Coccidia of the endangered South Island Takahē (*Porphyrio hochstetteri*): Investigations of pathobiology and management

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Megan Jayne Jolly

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This thesis is dedicated to my Mum and Dad, as always I owe you everything, and also to absent friends.

Abstract

The South Island Takahē (*Porphyrio hochstetteri*) is a large flightless rail, endemic to New Zealand currently listed as endangered with a population numbering approximately 340 individuals. The intensive management programme for this species has seen a modest increase in the population and includes strategies such as captive rearing for release at protected sites and numerous translocations of birds between these sites each year to maximise genetic diversity. This interconnectedness of geographically dispersed populations and critical points of high stocking density contribute to the potential for spread of infectious disease. Coccidian oocysts have been detected in Takahē faecal samples for decades and sporadically coccidia were implicated in the death of Takahē. By early 2015 concerns were being raised about markedly elevated individual faecal oocyst counts and the apparent failures of treatment with toltrazuril. The potential for significant negative impacts of coccidia in terms of both clinical disease and sub-clinical effects on juvenile growth rates and fertility led to the investigations reported in this thesis into the coccidia affecting Takahē.

A novel *Eimeria* sp. is described from a Takahē host. Based on morphological characteristics this coccidian species is distinct from other *Eimeria* spp. described in hosts of the family Rallidae. A survey of stored historical faecal samples and contemporary routine screening samples demonstrated the presence of this *Eimeria* sp. across most of the fragmented conservation management network for Takahē. Modification of treatment protocols and management actions was closely associated temporally with a sustained reduction in the *Eimeria* sp. shedding rates at a breeding facility central to the Takahē population network. Preliminary results were supportive of the existence of a diurnal shedding pattern for the *Eimeria* sp. with a peak of oocyst shedding in the late afternoon after 3pm in winter, which has implications for the collection of screening samples and the interpretation of results collected at different times of day. Concurrent to these investigations into the biology of the Takahē coccidia, trials were carried out to establish the safety of the anti-coccidial medication, decoquinate, in Takahē. No clinically significant deleterious effects were found in the parameters examined.

The findings presented are initial investigations into an *Eimeria* sp. from a Takahē host, the effectiveness of control measures implemented and safety of potential management options. The crisis of regular extreme oocyst counts in Takahē, which prompted this research, was resolved, however the potential for a recurrence is ever present in a species conservation programme that relies on intensive breeding and translocation of individuals. This research is the foundation for future research into the characterisation and management of *Eimeria* spp. in Takahē.

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Permitting and Animal Ethics requirements

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The coccidiostat safety trial described in Chapter 5 was also approved by the Massey University Animal Ethics committee (Protocol 15-72). The remaining work did not involve the manipulation of live animals and therefore did not require Massey University Animal Ethics approval.

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Literature Review

1. Literature Review

1.1. Takahē Coccidiasis/Coccidiosis

The current species management programme for Takahē requires numerous translocations of birds every year (Grange *et al.*, 2014). Each of these translocations provides the opportunity for disease transmission between populations and locations. Consequently, routine disease screening, including faecal parasitology, is a central part of the relocation process and monitoring of young birds within the various highly interconnected populations. Coccidian oocysts were first detected in Takahē faecal samples in the 1980s (B. Pomfrey, pers comm.) and have been regularly detected since. In 2013, an adult Takahē from the major population breeding site was submitted to the Wildbase, Massey University, Pathology Service for necropsy. The bird had an apparent history of significant weight loss and was in poor body condition at time of post-mortem, faecal floatation revealed in 22,500 oocysts per gram but unfortunately severe autolysis of the gastrointestinal tract prevented coccidiosis being confirmed as a cause of death (Massey University Pathology database). Increased parasitological screening was carried out across the Takahē recovery programme, revealing intermittent markedly elevated individual oocyst counts and a perceived general trend to increasingly high counts over recent years, particularly at sites of long-term Takahē habitation (B. Gartrell, pers comm.).The potential for significant negative impacts of coccidia on the endangered Takahē, both in terms of clinical disease and sub-clinical impacts on growth rates and fertility, has led to the following investigations into the coccidia affecting Takahē.

1.2. Takahē

1.2.1. Species description/evolution and distribution

The South Island Takahē (*Porphyrio hochstetteri*) is a large bodied, flightless rail (Order: Gruiformes, Family: Rallidae) endemic to the South Island of New Zealand. A related species, the North Island Takahē (*Porphyrio mantelli*) was once present on the North Island of New Zealand but is now extinct (Trewick, 1996).

The Subfossil record indicates, prior to human arrival, the South Island Takahē (from here on referred to as Takahē) were once present throughout the South Island ranging through a variety of habitats and were most abundant along forest margins and streams (Trewick & Worthy, 2001). The population underwent a dramatic decline in numbers after the arrival of Polynesian settlers 800-1000 years ago, likely due to the combined effects of habitat alteration, introduction of mammalian predators and hunting (Bunin & Jamieson, 1995). Analysis of genetic diversity (Grueber & Jamieson, 2011) parallels with documented Maori legends, confirming that by the time of European arrival from the mid-1800s, Takahē were rare and confined largely to Fiordland (Wickes *et al.*, 2009). European naturalists collected only 4 specimens (the last in 1898) before the Takahē was considered to be extinct by 1930 (Grueber & Jamieson, 2011). In 1948 an expedition, led by Dr Geoffrey Orbell, rediscovered a remnant population of Takahē in the Murchison Mountains of Fiordland National Park (Watson, 2001).

1.2.2. Takahē biology

As world's largest living member of the Rallidae, adult Takahē are around 50cm tall and weigh up to 4kg (more commonly 2-3kg) (Lee & Jamieson, 2001; Taylor & van Perlo, 1998). The adult is an iridescent dark blue on the head, neck and underparts, olive-green on the back and wings with margins of lighter blue. The large beak is laterally compressed and together with the shield and stout legs is red in the adult and dull blackish orange in juvenile birds (Taylor & van Perlo, 1998). The vestigial wings are used in behavioural displays, when climbing over objects and held outward when running (Lee & Jamieson, 2001). Little is recorded about the internal anatomy and physiology of the Takahē, however one study (Suttie & Fennessy, 1992) did suggest they possess a shorter intestine and larger caecum when compared to the Pukeko (*Porphyrio melanotus*) an adaptation presumably related to the higher fibre content in the Takahē diet.

The diet of the Fiordland population during the summer consists predominantly of three species of snow tussock (*Chinochloa pallens, C. flavescens and C. crassiuscula*) (Wickes *et al.*, 2009). The target part of the plant is the base of the tiller and seeds taken directly from the inflorescence when they are available. During the winter birds move to more protected valleys and the rhizome of a fern (*Hypolepis millefollium*) becomes and

significant portion of the Takahē diet (Mills *et al.*, 1980). This high fibre diet has resulted in Takahē feeding for up to 19 hours a day to satisfy nutritional requirements and the continual production of fibrous elongated faecal deposits (Lee & Jamieson, 2001). The Takahē populations introduced to more temperate climates feed year around on a mixture of available native and introduced grasses (Wickes *et al.*, 2009). Birds in some managed populations also receive supplementation with a formulated pellet diet, fed in a variety of regimes (Phil Marsh, pers comm, August 2015). The only life stage that is not considered exclusively vegetarian is the very young chicks, who are reported to be fed insects by their parents for a short initial period (Lee & Jamieson, 2001).

The high fibre diet of the Takahē and a requirement for a large continuous supply of forage has contributed to the normal social structure of the species, with an adult bonded pair defending a large territorial range (Wickes *et al.*, 2009). The size of this range varies with food availability and quality, but not exclusively, as variations between home range size have not been able to be fully explained by differences in habitat quality (Ryan & Jamieson, 1998). Defended territories within home ranges are aggressively protected during the breeding season and differentiating the home range (which may shrink with increased population densities and better resource availability as seen on temperate islands) from a central breeding territory is necessary before the potential carrying capacities of new areas can be established. The behaviour of Takahē studied in the harsh and unpredictable climate of the Murchison Mountains may also not fairly represent the ecology of Takahē in more temperate climates where, at the very least, home ranges are documented to be considerably smaller, for example on predator free offshore islands (Ryan & Jamieson, 1998). This leads to birds being in closer proximity to a larger number of conspecifics and increasing the potential for aggressive interactions and contagious disease transmission.

It is rare for Takahē to breed before 2 years of age, by which time they usually form a long-lived pair bond, with very rare reports of polyandrous and polygynous trios (Clout & Craig, 1995; Lettink *et al.*, 2002). Genetic analysis of a small number of parent/chick groups has also conclude that extra-pair fertilisations would be rare or absent in the wild population (Lettink *et al.*, 2002). The previous year's offspring have been noted to remain in the territory till the following season, presumably to assist with the raising of

the next chick (Clout & Craig, 1995). However, the exact role of the sub-adult in alloparental care for the family group is not fully clear.

During the breeding season of September to December, the breeding pair build a nest on the ground, often within a clump of tussock or other vegetation. The females lay 1-2 eggs (very rarely up to 4) and both parents alternately share incubation duties (Wickes *et al.*, 2009). However, few pairs in the wild rear more than one chick and even fewer are able to repeat a successful chick rearing in the next season (Clout & Craig, 1995). After a failed nest some Takahē pairs will re-nest, however, in Fiordland this was only observed in exceptionally good seasons of higher temperatures and lower snow falls (Hegg *et al.*, 2012). Egg infertility in Takahē clutches has historically been high, estimated at 20-30% in the early 1990s (Clout & Craig, 1995) and may even be higher in some populations (McLelland *et al.*, 2011). This low fertility rate and poor early chick survival has resulted in estimates of breeding outputs in the range of 0.43 chicks (alive at 30 days of age) per breeding pair (Hegg *et al.*, 2012). Additionally, in the Murchison Mountains wild population, survival to 1 year of age varies between 27 and 71% (Clout & Craig, 1995). Cumulatively these factors produce a very low species reproduction rate that is sufficient to maintain populations in good years but when under challenge, such as when there were increased stoat numbers in the Murchison Mountains in 2007 (Hegg *et al.*, 2012), the population numbers drop rapidly and can be very slow to recover without intervention.

1.2.3. History of Takahē conservation

The Takahē population at the time of rediscovery in 1948 is not known but was later estimated by some to be greater than 560 individuals (Reid & Stack, 1974), while other authors suggest the population at this time was around 250 birds (Wickes *et al.*, 2009). However, by the mid-1970s population estimates of around 150 were considered more likely (Grueber & Jamieson, 2011). This apparent decline was at the time attributed to the effects of competition for limited food resources with introduced red deer (*Cervus elaphus*) and susceptibility to introduced mammalian predators, particularly stoats (*Mustela ermine*) (Clout & Craig, 1995). It has been suggested by others that the population was originally overestimated and the combined effects of red deer competition, mammalian predators, low fecundity of Takahē and the harsh marginal

environment of Fiordland saw a steady continued decline of a limited remnant population (Bunin & Jamieson, 1995). Irrespective of the causative factors, by 1981 the Takahē population reached a nadir of an estimated 112 birds and conservation efforts, that had commenced with the establishment of the 503km^2 Takahē Special Area soon after their rediscovery, were increased (Wickes *et al.*, 2009).

The introduced red deer were at very high population densities in the Fiordland National Park when the Takahē were rediscovered there in 1948 (Wickes *et al.*, 2009). When grazing the red deer have been shown to favour the same species as the Takahē and that recovery of the alpine plants after heaving browsing by deer is slow (Lee, 2001). Consequently, the deer are in direct competition with the Takahē for their preferred food source and have a negative impact on Takahē numbers. Ground and helicopter hunting programs have been carried out in the Murchison Mountains since the early 1960s, with intensity of their application varying with commercial value of deer products and Department of Conservation (DOC) policy (Wickes *et al.*, 2009).

The avifauna of New Zealand evolved without the selection pressure of mammalian predators and many aspects of their ecology, including ground nesting, flightlessness and a suggested lack of predator awareness, make them particularly sensitive to the introduction of these species (Bunin & Jamieson, 1995). Of the mammalian predators introduced with human arrivals in New Zealand the stoat is likely to have had the greatest impact on Takahē populations. Stoats are capable of preying upon all life stages of Takahē, from eggs to adults (Bunin & Jamieson, 1995; Wickes *et al.*, 2009). Examination of Takahē survival statistics over a period of high and low stoat numbers across areas of the Murchison Mountains with and without stoat control showed a significantly higher survival for adult Takahē when a trapping program was in place (Hegg *et al.*, 2012). This work also described the 2007/08 season when a decline in the Murchison Mountains Takahē population from 168 to 93 birds coincided with a significant stoat plague (Hegg *et al.*, 2012; Takahē Recovery Programme, 2017). As a result of the clear impact of high stoat populations on Takahē survival in the Murchison Mountains active stoat trapping is carried out across a portion of the Takahē Special Area (Wickes *et al.*, 2009).

Following years of research ascribing the poor reproductive performance of the wild Takahē population to high rates of infertility, a programme of nest manipulation was commenced in the early 1980s (Clout & Craig, 1995). Under this programme nests were monitored, infertile eggs removed and fertile eggs moved between nests in an effort to provide all breeding pairs with one fertile egg to hopefully hatch and rear in an effort to maximise chick output across the whole population (Eason & Willians, 2001). Subsequently the programme moved to a strategy of artificial incubation and rearing initially at the Te Anau Wildlife Park and subsequently at the Burwood Captive Rearing Unit (later the Burwood Takahē Centre, from now on referred to as Burwood) utilising eggs or chicks retrieved from the wild (Wickes *et al.*, 2009). Initially this programme involved hand-rearing of the chicks to full independence and release. However, concerns about the behavioural effects of this system led to pairs of adult Takahē being held at Burwood at act as foster parents to groups of up to six juveniles. This development aimed to avoid both sexual imprinting problems and to teach the artificially incubated chicks natural behaviours, such as digging for Hypolepis fern rhizomes (Eason & Willians, 2001). This program increased the reproductive output by 55% (Wickes *et al.*, 2009), and its aims currently include producing juveniles for release back into the Murchison Mountains population, the establishment of new populations on a number of predator free islands and populating the subsequently established captive breeding program at Burwood (2017; Wickes *et al.*, 2009).

Eggs and chicks are no longer collected from the wild for rearing, instead pairs of adult Takahē are maintained year round in 1.5-2 hectare enclosures at Burwood for captive breeding. Likewise, the eggs are no longer artificially incubated, rather nests are monitored and birds allowed to naturally incubate and rear chicks. In this managed captive population nest manipulation is carried out to remove infertile eggs and maximise second clutches similar to that carried out previously in the wild, the aim being to maximise the production of yearling juveniles for relocation. The number of captive breeding pairs was increased from 11 in 2011 to 25 in 2017 (Takahē Recovery Programme, 2017), with Burwood housing just under a third of the current Takahē population. In addition to nest manipulation, management of individuals at the breeding centre can involve cross-fostering of chicks to both maximise juvenile output and to

disseminate behavioural traits important to survival in the Murchison Mountains population, such as foraging for Hypolepis tubers. These management procedures often result in adult pairs remaining in the same enclosure for the majority of the season over repeated years. However, the juvenile birds may be moved between several enclosures and family groups over their first 12 months before they are removed from the family group before the subsequent nesting season. The enclosures at the breeding centre are managed with twice weekly pellet supplementation and weekly cleaning of water supply points. Accumulated faecal material remains in the enclosures and is allowed to degrade naturally.

Introduction of Takahē to predator free island sites commenced in 1984 with translocations of birds to Kāpiti, Mana, Maud and Tiritiri Matangi Islands (Clout & Craig, 1995). This had been expanded to other managed sites, with predator control and appropriate habitat, over the three decades since in a program to both increase breeding opportunities and to improve public awareness of the species. Breeding pairs are currently residing at Cape Sanctuary, Maungatautari Ecological Island, Tāwharanui Open Sanctuary and on Mana, Kapiti, Motutapu, Tiritiri Matangi, Rotoroa and two other privately owned islands. None of these sanctuary sites are large enough to support a self-sustaining population, and as a result they are managed as one large population to reduce the effects of inbreeding in an already genetically limited species (Grueber *et al.*, 2012). This requires the regular movement of birds between breeding sanctuaries, Burwood and the wild population, with its associated risks of capture, transport and disease transmission (Grange *et al.*, 2014). Birds that have retired from breeding or have been deemed infertile are housed at several advocacy sites including Pukaha/Mount Bruce Wildlife Centre, Willowbank Wildlife Reserve, Zealandia and Punanga Manu o Te Anau/Te Anau Bird Sanctuary (Takahē Recovery Programme, 2017).

1.2.4. Current situation

The intensive investment in the Takahē Recovery Program in all its aspects, from the early years of natural history research to the current intensively managed population has seen the population increase to 347 birds, 39% of which are in the Murchison Mountains, 26% at Burwood and the remainder in sanctuary sites (Takahē Recovery Programme, 2017). Due to steady population growth the Takahē was recently

reclassified from Nationally Critical to Nationally Vulnerable under the New Zealand Department of Conservation, conservation status of New Zealand Birds (Robertson *et al.*, 2017). However, the international threat status remains unchanged at Endangered on the IUCN Red List© (Birdlife International, 2016). The current Sanctuary and Burwood sites are considered to be at maximum stocking density therefore plans are currently underway for the creation of another mainland wild population. Whether this new enterprise is ultimately successful or not, it is clear that the pressures once responsible for almost driving the Takahē to extinction are still present today and the need for intensive conservation management of this species remains.

1.2.5. Known disease issues of Takahē

The long-term Takahē conservation programme has included investigations of disease occurrence and routine health monitoring. Most Takahē that die are submitted for post-mortem examination, however one retrospective study of records indicated that a cause of death could not be established in nearly half (46%) of cases of known age (McLelland *et al.*, 2011). This is likely due to prolonged delays between the time of death and recovery/submission of the body leading to advanced decomposition of the carcass, particularly for birds that die at remote sites. In some cases, post-mortem examination of Takahē has also been limited by cultural requirements of Ngai Tahu Maori and the desire to preserve museum quality specimens. Where cause of death could be established, identified problems in adult Takahē have included trauma, predation, age related degenerative diseases and infectious disease such as *Erysipelothrix rhusiopathiae* or *Escherichia coli* septicaemia. Takahē have been treated at veterinary hospitals across New Zealand for conditions including traumatic musculoskeletal injuries, developmental abnormalities and a variety of infectious processes, including parasitic infections. Of particular interest to this thesis is the coccidian parasites of Takahē.

1.3. Coccidia

1.3.1. Taxonomy of Coccidia

The phylum Apicomplexa includes an array of almost 4500 described species of obligate intracellular parasitic protozoa (Cowper *et al.*, 2012). The taxonomy of species within

this phylum is currently under discussion and as further molecular information is discovered and historical morphological groupings re-evaluated, classifications will likely continue to be revised. For the purposes of this review the taxonomic hierarchy described Taylor *et al.* (2015) for the classification of the protozoa kingdom has been used. Species of this phylum are all characterised by an apical complex of specialised organelles located at the anterior end of the invasive stages of their life cycle utilised for entrance into the host cell (Tenter *et al.*, 2002). The Orders Eucoccidiorida and Aconoidasida within this phylum include a spectrum of important pathogenic parasites from the numerous coccidian parasites to the haemoparasites *Plasmodium* and *Babesia* spp. (Taylor *et al.*, 2015).

All coccidian protozoa are included within the Order Eucoccidiorida which is further subdivided into lower classifications based upon morphology and life cycle characteristics such intracellular/extracellular stages and tissue tropism (Cowper *et al.*, 2012; Tenter *et al.*, 2002). Within this larger group the Suborder Eimeriorina contains parasites which mainly occur in vertebrate hosts, and the species of major veterinary importance fall within two families, the Sarcocystiidae and Eimeriidae (Taylor *et al.*, 2015).

