A field survey of BFDV in wild populations of *Cyanoramphus* parakeets in New Zealand and Norfolk Island

A thesis submitted in partial fulfilment of the requirements for the degree of

Master of Science in Conservation Biology

Massey University

New Zealand

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2022

GENERAL ABSTRACT

Psittaciformes is one of the most at-risk orders of birds, with 28% of parrot species being threatened with extinction as of 2016. Major threats to parrots include deforestation and habitat loss, predation by invasive mammals and poaching for the pet trade and collectors. Pathogens and disease are also a significant threat, particularly for species that are isolated, genetically depauperate and already at risk of extinction. Two parrot species that meet these conditions are the kākāpō (*Strigops habroptilus*) of New Zealand and the Norfolk parakeet (*Cyanoramphus cookii*) of Norfolk Island. Beak and feather disease virus (BFDV) stands out as a pathogen of concern for parrot conservation, with a global distribution, the ability to be transmitted between a wide range of parrot species and the potential to cause chronic and lethal infections. This thesis presents the results of testing 20 kākāriki (*Cyanoramphus spp.*) sympatric with kākāpō on Codfish Island/Whenua Hou from samples collected in 2019-2021 and 51 Norfolk parakeets sampled in 2015 for BFDV. This testing was conducted through PCR. BFDV was not detected in the kākāriki feather samples but four of the Norfolk parakeet samples tested positive for the virus. This is the most recent detection of BFDV in the sole population of Norfolk parakeets and the first to do so through PCR screening. The sample size of 20 kākāriki was determined to be insufficient to substantiate freedom from BFDV on Codfish Island. This thesis explores and discusses these findings in the context of other published studies to make conservation recommendations for the kākāpō and Norfolk parakeet with respect to BFDV and other pathogens of concern. Approaches to disease biosecurity and opportunities for future study are also discussed based off of the data presented and inspired by the challenges this study faced due to the global COVID-19 pandemic and ensuing national and regional lockdowns in New Zealand in 2021. Attached as an appendix is a scientific article that I helped to prepare for the journal Diversity presenting a retrospective analysis of screening five populations of parrots from Argentina, Australia and New Zealand for BFDV.

ACKNOWLEDGEMENTS

Thank you to my project supervisor Dr Luis Ortiz-Catedral for your introduction to the world of disease management for conservation, for answering my many questions and for the guidance and support you have provided throughout the completion of this thesis. This research would not have been possible without your insights, expertise and your willingness to share opportunities that have contributed to my journey in New Zealand's conservation space and my development as a research scientist.

I would like to thank the New Zealand Department of Conservation for their support. From clearing biosecurity in order to visit Codfish Island/Whenua Hou, to sharing accommodation and cooking and cleaning with rangers of the Kākāpō Recovery Team, every member of the DOC team that I met made me feel welcome. Thank you also for the board games and chocolate at the end of each day in the field.

Thank you to my family, you have always supported me and I love you all very much.

DECLARATION

I declare that this thesis is an accurate and original account of my research and that the contents have not previously been submitted for a degree at Massey University, nor any other tertiary institution. Except where acknowledged within, the material contained in this thesis has not been written or published by any other individual and to the extent of my knowledge, does not infringe upon copyright restrictions. The sampling of kākāriki (*Cyanoramphus spp.*) that occurred in April of 2021 was conducted under the supervision of Dr Luis Ortiz-Catedral in collaboration with the Department of Conservation and would have been part of an active collaboration between Massey University, the World Parrot Trust and the Department of Conservation had further fieldwork not been disrupted by the COVID-19 pandemic. Dr Luis Ortiz-Catedral allowed me to be involved with this fieldwork and subsequent analysis as the foundation of my thesis research project. The data on BFDV in the Norfolk parakeet (*Cyanoramphus cookii*) presented in this thesis is from a multi-year research project to benefit the conservation of the Norfolk parakeet that has been developed by Dr Luis Oritz-Catedral in collaboration with the Norfolk Island National Park authority, Island Conservation, the World Parrot Trust and BirdLife Australia since 2013. Dr Luis Ortiz-Catedral allowed me to publish and analyse this data to enable this thesis to present conservation recommendations for the management of the Norfolk parakeet. Dr Luis Ortiz-Catedral was the supervisor of my research and the concepts and ideas in this thesis were discussed and developed between my supervisor and I during in-person and online meetings. To the best of my knowledge the research presented in this thesis was conducted in accordance with the protocols of Massey University, the Department of the Environment Australia and the New Zealand Department of Conservation.

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Figure 1. A male juvenile Red-crowned parakeet (*Cyanoramphus novaezelandiae*) on Codfish Island/Whenua Hou. Photo: Connor Wallace.

BIODIVERSITY IN CRISIS

Biodiversity is in crisis as human actions push environments and ecosystems away from the operational thresholds to which species have adapted. A variety of human actions and human-induced environmental changes have been linked to the decline and extinction of species worldwide. Anthropogenic hunting was associated with the rapid extinction of species such as the moa (*Dinornis spp.*), Passenger pigeon (*Ectopistes migratorius*) and Steller's sea cow (*Hydrodamalis gigas*) (Halliday, 1980; Holdaway & Jacomb, 2000; Turvey & Risley, 2006). Invasive species spread by humans have contributed to the extinction of island species including New Zealand/Aotearoa's Bush wren (*Xenicus longipes*) and the Greater short-tailed bat (*Mystacina robusta*) (Bell et al., 2016). Pathogens spread by humans have also caused the extinction of species; this is poignantly demonstrated by Chytridiomycosis disease that is caused by the fungi *Batrachochytrium dendrobatidis* and *B. salamandivorans* and has recently been linked to the decline of 501 amphibian species and the presumed extinction of 90 species (O'Hanlon et al., 2018; Scheele et al., 2019; Skerratt et al., 2007). Habitat destruction and degradation is the most significant anthropogenic contributor to biodiversity decline, impacting 89% of threatened bird species and 83% of threatened mammal species as of 2007 (International Union for Conservation of Nature, 2007). Humans are not the sole cause of species extinction yet the way in which we have accelerated the process is alarming; modern extinction rates far outstrip background norms, with a conservative estimate recognising 100 times more vertebrate species going extinct between the years 1500 and 2014 than would be expected under normal rates of extinction (Barnosky et al., 2011; Ceballos et al., 2015).

Species do not exist in isolation but are components of larger ecosystems, interacting both with other species and the abiotic components of the environments in which they live (Byers et al., 2006; Grant-Hoffman et al., 2010; Naiman, 1988). For this reason, the loss or decline of species can have extensive impacts on ecosystem function and cause further

biodiversity loss in turn. This is well demonstrated by the impact that human overexploitation of Australasian snapper (*Chrysophrys auratus*) and Spiny rock lobsters (*Jasus edwardsii*) has had on the kelp forests of Auckland and Whangārei, New Zealand. In these marine environments Australasian snapper and Spiny rock lobsters are predators of kina (*Evechinus chloroticus*), a sea urchin that grazes on kelps such as *Ecklonia radiata* and are themselves popular foodstuffs of people (Babcock et al., 1999; Kerr & Grace, 2005; Shears & Babcock, 2002). Suppression of snapper and lobster populations by human harvesting can result in an increase in kina abundance and a subsequent decrease in kelp abundance (Babcock et al., 1999; Kerr & Grace, 2005; Shears & Babcock, 2002). This ultimately leads to the formation of 'kina barrens', swathes of the seafloor where the grazing pressure on kelp from kina is such that kelp forests are destroyed and supplanted by crustose coralline algae (Babcock et al., 1999; Kerr & Grace, 2005; Shears & Babcock, 2002). A 1999 study by Babcock et al. investigated this relationship by comparing the abundance of snapper, rock lobsters and kina, as well as the extent of kina barrens between two marine reserves and adjacent non-reserve areas. This study found that adult snapper were between 5.76 and 8.7 times more abundant in the reserves, that rock lobsters were 1.6 to 3.7 times more abundant in the reserves and that in one of the reserves kina abundance had decreased from 4.9 to 1.4 individuals per square metre since the reserve was established. The study also found that kina barrens comprised 40% of the non-reserve area surveyed and only 14% of the reserve area surveyed.

Human induced climate change already contributes to biodiversity loss and is set to accelerate rates of extinction as global temperatures rise (Alan Pounds et al., 2006; Urban, 2015). Due to the global reach of climate change, the vectors of this acceleration are many. A study by Cahill et al. (2013) compiled examples where phenomena induced by climate change had caused the local extinction or decline of species. Examples included the decline and local extinction of gobies (*Gobiodon spp.*) associated with coral bleaching, the population decline of

Quiver trees (*Aloe dichotoma*) associated with desiccation stress induced by decreased precipitation and increased mortality of Viviparous eelpout (*Zoarces viviparus*) associated with reduced oxygen solubility of water caused by warming (Foden et al., 2007; Munday, 2004; Pörtner & Knust, 2007). Recent studies have also identified the risk of climate change and rising global temperatures increasing the range and lethality of pathogens (Delgado-Baquerizo et al., 2020; Reverter et al., 2020). Mean annual temperatures share a positive correlation with the abundance of soil-borne fungal pathogens putting crop and wild plant species at increased risk of fungal diseases as temperatures rise (Delgado-Baquerizo et al., 2020). Climate change has also been shown to benefit fungal diseases of animals. *Batrachochytrium dendrobatidis* is one of the fungi that causes the disease chytridiomycosis, a fungal infection of amphibians that has a global distribution and has caused the decline and extinction of numerous frog species (O'Hanlon et al., 2018; Skerratt et al., 2007). In the mountain ranges of Costa Rica climate change is increasing cloud cover, shielding the chytrid fungus from temperatures ≥30ºC and keeping it viable to the detriment of frog populations (Alan Pounds et al., 2006). Increasing temperatures are also predicted to expand the ranges of mosquitoes (*Culicoides spp.*) and with them the range of arboviral viruses such as the West Nile virus which can be pathogenic for both humans and animals (Whitehorn & Yacoub, 2019).

Human actions can also reverse biodiversity decline and prevent biodiversity loss. This is well exemplified by the control or eradication of rat species such as the Black rat (*Rattus rattus*), Norway rat (*Rattus norvegicus*) and the Pacific rat (*Rattus exulans*) in environments where they are invasive. Many studies demonstrate that seabird populations increase following the removal of invasive rats (Brooke et al., 2018; Le Corre et al., 2015; Whitworth & Carter, 2018; Whitworth et al., 2013). A 2018 study of 181 seabird populations across 69 distinct bird species revealed that 83.4% of the populations exhibited positive growth rates following the removal of invasive mammals (Brooke et al., 2018). While this study reveals a general trend,

another 2018 study provides more insight into rates of recovery. Anacapa Island in California, the United States, is home to a breeding colony of Scripps's murrelet (*Synthliboramphus scrippsi*), a small seabird that is vulnerable to extinction as of 2021 (International Union for Conservation of Nature, 2021b; Whitworth & Carter, 2018; Whitworth et al., 2013). Black rats were eliminated on the island in 2002 and between 2001 and 2014 the number of occupied Scripps's murrelet nests increased by 445% (Whitworth & Carter, 2018). Hatching success rates also increased from 30% in 2001-2002 to 85% in 2003-2010 (Whitworth & Carter, 2018). Another example of a human action that can reverse biodiversity loss is the cessation of fishing activities through the establishment of no-take marine reserves. With the elimination of human fishing pressure, the species richness and species abundance within such reserves increases (Mello et al., 2020; Russ & Alcala, 2011). This increased biodiversity can also 'spill over' into non-protected areas through the movement of adults and juveniles, as well as by the dispersal of larvae, increasing biodiversity in these areas too. (Christie et al., 2010; Russ & Alcala, 2011). A 2010 study by Christie et al. demonstrated the larval dispersal of Yellow tangs (*Zebrasoma flavescens*) by determining parentage through genetic analysis of tissue samples taken from adult and juvenile fish in Hawai'i. Their research revealed offspring of Yellow tangs from a marine protected area in Miloli'i in two unprotected areas, Ho'okena and Wawaloli.

There are both economic and cultural incentives to conserve biodiversity and humanity depends upon the ecosystem services biodiversity enables (Gamfeldt et al., 2008; Guo et al., 2010; Patterson, 1998; Pimentel et al., 1997). This culminates in an obligation to undertake conservation. Constrained by funding and often time sensitive, conservation efforts must strive to be as effective as possible (Capizzi et al., 2010; Ricketts et al., 2005; Wilson et al., 2009) Not all species are threatened with extinction equally (International Union for Conservation of Nature, 2021a). Numerous studies have investigated the factors and life history characteristics that determine the vulnerability of species to population decline and extinction. A study of

sympatric rattlesnake species by Waldron et al. (2006) indicated that habitat specificity can contribute to a species' vulnerability to population decline. An analysis of 5491 mammal species in the IUCN database by González-Suárez et al. (2013) similarly found that ecologically specialised mammals are most threatened by pressures such as habitat loss and fragmentation. A comprehensive study by Olah et al. (2016) also used the IUCN database to investigate factors that influence extinction risk in parrots and determined that the risk of being classified as threatened was increased for parrots with larger body length, longer generation times and a high dependence on forests. This study also found that the severity of extinction risk amongst parrots was positively associated with the gross domestic product of the country in which parrot species are found and by species endemism. The disparity of extinction risk faced by species is an important consideration for conservationists and conservation-backers looking to target their efforts. According to figures from the IUCN Red List, 33.9% of amphibians, 22.3% of mammals, 17.2% of reptiles, 14.6% of fish and 13.3% of birds are threatened with extinction globally as of 2021 (International Union for Conservation of Nature, 2021c) (Table 1.). With a limited diversity of amphibians and mammals, these figures do not translate directly to conservation priorities in New Zealand. New Zealand is home to two native species of terrestrial mammals, four native amphibians, over 100 species of native reptiles and greater than 200 species of native birds (Department of Conservation, n.d.-b; New Zealand Herpetological Society, 2021; The Royal Forest & Bird Protection Society of New Zealand, 2018) The dominance of birds in New Zealand ecosystems along with the presence of charismatic and bizarre species such as the kiwi (*Apteryx spp.*) and kākāpō (*Strigops habroptilus*) has produced a conservation landscape where many environmental projects seek to benefit bird species either directly or indirectly. Seabirds and parrots are amongst the most threatened of bird taxa (BirdLife International, 2018; Croxall et al., 2012; Olah et al., 2016). This holds true in New Zealand where 90% of native seabirds and 67% of native parrot species

are threatened with extinction (Ministry for the Environment & Statistics New Zealand, 2016; Olah et al., 2018).

Table 1. Percentage of Red List species threatened with extinction by taxa. Data sourced from International Union for Conservation of Nature (2021c).

Taxa	Number of Red List Species	Number of Species Threatened with Extinction	Percentage of Species Threatened with Extinction
Amphibians	7212	2442	33.86%
Birds	11158	1481	13.27%
Fish	22005	3210	14.58%
Mammals	5940	1323	22.27%
Reptiles	8492	1458	17.16%

THE ORDER PSITTACIFORMES

Often colourful, gregarious, long-lived and intelligent members of the order Psittaciformes are charismatic species that have long captured the attention of people (Auersperg & von Bayern, 2019; Berg & Bennett, 2010; Burtt et al., 2011; Pires, 2012; Toft & Wright, 2015a; Wirthlin et al., 2018). This fascination has found many outlets, including but not limited to, cultural representation, collecting specimens both live and dead, a prolific pet trade and in recent decades conservation efforts too (Briceño-Linares et al., 2011; Eda et al., 2019; Pires, 2012; Snyder, 2004; World Parrot Trust, 2021). Psittaciformes is divided into three superfamilies, the basal Strigopoidea (New Zealand parrots), Cacatuoidea (cockatoos) and Psittacoidea (true parrots) (Provost et al., 2018; Schweizer et al., 2010; Wright et al., 2008). Although not all are

represented by natives, each of these three superfamilies are present in New Zealand (Miskelly et al., 2008). In New Zealand the genus *Cyanoramphus* is represented by the Antipodes Island parakeet (*C. unicolor*), Forbes' parakeet (*C. forbesi*), Orange-fronted parakeet (*C. malherbi*), Red-crowned parakeet (*C. novaezelandiae*), Reischek's parakeet (*C. hochstetteri*) and the Yellow-crowned parakeet (*C. auriceps*) (New Zealand Birds Online, 2013f). New Zealand's other native parrots are the only extant members of Strigopoidea, the kaka (*N. meridionalis*) and kea (*N. notabilis*) of the genus Nestor and the kākāpō (*S. habroptilus*) of the genus *Strigops*. All of these native parrot species are endemic and have been joined by five introduced parrot species that have since become naturalised: the Crimson rosella (*Platycercus elegans*), Eastern rosella (*Platycercus eximius*) and the Rainbow lorikeet (*Trichoglossus haematodus*) as well as the Galah (*Eolophus roseicapillus*) and Sulphur-crested cockatoo (*Cacatua galerita*) of the superfamily Cacatuoidea (Miskelly et al., 2008).

Morphologically parrots can be characterised by their downturned and curved beaks wherein the maxilla extends beyond the mandible and by their truly zygodactyl feet, distinct from the semi-zygodactyl feet of birds such as ospreys (*Pandionidae*) and owls (*Strigiformes*) (Botelho et al., 2015; Homberger, 2006). Many parrots possess brightly coloured feathers with a wide variety of colours including pinks, reds, oranges, yellows, greens and blues being exhibited both across species and within individuals (Berg & Bennett, 2010; Burtt et al., 2011; Carballo et al., 2020). The orange, pink, red and yellow pigments present in parrot feathers are unique amongst birds, being endogenously synthesised psittacofulvin pigments rather than carotenoid pigments accumulated from foodstuffs (Berg & Bennett, 2010; Burtt et al., 2011; Isaksson, 2009; McGraw & Nogare, 2005). Most parrots are primarily herbivorous with different species exhibiting varying levels of granivory, frugivory, nectivory and florivory (Toft & Wright, 2015c). Some parrots will also eat insects and a few species, such as the kea and Antipodes Island parakeet, also scavenge meat from carcasses and actively hunt smaller

birds (New Zealand Birds Online, 2013a, 2013b; Toft & Wright, 2015c). The majority of parrots are socially and genetically monogamous, establishing long-term pair bonds with just one partner, maintaining these bonds across multiple breeding seasons and producing offspring exclusively with this partner (Toft & Wright, 2015b). Some exceptions do exist with 7.7% of 827 Green-rumped parrotlet (*Forpus* passerines) chicks from six wild populations in Venezuela being determined to have been the product of extra-pair mating across three breeding seasons in 1995-1997 (Melland, 2000). Divorce and subsequently forming pair bonds with new partners has also been observed in parrots including cockatiels (*Nymphicus hollandicus*), who switched away from mates to form more reproductively successful pairings (Spoon et al., 2007). The kākāpō and Vasa parrot (*Caracopsis vasa*) also deviate from the norm by employing lek and polyandrous mating systems respectively (Toft & Wright, 2015b). Most parrots nest in pre-existing tree cavities, which along with their diet, creates a dependence on wooded forest environments (Toft & Wright, 2015a).

