Measuring the effects of supplementary feeding and biosecurity on the trajectory of a threatened avian population

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"I want to take everything I've seen and thought and learned and reduce them and relate them and refine them until I have something of meaning, something of use."

John Steinbeck

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All chapters were written by Deborah Fogell with guidance, editorial suggestions and comments from supervisors Jim Groombridge and John Ewen. Chapters 1 and 3 have been published in the scientific literature and Chapter 2 was under peer review at the time of submission. All research within this thesis was approved by the University of Kent Ethics Committee (0018-DF-16). All samples were obtained and imported into the United Kingdom with permissions from the relevant local wildlife authorities, conservation nongovernmental and research organizations.

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Contents

Abstract

When faced with emerging infectious diseases in wild populations, conservationists are often forced to respond rapidly, with decisions based in uncertainty. Clear decision-making processes are rarely followed and subsequent monitoring and evaluation as to the efficacy of the chosen solution is often neglected. This thesis interrogates the interaction between disease transmission and population management solutions for the recovery of the endangered Mauritius parakeet (*Psittacula eques*), with a particular focus on nest sites, supplementary feeding hoppers and biosecurity. Beak and feather disease virus (BFDV; Circoviridae), the etiological agent of Psittacine beak and feather disease (PBFD), is widely infectious and fatal. PBFD is considered the most common viral disease in wild parrots and was first detected in Mauritius parakeets in 2005. Here I apply a combination of field-based experiments and molecular genetic techniques to screen both host and environmental DNA for BFDV, alongside observational, demographic and breeding data to address three key research questions. I assess the influence of (i) the wildlife trade in the global spread of BFDV, (ii) artificial nest sites and supplementary feeding hoppers on the prevalence of BFDV in Mauritius parakeets, and (iii) sociality at supplementary feeding hoppers on the transmission of BFDV. The key aim of this thesis is to provide practical and implementable management solutions that are relevant to any conservationists managing wild populations affected by BFDV.

I detected BFDV in wild parrots from eight new countries, as well as from birds seized from illegal trafficking. Phylogenetic associations between geographically distant regions highlight the impacts of the wildlife trade in the spread of infectious disease globally. With regards to population management, I found that there is currently no observable relationship between nest site placement and either BFDV prevalence or fecundity, but the relationship between BFDV prevalence and nest altitude may be of greater relevance under future climate change scenarios. Whilst biosecurity protocols applied at nest sites successfully reduced BFDV prevalence in nestlings, upscaled disinfection of hoppers had no significant effect. However, both forms of biosecurity unintentionally and significantly hindered Mauritius parakeet breeding success. Finally, I determined that the relationship between BFDV prevalence and hoppers was better attributed to the artificially altered frequency of social interaction between individuals at these centralised hubs.

These results have both increased our knowledge of BFDV occurrence globally, covering some highly biodiverse but data deficient regions, and provided an evidence-based approach to the evaluation of *in situ* pathogen management. Management for wildlife conservation should be critically evaluated through targeted monitoring and experimental manipulation, and this evaluation should always focus on the fundamental objective of conservation.

Chapter 1

Introduction

The latest statistics from the Living Planet Index, indicating that wildlife populations have declined by 60% since 1970 (WWF 2018), clearly show that global biodiversity is in a crisis. Conservation managers are faced with the challenges of recovering populations under increasing threats from local anthropogenic pressures (Joppa *et al.* 2016; WWF 2018; Dirzo *et al.* 2014). Approximately 46% of all avian species are listed by the IUCN as being in population decline, with 15% listed in threatened categories or extinct, and a further 9% classified as near threatened (IUCN 2019).

Bird populations are naturally regulated by relatively few variables including food, availability of safe nests sites, predation, competition and disease (Newton 1998). In situations where one or a combination of these factors drive population decline, then targeted mitigation may allow for recovery. These may include, supplementary feeding to correct food shortages (Oro *et al.* 2008; Cole and Batzli 1978; Walker *et al.* 2013), invasive predator control to reduce pressure from novel predators, and captive breeding (Cade and Jones 1993; Andrew *et al.* 2018) combined with conservation translocation (Seddon *et al.* 2014) to overcome any combination of these population threats.

1.1 DECISION MAKING IN SPECIES RECOVERY

When faced with the need for intervention to prevent further wildlife population declines, conservationists have a number of integrated qualitative and quantitative strategies available to them (Linkov *et al.* 2006). The degree to which risks can be mitigated and management outcomes can be improved relies heavily on how successfully conservationists combine knowledge and information from a variety of different sources (e.g experts, stakeholders, observational data; Gore *et al.* 2009). However, decisions regarding the best management techniques to apply (e.g. captive breeding, translocations) are often made in uncertainty, with incomplete knowledge of the biology or ecology of the system (Canessa *et al.* 2016). Consequently managers frequently have to decide between those actions that are expected to improve the status of the population, and those that will improve knowledge of the system (Westgate, Likens and Lindenmayer 2013). Given that there is often a limited capacity to collect information on the target species within the short time-frames required for mitigation, an adaptive management approach allows for gradual improvements to be made to the selected intervention(s), as more knowledge is gathered (Canessa *et al.* 2016). Despite the advantages that adaptive management offers when there is a need to rapidly implement conservation actions, its application in conservation is uncommon and usually poor (Westgate, Likens and Lindenmayer 2013; Canessa *et al.* 2016). Similarly, post-hoc monitoring of conservation actions is typically unfocused or lacking entirely (Ewen, Soorae and Canessa 2014) and experimental

approaches to assessing the efficacy of population recovery interventions are severely lacking in the published literature. One of these targeted mitigation interventions, supplementary feeding, is widely applied in endangered species recovery programmes and, along with nest site management, is a key focus of this thesis. However the application of supplementary feeding in population recovery is often not based in sound scientific theory with carefully evaluated costs and benefits (Ewen *et al.* 2014).

1.2 SUPPLEMENTARY FEEDING FOR SPECIES RECOVERY

1.2.1 Benefits to productivity and health

Feeding of wild birds is a widespread pastime across millions of households in the UK and the US (Jones 2011; Robb *et al.* 2008). This hobby has been shown to benefit numerous populations of avian species present in anthropogenically modified habitats and urban areas (O'Leary and Jones 2006; Jones 2011). As desired when implementing supplementary feeding in wildlife recovery programmes, domestic provisioning has been shown to positively influence all stages of offspring production including clutch size, egg quality, hatching success, growth rate and fledging success (Robb *et al.* 2008). This is due in part to the role that female condition has in the allocation of resources to egg production (Metcalfe and Monaghan 2001) where good condition can increase early growth and offspring survival (Verboven *et al.* 2003). Supplementary feeding can also increase the probability of survival for overwintering birds (Jones 2011), enhance resistance to disease or pathogens (Brittingham and Temple 1988), provide a central means by which vaccinations or medication can be dispensed (Cross, Buddle and Aldwell 2007) or provide a source of food that is guaranteed free of drugs and poisons (Oro *et al.* 2008).

1.2.2 Behavioural side effects

Access to artificial food sources alters both the feeding ecology and behaviour of a population. Reducing scarcity of resources may change territorial behaviour and reduce the need for mixed-flock social foraging strategies (Robb *et al.* 2008). Changes to territoriality could be negative where a single, high density source of food becomes costly to birds when they feel a greater need to defend territories in close proximity (Strain and Mumme 1988). However, this stressor could be avoided or reduced by providing supplementation in more, smaller proportions over a greater area, thus diminishing the ability of larger or more aggressive individuals or species to dominate (Donázar, Cortés-Avizanda and Carrete 2010). Provisioning does however pose the risk of creating dependency of a species or population on this artificial resource, removing their ability to be sustained on natural resources alone. This could be due to either anthropogenic changes to the natural landscape (Friend, Mclean and Dein 2001; Cortés-Avizanda, Carrete and Donázar 2010) or dependence for overwintering survival (Orell 2008).

1.2.3 Costs to fitness and immune function

Despite the potential benefits to productivity, supplementary feeding of wild populations can also have unintentional adverse effects. Provisioning increases the density of individuals around a central resource (Sorensen, van Beest and Brook 2013; Robb *et al.* 2008), a common scenario for a population that would – without intervention - have naturally dispersed to feed throughout their available habitat. This increased density also increases the contact rate between individuals, both directly and indirectly facilitating the spread of pathogens between individuals and increasing exposure to infection (Lawson *et al.* 2012; Sorensen, van Beest and Brook 2013). If adequate biosecurity protocols are not thoroughly adhered to at supplementary feeders, then congregating individuals may be exposed to the pathogens shed from a single infected individual (Corn and Nettles 1995). There are many examples of disease outbreaks in avian populations that can be linked to supplementary feeding such as: Mycoplasmal conjunctivitis (Dhondt *et al.* 2005; Hotchkiss *et al.* 2005), avian pox (Lawson *et al.* 2012), trichomoniasis (Robinson *et al.* 2010) and salmonellosis (van Andel *et al.* 2015).

1.3 NEST SITE PROVISION TO IMPROVE BREEDING SUCCESS

The availability of safe nesting sites is critical in the maintenance of avian populations (Finch *et al.* 2019). Many bird species are dependent on nesting in cavities, and are vulnerable to decline if suitable sites become scarce (Sherley *et al.* 2012). In such cases the provision of artificial nest boxes is often reported as a highly successful management tool, from burrowing seabirds such as penguins (Sherley *et al.* 2012), to forest species such as tree swallows (Norris *et al.* 2018) and cockatoos (Berris *et al.* 2018). In parrots, where 70% of species are secondary tree cavity nesters, the loss of nesting sites through deforestation is considered a primary driver of population declines (Olah *et al.* 2016; Newton 1994). Consequently, conservationists frequently deploy artificial nest boxes as a means to increase breeding success (e.g. Beissinger et al. 1998; Downs 2005; Larson et al. 2015).

However, artificial nest boxes may have negative population effects if they are not an adequate functional substitute for natural cavities (Maziarz, Broughton and Wesołowski 2017). The differences in breeding success between natural and artificial nest sites may be due to variations in accessibility to predators (either increased or reduced, Møller 1989; Wesołowski 2011) and insulation from changes in ambient temperature (Maziarz, Broughton and Wesołowski 2017). As natural cavities vary in both size and location, their microclimates would vary accordingly (Coombs, Bowman and Garroway 2010; Maziarz and Wesołowski 2013). Therefore, the managers of avian populations should consider that the provision of consistently sized artificial nest sites, in areas that may not have otherwise been preferentially selected in an unmodified environment, may not necessarily result in positive breeding outcomes for their target species.

1.4 EMERGING INFECTIOUS DISEASES (EIDs) IN WILDLIFE

Disease causing pathogens have an important regulatory role within ecosystems when they are native. They alter species composition, influence host genetic diversity and act as powerful agents of natural selection (Altizer, Harvell and Friedle 2003), regulating host populations in the same manner as predation or competition (Lyles and Dobson 1993). However, emerging infectious diseases (EIDs) are concerning due to their ability to modify ecological balance (Artois *et al.* 2001). EIDs are defined as diseases that are newly evolved, recently discovered, have increased in incidence, shifted host population or have undergone geographical expansion (Daszak, Cunningham and Hyatt 2001; Morens, Folkers and Fauci 2004; Smith, Sax and Lafferty 2006). Whilst increased incidence of EIDs may be a product of better reporting and surveillance in recent years, emerging pathogens have been detected globally within every major ecosystem (Dobson and Foufopoulos 2001) and are responsible for reducing biodiversity and threatening populations or entire species with extinction (Lips *et al.* 2006). Disease outbreaks in wildlife populations present a unique challenge to conservationists, who are often ill-equipped to deal with unexpected epidemics (Woodroffe 1999) and often the response to EIDs in wildlife has been superficial in comparison to those affecting domestic animals and humans (Friend, Mclean and Dein 2001).

Pathogens are capable of rapid evolution and adaptation to novel hosts, thus bypassing any defence or immunity harboured by the original host in a natural system (Altizer, Harvell and Friedle 2003). Those responsible for wildlife epidemics are generally able to persist in a wide range of environments and are frequently characterised by their requirement for only a single host for complete development (Dobson and Foufopoulos 2001). A host organism may not present with clinical symptoms of infection with a pathogen if it is not infected at the appropriate life-stage or in a weakened physiological state (Cunningham 1996). However, it may instead act as a reservoir or vector for the pathogen, transmitting it to more susceptible hosts (Artois *et al.* 2001). Viruses are one group of pathogens whose transmission may be unintentionally facilitated by conservation action. Viruses are responsible for over 40% of all recently surveyed wildlife EIDs (Dobson and Foufopoulos 2001; Tompkins *et al.* 2015), and have thus been highlighted as a particular threat to wildlife. The threats from viruses are in part due to their ability to adapt rapidly to novel hosts (Altizer, Harvell and Friedle 2003; Jones *et al.* 2008), conferring the capacity to become infectious across a wide host range (Altizer, Harvell and Friedle 2003).

1.4.1 Management of EIDs in wildlife populations

Assessing the prevalence and impacts of disease in wildlife populations poses a number of challenges, especially with novel pathogens. Free-living animals are generally difficult to access, collect samples from and assess (Artois *et al.* 2001). Additionally, often little is known about the species concerned and their natural pathogen complement (Robinson *et al.* 2010). This lack of

knowledge complicates management decisions as detection of a previously unknown pathogen does not necessarily confirm its novelty in the host (Rachowicz *et al.* 2005). Broadly speaking, management of EIDs can be broken down into three main types of strategies. First, those that target direct treatment or vaccination of the infected host, such as anti-fungal treatment of amphibians affected by *Batrachochytrium dendrobatidis* (Hudson *et al.* 2016; Bosch *et al.* 2015) or the inoculation of black-footed ferrets against canine distemper virus (Thorne and Williams 1988). Second, strategies that aim to prevent interaction between disease vectors and the focal host, such as pesticide application for reducing tick populations that are responsible for the spread of Lyme disease (Stafford III 1997). Third, strategies that aim to reduce the risk of transmission through hygiene, biosecurity or direct treatment of environmental reservoirs (Wobeser 2002). For example, the disinfection of water bodies associated with the spread of avian cholera (Gershman *et al.* 1964) and liming around feeding stations to reduce the prevalence of lungworms in hares (Skrjabin 1970). Various combinations of these strategies have been broadly applied across taxonomic groups. In extreme cases these disease management strategies can be combined with the removal of surviving individuals to captivity (Zippel *et al.* 2011).

Management actions aimed at reducing EID transmission *in situ* are mostly reactive and the efficacy of only a few have been thoroughly assessed (Wobeser 2002; Woodroffe, Frost and Clifton-Hadley 1999; Artois *et al.* 2001). These management actions are often modified versions of those used in clinical settings and based on expert knowledge of wildlife health specialists. Importantly however, their application is rarely backed by critical evaluation of their ability to reduce transmission and aid recovery of the threatened host species (the fundamental objective). This lack of critical evaluation raises a dual concern that conservation management may continue despite an intervention being ineffective or even detrimental to endangered species recovery, and that this may add unnecessary financial and logistical burdens to management.

1.5 PSITTACINE BEAK AND FEATHER DISEASE

One such infectious disease that is currently a concern for conservationists is Psittacine beak and feather disease (PBFD), considered to be the most common viral disease in wild psittaciformes (Khalesi *et al.* 2005). Its etiological agent, Beak and feather disease virus (BFDV), belongs to the Circoviridae family; comprising a circular, single-stranded, approximately 2000 nucleotide long DNA genome which lacks a non-coding region (Ritchie *et al.* 1989a). Both its size and structure make BFDV a relatively simple pathogen for studying molecular variation in the context of disease ecology and drivers of spread (Sarker *et al.* 2014). The genome consists of a highly conserved replication associated protein (*Rep*) (Kondiah, Albertyn and Bragg 2006; Kundu *et al.* 2012; Peters *et al.* 2014) and viral encapsidation protein (*Cap*) responsible for and host cell penetration (Heath *et al.* 2004; Kundu *et al.* 2012). BFDV is transmissible horizontally, through

contact with contaminated feather dust, surfaces or objects (Ritchie, Anderson and Lambert 2003), and vertically, from a female to her offspring (Rahaus *et al.* 2008). PBFD has been reported in both wild and captive parrot populations since the mid-1970s and has been found to be widely infectious and often fatal, known to affect 60 Old and 18 New World psittacine species globally (Fogell, Martin and Groombridge 2016).

Due to its pathogenicity, viral infectiousness and wide global distribution it has been suggested that PBFD should now be classified as an EID (Fogell, Martin and Groombridge 2016). Most commonly affecting immature and fledgling birds, classical symptoms of PBFD include symmetrical loss of contour, tail and down feathers before replacement by dystrophic and necrotic feathers that fail to grow soon after emergence from the follicle (Perry 1981; Ritchie *et al.* 1991; Pass and Perry 1984). Beak deformities such as fractures, abnormal elongation and palatine necrosis are also typical symptoms of PBFD but their presence and severity vary from species to species (Ritchie *et al.* 1989b). Other clinical symptoms include lethargy, depression, diarrhoea and immunosuppression, which are individually variable, sometimes lead to death and may depend on the virulence of the viral strain or the route of viral exposure (Ritchie *et al.* 1989a).

1.6 FACTORS CONTRIBUTING TO BFDV TRANSMISSION

Numerous abiotic, biotic and management factors are responsible for determining whether a susceptible individual becomes infected with BFDV. Figure 1.1 depicts a simplified representation of the interactions and influence of each of these key factors in a managed parrot population. When conservationists face managing wild populations in the face of BFDV, it is vital to assess where management effort may be best focused or improved to reduce the anthropogenic spread of infection.

This thesis explores relationships between ten of these influential factors and the dynamics of BFDV infection and transmission within a managed population of endangered parrots. I include the abiotic influences of the international wildlife trade (and the consequential biotic influence of contact with introduced reservoir hosts species) and microclimate variation. I also focus on four management related factors, including the provision of artificial nest sites, the provision of supplementary feeding stations (and their specific placement within a degraded habitat to best support population recovery) and the biosecurity regimes implemented as a means to control the spread of infection. I also consider how these abiotic and management factors interlink with other biotic factors such as BFDV viral strain, changes in host density and the environmental accumulation of virions that individuals may be exposed to as a result.

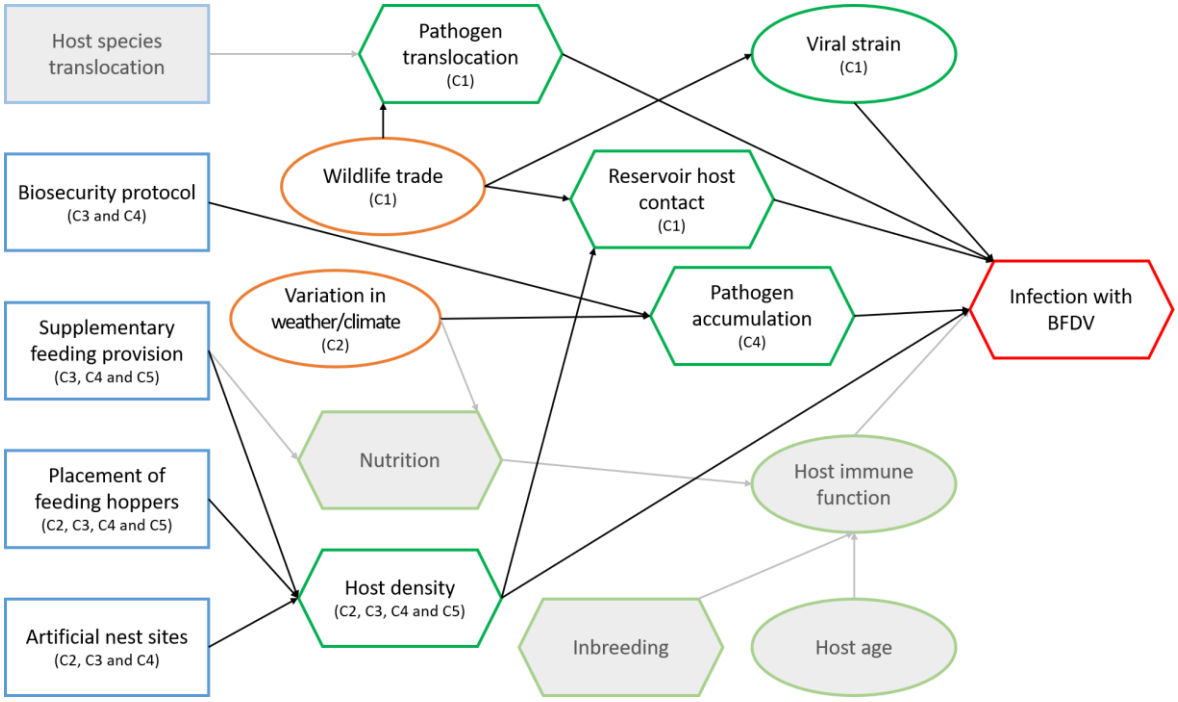

Figure 1.1 The influence of conservation management interventions (blue), abiotic (orange) and biotic (green) factors on the transmission of Beak and feather disease virus (BFDV) in a managed population of susceptible parrot hosts. Shaded cells have not been addressed in this thesis and annotations in brackets within unshaded cells indicate which chapters address each of these key factors.

1.6.1 International wildlife trade and contact with reservoir hosts

Parrots are among the most threatened bird groups (Olah *et al.* 2016) and are susceptible to a number of infectious diseases (Ritchie 1995). Parrots are also among the most frequently traded birds listed on the appendices of the Convention on International Trade in Endangered Species (CITES) (Pain *et al.* 2006), and the pet trade has driven cross-border movements of over 19 million parrots since 1975 (CITES 2016). This international trade has already been implicated in the spread of BFDV globally (Harkins *et al.* 2014).

Increasing reports of BFDV infections in wild parrot populations, both native and introduced, and including several populations of threatened species, have led to concerns over the conservation implications of the spread of infection (Kundu et al. 2012; Regnard et al. 2014; Jackson et al. 2015). The rapid adaptability and successful establishment of rose-ringed parakeets (*Psittacula krameri*) globally (Tayleur 2010; Menchetti, Mori and Angelici 2016) is particularly notable, as invasive populations and captive individuals of this species have previously tested positive for BFDV (Kundu *et al.* 2012; Julian *et al.* 2013; Sa *et al.* 2014). Therefore, rose-ringed parakeets may be a high-risk reservoir host and vector for BFDV, particularly where its distribution overlaps with that of vulnerable species. However, due to the recent detection of BFDV in a number of other non-psittacine hosts (Amery-Gale *et al.* 2017; Sarker *et al.* 2016; Sarker *et al.* 2015), parrots may also become vulnerable to transmission from other abundant sympatric reservoir host species.

1.6.2 Climate variation

Pathogens are generally able adapt to their environment locally and may therefore tolerate substantial variation in climate (Lafferty 2009). Like other circoviruses, BFDV is thought to be highly persistent and stable outside of the host (Ritchie 1995; Amery-Gale *et al.* 2017; Jackson *et al.* 2015), remaining viable for months after an infected bird has shed virions and even after short periods at high temperatures (Bougiouklis 2007). However, this assumption has not been empirically tested through detailed studies on BFDV and is based on the response of porcine circovirus (Allan *et al.* 1994; Bougiouklis 2007). To date, little is known about how regional climate or microclimates may affect the prevalence and transmission of BFDV infection within a wild population and, therefore, whether this may have differing effects across wild populations known to be susceptible. Altitude and aspect have been successfully used as climate proxies to model changes in parasite and pathogen prevalence or abundance (Gilbert 2010; Bødker *et al.* 2003), and may therefore prove to be valuable proxies for climatic variation in making predictions about management of BFDV.

1.6.3 Management factors

The risk of increasing the transmission of infectious disease with the use of supplementary feeding hoppers to support wild populations has already been discussed extensively above. As young birds appear to be particularly vulnerable to disease (Ritchie *et al.* 1989a), nest sites create a second high risk point for the transmission of BFDV, where infection of entire broods may occur vertically or horizontally, either naturally from parental contact or accidentally when managing parrot populations. Consequently, artificial nesting sites may present both a concern for conservation managers (i.e. where inappropriate use may increase disease risks) but also an opportunity for disease mitigation. Few management actions have been developed and tested to manage the transmission of BFDV *in situ*, most of which have focused on hygiene and biosecurity. In Australia, for example, a detailed Threat Abatement Plan for BFDV includes the use of disinfectants in nest and transport boxes (Department of the Environment and Heritage 2005). However, the same Threat Abatement Plan also notes that there is no assurance as to whether recommended actions will actually reduce transmission.

1.6.4 Environmental viral accumulation

Pathogens responsible for illness in both humans (Cheesbrough et al. 2000; Li et al. 2013) and domestic stock (Andraud et al. 2013; Li et al. 2013) have shown to accumulate and persist outside of a host whilst still remaining infectious. Quantitative Real Time PCR (RT-PCR) has been successfully conducted on environmental swabs for both the detection of Norwalk-like viruses (Cheesbrough *et al.* 2000) and to test the efficacy of multiple disinfectant products on the presence of the avian influenza virus in live bird markets (Suarez *et al.* 2003). RT-PCR methods to detect BFDV

were first developed for African grey parrots (Raue *et al.* 2004) and have recently been used for assessing viral load at the level of the individual (e.g. Eastwood et al., 2015; Regnard et al., 2015; Fogell et al. 2019). Despite the theory that BFDV is environmentally stable outside of the host (Ritchie 1995; Todd 2000; Jackson *et al.* 2014), this has not yet been conclusively evaluated.

1.7 STUDY SYSTEM

Mauritius parakeets (*Psittacula eques*) were once widely distributed across the tropical rainforest habitat of Mauritius. They are now confined to the Black River Gorges National Park (BRGNP), in the southwest (Figure 1.2), and a newly established sub-population in a private nature reserve (Le Vallée de Ferney), to the east of the island. Within the BRGNP the parakeet population is divided into two sub-populations, isolated by distance (Raisin *et al.* 2012), with one in the north of the reserve and one in the south (Figure 1.2).

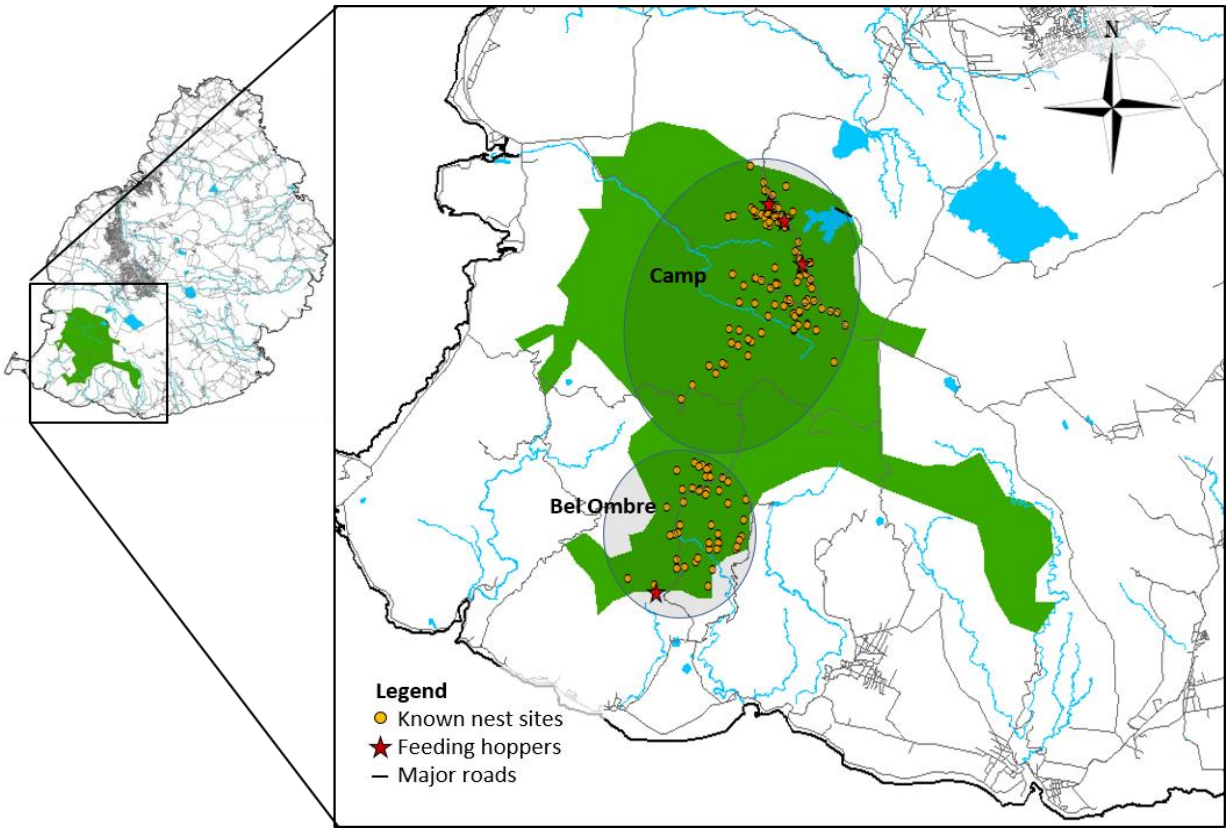

Figure 1.2 The distribution of Mauritius parakeet breeding sites within the Black River Gorges National Park in the south-west of Mauritius.

Mauritius parakeets were once the world's rarest parrot, numbering fewer than 20 individuals in the early 1980s (Duffy 1993). Intensive management including brood manipulation, supplementary feeding, provision of artificial nest sites, captive-breeding, reintroduction, and control of invasive alien predators (Bunbury *et al.* 2007; Tatayah *et al.* 2007) has increased their abundance to 136 breeding pairs in 2017 (Henshaw *et al.* 2018). However, these efforts were interrupted by an outbreak of PBFD in 2005 (Kundu *et al.* 2012). The disease outbreak was

considered a threat to the parakeet's recovery, prompting the immediate cessation of some elements of their management such as the transfer of individuals and eggs between nest sites, whilst the provision of artificial nest boxes, control of alien predators, the use of supplementary feeding hoppers and a minimal regime of visits to nest sites for monitoring purposes remained in place (Tollington *et al.* 2013). However, two management activities were considered high risk for continued spread of infection: supplementary feeding hoppers and nest box maintenance. Therefore, since 2005, the Mauritius parakeet field team has attempted to reduce or eliminate any potential human-mediated transmission of BFDV through biosecurity.

1.8 RESEARCH AIMS AND OBJECTIVES

The key aim of this thesis is to interrogate the interaction between human facilitated disease transmission and population management solutions implemented for the ongoing recovery and support of the Mauritius parakeet population. Fundamentally, I hope not just to provide a set of research papers that are of interest to the wider academic community. I use empirical assessments of current recovery tools and biosecurity protocols to provide practical and implementable management solutions that are relevant to any conservationists managing wild populations affected by BFDV.

In Chapter 2, I aimed to determine the presence and viral structure of BFDV not only in Mauritius, but also in native and introduced wild parrot populations from data deficient global regions and taxa. This research aimed to establish phylogenetic and biogeographic associations of BFDV among wild and captive populations from three continents, providing a broader context for the introduction of BFDV to Mauritius.

In Chapter 3, using long-term nesting data, I aimed to determine the influence of nest site location within the forest on the prevalence and viral load of BFDV in annually produced nestlings. I consider a range of abiotic factors such as their altitude and aspect, as well as their density within the forest and proximity to supplementary feeding stations.

Chapters 4 and 5 comprise iterative experimental designs to assess the efficacy of current biosecurity protocols in place at both nest sites and supplementary feeding hoppers. I aimed to determine whether current biosecurity successfully reduced the transmission of BFVD to nestlings *in situ* and whether these protocols improved nestling body condition and fecundity.

Finally, in Chapter 6, I aimed to determine whether the social contact network of Mauritius parakeets attending supplementary feeding hoppers changed temporally over the course of the breeding season and whether parental network centrality influenced the prevalence of BFDV infection in their nestlings.

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Chapter 2

Trade and conservation implications of new Beak and feather disease virus detection in native and introduced parrots

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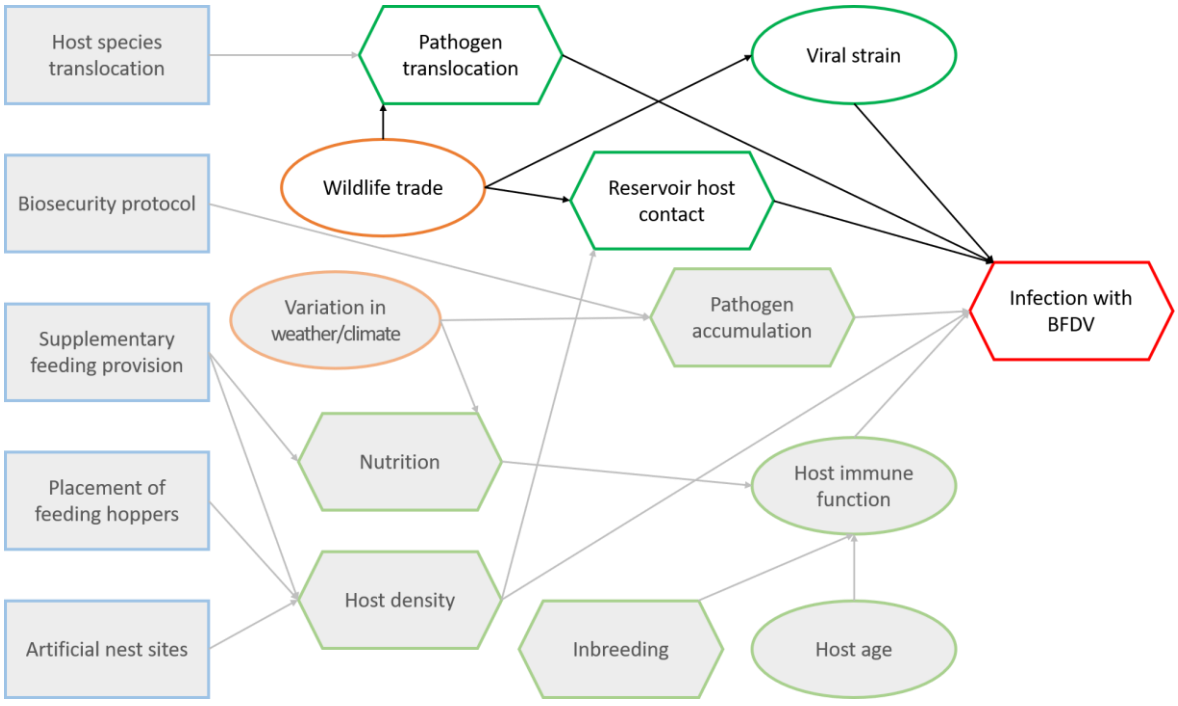

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2.1 ABSTRACT

Psittacine beak and feather disease (PBFD), caused by *Beak and feather disease virus* (BFDV), has spread rapidly around the world, raising concerns for threatened species conservation and biosecurity associated with the global pet bird trade. The virus has been reported in several wild parrot populations, but data are lacking for many taxa and geographical areas with high parrot endemism. We aimed to advance understanding of BFDV distribution in many data deficient areas and determine phylogenetic and biogeographic associations of the virus in five parrot species across Africa, the Indian Ocean islands, Asia, and Europe, with a specific focus on the highly traded and invasive *Psittacula krameri*. Blood, feather and tissue samples were screened for BFDV through standard PCR. Isolates obtained from positive individuals were then analysed in a maximum likelihood phylogeny along with all other publicly available global BFDV sequences. We detected BFDV in eight countries where it was not known to occur previously, indicating the virus is more widely distributed than currently recognized. We documented for the first time the presence of BFDV in wild populations of *P. krameri* within its native range in Asia and Africa. We detected BFDV among introduced *P. krameri* on Mauritius and the Seychelles, raising concerns for island endemic species in the region. Phylogenetic relationships between viral sequences showed likely pathways of transmission between populations in southern Asia and Western Africa, as well as between Seychelles and the United Kingdom. A high degree of phylogenetic relatedness between viral variants from geographically distant populations suggests recent introductions, likely driven by global trade. These findings highlight the need for effective regulation of international trade in live parrots, particularly in regions with high parrot endemism or vulnerable taxa where *P. krameri* could act as a reservoir host.

2.2 INTRODUCTION

The global spread of pathogens poses an increasing threat to biodiversity (Daszak, Cunningham and Hyatt 2000) and has been linked to wildlife-population collapse and multiple species extinctions (Cunningham, Daszak and Wood 2017). Parrots are among the most threatened bird groups (Olah *et al.* 2016) and are susceptible to a number of infectious diseases (Ritchie 1995). parrots are also among the most frequently traded birds listed on the appendices of the Convention on International Trade in Endangered Species (CITES) (Pain *et al.* 2006), and the pet trade has driven cross-border sarmovements of over 19 million parrots since 1975 (CITES 2016). This movement has exacerbated the establishment of numerous introduced populations, most notably the highly invasive Rose-ringed parakeet (*Psittacula krameri*), which has breeding populations in over 35 countries across five continents (Tayleur 2010; Menchetti, Mori and Angelici 2016).

Psittacine beak and feather disease (PBFD), caused by the Beak and feather disease virus (BFDV), is a commonly reported infectious disease of captive parrots. First described in the 1970s (Pass and Perry 1984) in the South Pacific (Ritchie et al. 1989; Heath et al. 2004; Harkins et al. 2014), PBFD is thought to have post-Gondwanan origins due to the paucity of ancestral non-Australian clades and infrequent observations across other regions where parrot endemism is high, such as Africa and South America (Raidal, Sarker and Peters 2015). All psittaciformes are susceptible to infection (Sarker *et al.* 2014), and PBFD is typically characterized by chronic symmetrical feather abnormalities, dystrophy, and severe claw and beak deformities (Latimer et al. 1991; Bassami et al. 1998). The immunosuppressant nature of BFDV increases host susceptibility to secondary infection (Ritchie *et al.* 1989; Ritchie, Anderson and Lambert 2003). The spread of BFDV may be facilitated by the global trade in live parrots (e.g. Varsani et al. 2011; Harkins et al. 2014) and its high environmental persistence and transmissibility between closely related host species (Peters *et al.* 2014; Sarker *et al.* 2014). To date BFDV or PBFD have been recorded in 78 species and five subspecies (Fogell, Martin and Groombridge 2016). Infection of parrots in captivity has been reported in at least 33 countries, whereas the virus occurs in comparatively few wild populations outside Oceania, where BFDV originated (Fogell, Martin and Groombridge 2016; Raidal, Sarker and Peters 2015).

Increasing reports of BFDV infections in wild populations, both native and introduced, including several populations of threatened species, have led to concerns over the conservation implications of the spread of infection (Kundu et al. 2012; Regnard et al. 2014; Jackson et al. 2015a). Although invasive populations and captive individuals of Rose-ringed parakeets have tested positive for BFDV (Kundu *et al.* 2012; Julian *et al.* 2013; Sa *et al.* 2014), to date no BFDV screening of Rose-ringed parakeets has been conducted on any free-living populations across their extensive native range (Fogell, Martin and Groombridge 2016). The rapid adaptability and successful establishment

of Rose-ringed parakeets globally makes it a high-risk reservoir host and vector for BFDV, particularly where its distribution overlaps with that of vulnerable species. These concerns have prompted actions such as the eradication of Rose-ringed parakeets on the island of Mahé, Seychelles, to minimize threats to the endemic Seychelles black parrot (*Coracopsis barklyi*). This eradication campaign was launched in 2013 in response to concerns over biosecurity (Seychelles Islands Foundation 2013), particularly in light of the similar BFDV-affected parakeet populations in Mauritius (Kundu *et al.* 2012).

Despite increasing surveillance effort over recent years (Fogell et al. 2016), there remains a paucity of information on BFDV distribution, notably in regions of high parrot endemism in Africa, Asia, and South America (Fogell, Martin and Groombridge 2016) and from parrots seized from illegal trade. Insufficient knowledge of the distribution of the virus among native and introduced populations and within trade hampers understanding of the biogeography and origins of BFDV, the potential conservation impacts of PBFD, and impedes the development of effective approaches to prevent BFDV spread.

We aimed to determine the presence of BFDV in native and introduced wild parrot populations in data deficient regions and taxa across three continents and to establish phylogenetic and biogeographic associations of the virus among wild and captive populations and parrots in illegal trade based on viral sequence analysis. We screened samples obtained from native and introduced populations of parrots from Africa, Asia, and Europe of Seychelles black parrots, Mauritius "echo" parakeets (*Psittacula eques*), Grey-headed parakeets (*Psittacula finschii*), Rose-ringed parakeets, and Timneh parrots (*Psittacus timneh*) for the presence of BFDV. We focused on the Rose-ringed parakeet because of its potential to act as a reservoir host across its native and invasive range.