The Sarcocystidae have heteroxenous life cycles with the asexual and sexual stages occurring within intermediate and final hosts, respectively. Many species within this family are largely non-pathogenic to their final host, with the cystic asexual stages in the intermediate hosts causing greater pathology (Taylor *et al.*, 2015). Species within the Sarcocystidae are capable of utilising a huge range of cells types within vertebrate species as either intermediate or final hosts and can vary from highly specific to broadly generalist and capable of infecting almost any nucleated cell within a warm blooded vertebrate (Tenter *et al.*, 2002).

The family Eimeriidae includes 16 genera differentiated based on the number of sporocysts in each oocyst and sporozoites within each sporocyst. The genera of this family that are of most interest to veterinary medicine are *Eimeria* and *Isospora/Cystisospora* (Taylor *et al.*, 2015). *Isospora/Cystiospora* are characterised by oocysts with two sporocysts each containing four sporozoites. Until recently the

Isospora genus was made up of around 200 described species, however those species with mammalian hosts that lack a Steida body in their sporocysts, have recently been reclassified into a new genus of *Cystiospora* (Chapman *et al.*, 2013; Taylor *et al.*, 2015). Those species remaining in the *Isospora* genus largely affect birds (most often described in but not confined to passerines), some causing considerable clinical disease and are less host and cell specific than those in the genus *Eimeria*. The number of *Eimeria* spp. currently described exceeds 1800 and some suggest that there remain tens of thousands yet to be described (Chapman *et al.*, 2013; Cowper *et al.*, 2012). *Eimeria* have been described from all classes of vertebrates, and some invertebrates (Berto *et al.*, 2014; Cowper *et al.*, 2012), and are traditionally considered highly host specific or at least to infect only closely related host species (Blake, 2015; Chapman *et al.*, 2013). The genus *Eimeria* is differentiated from other Eimeriidae by having a monoxenous lifecycle and oocysts with four sporocysts (tetrasporic) each containing two sporozoites (dizoic) (Berto *et al.*, 2014; Taylor *et al.*, 2015). Historically, *Eimeria* species have been identified based on an oocyst morphology, host species, pathology and geographic distribution (Yang *et al.*, 2014). The broad definition of *Eimeria* has produced a large genus that is increasing being recognised as a polyphyletic taxonomic group and the genus may include as many as four identifiable lineages. As research illuminates nuclear and mitochondrial sequences of more species and this information is interpreted together with oocyst structure, host species, tissue tropisms and details of the parasites' life cycle, it is likely that what is currently the *Eimeria* genus will be subdivided into several smaller genera (Chapman *et al.*, 2013).

1.3.2. *Eimeria* life cycle

Eimeria sp. have a direct monoxenous lifecycle with asexual and sexual reproduction occurring within one host and the maturation of the oocyst (sporulation) occurring in the environment (Yabsley, 2008). Unsporulated oocysts, a nucleated mass of protoplasm enclosed in a resistant wall, are passed in the faeces of the host. If exposed to appropriate environmental conditions the oocyst develops into the characteristic 4:2 sporulated oocyst which is the infective stage of the life cycle. Environmental variables including temperature, humidity and oxygenation, regulate the sporulation process. The optimum conditions vary between *Eimeria* species but generally sufficient oxygen, high

humidity and temperatures around 27^{o}C favour rapid sporulation (Taylor *et al.*, 2015). The minimum time for sporulation is a significant determining factor in the prepatent period which for some of the highly studied *Eimeria* spp. of domestic poultry it is known to be as short as 2-3 days (Eckert *et al.*, 1995).

The oocysts for most species of *Eimeria* are considered highly resistant in the environment, however, temperatures above 35^{o}C and humidity below 25% and sunlight for 4 hours are likely to be fatal to most (Parker & Jones, 1990). Oocysts of some *Eimeria* spp. have also been reported to remain infective over winter in Canada and Belarus despite snow cover and sub-zero temperatures, albeit at a lower rates of sporulation than during the summer months (Skene *et al.*, 1981). In artificial conditions the oocysts recovered from most terrestrial hosts can remain viable in the refrigerator (temperature not specified) for 3-4 years, while those from aquatic hosts appear to be much less resistant (Duszynski & Wilber, 1997). This disparity is potentially due to differences in oocyst wall thickness and related adaptations to desiccation. The oocyst wall of terrestrial species that have been studied is composed of 90% protein and is bilayered, with the separation of the two layers varying between parasite species and the age/stage of sporulation of the oocyst (Chapman *et al.*, 2013). The wall structure of those from aquatic hosts is yet to be characterised.

A host is infected by ingesting a sporulated oocyst which ruptures in response to physical and chemical insults that are not fully understood and probably vary with parasite and host species (Yabsley, 2008). The liberated sporozoites utilise their apical complexes to invade target host cells, most often, but not always, epithelial cells of the gastrointestinal tract. Once in the cell the parasite undergoes asexual reproduction (merogony) to produce merozoites within a structure called a meront, which once mature causes lysis of the host cell and release of the merozoites to invade neighbouring cells. Merogony can then be repeated for a finite number of cycles, varying with the species of parasite (Conway & McKenzie, 2008c). Once the appropriate number of cycles has been completed the invading merozoites switch, by triggers not fully understood, to sexual reproduction (gametogony), whereby they produce either macrogametes (one large female gamete per host cell) or microgametes (multiple flagellated organisms per cell). The motile microgametes exit the host cell in which they developed to fuse with

and fertilise a macrogamete, the resulting zygote developing into an oocyst that ruptures the host cell and is shed unsporulated in the faeces completing the lifecycle (Taylor *et al.*, 2015; Yabsley, 2008).

Pathological effects of the *Eimeria* life cycle on the host are related to the lysis of cells and the host inflammatory response to the presence of the parasite. Host pathology can vary from sub-clinical self-limiting infections to severe disease and death (Friend & Franson, 1999; Yabsley, 2008). Losses due to coccidian infection, in terms of both lost production and costs of control is a significant economic burden to livestock industries worldwide. With costs to the global poultry industry alone estimated to exceed £2 billion in 2006 (Blake, 2015). *Eimeria* are also known to infect a diversity of vertebrate wildlife hosts and while they are rarely reported to cause disease in free-living individuals (Yabsley, 2008), there is increasing concern over their impact on captive breeding and endangered species management (Blake, 2015).

1.3.3. *Eimeria* oocyst morphology

The sporulated oocyst is a resilient structure and while different species appear to have preferred environmental conditions for sporulation the oocyst is capable of remaining infective outside that range. Additionally, the oocyst is highly resistant to chemical deactivation, as indicated by the use of concentrated sulphuric acid in one described method of cleaning oocysts for in-vitro studies where the oocyst is still capable of sporulation and infection afterward (Fayer, 1980). Being exogenous the oocyst is readily available for examination outside the host. Consequently, as previously mentioned the morphology of the characteristic tetrasporic dizoic oocyst is the historical basis of Eimeria species differentiation. Standardised species descriptions include a detailed evaluation of the appearance under light microscopy of the of sporulated oocysts (Duszynski & Wilber, 1997).

The maximum length and the equatorial width of oocysts that can be examined in a longitudinal plane are key measurements which can be used to calculate the length:width ratio, an assessment of the overall shape (Duszynski & Wilber, 1997). A ratio of 1.0 indicates a spherical shape, 0-1.1 is considered sub-spherical and >1.1 ellipsoidal. The latter requires further description (Berto *et al.*, 2014).

The oocyst wall is a protein rich coat that gives the oocyst much of its resiliency in the environment. The wall consists of two distinct layers that may fuse or remain evidently separate under light microscopy depending on the species (Berto *et al.*, 2014). Wall thickness and texture (smooth vs rough, with or without protruding surface structures) are also characteristics to be noted (Duszynski & Wilber, 1997). In the past the colour of the oocyst wall was considered significant, however, this was found to be highly variable with storage time and media as well as light source type and intensity (Berto *et al.*, 2014).

The micropyle is a discontinuity in the oocyst wall, that may or may not be covered with a structure called the micropyle cap. The presence or absence of these structures and their dimensions if applicable should be recorded (Duszynski & Wilber, 1997). The micropyle could be a deficit in either the inner or outer wall layer or the full thickness of the wall, depending on the species. The role of the micropyle is unknown (Berto *et al.*, 2014).

The polar granule is a dense structure within the oocyst that is highly variable in size and shape between species (Berto *et al.*, 2014). Also within the oocyst but outside the sporocysts may be an oocyst residuum (Duszynski & Wilber, 1997). This accumulation of cellular debris from the sporulation can vary in size between species and the structure can vary from compact and dense to a loose collection of granular material (Berto *et al.*, 2014; Taylor *et al.*, 2015).

The length and width of the four sporocysts should be measured, using only sporozoites that are transected in an appropriate plane in each field of view. From these measurements an average for the oocyst can be determined. Similar to the overall oocyst, a length: width ratio is used as an indicator of relative shape (Duszynski & Wilber, 1997). The presence or absence and shape of a Stieda body, a thickening of one end of the sporocyst, should be noted. Berto et al. (2014) have proposed a set of standardised shape descriptions for the Stieda body and sub-Stieda body, a variably present mass located inside the Steida body. A parasteida body is a structure similar in appearance to the sub-steida body that is occasionally found at the opposite pole of the sporocyst (Duszynski & Wilber, 1997). Between the sporozoites there may be an evident sporocyst

residuum and if present the size, shape, structure and whether it appears to be bound to the sporocyst membrane should be recorded. Additional structures, including surface projections or filaments, are variably present associated with the sporocyst wall (Duszynski & Wilber, 1997).

Within each sporocyst the two sporozoites can often be tightly confined which can make measurement and assessment of structures difficult. The length and width of individual sporozoites, together with the variable presence of other structures such as multiple refractile bodies, striations or a nucleus can be important identifying features (Berto *et al.*, 2014).

Described in a standardised fashion the morphology of the sporulated oocyst remains an important part of species identification, even as molecular techniques become more widely utilised.

1.4. Avian Coccidiosis

Coccidiosis broadly refers to clinical disease caused by infection with protozoal parasites of the family Eimeriidae, including *Eimeria* and *Isospora*, in a variety of vertebrate host species (Schoener *et al.*, 2013; Taylor *et al.*, 2015). However, some researchers, particularly those working exclusively with commercial poultry, restrict this definition to disease caused only by protozoa of the genus *Eimeria* (Chapman *et al.*, 2013). This term is also sometimes erroneously used to describe infection with these protozoal parasites without causing overt clinical disease, which is more correctly referred to as coccidiasis (Conway & McKenzie, 2008c; Yabsley, 2008). Despite other genera including *Isospora* and *Atoxoplasma* causing significant pathology in avian hosts, the following review of coccidiosis in avian host species will focus on that caused by the genus *Eimeria*.

1.4.1. Host range and specificity

Over 200 species of *Eimeria* have been formally described from 17 orders of avian hosts (Yabsley, 2008; Yang *et al.*, 2014) and there are many more reports of tetrasporic dizoic oocysts recovered from an array of avian species and ascribed under the current system to *Eimeria* (Jankovsky *et al.*, 2017; Panigraphy *et al.*, 1981; Yang *et al.*, 2016).

A characteristic of *Eimeria* spp. is a high degree of host specificity (Chapman, 2014). However, some species are known to complete their life cycle in multiple host species, for example *E. dispersa* is capable of infecting turkeys (*Meleagris gallopavo*), chickens (*Gallus gallus domesticus*), ring-necked pheasants (*Phasianus colchicus*) and Chukar (*Alectoris chukar*) (Yabsley, 2008), while *E. gruis* and *E. reichenowi* are known to infect Whooping (*Grus Americana*) and Sandhill cranes (*Grus canadensis*) (Bertram *et al.*, 2015). In the majority of documented *Eimeria* sp. with multiple potential hosts, the host species involved are of the same genus or at least within the same order (Duszynski & Wilber, 1997). It is possible that these reported differences in host specificity may correlate with different genetic lineages of *Eimeria* spp. with similar morphology and the true definition of the host specificity of the organisms may depend on molecular identification of cryptic species, and awaits a future re-classification of this genus (Tenter *et al.*, 2002).

1.4.2. Tissue Tropism

The vast majority of *Eimeria* sp. undergo both asexual and sexual development in the epithelial cells of the host's gastrointestinal tract. A smaller group of *Eimeria* sp. are capable of invading other host cells either in addition to or instead of intestinal cells (Cowper *et al.*, 2012).

Renal *Eimeria*, where either one stage or the whole life cycle occurs within the epithelial cells of renal tubules, has been reported in Kiwi (*Apteryx sp*.), Great-Horned Owls (*Bubo virginianus*), Little Penguins (*Eudyptula minor*) and a variety of Anseriformes among others (Jankovsky *et al.*, 2017; Morgan *et al.*, 2013; Obendorf & McColl, 1980; Yabsley, 2008). The clinical presentations seen with renal associated *Eimeria* spp. vary from an incidental finding to renal failure or sudden death.

Hepatic coccidiosis, with parasite replication in the epithelial cells of the bile ducts has been reported in several mammalian host species, and is a particular concern as a cause of high mortalities in young meat rabbits (Sivajothi *et al.*, 2016). One case report exists of bile duct coccidiosis in a free living magpie-lark (*Grallina cyanoleuca*). In this case all stages of the protozoal lifecycle were seen in the affected duct tissue and oocysts of the tetrasporic dizoic for characteristic of *Eimeria* spp. were passed already sporulated in

the faeces (Reece, 1989). A new *Eimeria* species was proposed but has yet to be fully described. Endogenous sporulation, while not completely unknown in avian *Eimeria* is unusual (Yabsley, 2008) and that together with the extra-intestinal tissue tropism may indicate this coccidian is only distantly related to the rest of the *Eimeria*. Further research is required. Hepatic coccidiosis has also been reported in Kiwi (*Apteryx* spp), where areas of protozoal replication and surrounding necrosis was seen within the parenchyma of the liver, rather than the bile duct epithelium. In some of these cases there was concurrent intestinal and/or renal coccidiosis and localised splenic or pulmonary necrosis containing protozoa (Morgan *et al.*, 2013). Many of these lesions were noted to be located near blood vessels, suggesting possible haematological spread of a disseminated form of coccidiosis.

Disseminated visceral coccidiosis caused by two species, *E. reichenowi* and *E. gruis*, has been described in the endangered Whooping crane and Sandhill cranes since the 1970s (Carpenter *et al.*, 1979). Asexual reproduction of the parasite has been detected in a variety of tissues throughout the host, while sexual reproduction is confined to the intestinal and respiratory tracts (Novilla & Carpenter, 2004). Both of these species of *Eimeria* are regularly detected in wild cranes and the cost to the wild populations are unknown. However, significant morbidity and mortality has occurred in captive breeding facilities and challenge experiments, particularly in young chicks (Novilla & Carpenter, 2004). As the recovery of deceased wild nestlings is difficult and cause of death rarely established it is possible that disseminated visceral coccidiosis is a significant population regulator for the endangered Whooping crane (Bertram *et al.*, 2015).

Extra-intestinal *Eimeria* remains uncommon and the considerable majority of *Eimeria* spp. infecting birds replicate within the gastrointestinal tract.

1.4.3. Intestinal Coccidiosis

A significant proportion of the *Eimeria* spp. recovered from avian hosts are described solely on the morphology of the exogenous sporulated oocyst. However, a few *Eimeria* sp. have been studied in great detail, including the seven species that target the intestinal tract of domestic poultry (Conway & McKenzie, 2008c). Knowledge of these species is often extrapolated to lesser studied host-parasite interactions (Yabsley, 2008).

Within the group of enterotropic *Eimeria* different species are known to target different sections of the intestine, for example *E. tenella* invades cells of the caeca of domestic poultry while *E. acervulina* and *E. praecox* target the duodenum of the same host (Conway & McKenzie, 2008c). Confusion can arise with mixed parasite infections and species with overlapping or more disseminated target regions. As well as variable gross site tropism, *Eimeria* sp. vary in the cell type parasitised, some confined to epithelial cells of the tips of intestinal villi while others target crypt or subepithelial cells (Conway & McKenzie, 2008c). The target cell type may also vary with the stage of the parasite's lifecycle, with different cycles of asexual reproduction and sexual reproduction taking place in different host cell types. Additionally, the location within the host cell, where parasite replication occurs, can vary from different areas of the cytoplasm to within the nucleus (Taylor *et al.*, 2015).

1.4.3.1. Clinical signs

At each stage of the parasite lifecycle, for a species specific number of cycles, the development of the parasite will result in host cell rupture. The occurrence of clinical disease is dependent on a balance between the rate of cell loss and replacement, and the host response to infection (Friend & Franson, 1999). Infection with low numbers of parasites is often self-limiting and sub-clinical, with the parasites destroying a limited number of host cells that are rapidly replaced, and this is often seen in wild birds who shed oocysts but rarely present with overt disease (Yabsley, 2008). However, exposure of a naïve host to overwhelming numbers of infective oocysts can result in significant pathology (Ruff & Wilkins, 1987).

High-intensity infections result in the rapid loss of large numbers of host cells before they can be replaced and can alter the structure and function of the gastrointestinal tract. The result of this damage is decreased nutrient absorption, enteric loss of fluid, haemorrhage and altered gut motility. The altered gut structure and function can also predispose the host to secondary infections with pathogens such as *Clostridium perfringens* and *Salmonella* spp. (Yabsley, 2008). The host immune response to the presence of the parasite can also contribute to pathology. Various life stages of the *Eimeria* can induce significant lymphocytic inflammatory responses from the host that can lead to inhibition of the parasite and subsequent development of immunity.

However, the inflammatory response, if excessive, can temporarily contribute to poor nutrient absorption and, in some chronic cases, caseous caecal cores or surface plaques can permanently alter the structure of the gastrointestinal tract (Clarke, 1979).

Affected birds can present with diarrhoea (with or without haematochezia), anaemia, inappetance, emaciation, weakness, lack of coordination and, in severe cases, rapid death (Yabsley, 2008). In more moderate cases a decline in food intake and digestive functions can result in slowed growth rates, weight loss and declines in egg production and fertility (Ruff & Wilkins, 1987; Yabsley, 2008). For free-living wild host species, the depression associated with coccidiosis could predispose them to increased predation (Barker *et al.*, 1984).

1.4.3.2. Factors affecting pathogenicity

Pathogenicity has been shown to be dose dependent in a variety of *Eimeria* sp./host relationships. Exposure to higher numbers of infective oocysts produces more severe disease (Ruff & Wilkins, 1987). This becomes a concern in livestock production and wildlife captive breeding programs where the confinement of a large number of potential hosts can result in the parasite burden in the environment magnifying to considerable levels, increasing the probability of severe clinical disease. This effect can be compounded by environmental conditions that favour the sporulation and survival of oocysts, such as humidity >75% and no exposure to sunlight (Fayer, 1980).

In immune competent birds, a light infection is capable of rapidly producing a high level of immunity and resistance to reinfection with that species of *Eimeria* (Rose & Hesketh, 2009). This is likely responsible for the apparent increased resistance to infection seen with increasing age in most hosts, with young birds being more susceptible than adults (Yabsley, 2008). So rather than being a true “age effect”, it is an effect of previous exposure.

Different species of *Eimeria*, and even strains within the same species, are variably pathogenic, and this pathogenicity is often related to variation in the inherent reproductive potential of the protozoan (Fayer, 1980). Likewise there are demonstrable genetic differences between hosts in their resistance to both infection with *Eimeria* and clinical coccidiosis (Bumstead & Millard, 1992; Pinard-van der Laan *et al.*, 2009).

1.4.3.3. Diagnosis

Diagnosis of coccidiosis is reliant on the identification of *Eimeria* spp. oocysts in collected faecal samples along with clinical signs of disease. Oocysts can be detected by microscopy of a fresh faecal preparation in cases with extremely high rates of shedding. But initial concentration of the shed oocysts by flotation or centrifugation in 33% zinc sulphate solution or Sheather's sugar solution is recommended (Zajac & Conboy, 2012).