A dependence on forest environments has made the human destruction of habitat the primary contributor to the decline of parrot species globally (Olah et al., 2016; Vergara-Tabares et al., 2020). Habitat loss can cause the decline of parrot populations in a variety of ways. The loss of habitat is often associated with the loss of foodstuffs as is seen with the decline of the endangered South-eastern red-tailed black cockatoo (*Calyptorhynchus banksii graptogyne*) which depends on seeds from the buloke tree (*Allocasuarina luehmannii*) which is itself threatened by clearing for agriculture (Maron et al., 2010). Habitat loss can also limit access to tree cavities used for nesting, an issue that is exacerbated by the long generation time of tree cavities which limits the ability of young forests to supplement this resource (Manning et al., 2013). Another significant contributor to the decline of wild parrot populations is poaching for the pet trade and for collectors (Olah et al., 2016; Pires, 2012). People have a long history of catching parrots, with the Aztec empire using parrot feathers for decoration as well as both

eating parrots and keeping them as pets (Pires, 2012). Parrots can be negatively impacted by poaching in many ways, non-exhaustively: eggs can be broken, young can be separated from their parents, nest sites can be damaged and those birds that are removed from the wild are not available as mates to remaining wild birds (Dahlin et al., 2018; Juniper, 2004). These pressures can be particularly significant on parrots that make for popular pets. A recent study by Dahlin et al. (2018) assessed breeding biology and nesting success of wild Yellow-naped Amazons (*Amazona auropalliata*) nests from 1999-2008. Of 128 total nests, the researchers were able to identify the nesting outcomes, either successful fledging or nest failure, of 66 nests and found that 89.4% of these nests failed and that 64% of these failed nests failed due to poaching. The way in which multiple human induced pressures can come together to cause the decline of parrot species is well demonstrated by the plight of the Spix's macaw (*Cyanopsitta spixii*), officially recognised as extinct in the wild since 2019 (International Union for Conservation of Nature, 2019). Over a 300-year history of pastoral usage, the habitat of the Spix's macaw was reduced by 99.99% from roughly 300,000 square kilometres to only 30 square kilometres as of 1990 (Juniper & Yamashita, 1990). The population decline associated with this dramatic reduction in habitat was augmented by poaching which saw only one pair of Spix's macaws remaining in the wild as of 1987 until the female of this pair was captured later that year (Juniper, 2004). Parrots are also threatened by invasive species. Two ways through which invasive species can negatively impact parrots is through competition for resources and through predation. One form of interspecific competition associated with the decline of parrots is competition over tree cavities used for nesting. A study by Grarock et al. (2013) investigated nest competition between the invasive Common myna (*Acridotheres tristis*) and native Crimson and Eastern rosellas (*Platycercus elegans*, *Platycercus eximius*) in Canberra, Australia. The researchers established 225 artificial nest boxes in nature reserves featuring different densities of trees and observed the occupancy of these boxes. On average Common

mynas were found to occupy 26.5% of the nest boxes and used a further 6.8% of the boxes to construct ‘fake nests’ that weren’t used for egg laying. The mynas were also observed to interrupt both Crimson and Eastern rosella nesting, usurping 16 nest boxes to establish their own nests or ‘fake nests’. The researchers also identified that the proportion of nest occupancy by mynas had a significant negative relationship ($P < 0.001$) with the abundance of Crimson rosellas. The impact of predation on parrots by invasive species has been especially well demonstrated by the plight of New Zealand parrots. Endemic birds in New Zealand evolved in the absence of mammalian predators and many have proven vulnerable to predation by invasive mammals (Brown, 1997; Carpenter et al., 2021; Department of Conservation, n.d.-b; Dowding & Murphy, 2001; Innes et al., 2010). These invasive predators include the Black rat (*Rattus rattus*), Norway rat (*Rattus norvegicus*), Polynesian rat (*Rattus exulans*), European hedgehog (*Erinaceus europaeus*), Common brushtail possum (*Trichosurus vulpecula*), mustelids and cats (*Felis catus*) (Department of Conservation, n.d.-a). All endemic mainland parrot species in New Zealand have suffered predation from at least one of these invasive species (Department of Conservation, n.d.-g; Kemp et al., 2018; Lloyd & Powlesland, 1994; Moorhouse et al., 2003). Along with habitat destruction, this pressure is such that the kākāpō, now only survives on predator-free islands (Clout & Merton, 1998; Department of Conservation, n.d.-e). A study by Moorhouse et al. (2003) found that the implementation of predator control more than doubled the number of fledglings produced by wild kaka over a four year period when compared to sites lacking predator control. A more recent study by Kemp et al. (2018) investigated the impact of aerial 1080 (a sodium fluoroacetate mammal toxin) application on kea reproductive success in 30,000 hectares of native bush. The researchers recorded a sharp decline in the abundance of rats and stoats after the application of 1080 and the nest survival rate of kea chicks increased from 46.6% pre-1080 to 84.8% post-1080. Pathogens and disease pose yet another threat to parrots and one that is an emerging topic in scientific research (Fogell

et al., 2016; Ortiz-Catedral et al., 2019). Pathogens and disease are particularly substantial threats to small, isolated and genetically depauperate populations of parrots. This is especially significant where human induced pressures, such as those described above, have resulted in many parrot populations existing in this state. There are multiple ways through which pathogens and disease can cause the decline of parrot populations. Birds can be killed directly by an infection, as has been demonstrated by 17 captive parrot populations that lost birds to *Haemoproteous minutus* infections in Denmark, Germany and Great Britain (Ortiz-Catedral et al., 2019). Four of these were captive populations of Red-crowned parakeets in Germany. Some pathogens such as the Beak and feather disease virus (henceforth referred to as 'BFDV') can also cause immunosuppression, making secondary infections lethal (Todd, 2000). Even when not fatal, some infections can negatively impact parrots in ways that likely reduce reproductive fitness, inducing symptoms such as weight loss and the malformation of beaks and feathers (Pass & Perry, 1984). Smaller populations are particularly vulnerable to the impact of pathogens and disease as the reduced genetic diversity inherent in such populations can be associated with reduced diversity of immune-genes. A recent study by Morrison et al. (2020) identified exactly this in the critically endangered Orange-bellied parrot (*Neophema chrysogaster*). This study assessed the genetic diversity of six toll-like receptors, genes associated with immune response, in twenty Orange-bellied parrots and found that the birds were monomorphic for three of the receptors and lowly polymorphic for the other three. A key example of a pathogen that has recently caused the decline of a critically endangered and genetically depauperate parrot is *aspergillosis* fungus. A 2019 outbreak on Codfish Island/Whenua Hou resulted in the infection of 21 birds, roughly 10% of the total kākāpō population at the time, and nine of the birds infected died of the infection (Department of Conservation, 2019, 2020).

THE IMPACTS OF DISEASE ON PARROT CONSERVATION

Although pathogens and disease pose a serious threat to parrots, only in recent years has scientific research focused on the links between conservation and pathogen studies. The Echo parakeet (*Psittacula eques*), also known as the Mauritius parakeet, is a species endemic to Mauritius that was the rarest parrot in the world in the 1980s with a wild population of less than 12 birds (Raisin et al., 2012; Venning, 1993). Raisin et al. (2012) notes that efforts to save the species began with population monitoring that started in 1973 and intensified in 1987 before the first release of captive birds took place in 1997. Between 2001 and 2005 intensive conservation action took place involving the translocation of parakeets between wild sites and between the wild and captivity, captive breeding and the provision of supplementary foodstuffs (Fogell et al., 2019; Raisin et al., 2012). These efforts were disrupted in 2005 by an outbreak of Psittacine beak and feather disease (henceforth referred to as 'PBFD'), a highly contagious and potentially lethal disease caused by BFDV (Doneley, 2003; Kundu et al., 2012; Pass & Perry, 1984; Raisin et al., 2012). While the specific source of infection for this population remains ambiguous, it is suspected that conservation actions such as translocations of birds between sites, eggs between nests and the establishment of feeders around which birds would congregate had facilitated the spread of the virus (Fogell et al., 2019; Kundu et al., 2012; Raisin et al., 2012). As cited by Ortiz-Catedral et al. (2009), BFDV was ultimately identified in 18% of Echo parakeets circa 2008 and infected birds exhibited a mortality rate of 83%. In response to the outbreak, hygiene and biosecurity protocols were adopted that included conservationists wearing medical barrier suits when accessing nests, treating nests with anti-viral solution and discarding nest materials at the end of each season (Fogell et al., 2019). These methods decreased the prevalence of BFDV in Echo parakeet nestlings but not the viral load in individual nestlings (Fogell et al., 2019).

The plight and conservation of the Orange-bellied parrot (*Neophema chrysogaster*) shares many similarities to that of the Echo parakeet; both species suffered precipitous population decline, have been subject to intensive management involving captive breeding and both were threatened by PBFD while already at risk of extinction (BirdLife Australia, 2010; International Union for Conservation of Nature, 2018b; Kundu et al., 2012; Peters et al., 2014; Raisin et al., 2012; Venning, 1993). While the Echo parakeet has since rebounded with approximately 800 birds existing in the wild as of 2020, the outlook for the Orange-bellied parrot is grim (BirdLife International, 2020). Less than 150 Orange-bellied parrots were thought to exist in the wild as of 2002, this dropped to less than 50 birds as of 2010 and down to roughly 20 individuals as of 2018 (BirdLife Australia, 2010; International Union for Conservation of Nature, 2018b). Efforts to save the species have included habitat restoration, supplementary feeding, captive breeding and the annual release of captive-bred birds into the wild (BirdLife Australia, 2010; Das et al., 2015; Peters et al., 2014). The 1985 establishment of the captive breeding programme located in Bridgewater, Tasmania was hindered by a PBFD outbreak that was exacerbated by the cold and damp winters experienced at the site (Peters et al., 2014). This was remedied in 1989 by relocating the captive population to Taroona, Tasmania in pursuit of better weather conditions (Peters et al., 2014). The outbreak saw the captive population regularly screened for BFDV since 1994 and translocated birds regularly screened since 2000 (Peters et al., 2014; Sarker et al., 2014). PBFD re-emerged in the captive population in 2006 and BFDV was detected in the wild population in 2011 and 2015, placing both already strained populations in jeopardy (Das et al., 2020; Peters et al., 2014). A recent study by Das et al. (2020) used genetic analysis of BFDV samples to identify that at least three spillover events had occurred each reintroducing BFDV to the Orange-bellied parrot and that cockatoos (*Cacatuidae*) were likely the vector in at least two of these introductions. Taken together, the conservation of the Echo parakeet and Orange-bellied parrot provide strong

evidence that pathogens such as BFDV can have a severe impact on vulnerable species and that the detection and management of pathogens and disease must be a continuous consideration in the effective management of parrots.

STUDY SPECIES

Study species: Kākāpō

As the only lek breeding parrot species, the only flightless parrot, one of three species of nocturnal parrot and the world's largest parrot by weight, the kākāpō (*Strigops habropotilus*) is remarkably unique (Chambers & Worthy, 2013; Clout & Merton, 1998; Livezey, 1992). Kākāpō are large stocky parrots that exhibit sexual dimorphism, with male birds weighing up to 3.7 kg and females up to 2.2 kg (Department of Conservation, n.d.-f; Livezey, 1992). The feathers that cover the forehead, crown, nape, mantle and rump of the kākāpō are predominantly a bright green in colour that is interspersed with dark browns and pale yellows producing an overall mottled appearance. The feathers of the tail and wing are predominantly a pale brown colour that is interspersed with light green and pale yellow and the feathers on the belly are a mix of pale green and brown. Kākāpō also exhibit a distinctive facial disc of pale brown hair-like feathers that surrounds a broad beak and fleshy raised nostrils (Figure 2.). Kākāpō are herbivorous, eating native fruits, berries and seeds as well as other plant materials such as vines, tubers and rhizomes (Department of Conservation, n.d.-d; Lloyd & Powlesland, 1994; Merton et al., 1984). Kākāpō will forage for foodstuffs on the ground but are also capable climbers, scaling trees in the pursuit of foodstuffs and even reaching the canopy of trees 30m high (Powlesland et al., 2006). The reproductive cycle and output of the kākāpō is inextricably linked to the availability of foodstuffs, with birds only reproducing during mast years when podocarps and/or beech trees produce seeds and fruit en masse (Powlesland et al., 1992). This means that, despite exhibiting an annual gonadal cycle, kākāpō rarely breed in consecutive

years, breeding at two to five-year intervals (Cockrem & Rounce, 1995; Elliott et al., 2001; Powlesland et al., 1992). As a lek breeding species, males congregate between September and May to compete for females, digging themselves bowls and tracks in the dirt and using these bowls to amplify their booming calls and display to females (Eason et al., 2006; Merton et al., 1984). Mating has been observed occurring from December through January and after mating females will, on average, lay three eggs, incubate these eggs for 30 days each and provide all of the parental care (Eason et al., 2006). Male kākāpō are first capable of mating at five years of age and females will first mate at roughly ten years of age (Eason et al., 2006).

Figure 2. ‘Hoki’ pictured left, and ‘Sinbad’ pictured right, two of the kākāpō (*Strigops habropotilus*) hatched as part of the Department of Conservation’s kākāpō recovery project. Photos: Luis Ortiz-Catedral.

The threats faced by kākāpō are many and saw the total population of the species dwindle to approximately 54 individuals as of 1997 (Clout & Merton, 1998). As large flightless parrots that did not coexist with mammalian predators before the arrival of humans in New Zealand, kākāpō have proven vulnerable to predation by invasive mammals such as cats (*Felis catus*), rats (*Rattus spp.*) and mustelids (Clout & Merton, 1998; Elliott et al., 2001). Kākāpō populations have also suffered from disease, inbreeding, a male-skewed sex ratio and the destruction of habitat (Clout et al., 2002; Elliott et al., 2001; Gartrell et al., 2005; White et al., 2015). Although kākāpō once possessed a nationwide distribution, since the 1970s intensive conservation efforts have seen the translocation of all surviving kākāpō to the predator-free offshore islands of Anchor Island/Pukenui, Chalky Island/Te Kākahu-o-Tamatea, Codfish Island/Whenua Hou and Little Barrier Island/Te Hauturu-o-Toi (Clout & Merton, 1998; Department of Conservation, n.d.-e). Kākāpō are critically endangered with a total population of 201 birds as of September of 2021 (Department of Conservation, 2021). With the threats of invasive predators and habitat loss largely mitigated by life on predator-free islands, disease has emerged as a serious threat to the survival of this charismatic species. This is perhaps most strongly demonstrated by *aspergillosis* (see 'The Order Psittaciformes' above) however kākāpō have also suffered from *Erysipelothrix rhusiopathiae* bacterial infections that resulted in the death of three birds in 2004 (Gartrell et al., 2005). The infection is suspected to have been contracted from local seabirds following a translocation to Chalky Island/Te Kākahu-o-Tamatea, a real-world demonstration of the potential value in screening birds sympatric with kākāpō for pathogens (Gartrell et al., 2005).

Study species: Kākāriki

Literally 'small parrot' from the te reo word 'kākā' (parrot) and modifier 'riki' (small, little), kākāriki is both the te reo word for the colour green and the name of the five New Zealand

parakeets of the genus *Cyanoramphus*: the Forbes' parakeet (*C. forbesi*), Orange-fronted parakeet (*C. malherbi*), Red-crowned parakeet (*C. novaezelandiae*), Reischek's parakeet (*C. hochstetteri*) and the Yellow-crowned parakeet (*C. auriceps*) (Department of Conservation, n.d.-g; Māori Dictionary, n.d.-a, n.d.-b, n.d.-c). Three species of kākāriki are included in the scope of this study, these being the Orange-fronted, Red-crowned and Yellow-crowned parakeets as each are sympatric with the present range of kākāpō (*Strigops habropotilus*). These species are predominantly a vivid green in colour, exhibiting light green feathers on the nape, mantle, rump and tail and yellowish-green feathers on the belly, breast, throat and chin (Figure 3.). The wings of these species too are green bar the primary wing feathers that comprise a violet-blue wing flash (Chambers, 2007). Red-crowned and Yellow-crowned parakeets feature a red patch of feathers on each flank that can be concealed by the wings, on Orange-fronted parakeets these patches are orange (New Zealand Birds Online, 2013c, 2013d, 2013e). Another visual difference between these species can be found in the feathers of the crown, forehead and lore; the Red-crowned parakeet exhibits a crown of red feathers that joins with a band of red feathers across the lore and backwards past each eye, the Yellow-crowned parakeet exhibits a crown of yellow feathers that joins with a band of red feathers across the lore that reaches to each eye and the Orange-fronted parakeet exhibits a crown of yellow feathers that joins with a band of orange feathers across the lore that reaches to each eye. The Red-crowned parakeet is the largest of these species reaching up to 28 cm in length while Yellow-crowned parakeets can reach up to 25 cm in length and Orange-fronted parakeets up to 22cm (New Zealand Birds Online, 2013c, 2013d, 2013e). These species exhibit a broad diet, consuming a variety of plant materials, both native and invasive plant species and invertebrates (Greene, 1998; Kearvell et al., 2002; Ortiz–Catedral & Brunton, 2009). Invertebrates are a significant component of the diet of these parakeets being observed comprising roughly 20% of Red-crowned parakeet feeding events, 60% of Yellow-crowned parakeet feeding events and

75% of Orange-fronted parakeet feeding events in Spring (Greene, 1998; Kearvell et al., 2002). These Orange-fronted parakeet observations were made in a South Island beech forest in 1998 and 1999 however a more recent study by Ortiz–Catedral and Brunton (2009) found invertebrates to comprise only 4% of the diet of the Orange-fronted parakeet on Maud Island, based off of opportunistic sampling from 17 visits to the island between March of 2007 and January of 2009. This variability in diet composition between sites, along with the consumption of non-native plant species, suggests that these parakeets are food generalists that can adapt their diet to different environments. Kākāriki are also opportunistic with breeding, being capable of breeding year-round when foodstuffs are plentiful, as has been demonstrated by the Yellow-crowned parakeet during mast years (Elliott et al., 1996). Red-crowned parakeets have been observed breeding from October through March and active Orange-fronted parakeet nests have been identified in January through April as well as in November (Greene, 2003; Ortiz-Catedral et al., 2010; Ortiz-Catedral & Brunton, 2009). Nesting occurs in tree cavities as well as in holes in the ground and abandoned Sacred kingfisher (*Todiramphus sanctus*) burrows (Elliott et al., 1996; Greene, 2003; Ortiz-Catedral et al., 2010). Red-crowned, Yellow-crowned and Orange-fronted parakeets lay seven, six and five eggs on average respectively and females provide all of the incubation (Elliott et al., 1996; Greene, 2003; Ortiz-Catedral et al., 2010). Of these three kākāriki species only the Orange-fronted parakeet is threatened with extinction and is currently critically endangered (International Union for Conservation of Nature, 2016, 2018a, 2018c). Despite this disparity in vulnerability all three species have been impacted by predation from invasive mammals and habitat destruction (Department of Conservation, n.d.-g; Elliott et al., 1996). Pathogens and disease have also emerged as threats to these parakeets, with feather loss in wild Red-crowned parakeets on Tiritiri Matangi Island, New Zealand being attributed to infections by *Procnemidocoptes janssensi* mites and BFDV on Te Hauturu-o-Toi/Little Barrier Island (Jackson, 2014; Ortiz-Catedral et al., 2009). The Red-crowned

parakeet used to be a common appearance throughout New Zealand but now exhibits a fragmented mainland distribution only being commonly found on predator-free islands (Elliott et al., 1996; Greene, 1998; Ortiz-Catedral & Brunton, 2010). The Orange-fronted parakeet is now confined to just three valleys in North Canterbury as well as four predator-free offshore islands (Kearvell & Steeves, 2015). Yellow-crowned parakeets possess the largest mainland distribution and abundance of these three species, although rare overall they are common in some forested areas in both the North and South Islands (Chambers, 2007; Department of Conservation, n.d.-g; Elliott et al., 1996).

Figure 3. Top to bottom, an Orange-fronted parakeet (*Cyanoramphus malherbi*), a Red-crowned parakeet (*C. novaezelandiae)* and a Yellow-crowned parakeet (*C. auriceps*). Orange-fronted and Yellow-crowned parakeet photos: Luis Ortiz-Catedral. Red-crowned parakeet photo: Connor Wallace.

Study species: The Norfolk parakeet

The Norfolk parakeet (Also known as the Tasman parakeet and the Norfolk Island green parrot, *Cyanoramphus cookii*) is a relative of New Zealand's kākāriki (*Cyanoramphus spp.*) and is endemic to Norfolk Island located between Australia and New Zealand (Hill, 2002; Waldmann, 2016). Visually the Norfolk parakeet is very similar to the Red-crowned parakeet (*Cyanoramphus novaezelandiae*) with vivid light green feathers on the nape, mantle, rump, tail and wing and yellowish-green feathers on the belly, breast, throat and chin. The Norfolk parakeet also features a violet-blue wing flash and a crown of red feathers that joins with a band of red feathers across the lore and backwards past each eye (Figure 4.). The Norfolk parakeet grows to be slightly larger than the Red-crowned parakeet, reaching a length of 30cm compared to the Red-crowned parakeet's 28cm (New Zealand Birds Online, 2013d; World Parrot Trust, n.d.). Only one population of Norfolk parakeets exists and the species was recognised as one of the most endangered bird species in Australia in 2002 with just 160 individuals remaining (Hill, 2002; Waldmann, 2016). Two major threats to the Norfolk parakeet are predation from invasive cats (*Felis catus*) and rats (*rattus spp.*) and competition for nesting sites with invasive species such as the Crimson rosella (*Platycercus elegans*) (Ortiz-Catedral et al., 2018; Skirrow, 2018). Through intensive conservation efforts, including the culling of Crimson rosellas, baiting for rats and trapping cats, the Norfolk parakeet had increased in abundance to approximately 438 birds as of 2017 (Ortiz-Catedral et al., 2018; Skirrow, 2018). Researchers from Massey University's School of Natural and Computational Sciences, Auckland, New Zealand have been extensively involved with the recovery and study of the Norfolk parakeet. A recent Master of Science study by Waldmann (2016) investigated the diet and foraging behaviour of the Norfolk parakeet to inform a potential translocation of the species to Philip Island, just south of Norfolk Island. A team of observers recorded a variety of data including flock size, foraging height and the species and type (fruit, seeds etc.) of

foodstuff consumed by Norfolk parakeets. Data collection occurred in 2013 and 2019 and observations were made with the naked eye and through binoculars as the observers walked through the Mount Pitt section of Norfolk Island National Park. Waldmann (2016) identified that the diet of the Norfolk parakeet includes 30 different plant species and made no observations of insects being a prey item. Interestingly, while the native Norfolk pine (*Araucaria heterophylla*) comprised the largest proportion of feeding events at 24.6% of the observations, the invasive African olive (*Olea europea cuspidata*) comprised the second largest portion at 22.7%, a testament to the diet flexibility and adaptability of *Cyanoramphus* parakeets. A recent study by Brett and Ortiz-Catedral (2021) investigated the behaviour of this same population of Norfolk parakeets in autumn and winter in 2015 and 2016. Observed behaviours were placed into eight categories: agnostic behaviour, courtship feeding, climbing or walking, feeding, flying, preening, resting or resting alert. The frequency of behavioural states did not differ significantly between seasons; however, the parakeets were observed at significantly ($p < 0.05$) lower perches during winter (6.1 ± 4.3m) than in autumn (8.5 ± 8m). Norfolk parakeets reach sexual maturity early, with juvenile birds capable of nesting within months of fledging and can, as cited by Ortiz-Catedral et al. (2018) and Skirrow (2018), lay clutches of up to eight eggs (Ortiz-Catedral et al., 2018).

Figure 4. A photograph of an adult male Norfolk parakeet (*Cyanoramphus cookii*). Photo: Luis Ortiz-Catedral.

Pathogens of concern: Psittacine beak and feather disease virus

Psittacine beak and feather disease (PBFD) poses a significant threat to the conservation of parrots (Fogell et al., 2016). PBFD is caused by the beak and feather disease virus (BFDV), a virus that belongs to the genus Circovirus in the family Circoviridae characterised by single-stranded and circular DNA (Crowther et al., 2003). The genomes of Circoviridae viruses are the smallest of all animal viruses and that of BFDV consists of roughly 2000 base pairs that code for only two genes; rep, which is associated with replication, and cp which encodes a capsid protein (Crowther et al., 2003; Varsani et al., 2011). Physically BFDV particles are icosahedral in shape, roughly 14-16 nm in diameter and lack a lipid bilayer envelope (Ritchie

et al., 1989). Non-enveloped viruses are particularly robust to environmental conditions and experimentation has demonstrated BFDV withstanding environments with a pH of 6 through 10 as well as exposure to temperatures of up to 80ºC for 30 minutes (Raidal & Cross, 1994; Todd, 2000). The durability of BFDV allows for it to persist once shed into an environment through the crop secretions, faeces and feather dander of infected parrots (Jackson et al., 2015). Transmission of BFDV between parrots can occur horizontally through the ingestion of such secretions and can also occur vertically, being detected in the embryos of eggs laid by infected parents (Rahaus et al., 2008).