2.3 METHODS

2.3.1 Wild parrot sampling

Blood, muscle tissue, and feather samples were collected from wild, wild-caught captive, and seized parrots across 13 countries (Table 2.1, Figure 2.1). Samples were obtained from nestlings as part of ongoing Mauritius parakeet management from 1993 to 2015 (n = 894). Rose-ringed parakeets on Mauritius were mist-netted from 2009 to 2012 (n = 31). Samples from the Seychelles were obtained postmortem from Rose-ringed parakeets in 2014 (n = 23) and as part of long-term Seychelles Black parrot monitoring from 2009 to 2012 (n = 24). Further samples obtained from 2013 to 2016 from wild populations of Rose-ringed parakeets in the United Kingdom (Kent, n = 6), Germany (n = 20), Senegal (n = 10), Nigeria (n = 11), South Africa (n = 4), Japan (n = 15), Pakistan (n = 14), and Bangladesh (n = 29) were screened for BFDV where possible at the Durrell Institute of Conservation and Ecology (DICE) (University of Kent, United Kingdom) as part of a separate whole-

genome sequencing project. Under the same project, samples were obtained from sub adult (<3 years) captive Rose-ringed parakeets collected from nests in Gambia in 2014 (n = 3) and from wild Grey-headed parakeets in Vietnam in 2015 (n = 6). Samples were also obtained from an illegal shipment of parrots seized in 2015, including Timneh parrots (n = 8), thought to have originated in Ivory Coast, and from Rose-ringed parakeets (n = 5), thought to have originated in Senegal. Samples were collected postmortem from two Rose-ringed parakeets in 2012 and 2013 from the United Kingdom (Greater London). One of these birds had plumage abnormalities characteristic of PBFD, and disease was confirmed through histopathological examination. The second bird had normal plumage. Samples from both cases were screened with a real-time polymerase chain reaction (PCR) assay, and both were BFDV positive (Sa *et al.* 2014). Samples from these cases were subsequently sent to DICE for viral characterization.

This research was conducted under the University of Kent ethical guidelines (0018-DF-16). Sampling was undertaken in collaboration with local wildlife authorities, conservation nongovernmental and research organizations, and samples were imported to the United Kingdom under the following license numbers: TARP/2015/052, TARP/2013/210, TARP/2015/213, TARP/2015/243, TARP/2015/212, TARP/2015/055, ITIMP17.0656, TARP/2013/307, TARP/2015/228, TARP/2012/292, TARP/2016/105, TARP/2013/182, TARP/2015/085A.

2.3.2 DNA extraction and screening

An ammonium acetate DNA extraction method was used to extract bird and viral DNA prior to BFDV screening (Bruford *et al.* 1998). Samples were extracted in batches specific to geographic origin to reduce the risk of contamination between samples from different regions. For blood approximately 50–100 µL of whole blood was used from each sample and digested in 250 µL of DIGSOL lysis buffer with 10 µL of 10 mg/mL proteinase K. For skin and muscle tissue, approximately 4 mm^2 of tissue was used from each sample and digested in 250 µL of DIGSOL lysis buffer with 20 µL of 10 mg/mL proteinase K. For feather extractions, feather barbs were removed and the calamus was chopped finely prior to digestion in 250 µL of DIGSOL lysis buffer with 40 µL of 10 mg/mL proteinase K and 70 µL of 1M dithiothreitol. Extractions were quantified using a Qubit dsDNA Assay Kit (Thermo Fisher Scientific, Waltham) and standardized to approximately 25 ng/µL prior to BFDV screening where possible because of high yields. The only exception to this protocol was one of the U.K. Rose-ringed parakeet samples, from which DNA was extracted prior to its being sent to DICE for analysis.

We used BFDV-specific primers to determine presence of viral DNA within the host. Screening was carried out through PCR assays targeting a 717-bp region of *rep* (Ypelaar *et al.* 1999). The DNA from a BFDV-infected Mauritius parakeet was included as a positive control (Kundu *et al.* 2012). Reactions comprised 1 µL of extracted DNA template, 5 µL MyTaq HS Red Mix (Bioline,

London), and 0.2 µL each of the forward and reverse primers at 10 pmol/µL and were made up to 10 µL with double-distilled water. The PCR annealing temperature was 60 °C for 30 cycles, and products were visualized on a 1.5% agarose gel. A negative control of molecular-grade water was included in each PCR batch. All positive PCR products were sent to Macrogen Europe (Amsterdam) for sequencing. The single samples from Rose-ringed parakeets that tested positive for BFDV from Japan and Nigeria (Table 2.1) did not yield sequences of sufficient quality for further analysis. Population-prevalence estimates based on sample size were calculated. These estimates included a 0.9 test-sensitivity assumption that we derived with Epitools (Sergeant 2018).

2.3.3 BFDV phylogeny

We used GENEIOUS version 8.1.7 (Kearse *et al.* 2012) to align and edit the DNA sequences from this study with all *rep* gene sequences available in GenBank (downloaded 29 July 2016) for phylogenetic comparison and analysis (Supplementary Table 2.1). This global *rep* alignment was used to infer the best-fit substitution model with JModelTest version 2.1.7 (Posada 2008). We constructed a maximum likelihood (ML) phylogenetic tree with RAxML version 8 (Stamatakis 2014), which applies a gamma substitution model and a rapid bootstrapping (RBS) heuristic procedure (Stamatakis, Hoover and Rougemont 2008). We collapsed branches with <50% bootstrap support in TreeGraph 2 (Stöver and Müller 2010) and edited and annotated the final tree in FigTree version 1.4.2 (Rambaut 2009).

2.4 RESULTS

All individuals screened for BFDV from Bangladesh (95% CI 88.3–100%) and The Gambia (95% CI 43.9–100%) were infected. The virus was not detected in endemic Black parrots in the Seychelles (95% CI 0–13.8%) or in Rose-ringed parakeet populations in Germany (95% CI 0–16.1%), South Africa (95% CI 0–49.0%), or in Kent (95% CI 0–39.0%), despite being present in the adjoining Greater London Area. We detected BFDV in both the native (26.1%, 95% CI 23.3–29.0%) and invasive parakeet (16.1%, 95% CI 7.1–32.6%) species in Mauritius. We detected BFDV in Rose-ringed parakeet samples from Pakistan (71.4%, 95% CI 45.4–88.3%), Japan (6.7%, 95% CI 1.2–29.8%), Nigeria (9.1%, 95% CI 1.6–37.7%), and Senegal (50%, 95% CI 23.7–76.3%) and in individuals seized from trade in Western Africa (20%, 95% CI 3.6–62.5%). Grey-headed parakeets from Vietnam (66.7%, 95% CI 30.0–90.3%) and Timneh parrots seized in Western Africa (62.5%, 95% CI 30.6–86.3%) were also positive for BFDV.

2.4.1 BFDV in Western Africa

The ML phylogeny (Figure 2.2) showed possible multiple introductions of BFDV to Western Africa. Viral variants isolated from wild Rose-ringed parakeets in Senegal formed a monophyletic clade with the single positive individual seized from illegal trade in Western Africa. In contrast, the

sequences isolated from Timneh parrots confiscated during the same seizure incident and housed in an adjacent enclosure to the Rose-ringed parakeets were more closely related to those identified in a captive African grey parrot and Blue-and-yellow macaw from Taiwan (Figure 2.2, Supplementary Table 2.1). Isolates from wild Rose-ringed parakeets from southern Asia and the captive wild-caught individual from The Gambia were found to be closely related (Figure 2.2, Supplementary Table 2.1).

2.4.2 BFDV on the Indian Ocean Islands and in the UK

Isolates from Rose-ringed parakeets on the Seychelles and those in introduced Rose-ringed parakeets in Greater London were the most closely related (Figure 2.2). These sequences were distantly related to the two isolates available from captive parrots from the United Kingdom, which instead clustered into a diverse clade of isolates obtained from captive hosts across Europe, the United States, Oceania, and Southern and Southeast Asia (Figure 2.2, Supplementary Table 2.1). The BFDV isolates in both native Mauritius parakeets and invasive Rose-ringed parakeets on Mauritius formed a monophyletic clade with little genetic variation, consistent with a single-introduction founder effect. This Mauritius clade was sister to both the clade of isolates from wild Grey-headed parakeets in Vietnam and those obtained from wild Crimson Rosellas (*Platycercus elegans*) in Australia.

2.4.3 BFDV in Southern and Southeastern Asia

The majority of the isolates obtained from Rose-ringed parakeets in their Asian native range, from both Pakistan and Bangladesh, were most closely related to one another and to the aforementioned isolate from a wild-caught captive individual from Western African (Figure 2.2). Conversely, the isolates obtained from Grey-headed parakeets in Vietnam clustered into a monophyletic clade.

2.4.4 Wider phylogeographical patterns

The BFDV *rep* gene phylogenetic tree consisted of a high proportion of clades that were monophyletic by location (>70% branch support) and had founder-effect type low genetic variation, including groups of isolates from captive flocks in Thailand and a number of captive and wild host clades from Australia, Brazil, New Caledonia, and New Zealand (Figure 2.2). Sequences from captive hosts in Italy, Poland, South Africa, Japan, and Australia were widely dispersed throughout the phylogeny, which suggested multiple introductions of BFDV to these countries. The distribution of BFDV isolates from captive and wild parrots in New Caledonia differed substantially, which suggested the virus in captive populations was likely introduced from European captive stocks, whereas the strain in wild populations was instead most closely related to isolates from Australia and New Zealand.

2.5 DISCUSSION

We report the presence of BFDV in wild populations from eight countries where the virus had not been detected previously, showing the virus is more widespread than currently recognized and may pose a risk to several threatened species. We also found the first record of BFDV in wild Rose-ringed parakeets within their African and Asian native ranges and in Grey-headed parakeets in Southeastern Asia, invasive Rose-ringed parakeets in the Seychelles and Japan, and wild parrots in trade within Africa. Our phylogenetic analysis revealed multiple introduction events to Western Africa and close phylogenetic relationships between sequences from wild populations across geographically distinct global regions. These findings suggest the global trade in live birds and the establishment of invasive populations play a key role in the spread of infectious disease.

2.5.1 Conservation implications for infected native host populations

The relationship between the spread of BFDV and the global pet trade is most evident in Western Africa. Specifically, this influence can be seen in the identification of a BFDV isolate from The Gambia clustering with those originating from Southern Asia, and only distantly related to those isolated from neighboring Senegal. Because this isolate was detected in a wild-harvested captive individual, it is unknown whether infection occurred prior to its capture or in captivity. This finding emphasizes the need for further intensive sampling of wild parrot populations in this region as The Gambia is geographically encompassed by Senegal and the native distribution of Rose-ringed parakeets extends through both countries (BirdLife International 2016). Therefore, these isolates would be expected to form a single clade.

The presence of markedly different BFDV strains in the Rose-ringed parakeet and Timneh parrots seized from illegal trafficking is noteworthy because both were housed in high-density enclosures at a single wildlife trader's holding facility. Despite their close proximity, it appears horizontal transmission did not occur and that these birds became infected with BFDV from at least two different sources. None of these birds showed clinical signs of disease when examined by an experienced avian veterinarian. The similarity between the isolate from the Rose-ringed parakeet from this seizure and those from wild populations in Senegal suggests that either this individual became infected prior to capture or that wild parakeets in Senegal may have become infected by BFDV-positive parakeets that escaped captivity.

It is of conservation concern that multiple variants of BFDV occur in Western Africa because this could increase the risk of formation of novel, highly virulent strains through viral recombination (B. Jackson *et al.* 2015; Julian *et al.* 2013). Grey and Timneh parrots are among the most traded of all CITES-listed birds (Martin 2018a, b), and increased restrictions on their international movement due to their recent listing on CITES Appendix I may help limit the spread of BFDV. However, Rose-ringed parakeets are abundant across their native range and their population sizes are increasing

(BirdLife International 2016). The confirmed presence of BFDV in these hosts highlights a risk of spill over into other sympatrically distributed species that are susceptible to PBFD (Varsani *et al.* 2011; Fogell, Martin and Groombridge 2016), such as globally endangered Grey (*Psittacus erithacus*) and Timneh parrots (BirdLife International 2012; BirdLife International 2017b).

Asia has 112 parrot species, of which approximately 15% are listed on the IUCN Red List of threatened species (IUCN 2016). Over 50% of these species are declining (IUCN 2016), and little research has been conducted on the presence of BFDV in wild Asian hosts, except for a single Red Lory (*Eos bornea*) sampled from Indonesia (Sarker *et al.* 2013). As noted with infected species in Australia (Sarker *et al.* 2015), Rose-ringed parakeets in Asia appear to be endemically infected at high prevalence within a monophyletic clade, making them an abundant reservoir host. The identification of BFDV in Bangladesh and Pakistan highlights the risk of spillover into vulnerable sympatric species such as Red-breasted parakeets (*Psittacula alexandri*) and Blossom-headed parakeets (*Psittacula roseata*). The identification of BFDV in Grey-headed parakeets in Vietnam is also of conservation concern because their populations are declining due to trapping for the bird trade and widespread habitat loss, which have resulted in their up-listing from *Least concern* to *Near threatened* on the IUCN Red List of Threatened Species in 2013 (BirdLife International 2017a).

2.5.2 Patterns of viral host switching

The close relationship between BFDV *rep* sequences from the Seychelles and Rose-ringed parakeets from the United Kingdom is notable, as phylogenetic analysis suggests this invasive population is of Southern Asian ancestry (H. Jackson *et al.* 2015); therefore, it is expected that BFDV would be introduced from the same region. However, since establishment of the invasive population in 1996, there have been five CITES-listed imports of psittacines to the Seychelles (CITES 2016), and anecdotal reports of a feral Sulphur-crested Cockatoo (*Cacatua galerita*) on Mahé (N. Bunbury, personal communication). Any of these or imports of other non-CITES-listed parrot species into the Seychelles could have introduced BFDV, posing a high risk to the small remaining endemic population of Seychelles Black parrots on Praslin. Both inferences that BFDV is spread through trade and that the virus displays host generality are supported by the relationship between this UK–Seychelles clade and the clade of isolates derived from Polish, South African, and Brazilian Old and New World parrots.

Our results suggest a single introduction of BFDV to Mauritius, and this strain is shared by the native Mauritius parakeets and invasive Rose-ringed parakeets. Since the introduction of BFDV to Mauritius, there has been some diversification. Isolates present in more recent samples from both parakeet populations differ from those in Mauritius parakeets when PBFD was first observed in 1994. The Mauritius parakeet is the last remaining of ten Mascarene Island parrot species (Hume 2007) and has only recently recovered from a bottleneck of fewer than 20 known individuals (Duffy

1993). An outbreak of BFDV in 2005 caused the failure of a translocation attempt for further population recovery (Tollington *et al.* 2013) and decreased hatching success (Tollington *et al.* 2015). Despite the concerns of conservation managers when PBFD was first detected, Mauritius parakeets have continued to recover. Nevertheless, as with the risk to the Seychelles Black parrot, the pet bird trade substantially increases the likelihood of introducing novel or recombinant BFDV variants that may have higher pathogenicity than the strain currently in Mauritius.

The virus is highly prevalent in captive-breeding facilities (Julian *et al.* 2013), which are a large source of pet birds exported internationally and a likely source of infection worldwide (Harkins *et al.* 2014). The virus also has the potential to substantially impact the pet bird trade economically. For example, it was estimated that in the past commercial aviculturists in South Africa lost up to 20% of their flocks to PBFD annually (Heath *et al.* 2004). However, the benefits of conserving global parrot biodiversity within their native ranges and managing infectious disease within these populations extend far beyond their captive market value. Rose-ringed parakeets have established invasive populations across Europe (BirdLife International 2012; Jackson et al. 2015b), and, given that captive parrots in Germany, Portugal, Spain, Italy, and Poland have tested positive for BFDV (De Kloet & De Kloet 2004; Raue et al. 2004; Julian et al. 2013), the virus is presumably also present in other European wild flocks outside the United Kingdom. Although the presence of BFDV in invasive populations across Europe poses little direct threat to wild parrot populations globally, it is valuable epidemiological data and will aid the identification of viral movement pathways and guide the development of national policies (Harkins *et al.* 2014).

The absence of BFDV in samples from wild Rose-ringed parakeets in South Africa is likely due to the inadequacies of small sample sizes. Subsequent to the collection of these feather samples, clinical signs of PBFD were observed in Rose-ringed parakeets in Randburg (C. Symes and D. Hernández Brito, personal communication). It is possible that these signs are not linked to PBFD or that the sampled feathers were grown in prior to the establishment of novel infection in the population. The virus is already present in endemic Cape parrots (*Poicephalus robustus*) in eastern South Africa (Regnard *et al.* 2014), and, although the distribution of Rose-ringed parakeets in South Africa does not yet overlap with that of Cape parrots, their rapid population growth may soon increase the risk of introducing a novel strain to an already-infected *Vulnerable* endemic species. Consequently, we recommend more intensive surveillance of invasive Rose-ringed parakeet populations in South Africa.

2.5.3 Value of large-scale BFDV surveillance

Our results illustrate the value of disease screening samples gathered for genetic studies or over the course of long-term population monitoring. However, data sets comprising a large number of random samples are required to support the absence of infection with statistical confidence

(DiGiacomo & Koepsell 1986). It should also be considered that BFDV detection is improved by using multiple sample types (e.g. Raue et al. 2004; Robino et al. 2014). Feathers typically produce low DNA yields, particularly those that have been cut off from the blood supply once fully grown (De Volo *et al.* 2008). Blood or muscle tissue samples, however, can produce high-quality, high-concentration DNA extracts (D. Fogell Pers. Obs.), but BFDV may be undetectable in the blood, whilst virions are still present in feathers or shed in feces (Hess, Scope and Heincz 2004). Therefore, in the case of long-term population studies, mixed sampling regimes may provide more robust assessments of global or regional infection occurrence and allow for estimates of prevalence in entire populations.

The first detection of BFDV in wild parrots native to Southern and Southeast Asia and Western Africa highlights the need for further research in these regions and has implications for the conservation of vulnerable sympatric species. Most of the African continent is data deficient for BFDV presence because, to our knowledge, no screening of wild populations has occurred outside southern Africa (Fogell, Martin and Groombridge 2016). Similarly, little work has been conducted in Asia outside Southeastern Asian cockatoo species. Many of our results were obtained from opportunistic samples, rather than through systematic random sampling designed to provide statistical and epidemiological confidence. As noted with Rose-ringed parakeets in South Africa, these samples may therefore not provide a current picture of geographic occurrence of BFDV. Further screening of wild parrot populations would provide better insight into where BFDV occurs globally. This information could be used to inform conservation and management and provide a foundation for advanced studies of host immunity and susceptibility to infection.

We emphasize that dissemination of both BFDV-positive and -negative screening results are required due to the evidence that some species, such as Cockatiels (*Nymphicus hollandicus*), may be less susceptible to infection (Shearer *et al.* 2008). It should also be considered that the presence of infection is not always reflected in clinical signs of disease (McCallum and Dobson 2008). Therefore, once infection within a wild population is detected, the clinical signs and severity of PBFD should be noted because they differ among species. For example, diseased Mauritius parakeets do not present with beak deformities (D. Fogell, personal observation). Despite the more thoroughly documented presence of BFDV in threatened wild native parrot populations in South Africa (Regnard *et al.* 2014), Mauritius (Kundu *et al.* 2012), New Zealand (B. Jackson *et al.* 2015), and Australia (Peters *et al.* 2014), the interspecific variation and long-term population impacts of PBFD are still largely unknown. Conservationists therefore need to apply a precautionary principle when managing populations at risk of infection with BFDV until risks to individual populations are better assessed.

Our data provide support for a global assessment of captive-breeding activities and strict regulation of the trade and import of parrots (H. Jackson *et al.* 2015). We suggest decisions concerning the movements of parrots should include a disease risk analysis, evaluating the probability of previous exposure or infection and the potential risk posed to wild populations. It is particularly important that these risks to biosecurity are considered in regions of high conservation importance, both for threatened parrots and other avian taxa at risk of infection (e.g. Sarker et al. 2015b; Amery-Gale et al. 2017). Screening for BFDV through standard and real-time PCR is quick and easy, and the evidence-base for decisions will be improved with additional information on the extent of viral distribution and transmission pathways. We therefore recommend that consideration be given to the systematic screening of parrots in legal and illegal trade and urge conservation practitioners, parrot breeders, enforcement agencies and others who work with threatened parrots to increase efforts to sample wild and captive parrot populations globally.

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2.7 TABLES AND FIGURES

Table 2.1 parrot host species, country or region of origin, sample type used, and Genbank accession numbers of samples screened for Beak and feather disease virus (BFDV).

Country or Region	**Sampling location**	**Species**	**Common name**	**Native or invasive**	**Wild or captive**	**No. of individuals tested**	**Positive for BFDV (%) (95% CI)**	**Sample tissue**	**Sampling year**	**Accession no.**
Bangladesh	26.270869; 88.595175	*Psittacula krameri*	Rose-ringed parakeet	native	wild	29	100 (88.3 – 100)	blood	2013	KT725792 – 95; KX641203 – 27
The Gambia	13.6666; -15.05	*Psittacula krameri*	Rose-ringed parakeet	native	captive	3	100 (43.9 – 100)	blood	2014	KT725790
Germany	49.39381; 8.6952	*Psittacula krameri*	Rose-ringed parakeet	invasive	wild	20	0 (0 – 16.1)	blood	2007 - 2010	
Japan	35.689488; 139.69171	*Psittacula krameri*	Rose-ringed parakeet	invasive	wild	15	6.7 (1.2 – 29.8)	feather	2015	
Mauritius	-20.36937; 57.40602	*Psittacula krameri*	Rose-ringed parakeet	invasive	wild	31	16.1 (7.1 – 32.6)	blood	2009 – 2011	KT753489 - 93
	-20.38473; 57.44451	*Psittacula eques*	Mauritius parakeet	native	wild	894	26.1 (23.3 – 29.0)	blood	1994 - 2016	KT753401 - 88, KT753494 – 526; KX641202; KX641228 – 32

Nigeria	9.92849; 8.89212	*Psittacula krameri*	Rose-ringed parakeet	native	wild	11	9.1 (1.6 – 37.7)	blood	2014	
Pakistan	33.242722; 73.225929	*Psittacula krameri*	Rose-ringed parakeet	native	wild	14	71.4 (45.4 – 88.3)	blood	2014	KT725800 – 03; KX641233 – 39
Senegal	14.6937; -17.44406	*Psittacula krameri*	Rose-ringed parakeet	native	wild	10	50 (23.7 – 76.3)	blood	2014	KT725796 - 99
Seychelles	-04.6300222; 55.4568139	*Psittacula krameri*	Rose-ringed parakeet	invasive	wild	23	47.8 (29.2 – 67.0)	muscle[a]	2014	KU888682 – 83, MF669120 – 23, MF681683
	-04.330056; 55.73839	*Coracopsis barklyi*	Black parrot	native	wild	24	0 (0 – 13.8)	blood	2009 - 2012	
South Africa	-26.12346; -28.00836	*Psittacula krameri*	Rose-ringed parakeet	invasive	wild	4	0 (0 – 49.0)	feather	2015	
United Kingdom	51.4352361; 00.3325417	*Psittacula krameri*	Rose-ringed parakeet	invasive	wild	6[b]/ 2[c]	0 (0 – 39.0)[b] / na[c]	feather[b] / feather follicle[c]	2013 - 2015	KT725791, KU888693
Vietnam	19.0636028; 104.7520944	*Psittacula finschii*	Grey-headed parakeet	native	wild	6	66.7 (30.0 – 90.3)	blood	2015	KU888690 - 93
Western Africa[d]		*Psittacula krameri*	Rose-ringed parakeet	native	captive	5	20 (3.6 – 62.5)	blood	2015	KU888684

	Psittacus timneh	Timneh parrot	native	captive	8	62.5 (30.6 – 86.3)	blood	2015	KU888685 - 89

[a] Samples obtained post-mortem.

[b] Samples obtained from live birds in Kent, UK.

[c] Non-random samples obtained postmortem from Psittacine beak and feather disease diagnosed parakeets in Greater London, UK.

[d] Samples obtained from parrots seized by trade authorities

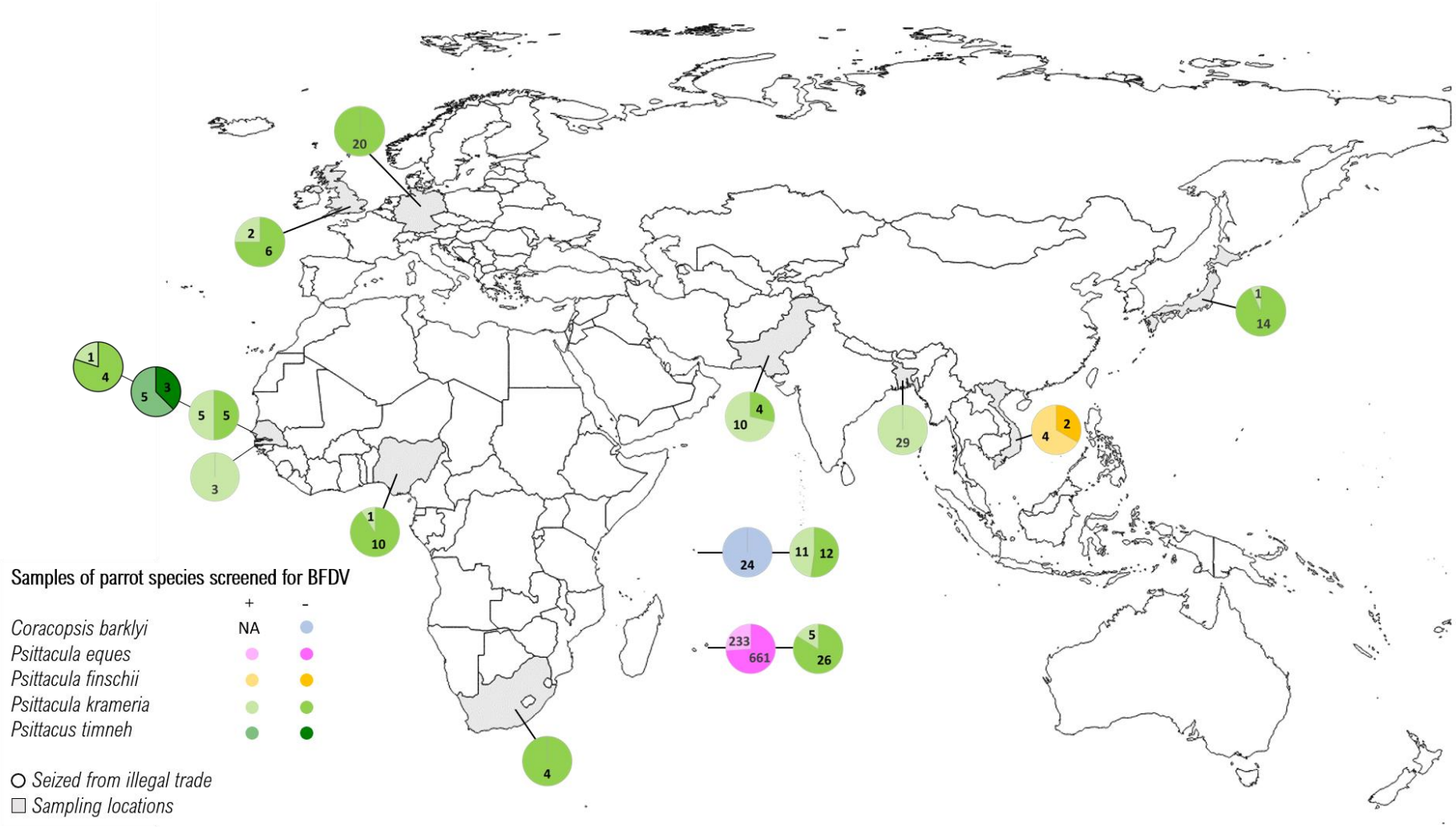

Figure 2.1 Sampling locations of parrot species screened for *Beak and feather disease virus* (BFDV) and the number of individuals testing positive and negative in each study location.

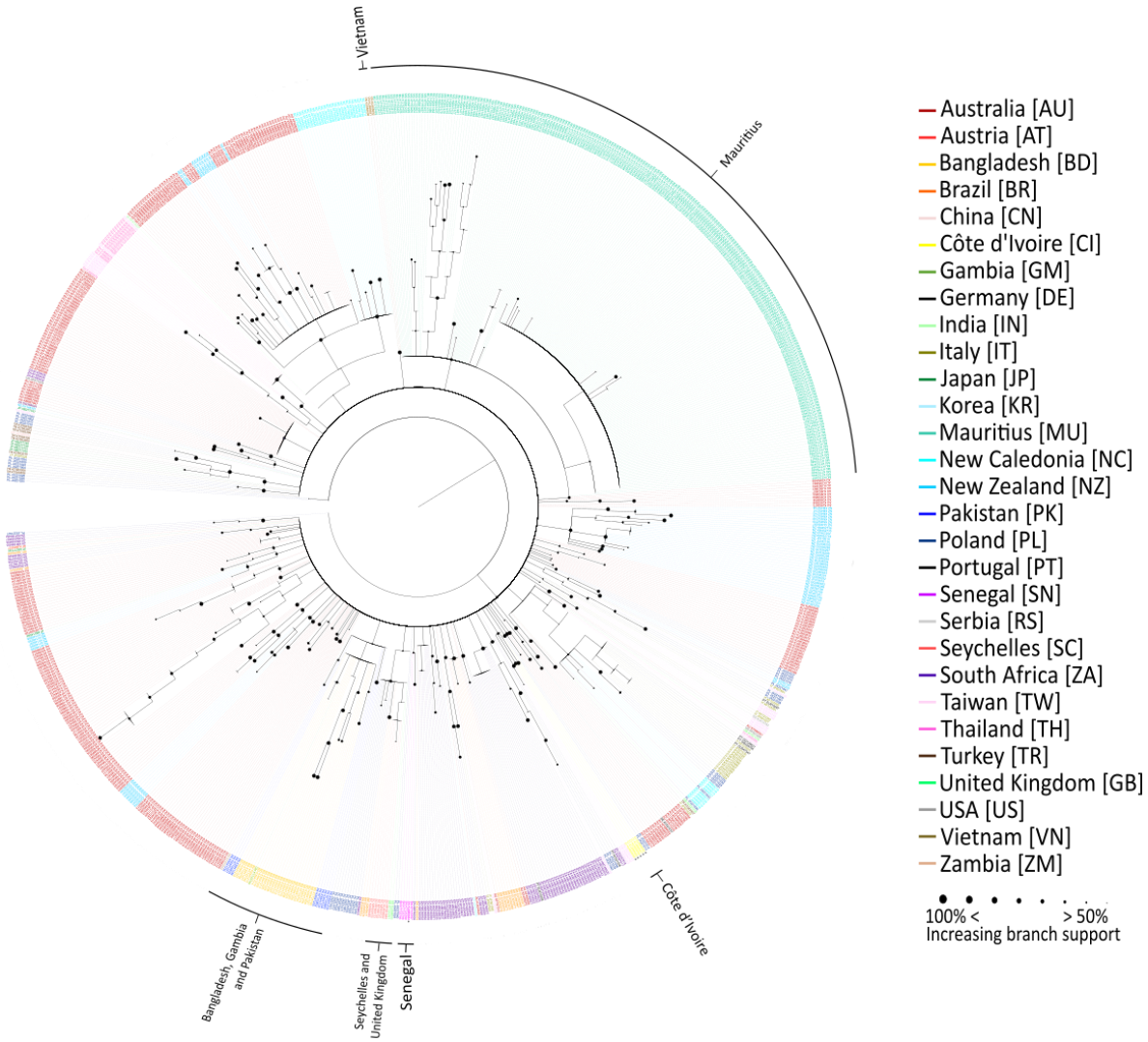

Figure 2.2 Maximum likelihood phylogenetic tree denoting relationships between Beak and feather disease virus (BFDV) *rep* sequences. Variants sequenced for this study are highlighted and labelled, and sequences derived from birds in trade are marked with an asterisk. Branches with <50% branch support are collapsed and branch support is indicated with proportionally increasing filled circles. Branches are coloured based on country of sampling as denoted in the key.

2.8 SUPPLEMENTARY INFORMATION

Supplementary Table 2.1 Details of all global BFDV Rep sequences obtained from Genbank and analysed in this study.

Accession #	Country	Species	Accession #	Country	Species	Accession #	Country	Species
AJ605577	AT	*Melopsittacus undulatus*	KM823543	AU	*Merops ornatus*	KF768552	NC	*Trichoglossus haematodus deplanchii*
AF080560	AU	*Cacatua galerita*	KM823544	AU	*Merops ornatus*	KF768553	NC	*Trichoglossus haematodus deplanchii*
AF311295	AU	*Northiella haematogaster*	KM823545	AU	*Merops ornatus*	AY148285	NZ	*Cacatua galerita*
AF311296	AU	*Agapornis roseicollis*	KM823546	AU	*Merops ornatus*	AY148287	NZ	*Cacatua galerita*
AF311297	AU	*Cacatua tenuirostris*	KM823547	AU	*Merops ornatus*	AY148288	NZ	*Cacatua galerita*
AF311298	AU	*Eolophus roseicapilla*	KM823548	AU	*Merops ornatus*	AY148289	NZ	*Cacatua tenuirostris*
AF311299	AU	*Trichogolossus haematodus*	KM887916	AU	*Trichoglossus haematodus*	AY148290	NZ	*Cacatua galerita*
AF311300	AU	*Cacatua leadbeateri*	KM887917	AU	*Glossopsitta concinna*	AY148291	NZ	*Trichoglossus rubritorquis*
AF311301	AU	*Cacatua galerita*	KM887918	AU	*Trichoglossus haematodus*	AY148292	NZ	*Lorius chlorocercus*

Accession #	Country	Species	Accession #	Country	Species	Accession #	Country	Species
AF311302	AU	*Cacatua galerita*	KM887919	AU	*Trichoglossus haematodus*	AY148293	NZ	*Trichogolossus haematodus*
DQ016388	AU	*Trichogolossus haematodus*	KM887920	AU	*Trichoglossus chlorolepidotus*	AY148294	NZ	*Trichogolossus haematodus*
DQ016389	AU	*Trichogolossus haematodus*	KM887921	AU	*Trichoglossus chlorolepidotus*	AY148295	NZ	*Trichogolossus haematodus*
DQ016390	AU	*Lathamus discolor*	KM887922	AU	*Trichoglossus haematodus*	AY148296	NZ	*Eos reticulata*
DQ016391	AU	*Lathamus discolor*	KM887923	AU	*Trichoglossus haematodus*	AY148297	NZ	*Eos reticulata*
DQ016392	AU	*Trichogolossus haematodus*	KM887924	AU	*Trichoglossus chlorolepidotus*	AY148298	NZ	*Psitteuteles goldiei*
DQ016393	AU	*Glossopsitta concinna*	KM887925	AU	*Trichoglossus haematodus*	AY148299	NZ	*Lorius chlorocercus*
DQ016394	AU	*Trichogolossus haematodus*	KM887926	AU	*Trichoglossus haematodus*	AY148300	NZ	*Trichogolossus haematodus*
DQ016395	AU	*Trichoglossus rubritorquis*	KM887927	AU	*Trichoglossus haematodus*	AY148301	NZ	*Melopsittacus undulatus*

Accession #	Country	Species	Accession #	Country	Species	Accession #	Country	Species
DQ016396	AU	*Trichogolossus haematodus*	KM887928	AU	*Trichoglossus haematodus*	GQ396652	NZ	*Cyanoramphus novaezelandiae*
EF457974	AU	*Nymphicus hollandicus*	KM887929	AU	*Trichoglossus haematodus*	GQ396653	NZ	*Cyanoramphus novaezelandiae*
EF457975	AU	*Nymphicus hollandicus*	KM887930	AU	*Trichoglossus haematodus*	GQ396654	NZ	*Cyanoramphus novaezelandiae*
JX049195	AU	*Trichogolossus haematodus*	KM887931	AU	*Trichoglossus haematodus*	GQ396655	NZ	*Cyanoramphus novaezelandiae*
KC693651	AU	*Neophema chrysogaster*	KM887932	AU	*Trichoglossus haematodus*	GQ396656	NZ	*Cyanoramphus novaezelandiae*
KC693652	AU	*Neophema chrysogaster*	KM887933	AU	*Trichoglossus haematodus*	GU936287	NZ	*Platycercus eximius*
KC693653	AU	*Neophema chrysogaster*	KM887934	AU	*Trichoglossus haematodus*	GU936288	NZ	*Cyanoramphus novaezelandiae*
KF188681	AU	*Neophema chrysogaster*	KM887935	AU	*Trichoglossus haematodus*	GU936289	NZ	*Cyanoramphus novaezelandiae*
KF188682	AU	*Neophema chrysogaster*	KM887936	AU	*Trichoglossus haematodus*	GU936290	NZ	*Cyanoramphus novaezelandiae*
KF188683	AU	*Neophema chrysogaster*	KM887937	AU	*Trichoglossus haematodus*	GU936291	NZ	*Cyanoramphus novaezelandiae*
KF188684	AU	*Neophema chrysogaster*	KM887938	AU	*Trichoglossus haematodus*	GU936292	NZ	*Cyanoramphus novaezelandiae*
KF188685	AU	*Neophema chrysogaster*	KM887939	AU	*Trichoglossus haematodus*	GU936293	NZ	*Cyanoramphus novaezelandiae*

Accession #	Country	Species	Accession #	Country	Species	Accession #	Country	Species
KF188686	AU	*Neophema chrysogaster*	KM887940	AU	*Trichoglossus haematodus*	GU936294	NZ	*Cyanoramphus novaezelandiae*
KF188687	AU	*Neophema chrysogaster*	KM887941	AU	*Trichoglossus chlorolepidotus*	GU936295	NZ	*Cyanoramphus novaezelandiae*
KF188688	AU	*Neophema chrysogaster*	KM887942	AU	*Trichoglossus haematodus*	GU936296	NZ	*Cyanoramphus novaezelandiae*
KF188689	AU	*Neophema chrysogaster*	KM887943	AU	*Trichoglossus haematodus*	GU936297	NZ	*Cyanoramphus novaezelandiae*
KF188690	AU	*Neophema chrysogaster*	KM887944	AU	*Trichoglossus chlorolepidotus*	JF519618	NZ	*Cyanoramphus novaezelandiae*
KF188691	AU	*Neophema chrysogaster*	KM887945	AU	*Trichoglossus haematodus*	JF519619	NZ	*Platycercus eximius*
KF188692	AU	*Neophema chrysogaster*	KM887946	AU	*Trichoglossus chlorolepidotus*	JQ782196	NZ	*Platycercus eximius*
KF188693	AU	*Neophema chrysogaster*	KM887947	AU	*Melopsittacus undulatus*	JQ782197	NZ	*Platycercus eximius*
KF188694	AU	*Neophema chrysogaster*	KM887948	AU	*Melopsittacus undulatus*	JQ782198	NZ	*Platycercus eximius*
KF188695	AU	*Neophema chrysogaster*	KM887949	AU	*Melopsittacus undulatus*	JQ782199	NZ	*Platycercus eximius*
KF188696	AU	*Neophema chrysogaster*	KM887950	AU	*Melopsittacus undulatus*	JQ782200	NZ	*Platycercus eximius*

Accession #	Country	Species	Accession #	Country	Species	Accession #	Country	Species
KF188697	AU	*Neophema chrysogaster*	KM887951	AU	*Melopsittacus undulatus*	JQ782201	NZ	*Cyanoramphus auriceps*
KF188698	AU	*Neophema chrysogaster*	KM978921	AU	*Trichoglossus haematodus*	JQ782202	NZ	*Cyanoramphus auriceps*
KF188699	AU	*Neophema chrysogaster*	KM978922	AU	*Trichoglossus haematodus*	JQ782203	NZ	*Cyanoramphus auriceps*
KF188700	AU	*Neophema chrysogaster*	KM978923	AU	*Trichoglossus haematodus*	JQ782204	NZ	*Cyanoramphus auriceps*
KF188701	AU	*Neophema chrysogaster*	KP795105	AU	*Trichoglossus haematodus*	JQ782205	NZ	*Cyanoramphus auriceps*
KF188702	AU	*Neophema chrysogaster*	KP795106	AU	*Trichoglossus haematodus*	JQ782206	NZ	*Cyanoramphus auriceps*
KF188703	AU	*Neophema chrysogaster*	KT008265	AU	*Ninox strenua*	JQ782207	NZ	*Cyanoramphus auriceps*
KF197006	AU	*Neophema chrysogaster*	KT008266	AU	*Cacatua galerita*	JQ782208	NZ	*Cyanoramphus auriceps*
KF197007	AU	*Neophema chrysogaster*	EU093967	BR	*Serinus canaria*	KF467251	NZ	*Platycercus eximius*
KF197008	AU	*Neophema chrysogaster*	EU093968	BR	*Serinus canaria*	KF467252	NZ	*Platycercus eximius*
KF197009	AU	*Neophema chrysogaster*	EU093969	BR	*Melopsittacus undulatus*	KF467253	NZ	*Platycercus eximius*
KF197010	AU	*Neophema chrysogaster*	EU093970	BR	*Serinus canaria*	KF467254	NZ	*Platycercus eximius*
KF197011	AU	*Neophema chrysogaster*	EU093971	BR	*Amazona amazonica*	KM452734	NZ	*Cyanoramphus novaezelandiae*
KF197012	AU	*Neophema chrysogaster*	EU093972	BR	*Psittacula krameri*	KM452735	NZ	*Cyanoramphus novaezelandiae*

