the UK and stored at -80°C. Samples were sent to the Centre for Evolutionary Hologenomics, University of Copenhagen, for DNA extraction, sequencing, and bioinformatic processing at the EHI.

5.3.3 DNA extraction

Lab protocols are described in detail by the Earth Hologenome Initiative (<https://www.earthhologenome.org/laboratory/>). Briefly, samples were first homogenised and digested using a lysing matrix and by bead-beating with ceramic, silica, and glass beads in a TissueLyser II (Qiagen, Manchester, UK). DNA was extracted from the sample using silica magnetic beads combined with solid-phase reversible immobilisation (SPRI) to remove inhibitors. DNA extracts were subsequently sheared using a Covaris LE220 Focused Ultrasonicator System (Covaris Corporation, Woburn, MA, USA) for optimal short-read sequencing.

5.3.4 DNA sequencing

The sheared DNA extracts were processed for shotgun metagenomics, through adaptor-ligation based preparation. Sequencing adaptors and the fragmented DNA were mixed, and a DNA ligase enzyme used to covalently join adaptors to the ends of the DNA fragments. This involved an end-repair reaction, ligation reaction, and fill-in reaction.

A reaction enhancer mix was prepared with 500 µl PEG 4000 50%, 100 µl BSA, 80 µl NaCl, and 320 µl ddH₂O. For the end-repair reaction, 24 µl of DNA fragment was mixed with 3 µl T4 DNA ligase buffer, 0.3 µl dNTPs, 0.75 µl T4 PNK, 0.3 µl T4 DNA polymerase, and 1.5 µl of the reaction enhancer and placed in a thermocycler for 30 min at 20°C followed by 30 min at 65°C, then cooled to 4°C. For the ligation reaction, 1.5 µl of BEDC3 blunt-end adapters, 0.75 µl T4 DNA ligase buffer, 0.75 µl T4 DNA ligase, and 4.5 µl PEG 4000 50% were added to each reaction and mixed by pipetting. The reactions were incubated in the thermocycler for 30 min at 20°C followed by 10 min at 64°C and cooling to 4°C. For the fill-in reaction, 1.5 µl Isothermal Amp buffer, 0.76 µl dNTPs, 1.2 µl Bts 2.0 WarmStart polymerase, and 4.2 µl ddH₂O were added to each reaction and mixed by pipetting. The reactions were incubated for 15 min at 65°C followed by 15 min at 80°C and cooled to 4°C. The final products were purified through magnetic bead-cleaning, using MagBio or SPRI beads.

The library was then amplified using primers containing unique identifiers, to increase the molarity of the library for sequencing and to assign sample-specific molecular tags to each library. Each library was qPCR screened to find the optimal number of PCR cycles the libraries should be subject to for optimal library preparation. PCR amplification was carried out in reaction mixes containing 5 µl PCR Gold buffer, 5 µl MgCl₂ Solution, 0.4 µl

dNTPs, 1 µl AmpliTaq GOLD DNA polymerase, 26.6 µl ddH₂O, 1 µl of each uniquely indexed primer, and 10 µl of DNA library product. A PCR program was carried out according to the results of the qPCR screening.

The final indexed library product was purified through magnetic bead-cleaning to remove enzymes and buffers added during PCR amplification. Each indexed library product was analysed for fragment size distribution with capillary electrophoresis. Indexed libraries were then pooled into a single pool, and a final bead-cleaning step was completed to remove any short fragment remains.

5.3.5 Bioinformatics pipeline

The bioinformatics workflow was carried out by the Earth Hologenome Initiative (EHI). The pipeline is described in detail at <https://www.earthhologenome.org/bioinformatics/>. Using this pipeline, raw sequencing data was pre-processed to filter out low-quality reads, adapters, and contaminants. Host and non-host data were separated by mapping reads against a host reference genome using Bowtie2, effectively isolating the metagenomic fraction of reads (Langmead & Salzberg, 2012). Subsequently, non-host reads were assembled into contigs using MEGAHIT (Li et al., 2015), resulting in a set of contigs representing the genetic material of the microbial community. These contigs were then binned into metagenome-assembled genomes (MAGs) based on sequence composition, coverage, and other characteristics, using MaxBin2 (Wu et al., 2016), Metabat (Kang et al., 2015), and CONCOCT (Alneberg et al., 2014).

Taxonomic identity of MAGs was determined using the Genome Taxonomy Database (GTDB) (Parks et al., 2020) and associated taxonomic classification toolkit (GTDB-Tk) (Chaumeil et al., 2020), while functional profiling was achieved through DRAM (which

performs functional annotation using Pfam, KEGG, UniProt, CAZY, and MEROPS databases) (Shaffer et al., 2020). To minimise redundancy, MAGs were dereplicated using dRep (Olm et al., 2017). Finally, a compositional overview of taxa in each sample was generated by mapping pre-processed reads against the final MAG catalogue. This allowed the abundance of each MAG to be quantified within each sample, using bowtie2 and CoverM (Aroney et al., 2024).

A phylogenetic tree of the MAG catalogue was constructed by placing the MAG sequences within reference archaeal and bacterial trees using GTDBK. Quality assessment was performed based on MAG completeness and contamination using CheckM, based on the presence of single-copy core genes in the MAGs. Functional annotation was achieved by annotating each MAG using the KEGG catalogue. MAGs were then ordinated according to functional annotation to assess how functionally close or diverse groups of MAGs were.

Sequencing depth was compared across all samples to assess the distribution of low-quality data (discarded in the data preprocessing and filtering steps), host genome data, metagenomic data, and unmapped data (which can include, for example, dietary items, viruses and other organisms, or potential prokaryotic DNA that could not be reconstructed). For the most representative results, the number of reads mapped to the MAG catalogue should be even across samples. However, the evenness can be influenced by uneven sequencing depth, microbiome complexity, and the amount of host or non-microbial DNA reads.

5.3.6 Alpha diversity

Calculation of alpha diversity metrics followed the Earth Hologenome Initiative data analysis workflow (<https://www.earthhologenome.org/analysis/>). Alpha diversity, which represents the species diversity within a single habitat or sample, was calculated using Hill numbers with different q values (Alberdi & Gilbert, 2019). These metrics include: (i) richness ($q=0$, accounting for presence/absence only), (ii) neutral diversity ($q=1$, exponential Shannon index, weighing species by relative abundance), (iii) phylogenetic diversity ($q=1$, incorporating evolutionary relationships through phylogenetic tree branch lengths), and (iv) functional diversity ($q=1$, considering functional differences between species). These measures were calculated using Hilldiv2 (Alberdi & Gilbert, 2019). The Hill pairwise dissimilarity index considers both species presence and abundance (Legendre & De Cáceres, 2013). Alpha diversity metrics were compared between populations (North and South) and age group (adult and chick) using Wilcoxon signed-rank tests

We investigated the relationship between alpha diversity and distance to supplementary feeding stations in the North and South. The populations have different spatial arrangements of feeding stations, with distances ranging from 0.0-3.3 km in the North population ($n=25$) and 1.5-3.7 km in the South population ($n=9$). To account for the non-normal distribution of alpha diversity values, we used generalized linear models with a Gamma distribution and log link function. We first analysed each population separately and then used a combined model with population as a factor to test for population-specific effects while accounting for the limited sample size in the South population.

5.3.7 Beta diversity

Beta diversity analysis, which measures the variation in species composition between different samples, followed the Earth Hologenome Initiative data analysis workflow (<https://www.earthhologenome.org/analysis/>). To examine community composition differences between populations, pairwise beta diversities were calculated using 'hilldiv2' (Alberdi & Gilbert, 2019). The beta diversity metric employed was the Hill pairwise dissimilarity index ($q = 1$) and the Sørensen metric. The Sørensen metric is a similarity index which calculates dissimilarity as the ratio of twice the number of species occurring in only one of the two samples to the total number of species in both samples (Sørensen, 1948).

A PERMANOVA was performed with 999 permutations to test for differences in beta diversity by population, age, season (sample collected in 2020-2021 or 2021-2022), and distance to feeding station (given significant differences observed previously with distance to feeder in this population), using the 'adonis2' function within the 'vegan' package (Oksanen et al., 2022). Non-metric multidimensional scaling (NMDS) was used to visualise community compositions.

5.3.8 Functional analysis

Functional analysis followed a similar workflow to Hernández et al. (2024). Functional analysis was performed using 'distillR' (Alberdi, 2025). KEGG module annotations from MAGs were converted to KO (KEGG Orthology) numbers using 'KEGGREST' (Tenenbaum, 2017). These were then processed to assign Genomic International Functional Traits (GIFTs) at both element and function levels. Community-weighted averages of functions were calculated using normalised abundance data. Differences in functional traits

between populations were tested using linear models, with population, age, and distance to feeder as predictor variables. Distance to feeder was chosen as a proxy for supplementary food, as it has been shown to have strong correlation with proportional supplementary food consumption (Tollington et al., 2019) and was available for all samples (proportional supplementary food consumption was only available for 28 of 36 samples).

5.4 Results

5.4.1 MAG catalogue and quality

A total of 212 MAGs were assembled from the metagenomic data, of which 122 are new species. The MAG catalogue phylogenetic tree is shown in **Figure 5.2**, followed by a plot of the MAG quality in **Figure 5.3** and a plot of the functional ordination of the MAGs in **Figure 5.4**.

5.4.2 Sequencing depth

The total mapped sequencing depth was 459.7 million reads, or 65.75 GB (gigabases). The average mapped sequencing depth was 12.77 ± 14.6 million reads, or 1.83 ± 4.38 GB. The variation in sequencing depth across samples is shown in **Figure 5.5**.

5.4.3 Taxonomic composition

The phylum-level taxonomic composition of each sample is shown in **Figure 5.6**, separated by population (North and South) and age (adult and chick). The most common genera found in each population are shown in **Supplementary Figure 1** and their possible functions in the microbiome described in **Supplementary Table 2**.

5.4.4 Alpha diversity

Each measure of alpha diversity (neutral, phylogenetic, and functional) is shown in **Figure 5.7**. Age-related differences in gut microbiota were detected in the North population, where all three diversity metrics were significantly lower in adults compared to chicks (Wilcoxon rank-sum test, $n=20$ chicks, $n=6$ adults; neutral diversity: $W=10.5$, $p=0.0027$; phylogenetic diversity: $W=10.5$, $p=0.0027$; functional diversity: $W=10.5$, $p=0.0027$). In contrast, no significant age-related differences were found in the South population (Wilcoxon rank-sum test, $n=8$ chicks, $n=2$ adults; neutral diversity: $W = 5$, $p = 0.53$; phylogenetic diversity: $W = 7$, $p = 0.89$; functional diversity: $W = 9$, $p = 0.89$).

Population differences were significant for adults across all three metrics ($n=6$ North, $n=2$ South; $W=0$, $p=0.036$ for neutral, phylogenetic, and functional diversity), with consistently higher diversity in the South population. For chicks, population differences were also significant for all diversity metrics ($n=20$ North, $n=8$ South; neutral diversity: $W=38$, $p=0.033$; phylogenetic diversity: $W=41$, $p=0.049$; functional diversity: $W=37$, $p=0.028$) (**Figure 5.8**).

Distance to supplementary feeding stations had a consistent positive trend with alpha diversity across both populations (**Figure 5.9**), though this relationship was not statistically significant. In the North population, diversity increased with distance from feeders ($\beta = 0.286$, $SE = 0.200$, $p = 0.153$), while a similar but slightly weaker trend was observed in the South population ($\beta = 0.195$, $SE = 0.175$, $p = 0.264$). The combined model revealed no significant interaction between population and distance to feeders ($\beta = -0.090$, $SE = 0.437$, $p = 0.836$), suggesting the relationship between diversity and distance to feeders may be consistent across populations.

5.4.5 Beta diversity

Beta diversity differed significantly between populations (PERMANOVA: $F = 3.1$, $R^2 = 0.08$, $p = 0.009$) and age categories ($F = 3.2$, $R^2 = 0.08$, $p = 0.008$), while season and distance to feeder showed no significant effects (**Table 5.1**). The NMDS ordination (**Figure 5.10**) visualizes these population differences.

5.4.6 Functional analysis

The heatmap visualisation (**Figure 5.11**) shows consistent patterns of functional traits across both populations, with high abundance (gift score > 0.75) of nucleic acid biosynthesis, amino acid biosynthesis, vitamin biosynthesis, organic anion biosynthesis, and xenobiotic degradation functions in both populations. However, linear models revealed significant effects of both population and distance to feeding station on functional traits (**Table 5.2**).