BFDV is a host generalist, being able to be transmitted between different species of parrots and with individual parrot species being susceptible to a variety of different BFDV strains (Sarker et al., 2015a). PBFD was originally described in Australia in the 1980s, but the international parrot trade has since given BFDV a global distribution, being present on every continent bar Antarctica and even in countries that lack native parrots such as Poland and the United Kingdom (Fogell et al., 2016; Julian et al., 2013; Pass & Perry, 1984). This wide geographical distribution in tandem with the host flexibility of BFDV and the way in which birds of different species and different countries of origin can be exposed to each other through the international parrot trade has contributed to the genetic diversity of BFDV through numerous recombination events, particularly along the intergenic region of the virus' genome and the open reading frame of the cp gene (Julian et al., 2013; Massaro et al., 2012; Sarker et al., 2015a; Varsani et al., 2011). This recombination activity can further increase the ability of BFDV to be transmitted between different parrot species (Massaro et al., 2012). Originally thought of as exclusive to parrots, novel research has also demonstrated that even non-psittacine birds can become infected with BFDV, having detected BFDV DNA in a variety of Australian birds including two species of kingfisher (*Coraciiformes*), two species of owl (*Strigiformes*) and a species of corvid amongst others (Amery-Gale et al., 2017). This reveals

another aspect to the management and control of BFDV for parrot conservation if BFDV can be transmitted not just between parrot species but between parrots and non-psittacine birds also.

BFDV infects the lymphoid tissues causing immunosuppression and can be found in the digestive organs, heart, liver and testes of infected parrots (Rahaus et al., 2008; Todd, 2000). Not all parrots infected with BFDV develop PBFD, some can be asymptomatic or subclinical carriers of the virus (Martens et al., 2020a). Of the birds that develop PBFD there are two general categories, those that develop a peracute or acute condition and those that develop a chronic condition. The peracute and acute forms of PBFD are most prevalent in young parrots, developing in neonates, nestlings, fledglings and juveniles (Doneley, 2003; Todd, 2000). The peracute form results in sudden death while the acute form has symptoms that include diarrhoea, feather abnormalities, secondary infection due to immunosuppression, weight loss, vomiting, and lethargy and can also result in death (Doneley, 2003; Pass & Perry, 1984; Sarker et al., 2014). The most common expression of PBFD is the chronic condition which entails immunosuppression as well as irreversible and roughly symmetrical feather loss as normal feathers moult only to be replaced by feathers that remain enclosed in their sheathes and develop bent or clubbed (Pass & Perry, 1984; Todd, 2000). In some cases the chronic form of PBFD also results in the malformed elongation of the beak and claws as well as claw loss (Pass & Perry, 1984). Parrots that suffer from the chronic condition can survive for many years but are often killed by a secondary bacterial or fungal infection (Todd, 2000).

BFDV has been present in wild populations of exotic parrots in the North Island of New Zealand since at least the early 2000s, being detected in Eastern rosellas (*Platycercus eximius*) from the Bay of Plenty and Wellington as well as in Sulphur-crested cockatoos (*Cacatua galerita*) near Whanganui (Ha et al., 2007). BFDV was first detected in a wild population of native New Zealand parrots in 2008, being identified in 15 Red-crowned parakeets

(*Cyanoramphus novaezelandiae*) from Te Hauturu-o-Toi/Little Barrier Island, two of which exhibited feather loss and abnormal feather morphologies, clinical symptoms of PBFD (Ortiz-Catedral et al., 2009). A later study by Massaro et al. (2012) reconfirmed the presence of BFDV in this Red-crowned parakeet population and also detected the virus in 32 Eastern rosellas from the Auckland region and in eight Yellow-crowned parakeets (*Cyanoramphus auriceps*) from a population in Eglinton Valley in the South Island. Molecular characterisation revealed that the BFDV isolates detected in Red-crowned parakeets and Eastern rosellas shared 94.9-97.9% sequence identity, that those detected in Red and Yellow-crowned parakeets shared 92.7-93.4% sequence identity and that those detected in Yellow-crowned parakeets and Eastern rosellas shared only 92-94% sequence identity (Massaro et al., 2012). Massaro et al. (2012) also determined that the BFDV isolates detected in Red-crowned parakeets and Eastern rosellas form a monophyletic BFDV-A clade while that detected in Yellow-crowned parakeets was a novel strain, BFDV-O that forms a clade with BFDV isolates from South Africa and Europe. The genetic distinctiveness between the Red and Yellow-crowned parakeet BFDV isolates indicates two separate BFDV incursions into New Zealand.

Pathogens of concern: Plasmodium and Haemoproteus

The protozoa of the genera *Plasmodium* and *Haemoproteus* are the causative agents of avian malaria and pseudo-malaria respectively (Ortiz-Catedral et al., 2019; Sá, 2011). *Plasmodium* (*spp.*) and *Haemoproteus* (*spp.*) are closely related and are both parasites that are transmitted to bird species through bites from blood sucking arthropods such as mosquitoes (*Culicoides spp.*) (Maharana & Kumar, 2017; Ortiz-Catedral et al., 2019; van Riper et al., 1986). While being carried by a bird host, these parasites have both intraerythrocytic stages, which can cause anemia and hemolysis, and exo-erythrocytic stages, which can physically disrupt body tissues and cause haemorrhaging through the formation of meronts or megalomeronts (Dinhopl et al.,

2015; Ortiz-Catedral et al., 2019). Avian malaria infections have both an acute stage, denoted by a large presence of plasmodia in the blood, and a chronic stage, denoted by a low presence of plasmodia in the blood (Howe et al., 2012; Schoener et al., 2014). While both stages can impair host fitness the chronic stage can also be associated with anorexia, dyspnoea and death (Howe et al., 2012; Lachish et al., 2011; Schoener et al., 2014). These pathogens can be devastating to naïve populations as has been demonstrated by the introduction of *Plasmodium relictum* to Hawai'i. van Riper et al. (1986) cite that the *Plasmodium* vector, the Southern house mosquito (*Culex quinquefasciatus*) arrived in Hawai'i in the 19th century and this was followed by *Plasmodium relictum* in the 20th century, with the latter likely coinciding with the release of non-native passerines by bird enthusiasts (Atkinson & LaPointe, 2009). Since this second introduction, the greatest species diversity and density of Hawai'i's native avifauna has been restricted to high elevation forests (LaPointe et al., 2010; van Riper et al., 1986). This dynamic was thought to be the result of Southern house mosquitoes being confined to lower elevations yet has more recently been suggested to be the product of the thermal conditions required for the sporogonic development of *Plasmodium relictum* not being met when higher elevation environments dip below 12.97º Celsius (LaPointe et al., 2010; van Riper et al., 1986). The influence of climate change may alter this restriction, further endangering Hawai'i's endemic birds. A recent study by Ortiz-Catedral et al. (2019) identified *Haemoproteus minutus* as the cause of 17 lethal disease outbreaks in parrot aviaries in Denmark, Germany and Great Britain, including four aviaries of Red-Crowned parakeets (*Cyanoramphus novaezelandiae*) in Germany. This study also identified that the *Haemoproteus minutus* parasites responsible for these outbreaks came from two lineages, TUPHI01 and TURDUS2 which commonly infect the Common blackbird (*Turdus merula*) and Song thrush (*Turdus philomelos*). With Red-crowned parakeets known to be fatally susceptible to infection by TURDUS2 *Haemoproteus minutus* and both the Common blackbird and Song thrush being introduced and naturalised in

New Zealand, there is the potential for these parasites to pose a threat to naïve native parrots if they and a suitable vector are introduced too. Ortiz-Catedral et al. (2019) did not detect any *Haemoproteus* species in 197 Red-crowned parakeets, 44 Common blackbirds and 19 Song thrushes sampled in 2009 but did detect two *Plasmodium* lineages, GRW6 and CYNOV1, in the Red-crowned parakeets. *Plasmodium spp.* have been detected in a variety of native New Zealand bird species including the Great spotted kiwi (*Apteryx haastii*), Brown kiwi (*A. mantelli*), kererū (*Hemiphaga novaeseelandiae*), kōkako (*Callaeas wilsoni*), hihi (*Notiomystis cincta*) and both North and South Island saddleback (*Philesturnus rufusater, P. carunculatus*) and fatal cases have been recorded in Great spotted kiwi, hihi and South Island saddleback amongst others (Alley et al., 2010; Castro et al., 2011; Howe et al., 2012; Schoener et al., 2014). With *Plasmodium spp.* being present on both the north and south islands of New Zealand and with *Cyanoramphus* parakeets being fatally susceptible to the closely related *Haemoproteus* parasites, both *Plasmodium* and *Haemoproteus* parasites could pose a serious threat to New Zealand's already vulnerable parrots.

THE PRESENT STUDY

Thesis Aims

My study aims to contribute to the knowledge of psittacine pathogens of concern in New Zealand and on Norfolk Island to benefit the conservation of parrots. A particular focus of this study is to inform the species management of the critically endangered kākāpō (*Strigops habroptilus*) of New Zealand and the endangered Norfolk parakeet (*Cyanoramphus cookii*) of Norfolk Island with respect to BFDV and other pathogens of concern. To this end feather samples were collected from kākāriki (*Cyanoramphus spp.*) sympatric with kākāpō on Codfish Island/Whenua Hou in April of 2021 and screened for BFDV through PCR analysis. This study also analyses and presents the results of screening 51 Norfolk parakeets for BFDV from feather

samples collected in 2015. Both sets of data are discussed in the context of other literature on BFDV to make conservation recommendations that address knowledge gaps about the role and prevalence of BFDV and other pathogens of concern, particularly for island parrot species such as New Zealand parrots.

COVID-19 declaration

The project presented here was originally intended to involve collecting blood and feather samples from kākāriki (*Cyanoramphus spp.*) on Anchor Island/Pukenui, Chalky Island/Te Kākahu-o-Tamatea, Codfish Island/Whenua Hou and Little Barrier Island/Te Hauturu-o-Toi. All sampling for this study would have been conducted as part of an active collaboration between Massey University, the World Parrot Trust and the Department of Conservation Kākāpō Recovery Team following approval protocols for animal handling and sampling of native species by the Department of Conservation (Uddstrom & Ortiz-Catedral, pers. comm.). This fieldwork was planned to begin in April of 2021 and to resume in October of 2021 and continue through summer. The samples collected were also going to be screened for both beak and feather disease virus and for *Plasmodium* (*spp.*). Due to the Auckland COVID-19 outbreak and the resulting lockdown that began in August of 2021, only April sampling on Codfish Island was able to occur. This excluded other populations of kākāriki from being sampled under this project and meant that sampling could not occur in spring or summer when *Cyanoramphus* parakeets are most active. These limitations greatly reduced the number of samples that could be collected. Ultimately blood and feather samples were only taken from four live birds however this was augmented by tissue and feather samples taken from fifteen deceased kākāriki from Codfish Island that the Department of Conservation gave me and my supervisor access to. As the Massey University Equine Parentage and Animal Genetics Services Centre does not

test for *Plasmodium* (*spp.*) from tissue or feather samples this removed screening for *Plasmodium* from this research also.

My supervisor and I discussed how to adapt my project in the face of these restrictions. Ultimately conservation recommendations for the kākāpō were made from a review of current literature in combination with the findings of this study and the challenges it faced. Additionally, my supervisor gave me access to the results of screening 51 Norfolk parakeets (*Cyanoramphus cookii*) for BFDV in 2015. This data was previously unpublished and enabled this report to better contribute to the conservation of parrots outside of New Zealand. My supervisor also invited me to contribute to a manuscript titled 'A PCR-Based Retrospective Study for Beak and Feather Disease Virus (BFDV) in Five Wild Populations of Parrots from Australia, Argentina and New Zealand' that was published in the journal Diversity on the 18th of February 2022 (Ortiz-Catedral et al., 2022) (Appendix I). This was an opportunity to collaborate with experts in ecology and disease management for conservation from around the world.

THESIS OUTLINE

Structure and appendix

This thesis consists of four chapters that are presented in the standard thesis format. Attached as an appendix is a manuscript that I contributed to while completing my thesis (Appendix I). This manuscript documents the PCR screening of five populations of parrots for BFDV: Burrowing parrots (*Cyanoliseus patagonus*) and Monk parakeets (*Myiopsitta monachus*) from Argentina, Crimson rosellas (*Platycercus elegans*) and Eclectus parrots (*Eclectus roratus*) from Australia and Forbes' parakeets from New Zealand (*Cyanoramphus forbesi*). A total of 612 blood samples were collected from these species between 1993-2014 for PCR testing. This manuscript relates to my thesis as both involved the PCR screening of wild populations of

parrots for BFDV to inform parrot conservation. With my supervisor I updated drafts of this manuscript to reflect advancements in scientific knowledge of BFDV that had been made since work on the manuscript begun.

Chapter one

Chapter one consists of a general introduction that outlines the ongoing biodiversity crisis and provides some background information on the order Psittaciformes, the impacts of disease on parrot conservation and specifics about my study species, the kākāpō (*Strigops habroptilus*), kākāriki (*Cyanoramphus spp.*) and Norfolk parakeet (*Cyanoramphus cookii*), and three pathogens of concern: BFDV, *Plasmodium* (*spp.*) and *Haemoproteus* (*spp.*). Chapter one also features a declaration on the impact of COVID-19 on this study and a project overview.

Chapter two

Chapter two provides some background information about the two locations where the sampling for this study took place, Codfish Island/Whenua Hou and Norfolk Island. Chapter two also details the sampling methods used at both locations, the PCR lab protocol that was used to screen for BFDV and the methods of data analysis. Additionally, this chapter presents the results of screening kākāriki on Codfish Island and the Norfolk parakeet for BFDV and the analysis of this data.

Chapter three

Chapter three provides a summary of the status of BFDV in New Zealand and on Norfolk Island. These summaries are based on a review of current literature and the findings of this study. Chapter three then builds on these summaries to make recommendations for the

conservation and species management of the critically endangered kākāpō (*Strigops habroptilus*) and endangered Norfolk parakeet (*Cyanoramphus cookii*).

Chapter four

Chapter four details possible avenues of future study. Parrot conservation could benefit from a less resource intensive and more reliable form of screening for BFDV, testing nests rather than birds for the virus is a potential option but research is lacking. The conservation of parrot species in New Zealand could benefit from an improved understanding of the distribution and diet of hematophagous arthropods that have the potential to act as disease vectors and of the diversity, abundance and distribution of pathogens of concern in captive parrots.

Chapter Two: Fieldwork and Analysis of Results

ABSTRACT

In this chapter I introduce Codfish Island/Whenua Hou and Norfolk Island where the sampling for this study took place. This chapter describes the sampling methods that were used, along with the lab protocol for PCR screening of feathers for BFDV and the data analysis tools used to assess disease freedom and the sample sizes required of future studies to establish disease freedom. The results of this sampling and analysis are detailed in this chapter, including the most recent detection of BFDV in the sole population of Norfolk parakeets (*Cyanoramphus cookii*) and the first through the use of PCR screening. Four of the 51 Norfolk parakeet feather samples collected in 2015 tested positive for BFDV, 7.84% of the Norfolk parakeets sampled. Using this data to substantiate disease freedom through Epitools FreeCalc by Ausvet yielded a 0.0017 probability of disease freedom, this indicates with statistical significance that BFDV was present amongst the population of Norfolk parakeets as of 2015. Three samplers spent 16 hours and 21 minutes mist netting on Codfish Island in April of 2021 and caught and sampled four Red-crowned parakeets (*Cyanoramphus novaezelandiae*). This means that four birds were caught in 49 hours and three minutes of sampling effort. Symptomatic expressions of PBFD such as feather loss and feather malformation were not apparent in the four Red-crowned parakeets caught in mist nets in 2021 or in the 14 deceased Red-crowned parakeet and one deceased Yellow-crowned parakeet collected by the Department of Conservation in 2019-2021. The feather samples taken from these 19 parakeets, as well as one feather that was found loose on Codfish Island, and could have originated from either a Red or Yellow-crowned parakeet (*Cyanoramphus auriceps*), all tested negative for BFDV through PCR analysis. To substantiate disease freedom through Epitools FreeCalc by Ausvet, a variety of size estimates ranging from 1430 to 116985 individuals were made for the population of Red and Yellow-crowned parakeets on Codfish Island based off of studies of other island kākāriki populations. With a sample size of 20 kākāriki the sampling undertaken in this study was insufficient to

substantiate freedom from BFDV amongst the population of Red and Yellow-crowned parakeets on Codfish Island for any of the population estimates used. Epitools FreeCalc by Ausvet was used to determine the sample sizes required to substantiate disease freedom amongst the population of Red and Yellow-crowned parakeets on Codfish Island based off of the same estimations of population size paired with BFDV test sensitivities of 0.9 and 0.95. These findings suggest that future screening of the kākāriki on Codfish Island for BFDV should sample between 130 and 166 birds.

INTRODUCTION

Kākāpō (*Strigops habroptilus*) are critically endangered with a total population of 201 birds as of September of 2021 (Department of Conservation, 2021). All kākāpō undergo regular health checks as part of their management by the New Zealand Department of Conservation, this approach enables the detection of diseases already afflicting kākāpō but does little to identify those that could pose a threat in future (Department of Conservation, n.d.-c). The detection of pathogens of concern, such as BFDV, before a kākāpō becomes infected would allow for preventative, rather than reactive, disease control. BFDV is host flexible, being able to be transmitted between different species of parrots, therefore parrots sympatric with kākāpō, like kākāriki (*Cyanoramphus spp.*), are a potential source of infection (Sarker et al., 2015a). The value of detecting pathogens before the infection of a kākāpō was demonstrated by the death of three kākāpō caused by *Erysipelothrix rhusiopathiae* bacterial infections suspected to have been contracted from local seabirds following a translocation to Chalky Island/Te Kākahu-o-Tamatea in 2004 (Gartrell et al., 2005).

The 2002 Recovery Plan for the Norfolk Island Green Parrot by the Natural Heritage Trust of Australia cites an unpublished report by Stevenson et al. (1995) that notes the presence of BFDV amongst the wild population of Norfolk parakeets (*Cyanoramphus cookii*) (Hill,

2002). This population of Norfolk parakeets is the only population of the species that exists, making it of very high conservation value. With between 46-93 individual Norfolk parakeets remaining as of 2013, the species has since benefited from a multi-year research project developed by Dr Luis Ortiz-Catedral in collaboration with the Norfolk Island National Park authority, Island Conservation, the World Parrot Trust and BirdLife (Massey University, 2014). One component of this project is to screen the Norfolk parakeet for BFDV using PCR analysis, a highly accurate method of virus detection, that has not been used on the Norfolk parakeet previously. Reconfirming the presence of BFDV in the Norfolk parakeet, along with infection prevalence data, will inform conservation priorities and actions for the species and also aligns with an objective of the 2005 PBFD Threat Abatement Plan by the Australian Government Department of Environment and Heritage and Natural Heritage Trust, to assess the risk BFDV poses to species identified as conservation priorities such as the Norfolk parakeet (Natural Heritage Trust & Australian Government Department of the Environment and Heritage, 2005).

To surveil kākāriki that are sympatric with kākāpō for BFDV samples need to be taken from kākāriki that live on the island refuges of the kākāpō. Only sampling on Codfish Island/Whenua Hou in April of 2021 could occur as a part of this research project due to the COVID-19 pandemic and ensuing lockdowns in Auckland, New Zealand. To screen Norfolk parakeets for BFDV samples need to be taken from the sole population of the species, this sampling took place in 2015.

STUDY LOCATIONS

Study site: Codfish Island/Whenua Hou

Codfish Island/Whenua Hou (46.7733º S, 167.6321º E, Figure 5.) is a small island below the South Island of New Zealand that is positioned three kilometres off of the northwest coast of

Rakiura/Stewart Island (Department of Conservation, 2012). The highest point on Codfish Island is 250m above sea level and the island is 1396 hectares in size, making it the second largest offshore island in the Rakiura/Stewart Island group (Department of Conservation, 2012). Codfish Island is surrounded by small islets and rock stacks and features a rocky coastline barring a sandy beach on the northeast of the island at Sealers' Bay (Department of Conservation, 2012). Podocarp and coastal forests are the predominant habitat type on Codfish but the island also features pakihi-type vegetation, the result of wet and infertile soil lacking peat, and sand dunes which are present at Sealers' Bay (Department of Conservation, 2012; Manaaki Whenua Landcare Research, 2021).

Codfish Island has a long history of human habitation with archaeological sites dating back to the 13th century (Department of Conservation, 2012). The island is of cultural significance to Ngāi Tahu, Ngāti Mamoe and Waitaha iwi, serving as a place for mutton-birders to rest during their hunting expeditions and being home to a settlement of European sealers and their Ngāi Tahu wives in the 19th century (Brankin, 2017; Department of Conservation, 2012). This settlement was also the earliest permanent association between Māori and Europeans in southern New Zealand, adding to its cultural and archaeological value (Department of Conservation, 2012). Codfish Island is free of invasive mammalian predators as possums (*Trichosurus vulpecula*) were removed in 1987 and Polynesian rats (*Rattus exulans*) in 1998 (Department of Conservation, 2012). Along with being closed to access from the general public, Codfish's predator-free status has made it a safe haven for vulnerable native species such as the Southern lesser short-tailed bat (*Mystacina tuberculata tuberculata*) and Yellow-crowned parakeets (*Cyanoramphus auriceps*) (Department of Conservation, 2012). Kākāpō (*Strigops habroptilus*) were first translocated to Codfish Island in 1997 and are monitored year-round by Department of Conservation staff (Department of Conservation, n.d.-e).