Accession #	Country	Species	Accession #	Country	Species	Accession #	Country	Species
KF197013	AU	*Neophema chrysogaster*	EU093973	BR	*Amazona aestiva*	KM452736	NZ	*Cyanoramphus novaezelandiae*
KF197014	AU	*Neophema chrysogaster*	EU093974	BR	*Psittacula krameri*	KM452737	NZ	*Cyanoramphus novaezelandiae*
KF197015	AU	*Neophema chrysogaster*	EU093976	BR	*Guarouba guarouba*	KM452738	NZ	*Cyanoramphus novaezelandiae*
KF197017	AU	*Neophema chrysogaster*	EU093977	BR	*Guarouba guarouba*	KM452739	NZ	*Cyanoramphus novaezelandiae*
KF197018	AU	*Neophema chrysogaster*	EU093978	BR	*Amazona aestiva*	KM452740	NZ	*Cyanoramphus novaezelandiae*
KF197019	AU	*Neophema chrysogaster*	EU093979	BR	*Melopsittacus undulatus*	KM452741	NZ	*Cyanoramphus novaezelandiae*
KF197020	AU	*Neophema chrysogaster*	JQ649409	BR	*Amazona aestiva*	KM452742	NZ	*Cyanoramphus novaezelandiae*
KF197021	AU	*Neophema chrysogaster*	JQ649410	BR	*Amazona aestiva*	KM452743	NZ	*Cyanoramphus novaezelandiae*
KF197022	AU	*Neophema chrysogaster*	JQ649411	BR	*Psittacula krameri*	KM452744	NZ	*Cyanoramphus novaezelandiae*
KF197023	AU	*Neophema chrysogaster*	GQ386944	CN	*Melopsittacus undulatus*	AY521236	PL	*Psittacus erithacus*
KF385399	AU	*Calyptorhynchus banksii*	AY521237	DE	*Psittacus erithacus*	EU810207	PL	*Psittacus erithacus*
KF385400	AU	*Calyptorhynchus banksii*	KF673337	ID	*Eos bornea*	EU810208	PL	*Psittacus erithacus*
KF385401	AU	*Callocephalon fimbriatum*	JF501523	IT	*Aratinga solstitialis*	GQ120621	PL	*Psittacus erithacus*
KF385402	AU	*Callocephalon fimbriatum*	JF501524	IT	*Melopsittacus undulatus*	GQ329705	PL	*Psittacus erithacus*

Accession #	Country	Species	Accession #	Country	Species	Accession #	Country	Species
KF385403	AU	*Callocephalon fimbriatum*	JF501525	IT	*Agapornis roseicollis*	GU047347	PL	*Psittacus erithacus*
KF385404	AU	*Callocephalon fimbriatum*	JF501526	IT	*Agapornis roseicollis*	JX221001	PL	*Psittacula krameri*
KF385405	AU	*Callocephalon fimbriatum*	JF501527	IT	*Cacatua alba*	JX221002	PL	*Psittacula krameri*
KF385406	AU	*Cacatua leadbeateri*	JF501528	IT	*Psittacus erithacus*	JX221003	PL	*Psittacula krameri*
KF385407	AU	*Cacatua leadbeateri*	JF501529	IT	*Psittacus erithacus*	JX221004	PL	*Melopsittacus undulatus*
KF385408	AU	*Calyptorhynchus lathami*	JF501530	IT	*Psittacus erithacus*	JX221005	PL	*Melopsittacus undulatus*
KF385409	AU	*Calyptorhynchus lathami*	JF501531	IT	*Psittacus erithacus*	JX221006	PL	*Platycercus elegans*
KF385410	AU	*Calyptorhynchus lathami*	JF501532	IT	*Psittacus erithacus*	JX221007	PL	*Psittacula krameri*
KF385411	AU	*Calyptorhynchus lathami*	JF827600	IT	*Psittacus erithacus*	JX221008	PL	*Psittacula krameri*
KF385412	AU	*Calyptorhynchus lathami*	JF827601	IT	*Psittacus erithacus*	JX221009	PL	*Melopsittacus undulatus*
KF385413	AU	*Cacatua galerita*	KF723384	IT	*Psittacus erithacus*	JX221010	PL	*Psittacula krameri*
KF385414	AU	*Cacatua galerita*	KF723385	IT	*Psittacus erithacus*	JX221011	PL	*Psittacula krameri*
KF385415	AU	*Cacatua galerita*	KF723386	IT	*Psittacus erithacus*	JX221012	PL	*Melopsittacus undulatus*
KF385416	AU	*Cacatua galerita*	KF723387	IT	*Psittacus erithacus*	JX221013	PL	*Poicephalus robustus*

Accession #	Country	Species	Accession #	Country	Species	Accession #	Country	Species
KF385417	AU	*Cacatua galerita*	KF723388	IT	*Psittacus erithacus*	JX221014	PL	*Melopsittacus undulatus*
KF385418	AU	*Cacatua galerita*	KF723389	IT	*Psittacus erithacus*	JX221015	PL	*Aprosmictus erythropterus*
KF385419	AU	*Cacatua galerita*	KF723390	IT	*Psittacus erithacus*	JX221016	PL	*Aprosmictus erythropterus*
KF385420	AU	*Cacatua tenuirostris*	KF723391	IT	*Psittacus erithacus*	JX221017	PL	*Psittacula krameri*
KF385421	AU	*Cacatua tenuirostris*	KF723392	IT	*Psittacus erithacus*	JX221018	PL	*Psittacus erithacus*
KF385422	AU	*Cacatua tenuirostris*	KF723393	IT	*Psittacus erithacus*	JX221019	PL	*Psittacula krameri*
KF385423	AU	*Cacatua tenuirostris*	AB277746	JP	*Melopsittacus undulatus*	JX221020	PL	*Psittacus erithacus*
KF385424	AU	*Cacatua tenuirostris*	AB277747	JP	*Melopsittacus undulatus*	JX221021	PL	*Amazona amazonica*
KF385425	AU	*Cacatua tenuirostris*	AB277748	JP	*Melopsittacus undulatus*	JX221022	PL	*Psittacus erithacus*
KF385426	AU	*Cacatua tenuirostris*	AB277749	JP	*Melopsittacus undulatus*	JX221023	PL	*Psittacus erithacus*
KF385427	AU	*Cacatua tenuirostris*	AB277750	JP	*Melopsittacus undulatus*	JX221024	PL	*Forpus coelestis*
KF385428	AU	*Cacatua tenuirostris*	AB277751	JP	*Melopsittacus undulatus*	JX221025	PL	*Cacatua alba*
KF385429	AU	*Cacatua tenuirostris*	AB514568	JP	*Neophema chrysogaster*	JX221026	PL	*Melopsittacus undulatus*
KF385430	AU	*Eolophus roseicapilla*	KM409545	KR	*Ara ararauna*	JX221027	PL	*Melopsittacus undulatus*

Accession #	Country	Species	Accession #	Country	Species	Accession #	Country	Species
KF385431	AU	*Eolophus roseicapilla*	HQ641457	MU	*Psittacula krameri*	JX221028	PL	*Melopsittacus undulatus*
KF385432	AU	*Eolophus roseicapilla*	HQ641458	MU	*Psittacula krameri*	JX221029	PL	*Alisterus scapularis*
KF385433	AU	*Eolophus roseicapilla*	HQ641459	MU	*Psittacula krameri*	JX221030	PL	*Poicephalus senegalus*
KF385434	AU	*Eolophus roseicapilla*	HQ641460	MU	*Psittacula krameri*	JX221031	PL	*Poicephalus senegalus*
KF385435	AU	*Eolophus roseicapilla*	HQ641461	MU	*Psittacula krameri*	JX221032	PL	*Psittacus erithacus*
KF385436	AU	*Eolophus roseicapilla*	HQ641462	MU	*Psittacula krameri*	JX221033	PL	*Alisterus scapularis*
KF495566	AU	*Callocephalon fimbriatum*	HQ641463	MU	*Psittacula krameri*	JX221034	PL	*Melopsittacus undulatus*
KF495567	AU	*Calyptorhynchus lathami*	HQ641464	MU	*Psittacula krameri*	JX221035	PL	*Platycercus eximius*
KF495568	AU	*Cacatua leadbeateri*	HQ641465	MU	*Psittacula krameri*	JX221036	PL	*Psittacula eupatria*
KF495569	AU	*Cacatua galerita*	HQ641466	MU	*Psittacula krameri*	JX221037	PL	*Psittacus erithacus*
KF495570	AU	*Cacatua galerita*	HQ641467	MU	*Psittacula krameri*	JX221038	PL	*Psittacus erithacus*
KF495571	AU	*Cacatua galerita*	HQ641468	MU	*Psittacula krameri*	JX221039	PL	*Psittacus erithacus*
KF495572	AU	*Cacatua galerita*	HQ641469	MU	*Psittacula krameri*	JX221040	PL	*Amazona aestiva*
KF495573	AU	*Cacatua galerita*	HQ641470	MU	*Psittacula krameri*	JX221041	PL	*Psittacus erithacus*

Accession #	Country	Species	Accession #	Country	Species	Accession #	Country	Species
KF495574	AU	*Cacatua galerita*	HQ641471	MU	*Psittacula krameri*	JX221042	PL	*Psittacula eupatria*
KF495575	AU	*Cacatua galerita*	HQ641472	MU	*Psittacula krameri*	JX221043	PL	*Platycercus elegans*
KF495576	AU	*Cacatua galerita*	HQ641473	MU	*Psittacula krameri*	KJ413143	RS	Unknown
KF495577	AU	*Cacatua galerita*	HQ641474	MU	*Psittacula krameri*	FJ685978	TH	*Cacatua galerita*
KF495578	AU	*Cacatua galerita*	HQ641475	MU	*Psittacula krameri*	FJ685979	TH	*Cacatua sulphurea*
KF495579	AU	*Cacatua galerita*	HQ641476	MU	*Psittacula krameri*	FJ685980	TH	*Ara ararauna*
KF495580	AU	*Cacatua galerita*	HQ641477	MU	*Psittacula krameri*	FJ685985	TH	*Agapornis sp.*
KF495581	AU	*Cacatua galerita*	HQ641478	MU	*Psittacula krameri*	FJ685989	TH	*Cacatua moluccensis*
KF495582	AU	*Cacatua galerita*	HQ641479	MU	*Psittacula krameri*	GU015012	TH	*Psittacus erithacus*
KF495583	AU	*Cacatua galerita*	HQ641480	MU	*Psittacula krameri*	GU015013	TH	*Psittacus erithacus*
KF495584	AU	*Cacatua galerita*	HQ641481	MU	*Psittacula krameri*	GU015014	TH	*Psittacula eupatria*
KF495585	AU	*Cacatua galerita*	HQ641482	MU	*Psittacula krameri*	GU015015	TH	*Psittacula eupatria*
KF495586	AU	*Cacatua galerita*	HQ641483	MU	*Psittacula krameri*	GU015016	TH	*Psittacula eupatria*
KF495587	AU	*Cacatua galerita*	HQ641484	MU	*Psittacula krameri*	GU015017	TH	*Ara severus*

Accession #	Country	Species	Accession #	Country	Species	Accession #	Country	Species
KF495588	AU	*Cacatua galerita*	HQ641485	MU	*Psittacula krameri*	GU015018	TH	*Diopsittaca nobilis*
KF495589	AU	*Cacatua tenuirostris*	HQ641486	MU	*Psittacula krameri*	GU015019	TH	*Eclectus roratus*
KF495590	AU	*Cacatua tenuirostris*	HQ641487	MU	*Psittacula krameri*	GU015020	TH	*Eclectus roratus*
KF495591	AU	*Cacatua tenuirostris*	HQ641488	MU	*Psittacula krameri*	GU015021	TH	*Ara chloropterus*
KF495592	AU	*Cacatua tenuirostris*	HQ641489	MU	*Psittacula krameri*	GU015022	TH	*Probosciger aterrimus*
KF495593	AU	*Cacatua tenuirostris*	HQ641490	MU	*Psittacula krameri*	GU015023	TH	*Ara ambiguus*
KF495594	AU	*Cacatua tenuirostris*	HQ641491	MU	*Psittacula eques*	KT583302	TR	*Melopsittacus undulatus*
KF495595	AU	*Cacatua tenuirostris*	HQ641492	MU	*Psittacula eques*	KT583303	TR	*Melopsittacus undulatus*
KF495596	AU	*Cacatua tenuirostris*	HQ641493	MU	*Psittacula eques*	KT583304	TR	*Melopsittacus undulatus*
KF495597	AU	*Eolophus roseicapillus*	HQ641494	MU	*Psittacula eques*	KT583305	TR	*Melopsittacus undulatus*
KF495598	AU	*Eolophus roseicapillus*	HQ641495	MU	*Psittacula eques*	KT583306	TR	*Melopsittacus undulatus*
KF495599	AU	*Eolophus roseicapillus* x *Cacatua sanguinea*	HQ641496	MU	*Psittacula eques*	KT583307	TR	*Melopsittacus undulatus*
KF495600	AU	*Eolophus roseicapillus*	HQ641497	MU	*Psittacula eques*	KT583308	TR	*Melopsittacus undulatus*

Accession #	Country	Species	Accession #	Country	Species	Accession #	Country	Species
		x *Cacatua sanguinea*						
KF499120	AU	*Calyptorhynchus banksii*	HQ641498	MU	*Psittacula eques*	KT583309	TR	*Melopsittacus undulatus*
KF499121	AU	*Calyptorhynchus banksii*	HQ641499	MU	*Psittacula eques*	KT583310	TR	*Melopsittacus undulatus*
KF499122	AU	*Calyptorhynchus banksii*	HQ641500	MU	*Psittacula eques*	DQ304738	TW	*Eos reticulata*
KF499123	AU	*Calyptorhynchus banksii*	HQ641501	MU	*Psittacula eques*	DQ304739	TW	*Eos reticulata*
KF499124	AU	*Calyptorhynchus banksii*	HQ641502	MU	*Psittacula eques*	DQ304740	TW	*Psittacus erithacus*
KF499125	AU	*Calyptorhynchus banksii*	HQ641503	MU	*Psittacula eques*	DQ304741	TW	*Psittacus erithacus*
KF499126	AU	*Callocephalon fimbriatum*	HQ641504	MU	*Psittacula eques*	DQ304742	TW	*Ara ararauna*
KF499127	AU	*Callocephalon fimbriatum*	HQ641505	MU	*Psittacula eques*	DQ304743	TW	*Psittacus erithacus*
KF499128	AU	*Callocephalon fimbriatum*	HQ641506	MU	*Psittacula eques*	DQ304744	TW	*Amazona auropalliata*
KF499129	AU	*Callocephalon fimbriatum*	HQ641507	MU	*Psittacula eques*	DQ304745	TW	*Psittacus erithacus*
KF499130	AU	*Callocephalon fimbriatum*	HQ641508	MU	*Psittacula eques*	DQ304746	TW	*Psephotus haematonotus*
KF499131	AU	*Callocephalon fimbriatum*	HQ641509	MU	*Psittacula eques*	DQ304747	TW	Unknown
KF499132	AU	*Callocephalon fimbriatum*	HQ641510	MU	*Psittacula eques*	DQ304748	TW	Unknown

Accession #	Country	Species	Accession #	Country	Species	Accession #	Country	Species
KF499133	AU	*Callocephalon fimbriatum*	HQ641512	MU	*Psittacula eques*	DQ304749	TW	Unknown
KF499134	AU	*Callocephalon fimbriatum*	HQ641513	MU	*Psittacula eques*	DQ304750	TW	Unknown
KF499135	AU	*Cacatua galerita*	HQ641514	MU	*Psittacula eques*	DQ304751	TW	Unknown
KF499136	AU	*Cacatua tenuirostris*	HQ641515	MU	*Psittacula eques*	DQ304752	TW	Unknown
KF499137	AU	*Cacatua tenuirostris*	HQ641516	MU	*Psittacula eques*	DQ304753	TW	Unknown
KF499138	AU	*Cacatua tenuirostris*	HQ641517	MU	*Psittacula eques*	DQ304754	TW	Unknown
KF499139	AU	*Callocephalon fimbriatum*	HQ641518	MU	*Psittacula eques*	DQ304755	TW	Unknown
KF499140	AU	*Callocephalon fimbriatum*	HQ641519	MU	*Psittacula eques*	DQ304756	TW	Unknown
KF561250	AU	*Neophema chrysogaster*	HQ641520	MU	*Psittacula eques*	DQ304757	TW	Unknown
KF673335	AU	*Lathamus discolor*	HQ641521	MU	*Psittacula eques*	DQ304758	TW	Unknown
KF673336	AU	*Lathamus discolor*	HQ641522	MU	*Psittacula eques*	KC980909	TW	*Cacatua ophthalmica*
KF688548	AU	*Barnardius zonarius semitorquatus*	HQ641523	MU	*Psittacula eques*	AY521235	GB	*Agapornis roseicollis*

Accession #	Country	Species	Accession #	Country	Species	Accession #	Country	Species
KF688549	AU	*Barnardius zonarius semitorquatus*	HQ641524	MU	*Psittacula eques*	AY521238	GB	*Psittacus erithacus*
KF688550	AU	*Barnardius zonarius semitorquatus*	HQ641525	MU	*Psittacula eques*	AF071878	US	Unknown
KF688551	AU	*Neopsephotus bourkii*	HQ641526	MU	*Psittacula eques*	AY521234	US	*Psittacula krameri*
KF688552	AU	*Psephotus chrysopterygius*	HQ641527	MU	*Psittacula eques*	AY450434	ZA	*Pionites leucogaster*
KF688553	AU	*Psephotus dissimilis*	HQ641528	MU	*Psittacula eques*	AY450435	ZA	*Psittacus erithacus*
KF688554	AU	*Trichoglossus haematodus*	HQ641529	MU	*Psittacula eques*	AY450436	ZA	*Cacatua alba*
KF688555	AU	*Trichoglossus haematodus*	HQ641530	MU	*Psittacula eques*	AY450437	ZA	*Poicephalus robustus*
KF688556	AU	*Trichoglossus haematodus*	HQ641531	MU	*Psittacula eques*	AY450438	ZA	*Poicephalus robustus*

Accession #	Country	Species	Accession #	Country	Species	Accession #	Country	Species
KF688557	AU	*Trichoglossus haematodus*	HQ641532	MU	*Psittacula eques*	AY450439	ZA	*Poicephalus rueppellii*
KF688558	AU	*Trichoglossus haematodus*	HQ641533	MU	*Psittacula eques*	AY450440	ZA	*Poicephalus rufiventris*
KF688559	AU	*Trichoglossus haematodus*	HQ641534	MU	*Psittacula eques*	AY450441	ZA	*Poicephalus gulielmi*
KF688560	AU	*Trichoglossus haematodus*	HQ641535	MU	*Psittacula eques*	AY450443	ZA	*Psittacus erithacus*
KF688561	AU	*Trichoglossus haematodus*	HQ641536	MU	*Psittacula eques*	DQ384621	ZA	*Psittacula krameri*
KF688562	AU	*Trichoglossus haematodus*	HQ641537	MU	*Psittacula eques*	DQ384622	ZA	*Psittacula krameri*
KF688563	AU	*Trichoglossus haematodus*	HQ641538	MU	*Psittacula eques*	DQ384623	ZA	*Melopsittacus undulatus*

Accession #	Country	Species	Accession #	Country	Species	Accession #	Country	Species
KF688564	AU	*Psittacula krameri manillensis*	HQ641539	MU	*Psittacula eques*	DQ384624	ZA	*Melopsittacus undulatus*
KF688565	AU	*Poicephalus gulielmi*	HQ641540	MU	*Psittacula eques*	DQ384625	ZA	*Melopsittacus undulatus*
KF688566	AU	*Platycercus elegans*	HQ641541	MU	*Psittacula eques*	DQ384626	ZA	*Poicephalus gulielmi*
KF688567	AU	*Platycercus elegans*	HQ641542	MU	*Psittacula eques*	DQ397816	ZA	Unknown
KF688568	AU	*Amazona oratrix*	HQ641543	MU	*Psittacula eques*	DQ397817	ZA	Unknown
KF688569	AU	*Melopsittacus undulatus*	HQ641544	MU	*Psittacula eques*	DQ397818	ZA	*Poicephalus robustus*
KF688570	AU	*Melopsittacus undulatus*	HQ641545	MU	*Psittacula eques*	GQ165756	ZA	*Melopsittacus undulatus*
KF688571	AU	*Melopsittacus undulatus*	HQ641546	MU	*Psittacula eques*	GQ165757	ZA	*Melopsittacus undulatus*
KF688572	AU	*Eclectus roratus*	HQ641547	MU	*Psittacula eques*	GQ165758	ZA	*Melopsittacus undulatus*
KF688573	AU	*Eclectus roratus*	HQ641548	MU	*Psittacula eques*	HM748918	ZA	*Poicephalus robustus*
KF850537	AU	*Polytelis anthopeplus*	HQ641549	MU	*Psittacula eques*	HM748919	ZA	*Poicephalus gulielmi*
KJ634410	AU	*Neophema chrysogaster*	HQ641550	MU	*Psittacula eques*	HM748920	ZA	*Psittacus erithacus*
KJ634411	AU	*Neophema chrysogaster*	HQ641551	MU	*Psittacula eques*	HM748921	ZA	*Poicephalus gulielmi*

Accession #	Country	Species	Accession #	Country	Species	Accession #	Country	Species
KJ634412	AU	*Neophema chrysogaster*	HQ641552	MU	*Psittacula eques*	HM748922	ZA	*Poicephalus gulielmi*
KJ634413	AU	*Neophema chrysogaster*	HQ641553	MU	*Psittacula eques*	HM748923	ZA	*Poicephalus gulielmi*
KJ634414	AU	*Neophema chrysogaster*	HQ641554	MU	*Psittacula eques*	HM748924	ZA	*Amazona sp.*
KJ634415	AU	*Trichoglossus haematodus*	HQ641555	MU	*Psittacula eques*	HM748925	ZA	*Amazona sp.*
KJ634416	AU	*Trichoglossus haematodus*	HQ641556	MU	*Psittacula eques*	HM748926	ZA	*Eclectus roratus*
KJ634417	AU	*Trichoglossus haematodus*	HQ641557	MU	*Psittacula eques*	HM748927	ZA	*Psittacula krameri*
KJ634418	AU	*Trichoglossus rubritorquis*	HQ641558	MU	*Psittacula eques*	HM748928	ZA	*Psittacula krameri*
KJ634419	AU	*Trichoglossus rubritorquis*	HQ641559	MU	*Psittacula eques*	HM748929	ZA	*Psittacula krameri*
KJ634420	AU	*Trichoglossus rubritorquis*	HQ641560	MU	*Psittacula eques*	HM748930	ZA	*Poicephalus robustus*
KJ634421	AU	*Trichoglossus rubritorquis*	HQ641561	MU	*Psittacula eques*	HM748931	ZA	*Psittacus erithacus*
KJ634422	AU	*Trichoglossus rubritorquis*	HQ641562	MU	*Psittacula eques*	HM748932	ZA	*Poicephalus robustus*

Accession #	Country	Species	Accession #	Country	Species	Accession #	Country	Species
KJ634423	AU	*Cacatua galerita*	HQ641563	MU	*Psittacula eques*	HM748933	ZA	*Poicephalus robustus*
KJ634424	AU	*Cacatua galerita*	JX049196	NC	*Trichoglossus haematodus deplanchii*	HM748934	ZA	*Poicephalus robustus*
KJ634425	AU	*Eolophus roseicapillus*	JX049197	NC	*Trichoglossus haematodus deplanchii*	HM748935	ZA	*Poicephalus robustus*
KJ634426	AU	*Cacatua galerita*	JX049198	NC	*Trichoglossus haematodus deplanchii*	HM748936	ZA	*Poicephalus robustus*
KJ866054	AU	*Barnardius zonarius*	JX049199	NC	*Trichoglossus haematodus deplanchii*	HM748937	ZA	*Poicephalus robustus*
KJ953846	AU	*Platycercus elegans*	JX049200	NC	*Trichoglossus haematodus deplanchii*	HM748938	ZA	*Poicephalus robustus*
KJ953852	AU	*Platycercus elegans*	JX049201	NC	*Trichoglossus haematodus deplanchii*	HM748939	ZA	*Poicephalus robustus*

Accession #	Country	Species	Accession #	Country	Species	Accession #	Country	Species
KJ953853	AU	*Platycercus elegans*	JX049202	NC	*Trichoglossus haematodus deplanchii*	KM188440	ZA	*Poicephalus robustus*
KJ953854	AU	*Platycercus elegans*	JX049203	NC	*Trichoglossus haematodus deplanchii*	KM188441	ZA	*Poicephalus robustus*
KJ953855	AU	*Platycercus elegans*	JX049204	NC	*Trichoglossus haematodus deplanchii*	KM188442	ZA	*Poicephalus robustus*
KJ953857	AU	*Platycercus elegans adelaidae*	JX049205	NC	*Trichoglossus haematodus deplanchii*	KM188443	ZA	*Poicephalus robustus*
KJ953858	AU	*Platycercus elegans*	JX049206	NC	*Trichoglossus haematodus deplanchii*	KM188444	ZA	*Poicephalus robustus*
KJ953859	AU	*Platycercus elegans adelaidae*	JX049207	NC	*Trichoglossus haematodus deplanchii*	KM188445	ZA	*Poicephalus robustus*
KJ953860	AU	*Platycercus elegans*	JX049208	NC	*Trichoglossus haematodus deplanchii*	KM188446	ZA	*Poicephalus robustus*

Accession #	Country	Species	Accession #	Country	Species	Accession #	Country	Species
KJ953861	AU	*Platycercus elegans adelaidae*	JX049209	NC	*Trichoglossus haematodus deplanchii*	KM188447	ZA	*Poicephalus robustus*
KJ953863	AU	*Platycercus elegans flaveolus*	JX049210	NC	*Trichoglossus haematodus deplanchii*	KM188448	ZA	*Poicephalus robustus*
KJ953864	AU	*Platycercus elegans adelaidae*	JX049211	NC	*Trichoglossus haematodus deplanchii*	KM188449	ZA	*Poicephalus robustus*
KJ953865	AU	*Platycercus elegans*	JX049212	NC	*Trichoglossus haematodus deplanchii*	KM188450	ZA	*Poicephalus robustus*
KJ953866	AU	*Platycercus elegans flaveolus*	JX049213	NC	*Eclectus roratus*	KM188451	ZA	*Poicephalus robustus*
KJ953867	AU	*Platycercus elegans adelaidae*	JX049214	NC	*Eclectus roratus*	KM188452	ZA	*Poicephalus robustus*
KJ953868	AU	*Platycercus elegans flaveolus*	JX049215	NC	*Eclectus roratus*	KM188453	ZA	*Poicephalus robustus*

Accession #	Country	Species	Accession #	Country	Species	Accession #	Country	Species
KJ953869	AU	*Platycercus elegans adelaidae*	JX049216	NC	*Eclectus roratus*	KM188454	ZA	*Poicephalus robustus*
KJ953871	AU	*Platycercus elegans adelaidae*	JX049217	NC	*Eclectus roratus*	KM188455	ZA	*Poicephalus robustus*
KJ953872	AU	*Platycercus elegans flaveolus*	JX049218	NC	*Eclectus roratus*	KM188456	ZA	*Poicephalus robustus*
KJ953873	AU	*Platycercus elegans flaveolus*	JX049219	NC	*Psephotus haematonotus*	KM188457	ZA	*Poicephalus robustus*
KJ953874	AU	*Platycercus elegans flaveolus*	JX049220	NC	*Cyanoramphus saisseti*	KM188458	ZA	*Poicephalus robustus*
KJ953876	AU	*Platycercus elegans flaveolus*	JX049221	NC	*Psittacula krameri*	KM188459	ZA	*Poicephalus robustus*
KJ953877	AU	*Platycercus elegans flaveolus*	KF768545	NC	*Trichoglossus haematodus deplanchii*	KM188460	ZA	*Poicephalus robustus*

Accession #	Country	Species	Accession #	Country	Species	Accession #	Country	Species
KJ953879	AU	*Platycercus elegans flaveolus*	KF768546	NC	*Trichoglossus haematodus deplanchii*	KM188461	ZA	*Poicephalus robustus*
KJ953881	AU	*Platycercus elegans*	KF768547	NC	*Trichoglossus haematodus deplanchii*	KM188462	ZA	*Poicephalus robustus*
KJ953882	AU	*Platycercus elegans*	KF768548	NC	*Trichoglossus haematodus deplanchii*	KM188463	ZA	*Poicephalus robustus*
KJ953883	AU	*Platycercus elegans*	KF768549	NC	*Trichoglossus haematodus deplanchii*	KM188464	ZA	*Poicephalus robustus*
KJ953885	AU	*Platycercus elegans*	KF768550	NC	*Trichoglossus haematodus deplanchii*	KM188465	ZA	*Poicephalus robustus*
KM823542	AU	*Merops ornatus*	KF768551	NC	*Trichoglossus haematodus deplanchii*	AY450442	ZM	*Agapornis nigrigenis*

Chapter 3

The potential for managing infectious disease through selective placement of artificial nest sites

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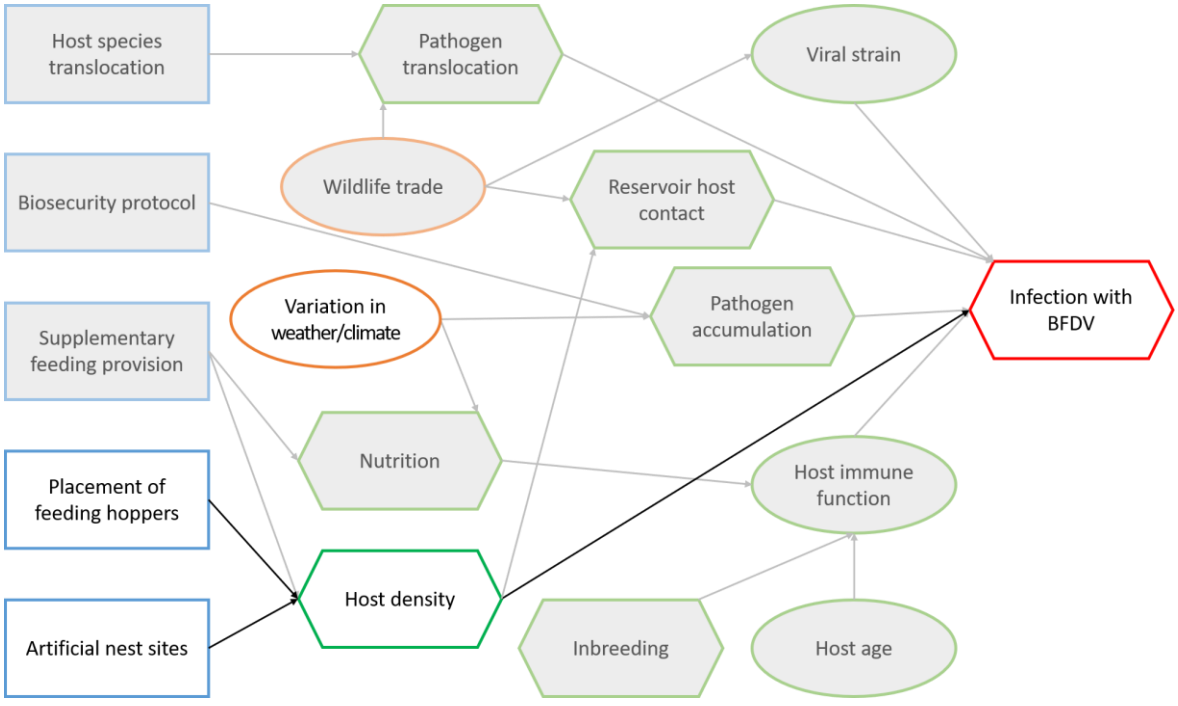

3.1 ABSTRACT

Approximately 46% of all avian species are declining according to the IUCN. Parrots are particularly vulnerable, due primarily to the dependency of over 70% of species on secondary tree cavities for breeding in an era of mass global deforestation. Artificial nest box provision is a highly successful management tool to mitigate against depletion of nesting sites, and is frequently used in the recovery of parrot populations. However, nesting sites can also be a key point for transmission of disease to occur when offspring are particularly vulnerable. Globally, parrots are threatened by an emergent and highly infectious disease, Psittacine beak and feather disease (PBFD), caused by the Beak and feather disease virus (BFDV), which can have implications for conservation management of endangered parrots. Here we test whether abiotic factors associated with nest site location, such as aspect and altitude, impact on nest productivity and the prevalence of BFDV in endangered Mauritius parakeet (*Psittacula eques*) nestlings. We found an observable, but non-significant relationship between nest altitude and the distance nests were located from a supplementary feeding hopper, where prevalence was greatest closest to feeding hoppers and was observed to increase in nests at higher altitudes located further away from hoppers. No abiotic impacts on productivity were observed other than the previously described positive relationship between total number of fledglings produced and proximity to feeding hoppers. Population management should remain a dynamic process and it is possible that the relationship between BFDV and altitude may become more relevant under future climate change scenarios. However, in the current case of Mauritius parakeets, to achieve the fundamental management objective of population recovery, nest site placement should continue to focus on proximity to supplementary feeding hoppers.

3.2 INTRODUCTION

Global biodiversity is in crisis, with the latest statistics from the Living Planet Index demonstrating that wildlife populations have declined by 60% since 1970 (WWF 2018). Conservationists are challenged with recovering populations under increasing threats from local anthropogenic pressures (Joppa *et al.* 2016; WWF 2018; Dirzo *et al.* 2014). Approximately 46% of all avian species are listed by the IUCN as being in population decline, with 15% listed in threatened categories or extinct and a further 9% classified as near threatened (IUCN 2019). One avian order, the parrots (Psittaciforms) appear particularly vulnerable (Fogell *et al.* 2018; Olah *et al.* 2016), with more than a quarter of known species considered threatened (IUCN 2019).

Bird populations are naturally regulated by relatively few variables including food, availability of safe nests sites, predation, competition and disease (Newton 1998). In situations where one or a combination of these factors drive population decline, then targeted mitigation may allow for recovery. These may include supplementary feeding to correct food shortages (Oro *et al.* 2008; Cole and Batzli 1978; Walker *et al.* 2013), invasive predator control to reduce pressure from novel predators, and captive breeding (Cade and Jones 1993; Andrew *et al.* 2018) combined with conservation translocation (Seddon *et al.* 2014) to overcome any combination of these population threats. The availability of safe nesting sites is also critical (Finch *et al.* 2019). Many bird species are dependent on nesting in cavities, and are therefore vulnerable to decline if suitable sites become scarce (Sherley *et al.* 2012). In parrots, where 70% of species are secondary tree cavity nesters, the loss of nesting sites through deforestation is considered a primary driver of population declines (Olah *et al.* 2016; Newton 1994). In such cases the provision of artificial nest boxes is often reported as a highly successful management tool in a wide variety of species ranging from burrowing seabirds such as penguins (Sherley *et al.* 2012), to forest species such as tree swallows (Norris *et al.* 2018) and cockatoos (Berris *et al.* 2018). Consequently, conservationists frequently deploy artificial nest boxes as a means to increase breeding success (e.g. Beissinger et al. 1998; Downs 2005; Larson et al. 2015).

Globally, parrots are now facing an additional threat through an emergent and highly infectious disease, Psittacine beak and feather disease (PBFD), caused by the Beak and feather disease virus (BFDV; Circoviridae). PBFD is thought to be the most common disease in wild psittaciformes (Khalesi *et al.* 2005), and is implicated in the decline of many species, including South African Cape parrots (*Poicephalus robustus*) (Regnard *et al.* 2015) and Australian Orange-bellied parrots (*Neophema chrysogaster*) (Peters *et al.* 2014). The virus is believed to have originated in Oceania (Raidal, Sarker and Peters 2015; Fogell, Martin and Groombridge 2016) and has rapidly spread across the world via the international trade in pet birds (Fogell *et al.* 2018; Harkins *et al.* 2014). Like other circoviruses, BFDV is thought to be highly persistent and stable outside of the host

(Ritchie 1995; Amery-Gale *et al.* 2017; Jackson *et al.* 2015), remaining viable for months after an infected bird has shed virions (Bougiouklis 2007). Young birds appear to be particularly vulnerable to disease (Ritchie *et al.* 1989). Therefore nesting sites may be a high risk point where infection of entire broods occurs through direct vertical transmission from parent to offspring (Ritchie *et al.* 1989; Kundu *et al.* 2012) and horizontally through the accumulation of feather dust and contaminated nesting material (Ritchie, Anderson and Lambert 2003).

Consequently, artificial nesting sites may present both a concern for conservation managers (i.e. where inappropriate use may increase disease risks) but also an opportunity for disease mitigation. Mitigation, facilitated by using artificial nests, could include three options; (i) biosecurity, e.g. frequency and ease of cleaning and disinfection (Fogell et al. 2019, Chapter 3), (ii) direct treatment of occupants, e.g. possible vaccination (currently not available for BFDV; Raidal et al. 1993; Shearer et al. 2009), and (iii) nest box placement. The latter option, selective nest placement may allow reduced exposure to BFDV, particularly where nest boxes can be situated away from highly contaminated areas, such as supplementary feeding hoppers or other nests, or when their position is influenced by abiotic factors that may reduce BFDV load or viability (e.g. altitude or aspect; both of which are known to influence infection prevalence from vector-borne pathogens Bødker et al. 2003; Gilbert 2010).

Here we use the detailed long-term BFDV monitoring dataset available for Mauritius 'echo' parakeets (*Psittacula eques*) to determine whether the prevalence and viral load of BFDV in annually produced nestlings is influenced by the location of a nest site within the forest. We consider a range of abiotic factors such as their altitude and aspect, as well as their density within the forest and proximity to supplementary feeding stations. We predict that BFDV prevalence will be lower when nests are at lower densities and further away from supplementary feeding hoppers (the latter has been previously shown; Fogell *et al*. 2019, Chapter 3). We also predict that infection prevalence and load may be higher in those nest sites located on cooler southern and south-eastern slopes, and at higher altitudes on the assumption that BFDV may be unstable when exposed to prolonged periods of high temperature (Ritchie 1995). However, the optimal nest site management choices will not only depend on the presence of BFDV at nest sites but, justifiably, should focus on improving reproductive success. Reproductive success is a key population vital rate and the fundamental purpose of providing nest boxes for Mauritius parakeets. Here we predict that proximity to feeding hoppers will improve reproductive success (previously shown; Fogell *et al*. 2019, Chapter 3). Reproductive success can also be influenced by abiotic factors such as altitude (Kleindorfer 2007; Johnson *et al.* 2006) and we wish to test for any such relationship in Mauritius parakeets. Together this will provide conservation managers with the detail required for strategic nest placement.

3.3 METHODS

3.3.1 Study system and sample collection

The endangered Mauritius parakeet is an intensively studied, island-endemic species that is successfully recovering through long-term collaborative conservation and monitoring efforts (Raisin *et al.* 2012; Tollington *et al.* 2013; Jones and Duffy 1993). Mauritius parakeets were once widely distributed across the tropical rainforest habitat of Mauritius but are now confined to the Black River Gorges National Park in the south-west (Figure 3.1) and a newly established subpopulation in a private nature reserve on the eastern side of the island. Since 2000, artificial nest boxes have been supplied throughout the forest in order to overcome a shortage of nesting sites due to a lack of natural tree cavities (Tatayah *et al.* 2007). In the 2006/07 breeding season it was estimated that 73% of all eggs produced were laid in artificial nest boxes (Tatayah *et al.* 2007) and in the 2016/17 breeding season this had increased to 87% (representing 94% of all hatchlings; S. Henshaw, Pers. Obs.).

However, since 2005, the Mauritius parakeet population has been affected by the presence of BFDV (Kundu *et al.* 2012). Despite the presence of this infectious disease, the Mauritius parakeet population has continued to steadily recover. Following the outbreak, a number of biosecurity protocols have been implemented at both Mauritius parakeet supplementary feeding hoppers and nest sites in an attempt to reduce the transmission of infection between individuals (Fogell *et al.* 2019, Chapter 3). Additionally, prevalence within the population has been continuously monitored by taking blood samples from all 45-day old nestlings produced annually. At the time of sampling each nestling is also given a unique combination of leg bands, assigned a Studbook ID and has morphometric data collected. We used all available nestling data from the 2009/10 to 2012/13 breeding seasons, and a partial dataset from the 2013/14 to 2016/17 breeding seasons excluding any treatment nest sites incorporated into concurrent experiments that may have influenced nest site BFDV prevalence.