For some parasite/host relationships, for example *Eimeria* spp. of the domestic chicken, oocysts are shed at approximately the same rate throughout the day. However, in some infections the number of oocysts shed in faeces shows significant diurnal variation, often with an increased level of shedding in the afternoon (Villanúa *et al.*, 2006). Knowledge about whether there is inherent temporal variation in shedding of oocysts in the host/parasite relationship being studied is important to the interpretation of the results of faecal analysis for parasites.

Post-mortem examination is used in the commercial poultry industry for both diagnosis and speciation of *Eimeria* infections and outbreaks (Chapman *et al.*, 2013). Gross lesions will vary with species of parasite and severity of infection. Lesions seen can include distended sections of intestine filled with mucoid or haemorrhagic material and with variable discolouration, haemorrhage and or thickening of the intestinal wall. For some chronic infections caseous luminal plugs or plaques on the mucosal surface are seen (Yabsley, 2008). For some well-studied *Eimeria* spp. the location of gross lesions in the gastrointestinal tract may be sufficient to determine the species of *Eimeria* involved (Conway & McKenzie, 2008c).

In cases of clinical disease, histological preparation of sections from the intestinal tract will reveal large numbers of protozoa in various life stages within the host cells and possibly concurrent lymphocytic infiltration (Yabsley, 2008). Cases of coccidiasis with fewer protozoa present may be harder to identify. The rapid degradation of intestinal mucosa post-mortem may also complicate the interpretation of both gross and histopathological signs in hosts that have been deceased for some time.

Several molecular assays that can detect and differentiate all seven of the economically important *Eimeria* spp. of the domestic chicken are now available and are used in

research and vaccination production (Chapman *et al.*, 2013). However, they are as yet not widely available commercially and molecular analysis of the remaining hundreds of species of *Eimeria* identified in birds is only in the early stages (Clark *et al.*, 2017). Diagnosis of the presence of *Eimeria* and potentially, differentiation to species level, may be available in the future once more species are described and some of the inherent problems with extracting genetic material from the resilient oocyst are overcome (Chapman *et al.*, 2013). Molecular differentiation of coccidia was not used in the investigations documented in this thesis, and so this will not be discussed further.

1.4.3.4. Treatment and control

Early extensive losses to coccidiosis lead to the development and application of a variety of treatment and control measures in the global commercial poultry industry. The cost of both the disease and the treatments used are considerable. While manifestations of clinical disease due to intestinal coccidiosis are rare in caged or wild birds there are occasions when control measures are required and those protocols used in the commercial poultry industry provide a framework from which treatment options in other species of birds are often extrapolated.

Sound husbandry practices play an important role in limiting oocyst sporulation and reducing the re-exposure of hosts. Key considerations include restricting bird access to faeces, controlling ventilation and moisture levels in the substrate, replacement or cleaning of the substrate between groups of birds and avoiding faecal contamination of food and water sources (Blake & Tomley, 2014). Careful management of the introduction of naïve birds to established groups of birds or contaminated holding areas is required, as is taking steps to limit over-crowding, stress or concurrent infections that may compromised the immune resistance of potential hosts (Yabsley, 2008).

The development and widespread use of anticoccidial medications is credited, by some, with the creation of the modern commercial poultry industry (Chapman *et al.*, 2013). Since the addition of sulphaquinoxaline to chicken feed in the late 1940s was shown to control coccidiosis, numerous drugs have been developed intended for the continual, seasonal or pulse inclusion in pelleted poultry diets to suppress coccidian oocyst production and these medications are usually called coccidiostats (Chapman *et al.*, 2013;

Grumbles *et al.*, 1948). Despite their widespread use the mechanism of action for many of the anticoccidial drugs that have been used in poultry are unknown. The quinolone group of drugs, including decoquinate, are suspected to have two modes of action, preventing mitochondrial electron transport in the early life cycle stages of the parasite and inhibiting chromosome re-assortment and consequently sporulation (Del Cacho *et al.*, 2006). Resistance has developed in the coccidia of poultry to most of the available drugs. However, they still contribute to effective coccidiosis control by partially suppressing the parasite and allowing the host to develop immunity (Quiroz-Castaneda & Dantan-Gonzalez, 2015). Some medications are not used prophylactically, but rather as treatment in the event of an outbreak of clinical disease. One such medication, toltrazuril, a triazine-based drug, is widely used in the treatment and control of protozoal infections across a variety of species, particularly infections with apicomplexan parasites in poultry and pigs (Kandeel, 2011). The mechanism of action of toltrazuril, and its metabolite ponazuril, is unclear but what is known is the efficacy of these medications against all intracellular life stages of the parasite (Greif, 2000). As for many other coccidiostats, resistance has been noted against toltrazuril, particularly in the production industries (Sykes & Papich, 2014). It has also been noted that stages of the parasite damaged by toltrazuril remain within host cells for an extended period, allowing the host immune system time to develop a response. As a result, a treatment with toltrazuril has been used in vaccination programs for poultry allowing the use of more virulent vaccine strains (Greif, 2000).

The knowledge that low intensity infections with eimerian protozoa will lead to the development of protective immunity in the host has led to the development of several vaccine protocols. Initial vaccines used low doses of wild-type oocysts that were still pathogenic, this produced an effective immunity but had the potential to cycle through the host and build up to levels that caused clinical disease (Chapman & Cherry, 1997). The next development was the use of attenuated strains of oocysts that were equally as immunogenic to the host but less pathogenic and not capable of inducing coccidiosis (Chapman *et al.*, 2013). For both the original wild-type vaccines and the subsequent attenuated ones, protective immunity does require cycling of the vaccine parasite through the host and may take several weeks to develop. A sub-unit vaccine has also

been used to immunize hens that subsequently pass maternal immunity via the egg to the next generation. This process is effective in reducing the impact of coccidiosis in the first few weeks of life, but the development of full immunity in the chicks requires exposure to oocysts from the environment (Chapman *et al.*, 2013).

There is increasing pressure on food producing industries to reduce or eliminate prophylactic antimicrobial use. Consequently, plant, fungi and microbial based alternatives to coccidiostat medications have and continue to be investigated. Some products tested in combination have been shown to reduce the severity of coccidiosis lesions but did not prevent the shedding of oocysts. At present there are no such products are considered economically viable or are commercially available (Quiroz-Castaneda & Dantan-Gonzalez, 2015).

1.4.4. Coccidia and wildlife

Eimerian infections in wildlife are usually intestinal, self-limiting and are widely believed to have little impact at the population level (Yabsley, 2008). However, outbreaks of disease in wild-living birds have been reported across a variety of species, with some host-parasite relationships considered to be significant contributor toward extinction of the host (Bertram *et al.*, 2015). In addition to mortality events and decreased survival of offspring, less overt effects such as increased predation of birds weakened by parasite has been suggested (Watson, 2013). Other researchers have claimed a positive relationship between the parasite burden (including coccidia in some cases) and the fitness of the host. These authors have proposed that some males were better able to maintain good feather quality and secondary sexual ornamentation in the face of high parasite burdens because evolution had indirectly selected for resistance to parasites (Buchholz, 1995; Hamilton & Zuk, 1982). However, other work related to variations in sample collection time and technique from wild birds has led to the methodology of some of the former work being questioned (Brawner & Hill, 1999). Irrespective of the unclear significance of parasites in free-living wild birds, the potential for parasite infections complicating captive management programmes for wildlife is well established.

Captive breeding facilities and translocation of individuals or populations are cornerstones of intensive wildlife conservation management and both these strategies have the potential to be impacted by disease, including parasites. The congregation of hosts during capture and transport or holding the hosts in a captive facility increases the transmission of parasites and environmental concentration of resistant organisms like *Eimeria* spp. (Lebarbenchon *et al.*, 2006). Therefore, pre-translocation disease screening and monitoring of captive populations for parasites and pathogens is an important aspect of intensive host conservation efforts. Recently there is increasing interest in the conservation of not only the host but any dependent species, including species-specific parasites such as many eimerian parasites (Gomez & Nichols, 2013). Therefore, while the focus of conservation efforts may be directed at the host, it is suggested that any dependent species should also be considered in management decisions, for example when to treat with anticoccidial medications (Moir *et al.*, 2012). In addition to the inherent conservation value of the parasite species, the value of the presence of a parasite to the maintenance of immunity in the host should also be considered. If captive birds are being bred for release, raising them without exposure to the appropriate parasites may produce immunologically naïve hosts that can be severely affected by infection once exposed in the wild (Cunningham, 1996).

1.5. Investigations into Takahē coccidiosis – objectives of the current research

The survival of the South Island Takahē is dependent on intensive management of wild populations and captive breeding of juveniles for release (Takahē Recovery Programme, 2017). This intensive management necessitates the keeping of Takahē at breeding and advocacy sites at much higher densities than would be the case in the free-ranging population. This intensification allows parasites, including coccidia, to build up in the environment and may result in these coccidia moving from an apparently benign mild infection to a potentially serious pathogen. Faecal samples taken from a variety of Takahē populations over the last few years have regularly shown very high levels of coccidia, particularly in sites that have been populated with Takahē for some time.

Management of the Takahē population includes the movement of birds between breeding, island and the free-ranging wild populations in order to maintain genetic

diversity (Grange *et al.*, 2014). In relation to coccidian parasites, this movement of birds raises two concerns; the potential of introduction of coccidia to a site with by a relocated bird and conversely the introduction of a naïve and highly susceptible bird to a location with significant environmental parasite burden. A better understanding of the relationship between Takahē and their coccidia is required to minimise the impact of the pathogen on the survival of the host. Additionally, the high value of each individual Takahē in a population of only 340 birds, means that the avoidable loss of any bird is unacceptable. Consequently, this research project was established with two distinct, but complementary aims; to initiate investigations into the host-parasite relationship and concurrently develop coccidiosis management protocols for Takahē.

The starting point of investigations into the host-parasite relationship was to establish a morphological description of the coccidian oocysts isolated from the faeces of Takahē (Chapter 2). Archived historical faecal samples from all locations currently inhabited by Takahē underwent parasitological evaluation to determine the geographical distribution of protozoal parasites across the host population. A more focused assessment of faecal protozoal burdens was carried out at a central breeding site to investigate variations in coccidian presence in across the years 2015-2017, inclusive. Collectively these surveys (Chapter 3) provided information into the distribution of the parasite of interest amongst the Takahē population and possible impacts of management changes.

Working within the confines of experimentation involving an endangered host species, trials were designed to evaluate variation in rate of oocyst shedding across the daylight hours (Chapter 4) and to evaluate the safety of a coccidiostat feed-additive, decoquinate, in this unique host (Chapter 5). This work aimed to provide a basis for future management protocols for coccidia in Takahē. The findings of these investigations are described in the following chapters.

Chapter Two:

Morphological characterisation of a novel *Eimeria* sp. parasite in South Island Takahē (*Porphyrio hochstetteri*)

2. Morphological characterisation of a novel *Eimeria* sp. parasite in South Island Takahē (*Porphyrio hochstetteri*)

2.1. Introduction

The South Island Takahē (*Porphyrio hochstetteri*) is the largest extant member of the Rallidae and endemic to New Zealand. After being assumed extinct on two occasions, the latest in 1930, the species was rediscovered in a remote valley in the Fiordland National Park in 1948 (Trewick, 1996). Extensive conservation efforts have increased the Takahē population to around 340, however they remain internationally classified as endangered (Birdlife International, 2016). Since the 1980s one aspect of the conservation management program for the Takahē has been captive rearing and latterly captive breeding, with numerous translocations of birds every year (Grange *et al.*, 2014). These practices, while critical to the survival of the species, increase host population density for varying lengths of time and increases the potential for pathogen/parasite transmission between birds. In order to limit the risk of significant disease outbreaks, translocation of Takahē is accompanied by pre-translocation testing. Routine testing includes faecal flotation for identification of enteric parasites. Coccidia oocysts have been regularly identified on routine faecal examination, but to date this species has not been formally described.

Thirteen coccidia of the genus *Eimeria* have been reported, named and described to some extent from hosts of the order; Gruiformes (Yabsley, 2008), eight were identified from the family Gruidae and five from the family Rallidae (Duszynski *et al.*, 1999). No coccidia have previously been described from Takahē. The aim of this study was describe the morphological features of a coccidia species from the endangered South Island Takahē.

2.2. Materials and Methods

2.2.1. Sample collection

Faecal samples were collected from multiple birds at two locations with managed Takahē populations; Burwood Takahē Breeding Centre (hereafter Burwood) and Cape Sanctuary. Samples were collected immediately after being passed and were stored in sterile sealed containers in a cooled transport box until they could be refrigerated prior to processing.

2.2.2. Faecal sample preparation and measurement

All samples were subjected to faecal floatation within 5 days of collection using a standard veterinary protocol (Zajac & Conboy, 2012). Briefly, the large fibrous faecal sample was broken up to allow more representative sub-sampling. One gram of the faecal material was suspended in approximately 10 mL of 33% zinc sulphate solution (S.G. 1.2) and agitated till all visible plant fibres were separated. The suspension was then strained through a fine nylon mesh sieve to remove large particles and placed into a 15 mL centrifuge tube. Additional zinc sulphate solution was added to fill the test tube and a coverslip was placed on the top of the test tube, ensuring a total layer of contact between coverslip and suspension. Samples were then centrifuged in a swinging bucket centrifuge (ThermoScientific Megafuge 40) at 177 g for 5 minutes. The coverslip was then placed on a microscope slide and examined under light microscopy (Olympus CX41) at 300x magnification for the presence of coccidia.

Samples that had identifiable oocysts present were subsequently subjected to sporulation. An additional one gram sub-sample of faeces were suspended in 5mL of sterile water in a test tube and agitated until all plant fibres were separated. This faeces/water suspension was then placed in a sterile disposable petri dish to form a shallow uniform layer and covered. The prepared samples were then stored at 25^{o}C and assessed by sub-sampling a drop and examining under light microscopy from day 3. The majority of oocysts appeared sporulated by day 5 and the faecal flotation process described above was carried out to concentrate the oocysts. Once the coverslip was placed on the microscope slide it was allowed to rest at room temperature for 10 mins, until crystallisation of the zinc sulphate solution began around the periphery of the

coverslip. The edges of the coverslip were then sealed with clear, quick set nail vanish (Sally Hansen Rapid Set) to prolong the time available to examine and measure the oocysts in the sample.

Samples were examined under light microscopy with a Leica DM750 and both still and video images captured with Leica ICC50 W (Leica Microsystems, Germany) at 100x magnification under oil immersion. Fully sporulated oocysts positioned in a longitudinal transverse plane were located and imaged. Images were processed using Leica LAS EZ software. Measurements (µm) and observations were made on each suitable oocyst in line with the method of Duszynski and Wilber (1997). Not all sporocysts or sporozoites were measureable in each oocyst, consequently numerical averages were calculated for each oocyst measured. Morphometric ratios of oocyst and sporocyst length:width were calculated. A composite drawing of one representative oocyst was made using a video progression through focus planes as reference and created with Adobe InDesign software (Adobe Systems, USA).

Statistical analysis was conducted using SPSS v24 (IBM). A linear regression analysis of oocyst width against length was performed as reviewed in Berto et al. (Berto *et al.*, 2014). ANOVA was used to assess for differences in oocyst morphology between sample sites. Subsequently means, standard deviations and size ranges were calculated for each parameter measured.

2.2.3. Revision of archived material

A review was undertaken of records in the Massey University Post-Mortem database, to identify Takahē that were positive on parasitological examination of faecal or gastrointestinal contents for coccidia. Archived tissues, preserved in 10% neutral buffered formalin, for each case of interest were examined for the presence of gastrointestinal tissues. When gastrointestinal tissues were present, multiple transverse sections of the intestinal tract were taken for histology and identified to the anatomical location within the intestinal tract where possible.

Tissue sections were submitted for routine histological processing and staining with Eosin and Haematoxylin. The returned slides were then examined under light microscopy (Leica DM750) at 1000x under oil emersion. The entirety of all tissue

sections was examined with particular emphasis on the mucosal and sub-mucosal layers. When areas of interested were identified photomicrographs were taken using (Leica ICC50 W) and processed with (Leica LAS EZ software).

2.3. Results

2.3.1. Evaluation for consistency amongst oocysts

A linear regression of oocyst width (μm) over oocyst length (μm) was carried out to evaluate if the oocysts measured were likely to represent a single species across the two evaluated sampling locations. The calculated regression is described in Figure 2-1.

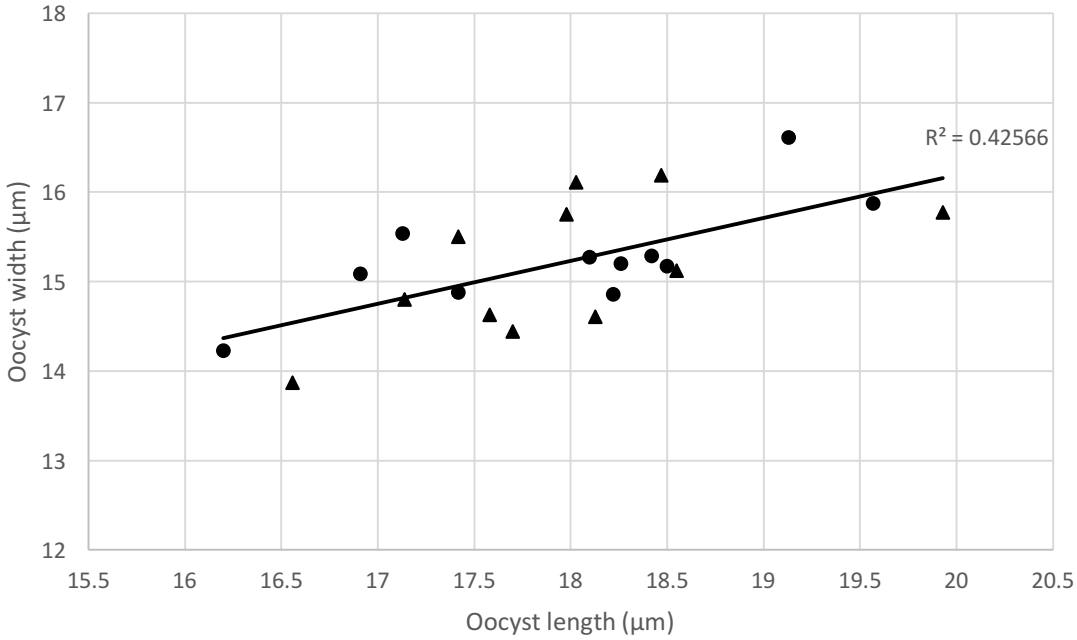

Figure 2-1: Linear regression illustrating the variation in oocyst width and length (y=0.4795x + 6.6012, R^2=0.43). Data points from different sampling locations indicated, Burwood (triangles) and Cape Sanctuary (dots).

The R^2 value calculated for the linear regression in Figure 2-1 was 0.43 and therefore below the standard R^2>0.5 used to indicate variation within a single population of oocysts. This may suggest more than one species of Eimeria were present, however, graphically data points from both sites were scattered around the line of best fit, therefore if there is more than one Eimeria sp. present it was present at both sites.

2.3.2. Morphological oocyst measurements

Table 2.1 presents the standard measurements taken when morphologically describing a coccidian oocyst. These include measurements of length and width of oocyst, sporocysts, sporozoites and polar granules, in addition to oocyst wall thickness measured at the equator. These measurements are then used to calculate length over width ratios for the oocyst and sporocyst. All data is presented as mean ± standard deviation for both sampling sites and total oocysts measured.