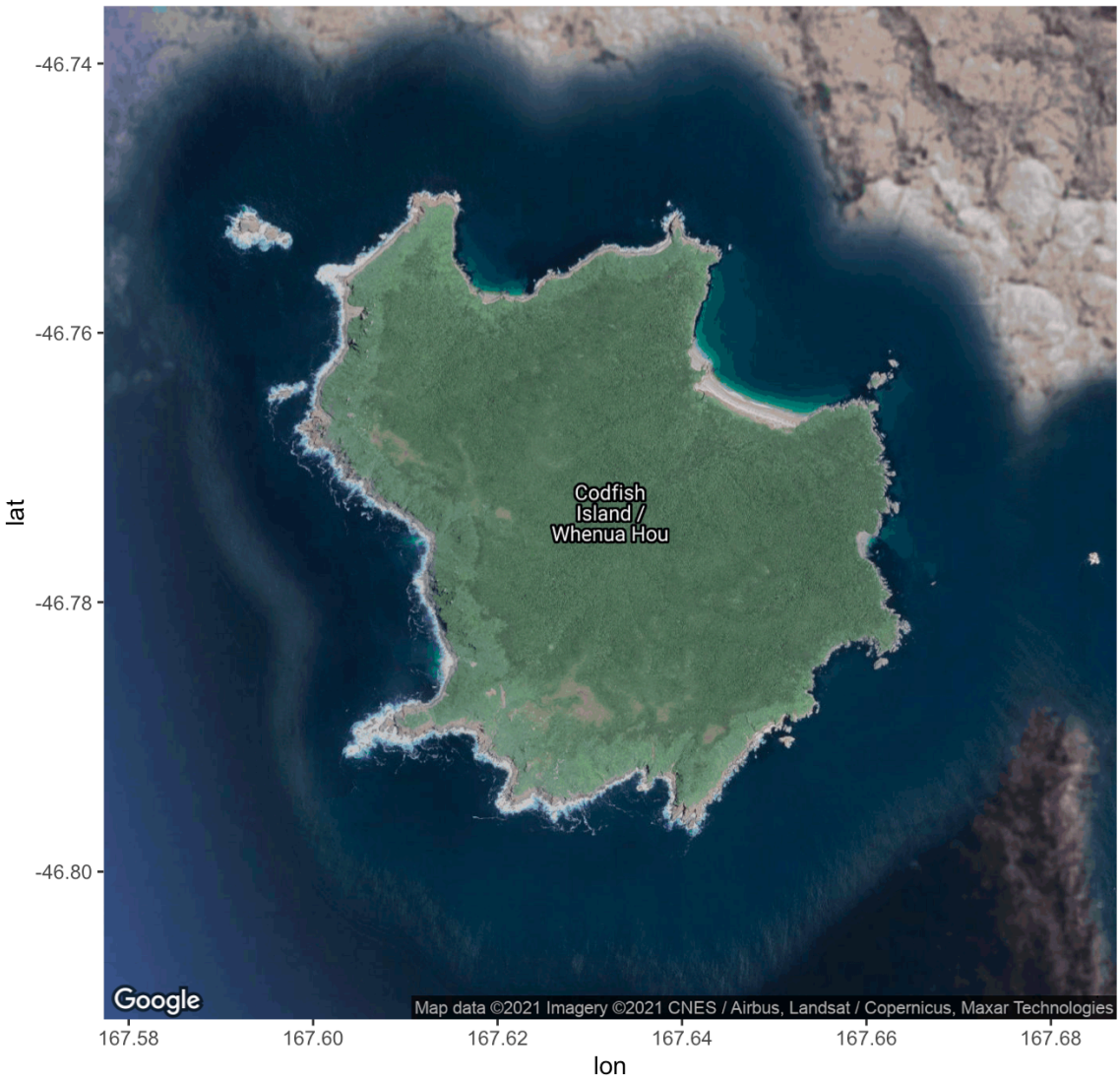

Figure 5 . A map depicting an aerial view of Codfish Island/Whenua Hou.

Study site: Norfolk Island

Norfolk Island (29.0408º S, 167.9547º E, Figure 6.) is an Australian territory in the South Pacific Ocean that is east of the Australian mainland and roughly 1700 kilometres from Sydney, Australia and 1100 kilometres from Auckland, New Zealand (Director of National Parks, 2018). The Territory of Norfolk Island consists of Norfolk Island and the smaller and uninhabited Nepean and Philip Islands to the South (Director of National Parks, 2018). Norfolk

Island is comprised primarily of olivine basaltic lava, the product of a series of volcanic eruptions from roughly 3.05 to 2.3 million years ago (Jones & McDougall, 1973). Norfolk Island is 3455 hectares in size and features a coastline that is entirely comprised of cliffs apart from a 1.5 kilometre stretch of shoreline at Kingston (Director of National Parks, 2018; Jones & McDougall, 1973). These coastal cliffs are taller in the northwest of the island, falling up to 90 metres, than they are in the south and east where they fall less than 60 metres (Jones & McDougall, 1973). Most of the island consists of a large southern plateau that stands at 90-120 metres above sea level and is separated from a much smaller northwest plateau by Mounts Pitt and Bates, the highest points of the island that reach 316 and 318 metres high respectively (Director of National Parks, 2010; Jones & McDougall, 1973). Norfolk features an average annual rainfall of 1320 millimetres and little surface water, lacking lakes entirely and with only a few wetlands and three permanently flowing streams (Director of National Parks, 2010). The average daily maximum temperature varies from 15.8º to 26.5º Celsius (Director of National Parks, 2010).

Norfolk Island has a unique history of human settlement and is home to a permanent population of roughly 1800 (Director of National Parks, 2018). Norfolk was first settled by Polynesians between the tenth and fifteenth centuries but was uninhabited by the time that it was rediscovered by Captain Cook in 1774 (Director of National Parks, 2018; McEvoy et al., 2010). The island was subsequently settled by the British Empire in 1788 and remained a British settlement until 1814 (Director of National Parks, 2018). This settlement was intended to support the new colony of Port Jackson, Sydney, Australia and convicts were used to harvest resources such as Norfolk pines (*Araucaria heterophylla*) and flax (*Phormium spp.*) (Director of National Parks, 2010, 2018). A penal settlement was established in 1825 and operated for thirty years until 1855 (Director of National Parks, 2010, 2018). In 1856, one year after the closure of the penal settlement, Norfolk was settled by the Pitcairn Islanders, descendants of

nine mutineers from the HM Armed Vessel Bounty and 12 Tahitian women (Director of National Parks, 2018; McEvoy et al., 2010). These Pitcairn settlers cultivated the land which had already been cleared through convict labour and their descendants still live on Norfolk today (Green, 1994; McEvoy et al., 2010).

When rediscovered by Captain Cook, Norfolk Island featured a dense subtropical rainforest but this was mostly cleared through European settlement and today the largest remnant forest is confined to Norfolk Island National Park (Director of National Parks, 2010; Green, 1994). There are two components to the national park, 460 hectares of land that surrounds and encompasses Mounts Pitt and Bates as well as the offshore Phillip Island, which contributes another 190 hectares of park land (Director of National Parks, 2010). The 'mainland' component of the national park features the 5.5 hectare Norfolk Island Botanic Garden, a reserve which is readily accessible to the public to showcase Norfolk's flora (Director of National Parks, 2010). A further 237 hectares of the Territory of Norfolk Island is protected as public reserve land, with twenty such reserves being found across the main island and Nepean Island (Director of National Parks, 2010). A recent Master of Science study completed by Simmonds (2019) sought to characterise the vegetation of the Mount Pitt section of Norfolk Island National Park. Simmonds recorded which woody plant species of fruiting age were within a 10 metre radius of 988 rodent bait stations within the national park. This study identified 80 woody plant species distributed across nine distinct habitat types: guava forest, forest edge, forestry, niau forest, olive forest, paddock, regenerating vegetation, remnant hardwood habitat and remnant Norfolk pine forest. The most abundant habitat type was forest edge, comprising 465 of the samples taken. This habitat type was not restricted to the borders of the national park and was found to coincide with roadsides and tracks for visitors and park maintenance. The vegetation on Norfolk Island boasts a high degree of endemism, exhibiting 445 species of vascular plants, of which 171 are native including 47 that are endemic (Green,

1994). The island is also home to two endemic plant genera, Ungeria and Streblorrhiza (Green, 1994).

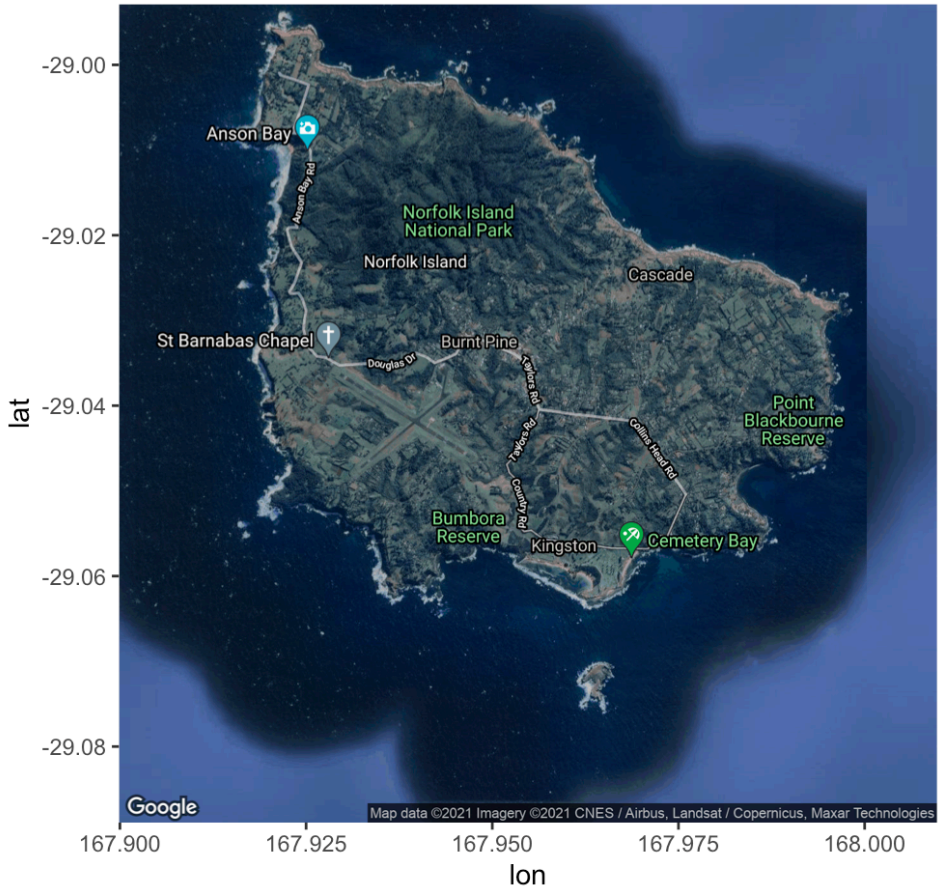

Figure 6. A map depicting an aerial view of Norfolk Island.

METHODS

Data collection: Codfish Island

Sampling occurred on Codfish Island/Whenua Hou from the 14th to the 21st of April 2021. Birds were caught using mist nest of three different lengths: 6, 9 and 12 metres long. At each sample site nets were suspended on bamboo poles so that they were in the flight path of low-flying Red and Yellow-crowned parakeets (*Cyanoramphus novaezelandiae, C. auriceps*) and

clear of short-reaching vegetation such as flaxes (*Phormium spp.*). The base and top of each net was roughly 1.5 and 4 metres above the ground respectively. The exact height of the nets varied between sites based on the surrounding environment and in one site extra bamboo was lashed together so that two nets could share the same poles, one directly atop the other, to produce a double-high net. A generalised sampling schedule is as follows: nets were opened at 7am each morning, closed at 10:30am, reopened at 3:30pm and closed again at 5:30pm. This schedule was altered when necessary, with hours being shortened when rain and wind made netting unsafe and extended when parakeets were sighted as the nets were about to be closed. Due to the sensitivity of the Department of Conservation's kākāpō (*Strigops habroptilus*) recovery project the exact Codfish Island sampling sites used in this project cannot be recorded, Figure 9. is a map that depicts obscured sampling locations.

Figure 7. A mist net on Codfish Island/Whenua Hou. Photo: Connor Wallace.

Figure 8. Photographs of weighing and measuring a juvenile male Red-crowned parakeet (*Cyanoramphus novaezelandiae*) on Codfish Island/Whenua Hou.

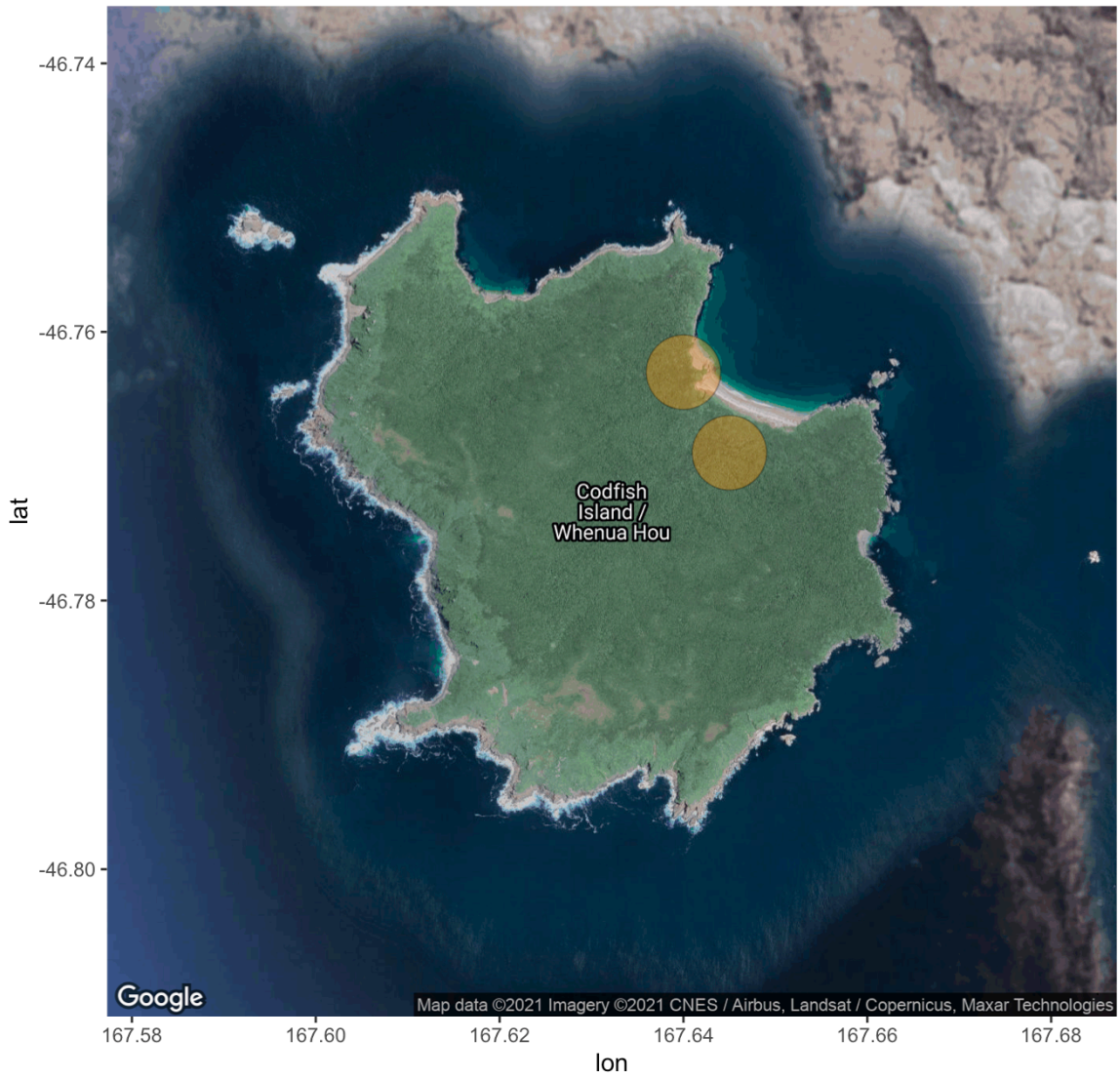

Figure 9. A map of Codfish Island/Whenua Hou displaying the generalised location of sample sites.

When birds flew into the nets, they were quickly removed by samplers to minimise the risk of stress and injury. Non-target species were released at this point while Red-crowned parakeets were placed into opaque bags of black cloth. To reduce the likelihood of sample cross-contamination each bag was used only once and then washed with Trigene®. These bags were weighed both while empty and while containing parakeets using portable scales. The culmen length, width and depth of each parakeet caught was measured using a calliper as was

the length of the wing, tarsus and tail. The Red-crowned parakeets were then banded according to the protocols approved by the Department of Conservation. After this a 40 µl blood sample was taken from the brachial vein. The vein was pricked with a sterile syringe, blood was collected using a capillary tube and then stored in 96% ethanol. A sterile cotton pad was then dabbed on the puncture. This pad along with one or two feathers that had dislodged during handling were collected and stored in paper envelopes. A photo of each Red-crowned parakeet sampled was taken using the wide-angle lens of an iPhone 12 Pro. These photos were taken in front of a cardboard envelope that featured the day and location of the capture as well as the identification number of the band applied to each bird. Ultimately four Red-crowned parakeets were captured, sampled, banded and photographed. The capture and handling of birds was conducted according to protocols approved by the Department of Conservation.

One feather, belonging to either a Red or Yellow-crowned parakeet was found near the Department of Conservation accommodation on Codfish Island and was also stored for analysis. The Department of Conservation also provided my supervisor and I with access to fifteen deceased kākāriki (*Cyanoramphus spp.*) specimens that their rangers had found on Codfish Island between 2019-2021 and stored in a freezer. Fourteen of these specimens were Red-crowned parakeets while one was a Yellow-crowned parakeet. Feather and toepad samples were collected from these parakeets for analysis. Feathers were removed from the breast and a slice of toepad tissue was removed using a scalpel that was sterilised between each bird with fire from a handheld lighter. Some of these deceased parakeets had been banded during previous expeditions to Codfish Island and where this was the case their band identification numbers were recorded. Two of the deceased parakeets had decayed to a point where only feather samples were taken.

Data collection: Norfolk Island

Sampling occurred on Norfolk Island in 2015-2016. All of the Norfolk parakeets (*Cyanoramphus cookii*) sampled were nestlings between 29 and 62 days of age with an average age of 46 days. When a nest was located the nestlings were extracted by hand and a blood sample was taken. A maximum of 40 µl of blood was taken through venepuncture of the right brachial vein and collected in non-heparinised capillary tubes before being stored in 96% ethanol. Two feather samples were also taken from each chick and were stored for analysis.

Figure 10. A series of photos displaying the sampling process for Norfolk parakeets on Norfolk Island. Photos: Luis Ortiz-Catedral.

Lab Protocols: Testing for BFDV

Testing of the Red and Yellow-crowned parakeet (*Cyanoramphus novaezelandiae, C. auriceps*) and Norfolk parakeet (*Cyanoramphus cookii*) feather samples for BFDV was conducted by the Massey University Equine Parentage and Animal Genetics Services Centre in Palmerston North, New Zealand. Testing of the Norfolk parakeet samples, that were collected on Norfolk Island in 2015-2016, occurred in November of 2016. Testing of the kākāriki samples from Codfish Island/Whenua Hou that were collected in April of 2021 as part of this study, as well as of the samples taken from deceased kākāriki collected by the Department of Conservation on the island in 2019-2021, occurred in November of 2021.

DNA was extracted from the feather samples for analysis according to the standard operating procedures of the Equine Parentage and Animal Genetics Services Centre. These procedures are set out in the H-Avian Manual, H.3 Sample Preparation-Extraction of DNA from samples (Kenny, 2019a). DNA was extracted by removing 3mm from the proximal end of each feather and submerging this 3mm segment in 50µL of MiliQ water in an Eppendorf tube. This was left to stand for at least five minutes before being centrifuged at 12000rpm for three minutes with the hinge of each Eppendorf tube facing outwards. 40µL of the resulting supernatant was removed from the side opposite the hinge of each tube. At this point InstaGene™ Matrix was removed from fridge storage and placed on a stirrer. 40µL of InstaGene™ Matrix was extracted while the matrix was still mixing and dropped into each Eppendorf tube via a wide-bore pipette. 1µL of Proteinase K was added also. This mixture was then vortexed for 10 seconds, incubated at 56º C for 30 minutes, boiled for 8 minutes and vortexed for another 10 seconds before being centrifuged for three minutes at 12000 rpm.

Polymerase chain reactions for the DNA extracted from the feather samples was conducted according to the standard operating procedures of the Equine Parentage and Animal Genetics Services Centre. These procedures are set out in the H-Avian Manual, H.4 Polymerase

Chain Reaction (PCR) (Kenny, 2019b). A small PCR tube for each sample as well as positive and negative controls were appropriately labelled. A PCR Master Mix was made up and 9µL of this mix was aliquoted into each PCR tube. For each feather sample, 1µL of the DNA extracted was placed into the corresponding labelled PCR tube. These tubes were then briefly vortexed before being placed in a PCR machine on a PBFD setting with the following primers:

PBFD F	TTA ACA ACC CTA CAG ACG GCG A
PBFD R	GGC GGA GCA TCT CGC AAT AAG

A Master Mix of digesting enzyme was then prepared and 2µL of this was aliquoted into each sample before the samples were incubated at 37º C for at least two hours. Once incubated the samples were loaded and run on a 1.5% agarose gel.