3.3.2 Laboratory analysis

Two methods, previously described in detail in Fogell *et al.* (2019), were used in the laboratory to provide both the viral prevalence dataset as well as an assessment of individual nestling viral load. In brief, host and viral DNA, where present, were extracted from host whole blood using a combination of DIGSOL extraction buffer and 10 mg/mL proteinase K (Bruford *et al.* 1998). Extractions were quantified using a Qubit dsDNA Assay Kit and standardised to approximately 25 ng/µl prior to screening for BFDV through standard PCR (with a known false-negative error rate of 49.5% (CI 39.8 – 60.2%); D. Fogell, Unpublished data), and to 10 ng/µl for quantification using real-time PCR (rtPCR) (with an estimated minimum detection threshold of 1×10^2 copies of viral DNA per reaction; Katoh, Ohya and Fukushi 2008).

Standard PCR protocols used to detect BFDV infection status of an individual were as detailed in Kundu *et al.* (2012) using a PCR assay targeting a 717-bp region of the replicase gene (Ypelaar *et al.* 1999) with the PCR annealing temperature adjusted to 60^{o}C, as per manufacturer's guidelines, for 30 cycles. A negative control was included in each PCR batch to ensure no contamination was present and products were visualized on a 1.5% agarose gel.

Quantitative rtPCR protocols used to assess individual viral load targeted a 120-bp region of the replicase gene (Tollington *et al.* 2018) with the annealing temperature set to 60°C for 40 cycles. All 96-well plates included two positive controls from a high viral load Mauritius parakeet individual (amplification at ~10 cycles) for the purposes of standardisation between runs and two negative controls to ensure no contamination was present. Each individual was run in duplicate. If the repeats did not amplify within one PCR cycle of one another, a third replicate was performed. The averaged CT values for each individual were then converted into a relative estimate of viral load (Eastwood *et al.* 2015) using the equation: Viral load = $2^{(-\Delta CT)}$

3.3.3 Data Analysis

3.3.3.1 BFDV in nestlings

Using the data generated from standard PCR, generalised linear mixed models (GLMMs) were run with the lme4 (Bates *et al.* 2015) package in R version 3.5.2 (R Core Team 2018) using a binomial response variable accounting for the number of BFDV-positive and -negative nestlings per nest site and setting a binomial error distribution and a logit link function (Tollington *et al.* 2013, Chapter 3). The resulting dataset comprised 781 nestlings from 382 clutches, across 135 nest sites. The evaluated set of candidate models investigated the effects of four variables directly attributed to their location within the forest on the probability of nestlings becoming infected with BFDV. The first of these was the distance to the nearest supplementary feeding hopper (km), which has previously been found to be a significant predictor of BFDV infection in Mauritius parakeet nestlings (Fogell *et al.* 2019, Chapter 3). The second was distance to the nearest neighbouring nest site (km) to account for nest site density. The final variables were both the aspect and altitude (m) of the nest sites, calculated from a digital elevation model of Mauritius (USGS 2018) in QGIS 2.18 (QGIS Development Team 2018). Nest site and breeding season were used as random intercept effects to account for both the vertical and horizontal viral transmission pathways (as females generally nest at the same site year on year) and for any annual climate variation between breeding seasons.

For the rtPCR viral load data, GLMMs were run across all available nestling data for the 2013/14 to 2016/17 breeding seasons, resulting in a dataset comprising 640 nestlings. The models were run using a Gaussian distribution, with the same independent variables as for the standard PCR diagnostic dataset. The response variable consisted of the logged viral load values and both

nest site and breeding season were included as random intercept effects to account for both the vertical and horizontal viral transmission pathways and for any annual climate variation between breeding seasons.

3.3.3.2 Nest productivity

To assess the potential impacts of nest site placement on reproductive success, GLMMs were run on the total number of fledglings produced per nesting attempt (n = 808 nestlings from 445 nesting attempts). The set of 21 candidate models evaluated the effects nest site altitude (m), aspect, distance to the nearest neighbouring nest site (km), distance to the nearest supplementary feeding hopper (km) and both the linear and quadratic terms for dam age, using a Gaussian distribution and with nest site and breeding season used as random intercept effects.

3.4 RESULTS

Of the 17 models constructed to assess the probability of BFDV infection in nestlings, we found four equally plausible models, including the factors of distance to nearest supplementary feeding hopper, distance to the nearest neighbouring nest site, nest site altitude and the interaction between nest site altitude and the distance to nearest feeding hopper (Table 3.1, Figure 3.2). As previously found by Fogell *et al.* (2019), distance to nearest feeding hopper was found to have a significant negative effect on the probability of nestling infection at a nest site, where probability was found to decrease with increasing distance. Whilst not significant (odds ratio crossed 1), an observable positive interaction was present between nest site altitude and the distance a nest was located from a feeding hopper. The probability of BFDV infection in nestlings was found to increase with increasing altitude, with a more pronounced effect at nest sites located further away from feeding hoppers (Figure 3.3). Those nest sites located within 0.1 km of a feeding hopper across altitudes had an approximately similar predicted 20% (0.2) probability of nestlings becoming infected with BFDV (Figure 3.3). Similarly, those nest sites located 1 km away from a feeding hopper had an approximate predicted infection probability of 0.15 across altitudes. However, at 4.5 km away from a feeding hopper, the predicted probability of infection with BFDV varied between approximately 0.05 at lower altitude nest sites and 0.1 at higher altitude nest sites (Figure 3.3). The geographical aspect of a nest site was not found to influence BFDV prevalence.

When assessing predictors of viral load in nestlings the null model was found to be the most parsimonious (Table 3.1). Therefore, we determined that none of the nest site factors assessed influenced the viral load of nestlings at the point of sampling.

Of the 21 models constructed to assess nest site placement effects on reproductive success, we found a single top model, including the factors of distance to the nearest feeding hopper and the linear and quadratic terms for dam age (Table 3.2), both of which were found to be significant.

Fledge success improved with reduced distance to feeding hoppers and increasing dam age, until female senescence. Distance to nearest neighbour, altitude and aspect were not found to have an impact on productivity.

3.5 DISCUSSION

Our study tested whether the location of Mauritius parakeet nesting boxes had any influence on two key management objectives. The first of these relates to whether managers are able to reduce the prevalence and load of BFDV infection in nestlings through the manipulation of nest site placement. The strong influence of the proximity of nest sites to supplementary feeding hoppers on BFDV prevalence is not surprising as this relationship has been previously described (see Fogell *et al.* 2019, Chapter 3). It is suspected that the increased aggregation of individuals at these feeding hoppers may become an important mechanism through which transmission of virus can occur. It has been shown that a linear relationship exists between the distance to these feeding hoppers and the proportion of supplementary food consumed by nestlings; this finding implies that those parents nesting closer to feeding hoppers attend them far more frequently than those that nest at sites located further away (Tollington *et al.* 2018). However, none of the variation in viral load present within our dataset was explained by any of the assessed variables and to date little is known about the factors that influence individual viral load and the patterns of infection within a brood (Tollington *et al.* 2018; Eastwood *et al.* 2019).

When assessing the second objective of whether nest site placement impacted on reproductive success, we similarly found only the relationships described previously by Fogell *et al.* (2019), namely that the total number of chicks produced was higher in nest sites located closer to supplementary feeding stations and increased with increasing dam age. Whilst it is evident that increasing the distance between nest sites and supplementary feeding hoppers would be beneficial in reducing the prevalence of BFDV in Mauritius parakeet nestlings, it is important for managers to remain focused on population recovery. Nest boxes are being used very effectively to promote successful breeding and our findings suggest nest placement is important in maximising this potential. Therefore, BFDV is important more as a means objective and should be carefully monitored to determine whether it influences population reproductive success and juvenile or adult survival. To date such evidence is limited.

Currently the high inter-annual variation observed across the long-term BFDV prevalence dataset from Mauritius cannot be attributed to large-scale global climatic patterns such as El Niño (National Oceanic and Atmospheric Administration 2019)(Figure S3.1). Similarly, it is unlikely that this variation is due to host-parasite co-evolution, given the generation time of Mauritius parakeets (age of first reproduction is 2 years; Tollington *et al.* 2013). However, abiotic patterns at a more local scale may exist. Despite the confidence limits of the odds ratio estimate crossing 1 for the

interaction between nest site altitude and the distance it is located away from a feeding hopper (Figure 3.2), there is a trend of a positive relationship between these variables. Variation in nest microclimate could increase the probability of nestling infection with BFDV when produced at higher altitudes, further away from feeding hoppers. Although we have not directly measured nest microclimate we hypothesise that factors such as atmospheric temperature, known to decrease consistently with increasing altitude (Körner 2007), could be important. Mauritius is predicted to become hotter and drier under future climate change scenarios (IPCC 2014). Therefore, if the lower probability of infection with BFDV prevalence in nestlings at lower altitudes is due primarily to nest microclimate, this may mean future changes in climate could impact on nestling infection prevalence. Whilst detailed climate prediction models are beyond the scope of this analysis, and location specific microclimate data were not available for assessment, it would be beneficial for future research to address whether patterns in the annually recorded BFDV prevalence can be attributed to fluctuations in microclimate parameters such as average or maximum temperature over the breeding seasons.

We noted that there were three ways that mitigation of PBFD could be implemented with artificial nest sites. Our previous work showed that nest site biosecurity was effective at reducing BFDV prevalence in nestlings, but at the expense of fledging success (Fogell *et al.* 2019, Chapter 3). Here we expand on this work by showing that informed placement of nest sites could potentially reduce BFDV prevalence in nestlings if positioned at lower altitudes, and further away from feeding hoppers. Combined, under current climate conditions, this could potentially reduce BFDV prevalence by about 7.6% in those nest sites 4.5 km away from feeding hoppers (between nest sites located at 665 m vs. those located at 243 m above sea level). Here we have chosen to restrict our focus to exclude the third approach, direct treatment, as there has been limited success with the development of a vaccination to date (Raidal, Firth and Cross 1993; Shearer *et al.* 2009). However, we acknowledge that nest sites are only one aspect of conservation management where mitigation could occur in threatened parrot populations. Management of infection transmission at feeding hoppers is another obvious area of potential and is the current focus of our ongoing work (Chapter 4). The optimal combination of management actions is dependent on the importance of reducing BFDV in the affected target species, whilst still ensuring that any other key management objectives are considered. These assessments should be made with as much future-proofing as possible, especially given the changing climates within which these populations exist.

Managing populations for conservation is a dynamic process, where conservationists need to be clear about their management objectives and alternatives. Continuous learning about management-sensitive uncertainty (i.e. uncertainty that, if reduced, would influence which management alternative best allows us to achieve stated objectives) allows us to make the most appropriate decisions and provides us with the framework for adaptive management. In the case

of Mauritius parakeets, where the fundamental management objective is population recovery then nest site placement should continue to focus on proximity to supplementary feeding hoppers. Whilst we show that nest placement can be used to reduce BFDV prevalence, infection does not currently appear to limit reproductive success. BFDV is a highly infectious and globally distributed pathogen of parrots (Fogell *et al.* 2018; Harkins *et al.* 2014; Fogell, Martin and Groombridge 2016) and our work with Mauritius parakeets provides information that is relevant to managers responsible for any parrot recovery programme. We encourage the managers of such programmes to carefully consider their disease mitigation options and what they may achieve.

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3.7 TABLES AND FIGURES

Table 3.1 A comparison of the 17 generalised linear mixed effect candidate models analysing a.) the prevalence of BFDV in 45-day old Mauritius parakeet nestlings over eight breeding seasons (2009/10 and 2016/17), and b.) individual BFDV load in 45-day old Mauritius parakeet nestlings over four breeding seasons (2013/14 and 2016/17). Factors assessed include nest site altitude (AL), aspect (AS), distance to the nearest neighbouring nest site (NN) and distance to the nearest supplementary feeding hopper (SF) based on Akaike's information criterion corrected for finite sample size (AIC_c) and weights (AIC_c weights). All models were run with the nest site and breeding season as fixed intercept effects. K denotes the number of parameters in each model and models are ranked according to their ΔAIC_c.

Rank	Model	K	AIC_c	ΔAIC_c	AIC_c weights
a.)	**Prevalence of BFDV**				
1	SF	4	542.76	0.00	0.27
2	SF + NN	5	543.80	1.04	0.16
3	AL + SF	5	543.81	1.04	0.16
4	AL + SF + (AL*SF)	6	543.92	1.16	0.15
5	AL + SF + NN	6	544.95	2.19	0.09
6	AL	4	545.40	2.63	0.07
7	AL + NN	5	546.31	3.54	0.05
8	Null model	3	548.21	5.45	0.02
9	NN	4	548.25	5.49	0.02
10	AS + SF	11	553.12	10.35	0.00
11	AS + SF + NN	12	554.23	11.47	0.00
12	AL + AS	11	554.89	12.12	0.00
13	AL + AS + SF + NN + (AL*SF)	14	555.08	12.32	0.00
14	AL + AS + SF + NN	13	555.52	12.76	0.00
15	AL + AS + NN	12	555.93	13.16	0.00
16	AS	10	556.36	13.59	0.00
17	AS + NN	11	556.84	14.08	0.00
b.)	**BFDV load**				
1	Null Model	4	-2202.89	0.00	0.96
2	SF	5	-2195.34	7.55	0.02
3	AL	5	-2194.68	8.21	0.02
4	AL + SF	6	-2185.22	17.67	0.00
5	AL + SF + AL*SF	7	-2173.92	28.97	0.00

6	AS	11	-2143.04	59.85	0.00
7	AS + SF	12	-2135.33	67.56	0.00
8	AL + AS	12	-2133.70	69.19	0.00
9	NN	5	-2095.69	107.20	0.00
10	SF + NN	6	-2087.69	115.20	0.00
11	AL + NN	6	-2086.82	116.07	0.00
12	AL + SF + NN	7	-2077.42	125.47	0.00
13	AS + NN	12	-2036.39	166.50	0.00
14	AS + SF + NN	13	-2028.11	174.78	0.00
15	AL + AS + NN	13	-2026.51	176.38	0.00
16	AL + AS + SF + NN	14	-2017.20	185.70	0.00
17	AL + AS + SF + NN + (AL*SF)	15	-2006.58	196.31	0.00

Table 3.2 A comparison of the 21 generalised linear mixed effect candidate models analysing the impacts of nest site altitude (AL), aspect (AS), distance to the nearest neighbouring nest site (NN), distance to the nearest supplementary feeding hopper (SF) and both the linear and quadratic terms for dam age (FA and F2) on the total number of 45-day old Mauritius parakeet nestlings produced over the 2009/10 to 2016/17 breeding seasons. Model selection was based on Akaike's information criterion corrected for finite sample size (AIC_c) and weights (AIC_c weights). All models were run with the nest site and breeding season as fixed intercept effects. K denotes the number of parameters in each model and models are ranked according to their ΔAIC_c.

Rank	Model	K	AIC_c	ΔAIC_c	AIC_c weights
1	SF + FA + F2	7	1213.87	0.00	0.89
2	SF + AL + FA + F2	8	1218.12	4.26	0.11
3	AL + FA + F2	7	1224.58	10.71	0.00
4	FA + F2	6	1226.22	12.35	0.00
5	SF + AL + NN + AS + FA + F2	16	1228.13	14.26	0.00
6	AL + NN + AS + FA + F2	15	1229.32	15.45	0.00
7	SF + AL + NN + AS + FA + F2 + (AL*SF)	17	1229.65	15.78	0.00
8	NN + AS + FA + F2	14	1230.64	16.78	0.00
9	AS + FA + F2	13	1239.39	25.52	0.00
10	SF + AL + NN + (AL*SF)	8	1275.86	62.00	0.00
11	SF	5	1280.46	66.59	0.00
12	SF + AL + (AL*SF)	7	1283.17	69.30	0.00
13	SF + AL	6	1283.73	69.87	0.00
14	NN	5	1284.45	70.58	0.00
15	AL	5	1289.42	75.55	0.00
16	Null Model	4	1292.76	78.89	0.00
17	SF + AL + NN + AS	14	1295.02	81.15	0.00
18	NN + AS	12	1297.99	84.12	0.00
19	AS + (AL*SF)	14	1300.04	86.17	0.00
20	SF + AS + (AL*SF)	14	1300.04	86.17	0.00
21	AS	11	1306.84	92.98	0.00

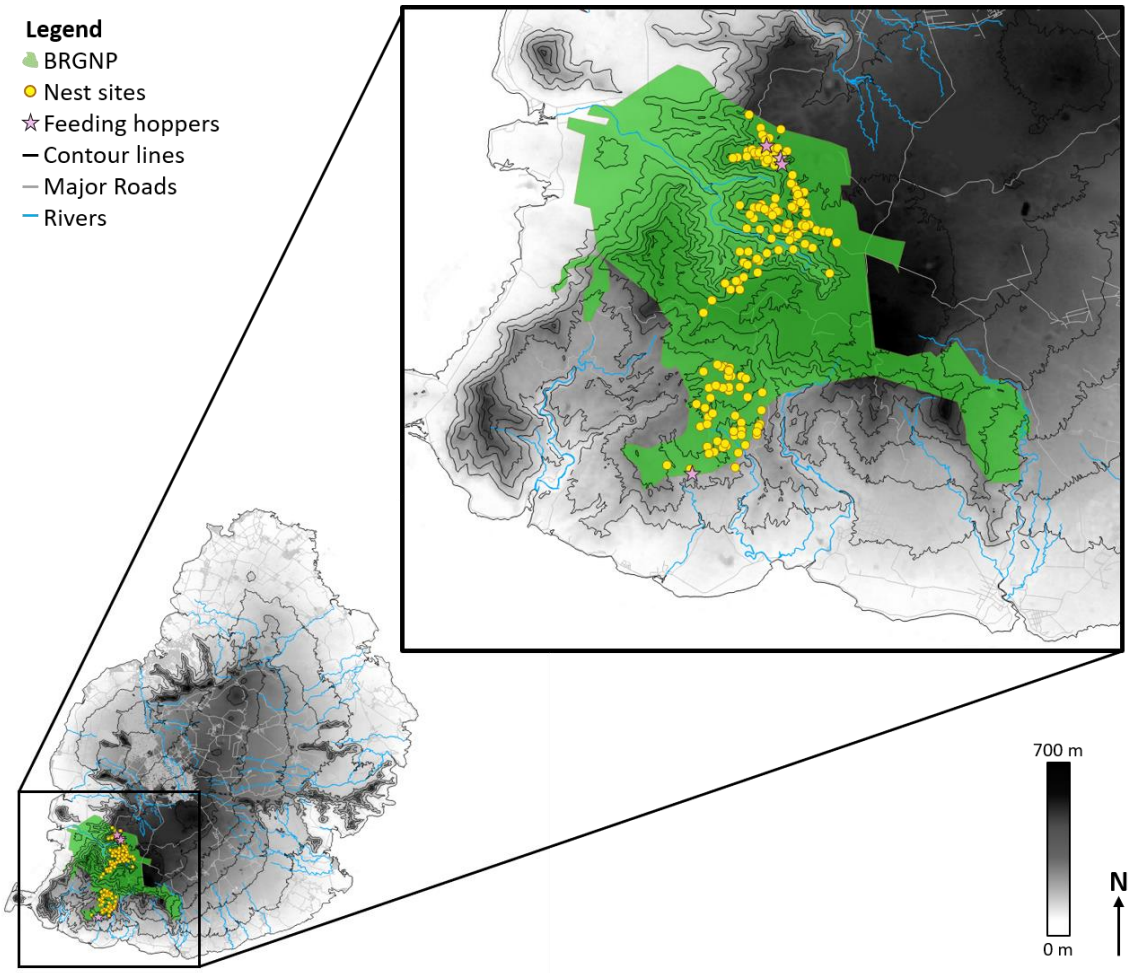

Figure 3.1 Location and distribution of nest sites and supplementary feeding hoppers used by the Mauritius "echo" parakeet population breeding within the Black River Gorges National Park (BRGNP) in the south west of Mauritius. Greyscale shading and contour lines mark the altitudinal gradient present across the island, with lines spaced at 100 m intervals above sea-level.

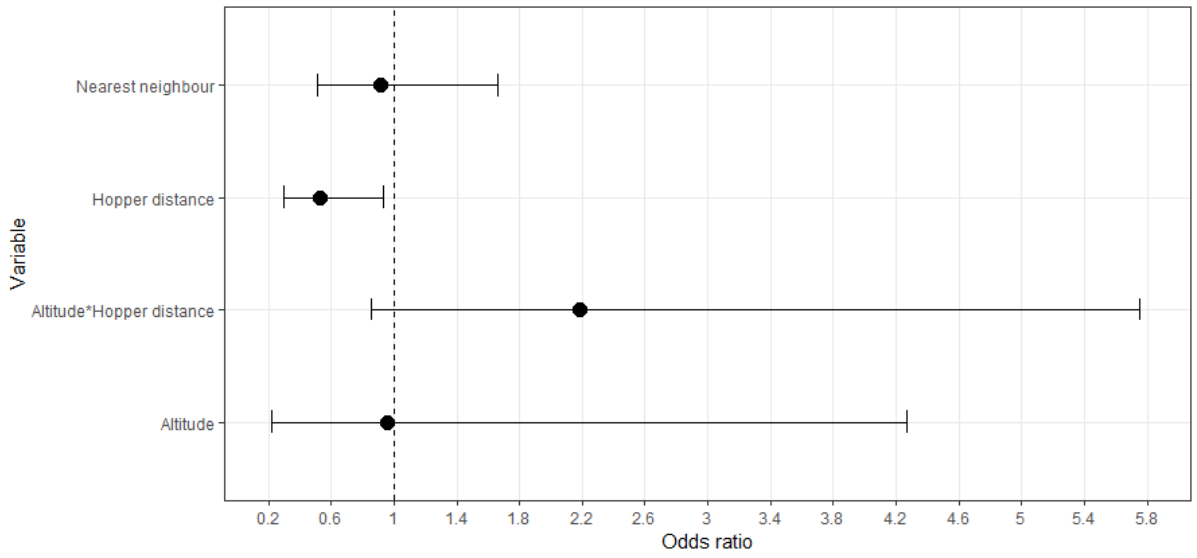

Figure 3.2 The association of nest site altitude, distance to the nearest supplementary feeding hopper, distance to the nearest neighbouring nest site and the interaction between nest site altitude and distance to the nearest supplementary feeding hopper with the probability of BFDV infection in 45-day old Mauritius parakeet nestlings produced over eight breeding seasons. Variable specific odds ratios are denoted by the filled circles along with their associated 95% CIs.

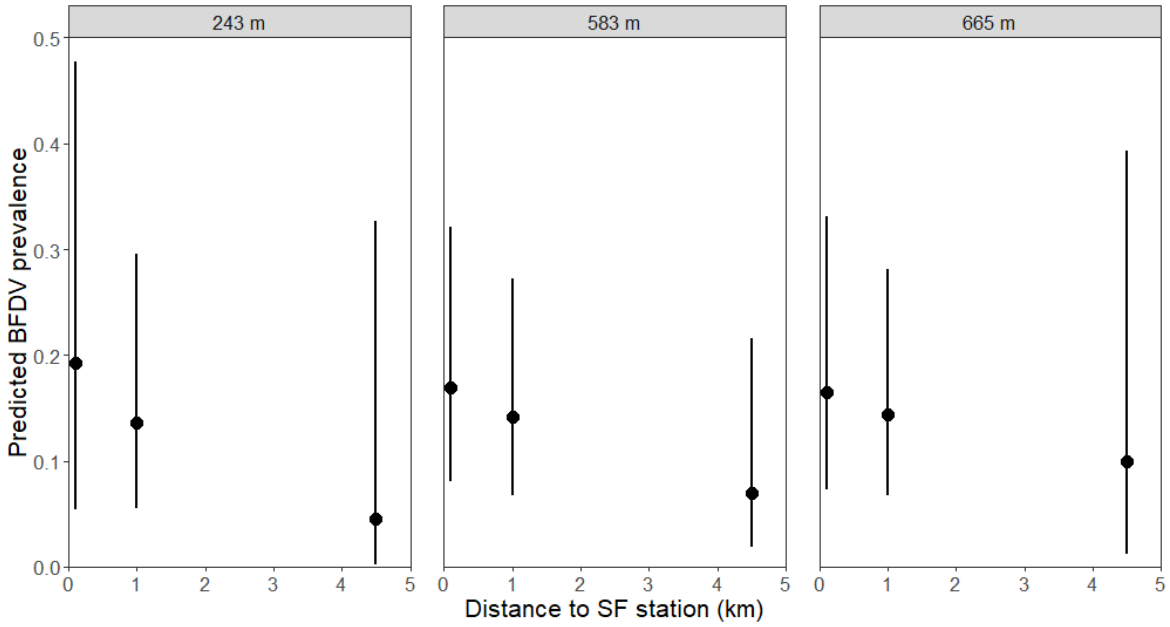

Figure 3.3 The estimated difference in the probability of Mauritius parakeet nestling infection with BFDV as a result of increasing nest site altitude and distance from the nearest feeding hopper, presented with 95% prediction intervals. Plots illustrate these relationships at the minimum, median and maximum recorded Mauritius parakeet nest site altitudes and hopper distances.

3.8 SUPPLEMENTARY INFORMATION

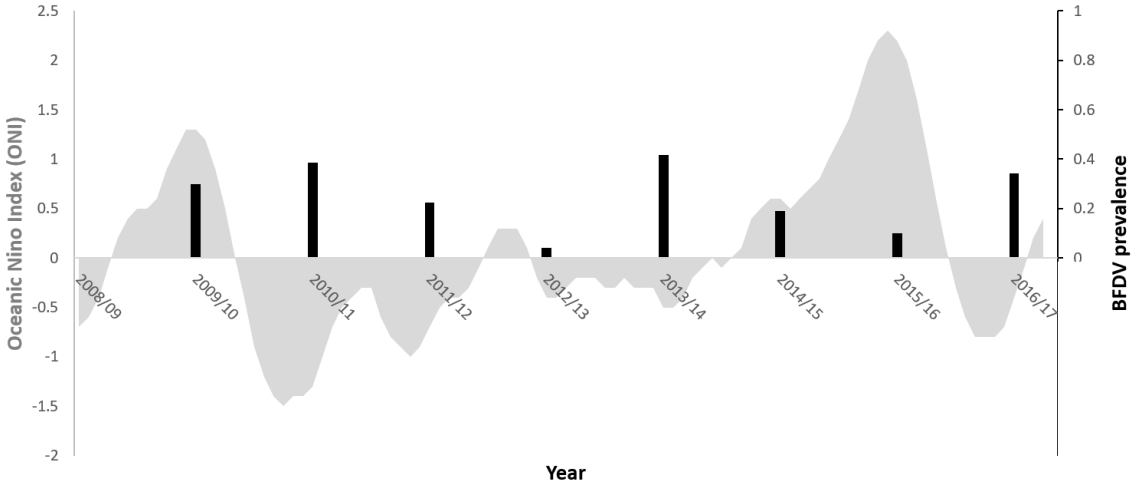

Figure S3.1 The annual fluctuations in Oceanic Nino Index (ONI) compared to the annual variation in BFDV prevalence in Mauritius parakeet nestlings screened through a diagnostic PCR, where no observable pattern exists between these datasets.

Chapter 4

Hygiene and biosecurity protocols reduce infection prevalence but do not improve fledging success in an endangered parrot

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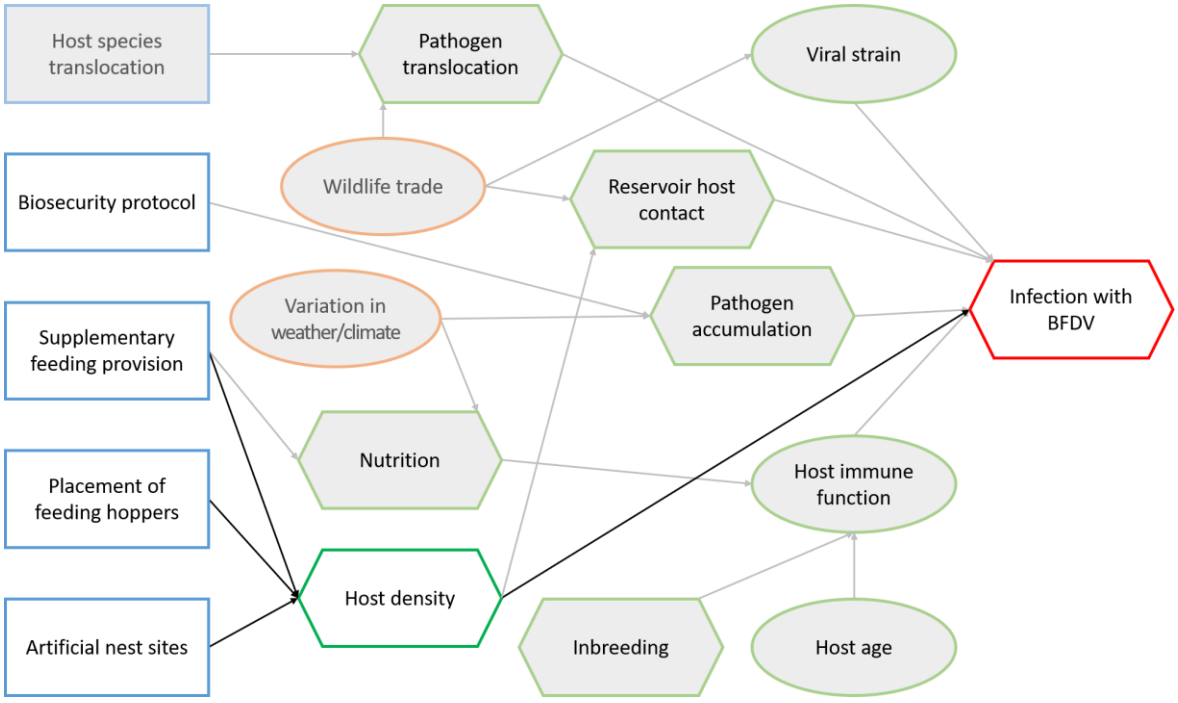

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4.1 ABSTRACT

Emerging Infectious Diseases (EIDs) are recognised as global extinction drivers of threatened species. Unfortunately, biodiversity managers have few tested solutions to manage them when often the desperate need for solutions necessitates a response. Here we test *in situ* biosecurity protocols to assess the efficacy of managing Psittacine beak and feather disease (PBFD), one of the most common and emergent viral disease in wild parrots (Psittaciformes) that is currently affecting numerous threatened species globally. In response to an outbreak of PBFD in Mauritius "echo" parakeets (*Psittacula eques*), managers implemented a set of biosecurity protocols to limit transmission and impact of Beak and feather disease virus (BFDV). Here we used a reciprocal design experiment on the wild population to test whether BFDV management reduced viral prevalence and viral load, and improved nestling body condition and fledging success. Whilst management reduced the probability of nestling infection by approximately 11% there was no observed impact on BFDV load and nestling body condition. In contrast to expectations there was lower fledging success in nests with added BFDV biosecurity (83% in untreated vs. 79% in treated nests). Our results clearly illustrate that management for wildlife conservation should be critically evaluated through targeted monitoring and experimental manipulation, and this evaluation should always focus on the fundamental objective of conservation.

4.2 INTRODUCTION

Emerging infectious diseases (EIDs) are key contributors to the current global biodiversity crisis (Yap *et al.* 2015; Brooks and Ferrao 2005). While population biologists recognize infectious pathogens as an integral and constant mechanism for evolutionary change within natural populations (Lyles and Dobson 1993), the emergence of novel pathogens may increase the risk of extinction for vulnerable species and populations (Lips *et al.* 2006). Viruses are responsible for over 40% of all recently surveyed wildlife EIDs (Dobson and Foufopoulos 2001; Tompkins *et al.* 2015), and have thus been highlighted as a particular threat to wildlife. The threats from viruses are in part due to their ability to adapt rapidly to novel hosts (Altizer, Harvell and Friedle 2003; Jones *et al.* 2008), conferring the capacity to become infectious across a wide host range (Altizer, Harvell and Friedle 2003).

Conservationists have struggled in the face of EIDs. Broadly speaking, management of EIDs can be broken down into three main types of strategies. First, those that target direct treatment or vaccination of the infected host, such as anti-fungal treatment of amphibians affected by *Batrachochytrium dendrobatidis* (Hudson *et al.* 2016; Bosch *et al.* 2015) or the inoculation of black-footed ferrets against canine distemper virus (Thorne and Williams 1988). Second, strategies that aim to prevent interaction between disease vectors and the focal host, such as pesticide application for reducing tick populations that are responsible for the spread of Lyme disease (Stafford III 1997). Third, strategies that aim to reduce the risk of transmission through hygiene, biosecurity or direct treatment of environmental reservoirs (Wobeser 2002). For example, the disinfection of water bodies associated with the spread of avian cholera (Gershman *et al.* 1964) and liming around feeding stations to reduce the prevalence of lungworms in hares (Skrjabin 1970). Various combinations of these strategies have been broadly applied across taxonomic groups. In extreme cases these disease management strategies can be combined with the removal of surviving individuals to captivity (Zippel *et al.* 2011).

Management actions aimed at reducing EID transmission *in situ* are mostly reactive and the efficacy of only a few have been thoroughly assessed (Wobeser 2002; Woodroffe, Frost and Clifton-Hadley 1999; Artois *et al.* 2001). These management actions are often modified versions of those used in clinical settings and based on expert knowledge of wildlife health specialists. However, their application is rarely backed by critical evaluation of their ability to reduce transmission (the means to threatened host species recovery) and aid recovery of the threatened host species (the fundamental objective). This raises a dual concern that conservation management may continue despite it being ineffective or even detrimental to endangered species recovery, and that this may add unnecessary financial and logistical burdens to management.

Psittaciformes (parrots) are one of the most vulnerable avian orders, with over a quarter of all extant species recognised as in need of conservation action and 75% of species in population decline (IUCN 2015). One major threat to parrots has been the emergence and global spread of Psittacine beak and feather disease (PBFD), one of the most common viral diseases in wild parrots (Julian *et al.* 2012; Černíková, Vitásková and Nagy 2017; Fogell, Martin and Groombridge 2016). PBFD was first described in the mid-1970s, originating in the South Pacific and is spreading rapidly across the world (Ritchie *et al.* 1989a; Fogell, Martin and Groombridge 2016; Fogell *et al.* 2018). PBFD is caused by the Beak and feather disease virus (BFDV) and the disease has been implicated in the decline of many wild parrot populations, including the endangered Cape parrot (*Poicephalus robustus*) of South Africa (Regnard *et al.* 2015), the Australian orange-bellied parrot (*Neophema chrysogaster*) (Peters *et al.* 2014) and the Mauritius "echo" parakeet (*Psittacula eques*) (Kundu *et al.* 2012). Concern about the threat of PBFD in Australia has led to it being listed as a "Key Threatening Process" to biodiversity (Department of the Environment and Heritage 2005). The emergence of PBFD has directly impacted species recovery programmes by altering how and what management tools are used (e.g. captive breeding, translocation, cross fostering; Tollington *et al.* 2015; Jackson *et al.* 2015).

Despite calls to more directly manage PBFD only a limited range of management actions have been developed, most focussing on hygiene and biosecurity. In Australia, for example, a detailed Threat Abatement Plan for BFDV includes the use of disinfectants in nest and transport boxes (Department of the Environment and Heritage 2005). However, the same Threat Abatement Plan also notes that there is no assurance as to whether recommended actions will actually reduce transmission. To our knowledge, there are no studies that provide empirical evidence for the effectiveness of *in situ* biosecurity management actions to reduce BFDV transmission. Research into the efficacy of biosecurity interventions is therefore paramount to improve our ability to carry out evidence-based management of endangered parrot species in the face of BFDV.

In this study, we experimentally test the performance of nest site biosecurity for reducing BFDV transmission and enhancing Mauritius parakeet population recovery. Mauritius parakeets were once the world's rarest parrot, numbering fewer than 20 individuals in the early 1980s (Duffy 1993). Intensive management has increased their abundance to 136 breeding pairs in 2017 (Henshaw *et al.* 2018). However, these efforts were interrupted by an outbreak of PBFD in 2005 (Kundu *et al.* 2012). Unfortunately, management actions for Mauritius parakeets such as cross-fostering offspring between nests, captive rearing and release of chicks between subpopulations, and the aggregation of individuals at supplementary feeding hoppers are thought to increase horizontal BFDV transmission (Raisin *et al.* 2012). Consequently, management actions including the movement of eggs and individuals between sites were ceased and additional, rigorous biosecurity

was implemented at nest sites. Supplementary feeding, however, has been maintained as it is demonstrated to improve fecundity (Tollington *et al.* 2015). Nest site management comprises three elements: (i) wearing medical barrier suits whilst accessing nests, (ii) disinfecting nest sites with an anti-viral solution and (iii) disposing of all nesting material at the end of each season. We test the hypothesis that management will reduce the transmission of BFDV to nestlings by using a reciprocal repeated measures experimental design implemented *in situ*. We also test whether management improves nestling body condition and fledging success.

4.3 METHODS

4.3.1 PBFD and the transmission of BFDV

PBFD is typically characterized by chronic symmetrical feather abnormalities and dystrophy but can also induce severe claw and beak deformities (Latimer *et al.* 1991; Bassami *et al.* 1998; Kondiah, Albertyn and Bragg 2006) and its immunosuppressant nature increases host susceptibility to secondary infection (Ritchie, Anderson and Lambert 2003; Ritchie *et al.* 1989a). BFDV, a member of the Circoviridae family (Ritchie *et al.* 1989a), is considered to demonstrate high environmental persistence owing to its ability to infect a broad range of closely related host species (Peters *et al.* 2014) and is transmissible both horizontally (through contact with contaminated feather dust, surfaces or objects (Ritchie, Anderson and Lambert 2003)), and vertically (from a female to her offspring; Ritchie *et al.* 1989b; Kundu *et al.* 2012). Whilst PBFD can be fatal and most commonly affects birds up to three years of age (Ritchie *et al.* 1989a), infected individuals can recover from acute presentation of the disease (Todd 2000). Other individuals may not display any clinical signs of infection despite carrying the virus (Ritchie *et al.* 1989a). BFDV within Mauritius parakeet nestlings has been continuously monitored by taking blood samples from all 45-day old nestlings produced annually since 2005 (Raisin *et al.* 2012; Tollington *et al.* 2013). In addition to collection of a blood sample in the field, each nestling is given a unique combination of leg bands, is assigned a Studbook ID and has morphometric data collected, including body mass, wing length and tail length.

4.3.2 Experimental design

Two experimental groups were allocated based on natural geographic separation of the population into two sub-populations (Bel Ombre in the South and Camp in the North, Figure 4.1). There is little evidence of natural parakeet dispersal between these subpopulations (Raisin *et al.* 2012) despite regular artificial movements during cross-fostering, captive breeding and release management prior to the initial outbreak of PBFD in 2005. Both sub-populations are found in similar forested and protected habitat within the Black River Gorges National Park and are assumed to face similar climatic conditions, as they are separated by only about 1.8 km. A key difference, however, is that the number of birds is much greater within the northern Camp group (87 vs. 39 known active

natural and artificial nest sites in the 2015/16 breeding season), probably due to the longer and more intense management focus that area has received.

We implemented an experiment over three breeding seasons (2013/14, 2014/15 and 2015/16). This experiment was conducted under the University of Kent ethical guidelines (0018-DF-16) with veterinary consultation and supervision by A. Greenwood, and approved by both the Mauritian Wildlife Foundation and the Mauritius National Parks and Conservation Services. In breeding season one we undertook standard PBFD management in Camp (n = 73 nest sites), involving wearing medical barrier suits whilst accessing the nests, disposing of all old nesting material and disinfecting these nest boxes with a hospital-grade disinfectant selected due to their virucidal efficacy (Royer *et al.* 2001; Martin, Le Potier and Maris 2008) (Virex, comprising a quaternary ammonium chloride base or Virkon, comprising a potassium peroxymonosulphate base, depending on availability) prior to the breeding season. No management measures were applied in Bel Ombre (n = 29 nest sites; Figure 4.1b). In breeding season two these treatments were swapped in a reciprocal design so that PBFD management was undertaken in Bel Ombre (n = 33 nest sites) but not in Camp (n = 74 nest sites; Figure 4.1c). In the final breeding season, 31 nest sites (25% of all active sites) across both sub-populations were selected for treatment to account for any variation between these two groups (Figure 4.1d). In this experiment our treatment refers to where PBFD management is used compared to our control where PBFD management is not. Across both groups all other management actions, including supplementary feeding, remained as normal (Henshaw *et al.* 2014; Henshaw *et al.* 2015; Henshaw *et al.* 2016).