Table 2.1 Oocyst measurements

	Burwood (n=11)	Cape Sanctuary (n=11)	Total (n=22)	Sig.	η^2
Oocyst length (µm)	18.0 ±1.0	18.0 ±0.9	18.0 ±0.9	NS	NS
Oocyst width (µm)	15.3 ±0.6	15.2 ±0.8	15.2 ±0.7	NS	NS
Oocyst L:W ratio	1.2 ±0.1	1.2 ±0.1	1.2 ±0.1	NS	NS
Wall thickness (µm)	1.0 ±0.1	0.9 ±0.1	0.9 ±0.1	0.03	0.21
Polar granule length (µm)	1.8 ±0.3	1.7 ±0.2	1.8 ±0.2	NS	NS
Polar granule width (µm)	1.2 ±0.2	1.1 ±0.2	1.2 ±0.2	NS	NS
Sporocyst length (µm)	9.3 ±1.2	8.6 ±0.7	9.0 ±1.0	NS	NS
Sporocyst width (µm)	6.1 ±0.7	6.2 ±0.7	6.2 ±0.7	NS	NS
Sporocyst L:W ratio	1.5 ±0.2	1.4 ±0.2	1.5 ±0.2	NS	NS
Sporozoite length (µm)	7.7 ±1.2	6.9 ±0.4	7.3 ±0.9	NS	NS
Sporozoites width (µm)	3.1 ±0.3	3.3 ±0.4	3.2 ±0.4	NS	NS

Mean ± standard deviation, with ANOVA significance and Eta^2 for significant (P-value <0.05 or η^2>0.14) interactions between location of sampling and the given measurement. NS = not significant

Oocyst wall thickness was the only measurement where there was statistically significant variation between the oocysts collected from the two different locations, Burwood and Cape Sanctuary (Table 2.1). In all other parameters there was no variation between sampling sites.

2.3.3. Histological survey

Between 2008 and 2017 post-mortem reports were available for 103 Takahē submissions, excluding embryos and neonates. Of these 26 were positive for coccidian oocysts on parasitological examination of faecal or gastrointestinal contents. Stored segments or complete gastrointestinal tracts were preserved for ten of these cases. In eight cases the mucosa of the gastrointestinal tract was severely autolysed and no normal tissue architecture could be located. In one out of the two remaining cases microgamonts were identified in epithelial cells of the caudal ileum and caecum (Figure 2-2).

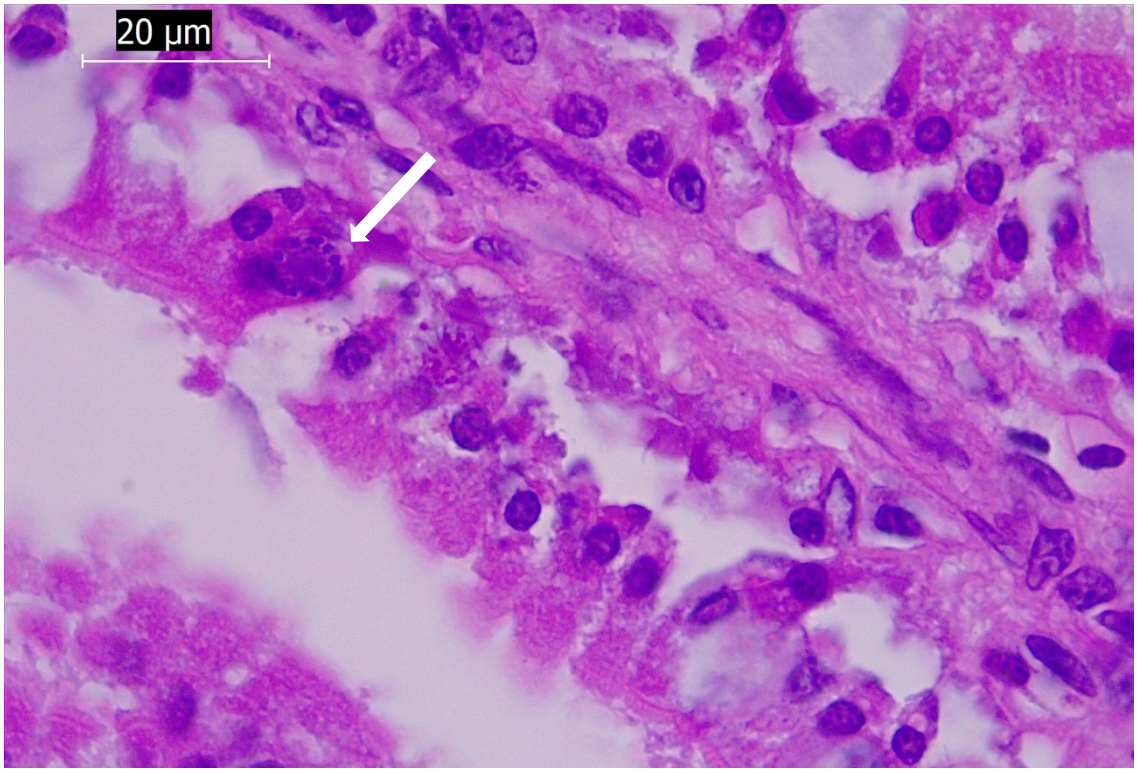

Figure 2-2: Micrograph of Takahē intestinal mucosa of caudal ileum, arrow indicating microgamont within host cell (H&E, 100x).

2.3.4. Morphological description

Type host: *Porphyrio hochstetteri* (Aves, Gruiformes, Rallidae)

Type locality: Burwood Takahē Breeding Centre, Burwood Bush, South Island, New Zealand (45°33'54"S, 168°04'18"E)

Prevalence: Unknown.

Sporulation: Unknown.

Site of infection: Unknown. Microgamonts detected in caudal ileum and caecum, site of asexual reproduction not identified.

Description of sporulated oocysts: Tetrasporic dizoic sporulated oocysts characteristic of the genus *Eimeria*. Measurements are presented as Mean ± standard deviation (range). Shape of oocyst was ovoid, L:W ratio 1.2±0.1 (1.1-1.3). Oocyst dimensions were 18.0±0.9 x 15.2±0.7 (16.2–19.9 x 13.9–16.6) µm. Wall was smooth, double layered and of thickness 0.9±0.1 (0.7–1.1) µm. The micropyle was absent. The oocyst residiuum was present and formed one granular spherical mass. A large, smooth polar granule was consistently present with some variation in shape from spherical to broadly obovate and measuring 1.2±0.2 x 1.8±0.2 (0.7-1.5 x 1.4-2.3) µm.

Description of sporocysts and sporozoites: Sporocysts were ovoid to elongate 9.0±1.0 x 6.2±0.7 (7.4–11.0 x 5.4-8.0) µm and L:W ratio 1.5±0.2 (1.0-1.8). A granular sporocyst residuum was present as was a Steida body, however, sub-Steida body or parasteida bodies were absent. Each sporocyst contained 2 ovoid to elongate sporozoites 7.3±0.9 x 3.2±0.4 (6.0-9.5 x 2.8-4.2) µm. One large spherical posterior and one small spherical anterior refractile body was present within each sporozoite.

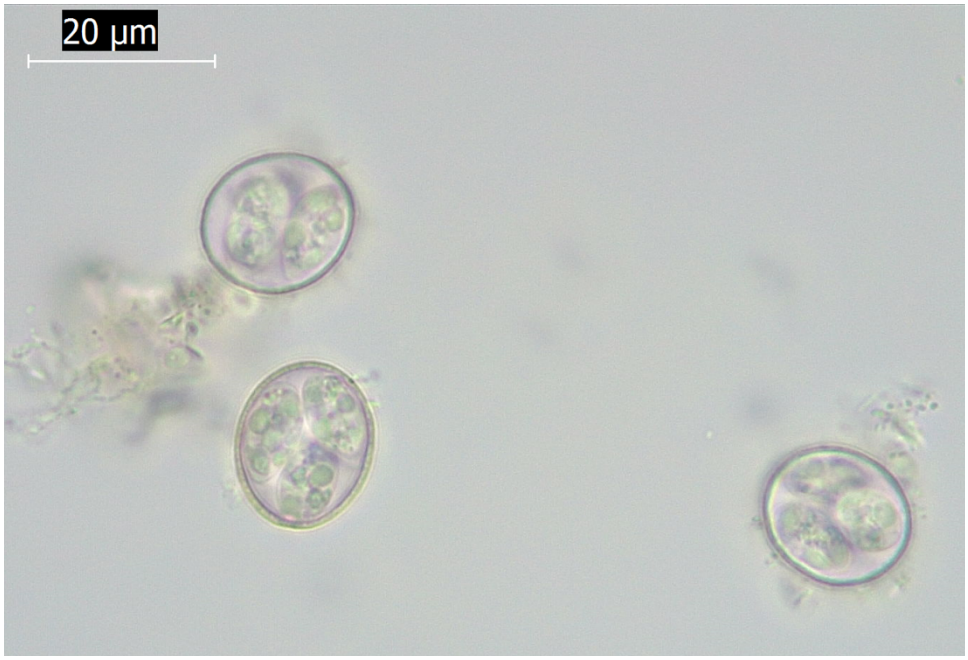

Figure 2-3: Micrograph of three sporulated oocysts (x100) of a novel *Eimeria* sp. isolated from a Takahē host.

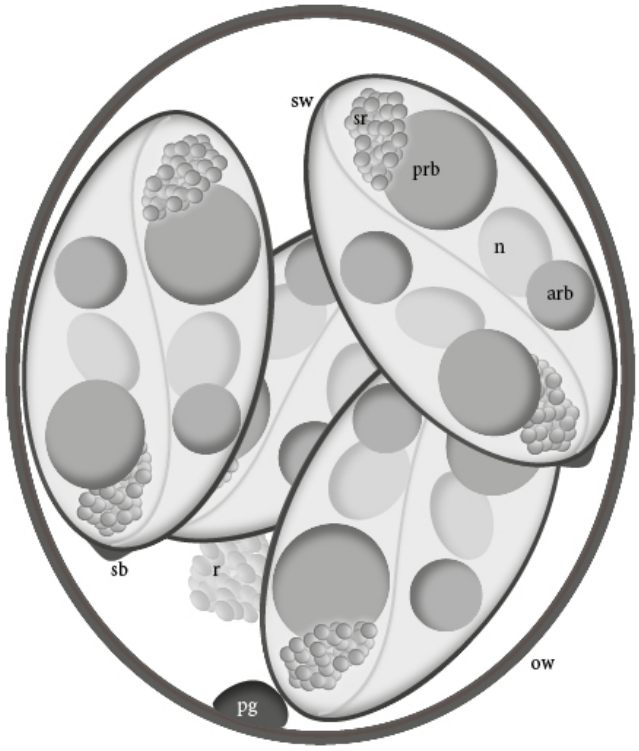

Figure 2-4: Annotated schematic drawing of a sporulated oocyst of a novel *Eimeria* sp. isolated from Takahē.

Labels indicating oocyst wall (ow), polar granule (pg), oocyst residium (r), Steida body (sb), sporocyst wall (sw), sporozoite residium (sr), posterior refractile body (prb), sporozoite nucleus (n) and anterior refractile body (arb).

2.4. Discussion

This is the first description of an *Eimeria* sp. protozoa from a Takahē host. In line with currently accepted guidelines, new species descriptions should be compared with those isolated from host species within the same genus or family (Duszynski & Wilber, 1997). There are five *Eimeria* species described from hosts in the family Rallidae, key features of the description of 3 of these species is presented in Table 2.2. Unfortunately, the descriptions of the remaining two species are not published in English and weren't available for comparison. *Eimeria paludosa* has been isolated from several hosts within this family including the Purple Swamphen (*Porphyrio porphyrio*) which belongs to the same genus as the Takahē. A major morphological characteristic of *E. paludosa* is the presence of two distinct micropyles (McAllister & Upton, 1990). However, in the newly described oocyst the micropyle is absent. A further distinction is *E. paludosa* has a single refractile body per sporozoite in contrast to the species reported here which has two. *Eimeria porphyrulae* and *E. neinei* oocysts are considerably larger than the currently described species. There is some overlap between the size the new species and *E. crecis*, described from the corncrake (*Crex crex*) (Jeanes *et al.*, 2013). Like the new species the micropyle is absent, however *E. crecis* also lacks an oocyst residuum and was spherical in shape (L:W 1.1) in direct contrast to this new ovoid species. Consequently, this morphological description of an *Eimeria* sp. from the Takahē host is distinct from other species previously described from family: Rallidae. Historically description of new protozoal species was based on descriptive characteristics of the sporulated oocyst and host factors, as described here for the proposed new Takahē coccidia. However, increasingly molecular description is being added to morphological description for the characterisation of species (Adl *et al.*, 2007; Jeanes *et al.*, 2013) Molecular analysis was outside the scope of the current project but would now be required to complete the description of this new *Eimeria* species.

Initial observations on the Takahē *Eimeria* sp. suggest that oocysts sporulated rapidly and despite exposure to severely cold temperatures. Anecdotally oocysts were often partially sporulated by the time they arrived at testing locations within days of collection and faecal samples that were frozen at the time of collection have been reported to produce oocysts capable of subsequent sporulation. This marked cold adaption of the

Table 2.2 Eimeria sp. coccidia described from Rallidae hosts

Comparative morphology of some *Eimeria* sp. described from hosts of the family Rallidae

Coccidia	Hosts	Reference	Oocysts					Sporocysts				
			Shape	Measurements	L:W ratio	Wall	Micropyle	Shape	Measurements	Steida body	Substeida body	Sporozoite refractile body
E. paludosa	American coot (*Fulica americana*) White-breasted waterhen (*Amaurornis phoenicurus*) Common coot (*Fulica atra*) Common gallinule (*Gallinula chloropus*) Purple swamphen (*Porphyrio porphyrio*) Dusky Moorhen (*Gallinula tenebrosa*)	(McAllister & Upton, 1990; Yang *et al.*, 2014)	ovoid	17.3 x 13.3 (16.3-17.9 x 12.7-13.9)	1.3	1.0	present -2 visible with central polar granule	elongate ovoid	8.4 x 5.1 (8.0-8.9 x 4.9-5.5)	present	present	one
E. crecis	Corncrake (*Crex crex*)	(Jeanes *et al.*, 2013)	spherical	15.3 x 14.3 (13-18 x 12-16)	1.1	1.1	absent	ellipsoid	9.2 x 5.6 (8-10 x 5-7)	present	present	two (AP*)
E neinei	Corncrake (*Crex crex*)	(Jeanes *et al.*, 2013)	ellipsoidal	23.6 x 18.1 (21-26 x 17-20)	1.3	1.4	present	Elongate ovoid	12.6 x 6.5 (11-14 x 6-8)	present	present	two (AP*)
E. porphyrulae	Purple gallinule (*Porphyrula martinica*)	(Lainson, 1994)	ellipsoidal	22.4 x 17.7 (20.0-23.7 x 16.2-18.7)	1.3	1.25	present	Elongate piriform	17.5 x 9.0 (17.0-19.0 x 8.0-10.0)	present	present	two (AP*)
novel *Eimeria* sp.	South Island Takahē (*Porphyrio hochstetterii*)	This study	ovoid	18.0 x 15.2 (16.2-19.9 x 13.9-16.6)	1.2	0.9	absent	Elongate ovoid	9.0 x 6.2 (7.4-11.0 x 5.4-8.0)	present	absent	two (AP*)

*AP= refractile bodies located anterior and posterior to sporozoite nucleus.

Takahē *Eimeria* warrants further investigation. The conditions in which oocysts are stored during sporulation can potentially alter morphological measurements (Berto *et al.*, 2014; Duszynski & Gardner, 1991). Therefore, fresh faecal samples were obtained that could be treated in a standardised manner prior to and during sporulation in order to minimise this contribution to polymorphism. Fresh samples with sufficient oocysts for analysis were obtained from two different geographic locations, Burwood and Cape Sanctuary. All Takahē populations are highly interconnected, with birds regularly moving between sites and particularly through the central breeding centre at Burwood (Grange *et al.*, 2014). Therefore, it is assumed that the parasite species would be consistent across the whole Takahē population. However, it was necessary to evaluate the data for differences between and across the two locations, in case the data represented more than one species of *Eimeria*.

Linear regression of oocyst width on length has been used to differentiate populations of different oocyst species within the same species of host or data set. In this analysis R^2 values of >0.5 indicate that the data points are all located close to the regression line and therefore likely represent variation within a single species population. For the presented data, R^2 was calculated at 0.43, potentially indicating an unexpected level of dispersion from the linear model. However, it has been suggested that ellipsoid *Eimeria* sp., with L:W ratios of greater than 1.1 (1.2 in this study) are likely to have a greater variation in R^2 due to slight variations in the transect observed under light microscopy (Berto *et al.*, 2014). ANOVA analysis failed to find significant differences between the oocysts assessed from Burwood verse Cape Sanctuary in any parameter except wall thickness. The Eta^2 measure of association suggests a large statistical effect of location on wall thickness, however, given the strong positive bias for Eta^2 when the sample size is less than 50, this apparent statistically significant effect is likely within the margin of error. The strong consistency in descriptive morphology amongst all oocysts, across both locations and all hosts is most consistent with a single species of *Eimeria*.

Until this current review of archived formalin fixed tissues, the coccidia parasite had not been visualised within the tissues of the Takahē. This is despite the large number of the deceased Takahē being sent for post mortem and exceptionally high faecal oocyst counts at some sites over this same period. This is likely due to the poor quality of

submitted specimens, with the majority being in a severely advanced state of decomposition at the time they are presented for post-mortem examination (McLelland *et al.*, 2011). It is not uncommon for there to be a delay of up to two months between estimated date of death and submission for pathology. For birds that die in the remote areas of the Murchison Mountains this delay can extend to many months, especially over winter. The target tissue for the parasite is expected to be the cells lining the gastrointestinal tract, which degrade rapidly post-mortem and were rarely intact in submitted specimens. The two specimens examined that had partially intact intestinal mucosa were both euthanased locally to the pathology facility and the gastrointestinal tract was promptly washed and fixed in formalin for histological examination. Even with this almost ideal preparation only very few parasitic organisms could be seen within host cells. This is consistent with this bird having coccidiasis, or a low intensity infection not causing clinical disease, with low numbers of parasites and host cells affected. Despite its limitations the review of archived tissues successfully demonstrated asexual replication of the parasite within mucosal cells of the aboral ileum and caecum of a Takahē with coccidiasis. The endangered status of the Takahē prevents consideration of experiments requiring deliberate infection and culling of birds that would be required to further examine the lifecycle of the parasite. Consequently, a full understanding may never be achieved and fragments of information available from opportunistic sampling will be all that is available.

2.5. Conclusion

A novel *Eimeria* sp. is described for the first time from the endangered Takahē (*Porphyrio hochstetteri*). Morphologically similar oocysts were isolated from four different individuals across two geographic locations linked by extensive bird translocation. Oocysts were ovoid (L:W ratio 1.2) and average dimensions 18.0 x 15.2 μm, with walls 1.0 μm thick at the equator. A micropyle was absent and the oocyst residuum was present as a granular spherical mass. Four sporocysts elongate ovoid in shape (L:W ratio 1.4), average dimensions 9.0 x 6.2 μm and each containing two sporozoites with anterior and posterior refractile bodies. This morphological description is distinct from other *Eimeria* spp. described in hosts of the family Rallidae.

Chapter Three:

A review of fresh and archived faecal samples to establish the geographic distribution of *Eimeria* sp. in South Island Takahē (*Porphyrio hochstetteri*) and variation in faecal oocysts counts over three years at a central breeding location

3. A review of fresh and archived faecal samples to establish the geographic distribution of *Eimeria* sp. in South Island Takahē (*Porphyrio hochstetteri*) and variation in faecal oocysts counts over three years at a central breeding location

3.1. Chapter Introduction

The South Island Takahē (*Porphyrio hochstetteri*), hereafter referred to as Takahē, is a large flightless rail endemic to New Zealand and once thought to be extinct. Since the rediscovery of the species in 1948, conservation efforts have aimed to stabilise the decline and increase the population. Conservation efforts have included predator and competitor control in the wild range of the Murchison Mountains, management of the remnant wild population, translocation of birds to predator-free areas, captive rearing and subsequently breeding for release (Wickes *et al.*, 2009). The combined effects of this conservation management has seen the current Takahē population rise to around 340 individuals spread across the wild Fiordland population, predator-free islands or mainland reserves and the central breeding unit established at Burwood Bush Reserve in the 1980s (Takahē Recovery Programme, 2017). The metapopulation of a few hundred individuals is intensively managed to maintain as genetically diverse a population as possible. As a result there are a large number of translocations of individuals each year with a consequent high level of interconnectedness between populations and a significant portion of the population passing through a central hub, the Burwood Takahē Breeding Centre (hereafter referred to as Burwood) at least once in their lifetime (Grange *et al.*, 2014).