Data Preparation: Estimating Population Size

Substantiating disease freedom through Epitools by Ausvet requires knowing or estimating the size of the population being sampled for pathogens (Ausvet, 2022a). An approximation of a given species' population size can be calculated by multiplying its population density by the size of the area in which it lives. The density of the population of Norfolk parakeets (*Cyanoramphus cookii*) on Norfolk Island in 2015, when sampling for BFDV took place, was estimated at 0.816 birds per hectare by Skirrow (2018) through a fixed-point survey in spring. As the 2021 COVID-19 pandemic and ensuing Auckland lockdown prevented a second expedition to Codfish Island/Whenua Hou and therefore the practical estimation of the Red and Yellow-crowned parakeet (*Cyanoramphus novaezelandiae, C. auriceps*) population densities on the island, estimates were drawn from kākāriki studies undertaken on comparable islands. To increase the likelihood of approximating the true number of Red and Yellow-crowned

parakeets on Codfish Island a range of population densities were incorporated into this study. A lower population density estimate of 0.512 was taken from Skirrow et al. (2021) who conducted a fixed-point survey of Orange-fronted parakeets (*Cyanoramphus malherbi*) on Blumine Island/Oruawairua in 2015. For an intermediate density estimate, the 2015 Norfolk parakeet data from Skirrow (2018) was again used. A high population density estimate was taken from Bliss (2016), an unpublished report that used distance sampling to estimate the population size of Forbes' parakeets (*Cyanoramphus forbesi*) on Mangere Island as 800 birds as of 2015. This was divided by the size of Mangere Island in hectares (113 ha) to yield a population density of 7.08 birds per hectare. The highest population density estimate was taken from Greene et al. (2004) who used distance sampling to determine the density of Kermadec Red-crowned parakeets (*Cyanoramphus novaezelandiae cyanurus*) as 41.9 birds per hectare in a 73 hectare region of Macauley Island in 2002. Each of these population densities were multiplied by respective habitat size to produce population estimates. The populations of Red and Yellow-crowned parakeets on Codfish Island were treated as one population due to the limited sample size of this study. The population estimates for sampling on Codfish Island were doubled to reflect this (Table 2.).

Table 2. Parakeet density and population estimates. Density data sourced from Bliss (2016), Greene et al. (2004), Skirrow (2018) and Skirrow et al. (2021).

Population Density (parakeets per hectare)	Island	Habitat Size (hectares)	Estimated Population Size per Species	Number of Parakeet Species Sampled	Estimated Total Population Size
0.816	Norfolk Island	310	252.96	1	252.96
0.512	Codfish Island	1396	714.75	2	1429.5
0.816	Codfish Island	1396	1139.14	2	2278.27
7.08	Codfish Island	1396	9883.68	2	19767.36
41.9	Codfish Island	1396	58492.4	2	116984.8

Data Analysis: Epitools by Ausvet

In order to substantiate whether or not the sampled Red and Yellow-crowned parakeet (*Cyanoramphus novaezelandiae, C. auriceps*) and Norfolk parakeet (*Cyanoramphus cookii*) populations are free from BFDV a series of 1-Stage Freedom analysis calculations were performed. These calculations were made through the Epitools FreeCalc utility (Ausvet, 2022a). The 1-Stage Freedom analysis calculations utilise a formula developed by Cameron and Baldock (1998a) (Equation 1.).

Equation 1. The modified hypergeometric distribution formula developed by Cameron and Baldock (1998a) to substantiate freedom from disease.

$$P(T^+ = x) = \sum_{y=0}^{d} \frac{\binom{d}{y}\binom{N-d}{n-y}}{\binom{N}{n}} \sum_{j=0}^{\min(x,y)} \binom{y}{j} Se^j (1-Se)^{y-j} \binom{n-y}{x-j} \times (1-Sp)^{x-j} Sp^{n-x-y+j}$$

This formula is a modification of a hypergeometric distribution formula that has been altered to allow for finite population sizes and non-perfect tests. These modifications are necessary as animal populations are not infinite and tests that screen for pathogens can give both false positives and negatives (Cameron & Baldock, 1998a, 1998b). In the context of screening for BFDV in parakeets, '$P(T^+ = x)$' denotes the probability of finding a given number 'x' of parakeets that test positive for BFDV 'T^+'. A parakeet that tests positive for BFDV might not be genuinely positive for the virus if a positive result is a false positive. 'd' denotes the number of parakeets that are genuinely BFDV-positive in the population from which samples were taken and 'y' denotes the number of genuinely BFDV-positive parakeets in the sample. 'Se' denotes the test sensitivity, the ability of the BFDV lab test to correctly identify BFDV-positive samples. 'Sp' denotes the test specificity, the ability of the BFDV lab test to correctly identify BFDV-negative samples. 'j' denotes true positives, parakeets that have tested positive for BFDV and are genuinely positive for BFDV, and '$x - j$' denotes false positives. 'N' denotes the size of the parakeet population from which samples were taken and 'n' denotes the sample size. The input variables for the Epitools FreeCalc 1-Stage Freedom analysis calculator were the test sensitivity, test specificity, population size, sample size and the number of test-positive samples. Population estimates were rounded to the nearest integer value. For each estimate of population size two analyses were performed, one with a test sensitivity of 0.9 and one with a

test sensitivity of 0.95. This was done to reflect that the precise ability of the BFDV test performed by the Massey University Equine Parentage and Animal Genetics Services Centre to correctly identify BFDV-positive samples is unknown. For each analysis the test specificity was set at 0.99. The design prevalence and Target Type I and Target Type II errors were kept at their default values of 0, 0.05 and 0.05 respectively.

To inform future study, minimum sample sizes required to substantiate a 0.95 freedom from disease with a P-value of less than 0.05 for the population of Red and Yellow-crowned parakeets on Codfish Island were determined through a series of FreeCalc sample size calculations (Ausvet, 2022b). For population sizes of less than 10,000 individuals this FreeCalc utility uses a rearrangement of the modified hypergeometric formula to solve for the sample size 'n' (Equation 1.). For population sizes of 10,000 individuals or more this FreeCalc utility utilises a rearrangement of a binomial distribution that assumes an infinite population to solve for the sample size 'n' (Cameron & Baldock, 1998a) (Equation 2.).

Equation 2. Binomial distribution formula used to approximate disease freedom. Formula sourced from (Cameron & Baldock, 1998a).

$$P(T^+ = x) = \binom{n}{x} [pSe + (1-p)(1-Sp)]^x [p(1-Se) + (1-p)Sp]^{n-x}$$

In this formula 'p' denotes disease prevalence, the other abbreviations are the same as for the modified hypergeometric formula (Equation 1.). The input variables for the Epitools 'FreeCalc: Calculate sample size for freedom testing with imperfect tests' calculator were the population size, test sensitivity, test specificity and design prevalence. Population estimates were rounded to the nearest integer value. For each sample size estimate two analyses were performed, one with a test sensitivity of 0.9 and one with a test sensitivity of 0.95. This was done to reflect that

the precise ability of the BFDV test performed by the Massey University Equine Parentage and Animal Genetics Services Centre to correctly identify BFDV-positive samples is unknown. For each analysis the test specificity was set at 0.99 and the design prevalence was set at 0.05. The Target Type I and Target Type II errors were kept at their default values of 0.05 and 0.05 respectively.

RESULTS

Sampling Effort and Catching Success

Sampling occurred on Codfish Island/Whenua Hou from the 14th to the 21st of April 2021. This sampling involved three samplers and 16 hours and 21 minutes of fieldwork, for a total of 49 hours and 3 minutes of sampling effort. In this time four Red-crowned parakeets (*Cyanoramphus novaezelandiae*) were caught and sampled, roughly one parakeet per 12 hours and 15 minutes of effort.

Table 3. Sampling efforts and success per island.

Island	Field Hours	Number of Samplers	Total Hours of Effort	Red-Crowned Parakeets Sampled
Anchor Island	-	-	-	-
Chalky Island	-	-	-	-
Codfish Island	16:21:00	3	49:03:00	4
Little Barrier Island	-	-	-	-
Total	16:21:00	3	49:03:00	4

Visual Assessment of Birds

Symptomatic expressions of PBFD can include feather, weight and claw loss and beak malformation (Pass & Perry, 1984). A visual assessment of the four Red-crowned parakeets

(*Cyanoramphus novaezelandiae*) sampled on Codfish Island/Whenua Hou in April of 2021 found none of these symptoms. These symptoms also were not apparent in the 14 deceased Red-crowned parakeets or the one deceased Yellow-crowned parakeet that the Department of Conservation had collected on Codfish Island in 2019-2021. A photo comparison with Red-crowned parakeets from Little Barrier Island/Te Hauturu-o-Toi that were symptomatic for PBFD and determined to be BFDV positive by Ortiz-Catedral et al. (2009) reinforces this assessment (Figure 11., Figure 12.).

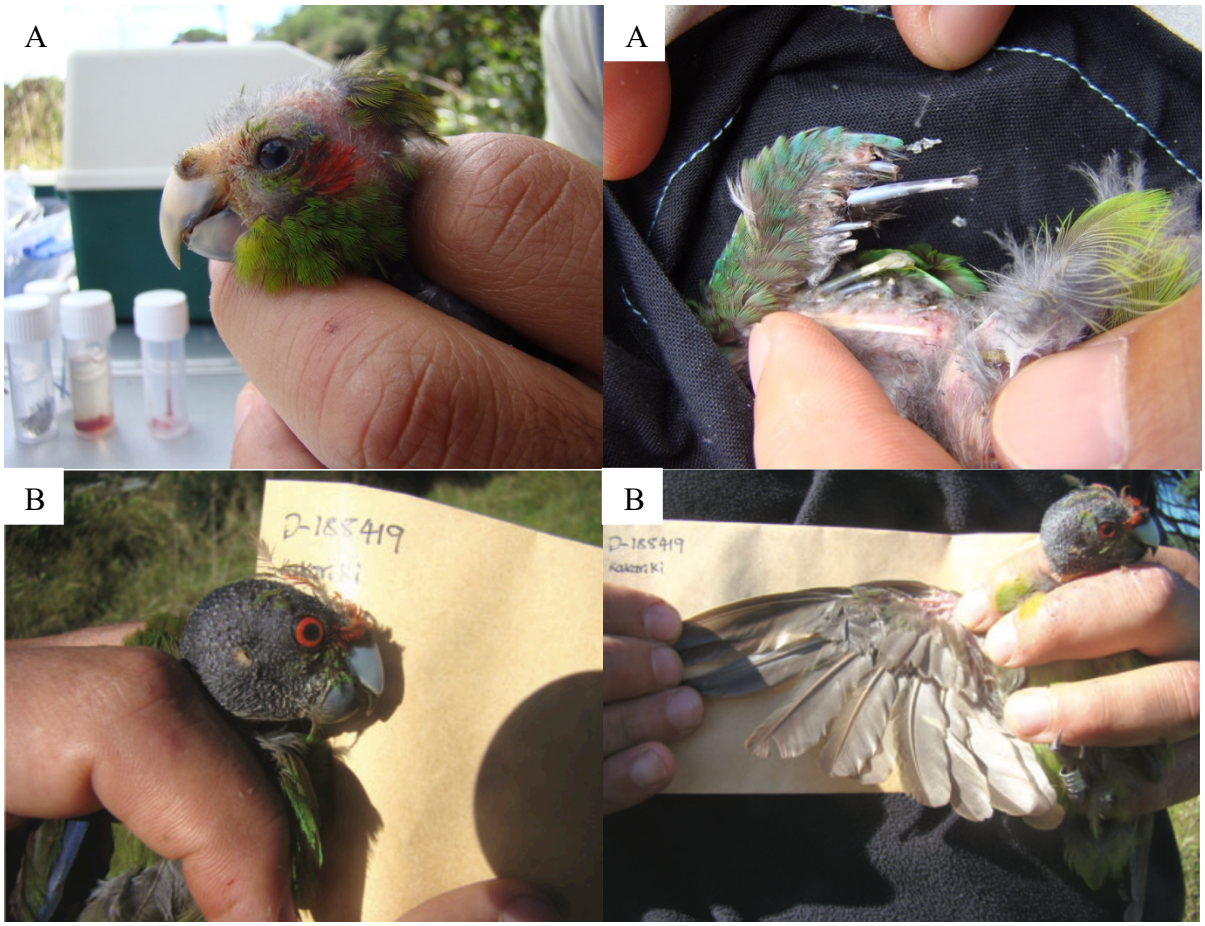

Figure 11. Two Red-crowned parakeets (*Cyanoramphus novaezelandiae*) exhibiting symptoms of chronic PBFD on Little Barrier Island/ Te Hauturu-o-Toi (Ortiz-Catedral et al., 2009). Photos: Luis Ortiz-Catedral.

Figure 12. The four Red-crowned parakeets (*Cyanoramphus novaezelandiae*) sampled for BFDV on Codfish Island in April of 2021. Photos: Connor Wallace.

Testing for BFDV

Of the 51 Norfolk parakeet (*Cyanoramphus cookii*) feather samples collected on Norfolk Island in 2015 and processed by the Massey University Equine Parentage and Animal Genetics Services Centre in 2016, four tested positive for BFDV (Table 4.). This suggests that BFDV was present in the sole population of Norfolk parakeets as of 2015. Of the 18 Red-crowned parakeet (*Cyanoramphus novaezelandiae*) and the single Yellow-crowned parakeet (*C.*

auriceps) feather samples collected from Codfish Island/Whenua Hou in 2019-2021 and processed in 2021, none tested positive for BFDV (Table 4.). The feather that was found near the Department of Conservation accommodation on Codfish Island, and could therefore belong to either a Red or Yellow-crowned parakeet, also did not test positive for BFDV. These findings suggest that if BFDV is present on Codfish Island it is not so prevalent that every kākāriki was infected with the virus as of 2019-2021.

Table 4. The results of screening Norfolk parakeets (*Cyanoramphus cookii*) on Norfolk Island in 2015 and Red and Yellow-Crowned parakeets (*Cyanoramphus novaezelandiae, C. auriceps*) on Codfish Island in 2019-2021 for BFDV.

Cyanoramphus Species	Island	Year of Sampling	Sample Size	Number of Parakeets that Tested BFDV-Positive	Percentage of Parakeets that Tested BFDV-Positive
C. cookii	Norfolk Island	2015	51	4	7.84%
C. auriceps and *C. novaezelandiae*	Codfish Island	2019-2021	20	0	0%

Disease Freedom Analysis

Substantiating disease freedom through Epitools FreeCalc by Ausvet found that the probability that the population of Norfolk parakeets (*Cyanoramphus cookii*) on Norfolk Island was free of BFDV as of 2015 was 0.0017 at test sensitivities of both 0.9 and 0.95 (Ausvet, 2022a) (Table 5.). This suggests that BFDV was present in the sole population of Norfolk parakeets as of 2015. The Epitools FreeCalc utility for substantiating disease freedom determined that the sample size for the population of Red and Yellow-crowned parakeets (*Cyanoramphus novaezelandiae, C. auriceps*) on Codfish Island/Whenua Hou in 2019-2021 was insufficient to substantiate a probability of freedom from BFDV (Table 5.). This held true for all estimates of

population size in combination with test sensitivities of 0.9 and 0.95 and also when a calculation was performed solely for the Red-crowned parakeet samples with the population estimate halved accordingly.

Table 5. The results of substantiating freedom from BFDV for the population of Norfolk parakeets (*Cyanoramphus cookii*) on Norfolk Island in 2015 and the population of Red and Yellow-Crowned parakeets (*Cyanoramphus novaezelandiae, C. auriceps*) on Codfish Island in 2019-2021.

Cyanoramphus Species	Island	Sample Size	Estimated Population Size	Test Sensitivity	Probability the Population is Free of BFDV
C. cookii	Norfolk Island	51	253	0.9	0.0017
C. cookii	Norfolk Island	51	253	0.95	0.0017
C. novaezelandiae C. auriceps	Codfish Island	20	1430	0.9	Insufficient sample size
C. novaezelandiae C. auriceps	Codfish Island	20	1430	0.95	Insufficient sample size
C. novaezelandiae C. auriceps	Codfish Island	20	2278	0.9	Insufficient sample size
C. novaezelandiae C. auriceps	Codfish Island	20	2278	0.95	Insufficient sample size
C. novaezelandiae C. auriceps	Codfish Island	20	19767	0.9	Insufficient sample size
C. novaezelandiae C. auriceps	Codfish Island	20	19767	0.95	Insufficient sample size
C. novaezelandiae C. auriceps	Codfish Island	20	116985	0.9	Insufficient sample size
C. novaezelandiae C. auriceps	Codfish Island	20	116985	0.95	Insufficient sample size

Target Sample Size Analysis

Determining the minimum sample sizes required to substantiate a 0.95 freedom from disease with a P-value of less than 0.05 for the population of Red and Yellow-crowned parakeets (*Cyanoramphus novaezelandiae, C. auriceps*) on Codfish Island/Whenua Hou through the Epitools FreeCalc utility yielded different results for different combinations of estimated population size and test sensitivity (Ausvet, 2022b). These target sample sizes ranged from 130 birds, with an estimated population size of 1430 individuals and a test sensitivity of 0.95, to 166 birds with an estimated population size of 116985 individuals and a test sensitivity of 0.9 (Table 6.).

Table 6. Target sample sizes to substantiate disease freedom by population size and test sensitivity.

Cyanoramphus Species	Island	Estimated Population Size	Test Sensitivity	Target Sample Size	Cut-point Number of Positives
C. novaezelandiae C. auriceps	Codfish Island	1430	0.9	137	3
C. novaezelandiae C. auriceps	Codfish Island	1430	0.95	130	3
C. novaezelandiae C. auriceps	Codfish Island	2278	0.9	163	4
C. novaezelandiae C. auriceps	Codfish Island	2278	0.95	132	3
C. novaezelandiae C. auriceps	Codfish Island	19767	0.9	166	4
C. novaezelandiae C. auriceps	Codfish Island	19767	0.95	134	3
C. novaezelandiae C. auriceps	Codfish Island	116985	0.9	166	4
C. novaezelandiae C. auriceps	Codfish Island	116985	0.95	134	3

Chapter Three: Conservation Implications for the Kākāpō and Norfolk parakeet

ABSTRACT

This chapter presents the status of BFDV in New Zealand and on Norfolk Island based off of the findings of this research project and other published studies and material. With samples from twenty kākāriki (*Cyanoramphus spp.*) being insufficient to substantiate freedom from BFDV for the kākāriki population on Codfish Island/Whenua Hou, this wider context is used to make conservation recommendations for kākāpō (*Strigops habroptilus*) with respect to BFDV and other pathogens of concern. BFDV is yet to be observed in a kākāpō and this is despite the kākāpō population on Little Barrier Island/Te Hauturu-o-Toi coexisting with BFDV-positive Red-crowned parakeets (*Cyanoramphus novaezelandiae*). Establishing the ease with which kākāpō can become infected with BFDV, if the species can contract the virus at all, as well as the severity of such an infection remains a critical unknown towards assessing the risk that BFDV poses to the conservation and recovery of the kākāpō. This chapter discusses this knowledge gap and suggests possible avenues for future research and also recommends a novel sampling regime to detect pathogens of concern that could threaten the kākāpō. This regime involves continuous 'low effort' sampling, efforts such as collecting loose feathers and faeces that could be undertaken by conservation staff during the completion of their other duties, to build a library of samples for the detection of BFDV and other pathogens of concern. This low effort sampling would be punctuated by less frequent 'high effort' sampling, activities such as mist netting and collecting blood and feather samples, which would occur biennially unless triggered earlier by the detection of a pathogen of concern through low effort sampling. The advantages of such a model are discussed in this chapter and a similar model is recommended for the conservation of the Norfolk parakeet (*Cyanoramphus cookii*) also. With this study reconfirming the presence of BFDV amongst the Norfolk parakeet the management of the species must look to manage the impact of the virus on the species. Such management must be proportionate as conservation funding is not infinite and the Norfolk

parakeet has other management needs such as the control of mammalian predators. There is a possibility that the Norfolk parakeet has coexisted with BFDV infections for 20-30 years, even while experiencing a significant population increase coinciding with pest control and other conservation measures. For this reason, quantifying the risk that BFDV poses to the Norfolk parakeet should be an important step towards allocating conservation funding for the species. This chapter discusses this concept and makes suggestions for future research and conservation actions.

INTRODUCTION

This research project involved sampling kākāriki (*Cyanoramphus spp.*) on Codfish Island/Whenua Hou for BFDV, but samples were not taken from the other islands where kākāpō (*Strigops habroptilus*) are present. This was a limitation caused by the COVID-19 pandemic and ensuing lockdowns in Auckland, New Zealand. Fortunately, this project is not the first to investigate the prevalence of BFDV in New Zealand or of BFDV amongst kākāriki sympatric with kākāpō. Of particular note, the studies by Ortiz-Catedral et al. (2009) and Jackson et al. (2015), which reveal and then reconfirm the presence of BFDV in kākāriki sympatric with kākāpō on Little Barrier Island/Te Hauturu-o-Toi, enable discussion of the implications of the presence of BFDV within the current geographical distribution of the kākāpō. This wider context, in combination with the findings and challenges faced by my research project, enable recommendations to be made for the conservation and species management of the kākāpō with respect to BFDV and other pathogens of concern.

The results of sampling 51 Norfolk parakeets (*Cyanoramphus cookii*) in 2015 that are presented in this thesis reconfirms the presence of BFDV amongst the sole population of the species. This data enables conservation and species management recommendations to be made for the Norfolk parakeet with respect to BFDV. Additionally, these findings in combination

with the 2002 Recovery Plan for the Norfolk Island Green Parrot, which cites an unpublished report by Stevenson et al. (1995) noting the presence of BFDV amongst the wild population of Norfolk parakeets, suggests that the species has been coexisting with BFDV infections for roughly 20-30 years (Hill, 2002). This possibility, along with the significant population increase that the Norfolk parakeet has experienced since the conservation efforts implemented in 2013, raises questions about the impact that BFDV has on Norfolk parakeets that can inform the conservation of the species and future research (Skirrow, 2018).

THE STATUS OF BFDV IN NEW ZEALAND

BFDV is present in captive parrots in New Zealand, having been detected in at least seven caged species including Rainbow lorikeets (*Trichoglossus haematodus*), Yellow-bib lorikeets (*Lorius chlorocercus*) and Longbill corellas (*Cacatua tenuirostris*) (Ritchie et al., 2003). BFDV is also present in naturalised parrots in New Zealand, having been detected in wild populations of Sulphur-crested cockatoos (*Cacatua galerita*) near Whanganui and in wild populations of Eastern rosellas (*Platycercus eximius*) in Auckland, Te Puke and Wellington (Ha et al., 2007; Massaro et al., 2012). BFDV also exists in New Zealand's wild native parrots in both the North and South Islands, first being detected in 16 Red-crowned parakeets (*Cyanoramphs novaezelandiae*) on Little Barrier Island/Te Hauturu-o-Toi in 2008 (Ortiz-Catedral et al., 2009). BFDV was subsequently detected in eight Yellow-crowned parakeets (*Cyanoramphus auriceps*) from the Eglington region that were sampled in 2011 and 2012 as well as in two additional populations of Red-crowned parakeets, from Kapiti Island and Zealandia-Karori sanctuary in Wellington, sampled between 2010 and 2012 (Jackson et al., 2015; Massaro et al., 2012). BFDV was again detected in Red-crowned parakeets on Little Barrier Island that were sampled in 2013 (Jackson et al., 2015).