4.3.3. Laboratory analysis

Two methods were used in the laboratory to provide both a viral prevalence dataset as well as an assessment of individual nestling viral load from the nestling blood samples collected. Host and viral DNA, where present, were extracted from 50 to 100 µl of host whole blood using a combination of DIGSOL extraction buffer and 10 mg/mL proteinase K (Bruford *et al.* 1998). Extractions were quantified using a Qubit dsDNA Assay Kit and standardised to approximately 25 ng/µl prior to screening for BFDV through standard PCR (with a known false-negative error rate of 49.5% (CI 39.8 – 60.2%); D. Fogell, Unpublished data), and to 10 ng/µl for quantification using real-time PCR (rtPCR) (with an estimated minimum detection threshold of 1×10^2 copies of viral DNA per reaction; Katoh, Ohya and Fukushi 2008).

Standard PCR protocols used to detect BFDV infection status of an individual were as detailed in Kundu et al. (2012). In brief, the PCR assay targeted a 717-bp region of the r*eplicase* gene (Ypelaar *et al.* 1999) and comprised 1 µl of extracted host DNA template, 5 µl MyTaq™ HS Red Mix (Bioline), 0.2 µl each of the forward and reverse primers at 10 pmol/µl and was made up to

10 µl with double-distilled water. PCR annealing temperature was adjusted to 60^{o}C, as per manufacturer's guidelines, for 30 cycles and products were visualized on a 1.5% agarose gel. Both a known BFDV positive Mauritius parakeet sample and a negative control were included in each PCR batch.

For rtPCR protocols an assay also targeting the *replicase* gene was used to quantify individual viral load (Tollington *et al.* 2018), with each reaction consisted of 10 µl iTaq Universal Probes Supermix (Bio-Rad Inc.), 0.8 µl of each of the forward (5'-TGGGTGGCTACCTTATTG-3') and reverse (5'-GGCTTATTGCTCGTGATAA-3') primers, 0.2 µl of a FAM-labelled fluorescent probe (5'FAM-CTCTGCGACCGTTACCCACA-3'TAM), 5 µl of DNA template and made up to 20 µl with double-distilled water. Cycle conditions were as follows: initial denaturation of 5 min at 95°C; followed by 40 cycles of: 5 s at 95°C and 30 s at 60°C. All 96-well plates included two positive controls from a high viral load Mauritius parakeet individual (amplification at ~10 cycles) for the purposes of standardisation between runs and two negative controls to ensure no contamination was present. Each individual was run in duplicate. If the repeats did not amplify within one PCR cycle of one another, a third replicate was performed. The averaged C_T values for each individual were then converted into a relative estimate of viral load (Eastwood *et al.* 2015) using the equation: Viral load = $2^{(-\Delta CT)}$

4.3.4 Data analysis

4.3.4.1 Viral prevalence

Using the data generated from standard PCR, generalised linear mixed models (GLMMs) were run with the lme4 (Bates *et al.* 2015) package in R version 3.4.3 (R Core Team 2017) using a binomial response variable accounting for the number of BFDV-positive and -negative nestlings per nest site, and setting a binomial error distribution and a logit link function (Tollington *et al.* 2015). To thoroughly investigate efficacy of management we also included the long-term data on nestling infection with BFDV systematically collected across both sub-populations between 2009 and 2013, where BFDV management was always applied (Supplementary Table 4.2). We evaluated a set of candidate models investigating the effects of three management related factors on the proportion of BFDV infected nestlings per brood (binomial response variable given by number of BFDV-positive nestlings to the number of negative nestlings tested): distance to the nearest feeding hopper (km), distance to the nearest neighbouring nest site (km) and our experimental treatment. Female parent and breeding season were used as random intercept effects to account for both the vertical and horizontal viral transmission pathways (as females generally nest at the same site year on year) and for any abiotic variation between breeding seasons. We were aware that each sub-population had a different placement of feeding hoppers relative to nests sites, resulting in differences in the

likelihood that breeders would use them (Camp, mean distance nest to feeding hopper = 0.76 ± 0.08 km (SE); Bel Ombre, mean distance nest to feeding hopper = 2.38 ± 0.14 km (SE); $t(240) = 18.06$, $p < 0.001$; Figure 4.1) (Tollington *et al.* 2013). Given the difference in proximity to feeding hoppers between sub-populations and previous indications that feeding hoppers are another potential site of human-influenced BFDV transmission, we included an interaction between treatment and distance to nearest feeding hopper in the candidate model set. Sub-population, controlled for in the experimental design, was inherently linked with year and treatment so was therefore not included as a factor in the model set. We selected the most parsimonious model based on the lowest Akaike's information criterion corrected for finite sample size (AIC_C). As more than one model was within 2 delta AIC_c, and therefore equally plausible, we used model averaging (*AICcmodavg* package; Mazerolle 2016) to estimate predicted parameter values.

4.3.4.2 Individual viral load

For the assessment of individual viral load derived from the qPCR data, GLMMs were run using the same response variables as for the viral prevalence dataset and spanned the three experimental breeding seasons from 2013 to 2016. Viral load values were logged and a Gaussian distribution was used, including both female parent and breeding season as random intercept effects (Chapter 3). We selected the most parsimonious model based on the lowest AIC_c.

4.3.4.3 Nestling fitness impacts

GLMMs were run on two parameters to assess potential population impacts of biosecurity protocols on productivity and individual fitness across the three experimental breeding seasons (Supplementary Table 4.3). The first set of candidate models evaluated the effects of distance to nearest feeding hopper (km), treatment and both the linear and quadratic terms for dam age on the proportion of nestlings fledged (n = 311 nest sites), using a Gaussian distribution and with female parent and breeding season used as random intercept effects. Viral load was not assessed as a factor to avoid bias in results due to the deficit of data from nestlings that didn't survive to the point of sampling. We developed a second set of candidate models to assess the impacts of distance to nearest feeding hopper (km), treatment, both the linear and quadratic terms for dam age and logged viral load on body mass (g) (n = 559 fledglings), with wing length (cm) used to correct for body size (Bergan and Smith 1993). Female parent and breeding season were used as random intercept effects to account for variability across broods due to abiotic or genetic factors.

4.4 RESULTS

4.4.1 Viral prevalence and load

For the binomial probability of infection in nestlings we found two equally supported candidate models that included the additive effects of treatment, distance to nearest feeding hopper, the interaction between these two factors, as well as the additive effect of distance to the nearest neighbour (Table 4.1a, Figure 4.2). When the interaction between treatment and distance to nearest feeding hopper was explored, it indicated that the probability of nestling infection with BFDV was lower both when the distance to supplementary feeding hopper was greater and when nest site management is done (Figure 4.3). Prevalence of BFDV-infected nestlings across years and with current BFDV nest site management was 13.9% (SE ± 5.31%) and our experimental models estimated this to be, on average, 11% lower than if no management was applied. However, we found no strong links between management actions and individual nestling viral load, with the null model as most parsimonious (Table 4.1b, Supplementary Table 4.1b). So, whilst management reduced the proportion of nestlings infected with BFDV it had no apparent impact on individual infection intensity.

4.4.2 Nestling fitness impacts

Fledging success was determined by the additive effects of treatment, distance to nearest feeding hopper and dam age (two equally supported models; Table 4.2). Counter to expectations there was a greater proportion of chicks fledged from control nests (i.e. those not managed with BFDV biosecurity; 83% vs. 79%), although only the interaction between treatment and the distance to nearest feeding hopper was found to be a significant predictor of the probability of fledging (Odds ratio = 0.49, 95% CI 0.29 – 0.80; Figure 4.4, Supplementary Table 4.1c). Whereas there was a clear decline in the probability of fledging success with distance away from feeding hoppers in managed nests this was not apparent in control nests. This pattern was found to be consistent across age cohorts, but with older females experiencing a steeper decline with increasing distance from feeding hopper in treatment sites, and an overall lower probability of fledging success than younger females (Figure 4.4). Only a single model determined fledgling body condition. This model included all of the assessed variables (Table 4.3), none of which were found to be predictive of nestling body condition (95% CIs overlap 0, Supplementary Table 4.1d).

4.5 DISCUSSION

Our results illustrate the complexity of applying disease management strategies in the context of endangered species conservation, and the vital importance of critically evaluating the effectiveness of actions. We found evidence that nest site management led to a small reduction in the probability of a brood becoming infected with BFDV, although the same management was not

found to affect BFDV load or the body condition of chicks. Conversely, we found that nest management does not enhance Mauritius parakeet recovery and may even hinder it (albeit by a small amount on otherwise high fecundity). Our experiment does not provide an explanation for the lower fecundity in managed nests, but we suggest two possibilities; that the chemical treatments, as used, may negatively affect parakeet eggs and nestlings or, perhaps, that the longer processing times required with the biosecurity protocols add to nest disturbance. Indeed, both Virkon specifically and quaternary ammonia-based disinfectants have been shown to impact on shell porosity when applied directly to eggs, thus reducing their hatchability (Wilson 2009; Scott, Swetnam and Kinsman 1993). Given our results, we recommend a change to current management, possibly beginning with an experimental reduction in the number of nests managed, or with a shortening of biosecurity protocols to reduce potential stress. However, since the results are relative to the conditions of our study, we also caution against a general interpretation that biosecurity is not important. Rather we suggest that the current method is not achieving its intended purpose.

It is clear that there may be benefits to population productivity in increasing the number of supplementary feeding hoppers available, and thus decreasing the average distance between nest sites and hoppers. However, BFDV prevalence was also driven partly by the proximity of nests to feeding hoppers. Parents nesting closer to feeding hoppers and aggregating around them may be facilitating BFDV transmission through increased contact rates (Tollington *et al.* 2013). Supplementary feeding stations are known to facilitate pathogen transmission across a broad range of host species globally and their use should be carefully managed to ensure they are beneficial in species recovery (Murray *et al.* 2016; Ewen *et al.* 2014).

The value of assessing EID management options through experimental evaluation is also illustrated by a handful of recent attempts at *in situ* management of amphibian chytridiomycoses. For example, despite the initial success of trials to reduce mortality through repeated anti-fungal treatment of *Batrachochytrium dendrobatidis* infection in the mountain chicken frog (*Leptodactylus fallax*), these benefits were lost on cessation of treatment (Hudson *et al.* 2016). Whilst the main objective of clearing infection was temporarily met, from the broader conservation perspective the fundamental objective of population recovery was unachievable in the long term. Conversely, in a simplified system with a single host and the ability to also treat the surrounding environment, experimental evaluation showed the beneficial outcomes of *B. dendrobatidis* management might be sustainable in Mallorcan midwife toads (*Alytes muletensis*) (Bosch *et al.* 2015). When considering management options for *Batrachochytrium salamandrivorans* in fire salamanders (*Salamandra salamandra*), models showed that even treatment actions that led to considerable increases in survival or reductions in transmission were unlikely to be effective in the long term and, in fact,

prolonging survival of infected individuals may instead encourage pathogen transmission and worsen population-level impacts (Canessa *et al.* 2018). Our experimental results and these examples clearly illustrate two important messages related to the management of EIDs in wildlife conservation.

Firstly, in the crisis scenarios commonly faced by critically endangered species, initial decisions about disease risk management inevitably draw on available knowledge and expert opinion from wildlife health professionals (Sainsbury and Vaughan-Higgins 2012). Advisory panels often combine very different experiences (such as zoo veterinarians and field rangers), and actions may be extrapolated from different contexts (e.g. *ex-situ* treatments applied in the wild) (Hartley and Sainsbury 2017). For example, in Mauritius parakeets, the initial decision of applying biosecurity and feeding was made under the assumptions that treatments known to reduce infection would be beneficial for population persistence. Given the critical status of the species and the potentially severe threat posed by BFDV, the initial decision to apply disinfection protocols was urgently required and therefore necessarily conservative.

Although such limitations are a necessity when initiating recovery programs, decisions can be re-evaluated critically by monitoring the outcomes of implemented actions (Jakob-Hoff *et al.* 2014), yet such re-evaluations are surprisingly rare (Hudson *et al.* 2016; Bosch *et al.* 2015; Canessa *et al.* 2018). Not measuring the efficacy of actions thought to reduce transmission of EIDs reflects a general pattern of poor integration of strategic monitoring in management (Ewen, Soorae and Canessa 2014; Nichols and Williams 2006); something that frequently leads to suboptimal conservation and the development of conservation dogmas (Martínez-Abraín and Oro 2013). In our study system, the evaluation of nest management provided by this study has led us to reconsider whether to continue the intensive biosecurity protocols, which we had assumed were necessary for Mauritius parakeet persistence.

Secondly, monitoring the effectiveness of management must maintain focus on the fundamental objective of that management. In our case, management aimed to reduce the transmission of BFDV. However, BFDV in itself was considered important because of its potential negative effects on the fundamental management objective, the recovery of the threatened host species. In this sense, reducing the prevalence and load of BFDV represents a means objective to species recovery, but one that is surrounded by substantial uncertainty in the way BFDV is transmitted, the risks it poses to the Mauritius parakeets and our ability to manage it. Our experiment suggested nest management could provide a small (on average 11%) reduction in the probability of infection of a brood with BFDV. If the evaluation focused exclusively on the target of BFDV prevalence, nest management may thus appear desirable. However, this marginal benefit might be offset by the tendency of managed nests to have lower fledging success (a component

vital rate of population growth). Such a trade-off clearly illustrates why incorrectly focusing monitoring on means objectives can increase the risk of suboptimal conservation outcomes (Ewen, Soorae and Canessa 2014).

Both poor monitoring of management outcomes and a tendency to focus on means objectives can be addressed through a better placement of science within management decision making. The emergence of BFDV in numerous wild populations has led to a substantial contribution of interesting and valuable research (Jackson *et al.* 2014; Regnard *et al.* 2014; Peters *et al.* 2014), yet managers remain uncertain on how best to respond. Our experiment was a direct response to manager requests to critically review long-running and increasingly demanding nest site management (over 13 years with a population that increased in size from 39 known breeding pairs in 2004 to 102 pairs by the start of our experiment (Henshaw *et al.* 2014)). We have not explicitly considered the logistic and financial cost of management but, as this is a substantial burden on the recovery program, it should be rewarded with improved conservation outcomes. Rather than simply measure BFDV, we also distinguished the means and fundamental objectives driving management of this EID. Structuring conservation science within management decision making ensures research findings are not only interesting, but relevant.

Faced with an increasing frequency of EIDs, managers need to make hard decisions about whether to alter management to reduce their spread or impact. Frustratingly, in the crisis scenarios that many endangered species face, these choices often need to be made quickly and in the face of substantial uncertainty. Given the high risks to populations or species from making the wrong choice (e.g. extinction) it is essential to evaluate whether management is achieving predicted outcomes. Targeted monitoring and, where possible, manipulation of the focal systems provides a powerful framework to advance threatened species conservation. When making these choices managers should carefully compare consequences against fundamentally important objectives, usually linked to the recovery of the host species.

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4.7 TABLES AND FIGURES

Table 4.1 A comparison of the ten generalised linear models analysing a.) the predicted probability of BFDV infection in 45-day old Mauritius parakeet nestlings over seven breeding seasons (2009/10 to 2015/16), and b.) individual BFDV load in 45-day old Mauritius parakeet nestlings over the three experimental breeding seasons (2013/14 to 2015/16). Management factors related to BFDV prevalence include treatment (T), distance to the nearest supplementary feeding station (SF) and distance to nearest neighbouring nest site (NN) based on Akaike's information criterion corrected for finite sample size (AIC_c) and weights (AIC_c weights). All models were run with the nesting female and breeding season as random intercept effects. K denotes the number of parameters in each model and models are ranked according to their ΔAIC_c.

Rank	Model	K	AIC_c	ΔAIC_c	AIC_c weights
a.)					
1	T + SF + NN	6	764.25	0.00	0.66
2	T + SF + NN + T*SF	7	766.18	1.94	0.25
3	T + NN	5	769.43	5.19	0.05
4	T + SF	5	771.14	6.89	0.02
5	SF + NN	5	771.95	7.70	0.01
6	SF + T + T*SF	6	773.16	8.91	0.01
7	NN	4	775.27	11.03	0.00
8	SF	4	778.97	14.73	0.00
9	T	4	779.83	15.58	0.00
10	Null model	3	785.42	21.17	0.00
b.)					
1	Null model	4	-1817.35	0.00	0.98
2	SF	5	-1808.64	8.72	0.01
3	T	5	-1806.27	11.08	0.00
4	NN	5	-1806.07	11.28	0.00
5	T + SF	6	-1797.50	19.86	0.00
6	SF + NN	6	-1796.81	20.54	0.00
7	T + NN	6	-1794.96	22.40	0.00
8	SF + T + T*SF	7	-1786.76	30.59	0.00
9	T + SF + NN	7	-1785.65	31.70	0.00
10	T + SF + NN + T*SF	8	-1774.94	42.42	0.00

Table 4.2 A comparison of the ten generalised linear models analysing the probability of fledging success of Mauritius parakeet nestlings over the three experimental breeding seasons (2013/14 to 2015/16). Factors related to fledging success include treatment (T), distance to the nearest supplementary feeding station (SF) and the linear (F) and quadratic terms (F2) of dam age based on Akaike's information criterion corrected for finite sample size (AIC_c) and weights (AIC_c weights). All models were run with the nesting female and breeding season as random intercept effects. K denotes the number of parameters in each model and models are ranked according to their ΔAIC_c.

Rank	Model	K	AIC_c	ΔAIC_c	AIC_c weights
1	SF + T + F + F2 + T*SF	8	446.90	0.00	0.42
2	SF + T + T*SF	6	448.22	1.32	0.22
3	F + F2	5	450.27	3.37	0.08
4	SF + F + F2	6	450.28	3.38	0.08
5	Null model	3	451.28	4.38	0.05
6	T + F + F2	6	451.46	4.56	0.04
7	SF	4	451.55	4.64	0.04
8	SF + T + F + F2	7	451.90	5.00	0.03
9	T	4	452.48	5.57	0.03
10	SF + T	5	453.12	6.22	0.02

Table 4.3 A comparison of the 16 generalised linear models analysing body condition ($^{mass}/_{wing\ length}$) of Mauritius parakeet nestlings over the three experimental breeding seasons (2013/14 to 2015/16). Factors related to body condition include treatment (T), distance to the nearest supplementary feeding station (SF), individual BFDV load (VL) and the linear (F) and quadratic terms (F2) of dam age based on Akaike's information criterion corrected for finite sample size (AIC_c) and weights (AIC_c weights). All models were run with the nesting female and breeding season as random intercept effects. K denotes the number of parameters in each model and models are ranked according to their ΔAIC_c.

Rank	Model	K	AIC_c	ΔAIC_c	AIC_c weights
1	T + VL + F + F2 + SF + T*SF	10	4672.95	0.00	0.79
2	T + VL + F + F2 + SF	9	4676.98	4.03	0.11
3	T + VL + F + F2	8	4678.43	5.47	0.05
4	VL + F + F2 + SF	7	4679.41	6.46	0.03
5	VL + F + F2	7	4680.71	7.76	0.02
6	T + F + F2 + SF + T*SF	9	4778.75	105.80	0.00
7	T + F + F2 + SF	8	4782.68	109.73	0.00
8	T + F + F2	8	4803.71	130.76	0.00
9	F + F2	6	4821.25	148.29	0.00
10	T + VL + SF + T*SF	8	4935.54	262.59	0.00
11	T + VL + SF	7	4939.14	266.19	0.00
12	T + VL	6	4939.79	266.83	0.00
13	VL	5	4942.37	269.42	0.00
14	T	5	5062.80	389.85	0.00
15	SF	5	5064.72	391.77	0.00
16	Null model	4	5082.66	409.71	0.00

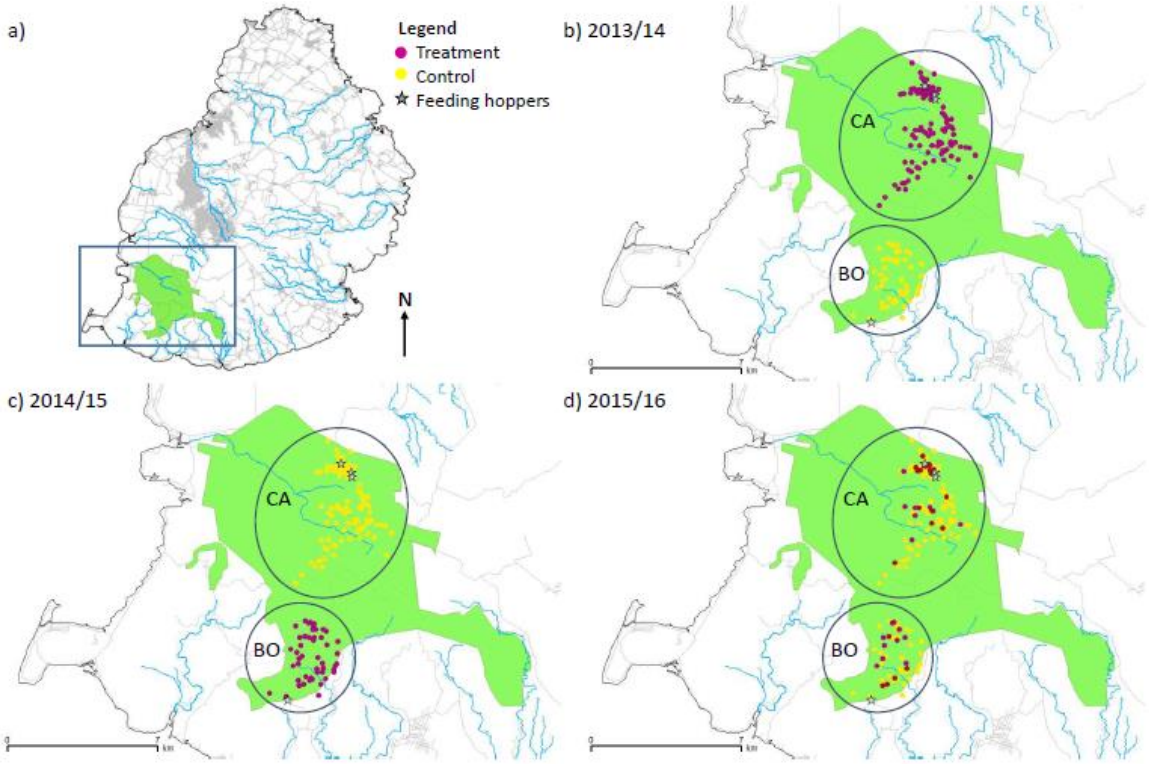

Figure 4.1 a.) The location of the remaining Mauritius parakeet breeding populations in the Black River Gorges National Park in the south-west of Mauritius, b.) the 2013/14 breeding season experimental design, c.) the 2014/15 breeding season reciprocal experimental design, and d.) the 2015/16 breeding season mixed experimental design. CA = Camp, BO = Bel Ombre.

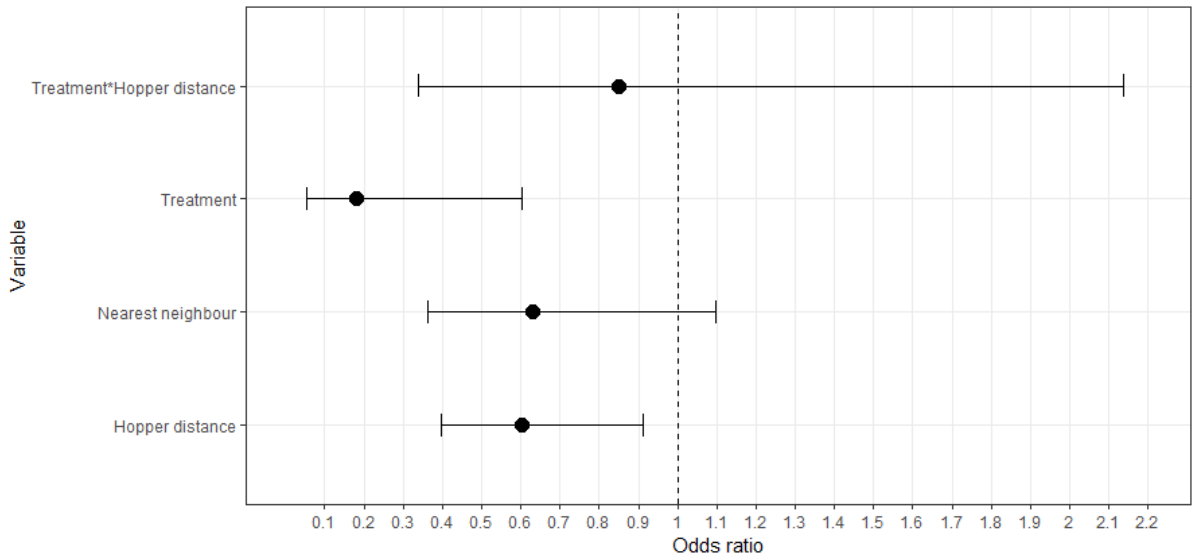

Figure 4.2 The association of treatment, distance to nearest feeding hopper, distance to nearest neighbouring nest site and the interaction between treatment and distance to nearest feeding hopper with the probability of BFDV infection in 45-day old Mauritius parakeet nestlings produced over the three experimental breeding seasons. Variable specific odds ratios are denoted by the filled circles along with their associated 95% CIs.

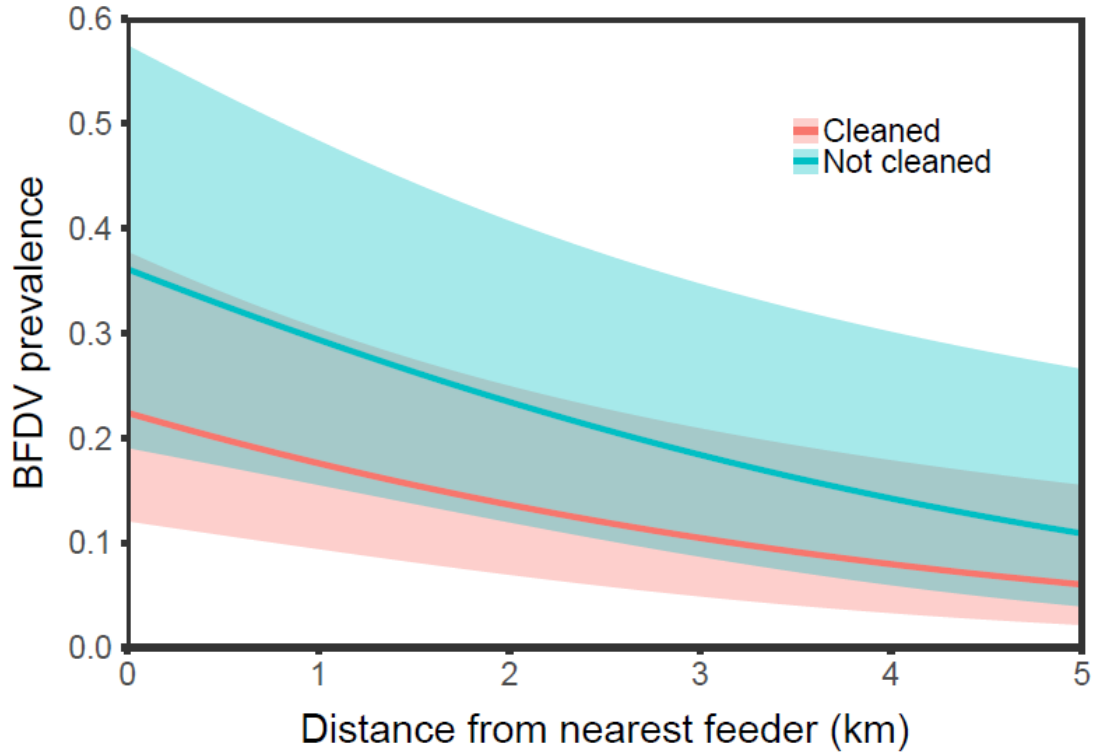

Figure 4.3 Predicted probability of Mauritius parakeet nestlings becoming infected with BFDV as a result of nest site treatment with increasing distance from the nearest feeding station, with female parent and breeding season specified as random intercept effects. Shaded areas are 95% prediction intervals.

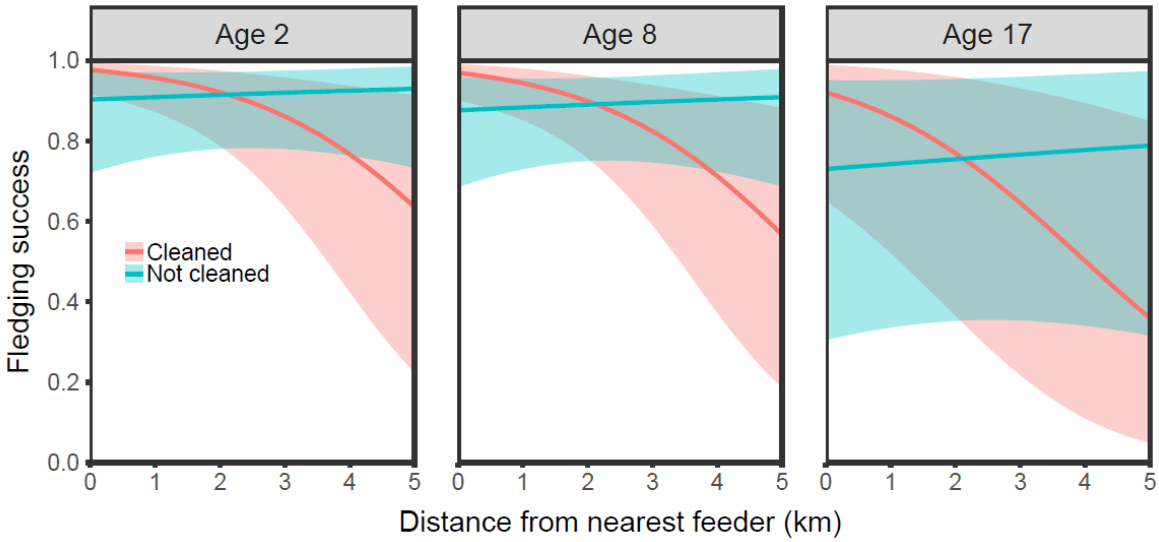

Figure 4.4 Predicted probability of Mauritius parakeet nestlings fledging as a function of nest site treatment and increasing distance from supplementary feeding hoppers. Panels indicate predicted probabilities over the experimental breeding seasons in breeding females across three discrete age cohorts (5, 7 and 11 years corresponding approximately to the 25th, 50th and 75th quantile of the distribution of age of birds in our dataset), with female parent and breeding season specified as random intercept effects. Shaded areas are 95% prediction intervals.

Supplementary Table 4.1 A summary of model averaged coefficients and effect sizes for the generalised linear mixed effect candidate models analysing a.) the probability of BFDV infection in 45-day old Mauritius parakeet nestlings over seven breeding seasons (2009/10 to 2015/16), b.) individual BFDV load, c.) probability of fledging success, and d.) body condition ($^{mass}/_{wing\ length}$) of Mauritius parakeet nestlings over the three experimental breeding seasons (2013/14 to 2015/16).

Factor	Model Estimate	95% Confidence Interval
a.		
Treatment	-0.74	-1.26 – -0.22
Nearest hopper	-0.22	-0.40 – -0.04
Nearest neighbour	-0.20	-0.44 – 0.04
Treatment*Nearest hopper	-0.07	-0.47 – 0.33
b.		
Treatment	0	-0.01 – 0.01
Nearest hopper	0	-0.01 – 0
Nearest neighbour	0	-0.01 – 0
Treatment*Nearest hopper	0	-0.01 – 0
c.		
Treatment	1.3	-0.04 – 2.63
Nearest hopper	0.02	-0.39 – 0.42
Treatment*Nearest hopper	-0.71	-1.2 – -0.22
Dam Age	1.12	-0.48 – 2.73
$(\text{Dam Age})^2$	-1.47	-3.08 – 0.15
d.		
Treatment	-1.95	-7.79 – 3.89
Nearest hopper	-2.86	-6.46 – 0.75
Treatment*Nearest hopper	4.36	-1.24 – 9.96
Log(Viral Load)	0.18	-2.59 – 2.95
Dam Age	5.92	-8.31 – 20.14
$(\text{Dam Age})^2$	-7.64	-21.75 – 6.46

Supplementary Table 4.2 A summary of the brood-level prevalence of BFDV in 45-day old Mauritius parakeet nestlings over seven breeding seasons (2009/10 to 2015/16) and individual Mauritius parakeet nestling BFDV load over the three experimental breeding seasons (2013/14 to 2015/16) broken down by subpopulation and treatment.

Breeding season	**Total nestlings screened**	**Total nest sites**	**Mean brood prevalence**		**Nestling viral load (Min – Max)**	
			Treated	**Untreated**	**Treated**	**Untreated**
Bel Ombre						
2009/10	27	13	0.10	*NA*	*NA*	*NA*
2010/11	24	14	0.35	*NA*	*NA*	*NA*
2011/12	19	12	0.28	*NA*	*NA*	*NA*
2012/13	46	23	0.00	*NA*	*NA*	*NA*
2013/14	53	23	*NA*	0.45	*NA*	1.03E-05 (7.05E-08 – 2.22E-04)
2014/15	58	28	0.03	*NA*	2.03E-03 (0.00E+00 – 1.19E-01)	*NA*
2015/16	73	35	0.00	0.00	6.48E-09 (0.00E+00 – 1.30E-07)	4.83E-09 (0.00E+00 – 1.05E-07)
Camp						
2009/10	104	48	0.30	*NA*	*NA*	*NA*
2010/11	98	48	0.40	*NA*	*NA*	*NA*
2011/12	97	49	0.25	*NA*	*NA*	*NA*
2012/13	102	54	0.05	*NA*	*NA*	*NA*

2013/14	101	46	0.39	*NA*	5.84E-02 (0.00E+00 – 2.07E+00)	*NA*
2014/15	141	63	*NA*	0.28	*NA*	3.00E-02 (0.00E+00 – 3.23E+00)
2015/16	131	64	0.04	0.08	3.49E-03 (0.00E+00 – 1.29E-01)	4.67E-02 (0.00E+00 – 2.24E+00)

Supplementary Table 4.3 A summary of the body condition ($^{mass}/_{wing\ length}$) and fledging success of Mauritius parakeet nestlings over the three experimental breeding seasons (2013/14 to 2015/16) broken down by treatment.

Breeding season	Body condition (Min – Max)		Fledging success	
	Treated	Untreated	Treated	Untreated
2013/14	1.32 (0.90 – 1.87)	1.24 (0.88 – 1.85)	0.67	0.81
2014/15	1.33 (0.92 – 3.14)	1.17 (0.86 – 1.83)	0.92	0.83
2015/16	1.38 (0.90 – 3.27)	1.31 (0.93 – 2.15)	0.97	0.83

Chapter 5

Limited success and unintentional consequences of *in situ* management of beak and feather disease virus in a highly threatened parrot

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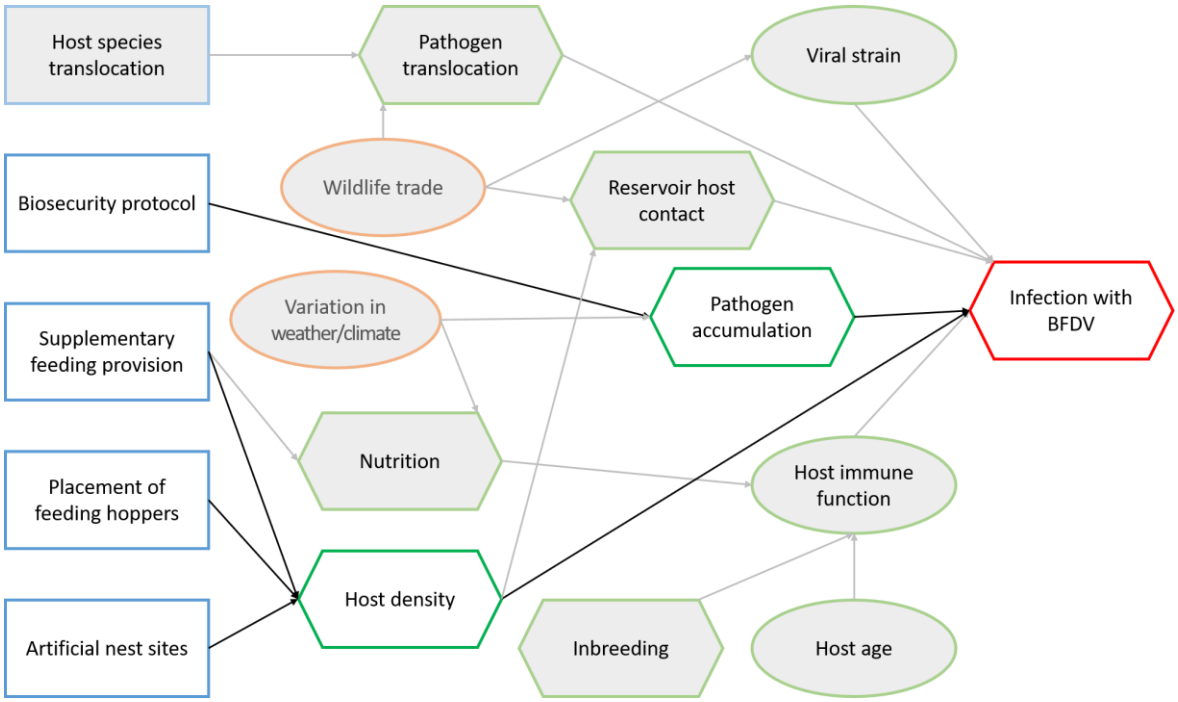

5.1 ABSTRACT

Conservationists are often forced to respond rapidly to emerging infectious diseases (EIDs) in wild populations. Decisions are regularly based in uncertainty and subsequent monitoring and evaluation of the selected response is often neglected. Using eDNA and RT-PCR techniques we present an evidence-based approach for assessing the individual and interacting effects of *in-situ* biosecurity management for Beak and feather disease virus infection (BFDV). BFDV, the etiological agent for Psittacine beak and feather disease, is one of the most common and emergent viruses in wild parrots (Psittaciformes) that affects numerous threatened species globally. We ran a crossed experimental design on biosecurity at two key points of management support in the Mauritius parakeet recovery programme; nest sites and supplementary feeding hoppers. We found that whilst disinfection of hoppers significantly reduced the BFDV viral load present on their surfaces, this effect was brief and all traceable quantities were only removed in 25% of applications. The intensification of disinfection at hoppers throughout the breeding season had a greater negative impact on the probability of eggs hatching (58% vs. 67% at lower intensity biosecurity hoppers) than a single disinfection of the nest site prior to the parakeets nesting (65% at disinfected vs. 68% at untreated sites). Our results illustrate the challenges faced by conservationists battling with reducing the transmission of infectious disease in wild populations, and highlight the vital importance of constant monitoring and evaluation of management strategies to avoid dogmatic approaches. Whilst biosecurity remains prudent within conservation, solutions targeted at BFDV remain a challenge.

5.2 INTRODUCTION

Emerging infectious diseases (EIDs) are key contributors to the current global biodiversity crisis (Yap *et al.* 2015; Brooks and Ferrao 2005). While infectious pathogens are an integral mechanism for evolutionary change within natural populations (Lyles and Dobson 1993), the emergence of novel pathogens may increase the risk of extinction for vulnerable species and populations (Lips *et al.* 2006). When faced with EIDs in wild populations, conservationists are often forced to respond rapidly, with decisions based on limited or uncertain information (Campbell Grant *et al.* 2017; Canessa *et al.* 2018). Whilst novel disease outbreak situations necessitate a response, a clear decision-making process is rarely followed when attempting mitigation. Subsequent monitoring and evaluation as to whether the selected response was the most appropriate solution is also often neglected (Fogell *et al.* 2019). However, monitoring the efficacy of response to an EID in a wildlife population is a necessity to provide sound, evidence-based management and increase our knowledge of *in situ* pathogen ecology. This allows conservationists to assess whether the current implemented measures have met the expected outcomes or could be improved to better meet the fundamental objectives of management (Campbell Grant *et al.* 2017; Canessa *et al.* 2018).

When the cause of population decline is unknown, a suite of exploratory intervention tools is available to conservation managers. Common tools used in the recovery and management of wildlife population include the control of introduced predators (Miskelly and Powlesland 2013; Jones *et al.* 2016), captive breeding, hand rearing and translocation (Jones 2004; Deguchi *et al.* 2014), reducing inbreeding depression (Weeks *et al.* 2011; Armstrong and Seddon 2008), provision of artificial breeding sites (Norris *et al.* 2018; Sherley *et al.* 2012; Tatayah *et al.* 2007) and the provision of supplementary food (Walker *et al.* 2013; Oro *et al.* 2008). Some of these recovery tools may (directly or indirectly) increase the contact rate between individuals, thereby altering the dynamics of infectious disease. Those that artificially alter the density of individuals within a landscape, such as the provision of food or water stations and breeding sites, may cause more frequent aggregation of individuals than if forage or breeding territory selection was natural (Lawson *et al.* 2012; Sorensen, van Beest and Brook 2013). If adequate biosecurity protocols are not thoroughly adhered to then congregating individuals may be exposed to the pathogens shed from a single infected individual (Corn and Nettles 1995). However, there is often substantial uncertainty in whether biosecurity reduces this risk or, worse, whether biosecurity measures move recovery projects further away from achieving their objectives. For example, in the situations where strict quarantine and health assessment for conservation translocation has to balance against increased stress that may reduce post-release survival (Dickens, Delehanty and Romero 2009; Armstrong and Seddon 2008).