Coccidian oocysts have been identified in faecal samples from Takahē since intermittent screening began in the 1980s. Unconfirmed involvement of coccidia in the death of an adult Takahē and an apparent rise in individual oocyst counts on routine parasitological screening has led to further enquiring into this host-parasite relationship. Having

identified this coccidian as a unique *Eimeria* sp. in Chapter 2, in this chapter a broad scale assessment of geographic distribution was undertaken of the distribution of the eimerian parasite across the locations used for current Takahē populations using fresh and archived samples. A longitudinal survey was conducted of faecal oocyst counts at the central Burwood site.

3.2. Materials and Methods

3.2.1. Source of samples

Individually identified faecal samples had been collected between March 2012 and April 2013 from five Takahē populations as described in the work by Grange et.al. (2017). The remaining sites were Burwood, Mana Island, Murchison Mountains, Tiritiri Matangi Island and a private island (the location of this island must remain confidential under agreement with the owners). Since collection the samples were stored in individually marked and sealed containers and refrigerated at 7^{o}C. For the current work faecal oocyst counts were carried out as described below on the homogenised faecal sample.

Faecal samples were submitted between April 2015 and July 2017 to the Massey University parasitology laboratory, as part of routine management practices at the Burwood. The reported oocyst counts per gram of faeces were made available for a longitudinal assessment of faecal oocyst shedding at this location. A subset of these samples were identified with time of sampling, which enabled an analysis of diurnal shedding of oocysts.

3.2.2. Faecal floatation technique

The stored Takahē faecal samples were subjected to faecal floatation using a standard veterinary protocol (Zajac & Conboy, 2012). Briefly, the large fibrous faecal sample was broken up to allow more representative sub-sampling. One gram of the faecal material suspended in 33% zinc sulphate solution (S.G. 1.2) and agitated till all visible plant fibres were separated. The suspension was then strained through a fine nylon mesh sieve to remove large particles and placed into a 15 mL centrifuge tube. Additional zinc sulphate solution was added to fill the test tube and a coverslip was placed on the top of the test tube, ensuring total contact between coverslip and the suspension. Samples were then

centrifuged in a swinging bucket centrifuge at 177g for 5 minutes. The coverslip was then placed on a microscope slide and examined under light microscopy (Olympus CX41) for the presence of coccidia. The entire coverslip was examined and oocysts counted at 300x magnification, except for slides with very high oocyst counts (> 20 oocysts per 20x field). For these concentrated samples the number of oocysts was counted in a central transect of the coverslip one field of view wide and the resulting number multiplied by 25 as previously calibrated on this microscope. Oocyst counts were carried out on the faecal samples submitted to the Massey University parasitology laboratory using the protocols as described above.

3.2.3. Data analysis

Data from the historical samples was initially evaluated as counts of oocysts per gram (opg) of faeces and found to not be normally distributed. Consequently, the relationship between opg and location or age at sampling were assessed using a non-parametric Kruskal-Wallis test. Data was subsequently converted to a binomial measure of presence or absence of oocysts. This presence/absence data was further analysed using Pearson's Chi-square test for independence and Fischer's exact one-sided test for the effects of host age category, sex or location of the population on the presence or absence of oocysts. Birds less than 12 months of age were considered juvenile for the purposes of this analysis.

3.3. Results

3.3.1. Geographic variation in coccidia shedding

There was a significant (P=0.009) difference in the distribution of opg results at the different locations Figure 3-1. *Post hoc* pairwise analysis with a Bonferroni correction indicated a significantly different distribution (p=0.022) between the Private Island and Mana Island.

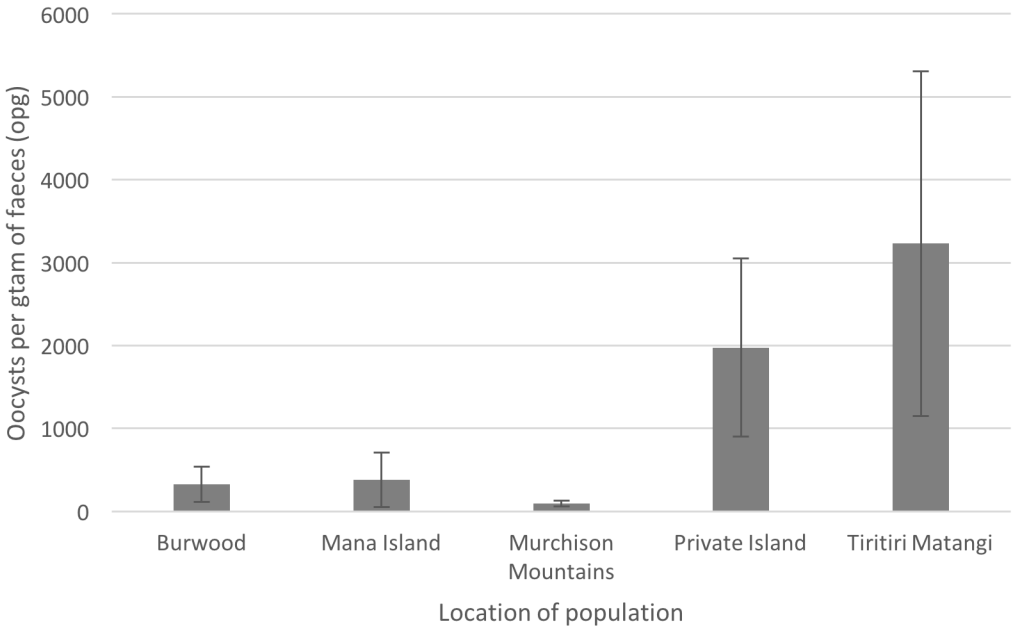

Figure 3-1: Coccidian oocyst counts in historical samples from Takahē over the period 2012-2013, measured in oocysts per gram of faeces at five collection locations (Mean +/- standard error)

The presence or absence of oocysts in individual faecal samples at each site is displayed in Figure 3-2. All sites assessed, except for Maud Island, had birds shedding *Eimeria* sp. oocysts.

There was a significant effect of location on the presence or absence of oocysts in the sample (Chi-squared P=0.007). Samples from the private island and Tiritiri Matangi Island had a higher proportion of positive results, while those from the Murchison mountains were more likely to be negative.

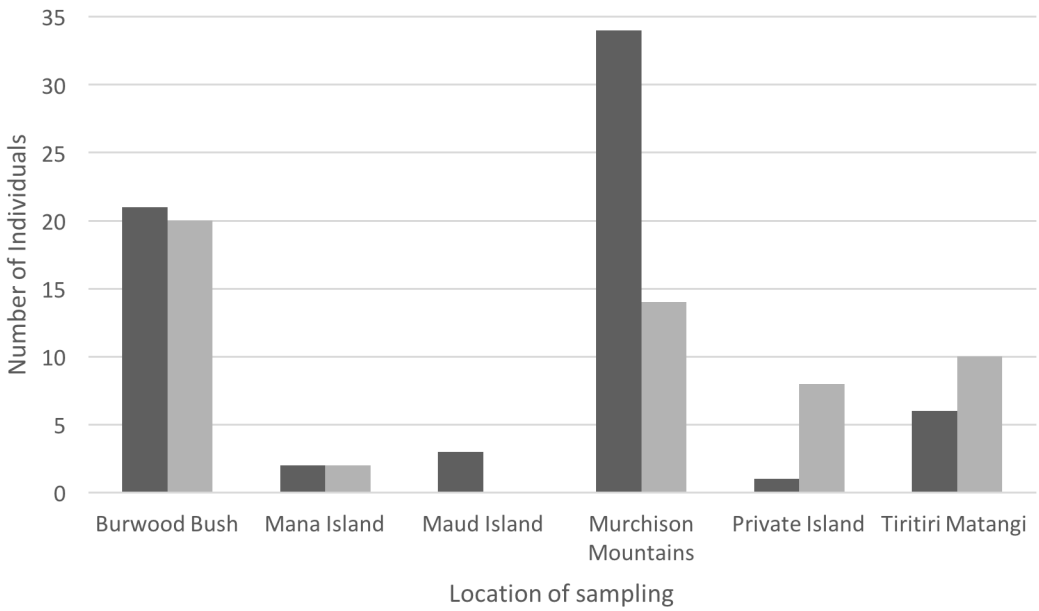

Figure 3-2: Coccidian presence in historical faecal samples from Takahē, with the number of individual samples from each site without (dark grey) and with (light grey) oocysts across each population location

Oocysts from some species of *Eimeria* are known to degrade over time, consequently it is normal practice to carry out parasitological examinations as soon as practical after collection of the faecal sample (Zajac & Conboy, 2012). To assess potential loss of oocysts during the prolonged storage of the historical samples the proportion of positive and negative samples was compared to months in storage before faecal floatation. There was no significant effect of the time the sample had been in storage on the presence or absence of oocysts and almost half the tested samples were positive when stored for the longest time, 57 months (Figure 3-3).

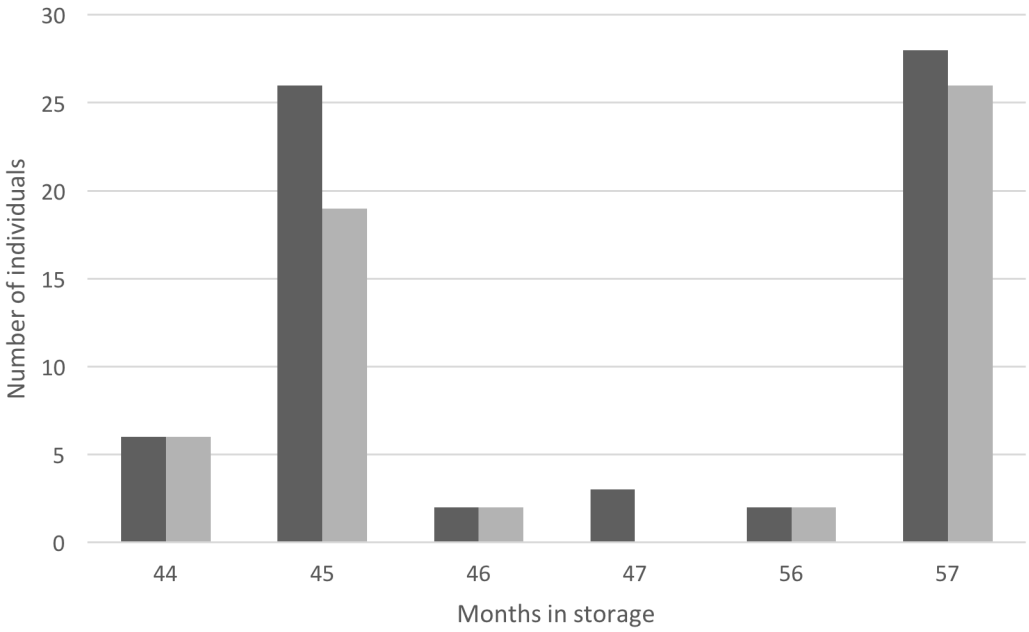

Figure 3-3: Results of analysis of historical faecal samples from Takahē showing negative (dark grey) and positive (light grey) results for coccidia oocysts categorised by different cold storage periods. There was no significant difference in the presence or absence of oocysts between the cold storage periods.

3.3.2. Annual variation in oocyst shedding

The oocyst counts from routine management samples from the Takahē breeding centre at Burwood showed significantly higher mean oocyst counts in 2015 compared to 2016 and 2017, while the latter were not significantly different from each other (Figure 3-4).

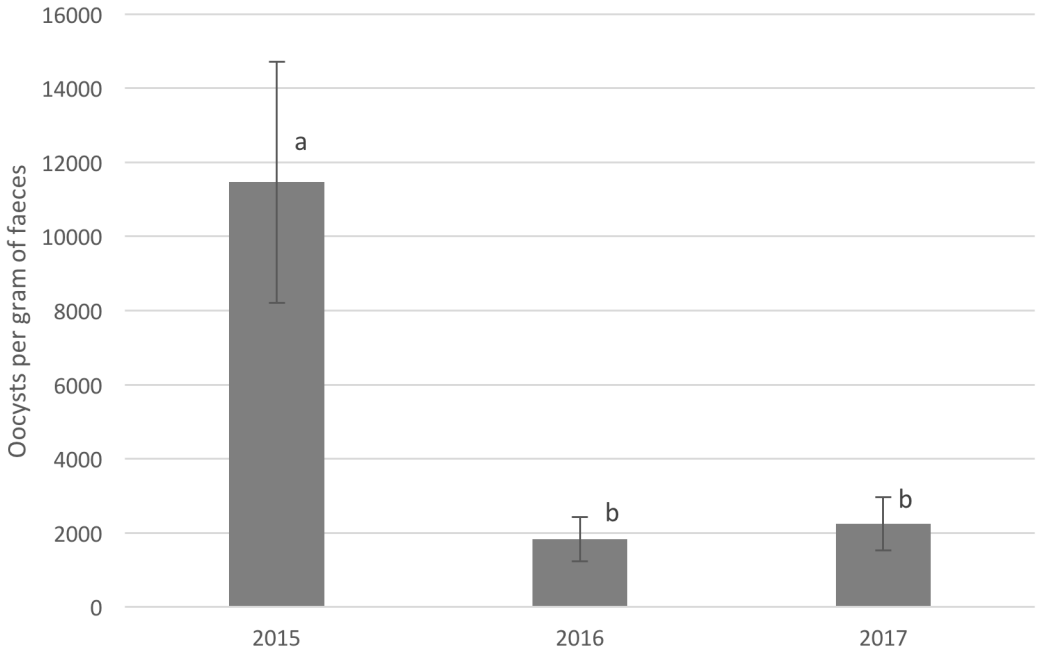

Figure 3-4: Annual variation in the faecal coccidian counts of Takahē held at Burwood, measured in mean oocysts per gram of faeces (different superscript letters indicate significant difference P<0.05)

When this time series data on faecal opg is further explored graphically (Figure 3-5) across these years a pattern of high frequency of higher counts in 2015 can be seen, with a reduction in opg closely associated with changes to management and treatment protocols in August 2015.

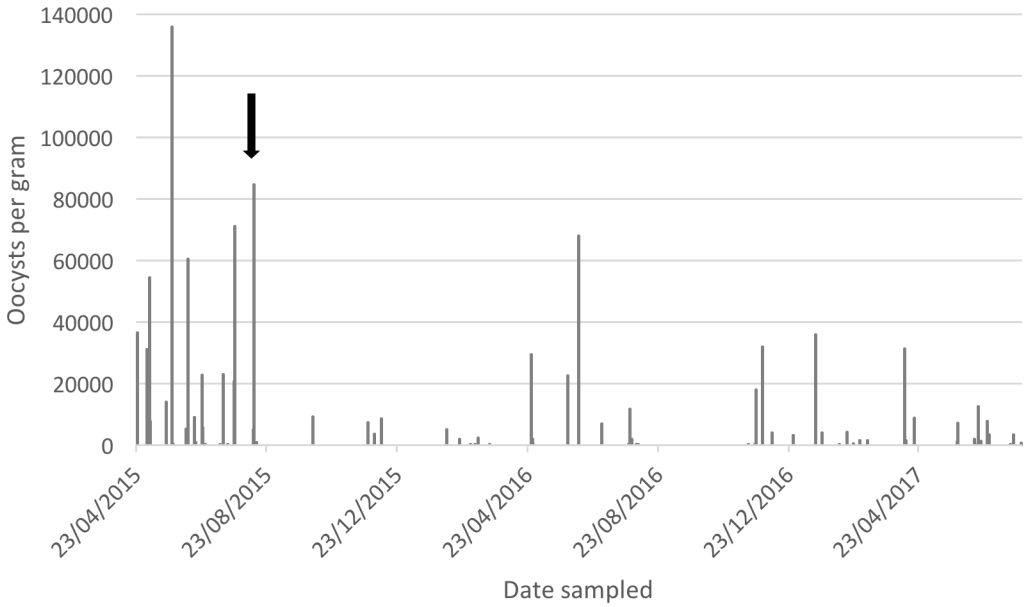

Figure 3-5: Time series of Takahē faecal coccidia counts from Burwood measured in oocysts per gram. The arrow indicates August 2015 when management and treatment changes to mitigate coccidiosis were instigated.

3.3.3. Diurnal variation in oocyst shedding

Samples that were identified with the diurnal time of collection were further analysed, initially with a graphical representation as a scatter plot (Figure 3-6) and subsequently using univariate analysis of variance. Due to unavoidable variation in collection times, for the purposes of analysis, times of collection were grouped into three intervals, "Early" before 10am, "Mid" between 10am and 2pm and "Late" after 2pm (Figure 3-7). There was a significant effect of the time of collection on the presence or absence of *Eimeria* sp. oocysts (Pearson Chi-squared, $P< 0.001$), with a higher likelihood of getting a positive result when a faecal sample is collected from a Takahē after 2pm.

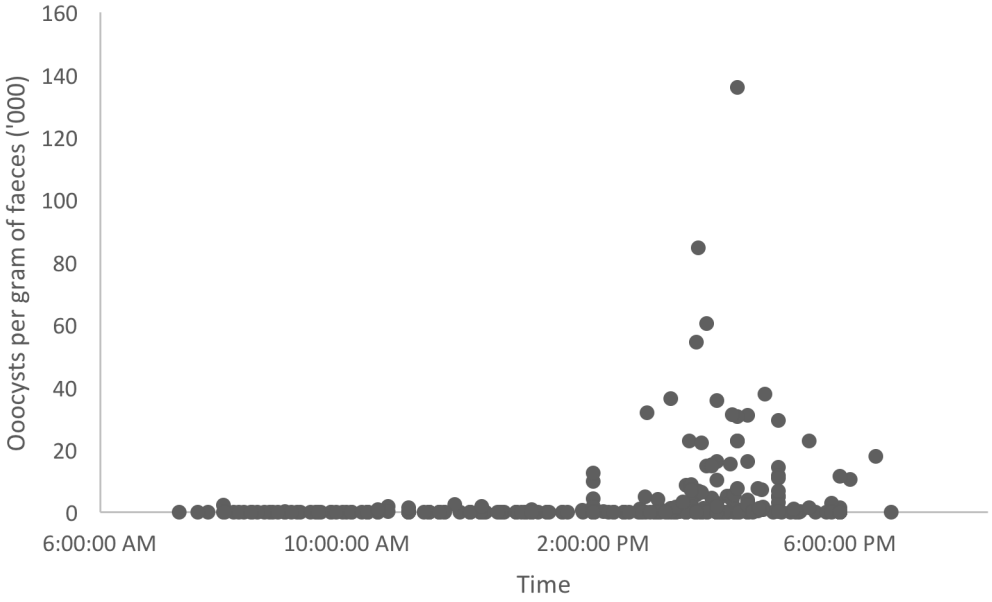

Figure 3-6: Burwood oocysts per gram for all samples identified by time of collection

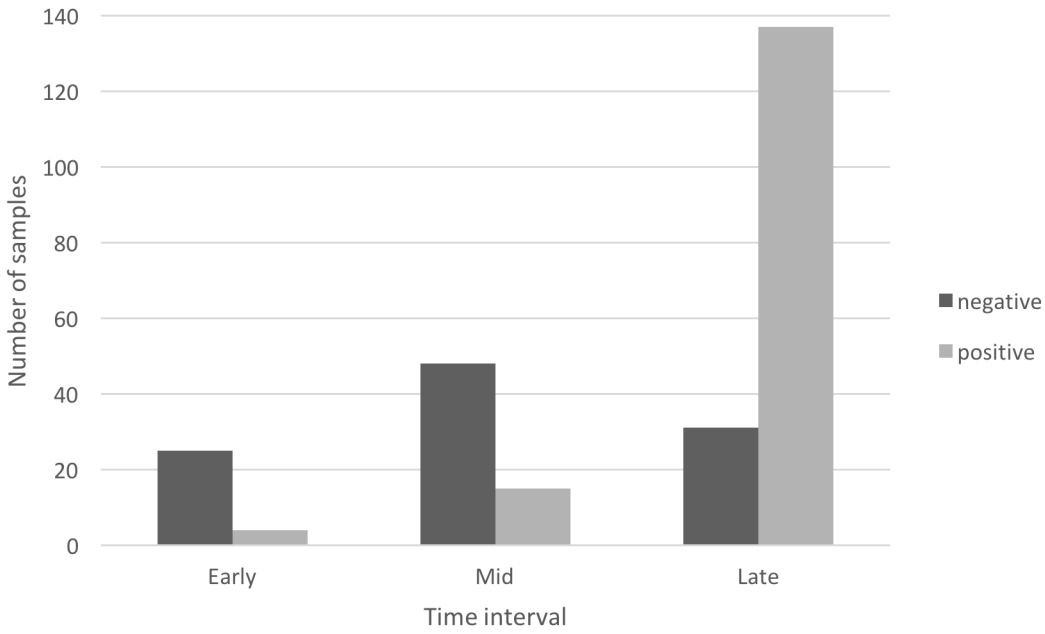

Figure 3-7: Individual Burwood Takahē faecal samples found to be positive and negative for Eimeria at different time intervals across the day

3.3.4. Age and sex effects on oocyst shedding

Within the limitations of the sample size, there was no significant statistical interactions between location, age and sex effects of faecal opg results. Hence, age and sex effects were analysed separately. There was a significant difference in the presence or absence of *Eimeria* sp. detected between juvenile and adult birds (Fisher's exact one-sided P=0.04). The results indicate that juvenile birds were more likely to be shedding oocysts than adult birds at Burwood over this time period (Figure 3-8).