This study did not detect BFDV in feather samples taken from twenty kākāriki (18 Red-crowned parakeets, one Yellow-crowned parakeet and one sample that could belong to either species) on Codfish Island/Whenua Hou in 2019-2021. Clinical symptoms of PBFD, such as feather loss, haemorrhagic feathers, claw loss and beak malformation were also not apparent in the kākāriki sampled (Ortiz-Catedral et al., 2009; Pass & Perry, 1984). Disease freedom analysis through Epitools FreeCalc by Ausvet revealed that, with a sample size of only twenty birds, this data is insufficient to state that the Red and Yellow-crowned parakeet population on Codfish Island is free from BFDV (Ausvet, 2022a). This assessment held true across multiple different estimates for the population size of Red and Yellow-crowned parakeets on Codfish Island. Despite the inconclusive nature of these results there are multiple conservation implications of the known range, characteristics, and potential risk of BFDV for the management of the critically endangered kākāpō (*Strigops habroptilus*) and recommendations that can be made based on this knowledge and the findings of this study.

RECOMMENDATIONS FOR THE CONSERVATION OF THE KĀKĀPŌ

Susceptibility of Kākāpō to BFDV

Given the presence of BFDV in New Zealand's wild and captive parrots, quantifying the risk that BFDV poses to kākāpō (*Strigops habroptilus*) should be a key consideration towards the allocation of conservation funding for the species. The risk that a pathogen poses to any given species is the product of a great variety of factors. For the sake of this discussion from a species management perspective these factors can be generalised into three primary elements: the vulnerability of the species to extinction, the ability of the species to be infected by the pathogen in question and the severity or consequences of infection. Assessing these elements in the context of BFDV and kākāpō should inform the management of the species by the Department of Conservation.

With a total population of 201 individuals as of September 2021, the kākāpō is critically endangered and is at risk of extinction (Department of Conservation, 2021). These 201 birds are spread across four offshore islands, Anchor Island/Pukenui, Chalky Island/Te Kākahu-o-Tamatea, Codfish Island/Whenua Hou and Little Barrier Island/Te Hauturu-o-Toi. With each island population being an isolated portion of the total number of kākāpō, the risk of extinction to each population from a localised threat, such as an invasion of mammalian predators or a pathogen outbreak, is increased but the risk of species extinction from such threats is reduced.

With a vulnerability to extinction established, the next consideration is the ability of kākāpō to contract BFDV infections. BFDV is host flexible and all parrot species are generally considered susceptible to infection (Martens et al., 2020a; Sarker et al., 2015a). In New Zealand the host flexibility of BFDV is demonstrated by the ~94.9-97.9% pairwise identity of the BFDV isolates detected in Red-crowned parakeets (*Cyanoramphus novaezelandiae*) and Eastern rosellas (*Platycercus eximius*) from Little Barrier Island and the Auckland region respectively (Massaro et al., 2012). Despite this host flexibility, the presence of BFDV in both the North and South Island and annual health checks of all kākāpō older than two years of age, as well as more frequent checks of birds younger than this, BFDV is yet to be detected in kākāpō (Department of Conservation, n.d.-c; Massaro et al., 2012). This is of particular note given the two-time detection of BFDV on Little Barrier Island. Fifteen Red-crowned parakeets, two of which exhibited feather loss symptomatic of chronic PBFD, were found to be BFDV-positive on Little Barrier Island in 2008 (Ortiz-Catedral et al., 2009). Two additional Red-crowned parakeets were found to be BFDV positive when 45 parakeets were sampled on the island in 2013 (Jackson et al., 2015). The BFDV isolates detected in the Red-crowned parakeets on Little Barrier Island in 2008 and 2013 were of two distinct clades, possible evidence of two separate introductions of BFDV to the island (Jackson et al., 2015). Little Barrier Island has a long history with the conservation of the kākāpō. A failed translocation attempt was made in 1903,

kākāpō were translocated to and then off of the island in the eighties and nineties and most recently nine kākāpō were translocated to the island in 2012 and four additional birds in 2017 (Ballance, 2010; Clout & Merton, 1998; Department of Conservation, 2017; Lloyd & Powlesland, 1994). Crucially, the 2012 translocation and detection of BFDV in 2013 suggests that the kākāpō on Little Barrier Island have been sympatric with Red-crowned parakeets that are BFDV-positive.

The many possible reasons why BFDV has not been detected in the kākāpō on Little Barrier Island can be categorised under three primary explanations: that the kākāpō have not been sufficiently exposed to the pathogen, that it is difficult for BFDV to infect kākāpō or random chance. These explanations are not mutually exclusive. The kākāpō on Little Barrier Island might have avoided BFDV exposure if the Red-crowned parakeets are not shedding the virus into the environment in large enough quantities and/or if there is little to no overlap between the habitat use of the parakeets and kākāpō on the island. BFDV is shed into the environment through crop secretions, faeces and feather dander (Jackson et al., 2015; Martens et al., 2020a). When BFDV was first identified in Red-crowned parakeets on Little Barrier Island in 2008 the virus was detected in feather samples, however in the 2013 sampling all of the feather samples taken tested negative for the virus while two blood sample tests were found to be BFDV-positive (Jackson et al., 2015; Ortiz-Catedral et al., 2009). With BFDV not being detected in feathers from the 2013 sampling this could potentially indicate a reduced quantity of virus being shed into the environment through feather dander. Such a discrepancy between the 2008 and 2013 sampling could be a reflection of the clade difference between the BFDV isolates detected, of different stress levels being experienced by the BFDV-positive birds sampled or of different stages of PBFD being experienced by the BFDV-positive birds at the time of sampling. There are also a variety of reasons that the kākāpō on Little Barrier Island might have avoided BFDV as a result of little to no overlap in habitat use between the kākāpō

and Red-crowned parakeets. Such a scenario could be born of the kākāpō being nocturnal and the Red-crowned parakeet being diurnal, the kākāpō being primarily ground-dwelling and the parakeet primarily arboreal or simply because of the small number of kākāpō on Little Barrier Island.

The Department of Conservation and/or university researchers should investigate the way in which BFDV-positive Red-crowned parakeets on Little Barrier Island shed the virus into the environment and the quantity of the virus that is shed also. Additionally, research should be done into the overlap in habitat use between the parakeets and kākāpō on the island. Together such information will produce evidence towards understanding the ability of BFDV to infect kākāpō, a significant component of assessing the risk that the virus poses to the species. The other outstanding element to this risk assessment is understanding the severity and consequences of a successful BFDV kākāpō infection. As the sole member of its genus, it is possible that this information will only come with a kākāpō becoming infected with BFDV. Some inference could potentially be made from a BFDV infection of the closest living relatives of the kākāpō, the kea (*Nestor notabilis*) and kaka (*N. meridionalis*), however BFDV has not been detected in these species either.

BFDV on Little Barrier Island

The presence of BFDV on Little Barrier Island/Te Hauturu-o-Toi poses a potential risk to the conservation of the kākāpō (*Strigops habroptilus*) but also provides an opportunity to study how the species interacts with the virus. The kākāpō population on Little Barrier Island has so far proven successful, with the female kākāpō 'Heather' and the male 'Dobbie' producing two chicks, 'Mahli' and 'Tohu' in 2014 (Department of Conservation, 2014; The New Zealand Herald, 2014). This population is also significant as the only North Island residence of the kākāpō. Pre-human settlement kākāpō had a nationwide distribution yet today only the kākāpō

on Little Barrier Island experience the warmer conditions and ecosystems typical of New Zealand's North Island (Lentini et al., 2018). This provides a unique study opportunity that will hopefully inform the translocation of kākāpō to other North Island locations in future.

As BFDV is yet to be detected in kākāpō the ability of the virus to infect kākāpō and the severity of such an infection remain largely unknown. While lacking this data, species management of the kākāpō should err on the side of caution given the endangerment and slow reproductive rate of the species (Cockrem & Rounce, 1995; Elliott et al., 2001; Powlesland et al., 1992). Kākāpō are also genetically depauperate, a characteristic that has been linked to a reduced diversity of immune-genes in the Orange-bellied parrot (*Neophema chrysogaster*) (Morrison et al., 2020; White et al., 2015). This could mean that should BFDV severely impact the fitness of a single kākāpō, the virus may have similar impacts on infected conspecifics increasing the threat posed by infection. Along with continued monitoring for BFDV in both the kākāpō and parakeets on Little Barrier Island, there are a variety of ways that the Department of Conservation can look to minimise the risk that is posed by the presence of BFDV on the island: the Department of Conservation could cull, or otherwise remove, the island's parakeet population, they could keep the number of kākāpō on the island minimal or they could remove kākāpō from the island entirely. The detection of two different clades of BFDV on Little Barrier Island in 2008 and 2013 suggest that culling the island's kākāriki population might not prevent the flow of BFDV to the island (Jackson et al., 2015). Additionally, the proximity of nearby Great Barrier Island/Aotea, roughly 18 kilometres east of Little Barrier Island, might cause a cull to be unsuccessful. The removal of kākāpō from Little Barrier Island entirely would mean forgoing an environment that has proven conducive to successful kākāpō reproduction as well as the only location where kākāpō can be studied under North Island climate and weather conditions. Maintaining a minimal, but still reproductively active, population of kākāpō on Little Barrier Island appears to be a sound

middle-of-the-road approach but confers the risk of one or multiple kākāpō contracting a BFDV infection. With careful management this risk could be harnessed as a conservation benefit, albeit a perhaps unconventional one.

The ongoing COVID-19 pandemic has demonstrated how difficult it can be to restrict the spread of a virus. Given the presence of BFDV in both the North and South Islands and the way that the virus is present in wild populations of native and naturalised parrots alike, if kākāpō are able to be infected by BFDV it is a safe assumption that such an infection will one day occur. The best way for this to transpire would be in a way that is anticipated, prepared for and can also serve to inform the conservation and management of the kākāpō. The Department of Conservation acknowledging that a kākāpō on Little Barrier Island could one day be infected with BFDV should allow for such an infection to be detected quickly and encourage research into the best possible response in terms of control and treatment protocols. Rapid detection of a BFDV infection could be achieved through increased frequency of testing of the kākāpō on Little Barrier Island and through thorough vigilance for the symptoms of PBFD. The early discovery of a BFDV infection would put the Department of Conservation in the best position to deal with a potential outbreak by affording as much time as possible to implement control and recovery actions such as isolation and medical observation and care. Such a scenario would also allow for a clear assessment of the consequences of a kākāpō becoming infected with BFDV and the success of medical intervention. Along with data on the ease with which kākāpō can become infected with BFDV this would enable an appropriate prioritisation of management funding for the species, benefiting the conservation of the kākāpō as a whole.

Monitoring for Pathogens of Concern

All kākāpō (*Strigops habroptilus*) that exist today reside on offshore island reserves where mammalian predators have been eradicated and where deforestation is prohibited. This

isolation has not eliminated the threat of pathogens and disease as has been demonstrated by the detection of BFDV on Little Barrier Island/Te Hauturu-o-Toi and by the loss of three kākāpō to *Erysipelothrix rhusiopathiae* bacterial infections following a translocation to Chalky Island/Te Kākahu-o-Tamatea in 2004 (Gartrell et al., 2005; Jackson et al., 2015; Ortiz-Catedral et al., 2009). For this reason, surveillance for pathogens of concern remains a critical biosecurity component of kākāpō conservation.

My study demonstrated one of the ways in which monitoring for pathogens can be disrupted. While a global pandemic is perhaps an extreme example, a variety of occurrences can disrupt the scientific process including, but not limited to, unfavourable weather, governmental, organisational and funding changes and the unpredictable elements of working with wildlife. To mitigate disruptions such as these I recommend a pathogen monitoring regime that incorporates continual 'low effort' monitoring punctuated by thorough 'high effort' monitoring events every two years or more frequently if prompted by circumstance. Low effort monitoring would refer to actions such as Department of Conservation staff collecting loose feathers as well as faeces from kākāpō and other bird species that are encountered in the field during the course of their other duties. Ideally samples collected would be appropriately stored in envelopes or specimen containers and marked with the date and location of collection as well as the relevant species name. Along with collecting deceased kākāriki (*Cyanoramphus spp.*), as is already practised by the Department of Conservation staff on Codfish Island/Whenua Hou, and deceased specimens from other bird species this would contribute to building up an archive of sample material that could be analysed by researchers, potentially including undergraduate students, for pathogen detection and education benefits. These samples could be analysed during periods when fieldwork is unrealistic and the durability of some pathogens, such as BFDV, could allow for samples to be viable years after collection (Raidal & Cross, 1994; Todd, 2000). High effort monitoring would refer to actions such as

directly capturing bird species sympatric with kākāpō, such as kākāriki, with mist nets and collecting blood and feather samples. These distinct sampling methods would work in tandem to form a comprehensive biosecurity model. If a pathogen of concern is detected through low effort sampling it could trigger an earlier bout of high effort sampling to gauge the extent of the issue more accurately. Additionally, the existence of a specimen archive built through low effort sampling could provide data towards the timeframe of an outbreak should a pathogen of concern be detected through high effort sampling. I recommend that this model be implemented on all of the kākāpō islands as well as on potential sites of future kākāpō translocations so as to detect potential pathogen risks before the introduction of kākāpō to a location. Theoretically, resources could be dedicated to enable the public to conduct low effort sampling throughout the country. If samples are appropriately labelled and fed into a specimen archive that is accessible to researchers, this could vastly improve our knowledge of New Zealand's avifaunal pathogens.

This study used the EpiTools target sample size calculator by Ausvet to determine the sample size required to assess freedom from BFDV for the Red and Yellow-crowned parakeet (*Cyanoramphus novaezelandiae, C. auriceps*) population on Codfish Island (Ausvet, 2022b). As the COVID-19 pandemic prevented the practical estimation of the size of the Red and Yellow-crowned parakeet population on Codfish Island, a variety of population estimates were made based on kākāriki density estimates taken from other studies. This analysis indicates that a sample size of 130 or 137 parakeets, with a test sensitivity of 0.95 or 0.9 respectively, would be sufficient for a population size of 1430 Red and Yellow-crowned parakeets and that a sample size of 134 of 166, with a test sensitivity of 0.95 or 0.9 respectively, would be sufficient for a population size of 116985 Red and Yellow-crowned parakeets on the island. As the target sample sizes determined for the lowest and highest estimates of the kākāriki population size on Codfish Island, these figures could be used to set sampling quotas for 'high effort' sampling in

future. This study was the second to screen the Red-crowned parakeets on Codfish Island for BFDV with a sample size of less than 130 birds, as a study from 2008-2010 sampled 32 parakeets on the island and also found no birds positive for BFDV (Massaro et al., 2012). The inability of these studies to capture sample sizes sufficient to determine freedom form disease for even the lowest estimated population size paired with the most accurate testing, provides support for the concept of continuous low effort sampling which could help to make the numerical difference.

THE STATUS OF BFDV ON NORFOLK ISLAND

Two species of parrot live on Norfolk Island, the introduced Crimson rosella (*Platycercus elegans*) and the endemic and endangered Norfolk parakeet (*Cyanoramphus cookii*). The population of Norfolk parakeets that live on Norfolk Island is the only wild population of the species that exists, making it of very high conservation value. As cited by the 2002 Natural Heritage Trust Recovery Plan for the Norfolk Island Green Parrot, an unpublished report by Hicks and Preece (1991) notes the presence of BFDV infections amongst Crimson rosellas on Norfolk Island and similarly an unpublished report by Stevenson et al. (1995) notes BFDV infections amongst the wild population of Norfolk parakeets (Hill, 2002). This study presents the results of sampling 51 Norfolk parakeets for BFDV in 2015. With four of the 51 parakeets sampled testing BFDV-positive the probability that the population is free from BFDV is only 0.0017 as determined through Epitools FreeCalc by Ausvet (Ausvet, 2022a). This is the most recent detection of BFDV infecting Norfolk parakeets and is the first screening of the population for the virus to utilise PCR analysis. From these findings multiple recommendations can be made for the conservation of the Norfolk parakeet.

RECOMMENDATIONS FOR THE CONSERVATION OF THE NORFOLK PARAKEET

Further BFDV Research

The data presented in this study provides statistically significant evidence ($P < 0.05$) that the only population of Norfolk parakeets (*Cyanoramphus cookii*) is not free from BFDV infection. This reconfirms the presence of BFDV in this population, with management plans for the Norfolk parakeet citing an unpublished report from 1995 as an early record of BFDV infection (Hill, 2002; Stevenson et al., 1995). With BFDV present amongst Norfolk parakeets, conservation actions for the species must manage the risk that is posed by the virus. To appropriately manage the risk that BFDV poses to the Norfolk parakeet it is important to quantify the level of threat so that conservation funding and other resources can be allocated accordingly. If we utilise the same method as was used to assess the risk that BFDV poses to the kākāpō (*Strigops habroptilus*) in the discussion above, there are fewer unknown variables associated with the Norfolk parakeet.

With just one population estimated to consist of roughly 438 individuals as of 2017, the Norfolk parakeet is endangered and threatened with extinction (Skirrow, 2018). With only one population the Norfolk parakeet is very vulnerable to localised threats, should the population on Norfolk Island be negatively impacted by invasive mammals or a disease outbreak there would be no unaffected population elsewhere. With BFDV-positive birds being recorded in 1995 and detected again through PCR analysis in 2016, the ability of the Norfolk parakeet to contract BFDV infections is established (Hill, 2002; Stevenson et al., 1995). The remaining unknown is the impact and consequences of BFDV infections of Norfolk parakeets. The Norfolk parakeet has come very close to extinction more than once and was recognised as one of the most endangered bird species in Australia in 2002 with just 160 individuals remaining (Hill, 2002; Ortiz-Catedral et al., 2018). This population count dopped even further with

between 46 and 93 birds remaining as of 2013 (Massey University, 2014). The primary causes of these declines were predation by rats (*Rattus spp.*) and cats (*Felis catus*) as well as the deterioration of nest sites exacerbated by nest hollow competition with the invasive Crimson rosella (*Platycercus elegans*) (Ortiz-Catedral et al., 2018). Through intensive conservation efforts, including the culling of Crimson rosellas, baiting for rats and trapping cats, the Norfolk parakeet has recently experienced a population rebound and steady population increases have been recorded from 2013 through 2017 (Massey University, 2014; Ortiz-Catedral et al., 2018; Skirrow, 2018) (Figure 13.). With the data in this study indicating the presence of BFDV in the Norfolk parakeet population in 2015 there is some overlap with the increase of the parakeet population that has been achieved through conservation efforts. With BFDV being recorded in the Norfolk parakeet as far back as the nineteen-nineties and again in 2015, there is also the potential that some level of BFDV infection has been a constant in the Norfolk parakeet population since at least the late nineties and that the virus has therefore been present during both the more recent population declines and the population rebound of the species (Hill, 2002; Stevenson et al., 1995). If this latter scenario holds true it would mean that the Norfolk parakeet has been coexisting with BFDV for roughly 20-30 years.

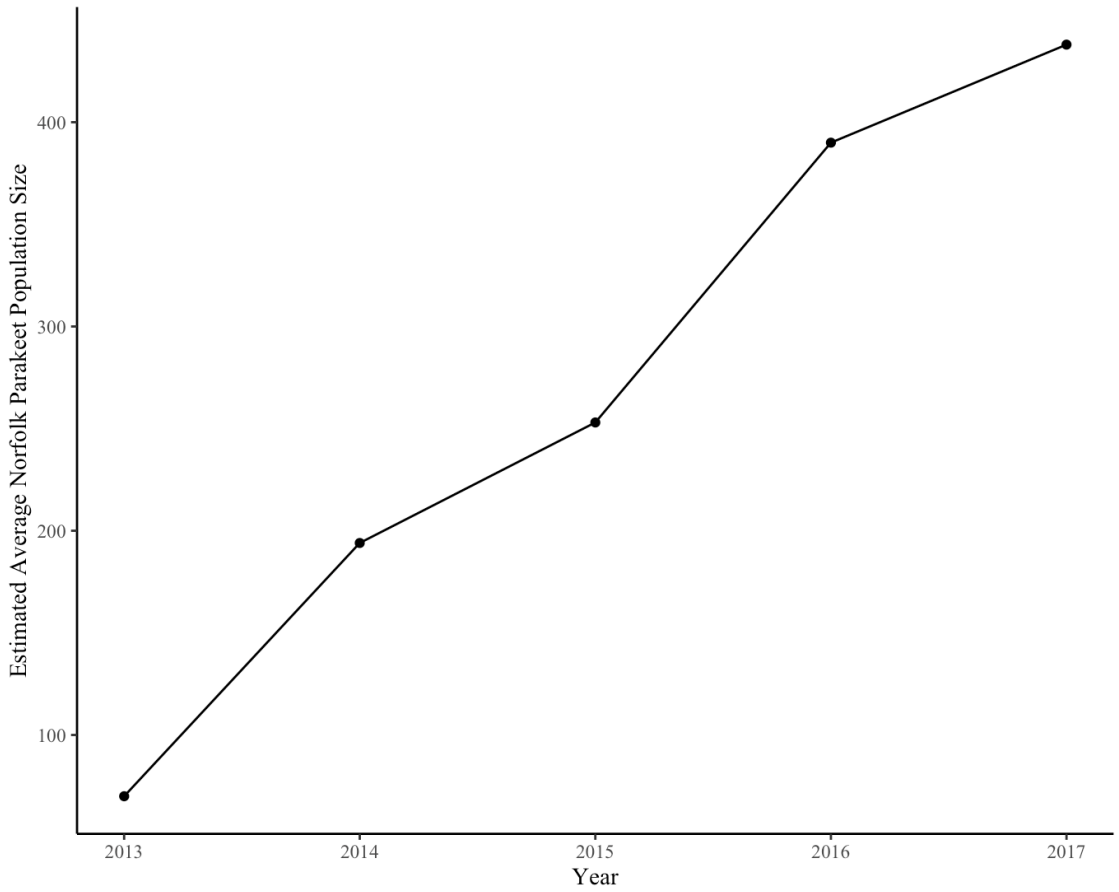

Figure 13. Line graph showing the population growth of the Norfolk parakeet from 2013 to 2017. Data sourced from Massey University (2014) and Skirrow (2018).