Population differences were most pronounced for antibiotic degradation, which showed higher abundance in the South population (estimate = $1.18\text{e-}03$, $p = 0.014$), with amino acid degradation showing a marginal effect (estimate = $1.84\text{e-}03$, $p = 0.05$). Distance to feeding station significantly affected multiple functional traits. With increasing distance from feeders, there was decreased abundance of antibiotic degradation (estimate = $-6.00\text{e-}04$, $p = 0.003$), vitamin biosynthesis (estimate = $-1.64\text{e-}03$, $p = 0.007$), and toxin biosynthesis (estimate = $-2.53\text{e-}04$, $p = 0.01$).

5.5 Discussion

This study provides the first insight into the gut microbiome composition of Mauritius parakeets (*Alexandrinus eques*), using genome-resolved metagenomics, revealing

distinct microbial communities between populations despite their geographic proximity. By assembling metagenome-assembled genomes (MAGs) from faecal samples, we identified significant differences in both taxonomic composition and functional potential between North and South populations, with consistently higher alpha diversity observed in the South.

The ability to reconstruct high-quality MAGs and analyse their encoded functions offers unprecedented insight into the metabolic capabilities of the gut microbiota in this species system, moving beyond simple taxonomic profiling to understand potential functional implications. As shown in other threatened species like the kākāpō (Waite et al., 2012; West et al., 2022), such detailed characterisation of the gut microbiome can provide valuable insights for conservation management by helping us to understand host-microbe relationships and their potential impacts on health and fitness. Of particular relevance to management, our finding that gut microbiome diversity and function varies with proximity to supplementary feeding stations warrants further investigation into how this management practice might influence host-microbe interactions and fitness.

5.5.1 Population differences in microbiome diversity and composition

The population-level differences in microbial diversity and function observed in Mauritius parakeets provide preliminary insights into their gut microbiome structure, though interpretation must be tempered by the relatively small sample size. While both populations shared core metabolic functions, particularly in nucleic acid biosynthesis, amino acid biosynthesis, and vitamin biosynthesis (**Figure 5.10**), the South population consistently exhibited higher taxonomic and functional diversity.

Population-level variation in gut microbiota has been observed in other threatened species, though the mechanisms and implications vary. For instance, in the eastern black rhinoceros, microbial communities varied significantly between zoo populations and were linked to breeding success through microbiota-mediated hormone production and metabolism (Antwis et al., 2019). Similarly, studies of the brown kiwi have shown that captivity can substantially reduce gut microbiome diversity (San Juan et al., 2020).

Our functional analysis revealed significantly higher abundance of antibiotic degradation ($p = 0.01$) and amino acid degradation ($p = 0.05$) functions in the South population. However, unlike the kākāpō, which shows remarkably low diversity dominated by *Escherichia-Shigella* across all populations (West et al., 2022b), the Mauritius parakeet populations maintain more diverse communities with distinct functional profiles.

The gut microbiota showed significant differences between chicks and adults in the North population (Wilcoxon test, $n=26$ chicks, $n=7$ adults, $p=0.0027$), though similar patterns were not detected in the South ($n=10$ chicks, $n=4$ adults, $p>0.5$). This age-related variation was evident across all diversity metrics. Age-related variation in gut microbiota has been documented in other avian species - for example, studies in other birds have shown distinct microbial communities between juveniles and adults, although these showed the opposite effect with microbial diversity increasing with age (Videvall et al., 2019; Rohrer et al., 2023). While our findings suggest potential developmental changes in the Mauritius parakeet gut microbiome, interpretation of these patterns requires caution due to the small sample sizes, particularly in the South population where the limited number of adult samples ($n=4$) reduces statistical power to detect age-related differences.

5.5.2 Microbiome composition, functional traits and supplementary feeding

Our beta diversity analysis revealed significant relationships between supplementary feeding and gut microbiome composition, aligning with research in other threatened species - for instance, West et al. (2022a) found that location and associated management practices, including supplementary feeding, accounted for 32% of microbiota variation in the threatened takahē. Our analysis of alpha diversity showed a consistent positive trend between distance from feeding station and diversity across both populations. Despite the different spatial scales and frequency of supplementary feeding between populations, the similar direction of effects suggests a potential relationship between feeding station proximity and both gut microbiome diversity and composition. This pattern aligns with previous findings in other species where artificial feeding influences gut microbiota composition, such as in kākāpō and brown kiwi (San Juan et al., 2020; West et al., 2022b).

While supplementary feeding is known to boost reproductive success in the Mauritius parakeet (Chapter 3; Tollington et al., 2019), the South population maintains both higher reproductive success (Chapters 2-4) and higher microbiome diversity despite lower supplementary food consumption. The distinct functional gene profiles near feeding stations - particularly genes associated with vitamin biosynthesis and antibiotic degradation - could potentially reflect microbial adaptation to processed food ingredients, though without temporal sampling or expression data, we cannot determine whether these represent stable, functionally relevant changes given the highly dynamic nature of gut microbiomes in wild animals (Marsh et al., 2024). The commercial pellets used to feed Mauritius parakeets contain multiple synthetic additives, preservatives, and

vitamin supplements (including vitamin A, B12, D3, E, and K), which could select for particular microbial functions. For example, the presence of preservatives and antimicrobial compounds in processed pellets might explain the higher antibiotic degradation capacity observed near feeding stations (Zhou et al., 2023). It's important to note that the presence of genes encoding these functions does not necessarily indicate active expression or functional relevance under natural conditions - these functional profiles represent genetic potential rather than confirmed metabolic activity (Aguiar-Pulido et al., 2016).

These differences in microbial communities could influence host fitness through multiple pathways: changes in microbiome diversity can affect nutrient absorption efficiency and immune function (Knutie, 2020), while altered functional profiles may impact disease susceptibility (van Veelen et al., 2020) and reproductive success (Martta et al., 2024). In relation to disease susceptibility, studies in domestic chickens have demonstrated a positive relationship between Bacillota (synonym: Firmicutes) abundance and immune system function (Liao et al., 2015; Zhang et al., 2015). We observed a high proportion of Bacillota in samples from the North population, with the adult samples being dominated by this phylum (**Figure 5.5**). Further study incorporating more samples and disease infection status would be required to understand if this abundance of Bacillota is related to disease resistance in the Mauritius parakeet.

5.5.3 MAGs as tools for taxonomic and functional characterisation

Metagenome-assembled genomes (MAGs) have revolutionised our understanding of microbial communities, particularly in environments like the gut microbiome (Frioux et al., 2020; Setubal, 2021). MAGs allow for identification and classification of microbial

taxa within the gut microbiome at a much finer resolution than traditional methods like 16S rRNA sequencing (Gehrig et al., 2022). The use of MAGs in this study enabled direct characterisation of both taxonomic composition and functional potential of the Mauritius parakeet gut microbiome. While 16S rRNA gene sequencing can be used to infer metabolic functions through pathway analysis, as demonstrated in studies of the kākāpō microbiome (West et al., 2022), MAGs allow direct identification of functional genes and metabolic pathways through analysis of encoded genes (Hernández et al., 2024).

This approach revealed not only population differences in taxonomic composition but also specific functional traits such as antibiotic degradation and vitamin biosynthesis pathways. This is important for understanding the role of the microbiome in host metabolism, nutrient processing, and overall gut health (Louca et al., 2018; Dodd & Grueber, 2021; Setubal, 2021). This information can also be used to explore interactions between different microbial taxa within the microbiome, and to identify potential metabolic dependencies or competitive interactions among microbial species.

However, the use of MAGs presents both advantages and challenges for studying threatened species' microbiomes. While offering higher resolution and functional insights, successful MAG assembly requires high-quality DNA and sufficient sequencing depth, as evidenced by the variable mapping rates in our study (**Figure 5.5**). These technical considerations are particularly important when working with limited samples from endangered species, where opportunities for repeat sampling may be restricted.

5.5.4 Conservation implications

While this study is preliminary due to limited sample sizes, our findings suggest several potential considerations for conservation management of the Mauritius parakeet. Recent

large-scale analyses have found that threatened species generally have lower gut bacterial alpha diversity compared to non-threatened species (Huang et al., 2022), making it particularly important to understand factors affecting microbiome diversity in at-risk populations. The consistent positive relationship between alpha diversity and distance from supplementary feeding stations across both populations, despite their different spatial arrangements, suggests that supplementary feeding might influence host-microbe interactions, though the functional significance and temporal stability of these patterns remain unknown. While maintaining microbiome diversity may be important for long-term population resilience, this needs to be balanced against the demonstrated benefits of supplementary feeding for reproductive success (Chapter 3; Tollington et al., 2019).

As highlighted in studies of other threatened species like the kākāpō, understanding these host-microbe relationships could provide new insights relevant to health and conservation management (West et al., 2022). The higher diversity and distinct functional gene profiles observed in the South population merit further investigation to determine whether these represent stable differences and whether they influence host health or fitness.

5.5.5 Limitations and future research directions

Several limitations of this study must be acknowledged. First, the small sample size, particularly from the South population, means our findings should be considered preliminary. Some samples showed relatively low proportions of MAGs mapped (**Figure 5.5**), indicating that deeper sequencing would be required to fully capture the functional diversity present. A larger study incorporating more individuals from both populations,

alongside reproductive fitness data, would be necessary to understand whether the observed differences in microbiome composition and function are representative of the whole population and to assess any relationship with host fitness.

Future research could also examine potential links between microbiome composition and disease susceptibility. The Mauritius parakeet population has been affected by psittacine beak and feather disease since 2005 (Fogell et al., 2021) and understanding how gut microbiota might influence or respond to disease could provide valuable insights for management. Studies in other species have shown that gut microbiota can influence immune function and disease susceptibility (Rosshart et al., 2017; Knutie, 2020; van Veelen et al., 2020), so this could be an important aspect to study in the Mauritius parakeet and other wild birds impacted by disease.

A critical limitation is our reliance on DNA-based inference of function. While MAGs reveal the genetic potential of microbial communities, they do not confirm gene expression, enzyme activity, or metabolic flux under natural conditions (Aguiar-Pulido et al., 2016). Additionally, gut microbiomes are highly dynamic systems, and our single-timepoint sampling cannot capture temporal variation or assess whether observed patterns represent stable community states (Marsh et al., 2024). Without controlled experiments or longitudinal sampling, we cannot determine the causal relationships between supplementary feeding and microbiome changes. Furthermore, the completeness of MAGs has a significant impact on functional inference, with incomplete genomes potentially leading to biased conclusions about metabolic capabilities (Eisenhofer et al., 2023).

5.6 Conclusion

Our study provides the first genome-resolved metagenomic analysis of the Mauritius parakeet gut microbiome, revealing distinct microbial communities between populations and possible relationships with supplementary feeding practices. The use of MAGs enabled detailed characterisation of both taxonomic composition and metabolic functions, demonstrating that while overall microbial diversity decreased near supplementary feeding stations, certain metabolic capabilities showed the opposite pattern. These results highlight the importance of considering both taxonomic and functional aspects when studying the gut microbiome.

While supplementary feeding has been crucial for population recovery in this species, our observations suggest potential associations between feeding practices and gut microbiome patterns that warrant further investigation to determine their stability and functional significance, and potential impact on population health. Future research should investigate links between microbiome composition and disease, as well as key fitness metrics like survival and reproductive success, particularly given the higher reproductive success observed in the South population despite lower supplementary food consumption. Understanding these relationships could eventually inform feeding practices, pending validation through longitudinal studies that confirm the stability and functional significance of these patterns, and controlled experiments that establish causal relationships between feeding practices and microbiome function.

While the small sample sizes limit our interpretation, this work establishes a starting point for understanding host-microbe relationships in the Mauritius parakeet and demonstrates how detailed microbiome characterisation might contribute to

conservation management of threatened species, pending validation of functional inferences and temporal stability.

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5.9 Tables and Figures

Table 5.1. Parameters influencing beta diversity of the gut microbiome.

Variable	Df	Sum of Squares	R ²	F-statistic	p-value
pop	1	0.91	0.08	3.079	0.009
age	1	0.94	0.08	3.187	0.008
season	1	0.25	0.02	0.86	0.508
distance to feeder	1	0.44	0.04	1.50	0.176
Residual	32	9.46	0.82	NA	NA
Total	35	11.57	1	NA	NA

Table 5.2. Significant (p-value < 0.05) linear model results comparing microbial functional traits between North and South populations and with increasing distance from feeding station. Estimates indicate the difference in trait abundance, with positive values indicating higher abundance in the North, or reduced abundance with increasing distance to feeder.