Viruses are one group of pathogens whose transmission may be unintentionally facilitated by conservation action. Viruses are responsible for over 40% of all recently surveyed wildlife EIDs (Dobson and Foufopoulos 2001; Tompkins *et al.* 2015), and have thus been highlighted as a particular threat to wildlife. Psittacine beak and feather disease (PBFD), caused by the Beak and feather disease virus (BFDV; Circoviridae), is thought to be the most common viral disease in wild psittaciformes (Khalesi *et al.* 2005). PBFD has been reported in both wild and captive parrot populations since the mid-1970s and has been found to be widely infectious and often fatal, known to affect 60 Old and 18 New World psittacine species globally (Fogell, Martin and Groombridge 2016). The virus has recently been detected in wild parrot populations across eight new countries (Fogell *et al.* 2018), as well as in a number of non-psittacine hosts (Amery-Gale *et al.* 2017; Sarker *et al.* 2016; Sarker *et al.* 2015).

Few management actions have been developed and tested to manage the transmission of BFDV *in situ*, most of which have focused on hygiene and biosecurity. In Australia, for example, a detailed Threat Abatement Plan for BFDV includes the use of disinfectants in nest and transport boxes (Department of the Environment and Heritage 2005). However, the same Threat Abatement Plan also notes that there is no assurance as to whether recommended actions will actually reduce transmission. A recent study by Fogell *et al.* (2019) provided the first empirical assessment of the efficacy of biosecurity protocols applied to nest sites in an attempt to reduce BFDV prevalence in the wild Mauritius parakeet (*Psittacula eques*) population. They found that, despite a reduction in the probability of nestling infection with BFDV, there was also an unintentional negative impact on breeding success, the fundamental objective of nest management. However, providing nest sites is only one of the suite of conservation interventions where increased disease transmission may occur, and could potentially be controlled. Supplementary feeding hoppers (henceforth referred to as hoppers), also provided for Mauritius parakeets, are subject to strict biosecurity controls targeting BFDV. As in all recovery programmes, the efficacy of biosecurity interventions at all points of application should be evaluated to ensure they are achieving stated conservation objectives.

Here we present an evidence-based approach for quantifying the risks of BFDV infection associated with the two points of management support in the Mauritius parakeet recovery programme. Furthermore, we experimentally assess the efficacy of biosecurity protocols to reduce this risk. Such an evaluation is extremely rare within conservation. Our approach is a combination of applying eDNA and RT-PCR techniques to quantify environmental pathogen accumulation at hoppers, alongside assessing reproductive success and BFDV prevalence in nestlings produced by Mauritius parakeets. Using a fully crossed experimental field design we are able to quantify the individual and interacting effects of biosecurity management at hoppers and nest sites.

5.3 METHODS

Mauritius parakeets (*Psittacula eques*) were once widely distributed across the tropical rainforest habitat of Mauritius. They are now confined to the Black River Gorges National Park (BRGNP), in the southwest, and a newly established sub-population in a private nature reserve (Le Vallée de Ferney), to the east of the island (Figure 5.1). Within the BRGNP the parakeet population is divided into two sub-populations, isolated by distance (Raisin *et al.* 2012), with one in the north of the reserve and one in the south (Figure 5.1). Mauritius parakeets have recovered from a severe population bottleneck, after declining to fewer than 20 individuals, through intensive management measures including brood manipulation, supplementary feeding, provision of artificial nest sites, captive-breeding, reintroduction, and control of invasive alien predators (Bunbury *et al.* 2007; Tatayah *et al.* 2007). However, these efforts were interrupted by an outbreak of PBFD in 2005 (Kundu *et al.* 2012). The disease outbreak was considered a threat to the parakeet's recovery, prompting the immediate cessation of some elements of their management such as the transfer of individuals and eggs between nest sites, whilst the provision of artificial nest boxes, control of alien predators, the use of hoppers and a minimal regime of visits to nest sites for monitoring purposes remained in place (Tollington *et al.* 2013). However, two management activities were considered high risk for continued spread of infection: hopper and nest box maintenance. Therefore, since 2005, the Mauritius parakeet field team has attempted to reduce or eliminate any potential human-mediated transmission of BFDV through biosecurity applied at nest sites and hoppers (see Supplementary Methods for details).

5.3.1 Supplementary feeding hopper design

Mauritius parakeets make use of three hopper designs. The first two are purpose built for parakeets; one is a small box-shaped PVC container with a hinged lid and attached to a wooden backing with a perch (Supplementary Figure 5.1a), the other is a "J-shape" dispenser made out of PVC drainpipe and a plastic lid to prevent water from entering. These hoppers are attached above wooden perches, in pairs, to a large PVC pipe with brackets (Supplementary Figure 5.1b), are easily disassembled for disinfection, and are filled with parrot pellets (KayteeExact Parrot Pellets; Kaytee Products Inc.) and maize. The third hopper is designed for use by the Mauritius pink pigeon (*Nesoenas mayeri*) and is supplied with wheat and maize. These hoppers consist of conical lids fitted over a cylindrical dispenser with a bowl-shaped base, made from galvanised metal, and attached to a wooden stand with a circular, rope-covered perch (Supplementary Figure 5.1c). Only the hoppers are easily disassembled for disinfection and the perches remain fixed to the stands.

Within the northern BRGNP subpopulation, hoppers are set up at three locations: Camp, Plateau Todd and Bris Fer (Figure 5.1). At Camp, both parakeet and pigeon hoppers are situated together in a large forest clearing and within field aviaries situated in this clearing, with food

provisioned throughout the year. Plateau Todd and Bris Fer are only utilised over the breeding season (from September to March) and consist of the first two, parakeet-only hoppers, which are located in small forest clearings. Within the southern BRGNP subpopulation, only a single location is used (Bel Ombre, Figure 5.1), with a mix of all three hoppers provided in both field aviaries and outside these aviaries within a forest clearing.

5.3.2 Experimental biosecurity protocol design

As previously discussed, biosecurity at nests since the BFDV outbreak has involved an annual, post-breeding, cleaning and disinfection of nest boxes (Supplementary Methods 1). Biosecurity at hoppers since the BFDV outbreak has involved disinfection of the two parakeet-only designs weekly and the pigeon design fortnightly (Supplementary Methods 2). In all cases, Virex or Virkon (depending on availability) is used for disinfection. Both disinfectants were selected for their virucidal efficacy (Royer *et al.* 2001; Martin, Le Potier and Maris 2008) and were made up according to manufacturer's guidelines (6 g/L solution of Virkon, comprising a potassium peroxymonosulphate base, or a 5 g/L solution of Virex, comprising a quaternary ammonium chloride base).

Our experiment was done over the 2016/2017 breeding season to test whether up-scaling biosecurity at hoppers would reduce the environmental accumulation of BFDV on their surfaces, and thus reduce the prevalence of infection in Mauritius parakeet nestlings. Hoppers were assigned to one of two treatments which were separated spatially. The first was an enhanced biosecurity protocol (experiment) applied at Camp and Plateau Todd (Figure 5.1) where hoppers were disinfected weekly and all aviaries were fully disinfected monthly (Supplementary Methods 2). The second was a reduced fortnightly hopper biosecurity protocol (control) applied at Bel Ombre and Bris Fer (Figure 5.1). The aviaries at Bel Ombre were not disinfected over the experimental period. Overlaid on the hopper treatment were nest boxes known to be in current use by parakeet breeding pairs (active), which were assigned to either the experimental group, where the standard biosecurity protocol was applied (n=47), or to the control group, where old nesting material was removed but no medical barrier suits were worn whilst accessing the nest and no disinfection solution was applied (n=57; Table 5.1). Therefore, where applied, nest disinfection occurred in the non-breeding season in 2016. Within each of the areas surrounding hoppers, nest boxes were matched as either disinfected (experiment) or not disinfected (control) by distance away from their nearest hopper. Matching nest boxes by distance to hoppers was important given the known relationships reported between BFDV prevalence and reproductive success, and proximity to hoppers (Fogell *et al.* 2019; Tollington *et al.* 2018). This created a fully crossed and balanced experimental design to investigate the effects of management on hoppers and nest boxes, both independently and in combination (Table 5.1). We did not include a true control of no hopper biosecurity because the current management framing assumes some level of biosecurity is

important and therefore our assessment was whether we could make things better as compared to current management. During both adjacent non-breeding seasons, status quo biosecurity was resumed at the Camp and Bel Ombre hoppers (Supplementary Methods 2).

5.3.3 Environmental sample collection and analysis

Environmental DNA (eDNA) samples were collected from experimental and control hoppers during the Mauritius parakeet breeding season from September 2016 to January 2017, as well as in the adjacent non-breeding seasons from active hoppers under status quo management from April to June 2016 and 2017 (Supplementary Methods 3). Sampling was conducted on Monday, Wednesday and Friday, in order to assess accumulation of BFDV over the course of the week, and samples were taken both immediately before and after disinfection. If disinfection days fell on standard sampling days then the pre-disinfection and day sample are the same (in total n=733 samples, Table 5.2). eDNA was collected using flocked swabs (eNat collection and preservation for nucleic acids, Copan, Italy) and all samples were stored at 5°C prior to DNA extraction.

An ammonium acetate DNA extraction method (Bruford *et al.* 1998) was used for all extractions by adding 250 µl of DIGSOL lysis buffer and 20 µl of 10 mg/ml proteinase K to each sample tube and all samples were eluted to 100 µl with ddH_2O at the final step. Extraction blanks (n = 10) were included on an ad hoc basis to ensure no contamination occurred during handling. RT-PCR has become the gold standard tool for the detection of pathogens due to its accuracy, sensitivity and generation of reproducible results; reducing the risk of false positives due to carry over contamination (Mackay, Arden and Nitsche 2002). Three repeats of each swab sample were screened for BFDV DNA using the RT-PCR protocol described by Tollington *et al.* (2018). In the event that any of these three sample replicates were not within one amplification cycle of one another, a further two replicates were performed to ensure consistency. Each reaction consisted of 10.0 µl iTaq Universal Probes Supermix (Bio-Rad Inc.), 0.8 µl of each of the forward (5'-TGGGTGGCTACCTTATTG-3') and reverse (5'-GGCTTATTGCTCGTGATAA-3') primers, 0.2 µl of a FAM-labelled fluorescent probe (5'FAM-CTCTGCGACCGTTACCCACA-3'TAM) (Tollington *et al.* 2018), 5 µl of DNA template and made up to 20 µl with ddH_2O. Cycle conditions included an initial denaturation step of 5 min at 95°C; followed by 50 cycles of: 5 s at 95°C and 30 s at 60°C. Viral load of each sample was determined using a standard curve of serial dilutions in triplicate using a blood extraction from an infected Mauritius parakeet individual of known viral DNA concentration and three negative controls per 96-well plate. The averaged C_T values for each sample were then converted into a relative estimate of viral load (Eastwood *et al.* 2015) using the equation: Viral load = $2^{(-\Delta CT)}$. As we experienced low levels of amplification in 36% of our field blanks, a minimum threshold was applied to all swab samples (under which they were considered to have a viral load of 0) to ensure that we only analysed true positive values.

5.3.4 Reproductive success, blood sampling and analysis

We quantified the effects of our experiment on Mauritius parakeet breeding success and BFDV infection in successfully produced nestlings. Three hundred and eighty-eight eggs were recorded in active nest boxes, with 241 known fledglings (Table 5.1). Blood samples (n = 217) were taken from the brachial vein from nestlings at approximately 45 days old and stored in ethanol. This research was conducted under the University of Kent ethical guidelines (0018-DF-16). Nest monitoring and nestling sampling was undertaken in collaboration with the Mauritius National Parks and Conservation Services and the Mauritian Wildlife Foundation (Henshaw *et al.* 2016) and samples were imported to the United Kingdom under the following license numbers: IMP/GEN/2014/02 and TARP/2016/105.

Prior to screening for BFDV, an ammonium acetate DNA extraction method was used to extract both host and viral DNA (Bruford et al. 1998). In brief, for all nestling samples approximately 50 to 100 µl of whole blood was used from each sample and digested in 250 µl of DIGSOL lysis buffer with 10 µl of 10 mg/mL proteinase K. Extractions were quantified using a Qubit dsDNA Assay Kit and standardized to approximately 10 ng/µl prior to screening for BFDV. For detection of BFDV in the blood sample extractions, two replicates were performed for each individual using the same RT-PCR primers and protocol as described above (Tollington *et al.* 2018). If the repeats did not amplify within one cycle of each other, a third replicate was performed. Each 96-well plate included two negatives and two positive controls from a high viral load Mauritius parakeet individual (amplification at ~10 cycles) for standardisation across runs. Each individual nestling was assigned a positive or negative infection status according to whether any viral amplification occurred. We do not consider BFDV viral load in more detail given our previous work shows substantial within brood variation; suggesting that inherent nestling characteristics such as immune fitness are swamping any external drivers of viral load variation (see Fogell *et al.* 2019).

5.3.5 Data analysis

Generalised linear models (GLMs) and generalised linear mixed models (GLMMs) were run with the lme4 (Bates *et al.* 2015) package in R version 3.4.3 (R Core Team 2017) for both the swab and nestling viral datasets. We selected the most parsimonious model based on the lowest AICc. Where more than one model was within 2 ΔAICc, and therefore equally plausible, we used weighted model averaging (*AICcmodavg* package, Mazerolle, 2016) to estimate predicted parameter values.

5.3.6 Analysis of eDNA swabs

Prior to analysis, all RT-PCR viral load values obtained for the eDNA samples were adjusted to viral load per cm swabbed. Swabs were classified according to whether they were taken immediately after disinfection of a hopper (A0 if from a weekly cleaning cycle; B0 if from a

fortnightly cleaning cycle) or from repeated sampling through time until the next disinfection. We grouped swabs into two-day bins relating to days since disinfection (e.g. days one and two, three and four, etc up to days thirteen and fourteen as the largest number of days before a disinfection event). Grouping into two-day bins helped to provide a balanced number of samples in each bin for subsequent analysis. Initially we tested whether a simple linear relationship existed between time and viral load, but this was not found to be significant (Supplementary Figure 5.2). We consequently simplified time into a binary variable of disinfected (A0 and B0) and dirty (for all other days) for further analysis. Thus, a candidate model set was created, using a Gaussian distribution, to assess the relationship between environmental BFDV viral load (log transformed) and five binary variables. These variables were hopper location (aviary or clearing), component swabbed (hopper or perch), hopper design (parakeet or pigeon), whether the sample was taken in the breeding season (0, 1) and whether the component had just been disinfected (0, 1) (Supplementary Table 5.1).

5.3.7 Analysis of reproductive success and nestling infection

We compiled candidate models to evaluate the effects of the distance a nest was located away from a hopper (km) and the two experimental treatments on brood prevalence (number nestlings in brood infected with BFDV/total number of nestlings sampled in brood; Supplementary Table 5.2). Models were run using a binomial error distribution and a logit link function (Tollington *et al.* 2015; Fogell *et al.* 2019).

Similarly, candidate model sets were run on three parameters to assess reproductive success across our treatments. To test the hypothesis that the chosen chemical treatment may impact on probability of successful hatching, the first set of candidate models evaluated the effects of both the linear and quadratic terms for dam age, distance to nearest hopper (km), the two experimental treatments and the interactions between treatments and distance on the proportion of nestlings hatched from the number of eggs a female had laid, using a binomial error distribution and a logit link function (Supplementary Table 5.3a) (Tollington *et al.* 2015; Fogell *et al.* 2019). To assess whether chemical treatment affected population growth the second set of candidate models evaluated the effects of both the linear and quadratic terms for dam age, distance to nearest hopper (km), the two experimental treatments and the interactions between treatments and distance on the total number of fledglings produced per brood, using a Gaussian distribution (Supplementary Table 5.3b). Finally, to test whether the experimental protocols influenced nestling body condition prior to fledging, a third set of candidate models assessed the impacts of both the linear and quadratic terms for dam age, infection status (positive or negative), distance to nearest hopper (km), the two experimental treatments and the interactions between treatments and distance on a scaled body condition score; calculated using the residuals derived from a linear

model of individual mass (g) and wing length (mm) (Supplementary Table 5.4). A Gaussian distribution was used, with female parent included as a random intercept effect.

5.4 RESULTS

5.4.1 Environmental viral accumulation

The presence of BFDV on hoppers was explained by two top models (within <2 ΔAICc values) including all five variables assessed (Supplementary Table 5.1). However, only three of these explanatory variables had statistically significant effects; the hopper component that had been swabbed, whether the component had just been disinfected and hopper design (Table 5.3a). Hoppers designed for pigeons were found to have higher viral loads than those designed for parakeets (Figure 5.2a) and all hoppers were found to have significantly higher viral loads than their perches (Figure 5.2b). Hoppers were found to have significantly lower viral loads after disinfection than over the subsequent seven to 14 days (Figure 5.2c). However, traces of BFDV were still detectable on 74.45% of all swab samples taken immediately following disinfection (n = 137). Therefore, whilst we found that disinfection removed most or all of the virions present from hoppers, this effect was brief. No significant differences were found in viral accumulation between the breeding and non-breeding seasons nor between those hoppers within aviaries and those located in forest clearings.

5.4.2 Impacts of biosecurity on nestling fitness

For the binary models analysing probability of BFDV infection in Mauritius parakeet nestlings, eight of the 13 models assessed fell within 2 ΔAICc (Supplementary Table 5.2). These were inclusive of the null model and all of the tested variables, none of which were found to be significant predictors of the probability of infection (Table 5.3b, Figure 5.3a). Therefore, neither biosecurity at nest sites nor increased cleaning regimes of hoppers had any strong effect on reducing BFDV infection in Mauritius parakeet nestlings.

Only a single model was found to be the most parsimonious when considering the probability of an egg successfully hatching. This model included both the linear and quadratic terms for dam age, as well as the experimental hopper treatment (Supplementary Table 5.3a), all of which were found to be significant (Table 5.3c). The probability of hatching was found to steadily increase with dam age. However, the experimental hopper treatment was found to significantly decrease the probability of eggs hatching, where nests closer to control hoppers had significantly higher mean probability of hatch success than those located closer to experimental hoppers (0.67 vs. 0.58, Figure 5.3b). Whilst the application of nest site biosecurity slightly decreased the probability of eggs hatching, this effect was not found to be statistically significant (Table 5.3c, Figure 5.3b).

When assessing impacts on the total number of Mauritius parakeet fledglings successfully produced, six of the 16 candidate models fell with 2 ΔAICc (Supplementary Table 5.3b). The top model set included all of the variables assessed, none of which were found to be significant. Of the 23 models run to assess the impacts of our tested variables on nestling body condition, the null model was found to be the most parsimonious (Supplementary Table 5.4). As with the probability of BFDV infection in nestlings, neither the hopper nor nest biosecurity experiments were seen to affect the number of fledglings produced or their condition prior to fledging (Table 5.3d and e, Figure 5.3c and d).

5.5 DISCUSSION

Our results demonstrate two key findings. The first is that environmental accumulation of BFDV outside of a host can be successfully measured through the application of eDNA molecular techniques. RT-PCR methods to detect BFDV were first developed for African grey parrots (Raue *et al.* 2004) and have recently been used for assessing viral load at the level of the individual (e.g. Eastwood *et al.*, 2015; Regnard *et al.* 2015; Fogell *et al.* 2019). Despite the theory that BFDV is environmentally stable outside of the host (Ritchie 1995; Todd 2000; Jackson *et al.* 2014), until now this had not been conclusively evaluated. The second is that upscaled biosecurity protocols applied to hoppers not only fails to achieve its intended purpose of reducing the transmission of BFDV in Mauritius parakeets, but also hinders their reproductive success more than nest site biosecurity alone (Fogell *et al.* 2019).

The use of eDNA protocols for aquatic- (e.g. Foote et al., 2012; Hunter et al., 2015; Buxton et al., 2017) and soil-based surveys for biodiversity (e.g. Lopez-Gutierrez et al., 2004; Martin and Rygiewicz, 2005) has grown substantially since their initial development but their application to other terrestrial wildlife remains under-developed. Through our eDNA sampling, we have been able to answer some key questions relating to how targeted biosecurity protocols are affecting the presence and persistence of BFDV. We found that the disinfection of hoppers significantly reduced the quantity of detectable virus on all components. However, the current disinfection protocol only removed all traceable quantities of BFDV in 25% of applications. This is not surprising as there are likely several challenges in taking a clinical disinfection protocol designed for a controlled laboratory setting and applying it in the wild. For example, field staff have no quarantine barriers between cleaning and using areas for equipment, and there are no available clean rooms in which to store components as they dry. Counter to expectations BFDV was not found to accumulate gradually over time following the disinfection of a hopper, which instead became quickly re-infected. We suggest that the lack of progressive accumulation may be due to exposure to wind and rain, which may naturally remove infected feather dust and faecal matter from the surfaces of the hoppers. It is also apparent that the density of individuals attending hoppers does not directly influence

environmental viral accumulation. Hoppers did not have significantly higher quantities of BDFV present over the breeding season, when the nutritional demands of the parakeet population are increased, and thus the frequency and volume of attendance at hoppers is greater than throughout the rest of the year (S Henshaw, Pers. Obs.; Chapter 4).

Our results also illustrate the challenges faced by conservationists battling with reducing the transmission of infectious disease. Our experimental design has proven the current approach, and an increased biosecurity alternative, are both ineffective at hoppers. Not only does intensified disinfection of hoppers not achieve its intended purpose of reducing the probability of BFDV infection in Mauritius parakeet nestlings, it significantly impacts on breeding success. Whilst, over the course of a decade, the disinfection of nest sites prior to the breeding season was determined to reduce fledge success marginally (83% of eggs surviving to fledgling stage in untreated vs. 79% in treated nests) (Fogell *et al.* 2019), this effect was not obvious within our single-season study. However, the intensification of disinfection at hoppers throughout the breeding season appears to have a greater negative impact on the probability of eggs hatching (58% vs. 67% at lower intensity biosecurity hoppers) than a single nest site disinfection prior to the parakeets nesting (65% at disinfected vs. 68% at untreated sites). Both Virkon specifically and quaternary ammonia-based disinfectants in general have been shown to reduce the hatchability of eggs in domestic fowl when applied to the eggs directly (Wilson 2009; Scott, Swetnam and Kinsman 1993). We suggest that the repeated and reinforced presence of these disinfectants brought back to the nest by parents regularly attending hoppers throughout the season reduces the viability of exposed eggs. However, once nestlings have hatched, their exposure to these chemicals does not appear to impact on their ability to fledge. Similarly, disinfection protocols for hoppers or nests had no effect on the body condition of nestlings.

When assessing accumulation on each of the hopper core components individually, we found that hoppers accumulated significantly more virions than their associated perches. This result was expected given that the hoppers have a much larger surface area exposed to the full bodies of infected individuals for the accumulation of feather dust and, in the case of the pigeon hoppers, faecal matter. Conversely perches present a relatively small area on which virions are able to settle and are generally only exposed to birds' feet. We also found that the pigeon hoppers harboured significantly more virus over the same period than parakeet hoppers. This result is likely due to the hopper design, where those intended for the pigeons are less complex for ease of access by the birds, and are far more sheltered than the parakeet hoppers to prevent water entering. However, during the study we observed that the design of the pigeon hoppers also allows the (much smaller) parakeets to enter them entirely, where they remain for an extended period whilst feeding. While the focus of this study is BFDV, we would expect that this finding reflects a general propensity for a range of pathogens to accumulate due to the sheltered nature afforded by this hopper design

(Mauritius species also frequently suffer from pathogens including *Trichomonas gallinae* (Bunbury *et al.* 2007; Swinnerton *et al.* 2005) and *Avipoxvirus* (Swinnerton *et al.* 2005)).

The results we present here highlight the vital importance of constant monitoring and evaluation of conservation management strategies. BFDV is one example of a global EID that is causing concern in a number of threatened parrot species. The results of the current study, along with those of our previous focus on nest sites (Fogell *et al.* 2019, Chapter 4), show that current biosecurity protocols are doing little to reduce BFDV in the Mauritius parakeet population, but are reducing breeding success. Moreover, the current protocol requires a considerable effort and, whilst we suspect some level of biosecurity is prudent, we remain uncertain as to its best form. Certainly, biosecurity targeted at BFDV remains a challenge.

We encourage those managing wildlife populations to carefully consider mitigation options, not only against pathogen control, but also the fundamental objectives of their recovery programmes. Not evaluating outcomes and then altering management accordingly is what leads to dogmatic approaches (Martínez-Abraín and Oro 2013) and the criticism that conservation effort is often inefficiently applied (Dasgupta 2016; Nichols and Williams 2006). In order to avoid negative outcomes against recovery objectives, conservationists should implement field trials of management solutions and adapt accordingly. These should be conducted in the same manner as would be developed for humans or livestock, whilst accounting for the additional complexity of partially understood and unobservable wild systems and remaining focused on the fundamental objective of management.

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5.7 TABLES AND FIGURES

Table 5.1 Total number of Mauritius parakeet nests, eggs, hatchlings and fledglings produced, and BFDV-screened nestlings over the 2016/2017 breeding season; where E represents those nest sites that were disinfected prior to the breeding season and those supplementary feeding hoppers with intensified cleaning regimes, and C represents those nests that were not disinfected prior to the breeding season and those hoppers with a fortnightly cleaning regime.

Total nests (%)		**Nest E**	**Nest C**
	Hopper E	27 (26.0)	23 (22.1)
	Hopper C	20 (19.2)	34 (32.7)
Total eggs (%)		**Nest E**	**Nest C**
	Hopper E	99 (25.5)	108 (27.8)
	Hopper C	78 (20.1)	103 (26.5)
Total hatchlings (%)		**Nest E**	**Nest C**
	Hopper E	65 (22.9)	71 (25.0)
	Hopper C	66 (23.2)	82 (28.9)
Total fledglings (%)		**Nest E**	**Nest C**
	Hopper E	57 (23.6)	49 (20.2)
	Hopper C	61 (25.2)	75 (31.0)
Total nestlings screened for BFDV (%)		**Nest E**	**Nest C**
	Hopper E	52 (24.0)	45 (20.6)
	Hopper C	49 (22.6)	71 (32.7)

Table 5.2 The location and number of eDNA swab samples taken over 20 weeks from two non-breeding (NB) periods and 14 weeks during the 2016/2017 Mauritius parakeet breeding season (BS)

Sub-population	Species	Location	Component	Material	Total samples BS	Total samples NB
Bel Ombre	*Mauritius parakeet*	Aviary	Hopper	PVC	48	61
			Hopper perch	Wood	34	44
	Pink pigeon		Hopper	Metal	21	16
			Hopper perch	Rope	15	14
		Clearing	Hopper	Metal	7	16
			Hopper perch	Rope	4	16
Bris Fer	*Mauritius parakeet*	Clearing	Hopper	PVC	27	NA
			Hopper perch	Wood	21	NA
Camp	*Mauritius parakeet*	Aviary	Hopper	PVC	27	33
			Hopper perch	Wood	26	32
		Clearing	Hopper	PVC	27	32
			Hopper perch	Wood	27	32
	Pink pigeon	Aviary	Hopper	Metal	27	15
			Hopper perch	Rope	27	18
		Clearing	Hopper	Metal	12	12
			Hopper perch	Rope	12	14
Plateau Todd	*Mauritius parakeet*	Clearing	Hopper	PVC	28	NA
			Hopper perch	Wood	28	NA
Total					418	355

Table 5.3 A summary of model averaged coefficients and effect sizes for the generalised linear mixed effect candidate models analysing the factors affecting a.) the environmental accumulation of BFDV on supplementary feeding hoppers between April 2016 and June 2017, as well as the b.) the probability of BFDV infection in 45-day old Mauritius parakeet nestlings c.) the probability of hatching success, d.) the total number of fledglings produced, and e.) scaled body condition score of Mauritius parakeet nestlings over the experimental breeding season (2016/2017).

Factor	Model Estimate	95% Confidence Interval
a.		
Location	-1.8×10^{-9}	$-4.6 \times 10^{-9} – 1.0 \times 10^{-9}$
Component	-6.9×10^{-9}	$-9.7 \times 10^{-09} – -4.1 \times 10^{-9}$
Breeding Season	1.8×10^{-9}	$-9.0 \times 10^{-10} – 4.6 \times 10^{-9}$
Species	4.6×10^{-9}	$1.7 \times 10^{-09} – 7.6 \times 10^{-9}$
Disinfected	6.8×10^{-9}	$3.1 \times 10^{-09} – 1.04 \times 10^{-8}$
b.		
Nest Experiment	0.08	-0.82 – 0.99
Hopper Experiment	-0.17	-1.29 – 0.95
Nearest Hopper	-0.31	-0.68 – 0.06
Nest Experiment*Nearest Hopper	0.38	-0.12 – 0.88
Hopper Experiment*Nearest Hopper	0.49	-0.01 – 1.00
c.		
Nest Experiment	-0.16	-1.03 – 0.71
Hopper Experiment	-0.71	-1.23 – -0.19
Nearest Hopper	-0.05	-0.33 – 0.23
Dam Age	1.37	0.2 – 2.53
$(\text{Dam Age})^2$	-1.64	-2.77 – -0.51
Nest Experiment*Nearest Hopper	0.25	-0.13 – 0.64
Hopper Experiment*Nearest Hopper	0.00	-0.39 – 0.39
Nest Experiment* Hopper Experiment	-0.26	-1.27 – 0.75
d.		
Nest Experiment	0.13	-0.49 – 0.75
Hopper Experiment	-0.35	-0.88 – 0.19
Nearest Hopper	-0.14	-0.37 – 0.09
Dam Age	0.77	-0.18 – 1.72
$(\text{Dam Age})^2$	-0.88	-1.82 – 0.07
Nest Experiment*Nearest hopper	0.21	-0.07 – 0.50
Hopper Experiment*Nearest hopper	-0.16	-0.44 – 0.13
Nest Experiment* Hopper Experiment	0.22	-0.53 – 0.97

e.

Nest Experiment	-5.19	-14.46 – 4.08
Hopper Experiment	0.02	-8.90 – 8.95
Nearest Hopper	1.79	-1.57 – 5.14
BFDV Positive	-0.56	-4.78 – 3.66
Dam Age	-4.86	-21.02 – 11.31
$(\text{Dam Age})^2$	4.93	-11.33 – 21.19
Nest Experiment*Nearest hopper	-1.86	-6.96 – 3.23
Hopper Experiment*Nearest hopper	-1.29	-6.27 – 3.70
Nest Experiment* Hopper Experiment	-0.97	-13.40 – 11.46

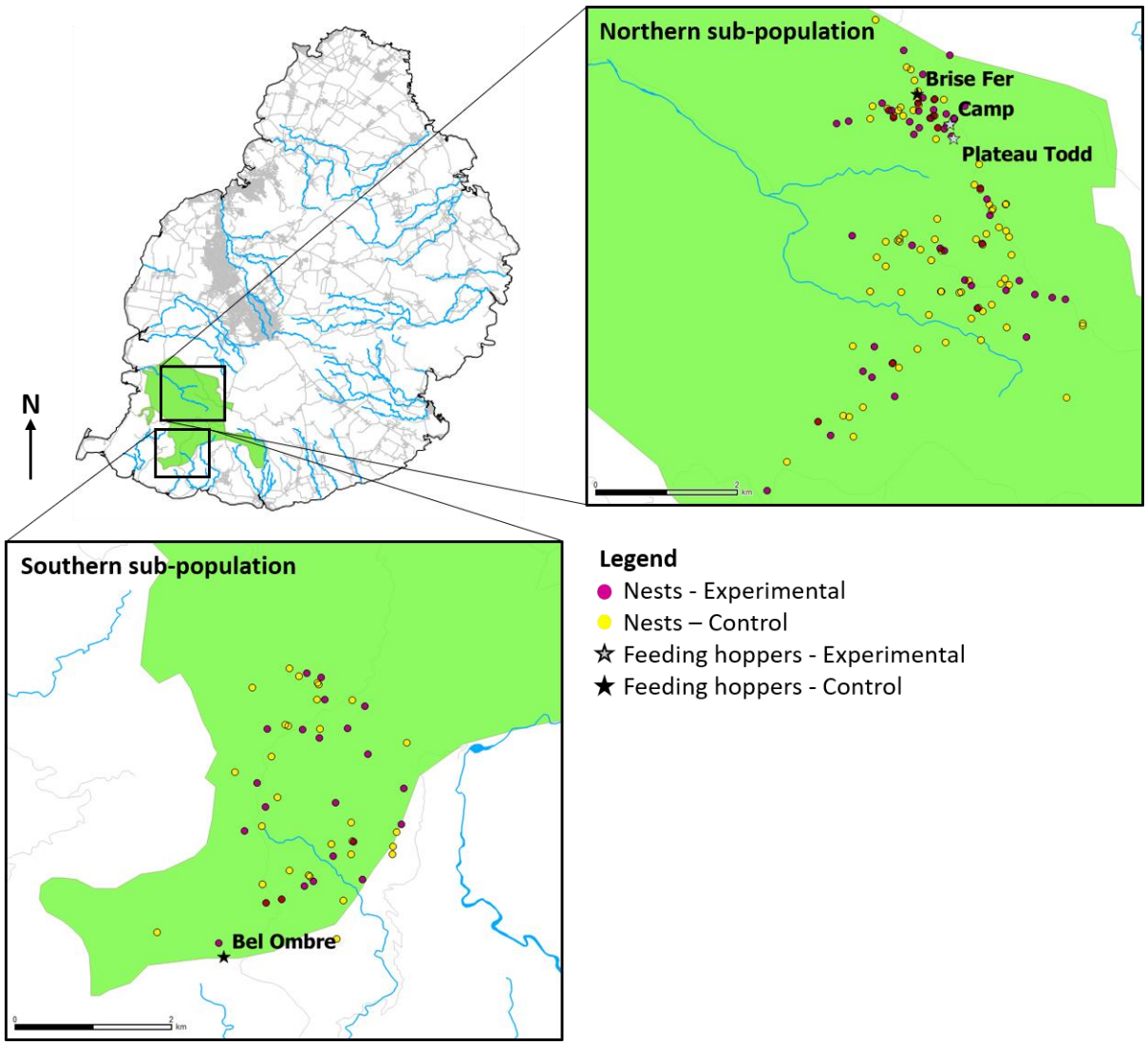


Figure 5.1 The location of the experimentally manipulated supplementary feeding hoppers and Mauritius parakeet nest sites for both the northern and southern sub-populations within the Black River Gorges National Park in the southwest of Mauritius

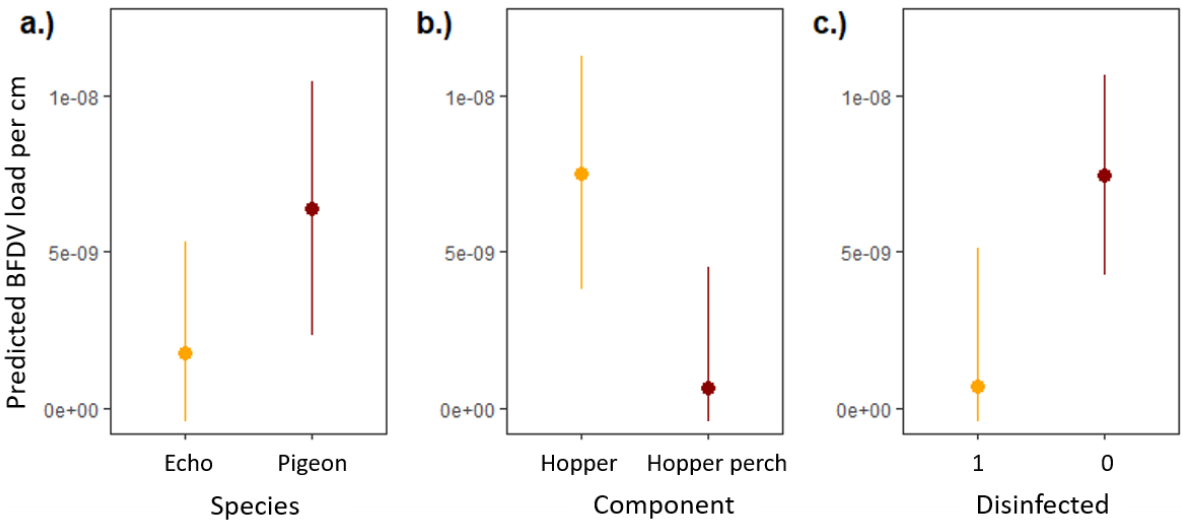


Figure 5.2 The predicted BFDV load for the three explanatory variables found to significantly influence environmental viral accumulation (presented with their corresponding 95% prediction intervals) where a.) pigeon hoppers accumulated significantly higher viral loads than parakeet hoppers, b.) perches accumulated significantly lower viral loads than hoppers and c.) disinfection treatment significantly reduced viral accumulation at hoppers.

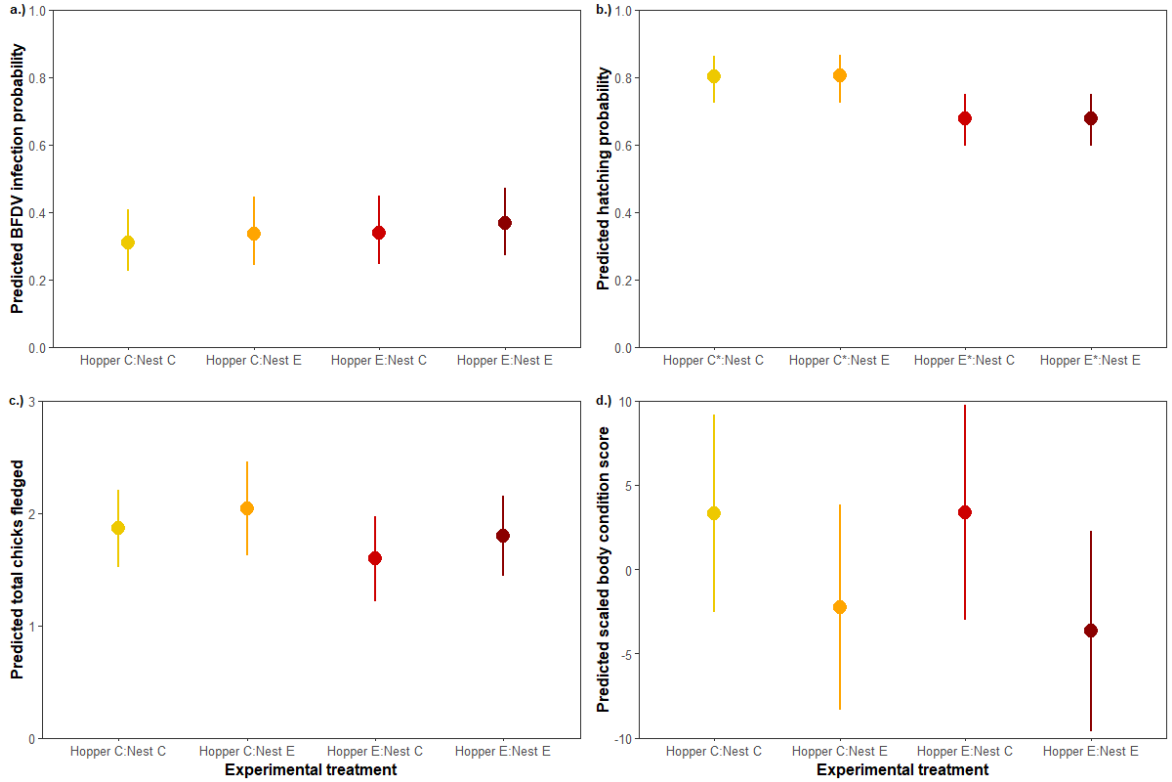


Figure 5.3 The impact of both nest site and supplementary feeding hopper experimental protocols over the 2016/2017 Mauritius parakeet breeding season (E – experiment, C - control) on the a.) probability of Mauritius parakeet nestling infection with BFDV at 45-days where neither treatment had any significant impact, b.) the probability of eggs hatching where the upscaled treatment of hoppers resulted in a significant reduction in hatching probability (* denotes a significant effect $p < 0.001$), c.) the total number of fledglings produced where neither treatment had any significant impact and d.) the scaled body condition score of nestlings (linear model residuals of mass ~ wing length) where neither treatment had any significant impact.

5.8 SUPPLEMENTARY INFORMATION

5.8.1 Supplementary Methods

5.8.1.1 Nest site biosecurity regime

Prior to the breeding season, the Mauritius parakeet field team access nest sites wearing medical barrier suits. All old nesting material is removed and a disinfection solution, selected for its virucidal efficacy (Royer *et al.* 2001; Martin, Le Potier and Maris 2008) is applied. This solution is made up according to manufacturer's guidelines and consists of either 6 g/L of Virkon (potassium peroxymonosulfate base), or 5 g/L of Virex (quaternary ammonium chloride base). Nests are then rinsed with clean water and closed until the start of the next breeding season.