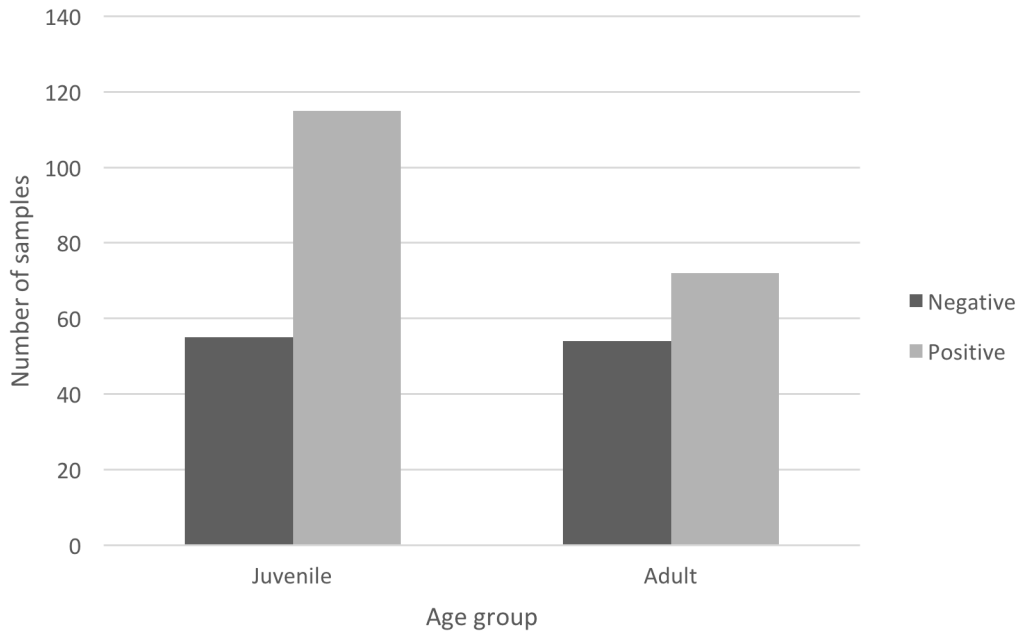

Figure 3-8: Proportion of samples positive or negative for *Eimeria* from juvenile or adult birds

Female Takahē including both adults and juveniles were significantly more likely to be shedding *Eimeria* sp. than males over the period of the study (Fisher's exact one-sided P=0.03) as described in Figure 3-9.

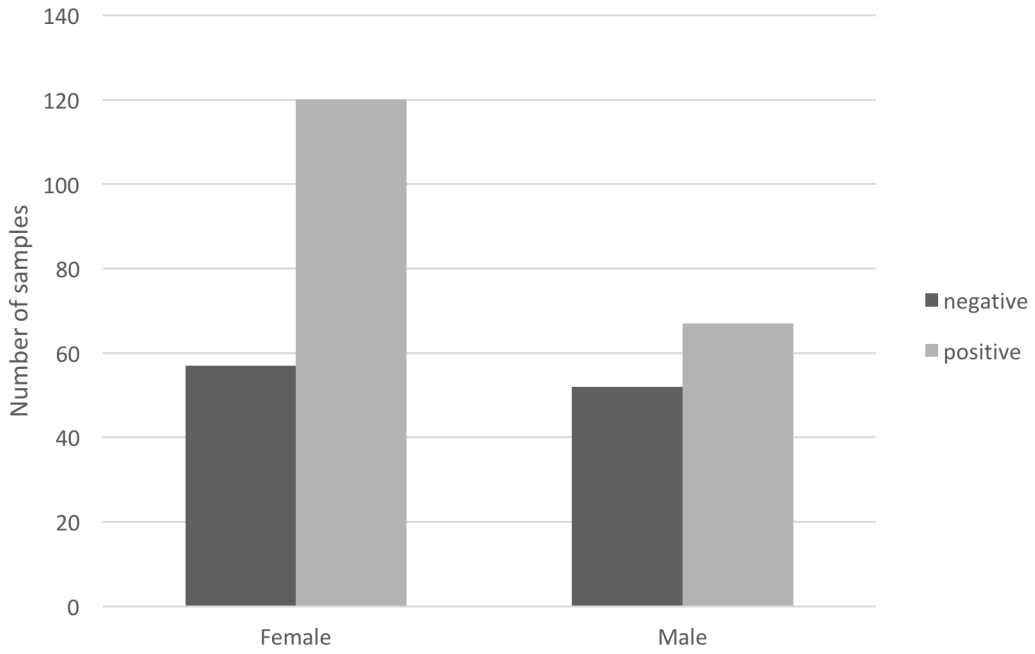

Figure 3-9: Proportion of samples positive and negative for *Eimeria* separated by gender of host

3.4. Discussion

The Takahē metapopulation is divided across a variety of locations and environment types throughout both main islands of New Zealand. However, this metapopulation is intensively managed as three population groups, Murchison Mountains, Burwood and all other sites collected together, within one larger sub-population. These populations are closely linked with numerous translocations of birds between sites each year in efforts to maintain genetic diversity and manage population densities. The analysis of the historically collected samples provides evidence that the *Eimeria* sp. being detected in Takahē has been present at almost all sites examined, with only Maud Island having no positive results. There were only 3 samples available from Maud Island and given the high level of interconnectedness between Takahē populations it is highly likely that *Eimeria* sp. is present on the Island but was not represented in the individuals sampled in 2012. Further samples from birds on the island would be required to confirm this. The current results are evidence that *Eimeria* sp. is likely present throughout wider the Takahē population. The historical samples also confirm that Eimeria sp. has been

present in the Takahē population since at least 2012 and is capable of replication and transmission between hosts in a diversity of environmental conditions. Given that the pre-hunting range of the Takahē covered the majority of the South Island (Bunin & Jamieson, 1995) any co-evolved parasites would have needed to be adaptable to a variety of environments. If the Takahē *Eimeria* sp. prove to be as host specific as is generally considered the case with coccidia of this genus, this parasite has also survived the near extinction of its host species. The decline in potential host population also coincided with the geographic reduction in the range of the hosts to a single valley in Fiordland National Park with overlapping home ranges and may have facilitated cycling of the parasite through the available individuals. The mild clinical impact of the parasite on the host is another characteristic of this host/parasite relationship that may have allowed the *Eimeria* sp. to survive through a period of reduced host availability (Best *et al.*, 2011).

The potential for loss of oocysts over time in storage means care must be taken in interpretation of the oocyst per gram of faeces results from the historical samples. It is possible that some results may be underestimates or that some negative results had low counts when collected. With this caveat, there were no significant differences in oocyst levels between the different locations assessed. However, there was a tendency for oocyst levels to be higher on Tiritiri Matangi and the private island. This would be consistent with previously published results from these samples related to *Salmonella* presence (Grange *et al.*, 2017). The increased prevalence of *Salmonella*, and from these results *Eimeria*, are likely due local conditions providing an environment conducive to longer survival and more efficient transmission of these pathogens. The private island at the time of sampling had a higher stocking density of Takahē compared to other sites and limited water access points (Grange *et al.*, 2017). *Eimeria* oocysts have a reported longer survival time in areas of higher moisture and low sunlight penetration (Parker & Jones, 1990), this longer environmental persistence together with increased concentration of potential hosts would effectively increase the transmission potential of the parasite amongst the Takahē population. The population density of Takahē on Tiritiri Matangi was not as high as on the private island when considered as the number of birds over the land area of the island. However, on the area of land utilised by the

Takahē on Tiritiri Matangi was limited to a few areas of grassland around areas of human habitation, effectively increasing the stocking density and again concentrating available hosts in a usually high rainfall environment.

The Burwood is a central hub of the larger Takahē population with birds moving to and from this location on a regular basis (Grange *et al.*, 2014). The faecal *Eimeria* counts that were made available from 2015 to 2017 demonstrate the prevalence of this parasite at this important site across this period. The high faecal *Eimeria* counts in multiple individuals in early 2015 was a major stimulus for investigation into the importance of *Eimeria* in the Takahē population and its potential impact on the host species conservation efforts. The repeated elevated counts, apparently despite treatment of some individuals with toltrazuril (Baycox® coccidiocide for piglets, 50mg/mL, Bayer Animal Health), prompted a re-evaluation of treatment and husbandry procedures in August 2015, indicated as an arrow in Figure 3-5. At this time the medication previously being used to treat any birds with counts in excess of 1,000opg was discarded and replaced with a new supply. The former medication used had become altered in consistency and colour despite being within the indicated shelf life of the product. The standard procedure at this time involved taking the whole bottle of medication into the field where the environmental extremes at some times of year were likely to have exceed the recommended storage range for the product and probably resulted in freezing. It was also noted that the medication was often viscous when being administered by crop tube and a visible proportion of the dose was seen to remain in the crop tube instead of passing into the bird, resulting in a less than optimal dose being delivered. In addition to replacing the altered medication, treatment recommendations were made to take only the required number of doses into the field, to dilute the required dose before administration into a 5mL of water to make it less viscous and to administer an additional 20mL of water after administering the dose of medication in order to flush any remnants from the crop tube. At the same time husbandry recommendations were made to reduce host exposure to large concentrations of infective eimerian oocysts. One of these strategies included relocating the pellet feeding stations in each pen on a weekly basis to a new location at least 5 metres from the previous location in an attempt to disperse faecal build up. The birds are known to

congregate around the feeding sites and the use of stationary supplementary feeding sites has been associated with build of parasite populations in other species (Blanco *et al.*, 2017). Establishment of watering points that could be more easily and thoroughly cleaned in all pens was also recommended. There is a strong temporal association with these management changes and the reduced frequency and maximum *Eimeria* sp. oocyst counts throughout late 2015 and the subsequent two years. A recommendation to allow pens that held birds with high coccidia counts to remain unused for 6-12 months was considered to be impractical by the staff of Burwood. Further environmental management of the site is limited by the terrain, vegetation and climate.

Temporal variation in oocyst shedding has been demonstrated in some host parasite relationships and not others (Brawner & Hill, 1999; Villanúa *et al.*, 2006). This pattern of diurnal shedding has substantial management implications, staffing and environmental limitations mean that standardisation of faecal collection times to late afternoon for disease screening across all Takahē populations is very difficult to achieve. Consequently, the time of day a faecal sample was produced and collected may be important to the interpretation of the result (Filipiak *et al.*, 2009). There was an uneven distribution of available results across the hours of the day with the majority of samples being collected in the afternoon, therefore the statistical analysis is less robust. However, the results presented here appear to confirm an earlier unpublished pilot investigation which suggested Takahē coccidia were shed in increased numbers in the late afternoon. However, the results of this survey were complicated not only by missing data points limiting the validity of the statistical methods applied, but also the procedure, during this period, of staff collecting faecal samples that were “fresh” in appearance and labelling them with the time of collection. Consequently, there may be more variability in the temporal clustering of the diurnal shedding than the results suggest. Despite these limitations, these preliminary results are supportive of the existence of a diurnal shedding pattern for the *Eimeria* sp. with a peak of shedding in the late afternoon.

Juvenile Takahē at Burwood are more likely to be positive for *Eimeria* sp. oocysts than adult birds. This is consistent with the pattern seen in other host parasite relationships where the young or less immunologically developed hosts, are more likely to be affected

by parasites (Moller *et al.*, 1990; Reed *et al.*, 2012). There may also be increased exposure to infective oocysts in the juvenile birds due to feeding on spilled pellets on the ground before they can physically reach the pellet hopper, this ground may be contaminated with faecal material. The apparent *Eimeria* sp. infection bias towards female Takahē may be due to sampling of a few female birds that returned initially high counts and had repeated monitoring samples submitted as part of their treatment program. An effect of host sex on coccidia oocyst shedding or infection has not been documented in other species and given the sample size of this study this result should be interpreted with care.

3.5. Conclusion

This investigation documented the wide geographical distribution of the *Eimeria* sp. across most of the fragmented conservation management network for Takahē. Despite the fragmentation and reduction of the host population, this presumed host-specific parasite appears to be thriving. These results are consistent with a highly co-evolved host parasite relationship, stable transmission pathways for the parasite and suggests a long association of the *Eimeria* sp. and the Takahē. Modification of treatment protocols and management actions for coccidia in Takahē at the central captive breeding centre of the Takahē conservation network was closely associated temporally with a sustained reduction in *Eimeria* sp. shedding rates at the facility. Preliminary results obtained here were supportive of the existence of a diurnal shedding pattern for the *Eimeria* sp. with a peak of shedding in the late afternoon.

Chapter Four:

Investigation of temporal variation in *Eimeria* sp. oocyst shedding by Takahē

4. Investigation of temporal variation in *Eimeria* sp. oocyst shedding by Takahē

4.1. Chapter Introduction

Coccidia morphologically similar to the genus *Eimeria* have been identified in the endangered South Island Takahē (*Porphyrio hochstetteri*) causing intestinal coccidiasis. Non-infective oocysts are passed in the faeces of infected birds, undergo sporulation in the environment to become infective before being ingested by another host. Demonstration of the unsporulated oocyst in faecal samples is the most common method of diagnosing host infection.

Temporal variation describes a characteristic pattern of oocyst shedding in host faeces across the day. Such variations have been reported for host-parasite relationships involving nematodes (Ngongeh, 2017; Wongrak *et al.*, 2015) and for a variety of bird hosts infected with the apicomplexan parasite, *Isospora* (Brawner & Hill, 1999; Misof, 2004). Temporal variation in oocyst shedding is considered an adaptive response by the parasite to ensure that peak shedding rates coincide with the host activity that will ensure optimal transmission rates or oocyst environmental survival (Wongrak *et al.*, 2015). Some species of the genus *Eimeria* are also reported to have characteristic patterns of temporal variation in oocyst shedding (Boughton, 1937; Villanúa *et al.*, 2006). Knowledge of the oocyst shedding pattern of a particular host parasite relationship is important for the standardisation of sampling and the interpretation of results, as samples taken at peak shedding periods will differ from those taken at other times.

An unpublished preliminary study carried out for the Department of Conservation in September 2014 suggested that there was variation in the number of oocysts shed by Takahē across the day (Phil Marsh, August 2015). This study suggested that Takahē coccidia were shed in greater numbers in the late afternoon. However, the study was based on the collection of samples from different individual birds at different times on different days and therefore did not take into account that some birds may have been

infected while others were not and that there may have been variations in parasite burdens between individuals.

The current trial was designed to collect repeated samples from the same birds across different times of day to establish if there was a pattern of higher oocyst shedding at a specific time of day.

4.2. Materials and Methods

4.2.1. Sample collection

Sampling periods were limited to winter to prevent disruption to Takahē conservation activities at other times of year. During each time period, sample collectors were permitted by the Takahē Recovery Group, Department of Conservation, to access 8 pens at the Burwood Takahē Breeding Centre (hereafter referred to as Burwood). Each pen contained a family group of Takahē, between 2 and 6 individual birds, that were housed and managed by Takahē recovery team rangers as part of the Recovery Plan breeding program. Each family group contained a combination of adult and juvenile birds less that 12 months of age. Prior to and after the collection period the birds received a supplementary formulated pellet diet on 2 days each week as part of their normal management. The collection periods were during the winter months of 2016 and 2017 at the request of the Takahē Recovery Team to limit the disturbance to the birds during the Spring to Autumn nesting and rearing seasons.

On each collection day the sample collector observed the birds in two adjacent pens and gradually fed out that days pellet ration to keep the birds within sight. Starting from sunrise (approximately 9am) till sunset (approximately 4:30pm) the birds were observed from a distance until they passed a faecal sample at which point the location of the sample was monitored until the bird moved away of its own accord before the collector quietly collected the sample. Samples were labelled with bird identification, date and time of collection. Samples were stored in refrigeration until they could be analysed.

This sampling protocol was repeated in each group of two pens once more over the course of ten days in either August 2016 or June 2017, with the exception of four pens in 2016 when heavy snow forced the cancellation of the second collection day.

4.2.2. Faecal floatation protocol

The stored Takahē faecal samples were subjected to faecal flotation using a standard veterinary protocol (Zajac & Conboy, 2012). Briefly, the large fibrous faecal sample was broken up to allow more representative sub-sampling. One gram of the faecal material was suspended in 33% Zinc Sulphate solution (S.G. 1.2) and agitated until all visible plant fibres were separated. The suspension was then strained through a fine nylon mesh sieve to remove large particles and placed into a 15 mL centrifuge tube. Additional zinc sulphate solution was added to fill the test tube and a coverslip was placed on the top of the test tube, ensuring total contact between coverslip and the suspension. Samples were then centrifuged in a swinging bucket centrifuge at 177g for 5 minutes. The coverslip was then placed on a microscope slide and examined under light microscopy (Olympus CX41) for the presence of coccidia. The entire coverslip was examined and oocysts counted at 300x magnification, except for slides with very high oocyst counts (> 20 oocysts per 20x field). For these concentrated samples the number of oocysts was counted in a central transect of the coverslip one field of view wide and the resulting number multiplied by 25 as previously calibrated on this microscope.

4.2.3. Statistical analysis

Samples from individual birds that were negative at all faecal collections and therefore were assumed to be free from coccidia were then removed from the data set. Time of collection was converted to a whole hour interval beginning at 9am each day. Univariate analysis of variance was used to analyse faecal coccidia counts, oocysts per gram of faeces for interaction between hour interval, year and day of collection. The results were converted to a positive or negative for frequency analysis of *Eimeria* sp. score. Pearson's Chi squared and Fischer's exact test were used to assess any significant effect of age group, sex, hours since 9am on the samples being positive or negative for *Eimeria* sp.

4.3. Results

Two hundred and forty three faecal samples identified to twenty nine individual birds and time of deposition were collected and analysed. The prevalence of *Eimeria* sp. oocysts in these samples was lower than expected at 13.5% across both years of collection. Of the 29 individual birds sampled, 19 were positive for *Eimeria* sp. oocysts

in at least one faecal sample. The high frequency of negative results prevented further statistical analysis of prevalence.