Predictor	Trait ID	Functional trait	Estimate	p-value
Pop (North)	D09	Antibiotic degradation	1.18E-03	0.01
	D05	Amino acid degradation	1.84E-03	0.05
Distance to feeder	D09	Antibiotic degradation	-6.00E-04	0.003
	B07	Vitamin biosynthesis	-1.64E-03	0.007
	B11	Toxin biosynthesis	-2.53E-04	0.01

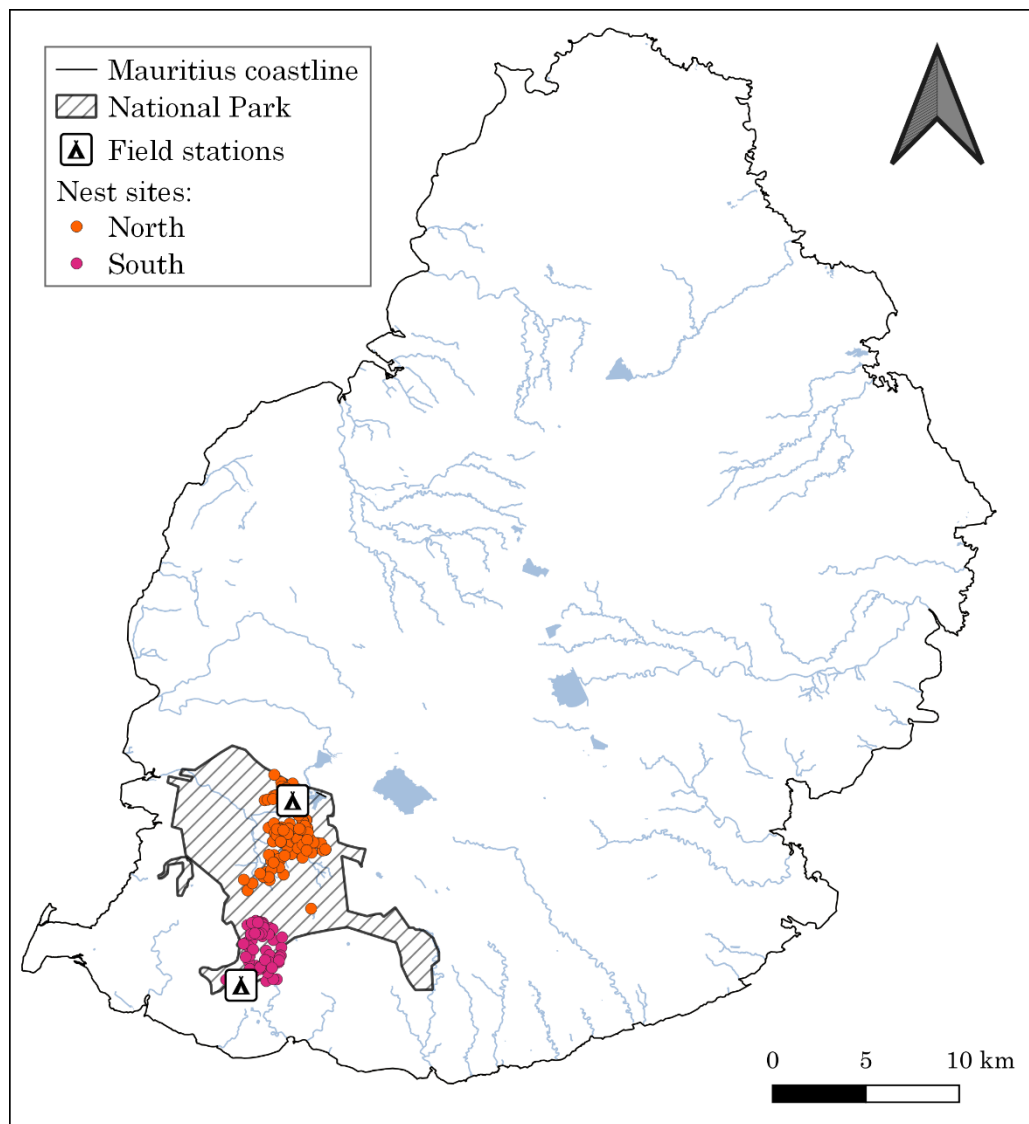


Figure 5.1. Distribution of Mauritius parakeet nest sites within the Black River Gorges National Park, Mauritius.

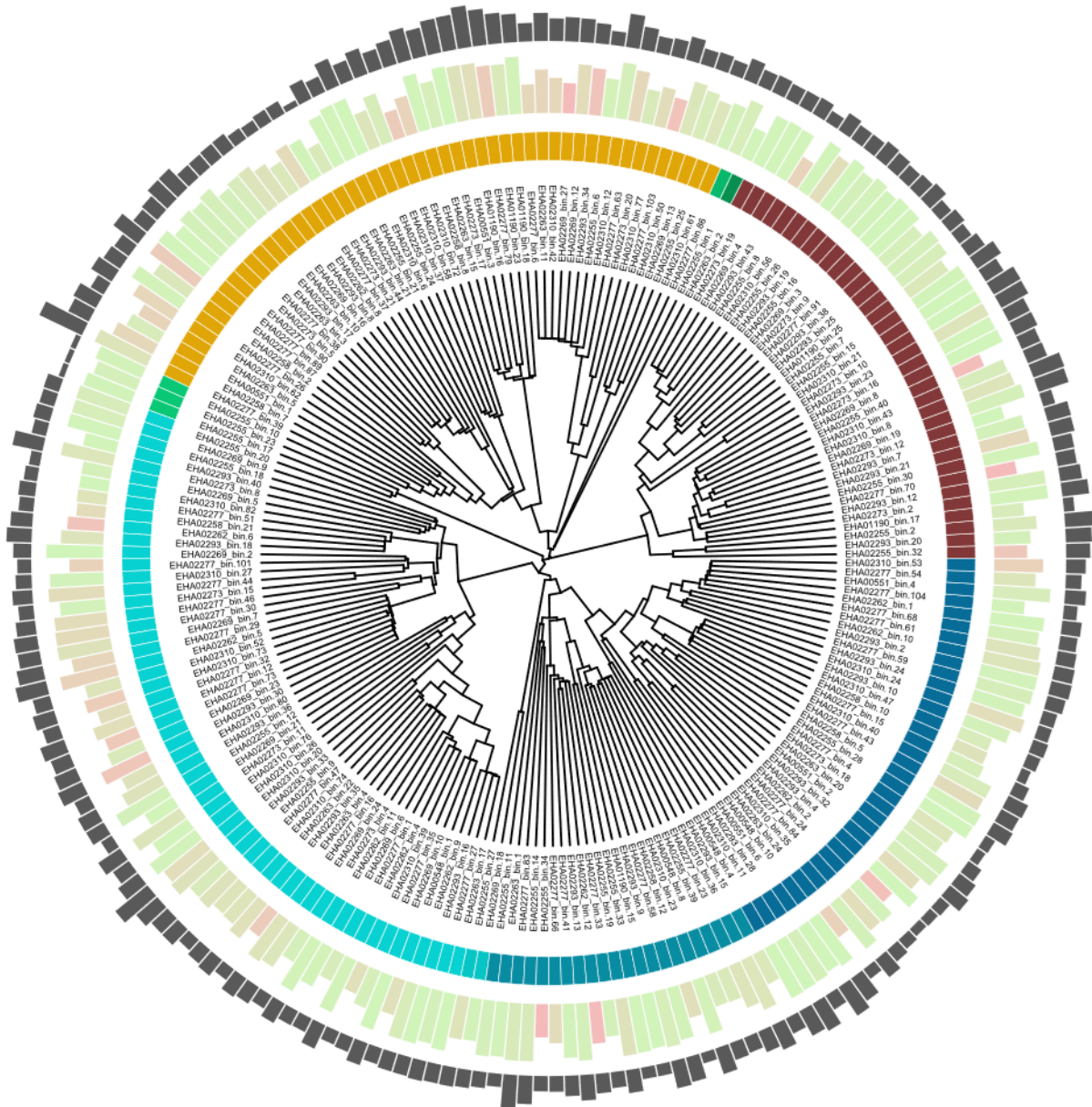


Figure 5.2. Circular tree visualising the phylogeny of the MAGs. The outer grey ring indicates the genome sizes of the MAGs. The middle ring shows an overview of the completeness (height) and contamination (green = low, red = high). The inner ring shoes the phylum.

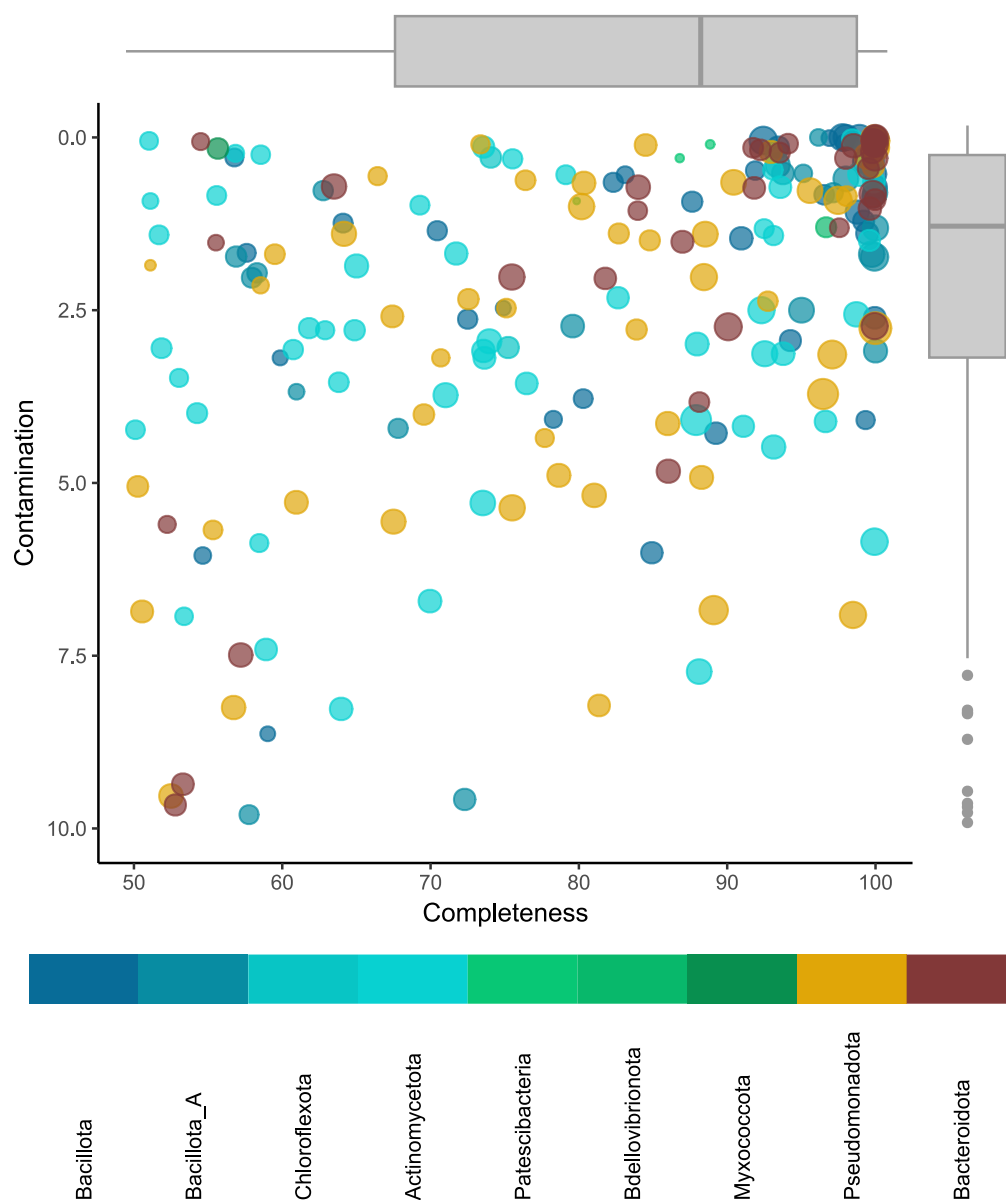


Figure 5.3. MAG quality plot. MAGs are ordinated according to their completeness and contamination scores. Dot size indicates genome size.