There is little published information on the severity of BFDV infections amongst Norfolk parakeets and how PBFD is expressed by the species. The PBFD Threat Abatement Plan published by the Natural Heritage Trust and Australian Government Department of the Environment and Heritage (2005) notes that the Norfolk parakeet has experienced clinical symptoms of PBFD or mortality from BFDV infection however does not provide more detailed case-history information than this. Information is also limited if we look to extrapolate from how PBFD is expressed in the other parakeets in the genus *Cyanoramphus*. In addition to the Norfolk parakeet, BFDV has also been recorded in the Red and Yellow-crowned parakeets of New Zealand (*C. novaezelandiae, C. auriceps*) and the New Caledonian parakeet (*C. saisseti*)

that is endemic to New Caledonia. Two of the 15 BFDV-positive Red-crowned parakeets detected through sampling on Little Barrier Island/Te Hauturu-o-Toi in 2008 exhibited chronic PBFD symptoms including extensive feather loss and feather malformation (Ortiz-Catedral et al., 2009). The reports of BFDV in eight Yellow-crowned parakeets and a single New Caledonian parakeet make no mention of clinical PBFD symptoms (Julian et al., 2012; Massaro et al., 2012).

With BFDV present in the sole population of Norfolk parakeets, the partnership between Norfolk Island National Park rangers, ecologists and Massey University researchers should investigate the way that PBFD is expressed by Norfolk parakeets. In addition to informing species management priorities and actions this research would align with two of the objectives detailed in the PBFD Threat Abatement Plan. This plan sets a variety of targets including addressing gaps in knowledge about PBFD and to add or remove species from the priority species list (Natural Heritage Trust & Australian Government Department of the Environment and Heritage, 2005). This is a list of Australian parrot species that potentially require prioritisation in the fight against PBFD and does include the Norfolk parakeet. Data about the severity of PBFD amongst Norfolk parakeets could reaffirm the position of the Norfolk parakeet on the priority species list or provide evidence that this status is unwarranted. One method to research the way that PBFD is expressed by Norfolk parakeets could be to monitor BFDV-positive and BFDV-negative parakeets over the course of multiple breeding seasons, recording what, if any, symptoms of chronic PBFD are developed as well as body condition indices and reproductive fitness. Depending on the timeframe available for such a study, lifespan could also be a recorded variable. I also recommend the molecular characterisation of the BFDV isolates present on Norfolk Island in both the Norfolk parakeet and Crimson rosellas. This would provide information about the flow of the virus between the Crimson rosella and Norfolk parakeet populations on Norfolk Island and relatedness

comparisons with BFDV isolates present on mainland Australia and elsewhere on the globe could provide insight into how long the Norfolk parakeets have been coexisting with the virus.

Translocation to Philip Island and Monitoring for Pathogens of Concern

Having just one population of Norfolk parakeets (*Cyanoramphus cookii*) puts the species at undue risk from localised threats. Should the predator control in Norfolk Island National Park lapse and cat (*Felis catus*) and rat (*Rattus spp.*) numbers within the park increase, the Norfolk parakeets on the island could be decimated resulting in the extinction of the species with no unaffected populations elsewhere. An outbreak of a potentially lethal pathogen such as avian malaria (*Plasmodium spp.*) could have a similar effect. Furthermore, given that BFDV is known to infect lymphoid tissues and cause immunosuppression, it is possible that BFDV being present amongst the Norfolk parakeet population could exacerbate the vulnerability of the species to such a threat (Todd, 2000). To reduce these risks a second population of Norfolk parakeets should be established on the nearby Philip Island and the Norfolk parakeets, Crimson rosellas (*Platycercus elegans*) and other avifauna on Norfolk and Philip Islands should be regularly monitored for disease.

Philip Island is 190 hectares in size and lies roughly 6 kilometres south of Norfolk Island (Director of National Parks, 2018; Priddel et al., 2010). Despite not being connected by land, the island became a part of Norfolk Island National Park in 1986 (Director of National Parks, 2018). Prior to becoming a component of the national park, Philip Island had lost much of its vegetation to introduced grazing animals such as pigs (*Sus scrofa*), goats (*Capra hircus*) and rabbits (*Oryctolagus cuniculus*) that were released to provide the British settlement on Norfolk Island with game (Coyne, 2010; Priddel et al., 2010). Both the pigs and goats on Philip Island eventually died out or were eradicated through hunting while the rabbits were exterminated through intensive pest control efforts involving the release of the lethal *Myxoma*

virus and use of 1080 (a sodium fluoroacetate mammal toxin) (Coyne, 2010). With the elimination of these invasive mammals the vegetation on Philip Island has been recovering naturally (Coyne, 2010). The idea of establishing a population of Norfolk parakeets on Philip Island is not novel and an unsuccessful attempt was made in 2017 (Simmonds, 2019). During this attempt eight pre-fledged juvenile parakeets were transferred to Philip Island and released into an aviary however the health of the birds declined shortly after arrival and the translocation was aborted (Simmonds, 2019). Simmonds (2019), who was going to monitor the dispersal of these parakeets notes that the cause of this decline in health is undetermined and that research into pathogens and potential diseases on Philip Island should be undertaken before another translocation is attempted. This ties in well with the idea of regularly monitoring the avifauna on Norfolk and Philip Islands for disease. Perhaps an approach utilising regular 'low effort' sampling and biennial 'high effort' sampling, as suggested above for detecting pathogens of concern on the kākāpō (*Strigops habroptilus*) islands, could be implemented on Norfolk Island. This method could be appropriate for Norfolk Island where park rangers already have duties in the field and where there is a community that could potentially assist with the low effort sampling. For the uninhabited Philip Island, perhaps a bout of high effort sampling could occur before the translocation of Norfolk parakeets to the island and on annual basis after this as low effort sampling might be less frequent and comprehensive due to the comparative inaccessibility of the site.

Chapter Four: Future Directions

ABSTRACT

This chapter discusses some avenues of future research inspired by the findings of this research project and the challenges it faced. Testing nests rather than live birds for BFDV could enable more productive fieldwork by reducing the unpredictable and chance elements of working with wildlife and could serve as a form of 'low effort' sampling under the biosecurity regime recommended in chapter three of this thesis. The COVID-19 pandemic and ensuing national and regional lockdowns in New Zealand prevented this project from screening kākāriki (*Cyanoramphus spp.*) sympatric with kākāpō (*Strigops habroptilus*) for *Plasmodium* (*spp.*) and *Haemoproteus* (*spp.*) leaving this as a valuable area of future research. Screening naturalised parrots in New Zealand and those kept in captivity for pathogens of concern could inform the conservation of the kākāpō and other endemic parrots throughout their ranges by enabling preventative rather than reactive disease control and improving understanding of the movement and range of avian pathogens between populations and geographical areas.

INTRODUCTION

The conservation recommendations presented in this thesis for both the kākāpō (*Strigops habroptilus*) and Norfolk parakeet (*Cyanoramphus cookii*) involve regimens of regularly screening for pathogens of concern through a combination of frequent 'low effort' sampling and less frequent 'high effort' sampling. For this discussion, low effort sampling refers to methods that are not resource intensive and that can occur regularly during the course of daily duties of someone like a park ranger or other conservation employee. Increasing the variety of low effort sampling methods should decrease the interference of such work with the regular duties of conservation employees, allowing for the most appropriate low effort sampling method to be chosen to compliment the other activities of a given day. This could also increase

the effectiveness of low effort sampling with the increased flexibility of sampling affording more sampling events and an increased variety of sample types presenting a wider array of vehicles for the detection of pathogens of concern. For these reasons future research should look to develop new methods of low effort sampling.

BFDV is not the only pathogen of conservation concern for parrots in New Zealand. Screening kākāriki (*Cyanoramphus spp.*) sympatric with kākāpō for the eukaryote pathogens *Plasmodium* (*spp.*) and *Haemoproteus* (*spp.*) was originally planned to be a component of this thesis research project but this was curtailed by the COVID-19 pandemic and ensuing lockdowns in Auckland, New Zealand. As *Plasmodium* (*spp.*) infections have been linked to the deaths of a variety of species of New Zealand native avifauna and fatal *Haemoproteus minutus* infections have been identified in captive Red-crowned parakeets (*Cyanoramphus novaezelandiae*) in Germany, this remains a valuable avenue of future research (Howe et al., 2012; Ortiz-Catedral et al., 2019; Schoener et al., 2014). As part of identifying pathogens of concern before the infection of a kākāpō or other at-risk species, future research should also assess the diversity and prevalence of pathogens present amongst populations of parrots that have become naturalised in New Zealand and those held in captivity. Given the genetic similarity of the BFDV isolates detected in Red-crowned parakeets and Eastern rosellas (*Platycercus eximius*) from Little Barrier Island and the Auckland region respectively, such research could also yield data towards the origin of BFDV in New Zealand (Massaro et al., 2012).

TESTING NESTS FOR BFDV

Biosecurity is an important component of the management and recovery plans for many parrot species. Due to a high dependency on forest habitats for foodstuffs and nesting, parrots are particularly vulnerable to the impacts of logging and deforestation (Olah et al., 2016; Toft &

Wright, 2015c; Vergara-Tabares et al., 2020). Population decline associated with deforestation has been demonstrated overseas by the Spix's macaw (*Cyanopsitta spixii*) and in New Zealand parrots including the kākāpō (*Strigops habroptilus*) and Red-crowned parakeet (*Cyanoramphus* novaezelandiae) which have both been relegated to small fractions of their geographic distributions pre-human settlement (Department of Conservation, n.d.-g; Juniper & Yamashita, 1990; Lentini et al., 2018). Due to their vivid colours, intelligence, vocal ability and rarity, parrots are also vulnerable to poaching for the pet trade and for collectors. This was poignantly demonstrated by the poaching of the sole remaining wild female Spix's macaw in 1987 (Juniper, 2004). Invasive mammalian predators and resource competition with invasive species are also threats to parrots (Clout & Merton, 1998; Elliott et al., 2001; Grarock et al., 2013; Moorhouse et al., 2003; Olah et al., 2016). For a variety of reasons, the particular biosecurity considerations for the kākāpō and Norfolk parakeet (*Cyanoramphus cookii*) differ. Kākāpō live exclusively on offshore island nature reserves that are free of invasive mammalian predators while the Norfolk parakeet lives only in a national park that borders a permanent human settlement and where invasive mammals are controlled but not entirely removed. Consequently, kākāpō biosecurity seeks to prevent the invasion of mammalian predators while biosecurity for the Norfolk parakeet must continually control invasive mammals that can invade from elsewhere on Norfolk Island. Biosecurity considerations with respect to BFDV also differ between the two species. Birds can be subclinical or asymptomatic carriers of BFDV (Martens et al., 2020a). BFDV is host flexible and can be transmitted by and between a wide variety of host species (Sarker et al., 2015a). BFDV infections can be lethal or can contribute to death through secondary infection (Doneley, 2003; Pass & Perry, 1984; Todd, 2000). Together these factors stress the importance of BFDV vigilance for the conservation of species such as the kākāpō, whose vulnerability to BFDV is suspected but yet to be fully determined. With the data presented in this study reconfirming the presence of BFDV amongst the Norfolk

parakeet, BFDV-biosecurity for the species should focus on quantifying and mitigating the risk posed by the virus.

My research contributed to biosecurity by sampling for BFDV through the capture and feather testing of wild parakeets, this method is widely used but is not without limitations. Successful mist netting depends heavily upon favourable weather and the chance likelihood of birds flying into nets. Capturing birds on and in natural nests and artificial nest boxes too depends upon the chance of birds being present at the time of sampling. These dependencies introduce opportunities for sampling to be disrupted, with an example from my fieldwork being when a heavy downpour on Wednesday April 21st would have made mist netting dangerous for birds, resulting in the postponement of sampling until the nets had dried. Both methods also run the risk of stressing the birds being sampled or of causing injury should a bird react unexpectedly or be poorly handled. BFDV is robust to environmental conditions and can be shed into an environment by infected birds through crop secretions, faeces and feather dander (Jackson et al., 2015; Martens et al., 2020a; Raidal & Cross, 1994; Sarker et al., 2015b; Todd, 2000). Together these characteristics enable an alternative method of sampling for BFDV, detecting the virus in swabs taken from nest sites. Testing nest sites rather than live birds is a relatively novel approach and has the potential to be a more reliable method of sampling for BFDV as it reduces the unpredictable and chance elements of working with wildlife. Testing nest sites could also be a more accessible method of BFDV sampling, involving less equipment, training and effort which could allow for the practise to become part of a regiment of continuous 'low effort' sampling as discussed above in 'Conservation Recommendations for the Kākāpō: Monitoring for Pathogens of Concern'.

A recent study by Martens et al. (2020b) investigated the efficacy of sampling nests for BFDV by testing artificial nest boxes and both nestling and adult Crimson and Eastern rosellas (*Platycercus elegans*, *Platycercus eximius*) for BFDV. Sampling took place in Victoria,

Australia before, during and after two breeding seasons from 2016 to 2018 and the researchers detected BFDV-positive birds in 11 of 82 nest boxes through cloacal swabs of adult birds and through blood tests of adult birds and nestlings. In one of these nest boxes both parent birds tested positive but in the other ten nests only one resident bird tested BFDV positive. Along with controls, these 11 nest boxes were also sampled for BFDV with a cotton-tipped swab being brushed along each of the internal walls and inserted into the nesting material. The swabs were tested for BFDV DNA through a modified ammonium acetate protocol. Of the 11 nest boxes known to contain at least one BFDV-positive parakeet, no nest boxes tested positive prior to the breeding season, four nest boxes tested positive during the breeding season and one of these four also tested positive after the breeding season. The researchers further analysed their results by categorising the BFDV-positive nest boxes by the method through which the resident parakeets had tested positive for BFDV. This revealed that while only 36.6% of the nest boxes containing BFDV-positive resident birds also tested positive, this was 66.7% of the boxes where resident adult birds had tested positive through blood samples, 66.7% of the boxes where resident adult birds had tested positive through cloacal swabs and 0% of the boxes where a nestling had tested positive for BFDV (Table 7.). Martens et al. (2020b) also tested the adult birds that tested BFDV-positive through blood tests, all of which were female, for antigen excretion through haemagglutination assays of chest feathers. This revealed that four of the five (80%) nest boxes containing resident birds known to be excreting antigens tested BFDV-positive. It is of note that the researchers also found that one of the ten unoccupied control nest boxes tested positive for BFDV which resulted in the likelihood of a nest box with a BFDV-positive resident bird testing positive for BFDV not being significantly different from the likelihood of an unoccupied nest box testing positive for the virus ($P > 0.1$). Martens et al. (2020b) posit that this occurrence may have been the product of a BFDV-positive bird visiting, but not settling in, the unoccupied nest box.

Table 7. Percentage of BFDV-positive nest boxes by method of detecting BFDV in resident birds. Data sourced from (Martens et al., 2020b).

Method of Resident BFDV Detection	BFDV-Positive Nest Boxes	BFDV-Negative Nest Boxes	Percentage of BFDV-Positive Nest Boxes
BFDV-positive blood samples	4	2	66.67%
BFDV-positive cloacal swabs	2	1	66.67%
BFDV-positive nestlings	0	5	0%
BFDV-positive blood samples, cloacal swabs or nestlings	4	7	36.36%

Given the small sample size of the study by Martens et al. (2020b) and the lack of a statistically significant difference between the likelihood of detecting BFDV in unoccupied nest boxes and those home to BFDV-positive birds, further research is warranted before testing of nest and nest boxes for BFDV is widely deployed. The appeal of a BFDV sampling method that is less stressful for birds and more accessible to researchers is strong. Perhaps the study by Martens et al. (2020b) can be repeated on a larger scale with the addition of nest box cameras to monitor control and non-control nest boxes alike. The addition of cameras would help verify the unoccupied status of unoccupied control nests and could also provide insight into the transmission of BFDV during nesting and competitive interactions. Further research should also investigate the effectiveness of screening nests for BFDV in natural nests and the likelihood of birds inhabiting the same nest sharing a BFDV positivity status. If a strong relationship between the BFDV status of birds sharing nests is determined it could enable a

method of approximating the abundance of BFDV in a population that requires the sampling of less individual birds. The population of Norfolk parakeets stands out as a potential subject for such research. BFDV is present amongst the Norfolk parakeet population and as such BFDV-biosecurity for the species should seek to monitor the impacts of the virus. Investigating the efficacy of sampling Norfolk parakeet nests for BFDV could compliment such efforts and also inform the reliability of the method in New Zealand given the relatedness of the Norfolk parakeet and New Zealand's kākāriki (*Cyanoramphus spp.*).

PLASMODIUM, HAEMOPROTEUS AND BITING INSECTS AS VECTORS OF DISEASE TRANSMISSION

This research project was originally planned to incorporate screening Orange-fronted, Red-crowned and Yellow-crowned parakeets (*Cyanoramphus malherbi, C. novaezelandiae, C. auriceps*) sympatric with kākāpō (*Strigops habroptilus*) for *Plasmodium* (*spp.*) and *Haemoproteus* (*spp.*) parasites as well as for BFDV. The global COVID-19 pandemic and ensuing national and regional lockdowns in New Zealand prevented this fieldwork from occurring, leaving this research as a valuable avenue of future study. Avian malaria and pseudo-malaria, caused by *Plasmodium* (*spp.*) and *Haemoproteus* (*spp.*) infections respectively, have both caused the death of New Zealand native birds including the Great spotted kiwi (*Apteryx haastii*) and Red-crowned parakeet (Howe et al., 2012; Ortiz-Catedral et al., 2019; Sá, 2011). *Plasmodium* (*spp.*) and *Haemoproteus* (*spp.*) are closely related eukaryotic parasites that are spread through bites from arthropods such as mosquitoes (*Culicoides spp.*) (Maharana & Kumar, 2017; Ortiz-Catedral et al., 2019; van Riper et al., 1986). With *Plasmodium* species having been detected in wild birds in New Zealand and with two bird species that are common *Haemoproteus* (*spp.*) hosts in Europe, the Common blackbird (*Turdus merula*) and Song thrush (*Turdus philomelos*), being naturalised in New Zealand there is a risk

that these parasites could cause further declines of critically endangered and genetically depauperate native bird species such as the kākāpō (Ortiz-Catedral et al., 2019; Schoener et al., 2014). Future research should screen kākāpō, kākāriki (*Cyanoramphus spp.*) and other native and naturalised bird species that are sympatric with kākāpō for these parasites to inform the species management of the kākāpō and our understanding of New Zealand's pathogen diversity.

The availability of transmission vectors is critical to the spread of vector-borne disease. *Plasmodium* (*spp.*) and *Haemoproteus* (*spp.*) are examples of pathogens that rely on hematophagous arthropods to infect avian hosts (Schoener et al., 2014). This dependency means that a comprehensive understanding of the hematophagous arthropods in New Zealand and their potential to act as disease vectors could be a valuable tool for managing disease risk amongst endangered species. New Zealand features a limited diversity of mosquitoes, with only 13 endemic species and three introduced species as of 2020 (Cane et al., 2020). A variety of these species are known to prey upon birds (Holder et al., 1999). A report on the animal disease significance of New Zealand's mosquitoes was published by the New Zealand Ministry for Primary Industries in 1999. This report notes that the status of nine of these 16 species as disease vectors was unknown and did not include a currently unnamed species that was discovered on the Chatham Islands in 2007 (Cane & Courtney, 2009; Cane et al., 2020; Holder et al., 1999). With so few mosquito species, future research could seek to determine the vector status of New Zealand's entire mosquito diversity as well as to characterise their feeding ecology to identify any prey species they might put at risk. If such a study were to incorporate sampling of wild mosquito populations, it could also prove valuable for providing further insight into New Zealand's pathogen diversity.

The geographic distribution of hematophagous arthropods is also a valuable topic of research as these vectors can only spread pathogens amongst species they can physically

access. The same 1999 Ministry for Primary Industries report also noted the distribution of mosquito species in New Zealand according to the biogeographical area code system devised by Landcare Research (Crosby et al., 1998; Holder et al., 1999). This system divides New Zealand into 37 regions with examples being 'AK' Auckland, 'TK' Taranaki and 'SI' Stewart Island (Crosby et al., 1998). This system is useful for understanding the broader distribution of species but, with bird refuge islands such as Rangitoto being categorised with Auckland and Little Barrier Island with Coromandel, a finer scale of data could prove beneficial to the management of species by conservationists should a mosquito-borne pathogen threaten bird species in New Zealand. Multiple studies have also investigated the distribution of mosquitoes within habitats and the way in which this is impacted by altitude and water availability. One such study by Derraik et al. (2005) sought to assess the vertical distribution of mosquitoes in native bush in Auckland, New Zealand by catching mosquitoes in traps at ground level and at a height of 10 metres in the canopy. The researchers caught five native species, *Coquillettidia iracunda, Culex asteliae, Culex pervigilans, Culiseta tonnoiri* and *Ochlerotatus antipodeus* as well as the exotic *Ochlerotatus notoscriptus* over five non-consecutive days in April of 2003. An interesting dynamic was observed where significantly ($P < 0.05$) more mosquitoes were observed at ground level than at the 10m canopy height but this was primarily the product of a high abundance of *O. antipodeus* specimens which comprised 78% of all the mosquitoes caught. All of the other native species were more frequently observed in the canopy than at ground level. Data of this nature adds yet another consideration to the potential for mosquitoes and other hematophagous arthropods to act as disease vectors and warrants further research.