Mean faecal oocysts per gram of faeces are graphed across time intervals in Figure 4-1. The mean oocyst per gram was higher in the 4pm interval, however this effect over time was not statistically significant.

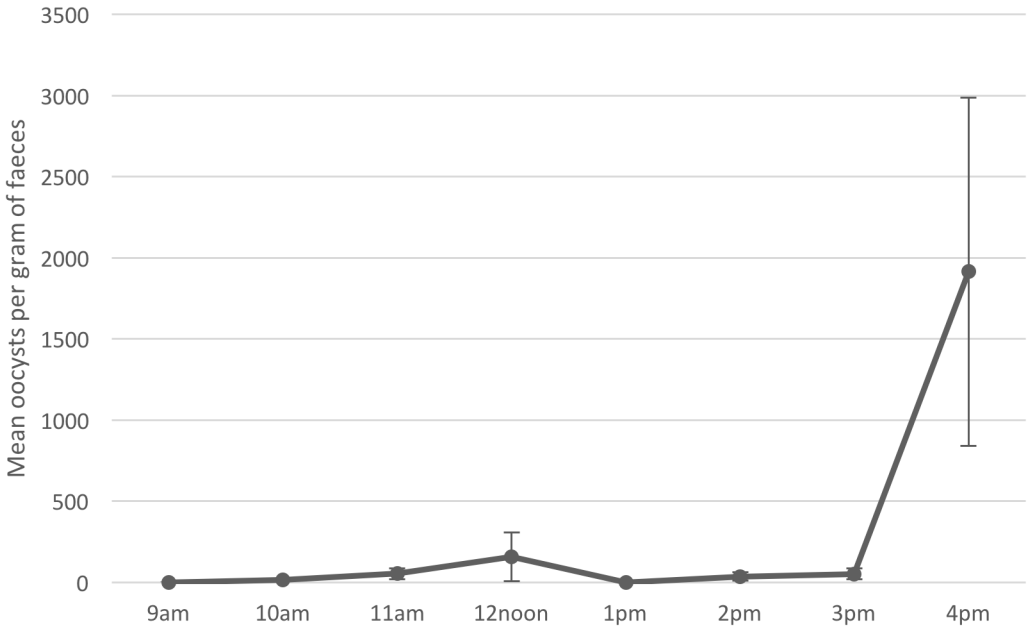

Figure 4-1: Mean oocysts per gram of faeces collated at hourly time intervals

The number of positive and negative samples in each hour interval is displayed graphically in Figure 4-2. While not a statistically significant effect a positive sample is more likely to occur collected after 3pm. There were no significant interactions with sex or age.

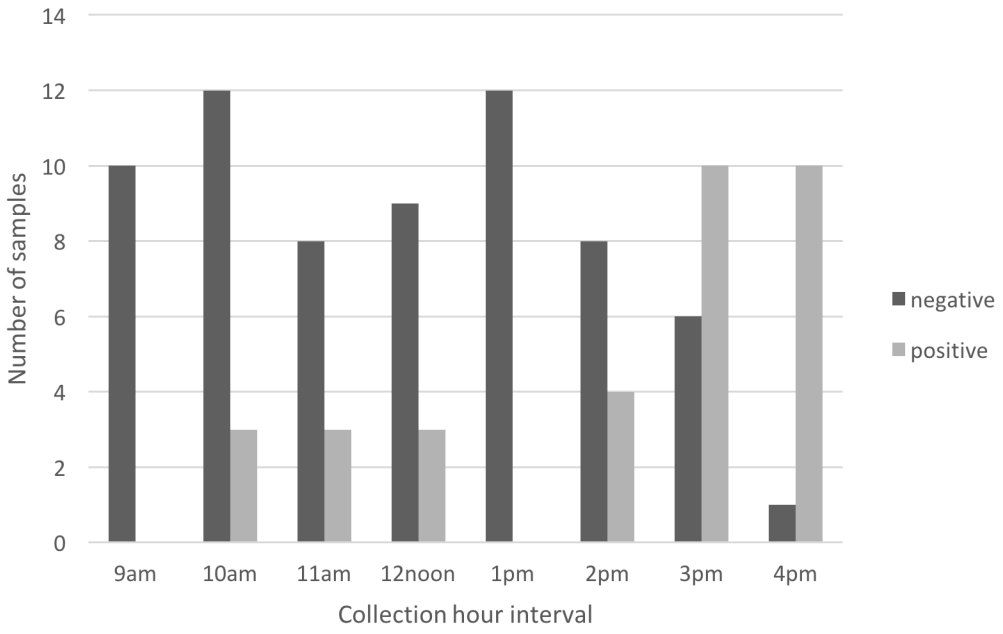

Figure 4-2: Number of faecal samples positive and negative for *Eimeria* sp. oocysts at different hourly intervals across the day

4.4. Discussion

Despite the limitations associated with research on a critically endangered species, this trial showed evidence of temporal variability of oocysts shedding with an observed peak and intensity of shedding towards the evening. However, the shedding rates over the nocturnal hours and in other seasons outside the winter period remain unknown.

The current trial did not find any statistically significant variation in oocyst shedding across the day when measured as either oocysts per gram of faeces or a positive vs negative binary. However, there was a general increase in the amount of oocysts shed and the likelihood of a positive sample being produced after 3pm. The small samples size and low prevalence contributed to the lack of statistical power in the analysis. The lower than anticipated prevalence may have been due to the collections occurring only in the winter months, with heavy snow and short day-length. Warm wet environmental conditions are more often associated with higher coccidia burdens (Conway & McKenzie, 2008b). However, collection during the Summer months was not possible due

to the importance of every breeding opportunity for an endangered species numbering only 340 individuals. Short day lengths at the collection times also resulted in the latest samples being collected around 4:30pm. After this time, darkness fell resulting in the birds rapidly returning to inaccessible roost areas and the birds could not be followed without disturbing them. It is possible that the current results represent the beginning of a crepuscular or nocturnal increase in oocyst shedding and later samples may have produced clearer results. However, one aim of this study was to provide information that would feed back into management decisions for timing of collection of faecal screening samples. At present all staff available to collect samples leave the Takahē breeding pens by 4pm, therefore a recommendation to collect faecal samples later would be impractical.

Temporal variation in oocyst shedding across the day has been demonstrated in a number of host-parasite relationships including nematodes in mice, goats and domestic poultry (Ngongeh, 2017; Wongrak *et al.*, 2015). It has been most widely studied in passerine birds infected with coccidia of the genus *Isospora*. For diurnal passerine hosts infected with *Isospora* sp. there is solid evidence that oocyst shedding increases in the late afternoon in some host-parasite relationships, specifically Eurasian Blackbirds (*Turdus merula*), Housefinches (*Haemorhous mexicanus*) and Greenfinches (*Chorlis chloris*) (Brawner & Hill, 1999; Brown *et al.*, 2001; Filipiak *et al.*, 2009; Martinaud *et al.*, 2009; Misof, 2004; Villanúa *et al.*, 2006). Temporal variation in oocyst shedding, if it occurs in a host-parasite relationship, is likely to be the result of long-term co-evolution with the parasite aiming to increase its chances of exposure to a new host and reduce its exposure to destructive environmental forces (Filipiak *et al.*, 2009; Wongrak *et al.*, 2015). It has been proposed that in diurnal host species there is an evolutionary advantage to the parasite to be shed at higher levels in the afternoon, when the host might be returning to a roost site and closer proximity to other potential hosts and at the same time be less susceptible to the effects of desiccation and UV radiation; thereby increasing the survival of the parasite to an infective sporulated life stage (Martinaud *et al.*, 2009; Wongrak *et al.*, 2015). Evolutionary selection pressures will be different on each host-parasite relationship and will have produced a variety of parasite adaptations including different shedding patterns across the day. Given this potential diversity and

the importance of faecal sampling in determining the infection status of the host, the normal pattern of temporal shedding should be determined for each host-parasite relationship to inform interpretation of faecal results.

Whether coccidia of the *Eimeria* genus exhibit temporal variation in oocyst shedding is less clear. Early work described increased shedding of *Eimeria* sp. oocysts in naturally infected wild pigeons with maximum shedding occurring around midday and lower shedding in early morning and late afternoon (Boughton, 1937). Eimerian parasite infection in domestic poultry has been intensely studied for over a century and temporal shedding is repeatedly referred to as not having been documented (Long, 1982; Yabsley, 2008). However, temporal variation in oocyst shedding without variations in faecal output has been described for five species of *Eimeria* in the domestic chicken host (Levine, 1942). In this early experimental report four *Eimeria* spp. showed peak oocyst output in the afternoon and evening, after 3pm while one species examined, *E. necatrix*, had maximum oocyst output around 9am in the morning. This early morning increase in oocyst shedding for *E. necatrix* was associated with an increased proportion of faecal samples during this interval appearing to contain caecal contents and is therefore unsurprising given the tissue tropism of the sexual life stages of this parasite species for caecal mucosa. Therefore, this result in poultry may not represent a true temporal variation by the parasite, rather a temporal variation in the section of the host gut being voided. The target tissue of the *Eimeria sp.* in Takahē is currently not known. It is also unknown if Takahē produce faecal pellets of differing proportions of caecal contents across the day. Until enlightened by further research, the role of caecal contents in apparent oocyst shedding variations in Takahē remains unknown.

As well as the timing of the peak oocyst output the intensity of the temporal variation also differed between parasite species in this study, with *E. hagani* displaying the largest difference between peak and low oocyst shedding, while *E. mitis* had a less distinct variation between peak and low periods and therefore a less distinct pattern of temporal shedding (Levine, 1942). Similar to E. mitis, E. maxima was also found to have less distinction between peak and low oocyst shedding periods, with the apparent peak happening at inconsistent time of day. The authors proposed that the species with the most marked and consistent pattern of temporal shedding were the parasite species

with short generation intervals and the ability to output large numbers of oocysts rapidly, potentially giving them a competitive advantage in mixed coccidia infections within the same host. Birds in that study were experimentally infected with a controlled low dose of the relevant *Eimeria* sp. and did not show clinical disease. This may account for the apparent discrepancy between these results and the general assumption that faecal oocyst levels do not vary across the day in *Eimeria* spp. infections of domestic poultry. Once oocyst production is at an intensity capable of inducing clinical disease there will be large numbers of oocysts shed in faeces irrespective of the time of day. However, subclinical infections may be missed if faecal sampling occurs at an inappropriate time of day.

Temporal variation in oocyst shedding has also been demonstrated in Red-legged partridge (*Alectoris rufa*) infected with an *Eimeria* sp. during a natural outbreak, a repeatable pattern was seen of increased oocyst shedding in the late afternoon (Villanúa *et al.*, 2006), but not as pronounced a trend as was seen in the previously mentioned passerine/*Isospora* infections (Brawner & Hill, 1999). Villanua et.al. (2006) suggested that this less pronounced effect may have been due to to infections in this trial being largely asymptomatic and of low intensity or to inherent differences in the parasite-host systems, particularly the difference in the significance of caeca to the relevant host species. Galliformes, including the partridge, have a larger caecum by volume than the passerines, this could lead to variable dilution of faecal samples or concentration of oocysts depending on the target mucosa of a particular parasite species. The *Isospora sp*. infecting blackbirds is known to target intestinal epithelium and would not be susceptible to variations in caecal output (Filipiak *et al.*, 2009). Nematode egg distribution is known to not be uniform throughout a given faecal sample, a factor increased as the volume and fibrous portion of a typical host faecal deposit increases (Wongrak *et al.*, 2015). If uneven distribution in fibrous faecal deposits is also true for coccidia oocysts it may complicate the accuracy of oocyst counts in species such as partridge and Takahē making temporal variations in shedding less distinct in these species.

None of the Takahē in this trial displayed any signs of clinical disease and oocyst counts were considerably lower than have previously been detected at the same location.

Therefore, Takahē *Eimeria* sp. infection would appear to be consistent with increased shedding in the afternoon evident in sub-clinical infections, as suggested but not confirmed by the results of the current work. However, the caveat on this conclusion is that our study did not extend into the nocturnal hours, and because our research period was restricted to winter, confined to the very short daylight hours of this season.

Research avenues to further investigate temporal variations in oocyst shedding by Takahē would include repeating the above trial with a host population of high coccidia burden, in a milder season when the number of oocysts being shed would presumably be higher and faecal collections could be made across a longer period of the day. While this situation was not available at the time of the current research, in the future an opportunity may arise at an advocacy or island site as the population of this endangered species increases or if access to a population of non-breeding pre-release young birds were to become available.

4.5. Conclusion

Although not statistically robust, the results of this trial do suggest that Eimeria sp. infections in Takahē are more likely to be detected in samples collected after 3pm. And given the apparent low prevalence of infection in the birds sampled it is recommended that multiple samples are collected from an individual at this time of day to have more confidence in the results.

Chapter Five:

Evaluation of decoquinate safety in endangered South Island Takahē (*Porphyrio hochstetteri*).

5. Evaluation of decoquinate safety in endangered South Island Takahē (*Porphyrio hochstetteri*).

5.1. Chapter Introduction

Coccidia similar in appearance to the *Eimeria* genus have been detected in Takahē since the 1980's but have yet to be formally described. Concerns were raised in early 2015 about very high oocyst counts being detected in routine faecal analysis and the unconfirmed involvement of the coccidian parasite in the death of one adult Takahē recently relocated to a site of long-term high population density. At this time the total population of Takahē was estimated at 250 breeding age birds and the loss of any individual amounted to a significant loss in genetic potential (Takahē Recovery Programme, 2017). Consequently, control measures were investigated and implemented to limit losses to coccidiosis, alongside research to better understand the host-parasite relationship. One control measure investigated was the addition of the anti-protozoal drug, decoquinate, to the supplementary diet offered to Takahē at many mainland and Island breeding sites.

The strategic use of treatment with anticoccidial medications, such as toltrazuril, in poultry and wildlife, has been used in high value populations when critical thresholds of oocyst counts have been reached or during introduction programs to new, uninfected locations (Blake & Tomley, 2014; Quiroz-Castaneda & Dantan-Gonzalez, 2015; Witcombe & Smith, 2014). Knowledge of the lifecycle and pathogenicity of an *Eimeria* sp., host response and pathology together with environmental conditions that may influence transmission, for example crowding of susceptible juvenile hosts, are considered when determining these critical treatment thresholds. The commercial poultry industry is increasingly using vaccination with traditional attenuated strains or sub-unit vaccines as a cornerstone of their coccidiosis control program. At present the very small population of Takahē and the need to have strain specific vaccines makes this control strategy impractical for the captive management of this endangered species.

A mainstay of coccidiosis control in the commercial poultry industry is the inclusion of coccidiostat medications within the formulated diets offered (Conway & McKenzie, 2008a; Witcombe & Smith, 2014) and this continues to be the case despite widespread resistance of coccidia to many of the medications used. The continued effectiveness of these in-feed medications is often put down to the medications suppressing the protozoa to allow the host immune system to respond appropriately (Blake & Tomley, 2014). Takahē at several locations around New Zealand are currently fed a supplementary pelleted diet at a variety of rates and feeding intervals. Therefore, the potential to include a coccidiostat medication in the Takahē pellets was considered.

Decoquinate is a quinolone anti-protozoal medication used in the commercial poultry industry at a recommended dose rate of 30mg per kilogram of feed (0.003%) (Australian Pesticides and Veterinary Medicines Authority, 2016). It has been used safely and effectively in Chukar partridge (*Alectoris chukar*) up to 0.008% of the diet (Gerhold *et al.*, 2016). The mechanism of action is unknown but possibly involves the disruption and rearrangement of protozoal genetic material during meiosis for final stage schizonts, in addition to the disruption of mitochondrial electron transport in sporozoites and first stage schizonts (Del Cacho *et al.*, 2006). Therefore, decoquinate will not stop protozoa penetrating the host cells, but may act at one of two further stages to prevent replication or to create a sporocyst that is subsequently incapable of sporulation and therefore not infective. There are no reported adverse effects on the host when used at the recommended dose in poultry species and safety assessments have deemed decoquinate a non-hazardous substance with no detrimental effects when fed at excessive dosages for extended periods (Australian Pesticides and Veterinary Medicines Authority, 2016). In poultry, decoquinate is also poorly absorbed systemically and any absorption beyond the mucosa of the gastrointestinal tract is rapidly cleared (Conway & McKenzie, 2008a).

Given the apparent safety in domestic poultry and the availability of decoquinate in New Zealand it was selected to assess the safety in Takahē for possible use as a coccidiostat in medicated supplementary pellets. Decoquinate is not registered for use in egg producing birds due to the potential for drug residues in eggs destined for human consumption. The effect of decoquinate on egg structure, fertility and hatchability is

unknown in domestic poultry, consequently this trial was carried out during the non-breeding period on a combination of adult and juvenile birds. The aim of this research was to evaluate the the safety of administering decoquinate as an in-feed coccidian prophylaxis in Takahē, using measures of body weight, haematology and plasma biochemistry the efficacy of this anticoccidial medication was not assessed in this work. This trial was carried out under a Department of Conservation, Wildlife Act Authority, Permit number 45809-FAU, a Massey University Animal Ethics Committee approval 15-72 and with the support of Ngāi Tahu.

5.2. Materials and Methods

5.2.1. Diet Preparation

A supplementary pelleted diet formulated for Takahē was commercially prepared at the same facility that produces supplies for Takahē holding facilities on the North and South Islands (Massey University Poultry Unit Feed Mill). A medicated pellet containing 0.002% decoquinate was prepared. This concentration was calculated to achieve as close to the 0.003% use in commercial poultry diets (Del Cacho *et al.*, 2006), while remaining under the 4.5 mg/kg maximum dose established for poultry (Australian Pesticides and Veterinary Medicines Authority, 2016), if a juvenile bird weighing approximately 1 kg was to consume all of the bi-weekly 200 g of supplied pellets at once.

5.2.2. Feeding protocol

Pens of birds to be used in the trial were selected by the Takahē Recovery Team based on maximising the number of birds sampled in a minimum number of pens and prior knowledge of individual bird behaviour making repeated capture for sampling likely.

The eight selected pens contained between 3 and 5 birds (16 birds total) and these birds were offered the medicated pellets at a rate of approximately 50 g per bird, in place of the un-medicated pellets that they had previously been receiving. The medicated pellets were offered for a period of 8 weeks and were supplied in the same feed hoppers that the birds were already accustomed to using. Due to the limited number of sample subjects that were available it was not possible to sample a control group for this trial, therefore baseline pre-medication samples were collected from all birds.

For the purposes of the analysis the sixteen individual birds included in the trial comprised 10 males and 6 females and there were 8 adults and 8 juveniles. In order to maximise the number of subjects, all available pens located at both major sites (Gorge Hill and Summer) at Burwood Takahē Breeding Centre (hereafter referred to as Burwood) were used. Evaluation for difference between these pens was carried out as there were minor management differences between the sites, specifically the Gorge Hill pens have a higher level of available browse and are therefore fed a slightly lower amount of pellets each week, however the amount of decoquinate was still within the desired dose range.

5.2.3. Sample collection and analysis

Birds were captured following standard operating protocol for Burwood and were handled by experienced Takahē handlers for the minimum amount of time possible. The birds were captured for weight measurement and 0.5 mL of blood was sampled from the medial metatarsal vein and placed into a lithium heparin sample vial (BD Microtainer®). Samples were taken prior to starting the medicated pelleted diet and repeated 4 and 8 weeks later. Due to the capture technique used at each sampling period it was not possible to get reliable serial faecal samples from the birds in the trial for parasitological analysis.

At each sampling period heparinised blood was used to measure packed cell volume and plasma total solids. The remaining blood was centrifuged at 2000g (Total Lab Systems, TLS C1301) for 5 minutes within 4 hours of collection and the plasma was separated and stored frozen until biochemical analysis could be carried out using VetScan VS2 (Abaxis Ltd) and the Avian/Reptilian Profile. Analytes measured included aspartate aminotransferase (AST), creatinine kinase (CK), albumin (ALB), bile acids (BA), glucose (Glu), uric acid (UA), total protein (TP), globulin (Glob), total calcium (Ca), phosphorus (P), potassium, (K) and sodium (Na).