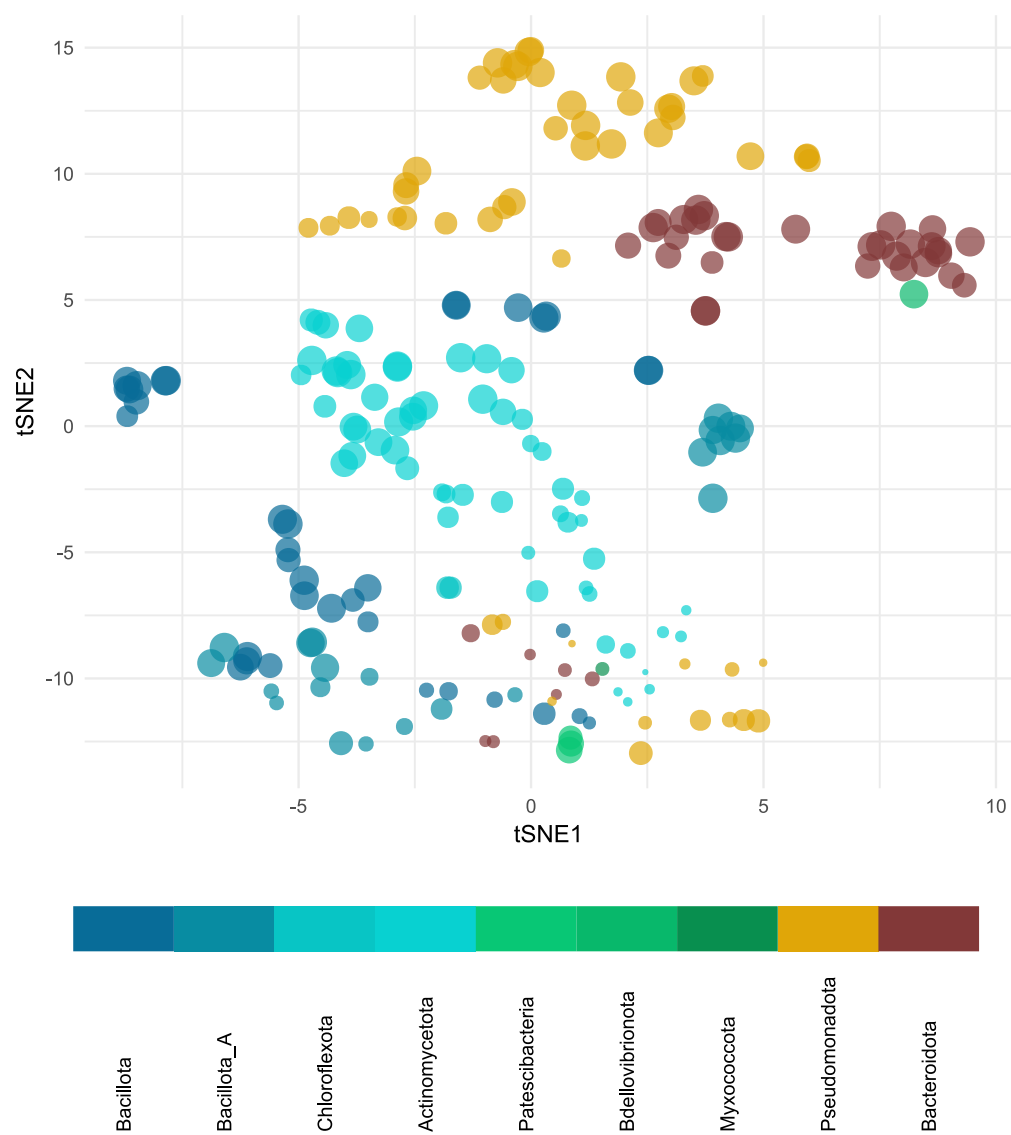


Figure 5.4. Functional ordination of MAGs. Dot size indicates genome size.

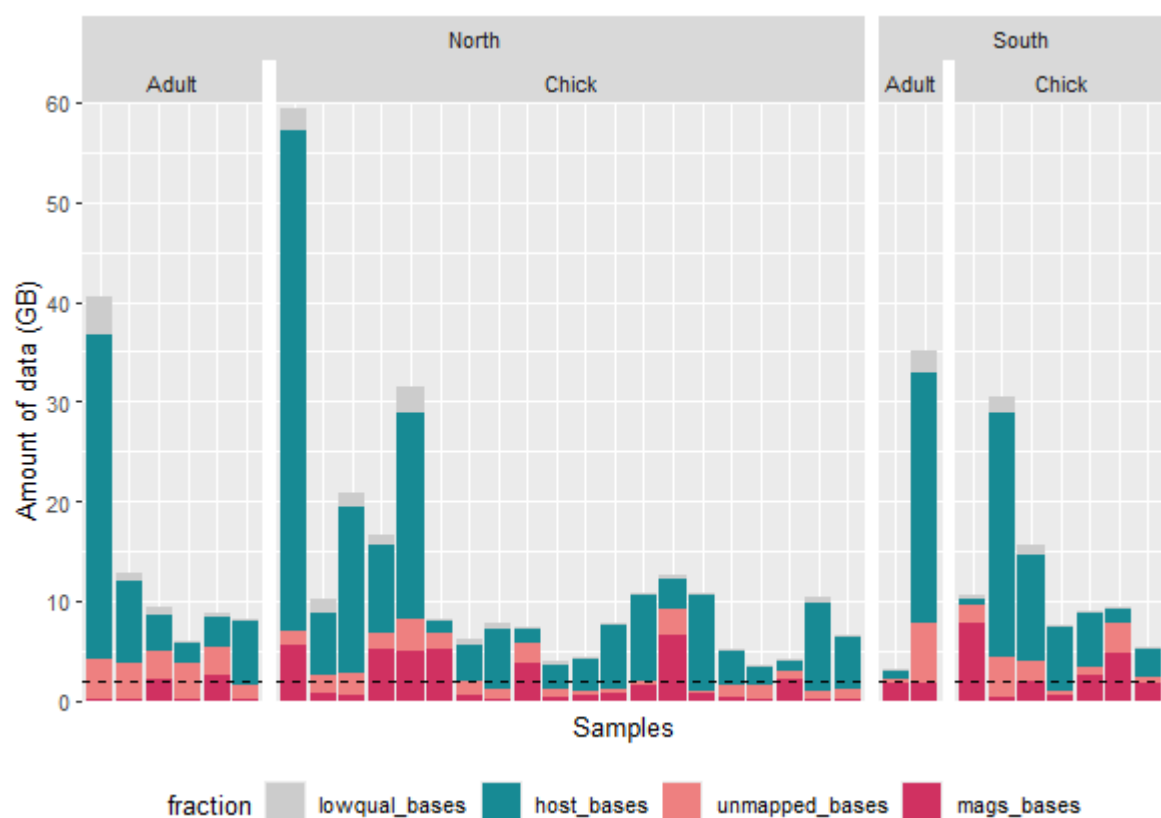


Figure 5.5. Sequencing data depth across chick faecal samples from the North and South, indicating the amount of host, unmapped, mapped, and low-quality bases. Dotted line indicates mean mags_bases.

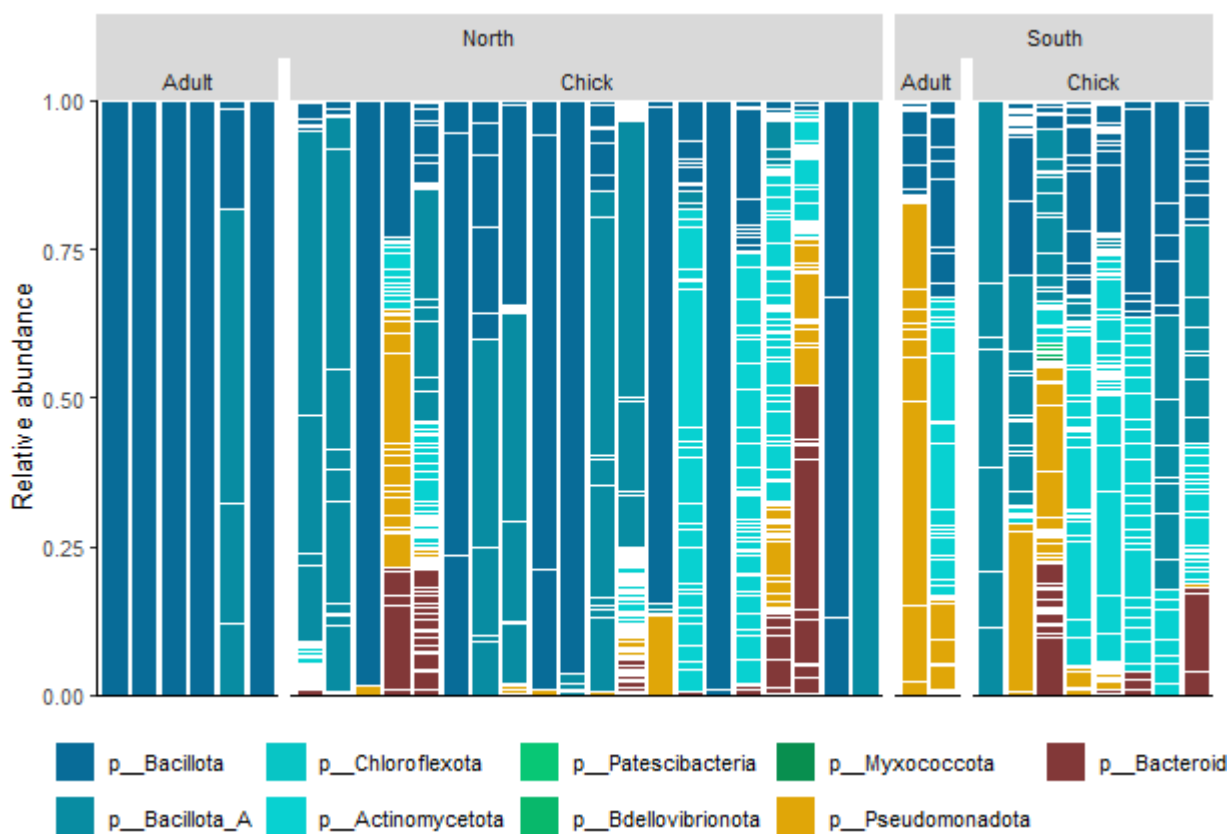


Figure 5.6. Relative abundance of phyla within each population. Each bar represents a different faecal sample.

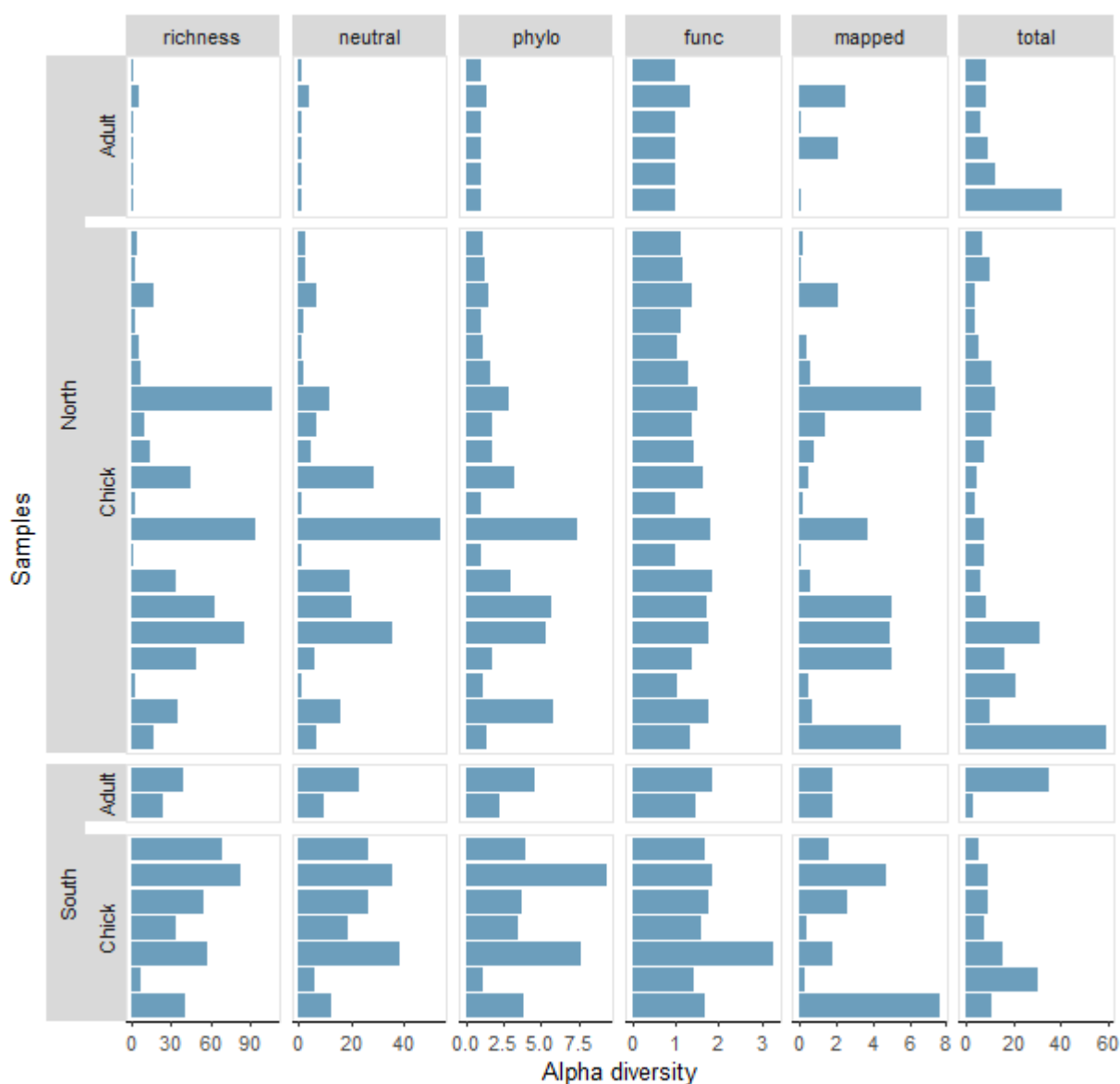


Figure 5.7. The alpha diversities, estimated as richness, neutral diversity, phylogenetic (phylo) diversity, and functional diversity, of all samples. The amount of mapped and total data (in GB – gigabases) for each sample is also shown.