PATHOGENS IN NATURALISED PARROTS AND AVICULTURE

Five introduced parrot species have become naturalised in New Zealand: the Crimson rosella (*Platycercus elegans*), Eastern rosella (*Platycercus eximius*), Rainbow lorikeet (*Trichoglossus*

haematodus), Galah (*Eolophus roseicapillus*) and Sulphur-crested cockatoo (*Cacatua galerita*) (Miskelly et al., 2008). These are joined by at least 20 other species of non-native parrots that are held and traded in aviculture (Parrot Society of New Zealand, n.d.-a). Both naturalised and captive parrots have the potential to act as disease reservoirs should they come into contact with native species and carry a pathogen that is sufficiently host flexible. As BFDV is the primary focus of my research project and is known to be transmissible between parrot species, this discussion will largely focus on BFDV but is also relevant to other avian pathogens such as *Plasmodium* (*spp.*) (Sarker et al., 2015a).

As of 2021 few studies have screened populations of wild naturalised parrots in New Zealand for BFDV. Ha et al. (2007) sampled Eastern rosellas from Te Puke, Wellington and Dunedin as well as Sulphur-crested cockatoos from Turakina Valley near Whanganui and Massaro et al. (2012) sampled Eastern rosellas from the Auckland region. These studies detected BFDV in 28% of the cockatoo samples and in 21.2%, 11.9% and 34.6% of the Auckland, Te Puke and Wellington Eastern rosella samples respectively (Table 8.). These results confirmed that BFDV is present in populations of at least two species of naturalised parrots in New Zealand, ranging as far north as Auckland and as far south as Wellington. With the most recent of these findings dating back to 2012, more up-to-date research is required to reaffirm these results. Ideally future research should also seek to sample all of New Zealand's populations of naturalised parrots for BFDV to identify the abundance and geographical range of the virus amongst these populations. Multiple studies, including Massaro et al. (2012), have performed full-genome sequence analysis of BFDV isolates to assess the relatedness of BFDV strains. Massaro et al. (2012) compared BFDV isolates from Eastern rosellas in Auckland with those from Red-fronted parakeets (*Cyanoramphus novaezelandiae*) on Little Barrier/Te Hauturu-o-Toi and found that the two form a monophyletic clade. Future efforts to screen

naturalised parrots for BFDV should build on this work so as to provide insight into the origin of BFDV in New Zealand and into patterns of BFDV spread between populations and species.

Table 8. Results of sampling naturalised parrots in New Zealand for BFDV as of 2021. Data sourced from Ha et al. (2007) and Massaro et al. (2012).

Species	Sample Size	Number of BFDV-Positive Birds	Percentage of BFDV-Positive Birds	Location of Sample Population	Mainland Island
Eastern rosellas	126	15	11.9%	Te Puke	North Island
Eastern rosellas	26	9	34.6%	Hutt Valley, Wellington	North Island
Eastern rosellas	10	0	0%	Dunedin	South Island
Eastern rosellas	33	7	21.2%	Auckland Region	North Island
Sulphur-crested cockatoo	255	70	28%	Turakina Valley	North Island

There is also a dearth of information on the abundance of BFDV in parrots held in captivity in New Zealand. Perhaps the most comprehensive study was conducted by Ritchie et al. (2003) who screened feather, blood and dried blood samples from captive parrots for BFDV. These samples were provided by aviculturists and veterinarians who suspected the sampled birds might be suffering from PBFD. This research, which dates back nearly two decades, detected BFDV in 21 of 25 specimens including Budgerigars (*Melopsittacus undulatus*), Blue-streak lorikeets (*Eos reticulata*), a Goldie's lorikeet (*Psitteuteles goldiei*), Rainbow lorikeets, a Red collared lorikeet (*Trichoglossus haematodus rubritorquis*), Yellow-bib lorikeets (*Lorius chlorocercus*), Longbill corellas (*Cacatua tenuirostris*) and Sulphur-crested cockatoos. Given

the age of this data it is clear that a contemporary study is required to modernise our understanding of BFDV in New Zealand's captive parrots. Future research should also work with aviculturists to incorporate the geographical location of captive birds that are screened for BFDV so as to yield data useful to conservation managers. A recent legal challenge by the Parrot Society of New Zealand against Auckland Council classifying Galahs, Sulphur-crested cockatoos, eastern rosellas, monk parakeets (*Myiopsitta monachus*) and rainbow lorikeets as pest species under the 2019-2029 Auckland Regional Pest Management Plan suggests that hobbyists might not always prioritise conservation issues (Block, 2021; Parrot Society of New Zealand, n.d.-b). This coupled with the potential economic impact of becoming known as a parrot breeder whose parrots have BFDV might make some aviculturists reluctant to contribute to the widespread screening of captive parrots in New Zealand. Despite these factors, the rules of the New Zealand Parrot Society do state the societal goal of 'liaising constructively with government and non-government bodies on all aspects affecting the keeping and breeding of parrots' (Parrot Society of New Zealand, 2019). On these grounds perhaps a future research project should seek to collaborate with aviculturists to devise a sampling programme that incentivises the screening of captive birds for BFDV and other pathogens. One possible approach could be to produce a map that displays regions known to be hotspots for BFDV in captive birds without specifying exact locations or the details of breeders. Such a product could still inform conservation decisions by denoting some regions as higher or lower-risk possible translocation sites for threatened species such as the kākāpō (*Strigops habroptilus*) and Orange-fronted parakeet (*Cyanoramphus malherbi*).

Figure 14. An Alexandrine parakeet (*Psittacula eupatria*) in captivity in Taranaki, New Zealand. Photo: Connor Wallace.

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Appendix

APPENDIX I

A PCR-Based Retrospective Study for Beak and Feather Disease Virus (BFDV) in Five Wild Populations of Parrots from Australia, Argentina and New Zealand

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A PCR-Based Retrospective Study for Beak and Feather Disease Virus (BFDV) in Five Wild Populations of Parrots from Australia, Argentina and New Zealand

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Abstract: The beak and feather disease virus (family *Circovirdae*) is a virus of concern in the conservation of wild Psittaciformes globally. We conducted a PCR screening for the beak and feather disease virus (BFDV) using samples collected during previous field studies (1993–2014) in five populations of parrots of the Southern Hemisphere: Eclectus parrots (*Eclectus roratus*) and Crimson rosellas (*Platycercus elegans*) from Australia, Burrowing parrots (*Cyanoliseus patagonus*) and Monk parakeets from Argentina (*Myiopsitta monachus*), and Forbes' parakeet from New Zealand (*Cyanoramphus forbesi*). A total of 612 samples were screened. BFDV was not detected in any of the sampled birds. Our results provide a retrospective screening, covering three different tribes of Old and New World parrots, including two of the most numerous species, and contributing a large set of negative results. Furthermore, our results suggest that geographical and temporal differences in BFDV distribution may exist and merit further research, as a critical component in the efforts to manage the disease and its epidemiological aspects. The results presented here hold the potential to provide a baseline for future studies investigating the temporal evolution and the spread of BFDV.

Keywords: BFDV; *Circoviridae*; infectious disease; Psittaciformes; surveillance; viral infection; vulnerable taxa; wild populations

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Citation: Ortiz-Catedral, L.; Wallace, C.J.; Heinsohn, R.; Krebs, E.A.; Langmore, N.E.; Vukelic, D.; Bucher, E.H.; Varsani, A.; Masello, J.F. A PCR-Based Retrospective Study for Beak and Feather Disease Virus (BFDV) in Five Wild Populations of Parrots from Australia, Argentina and New Zealand. *Diversity* **2022**, *14*, 148. https://doi.org/10.3390/d14020148

Academic Editors: Guillermo Blanco, José L. Tella and Martina Carrete

Received: 21 December 2021
Accepted: 13 February 2022
Published: 18 February 2022

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1. Introduction

Existing and emerging pathogens can drive rapid changes in population numbers and in the genetic diversity of the wild host population [1]. Pathogens have caused declines in previously large populations or even increased the rate of decline in endangered species [2–4]. Moreover, global pet trade and climate changes hold great potential to extend

current pathogen distributions and need to be considered as potential risk factors for the introduction of disease to wildlife [5–7]. For this reason, infectious disease has become a major challenge for conservation; thus, knowledge of the extent of infectious diseases in wildlife populations has become increasingly important for conservation work [8,9].

Parrots and cockatoos (Psittaciformes) have long been recognized as one of the most threatened orders of birds globally, with nearly a third of all known species classified as 'at risk of extinction', and a larger number facing population decline [10,11]. There are multiple factors associated with declining parrot populations, however, capture of wild parrots for the pet trade, intensified agriculture, hunting, and logging are the most frequent threats [10,11], with depredation by introduced species being a serious threat on islands [12]. Moreover, susceptibility to diseases substantially threatens some parrots e.g., Philippine cockatoo *Cacatua haematuropygia*, Cape parrots (*Poicepahlus robustus*), blue-headed racquettail *Prioniturus platenae*, orange-bellied parrot *Neophema chrysogaster* [13–15].

The potentially negative effects of diseases for the survival of endangered parrots have been widely acknowledged [11,16,17] and have triggered abundant research. Studies on diseases, health and pathogens of captive parrots are published regularly [15,18,19]. Nevertheless, there is limited information on pathogenic infection in free-living Psittaciformes [20–29]. This paucity of studies on pathogens and diseases among free-living parrots makes it clear that we only partially understand their role as a threatening factor.

The beak and feather disease virus (BFDV) is a small circular single stranded DNA virus in the family *Circoviridae* [30,31], often cited as a pathogen of conservation concern for parrots in the wild, as well as in captivity [6,8,29,32], given its immune-suppressive effect in infected birds [33,34]. Abnormal plumage and morphological development, anaemia, damage of the lymphoid tissue, feather loss and weight loss among infected birds are common symptoms associated with this viral infection [35].

BFDV infects predominantly Psittaciformes [35], and is reported to cause high mortalities in avicultural collections [36] and in at least two free-living populations [37–39]. Recent evidence indicates, however, that BFDV can also infect non-parrot species [40]. In general, the virus has been reported as infecting over 10% of known parrot species, a figure that comes mostly from studies on captive birds [8,18,41,42]. Despite a wealth of information on captive birds (e.g., [18,41,43,44]), the prevalence of the virus in wild populations remains largely unknown for most regions except Australia, Mauritius, New Caledonia and New Zealand [8,26–28,42,45–49].

The advances in molecular techniques to detect the virus (e.g., [28,46,50] open up an opportunity to conduct large scale surveys for BFDV among wild populations of Psittaciformes, and especially to screen large collections of blood samples from long term studies on parrots. Here, we present a retrospective study investigating the presence of BFDV among five wild populations of Psittaciformes belonging to three different tribes: (a) Psittaculini, the Eclectus parrot (*Eclectus roratus*) from tropical Australia, (b) Platycercini, the Crimson rosella (*Platycercus elegans*) from temperate Australia, and the Forbes' parakeet (*Cyanoramphus forbesi*) from the Chatham Islands, New Zealand, and (c) Arini, the Burrowing parrot (*Cyanoliseus patagonus*) from the Patagonian steppes and Monk parakeet (*Myiopsitta monachus*) from Central Argentina.

2. Methods

We used 612 blood samples collected during previous studies (Table 1), to investigate the presence of BFDV. Details on the sample and populations sizes for each species are given in Table 1. Every individual was sampled once.

Table 1. Details on blood samples from five wild populations of Psittaciformes in this study.

Species	Estimation of Population Size	Reference for Population Size	Year of Sample Collection	Blood Samples (n)		Total
				Adult	Nestling	
		Psittaculini				
Eclectus roratus	3000	[51]	1997–2007	24	291	315
		Platycercini				
Platycercuselegans	550	[52]	1993–1995	17	52	69
Cyanoramphus forbesi	1000	[53]	2014	95	–	95
		Arini				
Cyanoliseus patagonus	75,000	[54]	December 1998, December 1999	49t	55	104
Myopsitta monachus	500	[55] and E.H.B. unpubl. data	December 2000	29	–	29

Samples from Eclectus parrots were taken over the course of a long-term study (1997–2007) on Cape York Peninsula in northern Queensland Australia (12°45′ S, 143°17′ E) [56,57]. Most samples were taken from nestlings in nest hollows 15–25 m above the ground in rainforest trees. Adults were also captured using mist nets set at similar heights in the rainforest canopy. Approximately 100 µL of blood was taken from the brachial vein of each captured individual. Eclectus parrot blood was stored in 70% ethanol [57,58].

Samples from Crimson rosellas were collected from adult and nestling birds breeding in Black Mountain Nature Reserve, Australian Capital Territory (35°16′28″ S, 149°05′55″ E) [52]. Birds were captured in nest-boxes between 1993 and 1996; a small blood sample (50 to 100 µL) was taken from the brachial vein of each individual, and preserved in Queen's Buffer (10 mM Tris, 10 mM NaCl, 10 mM disodium EDTA, 1% n-lauroylsarcosine, pH 8.0) [59]. Blood samples were taken from adults on capture and from nestlings between 25 and 30 days of age.

Forbes' parakeets were captured using mist-nets on Mangere Island, Chatham Islands (44°26′ S, 176°29′ W), in March 2014. Blood samples (200 µL) were taken by puncture of the brachial vein immediately after capture and preserved in Queen's Buffer [59]. Only adults were sampled.

Burrowing parrots were captured at its major colony in El Cóndor, north-eastern Patagonia, Argentina (41°04′ S, 62°50′ W) during regular nest inspections in December 1998 and December 1999 [54]. Adults were sampled when found in the nest; nestlings were sampled between the age of 38 and 60 days. Monk parakeet samples were obtained in an area of 600 ha, situated near Jesús María, Córdoba, Argentina (31°05′ S, 64°11′ W) [55]. Monk parakeets were captured in their nests during December 2000. Blood samples (200 µL) of the adult and nestling burrowing parrots, as well as of adult monk parakeets, were taken by puncture of the brachial vein immediately after capture. The blood was stored in 70% ethanol [58].

In 2014, DNA was extracted from 10 µL of blood, which was added to 10 µL of 'lysis solution' from the Extract-n-AmpTM Blood PCR Kit (Sigma-Aldrich, St Louis, MO, USA) and incubated for 10 min at room temperature. Ninety microliters of this kit's 'neutralization solution' was subsequently added to yield crude total DNA. One microliter of the crude extract was used as template in the subsequent PCR [46]. Extracted DNA was stored at −20 °C. In addition, in 2014, as described in previously published studies [18,46,47,60], BFDV specific PCR screening was carried out using KAPA Blood PCR Kit Mix B (KAPA Biosystems, Wilmington, DE, USA) using the primer pair forward 5′-TTAACAACCCTACAGACGGCGA-3′ and reverse 5′-GGCGGAGCATCTCGCAATAAG-3′, which target a 605 bp region of the *rep* gene of BFDV [61]. The reaction volume was 25 µL with 1 µL of 10 µM F/R primer pair, 12.5 µL of the 2xKAPA Blood PCR Kit Mix, 1 µL of DNA templates and 10.5 µL of sterile molecular grade water. The PCR program contained an initial step of 94 °C for 5 min, which was followed by 25 cycles of 94 °C for 30 s, 56 °C

for 30 s and 72 °C for 45 s and with a final 1 min extension step at 72 °C and cooling to 4 °C for 10 min. DNA from a BFDV-infected red-fronted parakeet (*Cyanoramphus novaezelandiae*) from Little Barrier Island was used as a positive control [62]. The total DNA used as positive control was extracted from 60 µL of blood using the Qiagen QIAamp DNA minikit (Qiagen, Hilden, Germany) according to the manufacturer's protocols.

3. Results

We did not detect BFDV in any of the blood samples investigated by PCR.

4. Discussion

Surveillance for pathogens is a fundamental element for understanding the temporal and spatial prevalence of wildlife diseases and for understanding transmission pathways and effects on animal populations [63]. We applied a commonly used PCR screen [18,46,47,60] to detect viral DNA in blood samples collected during previous field studies of Eclectus parrots, Crimson rosellas, Forbes' parakeets, Burrowing parrots and Monk parakeets. Our negative results suggest that BFDV was not present in the studied populations at the time of sampling, and show some differences with previous studies, which could be related to temporal, geographical and captive versus wild population differences in BFDV prevalence and distribution. BFDV has previously been reported from captive Eclectus parrots [45,64,65]; however, the wild population here investigated is isolated from large human populations and parrots kept in captivity. Free-ranging Crimson rosellas on Norfolk Island and in Victoria, Australia, have been reported with BFDV [26–28,66], yet the samples in the current study originate from a population within and surrounding the city of Canberra, where a previous BFDV study found a very low number of potentially infected individuals [67]. BFDV has been reported on close relatives of Forbes' parakeets, including red-fronted parakeets and yellow-crowned parakeets (*Cyanoramphus auriceps*) [46], but has not been detected in other *Cyanoramphus* species in the wild. For Monk parakeets, the virus has been found in 37% of sampled individuals belonging to a feral population in Spain [68]. This high prevalence could be related to the origin of the birds, which accidentally escaped from captivity, where BFDV has been reported frequently [8,18,36]. To our knowledge, BFDV infection in Burrowing parrots is unknown for either captive or free-living individuals.

There are an increasing number of field studies with Psittaciformes worldwide; commonly, blood samples are collected. Those samples could be used to increase the range of species screened in the wild, allowing for a better understanding of the geographical distribution of BFDV. Moreover, Fogell et al. [8] pointed out that two biases currently exist in BFDV research, namely, the lack of (1) research in regions of the world such as South America and Southeast and Southern Asia, both characterised by a high parrot diversity, and (2) publications reporting negative results. Recent studies are starting to fill those gaps. Vaz et al. [29] using pathogen-specific PCR, evaluated the presence of BFDV. As in our study, Vaz et al. [29] detected no BFDV DNA in a large sample of 205 wild nestlings and 90 nestlings from the illegal trade. Moreover, we are confident that our study also makes a substantial contribution to BFDV research by providing further screening results for South American parrots, including two of the most numerous species, and by contributing a large screening with negative results, obtained with a methodology thoroughly tested [18,46,47,60]. Furthermore, our results suggest that geographical differences in BFDV distribution may exist and merit further research, as a critical component in the efforts to manage the disease and its epidemiological aspects. Lastly, the results presented here hold the potential to provide a baseline for future studies investigating the temporal evolution and the spreading of BFDV. However, two final cautionary remarks are needed. First, we acknowledge that there is a possibility that the nucleic acid may be damaged in storage and transport; this may impact the amplification of the target virus sequences in some of the samples. Second, the widely applied PCR protocol [18,46,47,60] used in this study has some limitation. BFDV is known for a high genetic diversity [68–70]; it cannot be

fully excluded that the primers used in this investigation might have missed some genetic variants. Thus, future studies should evaluate the presence of the virus based on any previous identification BFDV sequences from these hosts in captivity or introduction on new regions. Nonetheless, the primer pair we have used in this study binds with 100% complementarity to a BFDV sequence (GenBank Accession # MT303064) derived from the blood sample of Monk parakeets in Spain [68].

Author Contributions: Conceptualization, L.O.-C., R.H., A.V. and J.F.M.; methodology, L.O.-C., C.J.W., R.H., A.V., D.V., E.H.B. and J.F.M.; validation, L.O.-C. and A.V.; formal analysis, L.O.-C., C.J.W., R.H., A.V., D.V., E.H.B. and J.F.M.; investigation, L.O.-C., C.J.W., R.H., A.V., D.V., E.H.B. and J.F.M.; resources, L.O.-C., R.H., A.V., E.A.K., N.E.L., E.H.B. and J.F.M.; writing—original draft preparation, L.O.-C., C.J.W., R.H., A.V., E.H.B. and J.F.M.; writing—review and editing, L.O.-C., R.H., A.V. and J.F.M.; supervision, A.V. and J.F.M.; funding acquisition, L.O.-C., A.V., E.H.B. and J.F.M. All authors have read and agreed to the published version of the manuscript.

Funding: Funding for data analysis and manuscript preparation was provided by the National Council of Science and Technology of Mexico (CONACYT), Claude McCarthy Fellowship, New Zealand Vice-Chancellor's Committee and Mohamed bin Zayed Conservation Trust (to L.O.-C.). The Eclectus parrot research was funded by the Australian Research Council, National Geographic Society and Winifred Violet Scott Foundation. The molecular analyses were supported by an Early career grant (University of Canterbury) awarded to A.V. J.F.M. research was supported by the City Council of Viedma (Río Negro, Argentina), World Parrot Trust, Liz Claiborne Art Ortenberg Foundation, and Wildlife Conservation Society. E.H.B. research was partially funded by the US Fish and Wildlife Service, International Affairs division, and a co-operation grant between the IB of the BMBF of Germany (ARG 99/020) and the Argentinean SECyT (AL/A99-EXIII/003).

Institutional Review Board Statement: The Eclectus parrot research was conducted under license from the Queensland Government and the ANU Animal Ethics Committee (Permit No: C.R.E.35.04). Crimson Rosellas sampling was conducted in accordance with ANU Animal Ethics guidelines (ANU Animal Ethics Permit J.BTZ.22.93), an ACT Parks Capture and Release Permit LT96023, and an ABBBS banding permit 1778. Forbes' parakeets sampling was approved by the Department of Conservation, 19621-FAU, New Zealand. Burrowing Parrot sampling was carried out under permission of the Dirección de Fauna de la Provincia de Río Negro, Argentina (143089-DF-98). Monk parakeet sampling permit was granted by the Consejo National de Investigaciones Cientificas y Técnicas of Argentina (CONICET) to E.H.B.

Data Availability Statement: All data are available in the main text.

Acknowledgments: We especially thank C. Blackman, P. Huybers, E. Huybers, S. Legge, Anja Quellmalz and Pablo Manzano Baena for assistance with field work, Erio Curto for allowing us to sample nests in his property in Marull, Córdoba, Luke Martin, Florence Gaude and Kevin Parker for conducting sample collection for Forbes' parakeets, Tansy Bliss and Dave Houston for assistance with permits.

Conflicts of Interest: The authors declare no conflict of interest.

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