Fresh blood smears were made at the time of sample collection and stained using a commercial Romanovsky variant staining protocol (DiffQuik®) for manual differential white cell counts.

5.2.4. Statistical analysis

All statistical analysis was carried out using SPSS Statistic V23 (IBM). All parameters measured (body weight, haematological and biochemical parameters) were assessed for differences within and between individuals over time (month) using a repeated measures general linear model. The level of significance was 0.05.

5.3. Results

Medicated pellets were observed to be completely consumed in all pens at approximately the same rate as the un-medicated pellets, indicating no effect on palatability of pellets at this dose rate of decoquinate. There was no significant effect of decoquinate on the average body weight of the birds across the trial period (Figure 5-1). From the results of haematological and biochemical analysis of the blood (Table 5.1 and Table 5.2), there was a statistically significant decrease in estimated lymphocyte count ($p=0.01$) and albumin ($p=0.02$) over the 8 weeks of the trial. A statistically significant increase in plasma creatinine kinase ($p=0.04$), globulin ($p=0.02$) and plasma total calcium ($p=0.001$) was evident. Despite these significant variations over the course of the trial most blood parameters analysed remained within the limits of the normal ranges for the biochemical parameters (Youl, 2009). The exceptions were albumin that was consistently outside of the 10 – 18 g/L range published by Youl (2009) at all time points and phosphorus that has no published normal range in Takahē. There are no published normal haematological ranges for Takahē. Consequently, the published normal ranges for the Gough Moorhen (*Gallinula comeri*), a flightless rail from islands in the south Atlantic, were used as a point of comparison. By this extrapolated measure all parameters were within normal limits, except the packed cell volume which was consistently moderately above the expected range.

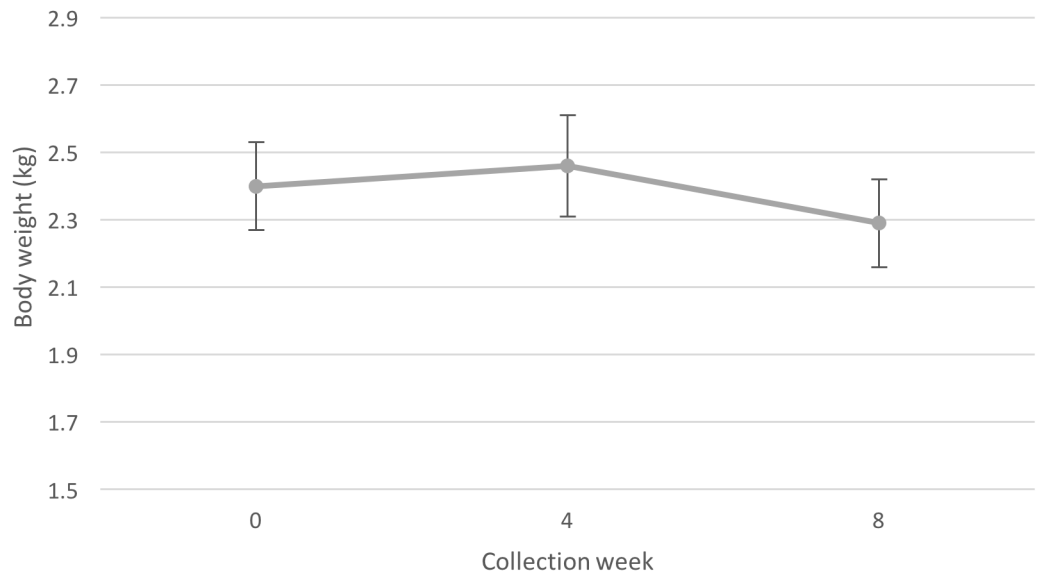

Figure 5-1: Body weight over 8 week decoquinate trial

Table 5.1: Results of haematological analysis over the three collection periods for Takahē being treated with decoquinate.

Test	Before	4 weeks	8 weeks	P-value	Reference range for Gough Moorhen (Dagleish *et al.*, 2017)
Packed cell volume (%)	47.60 ±0.93	48.00 ±0.95	47.63 ±1.3	0.79	21 - 41
White cell count ($x10^9$ cells/L)	13.04 ± 0.89	12.09 ± 0.77	11.36 ± 0.74	0.07	2.4 - 27
Heterophils ($x10^9$ cells/L)	7.88 ± 0.54	7.12 ± 0.37	7.59 ± 0.48	0.46	0.38 – 8.04
Lymphocytes ($x10^9$ cells/L)	**3.19 ± 0.48**	**3.57 ± 0.39**	**2.71 ± 0.24**	**0.01**	1.75 – 19.44
Monocytes ($x10^9$ cells/L)	1.10 ± 0.16	1.28 ± 0.16	0.99 ± 0.11	0.19	0 – 3.02
Eosinophils ($x10^9$ cells/L)	0.05 ± 0.02	0.06 ± 0.02	0.12 ± 0.02	0.23	0 – 0.33

Mean ± standard error with P-value of variation over the treatment period in a general linear model.

Table 5.2: Results of plasma biochemical analysis over the three collection periods for Takahē being treated with decoquinate.

Test	Before	4 weeks	8 weeks	P-value	Reference range (Youl, 2009)
AST (U/L)	345.80 ± 26.99	274.50 ± 24.20	327.20 ± 16.92	0.29	180 - 482
BA (μmol/L)	32.78 ± 4.06	24.7 ± 0.0	24.7 ± 0.0	0.09	17 - 61
CK (U/L)	**665.00 ± 76.10**	**676.38 ± 78.79**	**1063.40 ± 97.29**	**0.04**	330 - 3636
UA (μmol/L)	245.00 ± 43.79	284.07 ± 25.40	254.00 ± 40.25	0.18	172 - 858
Glu (mmol/L)	14.32 ± 2.72	11.80 ± 0.53	12.58 ± 0.90	0.40	2.3 - 20
TP (g/L)	**39.80 ± 2.44**	**39.63 ± 0.89**	**40.80 ± 1.53**	**0.71**	35 - 55
Alb (g/L)	**21.80 ± 1.53**	**20.75 ± 1.03**	**19.60 ± 1.12**	**0.02**	**10 - 18**
Glob (g/L)	**18.20 ± 1.46**	**18.63 ± 0.57**	**21.20 ± 0.86**	**0.02**	19 - 37
Ca (mmol/L)	**2.06 ± 0.09**	**2.37 ± 0.4**	**2.41 ± 0.03**	**0.001**	2.09 – 9.8
P (mmol/L)	1.19 ± 0.19	1.12 ± 0.15	0.94 ± 0.16	0.89	Not available
Na (mmol/L)	149.00 ± 5.31	142.75 ± 0.68	142.80 ± 0.86	0.98	136 - 152
K (mmol/L)	5.20 ± 0.31	4.57 ± 0.27	4.56 ± 0.46	0.55	2.6 – 8.9

Mean ± standard error with P-value of variation over the treatment period in a linear model.

5.4. Discussion

Management options utilised in the commercial poultry industries and those previously applied to wildlife populations elsewhere were used as a model for the Takahē coccidia control program. Techniques used in the poultry industry include maximising husbandry standards to reduce the environmental burden of infective oocysts, this includes minimising damp areas of substrate, avoiding overcrowding and taking steps to avoid faecal contamination of food and water resources (Blake & Tomley, 2014; Conway & McKenzie, 2008b; Yabsley, 2008). The largely successful coccidia control program in Whooping (*Grus americana*) and Sandhill (*Grus canadensis*) cranes included the previously mentioned steps as well as pen rotation and separation of birds by age class (Carpenter *et al.*, 2005). A further technique employed previously in both domestic poultry and wildlife breeding centres has been the addition of anti-coccidian

medications into pelletised diets. The safety of administrating one such medication, decoquinate, was evaluated in the endangered Takahē.

As assessed in this small number of birds (n=16 or 5% of the total species population), decoquinate had no deleterious effects in Takahē when fed at a rate of 0.002% of a pelletised diet. The small changes in some blood parameters that were statistically significant, with the exception of albumin, were still within the normal physiological ranges and are therefore unlikely to be significant to the birds' health. The mild increase in albumin was consistent across all collection times and may likely indicate a mild dehydration as a result of the capture process. This interpretation is also supported by the apparent elevation in packed cell volume. However, the latter could also be due to inherent differences between the reference moorhen species and the Takahē. The trend of decrease in body weight over the 8-week trial period was not statistically significant and may have been a combined effect of onset of winter on forage availability or air temperature and the decision by the Takahē Recovery Team during this period to reduce the pellet ration to some pens due to previous excessive weight gains.

In order to maximise the number of birds being offered the medicated pellets in this trial and at the same time minimise the number of birds disturbed for sample collection at monthly intervals, it was decided to not concurrently sample control birds fed un-medicated pellets. Instead the trial birds were sampled prior to starting the medicated pellets and acted as their own control group. A concurrently sampled control group may have allowed seasonal changes over the 8 week course of the trial to be accounted for.

Attempts were made to collect faecal samples from trial birds at each collection to concurrently assess efficacy in reducing the oocyst shedding rate. However, the capture technique of luring the birds to small holding pens for up to an hour before sampling, resulted in evacuation of the gut before faecal samples associated with specific individual birds could be collected. The supplementary feeding schedule at the Burwood is bi-weekly feeding of approximately 50 g of pellets per bird. Other sites maintaining Takahē have a variety of different feeding schedules from daily feeding of 50 g per bird to very occasional feeding of birds for luring purposes or when forage availability requires it. It has been demonstrated in domestic poultry that "pulse" or intermittent

feeding of medicated pellets can be effective, however, it can also contribute to the establishment of resistance of the coccidia to the medication (Witcombe & Smith, 2014). The potential for the feeding schedule to affect both the efficacy and the long-term usefulness of the medication needs to be considered when developing an efficacy evaluation trial and the potential widespread use of medicated pellets.

It would appear that decoquinate can safely be fed to non-breeding Takahē. However, the efficacy of the medication on the Takahē coccidia and the safety in feeding to egg laying birds remains to be evaluated. At the same time as this trial was being carried out, changes in husbandry and toltrazuril treatment protocols have resulted in an overall dramatic decline in the oocyst shedding rates by birds at the Takahē breeding centre and evidence for the coccidia causing notable disease in Takahē is limited (Chapter 3). There is increasing interest in preservation of parasites in their own right, as well as the endangered hosts to which they are specific (Moir *et al.*, 2012). Additionally, the Takahē coccidia has been identified in almost all locations with this host species and the eradication of the parasite from all breeding sites could result in birds that are immunologically naïve being released into environments with the parasite. Therefore it has to be considered if strict control or eradication of the Takahē *Eimeria* sp. at this time is desirable or necessary.

While the crisis of escalating faecal oocyst counts that precipitated this research has currently subsided, the potential remains for a recurrence of high parasite burdens in the captive breeding programme. This is particularly likely, given the pressures to maximise the output of juveniles from all breeding locations in order to speed numerical recovery of the endangered Takahē. A reluctance to spell pens and increasing stocking rates have the potential to contribute to a resurgence in environmental burdens of *Eimeria* and subsequently pathogenic effects in the Takahē host. The current work was a critical preliminary step in determining the safety of decoquinate administered in-feed in a pulse program to Takahē. Further work is required to assess the effectiveness of decoquinate against Takahē *Eimeria* sp. and to determine the appropriate pellet inclusion rate for pulse administration as current feeding regimes would allow. If an effective protocol can be determined it has the potential to become another tool alongside, husbandry and strategic toltrazuril treatment in the management of coccidia

in Takahē. The results also suggest that decoquinate is worth assessing as in in-feed medication for other endangered species captive breeding programmes that suffer losses from coccidia such as kiwi (*Apteryx* spp.) and whio/blue duck (*Hymenolaimus malacorhynchos*).

5.5. Conclusion

The addition of decoquinate at levels recommended for domestic poultry in the pelleted ration of Takahē was well tolerated by the birds and caused changes of minor clinical significance to the select group of biological parameters measured. Some parameters increased (creatinine kinase, globulin and total calcium) while other decreased (lymphocyte count and albumin) but all remained within the normal physiological ranges suggested for Takahē. Despite these minor changes, there were no major effects of decoquinate detected in body weight or other haematological or biochemical parameters measured that would suggest a concern for the continued use of this drug in this species. With the caveat of a necessarily small sample size in this trial, the results would suggest that decoquinate is safe for use in Takahē in the non-breeding season and with further research into its effectiveness it could be a useful additional tool to the management of disease in this species should more recent control measures prove to be ineffective in the longer term.

Chapter 6:

General Discussion

6. General discussion

The South Island Takahē has recovered from being thought extinct before 1948 to its current endangered status with the population estimated at 340 birds. The population of Takahē is distributed across wild, breeding centre and sanctuary sites and is managed as a metapopulation by the Takahē Recovery Program. Numerous translocations of birds between sites are carried out each year (Grange *et al.*, 2014) to maximise genetic diversity and breeding opportunities. These frequent bird movements together with the concentration of some subpopulations into high stocking density and the use of long term occupation sites, predispose the Takahē population to infectious disease. One such infectious disease agent that became a concern in the Takahē Recovery Program was a coccidian parasite.

Coccidian oocysts were first detected in Takahē faecal samples in the 1980s (B. Pomfrey, pers comm.) and have been regularly detected since. After a coccidian organism was implicated in the death of a recently translocated adult Takahē in 2013, increased parasitological screening was implemented to assess the presence of such parasites across the population. Between 2013 and 2015 there was a perceived general trend of increasingly high counts, particularly at sites of long-term Takahē habitation, and intermittent individual counts that were markedly elevated, some over 150,000 oocysts per gram of faeces. Clinical disease associated with coccidia has not been detected. Losses due to sub-clinical disease in-terms of fertility, juvenile growth rates and overall breeding output could not be defined but some degree of these pathological effects were likely at the levels of infection that were being detected. The regular exceptionally high oocyst counts and an apparent resistance of some infections to the recommended treatment with toltrazuril raised concerns about an impending crisis of coccidiosis in the Takahē population. Consequently, the research outlined in this thesis was commenced to initiate investigations into the Takahē coccidia, it's biology and potential management.

Included here is the first morphological description of an *Eimeria* sp. protozoa isolated from a Takahē host. In the current study, sporulated oocysts from Takahē faecal samples

was compared to *Eimeria* spp. isolated from other birds of the family Rallidae and were found to be distinct. Molecular analysis was outside the scope of the current work but would be required to complete the description of this new *Eimeria* species, *Eimeria hochstetteri* n.sp. This study was also the first to histologically identify protozoal life stages within the cells of a recently deceased Takahē host. The advanced state of decomposition of most submitted Takahē samples (McLelland *et al.*, 2011) has historically prevented the linkage of presence of oocysts in the intestinal contents and faecal samples with organisms within host cells. Coccidia from other hosts are known to have specific target tissues within the host with corresponding pathological effects, this could be different regions of the gut as occurs in domestic poultry (Conway & McKenzie, 2008c) or different organs systems as in Kiwi (Morgan *et al.*, 2013). The identification of protozoal life stages in the caudal gastrointestinal tract of Takahē suggests that the newly described *Eimeria* sp. is enterotropic, however further research is required to establish definitively the preferred target tissue and associated pathological effects. The standard approach of deliberate infection and culling of birds at predetermined time intervals to evaluate the lifecycle or pathogenic effects of a parasite are not available when working with an endangered species, like the Takahē. *Eimeria* sp. are generally considered largely host specific (Clark *et al.*, 2017), therefore infection of a more abundant model species, such as the Pūkeko (*Porphyrio melanotus*), is unlikely to be possible. Therefore, work to improve the quality of submitted samples and opportunistic sampling are the only avenues available to further describe the pathobiology of the Takahē *Eimeria* sp.

The Takahē population, while geographically dispersed, is highly interconnected with the vast majority of birds spending some time at the Burwood Takahē Breeding Centre (hereafter referred to as Burwood) before relocation to other sites (Grange *et al.*, 2014). Given this high level of interconnectedness between the fragmented populations it is not surprising that the newly described Takahē *Eimeria* sp. was found in the current study to be present throughout the vast majority of sites from which historical faecal samples were available. While the parasite may be already present at most sites currently holding Takahē there are still important considerations in relation to translocation of birds as part of species management. Handling, transport and re-

introduction involved in a translocation are all considerable stressors for any animal, but perhaps particularly the sensitive Takahē. The disease risks of translocation include exposure of a naïve translocated bird to a pathogen resident at the new site, recrudescence of existing disease in the translocated bird, and the introduction of a novel pathogen to a previously uncontaminated location (Jakob-Hoff *et al.*, 2014). The latter being particularly important as new sites for establishment of Takahē populations are developed.

Disease screening should be central to any translocation plan in addition to biological, environmental, logistical and political considerations. In the specific case of *Eimeria hochstetteri* n.sp and Takahē translocations, repeat faecal sampling would be required to establish the presence or absence of the parasite within the relocated bird and in the population at the intended site, if applicable. The collection and interpretation of such samples is complicated by the findings in Chapter 4 of a temporal variation in oocysts shedding rates across the day, with a potential increase in oocyst output after 3pm. The limitations imposed on the current work related to minimising disturbance on breeding pairs of an endangered species and included only collecting samples in the short days of winter from non-breeding birds and the use of small available samples sizes. These limitations make the findings of temporal variability of oocyst shedding less robust than would be desired. Research to repeat the temporal shedding trial, described in this thesis, using a high coccidia burden host population in a period of more practical day length and environmental conditions is required to further evaluate the phenomena of temporal variation in oocyst shedding. As they currently stand the findings do suggest that important pre-translocation screening faecal results should be interpreted with care as faeces passed during the hours of daylight and before 3pm may lead to false negative results.

The investigations into the *Eimeria* sp. affecting Takahē described in this thesis are the initial steps in describing the pathobiology of this host-parasite relationship. These investigations were often restricted by the endangered status of the host species, with each individual bird and potential breeding event being of critical importance to the survival of the species. For the same reason investigations into the host-parasite relationship needed to be concurrent with the development of management strategies

to prevent any further clinical or subclinical losses to coccidian infection and to prevent the envisaged crisis of escalating faecal oocyst counts. Modification of treatment protocols, medication handling and some husbandry actions at the central captive breeding centre were temporally associated with a sustained reduction in Eimeria sp. shedding rates at the facility. At the same time, through the current work, coccidia management techniques developed for other species started to be evaluated for its applicability to Takahē. Specifically, the practice in the commercial poultry industry, of including coccidiostatic medications into formulated diets to suppress coccidian replication or oocyst viability and therefore transmission. One such anticoccidial medication, decoquinate, was evaluated in a small sample of Takahē and found to cause no effects of clinical significance in the biological parameters tested. Further investigation is required into the effectiveness of decoquinate to reduce coccidian oocyst shedding and its safety in breeding birds, however, it's potential as a management tool is promising given its palatability in the pelleted ration and lack of measurable significant side effects on the host.

The research presented in this thesis are the preliminary investigations into an *Eimeria* sp. affecting the endangered Takahē, the distribution amongst the fragmented host population, effectiveness of control measures and safety of potential future management options. The impending crisis, which prompted this research, of regular extreme oocyst counts, increasing regularity of high burdens and coccidia associated deaths, was resolved with management changes. However, while the survival of the species is dependent on breeding centres with high population densities and translocation of individuals, there is the potential for such a crisis to return. Further research is recommended into the pathobiology of *Eimeria hochstetteri* n.sp and the management of the host-parasite relationship in current and future conservation programs. These studies on coccidia and Takahē clearly demonstrate the unintended consequences of intensive conservation management strategies on host-pathogen systems and that even highly host adapted parasites can cause problems when the host-parasite dynamic is unbalanced.

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