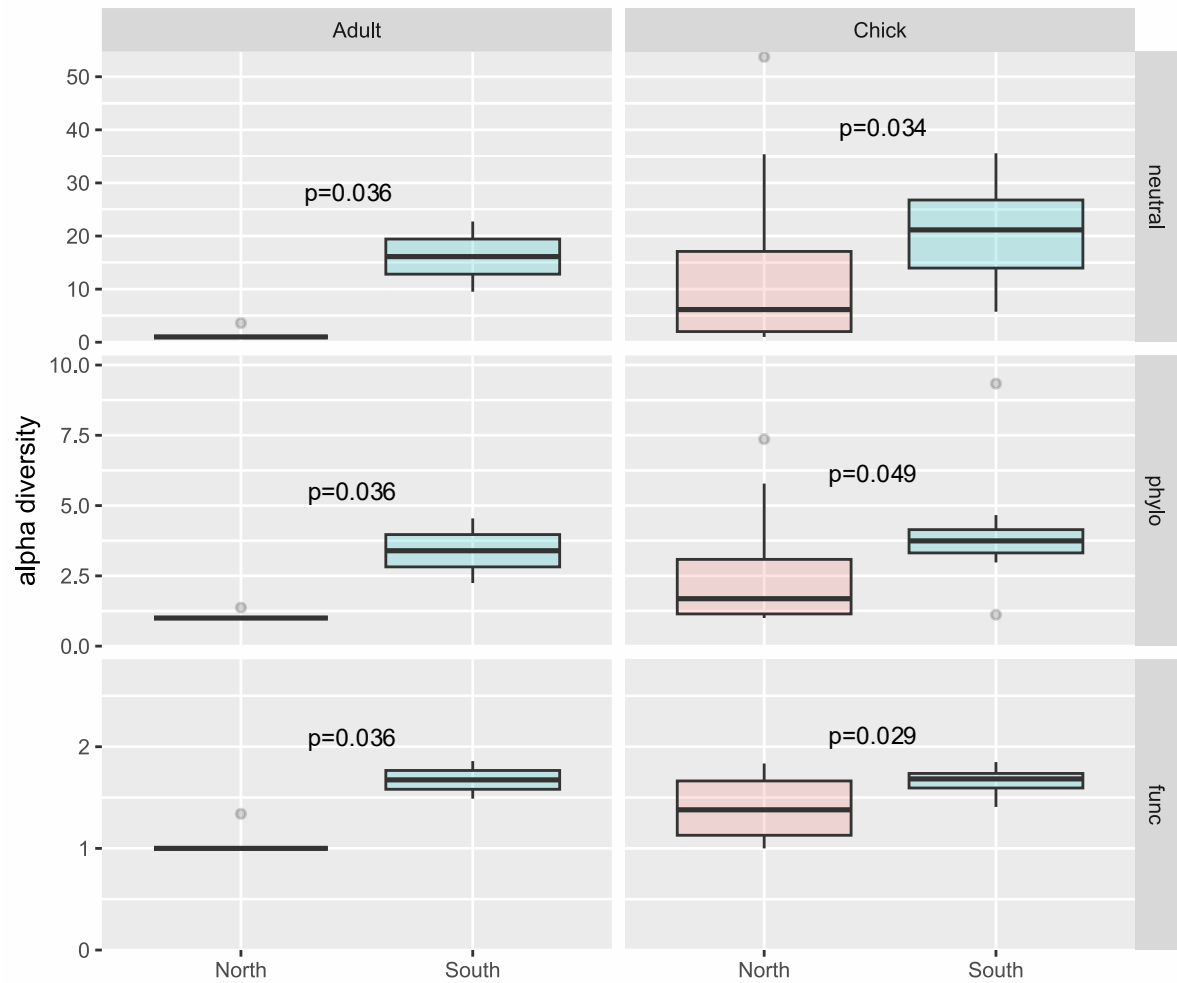


Figure 5.8. Alpha diversity measures across the two populations. Each measure was significantly higher in the South than the North (Wilcox test; p-values shown for population differences).

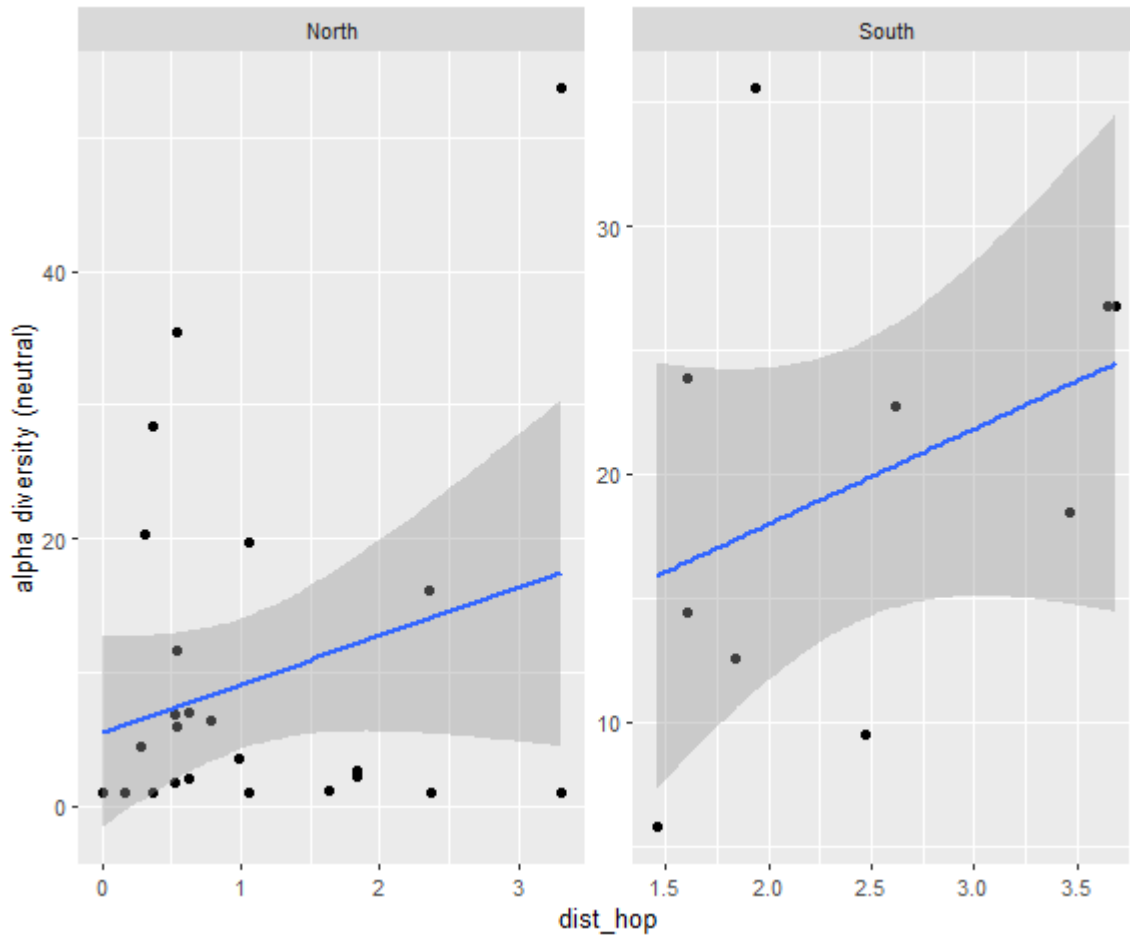


Figure 5.9. Positive but statistically insignificant relationship between alpha diversity and distance to supplementary feeding stations across both populations. The relationship between gut microbiome alpha diversity and distance to supplementary feeding stations (dist_hop, km) is shown for North (left panel, n=25) and South (right panel, n=9) populations. Blue lines represent fitted generalized linear models with Gamma distribution and log link function, with 95% confidence intervals shown in grey.

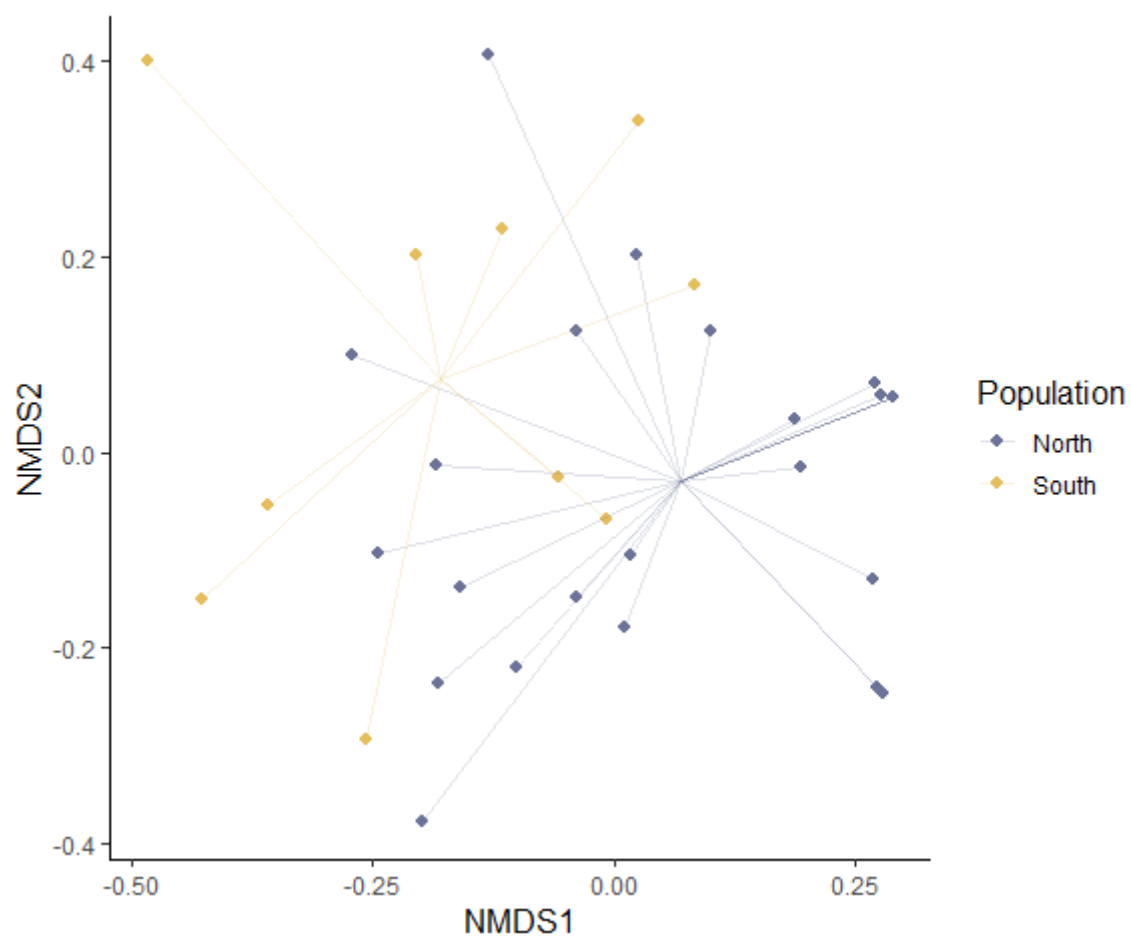


Figure 5.10. Non-metric multidimensional scaling (NMDS) ordination of microbial communities from North (n=20) and South (n=8) populations. Points represent individual samples, with distances between points indicating dissimilarity in community composition.