5.8.1.2 Supplementary feeding hopper disinfection protocol

Status quo parakeet hopper disinfection protocols throughout the year include the weekly rinsing of hoppers and perches at Camp, and only hoppers at Bel Ombre, in clean water before soaking all rinsed components in the disinfection solution, made up to manufacturer's guidelines, for 20 minutes. The disinfected components are then rinsed in clean water, dried and replaced. Full parakeet aviary disinfection, including the replacement of all perches, is conducted approximately bi-monthly at Camp and bi-annually at Bel Ombre; dependent primarily on field staff availability and workload. Pigeon hopper disinfection protocols are applied fortnightly and include the rinsing of hoppers under running water before scrubbing with disinfection solution and leaving for 20 minutes. The disinfected hoppers are then rinsed in clean water, dried and replaced. Full pigeon aviary disinfection occurs annually, during the pigeon moulting season, and includes the replacement of all removable perches.

Over the 2016/2017 breeding season, the weekly biosecurity protocols applied to both parakeet and pigeon hoppers at experimental hopper locations included the rinsing of all hoppers in clean water before soaking the rinsed elements in the disinfection solution for 20 minutes. The disinfected components were then rinsed in clean water, dried and replaced. All hopper perches were scrubbed with a hard brush and disinfection solution, left for 20 minutes, and then rinsed with clean water. Full parakeet and pigeon aviary disinfection occurred monthly at Camp and included the scrubbing of all internal walls and surfaces with a hard brush using either Virex or Virkon (made up to manufacturer's guidelines), leaving for 20 minutes and rinsing with clean water. All perches, were replaced. The fortnightly biosecurity protocols applied at control hopper locations, included only the disinfection of hoppers, using the same cleaning process as described for the experimental group, and none of their associated perches. Neither the parakeet nor pigeon aviaries were disinfected over the experimental period.

5.8.1.3 Swab sampling protocol

Three drops of sterile saline were added to moisten the tip of flocked swabs prior to sampling. Hoppers and their associated perches were swabbed individually and the length or circumference of the swabbed object was taken so that all quantified samples could later be standardised to a measure of viral load per centimetre. Field blanks were taken (n = 11) at the end of sampling on an ad hoc basis by adding three drops of sterile saline from the same tube used for a day's samples to the tip of a flocked swab to ensure no contamination occurred during handling. Pigeon hoppers located in the clearing at both Camp and Bel Ombre were removed during biannual screening for *Trichomonas gallinae*, so samples were not taken from these hoppers over this period.

5.8.2 Supplementary tables and figures

Supplementary Table 5.1 A comparison of the 18 generalised linear candidate models assessing the impact of the binary variables of supplemental feeder location (LC), component swabbed (C), which species the hopper was designed for (SP), whether the sample was taken in the breeding season (BS) and whether the component had just been disinfected (D) on environmental BFDV viral load, based on Akaike's information criterion corrected for finite sample size (AIC_c) and weights (AIC_c weights). K denotes the number of parameters in each model and models are ranked according to their ΔAIC_c.

Rank	Model	K	AICc	ΔAIC_c	AIC_c weights
1	SP + D + C + BS	6	-25244.94	0.00	0.53
2	SP + LC + D + C + BS	7	-25244.48	0.46	0.42
3	D + C	4	-25238.29	6.65	0.02
4	D + C + BS	5	-25237.52	7.42	0.01
5	LC + D + C + BS	6	-25237.32	7.63	0.01
6	SP + C	4	-25234.75	10.19	0.00
7	SP + LC + C	5	-25234.21	10.74	0.00
8	C	3	-25225.31	19.63	0.00
9	LC + C	4	-25225.11	19.83	0.00
10	LC + C + BS	5	-25224.19	20.75	0.00
11	C + BS	4	-25224.07	20.87	0.00
12	SP + LC + D	5	-25223.38	21.56	0.00
13	D	3	-25217.24	27.70	0.00
14	SP + LC	4	-25217.16	27.78	0.00
15	D + BS	4	-25216.52	28.43	0.00
16	LC	3	-25209.00	35.94	0.00
17	Null Model	2	-25208.89	36.06	0.00
18	BS	3	-25207.74	37.20	0.00

Supplementary Table 5.2 A comparison of the 13 general linear mixed candidate models analysing the probability of BFDV infection in 45-day old Mauritius parakeet nestlings over the 2016/17 experimental breeding season. Management factors related to BFDV prevalence and load include nest site treatment (NE), intensified treatment of the nearest supplementary feeding hopper (HE) and distance to the nearest supplementary feeding hopper (SF) based on Akaike's information criterion corrected for finite sample size (AICc) and weights (AICc weights). K denotes the number of parameters in each model and models are ranked according to their ΔAICc.

Rank	Model	K	AIC_c	ΔAIC_c	AIC_c weights
1	SF + HE + (SF * HE)	4	202.91	0.00	0.17
2	SF	2	203.00	0.09	0.16
3	Null model	1	203.80	0.89	0.11
4	SF +NE + (SF * NE)	4	204.22	1.31	0.09
5	SF + NE	3	204.28	1.37	0.08
6	SF + NE + HE + (SF * HE)	5	204.45	1.54	0.08
7	SF + HE	3	204.45	1.54	0.08
8	NE	2	204.83	1.92	0.06
9	HE	2	205.17	2.26	0.05
10	SF + NE + HE + (SF * NE)	5	205.90	2.99	0.04
11	SF + NE + HE	4	205.94	3.03	0.04
12	NE + HE	3	206.44	3.53	0.03
13	SF + NE + HE + (NE * HE)	5	208.14	5.23	0.01

Supplementary Table 5.3 A comparison of the a.) 18 generalised linear candidate models analysing the probability of hatch success from the eggs produced and b.) 16 generalised linear candidate models analysing the total number of fledglings produced by Mauritius parakeets nesting over the experimental 2016/17 breeding season. Factors related to hatch success and total fledglings produced include experimental treatment of nest sites (NE), experimentally intensified treatment of the nearest supplementary feeding hopper (HE), distance to the nearest supplementary feeding hopper (SF) and the linear (F) and quadratic terms (F2) of dam age based on Akaike's information criterion corrected for finite sample size (AIC_c) and weights (AIC_c weights). K denotes the number of parameters in each model and models are ranked according to their ΔAIC_c.

Rank	Model	K	AICc	ΔAIC_c	AIC_c weights
a.)					
1	HE + F + F2	4	271.60	0.00	0.80
2	SF + NE + F + F2 + HE + (NE*SF)	7	276.51	4.91	0.07
3	F + F2	3	277.63	6.03	0.04
4	SF + NE + F + F2 + HE + (NE*HE)	7	277.94	6.34	0.03
5	SF + NE + F + F2 + HE + (HE*SF)	7	278.19	6.60	0.03
6	SF + F + F2	4	279.72	8.12	0.01
7	NE + F + F2	4	279.73	8.13	0.01
8	SF + NE + F + F2	5	281.84	10.25	0.00
9	HE	2	296.00	24.40	0.00
10	SF + HE	3	296.27	24.68	0.00
11	NE + HE	3	297.75	26.16	0.00
12	SF + HE + (HE*SF)	4	298.29	26.70	0.00
13	NE + HE + (NE*HE)	4	299.45	27.86	0.00
14	Null Model	1	307.41	35.81	0.00
15	SF	2	308.32	36.72	0.00
16	NE	2	309.30	37.71	0.00
17	SF + NE	3	310.10	38.51	0.00
18	SF + NE + (NE*SF)	4	310.40	38.81	0.00
b.)					
1	SF + NE + F + FA + HE + (NE*SF)	8	382.56	0.00	0.24
2	HE + F + FA	5	383.19	0.63	0.18
3	SF + NE + F + FA + HE + (HE*SF)	8	383.58	1.03	0.14
4	SF + F + FA	5	383.86	1.30	0.13
5	SF + NE + F + FA	6	384.33	1.77	0.10
6	SF + NE + F + FA + HE + (NE*HE)	8	384.41	1.85	0.10

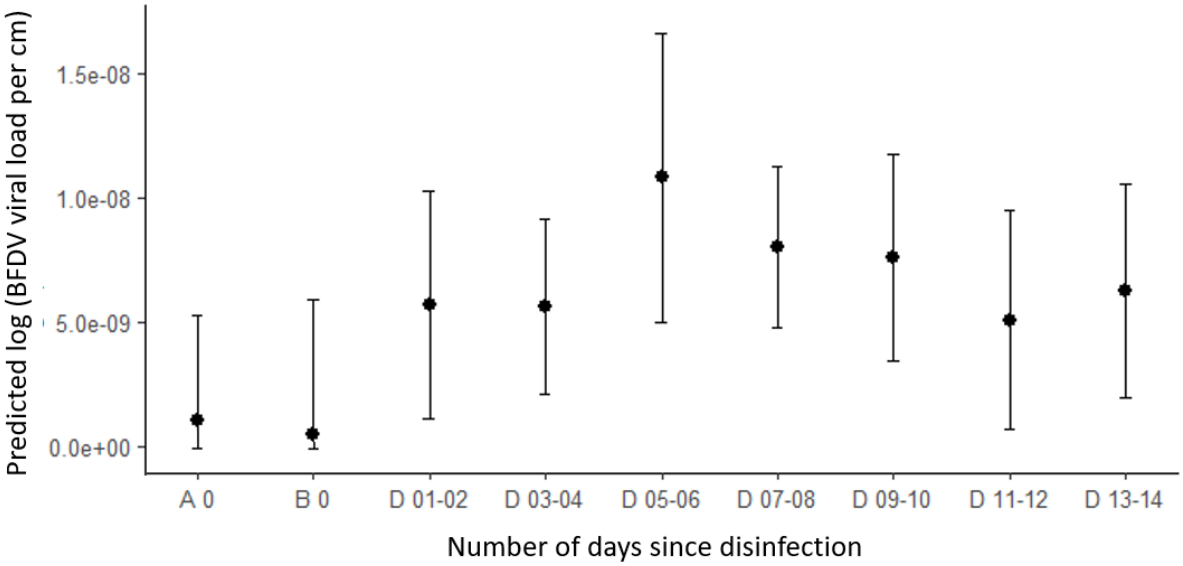
7	NE + F + FA	5	385.38	2.83	0.06
8	F + FA	4	385.54	2.99	0.05
9	SF + HE	4	410.37	27.82	0.00
10	HE	3	410.57	28.02	0.00
11	SF + HE + (HE*SF)	5	411.32	28.76	0.00
12	SF + NE	4	411.73	29.18	0.00
13	NE	3	411.92	29.36	0.00
14	SF + NE + (NE*SF)	5	412.65	30.09	0.00
15	SF	3	412.91	30.35	0.00
16	Null	2	413.78	31.22	0.00

Supplementary Table 5.4 A comparison of the 23 generalised linear candidate models analysing body condition ($^{mass}/_{wing\ length}$) of Mauritius parakeet nestlings over the experimental 2016/17 breeding season. Factors related to body condition included the experimental treatment of nest sites (NE), experimentally intensified treatment of the nearest supplementary feeding hopper (HE), distance to the nearest supplementary feeding hopper (SF), individual BFDV load (VL) and the linear (F) and quadratic terms (F2) of dam age based on Akaike's information criterion corrected for finite sample size (AIC_c) and weights (AIC_c weights). All models were run with the nesting female and breeding season as fixed effects. K denotes the number of parameters in each model and models are ranked according to their ΔAIC_c.

Rank	Model	K	AIC_c	ΔAIC_c	AIC_c weights
1	Null Model	3	-43.13	0	0.86
2	NE	4	-38.11	5.02	0.07
3	HE	4	-36.66	6.47	0.03
4	VL	4	-35.45	7.67	0.02
5	SF	4	-34.77	8.36	0.01
6	VL + NE	5	-30.3	12.83	0.00
7	VL + HE	5	-28.95	14.18	0.00
8	F + F2	5	-26.89	16.24	0.00
9	NE + F + F2	6	-21.95	21.18	0.00
10	NE + VL + SF	6	-21.9	21.22	0.00
11	HE + F + F2	6	-20.43	22.7	0.00
12	VL + F + F2	6	-18.97	24.16	0.00
13	HE + VL + SF + (HE*SF)	7	-18.29	24.84	0.00
14	NE + VL + SF + (NE*SF)	7	-16.68	26.45	0.00
15	NE + VL + F + F2	7	-13.96	29.17	0.00
16	NE + F + F2 + SF	7	-13.64	29.49	0.00
17	VL + F + F2 + SF	7	-10.69	32.44	0.00
18	NE + F + F2 + SF + (NE*SF)	8	-9.59	33.54	0.00
19	HE + F + F2 + SF + (HE*SF)	8	-9.06	34.07	0.00
20	NE + VL + F + F2 + SF	8	-5.64	37.49	0.00
21	NE + HE + VL + F + F2 + SF + (HE*SF)	10	3.81	46.94	0.00
22	NE + HE + VL + F + F2 + SF + (NE*SF)	10	4.7	47.83	0.00
23	NE + HE + VL + F + F2 + SF + (NE*HE)	10	5.73	48.86	0.00

a)  b)  c) 

Supplementary Figure 5.1 The three supplemental feeding hopper designs used by the Mauritius parakeet population where a.) consists of a plastic box with hinged lid, fitted onto a wooden backing with attached perch, b.) consists of a pair of PVC “J-shape” dispensers attached to a central stand and fitted with a hinged plastic lid and, c.) originally designed for use by the pink pigeons, consists of a galvanised metal conical lid, cylindrical dispenser and bowl-shaped base and is placed on top of a wooden stand with attached circular, rope covered perch.



Supplementary Figure 5.2 The pattern of BFDV viral accumulation on supplemental feeding hoppers over the course of a 14-day disinfection cycle, where A0 is the predicted viral load immediately after disinfection of a component after seven days of exposure and B0 is the predicted viral load immediately after disinfection of a component after 14 days of exposure.

Chapter 6

Parental social networks at supplementary feeding hoppers predict infectious disease prevalence in nestlings of an endangered parrot

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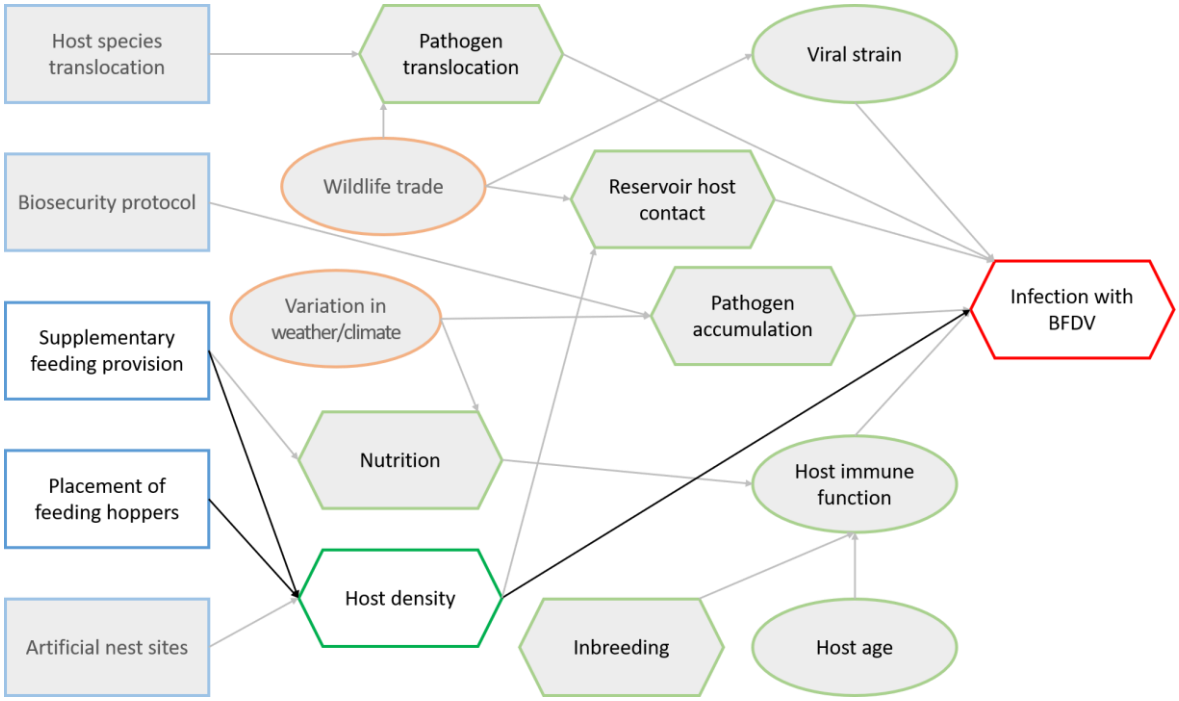

6.1 ABSTRACT

The transmission of infectious disease within a population is often complex, with social groups acting as the critical units for contact between infected and susceptible individuals. Population recovery tools, such as supplementary food provision, are available to conservationists but may directly or indirectly alter the dynamics of disease transmission. Social network analyses provide a means to quantify which individuals within a population are most central to the spread of infection. Here we quantify the changing social network interactions between Mauritius parakeets (*Psittacula eques*) foraging at supplementary feeding hoppers over the course of a breeding season, and how this influences Beak and feather disease virus (BFDV) prevalence in their offspring. All individuals attending two hoppers within the Black River Gorges National Park were recorded in hour-long sampling periods. Generalised linear models were run to assess the influence of frequency of parental attendance, network degree, closeness, betweenness and coreness on the prevalence of BFDV in nestlings. We found a strong negative correlation between the cumulative frequency of a breeding pair's attendance at a hopper and the distance of their nest site. Females were found to attend hoppers significantly less frequently early in the breeding season, due to sole incubation of broods by dams, whilst male attendance was found to significantly decrease later in the season. The interactive effect of both parents attending hoppers early in the breeding season was found to significantly predict brood BFDV prevalence. It is clear that supplementary feeding hoppers influence this host-pathogen relationship and, as such, the risks of potential infectious disease transmission need to be weighed against population recovery to ensure that management objectives are being met.

6.2 INTRODUCTION

According to the Living Planet Index, wildlife populations have declined by 60% since 1970 (WWF 2018), indicating that global biodiversity is in a crisis. While population biologists recognize infectious pathogens as an integral mechanism for evolutionary change within natural populations (Lyles and Dobson 1993), emerging infectious diseases (EIDs) are key contributors to this crisis (Yap *et al.* 2015; Brooks and Ferrao 2005) . The risk of extinction for vulnerable species and populations may be increased by the introduction or emergence of novel pathogens (Lips *et al.* 2006). The transmission of contagious pathogens within a population principally occurs through direct or indirect networks of interaction between infected and susceptible individuals (Corner, Pfeiffer and Morris 2003; Martínez-López, Perez and Sánchez-Vizcaíno 2009; Silk *et al.* 2017). These patterns of disease transmission within a population can be complex and differ with changes in behaviour and hierarchy (Böhm, Hutchings and White 2009), with social groups often acting as the critical units that enable spread of infection (Altizer, Harvell and Friedle 2003; Craft *et al.* 2009).

Empirical hypothesis testing on the association between social ecology and disease dynamics (Craft *et al.* 2011) is required to garner a better understanding of pathogen transmission (Silk *et al.* 2017; Craft *et al.* 2011). Therefore, identifying associations between individuals and their frequency of contact is key to unravelling aspects of behaviour that might drive patterns of infection across a population. Contact network analyses provide a means to quantify which individuals within a population are most central to the spread of infection (Corner, Pfeiffer and Morris 2003). Knowledge of such networks could aid in the prediction of infectious disease outbreaks (Rushmore *et al.* 2013), allowing for the inference of both direct contacts and indirect associations at either the individual or population level (Cross *et al.* 2012; Tiddi, Pfoh and Agostini 2019). Social networks have been demonstrated to successfully show the flow of tuberculosis between possums (Corner, Pfeiffer and Morris 2003), determine the effect of mating season on the connectivity between individuals for the spread of devil facial tumour disease in Tasmanian devils (Hamede *et al.* 2009), quantify how artificial food provisioning impacts host contact and parasite transmission in wild black capuchins (Tiddi, Pfoh and Agostini 2019) and assess the transmission dynamics of infectious disease between isolated, territorial populations of lions (Craft *et al.* 2009; Craft and Caillaud 2011). Networks can also be temporally dynamic structures that may be influenced by both climatic and reproductive seasonality (Silk *et al.* 2017). The shifting social interactions between individuals over time influences transmission, where the response of others towards infected or diseased individuals, or the behaviour of those that are sick, may change (Silk *et al.* 2017).

In a situation where the cause of a population decline is unknown, a suite of exploratory recovery tools is available to conservation managers. Some of these recovery tools may (directly or indirectly) increase the contact rate between individuals, thereby potentially changing the dynamics of infectious disease transmission. Common tools such as the provision of additional

breeding sites (Norris *et al.* 2018; Sherley *et al.* 2012; Tatayah *et al.* 2007) and supplementary food (Walker *et al.* 2013; Oro *et al.* 2008) artificially alter the density of individuals within a landscape. Their placement may cause more frequent aggregation than if foraging or breeding territory selection was natural (Lawson *et al.* 2012; Sorensen, van Beest and Brook 2013). Wildlife outbreaks of a range of disease-causing pathogens (viral, bacterial and parasitic) are linked to supplementary feeding, including for example, bovine tuberculosis (Brook *et al.* 2013) and brucellosis (Cross, Buddle and Aldwell 2007), mycoplasmal conjunctivitis (Dhondt *et al.* 2005; Hotchkiss *et al.* 2005), avian pox (Lawson *et al.* 2012), trichomoniasis (Bunbury *et al.* 2007; Robinson *et al.* 2010) and salmonellosis (van Andel *et al.* 2015). As viruses are responsible for over 40% of all recently surveyed wildlife EIDs (Dobson and Foufopoulos 2001; Tompkins *et al.* 2015), they are a particular risk to wildlife and managers of threatened populations should consider how their transmission may be unintentionally facilitated by conservation actions that artificially manipulate population density.

Psittacine beak and feather disease (PBFD), caused by the Beak and feather disease virus (BFDV; Circoviridae), is a widely infectious and often fatal disease that originated in Oceania (Raidal, Sarker and Peters 2015) and has since rapidly spread around the world due to the international wildlife trade (Harkins *et al.* 2014; Fogell *et al.* 2018). PBFD is thought to be the most common viral disease in wild psittaciformes (Khalesi *et al.* 2005), affecting at least 60 Old and 18 New World psittacine species globally (Fogell, Martin and Groombridge 2016). One such affected endangered and recovering species is the Mauritius parakeet (*Psittacula eques*), which was once widely distributed across the tropical rainforest habitat of Mauritius. The population has recently recovered from a severe population bottleneck, after declining to fewer than 20 individuals in the 1980s (Raisin *et al.* 2012; Duffy 1993; Fogell *et al.* 2019). The species is confined to the Black River Gorges National Park (BRGNP), in the southwest, and a newly established subpopulation in a private nature reserve to the east of the island. Within the BRGNP, the parakeet population is divided into two subpopulations, isolated by distance (Raisin *et al.* 2012), with one in the north of the reserve and one in the south (Figure 6.1). Their recovery is due to intensive management measures including brood manipulation, supplementary feeding, provision of artificial nest sites, captive-breeding, reintroduction, and control of invasive alien predators (Bunbury *et al.* 2007; Tatayah *et al.* 2007). However, these efforts were interrupted by an outbreak of Psittacine beak and feather disease (PBFD) in 2005 (Kundu *et al.* 2012).

The disease outbreak in Mauritius was considered a threat to the parakeet's recovery and prompted the immediate cessation of some elements of their management including the transfer of individuals and eggs between nest sites. The provision of nest boxes, control of alien predators, use of supplementary feeding hoppers (henceforth referred to as hoppers) and a minimal regime of visits to nest sites for monitoring purposes remained in place (Tollington *et al.* 2013). However,

recent research has indicated that the prevalence of BFDV infection in nestlings is significantly associated with their proximity to hoppers (Tollington et al. 2018; Fogell et al. 2019; Chapter 3, 4). Biosecurity was initially thought to be the primary means of reducing BFDV prevalence in nestlings by managing the exposure of breeders to contaminated surfaces (Chapter 4). However, to date the influence of social interactions between nesting individuals at hoppers has been largely ignored. Indeed, exposure by individuals to infected surfaces may only be a part of the story as sociality may be a key factor in transmission between parents and their offspring.

Consequently, here we explore and quantify the social network of Mauritius parakeets foraging at hoppers and assess demographic shifts in their social interactions, and subsequently BFDV transmission, over the course of the breeding season. We compare the prevalence of BFDV infection in broods of nestlings to the frequency of hopper attendance and network centrality of their parents. We predict that either nestling BFDV prevalence will be higher at those sites where parents attend hoppers more frequently, or in those where parents are more central to the social contact network, both of which will result in higher levels of exposure to and transmission of infection.

6.3 METHODS

6.3.1 Nest site monitoring

Mauritius parakeet nest site monitoring includes the annual banding of all known nestlings with a unique ring combination, resulting in the ability to identify the majority of the population individually at both hoppers and nest sites. Blood samples (n = 217) were taken from the brachial vein from nestlings produced over the 2016/17 breeding season and prior to fledging, at approximately 45-days old, and stored in ethanol. This research was conducted under the University of Kent ethical guidelines (0018-DF-16). Population monitoring and nestling sampling was undertaken in collaboration with the Mauritius National Parks and Conservation Services and the Mauritian Wildlife Foundation (Henshaw *et al.* 2016) and samples were imported to the United Kingdom under the following license numbers: IMP/GEN/2014/02 and TARP/2016/105.

6.3.2 Parakeet social network

We recorded the attendance of Mauritius parakeets at two hoppers - one in the north and one in the south of the BRGNP. Individuals were recorded twice daily (at dawn and again in the early afternoon) over the breeding season from September 2016 to January 2017, with weekly alternation between northern and southern subpopulations (Figure 6.1). Independent hour-long sampling periods began when two or more parakeets were in attendance at a hopper together ($N_{\text{northern subpopulation}}$ = 278 birds across 70 sampling periods; $N_{\text{southern subpopulation}}$ = 114 birds across 62 sampling periods), recording all identifiable individuals that attended over the hour. Observations were conducted both in person and from footage obtained by deploying two GoPro Hero 4 cameras

at the feeding hoppers. Video footage was found to be particularly beneficial when observing the southern subpopulation, as the birds in the north are provisioned in the clearing surrounding a main field station and were therefore more accustomed to a constant human presence. As only female Mauritius parakeets incubate their clutches (Jones *et al.* 1998), dams are hypothesised to be largely absent from the social networks early in the season. Consequently, we divided the association data for each subpopulation into two halves of the breeding season (early and late) to assess temporal changes in the network structure. All parakeets that attended the hoppers within an hour sampling period were assumed to be associated using the gambit of the group methodology (Franks, Ruxton and James 2010).

Previous stable isotope studies on the dietary composition of Mauritius parakeet nestlings indicated that the proportion of supplementary food consumed was proportional to the distance a nest site was located away from the nearest hopper (Tollington *et al.* 2018). Therefore, we ran a simple linear model testing whether the same relationship existed between the cumulative number of times both individuals from a nesting pair were recorded at a hopper and their nest site distance.

A set of four generalised linear mixed effect candidate models were run in R version 3.5.2 (R Core Team 2018) using a Gaussian distribution and the individual as a random intercept effect to account for non-independence of observations. The sex of the individuals (male, female or juveniles < 2 years old) constituting each network were compared to the frequency of attendance (response variable) to assess whether any demographic differences existed in total attendance at hoppers between the first half and second half of the breeding season. As more than one model was within 2 ΔAICc, and thus equally plausible, we used model averaging (*AICcmodavg* package, Mazerolle, 2016) to estimate predicted parameter values. Four undirected network visualisations (one per subpopulation, per half of the breeding season) were conducted in *igraph* (Csardi and Nepusz 2006) and *sna* (Butts and Carley 2001). We then used a two-tailed t-test to determine whether the observed patterns of sociality could have arisen by chance; using randomised network permutations (n=1000) on the raw data stream for each sample set (Farine 2013; Bejder, Fletcher and Brager 1998), to swap individuals between associations.

6.3.3 Incorporation of BFDV prevalence

Prior to screening for BFDV, an ammonium acetate DNA extraction method was used to extract both host and viral DNA (Bruford et al. 1998). In brief, for all nestling samples approximately 50 to 100 µl of whole blood was used from each sample and digested in 250 µl of DIGSOL lysis buffer with 10 µl of 10 mg/mL proteinase K. Extractions were quantified using a Qubit dsDNA Assay Kit and standardized to approximately 10 ng/µl prior to screening for BFDV. For detection of BFDV in the blood sample extractions, two replicates were performed for each individual using the same RT-PCR primers and protocol as described above (Tollington *et al.* 2018). If the repeats did not

amplify within one cycle of each other, a third replicate was performed. Each 96-well plate included two negatives and two positive controls from a high viral load Mauritius parakeet individual (amplification at ~10 cycles) for standardisation across runs. Each individual nestling was assigned a positive or negative infection status according to whether any viral amplification occurred.

Average BFDV prevalence for a nest site was then weighted on the basis of the number of chicks produced in the clutch and compared to five individual social network measures associated with their parents. These were calculated separately for both parents over each half of the breeding season and were as follows: total number of times recorded, degree centrality, closeness centrality, betweenness centrality and coreness. Degree centrality is an unweighted metric which directly measures the number of different associations an individual has (Aplin *et al.* 2013), closeness centrality is an estimate of how closely connected an individual is to all others within the network (where smaller values reflect more closely connected individuals) (Martínez-López, Perez and Sánchez-Vizcaíno 2009) and betweenness centrality is an estimated probability that shortest path between any pair of individuals within the network passes through a node (Martínez-López, Perez and Sánchez-Vizcaíno 2009). The coreness (or K-core) is a measure that identifies tightly interlinked groups of individuals within the network (Seidman 1983). Using these individual network metrics for both parents, we then ran a set of 31 candidate generalised linear models assessing the influence of each on the nestling weighted BFDV prevalence for their nest site (Table 6.1). As more than one model was within 2 ΔAICc, and thus equally plausible, we again used model averaging (*AICcmodavg* package, Mazerolle, 2016) to estimate predicted parameter values.

6.4 RESULTS

6.4.1 Associations between individuals and demographic structure

We found a strong negative correlation between the cumulative frequency of a breeding pair's attendance at a hopper and the distance their nest site was located away (Figure 6.2). Neither parent was recorded at a hopper for only four of the 87 nest sites in which BFDV prevalence was recorded, all from the southern subpopulation (average nest site distance from hopper = 2.89 ± SD 0.67 km). Only a single parent was recorded for 27 of the assessed nest sites, 41% of which were from the southern subpopulation (average nest site distance from hopper = 2.03 ± SD 0.85 km).

We found that across all of our sampling periods, the mean number of associations between individuals in the northern subpopulation were significantly higher than expected by chance (Figure S6.1, $P < 0.025$). Conversely, the mean number of associations across sampling periods for the southern subpopulation was found to be significantly lower than expected by chance within the second half of the breeding season (Figure S6.1, $P > 0.975$). In the first half of the breeding season, the associations within the southern subpopulation were also found to be low,

but these observations were found to fall just outside of the bounds of significance from those occurring by chance (Figure S6.1, P = 0.94). Across the social networks for both subpopulations, we found a significant change in their demographic structure between the first and second halves of the breeding season (Figure 6.3). When observing the southern subpopulation, we recorded a total of 53 males and 17 females during the first half versus a decrease in the number of males (n = 44) and increase in the number of females (n =31) during the second half of the breeding season. Juvenile numbers remained broadly similar (15 for the first half and 18 for the second half of the breeding season). During the northern subpopulation observations, we recorded a similar number of males and juveniles over the breeding season with a total of 124 males and 56 juveniles during the first half versus 125 and 55 respectively during the second half of the breeding season. Numbers of females increased from 41 to 66 from the first to second half of the breeding season. On average across both subpopulations for the first half of the breeding season females were predicted to attend hoppers 6.1 times, males 10.4 times and juveniles 1.7 times (Figure 6.4). Over the second half of the breeding season, the predicted average male attendance significantly decreased to 6.3 times ($z = -4.715$, $p < 0.001$), whilst females significantly increased to 8.6 times ($z = 6.921$, $p < 0.001$) and juveniles increased to 4.5 times ($z = 2.277$, $p = 0.184$) (Figure 6.4).

Across the breeding season the social network measures of degree, betweenness and coreness were found to be significantly correlated (correlation coefficient ≥ 0.8) with the total frequency of attendance at hoppers in both dams and sires. However, closeness was not found to be significantly correlated with frequency of attendance (Figure S6.2).

6.4.2 Social network position and relationship to BFDV

We found two equally plausible models (within 2 ΔAICc) explaining the relationship between BFDV prevalence (weighted according to the number of chicks produced in the nest) and the position of individuals within the social network at hoppers (Table 6.1). These were both the null model and the model comprising the interaction between the frequency of dam and sire attendance at feeding hoppers during the first half of the breeding season. The interaction between dam and sire total attendance was found to be significantly positively correlated with weighted BFDV prevalence of their brood; where those nest sites where both parents regularly attended feeding hoppers were found to have a higher brood BFDV prevalence than those where only a single parent attended during the first half of the season ($\beta = 2.68 \times 10^{-3}$, 95% CI = $5.10 \times 10^{-4} – 4.85 \times 10^{-3}$) (Figure 6.5).

6.5 DISCUSSION

Our results have provided three key insights into sociality of parrots in the presence of supplementary food, which may influence the transmission of infectious disease between parents and their offspring. The first is that the frequency of attendance at hoppers by a nesting pair of the

endangered Mauritius parakeet strongly relates to the distance they nest away from the provisioned resource. Despite previous inference of this relationship through stable isotope analyses on the proportional reliance of a brood on supplemental food (Tollington *et al.* 2018), observational evidence to support this hypothesis had not yet been conclusively evaluated. The second is that the frequency of attendance at hoppers over the breeding season is demographically dynamic in accordance with Mauritius parakeet ecology. Finally, we have successfully demonstrated that the prevalence of a highly infectious viral pathogen in parakeet nestlings can be attributed to the interactive effects of parental frequency of attendance at hoppers early in the breeding season.

The two constraints on parents foraging to provide for their offspring are time and energy (Weimerskirch, Prince and Zimmermann 2000). Tollington et al. (2018) demonstrated that those Mauritius parakeet pairs that rely more on hoppers during the breeding season have higher fecundity. This trade-off between convenient access to supplemental food and the time taken or energy expended to reach a hopper during the breeding season influences the frequency of attendance by breeding pairs. However, our study shows that it is not just the total frequency of attendance by a parent that determines nestling infection with BFDV, but rather that this effect is significantly amplified when both parents regularly interact with others attending the hoppers. The differences in the sociality of parakeets between subpopulations (Figure S6.2) is largely driven by the significantly higher average distance between nest sites and feeding hoppers in the south (northern subpopulation mean distance nest to feeding hopper = 0.76 ± 0.08 km (SE); southern subpopulation mean distance nest to feeding hopper = 2.38 ± 0.14 km (SE); $t(240) = 18.06$, $p < 0.001$). Due to the use of observational data from a single breeding season, data derived for the southern subpopulation is particularly limited. Therefore, whilst a significant pattern has been observed between parental sociality and BFDV infection in nestlings in this study, we acknowledge that these findings may be strengthened by an observational dataset spanning numerous breeding seasons.

The more frequent attendance of male parakeets and substantially lower overall number of females at hoppers in the first half of the breeding season is expected, as dams are solely responsible for incubation of the clutch (Jones *et al.* 1998). Once the chicks have hatched and become more independent during the latter half of the breeding season, dams return to the hoppers and presumably then share the burden of provisioning for their brood. Despite the increase in frequency of attendance by dams later in the breeding season, nestling infection is most strongly associated with early season attendance. This relationship suggests that, whilst some chicks may hatch already infected with BFDV due to vertical transmission from female to embryo (Rahaus *et al.* 2008; Ritchie *et al.* 1989), young broods are at greater risk of infection when dams return to the feeding stations. The significance of these parental individuals within the contact network may be

due to changes in their behaviour at hoppers driven by their own disease status, or may make them more susceptible to becoming infected with BFDV themselves through more frequent (direct or indirect) interaction with numerous other carriers of infection.

BFDV-infected individuals experience immunosuppression and are more susceptible to secondary infection (Peters *et al.* 2014). Breeding individuals experiencing primary or secondary disease may therefore attend hoppers for longer periods because of the easier access to food. Alternatively, those that are more closely connected within the network (i.e. social) run a higher risk of regularly encountering BFDV-infected infected individuals at hoppers, and thus become infected themselves. Regardless of which of these explanations is correct, these central individuals that attend hoppers most frequently may act as super-spreaders of infection within the Mauritius parakeet population (Silk *et al.* 2017; Lloyd-Smith *et al.* 2005), where they give rise to a disproportionately high number of secondary cases (Lloyd-Smith *et al.* 2005; Paull *et al.* 2013). The capacity of an individual to be a super-spreader is dependent on variations in susceptibility to infection (Cross *et al.* 2012) and is not necessarily reliant on their diseased status. Infected individuals may either behave as maintenance hosts (those maintaining steady infection with the potential to act as reservoirs) or non-maintenance hosts (those with transient infection) (Cleaveland *et al.* 2007; Craft *et al.* 2009).

As we are inferring transmission dynamics and do not have any infection data for the parents themselves, we cannot ascertain whether nestling infection is due to parental shedding of virions from their own active contracted infection, or to the frequent transport of virions from numerous other infected individuals back to the nest. Nevertheless, our study has identified that parental sociality in the first half of the breeding season drives the prevalence of infection in their nestlings. Whether those more socially connected are primarily givers or receivers of infection, the management response to reduce BFDV infection remains the same. In order to disrupt this network and reduce contact between infected and uninfected individuals, the distance between nest sites and hoppers should be increased. It is clear that supplementary feeding hoppers play a role in shaping this host-pathogen relationship and, as such, the risks of potential infectious disease transmission need to be weighed against population recovery. As current evidence suggests that the impacts of BFDV within the Mauritius parakeet population are limited (Fogell *et al.* 2019), management remains focused on recovery through the increased fecundity afforded by supplementary food provisioning. However, our findings suggest that conservationists managing recovering populations in the face of BFDV should be cautious about the use of supplementary feeding to ensure that their management objectives are being met.

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6.7 TABLES AND FIGURES

Table 6.1 A comparison of the 31 generalised linear candidate models assessing the relationship between the weighted BFDV brood prevalence and their parental network centrality of the first (H1) and second half (H2) of the breeding season. Extracted network metrics included Dam (D) and Sire (S) total frequency of attendance (total), degree, closeness (close), coreness (core) and betweenness (between). Models are assessed based on Akaike's information criterion corrected for finite sample size (AIC_c) and weights (AIC_c weights). K denotes the number of parameters in each model and models are ranked in order according to their ΔAIC_c.