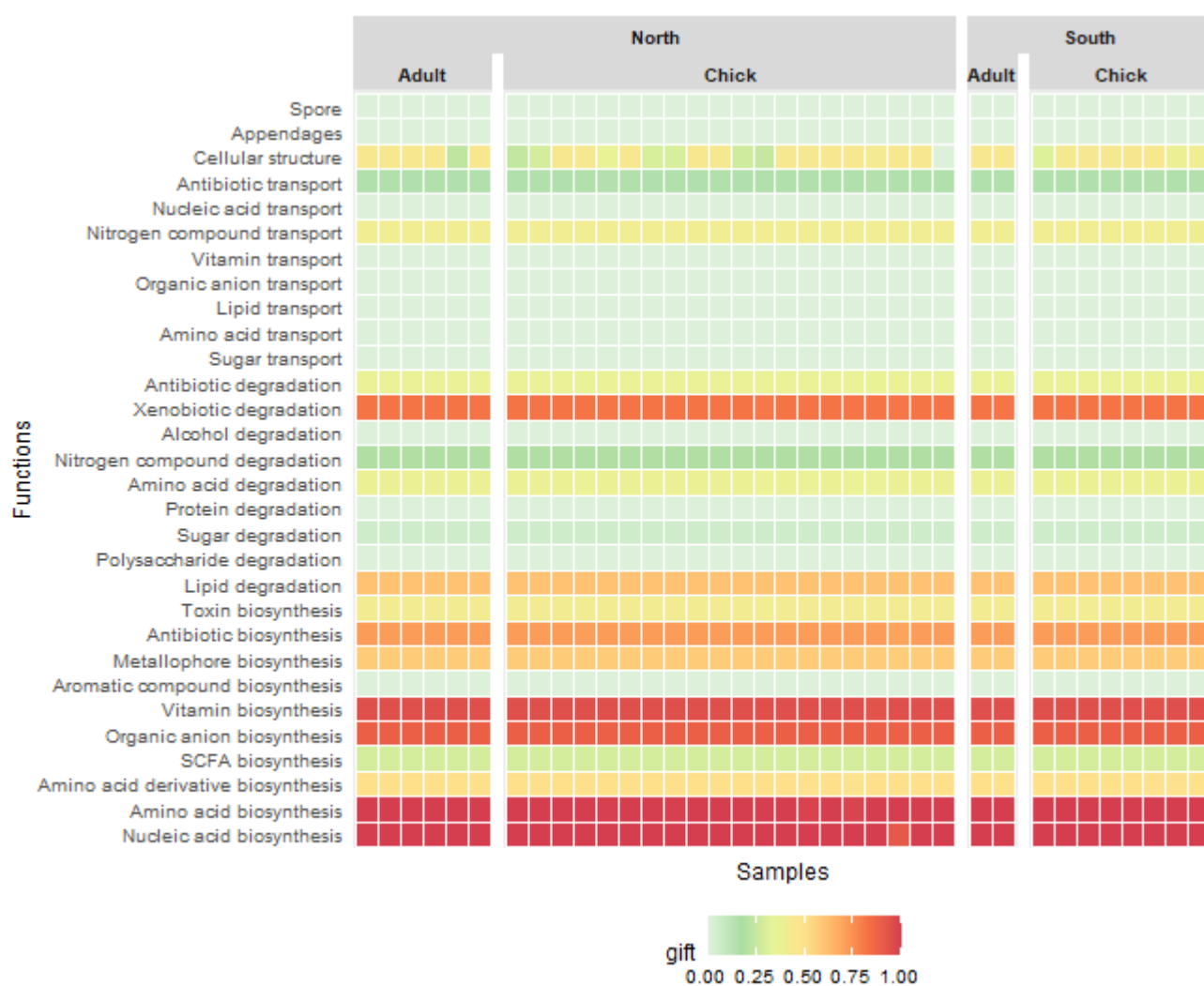
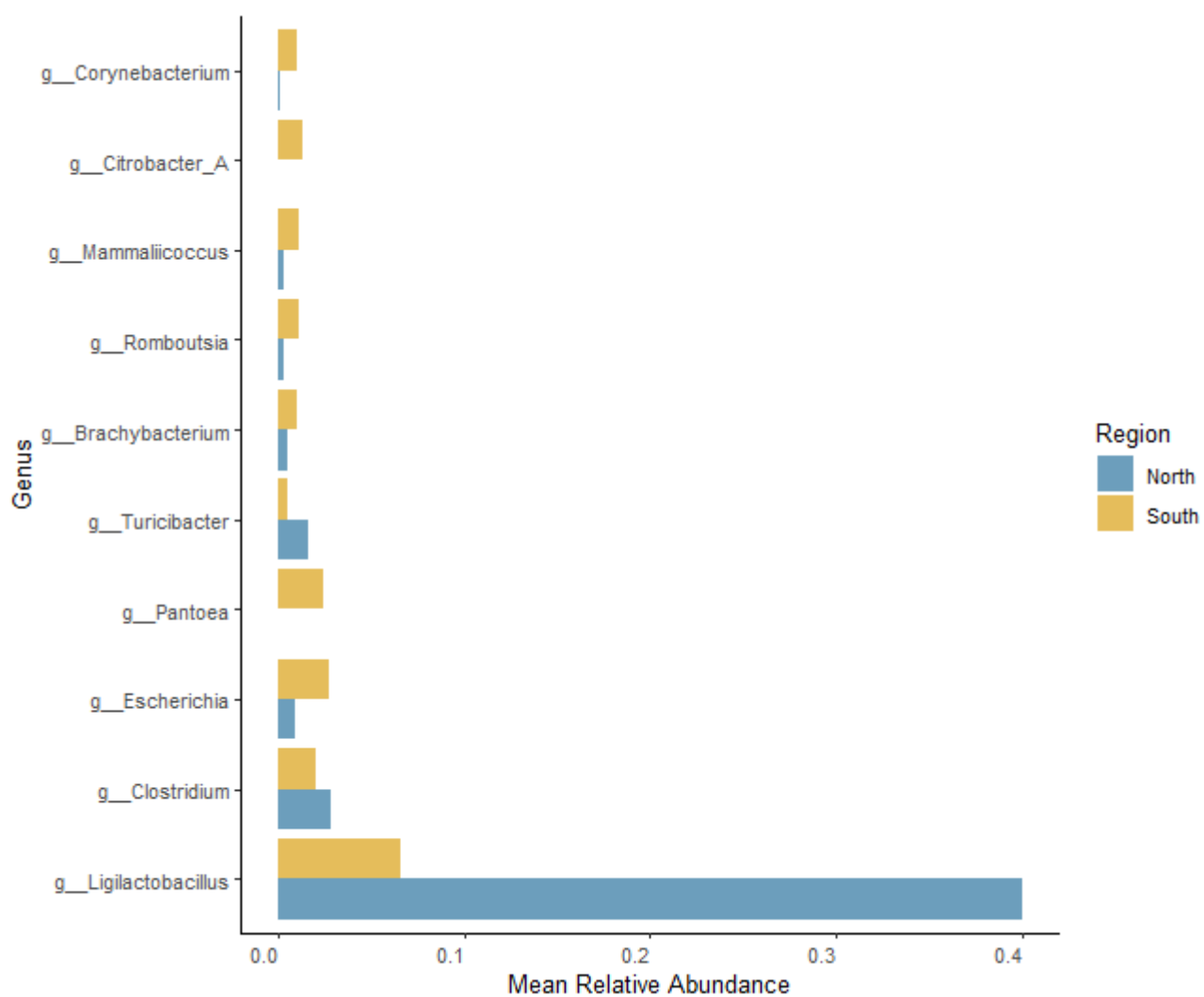


Figure 5.11. Heatmap showing functional trait abundance (gift scores) across individual samples from North and South populations. Gift scores range from 0 (light green) to 1 (dark red), indicating relative abundance of each functional trait. Each column represents one individual sample, and rows show different functional traits grouped into transport, degradation, and biosynthesis categories.

5.10 Supplementary information



Supplementary Figure 1. Mean relative abundance of most common genera in each population.

Supplementary Table 1. Complete ingredient composition of commercial parrot pellets provided as supplementary food to Mauritius parakeets (Kaytee Exact).

Category	Ingredients
Base Grains	Ground corn, ground wheat, ground oat groats, wheat middlings
Protein Sources	Corn gluten meal, dried whole egg, soybean meal
Fiber Sources	Dried molasses beet pulp, ground flax seed
Lipids	Soybean oil
Minerals	Calcium carbonate, dicalcium phosphate, salt, manganese proteinate, copper proteinate, ferrous sulfate, zinc oxide, manganous oxide, calcium iodate, cobalt carbonate, sodium selenite
Carbohydrates	Dextrose, wheat germ meal, dried cane molasses
Amino Acids	L-lysine, DL-methionine
Vitamins	Vitamin A supplement, choline chloride, vitamin E supplement, vitamin B12 supplement, riboflavin supplement, menadione sodium bisulfite complex (source of vitamin K activity), niacin, calcium pantothenate, thiamine mononitrate, pyridoxine hydrochloride, cholecalciferol (source of vitamin D3), beta-carotene, folic acid, biotin
Prebiotics	Fructooligosaccharide
Probiotics	Dried Bacillus licheniformis fermentation product, dried Bacillus subtilis fermentation product
Preservatives	Mixed tocopherols, rosemary extract, citric acid
Other Additives	Brewers dried yeast, yeast extract, Yucca schidigera extract, canthaxanthin (color), natural

Supplementary Table 2. Possible functions of most common genera identified in the Mauritius parakeet microbiome.

Genus	Possible function	Citation
Ligilactobacillus	Carbohydrate fermentation, lactic acid production, pathogen inhibition	(Aruwa et al., 2021)
Clostridium	Fiber digestion, short-chain fatty acid (SCFA) production, gut homeostasis; some pathogenic species (e.g., <i>C. perfringens</i>) cause necrotic enteritis	(Guo et al., 2020)
Escherichia	Vitamin K production, gut function; some pathogenic strains (<i>E. coli</i> , APEC)	(Aruwa et al., 2021)
Pantoea	Carbohydrate metabolism, nutrient processing; some opportunistic pathogens	(Aruwa et al., 2021)
Turicibacter	Carbohydrate metabolism, immune regulation, gut integrity maintenance	(Aruwa et al., 2021)
Brachybacterium	Protein degradation, lipid metabolism; role in birds unclear	(Aruwa et al., 2021)
Romboutsia	Carbohydrate metabolism, fermentation, SCFA production, gut health	(Bai et al., 2021)
Mammaliicoccus	Previously <i>Staphylococcus</i> , may play a role in skin/mucosal health; some opportunistic pathogens	(Aruwa et al., 2021)
Citrobacter	Some species assist in nutrient metabolism; others (e.g., <i>C. freundii</i>) are opportunistic pathogens	(Aruwa et al., 2021)
Corynebacterium	Some species aid in gut and skin health; some break down complex carbohydrates; others are opportunistic pathogens	(Aruwa et al., 2021)

Chapter 6 General discussion

Understanding the factors that influence the recovery of threatened populations is becoming increasingly critical as more species face extinction risk in human-modified landscapes. Small populations face multiple interacting threats that can accelerate their decline toward extinction, including genetic factors like inbreeding depression, ecological challenges such as limited resource availability, and demographic constraints (Frankham & Ralls, 1998; Mills, 2013).

Recent methodological advances have enhanced our ability to understand these challenges and their interactions. The standardisation of lethal equivalents has improved comparisons of inbreeding effects across species and life stages (Nietlisbach et al., 2019; Trask et al., 2021), while DNA metabarcoding and stable isotope analysis have enabled us to understand of dietary patterns and supplementary feeding effectiveness at an unprecedented resolution (Robb et al., 2011; Pompanon et al., 2012). Together, these approaches offer new opportunities to examine how multiple threats combine to influence population recovery.

The availability of long-term breeding data is crucial for understanding how these factors affect reproductive success and population recovery. The Mauritius parakeet provides an ideal study system, combining extensive monitoring data spanning over two decades with a population that has recovered from just 20 individuals in the 1980s to several hundred birds today. This species shows both the potential for successful recovery of a critically endangered species and the ongoing challenges of maintaining recovering populations. Its population is distributed across two distinct areas with different management intensities, offering a unique opportunity to examine how genetic and

ecological factors influence recovery under different conditions. By combining long-term breeding records with multiple analytical approaches – from genetic analysis to DNA metabarcoding to stable isotope analysis – this research revealed population-specific patterns in how inbreeding depression, diet composition, and supplementary feeding influence reproductive success.

Genetic analysis (Chapter 2) revealed that while inbreeding significantly impacted hatching success, its effects were not uniform across populations. Stable isotope analysis (Chapter 3) confirmed the positive effect of supplementary food consumption on reproductive success but again showed that the effect was not uniform across populations. Dietary analysis (Chapter 4) revealed unexpected patterns in foraging behaviour, with birds maintaining high native plant diversity in their diet despite habitat degradation, and, again, effects were not equal between populations. Finally, genome-resolved metagenomics provided the first characterisation of gut microbiota in this species, revealing population-specific differences in microbial diversity and function, as well as a potential relationship between supplementary feeding and reduced microbiome diversity (Chapter 5).

6.1 Main findings

6.1.1 Inbreeding depression and reproductive success (Chapter 2)

Chapter 2 provides the first comprehensive analysis of life stage-specific inbreeding depression in the Mauritius parakeet, contributing to a growing body of research demonstrating variable effects of genetic load across life stages in threatened species (Grueber et al., 2010; Huisman et al., 2016; Nietlisbach et al., 2019; Trask et al., 2021). Our finding of significant inbreeding effects on hatching success but not fledging success

aligns with patterns observed across multiple taxa, where genetic load often manifests most strongly in early development (Hemmings et al., 2012; White et al., 2015). The high hatching failure rates observed (30.2% North, 21.2% South) are similar to those seen in other bottlenecked island birds, such as takahe showing 31% egg failure rates (Jamieson & Ryan, 2000) and align with broader studies showing average hatching failures of 25.3% in populations bottlenecked below 150 individuals (Briskie & Mackintosh, 2004).

The lethal equivalents detected for hatching success (North: 2.40, South: 4.39) provide important context for understanding genetic load in small populations. While lower than values reported in some highly bottlenecked species, such as the Guam kingfisher with 8.43 LEs (Trask et al., 2021), they exceed those found in other island bird populations, such as the New Zealand hihi with 1.03 LEs (Brekke et al., 2010). These moderate values suggest that while inbreeding depression poses a concern, particularly for hatching success, the population may have maintained some resilience despite its severe historical bottleneck.

The absence of detectable inbreeding effects on fledging success could reflect masking effects of current management practices, particularly supplementary feeding. This aligns with research demonstrating that environmental conditions can modulate inbreeding depression (Fox & Reed, 2011). Supplementary food has been shown to boost productivity in Mauritius parakeets (Tollington et al., 2019 and Chapter 3), and this management intervention may be obscuring underlying genetic load. Alternatively, deleterious alleles affecting post-hatching survival may have already been purged through selection (Hedrick & Garcia-Dorado, 2016; Caballero et al., 2017).

6.1.2 Supplementary feeding and reproductive success (Chapter 3)

While previous research demonstrated the importance of supplementary feeding for reproductive success in this species during a single breeding season (Tollington et al., 2019), this study provides the first multi-year analysis of supplementary feeding effects, examining patterns across three breeding seasons (2014, 2020, and 2021). The substantial role of supplementary food in the Mauritius parakeet diet (mean 66.9%) demonstrates its importance as a conservation tool, but the patterns revealed through stable isotope analysis suggest its effectiveness varies by population. The marked difference in consumption between populations (North: 84.2%, South: 29.4%) reflects not only different provisioning schedules but potentially different resource needs and accessibility.

While supplementary food consumption positively predicted reproductive success across both populations, the North population did not show proportionally higher reproductive output despite significantly greater supplementary food use. This finding challenges assumptions about maximising supplementary food provision and suggests that other factors may constrain its benefits. Distance to feeding stations strongly predicted supplementary food consumption but not reproductive success, indicating complex spatial dynamics in how birds utilise these resources.

Our findings showed a positive but non-significant relationship between BFDV infection status and supplementary food consumption, suggesting that supplementary feeding is not increasing disease transmission risk to chicks in this population during the years studied (2014, 2020, and 2021). This is in contrast to findings of Fogell et al. (2019) where probability of BFDV infection in nestlings decreased with increasing distance from

feeding stations in the years 2013 to 2015. This adds important context to management decisions, given past concerns about feeding stations acting as potential disease transmission hotspots (Murray et al., 2016; Fogell et al., 2019).

6.1.3 Diet composition and reproductive success (Chapter 4)

This study is the first to utilise DNA metabarcoding to characterise the Mauritius parakeet diet, and to relate diet to reproductive success in this species. Our findings revealed distinct patterns in Mauritius parakeet diet across the two populations, particularly higher native plant diversity in the North (4.7 vs 1.9 species per sample) despite historical declines in native vegetation across both ranges. Our development of the DNA barcode reference library for mainland Mauritian plants was crucial to this analysis, enabling identification of 83 plant taxa compared to 38 species previously known from field observations. This reference library represents a significant contribution to conservation biology in Mauritius, providing a valuable resource for future studies of plant-animal interactions in this biodiversity hotspot.

DNA metabarcoding substantially expanded our understanding of Mauritius parakeet dietary breadth, identifying 83 plant taxa compared to 38 species previously known from field observations. The significant difference in native plant consumption between populations suggests differing foraging strategies between populations, that are likely influenced by differences in their habitat compositions and reflective of the frequency with which each population is provided supplementary food. Despite historical vegetation surveys showing higher non-native species dominance in the North (Virah-Sawmy et al., 2009; Florens et al., 2012), this population maintained higher native species richness in their diet (4.7 vs 1.9 species per sample).

The populations showed contrasting relationships between diet composition and reproductive success. In the North, supplementary food-related plant species positively correlated with fledgling numbers, while in the South, consumption of endemic *Gaertnera* sp. showed positive associations with reproductive success. The relatively low consumption of dominant invasive plants like *Psidium cattleianum* suggests selective foraging behaviour - despite comprising 57% of individual plants in parts of the North population's range (Virah-Sawmy et al., 2009), *P. cattleianum* appeared in only 21 of 156 faecal samples.

The relationship between CMAs and diet was more complex than expected. While initial analyses suggested CMAs increased overall plant species diversity in parakeet diet, they did not specifically enhance native species consumption and did not significantly predict fledging success in any of our models. This may reflect how Mauritius parakeets use the landscape - since their foraging ranges extend beyond CMA boundaries, birds may be accessing native plant resources across the habitat mosaic regardless of their nesting location or proximity to a CMA.

6.1.4 Gut microbiome analysis (Chapter 5)

Our metagenomic analysis revealed significant differences in gut microbiota between the North and South populations. The South population exhibited consistently higher alpha diversity across all metrics (richness, neutral, phylogenetic, and functional diversity). This pattern of higher diversity in the South aligns with their greater consumption of natural foods compared to supplementary feeding, following patterns seen in other bird species where diet diversity influences gut microbiome composition (McKenney et al., 2018; Bodawatta et al., 2022).

Age-related variation in gut microbiota was detected only in the North population, where adult birds showed significantly lower diversity than chicks. This contrasts with patterns observed in some other bird species, where microbial diversity typically increases with age (Grond et al., 2017; Videvall et al., 2019). The population-specific pattern could reflect the higher reliance on supplementary feeding in the North, similar to findings in house sparrows where restricted diets reduced microbial diversity (Florkowski et al., 2023), though larger sample sizes would be needed to confirm this relationship. The absence of age-related differences in the South population suggests that different feeding practices might influence how microbial communities develop and persist, though a larger-scale study would be required for us to confirm these differences.

The analysis of functional traits revealed decreased abundance of vitamin biosynthesis, antibiotic degradation, and toxin biosynthesis functions with increasing distance from feeding stations. While preliminary due to limited sample sizes, these findings suggest that supplementary feeding may influence not just taxonomic composition but also the metabolic capabilities of gut microbial communities, aligning with research showing how diet can shape microbiome function (West et al., 2019; Dodd & Grueber, 2021; Bodawatta et al., 2022). Recent studies have demonstrated that threatened species generally show lower gut bacterial diversity compared to non-threatened species (Huang et al., 2022), making it particularly important to understand factors affecting microbiome composition in at-risk populations.

These findings provide an important baseline for understanding host-microbe relationships in a threatened bird population. While the relationship between gut microbiota and reproductive success remains to be investigated, this characterisation of

population-specific patterns in microbial diversity and function raises questions about how conservation management might influence host-microbe interactions in recovering populations.

6.1.5 Population-specific effects

Our findings reveal population-specific patterns in how genetic and dietary factors influence reproductive success in Mauritius parakeets (**Figure 6.1**). While both populations show significant genetic load affecting hatching success through lethal equivalents, the South population exhibits nearly double the inbreeding load (4.39 LE vs 2.40 LE; Chapter 2) despite maintaining higher overall reproductive success. This apparent contradiction may be explained by the populations' different dietary strategies. The North population shows greater reliance on supplementary feeding (84.2% consumption) with a direct positive effect on reproductive success, while the South population, despite lower supplementary food consumption (29.4%) (Chapter 3), achieves higher reproductive success potentially through greater utilisation of native foods, particularly *Gaertnera* sp.

These contrasting patterns might also be explained by demographic differences between the populations. The North population's higher density (109 versus 52 breeding pairs in 2020; MWF, 2020) likely increases competition for resources and nest sites. In this context, supplementary feeding may be supporting breeding attempts of less fit individuals that would otherwise be selected against under more resource-limited conditions. This aligns with broader evidence that supplementary feeding can create localised areas of high animal density that influence both resource use and disease transmission patterns (Murray et al., 2016).

Supplementary food could be maintaining a broader range of individuals in the breeding population, potentially including those that might struggle to provision chicks effectively using natural food resources alone. This is supported by previous evidence that supplementary food was disproportionately boosting reproductive success in older females in the 2014 breeding season (Tollington et al., 2019), though we did not find evidence for this in the 2020 and 2021 breeding seasons (which could be explained by a decline in identification of females in recent years, therefore restricting the dataset available for dam ages). In contrast, the South population's lower density and reliance on natural foods may result in stronger selection pressure against less fit individuals, reflecting patterns seen in other species where density-dependent effects can influence reproductive success (Paradis et al., 2002; Carrete et al., 2006).

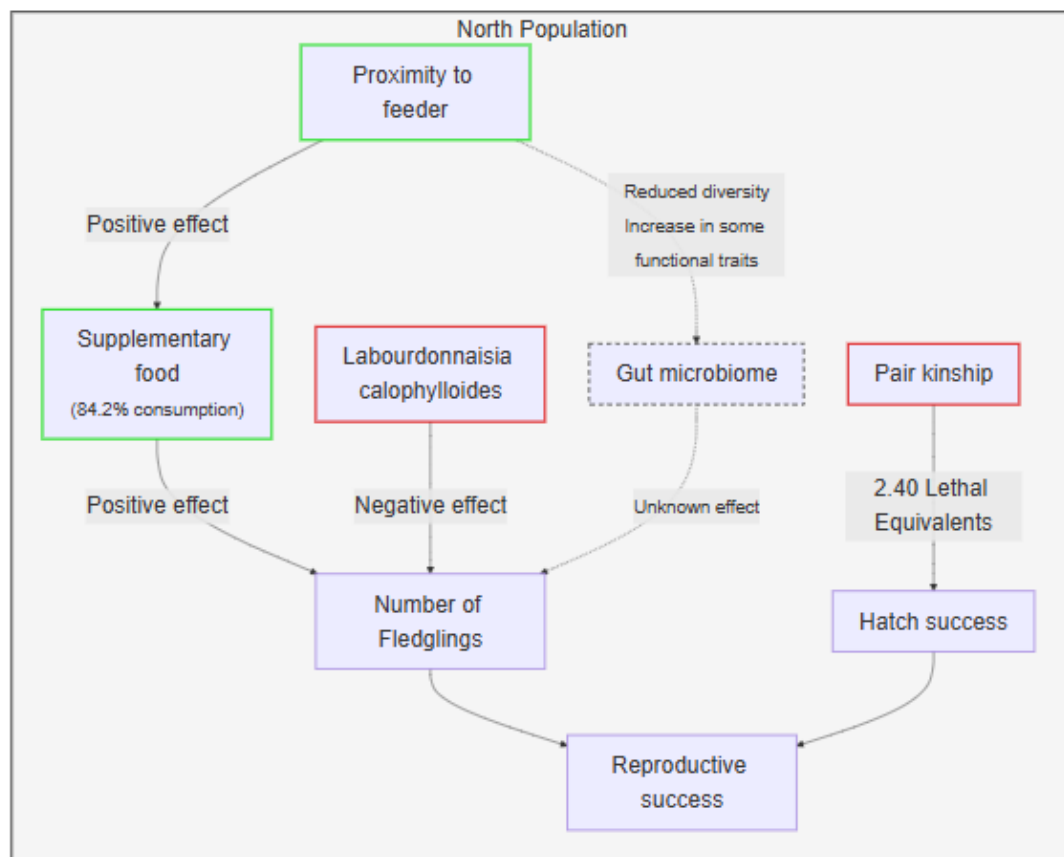
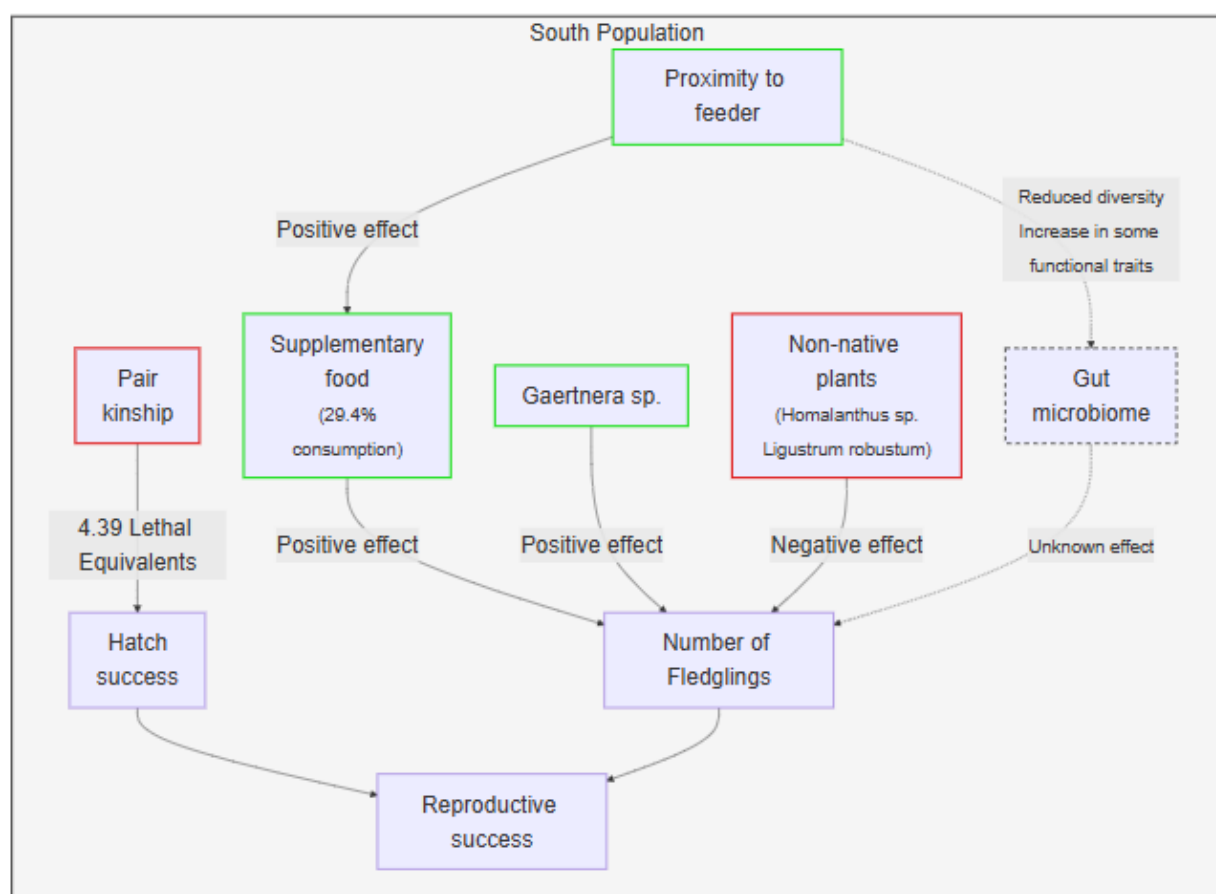