Rank	Model	K	AIC_c	ΔAIC_c	AIC_c Weights
1	Null model	2	-89.18	0.00	0.33
2	S total H1*D total H1	5	-89.00	0.17	0.30
3	S total H1 + D total H1	4	-85.33	3.84	0.05
4	S total H2 + D total H2	4	-85.07	4.10	0.04
5	S between H2 + D between H2	4	-84.80	4.38	0.04
6	S core H1*D core H1	5	-83.94	5.23	0.02
7	S degree H2 + D degree H2	4	-83.94	5.24	0.02
8	S core H2 + D core H2	4	-83.87	5.30	0.02
9	S total H2*D total H2	5	-83.74	5.44	0.02
10	S between H1*D between H1	5	-83.61	5.57	0.02
11	S between H1 + D between H1	4	-83.49	5.69	0.02
12	S degree H1*D degree H1	4	-83.31	5.86	0.02
13	S core H1*D core H1	4	-83.25	5.92	0.02

14	S degree H1* D degree H1	5	-82.70	6.48	0.01
15	S between H2* F between H2	5	-82.54	6.64	0.01
16	S degree H1* S degree H2	5	-82.44	6.74	0.01
17	S total H1 + S total H2 + D total H1 + D total H2	6	-82.00	7.18	0.01
18	S core H2*D core H2	5	-81.85	7.32	0.01
19	S total H1* S total H2 + D total H1*D total H2	8	-80.83	8.35	0.01
20	S core H1*D core H2 + S core H2*D core H2	8	-80.60	8.58	0.00
21	S between H1 + S between H2 + D between H1 + D between H2	6	-80.46	8.71	0.00
22	S degree H1 + S degree H2 + D degree H1 + D degree H2	6	-79.59	9.59	0.00
23	S core H1 + S core H2 + D core H1 + D core H2	6	-79.50	9.68	0.00
24	S degree H1*S degree H2 + D degree H1*D degree H2	8	-77.35	11.83	0.00
25	S between H1*S between H2 + D between H1*D between H2	8	-76.72	12.45	0.00
26	S close H2 + D close H2	4	-67.25	21.92	0.00
27	S close H2*D close H2	5	-64.92	24.26	0.00
28	S close H1 + D close H1	4	-24.71	64.46	0.00
29	S close H1*D close H1	5	-22.02	67.15	0.00
30	S close H1 + S close H2 + D close H1 + D close H2	6	-13.21	75.96	0.00
31	S close H1*S close H2 + D close H1*D close H2	8	-4.66	84.52	0.00

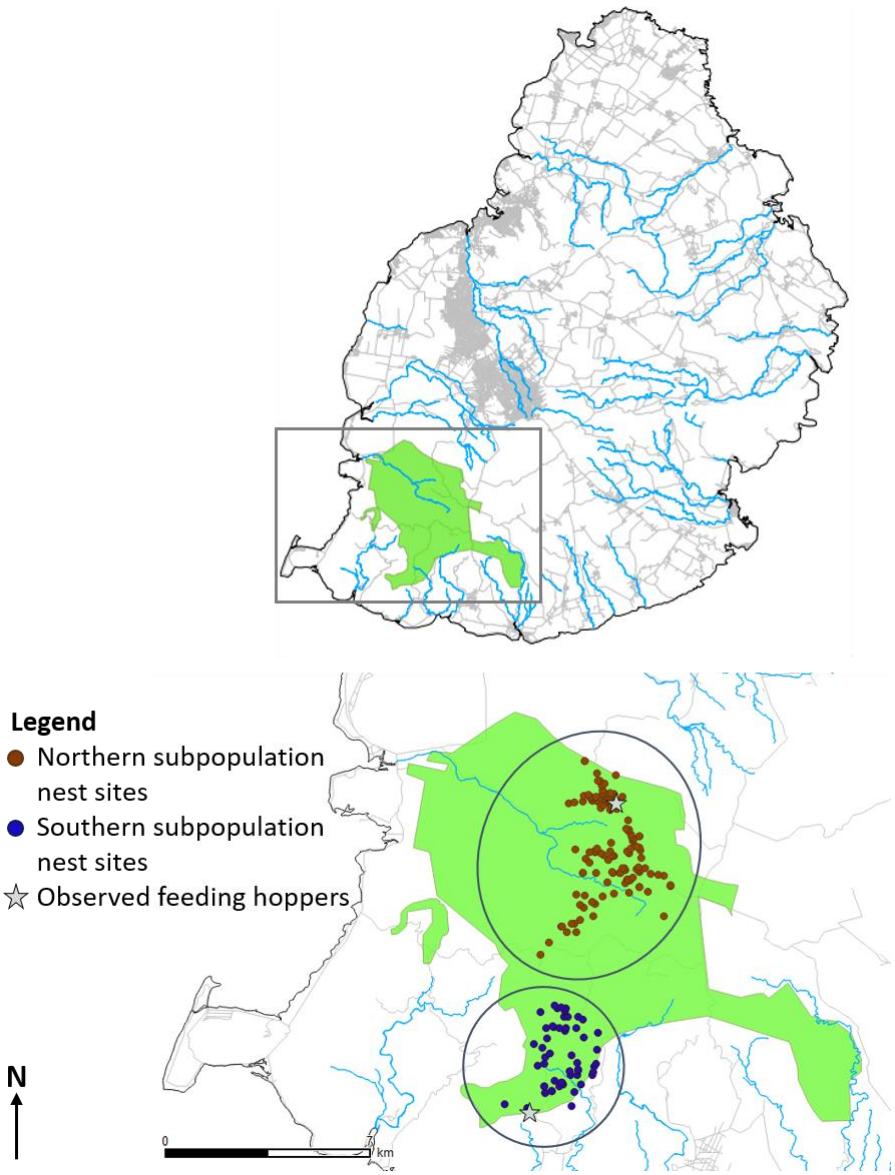

Figure 6.1 The location of the Mauritius parakeet nest sites and observed supplementary feeding hoppers within the Black River Gorges National Park in the southwest of Mauritius.

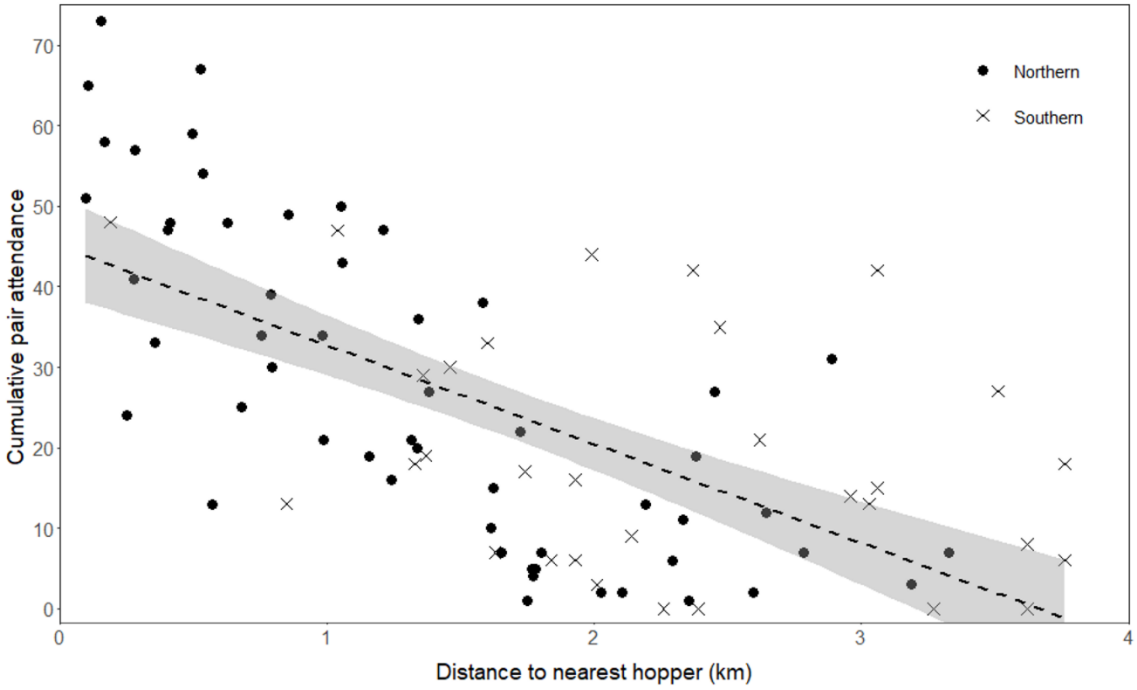

Figure 6.2 The observed cumulative total attendance of breeding pairs of Mauritius parakeets at supplementary feeding hoppers during the 2016/17 breeding season (September to January) plotted against the distance (km) their nest site is located away. The dotted line and corresponding shaded area depict the fitted linear model and corresponding 95% confidence intervals of the significant negative relationship between these two variables across the Mauritius parakeet breeding population within the Black River Gorges National Park (adjusted $r^2 = 0.41$). Filled circles and crosses represent those pairs from the northern and southern subpopulation respectively.

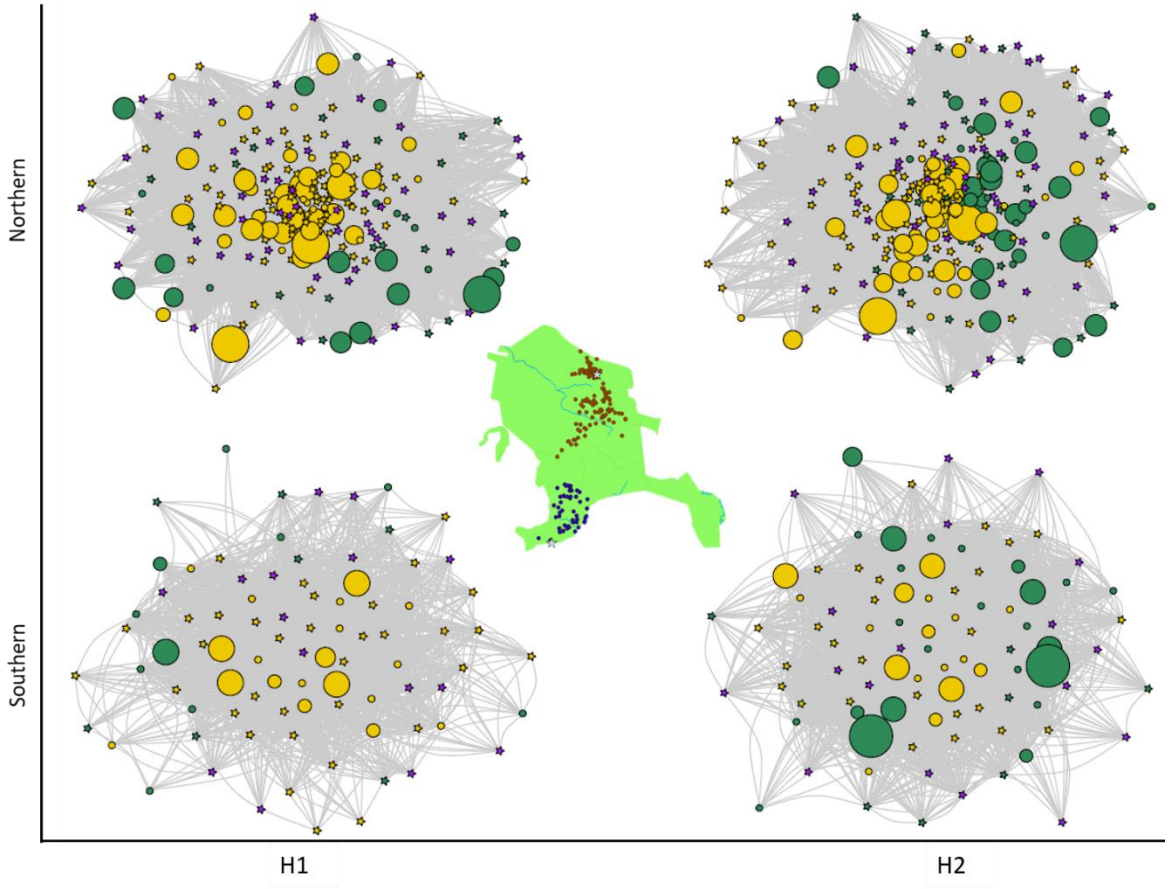

Figure 6.3 Social contact networks depicting the temporal changes in relationships between Mauritius parakeets attending supplementary feeding hoppers within the northern and southern subpopulations in the first half (H1) versus the second half (H2) of the 2016/17 breeding season. Circles represent breeding individuals and are sized according to the weighted prevalence of their brood. Stars represent non-breeding individuals. Males are coloured in gold, females are coloured in green and juveniles are coloured in purple. Female nodes increase in the second half of the breeding season across both subpopulations (from 41 to 66 in the northern and from 17 to 31 in the southern subpopulation), male nodes decrease in the second half of the breeding season within the southern subpopulation (from 53 to 44) and slightly increase (from 124 to 125) in the northern subpopulation) and juvenile nodes remain similar across the breeding season for both subpopulations (56 to 55 in the northern and 15 to 18 in the southern subpopulation respectively). Inset: the location of the supplementary feeding hoppers (grey stars) and Mauritius parakeet nest sites within the Black River Gorges National Park (filled circles).

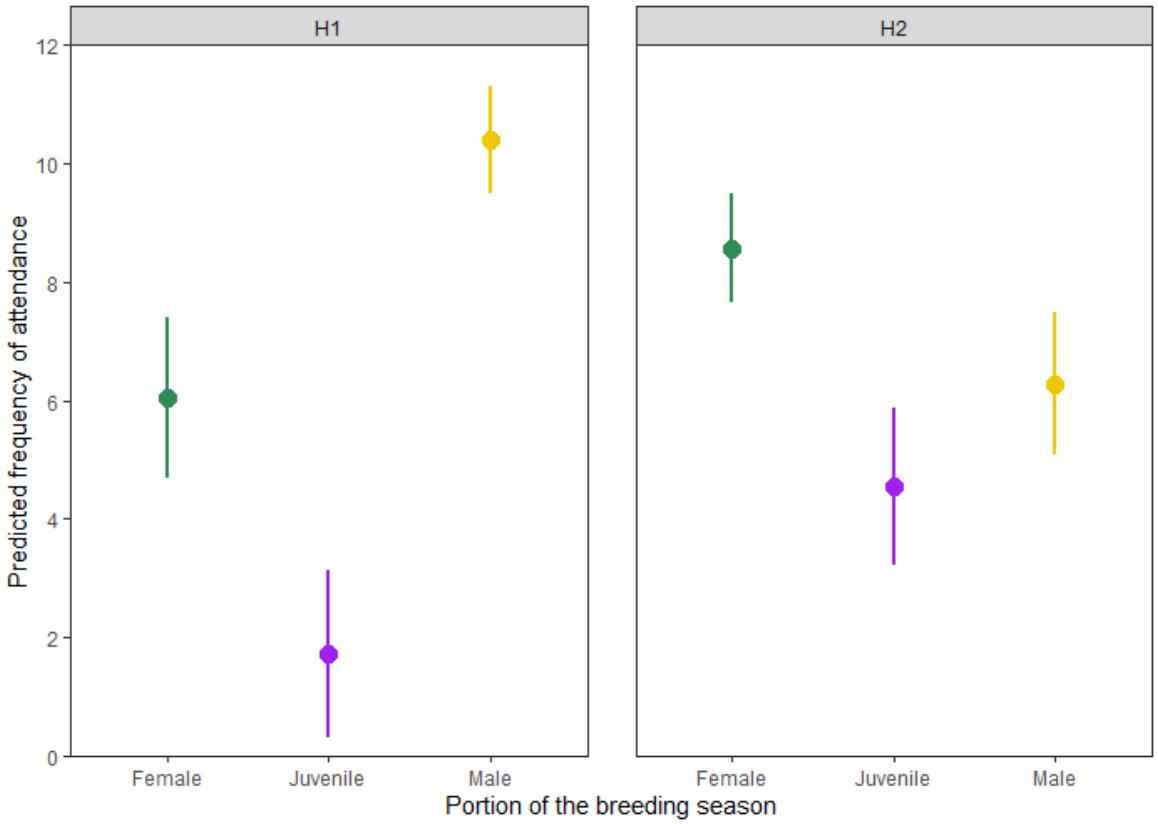

Figure 6.4 The predicted frequency of attendance of Mauritius parakeet females (green), juveniles under the age of 2 years (purple) and males (gold) over the course of the 2016/17 breeding season, displayed with their 95% prediction intervals. H1 = first half of the breeding season, H2 = second half of the breeding season

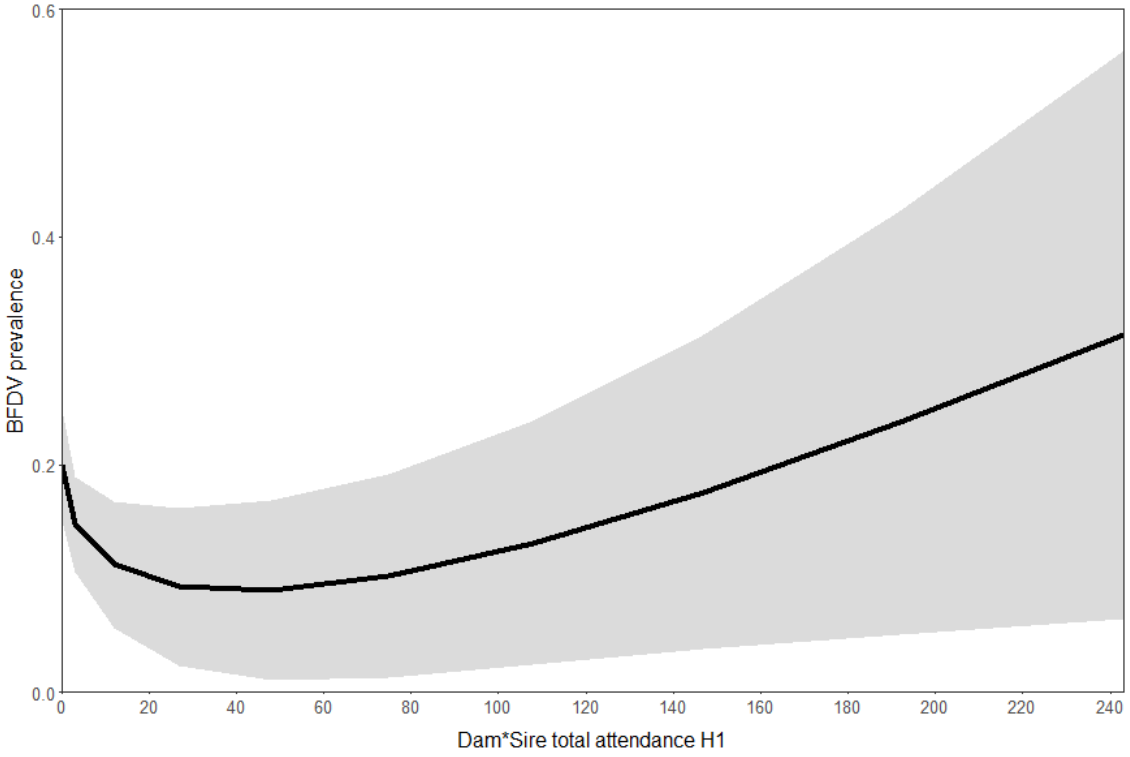

Figure 6.5 The interaction between sire and dam total attendance in the first half of the breeding season (H1) and the predicted prevalence of BFDV in nestlings (solid line) with their 95% prediction intervals (shaded area).

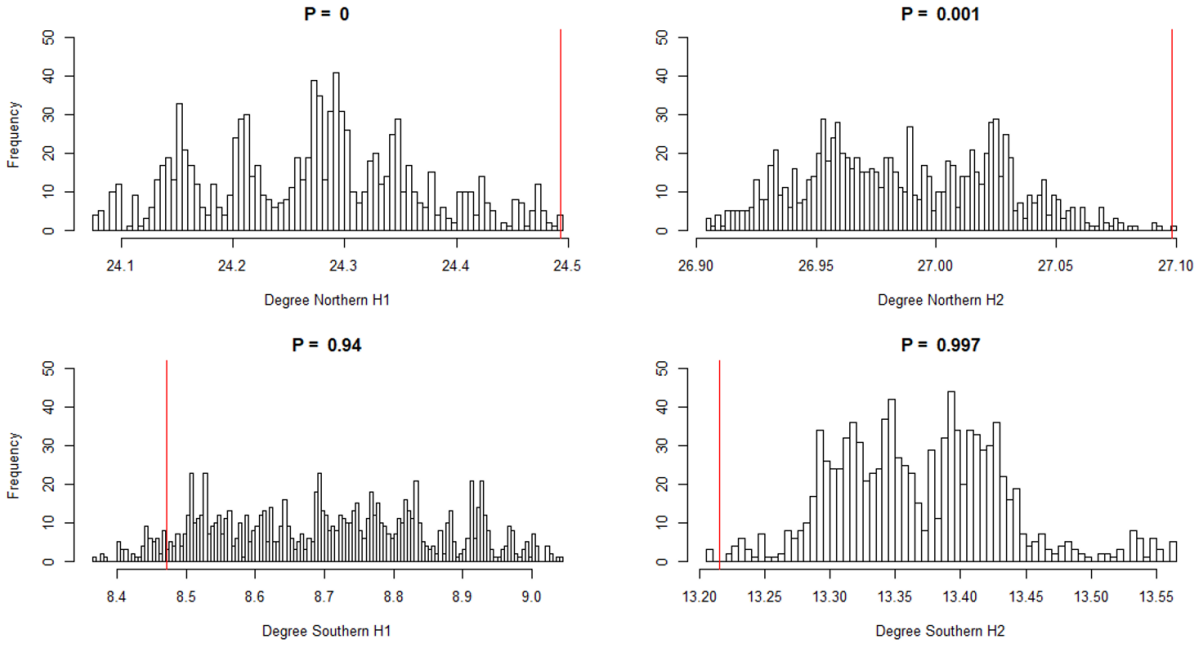

Figure S6.2 The comparison of 1000 random data stream permutations (bars) of observations within each sampling period with the observed degree (red line) of each Mauritius parakeet social network both in the first (H1) and second half (H2) of the breeding season across the northern and southern subpopulations. The strength of northern subpopulation associations (degree) were found to be significantly higher than if they occurred by chance throughout the breeding season. The strength of southern subpopulation associations (degree) were found to be significantly lower than if they occurred by chance during the second half of the breeding season.

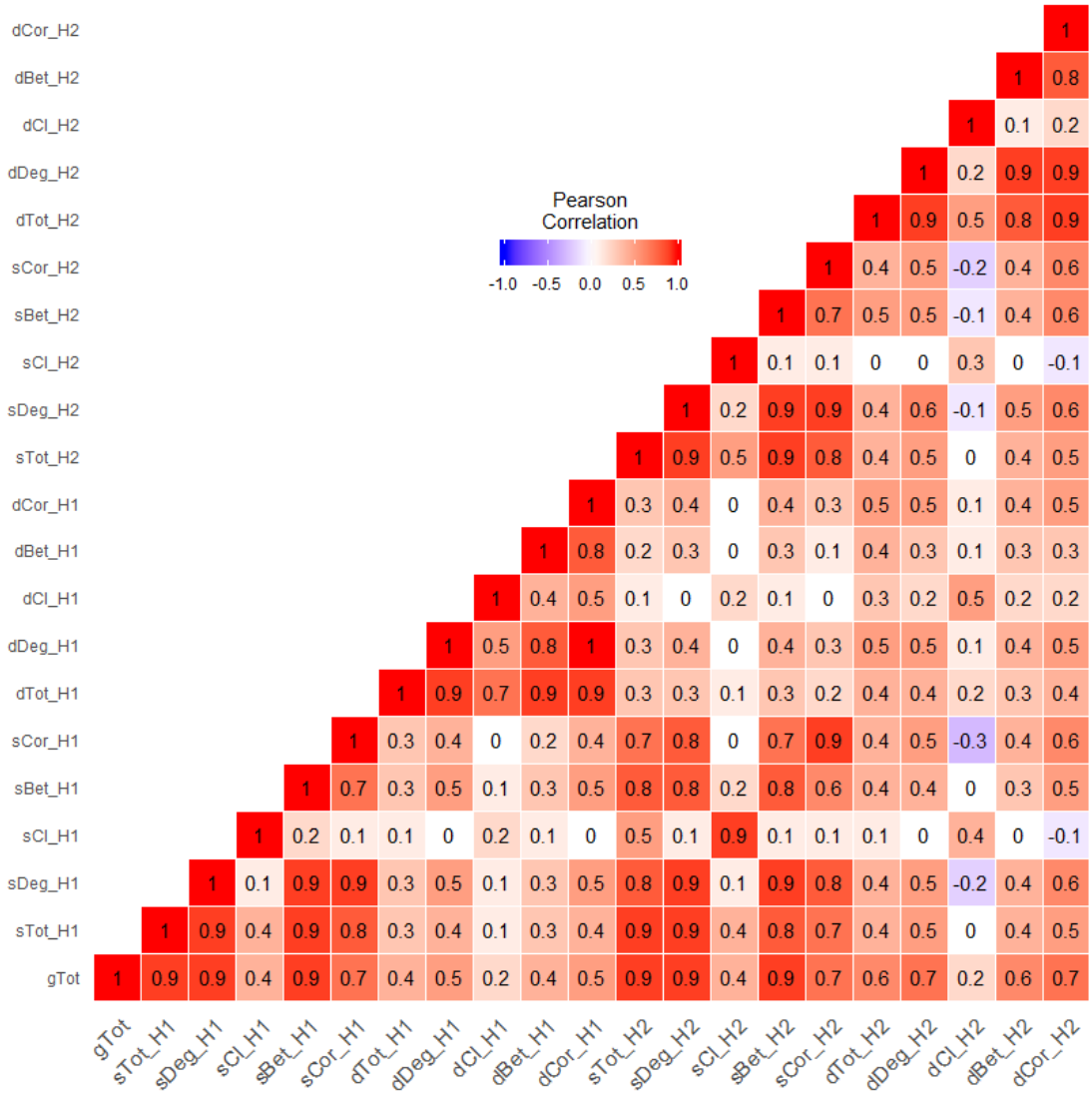

Figure S6.2 Pearson Correlation matrix of Mauritius parakeet social network metrics, z, where total attendance of both dams and sires is significantly correlated with degree, betweenness and coreness, but is not correlated to closeness. H1 = first half of the breeding season, H2 = second half of the breeding season, gTot = total pair attendance at feeding stations, s = sire, s = dam, Tot = total attendance, Deg = degree, Cl = closeness, Bet = betweenness and Cor = coreness.

Chapter 7

General discussion

Conservationists managing threatened wildlife populations are often required to act quickly in an attempt to halt declines and preserve biodiversity. Emerging infectious diseases (EIDs) have become an increasingly pressing issue in conservation and is one that is challenging to tackle due to the increased connectivity between distant geographic regions afforded by globalisation (Hoberg and Brooks 2015). The international wildlife trade has provided an ideal mechanism for not only the spread of invasive non-native species, but also the pathogens that they carry (Karesh *et al.* 2007; Karesh *et al.* 2005). In recent years the Beak and feather disease virus (BFDV) has become a pathogen of conservation concern and has been implicated in the decline of a number of threatened wild parrot populations, including the endangered Cape parrot (*Poicephalus robustus*) of South Africa (Regnard *et al.* 2015), the Australian orange-bellied parrot (*Neophema chrysogaster*) (Peters *et al.* 2014) and the Mauritius "echo" parakeet (*Psittacula eques*) (Kundu *et al.* 2012). The virus has been widely recognised in captive populations from across the world, and consequently there has been a call for better surveillance in wild populations to arm conservationists with a better understanding of the risks facing the parrot populations that they manage (Fogell, Martin and Groombridge 2016). Additionally, despite awareness by population mangers that stringent biosecurity protocols should be maintained in an attempt to limit the spread of this EID (e.g. Department of the Environment and Heritage 2005), there is a large amount of uncertainty surrounding the best methods with which to do this. The primary aim of this thesis was to provide some greater insight into the global distribution of BFDV, with a particular focus on geographic regions that lacked surveillance to date, and tackle some of the challenges associated with managing infection transmission within an affected recovering species.

7.1 THE ROLE OF THE WILDLIFE TRADE IN THE SPREAD OF BFDV

The theory that BFDV had spread around the world by the trade in pet birds had already been previously established (Harkins *et al.* 2014; Varsani *et al.* 2011) due to the widespread prevalence of the virus in captive parrots and distribution of similar viral haplotypes within distant global regions. The study I present in Chapter 1 successfully provides the first step towards some much-needed insight into BFDV incidence within wild populations from biodiverse, data deficient regions. Through collaboration with a network of conservationists and conservation organisations I have described the detection of BFDV in eight countries where it was previously unknown to occur, including within the native African and Asian ranges of Rose-ringed parakeets (*Psittacula krameri*). Rose-ringed parakeets are one of the most successful globally invasive species (Menchetti, Mori and Angelici 2016) and, therefore, could become an ideal infectious disease reservoir, especially as their native and invasive global distribution overlaps with numerous threatened parrot species.

The results of the phylogenetic analyses indicated that there had been numerous introductions of BFDV around the world, with close relationships between viral haplotypes from globally distant regions. Patterns of viral distribution such as these are of conservation concern due to the risks of formation of novel, highly virulent strains through viral recombination (Jackson *et al.* 2015; Julian *et al.* 2013). This may increase the impacts of disease in wild populations, inclusive of those in Oceania in which infection was already native. Phylogenetic relationships between viral sequences showed likely pathways of transmission between populations in southern Asia and Western Africa, as well as between Seychelles and the United Kingdom. Within the Seychelles, the presence of BFDV in introduced Rose-ringed parakeets was particularly pertinent due to the intensive control measures that were underway at the time of detection. The Seychelles Islands Foundation launched their Rose-ringed parakeet eradication campaign in 2013 in response to concerns over biosecurity (Seychelles Islands Foundation 2013), to minimize threats to the endemic Seychelles black parrot (*Coracopsis barklyi*). The results of this study provided scientific support that the concern for biosecurity was justified and Seychelles has since become the first country from which Rose-ringed parakeets have been extirpated (Bunbury *et al.* 2019). Within a global context, these findings have highlighted the need for effective regulation of international trade in live parrots, particularly in regions with high parrot endemism or vulnerable taxa where Rose-ringed parakeets could act as a reservoir host.

The BFDV isolates from both the native and invasive parakeet species present in Mauritius represent a large proportion of those sequenced from around the world. Whilst phylogenetic studies have been conducted before on a smaller subset of data than I present in Chapter 1, this research is the first time that the strain present on Mauritius has been placed in a global context. Despite the large representation of sequences from Mauritius within the Maximum Likelihood analysis, along with 708 further viral isolates accessioned to GenBank from captive and wild parrots globally, there is still little clarity on the likely origin of the strain present on the island.

7.2 THE INFLUENCE OF ABIOTIC FACTORS ON BFDV PREVALENCE

Artificial nest sites have become a frequently deployed management tool amongst conservationists managing avian populations as they have proven to successfully improve recovery outcomes for obligate cavity nesting avian species (Sherley *et al.* 2012; Norris *et al.* 2018; Berris *et al.* 2018). However, nest sites also pose a key risk point of contact for the spread of infectious disease, especially to individuals at a naïve life stage. As infection with BFDV is known to particularly impact juveniles under the age of three years old, it was pertinent to assess whether conservationists managing affected parrot populations could better inform nest placement to reduce nestling prevalence. The Mauritius parakeet study system has provided an ideal scenario on which this can be modelled, due to the availability of long-term data on nest site characteristics (i.e.

aspect and altitude of location), nesting success of each breeding pair and on BFDV prevalence within the population.

The results presented in Chapter 2 indicate that there is currently no need for managers to alter their approach to artificial nest site placement for continued species recovery. The proximity to supplementary feeding stations largely drives the patterns of nesting success and the prevalence of BFVD within the Mauritius parakeet population. Though future climate change scenarios of warming and drying within Mauritius (IPCC 2014) may mean that prevalence could increase at higher altitudes, the potential for this increase is only observable in those nest sites located furthest away from feeding hoppers, and is not currently a cause for concern. Similarly, neither altitude nor aspect of a nest have been demonstrated to alter the fecundity of this species. Whilst it appears that nest microclimate may not have a strong influence on BFDV prevalence, I have used altitude as a proxy for larger scale climatic effects. Therefore, it would be beneficial for future work to focus on how the variations in long-term prevalence within an affected population are influenced by more detailed factors such as temperature, humidity and rainfall.

7.3 THE COSTS AND BENEFITS OF BIOSECURITY TO MANAGE BFDV TRANSMISSION

It is clear from the two experiments described in Chapters 3 and 4 that there is still much to be learnt with regards to how to best manage the *in situ* transmission of BFDV in wild populations through biosecurity protocols. While it is obviously prudent to maintain good hygiene and biosecurity when managing any wild BFDV affected population, what form that protocol should take is still largely unknown. As discussed in Chapter 3, I found that a single disinfection of nest sites prior to the breeding season using Virex or Virkon did significantly reduce the prevalence of BFDV in nestlings, but this also impacted the proportion of eggs that were converted into fledglings for recruitment into the population. This management protocol was not only resource intensive (both labour and financially), but had an unintentional impact on fecundity - our fundamental objective for population recovery. These findings emphasise the value of constantly monitoring and evaluating the tools implemented in species recovery. Consistent continuous evaluation ensures that conservationists remain on the trajectory of achieving positive outcomes for their target species, and certainly allows managers to avoid, or at the very least mitigate, negative impacts such as the one I describe.

The strong negative relationship described between BFDV prevalence in nestlings and the distance those nestling were produced away from supplementary feeding hoppers suggested that biosecurity could instead be better targeted at these hubs of frequent contact between infected individuals and contaminated surfaces. Thus, through an adaptive management process, in collaboration and consultation with the Mauritian Wildlife Foundation, I designed an experiment focused on upscaling biosecurity at supplementary feeding hoppers. It was hoped that this form of

BFDV management would not only reduce the impact on reproductive success seen with disinfection at nest sites, but would also provide a less labour-intensive solution for field staff responsible for the monitoring of a steadily increasing Mauritius parakeet population.

The application of swabs to detect BFDV environmental DNA proved to be very successful. However, the challenges of avoiding DNA carry-over contamination when sampling in an area containing high levels of infected feather dust and faecal matter became apparent when I detected low levels of BFDV present in 36% of the field blanks obtained. This finding emphasises the need to ensure that blank samples are also taken whilst working with eDNA. They provide a means from which baseline thresholds for detection can be developed to maintain scientific rigour and avoid false positives. The accumulation of BFDV on hopper surfaces was not gradual and linear as was hypothesised, but instead surfaces became quickly re-infected after cleaning and often, despite significant reductions in viral accumulation, disinfection did not remove all traces of BFDV. I also found that a more hopper-focused approach to biosecurity proved to have a less substantial impact on BFDV prevalence than the single disinfection of nest sites (11% reduction over three breeding seasons vs no observable impact over a single breeding season). Additionally, counter to expectations, a far greater impact in reproductive success was observed with upscaled hopper disinfection than through nest site biosecurity, likely due to consistent reinforcement of chemical residues brought back to the nest by parents attending hoppers. However, as it was considered prudent to maintain a minimum level of hopper biosecurity during the breeding season it should be noted that, unlike the nest experiment, the experimental protocol applied to hoppers did not include a true control of no disinfection. As it was hypothesised in the discussion of Chapter 3 that the impacts on the number of fledglings produced was due to the selected chemical disinfectants reducing egg hatchability (Wilson 2009; Scott, Swetnam and Kinsman 1993), it was necessary to approach the statistical analysis differently in Chapter 4. Instead, I chose to model the proportion of eggs converted to nestlings within each experimental group and then whether the different protocols affected the total number of fledglings produced for recruitment into the population. This approach supported the hypothesis that eggs were being impacted and not nestlings.

These two experimental studies are highly novel and, as far as I am aware, represent the first time that any disease mitigation strategies have been empirically evaluated for wild avian populations. Indeed, critical evaluation of management actions aimed at reducing the transmission of pathogens across all wildlife taxa is exceptionally rare in the published literature, with only some exceptions for the treatment of *Batrachochytrium sp.* in amphibians (Hudson *et al.* 2016; Canessa *et al.* 2018), lungworms in hares (Skrjabin 1970) and tuberculosis in badgers (Woodroffe, Frost and Clifton-Hadley 1999). Given the findings of both Chapters 3 and 4, I would recommend that future experiments are designed to measure the effects of other disinfection solutions on both BFDV prevalence and fecundity. Non-chemical approaches such as UV sterilisation or heat treatments

may be a more appropriate means to maintaining *in situ* biosecurity when managing BFDV affected populations as they have been demonstrated to be effective in the inactivation of circoviruses or other similar ssDNA viruses (Nims and Plavsic 2012). Additionally, whilst the focus of this thesis has been BFDV, it is also clear that a large research gap exists on monitoring the effectiveness of all pathogen biosecurity applied within a wildlife context. This is concerning, particularly given the unintentional negative impacts detected through my research, and presents a need for wildlife health specialists and conservationists to change their behaviour with regards to monitoring to avoid dogmatic approaches to conservation.

7.4 THE INFLUENCE OF SOCIAL NETWORKS IN BFDV TRANSMISSION

It became apparent over the course of my research that the consistently significant relationship existing between the proximity of nest sites to supplementary feeding hoppers and nestling BFDV prevalence was not only attributed to parents frequently coming into contact with contaminated surfaces. Given that a more frequent regime for disinfection of these surfaces made no significant difference to the prevalence of BFDV in nestlings, I developed the hypothesis that the social interactions afforded by these artificial sources of food was influential to the dynamics of transmission between individuals. I found that, whilst the cumulative frequency of attendance at hoppers by a breeding pair negatively correlated with their nest site distance, this was not the key metric that influenced the BFDV prevalence of their nestlings. Instead, the factor that was most significant was the closeness of sires to other individuals within the social network (i.e. the fewest number of steps from their own direct contacts to indirectly associate with all other individuals within the network). As supplementary food provision is a necessary support tool for the continued recovery of Mauritius parakeets (Tollington *et al.* 2018), this analytical approach that focuses on social networks at hoppers provides a basis from which targeted disease management could be applied to specific individuals that are more central to the network. This could be achieved either through vaccination (should this management technique for BFDV be successfully developed in the future), or the provision of "hotel hoppers" closer to the nest sites of those central individuals, to reduce their potential for super-spreading of infection within the population.

Whilst many epidemiological studies exist in the literature on infectious disease transmission in wild populations, the Mauritius system has provided a unique approach to inferring super-spreaders by overlaying observational and prevalence data. It is rare to be able to identify the majority of individuals within a wildlife population, and the Mauritius parakeet population could provide ample future opportunities to further interrogate the relationships between management recovery tools, sociality and infectious disease. The use of nestling infection prevalence as a proxy for their parents' capacity to transmit infection is a minor pitfall of the study presented in Chapter 5, and would be improved by knowing the infection status of the breeding adults attending the

hoppers. These data would provide clarity as to whether those individuals which are more central to the contact networks at hoppers are also impacted by disease themselves, and thus rely on hoppers for a low effort food source, or have a higher propensity to be carriers of transient infection. However, access to these individuals to sample them is invasive and logistically challenging.

7.5 FUTURE DIRECTIONS AND RESEARCH QUESTIONS

Throughout this thesis I have placed strong emphasis on the importance of conservationists managing populations affected by BFDV remaining focused on the fundamental objectives of recovery, and how the presented management solutions can be manipulated to achieve the best possible outcomes. As discussed above, further research into non-chemical biosecurity solutions that risk less impact to fecundity would be valuable for the future management of all wild parrot populations vulnerable to BFDV. Additionally, now that there is evidence that viral prevalence is predicted by parental sociality at supplementary feeding hoppers, the stability of these networks and consistency in the relationships between parental sociality and nestling infection across multiple breeding seasons should be evaluated. The incorporation of the available long-term observational datasets would allow for a more thorough interrogation of these patterns temporally and may reveal relationships that were not initially apparent due to smaller sample sizes.

It is also evident from my analyses reported here and other published research (Tollington *et al.* 2018; Eastwood *et al.* 2019) that, aside from an age-related association, the factors that drive individual BFDV viral load are still poorly understood. Single samples taken from an individual may provide a relatively accurate binary assessment of infection status that can be used in analyses such as these. However, they only represent a solitary snapshot in time which is insufficient to determine key influences on viral load. Therefore, it would be beneficial for future research to focus on repeated sampling of juvenile individuals (the most susceptible to infection) to determine when infection occurs, how viral infection varies over time within an individual, and how long an individual takes to either display clinical signs of disease or clear infection. As assessed with BFDV prevalence (Knafler *et al.* 2016) it would also be of value to determine whether viral load can be attributed to immunogenetic traits such as genetic diversity within the Major Histocompatibility Complex or at toll-like receptors, given their links to vertebrate immune function and response (Alcaide and Edwards 2011).

7.6 CONCLUSIONS

The research compiled in this thesis has successfully provided a sound scientific basis for the continued refinement of management solutions implemented for the recovery of Mauritius parakeets. Although we are currently unable to infer where the strain of BFVD present in Mauritius

likely originated on the basis of phylogenetic relationships (Chapter 1), the combined isolate dataset obtained from both parakeet populations present on the island has become the most extensive of all of those publically available for BFDV on GenBank. Mauritius has become a global example of successful avian population recovery and the long-term monitoring of these systems has afforded an ideal opportunity to thoroughly and experimentally assess management solutions. It is reassuring that artificial nest site placement can remain focused solely on maximising reproductive output (Chapter 2), without concern that anthropogenic selection is increasing viral prevalence or impacting on fecundity and nestling condition. The results from the biosecurity experiments (Chapters 3 and 4) emphasise the necessity for conservationists to always monitor the outcomes of management and to follow the tenets of structured decision making (Canessa *et al.* 2016) to ensure that their focus remains on achieving their fundamental objectives. Significant logistical challenges exist in implementing *in situ* pathogen management versus within a laboratory environment and, consequently, it cannot be expected that the response of wild populations will be reflective of a controlled system. Conservationists need to be aware of the ways in which their population management tools may alter the sociality (Chapter 5), nesting and foraging strategies, and thus the dynamics of pathogen transmission, within their focal species.

BFDV has become a pathogen of conservation concern globally, as is evident by the linear increase in research intensity since the first description of PBFD (Fogell, Martin and Groombridge 2016). However, the methods and principals applied throughout this thesis can not only readily be transferred to other vulnerable BFDV affected populations, but are also applicable to other wildlife systems in which current management includes a form of disease mitigation. The research questions and experimental approaches taken to gather data for this thesis were developed in collaboration with the teams directly involved with the management of Mauritius parakeets, ensuring that the focus remained on science that was both relevant to ongoing conservation work and could be applied. In light of the current global biodiversity crisis, strengthening the link between research and management of wildlife populations is necessary to achieve the best conservation outcomes and avoid unsupported dogmatic approaches.

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