Figure 6.1. Population-specific effects of genetic and dietary factors on reproductive success, and possible links with the gut microbiome, in Mauritius parakeets.

6.1.6 Conservation management areas and potential habitat effects

CMAs were established to protect and restore native vegetation through invasive plant removal, leading to our prediction that CMAs would positively influence parakeet diet and reproductive success. However, our analyses found no significant effect of CMAs on either diet composition or breeding success. This lack of effect could be explained by several factors. First, our categorisation of nests as simply "inside" or "outside" CMAs, along with basic distance metrics, may have been too simplistic to capture the complex ways parakeets utilise these resources. Birds may access CMA resources regardless of nest location, making our spatial metrics ineffective measures of resource availability.

Additionally, the strong positive effect of supplementary feeding, particularly in the North population, may mask any potential benefits of nesting within or near CMAs. The relationship between CMAs and key food plants also remains unclear - for instance, while we found that *Gaertnera* sp. consumption positively influences fledging success in the South population, we lack data on how this species' distribution varies between CMAs and unmanaged areas. Systematic vegetation surveys comparing plant composition inside and outside CMAs would be crucial for understanding these patterns.

Understanding how CMAs influence resource availability and use would require integrating vegetation surveys with movement ecology data to map how birds actually utilise these managed areas within the broader landscape mosaic.

6.2 **Conservation management implications**

These findings highlight several key priorities for conservation management of small populations. Understanding life-stage specific inbreeding effects is crucial for targeting interventions effectively - in this case, focusing efforts on supporting successful hatching

rather than fledging survival. The moderate lethal equivalent values suggest ongoing genetic management and population monitoring remains important but may not require more intensive interventions like translocations to facilitate genetic rescue. However, the lethal equivalents calculated should be included in population simulations to better understand how inbreeding will impact this species in the long-term.

The contrasting relationships between diet and reproductive success in each population emphasise the importance of population-specific management approaches. While supplementary feeding remains crucial in the North, protecting and potentially expanding populations of key native species like *Gaertnera* sp. may be more beneficial in the South. The unexpected diversity of native plants in the diet, particularly in the North, suggests that maintaining habitat mosaics with even small patches of native vegetation is valuable for population persistence.

The differences in gut microbiome composition and function between populations highlight a potential unintended consequence of supplementary feeding. While supplementary feeding has proven crucial for reproductive success, particularly in the North population, our findings suggest it may influence host-microbe relationships in ways that are not yet fully understood. Our finding of lower microbial diversity nearer to supplementary feeding stations aligns with patterns seen in other avian species where artificial feeding influences gut microbiota composition (Knutie, 2020; West et al., 2022b; Florkowski et al., 2023). While the implications of these differences aren't yet fully understood in the Mauritius parakeet, studies in other species suggest gut microbiome diversity can influence disease susceptibility (Williams et al., 2018; van Veelen et al., 2020; Ottinger et al., 2024), immune function (Knutie, 2020), and reproductive success

(Martta et al., 2024; Ottinger et al., 2024). This suggests that while supplementary feeding remains an important management tool, its potential effects on gut microbiota warrant consideration in future management planning. For instance, future research could investigate whether feeding strategies could be optimized to maintain both nutritional benefits and microbial diversity

The findings of this thesis have broader implications for managing small populations in degraded landscapes. The population-specific patterns in both genetic and ecological factors demonstrate the importance of tailored conservation approaches based on local conditions. The integration of multiple analytical approaches - genetic analysis, DNA metabarcoding, stable isotope analysis, and metagenomics - provides an example of comprehensive population monitoring that could be applied to other threatened species facing similar challenges.

6.3 Future research directions

While this thesis has significantly advanced our understanding of factors influencing reproductive success in the Mauritius parakeet, several important knowledge gaps remain that warrant further investigation. These research priorities would not only enhance management of the Mauritius parakeet but could provide valuable insights for conservation of other threatened species facing similar challenges in modified landscapes.

6.3.1 Inbreeding depression across further life stages

Our analysis quantified genetic load effects on hatching and fledging success, but understanding inbreeding impacts across additional life stages is crucial for fully understanding the impact of inbreeding depression. Future research could examine

survival, recruitment to breeding population, and reproductive longevity. Such data would enable calculation of lethal equivalents across the full life cycle, similar to studies in song sparrows where cumulative effects increased from 1.4 LEs for early survival to 24.6 LEs for total fitness (Keller, 1998). Long-term monitoring focused on these later life stages could reveal important patterns in how genetic effects accumulate over time, particularly given the population-specific differences in genetic load we observed.

6.3.2 Vegetation surveys to improve dietary analysis

Current gaps in our understanding of resource availability limit interpretation of the dietary patterns revealed through DNA metabarcoding. Our DNA metabarcoding revealed unexpected patterns in native plant consumption, particularly in the North population, but without comprehensive data on plant availability, we cannot determine whether these patterns reflect active dietary selection or simply resource accessibility. These surveys should map both the distribution of key food plants identified in our study and quantify their relative abundance across different habitat types, including within and outside Conservation Management Areas. This information would be invaluable for habitat management decisions and understanding how landscape configuration influences resource use.

Investigation of temporal variation in diet composition would provide crucial insights for management. Our DNA metabarcoding focused on the breeding season, but understanding dietary shifts throughout the year would help ensure year-round resource availability. This could involve regular faecal and/or feather sampling across seasons combined with phenological monitoring of key food plants. Such data would be

particularly valuable given climate change may alter plant phenology and resource availability patterns.

6.3.3 Improvements to DNA metabarcoding methods

Several methodological refinements could enhance our understanding of Mauritius parakeet diet. While DNA metabarcoding successfully revealed a higher dietary breadth than was previously known, the ability to quantify relative abundances of different plant species could enhance our understanding of their resource use. Recent advances suggest potential for quantitative assessment of diet composition through quantitative PCR (Thomas et al., 2016; Lamb et al., 2019), though this remains debated in the field.

The DNA barcode reference library could be strengthened through the use of additional markers. Several potentially important food plants, particularly in the Ebenaceae family (e.g., multiple *Diospyros* species), were missing from our library due to poor amplification success with ITS2. Using a suite of complementary markers could improve species detection and identification (Adamowicz et al., 2019; Stenhouse et al., 2023), though this would significantly increase costs.

Temporal resolution of dietary patterns could be improved through systematic sampling throughout the breeding season. Our current analysis provides a snapshot of diet at approximately 45 days post-hatching, but diet composition likely varies across breeding stages and seasons. More frequent sampling at key nest sites could reveal these patterns, though this would need to be balanced against potential disturbance effects on breeding success. Future studies might focus intensive sampling on a subset of accessible nests to minimise disturbance while gathering more detailed temporal data.

6.3.4 Movement ecology

Understanding how Mauritius parakeets navigate their habitat requires detailed movement ecology studies. While our stable isotope analysis demonstrated clear spatial patterns in supplementary food use, we lack data on movement patterns between feeding areas, nest sites, and natural foraging locations. This could be studied through bird sighting data, or potentially through GPS tracking. This would help us to optimise the spatial distribution of conservation interventions like supplementary feeding, nest box placement, and planting of native food plants.

6.3.5 Temporal effects on reproductive success

While not a primary focus of this thesis, our analyses revealed a significant negative effect of later breeding year on reproductive success. This temporal decline in fledgling numbers could indicate increasing density-dependent effects as the population has grown. This pattern was particularly evident in the North population, which maintains higher breeding pair density (109 versus 52 pairs in the South in 2020). Such density-dependent effects have been documented in other recovering bird populations, where reproductive success often declines as populations approach carrying capacity (Paradis et al., 2002; Carrete et al., 2006).

Future research should examine whether this temporal decline reflects genuine density dependence or other factors such as habitat quality changes or shifts in management effectiveness. This understanding would be particularly valuable for long-term population management planning.

6.3.6 Gut microbiome, disease, and reproductive success

Our preliminary characterisation of the Mauritius parakeet gut microbiome revealed distinct microbial communities between populations, with the South population showing consistently higher alpha diversity despite lower supplementary food consumption. While sample sizes were limited, particularly from the South population, the analysis demonstrated how genome-resolved metagenomics can provide insights into both taxonomic composition and functional potential of gut microbial communities in threatened species. Furthermore, it has highlighted a further difference between the two populations of Mauritius parakeets and laid groundwork for future research.

The finding that supplementary feeding may influence microbiome diversity warrants further investigation with larger sample sizes. Studies in other threatened birds have shown important links between supplementary food and gut microbiota - for instance, research on the kākāpō found that supplementary feeding significantly affected microbiome composition (West et al., 2022), while studies in brown kiwi demonstrated that captivity and restricted diets reduced microbial diversity (San Juan et al., 2021). Given the Mauritius parakeet's history with psittacine beak and feather disease (Fogell et al., 2021), understanding potential relationships between gut microbiota, immune function, and disease susceptibility could provide valuable insights for population management. Future research with larger sample sizes and longitudinal sampling could help determine whether the observed patterns are stable over time and investigate potential links with reproductive success, disease resistances or susceptibility, and overall population health.

6.4 Conclusions

This thesis demonstrates how multiple analytical approaches can enhance our understanding of population recovery and improve conservation management. Long-term monitoring data combined with new methodological techniques revealed population-specific patterns in reproductive success, inbreeding depression, supplemental food use, and dietary composition. The striking differences between the two populations highlights the importance of evidence-based, locally tailored conservation strategies. The research questions were developed with conservation managers in Mauritius to ensure findings could help to inform management decisions.

These findings provide important information for the ongoing management of the Mauritius parakeet and demonstrate how the use of genetic analysis, stable isotope analysis, and DNA metabarcoding could be applied to understand the effects of key threats to small populations of other threatened species.

6.5 References

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